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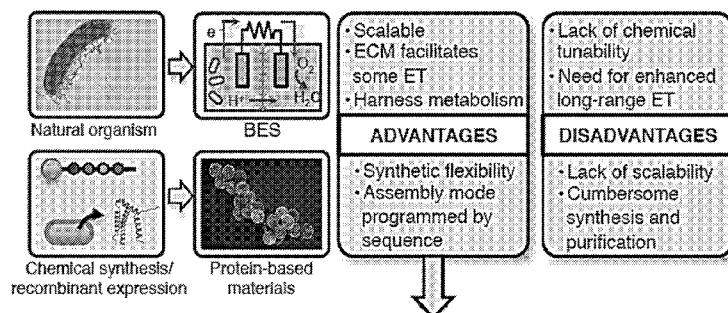
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(54) **Title:** ELECTRICALLY CONDUCTIVE PROTEIN NANOFIBERS AND BIOFILMS

Fig. 1A



(57) **Abstract:** Methods of making engineered protein-based materials, nanofibers, and biofilms from bacterial amyloid-based structures that are capable of mediating long-range electron transport are provided.

ELECTRICALLY CONDUCTIVE PROTEIN NANOFIBERS AND BIOFILMS

RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 62/257,441, filed on November 19, 2015, and U.S. Provisional Application No. 62/338,715, filed May 19, 2016, the entire contents of each of which are expressly incorporated herein by reference.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on November 17, 2016, is named 117823-13220_SeqLst.TXT and is 64 kilobytes in size.

STATEMENT OF GOVERNMENT INTERESTS

This invention was made with government support under ARO W911NF-13-1-0242 awarded by the Army Research Office. The government has certain rights in the invention.

FIELD

The technology described herein relates to engineered protein-based materials, nanofibers, and biofilms from bacterial amyloid structures known as curli fibers that are capable of mediating long-range electron transport.

BACKGROUND

Protein-based materials capable of mediating long-range electron transport have been investigated for their use in a wide range of modern optoelectronic technologies, including biosensors, light harvesting systems, microbial fuel cells, electrobiosynthetic devices, and other electronic devices. *See* Pant, D. *et al.* Bioelectrochemical systems (BES) for sustainable energy production and product recovery from organic wastes and industrial wastewaters. *RSC Adv.* 2, 1248–63 (2012). These materials can be classified into two categories with complementary advantages – naturally occurring systems and engineered biomolecular systems. Naturally occurring systems, like the conductive biofilms of *Geobacter sulfurreducens* and *Shewanella oneidensis* are useful for their ability to convert energy stored in chemical bonds into electrical energy (*i.e.*, microbial fuel cells) and vice

versa (*i.e.*, electrobiosynthesis). Pant, D. *et al.* Bioelectrochemical systems (BES) for sustainable energy production and product recovery from organic wastes and industrial wastewaters. *RSC Adv.* 2, 1248–1263 (2012); Erable, B., Duțeanu, N. M., Ghangrekar, M. M., Dumas, C. & Scott, K. Application of electroactive biofilms. *Biofouling* 26, 57–71 (2010); Michener, J. K., Thodey, K., Liang, J. C. & Smolke, C. D. Applications of genetically-encoded biosensors for the construction and control of biosynthetic pathways. *Metabolic Engineering* 14, 212–222 (2012). In order to mediate electron transfer to and from electrodes, these organisms have evolved highly specialized extracellular appendages containing periodically spaced chemical groups that facilitate electron transport. Vargas, M. *et al.* Aromatic Amino Acids Required for Pili Conductivity and Long-Range Extracellular Electron Transport in *Geobacter sulfurreducens*. *mBio* 4, e00105–13–e00105–13 (2013); Pirbadian, S. *et al.* *Shewanella oneidensis* MR-1 nanowires are outer membrane and periplasmic extensions of the extracellular electron transport components. *Proceedings of the National Academy of Sciences* 111, 12883–12888 (2014). However, despite their considerable advantages, these naturally occurring systems can be difficult to improve upon using rational design due to inherent difficulties in genetically engineering the host organisms, a lack of structural information regarding their conductive extracellular appendages, and their confinement to the use of naturally occurring amino acids as functional groups.

SUMMARY

In one aspect, provided herein is a method of producing a genetically modified bacterium comprising genetically altering a bacterium to include a nucleic acid sequence encoding a mutant amyloid polypeptide having a series of aligned aromatic groups, wherein the nucleic acid sequence is under the control of a promoter to express the mutant amyloid polypeptide.

In another aspect, provided herein is a method of producing a genetically modified bacterium comprising genetically altering a bacterium to include a nucleic acid sequence encoding a fusion protein comprising an amyloid polypeptide fused to a redox active domain, wherein the nucleic acid sequence is under the control of a promoter to express the fusion protein.

In one embodiment, the amyloid polypeptide is selected from the group consisting of CsgA, CspA, a beta-lactoglobulin, a lysozyme, Sup35, and an amyloid-beta peptide. In one embodiment, the amyloid polypeptide is CsgA.

In one embodiment, the redox active domain is selected from the group consisting of: a metalloprotein, a redox enzyme, a binding domain, and a stimuli-responsive peptide. In one embodiment, the redox active domain is selected from the group consisting of: plastocyanin, an oxygen-carrier protein, a microperoxidase, a cytochrome, a copper-binding protein, an iron-binding protein, a gold-binding domain, a metalloenzyme, rubredoxin, and a fragment thereof. In one embodiment, the redox active domain comprises plastocyanin or a fragment thereof. In one embodiment, the redox active domain comprises rubredoxin or a fragment thereof.

In one embodiment, the bacterium is *E. coli*. In one embodiment, the bacterium is non-pathogenic.

In one embodiment, the aromatic group is present on a standard amino acid or a nonstandard amino acid. In one embodiment, the aromatic group is present on a standard amino acid selected from the group consisting of phenylalanine, tryptophan, tyrosine and histidine. In one embodiment, the aromatic group is present on a nonstandard amino acid selected from the group consisting of 2-thienylalanine, 3-thienylalanine, acetylphenylalanine and azidophenylalanine.

In one embodiment, the mutant amyloid polypeptide further comprises a redox active domain.

In one embodiment, the methods described herein further provide genetically altering the bacterium to include second nucleic acid sequence encoding a fusion protein comprising an amyloid polypeptide fused to a redox active domain. In one embodiment, the amyloid polypeptide is selected from the group consisting of CsgA, CspA, a beta-lactoglobulin, a lysozyme, Sup35, and an amyloid-beta peptide. In one embodiment, the redox active domain is selected from the group consisting of: a metalloprotein, a redox enzyme, a binding domain, and a stimuli-responsive peptide. In one embodiment, the redox active domain is selected from the group consisting of: plastocyanin, an oxygen-carrier protein, a microperoxidase, a cytochrome, a copper-binding protein, an iron-binding protein, a gold-binding domain, a metalloenzyme, rubredoxin, and a fragment thereof. In one embodiment, the redox active domain comprises plastocyanin or a fragment thereof. In one embodiment, the redox active domain comprises rubredoxin or a fragment thereof.

In another aspect, provided herein is a method of making an electrically conductive biofilm comprising proliferating a genetically-altered bacterium comprising a nucleic acid sequence encoding a mutant amyloid polypeptide having a series of aligned aromatic groups, to produce a population of genetically-altered bacteria expressing the nucleic acid sequence,

and allowing the bacteria to form a biofilm comprising an amyloid-based structure formed from a plurality of mutant amyloid polypeptides, wherein the amyloid-based structure comprises a series of aligned aromatic groups forming electrically conductive pi-pi stacking along the length of the amyloid-based structure. In one embodiment, the amyloid-based structure comprises a curli fiber.

In yet another aspect, provided herein is a method of making an electrically conductive biofilm comprising proliferating a genetically-altered bacterium comprising a nucleic acid sequence encoding a fusion protein, wherein the fusion protein comprises an amyloid polypeptide fused to a redox active domain, to produce a population of genetically-altered bacteria expressing the nucleic acid sequence, and allowing the bacteria to form a biofilm comprising an amyloid-based structure formed from a plurality of fusion proteins, wherein the amyloid-based structure comprises a network of metal centers that mediate electron hopping between the metal centers. In one embodiment, the amyloid-based structure comprises a curli fiber.

In one embodiment, the amyloid polypeptide is selected from the group consisting of CsgA, CspA, a beta-lactoglobulin, a lysozyme, Sup35, and an amyloid-beta peptide. In one embodiment, the amyloid polypeptide is CsgA.

In one embodiment, the redox active domain is selected from the group consisting of a metalloprotein, a redox enzyme, a binding domain, and a stimuli-responsive peptide. In one embodiment, the redox active domain is selected from the group consisting of plastocyanin, an oxygen-carrier protein, a microperoxidase, a cytochrome, a copper-binding protein, an iron-binding protein, a gold-binding domain, a metalloenzyme, rubredoxin, and a fragment thereof. In one embodiment, the redox active domain comprises plastocyanin or a fragment thereof. In one embodiment, the redox active domain comprises rubredoxin or a fragment thereof.

In one embodiment, the bacterium is *E. coli*. In one embodiment, the bacterium is non-pathogenic.

In one embodiment, the aromatic group is present on a standard amino acid or a nonstandard amino acid. In one embodiment, the aromatic group is present on a standard amino acid selected from the group consisting of phenylalanine, tryptophan, tyrosine and histidine. In one embodiment, the aromatic group is present on a nonstandard amino acid selected from the group consisting of 2-thienylalanine, 3-thienylalanine, acetylphenylalanine and azidophenylalanine.

In one embodiment, the genetically modified bacterium further comprises a second nucleic acid sequence encoding a fusion protein comprising an amyloid polypeptide fused to a redox active domain.

In one embodiment, the aligned aromatic groups form a pi-pi stack aligned in a geometric configuration selected from the group consisting of sandwich-type pi-stacks, t-shaped pi-stacks, parallel-displaced pi-stacks.

In one aspect, provided herein is a method of making an electrically conductive amyloid-based structure from a genetically-modified bacterium comprising proliferating a genetically-altered bacterium, wherein the genetically-altered bacterium comprises a nucleic acid sequence encoding a mutant amyloid polypeptide having a series of aligned aromatic groups, to produce a population of genetically-altered bacteria expressing the nucleic acid sequence, and allowing the population of genetically-altered bacteria to produce an amyloid-based structure formed from a plurality of the mutant amyloid polypeptides, wherein the amyloid-based structure comprises a series of aligned aromatic groups forming electrically conductive pi-pi stacking along the length of the amyloid-based structures. In one embodiment, the amyloid-based structure comprises a curli fiber.

In another aspect, provided herein is a method of making an electrically conductive amyloid-based structure from a genetically-modified bacterium comprising proliferating a genetically-altered bacterium comprising a nucleic acid sequence encoding a fusion protein comprising an amyloid polypeptide fused to a redox active domain, to produce a population of genetically-altered bacteria expressing the nucleic acid sequence, allowing the population of genetically-altered bacteria to produce an amyloid-based structure formed from a plurality of the fusion proteins, wherein the amyloid-based structure comprises a network of metal centers that mediate electron hopping between the metal centers. In one embodiment, the amyloid-based structure comprises a curli fiber.

In one embodiment, the amyloid polypeptide is selected from the group consisting of CsgA, CspA, a beta-lactoglobulin, a lysozyme, Sup35, and an amyloid-beta peptide. In one embodiment, the amyloid polypeptide is CsgA.

In one embodiment, the redox active domain is selected from the group consisting of a metalloprotein, a redox enzyme, a binding domain, and a stimuli-responsive peptide. In one embodiment, the redox active domain is selected from the group consisting of plastocyanin, an oxygen-carrier protein, a microperoxidase, a cytochrome, a copper-binding protein, an iron-binding protein, a gold-binding domain, a metalloenzyme, rubredoxin, and a fragment thereof. In one embodiment, the redox active domain comprises plastocyanin or a fragment

thereof. In one embodiment, the redox active domain comprises rubredoxin or a fragment thereof.

In one embodiment, the bacterium is *E. coli*. In one embodiment, the bacterium is non-pathogenic.

In one embodiment, the aromatic group is present on a standard amino acid or a nonstandard amino acid. In one embodiment, the aromatic group is present on a standard amino acid selected from the group consisting of phenylalanine, tryptophan, tyrosine and histidine. In one embodiment, the aromatic group is present on a nonstandard amino acid selected from the group consisting of 2-thienylalanine, 3-thienylalanine, acetylphenylalanine and azidophenylalanine.

In one embodiment, the genetically-modified bacterium further comprises a second nucleic acid sequence encoding a fusion protein comprising an amyloid polypeptide fused to a redox active domain.

In one aspect, provided herein is a genetically-modified bacterium comprising a nucleic acid sequence encoding a mutant amyloid protein having a series of aligned aromatic groups, wherein the nucleic acid sequence is under the control of a promoter to express the mutant amyloid protein.

In another aspect, provided herein is a genetically-modified bacterium comprising a nucleic acid sequence encoding a fusion protein comprising an amyloid polypeptide fused to a redox active domain, wherein the nucleic acid sequence is under the control of a promoter to express the fusion protein.

In yet another aspect, provided herein is a non-naturally occurring mutant amyloid polypeptide having a series of aligned aromatic groups.

In one embodiment, the amyloid polypeptide is selected from the group consisting of CsgA, CspA, a beta-lactoglobulin, a lysozyme, Sup35, and an amyloid-beta peptide. In one embodiment, the amyloid polypeptide is CsgA.

In one embodiment, the aromatic group is present on a standard amino acid or a nonstandard amino acid. In one embodiment, the aromatic group is present on a standard amino acid selected from the group consisting of phenylalanine, tryptophan, tyrosine and histidine. In one embodiment, the aromatic group is present on a nonstandard amino acid selected from the group consisting of 2-thienylalanine, 3-thienylalanine, acetylphenylalanine and azidophenylalanine.

In one embodiment, the mutant amyloid polypeptide further comprises a redox active domain. In one embodiment, the redox active domain is selected from the group consisting

of a metalloprotein, a redox enzyme, a binding domain, and a stimuli-responsive peptide. In one embodiment, the redox active domain is selected from the group consisting of plastocyanin, an oxygen-carrier protein, a microperoxidase, a cytochrome, a copper-binding protein, an iron-binding protein, a gold-binding domain, a metalloenzyme, rubredoxin, and a fragment thereof. In one embodiment, the redox active domain comprises plastocyanin or a fragment thereof. In one embodiment, the redox active domain comprises rubredoxin or a fragment thereof.

In another aspect, provided herein is a fusion protein comprising an amyloid polypeptide fused to a redox active domain. In one embodiment, the amyloid polypeptide is selected from the group consisting of CsgA, CspA, a beta-lactoglobulin, a lysozyme, Sup35, and an amyloid-beta peptide. In one embodiment, the amyloid polypeptide is CsgA.

In one embodiment, the redox active domain is selected from the group consisting of a metalloprotein, a redox enzyme, a binding domain, and a stimuli-responsive peptide. In one embodiment, the redox active domain is selected from the group consisting of plastocyanin, an oxygen-carrier protein, a microperoxidase, a cytochrome, a copper-binding protein, an iron-binding protein, a gold-binding domain, a metalloenzyme, rubredoxin, and a fragment thereof. In one embodiment, the redox active domain comprises plastocyanin or a fragment thereof. In one embodiment, the redox active domain comprises rubredoxin or a fragment thereof.

In one embodiment, the amyloid polypeptide comprises a series of aligned aromatic groups. In one embodiment, the aromatic group is present on a standard amino acid or a nonstandard amino acid.

In one embodiment, the aromatic group is present on a standard amino acid selected from the group consisting of phenylalanine, tryptophan, tyrosine and histidine. In one embodiment, the aromatic group is present on a nonstandard amino acid selected from the group consisting of 2-thienylalanine, 3-thienylalanine, acetylphenylalanine and azidophenylalanine.

In one aspect, provided herein is a non-naturally occurring amyloid-based structure formed from a plurality of non-naturally occurring mutant amyloid polypeptides having a series of aligned aromatic groups, wherein the amyloid-based structure has a series of aligned aromatic groups forming electrically conductive pi-pi stacking along the length of the amyloid-based structure. In one embodiment, the amyloid-based structure comprises a curli fiber.

In another aspect, provided herein is a non-naturally occurring amyloid-based structure formed from a plurality of fusion proteins comprising an amyloid polypeptide fused to a redox active domain, wherein the amyloid-based structure comprises a network of metal centers that mediate electron hopping between the metal centers. In one embodiment, the amyloid-based structure comprises a curli fiber.

In yet another aspect, provided herein is a biofilm comprising a non-naturally occurring amyloid-based structure described herein. In one embodiment, the amyloid-based structure comprises a curli fiber.

In one aspect, provided herein is a nucleic acid sequence encoding a mutant amyloid polypeptide described herein.

In another aspect, provided herein is a nucleic acid sequence encoding a fusion protein described herein.

In one aspect, provided herein is a nucleic acid sequence encoding a non-naturally occurring mutant amyloid polypeptide having a series of aligned aromatic groups.

In one embodiment, the amyloid polypeptide is selected from the group consisting of CsgA, CspA, a beta-lactoglobulin, a lysozyme, Sup35, and an amyloid-beta peptide.

In one embodiment, the aromatic group is present on a standard amino acid or a nonstandard amino acid. In one embodiment, the aromatic group is present on a standard amino acid selected from the group consisting of phenylalanine, tryptophan, tyrosine and histidine. In one embodiment, the aromatic group is present on a nonstandard amino acid selected from the group consisting of 2-thienylalanine, 3-thienylalanine, acetylphenylalanine and azidophenylalanine.

In one embodiment, the amyloid polypeptide further comprising a redox active domain. In one embodiment, the redox active domain is selected from the group consisting of a metalloprotein, a redox enzyme, a binding domain, and a stimuli-responsive peptide. In one embodiment, the redox active domain is selected from the group consisting of plastocyanin, an oxygen-carrier protein, a microperoxidase, a cytochrome, a copper-binding protein, an iron-binding protein, a gold-binding domain, a metalloenzyme, rubredoxin, and a fragment thereof. In one embodiment, the redox active domain comprises plastocyanin or a fragment thereof. In one embodiment, the redox active domain comprises rubredoxin or a fragment thereof.

In another aspect, provided herein is a nucleic acid sequence encoding a fusion protein comprising an amyloid polypeptide fused to a redox active domain.

In one embodiment, the amyloid polypeptide is selected from the group consisting of CsgA, CspA, a beta-lactoglobulin, a lysozyme, Sup35, and an amyloid-beta peptide.

In one embodiment, the redox active domain is selected from the group consisting of a metalloprotein, a redox enzyme, a binding domain, and a stimuli-responsive peptide. In one embodiment, the redox active domain is selected from the group consisting of plastocyanin, an oxygen-carrier protein, a microperoxidase, a cytochrome, a copper-binding protein, an iron-binding protein, a gold-binding domain, a metalloenzyme, rubredoxin, and a fragment thereof. In one embodiment, the redox active domain comprises plastocyanin or a fragment thereof. In one embodiment, the redox active domain comprises rubredoxin or a fragment thereof.

In one embodiment, the amyloid polypeptide comprises a series of aligned aromatic groups. In one embodiment, the aromatic group is present on a standard amino acid or a nonstandard amino acid. In one embodiment, the aromatic group is present on a standard amino acid selected from the group consisting of phenylalanine, tryptophan, tyrosine and histidine. In one embodiment, the aromatic group is present on a nonstandard amino acid selected from the group consisting of 2-thienylalanine, 3-thienylalanine, acetylphenylalanine and azidophenylalanine.

In one aspect, provided herein is a vector comprising a nucleic acid sequence described herein. In one embodiment, the nucleic acid sequence is operably-linked to an inducible promoter. In one embodiment, the nucleic acid is operably-linked to a constitutive promoter.

In another aspect, provided herein is a bacterium comprising a nucleic acid sequence, or a vector comprising a nucleic acid sequence described herein.

In yet another aspect, provided herein is a biofilm comprising a bacterium expressing a nucleic acid sequence encoding a non-naturally occurring mutant amyloid polypeptide having a series of aligned aromatic groups.

In another aspect, provided herein is a biofilm comprising a bacterium expressing a nucleic acid sequence encoding a fusion protein comprising an amyloid polypeptide fused to a redox active domain.

Embodiments of the present disclosure are directed to engineered or genetically-altered microorganisms, such as *E. coli*, that produce amyloid-based structures that include moieties suited to electron transport through pi-stacking induced electron delocalization, such as amino acid sides having aromatic side chains. In one aspect, the genetically-altered microorganism is engineered to produce an amyloid fiber characterized by electron transfer

along the amyloid fiber. According to one aspect, *E. coli* is engineered to produce curli protein nanofibers characterized by electron transfer along the curli protein nanofibers. In order to generate electron transfer, amyloid fibers (*e.g.*, curli fibers) with π - π stacking are created. According to one aspect, amino acid residues in an amyloid polypeptide (*e.g.*, CsgA) are identified and mutated to amino acids having aromatic side chains without altering the basic amyloid structure of the curli fiber. Any amyloid protein may be used as described herein. Exemplary amyloid proteins include, but are not limited to CsgA, CspA, beta-lactoglobulin, lysozyme, Sup35, or an amyloid-beta peptide. In some embodiments, the amyloid protein is a protein capable of forming beta-helical amyloid assemblies. For example, in some embodiments, the amino acid residues identified for mutation are aligned along the five repeats of the beta-helix structure of CsgA. The conjugated side-chains of aligned aromatic residues, or their non-natural or nonstandard amino acid derivatives interact to form π -stacks and induce electron delocalization.

Useful amino acids may be standard amino acids or nonstandard amino acids, canonical amino acids or non-canonical amino acids. Exemplary nonstandard amino acids include but are not limited to a histidine or phenylalanine derivative, *e.g.*, 2-thienylalanine, 3-thienylalanine acetylphenylalanine, azidophenylalanine, or an amino acid comprising a side chain comprising a monomer of a polymer such as poly(3-hexylthiophene-2,5-diyl) (P3HT) or polyaniline. In some embodiments, the histidine or phenylalanine derivative comprises a side chain similar to histidine or phenylalanine (respectively) but with improved electron delocalization or electron transfer properties.

According to a certain aspect, the microorganism produces protein fibers using a biosynthetic pathway with the protein fibers having aromatic amino acids along the fiber length and oriented to provide pi-pi stacking for electron transport. As such, the protein fibers are electrically conductive. According to one aspect, the protein fibers are curli fibers produced by bacteria. According to aspects of the present disclosure, self-assembling amyloid-based curli fibers, which often grow to be >10 μ m in length, provide long-range electron transport between bacterial cells and with abiotic electrode surfaces. Aspects of the present disclosure may utilize materials and methods described in Barnhart, M. M. & Chapman, M. R. Curli Biogenesis and Function. *Annu. Rev. Microbiol.* 60, 131–147 (2006) hereby incorporated by reference in its entirety.

Embodiments of the present disclosure are directed to engineered or genetically altered microorganisms, such as *E. coli*, that produce amyloid-based structures that have attached thereto peptide domains including metal centers that facilitate transmission of

electrons from one metal center to the next. In some embodiments, the amyloid-based structure is formed by fusion proteins comprising an amyloid polypeptide (*e.g.*, CsgA) and a redox active domain. In some embodiments, the amyloid-based structure is a curli fiber, formed for example by CsgA fused to one or more peptide domains including metal centers. In some embodiments, the amyloid-based structure comprises a plurality of fusion proteins comprising an amyloid polypeptide and a redox active domain. In some embodiments, the redox active domain comprises a metal center. In some embodiments, the redox active domain does not comprise a metal-binding peptide. In some embodiments, the amyloid-based structure comprises a series of aligned metal centers which allow electrons to move from one metal center to another, thereby making the amyloid curli fiber electrically conductive. In some embodiments, the amyloid based fiber is a curli fiber formed by fusion proteins comprising a CsgA polypeptide and a redox active domain. In some embodiments, when the CsgA units are combined into a curli fiber, the one or more peptide domains including metal centers are aligned in a manner, given the structure of the curli fiber, to allow electrons to move from one metal center to the next, thereby making the curli fiber electrically conductive. According to one aspect, conductive peptides form a close-packed network allowing for electron hopping from one subunit to the next. Electron hopping or movement from one subunit to the next occurs between protein domains with metal coordination centers such as ferritin, cytochromes or heme-like proteins. According to one aspect, the protein domains with metal coordination centers are plastocyanin or rubredoxin.

In one aspect, provided herein are amyloid based structures formed by a plurality of mutant amyloid polypeptides having a series of aligned aromatic groups. According to one aspect, the basic unit of a curli fiber, for example CsgA, includes one or more aromatic groups, for example aromatic amino acids and when the CsgA units are combined into a curli fiber. In some embodiments, the one or more aromatic groups are aligned in a manner, given the structure of the amyloid-based structure (*e.g.*, a curli fiber), to create pi-pi stacking for electron transport. Aromatic rings form a π - π (pi-pi) stacking network along the amyloid-based structures (*e.g.*, curli fibers), which induces interchain electron delocalization. In such stacked aromatic systems, charges are spread (or delocalized) across p-orbitals of the rings, and across side chains. According to one aspect, an exogenous nucleic acid encoding the fiber subunit (*e.g.*, a mutant amyloid polypeptide) bearing the one or more aromatic amino acids, *e.g.*, a CsgA subunit for a curli fiber, is introduced into the bacteria for expression. According to one aspect, the endogenous nucleic acid encoding for the fiber subunit, *e.g.*, the curli fiber subunit CsgA, may be removed from the bacterium. According to one aspect, the

endogenous nucleic acid encoding for the fiber subunit, *e.g.*, the curli fiber subunit CsgA, may be genetically altered or modified or mutated to comprise one or more aromatic amino acids. In this context, the amyloid polypeptide (*e.g.*, CsgA) may be referred to as a mutant amyloid polypeptide a modified amyloid polypeptide or an altered amyloid polypeptide (*e.g.*, mutant CsgA or modified CsgA or altered CsgA). According to one aspect, a series of aligned residues in an amyloid polypeptide (*e.g.*, CsgA) are identified and mutated to aromatic amino acids within the helix structure of the amyloid polypeptide (*e.g.*, the five pseudo-repeats of the CsgA helix structure). The aromatic residues interact and form π -stacks leading to electron delocalization and optical phenomena. According to one aspect, biofilms comprising the mutant amyloid polypeptide or amyloid-based structures comprising the mutant amyloid polypeptide (*e.g.*, CsgA-based curli fibers) are included. According to one aspect, the mutant amyloid polypeptide or amyloid-based structures comprising the mutant amyloid polypeptide (*e.g.*, mutant CsgA-based curli fibers) are isolated from bacteria. According to one aspect, the amyloid-based structures (*e.g.*, mutant CsgA-based curli fibers) are screened for electrical conductivity using, for example, colorimetric detection of redox activity, conductive AFM (current (ORCA), electrostatic (EFM) or Kelvin-probe (KPFM)) or a current-voltage response of a biofilm comprising amyloid-based structures comprising the mutant amyloid polypeptides (*e.g.*, mutant CsgA-based curli fibers) in an electrode array. According to one aspect, the amyloid-based structures comprising a mutant amyloid polypeptide (*e.g.*, mutant CsgA-based curli fibers) may also include moieties providing charged, amphiphilic or hydrophobic properties. According to one aspect, the amyloid-based structures (*e.g.*, mutant CsgA-based curli fibers) may also include natural or non-natural amino acids having moieties suitable for covalent bonding, *e.g.*, a chemical moiety suitable for a click chemistry reaction with a non-natural amino acid.

In one aspect, provided herein is a mutant amyloid polypeptide comprising one or more aligned aromatic groups, wherein said amyloid polypeptides form amyloid-based structures having modified hydrophilicity or hydrophobicity, as compared to an amyloid-based structure formed by wild-type amyloid polypeptide. In some embodiments, the mutant amyloid polypeptide comprises several amino acid replacements, whereby aromatic amino acids are incorporated into the protein such that upon folding a side of the protein comprises aromatic amino acids. In some embodiments, one side of the protein comprises aligned aromatic amino acids and the other side of the protein is unchanged. In some embodiments, the mutant amyloid polypeptide forms an amyloid-based structure with modified fluorescent ability (*e.g.*, increased fluorescent ability) as compared to an amyloid-based structure formed

of wild-type amyloid polypeptide. In some embodiments, the mutant amyloid polypeptide forms an amyloid-based structure with modified charge (*e.g.*, positive charge or negative charge) as compared to an amyloid-based structure formed of wild-type amyloid polypeptide. In some embodiments, the mutant amyloid polypeptide forms an amyloid-based structure with modified fluorescent ability (*e.g.*, increased fluorescent ability) as compared to an amyloid-based structure formed of wild-type amyloid polypeptide. In some embodiments, the mutant amyloid polypeptide forms an amyloid-based structure with modified electric conductivity ability (*e.g.*, increased conductivity ability) as compared to an amyloid-based structure formed of wild-type amyloid polypeptide.

According to one aspect, mutant CsgA modified to include one or more aromatic groups, wherein the mutant CsgA modifies the hydrophilicity/hydrophobicity of a curli fiber comprising the mutant CsgA, are provided. According to one aspect, mutant CsgA is provided where a full side of the protein is mutated to aromatic residues (for example stacks 4 and 5 together, or stacks 1, 2 and 3 together) while the other side of the protein is unchanged. This produces amphiphilic fibers compared to wild type curli fibers.

According to one aspect, mutant CsgA modified to include one or more aromatic groups, wherein the mutant CsgA modifies the fluorescent ability of a curli fiber comprising the mutant CsgA, are provided. According to one aspect, mutant CsgA is provided which produce curli fibers having increased fluorescence as compared to the fluorescence of wild type curli fibers that do not comprise mutant CsgA.

According to one aspect, mutant CsgA modified to include charged groups, wherein the mutant CsgA modifies or creates a negative or positive charge in a curli fiber comprising the mutant CsgA as compared to the charge of a curli fiber that does not comprise the mutant CsgA are provided.

According to one aspect, an amyloid polypeptide (*e.g.*, CsgA) comprising a mutation to include one or more nonstandard amino acids are provided, whereby upon incorporation of the mutant amyloid polypeptide (*e.g.*, mutant CsgA) into an amyloid based structure (*e.g.*, a curli fiber), the nonstandard amino acids provide properties to the curli fibers that are different from the properties of amyloid-based structures formed by wild-type amyloid polypeptides (*e.g.*, wild-type curli fibers). For example, in some embodiments, curli fibers comprising CsgA mutated to contain azidophenylalanine and acetylphenylalanine may be used to perform bio-orthogonal covalent chemistry (*e.g.*, click chemistry reactions using azidophenylalanine, and reactions between the ketone group of acetylphenylalanine and alkoxyamine derivatives).

According to one aspect, a bacterium as described herein can proliferate and express the nucleic acid encoding the mutant amyloid polypeptide (*e.g.*, mutant CsgA) to produce amyloid-based structure (*e.g.*, curli fibers) having aromatic amino acids along the length of the amyloid-based structure (*e.g.*, curli fiber) and oriented in a manner to provide pi-pi stacking for electron transport along the length of the fiber. In one aspect, the amyloid-based structures comprising a mutant amyloid polypeptide or a fusion protein described herein form an electrically conductive biofilm. According to one aspect, the mutant amyloid polypeptide or fusion protein described herein may be attached, connected or fused to an electrical conductivity enhancing protein to further enhance the electrical conductivity of the amyloid-based structure beyond the pi-pi stacking of the aromatic groups along the length of the amyloid-based structure. According to one aspect, the curli fiber may form an electrically conductive biofilm. According to one aspect, the mutant CsgA may be attached, connected or fused to an electrical conductivity enhancing protein to further enhance the electrical conductivity of the curli fiber beyond the pi-pi stacking of the aromatic groups along the length of the curli fiber.

Embodiments of the present disclosure are directed to methods of genetically modifying a bacterium to include a nucleic acid that encodes a mutant amyloid polypeptide (*e.g.*, a mutant CsgA protein) including one or more aromatic amino acids not present in wild type CsgA for expression within the bacterium. In some embodiments, provided herein are methods of genetically modifying a bacterium to include a nucleic acid encoding a fusion protein comprising an amyloid polypeptide and a redox active domain. Such genetic modification may be accomplished by mutation of an endogenous nucleic acid sequence encoding the wild-type amyloid polypeptide (*e.g.*, wild-type CsgA) or introduction of an exogenous nucleic acid encoding a mutant amyloid polypeptide or a fusion protein described herein (*e.g.*, a mutant CsgA) using methods known to those of skill in the art.

Aspects of the present disclosure may utilize materials and methods described in: Cherny, I. & Gazit, E. Amyloids: Not Only Pathological Agents but Also Ordered Nanomaterials. *Angew Chem Int Edit* 47, 4062–4069 (2008); Culver, J. N. *et al.* Plant virus directed fabrication of nanoscale materials and devices. *Virology* 479-480, 200–212 (2015); Fischlechner, M. & Donath, E. Viruses as Building Blocks for Materials and Devices. *Angew Chem Int Edit* 46, 3184–3193 (2007); Ulijn, R. V. & Woolfson, D. N. Peptide and protein based materials in 2010: from design and structure to function and application. *Chem. Soc. Rev.* 39, 3349 (2010); Rodríguez-Ropero, F., Zanuy, D., Assfeld, X. & Alemán, C. Modeling an Electronic Conductor Based on Natural Peptide Sequences. *Biomacromolecules* 10, 2338–

2343 (2009); Hamley, I. W. *et al.* Self-Assembly of a Designed Amyloid Peptide Containing the Functional Thienylalanine Unit. *J. Phys. Chem. B* 114, 10674–10683 (2010), each of which are hereby incorporated by reference in their entirety, as synthetic biomolecular systems, such as amyloids, viruses, and those derived from other self-assembling protein scaffolds, offer embodiments of nanoscale spacing of chemical functional groups, including those particularly suited for electron transport and not represented in the naturally occurring amino acids.

According to the present disclosure, such systems can be modified with one or more molecules in order to enhance their electron transport properties. Aspects of the present disclosure may utilize Vargas, M. *et al.* Aromatic Amino Acids Required for Pili Conductivity and Long-Range Extracellular Electron Transport in *Geobacter sulfurreducens*. *mBio* 4, e00105–13–e00105–13 (2013); Malvankar, N. S., Tuominen, M. T. & Lovley, D. R. Comment on ‘On electrical conductivity of microbial nanowires and biofilms’ by S. M. Strycharz-Glaven, R. M. Snider, A. Guiseppi-Elie and L. M. Tender, *Energy Environ. Sci.*, 2011, 4, 4366. *Energy Environ. Sci.* 5, 6247 (2012); Strycharz-Glaven, S. M. & Tender, L. M. Reply to the ‘Comment on “On electrical conductivity of microbial nanowires and biofilms”’ by N. S. Malvankar, M. T. Tuominen and D. R. Lovley, *Energy Environ. Sci.*, 2012, 5, DOI: 10.1039/c2ee02613a. *Energy Environ. Sci.* 5, 6250 (2012); Strycharz-Glaven, S. M., Snider, R. M., Guiseppi-Elie, A. & Tender, L. M. On the electrical conductivity of microbial nanowires and biofilms. *Energy Environ. Sci.* 4, 4366 (2011) describing embodiments of “metallic-like” conductivity that arises from pi-stacked aromatic groups. According to one exemplary aspect, the conductive fibers and biofilms disclosed herein may be combined with other bacteria to produce hybrid biofilms with enhanced long-range conductivity so as to provide efficient bioelectrosynthetic systems and microbial fuel cells. Other applications of conductive curli fibers include biosensors, fabrication of conductive electrodes and materials (*e.g.*, free-standing electrodes, solution-processable electrodes, biocompatible electrodes, *etc.*), and fabrication of composites with other nanomaterials.

Further features and advantages of certain embodiments of the present invention will become more fully apparent in the following description of embodiments and drawings thereof, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing and other features and advantages of the present embodiments will be more fully understood from the following detailed description of illustrative embodiments

taken in conjunction with the accompanying drawings in which:

Figs. 1A-1B depict a biosynthetic conductive nanowire concept overview. **Fig. 1A** is a comparison between the electrically conductive systems produced by natural organisms, and chemical synthesis or recombinant expression. **Fig. 1B** schematically depicts variants of the major curli subunit (CsgA) containing periodically spaced aromatic amino acid residues (indicated with “*”). During biofilm formation, these variants will be secreted and assembled into functional curli fibers containing the aromatic side chains aligned to yield a network of conductive nanowires.

Figs. 2A-2D depict the Biofilm Integrated Nanofiber Display (BIND) as a method for analyzing curli fibers including mutant CsgA having one or more aromatic amino acids or aromatic group side chains. **Fig. 2A** depicts $\Delta csgA$ strain of *E. coli* transformed with a plasmid encoding for CsgA appended to a heterologous peptide domain. These domains are displayed throughout the biofilm and imbue it with non-natural functions. **Fig. 2B** depicts images of curli fibers displaying a silver binding peptide that can template the growth of silver nanoparticles (*middle, right*), whereas the wild-type curli network cannot (*left*). **Fig. 2C** depicts images of curli fibers displaying a steel binding peptide (*right*) that enable biofilm adhesion to a steel coupon, whereas cells producing wild-type curli (*middle*) or no curli (*left*) do not adhere. **Fig. 2D** schematically depicts curli fibers displaying a conjugation domain that can site selectively immobilize proteins or arbitrary size, including enzymes.

Figs. 3A-3B schematically depict a proposed plan for CsgA engineering. **Fig. 3A** is a schematic of a CsgA protein sequence. Alignment shows pseudo-repeat sequences with sites of proposed mutations (Series 1, 2). All five residues will be mutated simultaneously to one of the following aromatic amino acids: tyrosine, tryptophan, histidine, or 3-thienylalanine (through non-standard amino acid incorporation). **Fig. 3B** depicts a homology model of CsgA protein depicting a histidine mutant from Series 2. Adjacent CsgA monomers in an assembled curli fiber are depicted. Histidine residues are also depicted. The average distance between neighboring histidine residues is 5.1 Å.

Figs. 4A and 4B depict CsgA with a His-tag in the pET1d plasmid and the corresponding nucleic acid and amino acid sequences. **Fig. 4A** is a schematic representation of CsgA with his-tag in the pET21d plasmid. **Fig. 4B** is a schematic representation and corresponding nucleic acid and amino acid sequences of wild-type CsgA with a C-terminal His-tag. The depicted open reading frame also includes a SEC signal peptide, an N22 peptide, and a linker serine-glycine linker sequence (SEQ ID NOs: 1 and 2).

Figs. 5A, 5B, 5C, and 5D schematically depict a biomimetic approach for the

synthesis of conductive curli nanofibers.

Figs. 6A-6B shows Congo Red binding to CsgA mutants and fusions.

Fig. 7 shows electron microscopy of curli fibers produced by mutant and control bacteria.

Fig. 8 depicts the amounts of curli fibers produced by mutant and control bacteria, as quantitated by a Congo Red binding assay.

Fig. 9 depicts the amounts of curli fibers produced by mutant bacteria that express CsgA under the control of an inducible promoter.

Fig. 10 depicts a Congo Red spot test showing curli fiber formation by mutant and control bacteria.

Fig. 11 depicts the fluorescence intensity of wild-type and mutant curli fibers.

Fig. 12 shows electron microscopy of mutant and control curli fibers that have been isolated and purified.

Fig. 13 shows the residual impurities after purification of mutant and control curli fibers.

