(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
9 February 2006 (09.02.2006)

PCT

(10) International Publication Number
WO 2006/013552 A2

(51) International Patent Classification:
A61K 38/17 (2006.01) *C07K 14/47* (2006.01)

(21) International Application Number:
PCT/IL2005/000589

(22) International Filing Date: 5 June 2005 (05.06.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/592,523 2 August 2004 (02.08.2004) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) **Title:** ARTICLES OF PEPTIDE NANOSTRUCTURES AND METHOD OF FORMING THE SAME

(57) **Abstract:** A method of forming a fiber made of peptide nanostructures is disclosed. The method comprises: providing peptide nanostructures in solution, and fiberizing the solution thereby forming at least one fiber of the peptide nanostructures. Also disclosed are methods of forming films and other articles using the peptide nanostructures.

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ARTICLES OF PEPTIDE NANOSTRUCTURES AND METHOD OF FORMING THE SAME

FIELD AND BACKGROUND OF THE INVENTION

The present invention relates to articles made of nanostructures and, more particularly, to articles made of peptide nanostructures having sizes at least in the micrometer scale.

Material sciences involve the understanding of material characteristics as well as the development of new materials. Industrial and academic needs encourage material scientists to develop new materials having superior mechanical, electrical, optical and/or magnetic properties for many applications. Modern material sciences focus on the investigation of polymers, ceramics and semiconductors in many fluidic as well as solid forms including fibers, thin films, material bulks and the like.

Various manufacturing processes are known in the art for making synthetic fibers. Many synthetic fibers are produced by extrusion processes, in which a thick viscous liquid polymer precursor or composition is forced through one or more tiny holes of a spinneret to form continuous filaments of semi-solid polymer. As the filaments emerge from the holes of a spinneret, the liquid polymer converts first to a rubbery state which then is solidified. The process of extruding and solidifying filaments is generally known as spinning.

Wet spinning processes are typically employed with fiber-forming substances that have been dissolved in a solvent. Wet spinning techniques are preferred for spinning of high molecular weight polyamides. The spinnerets forming the filaments are submerged in a wet chemical bath, and as the filaments of the fiber-forming substances emerge from the spinnerets, they are induced to precipitate out of the solution and solidify.

In gel spinning, the polymer is not in a true liquid state during extrusion. The polymer chains are bound together at various points in liquid crystal form. This produces strong inter-chain forces in the resulting filaments that can significantly increase the tensile strength of the fibers. In addition, the liquid crystals are aligned along the fiber axis by the shear forces during extrusion. The filaments emerge with high degree of orientation relative to each other, further enhancing the strength. Typically, in gel spinning, the filaments first pass through air and then cooled in a liquid bath.

In dry spinning, the polymer is dissolved in a volatile solvent and the solution is pumped through the spinneret. As the fibers exit the spinneret, air is used to evaporate the solvent such that the fibers solidify and can be collected on a take-up wheel.

In melt spinning the polymer is melted and pumped through the spinneret. The molten fibers are cooled, solidified, and collected on a take-up wheel. Stretching of the fibers in both the molten and solid states provides for orientation of the polymer chains along the fiber axis.

Dispersion spinning is typically employed when the polymer having and infusible, insoluble and generally intractable characteristics. In this technique, the polymer is dispersed as fine particles in a chemical carrier that permit extrusion into fiber. The dispersed polymer is then caused to coalesce by a heating process and the carrier is removed by a thermal or chemical procedure.

Reaction spinning processes involve the formation of filaments from pre-polymers and monomers. The pre-polymers and monomers are further polymerized and cross-linked after the filament is formed. The reaction spinning process begins with the preparation of a viscous spinning solution, which is prepared by dissolving a low molecular weight polymer in a suitable solvent and a reactant. The spinning solution is then forced through the spinneret into a solution or being combined with a third reactant. The primary distinguishable characteristic of reaction spinning processes is that the final cross-linking between the polymer molecule chains in the filament occurs after the fibers have been spun. Post-spinning steps typically include drying and lubrication.

In tack spinning, a polymeric material in a tacky state is interposed between a foundation layer and a temporary anchorage surface. Being in a tacky state, the polymeric material adheres to the foundation layer and the temporary anchorage surface. The foundation layer is then separated from the temporary anchorage surface to produce fibers of the polymeric material. The fibers are hardened by thermal or chemical treatment, and separated from the temporary anchorage surface.

In electrospinning, a fine stream or jet of liquid is produced by pulling a small amount of charged liquefied polymer through space using electrical forces. The produced fibers are hardened and collected on a suitably located precipitation device to form a nonwoven article. In the case of a liquefied polymer which is normally solid

at room temperature, the hardening procedure may be mere cooling, however other procedures such as chemical hardening or evaporation of solvent may also be employed.

Other processes for manufacturing polymeric articles include film blowing and injection molding.

In film blowing, an extruder is used to melt the polymer and pump it into a tubular die. Air blown into the center of the tube causes the melt to expand in the radial direction. The melt in thus extended in both radial and down-stream direction. The formed film is then collected by an arrangement of rollers.

In injection molding, a reciprocating or rotating screw both melts polymer pellets and provides the pressure required to inject the melt into a cold mold. The cold mold provides the article the desired shape.

In the area of thin film production, a well-known method for producing and depositing monolayers is the Langmuir-Blodgett method. In this method a monolayer of amphiphilic molecules is formed at the surface of a tank filled with a liquid sub-phase such as water. Amphiphilic molecules are those having a hydrophobic first end and a hydrophilic second end lined up side by side in a particular direction. In the Langmuir-Blodgett method, a solution of amphiphilic molecules dissolved in a solvent which is not miscible with the sub-phase liquid in the tank is spread onto the liquid surface. When the solvent evaporates, a loosely packed monolayer is formed on the surface of the sub-phase. A transition of the monolayer thus formed from a state of gas or liquid to a solid state is then achieved by compressing surface area of the layer to a predetermined surface pressure. The resulting monolayer is deposited onto the surface of a substrate by passing the substrate through the compressed layer while maintaining the layer at a predetermined surface pressure during the period of deposition.

Another method for producing a monolayer is known as self-assembling of molecules. In this method, a monolayer film is generated as a result of adsorption and bonding of suitable molecules (*e.g.*, fatty acids, organic silicon molecules or organic phosphoric molecules) on a suitable substrate surface. The method typically involves solution deposition chemistry in the presence of water.

Over the years, extensive efforts were made to develop row materials which can be used for manufacturing fiber and films by the above techniques to provide

articles having enhanced and/or application-specific characteristics. For example, one of the most studied natural fibrillar system is silk [Kaplan DL, "Fibrous proteins - silk as a model system," Polymer degradation and stability, 59:25-32, 1998]. There are many forms of silk, of which spider silk of Nephila clavipas (the golden orb weaver) is regarded as nature's high performance fiber, with a remarkable combination of strength, flexibility, and toughness. Although assembled by non-covalent interactions, silk is stronger than steel per given fibrillar diameter but, at the same time, is much more flexible. Due to its superior mechanical properties, the spider silk can be used in many areas requiring the combination of high mechanical strength with biodegradability, *e.g.*, in tissue engineering applications [Kubik S., "High-Performance Fibers from Spider Silk," Angewandte Chemie International Edition, 41:2721-2723, 2002].

A known method of synthesizing spider silk material includes the introduction of a spider silk gene into a heterologous gene expression system and the secretion of spider silk protein therefrom. The protein is then processed, typically by electrospinning, to produce a fiber of enhanced mechanical properties [Jin HJ, Fridrikh SV, Rutledge GC and Kaplan DL, "Electrospinning Bombyx mori silk with poly(ethylene oxide)," Biomacromolecules, 3:1233-1239, 2002].

Recently, electrospinning has been employed to fabricate virus-based composite fibers hence to mimic the spinning process of silk spiders [Lee S and Belcher AM, "Virus-Based Fabrication of Micro- and Nanofibers Using Electrospinning," Nano letters, 4:388-390, 2004]. In this study, M13 virus was genetically modified to bind conductive and semiconductor materials, and was thereafter subjected to an electrospinning process to provide conductive and semiconductor fibers.

Other than synthesized spider silk, the electrospinning process can be applied on a diversity of polymers including polyamides, polyactides and water soluble polymer such as polyethyleneoxide [Huang ZM, Zhang YZ, Kotaki M and Ramakrishna S., "A review on polymer nanofibers by electrospinning and their applications in nanocomposites," Composites Science and technology, 63:2223-2253, 2003]. Heretofore, about 50 types of polymers have been successfully electrospun.

