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(54) Title: COMPOSITIONS COMPRISING FIBROUS POLYPEPTIDES AND POLYSACCHARIDES

(57) Abstract: Isolated polypeptides are disclosed comprising an amino acid sequence encoding a monomer of a fibrous polypeptide attached to a heterologous polysaccharide binding domain. Composites comprising same, methods of generating same and uses thereof are all disclosed.

COMPOSITIONS COMPRISING FIBROUS POLYPEPTIDES AND POLYSACCHARIDES

FIELD AND BACKGROUND OF THE INVENTION

The present invention, in some embodiments thereof, relates to compositions comprising fibrous polypeptides and polysaccharides and uses of same.

The most extensively investigated biological polymers for use in material science are polysaccharides due to their abundance and extremely diverse mechanical properties.

The polysaccharide cellulose is the most common biopolymer on earth. Although it is mostly found in plant biomass, it is also produced by animals, fungi and bacteria. Cellulose is a crystalline assembly of cellobiose subunits which are made from glucose. Due to its crystalline structure, cellulose has high tensile strength and elasticity approaching that of synthetic carbon fibers, and it has a very favorable strength/weight ratio compared to, for example, steel. In plant cell walls, cellulose is found as a composite with other polysaccharides such as hemicellulose, pectin, lignin, enzymes and structural proteins. These molecules link the cellulose microfibrils improving the mechanism of load transfer when the cell is subjected to mechanical stress whilst enhancing physical protection against pathogen attack.

The unique properties of natural biocomposites have prompted many scientists to produce composites of cellulose and synthetic polymer matrixes. For example, Favier *et al*, [Polymer engineering and science 37(10): 1732-1739] produced cellulose-latex composites resulting in increased shear modulus by more than three orders of magnitude of the latex rubbery state. Such biocomposites have been produced for the automotive industries and for production of biodegradable plastics.

The use of cellulose binding domains (CBD) for cellulose fiber modification is a well established technology [Shoseyov *et al,* Microbiol Mol Biol Rev. 70(2):283-95]. Recently, CBD was used for production of novel cellulose-protein composite materials when recombinant CBD or CBD dimers, CBD-CBD fusion proteins (CCP), were bound to paper resulting in improved mechanical and water repelling properties [Levy et al., Cellulose 9: 91-98]. Furthermore, a recombinant CBD-starch binding domain (CSCP) demonstrated cross-bridging ability in different model systems composed of insoluble or soluble starch and cellulose [Levy et al., Cellulose 9: 91-98].

In addition to polysaccharide research, biopolymer research has focused in recent years on fibrous proteins due to their unique mechanical properties. These proteins are distinguished by their repetitive amino acid sequences that confer mechanical strength or flexibility. Among these proteins are mammalian collagen and elastin and the arthropod proteins, silkworm silk (*Bombyx morii*), spider dragline silk and resilin. The unique repetitive sequence of each protein confers its mechanical properties. For instance, spider silk is extremely strong while resilin and elastin are extremely elastic and resilient with a rubber-like nature.

Resilin is found in specialized cuticle regions in many insects, especially in areas where high resilience and low stiffness are required, or as an energy storage system. It is best known for its roles in insect flight and the remarkable jumping ability of fleas and spittlebugs. The protein was initially identified in 1960 by Weis-Fogh who isolated it from cuticles of locusts and dragonflies and described it as a rubber-like material.

Resilin displays unique mechanical properties that combine reversible deformation with very high resilience. It has been reported to be the most highly efficient elastic material known. The elastic efficiency of the material is purported to be 97%; only 3% of stored energy is lost as heat (U.S. Patent Application 20070099231). Resilin shares similar mechanical properties with elastin which is produced in connective tissues of vertebrates. In humans, elastin is usually found at sites where elasticity is required, such as the skin and cartilage (often in association with collagen). Elastin-collagen composites also serve as a major component in arterial walls where it allows the blood vessels to smooth the pulsatile flow of blood from the heart into a continuous and steady flow.

In spite of their functional analogy, the sequence homology between resilin and elastin is very low, apart from the high abundance of glycine in both proteins. Nevertheless, the elasticity of both proteins results from their architecture of randomly coiled, crosslinked polypeptide chains. Resilin is synthesized in the insect cytoplasm and subsequently secreted to the cuticle where peroxidase enzymes catalyze its polymerization via formation of di/tri tyrosine bridges, resulting in assembly of a natural protein-carbohydrate composite material with cuticular chitin. Two *Drosophila melanogaster* Resilin mRNA variants have been identified - CG15920-RA and

CG15920-RB which differ in the truncation of their chitin binding domains (see Figure 1A). The major components that were annotated are the 17-amino acid long elastic repeats and the 35 amino acid-long chitin binding domain of type R&R.

Recently, Elvin et al., 2005, [Nature. 437: 999-1002] successfully expressed and polymerized a synthetic, truncated resilin-like gene in *E. coli*. The synthetic gene consists of the 17 repeats of the native gene. The protein, once expressed, undergoes photochemical crosslinking which casts it into a rubber-like biomaterial. U.S. Patent Application 20070099231 discloses hybrid resilins comprising resilin and structural polypeptides.

Silk proteins are produced by a variety of insects and arachnids, the latter of which form the strongest silk polymers on earth. The spider spins as many as seven different kinds of silks, each one being optimized to its specific biological function in nature. Dragline silk, used as the safety line and as the frame thread of the spider's web, is an impressive material with a combination of tensile strength and elasticity. Its extraordinary properties are derived from its composition as a semicrystalline polymer, comprising crystalline regions embedded in a less organized "amorphous" matrix. The crystalline regions consist of antiparallel β-pleated sheets of polyalanine stretches that give strength to the thread, while the predominant secondary structure of the amorphous matrix is the glycine-rich helix which provides elasticity. Most dragline silks consist of at least two different proteins with molecular masses of up to several hundred kDa. On the basis of sequence similarities, dragline silk proteins have been grouped into spidroin1-like (MaSp1) and spidroin2-like (MaSp2) proteins.

As opposed to silkworm silk, isolation of silk from spiders is not industrially feasible. Spiders produce silk in small quantities, and their territorial behavior prevents large amounts thereof from being harvested in adjacent quarters. Therefore, production of silk protein through recombinant DNA techniques is preferred. For such purposes, widespread use is made of synthetic genes based on a monomer consensus of the native spidroin sequences. These synthetic genes have been successfully expressed in the methyltropic yeast host, *Pichia pastoris*, in *E.coli* and in the *tobacco* and potato plants [Fahnestock SR., and Bedzyk LA Appl Microbiol biotechnol 47:33-39 (1997); Fahnestock SR., and Bedzyk LA, Appl Microbiol biotechnol 47:23-32 (1997), Sceller J. et al. Nature biotechnology 19:573-577 (2001)]. Through such means, laboratory scale

amounts of silk-like protein powders are readily available. The final hurdle on the way to the production of manmade silks lies in the development of an appropriate spinning technology capable of converting these powders into high performance fibers. The tendency of these proteins to aggregate *in-vitro*, bypassing the protein folding process, acts as a significant limitation toward successfully producing functional silk. The assembly of the proteins from a liquid crystalline form into a solid silk string is extremely complex, and duplication of the operational function of spider spinning glands remains a major challenge.

Several attempts have been reported on the preparation of cellulose–silk fibroin composites which were prepared by molecular blending and regeneration of solubilized cellulose and silkworm silk [Freddi G, et al., (1995), J Appl Polymer Sci 56: 1537-1545;Yang, G, et al., (2000) J Membr Sci 210: 177-153]. Recently, Noishiki *et al* [Noishiki Y, Nishiyama Y, Wada M, Kuga S, Magoshi J. (2002) J Appl Polymer Sci 86: 3425–3429] prepared composite cellulose-silk films from solid cellulose whiskers and regenerated silkworm silk, resulting in notably improved mechanical strength, with breaking strength and ultimate strain about five times those of the constituent materials alone.

SUMMARY OF THE INVENTION

According to an aspect of some embodiments of the present invention there is provided an isolated polypeptide comprising an amino acid sequence encoding a monomer of a fibrous polypeptide attached to a heterologous polysaccharide binding domain, with the proviso that the polysaccharide binding domain is not a cellulose binding domain.

According to an aspect of some embodiments of the present invention there is provided an isolated polypeptide comprising an amino acid sequence encoding a resilin or spider-silk polypeptide attached to a heterologous polysaccharide binding domain.

According to an aspect of some embodiments of the present invention there is provided an isolated polynucleotide comprising a nucleic acid sequence encoding an isolated polypeptide comprising an amino acid sequence encoding a monomer of a fibrous polypeptide attached to a heterologous polysaccharide binding domain, with the proviso that the polysaccharide binding domain is not a cellulose binding domain.

According to an aspect of some embodiments of the present invention there is provided an isolated polynucleotide comprising a nucleic acid sequence encoding an isolated polypeptide comprising an amino acid sequence encoding a resilin or spider-silk polypeptide attached to a heterologous polysaccharide binding domain.

According to an aspect of some embodiments of the present invention there is provided a nucleic acid construct, comprising a nucleic acid sequence encoding resilin and a cis-acting regulatory element capable of directing an expression of the resilin in a plant.

According to an aspect of some embodiments of the present invention there is provided a nucleic acid construct, comprising a nucleic acid sequence encoding spider silk and a cis-acting regulatory element capable of directing an expression of the spider silk in a plant.

According to an aspect of some embodiments of the present invention there is provided a nucleic acid construct, comprising the isolated polynucleotides of the present invention.

According to an aspect of some embodiments of the present invention there is provided a cell comprising the nucleic acid constructs of the present invention.

According to an aspect of some embodiments of the present invention there is provided a plant cell comprising the nucleic acid constructs of the present invention.

According to an aspect of some embodiments of the present invention there is provided an isolated composite comprising a fibrous polypeptide and a polysaccharide, the fibrous polypeptide being resilin or spider silk.

According to an aspect of some embodiments of the present invention there is provided an isolated composite comprising a fibrous polypeptide and a polysaccharide, the fibrous polypeptide comprising a heterologous polysaccharide binding domain, the composite being non-immobilized.

According to an aspect of some embodiments of the present invention there is provided an isolated composite comprising at least two non-identical fibrous polypeptides, wherein a first fibrous polypeptide of the at least two non-identical fibrous polypeptide is an isolated polypeptide comprising an amino acid sequence encoding a monomer of a fibrous polypeptide attached to a heterologous polysaccharide binding

domain, with the proviso that the polysaccharide binding domain is not a cellulose binding domain.

According to an aspect of some embodiments of the present invention there is provided an isolated composite comprising at least two non-identical fibrous polypeptides, wherein a first fibrous polypeptide of the at least two non-identical fibrous polypeptide is an isolated polypeptide comprising an amino acid sequence encoding a resilin or spider-silk polypeptide attached to a heterologous polysaccharide binding domain.

According to an aspect of some embodiments of the present invention there is provided a method of generating the isolated composites of the present invention, the method comprising contacting the fibrous polypeptide with the polysaccharide under conditions which allow binding between the fibrous polypeptide and the polysaccharide to generate the isolated composites of the present invention.

According to an aspect of some embodiments of the present invention there is provided a use of the isolated composite of the present invention for the manufacture of a medicament for the treatment of a cartilage or bone disease or condition.

According to an aspect of some embodiments of the present invention there is provided a use of the isolated composite of the present invention for the manufacture of a medicament for the treatment of urinary incontinence.

According to an aspect of some embodiments of the present invention there is provided a scaffold comprising the isolated composite of the present invention.

According to an aspect of some embodiments of the present invention there is provided a method of treating a cartilage or bone disease or condition, the method comprising administering to a subject in need thereof a therapeutically effective amount of the isolated composite of the present invention, thereby treating the cartilage disease or condition.

According to an aspect of some embodiments of the present invention there is provided a method of treating urinary incontinence, the method comprising administering to a subject in need thereof a therapeutically effective amount of the isolated composite of the present invention, thereby treating urinary incontinence.

According to an aspect of some embodiments of the present invention there is provided a pharmaceutical composition comprising the isolated composite of the present invention.

According to an aspect of some embodiments of the present invention there is provided a cosmetic composition comprising the isolated composite of the present invention.

According to some embodiments of the invention, the fibrous polypeptide is selected from the group consisting of resilin, elastin, spider silk, silk-worm silk, collagen and mussel byssus protein.

According to some embodiments of the invention, the fibrous polypeptide comprises resilin.

According to some embodiments of the invention, the fibrous polypeptide comprises spider silk.

According to some embodiments of the invention, the resilin comprises an amino acid sequence as set forth in SEQ ID NO: 8.

According to some embodiments of the invention, the resilin comprises an amino acid sequence as set forth in SEQ ID NO: 9

According to some embodiments of the invention, the polypeptide further comprises an amino acid sequence as set forth in SEQ ID NOs: 52 or 53.

According to some embodiments of the invention, the polysaccharide binding domain is selected from the group consisting of a chitin binding domain, a starch binding domain, a dextran binding domain, a glucan binding domain, a chitosan binding domain, an alginate binding domain and an hyaluronic acid binding domain.

According to some embodiments of the invention, the polysaccharide binding domain is selected from the group consisting of a chitin binding domain, a cellulose binding domain, a starch binding domain, a dextran binding domain, a glucan binding domain, a chitosan binding domain, an alginate binding domain and an hyaluronic acid binding domain.

According to some embodiments of the invention, the isolated polypeptide is as set forth in SEQ ID NOs: 11-13 and SEQ ID NOs. 32-36.

According to some embodiments of the invention, the spider silk comprises an amino acid sequence as set forth in SEQ ID NO: 16 or SEQ ID NO: 26.

According to some embodiments of the invention, the polynucleotide comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 17-22, 24, 28 and 29.

According to some embodiments of the invention, the nucleic acid construct further comprises at least one cis-acting regulatory element.

According to some embodiments of the invention, the cis-acting regulatory element is a plant promoter.

According to some embodiments of the invention, the plant promoter is a rbcS1 promoter.

According to some embodiments of the invention, the nucleic acid construct further comprises a nucleic acid sequence encoding a vacuolar signal sequence.

According to some embodiments of the invention, the cis-acting regulatory sequence is a terminator sequence.

According to some embodiments of the invention, the terminator sequence is a rbcS1 sequence.

According to some embodiments of the invention, the cell is a plant cell.

According to some embodiments of the invention, the polysaccharide is selected from the group consisting of chitin, cellulose, starch, dextran, glucan, chitosan, alginate and hyaluronic acid.

According to some embodiments of the invention, the fibrous polypeptide comprises a polysaccharide binding domain.

According to some embodiments of the invention, the polysaccharide binding domain is a heterologous polysaccharide binding domain.

According to some embodiments of the invention, the polysaccharide binding domain comprises a chitin binding domain, a cellulose binding domain, a chitosan binding domain, an alginate binding domain, a starch binding domain, a dextran binding domain, a glucan binding domain and an hyaluronic acid binding domain.

According to some embodiments of the invention, the fibrous polypeptide is selected from the group consisting of mussel byssus protein, resilin, silkworm silk protein, spider silk protein, collagen, elastin or fragments thereof.

According to some embodiments of the invention, the isolated composite further comprises an additional fibrous polypeptide, wherein the additional fibrous polypeptide

is different to the fibrous polypeptide, the additional fibrous polypeptide being selected from the group consisting of mussel byssus protein, resilin, silkworm silk protein, spider silk protein, collagen, elastin and fragments thereof.

According to some embodiments of the invention, the isolated composite is crosslinked.

According to some embodiments of the invention, the isolated composite is non-crosslinked.

According to some embodiments of the invention, the method further comprises crosslinking the composite following the contacting.

According to some embodiments of the invention, the crosslinking is affected by a method selected from the group consisting of photochemical crosslinking, enzymatic crosslinking, chemical crosslinking and physical crosslinking.

According to some embodiments of the invention, the method further comprises coating the composite with an additional fibrous polypeptide, the coating being effected following the crosslinking the composite.

According to some embodiments of the invention, the method further comprises binding the fibrous polypeptide with an additional fibrous polypeptide prior to the contacting.

According to some embodiments of the invention, the additional fibrous polypeptide is selected from the group consisting of a mussel byssus protein, spider silk protein, collagen, elastin, and fibronectin, and fragments thereof.

According to some embodiments of the invention, the polysaccharide is selected from the group consisting of a chitin, a cellulose, a starch, a dextran, a glucan, a chitosan, an alginate, a carboxymethyl cellulose and an hyaluronic acid.

According to some embodiments of the invention, the use is for cartilage repair, knee repair, meniscus repair a knee lubricant and disc repair.

According to some embodiments of the invention, the administering is effected locally.

According to some embodiments of the invention, the locally administering is effected by intra-articular administration.

According to some embodiments of the invention, the intra-articular administration comprises administration into a joint selected from the group consisting

of a knee, an elbow, a hip, a sternoclavicular, a temporomandibular, a carpal, a tarsal, a wrist, an ankle, an intervertebral disk and a ligamentum flavum.

According to some embodiments of the invention, the cartilage disease or condition is selected from the group consisting of osteoarthritis, limited joint mobility, gout, rheumatoid arthritis, osteoarthritis, chondrolysis, scleroderma, degenerative disc disorder and systemic lupus erythematosus.

According to some embodiments of the invention, the administering is effected by injection into an area surrounding the urethra.

According to some embodiments of the invention, the composition is formulated as a gel, a strip, an injectable, or a foam.

Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

Definitions of the specific embodiments of the invention as claimed herein follow.

According to a first embodiment of the invention, there is provided an isolated polypeptide comprising an amino acid sequence encoding a resilin attached to a heterologous polysaccharide binding domain.

According to a second embodiment of the invention, there is provided an isolated polynucleotide comprising a nucleic acid sequence encoding the polypeptide of the first embodiment.

According to a third embodiment of the invention, there is provided an isolated composite comprising resilin and a polysaccharide selected from the group consisting of cellulose, starch, dextran, glucan, chitosan, alginate and hyaluronic acid.

According to a fourth embodiment of the invention, there is provided a method of generating the isolated composite of the third embodiment, said method comprising the step of contacting said resilin with the polysaccharide under conditions which allow binding between said resilin and the polysaccharide to generate the isolated composite of the third embodiment.

According to a fifth embodiment of the invention, there is provided a method of treating a cartilage or bone disease or condition, said method comprising the step of administering to a subject in need thereof, a therapeutically effective amount of the isolated composite of the third embodiment, to thereby treat said cartilage or bone disease or condition.

According to a sixth embodiment of the invention, there is provided a method of treating urinary incontinence, said method comprising the step of administering to a subject in need thereof, a therapeutically effective amount of the isolated composite of the third embodiment, to thereby treat said urinary incontinence.

According to a seventh embodiment of the invention, there is provided a scaffold comprising the isolated composite of the third embodiment.

According to an eighth embodiment of the invention, there is provided a pharmaceutical composition comprising the isolated composite of the third embodiment.

According to a ninth embodiment of the invention, there is provided a cosmetic composition comprising the isolated composite of the third embodiment.

BRIEF DESCRIPTION OF THE DRAWINGS

Some embodiments of the invention are herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of embodiments of the invention. In this regard, the description taken with the drawings makes apparent to those skilled in the art how embodiments of the invention may be practiced.

In the drawings:

FIGs. 1A-B is a schematic illustration (FIG. 1A) and scan (FIG. 1B) illustrating the size and structure of the resilin gene from *D. melonogaster*. FIG. 1A illustrates the schematic structure of *Drosophila melanogaster* resilin variant A CG15920-RA gene; S.P.; cuticular signal peptide, ChBD R&R; chitin binding domain type R&R. Variant B CG15920-RB contains a truncated chitin binding domain. FIG. 1B illustrates RT-PCR results of amplification of the resilin gene from *D. melonogaster*. The resilin cDNA is

[Text continues on page 11]

highlighted by the red rectangle. The thick band was formed due to the presence of two resilin variants. The band in the control reaction lanes (–RT) indicates the genomic gene that contains one intron and therefore migrates slower than the RT-PCR product.

FIG. 2 is a scheme of the multiple cloning site of the pHis-parallel3 expression vector (SEQ ID NO: 54).

FIGs. 3A-B are scans of PCR results of CBD-Resilin (SEQ ID NO: 18) construction. FIG. 3A illustrates the first round PCR of the separate reactions of CBD (left) and resilin (right) sequence amplification. The CBD sequence contains a resilin-matching overhang on the 3' prime while the Resilin contains CBD-matching overhang on the 5' prime. FIG. 3B illustrates the PCR result of the second round following mixture of 1 μl of both products from round one. Note the increased molecular weight of the linked sequences.

FIG. 4 is a scan of a Coomassie blue-stained SDS PAGE analysis of a small scale batch purification of 6H-Res-ChBD (SEQ ID NO: 55) expressed in bacteria. S: soluble protein fraction of the lyzed cells; IB: inclusion bodies; UB: unbound fraction removed by centrifugation; W: wash; E1,E2: eluted protein with 0.4M imidazole. MW: protein molecular weight marker.

FIG. 5 is a scan of a Coomassie blue-stained SDS PAGE analysis illustrating the results of a cellulose and chitin binding assay with the affinity-purified 6H-Res-ChBD protein (SEQ ID NO: 55). T: Protein pulled down by HIS-Select® affinity product; UB: unbound fraction removed by centrifugation; W: wash fraction; B: bound fraction eluted from cellulose/chitin pellets by boiling with SDS-PAGE sample application buffer. MW: molecular weight marker.

FIGs. 6A-C are scans of Coomassie blue-stained SDS PAGE analyses illustrating the results of a cellulose and chitin binding assay of a crude extract comprising 6H-Res-ChBD (SEQ ID NO: 55). T: crude lysate; W: wash fraction UB; unbound fraction removed by centrifugation; B: bound fraction eluted from cellulose/chitin pellets by boiling with SDS-PAGE sample application buffer. B1:5: bound fraction diluted five time to the true initial load concentration. MW: protein molecular weight marker.

FIG. 7 is a photograph of an SDS PAGE analysis illustrating the results of a Res-ChBD (SEQ ID NO: 55) heat stability assay. UH: unheated protein. Lanes 2-4:

samples subjected to 85 °C for 15, 30, 60 minutes, respectively. MW: protein molecular weight marker.

FIGs. 8A-B illustrate a small scale affinity purification of 6H-Res-ChBD. FIG. 8A: Chromatogram illustrating purification of Res-ChBD on a Ni-NTA column. The observed peak was eluted with 220 mM imidazole at min 13.4. FIG. 8B: SDS-PAGE analysis of small scale Ni-NTA purification of 6H-Res-ChBD (SEQ ID NO: 55). 6-17: number of FPLC fractions loaded on the gel; FT: column flow through. Fractions 9-18 were collected for further analysis.

FIG. 9 is a scan of an SDS-PAGE analysis of photochemical polymerization of 6H-Res-ChBD (SEQ ID NO: 55). UH: unheated affinity purified 6H-Res-ChBD; H: purified 6H-Res-ChBD incubated at 85 °C for 15 minutes; P: 6H-Res-ChBD treated with $Ru(bpy)_3Cl_2 \cdot 6H_2O$ and ammonium persulfate prior to subjection to sunlight. The treatment resulted in high molecular weight products that could not get into the gel and remained in the loading wells (indicated by arrow).

FIGs. 10A-B illustrate a medium scale affinity purification of 6H-Res-ChBD (SEQ ID NO: 55). FIG. 10A: Chromatogram of 6H-Res-ChBD purification on a Ni-NTA column. The protein peak observed was eluted with 180 mM imidazole at min 22.7. FIG. 10B: Coomassie blue stained SDS-PAGE analysis of a medium scale Ni-NTA purification of 6H-Res-ChBD. 1-11: numbers of FPLC fractions loaded on the gel; FT: column flow through; W: column wash.

FIG. 11 is a scan of a Coomassie-blue stained SDS-PAGE analysis of a Ni-NTA-purified recombinant resilin (SEQ ID NO: 56). Lanes 1-8: FPLC fractions; FT: column flow through. Fractions 4-7 correspond to the purified resilin.

FIG. 12 is a scan of a Coomassie blue stained SDS-PAGE analysis of CBD-resilin (SEQ ID NO: 57), marked by the arrow, post lysis of the bacteria. The protein was detected almost exclusively in the inclusion bodies (IB).

FIG. 13 is a scan of a Coomassie blue stained SDS-PAGE analysis of the cellulose binding capacity of affinity purified CBD-resilin (SEQ ID NO: 57). T: Ni-NTA purified CBD-resilin; UB: unbound fraction removed by centrifugation; W: wash fraction; B: bound fraction eluted from cellulose/chitin pellets by boiling with SDS-PAGE sample application buffer.

FIG. 14 is a scan of a Coomassie blue stained SDS-PAGE analysis of CBD-resilin refolded via the Aktaprime™ Plus FPLC automated refolding system (SEQ ID NO: 57) bound to cellulose. T; Ni-NTA purified CBD-resilin; B: bound fraction eluted from cellulose pellets by boiling with SDS-PAGE sample application buffer; UB: unbound fraction removed by centrifugation.

FIG. 15 is a model of a composite of cellulose and spider silk.

FIG. 16 is a scan of a Coomassie-stained SDS-PAGE analysis of Ni-NTA purified recombinant resilin-CBD (SEQ ID NO: 58). Samples 9-17 were the FPLC-ÄKTAprime™ plus fractions. Fractions 15-16 correspond to the resilin-CBD peak as observed at O.D. 280 nm.

FIG. 17 is a scan of a Coomassie-stained SDS-PAGE analysis of resilin-CBD (SEQ ID NO: 58) following heat treatment and a cellulose binding assay. UH: Unheated protein; H: Protein incubated at 85 °C for 15 minutes; T: Total protein (affinity chromatography product); B: Bound fraction eluted by boiling the cellulose pellet with X2 SAB; UB: Unbound fraction removed by centrifugation.

FIG. 18 is a scan of a Coomassie-stained SDS-PAGE analysis of the solubility of resilin (SEQ ID NO: 56) and resilin-ChBD (SEQ ID NO: 55) proteins under different pH conditions, following gradual titration with 2M HCl.

FIG. 19 is a scan of a Coomassie-stained SDS-PAGE analysis of resilin samples that were subjected to light-induced polymerization under different pH conditions in the presence (+) or absence (-) of $Ru(bpy)3Cl_2 \cdot 6H_2O$ and APS. Control samples of Res-ChBD (SEQ ID NO: 55) proteins (pH 7.4) were subjected to similar crosslinking conditions. The arrow points out the high molecular weight products in samples containing the crosslinkers.

FIG. 20 is a scan of a Coomassie-stained SDS-PAGE analysis of resilin polymerized by the MCO method in either a phosphate buffer or water-based reaction solution. A high molecular weight product was formed both in phosphate buffer and H_2O. The reaction carried out in H_2O demonstrated a polymerization effect in the reaction with H_2O_2 only.

FIGs. 21A-B are photographs illustrating the generation of a composite of the present invention. FIG. 21A - Opening of the Teflon mold following photochemical crosslinking of the 6H-Res-ChBD-cellulose composites. FIG. 21B - left and middle are

resulting composite polymers of 150 and 75 µl samples of 6H-Res-ChBD-cellulose whiskers, respectively, while the sample on the right is that received from the 150 µl sample of pure 6H-Res-ChBD polymer casted in the absence of cellulose whiskers.

FIGs. 22A-B are scans illustrating SDS PAGE analyses of *E.Coli* proteins following overexpression of an exemplary spider silk of the present invention. FIG. 22A - Coomassie blue-stained SDS-PAGE analysis of total *E.coli* proteins. Proteins were stained with Coomassie blue. Lane 1- protein molecular weight marker, lane 2 - control bacteria transformed with empty vector, lane 3 – proteins collected from SpS (SEQ ID NO: 33)-expressing bacteria, lane 4 – proteins of SpS-CBD-expressing bacteria (SEQ ID NO: 34). FIG. 22B – and Instant blue-stained SDS-PAGE analysis of soluble (S) and insoluble (IB) *E.coli* proteins. Proteins were stained with Coomassie blue. Lane 1- protein molecular weight marker, lanes 2-3 – proteins of SpS (SEQ ID NO: 33)-expressing bacteria, S and IB, respectively. Lanes 4-5 – proteins of SpS-CBD (SEQ ID NO: 34)-expressing bacteria, S and IB, respectively.

FIGs. 23A-B are scans illustrating SDS-PAGE analyses of FPLC-purified 6H-SpS (SEQ ID NO: 33) and 6H-SpS-CBD (SEQ ID NO: 34) expressed in *E. Coli.* FIG. 23A - SDS-PAGE analysis of FPLC fractions of Ni-NTA-purified SpS proteins. Lane 1- protein molecular weight marker, lanes 2-4–soluble proteins of empty vector-transformed E. Coli control lysates, SpS (SEQ ID NO: 33) and SpS-CBD (SEQ ID NO: 34) samples, respectively, prior to Ni-NTA purification. Lanes 4-7 – purified protein fractions of control, SpS (SEQ ID NO: 33), SpS-CBD (SEQ ID NO: 34), respectively, following Ni-NTA purification. FIG. 23B – Western blot analysis of the same samples as described in FIG. 17A with anti-6His antibody.

FIGs. 24A-C are graphs illustrating FPLC purification of 6H-SpS (SEQ ID NO: 33) and 6H-SpS-CBD (SEQ ID NO: 34). FIG. 24A - Chromatogram of the purification of control *E.coli* proteins on a Ni-NTA column. FIG. 24B - Chromatogram of the purification of 6H-SpS (SEQ ID NO: 33), on Ni-NTA column. FIG. 24C - Chromatogram of the purification of 6H-SpS-CBD (SEQ ID NO: 34), on a Ni-NTA column.

FIG. 25 is a scan of an SDS-PAGE analysis of a qualitative cellulose binding assay of affinity-purified SpS (SEQ ID NO: 33) and SpS-CBD (SEQ ID NO: 34). Lane 1- protein molecular weight marker, Lanes 2-4 – spider silk cellulose binding assay:

lane 2-SpS after Ni-NTA purification, lane 3 – cellulose-bound protein, lane 4- unbound protein. The unbound protein is diluted 1:10 in comparison to protein concentration in lane 2. Lanes 5-7 – SpS-CBD cellulose binding assay: lane 5-SpS–CBD after Ni-NTA purification, lane 6 – cellulose-bound protein, lane 7- unbound protein. The unbound protein is diluted 1:10 in comparison to protein concentration in lane 5.

FIG. 26 is a graph of an adsorption/desorption isotherm. CBD*Clostridium cellulovorans* (CBDclos) (SEQ ID NO: 10), SpS (SEQ ID NO: 33) and SpS-CBD (SEQ ID NO: 34), at different concentrations, were allowed to adsorb to cellulose to the point of equilibrium (B). After equilibrium was reached, the highest protein concentration containing mixture was diluted to allow desorption (R).

FIGs. 27A-B are scans of Western blot analyses of lysates of CBD-SpS12 (SEQ ID NO:35) and SpS6-CBD-SpS6 (SEQ ID NO:36)-expressing plants, using anti-CBD antibody for immunodetection. FIG. 27A – Tobacco plants expressing and accumulating CBD-SpS12 (SEQ ID NO:35) in the apoplast. Lane 1- protein molecular weight marker, lane 2- wild type tobacco plant lysates, Lanes 3-8 – lysates of transgenic tobacco plant numbers 13.1-13.6, respectively. S-soluble proteins, P- insoluble proteins. FIG. 27B – Tobacco plants expressing SpS6-CBD-SpS6 (SEQ ID NO:36) in the cytoplasm. Lane 1- protein molecular weight marker, lane 2- wild type tobacco plant lysates, Lanes 3-8 – Lysates of transgenic tobacco plant numbers 6.1-6.6, respectively. S-soluble proteins, P- insoluble proteins.

FIGs. 28A-B are scans of Western blot analyses of the SpS6-CBD-SpS6 (SEQ ID NO: 36) purification procedure, using anti-CBD antibody for immunodetection. FIG. 28A - Lane 1- protein molecular weight marker, lane 2- soluble proteins of wild type tobacco plant extracts, Lane 3 - insoluble proteins of wild type tobacco, lane 4- soluble proteins of transgenic tobacco plant #6.4, lane 5 - insoluble proteins of transgenic tobacco plant #6.4, lane 6 - soluble proteins eluted from the insoluble fraction of #6.4 transgenic tobacco plant SpS6-CBD-SpS6 (SEQ ID NO: 36), lane 7 – insoluble proteins eluted from the insoluble fraction of 6.4 transgenic tobacco plant SpS6-CBD-SpS6 . FIG. 28B illustrates the heat stability and pH solubility of SpS6-CBD-SpS6 (SEQ ID NO: 36). Lane 1- protein molecular weight marker, lane 2-soluble proteins eluted from the insoluble fraction of the plant extract (as shown in FIG. 24A

lane 6), lanes 3-6-heat stability assay at 60, 70, 80 and 90 °C respectively. Lanes 7-12 – pH solubility test under pH=8, 7, 6, 5, 4, 3, respectively.

FIG. 29 is a Coomassie-stained SDS-PAGE analysis of metal-catalyzed polymerization of silk. Lane 1- protein molecular weight marker; lanes 2-5-reaction analysis of SpS (SEQ ID NO: 33) dialyzed against DDW: lane 2 – protein solution without H_2O_2 or $CuCl_2$, lane 3- polymerization reaction including H_2O_2 and $CuCl_2$, lane 4 – protein solution with addition of H_2O_2 only, lane 5 – protein solution with the addition of $CuCl_2$ only. Lanes 6-9: reaction analysis of SpS dialyzed against 50mM sodium phosphate (pH 7.5): lane 6 – protein solution without H_2O_2 or $CuCl_2$, lane 7-polymerization reaction including H_2O_2 and $CuCl_2$, lane 8 – protein solution with addition of H_2O_2 only, lane 9 – protein solution with the addition of $CuCl_2$ only.

FIG. 30 is a Coomassie-stained SDS-PAGE analysis of SpS sponge preparation. Lane 1- protein molecular weight marker, lane 2 – soluble protein before sponge preparation procedure, lane 3 - soluble protein after dialysis against 50 mM sodium phosphate (pH 7.5), lane 4 – soluble protein after dialysis against DDW, lane 5 – soluble protein after concentration to ~50 mg/ml. The sample was diluted x50 in order to confirm that there was no protein loss.

FIGs. 31A-C depict the results of DSC analysis of SpS-cellulose whisker sponges. A – DSC thermogram analysis of cellulose whiskers sponge; B – DSC thermogram analysis of SpS sponge; C - DSC thermogram analysis of 70 %whiskers/30 %SpS sponge.

DESCRIPTION OF EMBODIMENTS OF THE INVENTION

The present invention, in some embodiments thereof, relates to compositions comprising fibrous polypeptides and polysaccharides and uses of same. The fibrous polypeptides may comprise an endogenous polysaccharide binding domain or a heterologous polysaccharide binding domain.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not necessarily limited in its application to the details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways.

In a search to identify novel composite biomaterials with superior mechanical properties for use in medical, industrial and other applications, the present inventors have generated novel fibrous polypeptides that enable directional binding and polymerization on polysaccharides.

Whilst reducing the present invention to practice, the present inventors generated and purified both resilin and spider-silk fusion proteins. Exemplary fusion proteins generated include resilin-chitin binding domain (Res-ChBD) (Figures 4-10, 18 and 19); resilin-cellulose binding domain (Res-CBD) (Figures 12-14, 16-17) and; spider-silk-cellulose binding domain (Figures 23-28).

Thus, according to one aspect of the present invention, there is provided an isolated polypeptide comprising an amino acid sequence encoding a monomer of a fibrous polypeptide attached to a heterologous polysaccharide binding domain.

As used herein, the phrase "fibrous polypeptide" refers to a polypeptide that consists of a plurality of monomer chains arranged in a matrix so as to form fibers or sheets. Fibrous proteins are described in D. Voet & J. G. Voet, "Biochemistry" (2d ed., John Wiley & Sons, New York, 1995, pp. 153-162), incorporated herein by this reference.

Examples of fibrous polypeptides include, but are not limited to, resilin, elastin, spider silk, silk-worm silk, collagen and mussel byssus protein.

As used herein, the term "resilin" refers to an elastic polypeptide, capable of forming a fiber, wherein each monomer thereof comprises at least two repeating units of the sequence as set forth in SEQ ID NO: 45. According to one embodiment, the repeating unit comprises a sequence as set forth in SEQ ID NO: 8. GenBank Accession Nos. of non-limiting examples of resilin are listed in Table 1 below. A resilin of the present invention also refers to homologs (e.g. polypeptides which are at least 50 %, at least 55 %, at least 60 %, at least 65 %, at least 70 %, at least 75 %, at least 80 %, at least 85 %, at least 87 %, at least 89 %, at least 91 %, at least 93 %, at least 95 % or more say 100 % homologous to resilin sequences listed in Table 1 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). The homolog may also refer to a deletion, insertion, or substitution variant, including an amino acid substitution, thereof and biologically active polypeptide fragments thereof.

Table 1 below lists examples of resilin NCBI sequence numbers.

Table 1

Exemplary resilin NCBI sequence number	*Organism*
NP 995860	Drosophila melanogaster
NP 611157	Drosophila melanogaster
Q9V7U0	Drosophila melanogaster
AAS64829	Drosophila melanogaster
AAF57953	Drosophila melanogaster
XP 001817028	Tribolium castaneum
XP001947408	Acyrthosiphon pisum

According to one embodiment, the polypeptide sequence of resilin is set forth in SEQ ID NO: 9.

As used herein, the term "elastin" refers to an elastic polypeptide, capable of forming a fiber, wherein each monomer thereof comprises at least two repeating units of the sequence as set forth in SEQ ID NO: 46. GenBank Accession Nos. of non-limiting examples of elastin are listed in Table 2 below. An elastin of the present invention also refers to homologs (e.g., polypeptides which are at least 50 %, at least 55 %, at least 60 %, at least 65 %, at least 70 %, at least 75 %, at least 80 %, at least 85 %, at least 87 %, at least 89 %, at least 91 %, at least 93 %, at least 95 % or more say 100 % homologous to elastin sequences listed in Table 2 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). The homolog may also refer to a deletion, insertion, or substitution variant, including an amino acid substitution, thereof and biologically active polypeptide fragments thereof.

Table 2 below lists examples of elastin NCBI sequence numbers.

Table 2

Organism	*Exemplary elastin NCBI sequence number*
Bos taurus	NP786966
mouse	NP 031951
rat	NP 036854
Human	AAC98395
sheep	I47076

As used herein, the term "spider silk" refers to a polypeptide capable of forming a fiber which is comprised of spider silk, wherein each monomer thereof comprises at least two repeating units of the sequence set forth in SEQ ID NO: 26. According to one embodiment, the polypeptide chain comprises a spidroin 1 amino acid sequence. According to another embodiment, the polypeptide chain comprises a spidroin 2 amino

acid sequence. According to one embodiment, the spider silk is dragline spider silk. GenBank Accession Nos. of non-limiting examples of spidroins 1 and 2 are listed in Table 3 below. A spider silk polypeptide of the present invention also refers to homologs (e.g., polypeptides which are at least 50 %, at least 55 %, at least 60 %, at least 65 %, at least 70 %, at least 75 %, at least 80 %, at least 85 %, at least 87 %, at least 89 %, at least 91 %, at least 93 %, at least 95 % or more say 100 % homologous to spider silk sequences listed in Table 3 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). The homolog may also refer to a deletion, insertion, or substitution variant, including an amino acid substitution, thereof and biologically active polypeptide fragments thereof.

Table 3 below lists examples of spider silk NCBI sequence numbers.

Table 3

Spider silk polypeptide	*Exemplary spider silk NCBI sequence number*
Spidroin 1	P19837
Spidroin 1	AAC38957
Spidroin 2	ABR68858
Spidroin 2	AAT75317
Spidroin 2	P46804

According to one embodiment, the polypeptide sequence of the spider silk polypeptide is set forth in SEQ ID NO: 16 or SEQ ID NO: 38.

As used herein, the term "silkworm silk" refers to a silk polypeptide derived from silkworm, capable of forming a fiber. GenBank Accession Nos. of non-limiting examples of silkworm silk polypeptides are listed in Table 4 below. A silkworm silk polypeptide of the present invention also refers to homologs (e.g., polypeptides which are at least 50 %, at least 55 %, at least 60 %, at least 65 %, at least 70 %, at least 75 %, at least 80 %, at least 85 %, at least 87 %, at least 89 %, at least 91 %, at least 93 %, at least 95 % or more say 100 % homologous to silkworm silk sequences listed in Table 4 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). The homolog may also refer to a deletion, insertion, or substitution variant, including an amino acid substitution, thereof and biologically active polypeptide fragments thereof.

Table 4 below lists examples of silkworm silk NCBI sequence numbers.

Table 4

Exemplary silkworm silk NCBI sequence number
AAL83649
AAA27839
NP 001106733
NP001037488
Caa35180

As used herein, the term "collagen" refers to an assembled collagen trimer, which in the case of type I collagen includes two alpha 1 chains and one alpha 2 chain. A collagen fiber is collagen which is devoid of terminal propeptides C and N. Contemplated collagens include types I, II, III, V, XI, and biologically active fragments therefrom. The collagen may be comprised of procollagen, atelocollagen or telocollagen. A collagen of the present invention also refers to homologs (e.g., polypeptides which are at least 50 %, at least 55 %, at least 60 %, at least 65 %, at least 70 %, at least 75 %, at least 80 %, at least 85 %, at least 87 %, at least 89 %, at least 91 %, at least 93 %, at least 95 % or more say 100 % homologous to collagen sequences listed in Table 1 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). The homolog may also refer to a deletion, insertion, or substitution variant, including an amino acid substitution, thereof and biologically active polypeptide fragments thereof.

Table 5 below lists examples of collagen NCBI sequence numbers.

Table 5

Exemplary human collagen NCBI sequence number
P02452
P08123

As used herein, the phrase "mussel byssus protein" refers to the polypeptide found in the byssal threads of mussels comprising both collagen and elastin domains (e.g. Col-P or Col-D). A mussel byssus protein of the present invention also refers to homologs (e.g., polypeptides which are at least 50 %, at least 55 %, at least 60 %, at least 65 %, at least 70 %, at least 75 %, at least 80 %, at least 85 %, at least 87 %, at least 89 %, at least 91 %, at least 93 %, at least 95 % or more say 100 % homologous to mussel byssus sequences as set forth in NCBI sequence numbes AAB34042 and as

determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters).

The homolog may also refer to a deletion, insertion, or substitution variant, including an amino acid substitution, thereof and biologically active polypeptide fragments thereof.

As mentioned, the isolated polypeptides of the present invention comprise a monomer of a fibrous polypeptide attached to a heterologous polysaccharide binding domain.

As used herein, the qualifier "heterologous" when relating to the heterologous polysaccharide binding domains of the fibrous polypeptides of the present invention indicates that the heterologous polysaccharide binding domain is not naturally found in that fibrous polypeptide to which it is fused.

The phrase "polysaccharide binding domain" refers to an amino acid sequence which binds a polysaccharide with a minimal dissociation constant (K_d) of about 10 μM [Tomme P, Boraston A, McLean B, Kormos J, Creagh AL, Sturch K, Gilkes NR, Haynes CA, Warren RA, Kilburn DG (1998) Characterization and affinity applications of cellulose-binding domains. J Chromatogr B Biomed Sci Appl. 715(1):283-96, Boraston AB, Bolam DN, Gilbert HJ, Davies GJ. (2004) Carbohydrate-binding modules: fine-tuning polysaccharide recognition. Biochem J. 382(Pt 3):769-81]. Typically, the polysaccharide binding domain comprises at least a functional portion of a polysaccharide binding domain of a polysaccharidase or a polysaccharide binding protein.

It will be appreciated that the fibrous polypeptide may be joined directly to the polysaccharide binding domain or may be joined via a linker. Amino acid sequences of exempary linkers contemplated for the present invention are set forth in SEQ ID NOs: 52 and 53.

Exemplary polysaccharide binding domains include but are not limited to a chitin binding domain (examples of which are set forth in SEQ ID NO: 39 and 40), a starch binding domain (an example of which is set forth in SEQ ID NO: 41), a dextran binding domain (an example of which is set forth in SEQ ID NO: 42), a glucan binding domain, a chitosan binding domain (see for example Chen, HP; Xu, LL, (2005) J. of Integrative Plant Biology 47(4): 452-456), an alginate binding domain (an example of

which is set forth in SEQ ID NO: 43) and an hyaluronic acid binding domain (an example of which is set forth in SEQ ID NO: 44).

According to this aspect of the present invention, when the fibrous polypeptide comprises resilin or spider silk, the polysaccharide binding domain may also be a cellulose binding domain.

Table 6 below lists exemplary sources of polysaccharide binding domains which are contemplated for use in the present invention.

Table 6

Sources of polysaccharide binding domains

Binding Domain	Proteins Containing the Binding Domain
Cellulose Binding Domains[1]	β-glucanases (avicelases, CMCases, cellodextrinases) exoglucanses or cellobiohydrolases cellulose binding proteins xylanases mixed xylanases/glucanases esterases chitinases β-1,3-glucanases β-1,3-(β-1,4)-glucanases (β-)mannanases β-glucosidases/galactosidases cellulose synthases (unconfirmed)
Starch/Maltodextrin Binding Domains	α-amylases[2,3] β-amylases[4,5] pullulanases glucoamylases[6,7] cyclodextrin glucotransferases[8-10] (cyclomaltodextrin glucanotransferases) maltodextrin binding proteins[11]
Dextran Binding Domains	*(Streptococcal)* glycosyl transferases[12] dextran sucrases (unconfirmed) *Clostridial* toxins[13,14] glucoamylases[6] dextran binding proteins
β-Glucan Binding Domains	β-1,3-glucanases[15,16] β-1,3-(β-1,4)-glucanases (unconfirmed) β-1,3-glucan binding protein[17]
Chitin Binding Domains	chitinases

chitobiases
chitin binding proteins
(see *also* cellulose binding domains)
Heivein

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[15]Klebl *et al., J. Bacteriol.* (1989) 171:6259.
[16]Watanabe *et al., J. Bacteriol.* (1992) 174:186.
[17]Duvic *et al., J. Biol. Chem.* (1990) :9327.

Table 7 below lists an overview of enzymes with chitin binding domains which are contemplated for use as the polysaccharide domains of the present invention.

Table 7

Source (strain)	Enzyme	Accession No.	Ref.[1]
Bacterial enzymes			
Type I			
Aeromonas sp. (No10S-24)	Chi	D31818	1
Bacillus circulans (WL-12)	ChiA1	P20533/M57601/A38368	2
Bacillus circulans (WL-12)	ChiD	P27050/D10594	3
Janthinobacterium lividum	Chi69	U07025	4
Streptomyces griseus	Protease C	A53669	5
Type II			
Aeromonas cavia (K1)	Chi	U09139	6
Alteromonas sp (0-7)	Chi85	A40633/P32823/D13762	7
Autographa californica (C6)	NPH-128[a]	P41684/L22858	8
Serratia marcescens	ChiA	A25090/X03657/L01455/P07254	9
Type III			
Rhizopus oligosporus (IFO8631)	Chi1	P29026/A47022/D10157/S27418	10
Rhizopus oligosporus (IFO8631)	Chi2	P29027/B47022/D10158/S27419	10

Saccharomyces cerevisiae	Chi	S50371/U17243	11
Saccharomyces cerevisiae (DBY939)	Chi1	P29028/M74069	12
Saccharomyces cerevisiae (DBY918)	Chi2	P29029/M7407/B41035	12
Plant enzymes			
Hevein superfamily			
Allium sativum	Chi	M94105	13
Amaranthus caudatus	AMP-1[b]	P27275/A40240	14, 15
Amaranthus caudatus	AMP-2[b]	S37381/A40240	14, 15
Arabidopsis thaliana (cv. colombia)	ChiB	P19171/M38240/B45511	16
Arabidopsis thaliana	PHP[c]	U01880	17
Brassica napus	Chi	U21848	18
Brassica napus	Chi2	Q09023/M95835	19
Hevea brasiliensis	Hev1[d]	P02877/M36986/A03770/A38288	20,21
Hordeum vulgare	Chi33	L34211	22
Lycopersicon esculentum	Chi9	Q05538/Z15140/S37344	23
Nicotiana tabacum	CBP20[e]	S72424	24
Nicotiana tabacum	Chi	A21091	25
Nicotiana tabacum (cv. Havana)	Chi	A29074/M15173/S20981/S19855	26
Nicotiana tabacum (FB7-1)	Chi	JQ0993/S0828	27
Nicotiana tabacum (cv. Samsun)	Chi	A16119	28
Nicotiana tabacum (cv. Havana)	Chi	P08252/X16939/S08627	27
Nicotiana tabacum (cv. BY4)	Chi	P24091/X51599/X64519//S13322	26,27,29
Nicotiana tabacum (cv. Havana)	Chi	P29059/X64518/S20982	26
Oryza sativum (IR36)	ChiA	L37289	30
Oryza sativum	ChiB	JC2253/S42829/Z29962	31
Oryza sativum	Chi	S39979/S40414/X56787	32
Oryza sativum (cv. Japonicum)	Chi	X56063	33
Oryza sativum (cv. Japonicum)	Chi1	P24626/X54367/S14948	34
Oryza sativum	Chi2	P25765/S15997	35
Oryza sativum (cv. Japonicum)	Chi3	D16223	
Oryza sativum	ChiA	JC2252/S42828	30
Oryza sativum	Chi1	D16221	32
Oryza sativum (IR58)	Chi	U02286	36
Oryza sativum	Chi	X87109	37
Pisum sativum (cv. Birte)	Chi	P36907/X63899	38
Pisum sativum (cv. Alcan)	Chi2	L37876	39
Populus trichocarpa	Chi	S18750/S18751/X59995/P29032	40
Populus trichocarpa (H11-11)	Chi	U01660	41
Phaseolus vulgaris (cv. Saxa)	Chi	A24215/S43926/Jq0965/P36361	42
Phaseolus vulgaris (cv. Saxa)	Chi	P06215/M13968/M19052/A25898	43,44,45
Sambucus nigra	PR-3[f]	Z46948	46
Secale cereale	Chi	JC2071	47
Solanum tuberosum	ChiB1	U02605	48
Solanum tuberosum	ChiB2	U02606	48
Solanum tuberosum	ChiB3	U02607/S43317	48
Solanum tuberosum	ChiB4	U02608	48
Solanum tuberosum (cv. Maris Piper)	WIN-1[g]	P09761/X13497/S04926	49

Solanum tuberosum (cv. Maris Piper)	WIN-2[g]	P09762/X13497/S04927	49
Triticum aestivum	Chi	S38670/X76041	50
Triticum aestivum	WGA-1[h]	P10968/M25536/S09623/S07289	51,52
Triticum aestivum	WGA-2[h]	P02876/M25537/S09624	51,53
Triticum aestivum	WGA-3[h]	P10969/J02961/S10045/A28401	54
Ulmus americana (NPS3-487)	Chi	L22032	55
Urtica dioica	AGL[i]	M87302	56
Vigna unguiculata (cv. Red caloona)	Chi1	X88800	57

[a]NHP : nuclear polyhedrosis virus endochitinase like sequence; Chi : chitinase, [b]anti-microbial peptide, [c]pre-hevein like protein, [d]hevein, [e]chitin-binding protein, [f]pathogenesis related protein, [g]wound-induced protein, [h]wheat germ agglutinin, [i]agglutinin (lectin).