Figs. 14A and 14B depicts the purification of curli nanofiber thin films, and measurement of thin film electrical properties.

Figs. 15A and 15B depict dropcast films of curli fibers, or single curli fibers on substrates and electrodes. **Fig. 15A** depicts wild-type curli fibers dropcast on plasma-treated silicon wafers at decreasing concentrations (from left to right) of 1.5, 0.75, 0.15 and 0.015 mg/mL, from 1:1 HFIP:TFA mixtures. Prior to dropcasting, fibers were sonicated for 1 hr in HFIP and TFA. Samples were imaged using scanning electron microscopy (SEM). **Fig. 15B** depicts high magnification scanning electron microscopy (SEM) images showing single fibers for diluted samples.

Figs. 16A and 16B depict (SEM) images of dropcast films formed using dilute solutions of curli fibers comprising CsgA fused to either ferritin domain, plastocyanin or rubredoxin (**Fig. 16A**) or curli fibers comprising mutant CsgA (**Fig. 16B**) can be dissolved/disassembled in HFIP/TFA and reassembled on a substrate. SEM images of dispersed mutant and fusion curli fibers, after dissolution/disasassembly in 1:1 HFIP:TFA, and reassembly by dropcasting and solvent evaporation on a silicon substrate are shown.

Figs. 17A-17C depict curli fibers comprising CsgA fused to a 36 amino acid linker and-plastocyanin and 10 mM copper nitrate on 10 μ m polycarbonate membrane. Energy-dispersive X-ray spectroscopy (EDX) of CsgA-plastocyanin fusions showing the presence of copper in the samples. EDX was used to perform elemental mapping on fibers, after

filtration purification on polycarbonate filter membranes. Copper was detected at a weight percent of 0.49 for CsgA-plastocyanin fusions. Samples were coated with 5 nm of Pt/Pd prior to imaging. Fe signal is detected mainly in the pores of the filter membrane and comes from the SEM holder.

Figs. 18A and 18B depict wild-type curli fiber plus NiNTA on 10 μ m polycarbonate membrane. EDX of wild-type curli fibers with a His-tag that were purified via filtration, and incubated with NiNTA-Au nanoparticles. Copper was not detected in this sample, but gold (from the nanoparticles) was detected. Samples were coated with 5 nm of Pt/Pd prior to imaging. Residual salts from rinses with guanidine hydrochloride during filtration purification can explain the Cl signal.

Figs. 19A and 19B depict SEM images of dropcast films formed using curli fibers comprising CsgA fused to plastocyanin overlayed on commercial Micrux interdigitated electrodes. Samples were coated with 5nm of Pt/Pd prior to imaging. **Fig. 19A** depicts the top surface morphology of the film. **Fig 19B** depicts the scratch in the film at the film-electrode interface showing the fibrous cross-section and the good contact between the fibers and the gold electrode.

Figs. 20A and 20B depict SEM images of dropcast films formed using curli fibers comprising CsgA fused to plastocyanin on commercial Micrux interdigitated electrodes. Samples were not coated with Pt/Pd prior to imaging. **Fig. 20A.** depicts CsgA-plastocyanin fibers dropcast at a dilute 0.1 mg/mL concentration on interdigitated electrodes. SEM images show fibrous surface morphology. **Fig. 20B** depicts CsgA-plastocyanin fibers dropcast at 10 mg/mL on interdigitated electrodes. Full coverage of the electrode is observed. Fibers form a thick film.

Figs. 21A and 21B depict current-voltage curves for curli fibers comprising CsgA-fused to plastocyanin relative to wild-type curli fibers and control proteins. Protein fibers and control proteins at 5 mg/mL in 1:1 HFIP:TFA mixture were dropcast on commercial Micrux interdigitated electrodes with 10 μ m spacing. Prior to dropcasting, electrodes were cleaned with isopropanol and were plasma-treated. After drying of the fiber solution, electrodes were rinsed with deionized water and dried in air. A probe station was used to measure current-voltage curves. **Fig. 21A** depicts the current-voltage curves for curli fibers comprising CsgA fused to plastocyanin (“plastocyanin”), hexafluoro-2-propanol: trifluoroacetic acid solvent control (“HFIP:TFA”); curli fibers comprising wild-type CsgA (“WT”); milk control (“Milk”); or bovine serum albumin control (“BSA”); or non-coated electrodes (“Blank”). **Fig. 21B** depicts current-voltage curves for control proteins: curli fibers comprising wild-type

CsgA (“WT”); milk control (“Milk”); or bovine serum albumin control (“BSA”).

Figs. 22A-22C depict additional current-voltage curves for curli fibers comprising CsgA fused to plastocyanin. **Fig. 22A** depicts a current-voltage curve of a scan from -2 V to +2 V. **Fig. 22B** depicts a current-voltage curve of a scan from -20 V to +20 V. **Fig. 22C** depicts a current-voltage curve of a scan from 0 to 20 V, and back from 20 V to 0 V showing little hysteresis between the forward and reverse scans.

Fig. 23 depicts current-voltage curves for curli fibers comprising CsgA fused to plastocyanin and for curli fibers comprising CsgA fused to ferritin relative to wild-type curli fibers and control proteins dropcast on commercial Micrux electrodes (10 μ m spacing) from 1:1 hexafluoro-2-propanol: trifluoroacetic acid solvent control (HFIP:TFA), dried and rinsed with water. Curli fibers or control proteins were dropped at the indicated concentration (where indicated). Current-voltage curves were measured from 0 V to 20 V using a probe station. Current-voltage curves are shown for curli fibers comprising CsgA fused to plastocyanin (“plasto”) dropcast at the indicated concentration; curli fibers comprising CsgA fused to ferritin (“Ferri”); curli fibers comprising wild-type CsgA (“WT”); or bovine serum albumin control (“BSA”). Where the concentration of the curli fiber or protein is not specified, protein were dropcast at 5 mg/mL. Concentrations of curli fiber comprising CsgA fused to plastocyanin below 5 mg/mL caused a decrease in current.

Fig. 24 depicts current-voltage curves of various pi-stack-containing curli fibers. 5 mg/mL solution of curli fiber in 1:1 HFIP:TFA were dropcast on commercial Micrux interdigitated electrodes (10 μ m spacing), rinsed with water after drying, and dried in air. Current-voltage curves were measured from 0 V to 20 V using a probe station.

Fig. 25 depicts current voltage curves of curli fibers comprising CsgA mutant containing Trp at positions of stack 1 (“Trp stack 1”) relative to curli fibers comprising wild-type CsgA (“WT”) and BSA control (“BSA”). 5 mg/mL solution of fibers in 1:1 HFIP:TFA were dropcast on commercial micrux interdigitated electrodes (10 μ m spacing), rinsed with water after drying, and dried in air. Current-voltage curves were measured from 0 V to 20 V using a probe station.

DETAILED DESCRIPTION

Aspects of the present disclosure are directed to the use of a bacterium capable of producing an amyloid-based structure (*e.g.*, a bacterium with a curli fiber-producing capability) to produce amyloid-based structures (*e.g.*, curli fibers) having mutant amyloid units (*e.g.*, a fusion protein or mutant amyloid polypeptide described herein including mutant

CsgA units) which comprise one or more aromatic groups or aromatic side chains or aromatic amino acids. The aromatic groups are aligned along the length of the amyloid-based structure (*e.g.*, curli fiber) to allow for electrical conductance or for electron transporting via pi-pi stacking.

According to one aspect, methods of the present invention use the curli fiber production systems of a bacterium, such as *E. coli*. Curli fibers are the primary proteinaceous structural component of *E. coli* biofilms. They are highly robust functional amyloid nanofibers with a diameter of ~4-7 nm that exist as extended tangled networks encapsulating the cells. Curli fibers are formed from the extracellular self-assembly of CsgA, a small secreted 13-kDa protein. Chapman, M. R. *et al.* Role of *Escherichia coli* curli operons in directing amyloid fiber formation. *Science* 295, 851–855 (2002). A homologous outer-membrane protein, CsgB, nucleates CsgA assembly and also anchors the nanofibers to the bacterial surface. Detached curli fibers can also exist as non-cell associated structural components of the ECM. The curli genes exist as two divergently transcribed operons (*csgBAC* and *csgDEFG*), whose seven products mediate the structure (CsgA), nucleation (CsgB), processing (CsgE, F), secretion (CsgC, G), and direct transcriptional regulation (CsgD) of curli nanofibers. This curli secretion system is considered a distinct secretion system of its own in gram-negative bacterium and is named the Type-VIII secretion system (T8SS). See Desvaux *et al.*, *Trends Microbiol.* 17, 139-45 (2009) hereby incorporated by reference in its entirety. According to one aspect, other useful bacteria with suitable secretions systems known to those of skill in the art may be used to produce the electrically conductive curli fibers of the present disclosure.

As used herein, “CsgA” refers to the major structural subunit of curli. The sequences of CsgA and its homologs are known in a number of species, *e.g.* the sequence of *E. coli* CsgA is known (encoded by *csgA* (NCBI Gene ID NO: 949055); SEQ ID NO: 44 (polypeptide)).

CsgA polypeptide (NCBI Ref Seq: NP_415560)

mkllkvaiaaivfsgsalagvvpqyggggnhgggggnsgpnseiniyqygggnsalalqtd
arnsdltitqhgnggadvgqgsddssidltqrgfgnsatldqwnqknsemtvkqfgggnga
avdqtasnsnvntqvgfgnnatahgy (SEQ ID NO: 44)

In some embodiments, “CsgA” refers to *E. coli* CsgA. In some embodiments, “CsgA” refers to a polypeptide having at least 80% homology to SEQ ID NO: 44 (*e.g.*, 80% or greater homology, 90% or greater homology, or 95% or greater homology), *e.g.* naturally occurring mutations or variants of CsgA, homologs of CsgA, or engineered mutations or

variants of CsgA. In some embodiments, CsgA refers to a polypeptide comprising an amino acid sequence having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the amino acid sequence of SEQ ID NO: 44.

According to one aspect, self-assembling protein domains referred to herein as amyloid polypeptides, such as CsgA, are used to generate amyloid-based structures (*e.g.*, curli fibers) including a plurality of aromatic groups arranged in pi-pi stacking to promote electrical conductivity of the amyloid-based structure (*e.g.*, curli fiber) or electron transport along the amyloid-based structure (*e.g.*, curli fiber). π -stacks can occur when conjugated molecules are a few angstroms apart (*e.g.*, between 4 to 7 angstroms apart). For example, the spacing between the beta sheets of CsgA is in the range of 4.6 angstroms, which allows for proper spacing between the aromatic side chains to form a π -stack (see, *e.g.*, Duelholm *et al.* (2011) *Biochemistry* 50(39): 8281-90. Various types of π -stack geometric configurations are formed using the mutant amyloid polypeptides described herein (*e.g.*, mutant CsgA polypeptides) described herein. In some embodiments, mutation of an amyloid polypeptide (*e.g.*, CsgA) with at least one amino acid having an aromatic residue results in sandwich-type π -stacks (also known as parallel rings). In some embodiments, mutation of an amyloid polypeptide (*e.g.*, CsgA) with at least one amino acid having an aromatic residue results in t-shaped π -stacks (also known as perpendicular rings). In some embodiments, mutation of an amyloid polypeptide (*e.g.*, CsgA) with at least one amino acid having an aromatic residue results in parallel-displaced π -stacks (also known as offset rings). π -stacks can be formed using any combination of amino acid residues having an aromatic side chain. In some embodiments, the π -stack is formed using amino acid residues of the same type. In some embodiments, the π -stack is formed using amino acid residues of different types (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9 types of amino acid residues (*e.g.*, natural or non-natural amino acid residues)). In some embodiments, the amyloid-based structures (*e.g.*, curli fibers) can be deposited on pre-fabricated substrates (*e.g.*, interdigitated electrodes) to facilitate electrical conductivity measurements. In some embodiments, the amyloid-based structures (*e.g.*, curli fibers) are dissolved and disassembled in a solvent and dropcast onto a pre-fabricated substrate. In some embodiments, electrodes can be drawn directly onto filter membranes onto which the amyloid-based structures (*e.g.*, curli fibers) have been directly purified, as described herein.

According to one aspect, bacteria are modified to include a nucleic acid encoding a mutant amyloid polypeptide (*e.g.*, a mutant CsgA) having one or more aromatic groups or aromatic side chains or aromatic amino acids. The nucleic acid encoding a mutant amyloid

polypeptide (*e.g.*, a mutant CsgA) may be exogenous and introduced into the bacterium using methods known to those of skill in the art. The nucleic acid encoding a mutant amyloid polypeptide (*e.g.*, a mutant CsgA) may result from mutation of the endogenous nucleic acid encoding an amyloid polypeptide (*e.g.*, CsgA) using methods known to those of skill in the art. According to one aspect, the modified bacteria secrete the mutant amyloid polypeptide (*e.g.*, mutant CsgA) which results in the production of an amyloid-based structure (*e.g.* a curli fiber), and optionally the production of a biofilm comprising the mutant amyloid polypeptide (*e.g.*, mutant CsgA) being the subunit of the curli fiber. According to one aspect, the electrically conductive amyloid-based structures (*e.g.*, curli fibers) are produced by engineered or non-naturally occurring bacteria. According to one aspect, methods are provided for engineering a bacteria to produce the electrically conductive extracellular amyloid fibers. After secretion, the mutant amyloid polypeptide (*e.g.*, mutant CsgA) is nucleated to form an amyloid at the cell surface, and then continues to polymerize into long fibers that eventually encapsulate the cells and provide the biofilm with structural support, with the amyloid-based structure (*e.g.*, curli fiber) and the resulting biofilm being electrically conductive.

Aspects of the present disclosure are directed to a method of producing a genetically-modified bacterium including genetically altering a bacterium to include a nucleic acid sequence encoding a mutant amyloid polypeptide (*e.g.*, a CsgA protein) having a series of aligned aromatic groups, wherein the nucleic acid sequence is under the control of a promoter to express the mutant amyloid polypeptide (*e.g.*, CsgA protein). According to one aspect, the bacterium is *E. coli*. In one embodiment, the bacterium is non-pathogenic. In one embodiment, the bacterium is pathogenic. In one embodiment, the bacterium is an attenuated bacterium.

According to one aspect, the aromatic group is present on a standard amino acid or a nonstandard amino acid. According to one aspect, the aromatic group is present on a standard amino acid selected from the group consisting of phenylalanine, tryptophan, tyrosine and histidine. According to one aspect, the aromatic group is present on a nonstandard amino acid selected from the group consisting of 2-thienylalanine, 3-thienylalanine, acetylphenylalanine or azidophenylalanine. According to one aspect, the nucleic acid sequence further encodes a redox active domain (*e.g.*, one or more of a metalloprotein, redox enzyme, binding domain or stimuli-responsive peptide) fused to the mutant amyloid polypeptide (*e.g.*, a mutant CsgA protein). According to one aspect, the nucleic acid sequence further encodes one or more of plastocyanin, an oxygen-carrier protein (*e.g.*,

hemoglobin, myoglobin, hemerythrin, and hemocyanin), a microperoxidase, a cytochrome, a copper-binding protein (*e.g.*, azurin), an iron-binding protein (*e.g.*, ferritin, lactoferrin, and transferrin), a metal-binding domain (*e.g.*, a gold-binding domain), a metalloenzyme, or rubredoxin, or fragments thereof, fused to the mutant amyloid polypeptide (*e.g.*, a CsgA protein).

In other aspects, provided herein are methods of producing a genetically-modified bacterium whereby the bacterium is genetically altered to include a nucleic acid sequence encoding fusion protein, wherein the fusion protein comprises a redox active domain (*e.g.*, one or more of a metalloprotein, redox enzyme, binding domain or stimuli-responsive peptide) fused to an amyloid polypeptide (*e.g.*, a wild type or mutant CsgA protein (*e.g.*, a mutant CsgA protein described herein)). Exemplary redox active domains are described herein.

Aspects of the present disclosure are directed to a method of making an electrically conductive biofilm comprising proliferating a genetically-altered bacterial cell comprising a nucleic acid sequence encoding a mutant amyloid polypeptide (*e.g.*, a mutant CsgA protein) having a series of aligned aromatic groups to produce a population of genetically-altered bacterial cells expressing the nucleic acid sequence and forming a biofilm from curli fibers formed from a plurality of the mutant amyloid polypeptides (*e.g.*, mutant CsgA proteins), wherein the curli fibers have a series of aligned aromatic groups forming electrically conductive pi-pi stacking along the length of the curli fibers. In one embodiment, the bacterium is *E. coli*. In one embodiment, the bacterium is non-pathogenic. In one embodiment, the bacterium is pathogenic. According to one aspect, the aromatic group is present on a standard amino acid or a nonstandard amino acid. According to one aspect, the aromatic group is present on a standard amino acid selected from the group consisting of phenylalanine, tryptophan, tyrosine and histidine. According to one aspect, the aromatic group is present on a nonstandard amino acid selected from the group consisting of 2-thienylalanine, 3-thienylalanine, acetylphenylalanine or azidophenylalanine. According to one aspect, the nucleic acid sequence further encodes a redox active domain (*e.g.*, one or more of a metalloprotein, redox enzyme, binding domain or stimuli-responsive peptide) fused to the mutant amyloid protein (*e.g.*, the mutant CsgA protein). According to one aspect, the nucleic acid further encodes one or more of plastocyanin, an oxygen-carrier protein (*e.g.*, hemoglobin, myoglobin, hemerythrin, and hemocyanin), a microperoxidase, a cytochrome, a copper-binding protein (*e.g.*, azurin), an iron-binding protein (*e.g.*, ferritin, lactoferrin, and transferrin), a metal-binding domain (*e.g.*, a gold-binding domain), a metalloenzyme, or

rubredoxin, or fragments thereof, fused to the mutant amyloid polypeptide (*e.g.*, a mutant CsgA protein described herein).

Aspects of the present disclosure are directed to a method of making electrically conductive amyloid-based structures (*e.g.*, curli fibers) from a genetically-modified bacterium including providing the genetically modified bacterium in culture media conditions, wherein the genetically-modified bacterium includes a nucleic acid sequence encoding a mutant amyloid polypeptide (*e.g.*, a mutant CsgA protein) having a series of aligned aromatic groups, wherein the nucleic acid sequence is under the control of a promoter to express the mutant amyloid polypeptide (*e.g.*, a mutant CsgA protein), expressing the nucleic acid sequence to produce the mutant amyloid polypeptide (*e.g.*, the mutant CsgA protein), and forming an amyloid-based structure (*e.g.*, a curli fiber) from a plurality of the mutant amyloid polypeptides, wherein the amyloid-based structures (*e.g.*, curli fibers) have a series of aligned aromatic groups forming electrically-conductive pi-pi stacking along the length of the amyloid-based structures (*e.g.*, curli fibers). According to one aspect, the bacterium is *E. coli*. According to one aspect, the bacterium is non-pathogenic. According to one aspect, the aromatic group is present on a standard amino acid or a nonstandard amino acid. According to one aspect, the aromatic group is present on a standard amino acid selected from the group consisting of phenylalanine, tryptophan, tyrosine and histidine. According to one aspect, the aromatic group is present on a nonstandard amino acid selected from the group consisting of 2-thienylalanine, 3-thienylalanine, acetylphenylalanine or azidophenylalanine. According to one aspect, the nucleic acid sequence further encodes a redox active domain (*e.g.*, one or more of a metalloprotein, redox enzyme, binding domain or stimuli-responsive peptide) fused to the mutant amyloid polypeptide (*e.g.*, mutant CsgA protein). According to one aspect, the nucleic acid further encodes one or more of plastocyanin, an oxygen-carrier protein (*e.g.*, hemoglobin, myoglobin, hemerythrin, and hemocyanin), a microperoxidase, a cytochrome, a copper-binding protein (*e.g.*, azurin), an iron-binding protein (*e.g.*, ferritin, lactoferrin, and transferrin), a metal-binding domain (*e.g.*, a gold-binding domain), a metalloenzyme, or rubredoxin, or fragments thereof, fused to the mutant CsgA protein.

Exemplary amino acid sequences of redox active domains (*e.g.*, metalloproteins or domains thereof) are provided below:

Plastocyanin, PetE - *Prochlorococcus marinus* subsp. *marinus* str. CCMP1375 (NCBI Reference Sequence: NP_875473.1)

MISSLRALSACFALLLVLAFGVASAQAKTVEVKLGTDAGMLAFEPSSVTISTGDSVKFVNN
KLAPHNAVFEHGHEELSHPDIAFAPGESWQETFTAGTYDYCEPHRGAGMVGKVVVN (SEQ
ID NO: 55)

Ferritin, mitochondrial [*Cricetulus griseus* (Chinese hamster)] (NCBI Reference Sequence:
XP_003507127)

MLSGFWFFSKHIGPALMSLPRVLHRCTVPQCLASRYPLLPASPRLLASVASSQGSDGTARV
RHNHFHPDSEAAINHQINMELYASYVYLSMAYYFSRDDVALYNFSKSFRLRQSLEEREHAELM
KLQNQRGGRIQLQDIKKPEQDDWESGLRAMECALLLEKSVNQSLDLHTLASEKGDPHLCDF
LETHYLNQVKSIELGDHVNHLVTMGAPAVGLAEYLFDKHTLGSESKH (SEQ ID NO: 56)

Rubredoxin [*Pyrococcus furiosus* DSM 3638] (NCBI Reference Sequence:
WP_011012426.1)

MAKWVCKICGYIYDEDAGDPDNGISPGTKFEELPDDWVCPICGAPKSEFEKLED (SEQ ID
NO: 57)

Microperoxidase-8 (MP-8)
AQCHTVE (SEQ ID NO: 58)

Microperoxidase-9 (MP-9)
AQCHTVEK (SEQ ID NO: 59)

Microperoxidase-11 (MP-11)
QKCAQCHTVE (SEQ ID NO: 60)

In some embodiments, a polypeptide described herein (*e.g.*, a fusion protein or a mutant amyloid polypeptide described herein) comprises a redox active domain fused to the C-terminal of an amyloid polypeptide (*e.g.*, a wild-type or mutant CsgA protein described herein). In some embodiments, a polypeptide (*e.g.*, a fusion protein or a mutant amyloid polypeptide described herein) comprises a redox active domain fused to the N-terminal of an amyloid polypeptide (*e.g.*, a wild-type or mutant CsgA protein described herein). In some embodiments, a polypeptide described herein comprises a linker disposed between the amyloid polypeptide and the polypeptide to which it is fused (*e.g.*, a redox active domain). In some embodiments, a polypeptide described herein comprises a linker disposed between the amyloid polypeptide and the polypeptide to which it is fused (*e.g.*, a redox active domain). In some embodiments, the linker is a flexible linker. In some embodiments, the flexible linker

is a glycine-serine flexible linker. In some embodiments, the glycine-serine flexible linker is GSGGSGGSGGSG (SEQ ID NO: 61). In some embodiments, the linker is an inflexible linker. In some embodiments, the linker is between 5 and 100 amino acids in length. In some embodiments, the linker is 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acids in length. In some embodiments, the linker is 12, 24, 36, 48, 60, 72, or 96 amino acid residues in length. In some embodiments, the linker is 36 amino acid residues in length. In some embodiments, the linker is a linker described herein. The linker length may be modified to affect electron transfer within an amyloid-based structure (*e.g.*, a curli fiber).

In some embodiments, a polypeptide described herein (*e.g.*, a fusion protein or a mutant amyloid polypeptide described herein) comprises a signal sequence to facilitate transport of the protein to the extracellular milieu. In some embodiments, the signal peptide is disposed at the N-terminal of the polypeptide. Any signal peptide known in the art may be used to facilitate the export of the protein to the extracellular milieu. In some embodiments, the signal peptide is a Sec signal peptide. In some embodiments, the signal peptide is cleaved. In some embodiments, the signal peptide is not cleaved.

In some embodiments, a polypeptide described herein (*e.g.*, a fusion protein or a mutant amyloid polypeptide described herein) comprises a peptide tag. In some embodiments, the linker is a glycine-serine linker. In some embodiments, the peptide tag is a His-tag. In some embodiments, the peptide tag is a myc-tag. In some embodiments, the peptide tag is a GST-tag. In some embodiments, the peptide tag is a V5 tag. The peptide tag may be disposed at the N-terminal, C-terminal or within the mutant amyloid polypeptide or fusion protein described herein. In some embodiments, the mutant amyloid polypeptide (*e.g.*, mutant CsgA protein) and/or fusion protein (*e.g.*, CsgA protein fused to a redox active domain) described herein further comprises a linker disposed between the peptide tag and the mutant amyloid polypeptide and/or between the fusion protein and the peptide tag. In some embodiments, the linker is a flexible linker. In some embodiments, the linker is a non-flexible linker. Any linker described herein may be disposed between the peptide tag and the polypeptides described herein.

Methods of purifying curli fibers are known in the art and may be used to purify a curli fiber comprising a mutant CsgA protein described herein. Curli fiber purification methods include, for example, affinity chromatography purification (*e.g.* poly-histidine-tagged-based affinity purification), purification from SDS-PAGE gels, and salt precipitation and sequential differential centrifugation (see, *e.g.*, Chapman *et al.*, *Science*, 295(5556):851-

855 (2002); Zhou *et al.*, Experimental Manipulation of the Microbial Functional Amyloid Called Curli. In *Bacterial Cell Surfaces: Methods and Protocols*, Delcour, H. A., Ed. Humana Press: Totowa, NJ, 2013; pp 53-75; Collinson *et al.*, *J. Bact.* 173(15): 4773-81 (1991); Chapman *et al.*, *Science*, 295(5556):851-5 (2002), which are incorporated herein by reference in their entirety). In addition, curli fibers may be purified using vacuum filtration, as described, for example, in Dorval Courchesne *et al.* *ACS Biomaterials Science & Engineering* Article ASAP, doi: 10.1021/acsbiomaterials.6b00437 (2016), the entire contents of which are incorporated herein by reference.

Aspects of the present disclosure are directed to a genetically-modified bacterium comprising a nucleic acid sequence encoding a mutant amyloid polypeptide (*e.g.*, a mutant CsgA protein) having a series of aligned aromatic groups, wherein the nucleic acid sequence is under the control of a promoter to express the mutant amyloid polypeptide. Aspects of the present disclosure are directed to a non-naturally-occurring mutant amyloid polypeptides (*e.g.*, a mutant CsgA protein) having a series of aligned aromatic groups. Aspects of the present disclosure are directed to a non-naturally occurring amyloid-based structure (*e.g.*, a curli fiber) formed from a plurality of mutant amyloid polypeptides (*e.g.*, mutant CsgA proteins) forming a series of aligned aromatic groups, wherein the amyloid-based structure (*e.g.*, curli fiber) has a series of aligned aromatic groups forming electrically-conductive pi-pi stacking along the length of the amyloid-based structures. Aspects of the present disclosure are directed to a nucleic acid sequence encoding a non-naturally occurring mutant amyloid polypeptide (*e.g.*, a CsgA protein) having a series of aligned aromatic groups. Aspects of the present disclosure are directed to a vector comprising a nucleic acid sequence encoding a non-naturally occurring mutant amyloid polypeptide (*e.g.*, a mutant CsgA protein) having a series of aligned aromatic groups. Aspects of the present disclosure are directed to a bacterium including a nucleic acid sequence encoding a non-naturally occurring mutant amyloid polypeptide (*e.g.*, a mutant CsgA protein) having a series of aligned aromatic groups. Aspects of the present disclosure are directed to a bacterium including a vector comprising a nucleic acid sequence encoding a non-naturally occurring mutant amyloid polypeptide (*e.g.*, a mutant CsgA protein) having a series of aligned aromatic groups. Aspects of the present disclosure are directed to a bacterium expressing a nucleic acid sequence encoding a non-naturally occurring mutant amyloid polypeptide (*e.g.*, a mutant CsgA protein) having a series of aligned aromatic groups. Aspects of the present disclosure are directed to a biofilm including a bacterium expressing a nucleic acid sequence encoding a

non-naturally occurring mutant amyloid polypeptide (*e.g.*, a mutant CsgA protein) having a series of aligned aromatic groups.

A "vector" includes a nucleic acid construct designed for delivery to a host cell or transfer between different host cells. A vector can be viral or non-viral. Many vectors useful for transferring genes into target cells are available, *e.g.* the vectors may be episomal, *e.g.*, plasmids, virus derived vectors or may be integrated into the target cell genome, through homologous recombination or random integration. In some embodiments, a vector can be an expression vector. An "expression vector" can be a vector that has the ability to incorporate and express heterologous nucleic acid fragments in a cell. An expression vector may comprise additional elements, for example, the expression vector may have two replication systems, thus allowing it to be maintained in two organisms. The nucleic acid incorporated into the vector can be operatively linked to an expression control sequence when the expression control sequence controls and regulates the transcription and translation of that polynucleotide sequence.

In some embodiments, a nucleic acid encoding a mutant amyloid polypeptide (*e.g.*, a mutant CsgA protein) alone or being connected to a redox active domain (*e.g.*, a metalloprotein, redox enzyme, binding domain or stimuli-responsive peptide) can be present within a portion of a plasmid. In some embodiments, a nucleic acid encoding a fusion protein comprising an amyloid polypeptide fused to a redox active domain (*e.g.*, a metalloprotein, redox enzyme, binding domain or stimuli-responsive peptide) can be present within a portion of a plasmid. Plasmid vectors can include, but are not limited to, pBR322, pBR325, pACYC177, pACYC184, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which is hereby incorporated by reference), pQE, pIH821, pGEX, pET series (see Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," Gene Expression Technology, vol. 185 (1990), which is hereby incorporated by reference in its entirety).

A "viral vector" may be a nucleic acid vector construct that includes at least one element of viral origin and has the capacity to be packaged into a viral vector particle. The viral vector can contain a transgenic gene in place of non-essential viral genes. The vector and/or particle may be utilized for the purpose of transferring any nucleic acids into cells either *in vitro* or *in vivo*. Numerous viral vectors are known in the art and can be used as carriers of a nucleic acid into a cell, *e.g.* lambda vector system gt11, gt WES.tB, Charon 4.

In some embodiments, the nucleic acid encoding a polypeptide described herein can

be constitutively expressed. In some embodiments, the nucleic acid encoding a polypeptide described herein can be operably-linked to a constitutive promoter. In some embodiments, the nucleic acid encoding a polypeptide described herein can be operably-linked to a constitutive promoter. In some embodiments, the nucleic acid encoding a polypeptide described herein can be inducibly-expressed. In some embodiments, the nucleic acid encoding a a polypeptide described herein can be can be operably-linked to an inducible promoter. In some embodiments, the nucleic acid encoding a polypeptide described herein can be can be operably linked to an inducible promoter. In some embodiments, the nucleic acid encoding a polypeptide described herein can be can be operably linked to a native CsgA promoter.

An "inducible promoter" may be one that is characterized by initiating or enhancing transcriptional activity when in the presence of, influenced by, or contacted by an inducer or inducing agent than when not in the presence of, under the influence of, or in contact with the inducer or inducing agent. An "inducer" or "inducing agent" may be endogenous, or a normally exogenous compound or protein that is administered in such a way as to be active in inducing transcriptional activity from the inducible promoter. In some embodiments, the inducer or inducing agent, *e.g.*, a chemical, a compound or a protein, can itself be the result of transcription or expression of a nucleic acid sequence (*e.g.*, an inducer can be a transcriptional repressor protein), which itself may be under the control of an inducible promoter. Non-limiting examples of inducible promoters include but are not limited to, the lac operon promoter, a nitrogen-sensitive promoter, an IPTG-inducible promoter, a salt-inducible promoter, and tetracycline, steroid-responsive promoters, rapamycin responsive promoters and the like. Inducible promoters for use in prokaryotic systems are well known in the art, see, *e.g.* the beta.-lactamase and lactose promoter systems (Chang *et al.*, *Nature*, 275: 615 (1978, which is incorporated herein by reference); Goeddel *et al.*, *Nature*, 281: 544 (1979), which is incorporated herein by reference), the arabinose promoter system, including the araBAD promoter (Guzman *et al.*, *J. Bacteriol.*, 174: 7716-7728 (1992), which is incorporated herein by reference; Guzman *et al.*, *J. Bacteriol.*, 177: 4121-4130 (1995), which is incorporated herein by reference; Siegle and Hu, *Proc. Natl. Acad. Sci. USA*, 94: 8168-8172 (1997), which is incorporated herein by reference), the rhamnose promoter (Haldimann *et al.*, *J. Bacteriol.*, 180: 1277-1286 (1998), which is incorporated herein by reference), the alkaline phosphatase promoter, a tryptophan (trp) promoter system (Goeddel, *Nucleic Acids Res.*, 8: 4057 (1980), which is incorporated herein by reference), the PLtetO-1 and Plac/are-1 promoters (Lutz and Bujard, *Nucleic Acids Res.*, 25: 1203-1210 (1997), which is

incorporated herein by reference), and hybrid promoters such as the tac promoter. deBoer *et al.*, Proc. Natl. Acad. Sci. USA, 80: 21-25 (1983), which is incorporated herein by reference.

An inducible promoter useful in the methods and systems as disclosed herein can be induced by one or more physiological conditions, such as changes in pH, temperature, radiation, osmotic pressure, saline gradients, cell surface binding, and the concentration of one or more extrinsic or intrinsic inducing agents. The extrinsic inducer or inducing agent may comprise amino acids and amino acid analogs, saccharides and polysaccharides, nucleic acids, protein transcriptional activators and repressors, cytokines, toxins, petroleum-based compounds, metal containing compounds, salts, ions, enzyme substrate analogs, hormones, and combinations thereof. In specific embodiments, the inducible promoter is activated or repressed in response to a change of an environmental condition, such as the change in concentration of a chemical, metal, temperature, radiation, nutrient or change in pH. Thus, an inducible promoter useful in the methods and systems as disclosed herein can be a phage inducible promoter, nutrient inducible promoter, temperature inducible promoter, radiation inducible promoter, metal inducible promoter, hormone inducible promoter, steroid inducible promoter, and/or hybrids and combinations thereof. Appropriate environmental inducers can include, but are not limited to, exposure to heat (*i.e.*, thermal pulses or constant heat exposure), various steroidal compounds, divalent cations (including Cu^{2+} and Zn^{2+}), galactose, tetracycline, IPTG (isopropyl- β -D thiogalactoside), as well as other naturally occurring and synthetic inducing agents and gratuitous inducers.

Inducible promoters useful in the methods and systems as disclosed herein also include those that are repressed by "transcriptional repressors" that are subject to inactivation by the action of environmental, external agents, or the product of another gene. Such inducible promoters may also be termed "repressible promoters" where it is required to distinguish between other types of promoters in a given module or component of the biological switch converters described herein. Preferred repressors for use in the present invention are sensitive to inactivation by physiologically benign agent. Thus, where a lac repressor protein is used to control the expression of a promoter sequence that has been engineered to contain a lacO operator sequence, treatment of the host cell with IPTG will cause the dissociation of the lac repressor from the engineered promoter containing a lacO operator sequence and allow transcription to occur. Similarly, where a tet repressor is used to control the expression of a promoter sequence that has been engineered to contain a tetO operator sequence, treatment of the host cell with tetracycline will cause the dissociation of the tet repressor from the engineered promoter and allow transcription of the sequence

downstream of the engineered promoter to occur.

A bacterial cell of the methods and compositions described herein can be any of any species. Preferably, the bacterial cells are of a species and/or strain which is amenable to culture and genetic manipulation. In some embodiments, the bacterial cell can be a gram-positive bacterial cell. In some embodiments, the bacterial cell can be a gram-negative bacterial cell. In some embodiments, the parental strain of the bacterial cell of the technology described herein can be a strain optimized for protein expression. Non-limiting examples of bacterial species and strains suitable for use in the present technologies include *Escherichia coli*, *E. coli* BL21, *E. coli* Tuner, *E. coli* Rosetta, *E. coli* JM101, and derivatives of any of the foregoing. Bacterial strains for protein expression are commercially available, *e.g.* EXPRESS™ Competent *E. coli* (Cat. No. C2523; New England Biosciences; Ipswich, MA). In some embodiments, the cell is an *E. coli* cell.

In some embodiments, a bacterium as described herein includes a mutation and/or deletion of the wild-type amyloid polypeptide gene (*e.g.*, a gene encoding CsgA or CspA), *e.g.* such that the cell does not express wild-type amyloid polypeptide protein (*e.g.*, CsgA). In some embodiments, the nucleic acid encoding a polypeptide described herein is introduced into a cell by homologous recombination. In some embodiments, the nucleic acid encoding a mutant amyloid polypeptide (*e.g.*, a mutant CsgA protein alone or being connected to a redox active domain (*e.g.*, a metalloprotein, redox enzyme, binding domain or stimuli-responsive peptide) replaces the endogenous amyloid polypeptide-encoding gene in the cell.

In one aspect, provided herein is a biofilm comprising an engineered microbial cell comprising one or more mutant amyloid polypeptides or fusion proteins described herein (*e.g.*, a CsgA polypeptide having one or more aromatic groups that are not present in wild type CsgA polypeptide) and/or comprising a vector or nucleic acid encoding such a polypeptide. In one aspect, provided herein is a biofilm comprising an amyloid-based structure (*e.g.*, a curli fiber) comprising a mutant amyloid polypeptide or a fusion protein described herein. As used herein, a “biofilm” refers to a mass of microorganisms which can adhere to or is adhering to a surface. A biofilm comprises a matrix of extracellular polymeric substances, including, but not limited to extracellular DNA, proteins, glycopeptides, and polysaccharides. The nature of a biofilm, such as its structure and composition, can depend on the particular species of bacteria present in the biofilm. Bacteria present in a biofilm are commonly genetically or phenotypically different than corresponding bacteria not in a biofilm, such as isolated bacteria or bacteria in a colony.

In some embodiments, the technology described herein relates to a biofilm that is

produced by culturing an engineered microbial cell described herein under conditions suitable for the production of a biofilm. In some embodiments, the engineered bacterial cell expresses a recombinant mutant amyloid polypeptide having a series of aligned aromatic groups. In some embodiments, the engineered bacterial cell expresses a recombinant fusion protein comprising an amyloid polypeptide and a redox active domain. In some embodiments, the engineered bacterial cell comprises an engineered CsgA polypeptide (and/or comprising a vector or nucleic acid encoding such a polypeptide) under conditions suitable for the production of a biofilm. Conditions suitable for the production of a biofilm can include, but are not limited to, conditions under which the microbial cell is capable of logarithmic growth and/or polypeptide synthesis. Conditions may vary depending upon the species and strain of microbial cell selected. Conditions for the culture of microbial cells are well known in the art. Biofilm production can also be induced and/or enhanced by methods well known in the art, *e.g.* contacting cells with subinhibitory concentrations of beta-lactam or aminoglycoside antibiotics, exposing cells to fluid flow, contacting cells with exogenous poly-N-acetylglucosamine (PNAG), or contacting cells with quorum sensing signal molecules. In some embodiments, conditions suitable for the production of a biofilm can also include conditions which increase the expression and secretion of CsgA, *e.g.*, by exogenously expressing CsgD.