Electrospinning has also been used with carbon nanotubes to obtain super-though carbon-nanotube fibers [Dalton AB *et al.*, "Super-tough carbon-nanotube fibres

- These extraordinary composite fibres can be woven into electronic textiles," Nature, 423:703, 2003]. By modifying a familiar method for carbon nanotubes fibers [Vigolo *et al.*, "Macroscopic Fibers and Ribbons of Oriented Carbon Nanotubes," Science, 17:1331-1334, 2000] the researchers were able to spin a reel of nanotube gel fiber and then convert it into 100 m length of solid nanotube composite fiber. The resulting fibers were tougher than any other known natural or synthetic organic fiber.

However, carbon nanotubes in general and carbon-nanotube fibers in particular suffer from structural deviations. Although deviations in structure can be introduced in a "controlled" manner under specific conditions, frequent uncontrollable insertion of such defects result in spatial structures with unpredictable electronic, molecular and structural properties. In addition, the production process of carbon nanotubes is very expensive and presently stands hundreds of U.S. dollars per gram.

Other known nanostructures are peptide-based nanotubular structures, made through stacking of cyclic D-, L- peptide subunits. These peptides self-assemble through hydrogen-bonding interactions into nanotubules, which in-turn self-assemble into ordered parallel arrays of nanotubes. The number of amino acids in the ring determines the inside diameter of the nanotubes obtained. Such nanotubes have been shown to form transmembrane channels capable of transporting ions and small molecules [Ghadiri, M.R. et al., Nature 366, 324-327 (1993); Ghadiri, M.R. et al., Nature 369, 301-304 (1994); Bong, D.T. et al., Angew. Chem. Int. Ed. 40:988-1011, 2001].

More recently, surfactant-like peptides that undergo spontaneous assembly to form nanotubes with a helical twist have been discovered. The monomers of these surfactant peptides, like lipids, have distinctive polar and nonpolar portions. They are composed of 7-8 residues, approximately 2 nm in length when fully extended, and dimensionally similar to phospholipids found in cell membranes. Although the sequences of these peptides are diverse, they share a common chemical property, *i.e.*, a hydrophobic tail and a hydrophilic head. These peptide nanotubes, like carbon and lipid nanotubes, also have a very high surface area to weight ratio. Molecular modeling of the peptide nanotubes suggests a possible structural organization [Vauthey (2002) Proc. Natl. Acad. Sci. USA 99:5355; Zhang (2002) Curr. Opin. Chem. Biol. 6:865]. Based on observation and calculation, it is proposed that the cylindrical subunits are formed from surfactant peptides that self-assemble into

bilayers, where hydrophilic head groups remain exposed to the aqueous medium. Finally, the tubular arrays undergo self- assembly through non-covalent interactions that are widely found in surfactant and micelle structures and formation processes.

Peptide based bis(N-α-amido-glycyglycine)-1,7-heptane dicarboxylate molecules were also shown to be assembled into tubular structures [Matsui (2000) J. Phys. Chem. B 104:3383].

When the crystal structure of di-phenylalanine peptides was determined, it was noted that hollow nanometric channels are formed within the framework of the macroscopic crystal [Gorbitz *et al.*, Chemistry 7(23):5153-9, 2001]. However, no individual nanotubes could be formed by crystallization, as the crystallization conditions used in this study included evaporation of an aqueous solution at 80 °C. No formation of discrete nano-structures was reported under these conditions.

International Patent Application Nos. IL03/01045 and IL2004/000012 (see also Reches M and Gazit E, "Casting metal nanowires within discrete self-assembled peptide nanotubes," Science, 300:625-627, 2003), disclose a new procedure for making peptide nanostructures that show many ultrastructural and physical similarities to carbon nanotubes. These peptide nanostructures are self assembled by diphenylalanine, the core recognition motif of the β-amyloid peptide [Findeis *et al.*, "Peptide inhibitors of beta amyloid aggregation," Biochemistry, 38:6791, 1999; Tjernberg *et al.*, "Arrest of -Amyloid Fibril Formation by a Pentapeptide Ligand," J. Biol. Chem., 271:8545 - 8548 , 1996; and Soto *et al.*, "Beta-sheet breaker peptides inhibit fibrillogenesis in a rat brain model of amyloidosis: implications for Alzheimer's therapy," Nature Medicine, 4:822-826, 1998].

The self-assembled peptide nanostructures are well ordered assemblies of various shapes with persistence length on the order of micrometers. The formation of the peptide nanostructures is very efficient and the nanostructures solution is very homogeneous. Similar to carbon nanotubes, the peptide nanostructures are formed as individual entities. For industrial applications, the self-assembled peptide nanostructures are favored over carbon nanotubes and spider silk from standpoint of cost, production means and availability.

There is thus a widely recognized need for, and it would be highly advantageous to have macroscopic and microscopic articles exploiting the advantages of self-assembled peptide nanostructures.

SUMMARY OF THE INVENTION

According to one aspect of the present invention there is provided a method of forming a fiber made of peptide nanostructures, the method comprising providing peptide nanostructures in solution, and fiberizing the solution thereby forming at least one fiber of the peptide nanostructures.

According to further features in preferred embodiments of the invention described below, the fiberizing is by an electrospinning process, a wet spinning process, a dry spinning process, a gel spinning process, a dispersion spinning process, a reaction spinning process or a tack spinning process.

According to another aspect of the present invention there is provided a method of forming a film of peptide nanostructures, comprising: dissolving peptide molecules in an organic solvent; adding an aqueous solvent to the organic solvent such that an interface is formed between the organic solvent and the aqueous solvent; and incubating the organic and the aqueous solvents under conditions which allow the peptide molecules to form a film of peptide nanostructures in the interface.

According to still further features in the described preferred embodiments the organic solvent is an aromatic solvent, such as but not limitied to benzene. According to still further features in the described preferred embodiments the hydrophilic solvent is water.

According to yet another aspect of the present invention there is provided a method of forming at least one layer of peptide nanostructures, comprising: placing peptide nanostructures in an organic solvent; applying at least one droplet of the organic solvent onto a surface of an hydrophilic solvent; and applying pressure onto the at least one droplet of the organic solvent, so as to form at least one layer of peptide nanostructures on the surface of the hydrophilic solvent.

According to further features in preferred embodiments of the invention described below, the method further comprises transferring the at least one layer of the peptide nanostructures to a substrate.

According to still further features in the described preferred embodiments the transferring of the layer(s) to the substrate is effected by a Langmuir-Blodgett technique or a Langmuir-Schaeffer technique.

According to still another aspect of the present invention there is provided a method of forming an aligned array or film of peptide nanostructures. The method

comprising: dissolving peptide molecules in an organic solvent; applying the organic solvent on a substrate; and incubating the substrate and the organic solvent under conditions which allow the peptide molecules to form an aligned array or a film of peptide nanostructures on the substrate.

According to further features in preferred embodiments of the invention described below, the nanostructures are responsive to a magnetic field. According to still further features in the described preferred embodiments the method further comprises subjecting the substrate to a magnetic field.

According to further features in preferred embodiments of the invention described below, the nanostructures are responsive to an electric field. According to still further features in the described preferred embodiments the method further comprises subjecting the substrate to an electric field.

According to still another aspect of the present invention there is provided a method of forming a fiber made of peptide nanostructures, the method comprising subjecting peptide nanostructures, in solution, to an electric field so as to form at least one fiber of the peptide nanostructures.

According to further features in preferred embodiments of the invention described below, collecting the at least one fiber on a precipitation electrode.

According to still further features in the described preferred embodiments the collecting of the fiber(s) comprises rotating the precipitation electrode so as to wind the at least one fiber around the precipitation electrode.

According to still further features in the described preferred embodiments the collecting of the fiber(s) comprises moving the at least one fiber relative to the precipitation electrode so as to provide a nonwoven mat of peptide nanostructures.

According to still further features in the described preferred embodiments the method further comprises unwinding the at least one fiber of the peptide nanostructure off the precipitation electrode.

According to an additional aspect of the present invention there is provided a fiber comprising a plurality of peptide nanostructures as described herein, the fiber being at least 100 nm in length.

According to still an additional aspect of the present invention there is provided a nonwoven article comprising a plurality of electrospun fibers, wherein at least one of the plurality of electrospun fibers is the fiber described herein.

According to yet a further aspect of the present invention there is provided a thin film comprising at least one layer of peptide nanostructures as described herein, the thin film being at least 100 nm^2 in area size.