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Table 8 herein below provides an overview of proteins containing Streptocooai glucan-binding repeats (Cpl superfamily) which may be used as polysaccharide domains of the present invention.

Table 8
Overview of proteins containing Streptococcal glucan-binding repeats (Cpl superfamily)

Source	Protein	Accession No.	Ref.[2]
S. downei (sobrinus) (0MZ176)	GTF-I	D13858	1
S. downei (sobrinus) (MFe28)	GTF-I	P11001/M17391	2
S. downei (sobrinus) (MFe28)	GTF-S	P29336/M30943/A41483	3
S. downei (sobrinus) (6715)	GTF-I	P27470/D90216/A38175	4
S. downei (sobrinus)	DEI	L34406	5
S. mutants (Ingbritt)	GBP	M30945/A37184	6
S. mutants (GS-5)	GTF-B	A33128	7
S. mutants (GS-5)	GTF-B	P08987/M17361/B33135	8
S. mutants	GTF-B$^{3'\text{-ORF}}$	P05427/C33135	8
S. mutants (GS-5)	GTF-C	P13470/M17361/M22054	9
S. mutants (GS-5)	GTF-C	not available	10
S. mutants (GS-5)	GTF-D	M29296/A45866	11
S. salivarius	GTF-J	A44811/S22726/S28809 Z11873/M64111	12
S. salivarius	GTF-K	S22737/S22727/Z11872	13
S. salivarius (ATCC25975)	GTF-L	L35495	14
S. salivarius (ATCC25975)	GTF-M	L35928	14

S. pneumoniae R6	LytA	P06653/A25634/M13812	15
S. pneumoniae	PspA	A41971/M74122	16
Phage HB-3	HBL	P32762/M34652	17
Phage Cp-1	CPL-1	P15057/J03586/A31086	18
Phage Cp-9	CPL-9	P19386/M34780/JQ0438	19
Phage EJ-1	EJL	A42936	20
C. difficile (VPI 10463)	ToxA	P16154/A37052/M30307 X51797/S08638	21
C. difficile (BARTS W1)	ToxA	A60991/X17194	22
C. difficile (VPI 10463)	ToxB	P18177/X53138/X60984 S10317	23,24
C. difficile (1470)	ToxB	S44271/Z23277	25,26
C. novyi	a-toxin	S44272/Z23280	27
C. novyi	a-toxin	Z48636	28
C. acetobutylicum (NCIB8052)	CspA	S49255/Z37723	29
C. acetobutylicum (NCIB8052)	CspB	Z50008	30
C. acetobutylicum (NCIB8052)	CspC	Z50033	30
C. acetobutylicum (NCIB8052)	CspD	Z50009	30

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Table 9 below lists proteins containing putative β-1,3 glucan-binding domains which may be contemplated as the polysaccharide binding domains of the present invention.

Table 9
Overview of proteins containing putative

Source (strain)	Protein	accession No.	Ref[3]
Type I			
B. circulans (WL-12)	GLCA1	P23903/M34503/JQ0420	1
B. circulans (IAM 1165)	BglH	JN0772/D17519/S67033	2
Type II			
Actinomadura sp. (FC7)	XynII	U08894	3
Arthrobacter sp. (YCWD3)	GLCI	D23668	9
O. xanthineolytica	GLC	P22222/M60826/A39094	4
R. faecitabidus (YLM-50)	RP I	Q05308/A45053/D10753	5a,b
R. communis	Ricin	A12892	6
S. lividans (1326)	XlnA	P26514/M64551/JS07986	7
T. tridentatus	FactorGa	D16622	8

B. : *Bacillus, O.* : *Oerskovia, R. faecitabidus* : *Rarobacter faecitabidus, R. communis*: *Ricinus communis, S.* : *Streptomyces, T.* : *Tachypleus* (Horseshoe Crab)

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The term "polypeptide" as used herein encompasses native polypeptides (either degradation products, synthetically synthesized polypeptides or recombinant polypeptides) and peptidomimetics (typically, synthetically synthesized polypeptides), as well as peptoids and semipeptoids which are polypeptide analogs, which may have, for example, modifications rendering the polypeptides more stable while in a body or more capable of penetrating into cells. Such modifications include, but are not limited to N' terminus modification, C' terminus modification, polypeptide bond modification,

including, but not limited to, CH2-NH, CH2-S, CH2-S=O, O=C-NH, CH2-O, CH2-CH2, S=C-NH, CH=CH or CF=CH, backbone modifications, and residue modifications. Methods for preparing peptidomimetic compounds are well known in the art and are specified, for example, in Quantitative Drug Design, C.A. Ramsden Gd., Chapter 17.2, F. Choplin Pergamon Press (1992), which is incorporated by reference as fully set forth herein. Further details in this respect are provided hereinunder.

Polypeptide bonds (-CO-NH-) within the polypeptide may be substituted, for example, by N-methylated bonds (-N(CH3)-CO-), ester bonds (-C(R)H-C-O-O-C(R)-N-), ketomethylen bonds (-CO-CH2-), α-aza bonds (-NH-N(R)-CO-), wherein R is any alkyl, e.g., methyl, carba bonds (-CH2-NH-), hydroxyethylene bonds (-CH(OH)-CH2-), thioamide bonds (-CS-NH-), olefinic double bonds (-CH=CH-), retro amide bonds (-NH-CO-), polypeptide derivatives (-N(R)-CH2-CO-), wherein R is the "normal" side chain, naturally presented on the carbon atom.

These modifications can occur at any of the bonds along the polypeptide chain and even at several (2-3) at the same time.

Natural aromatic amino acids, Trp, Tyr and Phe, may be substituted for synthetic non-natural acids such as Phenylglycine, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (TIC), naphthylelanine (Nol), ring-methylated derivatives of Phe, halogenated derivatives of Phe or o-methyl-Tyr.

As mentioned, the amino acid sequences of polypeptides of fibrous proteins may either be the amino acid sequences of the polypeptides in naturally-occurring fibrous proteins or those that comprise either conservative or non-conservative substitutions.

The term "conservative substitution" as used herein, refers to the replacement of an amino acid present in the native sequence in the peptide with a naturally or non-naturally occurring amino or a peptidomimetics having similar steric properties. Where the side-chain of the native amino acid to be replaced is either polar or hydrophobic, the conservative substitution should be with a naturally occurring amino acid, a non-naturally occurring amino acid or with a peptidomimetic moiety which is also polar or hydrophobic (in addition to having the same steric properties as the side-chain of the replaced amino acid).

As naturally occurring amino acids are typically grouped according to their properties, conservative substitutions by naturally occurring amino acids can be easily

determined bearing in mind the fact that in accordance with the invention replacement of charged amino acids by sterically similar non-charged amino acids are considered conservative substitutions.

For producing conservative substitutions by non-naturally occurring amino acids it is also possible to use amino acid analogs (synthetic amino acids) well known in the art. A peptidomimetic of the naturally occurring amino acids is well documented in the literature known to the skilled practitioner.

When effecting conservative substitutions the substituting amino acid should have the same or a similar functional group in the side chain as the original amino acid.

The phrase "non-conservative substitutions" as used herein refers to replacement of the amino acid as present in the parent sequence by another naturally or non-naturally occurring amino acid, having different electrochemical and/or steric properties. Thus, the side chain of the substituting amino acid can be significantly larger (or smaller) than the side chain of the native amino acid being substituted and/or can have functional groups with significantly different electronic properties than the amino acid being substituted. Examples of non-conservative substitutions of this type include the substitution of phenylalanine or cycohexylmethyl glycine for alanine, isoleucine for glycine, or -NH-CH[(-CH_2)$_5$-COOH]-CO- for aspartic acid. Those non-conservative substitutions which fall within the scope of the present invention are those which still constitute a polypeptide being able to form a fibrous protein.

As used herein in the specification and in the claims section below, the term "amino acid" or "amino acids" is understood to include the 20 naturally occurring amino acids; those amino acids often modified post-translationally *in vivo*, including, for example, hydroxyproline, phosphoserine and phosphothreonine; and other unusual amino acids including, but not limited to, 2-aminoadipic acid, hydroxylysine, isodesmosine, nor-valine, nor-leucine and ornithine. Furthermore, the term "amino acid" includes both D- and L-amino acids.

Tables 10 and 11 below list naturally occurring amino acids (Table10) and non-conventional or modified amino acids (Table 11) which can be used with the present invention.

Table 10

Amino Acid	Three-Letter Abbreviation	One-letter Symbol
alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic Acid	Glu	E
glycine	Gly	G
Histidine	His	H
isoleucine	Iie	I
leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
tryptophan	Trp	W
tyrosine	Tyr	Y
Valine	Val	V
Any amino acid as above	Xaa	X

Table 11

Non-conventional amino acid	Code	Non-conventional amino acid	Code
α-aminobutyric acid	Abu	L-N-methylalanine	Nmala
α -amino- α -methylbutyrate	Mgabu	L-N-methylarginine	Nmarg
aminocyclopropane-	Cpro	L-N-methylasparagine	Nmasn
carboxylate		L-N-methylaspartic acid	Nmasp
aminoisobutyric acid	Aib	L-N-methylcysteine	Nmcys
aminonorbornyl-	Norb	L-N-methylglutamine	Nmgin
carboxylate		L-N-methylglutamic acid	Nmglu
cyclohexylalanine	Chexa	L-N-methylhistidine	Nmhis
cyclopentylalanine	Cpen	L-N-methylisolleucine	Nmile
D-alanine	Dal	L-N-methylleucine	Nmleu
D-arginine	Darg	L-N-methyllysine	Nmlys
D-aspartic acid	Dasp	L-N-methylmethionine	Nmmet
D-cysteine	Dcys	L-N-methylnorleucine	Nmnle
D-glutamine	Dgln	L-N-methylnorvaline	Nmnva
D-glutamic acid	Dglu	L-N-methylornithine	Nmorn
D-histidine	Dhis	L-N-methylphenylalanine	Nmphe
D-isoleucine	Dile	L-N-methylproline	Nmpro
D-leucine	Dleu	L-N-methylserine	Nmser
D-lysine	Dlys	L-N-methylthreonine	Nmthr
D-methionine	Dmet	L-N-methyltryptophan	Nmtrp
D-ornithine	Dorn	L-N-methyltyrosine	Nmtyr
D-phenylalanine	Dphe	L-N-methylvaline	Nmval
D-proline	Dpro	L-N-methylethylglycine	Nmetg
D-serine	Dser	L-N-methyl-t-butylglycine	Nmtbug
D-threonine	Dthr	L-norleucine	Nle
D-tryptophan	Dtrp	L-norvaline	Nva
D-tyrosine	Dtyr	α -methyl-aminoisobutyrate	Maib
D-valine	Dval	α -methyl-γ-aminobutyrate	Mgabu
D- α -methylalanine	Dmala	α ethylcyclohexylalanine	Mchexa
D- α -methylarginine	Dmarg	α -methylcyclopentylalanine	Mcpen
D- α -methylasparagine	Dmasn	α -methyl- α -napthylalanine	Manap
D- α -methylaspartate	Dmasp	α - methylpenicillamine	Mpen

D- α -methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
D- α -methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
D- α -methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn
D- α -methylisoleucine	Dmile	N- amino- α -methylbutyrate	Nmaabu
D- α -methylleucine	Dmleu	α -napthylalanine	Anap
D- α -methyllysine	Dmlys	N-benzylglycine	Nphe
D- α -methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
D- α -methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
D- α -methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
D- α -methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
D- α -methylserine	Dmser	N-cyclobutylglycine	Ncbut
D- α -methylthreonine	Dmthr	N-cycloheptylglycine	Nchep
D- α -methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
D- α -methyltyrosine	Dmty	N-cyclodecylglycine	Ncdec
D- α -methylvaline	Dmval	N-cyclododeclglycine	Ncdod
D- α -methylalnine	Dnmala	N-cyclooctylglycine	Ncoct
D- α -methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro
D- α -methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
D- α -methylasparatate	Dnmasp	N-(2,2-diphenylethyl)glycine	Nbhm
D- α -methylcysteine	Dnmcys	N-(3,3-diphenylpropyl)glycine	Nbhe
D-N-methylleucine	Dnmleu	N-(3-indolylyethyl) glycine	Nhtrp
D-N-methyllysine	Dnmlys	N-methyl-γ-aminobutyrate	Nmgabu
N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmet
D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpen
N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
N-(2-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nva
D-N-methyltyrosine	Dnmtyr	N-methyla-napthylalanine	Nmanap
D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
γ-aminobutyric acid	Gabu	N-(*p*-hydroxyphenyl)glycine	Nhtyr
L-*t*-butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
L-ethylglycine	Etg	penicillamine	Pen
L-homophenylalanine	Hphe	L- α -methylalanine	Mala
L- α -methylarginine	Marg	L- α -methylasparagine	Masn
L- α -methylaspartate	Masp	L- α -methyl-*t*-butylglycine	Mtbug
L- α -methylcysteine	Mcys	L-methylethylglycine	Metg
L- α thylglutamine	Mgln	L- α -methylglutamate	Mglu
L- α -methylhistidine	Mhis	L- α -methylhomo phenylalanine	Mhphe
L- α -methylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet
D-N-methylglutamine	Dnmgln	N-(3-guanidinopropyl)glycine	Narg
D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr
D-N-methylhistidine	Dnmhis	N-(hydroxyethyl)glycine	Nser
D-N-methylisoleucine	Dnmile	N-(imidazolylethyl)glycine	Nhis
D-N-methylleucine	Dnmleu	N-(3-indolylyethyl)glycine	Nhtrp
D-N-methyllysine	Dnmlys	N-methyl-γ-aminobutyrate	Nmgabu
N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmet
D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpen
N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
D-N-methyltyrosine	Dnmtyr	N-methyla-napthylalanine	Nmanap
D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
γ-aminobutyric acid	Gabu	N-(*p*-hydroxyphenyl)glycine	Nhtyr
L-*t*-butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
L-ethylglycine	Etg	penicillamine	Pen

L-homophenylalanine	Hphe	L- α -methylalanine	Mala
L- α -methylarginine	Marg	L- α -methylasparagine	Masn
L- α -methylaspartate	Masp	L- α -methyl-*t*-butylglycine	Mtbug
L- α -methylcysteine	Mcys	L-methylethylglycine	Metg
L- α -methylglutamine	Mgln	L- α -methylglutamate	Mglu
L- α ethylhistidine	Mhis	L- α -methylhomophenylalanine	Mhphe
L- α thylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet
L- α -methylleucine	Mleu	L- α -methyllysine	Mlys
L- α -methylmethionine	Mmet	L- α -methylnorleucine	Mnle
L- α -methylnorvaline	Mnva	L- α -methylornithine	Morn
L- α -methylphenylalanine	Mphe	L- α -methylproline	Mpro
L- α -methylserine	mser	L- α -methylthreonine	Mthr
L- α ethylvaline	Mtrp	L- α -methyltyrosine	Mtyr
L- α -methylleucine	Mval nbhm	L-N-methylhomophenylalanine	Nmhphe
N-(N-(2,2-diphenylethyl)		N-(N-(3,3-diphenylpropyl)	
carbamylmethyl-glycine	Nnbhm	carbamylmethyl(1)glycine	Nnbhe
1-carboxy-1-(2,2-diphenyl hylamino)cyclopropane	Nmbc		

Table 11 Cont.

Amino acid sequences of exemplary polypeptides of the present invention are set forth in SEQ ID NOs: 11-13, 55, 57, 58 and SEQ ID NOs. 32-36.

Recombinant techniques are preferably used to generate the polypeptides of the present invention since these techniques are better suited for generation of relatively long polypeptides (e.g., longer than 20 amino acids) and large amounts thereof. Such recombinant techniques are described by Bitter et al., (1987) Methods in Enzymol. 153:516-544, Studier et al. (1990) Methods in Enzymol. 185:60-89, Brisson et al. (1984) Nature 310:511-514, Takamatsu et al. (1987) EMBO J. 6:307-311, Coruzzi et al. (1984) EMBO J. 3:1671-1680, Brogli et al., (1984) Science 224:838-843, Gurley et al. (1986) Mol. Cell. Biol. 6:559-565 and Weissbach & Weissbach, 1988, Methods for Plant Molecular Biology, Academic Press, NY, Section VIII, pp 421-463.

To produce a polypeptide of the present invention using recombinant technology, a polynucleotide encoding a polypeptide of the present invention is ligated into a nucleic acid expression vector, which comprises the polynucleotide sequence under the transcriptional control of a *cis*-regulatory sequence (e.g., promoter sequence) suitable for directing constitutive, tissue specific or inducible transcription of the polypeptides of the present invention in the host cells.

An example of an isolated polynucleotide which can be used to express resilin is as set forth in SEQ ID NO: 15. Examples of isolated polynucleotide sequences which can be used to express spider silk are as set forth in SEQ ID NOs: 23 and 27. An example of an isolated polynucleotide which can be used to express a cellulose binding

domain is set forth in SEQ ID NO: 25. Exemplary polynucleotide sequences which can be used to express the polypeptides of the present invention are set forth in SEQ ID NO: 17-22, 24, 28 and 29.

The phrase "an isolated polynucleotide" refers to a single or double stranded nucleic acid sequence which is isolated and provided in the form of an RNA sequence, a complementary polynucleotide sequence (cDNA), a genomic polynucleotide sequence and/or a composite polynucleotide sequences (e.g., a combination of the above).

As used herein, the phrase "complementary polynucleotide sequence" refers to a sequence which results from reverse transcription of messenger RNA using a reverse transcriptase or any other RNA-dependent DNA polymerase. Such a sequence can be subsequently amplified *in vivo* or *in vitro* using a DNA-dependent DNA polymerase.

As used herein, the phrase "genomic polynucleotide sequence" refers to a sequence derived (isolated) from a chromosome and thus represents a contiguous portion of a chromosome.

As used herein, the phrase "composite polynucleotide sequence" refers to a sequence, which is at least partially complementary and at least partially genomic. A composite sequence can include some exonal sequences required to encode the polypeptide of the present invention, as well as some intronic sequences interposing therebetween. The intronic sequences can be of any source, including of other genes, and typically will include conserved splicing signal sequences. Such intronic sequences may further include *cis*-acting expression regulatory elements.

The polynucleotides of the present invention may further comprise a signal sequence encoding a signal peptide for the secretion of the fibrous polypeptide. An exemplary signal sequence that may be used in the constructs of the present invention (for plant transfection) is a vacuolar signal sequence.

Following expression and secretion, the signal peptides are typically removed from the precursor proteins resulting in the mature proteins.

Polynucleotides of the present invention may be prepared using PCR techniques as described in Example 1 and Example 7 herein below, or any other method or procedure known in the art for ligation of two different DNA sequences. See, for example, "Current Protocols in Molecular Biology", eds. Ausubel et al., John Wiley & Sons, 1992.

As mentioned hereinabove, polynucleotide sequences of the present invention are inserted into expression vectors (i.e., a nucleic acid construct) to enable expression of the recombinant polypeptide. The expression vector of the present invention includes additional sequences which render this vector suitable for replication and integration in prokaryotes, eukaryotes, or preferably both (e.g., shuttle vectors). Typical cloning vectors contain transcription and translation initiation sequences (e.g., promoters, enhances) and transcription and translation terminators (e.g., polyadenylation signals).

Eukaryotic promoters typically contain two types of recognition sequences, the TATA box and upstream promoter elements. The TATA box, located 25-30 base pairs upstream of the transcription initiation site, is thought to be involved in directing RNA polymerase to begin RNA synthesis. The other upstream promoter elements determine the rate at which transcription is initiated.

Enhancer elements can stimulate transcription up to 1,000-fold from linked homologous or heterologous promoters. Enhancers are active when placed downstream or upstream from the transcription initiation site. Many enhancer elements derived from viruses have a broad host range and are active in a variety of tissues. For example, the SV40 early gene enhancer is suitable for many cell types. Other enhancer/promoter combinations that are suitable for the present invention include those derived from polyoma virus, human or murine cytomegalovirus (CMV), the long term repeat from various retroviruses such as murine leukemia virus, murine or Rous sarcoma virus and HIV. See, Enhancers and Eukaryotic Expression, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. 1983, which is incorporated herein by reference.

In the construction of the expression vector, the promoter is preferably positioned approximately the same distance from the heterologous transcription start site as it is from the transcription start site in its natural setting. As is known in the art, however, some variation in this distance can be accommodated without loss of promoter function.

In addition to the elements already described, the expression vector of the present invention may typically contain other specialized elements intended to increase the level of expression of cloned nucleic acids or to facilitate the identification of cells that carry the recombinant DNA. For example, a number of animal viruses contain DNA sequences that promote the extra chromosomal replication of the viral genome in

permissive cell types. Plasmids bearing these viral replicons are replicated episomally as long as the appropriate factors are provided by genes either carried on the plasmid or with the genome of the host cell.

The vector may or may not include a eukaryotic replicon. If a eukaryotic replicon is present, then the vector is amplifiable in eukaryotic cells using the appropriate selectable marker. If the vector does not comprise a eukaryotic replicon, no episomal amplification is possible. Instead, the recombinant DNA integrates into the genome of the engineered cell, where the promoter directs expression of the desired nucleic acid.

The expression vector of the present invention can further include additional polynucleotide sequences that allow, for example, the translation of several proteins from a single mRNA such as an internal ribosome entry site (IRES) and sequences for genomic integration of the promoter-chimeric polypeptide.

Examples for mammalian expression vectors include, but are not limited to, pcDNA3, pcDNA3.1(+/-), pGL3, pZeoSV2(+/-), pSecTag2, pDisplay, pEF/myc/cyto, pCMV/myc/cyto, pCR3.1, pSinRep5, DH26S, DHBB, pNMT1, pNMT41, pNMT81, which are available from Invitrogen, pCI which is available from Promega, pMbac, pPbac, pBK-RSV and pBK-CMV which are available from Strategene, pTRES which is available from Clontech, and their derivatives.

Expression vectors containing regulatory elements from eukaryotic viruses such as retroviruses can be also used. SV40 vectors include pSVT7 and pMT2. Vectors derived from bovine papilloma virus include pBV-1MTHA, and vectors derived from Epstein Bar virus include pHEBO, and p2O5. Other exemplary vectors include pMSG, pAV009/A$^+$, pMTO10/A$^+$, pMAMneo-5, baculovirus pDSVE, and any other vector allowing expression of proteins under the direction of the SV-40 early promoter, SV-40 later promoter, metallothionein promoter, murine mammary tumor virus promoter, Rous sarcoma virus promoter, polyhedrin promoter, or other promoters shown effective for expression in eukaryotic cells.

Viruses are very specialized infectious agents that have evolved, in many cases, to elude host defense mechanisms. Typically, viruses infect and propagate in specific cell types. The targeting specificity of viral vectors utilizes its natural specificity to specifically target predetermined cell types and thereby introduce a recombinant gene

into the infected cell. Thus, the type of vector used by the present invention will depend on the cell type transformed.

Recombinant viral vectors may be useful for expression of the polypeptides of the present invention since they offer advantages such as lateral infection. Lateral infection is inherent in the life cycle of, for example, retrovirus and is the process by which a single infected cell produces many progeny virions that bud off and infect neighboring cells. The result is that a large area becomes rapidly infected, most of which was not initially infected by the original viral particles. This is in contrast to vertical-type infection in which the infectious agent spreads only through daughter progeny. Viral vectors can also be produced that are unable to spread laterally. This characteristic can be useful if the desired purpose is to introduce a specified gene into only a localized number of targeted cells.

A variety of prokaryotic or eukaryotic cells can be used as host-expression systems to express the polypeptides of the present invention. These include, but are not limited to, microorganisms, such as bacteria (for example, E. coli including but not limited to E. coli strains BL21 (DE3) plysS, BL21;(DE3)RP and BL21* and B. subtilis) transformed with a recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vector containing the polypeptide coding sequence; yeast transformed with recombinant yeast expression vectors containing the polypeptide coding sequence; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors, such as Ti plasmid, containing the polypeptide coding sequence.

Various methods can be used to introduce the expression vector of the present invention into the cells of the host expression system. Such methods are generally described in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Harbor Laboratory, New York (1989, 1992), in Ausubel et al., Current Protocols in Molecular Biology, John Wiley and Sons, Baltimore, Md. (1989), Chang et al., Somatic Gene Therapy, CRC Press, Ann Arbor, Mich. (1995), Vega et al., Gene Targeting, CRC Press, Ann Arbor Mich. (1995), Vectors: A Survey of Molecular Cloning Vectors and Their Uses, Butterworths, Boston Mass. (1988) and Gilboa et at. [Biotechniques 4 (6): 504-512, 1986] and include, for example, stable or transient transfection, lipofection,

electroporation and infection with recombinant viral vectors. In addition, see U.S. Pat. Nos. 5,464,764 and 5,487,992 for positive-negative selection methods.

Introduction of nucleic acids by viral infection offers several advantages over other methods such as lipofection and electroporation, since higher transfection efficiency can be obtained due to the infectious nature of viruses.

According to one embodiment, the polypeptides of the present invention are expressed in plants.

The term "'plant" as used herein encompasses whole plants, ancestors and progeny of the plants and plant parts, including seeds, shoots, stems, roots (including tubers), and plant cells, tissues and organs. The plant may be in any form including suspension cultures, embryos, meristematic regions, callus tissue, leaves, gametophytes, sporophytes, pollen, and microspores. Plants that are particularly useful in the methods of the invention include all plants which belong to the superfamily Viridiplantee, in particular monocotyledonous and dicotyledonous plants including a fodder or forage legume, ornamental plant, food crop, tree, or shrub selected from the list comprising Acacia spp., Acer spp., Actinidia spp., Aesculus spp., Agathis australis, Albizia amara, Alsophila tricolor, Andropogon spp., Arachis spp, Areca catechu, Astelia fragrans, Astragalus cicer, Baikiaea plurijuga, Betula spp., Brassica spp., Bruguiera gymnorrhiza, Burkea africana, Butea frondosa, Cadaba farinosa, Calliandra spp, Camellia sinensis, Canna indica, Capsicum spp., Cassia spp., Centroema pubescens, Chacoomeles spp., Cinnamomum cassia, Coffea arabica, Colophospermum mopane, Coronillia varia, Cotoneaster serotina, Crataegus spp., Cucumis spp., Cupressus spp., Cyathea dealbata, Cydonia oblonga, Cryptomeria japonica, Cymbopogon spp., Cynthea dealbata, Cydonia oblonga, Dalbergia monetaria, Davallia divaricata, Desmodium spp., Dicksonia squarosa, Dibeteropogon amplectens, Dioclea spp, Dolichos spp., Dorycnium rectum, Echinochloa pyramidalis, Ehraffia spp., Eleusine coracana, Eragrestis spp., Erythrina spp., Eucalypfus spp., Euclea schimperi, Eulalia vi/losa, Pagopyrum spp., Feijoa sellowlana, Fragaria spp., Flemingia spp, Freycinetia banksli, Geranium thunbergii, GinAgo biloba, Glycine javanica, Gliricidia spp, Gossypium hirsutum, Grevillea spp., Guibourtia coleosperma, Hedysarum spp., Hemaffhia altissima, Heteropogon contoffus, Hordeum vulgare, Hyparrhenia rufa, Hypericum erectum, Hypeffhelia dissolute, Indigo incamata, Iris spp., Leptarrhena pyrolifolia, Lespediza spp., Lettuca spp., Leucaena

leucocephala, Loudetia simplex, Lotonus bainesli, Lotus spp., Macrotyloma axillare, Malus spp., Manihot esculenta, Medicago saliva, Metasequoia glyptostroboides, Musa sapientum, Nicotianum spp., Onobrychis spp., Ornithopus spp., Oryza spp., Peltophorum africanum, Pennisetum spp., Persea gratissima, Petunia spp., Phaseolus spp., Phoenix canariensis, Phormium cookianum, Photinia spp., Picea glauca, Pinus spp., Pisum sativam, Podocarpus totara, Pogonarthria fleckii, Pogonaffhria squarrosa, Populus spp., Prosopis cineraria, Pseudotsuga menziesii, Pterolobium stellatum, Pyrus communis, Quercus spp., Rhaphiolepsis umbellata, Rhopalostylis sapida, Rhus natalensis, Ribes grossularia, Ribes spp., Robinia pseudoacacia, Rosa spp., Rubus spp., Salix spp., Schyzachyrium sanguineum, Sciadopitys vefficillata, Sequoia sempervirens, Sequoiadendron giganteum, Sorghum bicolor, Spinacia spp., Sporobolus fimbriatus, Stiburus alopecuroides, Stylosanthos humilis, Tadehagi spp, Taxodium distichum, Themeda triandra, Trifolium spp., Triticum spp., Tsuga heterophylla, Vaccinium spp., Vicia spp., Vitis vinifera, Watsonia pyramidata, Zantedeschia aethiopica, Zea mays, amaranth, artichoke, asparagus, broccoli, Brussels sprouts, cabbage, canola, carrot, cauliflower, celery, collard greens, flax, kale, lentil, oilseed rape, okra, onion, potato, rice, soybean, straw, sugar beet, sugar cane, sunflower, tomato, squash tea, trees. Alternatively, algae and other non-Viridiplantae can be used for the methods of the present invention.

It will be appreciated that in order to express the polypeptides of the present invention in plants, the constructs encoding same typically comprise a plant-expressible promoter.

As used herein, the phrase "plant-expressible" refers to a promoter sequence, including any additional regulatory elements added thereto or contained therein, is at least capable of inducing, conferring, activating or enhancing expression in a plant cell, tissue or organ, preferably a monocotyledonous or dicotyledonous plant cell, tissue, or organ. One exemplary promoter that may be useful in the constructs of the present invention is the RbcS1 promoter (SEQ ID NO: 30), either in addition to or in absence of SEQ ID NO: 31, as exemplified in the Examples section herein under. Of note, other sequences may also be used for plant expression such as set forth in SEQ ID NOs: 48 and 50.

Nucleic acid sequences of the polypeptides of the present invention may be optimized for plant expression. Examples of such sequence modifications include, but are not limited to, an altered G/C content to more closely approach that typically found in the plant species of interest, and the removal of codons atypically found in the plant species commonly referred to as codon optimization.

The phrase "codon optimization" refers to the selection of appropriate DNA nucleotides for use within a structural gene or fragment thereof that approaches codon usage within the plant of interest. Therefore, an optimized gene or nucleic acid sequence refers to a gene in which the nucleotide sequence of a native or naturally occurring gene has been modified in order to utilize statistically-preferred or statistically-favored codons within the plant. The nucleotide sequence typically is examined at the DNA level and the coding region optimized for expression in the plant species determined using any suitable procedure, for example as described in Sardana *et al.* (1996, Plant Cell Reports 15:677-681). In this method, the standard deviation of codon usage, a measure of codon usage bias, may be calculated by first finding the squared proportional deviation of usage of each codon of the native gene relative to that of highly expressed plant genes, followed by a calculation of the average squared deviation. The formula used is: 1 SDCU = n = 1 N [(Xn - Yn) / Yn] 2 / N, where Xn refers to the frequency of usage of codon n in highly expressed plant genes, where Yn to the frequency of usage of codon n in the gene of interest and N refers to the total number of codons in the gene of interest. A table of codon usage from highly expressed genes of dicotyledonous plants is compiled using the data of Murray *et al.* (1989, Nuc Acids Res. 17:477-498).

One method of optimizing the nucleic acid sequence in accordance with the preferred codon usage for a particular plant cell type is based on the direct use, without performing any extra statistical calculations, of codon optimization tables such as those provided on-line at the Codon Usage Database through the NIAS (National Institute of Agrobiological Sciences) DNA bank in Japan (www.kazusa.or.jp/codon/). The Codon Usage Database contains codon usage tables for a number of different species, with each codon usage table having been statistically determined based on the data present in Genbank.

By using the above tables to determine the most preferred or most favored codons for each amino acid in a particular species (for example, rice), a naturally-occurring nucleotide sequence encoding a protein of interest can be codon optimized for that particular plant species. This is effected by replacing codons that may have a low statistical incidence in the particular species genome with corresponding codons, in regard to an amino acid, that are statistically more favored. However, one or more less-favored codons may be selected to delete existing restriction sites, to create new ones at potentially useful junctions (5' and 3' ends to add signal peptide or termination cassettes, internal sites that might be used to cut and splice segments together to produce a correct full-length sequence), or to eliminate nucleotide sequences that may negatively effect mRNA stability or expression.

The naturally-occurring encoding nucleotide sequence may already, in advance of any modification, contain a number of codons that correspond to a statistically-favored codon in a particular plant species. Therefore, codon optimization of the native nucleotide sequence may comprise determining which codons, within the native nucleotide sequence, are not statistically-favored with regards to a particular plant, and modifying these codons in accordance with a codon usage table of the particular plant to produce a codon optimized derivative. A modified nucleotide sequence may be fully or partially optimized for plant codon usage provided that the protein encoded by the modified nucleotide sequence is produced at a level higher than the protein encoded by the corresponding naturally occurring or native gene. Construction of synthetic genes by altering the codon usage is described in for example PCT Patent Application 93/07278.

Thus, the present invention encompasses nucleic acid sequences described hereinabove; fragments thereof, sequences hybridizable therewith, sequences homologous thereto, sequences orthologous thereto, sequences encoding similar polypeptides with different codon usage, altered sequences characterized by mutations, such as deletion, insertion or substitution of one or more nucleotides, either naturally occurring or man induced, either randomly or in a targeted fashion.

Exemplary polynucleotide sequences that may be used to express the polypeptides of the present invention in plants are set forth in SEQ ID NOs: 20-22.

Plant cells may be transformed stably or transiently with the nucleic acid constructs of the present invention. In stable transformation, the nucleic acid molecule of the present invention is integrated into the plant genome and as such it represents a stable and inherited trait. In transient transformation, the nucleic acid molecule is expressed by the cell transformed but it is not integrated into the genome and as such it represents a transient trait.

There are various methods of introducing foreign genes into both monocotyledonous and dicotyledonous plants (Potrykus, I., Annu. Rev. Plant. Physiol., Plant. Mol. Biol. (1991) 42:205-225; Shimamoto et al., Nature (1989) 338:274-276).

The principle methods of causing stable integration of exogenous DNA into plant genomic DNA include two main approaches:

(i) Agrobacterium-mediated gene transfer: Klee et al. (1987) Annu. Rev. Plant Physiol. 38:467-486; Klee and Rogers in Cell Culture and Somatic Cell Genetics of Plants, Vol. 6, Molecular Biology of Plant Nuclear Genes, eds. Schell, J., and Vasil, L. K., Academic Publishers, San Diego, Calif. (1989) p. 2-25; Gatenby, in Plant Biotechnology, eds. Kung, S. and Arntzen, C. J., Butterworth Publishers, Boston, Mass. (1989) p. 93-112.

(ii) direct DNA uptake: Paszkowski et al., in Cell Culture and Somatic Cell Genetics of Plants, Vol. 6, Molecular Biology of Plant Nuclear Genes eds. Schell, J., and Vasil, L. K., Academic Publishers, San Diego, Calif. (1989) p. 52-68; including methods for direct uptake of DNA into protoplasts, Toriyama, K. et al. (1988) Bio/Technology 6:1072-1074. DNA uptake induced by brief electric shock of plant cells: Zhang et al. Plant Cell Rep. (1988) 7:379-384. Fromm et al. Nature (1986) 319:791-793. DNA injection into plant cells or tissues by particle bombardment, Klein et al. Bio/Technology (1988) 6:559-563; McCabe et al. Bio/Technology (1988) 6:923-926; Sanford, Physiol. Plant. (1990) 79:206-209; by the use of micropipette systems: Neuhaus et al., Theor. Appl. Genet. (1987) 75:30-36; Neuhaus and Spangenberg, Physiol. Plant. (1990) 79:213-217; glass fibers or silicon carbide whisker transformation of cell cultures, embryos or callus tissue, U.S. Pat. No. 5,464,765 or by the direct incubation of DNA with germinating pollen, DeWet et al. in Experimental Manipulation of Ovule Tissue, eds. Chapman, G. P. and Mantell, S. H. and Daniels,

W. Longman, London, (1985) p. 197-209; and Ohta, Proc. Natl. Acad. Sci. USA (1986) 83:715-719.

Although stable transformation is presently preferred, transient transformation of leaf cells, meristematic cells or the whole plant is also envisaged by the present invention.

Transient transformation can be effected by any of the direct DNA transfer methods described above or by viral infection using modified plant viruses.

Viruses that have been shown to be useful for the transformation of plant hosts include CaMV, TMV and BV. Transformation of plants using plant viruses is described in U.S. Pat. No. 4,855,237 (BGV), EP-A 67,553 (TMV), Japanese Published Application No. 63-14693 (TMV), EPA 194,809 (BV), EPA 278,667 (BV); and Gluzman, Y. et al., Communications in Molecular Biology: Viral Vectors, Cold Spring Harbor Laboratory, New York, pp. 172-189 (1988). Pseudovirus particles for use in expressing foreign DNA in many hosts, including plants, is described in WO 87/06261.

Independent of the host cell system, it will be appreciated that other than containing the necessary elements for the transcription and translation of the inserted coding sequence (encoding the polypeptide), the expression construct of the present invention can also include sequences engineered to optimize stability, production, purification, yield or activity of the expressed polypeptide.

Transformed cells are cultured under effective conditions, which allow for the expression of high amounts of recombinant polypeptide. Effective culture conditions include, but are not limited to, effective media, bioreactor, temperature, pH and oxygen conditions that permit protein production. An effective medium refers to any medium in which a cell is cultured to produce the recombinant polypeptide of the present invention. Such a medium typically includes an aqueous solution having assimilable carbon, nitrogen and phosphate sources, and appropriate salts, minerals, metals and other nutrients, such as vitamins. Cells of the present invention can be cultured in conventional fermentation bioreactors, shake flasks, test tubes, microtiter dishes and petri plates. Culturing can be carried out at a temperature, pH and oxygen content appropriate for a recombinant cell. Such culturing conditions are within the expertise of one of ordinary skill in the art.

Depending on the vector and host system used for production, resultant polypeptides of the present invention may either remain within the recombinant cell, secreted into the fermentation medium, secreted into a space between two cellular membranes, such as the periplasmic space in *E. coli*; or retained on the outer surface of a cell or viral membrane.

Following a predetermined time in culture, recovery of the recombinant polypeptide is effected.

The phrase "recovering the recombinant polypeptide" used herein refers to collecting the whole fermentation medium containing the polypeptide and need not imply additional steps of separation or purification.

Thus, polypeptides of the present invention can be purified using a variety of standard protein purification techniques, such as, but not limited to, affinity chromatography, ion exchange chromatography, filtration, electrophoresis, hydrophobic interaction chromatography, gel filtration chromatography, reverse phase chromatography, concanavalin A chromatography, chromatofocusing and differential solubilization.

To facilitate recovery, the expressed coding sequence can be engineered to encode the polypeptide of the present invention and fused cleavable moiety e.g. histidine. Such a fusion protein can be designed so that the polypeptide can be readily isolated by affinity chromatography; e.g., by immobilization on a column specific for the cleavable moiety. Examples 3-5 and 8 describe purification of resilin and spidersilk polypeptides of the present invention.

Where a cleavage site is engineered between the polypeptide and the cleavable moiety, the polypeptide can be released from the chromatographic column by treatment with an appropriate enzyme or agent that specifically cleaves the fusion protein at this site [e.g., see Booth *et al.*, Immunol. Lett. 19:65-70 (1988); and Gardella *et al.*, J. Biol. Chem. 265:15854-15859 (1990)].

The polypeptide of the present invention is preferably retrieved in a "substantially pure" form.

As used herein, the phrase "substantially pure" refers to a purity that allows for the effective use of the protein in the applications described herein.

In addition to being synthesizable in host cells, the polypeptide of the present invention can also be synthesized using *in vitro* expression systems. These methods are well known in the art and the components of the system are commercially available.

Following expression and optional purification of the polypeptides of the present invention, the polypeptides may be polymerized to form an insoluble material from a solution, preferably one with a relatively high concentration of polypeptide. According to one embodiment, the critical concentration of a resilin polypeptide of the present invention is about 50 mg/ml. According to one embodiment, the polypeptide is concentrated by ultracentrifugation.

Generally, crosslinking of proteins can be performed using standard crosslinking agents such as gluteraldahyde, di-isocyanate and Genipin. Exemplary polymerization conditions for particular fibrous polypeptide monomers are presented herein below.

Crosslinking conditions for Resilin

According to a preferred embodiment, the crosslinking is such that dityrosine bonds are formed. These methods are well known to the person skilled in the art and are discussed by Malencik and Anderson (Biochemistry 1996, 35, 4375-4386), the contents of which are incorporated herein by reference.

In an embodiment, enzyme-mediated cross-linking in the presence of $Ru(bpy)3Cl_2 \cdot 6H_2O$ may be employed. Exemplary peroxidases that may be used to crosslink resilin include, but are not limited to horseradish peroxidase, Arthromyces peroxidase, Duox peroxidase from Caenorhabditis elegans, Sea urchin ovoperoxidases and Chorion.

Following irradiation, a Ru(III) ion is formed, which serves as an electron abstraction agent to produce a carbon radical within the polypeptide, preferentially at a tyrosine residue, and thus allows dityrosine link formation. This method of induction allows quantitative conversion of soluble resilin or pro-resilin fragments to a very high molecular weight aggregate. Moreover this method allows for convenient shaping of the bioelastomer by introducing recombinant resilin into a glass tube of the desired shape and irradiating the recombinant resilin contained therein.

In another embodiment, UV irradiation is effected in order to crosslink the resilin polypeptides of the present invention [Lehrer SS, Fasman GD. (1967) Biochemistry. 6(3):757-67; Malencik DA, Anderson SR. (2003) Amino Acids. 25(3-4):233-47],

although care must be taken not to damage the protein through exposure to this radiation. UVB radiation cross-linking may also be undertaken in the presence or absence of riboflavin. In the absence of riboflavin, a substantial amount of cross-linking takes place within one hour of exposure. The crosslinking time is substantially reduced if riboflavin is present. Still further, cross-linking may be effected with ultra-violet light in the presence of coumarin or by white light in the presence of fluorescein. An analysis of the dityrosine may be performed using conventional methods such as high performance liquid chromatography measurements in order to ascertain the extent of dityrosine cross-link formation.

Metal ions and H_2O_2 may also be used to induce dytirosine formation through Fenton's reaction [Ali FE, J Inorg Biochem. 98(1):173-84].

Crosslinking conditions for elastin:

Following heating above the transition temperature (Tm), elastin may be crosslinked using the following oxidizing agents: lysil oxidase bis(sulfosuccimidyl) suberate, pyrroloquinoline quinine (PQQ), catechol/peroxidase reagent, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide in the presence of *N*-hydroxybenzotriazole, *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC), 1-hydroxybenzotriazole hydrate (HOBt); 1,6-diisocyanatohexane (HOBt); glutaraldehyde; *N*-hydroxysuccinimide (NHS), genipin.

Elastin may also be crosslinked by γ-irradiation, or, following functionalization with methacrylate, it may also be photo-crosslinked.

Crosslinking conditions for silk:

Silk polypeptides, such as spider silk and silkworm silk may be polymerized into ß sheets using organic solvents, such as methanol. Alternatively, the silk polypeptides may be solubilized in water followed by dehydration in order to form ß sheets.

Crosslinking conditions for collagen:

Collagen may be crosslinked by glutaraldehyde and other chemical crosslinking agents, by glycation using different sugars, by Fenton reaction using metal ions such as cupper, by lysine oxidase or by UV radiation.

To determine the effect of cross-links and the optimal number of cross-links per monomer unit, the resilience of a cross-linked polymer can be measured using methods

known in the art. The level of cross-linking can vary provided that the resulting polymer displays the requisite resilient properties. For example, when the cross-linking is by gamma-irradiation, the degree of cross-linking is a function of the time and energy of the irradiation. The time required to achieve a desired level of cross-linking may readily be computed by exposing non-cross-linked polymer to the source of radiation for different time intervals and determining the degree of resilience (elastic modulus) of the resulting cross-linked material for each time interval. By this experimentation, it will be possible to determine the irradiation time required to produce a level of resiliency appropriate for a particular application.

The extent of cross-linking may be monitored during the reaction or pre-determined by using a measured amount of reactants. For example, in the case of resilin polypeptides, since-the dityrosine cross-link is fluorescent, the fluorescence spectrum of the reactant mixture may be monitored during the course of a reaction to determine the extent of cross-linking at any particular time. Once the desired level of cross-linking is achieved (indicated by reaching a predetermined fluorescence intensity) a peroxidase-catalysed reaction may be quenched by for example the addition of glutathione.

The polypeptides of the present invention may be used as are or they may be blended with polysaccharides in order to generate novel composite materials.

Thus, according to another aspect of the present invention, there is provided an isolated composite comprising a fibrous polypeptide and a polysaccharide.

As used herein the term "composite" refers to a a substantially solid material that is composed of two or more discrete materials, one being the fibrous polypeptide, the other the polysaccharide, each of which retains its identity, e.g., physical characteristics, while contributing desirable properties to the composite.

The term "isolated" as used herein refers to the composite being substantially free from other substances (e.g., other cells, proteins, nucleic acids, etc.) in its *in-vivo* environment (e.g in the case of resilin-chitin composites, removed from other insect wing components). According to another embodiment, the composites are also isolated from (i.e. removed from) solid supports (i.e. are non-immobilized).

Exemplary polysaccharides contemplated for the composites of the present invention include, but are not limited to chitin, cellulose, starch, dextran, glucan, chitosan, alginate and hyaluronic acid.

The cellulose may be in the form of powder such as Sigmacell, cellulose whiskers, cellulose threads or 3D structures such as paper or scaffolds. Whisker preparation is typically performed by hydrolysis of cellulose with 60 % H_2SO_2 for 1 to 6 hours at 60 °C followed by sonication. The suspension is then diluted in double distilled H_2O (DDW) followed by repeated cycles (at leats 5) of resuspension with DDW and centrifugation to remove the acid. Finally, the whiskers pellet is dialyzed against DDW while monitoring the pH up to 7. Whiskers quality may be monitored by Transfer Electron Microscopy (TEM).

According to one embodiment of this aspect of the present invention, the monomers of the fibrous polypeptides of the present invention comprise polysaccharide binding domains (e.g. heterologous polysaccharide binding domains). Such polysaccharide binding domains allows directional binding between the polysaccharide and the fibrous polypeptide at defined points of contact. Furthermore, the affinity of the fibrous polypeptide for the polysaccharide may be adjusted according to the polysaccharide binding domain.

Other composites which are contemplated by the present invention include those comprising two fibrous polypeptides wherein at least one of which comprises a heterologous polysaccharide binding domain.

Such composites may also comprise polysaccharides. Thus composites of two fibrous polypeptides and a polysaccharide are also contemplated by the present invention.

It is expected that the composites of the present invention comprise enhanced characteristics (e.g. increased strength) compared to the constituent fibrous polypeptide since the flat and ordered surface of the polysaccharide (e.g. cellulose whisker) may serve as a template for assembly of the fibrous polypeptide that usually requires shearing and elongation stress.

In order to generate the composites of the present invention, suspensions of monomers of the fibrous polypeptides and the polysaccharides (e.g. cellulose whiskers) – for example at approximately 2 % solid content, are blended together.

Exemplary ratio of the component suspension include: 100/0, 90/10, 80/20, 70/30, 60/40, 50/50, 40/60, 30/70, 20/80, 10/90, and 0/100.

The mixed solutions may be cast onto suitable molds (e.g. Teflon or polystyrene) following which appropriate assembly and crosslinking is optionally effected.

As mentioned above, the type of crosslinking depends on the fibrous polypeptide of the composite. The crosslinking may be effected in the presence of other fibrous polypeptides to generate the two fibrous polypeptide/polysaccharide composites described herein above.

The present invention also contemplates coating the novel composites. According to one embodiment, the coating is comprised of fibrous polypeptides. In this method, following the crosslinking of the composites, dipping in solutions of other fibrous polypeptides may be carried out. The fibrous proteins in the coating will typically be absorbed into the composite. Following coating, a suitable polymerization method may be used depending on the actual fibrous polypeptide of the coating. For example, a cellulose-resilin composite may be dipped in a solution containing silk monomers. Subsequently the composite may be transferred into 90 % methanol solution which promotes silk ß-sheet formation resulting in a cellulose-resilin-silk composite material.

The composites of the present invention may be combined with other polymers in blends and adducts to manipulate the degradation and mechanical properties of the material. Practically any biocompatible polymer may be combined with the composites. In a preferred embodiment, the added polymer is biodegradable. Exemplary biodegradable polymers include natural polymers and their synthetic analogs, including polysaccharides, proteoglycans, glycosaminoglycans, collagen-GAG, collagen, fibrin, and other extracellular matrix components, such as elastin, fibronectin, vitronectin, and laminin. Hydrolytically degradable polymers known in the art include, for example, certain polyesters, polyanhydrides, polyorthoesters, polyphosphazenes, and polyphosphoesters. Biodegradable polymers known in the art, include, for example, certain polyhydroxyacids, polypropylfumerates, polycaprolactones, polyhydroxyalkanoates, poly (amide-enamines), polyamides, poly (amino acids), polyacetals, polyethers, biodegradable polycyanoacrylates, biodegradable polyurethanes and polysaccharides. For example, specific biodegradable polymers that may be used in the present invention include but are not limited to, polylysine, poly (lactic acid) (PLA),

poly (glycolic acid) (PGA), copolymers and mixtures of PLA and PGA, e. g. , poly (lactide-co-glycolide) (PLG), poly (caprolactone) (PCL), poly (lactide-co-caprolactone) (PLC), and poly (glycolide-co-caprolactone) (PGC).