In some embodiments, the biofilm can comprise a cell which produced the biofilm. In some embodiments, the biofilm comprises a mutant amyloid polypeptide described herein. In some embodiments, the biofilm comprises a fusion protein comprising an amyloid polypeptide fused to a redox active domain. In some embodiments, the biofilm comprises an amyloid-based structure formed by a plurality of mutant amyloid polypeptides described herein. In some embodiments, the biofilm comprises an amyloid-based structure comprising a mutant amyloid polypeptide described herein. In some embodiments, the biofilm comprises a fusion protein comprising an amyloid polypeptide fused to a redox active domain. In some embodiments, the biofilm comprises an amyloid-based structure formed by a plurality of fusion proteins, wherein each fusion protein comprises an amyloid polypeptide fused to a redox active domain. In some embodiments, the biofilm comprises an amyloid-based structure comprising a fusion protein described herein. In some embodiments, a composition includes an engineered CsgA polypeptide which includes CsgA having one or more aromatic groups not present in the wild type CsgA, as described herein. When expressed by a cell capable of forming curli, *e.g.* a cell expressing CsgA, CsgB, CsgC, CsgD, CsgE, CsgF, and CsgG or some subset thereof, CsgA units will be assembled to form curli filaments, *e.g.*

polymeric chains of CsgA. In some embodiments, filaments of the polypeptide can be present in the composition. In some embodiments, the filaments can be part of a proteinaceous network, *e.g.* multiple filaments which can be, *e.g.* interwoven, overlapping, and/or in contact with each other. In some embodiments, the proteinaceous network can comprise additional biofilm components, *e.g.* materials typically found in an *E. coli* biofilm. Non-limiting examples of biofilm components can include biofilm proteins (*e.g.* FimA, FimH, Ag43, AidA, and/or TibA) and/or non-proteinaceous biofilm components (*e.g.* cellulose, PGA and/or colonic acid). In some embodiments, the composition can further comprise an engineered microbial cell comprising an engineered CsgA polypeptide and/or comprising a vector or nucleic acid encoding such a polypeptide, as described herein.

As used herein, the terms "protein" and "polypeptide" are used interchangeably herein to designate a series of amino acid residues, connected to each other by peptide bonds between the alpha-amino and carboxy groups of adjacent residues. The terms "protein", and "polypeptide" refer to a polymer of amino acids, including modified amino acids (*e.g.*, phosphorylated, glycosylated, glycosylated, etc.) and amino acid analogs, regardless of its size or function. "Protein" and "polypeptide" are often used in reference to relatively large polypeptides, whereas the term "peptide" is often used in reference to small polypeptides, but usage of these terms in the art overlaps. The terms "protein" and "polypeptide" are used interchangeably herein when referring to a gene product and fragments thereof. Thus, exemplary polypeptides or proteins include gene products, naturally occurring proteins, homologs, orthologs, paralogs, fragments and other equivalents, variants, fragments, and analogs of the foregoing.

An "amyloid polypeptide" refers to an polypeptide capable of aggregating to form a structure having fibrillar morphology. In some embodiments, the amyloid polypeptides form an amyloid-based structure which typically consists of β -strands which are perpendicular to the fiber axis and β sheets which stack parallel to the fiber axis. In some embodiments, the amyloid polypeptide is selected from the group consisting of CsgA, CspA, a beta-lactoglobulin, a lysozyme, Sup35, and an amyloid-beta peptide, or a functional fragment thereof.

An "amyloid-based structure" or "amyloid-based fiber" as used herein refers to an polymeric aggregate of amyloid polypeptides. In some embodiments, the amyloid-based structure forms a structure of fibrillary morphology. In some embodiments, the amyloid-based structure is a curli fiber. In some embodiments, the amyloid-based structure is formed by a heterogeneous population of amyloid polypeptides. In some embodiments, the amyloid-

based structure is formed by a homogenous population of amyloid polypeptides. An amyloid-based structure may be formed by any population of amyloid polypeptides, including but not limited to, CsgA, CspA, a beta-lactoglobulin, a lysozyme, Sup35, and an amyloid-beta peptide.

A “nucleic acid” or “nucleic acid sequence” may be any molecule, preferably a polymeric molecule, incorporating units of ribonucleic acid, deoxyribonucleic acid or an analog thereof. The nucleic acid can be either single-stranded or double-stranded. A single-stranded nucleic acid can be one nucleic acid strand of a denatured double-stranded DNA. Alternatively, it can be a single-stranded nucleic acid not derived from any double-stranded DNA. In one aspect, the nucleic acid can be DNA. In another aspect, the nucleic acid can be RNA. Suitable nucleic acid molecules are DNA, including genomic DNA or cDNA. Other suitable nucleic acid molecules are RNA, including mRNA.

Definitions of common terms in cell biology and molecular biology can be found in The Encyclopedia of Molecular Biology, published by Blackwell Science Ltd., 1994 (ISBN 0-632-02182-9); Benjamin Lewin, Genes X, published by Jones & Bartlett Publishing, 2009 (ISBN-10: 0763766321); Kendrew *et al.* (eds.), Molecular Biology and Biotechnology: a Comprehensive Desk Reference, published by VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8) and Current Protocols in Protein Sciences 2009, Wiley Intersciences, Coligan *et al.*, eds.

Unless otherwise stated, the present invention was performed using standard procedures, as described, for example in Sambrook *et al.*, Molecular Cloning: A Laboratory Manual (3 ed.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., USA (2001); Davis *et al.*, Basic Methods in Molecular Biology, Elsevier Science Publishing, Inc., New York, USA (1995); or Methods in Enzymology: Guide to Molecular Cloning Techniques Vol. 152, S. L. Berger and A. R. Kimmel Eds., Academic Press Inc., San Diego, USA (1987); and Current Protocols in Protein Science (CPPS) (John E. Coligan, *et al.*, ed., John Wiley and Sons, Inc.), which are all incorporated by reference herein in their entireties.

The description of embodiments of the disclosure is not intended to be exhaustive or to limit the disclosure to the precise form disclosed. While specific embodiments of, and examples for, the disclosure are described herein for illustrative purposes, various equivalent modifications are possible within the scope of the disclosure, as those skilled in the relevant art will recognize. For example, while method steps or functions are presented in a given order, alternative embodiments may perform functions in a different order, or functions may be performed substantially concurrently. The teachings of the disclosure provided herein can

be applied to other procedures or methods as appropriate. The various embodiments described herein can be combined to provide further embodiments. Aspects of the disclosure can be modified, if necessary, to employ the compositions, functions and concepts of the above references and application to provide yet further embodiments of the disclosure. Moreover, due to biological functional equivalency considerations, some changes can be made in protein structure without affecting the biological or chemical action in kind or amount. These and other changes can be made to the disclosure in light of the detailed description. All such modifications are intended to be included within the scope of the appended claims.

Specific elements of any of the foregoing embodiments can be combined or substituted for elements in other embodiments. Furthermore, while advantages associated with certain embodiments of the disclosure have been described in the context of these embodiments, other embodiments may also exhibit such advantages, and not all embodiments need necessarily exhibit such advantages to fall within the scope of the disclosure.

The following examples are set forth as being representative of the present disclosure. These examples are not to be construed as limiting the scope of the present disclosure as these and other equivalent embodiments will be apparent in view of the present disclosure, figures and accompanying claims.

EXAMPLE I

Biofilm Integrated Nanofiber Display (BIND)

A method has been developed to repurpose the amyloid curli fibers of *E. coli* as a customizable nanomaterial, such as an electrically conductive fiber material or biofilm. This method, called Biofilm Integrated Nanofiber Display (BIND) uses straightforward genetic manipulation to append functional heterologous sequences to the CsgA protein, or a mutant as described herein, such as a redox active domain (*e.g.*, a metalloprotein, redox enzyme, binding domain or stimuli-responsive peptide) fused to the mutant CsgA protein (Fig. 2A). These heterologous domains are chosen such that they convey non-natural or augmented functions to the biofilm as a whole. The result is a programmable and completely biosynthetic system for creating living materials with a wide range of defined functions, including electrical conductivity. Examples of functions include enhanced adhesion to abiotic surfaces, the ability to template the growth of inorganic nanoparticles, and the ability to site-specifically immobilize enzymes to yield a catalytic surface (Figs. 2B - 2D). See Botyanszki, Z., Tay, P. K. R., Nguyen, P. Q., Nussbaumer, M. G. & Joshi, N. S. Engineered

catalytic biofilms: Site-specific enzyme immobilization onto *E. coli* curli nanofibers. *Biotechnol. Bioeng.* 112 (10): 2016–24 (2015); Nguyen, P. Q., Botyanszki, Z., Tay, P. K. R. & Joshi, N. S. (2014) Programmable biofilm-based materials from engineered curli nanofibers. *Nature Communications* 5:4945. Others functions include enhanced conduction of a curli-based biofilm programmed to specifically bind to gold nanoparticles. Zhong, C. *et al.* Strong underwater adhesives made by self-assembling multi-protein nanofibers. *Nature Nanotech* 1–9 (2014); Van Gerven, N. *et al.* Secretion and functional display of fusion proteins through the curli biogenesis pathway. *Molecular Microbiology* 91, 1022–1035 (2014); Chen, A. Y. *et al.* Synthesis and patterning of tunable multiscale materials with engineered cells. *Nature Materials* 13: 515–23 (2014).

EXAMPLE II

Production of Biosynthetic Amyloid Networks Containing Regularly Spaced Standard and Non-standard Aromatic Amino Acids

According to one aspect of the current disclosure, methods are included to create mutants of the CsgA protein that contain multiple periodic mutations. The CsgA sequence already consists mainly of 5 pseudo-repeats of a consensus sequence based on a Ser-X₅-Gln-X₄-Asn-X₅-Gln motif (SEQ ID NO: 90; Fig. 3A). The CsgA structure is quite malleable in terms of preserving its ability to assemble into amyloid fibers and can even participate in cross-seeding with a number of other amyloidogenic proteins. In one embodiment, the conserved residues in the above motif represent “gate-keeper” residues that are responsible for maintaining key intramolecular interactions, while several of the other residues can be altered without abolishing assembly function. Furthermore, some (but not all) permutations that shuffle the order the repeats R1-R5 preserve assembly as well. According to one aspect, a mutant CsgA protein is provided which includes aromatic groups on the outer face of the β -helix.

According to one aspect, a series of mutants that contain up to 5 mutations (1 per repeat) is created that result in the alignment of aromatic amino acids on the exterior of the CsgA β -helix (Fig. 3B). A representative example of one mutant containing 5 aligned histidine residues demonstrates their alignment in two consecutive CsgA monomers. Histidine is used as an example because it is isosteric with 3-thienylalanine, but other aromatic amino acids that are purported to be involved in long range EET in the *G. sulfurreducens* PilA nanowire (*i.e.*, tyrosine and tryptophan) are also exemplary. The CsgA mutants are expressed recombinantly from a system developed that includes a plasmid

containing a synthetic curli operon in an engineered strain containing the entire curli operon deleted. The amyloid production in each mutant is monitored using a standard plate-based Congo Red assay. A diagnostic device has been created to enable simultaneous monitoring of ~100s of bacterial colonies using time-lapse photography, facilitating the rapid identification of “hits” from relatively large libraries of *E. coli* variants. The hits are subsequently characterized using whole cell ELISA, TEM, and SEM on intact biofilms to confirm the identity and morphology of the mutant amyloid fibers. AFM of purified curli fibers is also conducted to confirm fiber dimensions. The variants are subjected to growth curve analyses to approximate the effects of the mutations on organismal fitness. Growth rates comparable to cells recombinantly producing the wild-type CsgA sequence are considered acceptable. According to one aspect, natural aromatic amino acids are sufficient to convey a base level of electronic conductance. According to one aspect, non-standard amino acid residues (NSAAs) are sufficient to convey a base level of electronic conductance. According to this aspect, an orthogonal set of tRNAs and aminoacyl-tRNA synthetases (aaRSs) are used to specifically incorporate synthetic amino acids. See Xiao, H. *et al.* Genetic Incorporation of Histidine Derivatives Using an Engineered PyrrolysyltRNA Synthetase. *ACS Chemical Biology* 9, 1092–1096 (2014) hereby incorporated by reference in its entirety. This requires the reassignment of the UAG stop codon (or another codon) to code for the NSAA. An MG1655-derived *E. coli* strain is specifically engineered to enable high-fidelity site-specific NSAA incorporation by having all of the genomic copies of the UAG stop codons removed. See Lajoie, M. J., Rovner, A. J., Goodman, D. B. & Aerni, H. R. Genomically recoded organisms expand biological functions. *Science* (2013) hereby incorporated by reference in its entirety.

The system is used to incorporate 3-thienylalanine (3-Th) into the permissive sites in the CsgA sequence, as determined by the above mutagenesis experiments. 3-Th is advantageous because of its similarity to the thiophene functional groups that pervade the field of conjugated conductive polymers, but several other non-standard amino acids are useful, including fluorinated phenylalanine analogs. See Mottishaw, J. D. & Sun, H. Effects of Aromatic Trifluoromethylation, Fluorination, and Methylation on Intermolecular π - π Interactions. *J. Phys. Chem. A* 117, 7970–9 (2013) hereby incorporated by reference in its entirety. Successful NSAA incorporation is determined by trypsin digest followed by LC/MS/MS analysis. Amyloid fiber formation ability for the 3-Th CsgA mutants is assayed as described above.

EXAMPLE III

Screening of Curli Library for Electrical Conductivity

Successful mutants or “hits” that emerge from the screening protocol above are confirmed to secrete and assemble CsgA variants into microns long amyloid fibers containing aligned, consecutive aromatic amino acids where the synthetic amyloids exhibit measurable electrical conductivity. See Amit, M., Cheng, G., Hamley, I. W. & Ashkenasy, N. Conductance of amyloid β based peptide filaments: structure–function relations. *Soft Matter* 8, 8690 (2012); Del Mercato, L. L. *et al.* Charge transport and intrinsic fluorescence in amyloid-like fibrils. *Proceedings of the National Academy of Sciences* 104, 18019–18024 (2007); Amdursky, N. Enhanced solid-state electron transport via tryptophan containing peptide networks. *Phys. Chem. Chem. Phys.* 15, 13479 (2013) each of which are hereby incorporated by reference in its entirety. Computational studies of amyloid folds containing stacked thienyl moieties indicate that a spacing on the order of 4-6 Å is sufficient to allow for delocalization. See Hamley, I. W. *et al.* Self-Assembly of a Designed Amyloid Peptide Containing the Functional Thienylalanine Unit. *J. Phys. Chem. B* 114, 10674–10683 (2010) hereby incorporated by reference in its entirety. Based on a computationally derived structure for CsgA, the proposed mutations exhibit an average distance of 5.11 Å between their respective β -carbons. In order to screen the narrowed library for conductive properties, the modified curli fibers are purified using standard protocols and measure their absorbance and fluorescence spectra under oxidizing and reducing conditions. See Tian, P. *et al.* Structure of a Functional Amyloid Protein Subunit Computed Using Sequence Variation. *J Am Chem Soc* 137, 22–25 (2015); Wang, X. & Chapman, M. R. Sequence Determinants of Bacterial Amyloid Formation. *J Mol Biol* 380, 570–580 (2008) hereby incorporated by reference in its entirety. The readout for delocalization is red-shifted in emission wavelength coupled with a decrease in intensity. Conductivity is measured directly by conductive AFM (ORCA) measurements on purified curli fibers. Finally, 96-well plates with an embedded array of addressable electrodes are used (Applied Biophysics, Inc.) to monitor bulk conductivity in intact biofilms.

EXAMPLE IV

Assessing the Ability of Conductive Fibers to Enhance EET Between Electrodes and Bacteria

Biosynthetically produced conductive nanofibers are useful for several applications independently. Biosynthetically produced conductive nanofibers are also useful to mediate

electron transport between electrodes and bacteria with the ability to use electrons directly as terminal electron acceptors in their metabolism. This is accomplished by using methods analogous to genetic engineering of the pili synthesized by microbes naturally, heterologously expressing the conductive curli fibers in the electroactive bacteria, or co-culturing two different bacterial species. According to one aspect of hybrid biofilm formation, the conductive matrix is created by engineered *E. coli* in an initial step, followed by colonization of the matrix with a different *E. coli* strain that has been specifically engineered to use external electrodes as terminal acceptors in its metabolism. See Jensen, H. M. *et al.* Engineering of a synthetic electron conduit in living cells. *Proc. Natl. Acad. Sci. USA* 107, 19213–8 (2010) hereby incorporated by reference in its entirety.

EXAMPLE V

Cell Strains and Plasmids

All cloning was performed in Mach1 cells.

For fibers containing only natural amino acids, the CsgA gene was isolated from *E. coli* K12 and cloned in a pET21d plasmid, under the control of the T7 promoter. A six his tag was added to the N-terminus of CsgA to allow for protein purification. Aromatic amino acid mutations were introduced in CsgA using gBlocks gene fragments (Integrated DNA Technologies) containing the point mutations, and isothermal Gibson assembly. Protein expression was performed in PQN4, an *E. coli* strain derived from LSR10, in which the curli operon was knocked out to prevent any background expression level of wild-type curli fibers.

For fibers containing non-natural amino acids, the CsgA gene was cloned in a pBbB8k or pBbB8a vector under the control of a PBAD vector, and a six his tag was added to the N-terminus of CsgA. Mutations were introduced in CsgA using gBlocks gene fragments with UAG codons at the desired mutation sites, and isothermal assembly was used to generate the mutant plasmids. Protein expression was performed in C321.ΔA.exp (obtained from Addgene, Bacterial strain #49018), an *E. coli* strain in which UAG codons have been removed from the genome to allow for non-natural amino acid incorporation. C321.ΔA.exp are co-transformed with a plasmid coding for CsgA, along with a plasmid coding for the aminoacyl-tRNA synthetase and tRNA specific for a non-natural amino acid of interest. A plasmid specific for 2-thienylalanine or 3-thienylalanine (pKB-pylHRS plasmid) was used and plasmids specific for p-acetylphenylalanine (pEVOL-pAcF plasmid), and p-azidophenylalanine (pEVOL-pAzF plasmid) were used. For protein expression, the non-natural amino acid of interest is added to the culture medium at induction.

EXAMPLE VI

Designing Conductive CsgA Proteins

Mutations introduced in CsgA are referred to by residue number for single mutations, or by stacks for groups of four or five residues mutated together. The residues selected for mutations corresponding to each stack are listed below. The numbering begins at the first glycine residues in the N22 peptide (it excludes the Sec peptide). Residues selected for mutations face outward (exposed to the solvent), and are located in the β -sheets of CsgA. Each stack represents an aligned series of residues useful in forming pi-stacks.

Stack 1: Glu 24, Asp 47, Ser 69, Glu 92, Ser 114 (SEQ ID NO:45)

Stack 2: Asn 26, Thr 49, Asp 71, Thr 94, Asn 116 (SEQ ID NO:46)

Stack 3: Thr 51, Thr 73, Lys 96, Thr 118 (SEQ ID NO:47)

Stack 4: Ala 38, Asp 60, Thr 82, Ala 105, Thr 127 (SEQ ID NO:48)

Stack 5: Gln 40, Gly 62, Asp 84, Asp 107 (SEQ ID NO:49)

The residues in each stack were simultaneously mutated to tyrosine. Then, for stack 1, the residues were also simultaneously mutated to tryptophan, phenylalanine or histidine, or to a non-natural amino acid. In addition, all five stacks were mutated together to tyrosine (23 mutations) in a single mutant.

Table 1. gBlock gene fragments used to produce the CsgA mutants with stacked aromatic residues or fusions comprising redox active domains (*e.g.*, metalloproteins or domains thereof), and corresponding primers used to linearize the plasmids. NSAA stands for non-standard amino acid.

CONSTRUCT	GBLOCK GENE FRAGMENT (5' TO 3')	PRIMERS USED TO LINEARIZE THE PLASMID BEFORE GIBSON ASSEMBLY (5' TO 3')
Stack 1 - Tyr	ACGGTGGTGGCGGTAATAATAGCGGCCCAAATTCTTA TCTGAACATTTACCAGTACGGTGGCGGTAACCTCTGCA CTTGCTCTGCAAACTGATGCCCGTAACTCTTACTTGAC TATTACCCAGCATGGCGGCGGTAATGGTGCAGATGTT GGTCAGGGCTCAGATGACAGCTATATCGATCTGACCC AACGTGGCTTCGGTAACAGCGCTACTCTTGATCAGTG GAACGGCAAAAATTCTTATATGACGGTTAAACAGTTC GGTGGTGGCAACGGTGCTGCAGTTGACCAGACTGCAT CTAACTCCTACGTCAACGTGACTCAGGTTGGCTTTGG TAAC (SEQ ID NO: 3)	Fwd: GTGACTCAGGTT GGCTTTGGTAAC AACGCGACCG (SEQ ID NO: 82) Rev: GAATTTGGGCCG CTATTATTACCG CCACCA (SEQ ID NO: 83)
Stack 2 - Tyr	GGTAATAATAGCGGCCCAAATTCTGAGCTGTACATT	Fwd:

	ACCAGTACGGTGGCGGTAACCTCTGCACTTGCTCTGCA AACTGATGCCCCGTAACCTCTGACTTGATATTACCCAG CATGGCGGCGGTAATGGTGCAGATGTTGGTCAGGGCT CAGATGACAGCTCAATCTATCTGACCCAACGTGGCTT CGGTAACAGCGCTACTCTTGATCAGTGGAACGGCAAA AATTCTGAAATGTATGTTAAACAGTTCGGTGGTGGCA ACGGTGCTGCAGTTGACCAGACTGCATCTAACTCCTC CGTCTACGTGACTCAGGTTGGCTTTGGTAACAACGCG (SEQ ID NO: 4)	GTGACTCAGGTT GGCTTTGGTAAC AACGCGACCG (SEQ ID NO: 82) Rev: GAATTTGGGCCG CTATTATTACCG CCACCA (SEQ ID NO: 83)
Stack 3 - Tyr	ACTGATGCCCCGTAACCTCTGACTTGACTATTTACCAGC ATGGCGGCGGTAATGGTGCAGATGTTGGTCAGGGCTC AGATGACAGCTCAATCGATCTGTACCAACGTGGCTTC GGTAACAGCGCTACTCTTGATCAGTGGAACGGCAAAA ATTCTGAAATGACGGTTTATCAGTTCGGTGGTGGCAA CGGTGCTGCAGTTGACCAGACTGCATCTAACTCCTCC GTCAACGTGTATCAGGTTGGCTTTGGTAACAACGCGA CCGCT (SEQ ID NO: 5)	Fwd: CAGGTTGGCTTT GGTAACAACGCG ACCGCTCATCAG (SEQ ID NO: 84) Rev: TAGTCAAGTCAG AGTTACGGGCAT CAGTTT (SEQ ID NO: 85)
Stack 4 - Tyr	TACCAGTACGGTGGCGGTAACCTCTGCACTTTATCTGC AACTGATGCCCCGTAACCTCTGACTTGACTATTACCCA GCATGGCGGCGGTAATGGTGCATATGTTGGTCAGGGC TCAGATGACAGCTCAATCGATCTGACCCAACGTGGCT TCGGTAACAGCGCTTATCTTGATCAGTGGAACGGCAA AAATTCTGAAATGACGGTTAAACAGTTCGGTGGTGGC AACGGTGCTTATGTTGACCAGACTGCATCTAACTCCT CCGTCAACGTGACTCAGGTTGGCTTTGGTAACAACGC GTATGCTCATCAGTACGGcTCTGGTGGcTCTGGT (SEQ ID NO: 6)	Fwd: CAGTACGGCTCT GGTGGCTCTGGT GGCTCT (SEQ ID NO: 86) Rev: AAGTGCAGAGTT ACCGCCACCGTA CTGG (SEQ ID NO: 87)
Stack 5 - Tyr	TACGGTGGCGGTAACCTCTGCACTTGCTCTGTATACTG ATGCCCCGTAACCTCTGACTTGACTATTACCCAGCATGG CGGCGGTAATGGTGCAGATGTTTATCAGGGCTCAGAT GACAGCTCAATCGATCTGACCCAACGTGGCTTCGGTA ACAGCGCTACTCTTTATCAGTGGAACGGCAAAAATTTC TGAAATGACGGTTAAACAGTTCGGTGGTGGCAACGGT GCTGCAGTTTACCAGACTGCATCTAACTCCTCCGTCA ACGTG (SEQ ID NO: 7)	Fwd: GACTGCATCTAA CTCCTCCGTCAA CGTGAC (SEQ ID NO: 88) Rev: AAGTGCAGAGTT ACCGCCACCGTA CTGG (SEQ ID NO: 87)
Stack 1 - Trp	ACGGTGGTGGCGGTAATAATAGCGGCCCAAATTCTTG GCTGAACATTTACCAGTACGGTGGCGGTAACCTCTGCA CTTGCTCTGCAAACTGATGCCCCGTAACCTCTGGTTGAC TATTACCCAGCATGGCGGCGGTAATGGTGCAGATGTT GGTCAGGGCTCAGATGACAGCTGGATCGATCTGACCC AACGTGGCTTCGGTAACAGCGCTACTCTTGATCAGTG GAACGGCAAAAATTCTTGATGACGGTTAAACAGTTC GGTGGTGGCAACGGTGCTGCAGTTGACCAGACTGCAT CTAACTCCTGGGTCAACGTGACTCAGGTTGGCTTTGG TAAC (SEQ ID NO: 8)	Fwd: GTGACTCAGGTT GGCTTTGGTAAC AACGCGACCG (SEQ ID NO: 82) Rev: GAATTTGGGCCG CTATTATTACCG CCACCA (SEQ ID

		NO: 83)
Stack 2 - Trp	GGTAATAATAGCGGCCCAAATTCTGAGCTGTGGA TTTACCAGTACGGTGGCGGTAACCTCTGCACTTGC TCTGCAAACCTGATGCCCCGTAACCTCTGACTTGTGG ATTACCCAGCATGGCGGCGGTAATGGTGCAGATG TTGGTCAGGGCTCAGATGACAGCTCAATCTGGCT GACCCAACGTGGCTTCGGTAACAGCGCTACTCTT GATCAGTGGAACGGCAAAAATTCTGAAATGTGG GTTAAACAGTTCGGTGGTGGCAACGGTGCTGCAG TTGACCAGACTGCATCTAACTCCTCCGTCTGGGT GACTCAGGTTGGCTTTGGTAACAACGCG (SEQ ID NO: 62)	Fwd: GTGACTCAGGT TGGCTTTGGTA ACAACGCGACC G (SEQ ID NO: 82) Rev: GAATTTGGGCC GCTATTATTAC CGCCACCA (SEQ ID NO: 83)
Stack 3 - Trp	ACTGATGCCCCGTAACCTCTGACTTGACTATTTGGC AGCATGGCGGCGGTAATGGTGCAGATGTTGGTCA GGGCTCAGATGACAGCTCAATCGATCTGTGGCAA CGTGGCTTCGGTAACAGCGCTACTCTTGATCAGT GGAACGGCAAAAATTCTGAAATGACGGTTTGGC AGTTCGGTGGTGGCAACGGTGCTGCAGTTGACCA GACTGCATCTAACTCCTCCGTCAACGTGTGGCAG GTTGGCTTTGGTAACAACGCGACCGCT (SEQ ID NO: 63)	Fwd: CAGGTTGGCTT TGGTAACAACG CGACCGCTCAT CAG (SEQ ID NO: 89) Rev: TAGTCAAGTCA GAGTTACGGGC ATCAGTTT (SEQ ID NO: 85)
Stack 4 - Trp	TACCAGTACGGTGGCGGTAACCTCTGCACTTTGGC TGCAAACCTGATGCCCCGTAACCTCTGACTTGACTAT TACCCAGCATGGCGGCGGTAATGGTGCATGGGTT GGTCAGGGCTCAGATGACAGCTCAATCGATCTGA CCCAACGTGGCTTCGGTAACAGCGCTTGGCTTGA TCAGTGGAACGGCAAAAATTCTGAAATGACGGTT AAACAGTTCGGTGGTGGCAACGGTGCTTGGGTTG ACCAGACTGCATCTAACTCCTCCGTCAACGTGAC TCAGGTTGGCTTTGGTAACAACGCGTGGGCTCAT CAGTACGGcTCTGGTGGcTCTGGT (SEQ ID NO: 64)	Fwd: CAGTACGGCTC TGGTGGCTCTG GTGGCTCT (SEQ ID NO: 86) Rev: AAGTGCAGAGT TACCGCCACCG TACTGG (SEQ ID NO: 87)
Stack 5 - Trp	TACGGTGGCGGTAACCTCTGCACTTGCTCTGTGGA CTGATGCCCCGTAACCTCTGACTTGACTATTACCCA GCATGGCGGCGGTAATGGTGCAGATGTTTGGCAG GGCTCAGATGACAGCTCAATCGATCTGACCCAAC GTGGCTTCGGTAACAGCGCTACTCTTTGGCAGTG GAACGGCAAAAATTCTGAAATGACGGTTAAACA GTTTCGGTGGTGGCAACGGTGCTGCAGTTTGGCAG ACTGCATCTAACTCCTCCGTCAACGTG (SEQ ID NO: 65)	Fwd: GACTGCATCTA ACTCCTCCGTC AACGTGAC (SEQ ID NO: 88) Rev: AAGTGCAGAGT TACCGCCACCG TACTGG (SEQ ID NO: 87)
Stack 1 - Phe	ACGGTGGTGGCGGTAATAATAGCGGCCCAAATTCTTT TCTGAACATTTACCAGTACGGTGGCGGTAACCTCTGCA CTTGCTCTGCAAACCTGATGCCCCGTAACCTTTCTTGAC	Fwd: GTGACTCAGGTT GGCTTTGGTAAC

	TATTACCCAGCATGGCGGCGGTAATGGTGCAGATGTT GGTCAGGGCTCAGATGACAGCTTTATCGATCTGACCC AACGTGGCTTCGGTAACAGCGCTACTCTTGATCAGTG GAACGGCAAAAATTCTTTTATGACGGTTAAACAGTTC GGTGGTGGCAACGGTGCTGCAGTTGACCAGACTGCAT CTAACTCCTTCGTCAACGTGACTCAGGTTGGCTTTGGT AAC (SEQ ID NO: 9)	AACGCGACCG (SEQ ID NO: 82) Rev: GAATTTGGGCCG CTATTATTACCG CCACCA (SEQ ID NO: 83)
Stack 1 – His	ACGGTGGTGGCGGTAATAATAGCGGCCCAAATTCTCA TCTGAACATTTACCAGTACGGTGGCGGTAACCTCTGCA CTTGCTCTGCAAACCTGATGCCCCGTAACCTCTACTTGAC TATTACCCAGCATGGCGGCGGTAATGGTGCAGATGTT GGTCAGGGCTCAGATGACAGCCATATCGATCTGACCC AACGTGGCTTCGGTAACAGCGCTACTCTTGATCAGTG GAACGGCAAAAATTCTCATATGACGGTTAAACAGTTC GGTGGTGGCAACGGTGCTGCAGTTGACCAGACTGCAT CTAACTCCACGTCAACGTGACTCAGGTTGGCTTTGG TAAC (SEQ ID NO: 10)	Fwd: GTGACTCAGGTT GGCTTTGGTAAC AACGCGACCG (SEQ ID NO: 82) Rev: GAATTTGGGCCG CTATTATTACCG CCACCA (SEQ ID NO: 83)
Stack 1,2,3,4 and 5 - Tyr	ACGGTGGTGGCGGTAATAATAGCGGCCCAAATTCTTA TCTGTATATTTACCAGTACGGTGGCGGTAACCTCTGCA CTTTATCTGTATACTGATGCCCCGTAACCTTACTTGTA TATTTATCAGCATGGCGGCGGTAATGGTGCATATGTT TATCAGGGCTCAGATGACAGCTATATCTATCTGTATC AACGTGGCTTCGGTAACAGCGCTTATCTTTATCAGTG GAACGGCAAAAATTCTTATATGTATGTTTATCAGTTC GGTGGTGGCAACGGTGCTTATGTTTATCAGACTGCAT CTAACTCCTACGTCTATGTGTATCAGGTTGGCTTTGGT AACAACGCGTATGCTCATCAGTACGGcTCTGGTGGcTC TGGT (SEQ ID NO: 11)	Fwd: CAGTACGGCTCT GGTGGCTCTGGT GGCTCT (SEQ ID NO: 86) Rev: GAATTTGGGCCG CTATTATTACCG CCACCA (SEQ ID NO: 83)
Stack 1 – NSAA	ACGGTGGTGGCGGTAATAATAGCGGCCCAAATTCTTA GCTGAACATTTACCAGTACGGTGGCGGTAACCTCTGCA CTTGCTCTGCAAACCTGATGCCCCGTAACCTTAGTTGAC TATTACCCAGCATGGCGGCGGTAATGGTGCAGATGTT GGTCAGGGCTCAGATGACAGCTAGATCGATCTGACCC AACGTGGCTTCGGTAACAGCGCTACTCTTGATCAGTG GAACGGCAAAAATTCTTAGATGACGGTTAAACAGTTC GGTGGTGGCAACGGTGCTGCAGTTGACCAGACTGCAT CTAACTCCTAGGTCAACGTGACTCAGGTTGGCTTTGG TAAC (SEQ ID NO: 12)	Fwd: GTGACTCAGGTT GGCTTTGGTAAC AACGCGACCG (SEQ ID NO: 82) Rev: GAATTTGGGCCG CTATTATTACCG CCACCA (SEQ ID NO: 83)
Microperoxid ase-8 fusion	GTAACAACGCGACCGCTCATCAGTACGGTGGATC TGGTAGCAGCGGCTCTGGTGGTTCTGGGGGCGGA AGTGGCTCCTCTGGGAGCGGGGGGTCGGGTGGT GGCTCGGGTTCATCTGGTAGTGGCGGTTTCGGGT GCGCGCAGTGCCATACCGTGGAAGGcTCTGGTGG cTCTGGTGGcTCTGGcGGCAGCGGGCAcCACCACC ACCATCATTAATACATCATTTGTATTACAGAAAC AGGGC (SEQ ID NO: 66)	
Microperoxid ase-9 fusion	TAACAACGCGACCGCTCATCAGTACGGTGGATCT GGTAGCAGCGGCTCTGGTGGTTCTGGGGGCGGA AGTGGCTCCTCTGGGAGCGGGGGGTCGGGTGGT	

	GGCTCGGGTTCATCTGGTAGTGGCGGTTTCGGGTT GCGCGCAGTGCCATACCGTGGA AAAAGGcTCTGG TGGcTCTGGTGGcTCTGGcGGCAGCGGGCAcCACC ACCACCATCATTAAATACATCATTTGTATTACAGA AACAGGGC (SEQ ID NO: 67)	
Microperoxidase-11 fusion	ACAACGCGACCGCTCATCAGTACGGTGGATCTGG TAGCAGCGGCTCTGGTGGTTCTGGGGGCGGAAGT GGCTCCTCTGGGAGCGGGGGGTCGGGTGGTGGCT CGGGTTCATCTGGTAGTGGCGGTTTCGGGTGTGCA GAAATGCGCGCAGTGCCATACCGTGGAAGGcTCT GGTGGcTCTGGTGGcTCTGGcGGCAGCGGGCAcCA CCACCACCATCATTAAATACATCATTTGTATTACA G (SEQ ID NO: 68)	
Plastocyanin fusion	ACAACGCGACCGCTCATCAGTACGGTGGATCTGG TAGCAGCGGCTCTGGTGGTTCTGGGGGCGGAAGT GGCTCCTCTGGGAGCGGGGGGTCGGGTGGTGGCT CGGGTTCATCTGGTAGTGGCGGTTTCGGGTATGAT TTCCTCGCTTCGCTCAGCGCTATCTGCATGTTTTG CATTGCTTTTGGTGCTTGCCTTTGGAGTTGCATCC GCACAAGCTAAAACAGTTGAAGTAAACTAGGA ACTGATGCTGGAATGCTTGCATTCGAACCCAGCT CTGTAACCATAAGTACTGGCGACTCTGTGAAGTT TGTCAACAACAACTTGCTCCTCACAATGCAGTT TTTGAAGGTCATGAAGAATTAAGTCATCCTGACC TTGCTTTTGCTCCTGGCGAAAGCTGGCAAGAAAC TTTTACAGAAGCAGGTACATATGACTATTACTGC GAGCCTCATAGAGGAGCAGGGATGGTCGGGAAA GTAGTTGTTAACGGcTCTGGTGGcTCTGGTGGcTC TGGcGGCAGCGGGCAcCACCACCACCATCATTAAAT ACATCATTTGTATTACAGAAACAGGGC (SEQ ID NO: 69)	
Mitochondrial ferritin fusion	AACGCGACCGCTCATCAGTACGGTGGATCTGGTA GCAGCGGCTCTGGTGGTTCTGGGGGCGGAAGTG GCTCCTCTGGGAGCGGGGGGTCGGGTGGTGGCTC GGGTTCATCTGGTAGTGGCGGTTTCGGGTATGCTG TCTGGTTTCTGGTTCTTCTCCAAGCACATCGGCCC TGCATTGATGTCCTTGCCCCGTGTGCTACACAGG TGCACTGTCCACAGTGTTTGGCCTCCAGGTATC CTTTACTACCCGCCTCCCCTCGACGTCTGCTGGCC TCGGTGGCCTCCTCCCAGGGCTCTGATGGAAGTG CCAGGGTGCGCCACAACCTTTCACCCAGACTCTGA GGCAGCCATCAACCACCAAATCAACATGGAGCTT TACGCATCCTACGTGTACCTGTCCATGGCCTACT ACTTCTCCAGGGATGATGTGGCCTTGTACAACCT CTCCAAGTCTTTCTTCCTTCGCCAGTCGCTGGAGGAG AGGGAGCATGCAGAGAAGCTAATGAAGCTGCAG AACCAACGCGGAGGCCGGATCTGCCTCCAGGAT ATCAAGAAGCCAGAGCAAGATGACTGGGAGAGC GGACTGCGGGCCATGGAATGTGCTCTGCTCCTGG AAAAGAGTGTAACACAGTCGCTGCTGGACCTGC	

	ATACTCTGGCCTCAGAAAAAGGAGATCCTCATTT GTGCGACTTTCTGGAAACACACTACCTGAATGAG CAGGTGAAGTCTATCAAAGAATTAGGTGACCAC GTGCACAACTTAGTCACCATGGGGGCTCCAGCTG TTGGCCTAGCGGAGTACCTTTTTTGACAAGCACAC CCTTGGAAGTGAGAGCAAGCACGGcTCTGGTGGc TCTGGTGGcTCTGGcGGCAGCGGGCAcCACCACCA CCATCATTAATACATCATTTGTATTAC (SEQ ID NO: 70)	
Rubredoxin fusion	AACGCGACCGCTCATCAGTACGGTGGATCTGGTA GCAGCGGCTCTGGTGGTTCTGGGGGCGGAAGTG GCTCCTCTGGGAGCGGGGGGTCGGGTGGTGGCTC GGGTTCATCTGGTAGTGGCGGTTCGGGTATGGCA AAGTGGGTTTGTAAGATATGCGGATACATATATG ATGAAGATGCAGGAGATCCAGACAATGGTATTTTC TCCTGGAAC TAAGTTTGAGGAGCTACCAGATGAT TGGGTTTGCCCCATTTGTGGGGCTCCAAAAGTG AATTTGAAAAGTTAGAAGATGGcTCTGGTGGcTCT GGTGGcTCTGGcGGCAGCGGGCAcCACCACCACCA TCATTAATACATCATTTGTATTACAGAAACAGGG C (SEQ ID NO: 71)	

Table 2. Protein sequence and expected molecular weight of the mutants containing stacked aromatic amino acids or fusions comprising redox active domains (*e.g.*, metalloproteins or domains thereof). The Sec peptide (underlined) has a molecular weight of 1974.5 Da, which should be subtracted from the molecular weight of the protein after CsgA is secreted outside of the cell, as Sec is cleaved. The molecular weight of wild-type CsgA with his-tag and Sec peptide is 16677 Da. The Sec peptide in each of the protein sequences below is underlined. His-tags (and corresponding flexible linkers) are italicized. The flexible linkers between fusion proteins are also italicized.

