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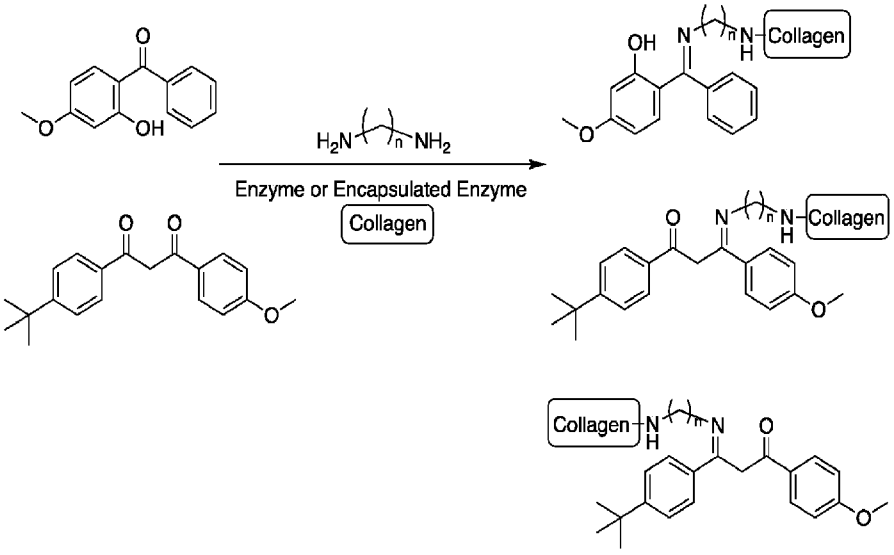
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(54) **Title:** TRANSGLUTAMINASE VARIANTS AND APPLICATIONS OF USE THEREOF

Fig. 1



(57) **Abstract:** Variants of the transglutaminase enzyme of Streptomyces mobaraensis are provided. The disclosed variants exhibit at least about 2-fold increased enzymatic activity versus the wild-type enzyme. Methods and compositions are provided for biocidal applications of use and for covalently binding small organic molecules to a protein or material of interest. Methods are provided for increasing the self-life of products, such as personal care, household and industrial products, by incorporating an effective amount of the disclosed variant enzymes into the product. The transglutaminase variants may also be used to covalently bind functional ingredients, such as UV-blocking molecules, dyes, or pigments to proteins. The transglutaminase enzymes and functional ingredients may be incorporated into a cosmetic formulation for modifying skin, hair, or nail proteins or skin-derived proteins, such as collagen, keratin, and/or elastin.

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TRANSGLUTAMINASE VARIANTS AND APPLICATIONS OF USE THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[01] This application claims the benefit of U.S. Provisional Application Nos. 63/024,398, filed on May 13, 2020, and 63/074,288, filed on September 3, 2020, both of which are incorporated by reference herein in their entireties.

FIELD OF THE INVENTION

[02] The present invention relates to engineered variant microbial transglutaminase polypeptides comprising two or more amino acid substitutions, and the nucleic acids encoding them. The variants may be used for conjugating proteins, peptides, or small molecules with increased specific activity compared to wild-type *Streptomyces mobaraensis* transglutaminase, such as in topical applications of use. The variants may also be used as active biocidal enzymes and in formulations thereof for use as agents for broad spectrum microbial control.

BACKGROUND

[03] Transglutaminases (Tgase, EC 2.3.2.13) are enzymes capable of catalyzing an acyl transfer reaction in which a γ-carboxy-amide group of a peptide bound glutamine residue is the acyl donor. Primary amino groups in a variety of compounds may function as acyl acceptors with the subsequent formation of monosubstituted γ-amides of peptide bound glutamine. When the ε-amino group of a lysine residue in a peptide chain serves as the acyl acceptor, the Tgases form intramolecular or intermolecular γ-glutamyl-ε-lysyl crosslinks. The catalytic reaction proceeds via glutamine deamination and formation of a protein–glutamyl–thioester at the active site of the enzyme. Nucleophilic attack by a lysyl ε-amino group of a second protein at the carbonyl moiety of the thioester intermediate generates isopeptide-crosslinked proteins that are largely resistant to proteolysis by common peptidases (Mariniello, et al. (2007) *J Agr Food Chem.* 55:4717–4721).
Bonds formed by a Tgase exhibit high resistance to proteolytic degradation (proteolysis). Tgases from microbial origin are calcium-independent, which represents a major advantage for their practical use.

[05] Tgase has found many applications in biotechnology and in the food processing industry, where it has earned the moniker “meat glue.” The peptide crosslinking activity has

shown useful for a variety of industrial purposes ranging from food processing, biotechnology, pharmaceuticals, medical devices, personal and household goods, and leather and textile treatment. The most commonly used Tgase is microbial transglutaminase from *Streptomyces mobaraensis*, having the amino acid sequence depicted in SEQ ID NO:1 and referred to hereinafter as Tgase.

[06] Lysyl oxidases (LOX) (also known as protein-lysine 6-oxidase) are copper-dependent enzymes that catalyze formation of aldehydes from lysine residues in collagen and elastin precursors. These aldehydes are highly reactive, and undergo spontaneous chemical reactions with other lysyl oxidase-derived aldehyde residues, or with unmodified lysine residues. This results in cross-linking collagen and elastin. LOX proteins have been identified in animals, other eukaryotes, and in bacteria and archaea (reviewed in Grau-Bove, et al. (2015) *Scientific Reports* 5: Article number: 10568).

[07] Semi-permanent personal care products are increasingly popular among consumers who want to put less effort into their daily routines. Furthermore, waterproof and sweat-proof cosmetics have been long sought after by consumers and product formulators to achieve long-lasting effects of applied makeup and dyes. The use of hydrophobic silicones has been extensively used to achieve longer lasting effects in this context. A disadvantage of cosmetics is the inability to sustain an initial or freshly applied look after application. The applied product in liquid and powder makeup sits on top of the skin and therefore has the tendency to transfer easily from the skin, lips, and/or hair onto objects with which it is brought into contact, such as glassware, cups, fabrics, or other skin. Long-lasting lip sticks, glosses, and stain utilize bromoacids to stain the skin, which can leave the skin dry and irritated. Moreover, the mobility of the applied product often allows the product to migrate and/or concentrate easily into the fine lines, wrinkles, folds, and/or pores of the skin and/or lips, resulting in an undesirable non-uniform appearance.

[08] Conventional cosmetic foundations are typically sold in a liquid, semi-liquid or cream, or powder form. Powder-type formulations may be perceived as having a shorter useful life than the liquid-type formulations (*i.e.*, do not provide the desired cosmetic benefit for as long) and/or are more susceptible to undesirable transfer from the skin to another surface. This is due to the fact that powder-type foundation products are essentially individual solid particles lying on the skin surface with little to prevent them from being rubbed or wiped away. Throughout the course of the day, the product is exposed to sebum (produced by the skin), moisture (sweat, tears, humidity, rain,

washing) and oils (skin care products, other cosmetic products). As such, conventional liquid and semi-liquid products are not suited for staying on the skin for longer than one day. One drawback shared by conventional concealers and foundations is that they may be unintentionally transferred to other surfaces (*e.g.*, clothes, furniture, hair, and other areas of the body). Such transfers may result in clothing, furniture or other surfaces being soiled with makeup, and/or it may reduce the effective life of the makeup. A variety of mechanisms may contribute to the undesired transfer of makeup from the skin. For example, sebum or other waxy/oily substances found on the skin can impede the ability of a foundation or concealer to adhere to the skin. In some instances, rubbing against a surface may abrade the concealer or foundation from the skin. In order to overcome the drawback of undesirable transfer and/or to increase the effective life of a product, "long-wear" or "transfer-free" products are known. However, even conventional long-wear or transfer-free products may not provide a suitable level of coverage and/or beauty enhancement for more than 24 hours or provide suitable resistance to the abrasion encountered by skin during typical daily activities.

[09] Moreover, cosmetics and other personal care products are typically applied and removed during the same day. This leaves many time-points in consumers' lives when they are not able to enjoy the benefits of the product.

[10] Accordingly, it would be desirable to provide a semi-permanent cosmetic product that can withstand the environmental insults typically faced by a product in use for more than twenty-four hours. It would further be desirable to provide a semi-permanent cosmetic formulation that exhibits good abrasion resistance. It would also be desirable to provide a semi-permanent cosmetic formulation that can withstand exposure to water and soap during showering and facial cleansing.

[11] A new category of cosmetic and personal care products is desired: a semi-permanent technology that provides the same effect as when the product is initially applied, for multiple hours or days. Such a product does not require daily application or the frequent touch-ups of conventional technologies.

[12] There is also considerable interest in binding functional or active ingredients to skin for long-lasting effects. One such application is to extend UV protection by binding of sunscreen ingredients to skin proteins or exogenous proteins for long-lasting protection and reduced skin penetration of the ingredient.

[13] There is also considerable interest in preventing topical active or functional ingredients from penetrating the skin by anchoring these ingredients to a high molecular weight, cosmetically acceptable peptide or protein. For example, binding chemical sunscreen molecules to a protein or peptide prior to or at the point of topical application will prevent these ingredients' penetration and absorption into the bloodstream (**Fig. 1**). A recent FDA monograph ruling indicated twelve chemical sunscreens demonstrated higher than acceptable levels in the bloodstream after a single application (https://www.fda.gov/news-events/fda-voices/shedding-more-light-sunscreen-absorption). Anchoring these sunscreen ingredients to a high molecular weight protein or peptide will prevent their absorption through the skin and uptake into the bloodstream, enhancing the safety of chemical sunscreens for consumers and meeting regulatory requirements.

[14] There is also considerable interest in adding color to or dying animal-based products which are comprised of the same proteins as those found in human skin (*e.g.*, collagen, keratin, elastin) or similar naturally derived structural proteins (*e.g.*, silk). For example, the ability to permanently bond color to leather, silk, wool, or fabric blends thereof, without using traditional dying techniques which have the potential to damage these natural fibers by causing undesirable shrinkage, crimping, or elongation, would be desirable. In addition, the process of dying these fabrics is a large source of environmental pollutants and is driving the textile and fashion industries to identify suitable alternative methods. It is similarly important for manufacturers to be able to dye these fabrics to design clothes and other textiles which are fashionably and visually appealing.

[15] There is also considerable interest in the ability to enhance the color of food products with natural dyes which do not transfer or bleed in the final prepared product, especially as it pertains to meat and processed meat products. Edible food casings made of a skin fiber composition of animal origin (collagen) are increasingly used for the production of frankfurters and Vienna sausages, and their manufacture is described, for example, in German Pat. No. 972,854. Such collagen sausage casings range from practically colorless to pale yellow, which is not visually appealing or appetizing. In sausages where the casings are intended to be consumed, and especially in the case of various scalding sausages such as bockwurst, an increasing consumer preference towards a strongly colored sausage has become evident. Thus, in some countries such as USA, France, and the UK, certain sausages such as hot dogs are marketed dyed red.

[16] Such a method of dying has several disadvantages. First, uniform dying can be obtained only with great difficulty. The casing shows regions which are dyed more weakly or not at all, depending on how close together the sausages were arranged and how adequately they were wetted. Furthermore, the dyestuff tends to both bleed out and also to migrate into the sausage material. An improved method for dying sausage casings would be desirable.

BRIEF SUMMARY OF THE INVENTION

[17] Transglutaminase (Tgase) enzymes are provided herein. The enzymes are variants of *Streptomyces mobaraensis* Tgase (SEQ ID NO:1). Some of the variants demonstrate improvement in transamidation activity that at least about 1.2-fold or at least about 2-fold, *e.g.*, about 1.2-fold to about 10-fold greater than the wild-type *Streptomyces mobaraensis* enzyme (at least about or greater than any of about 20%, 50%, 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500%, or 1000% improvement in enzymatic activity).

[18] It is desirable to have high specific activity of transglutaminase to allow for lower quantities of enzyme for cross-linking glutamine-donor substrates with amine substrates in the transamidation reaction, to allow for lower cost of product development. Additionally, it is beneficial to identify mutational variants of transglutaminase that exhibit higher initial rates to a deliver shorter reaction times. Such variants may promote rapid cross-linking for applications of use such as microbial control. One example is preservation, where rapid crosslinking of proteins, *e.g.*, cellular surface proteins, leads to superior microbial control, such as, but not limited to, faster or more effective microbial kill rate. In other embodiments, the transglutaminase variants may be use in applications such as bonding of dye molecules or pigments to collagen, keratin, elastin, and/or other structural or accessory skin, hair, or nail proteins or peptides, which may be found in a product formulation or on the surface of skin, hair, or nails, such as bonding agents for semi-permanent or permanent application of functional ingredients, color, dye, or pigment in long-lasting topically applied products.

[19] In one aspect, variants of the *Streptomyces mobaraensis* Tgase enzyme are provided. In some embodiments, the variant comprises or consists of : (i) S199G and S299V (SEQ ID NO:2); (ii) H289V and S299A (SEQ ID NO:3); (iii) N292M, H289T, and S299V (SEQ ID NO:4); (iv) N282E, H289V, and S299K (SEQ ID NO:5); (v) S284D, H289L, and S299K (SEQ ID NO:6); (vi) N282E, H289I, and S299K (SEQ ID NO:7); (vii) N282K, G283A, and

S299V (SEQ ID NO:8); (viii) N282Q, S284P, H289E, and S299V (SEQ ID NO:9); (ix) N282K, G283A, S284P, and S299V (SEQ ID NO:10); (x) N282R, G283A, S284E, H289Q, and S299V (SEQ ID NO:11); (xi) S199A and S299A (SEQ ID NO:12); (xii)S199A and S299E (SEQ ID NO:13); (xiii) S199A and S299K (SEQ ID NO:14); (xiv) S199A and S299V (SEQ ID NO:15); (xv) S199G and S299A (SEQ ID NO:16); (xvi)S199G and S299K (SEQ ID NO:17); (xvii) S2P, S199A, and S299A (SEQ ID NO:18); (xviii) S2P, S199A, and S299E (SEQ ID NO:19); (xix) S2P, S199A, and S299K (SEQ ID NO:20); (xx) S2P, S199A, and S299V (SEQ ID NO:21); (xxi) S2P, S199G, and S299A (SEQ ID NO:22); (xxii) S2P, S199G, and S299E (SEQ ID NO:23); (xxiii) S2P, S199G, and S299K (SEQ ID NO:24); (xxiv) S2P, S199G, and S299V (SEQ ID NO:25); (xxv) N292D, G283A, S284A, and S299V (SEQ ID NO:26); or (xxvi) S199G and S299E (SEQ ID NO:27), relative to the wild-type enzyme sequence (SEQ ID NO:1), or has the disclosed mutations and one or more conservative substitution within the remainder of the amino acid sequence, *e.g.*, having the disclosed mutations and at least about 90%, at least about 95%, or at least about 98% sequence identity to the disclosed amino acid sequence, or is a circular permutant of a variant having the disclosed mutations.

[20] Variants of the Tgase sequence depicted in SEQ ID NO:1 are provided that comprise or consist of substitutions of amino acid residue A or G at position 199 and amino acid residue A, E, K, or V at position 299, or has the disclosed mutations and one or more conservative substitution within the remainder of the amino acid sequence, *e.g.*, having the disclosed mutations and at least about 90%, at least about 95%, or at least about 98% sequence identity to the disclosed amino acid sequence, or is a circular permutant of a variant having the disclosed mutations.

[21] Variants of the Tgase sequence depicted in SEQ ID NO:1 are provided that comprise or consist of substitutions of amino acid residue C, D, E, F, H, I, K, L, M, N, P, Q, R, T, V, W, or Y at position 2, A or G at position 199 and amino acid residue A, E, K, or V at position 299, or has the disclosed mutations and one or more conservative substitution within the remainder of the amino acid sequence, *e.g.*, having the disclosed mutations and at least about 90%, at least about 95%, or at least about 98% sequence identity to the disclosed amino acid sequence, or is a circular permutant of a variants having the disclosed mutations.

[22] Variants of the Tgase sequence depicted in SEQ ID NO:1 are provided that comprise or consist of substitutions of amino acid residue E or M at position 282, amino acid residue I, T, or V at position 289, and amino acid residue K or V at position 299, or has the

disclosed mutations and one or more conservative substitution within the remainder of the amino acid sequence, *e.g.*, having the disclosed mutations and at least about 90%, at least about 95%, or at least about 98% sequence identity to the disclosed amino acid sequence, or is a circular permutant of a variant having the disclosed mutations.

[23] In some embodiments, a variant as described herein further comprises a C-terminal polyhistidine sequence. In some embodiments, a variant as described herein further comprises an N-terminal methionine residue. In some embodiments, the transglutaminase is a circular permutant of any of the amino acid sequences described herein, optionally further comprising an N-terminal methionine residue. In some embodiments, the transglutaminase enzyme further includes a pro-sequence. In some embodiments, a variant as disclosed herein, or a variant having disclosed mutations and at least about 90%, at least about 95%, or at least about 98% sequence identity to the disclosed amino acid sequence, or a variant that is a circular permutant of a variant having disclosed mutations, has at least about 2-fold greater transglutaminase enzyme activity than the wild type enzyme having the amino acid sequence depicted in SEQ ID NO:1.

[24] In another aspect, methods are provided for increasing the shelf life and/or eliminating odor of a product. The methods include incorporating a transglutaminase variant as described herein into the product in an amount effective to prevent or decrease growth of one or more microbe in comparison to an identical product that does not include the composition.

[25] In another aspect, products are provided that include a transglutaminase variant as described herein in an effective amount to increase the shelf life of the product, in comparison to an identical product that does not include the enzyme. For example, the product may be a personal care, household, industrial, food, pharmaceutical, cosmetic, healthcare, marine, paint, coating, energy, plastic, packaging, or agricultural product. In some embodiments, the product bar soap, liquid soap, hand sanitizer, preoperative skin disinfectant, cleansing wipes, disinfecting wipes, body wash, acne treatment products, antifungal diaper rash cream, antifungal skin cream, shampoo, conditioner, cosmetics deodorant, antimicrobial creams, body lotion, hand cream, topical cream, aftershave lotion, skin toner, mouth wash, toothpaste, or sunscreen lotion. In other embodiments, the product is a wound care product selected from wound healing ointments, creams, and lotions, wound coverings, burn wound cream, bandages, tape, or steri-strips.

[26] In another aspect, an enzyme composition is provided that includes: (i) a transglutaminase variant enzyme as described herein, a transglutaminase enzyme comprising or consisting of the amino acid sequence depicted in SEQ ID NO:1, a transglutaminase enzyme comprising or consisting of a ; and (ii) a substrate for the transglutaminase enzyme, such as a sunscreen molecule, functional ingredient, a pigment, or a dye molecule. In some embodiments, the sunscreen molecule, functional ingredient, pigment or dye molecule is conjugated to a molecule that includes a free amino group (Fig. 2). For example, the molecule that includes a free amino group may be derived from derivatization with a linker such as lysine, cadaverine, putrescine, hydrazine, adipic acid dihydrazide, sebacic dihydrazide, or hexamethylenediamine **(Fig. 4)**.

[27] In some embodiments, the molecule that includes a free amino group is derived from an aliphatic amine of formula $R(CH_2)_n(NH_2)$, wherein n is an integer between 1 and 30, or n is an integer between 5 and 10, and R is a functional ingredient. Previous attempts at this chemical functionality have focused on preparing carbamates at the phenolic oxygen, formula presented in **Fig. 4A**. (*See, e.g.,* U.S. Patent No. 5,490,980). However, these compounds have not been shown to be substrates for transglutaminase. The present disclosure has successfully demonstrated linkers via Schiff Base reactions at the carbonyl of oxybenzone or avobenzone with diamines separated by $(CH2)_n$ (Formula in **Figs. 4B-4C**) where n is an integer between 1 and 30, or between 5 and 10. The present disclosure has demonstrated that these amine containing sunscreen molecules are substrates for transglutaminase.

[28] In some embodiments, the sunscreen molecule, pigment, or dye molecule is conjugated to an amino acid, peptide, or protein with a free glutamine side chain **(Fig. 3)**. Cosmetic compositions that include the enzyme composition are also provided.

[29] In another aspect, methods are provided for bonding color to a material or protein of interest. The methods include contacting the material or protein of interest with a transglutaminase variant enzyme as described herein and a pigment or dye molecule, wherein the transglutaminase variant enzyme is present in an amount effective to covalently bind the pigment or dye molecule to the material or protein of interest **(Fig. 7** and **Fig. 8)**. In one embodiment, the protein of interest is a protein that is present in skin. For example, the protein that is present in skin may be collagen, keratin, and/or elastin.

[30] In another aspect, products are provided that include a transglutaminase variant enzyme as described herein in an effective amount to add a color molecule onto a protein or

a protein-, peptide-, or amino acid-containing material of interest when contacted with the product. In some embodiments, the product may be a personal care, cosmetic, leather, food, or agricultural product. Methods for modifying the color of a protein or material of interest are also provided, which include contacting the protein or material of interest with the product.