Those skilled in the art will recognize that this is an exemplary, not comprehensive, list of biodegradable polymers. The properties of these and other polymers and methods for preparing them are further described in the art. See, for example, U. S. Patents Nos. 6,123, 727; 5,804, 178; 5,770, 417; 5,736, 372; 5,716, 404 to Vacanti ; 6,095, 148; 5,837, 752 to Shastri ; 5,902, 599 to Anseth; 5,696, 175; 5,514, 378; 5,512, 600 to Mikos; 5,399, 665 to Barrera; 5,019, 379 to Domb; 5,010, 167 to Ron; 4,806, 621; 4,638, 045 to Kohn; and 4,946, 929 to d'Amore; see also Wang et al., J. Am. Chem. Soc. 123: 9480, 2001 ; Lim et al., J. Am. Chem. Soc. 123: 2460,2001 ; Langer, Acc. Chen7. Res. 33: 94,2000 ; Langer, J. Control Release 62: 7,1999 ; and Uhrich et al., Chem. Rev. 99: 3181,1999.

The composites of the present invention may also be combined with non-biodegradable polymers. For example, polypyrrole, polyanilines, polythiophene, and derivatives thereof are useful electrically conductive polymers that can provide additional stimulation to seeded cells or neighboring tissue. Exemplary non-biodegradable polymers include, but are not limited to, polystyrene, polyesters, non-biodegradable polyurethanes, polyureas, poly (ethylene vinyl acetate), polypropylene, polymethacrylate, polyethylene, polycarbonates, and poly (ethylene oxide).

The importance of biopolymer based biomaterials is constantly increasing in the field of reconstructive medicine. In the recent years, the focus of this field has turned from the search for inert materials for implantation to development of biopolymer based materials that interact with the tissue and promote its correct regeneration. Furthermore, synthetic implants often fail the test of long term biocompatibility requiring their replacement during the lifetime of a patient which is a major drawback. With respect to reconstructive medicine, polysaccharides and protein polymers have been extensively investigated.

When used in vivo, and in particular inside the body of a subject, e.g., a human patient, it is important that the composites of the present invention be biocompatible. A "biocompatible" material is not substantially mutagenic, antigenic, inflammatory, pyrogenic, or hemolytic. Furthermore, it must neither exhibit substantial cytotoxicity,

acute systemic toxicity, or intracutaneous toxicity, nor significantly decrease clotting time. In vivo and in vitro tests for these undesirable biological activities are well known in the art; examples of such assays are given, for example, in U.S. Pat. No. 5,527,610, the contents of which are incorporated by reference. Also, when used in vivo, the materials may be biogradable.

In the event that toxicity or immunogenicity, for example, occurs in a relevant composite, methods for modulating these undesirable effects are known in the art. For example, "tanning" of the composite by treating it with chemicals such as aldehydes (e.g., glutaraldehyde) or metaperiodate will substantially decrease both toxicity and immunogenicity. Preferably, the composites used to make devices for in vivo use are also sterilizable.

As mentioned, the composites of the present invention may be used in the field of reconstructive medicine such as for the generation of scaffolds.

As used herein, the term "scaffold" refers to a 3D matrix upon which cells may be cultured (i.e., survive and preferably proliferate for a predetermined time period).

The scaffold may be fully comprised of the composites of the present invention, or may comprise a solid support on which is layered the composites of the present invention.

A "solid support," as used refers to a three-dimensional matrix or a planar surface (e.g. a cell culture plate) on which cells may be cultured. The solid support can be derived from naturally occurring substances (i.e., protein based) or synthetic substances. Suitable synthetic matrices are described in, e.g., U.S. Pat. Nos. 5,041,138, 5,512,474, and 6,425,222. For example, biodegradable artificial polymers, such as polyglycolic acid, polyorthoester, or polyanhydride can be used for the solid support. Calcium carbonate, aragonite, and porous ceramics (e.g., dense hydroxyapatite ceramic) are also suitable for use in the solid support. Polymers such as polypropylene, polyethylene glycol, and polystyrene can also be used in the solid support.

Therapeutic compounds or agents that modify cellular activity can also be incorporated (e.g. attached to, coated on, embedded or impregnated) into the scaffold composite material or a portion thereof. In addition, agents that act to increase cell attachment, cell spreading, cell proliferation, cell differentiation and/or cell migration

in the scaffold may also be incorporated into the scaffold. Such agents can be biological agents such as an amino acid, peptides, polypeptides, proteins, DNA, RNA, lipids and/or proteoglycans.

Suitable proteins which can be used along with the present invention include, but are not limited to, extracellular matrix proteins [e.g., fibrinogen, collagen, fibronectin, vimentin, microtubule-associated protein 1D, Neurite outgrowth factor (NOF), bacterial cellulose (BC), laminin and gelatin], cell adhesion proteins [e.g., integrin, proteoglycan, glycosaminoglycan, laminin, intercellular adhesion molecule (ICAM) 1, N-CAM, cadherin, tenascin, gicerin, RGD peptide and nerve injury induced protein 2 (ninjurin2)], growth factors [epidermal growth factor, transforming growth factor-α, fibroblast growth factor-acidic, bone morphogenic protein, fibroblast growth factor-basic, erythropoietin, thrombopoietin, hepatocyte growth factor, insulin-like growth factor-I, insulin-like growth factor-II, Interferon-β, platelet-derived growth factor, Vascular Endothelial Growth Factor and angiopeptin], cytokines [e.g., M-CSF, IL-1beta, IL-8, beta-thromboglobulin, EMAP-II, G-CSF and IL-10], proteases [pepsin, low specificity chymotrypsin, high specificity chymotrypsin, trypsin, carboxypeptidases, aminopeptidases, proline-endopeptidase, Staphylococcus aureus V8 protease, Proteinase K (PK), aspartic protease, serine proteases, metalloproteases, ADAMTS17, tryptase-gamma, and matriptase-2] and protease substrates.

Additionally and/or alternatively, the scaffolds of the present invention may comprise an antiproliferative agent (e.g., rapamycin, paclitaxel, tranilast, Atorvastatin and trapidil), an immunosuppressant drug (e.g., sirolimus, tacrolimus and Cyclosporine) and/or a non-thrombogenic or anti-adhesive substance (e.g., tissue plasminogen activator, reteplase, TNK-tPA, glycoprotein IIb/IIIa inhibitors, clopidogrel, aspirin, heparin and low molecular weight heparins such as enoxiparin and dalteparin).

The scaffolds of the present invention may be administered to subjects in need thereof for the regeneration of tissue such as connective tissue, muscle tissue such as cardiac tissue and pancreatic tissue. Examples of connective tissues include, but are not limited to, cartilage (including, elastic, hyaline, and fibrocartilage), collagen, adipose tissue, reticular connective tissue, embryonic connective tissues (including

mesenchymal connective tissue and mucous connective tissue), tendons, ligaments, and bone.

The composites of the present invention may thus be used for treating a cartilage or bone disease or condition.

Exemplary cartilage conditions include, but are not limited to osteoarthritis, limited joint mobility, gout, rheumatoid arthritis, osteoarthritis, chondrolysis, scleroderma, degenerative disc disorder and systemic lupus erythematosus.

As used herein, the term "treating" refers to inhibiting or arresting the development of a disease, disorder or condition and/or causing the reduction, remission, or regression of a disease, disorder or condition in an individual suffering from, or diagnosed with, the disease, disorder or condition. Those of skill in the art will be aware of various methodologies and assays which can be used to assess the development of a disease, disorder or condition, and similarly, various methodologies and assays which can be used to assess the reduction, remission or regression of a disease, disorder or condition.

As used herein, the term "subject" refers to mammals, including, but not limited to, humans, canines and horses.

It will be appreciated that the composites of the present invention comprises a myriad of medical uses other than for tissue regeneration and for treating cartilage and bone diseases including, but not limited to treatment of urinary incontinence (e.g. urethral bulking), as a healing aid for burn patients and as a dressing to prevent bleeding.

In addition, other medical applications may also benefit from the elasticity, biodegradability and/or biovavailabiliy of the composites of the present invention. For example, after abdominal surgery, the intestines and other abdominal organs tend to adhere to one another and to the abdominal wall. It is thought that this adhesion results from post-surgical inflammation, however, anti- inflammatory drugs delivered directly to the abdominal region dissipate quickly. The composites of the present invention (e.g. those comprising resilin) may be used to deliver anti-inflammatory drugs to the abdominal region.

A soft and flexible composite may be implanted between the abdominal wall and internal organs, for example, by attaching it to the abdominal wall, without cutting

internal organs, which would lead to infection. The anti-inflammatory drug can be released from the composite over a period of months. While previous researchers have attempted to use hydrogels, hyaluronic acid-based membranes, and other materials to solve these problems, such materials tend to degrade quickly in the body; a longer resident period is necessary to prevent adhesion.

In another embodiment, the composites of the present invention may be used to coat a metallic stent. Because the composites may be made flexible, they will expand with the stent without ripping, while the stiffness of the metal stent will prevent the composites from elastically assuming its previous shape. The composites being highly bioavailable may release heparin or other anti-coagulants or anti- inflammatory agents to prevent the formation of clots or scar tissue, which could close off the blood vessel or throw off a thrombus that could cause circulatory problems, including stroke, elsewhere in the body. Alternatively or in addition, angiogenic agents may be used to promote the remodeling of the blood vessel surrounding the stent. Indeed, any biomolecule, small molecule, or bioactive agent may be combined with the composites of the present invention. Such molecules may be covalently or non-covalently linked with the composites.

The composites of the present invention may also be used to prepare "long term" medical devices. Unlike typical permanent medical devices, the composites of the present invention will degrade over time. For example, the material may be fabricated into a biodegradable cardiac stent. Preferably, the composites are combined with a harder polymer that plastically forms for the production of stents. Exemplary polymers include any of the polymers listed above, preferably biodegradable polymers. The bio-rubber acts as a plasticizer that enables the stent to expand into the desired shape after implantation. The stent increases the diameter of the blood vessel to allow easier circulation, but, because the stent is biodegradable, surrounding blood vessels increase in diameter without thrombosis or covering the stent with scar tissue, which would reclose the blood vessel. The time the stent should remain in place and retain its shape before degradation will vary from patient to patient and depend partially on the amount of blockage and the age of the patient (e. g. , older patients require more time to heal). One skilled in the art will easily be able to adjust the molecular weight and cross-link density of the composites in the stent to adjust the

degradation rate. As for the coated stent, the degradable stent may release biomolecules, small molecules, bioactive agents, or some combination of these in situ.

The composites of the present invention may also be used to support in vivo sensors and catheters. The composites may be constructed into a chamber for an optical fiber-based sensor or a coating for a catheter that is inserted into the area of interest. In a sensor, the chamber contains a specific chromophore-bonded receptor for the molecule of interest. When an analyte attaches to the receptor, the chromophore will either emit or absorb light at an specific wavelength. The absorption or emission may be detected by an apparatus connected to the optical fiber. The sensor may be used for short term, continuous monitoring, for example, for ten to fifteen days. Likewise, a catheter may be used to periodically deliver drugs or other small molecules or bioactive agents to a specific site or intravenously. Use of biodegradable composites of the present invention reduces the formation of scar tissue which would ordinarily form around a shunt or other implant that is used for more than two weeks. The degradation rate of the composite should be optimized so that there is not significant degradation of the material while it is in place in the patient.

The composites of the present invention may also be used for other wounds that are hard to close or that fail to heal properly through normal physiologic mechanisms. For example, diabetics often get skin injuries ("diabetic ulcers"), especially in the lower extremities, which take a long time to heal or fail to heal properly due to poor circulation. The use of the present composites to deliver antibiotics or anti-inflammatory agents to these wounds will aid healing and provide a cover for the wound.

Other implantable medical devices which may be fabricated from the composites of the present invention include artificial blood vessels, catheters and other devices for the removal or delivery of fluids to patients, artificial hearts, artificial kidneys, orthopedic pins, plates and implants; catheters and other tubes (including urological and biliary tubes, endotracheal tubes, peripherably insertable central venous catheters, dialysis catheters, long term tunneled central venous catheters peripheral venous catheters, short term central venous catheters, arterial catheters, pulmonary catheters, Swan-Ganz catheters, urinary catheters, peritoneal catheters), urinary devices (including long term urinary devices, tissue bonding urinary devices, artificial

urinary sphincters, urinary dilators), shunts (including ventricular or arterio-venous shunts); prostheses (including breast implants, penile prostheses, vascular grafting prostheses, aneurysm repair devices, heart valves, artificial joints, artificial larynxes, otological implants), anastomotic devices, vascular catheter ports, clamps, embolic devices, wound drain tubes, hydrocephalus shunts, pacemakers and implantable defibrillators, and the like.

Of note, the cellulose produced by *Gluconacetobacter xylinus* is most suitable for medical applications. The Bacterial cellulose (BC) produced by these bacteria has high mechanical strength combined with negligible foreign body and inflammatory responses that make it an attractive material for development of medical applications. BC has excellent water retaining properties which make it suitable for production of chronic wound burn dressings and even artificial skin. Furthermore BC and BC composites can be shaped into almost any desired three-dimensional structure.

The composites of the present invention may be formulated as pharmaceutical and/or cosmetic compositions.

The term "cosmetic composition" as used herein refers to a composition formulated for external application to human or animal skin, nails, or hair for the purpose of beautifying, coloring, conditioning, or protecting the body surface. The present cosmetic composition can be in any form including for example: a gel, cream, lotion, makeup, colored cosmetic formulations, shampoo, hair conditioner, cleanser, toner, aftershave, fragrance, nail enamel, and nail treatment product.

The phrase "colored cosmetic formulation" refers to cosmetics containing pigment including for example eye shadow, lipsticks and glosses, lip and eye pencils, mascara, and blush.

As mentioned, the composites of the present invention may also be used as cosmetic agents for treatment of skin and hair.

Thus, the present invention contemplates the composites (e.g. comprising collagen) of the present invention as a substance which can be topically applied, optionally in combination with other active substance such as for example a vitamin (vitamin A, C, E or their mixtures) or other topically active substances including but not limited to avarol, avarone or plant extracts, such as Extr. Cepae or Extr. Echinaceae pallidae. The composites of the present invention may be formulated as topical agents

in the form of creams, ointments, lotions or gels such as a hydrogels e.g. on the basis of polyacrylate or an oleogel e.g. made of water and Eucerin.

Oleogels comprising both an aqueous and a fatty phase are based particularly on Eucerinum anhydricum, a basis of wool wax alcohols and paraffin, wherein the percentage of water and the basis can vary. Furthermore additional lipophilic components for influencing the consistency can be added, e.g. glycerin, polyethylene glycols of different chain length, e.g. PEG400, plant oils such as almond oil, liquid paraffin, neutral oil and the like. The hydrogels of the present invention can be produced through the use of gel-forming agents and water, wherein the first are selected especially from natural products such as cellulose derivatives, such as cellulose ester and ether, e.g. hydroxyethyl-hydroxypropyl derivatives, e.g. tylose, or also from synthetic products such as polyacrylic acid derivatives, such as Carbopol or Carbomer, e.g. P934, P940, P941. They can be produced or polymerized based on known regulations, from alcoholic suspensions by adding bases for gel formation.

The cosmetic compositions may comprise other agents capable of conditioning the body surface including, for example humectants; emollients; oils including for example mineral oil; and shine enhancers including for example dimethicone and cyclomethicone. The present conditioning agents may be included in any of the present pharmacological and/or cosmetic compositions.

As used herein a "pharmaceutical composition" refers to a preparation of one or more of the active ingredients described herein with other chemical components such as physiologically suitable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.

Herein the term "active ingredient" refers to the collagen accountable for the biological effect.

Hereinafter, the phrases "physiologically acceptable carrier" and "pharmaceutically acceptable carrier" which may be interchangeably used refer to a carrier or a diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound. An adjuvant is included under these phrases.

Herein the term "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of an active ingredient. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

Techniques for formulation and administration of drugs may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition, which is incorporated herein by reference.

Suitable routes of administration may, for example, include oral, rectal, transmucosal, especially transnasal, intestinal or parenteral delivery, including intramuscular, subcutaneous and intramedullary injections as well as intrathecal, direct intraventricular, intracardiac, e.g., into the right or left ventricular cavity, into the common coronary artery, intravenous, inrtaperitoneal, intranasal, or intraocular injections.

Alternately, one may administer the pharmaceutical composition in a local rather than systemic manner, for example, via injection of the pharmaceutical composition directly into a tissue region of a patient. Thus, for treatment of urinary incontinence, the compositions of the present invention may be administered directly to the area surrounding the urethra. For treatment of cartilage diseases, the compositions of the present invention may be administered by intra-articular administration via a joint (e.g. directly into the knee, elbow, hip, sternoclavicular, temporomandibular, carpal, tarsal, wrist, ankle, intervertebral disk or a ligamentum flavum. For disc replacement, the pharmaceutical compositions of the present invention may als be administered directly into the pulposus.

According to a particular embodiment of this aspect of the present invention, the composites of the present invention may be administered directly into the discs for total disc replacement, total disc nucleus pulposus replacement or disc nucleus polposus augmentation and repair or directly into the breast for breast augmentation. According to this embodiment, the composites may be comprised in injectable non-crosslinked formulations. Following injections of such formulations, photopolymerization may be initiated in situ. This may be effected using classical crosslinking techniques including gluteraldehyde, or crosslinking via sugar molecules.

According to one embodiment, in-situ crosslinking of the injectable formulation may be affected by addition of an appropriate buffer (e.g. PBS pH 7.4) together with 200 μM of $CuCl_2$ and 10 mM of H_2O_2 so as to generate dityrosine formation.

According to another embodiment, in situ crosslinking is effected using the same components described herein above, but the pH is maintained at 5.2. This leads to modification of the tyrosines into DOPA. Following injection of the materials 0.1-0.5 mM of Sodioum periodate may be added to form DOPA-DOPA bridges resulting in crossliniking of the fibrous polypeptides.

According to another embodiment, in situ crosslinking is effected using tyrosine crosslinking techniques involving H_2O_2 and radiation of the injected material with UV.

Pharmaceutical compositions of the present invention may be manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with the present invention thus may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active ingredients into preparations which, can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the active ingredients of the pharmaceutical composition may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological salt buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the pharmaceutical composition can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by nasal inhalation, the active ingredients for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from a pressurized pack or a nebulizer with the use of a suitable

propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane or carbon dioxide.

The pharmaceutical composition described herein may be formulated for parenteral administration, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multidose containers with optionally, an added preservative. The compositions may be suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical compositions for parenteral administration include aqueous solutions of the active preparation in water-soluble form. Additionally, suspensions of the active ingredients may be prepared as appropriate oily or water based injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acids esters such as ethyl oleate, triglycerides or liposomes. Aqueous injection suspensions may contain substances, which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the active ingredients to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water based solution, before use.

The pharmaceutical composition of the present invention may also be formulated in rectal compositions such as suppositories or retention enemas, using, e.g., conventional suppository bases such as cocoa butter or other glycerides.

Pharmaceutical compositions suitable for use in context of the present invention include compositions wherein the active ingredients are contained in an amount effective to achieve the intended purpose. More specifically, a therapeutically effective amount means an amount of active ingredients (composite) effective to prevent, alleviate or ameliorate symptoms of a disorder (e.g. cartilage or bone disease).

Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

For any preparation used in the methods of the invention, the therapeutically effective amount or dose can be estimated initially from in vitro and cell culture assays. For example, a dose can be formulated in animal models to achieve a desired concentration or titer. Such information can be used to more accurately determine useful doses in humans.

Toxicity and therapeutic efficacy of the active ingredients described herein can be determined by standard pharmaceutical procedures in vitro, in cell cultures or experimental animals. The data obtained from these in vitro and cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See e.g., Fingl, et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.1).

Dosage amount and interval may be adjusted individually to provide tissue levels of the active ingredient are sufficient to induce or suppress the biological effect (minimal effective concentration, MEC). The MEC will vary for each preparation, but can be estimated from in vitro data. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. Detection assays can be used to determine plasma concentrations.

Depending on the severity and responsiveness of the condition to be treated, dosing can be of a single or a plurality of administrations, with course of treatment lasting from several days to several weeks or until cure is effected or diminution of the disease state is achieved.

The amount of a composition to be administered will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc.

Compositions of the present invention may, if desired, be presented in a pack or dispenser device, such as an FDA approved kit, which may contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. The pack or dispenser may also be accommodated by a notice associated with the container in a form prescribed by a

governmental agency regulating the manufacture, use or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the compositions or human or veterinary administration. Such notice, for example, may be of labeling approved by the U.S. Food and Drug Administration for prescription drugs or of an approved product insert. Compositions comprising a preparation of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition, as is further detailed above.

The terms "comprises", "comprising", "includes", "including", “having” and their conjugates mean "including but not limited to".

The term “consisting of means “including and limited to”.

The term "consisting essentially of" means that the composition, method or structure may include additional ingredients, steps and/or parts, but only if the additional ingredients, steps and/or parts do not materially alter the basic and novel characteristics of the claimed composition, method or structure.

As used herein, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a polyeptide" or "at least one polypeptide" may include a plurality of compounds, including mixtures thereof.

Throughout this application, various embodiments of this invention may be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

As used herein the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known

manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination or as suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

Various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below find support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions, illustrate the invention in a nonlimiting fashion.

Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific American Books, New York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Culture of Animal Cells – A Manual of Basic Technique" by Freshney, Wiley-Liss, N. Y. (1994), Third Edition; "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Selected

Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available immunoassays are extensively described in the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait, M. J., ed. (1984); "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins S. J., eds. (1984); "Animal Cell Culture" Freshney, R. I., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic Press, San Diego, CA (1990); Marshak et al., "Strategies for Protein Purification and Characterization – A Laboratory Course Manual" CSHL Press (1996); all of which are incorpotaed by reference as if fully set forth herein. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader. All the information contained therein is incorporated herein by reference.

EXAMPLE 1

Construction of resilin chimeric genes

Preparation of resilin cDNA: According to Elvin *et al* [Nature. 437: 999-1002, 2005] resilin is mostly expressed at the pupa level in *D. melanogaster*. Therefore, RNA was extracted from this stage for cDNA preparation. RNA was extracted from *D. melanogaster* pupas using TRI® Reagent (Sigma, St. Louis, MO). Reverse transcription of the resilin cDNA was performed with M-MLV RT (H–) (Promega corporation, Madison WI) with oligo$(dT)_{15}$ primer according to the manufacturers instructions.

Construction of Resilin fusion proteins: Four resilin genes were designed for expression in *E. coli;*

1. Resilin 17 elastic repeats including the native putative chitin binding domain (gi|45550440, nucleotides 698-1888) referred to as Res-ChBD gene. (Protein sequence: SEQ ID NO: 11, 55; polynucleotide sequence: SEQ ID NO:17)

2. Resilin 17 elastic repeats and the native linker for N-terminal fusions and sole expression of a gene similar to Elvin *et al,* [Nature. 437: 999-1002, 2005] (nucleotides 698-1666) referred to as Resilin (Protein sequence: SEQ ID NO: 14, 56; polynucleotide sequence: SEQ ID NO: 15).
3. *Clostridium cellulovorans* CBD (CBDclos) fused to Resilin 17 elastic repeats referred to as CBD-resilin (Protein sequence: SEQ ID NO: 12, 57; polynucleotide sequence: SEQ ID NO: 18).
4. Resilin (Gene No. 2) fused to CBD, referred to as Resilin-CBD (Protein sequence: SEQ ID NO: 13, 58; polynucleotide sequence: SEQ ID NO: 19).

PCR primers were designed in order to construct the genes mentioned herein above as detailed in Table 12, herein below. A standard PCR method was designed suitable for all reactions: 94 °C for 4 minutes, 35 cycles of 94 °C for 1 minute, 56 °C for 1 minute, 72 °C for 1 minute and 72 °C for 4 minutes. All DNA products were separated on a 1 % agarose gel. Appropriate bands were excised with a scalpel and the DNA was purified with HiYield™ Gel/PCR DNA extraction kit (RBC Taipei, Taiwan).

Table 12

No	*Primer name*	*Sequence*	*Description*	*Tm° C*
1	resCBD1.1	CCATGGGACCGGAGCCACCAG TTAACTC (SEQ ID NO: 1)	Forward primer of resilin + NcoI site	55
2	resCHBDRev	GGATCCTTAAGGACCGCTGGG ACCACTG (SEQ ID NO: 2)	Reverse primer of resilin + chitin binding domain + BamHI site	57
3	resbmh1_rev	GGATCCCTCATCGTTATCGTAG TCAGCG (SEQ ID NO: 3)	Reverse primer of resilin 17 repeats + BamHI site for N-terminal fusion	56
4	CBD6Pl1	GTCTAGAAATAATTTTGTTTAA CTTTAAGAAGGAG (SEQ ID NO: 4)	Forward primer of CBD using pET-CSCP[37] as template + XbaI site	56
5	CBDRes2	AACTGGTGGCTCCGGCATATCA AATGTTGCAGAAGTAGGATTA ATTATTG (SEQ ID NO: 5)	Reverse primer of CBD + resilin overhang (pink) for PCR fusion cloning	56
6	CBDRes3	TTCTGCAACATTTGATCCGGAG CCACCAGTTAACTC (SEQ ID NO: 6)	Forward primer of resilin + CBD overhang (blue) for PCR fusion cloning	56
7	CBDRes4	GGATCCTTACTCATCGTTATCG TAGTCAGCG (SEQ ID NO: 7)	Reverse primer of resilin 17 repeats + stop codon and BamHI site	56

Construction of Res-ChBD: Res-ChBD was the first gene that was constructed directly from the cDNA and served as PCR template for cloning of all the other resilin

genes. The PCR was performed according to Table 13 herein below. Ex Taq™ (Takara, Madison WI) is a proofreading enzyme suitable for TA cloning.

Table 13

Ingredient	*Volume (µl)*
TaKaRa Ex Taq™ (5 units/µl)	0.25
10× Ex Taq Buffer (Mg^{2+} plus)	5
dNTP Mixture (2.5 mM each)	4
Resilin cDNA	1
Primer 1 resCBD1.1 (10 µmol)	1
Primer 2 resCHBDRev (10 µmol)	1
Sterilized distilled water	Up to 50

The 1200 base pair product (Figure 1B) was purified and cloned into pGEM-T Easy vector (Promega Corporation, Madison WI). The presence of resilin-ChBD was verified by sequencing. The sequencing was performed using T7 and Sp6 primers that are complimentary to pGEM-T Easy vector. The sequencing results confirmed the cloning of the two resilin variants according to Ardelll and Anderson [Insect Biochem Mol Biol. 31: 965-70, 2002]. Variant A was chosen for further work.

Finally, the Res-ChBD was digested with NcoI, NotI restriction enzymes and cloned into pHis-parallel3 vector (Figure 2) which contains an N-terminal His tag and a rTev cleavage site enabling purification of the protein on Ni-NTA column and removal of the His tag if desired.

Construction of CBD-resilin: This gene was constructed by PCR-fusion method [Hobert O. (2002) Biotechniques. 32: 728-30]. A pET-CSCP vector [Levy et al., 2004, Biomaterials 25: 1841–1849] was used as template for CBD amplification by PCR and the Res-ChBD product described herein above was used as template for resilin amplification. In the first round, two separate PCRs were performed. The CBD was amplified using primers No. 4 & 5. The resilin was amplified with primers 6 & 7. The first amplification was performed with Deep $Vent_R$ DNA Polymerase (NEB Inc. Ipswich, MA). By the end of the reactions, 1 µl of each product (Figure 3A) were mixed to serve as a template for the second step PCR. In this step, primers 4 and 7 were used. The PCR was performed under the same conditions except the usage of Ex Taq™ (Takara, Madison WI) to allow TA cloning. The 1600 base pair product was purified and cloned into pBluescript SK+ (Ferments, MD) (Figure 3B). The presence of CBD-

resilin was verified by sequencing with T7 and T3 primers. The complete gene was digested with NcoI and NotI enzymes and cloned into pHis-parallel3 vector.

Construction of Resilin-CBD and Resilin (Genes 2 & 4): The resilin gene was amplified using primers No. 1 and 3. The enzyme used for amplification was PfuTurbo® (Stratagene corporation, LA Jolla CA). The PCR mixture used for generating DNA encoding Resilin-CBD and Resilin (genes 2 and 4) is described in Table 14, herein below.

Table 14

Ingredient	*Volume (µl)*
PfuTurbo® (Stratagene corporation, LA Jolla CA)	1
10× cloned Pfu DNA polymerase reaction buffer	5
dNTP Mixture (2.5 mM each)	1
pGEM-T-ResCHBD (10ng/µl)	1
Primer 1 resCBD1.1 (10 µmol)	1
Primer 3 resbmh1_rev (10 µmol)	1
Sterilized distilled water	Up to 30

Following the PCR reaction described herein above, 7 µl of 10x Taq polymerase reaction buffer, 1 µl of Taq polymerase (Bio-lab, Israel), 1 µl of dNTP mixture and sterilized distilled water to a volume of 100 µl was added to the reaction tube. The tube was then incubated at 72 °C for 30 minutes in order to add A nucleotides to the PCR product. The final product was purified and cloned into pGEM-T Easy vector (Promega corporation, Madison WI). The presence of the resilin gene was verified by sequencing as described above.

For construction of Resilin-CBD gene, the resilin fragment was digested with NcoI, BamHI and cloned into pET3d (Novagen, EMD Chemicals, Inc. CA) upstream to the CBDclos gene followed by digestion of the Resilin-CBD with NcoI, EcoRI and cloning into pHis-parallel3 vector digested with the same enzymes.

Resilin expression vector (gene 2) was constructed by digestion of pGEM-T Easy-Resilin with NcoI, NotI. In this way a stop codon was added to the gene that allowed its direct expression. The gene was subsequently cloned into pHis-parallel3 digested with the same enzymes.

EXAMPLE 2

Expression of resilin chimeric genes

All four vectors were transformed into BL21(DE3) (Novagen, EMD Chemicals, Inc. CA). 5 ml of overnight cultures were grown in LB medium with 100 mg/L ampicillin at 37 °C rotary shaker. These starters were used for inoculation of 250 to 350 ml of LB with 100 mg/L ampicillin at a ratio of 1/100 of starter to culture volume. At O.D.600 of 0.8 to 0.9 expression was induced with 1mM IPTG. Four hours after induction, bacteria were harvested by centrifugation. 6H-Res-ChBD pellet was divided to 50 ml aliquots for initial analysis and the pellets were stored at -80 °C.

EXAMPLE 3

Purification of resilin-ChBD and characterization thereof

Small scale batch purification of 6H-Res-ChBD: Bacterial pellet of 50 ml was re-suspended in 2 ml of 100 mM Tris pH 7.5, 0.1 % Triton® X-100, Complete™ (Roche, Basel Switzerland). Bacteria were lyzed by sonication with pulsed bursts for 2 minutes on ice. The soluble and bacterial precipitates were separated by centrifugation at 15000 RPM for 10 minutes at 4 °C. SDS-PAGE analysis revealed that the Res-ChBD product is mostly found in the soluble fraction (Figure 4 lanes 1, 2). 500 µl of lysate were added into 1.5 ml eppendorf tube containing 75 µl pre-equilibrated HIS-Select® Nickel Affinity Gel, (Sigma, St. Louis, MO). Purification was performed according to the product manual. Final elution was repeated twice with 100 µl elution buffer containing 0.4 M imidazole.

Binding assay of purified 6H-Res-ChBD to cellulose and chitin: 25 mg of chitin (Sigma) and 50 mg of cellulose (Sigmacell) were added to two separate 1.5 ml eppendorf tubes. The materials were washed with PBS followed by addition of 50 µl of affinity purified protein solution. 450 µl of PBS were added to each tube to a total reaction volume of 500 µl. A third tube containing chitin only was supplemented with 500 µl of PBS as negative control since practical grade chitin (Sigma Cat No. C7170) that contained proteins was used. The tubes were incubated under gentle spinning for 30 minutes at RT followed by centrifugation. The supernatant was removed (unbound fraction) and the pellets were washed 3 times with 500 µl of PBS. The final pellets were boiled with 50 µl of 2x sample application buffer (SAB). Samples of unbound and wash

fractions from each tube were also boiled with SAB. Samples were loaded on 12.5 % SDS-PAGE gel.

Binding assay of crude extracts of 6H-Res-ChBD to cellulose and chitin: Bacterial lysates were produced from 50 ml pellets as described above. Cellulose and chitin binding assays were performed with 3 increasing lysate volumes as described in Table 15 below, in 2 ml eppendorf tubes.

Table 15

Tube No.	*Lysate volume (µl)*	*10x PBS(µl)*	*DDH_2O(µl)*	*Carbohydrate*
1	50	50	400	Cellulose 50 mg
2	125	50	325	Cellulose 50 mg
3	250	50	200	Cellulose 50 mg
4	50	50	400	Chitin 25 mg
5	125	50	325	Chitin 25 mg
6	250	50	200	Chitin 25 mg

6H-Res-ChBD Thermostability assay: 15 µl of affinity purified protein were added to 3 0.5 ml eppendorf tubes. The tubes were incubated at 85 °C for 15, 30, 60 minutes. By the end of the incubation the tubes were transferred to ice and centrifuged at 14000 rpm for 10 minutes. Subsequently, the samples were boiled with 2x SAB and loaded on 12.5 % SDS-PAGE gel.

Small-scale FPLC purification of 6H-Res-ChBD: Bacterial lysates were produced from 50 ml pellets as described above. The lysate was filtered with a syringe filter of 0.45 µm for the purpose of FPLC (GE, Uppsala Sweden) purification on HisTrap™ HP (GE, Uppsala Sweden) Ni-NTA 1 ml column pre-equilibrated according to the user manual.

The purification program was run as follows:

Binding buffer; 20 mM $NaHPO_4$, 0.5 M NaCl, 10 mM imidazole

Elution buffer; 20 mM $NaHPO_4$, 0.5 M NaCl, 0.5 M imidazole

1. 5 column volumes (CV) of binding buffer at 1ml/min.
2. 5 ml injection of the lysate at 1 ml/min
3. 5 CV wash with the binding buffer.
4. linear gradient up to 500 mM imidazole for 10 min at 0.7 ml/min with the elution buffer
5. Equilibration with 5 CV of binding buffer at 1ml/min.

Eluted proteins were detected at O.D.280. 400 µl fractions were collected and 10 µl of samples boiled with SAB were loaded on a 12.5 % SDS-PAGE gel.

Production of soluble high molecular weight 6H-res-ChBD: FPLC fractions 9 to 18 were collected to a total volume of 2 ml and imidazole was removed by three dialyses against 200 ml of polymerization buffer; 15 mM NaH_2PO_4, 150 mM NaCl pH 7.5. 500 µl of dialyzed protein was incubated at 85 °C for 10 min followed by O.N. incubation at 4 °C. Polymerization was performed by adding 20 µl of 40 mM ammonium persulfate and 20 µl of 0.5 mM $Ru(bpy)3Cl_2 \cdot 6H_2O$ (Sigma) dissolved in the polymerization buffer to an Eppendorf tube containing 40 µl of the purified protein. The samples were subjected to sun light for 5 minutes followed by boiling with 2x SAB. Samples were loaded on 12.5% SDS-PAGE gel.

M*edium-scale FPLC purification of 6H-Res-ChBD:* Bacterial pellets from 200 ml culture were resuspended in 15 ml of lysis buffer as described above. Bacteria were lyzed by sonication with pulsed bursts for 5 minutes in an ice bath. The soluble and bacterial precipitates were separated by centrifugation at 15000 RPM for 10 minutes at 4 °C. The purification was performed with FPLC using the same method as described above. Eluted proteins were detected at O.D.280. 400 µl fractions were collected and 10 µl of samples boiled with SAB were loaded on a 12.5 % SDS-PAGE gel.

Production of solid 6H-Res-ChBD: Following medium-scale FPLC purification described herein above, the fractions were collected into two different dialysis bags; concentrated fractions No. 4 to 7 (6ml) and diluted fractions No. 3, 8-12. The dialysis was performed as described above. The concentration of the concentrated peak was 10 mg/ml by O.D. 280 nm measurement. The sample was loaded on a Vivaspin 6 10,000 MWCO (Sartorius Stedim Biotech, Aubagne, France) ultrafiltration tube and centrifuged at 5000 g for 40 minutes. The final product gave around 500 µl at protein concentration of 160 mg/ml. 40 µl of concentrated protein were pipette into an eppendorf tube that was added 4 µl of 250 mM ammonium persulfate and 1 µl of 0.5mM $Ru(bpy)_3Cl_2 \cdot 6H_2O$. Immediately following the exposure of the tube to sunlight a solid polymer formed in the tube. The reaction was stopped after 5 minutes by washing the polymer with water when no more polymerization could be observed.

RESULTS

Small-scale batch purification of 6H-Res-ChBD: Purification was effected as illustrated in Figure 4 lanes 3-7.

Binding assay of purified 6H-Res-ChBD to cellulose and chitin: Coomassie blue staining of the proteins revealed that 6H-Res-ChBD binds both to chitin and cellulose with a higher affinity to chitin (Figure 5). The presence of protein in the unbound fraction is explained due the saturation chitin/cellulose with Res-ChBD protein.

Binding assay of crude extracts of 6H-Res-ChBD to cellulose and chitin: Coomassie blue staining of the gels revealed that no cellulose binding was detected in crude lysates comprising the 6H-Res-ChBD (Figure 6A, 6B lanes 2-5), contrasting the binding results following affinity chromatography AC purification. Nevertheless, the affinity of the protein to chitin remained high as displayed by the crude lysates results. At 50 and 125 µl of crude lysate loaded on 25 mg of chitin, nearly 100 % of the protein precipitated and very little protein remained in the unbound fraction (Figure 6B lanes 7-10, Figure 6C lanes 2-5). When 250 µl of lysate were applied, the amount of bound protein continued to increase but a larger band was detected in the unbound fraction probably due to saturation of the binding sites (Figure 6C lanes 6-9).

6H-Res-ChBD Thermostability assay: Heat treatment displayed that 6H-Res-ChBD is stable at 85 °C for 1 hour (Figure 7). As indicated in the Materials and Methods, the proteins were immediately transferred to ice following the heat treatment. This could explain the band shift observed in the gel due to initiation of coacervation process.

Small-scale FPLC purification of 6H-Res-ChBD: The results of the purification process are illustrated in Figures 8A-B.

Production of soluble high molecular weight 6H-Res-ChBD: The results of the solubilization process are illustrated in Figure 9.

Medium-scale *FPLC purification of 6H-Res-ChBD:* The results of the purification process are illustrated in Figures 10A-B.

EXAMPLE 4

Expression and Purification of 6H- resilin 17 elastic repeats without any polysaccharide binding domain (PBD) (SEQ ID NO: 56)

Following expression of the resilin of SEQ ID NO: 56 in E.coli, the soluble protein was purified on a Ni-NTA column as illustrated in Figure 11. In addition, the protein was found to be thermostable and was polymerized into solid resilin in the same manner as resilin-ChBD.

EXAMPLE 5

Purification of CBD-resilin (SEQ ID NO: 57) and characterization thereof

Following expression of CBD-resilin in bacteria, it was found to be expressed in inclusion bodies (Figure 12).

Cells were lyzed by sonication in 0.1% Triton® X-100, Complete™ (Roche, Basel Switzerland).

The insoluble fraction was precipitated by centrifugation.

The supernatant was removed and the inclusion bodies were washed as follows:

1. Resuspension with PBS buffer, 1 % Triton® X-100, 1 mM EDTA, for 30 minutes with gentle shaking followed by centrifugation.
2. Resuspension with PBS buffer, 1 % Triton® X-100, for 30 minutes with gentle shaking followed by centrifugation.
3. Resuspension with PBS buffer for 30 minutes with gentle shaking followed by centrifugation.

From that stage, purification of the inclusion bodies was performed by one of two methods.

1. Ni-NTA purification under denaturizing conditions. IBs were solubilzed in 20 mM phosphate buffer pH 7.5, 20 mM imidazole, 0.5 M NaCl, 6 M GuHCl. The proteins were loaded on pre-equilibrated Ni-NTA column and the proteins were eluted with a linear gradient of 20 mM phosphate buffer pH 7.5, 0.5 M imidazole, 0.5 M NaCl, 6 M GuHCl. The fractions containing the peak that was detected at O.D. 280 nm were collected and were refolded by dialysis against 50 mM Tris pH 7.5 buffer. The proteins were analyzed by SDS-PAGE. Refolding of the protein was assayed by cellulose binding assay (Figure 13).

2. Washed IB were solubilzed in 20 mM phosphate buffer pH 7.5, 20 mM imidazole, 0.5 M NaCl, 6 M GuHCl. The proteins were then injected to the ÄKTAprime™ plus (GE Healthcare, Uppsala Sweden) loaded with Ni-NTA column and purified using an automated refolding protocol that is programmed in the machine. The fractions containing the refolded proteins were collected (Figure 14) followed by cellulose binding assay. The automated refolded CBD-resilin protein was found mostly in the bound fraction similar to the proteins refolded via standard protocols, involving dialysis of samples purified in the presence of 6M GuHCl or 8M urea, indicating that this method can be applied since it is highly efficient and time saving.

EXAMPLE 6

Cloning and expression of resilin-CBD (SEQ ID NO: 58)

A DNA fragment coding for resilin 17 elastic repeats + putative resilin linker was cloned upstream to a vector containing the CBD to generate a polynucleotide of SEQ ID NO: 19. The correct insertion was verified by sequence followed by cloning of the gene into pHis parallel3 for protein expression. Expression was performed in BL21 bacteria similarly to all the other proteins. Following protein expression the bacteria were centrifuged and lyzed as described for CBD-resilin. The soluble and insoluble fractions were separated by centrifugation. SDS-PAGE analysis revealed that about 50 % of the recombinant protein was found in the soluble fraction. A cellulose binding assay was performed directly on resilin-CBD crude lysates resulting in high affinity binding of resilin-CBD to cellulose (see Figure 17).

EXAMPLE 7

Purification of resilin-CBD (SEQ ID NO: 58)

Following resilin-CBD expression, BL21 bacteria were centrifuged and lyzed as described for the other proteins. The soluble and insoluble fractions were separated by centrifugation. The lysate was filtered with a syringe filter of 0.45 μm. Proteins were then loaded on to a preequilibrated Ni-NTA column and were eluted with a linear gradient of 20 mM phosphate buffer (pH 7.5, 0.5 M imidazole, 0.5 M NaCl). The fractions containing the peak that was detected at O.D. 280nm were pooled and dialyzed

three times against phosphate buffer saline (PBS) to remove the imidazole. The proteins were boiled with X2 sample application buffer (SAB) and analyzed by Coomassie-stained SDS-PAGE (Figure 16).

Table 16 herein below summarizes the cloned resilin proteins described herein.

Table 16

Protein	*Sequence*	*Number of elastic repeats*	*Expression vector*	*Expressed in*
Resilin	SEQ ID NO: 56	17	pHis-parallel3	BL21(DE3)
Resilin-ChBD	SEQ ID NO: 55	17	pHis-parallel3	BL21(DE3)
CBD-Resilin	SEQ ID NO: 57	17	pHis-parallel3	BL21(DE3)
Resilin-CBD	SEQ ID NO: 58	17	pHis-parallel3	BL21(DE3)

EXAMPLE 8

Heat resistance and cellulose binding assay of resilin-CBD (SEQ ID NO: 58)

A sample solution containing the purified resilin-CBD protein was incubated at 85 °C for 15 minutes followed by centrifugation for 15 minutes at 14,000 rpm. 50µl of the heated protein solution was added to 30 mg of cellulose powder (Sigmacell) for the purpose of cellulose binding assay as described in Example 3. The cellulose binding assay was also performed with a non-heated resilin-CBD solution as control. As shown in Figure 17, the resilin-CBD protein displays both heat resistance and efficient binding capacity to cellulose that was not compromised by the heat treatment.

EXAMPLE 9

Solubility of resilin proteins in solutions of different pH

MATERIALS AND METHODS

There is increasing evidence that reactive oxygen species (ROS)-induced oxidative stress resulting from enzymatic or metal-catalyzed oxidation (MCO) reactions, can highly affect protein side chains and overall character. Tyrosine is one of the most ROS-sensitive residues in proteins. Its oxidation products include 3,4-dihydroxyphenylalanine (DOPA), dopamine, dopamine quinine, dityrosine (DT) and isoDT. In addition, DOPA is the major product of hydroxyl radical treatment of tyrosine (Ali F.E. et al., Journal of inorganic biochemistry 2004, 98, 173-184). According to Ali *et al* (2004), MCO of tyrosine in solutions of varying pHs results in varying products such as dityrosine and 3,4-dihydroxyphenylalanine (DOPA).

In order to use the MCO system to achieve these modifications on the resilin proteins, their stability under such pH conditions was analyzed.

Protein solutions of resilin and resilin-ChBD (pH ~7.5) were gently titered with 2M HCl to pH 5.6 or pH 5.4,. During the titration, 200 µl samples, representing different pH between the starting point, and the final pH were collected. The samples were incubated at 4 °C for 72 hours to allow for protein precipitation and then centrifuged for 15 minutes at 14000 rpm. The soluble proteins were detected on a Coomassie-stained SDS-PAGE.

RESULTS

In both cases, massive protein precipitation was observed at approximately pH 5. As illustrated in Figure 18, the proteins remained in solutions of pH up to 5.6 and 5.4, respectively, demonstrating the pH range of solubility of these recombinant proteins. With these fundamental determinations, the effect of MCO on resilin side chains can be studied.

EXAMPLE 10

Light induced polymerization of resilin proteins products in different pH.

MATERIALS AND METHODS

Resilin and resilin-ChBD protein solutions (50µl) at varying pH, containing 0.5 mM of $Ru(bpy)3Cl_2 \cdot 6H_2O$ and 2.5mM of ammonium persulfate (APS) were subjected to sunlight for 10 minutes followed by protein separation and detection on a Coomassie-stained SDS-PAGE. Protein samples without $Ru(bpy)3Cl_2 \cdot 6H_2O$ and APS were used as control.

RESULTS

In all the samples containing the $Ru(bpy)3Cl_2 \cdot 6H_2O$ and APS, high molecular weight products were formed. Nevertheless, the pattern of the seemingly crosslinked products differed according to the pH (Figure 19, see arrow).

EXAMPLE 11

Metal-catalyzed polymerization of Resilin

MATERIALS AND METHODS

Purified resilin was dialyzed three times against either 50 mM phosphate buffer (pH 7.5) or deionized water. Following the dialysis, the proteins were incubated at 85 °C for 15 minutes and subsequently centrifuged for 30 minutes at 10000 rpm. Generally, the polymerization was performed according to the MCO method reported by Kato *et al* (2001) (Kato Y, Kitamoto N, Kawai Y, Osawa T. (2001) The hydrogen peroxide/copper ion system, but not other metal-catalyzed oxidations systems, produces protein-bound dityrosine. Free Radical Biology & Medicine, 31,(5), 624–632*)* and Ali *et al* (Ali FE, Barnham KJ, Barrow CJ, Separovic F. (2004) Metal catalyzed oxidation of tyrosine residues by different oxidation systems of copper/hydrogen peroxide. J Inorg Biochem. 98(1):173-84). All the reactions were performed at a final volume of 250 µl in 1.5 ml eppendorf tubes. The MCO polymerization was performed by adding 4 mmol H_2O_2 (1 µl of 30 % H_2O_2) and 200 µM $CuCl_2$ (2.5 µl of 20 mM $CuCl_2$ dissolved in H_2O) followed by O.N. incubation at 37 °C. Tubes with protein solutions only, protein solutions with H_2O_2 only or $CuCl_2$ only were used as negative controls. The reactions were terminated by adding 1 mM EDTA. Finally, the samples were boiled in X2 SAB and were analyzed by SDS-PAGE.

RESULTS

Polymerization was achieved in both phosphate buffer and water, as displayed in Figure 20. Further analysis of these results is under way.

EXAMPLE 12

Preparation of recombinant resilin-cellulose whisker composites

MATERIALS AND METHODS

His tag-purified protein solutions containing 10 mg/ml of 6H-Res-ChBD (SEQ ID NO: 55) were mounted onto a 10 kDa cutoff Vivaspin Centrifugal Concentrator (Sartorius, UK) and centrifuged at 6000 rpm to a concentration of 100mg/ml. At this stage, a 200 µl sample was removed and stored for later analysis, while the rest of the solution was further concentrated to 200 mg/ml concentration.

6H-Res-ChBD-cellulose whiskers composites were produced by casting equal volumes of 200 mg/ml 6H-Res-ChBD-cellulose whiskers solution and cellulose whiskers solution (prepared as describe in Bondeson D, Mathew A, Oksman K. (2006) Cellulose 13:171–180) into 150 µl and 75 µl Teflon molds resulting in final protein concentration of 100 mg/ml. 150 µl of a 100 mg/ml pure 6H-Res-ChBD solution was poured into a similar mold as control. Subsequently 250 µM of $Ru(bpy)_3$ and 2.5 mM of ammonium persulfate (APS) were added to each sample solution. The mixtures were homogenized in the molds by pipeting, followed by polymerization by induced crosslinking via exposure to a 500 W tungsten light for 5 seconds.

RESULTS

The 150 µl 6H-Res-ChBD sample (Figure 21B – far right) and the 75 and 150 µl 6H-Res-ChBD-cellulose whiskers sample composites (Figure 21B – middle and left, respectively) were removed from the mold and sent to Differential Scanning Calorimetry (DSC) for further analysis.

