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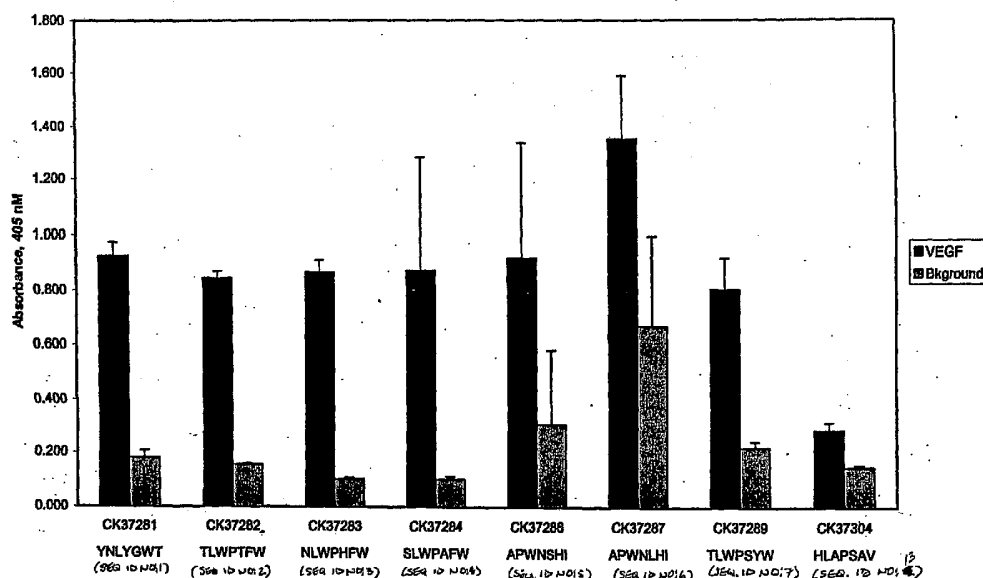
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[Continued on next page]

- (54) Title: PERSONAL CARE COMPOSITIONS AND METHODS FOR THEIR USE



- (57) Abstract: The present invention provides peptides and supported peptides for treating various diseases and conditions. In particularly preferred embodiments, the present invention provides compositions and methods for personal care. In some embodiments, the present invention provides compositions for use in skin and/or hair care, as well as cosmetic compositions. In alternative particularly preferred embodiments, the present invention provides peptides and supported peptides for treating diseases of the skin, such as rosacea. In some particularly preferred embodiments, the supported peptides of the present invention are anti-VEGF peptides. In alternative particularly preferred embodiments, the anti-VEGF peptides are expressed on a scaffold protein. In some most preferred embodiments, the scaffold protein comprises BBI.



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PERSONAL CARE COMPOSITIONS AND METHODS FOR THEIR USE

FIELD OF THE INVENTION

5 The present invention provides peptides and supported peptides for treating various diseases and conditions. In particularly preferred embodiments, the present invention provides compositions and methods for personal care. In some embodiments, the present invention provides compositions for use in skin and/or hair care, as well as cosmetic compositions. In alternative particularly preferred embodiments, the present invention provides peptides and supported peptides for treating diseases of the skin, such as
10 rosacea. In some particularly preferred embodiments, the supported peptides of the present invention are anti-VEGF peptides. In alternative particularly preferred embodiments, the anti-VEGF peptides are expressed on a scaffold protein. In some most preferred embodiments, the scaffold protein comprises BBI.

BACKGROUND OF THE INVENTION

15 Angiogenesis is the development of a blood supply to a given area of tissue. Angiogenesis is part of normal embryonic development and revascularization of wound beds, as well as due to the stimulation of vessel growth by inflammatory or malignant cells. Angiogenesis is also the process through which tumors or inflammatory conditions derive a blood supply through the generation of microvessels.

 Angiogenesis is regulated in normal and malignant cancer tissues by the balance of angiogenic
20 stimuli and angiogenic inhibitors that are produced in the target tissue and at distant sites (*See*, Fidler *et al.*, [1998]; and McNamara *et al.*, [1998]). Vascular endothelial growth factor-A (VEGF, also known as vascular permeability factor, "VPF") is a primary stimulant of angiogenesis. VEGF is a multifunctional cytokine that is induced by hypoxia and oncogenic mutations and can be produced by a wide variety of tissues (*See*, Kerbel *et al.*, [1998]; and Mazure *et al.*, [1996]).

25 The recognition of VEGF as a primary stimulus of angiogenesis in pathological conditions has led to various attempts to block VEGF activity. Inhibitory anti-VEGF receptor antibodies, soluble receptor constructs, antisense strategies, RNA aptamers against VEGF and low molecular weight VEGF receptor tyrosine kinase (RTK) inhibitors have all been proposed for use in interfering with VEGF signaling (*See*, Siemeister *et al.*, [1998]). In fact, monoclonal antibodies against VEGF have been shown to inhibit human
30 tumor xenograft growth and ascites formation in mice (*See*, Kim *et al.*, [1993]; Asano *et al.*, [1998]; Mesiano *et al.*, [1998]; Luo *et al.*, [1998a] and [1998b]; and Borgstrom *et al.*, [1996] and [1998]).

 RTKs comprise a large family of transmembrane receptors for polypeptide growth factors with diverse biological activities. The intrinsic function of RTKs is activated upon ligand binding, which results in phosphorylation of the receptor and multiple cellular substrates, and subsequently in a variety of cellular
35 responses. (*See*, Ullrich & Schlessinger, Cell 61:203-212 [1990]).

Angiogenesis, involving VEGF and RTKs is not only involved in cancer development, as many other diseases or conditions affecting different physiological systems are angiogenesis-dependent, such as arthritis and atherosclerotic plaques (bone and ligaments), diabetic retinopathy, neovascular glaucoma, macular degeneration, ocular herpes, trachoma and corneal graft neovascularization (eye), psoriasis, scleroderma, rosacea, hemangioma and hypertrophic scarring (skin), vascular adhesions and angiofibroma (blood system).

VEGF is an angiogenesis factor of major importance for skin vascularization (Detmar [2000]). VEGF expression is upregulated in the hyperplastic epidermis of psoriasis (Detmar and Yeo *et al.* [1995]), in healing wounds and in other skin diseases characterized by enhanced angiogenesis (Detmar [2000], *supra*). Targeted overexpression of VEGF in the epidermis of transgenic mice was reported to result in enhanced skin vascularization with equal numbers of tortuous and leaky blood vessels (*See e.g.*, Brown *et al.* [1998]). Also, chronic synthesis of VEGF in mouse skin leads to the first histologically equivalent murine model of human psoriasis (Xia *et al.* [2003]) that is reversible by binding agents specific for VEGF.

Proteases are involved in a wide variety of biological processes. Disruption of the balance between proteases and protease inhibitors is often associated with pathologic tissue destruction. Indeed, various studies have focused on the role of proteases in tissue injury, and it is thought that the balance between proteases and protease inhibitors is a major determinant in maintaining tissue integrity. Serine proteases from inflammatory cells, including neutrophils, are implicated in various inflammatory disorders, such as pulmonary emphysema, arthritis, atopic dermatitis and psoriasis.

Proteases also appear to function in the spread of certain cancers. Normal cells exist in contact with a complex protein network, called the extracellular matrix (ECM). The ECM is a barrier to cell movement and cancer cells must devise ways to break their attachments, degrade, and move through the ECM in order to metastasize. Proteases are enzymes that degrade other proteins and have long been thought to aid in freeing the tumor cells from their original location by chewing up the ECM. Recent studies have suggested that they may promote cell shape changes and motility through the activation of a protein in the tumor cell membrane called Protease-Activated Receptor-2 (PAR2). This leads to a cascade of intracellular reactions that activates the motility apparatus of the cell. Thus, it is hypothesized that one of the first steps in tumor metastasis is a reorganization of the cell shape, such that it forms a distinct protrusion at one edge facing the direction of migration. The cell then migrates through a blood vessel wall and travels to distal locations, eventually reattaching and forming a metastatic tumor. For example, human prostatic epithelial cells constitutively secrete prostate-specific antigen (PSA), a kallikrein-like serine protease, which is a normal component of the seminal plasma. The protease acts to degrade the extracellular matrix and facilitate invasion of cancerous cells.

Synthetic and natural protease inhibitors have been shown to inhibit tumor promotion *in vivo* and *in vitro*. Previous investigations have indicated that certain protease inhibitors belonging to a family of structurally-related proteins classified as serine protease inhibitors or SERPINS, are known to inhibit several

proteases including trypsin, cathepsin G, thrombin, and tissue kallikrein, as well as neutrophil elastase. The SERPINS are extremely effective at preventing/suppressing carcinogen-induced transformation *in vitro* and carcinogenesis in animal model systems. Systemic delivery of purified protease inhibitors apparently reduces joint inflammation and cartilage and bone destruction as well.

5 Topical administration of protease inhibitors finds use in such conditions as atopic dermatitis, a common form of inflammation of the skin, which may be localized to a few patches or involve large portions of the body. The depigmenting activity of protease inhibitors and their capability to prevent ultraviolet-induced pigmentation have been demonstrated both *in vitro* and *in vivo* (*See e.g.*, Paine *et al.*, J. Invest. Dermatol., 116:587-595 [2001]). Protease inhibitors have also been reported to facilitate wound healing.
10 For example, secretory leukocyte protease inhibitor was demonstrated to reverse the tissue destruction and speed the wound healing process when topically applied. In addition, serine protease inhibitors can also help to reduce pain in lupus erythematosus patients (*See e.g.*, US Patent No. 6537968).

 The Bowman-Birk protease inhibitor (BBI) is a designation of a family of stable, low molecular weight trypsin and chymotrypsin enzyme inhibitors found in soybeans and various other seeds, mainly
15 leguminous seeds and vegetable materials. BBI comprises a family of disulfide bonded proteins with a molecular weight of about 8 kD (*See e.g.*, Chou *et al.*, Proc. Natl. Acad. Sci. USA 71:1748-1752 [1974]; Yavelow *et al.*, Proc. Natl. Acad. Sci. USA 82:5395-5399 [1985]; and Yavelow *et al.*, Cancer Res. (Suppl.) 43:2454s-2459s [1983]). BBI has a pseudo-symmetrical structure of two tricyclic domains each containing an independent native binding loop, the native loops containing binding sites for both trypsin and
20 chymotrypsin (*See*, Liener, *in* Summerfield and Bunting (eds), Advances in Legume Science, Royal Bot. Gardens, Kew, England). These binding sites each have a canonical loop structure, which is a motif found in a variety of serine proteinase inhibitors (Bode and Huber, Eur. J. Biochem., 204:433-451 [1992]). Commonly, as in one of the soybean inhibitors, one of the native loops inhibits trypsin and the other inhibits chymotrypsin (*See*, Chen *et al.*, J. Biol. Chem., 267:1990-1994 [1992]; Werner & Wemmer, Biochem.,
25 31:999-1010 [1992]; Lin *et al.*, Eur. J. Biochem., 212:549-555 [1993]; and Voss *et al.*, Eur. J. Biochem., 242:122-131 [1996]) though in other organisms (*e.g.*, *Arabidopsis*), both loops are specific for trypsin.

 STI inhibits the proteolytic activity of trypsin by the formation of a stable stoichiometric complex (*See e.g.*, Liu, Chemistry and Nutritional Value of Soybean Components, *In: Soybeans, Chemistry, Technology and Utilization*, pp. 32-35, Aspen Publishers, Inc., Gaithersburg, Md., [1999]). STI consists of
30 181 amino acid residues with two disulfide bridges and is roughly spherically shaped (*See e.g.*, Song *et al.*, J. Mol. Biol., 275:347-63 [1998]). The trypsin inhibitory loop lies within the first disulfide bridge. The Kunitz-type soybean trypsin inhibitor (STI) has played a key role in the early study of proteinases, having been used as the main substrate in the biochemical and kinetic work that led to the definition of the standard mechanism of action of proteinase inhibitors.

35 Eglin C is a small monomeric protein that belongs to the potato chymotrypsin inhibitor family of serine protease inhibitors. The proteins that belong to this family are usually small (60-90 amino acid

residues in length) and contain no disulfide bonds. Eglin C, however, is highly resistant to denaturation by acidification or heat regardless of the lack of disulfide bonds to help stabilize its tertiary structure. The protein occurs naturally in the leech *Hirudo medicinalis*.

As noted above, protease inhibitors interfere with the action of proteases. Naturally occurring protease inhibitors can be found in a variety of foods such as cereal grains (oats, barley, and maize), Brussels sprouts, onion, beetroot, wheat, finger millet, and peanuts. One source of interest is the soybean. The average level of protease inhibitors present in soybeans is around 1.4 percent and 0.6 percent for Kunitz and Bowman-Birk respectively, two of the most important protease inhibitors. Notably, these low levels make it impractical to isolate the natural protease inhibitor for clinical and other applications. Indeed, despite much research in the personal care arena, there remains a need in the art for personal care compositions that have desired characteristics without undesirable chemical modification of the proteins. There also remains a need in the art for a method of delivering a protein into a personal care composition so as to effectively deliver the protein in a useable form.

SUMMARY OF THE INVENTION

The present invention provides peptides and supported peptides for treating various diseases and conditions. In particularly preferred embodiments, the present invention provides compositions and methods for personal care. In some embodiments, the present invention provides compositions for use in skin and/or hair care, as well as cosmetic compositions. In alternative particularly preferred embodiments, the present invention provides peptides and supported peptides for treating diseases of the skin, such as rosacea. In some particularly preferred embodiments, the supported peptides of the present invention are anti-VEGF peptides. In alternative particularly preferred embodiments, the anti-VEGF peptides are expressed on a scaffold protein. In some most preferred embodiments, the scaffold protein comprises BBI.

In some preferred embodiments, the present invention provides cosmetic and/or pharmaceutical compounds suitable for improving the appearance of skin. The present invention further provides peptides that block binding of a protein. In some preferred embodiments, the protein is VEGF. In some particularly preferred embodiments, the peptide is expressed in a protease-resistant scaffold. In some especially preferred embodiments, the scaffold is a protease inhibitor (*e.g.*, BBI, STI, or Eglin chymotrypsin inhibitor). In some most preferred embodiments, the protease inhibitor is BBI.

In some embodiments, the present invention provides personal care compositions comprising a scaffold, wherein the scaffold comprises at least one protease inhibitor and at least one peptide selected from the group consisting of SEQ ID NOS:1-17. In alternative embodiments, any of the additional peptide sequences provided herein find use in the present invention. In further embodiments, additional sequences find use in the present invention, including but not limited to SEQ ID NOS:20-25, 31, 32-34, 43, and 238. In yet further embodiments, the at least one peptide

sequence is selected from the group consisting of YNLYGWT (SEQ ID NO:1), KYLYLYWW (SEQ ID NO:239), WYTLYKW (SEQ ID NO:240), TYRLYWW (SEQ ID NO:241), RYSLYYW (SEQ ID NO:242), YYLYYW (SEQ ID NO:243), NYQLYGW (SEQ ID NO:244), TLWKSYYW (SEQ ID NO:245), TKWPSYYW (SEQ ID NO:246), PLWPSYYW (SEQ ID NO:247), RLWPSYYW (SEQ ID NO:248), TLWPKYW (SEQ ID NO:249), KYDLYWW (SEQ ID NO:33), RYDLYWW (SEQ ID NO:250), DYRLYWW (SEQ ID NO:251), DYKLYWW (SEQ ID NO:34), EYKLYWW (SEQ ID NO:252), and RYPLYWW (SEQ ID NO:253). In some particularly preferred embodiments, the peptide sequence comprises the motif set forth in SEQ ID NO:31. In yet further embodiments, the scaffold comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:22-25.

In yet additional embodiments, the protease inhibitor is selected from the group consisting of Bowman-Birk inhibitor, soybean trypsin inhibitor, and Elgin chymotrypsin inhibitor. In some particularly preferred embodiments, the Bowman-Birk inhibitor is a modified Bowman-Birk inhibitor. In further embodiments, the scaffold comprises from about 0.001 weight percent to about 5 weight percent of the personal care composition, while in alternative embodiments, the scaffold comprises from about 0.01 weight percent to about 2.0 weight percent of the personal care composition, and in yet additional embodiments, the scaffold comprises from about 0.01 weight percent to about 1 weight percent of the personal care composition.

The present invention also provides personal care compositions comprising skin care compositions. In some preferred embodiments, the skin care compositions are selected from the group consisting of skin creams, lotions, sprays, emulsions, colloidal suspensions, foams, aerosols, liquids, gels, sera, and solids. In additional embodiments, the skin care compositions are moisturizing body washes, body washes, antimicrobial cleansers, skin protective creams, body lotions, facial creams, moisturizing creams, facial cleansing emulsions, facial gels, facial sera, surfactant-based facial cleansers, facial exfoliating gels, anti-acne treatments, facial toners, exfoliating creams, facial masks, after shave balms, pre-shave balms, tanning compositions, skin lightening compositions, skin redness reduction compositions, sunscreens, depilatories, hair growth inhibitors, and radioprotectives. In additional embodiments, the skin care compositions comprise topically applied over-the-counter compositions, anti-fungal treatments, anti-acne treatments, skin protectants, sunscreens, deodorants, and antiperspirants.

In some preferred embodiments, the skin care compositions are capable of lightening the skin tone, while in alternative embodiments the skin care compositions are capable of reducing redness in skin tone. In yet further embodiments, the skin care compositions are capable of preventing skin tone darkening, while in additional embodiments, the skin care compositions are capable of preventing skin color development. In some preferred embodiments, the skin care compositions are radioprotective. In alternative embodiments, the skin care compositions comprise at least one radioprotective. In some particularly preferred embodiments, the radioprotectives are selected from the group consisting of sunscreens. In some most

preferred embodiments, the sunscreens are selected from non-water-resistant sunscreens, very water-resistant sunscreens, and water-in-silicone sunscreens.

The present invention also provides personal care compositions that are capable of preventing hair growth. In some embodiments, the hair is selected from the group consisting of facial hair, leg hair, arm
5 hair, and torso hair.

The present invention also provides personal care compositions that are hair care compositions. In some embodiments, the hair care composition is selected from the group consisting of shampoos, conditioners, hair styling compositions, hair colorants, permanent wave formulations, creams, gels, mousses, sprays, emulsions, colloidal suspensions, liquids, foams, and solids. In further embodiments, the hair care
10 compositions comprise at least one radioprotectant. In some preferred embodiments, the radioprotectant is a sunscreen selected from non-water-resistant sunscreens, very water-resistant sunscreens, and water-in-silicone sunscreens. In some embodiments, the hair care composition is radioprotective.

The present invention further provides personal care compositions that are oral care compositions. In some preferred embodiments, the oral care compositions are selected from the group consisting of
15 toothpastes, tooth gels, mouth rinses, mouthwashes, anti-caries compositions, tooth whitening compositions, chewing gums, denture adhesives, and breath fresheners.

The present invention also provides personal care compositions that are cosmetic compositions. In some preferred embodiments, the cosmetic compositions are selected from eye gels, eye shadows, high-melting point lipsticks, lipsticks, lip glosses, lip balms, mascaras, eyeliners, pressed powder formulations,
20 and foundations. In some preferred embodiments, the makeup compositions comprise at least one pigment.

In some preferred embodiments, the makeup composition comprising at least one pigment is a mascara selected from non-waterproof mascaras, waterproof mascaras, volumizing mascaras, lengthening mascaras, curling mascaras, anhydrous waterproof mascaras, water-based mascaras, and eyelash or eyebrow treatments.

In yet additional embodiments, the makeup compositions are pressed powder formulations selected from loose powders, blushes, eye shadows, and bronzing powders. In still further embodiments, the makeup compositions are foundations selected from water-in-oil foundations, water-in-silicone foundations, oil-in-water foundations, anhydrous makeup sticks, and cream-to-powder foundations.

The present invention further provides personal care compositions having a scaffold, wherein the
30 scaffold comprises the amino acid sequence set forth in SEQ ID NO:19. In additional embodiments, the scaffold further comprises the amino acid sequence(s) set forth in SEQ ID NOS:20 and/or 21. In alternative embodiments, the amino acid sequence(s) set forth in SEQ ID NOS: 20 and/or 21 is/are replaced by at least one peptide having an amino acid sequence selected from the group consisting of SEQ ID NOS:1-17. In alternative embodiments, any of the peptide sequences provided herein find use as a replacement of SEQ ID
35 NOS:20 and/or 21. In alternative embodiments, any of the additional peptide sequences provided herein find use in the present invention. In further embodiments, additional sequences find use in the present

invention, including but not limited to SEQ ID NOS:20-25, 31, 32-34, 43, and 238. In yet further embodiments, the at least one peptide sequence is selected from the group consisting of YNLYGWT (SEQ ID NO:1), KYLYYW (SEQ ID NO:239), WYTLYKW (SEQ ID NO:240), TYRLYW (SEQ ID NO:241), RYSLYW (SEQ ID NO:242), YYLYWK (SEQ ID NO:243), NYQLYGW (SEQ ID NO:244),
5 TLWKS YW (SEQ ID NO:245), TKWPS YW (SEQ ID NO:246), PLWPS YW (SEQ ID NO:247), RLWPS YW (SEQ ID NO:248), TLWPK YW (SEQ ID NO:249), KYDLYW (SEQ ID NO:33), RYDLYW (SEQ ID NO:250), DYRLYW (SEQ ID NO:251), DYKLYW (SEQ ID NO:34), EYKLYW (SEQ ID NO:252), and RYPLYW (SEQ ID NO:253). In some particularly preferred embodiments, the peptide sequence comprises the motif set forth in SEQ ID NO:31. In yet further
10 embodiments, the scaffold comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:22-25.

The present invention also provides methods for making the personal care compositions of the present invention, comprising combining an effective amount of the scaffold and at least one physiologically acceptable carrier or excipient.

15 The present invention further provides methods for modifying the skin tone of a subject, comprising the steps of: providing at least one composition comprising a personal care composition of the present invention; ii) providing a subject to be treated; and applying the composition to the subject in an area in which modifications to the subject's skin tone is desired. In some embodiments, the modification of skin tone comprises lightening the subject's skin tone. In some alternative embodiments, the modification of skin
20 tone comprises reducing redness in the subject's skin tone. In yet additional embodiments, the methods comprise a personal care composition comprising the amino acid sequence set forth in SEQ ID NO:19. In alternative embodiments of the methods, the personal care composition comprises at least one of the amino acid sequences set forth in SEQ ID NOS:20 and 21. In yet further embodiments of the methods, at least one of the amino acid sequences set forth in SEQ ID NOS:20 and 21 is replaced by at least one peptide having
25 an amino acid sequence selected from the group consisting of SEQ ID NOS:1-17. In alternative embodiments, any of the additional peptide sequences provided herein find use as a replacement of SEQ ID NOS:20 and/or 21. In further embodiments, additional sequences find use in the present invention, including but not limited to SEQ ID NOS:20-25, 31, 32-34, 43, and 238. In yet further embodiments, the at least one peptide sequence is selected from the group consisting of YNLYGWT (SEQ ID NO:1),
30 KYLYYW (SEQ ID NO:239), WYTLYKW (SEQ ID NO:240), TYRLYW (SEQ ID NO:241), RYSLYW (SEQ ID NO:242), YYLYWK (SEQ ID NO:243), NYQLYGW (SEQ ID NO:244), TLWKS YW (SEQ ID NO:245), TKWPS YW (SEQ ID NO:246), PLWPS YW (SEQ ID NO:247), RLWPS YW (SEQ ID NO:248), TLWPK YW (SEQ ID NO:249), KYDLYW (SEQ ID NO:33), RYDLYW (SEQ ID NO:250), DYRLYW (SEQ ID NO:251), DYKLYW (SEQ ID NO:34),
35 EYKLYW (SEQ ID NO:252), and RYPLYW (SEQ ID NO:253). In some particularly preferred embodiments, the peptide sequence comprises the motif set forth in SEQ ID NO:31. In yet further

embodiments, the scaffold comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:22-25.

The present invention also provides methods for modifying the hair growth of a subject, comprising the steps of: providing the personal care composition of the present invention; providing a subject to be
 5 treated; and applying the composition to the subject in an area in which modifications to the subject's hair growth is desired. In some embodiments, the modification of hair modification of hair growth comprises inhibiting the growth of the subject's hair, wherein the hair to be inhibited is selected from the group consisting of facial air, underarm hair, leg hair, torso hair, and arm hair, and head hair. In yet additional
 10 embodiments, the methods comprise a personal care composition comprising the amino acid sequence set forth in SEQ ID NO:19. In alternative embodiments of the methods, the personal care composition comprises at least one of the amino acid sequences set forth in SEQ ID NOS:20 and 21. In yet further embodiments of the methods, at least one of the amino acid sequences set forth in SEQ ID NOS:20 and 21 is replaced by at least one peptide having an amino acid sequence selected from the group consisting of SEQ
 15 ID NOS:1-17. In some alternative embodiments, any of the additional peptide sequences provided herein find use as a replacement of SEQ ID NOS:20 and/or 21. In further embodiments, additional sequences find use in the present invention, including but not limited to SEQ ID NOS:20-25, 31, 32-34, 43, and 238. In yet further embodiments, the at least one peptide sequence is selected from the group consisting of
 YNLYGWT (SEQ ID NO:1), KYLYWW (SEQ ID NO:239), WYTLYKW (SEQ ID NO:240),
 TYRLYWW (SEQ ID NO:241), RYSLYWW (SEQ ID NO:242), YYLYWW (SEQ ID NO:243),
 20 NYQLYGW (SEQ ID NO:244), TLWKSYY (SEQ ID NO:245), TKWPSYY (SEQ ID NO:246),
 PLWPSYY (SEQ ID NO:247), RLWPSYY (SEQ ID NO:248), TLWPKYY (SEQ ID NO:249),
 KYDLYWW (SEQ ID NO:33), RYDLYWW (SEQ ID NO:250), DYRLYWW (SEQ ID NO:251),
 DYKLYWW (SEQ ID NO:34), EYKLYWW (SEQ ID NO:252), and RYPLYWW (SEQ ID NO:253). In
 some particularly preferred embodiments, the peptide sequence comprises the motif set forth in SEQ ID
 25 NO:31. In yet further embodiments, the scaffold comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:22-25.

In some embodiments, the present invention provides cosmetic and/or pharmaceutical compounds for improving the appearance of skin comprising at least one polypeptide or a peptide. In some preferred
 30 embodiments, the polypeptide or peptide binds to VEGF. In alternative embodiments, the binding of the polypeptide or peptide to VEGF blocks the downstream activity of VEGF. In some embodiments, the compounds comprise at least one peptide, while in other embodiments, the compounds comprise at least one polypeptide. In some preferred embodiments, the peptide has an amino acid sequence selected from the group consisting of SEQ ID NOS:1-17. In still further embodiments, the peptide has an amino acid
 35 sequence selected from the group consisting of KYDLYWW (SEQ ID NO:33) and DYKLYWW (SEQ ID NO:34). In additional preferred embodiments, the peptide has a conserved binding sequence, the sequence

being XXLWPXWC (SEQ ID NO:15). In some preferred embodiments, the sequence comprises SEQ ID NO:15. In further embodiments, the sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:1-17. In further embodiments, the sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:22-25. In alternative preferred embodiments, the compounds have a sequence, the sequence being at least 70%, preferably 80%, more preferably 90%, and most preferably 95% homologous to the sequences set forth herein. In some preferred embodiments, the polypeptide has a molecular weight that is preferably between 500 Daltons and 30,000 Daltons, more preferably between 1000 Daltons and 10,000 Daltons, and most preferably from 1500 Daltons to 8,000 Daltons.

In some preferred embodiments, the compounds find use in the improvement of skin in an organism (*i.e.*, subject) having a skin disorder. In some preferred embodiments, the skin disorder is an angiogenic skin disorder. In additional preferred embodiments, the skin disorder is at least one selected from the group consisting of psoriasis, venous ulcers, acne, rosacea, warts, eczema, hemangiomas and lymphangiogenesis, etc. In some particularly preferred embodiments, the skin disorder is rosacea.

In other preferred embodiments, the present invention provides cosmetic and/or pharmaceutical compounds for improving the appearance of skin. In these preferred embodiments, the compounds comprise at least one peptide or polypeptide and at least one scaffold, the peptide or polypeptide being expressed in the scaffold. In some particularly preferred embodiments, the at least one peptide or polypeptide is a loop. In other particularly preferred embodiments, the loop is closed by a disulfide bond. In some preferred embodiments, the polypeptide or peptide binds to VEGF. In alternative embodiments, the binding of the polypeptide or peptide to VEGF blocks the downstream activity of VEGF. In some particularly preferred embodiments, the peptide is expressed in a protease-resistant scaffold. In some especially preferred embodiments, the scaffold is a protease inhibitor (*e.g.*, BBI, STI, or Eglin chymotrypsin inhibitor). In some most preferred embodiments, the protease inhibitor is BBI.

In some preferred embodiments, the compounds further comprise at least one peptide. Preferably, the peptide has an amino acid sequence selected from the group consisting of SEQ ID NOS: 1-17. Most preferably, the compounds comprise an amino acid sequence selected from the group consisting of SEQ ID NOS:22-25. In some preferred embodiments, the peptide has a conserved binding sequence, the sequence being XXLWPXWC (SEQ ID NO:15). In some preferred embodiments, the compounds have a sequence, the sequence being at least 70%, preferably 80%, more preferably 90%, and most preferably 95% identical to the sequences set forth herein. The peptide molecular weight is preferably between 500 Daltons and 45,000 Daltons, more preferably between 1000 Daltons and 12,000 Daltons, and most preferably from 1500 Daltons to 10,000 Daltons. In some preferred embodiments, the compounds comprise at least one polypeptide.

The present invention provides compositions comprising at least one peptide selected from the group consisting of SEQ ID NOS:1-17, wherein the peptide binds to a vascular endothelial growth factor. In

some preferred embodiments, the peptide is expressed in a protease resistant scaffold. In alternative preferred embodiments, the scaffold comprises a protease inhibitor. In some more preferred embodiments, the protease inhibitor is selected from the group consisting of Bowman-Birk Inhibitor, soybean trypsin inhibitor, and Eglin chymotrypsin inhibitor. In some most preferred embodiments, the scaffold is Bowman-Birk inhibitor. In still further embodiments, the protease resistant scaffold and the peptide comprise a fusion protein. In some particularly preferred embodiments, the composition comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:22-25. In additional embodiments, the scaffold comprises the amino acid sequence set forth in SEQ ID NO:19. In still further embodiments, the scaffold comprises at least one of the amino acid sequences set forth in SEQ ID NOS:20 and 21. In yet additional embodiments, at least one of the amino acid sequences set forth in SEQ ID NOS:20 and 21 is replaced by at least one peptide having an amino acid sequence selected from the group consisting of SEQ ID NOS:1-17.

The present invention also provides cosmetic and/or pharmaceutical compositions comprising the at least one peptide that binds to a vascular endothelial growth factor. In some embodiments, the composition is capable of modulating angiogenesis. In additional embodiments, the composition further comprises a scaffold comprising a protease inhibitor. In some preferred embodiments, the protease inhibitor is selected from the group consisting of Bowman-Birk Inhibitor, soybean trypsin inhibitor, and Eglin chymotrypsin inhibitor. In some preferred embodiments, the scaffold is Bowman-Birk inhibitor. In some particularly preferred embodiments, the scaffold comprises the amino acid sequence set forth in SEQ ID NO:19. In some alternative embodiments, the scaffold comprises at least one of the amino acid sequences set forth in SEQ ID NOS:20 and 21. In further preferred embodiments, at least one of the amino acid sequences set forth in SEQ ID NOS:20 and 21 is replaced by at least one peptide having an amino acid sequence selected from the group consisting of SEQ ID NOS:1-17.

The present invention also provides methods for modulating angiogenesis comprising: i) providing a composition comprising a peptide contained within a scaffold; ii) providing a subject to be treated; and iii) applying the composition to the subject in an area in which angiogenesis modulation is desired. In some embodiments, the peptide binds to a vascular endothelial growth factor (VEGF). In some preferred embodiments, the vascular endothelial growth factor (VEGF) is VEGF-A. In further preferred embodiments, the scaffold is selected from the group consisting of Bowman-Birk inhibitor, soybean trypsin inhibitor, and Eglin chymotrypsin inhibitor. In some particularly preferred embodiments, the scaffold is Bowman-Birk inhibitor. In some further embodiments, the scaffold comprises the amino acid sequence set forth in SEQ ID NO:19. In still further embodiments, the scaffold comprises at least one of the amino acid sequences set forth in SEQ ID NOS:20 and 21. In some particularly preferred embodiments, at least one of the amino acid sequences set forth in SEQ ID NOS:20 and 21 is replaced by at least one peptide having an amino acid sequence selected from the group consisting of SEQ ID NOS:1-17. In still further particularly preferred embodiments, the scaffold and the peptide are encoded by an amino acid sequence selected from the group consisting of SEQ ID NOS:22-25.

The present invention also provides methods for decreasing the activity of a vascular endothelial growth factor comprising the steps of: i) providing a subject; and ii) administering the composition comprising at least one peptide that binds to the vascular endothelial growth factor to the subject, under conditions such that the activity of the vascular endothelial growth factor is decreased. In some
5 embodiments, the vascular endothelial growth factor (VEGF) is VEGF-A. In some particularly preferred embodiments, the composition comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:22-25.

In some additional preferred embodiments, the compounds are used for the improvement of skin in an organism (*i.e.*, a subject) having a skin disorder. In additional preferred embodiments, the skin disorder
10 is at least one selected from the group consisting of psoriasis, venous ulcers, acne, rosacea, warts, eczema, hemangiomas and lymphangiogenesis, etc. In some particularly preferred embodiments, the skin disorder is rosacea.

In yet further embodiments, the present invention provides cosmetic and/or pharmaceutical compositions comprising at least one polypeptide or peptide, as set forth herein, and a physiologically
15 acceptable carrier or excipient. Preferably, the compound is present in an amount of about 0.0001% to about 5% by weight based on the total weight of the composition. Also preferably, the compound is present in an amount of about 0.001% to about 0.5% by weight based on the total weight of the composition. The composition may be in the form of an emulsified vehicle, such as a nutrient cream or lotion, a stabilized gel or dispersion system, a treatment serum, a liposomal delivery system, a topical pack or mask, a surfactant-
20 based cleansing system such as a shampoo or body wash, an aerosolized or sprayed dispersion or emulsion, a hair or skin conditioner, styling aid, or a pigmented product such as makeup, as well as other suitable make-up and cosmetic preparations. In some embodiments, the carrier is preferably at least one selected from the group consisting of water, propylene glycol, ethanol, propanol, glycerol, butylene glycol and polyethylene glycol.

In yet further embodiments, the present invention provides means for decreasing VEGF activity and/or levels. In some preferred embodiments, the VEGF activity and/or levels are decreased in the epidermis. In some embodiments, the method comprising applying an effective amount of at least one of the compounds described herein to an organism in need thereof.

In additional embodiments, the present invention provides applications for hair and/or skin
30 treatment, as well as applications wound healing, treatment of proliferative diseases, etc. Thus, the present invention provides compositions and methods suitable for application in/on humans and other animals.

DESCRIPTION OF THE FIGURES

Figure 1 provides a sequence summary of VEGF binding phage clones (SEQ ID NOS:1-12).
35 Twenty-four phage clones were sequenced after 3 rounds of panning. The sequence alignment tree indicates

a highly conserved sequence motif ACXLWPXXWC (SEQ ID NO:14). The number in parentheses represents the frequency of that sequence within the 24 clones sequenced after the third round of panning.

Figure 2 provides results of a phage ELISA to demonstrate the binding of unique clones to VEGF and not to BSA. Equivalent amounts of phage were evaluated to determine their relative binding affinity to hVEGF₁₆₅. The clone number and randomized sequence (SEQ ID NOS:1-7, and 13) are indicated below each symbol. Target-bound phage were detected with anti-M13-HRP. The HRP was monitored with ABTS substrate at an A_{405nm} after 30 minutes (n=3).

Figure 3 provides results of a BIACORE binding analysis of VEGF binding peptides. Binding curves were obtained as described in the Examples. Data were fit to a two state reaction model with conformation change: Analyte (A) binds to ligand (B) to form complex AB. Complex AB changes to AB* which cannot dissociate directly to A + B. Panel A provides results for biotinylated peptide CK37281. $ka_1=2.84e^3 \text{ M}^{-1}\text{s}^{-1}$, $kd_1=0.0122 \text{ s}^{-1}$, $ka_2=1.5e-3 \text{ s}^{-1}$, $kd_2=3.36e-3 \text{ s}^{-1}$, $K_D=1.92e^{-6} \text{ M}$. Panel B provides results for CK37283 (6000 RU VEGF, 3500 RU TNF α no buffer only subtraction); $ka_1=1.24e^4 \text{ M}^{-1}\text{s}^{-1}$, $kd_1=0.318 \text{ s}^{-1}$, $ka_2=6.34e-3 \text{ s}^{-1}$, $kd_2=1.23e-3 \text{ s}^{-1}$, $K_D=4.90e^{-6} \text{ M}$. Panel C provides results for v114 control peptide (1000 RU VEGF, 850 RU TNF α). Data were fit to a 1:1 Langmuir binding $ka_1=7.51e^5 \text{ M}^{-1}\text{s}^{-1}$, $kd_1=0.167 \text{ s}^{-1}$, $K_D=2.23e^{-7} \text{ M}$.

Figure 4 provides plasmid maps used in the Examples. Panel A provides the map for pCB04WT expression phagemid for expression of C-terminal His6X tagged beta-lactamase. Panel B provides the map for pME22 N-terminal stuffer phagemid for cloning using BbsI restriction sites. Panel C provides the map for pCM01 N-terminal aVEGF-BLA fusion expression phagemid.

Figure 5 provides a summary of N-terminal fusion cloning strategy using BbsI cloning sites (SEQ ID NOS:227-237).

Figure 6 provides an SDS-PAGE gel of His-tag purified beta-lactamase fusions with peptides. IMAC purified BLA versions and different peptides were concentrated and loaded onto an SDS PAGE gel (4-12%). Lanes 1 & 10: MW markers. Lane 2: pCB04 (WT with 6Xhis tag); Lanes 3,4,5,6: pCM01 aVEGF-BLA N-terminal fusion protein scaffold; and Lanes 7,8: pCM02 achymotrypsin-BLA N-terminal fusion protein.

Figure 7 provides a graph showing that aVEGF peptide-BLA fusion binds specifically to VEGF. Increasing concentrations of pCM01 (aVEGF peptide-BLA fusion) and pCB04 (WT) were added to VEGF coated wells of a microtiter plate. Residual bound nitrocefin activity was measured after washing 5X with nitrocefin assay buffer (0.125% n-octyl-beta-D-glucopyranoside in PBS).

Figure 8 provides a graph showing inhibition of VEGF-induced HUVEC proliferation by anti-VEGF peptide (filled circles). Proliferation was monitored by radioactive incorporation of ³H thymidine (n = 3). Anti-VEGF antibody (open circles) was used as a positive control, as described in the Examples.

Figure 9 provides the BBI gene and amino acid sequences (SEQ ID NOS:18 and 19, respectively) designed for efficient cloning. This sequence comprises the expression cassette used in *E.coli*. It codes for

the pro-BBI protein with a C-terminal His tag and has extra sequences at the 5' and 3' ends for cloning into the *E. coli* expression vector. The protein signal sequence is italicized while the trypsin loop (CTKSNPPQC; SEQ ID NO:20) and chymotrypsin loop (CALSYPAC; SEQ ID NO:21) are highlighted in bold and boxed.

Figure 10 provides an SDS PAGE gel showing the results of refolding anti-VEGF BBI. Anti-VEGF BBI was refolded in the presence or absence of subtilisin BPN' Y217L. The lanes are as follows: Lane 1: Hampton Foldit 11, refolding buffer, -subtilisin; Lane 2: Hampton Foldit 11 refolding buffer, +subtilisin; Lane 3: Hampton Foldit 13 refolding buffer, -subtilisin; Lane 4, Hampton Foldit 13 refolding buffer, + subtilisin; and Lane 5, molecular weight markers.

Figure 11 provides a graph showing that BBI-VEGF1 (SEQ ID NO:22) binds specifically to VEGF.

Figure 12 provides a graph showing HUVEC results for designated peptides.

Figure 13 provides sequences of three BBI-VEGF fusions, BBI-VEGF1 (SEQ ID NO:22), BBI-VEGF2 (SEQ ID NO:23) and BBI-VEGF12 (SEQ ID NO:24). Fusions BBI-VEGF1 and BBI-VEGF2 have only one of the binding loops replaced; fusion BBI-VEGF12 has both of the binding loops replaced.

Figure 14 provides the DNA and amino acid sequences of the *aprE*-BCE103-BBI-Histag expression cassette (*EcoRI*-*HindIII*) cloned into the pJM103 integration vector (SEQ ID NOS:35 and 36).

Figure 15 provides a schematic map of the pJM103BBIhis expression vector.

Figure 16 provides the DNA and amino acid sequences of 12BBICK81 from the BCE103 fusion site (at the *Bam*HI) to the end of the gene (SEQ ID NOS:37 and 38). The CK37281 peptide sequences (ACYNLYGWTC (SEQ ID NO:43)) are inserted into both the trypsin and chymotrypsin inhibitory loops.

Figure 17 provides a graph showing titers of active versus inactive 2BBICK81 (by trypsin inhibition) and the ratio of the activities of BCE103 cellulase to 2BBICK81 with various thiol reducing agents added during the growth of the culture. In this Figure, BME = 2-mercaptoethanol, Cyt = cysteine, Glut = reduced glutathione, DTT = dithiothreitol).

Figure 18 provides a graph showing activation of BCE-Ink2-2BBICK81 with 2-mercaptoethanol (bME) after partial purification by ion exchange chromatography.

Figure 19 provides a graph showing results from a competition analysis of 2BBICK81 versus anti-VegF antibody binding to VegF.

Figure 20 provides the sequence of the synthetic DNA fragment carrying the *H. insolens* PDI (hiPDI) that was inserted into the *B. subtilis* BBI expression vector, as well as the amino acid sequence (SEQ ID NOS:39 and 40)

Figure 21 provides the DNA and amino acid sequences of the *aprE*-cutinase expression cassette that was ligated into the *EcoRI*-*Bam*HI sites of p2JM103-Ink2-2BBICK81 (SEQ ID NOS:41 and 42).

DESCRIPTION OF THE INVENTION

The present invention provides peptides and supported peptides for treating various diseases and conditions. In particularly preferred embodiments, the present invention provides compositions and methods for personal care. In some embodiments, the present invention provides compositions for use in skin and/or hair care, as well as cosmetic compositions. In alternative particularly preferred embodiments, the present invention provides peptides and supported peptides for treating diseases of the skin, such as rosacea. In some particularly preferred embodiments, the supported peptides of the present invention are anti-VEGF peptides. In alternative particularly preferred embodiments, the anti-VEGF peptides are expressed on a scaffold protein. In some most preferred embodiments, the scaffold protein comprises BBI.

As described in greater detail herein, the present invention provides compositions for use in numerous aspects of personal care, including but not limited to hair and skin care, as well as cosmetics (*e.g.*, make-up). For example, the present invention provides compositions that find use in daily personal care, skin care, sun care (*e.g.*, sunscreens, as well as tanners), hair care (*e.g.*, shampoos, leave-on and/or rinse off conditioners, hair tonics, hair sprays, gels, foams, mousses, setting products, hair colorants, permanent formulations, other styling and cleaning products, etc.), after-sun care for skin, hair and lips, oral care (*e.g.*, toothpastes and gels, mouthwashes, rinses, etc.), bathing (*e.g.*, washes, shower soaps, bath soaps, salts, pearls, etc.), skin lighteners, cleansing treatments for skin conditions (*e.g.*, pimples, acne, skin toners, etc.), depilatories, wet wipes, deodorants, anti-perspirants, facial masks, shaving (*e.g.*, shaving creams, gels, etc.), after-shave, skin peeling (*e.g.*, exfoliants), intimate care products (*e.g.*, feminine hygiene products), personal fresheners, and foot care. The present invention also provides compositions that find use in cosmetics (*e.g.*, foundations, mascara, eye shadows, eye liners, lipsticks, lip glosses, blushers, etc.). It is contemplated that the compositions of the present invention will find use various forms, including but not limited to solids, liquids, colloidal suspensions, emulsions, oils, gels, aerosols, foams, powders, pump sprays, etc., as well as being used in conjunction with items such as wet wipes, etc. Indeed, it is contemplated that the present invention will find use in any suitable form for the intended use(s).

Unless otherwise indicated, the practice of the present invention involves conventional techniques commonly used in molecular biology, microbiology, and recombinant DNA, which are within the skill of the art. Such techniques are known to those of skill in the art and are described in numerous texts and reference works (See *e.g.*, Sambrook *et al.*, "Molecular Cloning: A Laboratory Manual", Second Edition (Cold Spring Harbor), [1989]); and Ausubel *et al.*, "Current Protocols in Molecular Biology" [1987]). All patents, patent applications, articles and publications mentioned herein, both *supra* and *infra*, are hereby expressly incorporated herein by reference.

Unless defined otherwise herein, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. For example, Singleton and Sainsbury, *Dictionary of Microbiology and Molecular Biology*, 2d Ed., John Wiley and Sons, NY (1994); and Hale and Marham, *The Harper Collins Dictionary of Biology*, Harper Perennial, NY (1991) provide those of skill in the art with a general dictionaries of many of the terms used in the

invention. Although any methods and materials similar or equivalent to those described herein find use in the practice of the present invention, the preferred methods and materials are described herein. Accordingly, the terms defined immediately below are more fully described by reference to the Specification as a whole. Also, as used herein, the singular "a," "an," and "the" includes the plural reference unless the context clearly indicates otherwise. Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, nucleic acids are written left to right in 5' to 3' orientation; amino acid sequences are written left to right in amino to carboxy orientation, respectively. It is to be understood that this invention is not limited to the particular methodology, protocols, and reagents described, as these may vary, depending upon the context they are used by those of skill in the art.

Furthermore, the headings provided herein are not limitations of the various aspects or embodiments of the invention which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification as a whole. Nonetheless, in order to facilitate understanding of the invention, a number of terms are defined below.

Definitions

As used herein, the term "scaffold" refers to a protease inhibitor having a heterologous and/or modified peptide sequence incorporated therein. In preferred embodiments, the term "scaffold" refers to a wild-type protein sequence into which a variant sequence is introduced. In some embodiments, the scaffold has portions (*e.g.*, parts or all of one or both loops), that are replaced with heterologous sequence(s). For example, the BBI sequences having anti-VEGF (AV) sequences incorporated as provided herein, find use as scaffolds. Indeed, the present invention encompasses BBI-based sequences, but which have structural and functional differences from wild-type BBI.

As used herein, the term "vascular endothelial growth factor" (VEGF) refers to proteins with the ability to stimulate vascular growth, including those designated "VEGF-A" known to those of skill in the art.

As used herein, the term "anti-VEGF" ("aVEGF" and "AV") refers to peptides and other compositions that recognize (*i.e.*, bind) to VEGF. In preferred embodiments, these peptides/compositions modulate VEGF activity.

The term "angiogenesis" refers to the biological processes which result in the development of blood vessels and/or increase in the vascularity of tissue in an organism. In particular embodiments herein, the term refers to the process through which tumors or other rapidly proliferating tissue derive a blood supply through the generation of microvessels.

The terms "angiogenic disease," "angiogenic disorder," and "angiogenic skin disorder," are used in reference to a disorder, generally a skin disorder or related disorder which occurs as a consequence of or which results in increased vascularization in tissue. Oftentimes, the etiology of the angiogenic disease is unknown. However, whether angiogenesis is an actual cause of a disease state or is simply a condition of the disease state is unimportant, but the inhibition of angiogenesis in treating or reversing the disease state or

condition is an important aspect of the present invention. Thus, it is not intended that the present invention be limited to any particular mechanisms of action. Examples of angiogenic skin disorders which are suitable for treatment utilizing compounds of the present invention include, but are not limited to psoriasis, acne, rosacea, warts, eczema, hemangiomas and lymphangiogenesis, Sturge-Weber syndrome, neurofibromatosis, tuberos sclerosis, chronic inflammatory disease, and arthritis. Any skin disorder which has as a primary or secondary characterization, increased vascularization, is considered an angiogenic skin disorder herein. Thus, the compounds provided by the present invention find use in treatment of a wide variety of diseases and/or conditions.

The term "rosacea" is used to describe acne, rosacea, or erythematosa characterized by vascular and follicular dilation typically involving the nose and contiguous portions of the cheeks. Rosacea may vary from very mild but persistent erythema to extensive hyperplasia of the sebaceous glands with deep-seated papules and pustules and be accompanied by telangiectasia at the affected erythematous sites. This condition is also referred to as "hypertrophic rosacea" or "rhinophyma," depending upon the severity of the condition. It is intended that the term encompass all of the various forms of the condition.

The term "wart" is used to describe a small, usually hard growth on the skin. Also known as a "verruca," warts are flesh-colored growths of the skin which are characterized by circumscribed hypertrophy of the papillae of the corium, with thickening of the malpighian, granulation and keratin layers of the epidermis. Verucca vulgaris, a subset of warts or verruca, is characterized by infection of the keratinocytes with human papillomavirus.

The term "psoriasis" is used to describe a skin condition which is characterized by the eruption of circumscribed, discrete and confluent, reddish, silvery-scaled maculopapules. Although it is not intended that the present invention be limited to any particular body area, psoriatic lesions typically occur on the elbows, knees, scalp and trunk. Microscopically, these lesions demonstrate characteristic parakeratosis and elongation of rete ridges.

The term "acne" is used to describe a condition of the skin characterized by inflammatory follicular, papular and pustular eruptions involving the sebaceous apparatus. Although there are numerous forms of acne, the most common form is known as acne simplex or acne vulgaris which is characterized by eruptions of the face, upper back and chest and is primarily comprised of comedones, cysts, papules and pustules on an inflammatory base. The condition occurs primarily during puberty and adolescence due to an overactive sebaceous apparatus which is believed to be affected by hormonal activity.

The term "eczema" is a generic term used to describe acute or chronic inflammatory conditions of the skin, typically erythematous, edematous, papular, vesicular and/or crusting. These conditions are often followed by lichenification, scaling and occasionally, by duskiness of the erythema and, infrequently, hyperpigmentation. Eczema is often accompanied by the sensation of itching and burning. Eczema vesicles form due to intraepidermal spongiosis. Eczema is sometimes referred to colloquially as "tetter," "dry tetter,"

and "scaly tetter." There are numerous subcategories of eczema, all of which are treated by one or more of the compounds according to the present invention.

As used herein, "CK" followed by an integer refers to a specific peptide. Various peptide sequences that find use with the present invention are provided herein (*See e.g.*, Figure 1). As an example, CK37281 refers to the peptide sequence YNLYGWT (SEQ ID NO:1) which is also included in various other sequences (*e.g.*, "ACYNLYGWTCGGG" (SEQ ID NO:238)).

As used herein, in some embodiments, the "compound" comprises the "complete" protein, (*i.e.*, in its entire length as it occurs in nature (or as mutated)), while in other embodiments it comprises a truncated form of a protein. Thus, in some embodiments, the compounds of the present invention are either truncated or be "full-length." In addition, in some embodiments, the truncation is located at the N-terminal end, while in other embodiments the truncation is located at the C-terminal end of the protein. In further embodiments, the compound lacks one or more portions (*e.g.*, sub-sequences, signal sequences, domains or moieties), whether active or not.

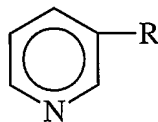
The term "organism" is used throughout the specification to describe an animal; preferably a human, to whom treatment, including prophylactic treatment, with the compounds according to the present invention is provided. For treatment of those infections, conditions or disease states which are specific for a specific animal such as a human patient, the term organism refers to that specific animal.

The "host cells" used in the present invention generally are prokaryotic or eukaryotic hosts which contain an expression vector and/or gene of interest. Host cells are transformed or transfected with vectors constructed using recombinant DNA techniques. Such transformed host cells are capable of either replicating vectors encoding the protein variants or expressing the desired protein variant. In the case of vectors which encode the pre- or prepro-form of the protein variant, such variants, when expressed, are typically secreted from the host cell into the host cell medium.

The term "effective amount" is used throughout the specification to describe concentrations or amounts of compounds according to the present invention which may be used to produce a favorable change in the disease or condition treated, whether that change is hair growth or prevention of hair growth.

As used herein, "active" (and "actives") refers to a composition that imparts a benefit to a subject being treated. For example, in preferred embodiments, the present invention provides personal care compositions comprising BBI-AV, a "primary active" which functions to provide benefit to the area to which it is applied. Thus, in some embodiments, BBI-AV is present in skin care formulations and serves to modify the skin tone of subjects to which is applied. It is not intended that the term be limited to BBI-AV, as there are additional constituents present in the personal care compositions of the present invention which impart benefits. In some preferred embodiments, these additional constituents are encompassed by the designation "secondary actives." Primary and secondary actives are collectively referred to as "actives" herein.

As used herein, "vitamin B₃ compound" means a compound having the formula:



wherein R is - CONH₂ (*i.e.*, niacinamide), - COOH (*i.e.*, nicotinic acid) or - CH₂OH (*i.e.*, nicotiny alcohol); derivatives thereof; and salts of any of the foregoing.

5 As used herein, "non-vasodilating" means that an ester does not commonly yield a visible flushing response after application to the skin in the subject compositions. It is contemplated that the majority of the general population would not experience a visible flushing response, although such compounds may cause vasodilation not visible to the naked eye.

As used herein, "retinoid" includes all natural and/or synthetic analogs of Vitamin A and/or retinol-like compounds which possess the biological activity of Vitamin A in/on the skin, as well as the geometric isomers and stereoisomers of these compounds. However, it is not intended that the term be limited to these compounds, as the term encompasses vitamin A alcohol (retinol) and its derivatives such as vitamin A aldehyde (retinal), vitamin A acid (retinoic acid) and vitamin A esters (*e.g.*, retinyl acetate, retinyl propionate and retinyl palmitate), etc. It is further intended that the term encompass all-trans-retinoic acids and 13-cis-retinoic acids. It is also intended that the term encompass compositions that are encapsulated, as well as provided for use in various forms. The terms "retinol" and "retinal" preferably comprise the all-trans compounds. The retinoid preferably used for the formulation of the present invention is all-trans-retinol, generally referred to as "retinol" herein.

As used herein, "carotenoid" is used in reference to β -carotene, lycopene, lutein, astaxanthin, zeaxanthin, cryptoxanthin, citranaxanthin, canthaxanthin, bixin, β -apo-4-carotenal, β -apo-8-carotenal, β -apo-8-carotenoic esters, alone, as well as in combination. Carotenoids which are preferably used are β -carotene, lycopene, lutein, astaxanthin, zeaxanthin, citranaxanthin and canthaxanthin. In some embodiments, carotenoids are utilized in crystalline form, as well as in formulations, including but not limited to dry powders (*See e.g.*, dry powders, as described in EP 0 065 193; hereby incorporated by reference).

In some embodiments, the preferred use in the case of lycopene, astaxanthin and canthaxanthin is of lycopene-, astaxanthin- and canthaxanthin-containing dry powders, for example LYCOVIT[®], LUCANTIN[®] Pink and LUCANTIN[®] Red (10% dry powders respectively of lycopene, astaxanthin and canthaxanthin, commercially available from BASF AG, Ludwigshafen, Germany).

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As used herein, the term "bioactivity" refers to a cause and effect relationship between a composition and a biological system. Thus, the term is used as by those skilled in the art of biotechnology

and biological sciences as the phrase that describes a cause and effect relationship between a molecular composition and living biological matter (*e.g.*, tissue, cells, etc.).

As used herein as a noun, the term “bioactive” refers a composition that exhibits bioactivity upon administration to living biological matter (*e.g.*, tissue, cells, etc.). The term is used synonymously with “bioactive compound.”

As used herein, “silicone gum” means high molecular weight silicones having an average molecular weight in excess of about 200,000 and preferably from about 200,000 to about 4,000,000. It is intended that the definition encompass non-volatile polyalkyl and polyaryl siloxane gums.

As used herein, the term “polypeptide” refers to a compound made up of a single chain of amino acid residues linked by peptide bonds. The term “protein” herein may be synonymous with the term “polypeptide” or may refer, in addition, to a complex of two or more polypeptides. The exact meaning is that known to those in the art.

As used herein, the terms “expression cassette” and “expression vector” refer to nucleic acid constructs generated recombinantly or synthetically, with a series of specified nucleic acid elements that permit transcription of a particular nucleic acid in a target cell. The recombinant expression cassette can be incorporated into a plasmid, chromosome, mitochondrial DNA, plastid DNA, virus, or nucleic acid fragment. Typically, the recombinant expression cassette portion of an expression vector includes, among other sequences, a nucleic acid sequence to be transcribed and a promoter. The term “expression cassette” may be used interchangeably herein with “DNA construct” and its grammatical equivalents.

As used herein, the terms “vector” and “cloning vector” refer to nucleic acid constructs designed to transfer nucleic acid sequences into cells.

As used herein, the term “expression vector” refers to a vector that has the ability to incorporate and express heterologous DNA fragments in a foreign cell. Many prokaryotic and eukaryotic expression vectors are commercially available. Selection of appropriate expression vectors is within the knowledge of those of skill in the art.

As used herein, the term “plasmid” refers to a circular double-stranded (ds) DNA construct used as a cloning vector, and which forms an extrachromosomal self-replicating genetic element in some eukaryotes or integrates into the host chromosomes.

As used herein, the term “expression” refers to the process by which a polypeptide is produced based on the nucleic acid sequence of the gene or the chemical synthetic peptide. The process includes both transcription and translation of the gene to produce polypeptide/protein.

As used herein, the term “gene” means the segment of DNA involved in producing a polypeptide chain that may or may not include regions preceding or following the coding region.

As used herein, the terms “nucleic acid molecule” and “nucleic acid sequence” include sequences of any form of nucleic acid, including, but not limited to RNA, DNA and cDNA molecules. It will be

understood that, as a result of the degeneracy of the genetic code, a multitude of nucleotide sequences encoding a given protein may be produced, in addition to mutant proteins.

As used herein, "codon" refers to a sequence of three nucleotides in a DNA or mRNA molecule that represents the instruction for incorporation of a specific amino acid into a polypeptide chain.

5 As used herein, the term "disulfide bridge" or "disulfide bond" refers to the bond formed between the sulfur atoms of cysteine residues in a polypeptide or a protein. In this invention, a disulfide bridge or disulfide bond may be non-naturally occurring and introduced by way of point mutation.

As used herein, the term "salt bridge" refers to the bond formed between oppositely charged residues, amino acids in a polypeptide or protein. In this invention, a salt bridge may be non-naturally
10 occurring and introduced by way of point mutation.

As used herein, an "enzyme" refers to a protein or polypeptide that catalyzes at least one chemical reaction.

As used herein, the term "activity" refers to any activity associated with a particular protein, such as enzymatic activity associated with a protease. In some embodiments, the activity is biological activity. In
15 further embodiments, activity encompasses binding of proteins to receptors which results in measurable downstream effects (*e.g.*, VEGF binding to its cognate receptor). "Biological activity" refers to any activity that would normally be attributed to that protein by one skilled in the art.

As used herein, the term "protease" refers to an enzyme that degrades peptide bonds.

As used herein, "peptide bond" refers to the chemical bond between the carbonyl group of one
20 amino acid and the amino group of another amino acid.

As used herein, "wild-type" refers to a sequence or a protein that is native or naturally occurring.

As used herein, "point mutations" refers to a change in a single nucleotide of DNA, especially where that change results in a sequence change in a protein.

As used herein, "mutant" refers to a version of an organism or protein where the version is other
25 than wild-type. The change may be effected by methods well known to one skilled in the art, for example, by point mutation in which the resulting protein may be referred to as a mutant.

As used herein, "mutagenesis" refers to the process of changing a composition (*e.g.*, protein) from a wild-type composition (*e.g.*, protein) into a mutant or variant composition (*e.g.*, protein).

As used herein, "substituted" and "substitutions" refer to replacement(s) of an amino acid residue or
30 nucleic acid base in a parent sequence. In some embodiments, the substitution involves the replacement of a naturally occurring residue or base.

As used herein, "modification" and "modify" refer to any change(s) in an amino acid or nucleic acid sequence, including, but not limited to deletions, insertions, interruptions, and substitutions. In some embodiments, the modification involves the replacement of a naturally occurring residue or base.

35 As used herein, "functional portion of a secreted polypeptide" and its grammatical equivalents refers to a truncated secreted polypeptide that retains its ability to fold into a normal, albeit truncated,

configuration. In some embodiments, it is contemplated that sufficient residues of a domain of the naturally secreted polypeptide must be present to allow it to fold in its normal configuration independently of the desired polypeptide to which it is attached. However, in most cases, the portion of the secreted polypeptide are both correctly folded and result in increased secretion as compared to its absence. Similarly, in most cases, the truncation of the secreted polypeptide means that the functional portion retains a biological function. In a preferred embodiment, the catalytic domain of a secreted polypeptide is used, although other functional domains may be used, for example, the substrate binding domains. Additionally preferred embodiments utilize the catalytic domain and all or part of the linker region.

As used herein, "loop" refers to a sequence of amino acids, for example 3-20 amino acids, more preferably 5-15 amino acids, even more preferably 5-10 amino acids, and most preferably 7-9 amino acids, which connects structural elements of a protein. Such elements include, but are not limited to beta sheets and helical elements and the connecting loop of a beta-hairpin. In some embodiments, the loop is further stabilized through the use of covalent linkages. In some preferred embodiments, the covalent linkages comprise disulfide bonds, especially as provided herein. In alternative embodiments, the loops are stabilized by the use of other means, including but not limited to amides, hydrogen bonds, and/or salt bridges. In most embodiments, the loops are located on the surface of proteins and may be altered, as provided herein, to confer additional (*e.g.*, desirable) properties to the requisite proteins.

As used herein, "oligonucleotide" refers to a short nucleotide sequence which may be used, for example, as a primer in a reaction used to create mutant proteins.

As used herein, the terms "an oligonucleotide having a nucleotide sequence encoding a gene" and "polynucleotide having a nucleotide sequence encoding a gene," means a nucleic acid sequence comprising the coding region of a gene or in other words the nucleic acid sequence which encodes a gene product. The coding region may be present in either a cDNA, genomic DNA or RNA form. When present in a DNA form, the oligonucleotide or polynucleotide may be single-stranded (*i.e.*, the sense strand) or double-stranded. Suitable control elements such as enhancers/promoters, splice junctions, polyadenylation signals, *etc.* may be placed in close proximity to the coding region of the gene if needed to permit proper initiation of transcription and/or correct processing of the primary RNA transcript. Alternatively, the coding region utilized in the expression vectors of the present invention may contain endogenous enhancers/promoters, splice junctions, intervening sequences, polyadenylation signals, *etc.* or a combination of both endogenous and exogenous control elements.

As used herein, the term "primer" refers to an oligonucleotide, whether occurring naturally as in a purified restriction digest or produced synthetically, which is capable of acting as a point of initiation of synthesis when placed under conditions in which synthesis of a primer extension product which is complementary to a nucleic acid strand is induced, (*i.e.*, in the presence of nucleotides and an inducing agent such as DNA polymerase and at a suitable temperature and pH). The primer is preferably single stranded for maximum efficiency in amplification, but may alternatively be double stranded. If double stranded, the

primer is first treated to separate its strands before being used to prepare extension products. Preferably, the primer is an oligodeoxyribonucleotide. The primer must be sufficiently long to prime the synthesis of extension products in the presence of the inducing agent. The exact lengths of the primers will depend on many factors, including temperature, source of primer and the use of the method.

5 As used herein, the term "probe" refers to an oligonucleotide (*i.e.*, a sequence of nucleotides), whether occurring naturally as in a purified restriction digest or produced synthetically, recombinantly or by PCR amplification, which is capable of hybridizing to another oligonucleotide of interest. A probe may be single-stranded or double-stranded. Probes are useful in the detection, identification and isolation of particular gene sequences. It is contemplated that any probe used in the present invention will be labeled
10 with any "reporter molecule," so that is detectable in any detection system, including, but not limited to enzyme (*e.g.*, ELISA, as well as enzyme-based histochemical assays), fluorescent, radioactive, and luminescent systems. It is not intended that the present invention be limited to any particular detection system or label.

As used herein, the term "target," when used in reference to the polymerase chain reaction, refers to
15 the region of nucleic acid bounded by the primers used for polymerase chain reaction. Thus, the "target" is sought to be sorted out from other nucleic acid sequences. A "segment" is defined as a region of nucleic acid within the target sequence.

As used herein, the term "polymerase chain reaction" ("PCR") refers to the method well-known in the art (*See e.g.*, U.S. Patent Nos. 4,683,195 4,683,202, and 4,965,188, hereby incorporated by reference),
20 for increasing the concentration of a segment of a target sequence in a mixture of genomic DNA without cloning or purification. This process for amplifying the target sequence consists of introducing a large excess of two oligonucleotide primers to the DNA mixture containing the desired target sequence, followed by a precise sequence of thermal cycling in the presence of a DNA polymerase. The two primers are complementary to their respective strands of the double stranded target sequence. To effect amplification,
25 the mixture is denatured and the primers then annealed to their complementary sequences within the target molecule. Following annealing, the primers are extended with a polymerase so as to form a new pair of complementary strands. The steps of denaturation, primer annealing and polymerase extension can be repeated many times (*i.e.*, denaturation, annealing and extension constitute one "cycle"; there can be numerous "cycles") to obtain a high concentration of an amplified segment of the desired target sequence.
30 The length of the amplified segment of the desired target sequence is determined by the relative positions of the primers with respect to each other, and therefore, this length is a controllable parameter. By virtue of the repeating aspect of the process, the method is referred to as the "polymerase chain reaction" (hereinafter "PCR"). Because the desired amplified segments of the target sequence become the predominant sequences (in terms of concentration) in the mixture, they are said to be "PCR amplified."

35 As used herein, the terms "PCR product," "PCR fragment," and "amplification product" refer to the resultant mixture of compounds after two or more cycles of the PCR steps of denaturation, annealing and

extension are complete. These terms encompass the case where there has been amplification of one or more segments of one or more target sequences.

As used herein, the term "amplification reagents" refers to those reagents (deoxyribonucleotide triphosphates, buffer, etc.), needed for amplification except for primers, nucleic acid template and the amplification enzyme. Typically, amplification reagents along with other reaction components are placed and contained in a reaction vessel (test tube, microwell, etc.).

As used herein, the term "RT-PCR" refers to the replication and amplification of RNA sequences. In this method, reverse transcription is coupled to PCR, most often using a one enzyme procedure in which a thermostable polymerase is employed, as described in U.S. Patent No. 5,322,770, herein incorporated by reference. In RT-PCR, the RNA template is converted to cDNA due to the reverse transcriptase activity of the polymerase, and then amplified using the polymerizing activity of the polymerase (*i.e.*, as in other PCR methods).

As used herein, the term "hybridization" refers to the process by which a strand of nucleic acid joins with a complementary strand through base pairing, as known in the art.

As used herein, "maximum stringency" refers to the level of hybridization that typically occurs at about $T_m - 5^\circ\text{C}$ (5°C below the T_m of the probe); "high stringency" at about 5°C to 10°C below T_m ; "intermediate stringency" at about 10°C to 20°C below T_m ; and "low stringency" at about 20°C to 25°C below T_m . As will be understood by those of skill in the art, a maximum stringency hybridization can be used to identify or detect identical polynucleotide sequences while an intermediate or low stringency hybridization can be used to identify or detect polynucleotide sequence homologs.

The phrases "substantially similar" and "substantially identical" in the context of two nucleic acids or polypeptides typically means that a polynucleotide or polypeptide comprises a sequence that has at least 75% sequence identity, preferably at least 80%, more preferably at least 90%, still more preferably 95%, most preferably 97%, sometimes as much as 98% and 99% sequence identity, compared to the reference (*i.e.*, wild-type) sequence. Sequence identity may be determined using known programs such as BLAST, ALIGN, and CLUSTAL using standard parameters. (*See e.g.*, Altschul, *et al.*, J. Mol. Biol. 215:403-410 [1990]; Henikoff *et al.*, Proc. Natl. Acad. Sci. USA 89:10915 [1989]; Karin *et al.*, Proc. Natl. Acad. Sci. USA 90:5873 [1993]; and Higgins *et al.*, Gene 73:237 - 244 [1988]). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. Also, databases may be searched using FASTA (Pearson *et al.*, Proc. Natl. Acad. Sci. USA 85:2444-2448 [1988]).

As used herein, "equivalent residues" refers to proteins that share particular amino acid residues. For example, equivalent residues may be identified by determining homology at the level of tertiary structure for a protein (*e.g.*, VEGF) whose tertiary structure has been determined by x-ray crystallography. Equivalent residues are defined as those for which the atomic coordinates of two or more of the main chain atoms of a particular amino acid residue of the protein having putative equivalent residues and the protein of

interest (N on N, CA on CA, C on C and O on O) are within 0.13 nm and preferably 0.1 nm after alignment. Alignment is achieved after the best model has been oriented and positioned to give the maximum overlap of atomic coordinates of non-hydrogen protein atoms of the proteins analyzed. The preferred model is the crystallographic model giving the lowest R factor for experimental diffraction data at the highest resolution available, determined using methods known to those skilled in the art of crystallography and protein characterization/analysis.

In some embodiments, modification is preferably made to the "precursor DNA sequence" which encodes the amino acid sequence of the precursor enzyme, but can be by the manipulation of the precursor protein. In the case of residues which are not conserved, the replacement of one or more amino acids is limited to substitutions which produce a variant which has an amino acid sequence that does not correspond to one found in nature. In the case of conserved residues, such replacements should not result in a naturally-occurring sequence. Derivatives provided by the present invention further include chemical modification(s) that change the characteristics of the protein.

In some preferred embodiments, the protein gene is ligated into an appropriate expression plasmid. The cloned protein gene is then used to transform or transfect a host cell in order to express the protein gene. In some embodiments, this plasmid replicates in the hosts, in the sense that it contains the well-known elements necessary for plasmid replication or the plasmid may be designed to integrate into the host chromosome. The necessary elements are provided for efficient gene expression (*e.g.*, a promoter operably linked to the gene of interest). In some embodiments, these necessary elements are supplied as the gene's own homologous promoter if it is recognized, (*i.e.*, transcribed, by the host), a transcription terminator (a polyadenylation region for eukaryotic host cells) which is exogenous or is supplied by the endogenous terminator region of the protein gene. In some embodiments, a selection gene such as an antibiotic resistance gene that enables continuous cultural maintenance of plasmid-infected host cells by growth in antimicrobial-containing media is also included.

As used herein, the terms "restriction endonucleases" and "restriction enzymes" refer to bacterial enzymes, each of which cut double-stranded DNA at or near a specific nucleotide sequence.

As used herein, the term "recombinant DNA molecule" as used herein refers to a DNA molecule which is comprised of segments of DNA joined together by means of molecular biological techniques.

The term "recombinant protein" or "recombinant polypeptide" as used herein refers to a protein molecule which is expressed from a recombinant DNA molecule.

The term "native protein" as used herein to indicate that a protein does not contain amino acid residues encoded by vector sequences; that is the native protein contains only those amino acids found in the protein as it occurs in nature. A native protein may be produced by recombinant means or may be isolated from a naturally occurring source.

As used herein the term "portion" when in reference to a protein (as in "a portion of a given protein") refers to fragments of that protein. The fragments may range in size from four amino acid residues to the entire amino acid sequence minus one amino acid.

As used herein, the term "fusion protein" refers to a chimeric protein containing the protein of interest (*i.e.*, VEGF and fragments thereof) joined to an exogenous protein fragment (the fusion partner which consists of a non-VEGF protein). In some embodiments, the fusion partner enhances solubility of the VEGF protein as expressed in a host cell, may provide an affinity tag to allow purification of the recombinant fusion protein from the host cell or culture supernatant, or both. If desired, the fusion protein may be removed from the protein of interest (*i.e.*, VEGF and/or fragments thereof) by a variety of enzymatic or chemical means known to the art.

The terms "in operable combination," "in operable order," and "operably linked" as used herein refer to the linkage of nucleic acid sequences in such a manner that a nucleic acid molecule capable of directing the transcription of a given gene and/or the synthesis of a desired protein molecule is produced. The term also refers to the linkage of amino acid sequences in such a manner so that a functional protein is produced.

As used herein the term "coding region" when used in reference to structural gene refers to the nucleotide sequences which encode the amino acids found in the nascent polypeptide as a result of translation of a mRNA molecule. The coding region is bounded, in eukaryotes, on the 5' side by the nucleotide triplet "ATG" which encodes the initiator methionine and on the 3' side by one of the three triplets which specify stop codons (*i.e.*, TAA, TAG, TGA).

As used herein, the term "structural gene" refers to a DNA sequence coding for RNA or a protein. In contrast, "regulatory genes" are structural genes which encode products which control the expression of other genes (*e.g.*, transcription factors).

As used herein, the term "purified" or "to purify" refers to the removal of contaminants from a sample. For example, recombinant VEGF or aVEGF polypeptides are expressed in host cells and the polypeptides are purified by the removal of host cell proteins; the percent of recombinant VEGF or aVEGF polypeptides is thereby increased in the sample.

As used herein, the term "substantially pure" when applied to the proteins or fragments thereof of the present invention means that the proteins are essentially free of other substances to an extent practical and appropriate for their intended use. In particular, the proteins are sufficiently pure and are sufficiently free from other biological constituents of the host cells so as to be useful in, for example, protein sequencing, and/or producing pharmaceutical preparations.

As used herein, the term "target protein" refers to protein (*e.g.*, enzyme, hormone, etc.), whose action would be blocked by the binding of the variant inhibitors provided for herein.

As used herein, the terms "variant sequence" and "variant sequences" refer to the short polypeptide sequence(s) that replace the binding loops of the wild-type protease inhibitor. The variant sequence does not need to be of the same length as the binding loop sequence it is replacing in the wild-type protease inhibitor.

The term "isolated" when used in relation to a nucleic acid, as in "an isolated oligonucleotide" or "isolated polynucleotide" refers to a nucleic acid sequence that is identified and separated from at least one contaminant nucleic acid with which it is ordinarily associated in its natural source. Isolated nucleic acid is such present in a form or setting that is different from that in which it is found in nature. In contrast, non-isolated nucleic acids as nucleic acids such as DNA and RNA found in the state they exist in nature. For example, a given DNA sequence (*e.g.*, a gene) is found on the host cell chromosome in proximity to neighboring genes; RNA sequences, such as a specific mRNA sequence encoding a specific protein, are found in the cell as a mixture with numerous other mRNA s which encode a multitude of proteins. However, isolated nucleic acid encoding a VEGF protein includes, by way of example, such nucleic acid in cells ordinarily expressing a VEGF protein where the nucleic acid is in a chromosomal location different from that of natural cells, or is otherwise flanked by a different nucleic acid sequence than that found in nature. The isolated nucleic acid, oligonucleotide, or polynucleotide may be present in single-stranded or double-stranded form. When an isolated nucleic acid, oligonucleotide or polynucleotide is to be utilized to express a protein, the oligonucleotide or polynucleotide will contain at a minimum the sense or coding strand (*i.e.*, the oligonucleotide or polynucleotide may single-stranded), but may contain both the sense and anti-sense strands (*i.e.*, the oligonucleotide or polynucleotide may be double-stranded).

As used herein, a "composition comprising a given polynucleotide sequence" refers broadly to any composition containing the given polynucleotide sequence. The composition may be in any form, particularly a form that is suitable for administration.

As used herein, a compound is said to be "in a form suitable for administration" when the compound may be administered to a human or other animal by any desired route (*e.g.*, topical, oral, etc.).

As used herein, "safe and effective amount" refers to a sufficient amount of a material that significantly induces a positive modification to the area upon which the material is applied and also does not result in the production of serious side effects (at a reasonable risk/benefit ratio). The safe and effective amount of the material may vary with the particular skin or other body part being treated, the age of the subject being treated, the severity of the condition being treated, the duration of treatment, the nature of concurrent therapy, the specific material used, the particular carrier utilized, etc. Those of skill in the art are capable of adjusting the concentration of the personal care compositions provided herein for the desired application of the compositions.

As used herein, the term "personal care composition" refers to a product for application to the skin, hair, nails, oral cavity and related membranes for the purposes of improving, cleaning, beautifying, treating, and/or caring for these surfaces and membranes.

In some embodiments, the personal care composition is in the form of an emulsified vehicle, such as a nutrient cream or lotion, a stabilized gel or dispersion system, such as skin softener, a nutrient emulsion, a nutrient cream, a massage cream, a treatment serum, a liposomal delivery system, a topical facial pack or mask, a surfactant-based cleansing system such as a shampoo or body wash, an aerosolized or

sprayed dispersion or emulsion, a hair or skin conditioner, styling aid, or a pigmented product such as makeup in liquid, cream, solid, anhydrous or pencil form. However, it is not intended that the present invention be limited to any particular form, as various forms find use in the present invention.

Personal care products can be classified/described as cosmetic, over-the-counter ("OTC") compounds that find use in personal care applications (*e.g.*, cosmetics, skin care, oral care, hair care, nail care). In some embodiments, the repeat sequence protein polymer is added to a personal care composition such as a hair care composition, a skin care composition, a nail care composition, a cosmetic composition, or any combinations thereof.

As used herein, "cosmetic composition" refers to compositions that find use in the cosmetics. The Food Drug and Cosmetic Act (FD&C Act) definition is used herein. Thus, cosmetics are defined by their intended use, as articles intended to be rubbed, poured, sprinkled, or sprayed on, introduced into, or otherwise applied to the human body for cleansing, beautifying, promoting attractiveness, or altering appearance. These compositions provide non-therapeutic benefits and are not regulated as pharmaceuticals. However, in some situations, cosmetic compositions are incorporated into pharmaceutical compositions to provide cosmetic benefits (*e.g.*, products that treat skin or hair diseases, but also contain cosmetic compositions for their coloring or other benefits). Also, it is intended that the present invention encompass the use of cosmetics on animals other than humans.

As used herein, the terms "pharmaceutical compositions" and "therapeutic compositions" refer to compositions such as drugs that provide medical benefits, rather than solely cosmetic benefits. In the United States, pharmaceutical and therapeutic compositions are approved by the Food and Drug Administration for treatment and/or prevention of particular conditions.

As used herein, the term "drug" is defined as it is in the FD&C Act definition. Thus, drugs are defined as articles intended for use in the diagnosis, cure, mitigation, treatment or prevention of disease, and articles (other than food) intended to affect the structure or any function of the body of man or other animals.

As used herein, "leave-on" refers to a composition that is applied to a subject and not removed (*e.g.*, cleansed by washing, rinsing, etc.) for a period of typically at least several hours (*e.g.*, 4-12 hours) before the area exposed to the composition is cleansed.

As used herein, a "rinse-off" composition is a composition that is applied and cleansed (*e.g.*, by washing, rinsing, etc.) soon after its application (generally within about 30 minutes of application). In some preferred embodiments, rinse-off compositions are formulated so as to deposit an effective amount of active(s) on the area treated.

As used herein, the term "cosmetic benefit" refers to a desired cosmetic change that results from the administration of a personal care composition. Cosmetic benefits include but are not limited to improvements in the condition of skin, hair, nails, and the oral cavity. In preferred embodiments, at least

one cosmetic benefit is provided by the skin care, hair care, nail care, and makeup compositions of the present invention.

As used herein, "cosmetically acceptable" refers to materials that are suitable for use in contact with tissues of humans and/or other animals, without undue toxicity, incompatibility, instability, irritation, allergic responses, etc., commensurate with a reasonable benefit/risk ratio.

As used herein, "skin care composition" refers to compositions that are applied to skin in order to provide beneficial properties, including but not limited to wrinkle minimizing, wrinkle removal, decoloring, coloring, skin softening, skin smoothing, depilation, cleansing, etc. In some particularly preferred embodiments, the present invention provides skin care compositions that improve skin tone. In these embodiments, the improvement comprises lessening of wrinkles, smoothing skin texture, modifying skin coloration, and other desired cosmetic benefits. In further embodiments, the skin care composition is in a form selected from the group consisting of body washes, moisturizing body washes, deodorant body washes, antimicrobial cleansers, skin protecting treatments, body lotions, facial creams, moisturizing creams, facial cleansing emulsions, surfactant-based facial cleansers, facial exfoliating gels, facial toners, exfoliating creams, facial masks, after shave lotions, balms, and/or radioprotective compositions (*e.g.*, sunscreens).

As used herein, "improving the visual appearance of skin" refers to any benefit achieved through use of the personal care compositions of the present invention. Examples of benefits include but are not limited to reducing the visual appearance of pores (*e.g.*, by reducing pore size), reducing imperfections and/or blemishes in skin color, including lightening hyperpigmented regions of skin and/or evening skin pigmentation, relieving dryness, eliminating rough, dry spots, improving the skin's ability to retain moisture and/or protect itself from environmental stresses, reducing the appearance of fine lines and wrinkles, improving appearance and skin tone, increasing skin firmness and/or suppleness, decreasing sagging of skin, increasing skin glow and clarity, increasing the skin renewal process, and/or removing vellus hair. Improving the visual appearance of skin also encompasses regulating wrinkles, atrophy, skin lightening, skin darkening, skin smoothness, and/or reducing the visual appearance of pores.

As used herein, "modifying skin tone" refers to a change in skin color, either darkening or lightening the color of the skin. However, in other contexts, the term is also used in reference to modifications in the muscular and connective tissue health of the skin (*i.e.*, it is not related to the color of the skin, but the firmness of the skin).

As used herein, "lightening skin tone" and "lightening skin" refer to a decrease in skin darkness visualized by eye and/or mechanical means. It is intended that the term encompass any range of observable lightening (*i.e.*, "whitening") of the skin tone. In some embodiments, the term encompasses decreasing the concentration of melanin present in the skin, including skin areas with hyperpigmentation due to the presence of age spots, melasma, chloasma, freckles, post-inflammatory hyperpigmentation, and/or skin-induced hyperpigmentation.

As used herein, "hyperpigmented region" refers to a localized region of darker skin relative to the base skin tone of a particular individual. For example, in preferred embodiments, hyperpigmented regions are areas with localized increases in melanin.

As used herein, "preventing skin darkening," "preventing darkening of skin tone," "preventing skin tone darkening," "preventing skin color development," and other equivalent phrases refer to skin tone that is observed as less skin pigmentation or darkness compared to untreated skin after UV radiation and/or sun exposure. Thus, in some particularly preferred embodiments, the compositions of the present invention provide a benefit by preventing the skin darkening effects associated with exposure to sun and/or UV radiation. It is intended that the term encompass any range of observable difference between sun-exposed skin that is protected from darkening and sun-exposed skin that is not protected.