According to further features in preferred embodiments of the invention described below, each of the peptide nanostructures comprises no more than 4 amino acids, at least one of the 4 amino acids being an aromatic amino acid.

According to still further features in the described preferred embodiments each of the 4 amino acids is independently selected from the group consisting of naturally occurring amino acids, synthetic amino acids, β-amino acids, Peptide Nucleic Acid (PNA) and combinations thereof.

According to still further features in the described preferred embodiments at least one of the 4 amino acids is a D-amino acid.

According to still further features in the described preferred embodiments at least one of the 4 amino acids is an L-amino acid.

According to still further features in the described preferred embodiments at least one of the peptide nanostructures comprises at least two aromatic moieties.

According to still further features in the described preferred embodiments at least one of the peptide nanostructures is a homodipeptide.

According to still further features in the described preferred embodiments each of the amino acids is the homodipeptide comprises an aromatic moiety, such as, but not limited to, substituted naphthalenyl, unsubstituted naphthalenyl, substituted phenyl or unsubstituted phenyl.

According to still further features in the described preferred embodiments the substituted phenyl is selected from the group consisting of pentafluoro phenyl, iodophenyl, biphenyl and nitrophenyl.

Thus, representative examples of the amino acids in the homopeptide include, without limitation, naphthylalanine, *p*-nitro-phenylalanine, iodo-phenylalanine and fluro-phenylalanine.

According to still further features in the described preferred embodiments the homodipeptide is selected from the group consisting of naphthylalanine-naphthylalanine dipeptide, (pentafluro-phenylalanine)-(pentafluro-phenylalanine) dipeptide, (iodo-phenylalanine)-(iodo-phenylalanine) dipeptide, (4-phenyl

phenylalanine)-(4-phenyl phenylalanine) dipeptide and (*p*-nitro-phenylalanine)-(*p*-nitro-phenylalanine) dipeptide.

According to still further features in the described preferred embodiments each of the peptide nanostructures comprises a plurality of polyaromatic peptides.

According to still further features in the described preferred embodiments each of the plurality of polyaromatic peptides comprises a component selected from the group consisting of a polyphenylalanine peptide, a polytriptophane peptide, a polytyrosine peptide, a non-natural derivatives thereof and a combination thereof.

According to still further features in the described preferred embodiments each of the plurality of polyaromatic peptides comprises at least 30 amino acids.

According to still further features in the described preferred embodiments the nanostructures at least partially enclose a material therein.

According to still further features in the described preferred embodiments the material is in a gaseous state.

According to still further features in the described preferred embodiments the material is in a condensed state.

According to still further features in the described preferred embodiments the material is selected from the group consisting of a conducting material, a semiconducting material, a thermoelectric material, a magnetic material, a light-emitting material, a biomineral, a polymer and an organic material.

According to still further features in the described preferred embodiments the conducting material is selected from the group consisting of silver, gold, copper, platinum, nickel and palladium.

According to still further features in the described preferred embodiments the semiconducting material is selected from the group consisting of CdS, CdSe, ZnS and SiO_2.

According to still further features in the described preferred embodiments the magnetic material is a paramagnetic material.

According to still further features in the described preferred embodiments the paramagnetic material is selected from the group consisting of cobalt, copper, nickel and platinum.

According to still further features in the described preferred embodiments the magnetic material is a ferromagnetic material.

According to still further features in the described preferred embodiments the ferromagnetic material is selected from the group consisting of magnetite and NdFeB.

According to still further features in the described preferred embodiments the light-emitting material is selected from the group consisting of dysprosium, europium, terbium, ruthenium, thulium, neodymium, erbium, ytterbium and any organic complex thereof.

According to still further features in the described preferred embodiments the biomineral comprises calcium carbonate.

According to still further features in the described preferred embodiments the polymer is selected from the group consisting of polyethylene, polystyrene polyvinyl chloride and a thermoplastic polymer.

According to still further features in the described preferred embodiments the thermoelectric material is selected from the group consisting of bismuth telluride, bismuth selenide, bismuth antimony telluride and bismuth selenium telluride.

According to still further features in the described preferred embodiments the polymer is selected from the group consisting of a polynucleotide and a polypeptide.

The present invention successfully addresses the shortcomings of the presently known configurations by providing a fiber, film, article and method of manufacturing the same. The fiber, film, article and method of the present invention enjoy properties far exceeding the prior art.

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

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only, and

are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for a fundamental understanding of the invention, the description taken with the drawings making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

In the drawings:

FIG. 1 is a flowchart diagram of a method suitable for forming a fiber made of peptide nanostructures, according to a preferred embodiment of the present invention;

FIG. 2 is a flowchart diagram of a method suitable for forming a film of peptide nanostructures, according to a preferred embodiment of the present invention;

FIG. 3 is a flowchart diagram of a method suitable for forming one or more layers of peptide nanostructures, according to another preferred embodiment of the present invention;

FIG. 4 is a flowchart diagram of a method suitable for forming an array or film of peptide nanostructures on a substrate, according to another preferred embodiment of the present invention;

FIGs. 5A-C show a process of manufacturing a thin film in accordance with preferred embodiments of the present invention;

FIG. 6 is a pressure-area isotherm obtained during manufacturing of a thin film using Langmuir-Blodgett technique and in accordance with preferred embodiments of the present invention;

FIGa. 7A-B are high-resolution scanning electron microscope images, showing a low magnification (Figure 7A) and a high magnification (Figure 7B) of an aligned array of peptide nanotubes formed on a substrate, according to the teaching of various exemplary embodiments of the present invention;

FIG. 8 is a schematic illustration of a chemical structure of a naphthylalanine-naphthylalanine (Nal-Nal) dipeptide;

FIG. 9 is an electron microscope image of Nal-Nal tubular nanostructures; and

FIGs. 10A-D are electron microscope images of tubular and planar nanostructures assembled from the following aromatic-homodipeptides: (pentafluro-phenylalanine)-(pentafluro-phenylalanine) (Figure 10A), (iodo-phenylalanine)-(iodo-

phenylalanine) (Figure 10B), (4-phenyl phenylalanine)-(4-phenyl phenylalanine) (Figure 10C), and (p-nitro-phenylalanine)-(p-nitro-phenylalanine) (Figure 10D).

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is of a method which can be used for manufacturing articles of peptide nanostructures. Specifically, the present invention can be used to manufacture fibers, films and other articles having sizes at least in the micrometer scale. The present invention is further of fibers, films and other articles made of peptide nanostructures.

The principles and operation of a method according to the present invention may be better understood with reference to the drawings and accompanying descriptions.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to the details of construction and the arrangement of the components set forth in the following description or illustrated in the drawings. The invention is capable of other embodiments or of being practiced or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

Referring now to the drawings, Figure 1 is a flowchart diagram of a method suitable for forming a fiber made of peptide nanostructures. The method begins at step **10** and continues to step **12** in which peptide nanostructures in solution are provided.

As used herein the phrase "nanostructure" refers to a structure having a diameter or a cross-section of less than 1 μm (preferably less than 500 nm, more preferably less than about 50 nm, even more preferably less than about 5 nm). The length of the nanostructure of the present embodiments is preferably at least 1 nm, more preferably at least 10 nm, even more preferably at least 100 nm and even more preferably at least 500 nm. It will be appreciated, though, that the nanostructure of the present embodiments can be of infinite length (*i.e.*, macroscopic fibrous structures) and as such can be used in the fabrication of hyper-strong materials.

As used herein the term "about" refers to ± 10 %.

The solution can be prepared, for example, by placing or dissolving the nanostructures in an organic solvent, which is preferably an aromatic solvent, such as, but not limited to, benzene. Additionally, the solution may contain polymeric additives, or any other material suitable for forming fibers therefrom.

The method continues to step **14** in which the solution is fiberized to form at least one fiber of peptide nanostructures. The solution can be fiberized by any conventional process, such as, but not limited to, a spinning process, a blowing process, an injection process and the like. Contemplated spinning processes include, without limitation, wet spinning process, gel spinning process, dry spinning process, dispersion spinning process, reaction spinning process, tack spinning process and electrospinning process. These spinning processes are described in the Background section above and can be found in many text books and patents, see, *e.g.*, U.S. Patent Nos., 3,737,508, 3,950,478, 3,996,321, 4,189,336, 4,402,900, 4,421,707, 4,431,602, 4,557,732, 4,643,657, 4,804,511, 5,002,474, 5,122,329, 5,387,387, 5,667,743, 6,248,273 and 6,252,031 the contents of which are hereby incorporated by reference. Representative examples of spinning processes in various preferred embodiments of the present invention are further detailed hereinunder.