CONSTRUCT	PROTEIN SEQUENCE (INCLUDING SEC PEPTIDE) FOR HIS-TAGGED PROTEINS	EXPECTED MOLECULAR WEIGHT BEFORE CLEAVAGE OF THE SEC PEPTIDE (Da)
Stack 1 – Tyr Sec peptide is underlined; linker and His-tag sequences are italicized.	<u>MKLLKVA</u> <i>AAIAIV</i> <u>FGS</u> <u>SALAGVVPQYGGGGNHGG</u> <u>GGNNSGPNSYLN</u> <i>IQYGGGNSALALQTDARNSYLT</i> <u>ITQHGGGNGADV</u> <u>QGSDDSYIDLTQRGFGNSATLD</u> <u>QWNGKNSYMTVKQF</u> <u>GGGNGAAVDQTASNSYVNV</u> <u>TQVGFGNNATAHQYGS</u> <u>GGSGGSGGSGHHHHH*</u> (SEQ ID NO: 13)	169452

Stack 2 – Tyr Sec peptide is underlined; linker and His-tag sequences are italicized.	<u>MKLLKVA</u> <u>AAIAA</u> IVFSGSALAGVVPQYGGGGNHGG GGNNSGPNSELYIYQYGGGNSALALQTDARNSDLY ITQHGGGNGADVGGQGSDDSSIYLTQRGFGNSATLD QWNGKNSEMYVKQFGGGNGAAVDQTASNSSVYV TQVGFGNNATAH <i>QYGS</i> <i>GGSGGSGGSGHHHHHHH*</i> (SEQ ID NO: 14)	16947
Stack 3 – Tyr Sec peptide is underlined; linker and His-tag sequences are italicized.	<u>MKLLKVA</u> <u>AAIAA</u> IVFSGSALAGVVPQYGGGGNHGG GGNNSGPNSELNIYQYGGGNSALALQTDARNSDLT IYQHGGGNGADVGGQGSDDSSIDLYQRGFGNSATLD QWNGKNSEMTVYQFGGGNGAAVDQTASNSSVNV YQVGFGNNATAH <i>QYGS</i> <i>GGSGGSGGSGHHHHHHH*</i> (SEQ ID NO: 15)	16897
Stack 4 – Tyr Sec peptide is underlined; linker and His-tag sequences are italicized.	<u>MKLLKVA</u> <u>AAIAA</u> IVFSGSALAGVVPQYGGGGNHGG GGNNSGPNSELNIYQYGGGNSALYLQTDARNSDLT ITQHGGGNGAYVGGQGSDDSSIDLTQRGFGNSAYLD QWNGKNSEMTVKQFGGGNGAYVDQTASNSSVNV TQVGFGNNAYAH <i>QYGS</i> <i>GGSGGSGGSGHHHHHHH*</i> (SEQ ID NO: 16)	17033
Stack 5 – Tyr Sec peptide is underlined; linker and His-tag sequences are italicized.	<u>MKLLKVA</u> <u>AAIAA</u> IVFSGSALAGVVPQYGGGGNHGG GGNNSGPNSELNIYQYGGGNSALALYTDARNSDLT ITQHGGGNGADVYQGSDDSSIDLTQRGFGNSATLY QWNGKNSEMTVKQFGGGNGAAVYQTASNSSVNV TQVGFGNNATAH <i>QYGS</i> <i>GGSGGSGGSGHHHHHHH*</i> (SEQ ID NO: 17)	16914
Stack 1 – Trp Sec peptide is underlined; linker and His-tag sequences are italicized.	<u>MKLLKVA</u> <u>AAIAA</u> IVFSGSALAGVVPQYGGGGNHGG GGNNSGPNSWLNIYQYGGGNSALALQTDARNSWL TITQHGGGNGADVGGQGSDDSWIDLTQRGFGNSATL DQWNGKNSWMTVKQFGGGNGAAVDQTASN SWV NVTQVGFGNNATAH <i>QYGS</i> <i>GGSGGSGGSGHHHHHHH</i> * (SEQ ID NO: 18)	17060
Stack 2 – Trp Sec peptide is underlined; linker and His-tag sequences are italicized.	<u>MKLLKVA</u> <u>AAIAA</u> IVFSGSALAGVVPQYGGGGNHGG GGNNSGPNSELWYQYGGGNSALALQTDARNSDL WITQHGGGNGADVGGQGSDDSSIWLTQRGFGNSAT LDQWNGKNSEM WVK QFGGGNGAAVDQTASNSSV WVTQVGFGNNATAH <i>QYGS</i> <i>GGSGGSGGSGHHHHHHH</i> H (SEQ ID NO: 72)	17062
Stack 3 – Trp Sec peptide is underlined; linker and His-tag sequences are italicized.	<u>MKLLKVA</u> <u>AAIAA</u> IVFSGSALAGVVPQYGGGGNHGG GGNNSGPNSELNIYQYGGGNSALALQTDARNSDLT IWQHGGGNGADVGGQGSDDSSIDLWQRGFGNSATL	16990

	<u>DQWNGKNSEMTVWQFGGGNGAAVDQTASNSSVN</u> <u>VWQVGFGNNATAHQYGS</u> <u>GGSGGSGGSGHHHHH</u> (SEQ ID NO: 73)	
Stack 4 – Trp	<u>MKLLKVA</u> <u>AAIAIV</u> <u>FGS</u> <u>ALAGVVPQYGGGGNHGG</u> <u>GGNSGP</u> <u>NSELNIYQYGGGNSALWLQTDARNSDL</u> <u>TITQHGGGNGAWVGQGSDDSSIDLTQRGFGNSAW</u> <u>LDQWNGKNSEMTVKQFGGGNGAWVDQTASNSSV</u> <u>NVTQVGFGNNAWAHQYGS</u> <u>GGSGGSGGSGHHHHH</u> <i>H</i> (SEQ ID NO: 74)	17148
Stack 5 - Trp	<u>MKLLKVA</u> <u>AAIAIV</u> <u>FGS</u> <u>ALAGVVPQYGGGGNHGG</u> <u>GGNSGP</u> <u>NSELNIYQYGGGNSALALWTDARNSDL</u> <u>TITQHGGGNGADVWQGSDDSSIDLTQRGFGNSATL</u> <u>WQWNGKNSEMTVKQFGGGNGAAVWQTASNSSV</u> <u>NVTQVGFGNNATAHQYGS</u> <u>GGSGGSGGSGHHHHH</u> (SEQ ID NO: 75)	17006
Stack 1 – Phe Sec peptide is underlined; linker and His-tag sequences are italicized.	<u>MKLLKVA</u> <u>AAIAIV</u> <u>FGS</u> <u>ALAGVVPQYGGGGNHGG</u> <u>GGNSGP</u> <u>NSFLNIYQYGGGNSALALQTDARNSFLTI</u> <u>TQHGGGNGADVGGQGSDDSFIDLTQRGFGNSATLD</u> <u>QWNGKNSFMTVKQFGGGNGAAVDQTASN</u> <u>SFVNV</u> <u>TQVGFGNNATAHQYGS</u> <u>GGSGGSGGSGHHHHH</u> <i>H*</i> (SEQ ID NO: 19)	16865
Stack 1 – His Sec peptide is underlined; linker and His-tag sequences are italicized.	<u>MKLLKVA</u> <u>AAIAIV</u> <u>FGS</u> <u>ALAGVVPQYGGGGNHGG</u> <u>GGNSGP</u> <u>NSHLNIYQYGGGNSALALQTDARNSHLT</u> <u>ITQHGGGNGADVGGQGSDDSHIDLTQRGFGNSATLD</u> <u>QWNGKNSHMTVKQFGGGNGAAVDQTASN</u> <u>SHVNV</u> <u>TQVGFGNNATAHQYGS</u> <u>GGSGGSGGSGHHHHH</u> <i>H*</i> (SEQ ID NO: 20)	16815
Stack 1,2,3,4 and 5 – Tyr Sec peptide is underlined; linker and His-tag sequences are italicized.	<u>MKLLKVA</u> <u>AAIAIV</u> <u>FGS</u> <u>ALAGVVPQYGGGGNHGG</u> <u>GGNSGP</u> <u>NSYLYIYQYGGGNSALYLYTDARNSYL</u> <u>YIYQHGGGNGAYVYQGSDDSYIYLYQRGFGNSAY</u> <u>LYQWNGKNSYMYVYQFGGGNGAYVYQTASN</u> <u>SYV</u> <u>YVYQVGFGNNAYAHQYGS</u> <u>GGSGGSGGSGHHHHH</u> <i>H*</i> (SEQ ID NO: 21)	18031
Microperoxidase-8 fusion	<u>MKLLKVA</u> <u>AAIAIV</u> <u>FGS</u> <u>ALAGVVPQYGGGGNHGG</u> <u>GGNSGP</u> <u>NSELNIYQYGGGNSALALQTDARNSDLT</u> <u>ITQHGGGNGADVGGQGSDDSSIDLTQRGFGNSATLD</u> <u>QWNGKNSEMTVKQFGGGNGAAVDQTASNSSVNV</u> <u>TQVGFGNNATAHQYGS</u> <u>GGSGSSGSGGSGGGSGSSGSG</u> <u>GSGGGSGSSGSGSGCAQCHTVEGSGGSGGSGGSG</u> <u>HHHHHH</u> (SEQ ID NO: 76)	20052
Microperoxidase-9 fusion	<u>MKLLKVA</u> <u>AAIAIV</u> <u>FGS</u> <u>ALAGVVPQYGGGGNHGG</u> <u>GGNSGP</u> <u>NSELNIYQYGGGNSALALQTDARNSDLT</u> <u>ITQHGGGNGADVGGQGSDDSSIDLTQRGFGNSATLD</u>	20181

	QWNGKNSEMTVKQFGGGNGAAVDQTASNSSVNV TQVGFGNNAHAHQYGGSGSSGSGSGSGSGSGSGSG GSGGGSGSSGSGSGSCAQCHTVEKSGSGSGSGSGSG GHHHHHH (SEQ ID NO: 77)	
Microperoxidase-11 fusion	<u>MKLLKVAIAAIVFSGSALAGVVPQYGGGGNHGG</u> GGNNSGPNSELNIYQYGGGNSALALQTDARNSDLT ITQHGGGNGADVGGQSDSSIDLTQRGFGNSATLD QWNGKNSEMTVKQFGGGNGAAVDQTASNSSVNV TQVGFGNNAHAHQYGGSGSSGSGSGSGSGSGSGSG GSGGGSGSSGSGSGSVQKCAQCHTVEGSGSGSGSGSG GSGHHHHHH (SEQ ID NO: 78)	20408
Plastocyanin fusion	<u>MKLLKVAIAAIVFSGSALAGVVPQYGGGGNHGG</u> GGNNSGPNSELNIYQYGGGNSALALQTDARNSDLT ITQHGGGNGADVGGQSDSSIDLTQRGFGNSATLD QWNGKNSEMTVKQFGGGNGAAVDQTASNSSVNV TQVGFGNNAHAHQYGGSGSSGSGSGSGSGSGSGSG GSGGGSGSSGSGSGSMISLRSALSACFALLLVLA FGVASAQAKTVEVKLGTDAGMLAFEPSSVTISTGDSV KFVNNKLAPHNAVFEHGHEELSHPDLAFAFGESWQE TFTEAGTYDYYCEPHRGAGMVGKVVVNGSGSGSG SGSGHHHHHH (SEQ ID NO: 79)	31772
Mitochondrial ferritin fusion	<u>MKLLKVAIAAIVFSGSALAGVVPQYGGGGNHGG</u> GGNNSGPNSELNIYQYGGGNSALALQTDARNSDLT ITQHGGGNGADVGGQSDSSIDLTQRGFGNSATLD QWNGKNSEMTVKQFGGGNGAAVDQTASNSSVNV TQVGFGNNAHAHQYGGSGSSGSGSGSGSGSGSGSG GSGGGSGSSGSGSGMLSGFWFFSKHIGPALMSLPR VLHRCTVPQCLASRYPLLPASPRLLASVASSQGS GTARVRHNFHPDSEAAINHQINMELYASYVYLSMA YYFSRDDVALYNFSKSFLRQSLEEREHAELMKLQ NQRGGRICLQDIKKPEQDDWESGLRAMECALLLEK SVNQSLDLHTLASEKGDPHLCDFLETHYLNEQVK SIKELGDHVHNLVTMGAPAVGLAEYLFDKHTLGSE SKHSGSGSGSGSGSGHHHHHH (SEQ ID NO: 80)	45821
Rubredoxin fusion	<u>MKLLKVAIAAIVFSGSALAGVVPQYGGGGNHGG</u> GGNNSGPNSELNIYQYGGGNSALALQTDARNSDLT ITQHGGGNGADVGGQSDSSIDLTQRGFGNSATLD QWNGKNSEMTVKQFGGGNGAAVDQTASNSSVNV TQVGFGNNAHAHQYGGSGSSGSGSGSGSGSGSGSG GSGGGSGSSGSGSGMAKWVCKICGYIYDEDAGDP DNGISPGTKFEELPDDWVCPICGAPKSEFEKLEDGS GGSGSGSGSGHHHHHH (SEQ ID NO: 81)	25190

In an alternative embodiment, the mutant may lack the Sec peptide (underlined sequence). In an alternative embodiment, the mutant may lack the His tag.

EXAMPLE VII

Curli-Producing Biofilm Formation

For fibers containing only natural amino acids, biofilms producing curli can be grown on agar plates, or in liquid medium. Transformed PQN4 cells are streaked onto Luria-Bertani (LB) agar plates containing 100ug/mL carbenicillin and 0.5% glucose (to prevent activation of the T7 promoter). Colonies are picked from the plates and 5mL cultures are inoculated (in LB containing and 100ug/mL carbenicillin). Cultures are grown overnight at 37°C. The overnight cultures are diluted 100 times in fresh LB medium with 100ug/mL carbenicillin and 2% glucose, and grown at 37°C until they reach an optical density (OD) at 600nm of 0.6 to 0.8. Cells are pelleted at 4000xg, and gently resuspended in an induction medium (LB without glucose, containing 0.4 mM IPTG and 100 ug/mL carbenicillin). Protein expression is allowed to occur at 30°C overnight.

For fibers containing non-natural amino acids, biofilm formation in solution follows the same procedure as for fibers containing only natural amino acids, except that no glucose is used at any step, and carbenicillin is replaced by a combination of two antibiotics selected specifically for each vector used. Carbenicillin or ampicillin is used for pBbB8a, and kanamycin is used for pBbB8k plasmids, in combination with the appropriate selection marker for pKB-pylHRS (kanamycin) or pEVOL (spectinomycin) plasmids. In addition, at the point of induction, the non-natural amino acid of interest is added to the culture medium at a final concentration ranging between 1mM and 10mM.

EXAMPLE VIII

Assessment and Quantification of Curli Fiber Production

Two types of Congo red assays were performed. 1) Agar plates containing LB, Congo red, coomassie blue and carbenicillin, with or without IPTG, were prepared. 5 to 10 ul of biofilm-forming cultures were spotted onto the plates at an OD_{600nm} of 0.6 to 0.8 and incubated for one to two days. The incubation was done at room temperature for plates with IPTG (induced expression), and at 37°C for plates without IPTG. 2) Congo red spin-down assay were performed to quantify the relative expression levels of the mutants. 1 mL of cultures that were induced with IPTG or simply grown overnight were centrifuged at 4000xg for 10 minutes and resuspended gently in phosphate buffer. 100 ul of a Congo red solution at 0.015% was added to each suspension and incubated 5 minutes. The suspensions were then centrifuged at maximum speed to 10 minutes. 100 ul of the supernatant was transferred to a 96-well plate and the absorption at 490nm was recorded. This reading corresponds to Congo

red molecules that are not bound to the biofilm. As controls, wild-type cultures were included in the experiment, as well as bacteria producing no CsgA but a maltose-binding protein instead.

To quantitatively detect the presence of CsgA, an adapted whole-cell ELISA was also performed. The same cultures used for Congo assays were diluted to an OD 600 nm of 0.3 in Tris-buffered saline (TBS), and filtered on a 96-well filter plate (as described in Nguyen *et al.*, Nature Communications, 2014). The filtered cultures were successively incubated with a blocking solution, an anti-his antibody and a secondary antibody, with several wash steps in between each incubation. Finally, an ELISA substrate was added to each well and incubated 5 min. 100 ul of this reaction mixture was transferred to a 96-well plate and the absorbance at 450 and 650nm was recorded.

EXAMPLE IX

Purification of Curli Fibers via His-tag Affinity Purification

Curli fibers can be separated from bacteria using cell lysis and his-tag affinity purification, or using constructs without CsgB proteins to secrete CsgA directly in the culture medium and collecting the produced CsgA using Ni NTA beads. 1) For purification with cell lysis: After expression, cells are harvested by centrifugation at 4000xg for 20 minutes and resuspended in a lysis buffer containing 7M guanidine chloride. A protease inhibitor is added, and the suspension is stored at -20°C overnight. The suspension is thawed on ice, and sonicated to lyse the cells completely (40% amp, 3x25s ON, 35 OFF). The cell debris is pelleted at 10000xg for 30 minutes. 4mL of Ni NTA beads are added to the supernatant, along with 10mM imidazole, and the mixture is incubated with agitation at room temperature for 2 hours. The beads are added to a column, and rinsed three times with phosphate buffer to remove residual imidazole. The column is then washed five times with one column volume with phosphate buffer containing 40 mM imidazole. CsgA is then eluted with phosphate buffer containing 500 mM imidazole. 2) For purification of secreted CsgA: Ni NTA beads are added directly to the culture medium at the time of induction. After overnight expression, the beads are allowed to settle down in a conical flask, and then separated from the supernatant and bacteria by pipetting the bottom layer. As described before, the beads are added to a column, from which CsgA can be eluted.

EXAMPLE X

Characterization Via Electron Microscopy

Scanning electron microscopy (SEM) samples are prepared by filtering biofilms in suspension onto Nucleopore filters (0.22 μ m pore size, GE Healthcare Bio-Sciences) under vacuum. The filters are then washed with 0.1M sodium cacodylate buffer, and fixed with 2% glutaraldehyde and 2% paraformaldehyde for 2h at room temperature. They are then washed with water, and an increasing ethanol step gradient, before drying in a critical point dryer and sputtering with 5nm of Pt/Pd.

EXAMPLE XI

CsgA Proteins Engineered to Allow for Electron Transfer

Fig. 5A shows small peptide domains containing metal centers fused to CsgA (*e.g.*, via SpyCatcher-SpyTag binding), thereby forming a close-packed network of conductive peptides allowing for electron hopping.

Fig. 5B shows CsgA mutants wherein aromatic rings form a π - π stacking network along the fibers, which induces interchain electron delocalization.

Fig. 5C depicts an example of a fusion protein of CsgA with a small protein or domain containing a metal center, in this case, CsgA fused to plastocyanin.

Fig. 5D depicts the series or stacks of aligned amino acid residues in the five pseudo-repeats of the CsgA β -helix structure that have been identified and mutated to aromatic amino acids. The aromatic residues are expected to interact and form π -stacks, leading to electron delocalization and optical phenomena.

EXAMPLE XII

CsgA Fusions and Mutants Are Expressed and Form Curli Nanofibers

As depicted in Fig. 6A, an amyloid-specific dye, Congo Red, binds to curli nanofibers produced by bacteria having CsgA mutations and CsgA fusions (CsgA-plastocyanin and CsgA-rubredoxin), indicating the formation of curli nanofibers. Fig. 6B illustrates that biofilm formation was observed with all CsgA-fusions and mutants.

EXAMPLE XIII

CsgA Fusions and Mutants Are Expressed and Form Curli Nanofibers

As depicted in Fig. 7, electron microscopy reveals the presence of mutant curli fibers with morphology similar to wild-type curli fibers.

Congo Red, a dye that binds specifically to amyloid fibers, was also used to detect the formation of curli fibers. As shown in Fig. 8, all biofilms with aromatic amino acid mutations express curli fibers significantly more than control biofilms without plasmid encoding CsgA.

Fig. 9 shows that all bacteria with aromatic amino acid mutations produce curli nanofibers, whether or not a CsgA inducible promoter was induced, based on a Congo Red pull-down assay and ELISA using an anti-his antibody. Expression levels vary with or without induction.

Similarly, as depicted in Fig. 10, a Congo Red spot test shows that mutant and control bacteria produce curli fibers. Curli fibers were produced, whether or not CsgA was under the control of an IPTG-inducible promoter, and the promoter was induced.

EXAMPLE XVI

Mutant Curli Fibers Exhibit Increased Fluorescence

As depicted in Fig. 11, curli fibers composed of CsgA mutants, *e.g.*, stack 1 tyrosine or tryptophan mutants, exhibit increase fluorescence relative to wild-type control curli fibers.

EXAMPLE XV

Mutant Curli Fibers Isolated from Bacteria

His-tagged CsgA monomers and fibers were isolated using an affinity column, as described herein. About 1-2 mg of proteins was purified per 500 ml of culture. Isolated CsgA monomers spontaneously re-assemble into fibers in buffer. Electron microscopy was used to assess morphology of isolate, purified mutant curli fibers relative to a wild-type control. As shown in Fig. 12, purified mutant fibers exhibit slightly different aggregation behavior relative to wild-type control.

SDS-Page and Western blots using anti-His antibody were used to determine the impurity levels remaining after the purification process. Fig. 13 shows that some impurities remain after purification of mutant and control curli fibers.

EXAMPLE XVI

Purification of Curli Nanofiber Thin Films and Measurement of Electrical Properties

As depicted in Fig. 14A, filtration purification was used to purify wild-type and mutant curli nanofiber thin films, and curli nanofiber films made from CsgA fusions, including CsgA-plastocyanin fusion. The electrical properties of these thin films were then

measured. As shown in Fig. 14B, silver interdigitated electrodes were drawn directly onto dried curli films deposited on filter membranes. Resistance and current-voltage curves were then measured to determine which of the protein films were conductive. Purified films made using CsgA fusions to rubredoxin, plastocyanin, and mitochondrial ferritin were tested, as were purified films made using CsgA mutants, *e.g.*, tyrosine stack mutants and tryptophan stack mutants.

EXAMPLE XVII

Dropcasting of Curli Fiber Comprising Mutant CsgA or CsgA Fused to a Redox Active Domain to Form Thin Films On Substrates and Electrodes

To demonstrate that curli fibers comprising mutant CsgA or CsgA fused to a redox active domain may be used to form thin films, curli fibers comprising wild-type CsgA or curli fibers comprising mutant CsgA or CsgA fused to a redox active domain were dropcast from a 1:1 HFIP:TFA solution onto substrates to form thin films. After dropcasting, samples were rinsed with DI water and dried in air. The morphology of some amyloid fibers has been studied and described previously (see, *e.g.*, Jordens *et al.* (2011) *Biomacromolecules* 12(1): 187-93; Abraham *et al.* (2015) *European Polymer Journal* 65: 268–275; Adamcik *et al.* (2016) *Angew. Chem. Int. Ed.* 55: 618-22; Assenza *et al.* (2014) *Phys. Rev. Lett.* 113: 268103; Usov and Mezzenga (2014) *ACS Nano* 8 (11): 11035-41; and Zhong *et al.* (2014) *Nature Nanotechnology* 9: 858–866 ; the entire contents of each of which are incorporated herein by reference). As shown in Fig. 15A, thin films formed by dropcasting curli fibers comprising wild-type CsgA onto plasma-treated silicon wafers at decreasing concentrations led to the formation of thin films and dispersed curli fibers. For high concentrations (0.75 mg/mL and above), fibrous thin films were obtained. For lower concentrations, dispersed fiber aggregates, and dispersed single fibers were obtained. High magnification scanning electron microscopy (SEM) images of individual curli fibers are shown in Fig. 15B. Curli fibers comprising CsgA fused to either ferritin domain, plastocyanin or rubredoxin, or curli fibers comprising mutant CsgA were also dropcast onto silicon substrate to form thin films as described above, and characterized using SEM. As shown in Fig. 16A and 16B, thin films were successfully formed using both types of curli fibers. The films were also further deposited on glass and stained with Congo Red to confirm the presence of amyloid fibers.

EXAMPLE XVIII

Measurement of Electrical Properties of Thin Films Formed by Dropcasting of Curli Fiber Comprising Mutant CsgA or CsgA Fused to a Redox Active Domain

To characterize the electric conductivity of thin films of curli fibers comprising mutant CsgA or CsgA fused to a redox active domain were formed by dropcasting the disassembled curli fibers from a 1:1 HFIP:TFA solution onto electrodes, and allowing them to reassemble by as the solvent evaporates. Thin films formed using curli fibers comprising CsgA fused to plastocyanin were overlayed onto commercial Micrux interdigitated electrodes. Samples were coated with 5 nm of Pt/Pd prior to SEM imaging. As shown in Fig. 19A, film formed by protein fibers was continuous, and revealed some porosity and nanostructured fibrous morphology. Moreover, as shown in Fig 19B, SEM images of the interface between the film and the electrode showed a fibrous cross-section and good contact between the fibers and the gold electrode. The surface morphology of the thin films formed on interdigitated electrodes was further characterized using SEM with samples that were not coated with Pt/Pd prior to imaging. As shown in Figs. 20A and 20B, thin films formed using curli fibers comprising CsgA fused to plastocyanin formed thick films that fully covered the electrode.

To characterize the conductivity of thin films using curli fiber comprising mutated CsgA or CsgA fused to a redox active domain, a probe station was used to perform voltage sweeps from 0 to 20 V and the current response was measured (Figures 21, 23, 24, 25). The engineered curli fibers with the highest current at a given voltage or steepest curves correspond to curli fibers with the highest conductivity. For CsgA-plastocyanin fusions, -2 V to +2 V, and -30 V to +30 V sweeps were also performed (Figures 22A and 22B). A forward and reverse sweep from 0 V to 20 V, and 20 V to 0 V was also recorded and shows little hysteresis between forward and reverse measurements (Figure 22C).

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Claims

1. A method of producing a genetically modified bacterium comprising genetically altering a bacterium to include a nucleic acid sequence encoding a mutant amyloid polypeptide having a series of aligned aromatic groups, wherein the nucleic acid sequence is under the control of a promoter to express the mutant amyloid polypeptide.
2. A method of producing a genetically modified bacterium comprising genetically altering a bacterium to include a nucleic acid sequence encoding a fusion protein comprising an amyloid polypeptide fused to a redox active domain, wherein the nucleic acid sequence is under the control of a promoter to express the fusion protein.
3. The method of claim 1 or claim 2, wherein the amyloid polypeptide is selected from the group consisting of CsgA, CspA, a beta-lactoglobulin, a lysozyme, Sup35, and an amyloid-beta peptide.
4. The method of any one of claims 1-3, wherein the amyloid polypeptide is CsgA.
5. The method of claim 2, wherein the redox active domain is selected from the group consisting of: a metalloprotein, a redox enzyme, a binding domain, and a stimuli-responsive peptide.
6. The method of claim 2 or claim 5, wherein the redox active domain is selected from the group consisting of: plastocyanin, an oxygen-carrier protein, a microperoxidase, a cytochrome, a copper-binding protein, an iron-binding protein, a gold-binding domain, a metalloenzyme, rubredoxin, and a fragment thereof.
7. The method of any one of claims 1-6, wherein the bacterium is *E. coli*.
8. The method of any one of claims 1-7, wherein the bacterium is non-pathogenic.
9. The method of claim 1, wherein the aromatic group is present on a standard amino acid or a nonstandard amino acid.

10. The method of claim 1 or claim 9, wherein the aromatic group is present on a standard amino acid selected from the group consisting of phenylalanine, tryptophan, tyrosine and histidine.
11. The method of claim 1 or claim 9, wherein the aromatic group is present on a nonstandard amino acid selected from the group consisting of 2-thienylalanine, 3-thienylalanine, acetylphenylalanine and azidophenylalanine.
12. The method of any one of claims 1 or 9-11, wherein the mutant amyloid polypeptide further comprises a redox active domain.
13. The method of any one of claims 1 or 9-11, further comprising genetically altering the bacterium to include second nucleic acid sequence encoding a fusion protein comprising an amyloid polypeptide fused to a redox active domain.
14. The method of claim 12 or claim 13, wherein the amyloid polypeptide is selected from the group consisting of CsgA, CspA, a beta-lactoglobulin, a lysozyme, Sup35, and an amyloid-beta peptide.
15. The method of any one of claims 12-14, wherein the redox active domain is selected from the group consisting of: a metalloprotein, a redox enzyme, a binding domain, and a stimuli-responsive peptide.
16. The method of any one of claims 12-14, wherein the redox active domain is selected from the group consisting of: plastocyanin, an oxygen-carrier protein, a microperoxidase, a cytochrome, a copper-binding protein, an iron-binding protein, a gold-binding domain, a metalloenzyme, rubredoxin, and a fragment thereof
17. A method of making an electrically conductive biofilm comprising
proliferating a genetically-altered bacterium comprising a nucleic acid sequence encoding a mutant amyloid polypeptide having a series of aligned aromatic groups, to produce a population of genetically-altered bacteria expressing the nucleic acid sequence,
and allowing the bacteria to form a biofilm comprising an amyloid-based structure formed from a plurality of mutant amyloid polypeptides, wherein the amyloid-based structure

comprises a series of aligned aromatic groups forming electrically conductive pi-pi stacking along the length of the amyloid-based structure.

18. A method of making an electrically conductive biofilm comprising proliferating a genetically-altered bacterium comprising a nucleic acid sequence encoding a fusion protein, wherein the fusion protein comprises an amyloid polypeptide fused to a redox active domain, to produce a population of genetically-altered bacteria expressing the nucleic acid sequence,
and allowing the bacteria to form a biofilm comprising an amyloid-based structure formed from a plurality of fusion proteins, wherein the amyloid-based structure comprises a network of metal centers that mediate electron hopping between the metal centers.
19. The method of claim 17 or claim 18, wherein the amyloid polypeptide is selected from the group consisting of CsgA, CspA, a beta-lactoglobulin, a lysozyme, Sup35, and an amyloid-beta peptide.
20. The method of any one of claims 17-19, wherein the amyloid polypeptide is CsgA.
21. The method of claim 18 or claim 19, wherein the redox active domain is selected from the group consisting of a metalloprotein, a redox enzyme, a binding domain, and a stimuli-responsive peptide.
22. The method of claim 18 or claim 19, wherein the redox active domain is selected from the group consisting of plastocyanin, an oxygen-carrier protein, a microperoxidase, a cytochrome, a copper-binding protein, an iron-binding protein, a gold-binding domain, a metalloenzyme, rubredoxin, and a fragment thereof.
23. The method of any one of claims 17-22, wherein the bacterium is *E. coli*.
24. The method of any one of claims 17-23, wherein the bacterium is non-pathogenic.
25. The method of claim 17, wherein the aromatic group is present on a standard amino acid or a nonstandard amino acid.

26. The method of claim 17 or claim 25, wherein the aromatic group is present on a standard amino acid selected from the group consisting of phenylalanine, tryptophan, tyrosine and histidine.
27. The method of claim 17 or claim 25, wherein the aromatic group is present on a nonstandard amino acid selected from the group consisting of 2-thienylalanine, 3-thienylalanine, acetylphenylalanine and azidophenylalanine.
28. The method of any one of claims 17 or 25-27, wherein the genetically modified bacterium further comprises a second nucleic acid sequence encoding a fusion protein comprising an amyloid polypeptide fused to a redox active domain.
29. The method of any one of claims 17 or 25-28, wherein the aligned aromatic groups form a pi-pi stack aligned in a geometric configuration selected from the group consisting of sandwich-type pi-stacks, t-shaped pi-stacks, parallel-displaced pi-stacks.
30. A method of making an electrically conductive amyloid-based structure from a genetically-modified bacterium comprising
proliferating a genetically-altered bacterium, wherein the genetically-altered bacterium comprises a nucleic acid sequence encoding a mutant amyloid polypeptide having a series of aligned aromatic groups, to produce a population of genetically-altered bacteria expressing the nucleic acid sequence,
and allowing the population of genetically-altered bacteria to produce an amyloid-based structure formed from a plurality of the mutant amyloid polypeptides,
wherein the amyloid-based structure comprises a series of aligned aromatic groups forming electrically conductive pi-pi stacking along the length of the amyloid-based structures.
31. A method of making an electrically conductive amyloid-based structure from a genetically-modified bacterium comprising
proliferating a genetically-altered bacterium comprising a nucleic acid sequence encoding a fusion protein comprising an amyloid polypeptide fused to a redox active domain, to produce a population of genetically-altered bacteria expressing the nucleic acid sequence,

allowing the population of genetically-altered bacteria to produce an amyloid-based structure formed from a plurality of the fusion proteins,

wherein the amyloid-based structure comprises a network of metal centers that mediate electron hopping between the metal centers.

32. The method of claim 30 or claim 31, wherein the amyloid polypeptide is selected from the group consisting of CsgA, CspA, a beta-lactoglobulin, a lysozyme, Sup35, and an amyloid-beta peptide.

33. The method of any one of claims 30-32, wherein the amyloid polypeptide is CsgA.

34. The method of any one of claims 31-33, wherein the redox active domain is selected from the group consisting of a metalloprotein, a redox enzyme, a binding domain, and a stimuli-responsive peptide.

35. The method of any one of claims 31-33, wherein the redox active domain is selected from the group consisting of plastocyanin, an oxygen-carrier protein, a microperoxidase, a cytochrome, a copper-binding protein, an iron-binding protein, a gold-binding domain, a metalloenzyme, rubredoxin, and a fragment thereof.

36. The method of any one of claims 30-35, wherein the bacterium is *E. coli*.

37. The method of any one of claims 30-36, wherein the bacterium is non-pathogenic.

38. The method of claim 30, wherein the aromatic group is present on a standard amino acid or a nonstandard amino acid.

39. The method of claim 30 or claim 38, wherein the aromatic group is present on a standard amino acid selected from the group consisting of phenylalanine, tryptophan, tyrosine and histidine.

40. The method of claim 30 or claim 38, wherein the aromatic group is present on a nonstandard amino acid selected from the group consisting of 2-thienylalanine, 3-thienylalanine, acetylphenylalanine and azidophenylalanine.

41. The method of any one of claims 30 or 38-40, wherein the genetically-modified bacterium further comprises a second nucleic acid sequence encoding a fusion protein comprising an amyloid polypeptide fused to a redox active domain.
42. A genetically-modified bacterium comprising a nucleic acid sequence encoding a mutant amyloid protein having a series of aligned aromatic groups, wherein the nucleic acid sequence is under the control of a promoter to express the mutant amyloid protein.
43. A genetically-modified bacterium comprising a nucleic acid sequence encoding a fusion protein comprising an amyloid polypeptide fused to a redox active domain, wherein the nucleic acid sequence is under the control of a promoter to express the fusion protein.
44. A non-naturally occurring mutant amyloid polypeptide having a series of aligned aromatic groups.
45. The mutant amyloid polypeptide of claim 44, wherein the amyloid polypeptide is selected from the group consisting of CsgA, CspA, a beta-lactoglobulin, a lysozyme, Sup35, and an amyloid-beta peptide.
46. The mutant amyloid polypeptide of claim 44 or claim 45, wherein the amyloid polypeptide is CsgA.
47. The mutant amyloid polypeptide of any one of claims 44-46, wherein the aromatic group is present on a standard amino acid or a nonstandard amino acid.
48. The mutant amyloid protein of any one of claims 44-47, wherein the aromatic group is present on a standard amino acid selected from the group consisting of phenylalanine, tryptophan, tyrosine and histidine.
49. The mutant amyloid polypeptide of any one of claims 44-47, wherein the aromatic group is present on a nonstandard amino acid selected from the group consisting of 2-thienylalanine, 3-thienylalanine, acetylphenylalanine and azidophenylalanine.

50. The mutant amyloid polypeptide of any one of claims 44-49, further comprising a redox active domain.
51. The mutant amyloid polypeptide of claim 50, wherein the redox active domain is selected from the group consisting of a metalloprotein, a redox enzyme, a binding domain, and a stimuli-responsive peptide.
52. The mutant amyloid polypeptide of claim 50, wherein the redox active domain is selected from the group consisting of plastocyanin, an oxygen-carrier protein, a microperoxidase, a cytochrome, a copper-binding protein, an iron-binding protein, a gold-binding domain, a metalloenzyme, rubredoxin, and a fragment thereof.
53. A fusion protein comprising an amyloid polypeptide fused to a redox active domain.
54. The fusion protein of claim 53, wherein the amyloid polypeptide is selected from the group consisting of CsgA, CspA, a beta-lactoglobulin, a lysozyme, Sup35, and an amyloid-beta peptide.
55. The fusion protein of claim 53 or claim 54, wherein the amyloid polypeptide is CsgA.
56. The fusion protein of any one of claims 53-55, wherein the redox active domain is selected from the group consisting of a metalloprotein, a redox enzyme, a binding domain, and a stimuli-responsive peptide.
57. The fusion protein of any one of claims 53-55, wherein the redox active domain is selected from the group consisting of plastocyanin, an oxygen-carrier protein, a microperoxidase, a cytochrome, a copper-binding protein, an iron-binding protein, a gold-binding domain, a metalloenzyme, rubredoxin, and a fragment thereof.
58. The fusion protein of any one of claims 53-57, wherein the amyloid polypeptide comprises a series of aligned aromatic groups.
59. The fusion protein of claim 58, wherein the aromatic group is present on a standard amino acid or a nonstandard amino acid.

60. The fusion protein of claim 58 or claim 59, wherein the aromatic group is present on a standard amino acid selected from the group consisting of phenylalanine, tryptophan, tyrosine and histidine.
61. The fusion protein of claim 58 or claim 59, wherein the aromatic group is present on a nonstandard amino acid selected from the group consisting of 2-thienylalanine, 3-thienylalanine, acetylphenylalanine and azidophenylalanine.
62. A non-naturally occurring amyloid-based structure formed from a plurality of non-naturally occurring mutant amyloid polypeptides having a series of aligned aromatic groups, wherein the amyloid-based structure has a series of aligned aromatic groups forming electrically conductive pi-pi stacking along the length of the amyloid-based structure.
63. A non-naturally occurring amyloid-based structure formed from a plurality of fusion proteins comprising an amyloid polypeptide fused to a redox active domain, wherein the amyloid-based structure comprises a network of metal centers that mediate electron hopping between the metal centers.
64. A nucleic acid sequence encoding the mutant amyloid polypeptide of any one of claims 45-52.
65. A nucleic acid sequence encoding the fusion protein of any one of claims 53-61.
66. A nucleic acid sequence encoding a non-naturally occurring mutant amyloid polypeptide having a series of aligned aromatic groups.
67. The nucleic acid sequence of claim 66, wherein the amyloid polypeptide is selected from the group consisting of CsgA, CspA, a beta-lactoglobulin, a lysozyme, Sup35, and an amyloid-beta peptide.
68. The nucleic acid sequence of claim 66 or claim 67, wherein the aromatic group is present on a standard amino acid or a nonstandard amino acid.