As used herein, "evening skin tone" refers to the evening of skin color in an application area. In preferred embodiments, the phrase refers to the use of compositions of the present invention to make the skin color the same or provide less of a contrast in the skin color in one or more skin region.

As used herein, "reducing redness in skin tone" refers to a lessening of red color in skin, as observed visually or by other means.

As used herein, "inhibiting hair growth" and "inhibition of hair growth" refer to an observed lessening of hair length and/or thickness. Thus, in some preferred embodiments, application of a personal care composition of the present invention provides a benefit in lessening hair length and/or thickness as compared to an area in which a personal care composition of the present invention has not been applied. In some embodiments, the observed reduction of hair growth and/or thickness is a range from less than 1% to more than 99%, as compared to untreated areas, while in other embodiments, the observed reduction is from about 100% to about 90%, from about 90% to about 80%, from about 80% to about 70%, from about 70% to about 60%, from about 60% to about 50%, from about 50% to about 40%, from about 40% to about 30%, from about 30% to about 20%, from about 20% to about 10%, from about 10% to about 1%. Indeed, it is not intended that the term be limited to any particular percentage reduction, as long as the reduction is observable by visual (*i.e.*, by eye) or other means. It is also intended that the term encompass "preventing hair growth" to any degree, as described above. It is not intended that the term be limited to the complete prevention of hair growth (*i.e.*, there is no observed growth of hair).

As used herein, "hair care composition" refers to compositions that are applied to hair to provide beneficial properties such as thickening, thinning, coloring, decoloring, cleansing, conditioning, softening, shaping, etc. In some embodiments, the hair care composition is in a form selected from the group consisting of shampoos, conditioners, anti-dandruff treatments, styling aids, styling conditioners, hair repair or treatment sera, lotions, creams, pomades, and chemical treatments. In other embodiments, the styling aids are selected from the group consisting of sprays, mousses, rinses, gels, foams, and combinations thereof. In further embodiments, the chemical treatments are selected from the group consisting of permanent waves, relaxers, and permanents, semi-permanents, temporary color treatments and combinations thereof.

As used herein, "makeup compositions" refer to cosmetic preparations that are used to beautify, caring for, maintaining, or augment the appearance of a human or other animal. "Makeup compositions" include, but are not limited to color cosmetics, such as mascaras, lipsticks, lip liners, eye shadows, eye-liners, rouges, face powders, foundations, blushes, and nail polish. In some embodiments, the cosmetic composition of the present invention is in a form selected from the group consisting of eye gels, eye shadows, high-melting point lipsticks, lipsticks, lip glosses, lip balms, mascara, brow liners, eyeliners, pressed powder formulations, foundations, protein coated pigments, and combinations thereof. In further embodiments, the cosmetic compositions comprise makeup compositions. In yet another embodiment, the nail care composition is in a form selected from the group consisting of nail enamel, cuticle treatment, nail polish, nail treatment, and polish remover.

As used herein, "oral care compositions" refer to personal care compositions suitable for use in the mouth, including but not limited to forms selected from the group consisting of toothpastes, tooth gels, mouth rinses, breath fresheners, whitening treatments, and inert carrier substrates.

As used herein, the term "dispersed phase" is used as by those of skill in the art of emulsion technology as the phase that exists as small particles or droplets suspended in and surrounded by a continuous phase. The dispersed phase is also known as the "internal" or "discontinuous" phase.

As used herein, "penetration enhancers" refer to compositions that facilitate penetration through the upper stratum corneum barrier to the deeper skin layers. Examples of penetration enhancers include, but are not limited to, propylene glycol, azone, ethoxydiglycol, dimethyl isosorbide, urea, ethanol, dimethyl sulfoxide, microemulsions, liposomes, and nanoemulsions.

As used herein, the terms "emulsifier" and "surfactant" refer to compounds that disperse and suspend the dispersed phase within the continuous phase of a material. Surfactants find particular use in products intended for skin and/or hair cleansing. In particular embodiments, the term "surfactant(s)" is used in reference to surface-active agents, whether used as emulsifiers or for other surfactant purposes such as skin cleansing.

In various embodiments, the present invention also includes "protectants" such as UV absorbers (*e.g.*, octyl methoxycinnamate, benzophenone-3, titanium dioxide, and octyl salicylate); film-forming agents (*e.g.*, VP/Eicosene copolymer); cosmeceutical agents (*e.g.*, peptides and proteins, alpha hydroxy acids, and retinol and retinoic acid derivatives); antioxidants (*e.g.*, tocopherol and derivatives thereof and ascorbic acid and derivatives thereof); vitamins (*e.g.*, B, D, K and their derivatives); antiperspirant actives (*e.g.*, aluminum hydroxide and zirconium hydroxide); depilating agents (*e.g.*, thioglycolate salts); anti-acne agents (*e.g.*, salicylic acid and benzoyl peroxide); abrasives and exfoliants (*e.g.*, silicates, pumice, and polyethylene); and extracts of plant, fruit, vegetable and/or marine sources.

Thus, in some embodiments, the present invention provides compositions comprising any organic UV-A and UV-B filter, for example but not limited to the following:

Nr.	Compound	CAS-Nr. (=Acid)
1	4-Aminobenzoicacid	150-13-0
2	3-(4'-Trimethylammonium)-benzylidenbornan-2-on-methylsulfate	52793-97-2
3	3,3,5-Trimethyl-cyclohexyl-salicylate (Homosalatum)	118-56-9
4	2-Hydroxy-4-methoxy-benzophenon (Oxybenzonum)	131-57-7
5	2-Phenylbenzimidazol-5-sulfonic acid and their Calcium-, Sodium- and Triethanolaminosalts	27503-81-7
6	3,3'-(1,4-Phenylendimethin)-bis(7,7-dimethyl- 2-oxobicyclo[2.2.1]heptan-1-methansulfonicacid) and salts thereof	90457-82-2
7	4-Bis(polyethoxy)amino-benzoessäurepolyethoxy-ethylester	113010-52-9
8	4-Dimethylamino-benzoicacid-2-ethylhexylester	21245-02-3
9	Salicylicacid-2-ethylhexylester	118-60-5
10	4-Methoxy-cinnamicacid-2-isoamylester	71617-10-2
11	4-Methoxy-cinnamicacid -2-ethylhexylester	5466-77-3
12	2-Hydroxy-4-methoxy-benzophenon-5-sulfonicacid- (Sulisobenzonum) and the sodiumsalt	4065-45-6
13	3-(4'-Sulfobenzyliden)-bornan-2-on and salts thereof	58030-58-6
14	3-Benzylidenbornan-2-on	16087-24-8
15	1-(4'-Isopropylphenyl)-3-phenylpropan-1,3-dion	63260-25-9
16	4-Isopropylbenzylsalicylat	94134-93-7
17	3-Imidazol-4-yl-acrylicacid und ihr Ethylester	104-98-3
18	2-Cyano-3,3-diphenylacrylicacidethylester	5232-99-5
19	2-Cyano-3,3-diphenylacrylicacid-2'-ethylhexylester	6197-30-4
20	Menthyl-o-aminobenzoat oder: 5-Methyl-2-(1-methylethyl)-2-aminobenzoat	134-09-8
21	Glyceryl p-aminobenzoat oder: 4-Aminobenzoicacid-1-glyceryl-ester	136-44-7
22	2,2'-Dihydroxy-4-methoxybenzophenon (Dioxybenzone)	131-53-3
23	2-Hydroxy-4-methoxy-4-methylbenzophenon (Mexenon)	1641-17-4
24	Triethanolamin Salicylat	2174-16-5
25	Dimethoxyphenylglyoxalsäure oder: 3,4-dimethoxy-phenyl-glyoxal-saures Natrium	4732-70-1
26	3-(4'Sulfobenzyliden)-bornan-2-on und seine Salze	56039-58-8
27	4-tert.-Butyl-4'-methoxy-dibenzoylmethan	70356-09-1
28	2,2',4,4'-Tetrahydroxybenzophenon	131-55-5
29	2,2'-Methylen-bis-[6-(2H-benzotriazol-2-yl)-4-(1,1,3,3,-	103597-45-1

Nr.	Compound	CAS-Nr. (=Acid)
	tetramethylbutyl]phenol]	
30	2,2'-(1,4-Phenylene)-bis-1H-benzimidazol-4,6-disulfonicacid, Sodiumsalt	180898-37-7
31	2,4-bis-[4-(2-Ethylhexyloxy)-2-hydroxy]phenyl-6-(4-methoxyphenyl)-(1,3,5)-triazin	187393-00-6
32	3-(4-Methylbenzyliden)-campher	36861-47-9
33	4-Bis(polyethoxy)paraaminobenzoicacidpolyethoxyethylester	113010-52-9
34	2,4-Dihydroxybenzophenon	131-56-6
35	2,2'-Dihydroxy-4,4'-dimethoxybenzophenon-5,5'-disodiumsulfonat	3121-60-6
36	Benzoicacid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester	302776-68-7
37	2-(2H-Benzotriazol-2-yl)-4-methyl-6-[2-methyl-3-[1,3,3,3-tetramethyl-1-[(trimethylsilyl)oxy]disiloxanyl]propyl]phenol	155633-54-8
38	1,1-[(2,2'-Dimethylpropoxy)carbonyl]-4,4-diphenyl-1,3-butadien	363602-15-7

In some embodiments, the present invention provides compositions comprising pigments, including, but not limited to inorganic pigments based on metaloxides and/or other in water slightly soluble or insoluble metal compounds such as zinc oxides (ZnO), titanium (TiO₂), iron (*e.g.*, Fe₂O₃), zirconium (ZrO₂), silica (SiO₂), manganese (*e.g.*, MnO), aluminium (Al₂O₃), cer (*e.g.*, Ce₂O₃), and mixed compositions of these oxides, as well as blends thereof. In some preferred embodiments, the metaloxides are microfine, while in alternative preferred embodiments, the metaloxides are pigment grade. In yet additional embodiments, the pigments are "coated" such that they are surface treated. In some preferred embodiments, the coating comprises a thin, hydrophobic film layer, while in other embodiments, the coating comprises a thin, hydrophilic film layer.

As used herein, the terms "pigment," "color pigment," and "dye" used in reference to the compositions of the present invention encompasses any compound that provides a color to the composition and/or imparts a color to the surface (*e.g.*, skin and/or hair) to which the composition is applied. In some embodiments, the dyes and pigments are chosen from the list of cosmetic colorants provided by the Cosmetics Directive or the EC. In most cases, these dyes and pigments are identical to the dyes approved for foods. Preferred pigments/dyes include for example, titanium dioxide, mica, iron oxides (*e.g.*, Fe₂O₃, Fe₃O₄, FeO(OH)) and/or tin oxide. Advantageous pigments/dyes include for example, carmine, Berlin blue, chrome oxide green, ultramarine blue and/or manganese violet. In some preferred embodiments, the pigments/dyes include, but are not limited to those in the following table. The Colour Index Numbers (CIN) those known in the art (*See*, Society of Dyers and Colourists, Rowe Colour Index, 3rd Edition, Bradford, England, [1971]).

CHEMICAL OR OTHER NAME	CIN	COLOR
Pigment Green	10006	green
Acid Green 1	10020	green
2,4-Dinitrohydroxynaphthalene-7-sulfonic acid	10316	yellow
Pigment Yellow 1	11680	yellow
Pigment Yellow 3	11710	yellow
Pigment Orange 1	11725	orange
2,4-Dihydroxyazobenzene	11920	orange
Solvent Red 3	12010	red
1-(2'-Chloro-4'-nitro-1'-phenylazo)-2-hydroxynaphthalene	12085	red
Pigment Red 3	12120	red
Ceres red; Sudan red; Fat Red G	12150	red
Pigment Red 112	12370	red
Pigment Red 7	12420	red
Pigment Brown 1	12480	brown
4-(2'-Methoxy-5'-sulfodiethylamido-1'-phenylazo)-3-hydroxy-5''-chloro-2'',4''-dimethoxy-2-naphthanilide	12490	red
Disperse Yellow 16	12700	yellow
1-(4-Sulfo-1-phenylazo)-4-aminobenzene-5-sulfonic acid	13015	yellow
2,4-Dihydroxyazobenzene-4'-sulfonic acid	14270	orange
2-(2,4-Dimethylphenylazo-5-sulfo)-1-hydroxynaphthalene-4-sulfonic acid	14700	red
2-(4-Sulfo-1-naphthylazo)-1-naphthol-4-sulfonic acid	14720	red
2-(6-Sulfo-2,4-xylylazo)-1-naphthol-5-sulfonic acid	14815	red
1-(4'-Sulfophenylazo)-2-hydroxynaphthalene	15510	orange
1-(2-Sulfo-4-chloro-5-carboxy-1-phenylazo)-2-hydroxynaphthalene	15525	red
1-(3-Methylphenylazo-4-sulfo)-2-hydroxynaphthalene	15580	red
1-(4', (8'))-Sulfonaphthylazo)-2-hydroxynaphthalene	15620	red
2-Hydroxy-1,2'-azonaphthalene-1'-sulfonic acid	15630	red
3-Hydroxy-4-phenylazo-2-naphthylcarboxylic acid	15800	red
1-(2-Sulfo-4-methyl-1-phenylazo)-2-naphthyl- carboxylic acid	15850	red
1-(2-Sulfo-4-methyl-5-chloro-1-phenylazo)-2-hydroxynaphthalene-3-carboxylic acid	15865	red
1-(2-Sulfo-1-naphthylazo)-2-hydroxynaphthalene-3-carboxylic acid	15880	red
1-(3-Sulfo-1-phenylazo)-2-naphthol-6-sulfonic acid	15980	orange
1-(4-Sulfo-1-phenylazo)-2-naphthol-6-sulfonic acid	15985	yellow
Allura Red	16035	red
1-(4-Sulfo-1-naphthylazo)-2-naphthol-3,6-disulfonic acid	16185	red
Acid Orange 10	16230	orange
1-(4-Sulfo-1-naphthylazo)-2-naphthol-6,8-disulfonic acid	16255	red
1-(4-Sulfo-1-naphthylazo)-2-naphthol-3,6,8-trisulfonic acid	16290	red
8-Amino-2-phenylazo-1-naphthol-3,6-disulfonic acid	17200	red
Acid Red 1	18050	red
Acid Red 155	18130	red
Acid Yellow 121	18690	yellow
Acid Red 180	18736	red
Acid Yellow 11	18820	yellow
Acid Yellow 17	18965	yellow
4-(4-Sulfo-1-phenylazo)-1-(4-sulfophenyl)-5-hydroxy-pyrazolone-3-	19140	yellow

CHEMICAL OR OTHER NAME	CIN	COLOR
carboxylic acid		
Pigment Yellow 16	20040	yellow
2,6-(4'-Sulfo-2'', 4''-dimethyl)bisphenylazo)-1,3-dihydroxybenzene	20170	orange
Acid Black 1	20470	black
Pigment Yellow 13	21100	yellow
Pigment Yellow 83	21108	yellow
Solvent Yellow	21230	yellow
Acid Red 163	24790	red
Acid Red 73	27290	red
2-[4'-(4''-Sulfo-1''-phenylazo)-7'-sulfo-1'-naphthylazo]-1-hydroxy-7-aminonaphthalene-3,6-di-sulfonic acid	27755	black
4'-[(4''-Sulfo-1''-phenylazo)-7'-sulfo-1'-naphthylazo]-1-hydroxy-8-acetylaminonaphthalene-3,5-disulfonic acid	28440	black
Direct Orange 34, 39, 44, 46, 60	40215	orange
Food Yellow	40800	orange
trans- β -Apo-8'-carotinaldehyde (C ₃₀)	40820	orange
trans-Apo-8'-carotinic acid (C ₃₀)-ethyl ester	40825	orange
Canthaxanthin	40850	orange
Acid Blue 1	42045	blue
2,4-Disulfo-5-hydroxy-4'-4''-bis(diethylamino)triphenylcarbinol	42051	blue
4-[(4-N-Ethyl-p-sulfobenzylamino)phenyl(4-hydroxy-2-sulfophenyl)(methylene)-1-(N-ethyl-N-p-sulfobenzyl)-2,5-cyclohexadienimine]	42053	green
Acid Blue 7	42080	blue
(N-Ethyl-p-sulfobenzylamino)phenyl(2-sulfophenyl)methylene-(N-ethyl-N-p-sulfobenzyl)D ^{2,5} -cyclohexadienimine	42090	blue
Acid Green 9	42100	green
Diethyldisulfobenzyl-di-4-amino-2-chloro-di-2-methyl-fuchsonimmonium	42170	green
Basic Violet 14	42510	violet
Basic Violet 2	42520	violet
2'-Methyl-4'-(N-ethyl-N-m-sulfobenzyl)amino-4''-(N-diethyl)amino-2-methyl-N-ethyl-N-m-sulfobenzylfuchsonimmonium	42735	blue
4'-(N-Dimethyl)amino-4''-(N-phenyl)aminonaphtho-N-dimethyl-fuchsonimmonium	44045	blue
2-Hydroxy-3,6-disulfo-4,4'-bisdimethylaminonaphtho-fuchsonimmonium	44090	green
Acid Red 52	45100	red
3-(2'-Methylphenylamino)-6-(2'-methyl-4'-sulfophenylamino)-9-(2''-carboxyphenyl)xanthenium salt	45190	violet
Acid Red 50	45220	red
Phenyl-2-oxyfluorone-2-carboxylic acid	45350	yellow
4,5-Dibromofluorescein	45370	orange
2,4,5,7-Tetrabromofluorescein	45380	red
Solvent Dye	45396	orange
Acid Red 98	45405	red
3',4',5',6'-Tetrachloro-2,4,5,7-tetrabromofluorescein	45410	red
4,5-Diiodofluorescein	45425	red
2,4,5,7-Tetraiodofluorescein	45430	red
Quinophthalone	47000	yellow
Quinophthalonedisulfonic acid	47005	yellow

CHEMICAL OR OTHER NAME	CIN	COLOR
Acid Violet 50	50325	violet
Acid Black 2	50420	black
Pigment Violet 23	51319	violet
1,2-Dioxyanthraquinone, calcium-aluminum complex	58000	red
3-Oxypyrene-5,8,10-sulfonic acid	59040	green
1-Hydroxy-4-N-phenylaminoanthraquinone	60724	violet
1-Hydroxy-4-(4'-methylphenylamino)anthraquinone	60725	violet
Acid Violet 23	60730	violet
1,4-Di(4'-methylphenylamino)anthraquinone	61565	green
1,4-Bis(o-sulfo-p-toluidino)anthraquinone	61570	green
Acid Blue 80	61585	blue
Acid Blue 62	62045	blue
N,N'-Dihydro-1,2,1',2'-anthraquinone azine	69800	blue
Vat Blue 6; Pigment Blue 64	69825	blue
Vat Orange 7	71105	orange
Indigo	73000	blue
Indigo-disulfonic acid	73015	blue
4,4'-Dimethyl-6,6'-dichlorothioindigo	73360	red
5,5'-Dichloro-7,7'-dimethylthioindigo	73385	violet
Quinacridone Violet 19	73900	violet
Pigment Red 122	73915	red
Pigment Blue 16	74100	blue
Phthalocyanine	74160	blue
Direct Blue 86	74180	blue
Chlorinated Phthalocyanines	74260	green
Natural Yellow 6,19; Natural Red 1	75100	yellow
Bixin, Nor-Bixin	75120	orange
Lycopene	75125	yellow
trans-alpha-, beta- and gamma-carotene	75130	orange
Keto- and/or hydroxyl derivates of carotene	75135	yellow
Guanine or pearlizing agent	75170	white
1,7-Bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione	75300	yellow
Complex salt (Na, Al, Ca) of carminic acid	75470	red
Chlorophyll a and b; copper compounds of chlorophylls and Chlorophyllins	75810	green
Aluminum	77000	white
Hydrated alumina	77002	white
Hydrous aluminum silicates	77004	white
Ultramarine	77007	blue
Pigment Red 101 und 102	77015	red
Barium sulfate	77120	white
Bismuth oxychloride and its mixtures with mica	77163	white
Calcium carbonate	77220	white
Calcium sulfate	77231	white
Carbon	77266	black
Pigment Black 9	77267	black
Carbo medicinalis vegetabilis	77268	black
Chromium oxide	77288	green
Chromium oxide, hydrous	77289	green
Pigment Blue 28, Pigment Green 14	77346	green
Pigment Metal 2	77400	brown

CHEMICAL OR OTHER NAME	CIN	COLOR
Gold	77480	brown
Iron oxides and hydroxides	77489	orange
Iron oxide	77491	red
Iron oxide, hydrated	77492	yellow
Iron oxide	77499	black
Mixtures of iron (II) and iron(III)hexacyano-ferrate	77510	blue
Pigment White 18	77713	white
Manganese ammonium diphosphate	77742	violet
Manganese phosphate; $Mn_3(PO_4)_2 \cdot 7 H_2O$	77745	red
Silver	77820	white
Titanium dioxide and its mixtures with mica	77891	white
Zinc oxide	77947	white
6,7-Dimethyl-9-(1'-D-ribityl)-isoalloxazine, lactoflavine		yellow
Sugar coloring		brown
Capsanthin, capsorubin		orange
Betanin		red
Benzopyrylium salts, Anthocyan		red
Aluminum, zinc, magnesium and calcium stearate		white
Bromothymol blue		blue
Bromocresol green		green
Acid Red 195		red

In yet further embodiments, compositions of the present invention further comprise one or more substances from the following group: 2,4-dihydroxyazobenzene, 1-(2'-chloro-4'-nitro-1'-phenylazo)-2-hydroxynaphthalene, Ceres Red, 2-(4-sulfo-1-naphthylazo)-1-naphthol-4-sulfonic acid, calcium salt of 2-hydroxy-1,2'-azonaphthalene-1'-sulfonic acid, calcium and barium salts of 1-(2-sulfo-4-methyl-1-phenylazo)-2-naphthylcarboxylic acid, calcium salt of 1-(2-sulfo-1-naphthylazo)-2-hydroxynaphthalene-3-carboxylic acid, aluminum salt of 1-(4-sulfo-1-phenylazo)-2-naphthol-6-sulfonic acid, aluminum salt of 1-(4-sulfo-1-naphthylazo)-2-naphthol-3,6-disulfonic acid, 1-(4-sulfo-1-naphthylazo)-2-naphthol-6,8-disulfonic acid, aluminum salt of 4-(4-sulfo-1-phenylazo)-1-(4-sulfophenyl)-5-hydroxypyrazolone-3-carboxylic acid, aluminum and zirconium salts of 4,5-dibromofluorescein, aluminum and zirconium salts of 2,4,5,7-tetrabromofluorescein, 3',4',5',6'-tetrachloro-2,4,5,7-tetrabromofluorescein and its aluminum salt, aluminum salt of 2,4,5,7-tetraiodofluorescein, aluminum salt of quinophthalone disulfonic acid, aluminum salt of indigo disulfonic acid, red and black iron oxide (CIN: 77 491 (red) and 77 499 (black)), iron oxide hydrate (CIN: 77 492), manganese ammonium diphosphate and titanium dioxide.

In yet further embodiments, oil-soluble natural dyes, such as, for example, paprika extracts, β -carotene or cochénille find use in the present invention.

In yet additional embodiments, gel cream compositions of the present invention comprise pearlescent pigments. In some preferred embodiments, various pearlescent pigments find use in the present invention, including but not limited to "natural pearlescent pigments" (*e.g.*, "pearl essence"

[guanine/hypoxanthine mixed crystals from fish scales], "mother of pearl" [ground mussel shells]), and

“monocrystalline pearlescent pigments” (*e.g.*, bismuth oxychloride [BiOCl]); and “layer substrate pigments” (*e.g.* mica/metal oxide).

Bases for pearlescent pigments include, but are not limited to pulverulent pigments, castor oil dispersions of bismuth oxychloride and/or titanium dioxide, bismuth oxychloride and/or titanium dioxide on mica. The luster pigment listed under CIN 77163, for example, is particularly advantageous.

An additional group of pearlescent pigments based on mica/metal oxide find particular use in the present invention is provided below.

GROUP	COATING/LAYER THICKNESS	COLOR
Silver-white pearlescent pigments	TiO ₂ : 40 – 60 nm	silver
Interference pigments	TiO ₂ : 60 – 80 nm	yellow
	TiO ₂ : 80 – 100 nm	red
	TiO ₂ : 100 – 140 nm	blue
	TiO ₂ : 120 – 160 nm	green
Color luster pigments	Fe ₂ O ₃	bronze
	Fe ₂ O ₃	copper
	Fe ₂ O ₃	red
	Fe ₂ O ₃	red-violet
	Fe ₂ O ₃	red-green
	Fe ₂ O ₃	black
Combination pigments	TiO ₂ / Fe ₂ O ₃	gold shades
	TiO ₂ / Cr ₂ O ₃	green
	TiO ₂ / Berlin blue	deep blue
	TiO ₂ / carmine	red

In some preferred embodiments, the pearlescent pigments available from Merck under the trade names Timiron, Colorona or Dichrona find use in the present invention. However, it is not intended that the present invention be limited to the specific pigments listed herein. Indeed, pearlescent pigments that find use in the present invention are obtainable from numerous sources. For example, other substrates apart from mica can be coated with further metal oxides, such as, for example, silica and the like. SiO₂ particles coated with, for example, TiO₂ and Fe₂O₃ (“ronaspheres”), which are sold by Merck and are particularly suitable for the optical reduction of fine lines find use in the present invention.

In alternative embodiments, the substrate (*e.g.*, mica) is not included. In some preferred embodiments, particular preference is given to pearlescent pigments prepared using SiO₂. Such pigments, which may also additionally have goniochromatic effects, are available, for example, under the trade name Sicopearl Fantastico, available from BASF.

In additional embodiments, pigments obtained from Engelhard / Mearl based on calcium sodium borosilicate which have been coated with titanium dioxide also find use. These are available under the name

Reflecks. In addition to the color, as a result of their particle size of from 40 nm to 180 nm, they have a glitter effect.

In yet further embodiments, effect pigments which are available under the trade name Metasomes Standard/Glitter in various colors (yellow, red, green, blue) from Flora Tech find use in the compositions of the present invention. The glitter particles are present here in the mixtures with various auxiliaries and dyes (such as, for example, the dyes with the Colour Index (CI) Numbers 19140, 77007, 77289, 77491).

In some embodiments, the dyes and pigments are present either individually or in a mixture. In alternative embodiments, they are mutually coated with one another, different coating thicknesses generally giving rise to different color effects. In some embodiments, the total amount of dyes and color-imparting pigments is chosen from a range of concentrations (*e.g.*, from about 0.1% by weight to about 30% by weight; preferably from about 0.5 to about 15% by weight; and most preferably from about 1.0 to about 10% by weight, in each case based on the total weight of the preparations).

The yet further embodiments, the present invention provides methods for the preparation of the compositions of the present invention. In some embodiments, these methods include combining and heating the constituents of the oil phase and/or the water phase separately, and then combining them together with stirring. In some preferred embodiments, the phases are homogenized. In some particularly preferred embodiments, the compositions are stirred with moderate to high input of energy, advantageously using a gear rim dispersing machine at a rotary number up to at most 10000 rpm (preferably in the range from about 2500 to about 7700 rpm).

Cosmetic Formulations Comprising the Present Invention

It is contemplated that the present invention will find use in numerous personal care compositions. It is not intended that the present invention be limited to any particular format or type of composition. The following description provides exemplary, not limiting compositions comprising the following invention.

Emulsions comprises one group of customary, commonly-used cosmetics. The term "emulsion" is generally used in reference to a heterogeneous system of two liquids which are immiscible or miscible only to a limited extent with one another, which are usually referred to as "phases." One phase is typically in the form of droplets (*i.e.*, the "dispersed," "discontinuous" or "internal" phase), while the other liquid forms a continuous (*i.e.*, "coherent" or "external") phase. Less common forms of application include multiple emulsions (*i.e.* those in which the droplets of the dispersed [or discontinuous] phase, comprise for their part droplets of a further dispersed phase, such as water/oil/water [W/O/W] emulsions and oil/water/oil [O/W/O] emulsions).

If the oil phase is finely distributed in the water phase, then this is an oil-in-water emulsion (O/W emulsion; *e.g.* milk). The basic character of an O/W emulsion is determined by the water. These emulsions are

generally less greasy on the skin, are rather matting, and absorb more rapidly into the skin than W/O (water-in-oil) emulsions.

Those of skill in the art are familiar with a large number of options of formulating stable W/O preparations for cosmetic and/or dermatological uses, including such formulations as creams and ointments, which are spreadable in the range from room temperature to skin temperature, as well as lotions and milks, which are more flowable in this temperature range.

The stability of emulsions is dependent on their viscosity, in particular on the viscosity of the external phase. An emulsion becomes unstable when the finely dispersed particles collect together to form relatively large aggregates, and the droplets which are in contact coalesce. This process is referred to as "coalescence." The more viscous the external phase of the emulsion, the slower the process of coalescence. Emulsions of "liquid" (= flowable) consistency are used in various cosmetics (*e.g.*, skin care lotions, cleansing lotions, face lotions, hand lotions, etc.). These compositions generally have a viscosity of from about 2000 mPa·s to about 10,000 mPa·s. The stability of flowable emulsions is deserving of particular attention since the considerably greater mobility of the particles promotes more rapid coalescence.

It is known that liquid emulsions typically presently in use generally comprise thickeners and are not stable toward relatively high electrolyte concentrations. This is manifested in phase separation of the compositions. However, in some embodiments, it is desirable to use certain electrolytes (*e.g.*, water-soluble UV filters), in order to be able to utilize the other physical, chemical or physiological properties thereof. Although in many cases appropriate choice of the emulsifier system can provide remedies to a certain extent, other disadvantages then arise just as often.

For example, some disadvantages result due to the fact that emulsifiers, like ultimately any chemical substance, may trigger allergic reactions or reactions based on oversensitivity (*i.e.*, hypersensitivity) of the user. The use of customary cosmetic emulsifiers is generally entirely without risk, although for some individuals, "hypoallergenic" compositions are necessary and/or preferred. Indeed, in some particularly sensitive individuals, certain dermatoses are triggered by exposure to certain emulsifiers and simultaneous exposure to sunlight. Thus, as known to those in the art, in some compositions, particular emulsifiers are less preferred and/or are avoided.

It is possible to prepare emulsifier-free preparations. For example, some preparations have an oily phase which contains dispersed water droplets (*i.e.*, it is similar to a W/O emulsion). Such systems are sometimes called "hydrodispersions" or "oleodispersions," depending upon which is the disperse phase and which is the continuous phase.

For cosmetic technology, it is, however, neither necessary nor possible to dispense with emulsifiers altogether, especially since there is a certain choice of particularly mild emulsifiers. However, the emulsifiers in current use lack a satisfactorily broad range of choices. Thus, the application spectrum of correspondingly mild cosmetic preparations which are tolerated by the skin is limited.

In addition to the deleterious effects of some emulsifiers, exposure to other factors is known to harm skin and hair. For example, the harmful effect of the ultraviolet portion of solar radiation on the skin is generally known. While rays having a wavelength of less than 290 nm (*i.e.*, the UVC region) are absorbed by the ozone layer in the earth's atmosphere, rays in the range between 290 nm and 320 nm (*i.e.*, the UVB region), cause erythema, simple sunburn or even burns of varying severity. The erythema activity maximum of sunlight is given as the relatively narrow region around 308 nm.

Numerous compounds are known to provide protection against harmful UVB radiation. Most commonly, these compounds are derivatives of 3-benzylidenecamphor, of 4-aminobenzoic acid, of cinnamic acid, of salicylic acid, of benzophenone, and of 2-phenyl-benzimidazole.

It is also important to have available filter substances for the range between about 320 nm and about 400 nm, the UVA region, since its rays can also cause damage. For a long time it was incorrectly assumed that the long-wave UV-A radiation having a wavelength of between 320 nm and 400 nm had only a negligible biological action and that, accordingly, the UV-B rays were responsible for most photodamage to the human skin. However, numerous recent studies have shown that UV-A radiation is much more harmful than UV-B radiation with regard to the triggering of photodynamic, specifically phototoxic, reactions and chronic changes in the skin. In addition, the harmful effects of UV-B radiation can be further intensified by exposure to UV-A radiation.

It has been shown that UV-A radiation by itself and under very normal everyday conditions, is sufficient to damage collagen and elastin fibers, which are of essential importance for the structure and strength of the skin, within a short period. This leads to chronic light-induced changes in the skin, such that the skin prematurely "ages." The clinical appearance of skin aged by light typically includes increased wrinkles and lines, and an irregular, furrowed relief. In addition, the skin areas affected by light-induced skin aging often show irregular pigmentation. In some cases, brown patches, keratoses, carcinomas, or malignant melanomas arise. Skin prematurely aged as a result of everyday UV exposure is also characterized by having lowered activity of the Langerhans cells and slight, chronic inflammation.

Approximately 90% of the ultraviolet radiation which reaches the Earth consists of UV-A rays. While amount of UV-B radiation reaching Earth varies widely depending on numerous factors (*e.g.*, time of year and day and/or degree of latitude), the UV-A radiation levels that reach Earth remain relatively constant on a daily basis, irrespective of the time of year and day or geographical factors. Additionally, the majority of UV-A radiation penetrates the living epidermis, while about 70% of the UV-B rays are retained by the horny layer. Preventive protection against UV-A rays, for example by applying light protection filter substances in the form of a cosmetic or dermatological formulation to the skin, is therefore of fundamental importance.

In general, the light absorption behavior of light protection filter substances is very well known and documented, largely due to the fact that most industrialized countries have positive lists for the use of such substances, which impose very strict standards on the documentation that accompanies each product which

incorporates these substances. For the concentration of the substances in the finished formulations, the absorbance values provide a guide, since interaction with substances within the skin or the surface of the skin itself often presents variables that may impact how well the compositions perform on each individual. However, it is usually difficult to estimate beforehand, how uniformly and thickly the filter substance is distributed in and on the horny layer of the skin.

To test UV-A protection performance, use is usually made of the IPD method (IPD 5 immediate pigment darkening) known to those in the art. This method is similar to the determination of the sun protection factor, and provides a method which indicates how much longer skin protected with the light protection composition can be irradiated with UV-A radiation before the pigmentation which occurs is the same as that produced for unprotected skin.

Another test method which has become established throughout Europe is the Australian standard AS/NZS 2604:1997. In this method, the absorption of the preparation in the UV-A region is measured. In order to satisfy the standard, the preparation must absorb at least 90% of the UV-A radiation in the region 320-360 nm.

Of concern in the formulation of sunscreen compositions is that the use concentration of known light protection filter substances which also exhibit high filter action in the UV-A region are often limited by the very fact that they are combined with other substances which are in the form of solids. Thus, there are certain formulation difficulties associate with achieving relatively high sun protection factors and UV-A protection performance. However, those of skill in the art are generally aware of means to overcome and/or compensate for these difficulties.

As light protection filter substances are generally expensive and some light protection filter substances are additionally difficult to incorporate into cosmetic and/or dermatological preparations in relatively high concentrations, some embodiments of the present invention were designed to provide simple and cost-effective preparations which, despite having unusually low concentrations of conventional UV-A light protection filter substances, nevertheless achieve acceptable or even high UV-A protection performance.

However, as known in the art, UV radiation can also lead to photochemical reactions which produce products that interfere with the skin's metabolism. These photochemical reaction products are predominantly free-radical compounds (*e.g.*, hydroxyl radicals). Undefined free-radical photoproducts which form in the skin itself can also exhibit uncontrolled secondary reactions as a result of their high reactivity. However, singlet oxygen, a non-free-radical excited state of the oxygen molecule, can also arise during UV irradiation, as can short-lived epoxides and many others. Singlet oxygen, for example, differs from normal triplet oxygen (free-radical ground state) by virtue of its increased reactivity. However, excited, reactive "free-radical" triplet states of the oxygen molecule also exist. Thus, in order to prevent these reactions, antioxidants and/or free-radical scavengers find use in cosmetic and/or dermatological formulations.

The compounds which are commonly used as light protection agents in cosmetic and/or dermatological light protection formulations are generally characterized as providing good light protection. However, they have the disadvantage that it is sometimes difficult to incorporate them into the desired formulations in a satisfactory manner.

5 As indicated above, the sun protection factor (SPF) indicates how much longer the skin protected with the light protection composition can be irradiated before the erythema reaction which occurs is the same as for unprotected skin (*i.e.*, ten times as long compared with unprotected skin for an SPF = 10). Consumers are very aware of the meaning of "SPF" and choose skin and/or hair care products based on the SPF values indicated on products. Consumers expect to receive reliable information from manufacturers
10 regarding the sun protection factor, largely due to increased public awareness of the association between excess sun exposure and skin cancer, as well as premature aging. In addition, in some parts of the world, the degradation of the ozone layer is a major concern. Depending upon the skin type and the sun exposure expected, consumers choose products with a lower or a higher SPF. However, there appears to be a tendency for consumers to select relatively high SPF factors, particularly for products to be applied to
15 children and those with fair skin. In some embodiments, the present invention provides compositions with relatively low concentrations of conventional light protection filter substances, yet with SPF values that are acceptable to consumers.

In some preferred embodiments, the basic constituents of the sunscreen preparations provided by the present invention include: water or aqueous solutions; aqueous ethanolic solutions; natural oils and/or
20 chemically modified natural oils and/or synthetic oils; fats, waxes and other natural and synthetic fatty substances, preferably esters of fatty acids with alcohols of low carbon number (*e.g.*, with isopropanol, propylene glycol or glycerol), or esters of fatty alcohols with alkanolic acids of low carbon number or with fatty acids; alcohols, diols or polyols of low carbon number, and ethers thereof, preferably ethanol, isopropanol, propylene glycol, glycerol, ethylene glycol, ethylene glycol monoethyl or monobutyl ether,
25 propylene glycol monomethyl, monoethyl or monobutyl ether, diethylene glycol monomethyl or monoethyl ether and analogous products. In alternatively preferred embodiments, mixtures of two or more of these constituents find use in the present invention.

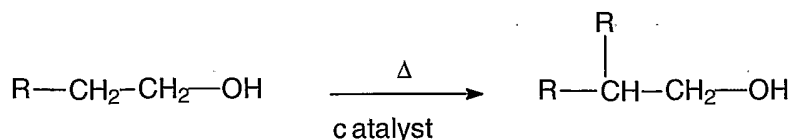
The term "lipid" is often used as a generic term to refer to fats, oils, waxes and the like. In addition, the terms "oil phase" and "lipid phase" are also used synonymously. However, oils and fats differ from one
30 another in their polarity, which is difficult to define. It has been proposed to adopt the interfacial tension toward water as a measure of the polarity index of an oil or of an oily phase. Thus, it is contemplated that the interfacial tension be regarded as a suitable measure of the polarity of a given oil component. The "interfacial tension" is the force which acts on an imaginary line one meter in length in the interface between two phases. In this measurement, the lower the interfacial tension between the oily phase and
35 water, the greater the polarity of the oily phase being analyzed. The physical unit for this interfacial tension is conventionally calculated from the force/length relationship and is usually expressed in mN/m

(millinewtons divided by meters). It has a positive sign if it endeavours to reduce the interface. In the converse case, it has a negative sign. As used herein, lipids are regarded as "polar," if their interfacial tension toward water is less than 30 mN/m.

"Polar oils" include those from the group of lecithins and of fatty acid triglycerides, namely the triglycerol esters of saturated and/or unsaturated, branched and/or unbranched alkane carboxylic acids having a chain length of from 8 to 24, in particular 12 to 18, carbon atoms. In some embodiments, the fatty acid triglycerides are chosen from the group consisting of synthetic, semi-synthetic and natural oils (*e.g.*, olive oil, sunflower oil, soya oil, groundnut oil, rapeseed oil, almond oil, palm oil, coconut oil, castor oil, wheatgerm oil, grapeseed oil, thistle oil, evening primrose oil, macadamia nut oil and the like). However, is it not intended that the present invention be limited to compositions that contain particular polar oils.

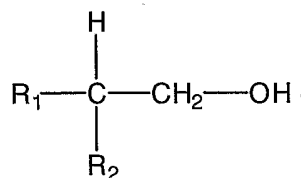
Additional examples of polar oils that find use in the present invention include the group of esters of saturated and/or unsaturated, branched and/or unbranched alkane carboxylic acids having a chain length of from 3 to 30 carbon atoms and saturated and/or unsaturated, branched and/or unbranched alcohols having a chain length of from 3 to 30 carbon atoms, and from the group of esters of aromatic carboxylic acids and saturated and/or unsaturated, branched and/or unbranched alcohols having a chain length of from 3 to 30 carbon atoms. In some embodiments, such ester oils are chosen from the group consisting of isopropyl myristate, isopropyl palmitate, isopropyl stearate, isopropyl oleate, n-butyl stearate, n-hexyl laurate, n-decyl oleate, isooctyl stearate, isononyl stearate, isononyl isononanoate, 2-ethylhexyl palmitate, 2-ethylhexyl laurate, 2-hexyldecyl stearate, 2-octyldodecyl palmitate, oleyl oleate, oleyl erucate, erucyl oleate, erucyl erucate and synthetic, semi-synthetic and natural mixtures of such esters (*e.g.*, jojoba oil).

In addition, in some embodiments, the oily phase is chosen from the group consisting of dialkyl ethers, as well as saturated or unsaturated, and branched or unbranched alcohols. In some particularly preferred embodiments, the oily phase of the compositions of the preferred embodiments also contains C₁₂₋₁₅-alkyl benzoate, while in alternative embodiments, the preferred embodiments contains only the latter. In yet additional embodiments, the oil phase is chosen from the group of Guerbet alcohols (*i.e.*, the group of alcohols named after Marcel Guerbet who first described their preparation). These alcohols are formed according to the equation:

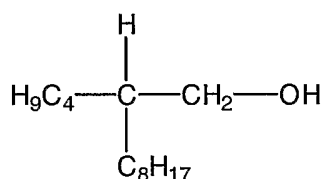


by oxidation of an alcohol to an aldehyde, by aldol condensation of the aldehyde, elimination of water from the aldol and hydrogenation of the allyl aldehyde. Guerbet alcohols are liquid even at low temperatures and result in virtually no skin irritations. Thus, they find use as fatting, superfatting and also refatting constituents in skincare and hair care compositions. Indeed, the use of Guerbet alcohols is known in the

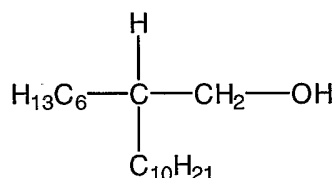
cosmetic art. In these applications, the species are generally characterized as having the following structure:



In this structure, R₁ and R₂ are usually unbranched alkyl radicals. In some preferred embodiments of the present invention the following Guerbet alcohols in which R₁ is propyl, butyl, pentyl, hexyl, heptyl or octyl and/or R₂ is hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl, tridecyl or tetradecyl find use in the present invention. In additional embodiments, preferred Guerbet alcohols include 2-butyloctanol with the following chemical structure:



which is commercially available, for example, under the trade name ISOFOL[®] 12 (Condea Chemie GmbH), and 2-hexyldecanol with the following chemical structure:



which is commercially available, for example, under the trade name ISOFOL[®] 16 (Condea Chemie GmbH).

In additional embodiments, mixtures of Guerbet alcohols find use in compositions of the present invention. For example, mixtures of 2-butyloctanol and 2-hexyldecanol find use in some embodiments. The total amount of Guerbet alcohols in the finished cosmetic or dermatological preparations is selected from the of range up to about 25.0% by weight, preferably about 0.5 to about 15.0% by weight, based on the total weight of the preparations. However, it is not intended that the present invention be limited to any particular concentration nor range of concentrations, as those of skill in the art know how to prepare compositions having suitable concentrations for the desired compositions and their use(s). In addition, it is contemplated that any mixtures of oil and/or wax components will find use in the present invention. For example, in some embodiments, waxes (*e.g.*, cetyl palmitate) find use as the sole lipid component of the oil phase. In additional embodiments, nonpolar oils (*e.g.*, those which are chosen from the group of branched

and unbranched hydrocarbons and hydrocarbon waxes, in particular VASELINE® [*i.e.*, petrolatum], paraffin oil, squalane and squalene, polyolefins and hydrogenated polyisobutenes find use in the present invention. In some embodiments containing polyolefins, polydecenes are the preferred substances.

Fatty and/or wax components which find use in embodiments of the present invention include but are not limited to vegetable waxes, animal waxes, mineral waxes and petrochemical waxes. Examples which particularly preferred waxes include candelilla wax, carnauba wax, japan wax, esparto grass wax, cork wax, guaruma wax, rice germ oil wax, sugar cane wax, berry wax, ouricury wax, montan wax, jojoba wax, shea butter, beeswax, shellac wax, spermaceti, lanolin (wool wax), uropygial grease, ceresin, ozokerite (earth wax), paraffin waxes and microcrystalline waxes.

Additional fatty and/or wax components that find use in the present invention include chemically modified waxes and/or synthetic waxes (*e.g.*, those commercially available under the trade names SYNCROWAX® HRC [glyceryl tribehenate] and SYNSCROWAX® AW 1C [C_{18} - C_{36} fatty acid], which are available from CRODA GmbH), and montan ester waxes, Sasol waxes, hydrogenated jojoba waxes, synthetic or modified beeswaxes (*e.g.*, dimethicone copolyol beeswax and/or C_{30-50} alkyl beeswax), polyalkylene waxes, polyethylene glycol waxes, as well as chemically modified fats (*e.g.*, hydrogenated vegetable oils, such as hydrogenated castor oil and/or hydrogenated coconut fatty glycerides), triglycerides (*e.g.*, trihydroxystearin, fatty acids, fatty acid esters, and glycol esters, such as, C_{20} - C_{40} -alkyl stearate, C_{20} - C_{40} -alkylhydroxystearoyl stearate and/or glycol montanate). In further embodiments, the present invention comprises certain organosilicone compounds, which have similar physical properties to the specified fatty and/or wax components (*e.g.*, stearoxytrimethylsilane). In additional embodiments, the fatty and/or wax components are provided individually, while in still further embodiments, they are provided as a mixture. Indeed, it is intended that any desired mixture of such oil and/or wax components will find use in various embodiments of the present invention.

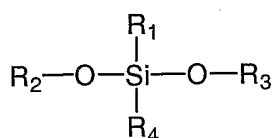
In some embodiments, the oily phase is selected from the group consisting of 2-ethylhexyl isostearate, octyldodecanol, isotridecyl isononanoate, isoeicosane, 2-ethylhexyl cocoate, C_{12} - C_{15} -alkyl benzoate, caprylic/capric triglyceride, and dicaprylyl ether. In alternative embodiments, mixtures of various oily phases are provided, including but not limited to mixtures comprising one or more of octyldodecanol, caprylic/capric triglyceride, dicaprylyl ether, C_{12} - C_{15} -alkyl benzoate, 2-ethylhexyl isostearate, isotridecyl isononanoate. The following table provides a list of lipids which find use alone or in combination with other lipids in various embodiments of the present invention. The corresponding interfacial tensions toward water are given in the last column. However, it is not intended that the present invention be limited to these specific components, as other components find use in various embodiments of the present invention, including mixtures of greater or lesser polar components and the like.

LIPIDS		
Trade name	INCI name	(m/Nm)
ISOFOL [®] 14 T	Butyl Decanol + Hexyl Decanol + Hexyl Octanol + Butyl Octanol	27.6
ISOFOL [®] 16	Hexyl Decanol	24.3
EUTANOL [®] G	Octyldodecanol	24.8
CETIOL [®] OE	Dicaprylyl Ether	22.1
MIGLYOL [®] 812	Caprylic/Capric Triglyceride	21.3
CEGESOFT [®] C24	Octyl Palmitate	23.1
Isopropyl stearate	Isopropyl Stearate	21.9
ESTOL [®] 1540 EHC	Octyl Octanoate	30.0
FINSOLV [®] TN	C ₁₂ -C ₁₅ Alkyl Benzoate	21.8
CETIOL [®] SN	Cetearyl Isonanoate	28.6
DERMOFEEL [®] BGC	Butylene Glycol Dicaprylate/Dicapate	21.5
TRIVENT [®] OCG	Tricaprylin	20.2
MOD	Octyldodeceyl Myristate	22.1
COSMACOL [®] ETI	Di-C ₁₂ -C ₁₃ Alkyl Tartrate	29.4
MIGLYCOL [®] 829	Caprylic/Capric Diglyceryl Succinate	29.5
PRISORINE [®] 2036	Octyl Isostearate	29.7
TEGOSOFT [®] SH	Stearyl Heptanoate	28.7
ABIL [®] Wax 9840	Cetyl Dimethicone	25.1
CETIOL [®] LC	Coco-Caprylate/Caprato	24.8
IPP	Isopropyl Palmitate	22.5
LUVITOL [®] EHO	Cetearyl Octanoate	28.6
CETIOL [®] 868	Octyl Stearate	28.4

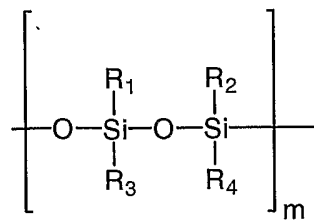
In some embodiments, some or all of the oil phase of the preparations are selected from the group consisting of cyclic and/or linear silicones which are also often referred to as "silicone oils." In some

5 embodiments, these silicones or silicone oils are present as monomers which are generally characterized by structural elements as follows:

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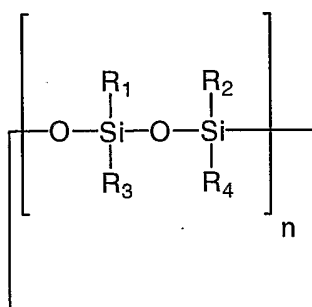


Silicones having two or more siloxyl units which find use in some embodiments of the present invention are generally characterized by structural elements as follows:



- 5 where the silicon atoms may be substituted by identical or different alkyl radicals and/or aryl radicals, which are represented in general terms by the radicals R_1 to R_4 , where the number of different radicals is not necessarily limited to 4 and may assume values from 2 to 200,000.

Cyclic silicones to be used advantageously according to the invention are generally characterized by the structural elements as follows



10 where the silicon atoms may be substituted by identical or different alkyl radicals and/or aryl radicals, which are represented here in general terms by the radicals R_1 to R_4 , where the number of different radicals is not necessarily limited to 4. n can assume values of $3/2$ to 20. Fractional values for " n " take into consideration that uneven numbers of siloxyl groups may be present in the cycle.

- 15 In some embodiments, phenyltrimethicone is selected as silicone oil. Other silicone oils suitable for use in various embodiments of the present invention include, but are not limited to dimethicone, phenyldimethicone, cyclomethicone (octamethylcyclotetrasiloxane), hexamethylcyclotrisiloxane, polydimethylsiloxane, poly(methylphenylsiloxane), cetyldimethicone, and behenoxydimethicone. In alternative embodiments, mixtures of these compounds find use in the present invention, including but not
- 20 limited to mixtures of cyclomethicone and isotridecyl isononanoate, and mixtures of cyclomethicone and 2-ethylhexyl isostearate. In yet additional embodiments, silicone oils of similar constitution, such as the compounds referred to above whose organic side chains have been derivatized (*e.g.*, polyethoxylated and/or polypropoxylated) find use in the present invention. These include, but are not limited to such compounds as

polysiloxane-polyalkyl-polyether copolymers such as cetyldimethicone copolyol (*i.e.*, cetyldimethicone copolyol (and) polyglyceryl-4 isostearate (and) hexyl laurate). Indeed, it is not intended that the present invention be limited to any specific silicone oil nor mixture of silicone oils, as various oils find use in various embodiments of the present invention.

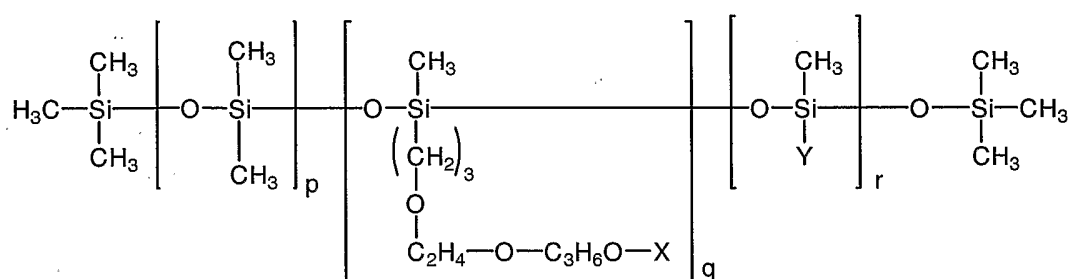
5 In additional embodiments, water in oil (W/O) emulsions find use in the present invention. In some embodiments, W/O emulsifiers are used with or without additional co-emulsifiers. In still further embodiments, W/O emulsions of the present further comprise one or more emulsifiers, including, but not limited to one or more of the following compounds: lecithin, lanolin, microcrystalline wax (Cera microcrystallina) in a mixture with paraffin oil (Paraffinum liquidum), ozokerite, hydrogenated castor oil, 10 polyglyceryl-3 oleate, wool wax acid mixtures, wool wax alcohol mixtures, pentaerythrithyl isostearate, polyglyceryl-3 diisostearate, beeswax (Cera alba) and stearic acid, sodium dihydroxycetylphosphate in a mixture with isopropyl hydroxycetyl ether, methylglucose dioleate, methylglucose dioleate in a mixture with hydroxystearate and beeswax, mineral oil in a mixture with petrolatum and ozokerite and glyceryl oleate and lanolin alcohol, petrolatum in a mixture with ozokerite and hydrogenated castor oil and glyceryl isostearate 15 and polyglyceryl-3 oleate, PEG-7 hydrogenated castor oil, ozokerite and hydrogenated castor oil, polyglyceryl-4 isostearate, polyglyceryl-4 isostearate in a mixture with cetyldimethicone copolyol and hexyl laurate, laurylmethicone copolyol, cetyldimethicone copolyol, acrylate/C₁₀-C₃₀-alkyl acrylate crosspolymer, Poloxamer 101, polyglyceryl-2 dipolyhydroxystearate, polyglyceryl-3 diisostearate, polyglyceryl-4 dipolyhydroxystearate, PEG-30 dipolyhydroxystearate, diisostearyl polyglyceryl-3 diisostearate, 20 polyglyceryl-2 dipolyhydroxystearate, polyglyceryl-3 dipolyhydroxystearate, polyglyceryl-4 dipolyhydroxystearate, polyglyceryl-3 dioleate.

In yet additional embodiments of the present invention, W/O emulsions of the present invention comprise one or more coemulsifiers, including, but not limited to the following:

glyceryl stearate in a mixture with cetareth-20, cetareth-25, cetareth-6 in a mixture with stearyl alcohol, 25 cetylstearyl alcohol in a mixture with PEG-40 castor oil and sodium cetylstearyl sulfate, tricetareth-4 phosphate, sodium cetylstearyl sulfate, lecithin trilaureth-4 phosphate, laureth-4 phosphate, stearic acid, propylene glycol stearate SE, PEG-25 hydrogenated castor oil, PEG-54 hydrogenated castor oil, PEG-6 caprylic/capric glycerides, glyceryl oleate in a mixture with propylene glycol, ceteth-2, ceteth-20, polysorbate 60, glyceryl stearate in a mixture with PEG-100 stearate, laureth-4, cetareth-3, isostearyl 30 glyceryl ether, cetylstearyl alcohol in a mixture with sodium cetylstearyl sulfate, laureth-23, steareth-2, glyceryl stearate in a mixture with PEG-30 stearate, PEG-40 stearate, glycol distearate, PEG-22 dodecyl glycol copolymer, polyglyceryl-2 PEG-4 stearate, cetareth-20, methylglucose sesquistearate, steareth-10, PEG-20 stearate, steareth-2 in a mixture with PEG-8 distearate, steareth-21, steareth-20, isosteareth-20, PEG-45/dodecyl glycol copolymer, methoxy-PEG-22/dodecyl glycol copolymer, PEG-20 glyceryl stearate, 35 PEG-8 beeswax, polyglyceryl-2 laurate, isostearyl diglyceryl succinate, stearamidopropyl PG dimonium chloride phosphate, glyceryl stearate SE, ceteth-20, triethyl citrate, PEG-20 methylglucose sesquistearate,

ceteareth-12, glyceryl stearate citrate, cetyl phosphate, triceteareth-4 phosphate, trilaureth-4 phosphate, polyglyceryl methylglucose distearate, potassium cetyl phosphate, isosteareth-10, polyglyceryl-2 sesquiisostearate, ceteth-10, oleth-20, isoceteth-20, glyceryl stearate in a mixture with ceteareth-20, ceteareth-12, cetylstearyl alcohol and cetyl palmitate, cetylstearyl alcohol in a mixture with PEG-20 stearate, PEG-30 stearate, PEG-40 stearate, and PEG-100 stearate.

In yet additional embodiments in which the oil phase of the preparations consists at least partially of silicone oils, silicone emulsifiers find use. In some embodiments, the silicone emulsifiers are selected from the group of interface-active substances, alkylmethicone copolyols, and/or alkyl dimethicone copolyols, particularly from the group of compounds characterized by the following chemical structure:



in which X and Y, independently of one another, are chosen from the group H and the branched and unbranched alkyl groups, acyl groups and alkoxy groups having 1 to 24 carbon atoms, p is a number from 0 to 200, q is a number from 1 to 40, and r is a number from 1 to 100. Some examples of silicone emulsifiers which find use in the present invention include, but are not limited to dimethicone copolyols (*e.g.*, ABIL® B 8842, ABIL® B 8843, ABIL® B 8847, ABIL® B 8851, ABIL® B 8852, ABIL® B 8863, ABIL® B 8873, and ABIL® B 88183, all of which are commercially available from Th. Goldschmidt AG). An additional example of an interface-active substances which finds use in the present invention includes cetyldimethicone copolyol (ABIL® EM 90), as well as cyclomethiconedimethicone copolyol (ABIL® EM 97), both of which are commercially available from Th. Goldschmidt AG. An additional emulsifier which has proven useful in various compositions that finds use in embodiments of the present invention is laurylmethicone copolyol (Dow Corning® 5200 Formulation Aid), which is commercially available from Dow Corning Ltd.

In preferred embodiments of the present invention, the total amount of emulsifiers used in the personal care compositions (*e.g.*, cosmetic or skin care preparations) are present in the range from about 0.1 to about 10.0% by weight, preferably about 0.5 to about 5.0% by weight, based on the total weight of the preparations. However, it is not intended that the present invention be limited to any specific concentration of emulsifier and/or co-emulsifier, as various embodiments of the present invention have different preferred concentrations and/or concentration ranges.

In some embodiments, the present invention provides emulsions in various forms, including skin protection creams, skin lotions, cosmetic milks, sunscreen creams, and sun protection milks. In some preferred embodiments, these compositions comprise fats, oils, waxes, and/or other fatty substances, as well

as water, and one or more emulsifiers as are customarily used for such a type of formulation.

In addition to the liquid and somewhat more solid emulsions of the cosmetic cleansing lotions and/or cleansing creams of the present invention, the present invention also provides sprayable cleansing preparations ("cleansing sprays"), which are used, for example, for removing make-up or as mild washing lotion. In addition, these cleansing sprays find use in applications for treatment of blemished skin. These cleansing preparations also find use as "rinse-off preparations" (*i.e.*, products which are rinsed off the skin following application).

In addition to the above constituents, various embodiments of the present invention include additional components, such as auxiliaries and additives, including but not limited to bodying agents, fillers, perfume, dyes, emulsifiers, additional active ingredients (*e.g.*, vitamins and proteins), light protection agents, stabilizers, insect repellents, alcohol, self-tanning substances, water, salts, antimicrobials, proteases, and/or keratinase, etc. Indeed, it is not intended that the present invention be limited to any particular components, as long as the active component comprising a scaffold and a peptide is included. It is further contemplated that the present invention will find use in numerous and various medicinal preparations.

In some preferred embodiments, the present invention provides cosmetic and/or topical dermatological preparations suitable for use as skin protection creams, cleansing milks, sun screen lotions, nourishing creams, day creams, night creams etc. In some embodiments, the present invention finds use a components of drug (*i.e.*, pharmaceutical) compositions. In additional embodiments, the present invention finds use in decorative cosmetics (*e.g.*, make-up formulations).

In some particularly preferred embodiments, the present invention provides sunscreens useful in cosmetic and/or skin care preparations. In addition to the active ingredient used according to the embodiments of the present invention, in some embodiments, these preparations preferably additionally comprise at least one broadband filter and/or at least one UVA filter substance and/or at least one UVB filter substance and/or at least one inorganic pigment.

In yet further embodiments, the present invention provides personal care compositions which have UV protection components, but which are not primarily sunscreens. For example, in some embodiments, UV-A and/or UV-B filter substances are incorporated into day creams and/or hair care compositions.

In additional embodiments, the personal care compositions of the present invention comprise cosmetically active ingredients, auxiliaries and/or additives, as are customarily used in such preparations (*e.g.*, antioxidants, preservatives, bacteriocides, perfumes, antifoams, dyes, pigments which have a coloring action, thickeners, surface-active substances, emulsifiers, emollients, moisturizers and/or humectants, fats, oils, waxes or other customary constituents of a cosmetic or dermatological formulation, such as alcohols, polyols, polymers, foam stabilizers, electrolytes, organic solvents or silicone derivatives). Indeed it is contemplated that various compounds will find use in the various embodiments of the present invention, as appropriate for the product and the user.

In still further embodiments, preservatives, such as those used in food and feed applications find use

in various compositions of the present invention. The following table provides a list of such compounds, as well as the E number for each compound. However, it is not intended that the present invention be limited to these specific preservatives, as it is contemplated that additional preservatives will find use in various embodiments of the present invention.

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Examples of Food Grade Preservatives That Find Use in Embodiments of the Present Invention			
200	Sorbic acid	227	Calcium hydrogen sulfite
201	Sodium sorbate	228	Potassium hydrogen sulfite
202	Potassium sorbate	230	Biphenyl (Diphenyl)
203	Calcium sorbate	231	Orthophenylphenol
210	Benzoic acid	232	Sodium orthophenylphenoxide
211	Sodium benzoate	233	Thiabendazole
212	Potassium benzoate	235	Natamycin
213	Calcium benzoate	236	Formic acid
214	Ethyl p-hydroxybenzoate	237	Sodium formate
215	p-hydroxybenzoic ethyl ester Na salt	238	Calcium formate
216	n-propyl p-hydroxybenzoate	239	Hexamethylenetetramine
217	p-hydroxybenzoic-n-propyl ester Na salt	249	Potassium nitrite
218	methyl p-hydroxybenzoate	250	Sodium nitrite
219	p-hydroxybenzoic methyl ester Na salt	251	Sodium nitrate
220	Sulfur dioxide	252	Potassium nitrate
221	Sodium sulfite	280	Propionic acid
222	Sodium hydrogensulfite	281	Sodium propionate
223	Sodium disulfite	282	Calcium propionate
224	Potassium disulfite	283	Potassium propionate
226	Calcium sulfite	290	Carbon dioxide

Additional preservatives that find use in various embodiments include but are not limited to dibromodicyanobutane (2-bromo-2-bromomethylglutarodinitrile), 3-iodo-2-propinylbutylcarbamate, 2-bromo-2-nitropropane-1,3-diol, imidazolidinylurea, 5-chloro-2-methyl-4-isothiazolin-3-one, 2-chloroacetamide, benzalkonium chloride, benzyl alcohol, and formaldehyde donors. Further preservatives that find use in various embodiments of the present invention include phenyl hydroxyalkyl ethers, in particular the compounds known as "phenoxyethanol," due to their bactericidal and fungicidal effects on a number of microorganisms.

Yet other antimicrobial agents are likewise suitable for use in various embodiments of the present invention, including but not limited to 2,4,4'-trichloro-2'-hydroxydiphenyl ether (*i.e.*, IRGASAN®), 1,6-di(4-chlorophenylbiguanido)hexane (*i.e.*, CHLORHEXIDIN), 3,4,4'-trichlorocarbaniide, quaternary ammonium compounds, oil of cloves, mint oil, thyme oil, triethyl citrate, FARNESOL® (3,7,11-trimethyl-2,6,10-dodecatrien-1-ol) and the active ingredients and/or active ingredient combinations described in DE-37 40 186, DE-39 38 140, DE-42 04 321, DE-42 29 707, DE-43 09 372, DE-44 11 664, DE-195 41 967, DE-195 43 695, DE-195 43 696, DE-195 47 160, DE-196 02 108, DE-196 02 110, DE-196 02 111, DE-196 31 003, DE-196 31 004, DE-196 34 019, DE-42 29 737, DE-42 37 081, DE-43 24 219, DE-44 29 467, DE-44 23 410, and DE-195 16 705, all of which are hereby incorporated by reference. In still further embodiments, sodium hydrogencarbonate is also included in some compositions of the present invention. However, it is not intended that the present invention be limited to any particular antimicrobial nor combination of antimicrobial, as various compounds having such effects will find use in various embodiments of the present invention.

In additional embodiments of the personal care compositions of the present invention, compounds such as anti-irritants and/or anti-inflammatory actives are included. In some embodiments, batyl alcohol (a-octadecyl glyceryl ether), selachyl alcohol (a-9-octadecenyl glyceryl ether), chimyl alcohol (a-hexadecyl glyceryl ether), bisabolol, and/or panthenol are included. However, it is not intended that the present invention be limited to the incorporation of any specific anti-irritant(s) and/or anti-inflammatory(ies), as various compounds suitable for such applications find use in the present invention.

In still further embodiments of the present invention, antioxidants are incorporated in the personal care compositions. It is contemplated that any suitable antioxidants will find use in the personal care compositions of the present invention. Suitable antioxidants include, but are not limited to amino acids (*e.g.*, glycine, histidine, tyrosine, and tryptophan) and derivatives thereof, imidazoles (*e.g.*, urocanic acid) and derivatives thereof, peptides (*e.g.*, D,L-carnosine, D-carnosine, and L-carnosine) and derivatives thereof (*e.g.*, anserine), carotenoids, carotenes (*e.g.*, α -carotene, β -carotene, and γ -lycopene) and derivatives thereof, chlorogenic acid and derivatives thereof, aurothioglucose, propylthiouracil and other thiols (*e.g.*, thioredoxin, glutathione, cysteine, cystine, cystamine and the glycosyl, N-acetyl, methyl, ethyl, propyl, amyl, butyl and lauryl, palmitoyl, oleyl, g-linoleyl, cholesteryl and glyceryl esters thereof) and salts thereof, dilauryl thiodipropionate, distearyl thiodipropionate, thiodipropionic acid and derivatives thereof (*e.g.*,

esters, ethers, peptides, lipids, nucleotides, nucleosides and salts), and sulfoximine compounds (*e.g.* buthionine sulfoximines, homocysteine sulfoximine, buthionine sulfones, penta-, hexa-, and heptathionine sulfoximine) in very small tolerated doses (*e.g.*, typically pmol to mmol/kg), chelating agents (*e.g.*, α -hydroxy fatty acids, palmitic acid, phytic acid, and lactoferrin), α -hydroxy acids (*e.g.* citric acid, lactic acid, and malic acid), humic acid, bile acid, bile extracts, bilirubin, biliverdin, EDTA, EGTA and derivatives thereof, unsaturated fatty acids and derivatives thereof (*e.g.*, linolenic acids, linoleic acid, oleic acid), folic acid and derivatives thereof, furfurylidenesorbitol and derivatives thereof, ubiquinone and ubiquinol and derivatives thereof, vitamin C and derivatives thereof (*e.g.*, sodium ascorbyl phosphate, ascorbyl palmitate, Mg ascorbyl phosphate, and ascorbyl acetate), tocopherols and derivatives (*e.g.*, vitamin E acetate), coniferyl benzoate of benzoin resin, ferulic acid, furfurylidene-glucitol, carnosine; butylhydroxytoluene, butylhydroxyanisole, nordihydroguaiacic acid, nordihydroguaiaretic acid, trihydroxybutyrophenone, uric acid and derivatives thereof, mannose and derivatives thereof, zinc and derivatives thereof (*e.g.*, ZnO, ZnSO₄), selenium and derivatives thereof (*e.g.*, selenomethionine), stilbenes and derivatives thereof (*e.g.*, stilbene oxide, trans-stilbene oxide) and the derivatives thereof (*e.g.*, salts, esters, ethers, sugars, nucleotides, nucleosides, peptides and lipids) of said active ingredients which are suitable for the intended use of the particular embodiment(s) of the present invention.

In some embodiments, the concentration of one or more antioxidant in the compositions of the present invention is preferably from about 0.001 to about 30% by weight, particularly preferably from about 0.05 to about 20% by weight, and more preferably from about 1 to about 10% by weight, based on the total weight of the preparation. In additional embodiments, in which vitamin E and/or its derivatives are utilized as anti-oxidant(s), the preferred range is from about 0.001 to about 10% by weight, based on the total weight of the formulation. However, it is not intended that the present invention be limited to any specific antioxidant concentration(s), as various concentrations will find use in the various embodiments of the present invention.

In yet additional embodiments, the present invention provides preparations suitable for use as deodorants and/or antiperspirants. It is contemplated that any of the active ingredients which commonly find use in such preparations will also find use in various embodiments of the present invention. Additional components that are commonly used in such preparations also find use in various embodiments of the present invention. Examples of such actives and inactive compounds include, but are not limited to odor maskers (*e.g.*, perfumes), odor absorber (*e.g.*, phyllosilicates described in DE-P 40 09 347); as well as montmorillonite, kaolinite, illite, beidellite, nontronite, saponite, hectorite, bentonite, smectite, and zinc salts of ricinoleic acid. In some embodiments of the present invention, the range of active ingredients (*i.e.*, one or more compounds) in such preparations is preferably from about 0.001 to about 30% by weight; more preferably from about 0.05 to about 20% by weight; and most particularly in the range of from about 1 to about 10% by weight, based on the total weight of the preparation.

In some embodiments of the present invention, the water phase has a gel character which, in

addition to an effective content of compounds and solvents (as appropriate) preferably comprises water, further organic and/or inorganic thickeners, and/or hydrocolloids.

In some embodiments, inorganic thickeners are selected from the group consisting of modified, unmodified, naturally occurring, and synthetic phyllosilicates. Although it is generally preferable to use pure components, in some embodiments, mixtures of different modified and/or unmodified phyllosilicates find use in various compositions of the present invention. As generally known in the art, phyllosilicates are silicates and aluminosilicates in which the silicate or aluminate units are linked together via three Si-O- or Al-O- bonds and form a wavy sheet or layer structure. The fourth Si-O- or Al-O- valence is saturated by cations. Relatively weak electrostatic interactions (*e.g.* hydrogen bridge bonds), exist between the individual layers. The layer structure is largely defined by strong, covalent bonds. The stoichiometry of the sheet silicates is $(\text{Si}_2\text{O}_5^{2-})$ for pure silicate structures and $(\text{Al}_m\text{Si}^{2-m}\text{O}_5^{(2+m)-})$ for aluminosilicates, wherein "m" is a number greater than zero and less than 2. In some embodiments in which aluminosilicates are present in the absence of pure silicates, each Si^{4+} group replaced by Al^{3+} requires another singly charged cation to neutralize the charge. The charge balance is preferably evened out by H^+ , alkali metal ions or alkaline earth metal ions. In alternative embodiments, aluminum is used as a counterion. In contrast to the aluminosilicates, these compounds are referred to as "aluminum silicates." "Aluminum aluminosilicates," in which aluminum is present both in the silicate network, and also as counterion, also find use in some embodiments of the present invention.

Phyllosilicates are well known in the art (*See e.g.*, Hollemann *et al.*, Lehrbuch der Anorganischen Chemie [Textbook of Inorganic Chemistry], 91st-100th Ed., Walter de Gruyter - Verlag [1985]; Remy, Lehrbuch der Anorganischen Chemie, 12th Ed., Akademische Verlagsgesellschaft, Leipzig [1965]). The layer structure of montmorillonite is also known (*See*, Römpps Chemie-Lexikon, Franckh'sche Verlagshandlung, W. Keller & Co., Stuttgart, 8th Ed., [1985], p. 2668 f). Examples of phyllosilicates include the following (montmorillonite is the main mineral comprising the naturally-occurring bentonites);

Montmorillonite	$\text{Na}_{0.33}((\text{Al}_{1.67}\text{Mg}_{0.33})(\text{OH})_2(\text{Si}_4\text{O}_{10}))$
often simplified:	$\text{Al}_2\text{O}_3 \cdot 4\text{SiO}_2 \cdot \text{H}_2\text{O} \cdot n\text{H}_2\text{O}$ or $\text{Al}_2[(\text{OH})_2/\text{Si}_4\text{O}_{10}] \cdot n\text{H}_2\text{O}$
Kaolinite	$\text{Al}_2(\text{OH})_4(\text{Si}_2\text{O}_5)$
Illite	$(\text{K}, \text{H}_3\text{O})_y(\text{Mg}_3(\text{OH})_2(\text{Si}_{4-y}\text{Al}_y\text{O}_{10}))$ or $(\text{K}, \text{H}_3\text{O})_y(\text{Al}_2(\text{OH})_2(\text{Si}_{4-y}\text{Al}_y\text{O}_{10}))$ where $y = 0.7 - 0.9$
Beidellite	$(\text{Ca}, \text{Na})_{0.3}(\text{Al}_2(\text{OH})_2(\text{Al}_{0.5}\text{Si}_{3.5}\text{O}_{10}))$

Nontronite	$\text{Na}_{0.33}(\text{Fe}_2(\text{OH})_2(\text{Al}_{0.33}\text{Si}_{3.67}\text{O}_{10}))$
Saponite	$(\text{Ca},\text{Na})_{0.33}((\text{Mg},\text{Fe})_3(\text{OH})_2(\text{Al}_{0.33}\text{Si}_{3.67}\text{O}_{10}))$
5 Hectorite	$\text{Na}_{0.33}((\text{Mg},\text{Li})_3(\text{OH},\text{F})_2(\text{Si}_4\text{O}_{10}))$

In some preferred embodiments, inorganic gel formers including but not limited to aluminum silicates, such as the montmorillonites (bentonites, hectorites and derivatives thereof, such as quaternium-18 bentonite, quaternium-18 hectorites, stearylaluminum bentonites and stearylaluminum hectorites), and also magnesium-aluminum silicates (VEEGUM® grades), and sodium-magnesium silicates (LAPONITE® grades) find use in the present invention.

Montmorillonites represent clay minerals which are a type of dioctahedral smectites, and are masses which swell in water, but do not become plastic. The layer packets in the three-layer structure of the montmorillonites can swell as a result of reversible incorporation of water (in a 2- to 7-fold amount) and other substances, such as, for example, alcohols, glycols, pyridine, picoline, ammonium compounds, hydroxy-aluminosilicate ions etc. The chemical formula given above provides just an approximation of the formula, as montmorillonites have a large capacity for ion exchange. For example, Al can be replaced by Mg, Fe²⁺, Fe³⁺, Zn, Pb (e.g., from harmful substances in waste waters), Cr, Cu and other elements. The resulting negative charge of the octahedral layers is compensated for by the presence of cations, in particular Na⁺ (i.e., sodium montmorillonite) and Ca²⁺ (i.e., calcium montmorillonite, a compound that is only swellable to a very small extent) in interlayer positions.

In alternative embodiments, synthetic magnesium silicates and/or bentonites find use in the present invention, including but not limited to such commercially available compounds as OPTIGEL® (Süd-Chemie). As indicated above, in some embodiments, aluminum silicates such as the commercially available VEEGUM® (R.T. Vanderbilt Comp., Inc), find use in the present invention. Various VEEGUM® grades which find use in various embodiments of the present invention are provided below.

VEEGUM® Grades

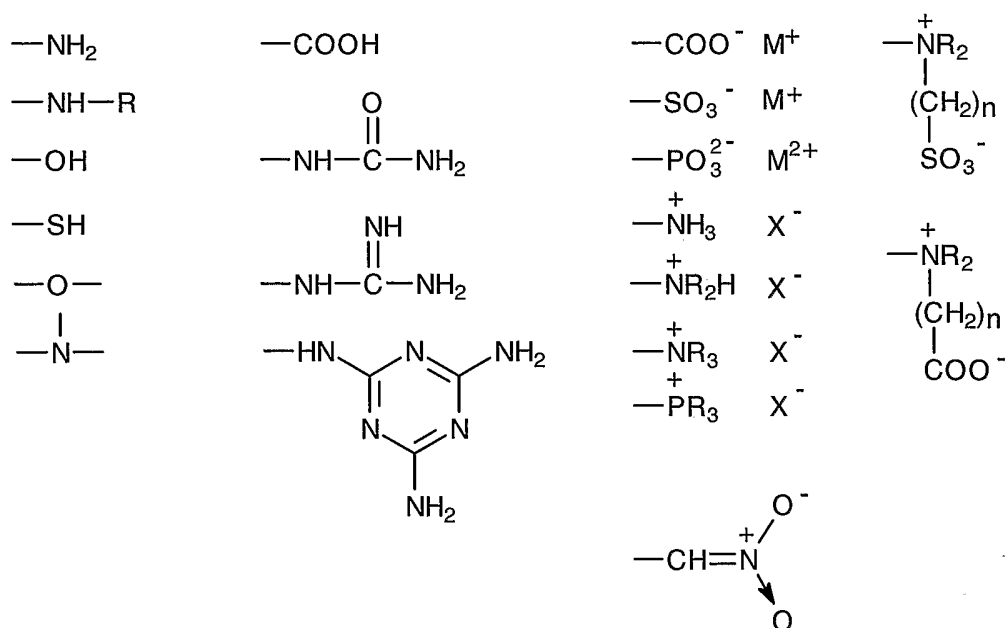
	Regular Grade	HV	K	HS	S-728
SiO ₂	55.5	56.9	64.7	69.0	65.3
MgO	13.0	13.0	5.4	2.9	3.3
Al ₂ O ₃	8.9	10.3	14.8	14.7	17.0
Fe ₂ O ₃	1.0	0.8	1.5	1.8	0.7
CaO	2.0	2.0	1.1	1.3	1.3
Na ₂ O	2.1	2.8	2.2	2.2	3.8
K ₂ O	1.3	1.3	1.9	0.4	0.2
Ashing loss	11.1	12.6	7.6	5.5	7.5

The above products swell in water to form viscous gels, which have an alkaline reaction. The organophilization of montmorillonite or bentonites (exchange of the interlayer cations for quaternary alkylammonium ions) produces products (bentonates) which are preferably used for dispersion in organic solvents and oils, fats, ointments, inks, surface coatings and in detergents.

BENTONE® is a trade name for various neutral and chemically inert gelling agents which are constructed from long-chain, organic ammonium salts and specific types of montmorillonite. BENTONE® gelling agents swell in organic media, which cause the media to also swell. The gels are resistant to diluted acids and alkalis, although they partially lose their gelling properties upon prolonged contact with strong acids and alkalis. Because of their organophilic character, BENTONE® gelling agents are only wettable by water with difficulty. There are various BENTONE® gelling agent grades commercially available, including those sold by Kronos Titan: BENTONE® 27, an organically modified montmorillonite; BENTONE® 34 (dimethyldioctylammonium bentonite; prepared in accordance with US Patent Nos. 2,531,427, incorporated herein by reference, which because of its lipophilic groups, swells more readily in lipophilic medium than in water); BENTONE® 38, an organically modified montmorillonite, available as a cream-colored to white powder; BENTONE® LT, a purified clay mineral; BENTONE® Gel MIO, an organically modified montmorillonite which is supplied as a very fine suspension in mineral oil (SUS-71) (10% bentonite, 86.7% mineral oil and 3.3% wetting agent); BENTONE® Gel IPM, an organically modified bentonite which is suspended in isopropyl myristate (10% bentonite, 86.7% isopropylmyristate, 3.3% wetting agent); BENTONE® Gel CAO, an organically modified montmorillonite which is taken up in castor oil (10% bentonite, 86.7% castor oil and 3.3% wetting agent); BENTONE® Gel Lantrol, an organically modified montmorillonite which, in paste form, is intended for the further processing, in particular for the preparation, of cosmetic compositions; 10% bentonite, 64.9 LANTROL® (wool wax oil), 22.0 isopropyl myristate, 3.0 wetting agent and 0.1 propyl p-hydroxybenzoate; BENTONE® Gel Lan I, a 10% strength BENTONE® 27 paste in a mixture of wool wax USP and isopropyl palmitate; BENTONE® Gel Lan II, a

bentonite paste in pure liquid wool wax; BENTONE® Gel NV, a 15% strength BENTONE® 27 paste in dibutyl phthalate; BENTONE® Gel OMS, a bentonite paste in Shellsol T.; BENTONE® Gel OMS 25, a bentonite paste in isoparaffinic hydrocarbons (IDOPAR® H); and BENTONE® Gel IPP, a bentonite paste in isopropyl palmitate.

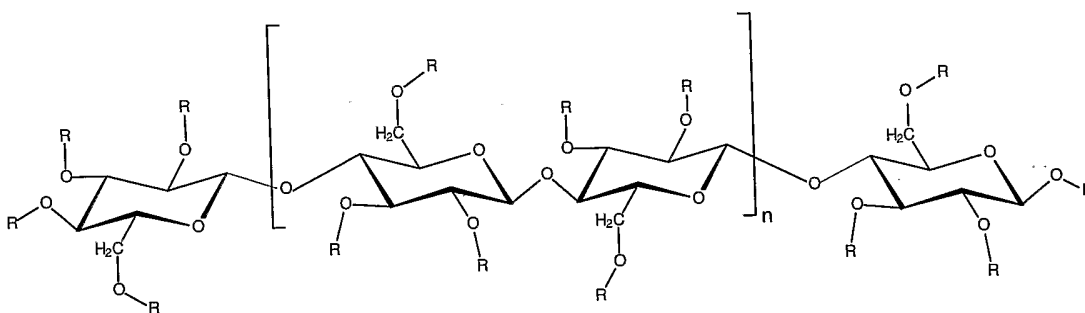
- 5 “Hydrocolloid” is the technological abbreviation for the more correct name “hydrophilic colloid.” Hydrocolloids are macromolecules which have a largely linear structure and intermolecular forces of interaction which permit secondary and primary valence bonds between the individual molecules to form a reticular structure. Some hydrocolloids are water-soluble natural or synthetic polymers which, in aqueous systems, form gels or viscous solutions. These compounds increase the viscosity of water by either binding
- 10 water molecules (hydration), or by absorbing and encapsulating the water into their interwoven macromolecules, while restricting the mobility of water. These water-soluble polymers represent a large group of natural and synthetic polymers that are chemically very different, but which share a common feature in their solubility in water or aqueous media. A prerequisite for this is that these polymers have a number of hydrophilic groups sufficient for solubility in water and are not too greatly crosslinked. These
- 15 hydrophilic groups can be nonionic, anionic or cationic in nature, for example as follows:



- In some preferred embodiments, the group of the cosmetically and dermatologically relevant
- 20 hydrocolloids are divided into the following groups: organic, natural compounds (*e.g.*, agar agar, carrageen, tragacanth, gum arabic, alginates, pectins, polyoses, guar flour, carob bean flour, starch, dextrans, gelatins, and casein); organic, modified natural substances (*e.g.*, carboxymethylcellulose and other cellulose ethers, hydroxyethylcellulose and hydroxypropylcellulose and microcrystalline cellulose); organic, completely

synthetic compounds (*e.g.*, polyacrylic and polymethacrylic compounds, vinyl polymers, polycarboxylic acids, polyethers, polyimines, polyamides, and polyurethanes); and inorganic compounds (*e.g.*, polysilicic acids, clay minerals, such as montmorillonites, zeolites, and silicas).