The method ends at step **16**.

Peptide nanostructures which can be used according to the present embodiments include nanostructures composed of surfactant like peptides and cyclic D-, L-peptide subunits. The nanostructures can be self-assembled from a plurality of peptides. Preferably, but not obligatorily, the peptides include no more than 4 amino acids of which at least one is an aromatic amino acid.

In various exemplary embodiments of the embodiments the peptides comprise a dipeptide or a tripeptide. The shape of the nanostructures depends on the rigidity of the molecular structure of the peptide used. For example a plurality of diphenylglycine peptides which offer similar molecular properties as diphenylalenine peptides albeit with a lower degree of rotational freedom around the additional C-C bond and a higher steric hindrence self-assemble into nano-spheres, while a plurality of diphenylalenine peptides self-assemble into nano-tubes.

The present embodiments also envisages nanostructures which are composed of a plurality of polyaromatic peptides being longer than the above described (e.g., 50-136 amino acids).

As used herein the phrase "polyaromatic peptides" refers to peptides which include at least 80 %, at least 85 % at least 90 %, at least 95 % or more, say 100 % aromatic amino acid residues. These peptides can be homogenic (e.g., polyphenylalanine) or heterogenic of at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 100, at least 105, at least 110, at least 120, at least 125, at least 130, at least 135, at least 140, at least 145, at least 150, at least 155, at least 160, at least 170, at least 190, at least 200, at least 300 or at least 500 amino acids.

The term "peptide" as used herein encompasses native peptides (either degradation products, synthetically synthesized peptides or recombinant peptides) and peptidomimetics (typically, synthetically synthesized peptides), as well as peptoids and semipeptoids which are peptide analogs, which may have, for example, modifications rendering the peptides more stable while in a body or more capable of penetrating into cells. Such modifications include, but are not limited to N terminus modification, C terminus modification, peptide bond modification, including, but not limited to, CH2-NH, CH2-S, CH2-S=O, O=C-NH, CH2-O, CH2-CH2, S=C-NH, CH=CH or CF=CH, backbone modifications, and residue modification. Methods for preparing peptidomimetic compounds are well known in the art and are specified, for example, in Quantitative Drug Design, C.A. Ramsden Gd., Chapter 17.2, F. Choplin Pergamon Press (1992), which is incorporated by reference as if fully set forth herein. Further details in this respect are provided hereinunder.

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

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

Natural aromatic amino acids, Trp, Tyr and Phe, may be substituted for synthetic non-natural acid such as Phenylglycine, TIC, naphthylalanine (Nal), ring-

methylated derivatives of Phe, halogenated derivatives of Phe or O-methyl-Tyr and β amino-acids

In addition to the above, the peptides of the present embodiments may also include one or more modified amino acids (*e.g.*, thiolated or biotinylated amino acids) or one or more non-amino acid monomers (*e.g.*, fatty acids, complex carbohydrates *etc.*). Also contemplated are homodipeptides, and more preferably aromatic homodipeptides in which each of the amino acids comprises an aromatic moiety, such as, but not limited to, substituted or unsubstituted naphthalenyl and substituted or unsubstituted phenyl. The aromatic moiety can alternatively be substituted or unsubstituted heteroaryl such as, for example, indole, thiophene, imidazole, oxazole, thiazole, pyrazole, pyridine, pyrimidine, quinoline, isoquinoline, quinazoline, quinoxaline, and purine

When substituted, the phenyl, naphthalenyl or any other aromatic moiety includes one or more substituents such as, but not limited to, alkyl, trihaloalkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, heteroalicyclic, halo, nitro, azo, hydroxy, alkoxy, thiohydroxy, thioalkoxy, cyano, and amine.

As used herein, the term "alkyl" refers to a saturated aliphatic hydrocarbon including straight chain and branched chain groups. Preferably, the alkyl group has 1 to 20 carbon atoms. The alkyl group may be substituted or unsubstituted. When substituted, the substituent group can be, for example, trihaloalkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, heteroalicyclic, halo, nitro, azo, hydroxy, alkoxy, thiohydroxy, thioalkoxy, cyano, and amine.

A "cycloalkyl" group refers to an all-carbon monocyclic or fused ring (*i.e.*, rings which share an adjacent pair of carbon atoms) group wherein one of more of the rings does not have a completely conjugated pi-electron system. Examples, without limitation, of cycloalkyl groups are cyclopropane, cyclobutane, cyclopentane, cyclopentene, cyclohexane, cyclohexadiene, cycloheptane, cycloheptatriene, and adamantane. A cycloalkyl group may be substituted or unsubstituted. When substituted, the substituent group can be, for example, alkyl, trihaloalkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, heteroalicyclic, halo, nitro, azo, hydroxy, alkoxy, thiohydroxy, thioalkoxy, cyano, and amine.

An "alkenyl" group refers to an alkyl group which consists of at least two carbon atoms and at least one carbon-carbon double bond.

An "alkynyl" group refers to an alkyl group which consists of at least two carbon atoms and at least one carbon-carbon triple bond.

An "aryl" group refers to an all-carbon monocyclic or fused-ring polycyclic (*i.e.*, rings which share adjacent pairs of carbon atoms) groups having a completely conjugated pi-electron system. Examples, without limitation, of aryl groups are phenyl, naphthalenyl and anthracenyl. The aryl group may be substituted or unsubstituted. When substituted, the substituent group can be, for example, alkyl, trihaloalkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, heteroalicyclic, halo, nitro, azo, hydroxy, alkoxy, thiohydroxy, thioalkoxy, cyano, and amine.

A "heteroaryl" group refers to a monocyclic or fused ring (*i.e.*, rings which share an adjacent pair of atoms) group having in the ring(s) one or more atoms, such as, for example, nitrogen, oxygen and sulfur and, in addition, having a completely conjugated pi-electron system. Examples, without limitation, of heteroaryl groups include pyrrole, furane, thiophene, imidazole, oxazole, thiazole, pyrazole, pyridine, pyrimidine, quinoline, isoquinoline and purine. The heteroaryl group may be substituted or unsubstituted. When substituted, the substituent group can be, for example, alkyl, trihaloalkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, heteroalicyclic, halo, nitro, azo, hydroxy, alkoxy, thiohydroxy, thioalkoxy, cyano, and amine.

A "heteroalicyclic" group refers to a monocyclic or fused ring group having in the ring(s) one or more atoms such as nitrogen, oxygen and sulfur. The rings may also have one or more double bonds. However, the rings do not have a completely conjugated pi-electron system. The heteroalicyclic may be substituted or unsubstituted. When substituted, the substituted group can be, for example, lone pair electrons, alkyl, trihaloalkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, heteroalicyclic, halo, nitro, azo, hydroxy, alkoxy, thiohydroxy, thioalkoxy, cyano, and amine. Representative examples are piperidine, piperazine, tetrahydro furane, tetrahydropyrane, morpholino and the like.

A "hydroxy" group refers to an -OH group.

An "azide" group refers to a -N=N≡N group.

An "alkoxy" group refers to both an -O-alkyl and an -O-cycloalkyl group, as defined herein.

An "aryloxy" group refers to both an -O-aryl and an -O-heteroaryl group, as defined herein.

A "thiohydroxy" group refers to an -SH group.

A "thioalkoxy" group refers to both an -S-alkyl group, and an -S-cycloalkyl group, as defined herein.

A "thioaryloxy" group refers to both an -S-aryl and an -S-heteroaryl group, as defined herein.

A "halo" group refers to fluorine, chlorine, bromine or iodine.

A "trihaloalkyl" group refers to an alkyl substituted by three halo groups, as defined herein. A representative example is trihalomethyl.

An "amino" group refers to an –NR'R'' group where R' and R'' are hydrogen, alkyl, cycloalkyl or aryl.

A "nitro" group refers to an $-NO_2$ group.

A "cyano" group refers to a -C≡N group.

Representative examples of homodipeptides which can be used include, without limitation, naphthylalanine-naphthylalanine (nal-nal), (pentafluro-phenylalanine)-(pentafluro-phenylalanine), (iodo-phenylalanine)-(iodo-phenylalanine), (4-phenyl phenylalanine)-(4-phenyl phenylalanine) and (p-nitro-phenylalanine)-(p-nitro-phenylalanine), see Examples 4, 5 and Figures 8-10.