69. The nucleic acid sequence of any one of claims 66-68, wherein the aromatic group is present on a standard amino acid selected from the group consisting of phenylalanine, tryptophan, tyrosine and histidine.
70. The nucleic acid sequence of any one of claims 66-68, wherein the aromatic group is present on a nonstandard amino acid selected from the group consisting of 2-thienylalanine, 3-thienylalanine, acetylphenylalanine and azidophenylalanine.
71. The nucleic acid sequence of any one of claims 66-70, wherein the amyloid polypeptide further comprising a redox active domain.
72. The nucleic acid sequence of claim 71, wherein the redox active domain is selected from the group consisting of a metalloprotein, a redox enzyme, a binding domain, and a stimuli-responsive peptide.
73. The nucleic acid sequence of claim 71, wherein the redox active domain is selected from the group consisting of plastocyanin, an oxygen-carrier protein, a microperoxidase, a cytochrome, a copper-binding protein, an iron-binding protein, a gold-binding domain, a metalloenzyme, rubredoxin, and a fragment thereof.
74. A nucleic acid sequence encoding a fusion protein comprising an amyloid polypeptide fused to a redox active domain.
75. The nucleic acid sequence of claim 74, wherein the amyloid polypeptide is selected from the group consisting of CsgA, CspA, a beta-lactoglobulin, a lysozyme, Sup35, and an amyloid-beta peptide.
76. The nucleic acid sequence of claim 74 or claim 75, wherein the redox active domain is selected from the group consisting of a metalloprotein, a redox enzyme, a binding domain, and a stimuli-responsive peptide.
77. The nucleic acid sequence of claim 74 or claim 75, wherein the redox active domain is selected from the group consisting of plastocyanin, an oxygen-carrier protein, a

microperoxidase, a cytochrome, a copper-binding protein, an iron-binding protein, a gold-binding domain, a metalloenzyme, rubredoxin, and a fragment thereof.

78. The nucleic acid sequence of any one of claims 74-77, wherein the amyloid polypeptide comprises a series of aligned aromatic groups.

79. The nucleic acid sequence of claim 78, wherein the aromatic group is present on a standard amino acid or a nonstandard amino acid.

80. The nucleic acid sequence of claim 78 or claim 79, wherein the aromatic group is present on a standard amino acid selected from the group consisting of phenylalanine, tryptophan, tyrosine and histidine.

81. The nucleic acid sequence of claim 78 or claim 79, wherein the aromatic group is present on a nonstandard amino acid selected from the group consisting of 2-thienylalanine, 3-thienylalanine, acetylphenylalanine and azidophenylalanine.

82. A vector comprising the nucleic acid sequence of any one of claims 64-81.

83. The vector of claim 82, wherein the nucleic acid sequence is operably-linked to an inducible promoter.

84. The vector of claim 83, wherein the nucleic acid is operably-linked to a constitutive promoter.

85. A bacterium comprising the nucleic acid sequence of any one of claims 64-81, or a vector of any one of claims 82-84.

86. A biofilm comprising a bacterium expressing a nucleic acid sequence encoding a non-naturally occurring mutant amyloid polypeptide having a series of aligned aromatic groups.

87. A biofilm comprising a bacterium expressing a nucleic acid sequence encoding a fusion protein comprising an amyloid polypeptide fused to a redox active domain.

88. A biofilm comprising the non-naturally occurring amyloid-based structure of claim 62 or claim 63.
89. The method of any of claims 2, 18 or 31, wherein the redox active domain comprises plastocyanin or a fragment thereof.
90. The method of any of claims 2, 18 or 31, wherein the redox active domain comprises rubredoxin or a fragment thereof.
91. The method of any one of claims 17, 18, 30 or 31, wherein the amyloid-based structure comprises a curli fiber.
92. The amyloid-based structure of claim 62 or claim 63, wherein the amyloid-based structure comprises a curli fiber.

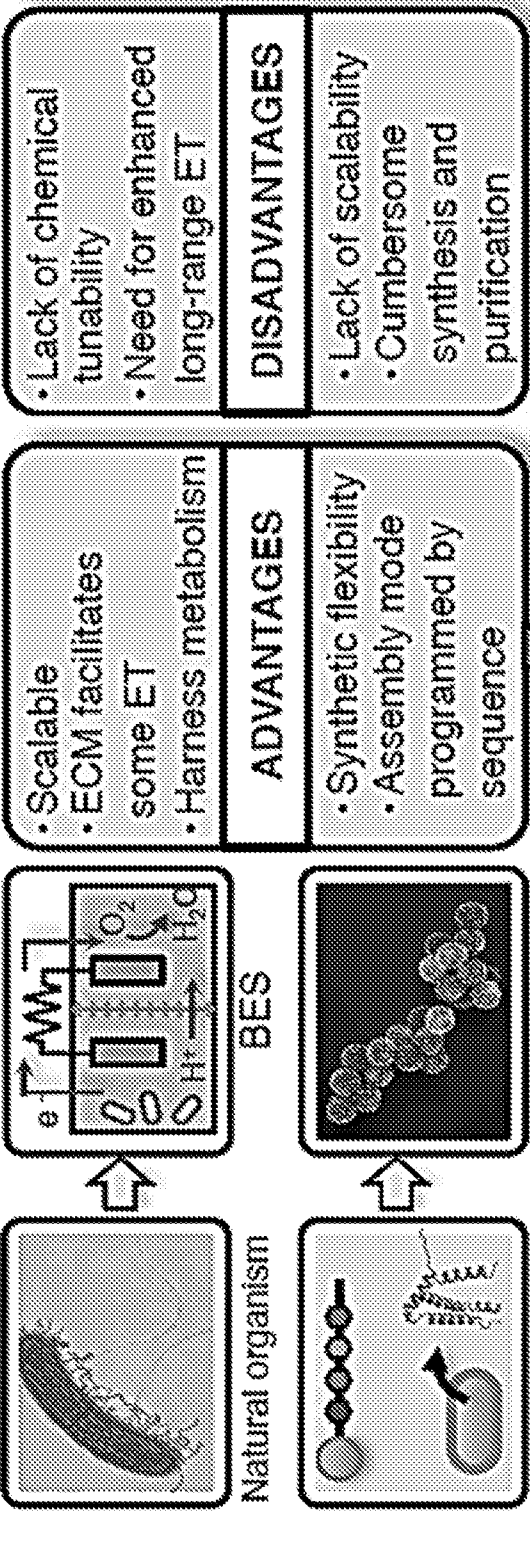


Fig. 1A

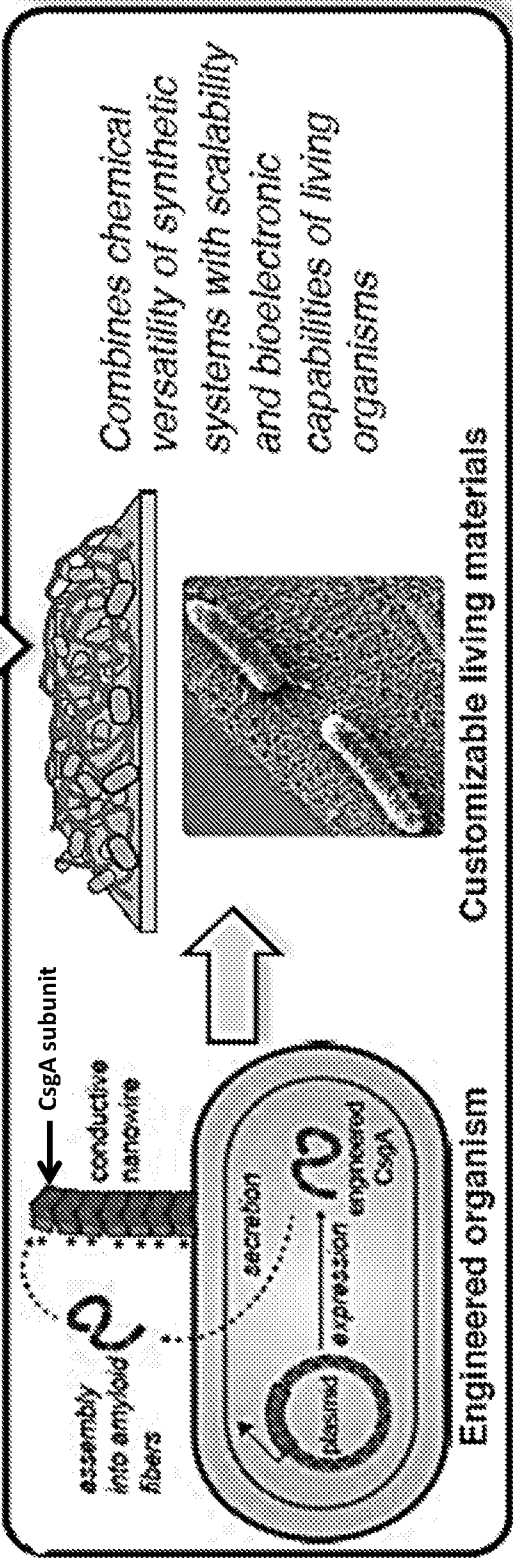
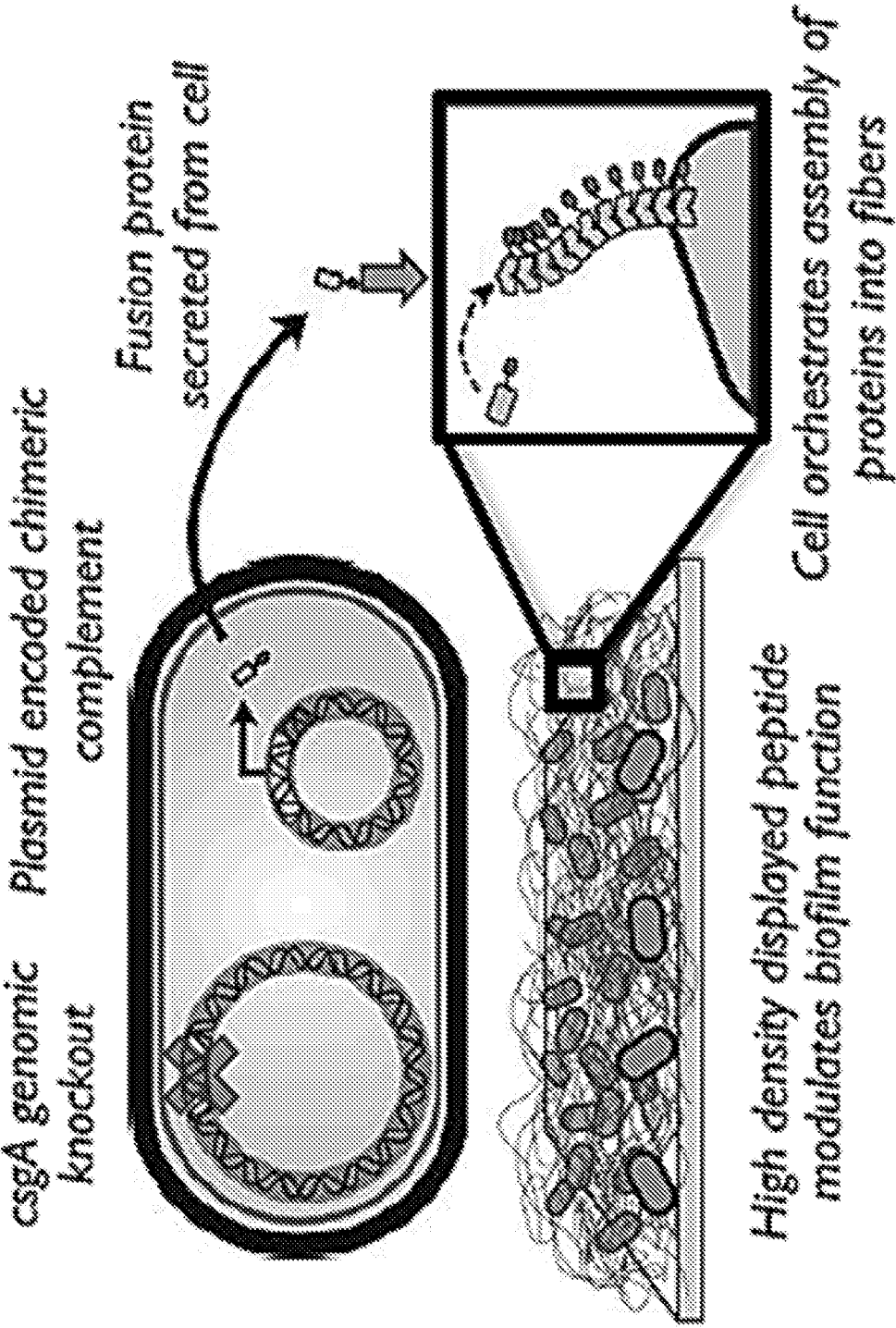


Fig. 1B

Fig. 2A



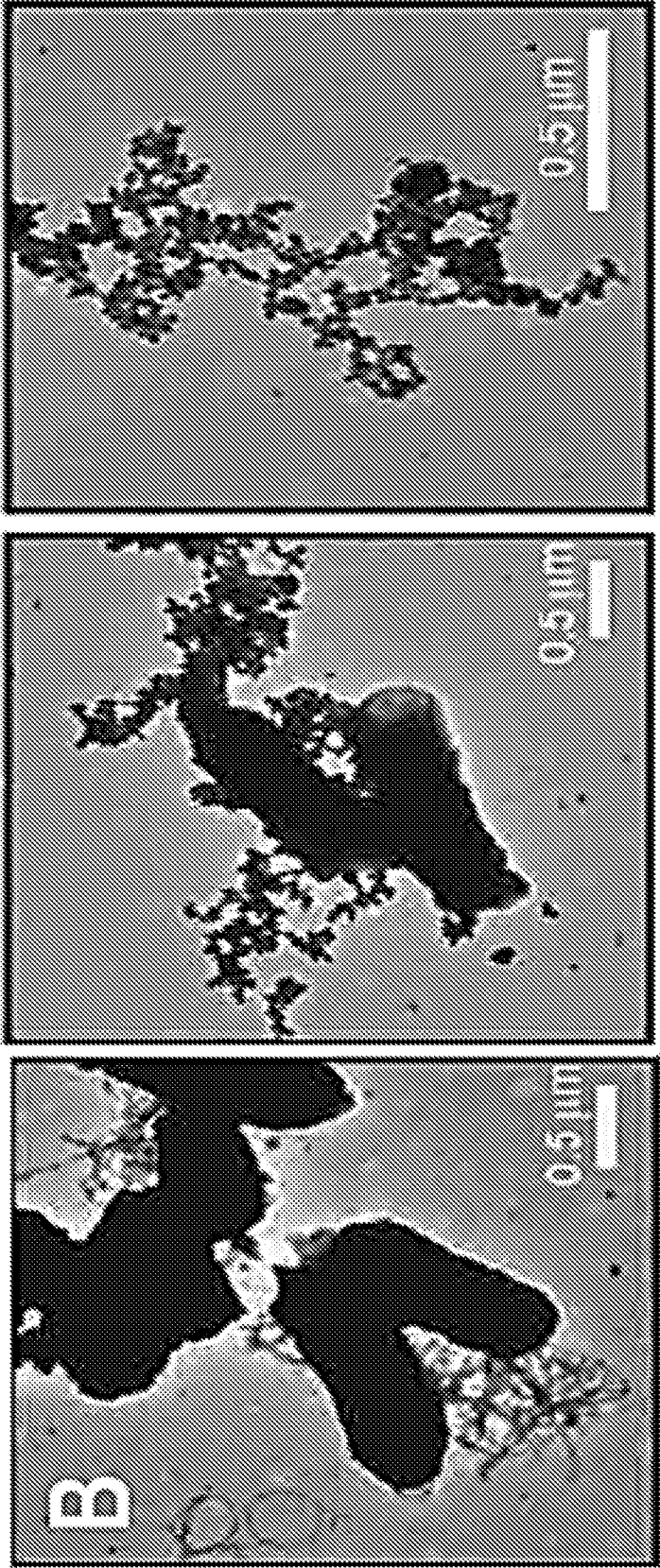


Fig. 2B

Fig. 2C

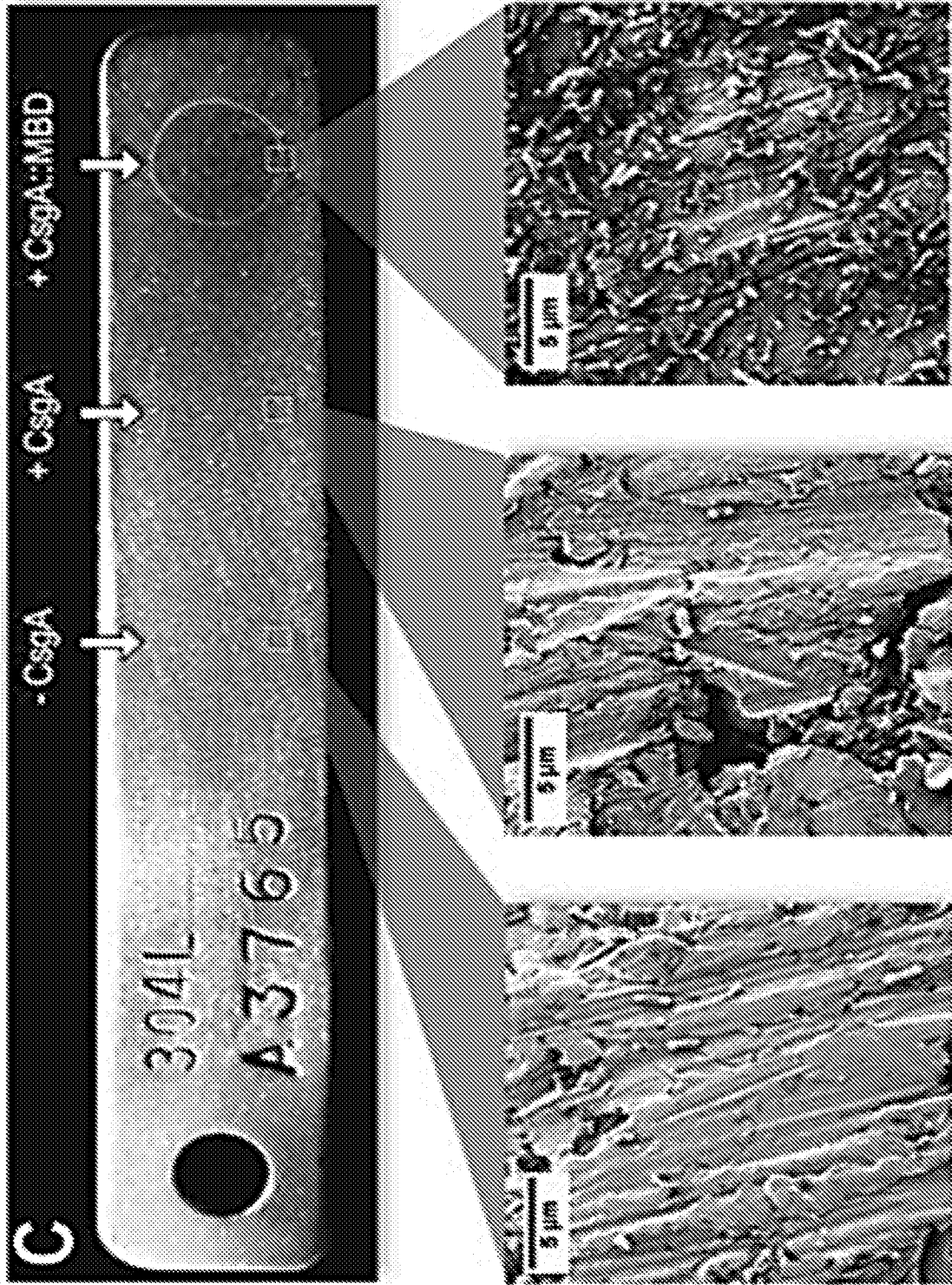


Fig. 2D

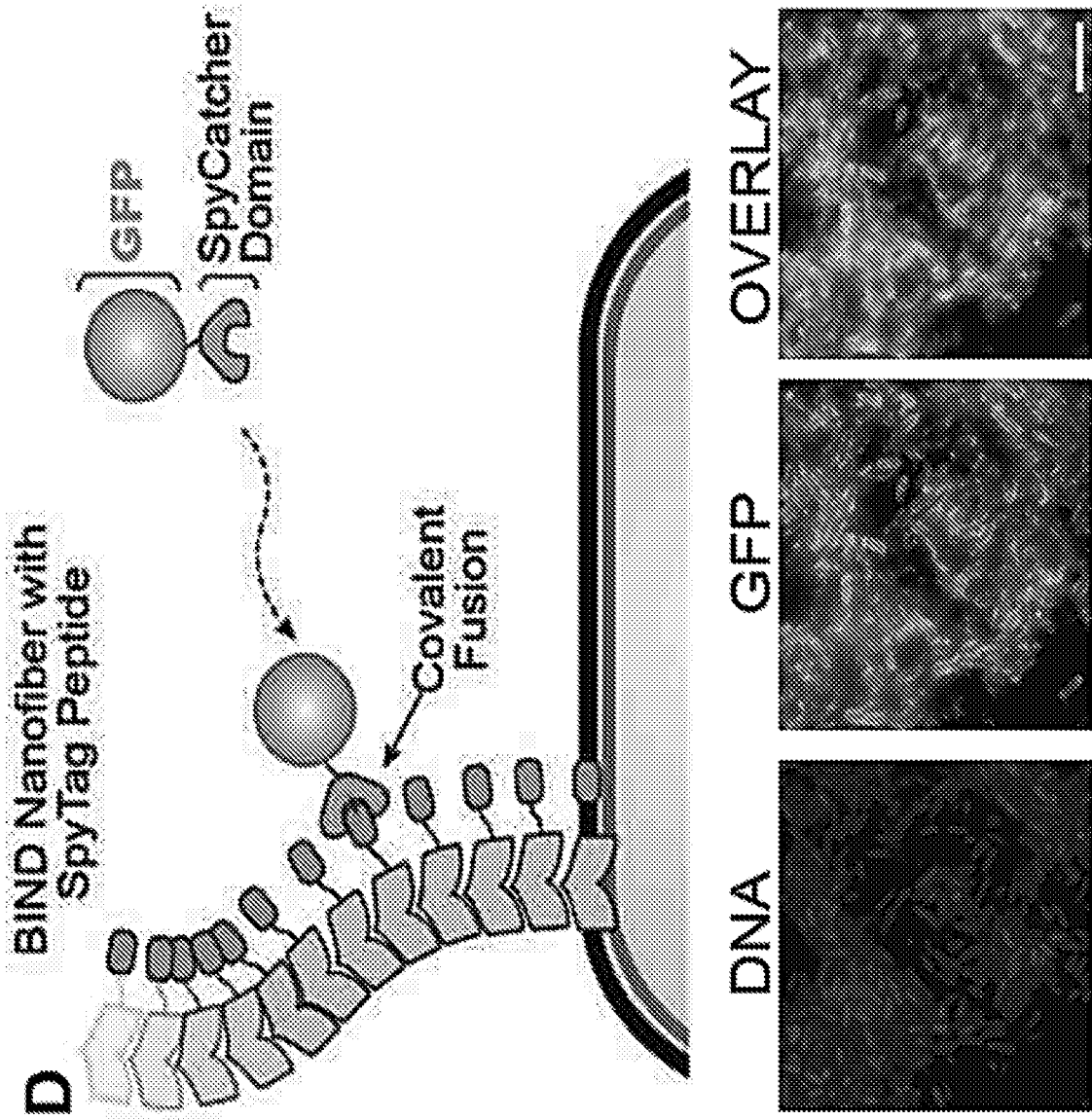


Fig. 3A

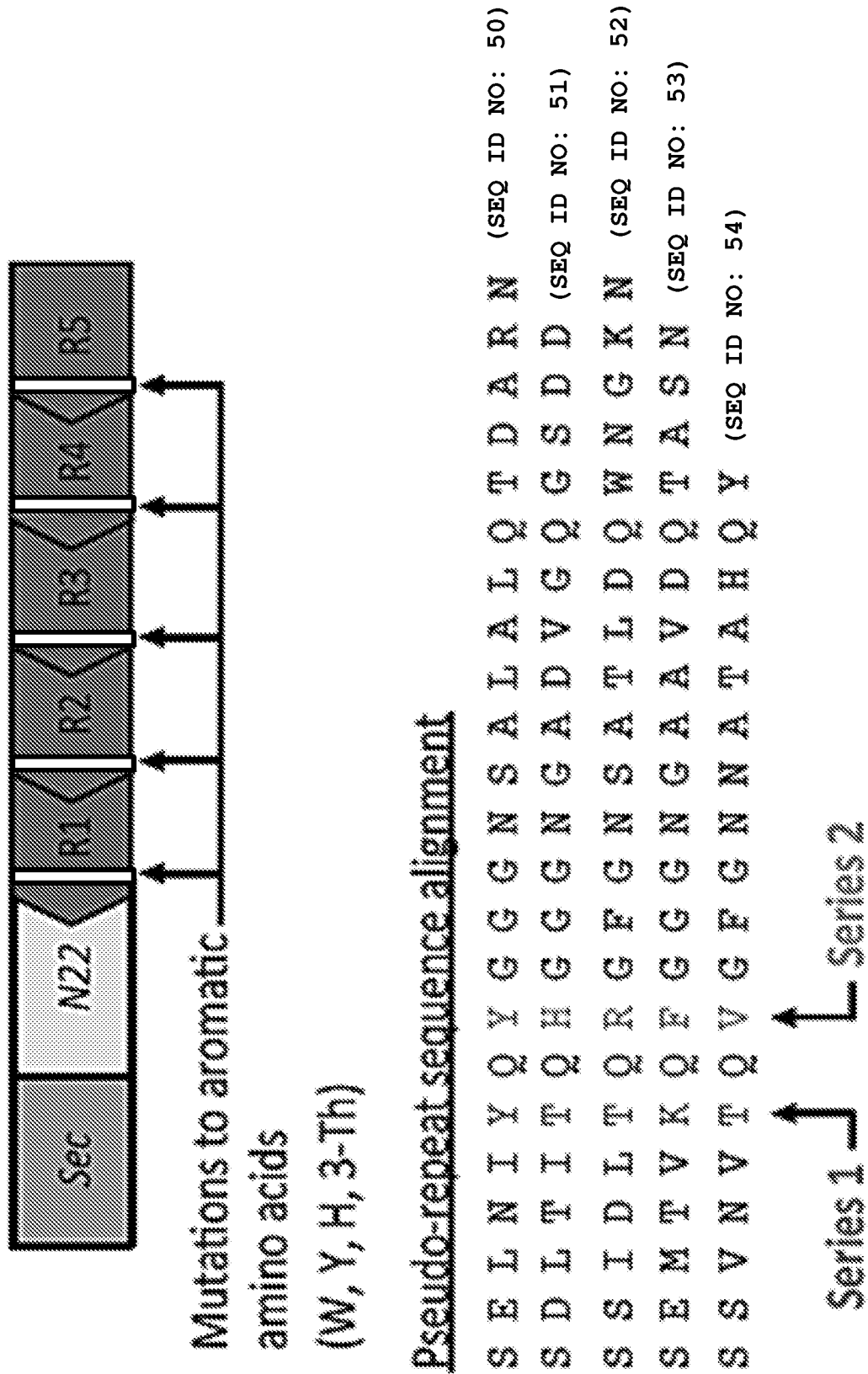


Fig. 3B

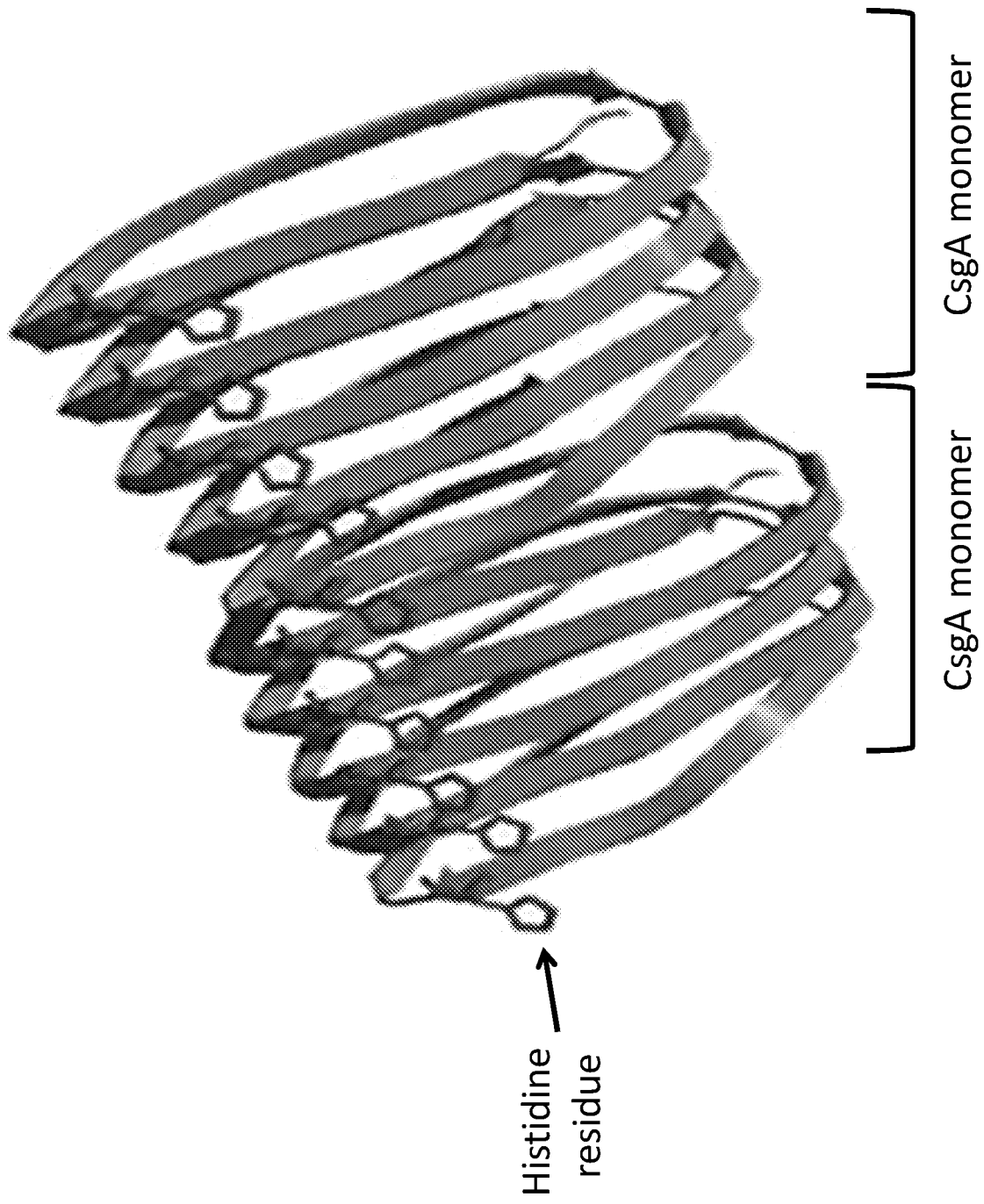


Fig. 4A

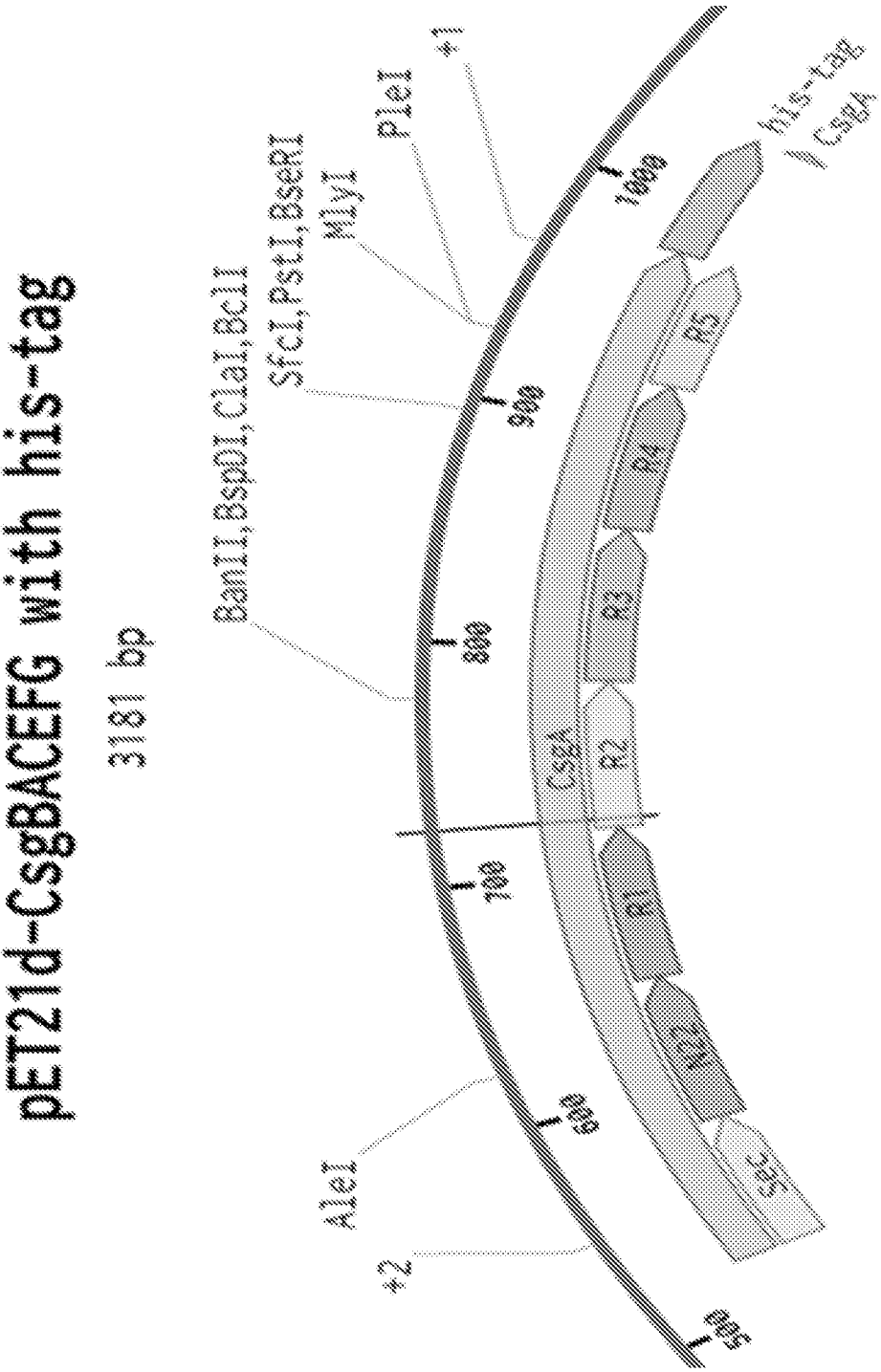
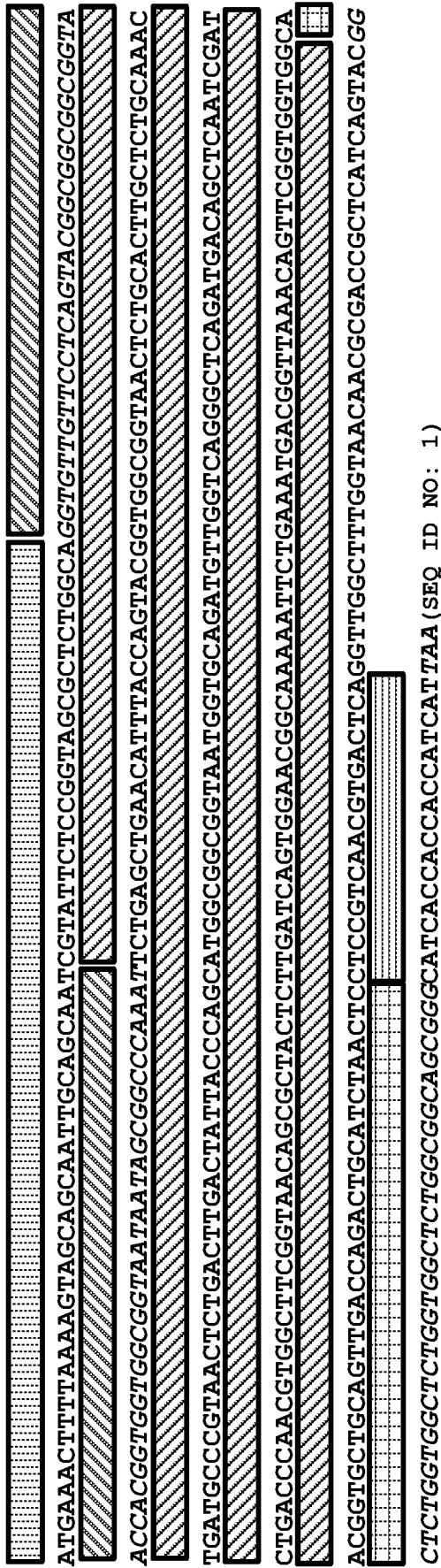


Fig. 4B

Wild-type CsgA with His-tag at the N-terminus nucleic acid sequence:



Wild-type CsgA with His-tag at the N-terminus amino acid sequence:

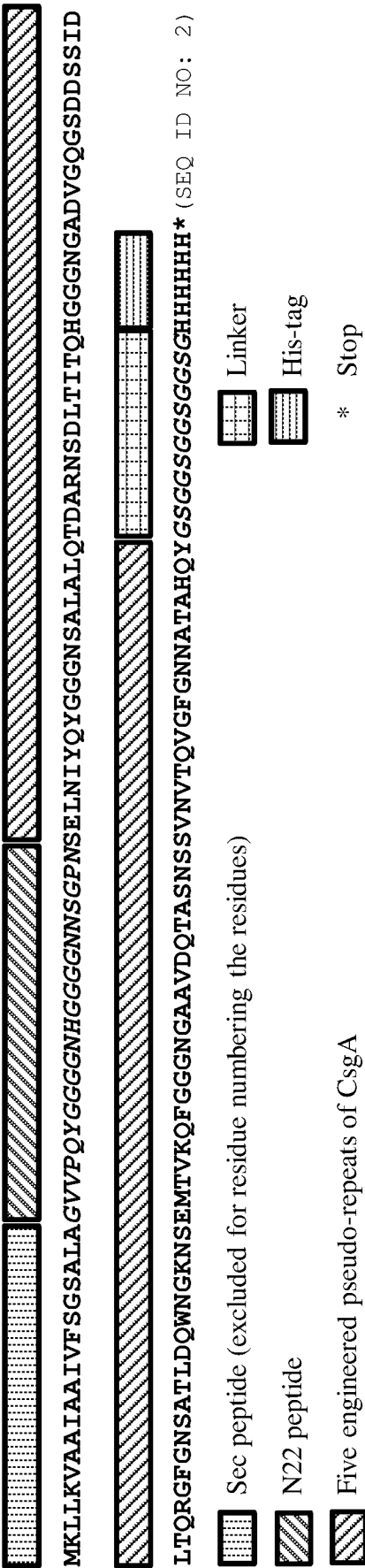


Fig. 5A

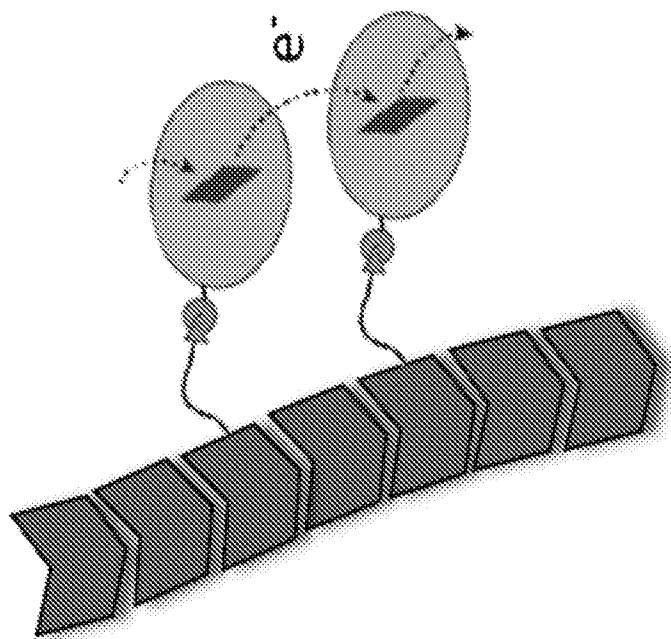
Electron hopping

Fig. 5B

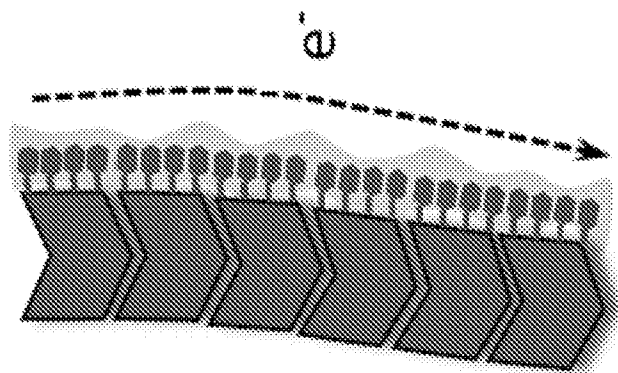
Electron delocalization

Fig. 5D

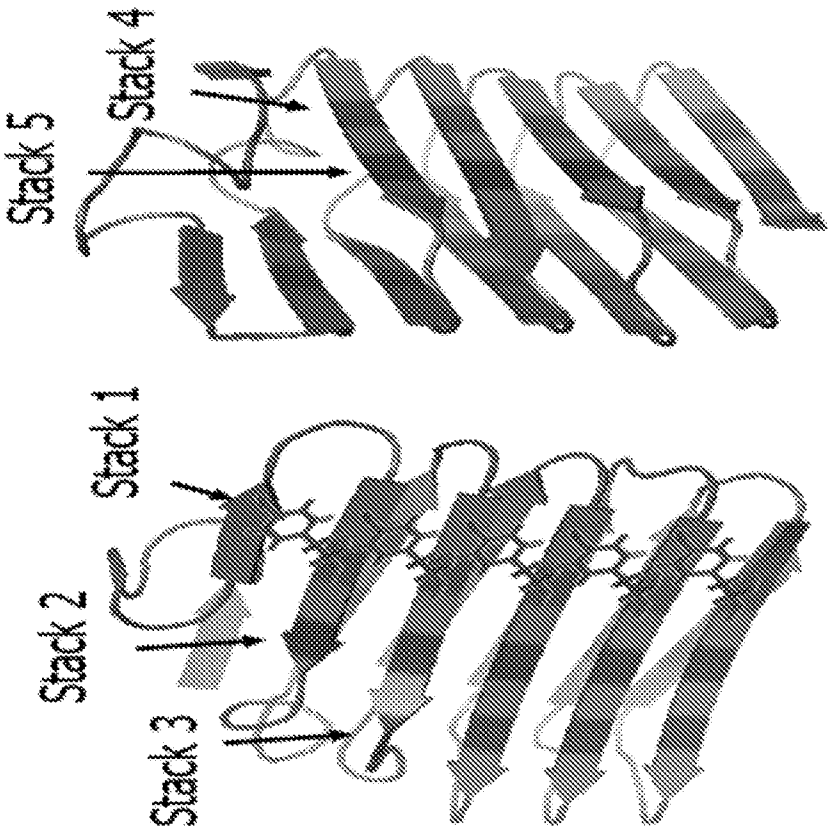
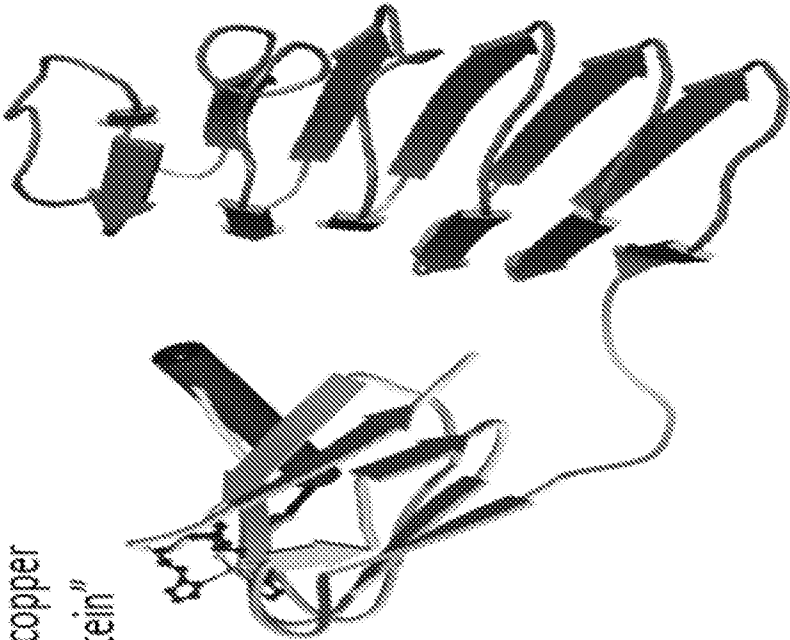


Fig. 5C

Plastocyanin
"blue copper
protein"



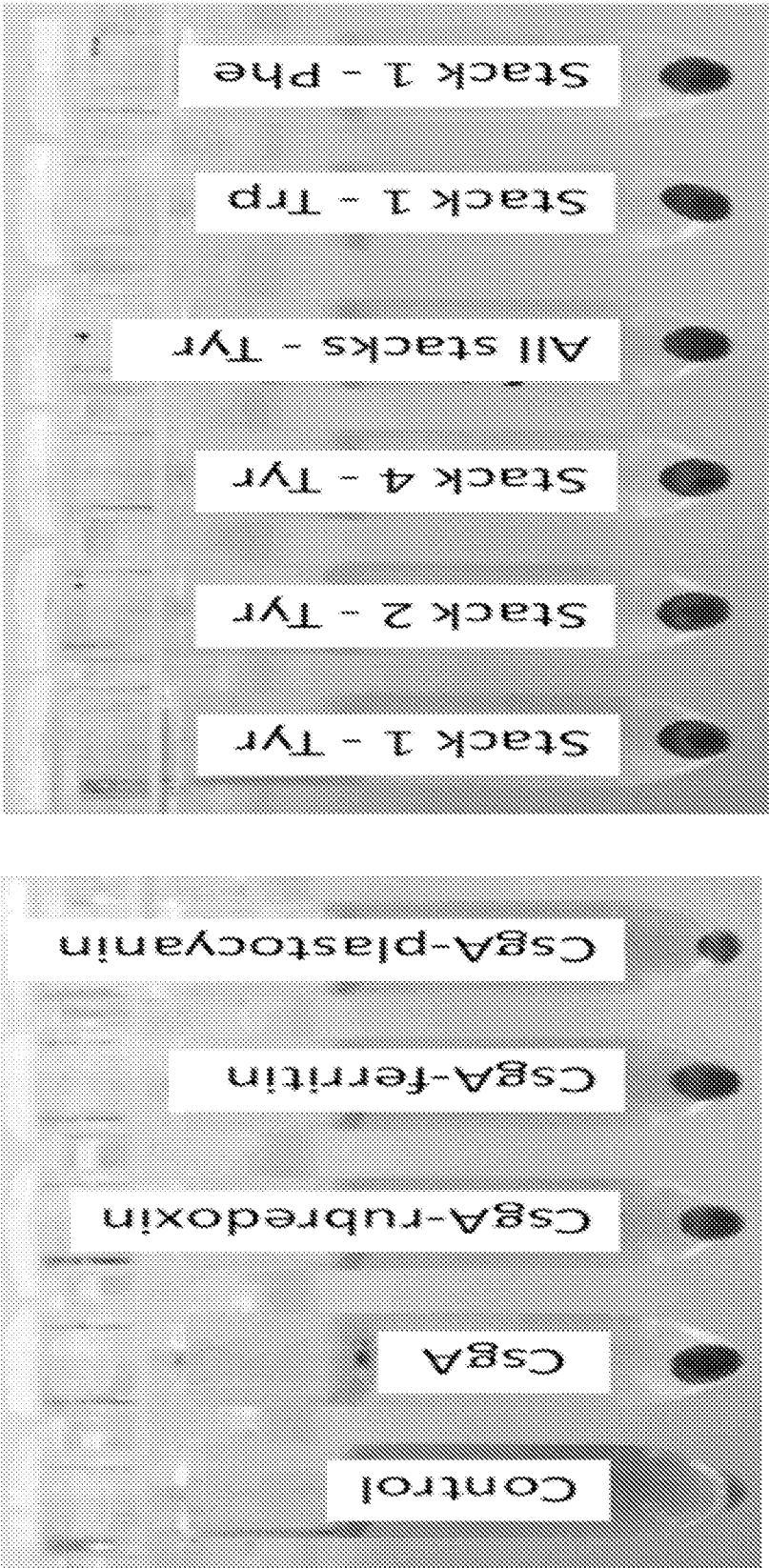


Fig. 6A



Fig. 6B

Fig. 7

Electron microscopy reveals the presence of mutant fibers with morphology similar to wild-type fibers

Scale bars: 500 nm

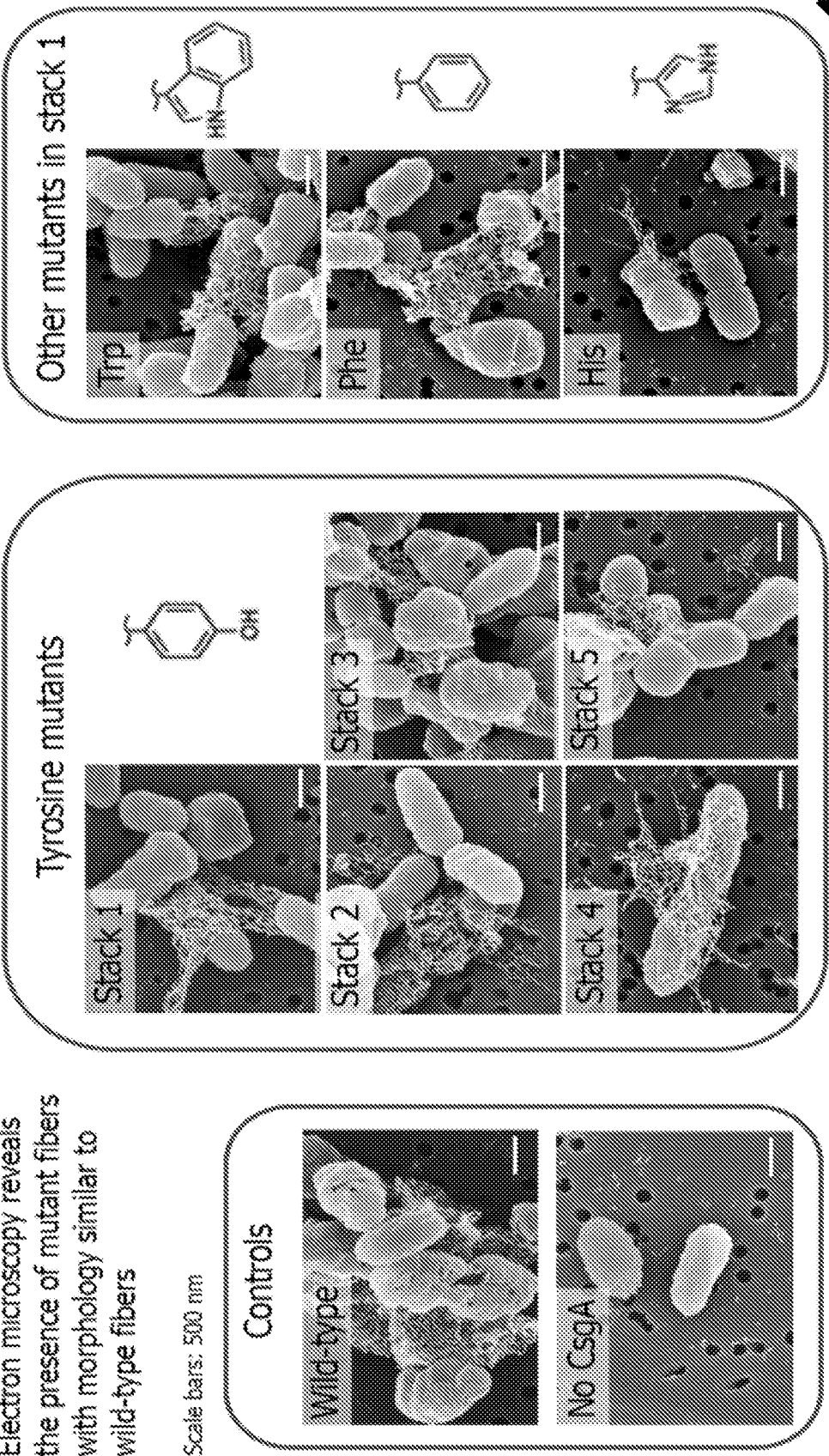


Fig. 8

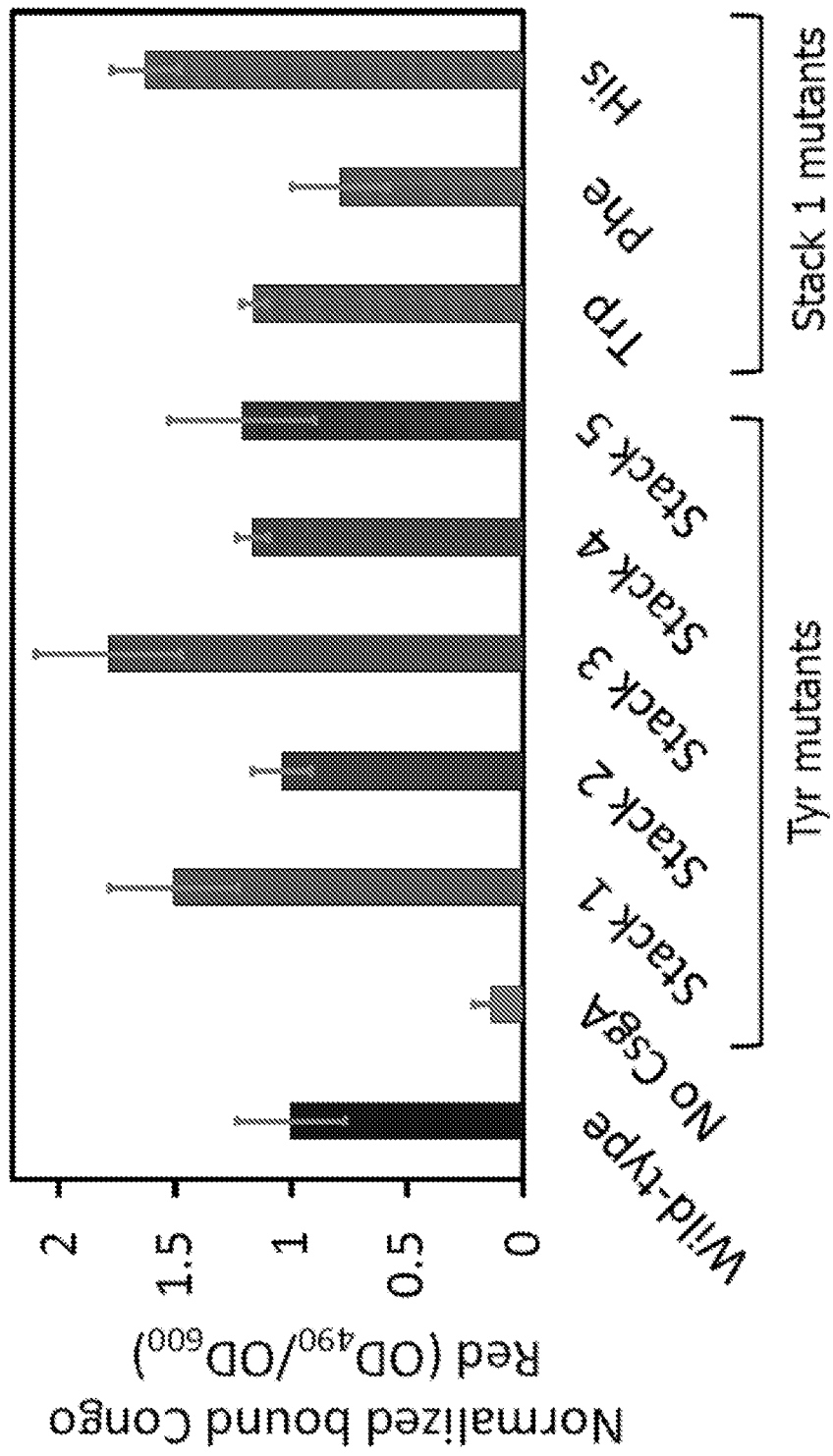


Fig. 9

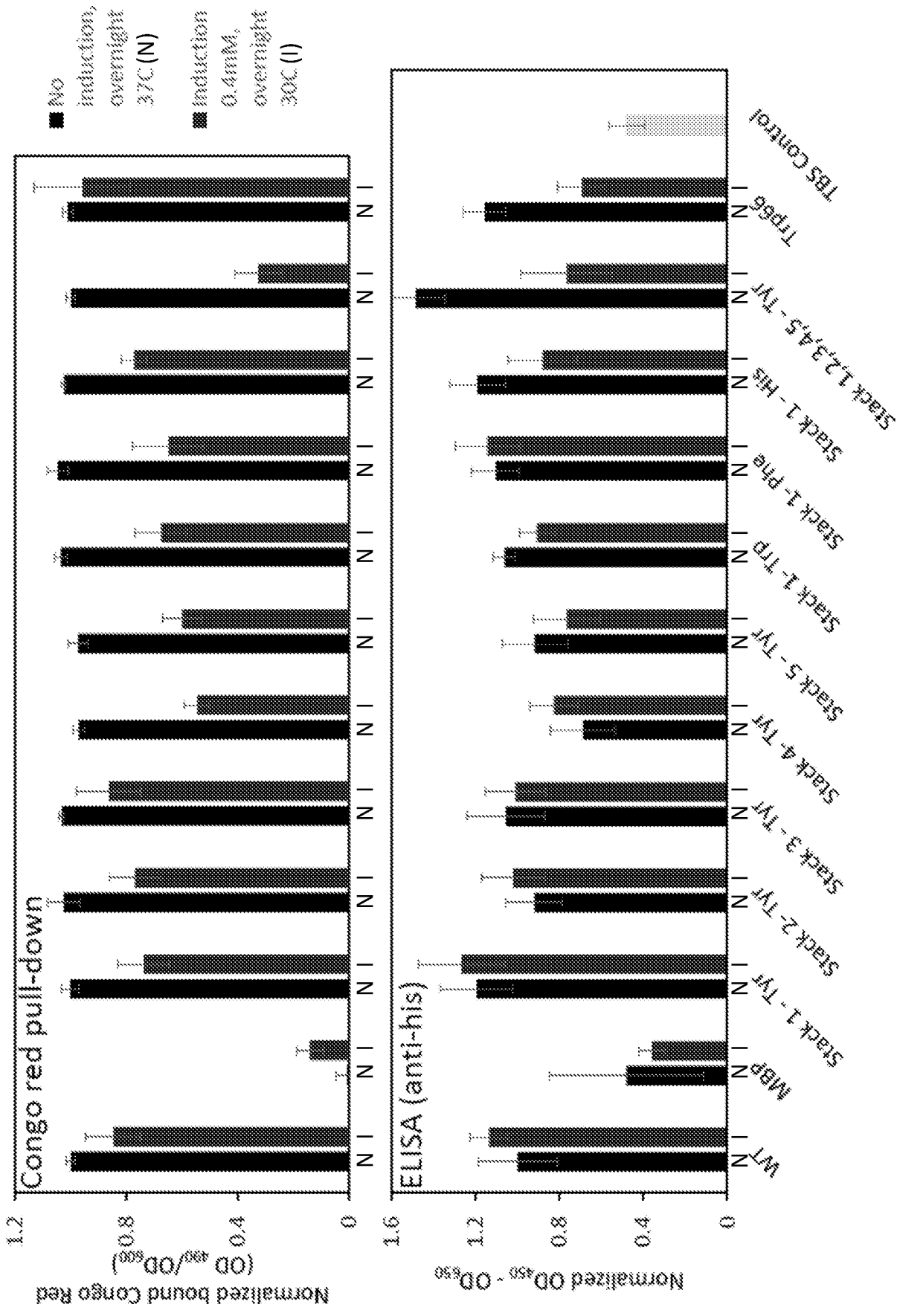
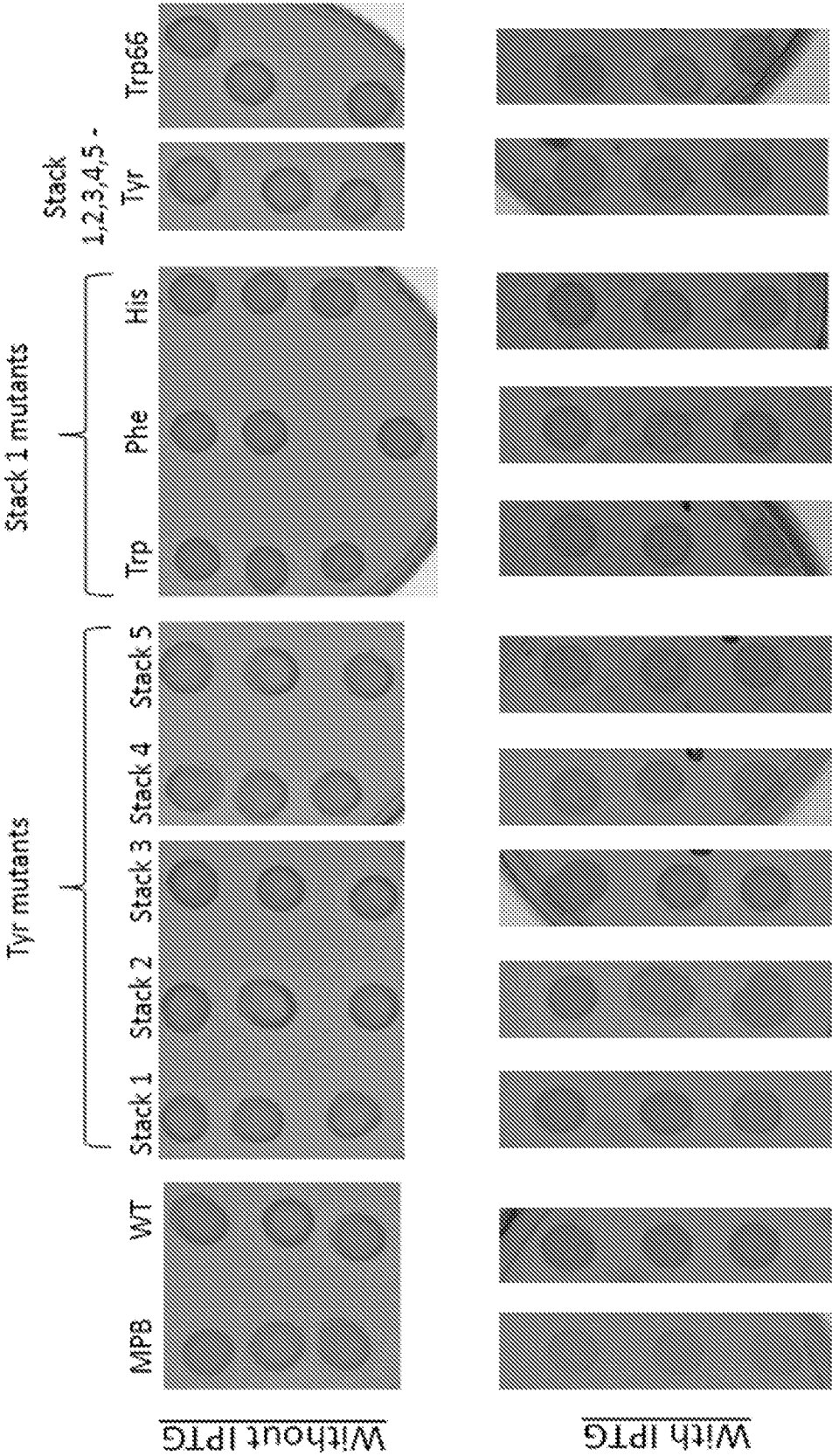


Fig. 10



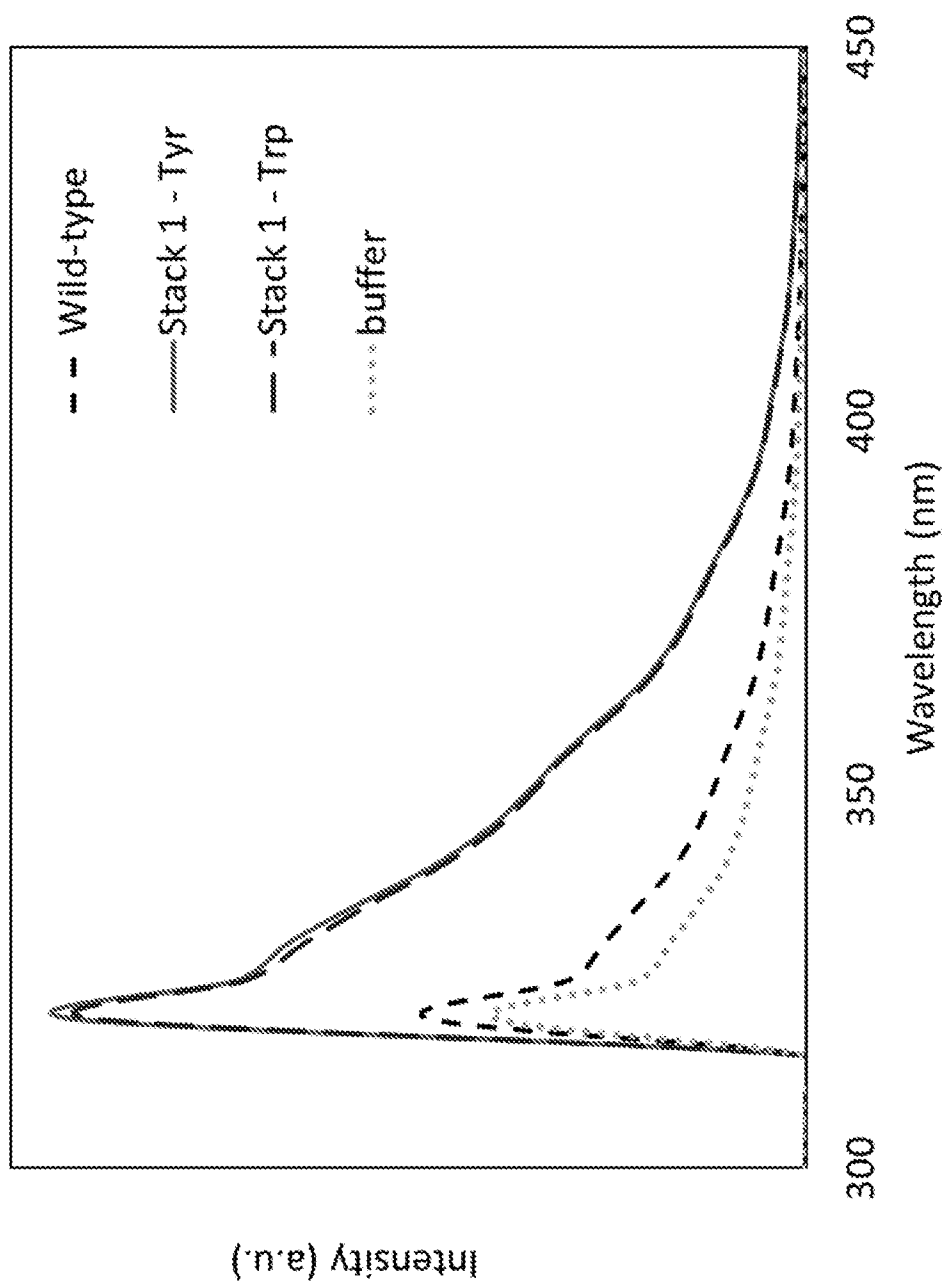


Fig. 11

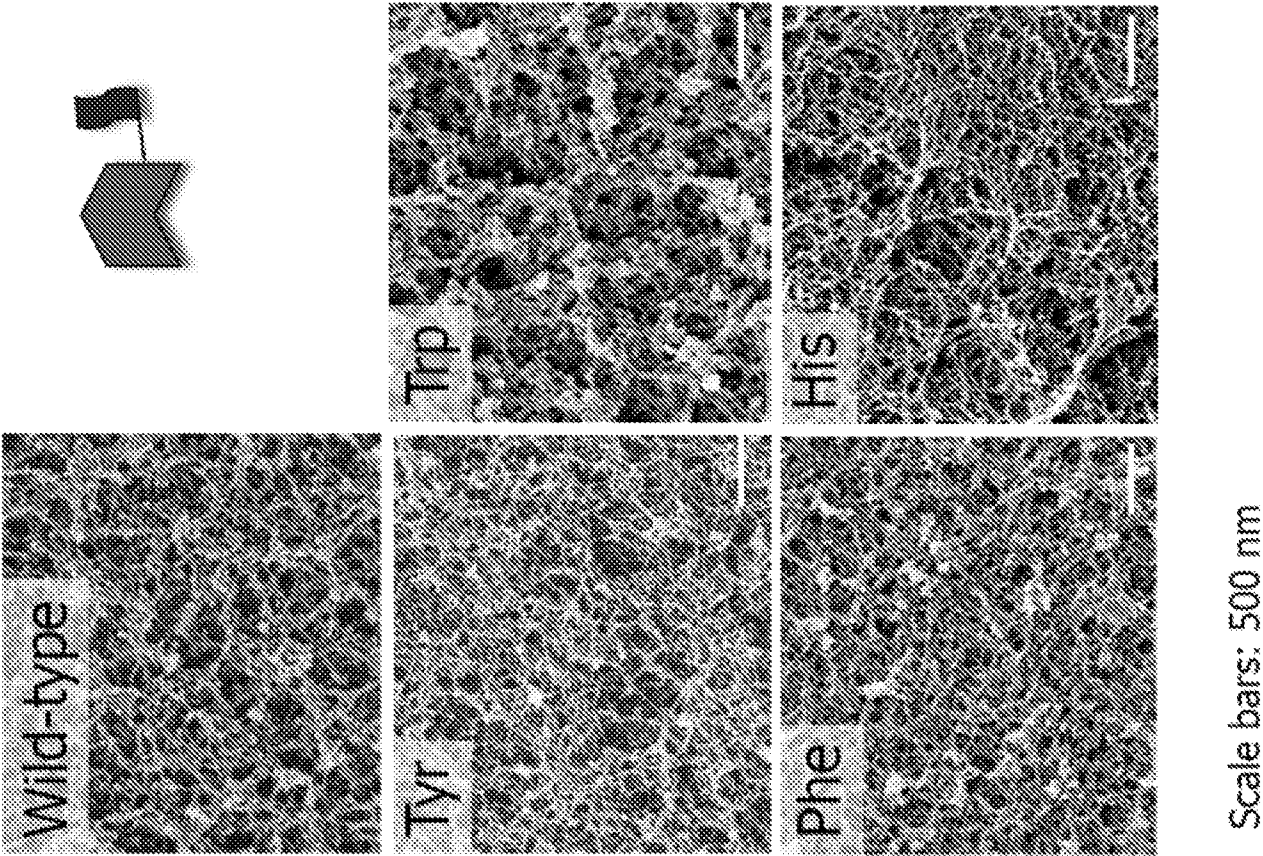


Fig. 12

Fig. 13

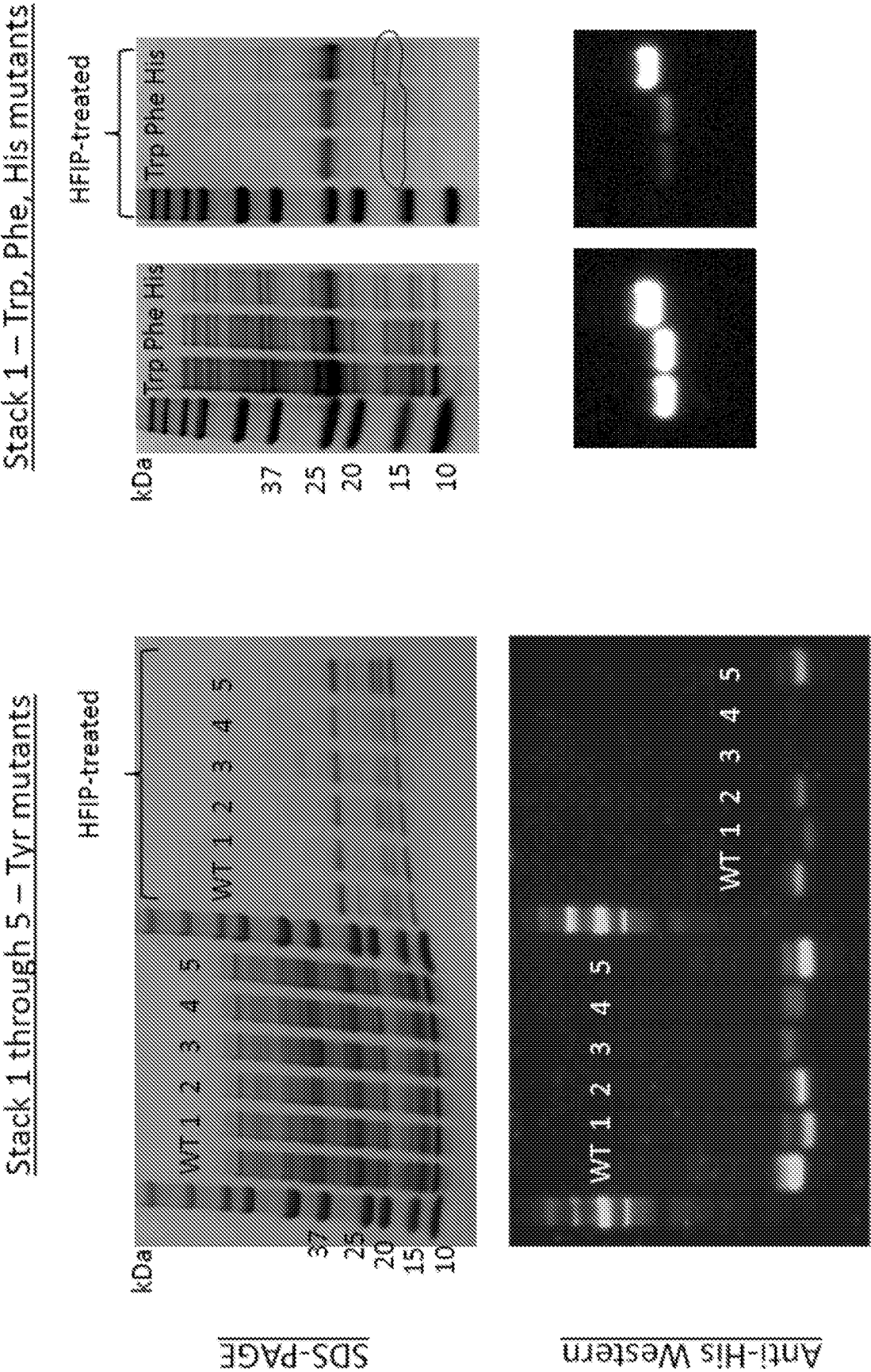


Fig. 14A

Assembly of conductive curli nanofiber films via filtration

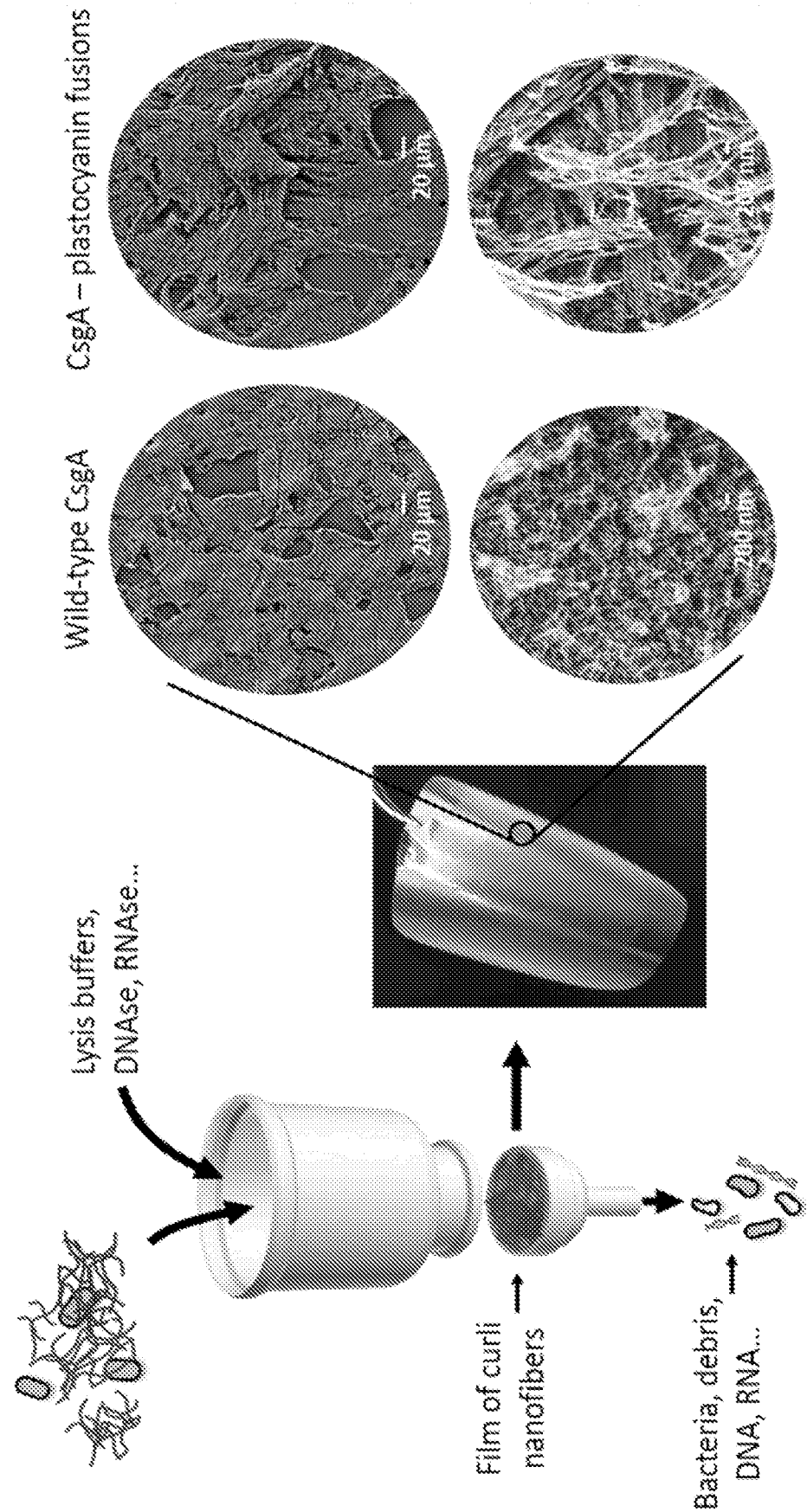
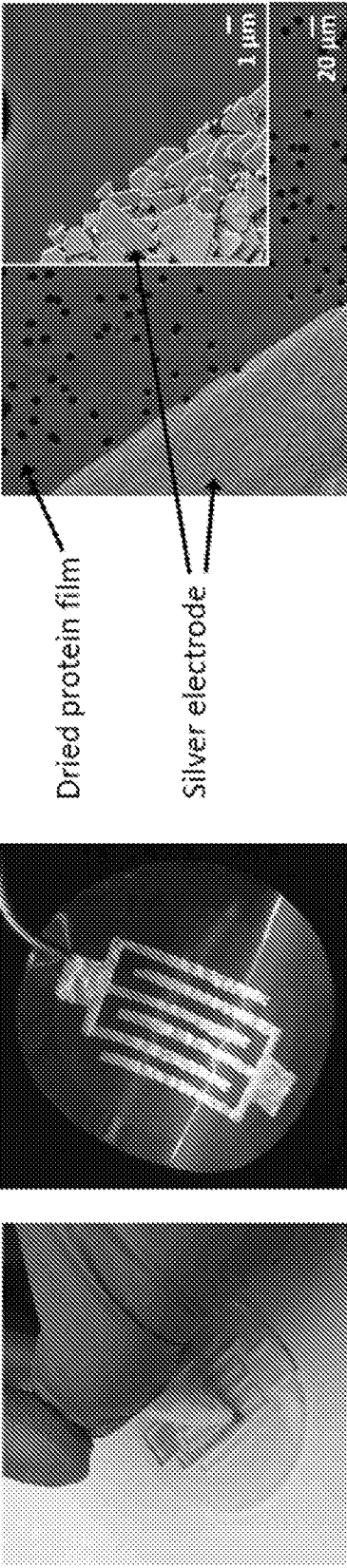