In alternative embodiments, ethylcelluloses find use in compositions of the present invention as stabilizers. Ethylcelluloses are characterized by the following structure. In this structure, the Rs are either ethyl groups or hydrogen atoms.



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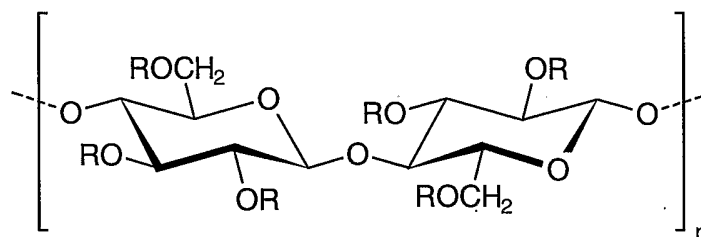
In some preferred embodiments, the degree of ethylation in the ethylcellulose is from about 2.0 to about 3.0, corresponding to about 40 to about 55%, and more preferably about 48.0 to about 49.5% ethylation. The average molecular mass is preferably chosen such that the viscosity of an approximately 5% strength solution in a mixture of 80 parts of toluene and 20 parts of ethanol at 25°C is 3 to 110 mPas, and more preferably 9 to 11 mPas. In some particularly preferred embodiments, the average molar mass is from about 100,000 to about 400,000 g/mol. In some preferred embodiments, the ethylcellulose concentration in compositions of the present invention ranges from about 0.1 to about 10% by weight, based on the total weight of the preparations. Various ethylcelluloses find use in the present invention, including but not limited to those that are commercially available (*e.g.*, ETHOCEL® Standard 10 Premium; Dow Chemicals).

In yet additional embodiments, microcrystalline cellulose finds use as hydrocolloid in compositions of the present invention. Various microcrystalline cellulose preparations find use in the present invention, including but not limited to those that are commercially available (*e.g.*, AVICEL®, such as AVICEL® RC-591, as well as AVICEL® RC/CL; AVICEL® CE-15; and AVICEL® 500; FMC Corporation Food and Pharmaceutical Products). In some particularly preferred embodiments, AVICEL® RC-591 (a modified microcrystalline cellulose which is made up of 89% microcrystalline cellulose and 11% sodium carboxymethylcellulose) finds use in the present invention.

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Additional hydrocolloids that find use in the present invention include methylcelluloses (*i.e.*, methylesters of cellulose). These compounds are characterized by the following structural formula

59



in which R can be a hydrogen or a methyl group.

Cellulose mixed ethers (generally referred to as methylcelluloses, which contain, in addition to a predominating content of methyl groups, also 2-hydroxyethyl, 2-hydroxypropyl or 2-hydroxybutyl groups) also find use in some embodiments of the present invention. In some preferred embodiments, hydroxypropyl)methyl-celluloses (e.g., METHOCEL® E4M; Dow Chemical Co.) find use in the present invention.

In yet further embodiments sodium carboxymethylcellulose (i.e., the sodium salt of the glycolic ether of cellulose, for which R in the above structural formula may be hydrogen and/or $\text{CH}_2\text{-COONa}$) finds use in the present invention. In some preferred embodiments, sodium carboxymethylcellulose, also sometimes referred to as “cellulose gum” (e.g., NATROSOL® Plus 330 CS; Aqualon) finds use in the present invention.

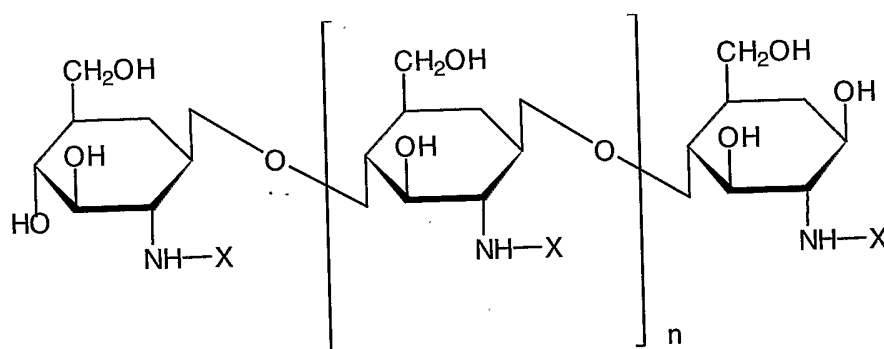
In additional embodiments, xanthan (CAS No. 11138-66-2), (i.e., xanthan gum), an anionic heteropolysaccharide generally formed by fermentation from maize sugar and isolated as potassium salt finds use in the present invention. It is produced by *Xanthomonas campestris* and some other species under aerobic conditions and has a molecular weight of from 2×10^6 to 24×10^6 . Xanthan is formed from a chain having cellulose with side chains. The structure of the subgroups consists of glucose, mannose, glucuronic acid, acetate and pyruvate. The number of pyruvate units determines the viscosity of the xanthan.

In still further embodiments, carrageen is used as a gel former in compositions of the present invention. This compound is an extract from North Atlantic red algae (*Florideae*; *Chondrus crispus* and *Gigartina stellata*) that has a structure similar to that of agar. The term “carrageen” is frequently used in reference to a dried algae product and “carrageenan” is used in reference to the extract thereof. The carrageen precipitated from the hot-water extract of the algae is a colorless to sand-colored powder with a molecular weight range from about 100,000 to about 800,000 and a sulfate content of about 25%. Carrageen, which is very readily soluble in warm water, forms a thixotropic gel upon cooling, even if the water content is 95–98%. The rigidity of the gel is effected by the double helix structure of the carrageen.

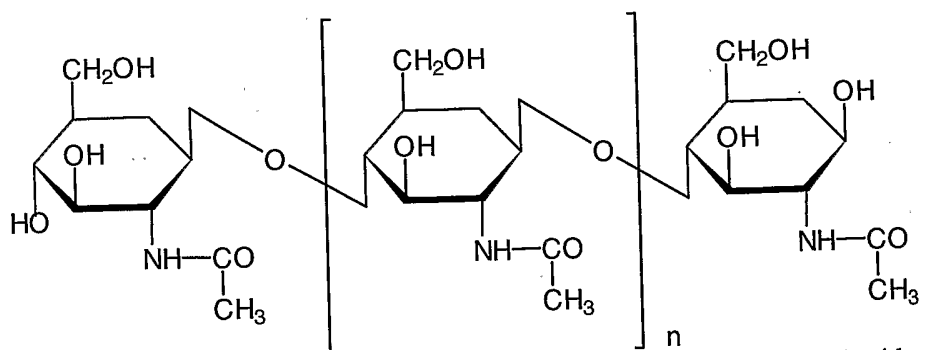
In the case of carrageenan, three principle constituents are differentiated. The gel-forming “κ fraction” consists of D-galactose 4-sulfate and 3,6-anhydro-α-D-galactose, which has alternate glycoside bonds in the 1,3- and 1,4 position (in contrast, agar contains 3,6-anhydro-α-L-galactose). The nongelling “λ fraction” is composed of 1,3-glycosidically linked D-galactose 2-sulfate and 1,4-bonded D-galactose-2,6-disulfate radicals, and is readily soluble in cold water. Finally, “ι-carrageenan,” composed of D-galactose 4-

sulfate in 1,3 bond and 3,6-anhydro- α -D-galactose 2-sulfate in 1,4 bond, is both water-soluble and also gel-forming. The nature of any cations which are present (K^+ , NH_4^+ , Na^+ , Mg^{2+} , Ca^{2+}) also influences the solubility of the carrageens.

In yet additional embodiments, chitosan (*i.e.*, partially deacetylated chitin) finds use in various compositions of the present invention. Chitosan has film-forming properties and is characterized as having a silky feel on the skin. One disadvantage for some uses, is its severe stickiness on the skin which occurs in temporarily (usually) during application. Due to this stickiness, some preparations are not acceptable to consumers. However, chitosan finds use in some preparations, including hair care compositions, as it is better than chitin in thickening and/or stabilizing, as well as improving the adhesion and water resistance of polymeric films. The use of chitosan is well-known to those of skill in the personal care art (*See e.g.*, Fiedler, Lexikon der Hilfsstoffe für Pharmazie, Kosmetik und angrenzende Gebiete, [Lexikon of auxiliaries for pharmacy, cosmetics and related fields], 3rd edition, Editio Cantor, Aulendorf, [1989], p. 293). Chitosan is characterized by the following structural formula:



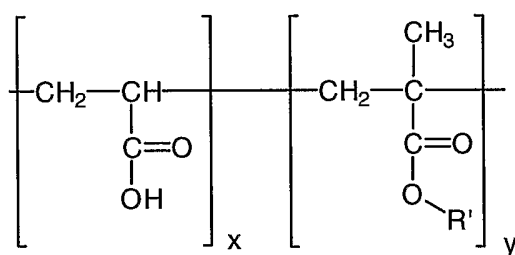
where n assumes values up to about 10 000, and X is either the acetyl radical or hydrogen. Chitosan forms by deacetylation and partial depolymerization (hydrolysis) of chitin, which is characterized by the structural formula



Chitin is an essential constituent of the arthropod (*e.g.* insects, crabs, and spiders) ectoskeleton, and is also found in the connective and/or supporting tissues of other organisms (*e.g.* mollusks, algae, and fungi). In the region of about $pH < 6$, chitosan is positively charged and in that range is also soluble in

aqueous systems. It is incompatible with anionic raw materials. For this reason, in order to prepare chitosan-containing oil-in-water emulsions, the use of nonionic emulsifiers is appropriate (*See e.g.*, EP 776 657). In some preferred embodiments, the compositions of the present invention contain at least one chitosans with a degree of deacetylation of at least about > 25% , and more preferably, a range of more than about 55 to about 99% (as determined by means of $^1\text{H-NMR}$). In some embodiments, chitosans of molecular weights between about 10,000 and about 1,000,000, in particular those with molecular weights between 100,000 and 1,000,000 (determined by means of gel permeation chromatography) find use in the present invention.

In yet further embodiments, polyacrylates find use as gelling agents in some compositions of the present invention. Suitable polyacrylates include but are not limited to acrylate-alkyl acrylate copolymers, in particular those chosen from the group of carbomers or CARBOPOL® copolymers (B. F. Goodrich Co.). In particular, the acrylate-alkyl acrylate copolymers that find use in some embodiments of the present invention have the following structure:



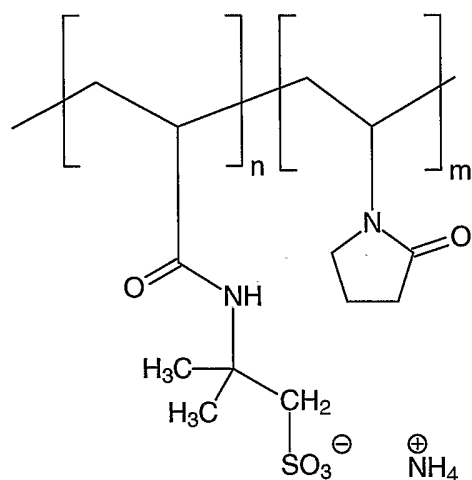
where R' is a long-chain alkyl radical, and x and y represent numbers which symbolize the respective stoichiometric proportion of each of the comonomers.

In some embodiments, acrylate copolymers and/or acrylate-alkyl acrylate copolymers, include but are not limited to those that are commercially available (*e.g.*, CARBOPOL® 1382, CARBOPOL® 981, and CARBOPOL® 5984; B. F. Goodrich Co., and in particular, polyacrylates from the group of CARBOPOL grades 980, 981, 1382, 2984, 5984 and Carbomer 2001). In additional embodiments, copolymers of C₁₀₋₃₀-alkyl acrylates and one or more monomers of acrylic acid, of methacrylic acid or esters thereof which are crosslinked with an allyl ether of sucrose or an allyl ether of pentaerythritol find use in some embodiments of the present invention.

Compounds which carry the INCI name "Acrylates/C₁₀₋₃₀ Alkyl Acrylate Crosspolymer" also find use in some embodiments of the present invention. In some embodiments, commercially available polymers (*e.g.*, PEMULEN® TR1 and PEMULEN® TR2; B. F. Goodrich Co.) find use in some embodiments of the present invention, although it is not intended that the present invention be limited to any specific acrylate-containing composition.

In yet additional embodiments, compounds which carry the INCI name "ammonium

acryloyldimethyltaurates/vinylpyrrolidone copolymers“ find use in the present invention. These ammonium acryloyldimethyl taurate/vinylpyrrolidone copolymers have the empirical formula $[C_7H_{16}N_2SO_4]_n$ $[C_6H_9NO]_m$, which corresponds to the following structure:



Preferred species of this compound are listed in Chemical Abstracts under the Registry numbers 58374-69-9, 13162-05-5 and 88-12-0, and are commercially available (*e.g.*, ARISTOFLEX®; Clariant GmbH). However, it is not intended that the present invention be limited to any particular species. In yet additional embodiments of the present invention, copolymers/crosspolymers comprising acryloyldimethyl taurate (*e.g.*, SIMUGEL® EG and SIMUGEL® EG; Seppic S.A.) find use in some compositions of the present invention.

Additional completely synthetic hydrocolloids that find use in the present invention include, but are not limited to anionic polyurethanes which are soluble or dispersible in water and which are advantageously obtainable from:

- Aa) at least one compound which contains two or more active hydrogen atoms per molecule,
- Ab) at least one diol containing acid or salt groups, and
- Ac) at least one diisocyanate.

In some preferred embodiments, the component Aa) is, in particular, a diol, aminoalcohol, diamine, polyesterol, polyetherol with a number-average molecular weight of in each case up to 3000, or mixtures thereof, where up to 3 mol% of said compounds may be replaced by triols or triamines. Preference is given to diols and polyesterdiols. In particular, the component Aa) comprises at least 50% by weight, based on the total weight of the component Aa), of a polyesterdiol. Suitable polyesterdiols are all those which are

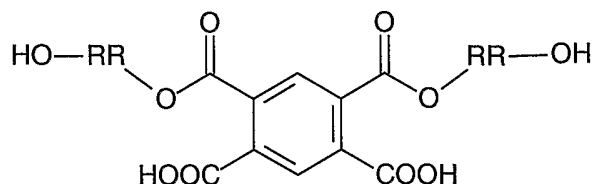
customarily used for the preparation of polyurethanes, in particular the reaction products of phthalic acid and diethylene glycol, isophthalic acid and 1,4-butanediol, isophthalic acid/adipic acid and 1,6-hexanediol, and adipic acid and ethylene glycol or 5-NaSO₃-isophthalic acid, phthalic acid, adipic acid and 1,6-hexanediol.

Examples of diols which find use in some embodiments of the present invention include, but are not limited to ethylene glycol, propylene glycol, butylene glycol, neopentyl glycol, polyetherols (*e.g.*, polyethylene glycols having molecular weights up to 3000), block copolymers of ethylene oxide and propylene oxide with number-average molecular weights of up to 3000, and block copolymers of ethylene oxide, propylene oxide and butylene oxide which contain the copolymerized alkylene oxide units in randomly distributed manner or in the form of blocks. Preference is given to ethylene glycol, neopentyl glycol, di-, tri-, tetra-, penta- or hexaethylene glycol. Other diols which find use include poly(α -hydroxycarboxylic acid)diols.

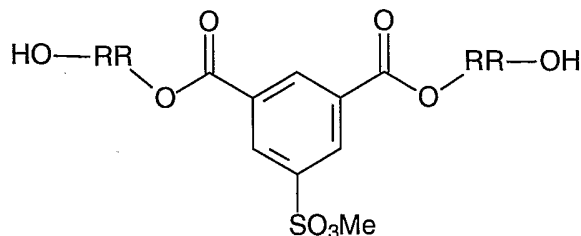
Suitable aminoalcohols that find use in some embodiments of the present invention include but are not limited to 2-aminoethanol, 2-(N-methylamino)ethanol, 3-aminopropanol, and 4-aminobutanol.

In some embodiments, diamines such as ethylenediamine, propylenediamine, 1,4-diaminobutan, 1,6-diaminohexane, and α,ω -diamines which can be prepared by amination of polyalkylene oxides with ammonia find use in some compositions of the present invention.

Component Ab) is, in particular, dimethylolpropanoic acid or a compound with the formula:



and



where RR is in each case a C₂-C₁₈-alkylene group and Me is Na or K.

Component Ac) is, in particular, hexamethylene diisocyanate, isophorone diisocyanate, methyldiphenyl isocyanate (MDI), and/or tolylene diisocyanate.

In some embodiments, the polyurethanes are obtained by reacting the compounds of groups Aa) and

Ab) under an inert-gas atmosphere in an inert solvent at temperatures of from 70 to 130°C with the compounds of group Ac). This reaction can be carried out, where appropriate, in the presence of chain extenders in order to prepare polyurethanes with relatively high molecular weights. As is customary in the preparation of polyurethanes, the components [(Aa)+(Ab)]:Ac are advantageously used in the molar ratio of from 0.8 to 1.1 : 1. The acid number of the polyurethanes is determined by the composition and the concentration of the compounds of component (Ab) in the mixture of components (Aa) and (Ab).

In some embodiments, the polyurethanes have K values according to H. Fikentscher (determined in 0.1% strength by weight solutions in N-methylpyrrolidone at 25°C and pH 7) of from about 15 to about 100, and preferably about 25 to about 50. The K value (*i.e.*, "intrinsic viscosity"), is a parameter which is easy to determine by means of viscosity measurements of polymer solutions and is therefore frequently used in the industrial sector for characterizing polymers. Polyurethanes containing acid groups that find use in some embodiments of the present invention include, but are not limited to polyurethanes that are water-soluble or dispersible without the aid of emulsifiers after partial or complete neutralization. The salts of the polyurethanes generally have better solubility or dispersibility in water than the unneutralized polyurethanes. Bases which find use for the neutralization of the polyurethanes include alkali metal bases (*e.g.*, sodium hydroxide solution, potassium hydroxide solution, soda, sodium hydrogencarbonate, potassium carbonate or potassium hydrogen carbonate) and alkaline earth metal bases (*e.g.*, calcium hydroxide, calcium oxide, magnesium hydroxide or magnesium carbonate, and ammonia and amines). In some embodiments, 2-amino-2-methylpropanol, diethylaminopropylamine and triisopropanolamine find particular use in the neutralization of the polyurethanes containing acid groups. In yet additional embodiments, the neutralization of the polyurethanes containing acid groups is carried out using mixtures of two or more bases (*e.g.* mixtures of sodium hydroxide solution and triisopropanolamine). Depending on the intended use, neutralization is partial (*e.g.* about 20 to about 40%) or complete (*i.e.*, 100%). These polymers and their preparation are described in more detail in DE-A-42 25 045, incorporated herein by reference.

B. Water-soluble or -dispersible cationic polyurethanes and polyureas of:

Ba) at least one diisocyanate, which may have already been reacted beforehand with one or more compounds which contain two or more active hydrogen atoms per molecule, and

Bb) at least one diol, primary or secondary amino alcohol, primary or secondary diamine or primary or secondary triamine with one or more tertiary, quaternary or protonated tertiary amino nitrogen atoms.

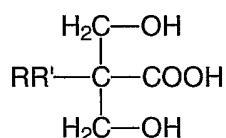
Preferred diisocyanates are as given above under A). Compounds with two or more active hydrogen atoms are diols, aminoalcohols, diamines, polyesterols, polyamidediamines and polyetherols. Suitable

compounds of this type are as given above under A).

The polyurethanes are prepared as described above under A). Charged cationic groups can be produced in the polyureas from the tertiary amino nitrogen atoms present either by protonation, (*e.g.*, with carboxylic acids, such as lactic acid), or by quaternization (*e.g.* with alkylating agents, such as C₁ to C₄-alkyl halides) or sulfates. Examples of such alkylating agents include, but are not limited to ethyl chloride, ethyl bromide, methyl chloride, methyl bromide, dimethyl sulfate and diethyl sulfate. These polymers and their preparation are described in more detail in DE-A-42 41 118, which is incorporated herein by reference.

C. Linear polyurethanes with carboxylate groups of:

Ca) a 2,2-hydroxymethyl-substituted carboxylic acid of the formula



in which RR' is a hydrogen atom or a C₁-C₂₀-alkyl group, which is used in an amount which suffices for about 0.35 to about 2.25 milliequivalents of carboxyl groups to be present in the polyurethane per g of polyurethane,

Cb) about 10 to about 90% by weight, based on the weight of the polyurethane, of one or more organic compounds with not more than two active hydrogen atoms and

Cc) one or more organic diisocyanates.

In some preferred embodiments, the carboxyl groups present in the polyurethane are, finally, at least partially neutralized with a suitable base. These polymers and their preparation are described in EP-A-619 111, incorporated herein by reference.

D. Carboxyl-containing polycondensation products of anhydrides of tri- or tetracarboxylic acids and diols, diamines or aminoalcohols (polyesters, polyamides or polyester amides). These polymers and their preparation are described in more detail in DE-A-42 24 761, incorporated herein by reference.

E. Polyacrylates and polymethacrylates, as are described in more detail in DE-A-43 14 305, 36 27 970 and 29 17 504, all of which are incorporated herein by reference.

The polymers used in some embodiments of the present invention have a K value of from about 15 to about 100, and more preferably from about 25 to about 50. The polymers are generally present in the composition in an amount in the range from about 0.2 to about 20% by weight, based on the total weight of the compositions. The salt is used in an amount effective for improving the exchangeability of the polymers.

5 The salt is generally used in an amount of from about 0.02 to about 10% by weight, and more preferably from about 0.05 to about 5% by weight, and in particular, from about 0.1 to about 3% by weight, based on the total weight of the composition.

10 The total amount of one or more hydrocolloids in some embodiments of the personal care compositions of the present invention is less than about 5% by weight, preferably between about 0.05 and about 3.0% by weight, and more preferably between about 0.1 and about 1.0% by weight, based on the total weight of the preparations.

In some additional embodiments, interface- and/or surface-active agents are included in some personal care compositions of the present invention, including but not limited to cationic emulsifiers (*e.g.*, quaternary surfactants).

15 Quaternary surfactants that contain at least one N atom which is covalently bonded to 4 alkyl or aryl groups. This leads, irrespective of the pH, to a positive charge. Alkylbetain, alkylamidopropylbetain and alkylamidopropylhydroxysultaine are examples of quaternary surfactants that find use in some embodiments of the present invention.

20 The cationic surfactants provided in some embodiments of the present invention also include, but are not limited to quaternary ammonium compounds, in particular benzyltrialkylammonium chlorides or bromides (*e.g.*, benzyltrimethylstearyl ammonium chloride), alkyltrialkylammonium salts (*e.g.*, cetyltrimethylammonium chloride or bromide), alkyltrimethylhydroxyethylammonium chlorides or bromides, dialkyltrimethylammonium chlorides or bromides, alkylamidoethyltrimethylammonium ether sulfates, alkylpyridinium salts (*e.g.*, lauryl- or cetylpyridinium chloride), imidazoline derivatives, and
25 compounds with a cationic character, such as amine oxides (*e.g.*, alkyltrimethylamine oxides or alkylaminoethyltrimethylamine oxides). In some preferred embodiments, cetyltrimethylammonium salts find use in some personal care compositions of the present invention.

In yet additional embodiments, cationic polymers (*e.g.*, JAGUAR® C 162 [hydroxypropyl guar hydroxypropyltrimonium chloride]), modified magnesium aluminum silicates (*e.g.*, quaternium-18-hectorite,
30 which is commercially available (*e.g.*, BENTONE® 38; Rheox), and/or stearylalkonium hectorite, which is commercially available (*e.g.*, SOFTISAN® gel; Hüls AG) find use in some personal care compositions of the present invention. However, it is not intended that the present invention be limited to any particular cationic polymer.

35 In some yet further embodiments, some compositions of the present invention comprise oil thickeners in order to improve the tactile properties of emulsions. Preferred oil thickeners include, but are not limited to other solids (*e.g.*, hydrophobic silicon oxides of the AEROSIL® type, which are available

from Degussa AG). Examples of advantageous AEROSIL® oxide grades include AEROSIL® OX50, AEROSIL® 130, AEROSIL® 150, AEROSIL® 200, AEROSIL® 300, AEROSIL® 380, AEROSIL® MOX 80, AEROSIL® MOX 170, AEROSIL® COK 84, AEROSIL® R 202, AEROSIL® R 805, AEROSIL® R 812, AEROSIL® R 972, AEROSIL® □ R 974 and AEROSIL® R976.

5 In some additional embodiments, some personal care compositions of the present invention comprise at least one "metal soap" (*i.e.*, a salt of a higher fatty acid, with the exception of alkali metal salt), which are function as oil thickeners. Examples of such metal soaps include, but are not limited to aluminum stearate, zinc stearate and/or magnesium stearate.

10 In some further embodiments, some personal care compositions comprise at least one amphoteric and/or zwitterionic surfactant (*e.g.*, cocamidopropylbetain) and/or moisturizer (*e.g.*, betain). Examples of amphoteric surfactants that find use in such embodiments of the present invention include but are not limited to acyl/dialkylethylenediamine (*e.g.*, sodium acylamphoacetate), disodium acylamphodipropionate, disodium alkylamphodiacetate, sodium acylamphohydroxypropylsulfonate, disodium acylamphodiacetate, sodium acylamphopropionate, N-alkylamino acids, for example aminopropylalkylglutamide, 15 alkylaminopropionic acid, sodium alkylimidodipropionate, and lauroamphocarboxyglycinate.

In some embodiments, the amount of surface- or interface-active substances (one or more compounds) in the preparations is preferably between about 0.001 and about 30% by weight, and more preferably between about 0.05 and about 20% by weight, in most preferably between about 1 and about 10% by weight, based on the total weight of the preparation.

20 In some yet additional embodiments, the active ingredients (one or more compounds) comprise at least one lipophilic active ingredient. In some embodiments, these lipophilic active ingredients are selected from the group consisting of acetylsalicylic acid, atropine, azulene, hydrocortisone and derivatives thereof (*e.g.*, hydrocortisone-17-valerate), B vitamins, D vitamins, vitamin B₁, vitamin B₁₂, vitamin D₁, retinoid, bisabolol, unsaturated fatty acids (*e.g.*, the essential fatty acids often also referred to as "vitamin F"), γ- 25 linolenic acid, oleic acid, eicosapentenoic acid, docosahexenoic acid and derivatives thereof, chloramphenicol, caffeine, prostaglandins, thymol, camphor, extracts or other products of a vegetable and animal origin (*e.g.* evening primrose oil, borraige oil or currant seed oil, fish oils, cod-liver oil), and ceramides and ceramide-like compounds, etc. In some embodiments, the active ingredient(s) are refatting substances (*e.g.*, purcellin oil, EUCERIT® and/or NERO CERIT®).

30 In some yet further embodiments, the active ingredient(s) comprise NO synthetase inhibitors. These embodiments find particular use in treatment and/or prophylaxis of the signs and symptoms associated intrinsic and/or extrinsic skin aging, as well as for the treatment and/or prophylaxis associated with the harmful effects of ultraviolet radiation on the skin. In some preferred embodiments, the NO synthase inhibitor is nitroarginine.

35 In yet some additional embodiments, the active ingredient(s) is/are catechins, bile esters of catechins, and/or aqueous or organic extracts from plants or sections of plants which have a content of

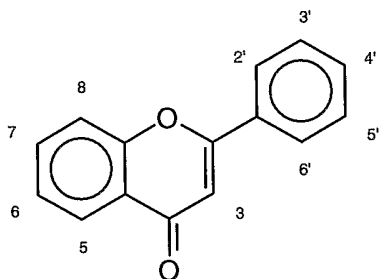
catechins or bile esters of catechins (*e.g.*, the leaves of the *Theaceae* plant family, in particular of the species *Camellia sinensis* [green tea]). Their typical ingredients (*e.g.*, polyphenols or catechins, caffeine, vitamins, sugars, minerals, aminoacids, lipids) find particular use in some embodiments of the present invention.

In some embodiments, catechins find use in the present invention. Catechins are a group of compounds which are regarded as hydrogenated flavones or anthocyanidines, and are derivatives of "catechin" (catechol, 3,3',4',5,7-flavanpentol, 2-(3,4-dihydroxyphenyl)chroman-3,5,7-triol). Epicatechin ((2R,3R)-3,3',4',5,7-flavanpentol) is also an active ingredient that finds use in some embodiments of the present invention.

In yet additional embodiments, plant extracts with a content of catechin, in particular extracts of green tea (*e.g.*, extracts from leaves of the plants of the genus *Camellia*, in particular those used for tea, such as *C. sinensis*, *C. assamica*, *C. taliensis*, and *C. irrawadiensis* and hybrids of these species with other species, such as *C. japonica*) find use in some personal care compositions of the present invention.

In some further embodiments, preferred active ingredients include polyphenols and catechins from the group (-)-catechin, (+)-catechin, (-)-catechin gallate, (-)-gallocatechin gallate, (+)-epicatechin, (-)-epicatechin, (-)-epicatechin gallate, (-)-epigallocatechin, and (-)-epigallocatechin gallate.

In some additional embodiments of the compositions of the present invention flavone and its derivatives (also often collectively called "flavones") find used. These compounds have the following basic structure (substitution positions are shown):



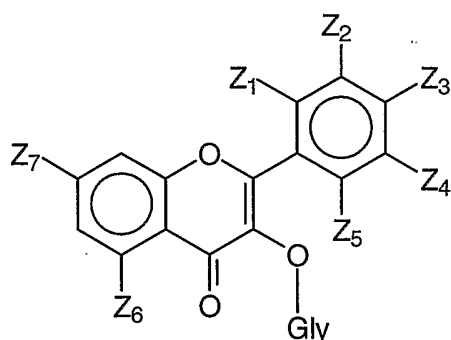
Some of the more important flavones which find use in some personal care compositions of the present invention are provided below. However, it is not intended that the present invention be limited to any particular flavone.

FLAVONES								
	OH Substitution Positions							
	3	5	7	8	2'	3'	4'	5'
Flavone	-	-	-	-	-	-	-	-
Flavonol	+	-	-	-	-	-	-	-
Chrysin	-	+	+	-	-	-	-	-
Galangin	+	+	+	-	-	-	-	-

FLAVONES								
	OH Substitution Positions							
Apigenin	-	+	+	-	-	-	+	-
Fisetin	+	-	+	-	-	+	+	-
Luteolin	-	+	+	-	-	+	+	-
Kaempferol	+	+	+	-	-	-	+	-
Quercetin	+	+	+	-	-	+	+	-
Morin	+	+	+	-	+	-	+	-
Robinetin	+	-	+	-	-	+	+	+
Gossypetin	+	+	+	+	-	+	+	-
Myricetin	+	+	+	-	-	+	+	+

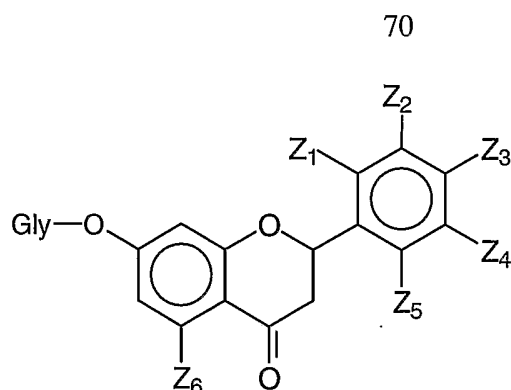
In nature, flavones are usually present in glycosylated form.

In some further embodiments, the personal care compositions of the present invention comprise at
 5 least one flavonoids having generic structural formula:



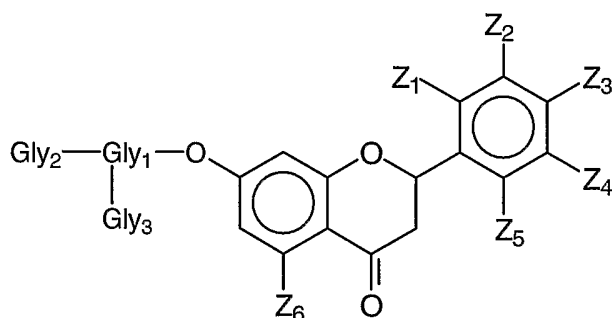
10 where Z₁ to Z₇, independently of one another, are chosen from the group consisting of H, OH, alkoxy and hydroxyalkoxy, where the alkoxy and hydroxyalkoxy groups can be branched or unbranched and have 1 to 18 carbon atoms, and where Gly is chosen from the group of mono- and oligoglycoside radicals.

In some alternative embodiments, the personal care compositions of the present invention comprise
 15 at least one flavonoids having the generic structural formula:



where Z_1 to Z_6 , independently of one another, are chosen from the group consisting of H, OH, alkoxy and hydroxyalkoxy, where the alkoxy and hydroxyalkoxy groups may be branched or unbranched and have 1 to 18 carbon atoms, where Gly is chosen from the group mono and oligoglycoside radicals.

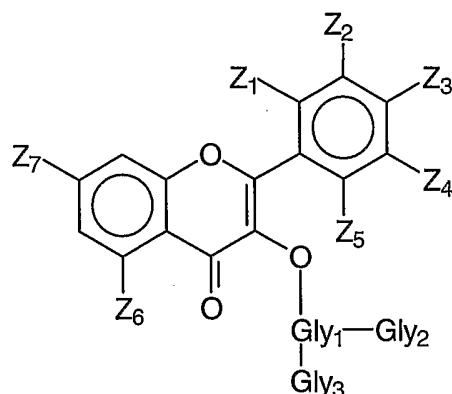
5 In some preferred embodiments, the composition has the generic structural formula



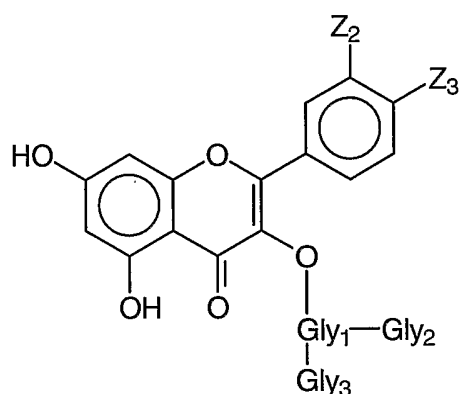
where Gly_1 , Gly_2 and Gly_3 , independently of one another, are monoglycoside radicals. Gly_2 and Gly_3 may also, individually or together, represent saturations by hydrogen atoms. In some preferred embodiments, Gly_1 , Gly_2 and Gly_3 , independently of one another, are selected from the group of hexosyl radicals, in particular the rhamnosyl radicals and glucosyl radicals. However, hexosyl radicals, for example allosyl, altrosyl, galactosyl, gulosyl, idosyl, mannosyl and talosyl, also find use in some embodiments of the present invention. In yet additional embodiments, pentosyl radicals find use in some personal care compositions of the present invention.

15 In some embodiments, Z_1 to Z_5 are, independently of one another, advantageously chosen from the group consisting of H, OH, methoxy, ethoxy and 2-hydroxyethoxy, and the flavone glycosides have the structure:

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In some embodiments, the flavone glycosides provided in some of the personalcare compositions of the present invention have the following structure:



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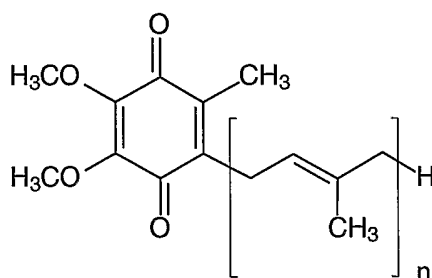
where Gly₁, Gly₂ and Gly₃, independently of one another, are monoglycoside radicals. Gly₂ and Gly₃ can also, individually or together, represent saturations by hydrogen atoms. In alternative embodiments, Gly₁, Gly₂ and Gly₃, independently of one another, are selected from the group of hexosyl radicals, in particular of rhamnosyl radicals and glucosyl radicals. However, other hexosyl radicals, for example allosyl, altrosyl, galactosyl, gulosyl, idosyl, mannosyl and talosyl, find use in some embodiments of the present invention. In addition, in some embodiments, pentosyl radicals find use in the present invention. In some preferred embodiments, the personal care compositions of the present invention comprise one or more flavone glucoside selected from the group consisting of a-glucosylrutin, a-glucosylmyricetin, a-glucosylisoquercitrin, a-glucosylisoquercetin and a-glucosylquercitrin. In some particularly preferred

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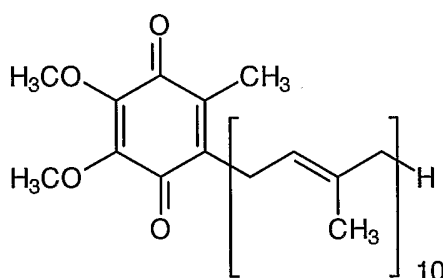
embodiments, the flavone glucoside is a-glucosylrutin.

In yet some additional embodiments, the personal care compositions of the present invention comprise at least one naringin (*e.g.*, aurantin, naringenin-7-rhamno-glucoside), hesperidin 3',5,7-trihydroxy-4'-methoxyflavanone-7-rutinoside, hesperidoside, hesperetin-7-O-rutinoside), rutin (3,3',4',5,7-pentahydroxyflavone-3-rutinoside, quercetin-3-rutinoside, sophorin, birutan, rutabion, taurutin, phytomelin,

melin), troxerutin (3,5-dihydroxy-3',4',7-tris(2-hydroxyethoxy)flavone-3-(6-O-(6-deoxy- α -L-mannopyranosyl)-b-D-glucopyranoside)), monoxerutin (3,3',4',5-tetrahydroxy-7-(2-hydroxyethoxy)flavone-3-(6-O-(6-deoxy- α -L-mannopyranosyl)-b-D-glucopyranoside)), dihydrorobinetin (3,3',4',5',7-pentahydroxyflavanone), taxifolin (3,3',4',5,7-pentahydroxyflavanone), eriodictyol-7-glucoside (3',4',5,7-tetrahydroxyflavanone-7 glucoside), flavanomarein (3',4',7,8-tetrahydroxyflavanone-7 glucoside), and/or isoquercetin (3,3',4',5,7-pentahydroxyflavanone-3-(b-D-glucopyranoside). In some yet further embodiments, the active ingredient is selected from the group consisting of ubiquinones and plastoquinones. Ubiquinones are characterized by the structural formula:

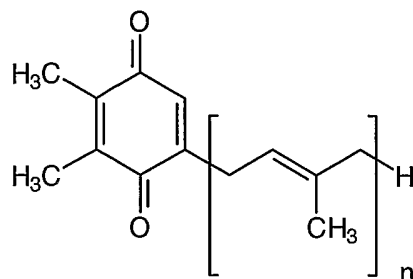


Ubiquinones are the most widespread and the most investigated bioquinones. Ubiquinones are referred to, depending on the number of isoprene units linked in the side chain, as Q-1, Q-2, Q-3 etc., or according to the number of carbon atoms, as U-5, U-10, U-15 etc. They preferably arise with certain chain lengths (*e.g.* in some microorganisms and yeasts where $n=6$). In most mammals, including humans, Q10 predominates. Coenzyme Q10 finds particular use in some embodiments of the present invention. Its structural formula is:



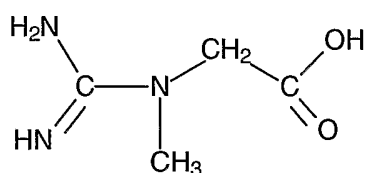
Plastoquinones have the general structural formula:

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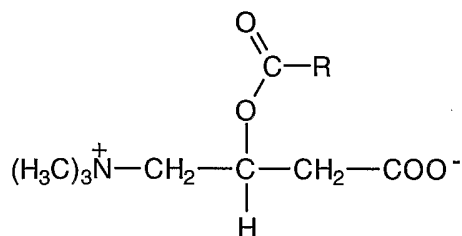
Plastoquinones differ in the number n of isoprene radicals and are referred to accordingly (*e.g.* PQ-9 [n=9]). In addition, other plastoquinones with varying substituents on the quinone ring exist in some embodiments.

In some still further embodiments, the present invention comprises at least one creatine and/or creatine derivative. Creatine has the following structure:



In some preferred embodiments of the personal care compositions of the present invention creatine phosphate, creatine sulfate, creatine acetate, creatine ascorbate, and/or derivatives esterified at the carboxyl group with mono- or polyfunctional alcohols find use.

In some additional embodiments, the personal care compositions of the present invention contain L-carnitine [3-hydroxy-4-(trimethylammonio)butyrobetaine]. Acylcarnitines have the following general structure:



where R is chosen from the group of branched and unbranched alkyl radicals having up to 10 carbon atoms, and find use in some embodiments of the present invention. In some preferred embodiments, propionylcarnitine and/or acetylcarnitine find use. Both enantiomers (D and L form), as well as mixtures and racemates of the D- and L- forms find use in some personal care compositions of the present invention.

In some further embodiments, the active ingredients of the present invention include, but are not limited to sericoside, pyridoxol, vitamin K, biotin, and aroma substances. In addition, it is not intended that the active ingredients present in the personal care compositions of the present invention be limited to any particular constituent and/or mixture(s) of actives. Indeed, it is intended that various actives and mixtures of actives will find use in various embodiments of the present invention. It is also not intended that the concentration(s) of such actives be limited to any particular level. In some embodiments, the concentration is from about 0.001 to about 30% by weight, while in other embodiments it is from about 0.05 to about 20% by weight, and in still further embodiments, it is from about 0.1 to about 10% by weight, based on the total weight of the preparation. It is further contemplated that those of skill in the art will formulate personal care compositions of the present invention with active(s) concentrations that are suitable for the intended use of the compositions.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides peptides and supported peptides for treating various diseases and conditions. In particularly preferred embodiments, the present invention provides compositions and methods for personal care. In some embodiments, the present invention provides compositions for use in skin and/or hair care, as well as cosmetic compositions. In alternative particularly preferred embodiments, the present invention provides peptides and supported peptides for treating diseases of the skin, such as rosacea. In some particularly preferred embodiments, the supported peptides of the present invention are anti-VEGF peptides. In alternative particularly preferred embodiments, the anti-VEGF peptides are expressed on a scaffold protein. In some most preferred embodiments, the scaffold protein comprises BBI.

In some preferred embodiments, the present invention provides cosmetic and/or pharmaceutical compounds suitable for improving the appearance of skin. The present invention further provides peptides that block binding of a protein. In some preferred embodiments, the protein is VEGF. In some particularly preferred embodiments, the peptide is expressed in a protease-resistant scaffold. In some especially preferred embodiments, the scaffold is a protease inhibitor (*e.g.*, BBI, STI, or Eglin chymotrypsin inhibitor). In some most preferred embodiments, the protease inhibitor is a BBI that has been functionally and/or structurally modified.

As indicated above, two protein protease inhibitors have been isolated from soybeans, the Kunitz-type trypsin inhibitor (soybean trypsin inhibitor, STI) and the Bowman-Birk protease inhibitor (BBI) (*See e.g.*, Birk, *Int. J. Pept. Protein Res.*, 25:113-131 [1985]; and Kennedy, *Am. J. Clin. Nutr.*, 68:1406S-1412S [1998]). These inhibitors serve as a beginning scaffold for the variant sequences provided herein to produce the protease inhibitor in combination with at least one peptide sequence that has been modified and/or substituted in the sequence (*e.g.*, BBI-AV or STI-AV). In addition to alterations in the scaffold comprising the variant sequences, other desired proteins used herein include the addition of six histidine residues at the C-terminus (*See*, Figures 1 and 2).

Soybean Trypsin Inhibitor (STI)

STI inhibits the proteolytic activity of trypsin by the formation of a stable stoichiometric complex (See e.g., Liu, Chemistry and Nutritional Value of Soybean Components, *In: Soybeans, Chemistry, Technology and Utilization*, pp. 32-35, Aspen Publishers, Inc., Gaithersburg, Md., [1999]). STI consists of 181 amino acid residues with two disulfide bridges and is roughly spherically shaped (See e.g., Song *et al.*, J. Mol. Biol., 275:347-63 [1998]). The trypsin inhibitory loop lies within the first disulfide bridge. The Kunitz-type soybean trypsin inhibitor (STI) has played a key role in the early study of proteinases, having been used as the main substrate in the biochemical and kinetic work that led to the definition of the standard mechanism of action of proteinase inhibitors.

Bowman-Birk Inhibitor (BBI)

Bowman-Birk inhibitor proteins are a kinetically and structurally well-characterized family of small proteins (60-90 residues) isolated from leguminous seeds, as well as other plants, including various grasses. They typically have a symmetrical structure of two tricyclic domains each containing an independent binding loop, although some have one domain and some have more than two domains. The major ~ 8 kDa Bowman-Birk inhibitor isolated from soybeans (BBI) has two separate reactive site loops, loop I inhibits proteases having trypsin-like specificity and loop II inhibits proteases with chymotrypsin-like specificity (See e.g., Chen *et al.*, J. Biol. Chem., 267:1990-1994 [1992]; Werner and Wemmer, Biochem., 31:999-1010 [1992]; Lin *et al.*, Eur. J. Biochem., 212:549-555 [1993]; Voss *et al.*, Eur. J. Biochem., 242:122-131 [1996]; and Billings *et al.*, Pro. Natl. Acad. Sci., 89:3120-3124 [1992]). These binding regions each contain a "canonical loop" structure, which is a motif found in a variety of serine proteinase inhibitors (Bode and Huber, Eur. J. Biochem., 204:433-451 [1992]). STI and BBI are found only in the soybean seed, and not in any other part of the plant (See e.g., Birk, Int. J. Pept. Protein Res., 25:113-131 [1985]).

Although numerous isoforms of BBI have been characterized, SEQ ID NO:47 shows the amino acid sequence of the BBI backbone used in some experiments described herein comprising approximately 71 amino acid residues (See Example 16).

In soybeans, BBI is produced as a pro-protein with an N-terminal pro-peptide that is 19 amino acids in length. Thus, in some embodiments, BBI is produced with all or at least a portion of the propeptide. In some embodiments, BBI is truncated, with as many as 10 amino acid residues being removed from either the N- or C- terminal. For example, upon seed desiccation, some BBI molecules have the C-terminal 9 or 10 amino acid residues removed. Thus, proteolysis is generally highly tolerated prior to the initial disulfide and just after the terminal disulfide bond, the consequences of which are usually not detrimental to the binding to target protein. However, it will be appreciated that any one of the isoforms or truncated forms find use in various embodiments of the present invention.

Protease Inhibitor Variants

As indicated above, the STI and BBI protease inhibitors have binding loops that inhibit proteases. The present invention provides protease inhibitor variants with alterations in one or more reactive sites (*e.g.*, Loop I and/or Loop II of BBI). In some preferred embodiments, the loops are replaced with sequences that
5 interact with a target protein.

For example, in some embodiments, the loops are replaced with sequences derived from VEGF binding proteins, inhibitors of the complement pathway such as C2, C3, C4 or C5 inhibitors, Compstatin, cytokines, other proteins of interest, etc. Indeed, it is not intended that the present invention be limited to any particular sequence substituted into either of these loops, as any suitable sequence finds use in the
10 present invention.

In some embodiments, variant sequences are selected by various methods known in the art, including but not limited to phage display and other suitable screening methods. For example, a random peptide gene library is fused with phage PIII gene so the peptide library will be displayed on the surface of the phage. Subsequently, the phage display library is exposed to the target protein and washed with buffer
15 to remove non-specific binding (this process is sometimes referred to as panning). Finally, the binding phage and PCR the DNA sequence for the peptide encoded are isolated.

In most embodiments, one of the loops is replaced with a variant sequence (*i.e.*, peptides; often 3 to 14 amino acids in length, with 5 to 10 amino acids being preferred). Longer sequences find use in the present invention, as long as they provide the binding and/or inhibition desired. In addition, peptides
20 suitable for use as replacements of the binding loop(s) preferably adopt a functional conformation when contained within a constrained loop (*i.e.*, a loop formed by the presence of a disulfide bond between two cysteine residues). In some specific embodiments, the peptides are between 7 and 9 amino acids in length. These replacement sequences also provide protease inhibition or binding to the targeted proteins. In some
25 embodiments, it is advantageous to alter a single amino acid.

Fusion Proteins

In preferred embodiments, each protease inhibitor or variant thereof is expressed as a fusion protein by the host bacterial cell. Although cleavage of the fusion polypeptide to release the desired protein will often be useful, it is not necessary. Protease inhibitors and variants thereof expressed and secreted as fusion
30 proteins surprisingly retain their function.

The above-defined DNA sequences encoding the corresponding amino acid sequences are combined to form a "fusion DNA sequence." Such fusion DNA sequences are assembled in proper reading frame from the 5' terminus to 3' terminus in the order of first, second, third and fourth DNA sequences. As so
35 assembled, the DNA sequence encodes a "fusion polypeptide" encoding from its amino-terminus a signal peptide functional as a secretory sequence in a bacterial species, a secreted polypeptide or portion thereof normally secreted from a bacterial species, a cleavable linker peptide and a desired polypeptide (*e.g.*, a

protease inhibitor and variants thereof). Various methods are known to those in the art for the production of fusion proteins (*See e.g.*, US Patents 5,411,873, 5,429,950, and 5,679,543, all of which are incorporated herein by reference). Thus, it is intended that any suitable method will find use in the present invention.

5 Expression of Recombinant Protease Inhibitors

To the extent that the present invention depends on the production of fusion proteins, it relies on routine techniques in the field of recombinant genetics. Basic texts disclosing the general methods of use in this invention include Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual* ((2nd ed.) [1989]); Kriegler, *Gene Transfer and Expression: A Laboratory Manual* (1990); and Ausubel *et al.*, (eds.), *Current*
10 *Protocols in Molecular Biology* (1994).

The present invention provides bacterial host cells which have been transduced, transformed or transfected with an expression vector comprising a protease inhibitor-encoding nucleic acid sequence. The culture conditions, such as temperature, pH and the like, are those previously used for the parental host cell prior to transduction, transformation or transfection are apparent to those skilled in the art.

15 Basically, a nucleotide sequence encoding a fusion protein is operably linked to a promoter sequence functional in the host cell. This promoter-gene unit is then typically cloned into intermediate vectors before transformation into the host cells for replication and/or expression. These intermediate vectors are typically prokaryotic vectors (*e.g.*, plasmids, or shuttle vectors). However, it is not intended that the present invention be limited to the use of intermediate vectors, as this step is omitted in some preferred
20 embodiments.

In one approach, a bacterial culture is transformed with an expression vector having a promoter or biologically active promoter fragment or one or more (*e.g.*, a series) of enhancers which functions in the host cell, operably linked to a nucleic acid sequence encoding a protease inhibitor, such that the a protease is expressed in the cell. In some preferred embodiments, the DNA sequences encode a protease inhibitor or
25 variant thereof. In another preferred embodiment, the promoter is a regulatable one.

Nucleic Acid Constructs/Expression Vectors.

Natural or synthetic polynucleotide fragments encoding a protease inhibitor (*i.e.*, "PI-encoding nucleic acid sequences") may be incorporated into heterologous nucleic acid constructs or vectors, capable
30 of introduction into, and replication in, a bacterial cell. The vectors and methods disclosed herein are suitable for use in various host cells for the expression of protease inhibitors and variants thereof. Any vector may be used as long as it is replicable and viable in the cells into which it is introduced. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available. Appropriate cloning and expression vectors are also described in various references known to
35 those in the art (*See e.g.*, Sambrook *et al.*, *supra* and Ausubel *et al.*, *supra*, expressly incorporated by reference herein). The appropriate DNA sequence is inserted into a plasmid or vector (collectively referred

to herein as “vectors”) by any suitable method. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by standard procedures known to those in the art.

Appropriate vectors are typically equipped with a selectable marker-encoding nucleic acid sequence, insertion sites, and suitable control elements, such as termination sequences. In some embodiments, the vectors comprise regulatory sequences, including, for example, control elements (*i.e.*, promoter and terminator elements or 5' and/or 3' untranslated regions), effective for expression of the coding sequence in host cells (and/or in a vector or host cell environment in which a modified soluble protein coding sequence is not normally expressed), operably linked to the coding sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, many of which are commercially available and known to those in the art.

Exemplary promoters include both constitutive promoters and inducible promoters. Such promoters are well known to those of skill in the art. Those skilled in the art are also aware that a natural promoter can be modified by replacement, substitution, addition or elimination of one or more nucleotides without changing its function. The practice of the present invention encompasses and is not constrained by such alterations to the promoter. The choice of promoter used in the genetic construct is within the knowledge of one skilled in the art.

The choice of the proper selectable marker will depend on the host cell. Appropriate markers for different bacterial hosts are well known in the art. Typical selectable marker genes encode proteins that (a) confer resistance to antibiotics or other toxins (*e.g.*, ampicillin, methotrexate, tetracycline, neomycin mycophenolic acid, puromycin, zeomycin, or hygromycin; or (b) complement an auxotrophic mutation or a naturally occurring nutritional deficiency in the host strain.

In some embodiments, a selected PI coding sequence is inserted into a suitable vector according to well-known recombinant techniques and used to transform a cell line capable of PI expression. Due to the inherent degeneracy of the genetic code, other nucleic acid sequences which encode substantially the same or a functionally equivalent amino acid sequence may be used to clone and express a specific protease inhibitor, as further detailed above. Therefore it is appreciated that such substitutions in the coding region fall within the sequence variants covered by the present invention. Any and all of these sequence variants can be utilized in the same way as described herein for a parent PI-encoding nucleic acid sequence. Those skilled in the art recognize that differing PIs will be encoded by differing nucleic acid sequences.

In some embodiments, once the desired form of a protease inhibitor nucleic acid sequence, homologue, variant or fragment thereof, is obtained, it is modified by any number of ways. Where the sequence involves non-coding flanking regions, the flanking regions may be subjected to resection, mutagenesis, etc. Thus, transitions, transversions, deletions, and insertions may be performed on the naturally occurring sequence.

In some preferred embodiments, heterologous nucleic acid constructs include the coding sequence for at least one protease inhibitor, or variant(s), fragment(s) or splice variant(s) thereof: (i) in isolation; (ii)

in combination with additional coding sequences; such as fusion protein or signal peptide coding sequences, where the PI coding sequence is the dominant coding sequence; (iii) in combination with non-coding sequences, such as control elements, such as promoter and terminator elements or 5' and/or 3' untranslated regions, effective for expression of the coding sequence in a suitable host; and/or (iv) in a vector or host environment in which the PI coding sequence is a heterologous gene.

In some embodiments, heterologous nucleic acid containing the appropriate nucleic acid coding sequence, together with appropriate promoter and control sequences, is employed to introduced into bacterial host cells to permit the cells to express at least one protease inhibitor or variant thereof.

In some embodiments of the present invention, a heterologous nucleic acid construct is employed to transfer a PI-encoding nucleic acid sequence into a cell *in vitro*. In some preferred embodiments, the host cells stably integrate the nucleic acid sequences of the present invention. Thus, any suitable method for effectively generating stable transformants finds use in the present invention.

In additional embodiments of the present invention, the first and second expression cassettes are present on a single vector, while in other embodiments these cassettes are present on separate vectors.

In some preferred embodiments, in addition to a promoter sequence, the expression cassette also contains a transcription termination region downstream of the structural gene to provide for efficient termination. In some embodiments, the termination region is obtained from the same gene as the promoter sequence, while in other embodiments it is obtained from another gene. The selection of suitable transcription termination signals is well-known to those of skill in the art.

In addition, it is contemplated that any suitable expression vector will find use in the present invention. Indeed, it is contemplated that various conventional vectors used for expression in eukaryotic or prokaryotic cells will be suitable and find use with the present invention. Standard bacterial expression vectors include bacteriophages λ and M13, as well as plasmids such as pBR322 based plasmids, pSKF, pET23D, and fusion expression systems such as MBP, GST, and LacZ. In further embodiments, epitope tags are added to recombinant proteins, in order to provide convenient methods of isolation (*e.g.*, c-myc).

Additional elements typically included in expression vectors are replicons, a gene encoding antibiotic resistance to permit selection of bacteria that harbor recombinant plasmids, and unique restriction sites in nonessential regions of the plasmid to allow insertion of heterologous sequences. The particular antibiotic resistance gene chosen is not critical, any of the many resistance genes known in the art are suitable.

Introduction Of A Protease Inhibitor-Encoding Nucleic Acid Sequence Into Host Cells.

In some preferred embodiments, the methods of the present invention provide host cells that contain a stably integrated sequence of interest (*i.e.*, PI-encoding nucleic acid). However, in alternative embodiments, the methods of the present invention provide for maintenance of a self-replicating extra-chromosomal transformation vector.

The invention further provides cells and cell compositions which have been genetically modified to comprise an exogenously provided PI-encoding nucleic acid sequence. In some embodiments, a parental host cell is genetically modified by an expression vector. In some embodiments, the vector is a plasmid, while in other embodiments the vector is a viral particle, phage, naked DNA, etc. Thus, it is not intended that the form of the vector be limited to any particular type of vector, as various vectors will find use in the present invention.

Various methods may be employed for delivering an expression vector into cells *in vitro*. Methods of introducing nucleic acids into cells for expression of heterologous nucleic acid sequences are also known to the ordinarily skilled artisan, including, but not limited to electroporation; protoplast fusion with intact cells; transduction; high velocity bombardment with DNA-coated microprojectiles; infection with modified viral (*e.g.*, phage) nucleic acids; chemically-mediated transformation, competence, etc. In addition, in some embodiments, heterologous nucleic acid constructs comprising a PI-encoding nucleic acid sequence are transcribed *in vitro*, and the resulting RNA introduced into the host cell by any of the suitable methods known in the art.

Following introduction of a heterologous nucleic acid construct comprising the coding sequence for a protease inhibitor, the genetically modified cells are cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants, and/or amplifying expression of a PI-encoding nucleic acid sequence. The culture conditions, such as temperature, pH and the like, are those previously used for the host cell selected for expression, and are apparent to those skilled in the art.

The progeny of cells into which such heterologous nucleic acid constructs have been introduced are generally considered to comprise the PI-encoding nucleic acid sequence found in the heterologous nucleic acid construct.

Bacterial Hosts and Expression

Appropriate host cells include any suitable bacterial species. In some embodiments, the bacterial hosts serve both as the expression hosts and the source of the first and second nucleic acids. Using the present inventive methods and host cells, surprising levels of expression have been obtained. The system utilized herein has achieved levels of expression and secretion of greater than 0.5 g/l of protease inhibitor.

After the expression vector is introduced into the host cells, the transfected host cells are cultured under conditions favoring expression of gene encoding the desired protein. Large batches of transformed cells can be cultured as described above. Finally, product is recovered from the culture using techniques known in the art.

Accessory proteins such as thiol-disulfide oxidoreductases or chaperones find use in some embodiments, as they may be beneficial to help fold the secretory protein into its active conformation. Thiol-disulfide oxidoreductases and protein disulfide isomerases catalyze the formation of the correct disulfide bonds in the protein. Overexpression of the *bdbDC* operon in *B. subtilis* has been shown to be

beneficial for the production of a protein with disulfide bonds (See *e.g.*, Meima *et al.*, J. Biol. Chem., 277:6994-7001, [2002]). Chaperones help the secretory protein to fold by binding to exposed hydrophobic regions in the unfolded states and preventing unfavorable interactions and prolyl-peptidyl cis-trans isomerases assist in formation of the proper conformation of the peptide chain adjacent to proline residues.

5 In some embodiments of the present invention, the host cells are transformed with an expression vector encoding at least one thiol-disulfide oxidoreductase or chaperone. It is not intended that the present invention be limited to any particular thiol-disulfide oxidoreductase or chaperone, as any suitable thiol-disulfide oxidoreductase or chaperone known to those skilled in the art will find use in the present invention.

10 In some embodiments of the present invention, the fraction of properly folded secretory protein is increased by the addition of chemicals to the growth medium that reduce/oxidize disulfide bonds, and/or alter the general redox potential, and/or chemicals that alter solvent properties thus affecting protein conformation and aggregation. In particularly preferred embodiments, a reagent that reduces disulfide bonds, such as 2-mercaptoethanol, is preferred to increase the fraction of correctly folded protein. However, in other embodiments and depending on the medium used, other
15 disulfide reducing or oxidizing agents (*e.g.*, DTT, TCEP, reduced and oxidized glutathione, cysteine, cystine, cysteamine, thioglycolate, $S_2O_3^{2-}$, $S_2O_4^{2-}$, $S_2O_5^{2-}$, SO_3^{2-} , $S_2O_7^{2-}$, Cu^{+} , etc.), either used alone or in combination, find use in the present invention. It is contemplated that other adjuvants that alter solvent properties, (*e.g.*, urea, DMSO, TWEEN®-80, etc.), either added to the growth medium alone or preferably in combination with disulfide reducing/oxidizing agents, such
20 as β ME, will also increase the fraction of correctly folded secretory protein and find use in various embodiments of the present invention. In some preferred embodiments, the β ME is used at concentrations ranging from 0.5 to 4 mM, while in other embodiments, the concentrations range from 0.1 mM to 10 mM. Indeed, those of skill in the art know how to select the best growth medium and growth conditions to optimize the effects of the added thiol reducing/oxidizing agents
25 and/or other adjuvants, as well as the concentration of thio reducing/oxidizing agents and/or other adjuvants to use. It is not intended that the present invention be limited to any particular disulfide reducing/oxidizing agent or adjuvant, as any suitable reagents known to those skilled in the art find use in the present invention.

30 Fermentation Parameters

The present invention relies on fermentation procedures for culturing bacterial species. Fermentation procedures for production of heterologous proteins by bacterial species are well known in the art. Culturing is accomplished in a growth medium comprising an aqueous mineral salts medium, organic

growth factors, the carbon and energy source material, molecular oxygen (for aerobic and facultative bacteria), and, of course, a starting inoculum of one or more particular microorganism species to be employed.

5 In addition to the carbon and energy source, oxygen, assimilable nitrogen, and an inoculum of the microorganism, it is necessary to supply suitable amounts in proper proportions of mineral nutrients to assure proper microorganism growth, maximize the assimilation of the carbon and energy source by the cells in the microbial conversion process, and achieve maximum cellular yields with maximum cell density in the fermentation medium.

10 Various culture media find use in the present invention, as known to those of skill in the art. However, standard bacterial culture media find use in the present invention. In some preferred media formulations, the media include, in addition to nitrogen, suitable amounts of phosphorus, magnesium, calcium, potassium, sulfur, and sodium, in suitable soluble assimilable ionic and combined forms, and also present preferably should be certain trace elements such as copper, manganese, molybdenum, zinc, iron, boron, and iodine, and others, again in suitable soluble assimilable form, all as known in the art.

15 In some embodiments, the fermentation reaction involves an aerobic process in which the molecular oxygen needed is supplied by a molecular oxygen-containing gas such as air, oxygen-enriched air, or even substantially pure molecular oxygen, provided to maintain the contents of the fermentation vessel with a suitable oxygen partial pressure effective in assisting the microorganism species to grow in a thriving fashion. In effect, by using an oxygenated hydrocarbon substrate, the oxygen requirement for growth of the
20 microorganism is reduced. Nevertheless, molecular oxygen must be supplied for growth of aerobic and to a lesser extent, facultative organisms.

Although the aeration rate can vary over a considerable range, aeration generally is conducted at a rate which is in the range of about 0.5 to 10, preferably about 0.5 to 7, volumes (at the pressure employed and at 25°C.) of oxygen-containing gas per liquid volume in the fermentor per minute. This amount is based
25 on air of normal oxygen content being supplied to the reactor, and in terms of pure oxygen the respective ranges would be about 0.1 to 1.7, or preferably about 0.1 to 1.3, volumes (at the pressure employed and at 25°C.) of oxygen per liquid volume in the fermentor per minute.

The pressure employed for the microbial conversion process can range widely. Pressures generally are within the range of about 0 to 50 psig, presently preferably about 0 to 30 psig, more preferably at least
30 slightly over atmospheric pressure, as a balance of equipment and operating cost versus oxygen solubility achieved. Greater than atmospheric pressures are advantageous in that such pressures do tend to increase a dissolved oxygen concentration in the aqueous ferment, which in turn can help increase cellular growth rates. At the same time, this is balanced by the fact that high atmospheric pressures do increase equipment and operating costs.

35 The fermentation temperature can vary somewhat, but for most bacterial species used in the present invention, the temperature generally will be within the range of about 20°C to 40°C, generally preferably in

the range of about 28°C to 37°C, depending on the strain of microorganism chosen, as known to those skilled in the art.

The microorganisms also require a source of assimilable nitrogen. The source of assimilable nitrogen can be any nitrogen-containing compound or compounds capable of releasing nitrogen in a form suitable for metabolic utilization by the microorganism. While a variety of organic nitrogen source compounds, such as protein hydrolysates, can be employed, usually cheap nitrogen-containing compounds such as ammonia, ammonium hydroxide, urea, and various ammonium salts such as ammonium phosphate, ammonium sulfate, ammonium pyrophosphate, ammonium chloride, or various other ammonium compounds can be utilized. Ammonia gas itself is convenient for large scale operations, and can be employed by bubbling through the aqueous ferment (fermentation medium) in suitable amounts. At the same time, such ammonia can also be employed to assist in pH control.

The pH range in the aqueous microbial ferment (fermentation admixture) should be in the exemplary range of about 2.0 to 8.0. However, pH range optima for certain microorganisms are dependent on the media employed to some extent, as well as the particular microorganism, and thus change somewhat with change in media as known to those skilled in the art.

While the average retention time of the fermentation admixture in the fermentor can vary considerably, depending in part on the fermentation temperature and culture employed, as known in the art.

In some embodiments, the fermentation is preferably conducted in such a manner that the carbon-containing substrate can be controlled as a limiting factor, thereby providing good conversion of the carbon-containing substrate to cells and avoiding contamination of the cells with a substantial amount of unconverted substrate. The latter is not a problem with water-soluble substrates, since any remaining traces are readily removed. It may be a problem, however, in the case of non-water-soluble substrates, and require added product-treatment steps such as suitable washing steps. The time needed to reach this limiting substrate level is not critical and may vary with the particular microorganism and fermentation process being conducted. However, it is well known in the art how to determine the carbon source concentration in the fermentation medium and whether or not the desired level of carbon source has been achieved.

Although in some embodiments, the fermentation is conducted as a batch or continuous operation, fed batch operation is generally preferred for ease of control, production of uniform quantities of products, and most economical uses of all equipment.

If desired, part or all of the carbon and energy source material and/or part of the assimilable nitrogen source such as ammonia can be added to the aqueous mineral medium prior to feeding the aqueous mineral medium into the fermentor. Indeed, each of the streams introduced into the reactor preferably is controlled at a predetermined rate, or in response to a need determinable by monitoring such as concentration of the carbon and energy substrate, pH, dissolved oxygen, oxygen or carbon dioxide in the off-gases from the fermentor, cell density measurable by light transmittancy, or the like. The feed rates of the various materials can be varied so as to obtain as rapid a cell growth rate as possible, consistent with efficient utilization of the

carbon and energy source, to obtain as high a yield of microorganism cells relative to substrate charge as possible, but more importantly to obtain the highest production of the desired protein per unit volume.

In either a batch, or the preferred fed batch operation, all equipment, reactor, or fermentation means, vessel or container, piping, attendant circulating or cooling devices, and the like, are initially sterilized, usually by employing steam such as at about 121°C for at least about 15 minutes. The sterilized reactor then is inoculated with a culture of the selected microorganism in the presence of all the required nutrients, including oxygen, and the carbon-containing substrate. The type of fermentor employed is not critical, though in some embodiments, the 15L Biolafitte (Saint-Germain-en-Laye, France) is preferred.

10 Protein Separations

In some particularly preferred embodiments, once the desired protein is expressed, the secreted protein is recovered. The present invention provides methods of separating a desired protein from its fusion analog. It is specifically contemplated that the methods described herein will find use in the separation of proteinase inhibitor and variants from the fusion analog.

The collection and purification of the desired protein from the fermentation broth can also be achieved using procedures known to those of skill in the art. The fermentation broth will generally contain cellular debris, including cells, various suspended solids and other biomass contaminants, as well as the desired protein product, which are preferably removed from the fermentation broth by means known in the art. Suitable processes for such removal include conventional solid-liquid separation techniques (*e.g.*, centrifugation, filtration, dialysis, microfiltration, rotary vacuum filtration, or other known processes), to produce a cell-free filtrate. In some embodiments, it is preferable to further concentrate the fermentation broth or the cell-free filtrate prior to the purification and/or crystallization process using techniques such as ultrafiltration, evaporation and/or precipitation.

Precipitating the proteinaceous components of the supernatant or filtrate may be accomplished by means of a salt (*e.g.*, ammonium sulfate) or low pH (typically less than 3), followed by purification by a variety of chromatographic procedures (*e.g.*, ion exchange chromatography, affinity chromatography, hydrophobic interaction chromatography, hydrophobic charge induction chromatography etc.) or similar art recognized procedures. It is not intended that the present invention be limited to any particular separation method, as it is contemplated that any method will find use in the present invention.

In certain preferred embodiments, when the expressed desired polypeptide is secreted from the bacterial cells, the polypeptide is purified from the growth media. In preferred embodiments, the expression host cells are removed from the media before purification of the polypeptide (*e.g.* by centrifugation).

When the expressed recombinant desired polypeptide is not secreted from the host cell, the host cell is preferably disrupted and the polypeptide released into an aqueous "extract" which is the first stage of purification. Preferably, the expression host cells are collected from the media before the cell disruption (*e.g.* by centrifugation). The cell disruption may be performed by using any suitable means known in the art,

such as by lysozyme or beta-glucanase digestion or by forcing the cells through high pressure (*See e.g.*, Scobes, Protein Purification, Second edition, Springer-Verlag)

In some embodiments, the addition of six histidine residues (*i.e.*, a “His Tag”) to the C-terminus is used as an aid in the purification of the desired protein and its fusion analog. Use of the His tags as a purification aid is well known in the art (*See e.g.*, Hengen, TIBS 20:285-286 [1995]). The 6x his-tagged proteins are easily purified using Immobilized Metal ion Affinity Chromatography (IMAC), as known to those skilled in the art.

Purity

For some applications, it is of great importance that the protease inhibitors produced using the present invention be very highly pure (*e.g.*, having a purity of more than 99%). This is particularly true whenever the desired protein is to be used as a therapeutic, but is also necessary for other applications. The methods described herein provide a way of producing substantially pure desired proteins. The desired proteins described herein are useful in pharmaceutical and personal care compositions. However, it is contemplated that proteins of varying purity levels will be produced using the methods of the present invention and it is not intended that the proteins produced using the present invention be limited to any particular level of purity.

Activation of BBI During Purification

In some embodiments of the present invention, after growth during the purification process, the activity of the protein is increased by the addition of chemicals that reduce/oxidize disulfide bonds and/or alter the general redox potential, and/or chemicals that alter solvent properties thus affecting protein conformation and aggregation. In some particularly preferred embodiments, addition of a reagent that reduces disulfide bonds, such as 2-mercaptoethanol, is used to increase activity of the protein. However, as those skilled in the art appreciate, depending purity and buffer composition, other disulfide reducing or oxidizing agents (*e.g.*, DTT, TCEP, reduced and oxidized glutathione, cysteine, cystine, cysteamine, thioglycolate, $S_2O_3^{2-}$, $S_2O_4^{2-}$, $S_2O_5^{2-}$, SO_3^{2-} , $S_2O_7^{2-}$, Cu^{+} , protein disulfide isomerases, protein thiol-disulfide oxidoreductases, etc.), either used alone or in combination, find use in the present invention. Other adjuvants that alter solvent properties, (*e.g.* ethanolamine, DMSO, TWEEN®-80, arginine, urea, *etc.*), either added alone or preferably in combination with disulfide reducing/oxidizing agents, such as β ME, during the purification process also find use in the present invention by increasing the activity of the protein. In certain preferred embodiments, partially purified protein is diluted in buffer (in some particularly preferred embodiments, a zwitterionic buffer with TWEEN®-80 at basic pH) and activated with β ME and a disulfide oxidizing agent (in alternative preferred embodiments, oxidized glutathione or sodium sulfite).

In addition, it is contemplated that conditions will be screened in order to determine the optimal activation of the protein, if desired. For example, various β ME concentrations (0.1 – 10 mM), oxidizing agent concentrations (0 to 1/20 to 20 times the β ME concentration) pH (7.5 – 9.5), temperatures (15 – 40°C), dilutions (1 – 20 fold), incubation times (12 – 72 h), aeration (incubations under inert gas to vigorous mixing under oxygen containing gases), buffer types (Tris, CHES, CAPS, Tricine, TAPS, other zwitterionic buffers, etc.), buffer concentrations (0.1 – 1 M), and the addition of various adjuvants known to alter solvent properties thereby affecting protein conformation and aggregation (*e.g.*, ethanolamine, DMSO, TWEEN®-80, arginine, urea, etc.) are tested in order to determine the optimum conditions for the expression system used. It is not intended that the present invention be limited to any particular disulfide reducing/oxidizing agent, dilution, temperature, pH, buffer type or composition, or adjuvant, as any suitable reagents known to those skilled in the art find use in the present invention.

Personal Care Compositions

In particularly preferred embodiments, the present invention provides cosmetic and/or pharmaceutical compounds for improving the appearance of skin comprising at least one polypeptide or a peptide. In some preferred embodiments, the polypeptide or peptide binds to VEGF. In alternative embodiments, the binding of the polypeptide or peptide to VEGF blocks the downstream activity of VEGF. In some embodiments, the compounds comprise at least one peptide, while in other embodiments, the compounds comprise at least one polypeptide. In some preferred embodiments, the peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:1-7, 16, and 17. In additional preferred embodiments, the peptide has a conserved binding sequence, the sequence being XXLWPXWC (SEQ ID NO:15). In some preferred embodiments, the peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:22-24. In alternative preferred embodiments, the compounds have a sequence, the sequence being at least 70%, preferably 80%, more preferably 90%, and most preferably 95% homologous to the sequences set forth herein. In some preferred embodiments, the polypeptide has a molecular weight that is preferably between 500 Daltons and 30,000 Daltons, more preferably between 1000 Daltons and 10,000 Daltons, and most preferably from 1500 Daltons to 8,000 Daltons.

In some preferred embodiments, the compounds find use in the improvement of skin in an organism (*i.e.*, subject) having a skin disorder. In some preferred embodiments, the skin disorder is an angiogenic skin disorder. In additional preferred embodiments, the skin disorder is at least one selected from the group consisting of psoriasis, venous ulcers, acne, rosacea, warts, eczema, hemangiomas and lymphangiogenesis, etc. In some particularly preferred embodiments, the skin disorder is rosacea.

It is not intended that the present invention be limited to any particular skin treatment or condition. For example, skin coloring has been of concern to human beings for many years. In particular, there is a desire to remove the hyperpigmentation associated with age spots, freckles, and other areas of darkening

skin due to age or other factors. A uniformly colored complexion (*i.e.*, without areas of redness, darkness or white) is preferred by many individuals. In addition, in some areas, whitening of the skin is a desired effect, while in others tanned skin is preferred. Various compounds have been used to achieve depigmentation, including kojic acid, hydroxyquinone, and retinoids. Much research has been devoted to the production of tanned skin without the use of radiation in order to avoid photodamage. Compounds that have found use in tanning skin without sun exposure include dihydroxyacetone and similar chemicals. However, the results obtained with these products are usually not optimal, as even and precise application is required in order to achieve the desired result. In addition, many of these compounds are skin irritants.

The chemical and enzymatic basis of melanogenesis is well known. Melanocytes migrate from the embryonal neural crest into the skin to produce the secretory granules known as melanosomes, which produce melanin via melanogenesis. The melanin produced by these cells is then distributed to keratinocytes via melanocyte dendrites. The key enzyme in melanogenesis is tyrosinase, which initiates a cascade of reactions which convert tyrosine to melanin, which is a polymer. Two tyrosinase-related proteins (TRP's) are known, which share about 40% homology with tyrosinase. These proteins (TRP-1 and TRP-2) have catalytic activities as well as regulatory roles in melanogenesis. TRP-1 is reported to be the most abundant glycoprotein in melanocytes.

Although the chemical and enzymatic basis of melanogenesis is well-documented, its regulation at the cellular level is only partially understood. Tyrosinase and the TRP's share structural and biological properties with the lysosomal-associated membrane protein (LAMP) gene family. Thus, it has been contemplated that their targeting to the melanosomal membrane might induce their activation. It is also contemplated that phosphorylation/dephosphorylation reaction occurring at the cytoplasmic tails of these proteins could be involved in the regulation of melanogenesis. The beta isoform of the Protein Kinase C (PKC) family has been shown to regulate human melanogenesis through tyrosinase activation. Gene expression of tyrosinase, TRP-1 and TRP-2 is coordinated. In addition, all three enzymes are expressed in human epidermis.

The Protease-activated receptor-2 (PAR-2) is a seven transmembrane G-protein-coupled receptor, that is related to, but distinct from the thrombin receptors (TR also named PAR-1, and PAR-3) in its sequence. Both receptors are activated proteolytically by an arginine-serine cleavage at the extracellular domain. The newly created N-termini then activate these receptors as tethered ligands. Both receptors may be activated by trypsin, but only the TRs are activated by thrombin. In addition, only PAR-2 is activated by mast cell tryptase. Both receptors may also be activated by the peptides that correspond to their new N-termini, independent of receptor cleavage. In addition, a peptide with the sequence SLIGRL (SEQ ID NO:32), known to be a mouse PAR-2 activating peptide, is equipotent in the activation of the human receptor. Although the TR functions are well documented, the biology of the PAR-2 system has not yet been fully identified. However, a role for PAR-2 activation in the inhibition of keratinocyte growth and differentiation has been recently described (Derian *et al.*, Cell Growth Different., 8:743-749 [1997]).

In other preferred embodiments, the present invention provides cosmetic and/or pharmaceutical compounds for improving the appearance of skin. In these preferred embodiments, the compounds comprise at least one peptide or polypeptide within at least one scaffold, the peptide or polypeptide being expressed in the scaffold. In some particularly preferred embodiments, the at least one peptide or polypeptide is a loop.

- 5 In other particularly preferred embodiments, the loop is closed by a disulfide bond. In some preferred embodiments, the polypeptide or peptide binds to VEGF. In alternative embodiments, the binding of the polypeptide or peptide to VEGF blocks the downstream activity of VEGF. In some particularly preferred embodiments, the peptide is expressed in a protease-resistant scaffold. In some especially preferred embodiments, the scaffold comprises a protease inhibitor (*e.g.*, BBI, STI, or Eglin chymotrypsin inhibitor).
- 10 In some most preferred embodiments, the protease inhibitor is BBI.

- In some preferred embodiments, the compounds further comprise at least one peptide. Preferably, the peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 1-7, 16, and 17. In some alternative the peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 14). In some embodiments, the compounds comprise an amino acid sequence
- 15 selected from the group consisting of SEQ ID NOS:22-24. Most preferably, the compounds comprise SEQ ID NO:22. In some preferred embodiments, the peptide has a conserved binding sequence, the sequence being XXLWPXWC (SEQ ID NO:15). In some preferred embodiments, the compounds have a sequence, the sequence being at least 70%, preferably 80%, more preferably 90%, and most preferably 95% identical to the sequences set forth herein. The peptide molecular weight is preferably between 500 Daltons and
- 20 45,000 Daltons, more preferably between 1000 Daltons and 12,000 Daltons, and most preferably from 1500 Daltons to 10,000 Daltons. In some preferred embodiments, the compounds comprise at least one polypeptide.

- In some preferred embodiments, the compounds are used for the improvement of skin in an organism (*i.e.*, a subject) having a skin disorder. In additional preferred embodiments, the skin disorder is at
- 25 least one selected from the group consisting of psoriasis, venous ulcers, acne, rosacea, warts, eczema, hemangiomas and lymphangiogenesis, etc. In some particularly preferred embodiments, the skin disorder is rosacea.

- In yet further embodiments, the present invention provides cosmetic and/or pharmaceutical compositions comprising at least one polypeptide or peptide, as set forth herein, and a physiologically
- 30 acceptable carrier or excipient. Preferably, the compound is present in an amount of about 0.0001% to about 5% by weight based on the total weight of the composition. Also preferably, the compound is present in an amount of about 0.001% to about 0.5% by weight based on the total weight of the composition. The composition may be in the form of an emulsified vehicle, such as a nutrient cream or lotion, a stabilized gel or dispersion system, a treatment serum, a liposomal delivery system, a topical pack or mask, a surfactant-
- 35 based cleansing system such as a shampoo or body wash, an aerosolized or sprayed dispersion or emulsion, a hair or skin conditioner, styling aid, or a pigmented product such as makeup, as well as other suitable

make-up and cosmetic preparations. In some embodiments, the carrier is preferably at least one selected from the group consisting of water, propylene glycol, ethanol, propanol, glycerol, butylene glycol and polyethylene glycol.

5 In additional embodiments, the present invention provides cosmetic and/or pharmaceutical compounds comprising at least one polypeptide or a peptide suitable for modulating hair growth. In some preferred embodiments, the compounds comprise at least one polypeptide.

10 In some preferred embodiments, modulation comprises treatment of at least one disease or condition that involves loss of hair. In some preferred embodiments, the disease or condition is at least one selected from the group consisting of inflammatory alopecias, pseudopelade, scleroderma, tick bites, lichen planus, psoriasis, lupus, seborrheic dermatitis, loose hair syndrome, hemochromatosis, androgenic alopecia, alopecia areata, cancer, conditions that affect defective hair fiber production, and environmental factors that affect hair production. In some preferred embodiments, the disease is androgenic alopecia or alopecia areata.