[31] In another aspect, compositions are provided that include a transglutaminase variant enzyme as described herein in combination with one or more antimicrobial enzyme, peptide, or protein, wherein the composition possesses a preservative, biocidal, antimicrobial, or virucidal activity. In some embodiments, the antimicrobial enzyme, peptide, or protein is lysozyme, chitinase, lipase, lysin, lysostaphin, glucanase, DNase, RNase, lactoferrin, glucose oxidase, peroxidase, lactoperoxidase, lactonase, acylase, dispersin B, a-amylase, cellulase, nisin, bacteriocin, siderophore, polymyxin, or defensin.

[32] In another aspect, a bacteriophage is provided, which includes a nucleic acid sequence that encodes a transglutaminase variant enzyme as described herein. In one embodiment, a composition that includes the bacteriophage provides antimicrobial activity. The composition may further include a pharmaceutically acceptable excipient.

BRIEF DESCRIPTION OF THE DRAWINGS

[33] The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate several embodiments of the disclosed methods and compositions and together with the description, serve to explain the principles of the disclosed methods and compositions.

[34] **Figure 1** schematically shows covalent addition of sunscreen conjugated to an aliphatic diamine $(CH2)_n(NH_2)_2$, where the sunscreen is oxybenzone or avobenzone, to an example protein, collagen, catalyzed by transglutaminase or lysyl oxidase to yield sunscreen-linker-collagen. The enzyme catalyzes cross-linking between an amine group on the sunscreen molecule and either a Gln sidechain (transglutaminase) or Lys sidechain (lysyl oxidase) on collagen. The enzyme can be encapsulated or unencapsulated.

[35] **Figure 2** schematically shows covalent addition of sunscreen-linker-NH_2 to an example protein, collagen, catalyzed by transglutaminase to yield sunscreen-linker-collagen. The enzyme catalyzes cross-linking between an amine group on the linker molecule and a Gln sidechain on collagen. The enzyme can be encapsulated or unencapsulated.

[36] **Figure 3** schematically shows covalent addition of sunscreen-linker-Gln to an example protein, collagen, catalyzed by transglutaminase to yield sunscreen-linker-collagen. The enzyme catalyzes cross-linking between the Gln group on the linker molecule and an available amine on a Lys residue on collagen. The enzyme can be encapsulated or unencapsulated.

[37] **Figures 4A-4C** illustrate formulas of sunscreen and linker molecules which may be combined to produce sunscreen-linker adducts. **Fig. 4A** shows a method for functionalizing oxybenzone via carbamate formation at the phenolic alcohol but not demonstrated to have reactivity with transglutaminase. **Fig. 4B** shows imine formation via the carbonyl of oxybenzone produces a transglutaminase compatible amine donor. **Fig. 4C** shows imine formation via the carbonyl of avobenzone produces a mixture of two transglutaminase compatible amine donors.

[38] **Figure 5** illustrates the reaction of 1,6-hexane diamine (HMDA) with oxybenzone followed by covalent addition of the oxybenzone-linker adduct to an example protein, Cbz-Gln-Gly, catalyzed by transglutaminase to yield sunscreen-linker-protein. The enzyme catalyzes cross-linking between a Gln sidechain on dipeptide and an available amine group on the sunscreen-linker. The enzyme can be encapsulated or unencapsulated.

[39] **Figure 6** shows characterization of the reaction product of Tgase catalyzed coupling of oxybenzone-linker to Cbz-Gln-Gly. This figure compares the amount of sunscreen-linker-protein product between wild-type Tgase, Tgase variant SEQ ID NO:28, and a negative control in the absence of Tgase. Tgase SEQ ID NO:28 contains 11-fold more product than wild-type Tgase. No sunscreen-linker-protein product is observed in the absence of Tgase.

[40] **Figure 7** schematically shows covalent addition of Dye-NH_2 (TAMRA-cadaverine) to Collagen catalyzed by transglutaminase to yield Dye-Collagen. The enzyme catalyzes cross-linking between an amine group on the dye molecule and a Gln sidechain on collagen. The enzyme can be encapsulated or unencapsulated.

[41] **Figure 8** schematically shows covalent addition of Dye-Gln to Collagen catalyzed by transglutaminase to yield Dye-Collagen. The enzyme catalyzes cross-linking between a Gln sidechain on the dye (ZQG-TAMRA) and a Lys, N-terminus, or other amine group on collagen. The enzyme can be encapsulated or unencapsulated.

[42] **Figure 9** shows an enzyme dose response of TAMRA-cadaverine (1.7 g/L) in solution covalently bound to a collagen plate catalyzed by Tgase variant SEQ ID NO:28 (0

to 0.33%w/v). Dye (TAMRA-cadaverine) was bound to the collagen plate by increasing amounts of Tgase variant SEQ ID NO:28 (left to right). As a negative control in the last column, Tgase was replaced by bovine serum albumin (BSA, 0.33% w/v). Bottom row shows the collagen-bound dye after removing residual, unbound dye through a washing process with phosphate buffered saline (PBS).

[43] **Figure 10** shows covalent addition of the short dye labeled peptide Cbz-Gln-Gly-TAMRA (2.5 g/L) in solution to a collagen plate catalyzed by Tgase variant SEQ ID NO:28. Column 1 illustrates the Dye (Cbz-Gln-Gly-TAMRA) in buffer added to the collagen plate in the presence or absence of Tgase. Column 2 illustrates removal of the excess reaction mixture after incubation. Column 3 represents a washing step with PBS to remove unbound Dye. Column 4 represents Dye bound to collagen after removal of excess unbound Dye. Dye was bound to the collagen in the presence of Tgase, and minimal or no binding was observed in the absence of Tgase.

DETAILED DESCRIPTION

[44] Tgase enzymes are provided herein. The enzymes are variants of Tgase (SEQ ID NO:1), and demonstrate improvements in transamidation activity that is at least about 1.2-fold, at least about 2-fold, at least about 10-fold, or at least about 11-fold greater than the wild-type *Streptomyces mobaraensis* enzyme.

[45] Also provided herein are applications of use for lysyl oxidase (LOX) enzymes, which may be utilized to form reactive aldehydes, for example, on cosmetically relevant proteins, such as collagen, to react with functional ingredients containing a nucleophile, such as a free $R\text{-}NH_2$.

[46] The cross-linking enzymes disclosed herein may be employed as novel biocidal agents for microbial control, with applications in healthcare products, personal care or cosmetic formulations, packaging (*e.g.*, food, cosmetic, and pharmaceuticals), textile and leather production, paints and coatings, and marine applications including water treatment and purification. In some embodiments, Tgase enzymes disclosed herein may be employed for permanently modifying proteins of interest, such as, but not limited to, keratin and collagen, with dyes or proteins. In some embodiments, the Tgase enzymes may be used as preservatives.

[47] Tgase enzymes that are mutant forms of the *Streptomyces mobaraensis* Tgase are disclosed herein. Specifically, the enzymes described herein are proteins obtained by

mutating at least two amino acids in the polypeptide sequence of the wild-type Tgase, or circular permutants thereof, and observing transglutaminase transamidation activity between a glutamine amino acid residue and an amine (or hydroxylamine) acceptor.

[48] Methods for recombinant expression of proteins with mutational substitutions have been described previously, for example, Molecular Cloning, A Laboratory Manual 4th ed., Cold Spring Harbor Press (1989), Current Protocols in Molecular Biology, John Wiley & Sons (1987-1997) and the like. Point mutant enzymes can be generated using site-directed mutagenesis or any other methods known in the art. Such methods can include, but are not limited to, using kits and commercially available reagents such as the Kunkel method, KLD method, or Gapped duplex method, and examples of the kit, for example, QuickChange ™ Site-Directed Mutagenesis Kit (Stratagene), GeneArt™ Site-Directed Mutagenesis System (Invitrogen), Q5®, Site-Directed Mutagenesis System (New England Biolabs), TaKaRa Site-Directed Mutagenesis System (Prime STAR® Mutagenesis Basal kit, or Muta-Direct ™ Site Directed Mutagenesis Kit (iNtRON), and the like.

[49] Compositions and methods are provided herein for covalent bonding of functional ingredients such as UV-blocking molecules and/or color producing molecules (such as dye and pigment molecules) to proteins and peptides, in applications of use such as cosmetic and/or sunscreen products, for sunscreen and/or color application and binding to skin and skin-derived proteins and peptides, such as collagen, keratin, and/or elastin, and for color application and binding to food products such as edible food casings.

I. DEFINITIONS

[50] 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.

[51] Numeric ranges provided herein are inclusive of the numbers defining the range.

[52] "A," "an," and "the" include plural references unless the context clearly dictates otherwise.

[53] The term "about" is used herein to mean plus or minus ten percent (10%) of a value. For example, "about 100" refers to any number between 90 and 110.

[54] The phrase "and/or," as used herein in the specification and in the claims, should be understood to mean "either or both" of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Other elements

may optionally be present other than the elements specifically identified by the "and/or" clause, whether related or unrelated to those elements specifically identified unless clearly indicated to the contrary. Thus, as a non-limiting example, a reference to "A and/or B," when used in conjunction with open-ended language such as "comprising" can refer, in one embodiment, to A without B (optionally including elements other than B); in another embodiment, to B without A (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

[55] The term "amino acid" refers to a molecule containing both an amine group and a carboxyl group that are bound to a carbon, which is designated the alpha-carbon. Suitable amino 30 acids include, without limitation, both the D- and L-isomers of the naturally occurring amino acids, as well as non-naturally occurring amino acids prepared by organic synthesis or other metabolic routes. In some embodiments, a single "amino acid" might have multiple sidechain moieties, as available per an extended aliphatic or aromatic backbone scaffold. Unless the context specifically indicates otherwise, the term amino acid, as used herein, is intended to include amino acid analogs.

[56] As used herein, "antimicrobial" refers to a substance that is intended to kill or inhibit the growth of bacteria, fungi, and viruses, for example, according to the Environmental Protection Agency (EPA).

[57] The term "base pair" or "bp" as used herein refers to a partnership (i.e., hydrogen bonded pairing) of adenine (A) with thymine (T), or of cytosine (C) with guanine (G) in a double stranded DNA molecule. In some embodiments, a base pair may include A paired with Uracil (U), for example, in a DNA/RNA duplex.

[58] A "bead" refers to a solid particle, comprising or consisting of a polymer as described herein.

[59] As used herein, "biocide" refers to a substance that kills microorganisms, for example, according to the Environmental Protection Agency (EPA).

[60] "Biodegradable" refers to a substance that is capable of decomposition by microbes (*e.g.*, bacteria) or other living organisms."

[61] "Cbz" is an abbreviation for benzyloxycarbonyl.

[62] The term "catalyst" refers to a chemical actor, such as a molecule or macromolecular structure, which accelerates the speed at which a chemical reaction occurs where a reactant or reactants is converted into a product or products, while the catalyst is not turned into a product itself, or otherwise changed or consumed at the completion of the

chemical reaction. After a catalyst participates in one chemical reaction, because it is unchanged, it may participate in further chemical reactions, acting on additional reactants to create additional products. To accelerate a chemical reaction a catalyst decreases the activation energy barrier across the reaction path allowing it to occur at a colder temperature, or faster at a given temperature. In this way, a more rapid approach of the system to chemical equilibrium may be achieved. Catalysts subsume enzymes, which are protein catalysts.

[63] A "circular permutant" refers to a protein that has a changed order of amino acids in its amino acid sequence in comparison with a reference sequence. The result is a protein structure with different connectivity, but overall similar three-dimensional (3D) shape in comparison to the reference protein. For example, an N-terminal fragment may be moved to the C-terminal end of the protein amino acid sequence.

[64] A "coloring agent" or "color producing molecule" refers to a molecule or compound that imparts a color to mammalian (*e.g.*, human) skin, hair, or nails. Coloring agents may include dyes and/or pigments. Nonlimiting examples of coloring agents include phenols, naphthols, and hydroxy azo derivatives. As discussed herein, a coloring agent may be modified to include an amino group (*e.g.*, an alkylamino, alkylhydrazine, alkylhydrazide, or alkoxyamine moiety), attached either directly to the coloring agent or indirectly via a linker that is attached to the coloring agent.

[65] As used herein, the term "composition" refers to a combination of two or more substances, for example, a combination that includes one or more cross-linking enzyme as described herein and one or more chromophores, such as UV-blocking molecules, dyes, pigments, or other color-producing molecules.

[66] A "conservative modification" or "conservative substitution" means, in respect of a polypeptide, the replacement of an amino acid therein with another amino acid having a similar side chain. Families of amino acids having similar side chains are known in the art. Such families include amino acids with basic side chains (lysine, arginine, histidine), acidic side chains (aspartic acid, glutamic acid), uncharged polar side chains (asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), branched side chains (threonine, valine, isoleucine), small side chains (glycine, alanine, serine), chain orientation changing side chains (glycine, proline) and aromatic side chains (tyrosine, phenylalanine, tryptophan).

[67] The term "derived from" encompasses the terms "originated from," "obtained from," "obtainable from," "isolated from," "purified from," and "created from," and generally indicates that one specified material finds its origin in another specified material or has features that can be described with reference to another specified material.

[68] The term "duplex" herein refers to a region of complementarity that exists between two polynucleotide sequences. The term "duplex region" refers to the region of sequence complementarity that exists between two oligonucleotides or two portions of a single oligonucleotide.

[69] A "dye" refers to a colored substance, *e.g.*, a natural or synthetic substance used to add a color to or change the color of something, which is typically a water-soluble organic molecule.

[70] "Effective amount" as used herein refers to an amount (*e.g.*, minimum inhibitory concentration (MIC)) of a preservative composition as disclosed herein that is sufficient to prevent or inhibit microbial growth. The preservative compositions described herein may be active against Gram positive bacteria, Gram negative bacteria, yeasts, and/or molds.

[71] "Emollients" are externally applied agents that soften or soothe skin, and are generally known in the art and listed in compendia, such as the "Handbook of Pharmaceutical Excipients", 4th Ed., Pharmaceutical Press, 2003.

[72] "Emulsifiers" are surface active substances which promote the suspension of one liquid in another and promote the formation of a stable mixture, or emulsion, of hydrophobic and hydrophilic substances, such as oil and water.

[73] "Encapsulate" or "encapsulation" as used herein refers to the entrapment or enclosure of an enzyme in a matrix. The matrix can be polymer alone or polymer with a cross-linking agent to covalently bind the enzyme to the polymer or to a porous polymeric network structure of the matrix or to a semi-permeable membrane coating containing the enzyme.

[74] As used herein, the term "expression" refers to the process by which a polypeptide is produced based on the nucleic acid sequence of a gene. The process includes both transcription and translation.

[75] "Functional ingredient" refers to an ingredient which performs or fulfills a specific function within a product to deliver a benefit to the consumer. For example, sunscreen molecules block UV radiation and pigment or dye molecules modify skin, hair, or nail color.

[76] A "gene" refers to a DNA segment that is involved in producing a polypeptide and includes regions preceding and following the coding regions as well as intervening sequences (introns) between individual coding segments (exons).

[77] "Household products" are products, other than personal care products, that would be used by individual consumers.

[78] "Hybridization" and "annealing" refer to a reaction in which one or more polynucleotides react to form a complex that is stabilized via hydrogen bonding between the bases of the nucleotide residues. The hydrogen bonding may occur by Watson Crick base pairing, Hoogstein binding, or in any other sequence specific manner. The complex may include two nucleic acid strands forming a duplex structure, three or more strands forming a multi-stranded complex, a single self-hybridizing strand, or any combination of these. A hybridization reaction may constitute a step in a more extensive process, such as the initiation of polymerase chain reaction (PCR), ligation reaction, sequencing reaction, or cleavage reaction, e.g., enzymatic cleavage of a polynucleotide by a ribozyme. A first nucleic acid sequence that can be stabilized via hydrogen bonding with the bases of the nucleotide residues of a second sequence is said to be "hybridizable" to the second sequence. In such a case, the second sequence can also be said to be hybridizable to the first sequence. The term "hybridized" refers to a polynucleotide in a complex that is stabilized via hydrogen bonding between the bases of the nucleotide residues.

[79] "Industrial products" refers to products that are used in industry.

[80] The terms "isolated," "purified," "separated," and "recovered" as used herein refer to a material (e.g., a protein, nucleic acid, or cell) that is removed from at least one component with which it is naturally associated, for example, at a concentration of at least 90% by weight, or at 15 least 95% by weight, or at least 98% by weight of the sample in which it is contained. For example, these terms may refer to a material which is substantially or essentially free from components which normally accompany it as found in its native state, such as, for example, an intact biological system. An isolated nucleic acid molecule includes a nucleic acid molecule contained in cells that ordinarily express the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

[81] A "mature" polypeptide, protein or enzyme refers to the activated form of a zymogen or proprotein following cleavage of its pro-sequence or in the absence of the pro-sequence. In some embodiments, the mature enzyme may be produced as a separate

polypeptide from the pro-sequence in order to eliminate a post-translational processing (activation) step.

[82] A "microbead" refers to a bead that is less than one millimeter in its largest dimension.

[83] The terms "microorganism" and "microbe" can include bacteria, protozoa, fungi, algae, amoebas, viruses, and molds life forms.

[84] The term "mutation" herein refers to a change introduced into a parental sequence, including, but not limited to, substitutions, insertions, and deletions (including truncations), thereby producing a "mutant." The consequences of a mutation include, but are not limited to, the creation of a new character, property, function, phenotype or trait not found in the protein encoded by the parental sequence.

[85] The term "nucleotide" herein refers to a monomeric unit of DNA or RNA consisting of a sugar moiety (pentose), a phosphate, and a nitrogenous heterocyclic base. The base is linked to the sugar moiety via the glycosidic carbon (1′ carbon of the pentose) and that combination of base and sugar is a nucleoside. When the nucleoside contains a phosphate group bonded to the 3′ 30 or 5′ position of the pentose it is referred to as a nucleotide. A sequence of polymeric operatively linked nucleotides is typically referred to herein as a "base sequence," "nucleotide sequence," "polynucleotide sequence," "oligonucleotide sequence", or nucleic acid or polynucleotide "strand," and is represented herein by a formula whose left to right orientation is in the conventional direction of 5′-terminus to 3′-terminus, referring to the terminal 5' phosphate group and the terminal 3' hydroxyl group at the "5'" and "3'" ends of the polymeric sequence, respectively.

[86] "Optional" or "optionally" means that the subsequently described event, circumstance, or material may or may not occur or be present, and that the description includes instances where the event, circumstance, or material occurs or is present and instances where it does not occur or is not present.

[87] As used herein in the specification and in the claims, "or" should be understood to have the same meaning as "and/or" as defined above. For example, when separating items in a list, "or" or "and/or" shall be interpreted as being inclusive, i.e., the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as "only one of" or "exactly one of," or, when used in the claims, "consisting of," will refer to the inclusion of exactly one element of a number or list of elements. In general, the term "or" as used

herein shall only be interpreted as indicating exclusive alternatives (i.e. "one or the other but not both") when preceded by terms of exclusivity, such as "either," "one of," "only one of," or "exactly one of." "Consisting essentially of," when used in the claims, shall have its ordinary meaning as used in the field of patent law.

[88] As used herein, "pathogen" refers to microorganisms (*e.g.*, bacteria, viruses, or parasites) that can cause disease in humans, animals, and/or plants.

[89] "Peptide" refers to a compound consisting of two or more amino acids linked in a chain, the carboxyl group of each acid being joined to the amino group of the next by a bond of the type R-OC-NH-R', for example, about 2 to about 50 amino acids.

[90] As used herein, "permanent" refers to the bonded application of either a sunscreen molecule or a color molecule, such as a dye or pigment molecule, on a surface or a protein molecule through a chemical covalent bond rather than a physical deposition or adsorption.

[91] A "pigment" refers to a material that provides color, *e.g.*, a material that changes the color of reflected or transmitted light as the result of wavelength-selective absorption, which is typically a water insoluble inorganic substance, such as, but not limited to, a mineral or a metal salt.

[92] The term "polymerase" herein refers to an enzyme that catalyzes the polymerization of nucleotides (i.e., the polymerase activity). The term polymerase encompasses DNA polymerases, RNA polymerases, and reverse transcriptases. A "DNA polymerase" catalyzes the polymerization of deoxyribonucleotides. An "RNA polymerase" catalyzes the polymerization of ribonucleotides. A "reverse transcriptase" catalyzes the polymerization of deoxyribonucleotides that are complementary to an RNA template.