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

The nanostructures which can be used in the present embodiments are preferably generated by allowing a highly concentrated hydrophilic solution of the peptides of the present embodiments to self-assemble under mild conditions. The resulting nanostructures are preferably stable under acidic and/or basic pH conditions, a wide range of temperatures (*e.g.*, 4-400 °C, more preferably 2-200 °C) and/or proteolytic conditions (*e.g.*, proteinase K).

It was found by the present Inventor that the peptide nanostructures are sufficiently stable to allow integration of the peptide nanostructures into polymer fibers during manufacturing process of fibers or other articles.

Depending on the number and type of amino acids used, the nanostructure can be insulators, conductors or semiconductors. The nanostructure of the present embodiments can also be utilized as carriers onto which atoms of different materials (*e.g.*, conductive materials, chemical or biological agents, *etc.*) may be incorporated.

According to preferred embodiments of the present invention, the nanostructures are filled or partially filled with at least one material (*i.e.*, the nanostructures enclose or partially enclose the material). The material can be composed of a conducting material, a semiconducting material, a thermoelectric material, a magnetic material (paramagnetic, ferromagnetic or diamagnetic), a light-emitting material, a gaseous material, a biomineral, a polymer and/or an organic material.

For example, the nanostructures may enclose conducting or semiconducting materials, including, without limitation, inorganic structures such as Group IV, Group III/Group V, Group II/Group VI elements, transition group elements, or the like.

As used herein, the term "Group" is given its usual definition as understood by one of ordinary skill in the art. For instance, Group II elements include Zn, Cd and Hg; Group III elements include B, Al, Ga, In and Tl; Group IV elements include C, Si, Ge, Sn and Pb; Group V elements include N, P, As, Sb and Bi; and Group VI elements include O, S, Se, Te and Po.

Thus, for conducting materials, the nanostructures may enclose, for example, silver, gold, copper, platinum, nickel or palladium. For semiconducting materials the nanostructures may enclose, for example, silicon, indium phosphide, gallium nitride and others.

The nanostructures may also encapsulate, for example, any organic or inorganic molecules that are polarizable or have multiple charge states. For example, the nanostructures may include main group and metal atom-based wire-like silicon, transition metal-containing wires, gallium arsenide, gallium nitride, indium phosphide, germanium, or cadmium selenide structures.

Additionally, the nanostructure of the present embodiments may enclose various combinations of materials, including semiconductors and dopants.

Representative examples include, without limitations, silicon, germanium, tin, selenium, tellurium, boron, diamond, or phosphorous. The dopant may also be a solid solution of various elemental semiconductors, for example, a mixture of boron and carbon, a mixture of boron and P, a mixture of boron and silicon, a mixture of silicon and carbon, a mixture of silicon and germanium, a mixture of silicon and tin, or a mixture of germanium and tin. In some embodiments, the dopant or the semiconductor may include mixtures of different groups, such as, but not limited to, a mixture of a Group III and a Group V element, a mixture of Group III and Group V elements, a mixture of Group II and Group VI semiconductors. Additionally, alloys of different groups of semiconductors may also be possible, for example, a combination of a Group II-Group VI and a Group III-Group V semiconductor and a Group I and a Group VII semiconductor.

Specific and representative examples of semiconducting materials which can be encapsulated by the nanostructure of the present embodiments include, without limitation, CdS, CdSe, ZnS and SiO_2.

The nanostructure of the present embodiments may also enclose a thermoelectric material that exhibits a predetermined thermoelectric power. Preferably, such a material is selected so that the resulting nanostructure composition is characterized by a sufficient figure of merit. Such composition may be used in thermoelectric systems and devices as heat transfer media or thermoelectric power sources. According to a preferred embodiment of the present invention the thermoelectric material which can be encapsulated in the nanostructure of the present embodiments may be a bismuth-based material, such as, but not limited to, elemental bismuth, a bismuth alloy or a bismuth intermetallic compound. The thermoelectric material may also be a mixture of any of the above materials or other materials known to have thermoelectric properties. In addition the thermoelectric material may also include a dopant. Representative examples include, without limitation, bismuth telluride, bismuth selenide, bismuth antimony telluride, bismuth selenium telluride and the like. Other materials are disclosed, for example, in U.S. Patent Application No. 20020170590.

As stated, the nanostructure of the present embodiments may also enclose magnetic materials. Generally, all materials in nature posses some kind of magnetic properties which are manifested by a force acting on a specific material when present

in a magnetic field. These magnetic properties, which originate from the sub-atomic structure of the material, are different from one substrate to another. The direction as well as the magnitude of the magnetic force is different for different materials.

Whereas the direction of the force depends only on the internal structure of the material, the magnitude depends both on the internal structure as well as on the size (mass) of the material. The internal structure of the materials in nature, to which the magnetic characteristics of matter are related, is classified according to one of three major groups: diamagnetic, paramagnetic and ferromagnetic materials, where the strongest magnetic force acts on ferromagnetic materials.

In terms of direction, the magnetic force acting on a diamagnetic material is in opposite direction than that of the magnetic force acting on a paramagnetic or a ferromagnetic material. When placed in external magnetic field, a specific material acquires a non-zero magnetic moment per unit volume, also known as a magnetization, which is proportional to the magnetic field vector. For a sufficiently strong external magnetic field, a ferromagnetic material, due to intrinsic non-local ordering of the spins in the material, may retain its magnetization, hence to become a permanent magnet. As opposed to ferromagnetic materials, both diamagnetic and paramagnetic materials loose the magnetization once the external magnetic field is switched off.

Representative examples of paramagnetic materials which can be enclosed by the nanostructure of the present embodiments include, without limitation, cobalt, copper, nickel and platinum. Representative examples of ferromagnetic materials include, without limitation, magnetite and NdFeB.

Other materials which may be encapsulated by the nanostructure of the present embodiments include, without limitation, light-emitting materials (*e.g.*, dysprosium, europium, terbium, ruthenium, thulium, neodymium, erbium, ytterbium or any organic complex thereof), biominerals (*e.g.*, calcium carbonate), polymers (*e.g.*, polyethylene, polystyrene, polyvinyl chloride, polynucleotides and polypeptides, thermoplastics, fluorescent materials or other colored materials.

In order to generate the filled nanostructure of the present embodiments, the foreign material is introduced into the internal cavity of the nanostructure, to encapsulate the material in nanostructure.

Exemplified methods for filling nanostructure are described in "Capillarity-induced filling of carbon nanotubes", PM Ajayan et al., Nature, vol. 361, 1993, pp. 333-334; "A simple chemical method of opening and filling carbon nanotubes", SC Tsang et al., Nature, vol. 372, 1994, pp. 159-162; and U.S. Pat. Nos. 5,916,642 and 6,361,861.

Following are representative examples of spinning methods which can be used in various preferred embodiments of the present invention.

In the embodiment in which electrospinning is employed, the solution with the peptide nanostructures is extruded, for example under the action of hydrostatic pressure, through capillary apertures of a dispenser, which is spaced apart from a precipitation electrode. The dispenser and precipitation electrode are kept at different electrical potentials thus forming an electric field therebetween. Under the effect of electrical force, jets depart from the dispenser and travel towards the precipitation electrode. Moving with high velocity in the inter-electrode space, the jet cools or solvent therein evaporates, thus forming fibers which are collected on the surface of the precipitation electrode.

In the embodiment in which a wet spinning process is employed, the solution with the peptide nanostructure of the present embodiments is extruded through a spinneret, under the action of mechanical forces (*e.g.*, pressure, gravity). The formed fiber(s) can then be collected using a suitable take up device, *e.g.*, a drum.

In the embodiment in which a dry spinning process is employed, the solution with the peptide nanostructures of the present embodiments is extruded through a spinneret, and solvent therein is rapidly evaporated by inert gas. Similarly to the above, the formed fiber(s) can be collected using a take up device.

In the embodiment in which tack spinning is employed, the solution is preferably prepared in a tack state. This can be done, for example, by mixing the solution with certain polymeric additives which facilitate the adherence of the solution to a surface. Once prepared, the solution with the peptide nanostructures is pressed against a heated surface, such as, but not limited to, a heated roll. The solution can then be separated from the surface and cooled by blowing cold air or another cooling medium into a nip formed between the heated surface and the solution as the solution is separated from the surface. The separation and cooling of the solution result in drawing of fibrils out from the surface.

In any event, once the fibers are formed and collected, they can be unwound off the take up device, if desired, for example, for packing or storage purposes or for uploading to another apparatus.