15 In some preferred embodiments, modulation comprises hair growth inhibition and/or hair removal for treatment of at least one disease or condition for which decreased hair growth is desirable. In some preferred embodiments, inhibition and/or removal comprises depilation.

20 In some preferred embodiments, the invention provides cosmetic and/or pharmaceutical compounds for modulating hair growth comprising at least one peptide or polypeptide and at least one scaffold, the peptide or polypeptide being contained within the scaffold, preferably the peptide or polypeptide being a loop, and most preferably, the loop being closed by a disulfide bond. In some preferred embodiments, the scaffold comprises STI, Eglin or BBI. In particularly preferred embodiments, the scaffold comprises BBI. In further preferred embodiments, the peptide or polypeptide comprises a polypeptide.

25 In yet further embodiments, the present invention provides means for decreasing VEGF activity and/or levels. In some preferred embodiments, the VEGF activity and/or levels are decreased in the epidermis. In some embodiments, the method comprising applying an effective amount of at least one of the compounds described herein to an organism in need thereof.

In additional embodiments, the present invention provides applications for hair and/or skin treatment, as well as applications wound healing, treatment of proliferative diseases, etc. Thus, the present invention provides compositions and methods suitable for application in/on humans and other animals.

30 In additional preferred embodiments, the present invention is directed to at least one protease-resistant scaffold comprising at least one peptide or polypeptide and at least one loop. Flexible native loops are found on the surface of most protein modules and exist as short stretches of amino acids that connect regions of defined secondary structure. Although crystallographic and NMR (nuclear magnetic resonance) studies show that native loops are usually less well defined than alpha-helices and beta-sheets, their conformational freedom is normally restricted substantially compared with free peptides. Consequently, the

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binding activities of native loops in proteins usually differ significantly from those of the corresponding linear amino acid sequence. However, it is not intended that the present invention be limited to any specific mechanism.

The loops provided by the present invention bind proteins such as VEGF (*e.g.*, VEGF-A). Binding the loop to the protein prevents the protein from binding to its target. Thus, binding interactions are thought to be disrupted by binding the loop to the protein. As a result, bioactivity can be altered as desired. However, it is not intended that the present invention be limited to any particular mechanism.

The present invention further provides protease inhibitors to stabilize the loops. STI, BBI and Eglin C have native loops that bind to and inhibit proteases. In some embodiments, STI and BBI native loops are replaced with the polypeptides and/or peptides of the invention. In some embodiments, these sequences are replaced with inhibitors or enhancers of any VEGF, while in other embodiments, the sequences are replaced with inhibitors or enhancers of VEGF-A. In alternative embodiments, the sequences are replaced with inhibitors of FGF-5, TGFB-1 and TGFB-2, as well as inhibitors of the complement pathway such as C2, C3, C4 or C5 inhibitors and Compstatin, etc. In additional embodiments, STI and BBI native loops are replaced with sequences that have been isolated using various techniques known in the art (*e.g.*, phage display), such as the VEGF binding proteins described herein.

In some embodiments, a native loop is replaced with a loop which is 3 to 20 amino acids in length, preferably 5 to 15 amino acids in length, and more preferably 5 to 10 amino acids in length. In alternative embodiments, longer sequences find use, as long as they provide binding and/or inhibition. In addition, peptides suitable for use as replacements of the native loop(s) can form constrained loops (*i.e.*, a loop formed by the presence to a disulfide bond between two cysteine residues). In some particularly preferred embodiments, the peptides are between 7 and 9 amino acids in length.

There are several advantages to using scaffolds to stabilize peptide sequences. In some preferred embodiments, the biological activity of the peptide is higher and/or effectively modulates biological function as a result of limiting peptide flexibility and reducing the entropic cost of fixing the polypeptide sequence in the bioactive conformation. In addition, structural information obtained by x-ray crystallography finds use in guiding affinity maturation. Furthermore, in some embodiments, the sequence presented on a structural scaffold is more resistant to proteolytic degradation in different biological applications. In still further embodiments, the chimeric construction is obtained in large amount in low cost biological expression systems for industrial applications.

BBI represents a class of protein scaffolds whose binding to proteases is mediated by an exposed native loop that is fixed in a characteristic canonical conformation and which fits into the active site in a manner thought to be similar to that of a substrate (Laskowski and Kato, *Ann. Rev. Biochem.*, 49:593-626 [1980]; and Bode & Huber, *supra*). The native loop is frequently constrained by the presence of disulfide bridges and/or extensive hydrogen-bonding networks that act to lock the structure into the correct canonical structure. The sequence of this loop determines the specificity of the inhibition, which mirrors the

specificity of proteases for their substrates. For example, most trypsin inhibitors have Arg or Lys as their P1 residue. Inhibitors of the BBI family have a network of conserved disulfide bridges that help form a symmetrical structure of two tricyclic domains (Chen *et al.*, *supra*; Werner and Wemmer, *supra*; and Liu *et al.*, *supra*), each containing an independent serine protease binding site. The native binding loop is contained within a region joined by disulfide bridges formed between cysteine residues. The identity of the amino acid residue at the P1 site on each domain is the main determinant of the serine protease inhibited. Native domains possess lysine or arginine for trypsin, leucine or tyrosine for chymotrypsin and alanine for elastase (Tsunogae *et al.*, J. Biochem. (Tokyo) 100:243-246 [1986]). In addition, serine is highly conserved at the P'1 position and proline at the P'3 position. The individual native loop regions of BBI are well suited for protein loop grafting of previously identified cysteine constrained peptides that bind to targets selectively, as described herein.

Numerous isoforms of BBI have been characterized. For example, the sequence DDESSKPCCDQCACTKSNPPQCRCSDMRLNSCHSACKSCICALSYPAQCFCVDITDFCYE PCKPSEDDKEN (SEQ ID NO:25) provides the amino acid sequence of a BBI backbone described herein. In addition, in some embodiments BBI is truncated with as many as 10 amino acid residues being removed from either the N- or C- terminal. Any of the isoforms described herein, as well as those additional ones known in the art, find use as scaffolds in the present invention.

The present invention provides several advantages over creation of, for example, chimeric proteins. Transfer of an entire protein can be difficult when, for example, a protein domain of interest carries more than one important biological activity. Maintaining one activity (*e.g.* functionally significant domain-domain interactions) while altering another (*e.g.* high affinity binding to a co-factor or receptor) can be problematic. The present invention, as indicated herein, overcomes that limitation, as in preferred embodiments the loops are transferred, instead of entire domains.

In addition, in some embodiments, the compounds of the present invention comprise at least one mutation in addition to those set out above. Other mutations, such as deletions, insertions, substitutions, transversions, transitions and inversions, at one or more other locations, also find use in the present invention.

In some embodiments, the compounds of the present invention also comprise a conservative substitution that may occur as a like-for-like substitution (*e.g.*, basic for basic, acidic for acidic, polar for polar etc.). In additional embodiments, non-conservative substitutions are provided (*i.e.*, from one class of residue to another or alternatively involving the inclusion of unnatural amino acids such as ornithine, diaminobutyric acid ornithine, norleucine ornithine, pyriylalanine, thienylalanine, naphthylalanine and phenylglycine).

In some embodiments, the sequences also have deletions, insertions and/or substitutions of amino acid residues that produce a silent change and result in a functionally equivalent substance.

In some embodiments, deliberate amino acid substitutions are made on the basis of similarity in amino acid properties (*e.g.*, polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues) and it is therefore useful to group amino acids together in functional groups. Amino acids can be grouped together based on the properties of their side chain alone. However it is more useful to include mutation data as well. The sets of amino acids thus derived are likely to be conserved for structural reasons. These sets can be described in the form of a Venn diagram (*See e.g.*, Livingstone and Barton, *Comput. Appl. Biosci.*, 9:745-756 [1993]; and (Taylor, *J. Theor. Biol.*, 119:205-218 [1986])). In some embodiments, conservative substitutions are made, for example according to the table below that describes a generally accepted Venn diagram grouping of amino acids.

Set		Sub-set	
Hydrophobic	F W Y H K M I L V A G C	Aromatic	F W Y H
		Aliphatic	I L V
Polar	W Y H K R E D C S T N Q	Charged	H K R E D
		Positively charged	H K R
		Negatively charged	E D
Small	V C A G S P T N D	Tiny	A G S

In some embodiments, variant amino acid sequences of the present invention also include suitable spacer groups inserted between any two amino acid residues of the sequence including alkyl groups such as methyl, ethyl or propyl groups in addition to amino acid spacers such as glycine or β -alanine residues. A further form of variation involves the presence of one or more amino acid residues in peptoid form.

In some embodiments, homology comparisons find use in identifying homologous sequences that find use in the present invention. Homology comparisons can be conducted by eye, or more usually, with the aid of readily available sequence comparison programs. Available computer programs can calculate the percent homology between two or more sequences. Additionally, percent homology may be calculated over contiguous sequences (*i.e.*, one sequence is aligned with the other sequence and each amino acid in one sequence is directly compared with the corresponding amino acid in the other sequence one residue at a time). This is called an "ungapped" alignment. Typically, such ungapped alignments are performed only over a relatively short number of residues.

Although this is a very simple and consistent method, it fails to take into consideration that, for example, in an otherwise identical pair of sequences, one insertion or deletion will cause following amino acid residues to be put out of alignment, thus potentially resulting in a large reduction in % homology when a global alignment is performed. Consequently, most sequence comparison methods are designed to produce optimal alignments that take into consideration possible insertions and deletions without penalizing unduly

the overall homology score. This is achieved by inserting "gaps" in the sequence alignment to try to maximize local homology.

However, these more complex methods assign "gap penalties" to each gap that occurs in the alignment, so that for the same number of identical amino acids, a sequence alignment with as few gaps as possible (*i.e.*, reflecting higher relatedness between the two compared sequences) will achieve a higher score than one with many gaps. "Affine gap costs" are typically used that charge a relatively high cost for the existence of a gap and a smaller penalty for each subsequent residue in the gap. This is one of the most commonly used gap scoring system. High gap penalties will of course produce optimized alignments with fewer gaps. Most alignment programs allow the gap penalties to be modified. However, it is preferred to use the default values when using such software for sequence comparisons. For example when using the GCG Wisconsin Bestfit package the default gap penalty for amino acid sequences is -12 for a gap and -4 for each extension.

Calculation of maximum % homology therefore firstly requires the production of an optimal alignment, taking into consideration gap penalties. A suitable computer program for carrying out such an alignment is the GCG Wisconsin Bestfit package (*See e.g.*, Devereux *et al.*, Nuc. Acids Res., 12:387 [1984]). Examples of other software packages that can perform sequence comparisons include, but are not limited to, the BLAST package FASTA, and the GENWORKS suite of comparison tools, all of which are well-known to those in the art. Both BLAST and FASTA are available for offline and online searching. However, for some applications, it is preferred to use the GCG Bestfit program. The BLAST 2 Sequence package is also available for comparing protein and nucleotide sequences.

Although the final percent homology can be measured in terms of identity, the alignment process itself is typically not based on an all-or-nothing pair comparison. Instead, a scaled similarity score matrix is generally used that assigns scores to each pair-wise comparison based on chemical similarity or evolutionary distance. An example of such a matrix commonly used is the BLOSUM62 matrix - the default matrix for the BLAST suite of programs. GCG Wisconsin programs generally use either the public default values or a custom symbol comparison table if supplied. For some applications, it is preferred to use the public default values for the GCG package, or in the case of other software, the default matrix, such as BLOSUM62.

Alternatively, percentage homologies may be calculated using the multiple alignment feature in DNASIS™ (Hitachi Software), based on an algorithm, analogous to CLUSTAL (*See e.g.*, Higgins and Sharp, Gene 73:237-244 [1988]).

Once the software has produced an optimal alignment, it is possible to calculate the percent of homology, and more preferably, the percent of sequence identity. The software typically does this as part of the sequence comparison and generates a numerical result.

In some embodiments, the present invention provides nucleic acids encoding any of the compounds described herein, as well as complements thereof. In additional preferred embodiments, the invention

provides vectors comprising a compound, as disclosed herein, cells comprising the compound and methods of expressing the compound.

Those of skill in the art appreciate the relationship between nucleic acid sequences and polypeptide sequences, in particular as relate to the genetic code and the degeneracy of this code, and will be able to construct such nucleic acids without difficulty. For example, one skilled in the art is aware that for each amino acid substitution in a sequence there may be one or more codons that encode the substitute amino acid. Accordingly, it is evident that, depending on the degeneracy of the genetic code with respect to that particular amino acid residue, one or more nucleic acid sequences may be generated corresponding to that polypeptide sequence.

Mutations in amino acid sequence and nucleic acid sequence may be made by any of a number of techniques, as known in the art. In particularly preferred embodiments, the mutations are introduced into parent sequences by means of PCR (polymerase chain reaction) using appropriate primers. In some embodiments, the parent enzymes are modified at the amino acid level, while in other embodiments, the enzymes are modified at the nucleic acid level, in order to generate the sequences described herein. In some preferred embodiments, the present invention provides for the generation of compounds by introducing one or more corresponding codon changes in the nucleotide sequence encoding a compound. It will be appreciated that the above codon changes will find use in various nucleic acid sequences of the present invention. For example, in some embodiments, sequence changes are made to any of the homologous sequences described herein.

As indicated above, in some embodiments, the "compound" comprises the "complete" protein, (*i.e.*, in its entire length as it occurs in nature (or as mutated)), while in other embodiments it comprises a truncated form of a protein. Thus, the compounds of the present invention are either truncated or be "full-length." In addition, in some embodiments, the truncation is located at the N-terminal end, while in other embodiments the truncation is located at the C-terminal end of the protein. In further embodiments, the compound lacks one or more portions (*e.g.*, sub-sequences, signal sequences, domains or moieties), whether active or not.

In yet further alternative embodiments, the nucleotide sequences encoding the compounds are prepared synthetically by established standard methods (*e.g.* the phosphoroamidite method described by Beucage *et al.*, Tetrahedr. Lett., 22:1859-1869 [1981]; or the method described by Matthes *et al.*, EMBO J., 3:801-805 [1984]). In the phosphoroamidite method, oligonucleotides are synthesized (*e.g.*, in an automatic DNA synthesizer), purified, annealed, ligated and cloned in appropriate vectors.

In some embodiments of the present invention, the nucleotide sequences are either of mixed genomic and synthetic origin, mixed synthetic and cDNA origin or mixed genomic and cDNA origin, prepared by ligating fragments of synthetic, genomic or cDNA origin, in accordance with standard techniques. Each ligated fragment corresponds to various parts of the entire nucleotide sequence. In some

embodiments, the DNA sequence is prepared by polymerase chain reaction (PCR) using specific primers, as known in the art.

In some embodiments, the nucleotide sequences described here and suitable for use in the methods and compositions described here include within them synthetic or modified nucleotides. A number of different types of modification to oligonucleotides are known in the art. These include, but are not limited to methylphosphonate and phosphorothioate backbones and/or the addition of acridine or polylysine chains at the 3' and/or 5' ends of the molecule. However, it is not intended that the present invention be limited to any particular method, as any suitable method known to those in the art for modifying nucleotide sequences find use in the present invention. In some embodiments, these modifications are performed in order to enhance the *in vivo* activity and/or life span of nucleotide sequences.

In some preferred embodiments, the present invention provides nucleotide sequences and methods for using nucleotide sequences that are complementary to the sequences presented herein, as well as derivatives and/or fragments of these sequences.

In some embodiments, the polynucleotides of the present invention find use in the production of primers and/or probes. Thus, in some embodiments, the polynucleotide sequences are used to produce PCR primers, primers for other amplification methods as known in the art, labeled probes, and/or for cloning methods. In preferred embodiments, these primers, probes and other fragments are at least 15, preferably at least 20, and in some more preferable embodiments, at least 25, 30 or 40 nucleotides. In addition, these primers, probes and fragments are encompassed by the term "polynucleotide."

In some embodiments, polynucleotides such as DNA polynucleotides and probes are produced recombinantly, while in other embodiments they are produced synthetically. In additional embodiments, these sequences are cloned using standard methods. In general, primers are produced by synthetic means, involving a stepwise manufacture of the desired nucleic acid sequence one nucleotide at a time. Techniques for accomplishing this using automated techniques are readily available in the art. However, it is not intended that the present invention be limited to production of polynucleotides using any particular method, as any suitable method known to those in the art finds use in the present invention.

In some embodiments, longer polynucleotides are generally be produced using recombinant means, for example using PCR cloning techniques, as known in the art. In such embodiments, the primers are typically designed to contain suitable restriction enzyme recognition sites so that the amplified DNA can be readily cloned into a suitable cloning vector. Preferably, the variant sequences are at least as biologically active as the sequences presented herein.

In some preferred embodiments, sequences that are provided that are complementary to the compound or sequences that are capable of hybridizing to the nucleotide sequences of the compounds (including complementary sequences of those presented herein), as well as nucleotide sequences that are complementary to sequences that can hybridize to the nucleotide sequences of the compounds (including complementary sequences of those presented herein). In some preferred embodiments, polynucleotide

sequences that are capable of hybridizing to the nucleotide sequences presented herein under conditions of intermediate to maximal stringency are provided.

In some preferred embodiments, nucleotide sequences that can hybridize to the nucleotide sequence of the compound nucleic acid, or the complement thereof, under stringent conditions (*e.g.*, 50°C and 0.2xSSC) are provided. More preferably, the nucleotide sequences can hybridize to the nucleotide sequence of the compound, or the complement thereof, under more highly stringent conditions (*e.g.*, 65°C and 0.1xSSC).

In some embodiments, it is desirable to mutate the sequence in order to prepare a compound. Accordingly, in some embodiments, mutants are prepared from the compounds provided herein. In some embodiments, mutations are introduced using synthetic oligonucleotides. These oligonucleotides contain nucleotide sequences flanking the desired mutation sites. Various methods known in the art find use in this embodiment (*See e.g.*, Morinaga *et al.*, *Biotechnol.*, 2:646-649 [1984]; Nelson and Long, *Anal. Biochem.*, 180:147-151 [1989]; and Sarkar and Sommer, *Biotechn.*, 8:404-407 [1990]). However, additional methods find use in the present invention and it is not intended that the present invention be limited to any particular method.

In some preferred embodiments, the sequences used in the methods and compositions described herein is a recombinant sequence (*i.e.*, a sequence that has been prepared using recombinant DNA techniques produced using any suitable method known in the art).

In additional embodiments, the present invention provides vectors comprising the compound, cells comprising the compound, and methods of expressing the compound. In some embodiments, the nucleotide sequences used in the methods and compositions described herein are incorporated into a recombinant replicable vector. In some embodiments, the vector is used to replicate and express the nucleotide sequence, in enzyme form, in and/or from a compatible host cell. In some embodiments, expression is controlled using control sequences (*e.g.*, regulatory sequences). In some embodiments, proteins produced by a host cell by expression of the nucleotide sequence are secreted (*i.e.*, into the growth medium), while in other embodiments, the proteins are contained intracellularly within the host cell. In some embodiments, the coding sequences are designed to include signal sequences which direct secretion of the substance coding sequences through a particular prokaryotic or eukaryotic cell membrane. In further embodiments, polynucleotides are incorporated into a recombinant replicable vector. In additional embodiments, the vector is used to replicate the nucleic acid in a compatible host cell. In preferred embodiments, the vector comprising the polynucleotide sequence is transformed into a suitable host cell. While any suitable host finds use in the present invention, in some preferred embodiments, the hosts are selected from the group consisting of bacterial, yeast, insect, fungal, and mammalian cells.

In some embodiments, compounds and their polynucleotides are expressed by introducing a polynucleotide into a replicable vector, introducing the vector into a compatible host cell, and growing the

host cell under conditions which bring about replication of the vector. In some embodiments, the vector is recovered from the host cell.

5 In additional embodiments, the compound nucleic acid is operatively linked to transcriptional and translational regulatory elements active in the host cell. In some embodiments, the compound nucleic acid also encodes a fusion protein comprising at least one signal sequence (*e.g.*, those derived from the glucoamylase gene from *Schwanniomyces occidentalis*, α -factor mating type gene from *Saccharomyces cerevisiae* and the TAKA-amylase from *Aspergillus oryzae*). In further alternative embodiments, the compound nucleic acid encodes a fusion protein comprising a membrane binding domain.

10 In some preferred embodiments, the compound is expressed at the desired levels in a host organism using an expression vector. It is contemplated that any expression vector comprising a compound nucleic acid that is capable of expressing the gene encoding the compound nucleic acid in the selected host organism will find use in the present invention. The choice of vector depends upon the host cell into which it is to be introduced. Thus, in some embodiments, the vector is an autonomously replicating vector (*i.e.*, a vector that exists as an episomal entity, the replication of which is independent of chromosomal replication, 15 such as, for example, a plasmid, a bacteriophage or an episomal element, a minichromosome or an artificial chromosome). Alternatively, in some embodiments, the vector integrates into the host cell genome and replicates together with the chromosome.

In some preferred embodiments, the expression vector includes the components of a cloning vector, including but not limited to such components as an element that permits autonomous replication of the 20 vector in the selected host organism and one or more phenotypically detectable markers for selection purposes. In preferred embodiments, the expression vector further comprises control nucleotide sequences encoding a promoter, operator, ribosome binding site, translation initiation signal, and optionally, a repressor gene or one or more activator genes. Additionally, in some embodiments, the expression vector comprises a sequence coding for an amino acid sequence capable of targeting the compound to a host cell 25 organelle such as a peroxisome or to a particular host cell compartment. Such a targeting sequence includes but is not limited to the sequence SKL. For expression under the direction of control sequences, the nucleic acid sequence encoding the compound is operably linked to the control sequences in proper manner with respect to expression.

30 In some preferred embodiments, the polynucleotide in a vector is operably linked to a control sequence that is capable of providing for the expression of the coding sequence by the host cell (*i.e.*, the vector is an expression vector). In some embodiments, the control sequences are modified (*e.g.*, by the addition of further transcriptional regulatory elements) in order to make the level of transcription directed by the control sequences more responsive to transcriptional modulators. In some preferred embodiments, the control sequences comprise promoters.

In some preferred embodiments of the vectors, the nucleic acid sequence encoding for the compound is operably combined with a suitable promoter sequence. The promoter can be any DNA sequence having transcription activity in the host organism of choice and can be derived from genes that are homologous or heterologous to the host organism. Examples of suitable promoters for directing the transcription of the modified nucleotide sequence, such as compound nucleic acids, in a bacterial host include, but are not limited to the promoter of the *lac* operon of *E. coli*, the *Streptomyces coelicolor* agarase gene *dagA* promoters, the promoters of the *Bacillus licheniformis* α -amylase gene (*amyL*), the *aprE* promoter of *Bacillus subtilis*, the promoters of the *Bacillus stearothermophilus* maltogenic amylase gene (*amyM*), the promoters of the *Bacillus amyloliquefaciens* α -amylase gene (*amyQ*), the promoters of the *Bacillus subtilis* *xylA* and *xylB* genes and a promoter derived from a *Lactococcus* sp.-derived promoter including the PI70 promoter. When the gene encoding the compound is expressed in a bacterial species such as *E. coli*, a suitable promoter can be selected, for example, from a bacteriophage promoter including a T7 promoter and a phage lambda promoter. For transcription in a fungal species, examples of useful promoters are those derived from the genes encoding the *Aspergillus oryzae* TAKA amylase, *Rhizomucor miehei* aspartic proteinase, *A. niger* neutral α -amylase, *A. niger* acid stable α -amylase, *A. niger* glucoamylase, *Rhizomucor miehei* lipase, *A. oryzae* alkaline protease, *A. oryzae* triose phosphate isomerase, and *A. nidulans* acetamidase. Examples of suitable promoters for the expression in a yeast species include but are not limited to the Gal 1 and Gal 10 promoters of *Saccharomyces cerevisiae* and the *Pichia pastoris* AOX1 or AOX2 promoters.

Examples of suitable bacterial host organisms are Gram positive species, including, but not limited to members of the *Bacillaceae*, (e.g., *B. subtilis*, *B. licheniformis*, *B. lentus*, *B. brevis*, *B. stearothermophilus*, *B. alkalophilus*, *B. amyloliquefaciens*, *B. coagulans*, *B. lautus*, *B. megaterium* and *B. thuringiensis*), *Streptomyces* species (e.g., *S. murinus* and *S. lividans*) lactic acid bacteria (e.g., *Lactococcus* spp. such as *Lactococcus lactis*; *Lactobacillus* spp. including *Lactobacillus reuteri*; *Leuconostoc* spp.; *Pediococcus* spp.; and *Streptococcus* spp. Alternatively, strains of Gram-negative species belonging to *Enterobacteriaceae* (e.g., *E. coli*) or members of the *Pseudomonadaceae* find use in the present invention.

In some embodiments, a suitable yeast host organism is selected from various biotechnologically useful yeasts species, including but not limited to *Pichia* sp., *Hansenula* sp or *Kluyveromyces*, *Yarrowinia*, *Saccharomyces* (e.g., *Saccharomyces cerevisiae*), *Schizosaccharomyce* (e.g., *S. pombe*). In some embodiments, strains of the methylotrophic yeast species *Pichia pastoris* are used as the host organism, while in other embodiments, the host organism is a *Hansenula* species. Suitable host organisms among filamentous fungi include species of *Aspergillus* (e.g., *A. niger*, *A. oryzae*, *A. tubigensis*, *A. awamori* and *Aspergillus nidulans*). Alternatively, strains of *Fusarium* species (e.g. *F. oxysporum*) and *Rhizomucor* (e.g., *Rhizomucor miehei*) find used as the host organism. Additional suitable strains include, but are not limited to *Thermomyces* and *Mucor* species.

In some preferred embodiments, host cells comprising polynucleotides are used to express polypeptides, such as the compounds disclosed herein, fragments, homologues, variants or derivatives thereof. Host cells are cultured under suitable conditions which allow expression of the proteins. In some embodiments, expression of the polypeptides is constitutive (*i.e.*, the peptides are continually produced), while in other embodiments, expression is inducible. In the case of inducible expression, protein production is initiated when required by addition of an inducer substance to the culture medium (*e.g.*, dexamethasone or IPTG). Polypeptides can be extracted from host cells by a variety of techniques known in the art, including enzymatic, chemical, and/or osmotic lysis and physical disruption. Indeed, it is not intended that the present invention be limited to any particular means of harvesting expressed polypeptides.

In alternative embodiments, polypeptides are produced recombinantly in any suitable (including commercially available) *in vitro* cell-free system, such as the TnTTM (Promega) rabbit reticulocyte system.

In additional preferred embodiments, the present invention provides cosmetic and/or pharmaceutical compositions comprising at least one polypeptide or peptide, as set forth herein, and a physiologically acceptable carrier or excipient. Preferably, the compound is present in an amount of about 0.0001% to about 5% by weight, based on the total weight of the composition. Also preferably, the compound is present in an amount of about 0.001% to about 0.5% by weight based on the total weight of the composition. The composition may be in the form of an emulsified vehicle, such as a nutrient cream or lotion, a stabilized gel or dispersion system, a treatment serum, a liposomal delivery system, a topical pack or mask, a surfactant-based cleansing system such as a shampoo or body wash, an aerosolized or sprayed dispersion or emulsion, a hair or skin conditioner, styling aid, or a pigmented product such as makeup. Preferably, the carrier is at least compound selected from the group consisting of water, propylene glycol, ethanol, propanol, glycerol, butylene glycol and polyethylene glycol.

In some liposomal embodiments, the liposomes comprise water and one or more ingredients capable of forming lipid bilayer vesicles that can hold one or more functional or active ingredient(s). Non-limiting examples of ingredients capable of forming lipid bilayer vesicles include: phospholipids, hydrogenated phosphatidylcholine, lecithin, cholesterol and sphingolipids. Preferred liposomes include, without limitation: a) lipid liposome 0003 (composed of water and lecithin and glycerin); b) lipid liposome 0300 (composed of water and phosphatidylcholine); c) lipid liposome 0111 (composed of water, *Ginkgo biloba* leaf extract, denatured alcohol, hydrogenated lecithin and cholesterol); d) anti-irritant liposomes (composed of water, *cola acuminata* seed extract, bisabolol and phospholipids); e) vitamin C and E liposomes (composed of water, phospholipids, tocopheryl acetate and ascorbyl palmitate); f) firming liposomes (composed of water, butylene glycol, *pyrus malus* (Apple) fruit extract, phospholipids, tocopheryl acetate and carbomer); and g) moisturizing liposomes (composed of water, sodium PCA, tocopheryl acetate, xanthan gum, arginine, lysine, glycine and proline).

In other embodiments, the personal care composition of the present invention further comprise at least one active ingredient in addition to the scaffolds provide herein. There are numerous active ingredients

known to those of skill in the art that find use in the personal care compositions of the present invention. Indeed, it is contemplated that any suitable active ingredient or combination of suitable active ingredients will find use in the present invention (*See e.g.*, McCutcheon's Functional Materials, North American and International Editions, published by MC Publishing Co. [2003]). For example, in some embodiments, the personal care compositions herein comprise a skin care active ingredient at a level from about 0.0001% to about 20%, by weight of the composition. In another embodiment, the personal care compositions comprise a skin care active ingredient from about 0.001% to about 5%, by weight of the composition. In yet another embodiment, the personal care compositions comprise a skin care active ingredient from about 0.01% to about 2%, by weight of the composition.

Non-limiting examples of functional or active ingredients that can be delivered via liposomes include: vitamins and their derivatives, antioxidants, proteins and peptides, keratolytic agents, bioflavonoids, terpenoids, phytochemicals, and extracts of plant, marine or fermented origin. In a preferred embodiment, the composition further comprises a skin care or hair care active. Active ingredients can include any of a wide variety of ingredients such as are known in the art. (*See e.g.*, McCutcheon's Functional Materials, North American and International Editions, (2003), published by MC Publishing Co.). Preferably, such actives include but are not limited to antioxidants, such as tocopheryl and ascorbyl derivatives, bioflavonoids, terpenoids, synthetics and the like, vitamins and vitamin derivatives, hydroxyl- and polyhydroxy acids and their derivatives, such as AHAs and BHAs and their reaction products, peptides and polypeptides and their derivatives, such as glycopeptides and lipophilized peptides, heat shock proteins and cytokines, enzymes and enzymes inhibitors and their derivatives, such as proteases, MMP inhibitors, catalases, glucose oxydase and superoxide dismutase, amino acids and their derivatives, bacterial, fungal and yeast fermentation products and their derivatives, including mushrooms, algae and seaweed and their derivatives, phytosterols and plant and plant part extracts and their derivatives and phospholipids and their derivatives, anti-dandruff agents such as zinc pyrithione and delivery systems containing them, as provided herein and/or known in the art.

In some preferred embodiments, the skin care active is selected from the group consisting of a Vitamin B3 component, panthenol, Vitamin E, Vitamin E acetate, retinol, retinyl propionate, retinyl palmitate, retinoic acid, Vitamin C, theobromine, alpha-hydroxyacid, farnesol, phytrantriol, salicylic acid, palmityl peptide-3 and mixtures thereof. In some preferred embodiments, the Vitamin B3 component is niacinamide. In some embodiments, the compositions provided herein comprise a skin care active at a level from about 0.0001% to about 20%, preferably from about 0.001% to about 5%, more preferably from about 0.01% to about 2%, by weight.

Exemplary derivatives of the foregoing vitamin B3 compounds include nicotinic acid esters, including non-vasodilating esters of nicotinic acid, nicotinyl amino acids, nicotinyl alcohol esters of carboxylic acids, nicotinic acid N-oxide and niacinamide N-oxide. Suitable esters of nicotinic acid include

nicotinic acid esters of C₁-C₂₂, preferably C₁-C₁₆, more preferably C₁-C₆ alcohols. In these embodiments, the alcohols are suitably straight-chain or branched chain, cyclic or acyclic, saturated or unsaturated (including aromatic), and substituted or unsubstituted. The esters are preferably non-vasodilating.

Non-vasodilating esters of nicotinic acid include tocopherol nicotinate and inositol hexanicotinate; 5 tocopherol nicotinate are preferred. A more complete description of vitamin B₃ compounds is provided in WO 98/22085. Preferred vitamin B₃ compounds include niacinamide and tocopherol nicotinate.

In additional embodiments, the skin care active comprises at least one retinoid. The retinoid is preferably retinol, retinol esters (*e.g.*, C₂ - C₂₂ alkyl esters of retinol, including retinyl palmitate, retinyl acetate, retinyl propionate), retinal, and/or retinoic acid (including all-trans retinoic acid and/or 13-cis- 10 retinoic acid), more preferably retinoids other than retinoic acid. These compounds are well known in the art and are commercially available from a number of sources (*e.g.*, Sigma and Boehringer Mannheim). Preferred retinoids include retinol, retinyl palmitate, retinyl acetate, retinyl propionate, retinal, retinoic acid and combinations thereof. More preferred are retinol, retinoic propionate, retinoic acid and retinyl palmitate. In some embodiments, the retinoid is included as a substantially pure material, while in other 15 embodiments, it is provided as an extract obtained by suitable physical and/or chemical isolation from natural (*e.g.*, plant) sources. When a retinoid is included in the compositions herein, it preferably comprises from about 0.005% to about 2%, preferably from about 0.01% to about 1% retinoid. Retinol is preferably used in an amount of from about 0.01% to about 0.15%; retinol esters are preferably used in an amount of from about 0.01% to about 2% (*e.g.*, about 1%).

20 In some embodiments, the compositions of the present invention comprise safe and effective amounts of a dermatologically acceptable carrier that is suitable for topical application to the skin or hair within which the essential materials and optional other materials are incorporated to enable the essential materials and optional components to be delivered to the skin or hair at an appropriate concentration. Thus, in some embodiments, the carrier acts as a diluent, dispersant, solvent or the like for the essential 25 components, ensuring that these components can be applied and distributed evenly over the selected target at an appropriate concentration.

In further embodiments, an effective amount of one or more compounds described herein is also be included in compositions to be applied to keratinous materials such as nails and hair, including but not limited to those useful as hair spray compositions, hair styling compositions, hair shampooing and/or 30 conditioning compositions, compositions applied for the purpose of hair growth regulation and compositions applied to the hair and scalp for the purpose of treating seborrhea, dermatitis and/or dandruff.

In yet additional embodiments, an effective amount of one or more compounds described herein is included in compositions suitable for topical application to the skin or hair. These compositions are provided in any suitable form, including but not limited to creams, lotions, gels, suspensions dispersions, 35 microemulsions, nanodispersions, microspheres, hydrogels, emulsions (*e.g.*, oil-in-water and water-in-oil, as

well as multiple emulsions), and multilaminar gels and the like (*See e.g.*, Schlossman *et al.*, The Chemistry and Manufacture of Cosmetics, [1998], incorporated by reference, herein). In some embodiments, the compositions are formulated as aqueous or silicone compositions, while in other embodiments they are formulated as emulsions of one or more oil phases in an aqueous continuous phase (or an aqueous phase in an oil phase).

The type of carrier utilized in the present invention depends on the type of product form desired for the composition. The carrier can be solid, semi-solid or liquid. Suitable carriers include liquids, semi-solids (*e.g.*, creams, lotions, gels, sticks, ointments, and pastes), sprays and mousses. Preferably the carrier is in the form of a lotion, cream or a gel, more preferably one which has a sufficient thickness or yield point to prevent the particles from sedimenting. In some embodiments, the carrier is inert, while in other embodiments it provides dermatological benefits. In some embodiments, the carrier is applied directly to the skin and/or hair, while in other embodiments, it is applied via a woven or non-woven wipe or cloth. In yet other embodiments, it is in the form of a patch, mask or wrap. In still further embodiments, it is aerosolized or otherwise sprayed or pumped onto the skin and/or hair. The carrier chosen is physically and chemically compatible with the essential components described herein, and should not unduly impair stability, efficacy or other use benefits associated with the compositions of the present invention.

Preferred carriers contain a dermatologically acceptable, hydrophilic diluent. Suitable hydrophilic diluents include water, organic hydrophilic diluents such as $C_2 - C_{10}$, preferably $C_2 - C_6$, preferably, $C_3 - C_6$ monohydric alcohols and low molecular weight glycols and polyols, including propylene glycol, polyethylene glycol polypropylene glycol, glycerol, butylene glycol, 1,2,4-butanetriol, sorbitol esters, 1,2,6-hexametriol, pentylene glycol, hexylene glycol, sorbitol esters, ethoxylated ethers, propoxylated ethers, and combinations thereof. The diluent is preferably liquid. Water is a preferred diluent. The composition preferably comprises at least about 20% of the hydrophilic diluent.

In some embodiments, suitable carriers also comprise an emulsion comprising a hydrophilic phase, especially an aqueous phase, and a hydrophobic phase (*e.g.*, a lipid, oil or oily material). As well known to those skilled in the art, the hydrophilic phase is dispersed in the hydrophobic phase, or vice versa, to form respectively hydrophilic or hydrophobic dispersed and continuous phases, depending on the composition of ingredients. The term "dispersed phase" is a term well-known to one skilled in the art of emulsion technology, used in reference to the phase which exists as small particles or droplets that are suspended in and surrounded by a continuous phase. The dispersed phase is also known as the internal or discontinuous phase. The emulsion may be or comprise (*e.g.*, in a triple or other multi-phase emulsion) an oil-in-water emulsion or a water-in-oil emulsion such as a water-in-silicone emulsion. Oil-in-water emulsions typically comprise from about 1% to about 60% (preferably about 1% to about 30%) of the dispersed hydrophobic phase and from about 1% to about 99% (preferably from about 10% to about 90%) of the continuous hydrophilic phase, while water-in-oil emulsions typically comprise from about 1% to about 98% (preferably

from about 40% to about 90%) of the dispersed hydrophilic phase and from about 1% to about 50% (preferably about 1% to about 30%) of the continuous hydrophobic phase.

In further embodiments, the carrier also includes one or more components that facilitate penetration through the upper stratum corneum barrier to the lower levels of the skin. Examples of penetration enhancers include, but are not limited to, propylene glycol, azone, ethoxydiglycol, dimethyl isosorbide, urea, ethanol and dimethyl sulfoxide, as well as microemulsions, liposomes and nanoemulsions.

In some additional embodiments, the compositions of the present invention comprise humectants which are preferably present at a level of from about 0.01% to about 20%, preferably from about 0.1% to about 15% and preferably from about 0.5% to about 10%. Preferred humectants include, but are not limited to, compounds selected from polyhydric alcohols, sorbitol, glycerol, urea, betaine, D-panthenol, DL-panthenol, calcium pantothenate, royal jelly, panthetine, pantotheine, panthenyl ethyl ether, pangamic acid, pyridoxin, pantoyl lactose Vitamin B complex, sodium pyrrolidone carboxylic acid, hexane - 1, 2, 6, - triol, guanidine or its derivatives, and mixtures thereof.

Suitable polyhydric alcohols for use herein include, but are not limited to polyalkylene glycols and preferably alkylene polyols and their derivatives, including propylene glycol, dipropylene glycol, polypropylene glycol, polyethylene glycol and derivatives thereof, sorbitol, hydroxypropyl sorbitol, erythritol, threitol, pentaerythritol, xylitol, glucitol, mannitol, pentylene glycol, hexylene glycol, butylene glycol (e.g., 1,3-butylene glycol), hexane triol (e.g., 1,2,6-hexanetriol), trimethylol propane, neopentyl glycol, glycerine, ethoxylated glycerine, propane-1,3 diol, propoxylated glycerine and mixtures thereof. The alkoxylated derivatives of any of the above polyhydric alcohols are also suitable for use herein. Preferred polyhydric alcohols of the present invention are selected from glycerine, butylene glycol, propylene glycol, pentylene glycol, hexylene glycol, dipropylene glycol, polyethylene glycol, hexane triol, ethoxylated glycerine and propoxylated glycerine and mixtures thereof.

Suitable humectants useful herein are sodium 2-pyrrolidone-5-carboxylate (NaPCA), guanidine; glycolic acid and glycolate salts (e.g., ammonium and quaternary alkyl ammonium); lactic acid and lactate salts (e.g., ammonium and quaternary alkyl ammonium); aloe vera in any of its variety of forms (e.g., aloe vera gel); hyaluronic acid and derivatives thereof (e.g., salt derivatives such as sodium hyaluronate); lactamide monoethanolamine; acetamide monoethanolamine; urea; betaine, panthenol and derivatives thereof; and mixtures thereof.

In some embodiments, at least part (up to about 5% by weight of composition) of a humectant is incorporated into the compositions of the present invention in the form of an admixture with a particulate cross-linked hydrophobic acrylate or methacrylate copolymer, itself preferably present in an amount of from about 0.1% to about 10%, which can be added either to the aqueous or disperse phase. This copolymer is particularly valuable for reducing shine and controlling oil while helping to provide effective moisturization benefits and is described in further detail in WO96/03964, incorporated herein by reference.

In some embodiments, the oil-in-water and water-in-oil compositions of the present invention comprise from about 0.05% to about 20%, preferably from about 1% to about 15%, preferably from about 2% to about 10%, preferably from about 2% to about 5% of a dermatologically acceptable emollient.

Emollients tend to lubricate the skin, increase the smoothness and suppleness of the skin, prevent or relieve dryness of the skin and/or protect the skin. Emollients are typically water-immiscible, oily or waxy materials and emollients can confer aesthetic properties to a topical composition. A wide variety of suitable emollients are known (*See e.g.*, Sagarin, Cosmetics, Science and Technology, 2nd Edition, Vol. 1, pp. 32-43 [1972]; and WO 00/24372), and find use herein, contains numerous examples of materials suitable as emollients. Additional emollients include, but are not limited to the following:

i) Straight and branched chain hydrocarbons having from about 7 to about 40 carbon atoms, such as mineral oils, dodecane, squalane, cholesterol, hydrogenated polyisobutylene, isohexadecane, isoeicosane, isooctahexacontane, isohexapentacontahexane, and the C₇-C₄₀ isoparaffins, which are C₇-C₄₀ branched hydrocarbons. Suitable branched chain hydrocarbons for use herein are selected from isopentacontaoctadecane, petrolatum and mixtures thereof;

ii) C₁-C₃₀ fatty acid esters of C₁-C₃₀ carboxylic acids, C₁₂₋₁₅ alkyl benzoates and of C₂-C₃₀ dicarboxylic acids, *e.g.* isononyl isononanoate, isostearyl neopentanoate, isodecyl octanoate, isodecyl isononanoate, tridecyl isononanoate, myristyl octanoate, octyl pelargonate, octyl isononanoate, myristyl myristate, myristyl neopentanoate, myristyl octanoate, isopropyl myristate, myristyl propionate, isopropyl stearate, isopropyl isostearate, methyl isostearate, behenyl behenate, dioctyl maleate, diisopropyl adipate, and diisopropyl dilinoleate and mixtures thereof also find use in the present invention;

iii) C₁-C₃₀ mono- and poly- esters of sugars and related materials. These esters are derived from a sugar or polyol moiety and one or more carboxylic acid moieties. Depending on the constituent acid and sugar, these esters can be in either liquid or solid form at room temperature. Examples include: glucose tetraoleate, the galactose tetraesters of oleic acid, the sorbitol tetraoleate, sucrose tetraoleate, sucrose pentaoleate, sucrose hexaoleate, sucrose heptaoleate, sucrose octaoleate, sorbitol hexaester. Other materials include cottonseed oil or soybean oil fatty acid esters of sucrose. Other examples of such materials are described in WO 96/16636, incorporated by reference herein;

iv) Vegetable oils and hydrogenated vegetable oils. Examples of vegetable oils and hydrogenated vegetable oils include safflower oil, grapeseed oil, coconut oil, cottonseed oil, menhaden oil, palm kernel oil, palm oil, peanut oil, soybean oil, rapeseed oil, linseed oil, rice bran oil, pine oil, nut oil, sesame oil, sunflower seed oil, partially and fully hydrogenated oils from the foregoing sources and mixtures thereof;

v) Soluble or colloiddally-soluble moisturizing agents. Examples include hyaluronic acid and chondroitin sulfate.

In some embodiments, the compositions of the present invention contain an emulsifier and/or surfactant, generally to help disperse and suspend the disperse phase within the continuous aqueous phase. A surfactant may also be useful if the product is intended for skin or hair cleansing. For convenience hereinafter, "emulsifiers" are encompassed by the term "surfactants." Thus, as used herein, the term "surfactant(s)" refers to surface active agents, whether used as emulsifiers or for other surfactant purposes such as skin cleansing. Known, including conventional surfactants find use in the present invention, provided that the selected agent is chemically and physically compatible with essential components of the composition and provides the desired characteristics (*See e.g.*, WO 00/24372). Suitable surfactants include non-silicone derived materials, silicone-derived materials, and mixtures thereof.

In further embodiments, the compositions of the present invention comprise preferably from about 0.05% to about 30%, more preferably from about 0.5% to 15%, and most preferably from about 1% to 10% of a surfactant or mixture of surfactants. The exact surfactant or surfactant mixture chosen depends upon the pH of the composition, the other components present and the desired final product aesthetics.

Among the nonionic surfactants that are useful herein are those that can be broadly defined as condensation products of long chain alcohols (*e.g.*, C₈₋₃₀ alcohols), with sugar or starch polymers (*e.g.*, glycosides). Other useful nonionic surfactants include the condensation products of alkylene oxides with fatty acids (*i.e.*, alkylene oxide esters of fatty acids). These materials have the general formula RCO(X)_nOH wherein R is a C₁₀₋₃₀ alkyl group, X is -OCH₂CH₂- (*i.e.*, derived from ethylene glycol or oxide) or -OCH₂CHCH₃- (*i.e.*, derived from propylene glycol or oxide) and n is an integer from about 6 to about 200.

Other nonionic surfactants are the condensation products of alkylene oxides with 2 moles of fatty acids (*i.e.*, alkylene oxide diesters of fatty acids). These materials have the general formula RCO(X)_nOOCR wherein R is a C₁₀₋₃₀ alkyl group, X is -OCH₂CH₂- (*i.e.*, derived from ethylene glycol or oxide) or -OCH₂CHCH₃- (*i.e.*, derived from propylene glycol or oxide) and n is an integer from about 6 to about 100. In some embodiments, an emulsifier for use herein is preferably a fatty acid ester blend based on a mixture of sorbitan fatty acid ester and sucrose fatty acid ester, especially a blend of sorbitan stearate and sucrose cocoate. Further suitable examples include a mixture of cetearyl alcohols and cetearyl glucosides. However, it is not intended that the present invention be limited to any particular emulsifier, as various suitable emulsifiers are known in the art.

In additional embodiments, the hydrophilic surfactants useful herein alternatively or additionally include any of a wide variety of cationic, anionic, zwitterionic, and amphoteric surfactants such as are known in the art (*See, e.g.*, McCutcheon's, Emulsifiers and Detergents, North American and International Editions, MC Publishing Co. [2003]; U.S. Patent No. 5,011,681 U.S. Patent No. 4,421,769; and U.S. Patent No. 3,755,560).

A variety of anionic surfactants are also useful herein (*See e.g.*, U.S. Patent No. 3,929,678). Exemplary anionic surfactants include, but are not limited to alkoyl isethionates (*e.g.*, C₁₂ - C₃₀), alkyl and alkyl ether sulfates and salts thereof, alkyl and alkyl ether phosphates and salts thereof, alkyl methyl taurates (*e.g.*, C₁₂ - C₃₀), and soaps (*e.g.*, substituted alkylamine and alkali metal salts, *e.g.*, sodium or potassium salts) of fatty acids.

Amphoteric and zwitterionic surfactants are also useful herein. Examples of preferred amphoteric and zwitterionic surfactants which find use in the compositions of the present invention are those which are broadly described as derivatives of aliphatic secondary and tertiary amines in which the aliphatic radical can be straight or branched chain and wherein one of the aliphatic substituents contains from about 8 to about 22 carbon atoms (preferably C₈ - C₁₈) and one contains an anionic water solubilizing group (*e.g.*, carboxy, sulfonate, sulfate, phosphate, or phosphonate). Examples, include but are not limited to alkyl imino acetates and iminodialkanoates and aminoalkanoates, imidazolinium and ammonium derivatives. Other suitable amphoteric and zwitterionic surfactants are those selected from the group consisting of betaines, sultaines, hydroxysultaines, and branched and unbranched alkanoyl sarcosinates, and mixtures thereof.

In further embodiments, some emulsions of the present invention include a silicone containing emulsifier or surfactant. A wide variety of silicone emulsifiers find use herein. These silicone emulsifiers are typically organically modified organopolysiloxanes, also known to those skilled in the art as silicone surfactants. Useful silicone emulsifiers include, but are not limited to dimethicone copolyols. These materials are polydimethyl siloxanes which have been modified to include polyether side chains such as polyethylene oxide chains, polypropylene oxide chains, mixtures of these chains and polyether chains containing moieties derived from both ethylene oxide and propylene oxide. Other examples include alkyl-modified dimethicone copolyols (*i.e.*, compounds which contain C₂-C₃₀ pendant side chains). Still other useful dimethicone copolyols include materials having various cationic, anionic, amphoteric, and zwitterionic pendant moieties.

In some embodiments, the compositions of the present invention comprise at least one polymeric thickening agent. The polymeric thickening agents useful herein preferably have a number average molecular weight of greater than about 20,000, more preferably greater than about 50,000, and most preferably greater than about 100,000. In some embodiments, the compositions of the present invention comprise from about 0.01% to about 10%, preferably from about 0.1% to about 8% and more preferably from about 0.2% to about 5% by weight of the composition of the polymeric thickening agent or mixtures thereof.

Preferred polymer thickening agents for use herein include, but are not limited to non-ionic thickening agents and anionic thickening agents or mixtures thereof. Suitable non-ionic thickening agents include, but are not limited to polyacrylamide polymers, crosslinked poly(N-vinylpyrrolidones), polysaccharides, natural or synthetic gums, polyvinylpyrrolidone and polyvinylalcohol. Suitable anionic

thickening agents include, but are not limited to acrylic acid/ethyl acrylate copolymers, carboxyvinyl polymers and crosslinked copolymers of alkyl vinyl ethers and maleic anhydride. Commercially available thickeners (*e.g.*, Carbopol; Noveon) find use in some embodiments of the present invention. Suitable Carbopol resins may be hydrophobically modified, and other suitable resins are described in WO98/22085, or mixtures thereof.

In some embodiments, the present compositions comprise at least one silicone oil phase. Silicone oil phase(s) generally comprises from about 0.1% to about 20%, preferably from about 0.5% to about 10%, and more preferably from about 0.5% to about 5%, of the composition. The silicone oil phase preferably comprises one or more silicone components.

In some embodiments, silicone components are fluids, including straight chain, branched and cyclic silicones. Suitable silicone fluids useful herein include silicones inclusive of polyalkyl siloxane fluids, polyaryl siloxane fluids, cyclic and linear polyalkylsiloxanes, polyalkoxylated silicones, amino and quaternary ammonium modified silicones, polyalkylaryl siloxanes or a polyether siloxane copolymer and mixtures thereof. Volatile, as well as non-volatile silicone fluids find use herein. Silicone fluids generally have an average molecular weight of less than about 200,000. In preferred embodiments, suitable silicone fluids have a molecular weight of about 100,000 or less, preferably about 50,000 or less, and more preferably about 10,000 or less. Preferably the silicone fluid is selected from silicone fluids having a weight average molecular weight in the range from about 100 to about 50,000 and preferably from about 200 to about 40,000. Typically, silicone fluids have a viscosity ranging from about 0.65 to about 600,000 mm²s⁻¹, preferably from about 0.65 to about 10,000 mm²s⁻¹ at 25°C. The viscosity can be measured by means of a glass capillary viscometer as set forth in Dow Corning Corporate Test Method CTM0004, July 29, 1970. Suitable polydimethyl siloxanes that can be used herein include commercially available compounds (*e.g.*, from the General Electric Company and Dow Corning). Also useful are essentially non-volatile polyalkylarylsiloxanes, for example, polymethylphenylsiloxanes, having viscosities of about 0.65 to 30,000 mm²s⁻¹ at 25°C (General Electric Company or from Dow Corning). Cyclic polydimethylsiloxanes suitable for use herein are those having a ring structure incorporating from about 3 to about 7 (CH₃)₂SiO moieties, preferably about 5 or more.

In additional embodiments, silicone gums find use herein. In some preferred embodiments, a silicone oil phase comprises a silicone gum or a mixture of silicones including the silicone gum. Typically, silicone gums have a viscosity at 25°C in excess of about 1,000,000 mm²s⁻¹. The silicone gums include dimethicones as known in the art (*See e.g.*, U.S. Pat. No. 4,152,416; and Noll, Chemistry and Technology of Silicones, Academic Press, New York [1968]). Silicone gums such as those described in General Electric Silicone Rubber Product Data Sheets SE 30, SE 33, SE 54 and SE 76, also find use in the present invention. Specific examples of silicone gums include polydimethylsiloxane, (polydimethylsiloxane) (methylvinylsiloxane) copolymer, poly(dimethylsiloxane)(diphenyl)(methylvinylsiloxane) copolymer and

mixtures thereof. Preferred silicone gums for use herein are silicone gums having a molecular weight of from about 200,000 to about 4,000,000 selected from dimethiconol, dimethicone copolyol, dimethicone and mixtures thereof.

In some embodiments, a silicone phase herein preferably comprises a silicone gum incorporated into the composition as part of a silicone gum-fluid blend. When the silicone gum is incorporated as part of a silicone gum-fluid blend, the silicone gum preferably constitutes from about 5% to about 40%, especially from about 10% to 20% by weight of the silicone gum-fluid blend. Suitable silicone gum-fluid blends herein are mixtures consisting essentially of:

(i) a silicone having a molecular weight of from about 200,000 to about 4,000,000 selected from dimethiconol, fluorosilicone and dimethicone and mixtures thereof; and

(ii) a carrier which is a silicone fluid, the carrier having a viscosity from about $0.65 \text{ mm}^2\text{s}^{-1}$ to about $100 \text{ mm}^2\text{s}^{-1}$,

wherein the ratio of i) to ii) is from about 10:90 to about 20:80 and wherein said silicone gum-based component has a final viscosity of from about $100 \text{ mm}^2\text{s}^{-1}$ to about $100,000 \text{ mm}^2\text{s}^{-1}$, preferably from $500 \text{ mm}^2\text{s}^{-1}$ to about $10,000 \text{ mm}^2\text{s}^{-1}$.

Further silicone components suitable for use in a silicone oil phase herein include crosslinked polyorganosiloxane polymers, optionally dispersed in a fluid carrier. In general, when present the crosslinked polyorganosiloxane polymers, together with its carrier (if present) comprises from about 0.1% to about 20%, preferably from about 0.5% to about 10%, and more preferably from about 0.5% to about 5% of the composition. Such polymers comprise polyorganosiloxane polymers crosslinked by a crosslinking agent (*See e.g.*, WO98/22085). Examples of suitable polyorganosiloxane polymers for use herein include, but are not limited to methyl vinyl dimethicone, methyl vinyl diphenyl dimethicone and methyl vinyl phenyl methyl diphenyl dimethicone.

Another class of silicone components suitable for use in a silicone oil phase herein includes polydiorganosiloxane-polyoxyalkylene copolymers containing at least one polydiorganosiloxane segment and at least one polyoxyalkylene segment (*See e.g.*, WO98/22085). Suitable polydiorganosiloxane-polyalkylene copolymers are available commercially under the tradenames BELSIL® from Wacker-Chemie GmbH. A particularly preferred copolymer fluid blend for use herein includes Dow Corning DC3225C which has the CTFA designation Dimethicone/Dimethicone copolyol.

In further embodiments, compositions of the present invention comprise an organic sunscreen. In some embodiments, suitable sunscreens have UVA absorbing properties, while others have UVB absorbing properties, and still others comprise a mixture thereof. The exact amount of the sunscreen active varies, depending upon the desired Sun Protection Factor (*i.e.*, the "SPF") of the composition, as well as the desired level of UV protection. SPF is a commonly used measure of photoprotection of a sunscreen against erythema. The SPF is defined as a ratio of the ultraviolet energy required to produce minimal erythema on

protected skin to that required to produce the same minimal erythema on unprotected skin in the same individual. Amounts of the sunscreen used are preferably from about 2% to about 20%, and more preferably from about 4% to about 14%. Suitable sunscreens include, but are not limited to those approved for use in the United States, Japan, Europe and Australia. The compositions of the present invention preferably
5 comprise an SPF of about 2 to about 30, preferably about 4 to about 30, and more preferably about 4 to about 15.

In some embodiments, the compositions of the present invention comprise one or more UVA absorbing sunscreen actives that absorb UV radiation having a wavelength of from about 320nm to about 400nm. Suitable UVA absorbing sunscreen actives include, but are not limited to dibenzoylmethane (*See*
10 *e.g.*, Lowe and Shaath (eds.), Sunscreens: Development, Evaluation, and Regulatory Aspects, Marcel Dekker, Inc.) derivatives, anthranilate derivatives such as methylanthranilate and homomethyl, 1-N-acetylanthranilate, and mixtures thereof. The UVA absorbing sunscreen active is preferably present in an amount sufficient to provide broad spectrum UVA protection either independently, or in combination with, other UV protective actives which may be present in the composition.

15 Suitable UVA sunscreen actives include dibenzoylmethane sunscreen actives and their derivatives. They include, but are not limited to, those selected from 2-methyldibenzoylmethane, 4-methyldibenzoylmethane, 4-isopropyldibenzoylmethane, 4-tert-butyldibenzoylmethane, 2, 4-dimethyldibenzoylmethane, 2, 5-dimethyldibenzoylmethane, 4, 4'-diisopropylbenzoylmethane, 4-(1, 1-dimethylethyl)-4'-methoxydibenzoylmethane, 2-methyl-5-isopropyl-4'-methoxydibenzoylmethane, 2-
20 methyl-5-tert-butyl-4'-methoxydibenzoylmethane, 2, 4-dimethyl-4'-methoxydibenzoylmethane, 2, 6-dimethyl-4'-tert-butyl-4'-methoxydibenzoylmethane, and mixtures thereof. Preferred dibenzoyl sunscreen actives include those selected from 4-(1, 1-dimethylethyl)-4'-methoxydibenzoylmethane, 4-isopropyldibenzoylmethane, and mixtures thereof. A preferred sunscreen active is 4-(1, 1-dimethylethyl)-4'-methoxydibenzoylmethane.

25 The sunscreen active 4-(1, 1-dimethylethyl)-4'-methoxydibenzoylmethane, which is also known as butyl methoxydibenzoylmethane or "avobenzone," is commercially available under the names of Parsol® 1789 from Givaudan Roure (International) S. A., and Eusolex® 9020 from Merck & Co., Inc. The sunscreen 4-isopropyldibenzoylmethane, which is also known as isopropyldibenzoylmethane, is commercially available from Merck under the name of Eusolex® 8020.

30 In some embodiments, the compositions of the present invention further include one or more UVB sunscreen actives that absorb(s) UV radiation having a wavelength of about 290nm to about 320nm. The compositions comprise an amount of the UVB sunscreen active that is safe and effective in providing UVB protection either independently, or in combination with, other UV protective actives which may be present in the compositions. The compositions comprise from about 0.1% to about 20%, preferably from about

0.1% to about 12%, and more preferably from about 0.5% to about 8% by weight, of each UVB absorbing organic sunscreen, or as mandated by the relevant regulatory authority(s).

A variety of UVB sunscreen actives are suitable for use herein (See e.g., U.S. Pat. No. 5,087,372; U.S. Pat. No. 5,073,371; U.S. Pat. No. 5,073,372; U.S. Pat. No. 4,937,370; and U.S. Pat. No. 4,999,186).

5 Preferred UVB sunscreen actives are selected from 2-ethylhexyl-2-cyano-3, 2-ethylhexyl N,N-dimethyl-p-aminobenzoate, p-aminobenzoic acid, oxybenzone, homomenthyl salicylate, octyl salicylate, 4,4'-methoxy-t-butylidibenzoylmethane, 4-isopropyl dibenzoylmethane, 3-benzylidene camphor, 3-(4-methylbenzylidene) camphor, 3-diphenylacrylate, 2-phenyl-benzimidazole-5-sulphonic acid (PBSA), cinnamate esters and their derivatives such as 2-ethylhexyl-p-methoxycinnamate, salicylate esters and their derivatives such as
10 triethanolamine salicylate, ethylhexyl salicylate, octyldimethyl para-aminobenzoic acid, camphor derivatives and their derivatives, and mixtures thereof. Preferred organic sunscreen actives include 2-ethylhexyl-2-cyano-3, 3-diphenylacrylate, 2-phenyl-benzimidazole-5-sulphonic acid (PBSA), octyl-p-methoxycinnamate, and mixtures thereof. Salt and acid neutralized forms of the acidic sunscreens are also useful herein.

In some embodiments, at least one agent is added to any of the compositions useful in the present
15 invention to stabilize the UVA sunscreen to prevent it from photo-degrading on exposure to UV radiation and thereby maintaining its UVA protection efficacy. A wide range of compounds are reported to have these stabilizing properties and should be chosen to complement both the UVA sunscreen and the composition as a whole (See e.g., U.S. Pat. Nos 5,972,316; 5,968,485; 5,935,556; 5,827,508; and WO
20 00/06110). Preferred examples of stabilizing agents for use in the present invention include 2-ethylhexyl-2-cyano-3, 3-diphenylacrylate, ethyl-2-cyano-3, 3-diphenylacrylate, 2-ethylhexyl-3, 3-diphenylacrylate, ethyl-3, 3-bis(4-methoxyphenyl)acrylate, diethylhexyl 2,6 naphthalate and mixtures thereof (Symrise Chemical Company).

In some embodiments, at least one agent is added to any of the compositions useful in the present invention to improve the skin substantivity of those compositions, particularly to enhance their resistance to
25 being washed off by water or rubbed off. Examples include, but are not limited to, acrylates/C₁₂₋₂₂ alkylmethacrylate copolymer, acrylate/acrylate copolymer, dimethicone, dimethiconol, graft-copoly (dimethylsiloxane/i-butyl methacrylate), lauryl dimethicone, PVP/Hexadecane copolymer, PVP/Eicosene copolymer, tricontanyl PVP and trimethoxysiloxysilicate.

In addition to organic sunscreens, in some embodiments, the compositions of the present invention
30 additionally comprise inorganic physical sunblocks (See e.g., TFA International Cosmetic Ingredient Dictionary, 6th Edition, pp. 1026-28 and 1103 [1995]; Sayre *et al.*, J. Soc. Cosmet. Chem., 41:103-109 [1990]; and Lowe *et al.*, *supra*). Preferred inorganic physical sunblocks include zinc oxide and titanium dioxide and mixtures thereof.

When used in preferred embodiments, the physical sunblocks are present in an amount such that the
35 present compositions are transparent on the skin (*i.e.*, non-whitening), preferably from about 0.5% to about 20%, preferably from about 0.5% to about 10%, and more preferably from about 0.5% to 5% by weight.

When titanium dioxide is used, it can have an anatase, rutile or amorphous structure. Manufacturers of micronized grade titanium dioxide and zinc oxide for sunscreen use include, but are not limited to Tayca Corporation, Uniqema, Shinetsu Chemical Corporation, Kerr-McGee, Nanophase, Nanosource, Sachtleben, Elementis, and BASF Corporation, as well as their distribution agents and those companies that further process the material for sunscreen use. Physical sunblock particles (*e.g.*, titanium dioxide and zinc oxide) can be uncoated or coated with a variety of materials including but not limited to amino acids, aluminum compounds such as alumina, aluminum stearate, aluminum laurate, and the like; carboxylic acids and their salts (*e.g.*, stearic acid and its salts); phospholipids, such as lecithin; organic silicone compounds; inorganic silicone compounds such as silica and silicates and mixtures thereof. In some preferred embodiments, the compositions of the present invention comprise from about 0.1% to about 15%, preferably from about 0.1% to about 7%, and more preferably from about 0.5% to about 5%, by weight, of inorganic sunscreen.

In some preferred embodiments, the composition of the present invention also includes preservatives. Such preservatives include, but are not limited to pentylene glycol, ethylene diamine tetra acetate (EDTA) and their salts, chlorhexidine (and its diacetate, dihydrochloride, digluconate derivatives), 1,1,1-trichloro-2-methyl-2-propanol, parachloro metaxyleneol, polyhexamethylenebiguanide hydrochloride, dehydroacetic acid, diazolidinyl urea, 2,4-dichlorobenzyl alcohol, 4,4-dimethyl-1,3-oxazolidine, formaldehyde (*e.g.*, 37% aqueous solution, with 10-15% methanol to avoid polymerization), glutaraldehyde, dimethylidantoin, imidazolidinyl urea, 5-Chloro-2-methyl-4-isothiazolin-3-one, ortho-phenylphenol, 4-hydroxybenzoic acid esters (*e.g.*, "paraben") and its methyl-, ethyl-, propyl-, isopropyl-, butyl-, and isobutyl-esters, trichlosan, 2-phenoxyethanol, phenyl mercuric acetate, borate, nitrate, quaternium-15, salicylate, salicylic acid and its salts, calcium, calcium sorbate, sorbic acid and its salts, iodopropanyl butylcarbamate zinc pyrithione, benzyl alcohol, 5-bromo-5nitro-1,3-dioxane, 2-bromo-2-nitropropane-1,3-diol, benzoic acid and its salts, sulfites, bisulfites, phenoxyethanol, chloroxylenol, diazolidinyl urea, methylparabens, propylparabens, isopropylparabens, isobutylparabens, butylparabens, ethylparaben, phenoxyethanol PG, and benzalkonium chloride.

A variety of optional ingredients such as neutralizing agents, perfumes and perfume solubilizing agents, and coloring agents, also find use in some of the compositions herein. It is preferred that any additional ingredients enhance the skin softness/smoothness benefits of the product. In addition it is preferred that any such ingredients do not negatively impact the aesthetic properties of the product.

Other optional materials include keratolytic agents, as well as water-soluble and/or solubilizable preservatives preferably at a level of from about 0.1% to about 5% (*e.g.*, Germall 115, methyl, ethyl, propyl and butyl esters of hydroxybenzoic acid, benzyl alcohol, DMDM hydantoin iodopropanyl butylcarbanate available under the trade name Glydant Plus from Lonza; EDTA, EUXYL® K400, Bromopol (2-bromo-2-nitropropane-1,3-diol) and phenoxypropanol); anti-bacterials (*e.g.*, IRGASAN®) and phenoxyethanol (preferably at levels of from about 0.1% to about 5%); as well as soluble or colloiddally-soluble moisturizing agents such as hyaluronic acid, chondroitin sulfate, and starch-grafted sodium polyacrylates (*e.g.*,

SANWET® IM-1000, IM-1500 and IM-2500, available from Celanese Superabsorbent Materials, Portsmouth, VA, *See e.g.*, U.S. Pat. No. 4,076,663; vitamins such as vitamin A, vitamin C, vitamin E and derivatives thereof and building blocks thereof such as phytantriol, and vitamin K and components thereof such as the fatty alcohol dodecatrienol; alpha and beta hydroxyacids; aloe vera; sphingosines and phytosphingosines, cholesterol; skin whitening agents; N-acetyl cysteine; colouring agents; antibacterial agents such as TCC/TCS, also known as triclosan and trichlorocarbon; perfumes and perfume solubilizers. Examples of alpha hydroxy acids include glycolic acid, lactic acid, malic acid, citric acid, glycolic acid in conjunction with ammonium glycolate, alpha-hydroxy ethanoic acid, alpha-hydroxyoctanoic acid, alpha-hydroxycaprylic acid, hydroxycaprylic acid, mixed fruit acid, tri-alpha hydroxy fruit acids, triple fruit acid, sugar cane extract, alpha hydroxy and botanicals, l-alpha hydroxy acid and glycomer in crosslinked fatty acids (*e.g.*, alpha nutrium). Preferred examples of alpha hydroxy acids are glycolic acid and lactic acid. It is preferred that alpha hydroxy acids are used in levels of up to about 10%. It is not intended that the present invention be limited to any particular compound derived from any particular source, as any suitable additive compound, whether obtained from natural sources or through synthesis in the laboratory find use in the present invention.

Other optional materials include water-soluble or solubilizable preservatives preferably at a level of from about 0.1% to about 5% each, such as Germall 115, methyl, ethyl, propyl and butyl esters of hydroxybenzoic acid, benzyl alcohol, DMDM hydantoin iodopropanyl butylcarbanate available under the trade name Glydant Plus from Lonza, EDTA, Euxyl (RTM) K400, Bromopol (2-bromo-2-nitropropane-1,3-diol), pentylene glycol and phenoxypropanol; anti-bacterials such as Irgasan (RTM) and phenoxyethanol (preferably at levels of from 0.1% to about 5%). Antibacterial agents such as TCC/TCS, also known as triclosan and trichlorocarbon are also useful in compositions of the present invention.

Neutralizing agents suitable for use in neutralizing acidic group containing hydrophilic gelling agents herein include sodium hydroxide, potassium hydroxide, ammonium hydroxide, monoethanolamine, diethanolamine, amino methyl propanol, tris-buffer and triethanolamine.

Other optional materials that find use in the present invention include any of the numerous functional and/or active ingredients known to those skilled in the art (*See e.g.*, McCutcheon's Functional Materials, North American and International Editions, MC Publishing Co. [2003]) As indicated above, non-limiting examples include keratolytic agents; soluble or colloiddally-soluble moisturizing agents such as hyaluronic acid and chondroitin sulfate; vitamins such as vitamin A, vitamin C, vitamin E, vitamin K and derivatives thereof and building blocks thereof; phytantriol; fatty alcohols such as dodecatrienol; alpha and beta hydroxyacids; aloe vera; sphingosines and phytosphingosines, cholesterol; skin whitening agents; N-acetyl cysteine; coloring agents; Examples of alpha hydroxy acids include glycolic acid, lactic acid, malic acid, and citric acid (whether derived synthetically or from natural sources and whether used alone or in combination) and their esters or relevant buffered combinations. Other examples of alpha-hydroxy acids include: alpha-hydroxy ethanoic acid, alpha-hydroxyoctanoic acid, alpha-hydroxycaprylic acid, and

hydroxycaprylic acid. Preferred examples of alpha hydroxy acids include glycolic acid and lactic acid. It is preferred that alpha hydroxy acids are used in levels of up to about 10%.

Optional materials include pigments that, where water-insoluble, contribute to and are included in the total level of oil phase ingredients. Pigments suitable for use in the compositions of the present invention can be organic and/or inorganic. Also included within the term "pigment" are materials having a low color or luster, such as matte finishing agents, light scattering agents, and formulation aids such as micas, seracites, and carbonate salts. Further examples of suitable pigments include titanium dioxide, iron oxides, glutamate iron oxides, zinc oxide, bismuth oxychloride, ultramarine blue (all of which may be either pre-dispersed and/or pre-coated or not) D&C dyes and lakes, FD&C colors, natural color additives such as carmine, and mixtures thereof. Depending upon the type of composition, a mixture of pigments is usually used in preferred embodiments of the present invention. Preferred pigments for use herein from the viewpoint of moisturization, skin feel, skin appearance and emulsion compatibility are treated pigments. In some embodiments, the pigments are treated with compounds, including but not limited to amino acids, silicones, lecithin and ester oils.

In preferred embodiments, the pH of the compositions herein is in the range from about 3.5 to about 10, preferably from about 4 to about 8, and more preferably from about 5 to about 7, wherein the pH of the final composition is adjusted by addition of acidic, basic or buffer salts as necessary, depending upon the composition of the forms and the pH-requirements of the compounds.

The compositions of the present invention are prepared by standard techniques well known to those skilled in the art. In general the aqueous phase and/or the oil phase are prepared separately, with materials of similar phase partitioning being added in any order. If the final product is an emulsion, the two phases are then combined with vigorous stirring and/or homogenization as necessary, to reduce the size of the internal phase droplets. Any ingredients in the formulation with high volatility, or which are susceptible to hydrolysis or decomposition at high temperatures, are added with gentle stirring towards the end of the process, post emulsification if applicable. Dosage frequency and amount will depend upon the desired performance criteria.

In some embodiments of the present invention, method of decreasing VEGF activity are provided. In these embodiments, the methods comprise applying to an organism in need thereof an effective amount of any one of the compounds set forth herein. In additional preferred embodiments, the present invention provides compounds for treatment of an organism in need thereof, including humans and other animals.

EXPERIMENTAL

The following Examples serve to illustrate certain preferred embodiments and aspects of the present invention and are not to be construed as limiting the scope thereof.

In the experimental disclosure which follows, the following abbreviations apply: PI (proteinase inhibitor), BBI (Bowman-Birk inhibitor), STI (Soybean Trypsin inhibitor); ppm (parts per million); VEGF

and VegF (vascular endothelial growth factor); M (molar); mM (millimolar); μ M (micromolar); nM (nanomolar); mol (moles); mmol (millimoles); μ mol (micromoles); nmol (nanomoles); gm (grams); mg (milligrams); μ g (micrograms); pg (picograms); L (liters); ml and mL (milliliters); μ l and μ L (microliters); cm (centimeters); mm (millimeters); μ m (micrometers); nm (nanometers); U (units); V (volts); MW (molecular weight); sec (seconds); min(s) (minute/minutes); hr(s) (hour/hours); °C (degrees Centigrade); QS (quantity sufficient); ND (not done); SA (see able); NA (not applicable); rpm (revolutions per minute); H₂O (water); dH₂O (deionized water); HCl (hydrochloric acid); aa (amino acid); bp (base pair); kb (kilobase pair); kD (kilodaltons); cDNA (copy or complimentary DNA); DNA (deoxyribonucleic acid); ssDNA (single stranded DNA); dsDNA (double stranded DNA); dNTP (deoxyribonucleotide triphosphate); RNA (ribonucleic acid); MgCl₂ (magnesium chloride); NaCl (sodium chloride); w/v (weight to volume); v/v (volume to volume); g (gravity); OD (optical density); A₄₀₅ (absorbance at 405 nm); V_{max} (the maximum initial velocity of an enzyme catalyzed reaction); FGFR1(IIIc) (FGF-5 receptor); Dulbecco's phosphate buffered solution (DPBS); SOC (2% Bacto-Tryptone, 0.5% Bacto Yeast Extract, 10 mM NaCl, 2.5 mM KCl); Terrific Broth (TB; 12 g/l Bacto Tryptone, 24 g/l glycerol, 2.31 g/l KH₂PO₄, and 12.54 g/l K₂HPO₄); OD₂₈₀ (optical density at 280 nm); OD₆₀₀ (optical density at 600 nm); PAGE (polyacrylamide gel electrophoresis); PBS (phosphate buffered saline [150 mM NaCl, 10 mM sodium phosphate buffer, pH 7.2]); PBST (PBS+0.25% Tween® 20); PEG (polyethylene glycol); PCR (polymerase chain reaction); RT-PCR (reverse transcription PCR); SDS (sodium dodecyl sulfate); β ME, BME and β ME (beta-mercaptoethanol or 2-mercaptoethanol); Tris-HCl (tris[Hydroxymethyl]aminomethane-hydrochloride); Tricine (N-[tris-(hydroxymethyl)-methyl]-glycine); CHES (2-(N-cyclo-hexylamino) ethane-sulfonic acid); TAPS (3-[tris-(hydroxymethyl)-methyl]-amino}-propanesulfonic acid); CAPS (3-(cyclo-hexylamino)-propane-sulfonic acid); DMSO (dimethyl sulfoxide); DTT (1,4-dithio-DL-threitol); Glut and GSH (reduced glutathione); GSSG (oxidized glutathione); TCEP (Tris[2-carboxyethyl] phosphine); Tris (tris(hydroxymethyl)aminomethane); HEPES (N-[2-Hydroxyethyl]piperazine-N-[2-ethanesulfonic acid]); HBS (HEPES buffered saline); SDS (sodium dodecylsulfate); Tris-HCl (tris[Hydroxymethyl]aminomethane-hydrochloride); Ci (Curies) mCi (milliCuries); μ Ci (microCuries); TLC (thin layer chromatography); O/W (oil in water emulsion); W/O (water in oil emulsion); W/S (water in silicon emulsion); pickering emulsion (emulsion stabilized with a solid compound); hydrodispersion (emulsifier-free formulations); PIT (phase inversion temperature technology used to manufacture special emulsions); sticks (any product that is provided in a stick format, including but not limited to lipsticks, anti-perspirants, deodorants); Ts (tosyl); Bn (benzyl); Ph (phenyl); Ms (mesyl); Et (ethyl), Me (methyl); Klenow (DNA polymerase I large (Klenow) fragment); rpm (revolutions per minute); EGTA (ethylene glycol-bis(β -aminoethyl ether) N, N, N', N'-tetraacetic acid); EDTA (ethylenediaminetetracetic acid); bla (β -lactamase or ampicillin-resistance gene); PDS (plasma-derived bovine serum that has been dialyzed to remove growth factors; dialysis of defibrinated bovine plasma is performed against DMEM for about 6 hours at 4°C, with stirring, the media is changed and

dialysis is continued overnight; the dialyzed PDS is collected after 24 hours, and sterile filtered twice through a 0.2 μ m filter); FCS and FBS (fetal calf serum); GE Healthcare (GE Healthcare, Chalfont St. Giles, United Kingdom); DNA2.0 (DNA2.0, Menlo Park, CA); OXOID (Oxoid, Basingstoke, Hampshire, UK); Megazyme (Megazyme International Ireland Ltd., Bray Business Park, Bray, Co., Wicklow, Ireland);
5 Corning (Corning Life Sciences, Corning, NY); (NEN (NEN Life Science Products, Boston, MA); Pharma AS (Pharma AS, Oslo, Norway); Dynal (Dynal, Oslo, Norway); Bio-Synthesis (Bio-Synthesis, Lewisville, TX); ATCC (American Type Culture Collection, Rockville, MD); Gibco/BRL (Gibco/BRL, Grand Island, NY); Sigma (Sigma Chemical Co., St. Louis, MO); Pharmacia (Pharmacia Biotech, Piscataway, NJ); NCBI (National Center for Biotechnology Information); Applied Biosystems (Applied Biosystems, Foster City,
10 CA); Clontech (CLONTECH Laboratories, Palo Alto, CA); Difco (Difco Laboratories, Detroit, MI); Oxoid (Oxoid Inc., Ogdensburg, NY); GIBCO BRL or Gibco BRL (Life Technologies, Inc., Gaithersburg, MD); Millipore (Millipore, Billerica, MA); Bio-Rad (Bio-Rad, Hercules, CA); Invitrogen (Invitrogen Corp., San Diego, CA); NEB (New England Biolabs, Beverly, MA); Cambrex (Cambrex Bioproducts, East Rutherford, NJ); Sigma (Sigma Chemical Co., St. Louis, MO); Pierce (Pierce Biotechnology, Rockford, IL); Takara
15 (Takara Bio Inc. Otsu, Japan); Roche (Hoffmann-La Roche, Basel, Switzerland); EM Science (EM Science, Gibbstown, NJ); Qiagen (Qiagen, Inc., Valencia, CA); Biodesign (Biodesign Intl., Saco, Maine); Biosource (Biosource, Intl., Camarillo, CA); Aptagen (Aptagen, Inc., Herndon, VA); Molecular Devices (Molecular Devices, Corp., Sunnyvale, CA); R&D Systems (R&D Systems, Minneapolis, MN); Stratagene (Stratagene Cloning Systems, La Jolla, CA); Marsh (Marsh Biosciences, Rochester, NY); Bio-Tek (Bio-Tek
20 Instruments, Winooski, VT); (Biacore (Biacore, Inc., Piscataway, NJ); PeproTech (PeproTech, Rocky Hill, NJ); SynPep (SynPep, Dublin, CA); Chemicon (CHEMICON, Temecula, CA); Clinical Research Laboratories, (Clinical Research Laboratories, Inc., Piscataway, NJ); and Microsoft (Microsoft, Inc., Redmond, WA).