Fig. 14B

Measuring electrical properties of curli nanofiber films

Silver interdigitated electrodes are directly drawn onto dried curli films deposited on filter membranes



Resistance and current-voltage curves are measured to determine which protein films are conductive

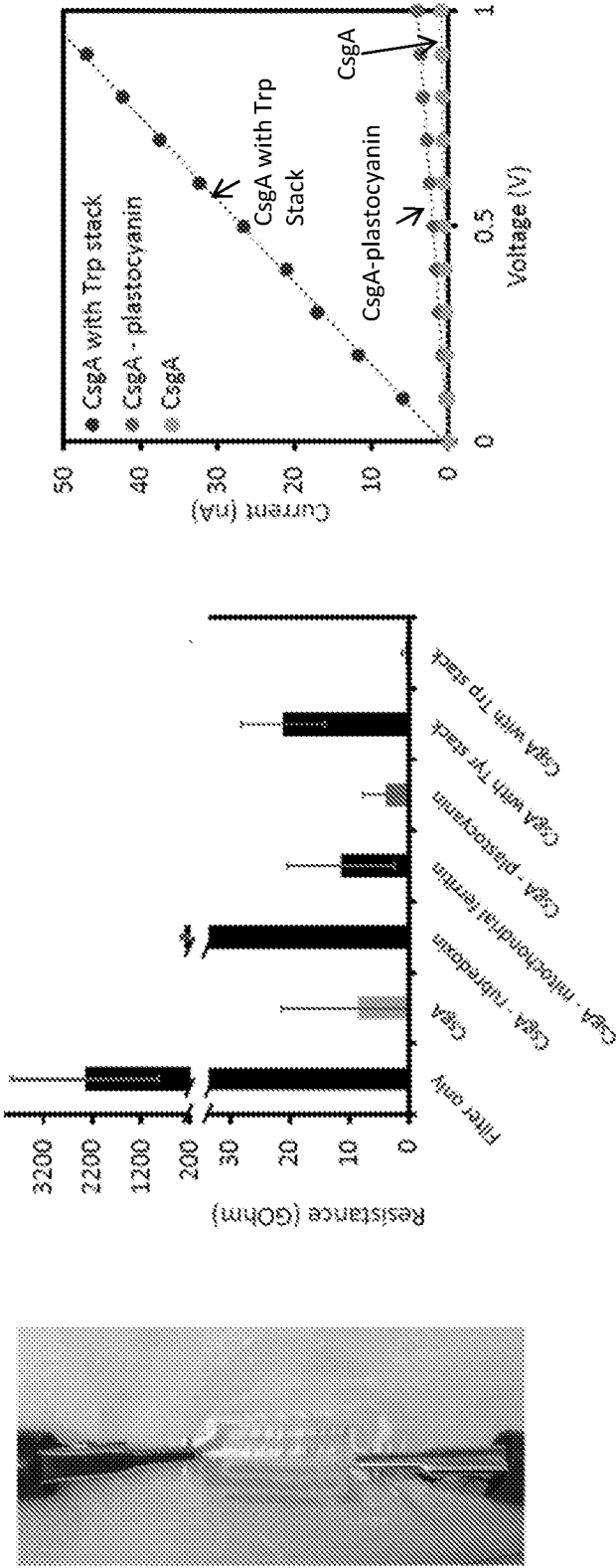


Fig. 15A

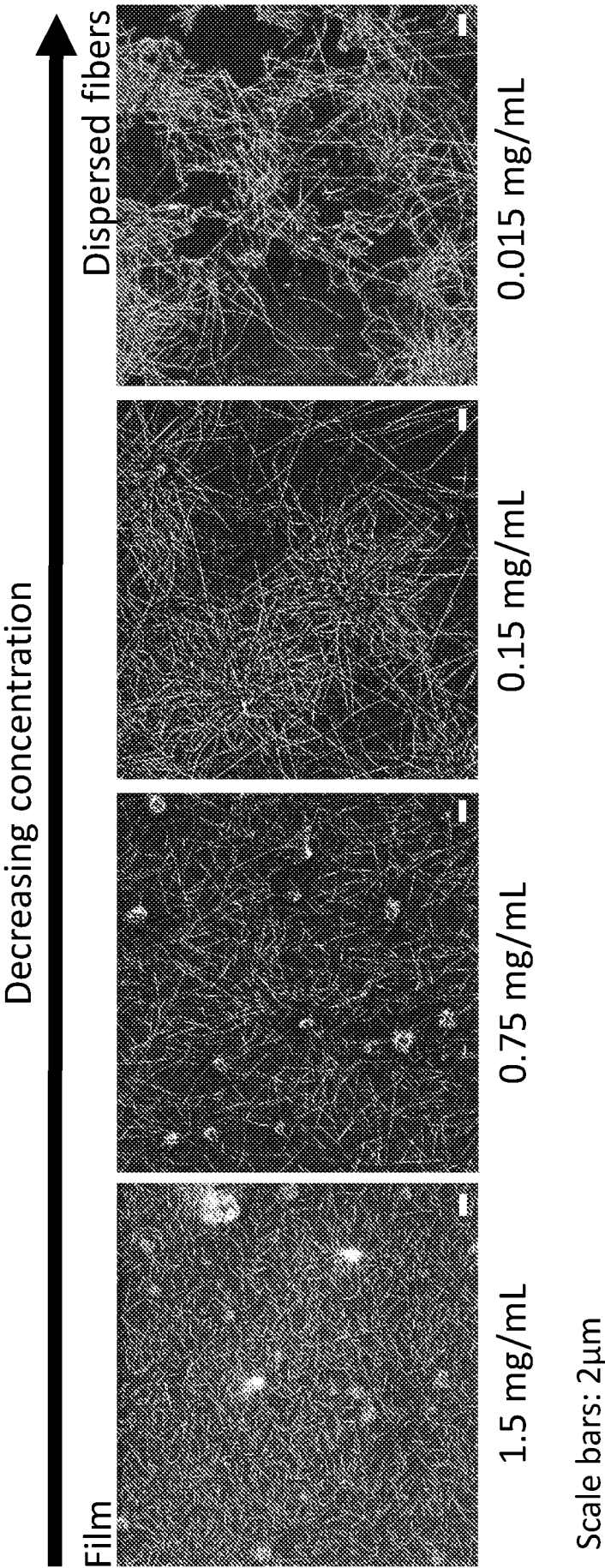


Fig. 15B

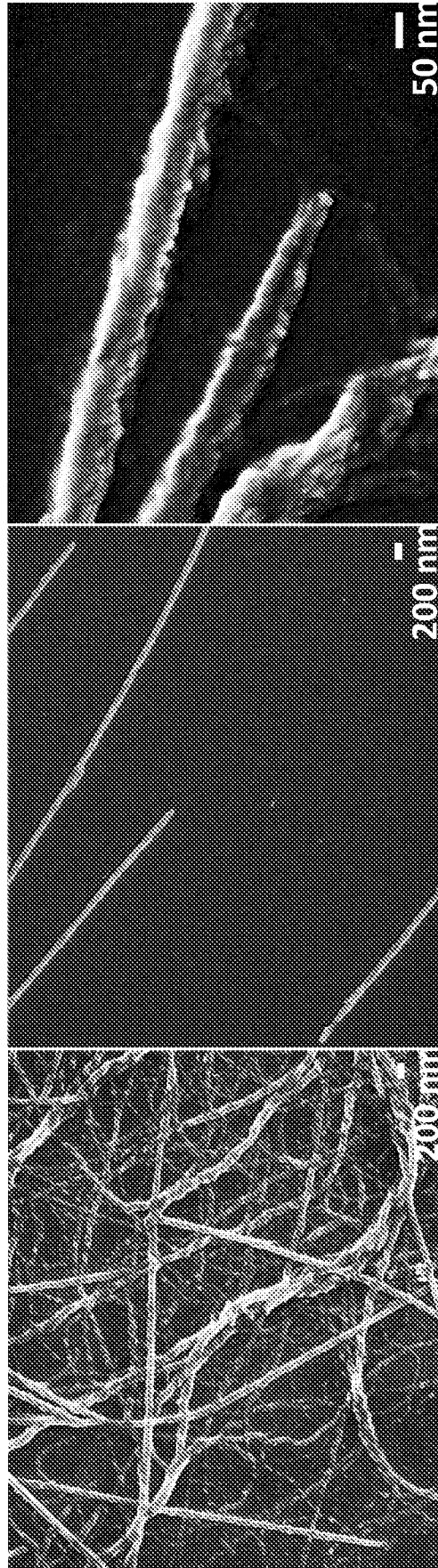
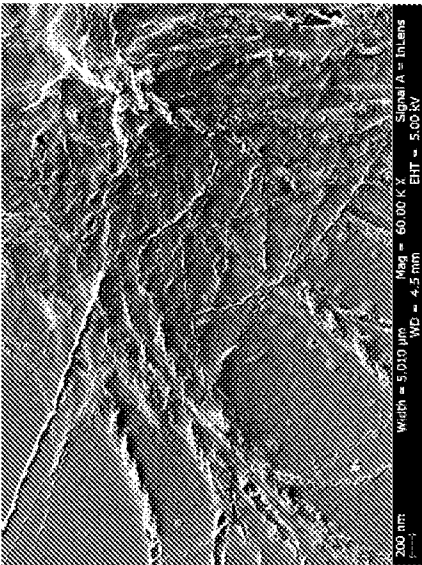


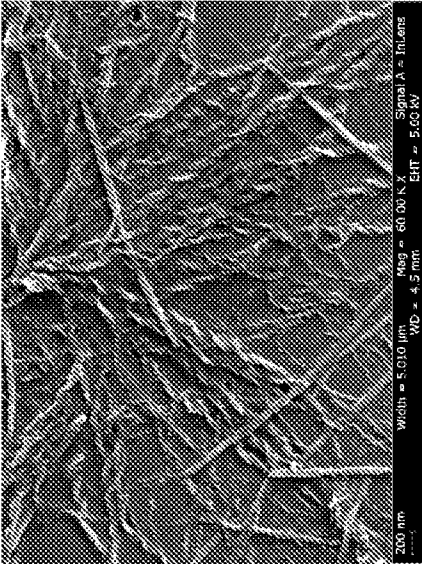
Fig. 16A

Metalloprotein fusions

Ferritin domain



Plastocyanin



Rubredoxin

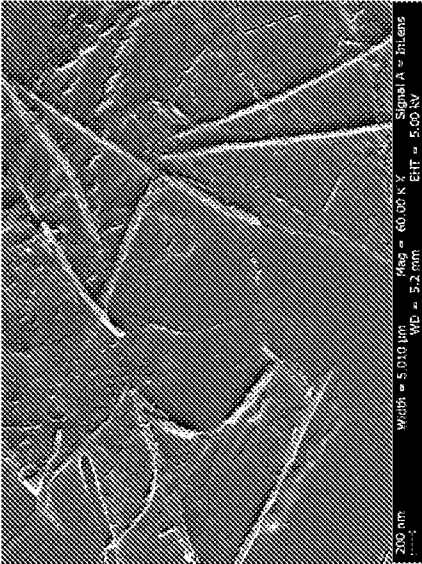
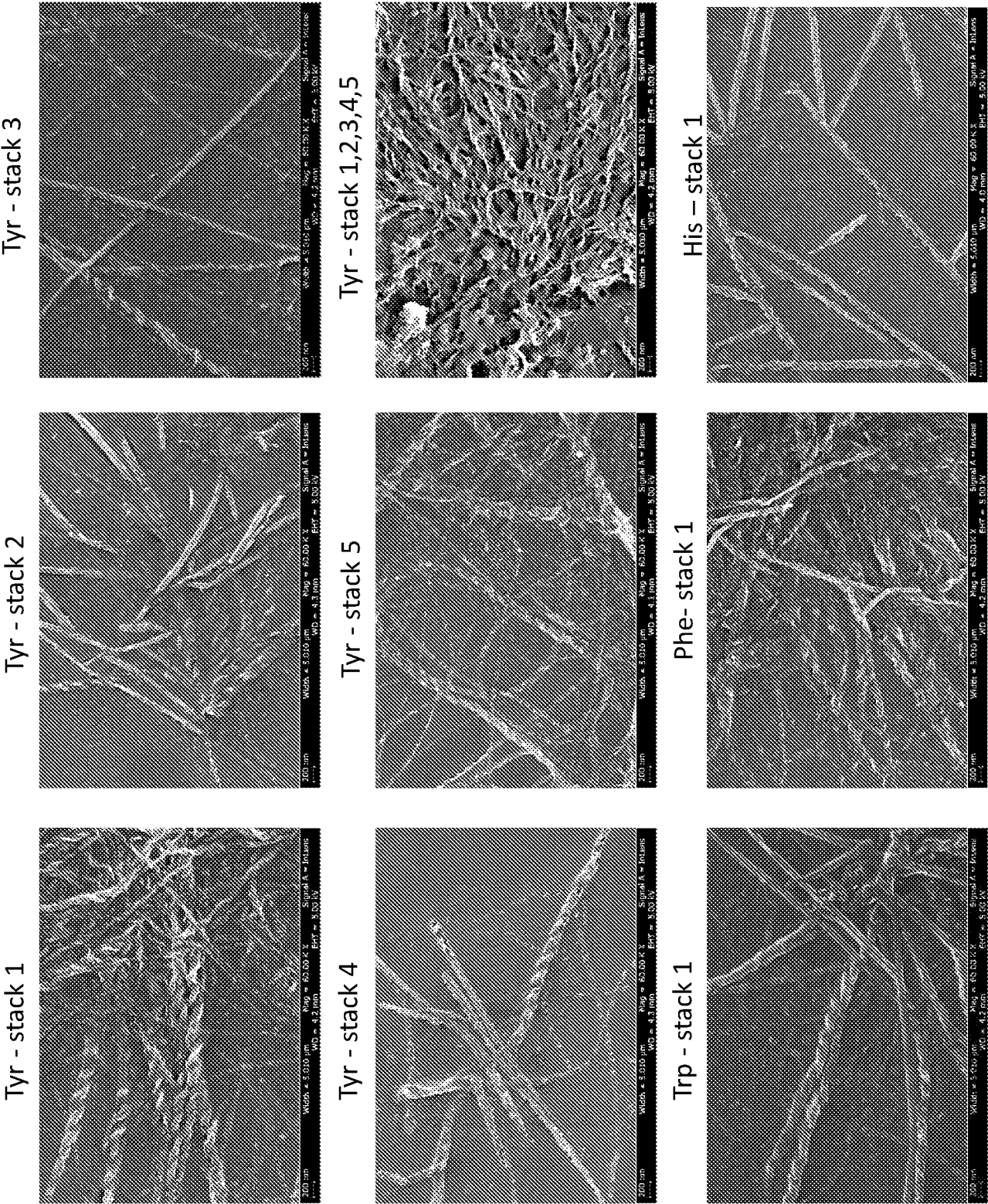


Fig. 16B



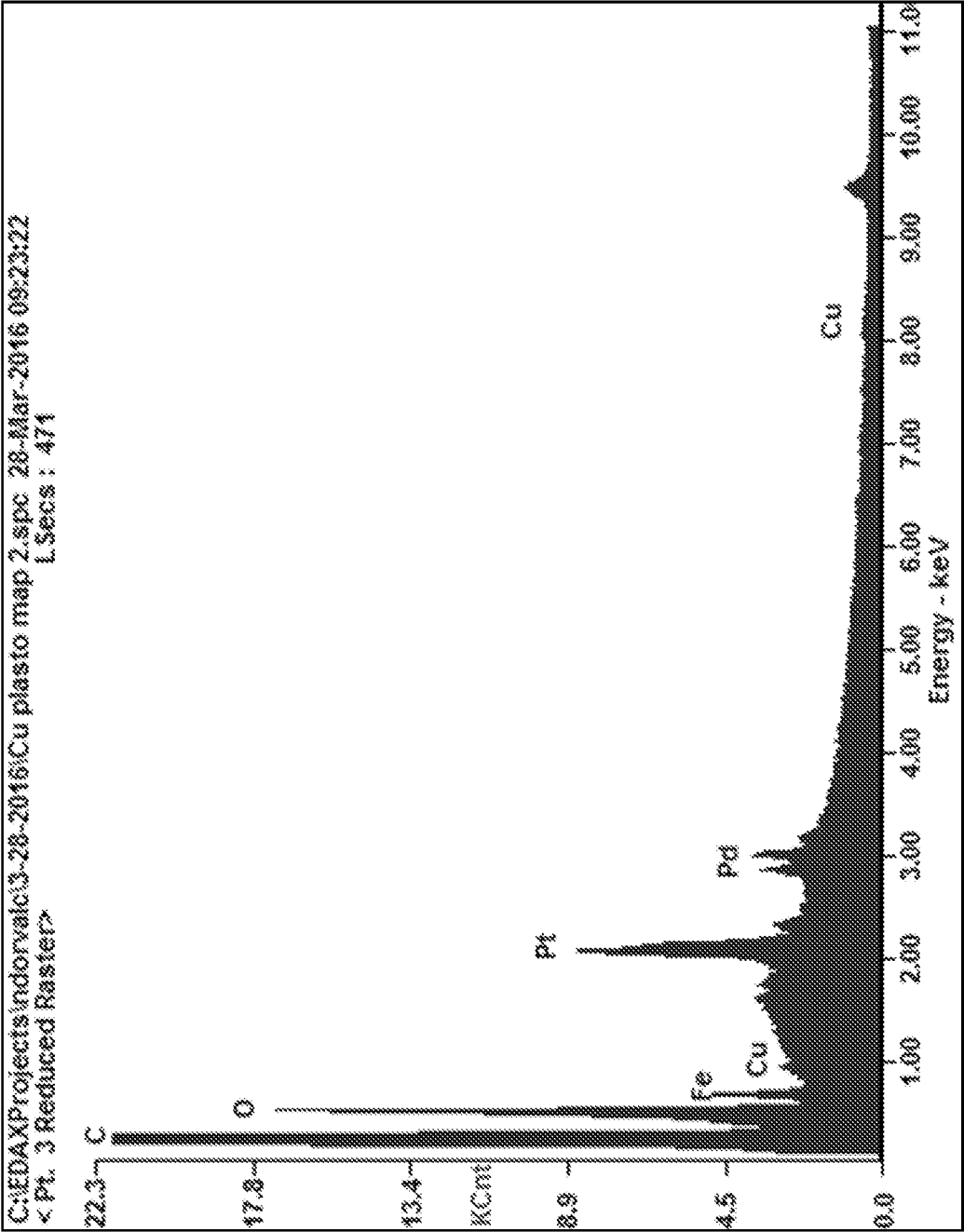
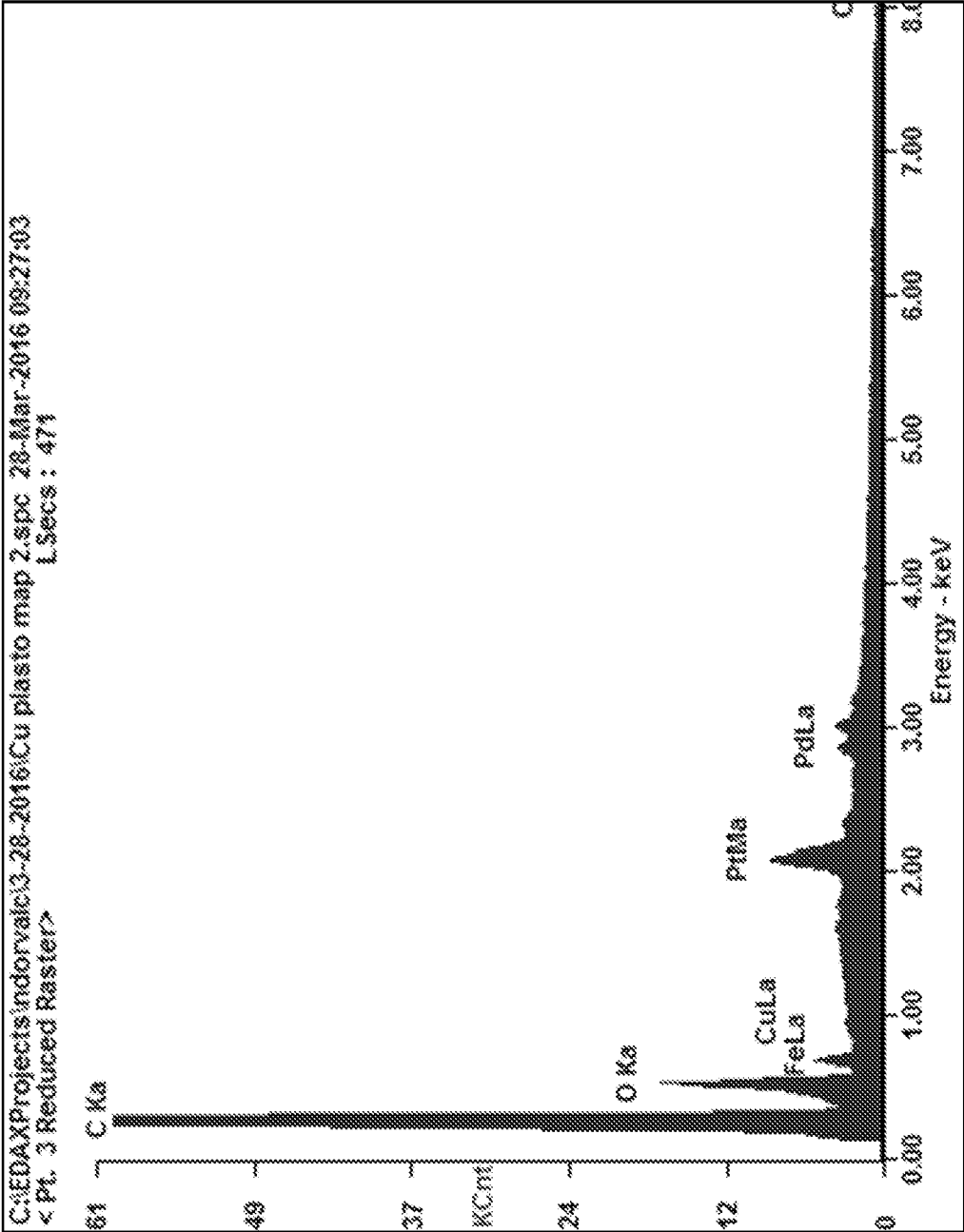


Fig. 17A

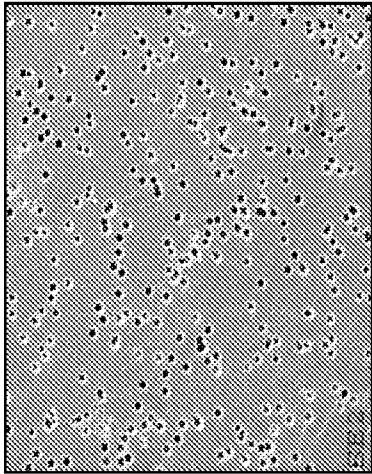
Fig. 17B



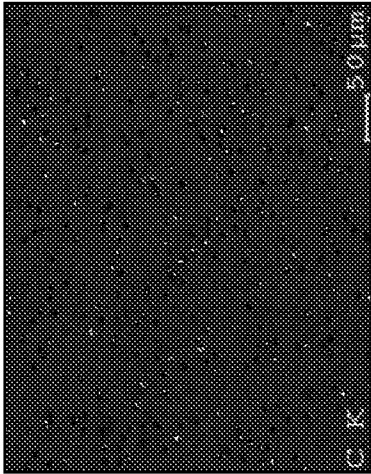
Elem	Wt %	At %	K-Ratio	Z	A	F
C K	69.54	80.33	0.4025	1.0220	0.5663	1.0002
O K	18.55	16.08	0.0319	1.0050	0.1710	1.0001
Fe K	3.95	2.88	0.0073	0.9446	0.1956	1.0000
Pt M	6.44	0.46	0.0594	0.6873	1.3416	1.0000
Pd L	1.04	0.14	0.0088	0.7585	1.1115	1.0000
Cu K	0.49	0.11	0.0042	0.8197	1.0130	1.0478

Atom ratio Cu/C = 0.11/80.33 = 0.13%

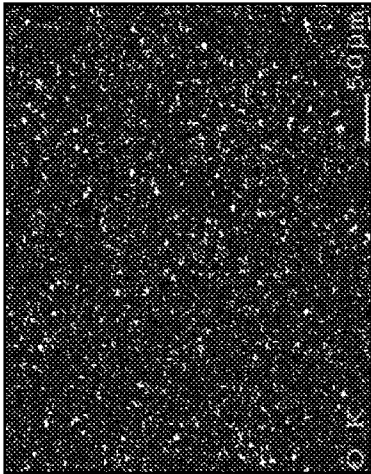
SE2



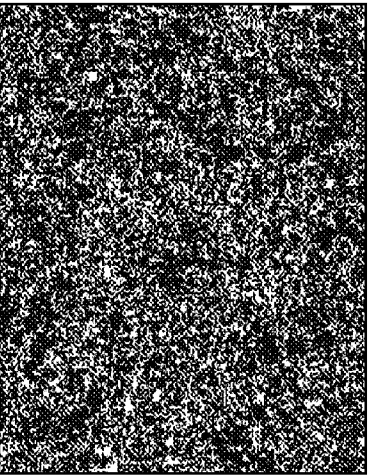
CK



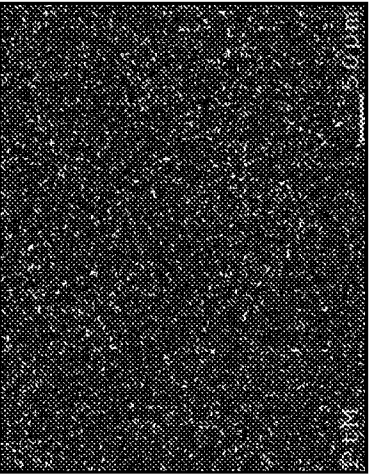
OK



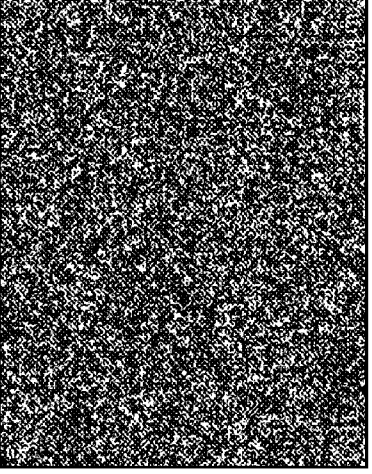
FeL



PtM



PdL



CuK

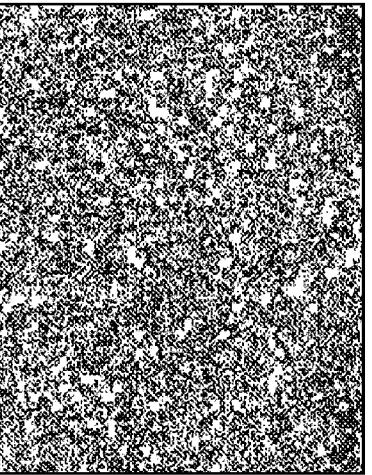
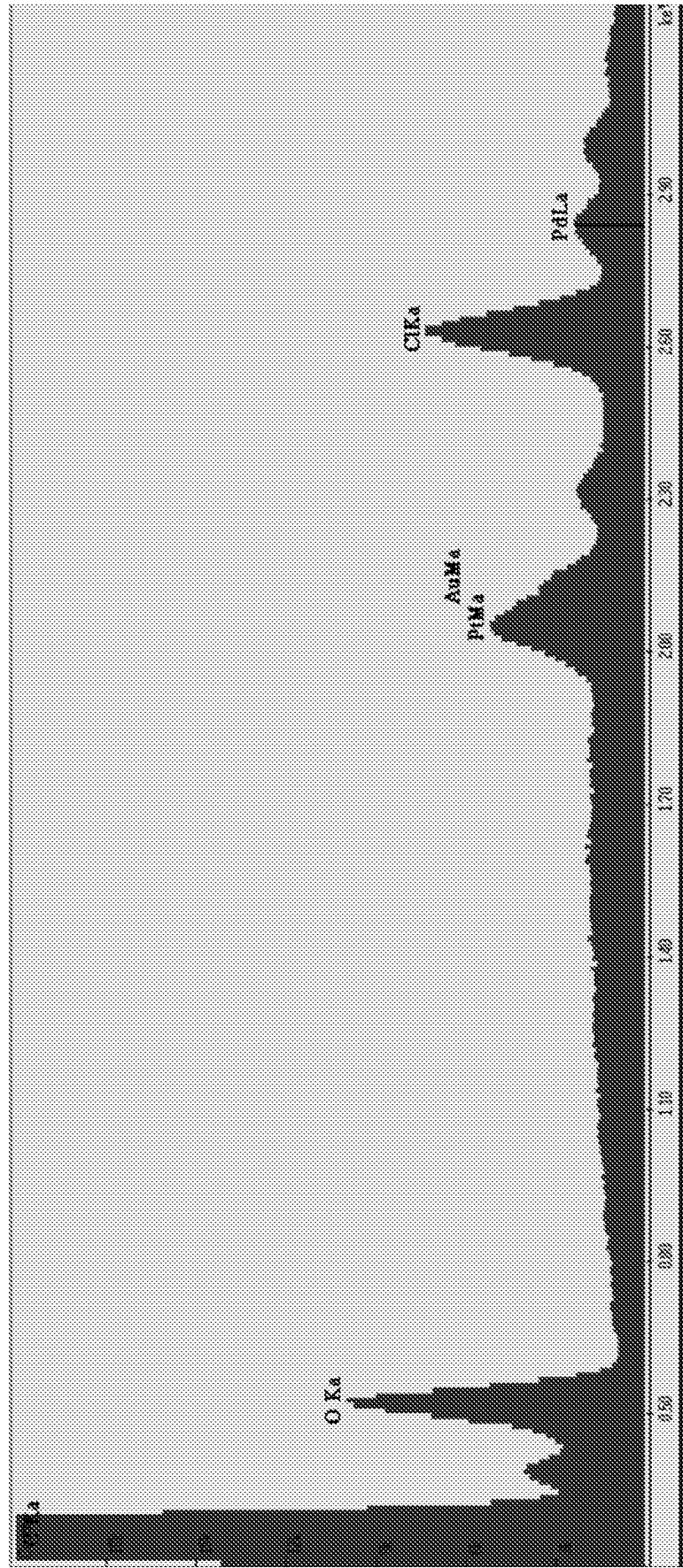


Fig. 17C

Fig. 18A



Element	Wt %	At %	Net Inte	Bgnd
CK	65.64	80.11	947.91	12.64
OK	18.88	17.29	171.35	15.96
PtM	7.40	0.56	117.94	58.77
ClK	4.07	1.68	169.02	49.50
PdL	0.95	0.13	14.70	48.71
AuL	3.06	0.23	4.70	5.06