It is expected that during the life of this patent many relevant spinning processes will be developed and the scope of the term spinning processes is intended to include all such new technologies *a priori*.

Performing the above method according to present embodiments successfully produces one or more fibers. Therefore, according to another aspect of the present invention there is provided a fiber of peptide nanostructures. In accordance with preferred embodiments of the present invention the fiber is at least 100 nm, more preferably at least 1 μm, more preferably at least 10 μm in length. The fiber can contain any of the aforementioned peptide nanostructures.

The fibers of the present embodiments can be used for forming nonwoven articles. This can be done, for example, by repeating the selected spinning process a plurality of times and allowing the formed fiber to precipitate on a suitable precipitation device thus forming the nonwoven article thereupon. As will be appreciated by one ordinarily skilled in the art, when a relative motion is established between the formed fiber(s) and the precipitation device, a nonwoven mat of fibers made of peptide nanostructures is formed.

Reference is now made to Figure 2 which is a flowchart diagram of a method suitable for forming a film of peptide nanostructures, according to a preferred embodiment of the present invention. The method begins at step **20** and continues to step **22** in which peptide molecule are placed in an organic solvent. The method continues to step **24** in which an hydrophilic solvent (*e.g.*, water) is added to the organic solvent, such that an interface is formed between the organic and hydrophilic solvents. The method then proceeds to step **26** in which the organic and hydrophilic solvents are incubated under conditions which allow the peptide molecules to form a film of peptide nanostructures in the formed interface. The incubation conditions are such that the nanostructures are self-assembled as further detailed hereinabove.

The method ends at step **28**.

Reference is now made to Figure 3 which is a flowchart diagram of a method suitable for forming one or more layers of peptide nanostructures, according to another

preferred embodiment of the present invention. The method begins at step **30** and continues to step **32** in which peptide nanostructures are placed in an organic solvent.

The method continues to step **34** in which one or more droplets of the organic solvent are applied onto a surface of an hydrophilic solvent. The method then continues to step **36** in which pressure is applied on the droplet of organic solvent, so as to form a layer of peptide nanostructures on the surface.

The application of pressure is preferably by moving barriers which can be made, for example, from Teflon®. The barriers reduce the surface area of the film and as a consequence the surface pressure of the layer increases. The pressure-area isotherm can be monitored continuously, *e.g.*, by monitoring the surface are of the layer and measuring the force applied by the barrier. When pressure reaches some predetermined level, the barriers are stopped such that the layer is substantially in a steady state.

The layers can then be transferred to a substrate by any way known in the art, for example, the Langmuir-Blodgett technique or the Langmuir-Schaeffer technique.

When a Langmuir-Blodgett technique is employed, the substrate is preferably immersed a vertically through the layer. The substrate is then pulled up and the layer is transferred onto the substrate by lateral compression.

When a Langmuir-Schaeffer is employed, the substrate is descended horizontally onto the layer. Once a contact is made between the layer and the substrate, the substrate is extracted with the layer on it.

According to a preferred embodiment of the present invention the film can be subjected to a doping procedure. Doping procedures are known in the art and are found, for example, in the Handbook for conductive polymers, Edited by Terje A. Skotheim Vol. 1, 1986. This embodiment is particularly useful when it is desired to form a film with conductive properties.

Reference is now made to Figure 4 which is a flowchart diagram of a method suitable for forming an array or film of peptide nanostructures on a substrate, according to a preferred embodiment of the present invention. The method begins at step **40** and continues to step **42** in which peptide molecule are placed in an organic solvent, such as, but not limited to, Hexafloropropanol. The method continues to step **44** in which the organic solvent is applied on a substrate, such as, but not limited to, a siliconized glass an ITO glass and the like. The method then proceeds to step **46** in

which the substrate is incubated under conditions which allow the peptide molecules to form an array or film of peptide nanostructures on the substrate. The incubation conditions are preferably such that the nanostructures are self-assembled as further detailed hereinabove.

When the nanostructures are responsive to a magnetic and/or electric field (*i.e.*, when a magnetic and/or electric field exerts a force on the nanostructures), the method preferably continues to step **48** in which the substrate is subjected to a magnetic and/or electric field. The advantage of this embodiment, is that the forces can facilitate the assembling and/or alignment of the nanostructures on the substrate. Step **48** can be executed subsequently or contemporaneously with step **46**.

The method ends at step **49**.

Performing one or more of the above methods according to present embodiments successfully produces a thin film of peptide nanostructures. In accordance with preferred embodiments of the present invention, the thin film is at least 100 nm^2, more preferably at least 1 μm^2, more preferably at least 10 μm^2 in area size.

Additional objects, advantages and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

EXAMPLES

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

EXAMPLE 1

Thin film of peptide nanostructures was manufactured, in accordance with preferred embodiments of the present invention.

The manufacturing process is shown in Figures 5A-C. In a first step, shown in Figure 5A, Boc-FF-COOH dipeptide was dissolved in benzene to a concentration of 10 mg/ml and placed in a reaction beaker. In a second step, shown in Figure 5B, a water solution was added into the reaction beaker. As shown in Figure 5B, an

interface was formed between the benzene and the water solution. The benzene and the water solution were incubated for several hours. Figure 5C shows a film of Boc-FF-COOH dipeptide which was fromed at the interface after incubation.

EXAMPLE 2

The Langmuir-Blodgett technique was employed to manufacture a thin film of peptide nanostructures, in accordance with preferred embodiments of the present invention.

A droplet of Boc- FF-COOH dipeptide was dissolved in chloroform-methanol (9:1) to a concentration of 1 mg/ml. The resulted solution was then applied on a surface of water in a Langmuir-Blodgett trough. Moving barriers, positioned at the ends of the trough were used to apply pressure on the droplet, and a film of nanostructures was formed.

Figure 6 shows a pressure-area isotherm obtained while applying the pressure. As shown in Figure 6, the surface tension increases when the surface area is decreased, and peptide molecules are assembled together into a monolayer, which is further compressed into a film.

EXAMPLE 3

An array of peptide nanostructures was manufactured on a substrate, in accordance with preferred embodiments of the present invention.

Diphenylalanine peptide was dissolved to a concentration of 100 mg/ml in 1,1,1,3,3,3-Hexafluoropropanol. 30 µl of the solution were applied onto siliconized glass and let to dry in room temperature.

Figures 7A-B are a low magnification (Figure 7A) and a high magnification (Figure 7B) images of the substrate, obtained using a high-resolution scanning electron microscope. As shown in Figures 7a-b, an aligned array of peptide nanotubes was formed on the substrate.

EXAMPLE 4

Tubular nanostructures were formed from naphthylalanine-naphthylalanine (Nal-Nal) dipeptides, in accordance with preferred embodiment of the present

invention. The Chemical structure of the (Nal-Nal) dipeptide is schematically shown in Figure 8.

Fresh stock solutions of Nal-Nal dipeptides were prepared by dissolving lyophilized form of the peptides in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP, Sigma) at a concentration of 100 mg/mL. To avoid any pre-aggregation, fresh stock solutions were prepared for each experiment.

The peptides stock solutions were diluted into a final concentration of 2 mg/mL in double distilled water, then the samples were placed on 200 mesh copper grid, covered by carbon stabilized formvar film. Following 1 minute, excess fluid was removed and the grid was negatively stained with 2 % uranyl acetate in water. Following 2 minutes of staining, excess fluid was removed from the grid. Samples were viewed in JEOL 1200EX electron microscope operating at 80 kV.

Figure 9 is an electron microscope image of the samples, captured a few minutes after the dilution of the peptide stock into the aqueous solution. As shown, the dipeptides form thin (from several nanometers to a few tens of nanometers in diameter) and elongated (several microns in length) tubular structures.

EXAMPLE 5

Tubular and planar nanostructures were formed from by four different dipeptides, in accordance with preferred embodiment of the present invention.

The following dipeptides were used: (Pentafluro-Phenylalanine)-(Pentafluro-Phenylalanine), (Iodo-Phenylalanine)-(Iodo-Phenylalanine), (4-Phenyl phenylalanine)-(4-Phenyl phenylalanine) and (P-nitro-Phenylalanine)-(P-nitro-Phenylalanine).

For the first two dipeptides [(Pentafluro-Phenylalanine)-(Pentafluro-Phenylalanine) and (Iodo-Phenylalanine)-(Iodo-Phenylalanine)] fresh stock solutions were prepared by dissolving lyophilized form of the peptides in DMSO at a concentration of 100 mg/mL.