[93] The terms "polynucleotide," "nucleic acid," and "oligonucleotide" are used interchangeably. They refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof. Polynucleotides may have any three-dimensional structure, and may perform any function, known or unknown, may be single-or multi-stranded (*e.g.*, single-stranded, double-stranded, triple-helical, etc.), and may contain deoxyribonucleotides, ribonucleotides, and/or analogs or modified forms of deoxyribonucleotides or ribonucleotides, including modified nucleotides or bases or their analogs. Because the genetic code is degenerate, more than one codon may be used to encode a particular amino acid, and the present invention encompasses polynucleotides which encode a particular amino acid sequence. Any type of modified nucleotide or nucleotide analog may be used, so long as the polynucleotide retains the desired

functionality under conditions of use, including modifications that increase nuclease resistance (*e.g.*, deoxy, 2'-O-Me,phosphorothioates, etc.). Labels may also be incorporated for purposes of detection or capture, for example, radioactive or nonradioactive labels or anchors, *e.g.*, biotin. The term polynucleotide also includes peptide nucleic acids (PNA). Polynucleotides may be naturally occurring or non-naturally occurring. Polynucleotides may contain RNA, DNA, or both, and/or modified forms and/or analogs thereof. A sequence of nucleotides may be interrupted by non-nucleotide components. One or more phosphodiester linkages may be replaced by alternative linking groups. These alternative linking groups include, but are not limited to, embodiments wherein phosphate is replaced by P(O)S ("thioate"), P(S)S ("dithioate"), (O)NR2 ("amidate"), P(O)R, P(O)OR', CO or CH2 ("formacetal"), in which each R or R' is independently H or substituted or unsubstituted alkyl (1-20 C) optionally containing an ether (--O--) linkage, aryl, alkenyl, cycloalkyl, cycloalkenyl or araldyl. The following are nonlimiting examples of polynucleotides: coding or non-coding regions of a gene or gene fragment, intergenic DNA, loci (locus) defined from linkage analysis, exons, introns, messenger RNA (mRNA), transfer RNA, ribosomal RNA, short interfering RNA (siRNA), short-hairpin RNA (shRNA), micro-RNA (miRNA), small nucleolar RNA, ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, adapters, and primers. A polynucleotide may include modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after polymerization, such as by conjugation with a labeling component, tag, reactive moiety, or binding partner. Polynucleotide sequences, when provided, are listed in the 5' to 3' direction, unless stated otherwise.

[94] As used herein, "polypeptide" refers to a composition comprised of amino acids and recognized as a protein by those of skill in the art. The conventional one-letter or three-letter code for amino acid residues is used herein. The terms "polypeptide" and "protein" are used interchangeably herein to refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation,

lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also, included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), as well as other modifications known in the art.

[95] As used herein, "preservative" is an agent added to a product as described herein to prevent (for some period of time) the growth of microorganisms, or the occurrence of undesirable chemical reactions (such as oxidation or odor generation), that spoil or deteriorate, including deterioration of one or more utility, of the product.

[96] As used herein, the term "product" is intended to refer to a preparation or composition that has a specific utility, such as a consumer packaged good. Examples include, but are not limited to, personal care products, household products, cosmetics, over the counter therapeutics, pharmaceutical preparations, paints, coatings, adhesives, and formulations for purchase by a consumer. In certain embodiments, a product includes a composition for bonding a sunscreen molecule (chromophore), dye, or pigment or modifying a protein with a sunscreen or color-producing molecule, such as a cosmetic or topically applied product.

[97] A "promoter" refers to a regulatory sequence that is involved in initiating transcription of a gene by RNA polymerase. A promoter may be an inducible promoter or a constitutive 5 promoter. An "inducible promoter" is a promoter that is active under environmental or developmental regulatory conditions.

[98] A "pro-sequence" refers to a polypeptide sequence within an expressed protein, *e.g.*, a zymogen or proprotein, such as transglutaminase, which is typically cleaved from the protein to produce an active protein, such as an enzyme. In some embodiments, a pro-sequence may be essential for correct folding of the protein. In some embodiments, cleavage of the pro-sequence results in transition of an inactive enzyme to active enzyme. In some embodiments, the pro-sequence may be cleaved at multiple positions within the proprotein sequence, which may result in improved enzyme activity or stability relative to the native mature enzyme sequence.

[99] The term "recombinant," refers to genetic material (i.e., nucleic acids, the polypeptides they encode, and vectors and cells comprising such polynucleotides) that has been modified to alter its sequence or expression characteristics, such as by mutating the coding sequence to produce an altered polypeptide, fusing the coding sequence to that of another gene, placing a gene under the control of a different promoter, expressing a gene in

a heterologous organism, expressing a gene at a decreased or elevated levels, expressing a gene conditionally or constitutively in manner different from its natural expression profile, and the like. Generally recombinant nucleic acids, polypeptides, and cells based thereon, have been manipulated such that they are not identical to related nucleic acids, polypeptides, and cells found in nature. A recombinant cell may also be referred to as "engineered."

[100] A "reversibly soluble polymer" refers to a polymer which can phase transition from a soluble to insoluble material in solution in response to controllable stimuli in the environment, such as, but not limited to, pH, temperature, or ionic strength. This transition process can be repeatably cycled between phases.

[101] "Shelf life" refers to the length of time for which an item (e.g., a product as described herein) remains usable, fit for consumption, or saleable.

[102] The phrases "substantially similar" and "substantially identical" in the context of at least two nucleic acids typically means that a polynucleotide includes a sequence that has at least about 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or even 99.5% sequence identity, in comparison with a reference (e.g., wild-type) polynucleotide or polypeptide. Sequence identity may be determined using known programs such as BLAST, ALIGN, and CLUSTAL using standard parameters. (See, e.g., Altshul et al. (1990) J. Mol. Biol. 215:403-410; Henikoff et al. (1989) Proc. Natl. Acad. Sci. 89:10915; Karin et al. (1993) Proc. Natl. Acad. Sci. 90:5873; and Higgins et al. (1988) Gene 73:237). 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. (1988) *Proc. Natl. Acad. Sci.* 85:2444-2448.) In some embodiments, substantially identical nucleic acid molecules hybridize to each other under stringent conditions (e.g., within a range of medium to high stringency). Nucleic acid "synthesis" herein refers to any in vitro method for making a new strand of polynucleotide or elongating an existing polynucleotide (i.e., DNA or RNA) in a template dependent manner. Synthesis, according to the invention, can include amplification, which increases the number of copies of a polynucleotide template sequence with the use of a polymerase. Polynucleotide synthesis (e.g., amplification) results in the incorporation of nucleotides into a polynucleotide (e.g., extension from a primer), thereby forming a new polynucleotide molecule complementary to the polynucleotide template. The formed polynucleotide molecule and its template can be used as templates to synthesize additional polynucleotide molecules. "DNA synthesis," as

used herein, includes, but is not limited to, polymerase chain reaction (PCR), and may include the use of labeled nucleotides, e.g., for probes and oligonucleotide primers, or for polynucleotide sequencing. "Under transcriptional control" is a term well understood in the art that indicates that transcription of a polynucleotide sequence depends on its being operably linked to an element which contributes to the initiation of, or promotes transcription.

[103] "Surfactants" are surface-active agents that lower surface tension and thereby increase the emulsifying, foaming, dispersing, spreading and wetting properties of a product.

[104] The term "sunscreen" refers to an organic compound or mixture of organic compounds that can protect and block human skin from ultraviolet A (UVA) and/or ultraviolet B (UVB) radiation. Nonlimiting examples of UV-absorbing sunscreen compounds include benzoates, oxybenzones, and cinnamic acid. Exemplary organic sunscreens include, but are not limited to, para-aminobenzoic acid, trolamine salicylate, cinoxate, dioxybenzone, ensulizole, homosalate, meradimate, octinoxate, octisalate, octocrylene, padimate O, sulisobenzone, oxybenzone, and avobenzone. Sunscreens may be approved by the Food and Drug Administration (FDA) or other international regulatory bodies for the purpose of blocking UVA and/or UVB radiation. Sunscreens may also be molecules which provide UVA and/or UVB protection, but which are not yet approved by the FDA or other international regulatory bodies for the purpose of blocking UVA and/or UVB radiation. Sunscreens may also be molecules with analogous structures to those approved by the FDA or other international regulatory bodies and which also provide UVA and/or UVB protection (sunscreen analogs). Sunscreens may also be modified by linkers and/or other small molecules and still provide UVA and/or UVB protection, either with the linker attached or after hydrolysis (sunscreen-linker adducts). A "sunscreen" herein may refer to a sunscreen molecule, a sunscreen analog, and/or a sunscreen-linker adduct. As discussed herein, a sunscreen molecule may be modified to include an amino group (*e.g.*, an alkylamino, alkylhydrazine, alkylhydrazide, or alkoxyamine moiety), attached either directly to the sunscreen molecule or indirectly via a linker (*e.g.*, as part of a linker) that is attached to the sunscreen molecule.

[105] "TAMRA" is an abbreviation for carboxytetramethylrhodamine.

[106] Related (and derivative) proteins encompass "variant" proteins. Variant proteins differ from another (*i.e.*, parental) protein and/or from one another by a small number of

amino acid residues. A variant may include one or more amino acid mutations (*e.g.*, amino acid deletion, insertion or substitution) as compared to the parental protein from which it is derived. Alternatively or additionally, variants may have a specified degree of sequence identity with a reference protein or nucleic acid, e.g., as determined using a sequence alignment tool, such as BLAST, ALIGN, and CLUSTAL (see, infra). For example, variant proteins or nucleic acid may have at least about 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or even 99.5% amino acid sequence identity with a reference sequence.

[107] A "zymogen" or "proenzyme" refers to an inactive precursor of an enzyme, which may be converted into an active enzyme by catalytic action, such as via proteolytic cleavage of a pro-sequence.

[108] Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. The methods and techniques of the present disclosure are generally performed according to conventional methods well-known in the art. Generally, nomenclatures used in connection with, and techniques of biochemistry, enzymology, molecular and cellular biology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well-known and commonly used in the art. The methods and techniques of the present disclosure are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated.

II. TRANSGLUTAMINASE VARIANTS

[109] Tgase enzymes are provided herein that are variants of the Ca^{2+}-independent microbial transglutaminase (Tgase) *Streptomyces mobaraensis*. Tgase variants with at least about 1.2-fold (20%) or 2-fold (100%) improvement in enzyme activity, versus the wild-type enzyme from Streptomyces mobaraensis (SEQ ID NO:1), are disclosed herein.

[110] The amino acid sequence of wild-type *S. mobaraensis* Tgase is provided in SEQ ID NO:1. Tgase variants as described herein may be obtained by mutating at least two amino acids in the polypeptide sequence of the wild-type Tgase, and observing transglutaminase transamidation activity between a glutamine amino acid residue and an amine (or

hydroxylamine) acceptor. Methods for recombinant expression of proteins with mutational substitutions have been described previously and are well known in the art, for example, Molecular Cloning, A Laboratory Manual 4th ed., Cold Spring Harbor Press (1989), Current Protocols in Molecular Biology, John Wiley & Sons (1987-1997) and the like. Combinations of point mutations can be generated using a number of methods including error-prone PCR, gene shuffling, molecular breeding, and the like.

[111] The amino acid sequences of Tgase variants, relative to the wild-type amino acid sequence set forth in SEQ ID NO:1, are disclosed in Table 3, along with enzymatic activity improvement, relative to the wild-type enzyme (SEQ ID NO:1).

[112] One Tgase variant of this invention, designated M2, has a double mutation (S199G and S299V), relative to the sequence of the wild-type *S. mobaraensis* Tgase (SEQ ID NO: 1). The amino acid sequence of variant M2 is shown in SEQ ID NO: 2.

[113] One Tgase variant of this invention, designated M3, has a double mutation (H289V and S299A), relative to the sequence of the wild-type *S. mobaraensis* Tgase. The amino acid sequence of variant M3 is shown in SEQ ID NO: 3.

[114] One Tgase variant of this invention, designated M4, has a triple mutation (N282M, H289T and S299V), relative to the sequence of the wild-type *S. mobaraensis* Tgase. The amino acid sequence of variant M4 is shown in SEQ ID NO: 4.

[115] One Tgase variant of this invention, designated M5, has a triple mutation (N282E, H289V, and S299K), relative to the sequence of the wild-type *S. mobaraensis* Tgase. The amino acid sequence of variant M5 is shown in SEQ ID NO: 5.

[116] One Tgase variant of this invention, designated M6, has a triple mutation (S284D, H289L, and S299K), relative to the sequence of the wild-type *S. mobaraensis* Tgase. The amino acid sequence of variant M6 is shown in SEQ ID NO: 6.

[117] One Tgase variant of this invention, designated M7, has a triple mutation (N282E, H289I, and S299K), relative to the sequence of the wild-type *S. mobaraensis* Tgase. The amino acid sequence of variant M7 is shown in SEQ ID NO: 7.

[118] One Tgase variant of this invention, designated M8, has a quadruple mutation (N282K, G283A, and S299V), relative to the sequence of the wild-type *S. mobaraensis* Tgase. The amino acid sequence of variant M8 is shown in SEQ ID NO: 8.

[119] One Tgase variant of this invention, designated M9, has a quadruple mutation (N282Q, S284P, H289E, and S299V), relative to the sequence of the wild-type *S. mobaraensis* Tgase. The amino acid sequence of variant M9 is shown in SEQ ID NO: 9.

[120] One Tgase variant of this invention, designated M10, has a quadruple mutation (N282K, G283A, S284P, and S299V), relative to the sequence of the wild-type *S. mobaraensis* Tgase. The amino acid sequence of variant M10 is shown in SEQ ID NO: 10.

[121] One Tgase variant of this invention, designated M11, has a five-mutation (N282R, G283A, S284E, H289Q, and S299V), relative to the sequence of the wild-type *S. mobaraensis* Tgase. The amino acid sequence of variant M11 is shown in SEQ ID NO: 11.

[122] One Tgase variant of this invention, designated M12, has a double mutation (S199A and S299A), relative to the sequence of the wild-type *S. mobaraensis* Tgase. The amino acid sequence of variant M12 is shown in SEQ ID NO: 12.

[123] One Tgase variant of this invention, designated M13, has a double mutation (S199A and S299E), relative to the sequence of the wild-type *S. mobaraensis* Tgase. The amino acid sequence of variant M13 is shown in SEQ ID NO: 13.

[124] One Tgase variant of this invention, designated M14, has a double mutation (S199A and S299K), relative to the sequence of the wild-type *S. mobaraensis* Tgase. The amino acid sequence of variant M14 is shown in SEQ ID NO: 14.

[125] One Tgase variant of this invention, designated M15, has a double mutation (S199A and S299V), relative to the sequence of the wild-type *S. mobaraensis* Tgase. The amino acid sequence of variant M15 is shown in SEQ ID NO: 15.

[126] One Tgase variant of this invention, designated M16, has a double mutation (S199G and S299A), relative to the sequence of the wild-type *S. mobaraensis* Tgase. The amino acid sequence of variant M16 is shown in SEQ ID NO: 16.

[127] One Tgase variant of this invention, designated M17, has a double mutation (S199G and S299K), relative to the sequence of the wild-type *S. mobaraensis* Tgase. The amino acid sequence of variant M17 is shown in SEQ ID NO: 17.

[128] One Tgase variant of this invention, designated M18, has a triple mutation (S2P, S199A, and S299A), relative to the sequence of the wild-type *S. mobaraensis* Tgase. The amino acid sequence of variant M18 is shown in SEQ ID NO: 18.

[129] One Tgase variant of this invention, designated M19, has a triple mutation (S2P, S199A, and S299E), relative to the sequence of the wild-type *S. mobaraensis* Tgase. The amino acid sequence of variant M19 is shown in SEQ ID NO: 19.

[130] One Tgase variant of this invention, designated M20, has a triple mutation (S2P, S199A, and S299K), relative to the sequence of the wild-type *S. mobaraensis* Tgase. The amino acid sequence of variant M20 is shown in SEQ ID NO: 20.

[131] One Tgase variant of this invention, designated M21, has a triple mutation (S2P, S199A, and S299V), relative to the sequence of the wild-type *S. mobaraensis* Tgase. The amino acid sequence of variant M21 is shown in SEQ ID NO: 21.

[132] One Tgase variant of this invention, designated M22, has a triple mutation (S2P, S199G, and S299A), relative to the sequence of the wild-type *S. mobaraensis* Tgase. The amino acid sequence of variant M22 is shown in SEQ ID NO: 22.

[133] One Tgase variant of this invention, designated M23, has a triple mutation (S2P, S199G, and S299E), relative to the sequence of the wild-type *S. mobaraensis* Tgase. The amino acid sequence of variant M23 is shown in SEQ ID NO: 23.

[134] One Tgase variant of this invention, designated M24, has a triple mutation (S2P, S199G, and S299K), relative to the sequence of the wild-type *S. mobaraensis* Tgase. The amino acid sequence of variant M24 is shown in SEQ ID NO: 24.

[135] One Tgase variant of this invention, designated M25, has a triple mutation (S2P, S199G, and S299V), relative to the sequence of the wild-type *S. mobaraensis* Tgase. The amino acid sequence of variant M25 is shown in SEQ ID NO: 25.

[136] One Tgase variant of this invention, designated M26, has a quadruple mutation (N282D, G283A, S284A, and S299V), relative to the sequence of the wild-type *S. mobaraensis* Tgase. The amino acid sequence of variant M26 is shown in SEQ ID NO: 26.

[137] One Tgase variant of this invention, designated M27, has a double mutation (S199G and S299E), relative to the sequence of the wild-type *S. mobaraensis* Tgase. The amino acid sequence of variant M27 is shown in SEQ ID NO: 27.

[138] Variants M2-M27 can have conservative substitutions thereto, provided their respective distinctive substitutions: (i) S199G/S299V, (ii) H289V/S299A, (iii) N292M/H289T/S299V, (iv) N282E/H289V/S299K, (v) S284D/H289L/S299K, (vi) N282E/H289I/S299K, (vii) N282K/G283A/S299V, (viii) N282Q/S284P/H289E/S299V, (ix) N282K/G283A/S284P/S299V, (x) N282R/G283A/S284E/H289Q/S299V, (xi) S199A/S299A, (xii)S199A/S299E, (xiii) S199A/S299K, (xiv) S199A/S299V, (xv) S199G/S299A, (xvi) S199G/S299K, (xvii) S2P/S199A/S299A, (xviii) S2P/S199A/S299E, (xix) S2P/S199A/S299K, (xx) S2P/S199A/S299V, (xxi) S2P/S199G/S299A, (xxii) S2P/S199G/S299E, (xxiii) S2P/S199G/S299K, (xxiv) S2P/S199G/S299V, (xxv) N292D/G283A/S284A/S299V, or (xxvi) S199G/S299E, respectively, are preserved. Such conservatively modified versions of variants M2-M27 are included in the scope of this invention. Plural conservative substitutions/modifications may be present. Conservatively

modified versions of variants M2-M27 may be at least about 90%, 95%, or 98% identical to their respective unmodified sequences and may have at least about 2-fold greater transglutaminase enzymatic activity than the wild-type transglutaminase (SEQ ID NO:1).

[139] Tgase variants disclosed herein (*e.g.*, M2-M27) may further comprise a polyhistidine peptide extension at their C-terminus, as exemplified with amino acid residues 334-339 of SEQ ID NO:29. The polyhistidine peptide is a useful tag for purification purposes and does not affect enzymatic activity. Typically, the polyhistidine peptide is 6-8 residues long.

[140] Tgase variants disclosed herein (*e.g.*, M2-M27) may further comprise a methionine residue at their N-terminus. The mature wild-type *Streptomyces mobaraensis* Tgase enzyme lacks the N-terminal methionine residue encoded by the gene sequence that encodes the enzyme. In some embodiments, the Tgase variant is expressed as a variant of the mature *Streptomyces mobaraensis* Tgase without an N-terminal methionine residue. In other embodiments, the Tgase is expressed as the mature Tgase with an additional N-terminal methionine residue, which may be provided by an expression vector from which the Tgase is expressed.

[141] Tgase variants herein may further include a pro-sequence. In some embodiments, the variant is expressed with a pro-sequence, either as part of the variant polypeptide sequence (*e.g.*, an additional amino acid sequence as an extension of an amino acid sequence described in Table 3) or as a separate polypeptide. In some embodiments, the mature variant polypeptide is expressed in the presence of a polypeptide Tgase pro-sequence. In one embodiment, a DNA sequence that encodes the pro-sequence and the DNA sequence that encodes the mature Tgase variant are expressed as discrete polypeptide sequences from the same DNA template. In another embodiment, the DNA sequence that encodes the mature polypeptide is expressed from a first DNA template, and the DNA sequence that encodes the pro-sequence is expressed from a separate second DNA template. In another embodiment, the pro-sequence is synthesized chemically and added to an expression system prior to, during, or after expression of the mature polypeptide. In one example, the Tgase variant may be expressed in a cell free expression system, as disclosed in PCT Application No. US20/49226, which is incorporated by reference herein in its entirety.