EXAMPLE 13

Construction of spider silk-CBD fusion genes

MATERIALS AND METHOS

The spider silk (SpS) is a synthetic gene (SEQ ID NO: 23) optimized for expression in *E.coli*. Its sequence is a design of 15 repeats of a monomer consensus derived from the native sequence of the spidroin 1 sequence of Nephila clavipes (Accession P19837).

The SpS synthetic gene was provided in a pET30a vector, which contains an N and C terminal His tag and an Enterokinase cleavage site enabling purification of the protein on Ni-NTA column and removal of the N-terminal His tag if desired.

Construction of* SpS-*CBD fusion genes for expression in E.coli: *Clostridium cellulovorans* CBD (CBDclos) (SEQ ID NO: 25) was fused to the 3' of the spider silk synthetic gene. The fusion gene is referred to as SpS-CBD (SEQ ID NO: 24).

PCR primers were designed in order to construct the SpS-CBD fusion gene as summarized in Table 17 herein below. The PCR primers will add an N-terminal SpeI and a C-terminal XhoI restriction sites to the CBDclos gene template.

Table 17

SEQ ID No.	*Primer name*	*Sequence*	*description*	*Tm°C*
37	CBDSpeI_for	GACTAGTATGGCAGC GACATCATCAATGTC	Forward primer of CBD160 + SpeI site	56
47	CBDSXhoI_rev	CTCGAGATCAAATGT TGCAGAAGTAGGATT AATTATTG	Reverse primer of CBD160 + XhoI site	56

The CBDclos gene served as a PCR template for cloning of the fusion genes. A standard PCR was performed using Ex Taq™ (Takara, Madison WI), which is a proof reading enzyme suitable for TA cloning. The PCR product was purified from a 1 % agarose gel and was cloned into pGEM-T Easy vector (Promega Corporation, Madison WI). The presence of SpeI-CBDclos-XhoI was verified by sequencing.

Cloning of SpS-CBD - The SpeI-CBDclos-XhoI was cloned into SpeI and XhoI restriction sites on pET30a-SpS vector.

Construction of spider silk genes optimized for expression in tobacco plants: The synthetic dragline silk gene (GENEART GmbH Regensburg, Germany, SEQ ID NO: 27) is composed of a repeat unit, which was selected based on a consensus (GPGGQGPYGPGASAAAAAAGGYGPGYGQQGPGQQGPGQQ) SEQ ID NO:26 derived from the native sequence of the *Arenaus diadematus* ADF-3 gene (Accession U47855). Multimers encoding this repeat were developed by the use of the condensation method [Lewis et al., Protein Expression and Purification 7, 400-406 (1996)]. The synthetic gene includes the sequence of the monomer limited by the SmaI and NaeI restriction sites, which were used for the development of the multimers with the aid of another unique restriction site (AatII) on the pUC19 vector.

At the end of the spider silk monomer sequence there is an addition of the 3' non-repetitive sequence of the ADF-3 dragline gene. This sequence was shown to contribute to the solubility of the protein [Lazaris et al., Science 295: 472-476 (2002)]. At the 5' of the silk monomer a partial sequence of a synthetic CBDclos gene was added as described herein below.

Construction of 6 monomer (6mer) spider silk gene: In order to construct a 6mer spider silk gene a double digest was performed as follows:

1. Digest of the synthetic monomer (SEQ ID NO: 26) with SmaI and AatII.
2. Digest of the synthetic monomer (SEQ ID NO: 26) with NaeI and AatII.

The DNA products were purified on a 1 % agarose gel and the ligation of the purified fragments yielded a 2mer spider silk gene. Subsequently, a condensation of 2mers was performed to create a 4mer gene and a 4mer and a 2mer were condensed to form a 6mer gene.

Construction of 6mer-CBDclos fusion genes: The sequence of the CBDclos was optimized for expression in *tobacco* plants. The CBD synthetic DNA was fused to the 5' of the silk monomer. In order to construct a full length CBDclos-6mer fusion, a digest of BclI and NcoI restriction sites on the partial CBD-6mer gene and the full length non synthetic CBDclos was performed.

The fusion of the CBD to the 6mer gene was made in two orientations:

1. Two 6mer repeats were fused to the 3' terminal end of CBDclos to create CBDclos-SpS12 (SEQ ID NO: 28). The condensation of two 6mers was performed as described above.

2. CBDclos was fused in the middle of two 6mer repeats. The fusion gene is referred to as SpS6-CBD-SpS6 (SEQ ID NO: 29). The cloning of the two 6mers was performed by double digestion of one CBD-6mer plasmid with SmaI and NaeI and the other with StuI. The fragments were purified and ligated to form SpS6-CBD-SpS6.

Both CBD-12mer and SpS6-CBD- SpS6 were cloned into Rubisco's small subunit cassette (includes regulatory elements, such as the promoter ,terminator, 5'and 3' untranslated regions cloned from *Chrysanthemum sp.*) SEQ ID NOs: 30 and 31, on the pBINPLUS binary vector. Another expression cassette which was used includes the Cell signal peptide for secretion of the fusion proteins to the apoplast. This signal was fused to the 5' of the fusion genes before the 5'UTR of the Rubisco's small subunit gene.

Table 18 summarizes the cloned spider silk proteins described herein.

Table 18

Protein	***Number of monomer repeats***	***Expression vector/tag***	***Expressed in***
Spider silk (SpS) (SEQ ID NO: 33)	15	pET30a/His	BL21(DE3)
Spider silk-CBD (SpS-CBD) (SEQ ID NO: 34)	15	pET30a/His	BL21(DE3)
CBD-spider silk (CBD-SpS12) (SEQ ID NO:	12	pBINPLUS/Cell	N. tabacum-SR1

28)			
Spider silk-CBD-spider silk (SpS6-CBD-SpS6) (SEQ ID NO: 29)	12	pBINPLUS	N. tabacum-SR1

EXAMPLE 14

Expression and Purification of SpS-CBD fusion genes

MATERIALS AND METHODS

Expression of SpS and SpS-CBDclos proteins in E.coli: The pET30a-SpS and pET30a-SpS-CBDclos vectors were transformed into BL21(DE3) (Novagen, EMD Chemicals, Inc. CA). 5 ml of over night cultures were grown in LB medium with 50 mg/l kanamicin at 37 °C on a rotary shaker. These starters were used for inoculation of 250 to 350 ml of LB with 50 mg/l kanamycin at a ratio of 1/100 of starter to culture volume. At O.D.600 of 0.6 to 0.9, expression was induced with 1 mM IPTG. Following four hours from induction, bacteria were harvested by centrifugation. One pellet was divided to 50 ml aliquots for initial analysis and the pellets were stored at -80 °C.

FPLC purification of 6H-SpS and 6H-SpS-CBD: Bacterial pellet of 300 ml was re-suspended in 5 ml of 100 mM Tris pH 7.5, 0.1 % Triton® X-100, Complete™ (Roche, Basel Switzerland). Bacteria were lyzed by sonication with pulsed bursts for 5 minutes on an ice bath. The soluble and bacterial precipitates were separated by centrifugation at 15000 rpm for 10 minutes at 4 °C. The soluble fraction of the proteins was filtered with a syringe filter of 0.45 μm for the purpose of FPLC (GE, Uppsala Sweden) purification on HisTrap™ HP (GE, Uppsala Sweden) Ni-NTA 1 ml column pre-equilibrated according to the user manual.

The purification program was run as follows:

Binding buffer; 20 mM $NaHPO_4$, 0.5 M NaCl, 10 mM imidazole

Elution buffer; 20 mM $NaHPO_4$, 0.5 M NaCl, 0.5 M imidazole

1. 5 column volumes (CV) of binding buffer at 1ml/min.
2. 5 ml injection of the lysate at 1 ml/min
3. 10 CV wash with the binding buffer.
4. linear gradient up to 500 mM imidazole for 15 minutes at 1 ml/min with the elution buffer
5. Equilibration with 10 CV of binding buffer at 1ml/min.

Eluted proteins were detected at O.D.280. 500 µl fractions were collected and 20 µl of samples boiled with SAB were loaded on a 10% SDS-PAGE gel.

Expression of CBD-SpS12 and SpS6-CBD-SpS6 proteins in tobacco plants

Transformation of tobacco plants: The binary pBINPLUS vector including the Robisco's expression cassette and the fusion genes were introduced into A. tumefaciens strain LBA4404 for plant transformation. Leaf-disc transformation was performed with N. tabacum-SR1 plants as described previously (DeBlock et al., 1984 The EMBO Journal vol. 3 no.8 pp. 1681 - 1689, 1984). More than 15 independent tobacco transformants were generated for each construct, propagated in vitro and transferred to the greenhouse. The presence of the transgene was confirmed by PCR on genomic DNA using specific primers for the Robisco's cassette terminator/promoter. T1 seeds obtained by self-pollination of transformants were harvested and selected further on germination medium containing kanamycin (300 mg l-1). The sterilization treatment was for 30 secends in 70 % ethanol followed by 5 minutes 2.5 % NaOCl.

Expression of CBD-SpS12 and SpS6-CBD-SpS6 by T1 homozygous plants: Protein extraction was performed by grinding 90 mg of transgenic tobacco leaves with chilled extraction buffer (50 mM Tris-HCL pH=7.5, "complete"-protease inhibitor cocktail tablets. Roche-Cat# 1697498) in a tissueLyser (Retch Mixer Mill Type MM301 /220-240V 50/60HZ.cat# 20.741.0001). Separation of soluble and insoluble fractions was done by centrifugation at 15000 rpm for 10 minutes at 4 °C. Soluble and insoluble fractions were boiled with SAB.

Purification of CBD-SpS12 and SpS6-CBD-SpS6 from transgenic tobacco plants: 20mg of transgenic leaves in 40 ml purification buffer (50 mM Tris-HCL pH=7.5, 10 mM DTT, 0.5 gr cellulose Sigmacell20, PMSF 1 mM were ground in a blender till a uniform mixture was obtained. Separation of soluble and insoluble fractions was performed by centrifugation at 14000 rpm for 15 minutes at 4 °C. The insoluble fraction, which includes the bound CBD fusion proteins, was washed extensively twice in 30 ml extraction buffer each. The bound proteins were eluted from the cellulose pellet by suspension in elution buffer (50 mM Tris-HCL pH=12.5, 10mM DTT, 0.1 %Triton) for 1hour in a shaking rotor. Separation of the soluble fraction, which includes the eluted CBD fusion protein, was effected by centrifugation at 14000 RPM for 15 minutes at 4 °C.

Further purification of SpS6-CBD-SpS6: The eluted soluble protein from the procedure detailed above was dialyzed against 5 liter of heat stability test buffer (50 mM sodium phosphate pH=8, 10 mM DTT) over night. Then the sample was centrifuged at 14000 RPM for 10 minutes at 4 °C. The soluble protein was subjected to heat treatment in 60-90 °C for 10 minutes, followed by 20 minutes on ice, and centrifuging at a maximum speed for 10 minutes. The soluble protein was also tested for its solubility at a wide range of pHs from 8-2. The pH of the heat stability test buffer was adjusted with 2M HCL until the pH of the solution reached pH=2. For every pH coordinate, a sample was taken for analysis and incubated at 4°C overnight. To separate soluble from insoluble, the samples were centrifuged at a maximum speed for 10minutes. The soluble proteins were boiled with SDS-PAGE sample application buffer (SAB).

Qualitative binding assay of purified SpS and SpS-CBD to cellulose: 30 mg of cellulose (Sigmacell) were added to 1.5 ml eppendorf tubes. The materials were washed with PBS followed by addition of 50µl of affinity purified protein solution. 450 µl of PBS were added to each tube to a total reaction volume of 500 µl. The tubes were incubated under gentle spinning for 30 minutes at RT followed by centrifugation. The supernatant was removed (unbound fraction) and the pellets were washed for 3 times with 500 µl of PBS. The final pellets were boiled with 50 µl of SAB. Samples of unbound fraction from each tube were also boiled with SAB. Samples were loaded on 10% SDS-PAGE gel.

Quantitative binding reversibility assay of purified CBDclos, SpS and SpS-CBDclos to cellulose: 100 to 600 µg of SpS and SpS-CBD proteins in 500 µL PBS were adsorbed to 30 mg prewashed cellulose (Sigmacell) for 30 minutes at 25 °C. Desorption from the cellulose was performed, while the most concentrated protein:cellulose mixture (600 µg + 30mg cellulose) was diluted in individual test tubes to final protein quantity ranging from 600 to 100 µg, followed by mixing for an additional 30 minutes. After centrifugation at 13000 g for 10 minutes, the bound protein concentration was assayed by the Lowry method (The NaOH in the Lowry A solution elutes the bound proteins from the cellulose pellet).

RESULTS

Expression of SpS and SpS-CBDclos proteins in E.coli: The SpS and SpS-CBD proteins were successfully expressed in *E.coli* (Figure 22A). SDS-PAGE analysis of soluble and insoluble (IB content) proteins revealed that the SpS protein product is found in the soluble fraction, whereas SpS-CBD protein product is mostly found in the insoluble inclusion bodies (IB) fraction (Figure 22B).

FPLC purification of 6H-SpS and 6H-SpS-CBD: The SpS and SpS-CBD proteins were successfully purified on a Ni-NTA column (Figure 23A). The purified SpS and SpS-CBD were identified by anti-6HIS antibody (Figure 23B). When looking at the chromatogram of the purification on Ni-NTA (Figures 24A-C), a non specific protein peak can be observed in the control run (Figure 24A). The protein which was eluted is identified by literature as SlyD. This doesn't interfere with the SpS and SpS-CBD purification as SlyD elutes prior to the fusion proteins (Figure 24A lanes 5-7).

Qualitative binding assay of purified 15mer and 15mer-CBD to cellulose: Coomassie blue staining showed that the SpS-CBD was bound to cellulose, with no apparent protein revealed by Coomassie blue in the unbound fraction (Figure 25 lanes 5-7). The SpS is mostly found in the unbound fraction following the binding procedure (Figure 26 lanes 2-4). The SpS protein found in the bound fraction is nonspecifically adsorbed to cellulose. This phenomenon can be explained by the mechanism of proteins adsorption in solid/liquid interfaces [Haynes et al, Colloids and Surfaces B, Biointerfaces. 2:517-566 (1994)] as further demonstrated below.

Quantitative binding reversibility assay of purified CBDclos, SpS and SpS-CBDclos to cellulose: Adsorption/desorption experiments are critical tests to study the reversible nature of adsorption. A reversible adsorption process is defined if the departure from adsorption equilibrium is infinitesimally small, so that in the reverse process (desorption) the variables characterizing the state of the system return to the same values in the reverse order. Therefore in a reversible adsorption process, the ascending branch (increasing concentration in the solution) and the descending branch (decreasing concentration in the solution) of the isotherm must overlap. If the ascending and descending branches of the isotherm do not overlap, the process is defined as irreversible and the deviation between the ascending and descending branches is defined as hysteresis [Haynes et al, Colloids and Surfaces B, Biointerfaces. 2:517-566(1994)].

The desorption experiments of CBDclos and SpS-CBDclos revealed that a new equilibrium was established after dilution, which was not on the same isotherm (Figure 26). These results prove that the ascending and descending isotherms do not overlap, which is a prerequisite for irreversible binding. These results demonstrate that under the conditions tested, CBDclos and SpS-CBDclos display similar adsorption behavior and bind almost irreversibly to cellulose. The results also reveal that the ascending and descending branches of the SpS isotherm almost overlap, therefore it can be known for certain that the SpS adsorption to cellulose is not reversible but rather due to protein adsorption in solid/liquid interfaces. Table 19 herein below summarizes the results quantitative binding reversibility assay results.

Table 19

	Bound (μg)/10 mg cellulose			***Reverse binding (μg)/10 mg cellulose***		
Total protein (μg)	***CBD***	***SpS***	***SpSr-CBD***	***CBD***	***SpS***	***SpS-CBD***
100	99.21	14.05	94.15	474.56	3.41	408.13
150	138.67	23.28	120.92	467.57	13.02	402.88
300	237.38	41.57	231.47	442.22	77.71	416.87
600	462.17	101.33	419.58	462.17	101.33	419.58

Expression of CBD-SpS12 and SpS6-CBD-SpS6 by T1 homozygous plants: Four homozygous T1 plants, with elevated protein expression, were isolated:

1. Two plants of CBD-SpS12 number 13.7 and 13.8, which express and secrete CBD-SpS12 to the appoplast were identified, referred to herein as 13.7 and 13.8, respectively.

2. Two plants of SpS6-CBD-SpS6 number 6.4 and 6.8, which express 6mer-CBD-SpS6 in the cytoplasm were identified, referred to herein as 6.4 and 6.8, respectively.

SDS-PAGE analysis of protein extracts revealed that both CBD-SpS12 and SpS6-CBD-SpS6 bound cellulose and therefore were mostly found in the insoluble fraction (Figures 27A-B). With the addition of extra cellulose to the extraction procedure, all the soluble fraction of the CBD fusion proteins bound cellulose.

Purification of CBD-SpS12 and SpS6-CBD-SpS6 from transgenic tobacco plants: The purification of CBD-SpS12 and SpS6-CBD-SpS6 is based on the unique binding of the fusion CBD proteins to the plant's cell wall. This specific binding confirms that the CBD is active and serves as the first step of purification (Figure 28A).

CBD-containing proteins were shown to bind the cell wall and to precipitate along with the insoluble fractions of the cell extract. The pellet was then treated with elution buffer, leading to release of CBD-containing proteins to the soluble fraction of this elution process (Figure 28A, lane 6). Further purification of SpS6-CBD-SpS6 is based on the spider silk unique heat stability and solubility at a wide range of pHs. From SDS PAGE analysis it is clear that the SpS6-CBD-SpS6 is heat stable and soluble at a wide range of pHs (Figure 28B).

EXAMPLE 14

Metal catalyzed polymerization of spider silk

MATERIALS AND METHODS

Purified SpS protein (Example 8), containing 15 tyrosine residues, was dialyzed four times against either 50 mM phosphate buffer (pH 7.5) or deionized water. Following the dialysis, the protein was centrifuged for 10 minutes at 13000 rpm (Figure 29, lanes 2,3,4). The polymerization reaction was performed according to the MCO method reported by Kato *et al* (Kato Y, Kitamoto N, Kawai Y, Osawa T. (2001) The hydrogen peroxide/copper ion system, but not other metal-catalyzed oxidations systems, produces protein-bound dityrosine. Free Radical Biology & Medicine, 31,(5), 624–632) and Ali *et al* (Ali FE, Barnham KJ, Barrow CJ, Separovic F. (2004) Metal catalyzed oxidation of tyrosine residues by different oxidation systems of copper/hydrogen peroxide. J Inorg Biochem. 98(1):173-84). All the reactions were performed in 250 µl solution volume in 1.5 ml eppendorf tubes. The MCO polymerization was performed by adding 4 mmol H_2O_2 (1 µl of 30 % H_2O_2) and 200 µM $CuCl_2$ (2.5 µl of 20 mM $CuCl_2$ dissolved in H_2O) followed by O.N. incubation at 37 °C. Tubes with protein solution only, protein solution with H_2O_2 only or $CuCl_2$ only were used as negative controls. The reactions were terminated by adding 1 mM EDTA. Finally the samples were boiled in X2 SAB and analyzed by Coomassie-stained SDS-PAGE.

RESULTS

Polymerization was achieved in both phosphate buffer and water, as displayed in Figure 29, lanes 3 and 7.

EXAMPLE 15

Method for preparation of spider silk and cellulose whiskers sponges with/without CBD

MATERIALS AND METHODS

Aqueous protein solutions (5 wt %) were mixed with cellulose whiskers in a Teflon mold. After obtaining a homogenous solution, 100 % methanol was added to the protein-whiskers mixture to a final concentration of 15 % (stirring was manually performed). The mold was placed in a -80 °C freezer for more than 1 hour. The protein-whiskers frozen solution was freeze-dried to generate a sponge. This method is based on Nazarov R *et al.* Porous 3-D scaffolds from regenerated silk fibroin. Biomacromolecules (2004): 5, 718-726.

EXAMPLE 16

Preparation of recombinant a spider silk-cellulose whisker sponge

The purified SpS protein was dialyzed against water for 18 hours, changing the water four times (the first change after 12 hours and the following three changes every two hours). After dialysis, the protein aqueous solution was concentrated to 5 wt % (Figure 30, lanes 2 and 4 *vs.* 5). The concentrated SpS protein was then mixed with cellulose whiskers in a Teflon mold to yield a desired ratio of 100/0 %, 30/70 %, 0/100 %, respectively.

EXAMPLE 17

Determination of Tm of silk-whisker composites

MATERIALS AND METHODS

Sponges, generated according to the methods described in Example 15 and 16, were analyzed by differential scanning calorimetry (DSC). For each run, ~5 mg of sample was used, and the thermogram was recorded from 0-300 °C at a heating rate of 5 °C/min, under nitrogen.

RESULTS

The DSC analysis (Figures 31A-C) shows three different thermogram profiles. In the composite spider silk-cellulose whiskers thermogram the transition temperature peak 2 of spider silk and cellulose whiskers alone disappeared and a higher peak

appeared at 243.69 °C. Table 20 summarizes the transition temperature peaks from DSC thermograms of whiskers, silk and 70 % whiskers/30 % silk sponges. This analysis demonstrates that the silk-whisker combination leads to a significant increase in whiskers transition temperature peak2. Table 20 summarizes the transition temperature peaks from DSC thermograms of whiskers, silk and 70% whiskers/30% silk sponges.

Table 20

	Transition temp. peak1(°C)	Tg (°C)	Transition temp. peak2(°C)
Cellulose whiskers (figure 2A)	93.04	-	193.71
Silk (figure 2B)	81.07	175.44	267.66
70%whiskers/30%silk(figure 2C)	87.56	-	243.69

Sample	***Transition temp. peak1 (°C)***	***Transition temp. peak2 (°C)***
Cellulose whiskers	93.04	193.71
Silk	81.07	175.44 (Tg)
70%whiskers/30%silk	87.56	243.69

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention. To the extent that section headings are used, they should not be construed as necessarily limiting.

The term "comprise" and variants of the term such as "comprises" or "comprising" are used herein to denote the inclusion of a stated integer or stated integers but not to exclude any other integer or any other integers, unless in the context or usage an exclusive interpretation of the term is required.

Any reference to publications cited in this specification is not an admission that the disclosures constitute common general knowledge in Australia.

CLAIMS

1. An isolated polypeptide comprising an amino acid sequence encoding a resilin attached to a heterologous polysaccharide binding domain.

2. The isolated polypeptide of claim 1, wherein said resilin comprises an amino acid sequence as set forth in SEQ ID NO: 8 or SEQ ID NO: 9.

3. The isolated polypeptide of claim 1 or claim 2, wherein said polysaccharide binding domain is selected from the group consisting of a cellulose binding domain, a starch binding domain, a dextran binding domain, a glucan binding domain, a chitosan binding domain, an alginate binding domain and a hyaluronic acid binding domain.

4. The isolated polypeptide of claim 1 as set forth in SEQ ID NOs: 12 or 13.

5. An isolated polynucleotide comprising a nucleic acid sequence encoding the polypeptide of claim 1.

6. An isolated composite comprising resilin and a polysaccharide selected from the group consisting of cellulose, starch, dextran, glucan, chitosan, alginate and hyaluronic acid.

7. The isolated composite of claim 6, wherein said resilin comprises a polysaccharide binding domain.

8. The isolated composite of claim 7, wherein said polysaccharide binding domain is a heterologous polysaccharide binding domain.

9. The isolated composite of claim 7, wherein said polysaccharide binding domain is selected from the group consisting of a chitin binding domain, a cellulose binding domain, a chitosan binding domain, an alginate binding domain, a starch binding domain, a dextran binding domain, a glucan binding domain and a hyaluronic acid binding domain.

10. The isolated composite of any one of claims 6-9, further comprising an additional polypeptide that is different to resilin, wherein said additional polypeptide is selected from the group consisting of a mussel byssus protein, a silkworm silk protein, a spider silk protein, a collagen, an elastin, and fragments thereof.

11. The isolated composite of any one of claims 6-9, being crosslinked.

12. A method of generating the isolated composite of any one of claims 6-9, said method comprising the step of contacting said resilin with the polysaccharide under conditions which allow binding between said resilin and the polysaccharide to generate the isolated composite of any one of claims 6-9.

13. The method of claim 12, further comprising the step of crosslinking said composite following said contacting.

14. The method of claim 12, further comprising binding said resilin with an additional polypeptide that is different from resilin, prior to said contacting.

15. A method of treating a cartilage or bone disease or condition, said method comprising the step of administering to a subject in need thereof, a therapeutically effective amount of the isolated composite of any one of claims 6-11, to thereby treat said cartilage or bone disease or condition.

16. A method of treating urinary incontinence, said method comprising the step of administering to a subject in need thereof, a therapeutically effective amount of the isolated composite of any one of claims 6-11, to thereby treat said urinary incontinence.

17. A scaffold comprising the isolated composite of any one of claims 6-11.

18. A pharmaceutical composition comprising the isolated composite of any one of claims 6-11.

19. A cosmetic composition comprising the isolated composite of any one of claims 6-11.

20. An isolated polypeptide as defined in claim 1, and substantially as disclosed herein, with reference to the accompanying examples.

Dated: 4 October 2013

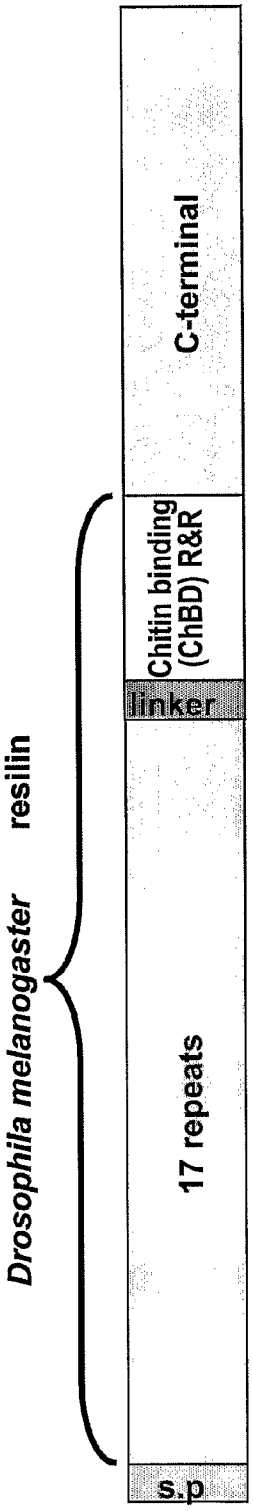

Drosophila melanogaster resilin
C-terminal
Chitin binding (ChBD) R&R
linker
17 repeats
s.p

FIG. 1A

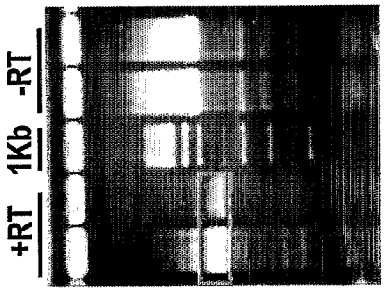

+RT
1Kb
-RT

FIG. 1B

pHIS-Parallel3
T7 Promoter->
Lac Operator
Xba I
Nde I
6xHis
Spacer Region
Hind III
Nco I
BamH III
EcoR I
Stu I
Sal I
Sst I
rTEV Protease
Cleavage Site
Spe I
Not I
Nsp V
Xho I
Pst I
Xho I
*Non-unique sites

FIG. 2

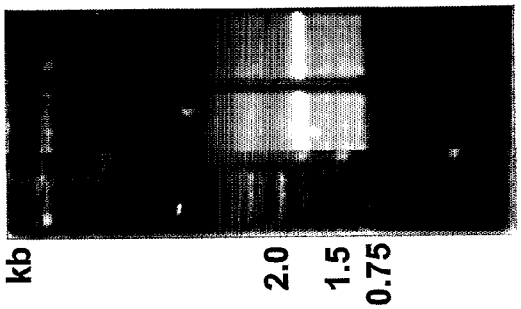


FIG. 3B

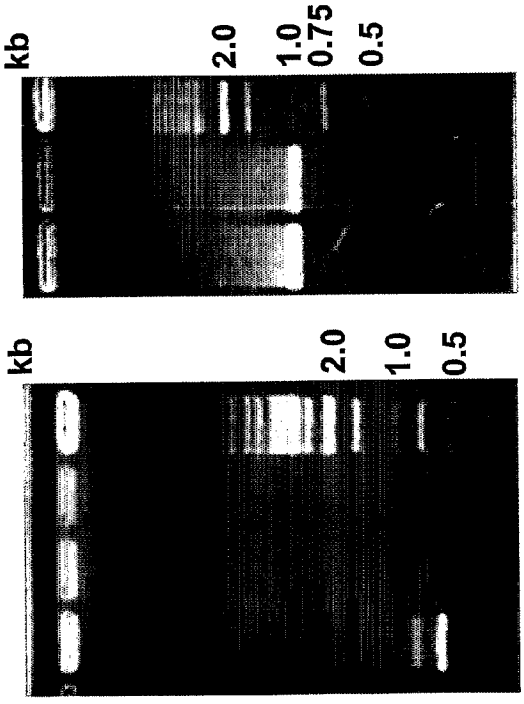


FIG. 3A

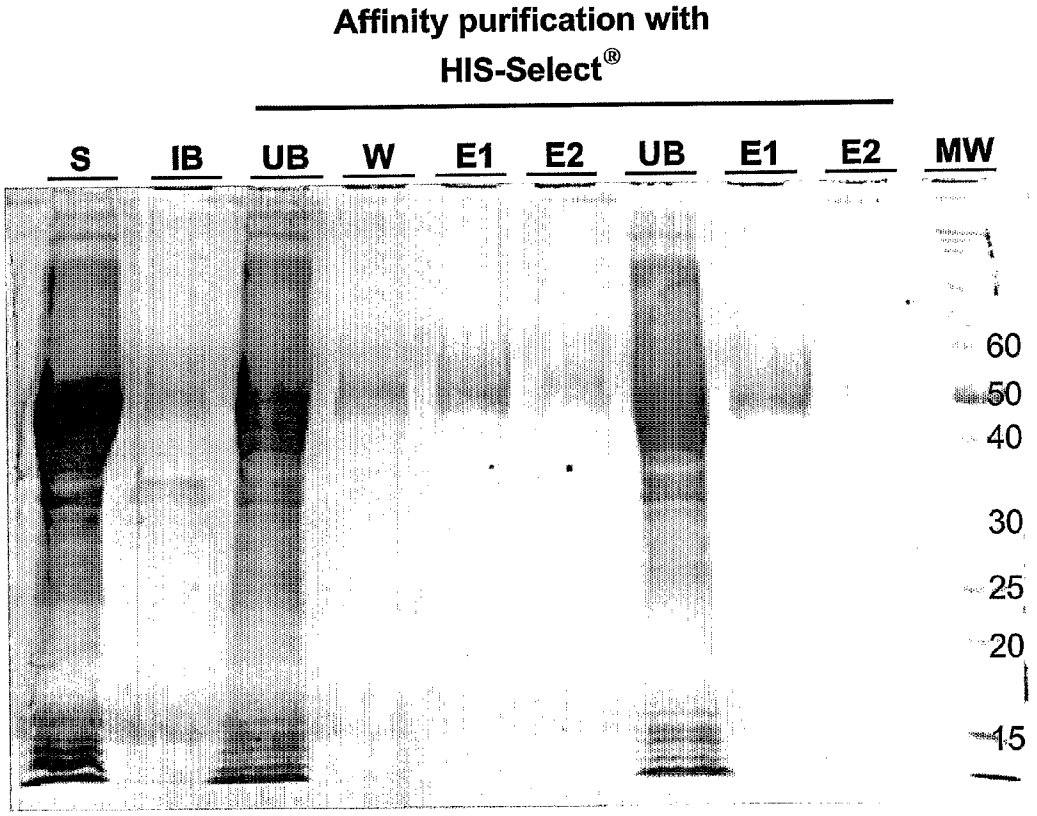

Affinity purification with
HIS-Select®
S
IB
UB
W
E1
E2
UB
E1
E2
MW
60
50
40
30
25
20
15

FIG. 4

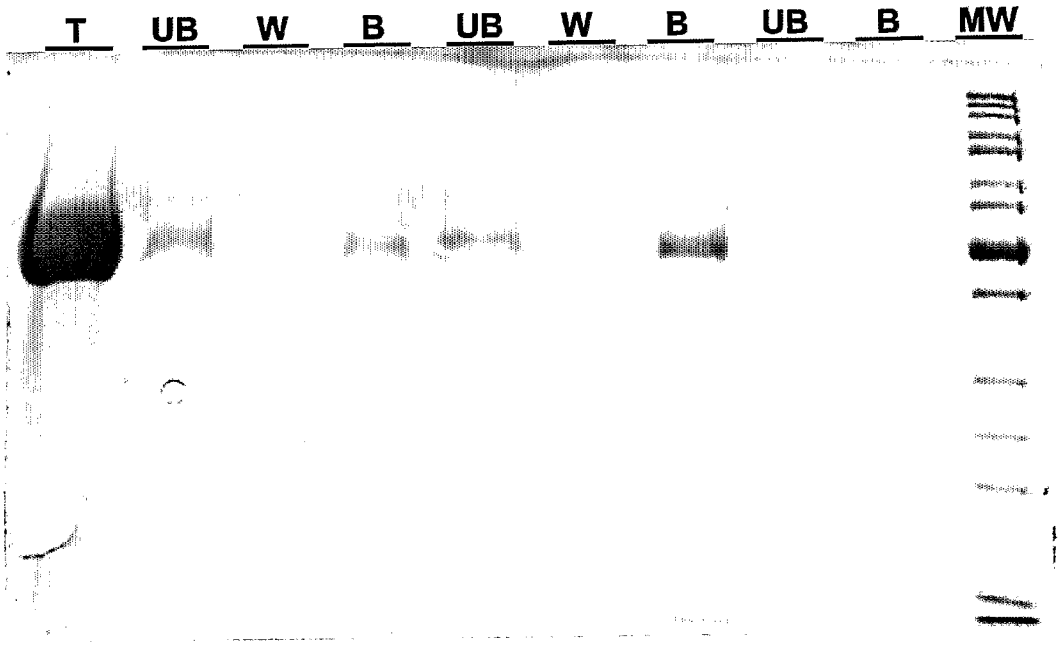


FIG. 5

FIG. 6A

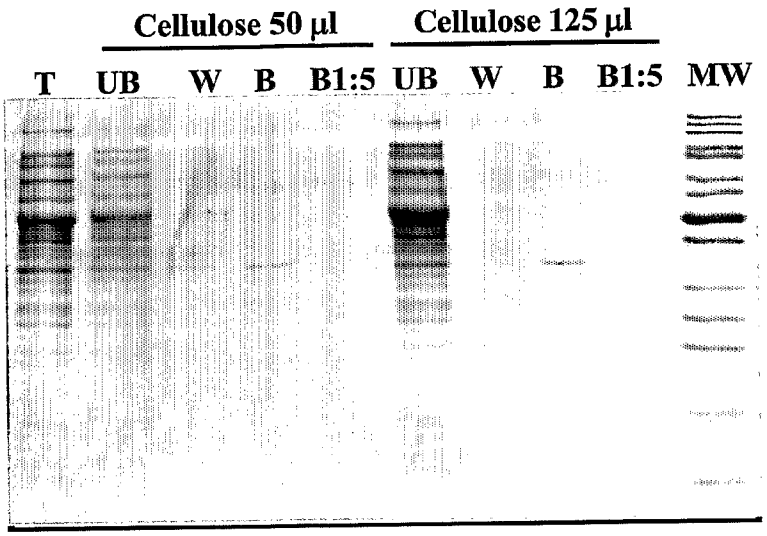


FIG. 6B

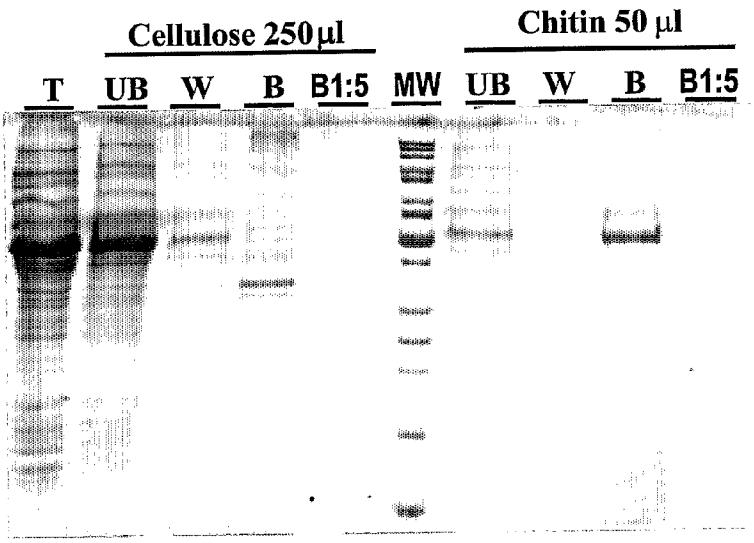
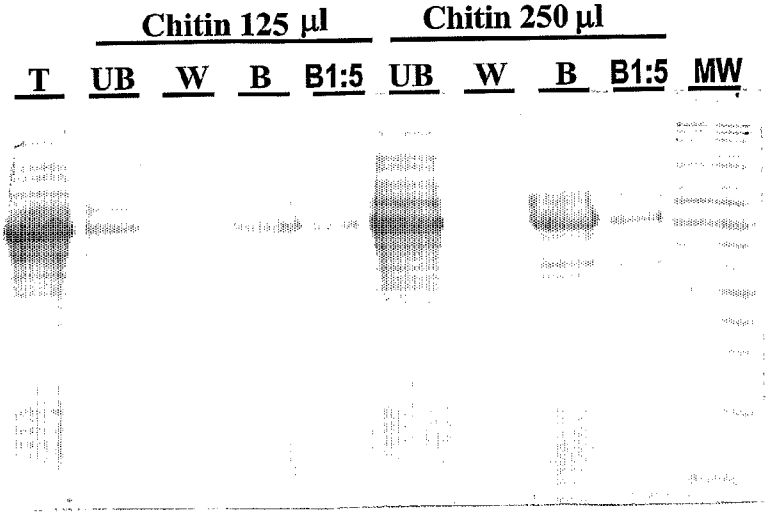


FIG. 6C



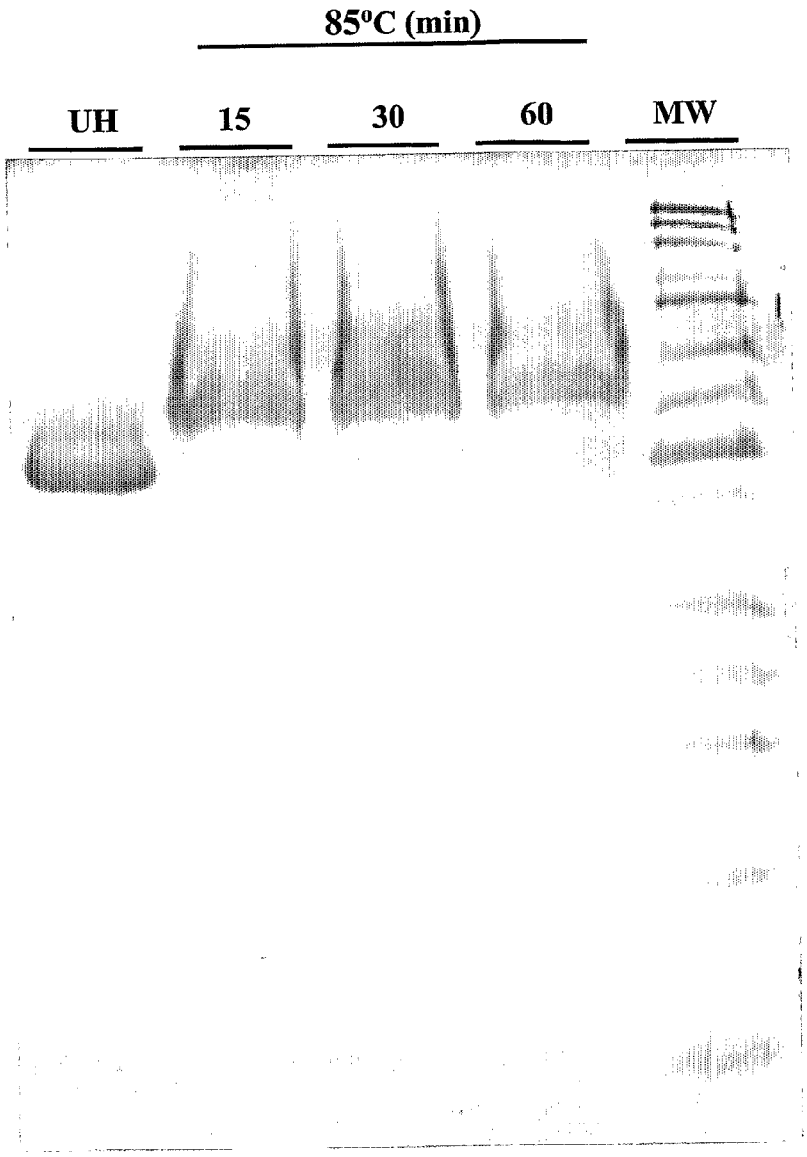


FIG. 7

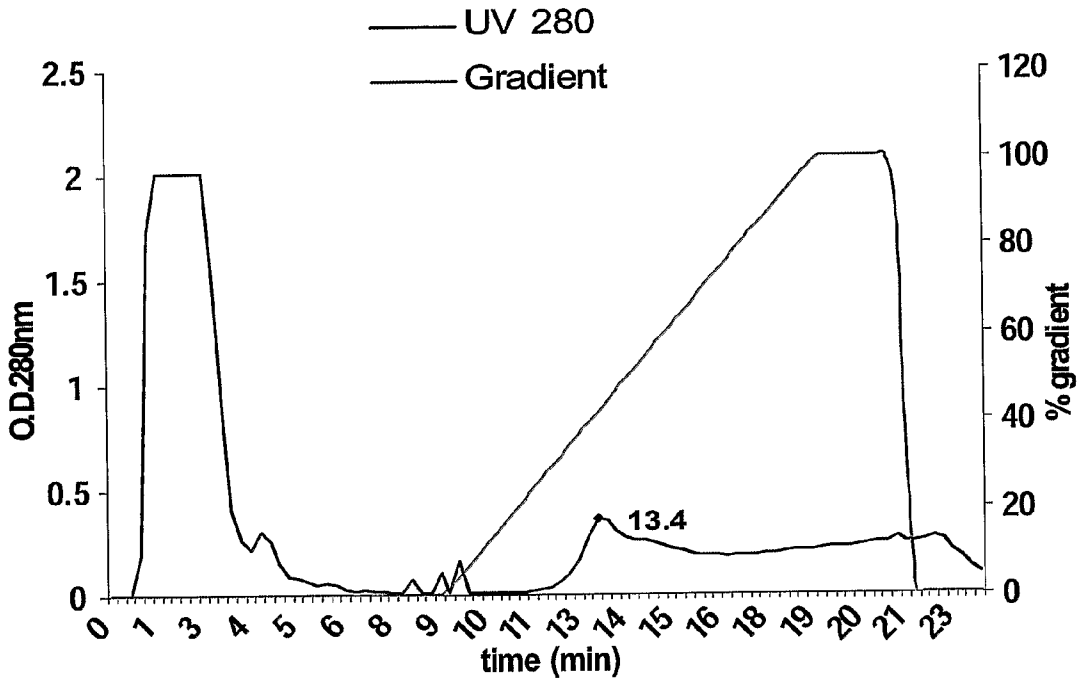

UV 280
Gradient
2.5
2
1.5
1
0.5
0
O.D.280nm
120
100
80
60
40
20
0
% gradient
13.4
0 1 3 4 5 6 8 9 10 11 13 14 15 16 18 19 20 21 23
time (min)