For the third and fourth dipeptides [(4-Phenyl phenylalanine)-(4-Phenyl phenylalanine) and (P-nitro-Phenylalanine)-(P-nitro-Phenylalanine)], fresh stock solutions were prepared by dissolving lyophilized form of the peptides in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP, Sigma) at a concentration of 100 mg/mL. To avoid any pre-aggregation, fresh stock solutions were prepared for each experiment.

The peptides stock solutions were diluted into a final concentration of 2 mg/mL in double distilled water.

In the case of (P-nitro-Phenylalanine)-(P-nitro-Phenylalanine) the final concentration was 5 mg/mL.

Subsequently, the samples were placed on 200 mesh copper grid, covered by carbon stabilized formvar film. Following 1 minute, excess fluid was removed and the grid was negatively stained with 2 % uranyl acetate in water. Following 2 minutes of staining, excess fluid was removed from the grid. Samples were viewed in JEOL 1200EX electron microscope operating at 80 kV.

Figures 10A-D are electron microscope images of the four samples, captured a few minutes after the dilution of the peptide stock into the aqueous solution.

Figure 10A shows tubular assemblies formed by the (Pentafluro-Phenylalanine)-(Pentafluro-Phenylalanine) dipeptide, Figure 10B shows tubular structures assembled by (Iodo-Phenylalanine)-(Iodo-Phenylalanine), Figure 10 C shows planar nanostructures formed by (4-Phenyl phenylalanine)-(4-Phenyl phenylalanine), and Figure 10D shows fibrilar assemblies of (P-nitro-Phenylalanine)-(P-nitro-Phenylalanine).

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims. All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application

shall not be construed as an admission that such reference is available as prior art to the present invention.

WHAT IS CLAIMED IS:

1. A method of forming a fiber made of peptide nanostructures, the method comprising providing peptide nanostructures in solution, and fiberizing said solution thereby forming at least one fiber of said peptide nanostructures.

2. The method of claim 1, wherein said fiberizing is by an electrospinning process.

3. The method of claim 1, wherein said fiberizing is by a wet spinning process.

4. The method of claim 1, wherein said fiberizing is by a dry spinning process.

5. The method of claim 1, wherein said fiberizing is by a gel spinning process.

6. The method of claim 1, wherein said fiberizing is by a dispersion spinning process.

7. The method of claim 1, wherein said fiberizing is by a reaction spinning process.

8. The method of claim 1, wherein said fiberizing is by a tack spinning process.

9. A method of forming a film of peptide nanostructures, comprising: dissolving peptide molecules in an organic solvent; adding an hydrophilic solvent to said organic solvent such that an interface is formed between said organic solvent and said hydrophilic solvent; and incubating said organic and said hydrophilic solvents under conditions which allow said peptide molecules to form a film of peptide nanostructures in said interface.

10. The method of claim 9, wherein said organic solvent is an aromatic solvent.

11. The method of claim 10, wherein said aromatic solvent comprises benzene.

12. The method of claim 10, wherein said hydrophilic solvent is water.

13. A method of forming at least one layer of peptide nanostructures, comprising: placing peptide nanostructures in an organic solvent; applying at least one droplet of said organic solvent onto a surface of an hydrophilic solvent; and applying pressure onto said at least one droplet of said organic solvent, so as to form at least one layer of peptide nanostructures on said surface of said hydrophilic solvent.

14. The method of claim 13, further comprising transferring said at least one layer of said peptide nanostructures to a substrate.

15. The method of claim 14, wherein said transferring of said at least one layer of said peptide nanostructures to said substrate is effected by a technique selected from the group consisting of a Langmuir-Blodgett technique and a Langmuir-Schaeffer technique.

16. A method of forming an aligned array or film of peptide nanostructures, comprising: dissolving peptide molecules in an organic solvent; applying said organic solvent on a substrate; and incubating said substrate and said organic solvent under conditions which allow said peptide molecules to form an aligned array or a film of peptide nanostructures on said substrate.

17. The method of claim 16, wherein said nanostructures are responsive to a magnetic field.

18. The method of claim 17, further comprising subjecting said substrate to a magnetic field.

19. The method of claim 16, wherein said nanostructures are responsive to an electric field.

20. The method of claim 19, further comprising subjecting said substrate to an electric field.

21. A method of forming a fiber made of peptide nanostructures, the method comprising subjecting peptide nanostructures, in solution, to an electric field so as to form at least one fiber of said peptide nanostructures.

22. The method of claim 21, further comprising collecting said at least one fiber on a precipitation electrode.

23. The method of claim 22, wherein said collecting said at least one fiber comprises rotating said precipitation electrode so as to wind said at least one fiber around said precipitation electrode.

24. The method of claim 22, wherein said collecting said at least one fiber comprises moving said at least one fiber relative to said precipitation electrode so as to provide a nonwoven mat of peptide nanostructures.

25. The method of claim 22, further comprising unwinding said at least one fiber of said peptide nanostructure off said precipitation electrode.

26. A fiber comprising a plurality of peptide nanostructures, wherein each peptide nanostructure of said plurality of peptide nanostructures comprises no more than 4 amino acids, at least one of said 4 amino acids being an aromatic amino acid, the fiber being at least 100 nm in length.

27. A fiber comprising a plurality of peptide nanostructures, each peptide nanostructure of said plurality of peptide nanostructures comprises a plurality of polyaromatic peptides, the fiber being at least 100 nm in length.

28. A nonwoven article comprising a plurality of electrospun fibers, wherein at least one of said plurality of electrospun fibers is the fiber of claim 26.

29. A nonwoven article comprising a plurality of electrospun fibers, wherein at least one of said plurality of electrospun fibers is the fiber of claim 27.

30. A thin film comprising at least one layer of peptide nanostructures, wherein each peptide nanostructure comprises no more than 4 amino acids, at least one of said 4 amino acids being an aromatic amino acid, the thin film being at least 100 nm^2 in area size.

31. A thin film comprising at least one layer of peptide nanostructures, wherein each peptide nanostructure comprises a plurality of polyaromatic peptides, the thin film being at least 100 nm^2 in area size.

32. The method, fiber, film or article of claim 1, 9, 13, 16, 16, 21, 22, 23, 25 or 26, wherein each of said peptide nanostructures comprises no more than 4 amino acids, at least one of said 4 amino acids being an aromatic amino acid.

33. The method, fiber, film or article of claim 32, wherein each of said 4 amino acids is independently selected from the group of naturally occurring amino acids, synthetic amino acids and combinations thereof.

34. The method, fiber, film or article of claim 32, wherein at least one peptide of said plurality of peptides comprises at least two aromatic amino acids.

35. The method, fiber, film or article of claim 34, wherein at least one peptide of said plurality of peptides is a homodipeptide.

36. The method, fiber, film or article of claim 35, wherein each of the amino acids is said homodipeptide comprises an aromatic moiety.

37. The method, fiber, film or article of claim 36, wherein said aromatic moiety is selected from the group consisting of substituted or unsubstituted naphthalenyl and substituted or unsubstituted phenyl.

38. The method, fiber, film or article of claim 37, wherein said substituted phenyl is selected from the group consisting of pentafluoro phenyl, iodophenyl, biphenyl and nitrophenyl.

39. The method, fiber, film or article of claim 37, wherein said homodipeptide is selected from the group consisting of naphthylalanine-naphthylalanine dipeptide, (pentafluro-phenylalanine)-(pentafluro-phenylalanine) dipeptide, (iodo-phenylalanine)-(iodo-phenylalanine) dipeptide, (4-phenyl phenylalanine)-(4-phenyl phenylalanine) dipeptide and (*p*-nitro-phenylalanine)-(*p*-nitro-phenylalanine) dipeptide.

40. The method, fiber, film or article of claim 32, wherein at least one of said 4 amino acids is a D-amino acid.

41. The method, fiber, film or article of claim 32, wherein at least one of said 4 amino acids is an L-amino acid.

42. The method, fiber, film or article of claim 32, wherein at least one of said peptide nanostructures comprises at least two aromatic moieties.

43. The method, fiber, film or article of claim 42, wherein at least one of said peptide nanostructures is a homodipeptide.

44. The method, fiber, film or article of claim 43, wherein each of the amino acids is said homodipeptide comprises an aromatic moiety.

45. The method, fiber, film or article of claim 44, wherein said aromatic moiety is naphthalenyl.

46. The method, fiber, film or article of claim 43, wherein said homodipeptide is a naphthylalanine-naphthylalanine dipeptide.