[142] In some embodiments, the Tgase variant is expressed, *e.g.*, expressed recombinantly, with a homologous pro-sequence, *i.e.,* the native pro-sequence for the Tgase enzyme, *i.e.*, the pro-sequence for the wild-type Tgase enzyme from the same organism. In other

embodiments, the Tgase variant is expressed, *e.g.*, expressed recombinantly, with a heterologous pro-sequence, *i.e.*, a pro-sequence for the same enzyme but from a different organism or a pro-sequence for a different enzyme from the same or different organism.

[143] In some embodiments, a Tgase variant may be a circular permutant of a Tgase variant described herein (*e.g.*, a variant described in Table 3). In some embodiments, the Tgase variant may be a circular permutant of a Tgase variant as described in Table 3, optionally further including an N-terminal methionine residue. The circular permutants may provide novel substrate specificities, product profiles, and reaction kinetics versus the parent enzyme, *i.e.*, the wild-type enzyme or a disclosed variant, *e.g.*, as depicted in Table 3. A circular permutant retains the same basic folding of the parent enzyme, but has the N-terminus in a different position, with the original N- and C-termini connected, optionally by a linking sequence. In a Tgase wild-type or variant circular permutant, the N-terminal residue of the wild-type or variant enzyme is positioned at a site in the protein other than the natural N-terminus.

III. ANTIMICROBIAL COMPOSITIONS

[144] Disclosed are compositions, *e.g.*, biocidal, preservative, antimicrobial, anti-bacterial, and anti-viral (virucidal) compositions that include one or more Tgase variant enzyme as described herein, such as any of the variants disclosed in Table 3, optionally with an N-terminal methionine residue, including circular permutants thereof, and optionally with a pro-sequence as described herein. Such a composition may be included in or with (*e.g.*, within or associated with) products to be preserved, *e.g.*, for microbial control. The Tgase variant enzyme may catalyze a reaction of amino acid residues on a protein, thereby effecting, for example, protein cross-linking or binding a molecule of interest to a protein. In some embodiments, the compositions include one or more Tgase variant enzyme, *e.g.*, comprising or consisting of one or more Tgase variant as disclosed herein, in an amount effective to inhibit microbial (*e.g.*, bacterial) growth, *e.g.*, inhibition of 80% to 100%, or any of at least about 80%, 85%, 90%, 95%, 98%, or 99% of microbial growth, in a product to be preserved.

[145] Preservatives are antimicrobial ingredients added to product formulations to maintain the microbiological safety of the products by inhibiting the growth of and reducing the amount of microbial contaminants. US Pharmacopeia has published protocols for acceptable microbial survival for preservatives in cosmetics and personal care products.

These tests include USP 51 (Antimicrobial Effectiveness Test) and USP 61 (Microbial Limits Test) (https://www.fda.gov/files/about%20fda/published/Pharmaceutical-Microbiology-Manual.pdf).

[146] The effectiveness of the preservative system disclosed herein is determined based on the MIC (minimum inhibitory concentration) against a variety of microbes, including, but not limited to, Gram positive bacteria, Gram negative bacteria, yeast and/or mold (*e.g. E. coli* DH10ß, *E. coli* ATCC 8739, *B. subtilis* BGSC 1A976, *C. albicans* ATCC 10231, and/or *A. brasiliensis* ATCC 16404). Minimum inhibitory concentrations (MICs) are defined as the lowest concentration of an antimicrobial that will inhibit the growth of a microorganism. Microbial growth may be determined, for example, by spectrophotometric methods (the optical density at 600 nm) or with a cell viability assay (BacTiter Glo, Promega).

[147] In some embodiments, the compositions include one or more additional biocidal enzymes, such as a cross-linking enzyme, oxidase, nuclease, hydrolase, protease, and/or lytic enzyme. In some embodiments, the composition further includes one or more biocidal chemical, such as, but not limited to, chitosan, polylysine, and/or quaternary ammonium compounds. Exemplary, but nonlimiting examples of biocidal enzymes, compositions, and formulations, and methods of use thereof, are disclosed in PCT/US20/21211, which is incorporated by reference herein in its entirety.

[148] Without wishing to be bound by theory, the use of a biocidal enzyme enhances the antimicrobial properties of a biocidal chemical by providing an additional mechanism of antimicrobial action. Chitosan, for example, ruptures the cell membrane and leads to spillage of the cell contents. The cross-linking Tgase enzyme can cross-link proteins vital for cell function both on the surface of the cell and within the cell. This combination of both materials together reduce the quantity of the materials needed and provide additional stability to the enzyme allowing for greater activity over time (less chitosan and less enzyme) and reduce the undesirable effects that may accompany the use of biocidal chitosan.

A. Biocidal proteins and peptides

[149] In some embodiments, the compositions include one or more antimicrobial peptides. Examples of antimicrobial peptides include, but are not limited to, nisin and pediocin.

[150] In some embodiments, the compositions include one or more antimicrobial proteins. Examples of antimicrobial proteins include, but are not limited to, casein.

[151] Nonlimiting examples of known biocidal enzymes and antimicrobial peptides, which may be included in combination with a Tgase variant enzyme as disclosed herein, are shown in Table 1. In some embodiments, a Tgase variant enzyme as described herein may be utilized in a biocidal, preservative, anti-bacterial, or anti-viral (virucidal) composition in combination with one or more of the antimicrobial enzymes, peptides, or proteins described in Table 1.

TABLE 1. Enzymes, Peptides and Proteins with Known Antimicrobial Properties

Mechanism	Enzyme	Description	Citation
Lytic	Lysozyme	Produced by animals as part of the innate immune system. Hydrolyzes the peptidoglycan subunits in the bacterial cell wall.	Ibrahim et al. (2001) *FEBS Letters* 506(1):27-32; Małaczewska et al. (2019) *BMC Vet. Res.* 15:318
	Chitinase	Secreted by soil bacteria including *Bacillus thuringiensis* to combat insects and fungi	Martínez-Zavala et al (2020) *Front. Microbiol.* 10:3032
	Lipase	Hydrolyzes extracellular lipids and polymers.	Prabhawathi et al. (2014) *PLoS One* 9(5)
	Lysin	Utilized by bacteriophages to hydrolyze the glycan component of bacterial cell wall	Hoops et al. (2008) *Appl. Environ. Microbiol.* 75:5, 1388-1394
	Lysostaphin	Metalloendopeptidase which cleaves the pentaglycine bridges found in cell wall peptidoglycan.	Kokai-Kun et al. (2003) *Antimicrob Agents Chermother* **47**(5):1589-1597
	Glucanase	Secreted by soil bacteria including *Bacillus* species to degrade the fungal cell wall. Has also been utilized as an algicide and for biofilm control.	Shafi et al. (2017) *Biotechnology & Biotechnological Equipment* 31:3 446-459
Nuclease	DNase	Hydrolyzes extracellular nucleic acids and viral genomic DNA.	Kaplan et al. (2012) *J. Antibiot. (Tokyo)* 65(2):73-77
	RNase	Hydrolyzes viral RNA.	Wirth (1992) WO1994000016A1
	Lactoferrin	Sequesters essential iron ions to prevent microbial growth. Also possesses nuclease activity and hydrolyzes biofilm polymers.	Niaz et al. (2019) *International Journal of Food Properties* 22:1 1626-1641
Oxidoreductase	Glucose Oxidase	Oxidizes glucose to D-glucono-δ-lactone and hydrogen peroxide.	Wong et al. (2008) *Appl Microbiol Biotechnol.* 78(6):927-938
	Peroxidase	Oxidizes inert substrates to form biocidal actives.	Ihalin et al. (2006) *Arch. Biochem. Biophys.* 445, 261-268
	Lactoperoxidase	Oxidizes inert substrates to form biocidal actives.	White et al. (1983) *Antimicrob Agents Chemother* **32**(2): 267-272
Quorum Quenching	Lactonase	Hydrolyzes quorum sensing lactones, preventing activation of biofilm- and	Schwab et al. (2019) *Front Microbiol.* 10:611

		pathogenesis-promoting pathways.	
	Acylase	Hydrolyzes quorum sensing lactones, preventing activation of biofilm- and pathogenesis-promoting pathways.	Vogel et al. (2020) *Front. Chem.* 8:54
Hydrolase	Dispersin B	Hydrolyzes biofilm polymers	Izano et al. (2007) *J Dent Res* 86(7):618-622
	α-amylase	Hydrolyzes extracellular polysaccharides.	Craigen et al. (2011) *Open Microbiol J.* 5: 21-31
	Cellulase	Hydrolyzes the cellulose component of biofilms and algal cell walls.	Loiselle et al. (2003) *Biofouling* 19(2):77-85
Antimicrobial Peptides	Nisin	Increases permeability of the microbial cell membrane.	Li et al. (2018) *Appl Environ Microbiol* 18(12)
	Bacteriocin	Modes of action include inhibition of cell wall synthesis and increasing cell membrane permeability.	Meade et al. (2020) *Antibiotics* 9(1):32
	Siderophore	Binds to and sequesters iron ions	Raaska et al. (1999) *J Indust Microbiol Biotechnol* 22, 27-32
	Polymyxin	Increases permeability of the microbial cell membrane.	Poirel et al. (2017) *Clin Microbiol Rev* 30:577-596
	Defensin	Increases permeability of the microbial cell membrane.	Gans (2003) *Nat Rev Immunol* **3**, 710-720

B. Biocidal chemicals

[152] In some embodiments, a Tgase variant as described herein may be formulated with one or more biocidal chemical, including, but not limited to chitosan, polylysine, or quaternary ammonium compounds, for example, for use as a biocidal, preservative, anti-bacterial, or anti-viral (virucidal) composition. Nonlimiting examples of biocidal chemicals are shown in Table 2.

TABLE 2. Examples of Biocidal Chemicals for Antimicrobial Applications

Classification	Chemical
Polymers	Chitosan
	N,N,N-trimethyl chitosan
	ε-poly-lysine
	Polyvinylbenzyl-dimethylbutyl ammonium chloride
	Polyvinylbenzyl trimethyl ammonium chloride
	Quaternary ammonium polyethyleneimine
	Quaternary amine compounds (QAC)
	Quaternary phosphonium modified epoxidized natural rubber
	Arginine-tryptophan-rich peptide
	Guanylated

	polymethacrylate
	Ammonium ethyl methacrylate homopolymers
	Metallo-terpyridine carboxymethyl cellulose
	Poly(*n*-vinylimidazole) modified silicone rubber
Quaternary Ammonium	Cocamidopropyl Betaine
	Myristamidopropyl-pg-dimonium Cl Phosphate
	Benzalkonium Chloride
	Quaternium-6
	Coco Betaine
Detergents	Sodium Lauryl Sulfate
	Dodecylbenzenesulfonic Acid
Chaotropic Agent	Polyamidopropyl biguanide
	Guanidinium chloride
Organic Acids	Lactic Acid
	Citric Acid
	Salicylic Acid
	Sorbic Acid
	Acetic Acid
	Dehydroacetic Acid
	Peracetic Acid
	Benzoic Acid
Phenols & Alcohols	Ethanol
	Isopropanol
	Dichlorobenzyl Alcohol
	Glycerol
	Caprylyl Glycol
	Ethylhexylglycerin
	Benzyl Alcohol
	2-Phenoxyethanol
Aldehydes & Aldehyde Releasers	Glutaraldehyde
	Formaldehyde
	Sodium Hydroxymethylglycerate
	DMDM Hydantoin
Base	Sodium Hydroxide
Oxidizers	Hydrogen Peroxide
Parabens	Methyl Paraben
	Ethyl Paraben
	Propyl Paraben
Misc	Natamycin
	Benzisothiazolinone
	Bronopol
	Sorbitan Caprylate
	Ethyl Lauroyl Arginate
	Methylisothiazolinone
	Cetylpyridinium Chloride
	Chlorphenesin
	Zinc Omamide
	Sodium Omamide
	N-(3-aminopropyl)-N-dodecylpropane-1,3-diamine

	Methylchloroisothiazolinone
	2,2-dibromo-3-nitrilopropionamide
	1-Octadecanaminium, N,N-dimethyl-N-[3-(trimethoxysilyl)propyl]-, chloride
	Saponin
	Sodium Benzoate

1. Quaternary Ammonium Compounds

[153] Quaternary ammonium compounds containing biopolymers, like chitosan and its more acetylated form chitin, are well known for their antimicrobial activity (Kong, et al. (2010) *Int. J. of Food Microbiol.* 144: 51-63). The antimicrobial activity of chitin, chitosan and their derivatives against different groups of microorganisms, such as bacteria, yeast, and fungi, is known.

[154] Quaternary ammonium compounds (non-limiting examples include, cetyl pyridinium chloride, benzethonium chloride, benzalkonium chloride, polyaminopropyl biguanide), have limited use for personal care industry due to specific incompatibilities with other cosmetic ingredients.

[155] Lonza's Geogard series of preservative blends avoids use of parabens in their new creations (Geogard 233S, Geogard 233S, Geogard 233S, Geogard 361) however, these antimicrobial compositions are based on cationic benzethonium chloride which gets deactivated by many anionic ingredients that form important part of topical personal care formulations.

2. Aldehydes & Aldehyde-Releasing Compounds

[156] Formaldehyde is classified as Category 3 CMR (carcinogenic, mutagenic and reproductive toxicity). However, it is interesting to note that a few antimicrobials that slowly release formaldehyde are still being used and being commercially manufactured. Due to the paucity of effective and well-accepted antimicrobials, the industry is forced to continue with the use of formaldehyde donors like DMDM hydantoin (CAS 6440-58-0), imidazolidinyl urea, and diazolidinyl urea (CAS 39236-46-9). The formaldehyde released by these substances is capable of reacting with several cosmetic ingredients via its very reactive aldehydic carbonyl functionality. For example, the only available and globally approved UV-A absorber, Avobenzone, reacts with formaldehyde that is released by

formaldehyde derivatives. This is a significant disadvantage for sunscreen formulations. Preservative blends, Clariant's Niapaguard PDU and Cognis's Elestab 305, ISP's Germaben II, Germaben H-E, exploit combinations of parabens with diazolidinyl urea. ISP's Germall Plus and Lonza's Glydant Plus utilize diazolidinyl urea along with iodopropynyl butyl carbamate (IPBC). McIntyre's Paragon series has DMDM hydantoin and other antimicrobials like paraben, phenoxy ethanol and IPBC. Symrise's Neo-Dragocide and Thor's Microcare IMP exploit synergy between parabens and imidazolidinyl urea.

3. Parabens

[157] Parabens are esters of p-hydroxy benzoic acid. Paraben compounds include in particular Methyl-paraben (CAS 99-76-3), Ethyl-paraben (CAS 120-47-8), Propyl-paraben (CAS 94-13-3), Butyl-paraben (CAS 94-26-8), Isopropyl-paraben (CAS 4191-73-5), and Benzyl-paraben (CAS 94-18-8). Clariant's 'Phenonip' is a blend of six antimicrobials out of which the five are parabens. The same company offers blends of only parabens as 'Nipastat' and 'Nipasept', Cognis's Elestab FL 15, Elestab 48, Elestab 50J, Elestab 305, Elestab 388, Elestab 3344, Elestab 4112, Elestab 4121, Elestab 4150 Lipo are all blends of antimicrobials with at least one paraben in them. Induchem's Uniphen P23, ISP's Germaben and LiquaPar series of blends contain several parabens. Galaxy Surfactants offers Galguard NK1 and Galguard NK2 blends that are based on four and five paraben blends respectively with phenoxy ethanol. Five blends by McIntyre/Rhodia from their 'Paragon' series have several parabens. Neolone MXP of Rohm and Haas has parabens with methyl isothiazolinone. Neo-Dragocide series of blends from Symrise has parabens. Euxyl K 300 of Schulke and Mayr has five parabens. Thor's Microcare PM4 and Microcare PM5 have four and five parabens respectively. Parabens are phenol derivatives; all phenolic antimicrobials have phenolic 'hydroxyl' group and that is a very reactive organic functionality with very acidic hydrogen with pKa of 10.

4. Halogenated Compounds

[158] Nalco's Merguard series (four blends) relies on halogenated molecules, methyl dirbromo glutaronitrile and 2-bromo-2-nitro-1,3-diol. Several blends of Euxyl series from Schulke and Mayr are based on chlorothiazolinones, methyl dibromo glutaronitrile, 2-bromo-2-nitro-1,3-diol and diazolidinyl urea. Microcare series from Thor employs parabens, 2-bromo-2-nitro-1,3-diol, iodopropynyl butylcarbamate (IPBC), imidazolidynyl urea, and diazolidinyl urea.

[159] The other examples of halogenated antimicrobials are chlorphenesin, and chlorhexidine. It is common knowledge that like phenolic compounds, the halogenated organic molecules exhibit significant levels of toxic effects. For example, IPBC has risk of thyroid hormonal disturbances due to its iodine content. It has not been allowed in Japan and in the EU is allowed only up to 0.02% in leave-on products. Similarly, the EU permits usage of methyl dirbromo glutaronitrile only up to 0.1% in rinse-off products only. Bronopol, 2-bromo-2-nitropropane-1,3-diol, is implicated in generation of carcinogenic nitrosoamines on interacting with some of the nitrogen containing cosmetic ingredients. The antimicrobial efficacy of methyl chloro isothiazolinone is so powerful that it is allowed only in rinse-off products at 15 ppm concentration. Chloromethyl isothiazolinone does have a very broad spectrum of anti-microbial activity, but the toxicity of such powerful anti-microbials is extremely high and hence cosmetic formulators do not prefer to use this kind of powerful antimicrobial in the cosmetics that remain on human skin for a long time. It is reasonable to expect that any strong bactericide at a low concentration (ppm level) is likely to be equally lethal to any other cells of a living organism, including human cells. This is the precise reason why in Japan chloromethyl isothiazolinone is not allowed for preservation if the product is going to come in contact with the mucous membrane.

[160] Halogenated compounds include 2,4-dichlorobenzyl-alcohol, Chloroxylenol (also known as 4-chloro-3,5-dimethyl-phenol, Bronopol (also known as 2-bromo-2-nitropropane-1,3-diol, iodopropynyl butyl carbamate.

C. Vector Delivery

[161] The compositions described herein may include vectors (*e.g.*, bacteriophage), for the delivery of genetic material encoding one or more biocidal enzyme(s) (*e.g.*, Tgase variant(s)) as described herein.

[162] As used herein, "bacteriophage" and "phage" are used interchangeably to refer to a bacteriophage isolate in which members of the isolate have substantially the same genetic makeup, such as sharing at least about any of 90%, 95%, 99%, 99.9% or more sequence identity in the genome. "Bacteriophage" or "phage" refers to the parent bacteriophage as well as the progeny or derivatives (such as genetically engineered versions) thereof. The bacteriophage can be a naturally occurring phage isolate, or an engineered phage, including vectors, or nucleic acids that encode at least all essential genes, or the full genome of a phage to carry out the life cycle of the phage inside a host bacterium.

IV. Compositions for Cross-linking Functional Molecules to Protein

[163] Disclosed are enzyme compositions that can be included in products to be used for long-lasting application of functional ingredients including UV-blocking sunscreens, and/or coloring agents, such as pigments or dyes. The disclosed compositions include one or more cross-linking enzyme, such as a Tgase enzyme (*e.g.*, a Tgase variant as disclosed herein) and/or a lysyl oxidase enzyme, for the purpose of reacting amino acid residues on skin protein or other protein-, peptide-, or amino acid-containing materials with a molecule of interest, such as a sunscreen, or color producing molecule, *e.g.*, a pigment or dye. In some embodiments, the composition includes any of the Tgase enzymes disclosed in SEQ ID NOs:1-29, and/or a lysyl oxidase enzyme. In some embodiments, the Tgase and/or lysyl oxidase enzyme(s) acts as a catalyst to crosslink the active or functional ingredient (*e.g.*, sunscreen molecule or coloring agent) to a protein or peptide, such as a protein or peptide of mammalian (*e.g.*, human) skin, hair, or nails. In some embodiments, the transglutaminase enzyme (e.g., Tgase variant enzyme) and/or lysyl oxidase enzyme(s) acts as a catalyst to crosslink an amino group (*e.g.*, an alkylamino, alkylhydrazine, alkylhydrazide, or alkoxyamine moiety) of the active or functional ingredient or of a linker with an amino acid (*e.g.*, side chain of glutamine or lysine residues) in skin, hair, or nail proteins or peptides.

[164] Examples of skin, hair, or nail proteins include but are not limited to collagen, keratin, elastin, and/or cornified cell envelope proteins including involucrin, loricrin, small proline-rich proteins, periplakin, envoplakin, and filaggrin. Examples of peptides include but are not limited to hydrolyzed collagen, hydrolyzed keratin, and/or hydrolyzed elastin. In some embodiments, the compositions include UV-blocking molecules such as sunscreens. In some embodiments, the compositions include coloring agents (color producing molecules), such as dye or pigment molecules. In some embodiments, the enzyme is immobilized on or encapsulated in a polymeric support. In some embodiments, the enzyme is a transglutaminase enzyme.