EXAMPLE 1

Personal Care Compositions

In this Example, various personal care compositions comprising any of the compounds of the present invention are provided as follows. In these formulations, the amounts are given as percentages of the total composition, unless otherwise indicated. Also, unless otherwise indicated in the following
30 formulations, the concentration of BBI-AV (referred to as "Compound" below) ranges from about 0.01% to about 1.0%. In some formulations, the preferred concentration is in the range of about 0.1% to about 0.2%, while in other formulations, the preferred concentration is in the range of about 0.05% to about 0.1% (*e.g.*, for some hair growth inhibition embodiments); from about 0.02 to 0.1% (*e.g.*, for some skin lightening
35 concentrations greater than about 0.1% (*e.g.*, for some rosacea treating embodiments). Those of skill in the art know how to determine the suitable (*i.e.*, optimum) concentration of BBI-AV for each product. In some

116

MOISTURIZING BODYWASH (pH 7)

RAW MATERIAL (INCI Designation)	Amount
Deionized Water	QS
Glycerin	4.0
PEG-6 Caprylic/Capric Glycerides	4.0
Palm Kernel Fatty acids	3.0
Sodium Laureth-3 Sulphate	45.0
Cocamide MEA	3.0
Sodium Lauroamphoacetate	25.0
Soybean Oil	10.0
Polyquaternium-10	0.70
Preservative, fragrance, color	QS
Compound	1000ppm

BODY WASH

RAW MATERIAL (INCI Designation)	pH 8	pH 6.5	pH 7
	Amount	Amount	Amount
Deionized water	QS	QS	QS
Sodium Laureth Sulphate	12	15	8
Cocamidopropyl Betaine	8	10	15
Decyl Glucoside	0	2	1
Polyquaternium-10	0.25	0	0
Polyquaternium-7	0	0	0.7
Preservative, fragrance, color	QS	QS	QS
Compound	250ppm	500ppm	1000ppm

BODY LOTION	pH 7	pH 7	pH 7.5	pH 7
RAW MATERIAL (INCI Designation)	Amount	Amount	Amount	Amount
Deionized Water	QS	QS	QS	QS
Glycerine	8	8	0	12
Isohexadecane	3	3	3	6
Niacinamide	0	3	5	6
Isopropyl Isostearate	3	3	3	3
Polyacrylamide (and) Isoparaffin (and) Laureth-7	3	3	3	3
Petrolatum	4	4	4	2
Nylon 12	2	2	2.5	2.5
Dimethicone	2	2	2.5	2.5
Sucrose Polycottonseed Oil	1.5	1.5	1.5	1.5
Stearyl Alcohol 97%	1	1	1	1
D Panthenol	1	1	1	1
DL-alphaTocopherol Acetate	1	1	1	1
Cetyl Alcohol 95%	0.5	0.5	0.5	1
Behenyl Alcohol	1	1	1	0.5
Cetearyl Alcohol (and) Cetearyl Glucoside	0.4	0.4	0.5	0.5
Stearic Acid	0.15	0.15	0.15	0.15
PEG-100-Stearate	0.15	0.15	0.15	0.15
Preservative, fragrance, color Compounds	QS 250 ppm	QS 500 ppm	QS 750 ppm	QS 1000ppm

**ULTRA-HIGH MOISTURIZING
EMULSION****pH 7****pH 7**

RAW MATERIAL (INCI Designation)	Amount	Amount
Deionized water	QS	QS
Glycerin	12	5
PEG 400	0	10
Niacinamide	5	7
Isohexadecane	5	5
Dimethicone	3	2
Polyacrylamide (and) Isoparaffin (and) Laureth-7	3	3
Isopropyl Isostearate	2	2
Polymethylsilsesquioxane	2	2
Cetyl Alcohol 95%	1	1
Sucrose polycottonseed oil	1	1
D-Panthenol	1	1
Tocopherol Acetate	1	1
Stearyl Alcohol 95%	0.5	0.5
Cetearyl Glucoside	0.5	0.5
Titanium dioxide	0.3	0.3
Stearic Acid	0.15	0.15
PEG-100-Stearate	0.15	0.15
Preservative, fragrance, color Compound	QS 250 ppm	QS 100 ppm

MOISTURIZING CREAM

	pH 7	pH 7	pH 7.5
RAW MATERIAL (INCI Designation)	Amount	Amount	Amount
Deionized water	QS	QS	QS
Glycerine	3	5	10
Petrolatum	3	3	0
Cetyl Alcohol 95%	1.5	1.5	1
Dimethicone Copolyol	2	2	2
Isopropyl Palmitate	1	1	0.5
Carbopol 954 (Noveon)	0.7	0.7	0.7
Dimethicone (350cs)	1	1	1
Stearyl Alcohol 97%	0.5	0.5	1
Stearic acid	0.1	0.1	0.1
Peg-100-stearate	0.1	0.1	0.1
Titanium Dioxide	0.3	0.3	0.3
Preservative, color, fragrance	QS	QS	QS
Compound	50ppm	250ppm	1000ppm

FACIAL CLEANSING EMULSION

RAW MATERIAL (INCI Designation)	Amount
Water	69.05
Disodium EDTA	0.1
Glyceryl polymethacrylate (and) Propylene glycol	1.0
Glycerin	2.0
Xanthan gum	0.5
Hydroxyethyl cellulose	0.5
Tridecyl neopentanoate	4.0
Isocetyl stearate	6.0
Octyl palmitate	8.0
Glyceryl dilaurate	4.0
PEG-20 stearate	2.0
Glyceryl stearate (and) Laureth-23	2.0
Lauryl pyrrolidone	0.5
Chamomile extract	0.2
Aloe vera (200x)	0.05
Fragrance, preservative	QS
Compound	SA

SURFACTANT-BASED FACIAL CLEANSER	
RAW MATERIAL (INCI Designation)	Amount
Water	62.55
Acrylates/Steareth-20 methacrylate copolymer	3.3
Disodium EDTA	0.05
Glycerin	2.0
Glyceryl polymethacrylate (and) Propylene glycol (and) PVM/MA copolymer	0.5
Sodium laureth sulfate (30%)	17.5
Cetearyl alcohol	1.0
Shea butter	1.0
Disodium oleamido PEG-2 sulfosuccinate	5.0
Cocoamidopropyl Betaine	3.0
Sodium lauroyl sarcosinate	1.0
PEG-7 glyceryl cocoate	1.0
Isodecyl oleate	1.5
Peppermint extract	0.25
Eucalyptus extract	0.25
Fragrance, preservative, color, pH adjust	QS
Compound	SA

FACIAL EXFOLIATING GEL	
RAW MATERIAL (INCI Designation)	Amount
Water	64.39
Disodium EDTA	0.05
Aloe vera (200x)	0.01
Benzophenone-4	0.25
Propylene glycol	1.0
Acrylates/C10-30 alkyl acrylate crosspolymer (2%)	20.0
Glyceryl polymethacrylate (and) Propylene glycol	10.0
Glyceryl polymethacrylate (and) Propylene glycol (and) PVM/MA copolymer	1.0
Hydrogenated jojoba oil	1.5
Fragrance, preservative, color, pH adjust	QS
Compound	SA

FACIAL TONER	
RAW MATERIAL (INCI Designation)	Amount
Water	93.99
Disodium EDTA	0.1
Butylene glycol	2.0
Aloe vera (200x)	0.1
Allantoin	0.1
Benzophenone-4	0.5
Witch hazel extract	0.3
Propylene glycol (and) Euphrasia extract (and) Golden seal root extract (and) Green tea extract	0.01
PEG-40 hydrogenated castor oil	0.5
Quaternium-22	0.5
Sandlewood oil	0.02
Fragrance, preservative, color, pH adjust	QS
Compound	SA

EXFOLIATING CREAM	
RAW MATERIAL (INCI Designation)	Amount
Water	68.80
Disodium EDTA	0.1
PVM/MA decadiene crosspolymer	1.0
Butylene glycol	3.0
PEG-20 stearate	1.0
Glyceryl stearate (and) Laureth-23	2.0
Diisopropyl adipate	2.0
Isodecyl oleate	2.0
Isocetyl stearyl stearate	5.0
Myristyl myristate	1.0
Glyceryl dilaurate	2.0
Sodium hydroxide, 10%	2.6
Glyceryl polymethacrylate (and) Propylene glycol	5.0
Glyceryl polymethacrylate (and) Propylene glycol (and) PVM/MA copolymer	0.5
Hydrogenated jojoba oil	3.0
Fragrance, preservative, color, pH adjust	QS
Compound	SA

FACIAL MASK	
RAW MATERIAL (INCI Designation)	Amount
Water	76.4
Disodium EDTA	0.1
Bentonite	12.5
Potassium C12-13 Alkyl Phosphate	5.0
Propylene glycol	4.0
Sodium Coco PG-Dimonium Chloride Phosphate	1.0
Fragrance, preservative, color, pH adjust	QS
Compound	SA

AFTER-SHAVE BALM	
RAW MATERIAL (INCI Designation)	Amount
Water	82.12
Disodium EDTA	0.1
Acrylate copolymer	2.0
Acrylate/Stareth-20 methacrylate copolymer	1.0
Propylene glycol	3.0
Sodium hydroxide (10%)	1.28
Glyceryl stearate (and) Cetyl alcohol (and) Stearyl alcohol (and) Behenyl alcohol (and) Palmitic acid (and) Stearic acid (and) Hydroxyethyl cetearamidopropyldimonium chloride	3.5
Isocetyl stearate	1.0
C12-15 alkyl lactate	1.5
Octyldodecyl stearate	3.0
Glyceryl polymethacrylate (and) Propylene glycol (and) PVM/MA copolymer	1.0
Polyquaternium-11	0.5
Fragrance, preservative, color, pH adjust	QS
Compound	SA

5

EYE GEL	
RAW MATERIAL (INCI Designation)	Amount
Water	89.14
VP/Acrylates/Lauryl methacrylate copolymer	0.5
Glycerin	5.0
Aminomethyl propanol	0.3
Aloe vera (200x)	0.05
Benzophenone-4	0.1
Glyceryl polymethacrylate (and) Propylene glycol (and) PVM/MA copolymer	0.2

Butylene glycol (and) Water (and) Witch hazel extract	0.5
Butylene glycol (and) Water (and) Cucumber extract	0.3
PEG-40 hydrogenated castor oil	0.01
Acrylates/Beheneth-25 methacrylate copolymer	2.4
Fragrance, preservative, color, pH adjust	QS
Compound	SA

HIGH MELTING POINT LIPSTICK	
RAW MATERIAL (INCI Designation)	Amount
Ozokerite wax	5.0
Candelilla wax	11.0
Octyl dodecanol	26.0
C30-45 alkyl methicone	5.0
Cyclomethicone	4.8
Petrolatum	3.0
Lanolin oil	9.0
Avocado oil	2.0
Oleyl alcohol	8.0
Pigment/cyclomethicone	25.0
Fragrance, preservative	QS
Compound	SA

5

LIPSTICK	
RAW MATERIAL (INCI Designation)	Amount
Candelilla wax	9.1
Isopropyl myristate	9.6
Lanolin	5.0
Beeswax	4.0
Paraffin (130/135)	2.0
Ozokerite wax	2.5
Castor oil	53.7
Carnauba wax	1.5
Pigments	7.5
Mineral oil	4.0
Fragrance, preservative	QS
Compound	SA

LIP GLOSS	
RAW MATERIAL (INCI Designation)	Amount
Bis-diglyceryl polyacyladipate-1	43.5
Bis-diglyceryl polyacyladipate-2	10
Glycerol ricinoleate	10
Polyisobutene 1000	13
Lanolin wax	10
Candelilla wax	2.5
Mica (and) titanium dioxide	3
d-Panthenol	5
Fragrance, preservative, color	QS
Compound	SA

LIP GLOSS WITH SUNSCREEN	
RAW MATERIAL (INCI Designation)	Amount
Triisostearyl Citrate	58.4
Candelilla wax	8.0
Myristyl lactate	7.5
Microcrystalline wax	5.0
Carnauba wax	2.0
Diisopropyl dimmer dilinoleate	10.0
Mica (and) Bismuth oxychloride (and) Carmine	6.0
Zinc oxide (microfine)	2.0
Fragrance, preservative	QS
Compound	SA

5

LIP BALM	
RAW MATERIAL (INCI Designation)	Amount
Petrolatum	47.3
Isopropyl lanolate	6.0
Ozokerite wax	16.5
Candelilla wax	4.5
Diisopropyl dilinoleate	25.0
Retinyl palmitate	0.5
Tocopherol acetate	0.2
Fragrance, preservative	QS
Compound	SA

WATERPROOF MASCARA	
RAW MATERIAL (INCI Designation)	Amount
Water	49.45
Propylene glycol	3.0
Triethanolamine (99%)	3.1
Acrylates/Octylacrylamine Copolymer	5.0
Diisostearyl trimethylolpropane siloxy silicate	5.0
Candelilla wax	4.5
Beeswax	5.5
Ozokerite wax	2.0
Carnauba wax	1.0
Cetyl alcohol	3.0
Stearic acid	5.0
Iron oxides	11.0
Fragrance, preservative	QS
Compound	SA

ANHYDROUS WATERPROOF MASCARA	
RAW MATERIAL (INCI Designation)	Amount
C9-11 Isoparaffin	30.95
Polyethylene	11.0
Candelilla wax	4.5
Hydroxylated lanolin	0.25
Pentaerythrityl rosinat	2.0
Zinc stearate	1.0
Silica silylate	1.0
Petroleum distillates (and) Quaternium-18 hectorite (and)	35.0
Propylene Carbonate	
Iron oxides	12.0
Fragrance, preservative	QS
Compound	SA

WATER-BASED MASCARA	
RAW MATERIAL (INCI Designation)	Amount
Water	43.32
Polyvinyl pyrrolidone (K30)	2.0
Hydroxyethyl cellulose	1.0
Triethanolamine (99%)	2.0
Disodium EDTA	0.1
Iron Oxides	10.0
Stearic acid	4.5
Glyceryl monostearate	2.0
Beeswax	7.0
Carnauba wax	4.5
Hydroxylated lanolin	1.0
Acrylates copolymer	20.0
Fragrance, preservative	QS
Compound	SA

LIQUID EYELINER	
RAW MATERIAL (INCI Designation)	Amount
Water	50 – 70
Gellant	0.5 – 1.5
Wetting agent(s)	1 – 3
Polyol	4 – 8
Colorants	10 – 20
Alcohol	5 – 10
Film former	3 – 8
Fragrance, preservative	QS
Compound	SA

5

NAIL ENAMEL	
RAW MATERIAL (INCI Designation)	Amount
Solvent(s)	40 – 70
Resin(s)	10 – 20
Plasticizer	3 – 12
Gellant	0 – 2
Colorants	0 – 3
Fragrance, preservative	QS
Compound	SA

CUTICLE TREATMENT	
RAW MATERIAL (INCI Designation)	Amount
<u>Petrolatum</u>	<u>34.8</u>
<u>Beeswax</u>	<u>7.2</u>
<u>Ozokerite wax</u>	<u>4.3</u>
<u>Candelilla wax</u>	<u>4.0</u>
<u>Cocoa butter</u>	<u>1.0</u>
<u>Shea butter</u>	<u>1.0</u>
<u>Glyceryl dilaurate</u>	<u>8.0</u>
<u>Ethylhexyl palmitate</u>	<u>20.0</u>
<u>C12-15 alkyl lactate</u>	<u>6.0</u>
<u>PVP/Eicosene copolymer</u>	<u>3.5</u>
<u>Diisopropyl adipate</u>	<u>2.0</u>
<u>Octinoxate</u>	<u>7.5</u>
<u>Retinyl palmitate</u>	<u>0.1</u>
<u>Tocopherol acetate</u>	<u>0.1</u>
Fragrance, preservative, color, pH adjust	QS
Compound	SA

PRESSED POWDER FORMULATIONS					
	Loose Powder	Pressed Powder	Foundation	Blush	Eye Shadow
Fillers (e.g., talc, mica, seracite)	70-95	40-90	40-80	40-80	40-80
Compression aids (e.g., metallic soaps, waxes)	0-2.5	3-5	2-5	2-7	2-10
Texture enhancers	10-40	5-40	10-40	10-40	0-30
Colorants (e.g., iron oxides, organic colors)	2-10	2-10	5-20	2-10	1-40
Pearls (e.g. titanated mica, bismuth oxychloride)	0-20	0-10	0-5	0-20	0-60
Wet binder (e.g., Octyldodecyl stearoyl stearate, di-PPG3 myristyl ether adipate, isocetyl stearate, cetyl dimethicone)	0-3	2-5	2-5	3-10	3-15
Dry binder (e.g., calcium silicate, kaolin)	0-2	2-5	2-5	3-8	3-8
Fragrance, preservative	QS	QS	QS	QS	QS
Compound	SA	SA	SA	SA	SA

WATER-IN-OIL FOUNDATION	
RAW MATERIAL (INCI Designation)	Amount
Cyclomethicone	12.0
Dimethicone	5.0
Cyclomethicone (and) Dimethicone copolyol	20.0
Laureth-7	0.5
Colorants (hydrophobically treated)	2.2
Titanium dioxide (and) methicone	8.5
Talc (and) methicone	3.3
Water	37.2
Sodium chloride	2.0
Propylene glycol	8.0
Fragrance, preservative	QS
Compound	SA

ANHYDROUS MAKEUP STICK	
RAW MATERIAL (INCI Designation)	Amount
Ozokerite wax	5.6
Polyethylene	5.3
Glyceryl dilaurate	5.5
Isostearyl neopentanoate	13.0
Octyldodecyl stearoyl stearate	12.0
Myristyl myristate	11.0
Ethylhexyl methoxycinnamate	7.5
PVP/Eicosene copolymer	0.5
Tocopherol acetate	0.1
Dimethicone (and) Trimethylsiloxysilicate	8.0
Cyclopentasiloxane	9.0
Mica	10.0
Talc	1.7
Titanium dioxide (and) Isopropyl titanium triisostearate	8.86
Iron oxides (and) Isopropyl titanium triisostearate	1.94
Fragrance, preservative	QS
Compound	SA

WATER-IN-SILICONE FOUNDATION	
RAW MATERIAL (INCI Designation)	Amount
Cetyl dimethicone copolyol	0.45
Polyglycerol-4 isostearate (and) Cetyl dimethicone copolyol (and) Hexyl laurate	1.75
Polyalkylene polysiloxane copolymer	0.9
Cetyl dimethicone	0.9
Beeswax	0.7
Castor wax (and) hydrogenated castor oil	0.35
Octyl palmitate	7.0
Cyclomethicone	7.95
Phenyl trimethicone	2.2
Titanium dioxide (and) Caprylyl silane	7.5
Iron oxides (and) Caprylyl silane	1.1
Talc (and) Caprylyl silane	3.8
Cyclomethicone	7.95
Dimethicone	1.3
Water	49.55
Sodium chloride	0.5
Propylene glycol	5.3
Fragrance, preservative	QS
Compound	SA

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10

15

20

OIL-IN-WATER FOUNDATION	
RAW MATERIAL (INCI Designation)	Amount
Water	59.85
Polyvinylpyrrolidone	5.0
Magnesium aluminum silicate	2.0
Xanthan gum	0.4
Trisodium EDTA	0.05
Glyceryl polymethacrylate (and) Propylene glycol (and) PVM/MA copolymer	1.0
Polysorbate 20	1.0
Kaolin	0.8
Butylene glycol	4.0
Titanium dioxide	6.05
Iron oxides	1.15
Dimethicone	6.0
Ethylhexyl palmitate	2.0
PEG/PPG-25/25 Dimethicone	1.0
Tocopherol acetate	0.1
Retinyl palmitate	0.1
Silica	3.0
Cyclopentasiloxane	5.0
Fragrance, preservative	QS
Compound	SA

SUNSCREEN FORMULAE		
RAW MATERIAL (INCI Designation)	Amount	
	SPF ~25	SPF~15
Water	52.65	71.10
PVM/MA decadiene crosspolymer	0.5	0.5
Butylene glycol	3.0	3.0
Disodium EDTA	0.1	0.1
PEG-20 stearate	1.5	1.5
Glyceryl stearate (and) Laureth-23	2.0	2.0
Isostearyl neopentanoate	1.0	1.0
Ethylhexyl palmitate	2.0	2.0
Glyceryl dilaurate	0.5	0.5
Octinoxate	7.5	7.5
Oxybenzone	2.0	2.0
Ethylhexyl salicylate	3.0	3.0
Sodium hydroxide (10%)	1.3	1.3
Glyceryl polymethacrylate (and) Propylene glycol	3.0	3.0
Glyceryl polymethacrylate (and) Propylene glycol (and) PVM/MA copolymer	0.5	0.5
Styrene/Acrylates copolymer (27% solids)	18.45	-
Fragrance, preservative	QS	QS
Compound	SA	SA

VERY WATER-RESISTANT SUNSCREEN FORMULAE	Amount	
RAW MATERIAL (INCI Designation)		
	SPF ~12	SPF~22
Water	65.16	46.53
Acrylates copolymer	3.0	3.0
Disodium EDTA	0.1	0.1
Butylene glycol	2.0	2.0
Glyceryl polymethacrylate (and) Propylene glycol (and) PVM/MA copolymer	1.0	1.0
Butylated PVP	0.05	0.05
Glyceryl stearate (and) Behenyl alcohol (and) Palmitic acid (and) Stearic acid (and) Lecithin (and) Lauryl alcohol	4.5	4.5
Tricontanyl PVP	1.0	1.0
Octyl palmitate	2.0	2.0
Octinoxate	7.5	7.5
Oxybenzone	2.0	2.0
Ethylhexyl salicylate	3.0	3.0
Tridecyl neopentanoate	3.0	3.0
Glyceryl dilaurate	0.5	0.5
Sodium hydroxide (10%)	1.89	1.89
Cyclopentasiloxane	2.0	2.0
Butylene glycol	1.0	1.0
Styrene/Acrylates copolymer (27% solids)	18.45	-
Fragrance, preservative	QS	QS
Compound	SA	SA

WATER-IN-SILICONE SUNSCREEN	Amount
RAW MATERIAL (INCI Designation)	
Cetyl PEG/PPG-15/15 butyl ether dimethicone	2.0
Mineral oil	3.0
Ethylhexyl palmitate	1.0
Ethylhexyl salicylate	5.0
Hydrogenated castor oil	0.5
Beeswax	0.5
Octinoxate	7.5
Polyethylene	1.0
PEG-30 dipolyhydroxystearate	2.0
Cyclopentasiloxane	5.0
Dimethicone	5.0
Sodium chloride	0.6
Acrylates/C12-22 alkylmethacrylate copolymer	0.5
Water	66.4
Fragrance, preservative	QS
Compound	SA

LEAVE-ON HAIR CONDITIONER

RAW MATERIAL (INCI Designation)	Amount
Deionized Water	QS
Isostearamidopropyl Morpholine Lactate	6.0
Hydroxyethylcellulose	1.0
Preservative, fragrance, color	QS
Compound	1000ppm

5 CREAM RINSE (pH 4)

RAW MATERIAL (INCI Designation)	Amount
Deionized Water	QS
Behentrimonium Chloride	2.0
Trilaureth-4 Phosphate	1.5
Cetyl alcohol	2.0
Citric acid	QS
Preservative, fragrance, color	QS
Compound	1000ppm

NOURISHING HAIR CONDITIONER /TREATMENT (pH 6)

RAW MATERIAL (INCI Designation)	Amount
Deionized Water	QS
Behentrimonium Methosulfate (and) Cetyl Alcohol	4.0
Wheat germ oil	1.0
Cetyl alcohol	0.5
Propylene glycol	5.0
PEG-60 Lanolin	1.0
Panthenol	2.0
Lupin amino acids	1.0
Cocodimonium Hydroxypropyl Hydrolyzed Wheat Protein	1.0
Fragrance, preservative, color	QS
Compound	1000ppm

CONDITIONING SHAMPOO

RAW MATERIAL (INCI Designation)	Amount
Deionized Water	QS
Sodium Laureth Sulfate 30%	27.0
Cocamidopropyl Betaine	3.7
Coco-Glucoside (and) Glyceryl Oleate	5.0
Coco-Glucoside (and) Glycol Distearate (and) Glycerine	3.0
Guar Hydroxypropyl Trimonium Chloride	0.1
Laureth-2	1.55
Fragrance, preservative, color	QS
Compound	1000ppm

5

ANTI-DANDRUFF SHAMPOO

RAW MATERIAL (INCI Designation)	Amount
Deionized Water	QS
Magnesium Aluminum Silicate	1.0
Hydroxypropyl Methylcellulose	0.8
Sodium Olefin Sulfate 40%	35.0
Lauramide DEA	4.0
Soyamide DEA	1.0
Quaternium-70 Hydrolyzed Collagen	2.0
Zinc Pyrithione 40%	4.0
Fragrance, preservative, color	QS
Compound	1000ppm

CLEAR SHAMPOO**Formulations**
(Amounts)

RAW MATERIAL (INCI Designation)	1	2	3	4	5
Texapon N 70	13.00	15.00	10.50	12.50	10.00
Dehyton PK 45	7.50	7.00	5.00	5.50	10.00
Cetiol HE	2.00	2.50	3.50	5.00	2.30
Fragrance	0.10	0.10	0.10	0.10	0.10
Compound	SA	SA	SA	SA	SA
D-Panthenol USP	1.00	1.50	1.80	1.70	1.40
Preservative	0.10	0.10	0.10	0.10	0.10
Citric Acid	0.10	0.10	0.10	0.10	0.10
Luviquat Ultra Care	1.50	1.00	1.50	1.20	1.10
Sodium Chloride	1.50	1.40	1.40	1.30	1.50
Water	QS (100)	QS (100)	QS (100)	QS (100)	QS (100)

SHAMPOO**Formulations**
(Amounts)

RAW MATERIAL (INCI Designation)	1	2	3	4	5
Texapon NSO	35.00	40.00	30.00	45.00	27.00
Plantacare 2000	5.00	5.50	4.90	3.50	7.00
Tego Betain L7	10.00	5.00	12.50	7.50	15.00
Fragrance	0.10	0.10	0.10	0.10	0.10
Compound	SA	SA	SA	SA	SA
D-Panthenol USP	0.50	1.00	0.80	1.50	0.50
Preservative	0.10	0.10	0.10	0.10	0.10
Citric Acid	0.10	0.10	0.10	0.10	0.10
Rewopal LA 3	0.50	2.00	0.50	0.50	2.00
Sodium Chloride	1.50	1.50	1.50	1.50	1.50
Water	QS (100)	QS (100)	QS (100)	QS (100)	QS (100)

**CLEAR
CONDITIONING
SHAMPOO****Formulations
(Amounts)**

RAW MATERIAL (INCI Designation)	1	2	3	4	5
Amphotensid GB 2009	10.00	15.00	20.00	12.00	17.00
Plantacare 2000	5.00	6.00	7.00	8.00	4.00
Tego Betain L7	15.00	12.00	10.00	18.00	20.00
Luviquat FC 550	0.30	0.20	0.20	0.20	0.30
Fragrance	0.10	0.10	0.10	0.10	0.10
Compound	SA	SA	SA	SA	SA
Cremophor PS 20	5.00	1.00	1.00	7.00	5.00
Preservative	0.10	0.10	0.10	0.10	0.10
Rewopal LA 3	2.00	1.00	0.50	2.00	2.00
Citric Acid	0.20	0.20	0.20	0.20	0.20
Stepan PEG 600 DS	3.00	2.00	2.00	3.00	2.50
Water	QS (100)	QS (100)	QS (100)	QS (100)	QS (100)

FOAM O/W-EMULSION	Formulations (Amounts)	
RAW MATERIAL (INCI Designation)	1	2
Stearic acid	5.00	1.00
Cetyl alcohol	5.50	
Cetylstearyl alcohol		2.00
PEG-40 Stearate	8.50	
PEG-20 Stearate		1.00
Caprylsäure/Caprinsäure triglyceride	4.00	2.00
C12-15 Alkylbenzoate	10.00	15.00
Cyclomethicone	4.00	
Dimethicone		0.50
Compound	SA	SA
Octylisostearate		5.00
Myristyl Myristate		2.00
Ceresin	1.50	
Glycerine		3.00
Filter		
Hydroxypropyl distärke phosphate	1.00	3.50
BHT		0.02
Disodium EDTA	0.50	0.10
Parfüm, Konservierungsmittel	QS	QS
Colorant	QS	QS
Potassium hydroxide	QS	QS
Water dem.	QS (100)	QS (100)

	pH adjusted to 6.5-7.5	pH adjusted to 5.0-6.0
Emulsion 1		
Emulsion 2		
Gas (Stickstoff)		
Gas (Helium)		

CONDITIONER SHAMPOO WITH PEARLESCENT

Formulations (Amounts)

RAW MATERIAL (INCI Designation)	1	2	3
Polyquarternium-10	0.50	0.50	0.40
Sodiumlaurethsulfat	9.00	8.50	8.90
Cocoamidopropylbetain	2.50	2.60	3.00
Benzophenon-4	1.50	0.50	1.00
Compound	SA	SA	SA
Pearlescent compound	2.00	2.50	
Disodium EDTA	0.10	0.15	0.05
Preservative, Perfume, thickener	QS	QS	QS
Water dem.	QS (100)	QS (100)	QS (100)

pH adjusted to 6.0

CLEAR CONDITIONING SHAMPOO

Formulations (Amounts)

RAW MATERIAL (INCI Designation)	1	2	3
Polyquarternium-10	0.50	0.50	0.50
Sodiumlaurethsulfat	9.00	8.50	9.50
Compound	SA	SA	SA
Benzophenon-3	1.00	1.50	0.50
Imidosuccinicacid, Na	0.20	0.20	0.80
Preservative, Perfume, thickener	QS	QS	QS
Water dem.	QS (100)	QS (100)	QS (100)

pH adjusted to 6.0

CLEAR CONDITIONING SHAMPOO WITH VOLUME EFFECT

Formulations (Amounts)

RAW MATERIAL (INCI Designation)	1	2	3
Natriumlaurethsulfat	10.00	10.50	11.00
Ethylhexyl Methoxycinnamat	2.00	1.50	2.30
Compound	SA	SA	SA
Cocoamidopropylbetain	2.50	2.60	2.20
Disodium EDTA	0.01	0.10	0.01
Preservative, Perfume, thickener	QS	QS	QS
Water dem.	QS (100)	QS (100)	QS (100)

pH adjusted to 6.0

CONDITIONING SHAMPOO WITH PEARLESCENT**Formulations
(Amounts)**

RAW MATERIAL (INCI Designation)	1	2	3
Polyquarternium-10	0.50	0.50	0.40
Sodiumlaurethsulfat	9.00	8.50	8.90
Cocoamidopropylbetain	2.50	2.60	3.00
Benzophenon-4	1.50	0.50	1.00
Compound	SA	SA	SA
Pearlescent compound	2.00	2.50	
Disodium EDTA	0.10	0.15	0.05
Preservative, Perfume, thickener	QS	QS	QS
Water dem.	QS (100)	QS (100)	QS (100)

pH adjusted to 6.0

CLEAR CONDITIONING SHAMPOO**Formulations
(Amounts)**

RAW MATERIAL (INCI Designation)	1	2	3
Polyquarternium-10	0.50	0.50	0.50
Sodiumlaurethsulfat	9.00	8.50	9.50
Compound	SA	SA	SA
Benzophenon-3	1.00	1.50	0.50
Imidosuccinicacid, Na	0.20	0.20	0.80
Preservative, Perfume, thickener	QS	QS	QS
Water dem.	QS (100)	QS (100)	QS (100)

pH adjusted to 6.0

CLEAR CONDITIONING SHAMPOO WITH VOLUME EFFECT**Formulations
(Amounts)**

RAW MATERIAL (INCI Designation)	1	2	3
Natriumlaurethsulfate	10.00	10.50	11.00
Ethylhexyl Methoxycinnamat	2.00	1.50	2.30
Compound	SA	SA	SA
Cocoamidopropylbetain	2.50	2.60	2.20
Disodium EDTA	0.01	0.10	0.01
Preservative, Perfume, thickener	QS	QS	QS
Water dem.	QS (100)	QS (100)	QS (100)

pH adjusted to 6.0

GEL CREME**Formulations
(Amounts)**

RAW MATERIAL (INCI Designation)	1	2	3	4
Acrylat/C10-30 Alkylacrylat Crosspolymer	0.40	0.35	0.40	0.35
Polyacrylicacid	0.20	0.22	0.20	0.22
Xanthan Gummi	0.10	0.13	0.10	0.13
Cetearylalkohol	3.00	2.50	3.00	2.50

C12-15 Alkylbenzoat	4.00	4.50	4.00	4.50
Caprylic/Capric Triglycerid	3.00	3.50	3.00	3.50
Aminobenzophenon (e.g., UVINUL A PLUS™)	2.00	1.50	0.75	1.00
UVASorb K2A		3.00		
Ethylhexyl Methoxycinnamat	3.00		1.00	
Bis-Ethylhexyloxyphenol methoxyphenyl Triazin		1.50		2.00
Butyl Methoxydibenzoylmethan			2.00	
Disodium Phenyl Dibenzimidazol Tetrasulfonat	2.50		0.50	2.00
Ethyhexyl Triazon	4.00		3.00	4.00
Octocrylen		4.00		
Diethylhexyl Butamido Triazon	1.00			2.00
Phenylbenzimidazol Sulfonsäure	0.50		3.00	
Methylen Bis-Benzotriazolyl	2.00		0.50	1.50
Tetramethylbutylphenol				
Ethylhexysalicylate			3.00	
Drometrizol Trisiloxan			0.50	
Terephthaliden Dicamphor Sulfonsäure		1.50		1.00
Diethylhexyl-2,6-naphthalate	3.50	4.00	7.00	9.00
Titanium dioxide- microfine	1.00		3.00	
Zincoxide- microfine				0.25
Compound	SA	SA	SA	SA
Cyclisches Dimethylpolysiloxane	5.00	5.50	5.00	5.50
Dimethicon Polydimethylsiloxane	1.00	0.60	1.00	0.60
Glycerine	1.00	1.20	1.00	1.20
Sodium hydroxide	QS.	QS	QS	QS
Preservative	0.30	0.23	0.30	0.23
Perfume	0.20		0.20	
Water	QS (100)	QS (100)	QS (100)	QS (100)
pH adjusted to 6.0				

GELCREME	Formulations (Amounts)			
RAW MATERIAL (INCI Designation)	1	2	3	4
Acrylat/C10-30 Alkylacrylat Crosspolymer	0.40	0.35	0.40	0.35
Polyacrylicacid	0.20	0.22	0.20	0.22
Xanthan Gummi	0.10	0.13	0.10	0.13
Cetearylalkohol	3.00	2.50	3.00	2.50
C12-15 Alkylbenzoat	4.00	4.50	4.00	4.50
Caprylic/Capric Triglycerid	3.00	3.50	3.00	3.50
Aminobenzophenon (e.g., UVINUL A PLUS™)	2.00	1.50	0.75	1.00
UVASorb K2A		3.00		
Ethylhexyl Methoxycinnamat	3.00		1.00	
Bis-Ethylhexyloxyphenol methoxyphenyl Triazin		1.50		2.00
Butyl Methoxydibenzoylmethan			2.00	
Disodium Phenyl Dibenzimidazol Tetrasulfonat	2.50		0.50	2.00
Ethyhexyl Triazon	4.00		3.00	4.00
Octocrylen		4.00		

Diethylhexyl Butamido Triazon	1.00			2.00
Phenylbenzimidazol Sulfonsäure	0.50		3.00	
Methylen Bis-Benzotriazolyl Tetramethylbutylphenol	2.00		0.50	1.50
Ethylhexysalicylat			3.00	
Drometrisol Trisiloxan			0.50	
Terephthaliden Dicamphor Sulfonsäure		1.50		1.00
Diethylhexyl-2,6-naphthalat	3.50	4.00	7.00	9.00
Titaniumdioxide- microfine	1.00		3.00	
Zincoxide- microfine				0.25
Compound	SA	SA	SA	SA
Cyclisches Dimethylpolysiloxan	5.00	5.50	5.00	5.50
Dimethicon Polydimethylsiloxan	1.00	0.60	1.00	0.60
Glycerin	1.00	1.20	1.00	1.20
Sodiumhydroxid	QS	QS	QS	QS
Preservative	0.30	0.23	0.30	0.23
Perfume	0.20		0.20	
Water	QS (100)	QS (100)	QS (100)	QS (100)
pH adjusted to 6.0				

**O/W SUNSCREEN
FORMULATION**
**Formulation
(Amounts)**

RAW MATERIAL (INCI Designations)	1	2	3	4	5	6	7
Glycerin monostearate SE	0.50	1.00	3.00			1.50	
Glycerl Stearate Citrate	2.00		1.00	2.00	4.00		
Stearic acid		3.00		2.00			
PEG-40 Stearate	0.50					2.00	
Cetyl Phosphate						1.00	
Cetearyl Sulfate							0.75
Stearyl Alcohol			3.00			2.00	0.60
Cetyl Alcohol	2.50	1.10		1.50	0.60		2.00
Compound	SA	SA	SA	SA	SA	SA	SA
Aminobenzophenon (e.g., UVINUL A PLUS™)	2.00	1.50	0.75	1.00	2.10	4.50	5.00
UVASorb K2A							
Ethylhexyl Methoxycinnamate					5.00	6.00	8.00
Bis-Ethylhexyloxyphenol methoxyphenyl Triazin		1.50		2.00	2.50		2.50
Butyl Methoxydibenzoylmethane			2.00		2.00	1.50	
Dinatrium Phenyl Dibenzimidazol Tetrasulfonate	2.50		0.50	2.00		0.30	
Ethylhexyl Triazone	4.00		3.00	4.00		2.00	
Octocrylen		4.00					7.50
Diethylhexyl Butamido Triazon	1.00			2.00	1.00		1.00
Phenylbenzimidazol Sulfonsäure	0.50		3.00				

**O/W SUNSCREEN
FORMULATION****Formulation
(Amounts)**

Methylen Bis-Benzotriazolyl Tetramethylbutylphenol	2.00		0.50	1.50	2.50		
Ethylhexysalicylat			3.00				5.00
Drometrizol Trisiloxan			0.50			1.00	
Terephthaliden Dicamphor Sulfonic Acid		1.50		1.00	1.00		0.50
Diethylhexyl-2,6-naphthalat	3.50		7.00		6.00	9.00	
Titandioxid- microfine	1.00		3.00		3.50		1.50
Zinkoxid- microfine				0.25		2.00	
C12-15 Alkyl Benzoate		0.25			4.00	7.00	
Dicapryl Ether			3.50		2.00		
Butylenglycol Dicaprylat/Dicaprat	5.00		6.00				
Cocoglyceride			6.00		2.00		
Dimethicon	0.50		1.00		2.00		
Cyclomethicone	2.00		0.50		0.50		
Shea Butter		2.00					
PVP Hexadecen Copolymer	0.20			0.50		1.00	
Glycerin	3.00	7.50		7.50	5.00		2.50
Xanthan Gum	0.15		0.05			0.30	
Sodium Carbomer		0.20		0.15	0.25		
Vitamin E Acetat	0.60		0.23		0.70	1.00	
Fucogel 1000		3.00	10.00				
Glycin Soja				0.50		1.50	1.00
Ethylhexyloxyglycin	0.30						
DMDM Hydantoin		0.60	0.40	0.20			
Glycil-L				0.18	0.20		
Methylparaben	0.15		0.25		0.50		
Phenoxyethanol	1.00	0.40			0.40	0.50	0.40
Trinatrium EDTA	0.02		0.05				
Iminosuccinicacid				0.25	1.00		
Ethanol	2.00	1.50		3.00		1.20	5.00
Perfume	0.10	0.25	0.30		0.40	0.20	
Water	QS (100)	QS (100)	QS (100)	QS (100)	QS (100)	QS (100)	QS (100)

HYDRODISPERSION**Formulations
(Amounts)**

RAW MATERIAL (INCI Designation)	1	2	3	4	5
Ceteaereth-20	1.00			0.50	
Cetyl Alkohol			1.00		
Sodium Carbomer		0.20		0.30	
Acrylat/C10-30 Alkyl Acrylat Crosspolymer	0.50		0.40	0.10	0.50
Xanthan Gummi		0.30	0.15		
Compound	SA	SA	SA	SA	SA
Aminobenzophenon (e.g., UVINUL A	2.00	1.50	0.75	1.00	2.10

HYDRODISPERSION

Formulations
(Amounts)

RAW MATERIAL (INCI Designation)	1	2	3	4	5
PLUS™)					
UVASorb K2A		3.50			
Ethylhexyl Methoxycinnamat					5.00
Bis-Ethylhexyloxyphenol methoxyphenyl Triazin		1.50		2.00	2.50
Butyl Methoxydibenzoylmethan			2.00		2.00
Dinatrium Phenyl Dibenzimidazol Tetrasulfonat	2.50		0.50	2.00	
Ethylhexyl Triazon	4.00		3.00	4.00	
Octocrylen		4.00			
Diethylhexyl Butamido Triazon	1.00			2.00	1.00
Phenylbenzimidazol Sulfonsäure	0.50		3.00		
Methylen Bis-Benzotriazolyl Tetramethylbutylphenol	2.00		0.50	1.50	2.50
Ethylhexylsalicylat			3.00		
Drometrizol Trisiloxan			0.50		
Terephthaliden Dicamphor Sulfonsäure		1.50		1.00	1.00
Diethylhexyl-2,6-naphthalat			7.00		9.00
Titaniumdioxide- microfine	1.00		3.00		3.50
Zincoxide- microfine				0.25	
C12-15 Alkyl Benzoat	2.00	2.50			
Dicapryl Ether		4.00			
Butylenglycol Dicaprylat/Dicaprat	4.00		2.00	6.00	
Dicapryl Carbonat		2.00	6.00		
Dimethicon		0.50	1.00		
Phenyltrimethicon	2.00		0.50		
Shea Butter		2.00		5.00	
PVP Hexadecen Copolymer	0.50			0.50	1.00
Tricontanyl PVP	0.50		1.00		
Ethylhexylglycerin			1.00		0.80
Glycerin	3.00	7.50		7.50	8.50
Glycin Soja			1.50		1.00
Vitamin E Acetat	0.50		0.25		1.00
Alpha-Glucosilrutin	0.60			0.25	
Fucogel 1000		2.50	0.50		2.00
DMDM Hydantoin		0.60	0.45	0.25	
Glycil-S	0.20				
Methylparaben	0.50		0.25	0.15	
Phenoxyethanol	0.50	0.40		1.00	
Trinatrium EDTA		0.01	0.05		0.10
Ethanol	3.00	2.00	1.50		7.00
Perfume	0.20		0.05	0.40	
Water	QS (100)	QS (100)	QS (100)	QS (100)	QS (100)

**W/O SUNSCREEN
EMULSION****Formulations
(Amounts)**

RAW MATERIAL (INCI Designation)	1	2	3	4	5
Cetyldimethicon Copolyol		2.50		4.00	
Polyglyceryl-2-dipolyhydroxystearat	5.00				4.50
PEG-30-dipolyhydroxystearat			5.00		
Compound	SA	SA	SA	SA	SA
Aminobenzophenon (e.g., UVINUL A PLUS™)	2.00	1.50	0.75	1.00	2.10
UVASorb K2A		2.00			
Ethylhexyl Methoxycinnamat					5.00
Bis-Ethylhexyloxyphenol methoxyphenyl Triazin		1.50		2.00	2.50
Butyl Methoxydibenzoylmethan			2.00		2.00
Dinatrium Phenyl Dibenzimidazol Tetrasulfonat	2.50		0.50	2.00	
Ethylhexyl Triazon	4.00		3.00	4.00	
Octocrylen		4.00			
Diethylhexyl Butamido Triazon	1.00			2.00	1.00
Phenylbenzimidazol Sulfonsäure	0.50		3.00		
Methylen Bis-Benzotriazolyl	2.00		0.50	1.50	2.50
Tetramethylbutylphenol					
Ethylhexylsalicylat			3.00		
Drometrizol Trisiloxan			0.50		
Terephthaliden Dicamphor Sulfonsäure		1.50		1.00	1.00
Diethylhexyl-2,6-naphthalat			7.00		4.00
Titaniumdioxide- microfine	1.00		3.00		3.50
Zincoxide- microfine				0,25	
Mineraloil		12.00	10.00		8.00
C12-15 Alkyl Benzoat				9.00	
Dicaprylyl Ether	10.00				7.00
Butylenglycol Dicaprylat/Dicaprat			2.00	8.00	4.00
Dicaprylyl Carbonat	5.00		6.00		
Dimethicon		4.00	1.00	5.00	
Cyclomethicon	2.00	2.50			2.00
Shea Butter			3.00		
Vaseline		4.50			
PVP Hexadecen Copolymer	0.50			0.50	1.00
Ethylhexylglycerin		0.30	1.00		0.50
Glycerin	3.00	7.50		7.50	8.50
Glycin Soja		1.00	1.50		1.00
MgSO4	1.00	0.50		0,50	
MgCl2			1.00		0.70
Vitamin E Acetat	0.50		0.25		1.00
Ascorbyl Palmitat	0.50			2.00	
Fucogel 1000				3.50	1.00
DMDM Hydantoin		0.60	0.40	0.20	
Methylparaben	0.50		0.25	0.15	

Phenoxyethanol	0.50	0.40		1.00	
Trisodium EDTA	0.12	0.05		0.30	
Ethanol	3.00		1.50		5.00
Perfume	0.20		0.40	0.35	
Water	QS (100)	QS (100)	QS (100)	QS (100)	QS (100)

STICKS**Formulations**
(Amounts)

RAW MATERIAL (INCI Designation)	1	2	3	4
Caprylic/Capric Triglycerid	12.00	10.00	6.00	
Octyldodecanol	7.00	14.00	8.00	3.00
Butylene Glycol Dicaprylat/Dicaprat				12.00
Pentaerythrityl Tetraisostearat	10.00	6.00	8.00	7.00
Polyglyceryl-3 Diisostearat	2.50			
Bis-Diglyceryl Polyacyladipate-2	9.00	8.00	10.00	8.00
Cetearyl Alcohol	8.00	11.00	9.00	7.00
Myristyl Myristate	3.50	3.00	4.00	3.00
Beeswax	5.00	5.00	6.00	6.00
Cera Carnauba	1.50	2.00	2.00	1.50
Cera Alba	0.50	0.50	0.50	0.40
C16-40 Alkyl Stearat		1.50	1.50	1.50
Compound	SA	SA	SA	SA
Aminobenzophenon (e.g., UVINUL A PLUS™)	2.00	1.50	0.75	9.00
UVASorb K2A		2.00		4.00
Ethylhexyl Methoxycinnamat		3.00		
Bis-Ethylhexyloxyphenol methoxyphenyl Triazin		1.50		2.00
Butyl Methoxydibenzoylmethan			2.00	
Dinatrium Phenyl Dibenzimidazol Tetrasulfonat	2.50		0.50	2.00
Ethylhexyl Triazon	4.00		3.00	4.00
Octocrylen		4.00		
Diethylhexyl Butamido Triazon	1.00			2.00
Phenylbenzimidazol Sulfonsäure	0.50		3.00	
Methylen Bis-Benzotriazolyl	2.00		0.50	1.50
Tetramethylbutylphenol				
Ethylhexysalicylat			3.00	
Drometrizol Trisiloxan			0.50	
Terephthaliden Dicapthor Sulfonsäure		1.50		1.00
Diethylhexyl-2,6-naphthalat			7.00	
Titaniumdioxide- microfine	1.00		3.00	
Zincoxide- microfine				0.25
Vitamin E Acetat	0.50	1.00		
Ascorbyl Palmitat	0.05		0.05	
Buxux Chinensis	2.00	1.00		1.00
Perfume, BHT	0.10	0.25		0.35
Ricinus Communis	QS (100)	QS (100)	QS (100)	QS (100)

PIT-EMULSION

Formulations
(Amounts)

RAW MATERIAL (INCI Designation)	1	2	3	4	5	6	7	8
Glycerinmonostearat SE	0.50	2.00	3.00	5.00		0.50	4.00	
Glyceryl Isostearat					3.50	4.00	2.00	
Isoceteth-20		0.50			2.00			
Ceteareth-12		5.00		1.00			3.50	5.00
Ceteareth-20		5.00		1.00				3.50
PEG-100 Stearat				2.80		2.30	3.30	
Cetyl Alkohol	5.20		1.20	1.00	1.30		0.50	0.30
Cetyl Palmitat	2.50	1.20		1.50		0.50		1.50
Cetyl Dimethicon Copolyol				0.50		1.00		
Polyglyceryl-2				0.75	0.30			
Compound	SA	SA	SA	SA	SA	SA	SA	SA
Aminobenzophenon (e.g., UVINUL A PLUS™)	2.00	1.50	0.75	1.00	2.10	4.50	5.00	2.10
UVASorb K2A			4.00				1.50	
Ethylhexyl Methoxycinnamat					5.00	6.00	8.00	5.00
Bis-Ethylhexyloxyphenol methoxyphenyl Triazin		1.50		2.00	2.50		2.50	2.50
Butyl Methoxydibenzoylmethan			2.00		2.00	1.50		2.00
Dinatrium Phenyl Dibenzimidazol Tetrasulfonat	2.50		0.50	2.00		0.30		
Ethylhexyl Triazon	4.00		3.00	4.00		2.00		
Octocrylen		4.00					7.50	
Diethylhexyl Butamido Triazon	1.00			2.00	1.00		1.00	1.00
Phenylbenzimidazol Sulfonsäure	0.50		3.00					
Methylen Bis-Benzotriazolyl Tetramethylbutylphenol	2.00		0.50	1.50	2.50			2.50
Ethylhexylsalicylat			3.00				5.00	
Drometrizol Trisiloxan			0.50			1.00		
Terephthaliden Dicamphor Sulfonsäure		1.50		1.00	1.00		0.50	1.00
Diethylhexyl-2,6-naphthalat			7.00		10.00	7.50		8.00
Titandioxid- microfine	1.00		3.00		3.50		1.50	3.50
Zinkoxid- microfine				0.25		2.00		
C12-15 Alkyl Benzoat	3.50			6.35				0.10
Cocoglyceride		3.00		3.00				1.00
Dicapryl Ether	4.50							
Dicaprylyl Carbonat		4.30		3.00				7.00
Dibutyl Adipate				0.50				0.30
Phenyltrimethicone	2.00			3.50		2.00		
Cyclomethicon		3.00						
Ethyl Galaktomannan		0.50			2.00			
Hydrierte Coco-Glyceride					3.00	4.00		
Abil Wax 2440						1.50	2.00	
PVP Hexadecen Copolymer				1.00	1.20			
Glycerin	4.00	6.00	5.00		8.00	10.00		
Vitamin E Acetat	0.20	0.30	0.40		0.30			

Shea Butter		2.00		3.60		2.00		
Iodopropyl Butylcarbamate	0.12				0.20			
Fucogel 1000				0.10				
DMDM Hydantoin	0.10				0.12		0.13	
Methylparaben		0.50	0.30		0.35			
Phenoxyethanol	0.50	0.40		1.00				
Octoxyglycerin		0.30			1.00		0.35	
Ethanol	2.00		2.00			5.00		
Trinatrium EDTA	0.40		0.15			0.20		
Perfume	0.20		0.20		0.24	0.16	0.10	0.10
Water	QS (100)	QS (100)	QS (100)	QS (100)	QS (100)	QS (100)	QS (100)	QS (100)

GEL CREME**Formulations**

(Amounts)

RAW MATERIAL (INCI Designation)	1	2	3	4
Acrylat/C10-30 alkylacrylat crosspolymer	0.40	0.35	0.40	0.35
Polyacrylic acid	0.20	0.22	0.20	0.22
Luvigel EM	1.50	2.50	2.80	3.50
Xanthan gum	0.10	0.13	0.10	0.13
Cetearylalkohol	3.00	2.50	3.00	2.50
C12-15 Alkylbenzoate	4.00	4.50	4.00	4.50
Caprylic/Capric Triglyceride	3.00	3.50	3.00	3.50
Titan dioxide- microfine	1.00		1.50	
Zinc oxide- microfine		2.00		0.25
Compound	SA	SA	SA	SA
Dihydroxyacetone			3.00	5.00
Cyclisches Dimethylpolysiloxan	5.00	5.50	5.00	5.50
Dimethicon Polydimethylsiloxan	1.00	0.60	1.00	0.60
Glycerine	1.00	1.20	1.00	1.20
Natrium hydroxide	QS	QS	QS	QS
Preservatives	0.30	0.23	0.30	0.23
Perfume	0.20		0.20	
Water	QS (100)	QS (100)	QS (100)	QS (100)
pH adjusted to 6.0				

O/W SELF TANNER**Formulations**

(Amounts)

RAW MATERIAL (INCI Designation)	1	2	3	4	5	6	7
Glycerin monostearate SE	0.50	1.00	3.00			1.50	
Glycerlstearate citrate	2.00		1.00	2.00	4.00		
Stearic acid		3.00		2.00			
PEG-40 stearate	0.50					2.00	
Cetyl phosphate						1.00	
Cetearyl sulfate							0.75
Stearyl alcohol			3.00			2.00	0.60

Cetyl alcohol	2.50	1.10		1.50	0.60		2.00
Compound	SA	SA	SA	SA	SA	SA	SA
Dihydroxy acetone			3.00	5.00		4	
Titanium dioxide- microfine	1.00				1.50		1.50
Zinc oxide- microfine				0.25		2.00	
C12-15 Alkyl benzoate		0.25			4.00	7.00	
Dicapryl ether			3.50		2.00		
Butylenglycol	5.00		6.00				
Dicaprylaet/Dicaprat							
Cocoglyceride			6.00		2.00		
Dimethicon	0.50		1.00		2.00		
Cyclomethicon	2.00		0.50		0.50		
Shea butter		2.00					
PVP hexadecen copolymer	0.20			0.50		1.00	
Glycerin	3.00	7.50		7.50	5.00		2.50
Xanthan gum	0.15		0.05			0.30	
Sodium carbomer		0.20		0.15	0.25		
Vitamin E acetate	0.60		0.23		0.70	1.00	
Fucogel 1000		3.00	10.00				
Glycin Soja				0.50		1.50	1.00
Ethylhexyloxy glycin	0.30						
DMDM hydantoin		0.60	0.40	0.20			
Glycil-L				0.18	0.20		
Methylparaben	0.15		0.25		0.50		
Phenoxyethanol	1.00	0.40			0.40	0.50	0.40
Trinatrium EDTA	0.02		0.05				
Iminobernsteinsäure				0.25	1.00		
Ethanol	2.00	1.50		3.00		1.20	5.00
Perfume	0.10	0.25	0.30		0.40	0.20	
Water	QS (100)	QS (100)	QS (100)	QS (100)	QS (100)	QS (100)	QS (100)

O/W MAKE UP

Formulations
(Amounts)

RAW MATERIAL (INCI Designation)	1	2	3	4	5	6	7
Glycerinmonostearat SE	0.50	1.00	3.00			1.50	
Glycerl Stearat Citrat	2.00		1.00	2.00	4.00		
Stearicacid		3.00		2.00			
PEG-40 Stearat	0.50					2.00	
Cetyl Phosphat						1.00	
Cetearyl Sulfat							0.75
Stearyl Alkohol			3.00			2.00	0.60
Cetyl Alkohol	2.50	1.10		1.50	0.60		2.00
Compound	SA	SA	SA	SA	SA	SA	SA
Titaniumoxide	10.00	12.00	9.00	8.50	11.00	9.50	10.00
Ironoxide	2.00	4.00	3.00	5.00	3.40	6.00	4.40
Zincoxide		4.00		2.00		3.00	

C12-15 Alkyl Benzoat		0.25			4.00	7.00	
Dicapryl Ether			3.50		2.00		
Butylenglycol	5.00		6.00				
Dicaprylat/Dicaprat							
Cocoglyceride			6.00		2.00		
Dimethicon	0.50		1.00		2.00		
Cyclomethicon	2.00		0.50		0.50		
Shea Butter		2.00					
PVP Hexadecen Copolymer	0.20			0.50		1.00	
Glycerin	3.00	7.50		7.50	5.00		2.50
Xanthan Gummi	0.15		0.05			0.30	
Sodium Carbomer		0.20		0.15	0.25		
Vitamin E Acetat	0.60		0.23		0.70	1.00	
Glycin Soja				0.50		1.50	1.00
Ethylhexyloxyglycin	0.30						
DMDM Hydantoin		0.60	0.40	0.20			
Glycil-L				0.18	0.20		
Methylparaben	0.15		0.25		0.50		
Phenoxyethanol	1.00	0.40			0.40	0.50	0.40
Trinatrium EDTA	0.02		0.05				
Iminosuccinicacid				0.25	1.00		
Ethanol	2.00	1.50		3.00		1.20	5.00
Perfume	0.10	0.25	0.30		0.40	0.20	
Water	QS (100)	QS (100)	QS (100)	QS (100)	QS (100)	QS (100)	QS (100)

**SELF TANNER
HYDRODISPERSION**
**Formulations
(Amounts)**

RAW MATERIAL (INCI Designation)	1	2	3	4	5
Ceteareth-20	1.00			0.50	
Cetyl Alcohol			1.00		
Luvigel EM		2.00		2.50	2.00
Acrylat/C10-30 Alkyl Acrylat Crosspolymer	0.50		0.40	0.10	0.50
Xanthan Gummi		0.30	0.15		
Compound	SA	SA	SA	SA	SA
Dihydroxyacetone			3.00	5.00	
Aminobenzophenon (e.g., UVINUL A PLUS™)	2.00	1.50	0.75	1.00	2.10
Titanioxid- microfine	1.00		1.00		1.00
Zinkoxid- microfine		1.90		0.25	
C12-15 Alkyl Benzoat	2.00	2.50			
Dicapryl Ether		4.00			
Butylenglycol Dicaprylat/Dicaprat	4.00		2.00	6.00	
Dicapryl Carbonat		2.00	6.00		
Dimethicon		0.50	1.00		
Phenyltrimethicon	2.00		0.50		

Shea Butter		2.00		5.00	
PVP Hexadecen Copolymer	0.50			0.50	1.00
Tricontanyl PVP	0.50		1.00		
Ethylhexylglycerin			1.00		0.80
Glycerin	3.00	7.50		7.50	8.50
Glycin Soja			1.50		1.00
Vitamin E Acetat	0.50		0.25		1.00
Alpha-Glucosilrutin	0.60			0.25	
DMDM Hydantoin		0.60	0.45	0.25	
Glycil-S	0.20				
Methylparaben	0.50		0.25	0.15	
Phenoxyethanol	0.50	0.40		1.00	
Trinatrium EDTA		0.01	0.05		0.10
Ethanol	3.00	2.00	1.50		7.00
Parfüm	0.20		0.05	0.40	
Water	QS (100)	QS (100)	QS (100)	QS (100)	QS (100)

**AFTER SUN
HYDRODISPERSION**
**Formulations
(Amounts)**

	1	2	3	4	5
Ceteareth-20	1.00			0.50	
Cetyl Alcohol			1.00		
Luvigel EM		2.00		2.50	2.00
Acrylat/C10-30 Alkyl Acrylat	0.50	0.30	0.40	0.10	0.50
Crosspolymer					
Xanthan Gummi		0.30	0.15		
Compound	SA	SA	SA	SA	SA
C12-15 Alkyl Benzoat	2.00	2.50			
Dicapryl Ether		4.00			
Butylenglycol Dicaprylat/Dicaprat	4.00		2.00	6.00	
Dicapryl Carbonat		2.00	6.00		
Dimethicon		0.50	1.00		
Phenyltrimethicon	2.00		0.50		
Tricontanyl PVP	0.50		1.00		
Ethylhexylglycerin			1.00		0.80
Glycerin	3.00	7.50		7.50	8.50
Glycin Soja			1.50		1.00
Vitamin E Acetat	0.50		0.25		1.00
Alpha-Glucosilrutin	0.60			0.25	
Trinatrium EDTA		0.01	0.05		0.10
Ethanol	1.00	10.00	8.00	12.00	9.00
Perfume	0.20		0.05	0.40	
Water	QS (100)	QS (100)	QS (100)	QS (100)	QS (100)

WO-EMULSIONS

Formulations

(Amounts)

RAW MATERIAL (INCI Designation)	1	2	3	4	5
Cetyldimethicon Copolyol		2.50		4.00	
Polyglyceryl-2-dipolyhydroxystearat	5.00				4.50
PEG-30-dipolyhydroxystearat			5.00		
Compound	SA	SA	SA	SA	SA
Aminobenzophenon (<i>e.g.</i> , UVINUL A PLUS™)	2.00	1.50	0.75	1.00	2.10
Titaniumdioxide- microfine	1.00		3.00		3.50
Zincoxide- microfine		0.90		0.25	
Mineralöl		12.00	10.00		8.00
C12-15 Alkyl Benzoat				9.00	
Dicaprylyl Ether	10.00				7.00
Butylenglycol Dicaprylat/Dicaprat			2.00	8.00	4.00
Dicaprylyl Carbonat	5.00		6.00		
Dimethicon		4.00	1.00	5.00	
Cyclomethicon	2.00	25.00			2.00
Shea Butter			3.00		
Vaseline		4.50			
PVP Hexadecen Copolymer	0.50			0.50	1.00
Ethylhexylglycerin		0.30	1.00		0.50
Glycerin	3.00	7.50		7.50	8.50
Glycin Soja		1.00	1.50		1.00
MgSO ₄	1.00	0.50		0.50	
MgCl ₂			1.00		0.70
Vitamin E Acetat	0.50		0.25		1.00
Ascorbyl Palmitat	0.50			2.00	
Fucogel 1000				3.50	7.00
DMDM Hydantoin		0.60	0.40	0.20	
Methylparaben	0.50		0.25	0.15	
Phenoxyethanol	0.50	0.40		1.00	
Trinatrium EDTA	0.12	0.05		0.30	
Ethanol	3.00		1.50		5.00
Perfume	0.20		0.40	0.35	
Water	QS (100)	QS (100)	QS (100)	QS (100)	QS (100)

**SOLID STABILIZED EMULSIONS
(PICKERING EMULSIONS)****Formulations
(Amounts)**

RAW MATERIAL (INCI Designation)	1	2	3	4	5
Mineral oil			16.00	16.00	
Octyldodecanol	9.00	9.00	5.00		
Caprylic/Capric Triglycerid	9.00	9.00	6.00		
C12-15 Alkyl Benzoat				5.00	8.00
Butylen Glycol Dicaprylat/Dicaprat					8.00
Dicaprylyl Ether	9.00			4.00	
Dicaprylyl Carbonat		9.00			
Hydroxyoctacosanyl Hydroxystearat	2.00	2.00	2.20	2.50	1.50
Disteardimonium Hectorit	1.00	0.75		0.50	0.25
Cera Microcristallina + Paraffinum Liquidum		0.35			5.00
Hydroxypropyl Methylcellulose			0.10		0.05
Dimethicon					3.00
Compound	SA	SA	SA	SA	SA
Titaniumdioxide + Alumina + Simethicon + Aqua		3.00			
Titaniumdioxide + Trimethoxycaprylylsilan		2.00	4.00	2.00	4.00
Silica Dimethyl Silylat	2.50			6.00	2.50
Bornitrid			1.00		
Stärke/-Natriummetaphosphat-Polymer	2.00				
Tapioca Stärke		0.50			
Sodium Chlorid	5.00	7.00	8.50	3.00	4.50
Glycerin				1.00	
Trinatrium EDTA	1.00	1.00	1.00	1.00	1.00
Vitamin E Acetat	5.00	10.00	3.00	6.00	10.00
Ascorbyl Palmitat	1.00	1.00		1.00	
Methylparaben		0.60			0.20
Propylparaben					0.20
Phenoxyethanol			0.20		
Hexamidin Diisethionat			0.40	0.50	0.40
Diazolidinyl Harnstoff					0.08
Ethanol			0.23	0.20	
Perfume	5.00		3.00	4.00	
Water	0.20		0.30	0.10	
	QS (100)	QS (100)	QS (100)	QS (100)	QS (100)

STICKS**Formulations**

(Amounts)

RAW MATERIAL (INCI Designation)	1	2	3	4
Caprylic/Capric Triglycerid	12.00	10.00	6.00	
Octyldodecanol	7.00	14.00	8.00	3.00
Butylene Glycol Dicaprylat/Dicaprat				12.00
Pentaerythrityl Tetraisostearat	10.00	6.00	8.00	7.00
Polyglyceryl-3 Diisostearat	2.50			
Bis-Diglyceryl Polyacyladipate-2	9.00	8.00	10.00	8.00
Cetearyl Alcohol	8.00	11.00	9.00	7.00
Myristyl Myristate	3.50	3.00	4.00	3.00
Beeswax	5.00	5.00	6.00	6.00
Cera Carnauba	1.50	2.00	2.00	1.50
Cera Alba	0.50	0.50	0.50	0.40
C16-40 Alkyl Stearat		1.50	1.50	1.50
Compound	SA	SA	SA	SA
Aminobenzophenon (e.g., UVINUL A PLUS™)	2.00	1.50	0.75	9.00
Titaniumdioxide- microfine	1.00		3.00	
Zincoxide- microfine		1.00		0.25
Vitamin E Acetat	0.50	1.00		
Ascorbyl Palmitat	0.05		0.05	
Buxux Chinensis	2.00	1.00		1.00
Perfume, BHT	0.10	0.25		0.35
Ricinus Communis	QS (100)	QS (100)	QS (100)	QS (100)

**SELF TANNER
PIT-EMULSION****Formulations**

(Amounts)

	1	2	3	4	5	6	7	8
Glycerinmonostearat SE	0.50	2.00	3.00	5.00		0.50	4.00	
Glyceryl Isostearat					3.50	4.00	2.00	
Isoceteth-20		0.50			2.00			
Ceteareth-12		5.00		1.00			3.50	5.00
Ceteareth-20		5.00		1.00				3.50
PEG-100 Stearat				2.80		2.30	3.30	
Cetyl Alkohol	5.20		1.20	1.00	1.30		0.50	0.30
Cetyl Palmitat	2.50	1.20		1.50		0.50		1.50
Cetyl Dimethicon Copolyol				0.50		1.00		
Polyglyceryl-2				0.75	0.30			
Compound	SA	SA	SA	SA	SA	SA	SA	SA
Dihydroxyaceton			3.00	5.00			4.00	
Aminobenzophenon (e.g., UVINUL A PLUS™)	2.00	1.50	0.75	1.00	2.10	4.50	5.00	2.10
Titandioxide- microfine	1.00		1.50		3.50		1.50	1.00
Zinkoxide- microfine		1.00		0.25		2.00		1.50
C12-15 Alkyl Benzoat	3.50			6.35				0.10

**SELF TANNER
PIT-EMULSION****Formulations
(Amounts)**

	1	2	3	4	5	6	7	8
Cocoglyceride		3.00		3.00				1.00
Dicapryl Ether	4.50							
Dicaprylyl Carbonat		4.30		3.00				7.00
Dibutyl Adipate				0.50				0.30
Phenyltrimethicone	2.00			3.50		2.00		
Cyclomethicon		3.00						
Ethyl Galaktomannan		0.50			2.00			
Hydrogenated Coco-Glyceride					3.00	4.00		
Abil Wax 2440						1.50	2.00	
PVP Hexadecen Copolymer				1.00	1.20			
Glycerin	4.00	6.00	5.00		8.00	10.00		
Vitamin E Acetat	0.20	0.30	0.40		0.30			
Shea Butter		2.00		3.60		2.00		
Iodopropyl Butylcarbamate	0.12				0.20			
DMDM Hydantoin	0.10				0.12		0.13	
Methylparaben		0.50	0.30		0.35			
Phenoxyethanol	0.50	0.40		1.00				
Octoxyglycerin		0.30			1.00		0.35	
Ethanol	2.00		2.00			5.00		
Trinatrium EDTA	0.40		0.15			0.20		
Perfume	0.20		0.20		0.24	0.16	0.10	0.10
Water	QS (100)	QS (100)	QS (100)	QS (100)	QS (100)	QS (100)	QS (100)	QS (100)

OILGELS**Formulations
(Amounts)**

RAW MATERIAL (INCI Designation)	1	2	3	4
Caprylic/Capric Triglycerid	12.00	10.00	6.00	
Octyldodecanol	7.00	14.00	8.00	3.00
Butylene Glycol				12.00
Dicaprylat/Dicaprat				
Pentaerythrityl Tetraisostearat	10.00	6.00	8.00	7.00
Polyglyceryl-3 Diisostearat	2.50			
Bis-Diglyceryl Polyacyladipate-2	9.00	8.00	10.00	8.00
Myristyl Myristate	3.50	3.00	4.00	3.00
Bentone -34	5.00	5.00	6.00	6.00
Propylencarbonat	15.00	20.00	18.00	19.50
Compound	SA	SA	SA	SA
Vitamin E Acetat	0.50	1.00		
Ascorbyl Palmitat	0.05		0.05	
Buxux Chinensis	2.00	1.00		1.00
Perfume, BHT	0.10	0.25		0.35
Ricinus Communis	QS (100)	QS (100)	QS (100)	QS (100)

In still further embodiments, the present invention comprises at least one inorganic pigment. In some preferred embodiments, these inorganic pigments are based on metaloxides and/or other water slightly soluble or insoluble metal compounds, including but not limited to compounds such as zinc oxides (ZnO), titanium (TiO₂), iron (*e.g.*, Fe₂O₃), zirconium (ZrO₂), silica (SiO₂), manganese (*e.g.*, MnO), aluminium (Al₂O₃), cer (*e.g.*, Ce₂O₃), and mixed oxides of these oxides, as well as blends thereof. In some embodiments, the metaloxides are microfine grade, while in other embodiments, the metaloxides are pigment grade. In further embodiments, the metaloxides are a mixture of microfine and pigment grades.

In additional embodiments, the inorganic pigments are coated (*i.e.*, they are treated on the surface).

In some particularly preferred embodiments, the surface is coated with a thin, hydrophobic film. In some other particularly preferred embodiments, the surface is coated with a thin, hydrophilic film. In yet additional embodiments, the present invention provides compositions comprising various make ups and make up constituents. For example, in some embodiments, the present invention provides various dyes and/or pigments. In some embodiments, useful pigments include, but are not limited to titanium dioxide, mica, iron oxides (*e.g.*, Fe₂O₃, Fe₃O₄, FeO(OH), etc.) and/or stannous oxide. The present invention further provides colorants, including but not limited to carmine, blue, chromooxide, ultramarine and/or purple manganese. The colorants and pigments of some most preferred embodiments are known to those in the art and provided previously (*See e.g.*, Colour Index Nummern (CIN), Rowe Colour Index, 3rd ed., Society of Dyers and Colourists, Bradford, England [1971]).

In additional embodiments, pearlescent pigments based on mica/metaloxide find use, as described above. However, it is not intended that the present invention be limited to these particular pigments, as additional pearlescent pigments find use in various embodiments of the present invention.

The following formulations provide additional examples of the use of the present invention.

RAW MATERIAL (INCI Designation)	Formulation (Amounts)				
	1	2	3	4	5
Sodium Carbomer		0.2			
Acrylates/C ₁₀ -C ₃₀ Alkyl Acrylate Crosspolymer	0.3	0.2	0.6		
Hydroxypropyl Cellulose				1.0	1.50
Xanthan Gummi		0.6	0.2	1.0	1.0
Compound	0.5	0.1	0.01	0.01	1.0
Diocetyl Butamidotriazon	2.0	2.0	1.0		
Ethylhexyl Triazon	4.0	4.0	5.0		
Aniso Triazin	1.0	0.5		2.0	2.5
Bisocetyltriazol				6.0	
Drometrizole Trisiloxane					
PhenylbenzimidazSulfonicacid	2.0			1.0	

	Formulation (Amounts)				
RAW MATERIAL (INCI Designation)	1	2	3	4	5
Bisimidazylate				1.0	
Terephthalylidene Dicamphor Sulfonic Acid				0.2	
Ethylhexyl Methoxycinnamat	7.5	10.0		5.0	
Octocrylen					5.0
Dimethicone-diethylbenzalmalonate				4.0	
Ethylhexyl Salicylate					
Homosalate					
Butyl Methoxydibenzoylmethan	1.0	1.0	4.0		
Titan dioxide	1.0	4.0			
Zinc oxide				4.0	
Caprylic/Capric Triglycerid			2.0		
Hydrogenated Coco-Glyceride			3.0		
C12-15 Alkyl Benzoat	2.0	2.5	3.0		
Dicaprylyl Ether		4.0			
Butylenglycol Dicaprylat/Dicaprat	4.0		2.0	6.0	
Dicaprylyl Carbonat		2.0			
Cetyl Dimethicon	2.0	0.5	1.0		
Shea Butter		2.0			
PVP Hexadecen Copolymer	0.5		0.05	0.5	
Glycerin	3.0	7.5		7.5	2.5
Tocopherol		0.5	0.75		0.2
Trisodium EDTA	1.0	0.5	0.5	1.0	1.5
Natriumcitrat		0.2			
Zitronensäure		0.1		0.1	0.1
DMDM Hydantoin		0.6		0.2	
Methylparaben	0.5		0.3	0.15	
Phenoxyethanol	0.5	0.4	0.4	1.0	0.60
Ethanol	3.0	2.0	3.0		1.0
Perfume	0.2			0.2	0.2
Water	QS (100)	QS (100)	QS (100)	QS (100)	QS (100)

	Formulations (Amounts)				
RAW MATERIAL (INCI Designation)	1	2	3	4	5
Sodium Carbomer	0.5			1.5	
Acrylates/C ₁₀ -C ₃₀ Alkyl Acrylate Crosspolymer		0.4	0.1		0.75
Hydroxypropyl Cellulose			0.5		0.25
Xanthan Gummi	0.2	0.4			
Compound	0.5	0.1	0.01	0.01	1.0
Diocetyl Butamidotriazon	1.0		2.0		

RAW MATERIAL (INCI Designation)	Formulations (Amounts)				
	1	2	3	4	5
Ethylhexyl Triazon		2.0		2.0	
Aniso Triazin	1.0	0.2	3.0	1.0	
Bisoctyltriazol					8.0
Drometrizole Trisiloxane					4.0
Phenylbenzimidazole Sulfonicacid		1.5			
Bisimidazylate			1.5		
Terephthalylidene Dicamphor Sulfonic Acid					0.5
Ethylhexyl Methoxycinnamat		7.5	5.0	10.0	
Octocrylen	10.0		5.0		5.0
Dimethicone-diethylbenzalmalonate					2.5
Ethylhexyl Salicylate			3.5	5.0	
Homosalate			4.0		
Butyl Methoxydibenzoylmethan	0.5				
Titandioxide	1.5	2.0	1.0		2.5
Zincoxide			1.0		0.5
Caprylic/Capric Triglycerid					
Hydrogenierte Coco-Glyceride					
C12-15 Alkyl Benzoat					5.0
Dicaprylyl Ether					7.5
Butylenglycol					
Dicaprylat/Dicaprat					
Dicaprylyl Carbonat		7.5			
Cetyl Dimethicon					
Shea Butter					3.0
PVP Hexadecen Copolymer	0.5		0.75		1.0
Glycerin	5.0		10.0		
Tocopherol	0.3		1.5		1.0
Trisodium EDTA	0.5		0.1	0.5	
Natriumcitrat			0.3		
Zitronensäure			0.15		
DMDM Hydantoin				0.3	0.15
Methylparaben		0.4			
Phenoxyethanol		1.0			
Ethanol	7.5		5.0		7.0
Perfume		0.25		0.2	
Water	QS (100)	QS (100)	QS (100)	QS (100)	QS (100)

RAW MATERIAL (INCI Designation)	Formulations (Amounts)					
	1	2	3	4	5	6
Sodium Carbomer	0.5	1.5	1.0			0.5
Acrylates/C ₁₀ -C ₃₀ Alkyl Acrylate Crosspolymer	1.0			0.75	1.0	
Hydroxypropyl Cellulose			0.4	1.0		1.0
Xanthan Gummi		0.6	0.2	1.0	1.0	
BBI	4.0	0.5	3.0	2.0	4.0	1.5
Dioctyl Butamidotriazon	2.0	2.0		2.0		1.0
Ethylhexyl Triazon		4.0	5.0	4.0		
Aniso Triazin	1.0			1.0	2.5	1.0
Bisocetyltriazol			4.0			
Drometrizole Trisiloxane		3.0				
Phenylbenzimidazole Sulfonicacid	2.0			1.0		
Bisimidazylate			1.5			3.5
Terephthalylidene Dicamphor Sulfonic Acid				0.2		1.0
Ethylhexyl Methoxycinnamat		10.0		5.0		
Octocrylen	10.0				5.0	
Dimethicone- diethylbenzalmalonate				4.0		
Ethylhexyl Salicylate						5.0
Homosalate				5.0		
Butyl Methoxy- dibenzoylmethan	1.0	1.0	4.0			0.5
Titandioxide	1.0	4.0				1.5
Zincoxide				4.0		
Caprylic/Capric Triglycerid			2.0			
Paraffinöl				1.0		
C ₁₂ -C ₁₅ Alkyl Benzoat	2.0	2.5	3.0			
Dicaprylyl Ether		4.0				
Isohexadecen	4.0		2.0	6.0		
Dicaprylyl Carbonat		2.0				
Dibutyl Adipat	2.0	0.5	1.0			
Cylomethicon				3.0		
Jojobaöl		2.0				
PVP Hexadecen Copolymer	0.5		0.05	0.5		0.5
Butylen Glycol	3.0	7.5		7.5	2.5	5.0
Ascorbyl-Palmitate		0.5	0.75		0.2	0.3

158

RAW MATERIAL (INCI Designation)	Formulations (Amounts)					
	1	2	3	4	5	6
Octoxyglycerin		1.0		0.5		1.0
Glycin Soja			2.0			1.5
Trisodium EDTA	1.0	0.5	0.5		1.5	0.5
Caustic acid	1.0	0.2	0.25			
Iodopropyl Butylcarbamate		0.6		0.2		
Phenoxyethanol		0.4		1.0		
Ethanol	5.0	2.0	7.0			
Perfume	0.2			0.2	0.2	
Water	QS (100)	QS (100)	QS (100)	QS (100)	QS (100)	QS (100)

The following formula provides an example of an after-shave product comprising the BBI-AV of the present invention.

5

AFTER SHAVE LOTION

	%	Ingredient	INCI
10	A	10.0 Luvitol EHO	Cetearyl Ethylhexanoate
		5.0 Vitamin E Acetate	Tocopheryl Acetate
		1.0 Bisabolol rac.	Bisabolol
		0.1 Perfume	
15		0.3 Carbopol Ultrez 21	Acrylates/C10-30 Alkyl Acrylate Crosspolymer
	B	15.0 Ethanol	Alcohol
		1.0 D-Panthenol USP	Panthenol
		3.0 Glycerin 87%	Glycerin
		0.1 Triethanolamine Care	Triethanolamine
20	SA	Compound	
	QS	Water dem.	Aqua dem.

Production: Weigh out the components of Phase A and mix them. Dissolve Phase B, stir it into Phase A and homogenize well.

25

Measure values:

Viscosity: 18 500mPa s Brookfield RVD II+
pH value: 5.8

30

The following formula provides an example of an after-shave product comprising the BBI-AV of the present invention.

PRE SHAVE

		%	Ingredient	INCI
5	A	80	Ethanol	Alcohol
		3.0	Vitamin E Acetate	Tocopheryl Acetate
		1.0	Bisabolol rac.	Bisabolol
		0.2	Perfume	
10		0.1	Menthol	Menthol
		4.0	Luvitol EHO	Cetearyl Ethylhexanoate
		2.0	Eutanol G	Octyldodecanol
		2.0	Miglyol 812	Caprylic/Capric Triglyceride
		2.0	D-Panthenol USP	Panthenol
15		2.0	Whitch Hazel Distillate	Hamamelis Virginiana (Whitch Hazel) Distillate
		2.0	Jojoba Oil	Simmondsia Chinensis (Jojoba) Seed Oil
		SA	Compound	

Production: Weigh out the components of Phase A and dissolve them clearly.

The after-shave and pre-shave formula provided above contain sufficient BBI-AV (Compound) to provide the desired effect(s). In some embodiments, the concentration of BBI-AV is in the range of about 1,000 ppm to about 10,000 ppm. In the following formulations, typical concentrations of BBI-AV used range from about 100 ppm to about 1,000 ppm or from about 1,000 ppm to about 10,000 ppm. However, it is not intended that the present invention be limited to this specific concentration range, as other concentrations find use in other embodiments of the present invention.

The following formula provides an example of an after-sun product comprising the BBI-AV of the present invention.

AFTER SUN LOTION

		%	Ingredient	INCI
35	A	0.4	Carbopol 1342	Acrylates/C10-30 Alkyl Acrylate Crosspolymer
		15.0	Luvitol EHO	Cetearyl Ethylhexanoate
		0.2	Bisabolol rac.	Bisabolol
		1.0	Vitamin E Acetate	Tocopheryl Acetate
		q.s.	Perfume	
40	B	1.0	D-Panthenol USP	Panthenol
		15.0	Ethanol 96%	Alcohol
		3.0	Glycerin 87%	Glycerin
		SA	Compound	
45		64.2	Water dem.	Aqua dem.
	C	0.2	Triethanolamine Care	Triethanolamine

Production: Mix the components of Phase A. Dissolve Phase B and stir it into Phase A whilst homogenizing. Neutralize with Phase C and homogenize again.

5 **Measure values:**

Viscosity: 7 500mPa s Haake Viscotester VT-02

pH value: 6.0

10 The following formula provides an example of a facial cleanser product comprising the BBI-AV of the present invention.

15 **FACIAL CLEANSER**

	%	Ingredient	INCI
20	A 10.0	Luvitol EHO	Cetearyl Ethylhexanoate
	10.0	Miglyol 812	Caprylic/Capric Triglyceride
	1.5	Dow Corning 345 Fluid	Cyclopentasiloxane, Cyclohexasilosane
	2.0	Cremophor CO 40	PEG-40 Hydrogenated Castor Oil
25	B 3.5	Luvigel EM	Caprylic/Capric Triglyceride, Sodium Acrylates Copolymer
30	C 1.0	Vitamin E Acetate	Tocopheryl Acetate
	0.2	Bisabolol rac.	Bisabolol
	QS	Preservative	
	QS	Perfume	
35	D 3.0	Luviquat Care	Polyquaternium-44
	0.5	Luviquat Mono LS	Cocotrimonium Methosulfate
	0.5	Cremophor A 25	Cetareth-25
	0.2	D-Panthenol 50 P	Panthenol, Propylene Glycol
	4.0	1,2 Propylene Glycol Care	Propylene Glycol
	0.1	Edeta BD	Disodium EDTA
	SA	Compound	
	QS	Water dem.	Aqua dem.

40 **Production:** Dissolve Phase A, then stir in Phase B. Fold in Phase C. Dissolve Phase D, stir it into the combined Phases A+B+C, homogenize and stir again for 15min.

Measure values:

Viscosity: 7 200mPa s Brookfield RVT
pH value: 5.8

5

The following formula provides an example of a daily care body spray product with SPF 8 comprising the BBI-AV of the present invention.

10 **DAILY CARE BODY SPRAY – SPF 8**

	%	Ingredient	INCI
15	A 3.0	Uvinul MC 80	Ethylhexyl Methoxycinnamate
	2.0	Uvinul A Plus™	Diethylamino Hydroxybenzoyl Hexyl Benzoate
	1.0	Luviquat UltraCare	Polyquaternium-44
	3.0	1,2 Propylenglycol Care	Propylene Glycol
	2.0	D-Panthenol 50 P	Panthenol, Propylene Glycol
20	1.0	Dow Corning 345 Fluid	Cyclopentasiloxane, Cyclohexasiloxane
	10.0	Eutanol G	Octyldodecanol
	0.5	Luviskol K 30	PVP
	10.0	Miglyol 812	Caprylic/Capric Triglyceride
	3.0	Finsolv TN	C12-15 Alkyl Benzoate
25	3.0	Glycerin 87%	Glycerin
	1.0	Vitamin E Acetate	Tocopheryl Acetate
	0.3	Bisabolol rac. Compound	Bisabolol
	QS	Ethanol	Alcohol

30 **Production:** Weigh out the components of Phase A and dissolve them clearly.

Measure values:

SPF: 8 Colipa Task Force "Sun Protection Measurement"

35

The following formula provides an example of a daily care sun care lotion product with SPF 27 comprising the BBI-AV of the present invention.

40

SUN CARE LOTION - SPF 27

		%	Ingredient	INCI
5	A	4.5	Uvinul MC 80	Ethylhexyl Methoxycinnamate
		2.0	Uvinul A Plus TM	Diethylamino Hydroxybenzoyl Hexyl Benzoate
		3.0	Uvinul N 539 T	Octocrylene
		2.5	Cosmacol EMI	Di-C12-13 Alkyl Malate
10		0.5	Vitamin E Acetate	Tocopheryl Acetate
		4.0	Tego Care 450	Polyglyceryl-3 Methyl Glucose Distearate
	B	3.5	Cetiol SN Deo	Cetearyl Isononanoate
		1.0	Ganex V-220	VP/Eicosene Copolymer
15		5.0	Isohexadecane	Isohexadecane
		2.5	Cosmacol EMI	Di-C12-13 Alkyl Malate
		3.0	Uvinul TiO ₂	Titanium Dioxide, Trimethoxycaprylylsilane
	C	5.0	Glycerin 87%	Glycerin
20		1.0	Lanette E	Sodium Cetearyl Sulfate
		0.5	Keltrol	Xanthan Gum
		60.7	Water dem.	Aqua dem.
	D	SA	Compound	
25		1.0	Phenonip	Phenoxyethanol, Methylparaben, Ethylparaben,
		0.3	Bisabolol rac.	Bisabolol

30 **Production:** Heat Phases A and B separately to about 80°C. Stir Phase B into Phase A whilst homogenizing. Heat Phase C to about 80°C and stir it into the combined Phases A+B whilst homogenizing. Cool to about 40°C add Phase D and homogenize again.