Matrix Correction: ZAF

Fig. 18B

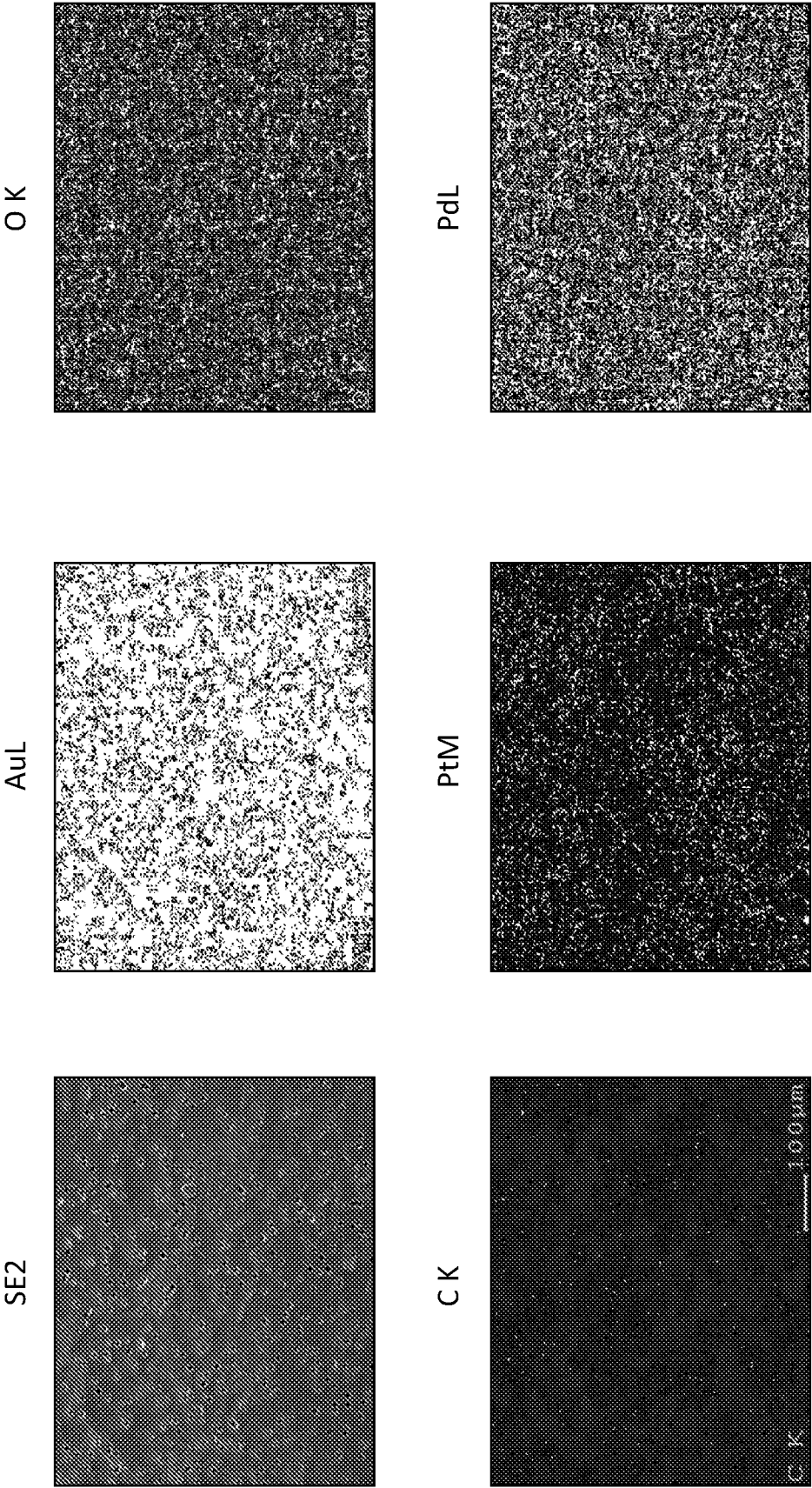


Fig. 19A

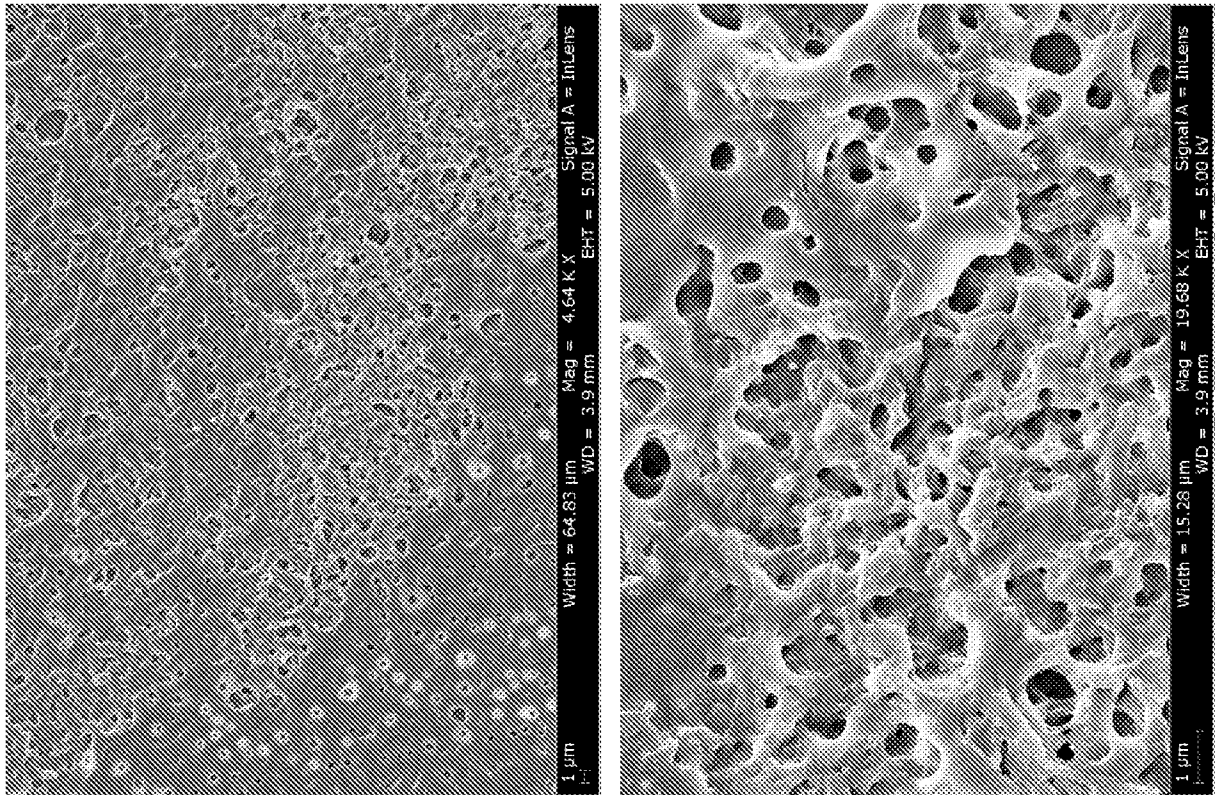


Fig. 19B

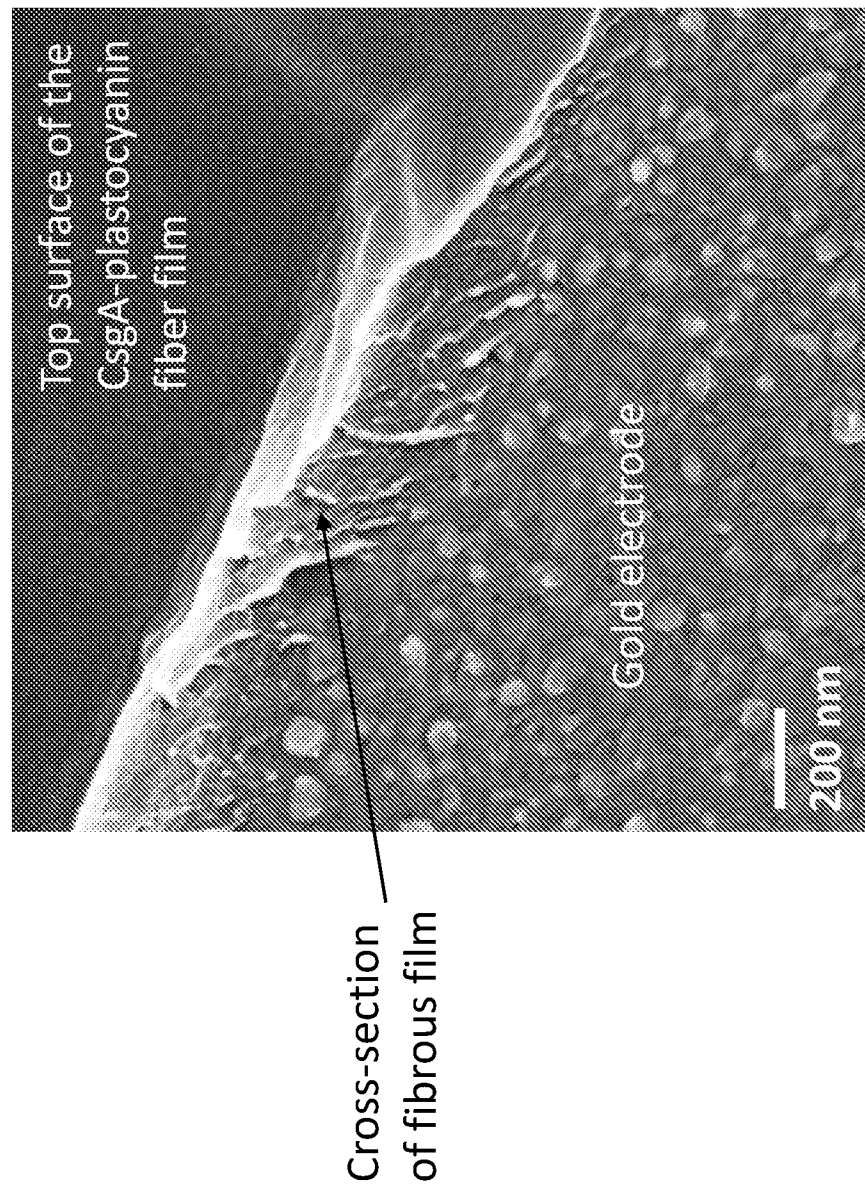


Fig. 20A

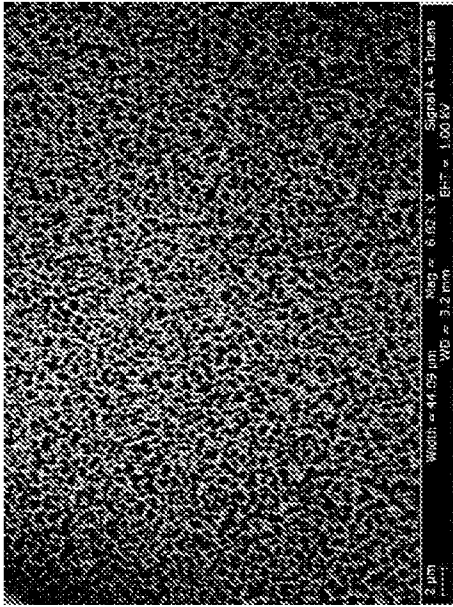
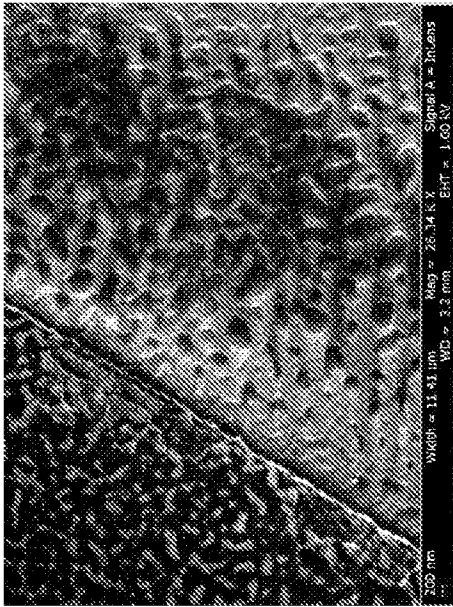
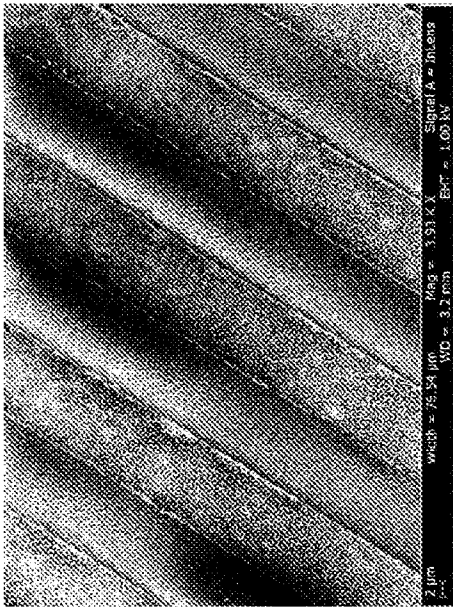
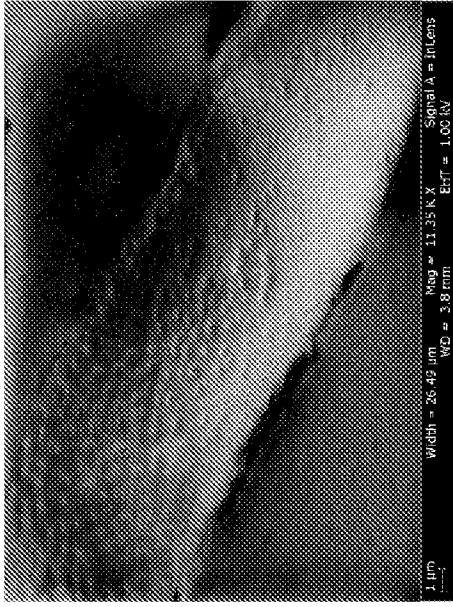
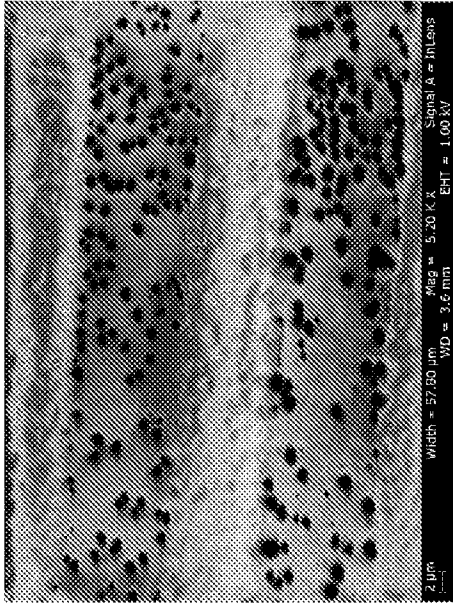
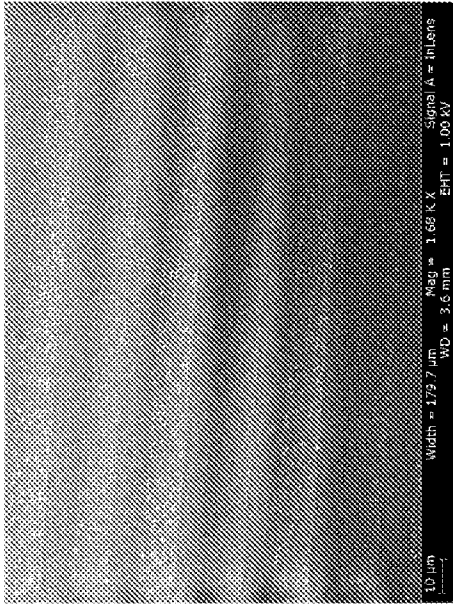


Fig. 20B



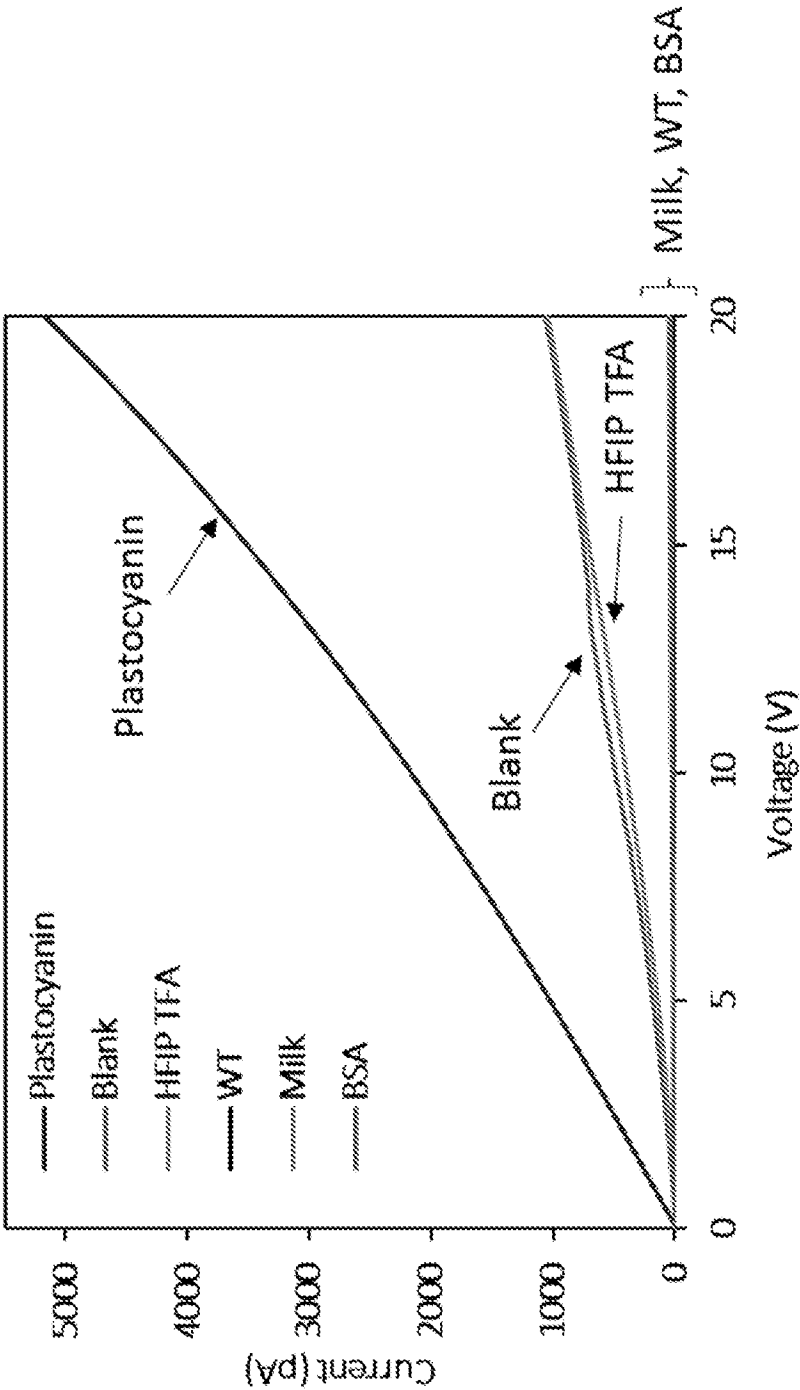


Fig. 21A

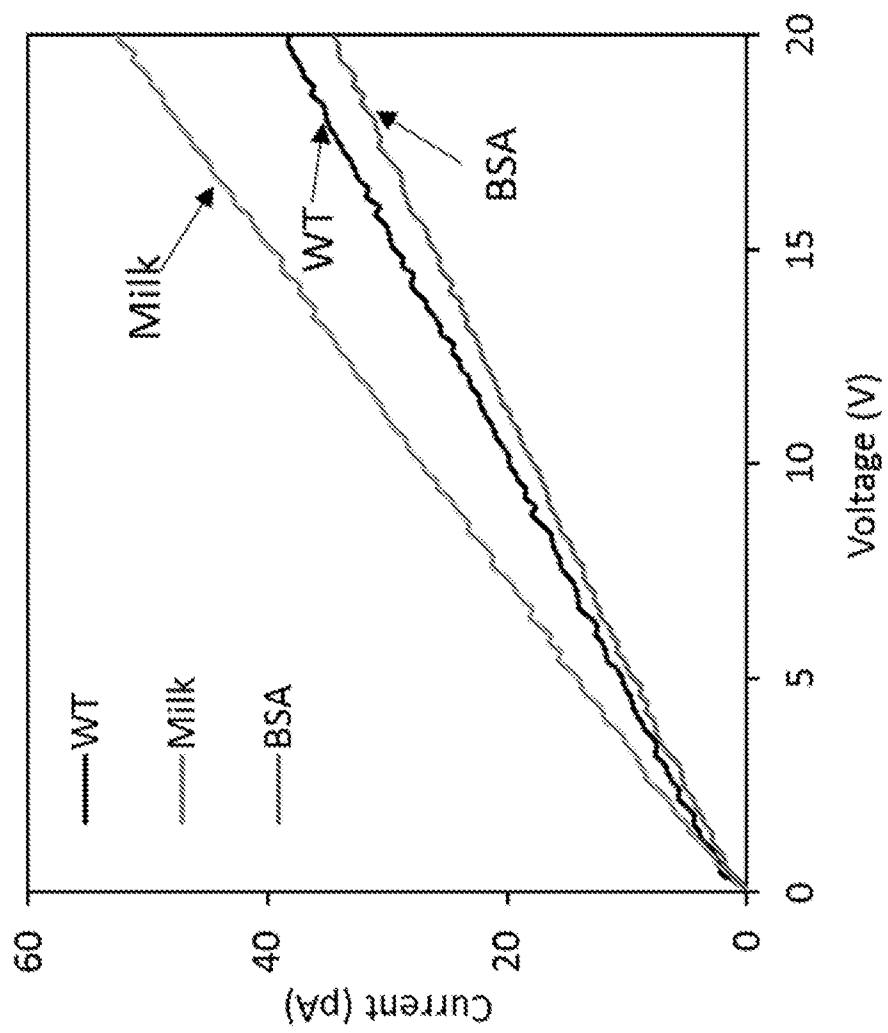


Fig. 21B

Fig. 22B

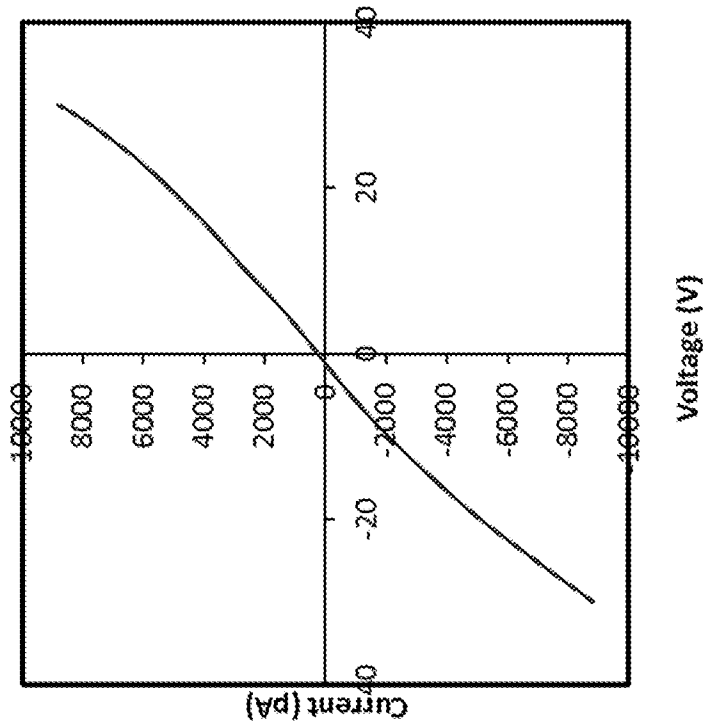


Fig. 22A

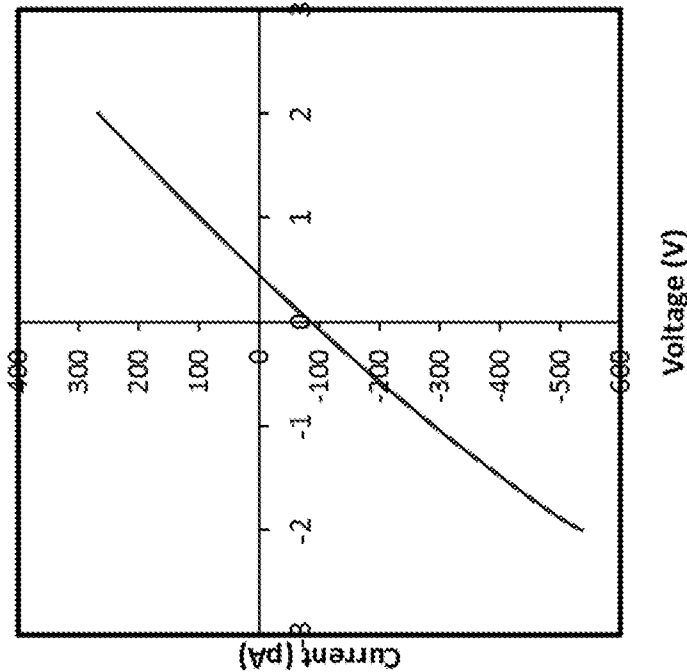
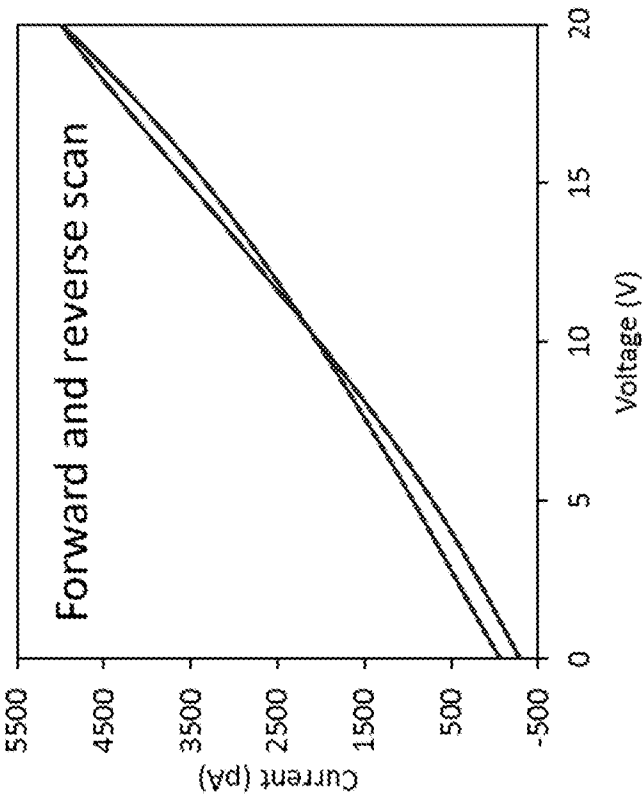


Fig. 22C



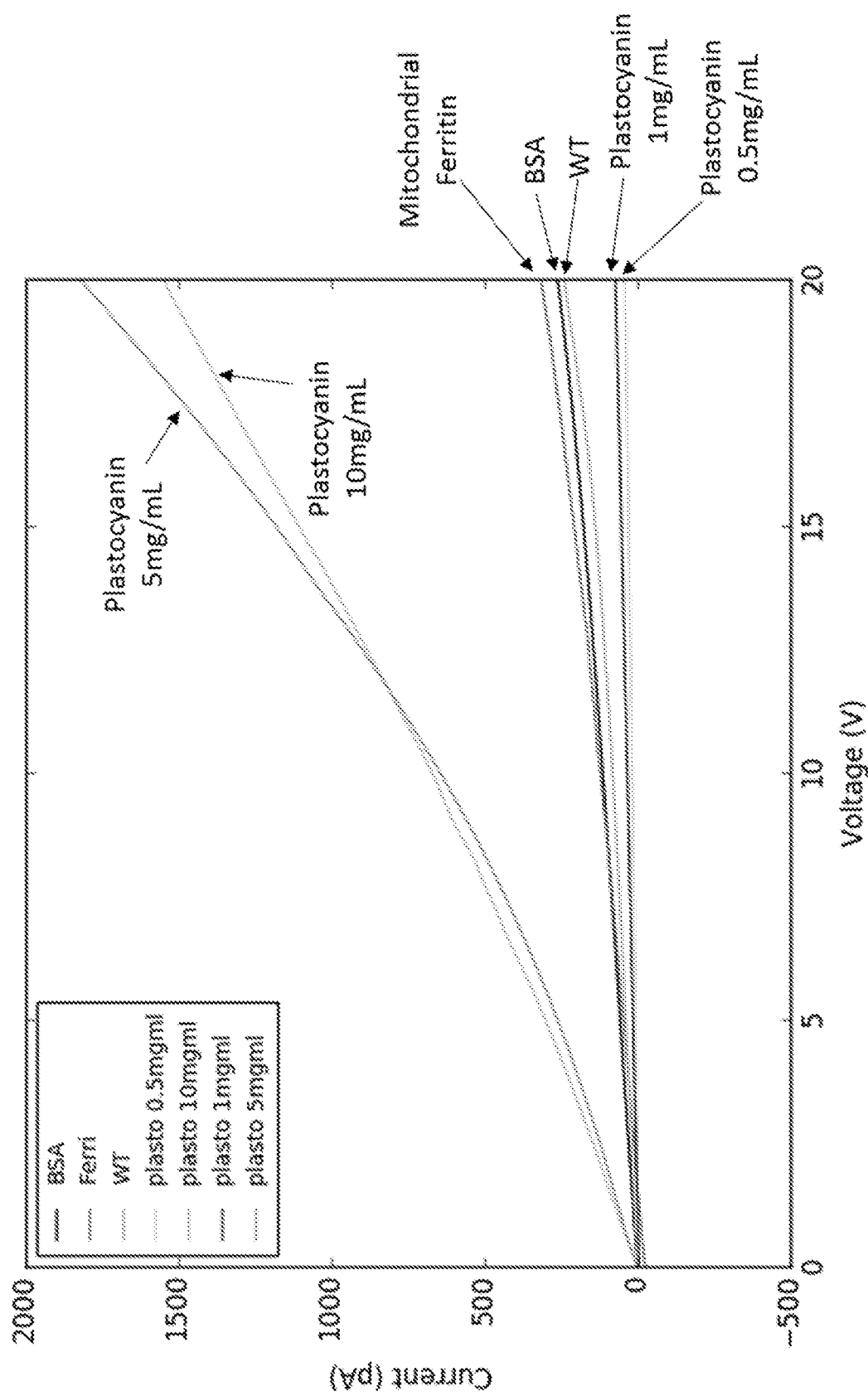


Fig. 23

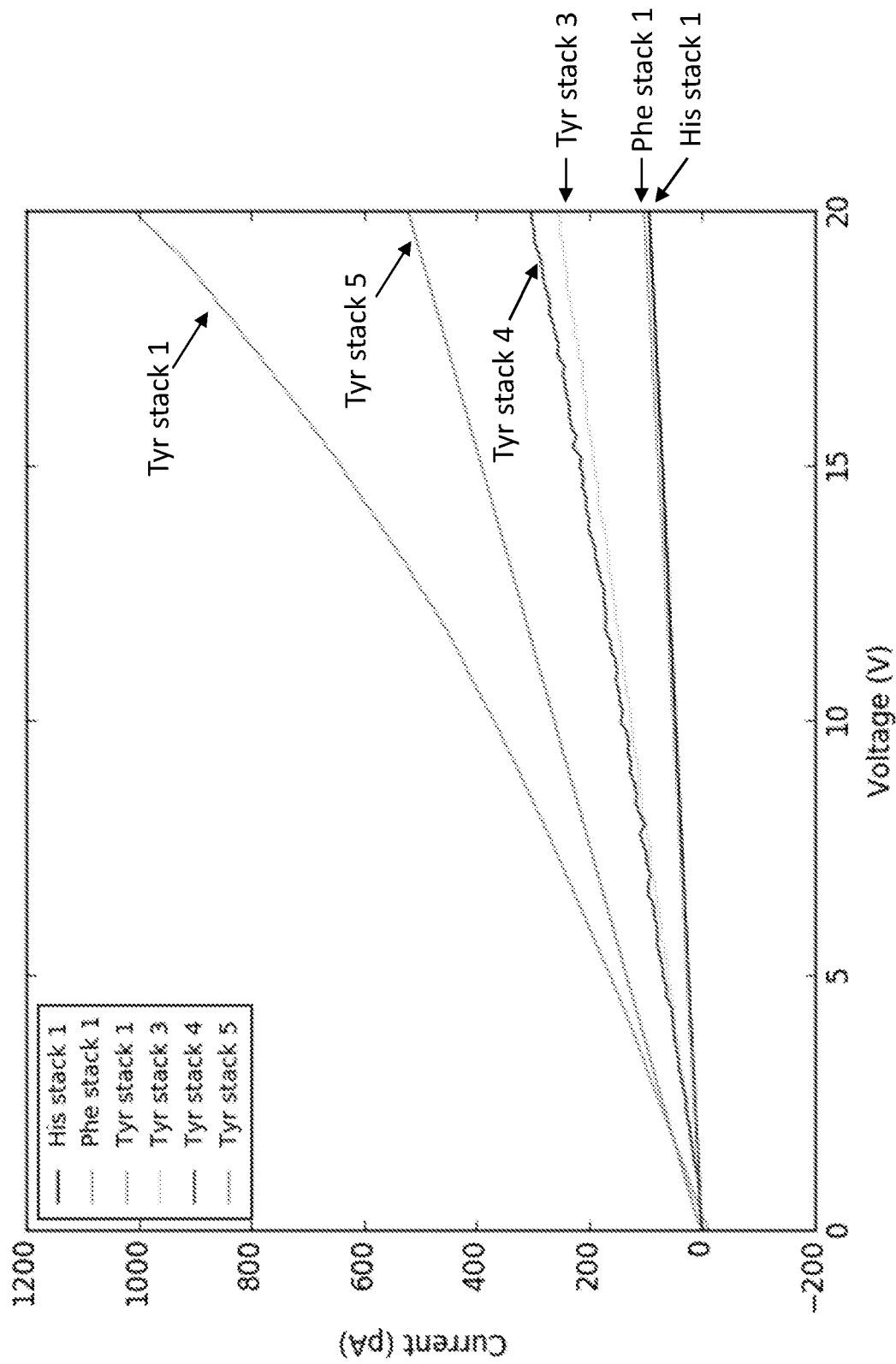


Fig. 24

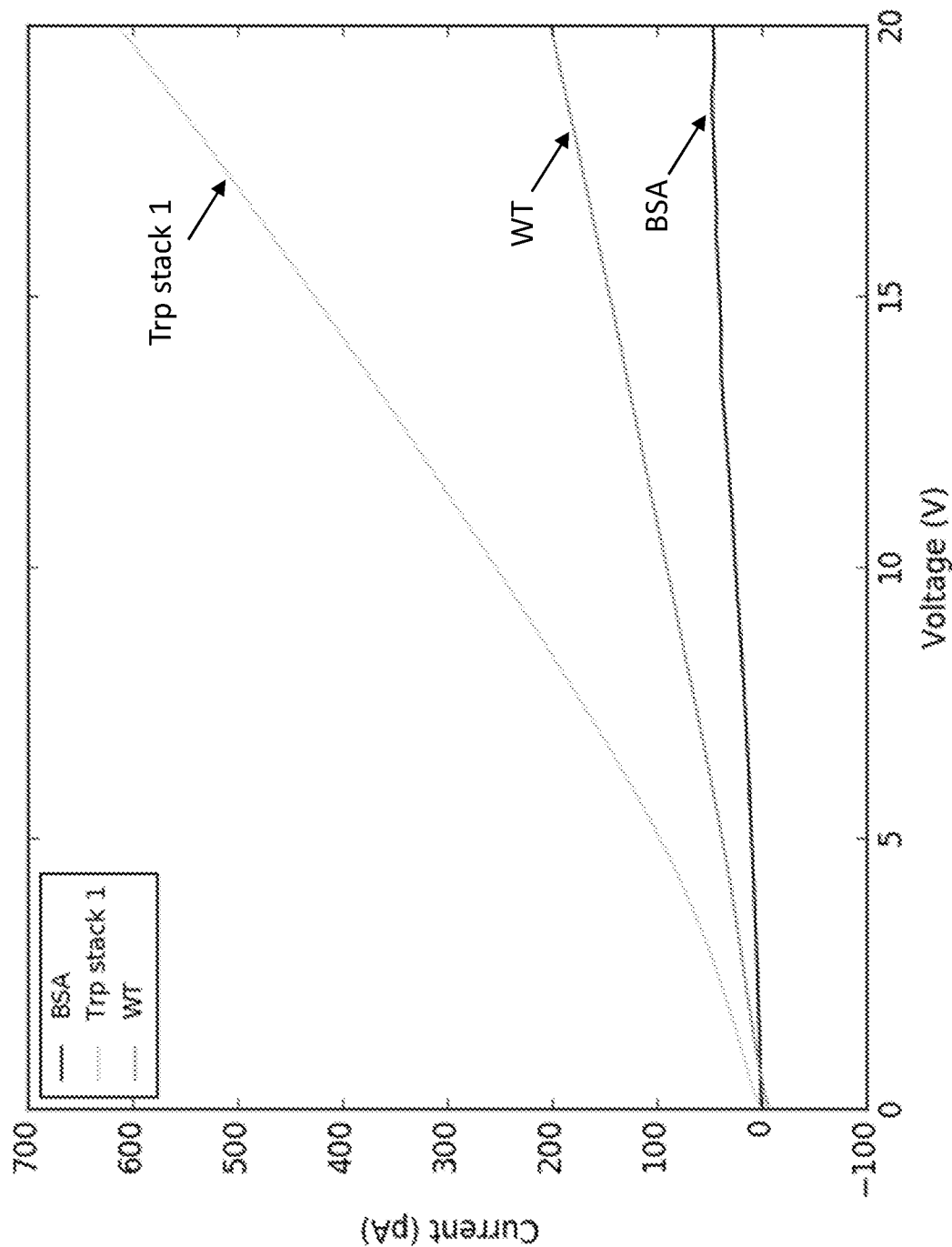


Fig. 25

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/62743

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C07K 14/245, 14/21 (2017.01)

CPC - C07K 14/245, 14/21

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

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

See Search History document

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	WO 2014/176311 A1 (PRESIDENT AND FELLOWS OF HARVARD COLLEGE) 30 October 2014; paragraphs [0004]-[0006], [0047], [00100], [00109]	1, 3/1, 9, 10/1, 10/9, 17, 19/17, 25, 26/17, 26/25, 30, 32/30, 38, 39/30, 39/38, 42, 44, 45, 46/44-45, 53, 54, 55/53-54, 62, 66, 67, 68/66-67, 86, 88/62, 91/17, 91/30, 92/62 11/1, 11/9, 27/17, 27/25, 40/30, 40/38
Y	US 2014/0336357 A1 (BOARD OF TRUSTEES OF MICHIGAN STATE UNIVERSITY) 13 November 2014; paragraphs [0007]-[0008], [0053]	11/1, 11/9, 27/17, 27/25, 40/30, 40/38

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

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

29 March 2017 (29.03.2017)

Date of mailing of the international search report

25 APR 2017

Name and mailing address of the ISA/

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/62743

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 4, 7, 8, 12-16, 20-24, 28, 29, 33-37, 41, 47-52, 56-61, 64, 65, 69-73, 78-85
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

-Please See Extra Sheet Below-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Claims 1, 3 (in-part), 9-11, 17, 19 (in-part), 25-27, 30, 32 (in-part), 38-40, 42, 44-46, 53-55, 62, 66-68, 86, 88 (in-part), 91 (in-part) and 92 (in-part)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US16/62743

---Continued from Box III: Observations where unity of invention is lacking---

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, Claims 1, 3 (in-part), 9-11, 17, 19 (in-part), 25-27, 30, 32 (in-part), 38-40, 42, 44-46, 53-55, 62, 66-68, 86, 88 (in-part), 91 (in-part) and 92 (in-part) are directed toward methods related to a genetically modified bacterium comprising a mutant amyloid polypeptide having a series of aligned aromatic groups; a genetically modified bacterium or biofilm related thereto; and nucleic acid related thereto.

Group II, Claims 2, 3 (in-part), 5, 6, 18 19 (in-part), 31, 32 (in-part), 43, 63, 74-77, 87, 88 (in-part), 89, 90, 91 (in-part) and 92 (in-part) are directed toward a genetically modified bacterium comprising an amyloid polypeptide fused to a redox active domain; a genetically modified bacterium or biofilm related thereto; and nucleic acid related thereto.

The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical features of Group I include a series of aligned aromatic groups, not present in Group II; the special technical features of Group II include a fused redox active domain, not present in Group I.

Groups I and II share the technical features including: a method of producing a genetically modified bacterium comprising genetically altering a bacterium to include a nucleic acid sequence encoding an amyloid polypeptide, wherein the nucleic acid sequence is under the control of a promoter to express the protein; a method of making an electrically conductive biofilm comprising proliferating a genetically-altered bacterium comprising a nucleic acid sequence encoding a protein, wherein the protein comprises an amyloid polypeptide, to produce a population of genetically-altered bacteria expressing the nucleic acid sequence, and allowing the bacteria to form a biofilm comprising an amyloid-based structure formed from a plurality of proteins; a method of making an electrically conductive amyloid-based structure from a genetically-modified bacterium comprising proliferating a genetically-altered bacterium comprising a nucleic acid sequence encoding a protein comprising an amyloid polypeptide, to produce a population of genetically-altered bacteria expressing the nucleic acid sequence, allowing the population of genetically-altered bacteria to produce an amyloid-based structure formed from a plurality of the proteins; a genetically-modified bacterium comprising a nucleic acid sequence encoding a protein comprising an amyloid polypeptide, wherein the nucleic acid sequence is under the control of a promoter to express the protein; a protein comprising an amyloid polypeptide; a non-naturally occurring amyloid-based structure formed from a plurality of proteins comprising an amyloid polypeptide, a nucleic acid sequence encoding a protein comprising an amyloid polypeptide; and a biofilm comprising a bacterium expressing a nucleic acid sequence encoding a protein comprising an amyloid polypeptide.

However, these shared technical features are previously disclosed by WO 2014/176311 A1 to President and Fellows of Harvard College (hereinafter 'Harvard').

Harvard discloses a method of producing a genetically modified bacterium (a method of producing a genetically modified *E. coli* (bacterium); paragraphs [0004], [0006]) comprising genetically altering a bacterium to include a nucleic acid sequence encoding an amyloid polypeptide (comprising genetically altering a bacterium to include a nucleic acid sequence encoding a CsgA (an amyloid) polypeptide; paragraphs [0004], [0006]), wherein the nucleic acid sequence is under the control of a promoter to express the protein (wherein the nucleic acid sequence is under the control of a promoter to express the protein; paragraph [0047]); a method of making an electrically conductive biofilm (a method of making an electrically conductive biofilm; paragraphs [0004], [0006], [00109]) comprising proliferating a genetically-altered bacterium (comprising proliferating a genetically-altered bacterium; paragraphs [0004], [0006]) comprising a nucleic acid sequence encoding a protein (comprising a nucleic acid sequence encoding a protein; paragraphs [0004], [0006]), wherein the protein comprises an amyloid polypeptide (wherein the protein comprises an amyloid polypeptide; paragraphs [0004], [0006]), to produce a population of genetically-altered bacteria expressing the nucleic acid sequence (to produce a population of genetically-altered bacteria expressing the nucleic acid sequence; paragraphs [0004], [0006]), and allowing the bacteria to form a biofilm comprising an amyloid-based structure formed from a plurality of proteins (allowing the bacteria to form a biofilm comprising an amyloid-based structure formed from a plurality of proteins; paragraphs [0004], [0006], [00109]); a method of making an electrically conductive (a method of making an electrically conductive; paragraphs [0004], [0006], [00109]) amyloid-based structure (amyloid-based structure; paragraph [0004]) from a genetically-modified bacterium (from a genetically-modified bacterium; paragraphs [0004], [0006]) comprising proliferating a genetically-altered bacterium comprising a nucleic acid sequence encoding a protein comprising an amyloid polypeptide (comprising proliferating a genetically-altered bacterium comprising a nucleic acid sequence encoding a protein comprising an amyloid polypeptide; paragraphs [0004], [0006]), to produce a population of genetically-altered bacteria expressing the nucleic acid sequence (to produce a population of genetically-altered bacteria expressing the nucleic acid sequence; paragraphs [0004], [0006]), allowing the population of genetically-altered bacteria to produce an amyloid-based structure formed from a plurality of the proteins (allowing the population of genetically-altered bacteria to produce an amyloid-based structure formed from a plurality of the proteins; paragraphs [0004], [0006]); a genetically-modified bacterium comprising a nucleic acid sequence encoding a protein comprising an amyloid polypeptide (a genetically-modified bacterium comprising a nucleic acid sequence encoding a protein comprising an amyloid polypeptide; paragraphs [0004], [0006]), wherein the nucleic acid sequence is under the control of a promoter to express the protein (wherein the nucleic acid sequence is under the control of a promoter to express the protein; paragraph [0047]); a protein comprising an amyloid polypeptide (a protein comprising an amyloid polypeptide; paragraph [0004]); a non-naturally occurring amyloid-based structure formed from a plurality of proteins comprising an amyloid polypeptide (a non-naturally occurring amyloid-based structure formed from a plurality of proteins comprising an amyloid polypeptide; paragraphs [0004], [00109]), a nucleic acid sequence encoding a protein comprising an amyloid polypeptide (a nucleic acid sequence encoding a protein comprising an amyloid polypeptide; paragraphs [0004], [0006]); and a biofilm comprising a bacterium expressing a nucleic acid sequence encoding a protein comprising an amyloid polypeptide (a biofilm comprising a bacterium expressing a nucleic acid sequence encoding a protein comprising an amyloid polypeptide; paragraphs [0004], [0006]).

Since none of the special technical features of the Groups I and II inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Harvard reference, unity of invention is lacking.