[165] In some embodiments, a composition described herein includes a pharmaceutically or cosmetically acceptable vehicle or carrier, *e.g.*, to act as a diluent or dispersant for the active or functional ingredient and the Tgase and/or lysyl oxidase enzyme(s), or cross-linked active or functional ingredient with proteins or peptides, in the composition, for example, to promote or facilitate distribution of the active or functional ingredient and the cross-linking enzyme, or cross-linked active or functional ingredient with proteins or peptides, when the composition is applied to the skin, hair, or nails of a subject. The

pharmaceutically or cosmetically acceptable vehicle or carrier may comprise or consist of water, and may include liquid or solid emollients, solvents, humectants, thickeners, and/or powders, and in some embodiments, may form about 10% to about 99.9%, or about 50% to about 99%, by weight of the composition. In some embodiments, the composition is in the form of an emulsion, which may contain an oil or oily material in an amount up to about 90%, or about 10% to about 80% by volume of the composition. In some embodiments, the composition includes one or more emulsifier and/or one or more surfactant.

[166] In some embodiments, the active or functional ingredient includes at least one alkyl-amino (-RNH_2), hydrazine, hydrazide, or hydroxylamine moiety, either directly on the active or functional ingredient, or indirectly on a linker attached (*e.g.*, covalently bound) thereto. For example, the alkyl (R) group may be an aliphatic hydrocarbon chain including 1 to 8 carbon atoms.

[167] In certain embodiments, a composition for application to mammalian (*e.g.*, human) skin, hair, or nails is provided, which includes: (a) an effective amount of at least one active or functional ingredient (such as, for example, a sunscreen molecule or coloring agent); and (b) one or more transglutaminase enzyme (*e.g.*, a Tgase variant as described herein or any of the Tgase enzymes disclosed in SEQ ID NOs:1-29, and/or a lysyl oxidase enzyme) in an amount effective to catalyze the crosslinking of the active or functional ingredient to a protein or peptide of mammalian (*e.g.*, human) skin, hair, or nails. In some embodiments, the composition is formulated for topical application to skin, hair, or nails of a mammalian (*e.g.*, human) individual, and in certain embodiments may contain: (c) a pharmaceutically or acceptable carrier in an amount effective to deliver the Tgase and/or lysyl oxidase enzyme(s) and the active or functional ingredient to the skin, hair, or nails of the individual. For example, the active or functional ingredient may include at least one alkylamino (-RNH_2), hydrazine, hydrazide, or hydroxylamine moiety, either directly on the active or functional ingredient, or indirectly on a linker attached (*e.g.*, covalently bound) thereto.

A. Color Molecules

[168] The compositions described herein may contain one or more color producing molecule, such as a dye or pigment molecule, for application and binding to a surface, such as binding to one or more protein on the surface of skin, such as collagen, keratin, and/or elastin, or binding to an edible casing for a food product, such as a sausage casing. Nonlimiting examples of color producing molecules are described in "Summary of Color Additives for Use in the United States in Foods, Drugs, Cosmetics, and Medical Devices,"

US Food and Drug Administration, https://www.fda.gov/industry/color-additive-inventories/summary-color-additives-use-united-states-foods-drugs-cosmetics-and-medical-devices.

B. Sunscreen Molecules and Linkers

[169] The compositions described herein may contain one or more UV-blocking molecule(s), such as a sunscreen molecule, for application and binding to a protein or peptide within the product formulation or on the surface of skin, such as collagen, keratin, elastin, hydrolyzed collagen, hydrolyzed keratin, and/or hydrolyzed elastin.

[170] Nonlimiting examples of sunscreen and/or sunscreen analog molecules include but are not limited to, para-aminobenzoic acid, trolamine salicylate, cinoxate, dioxybenzone, ensulizole, homosalate, meradimate, octinoxate, octisalate, octocrylene, padimate O, sulisobenzone, oxybenzone, avobenzone, and benzophenone hydrazone.

[171] In some embodiments, the sunscreen is functionalized with a linker molecule to provide a substrate handle for enzymatic binding to a protein or peptide. A non-limiting example of this functionalization may be accomplished through formation of a Schiff base between the sunscreen molecule and linker. A non-limiting example of this functionalization may be accomplished through formation of a carbamate linkage between the sunscreen molecule and linker. The linker may include an available amine for enzyme recognition in the form of a primary amine, hydrazine, hydrazide, or alkoxyamine moiety. The linker may also include a glutamine residue for enzyme recognition. The linker may consist of two functional chemical end groups linked by an aliphatic carbon chain of varying length for *in situ* formation of the sunscreen-linker molecule. Nonlimiting examples of linkers include cadaverine, putrescine, hydrazine, adipic acid dihydrazide, sebacic dihydrazide, and hexamethylenediamine.

[172] In some embodiments, the sunscreen-linker adduct is bound to a protein or peptide of interest and the sunscreen can be subsequently released by hydrolysis. In one embodiment, the sunscreen molecule is hydrolysable or otherwise releasable from the linker. In some embodiments, the sunscreen-linker adduct remains bound to a protein or peptide, *e.g.*, a protein or peptide present on skin, to provide UV-blocking protection.

C. Proteins and Peptides

[173] The compositions described herein may contain one or more proteins or peptides of interest for sunscreen, skin care, and/or cosmetic products or applications of use.

Nonlimiting examples of proteins and peptides of interest for skin care products and cosmetics are: collagen, hydrolyzed collagen, keratin, hydrolyzed keratin, elastin, hydrolyzed elastin, silk, hydrolyzed silk, silk fibroin peptide, acetyl hexapeptide-3, acetyl hexapeptide-8, acetyl tetrapeptide-5, acetyl tetrapeptide-9, acetylarginyltryptophyl diphenylglycine, copper tripeptide-1, CT-2, dipeptide-2, heptapeptide-7, hexanoyl dipeptide-3 norleucine acetate, hexapeptide-9, hexapeptide-11, manganese tripeptide-1, myristoyl hexapeptide-16, myristoyl hexapeptide-16, myristoyl pentapeptide-17, nonapeptide-1, palmitoyl dipeptide-5 diaminobutyroyl Hydroxythreonine, palmitoyl dipeptide-5 diaminohydroxybutyrate, palmitoyl hexapeptide-12, palmitoyl hexapeptide-14, palmitoyl hexapeptide-6, palmitoyl pentapeptide-4, palmitoyl tetrapeptide-7, palmitoyl tripeptide-1, palmitoyl tripeptide-3, palmitoyl tripeptide-38, pentapeptide-3, pentapeptide-18, sh-oligopeptide-1, sh-oligopeptide-2, sh-polypeptide-1, sh-polypeptide-11, sh-polypeptide-9, soybean peptide, tetrapeptide PKEK, tetrapeptide-21, tripeptide-1, tripeptide-10 citrulline, and modified hydrolysates of hemoglobin, rice, soy, wheat protein, corn, fibronectin, reticulin, serum protein, wheat gluten.

[174] The compositions described herein may contain one or more model peptides of interest. One non-limiting example of a model peptide of interest includes Cbz-Gln-Gly.

V. POLYMERIC SUPPORTS

[175] In certain embodiments, compositions described herein include a polymeric support. One or more Tgase enzyme (*e.g.*, Tgase variant enzyme) as described herein and/or lysyl oxidase enzyme is immobilized on the support, with or without a linker, or encapsulated within a polymeric support, such as a reversibly soluble polymer, including, but not limited to, chitosan, carboxymethylchitosan, or polylysine. Nonlimiting examples of polymeric supports include: chitin, chitosan, carboxymethylchitosan, oxidized cellulose, quaternary ammonium cellulose, alginates, pectin, and carboxycellulose. Examples of polymeric supports and immobilization or encapsulation of cross-linking enzymes therein are described in PCT/US20/21211, which is incorporated herein by reference in its entirety.

[176] In some embodiments, the Tgase (*e.g.*, Tgase variant enzyme as disclosed herein) and/or lysyl oxidase enzyme(s) are immobilized on particles, *e.g.*, chitosan particles, such as beads, *e.g.*, chitosan beads (*e.g.*, microbeads), or nanoparticles. For example , the beads (*e.g.*, microbeads) may be biodegradable. In some embodiments, the enzyme may be

immobilized by encapsulation with free monomers (*e.g.*, chitosan or carboxymethylchitosan monomers), for example, utilizing a linker.

[177] Chitosan is a linear aminopolysaccharide of glucosamine and N-acetylglucosamine units and is obtained by alkaline deacetylation of chitin extracted from the exoskeleton of crustaceans such as shrimps and crabs, as well from the cell walls of some fungi. Chitin is a linear polymer of (1→4)-linked 2-acetamido-2-deoxy-β-D-glucopyranose (GlcNAc; A-unit), which is insoluble in aqueous solvents. It also has many structural similarities with cellulose, such as conformation of the monomers and diequatorial glycosidic linkages. Chitosan may be considered as a family of linear binary copolymers of (1→4)-linked A-units and 2-amino-2-deoxy-β-D-glucopyranose (GlcN; D-unit). Carboxymethylchitosan (*e.g.*, of fungal origin), *e.g.*, N,O-carboxymethylchitosan, is > 80% substituted with carboxyl groups.

[178] Quaternary ammonium containing biopolymers, like chitosan and its more acetylated form chitin, are well known for their antimicrobial activity (Kong, et al. (2010) *Int. J. of Food Microbiol.* 144: 51-63). The antimicrobial activity of chitin, chitosan and their derivatives against different groups of microorganisms, such as bacteria, yeast, and fungi, is known.

[179] Chitin, chitosan, and other related polymers are excellent scaffolds to immobilize enzymes (Muzzarelli (1980) *Enzyme Microb. Technol.* 2:177-184). Tyrosinase has been immobilized on chitosan for dephenolization of industrial waste (Dinçer, et al. (2012) *Int. J. Biol. Macromol.* 50:815-820) and for optical detection of phenol compounds (Abdullah, et al. (2006) *Sensors and Actuators B: Chemical* 114:604-609). In these examples, the tyrosinase is either directly ligated to the chitosan support without a linker or using glutaraldehyde as a linker to immobilize the enzyme on chitosan. Additionally, tyrosinase-chitosan biocatalysts have been explored for the production of L-DOPA (Carvalho, et al., *Appl. Biochem. Biotechnol.* (2000) 84-86:791-800). Microbial transglutaminase has been immobilized on chitosan using glutaraldehyde as a linker for the purpose of deamidation of food proteins (Nonaka, et al. (1996) *Biosci, Biotechnol, and Biochem.* 60:532-533), using Chitopearl 3007, a microbead form of chitosan, for the polymer support, with glutaraldehyde as a linker. Examples of polymeric supports are provided in, for example, Nonaka, et al. (1996) *Biosci, Biotechnol, and Biochem.* 60:532-533 and Hayashi, T et al. (1991) *J Appl Polymer Sci* 42: 85-92, which is incorporated by reference herein in its entirety.

A. Linkers for Enzyme Immobilization on Polymeric Support

[180] In some embodiments, one or more Tgase enzyme (*e.g.*, Tgase variant enzyme as disclosed herein) and/or a lysyl oxidase enzyme is immobilized on a polymer, via a chemical linker, which covalently links the enzyme to the polymer. In some embodiments, the linker is an alkylene (*e.g.* methylene), a diimine (1,5-diimine), a diamine (1,5-diamine), dicarbonyl (e.g. 1,4-dicarbonyl), an amide bond, a polypeptide, an alkyl linker, or contains a phenyl group, a fused heterocycle, or an aromatic group.

[181] Examples of reagents which can be used to provide linkers include, but are not limited to: formaldehyde, glutaraldehyde, succinate anhydride, phenolic compounds, genipin, carbodiimide reagents, proteins or peptides (*e.g.*, zein, gelatin, collagen).

[182] In some embodiments the linking reagent is genepin, epichlorohydrin, formaldehyde, or glutaraldehyde.

[183] In some embodiments, the Tgase (e.g., Tgase variant enzyme) and/or lysyl oxidase enzyme(s) are covalently linked to a carrier (polymeric support), without the use of a linker.

VI. PRODUCTS

[184] In some embodiments, products disclosed herein include personal care products, household products, industrial food, pharmaceutical, cosmetic, healthcare, marine, paints, coatings, adhesives, energy, plastic, packaging, or agricultural products, optionally immobilized on or encapsulated in a polymeric support, which include an effective amount, for example, about 0.0001% w/v to about 5% w/v, of one or more Tgase variant enzyme as described herein, or a composition thereof as described herein, to act as an antimicrobial agent, *e.g.*, preservative, in the product.

[185] In some embodiments, products disclosed herein include cosmetics and personal care products which include compositions described herein, compositions that include one or more Tgase enzyme (*e.g.*, Tgase variant enzyme as described herein) and/or lysyl oxidase enzyme, optionally immobilized on or encapsulated in a polymeric support, and one or more active or functional ingredient which may include a sunscreen and/or color producing molecule, in an amount effective to bond a sunscreen molecule or color to a surface, such as covalently binding to one or more skin-derived protein or peptide either on the surface of skin or within the product formulation, *e.g.*, collagen, keratin, and/or elastin. In some embodiments, the product composition includes any of the Tgase enzymes disclosed in SEQ ID NOs:1-29, and/or a lysyl oxidase enzyme. In some embodiments, an effective amount of

the Tgase (*e.g.*, Tgase variant(s)) and/or lysyl oxidase is in a range of about 0.0001% to about 5% w/v, such as about 0.001% to about 1% or about 0.01% to 0.1%, by weight of the composition. In some embodiments, the active or functional ingredient (*e.g.*, sunscreen molecule or coloring agent) is present in the composition in an amount effective to provide a benefit, such as a UV protecting benefit or a cosmetic or aesthetic benefit, *e.g.*, to hair, skin, or nail proteins or peptides or to the hair, skin, or nails of an individual to whom the composition is topically applied. In some embodiments, the effective amount of the active or functional ingredient (*e.g.*, sunscreen molecule or coloring agent is in a range of about 0.1% to about 70%, such as about 1% to about 35%, by weight of the composition.

[186] In some embodiments, one or more Tgase variant as described herein is included in a personal care product, such as, but not limited to, bar soap, liquid soap (*e.g.*, hand soap), hand sanitizer (including rinse off and leave-on alcohol based and aqueous-based hand disinfectants), preoperative skin disinfectant, cleansing wipes, disinfecting wipes, body wash, acne treatment products, antifungal diaper rash cream, antifungal skin cream, shampoo, conditioner, cosmetics (including but not limited to liquid or powder foundation, liquid or solid eyeliner, mascara, cream eye shadow, tinted powder, "pancake" type powder to be used dry or moistened, make up removal products, etc.), deodorant, antimicrobial creams, body lotion, hand cream, topical cream, aftershave lotion, skin toner, mouth wash, toothpaste, sunscreen lotion, and baby products such as, but not limited to, cleansing wipes, baby shampoo, baby soap, and diaper cream. In some embodiments, one or more Tgase variant is included in a wound care item, such as, but not limited to, wound healing ointments, creams, and lotions, wound coverings, burn wound cream, bandages, tape, and steri-strips, and medical articles such as medical gowns, caps, face masks, and shoe-covers, surgical drops, etc. In some embodiments, one or more Tgase variant is included in an oral care product, such as mouth rinse, toothpaste, or dental floss coating, a veterinary or pet care product, a preservative composition, or a surface disinfectant, such as a disinfectant solution, spray or wipe.

[187] In some embodiments, one or more Tgase variant as described herein is incorporated into a household or industrial product, for example, as a preservative substance. For example, the Tgase variant(s) may be included in a household cleaner, such as concentrated a liquid cleaner or spray cleaner, cleaning wipes, dish washing liquid, dish washer detergent, spray-mop liquid, furniture polish, indoor paint, outdoor paint, dusting spray, laundry detergent, fabric softener, rug/fabric cleaner, window and glass cleaner, toilet bowl

cleaner, liquid/cream cleanser, etc. In some embodiments, one or more Tgase variant may be included in a food wash product, *e.g.*, designed to clean fruits and vegetables prior to consumption, packaging, and food coatings.

[188] Other products into which Tgase variants as described herein may be incorporated include, but are not limited to, food, pharmaceutical, cosmetic, healthcare, marine, paint, coating, energy (*e.g.*, fracking fluid), plastic, packaging, and agricultural products. In some embodiments, the Tgase variant may be incorporated into HVAC systems, cooling ponds, water purification systems, or may be used in an industrial application, such as, but not - limited to, pulp and paper processing.

[189] Products disclosed herein include cosmetics and personal care products which include a Tgase enzyme (*e.g.*, Tgase variant as described herein) and/or lysyl oxidase enzyme, or composition thereof as described herein, and one or more color producing molecule, in an amount effective to bond color to a surface, such as covalently binding to one or more protein of skin, *e.g.*, collagen, keratin, and/or elastin, or to a protein of a food product, such as an edible casing for a processed food product, *e.g.*, a sausage casing. In some embodiments, the product composition includes any of the Tgase enzymes disclosed in SEQ ID NOs:1-29, and/or a lysyl oxidase enzyme. In some embodiments, an effective amount of the Tgase enzyme (*e.g.*, Tgase variant enzyme) and/or lysyl oxidase enzyme is up to about 1% w/v.

[190] Products disclosed herein may include one or more Tgase variant enzyme(s), optionally immobilized on or encapsulated in a polymeric support, and one or more functional ingredients including a sunscreen and/or color producing molecule, in an amount effective to bond a sunscreen and/or color molecule to a surface. In some embodiments, the product covalently binds sunscreen molecules to skin-derived proteins, *e.g.* collagen, keratin, and/or elastin, found within the product formulation. In some embodiments, the product covalently binds sunscreen molecules to skin proteins, *e.g.* collagen, keratin, and/or elastin. In some embodiments, the product covalently binds color to skin proteins, *e.g.* collagen, keratin, and/or elastin. In some embodiments, the product contains the functional ingredient with a linker which is sufficient to react with the native enzymes on the skin's surface to crosslink to skin's proteins. In some embodiments, the product covalently binds color to a protein of a food product, such as an edible casing for a processed food product, *e.g.*, a sausage casing. In some embodiments, an effective amount of the cross-linking

enzyme (*e.g.*, a transglutaminase enzyme) is in a range of about 0.001 to about 20% w/v, such as about 0.01% to about 5% by weight of the composition

[191] In some embodiments, a product or composition which includes Tgase variant as described herein, further includes one or more additional enzymes selected from acyl transferases, alpha-amylases, beta-amylases, alpha-galactosidases, arabinosidases, aryl esterases, beta-galactosidases, carrageenases, catalases, cellobiohydrolases, cellulases, chondroitinases, cutinases, endo-beta-1,4-glucanases, endo-beta-mannases, esterases, exo-mannanases, galactanases, glucoamylases, hemicellulases, hyaluronidases, keratinases, laccases, lactases, ligninases, lipases, lipoxygenases, mannanases, oxidases, pectate lyases, pectin acetyl esterases, pectinases, pentosanases, peroxidases, phenoloxidases, phosphatases, phospholipases, phytases, polygalacturonases, beta-glucanases, tannases, xylan acetyl-esterases, xylanases, xyloglucanases, xylosidases, metalloproteases, serine proteases, or combinations thereof.

[192] In some embodiments, a Tgase variant enzyme, such as any of the variants disclosed in Table 3, optionally with an N-terminal methionine residue, including circular permutants thereof, and optionally with a pro-sequence as described herein, or a composition thereof as described herein, is included as an antimicrobial agent in any of the products disclosed herein at a concentration of any of at least about 0.0001% w/v, 0.0005% w/v, 0.001% w/v, 0.005% w/v, 0.01% w/v, 0.05% w/v, 0.1% w/v, 0.5% w/v, 1% w/v, 1.5% w/v, 2% w/v, 2.5% w/v, 3% w/v, 3.5% w/v, 4% w/v, 4.5% w/v, or 5% w/v. In some embodiments, the Tgase variant enzyme of composition thereof is included at a concentration of any of about 0.0001% w/v to about 0.0005% w/v, about 0.001% w/v to about 0.005% w/v, about 0.005% w/v to about 0.01% w/v, about 0.01% w/v to about 0.05% w/v, about 0.05% w/v to about 0.1% w/v, about 0.1% w/v to about 0.5% w/v, about 0.5% w/v to about 1% w/v, about 1% w/v to about 1.5% w/v, about 1.5% w/v to about 2% w/v, about 2% w/v to about 2.5% w/v, about 2.5% w/v to about 3% w/v, about 3% w/v to about 3.5% w/v, about 3.5% w/v to about 4% w/v, about 4% w/v to about 4.5% w/v, about 4.5% w/v to about 5% w/v, about 0.0001% w/v to about 0.001% w/v, about 0.001% w/v to about 0.01% w/v, about 0.01% w/v to about 0.1% w/v, about 0.1% w/v to about 1% w/v, about 1% w/v to about 2.5% w/v, about 2.5% w/v to about 5% w/v, or about 1% w/v to about 5% w/v.