Measure values:

35 Viscosity: 3 200mPa s Brookfield RVD II+
 pH value: 6.0
 SPF: 27 Colipa Task Force "Sun Protection Measurement"

163

SUN CARE LOTION – SPF 24

		%	Ingredient	INCI
5	A	2.0	Cremophor A 6	Ceteareth-6, Stearyl Alcohol
		2.0	Cremophor A 25	Ceteareth-25
		3.0	Syncrowax HRC	Tribehenin
		2.0	Lanette O	Cetearyl Alcohol
		2.0	Luvitol EHO	Cetearyl Ethylhexanoate
10		5.0	Uvinul MC 80	Ethylhexyl Methoxycinnamate
		1.0	Uvinul T 150	Ethylhexyl Triazone
		1.0	Ganex V-220	VP/Eicosene Copolymer
		7.0	Isopropyl Myristate	Isopropyl Myristate
15	B	5.0	Z-Cote HP-1	Zinc Oxide, Triethoxycaprylylsilane
	C	0.2	Keltrol	Hydroxyethyl Acrylate/Sodium Acryloyldimethyl Taurate Copolymer, Squalane, Polysorbate 60
		0.2	Edeta BD	Disodium EDTA
20		5.0	1,2 Propylene Glycol Care	Propylene Glycol
		0.5	D-Panthenol USP	Panthenol
		61.9	Water dem.	Aqua dem.
25	D	SA	Compound	
		0.5	Euxyl K 300	Phenoxyethanol, Methylparaben, Butylparaben, Ethylparaben, Propylparaben, Isobutylparaben
		1.0	Vitamin E Acetate	Tocopheryl Acetate
		0.2	Bisabolol rac.	Bisabolol

30

Production: Heat Phase A to 80°C, add Phase B and homogenize for 3min. Heat Phase C to about 80°C, and stir it into the combined Phases A+B whilst homogenizing. Cool to about 40°C, add Phase D, and homogenize.

35 **Measure values:**

Viscosity: 5 000mPa s Brookfield RVD II+
 pH value: 7.5
 SPF: 24 Colipa Task Force "Sun Protection Measurement"

40

The following formula provides an example of a sun screen emulsion product with SPF 28 comprising the BBI-AV of the present invention.

164

SUN SCREEN EMULSION – SPF 28

		%	Ingredient	INCI
5				
	A	3.5	Cremophor A 6	Ceteareth-6, Stearyl Alcohol
		1.5	Cremophor A 25	Ceteareth-25
		7.5	Uvinul MC 80	Ethylhexyl Methoxycinnamate
		2.0	Uvinul A Plus™	Diethylamino Hydroxybenzoyl Hexyl Benzoate
10		2.0	Dow Corning 345 Fluid	Cyclopentasiloxane, Cyclohexasiloxane
		0.5	Bees Wax 3044 PH	Bees Wax
		3.0	Lanette O	Cetearyl Alcohol
		10.0	Miglyol 812	Caprylic/Capric Triglyceride
15	B	5.0	T-Lite SF-S	Titanium Dioxide, Silica, Methicone, Alumina
	C	3.0	Glycerin 87%	Glycerin
		0.2	Edeta BD	Disodium EDTA
		0.3	Keltrol T	Xanthan Gum
20		1.0	Plantacare 2000	Decyl Glucoside
		2.0	D-Panthenol 50 P	Panthenol, Propylene Glycol
		57.3	Water dem.	Aqua dem.
25	D	SA	Compound	
		1.0	Vitamin E Acetate	Tocopheryl Acetate
		0.2	Bisabolol rac.	Bisabolol
		QS	Perfume	
		QS	Preservative	

30

Production: Heat Phase A to 80°C, add Phase B and homogenize for 3min. Heat Phase C to about 80°C, and stir it into the combined Phases A+B whilst homogenizing. Cool to about 40°C, add Phase D and homogenize.

35

Measure values:

Viscosity: 7 500mPa s Brookfield RVD II+
 pH value: 6.6
 SPF: 28 Colipa Task Force "Sun Protection Measurement"

40

The following formula provides an example of a foot balm product comprising the BBI-AV of the present invention.

45

165

FOOT BALM

		%	Ingredient	INCI
5	A	2.0	Cremophor A 6	Ceteareth-6, Stearyl Alcohol
		2.0	Cremophor A 25	Ceteareth-25
		5.0	Luvitol EHO	Cetearyl Ethylhexanoate
		4.0	Lanette 16	Cetyl Alcohol
		4.0	Cutina Gms	Glyceryl Stearate
10		5.0	Paraffin Oil	Mineral Oil
		0.2	Menthol	Menthol
		0.5	Camphor	Camphor
15	B	70.3	Water dem.	Aqua dem.
		QS	Preservative	
	C	SA	Compound	
		1.0	Bisabolol rac.	Bisabolol
20		1.0	Vitamin E Acetate	Tocopheryl Acetate
	D	5.0	Witch Hazel Extract	Witch Hazel Extract

25 **Production:** Heat Phases A and B to about 80°C separately. Stir Phase B into Phase A whilst homogenizing. Cool to about 40°C, add Phases C and D and homogenize again. Cool to room temperature.

Measure values:

Viscosity: 20 500mPa s Brookfield RVD II+
pH value: 6.0

30

The following formula provides an example of a refreshing foot gel product comprising the BBI-AV of the present invention.

REFRESHING FOOT GEL

		%	Ingredient	INCI
5				
	A	0.6	Carbopol Ultrez 21	Acrylates/C10-30 Alkyl Acrylate Crosspolymer
		45.9	Water dem.	Aqua dem.
	B	1.0	Bisabolol rac.	Bisabolol
10		0.5	Farnesol	Farnesol
		q.s.	Perfume	
		4.5	Cremophor CO 40	PEG-40 Hydrogenated Castor Oil
		1.0	Neutrol TE	Tetrahydroxypropyl Ethylenediamine
		1.5	Menthol	Menthol
15	SA		Compound	
		45.0	Ethanol 96%	Alcohol
	QS		FD&C Blue No. 1	C.I. 42 090, FD&C Blue No. 1

20 **Production:** Phase A: Intersperse the Carbopol and let it settle on the bottom of the beaker. Dissolve Phase B and stir it into Phase A.

Measure values:

Viscosity: 14 500mPa s Brookfield RVD II+

25 pH value: 7.5

The following formula provides an example of a skin conditioning gel product comprising the BBI-AV of the present invention.

30

Skin Conditioning Gel

		%	Ingredient	INCI
35				
	A	3.6	Cremophor CO 40	PEG-40 Hydrogenated Castor Oil
		15.0	Ethanol	Alcohol
		0.1	Bisabolol rac.	Bisabolol
		0.5	Vitamin E Acetate	Tocopheryl Acetate
40		QS	Perfume	
	B	3.0	D-Panthenol USP	Panthenol
		0.6	Carbopol 940	Carbomer
	SA		Compound	
45		76.4	Water dem.	Aqua dem.
	C	0.8	Triethanolamine Care	Triethanolamine

Production: Dissolve Phase A clearly. Allow Phase B to swell and neutralize with Phase C. Stir Phase A into the neutralized Phase B and homogenize.

5 **Measure values:**

Viscosity: 57 600mPa s Brookfield RVD II+
pH value: 7.7

10 The following formula provides an example of a W/O emulsion comprising the BBI-AV of the present invention.

W/O EMULSION

15		%	Ingredient	INCI
	A	6.0	Cremophor WO 7	PEG-7 Hydrogenated Castor Oil
		8.0	Luvitol EHO	Cetearyl Ethylhexanoate
		5.0	Isopropyl Myristate	Isopropyl Myristate
20		15.0	Paraffin Oil	Mineral Oil
		0.3	Magnesium Stearate	Magnesium Stearate
		0.3	Aluminum Stearate	Aluminum Stearate
		2.0	Elfacos ST9	PEG-45/Dodecyl Glycol Copolymer
25	B	5.0	Glycerin 87%	Glycerin
		0.7	Magnesium Sulfate-7-hydrate	Magnesium Sulfate
		56.6	Water dem.	Aqua dem.
30	C	SA	Compound	
		0.5	Vitamin E Acetate	Tocopheryl Acetate
		0.6	Bisabolol rac.	Bisabolol

Production: Heat Phases A and B separately to about 85°C. Stir Phase B into Phase A and homogenize.
35 Cool to about 40°C whilst stirring, add Phase C and homogenize again. Cool to room temperature.

Measure values:

Viscosity: 37 500mPa s Brookfield RVD II+

40

The following formula provides an example of a O/W emulsion product comprising the BBI-AV of the present invention.

O/W EMULSION

		%	Ingredient	INCI
5				
	A	1.7	Cremophor A 6	Ceteareth-6, Stearyl Alcohol
		0.7	Cremophor A 25	Ceteareth-25
		2.0	Uvinul A Plus TM	Diethylamino Hydroxybenzoyl Hexyl Benzoate
10		2.0	Abil B 8843	PEG-14 Dimethicone
		3.6	Lanette O	Cetearyl Alcohol
		6.0	Uvinul MC 80	Ethylhexyl Methoxycinnamate
		2.0	Cetiol B	Dibutyl Adipate
15	B	5.0	Glycerin 87%	Glycerin
		0.2	Edeta BD	Disodium EDTA
		1.0	D-Panthenol 75 W	Panthenol
		q.s.	Preservative	
		68.8	Water dem.	Aqua dem.
20	C	4.0	Luvigel EM	Caprylic/Capric Triglyceride, Sodium Acrylates Copolymer
25	D	0.2	Sodium Ascorbyl Phosphate	Sodium Ascorbyl Phosphate
		1.0	Vitamin E Acetate	Tocopheryl Acetate
		0.2	Bisabolol rac.	Bisabolol
	E	q.s.	Sodium Hydroxide 10% aq. w/w	Sodium Hydroxide
30	F	1.0	RetiSTAR	Caprylic/Capric Triglyceride, Sodium Ascorbate, Tocopherol, Retinol
	SA		Compound	

35 **Production:** Heat Phase A and B separately to about 80°C. Stir Phase B into Phase A and homogenize. Stir Phase C into the combined Phases A+B and homogenize. Cool to about 40°C, add Phase D, then adjust the pH value with Phase E to 6.5. Add Phase F and homogenize. Cool to room temperature.

Measure values:

40 Viscosity: 37 500mPa s Brookfield RVD II+
pH value: 6.3

The following formula provides an example of a protective day cream product comprising the BBI-
45 AV of the present invention.

PROTECTIVE DAY CREAM

		%	Ingredient	INCI
5				
	A	1.7	Cremophor A 6	Ceteareth-6, Stearyl Alcohol
		0.7	Cremophor A 25	Ceteareth-25
		2.0	Uvinul A Plus TM	Diethylamino Hydroxybenzoyl Hexyl Benzoate
10		2.0	Abil B 8843	PEG-14 Dimethicone
		3.6	Lanette O	Cetearyl Alcohol
		6.0	Uvinul MC 80	Ethylhexyl Methoxycinnamate
		2.0	Cetiol B	Dibutyl Adipate
15	B	5.0	Glycerin 87%	Glycerin
		0.2	Edeta BD	Disodium EDTA
		1.0	D-Panthenol 75 W	Panthenol
		QS	Preservative	
		69.6	Water dem.	Aqua dem.
20	C	4.0	Luvigel EM	Caprylic/Capric Triglyceride, Sodium Acrylates Copolymer
25	D	1.0	Sodium Ascorbyl Phosphate	Sodium Ascorbyl Phosphate
		1.0	Vitamin E Acetate	Tocopheryl Acetate
		SA	Compound	
		0.2	Bisabolol rac.	Bisabolol
30	E	QS	Sodium Hydroxide 10% aq. w/w	Sodium Hydroxide

Production: Heat Phase A and B separately to about 80°C. Stir Phase B into Phase A and homogenize. Stir Phase C into the combined Phases A+B and homogenize. Cool to about 40°C, add Phase D, then adjust the pH value with Phase E to 6.5 and homogenize. Cool to room temperature.

Measure values:

Viscosity: 24 000mPa s Brookfield RVD II+
pH value: 6.4

The following formulae provide examples of hair care products comprising the BBI-AV of the present invention.

HAIR CONDITIONERS

		%	Ingredients (INCI)
5			
	A	10.0	PVP/VA Copolymer
		0.2	Hydroxyethyl Cetyldimonium Phosphate
		0.2	Ceteareth-25
		0.5	Dimethicone Copolyol
10		QS	Perfume
		10.0	Alcohol
		SA	Compound
		68.1	Aqua dem.
15	B	10.0	Propane/Butane

		%	Ingredients (INCI)
20	A	10.0	PVP/VA Copolymer
		0.2	Hydroxyethyl Cetyldimonium Phosphate
		0.2	Ceteareth-25
		0.5	Dimethicone Copolyol
		QS	Perfume
25		10.0	Alcohol
		SA	Compound
		64.1	Aqua dem.
	B	10.0	Propane/Butane

30

Production: Add all compounds to Phase A and stir to homogenize. Fill into appropriate container and charge with Phase B.

35 FOAM CONDITIONERS

		%	Ingredients (INCI)
40	A	1.0	Polyquaternium-4
		0.5	Hydroxyethyl Cetyldimonium Phosphate
			Compound
		QS	Perfume
		QS	Preservative
45		91.5	Aqua dem.
	B	6.0	Propane/Butane

171

	%	Ingredients (INCI)
5	A 1.0	Polyquaternium-4
	0.5	Hydroxyethyl Cetyldimonium Phosphate Compound
	QS	Perfume
	QS	Preservative
	87.5	Aqua dem.
10	B 6.0	Propane/Butane

Production: Add all compounds to Phase A and stir to homogenize. Fill into appropriate container and charge with Phase B.

15

FOAM CONDITIONERS

20	%	Ingredients (INCI)
25	A 1.0	Polyquaternium-11
	0.5	Hydroxyethyl Cetyldimonium Phosphate Compound
	QS	Perfume
	QS	Preservative
	91.5	Aqua dem.
	B 6.0	Propane/Butane

30

	%	Ingredients (INCI)
35	A 1.0	Polyquaternium-11
	0.5	Hydroxyethyl Cetyldimonium Phosphate Compound
	SA	Compound
	QS	Perfume
	QS	Preservative
40	87.5	Aqua dem.
	B 6.0	Propane/Butane

Production: Add all compounds to Phase A and stir to homogenize. Fill into appropriate container and charge with Phase B.

STYLING FOAMS

		%	Ingredients (INCI)
5			
	A	0.5 QS	Laureth-4 Perfume
10	B	77.3 10.0 0.5 0.2 0.2 0.1 0.2	Aqua dem. Polyquaternium-28 Compound Dimethicone Copolyol Ceteareth-25 Panthenol PEG-25 PABA Hydroxyethylcellulose
15			
	C	10.0	HFC 152 A
20			

		%	Ingredients (INCI)
25			
	A	0.5 QS	Laureth-4 Perfume
30	B	73.3 10.0 SA 0.5 0.2 0.2 0.1 0.2	Aqua dem. Polyquaternium-28 Compound Dimethicone Copolyol Ceteareth-25 Panthenol PEG-25 PABA Hydroxyethylcellulose
35			
	C	10.0	HFC 152 A

40 **Production:** Weight out the compounds of Phase A and mix them. Dissolve Phase B, stir into Phase A and homogenize. Fill into appropriate container and charge with Phase C.

		%	Ingredients (INCI)
45			
	A	2.0 QS	Cocotrimonium Methosulfate Perfume
50	B	78.5 6.7	Aqua dem. Acrylates Copolymer

173

5		0.6	AMP
		SA	Compound
		0.5	Dimethicone Copolyol
		0.2	Ceteareth-25
		0.2	Panthenol
		0.1	PEG-25 PABA
		0.2	Hydroxyethylcellulose
10	C	10.0	HFC 152 A
		%	Ingredients (INCI)
15	A	2.0	Cocotrimonium Methosulfate
		QS	Perfume
20	B	74.5	Aqua dem.
		6.7	Acrylates Copolymer
		0.6	AMP
		SA	Compound
		0.5	Dimethicone Copolyol
		0.2	Ceteareth-25
25		0.2	Panthenol
		0.1	PEG-25 PABA
		0.2	Hydroxyethylcellulose
	C	10.0	HFC 152 A
30			

Production: Weight out the compounds of Phase A and mix them. Dissolve Phase B, stir into Phase A and homogenize. Fill into appropriate container and charge with Phase C.

35

STYLING FOAM

		%	Ingredients (INCI)
40	A	2.0	Cocotrimonium Methosulfate
		QS	Perfume
45	B	7.70	Polyquaternium-44
		SA	Compound
		QS	Preservative
		79.3	Aqua dem.
	C	10.0	Propane/Butane

50

Production: Weight out the compounds of Phase A and mix them. Dissolve Phase B, stir into Phase A and homogenize. Adjust pH to 6 –7. Fill into appropriate container and charge with Phase C.

5

STYLING FOAM

		%	Ingredients (INCI)
10	A	2.00 QS	Cocotrimonium Methosulfate Perfume
15	B	72.32 2.00 0.53 SA 0.20 0.50	Aqua dem. VP/Acrylates/Lauryl Methacrylate Copolymer AMP Compound Ceteareth-25 Panthenol
20		0.05 0.20 15.00	Benzophenone-4 Amodimethicone, Cetrimonium Chloride, Trideceth-12 Alcohol
25	C	0.20	Hydroxyethylcellulose
	D	6.00	Propane/Butane

		%	Ingredients (INCI)
30	A	2.00 QS	Cocotrimonium Methosulfate Perfume
35	B	68.32 2.00 0.53 SA 0.20 0.50	Aqua dem. VP/Acrylates/Lauryl Methacrylate Copolymer AMP Compound Ceteareth-25 Panthenol
40		0.05 0.20 15.00	Benzophenone-4 Amodimethicone, Cetrimonium Chloride, Trideceth-12 Alcohol
45	C	0.20	Hydroxyethylcellulose
	D	6.00	Propane/Butane

50 **Production:** Weight out the compounds of Phase A and mix them. Dissolve Phase B, stir into Phase A and homogenize. Add Phase C and homogenize again. Adjust pH to 6-7. Fill into appropriate container and charge with Phase D.

175

STYLING FOAMS

		%	Ingredients (INCI)
5	A	2.00 QS	Cetrimonium Chloride Perfume
	B	67.85	Aqua dem.
		7.00	Polyquaternium-46
10	SA		Compound
		0.20	Ceteareth-25
		0.50	Panthenol
		0.05	Benzophenone-4
		0.20	Amodimethicone, Cetrimonium Chloride, Trideceth-12
15		15.00	Alcohol
	C	0.20	Hydroxyethylcellulose
	D	6.00	Propane/Butane
20			

		%	Ingredients (INCI)
25	A	2.00 QS	Cetrimonium Chloride Perfume
	B	63.85	Aqua dem.
		7.00	Polyquaternium-46
30	SA		Compound
		0.20	Ceteareth-25
		0.50	Panthenol
		0.05	Benzophenone-4
35		0.20	Amodimethicone, Cetrimonium Chloride, Trideceth-12
		15.00	Alcohol
	C	0.20	Hydroxyethylcellulose
40	D	6.00	Propane/Butane

Production: Weight out the compounds of Phase A and mix them. Dissolve Phase B, stir into Phase A and homogenize. Add Phase C and homogenize again. Adjust pH to 6-7. Fill into appropriate container and charge with Phase D.

45

176

STYLING FOAMS

		%	Ingredients (INCI)
5	A	QS	PEG-40 Hydrogenated Castor Oil
		QS	Perfume
		85.5	Aqua dem.
	B	7.0	Sodium Polystyrene Sulfonate
10		SA	Compound
		0.5	Cetrimonium Bromide
		QS	Preservative
	C	6.0	Propane/Butane
15			
		%	Ingredients (INCI)
20	A	QS	PEG-40 Hydrogenated Castor Oil
		QS	Perfume
		81.5	Aqua dem.
25	B	7.0	Sodium Polystyrene Sulfonate
		SA	Compound
		0.5	Cetrimonium Bromide
		QS	Preservative
30	C	6.0	Propane/Butane

Production: Weight out the compounds of Phase A and mix them. Dissolve Phase B, stir into Phase A and homogenize. Adjust pH to 6-7. Fill into appropriate container and charge with Phase C.

35

STYLING FOAMS

5		%	Ingredients (INCI)
	A	QS	PEG-40 Hydrogenated Castor Oil
		QS	Perfume
		92.0	Aqua dem.
10	B	0.5	Polyquaternium-10
		1.0	Compound
		0.5	Cetrimonium Bromide
		QS	Preservative
15	C	6.0	Propane/Butane

		%	Ingredients (INCI)
20	A	QS	PEG-40 Hydrogenated Castor Oil
		QS	Perfume
		88.0	Aqua dem.
25	B	0.5	Polyquaternium-10
		5.0	Compound
		0.5	Cetrimonium Bromide
		QS	Preservative
30	C	6.0	Propane/Butane

Production: Weight out the compounds of Phase A and mix them. Dissolve Phase B, stir into Phase A and homogenize. Adjust pH to 6-7. Fill into appropriate container and charge with Phase C.

35

		%	Ingredients (INCI)
40	A	QS	PEG-40 Hydrogenated Castor Oil
		QS	Perfume
		82.5	Aqua dem.
45	B	10.0	Polyquaternium-16
		SA	Compound
		0.5	Hydroxyethyl Cetyldimonium Phosphate
		QS	Preservative
50	C	6.0	Propane/Butane

178

	%	Ingredients (INCI)
5	A QS	PEG-40 Hydrogenated Castor Oil
	QS	Perfume
	78.5	Aqua dem.
10	B 100	Polyquaternium-16
	SA	Compound
	0.5	Hydroxyethyl Cetyldimonium Phosphate
	QS	Preservative
	C 6.0	Propane/Butane

15 **Production:** Weight out the compounds of Phase A and mix them. Dissolve Phase B, stir into Phase A and homogenize. Adjust pH to 6-7. Fill into appropriate container and charge with Phase C.

20 STYLING FOAMS

	%	Ingredients (INCI)
25	A 2.0	Cocotrimonium Methosulfate
	QS	Perfume
30	B 84.0	Aqua dem.
	2.0	Chitosan
	SA	Compound
	0.5	Dimethicone Copolyol
	0.2	Ceteareth-25
	0.2	Panthenol
	0.1	PEG-25 PABA
35	C 10.0	HFC 152 A

	%	Ingredients (INCI)
40	A 2.0	Cocotrimonium Methosulfate
	QS	Perfume
45	B 80.0	Aqua dem.
	2.0	Chitosan
	SA	Compound
	0.5	Dimethicone Copolyol
	0.2	Ceteareth-25
	0.2	Panthenol
	0.1	PEG-25 PABA
50	C 10.0	HFC 152 A

Production: Weight out the compounds of Phase A and mix them. Dissolve Phase B, stir into Phase A and homogenize. Adjust pH to 6-7. Fill into appropriate container and charge with Phase C.

5

SHAMPOOS

	%	Ingredients (INCI)
10		
A	30.0	Sodium Laureth Sulfate
	6.0	Sodium Cocoamphoacetate
	6.0	Cocamidopropyl Betaine
	3.0	Sodium Laureth Sulfate, Glycol Distearate, Cocamide MEA, Laureth-10
15	SA	Compound
	7.7	Polyquaternium-44
	2.0	Amodimethicone
	QS	Perfume
	QS	Preservative
20	1.0	Sodium Chloride
	43.3	Aqua dem.

B QS Citric Acid

25

	%	Ingredients (INCI)
A	30.0	Sodium Laureth Sulfate
30	6.0	Sodium Cocoamphoacetate
	6.0	Cocamidopropyl Betaine
	3.0	Sodium Laureth Sulfate, Glycol Distearate, Cocamide MEA, Laureth-10
		Compound
	7.7	Polyquaternium-44
35	2.0	Amodimethicone
	QS	Perfume
	QS	Preservative
	1.0	Sodium Chloride
	39.3	Aqua dem.

40

B QS Citric Acid

Production: Weight out the compounds of Phase A and mix them. Adjust pH to 6-7 with citric acid.

45

180

SHOWER GELS

		%	Ingredients (INCI)
5			
	A	40.0	Sodium Laureth Sulfate
		5.0	Decyl Glucoside
		5.0	Cocamidopropyl Betaine
		SA	Compound
10		1.0	Panthenol
		QS	Perfume
		QS	Preservative
		2.0	Sodium Chloride
		46.0	Aqua dem.
15			
	B	QS	Citric Acid

20			
		%	Ingredients (INCI)
	A	40.0	Sodium Laureth Sulfate
		5.0	Decyl Glucoside
25		5.0	Cocamidopropyl Betaine
		SA	Compound
		1.0	Panthenol
		QS	Perfume
		QS	Preservative
30		2.0	Sodium Chloride
		42.0	Aqua dem.
	B	QS	Citric Acid

35

Production: Weight out the compounds of Phase A and mix them. Adjust pH to 6-7 with citric acid.

SHAMPOOS

5	%	Ingredients (INCI)
	A	40.0 Sodium Laureth Sulfate
		5.0 Sodium C12-15 Pareth-15 Sulfonate
10		5.0 Decyl Glucoside
		QS Perfume
		0.1 Phytantriol
		44.6 Aqua dem.
	SA	Compound
15		0.3 Polyquaternium-10
		1.0 Panthenol
	QS	Preservative
		1.0 Laureth-3
20		2.0 Sodium Chloride

	%	Ingredients (INCI)
25		
	A	40.0 Sodium Laureth Sulfate
		5.0 Sodium C12-15 Pareth-15 Sulfonate
		5.0 Decyl Glucoside
30		QS Perfume
		0.1 Phytantriol
		40.6 Aqua dem.
	SA	Compound
		0.3 Polyquaternium-10
35		1.0 Panthenol
	QS	Preservative
		1.0 Laureth-3
40		2.0 Sodium Chloride

Production: Weight out the compounds of Phase A and mix them. Adjust pH to 6-7 with citric acid.

SHAMPOOS

	%	Ingredients (INCI)
50	A	15.00 Cocamidopropyl Betaine
		10.00 Disodium Cocoamphodiacetate
		5.00 Polysorbate 20

182

		5.00	Decyl Glucoside
		QS	Perfume
		QS	Preservative
		SA	Compound
5		0.15	Guar Hydroxypropyltrimonium Chloride
		2.00	Laureth-3
		58.00	Aqua dem.
		QS	Citric Acid

10 **B** 3.00 PEG-150 Distearate

		%	Ingredients (INCI)
15	A	15.00	Cocamidopropyl Betaine
		10.00	Disodium Cocoamphodiacetate
		5.00	Polysorbate 20
		5.00	Decyl Glucoside
20		QS	Perfume
		QS	Preservative
			Compound
		0.15	Guar Hydroxypropyltrimonium Chloride
		2.00	Laureth-3
25		54.00	Aqua dem.
		QS	Citric Acid

B 3.00 PEG-150 Distearate

30

Production: Weight out the compounds of Phase A and mix them. Adjust pH to 6-7. Add Phase B and heat to max. 40°C.

35

BODY LOTIONS

40		%	Ingredients (INCI)
	A	2.0	Ceteareth-25
		2.0	Ceteareth-6, Stearyl Alcohol
		3.0	Cetearyl Ethylhexanoate
45		1.0	Dimethicone
		4.0	Cetearyl Alcohol
		3.0	Glyceryl Stearate SE
		5.0	Mineral Oil
		4.0	Simmondsia Chinensis (Jojoba) Seed Oil
50		3.0	Mineral Oil, Lanolin Alcohol

183

5	B	5.0	Propylene Glycol
	SA		Compound
	1.0		Panthenol
	0.5		Magnesium Aluminum Silicate
	QS		Preservative
	65.5		Aqua dem.
	C	QS	Perfume
10	D	QS	Citric Acid
15		%	Ingredients (INCI)
20	A	2.0	Ceteareth-25
		2.0	Ceteareth-6, Stearyl Alcohol
		3.0	Cetearyl Ethylhexanoate
		1.0	Dimethicone
		4.0	Cetearyl Alcohol
		3.0	Glyceryl Stearate SE
		5.0	Mineral Oil
25		4.0	Simmondsia Chinensis (Jojoba) Seed Oil
		3.0	Mineral Oil, Lanolin Alcohol
30	B	5.0	Propylene Glycol
	SA		Compound
	1.0		Panthenol
	0.5		Magnesium Aluminum Silicate
	QS		Preservative
	61.5		Aqua dem.
35	C	QS	Perfume
	D	QS	Citric Acid

Production: Heat Phases A and B separately to approx. 40°C. Add Phase B to Phase A and homogenize by stirring. Add Phase C to the combined Phase A and B and homogenize again. Adjust pH with Phase D to 6 - 7. Homogenize by stirring and cool to room temperature.

45 BODY LOTIONS

		%	Ingredients (INCI)
50	A	6.0	PEG-7 Hydrogenated Castor Oil

184

		10.0	Cetearyl Ethylhexanoate
		5.0	Isopropyl Myristate
		7.0	Mineral Oil
5		0.5	Shea Butter (Butyrospermum Parkii)
		0.5	Aluminum Stearate
		0.5	Magnesium Stearate
		0.2	Bisabolol
		0.7	Quaternium-18-Hectorite
10	B	5.0	Dipropylene Glycol
		0.7	Magnesium Sulfate
		QS	Preservative
		62.9	Aqua dem.
15	C	QS	Perfume
		SA	Compound

20		%	Ingredients (INCI)
	A	6.0	PEG-7 Hydrogenated Castor Oil
		10.0	Cetearyl Ethylhexanoate
		5.0	Isopropyl Myristate
25		7.0	Mineral Oil
		0.5	Shea Butter (Butyrospermum Parkii)
		0.5	Aluminum Stearate
		0.5	Magnesium Stearate
		0.2	Bisabolol
30		0.7	Quaternium-18-Hectorite
	B	5.0	Dipropylene Glycol
		0.7	Magnesium Sulfate
35		QS	Preservative
		58.9	Aqua dem.
	C	QS	Perfume
40		SA	Compound

Production: Heat Phases A and B separately to approx. 80°C. Add Phase B to Phase A and homogenize by stirring. Cool to 40°C and add Phase C. Homogenize again and cool to room temperature.

45

HAIR CONDITIONERS

		%	Ingredients (INCI)
5			
	A	10.0	PVP/VA Copolymer
		0.2	Hydroxyethyl Cetyldimonium Phosphate
		0.2	Ceteareth-25
		0.5	Dimethicone Copolyol
10		QS	Perfume
		10.0	Alcohol
		SA	Compound
		68.1	Aqua dem.
15	B	10.0	Propane/Butane

		%	Ingredients (INCI)
20			
	A	10.0	PVP/VA Copolymer
		0.2	Hydroxyethyl Cetyldimonium Phosphate
		0.2	Ceteareth-25
		0.5	Dimethicone Copolyol
25		QS	Perfume
		10.0	Alcohol
		SA	Compound
		64.1	Aqua dem.
30	B	10.0	Propane/Butane

Production: Add all compounds to Phase A and stir to homogenize. Fill into appropriate container and charge with Phase B.

35

FOAM CONDITIONERS

		%	Ingredients (INCI)
40			
	A	1.0	Polyquaternium-4
		0.5	Hydroxyethyl Cetyldimonium Phosphate
45		SA	Compound
		QS	Perfume
		QS	Preservative
		91.5	Aqua dem.
50	B	6.0	Propane/Butane

186

		%	Ingredients (INCI)
5	A	1.0	Polyquaternium-4
		0.5	Hydroxyethyl Cetyldimonium Phosphate
		SA	Compound
		QS	Perfume
		QS	Preservative
10		87.5	Aqua dem.
	B	6.0	Propane/Butane

15 **Production:** Add all compounds to Phase A and stir to homogenize. Fill into appropriate container and charge with Phase B.

20 FOAM CONDITIONERS

		%	Ingredients (INCI)
25	A	1.0	Polyquaternium-11
		0.5	Hydroxyethyl Cetyldimonium Phosphate
			Compound
		QS	Perfume
		QS	Preservative
		91.5	Aqua dem.
30	B	6.0	Propane/Butane

		%	Ingredients (INCI)
35	A	1.0	Polyquaternium-11
		0.5	Hydroxyethyl Cetyldimonium Phosphate
		SA	Compound
		QS	Perfume
40		QS	Preservative
		87.5	Aqua dem.
	B	6.0	Propane/Butane

Production: Add all compounds to Phase A and stir to homogenize. Fill into appropriate container and charge with Phase B.

5

STYLING FOAMS

10		%	Ingredients (INCI)
	A	0.5	Laureth-4
		QS	Perfume
15	B	77.3	Aqua dem.
		10.0	Polyquaternium-28
		SA	Compound
		0.5	Dimethicone Copolyol
		0.2	Ceteareth-25
20		0.2	Panthenol
		0.1	PEG-25 PABA
		0.2	Hydroxyethylcellulose
25	C	10.0	HFC 152 A
		%	Ingredients (INCI)
30	A	0.5	Laureth-4
		QS	Perfume
	B	73.3	Aqua dem.
		10.0	Polyquaternium-28
		SA	Compound
35		0.5	Dimethicone Copolyol
		0.2	Ceteareth-25
		0.2	Panthenol
		0.1	PEG-25 PABA
		0.2	Hydroxyethylcellulose
40	C	10.0	HFC 152 A

Production: Weight out the compounds of Phase A and mix them. Dissolve Phase B, stir into Phase A and
45 homogenize. Fill into appropriate container and charge with Phase C.

STYLING FOAMS

		%	Ingredients (INCI)
5	A	2.0 QS	Cocotrimonium Methosulfate Perfume
10	B	78.5 6.7 0.6 SA 0.5 0.2	Aqua dem. Acrylates Copolymer AMP Compound Dimethicone Copolyol Ceteareth-25
15		0.2 0.1 0.2	Panthenol PEG-25 PABA Hydroxyethylcellulose
20	C	10.0	HFC 152 A

		%	Ingredients (INCI)
25	A	2.0 QS	Cocotrimonium Methosulfate Perfume
30	B	74.5 6.7 0.6 SA 0.5 0.2	Aqua dem. Acrylates Copolymer AMP Compound Dimethicone Copolyol Ceteareth-25
35		0.2 0.1 0.2	Panthenol PEG-25 PABA Hydroxyethylcellulose
40	C	10.0	HFC 152 A

Production: Weight out the compounds of Phase A and mix them. Dissolve Phase B, stir into Phase A and homogenize. Fill into appropriate container and charge with Phase C.

STYLING FOAMS

5		%	Ingredients (INCI)
	A	2.0 QS	Cocotrimonium Methosulfate Perfume
10	B	7.70 SA QS 79.3	Polyquaternium-44 Compound Preservative Aqua dem.
15	C	10.0	Propane/Butane

		%	Ingredients (INCI)
20	A	2.0 QS	Cocotrimonium Methosulfate Perfume
	B	7.70 SA QS 75.3	Polyquaternium-44 Compound Preservative Aqua dem.
25	C	10.0	Propane/Butane

30 **Production:** Weight out the compounds of Phase A and mix them. Dissolve Phase B, stir into Phase A and homogenize. Adjust pH to 6–7. Fill into appropriate container and charge with Phase C.

STYLING FOAMS

35		%	Ingredients (INCI)
	A	2.00 QS	Cocotrimonium Methosulfate Perfume
40	B	72.32 2.00 0.53 SA	Aqua dem. VP/Acrylates/Lauryl Methacrylate Copolymer AMP Compound
45		0.20 0.50 0.05 0.20 15.00	Ceteareth-25 Panthenol Benzophenone-4 Amodimethicone, Cetrimonium Chloride, Trideceth-12 Alcohol
50	C	0.20	Hydroxyethylcellulose
	D	6.00	Propane/Butane

190

		%	Ingredients (INCI)
5			
	A	2.00	Cocotrimonium Methosulfate
		QS	Perfume
	B	68.32	Aqua dem.
10		2.00	VP/Acrylates/Lauryl Methacrylate Copolymer
		0.53	AMP
		SA	Compound
		0.20	Ceteareth-25
		0.50	Panthenol
15		0.05	Benzophenone-4
		0.20	Amodimethicone, Cetrimonium Chloride, Trideceth-12
		15.00	Alcohol
	C	0.20	Hydroxyethylcellulose
20	D	6.00	Propane/Butane

Production: Weight out the compounds of Phase A and mix them. Dissolve Phase B, stir into Phase A and
25 homogenize. Add Phase C and homogenize again. Adjust pH to 6-7. Fill into appropriate container and
charge with Phase D.

STYLING FOAMS

		%	Ingredients (INCI)
30			
	A	2.00	Cetrimonium Chloride
		QS	Perfume
35	B	67.85	Aqua dem.
		7.00	Polyquaternium-46
		SA	Compound
		0.20	Ceteareth-25
40		0.50	Panthenol
		0.05	Benzophenone-4
		0.20	Amodimethicone, Cetrimonium Chloride, Trideceth-12
		15.00	Alcohol
45	C	0.20	Hydroxyethylcellulose
	D	6.00	Propane/Butane

191

	%	Ingredients (INCI)
5	A	2.00 Cetrimonium Chloride
	QS	Perfume
10	B	63.85 Aqua dem.
		7.00 Polyquaternium-46
	SA	Compound
		0.20 Ceteareth-25
		0.50 Panthenol
		0.05 Benzophenone-4
		0.20 Amodimethicone, Cetrimonium Chloride, Trideceth-12
		15.00 Alcohol
15	C	0.20 Hydroxyethylcellulose
	D	6.00 Propane/Butane
20	Production: Weight out the compounds of Phase A and mix them. Dissolve Phase B, stir into Phase A and homogenize. Add Phase C and homogenize again. Adjust pH to 6-7. Fill into appropriate container and charge with Phase D.	

STYLING FOAMS

25		%	Ingredients (INCI)
30	A	QS	PEG-40 Hydrogenated Castor Oil
		QS	Perfume
		85.5	Aqua dem.
35	B	7.0	Sodium Polystyrene Sulfonate
		SA	Compound
		0.5	Cetrimonium Bromide
		QS	Preservative
	C	6.0	Propane/Butane
40		%	Ingredients (INCI)
45	A	QS	PEG-40 Hydrogenated Castor Oil
		QS	Perfume
		81.5	Aqua dem.
50	B	7.0	Sodium Polystyrene Sulfonate
		SA	Compound
		0.5	Cetrimonium Bromide
		QS	Preservative
	C	6.0	Propane/Butane

Production: Weight out the compounds of Phase A and mix them. Dissolve Phase B, stir into Phase A and homogenize. Adjust pH to 6-7. Fill into appropriate container and charge with Phase C.

5

STYLING FOAMS

10		%	Ingredients (INCI)
	A	QS QS 92.0	PEG-40 Hydrogenated Castor Oil Perfume Aqua dem.
15	B	0.5 1.0 0.5 QS	Polyquaternium-10 Compound Cetrimonium Bromide Preservative
20	C	6.0	Propane/Butane

25		%	Ingredients (INCI)
	A	QS QS 88.0	PEG-40 Hydrogenated Castor Oil Perfume Aqua dem.
30	B	0.5 5.0 0.5 QS	Polyquaternium-10 Compound Cetrimonium Bromide Preservative
35	C	6.0	Propane/Butane

Production: Weigh out the compounds of Phase A and mix them. Dissolve Phase B, stir into Phase A and homogenize. Adjust pH to 6-7. Fill into appropriate container and charge with Phase C.

40

STYLING FOAMS

5		%	Ingredients (INCI)
	A	QS QS 82.5	PEG-40 Hydrogenated Castor Oil Perfume Aqua dem.
10	B	10.0 SA 0.5 QS.	Polyquaternium-16 Compound Hydroxyethyl Cetyldimonium Phosphate Preservative
15	C	6.0	Propane/Butane

20		%	Ingredients (INCI)
	A	QS QS 78.5	PEG-40 Hydrogenated Castor Oil Perfume Aqua dem.
25	B	10.0 SA 0.5 QS	Polyquaternium-16 Compound Hydroxyethyl Cetyldimonium Phosphate Preservative
30	C	6.0	Propane/Butane

Production: Weight out the compounds of Phase A and mix them. Dissolve Phase B, stir into Phase A and
 35 homogenize. Adjust pH to 6-7. Fill into appropriate container and charge with Phase C.

STYLING FOAMS

40		%	Ingredients (INCI)
	A	2.0 QS	Cocotrimonium Methosulfate Perfume
45	B	84.0 2.0 SA 0.5 0.2	Aqua dem. Chitosan Compound Dimethicone Copolyol Ceteareth-25
50			

194

		0.2	Panthenol
		0.1	PEG-25 PABA
5	C	10.0	HFC 152 A
		%	Ingredients (INCI)
10	A	2.0	Cocotrimonium Methosulfate
		QS	Perfume
	B	80.0	Aqua dem.
		2.0	Chitosan
15	SA		Compound
		0.5	Dimethicone Copolyol
		0.2	Ceteareth-25
		0.2	Panthenol
		0.1	PEG-25 PABA
20	C	10.0	HFC 152 A

Production: Weight out the compounds of Phase A and mix them. Dissolve Phase B, stir into Phase A and
 25 homogenize. Adjust pH to 6-7. Fill into appropriate container and charge with Phase C.

SHAMPOOS

30		%	Ingredients (INCI)
	A	30.0	Sodium Laureth Sulfate
		6.0	Sodium Cocoamphoacetate
35		6.0	Cocamidopropyl Betaine
		3.0	Sodium Laureth Sulfate, Glycol Distearate, Cocamide MEA, Laureth-10
	SA		Compound
		7.7	Polyquaternium-44
		2.0	Amodimethicone
40	QS		Perfume
	QS		Preservative
		1.0	Sodium Chloride
		43.3	Aqua dem.
45	B	QS	Citric Acid

195

		%	Ingredients (INCI)
5	A	30.0	Sodium Laureth Sulfate
		6.0	Sodium Cocoamphoacetate
		6.0	Cocamidopropyl Betaine
		3.0	Sodium Laureth Sulfate, Glycol Distearate, Cocamide MEA, Laureth-10
		SA	Compound
10		7.7	Polyquaternium-44
		2.0	Amodimethicone
		SA	Perfume
		SA.	Preservative
		1.0	Sodium Chloride
15		39.3	Aqua dem.
	B	QS	Citric Acid

20

Production: Weight out the compounds of Phase A and mix them. Adjust pH to 6-7 with citric acid.

25 SHOWER GELS

		%	Ingredients (INCI)
30	A	40.0	Sodium Laureth Sulfate
		5.0	Decyl Glucoside
		5.0	Cocamidopropyl Betaine
		SA	Compound
		1.0	Panthenol
35		QS	Perfume
		QS	Preservative
		2.0	Sodium Chloride
		46.0	Aqua dem.
40	B	QS	Citric Acid

		%	Ingredients (INCI)
45	A	40.0	Sodium Laureth Sulfate
		5.0	Decyl Glucoside
		5.0	Cocamidopropyl Betaine
		SA	Compound
50		1.0	Panthenol
		QS	Perfume
		QS	Preservative

196

2.0 Sodium Chloride
42.0 Aqua dem.

B QS Citric Acid

Production: Weight out the compounds of Phase A and mix them. Adjust pH to 6-7 with citric acid.

SHAMPOOS

% **Ingredients (INCI)**

A 40.0 Sodium Laureth Sulfate
5.0 Sodium C12-15 Pareth-15 Sulfonate
5.0 Decyl Glucoside
QS Perfume
0.1 Phytantriol
44.6 Aqua dem.
SA Compound
0.3 Polyquaternium-10
1.0 Panthenol
QS Preservative
1.0 Laureth-3
2.0 Sodium Chloride

% **Ingredients (INCI)**

A 40.0 Sodium Laureth Sulfate
5.0 Sodium C12-15 Pareth-15 Sulfonate
5.0 Decyl Glucoside
QS Perfume
0.1 Phytantriol
40.6 Aqua dem.
SA Compound
0.3 Polyquaternium-10
1.0 Panthenol
QS Preservative
1.0 Laureth-3
2.0 Sodium Chloride

Production: Weight out the compounds of Phase A and mix them. Adjust pH to 6-7 with citric acid.

SHAMPOOS

5		%	Ingredients (INCI)
	A	15.00	Cocamidopropyl Betaine
		10.00	Disodium Cocoamphodiacetate
		5.00	Polysorbate 20
10		5.00	Decyl Glucoside
		QS	Perfume
		QS	Preservative
		SA	Compound
		0.15	Guar Hydroxypropyltrimonium Chloride
15		2.00	Laureth-3
		58.00	Aqua dem.
		QS	Citric Acid
	B	3.00	PEG-150 Distearate
20			

		%	Ingredients (INCI)
25	A	15.00	Cocamidopropyl Betaine
		10.00	Disodium Cocoamphodiacetate
		5.00	Polysorbate 20
		5.00	Decyl Glucoside
		QS	Perfume
30		QS	Preservative
		SA	Compound
		0.15	Guar Hydroxypropyltrimonium Chloride
		2.00	Laureth-3
		54.00	Aqua dem.
35		QS	Citric Acid
	B	3.00	PEG-150 Distearate

40 **Production:** Weight out the compounds of Phase A and mix them. Adjust pH to 6-7. Add Phase B and heat to max. 40°C.

45 BODY LOTIONS

		%	Ingredients (INCI)
50	A	2.0	Cetareth-25
		2.0	Cetareth-6, Stearyl Alcohol

198

5		3.0	Cetearyl Ethylhexanoate
		1.0	Dimethicone
		4.0	Cetearyl Alcohol
		3.0	Glyceryl Stearate SE
		5.0	Mineral Oil
		4.0	Simmondsia Chinensis (Jojoba) Seed Oil
		3.0	Mineral Oil, Lanolin Alcohol
10	B	5.0	Propylene Glycol
	SA		Compound
		1.0	Panthenol
		0.5	Magnesium Aluminum Silicate
	QS		Preservative
15		65.5	Aqua dem.
	C	QS	Perfume
	D	QS	Citric Acid

20

		%	Ingredients (INCI)
25	A	2.0	Ceteareth-25
		2.0	Ceteareth-6, Stearyl Alcohol
		3.0	Cetearyl Ethylhexanoate
		1.0	Dimethicone
		4.0	Cetearyl Alcohol
		3.0	Glyceryl Stearate SE
30		5.0	Mineral Oil
		4.0	Simmondsia Chinensis (Jojoba) Seed Oil
		3.0	Mineral Oil, Lanolin Alcohol
35	B	5.0	Propylene Glycol
		SA	Compound
		1.0	Panthenol
		0.5	Magnesium Aluminum Silicate
		QS	Preservative
		61.5	Aqua dem.
40	C	QS	Perfume
	D	QS	Citric Acid

45

Production: Heat Phases A and B separately to approx. 40°C. Add Phase B to Phase A and homogenize by stirring. Add Phase C to the combined Phase A and B and homogenize again. Adjust pH with Phase D to 6 - 7. Homogenize by stirring and cool to room temperature.

50

BODY LOTIONS

5		%	Ingredients (INCI)
	A	6.0	PEG-7 Hydrogenated Castor Oil
		10.0	Cetearyl Ethylhexanoate
		5.0	Isopropyl Myristate
10		7.0	Mineral Oil
		0.5	Shea Butter (Butyrospermum Parkii)
		0.5	Aluminum Stearate
		0.5	Magnesium Stearate
		0.2	Bisabolol
15		0.7	Quaternium-18-Hectorite
	B	5.0	Dipropylene Glycol
		0.7	Magnesium Sulfate
		QS	Preservative
20		62.9	Aqua dem.
	C	QS.	Perfume
		SA	Compound
25		%	Ingredients (INCI)
	A	6.0	PEG-7 Hydrogenated Castor Oil
		10.0	Cetearyl Ethylhexanoate
30		5.0	Isopropyl Myristate
		7.0	Mineral Oil
		0.5	Shea Butter (Butyrospermum Parkii)
		0.5	Aluminum Stearate
		0.5	Magnesium Stearate
35		0.2	Bisabolol
		0.7	Quaternium-18-Hectorite
	B	5.0	Dipropylene Glycol
		0.7	Magnesium Sulfate
40		QS	Preservative
		58.9	Aqua dem.
	C	QS	Perfume
45		SA	Compound

Production: Heat Phases A and B separately to approx. 80°C. Add Phase B to Phase A and homogenize by stirring. Cool to 40°C and add Phase C. Homogenize again and cool to room temperature.

DAILY SKIN CARE O/W

5			
		%	Ingredients (INCI)
	A	1.7	Ceteareth-6, Stearyl Alcohol
		0.7	Ceteareth-25
10		2.0	Diethylamino Hydroxybenzoyl Hexyl Benzoate
		2.0	PEG-14 Dimethicone
		3.6	Cetearyl Alcohol
		6.0	Ethylhexyl Methoxycinnamate
		2.0	Dibutyl Adipate
15	B	5.0	Glycerin
		0.2	Disodium EDTA
		1.0	Panthenol
		QS	Preservative
20		67.8	Aqua dem.
	C	4.0	Caprylic/Capric Triglyceride, Sodium Acrylates Copolymer
	D	0.2	Sodium Ascorbyl Phosphate
25		1.0	Tocopheryl Acetate
		0.2	Bisabolol
		1.0	Caprylic/Capric Triglyceride, Sodium Ascorbate, Tocopherol, Retinol
	SA		Compound
30	E	QS	Sodium Hydroxide
		%	Ingredients (INCI)
35	A	1.7	Ceteareth-6, Stearyl Alcohol
		0.7	Ceteareth-25
		2.0	Diethylamino Hydroxybenzoyl Hexyl Benzoate
		2.0	PEG-14 Dimethicone
40		3.6	Cetearyl Alcohol
		6.0	Ethylhexyl Methoxycinnamate
		2.0	Dibutyl Adipate
	B	5.0	Glycerin
45		0.2	Disodium EDTA
		1.0	Panthenol
		QS	Preservative
		63.8	Aqua dem.
50	C	4.0	Caprylic/Capric Triglyceride, Sodium Acrylates Copolymer

5	D	0.2	Sodium Ascorbyl Phosphate
		1.0	Tocopheryl Acetate
		0.2	Bisabolol
		1.0	Caprylic/Capric Triglyceride, Sodium Ascorbate, Tocopherol, Retinol
	SA		Compound
	E	QS	Sodium Hydroxide

10

Production: Heat Phase A and B separately to approx. 80°C. Add Phase B to Phase A and homogenize by stirring. Add Phase C to the combined Phase A and B and homogenize again. Cool to approx. 40°C and add Phase D. Adjust pH with Phase E to approx. 6.5. Homogenize by stirring and cool to room temperature.

15

PROTECTIVE DAY SKIN CREME O/W

		%	Ingredients (INCI)
20	A	1.7	Ceteareth-6, Stearyl Alcohol
		0.7	Ceteareth-25
		2.0	Diethylamino Hydroxybenzoyl Hexyl Benzoate
		2.0	PEG-14 Dimethicone
		3.6	Cetearyl Alcohol
25		6.0	Ethylhexyl Methoxycinnamate
		2.0	Dibutyl Adipate
30	B	5.0	Glycerin
		0.2	Disodium EDTA
		1.0	Panthenol
		QS	Preservative
		68.6	Aqua dem.
35	C	4.0	Caprylic/Capric Triglyceride, Sodium Acrylates Copolymer
40	D	1.0	Sodium Ascorbyl Phosphate
		1.0	Tocopheryl Acetate
		0.2	Bisabolol
		SA	Compound
	E	QS	Sodium Hydroxide

%Ingredients (INCI)

5	A	1.7	Ceteareth-6, Stearyl Alcohol
		0.7	Ceteareth-25
		2.0	Diethylamino Hydroxybenzoyl Hexyl Benzoate
		2.0	PEG-14 Dimethicone
		3.6	Cetearyl Alcohol
		6.0	Ethylhexyl Methoxycinnamate
10		2.0	Dibutyl Adipate
15	B	5.0	Glycerin
		0.2	Disodium EDTA
		1.0	Panthenol
		QS	Preservative
		64.6	Aqua dem.
	C	4.0	Caprylic/Capric Triglyceride, Sodium Acrylates Copolymer
20	D	1.0	Sodium Ascorbyl Phosphate
		1.0	Tocopheryl Acetate
		0.2	Bisabolol
			Compound
25	E	QS	Sodium Hydroxide

Production: : Heat Phase A and B separately to approx. 80°C. Add Phase B to Phase A and homogenize by stirring. Add Phase C to the combined Phase A and B and homogenize again. Cool to approx. 40°C and add Phase D. Adjust pH with Phase E to approx. 6.5. Homogenize by stirring and cool to room temperature.

30

FACIAL CLEANSER O/W

		%	Ingredients (INCI)
35	A	10.0	Cetearyl Ethylhexanoate
		10.0	Caprylic/Capric Triglyceride
		1.5	Cyclopentasiloxane, Cyclohexasilosane
		2.0	PEG-40 Hydrogenated Castor Oil
40	B	3.5	Caprylic/Capric Triglyceride, Sodium Acrylates Copolymer
	C	1.0	Tocopheryl Acetate
		0.2	Bisabolol
		QS	Preservative
45		QS	Perfume
50	D	3.0	Polyquaternium-44
		0.5	Cocotrimonium Methosulfate
		0.5	Ceteareth-25
		2.0	Panthenol, Propylene Glycol

203

5		4.0	Propylene Glycol
		0.1	Disodium EDTA
		SA	Compound
		60.7	Aqua dem.
		%	Ingredients (INCI)
10	A	10.0	Cetearyl Ethylhexanoate
		10.0	Caprylic/Capric Triglyceride
		1.5	Cyclopentasiloxane, Cyclohexasilosane
		2.0	PEG-40 Hydrogenated Castor Oil
15	B	3.5	Caprylic/Capric Triglyceride, Sodium Acrylates Copolymer
	C	1.0	Tocopheryl Acetate
0.2		Bisabolol	
QS		Preservative	
20		QS	Perfume
25	D	3.0	Polyquaternium-44
		0.5	Cocotrimonium Methosulfate
		0.5	Ceteareth-25
		2.0	Panthenol, Propylene Glycol
		4.0	Propylene Glycol
		0.1	Disodium EDTA
		SA	Compound
30		56.7	Aqua dem.

Production: Dissolve Phase A and add Phase B to Phase A and homogenize by stirring. Add Phase C to the combined Phase A and B and homogenize again. Add Phase D to the combined Phase A, B and C and homogenize again. Dissolve Phase D and add to Phase A, B, and C and homogenize again. Stir for 15 minutes.

DAILY CARE BODY SPRAY

40		%	Ingredients (INCI)
45	A	3.0	Ethylhexyl Methoxycinnamate
		2.0	Diethylamino Hydroxybenzoyl Hexyl Benzoate
		1.0	Polyquaternium-44
		3.0	Propylene Glycol
50		2.0	Panthenol, Propylene Glycol
		1.0	Cyclopentasiloxane, Cyclohexasiloxane
		10.0	Octyldodecanol
		0.5	PVP
		10.0	Caprylic/Capric Triglyceride

204

5	3.0	C12-15 Alkyl Benzoate
	3.0	Glycerin
	1.0	Tocopheryl Acetate
	0.3	Bisabolol
	SA	Compound
	59.2	Alcohol
10	%	Ingredients (INCI)
15	A 3.0	Ethylhexyl Methoxycinnamate
	2.0	Diethylamino Hydroxybenzoyl Hexyl Benzoate
	1.0	Polyquaternium-44
	3.0	Propylene Glycol
	2.0	Panthenol, Propylene Glycol
20	1.0	Cyclopentasiloxane, Cyclohexasiloxane
	10.0	Octyldodecanol
	0.5	PVP
	10.0	Caprylic/Capric Triglyceride
	3.0	C12-15 Alkyl Benzoate
25	3.0	Glycerin
	1.0	Tocopheryl Acetate
	0.3	Bisabolol
	SA	Compound
	55.2	Alcohol

Production: Weight all ingredients of Phase A and dissolve completely by stirring.

30

SKIN CARE GEL

35	%	Ingredients (INCI)
	A 3.6	PEG-40 Hydrogenated Castor Oil
	15.0	Alcohol
	0.1	Bisabolol
	0.5	Tocopheryl Acetate
40	QS	Perfume
45	B 3.0	Panthenol
	0.6	Carbomer
	SA	Compound
	75.4	Aqua dem,
50	C 0.8	Triethanolamine

% **Ingredients (INCI)**

205

5	A	3.6	PEG-40 Hydrogenated Castor Oil
		15.0	Alcohol
		0.1	Bisabolol
		0.5	Tocopheryl Acetate
		QS	Perfume
10	B	3.0	Panthenol
		0.6	Carbomer
		SA	Compound
		71.4	Aqua dem,
	C	0.8	Triethanolamine

15 **Production:** Dissolve Phase A. Swell Phase B and neutralize with Phase C. Add Phase A to Phase B and C and homogenize by stirring.

20 AFTER SHAVE LOTIONS

		%	Ingredients (INCI)
25	A	10.0	Cetearyl Ethylhexanoate
		5.0	Tocopheryl Acetate
		1.0	Bisabolol
		0.1	Perfume
		0.3	Acrylates/C10-30 Alkyl Acrylate Crosspolymer
30	B	15.0	Alcohol
		1.0	Panthenol
		3.0	Glycerin
		SA	Compound
		1.0	Triethanolamine
35		63.5	Aqua dem.
		%	Ingredients (INCI)
40	A	10.0	Cetearyl Ethylhexanoate
		5.0	Tocopheryl Acetate
		1.0	Bisabolol
		0.1	Perfume
		0.3	Acrylates/C10-30 Alkyl Acrylate Crosspolymer
45	B	15.0	Alcohol
		1.0	Panthenol
		3.0	Glycerin
		SA	Compound
		0.1	Triethanolamine
50		59.5	Aqua dem.

Production: Dissolve Phase A. Dissolve Phase B and add to Phase A. Homogenize by stirring.

5

AFTER SUN LOTIONS

		%	Ingredients (INCI)
10	A	0.4	Acrylates/C10-30 Alkyl Acrylate Crosspolymer
		15.0	Cetearyl Ethylhexanoate
		0.2	Bisabolol
		1.0	Tocopheryl Acetate
		QS.	Perfume
15	B	1.0	Panthenol
		15.0	Alcohol
		3.0	Glycerin
		SA	Compound
20		63.2	Aqua dem,
	C	0.2	Triethanolamine

25

		%	Ingredients (INCI)
	A	0.4	Acrylates/C10-30 Alkyl Acrylate Crosspolymer
		15.0	Cetearyl Ethylhexanoate
30		0.2	Bisabolol
		1.0	Tocopheryl Acetate
		QS	Perfume
	B	1.0	Panthenol
35		15.0	Alcohol
		3.0	Glycerin
		SA	Compound
		59.2	Aqua dem,
40	C	0.2	Triethanolamine

Production: Dissolve Phase A. Dissolve Phase B and add to Phase A. Homogenize by stirring. Neutralize Phase A and B by adding Phase C and homogenize again.

45

SUNSCREEN LOTIONS

		%	Ingredients (INCI)
50	A	4.5	Ethylhexyl Methoxycinnamate

207

5		2.0	Diethylamino Hydroxybenzoyl Hexyl Benzoate
		3.0	Octocrylene
		2.5	Di-C12-13 Alkyl Malate
		0.5	Tocopheryl Acetate
		4.0	Polyglyceryl-3 Methyl Glucose Distearate
10	B	3.5	Cetearyl Isononanoate
		1.0	VP/Eicosene Copolymer
		5.0	Isohexadecane
		2.5	Di-C12-13 Alkyl Malate
		3.0	Titanium Dioxide, Trimethoxycaprylylsilane
15	C	5.0	Glycerin
		1.0	Sodium Cetearyl Sulfate
		0.5	Xanthan Gum
		59.7	Aqua dem.
20	D	SA	Compound
		1.0	Phenoxyethanol, Methylparaben, Ethylparaben, Butylparaben, Propylparaben, Isobutylparaben
		0.3	Bisabolol
25		%	Ingredients (INCI)
30	A	4.5	Ethylhexyl Methoxycinnamate
		2.0	Diethylamino Hydroxybenzoyl Hexyl Benzoate
		3.0	Octocrylene
		2.5	Di-C12-13 Alkyl Malate
		0.5	Tocopheryl Acetate
		4.0	Polyglyceryl-3 Methyl Glucose Distearate
35	B	3.5	Cetearyl Isononanoate
		1.0	VP/Eicosene Copolymer
		5.0	Isohexadecane
		2.5	Di-C12-13 Alkyl Malate
		3.0	Titanium Dioxide, Trimethoxycaprylylsilane
40	C	5.0	Glycerin
		1.0	Sodium Cetearyl Sulfate
		0.5	Xanthan Gum
		55.7	Aqua dem.

5	D	SA	Compound
		1.0	Phenoxyethanol, Methylparaben, Ethylparaben, Butylparaben, Propylparaben, Isobutylparaben
		0.3	Bisabolol

Production: Heat Phase A and B separately to approx. 80°C. Add Phase B to Phase A and homogenize by stirring. Heat Phase C to 80°C and add to the combined Phase A and B and homogenize again. Cool to approx. 40°C and add Phase D. Homogenize again.

SUNSCREEN LOTIONS O/W

15		%	Ingredients (INCI)
20	A	2.0	Ceteareth-6, Stearyl Alcohol
		2.0	Ceteareth-25
		3.0	Tribehenin
		2.0	Cetearyl Alcohol
		2.0	Cetearyl Ethylhexanoate
		5.0	Ethylhexyl Methoxycinnamate
		1.0	Ethylhexyl Triazone
25		1.0	VP/Eicosene Copolymer
		7.0	Isopropyl Myristate
	B	5.0	Zinc Oxide, Triethoxycaprylylsilane
30	C	0.2	Xanthan Gum
		0.5	Hydroxyethyl Acrylate/Sodium Acryloyldimethyl Taurate Copolymer, Squalane, Polysorbate 60
		0.2	Disodium EDTA
		5.0	Propylene Glycol
		0.5	Panthenol
35		60.9	Aqua dem.
40	D	SA	Compound
		0.5	Phenoxyethanol, Methylparaben, Butylparaben, Ethylparaben, Propylparaben, Isopropylparaben
		1.0	Tocopheryl Acetate
		0.2	Bisabolol
45		%	Ingredients (INCI)
50	A	2.0	Ceteareth-6, Stearyl Alcohol
		2.0	Ceteareth-25
		3.0	Tribehenin
		2.0	Cetearyl Alcohol

209

5	2.0	Cetearyl Ethylhexanoate
	5.0	Ethylhexyl Methoxycinnamate
	1.0	Ethylhexyl Triazone
	1.0	VP/Eicosene Copolymer
	7.0	Isopropyl Myristate
B	5.0	Zinc Oxide, Triethoxycaprylylsilane
10	C 0.2	Xanthan Gum
	0.5	Hydroxyethyl Acrylate/Sodium Acryloyldimethyl Taurate Copolymer, Squalane, Polysorbate 60
	0.2	Disodium EDTA
	5.0	Propylene Glycol
	0.5	Panthenol
15	56.9	Aqua dem.
20	D SA	Compound
	0.5	Phenoxyethanol, Methylparaben, Butylparaben, Ethylparaben, Propylparaben, Isopropylparaben
	1.0	Tocopheryl Acetate
	0.2	Bisabolol

Production: Heat Phase A and B separately to approx. 80°C. Add Phase B to Phase A and homogenize by stirring. Heat Phase C to 80°C and add to the combined Phase A and B and homogenize again. Cool to approx. 40°C and add Phase D. Homogenize again.

SUNSCREEN LOTIONS O/W

30		% Ingredients (INCI)
35	A 3.5	Ceteareth-6, Stearyl Alcohol
	1.5	Ceteareth-25
	7.5	Ethylhexyl Methoxycinnamate
	2.0	Diethylamino Hydroxybenzoyl Hexyl Benzoate
	2.0	Cyclopentasiloxane, Cyclohexasiloxane
	0.5	Bees Wax
	3.0	Cetearyl Alcohol
40	10.0	Caprylic/Capric Triglyceride
	B 5.0	Titanium Dioxide, Silica, Methicone, Alumina
45	C 3.0	Glycerin
	0.2	Disodium EDTA
	0.3	Xanthan Gum
	1.0	Decyl Glucoside
	2.0	Panthenol, Propylene Glycol
	56.3	Aqua dem.
50	D SA	Compound

210

5		1.0	Tocopheryl Acetate
		0.2	Bisabolol
		QS	Parfümöl
		QS	Preservative
		%	Ingredients (INCI)
10	A	3.5	Ceteareth-6, Stearyl Alcohol
		1.5	Ceteareth-25
		7.5	Ethylhexyl Methoxycinnamate
		2.0	Diethylamino Hydroxybenzoyl Hexyl Benzoate
		2.0	Cyclopentasiloxane, Cyclohexasiloxane
15		0.5	Bees Wax
		3.0	Cetearyl Alcohol
		10.0	Caprylic/Capric Triglyceride
	B	5.0	Titanium Dioxide, Silica, Methicone, Alumina
20	C	3.0	Glycerin
		0.2	Disodium EDTA
		0.3	Xanthan Gum
		1.0	Decyl Glucoside
		2.0	Panthenol, Propylene Glycol
25		52.3	Aqua dem.
30	D	SA	Compound
		1.0	Tocopheryl Acetate
		0.2	Bisabolol
		QS	Perfume
		QS	Preservative

Production: Heat Phase A and B separately to approx. 80°C. Add Phase B to Phase A and homogenize by stirring. Heat Phase C to 80°C and add to the combined Phase A and B and homogenize again. Cool to approx. 40°C and add Phase D. Homogenize again.

FOOT BALM

40		%	Ingredients (INCI)
45	A	2.0	Ceteareth-6, Stearyl Alcohol
		2.0	Ceteareth-25
		5.0	Cetearyl Ethylhexanoate
		4.0	Cetyl Alcohol
		4.0	Glyceryl Stearate
50		5.0	Mineral Oil
		0.2	Menthol
		0.5	Camphor

211

	B	69.3 QS	Aqua dem. Preservative
5	C	1.0 1.0	Bisabolol Tocopheryl Acetate
10	D	SA 5.0	Compound Witch Hazel Extract

		%	Ingredients (INCI)
15	A	2.0	Ceteareth-6, Stearyl Alcohol
		2.0	Ceteareth-25
		5.0	Cetearyl Ethylhexanoate
		4.0	Cetyl Alcohol
		4.0	Glyceryl Stearate
20		5.0	Mineral Oil
		0.2	Menthol
		0.5	Camphor

25	B	65.3 QS	Aqua dem. Preservative
	C	1.0 1.0	Bisabolol Tocopheryl Acetate
30	D	SA 5.0	Compound Witch Hazel Extract

Production: Heat Phase A and B separately to approx. 80°C. Add Phase B to Phase A and homogenize by stirring. Cool to approx. 40°C and add Phase C and D. Homogenize by stirring and cool to room temperature

35

W/O

		%	Ingredients (INCI)
40	A	6.0	PEG-7 Hydrogenated Castor Oil
		8.0	Cetearyl Ethylhexanoate
		5.0	Isopropyl Myristate
		15.0	Mineral Oil
45		0.3	Magnesium Stearate
		0.3	Aluminum Stearate
		2.0	PEG-45/Dodecyl Glycol Copolymer
	B	5.0	Glycerin

212

		0.7	Magnesium Sulfate
		55.6	Aqua dem.
5	C	1.0	Compound
		0.5	Tocopheryl Acetate
		0.6	Bisabolol
10		%	Ingredients (INCI)
	A	6.0	PEG-7 Hydrogenated Castor Oil
		8.0	Cetearyl Ethylhexanoate
		5.0	Isopropyl Myristate
15		15.0	Mineral Oil
		0.3	Magnesium Stearate
		0.3	Aluminum Stearate
		2.0	PEG-45/Dodecyl Glycol Copolymer
20	B	5.0	Glycerin
		0.7	Magnesium Sulfate
		51.6	Aqua dem.
25	C	5.0	Compound
		0.5	Tocopheryl Acetate

Production: Heat Phase A and B separately to approx. 85°C. Add Phase B to Phase A and homogenize by stirring. Cool to approx. 40°C and add Phase C. Homogenize by stirring and cool to room temperature.

30

LIQUID MAKE-UP – TYPE O/W

35

		%	Ingredients (INCI)
40	A	2.0	Ceteareth-6, Stearyl Alcohol
		2.0	Ceteareth-25
		6.0	Glyceryl Stearate
		1.0	Cetyl Alcohol
		8.0	Mineral Oil
45		7.0	Cetearyl Ethylhexanoate
		0.2	Dimethicone
	B	3.0	Propylene Glycol
		1.0	Panthenol
		QS	Preservative
50		61.9	Aqua dem.

213

	C	0.1	Bisabolol
		SA	Compound
		QS	Perfume
5	D	5.7	C. I. 77 891, Titanium Dioxide
		1.1	Iron Oxides
10			
		%	Ingredients (INCI)
	A	2.0	Ceteareth-6, Stearyl Alcohol
		2.0	Ceteareth-25
15		6.0	Glyceryl Stearate
		1.0	Cetyl Alcohol
		8.0	Mineral Oil
		7.0	Cetearyl Ethylhexanoate
20		0.2	Dimethicone
	B	3.0	Propylene Glycol
		1.0	Panthenol
		QS	Preservative
25		57.9	Aqua dem.
	C	0.1	Bisabolol
		SA	Compound
		QS	Perfume
30	D	5.7	C. I. 77 891, Titanium Dioxide
		1.1	Iron Oxides

Production: Heat Phase A and B separately to approx. 80°C. Add Phase B to Phase A and homogenize by stirring. Cool to 40°C and add Phase C and D. Homogenize again and cool to room temperature.

FOUNDATION

40			
	<u>Ingredient</u>	<u>INCI Name</u>	<u>%</u>
	<u>Water Phase</u>		
	Dow Corning 9011	Cyclopentasiloxane, PEG-12 Dimethicone	
	Elastomer Blend	Copolymer	15.00
45	Dow Corning 245 Fluid	Cyclopentasiloxane	5.00
	Silcare 31 M50 SV	Caprylyl Trimethicone	6.35
	Propylparaben		0.05
	AS 5811	Titanium Dioxide, Triethoxycaprylylsilane	7.50
	AS 5131	Iron Oxides, Triethoxycaprylylsilane	0.70
50	AS 5146	Iron Oxides, Triethoxycaprylylsilane	0.05

214

AS 5126	Iron Oxides, Triethoxycaprylylsilane	0.35
AS 50230	Talc, Triethoxycaprylylsilane	3.50

Oil Phase

5	Deionized Water	53.30
	1.80 Butylene Glycol	6.00
	Methylparaben	0.20
	Benzoic Acid	0.20
10	Compound	SA

The pigments (AS 5811, 5131, 5146, 5126, and 50230; Color Techniques) and propylparaben are dispersed in Silcare 31 M50 SV (Clariant), stirring until wet. The mixture is then passed over a three roll mill at tight setting until particle size is <10 μ m. Then, DC 9011 Elastomer Blend (Dow Corning) and DC 245 Fluid are combined in finishing vessel, stirring until homogenous. The color grind is added with slow homogenizer agitation. The water is weighed into a separate vessel and Compound is gradually added with propeller agitation, stirring until dissolved. Methylparaben and benzoic acid are added to butylene glycol. The mixture is warmed slightly, and stirred until dissolved. The mixture is cooled to 30 °C and added to the Compound-containing solution. The water phase is added slowly to the oil phase with rapid agitation. When addition is complete, the preparation is homogenized for five minutes. This preparation is useful as a makeup foundation for application to skin.

MASCARA FORMULATION

The ingredients of both a control preparation and a mascara containing 2% are as follows:

<u>Trade Name</u>		<u>INCI Name</u>		<u>Control</u>	<u>%</u>
				1	2%Compound
<u>Phase # Water Phase</u>					
30	1 Deionized Water			42.96	42.96
35	2 Butylene Glycol			5.00	5.00
	2 Methylparaben			0.30	0.30
40	3 33-5198	(Black) Iron Oxides	(Sun)	10.00	10.00
	4 Natrosol 250 MR	Hydroxyethylcellulose (Aqualon)		0.20	0.20
	5 10% KOH	Potassium Hydroxide		0.01	0.01
45	6 Arlacel 165	Glyceryl Stearate, PEG-100 Stearate (Uniqema)		3.00	3.00
	7 10% Citric Acid			0.27	0.27

215

<u>Phase # Wax Phase</u>			
5	8 Arlacel 165	1.00	1.00
	8 Cerasynt SD Glyceryl Stearate (ISP)	3.50	3.50
	8 Beeswax, White SP 424 Beeswax (S&P)	7.50	7.50
	8 Carnauba #1 Copernica Cerifera (Carnauba) Wax (S&P)	4.80	4.80
	8 Propylparaben	0.10	0.10
10	9 Deionized Water	20.00	-
	9 10% Compound/Water	-	20.00
15	10 Deionized Water	1.00	1.00
	10 Glydant	<u>0.36</u>	<u>0.36</u>
	DMDM Hydantoin (Lonza)		
		100.00	100.00

To produce the mascara formulation, the wax phase 8 is combined and heated to 85-90°C with propeller mixing. The 10% Compound solution is prepared by adding Compound to water while propeller mixing. Phase 1 water is added to a tared stainless steel beaker (approximately 50 g excess is added to compensate for loss). Phase 2 methylparaben is added to butylene glycol and stirred while warming on top of a steam bath until dissolved, then added to the water with slow homomixer agitation. Then, the phase 4 black iron oxide is added, while maintaining agitation. Then, Natrosol is sprinkled in, while maintaining agitation. The 10% KOH is added, and heating is begun to 85°C, with the beaker covered as tightly as possible. When the Natrosol is dissolved, the 10% citric acid is added dropwise, maintaining temperature and agitation. Then, the Arlacel 165 is added slowly and mixed for at least 5 minutes to insure dissolution. At 85-90°C, the wax phase is slowly added to the water phase while homomixing. The temperature and agitation are maintained for 10 minutes. The batch is removed from the steam bath and allowed to cool while homomixing with occasional hand scraping of the beaker walls. At 55°C, the batch is weighed to check for water loss. Mixing is resumed and water is added back, if necessary. At 45°C, phases 9 and 10 are added. Cooling is continued using cold water to 30°C. At this point, continuous hand scraping of beaker walls is necessary.

In this preparation, the small amount of KOH (in Phase 5) is used to raise the pH to disperse the Natrosol which is coated with glyoxal to retard wetting, and prevent agglomeration. In phase 7, the citric acid is added slowly to adjust pH to ~5.5, below the isoelectric point of the iron oxides. In phases 7 and 8, the Arlacel 165 is split between the oil and water phases, as the emulsification is easier to accomplish with surfactant in both phases. In phase 9, the deionized water is added in the control batch instead of Compound. The Compound solution is prepared while the emulsion is being processed, so it is absolutely fresh. This preparation provides a formulation suitable for use as a mascara.

EXAMPLE 2

Skin Lightening Studies

In this Example, experiments conducted to assess the skin lightening capabilities of BBI-AV are described. Thirteen subjects, ages 18-65, with Fitzpatrick skin types III or IV participated in this 22-day study. The Fitzpatrick skin classification is based on the skin's unprotected response to the first 30 to 45 minutes of sun exposure following a winter season without sun exposure. The categories of skin types are:

- I Always burns easily; never tans
- II Always burns easily; tans minimally
- III Burns moderately; tans gradually
- IV Burns minimally; always tans well
- V Rarely burns; tans profusely
- VI Never burns; deeply pigmented

Five test sites of uniform color were marked on the lower back of each subject. The clinical grader assessed the degree of tan at each site using a 0-10 scale (0=very slight tan and 10=very deep tanning.). Each subject was pre-treated with one concentration of Molecule A, two concentrations of Molecule B, vehicle control buffer, and an untreated site for 11 consecutive days. Approximately 150uL of test materials (0.1% and 0.9% of BBI-AV) and vehicle control were dosed onto the lower back using occlusive patches. Occlusive patches consisted of a Webril® (non-woven cotton) pad held to the skin with a porous, hypoallergenic tape (*e.g.*, SCANPORE® or MICROPORE®). A strip of BLENDERM® tape equal in width of the cotton pad covered the outside of the tape to provide the occlusive patch. Patches remained in place until a clinician removed them the next day. This patching procedure was repeated for 11 days.

On day 12, the clinician removed the patches that were applied the previous day and the site was gently wiped to remove residual test material. The clinical grader assessed the degree of tan at each site using a 0-11 scale (0=no tan and 11=very deep tanning). The results are provided in the Table below. As indicated, there was a greater change in the tanning grade observed with the BBI-AV than the vehicle control.

Test Material	Average Day 1 Tanning Grade	Average Day 11 Tanning Grade	Change in Average Tanning Grade
Vehicle Control	4.92	4.82	-0.1
0.9% BBI-AV	4.96	4.69	-0.27

EXAMPLE 3

Reduction in Skin Pigmentation

In this Example, experiments conducted to test the ability of BBI-AV to reduce skin pigmentation induced by UVA exposure are described. This study was conducted with 13 individuals of skin type III and IV based on The Fitzpatrick skin classification.

On day one, each subject's minimal persistent pigment darkening dose (MPPD) was determined on the lower back of each individual, in an area that is equal in color to the area that received test material application. Subjects received five to seven exposures of UVA, with each exposure representing about a 25% increase over the previous exposure. Subjects returned to the clinic 3-4 hours after the MPPD exposures were complete. The test sites were scored for pigment darkening using the following scale:

- no visible pigment darkening
- ? questionable response; unclear
- + slight darkening over essentially the whole irradiation field
- ++ definite darkening over the whole irradiation field

The site treated with the lowest dose of UVA producing a score of '+' represented that subject's MPPD.

On day two, sites of uniform color were marked on the lower back. The clinical grader assessed the degree of tan at each site using a 0-10 scale (0=very slight tan and 10=very deep tanning.) Then the Test material (0.1% and 0.9% of BBI-AV) and the vehicle control applied under occlusion. A volume of 150 μ l was dosed onto each patch. The occlusive patches used hte these experiments were the same as described in Example 2. As described in Example 2, the patches remained in place until the next day, when a clinician removed them. This patching procedure was repeated for 11 days. On the days 12 – 14, the clinician removed the patches that were applied the previous day and the site was gently wiped to remove residual test material. The clinical grader assessed the degree of tan at each site using a 0-10 scale (0=no tan and 10=very deep tanning.) After grading was complete, each site was exposed to 1.0 MPPD of UVA as described for Day 1. New patches were applied after exposures were completed. On day 15, patches were removed and the degree of tan at each site was assessed using a 0-10 scale (0=no tan and 10=very deep tanning). The results are provided below.

Treatment	Day 12 Avg. Tanning Grade	Day 13 Avg. Tanning Grade	Day 14 Avg. Tanning Grade	Day 15 Avg. Tanning Grade
Buffer	4.82	5.29	5.59	5.98
0.1% BBI-AV	4.76	5.08	5.13	5.5
0.9% BBI-AV	4.69	4.92	5.04	5.5

5 The results showed that UVA induced pigmentation is decreased in case of BBI-AV exposed sites. This is further evident when the increase in tanning grade is tabulated in Table below. In this table, the tanning grade results are shown as the results obtained for day 12 subtracted from grades of days 13, 14 and 15.

10

Treatment	Change on Day 13	Change on Day 14	Change on Day 15
Buffer	0.41	0.77	1.16
0.1% BBI-AV	0.32	0.37	0.74
0.9% BBI-AV	0.23	0.35	0.81

EXAMPLE 4

15

Hair Growth Inhibition

In this Example, experiments to determine the ability of the compositions of the present invention to inhibit the growth of hair are described. In particular, these experiments are conducted in order to assess the ability of the compositions of the present invention to decrease hair growth after depilation by shaving or use of depilatory creams or waxing.

20

Facial Hair Experiments

25

In these experiments, a group (e.g., 5) male subjects with Fitzpatrick Skin Classification II are tested. Individuals are requested to use no topical facial treatment prior to beginning the experiments. On day 1, facial hair growth is visually evaluated and photographed. Following this evaluation and photography, the composition(s) to be tested, as well as a vehicle control are applied at the desired concentration(s). Beginning on day 2, the individuals apply the composition(s) immediately after shaving. No other pre- or post-shave treatment is used for the duration of the experiments. In most cases, the experiment continues for a time period of 30 to 45 days. Facial hair growth is visually evaluated and

photographed every third day during the experiments. The number of hairs, as well as the hair shaft length and width are measured using computerized image analysis. In preferred embodiments, there is a decrease in the number of hairs, hair thickness and/or hair length due to the application of the test compound(s).

5 Leg Hair Experiments

In these experiments, a group (*e.g.*, 5) female subjects with Fitzpatrick Skin Classification II are tested. Individuals are requested to use no topical leg treatment prior to beginning the experiments. On day 1, areas on both legs of each individual are marked and the hair growth is visually evaluated and photographed. Following this evaluation and photography, the composition(s) to be tested are applied at the
10 desired concentration(s). Following this evaluation and photography, the composition(s) to be tested (*i.e.*, test compounds containing a desired concentration of BBI-AV), as well as a vehicle control, are applied at the desired concentration(s). In some methods, each individual is provided with two tubes, one of which contains the BBI-AV and the other containing the vehicle control. These tubes are marked "left" and "right." Each day during the experiments, the subject applies the compositions in the two tubes the
15 respective legs. After 7 days of application, the individuals are visually evaluated and photographs are taken. Both legs are then shaved or exposed to a depilatory and the test individuals continue to apply the compositions as before. Hair growth is then evaluated visually and by photographing appropriate areas on the legs every 2 days. After 10 days, the legs are again shaved and the test subjects continue to apply the compositions as before. In some methods, the experiments are conducted for 3 cycles and the hair growth is
20 visually evaluated and photographs were taken. The experiments are then continued for an additional 8 days. In preferred embodiments, there is a decrease in the number of hairs, hair thickness and/or hair length due to the application of the test compound(s) in the marked area(s).