FIG. 8A

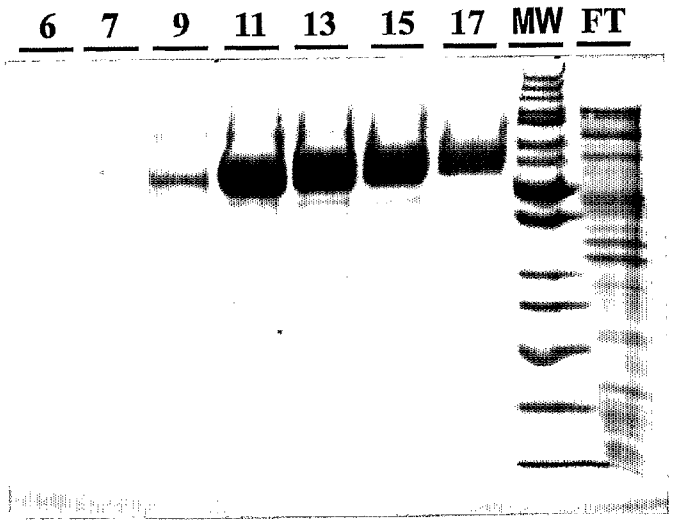

6 7 9 11 13 15 17 MW FT

FIG. 8B

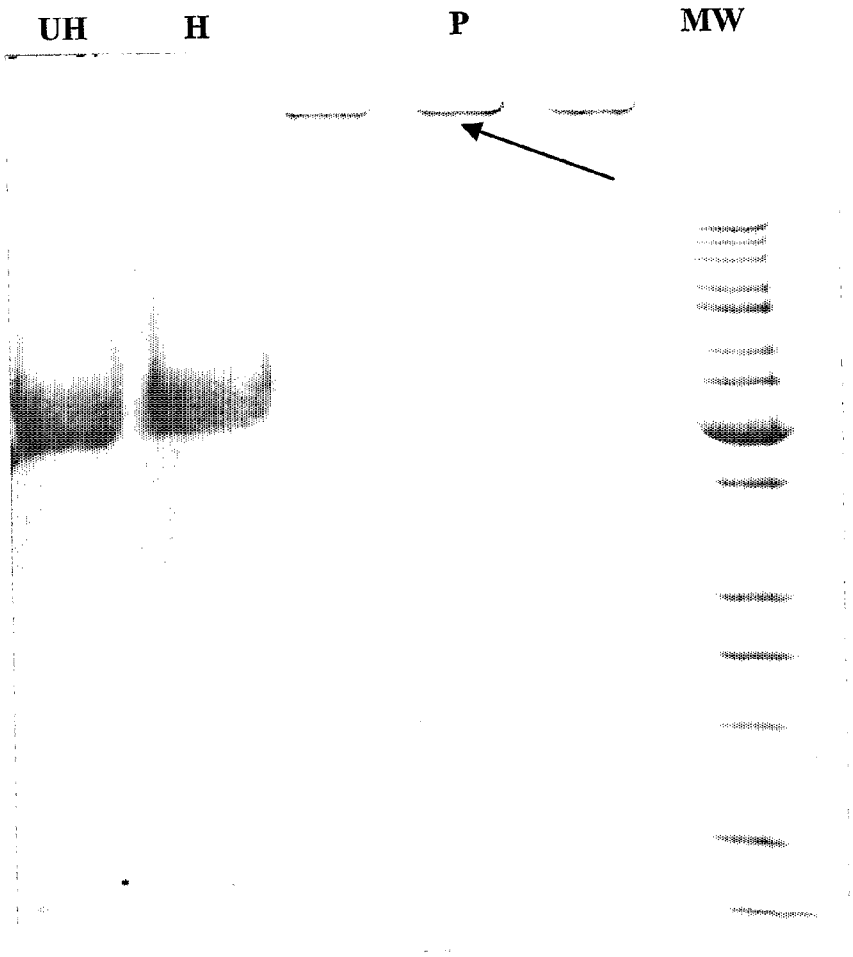


FIG. 9

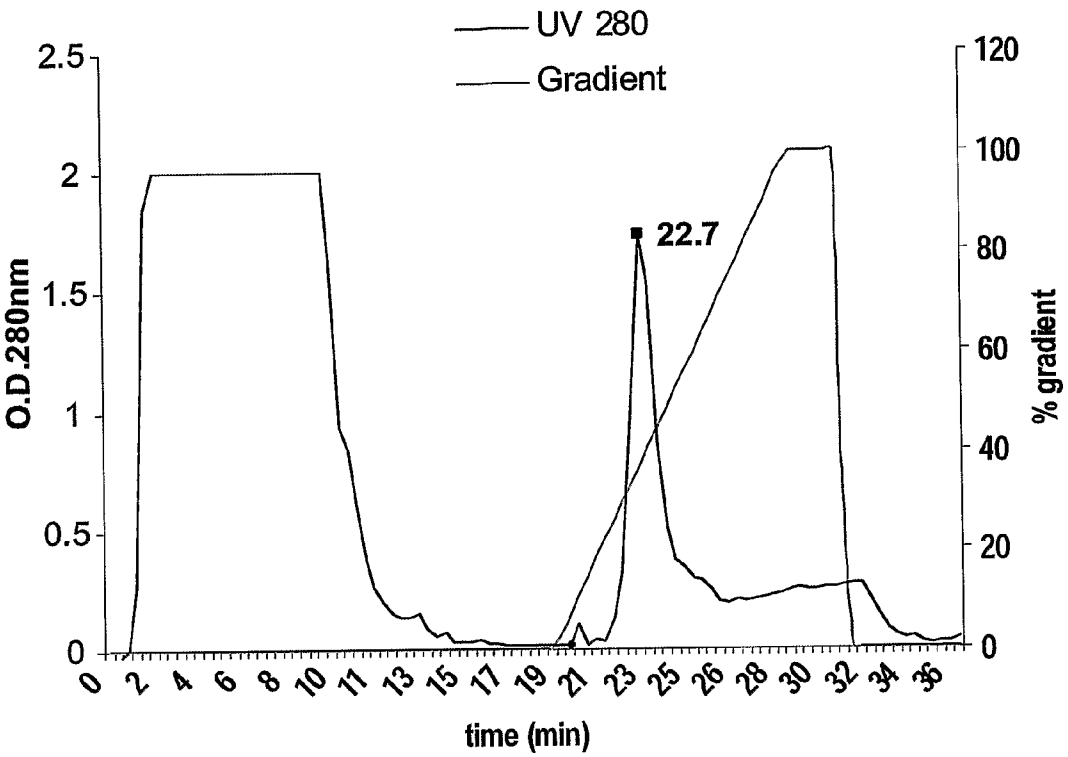


FIG. 10A

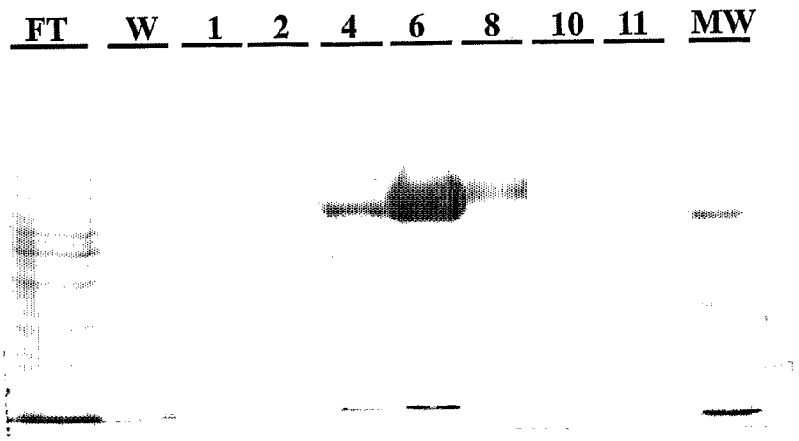


FIG. 10B

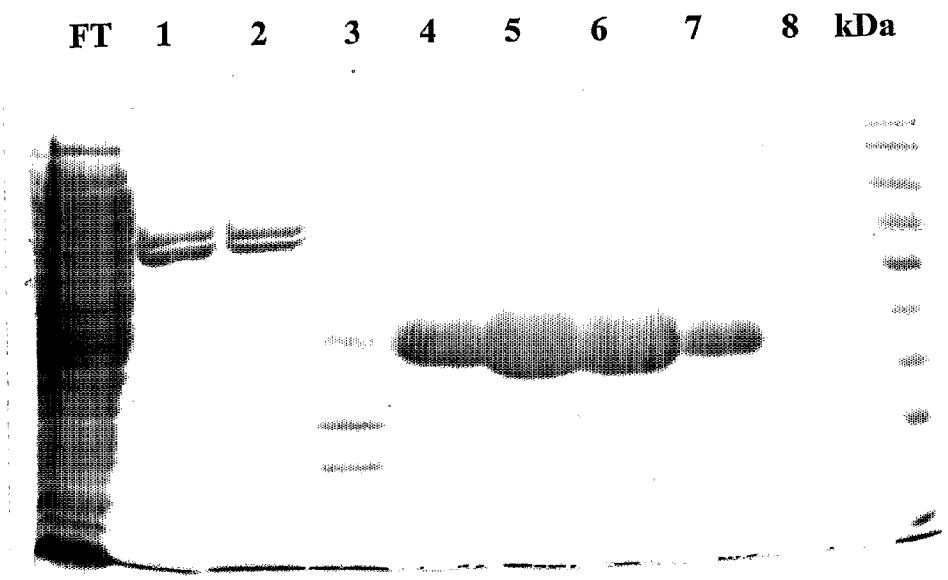


FIG. 11

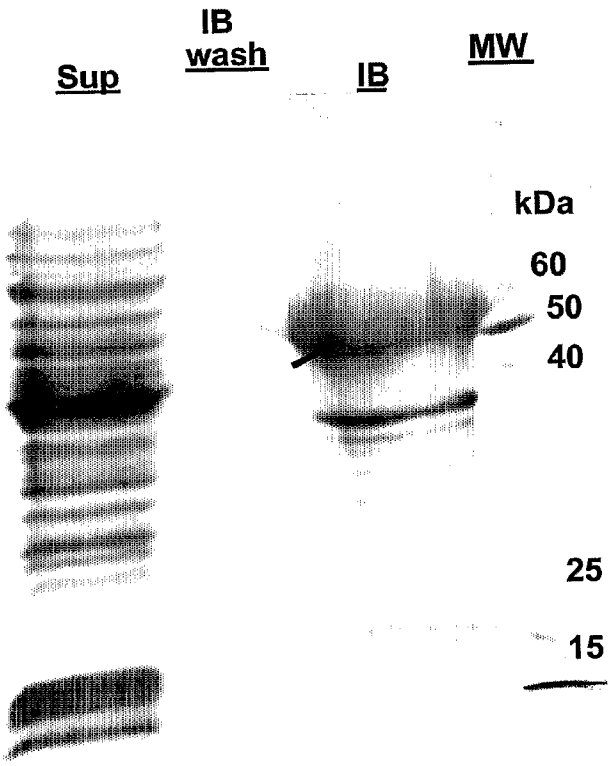


FIG. 12

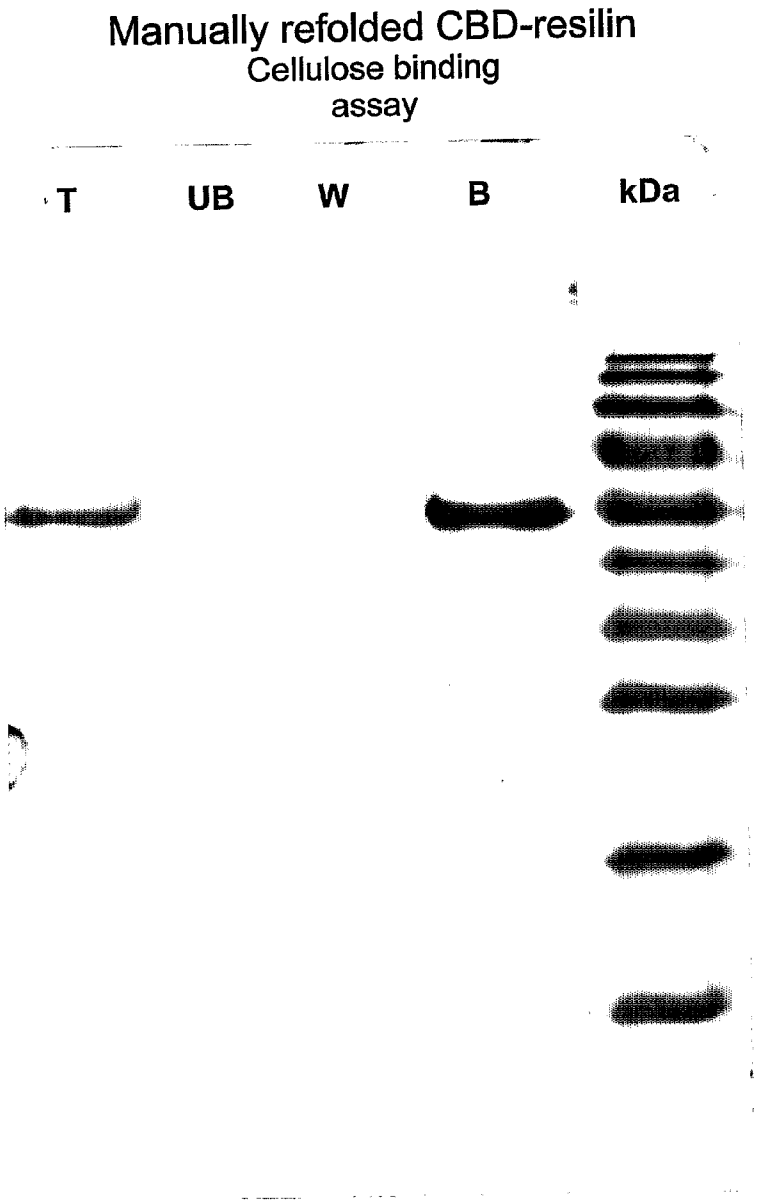

Manually refolded CBD-resilin
Cellulose binding
assay
T
UB
W
B
kDa

FIG. 13

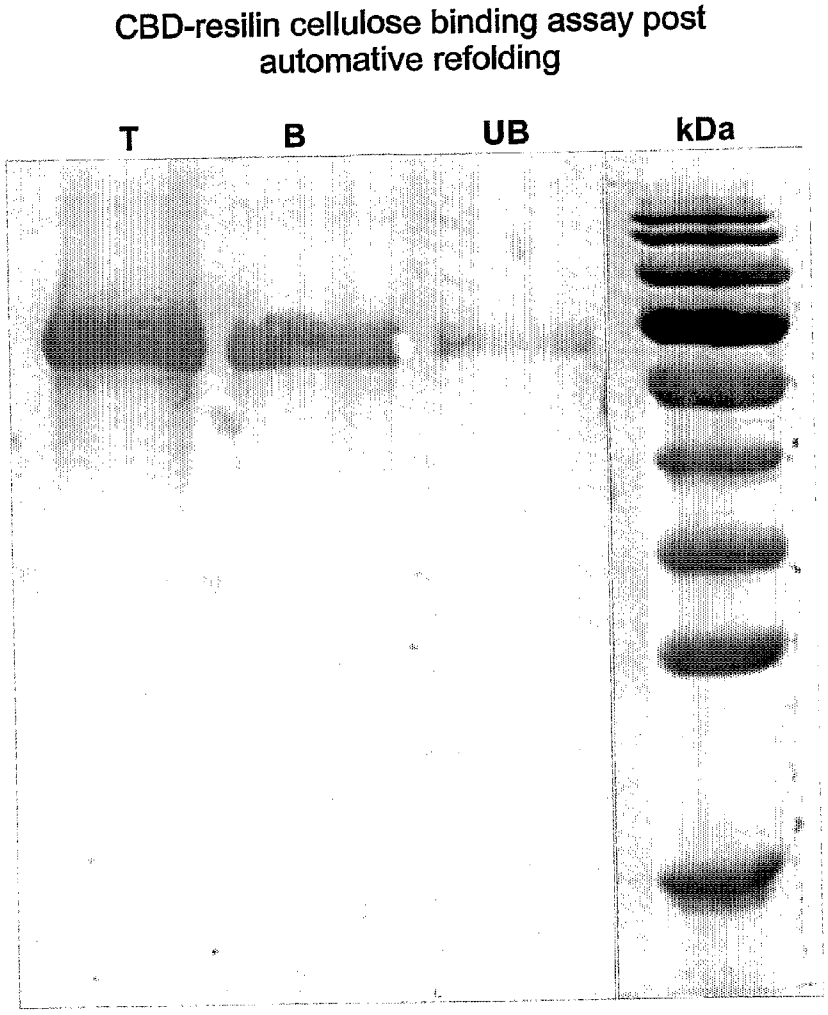

CBD-resilin cellulose binding assay post automative refolding
T
B
UB
kDa

FIG. 14

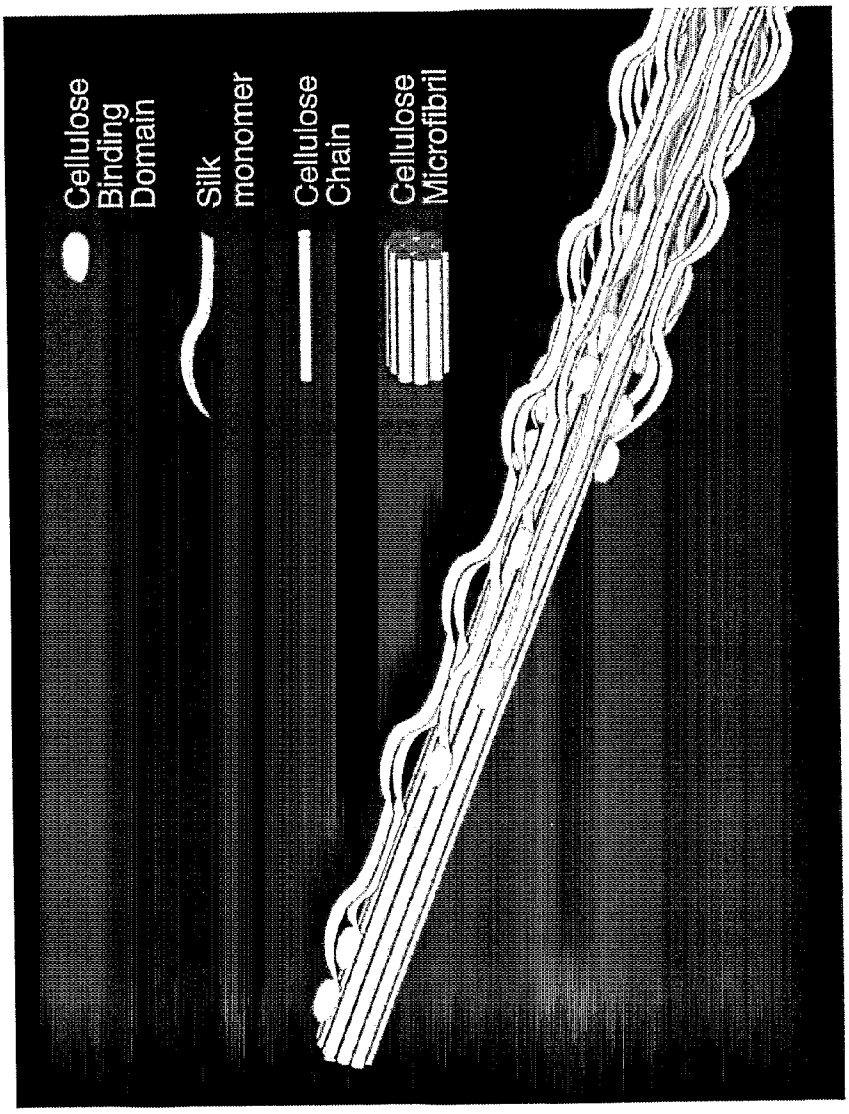

Cellulose Binding Domain
Silk monomer
Cellulose Chain
Cellulose Microfibril

FIG. 15

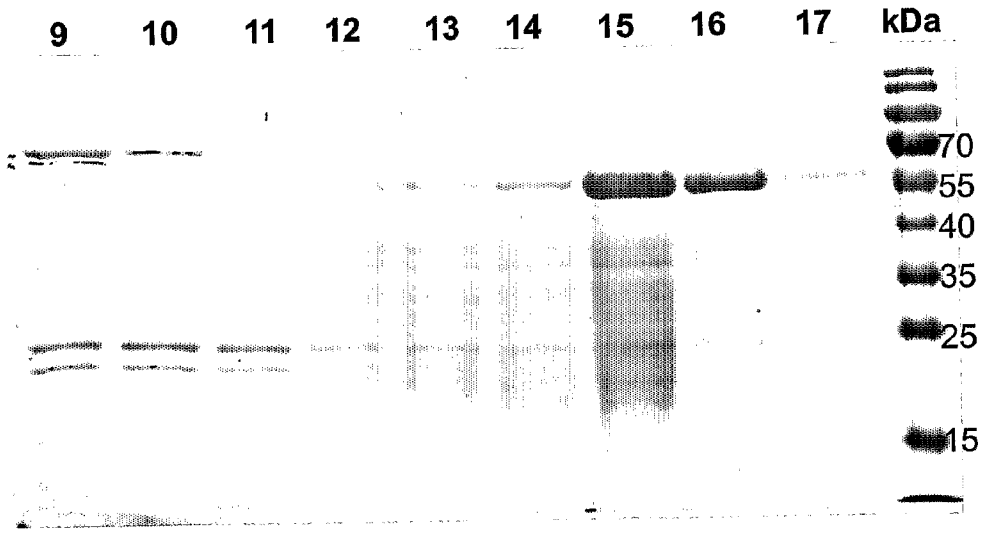


FIG. 16

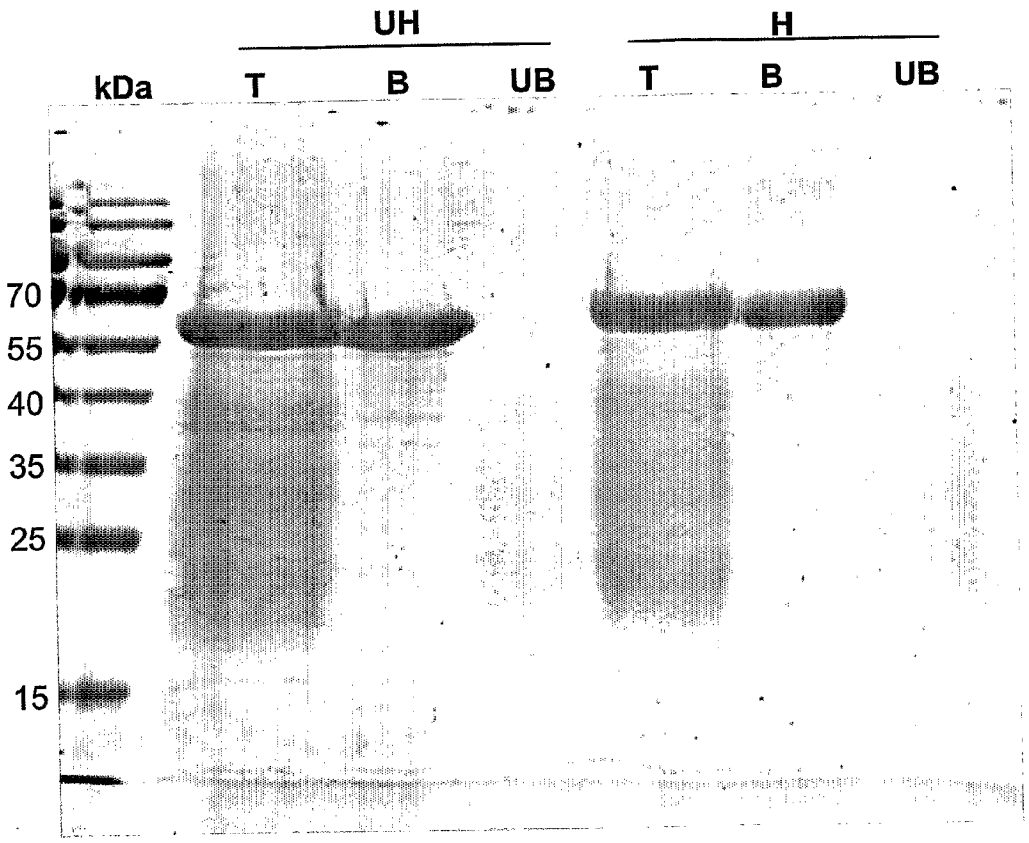


FIG. 17

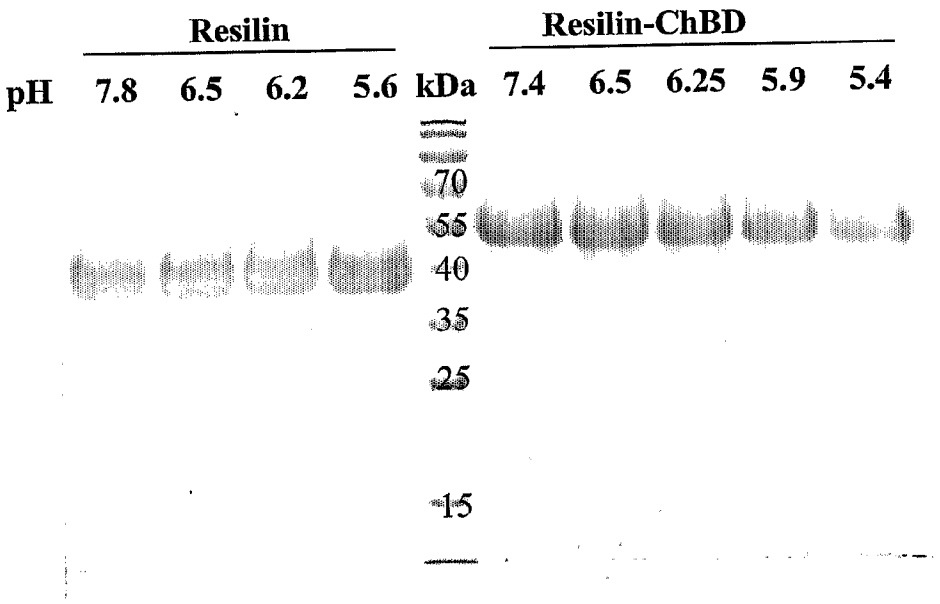

Resilin
Resilin-ChBD
pH
7.8
6.5
6.2
5.6
kDa
7.4
6.5
6.25
5.9
5.4
70
55
40
35
25
15

FIG. 18

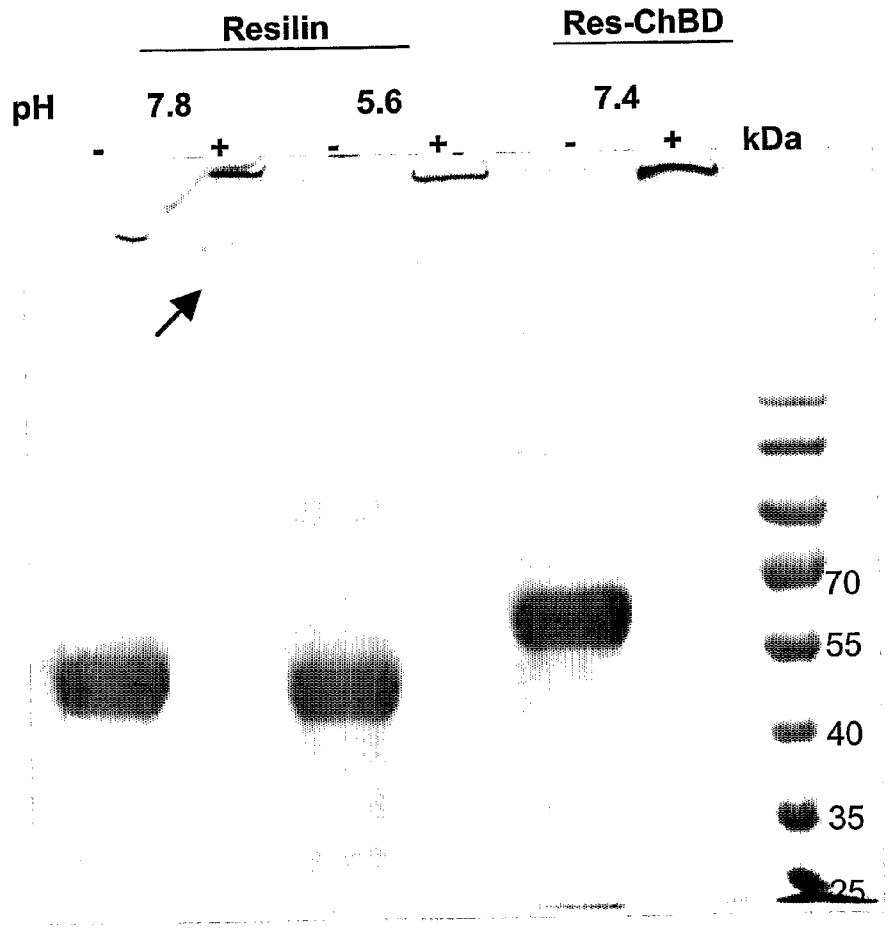


FIG. 19

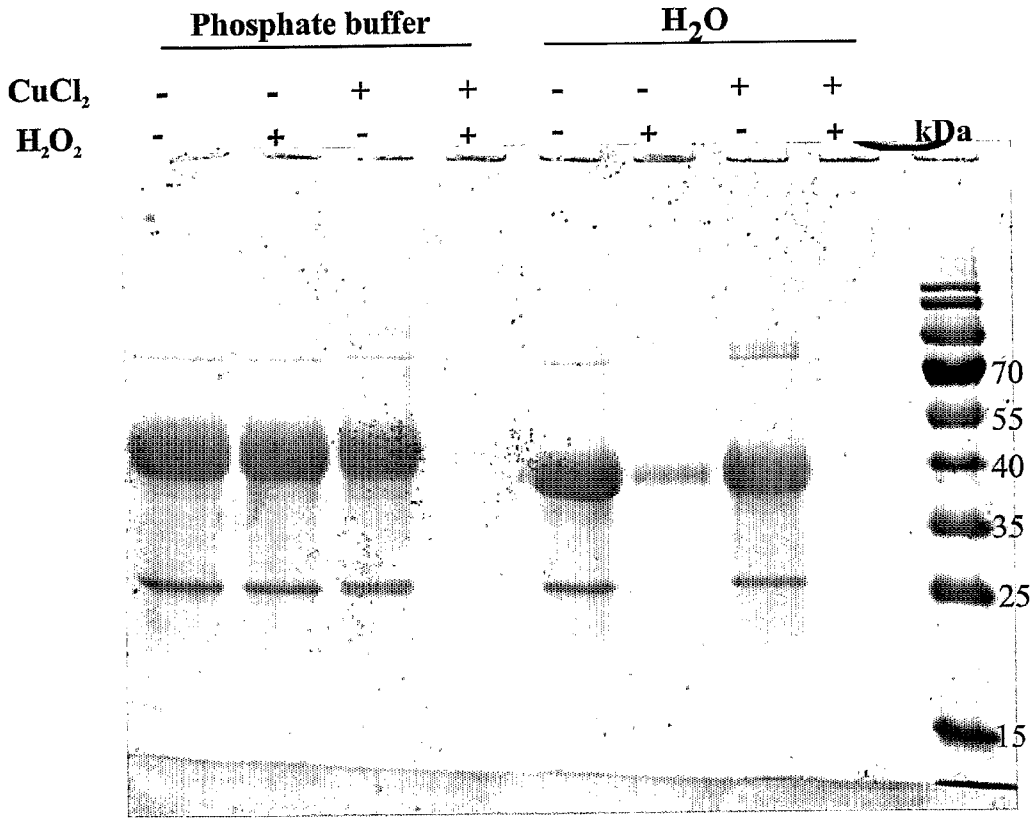


FIG. 20

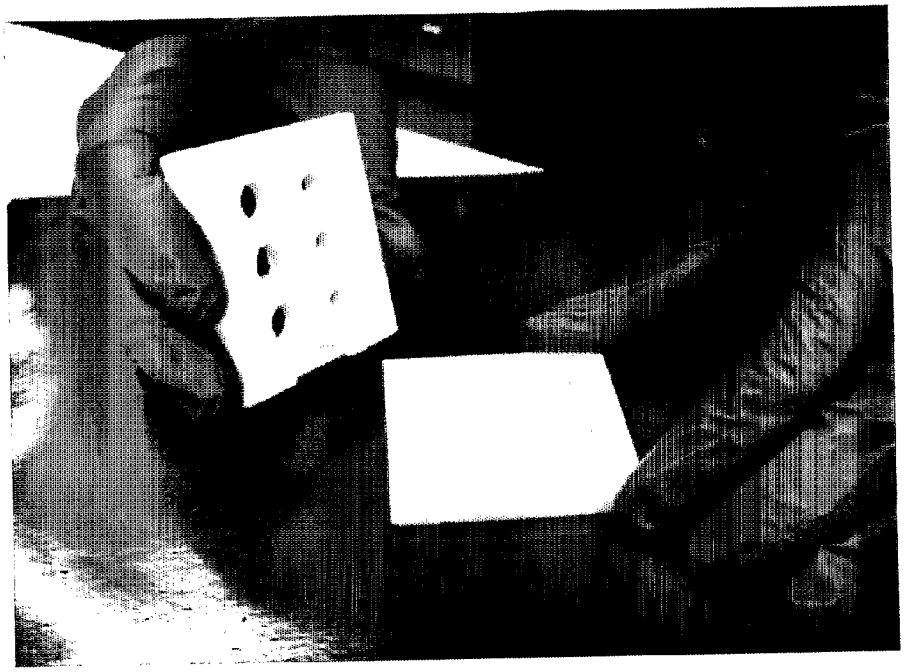


FIG. 21A

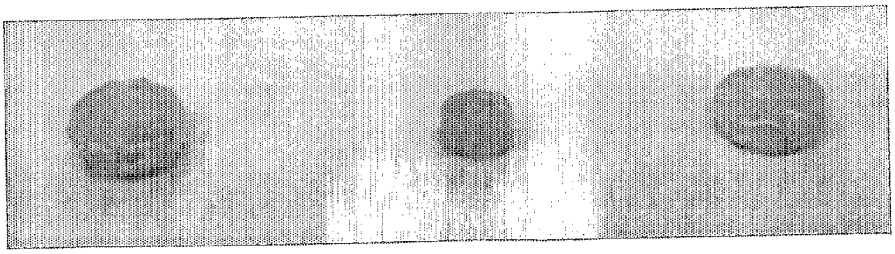


FIG. 21B

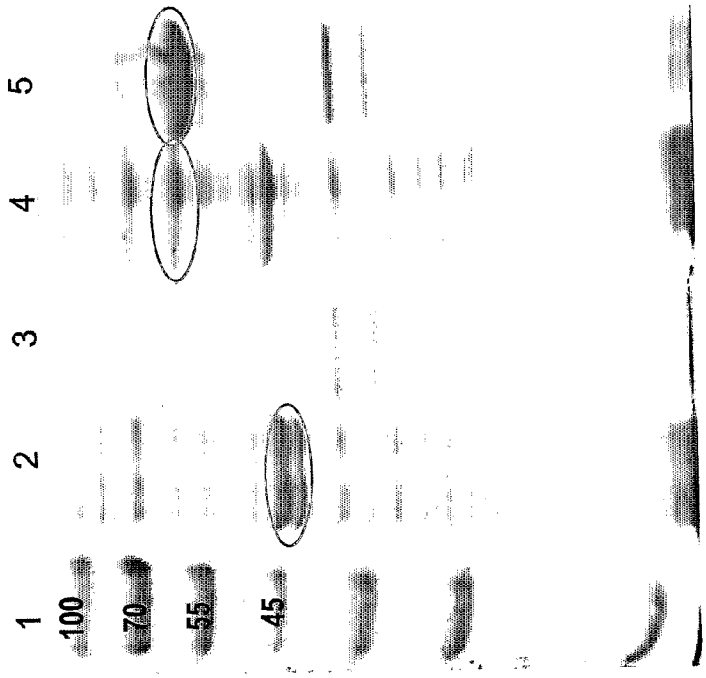


FIG. 22B

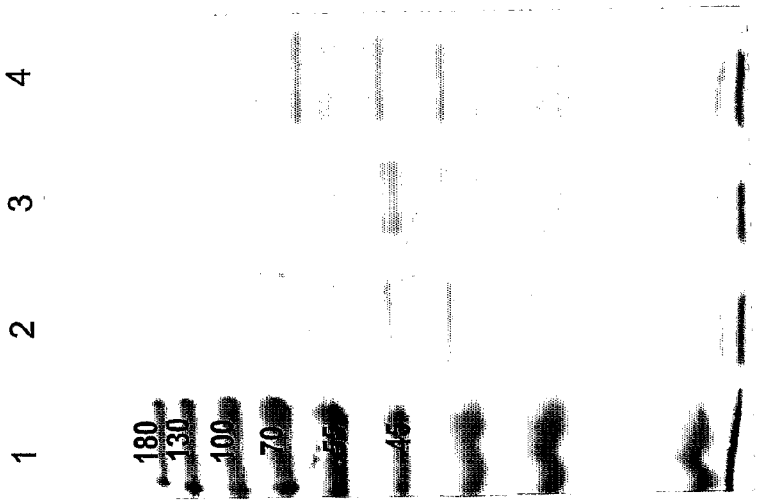


FIG. 22A

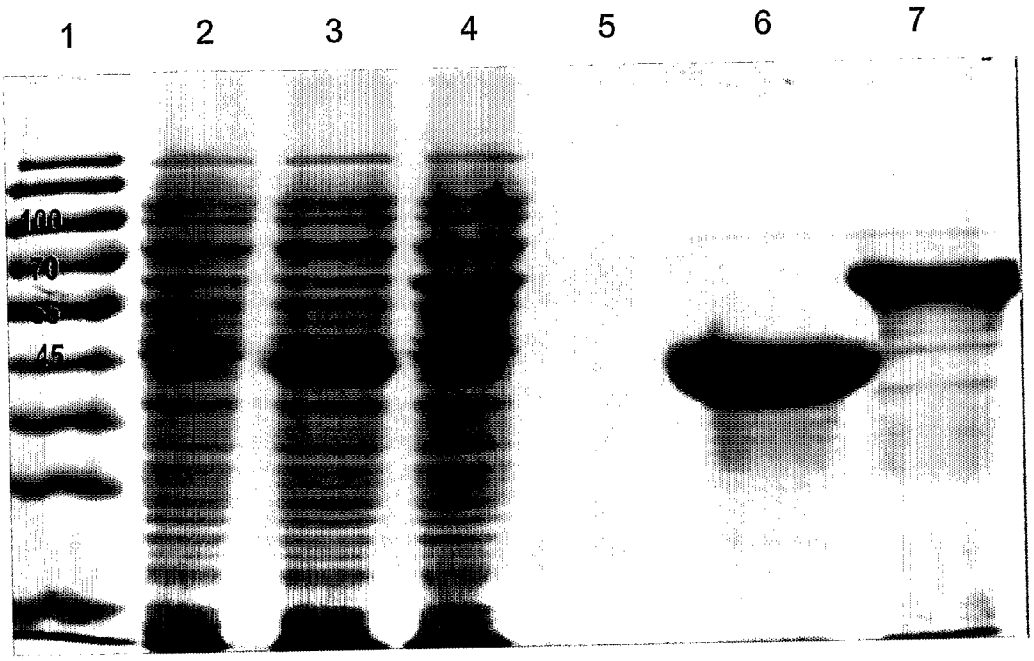


FIG. 23A

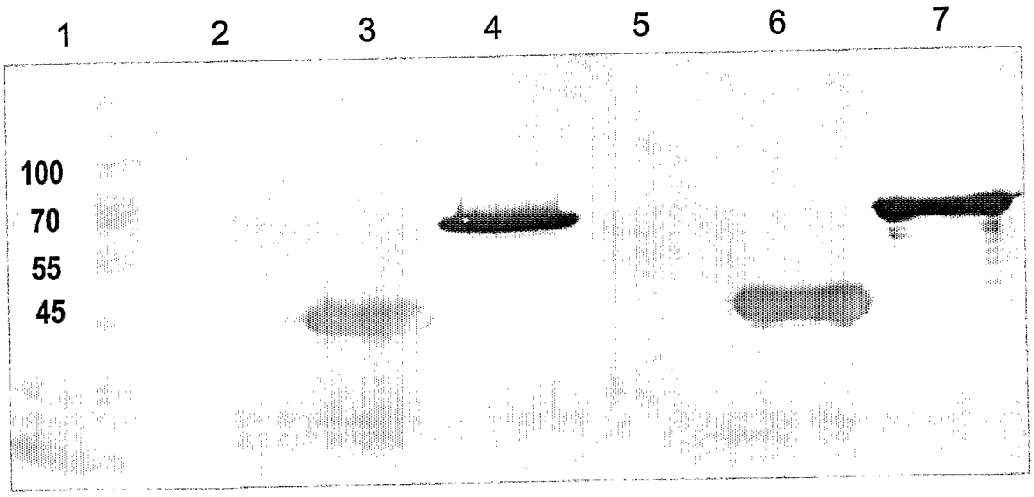


FIG. 23B

FIG. 24A

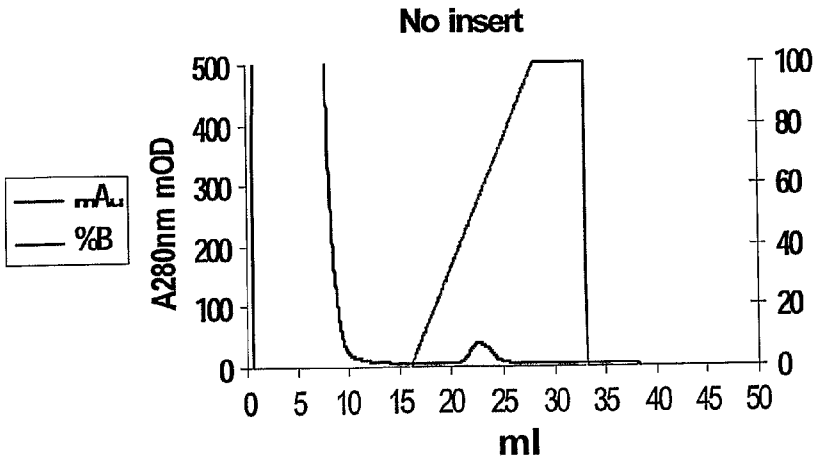


FIG. 24B

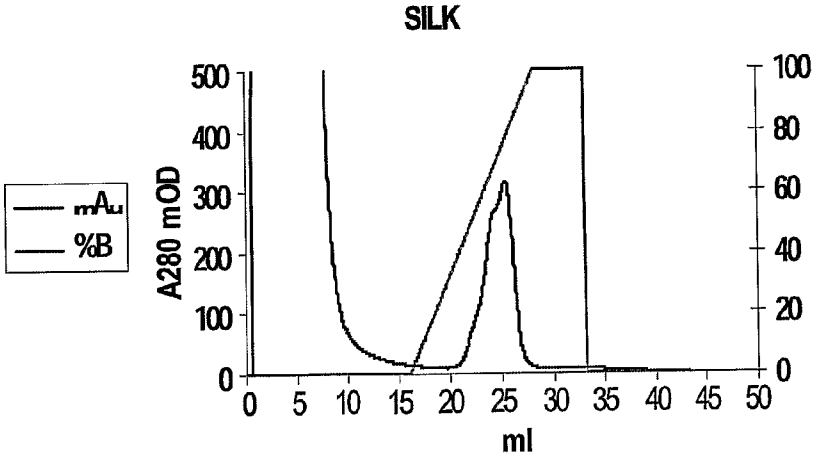
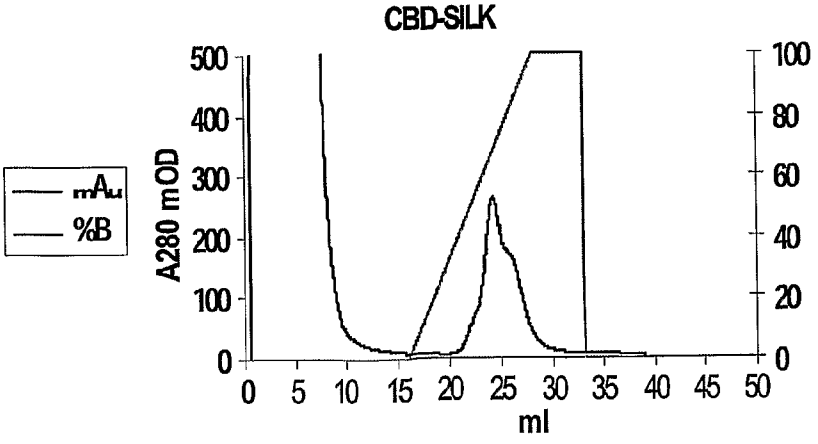


FIG. 24C



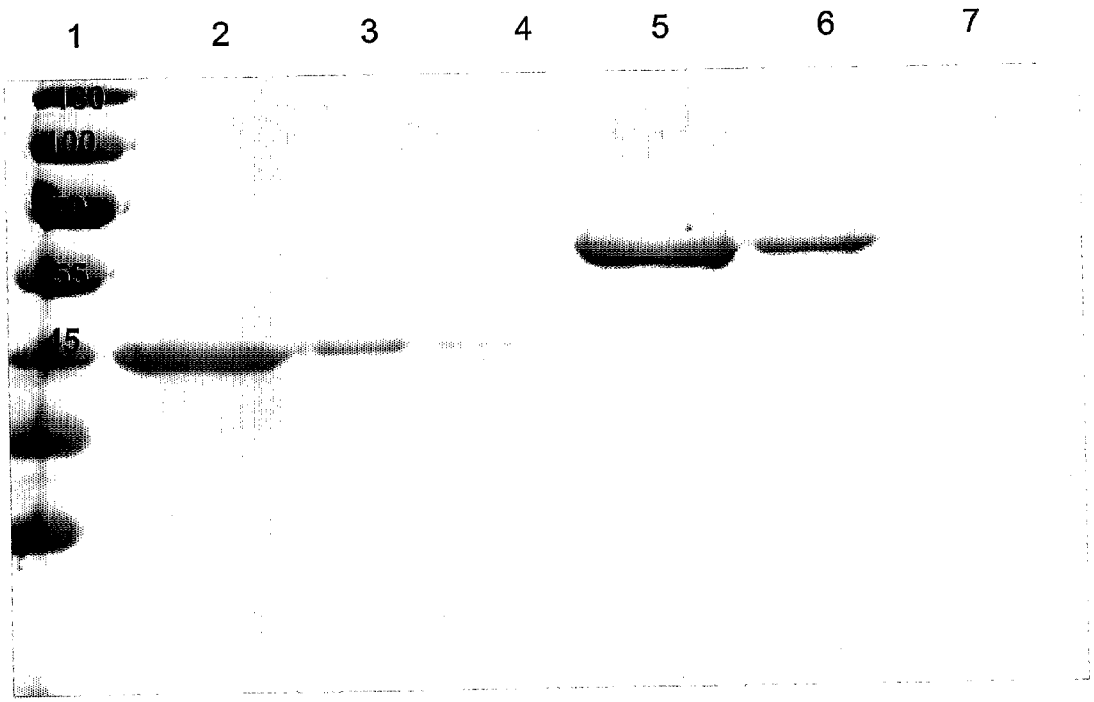


FIG. 25

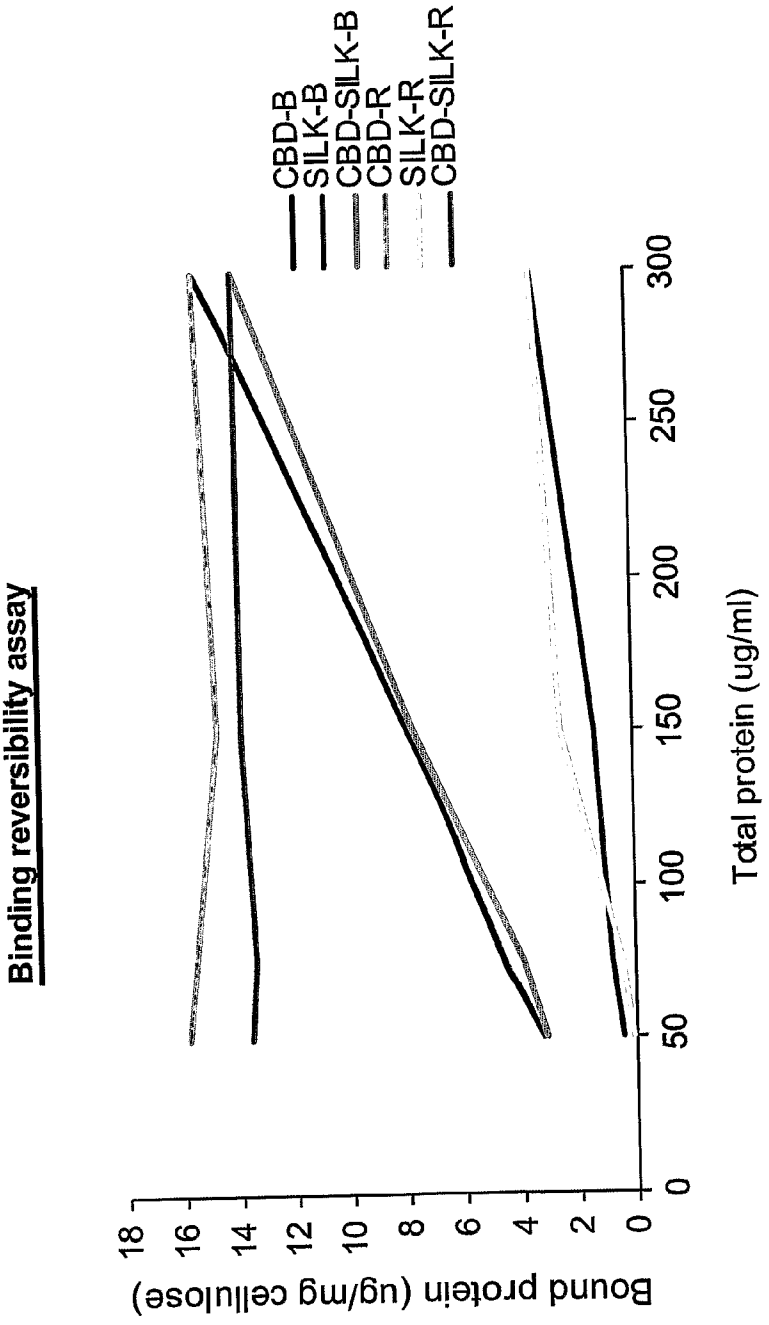

Binding reversibility assay
CBD-B
SILK-B
CBD-SILK-B
CBD-R
SILK-R
CBD-SILK-R
Bound protein (ug/mg cellulose)
Total protein (ug/ml)
0
2
4
6
8
10
12
14
16
18
0
50
100
150
200
250
300