[193] In some embodiments, products in which a Tgase variant enzyme or composition thereof as described herein is included as an antimicrobial agent do not include a petrochemically derived preservative substance, such as, but not limited to, parabens,

formaldehyde and formaldehyde releasers, isothiazolinones, phenoxyethanol, and/or organic acids (such as sodium benzoate). In some embodiments, a Tgase variant enzyme as described herein, alone or in combination with a biocidal chemical, *e.g.*, chitosan, is the only antimicrobial, *e.g.*, antibacterial or preservative, agent in the product. In some embodiments, a Tgase variant enzyme as described herein is included as an antimicrobial agent in combination with one or more additional antimicrobial agent(s), such as, but not limited to, one or more petrochemically derived preservative substance(s). In some embodiments, a Tgase variant enzyme as described herein is included as an antimicrobial agent in combination with one or more additional antimicrobial agent(s), such as, but not limited to, one or more petrochemically derived preservative substance(s).

[194] In some embodiments, preservative blends are compatible with products, stable towards oxidizing or reducing agents and to normal range of pH (4.5 to 8.0) of various products.

[195] Non-limiting examples of products in which the Tgase variants described herein may be incorporated are described in PCT Application No. PCT/US20/21211, and in U.S. Provisional Application No. 63/075,763, which are incorporated herein by reference in their entireties.

A. Personal care products

[196] A Tgase variant enzyme as described herein or composition thereof, *e.g.*, preservative composition, as described herein can be incorporated into any personal care product. Personal care products into which the disclosed Tgase variant enzymes compositions may be incorporated include, but are not limited to, bar soap, liquid soap (*e.g.*, hand soap), hand sanitizer (including rinse off and leave-on alcohol based and aqueous-based hand disinfectants), preoperative skin disinfectant, cleansing wipes, disinfecting wipes, body wash, acne treatment products, antifungal diaper rash cream, antifungal skin cream, shampoo, conditioner, cosmetics (including but not limited to liquid or powder foundation, liquid or solid eyeliner, mascara, cream eye shadow, tinted powder, "pancake" type powder to be used dry or moistened, make up removal products, etc.), deodorant, antimicrobial creams, body lotion, hand cream, topical cream, aftershave lotion, skin toner, mouth wash, toothpaste, sunscreen lotion, and baby products such as, but not limited to, cleansing wipes, baby shampoo, baby soap, and diaper cream. The present subject matter may also be applied to wound care items, such as, but not limited to, wound healing ointments, creams, and lotions, wound coverings, burn wound cream, bandages, tape, and

steri-strips, and medical articles such as medical gowns, caps, face masks, and shoe-covers, surgical drops, etc. Additional personal care products include, but are not limited to, oral products such as mouth rinse, toothpaste, dental floss coatings, veterinary and pet care products, preservative compositions, and surface disinfectants, including solutions, sprays or wipes.

[197] In general, a Tgase variant enzyme as disclosed herein can be incorporated into any suitable personal care product intended for use in modifying the appearance of skin, such as a cosmetic product (*e.g.*, lipstick, foundation, blush, or eye makeup). Cosmetic products into which the disclosed compositions may be incorporated include, but are not limited to, liquid or powder foundation, liquid or solid eyeliner, blush, eye shadow, tinted powder, "pancake" type powder to be used dry or moistened, lip color, or makeup setting sprays, etc. The disclosed compositions may also be incorporated into a bronzer or artificial tanning product. Additionally, the disclosed compositions may be incorporated into a sunscreen product, such as a chemical sunscreen, *e.g.*, to bind a sunscreen chromophore (such as, but not limited to, oxybenzone, avobenzone, octisalate, octocrylene, homosalate, or octinoxate, or a derivative thereof) to skin protein.

[198] In some embodiments, the personal care products that are protected from the microbial contamination by the disclosed enzymes and compositions can be of any type of such as emulsions, gels, serums, solutions, toners, lotions, creams, spray, gel, powder, stick and cleansers.

[199] The personal care product formulation typically includes a base formulation to which the enzyme composition of the present disclosure is added. The base formulation may contain numerous and different ingredients depending upon the end use application. The personal care product formulation, for instance, may contain solvents, surfactants, emulsifiers, consistency factors, conditioners, emollients, skin care ingredients, moisturizers, thickeners, lubricants, fillers, antioxidants, other preservatives, active ingredients, in particular dermatologically active ingredients, fragrances and the like, as well as mixtures thereof. Active ingredients as mentioned herein include, for example, anti-inflammatories, and optionally, anti-bacterials, antifungals and the like agents. In some embodiments, active ingredients suited for topical applications are included.

[200] In some embodiments, the personal care product does not contain any additional preservatives, such as a petrochemical derived preservative substance. In some embodiments, the personal care product includes one or more additional preservative

substance, such as a petrochemical derived preservative, in addition to the enzyme or enzyme/polymer composition described herein.

[201] In some embodiments, the personal care product does not include conventional anti-bacterial and/or antifungal "active agents" that are typically included in personal care products. Conventional anti-bacterials used in hand soap include: Cloflucarban, Fluorosalan, Hexachlorophene, Hexylresorcinol, Iodine complex (ammonium ether sulfate and polyoxyethylene sorbitan monolaurate), Iodine complex (phosphate ester of alkylaryloxy polyethylene glycol), Nonylphenoxypoly (ethyleneoxy) ethanoliodine, Poloxamer-iodine complex, Povidone, Undecoylium chloride iodine complex, Methylbenzethonium chloride, Phenol, Phenol 16, Secondary amyltricresols, Sodium oxychlorosene, Tribromsalan, Triclocarban, Triclosan, and Triple dye. Conventional antimicrobials used as preservatives in consumer product formulations include: parabens, formaldehyde and formaldehyde releasers, isothiazolinones, phenoxyethanol, and organic acids (such as sodium benzoate).

[202] In some embodiments, a Tgase variant enzyme as described herein, alone or in combination with (*e.g.*, blend) a biocidal chemical, including but not limited to, chitosan, is the only antibacterial, antifungal, antimicrobial, or preservative agent in the product. In some embodiments, the Tgase variant enzyme, alone or in combination (*e.g.*, blend) a biocidal chemical, such as but not limited to, chitosan, is combined with one or more additional preservative substance, such as one or more petrochemically derived preservative substance. In some embodiments, one or more biobased preservative (*i.e.*, Tgase variant enzyme or composition thereof as disclosed herein) is combined with one or more synthetic preservative (*e.g.*, petrochemical derived substance) and the preservative (*e.g.*, antimicrobial) effect achieved between the biobased and synthetic preservatives is additive or synergistic. In some embodiments, one or more biobased preservative (*i.e.*, Tgase variant enzyme or composition thereof as disclosed herein) is combined with one or more additional preservative substance, for example, a biocidal substance selected from polylysine, chitosan, benzoate, nisin, lysozyme, and chitosan, or any combination thereof, and the preservative (e.g., antimicrobial) effect achieved between the biobased preservative and the additional preservative substance(s) is additive or synergistic.

[203] In some embodiments, the personal care product may include emollients. Emollients include, without limitation, almond oil, castor oil, ceratonia extract, cetostearoyl alcohol, cetyl alcohol, cetyl esters wax, cholesterol, cottonseed oil, cyclomethicone, ethylene glycol

palmitostearate, glycerin, glycerin monostearate, glyceryl monooleate, isopropyl myristate, isopropyl palmitate, lanolin, lecithin, light mineral oil, medium-chain triglycerides, mineral oil and lanolin alcohols, petrolatum, petrolatum and lanolin alcohols, soybean oil, starch, stearyl alcohol, sunflower oil, xylitol and combinations thereof. In one embodiment, the emollients are ethylhexylstearate and ethylhexyl palmitate.

[204] Common emulsifiers are: metallic soaps, certain animal and vegetable oils, and various polar compounds. Suitable emulsifiers include acacia, anionic emulsifying wax, calcium stearate, carbomers, cetostearyl alcohol, cetyl alcohol, cholesterol, diethanolamine, ethylene glycol palmitostearate, glycerin monostearate, glyceryl monooleate, hydroxpropyl cellulose, hypromellose, lanolin, hydrous, lanolin alcohols, lecithin, medium-chain triglycerides, methylcellulose, mineral oil and lanolin alcohols, monobasic sodium phosphate, monoethanolamine, nonionic emulsifying wax, oleic acid, poloxamer, poloxamers, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, polyoxyethylene stearates, propylene glycol alginate, self-emulsifying glyceryl monostearate, sodium citrate dehydrate, sodium lauryl sulfate, sorbitan esters, stearic acid, sunflower oil, tragacanth, triethanolamine, xanthan gum and combinations thereof. In one embodiment, the emulsifier is glycerol stearate.

[205] Suitable non-ionic surfactants include emulsifying wax, glyceryl monooleate, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polysorbate, sorbitan esters, benzyl alcohol, benzyl benzoate, cyclodextrins, glycerin monostearate, poloxamer, povidone and combinations thereof. In one embodiment, the non-ionic surfactant is stearyl alcohol.

[206] Suitable antioxidants include, *e.g.*, sulfites (*e.g.*, sodium sulfite), tocopherol or derivates thereof, ascorbic acid or derivates thereof, citric acid, propyl gallate, chitosan glycolate, cysteine, N-acetyl cysteine plus zinc sulfate, thiosulfates (*e.g.* sodium thiosulfate), polyphenols glutathione, dithiothreitol (DTT), superoxide dismutase, catalase and the like.

[207] Chelators, such as ethylene diamine tetraacetic acid (EDTA), may also be included.

[208] Suitable thickeners include, *e.g.*, acrylates/steareth-20 methacrylate copolymer, carbomer, carboxymethyl starch, cera alba, dimethicone/vinyl dimethicone crosspolymer, propylene glycol alginate, hydroxyethylcellulose, hydroxypropyl methylcellulose, silica, silica dimethyl silylate, xanthan gum, and hydrogenated butylenes/ethylene/styrene copolymer.

[209] Suitable moisturizers include, *e.g.*, butylene glycol, cetyl alcohol, dimethicone, dimyristyl tartrate, glucose glycereth-26, glycerin, glyceryl stearate, hydrolyzed milk protein, lactic acid, lactose and other sugars, laureth-8, lecithin, octoxyglycerin, PEG- 12, PEG 135, PEG-150, PEG-20, PEG-8, pentylene glycol, hexylene glycol, phytantriol, poly quaternium-39 PPG-20 methyl glucose ether, propylene glycol, sodium hyaluronate, sodium lactate, sodium PCA, sorbitol, succinoglycan, synthetic beeswax, tri-C14-15 alkyl citrate, and starch.

[210] In general, an enzyme composition as disclosed herein (*e.g.*, a composition including one or more Tgase variant enzyme(s), optionally immobilized on or encapsulated in a polymeric support, and sunscreen or color producing molecule(s)), can be incorporated into any suitable personal care product intended for use in protecting the skin from UV exposure or in modifying the appearance of skin, such as a cosmetic product (*e.g.*, lipstick, foundation, blush, or eye makeup). Cosmetic products into which the disclosed compositions may be incorporated include, but are not limited to, liquid or powder foundation, liquid or solid eyeliner, blush, eye shadow, tinted powder, "pancake" type powder to be used dry or moistened, lip color, or makeup setting sprays, etc. The disclosed compositions may also be incorporated into a bronzer or artificial tanning product. Additionally, the disclosed compositions may be incorporated into a sunscreen product, such as a chemical sunscreen, *e.g.*, to bind a sunscreen chromophore (such as, but not limited to, oxybenzone, avobenzone, octisalate, octocrylene, homosalate, or octinoxate, or a derivative thereof) to skin protein.

B. Household/Industrial products

[211] Non-limiting embodiments of household/industrial products which may incorporate the disclosed Tgase variant enzymes or compositions thereof as disclosed herein as a preservative substance, either alone or in combination with one or more additional preservative substance, such as one or more petrochemically derived preservative substance, include, but are not limited to, householder cleaners, such as concentrated liquid cleaners and spray cleaners, cleaning wipes, dish washing liquid, dish washer detergent, spray-mop liquid, furniture polish, indoor paint, outdoor paint, dusting spray, laundry detergent, fabric softener, rug/fabric cleaner, window and glass cleaner, toilet bowl cleaner, liquid/cream cleanser, etc. In a particular embodiment, the compositions described herein may be used in a food wash product, *e.g.*, designed to clean fruits and vegetables prior to consumption. In some embodiments, one or more biobased preservative (*i.e.*, Tgase variant enzyme or

composition thereof as disclosed herein) is combined with one or more synthetic preservative (*e.g.*, petrochemically derived substance) and the preservative (*e.g.*, antimicrobial) effect achieved between the biobased and synthetic preservatives is additive or synergistic.

C. Leather

[212] In general, a Tgase enzyme, (*e.g.*, Tgase variant enzyme as described herein) and/or lysyl oxidase enzyme can be incorporated into any natural collagen containing product or used during leather processing to modify the leather such that color is covalently bound to one or more protein in leather, such as animal or non-animal derived collagen, keratin, silk, and/or elastin proteins.

D. Food products

[213] In general, a Tgase enzyme, (*e.g.*, Tgase variant enzyme as described herein) and/or lysyl oxidase enzyme can be incorporated into any food protein or used during food processing, to modify the color of food protein. Food products into which the Tgase and/or lysyl oxidase enzyme(s) may be incorporated include, but are not limited to, animal-derived products containing collagen or gelatin (hydrolyzed collagen). These include, but are not limited to, gelatin products, meat products or meat analogue products such as sausage casings, pork rinds, or any meat or marine product including the skin layer of the animal and/or collagen. In addition, the enzyme composition may be incorporated into non-animal derived collagen-containing products or any collagen-containing product.

E. Other products

[214] Other products into which the disclosed Tgase variant enzymes or compositions thereof as disclosed herein may be incorporated include, but are not limited to, food, pharmaceutical, cosmetic, healthcare, marine, paint, coating, adhesive, energy (*e.g.*, fracking fluid), plastic, packaging, and agricultural products. In some embodiments, the disclosed enzymes or enzyme-polymer compositions disclosed herein may be incorporated into HVAC systems, cooling ponds, water purification systems, or may be used in an industrial application, such as, but not limited to, pulp and paper processing.

[215] In some embodiments, a biocidal enzyme, *i.e.*, Tgase variant enzyme as disclosed herein, is combined with one or more additional preservative substance, such as one or more petrochemically derived preservative substance. In some embodiments, one or more biobased preservative (*i.e.*, Tgase variant enzyme or composition thereof as disclosed herein) is combined with one or more synthetic preservative (*e.g.*, petrochemically derived

substance) and the preservative (*e.g.*, antimicrobial) effect achieved between the biobased and synthetic preservatives is additive or synergistic.

VII. METHODS OF USE

[216] Methods are provided for use of the Tgase variants disclosed herein (including any of the variants disclosed in Table 3), optionally with an N-terminal methionine residue, including circular permutants thereof, and optionally with a pro-sequence as described herein) in various applications of use in which crosslinking of proteins or peptides is desired or beneficial.

[217] Tgase variants as described herein may be used in applications of use such as, but not limited to, preservative, antimicrobial, odor control, pharmaceutical, cosmetic, topical, industrial, energy, healthcare, or marine applications.

[218] The disclosed variants may be used as alternatives or in addition to conventional preservatives, such as, but not limited to, parabens, formaldehyde, and glutaraldehyde and conventional biocidal agents, including silver (used in wound care products), in various applications that require preservatives for example, personal care, household, industrial, food, pharmaceutical, cosmetic, healthcare, marine, paint, coating, energy, plastic, packaging, and agricultural products, or in any of the products or systems disclosed herein. The disclosed variants may be used as anti-microbial (*e.g.*, preservative) ingredients that inhibit the growth of potentially harmful bacteria, fungi, and/or other microbes, and accordingly, are added to the product to be preserved in an effective amount to inhibit bacterial, fungal, and/or microbial growth in these products. Nonlimiting examples of such applications of use are described, for example, in PCT/US20/21211, which is incorporated by reference herein in its entirety. The Tgase variants may be employed as antimicrobial agents with applications in healthcare products, personal care or cosmetic formulations, packaging (food, cosmetic, and pharmaceuticals), textile and leather production, paints and coatings, and marine applications including water treatment and purification.

[219] The Tgase variants may be employed for permanently modifying proteins of interest, such as, but not limited to, keratin and collagen, with functional ingredients, dyes, or proteins. Tgase enzymes (such as any of the disclosed variants) and/or lysyl oxidase enzymes may be incorporated into products to facilitate covalent bonding of color, dye, or pigment molecules to proteins or peptides. The methods include contacting a protein or material of interest with one or more Tgase enzyme (*e.g.*, Tgase variant as described herein)

and/or lysyl oxidase enzyme and one or more color-producing molecule, *e.g.*, a dye or pigment molecule. The Tgase and/or lysyl oxidase enzyme(s) are present in an amount that is sufficient (*i.e.*, effective) to covalently bind the color molecule(s) to the protein or material of interest. In some embodiments, the protein of interest is one or more protein present in skin, and the Tgase and/or lysyl oxidase enzyme(s) and/or color molecule(s) may be in the form of a cosmetic or personal care product. The protein present in skin may be collagen, keratin, and/or elastin. In some embodiments, the protein or material of interest is leather, a food product, or an agricultural product, or a protein of interest therein, and the Tgase enzyme(s) (*e.g.*, Tgase variant(s)) and/or lysyl oxidase) and color molecule(s) are in the form of a composition that is suitable for modifying or adding color to the leather, a food product, or an agricultural product, or a protein of interest therein.

A. Preservative methods

[220] A Tgase variant as described herein (*i.e.*, any of the variants disclosed in Table 3, optionally with an N-terminal methionine residue, including circular permutants thereof, and optionally with a pro-sequence as described herein) may be used as an alternative to or in addition to conventional preservatives, such as, but not limited to, parabens, formaldehyde, and glutaraldehyde and conventional biocidal agents, including silver (used in wound care products), in various applications that require preservatives for example, personal care, household, industrial, food, pharmaceutical, cosmetic, healthcare, marine, paint, coating, adhesive, energy, plastic, packaging, and agricultural products. A Tgase variant may be used as an antimicrobial (*e.g.*, preservative) ingredient that inhibits the growth of potentially harmful bacteria, fungi, and/or other microbes, and accordingly, is added to a product to be preserved in an effective amount to inhibit bacterial, fungal, and/or microbial growth in such a products. Nonlimiting examples of such applications of use are described, for example, in PCT/US20/21211, which is incorporated by reference herein in its entirety. In some embodiments, USP <51> passing criteria are achieved, *i.e.*, for Category 2 Products: Bacteria: No less than 2.0 log reduction from the initial calculated count at 14 days, and no increase from the 14 days' count at 28 days; for Yeast and Molds: No increase from the initial calculated count at 14 and 28 days. In some embodiments, the antimicrobial behavior of the enzymes and enzyme-biopolymer coformulations are characterized by MIC (minimum inhibitory concentration) against gram-positive and gram-negative bacteria as well as fungi, which results in reduction of microbial growth by

approximately 80 - 100%, or any of at least about 80%, 85%, 90%, 95%, 98%, or 99% of microbial growth.

[221] When combined with a product as described herein, *e.g.*, a personal care, household, industrial, food, pharmaceutical, cosmetic, healthcare, marine, paint, coating, adhesive, energy, plastic, packaging, or agricultural product, or in any of the products or systems disclosed herein, *e.g.*, in a formulation or incorporated into a product or system as a preservative, the composition may have effective broad spectrum preservation activity over a broad pH range.

[222] In some embodiments, the method includes adding a preservative composition as described herein (*e.g.*, a Tgase variant or a composition thereof as described herein) to a product or system, such as a personal care, household, industrial, food, pharmaceutical, cosmetic, healthcare, marine, paint, coating, adhesive, energy, plastic, packaging, or agricultural product, or in any of the products or systems disclosed herein, *e.g.*, in a formulation or incorporated into a product or system, wherein microbial growth is decreased and/or shelf life of the product is increased in comparison to an identical product that does not contain the preservative composition. In some embodiments, no other preservative is included in the product composition, such as, but not limited to formaldehyde and/or glutaraldehyde.

[223] In some embodiments, a method for increasing the shelf-life, integrity, or microbial free (*e.g.*, bacterial and/or fungal free) status of a product composition, such as a personal care, household or industrial product is provided, wherein the method includes incorporating an effective amount of a preservative composition as described herein into the product (*e.g.*, personal care, household or industrial product). In some embodiments, the effective amount may be an amount, referred to as the MIC (minimum inhibitory concentration), which results in reduction of microbial growth by approximately 80 - 100%, or any of at least about 80%, 85%, 90%, 95%, 98%, or 99% reduction of microbial growth as described herein.