Beginning on day 2, the individuals apply the composition(s) immediately after shaving. No other
25 pre- or post-shave treatment is used for the duration of the experiments. In most cases, the experiment continues for a time period of 30 to 45 days. Facial hair growth is visually evaluated and photographed every third day during the experiments. The number of hairs, as well as the hair shaft length and width are measured using computerized image analysis. In preferred embodiments, there is a decrease in the number of hairs, hair thickness and/or hair length due to the application of the test compound(s).

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EXAMPLE 5

Panning of a Phage Displayed Peptide Library

In this Example, experiments conducted to pan a phage displayed library are described. A
35 commercially available phage peptide library PhD C7C (NEB) was panned against hVEGF₁₆₅ (R&D

systems) for 3 rounds according to the manufacturer's instructions. This procedure yielded the sequence profiles summarized in Figure 1. Individual clones were confirmed using phage ELISA according to the manufacturer's instructions (See, Figure 2).

5

EXAMPLE 6

BIACore™ Binding Analysis of VEGF-Binding Peptides

In this Example, experiments conducted to assess the affinities of the peptides for VEGF are described. Affinities of the peptides for VEGF were measured using a BIACore™-3000 surface plasmon resonance system (Biacore). A CM5 sensor chip was conditioned with 50 mM NaOH, 0.1% HCl, 0.1% SDS, and 0.08% H₃PO₄, and activated for covalent coupling of VEGF using *N*-ethyl-*N'*-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) according to the supplier's instructions (Biacore). Human VEGF₁₆₅ (Biosource) was diluted to 5 µg/ml in 20 mM sodium acetate, pH 4.8 and injected at a flow rate of 2 µl/min to achieve approximately 1000 to 6000 response units (RU) of coupled protein. TNF-alpha (human TNF-alpha, Biosource, Int., Camarillo, CA) was similarly coupled to the CM5 sensor chip to approximately 850 to 3500 RU in the reference lane. A solution of 1 M ethanolamine was injected as a blocking agent. In some experiments, an additional solution of EDC and NHS were injected to improve baseline stability and a solution of 1 M ethanolamine injected as a blocking agent. The reference lane was activated with EDC and NHS and blocked with ethanolamine.

Peptides were synthesized using standard Fmoc chemistry, purified by reverse phase HPLC to >95% purity (SynPep), and stored at 10 mg/mL in DMSO. For kinetic measurements, twofold serial diluted peptides in HBS-EP buffer, 0.01 M HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% Surfactant P20 (Biacore), were injected at 25°C at a flow rate of 20 µL/min. Two-fold serial diluted DMSO samples and buffer samples were also injected for background subtraction. Kinetic parameters were calculated using BIAevaluation 3.1 software. Results from these experiments are provided in Figure 3.

EXAMPLE 7

Construction of Peptide-BLA Scaffolds

In this Example, methods used in the construction of anti-VEGF-BBI constructs are described. Plasmid pCM01 (5.1kb) encodes a 15-amino acid peptide sequence CK37281 fused to the N-terminus of *Enterobacter cloacae* β-lactamase (BLA) with a pIII signal sequence and C-terminal 6XHis tag, (See, Figure 4). The plasmid also carries a chloramphenicol resistance gene (CAT) as a selectable marker and expression is driven by a *lac* promoter (Plac). Plasmid pCM01 was constructed using a BbsI vector, pME30 constructed from pCB04. pCB04 was digested with *Dra*III and *Spe* I (NEB), resulting in 2.8 kb and 2.1kb

fragments. To make the inserts, the oligo pairs NtermStf2-F and NtermStf2-R (5 μ M) were combined in 50 μ l total volume in water, the mixture was heated at 95C in heat block for 5 minutes, and the block was allowed to cool to room temperature.

- 5 **Oligos:** NtermStf2-F and NtermStf2-R for stuffer vector insert:

NtermStf2-F

5'[Phos]

CTAGTGTCTTCGATCAAGTCGACAACAGCCTGTCTGCAGATCCTGAAGACTGGCGGAGGTGGTC
10 GCGAATACGATTACCCCGCTGATGAAAGCACAGA 3' (SEQ ID NO:26)

NtermStf2-R

5'[Phos]

GTGCTTTCATCAGCGGGGTAATCGTATTCGCGACCACCTCCGCCAGTCTTCAGGATCTGCAGAC
15 AGGCTGTTGTCGACTTGATCGAAGACA 3' (SEQ ID NO:27)

The 2.8kb fragment, 2.1kb fragment, and stuffer insert (100bp) were ligated overnight at 16°C in a 1:1:5 molar ratio respectively using 10 μ l of the DNA mix and 10 μ l of Takara solution I ligase. Ligations were purified using Zymo Research DNA clean kit and eluted in 2x 8 μ l of water. Then, 5 μ l of ligation mix
20 was transformed into 50 μ l Top 10 electrocompetent cells (Invitrogen), 250 μ l SOC was added and the cells grown for 1 hr at 37°C. The transformation mix was diluted 1/10 and plated on both LA + 5 ppm CMP and LA + 5 ppm CMP + 0.1 ppm CTX plates, followed by incubation overnight at 37 °C. 12 colonies were picked from CMP plates, grown in LB + 5 ppm CMP, DNA was isolated and digested with *Bbs*I enzyme (2 sites in stuffer plasmid). pCB04 (WT) was also digested as control. One clone had the correct sequence and
25 was designated pME22.

The VEGF peptide-BLA expression plasmid pCM01 was constructed from pME22 using the following primers for the BBS1 insert (*See*, Figure 5); Oligos VegF-F, VegF-5R, VegF-3RP for peptide insert.

30 **VegF-F**

5'ACTAGTCGTTCCCTTCTATTCTCACTCTGCTTGACCTGTGGCCGACCTTCTGGTGCGGTGGA
GGTTCGACGCCAGTGTGAGAAAAACAGCTG 3' (SEQ ID NO:28)

35 **VegF-5R**

222

5' AGCAGAGTGAGAATAGAAAGGAACGAC 3' (SEQ ID NO:29)

VegF-3RP

5 5' [Phos]CCGCCAGCTGTTTTTCTGACACTGG 3' (SEQ ID NO:30)

BLA-peptide fusion proteins pCM01 and pCB04 (WT) and a biased library pCM04 were expressed in *E. coli* (TOP10; Invitrogen) in 1-L shake flasks in the presence of 5 ppm CMP and 0.1 ppm cefotaxime antibiotic at 25°C for 40 hrs. Cell pastes were harvested from the 200 ml cell cultures by centrifugation at 10 3,000xg for 10 min. The pastes were then treated with 25 ml of B-PER reagent (Pierce) for 40 min with slow mixing. The extract was separated by centrifugation at 20,000xg for 20 min. BLA activity of all liquid fractions was assayed using nitrocefin and the concentration of fusion proteins in each fraction was calculated assuming the same specific activity as the WT enzyme. Fusion proteins were purified by IMAC chromatography. The imidazole-eluted BLA-active fractions were pooled and the purity was found better 15 than 95% as checked by SDS-PAGE (*See*, Figure 6).

EXAMPLE 8

Screening a Peptide-BLA Scaffold Library

In this Example, experiments to screen a peptide-BLA scaffold library are described. COSTAR 20 plates (96-well) were coated with 0.5 µg (100 µL of 5 µg/mL) hVEGF₁₆₅ (Preprotech) with gentle rocking at 4 °C overnight, followed by blocking with Superblock blocking buffer (Pierce) for several hours at room temperature. His-tag purified samples of pCM01 and pCM04 were diluted serially into BLA assay buffer and 100 µl portions were transferred to VEGF coated wells. After one hour, plates were washed six times with PBS, 0.05% TWEEN®-20 and 200 µL of nitrocefin assay buffer containing 0.1 mg/ml nitrocefin 25 (Oxoid) was added to measure residual bound beta-lactamase activity at Abs₄₉₀/min. Control wells contained pCB04 beta-lactamase as a control (*See*, Figure 7).

EXAMPLE 9

Inhibition of HUVE Cell Proliferation by aVEGF Peptides.

30 In this Example, experiments conducted to determine the effects of aVEGF peptides on HUVE cells are described. HUVE cells (human umbilical vein cells; Cambrex) were passaged 1-5 times and maintained according to manufacturer's instructions. HUVE cell growth was stimulated by 0.03 to 20 ng/ml VEGF with the highest proliferation at 10 ng/ml VEGF₁₆₅. This concentration was also used in subsequent experiments. A series of a VEGF peptides from 0.5 nM to 25 µM (and an anti-VEGF monoclonal antibody 35 control (R&D Systems)) were mixed with 10 ng/mL VEGF prior to addition to HUVE cells seeded in

triplicate in 96-well plates. Cell proliferation was measured by ^3H -thymidine incorporation (*See*, Figure 8). Significant inhibition was observed down to 0.4 μM anti-VEGF.

EXAMPLE 10

Inhibition of Blood Vessel Tube Formation by VEGF Peptide Conjugates

In this Example, experiments conducted to assess blood vessel tube formation are described. This *in vitro* angiogenesis assay was obtained as a kit from Chemicon and used according to the manufacturer's instructions.

This assay provides a simple model of angiogenesis in which the induction or inhibition of tube formation by exogenous signals can be monitored. An endothelial cell suspension of low passage HUVE cells was mixed with different concentrations of the inhibitor in the presence of 10 ng/mL VEGF, before adding the cells to "ECMatrix" (*i.e.*, a solution that is polymerized *in situ* and provides a solid gel of basement proteins prepared so that endothelial cells align and form hollow tube-like structures). Tube formation is a multi-step process involving cell adhesion, migration, differentiation and growth. The resulting tube formation was measured under an inverted light microscope at 20X-100X magnification. Significant inhibition of tubule formation was observed at concentrations above 1 μM peptide.

EXAMPLE 11

Construction of Phage-Displayed VEGF-Biased Peptide Libraries

In this Example, experiments conducted to constructed phage-display libraries are described. The affinity maturation libraries used for panning VEGF were constructed using the C7C gene III phage-display system known in the art (*See*, Noren and Noren [2001]). Oligonucleotides were synthesized and phosphorylated as known in the art. The oligonucleotides used to construct the libraries employ NNK (where N = G, A, T, C and K= G or T) codons. The NNK cloning scheme eliminates the potential for two stop codons and still encodes all twenty amino acids. The random peptide library displayed 9 random amino acids with two cysteines fixed at positions 2 and 9 (XC(X)₇CGGS; SEQ ID NO:31; X represents any amino acid). Seven CK37282 biased peptide libraries were created using the same methods as for the random library.

EXAMPLE 12

Construction of a VEGF Bowman Birk Inhibitor (BBI^{VEGF})

In this Example, construction of an anti-VEGF BBI (BBI-AV) construct is described. A synthetic gene coding for Bowman Birk Inhibitor (*See*, Figure 9) with appropriate restriction sites for introducing small peptide coding sequences into the trypsin loop (*SacI-EcoRI*) and/or chymotrypsin loop (*EcoRI-SalI*)

was cloned into pET-22b (Novagen) using *NdeI/XhoI* cloning sites according to standard procedures known in the art. The resulting vector, pET BBI, was used as a template to insert the sequences CK37281, CK37282 into BBI loops as double-stranded oligonucleotide cassettes (Operon). Constructs were transformed into BL-21(DE3) *E coli*, and plated on medium containing 50 µg/mL ampicillin. Plasmid DNA from individual clones was isolated using methods known in the art (Qiagen) and the correct inserts confirmed by DNA sequencing. Additional peptides of interest include PS-AV1 (1KSAIC-KYYLYWW-CF1V; SEQ ID NO:16) and PS-AV2 (1KSAIC-TLWKSYSW-CF1V; SEQ ID NO:17).

Fusion proteins and wild-type BBI were expressed in 14-L fermentors. Cell pastes were harvested and protein isolated from inclusion bodies using a modification of the FoldIt screening procedure (Hampton) (See, Figure 10).

EXAMPLE 13

BIACore™ Binding Analysis of BBI-VEGF

In this Example, experiments conducted to determine the binding affinity of constructs produced as indicated in Example 9 are described. Affinities of BBI-VEGF constructs for VEGF were measured using BIACore-3000 surface plasmon resonance (Biacore). A CM5 sensor chip was conditioned with 50 mM NaOH and activated for covalent coupling of VEGF using *N*-ethyl-*N'*-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) according to the supplier's instructions (Biacore). VEGF (human VEGF₁₆₅, Biosource) was diluted to 5µg/mL in 20mM sodium acetate, pH 4.8, and injected at a flow rate of 2 µL/min to achieve approximately 1000 to 6000 response units (RU) of coupled protein. Trypsin and chymotrypsin were similarly coupled to the CM5 sensor chip to approximately 850 to 3500 RU in remaining lanes. A solution of 1M ethanolamine was injected as a blocking agent. Selective binding affinity to VEGF of refolded BBI-VEGF is shown in Figure 11.

EXAMPLE 14

In vitro Cell Proliferation Assay to Test the Activity of hVEGF Inhibitory Peptides

In this Example, experiments conducted to determine the anti-proliferative activity of anti-VEGF peptides are described. The antiproliferative activity of VEGF inhibitory peptides was determined using human umbilical vein endothelial cells (HUVEC) as follows. An early passage (less than six) of HUVEC was seeded in 96-well plates at 5000 cells per well and starved for 18 hrs in 200 µl EBM medium (Cambrex) without growth factors and supplemented with 0.5% FBS, at 37 °C with 5% CO₂. The medium was replaced with 180 µl of growth medium containing EBM medium with 5% fetal bovine serum and 1% DMSO. Then, 20 µl of VEGF preincubated for one hour with varying peptide concentrations (the final DMSO concentration of all the wells was 1%) were added to the wells for a final VEGF concentration of 10 ng/ml. Human VEGF antibody (R & D Systems) was used as a positive control. Cells with 0.31 to 20 ng/ml

concentrations of VEGF alone in the growth medium were used to construct a standard growth curve. The cells were further incubated for 48 hrs, and the cell proliferation was measured using an MTS assay (CellTiter 96 Aqueous One Solution Cell Proliferation Assay Kit; Promega). Then, 40 μ l of the MTS tetrazolium solution was added to each well and after 3 and 4 hours incubation, the plates were read at 490 nM. The absorption of media alone was subtracted from all data points. The results indicated that the VEGF inhibitory peptides CK37281 and CK37283 have IC50 in the micromolar range.

EXAMPLE 15

Repeat Insult Patch Testing (RIPT) of an Anti-VEGF Peptide on Human Skin

In this Example, experiments conducted to determine the patch test result for anti-VEGF peptides are described. Samples of the aVEGF peptide CK37281 dosed at 0.5% (w/v) were formulated in a base formulation containing deionized water/butylene glycol. Approximately 0.2 mL of the formulation was applied to 200 human volunteers in a repeated insult patch test according to procedures designed by Clinical Research Laboratories, Inc. (Piscataway, NJ). The results indicated that there was no dermal irritation or sensitization on the skin of these volunteers.

Having described the preferred embodiments of the present invention, it will appear to those ordinarily skilled in the art that various modifications may be made to the disclosed embodiments, and that such modifications are intended to be within the scope of the present invention.

EXAMPLE 16

Production of BCE103-BBI Fusion Proteins in *B. subtilis*

In this Example, experiments conducted to produce BCE103-BBI fusion proteins in *B. subtilis* are described. The DNA sequence of the synthetic gene (Operon Technologies) coding for the pro-BBI protein with a C-terminal hexa-histidine tag used in these experiments is:

AACCTGCGTCTGTCTAAGCTTGGCCTGCTTATGAAATCAGACCATCAGCACAGCAATGACGATG
AGAGCTCTAAACCCTGTTGCGATCAATGCGCATGTACGAAATCAAATCCTCCACAGTGTCGGTG
TTCCGATATGCGTCTGAATAGCTGTCATAGTGCATGCAAAAGCTGTATCTGCGCCCTGAGTTAT
CCAGCTCAATGTTTTTTCGTCGACATCACGGACTTCTGCTATGAGCCATGTAAACCAAGCGAGG
ACGATAAAGAGAACCATCATCACCATCACCAT (SEQ ID NO:44)

The protein sequence of pro-BBI with a C-terminal hexa-histidine tagged coded for by the above synthetic gene is:

5 NLRLSKLGLLMKSDHQHSNDDDESSKPCCDQCACTKSNPPQCRCSDMRLNSCHSACKSCICALSYPA
QCFCVDITDFCYEPCKPSEDDKENHHHHHHH (SEQ ID NO:45)

The portion of the DNA sequence of the synthetic gene that codes for the major mature form of BBI is:

10 GACGATGAGAGCTCTAAACCCTGTTGCGATCAATGCGCATGTACGAAATCAAATCCTCCACAGT
GTCGGTGTTCGATATGCGTCTGAATAGCTGTCATAGTGCAATGCAAAAGCTGTATCTGCGCCCT
GAGTTATCCAGCTCAATGTTTTTTCGTCGACATCACGGACTTCTGCTATGAGCCATGTAAACCA
AGCGAGGACGATAAAGAGAAC (SEQ ID NO:46)

15 The protein sequence of the major mature form of BBI coded by the above synthetic gene is:

DDESSKPCCDQCACTKSNPPQCRCSDMRLNSCHSACKSCICALSYPAQCFCVDITDFCYEPCKPSED
DKEN (SEQ ID NO:47)

20 The PCR primers used to amplify the BBI gene for fusion to the BCE103 cellulase expression cassette in the pJ103 vector were:

BBI_{fusion}_FW: 5' CAGCACGGATCCAGACGATGAGAGCTCTAAACCC 3' (SEQ ID NO:48)

25 BBI_{HindIII}_RV: 5' CTGCAGAAGCTTAAAAATAAAAAACGGATTTCCTTCA
GGAAATCCGTCCTCTGTAACTTTTAGTTCTCTTTATCGTCCTCGC 3' (SEQ ID NO:49)

30 BBI_{HIS}-HindIII_RV: 5' CTGCAGAAGCTTAAAAATAAAAAACGGATT
TCCTTCAGGAAATCCGTCCTCTGTAACTTTTAATGGTGATGGTGATGATGGTTCTC 3'
(SEQ ID NO:50)

35 The sequence of the *aprE*-BCE103-BBI-HisTag expression cassette (*EcoRI*-*HindIII*) that was cloned into the pJM103 integration vector is provided in Figure 14. A schematic plasmid map of the pJM103BBIHis expression vector is provided in Figure 15.

The alkaline cellulase (BCE103) gene (*See*, van Soligen, U.S. Patent No. 6,063,611, hereby incorporated by reference) fused to the *B. subtilis aprE* promoter and signal sequence, was cloned from pUCAPR103 (Shaw *et al.*, J. Mol. Biol., 320:303-309 [2002]) as an *EcoRI*-*BamHI* fragment (*i.e.*, a fragment

that carries the coding sequence of the BCE103 catalytic domain and first cellulose binding domain linker only) into pJM103 (Perego, "Integrational vectors for genetic manipulation in *Bacillus subtilis*" In, *Bacillus subtilis* and Other Gram-positive Bacteria: Biochemistry, Physiology, and Molecular Genetics, Sonenshein, Hoch, and Losick (eds), American Society for Microbiology, Washington D.C., pp. 615-624 [1993]). A gene encoding the soybean Bowman-Birk protease inhibitor (BBI) (Swiss-Prot Accession # P01055; See, Odani and Ikenaka, J. Biochem., 71: 839-848 [1972]) with a C-terminal hexa-histidine tag (His-Tag) was synthesized by Operon Technologies (See, DNA sequence above). The BBI gene was amplified by PCR with primers (all primers were synthesized by MWG Biotech, Oligos Etc., or Operon Technologies) that generated a 5' *Bam*HI site in the correct reading frame with the BCE103 gene, and at the 3' end introduced a strong transcriptional terminator (LAT, from the *Bacillus licheniformis* α -amylase gene) after the end of the BBI gene with a 3' *Hind*III site for cloning into the pJM103 vector.

PCR fragments with or without a C-terminal His-Tag were generated with the primers BBIfusion_FW (SEQ ID NO:48) and BBIHISHindIII_RV (SEQ ID NO:50), or BBIfusion_FW (SEQ ID NO:48) and BBI-HindIII_RV(SEQ ID NO:49), respectively, using the synthetic BBI gene as a template. Unless indicated otherwise, PCR reactions were typically performed on a thermocycler for 30 cycles with High Fidelity Platinum *Taq* polymerase (Invitrogen) according to the instructions of the supplier (with an annealing temperature of 55°C). The PCR fragments were cloned as *Bam*HI-*Hind*III fragments into pJM103 carrying the *aprE*-BCE103 expression cassette. The correct gene sequence was verified by DNA sequencing.

Thus, as shown in Figure 14, the N-terminus of the mature coding region of the BBI gene (with or without the His-Tag) was fused in frame to the C-terminal coding region of the first CBD (cellulose binding domain) linker sequence coded by the BCE103 cellulase gene. Thereby, the two CBD's of BCE103 (Shaw *et al.*, *supra*) are replaced by BBI in the final expression vectors, pJM103BBI or pJM103BBIhis (See, Figure 15). The *aprE* promoter controls the expression of the BCE103-BBI gene fusions (See, Ferrari *et al.*, J. Bact., 170:289-295 [1988]; and Henner *et al.*, J. Bact., 170: 296-300 [1988]).

Competent *Bacillus subtilis* cells, BG3934comK, were transformed with the expression plasmids, pJM103BBI or pJM103BBIhis. The bacteria were made competent by the induction of the *comK* gene under control of a xylose inducible promoter (Hahn *et al.*, Mol. Microbiol., 21:763-775 [1996]). The transformants were selected on Luria Broth agar (LA) plates containing 5 μ g/ml chloramphenicol. To increase the expression by gene amplification, colonies were streaked and grown several times on LA plates with 25 μ g/ml chloramphenicol until the growth rate with the antibiotic was similar to growth rate in the absence of chloramphenicol. The BCE103-BBI fusion protein was produced by growth in shake flasks at 37 °C in TSB medium (Tryptone Soya Broth from OXOID, 30 g/L) or in MBD medium, a MOPS based defined medium. MBD medium was made essentially as described (Neidhardt *et al.*, J. Bacteriol., 119: 736-747 [1974]), except NH_4Cl_2 , FeSO_4 , and CaCl_2 were left out of the base medium, 3 mM K_2HPO_4 was used, and the base medium was supplemented with 60 mM urea, 75 g/L glucose, and 1 % soytone. Also, the

micronutrients were made up as a 100 X stock containing in one liter, 400 mg $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 100 mg $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 100 mg $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 50 mg $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, 100 mg $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 100 mg $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$, 100 mg $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$, 10 ml of 1M CaCl_2 , and 10 ml of 0.5 M sodium citrate.

5 BCE103–BBI fusion protein could be easily visualized in samples from cell free supernatants (after 24 h of growth in TSB medium or 48 h in MBD medium) as the major protein band on SDS-PAGE gels (10 % NuPAGE in MES buffer, run as described by the manufacturer, Invitrogen) running at ~ 44 kDa by using standard protein stains (*e.g.* GelCode Blue Stain Reagent; Pierce). The identity of the BCE103-BBI fusion protein was verified by immunoblots of SDS-PAGE gels using the protocols supplied by the manufacturer (BM Chromogenic Western Blotting Kit; Roche Applied Science using an anti-HisTag antibody or an anti-
10 BCE103 cellulase polyclonal antibody for detection).

To determine the BCE103 activity, cellulase degradation was assessed qualitatively on LA cellulase indicator plates (with 1% carboxymethylcellulose stained with 0.2 % Congo Red, or with 0.5 % azo-CM-cellulose, Megazyme), or quantitatively by a direct assay in Assay Buffer (100 mM Tris pH 8.6, 0.005 % Tween-80) on the culture broth using a the synthetic substrate, 4-nitrophenyl β -D-cellobioside (Sigma),
15 using methods known in the art (*See e.g.*, van Tilbeurgh *et al.*, *Meth. Enzymol.*, 160:45-59 [1988]).

Trypsin inhibitory assays were performed in Assay Buffer to determine the BBI activity. Specifically, a standard curve was generated by making eleven 1:1 serial dilutions (100 μL BBI + 100 μL Assay Buffer) of a 2 $\mu\text{g}/\text{mL}$ standard BBI solution. The BBI standard was purified from a 1 mg/ml Trypsin-Chymotrypsin Inhibitor (Sigma Cat. #T-9777) solution in 20 mM MES pH 6.0 using a hydrophobic
20 interaction column (POROS HP2, Phenyl column, Applied Biosystems). The column was equilibrated with 20mM MES pH 6.0, loaded with 5 mg of the inhibitor, washed with the equilibration buffer, and then the BBI was eluted with water. Unknown BBI samples to be tested in the inhibitory assay were diluted as necessary, so that two or more data points would fall within the standard curve (usually 1:10, 1:100, 1:200, 1:1000, 1:2000 sample dilutions were tested and then the dilutions fine tuned if necessary). Each diluted
25 BBI standard or sample, 20 μL , was added to 80 μL of 50 ng/ml bovine pancreatic trypsin (Worthington) (made by diluting a stock 1 mg/mL trypsin solution into Assay Buffer). For convenience, the standards and samples were arrayed in 96 well microtiter plates. The reactions were mixed and incubated 15 min at 25 °C. After the incubation, 100 μL of the 0.5 mg/ml trypsin substrate (diluted in Assay Buffer from a 100 mg/ml solution in DMSO), Suc-AAPR-pNA (succinyl-Ala-Ala-Pro-Arg-para-nitroanilide, Bachem), was added,
30 mixed and the OD (A_{405}) was monitored for 15 min, with 1 time point recorded every 12 sec using a Spectra Max 250 (Molecular Devices). The data points were used to determine the V_{max} for each reaction. The standard curve was generated by plotting V_{max} versus BBI concentration and was fitted to a four-parameter curve. All data fitting was done using software supplied by the manufacturer (Molecular Devices). The BBI concentration of the unknown samples was calculated from the standard curve. Alternatively, the BBI
35 activity was measured using the same protocol but by determining bovine pancreatic chymotrypsin

(Worthington) inhibition (chymotrypsin was used at the same concentration as trypsin and chymotrypsin activity was measured by adding 100 μ L of a 0.4 mg/ml chymotrypsin substrate, succinyl-Ala-Ala-Pro-Phe-para-nitroanilide, Bachem).

5 Titters from shake flask runs (500 ml MBD medium in 2.8 L Fernbach 6 baffled flasks, 37°C, 225 rpm, harvested 60 h after of growth) typically ranged from 0.4 – 0.9 mg/ml BCE activity and 40 - 150 μ g/ml BBI trypsin inhibitory activity. However, it is contemplated that titers likely could be improved further by optimizing the bacterial strain, culture medium and growth conditions (aeration, temperature, time of harvest, etc.).

10 In addition to the BCE103 fusion to wild-type BBI, fusion proteins to BBI variants and fusion proteins with various linkers between BCE103 and BBI were produced using the methods outlined above, as described in the following Examples. In addition, fusion proteins were also produced when the BBI was fused to the 2nd CBD linker (BCE-cbdD-BBI; *See*, Example 19) making it possible to use the 1st CBD to aid in the purification process.

EXAMPLE 17

Production of Peptides Substituted into the BBI Reactive Site Loops as BCE103-BBI Fusion Proteins

15 In this Example, experiments conducted to produce peptides substituted into the BBI reactive site loops as BCE103-BBI fusion proteins are described. The primers, as well as other sequences used in the various steps of these experiments are provided below. The sequence of 12BBick81 from the BCE103 fusion site (at the *Bam*HI site) to the end of the gene is provided in Figure 16. Peptides containing the CK37281 sequence (ACYNLYGWTC (SEQ ID NO:43) were inserted into both the trypsin and chymotrypsin inhibitory loops.

25 The primers used to introduce an *Eco*RI site in the BBI gene using QuikChange® site-directed mutagenesis (Stratagene) were:

BowBeco-F

5'-GATATGCGTCTGAATTCCTGTCATAGTGCAT (SEQ ID NO:51)

30 BowBeco-R

5'-ATGCACTATGACAGGAATTCAGACGCATATC (SEQ ID NO:52)

35 The sequences of the DNA oligonucleotides that were annealed and cloned in the BBI gene (*Sac*I-*Eco*RI) to replace the trypsin inhibitory loop with the VegF binding peptide CK37281 were:

1BBck81+
 5'-CTAAACCCTGTTGCGATCAATGCGCATGTTATAATTTGTATGGGTGGACTT
 GTCGCTGCAGCGATATGCGTCTG (SEQ ID NO:53)

1BBck81-
 5'-AATTCAGACGCATATCGCTGCAGCGACAAGTCCACCCATACAAATTATAA
 CATGCGCATTGATCGCAACAGGGTTTAGAGCT (SEQ ID NO:54)

The sequences of the DNA oligonucleotides that were annealed and cloned in the BBI gene (*EcoRI-SalI*) to replace the chymotrypsin inhibitory loop with the VegF binding peptide CK37281 were:

2BBck81+
 5'-AATTCCTGTCATAGTGCCTGCAAAAGCTGCGCATGTTATAACCTGTACGGG
 TGGACCTGTTTTTGCG (SEQ ID NO:55)

2BBck81-
 5'-TCGACGCAAAAACAGGTCCACCCGTACAGGTTATAACATGCGCAGCTTTT
 GCAGGCACTATGACAGG (SEQ ID NO:56)

The DNA sequences of the oligonucleotide pairs used to make cassettes to introduce peptides into the trypsin (*SacI* and *EcoRI* restriction sites) or chymotrypsin (*EcoRI* and *SalI* restriction sites) reactive site loops of the synthetic BBI gene are provided below. These peptide coding sequences were then moved into the p2JM103BBI expression vector as *SacI-SalI* fragments.

Comstatin (1st loop)
 CTAAACCCTGTTGCGATCAATGCGCATGTGTTGTTTCAGGACTGGGGTCACCACCGTTGTCGCTG
 CAGCGATATGCGTCTG (SEQ ID NO:57)
 and
 AATTCAGACGCATATCGCTGCAGCGACAACGGTGGTGACCCAGTCCTGAACAACACATGCGC
 ATTGATCGCAACAGGGTTTAGAGCT (SEQ ID NO:58)

Comstatin (2nd loop)
 CAAAAGCTGTATCTGCGTTGTTTCAGGACTGGGGTCACCACCGTTGTTTTTGCG (SEQ ID NO:59)
 and
 TCGACGCAAAAACAACGGTGGTGACCCAGTCCTGAACAACGCAGATACAGCTTTTGCATG
 (SEQ ID NO:60)

C2c (1st loop)
 CTAAACCCTGTTGCGATCAATGCAGCTGTGGTCGTAAATCCCGATCCAGTGTGCTGCAGCGA
 TATGCGTCTG (SEQ ID NO:61)
 and
 AATTCAGACGCATATCGCTGCAGCGACACTGGATCGGGATTTTACGACCACAGCTGCATTGATC
 GCAACAGGGTTTAGAGCT (SEQ ID NO:62)

C3c (1st loop)

CTAAACCCTGTTGCGATCAATGCGGTTGTGCTCGTTCTAACCTGGACGAATGTCGCTGCAGCGA
TATGCGTCTG (SEQ ID NO:63)

and

AATTCAGACGCATATCGCTGCAGCGACATTCGTCCAGGTTAGAACGAGCACAACCGCATTGATC
5 GCAACAGGGTTTAGAGCT (SEQ ID NO:64)

C4c (1st loop)

CTAAACCCTGTTGCGATCAATGCGGTTGTCAGCGTGCTCTGCCGATCCTGTGTCGCTGCAGCGA
10 TATGCGTCTG (SEQ ID NO:65)

and

AATTCAGACGCATATCGCTGCAGCGACACAGGATCGGCAGAGCACGCTGACAACCGCATTGAT
CGCAACAGGGTTTAGAGCT (SEQ ID NO:66)

C5c (1st loop)

15 CTAAACCCTGTTGCGATCAATGCCAGTGTGGTCGTCTGCACATGAAAACCTGTCGCTGCAGCGA
TATGCGTCTG (SEQ ID NO:67)

and

AATTCAGACGCATATCGCTGCAGCGACAGGTTTTTCATGTGCAGACGACCACACTGGCATTGATC
20 GCAACAGGGTTTAGAGCT (SEQ ID NO:68)

Xa1 (2nd loop)

AATTCCTGTCATAGTGCCTGCAAAAGCTGTATCTGCGCCCGTAGTTTGCCAGCTCAATGTTTTTG
25 CG (SEQ ID NO:69)

and

TCGACGCAAAAACATTGAGCTGGCAAACCTACGGGCGCAGATACAGCTTTTGCAGGCACTATGA
CAGG (SEQ ID NO:70)

hSCC1 (1st loop)

30 CTAAACCCTGTTGCGATCAATGCAACTGTACGTACTCAACCCCTCCACAGTGTCGCTGCAGCGA
TATGCGTCTG (SEQ ID NO:71)

and

AATTCAGACGCATATCGCTGCAGCGACACTGTGGAGGGGTTGAGTACGTACAGTTGCATTGATC
35 GCAACAGGGTTTAGAGCT (SEQ ID NO:72)

The DNA sequences of oligonucleotide primer pairs used to introduce peptide sequences into the
trypsin or chymotrypsin reactive site loops using a QuikChange® II XL site-directed mutagenesis kit
(Stratagene) are provided below. The reactions were performed as outlined by the manufacturer and
40 described in this Example. Twenty cycles were performed with extensions of 6 minutes at 68 °C,
denaturations of 50 s at 95 °C, and annealings at 55 °C for 50 s. After the cycles, a final extension was
performed at 68 °C for 20 minutes.

1A (2nd loop)

45 CTGTATCTGCAAACGCTCAAAATCTCGTGGCTGTTTTTGCGTGACATCAC (SEQ ID NO:73)

and

CGCAAAAACAGCCACGAGATTTTGAGCGTTTGCAGATACAGCTTTTGCATG (SEQ ID NO:74)

2B (2nd loop)

CTGTATCTGCTGGTATAATCAAATGACAACATGTTTTTTCGTCGACATCAC (SEQ ID NO:75)

and

5 CGCAAAAACATGTTGTCATTTGATTATACCAGCAGATACAGCTTTTGCATG (SEQ ID NO:76)

4A (2nd loop)

10 CTGTATCTGCCATCAACTTGGCCCGAATTCATGTTTTTTCGTCGACATCAC (SEQ ID NO:77)

and

CGCAAAAACATGAATTCGGGCCAAGTTGATGGCAGATACAGCTTTTGCATG (SEQ ID NO:78)

5A (2nd loop)

15 CTGTATCTGCCATCCGTGGGCACCGTATTCTTGTTTTTTCGTCGACATCAC (SEQ ID NO:79)

and

CGCAAAAACAAGAATACGGTGCCACGGATGGCAGATACAGCTTTTGCATG (SEQ ID NO:80)

6-1A (2nd loop)

20 CTGTATCTGCAATCTTCATTATCTTCAACAGTGTTTTTTCGTCGACATCAC (SEQ ID NO:81)

and

CGCAAAAACACTGTTGAAGATAATGAAGATTGCAGATACAGCTTTTGCATG (SEQ ID NO:82)

7A (2nd loop)

25 CTGTATCTGCACACCGTCTCTTTATCGCCCGTGTTTTTTCGTCGACATCAC (SEQ ID NO:83)

and

CGCAAAAACACGGGCGATAAAGAGACGGTGTGCAGATACAGCTTTTGCATG (SEQ ID NO:84)

8B (2nd loop)

30 CTGTATCTGCCTTACAGATCAATCTAAACCGTGTTTTTTCGTCGACATCAC (SEQ ID NO:85)

and

35 CGCAAAAACACGGTTTAGATTGATCTGTAAGGCAGATACAGCTTTTGCATG (SEQ ID NO:86)

9A (2nd loop)

CTGTATCTGCGTTACAACATCAATGGGCATGTGTTTTTTCGTCGACATCAC (SEQ ID NO:87)

and

40 CGCAAAAACACATGCCCATTTGATGTTGTAACGCAGATACAGCTTTTGCATG (SEQ ID NO:88)

10B (2nd loop)

CTGTATCTGCCGCGCATCACCGTATGATTGGTGTTTTTTCGTCGACATCAC (SEQ ID NO:89)

and

45 CGCAAAAACACCAATCATAACGGTGATGCGCGGCAGATACAGCTTTTGCATG (SEQ ID NO:90)

11-1A (2nd loop)

CTGTATCTGCTCAACACAAAAAATCCGCAATGTTTTTTCGTCGACATCAC (SEQ ID NO:91)

and

CGCAAAAACATTGCGGAATTTTTTGTGTTGAGCAGATACAGCTTTTGCATG (SEQ ID NO:92)

12B (2nd loop)

CTGTATCTGCACACAATTTTCGCTCTGCAACATGTTTTTTCGTCGACATCAC (SEQ ID NO:93)

and

5 CGCAAAAACATGTTGCAGAGCGAAATTGTGTGCAGATACAGCTTTTGCATG (SEQ ID NO:94)

13A (2nd loop)

CTGTATCTGCCCCGGATCATGTTCCGCATCTTTGTTTTTTCGTCGACATCAC (SEQ ID NO:95)

and

10 CGCAAAAACAAAGATGCGGAACATGATCCGGGCAGATACAGCTTTTGCATG (SEQ ID NO:96)

15-1A (2nd loop)

CTGTATCTGCTCAGGCTTTCCGCTTTCTACATGTTTTTTCGTCGACATCAC (SEQ ID NO:97)

and

15 CGCAAAAACATGTAGAAAGCGGAAAGCCTGAGCAGATACAGCTTTTGCATG (SEQ ID NO:98)

1A6 (1st loop)

TCAATGCGCATGTGAAGAGATCTGGACTATGCTTTGCCGGTGTTCCGATATGCGTC (SEQ ID NO:99)

and

20 CGGAACACCGGCAAAGCATAGTCCAGATCTCTTCACATGCGCATTGATCGCAACAGG (SEQ ID NO:100)

1A6 (2nd loop)

25 CAAAAGCTGTGCTTGTGAAGAGATCTGGACTATGCTTTGCTTTTTCGTCGACATCACGG (SEQ ID NO:101)

and

30 ACGCAAAAGCAAAGCATAGTCCAGATCTCTTCACAAGCACAGCTTTTGCATGCACTATG (SEQ ID NO:102)

1C2 (1st loop)

TCAATGCGCATGTTGGGCCCTTACTGTCAAAACATGCCGGTGTTCCGATATGCGTC (SEQ ID NO:103)

and

35 CGGAACACCGGCATGTTTTGACAGTAAGGGCCCAACATGCGCATTGATCGCAACAGG (SEQ ID NO:104)

1C2 (2nd loop)

40 CAAAAGCTGTGCTTGTGGGCCCTTACTGTCAAAACATGCTTTTTCGTCGACATCACGG (SEQ ID NO:105)

and

45 ACGCAAAAGCATGTTTTGACAGTAAGGGCCCAACAAGCACAGCTTTTGCATGCACTATG (SEQ ID NO:106)

2E2 (1st loop)

TCAATGCGCATGTCTTACAGTACTGTGGACTACATGCCGGTGTTCCGATATGCGTC (SEQ ID NO:107)

and

50 CGGAACACCGGCATGTAGTCCACAGTACTGTAAGACATGCGCATTGATCGCAACAGG (SEQ ID NO:108)

- 2E2 (2nd loop)
CAAAAGCTGTGCTTGTCTTACAGTACTGTGGACTACATGCTTTTGCCTCGACATCACGG (SEQ ID NO:109)
- 5 and
ACGCAAAAGCATGTAGTCCACAGTACTGTAAGACAAGCACAGCTTTTGCATGCACTATG (SEQ ID NO:110)
- 2E5 (1st loop)
10 TCAATGCGCATGTACTCTTTGGAACAGATCTCCTTGCCGGTGTTCGGATATGCGTC (SEQ ID NO:111)
and
CGGAACACCGGCAAGGAGATCTGTTCCAAAGAGTACATGCGCATTGATCGCAACAGG (SEQ ID NO:112)
- 15 2E5 (2nd loop)
CAAAAGCTGTGCTTGTACTCTTTGGAATCGATCTCCTTGCTTTTGCCTCGACATCACGG (SEQ ID NO:113)
and
20 ACGCAAAAGCAAGGAGATCGATTCCAAAGAGTACAAGCACAGCTTTTGCATGCACTATG (SEQ ID NO:114)
- FGFns (1st loop)
25 TCAATGCGCATGTACAAACATCGATTCTACTCCTTGCCGGTGTTCGGATATGCGTC (SEQ ID NO:115)
and
CGGAACACCGGCAAGGAGTAGAATCGATGTTTGTACATGCGCATTGATCGCAACAGG (SEQ ID NO:116)
- 30 FGFns (2nd loop)
CAAAAGCTGTGCTTGCACAAACATCGATTCTACTCCTTGTTTTTGCCTCGACATCACGG (SEQ ID NO:117)
and
35 ACGCAAAAACAAGGAGTAGAATCGATGTTTGTGCAAGCACAGCTTTTGCATGCACTATG (SEQ ID NO:118)
- FGFkr (1st loop)
40 TCAATGCGCATGTACAAAATCGATCGTACTCCTTGCCGGTGTTCGGATATGCGTC (SEQ ID NO:119)
and
CGGAACACCGGCAAGGAGTACGATCGATTTTTGTACATGCGCATTGATCGCAACAGG (SEQ ID NO:120)
- 45 FGFkr (2nd loop)
CAAAAGCTGTGCTTGCACAAAAATCGATCGTACTCCTTGTTTTTGCCTCGACATCACGG (SEQ ID NO:121)
and
ACGCAAAAACAAGGAGTACGATCGATTTTTGTGCAAGCACAGCTTTTGCATGCACTATG (SEQ ID NO:122)
- 50 FGFhl (1st loop)
TCAATGCGCATGTCACCTGCAGACAACTGAAACATGCCGGTGTTCGGATATGCGTC (SEQ ID NO:123)

and

CGGAACACCGGCATGTTTCAGTTGTCTGCAGGTGACATGCGCATTGATCGCAACAGG (SEQ ID NO:124)

5 FGFhl (2nd loop)

CAAAAGCTGTGCTTGCCACCTGCAGACAACCTGAAACATGTTTTTTCGTCGACATCACGG (SEQ ID NO:125)

and

10 ACGCAAAAACATGTTTCAGTTGTCTGCAGGTGGCAAGCACAGCTTTTGCATGCACTATG (SEQ ID NO:126)

FGFgy (1st loop)

TCAATGCGCATGTGGCTACTTCATCCCATCGATTTGCCGGTGTTCGATATGCGTC (SEQ ID NO:127)

15 and

CGGAACACCGGCAAATCGATGGGATGAAGTAGCCACATGCGCATTGATCGCAACAGG (SEQ ID NO:128)

FGFgy (2nd loop)

20 CAAAAGCTGTGCTTGCGGCTACTTCATCCCATCGATTTGTTTTTTCGTCGACATCACGG (SEQ ID NO:129)

and

ACGCAAAAACAAATCGATGGGATGAAGTAGCCGCAAGCACAGCTTTTGCATGCACTATG (SEQ ID NO:130)

25

MM005 (1st loop)

TCAATGCGCATGTTTACGTATCCTTGCTAACAAATGCCGGTGTTCGATATGCGTC (SEQ ID NO:131)

and

30 CGGAACACCGGCATTTGTTAGCAAGGATACGTAAACATGCGCATTGATCGCAACAGG (SEQ ID NO:132)

MM005 (2nd loop)

35 CAAAAGCTGTGCTTGCTTACGTATCCTTGCTAACAAATGTTTTTTCGTCGACATCACGG (SEQ ID NO:133)

and

ACGCAAAAACATTTGTTAGCAAGGATACGTAAAGCAAGCACAGCTTTTGCATGCACTATG (SEQ ID NO:134)

40 MM007 (1st loop)

GCGATCAATGCGCCTGCAGAACTCAACCATATCCTTTATGTCGGTGTTCGATATGCGTC (SEQ ID NO:135)

and

45 GGAACACCGACATAAAGGATATGGTTGAGTTCTGCAGGCGCATTGATCGCAACAGGGTTT (SEQ ID NO:136)

MM007 (2nd loop)

CAAAAGCTGTGCCTGCAGAACACAACCTTACCCACTTTGTTTTTTCGTCGACATCACGG (SEQ ID NO:137)

50 and

ACGCAAAAACAAAGTGGGTAAGGTTGTGTTCTGCAGGCACAGCTTTTGCATGCACTATG (SEQ ID NO:138)

MM009 (2nd loop)

CAAAAGCTGTGCCTGCCTGTAAACACCTACTCTTAACTGTTTTTTCGTCGACATCACGG (SEQ ID NO:139)

and

5 ACGCAAAAACAGTTAAGAGTAGGTGTAAACAGGCAGGCACAGCTTTTGCATGCACTATG (SEQ ID NO:140)

MM010 (1st loop)

10 TCAATGCGCATGCGCTCTTCCAACTCATTCTAACTGTCGGTGTTCGATATGCGTCT (SEQ ID NO:141)

and

CGGAACACCGACAGTTAGAATGAGTTGGAAGAGCGCATGCGCATTGATCGCAACAGG (SEQ ID NO:142)

15 MM010 (2nd loop)

CAAAAGCTGTGCCTGCGCGCTTCCTACACACTCTAACTGTTTTTTCGTCGACATCACGG (SEQ ID NO:143)

and

20 ACGCAAAAACAGTTAGAGTGTGTAGGAAGCGCGCAGGCACAGCTTTTGCATGCACTATG (SEQ ID NO:144)

MM017 (2nd loop)

25 CAAAAGCTGTGCCTGCCCTTTAGGCCTTTGCCACCTTGTTTTTTCGTCGACATCACGG (SEQ ID NO:145)

and

ACGCAAAAACAAGGTGGGCAAAGGCCTAAAGGGCAGGCACAGCTTTTGCATGCACTATG (SEQ ID NO:146)

FGFps1 (2nd loop)

30 AAGCTGTATCTGCTGGAACATCGATTCTACACCTTGTTTTTTCGTCGACATCACGG (SEQ ID NO:147)

and

35 ACGCAAAAACAAGGTGTAGAATCGATGTTCCAGCAGATACAGCTTTTGCATGCACT (SEQ ID NO:148)

FGFps2 (1st loop)

GCGATCAATGCATCTGTACTTGGATTGACAGTACTCCTTGTCGGTGTTCGATATGCGTC (SEQ ID NO:149)

and

40 GGAACACCGACAAGGAGTACTGTCAATCCAAGTACAGATGCATTGATCGCAACAGGGTTT (SEQ ID NO:150)

FGFps2 (2nd loop)

45 AAGCTGTATCTGCACATGGATCGATAGTACTCCTTGTTTTTTCGTCGACATCACGG (SEQ ID NO:151)

and

ACGCAAAAACAAGGTGTAGAATCGATCCATGTGCAGATACAGCTTTTGCATGCACT (SEQ ID NO:152)

50 FGFpsB (2nd loop)

AAGCTGTATCTGTACATGGATCGATTGGACACCTTGTTTTTTCGTCGACATCACGG (SEQ ID NO:153)

and

237

ACGCAAAAACAAGGTGTCCAATCGATCCATGTACAGATACAGCTTTTGCATGCACT (SEQ ID NO:154)

1A8 (2nd loop)

5 CAAAAGCTGCGCATGTGTTACTACAGATTGGATCGAATGTTTTTTCGTCGACATCACGG (SEQ ID NO:155)

and

ACGCAAAAACATTCGATCCAATCTGTAGTAACACATGCGCAGCTTTTGCATGCACTATG (SEQ ID NO:156)

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1A12 (2nd loop)

CAAAAGCTGTGCCTGCCCAACACTTTGGACTCATATGTGTTTTTTCGTCGACATCACGGAC (SEQ ID NO:157)

and

15 ACGCAAAAACACATATGAGTCCAAAGTGTTGGGCAGGCACAGCTTTTGCATGCACTATGAC (SEQ ID NO:158)

1E11 (2nd loop)

20 CAAAAGCTGCGCATGTACTACTCTCAATTCACCAATGTTTTTTCGTCGACATCACGG (SEQ ID NO:159)

and

ACGCAAAAACATTGGTGGAATTGAGAGTAGTAACATGCGCAGCTTTTGCATGCACTATG (SEQ ID NO:160)

25 TGFps1 (2nd loop)

CAAAAGCTGTCTTTGTCCGGAACGATAACGTTTCTCCTTGTAATTGCGTCGACATCACGGAC TTCTG (SEQ ID NO:161)

and

30 TGTGACGCAATTACAAGGAGAAACGTTATCGTTTTCCGGACAAAGACAGCTTTTGCATGCACT ATGAC (SEQ ID NO:162)

The DNA sequences of the oligonucleotide pairs used to make cassettes to introduce peptide sequences into the chymotrypsin reactive site loops of the p2JM103-Ink2-BBI expression vector are provided below. The cassettes were ligated into the *Sph*I and *Sal*I restriction sites in the vector.

MM021 (2nd loop)

CAAAAGCTGTGCTTGTAACACAACGTACGTCTTTTATGTTTTTGCG (SEQ ID NO:163)

and

40 TCGACGCAAAAACATAAAAGACGTACGTTGTGTTTACAAGCACAGCTTTTGCATG (SEQ ID NO:164)

VegT (2nd loop)

CAAATCTTGCGCGTGCACACTTTGGAAATCTTACTGGTGTTTTTTGCG (SEQ ID NO:165)

and

45 TCGACGCAAAAACACCAGTAAGATTTCCAAAGTGTGCACGCGCAAGATTTGCATG (SEQ ID NO:166)

VegK (2nd loop)

50 CAAATCTTGCGCATGTAAATATTACCTTTACTGGTGGTGTTTTTTGCG (SEQ ID NO:167)

and

TCGACGCAAAAACACCACCAGTAAAGGTAATATTTACATGCGCAAGATTGTCATG (SEQ ID NO:168)

VegKD (2nd loop)

5 CAAATCTTGCATCTGTAAATATGATCTTTACTGGTGGTGT TTTTGCG (SEQ ID NO:169)

and

TCGACGCAAAAACACCACCAGTAAAGATCATATTTACAGATGCAAGATTGTCATG (SEQ ID NO:170)

10 VegDK (2nd loop)

CAAATCTTGCATCTGTGATTATAAACTTTACTGGTGGTGT TTTTGCG (SEQ ID NO:171)

and

TCGACGCAAAAACACCACCAGTAAAGTTTATAATCACAGATGCAAGATTGTCATG (SEQ ID NO:172)

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Libraries made of cysteine constrained peptides are popular reagents (*e.g.* the commercially available PhD-C7C Phage Display Peptide Library Kit; NEB) for selecting peptides that bind to substrates of interest. BBI has two cysteine constrained reactive site loops that are structurally similar to the peptide loops displayed in various methods used to select peptide binders. So, once a cysteine constrained binding peptide has been selected, BBI is suitable for use as a scaffold to present the peptide in a binding reaction.

The VegF binding peptide CK37281 (*See e.g.*, co-pending U.S. Patent Application Ser. No. 10/984,270, filed November 8, 2004, incorporated herein by reference) was grafted into BBI by replacing the trypsin, chymotrypsin, or both reactive site loops, with the peptide sequence (ACYNLYGWTC)(SEQ ID NO:43) by using DNA oligonucleotide cassettes. To facilitate the construction, an *EcoRI* site was introduced in the coding region of the BBI gene (custom synthesized by Operon Technologies; *See*, Example 16) between the trypsin and chymotrypsin reactive site loops by QuikChange® site-directed mutagenesis, using methods described by the manufacturer (Stratagene) using the primers BowBeco-F and BowBeco-R, shown above (0.5 pmol of each primer was used in the QuikChange® reaction; after an initial denaturation step of 97°C for 3 minutes, 18 PCR cycles of 68°C for 12 minutes, 95°C for 30 seconds and 55°C for one minute, followed by a final extension reaction for 15 minutes at 68°C).

To replace the trypsin inhibitory peptide loop, two DNA oligonucleotides (1BBck81+ and 1BBck81-) were annealed and ligated into the *SacI* and *EcoRI* restriction sites. Likewise, to replace the chymotrypsin inhibitory peptide loop, *EcoRI* and *SalI* sites were used for insertion of a DNA cassette made by annealing the oligonucleotides (2BBck81+ and 2BBck81-). The (ACYNLYGWTC)(SEQ ID NO:43) peptide was grafted into both loops by inserting the peptide in the chymotrypsin loop (using the oligonucleotides (2BBck81+ and 2BBck81-) after the trypsin loop was first replaced by the peptide. BBI with the grafted peptide in the trypsin loop (1BBick81) was moved into the pJM103BBI expression vector as a *SacI-SphI* fragment. BBI with the grafted peptide in the chymotrypsin loop (2BBick81), or both loops (12BBick81), was moved into pJM103BBI as *SacI-SalI* fragments. The correct sequences were verified by DNA sequencing (the sequence of 12BBick81 gene is shown in Figure 16). The resulting vectors, pJM103-

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1BBick81, pJM103-2BBick81, or pJM103-12BBick81, were used to transform *B. subtilis* BG3934comK, and the production of the BCE fusion proteins was determined as in Example 16, above.

The fusion protein running at ~ 44 kDa was detected by SDS-PAGE to be the major protein present in the cell free broth. Although in some cases, there was significant degradation (up to 50 %) of the BBI moiety (especially after > 48 h of growth in MBD medium), as observed by the presence of a prominent protein band running at ~ 34 kDa corresponding to the BCE103 catalytic core. In these cases, the titers of the BCE103 cellulase were similar to that measured with fusions to the wild-type BBI (Example 16), but the activity of the BBI (trypsin inhibition with 2BBick81, or chymotrypsin inhibition with 1BBick81) was generally about two fold less.

To reduce the proteolytic degradation of BBI variants during growth (*i.e.* decrease the amount of BCE103 cellulase core present on SDS-PAGE gels in comparison to the fusion protein), a *Bacillus subtilis* strain with nine protease genes deleted, BG6006 (*degU^{Hy}32*, *oppA*, Δ *spoIIIE3501*, Δ *aprE*, Δ *nprE*, Δ *epi*, Δ *ispA*, Δ *bpr*, Δ *vpr* Δ *wprA*, Δ *mpr-ybjF*, Δ *nprB*, *amyE::xylRPxylAcomK-ermC*), was used as an expression host, and the growth temperature (35°C) and aeration (200 rpm) were reduced. With these changes, a major fusion protein band (~ 44 kDa) was observed on SDS-PAGE gels with an insignificant band present at the molecular weight expected for the BCE catalytic core protein (~ 34 kDa).

In addition to the above peptide, a number of other cysteine constrained peptides were produced when substituted into the trypsin and/or chymotrypsin reactive site loops of BBI fused to the C-terminus of the BCE103 cellulase. Specific examples included:

- (1) Peptides designed or selected as complement antagonists, compstatin introduced into the 1st or 2nd reactive site loops (*See*, Sahu *et al.*, J. Immunol., 157: 884-891, [1996]), C2c (1st loop), C3c (1st loop), C4c (1st loop) and C5c (1st loop); or peptides selected in a Factor B binding reaction 1B, 2B, 4A, 5A, 6-1A, 7A, 8B, 9A, 10B, 11-1A, 12B, 13A, and 15-1A (all in 2nd loop);
- (2) Peptides designed to bind to the proteases Factor Xa or stratum corenum chymotrypsin, Xa1 (2nd loop) or hSCC1 (1st loop), respectively;
- (3) Peptides selected in FGF5 binding reactions 1A6 (1st or 2nd loop), 1C2 (1st or 2nd loop), 2E2 (1st or 2nd loop), 2E5 (1st, 2nd or both loops), FGFns (1st or 2nd loop), FGFkr (1st or 2nd loop), FGFhl (1st or 2nd loop), FGFgy (1st or 2nd loop), MM005 (1st or 2nd loop), MM007 (1st, 2nd or both loops), MM009 (2nd loop), MM010 (1st, 2nd or both loops), MM017 (2nd loop), FGFps1 (2nd loop), FGFps2 (1st, 2nd or both loops), and FGFpsB (2nd loop); and
- (4) Peptides selected in TGFβ-1 binding reactions 1A8 (2nd loop), 1A12 (2nd loop), 1E11 (2nd loop), TGFps1 (2nd loop), and MM021 (2nd loop)
- (5) Peptides selected in VEGF binding reactions VegK (2nd loop), VegT (2nd loop), VegKD (2nd loop), and VegDK (2nd loop),

The oligonucleotides used to introduce these peptides into either the trypsin (1st loop) or chymotrypsin (2nd loop) reactive site loops, and methods used to graft these peptides into BBI, are provided above. In all

cases, fusion proteins were produced as determined by SDS-PAGE gels. However, with some substituted peptides, the amount of intact fusion protein was increased by reducing the proteolytic degradation as described above for the grafted (ACYNLYGWTC)(SEQ ID NO:43) peptide.

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EXAMPLE 18

Activation of BBI By Thiol Reducing/Oxidizing Agents

After growth, the activity of the BBI (by trypsin or chymotrypsin inhibition) is typically some 5 – 20 times lower than what would be expected from the activity of the BCE103 cellulase measured in the cell free supernatants (the two molecules should be present at a 1:1 molar ratio in the fusion protein). An increase in the activity of BBI (measured by either trypsin or chymotrypsin inhibition) in the BCE103-BBI fusion protein can be routinely obtained by adding bME, typically concentrations of 1 - 4 mM added to the MBD growth medium about 14 h after inoculation. The trypsin or chymotrypsin inhibitory activity of BBI in the fusion protein is also lower than expected when binding peptides (*e.g.* VegF binding peptide CK37281) replaced the chymotrypsin or trypsin reactive site loop, respectively. As with the wild-type BBI, the inhibitory activity can be increased by treatment with bME. Unexpectedly, other thiol reducing agents (*e.g.*, cysteine, reduced glutathione, DL-dithiothreitol and Tris[2-carboxyethyl] phosphine) had small or negligible effects on the activation of BBI during growth in these experiments. Also, additions of antioxidants (*e.g.*, ascorbic acid or DL- α -tocopherol acetate) or other adjuvants to the growth medium (*e.g.*, isoleucine, soybean oil, Tween-80), or growth at 30°C did not significantly improve the BCE103:BBI activity ratio.

Specifically, to determine the BBI activation during growth, cultures of *B. subtilis* BG6006 transformed with p2JM103-E3-2BBick81 (*See*, Example 19, below) were grown in 40 ml MBD medium in 250 ml shake flasks at 37°C for 13 h. Then, the thiol reducing agents indicated on the graph in Figure 17 were added and cell supernatants harvested after 62 h of growth. The reagents 2-mercaptoethanol (BME), cysteine (Cys), reduced glutathione (Glut), and DL-dithiothreitol (DTT) were added to the growth medium to the final concentrations indicated on the graph provided in Figure 17. Concentrations of 5 mM β ME can result in better BCE103:BBI activity ratios but typically result in an overall decrease in both BCE103 and BBI titers (*See*, Figure 17), at least partially due to the reduction in bacterial growth caused by the added reagent. Titers of BCE103 and 2BBick81 were determined using the assays described in Example 16.

BBI activation was also achieved after partial purification of the fusion proteins (*e.g.* BCE-Ink2-2BBick81; *See*, Example 19, below) by Q-Sepharose ion exchange chromatography.

The fusion protein was purified from cell free broth obtained from shake flasks or fermentor runs. The broth was filtered, diluted five to ten fold in water and the pH adjusted to pH 7.5 – 8.0. The diluted sample was loaded onto a column packed with Q-Sepharose resin (GE Healthcare). The column was washed

with 50 mM Tris pH 7.5 and then washed again in the same buffer containing 300 mM NaCl. The fusion protein was eluted in the same buffer with 700 mM NaCl.

To activate the BBI, the pooled fusion protein fractions were diluted ten fold in Assay Buffer then treated with 2 mM β ME and 0.2 mM oxidized glutathione (GSSG) with constant mixing on a stir plate or rocker platform for about 24 h at room temperature. The BBI could generally be activated to about 70 – 100 % of the expected trypsin inhibitory activity based on the measured concentration of the BCE103 cellulase. Although the activation method outlined above generally yielded the best results, in some cases, in order to maximize the activation of a given sample, screens were performed in 96-well plates to determine the optimal conditions. Initially, the typical conditions screened were the dilution in Assay Buffer (*e.g.*, a 2–50 fold dilution series), β ME concentration (*e.g.*, series between 0.5–5 mM) and oxidized glutathione concentration (*e.g.* 0 mM then a series of 1/20 to 1/2 the β ME concentration).

The activation of the fusion protein BCE-Ink2-2BBick81 is shown in Figure 18. In this specific example, the fusion protein from a Q-Sepharose purification was diluted 1:10 in Dulbecco's PBS (Mediatech) with 0.005 % TWEEN®-80. Beta-mercaptoethanol was added to a final concentration of 3 mM and incubated overnight at room temperature on a rocker. The sample was further incubated at room temperature for about 60 h with vigorous stirring on a magnetic stir plate. The titers of the BCE103 and 2BBick81 (before and after β ME treatment) were determined by cellulase assays and trypsin inhibitory assays, respectively.

In some embodiments, such as for activating BBI or its variants in cell free broth from large volume fermentations, it is desirable to reduce the dilution and β ME concentration in the activation reaction. This can be accomplished by using higher concentrations of buffer (500 mM Tris pH 8.6), or changing to zwitterionic buffers such as CHES (also CAPS, Tricine, TAPS, and other suitable zwitterionic buffers). For example, cell free broth (or fusion protein fractions purified by ion exchange chromatography) was diluted 1:1 in 375 mM CHES pH 8.6 with 0.005 % TWEEN®-80 then activated with 1 mM β ME and 10 mM Na_2SO_3 and incubated with stirring at room temperature for about 24 h. BBI or its variants, as BCE103 cellulase fusion proteins, were routinely activated by this method to 70 – 100 % of the expected value (based on BCE103 cellulase activities).

In some other embodiments, it is desirable to activate BBI or its variants in whole fermentation broth with the cells present. For example, in some experiments, whole broth was diluted 1:1 in 250 mM glycine pH 9.0 with 0.05 % TWEEN®-80, and then activated with 2 mM β ME and 10 mM Na_2SO_3 (or with 10 mM $\text{Na}_2\text{S}_2\text{O}_4$ alone) and incubated with stirring, at room temperature for about 24 h. BBI or its variants, as BCE103 cellulase fusion proteins, were routinely activated by this method to 30 – 100 % of the expected value (based on BCE103 cellulase activities).

EXAMPLE 19**Release of Free BBI/Variants by Cleavage of the BCE103-BBI Fusion Proteins**

This Example describes experiments developed to release free BBI or its variants by cleavage of the BCE103-BBI fusion proteins.

- 5 The sequences of the DNA oligonucleotide pairs that were annealed and ligated into the *Bam*HI and *Sac*I sites of pJM103-BBI to generate potential cleavage sites during culture growth between the BCE103 catalytic domain and BBI are provided below.

BCEsubBBI (a subtilisin-type sensitive peptide sequence)

- 10 GATCCAGGTGGAGCTGCTTTAGTTGACGATGAGAGCT (SEQ ID NO:173)
and
CTCATCGTCAACTAAAGCAGCTCCACCTG (SEQ ID NO:174)

BCEcbdLBBI (a portion of the 1st CBD)

- 15 GATCCAGGTGAACCTGACCCAACTCCTCCATCTGATCCTGGAGAATACCCAGCTTGGGACGATG
AGAGCT (SEQ ID NO:175)
and
CTCATCGTCCCAAGCTGGGTATTCTCCAGGATCAGATGGAGGAGTTGGGTCAGGTTACCTG
(SEQ ID NO:176)

20

BCEproBBI (the entire pro peptide of BBI)

- GATCCGGCGAACCTGCGTCTGTCTAAGCTTGGCCTGCTTATGAAATCAGACCATCAGCACAGCA
ATGACGATGAGAGCT (SEQ ID NO:177)
and
25 CTCATCGTCATTGCTGTGCTGATGGTCTGATTTTCATAAGCAGGCCAAGCTTAGACAGACGCAGG
TTCGCCG (SEQ ID NO:178)

BCEshortproBBI (a C-terminal portion of the pro peptide of BBI)

- GATCCAAAATCAGACCATCAGCACAGCAATGACGATGAGAGCT (SEQ ID NO:179)
30 and
CTCATCGTCATTGCTGTGCTGATGGTCTGATTTTG (SEQ ID NO:180)

- 35 The sequences of the DNA oligonucleotide pair that was annealed and ligated into the *Bam*HI and *Sac*I sites of p2JM103-BBI to fuse BBI to the 2nd CBD linker of BCE103 cellulase are provided below.

BCEcbdDBBI

GATCCAGGAGAACCGGACCCAACGCCCCAAGTGATCCAGGAGAGTATCCAGCATGGGATTCA
AATCAAATTTACACAAATGAAATTGTGTATCATAACGGTCAGTTATGGCAAGCGAAATGGTGG
ACACAAAATCAAGAGCCAGGTGACCCATACGGTCCGTGGGAACCACTCAAATCTGACCCAGAT
5 TCAGACGATGAGAGCT (SEQ ID NO:181)

and

CTCATCGTCTGAATCTGGGTCAGATTTGAGTGGTTCACGGACCGTATGGGTCACCTGGCTCT
TGATTTTGTGTCCACCATTTTCGCTTGCCATAACTGACCGTTATGATACACAATTTCAATTTGTGTA
AATTTGATTTGAATCCCATGCTGGATACTCTCCTGGATCACTTGGGGGCGTTGGGTCCGTTCTC
10 CTG (SEQ ID NO:182)

The peptide sequences susceptible to acid cleavage between aspartic acid and proline residues are provided below.

15

Linker 1 – WGDPHY (SEQ ID NO:183)(Lidell *et al.*, J. Biol. Chem. 278:13944-51 [2003])

Linker 2 – DNNDPI (SEQ ID NO:184)(Segalas *et al.*, FEBS Lett., 371:171-175 [1995])

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Linker 3 – VVADPN (SEQ ID NO:185)(Kemperman *et al.*, Appl. Env. Microbiol., 69:1589-1597 [2003])

Oligonucleotide primers used to introduce a *Bss*HII site into pJM103BBI by QuikChange® site-directed mutagenesis are provided below.

25

BCEbss-F

5'-TGCGGTTTCAGCAACATGAGCGCGCAGGCTGATGATTA (SEQ ID NO:186)

BCEbss-R

30 5'-TAATCATCAGCCTGCGCGCTCATGTTGCTGAACGCCA (SEQ ID NO:187)

Sequences of the DNA oligonucleotides that were annealed as a cassette (*Sal*I-*Hind*III) to introduce *Hind*III and *Xho*I sites after the stop codon of BBI, to introduce a *Pac*I site after the LAT, and remove the original *Hind*III site are provided below.

35

BCEterm+

5'-GACATCACGGACTTCTGCTATGAGCCATGTAAACCAAGCGAGGACGATAA
AGAGAACTAAAAGCTTAACTCGAGGTAAACAGAGGACGGATTTCTGAAGGAAATCCGTTTTT
40 TTATTTTAAATTAAG (SEQ ID NO:188)

BCEterm-

5'-AGCTCTTAATTAAAAATAAAAAACGGATTTCTTCAGGAAATCCGTCCTC
TGTTAACCTCGAGTTAAGCTTTTAGTTCTCTTATCGTCCTCGCTTGGTTTACATGGCTCATAGC
45 AGAAGTCCGTGATG (SEQ ID NO:189)

PCR primers used to generate the acid labile linkers provided above (*i.e.*, Linker 1, Linker 2, and Linker 3) inserted between the BCE103 catalytic domain and BBI are provided below.

BCE103coreBssHII_FW

5 5'-CAGCAACATGAGCGCGCAGGCTG (SEQ ID NO:190)

linkerWGDPHY_RV

10 5'-ATCGTCTGGATCCGGATAGTGGGGGTCTCCCCAAGATGCTGATTCTCTTAT
TTTTTCCC (SEQ ID NO:191)

linkerDNNDPI_RV

5'-ATCGTCTGGATCCGGTATGGGATCATTGTTGTCAGATGCTGATTCTCTTATT
TTTTCCC (SEQ ID NO:192)

linkerVVADPN_RV

15 5'-ATCGTCTGGATCCGGGTGGGATCTGCAACTACAGATGCTGATTCTCTTAT
TTTTTCCC (SEQ ID NO:193)

20 PCR primers used to generate the acid labile linkers provided above (*i.e.*, Linker 1, Linker 2, and Linker 3) inserted into the 1st CBD linker.

BCE103corePstI_FW

25 GCATAAGGAT GAGTCATCTG CAGCG (SEQ ID NO:194)

LplusWGDPHY_RV

5'-ATCGTCTGGATCCGGATAGTGGGGGTCTCCCCACGGTTCTCCTGGATCAGA
TGGCGG (SEQ ID NO:195)

LplusDNNDPI_RV

30 5'-ATCGTCTGGATCCGGTATGGGATCATTGTTGTCCGGTTCTCCTGGATCAGA
TGGCGG (SEQ ID NO:196)

LplusVVADPN_RV

35 5'-ATCGTCTGGATCCGGGTGGGATCTGCAACTACCGGTTCTCCTGGATCAGA
TGGCGG (SEQ ID NO:197)

40 Protein sequence of the acid labile linkers inserted between the BCE103 catalytic domain and BBI are provided below. The acid labile linkers are shown in bold type and the sequences from the first CBD domain are underlined.

Linker 1

BCE-**WGDPHY-PDP**-BBI (SEQ ID NO: 198)

45 Linker 2

BCE-**DNNDPI-PDP**-BBI (SEQ ID NO:199)

Linker 3

50 BCE-**VVADPN-PDP**-BBI (SEQ ID NO:200)

LinkerPlus 1

BCE-IPPSDPTPPSDPGEP-WGDPHY-PDP-BBI (SEQ ID NO:201)

LinkerPlus 2

5 BCE-IPPSDPTPPSDPGEP-DNNDPI-PDP-BBI (SEQ ID NO:202)

LinkerPlus 3

BCE-IPPSDPTPPSDPGEP-VVADPN-PDP-BBI (SEQ ID NO:203)

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The sequences of the DNA oligonucleotide pairs that were annealed and ligated into the *Bam*HI and *Sac*I sites of pJM103-BBI to generate potential cleavage sites between the BCE103 catalytic domain and BBI during the purification process are provided below.

15 BCEentBBI (Enteropeptidase cleavage site)

GATCCAGGTGGAGACGACGATGACAAAGACGATGAGAGCT (SEQ ID NO:204)

and

CTCATCGTCTTTGTCATCGTCGTCTCCACCTG (SEQ ID NO:205)

20 BCEgenen1BBI (Genenase I cleavage site)

GATCCAGGTGCTGCTCATTACGACGATGAGAGCT (SEQ ID NO:206)

and

CTCATCGTCGTAATGAGCAGCACCTG (SEQ ID NO:207)

25

The sequences of the DNA oligonucleotide pairs that were annealed and ligated into the *Bam*HI and *Sac*I sites of pJM103-lnk2-1BB1ck81 to generate potential cleavage sites between the BCE103 catalytic domain and BBI during the purification process are provided below.

30 BCEfurinBBI (Furin/Blisterase cleavage site)

GATCCACGTGCTAAAAGAGACGATGAGAGCT (SEQ ID NO:208)

and

CTCATCGTCTCTTTTAGCACGTG (SEQ ID NO:209)

35 BCEgenen2BBI (Genenase I cleavage site)

GATCCAGGCGCTGCACACTACAACGACGATGAGAGCT (SEQ ID NO:210)

and

CTCATCGTCGTTGTAGTGTGCAGCGCCTG (SEQ ID NO:211)

246

BCEfleBBI (Mpr cleavage site)

GATCCATTCCTTGAAGACGATGAGAGCT (SEQ ID NO:212)

and

CTCATCGTCTTCAAGGAATG (SEQ ID NO:213)

5

Sequences of the oligonucleotide primer pairs used to introduce the E and E3 linkers in Linker 2 by QuikChange site-directed mutagenesis (Stratagene) are provided below.

10 BCE-Elmk-BBI (Mpr cleavage site)

CCCATACCGGAGCCAGACGATGAGAGCTC (SEQ ID NO:214)

and

CATCGTCTGGCTCCGGTATGGGATCATTGTTG (SEQ ID NO:215)

15

The protein sequence of the E3 linker between the BCE103 catalytic domain and BBI was DNNDPIPEP**DD**ESFNMPIPEP (SEQ ID NO:216). In this sequence, the E Linker is underlined and the sequence generated by faulty recombination in *E. coli* is shown in bold type. Cleavage by Mpr (or V8 protease) can occur after any of the three glutamic acids present in the E3 Linker. Thus, the structure was

20 BCE-(SEQ ID NO:216)-BBI

The sequences of the DNA oligonucleotide pairs that were annealed and ligated into the *Bam*HI and *Sac*I sites of p2JM103-lnk2-2BBick81 to generate potential Genenase I cleavage sites between the BCE103 catalytic domain and BBI are provided below.

25

BCEgenen3BBI

GATCCAGGCGCTGCACACTACAAATCAGACCATCAGCACAGCAATGACGATGAGAGCT (SEQ ID NO:217)

and

30 CTCATCGTCATTGCTGTGCTGATGGTCTGATTTGTAGTGTGCAGCGCCTG (SEQ ID NO:218)

BCEgenen4BBI

GATCCAGGCGCTGCACACTACGTAGAATTTCAAGACGATGAGAGCT (SEQ ID NO:219)

and

35 CTCATCGTCTTGAAATTCTACGTAGTGTGCAGCGCCTG (SEQ ID NO:220)

The protein sequence of a Genenase I sensitive cleavage site (also acid and Mpr sensitive) inserted between the BCE103 catalytic domain and BBI was DNNDPIPDP**GAAHYVEFQ** (SEQ ID NO:221). The

seven amino acids of the BCE103 catalytic domain (underlined), linker 2 sequence (bold type), and 2BB1ck81 sequences are shown. The acid/heat labile Asp-Pro bonds are indicated with solid headed arrows and the Mpr sensitive bonds after glutamic acids are indicated with line headed arrows.

[illegible]

15 In order to isolate free BBI or its variants, the BBI moiety needs to be cleaved from the BCE103-BBI fusion protein. In some embodiments, this is accomplished during growth, by proteases intrinsically produced by *B. subtilis*. In some alternative embodiments, this cleavage occurs after growth, during the purification process (*e.g.* by acid/heat or proteolytic cleavage). Linkers potentially susceptible to cleavage during growth were designed (*See*, above, sub, cbdL, pro, shortpro, and cbdD) and cloned into the
20 pJM103BBI or p2JM103BBI expression vectors as *Bam*HI-*Sac*I cassettes. The production of fusion protein versus BCE103 catalytic domain was analyzed on SDS-PAGE gels as described in Example 16.

Little cleavage of the fusion protein was observed for all these linkers except with the pro linker, which was nearly completely cleaved so that very little intact fusion protein was observed on gels, although there was a large band corresponding to the BCE103 catalytic core. Unfortunately, this cleavage during growth resulted in negligible BBI activity measured in cell free supernatants and no BBI band could be identified on SDS-PAGE gels. Although it is not intended that the present invention be limited to any particular mechanism or theory, it is possible that the BBI is particularly sensitive to proteolytic degradation in its inactive form. Thus, cleavage during the purification process after activation is generally preferred.

In some embodiments, the bonds between aspartic acid and proline residues are cleaved by heat treatment at acidic pH as known in the art (*See e.g.*, Landon, Meth. Enzymol., 47:145-149 [1977]). The 1st CBD linker in the BCE103 cellulase has three Asp-Pro dipeptide sequences (*See*, Figure 14) with the potential to be cleaved by acid/heat treatment. However, cleavage by acid/heat treatment at these sites was found to be inefficient. Protein sequences that are especially labile to acid/heat have been described in the literature, three of such sequences are WGDPHY (SEQ ID NO:183), DNNDPI (SEQ ID NO:184), and VVADPN (SEQ ID NO:185) (*i.e.*, Linkers 1, 2 and 3).

Before these acid labile linkers were introduced into the BCE103-BBI expression vector, pJM103BBI, a *Bss*HII site was introduced by QuikChange® XL (Stratagene) mutagenesis (using the manufacturer's methods; and described in Example 17 above, except 8 minute extension and 1 minute

denaturation steps were used) in the *aprE* signal sequence coding region using the oligonucleotide primers BCEbss-F and BCEbss-R (provided above). Then, *Hind*III and *Xho*I sites were inserted in front of the LAT terminator (after the BBI stop codon) and a *Pac*I site was added after the terminator (the original *Hind*III site after the LAT terminator was removed) by inserting an oligonucleotide cassette (BCEterm+ and BCEterm-; provided above) into the *Sal*I and the original *Hind*III sites. This new vector was called "p2JM103BBI."

The acid labile linker fragments were generated by PCR, using forward primer BCE103coreBssHII_FW with each of the reverse primers, linker WGDPHY_RV (SEQ ID NO:191), linker DNNDPI_RV (SEQ ID NO:192), or linkerVVADPN_RV (SEQ ID NO:193) and p2JM103BBI as the template (See, Example 16, for the PCR protocol). The PCR fragments of 970 bp were digested with *Bam*HI and *Pst*I, the 154 bp fragments encoding the acid linker fragments were isolated from an agarose gel after electrophoresis, and ligated into the p2JM103 vector digested with *Bam*HI and *Pst*I that had also been purified from a gel. The linker sequences in the final expression vectors, p2JM103lnk1-BBI, p2JM103lnk2-BBI and p2JM103lnk3-BBI, were verified by DNA sequencing.

Competent *B. subtilis* strain BG3934comK or BG6006 were transformed with the plasmids, colonies selected on 5 µg/ml chloramphenicol LA plates and amplified to 25 µg/ml chloramphenicol as described in Example 16.

Similarly, the acid labile linkers were inserted into the first CBD linker. Specifically, PCR fragments were generated using the forward primer BCE103corePstI_FW (SEQ ID NO:194) with the reverse primers LplusWGDPHY_RV (SEQ ID NO:195), LplusDNNDPI_RV (SEQ ID NO:196), or LplusVVADPN_RV (SEQ ID NO:197) with p2JM103BBI as a template. The PCR fragments of about 150 bp were digested with *Bam*HI and *Pst*I, purified and ligated to the p2JM103BBI vector digested with *Bam*HI and *Pst*I. The correct sequences were verified by DNA sequencing and the plasmids p2JM103pllnk1-BBI, p2JM103pllnk2-BBI and p2JM103pllnk3-BBI were used to transform *B. subtilis* strains as described above.

After growth in MBD medium, the fusion proteins were purified by ion exchange chromatography essentially as described above (See, Example 17). The fusion protein was cleaved by treatment at 55°C for 16 h in 10% formic acid. The BCE103 catalytic domain precipitated during the acid treatment and was removed by centrifugation. The free BBI in the supernatant was dried overnight on a SpeedVac. The sample was suspended in 50 mM Tris pH 8 before loading on the SDS-PAGE gel. By analysis of the protein stained SDS-PAGE gels, it was observed that acid cleavage was much more efficient in the fusion proteins where Linker 2 was inserted between the BCE103 catalytic domain and BBI (BCE-DNNDPI-PDP-BBI; SEQ ID NO:199). This linker was found to be cleaved in a couple of hours at 75°C in 20 mM glycine pH 2.

In alternative embodiments, the fusion protein was cleaved by treatment with a protease during the purification process. Linkers were designed with cleavage sites for glutamic acid specific proteases (e.g., Mpr or V8 protease), furin/blisterase, genenase I, and enteropeptidase (enterokinase). These linkers were introduced as oligonucleotide cassettes (See above, for the sequences) between the BCE103 catalytic core and BBI in the expression vector using the *Bam*HI and *Sac*I sites (See, Figure 14). In the coding region of

the original expression vector (pJM103BBI), there is a glutamic acid residue in the 1st CBD domain and at the third residue in BBI (See, Figure 14), which is contemplated to be susceptible to cleavage by glutamic acid specific proteases such as *B. subtilis* Mpr (BsMpr) or V8 protease. However, neither BsMpr nor V8 protease were found to cleave the BCE-BBI fusion protein very efficiently at these sites. Thus, it was necessary to design other linkers that were susceptible to cleavage by these proteases.

The six acid labile linkers described above were tested for cleavage by BsMpr. These fusion proteins were cleaved by treatment for 16 h with 16 µg of BsMpr at room temperature. After cleavage, the BCE103 catalytic domain was precipitated by the addition of 10 % formic acid and removed by centrifugation. The free BBI in the supernatant was dried overnight on a SpeedVac. The sample was suspended in 50 mM Tris pH 8, before loading on the SDS-PAGE. Similar to the acid cleavage, the BCE-DNNDPI-PDP-BBI (Linker 2; SEQ ID NO:199) fusion protein was much more efficiently cleaved by BsMpr than any of the other linkers. Therefore, BBI and its variants were found to be effectively released from the BCE-DNNDPI-PDP-BBI (SEQ ID NO:199) fusion protein either by acid/heat treatment or proteolytic digestion with a glutamic acid specific protease such as BsMpr. Several other linkers designed for cleaved by Mpr (*e.g.*, E, E3 linker, and fle, provided above) were tested but none of them had any advantages over Linker 2 (the E3 linker was generated by faulty recombination in *E. coli* after transformation with the QuikChange® site-directed mutagenesis reaction designed to construct the E linker). As shown above, there are two acid/heat labile cleavage sites in Linker 2 and three sites sensitive to cleavage by Mpr.

Linkers designed for cleavage by furin or blisterase (NEB) (BCEfurinBBI), or enteropeptidase (enterokinase, NEB) (BCEentBBI) were tested, but none of these sequences were cleaved efficiently by the appropriate protease. Four linkers were also designed (BCEgenen1BBI, BCEgenen2BBI, BCEgenen3BBI, and BCEgenen4BBI) and tested for cleavage by genenase I (NEB). Efficient cleavage of the fusion protein was observed only with the Gen4 Linker (BCEgenen4BBI). BsMpr was also found to efficiently cleave the Gen4 linker.

After activation of the purified BCE-Ink2-2BBick81 fusion protein, cleavage by BsMpr does not go to completion as judged by SDS-PAGE gels. However, it was discovered that complete cleavage after activation of BCE-BBI fusion proteins with Linker 2 (or the Gen4 linker) can be accomplished by using the Mpr protease isolated from *Bacillus licheniformis* (BIMpr). While it is not intended that the present invention be limited to any particular mechanism, cleavage after the third amino acid in mature BBI appeared to be more sensitive to BIMpr while cleavage after the sixth amino acid from the C-terminus of BBI is more sensitive to BsMpr cleavage.