FIG. 26

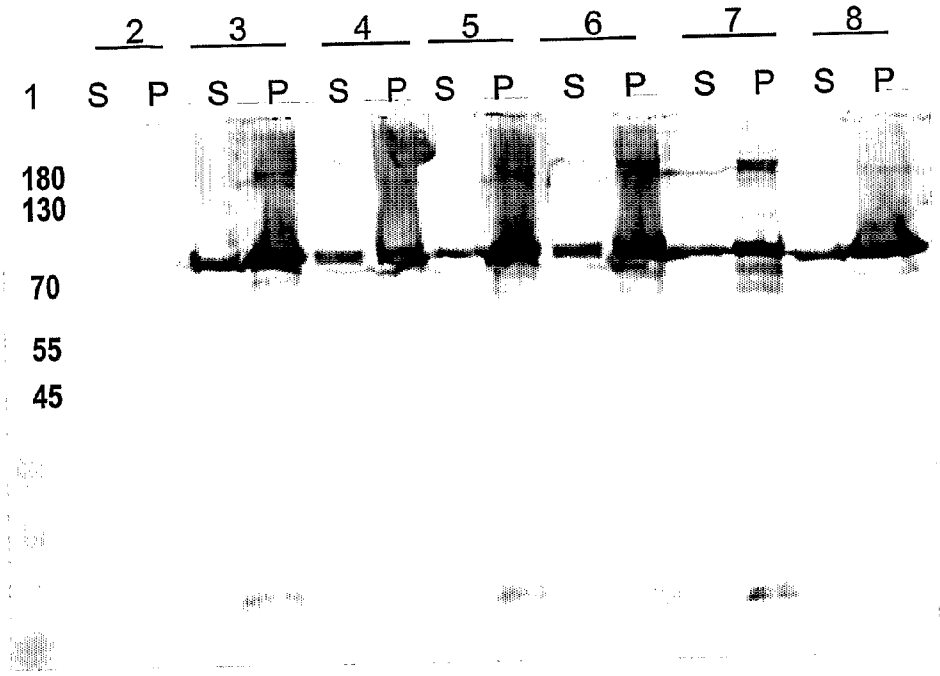


FIG. 27A

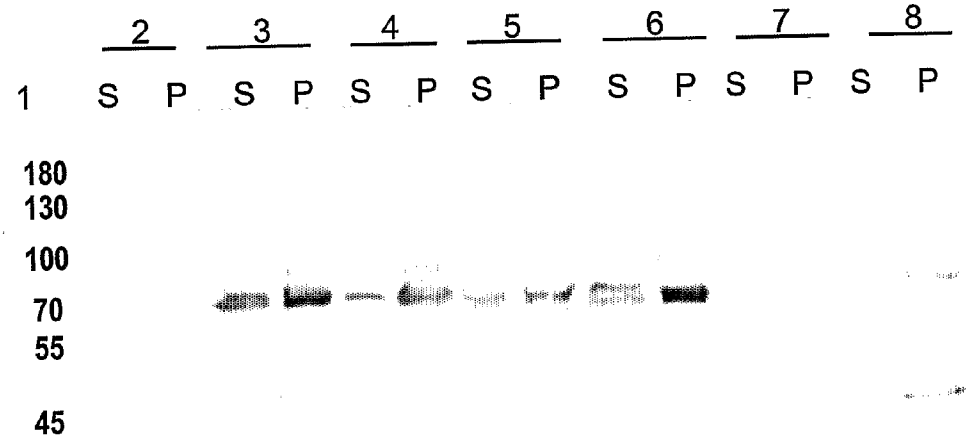


FIG. 27B

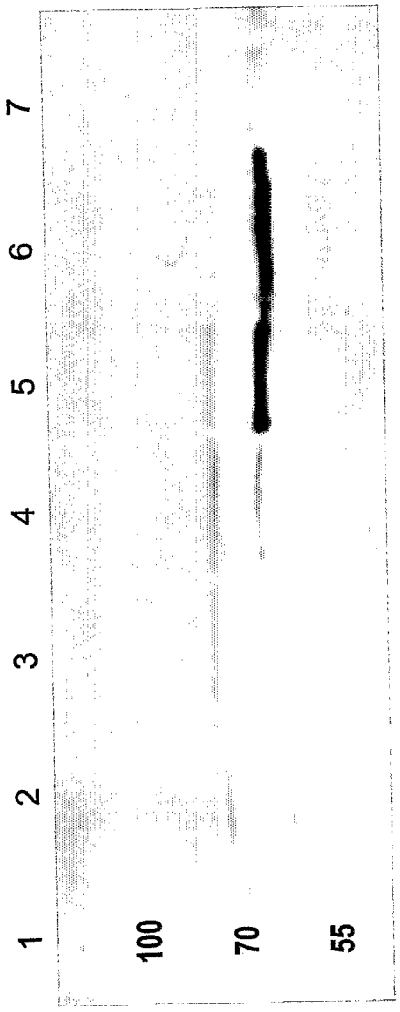


FIG. 28A

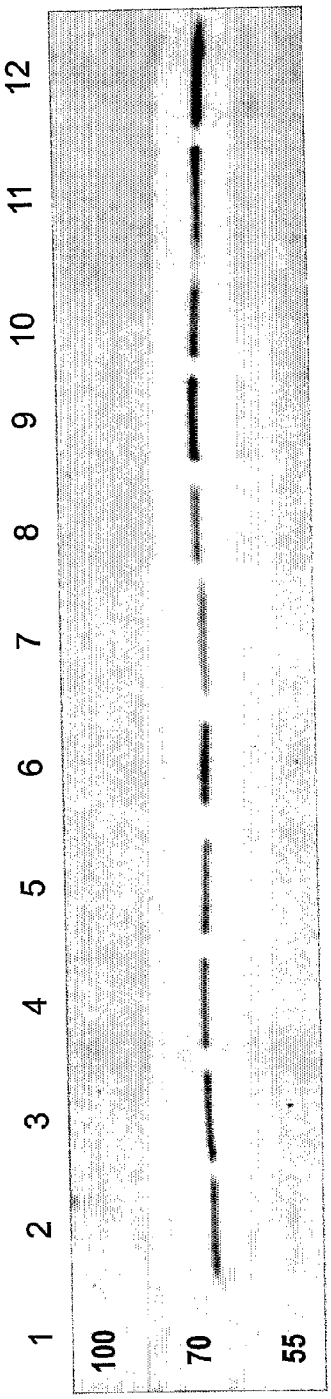


FIG. 28B

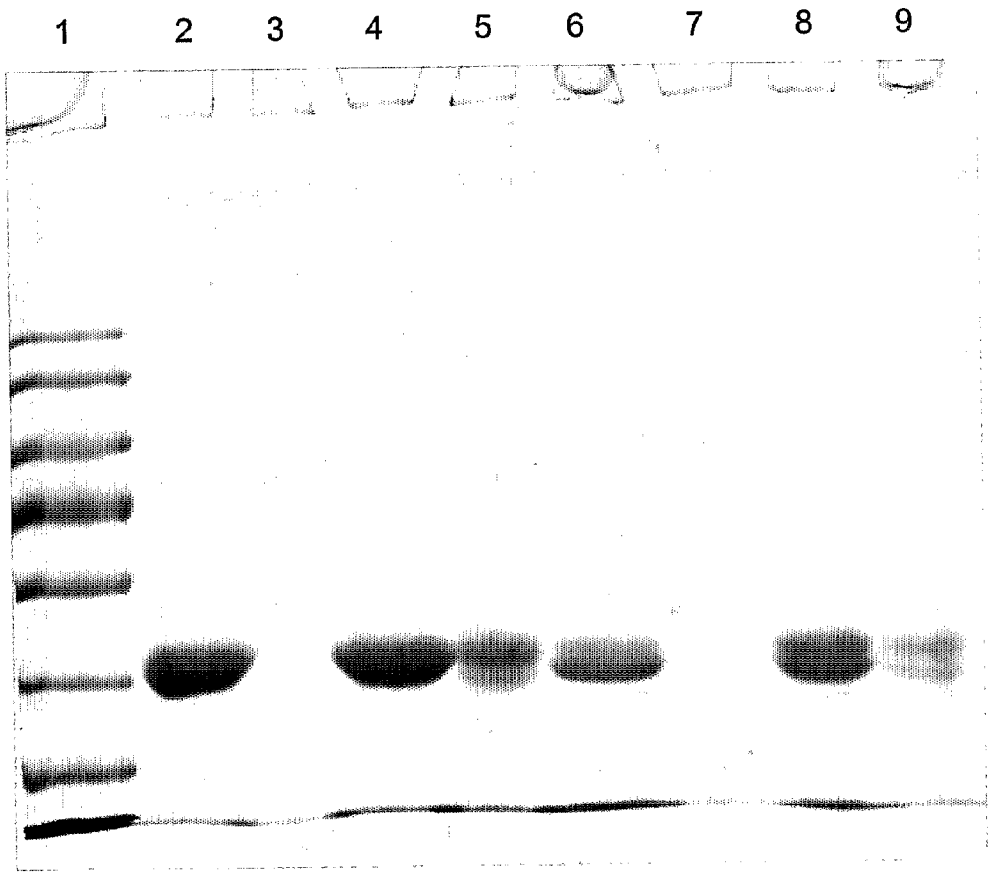


FIG. 29

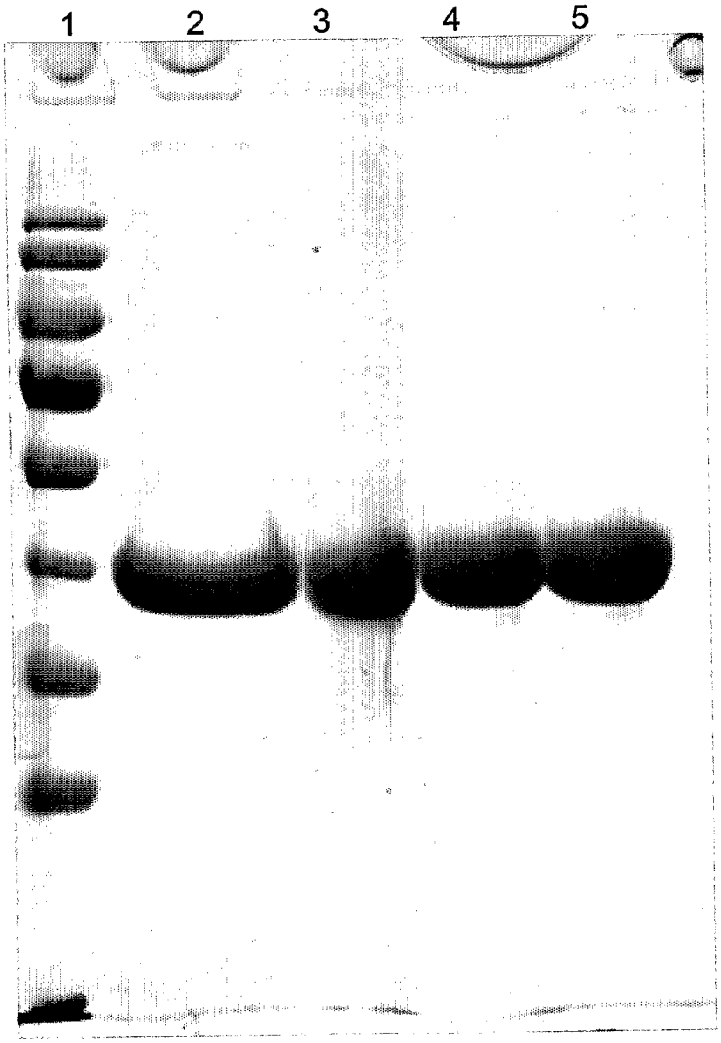


FIG. 30

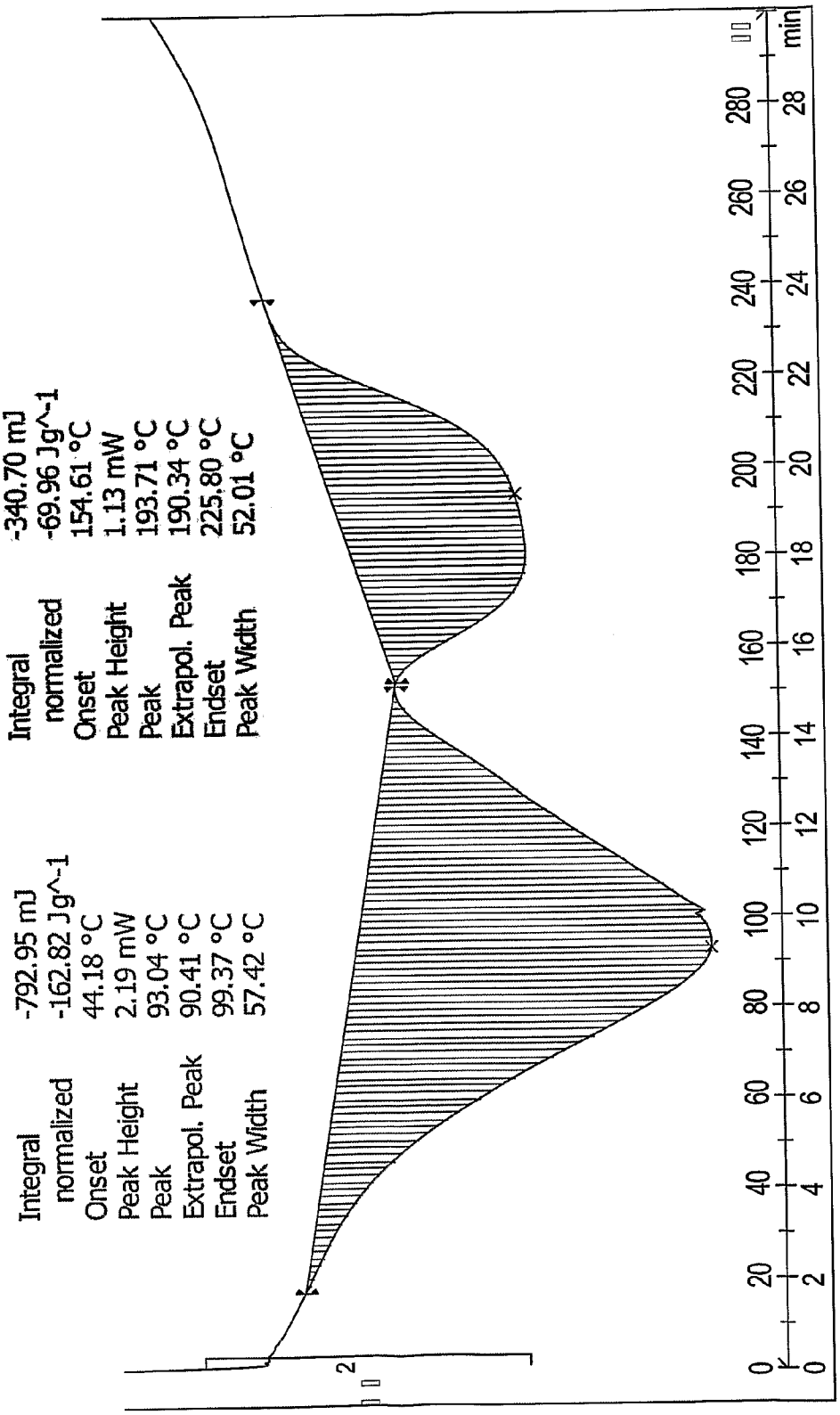

Integral -792.95 mJ
normalized -162.82 Jg^-1
Onset 44.18 °C
Peak Height 2.19 mW
Peak 93.04 °C
Extrapol. Peak 90.41 °C
Endset 99.37 °C
Peak Width 57.42 °C
Integral -340.70 mJ
normalized -69.96 Jg^-1
Onset 154.61 °C
Peak Height 1.13 mW
Peak 193.71 °C
Extrapol. Peak 190.34 °C
Endset 225.80 °C
Peak Width 52.01 °C
mW
min
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28

FIG. 31A

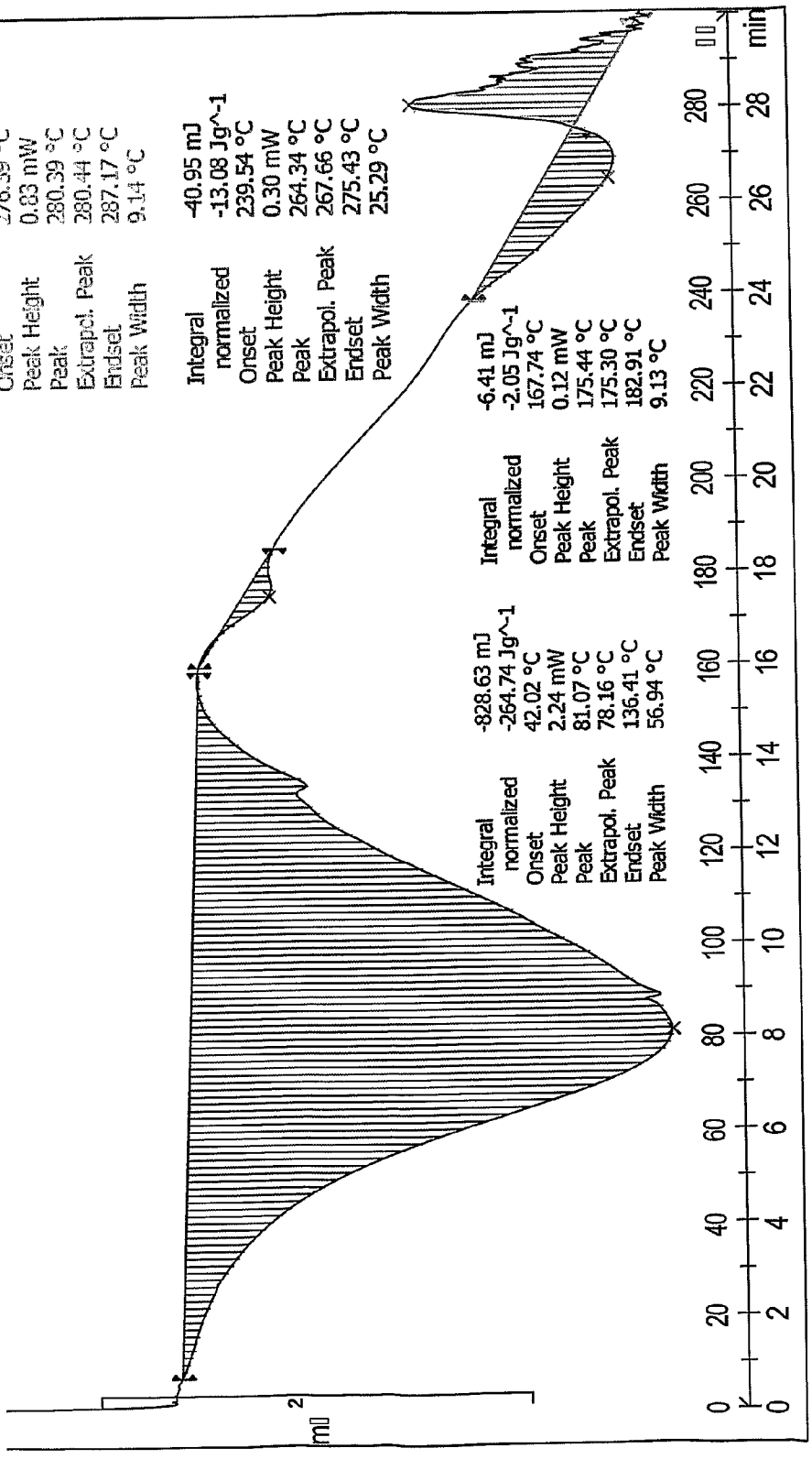

Integral 49.28 mJ
normalized 15.74 Jg^-1
Onset 276.39 °C
Peak Height 0.83 mW
Peak 280.39 °C
Extrapol. Peak 280.44 °C
Endset 287.17 °C
Peak Width 9.14 °C
Integral -40.95 mJ
normalized -13.08 Jg^-1
Onset 239.54 °C
Peak Height 0.30 mW
Peak 264.34 °C
Extrapol. Peak 267.66 °C
Endset 275.43 °C
Peak Width 25.29 °C
Integral -828.63 mJ
normalized -264.74 Jg^-1
Onset 42.02 °C
Peak Height 2.24 mW
Peak 81.07 °C
Extrapol. Peak 78.16 °C
Endset 136.41 °C
Peak Width 56.94 °C
Integral -6.41 mJ
normalized -2.05 Jg^-1
Onset 167.74 °C
Peak Height 0.12 mW
Peak 175.44 °C
Extrapol. Peak 175.30 °C
Endset 182.91 °C
Peak Width 9.13 °C
mW
min

FIG. 31B

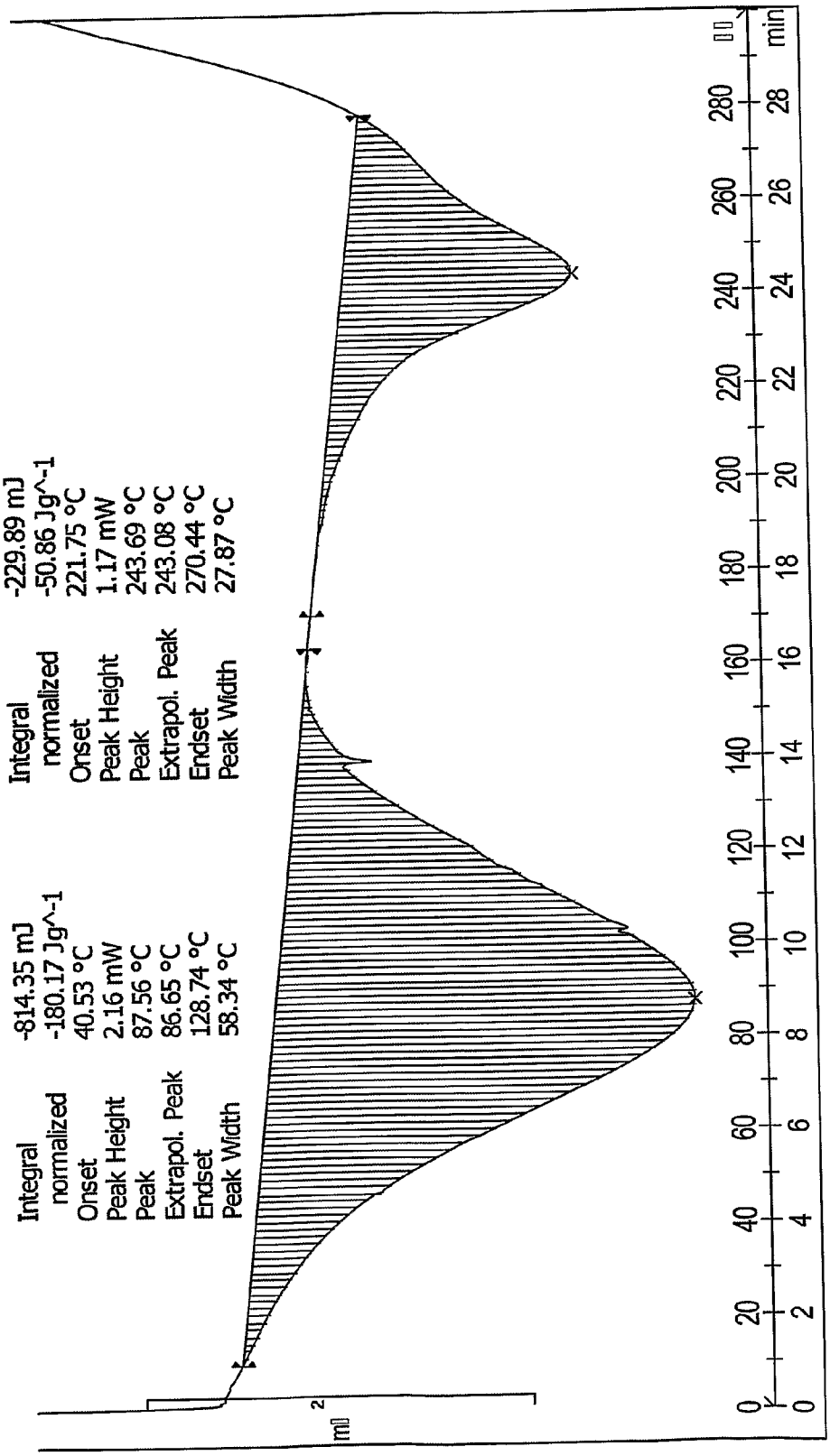

Integral -814.35 mJ
normalized -180.17 Jg^-1
Onset 40.53 °C
Peak Height 2.16 mW
Peak 87.56 °C
Extrapol. Peak 86.65 °C
Endset 128.74 °C
Peak Width 58.34 °C
Integral -229.89 mJ
normalized -50.86 Jg^-1
Onset 221.75 °C
Peak Height 1.17 mW
Peak 243.69 °C
Extrapol. Peak 243.08 °C
Endset 270.44 °C
Peak Width 27.87 °C
mJ
min

FIG. 31C

SEQUENCE LISTING

<110> Yissum Research Development Company of the Hebrew
University of Jerusalem
CollPlant Ltd.
Shoseyov, Oded
Lapidot, Shaul
Meirovitch, Sigal
Siegel, Daniel L.

<120> COMPOSITIONS COMPRISING FIBROUS POLYPEPTIDES AND POLYSACCHARIDES

<130> 45169

<150> US 60/996,581
<151> 2007-11-26

<150> US 61/071,968
<151> 2008-05-28

<160> 62

<170> PatentIn version 3.5

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<220>
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<210> 2
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<400> 2
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<210> 3
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<213> Artificial sequence

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<223> Single strand DNA oligonucleotide

<400> 3
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<213> Artificial sequence

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<212> DNA
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<220>
<223> Single strand DNA oligonucleotide

<400> 7
ggatccttac tcatcgttat cgtagtcagc g 31

<210> 8
<211> 15
<212> PRT
<213> Artificial sequence

<220>
<223> Elastic repeat unit of resilin

<400> 8

Gly Gly Arg Pro Ser Asp Ser Tyr Gly Ala Pro Gly Gly Gly Asn
1 5 10 15

<210> 9
<211> 325
<212> PRT
<213> Artificial sequence

<220>
<223> Minimal resilin polypeptide sequence derived from Drosophila

<400> 9

Met Gly Pro Glu Pro Pro Val Asn Ser Tyr Leu Pro Pro Ser Asp Ser
1 5 10 15

Tyr Gly Ala Pro Gly Gln Ser Gly Pro Gly Gly Arg Pro Ser Asp Ser
20 25 30

Tyr Gly Ala Pro Gly Gly Gly Asn Gly Gly Arg Pro Ser Asp Ser Tyr
35 40 45

Gly Ala Pro Gly Gln Gly Gln Gly Gln Gly Gln Gly Gln Gly Gly Tyr
50 55 60

```
Ala Gly Lys Pro Ser Asp Thr Tyr Gly Ala Pro Gly Gly Gly Asn Gly
65                  70                  75                  80

Asn Gly Gly Arg Pro Ser Ser Ser Tyr Gly Ala Pro Gly Gly Gly Asn
                85                  90                  95

Gly Gly Arg Pro Ser Asp Thr Tyr Gly Ala Pro Gly Gly Gly Asn Gly
            100                 105                 110

Gly Arg Pro Ser Asp Thr Tyr Gly Ala Pro Gly Gly Gly Gly Asn Gly
        115                 120                 125

Asn Gly Gly Arg Pro Ser Ser Ser Tyr Gly Ala Pro Gly Gln Gly Gln
    130                 135                 140

Gly Asn Gly Asn Gly Gly Arg Ser Ser Ser Ser Tyr Gly Ala Pro Gly
145                 150                 155                 160

Gly Gly Asn Gly Gly Arg Pro Ser Asp Thr Tyr Gly Ala Pro Gly Gly
                165                 170                 175

Gly Asn Gly Gly Arg Pro Ser Asp Thr Tyr Gly Ala Pro Gly Gly Gly
            180                 185                 190

Asn Asn Gly Gly Arg Pro Ser Ser Ser Tyr Gly Ala Pro Gly Gly Gly
        195                 200                 205

Asn Gly Gly Arg Pro Ser Asp Thr Tyr Gly Ala Pro Gly Gly Gly Asn
    210                 215                 220

Gly Asn Gly Ser Gly Gly Arg Pro Ser Ser Ser Tyr Gly Ala Pro Gly
225                 230                 235                 240

Gln Gly Gln Gly Gly Phe Gly Gly Arg Pro Ser Asp Ser Tyr Gly Ala
                245                 250                 255

Pro Gly Gln Asn Gln Lys Pro Ser Asp Ser Tyr Gly Ala Pro Gly Ser
            260                 265                 270

Gly Asn Gly Asn Gly Gly Arg Pro Ser Ser Ser Tyr Gly Ala Pro Gly
        275                 280                 285

Ser Gly Pro Gly Gly Arg Pro Ser Asp Ser Tyr Gly Pro Pro Ala Ser
    290                 295                 300

Gly Ser Gly Ala Gly Gly Ala Gly Gly Ser Gly Pro Gly Gly Ala Asp
305                 310                 315                 320

Tyr Asp Asn Asp Glu
                325
```

<210> 10
<211> 163
<212> PRT
<213> Artificial sequence

<220>

<223> Clostridium cellulovorans derived cellulose binding domain (CBD)

<220>
<221> misc_feature
<223> cellulose binding domain (CBD)

<400> 10

Met Ala Ala Thr Ser Ser Met Ser Val Glu Phe Tyr Asn Ser Asn Lys
1 5 10 15

Ala Ala Gln Thr Asn Ser Ile Thr Pro Ile Ile Lys Ile Thr Asn Thr
20 25 30

Ala Asp Ser Asp Leu Asn Leu Asn Asp Val Lys Val Arg Tyr Tyr Tyr
35 40 45

Thr Ser Asp Gly Thr Gln Gly Gln Thr Phe Trp Gly Asp His Ala Gly
50 55 60

Ala Leu Leu Gly Asn Ser Tyr Val Asp Asn Thr Gly Lys Val Thr Ala
65 70 75 80

Asn Phe Val Lys Glu Thr Ala Ser Pro Thr Ser Thr Tyr Asp Thr Tyr
85 90 95

Val Glu Phe Gly Phe Ala Ser Gly Ala Ala Thr Leu Lys Lys Gly Gln
100 105 110

Phe Ile Thr Ile Gln Gly Arg Ile Thr Lys Ser Asp Trp Ser Asn Tyr
115 120 125

Ala Gln Thr Asn Asp Tyr Ser Phe Asp Ala Ser Ser Ser Thr Pro Val
130 135 140

Val Asn Pro Lys Val Thr Gly Tyr Ile Gly Gly Ala Lys Val Leu Gly
145 150 155 160

Thr Ala Pro

<210> 11
<211> 399
<212> PRT
<213> Artificial sequence

<220>
<223> Resilin 17 elastic repeats including the native putative chitin binding domain (Res-CHBD)

<400> 11

Met Gly Pro Glu Pro Pro Val Asn Ser Tyr Leu Pro Pro Ser Asp Ser
1 5 10 15

Tyr Gly Ala Pro Gly Gln Ser Gly Pro Gly Gly Arg Pro Ser Asp Ser
20 25 30

Tyr Gly Ala Pro Gly Gly Gly Asn Gly Gly Arg Pro Ser Asp Ser Tyr
35 40 45

```
Gly Ala Pro Gly Gln Gly Gln Gly Gln Gly Gln Gly Gly Tyr
    50                  55                  60

Ala Gly Lys Pro Ser Asp Thr Tyr Gly Ala Pro Gly Gly Gly Asn Gly
65                  70                  75                  80

Asn Gly Gly Arg Pro Ser Ser Ser Tyr Gly Ala Pro Gly Gly Gly Asn
                85                  90                  95

Gly Gly Arg Pro Ser Asp Thr Tyr Gly Ala Pro Gly Gly Gly Asn Gly
            100                 105                 110

Gly Arg Pro Ser Asp Thr Tyr Gly Ala Pro Gly Gly Gly Gly Asn Gly
        115                 120                 125

Asn Gly Gly Arg Pro Ser Ser Ser Tyr Gly Ala Pro Gly Gln Gly Gln
    130                 135                 140

Gly Asn Gly Asn Gly Gly Arg Ser Ser Ser Ser Tyr Gly Ala Pro Gly
145                 150                 155                 160

Gly Gly Asn Gly Gly Arg Pro Ser Asp Thr Tyr Gly Ala Pro Gly Gly
                165                 170                 175

Gly Asn Gly Gly Arg Pro Ser Asp Thr Tyr Gly Ala Pro Gly Gly Gly
            180                 185                 190

Asn Asn Gly Gly Arg Pro Ser Ser Ser Tyr Gly Ala Pro Gly Gly Gly
        195                 200                 205

Asn Gly Gly Arg Pro Ser Asp Thr Tyr Gly Ala Pro Gly Gly Gly Asn
    210                 215                 220

Gly Asn Gly Ser Gly Gly Arg Pro Ser Ser Ser Tyr Gly Ala Pro Gly
225                 230                 235                 240

Gln Gly Gln Gly Gly Phe Gly Gly Arg Pro Ser Asp Ser Tyr Gly Ala
                245                 250                 255

Pro Gly Gln Asn Gln Lys Pro Ser Asp Ser Tyr Gly Ala Pro Gly Ser
            260                 265                 270

Gly Asn Gly Asn Gly Gly Arg Pro Ser Ser Ser Tyr Gly Ala Pro Gly
        275                 280                 285

Ser Gly Pro Gly Gly Arg Pro Ser Asp Ser Tyr Gly Pro Pro Ala Ser
    290                 295                 300

Gly Ser Gly Ala Gly Gly Ala Gly Gly Ser Gly Pro Gly Gly Ala Asp
305                 310                 315                 320

Tyr Asp Asn Asp Glu Pro Ala Lys Tyr Glu Phe Asn Tyr Gln Val Glu
                325                 330                 335
```

```
Asp Ala Pro Ser Gly Leu Ser Phe Gly His Ser Glu Met Arg Asp Gly
            340                 345                 350

Asp Phe Thr Thr Gly Gln Tyr Asn Val Leu Leu Pro Asp Gly Arg Lys
        355                 360                 365

Gln Ile Val Glu Tyr Glu Ala Asp Gln Gln Gly Tyr Arg Pro Gln Ile
    370                 375                 380

Arg Tyr Glu Gly Asp Ala Asn Asp Gly Ser Gly Pro Ser Gly Pro
385                 390                 395
```

<210> 12
<211> 503
<212> PRT
<213> Artificial sequence

<220>
<223> Clostridium cellulovorans CBD (CBDclos) fused to Resilin 17 elastic repeats (CBD-resilin)

<400> 12

```
Met Ala Ala Thr Ser Ser Met Ser Val Glu Phe Tyr Asn Ser Asn Lys
1               5                   10                  15

Ala Ala Gln Thr Asn Ser Ile Thr Pro Ile Ile Lys Ile Thr Asn Thr
            20                  25                  30

Ala Asp Ser Asp Leu Asn Leu Asn Asp Val Lys Val Arg Tyr Tyr Tyr
        35                  40                  45

Thr Ser Asp Gly Thr Gln Gly Gln Thr Phe Trp Gly Asp His Ala Gly
    50                  55                  60

Ala Leu Leu Gly Asn Ser Tyr Val Asp Asn Thr Gly Lys Val Thr Ala
65                  70                  75                  80

Asn Phe Val Lys Glu Thr Ala Ser Pro Thr Ser Thr Tyr Asp Thr Tyr
                85                  90                  95

Val Glu Phe Gly Phe Ala Ser Gly Ala Ala Thr Leu Lys Lys Gly Gln
            100                 105                 110

Phe Ile Thr Ile Gln Gly Arg Ile Thr Lys Ser Asp Trp Ser Asn Tyr
        115                 120                 125

Ala Gln Thr Asn Asp Tyr Ser Phe Asp Ala Ser Ser Ser Thr Pro Val
    130                 135                 140

Val Asn Pro Lys Val Thr Gly Tyr Ile Gly Gly Ala Lys Val Leu Gly
145                 150                 155                 160

Thr Ala Pro Gly Pro Asp Val Pro Ser Ser Ile Ile Asn Pro Thr Ser
                165                 170                 175

Ala Thr Phe Asp Pro Glu Pro Pro Val Asn Ser Tyr Leu Pro Pro Ser
            180                 185                 190
```

```
Asp Ser Tyr Gly Ala Pro Gly Gln Ser Gly Pro Gly Gly Arg Pro Ser
        195                 200                 205

Asp Ser Tyr Gly Ala Pro Gly Gly Gly Asn Gly Gly Arg Pro Ser Asp
    210                 215                 220

Ser Tyr Gly Ala Pro Gly Gln Gly Gln Gly Gln Gly Gln Gly Gln Gly
225                 230                 235                 240

Gly Tyr Ala Gly Lys Pro Ser Asp Thr Tyr Gly Ala Pro Gly Gly Gly
                245                 250                 255

Asn Gly Asn Gly Gly Arg Pro Ser Ser Ser Tyr Gly Ala Pro Gly Gly
            260                 265                 270

Gly Asn Gly Gly Arg Pro Ser Asp Thr Tyr Gly Ala Pro Gly Gly Gly
        275                 280                 285

Asn Gly Gly Arg Pro Ser Asp Thr Tyr Gly Ala Pro Gly Gly Gly Gly
    290                 295                 300

Asn Gly Asn Gly Gly Arg Pro Ser Ser Ser Tyr Gly Ala Pro Gly Gln
305                 310                 315                 320

Gly Gln Gly Asn Gly Asn Gly Gly Arg Ser Ser Ser Ser Tyr Gly Ala
                325                 330                 335

Pro Gly Gly Gly Asn Gly Gly Arg Pro Ser Asp Thr Tyr Gly Ala Pro
            340                 345                 350

Gly Gly Gly Asn Gly Gly Arg Pro Ser Asp Thr Tyr Gly Ala Pro Gly
        355                 360                 365

Gly Gly Asn Asn Gly Gly Arg Pro Ser Ser Ser Tyr Gly Ala Pro Gly
    370                 375                 380

Gly Gly Asn Gly Gly Arg Pro Ser Asp Thr Tyr Gly Ala Pro Gly Gly
385                 390                 395                 400

Gly Asn Gly Asn Gly Ser Gly Gly Arg Pro Ser Ser Ser Tyr Gly Ala
                405                 410                 415

Pro Gly Gln Gly Gln Gly Gly Phe Gly Gly Arg Pro Ser Asp Ser Tyr
            420                 425                 430

Gly Ala Pro Gly Gln Asn Gln Lys Pro Ser Asp Ser Tyr Gly Ala Pro
        435                 440                 445

Gly Ser Gly Asn Gly Asn Gly Gly Arg Pro Ser Ser Ser Tyr Gly Ala
    450                 455                 460

Pro Gly Ser Gly Pro Gly Gly Arg Pro Ser Asp Ser Tyr Gly Pro Pro
465                 470                 475                 480

Ala Ser Gly Ser Gly Ala Gly Gly Ala Gly Gly Ser Gly Pro Gly Gly
```

485 490 495

Ala Asp Tyr Asp Asn Asp Glu
500

<210> 13
<211> 494
<212> PRT
<213> Artificial sequence

<220>
<223> Resilin fused to CBD through a linker polypeptide

<400> 13

Met Gly Pro Glu Pro Pro Val Asn Ser Tyr Leu Pro Pro Ser Asp Ser
1 5 10 15

Tyr Gly Ala Pro Gly Gln Ser Gly Pro Gly Gly Arg Pro Ser Asp Ser
20 25 30

Tyr Gly Ala Pro Gly Gly Gly Asn Gly Gly Arg Pro Ser Asp Ser Tyr
35 40 45

Gly Ala Pro Gly Gln Gly Gln Gly Gln Gly Gln Gly Gln Gly Gly Tyr
50 55 60

Ala Gly Lys Pro Ser Asp Thr Tyr Gly Ala Pro Gly Gly Gly Asn Gly
65 70 75 80

Asn Gly Gly Arg Pro Ser Ser Ser Tyr Gly Ala Pro Gly Gly Gly Asn
85 90 95

Gly Gly Arg Pro Ser Asp Thr Tyr Gly Ala Pro Gly Gly Gly Asn Gly
100 105 110

Gly Arg Pro Ser Asp Thr Tyr Gly Ala Pro Gly Gly Gly Gly Asn Gly
115 120 125

Asn Gly Gly Arg Pro Ser Ser Ser Tyr Gly Ala Pro Gly Gln Gly Gln
130 135 140

Gly Asn Gly Asn Gly Gly Arg Ser Ser Ser Ser Tyr Gly Ala Pro Gly
145 150 155 160

Gly Gly Asn Gly Gly Arg Pro Ser Asp Thr Tyr Gly Ala Pro Gly Gly
165 170 175

Gly Asn Gly Gly Arg Pro Ser Asp Thr Tyr Gly Ala Pro Gly Gly Gly
180 185 190

Asn Asn Gly Gly Arg Pro Ser Ser Ser Tyr Gly Ala Pro Gly Gly Gly
195 200 205

Asn Gly Gly Arg Pro Ser Asp Thr Tyr Gly Ala Pro Gly Gly Gly Asn
210 215 220

Gly Asn Gly Ser Gly Gly Arg Pro Ser Ser Ser Tyr Gly Ala Pro Gly

225 230 235 240

Gln Gly Gln Gly Gly Phe Gly Gly Arg Pro Ser Asp Ser Tyr Gly Ala
245 250 255

Pro Gly Gln Asn Gln Lys Pro Ser Asp Ser Tyr Gly Ala Pro Gly Ser
260 265 270

Gly Asn Gly Asn Gly Gly Arg Pro Ser Ser Ser Tyr Gly Ala Pro Gly
275 280 285

Ser Gly Pro Gly Gly Arg Pro Ser Asp Ser Tyr Gly Pro Pro Ala Ser
290 295 300

Gly Ser Gly Ala Gly Gly Ala Gly Gly Ser Gly Pro Gly Gly Ala Asp
305 310 315 320

Tyr Asp Asn Asp Glu Gly Ile Pro Asp Pro Gly Met Ala Ala Thr Ser
325 330 335

Ser Met Ser Val Glu Phe Tyr Asn Ser Asn Lys Ser Ala Gln Thr Asn
340 345 350

Ser Ile Thr Pro Ile Ile Lys Ile Thr Asn Thr Ser Asp Ser Asp Leu
355 360 365

Asn Leu Asn Asp Val Lys Val Arg Tyr Tyr Tyr Thr Ser Asp Gly Thr
370 375 380

Gln Gly Gln Thr Phe Trp Cys Asp His Ala Gly Ala Leu Leu Gly Asn
385 390 395 400

Ser Tyr Val Asp Asn Thr Ser Lys Val Thr Ala Asn Phe Val Lys Glu
405 410 415

Thr Ala Ser Pro Thr Ser Thr Tyr Asp Thr Tyr Val Glu Phe Gly Phe
420 425 430

Ala Ser Gly Arg Ala Thr Leu Lys Lys Gly Gln Phe Ile Thr Ile Gln
435 440 445

Gly Arg Ile Thr Lys Ser Asp Trp Ser Asn Tyr Thr Gln Thr Asn Asp
450 455 460

Tyr Ser Phe Asp Ala Ser Ser Ser Thr Pro Val Val Asn Pro Lys Val
465 470 475 480

Thr Gly Tyr Ile Gly Gly Ala Lys Val Leu Gly Thr Ala Pro
485 490

<210> 14
<211> 329
<212> PRT
<213> Artificial sequence

<220>
<223> Resilin fused to a C' linker polypeptide (Elvin)

<400> 14

```
Met Gly Pro Glu Pro Pro Val Asn Ser Tyr Leu Pro Pro Ser Asp Ser
1               5                  10                  15

Tyr Gly Ala Pro Gly Gln Ser Gly Pro Gly Gly Arg Pro Ser Asp Ser
            20                  25                  30

Tyr Gly Ala Pro Gly Gly Gly Asn Gly Gly Arg Pro Ser Asp Ser Tyr
        35                  40                  45

Gly Ala Pro Gly Gln Gly Gln Gly Gln Gly Gln Gly Gln Gly Gly Tyr
    50                  55                  60

Ala Gly Lys Pro Ser Asp Thr Tyr Gly Ala Pro Gly Gly Gly Asn Gly
65                  70                  75                  80

Asn Gly Gly Arg Pro Ser Ser Ser Tyr Gly Ala Pro Gly Gly Gly Asn
                85                  90                  95

Gly Gly Arg Pro Ser Asp Thr Tyr Gly Ala Pro Gly Gly Gly Asn Gly
            100                 105                 110

Gly Arg Pro Ser Asp Thr Tyr Gly Ala Pro Gly Gly Gly Gly Asn Gly
        115                 120                 125

Asn Gly Gly Arg Pro Ser Ser Ser Tyr Gly Ala Pro Gly Gln Gly Gln
    130                 135                 140

Gly Asn Gly Asn Gly Gly Arg Ser Ser Ser Ser Tyr Gly Ala Pro Gly
145                 150                 155                 160

Gly Gly Asn Gly Gly Arg Pro Ser Asp Thr Tyr Gly Ala Pro Gly Gly
                165                 170                 175

Gly Asn Gly Gly Arg Pro Ser Asp Thr Tyr Gly Ala Pro Gly Gly Gly
            180                 185                 190

Asn Asn Gly Gly Arg Pro Ser Ser Ser Tyr Gly Ala Pro Gly Gly Gly
        195                 200                 205

Asn Gly Gly Arg Pro Ser Asp Thr Tyr Gly Ala Pro Gly Gly Gly Asn
    210                 215                 220

Gly Asn Gly Ser Gly Gly Arg Pro Ser Ser Ser Tyr Gly Ala Pro Gly
225                 230                 235                 240

Gln Gly Gln Gly Gly Phe Gly Gly Arg Pro Ser Asp Ser Tyr Gly Ala
                245                 250                 255

Pro Gly Gln Asn Gln Lys Pro Ser Asp Ser Tyr Gly Ala Pro Gly Ser
            260                 265                 270

Gly Asn Gly Asn Gly Gly Arg Pro Ser Ser Ser Tyr Gly Ala Pro Gly
        275                 280                 285
```

```
Ser Gly Pro Gly Gly Arg Pro Ser Asp Ser Tyr Gly Pro Pro Ala Ser
    290                 295                 300

Gly Ser Gly Ala Gly Gly Ala Gly Gly Ser Gly Pro Gly Gly Ala Asp
305                 310                 315                 320

Tyr Asp Asn Asp Glu Gly Ser Asn His
                325
```

<210> 15
<211> 1007
<212> DNA
<213> Artificial sequence

<220>
<223> Resilin fused a linker polynucleotide sequence

<400> 15

```
ccatgggacc ggagccacca gttaactcgt atctacctcc gtccgatagc tatggagcac       60

cgggtcagag tggtcccggc ggcaggccgt cggattccta tggagctcct ggtggtggaa      120

acggtggacg gccctcagac agctatggcg ctccaggcca gggtcaagga cagggacaag      180

gacaaggtgg atatgcaggc aagccctcag atacctatgg agctcctggt ggtggaaatg      240

gcaacggagg tcgtccatcg agcagctatg gcgctcctgg cggtggaaac ggtggtcgtc      300

cttcggatac ctacggtgct cctggtggcg gaaatggtgg acgcccatcg gacacttatg      360

gtgctcctgg tggtggtgga aatggcaacg gcggacgacc ttcaagcagc tatggagctc      420

ctggtcaagg acaaggcaac ggaaatggcg gtcgctcatc gagcagctat ggtgctcctg      480

gcggtggaaa cggcggtcgt ccttcggata cctacggtgc tcccggtggt ggaaacggtg      540

gtcgtccttc ggatacttac ggcgctcctg gtggcggcaa taatggcggt cgtccctcaa      600

gcagctacgg cgctcctggt ggtggaaacg gtggtcgtcc atctgacacc tatggcgctc      660

ctggtggcgg taacggaaac ggcagcggtg gtcgtccttc aagcagctat ggagctcctg      720

gtcagggcca aggtggattt ggtggtcgtc catcggactc ctatggtgct cctggtcaga      780

accaaaaacc atcagattca tatggcgccc ctggtagcgg caatggcaac ggcggacgtc      840

cttcgagcag ctatggagct ccaggctcag gacctggtgg ccgaccctcc gactcctacg      900

gacccccagc ttctggatcg ggagcaggtg gcgctggagg cagtggaccc ggcggcgctg      960

actacgataa cgatgaggga tccaatcact agtgaattcg cggccgc                   1007
```

<210> 16
<211> 513
<212> PRT
<213> Artificial sequence

<220>
<223> 15 sps spider silk polypeptide

<400> 16

```
Ser Gly Arg Gly Gly Leu Gly Gly Gln Gly Ala Gly Ala Ala Ala Ala
1               5                  10                  15

Ala Gly Gly Ala Gly Gln Gly Gly Tyr Gly Gly Leu Gly Ser Gln Gly
            20                  25                  30
```

```
Thr Ser Gly Arg Gly Gly Leu Gly Gly Gln Gly Ala Gly Ala Ala Ala
        35                  40                  45

Ser Ala Gly Gly Ala Gly Gln Gly Gly Tyr Gly Gly Leu Gly Ser Gln
    50                  55                  60

Gly Thr Ser Gly Arg Gly Gly Leu Gly Gly Gln Gly Ala Gly Ala Ala
65                  70                  75                  80

Ala Ala Ala Gly Gly Ala Gly Gln Gly Gly Tyr Gly Gly Leu Gly Ser
                85                  90                  95

Gln Gly Thr Ser Gly Arg Gly Gly Leu Gly Gly Gln Gly Ala Gly Ala
            100                 105                 110

Ala Ala Ala Ala Gly Gly Ala Gly Gln Gly Gly Tyr Gly Gly Leu Gly
        115                 120                 125

Ser Gln Gly Thr Ala Ser Gly Arg Gly Gly Leu Gly Gly Gln Gly Ala
    130                 135                 140

Gly Ala Ala Ala Ala Ala Gly Gly Ala Gly Gln Gly Gly Tyr Gly Gly
145                 150                 155                 160

Leu Gly Ser Gln Gly Thr Ala Ser Gly Arg Gly Gly Leu Gly Gly Gln
                165                 170                 175

Gly Ala Gly Ala Ala Ala Ala Ala Gly Gly Ala Gly Gln Gly Gly Tyr
            180                 185                 190

Gly Gly Leu Gly Ser Gln Gly Thr Ala Ser Gly Arg Gly Gly Leu Gly
        195                 200                 205

Gly Gln Gly Ala Gly Ala Ala Ala Ala Ala Gly Gly Ala Gly Gln Gly
    210                 215                 220

Gly Tyr Gly Gly Leu Gly Ser Gln Gly Thr Ala Ser Gly Arg Gly Gly
225                 230                 235                 240

Leu Gly Gly Gln Gly Ala Gly Ala Ala Ala Ala Ala Gly Gly Ala Gly
                245                 250                 255

Gln Gly Gly Tyr Gly Gly Leu Gly Ser Gln Gly Thr Ala Ser Gly Arg
            260                 265                 270

Gly Gly Leu Gly Gly Gln Gly Ala Gly Ala Ala Ala Ala Ala Gly Gly
        275                 280                 285

Ala Gly Gln Gly Gly Tyr Gly Gly Leu Gly Ser Gln Gly Thr Ala Ser
    290                 295                 300

Gly Arg Gly Gly Leu Gly Gly Gln Gly Ala Gly Ala Ala Ala Ala Ala
305                 310                 315                 320

Gly Gly Ala Gly Gln Gly Gly Tyr Gly Gly Leu Gly Ser Gln Gly Thr
```

325 330 335

Ala Ser Gly Arg Gly Gly Leu Gly Gly Gln Gly Ala Gly Ala Ala Ala
340 345 350

Ala Ala Gly Gly Ala Gly Gln Gly Gly Tyr Gly Gly Leu Gly Ser Gln
355 360 365

Gly Thr Ala Ser Gly Arg Gly Gly Leu Gly Gly Gln Gly Ala Gly Ala
370 375 380

Ala Ala Ala Ala Gly Gly Ala Gly Gln Gly Gly Tyr Gly Gly Leu Gly
385 390 395 400

Ser Gln Gly Thr Ala Ser Gly Arg Gly Gly Leu Gly Gly Gln Gly Ala
405 410 415

Gly Ala Ala Ala Ala Ala Gly Gly Ala Gly Gln Gly Gly Tyr Gly Gly
420 425 430

Leu Gly Ser Gln Gly Thr Ala Ser Gly Arg Gly Gly Leu Gly Gly Gln
435 440 445

Gly Ala Gly Ala Ala Ala Ala Ala Gly Gly Ala Gly Gln Gly Gly Tyr
450 455 460

Gly Gly Leu Gly Ser Gln Gly Thr Ala Ser Gly Arg Gly Gly Leu Gly
465 470 475 480

Gly Gln Gly Ala Gly Ala Ala Ala Ala Ala Gly Gly Ala Gly Gln Gly
485 490 495

Gly Tyr Gly Gly Leu Gly Ser Gln Gly Thr Ser Gly Ser Ala Arg Ala
500 505 510

Arg

<210> 17
<211> 1208
<212> DNA
<213> Artificial sequence

<220>
<223> Resilin 17 elastic repeats including the native putative chitin binding domain (Res-CHBD) polynucleotide sequence

<400> 17
ccatgggacc ggagccacca gttaactcgt atctacctcc gtccgatagc tatggagcac 60

cgggtcagag tggtcccggc ggcaggccgt cggattccta tggagctcct ggtggtggaa 120

acggtggacg gccctcagac agctatggcg ctccaggcca gggtcaagga cagggacaag 180

gacaaggtgg atatgcaggc aagccctcag atacctatgg agctcctggt ggtggaaatg 240

gcaacggagg tcgtccatcg agcagctatg gcgctcctgg cggtggaaac ggtggtcgtc 300

cttcggatac ctacggtgct cctggtggcg gaaatggtgg acgcccatcg gacacttatg 360

gtgctcctgg tggtggtgga aatggcaacg gcggacgacc ttcaagcagc tatggagctc 420

ctggtcaagg acaaggcaac ggaaatggcg gtcgctcatc gagcagctat ggtgctcctg 480

gcggtggaaa cggcggtcgt ccttcggata cctacggtgc tcccggtggt ggaaacggtg 540

gtcgtccttc ggatacttac ggcgctcctg gtggcggcaa taatggcggt cgtccctcaa 600

gcagctacgg cgctcctggt ggtggaaacg gtggtcgtcc atctgacacc tatggcgctc 660

ctggtggcgg taacggaaac ggcagcggtg gtcgtccttc aagcagctat ggagctcctg 720

gtcagggcca aggtggattt ggtggtcgtc catcggactc ctatggtgct cctggtcaga 780

accaaaaacc atcagattca tatggcgccc ctggtagcgg caatggcaac ggcggacgtc 840

cttcgagcag ctatggagct ccaggctcag gacctggtgg ccgaccctcc gactcctacg 900

gacccccagc ttctggatcg ggagcaggtg gcgctggagg cagtggaccc ggcggcgctg 960

actacgataa cgatgagccc gccaagtacg aatttaatta ccaggttgag gacgcgccca 1020

gcggactctc gttcgggcat tcagagatgc gcgacggtga cttcaccacc ggccagtaca 1080

atgtcctgtt gcccgacgga aggaagcaaa ttgtggagta tgaagccgac cagcagggct 1140

accggccaca gatccgctac gaaggcgatg ccaacgatgg cagtggtccc agcggtcctt 1200

aaggatcc 1208

<210> 18
<211> 1584
<212> DNA
<213> Artificial sequence

<220>
<223> Clostridium cellulovorans CBD (CBDclos) fused to Resilin 17 elastic repeats (CBD-resilin) polynucleotide sequence