[224] In some embodiments of the methods or compositions described herein, the Tgase variant enzyme may be included at a concentration of about .0001% w/v to about 1% w/v, .0001% w/v to about .01% w/v, about .0001% w/v to about 2.5% w/v, about .0001% w/v to about 5% w/v, about .0001% w/v to about .001% w/v, about .001% w/v to about .01% w/v, about .01% w/v to about .1% w/v, 0.01% w/v to about 5% w/v, or any of at least about 0.01% w/v, 0.05% w/v, 0.1% w/v, 0.5% w/v, 1% w/v, 1.5% w/v, 2% w/v, 2.5% w/v, 3%

w/v, 3.5% w/v, 4% w/v, 4.5% w/v, or 5% w/v, or any of about 0.01% w/v to about 0.05% w/v, about 0.1% w/v to about 0.5% w/v, about 1% w/v to about 1.5% w/v, about 1.5% w/v to about 2% w/v, about 2% w/v to about 2.5% w/v, about 2.5% w/v to about 3% w/v, about 3% w/v to about 3.5% w/v, about 3.5% w/v to about 4% w/v, about 4% w/v to about 4.5% w/v, about 4.5% w/v to about 5% w/v, about 0.01% w/v to about 0.1% w/v, about 0.1% w/v to about 1% w/v, about 1% to about 5% w/v, about 0.05% w/v to about 0.5% w/v, about 0.5% w/v to about 5% w/v, about 1% w/v to about 2.5% w/v, or about 2.5% w/v to about 5% w/v.

[225] Non-limiting examples of personal care products to which the preservative methods may be applied, utilizing the disclosed Tgase variants and compositions thereof, include bar soap, liquid soap (*e.g.*, hand soap), hand sanitizer (including rinse off and leave-on alcohol based and aqueous-based hand disinfectants), preoperative skin disinfectant, cleansing wipes, disinfecting wipes, body wash, acne treatment products, antifungal diaper rash cream, antifungal skin cream, shampoo, conditioner, cosmetics (including but not limited to liquid or powder foundation, liquid or solid eyeliner, mascara, cream eye shadow, tinted powder, "pancake" type powder to be used dry or moistened, make up removal products etc.) deodorant, antimicrobial creams, body lotion, hand cream, topical cream, aftershave lotion, skin toner, mouth wash, toothpaste, sunscreen lotion, and baby products such as, but not limited to, cleansing wipes, baby shampoo, baby soap, and diaper cream. The present subject matter may also be applied to wound care items, such as, but not limited to, wound healing ointments, creams, and lotions, wound coverings, burn wound cream, bandages, tape, and steri-strips, and medical articles such as medical gowns, caps, face masks, and shoe-covers, surgical drops, etc. Additional products include but are not limited to oral products such as mouth rinse, toothpaste, and dental floss coatings, veterinary and pet care products, preservative compositions, and surface disinfectants including solutions, sprays or wipes.

[226] Non-limiting examples of household/industrial products to which the preservative methods may be applied, utilizing the disclosed Tgase variants and compositions thereof, include householder cleaners such as concentrated liquid cleaners and spray cleaners, cleaning wipes, dish washing liquid, dish washer detergent, spray-mop liquid, furniture polish, indoor paint, outdoor paint, dusting spray, laundry detergent, fabric softener, rug/fabric cleaner, window and glass cleaner, toilet bowl cleaner, liquid/cream cleanser, etc. In a particular embodiment, the preservative methods of the present subject matter may be

used in a food wash product, designed to clean fruits and vegetables prior to consumption, packaging, and food coatings.

B. Protein Modification Methods

[227] In some embodiments, one or more Tgase enzyme, (*e.g.*, Tgase variant as disclosed herein) and/or lysyl oxidase enzyme may be included in a product to be used for long-lasting application of functional ingredients including UV-blocking sunscreens, and/or coloring agents, such as pigments or dyes. In some embodiments, any of the Tgase enzymes disclosed in SEQ ID NOs:1-29, and/or a lysyl oxidase enzyme is included in the product composition. For example, the Tgase (*e.g.*, Tgase variant) and/or lysyl oxidase enzyme(s) may be used in a composition for delivery of an active or functional ingredient to mammalian (*e.g.*, human) skin, hair, or nails, such as, but not limited to, permanent (covalent) color modification of the surface of hair fibers. In some embodiments, Tgase (*e.g.*, Tgase variant) and/or lysyl oxidase enzyme(s) may be incorporated in a product to be applied topically and which bonds to the skin of an individual, such as a UV-blocking (sunscreen) product, or a cosmetic product. In some embodiments, the Tgase (*e.g.*, Tgase variant) and/or lysyl oxidase enzyme(s) may be used to provide permanent application of color to the skin of an animal such as in leather processing. In some embodiments, the Tgase (*e.g.*, Tgase variant) and/or lysyl oxidase enzyme(s) may be used to provide a permanent application of color in food processing.

[228] Methods are provided herein for modifying or adding color to a protein or material of interest. The methods include contacting a protein, peptide, or material of interest with one or more Tgase (*e.g.*, Tgase variant as described herein) and/or lysyl oxidase enzyme(s) and one or more functional ingredient including a sunscreen and/or color-producing molecule, *e.g.*, a dye or pigment molecule. The Tgase (*e.g.*, Tgase variant) and/or lysyl oxidase enzyme(s) are present in an amount that is sufficient (*i.e.*, effective) to covalently bind the sunscreen and/or color molecule(s) to the protein, peptide, or material of interest.

[229] In some embodiments, the protein of interest is one or more protein present in skin, and the Tgase (*e.g.*, Tgase variant) and/or lysyl oxidase enzyme(s) and sunscreen(s) and/or color molecule(s) may be in the form of a cosmetic or personal care product. The protein present in skin may be collagen, keratin, and/or elastin.

[230] In some embodiments, the material of interest is one or more protein or peptide derived from skin, and the Tgase (*e.g.*, Tgase variant) and/or lysyl oxidase enzyme(s) and/or sunscreen(s) and/or color molecule(s) may be in the form of a cosmetic or personal

care product. The protein present in the product formulation may be collagen, keratin, and/or elastin. The peptide present in the product formulation may be hydrolyzed collagen, hydrolyzed keratin, and/or hydrolyzed elastin.

[231] In some embodiments, the protein or material of interest is leather, a food product, or an agricultural product, or a protein of interest therein, and the Tgase (*e.g.*, Tgase variant) and/or lysyl oxidase enzyme(s) and/or color molecule(s) are in the form of a composition that is suitable for modifying or adding color to the leather, a food product, or an agricultural product, or a protein of interest therein.

[232] In some embodiments, a method is provided for delivering an active or functional ingredient (such as a sunscreen molecule or coloring agent) to proteins or peptides of mammalian (*e.g.*, human) skin, hair, or nails. For example, the method may include application of a composition as described herein to proteins or peptides of mammalian (*e.g.*, human) skin, hair, or nails or topical application of the composition to skin, hair, or nails of a mammalian (*e.g.*, human) individual.

[233] In some embodiments, the method includes contacting proteins and/or peptides of mammalian (*e.g.*, human) skin, hair, or nails, with a composition that includes: (a) an effective amount of at least one active or functional ingredient (such as, for example, a sunscreen molecule or coloring agent); and (b) one or more Tgase (*e.g.*, Tgase variant) and/or lysyl oxidase enzyme(s) in an amount effective to catalyze the crosslinking of the active or functional ingredient to a protein or peptide of mammalian (*e.g.*, human) skin, hair, or nails. In some embodiments, the method includes topical application of the composition to the skin, hair, or nails of a mammalian (*e.g.*, human) individual, and in certain embodiments the composition may contain: (c) a pharmaceutically or acceptable carrier in an amount effective to deliver the Tgase variant enzyme and the active or functional ingredient to the skin, hair, or nails of the individual.

[234] For example, the active or functional ingredient may include at least one alkylamino (-RNH_2), hydrazine, hydrazide, or hydroxylamine moiety, either directly on the active or functional ingredient, or indirectly on a linker attached (*e.g.*, covalently bound) thereto, and the method includes catalysis by the transglutaminase enzyme of crosslinking (*e.g.*, formation of covalent bonds) between the amino groups of the active or functional ingredient and amino groups (*e.g.*, amino groups on glutamine and/or lysine amino acid residue side chains) in proteins or peptides of skin, hair, or nails.

[235] The following examples are intended to illustrate, but not limit, the invention.

EXAMPLES

Example 1. Tgase vector construction and mutagenesis

Cell Free Protein Synthesis (CFPS) vectors

[236] The genes coding for the pro-sequence and mature Tgase were codon optimized for expression in *E. coli* based on the published amino acid sequence (Kanaji, et al. (1993) *J. Biol. Chem.* 268(16):11565-11572), synthesized, and cloned onto pUC19-derived expression vectors as described in PCT/US20/49226.

E. coli vector

[237] The genes coding for the pro-sequence and mature Tgase were codon optimized for expression in E. coli based on the published amino acid sequence (Kanaji, et al. (1993) J. *Biol. Chem.* 268(16):11565-11572) and synthesized as described in PCT/US20/49226. The DNA was cloned onto the pET9a vector under control of the T7 promoter for expression in *E. coli*.

Creation of Tgase variants

[238] Mutations were introduced into the mature Tgase gene using site directed mutagenesis methods known in the art.

Example 2. Expression of Tgase variants using cell-free protein synthesis

[239] Pro-sequence and mature Tgase variants were expressed simultaneously in a commercially available cell-free protein synthesis kit following the manufacturer's instructions as described in PCT/US20/49226.

Example 3. Expression of Tgase variants in *E. coli*

[240] Single colonies of *E. coli* BL21(DE3) harboring the Tgase expression plasmid were picked and used to inoculate 1 mL of LB in 96 deep well plates. Starter cultures were grown overnight at 37 °C, 400 rpm. The following morning, 100 µL of starter culture was used to inoculate 1 mL of media and incubated at 30 °C, 400 rpm. After 6-8 hours, IPTG was added to a final concentration of 0.1 mM and the temperature was reduced to 20 °C. Growth continued overnight and cultures were lysed and stored at -80 °C.

Example 4. Measurement of Tgase specific activity

[241] Tgase specific activity was measured in the examples herein using a colorimetric hydroxamate activity assay (Folk and Cole (1965) *J Biol Chemistry* 240(7):2951-2960). Briefly, the hydroxamate assay uses N-carbobenzoxy-L-glutaminylglycine (Z-Gln-Gly or CBZ-Gln-Gly) as the amine acceptor substrate and hydroxylamine as an amine donor. In the presence of transglutaminase, the hydroxylamine is incorporated to form Z-glutamylhydroxamate-glycine, which develops a colored complex with iron (III), detectable at 525 nm after incubation at 37 °C for 5-60 minutes. The calibration was performed using L-glutamic acid γ -monohydroxamate (Millipore Sigma) as standard. One unit of Tgase is defined as the amount of enzyme, determined using a commercially available ELISA kit (Zedira E021) following the manufacturer's protocol, that catalyzes formation of 1μmol of the peptide derivative of γ-glutamylhydroxylamine per minute.

Example 5. Protein Functionalization Assays

[242] Protein functionalization by Tgase variants was determined by one of two methods: (1) the fluorogenic labeling of casein using monodansylcadaverine; and (2) covalently locking a fluorescent substrate onto a collagen plate. All results were normalized to Tgase concentration using ELISA.

Fluorogenic casein-labeling assay

[243] Initial rates of the active mutants for protein substrates were determined by measuring the increase in fluorescence over time associated with Tgase-catalyzed labeling of casein with dansylcadaverine (*e.g.*, a commercially available kit, Fluorogenic Activity Assay Kit, Zedira T036).

Fluorescent collagen-labeling assay

[244] Amine donor dye. TAMRA-cadaverine (1.7 g/L) was covalently bound to a collagen plate (Corning BioCoat Collagen I Multiwell Plates) in the presence of increasing amounts of Tgase, SEQ ID NO:28. Tgase variant (0-0.33% w/v) in phosphate buffered saline (PBS) at pH 7.4, total volume of 60 μL. A negative control containing bovine serum albumin (BSA, 0.33% w/v) in the absence of Tgase was run in parallel. The plate was incubated for 16 h at 37 °C.

[245] A scheme of the reaction is illustrated in **Fig. 7**. The results are shown in **Fig. 9**. The wells are shown pre-color removal (top row) and post-wash with PBS (bottom row).

[246] Glutamine donor dye. Cbz-Gln-Gly-TAMRA (2.5 g/L) was bonded to a collagen plate (Corning BioCoat Collagen I Multiwell Plates) in the presence of Tgase with the amino acid sequence depicted in SEQ ID NO:28 (0.05-0.3% w/v) in PBS at pH 7.4. A negative control containing BSA (0.33% w/v) and a negative control in the absence of BSA or Tgase were run in parallel. The plate was incubated for 16 h at 37 ºC. Following incubation, the plates were washed with PBS to remove any residual, unbound dye.

[247] A scheme of the reaction is illustrated in **Fig. 8**. The results are shown in **Fig. 10**. The wells are shown pre-color removal and post-wash with PBS.

Example 6. Antimicrobial Activity Assays

Minimum Inhibitory Concentration (MIC) assay

[248] Yeast or bacterial starter cultures were grown at 30 °C - 37 °C overnight. The following day, the cell density of the saturated cultures was calculated using OD_{600} and cultures were diluted to 10^5-10^8 cells per mL. Cultures (100 µL) were made from the dilute starters in 96 well plates. Mutant or wild-type Tgase was added to each culture at 0.0001-1 weight percent. The cultures were grown overnight at 30 °C - 37 °C and growth curves were measured by a BioTek Synergy Plate Reader. The following day, a cell viability assay such as BacTiter Glo (Promega following manufacturer's protocols) was used to assess cell survival rate following challenge with Tgase. A decrease in luminescence indicates a decrease in cell viability. Results are shown in Table 3.

Cell Growth (OD measurement)

[249] *E. coli* was cultured in the presence of wild-type Tgase or Tgase variants at concentrations ranging from 1 mg/L – 250 mg/L. Lower optical density (OD) measurements correlated to improvements in antibacterial efficacy relative to wild-type Tgase.

Table 3. Activity Assay Results for Tgase Variants

Variant Name	SEQ. ID. NO.	Mutations (Relative to SEQ. ID. NO. 1)	Specific Activity	Protein Functionalization Activity	Antimicrobial Activity
M2	2	S199G S299V	++	++	N/A
M3	3	H289V S299A	++	++	N/A
M4	4	N282M H289T S299V	++	N/A	N/A
M5	5	N282E H289V S299K	++	++	+
M6	6	S284D H289L S299K	++	N/A	N/A
M7	7	N282E H289I S299K	++	+++	+++
M8	8	N282K G283A S299V	+	+	+
M9	9	N282Q S284P H289E S299V	++	N/A	N/A

M10	10	N282K G283A S284P S299V	++	-	+++
M11	11	N282R G283A S284E H289Q S299V	+++	++	+
M12	12	S199A S299A	++	-	+++
M13	13	S199A S299E	++	++	N/A
M14	14	S199A S299K	++	++	+
M15	15	S199A S299V	+++	+++	+++
M16	16	S199G S299A	++	++	N/A
M17	17	S199G S299K	++	N/A	N/A
M18	18	S2P S199A S299A	+	N/A	N/A
M19	19	S2P S199A S299E	++	++	N/A
M20	20	S2P S199A S299K	++	++	+
M21	21	S2P S199A S299V	++	++	+
M22	22	S2P S199G S299A	++	+	+
M23	23	S2P S199G S299E	+	N/A	N/A
M24	24	S2P S199G S299K	++	++	+
M25	25	S2P S199G S299V	+++	+++	++
M26	26	N282D G283A S284A S299V	++	++	N/A
M27	27	S199G S299E	+++	++	+
M28	28	S2P	+	++	+

Variants of *Streptomyces mobaraensis* Tgase and improvements to activity relative to wild-type *S. mobaraensis* Tgase. Numbering of amino acid positions is in reference to the mature *S. mobaraensis* Tgase amino acid sequence depicted in SEQ ID NO:1. A "-" indicates a reduction in activity. A "+" indicates an improvement between 1.2- and 2-fold. A "++" indicates an improvement between 2- and 5-fold. A "+++" indicates an improvement greater than 5-fold.

Example 7. Formation of Sunscreen-Linker

[250] Oxybenzone (0.25 mol), toluene (100 mL), and glacial acetic acid (1 drop) were charged in a 250 mL three neck flask fitted with a mechanical agitator, thermocouple, and dean stark trap. Molten 1,6-hexanediamine (0.25 mol) was added to the flask and the reaction was refluxed overnight to remove water. The reaction was monitored by HPLC. The resulting oxybenzone-imine was isolated by rotary evaporation under reduced pressure and further dried under vacuum to deliver the imine product, 2-(((6-aminohexyl)imino)(phenyl)methyl)-5-methoxyphenol, as a yellow solid in nearly quantitative yield (m/z 327.3). The scheme is shown in **Fig. 5**.

Example 8. Covalent addition of sunscreen-linker to protein

[251] The imine product in Example 7 (1.6 g/L) and Cbz-Gln-Gly dipeptide (1.65 g/L) were dissolved in 0.1M Tris-HCl pH 8.0. To aid in dissolution of the imine, 10% 1:1 dichloromethane in dimethyl sulfoxide was employed. Tgase (0.01 wt%) was added to the

suspension and the reaction was incubated at 37 °C overnight with constant agitation. Tgase catalyzed, covalent addition of the dipeptide to the imine was confirmed by LCMS (m/z 647.25). In the absence of Tgase, no covalent addition was observed. The scheme is shown in **Fig. 5** and results are shown in **Fig. 6**. Tgase variant, SEQ ID NO:28 demonstrates 11-fold improvement in activity relative to wild-type Tgase. No product was observed in the absence of Tgase (negative control).

Amino Acid Sequences

SEQ ID NO:1 Wild-Type Tgase from *Streptomyces mobaraensis*
DSDDRVTPPAEPLDRMPDPYRPSYGRAETVVNNYIRKWQQVYSHRDGRKQQMTEEQREWLSYGCVGVTWVNSGQ
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SEQ ID NO:6 S284D H289L S299K
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SEQ ID NO:7 N282E H289I S299K
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SEQ ID NO:10 N282K G283A S284P S299V
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SEQ ID NO:12 S199A S299A
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NLKKELANGNDALRNEDARSPFYSALRNTPSFKERNGGNHDPSRMKAVIYAKHFWSGQDRSSSADKRKYGDPDA
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NWAEGYSDFDRGAYVITFIPKSWNTAPDKVKQGWP

SEQ ID NO:13 S199A S299E
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NWVEGYSDFDRGAYVITFIPKSWNTAPDKVKQGWP

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SEQ ID NO:17 S199G S299K
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DPDDRVTPPAEPLDRMPDPYRPSYGRAETVVNNYIRKWQQVYSHRDGRKQQMTEEQREWLSYGCVGVTWVNSGQ
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NLKKELANGNDALRNEDARSPFYSALRNTPSFKERNGGNHDPSRMKAVIYGKHFWSGQDRSSSADKRKYGDPDA
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SEQ ID NO:23 S2P S199G S299E
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NLKKELANGNDALRNEDARSPFYSALRNTPSFKERNGGNHDPSRMKAVIYGKHFWSGQDRSSSADKRKYGDPDA
FRPAPGTGLVDMSRDRNIPRSPTSPGEGFVNFDYGWFGAQTEADADKTVWTHGNHYHAPNGSLGAMHVYESKFR
NWEEGYSDFDRGAYVITFIPKSWNTAPDKVKQGWP

SEQ ID NO:28 S2P
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NLKKELANGNDALRNEDARSPFYSALRNTPSFKERNGGNHDPSRMKAVIYSKHFWSGQDRSSSADKRKYGDPDA
FRPAPGTGLVDMSRDRNIPRSPTSPGEGFVNFDYGWFGAQTEADADKTVWTHGNHYHAPNGSLGAMHVYESKFR
NWSEGYSDFDRGAYVITFIPKSWNTAPDKVKQGWP

SEQ ID NO:29 Wild-Type Tgase with polyhistidine tag.
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NLKKELANGNDALRNEDARSPFYSALRNTPSFKERNGGNHDPSRMKAVIYSKHFWSGQDRSSSADKRKYGDPDA
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NWSEGYSDFDRGAYVITFIPKSWNTAPDKVKQGWPLEHHHHHH

CLAIMS

We claim:

1. A variant of the transglutaminase enzyme amino acid sequence depicted in SEQ ID NO:1, comprising substitutions of amino acid residue A or G at position 199 and amino acid residue A, E, K, or V at position 299.

2. The transglutaminase variant of claim 1, further comprising an N-terminal methionine residue, a C-terminal histidine polyhistidine sequence, and/or a pro-sequence.

3. The transglutaminase variant of claim 1, further comprising substitution of amino acid residue C, D, E, F, H, I, K, L, M, N, P, Q, R, T, V, W, Y at position 2.

4. The transglutaminase variant of claim 3, further comprising an N-terminal methionine residue, a C-terminal histidine polyhistidine sequence, and/or a pro-sequence.