In some embodiments, after cleavage, the BBI is purified away from the BCE103 catalytic domain by selective acid precipitation (pH 3 or lower) of the BCE103 catalytic domain as described above, ion exchange chromatography (See, Example 20), or by selective binding of BBI on an anhydrotrypsin-agarose

(Sigma) column loaded in 50 mM Tris pH 8.0, washed with 50 mM Tris pH 8.0 with 150 mM NaCl, then eluting bound BBI with 50 mM glycine pH 2.2 with 300 mM NaCl).

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EXAMPLE 20 Binding of BBICK81 to VegF

In this Example, experiments conducted to assess the binding of BBICK81 to VegF are described. The BCE103-Ink2-2BBICK81 fusion protein was produced in *B. subtilis* as described in Example 17. The fusion protein was purified, and the BBI trypsin inhibitory activity was increased by treatment with β ME and oxidized glutathione as described in Example 18. The fusion protein was cleaved by BsMpr protease (See, Example 19) and the free 2BBICK81 was purified from the BCE103 catalytic domain by ion exchange chromatography using a Q-Sepharose column.

10

Briefly, after cleavage, the pH of the cleaved sample was adjusted to 5.5, the sample was then loaded onto the column (equilibrated with 25 mM MES pH 5.5). The free 2BBICK81 was washed through the column using 25 mM sodium acetate pH 5.0 while the BCE103 catalytic core remained bound to the resin. The 2BBICK81 fraction was concentrated by ultrafiltration and analyzed using an electrochemiluminescence (ECL) based binding assay (BioVeris). The Anti-VegF antibody (Santa Cruz) and VegF (PeproTech) were labeled with the electrochemiluminescent dye and biotin, respectively, as described by the manufacturer (BioVeris). All materials were in Dulbecco's PBS (Mediatech) supplemented with 0.1 % TWEEN®-80. An initial dilution series of Anti-VegF antibody (125, 250 and 500 ng/ml) and VegF (100, 150, 200 and 250 ng/ml) were tested in the binding assay to determine the concentrations of each that would give a robust ECL signal.

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For testing 2BBICK81 binding, 50 μ L of 500 ng/ml ECL labeled Anti-VegF antibody, 50 μ L of 250 ng/ml biotinylated VegF and 100 μ L 2BBICK81 (series of 12.5, 15, 31.25, 62.5, 125, 250 or 500 ng/ml) were incubated at room temperature for 2 h with shaking. Then, 50 μ L of 0.2 mg/ml streptavidin coated beads were added and the reaction was incubated at room temperature for 30 minutes. The ECL signal was measured using a BioVeris M8/384 Analyzer as described by the manufacturer (BioVeris). As shown in Figure 19, the ECL signal decreased as increasing concentrations of 2BBICK81 displaced more of the labeled Anti-VegF antibody bound to VegF attached to the magnetic beads.

Thus, the CK37281 peptide when grafted onto the chymotrypsin inhibitory loop of BBI (2BBICK81) competed with the Anti-VegF antibody for binding to VegF at micromolar concentrations. In fact, 2BBICK81 competed for VegF binding better than the synthesized CK37281 peptide itself (See, Figure 19). The CK37281 peptide inserted into the trypsin inhibitory loop, 1BBICK81, also competed with the Anti-VegF antibody in the BioVeris assay. Thus, BBI was found to be useful as part of a scaffold to present active binding peptides selected by various screening methods.

35

EXAMPLE 21**Use of Alternative Fusion Partners for the Production of 2BBICK81**

In this Example, experiments conducted to evaluate alternative fusion partners are described. The DNA sequence of the oligonucleotide primers used to amplify the *dsbC* gene (*E. coli*) from pET-40b(+) are provided below. These primers generate a *Bss*HII site at the 5' end and a *Bam*HI at the 3' end for cloning into pJM103-Gen4-2BBICK81.

DsbCBBI-F

AACATGAGCGCGCAGGCTGATGACGCGGCAATTCAACAAACGTTAG (SEQ ID NO:223)

DsbCBBI-R

TCGTCTGGATCCGGTATGGGATCATTGTTGTCACCAGAACCCTAGTTGATCCTTTACCGCTGG

TCATTTTTTGGTG (SEQ ID NO:224)

The DNA sequences of the oligonucleotides that were annealed together to make a cassette (*Alw*44I-*Bam*HI) for fusing the *P. mendocina* cutinase gene to BBI with Linker 2, are provided below.

CutinaseBBI+

TGCACTTCTCTGCTTTGGTCTGTTGAACGCAGAGGTCTTGACAACAATGATCCTATTCCG (SEQ ID NO:225)

CutinaseBBI-

GATCCGGAATAGGATCATTGTTGTCAAGACCTCTGCGTTCAACAGACCAAAGCAGAGAAG (SEQ ID NO:226)

Because the BBI moiety has seven disulfide bonds, it is contemplated that higher titers of active BBI will be obtained using fusion proteins other than the BCE103 cellulase catalytic domain. For example, in some embodiments, compositions such as thiol-disulfide oxidoreductases and/or protein disulfide isomerases find use as fusion proteins to help produce correctly folded BBI moieties. In this embodiment, no additional activation step is needed under most circumstances. In additional embodiments, other proteins produced at high titers in *B. subtilis* also find use as fusion partners. For example, the thermostable protein disulfide isomerase from the fungus *Humicola insolens* (hiPDI) has been used as a fusion partner to produce the light chain of immunoglobulin G (2 disulfides) in *Bacillus brevis* (See, Kajino *et al.*, Appl. Env. Microbiol., 66:638-642 [2000]).

To determine whether hiPDI could be a better fusion partner than BCE103 for the production of BBI, this hiPDI gene was synthesized (DNA2.0) and cloned into the expression vector, pJM103-lnk2-2BBICK81 (See, Example 19) as a *Bss*HII-*Sac*I fragment. In designing the synthetic gene, codons occurring with high frequency in highly expressed *B. subtilis* genes were selected except in cases where restriction sites were introduced or deleted. In the final construction, the N-terminus of the mature hiPDI gene was fused to the AprE signal sequence and the C-terminus was fused to a linker with an Enteropeptidase

cleavage site (Kajino *et al.*, Appl. Env. Microbiol., 66:638-642 [2000]), which in turn was fused to 2BBick81 (See, Figure 20). This expression vector, p2JM-PDI-EK-2BBick81, was used to transform *B. subtilis* BG6006 and the production of the fusion protein was determined in MBD medium (as described in Example 16) with or without 2 mM β ME added 14 h after inoculation.

As determined by SDS-PAGE gels, the production of the PDI-2BBick81 fusion protein was typically somewhat less than the BCE-2BBick81 grown under identical conditions. The BBI titers (trypsin inhibition) measured from the PDI-2BBick81 cell free supernatants were also typically less than the BCE-2BBick81 fusion. As with fusions to BCE103, the measured activities of BBI when fused to PDI were higher when grown in 2 mM β ME and the BBI activity was increased by the addition of β ME to the cell free supernatants after growth when grown in β ME-free medium (as described in Example 18). Thus, the thiol-disulfide oxidoreductase activity of PDI does not seem to significantly improve the titers of active 2BBick81 in the fusion protein or obviate the need for activation of the BBI molecule.

In order to increase the reduction potential of the fusion protein, which was contemplated to improve the BBI titers during growth, DsbC from *Escherichia coli* was used as a fusion partner for 2BBick81. The *dsbC* gene was amplified by PCR using Herculanse Enhanced DNA polymerase as described by the manufacturer (Stratagene) using DsbCBBI-F and DsbCBBI-R as primers (sequences shown above) and pET-40b(+) (Novagen) as a template. The isolated PCR fragment was cloned into the vector p2JM103-Gen4-2BBick81 (See, Example 19) as a *Bss*HIII-*Bam*HI fragment. The correct sequence of the fusion gene was verified by DNA sequencing. In this case, the titers of the DsbC-2BBick81 fusion protein were significantly lower than the BCE-2BBick81 fusion protein as judged on SDS-PAGE gels and the titers of the active 2BBick81 measured by trypsin inhibition were much lower as well.

Other proteins that are produced at high titers in *B. subtilis* find use as fusion partners for the production of BBI. One such protein is the cutinase from *Pseudomonas mendocina*, which has been expressed at high titers utilizing the *aprE* promoter from *B. subtilis* (See *e.g.*, U.S. Pat. No. 5,429,950, herein incorporated by reference). The *aprE*-cutinase gene fusion as an *Eco*RI-*Alw*44I fragment (from pAK-15) was ligated with an *Alw*44I-*Bam*HI linker oligonucleotide cassette (See, sequence above) into the p2JM103-linker2-2BBick81 (See, Example 19) that had been cut with *Eco*RI and *Bam*HI. This cutinase-linker2-2BBick81 expression vector (See, Figure 21 for the *Eco*RI-*Bam*HI *aprE*-cutinase-linker2 sequence) was used to transform *B. subtilis* BG6006 cells and the fusion protein was produced in MBD medium as described previously for the other fusion proteins (See, Example 16). In this case, the cutinase-linker2-2BBick81 fusion protein was not the major band observed on SDS-PAGE gels and the measured lipase titers (as measured using the methods provided in U.S. Pat. No. 5,429,950) and BBI titers were much less (ca. 20 fold) than found with the BCE-2BBick81 fusion protein. Also, the BBI titers in the cutinase fusion protein were not improved significantly when 3 mM β ME was added to the growth medium. Thus, the highest titers

of active 2BBick81 was consistently obtained by activation of the BCE-2BBick81 fusion protein. Nonetheless, it is contemplated that various fusion partners will find use in the present invention.

5 Those of skill in the art readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The molecular complexes and the methods, procedures, treatments, molecules, specific compounds described herein are presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. It will be readily apparent to one skilled in the art that varying substitutions and
10 modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

All patents and publications mentioned in the specification are indicative of the levels of those skilled in the art to which the invention pertains. All patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to
15 be incorporated by reference.

The invention illustratively described herein suitably may be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and
20 described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined herein.

25 The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

CLAIMS

1. A personal care composition comprising a scaffold, wherein said scaffold comprises a protease inhibitor and at least one peptide selected from the group consisting of SEQ ID NOS:1-17.

2. The personal care composition of Claim 1, wherein said protease inhibitor is selected from the group consisting of Bowman-Birk inhibitor, soybean trypsin inhibitor, and Elgin chymotrypsin inhibitor.

3. The personal care composition of Claim 2, wherein said Bowman-Birk inhibitor is a modified Bowman-Birk inhibitor.

4. The personal care composition of Claim 1, wherein said scaffold comprises from about 0.001 weight percent to about 5 weight percent of said personal care composition.

5. The personal care composition of Claim 4, wherein said scaffold comprises from about 0.01 weight percent to about 2.0 weight percent of said personal care composition.

6. The personal care composition of Claim 5, wherein said scaffold comprises from about 0.01 weight percent to about 1 weight percent of said personal care composition.

7. The composition of Claim 1, wherein said personal care composition is a skin care composition selected from the group consisting of skin creams, lotions, sprays, emulsions, colloidal suspensions, foams, aerosols, liquids, gels, sera, and solids.

8. The composition of Claim 7, wherein said personal care composition is a skin care composition selected from moisturizing body washes, body washes, antimicrobial cleansers, skin protective creams, body lotions, facial creams, moisturizing creams, facial cleansing emulsions, facial gels, facial sera, surfactant-based facial cleansers, facial exfoliating gels, anti-acne treatments, facial toners, exfoliating creams, facial masks, after shave balms, pre-shave balms, tanning compositions, skin lightening compositions, skin redness reduction compositions, sunscreens, depilatories, hair growth inhibitors, and radioprotectives.

9. The composition of Claim 7, wherein said skin care composition comprising topically applied over-the-counter compositions, anti-fungal treatments, anti-acne treatments, skin protectants, sunscreens, deodorants, and antiperspirants.

10. The composition of Claim 7, wherein said composition is capable of lightening the skin tone.

11. The composition of Claim 7, wherein said composition is capable of reducing redness in skin tone.

12. The composition of Claim 7, wherein said composition is capable of preventing skin tone darkening.

13. The composition of Claim 7, wherein said composition is capable of preventing skin color development.

14. The composition of Claim 7, wherein said skin care composition is a radioprotective.

15. The composition of Claim 14, wherein said radioprotective is a sunscreen selected from non-water-resistant sunscreens, very water-resistant sunscreens, and water-in-silicone sunscreens.

16. The composition of Claim 1, wherein said personal care composition is capable of preventing hair growth.

17. The composition of Claim 1, wherein said personal care composition is a hair care composition.

18. The hair care composition of Claim 17, wherein said hair care composition is selected from the group consisting of shampoos, conditioners, hair styling compositions, hair colorants, permanent wave formulations, creams, gels, mousses, sprays, emulsions, colloidal suspensions, liquids, foams, and solids.

19. The hair care composition of Claim 18, wherein said hair care composition further comprises a radioprotectant.

20. The composition of Claim 19, wherein said radioprotective is a sunscreen selected from non-water-resistant sunscreens, very water-resistant sunscreens, and water-in-silicone sunscreens.

21. The composition of Claim 1, wherein said personal care composition is an oral care composition.

22. The composition of Claim 21, wherein said oral care composition is selected from the group consisting of toothpastes, tooth gels, mouth rinses, mouthwashes, anti-caries compositions, tooth whitening compositions, chewing gums, denture adhesives, and breath fresheners.

5 23. The composition of Claim 1, wherein said personal care composition is a cosmetic composition.

10 24. The composition of Claim 23, wherein said cosmetic composition is elected from eye gels, eye shadows, high-melting point lipsticks, lipsticks, lip glosses, lip balms, mascaras, eyeliners, pressed powder formulations, and foundations.

25. The composition of Claim 24, wherein said makeup composition comprises at least one pigment.

15 26. The composition of Claim 25, wherein said makeup composition comprising at least one pigment is a mascara selected from non-waterproof mascaras, waterproof mascaras, volumizing mascaras, lengthening mascaras, curling mascaras, anhydrous waterproof mascaras, water-based mascaras, and eyelash or eyebrow treatments.

20 27. The composition of Claim 25, wherein said makeup composition is a pressed powder formulation selected from loose powders, blushes, eye shadows, and bronzing powders.

25 28. The composition of Claim 25, wherein said makeup composition is a foundation selected from water-in-oil foundations, water-in-silicone foundations, oil-in-water foundations, anhydrous makeup sticks, and cream-to-powder foundations.

29. The personal care composition of Claim 1, wherein said scaffold comprises the amino acid sequence set forth in SEQ ID NO:19.

30 30. The personal care composition of Claim 1, wherein said scaffold further comprises at least one amino acid sequence set forth in SEQ ID NOS:20 and 21.

35 31. The personal care composition of Claim 30, wherein at least one of said amino acid sequences set forth in SEQ ID NOS: 20 and 21 is replaced by at least one peptide having an amino acid sequence selected from the group consisting of SEQ ID NOS:1-17.

32. The personal care composition of Claim 1, wherein said scaffold comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:22-25.

33. A method for making the personal care composition of Claim 1, comprising combining an effective amount of said scaffold and at least one physiologically acceptable carrier or excipient.

34. A method for modifying the skin tone of a subject, comprising the steps of:

- i) providing a composition comprising the personal care composition of Claim 1;
- ii) providing a subject to be treated; and
- ii) applying said composition to said subject in an area in which modifications to said subject's skin tone is desired.

35. The method of Claim 34, wherein said modification of skin tone comprises lightening said subject's skin tone.

36. The method of Claim 34, wherein said modification of skin tone comprises reducing redness in said subject's skin tone.

37. The method of Claim 34, wherein said personal care composition comprises the amino acid sequence set forth in SEQ ID NO:19.

38. The method of Claim 34, wherein said personal care composition comprises at least one amino acid sequence set forth in SEQ ID NOS:20 and 21.

39. The method of Claim 34, wherein at least one of said amino acid sequences set forth in SEQ ID NOS:20 and 21 is replaced by at least one peptide having an amino acid sequence selected from the group consisting of SEQ ID NOS:1-17.

40. The method of Claim 34, wherein said personal care composition is encoded by an amino acid sequence selected from the group consisting of SEQ ID NOS:22-25.

41. A method for modifying the hair growth of a subject, comprising the steps of:

- i) providing the personal care composition of Claim 1;
- ii) providing a subject to be treated; and
- ii) applying said composition to said subject in an area in which modifications to said subject's hair growth is desired.

42. The method of Claim 41, wherein said modification of hair growth comprises inhibiting the growth of said subject's hair.

5 43. The method of Claim 41, wherein said modification of hair growth comprises inhibiting the growth of said subject's hair, wherein said hair to be inhibited is selected from the group consisting of facial air, underarm hair, leg hair, torso hair, and arm hair, and head hair.

10 44. The method of Claim 41, wherein said personal care composition comprises the amino acid sequence set forth in SEQ ID NO:19.

45. The method of Claim 41, wherein said personal care composition comprises at least one amino acid sequence set forth in SEQ ID NOS:20 and 21.

15 46. The method of Claim 45, wherein at least one of said amino acid sequences set forth in SEQ ID NOS:20 and 21 is replaced by at least one peptide having an amino acid sequence selected from the group consisting of SEQ ID NOS:1-17.

20 47. The method of Claim 38, wherein said personal care composition is encoded by an amino acid sequence selected from the group consisting of SEQ ID NOS:22-25.

CK37281 YNLYGWT- seq. ID NO: 1
 CK37282 -TLWPTFW seq. ID NO: 2
 CK37283 -NLWPHFW seq. ID NO: 3
 CK37284 -SLWPAFW seq. ID NO: 4
 CK37286 -APWNSHI seq. ID NO: 5
 CK37287 -APWNLHI seq. ID NO: 6
 CK37289 -TLWPSYW seq. ID NO: 7
 Consensus LWP W

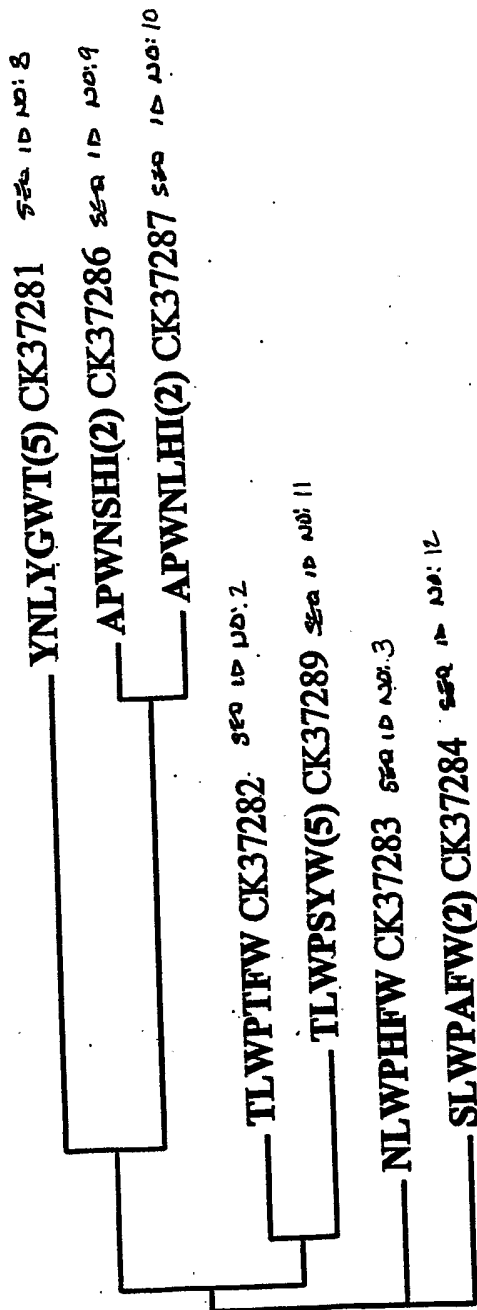
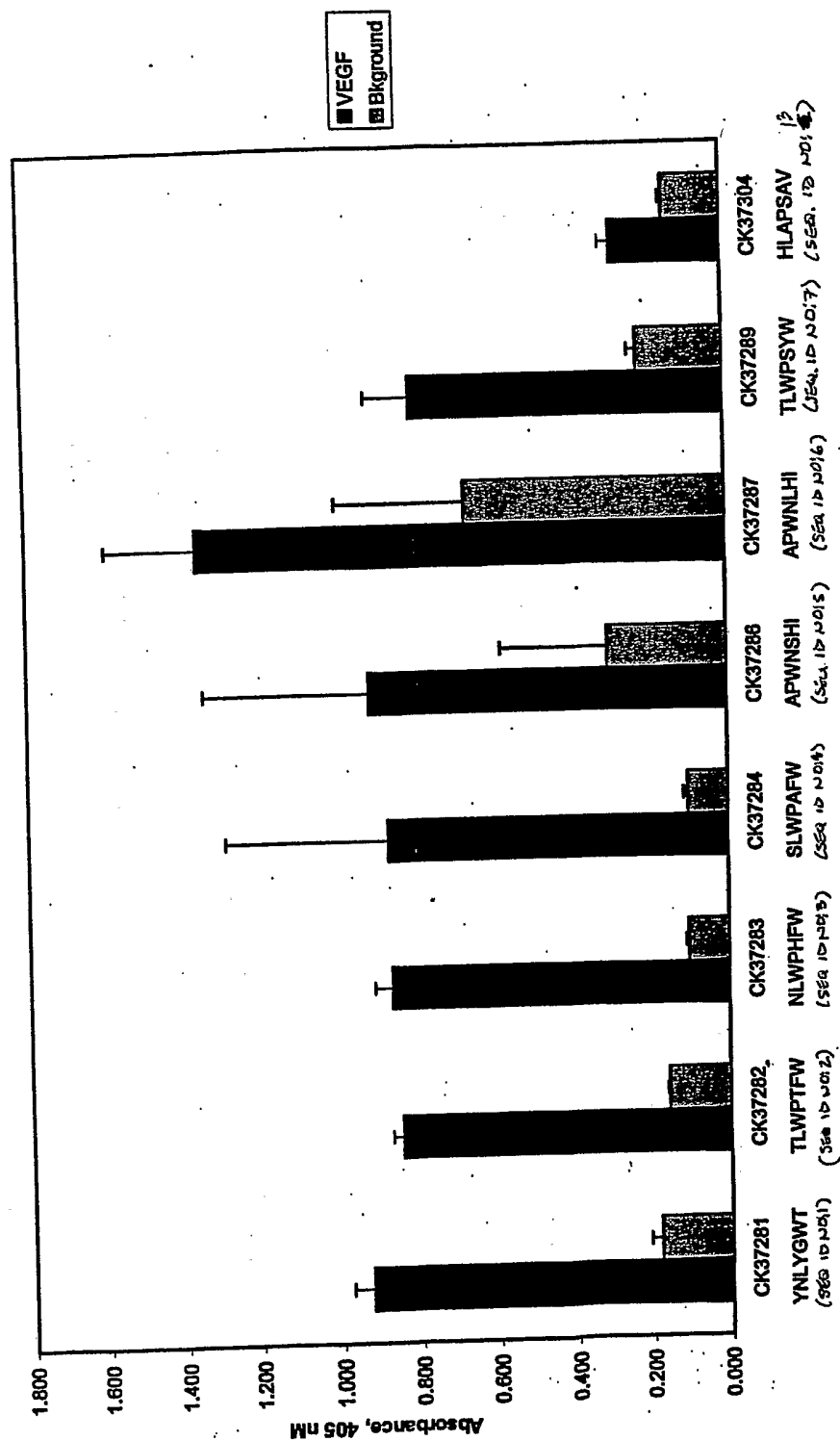


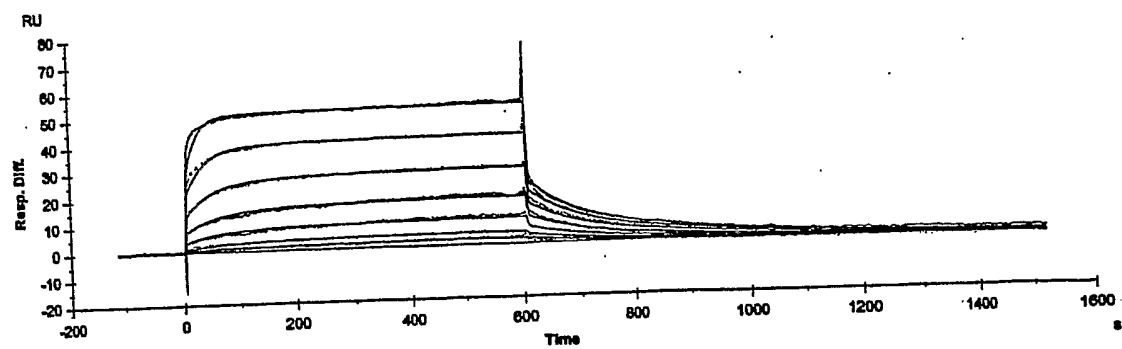
FIGURE 1

FIGURE 2

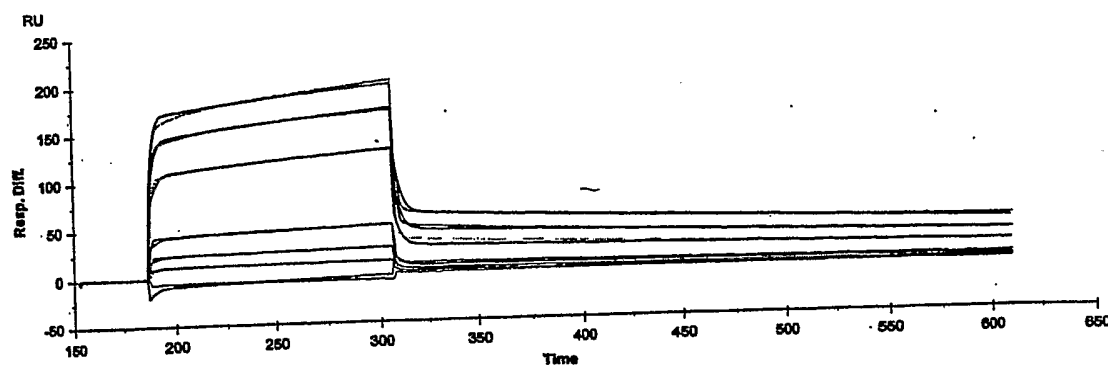


3/27

(A)



(B)



(C)

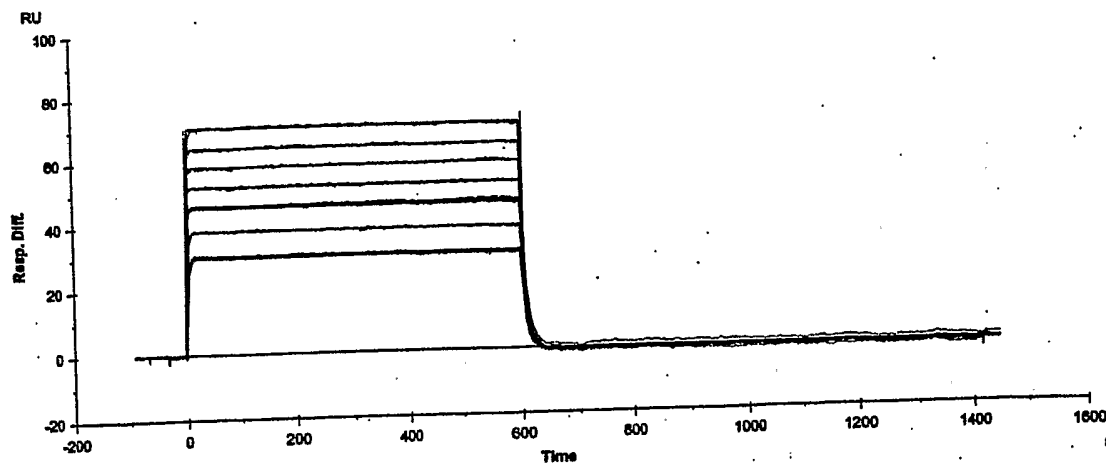


Figure 3

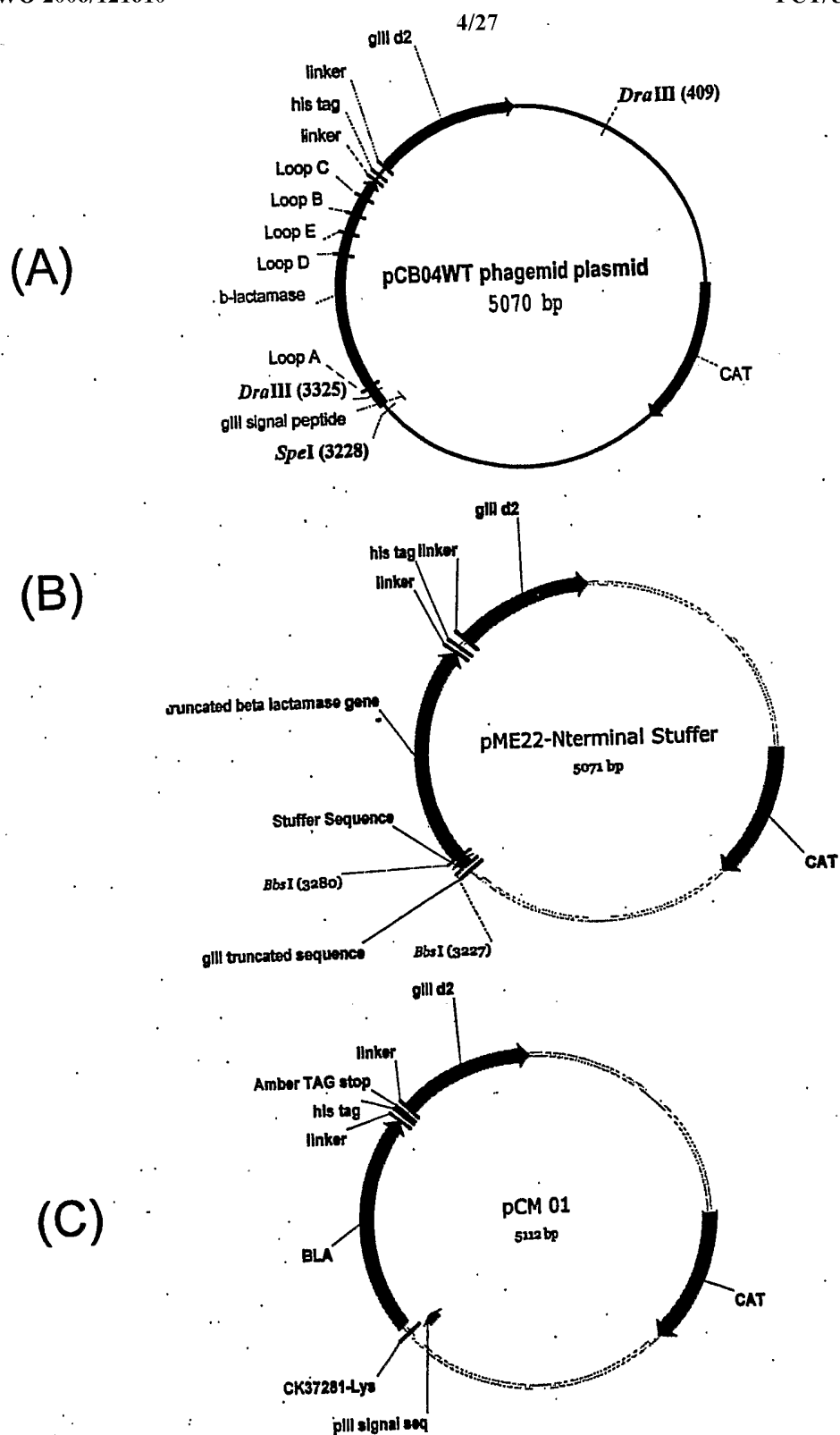


Figure 4

SeeBlue
TOP10/pCD04
TOP10/pCM01 fl 1/8
TOP10/pCM01 fl 1/12
TOP10/pCM01 f3 1/4
TOP10/pCM01 f3 1/6
TOP10/pCM02 1/8
TOP10/pCM02 1/12

SeeBlue

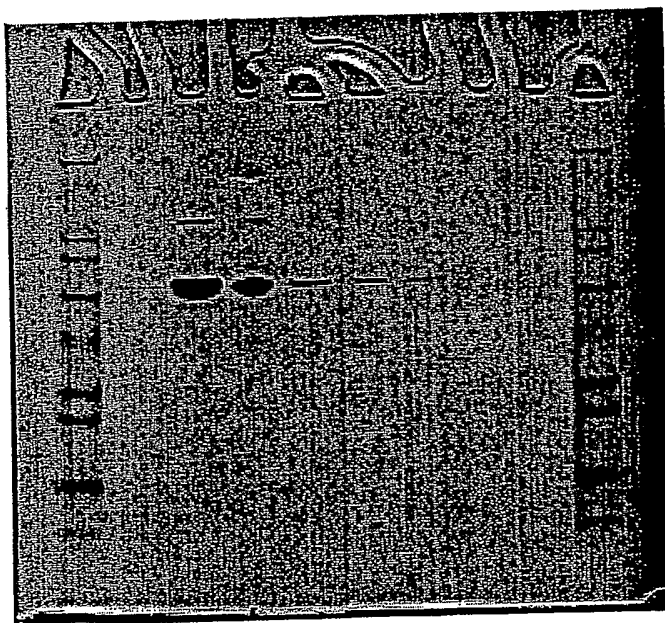


Figure 6

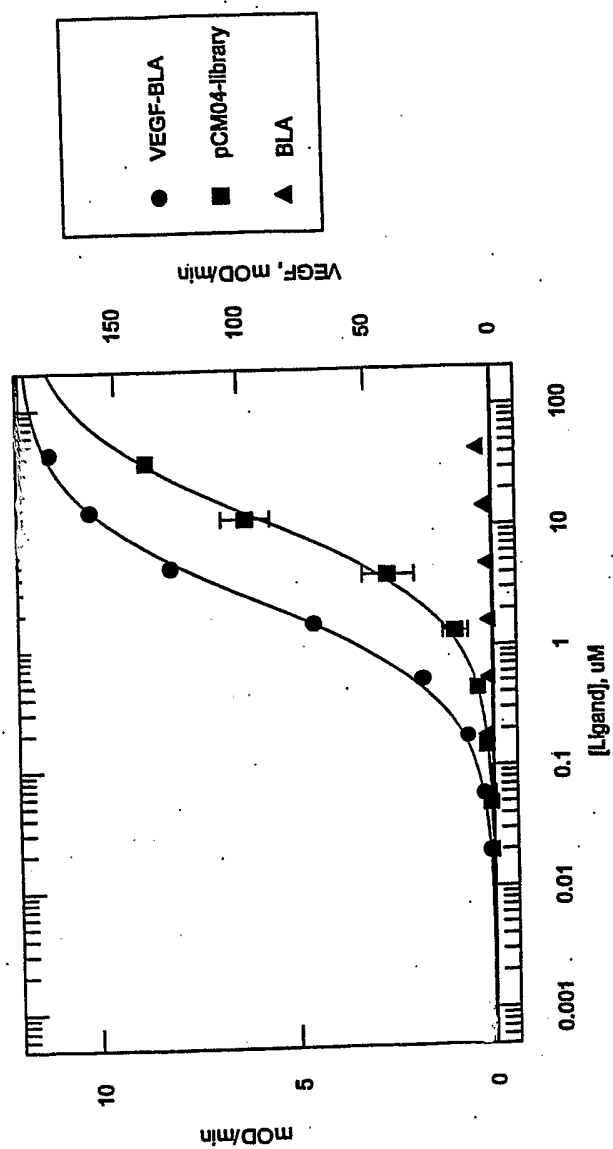


Figure 7

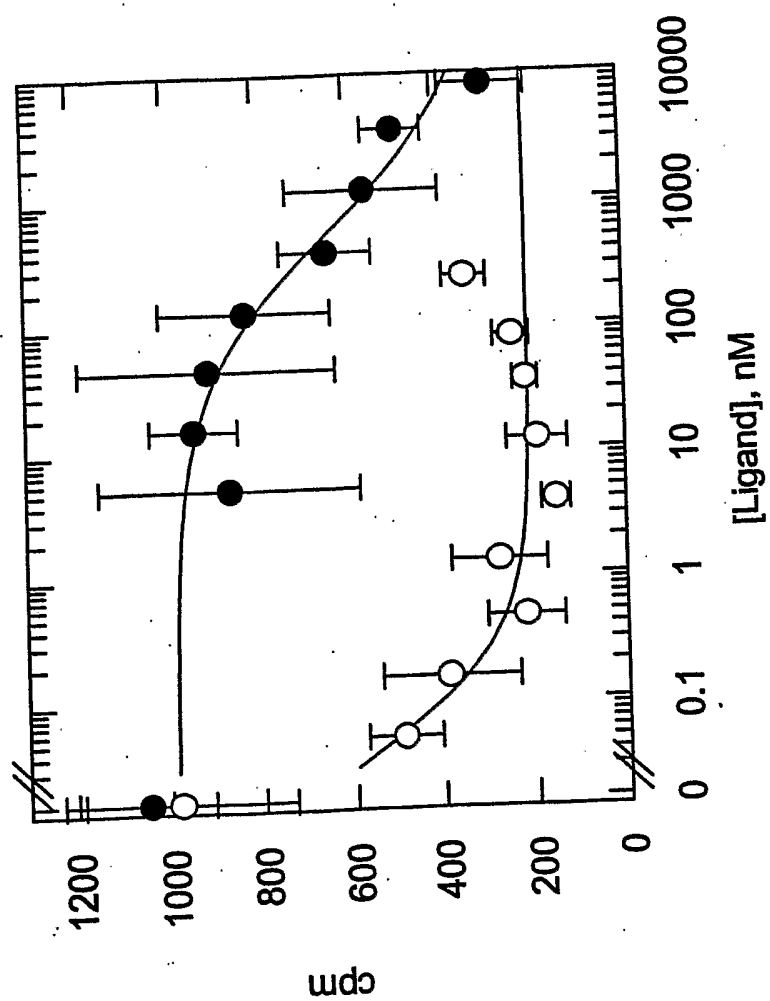


Figure 8

9/27

HindIII

NcoI

M G A N L R L S K L G L L M K S D H Q H S N D

CCATGGGTGC GAACCTGCGT CTGTCTAAGC TTGGCCTGCT TATGAAATCA GACCATCAG C ACAGCAATGA

SacI

D E S S K P C C D Q C A

BsrGI

C T K S N P P Q C

R C

PstI

CGATGAGAGC TCTAAACCCCT GTTGGCGATCA ATGGCGATGT ACAAATCAA ATCCTCCAC A GTGTCGGTGT

EcoRI

S D M R L N S C H S A C K S C I

SphI

C A L S Y P A Q

TCCGATATGC GTCIGAATTC CTGTCATAGT GCATGC AAAA GCTGTATCTG CGCCCTGAG T TATCCAGCTC

Sali

C F C V D I T D F C Y E P C K P S E D D K E N

AATGTTTTTG CGTCGACATC ACGGACTTCT GCTATGAGCC ATGTAAACCA AGCGAGGAC G ATAAAGAGAA

XhoI

H H H H H H *

CCATCATCAC CATCACCATT AACTCGAG

(Seq. 10 1201, 16)

(Seq. 10 1201, 19)

Figure 9.

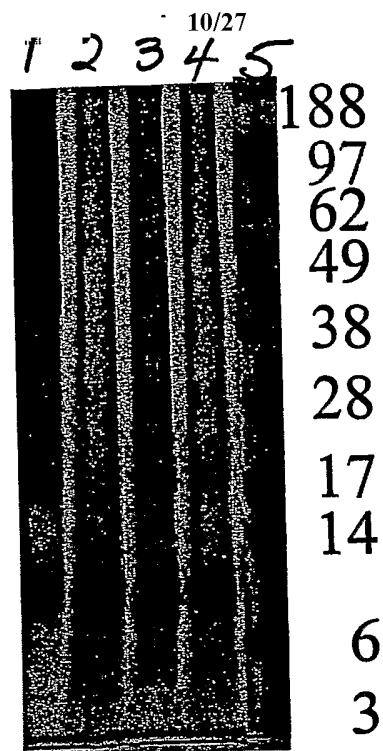


Figure 10

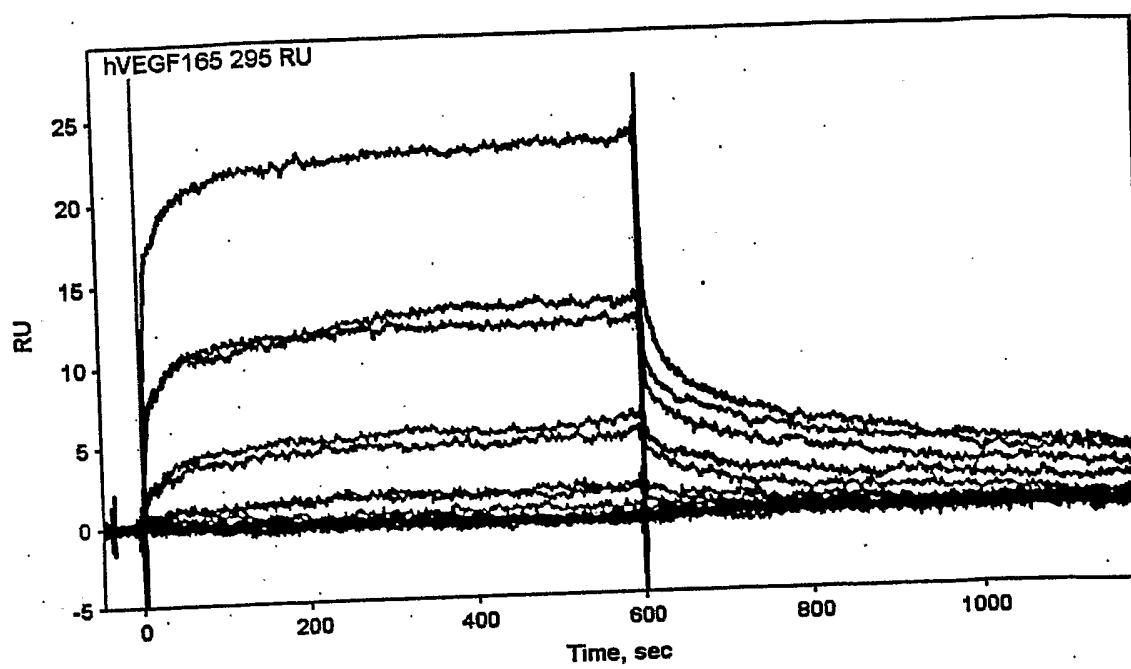


Figure 11

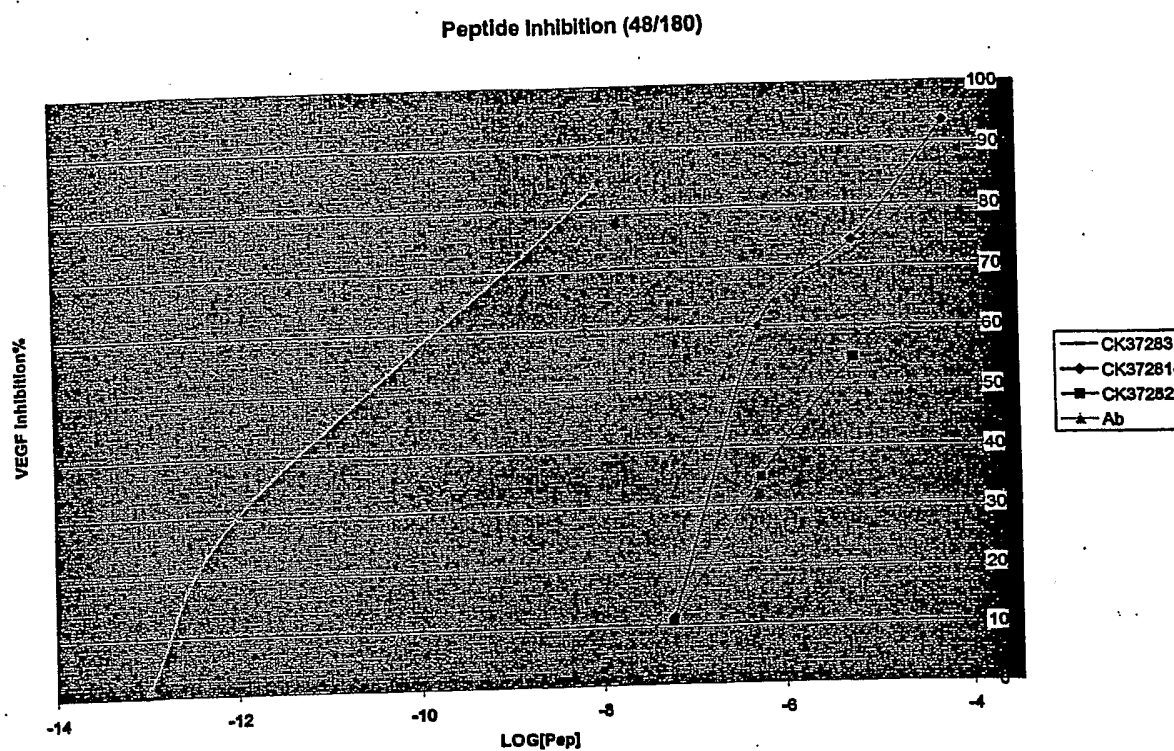


Figure 12

FIGURE 13

BBI-VEG1: ddesskpccdqacacynlygwtcrsdmrlnschsacksicalsyppaqcfvdltdfcyepckpseddken (563 10 MD:22)
 BBI-VEGF2: ddesskpccdqacaciksnppqcrsdmrlnschsacksacacynlygwtcfvdltdfcyepckpseddken (564 10 MD:23)
 BBI-VEGF12: ddesskpccdqacacynlygwtcrsdmrlnschsacksacacynlygwtcfvdltdfcyepckpseddken (565 10 MD:24)

Figure 13

FIGURE 14 A

```

      aprE promoter region
      ~~~~~
      EcoRI
      ~~~~~
1  AATTCTCCAT TTTCTTCTGC TATCAAATA ACAGACTCGT GATTTTCCAA

      aprE promoter region
      ~~~~~
51 ACGAGCTTTC AAAAAGCCT CTGCCCTTG CAAATCGGAT GCCTGTCTAT

      aprE promoter region
      ~~~~~
                                     EagI
                                     ~~~~~
                                     NotI
                                     ~~~~~
101 AAAATTCCCG ATATTGGTTA AACAGCGGCG CAATGGCGGC CGCATCTGAT

      aprE promoter region
      ~~~~~
151 GTCTTTGCTT GGCGAATGTT CATCTTATTT CTTCCTCCCT CTCAATAATT

      aprE promoter region
      ~~~~~
201 TTTTCATTCT ATCCCTTTTC TGTAAGTTT ATTTTTCAGA ATACTTTTAT

      aprE promoter region
      ~~~~~
251 CATCATGCTT TGAAAAATA TCACGATAAT ATCCATTGTT CTCACGGAAG

      aprE promoter region
      ~~~~~
301 CACACGCAGG TCATTGTAAC GAATTTTTC GACAGGAATT TGCCGGGACT

      aprE promoter region
      ~~~~~
351 CAGGAGCATT TAACCTAAAA AAGCATGACA TTTCAGCATA ATGAACATTT

      aprE promoter region
      ~~~~~
401 ACTCATGTCT ATTTTCGTC TTTTCTGTAT GAAATAGTT ATTTGAGTC

      aprE promoter region
      ~~~~~
451 TCTACGGAAA TAGCGAGAGA TGATATACCT AAATAGAGAT AAAATCATCT

      aprE promoter region
      ~~~~~
501 CAAAAAATG GGTCTACTAA AATATTATTC CATCTATTAC AATAAATTCA

      aprE promoter region
      ~~~~~
551 CAGAATAGTC TTTTAAGTAA GTCTACTCTG AATTTTTTTA AAAGGAGAGG

      AprE signal peptide
      ~~~~~
      aprE promoter region
      ~~~~~
      M R S K K L W I S L L F A L T
601 GTAAAGAGTG AGAAGCAAAA AATTGTGGAT CAGCTTGTG TTTGCGTTAA

```

¹³
FIGURE 14 (cont.)

AprE signal peptide

```

~~~~~
BCE103
~~~~~
  . L I F T M A F S N M S A Q A D D
651 CGTTAATCTT TACGATGGCG TTCAGCAACA TGTCTGCGCA GGCTGATGAT

BCE103
~~~~~
  Y S V V E E H G Q L S I S N G E L .
701 TATTCAGTTG TAGAGGAACA TGGGCAACTA AGTATTAGTA ACGGTGAATT

BCE103
~~~~~
NcoI
~~~~~
  . V N E R G E Q V Q L K G M S S H G .
751 AGTCAATGAA CGAGGCGAAC AAGTTCAGTT AAAAGGGATG AGTTCCTCATG

BCE103
~~~~~
NcoI
~~~~~
  . L Q W Y G Q F V N Y E S M K W L
801 GTTTCGAATG GTACGGTCAA TTTGTAACT ATGAAAGCAT GAAATGGCTA

BCE103
~~~~~
  R D D W G I T V F R A A M Y T S S .
851 AGAGATGATT GGGGAATAAC TGTATTCCGA GCAGCAATGT ATACCTCTTC

BCE103
~~~~~
  . G G Y I D D P S V K E K V K E T V .
901 AGGAGGATAT ATTGACGATC CATCAGTAAA GGAAAAAGTA AAAGAGACTG

BCE103
~~~~~
  . E A A I D L G I Y V I I D W H I .
951 TTGAGGCTGC GATAGACCTT GGCAATATG TGATCATTGA TTGGCATATC

BCE103
~~~~~
  L S D N D P N I Y K E E A K D F F .
1001 CTTTCAGACA ATGACCCGAA TATATATAAA GAAGAAGCGA AGGATTTCTT

BCE103
~~~~~
  . D E M S E L Y G D Y P N V I Y E I .
1051 TGATGAAATG TCAGAGTTGT ATGGAGACTA TCCGAATGTG ATATACGAAA

BCE103
~~~~~
  . A N E P N G S D V T W D N Q I K
1101 TTGCAAATGA ACCGAATGGT AGTGATGTTA CGTGGGACAA TCAAATAAAA

BCE103
~~~~~
  P Y A E E V I P V I R D N D P N N .
1151 CCGTATGCAG AAGAAGTGAT TCCGGTTATT CGTGACAATG ACCCTAATAA

```

FIGURE 14^C(cont.)

BCE103

~~~~~  
 . I V I V G T G T W S Q D V H H A A .  
 1201 CATTGTTATT GTAGGTACAG GTACATGGAG TCAGGATGTC CATCATGCAG

BCE103

~~~~~  
 . D N Q L A D P N V M Y A F H F Y
 1251 CCGATAATCA GCTTGCAGAT CCTAACGTCA TGTATGCATT TCATTTTAT

BCE103

~~~~~  
 A G T H G Q N L R D Q V D Y A L D .  
 1301 GCAGGAACAC ATGGACAAA TTTACGAGAC CAAGTAGATT ATGCATTAGA

BCE103

~~~~~  
 . Q G A A I F V S E W G T S A A T G .
 1351 TCAAGGAGCA GCGATATTG TTAGTGAATG GGGGACAAGT GCAGCTACAG

BCE103

~~~~~  
 . D G G V F L D E A Q V W I D F M  
 1401 GTGATGGTGG TGTGTTTTA GATGAAGCAC AAGTGTGGAT TGACTTTATG

BCE103

~~~~~  
 D E R N L S W A N W S L T H K D E .
 1451 GATGAAAGAA ATTTAAGCTG GGCCAACTGG TCTCTAACGC ATAAGGATGA

BCE103

PstI

~~~~~  
 . S S A A L M P G A N P T G G W T E .  
 1501 GTCATCTGCA GCGTTAATGC CAGGTGCAAA TCCAACGGT GGTGGACAG

BCE103

~~~~~  
 . A E L S P S G T F V R E K I R E
 1551 AGGCTGAAC TATCCATCT GGTACATTG TGAGGGAAAA AATAAGAGAA

BCE103

1st CBD Linker

~~~~~  
 S A S I P P S D P T P P S D P G E .  
 1601 TCAGCATCTA TTCCGCCAAG CGATCCAACA CCGCCATCTG ATCCAGGAGA

BBI

fusion site

1st CBD Linker

BamHI

SacI

~~~~~  
 . P D P D D E S S K P C C D Q C A C .
 1651 ACCGGATCCA GACGATGAGA GCTCTAAACC CTGTTGCGAT CAATGCGCAT

FIGURE 14^D(cont.)

```

      BBI
      ~~~~~
      . T K S N P P Q C R C S D M R L N
1701 GTACGAAATC AAATCCTCCA CAGTGTCGGT GTTCCGATAT GCGTCTGAAT

      BBI
      ~~~~~
      SphI
      ~~~~~
      S C H S A C K S C I C A L S Y P A
1751 AGCTGTCATA GTGCATGCAA AAGCTGTATC TGCGCCCTGA GTTATCCAGC

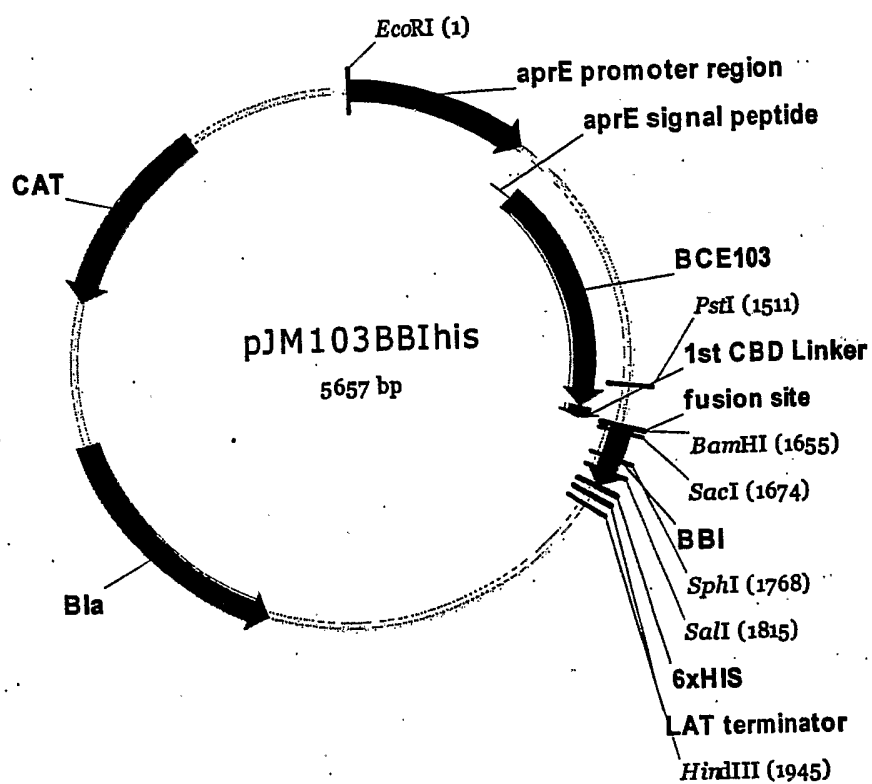
      BBI
      ~~~~~
      Sall
      ~~~~~
      . Q C F C V D I T D F C Y E P C K P
1801 TCAATGTTTT TGCGTCGACA TCACGGACTT CTGCTATGAG CCATGTAAAC

      6xHIS
      ~~~~~
      BBI
      ~~~~~
      . S E D D K E N H H H H H H H Stop (SEQ ID NO:2)
1851 CAAGCGAGGA CGATAAAGAG AACCATCATC ACCATCACCA TTAAAAGTTA

      LAT terminator
      ~~~~~
      HindIII
      ~~~~~
1901 ACAGAGGACG GATTCCTGA AGGAAATCCG TTTTATTATT TTTAAGCTTG (SEQ ID NO:1)

```

FIGURE 15



19/27

FIGURE 16

```

~~~~~
                                12BBick81
                                ~~~~~
BamHI                          SacI
~~~~~
      D P D D E S S K P C C D Q C A C Y .
1  GGATCCAGAC GATGAGAGCT CTAAACCCTG TTGCGATCAA TCGCATGTT
   CCTAGGTCCTG CTACTCTCGA GATTGGGAC AACGCTAGTT ACGCGTACAA

                                12BBick81
                                ~~~~~
                                PstI                      EcoRI
                                ~~~~~
      . N L Y G W T C R C S D M R L N S
51 ATAATTTGTA TGGGTGGACT TGTGCTGCA GCGATATGCG TCTGAATTC
   TATTAAACAT ACCCACCTGA ACAGCGACGT CGCTATACGC AGACTTAAG

                                12BBick81
                                ~~~~~
      C H S A C K S C A C Y N L Y G W T .
101 TGTCTAGTG CCTGCAAAAG CTGCGCATGT TATAACCTGT ACGGGTGGAC
   ACAGTATCAC GGACGTTTTC GACGCGTACA ATATTGGACA TGCCACCTG

                                12BBick81
                                ~~~~~
                                Sali
                                ~~~~~
      . C F C V D I T D F C Y E P C K P S .
151 CTGTTTTTGC GTCGACATCA CGGACTTCTG CTATGAGCCA TGTAACCAA
   GACAAAAACG CAGCTGTAGT GCCTGAAGAC GATACTCGGT ACATTGGTT

                                12BBick81
                                ~~~~~
      . E D D K E N * (SEQ ID NO: 35)
201 GCGAGGACGA TAAAGAGAAC TAA (SEQ ID NO: 37)
   CGCTCCTGCT ATTTCTCTTG ATT

```

FIGURE 17

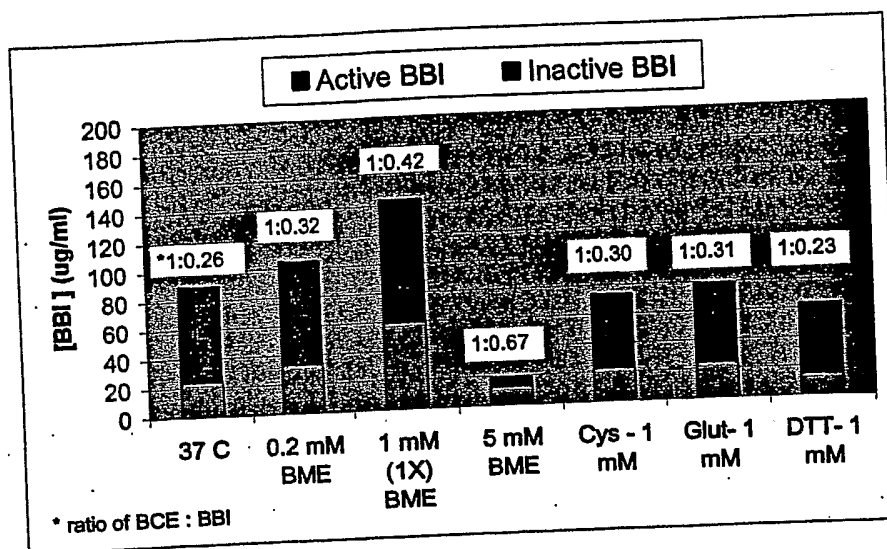


FIGURE 18

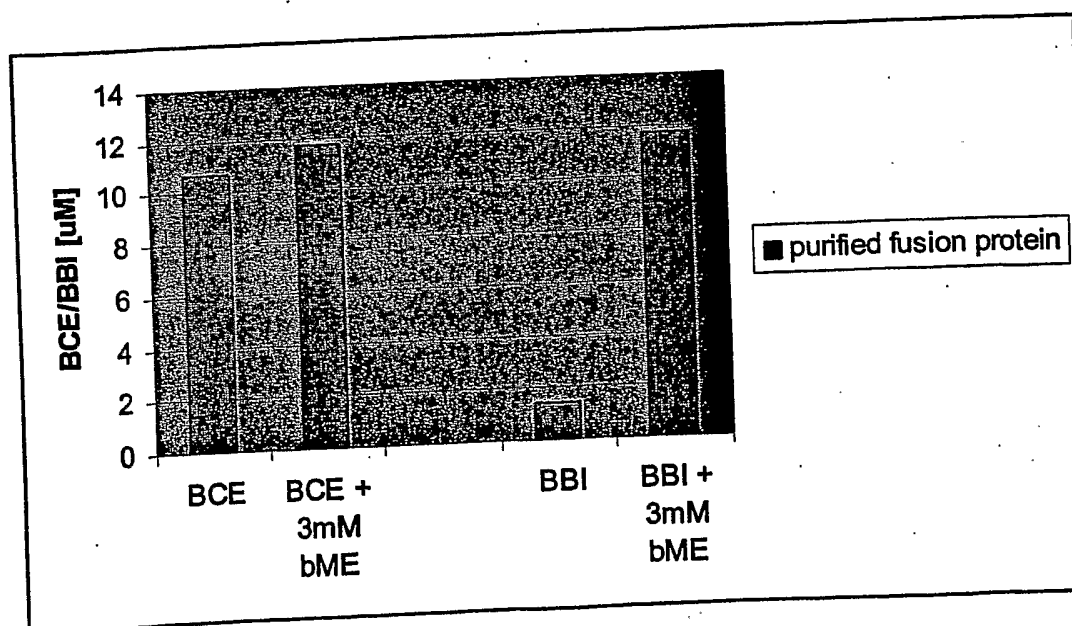


FIGURE 19

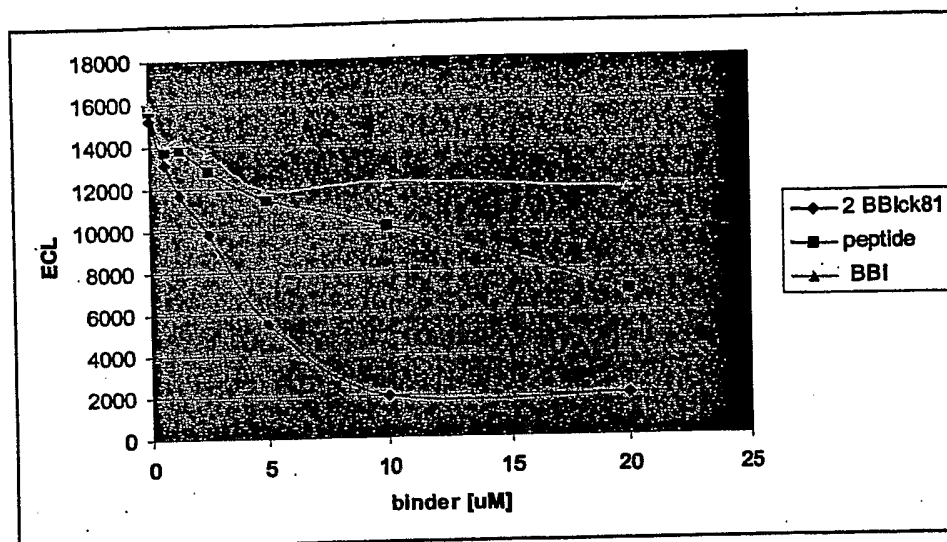


FIGURE 20 A

hiPDI

 AprE signal cleavage site

 BssHII NheI BsrGI

 S A Q A S D V V Q L K K D T F D D
 1 AGCGCGCAGG CTAGCGATGT TGTACAACTG AAAAAAGACA CTTTCGACGA
 TCGCGCGTCC GATCGCTACA ACATGTTGAC TTTTCTGT GAAAGCTGCT

hiPDI

 F I K T N D L V L A E F F A P W C
 51 CTTTCATCAA ACAATGACC TTGTTCTTGC TGAATTTTC GCGCCGTGGT
 GAAGTAGTTT TGTTTACTGG AACAAGAACG ACTTAAAAAG CGCGGCACCA

hiPDI

 G H C K A L A P E Y E E A A T T
 101 GCGGTCACTG CAAAGCTCTT GCTCCTGAGT ACGAGGAAGC TGCAACTACA
 CGCCAGTGAC GTTTCGAGAA CGAGGACTCA TGCTCCTTCG ACGTTGATGT

hiPDI

 L K E K N I K L A K V D C T E E T
 151 CTGAAAGAAA AGAACATCAA ACTTGCTAAA GTAGACTGCA CAGAAGAGAC
 GACTTTCTTT TCTGTAGTT TGAACGATTT CATCTGACGT GTCTTCTCTG

hiPDI

 D L C Q Q H G V E G Y P T L K V F
 201 TGATCTTTGC CAACAACATG GTGTTGAGGG CTACCCAACT CTTAAAGTTT
 ACTAGAAACG GTTGTGTAC CACAACCTCC GATGGGTTGA GAATTTCAA

hiPDI

 R G L D N V S P Y K G Q R K A A
 251 TCCGTGGCCT TGACAACGTA TCTCCTTACA AAGGTCAACG TAAAGCTGCT
 AGGCACCGGA ACTGTTGCAT AGAGGAATGT TTCCAGTTGC ATTCGACGA

hiPDI

 A I T S Y M I K Q S L P A V S E V
 301 GCAATCACTT CATACTGAT CAAACAATCT CTGCCTGCTG TATCTGAAGT
 CGTTAGTGAA GTATGTACTA GTTTGTTAGA GACGGACGAC ATAGACTTCA

hiPDI

 T K D N L E E F K K A D K A V L V
 351 TACAAAAGAC AACCTTGAAG AATTAAAAA AGCTGACAAA GCTGTTCTTG
 ATGTTTTCTG TTGGAACCTC TTAATTTTT TCGACTGTTT CGACAAGAAC

hiPDI

 A Y V D A S D K A S S E V F T Q
 401 TTGCTTATGT AGATGCTTCT GACAAAGCAT CTAGCGAAGT TTTCACTCAA
 AACGAATACA TCTACGAAGA CTGTTTCGTA GATCGCTTCA AAAGTGAGTT

hiPDI

 V A E K L R D N Y P F G S S S D A
 451 GTTGCTGAAA AACTGCGCGA TAACTACCCA TTCGGCTCTA GCTCTGATGC
 CAACGACTTT TTGACGCGCT ATTGATGGGT AAGCCGAGAT CGAGACTACG

B
FIGURE 20(cont.)

```

                                hiPDI
                                ~~~~~
501  • A L A E A E G V K A P A I V L Y K •
    T G C A C T G G C T   G A A G C T G A G G   G C G T T A A A G C   A C C T G C T A T T   G T T C T T T A C A
    A C G T G A C C G A   C T T C G A C T C C   C G C A A T T T C G   T G G A C G A T A A   C A A G A A A T G T

                                hiPDI
                                ~~~~~
551  • D F D E G K A V F S E K F E V E
    A A G A C T T T G A   T G A A G G T A A A   G C G G T T T T C T   C T G A A A A A T T   C G A A G T A G A G
    T T C T G A A A C T   A C T T C C A T T T   C G C C A A A A G A   G A C T T T T T A A   G C T T C A T C T C

                                hiPDI
                                ~~~~~
601  • A I E K F A K T G A T P L I G E I •
    G C A A T C G A A A   A A T T C G C T A A   A A C A G G T G C T   A C T C C A C T T A   T T G G C G A A A T
    C G T T A G C T T T   T T A A G C G A T T   T T G T C C A C G A   T G A G G T G A A T   A A C C G C T T T A

                                hiPDI
                                ~~~~~
651  • G P E T Y S D Y M S A G I P L A Y •
    C G G A C C T G A A   A C T T A C T C T G   A T T A C A T G T C   A G C T G G C A T C   C C T C T G G C A T
    G C C T G G A C T T   T G A A T G A G A C   T A A T G T A C A G   T C G A C C G T A G   G G A G A C C G T A

                                hiPDI
                                ~~~~~
                                SapI
                                ~~~~~
701  • I F A E T A E E R R E L S D K L
    A C A T T T T C G C   T G A A C A G C T   G A A G A G C G T A   A A G A A C T C A G   C G A C A A A C T T
    T G T A A A A G C G   A C T T T G T C G A   C T T C T C G C A T   T T C T T G A G T C   G C T G T T T G A A

                                hiPDI
                                ~~~~~
751  • K P I A E A Q R G V I N F G T I D •
    A A A C C A A T C G   C T G A A G C T C A   A C G T G G C G T T   A T T A A C T T T G   G T A C T A T T G A
    T T T G G T T A G C   G A C T T C G A G T   T G C A C C G C A A   T A A T T G A A A C   C A T G A T A A C T

                                hiPDI
                                ~~~~~
801  • A K A F G A H A G N L N L K T D K •
    C G C T A A A G C A   T T T G G T G C T C   A C G C T G G A A A   C C T G A A T C T G   A A A A C T G A C A
    G C G A T T T C G T   A A A C C A C G A G   T G C G A C C T T T   G G A C T T A G A C   T T T T G A C T G T

                                hiPDI
                                ~~~~~
851  • F P A F A I Q E V A K N Q K F P
    A A T T C C C T G C   T T T C G C A A T C   C A A G A A G T T G   C T A A A A C C A   A A A A T T C C C T
    T T A G G G A C G   A A A G C G T T A G   G T T C T T C A A C   G A T T T T T G G T   T T T A A G G G A

                                hiPDI
                                ~~~~~
901  • F D Q E K E I T F E A I K A F V D •
    T T T G A T C A A G   A A A A A G A A A T   T A C T T T T G A A   G C G A T C A A A G   C A T T C G T T G A
    A A A C T A G T T C   T T T T T C T T T A   A T G A A A A C T T   C G C T A G T T T C   G T A A G C A A C T

                                hiPDI
                                ~~~~~
951  • D F V A G K I E P S I K S E P I P •
    C G A T T T T G T T   G C T G G T A A A A   T C G A A C C A A G   C A T C A A A T C A   G A A C C A A T C C
    G C T A A A A C A A   C G A C C A T T T T   A G C T T G G T T C   G T A G T T T A G T   C T T G G T T A G G

                                hiPDI
                                ~~~~~
1001 • E K Q E G P V T V V V A K N Y N
    C T G A A A A C A A   A G A A G G T C C T   G T T A C T G T A G   T T G T A G C T A A   A A A C T A C A A T
    G A C T T T T T G T   T C T T C C A G G A   C A A T G A C A T C   A A C A T C G A T T   T T T G A T G T T A

```

C
FIGURE 20(cont.)

```

                                hiPDI
                                ~~~~~
1051  E I V L D D T K D V L I E F Y A P
      GAAATCGTTC TGGACGATAC TAAAGATGTA TTAATTGAAT TTTACGCTCC
      CTTTAGCAAG ACCTGCTATG ATTTCTACAT AATTAACCTA AAATGCGAGG

                                hiPDI
                                ~~~~~
1101  W C G H C K A L A P K Y E E L G A
      TTGGTGCGGT CACTGCAAG CTCTTGCTCC TAAATACGAA GAACCTGGTG
      AACCAAGCCA GTGACGTTTC GAGAACGAGG ATTTATGCTT CTTGAACCA

                                hiPDI
                                ~~~~~
1151  L Y A K S E F K D R V V I A K V
      CTCTGTATGC AAAAAGCGAG TTCAAAGACC GTGTTGTAAT TGCTAAAGTT
      GAGACATACG TTTTTCGCTC AAGTTTCTGG CACAACATTA ACGATTTCAA

                                hiPDI
                                ~~~~~
1201  D A T A N D V P D E I Q G F P T I
      GATGCAACAG CTAACGATGT TCCAGATGAA ATTCAGGAT TCCCTACTAT
      CTACGTTGTC GATTGCTACA AGGTCTACTT TAAGTTCCTA AGGGATGATA

                                hiPDI
                                ~~~~~
1251  K L Y P A G A K G Q P V T Y S G S
      CAACTATAC CCAGCTGGTG CAAAAGGTCA ACCTGTTACT TACTCTGGTT
      GTTTGATATG GGTGACCAAC GTTTCCAGT TGGACAAATGA ATGAGACCAA

                                hiPDI
                                ~~~~~
1301  R T V E D L I K F I A E N G K Y
      CACGCACTGT TGAAGACCTT ATCAAATTCA TTGCTGAAAA CGGTAAATAC
      GTGCGTGACA ACTTCTGGAA TAGTTTAAGT AACGACTTTT GCCATTTATG

                                hiPDI
                                ~~~~~
                                SpeI
                                ~~~~~
1351  K A A I S E D A E E T S S A T E T
      AAAGCTGCAA TCTCAGAAGA TGCTGAAGAG ACTAGTTCAG CAACTGAAAC
      TTTCGACGTT AGAGTCTTCT ACGACTTCTC TGATCAAGTC GTTGACTTTG

                                hiPDI
                                ~~~~~
1401  T T E T A T K S E E A A K E T A T
      AACTACAGAA ACTGCTACAA AGTCAGAAGA AGCTGCAAAA GAACTGCNA
      TTGATGTCTT TGACGATGTT TCAGTCTTCT TCGACGTTTT CTTTGACGTT

                                Enteropeptidase cleavage linker
                                ~~~~~
                                hiPDI                                N-term BBI
                                ~~~~~
1451  E H D E L G S G S G D D D D K D
      CAGAACACGA CGAACTTGGA TCTGGTTCCG GAGATGACGA TGACAAAGAC
      GTCTTGCTGCT GCTTGAACCT AGACCAAGGC CTCTACTGCT ACTGTTTCTG

                                N-term BBI
                                ~~~~~
                                SacI
                                ~~~~~
1501  D E S S
      GATGAGAGCT CT (SEQ ID NO:5)
      CTACTCTCGA GA (SEQ ID NO:6)

```

FIGURE 21A

```

      aprE promoter
      ~~~~~
EcoRI
      ~~~~~
1  GAATTCTCCA TTTTCTTCTG CTATCAAAT AACAGACTCG TGATTTTCCA
   CTTAAGAGGT AAAAGAAGAC GATAGTTTAA TTGTCTGAGC ACTAAAAGGT

      aprE promoter
      ~~~~~
51 AACGAGCTTT CAAAAAGCC TCTGCCCTT GCAAATCGGA TGCCTGTCTA
   TTGCTCGAAA GTTTTTTCGG AGACGGGGAA CGTTTAGCCT ACGGACAGAT

      aprE promoter
      ~~~~~
                                   NotI
                                   ~~~~~
101 TAAAATTCCC GATATTGGTT AAACAGCGGC GCAATGGCGG CCGCATCTGA
    ATTTTAAGGG CTATAACCAA TTTGTCGCCG CGTTACCGCC GGCCTAGACT

      aprE promoter
      ~~~~~
151 TGTCTTGTCT TGGCGAATGT TCATCTTATT TCTTCCTCCC TCTCAATAAT
    ACAGAAACGA ACCGCTTACA AGTAGAATAA AGAAGGAGGG AGAGTTATA

      aprE promoter
      ~~~~~
201 TTTTTCATTC TATCCCTTTT CTGTAAAGTT TATTTTTCAG AATACTTTTA
    AAAAGTAAG ATAGGGAAAA GACATTTCAA ATAAAAAGTC TTATGAAAT

      aprE promoter
      ~~~~~
251 TCATCATGCT TTGAAAAAAT ATCAGGATAA TATCCATTGT TCTCAGGAA
    AGTAGTACGA AACTTTTTTA TAGTGCTATT ATAGGTAACA AGAGTGCCTT

      aprE promoter
      ~~~~~
301 GCACACGCAG GTCATTTGAA CGAATTTTTT CGACAGGAAT TTGCCGGGAC
    CGTGTGCGTC CAGTAAACTT GCTTAAAAAA GCTGTCCTTA AACGGCCCTG

      aprE promoter
      ~~~~~
351 TCAGGAGCAT TTAACCTAAA AAAGCATGAC ATTCAGCAT AATGAACATT
    AGTCCTCGTA AATTGGATTT TTTCGTACTG TAAAGTCGTA TTAATTGTAA

      aprE promoter
      ~~~~~
401 TACTCATGTC TATTTTCGTT CTTTCTGTA TGAAAATAGT TATTTGAGT
    ATGAGTACAG ATAAAAGCAA GAAAAGACAT ACTTTTATCA ATAAAGCTCA

      aprE promoter
      ~~~~~
451 CTCTACGSA_ ATAGCGAGAG ATGATATACC TAAATAGAGA TAAATCATC
    GAGATGCCTT TATCGCTCTC TACTATATGG ATTTATCTCT ATTTAGTAG

      aprE promoter
      ~~~~~
501 TCAAAAAAAT GGTCTACTA AAATATTATT CCATCTATTA CAATAAATTC
    AGTTTTTTTA CCCAGATGAT TTTATAATAA GGTAGATAAT GTTATTTAAG

      aprE promoter
      ~~~~~
551 ACAGAATAGT CTTTAAAGTA AGTCTACTCT GAATTTTTTT AAAAGGAGAG
    TGTCTTATCA GAAAATTCAT TCAGATGAGA CTAAAAAA TTTTCCTCTC

      aprE promoter
      ~~~~~
      AprE signal peptide
      ~~~~~
Cutinase signal peptide
      ~~~~~

```

FIGURE 2 ^{*k*} (cont.)

```

      V R S K K L W I S L L F A L
601 GGTAAAGAGT GAGAAGCAAA AAATTGTGGA TCAGCTTGTT GTTTGCGTTA
    CCATTTCTCA CTCTTCGTTT TTTAACACCT AGTCGAACAA CAAACGCAAT

                                          Cutinase
                                          ~~~~~

      Cutinase signal peptide
      ~~~~~
      T L A A S C L S V C A T V A A A P .
651 ACGCTGGCGG CCTCTTGCCCT GTCCGTCTGT GCCACTGTG CCGCGGCTCC
    TGGCAGCCGC GGAGAACGGA CAGGCAGACA CGGTGACAGC GCCGCCGAGG

      Cutinase
      ~~~~~
      L P D T P G A P F P A V A N F D R .
701 CCTGCCGAT ACACCGGGAG CGCCATTTC GGCTGTGCGC AATTTCGACC
    GGACGGCCTA TGTGGCCCTC GCGGTAAAGG CCGACAGCGG TTAAAGCTGG

      Cutinase
      ~~~~~
      S G P Y T T S S Q S E G P S C R
751 GCAGTGGCCC CTACACCACC AGCAGCCAGA GCGAGGGGCC GAGCTGTGCG
    CGTCACCGGG GATGTGGTGG TCGTCGGTCT CGTCCCCGG CTCGACAGCG

      Cutinase
      ~~~~~
      I Y R P R D L G Q G G V R H P V I .
801 ATCTATCGGC CCCGCGACCT GGGTCAGGGG GCGGTGCGTC ATCCGGTGAT
    TAGATAGCCG GGGCGCTGGA CCCAGTCCCC CCGCACGCAG TAGGCCACTA

      Cutinase
      ~~~~~
      L W G N G T G A G P S T Y A G L L .
851 TCTCTGGGGC AATGGCACCG GTGCCGGGCC GTCCACCTAT GCCGGCTTGC
    AGAGACCCCG TTACCGTGGC CACGGCCCGG CAGGTGGATA CGGCCGAACG

      Cutinase
      ~~~~~
      S H W A S H G F V V A A A E T S
901 TATCGCACTG GGCAAGCCAC GGTTCGTGG TGGCGGCGGC GGAAACCTCC
    ATAGCGTGAC CCGTTCGGTG CCAAAGCACC ACCGCCCGCG CCTTGGAGG

      Cutinase
      ~~~~~
      N A G T G R E M L A C L D Y L V R .
951 AATGCCGTA CCGGGCGGGA AATGCTCGCC TGCCTGGACT ATCTGGTACG
    TTACGGCCAT GGCCCGCCCT TTACGAGCGG ACGGACCTGA TAGACCATGC

      Cutinase
      ~~~~~
      E N D T P Y G T Y S G K L N T G R .
1001 TGAGAACGAC ACCCCCTACG GCACCTATTC CGGCAAGCTC AATACCGGGC
    ACTCTTGCTG TGGGGGATGC CGTGGATAAG GCCGTTCGAG TTATGGCCCG

      Cutinase
      ~~~~~
      V G T S G H S Q G G G G S I M A
1051 GAGTCGGCAC TTCTGGGCAT TCCAGGGTG GTGGCGGCTC GATCATGGCC
    CTCAGCCGTG AAGACCCGTA AGGTCGCCAC CACCGCCGAG CTAGTACCGG

      Cutinase
      ~~~~~
      G Q D T R V R T T A P I Q P Y T L .
1101 GGGCAGGATA CGAGGGTGCG TACCACGGCG CCGATCCAGC CCTACACCCCT
    CCCGTCCTAT GCTCCACGC ATGGTGCCCG GGCTAGGTGC GGATGTGGGA

                                          Cutinase

```

FIGURE 2 (cont.)

1151
· G L G H D S A S Q R R Q Q G P M F ·
CGGCCTGGGG CACGACAGCG CCTCGCAGCG GCGGCAGCAG GGGCCGATGT
GCCGGACCCC GTGCTGTGCG GGAGCGTCGC CGCCGTCGTC CCCGGCTACA

Cutinase

1201
· L M S G G G D T I A F P Y L N A
TCCTGATGTC CGGTGGCGGT GACACCATCG CCTTCCCTA CCTCAACGCT
AGGACTACAG GCCACCGCCA CTGTGGTAGC GGAAAGGGAT GGAGTTGCCA

Cutinase

1251
Q P V Y R R A N V P V F W G E R R ·
CAGCCGGTCT ACCGGCGTGC CAATGTGCCG GTGTTCTGGG GCGAACGGCG
GTCGGCCAGA TGGCCGCAGC GTTACACGGC CACAAGACCC CGCTTGCCCG

Cutinase

1301
· Y V S H F E P V G S G G A Y R G P ·
TTACGTCAGC CACTTCGAGC CGGTCGGTAG CGGTGGGGCC TATCGCGGCC
AATGCAGTCG GTGAAGCTCG GCCAGCCATC GCCACCCCGG ATAGCGCCGG

Cutinase

1351
· S T A W F R F Q L M D D Q D A R
CGAGCACGGC ATGGTTCCGC TTCCAGCTGA TGGATGACCA AGACGCCCGC
GCTCGTGCCG TACCAAGGCG AAGGTCGACT ACCTACTGGT TCTGCGGGCG

Cutinase

Alw44I

1401
A T F Y G A Q C S L C T S L L W S ·
GCTACCTTCT ACGGCGCGCA GTGCAGTCTG TGCACTTCTC TGCTTTGGTC
CGATGGAAGA TGCCGCGCGT CACGTCAGAC ACGTGAAGAG ACGAAACCAG

Linker 2

Cutinase

BamHI

1451
· V E R R G L D N N D P I P D
TGTTGAACGC AGAGGTCTTG ACAACAATGA TCCTATTCG GATCC
ACAACCTGCG TCTCCAGAAC TGTTGTTACT AGGATAAGGC CTAGG

(SEQ ID NO: 7)
(SEQ ID NO: 8)

42