<400> 18
gattgtgagc ggataacaat tcccctctag aaataatttt gtttaacttt aagaaggaga 60

tataccatgg cagcgacatc atcaatgtca gttgaatttt acaactctaa caaagcagca 120

caaacaaact caattacacc aataatcaaa attactaaca cagctgacag tgatttaaat 180

ttaaatgacg taaaagttag atattattac acaagtgatg gtacacaagg acaaactttc 240

tggggtgatc atgctggtgc attattagga aatagctatg ttgataacac tggcaaagtg 300

acagcaaact tcgttaaaga aacagcaagc ccaacatcaa cctatgatac atatgttgaa 360

tttggatttg caagcggagc agctactctt aaaaaaggac aatttataac tattcaagga 420

agaataacaa aatcagactg gtcaaactac gctcagacaa atgactattc atttgatgca 480

agtagttcaa caccagttgt aaatccaaaa gttacaggat atataggtgg agctaaagta 540

cttggtacag caccaggtcc agatgtacca tcttcaataa ttaatcctac ttctgcaaca 600

tttgatccgg agccaccagt taactcgtat ctacctccgt ccgatagcta tggagcaccg 660

ggtcagagtg gtcccggcgg caggccgtcg gattcctatg gagctcctgg tggtggaaac 720

ggtggacggc cctcagacag ctatggcgct ccaggccagg gtcaaggaca gggacaagga 780

caaggtggat atgcaggcaa gccctcagat acctatggag ctcctggtgg tggaaatggc 840

aacggaggtc gtccatcgag cagctatggc gctcctggcg gtggaaacgg tggtcgtcct 900

tcggatacct acggtgctcc tggtggcgga aatggtggac gcccatcgga cacttatggt 960

gctcctggtg gtggtggaaa tggcaacggc ggacgacctt caagcagcta tggagctcct 1020

ggtcaaggac aaggcaacgg aaatggcggt cgctcatcga gcagctatgg tgctcctggc 1080

ggtggaaacg gcggtcgtcc ttcggatacc tacggtgctc ccggtggtgg aaacggtggt 1140

cgtccttcgg atacttacgg cgctcctggt ggcggcaata atggcggtcg tccctcaagc 1200

agctacggcg ctcctggtgg tggaaacggt ggtcgtccat ctgacaccta tggcgctcct 1260

ggtggcggta acggaaacgg cagcggtggt cgtccttcaa gcagctatgg agctcctggt 1320

cagggccaag gtggatttgg tggtcgtcca tcggactcct atggtgctcc tggtcagaac 1380

caaaaaccat cagattcata tggcgccccc ggtagcggca atggcaacgg cggacgtcct 1440

tcgagcagct atggagctcc aggctcagga cctggtggcc gaccctccga ctcctacgga 1500

cccccagctt ctggatcggg agcaggtggc gctggaggca gtggacccgg cggcgctgac 1560

tacgataacg atgagtaagg atcc 1584

<210> 19
<211> 1504
<212> DNA
<213> Artificial sequence

<220>
<223> Resilin fused to CBD through a linker coding sequence

<400> 19
ccatgggacc ggagccacca gttaactcgt atctacctcc gtccgatagc tatggagcac 60

cgggtcagag tggtcccggc ggcaggccgt cggattccta tggagctcct ggtggtggaa 120

acggtggacg gccctcagac agctatggcg ctccaggcca gggtcaagga cagggacaag 180

gacaaggtgg atatgcaggc aagccctcag atacctatgg agctcctggt ggtggaaatg 240

gcaacggagg tcgtccatcg agcagctatg gcgctcctgg cggtggaaac ggtggtcgtc 300

cttcggatac ctacggtgct cctggtggcg gaaatggtgg acgcccatcg gacacttatg 360

gtgctcctgg tggtggtgga aatggcaacg gcggacgacc ttcaagcagc tatggagctc 420

ctggtcaagg acaaggcaac ggaaatggcg gtcgctcatc gagcagctat ggtgctcctg 480

gcggtggaaa cggcggtcgt ccttcggata cctacggtgc tcccggtggt ggaaacggtg 540

gtcgtccttc ggatacttac ggcgctcctg gtggcggcaa taatggcggt cgtccctcaa 600

gcagctacgg cgctcctggt ggtggaaacg gtggtcgtcc atctgacacc tatggcgctc 660

ctggtggcgg taacggaaac ggcagcggtg gtcgtccttc aagcagctat ggagctcctg 720

gtcagggcca aggtggattt ggtggtcgtc catcggactc ctatggtgct cctggtcaga 780

accaaaaacc atcagattca tatggcgccc ctggtagcgg caatggcaac ggcggacgtc 840

cttcgagcag ctatggagct ccaggctcag gacctggtgg ccgaccctcc gactcctacg 900

gacccccagc ttctggatcg ggagcaggtg gcgctggagg cagtggaccc ggcggcgctg 960

actacgataa cgatgagggg atccccgacc ccggcatggc agcgacatca tcaatgtcag 1020

ttgaatttta caactctaac aaatcagcac aaacaaactc aattacacca ataatcaaaa 1080

ttactaacac atctgacagt gatttaaatt taaatgacgt aaaagttaga tattattaca 1140

caagtgatgg tacacaagga caaactttct ggtgtgacca tgctggtgca ttattaggaa 1200

atagctatgt tgataacact agcaaagtga cagcaaactt cgttaaagaa acagcaagcc 1260

caacatcaac ctatgataca tatgttgaat ttggatttgc aagcggacga gctactctta 1320

aaaaaggaca atttataact attcaaggaa gaataacaaa atcagactgg tcaaactaca 1380

ctcaaacaaa tgactattca tttgatgcaa gtagttcaac accagttgta aatccaaaag 1440

ttacaggata tataggtgga gctaaagtac ttggtacagc accataggat cgatccagat 1500

gtac 1504

<210> 20
<211> 1523
<212> DNA
<213> Artificial sequence

<220>
<223> CBD-resilin fusion construct, codon optimized for expression in plants

<400> 20
caattggcag cgacatcatc aatgtcagtt gaattttaca actctaacaa agcagcacaa 60

acaaactcaa ttacaccaat aatcaaaatt actaacacag ctgacagtga tttaaattta 120

aatgacgtaa aagttagata ttattacaca agtgatggta cacaaggaca aactttctgg 180

ggtgatcatg ctggtgcatt attaggaaat agctatgttg ataacactgg caaagtgaca 240

gcaaacttcg ttaaagaaac agcaagccca acatcaacct atgatacata tgttgaattt 300

ggatttgcaa gcggagcagc tactcttaaa aaaggacaat ttataactat tcaaggaaga 360

ataacaaaat cagactggtc aaactacgct cagacaaatg actattcatt tgatgcaagt 420

agttcaacac cagttgtaaa tccaaaagtt acaggatata taggtggagc taaagtactt 480

ggtacagcac caggtccaga tgtaccatct tcaataatta atcctacttc tgcaacattt 540

gatccggagc caccagttaa ctcgtatcta cctccgtccg atagctatgg agcaccgggt 600

cagagtggtc ccggcggcag gccgtcggat tcctatggag ctcctggtgg tggaaacggt 660

ggacggccct cagacagcta tggcgctcca ggccagggtc aaggacaggg acaaggacaa 720

ggtggatatg caggcaagcc ctcagatacc tatggagctc ctggtggtgg aaatggcaac 780

ggaggtcgtc catcgagcag ctatggcgct cctggcggtg gaaacggtgg tcgtccttcg 840

gatacctacg gtgctcctgg tggcggaaat ggtggacgcc catcggacac ttatggtgct 900

cctggtggtg gtggaaatgg caacggcgga cgaccttcaa gcagctatgg agctcctggt 960

caaggacaag gcaacggaaa tggcggtcgc tcatcgagca gctatggtgc tcctggcggt 1020

ggaaacggcg gtcgtccttc ggatacctac ggtgctcccg gtggtggaaa cggtggtcgt 1080

ccttcggata cttacggcgc tcctggtggc ggcaataatg gcggtcgtcc ctcaagcagc 1140

tacggcgctc ctggtggtgg aaacggtggt cgtccatctg acacctatgg cgctcctggt 1200

ggcggtaacg gaaacggcag cggtggtcgt ccttcaagca gctatggagc tcctggtcag 1260

ggccaaggtg gatttggtgg tcgtccatcg gactcctatg gtgctcctgg tcagaaccaa 1320

aaaccatcag attcatatgg cgcccctggt agcggcaatg gcaacggcgg acgtccttcg 1380

agcagctatg gagctccagg ctcaggacct ggtggccgac cctccgactc ctacggaccc 1440

ccagcttctg gatcgggagc aggtggcgct ggaggcagtg gacccggcgg cgctgactac 1500

gataacgatg agtaagcggc cgc 1523

<210> 21
<211> 1492

<212> DNA
<213> Artificial sequence

<220>
<223> Resilin-CBD fusion construct, codon optimized for expression in plants

<400> 21
caattgccgg agccaccagt taactcgtat ctacctccgt ccgatagcta tggagcaccg 60

ggtcagagtg gtcccggcgg caggccgtcg gattcctatg gagctcctgg tggtggaaac 120

ggtggacggc cctcagacag ctatggcgct ccaggccagg gtcaaggaca gggacaagga 180

caaggtggat atgcaggcaa gccctcagat acctatggag ctcctggtgg tggaaatggc 240

aacggaggtc gtccatcgag cagctatggc gctcctggcg gtggaaacgg tggtcgtcct 300

tcggatacct acggtgctcc tggtggcgga aatggtggac gcccatcgga cacttatggt 360

gctcctggtg gtggtggaaa tggcaacggc ggacgacctt caagcagcta tggagctcct 420

ggtcaaggac aaggcaacgg aaatggcggt cgctcatcga gcagctatgg tgctcctggc 480

ggtggaaacg gcggtcgtcc ttcggatacc tacggtgctc ccggtggtgg aaacggtggt 540

cgtccttcgg atacttacgg cgctcctggt ggcggcaata atggcggtcg tccctcaagc 600

agctacggcg ctcctggtgg tggaaacggt ggtcgtccat ctgacaccta tggcgctcct 660

ggtggcggta acggaaacgg cagcggtggt cgtccttcaa gcagctatgg agctcctggt 720

cagggccaag gtggatttgg tggtcgtcca tcggactcct atggtgctcc tggtcagaac 780

caaaaaccat cagattcata tggcgccccc ggtagcggca atggcaacgg cggacgtcct 840

tcgagcagct atggagctcc aggctcagga cctggtggcc gaccctccga ctcctacgga 900

cccccagctt ctggatcggg agcaggtggc gctggaggca gtggacccgg cggcgctgac 960

tacgataacg atgaggggat ccccgacccc ggcatggcag cgacatcatc aatgtcagtt 1020

gaattttaca actctaacaa atcagcacaa acaaactcaa ttacaccaat aatcaaaatt 1080

actaacacat ctgacagtga tttaaattta aatgacgtaa aagttagata ttattacaca 1140

agtgatggta cacaaggaca aactttctgg tgtgaccatg ctggtgcatt attaggaaat 1200

agctatgttg ataacactag caaagtgaca gcaaacttcg ttaaagaaac agcaagccca 1260

acatcaacct atgatacata tgttgaattt ggatttgcaa gcggacgagc tactcttaaa 1320

aaaggacaat ttataactat tcaaggaaga ataacaaaat cagactggtc aaactacact 1380

caaacaaatg actattcatt tgatgcaagt agttcaacac cagttgtaaa tccaaaagtt 1440

acaggatata taggtggagc taaagtactt ggtacagcac catagcggcc gc 1492

<210> 22
<211> 1208
<212> DNA
<213> Artificial sequence

<220>
<223> Resilin including the native putative chitin binding domain, codon optimized for expression in plants, polynucleotide sequence

<400> 22
caattgccgg agccaccagt taactcgtat ctacctccgt ccgatagcta tggagcaccg 60

ggtcagagtg gtcccggcgg caggccgtcg gattcctatg gagctcctgg tggtggaaac 120

ggtggacggc cctcagacag ctatggcgct ccaggccagg gtcaaggaca gggacaagga 180

caaggtggat atgcaggcaa gccctcagat acctatggag ctcctggtgg tggaaatggc 240

aacggaggtc gtccatcgag cagctatggc gctcctggcg gtggaaacgg tggtcgtcct 300

tcggatacct acggtgctcc tggtggcgga aatggtggac gcccatcgga cacttatggt 360

gctcctggtg gtggtggaaa tggcaacggc ggacgacctt caagcagcta tggagctcct 420

ggtcaaggac aaggcaacgg aaatggcggt cgctcatcga gcagctatgg tgctcctggc 480

ggtggaaacg gcggtcgtcc ttcggatacc tacggtgctc ccggtggtgg aaacggtggt 540

cgtccttcgg atacttacgg cgctcctggt ggcggcaata atggcggtcg tccctcaagc 600

agctacggcg ctcctggtgg tggaaacggt ggtcgtccat ctgacaccta tggcgctcct 660

ggtggcggta acggaaacgg cagcggtggt cgtccttcaa gcagctatgg agctcctggt 720

cagggccaag gtggatttgg tggtcgtcca tcggactcct atggtgctcc tggtcagaac 780

caaaaaccat cagattcata tggcgccccc ggtagcggca atggcaacgg cggacgtcct 840

tcgagcagct atggagctcc aggctcagga cctggtggcc gaccctccga ctcctacgga 900

cccccagctt ctggatcggg agcaggtggc gctggaggca gtggacccgg cggcgctgac 960

tacgataacg atgagcccgc caagtacgaa tttaattacc aggttgagga cgcgcccagc 1020

ggactctcgt tcgggcattc agagatgcgc gacggtgact tcaccaccgg ccagtacaat 1080

gtcctgttgc ccgacggaag gaagcaaatt gtggagtatg aagccgacca gcagggctac 1140

cggccacaga tccgctacga aggcgatgcc aacgatggca gtggtcccag cggtccttaa 1200

gcggccgc 1208

<210> 23
<211> 1485
<212> DNA
<213> Artificial sequence

<220>
<223> 15 sps spider silk synthetic gene

<400> 23
agcggtcgtg gcggtctggg tggccagggt gcaggtgctg ctgcggcagc aggcggtgct 60

ggccaaggtg gctacggtgg cctgggttct cagggtacta gcggtcgtgg cggtctgggt 120

ggccagggtg caggtgctgc tgcggcagca ggcggtgctg gccaaggtgg ctacggtggc 180

ctgggttctc agggtactag cggtcgtggc ggtctgggtg gccagggtgc aggtgctgct 240

gcggcagcag gcggtgctgg ccaaggtggc tacggtggcc tgggttctca gggtactagc 300

ggtcgtggcg gtctgggtgg ccagggtgca ggtgctgctg cggcagcagg cggtgctggc 360

caaggtggct acggtggcct gggttctcag ggtactagcg gtcgtggcgg tctgggtggc 420

cagggtgcag gtgctgctgc ggcagcaggc ggtgctggcc aaggtggcta cggtggcctg 480

ggttctcagg gtactagcgg tcgtggcggt ctgggtggcc agggtgcagg tgctgctgcg 540

gcagcaggcg gtgctggcca aggtggctac ggtggcctgg gttctcaggg tactagcggt 600

cgtggcggtc tgggtggcca gggtgcaggt gctgctgcgg cagcaggcgg tgctggccaa 660

ggtggctacg gtggcctggg ttctcagggt actagcggtc gtggcggtct gggtggccag 720

ggtgcaggtg ctgctgcggc agcaggcggt gctggccaag gtggctacgg tggcctgggt 780

tctcagggta ctagcggtcg tggcggtctg ggtggccagg gtgcaggtgc tgctgcggca 840

gcaggcggtg ctggccaagg tggctacggt ggcctgggtt ctcagggtac tagcggtcgt 900

ggcggtctgg gtggccaggg tgcaggtgct gctgcggcag caggcggtgc tggccaaggt 960

ggctacggtg gcctgggttc tcagggtact agcggtcgtg gcggtctggg tggccagggt 1020

gcaggtgctg ctgcggcagc aggcggtgct ggccaaggtg gctacggtgg cctgggttct 1080

cagggtacta gcggtcgtgg cggtctgggt ggccagggtg caggtgctgc tgcggcagca 1140

ggcggtgctg gccaaggtgg ctacggtggc ctgggttctc agggtactag cggtcgtggc 1200

ggtctgggtg gccagggtgc aggtgctgct gcggcagcag gcggtgctgg ccaaggtggc 1260

tacggtggcc tgggttctca gggtactagc ggtcgtggcg gtctgggtgg ccagggtgca 1320

ggtgctgctg cggcagcagg cggtgctggc caaggtggct acggtggcct gggttctcag 1380

ggtactagcg gtcgtggcgg tctgggtggc cagggtgcag gtgctgctgc ggcagcaggc 1440

ggtgctggcc aaggtggcta cggtggcctg ggttctcagg gtact 1485

<210> 24
<211> 2034
<212> DNA
<213> Artificial sequence

<220>
<223> 15 sps spider silk fused to CBD polynucleotide sequence

<400> 24
agcggtcgtg gcggtctggg tggccagggt gcaggtgctg ctgcggcagc aggcggtgct 60

ggccaaggtg gctacggtgg cctgggttct cagggtacta gcggtcgtgg cggtctgggt 120

ggccagggtg caggtgctgc tgcggcagca ggcggtgctg gccaaggtgg ctacggtggc 180

ctgggttctc agggtactag cggtcgtggc ggtctgggtg gccagggtgc aggtgctgct 240

gcggcagcag gcggtgctgg ccaaggtggc tacggtggcc tgggttctca gggtactagc 300

ggtcgtggcg gtctgggtgg ccagggtgca ggtgctgctg cggcagcagg cggtgctggc 360

caaggtggct acggtggcct gggttctcag ggtactagcg gtcgtggcgg tctgggtggc 420

cagggtgcag gtgctgctgc ggcagcaggc ggtgctggcc aaggtggcta cggtggcctg 480

ggttctcagg gtactagcgg tcgtggcggt ctgggtggcc agggtgcagg tgctgctgcg 540

gcagcaggcg gtgctggcca aggtggctac ggtggcctgg gttctcaggg tactagcggt 600

cgtggcggtc tgggtggcca gggtgcaggt gctgctgcgg cagcaggcgg tgctggccaa 660

ggtggctacg gtggcctggg ttctcagggt actagcggtc gtggcggtct gggtggccag 720

ggtgcaggtg ctgctgcggc agcaggcggt gctggccaag gtggctacgg tggcctgggt 780

tctcagggta ctagcggtcg tggcggtctg ggtggccagg gtgcaggtgc tgctgcggca 840

gcaggcggtg ctggccaagg tggctacggt ggcctgggtt ctcagggtac tagcggtcgt 900

ggcggtctgg gtggccaggg tgcaggtgct gctgcggcag caggcggtgc tggccaaggt 960

ggctacggtg gcctgggttc tcagggtact agcggtcgtg gcggtctggg tggccagggt 1020

gcaggtgctg ctgcggcagc aggcggtgct ggccaaggtg gctacggtgg cctgggttct 1080

cagggtacta gcggtcgtgg cggtctgggt ggccagggtg caggtgctgc tgcggcagca 1140

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tacggtggcc tgggttctca gggtactagc ggtcgtggcg gtctgggtgg ccagggtgca 1320

ggtgctgctg cggcagcagg cggtgctggc caaggtggct acggtggcct gggttctcag 1380

ggtactagcg gtcgtggcgg tctgggtggc cagggtgcag gtgctgctgc ggcagcaggc 1440

ggtgctggcc aaggtggcta cggtggcctg ggttctcagg gtactagtat ggcagcgaca 1500

tcatcaatgt cagttgaatt ttacaactct aacaaagcag cacaaacaaa ctcaattaca 1560

ccaataatca aaattactaa cacagctgac agtgatttaa atttaaatga cgtaaaagtt 1620

agatattatt acacaagtga tggtacacaa ggacaaactt tctggggtga tcatgctggt 1680

gcattattag gaaatagcta tgttgataac actggcaaag tgacagcaaa cttcgttaaa 1740

gaaacagcaa gcccaacatc aacctatgat acatatgttg aatttggatt tgcaagcgga 1800

gcagctactc ttaaaaaagg acaatttata actattcaag gaagaataac aaaatcagac 1860

tggtcaaact acgctcagac aaatgactat tcatttgatg caagtagttc aacaccagtt 1920

gtaaatccaa aagttacagg atatataggt ggagctaaag tacttggtac agcaccaggt 1980

ccagatgtac catcttcaat aattaatcct acttctgcaa catttgatct cgag 2034

<210> 25
<211> 542
<212> DNA
<213> Clostridium cellulovorans

<220>
<221> misc_feature
<223> Clostridium cellulovorans CBD (CBDclos) polynucleotide sequence

<400> 25
ccatggcagc gacatcatca atgtcagttg aattttacaa ctctaacaaa gcagcacaaa 60

caaactcaat tacaccaata atcaaaatta ctaacacagc tgacagtgat ttaaatttaa 120

atgacgtaaa agttagatat tattacacaa gtgatggtac acaaggacaa actttctggg 180

gtgatcatgc tggtgcatta ttaggaaata gctatgttga taacactggc aaagtgacag 240

caaacttcgt taaagaaaca gcaagcccaa catcaaccta tgatacatat gttgaatttg 300

gatttgcaag cggagcagct actcttaaaa aaggacaatt tataactatt caaggaagaa 360

taacaaaatc agactggtca aactacgctc agacaaatga ctattcattt gatgcaagta 420

gttcaacacc agttgtaaat ccaaaagtta caggatatat aggtggagct aaagtacttg 480

gtacagcacc aggtccagat gtaccatctt caataattaa tcctacttct gcaacatttg 540

at 542

<210> 26
<211> 39
<212> PRT
<213> Artificial sequence

<220>
<223> Spider silk repeated unit

<400> 26

Gly Pro Gly Gly Gln Gly Pro Tyr Gly Pro Gly Ala Ser Ala Ala Ala
1 5 10 15

Ala Ala Ala Gly Gly Tyr Gly Pro Gly Tyr Gly Gln Gln Gly Pro Gly
20 25 30

Gln Gln Gly Pro Gly Gln Gln
35

<210> 27
<211> 734
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic spider silk repeating unit (GENEART) polynucleotide sequence

<400> 27
gaattcaggc ctccaggcgc ccatatggct gctacttctt ctatgtctgt tgagttctac 60

aactccaaca aggctgctca gaccaactct attactccaa tcattaagat taccaacact 120

gccgattccg atttgaactt gaacgatgtt aaagttcgtt actactacac ttccgatgga 180

actcaaggtc aaactttctg gggtgatcat gctaccatgg cttctatgac tggtggtcag 240

cagatgggta gaattggatc cccaccaggt cccgggccag gtggtcaagg accttatggt 300

ccaggagctt ctgcagctgc tgcagccgct ggaggttatg gaccaggttc tggtcaacaa 360

ggtccaggac aacagggtcc tggtcaacaa gccggctctt ctgttccagt tgcttccgct 420

gttgcttcta gattgtcctc tccagctgct tcttccagag tttcctctgc tgtttcttct 480

ttggtttctt ctggtccaac taagcacgct gctttgtcca acactatttc ttccgttgtt 540

tctcaggttt ccgcttccaa tcctggtctt tctggttgtg atgttttggt tcaggctttg 600

ttggaagtgg tgtctgcttt ggtgtctatc ttgggctctt cctctattgg tcaaatcaac 660

tacggtgcct ctgctcagta tactcagatg gttggccaat ctgttgctca agctctagca 720

gcggccgcaa gctt 734

<210> 28
<211> 2411
<212> DNA
<213> Artificial sequence

<220>
<223> CBDclos fused to 12sps Spider silk polynucleotide sequence

<400> 28
gaattcaggc ctccaggcgc ccatatggct gctacttctt ctatgtctgt tgagttctac 60

aactccaaca aggctgctca gaccaactct attactccaa tcattaagat taccaacact 120

gccgattccg atttgaactt gaacgatgtt aaagttcgtt actactacac ttccgatgga 180

actcaaggtc aaactttctg gggtgatcat gctggtgcat tattaggaaa tagctatgtt 240

gataacactg gcaaagtgac agcaaacttc gttaaagaaa cagcaagccc aacatcaacc 300

tatgatacat atgttgaatt tggatttgca agcggagcag ctactcttaa aaaaggacaa 360

tttataacta ttcaaggaag aataacaaaa tcagactggt caaactacgc tcagacaaat 420

gactattcat ttgatgcaag tagttcaaca ccagttgtaa atccaaaagt tacaggatat 480

ataggtggag ctaaagtact tggtacagca ccaggtccag atgtaccatc ttcaataatt 540

aatcctactt ctgcaacatt tgatcccggt accatggctt ctatgactgg tggtcagcag 600

atgggtagaa ttggatcccc accaggtccc gggccaggtg gtcaaggacc ttatggtcca 660

ggagcttctg cagctgctgc agccgctgga ggttatggac caggttctgg tcaacaaggt 720

ccaggacaac agggtcctgg tcaacaagcc gggccaggtg gtcaaggacc ttatggtcca 780

ggagcttctg cagctgctgc agccgctgga ggttatggac caggttctgg tcaacaaggt 840

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ggagcttctg cagctgctgc agccgctgga ggttatggac caggttctgg tcaacaaggt 960

ccaggacaac agggtcctgg tcaacaagcc gggccaggtg gtcaaggacc ttatggtcca 1020

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gtttcttctg gtccaactaa gcacgctgct ttgtccaaca ctatttcttc cgttgtttct 2220

caggtttccg cttccaatcc tggtctttct ggttgtgatg ttttggttca ggctttgttg 2280

gaagtggtgt ctgctttggt gtctatcttg ggctcttcct ctattggtca aatcaactac 2340

ggtgcctctg ctcagtatac tcagatggtt ggccaatctg ttgctcaagc tctagcagcg 2400

gccgcaagct t 2411

<210> 29
<211> 2411
<212> DNA
<213> Artificial sequence

<220>
<223> Spider silk 6sps-CBD-6sps construct polynucleotide sequence

<400> 29
gaattcaggg ggccaggtgg tcaaggacct tatggtccag gagcttctgc agctgctgca 60

gccgctggag gttatggacc aggttctggt caacaaggtc caggacaaca gggtcctggt 120

caacaagccg ggccaggtgg tcaaggacct tatggtccag gagcttctgc agctgctgca 180

gccgctggag gttatggacc aggttctggt caacaaggtc caggacaaca gggtcctggt 240

caacaagccg ggccaggtgg tcaaggacct tatggtccag gagcttctgc agctgctgca 300

gccgctggag gttatggacc aggttctggt caacaaggtc caggacaaca gggtcctggt 360

caacaagccg ggccaggtgg tcaaggacct tatggtccag gagcttctgc agctgctgca 420

gccgctggag gttatggacc aggttctggt caacaaggtc caggacaaca gggtcctggt 480

caacaagccg ggccaggtgg tcaaggacct tatggtccag gagcttctgc agctgctgca 540

gccgctggag gttatggacc aggttctggt caacaaggtc caggacaaca gggtcctggt 600

caacaagccg ggccaggtgg tcaaggacct tatggtccag gagcttctgc agctgctgca 660

gccgctggag gttatggacc aggttctggt caacaaggtc caggacaaca gggtcctggt 720

caacaagccc ctccaggcgc ccatatggct gctacttctt ctatgtctgt tgagttctac 780

aactccaaca aggctgctca gaccaactct attactccaa tcattaagat taccaacact 840

gccgattccg atttgaactt gaacgatgtt aaagttcgtt actactacac ttccgatgga 900

actcaaggtc aaactttctg gggtgatcat gctggtgcat tattaggaaa tagctatgtt 960

gataacactg gcaaagtgac agcaaacttc gttaaagaaa cagcaagccc aacatcaacc 1020

tatgatacat atgttgaatt tggatttgca agcggagcag ctactcttaa aaaaggacaa 1080

tttataacta ttcaaggaag aataacaaaa tcagactggt caaactacgc tcagacaaat 1140

gactattcat ttgatgcaag tagttcaaca ccagttgtaa atccaaaagt tacaggatat 1200

ataggtggag ctaaagtact tggtacagca ccaggtccag atgtaccatc ttcaataatt 1260

aatcctactt ctgcaacatt tgatcccggt accatggctt ctatgactgg tggtcagcag 1320

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ccaggacaac agggtcctgg tcaacaagcc gggccaggtg gtcaaggacc ttatggtcca 1500

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caggtttccg cttccaatcc tggtctttct ggttgtgatg ttttggttca ggctttgttg 2280

gaagtggtgt ctgctttggt gtctatcttg ggctcttcct ctattggtca aatcaactac 2340

ggtgcctctg ctcagtatac tcagatggtt ggccaatctg ttgctcaagc tctagcagcg 2400

gccgcaagct t 2411

<210> 30
<211> 1017
<212> DNA
<213> Artificial sequence

<220>
<223> Rubisco's small subunit cassette including promoter and 5' UTR

<400> 30
aaatggcgcg ccaagcttag acaaacaccc cttgttatac aaagaatttc gctttacaaa 60

atcaaattcg agaaaataat atatgcacta aataagatca ttcggatcca atctaaccaa 120

ttacgatacg ctttgggtac acttgatttt tgtttcagta gttacatata tcttgtttta 180

tatgctatct ttaaggatct tcactcaaag actatttgtt gatgttcttg atggggctcg 240

gaagatttga tatgatacac tctaatcttt aggagatacc agccaggatt atattcagta 300

agacaatcaa attttacgtg ttcaaactcg ttatcttttc atttaatgga tgagccagaa 360

tctctataga atgattgcaa tcgagaatat gttcggccga tatccctttg ttggcttcaa 420

tattctacat atcacacaag aatcgaccgt attgtaccct ctttccataa aggaacacac 480

agtatgcaga tgctttttc ccacatgcag taacataggt attcaaaaat ggctaaaaga 540

agttggataa caaattgaca actatttcca tttctgttat ataaatttca caacacacaa 600

aagcccgtaa tcaagagtct gcccatgtac gaaataactt ctattatttg gtattgggcc 660

taagcccagc tcagagtacg tgggggtacc acatatagga aggtaacaaa atactgcaag 720

atagccccat aacgtaccag cctctcctta ccacgaagag ataagatata agacccaccc 780

tgccacgtgt cacatcgtca tggtggttaa tgataaggga ttacatcctt ctatgtttgt 840

ggacatgatg catgtaatgt catgagccac atgatccaat ggccacagga acgtaagaat 900

gtagatagat ttgattttgt ccgttagata gcaaacaaca ttataaaagg tgtgtatcaa 960

tacgaactaa ttcactcatt ggattcatag aagtccattc ctcctaagta tctaaac 1017

<210> 31
<211> 975
<212> DNA
<213> Artificial sequence

<220>
<223> Rubisco's small subunit cassette including 3' UTR and terminator

<400> 31
aaaaggatcc gcggccgcat aagttttact atttaccaag acttttgaat attaaccttc 60

ttgtaacgag tcggttaaat ttgattgttt agggttttgt attatttttt tttggtcttt 120

taattcatca ctttaattcc ctaattgtct gttcatttcg ttgtttgttt ccggatcgat 180

aatgaaatgt aagagatatc atatataaat aataaattgt cgtttcatat ttgcaatctt 240

tttttacaaa cctttaatta attgtatgta tgacattttc ttcttgttat attaggggga 300

aataatgtta aataaaagta caaaataaac tacagtacat cgtactgaat aaattaccta 360

gccaaaaagt acacctttcc atatacttcc tacatgaagg cattttcaac attttcaaat 420

aaggaatgct acaaccgcat aataacatcc acaaattttt ttataaaata acatgtcaga 480

cagtgattga aagattttat tatagtttcg ttatcttctt ttctcattaa gcgaatcact 540

acctaacacg tcattttgtg aaatattttt tgaatgtttt tatatagttg tagcattcct 600

cttttcaaat tagggtttgt ttgagatagc atttcagccg gttcatacaa cttaaaagca 660

tactctaatg ctggaaaaaa gactaaaaaa tcttgtaagt tagcgcagaa tattgaccca 720

aattatatac acacatgacc ccatatagag actaattaca cttttaacca ctaataatta 780

ttactgtatt ataacatcta ctaattaaac ttgtgagttt ttgctagaat tattatcata 840

tatactaaaa ggcaggaacg caaacattgc cccggtactg tagcaactac ggtagacgca 900

ttaattgtct atagtggacg cattaattaa ccaaaaccgc ctctttcccc ttcttcttga 960

agcttgagct ctttt 975

<210> 32
<211> 687
<212> PRT
<213> Artificial sequence

<220>
<223> 15 sps spider silk fused to CBD

<400> 32

Ser Gly Arg Gly Gly Leu Gly Gly Gln Gly Ala Gly Ala Ala Ala Ala
1 5 10 15

Ala Gly Gly Ala Gly Gln Gly Gly Tyr Gly Gly Leu Gly Ser Gln Gly
20 25 30

Thr Ser Gly Arg Gly Gly Leu Gly Gly Gln Gly Ala Gly Ala Ala Ala
35 40 45

Ser Ala Gly Gly Ala Gly Gln Gly Gly Tyr Gly Gly Leu Gly Ser Gln
50 55 60

Gly Thr Ser Gly Arg Gly Gly Leu Gly Gly Gln Gly Ala Gly Ala Ala
65 70 75 80

Ala Ala Ala Gly Gly Ala Gly Gln Gly Gly Tyr Gly Gly Leu Gly Ser
85 90 95

Gln Gly Thr Ser Gly Arg Gly Gly Leu Gly Gly Gln Gly Ala Gly Ala
100 105 110

Ala Ala Ala Ala Gly Gly Ala Gly Gln Gly Gly Tyr Gly Gly Leu Gly
115 120 125

Ser Gln Gly Thr Ala Ser Gly Arg Gly Gly Leu Gly Gly Gln Gly Ala
130 135 140

Gly Ala Ala Ala Ala Ala Gly Gly Ala Gly Gln Gly Gly Tyr Gly Gly
145 150 155 160

Leu Gly Ser Gln Gly Thr Ala Ser Gly Arg Gly Gly Leu Gly Gly Gln
165 170 175

```
Gly Ala Gly Ala Ala Ala Ala Gly Gly Ala Gly Gln Gly Gly Tyr
           180                 185                 190

Gly Gly Leu Gly Ser Gln Gly Thr Ala Ser Gly Arg Gly Gly Leu Gly
        195                 200                 205

Gly Gln Gly Ala Gly Ala Ala Ala Ala Ala Gly Gly Ala Gly Gln Gly
    210                 215                 220

Gly Tyr Gly Gly Leu Gly Ser Gln Gly Thr Ala Ser Gly Arg Gly Gly
225                 230                 235                 240

Leu Gly Gly Gln Gly Ala Gly Ala Ala Ala Ala Ala Gly Gly Ala Gly
                245                 250                 255

Gln Gly Gly Tyr Gly Gly Leu Gly Ser Gln Gly Thr Ala Ser Gly Arg
           260                 265                 270

Gly Gly Leu Gly Gly Gln Gly Ala Gly Ala Ala Ala Ala Ala Gly Gly
        275                 280                 285

Ala Gly Gln Gly Gly Tyr Gly Gly Leu Gly Ser Gln Gly Thr Ala Ser
    290                 295                 300

Gly Arg Gly Gly Leu Gly Gly Gln Gly Ala Gly Ala Ala Ala Ala Ala
305                 310                 315                 320

Gly Gly Ala Gly Gln Gly Gly Tyr Gly Gly Leu Gly Ser Gln Gly Thr
                325                 330                 335

Ala Ser Gly Arg Gly Gly Leu Gly Gly Gln Gly Ala Gly Ala Ala Ala
           340                 345                 350

Ala Ala Gly Gly Ala Gly Gln Gly Gly Tyr Gly Gly Leu Gly Ser Gln
        355                 360                 365

Gly Thr Ala Ser Gly Arg Gly Gly Leu Gly Gly Gln Gly Ala Gly Ala
    370                 375                 380

Ala Ala Ala Ala Gly Gly Ala Gly Gln Gly Gly Tyr Gly Gly Leu Gly
385                 390                 395                 400

Ser Gln Gly Thr Ala Ser Gly Arg Gly Gly Leu Gly Gly Gln Gly Ala
                405                 410                 415

Gly Ala Ala Ala Ala Ala Gly Gly Ala Gly Gln Gly Gly Tyr Gly Gly
           420                 425                 430

Leu Gly Ser Gln Gly Thr Ala Ser Gly Arg Gly Gly Leu Gly Gly Gln
        435                 440                 445

Gly Ala Gly Ala Ala Ala Ala Ala Gly Gly Ala Gly Gln Gly Gly Tyr
    450                 455                 460

Gly Gly Leu Gly Ser Gln Gly Thr Ala Ser Gly Arg Gly Gly Leu Gly
```

```
465                 470                 475                 480

Gly Gln Gly Ala Gly Ala Ala Ala Ala Gly Gly Ala Gly Gln Gly
                485                 490                 495

Gly Tyr Gly Gly Leu Gly Ser Gln Gly Thr Ser Met Ala Ala Thr Ser
            500                 505                 510

Ser Met Ser Val Glu Phe Tyr Asn Ser Asn Lys Ala Ala Gln Thr Asn
        515                 520                 525

Ser Ile Thr Pro Ile Ile Lys Ile Thr Asn Thr Ala Asp Ser Asp Leu
    530                 535                 540

Asn Leu Asn Asp Val Lys Val Arg Tyr Tyr Tyr Thr Ser Asp Gly Thr
545                 550                 555                 560

Gln Gly Gln Thr Phe Trp Gly Asp His Ala Gly Ala Leu Leu Gly Asn
                565                 570                 575

Ser Tyr Val Asp Asn Thr Gly Lys Val Thr Ala Asn Phe Val Lys Glu
            580                 585                 590

Thr Ala Ser Pro Thr Ser Thr Tyr Asp Thr Tyr Val Glu Phe Gly Phe
        595                 600                 605

Ala Ser Gly Ala Ala Thr Leu Lys Lys Gly Gln Phe Ile Thr Ile Gln
    610                 615                 620

Gly Arg Ile Thr Lys Ser Asp Trp Ser Asn Tyr Ala Gln Thr Asn Asp
625                 630                 635                 640

Tyr Ser Phe Asp Ala Ser Ser Ser Thr Pro Val Val Asn Pro Lys Val
                645                 650                 655

Thr Gly Tyr Ile Gly Gly Ala Lys Val Leu Gly Thr Ala Pro Gly Pro
            660                 665                 670

Asp Val Pro Ser Ser Ile Ile Asn Pro Thr Ser Ala Thr Phe Asp
        675                 680                 685
```

<210> 33
<211> 570
<212> PRT
<213> Artificial sequence

<220>
<223> 6His tagged 15sps spider silk polypeptide

<400> 33

```
Met His His His His His His Ser Ser Gly Leu Val Pro Arg Gly Ser
1               5                   10                  15

Gly Met Lys Glu Thr Ala Ala Ala Lys Phe Glu Arg Gln His Met Asp
            20                  25                  30

Ser Pro Asp Leu Gly Thr Asp Asp Asp Asp Lys Ala Met Ala Ser Gly
```

```
        35                  40                  45

Arg Gly Gly Leu Gly Gly Gln Gly Ala Gly Ala Ala Ala Ala Ala Gly
    50                  55                  60

Gly Ala Gly Gln Gly Gly Tyr Gly Gly Leu Gly Ser Gln Gly Thr Ser
65                  70                  75                  80

Gly Arg Gly Gly Leu Gly Gly Gln Gly Ala Gly Ala Ala Ala Ser Ala
                85                  90                  95

Gly Gly Ala Gly Gln Gly Gly Tyr Gly Gly Leu Gly Ser Gln Gly Thr
            100                 105                 110

Ser Gly Arg Gly Gly Leu Gly Gly Gln Gly Ala Gly Ala Ala Ala Ala
        115                 120                 125

Ala Gly Gly Ala Gly Gln Gly Gly Tyr Gly Gly Leu Gly Ser Gln Gly
    130                 135                 140

Thr Ser Gly Arg Gly Gly Leu Gly Gly Gln Gly Ala Gly Ala Ala Ala
145                 150                 155                 160

Ala Ala Gly Gly Ala Gly Gln Gly Gly Tyr Gly Gly Leu Gly Ser Gln
                165                 170                 175

Gly Thr Ala Ser Gly Arg Gly Gly Leu Gly Gly Gln Gly Ala Gly Ala
            180                 185                 190

Ala Ala Ala Ala Gly Gly Ala Gly Gln Gly Gly Tyr Gly Gly Leu Gly
        195                 200                 205

Ser Gln Gly Thr Ala Ser Gly Arg Gly Gly Leu Gly Gly Gln Gly Ala
    210                 215                 220

Gly Ala Ala Ala Ala Ala Gly Gly Ala Gly Gln Gly Gly Tyr Gly Gly
225                 230                 235                 240

Leu Gly Ser Gln Gly Thr Ala Ser Gly Arg Gly Gly Leu Gly Gly Gln
                245                 250                 255

Gly Ala Gly Ala Ala Ala Ala Ala Gly Gly Ala Gly Gln Gly Gly Tyr
            260                 265                 270

Gly Gly Leu Gly Ser Gln Gly Thr Ala Ser Gly Arg Gly Gly Leu Gly
        275                 280                 285

Gly Gln Gly Ala Gly Ala Ala Ala Ala Ala Gly Gly Ala Gly Gln Gly
    290                 295                 300

Gly Tyr Gly Gly Leu Gly Ser Gln Gly Thr Ala Ser Gly Arg Gly Gly
305                 310                 315                 320

Leu Gly Gly Gln Gly Ala Gly Ala Ala Ala Ala Ala Gly Gly Ala Gly
                325                 330                 335
```

```
Gln Gly Gly Tyr Gly Gly Leu Gly Ser Gln Gly Thr Ala Ser Gly Arg
            340                 345                 350

Gly Gly Leu Gly Gly Gln Gly Ala Gly Ala Ala Ala Ala Ala Gly Gly
        355                 360                 365

Ala Gly Gln Gly Gly Tyr Gly Gly Leu Gly Ser Gln Gly Thr Ala Ser
    370                 375                 380

Gly Arg Gly Gly Leu Gly Gly Gln Gly Ala Gly Ala Ala Ala Ala Ala
385                 390                 395                 400

Gly Gly Ala Gly Gln Gly Gly Tyr Gly Gly Leu Gly Ser Gln Gly Thr
                405                 410                 415

Ala Ser Gly Arg Gly Gly Leu Gly Gly Gln Gly Ala Gly Ala Ala Ala
            420                 425                 430

Ala Ala Gly Gly Ala Gly Gln Gly Gly Tyr Gly Gly Leu Gly Ser Gln
        435                 440                 445

Gly Thr Ala Ser Gly Arg Gly Gly Leu Gly Gly Gln Gly Ala Gly Ala
    450                 455                 460

Ala Ala Ala Ala Gly Gly Ala Gly Gln Gly Gly Tyr Gly Gly Leu Gly
465                 470                 475                 480

Ser Gln Gly Thr Ala Ser Gly Arg Gly Gly Leu Gly Gly Gln Gly Ala
                485                 490                 495

Gly Ala Ala Ala Ala Ala Gly Gly Ala Gly Gln Gly Gly Tyr Gly Gly
            500                 505                 510

Leu Gly Ser Gln Gly Thr Ala Ser Gly Arg Gly Gly Leu Gly Gly Gln
        515                 520                 525

Gly Ala Gly Ala Ala Ala Ala Ala Gly Gly Ala Gly Gln Gly Gly Tyr
    530                 535                 540

Gly Gly Leu Gly Ser Gln Gly Thr Ser Gly Ser Ala Arg Ala Arg Ala
545                 550                 555                 560

Ala Ala Leu Glu His His His His His His
                565                 570


<210>  34
<211>  741
<212>  PRT
<213>  Artificial sequence

<220>
<223>  6His tagged 15sps spider silk CBD fusion polypeptide

<400>  34

Met His His His His His His Ser Ser Gly Leu Val Pro Arg Gly Ser
1               5                   10                  15
```

```
Gly Met Lys Glu Thr Ala Ala Ala Lys Phe Glu Arg Gln His Met Asp
            20                  25                  30

Ser Pro Asp Leu Gly Thr Asp Asp Asp Asp Lys Ala Met Ala Ser Gly
        35                  40                  45

Arg Gly Gly Leu Gly Gly Gln Gly Ala Gly Ala Ala Ala Ala Ala Gly
    50                  55                  60

Gly Ala Gly Gln Gly Gly Tyr Gly Gly Leu Gly Ser Gln Gly Thr Ser
65                  70                  75                  80

Gly Arg Gly Gly Leu Gly Gly Gln Gly Ala Gly Ala Ala Ala Ser Ala
                85                  90                  95

Gly Gly Ala Gly Gln Gly Gly Tyr Gly Gly Leu Gly Ser Gln Gly Thr
            100                 105                 110

Ser Gly Arg Gly Gly Leu Gly Gly Gln Gly Ala Gly Ala Ala Ala Ala
        115                 120                 125

Ala Gly Gly Ala Gly Gln Gly Gly Tyr Gly Gly Leu Gly Ser Gln Gly
    130                 135                 140

Thr Ser Gly Arg Gly Gly Leu Gly Gly Gln Gly Ala Gly Ala Ala Ala
145                 150                 155                 160

Ala Ala Gly Gly Ala Gly Gln Gly Gly Tyr Gly Gly Leu Gly Ser Gln
                165                 170                 175

Gly Thr Ala Ser Gly Arg Gly Gly Leu Gly Gly Gln Gly Ala Gly Ala
            180                 185                 190

Ala Ala Ala Ala Gly Gly Ala Gly Gln Gly Gly Tyr Gly Gly Leu Gly
        195                 200                 205

Ser Gln Gly Thr Ala Ser Gly Arg Gly Gly Leu Gly Gly Gln Gly Ala
    210                 215                 220

Gly Ala Ala Ala Ala Ala Gly Gly Ala Gly Gln Gly Gly Tyr Gly Gly
225                 230                 235                 240

Leu Gly Ser Gln Gly Thr Ala Ser Gly Arg Gly Gly Leu Gly Gly Gln
                245                 250                 255

Gly Ala Gly Ala Ala Ala Ala Ala Gly Gly Ala Gly Gln Gly Gly Tyr
            260                 265                 270

Gly Gly Leu Gly Ser Gln Gly Thr Ala Ser Gly Arg Gly Gly Leu Gly
        275                 280                 285

Gly Gln Gly Ala Gly Ala Ala Ala Ala Ala Gly Gly Ala Gly Gln Gly
    290                 295                 300

Gly Tyr Gly Gly Leu Gly Ser Gln Gly Thr Ala Ser Gly Arg Gly Gly
```

```
305                 310                 315                 320

Leu Gly Gly Gln Gly Ala Gly Ala Ala Ala Ala Ala Gly Gly Ala Gly
                325                 330                 335

Gln Gly Gly Tyr Gly Gly Leu Gly Ser Gln Gly Thr Ala Ser Gly Arg
            340                 345                 350

Gly Gly Leu Gly Gly Gln Gly Ala Gly Ala Ala Ala Ala Ala Gly Gly
        355                 360                 365

Ala Gly Gln Gly Gly Tyr Gly Gly Leu Gly Ser Gln Gly Thr Ala Ser
    370                 375                 380

Gly Arg Gly Gly Leu Gly Gly Gln Gly Ala Gly Ala Ala Ala Ala Ala
385                 390                 395                 400

Gly Gly Ala Gly Gln Gly Gly Tyr Gly Gly Leu Gly Ser Gln Gly Thr
                405                 410                 415

Ala Ser Gly Arg Gly Gly Leu Gly Gly Gln Gly Ala Gly Ala Ala Ala
            420                 425                 430

Ala Ala Gly Gly Ala Gly Gln Gly Gly Tyr Gly Gly Leu Gly Ser Gln
        435                 440                 445

Gly Thr Ala Ser Gly Arg Gly Gly Leu Gly Gly Gln Gly Ala Gly Ala
    450                 455                 460

Ala Ala Ala Ala Gly Gly Ala Gly Gln Gly Gly Tyr Gly Gly Leu Gly
465                 470                 475                 480

Ser Gln Gly Thr Ala Ser Gly Arg Gly Gly Leu Gly Gly Gln Gly Ala
                485                 490                 495

Gly Ala Ala Ala Ala Ala Gly Gly Ala Gly Gln Gly Gly Tyr Gly Gly
            500                 505                 510

Leu Gly Ser Gln Gly Thr Ala Ser Gly Arg Gly Gly Leu Gly Gly Gln
        515                 520                 525

Gly Ala Gly Ala Ala Ala Ala Ala Gly Gly Ala Gly Gln Gly Gly Tyr
    530                 535                 540

Gly Gly Leu Gly Ser Gln Gly Thr Ser Met Ala Ala Thr Ser Ser Met
545                 550                 555                 560

Ser Val Glu Phe Tyr Asn Ser Asn Lys Ala Ala Gln Thr Asn Ser Ile
                565                 570                 575

Thr Pro Ile Ile Lys Ile Thr Asn Thr Ala Asp Ser Asp Leu Asn Leu
            580                 585                 590

Asn Asp Val Lys Val Arg Tyr Tyr Tyr Thr Ser Asp Gly Thr Gln Gly
        595                 600                 605
```

```
Gln Thr Phe Trp Gly Asp His Ala Gly Ala Leu Leu Gly Asn Ser Tyr
    610                 615                 620

Val Asp Asn Thr Gly Lys Val Thr Ala Asn Phe Val Lys Glu Thr Ala
625                 630                 635                 640

Ser Pro Thr Ser Thr Tyr Asp Thr Tyr Val Glu Phe Gly Phe Ala Ser
                645                 650                 655

Gly Ala Ala Thr Leu Lys Lys Gly Gln Phe Ile Thr Ile Gln Gly Arg
            660                 665                 670

Ile Thr Lys Ser Asp Trp Ser Asn Tyr Ala Gln Thr Asn Asp Tyr Ser
        675                 680                 685

Phe Asp Ala Ser Ser Ser Thr Pro Val Val Asn Pro Lys Val Thr Gly
    690                 695                 700

Tyr Ile Gly Gly Ala Lys Val Leu Gly Thr Ala Pro Gly Pro Asp Val
705                 710                 715                 720

Pro Ser Ser Ile Ile Asn Pro Thr Ser Ala Thr Phe Asp Leu Glu His
                725                 730                 735

His His His His His
            740
```