5. A variant of the transglutaminase enzyme amino acid sequence depicted in SEQ ID NO:1, comprising substitutions of amino acid residue D, E, K,M, R, or Q at position 282, amino acid residue E, I, L, T, Q, or V at position 289, and amino acid residue A, E, K, or V at position 299.

6. The transglutaminase variant of claim 5, further comprising an N-terminal methionine residue, a C-terminal histidine polyhistidine sequence, and/or a pro-sequence.

7. A variant of the transglutaminase enzyme amino acid sequence depicted in SEQ ID NO:1, comprising the amino acid sequence depicted in any of SEQ ID NOs: 2-27.

8. The transglutaminase variant of claim 7, further comprising an N-terminal methionine residue, a C-terminal histidine polyhistidine sequence, and/or a pro-sequence.

9. A transglutaminase enzyme, comprising a sequence having at least about 90% sequence identity to any of the amino acid sequences depicted in SEQ ID NOs:2-27 or a circular permutant of any of the amino acid sequences depicted in SEQ ID NOs: 2-27, wherein the variant comprises at least about 2-fold greater transglutaminase enzymatic activity than the transglutaminase enzyme comprising the amino acid sequence depicted in SEQ ID NO:1.

10. The transglutaminase variant of claim 9, further comprising an N-terminal methionine residue, a C-terminal histidine polyhistidine sequence, and/or a pro-sequence.

11. A method for increasing the shelf life of a product, comprising incorporating the transglutaminase variant of any of claims 1 to 10 into the product in an amount effective to prevent or decrease growth of one or more microbe in comparison to an identical product that does not comprise the variant.

12. A product comprising the transglutaminase variant of any of claims 1 to 10 in an effective amount to increase the shelf life of the product, in comparison to an identical product that does not comprise the variant.

13. The product of claim 12, wherein the product is a personal care, household, industrial, food, pharmaceutical, cosmetic, healthcare, wound care, marine, paint, coating, energy, plastic, packaging, textile, leather, or agricultural product.

14. The product of 13, wherein the product is a personal care product selected from bar soap, liquid soap, hand sanitizer, preoperative skin disinfectant, cleansing wipes, disinfecting wipes, body wash, acne treatment products, antifungal diaper rash cream, antifungal skin cream, shampoo, conditioner, cosmetic, deodorant, antimicrobial cream, body lotion, hand cream, topical cream, aftershave lotion, skin toner, mouth wash, toothpaste, and sunscreen lotion.

15. The product of claim 13, wherein the product is a wound care product selected from wound healing ointment, cream, and lotion, wound covering, burn wound cream, bandages, tape, and steri-strips.

16. An enzyme composition comprising: (i) the transglutaminase variant of any of claims 1 to 10, a transglutaminase enzyme comprising the amino acid sequence depicted in SEQ ID NO:1, 28, or 29, and/or a lysyl oxidase enzyme; and (ii) a substrate for the transglutaminase or lysyl oxidase enzyme, comprising a sunscreen molecule, a pigment or dye molecule, or a functional ingredient molecule.

17. The enzyme composition of claim 16, wherein the sunscreen molecule, pigment or dye molecule, or functional ingredient molecule is conjugated to a molecule that comprises a free amino group.

18. The enzyme composition of claim 17, wherein the molecule that comprises a free amino group is derived from an aliphatic amine of formula $R(CH_2)_n(NH_2)$, wherein n is an integer between 1 and 30 and R is a functional ingredient.

19. The enzyme composition of claim 18, wherein n is an integer between 5 and 10.

20. The enzyme composition of claim 17, wherein the molecule that comprises a free amino group is derived from lysine, cadaverine, putrescine, hydrazine, adipic acid dihydrazide, sebacic dihydrazide, and hexamethylenediamine.

21. The enzyme composition of claim 16, wherein the sunscreen molecule, pigment or dye molecule, or functional ingredient molecule is conjugated to an amino acid, peptide, or protein with a free glutamine side chain.

22. A cosmetic composition comprising the enzyme composition of claim 16.

23. A method for bonding color to a material or protein of interest, comprising contacting the material or protein of interest with the transglutaminase variant of any of claims 1 to 10, a transglutaminase enzyme comprising the amino acid sequence depicted in SEQ ID NO:1, 28, or 29, and/or a lysyl oxidase enzyme and a pigment or dye molecule, wherein the transglutaminase or lysyl oxidase enzyme is present in an amount effective to covalently bind the pigment or dye molecule to the material or protein of interest.

24. The method of claim 23, wherein the protein of interest is a protein that is present in skin.

25. The method of claim 24, wherein the protein that is present in skin comprises collagen, keratin, and/or elastin.

26. A product comprising the transglutaminase variant of any of claims 1 to 10, a transglutaminase enzyme comprising the amino acid sequence depicted in SEQ ID NO:1, 28, or 29, and/or a lysyl oxidase enzyme in an effective amount to add a functional ingredient or color molecule onto a protein or a protein-, peptide-, or amino acid-containing material of interest when contacted with the product.

27. The product of claim 26, wherein the product is a personal care, cosmetic, textile, leather, food, or agricultural product.

28. A method of modifying the color of a protein or material of interest, comprising contacting the protein or material of interest with the product according to claim 26.

29. A composition comprising the transglutaminase variant of any of claims 1 to 10 in combination with one or more antimicrobial enzyme, peptide, or protein, wherein the composition comprises preservative, biocidal, antimicrobial, or virucidal activity.

30. The composition of claim 29, wherein the antimicrobial enzyme, peptide, or protein is selected from lysozyme, chitinase, lipase, lysin, lysostaphin, glucanase, DNase, RNase, lactoferrin, glucose oxidase, peroxidase, lactoperoxidase, lactonase, acylase, dispersin B, a-amylase, cellulase, nisin, bacteriocin, siderophore, polymyxin, and defensin.

31. A bacteriophage, comprising a nucleic acid sequence that encodes the transglutaminase variant of any of claims 1 to 10.

32. A composition comprising the bacteriophage of claim 31, wherein the composition comprises antimicrobial activity.

33. The composition of claim 32, wherein the composition further comprises a pharmaceutically acceptable excipient.

Fig. 1

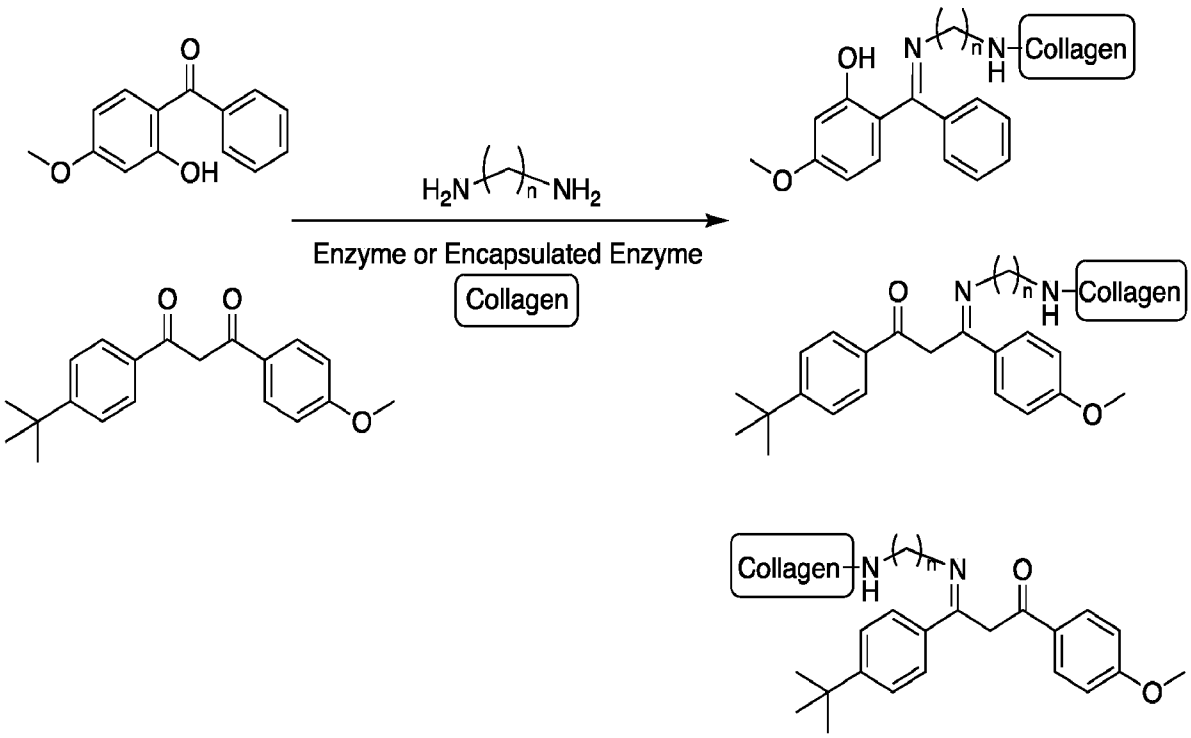


Fig. 2

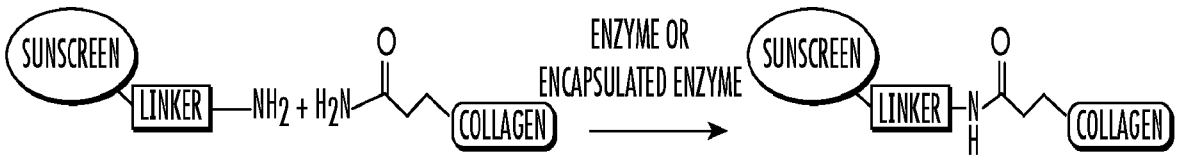


Fig. 3

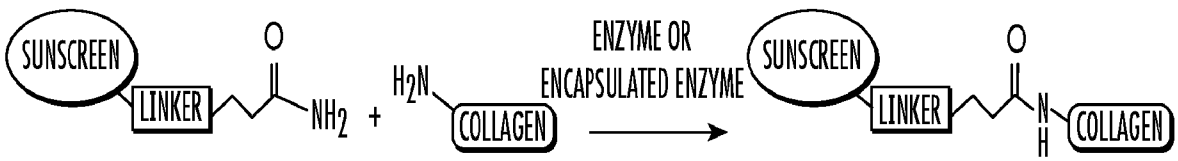


Fig. 4

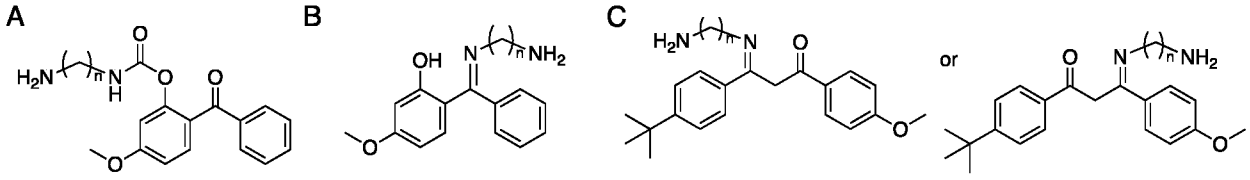


Fig. 5

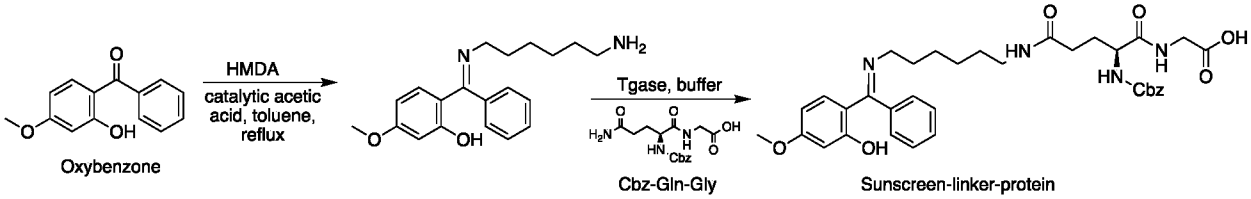


Fig. 6

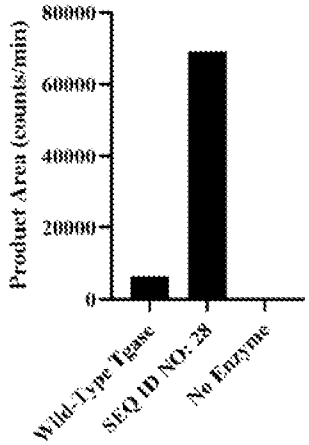


Fig. 7

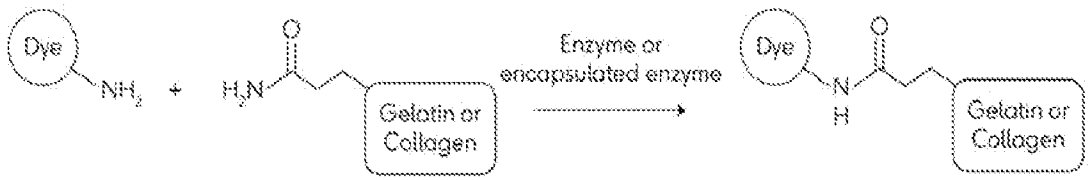


Fig. 8

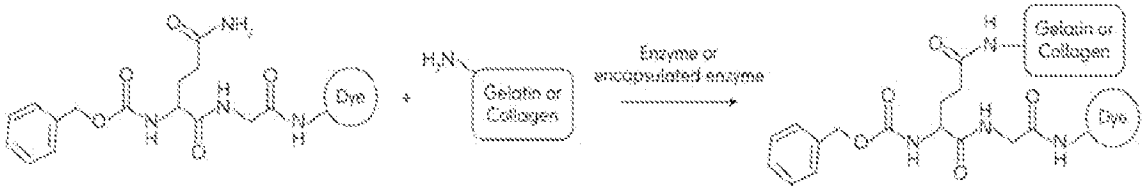
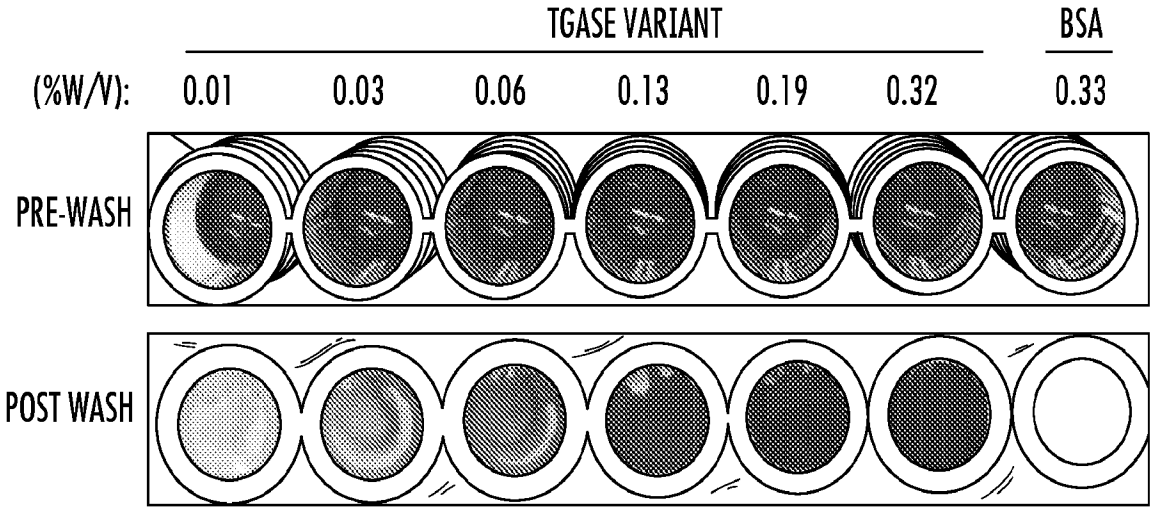


Fig. 9



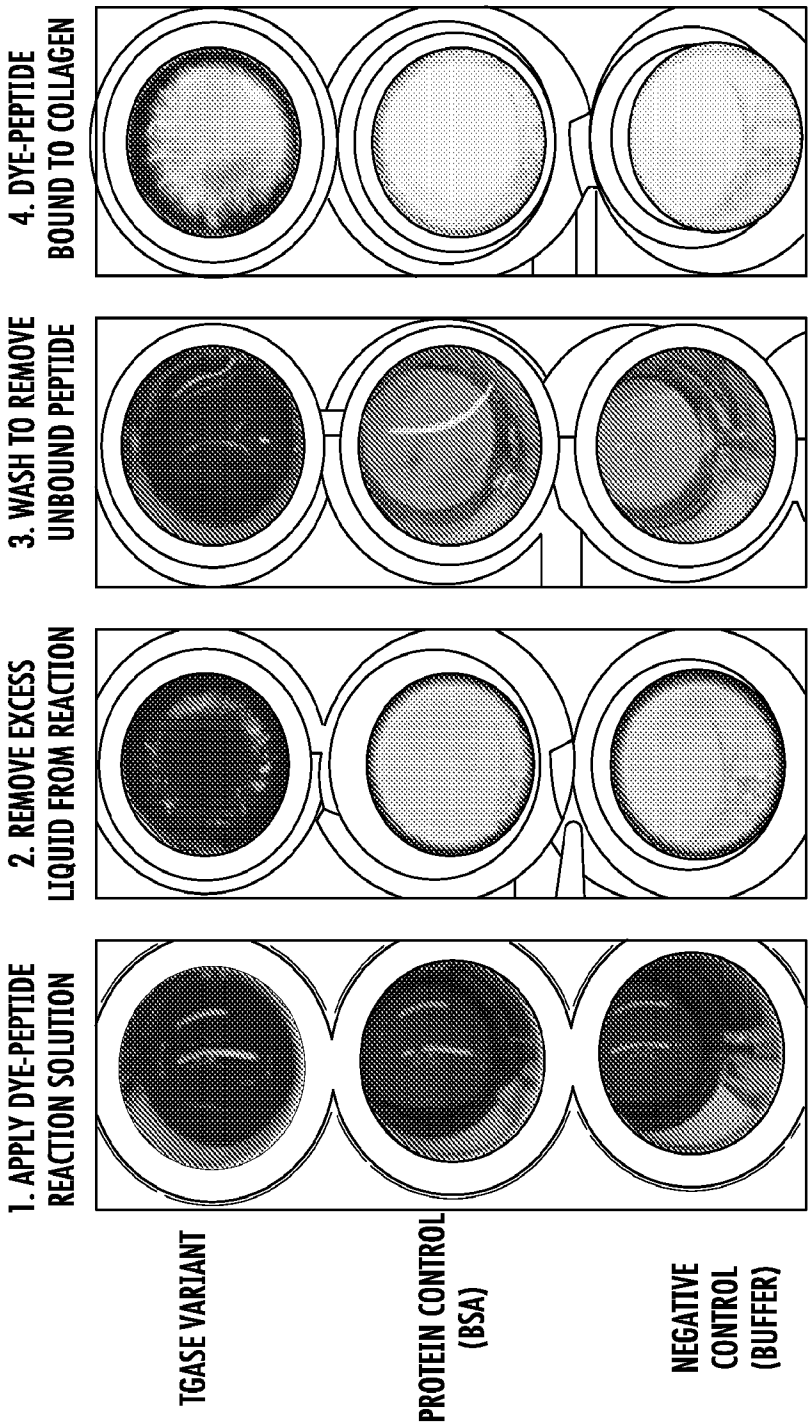

1. APPLY DYE-PEPTIDE REACTION SOLUTION
2. REMOVE EXCESS LIQUID FROM REACTION
3. WASH TO REMOVE UNBOUND PEPTIDE
4. DYE-PEPTIDE BOUND TO COLLAGEN
TGASE VARIANT
PROTEIN CONTROL (BSA)
NEGATIVE CONTROL (BUFFER)

FIG. 10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2021/032217

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12N 9/10; C12N 15/52 (2021.01)
CPC - C12N 9/1044; C12N 15/52; C12Y 203/02013 (2021.08)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
see Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
see Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
see Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 2019/107288 A1 (AMANO ENZYME, INC.) 06 June 2019 (06.06.2019) entire document	1, 2, 7-10, 31 --- 3, 4, 11-30, 32, 33
Y	US 2006/0275872 A1 (KASHIWAGI et al) 07 December 2006 (07.12.2006) entire document	3, 4
Y	US 2012/0027694 A1 (THIJSSEN et al) 02 February 2012 (02.02.2012) entire document	11-15
Y	US 2006/0104966 A1 (GREEN et al) 18 May 2006 (18.05.2006) entire document	16-28
Y	US 2005/0268405 A1 (BRUN et al) 08 December 2005 (08.12.2005) entire document	25
Y	US 2010/0322903 A1 (COLLINS et al) 23 December 2010 (23.12.2010) entire document	29, 30, 32, 33
A	US 2012/0021458 A1 (SUZUKI et al) 26 January 2012 (26.01.2012) entire document	1-33

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 August 2021	SEP 30 2021
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Harry Kim Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2021/032217

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☐ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☒ furnished subsequent to the international filing date for the purposes of international search only:

      ☒ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NOs:1-29 were searched.