<210> 35
<211> 774
<212> PRT
<213> Artificial sequence

<220>
<223> CBDclos 12sps spider silk fusion polypeptide

<400> 35

```
Asn Ser Ile Thr Pro Ile Ile Lys Ile Thr Asn Thr Ala Asp Ser Asp
1               5                   10                  15

Leu Asn Leu Asn Asp Val Lys Val Arg Tyr Tyr Tyr Thr Ser Asp Gly
            20                  25                  30

Thr Gln Gly Gln Thr Phe Trp Gly Asp His Ala Gly Ala Leu Leu Gly
        35                  40                  45

Asn Ser Tyr Val Asp Asn Thr Gly Lys Val Thr Ala Asn Phe Val Lys
    50                  55                  60

Glu Thr Ala Ser Pro Thr Ser Thr Tyr Asp Thr Tyr Val Glu Phe Gly
65                  70                  75                  80

Phe Ala Ser Gly Ala Ala Thr Leu Lys Lys Gly Gln Phe Ile Thr Ile
                85                  90                  95

Gln Gly Arg Ile Thr Lys Ser Asp Trp Ser Asn Tyr Ala Gln Thr Asn
            100                 105                 110
```

```
Asp Tyr Ser Phe Asp Ala Ser Ser Ser Thr Pro Val Val Asn Pro Lys
        115                 120                 125

Val Thr Gly Tyr Ile Gly Gly Ala Lys Val Leu Gly Thr Ala Pro Gly
    130                 135                 140

Pro Asp Val Pro Ser Ser Ile Ile Asn Pro Thr Ser Ala Thr Phe Asp
145                 150                 155                 160

Pro Gly Thr Met Ala Ser Met Thr Gly Gly Gln Gln Met Gly Arg Ile
                165                 170                 175

Gly Ser Pro Pro Gly Pro Gly Pro Gly Gly Gln Gly Pro Tyr Gly Pro
            180                 185                 190

Gly Ala Ser Ala Ala Ala Ala Ala Ala Gly Gly Tyr Gly Pro Gly Ser
        195                 200                 205

Gly Gln Gln Gly Pro Gly Gln Gln Gly Pro Gly Gln Gln Ala Gly Pro
    210                 215                 220

Gly Gly Gln Gly Pro Tyr Gly Pro Gly Ala Ser Ala Ala Ala Ala Ala
225                 230                 235                 240

Ala Gly Gly Tyr Gly Pro Gly Ser Gly Gln Gln Gly Pro Gly Gln Gln
                245                 250                 255

Gly Pro Gly Gln Gln Ala Gly Pro Gly Gly Gln Gly Pro Tyr Gly Pro
            260                 265                 270

Gly Ala Ser Ala Ala Ala Ala Ala Ala Gly Gly Tyr Gly Pro Gly Ser
        275                 280                 285

Gly Gln Gln Gly Pro Gly Gln Gln Gly Pro Gly Gln Gln Ala Gly Pro
    290                 295                 300

Gly Gly Gln Gly Pro Tyr Gly Pro Gly Ala Ser Ala Ala Ala Ala Ala
305                 310                 315                 320

Ala Gly Gly Tyr Gly Pro Gly Ser Gly Gln Gln Gly Pro Gly Gln Gln
                325                 330                 335

Gly Pro Gly Gln Gln Ala Gly Pro Gly Gly Gln Gly Pro Tyr Gly Pro
            340                 345                 350

Gly Ala Ser Ala Ala Ala Ala Ala Ala Gly Gly Tyr Gly Pro Gly Ser
        355                 360                 365

Gly Gln Gln Gly Pro Gly Gln Gln Gly Pro Gly Gln Gln Ala Gly Pro
    370                 375                 380

Gly Gly Gln Gly Pro Tyr Gly Pro Gly Ala Ser Ala Ala Ala Ala Ala
385                 390                 395                 400

Ala Gly Gly Tyr Gly Pro Gly Ser Gly Gln Gln Gly Pro Gly Gln Gln
```

405 410 415

Gly Pro Gly Gln Gln Ala Gly Pro Gly Gly Gln Gly Pro Tyr Gly Pro
420 425 430

Gly Ala Ser Ala Ala Ala Ala Ala Ala Gly Gly Tyr Gly Pro Gly Ser
435 440 445

Gly Gln Gln Gly Pro Gly Gln Gln Gly Pro Gly Gln Gln Ala Gly Pro
450 455 460

Gly Gly Gln Gly Pro Tyr Gly Pro Gly Ala Ser Ala Ala Ala Ala Ala
465 470 475 480

Ala Gly Gly Tyr Gly Pro Gly Ser Gly Gln Gln Gly Pro Gly Gln Gln
485 490 495

Gly Pro Gly Gln Gln Ala Gly Pro Gly Gly Gln Gly Pro Tyr Gly Pro
500 505 510

Gly Ala Ser Ala Ala Ala Ala Ala Ala Gly Gly Tyr Gly Pro Gly Ser
515 520 525

Gly Gln Gln Gly Pro Gly Gln Gln Gly Pro Gly Gln Gln Ala Gly Pro
530 535 540

Gly Gly Gln Gly Pro Tyr Gly Pro Gly Ala Ser Ala Ala Ala Ala Ala
545 550 555 560

Ala Gly Gly Tyr Gly Pro Gly Ser Gly Gln Gln Gly Pro Gly Gln Gln
565 570 575

Gly Pro Gly Gln Gln Ala Gly Pro Gly Gly Gln Gly Pro Tyr Gly Pro
580 585 590

Gly Ala Ser Ala Ala Ala Ala Ala Ala Gly Gly Tyr Gly Pro Gly Ser
595 600 605

Gly Gln Gln Gly Pro Gly Gln Gln Gly Pro Gly Gln Gln Ala Gly Pro
610 615 620

Gly Gly Gln Gly Pro Tyr Gly Pro Gly Ala Ser Ala Ala Ala Ala Ala
625 630 635 640

Ala Gly Gly Tyr Gly Pro Gly Ser Gly Gln Gln Gly Pro Gly Gln Gln
645 650 655

Gly Pro Gly Gln Gln Ala Gly Ser Ser Val Pro Val Ala Ser Ala Val
660 665 670

Ala Ser Arg Leu Ser Ser Pro Ala Ala Ser Ser Arg Val Ser Ser Ala
675 680 685

Val Ser Ser Leu Val Ser Ser Gly Pro Thr Lys His Ala Ala Leu Ser
690 695 700

```
Asn Thr Ile Ser Ser Val Val Ser Gln Val Ser Ala Ser Asn Pro Gly
705                 710                 715                 720

Leu Ser Gly Cys Asp Val Leu Val Gln Ala Leu Leu Glu Val Val Ser
                725                 730                 735

Ala Leu Val Ser Ile Leu Gly Ser Ser Ser Ile Gly Gln Ile Asn Tyr
            740                 745                 750

Gly Ala Ser Ala Gln Tyr Thr Gln Met Val Gly Gln Ser Val Ala Gln
        755                 760                 765

Ala Leu Ala Ala Ala Ala
    770
```

<210> 36
<211> 803
<212> PRT
<213> Artificial sequence

<220>
<223> 6sps-CBD-6sps fusion polypeptide

<400> 36

```
Met Gln Phe Arg Gly Pro Gly Gly Gln Gly Pro Tyr Gly Pro Gly Ala
1               5                   10                  15

Ser Ala Ala Ala Ala Ala Ala Gly Gly Tyr Gly Pro Gly Ser Gly Gln
            20                  25                  30

Gln Gly Pro Gly Gln Gln Gly Pro Gly Gln Gln Ala Gly Pro Gly Gly
        35                  40                  45

Gln Gly Pro Tyr Gly Pro Gly Ala Ser Ala Ala Ala Ala Ala Ala Gly
    50                  55                  60

Gly Tyr Gly Pro Gly Ser Gly Gln Gln Gly Pro Gly Gln Gln Gly Pro
65                  70                  75                  80

Gly Gln Gln Ala Gly Pro Gly Gly Gln Gly Pro Tyr Gly Pro Gly Ala
                85                  90                  95

Ser Ala Ala Ala Ala Ala Ala Gly Gly Tyr Gly Pro Gly Ser Gly Gln
            100                 105                 110

Gln Gly Pro Gly Gln Gln Gly Pro Gly Gln Gln Ala Gly Pro Gly Gly
        115                 120                 125

Gln Gly Pro Tyr Gly Pro Gly Ala Ser Ala Ala Ala Ala Ala Ala Gly
    130                 135                 140

Gly Tyr Gly Pro Gly Ser Gly Gln Gln Gly Pro Gly Gln Gln Gly Pro
145                 150                 155                 160

Gly Gln Gln Ala Gly Pro Gly Gly Gln Gly Pro Tyr Gly Pro Gly Ala
                165                 170                 175
```

```
Ser Ala Ala Ala Ala Ala Ala Gly Gly Tyr Gly Pro Gly Ser Gly Gln
            180                 185                 190

Gln Gly Pro Gly Gln Gln Gly Pro Gly Gln Gln Ala Gly Pro Gly Gly
        195                 200                 205

Gln Gly Pro Tyr Gly Pro Gly Ala Ser Ala Ala Ala Ala Ala Ala Gly
    210                 215                 220

Gly Tyr Gly Pro Gly Ser Gly Gln Gln Gly Pro Gly Gln Gln Gly Pro
225                 230                 235                 240

Gly Gln Gln Ala Pro Pro Gly Ala His Met Ala Ala Thr Ser Ser Met
                245                 250                 255

Ser Val Glu Phe Tyr Asn Ser Asn Lys Ala Ala Gln Thr Asn Ser Ile
            260                 265                 270

Thr Pro Ile Ile Lys Ile Thr Asn Thr Ala Asp Ser Asp Leu Asn Leu
        275                 280                 285

Asn Asp Val Lys Val Arg Tyr Tyr Tyr Thr Ser Asp Gly Thr Gln Gly
    290                 295                 300

Gln Thr Phe Trp Gly Asp His Ala Gly Ala Leu Leu Gly Asn Ser Tyr
305                 310                 315                 320

Val Asp Asn Thr Gly Lys Val Thr Ala Asn Phe Val Lys Glu Thr Ala
                325                 330                 335

Ser Pro Thr Ser Thr Tyr Asp Thr Tyr Val Glu Phe Gly Phe Ala Ser
            340                 345                 350

Gly Ala Ala Thr Leu Lys Lys Gly Gln Phe Ile Thr Ile Gln Gly Arg
        355                 360                 365

Ile Thr Lys Ser Asp Trp Ser Asn Tyr Ala Gln Thr Asn Asp Tyr Ser
    370                 375                 380

Phe Asp Ala Ser Ser Ser Thr Pro Val Val Asn Pro Lys Val Thr Gly
385                 390                 395                 400

Tyr Ile Gly Gly Ala Lys Val Leu Gly Thr Ala Pro Gly Pro Asp Val
                405                 410                 415

Pro Ser Ser Ile Ile Asn Pro Thr Ser Ala Thr Phe Asp Pro Gly Thr
            420                 425                 430

Met Ala Ser Met Thr Gly Gly Gln Gln Met Gly Arg Ile Gly Ser Pro
        435                 440                 445

Pro Gly Pro Gly Pro Gly Gly Gln Gly Pro Tyr Gly Pro Gly Ala Ser
    450                 455                 460

Ala Ala Ala Ala Ala Ala Gly Gly Tyr Gly Pro Gly Ser Gly Gln Gln
```

```
465                 470                 475                 480

Gly Pro Gly Gln Gln Gly Pro Gly Gln Gln Ala Gly Pro Gly Gly Gln
                485                 490                 495

Gly Pro Tyr Gly Pro Gly Ala Ser Ala Ala Ala Ala Ala Ala Gly Gly
            500                 505                 510

Tyr Gly Pro Gly Ser Gly Gln Gln Gly Pro Gly Gln Gln Gly Pro Gly
        515                 520                 525

Gln Gln Ala Gly Pro Gly Gly Gln Gly Pro Tyr Gly Pro Gly Ala Ser
    530                 535                 540

Ala Ala Ala Ala Ala Ala Gly Gly Tyr Gly Pro Gly Ser Gly Gln Gln
545                 550                 555                 560

Gly Pro Gly Gln Gln Gly Pro Gly Gln Gln Ala Gly Pro Gly Gly Gln
                565                 570                 575

Gly Pro Tyr Gly Pro Gly Ala Ser Ala Ala Ala Ala Ala Ala Gly Gly
            580                 585                 590

Tyr Gly Pro Gly Ser Gly Gln Gln Gly Pro Gly Gln Gln Gly Pro Gly
        595                 600                 605

Gln Gln Ala Gly Pro Gly Gly Gln Gly Pro Tyr Gly Pro Gly Ala Ser
    610                 615                 620

Ala Ala Ala Ala Ala Ala Gly Gly Tyr Gly Pro Gly Ser Gly Gln Gln
625                 630                 635                 640

Gly Pro Gly Gln Gln Gly Pro Gly Gln Gln Ala Gly Pro Gly Gly Gln
                645                 650                 655

Gly Pro Tyr Gly Pro Gly Ala Ser Ala Ala Ala Ala Ala Ala Gly Gly
            660                 665                 670

Tyr Gly Pro Gly Ser Gly Gln Gln Gly Pro Gly Gln Gln Gly Pro Gly
        675                 680                 685

Gln Gln Ala Gly Ser Ser Val Pro Val Ala Ser Ala Val Ala Ser Arg
    690                 695                 700

Leu Ser Ser Pro Ala Ala Ser Ser Arg Val Ser Ser Ala Val Ser Ser
705                 710                 715                 720

Leu Val Ser Ser Gly Pro Thr Lys His Ala Ala Leu Ser Asn Thr Ile
                725                 730                 735

Ser Ser Val Val Ser Gln Val Ser Ala Ser Asn Pro Gly Leu Ser Gly
            740                 745                 750

Cys Asp Val Leu Val Gln Ala Leu Leu Glu Val Val Ser Ala Leu Val
        755                 760                 765
```

Ser Ile Leu Gly Ser Ser Ser Ile Gly Gln Ile Asn Tyr Gly Ala Ser
770 775 780

Ala Gln Tyr Thr Gln Met Val Gly Gln Ser Val Ala Gln Ala Leu Ala
785 790 795 800

Ala Ala Ala

<210> 37
<211> 30
<212> DNA
<213> Artificial sequence

<220>
<223> Single strand DNA oligonucleotide

<400> 37
gactagtatg gcagcgacat catcaatgtc 30

<210> 38
<211> 212
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic spider silk repeating unit (GENEART) polypeptide

<400> 38

Ile Ile Lys Ile Thr Asn Thr Ala Asp Ser Asp Leu Asn Leu Asn Asp
1 5 10 15

Val Lys Val Arg Tyr Tyr Tyr Thr Ser Asp Gly Thr Gln Gly Gln Thr
20 25 30

Phe Trp Gly Asp His Ala Thr Met Ala Ser Met Thr Gly Gly Gln Gln
35 40 45

Met Gly Arg Ile Gly Ser Pro Pro Gly Pro Gly Pro Gly Gly Gln Gly
50 55 60

Pro Tyr Gly Pro Gly Ala Ser Ala Ala Ala Ala Ala Ala Gly Gly Tyr
65 70 75 80

Gly Pro Gly Ser Gly Gln Gln Gly Pro Gly Gln Gln Gly Pro Gly Gln
85 90 95

Gln Ala Gly Ser Ser Val Pro Val Ala Ser Ala Val Ala Ser Arg Leu
100 105 110

Ser Ser Pro Ala Ala Ser Ser Arg Val Ser Ser Ala Val Ser Ser Leu
115 120 125

Val Ser Ser Gly Pro Thr Lys His Ala Ala Leu Ser Asn Thr Ile Ser
130 135 140

Ser Val Val Ser Gln Val Ser Ala Ser Asn Pro Gly Leu Ser Gly Cys
145 150 155 160

Asp Val Leu Val Gln Ala Leu Leu Glu Val Val Ser Ala Leu Val Ser
165 170 175

Ile Leu Gly Ser Ser Ser Ile Gly Gln Ile Asn Tyr Gly Ala Ser Ala
180 185 190

Gln Tyr Thr Gln Met Val Gly Gln Ser Val Ala Gln Ala Leu Ala Ala
195 200 205

Ala Ala Ser Leu
210

<210> 39
<211> 74
<212> PRT
<213> *Drosophila melanogaster*

<220>
<221> misc_feature
<223> Resilin Chitin binding domain

<400> 39

Pro Ala Lys Tyr Glu Phe Asn Tyr Gln Val Glu Asp Ala Pro Ser Gly
1 5 10 15

Leu Ser Phe Gly His Ser Glu Met Arg Asp Gly Asp Phe Thr Thr Gly
20 25 30

Gln Tyr Asn Val Leu Leu Pro Asp Gly Arg Lys Gln Ile Val Glu Tyr
35 40 45

Glu Ala Asp Gln Gln Gly Tyr Arg Pro Gln Ile Arg Tyr Glu Gly Asp
50 55 60

Ala Asn Asp Gly Ser Gly Pro Ser Gly Pro
65 70

<210> 40
<211> 42
<212> PRT
<213> Arabidopsis thaliana

<220>
<221> misc_feature
<223> Basic endochitinase B Chitin binding domain

<400> 40

Glu Gln Cys Gly Arg Gln Ala Gly Gly Ala Leu Cys Pro Asn Gly Leu
1 5 10 15

Cys Cys Ser Glu Phe Gly Trp Cys Gly Asn Thr Glu Pro Tyr Cys Lys
20 25 30

Gln Pro Gly Cys Gln Ser Gln Cys Thr Pro
35 40

<210> 41

<211> 112
<212> PRT
<213> Aspergillus niger

<220>
<221> misc_feature
<223> Glucoamylase starch-binding domain

<400> 41

```
Ser Ser Thr Ser Cys Thr Thr Pro Thr Ala Val Ala Val Thr Phe Asp
1               5                  10                  15

Leu Thr Ala Thr Thr Thr Tyr Gly Glu Asn Ile Tyr Leu Val Gly Ser
            20                  25                  30

Ile Ser Gln Leu Gly Asp Trp Asp Thr Ser Asp Gly Ile Ala Leu Ser
        35                  40                  45

Ala Asp Lys Tyr Thr Ser Ser Asn Pro Leu Trp Tyr Val Thr Val Thr
    50                  55                  60

Leu Pro Ala Gly Glu Ser Phe Glu Tyr Lys Phe Ile Arg Ile Glu Ser
65                  70                  75                  80

Asp Asp Ser Val Glu Trp Glu Ser Asp Pro Asn Arg Glu Tyr Thr Val
                85                  90                  95

Pro Gln Val Cys Gly Glu Ser Thr Ala Thr Val Thr Asp Thr Trp Arg
            100                 105                 110
```

<210> 42
<211> 116
<212> PRT
<213> Streptococcus mutans

<220>
<221> misc_feature
<223> Dextran binding domain

<400> 42

```
Leu Gly Ile Asn Gly Asp Gln Val Trp Thr Tyr Ala Lys Lys Gly Asn
1               5                  10                  15

Asp Phe Arg Thr Ile Gln Leu Leu Asn Leu Met Gly Ile Thr Ser Asp
            20                  25                  30

Trp Lys Asn Glu Asp Gly Tyr Glu Asn Asn Lys Thr Pro Asp Glu Gln
        35                  40                  45

Thr Asn Leu Leu Val Thr Tyr Pro Leu Thr Gly Val Ser Met Ala Glu
    50                  55                  60

Ala Asp Arg Ile Ala Lys Gln Val Tyr Leu Thr Ser Pro Asp Asp Trp
65                  70                  75                  80

Leu Gln Ser Ser Met Ile Ser Leu Ala Thr Gln Ile Lys Thr Asn Glu
                85                  90                  95
```

```
Asn Gly Asp Pro Val Leu Tyr Ile Gln Val Pro Arg Leu Thr Leu Trp
            100                 105                 110

Asp Met Ile Tyr
        115

<210>  43
<211>  347
<212>  PRT
<213>  Sphingomonas sp.

<220>
<221>  misc_feature
<223>  Alginate binding domain

<400>  43

Lys Glu Ala Thr Trp Val Thr Asp Lys Pro Leu Thr Leu Lys Ile His
1               5                   10                  15

Met His Phe Arg Asp Lys Trp Val Trp Asp Glu Asn Trp Pro Val Ala
            20                  25                  30

Lys Glu Ser Phe Arg Leu Thr Asn Val Lys Leu Gln Ser Val Ala Asn
        35                  40                  45

Lys Ala Ala Thr Asn Ser Gln Glu Gln Phe Asn Leu Met Met Ala Ser
    50                  55                  60

Gly Asp Leu Pro Asp Val Val Gly Gly Asp Asn Leu Lys Asp Lys Phe
65                  70                  75                  80

Ile Gln Tyr Gly Gln Glu Gly Ala Phe Val Pro Leu Asn Lys Leu Ile
                85                  90                  95

Asp Gln Tyr Ala Pro His Ile Lys Ala Phe Phe Lys Ser His Pro Glu
            100                 105                 110

Val Glu Arg Ala Ile Lys Ala Pro Asp Gly Asn Ile Tyr Phe Ile Pro
        115                 120                 125

Tyr Val Pro Asp Gly Val Val Ala Arg Gly Tyr Phe Ile Arg Glu Asp
    130                 135                 140

Trp Leu Lys Lys Leu Asn Leu Lys Pro Pro Gln Asn Ile Asp Glu Leu
145                 150                 155                 160

Tyr Thr Val Leu Lys Ala Phe Lys Glu Lys Asp Pro Asn Gly Asn Gly
                165                 170                 175

Lys Ala Asp Glu Val Pro Phe Ile Asp Arg His Pro Asp Glu Val Phe
            180                 185                 190

Arg Leu Val Asn Phe Trp Gly Ala Arg Ser Ser Gly Ser Asp Asn Tyr
        195                 200                 205

Met Asp Phe Tyr Ile Asp Asn Gly Arg Val Lys His Pro Trp Ala Glu
```

```
    210                 215                 220

Thr Ala Phe Arg Asp Gly Met Lys His Val Ala Gln Trp Tyr Lys Glu
225                 230                 235                 240

Gly Leu Ile Asp Lys Glu Ile Phe Thr Arg Lys Ala Lys Ala Arg Glu
                245                 250                 255

Gln Met Phe Gly Gly Asn Leu Gly Gly Phe Thr His Asp Trp Phe Ala
            260                 265                 270

Ser Thr Met Thr Phe Asn Glu Gly Leu Ala Lys Thr Val Pro Gly Phe
        275                 280                 285

Lys Leu Ile Pro Ile Ala Pro Pro Thr Asn Ser Lys Gly Gln Arg Trp
    290                 295                 300

Glu Glu Asp Ser Arg Gln Lys Val Arg Pro Asp Gly Trp Ala Ile Thr
305                 310                 315                 320

Val Lys Asn Lys Asn Pro Val Glu Thr Ile Lys Phe Phe Asp Phe Tyr
                325                 330                 335

Phe Ser Arg Pro Gly Arg Asp Ile Ser Asn Phe
            340                 345

<210>  44
<211>  105
<212>  PRT
<213>  Rattus norvegicus

<220>
<221>  misc_feature
<223>  Hyaluronic acid binding domain

<400>  44

Gly Lys Arg Asp Phe Glu Arg Tyr Gly Ser Ser Asp Lys Ala Asn Arg
1               5                   10                  15

Met Glu Asp Ser Met Gly Gly Cys Gly Val Arg Thr Trp Gly Ser Gly
            20                  25                  30

Lys Asp Thr Ser Asp Thr Glu Pro Pro Ala Pro Met Glu Glu Thr Ser
        35                  40                  45

Met Met Glu Glu Cys Gln Gly Val Leu Asp Glu Glu Ser Ala Ser Lys
    50                  55                  60

Val Pro Glu Leu Glu Val Glu Glu Glu Asn Gln Val Gln Glu Met Thr
65                  70                  75                  80

Leu Asp Glu Trp Lys Asn Leu Gln Glu Gln Thr Arg Pro Lys Pro Glu
                85                  90                  95

Phe Asn Ile Arg Lys Pro Glu Ser Thr
            100                 105
```

<210> 45
<211> 7
<212> PRT
<213> Artificial sequence

<220>
<223> Repeating amino acid sequence in resilin

<220>
<221> misc_feature
<222> (2)..(3)
<223> Xaa can be any naturally occurring amino acid

<220>
<221> misc_feature
<222> (6)..(6)
<223> Xaa can be any naturally occurring amino acid

<400> 45

Ser Xaa Xaa Tyr Gly Xaa Pro
1 5

<210> 46
<211> 5
<212> PRT
<213> Artificial sequence

<220>
<223> Repeating amino acid sequence in elastin

<400> 46

Gly Val Gly Val Pro
1 5

<210> 47
<211> 38
<212> DNA
<213> Artificial sequence

<220>
<223> Single strand DNA oligonucleotide

<400> 47
ctcgagatca aatgttgcag aagtaggatt aattattg 38

<210> 48
<211> 138
<212> DNA
<213> Artificial sequence

<220>
<223> Vacuolar sorting signal coding polynucleotide

<400> 48
atggctcacg ctcgtgttct cctcctcgct ctcgctgttt tggcaacagc tgctgtggct 60

gtggcttcta gttcttcttt tgctgattca aaccctatta gacctgttac tgatagagca 120

gcttccactt tgcaattg 138

<210> 49
<211> 46
<212> PRT
<213> Artificial sequence

<220>

<223> Vacuolar sorting signal polypeptide

<400> 49

Met Ala His Ala Arg Val Leu Leu Leu Ala Leu Ala Val Leu Ala Thr
1 5 10 15

Ala Ala Val Ala Val Ala Ser Ser Ser Ser Phe Ala Asp Ser Asn Pro
20 25 30

Ile Arg Pro Val Thr Asp Arg Ala Ala Ser Thr Leu Gln Leu
35 40 45

<210> 50
<211> 127
<212> DNA
<213> Artificial sequence

<220>
<223> Apoplast sorting signal coding polynucleotide

<400> 50
gccatggcta ggaagtcttt gattttccca gtgattcttc ttgctgtgct tcttttctct 60

ccacctattt actctgctgg acacgattat agggatgctc ttaggaagtc atctatggct 120

caattgc 127

<210> 51
<211> 41
<212> PRT
<213> Artificial sequence

<220>
<223> apoplast sorting signal polypeptide

<400> 51

Met Ala Arg Lys Ser Leu Ile Phe Pro Val Ile Leu Leu Ala Val Leu
1 5 10 15

Leu Phe Ser Pro Pro Ile Tyr Ser Ala Gly His Asp Tyr Arg Asp Ala
20 25 30

Leu Arg Lys Ser Ser Met Ala Gln Leu
35 40

<210> 52
<211> 17
<212> PRT
<213> Artificial sequence

<220>
<223> Polypeptide used as linker between CBD and resilin

<400> 52

Gly Pro Asp Val Pro Ser Ser Ile Ile Asn Pro Thr Ser Ala Thr Phe
1 5 10 15

Asp

<210> 53
<211> 6

<212> PRT
<213> Artificial sequence

<220>
<223> Polypeptide used as linker between resilin and CBD

<400> 53

Gly Ile Pro Asp Pro Gly
1 5

<210> 54
<211> 324
<212> DNA
<213> Artificial sequence

<220>
<223> pHIS-Parallel3 fragment including Lac operator, 6XHis tag , a spacer region, rTEV cleavge site and a multiple cloning site

<400> 54
gaaattaata cgactcacta taggggaatt gtgagcggat aacaattccc ctctagaaat 60

aattttgttt aactttaaga aggagatata catatgtcgt actaccatca ccatcaccat 120

cacgattacg atatcccaac gaccgaaaac ctgtattttc agggcgccat ggggatccgg 180

aattcaaagg cctacgtcga cgagctcaac tagtgcggcc gctttcgaat ctagagcctg 240

cagtctcgag caccaccacc accaccactg agatccggct gctaacaaag cccgaaagga 300

agctgagttg gctgctgcca ccgc 324

<210> 55
<211> 424
<212> PRT
<213> Artificial sequence

<220>
<223> 6H-Res-ChBD

<400> 55

Met Ser Tyr Tyr His His His His His His Asp Tyr Asp Ile Pro Thr
1 5 10 15

Thr Glu Asn Leu Tyr Phe Gln Gly Ala Met Gly Pro Glu Pro Pro Val
20 25 30

Asn Ser Tyr Leu Pro Pro Ser Asp Ser Tyr Gly Ala Pro Gly Gln Ser
35 40 45

Gly Pro Gly Gly Arg Pro Ser Asp Ser Tyr Gly Ala Pro Gly Gly Gly
50 55 60

Asn Gly Gly Arg Pro Ser Asp Ser Tyr Gly Ala Pro Gly Gln Gly Gln
65 70 75 80

Gly Gln Gly Gln Gly Gln Gly Gly Tyr Ala Gly Lys Pro Ser Asp Thr
85 90 95

Tyr Gly Ala Pro Gly Gly Gly Asn Gly Asn Gly Gly Arg Pro Ser Ser
100 105 110

Ser Tyr Gly Ala Pro Gly Gly Gly Asn Gly Gly Arg Pro Ser Asp Thr

```
        115                 120                 125

Tyr Gly Ala Pro Gly Gly Gly Asn Gly Gly Arg Pro Ser Asp Thr Tyr
    130                 135                 140

Gly Ala Pro Gly Gly Gly Gly Asn Gly Asn Gly Gly Arg Pro Ser Ser
145                 150                 155                 160

Ser Tyr Gly Ala Pro Gly Gln Gly Gln Gly Asn Gly Asn Gly Gly Arg
                165                 170                 175

Ser Ser Ser Ser Tyr Gly Ala Pro Gly Gly Gly Asn Gly Gly Arg Pro
            180                 185                 190

Ser Asp Thr Tyr Gly Ala Pro Gly Gly Gly Asn Gly Gly Arg Pro Ser
        195                 200                 205

Asp Thr Tyr Gly Ala Pro Gly Gly Gly Asn Asn Gly Gly Arg Pro Ser
    210                 215                 220

Ser Ser Tyr Gly Ala Pro Gly Gly Gly Asn Gly Gly Arg Pro Ser Asp
225                 230                 235                 240

Thr Tyr Gly Ala Pro Gly Gly Gly Asn Gly Asn Gly Ser Gly Gly Arg
                245                 250                 255

Pro Ser Ser Ser Tyr Gly Ala Pro Gly Gln Gly Gln Gly Gly Phe Gly
            260                 265                 270

Gly Arg Pro Ser Asp Ser Tyr Gly Ala Pro Gly Gln Asn Gln Lys Pro
        275                 280                 285

Ser Asp Ser Tyr Gly Ala Pro Gly Ser Gly Asn Gly Asn Gly Gly Arg
    290                 295                 300

Pro Ser Ser Ser Tyr Gly Ala Pro Gly Ser Gly Pro Gly Gly Arg Pro
305                 310                 315                 320

Ser Asp Ser Tyr Gly Pro Pro Ala Ser Gly Ser Gly Ala Gly Gly Ala
                325                 330                 335

Gly Gly Ser Gly Pro Gly Gly Ala Asp Tyr Asp Asn Asp Glu Pro Ala
            340                 345                 350

Lys Tyr Glu Phe Asn Tyr Gln Val Glu Asp Ala Pro Ser Gly Leu Ser
        355                 360                 365

Phe Gly His Ser Glu Met Arg Asp Gly Asp Phe Thr Thr Gly Gln Tyr
    370                 375                 380

Asn Val Leu Leu Pro Asp Gly Arg Lys Gln Ile Val Glu Tyr Glu Ala
385                 390                 395                 400

Asp Gln Gln Gly Tyr Arg Pro Gln Ile Arg Tyr Glu Gly Asp Ala Asn
                405                 410                 415
```

```
Asp Gly Ser Gly Pro Ser Gly Pro
            420


<210>  56
<211>  354
<212>  PRT
<213>  Artificial sequence

<220>
<223>  6H-Resilin

<400>  56

Met Ser Tyr Tyr His His His His His His Asp Tyr Asp Ile Pro Thr
1               5                  10                  15


Thr Glu Asn Leu Tyr Phe Gln Gly Ala Met Gly Pro Glu Pro Pro Val
            20                  25                  30


Asn Ser Tyr Leu Pro Pro Ser Asp Ser Tyr Gly Ala Pro Gly Gln Ser
        35                  40                  45


Gly Pro Gly Gly Arg Pro Ser Asp Ser Tyr Gly Ala Pro Gly Gly Gly
    50                  55                  60


Asn Gly Gly Arg Pro Ser Asp Ser Tyr Gly Ala Pro Gly Gln Gly Gln
65                  70                  75                  80


Gly Gln Gly Gln Gly Gln Gly Gly Tyr Ala Gly Lys Pro Ser Asp Thr
                85                  90                  95


Tyr Gly Ala Pro Gly Gly Gly Asn Gly Asn Gly Gly Arg Pro Ser Ser
            100                 105                 110


Ser Tyr Gly Ala Pro Gly Gly Gly Asn Gly Gly Arg Pro Ser Asp Thr
        115                 120                 125


Tyr Gly Ala Pro Gly Gly Gly Asn Gly Gly Arg Pro Ser Asp Thr Tyr
    130                 135                 140


Gly Ala Pro Gly Gly Gly Gly Asn Gly Asn Gly Gly Arg Pro Ser Ser
145                 150                 155                 160


Ser Tyr Gly Ala Pro Gly Gln Gly Gln Gly Asn Gly Asn Gly Gly Arg
                165                 170                 175


Ser Ser Ser Ser Tyr Gly Ala Pro Gly Gly Gly Asn Gly Gly Arg Pro
            180                 185                 190


Ser Asp Thr Tyr Gly Ala Pro Gly Gly Gly Asn Gly Gly Arg Pro Ser
        195                 200                 205


Asp Thr Tyr Gly Ala Pro Gly Gly Gly Asn Asn Gly Gly Arg Pro Ser
    210                 215                 220


Ser Ser Tyr Gly Ala Pro Gly Gly Gly Asn Gly Gly Arg Pro Ser Asp
225                 230                 235                 240
```

```
Thr Tyr Gly Ala Pro Gly Gly Gly Asn Gly Asn Gly Ser Gly Gly Arg
                245                 250                 255

Pro Ser Ser Ser Tyr Gly Ala Pro Gly Gln Gly Gln Gly Gly Phe Gly
            260                 265                 270

Gly Arg Pro Ser Asp Ser Tyr Gly Ala Pro Gly Gln Asn Gln Lys Pro
        275                 280                 285

Ser Asp Ser Tyr Gly Ala Pro Gly Ser Gly Asn Gly Asn Gly Gly Arg
    290                 295                 300

Pro Ser Ser Ser Tyr Gly Ala Pro Gly Ser Gly Pro Gly Gly Arg Pro
305                 310                 315                 320

Ser Asp Ser Tyr Gly Pro Pro Ala Ser Gly Ser Gly Ala Gly Gly Ala
                325                 330                 335

Gly Gly Ser Gly Pro Gly Gly Ala Asp Tyr Asp Asn Asp Glu Gly Ser
            340                 345                 350

Asn His
```

<210> 57
<211> 528
<212> PRT
<213> Artificial sequence

<220>
<223> 6H-CBD-resilin

<400> 57

```
Met Ser Tyr Tyr His His His His His His Asp Tyr Asp Ile Pro Thr
1               5                   10                  15

Thr Glu Asn Leu Tyr Phe Gln Gly Ala Met Ala Ala Thr Ser Ser Met
            20                  25                  30

Ser Val Glu Phe Tyr Asn Ser Asn Lys Ala Ala Gln Thr Asn Ser Ile
        35                  40                  45

Thr Pro Ile Ile Lys Ile Thr Asn Thr Ala Asp Ser Asp Leu Asn Leu
    50                  55                  60

Asn Asp Val Lys Val Arg Tyr Tyr Tyr Thr Ser Asp Gly Thr Gln Gly
65                  70                  75                  80

Gln Thr Phe Trp Gly Asp His Ala Gly Ala Leu Leu Gly Asn Ser Tyr
                85                  90                  95

Val Asp Asn Thr Gly Lys Val Thr Ala Asn Phe Val Lys Glu Thr Ala
            100                 105                 110

Ser Pro Thr Ser Thr Tyr Asp Thr Tyr Val Glu Phe Gly Phe Ala Ser
        115                 120                 125
```

```
Gly Ala Ala Thr Leu Lys Lys Gly Gln Phe Ile Thr Ile Gln Gly Arg
    130                 135                 140

Ile Thr Lys Ser Asp Trp Ser Asn Tyr Ala Gln Thr Asn Asp Tyr Ser
145                 150                 155                 160

Phe Asp Ala Ser Ser Ser Thr Pro Val Val Asn Pro Lys Val Thr Gly
                165                 170                 175

Tyr Ile Gly Gly Ala Lys Val Leu Gly Thr Ala Pro Gly Pro Asp Val
            180                 185                 190

Pro Ser Ser Ile Ile Asn Pro Thr Ser Ala Thr Phe Asp Pro Glu Pro
        195                 200                 205

Pro Val Asn Ser Tyr Leu Pro Pro Ser Asp Ser Tyr Gly Ala Pro Gly
    210                 215                 220

Gln Ser Gly Pro Gly Gly Arg Pro Ser Asp Ser Tyr Gly Ala Pro Gly
225                 230                 235                 240

Gly Gly Asn Gly Gly Arg Pro Ser Asp Ser Tyr Gly Ala Pro Gly Gln
                245                 250                 255

Gly Gln Gly Gln Gly Gln Gly Gln Gly Gly Tyr Ala Gly Lys Pro Ser
            260                 265                 270

Asp Thr Tyr Gly Ala Pro Gly Gly Gly Asn Gly Asn Gly Gly Arg Pro
        275                 280                 285

Ser Ser Ser Tyr Gly Ala Pro Gly Gly Gly Asn Gly Gly Arg Pro Ser
    290                 295                 300

Asp Thr Tyr Gly Ala Pro Gly Gly Gly Asn Gly Gly Arg Pro Ser Asp
305                 310                 315                 320

Thr Tyr Gly Ala Pro Gly Gly Gly Gly Asn Gly Asn Gly Gly Arg Pro
                325                 330                 335

Ser Ser Ser Tyr Gly Ala Pro Gly Gln Gly Gln Gly Asn Gly Asn Gly
            340                 345                 350

Gly Arg Ser Ser Ser Ser Tyr Gly Ala Pro Gly Gly Gly Asn Gly Gly
        355                 360                 365

Arg Pro Ser Asp Thr Tyr Gly Ala Pro Gly Gly Gly Asn Gly Gly Arg
    370                 375                 380

Pro Ser Asp Thr Tyr Gly Ala Pro Gly Gly Gly Asn Asn Gly Gly Arg
385                 390                 395                 400

Pro Ser Ser Ser Tyr Gly Ala Pro Gly Gly Gly Asn Gly Gly Arg Pro
                405                 410                 415

Ser Asp Thr Tyr Gly Ala Pro Gly Gly Gly Asn Gly Asn Gly Ser Gly
```

420 425 430

Gly Arg Pro Ser Ser Ser Tyr Gly Ala Pro Gly Gln Gly Gln Gly Gly
435 440 445

Phe Gly Gly Arg Pro Ser Asp Ser Tyr Gly Ala Pro Gly Gln Asn Gln
450 455 460

Lys Pro Ser Asp Ser Tyr Gly Ala Pro Gly Ser Gly Asn Gly Asn Gly
465 470 475 480

Gly Arg Pro Ser Ser Ser Tyr Gly Ala Pro Gly Ser Gly Pro Gly Gly
485 490 495

Arg Pro Ser Asp Ser Tyr Gly Pro Pro Ala Ser Gly Ser Gly Ala Gly
500 505 510

Gly Ala Gly Gly Ser Gly Pro Gly Gly Ala Asp Tyr Asp Asn Asp Glu
515 520 525

<210> 58
<211> 519
<212> PRT
<213> Artificial sequence

<220>
<223> 6H-resilin-CBD

<400> 58

Met Ser Tyr Tyr His His His His His His Asp Tyr Asp Ile Pro Thr
1 5 10 15

Thr Glu Asn Leu Tyr Phe Gln Gly Ala Met Gly Pro Glu Pro Pro Val
20 25 30

Asn Ser Tyr Leu Pro Pro Ser Asp Ser Tyr Gly Ala Pro Gly Gln Ser
35 40 45

Gly Pro Gly Gly Arg Pro Ser Asp Ser Tyr Gly Ala Pro Gly Gly Gly
50 55 60

Asn Gly Gly Arg Pro Ser Asp Ser Tyr Gly Ala Pro Gly Gln Gly Gln
65 70 75 80

Gly Gln Gly Gln Gly Gln Gly Gly Tyr Ala Gly Lys Pro Ser Asp Thr
85 90 95

Tyr Gly Ala Pro Gly Gly Gly Asn Gly Asn Gly Gly Arg Pro Ser Ser
100 105 110

Ser Tyr Gly Ala Pro Gly Gly Gly Asn Gly Gly Arg Pro Ser Asp Thr
115 120 125

Tyr Gly Ala Pro Gly Gly Gly Asn Gly Gly Arg Pro Ser Asp Thr Tyr
130 135 140

Gly Ala Pro Gly Gly Gly Gly Asn Gly Asn Gly Gly Arg Pro Ser Ser

```
145                 150                 155                 160

Ser Tyr Gly Ala Pro Gly Gln Gly Gln Gly Asn Gly Asn Gly Gly Arg
                165                 170                 175

Ser Ser Ser Ser Tyr Gly Ala Pro Gly Gly Gly Asn Gly Gly Arg Pro
            180                 185                 190

Ser Asp Thr Tyr Gly Ala Pro Gly Gly Gly Asn Gly Gly Arg Pro Ser
        195                 200                 205

Asp Thr Tyr Gly Ala Pro Gly Gly Gly Asn Asn Gly Gly Arg Pro Ser
    210                 215                 220

Ser Ser Tyr Gly Ala Pro Gly Gly Gly Asn Gly Gly Arg Pro Ser Asp
225                 230                 235                 240

Thr Tyr Gly Ala Pro Gly Gly Gly Asn Gly Asn Gly Ser Gly Gly Arg
                245                 250                 255

Pro Ser Ser Ser Tyr Gly Ala Pro Gly Gln Gly Gln Gly Gly Phe Gly
            260                 265                 270

Gly Arg Pro Ser Asp Ser Tyr Gly Ala Pro Gly Gln Asn Gln Lys Pro
        275                 280                 285

Ser Asp Ser Tyr Gly Ala Pro Gly Ser Gly Asn Gly Asn Gly Gly Arg
    290                 295                 300

Pro Ser Ser Ser Tyr Gly Ala Pro Gly Ser Gly Pro Gly Gly Arg Pro
305                 310                 315                 320

Ser Asp Ser Tyr Gly Pro Pro Ala Ser Gly Ser Gly Ala Gly Gly Ala
                325                 330                 335

Gly Gly Ser Gly Pro Gly Gly Ala Asp Tyr Asp Asn Asp Glu Gly Ile
            340                 345                 350

Pro Asp Pro Gly Met Ala Ala Thr Ser Ser Met Ser Val Glu Phe Tyr
        355                 360                 365

Asn Ser Asn Lys Ser Ala Gln Thr Asn Ser Ile Thr Pro Ile Ile Lys
    370                 375                 380

Ile Thr Asn Thr Ser Asp Ser Asp Leu Asn Leu Asn Asp Val Lys Val
385                 390                 395                 400

Arg Tyr Tyr Tyr Thr Ser Asp Gly Thr Gln Gly Gln Thr Phe Trp Cys
                405                 410                 415

Asp His Ala Gly Ala Leu Leu Gly Asn Ser Tyr Val Asp Asn Thr Ser
            420                 425                 430

Lys Val Thr Ala Asn Phe Val Lys Glu Thr Ala Ser Pro Thr Ser Thr
        435                 440                 445
```

```
Tyr Asp Thr Tyr Val Glu Phe Gly Phe Ala Ser Gly Arg Ala Thr Leu
    450                 455                 460

Lys Lys Gly Gln Phe Ile Thr Ile Gln Gly Arg Ile Thr Lys Ser Asp
465                 470                 475                 480

Trp Ser Asn Tyr Thr Gln Thr Asn Asp Tyr Ser Phe Asp Ala Ser Ser
                485                 490                 495

Ser Thr Pro Val Val Asn Pro Lys Val Thr Gly Tyr Ile Gly Gly Ala
            500                 505                 510

Lys Val Leu Gly Thr Ala Pro
        515
```

<210> 59
<211> 1520
<212> DNA
<213> Artificial sequence

<220>
<223> 6H-Res-ChBD expressing sequence

<400> 59

```
gaaattaata cgactcacta taggggaatt gtgagcggat aacaattccc ctctagaaat       60

aattttgttt aactttaaga aggagatata catatgtcgt actaccatca ccatcaccat      120

cacgattacg atatcccaac gaccgaaaac ctgtattttc agggcgccat gggaccggag      180

ccaccagtta actcgtatct acctccgtcc gatagctatg gagcaccggg tcagagtggt      240

cccggcggca ggccgtcgga ttcctatgga gctcctggtg gtggaaacgg tggacggccc      300

tcagacagct atggcgctcc aggccagggt caaggacagg gacaaggaca aggtggatat      360

gcaggcaagc cctcagatac ctatggagct cctggtggtg gaaatggcaa cggaggtcgt      420

ccatcgagca gctatggcgc tcctggcggt ggaaacggtg gtcgtccttc ggatacctac      480

ggtgctcctg gtggcggaaa tggtggacgc ccatcggaca cttatggtgc tcctggtggt      540

ggtggaaatg gcaacggcgg acgaccttca agcagctatg gagctcctgg tcaaggacaa      600

ggcaacggaa atggcggtcg ctcatcgagc agctatggtg ctcctggcgg tggaaacggc      660

ggtcgtcctt cggataccta cggtgctccc ggtggtggaa acggtggtcg tccttcggat      720

acttacggcg ctcctggtgg cggcaataat ggcggtcgtc cctcaagcag ctacggcgct      780

cctggtggtg gaaacggtgg tcgtccatct gacacctatg gcgctcctgg tggcggtaac      840

ggaaacggca gcggtggtcg tccttcaagc agctatggag ctcctggtca gggccaaggt      900

ggatttggtg gtcgtccatc ggactcctat ggtgctcctg gtcagaacca aaaaccatca      960

gattcatatg gcgcccctgg tagcggcaat ggcaacggcg gacgtccttc gagcagctat     1020

ggagctccag gctcaggacc tggtggccga ccctccgact cctacggacc cccagcttct     1080

ggatcgggag caggtggcgc tggaggcagt ggacccggcg gcgctgacta cgataacgat     1140

gagcccgcca agtacgaatt taattaccag gttgaggacg cgcccagcgg actctcgttc     1200

gggcattcag agatgcgcga cggtgacttc accaccggcc agtacaatgt cctgttgccc     1260

gacggaagga agcaaattgt ggagtatgaa gccgaccagc agggctaccg gccacagatc     1320
```

cgctacgaag gcgatgccaa cgatggcagt ggtcccagcg gtccttaagg atccggaatt 1380

caaaggccta cgtcgacgag ctcaactagt gcggccgctt tcgaatctag agcctgcagt 1440

ctcgagcacc accaccacca ccactgagat ccggctgcta acaaagcccg aaaggaagct 1500

gagttggctg ctgccaccgc 1520

<210> 60
<211> 1275
<212> DNA
<213> Artificial sequence

<220>
<223> 6H-Resilin expressing sequence

<400> 60
gaaattaata cgactcacta taggggaatt gtgagcggat aacaattccc ctctagaaat 60

aattttgttt aactttaaga aggagatata catatgtcgt actaccatca ccatcaccat 120

cacgattacg atatcccaac gaccgaaaac ctgtattttc agggcgccat gggaccggag 180

ccaccagtta actcgtatct acctccgtcc gatagctatg gagcaccggg tcagagtggt 240

cccggcggca ggccgtcgga ttcctatgga gctcctggtg gtggaaacgg tggacggccc 300

tcagacagct atggcgctcc aggccagggt caaggacagg gacaaggaca aggtggatat 360

gcaggcaagc cctcagatac ctatggagct cctggtggtg gaaatggcaa cggaggtcgt 420

ccatcgagca gctatggcgc tcctggcggt ggaaacggtg gtcgtccttc ggatacctac 480

ggtgctcctg gtggcggaaa tggtggacgc ccatcggaca cttatggtgc tcctggtggt 540

ggtggaaatg gcaacggcgg acgaccttca agcagctatg gagctcctgg tcaaggacaa 600

ggcaacggaa atggcggtcg ctcatcgagc agctatggtg ctcctggcgg tggaaacggc 660

ggtcgtcctt cggataccta cggtgctccc ggtggtggaa acggtggtcg tccttcggat 720

acttacggcg ctcctggtgg cggcaataat ggcggtcgtc cctcaagcag ctacggcgct 780

cctggtggtg gaaacggtgg tcgtccatct gacacctatg gcgctcctgg tggcggtaac 840

ggaaacggca gcggtggtcg tccttcaagc agctatggag ctcctggtca gggccaaggt 900

ggatttggtg gtcgtccatc ggactcctat ggtgctcctg gtcagaacca aaaaccatca 960

gattcatatg gcgcccctgg tagcggcaat ggcaacggcg gacgtccttc gagcagctat 1020

ggagctccag gctcaggacc tggtggccga ccctccgact cctacggacc cccagcttct 1080

ggatcgggag caggtggcgc tggaggcagt ggacccggcg gcgctgacta cgataacgat 1140

gagggatcca atcactagtg aattcgcggc cgctttcgaa tctagagcct gcagtctcga 1200

gcaccaccac caccaccact gagatccggc tgctaacaaa gcccgaaagg aagctgagtt 1260

ggctgctgcc accgc 1275

<210> 61
<211> 1832
<212> DNA
<213> Artificial sequence

<220>
<223> 6H-CBD-resilin expressing sequence

<400> 61
gaaattaata cgactcacta taggggaatt gtgagcggat aacaattccc ctctagaaat 60

aattttgttt aactttaaga aggagatata catatgtcgt actaccatca ccatcaccat 120

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