47. The method, fiber, film or article of claim 1, 9, 13, 16, 16, 21, 22, 23, 25 or 26, wherein each of said peptide nanostructures comprises a plurality of polyaromatic peptides.

48. The method, fiber, film or article of claim 47, wherein each of said plurality of polyaromatic peptides comprises a component selected from the group consisting of a polyphenylalanine peptide, a polytriptophane peptide, a polytyrosine peptide, a non-natural derivatives thereof and a combination thereof.

49. The method, fiber, film or article of claim 48, wherein each of said plurality of polyaromatic peptides comprises at least 30 amino acids.

50. The method, fiber, film or article of claim 1, 9, 13, 16, 16, 21, 22, 23, 25 or 26, wherein said nanostructures at least partially enclose a material therein.

51. The method, fiber, film or article of claim 50, wherein said material is in a gaseous state.

52. The method, fiber, film or article of claim 50, wherein said material is in a condensed state.

53. The method, fiber, film or article of claim 50, wherein said material is selected from the group consisting of a conducting material, a semiconducting material, a thermoelectric material, a magnetic material, a light-emitting material, a biomineral, a polymer and an organic material.

54. The method, fiber, film or article of claim 53, wherein said conducting material is selected from the group consisting of silver, gold, copper, platinum, nickel and palladium.

55. The method, fiber, film or article of claim 53, wherein said semiconducting material is selected from the group consisting of CdS, CdSe, ZnS and SiO_2.

56. The method, fiber, film or article of claim 53, wherein said magnetic material is a paramagnetic material.

57. The method, fiber, film or article of claim 56, wherein said paramagnetic material is selected from the group consisting of cobalt, copper, nickel and platinum.

58. The method, fiber, film or article of claim 53, wherein said magnetic material is a ferromagnetic material.

59. The method, fiber, film or article of claim 58, wherein said ferromagnetic material is selected from the group consisting of magnetite and NdFeB.

60. The method, fiber, film or article of claim 53, wherein said light-emitting material is selected from the group consisting of dysprosium, europium, terbium, ruthenium, thulium, neodymium, erbium, ytterbium and any organic complex thereof.

61. The method, fiber, film or article of claim 53, wherein said biomineral comprises calcium carbonate.

62. The method, fiber, film or article of claim 53, wherein said polymer is selected from the group consisting of polyethylene, polystyrene, polyvinyl chloride and a thermoplastic polymer.

63. The method, fiber, film or article of claim 53, wherein said thermoelectric material is selected from the group consisting of bismuth telluride, bismuth selenide, bismuth antimony telluride and bismuth selenium telluride.

64. The method, fiber, film or article of claim 53, wherein said polymer is selected from the group consisting of a polynucleotide and a polypeptide.

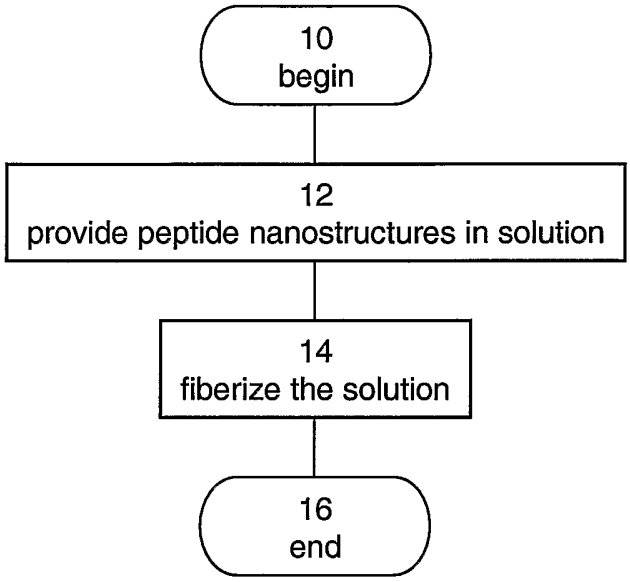

10
begin
12
provide peptide nanostructures in solution
14
fiberize the solution
16
end

Fig. 1

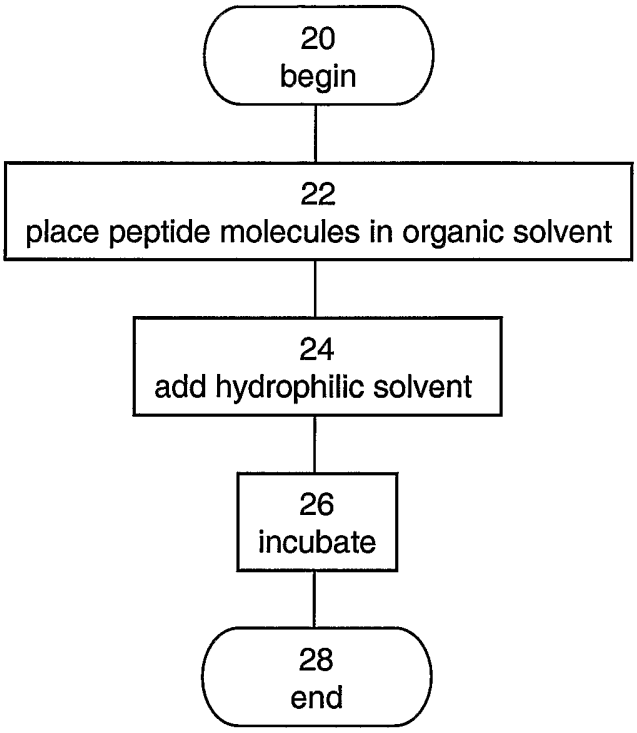

20
begin
22
place peptide molecules in organic solvent
24
add hydrophilic solvent
26
incubate
28
end

Fig. 2

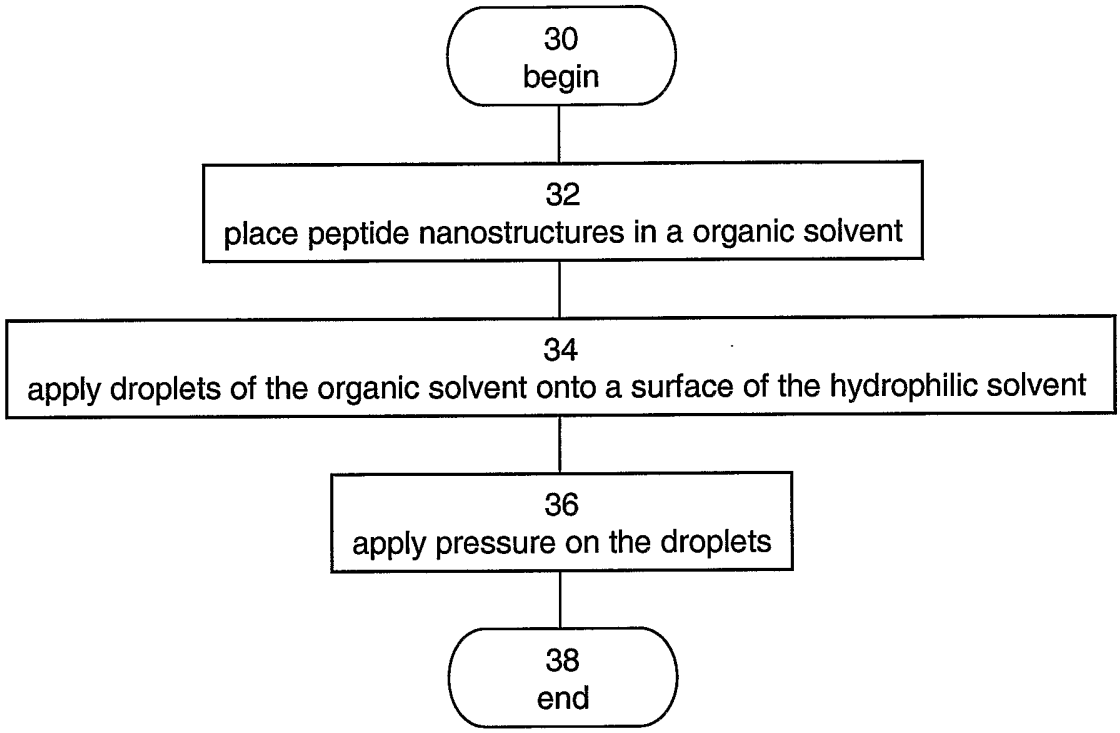

30
begin
32
place peptide nanostructures in a organic solvent
34
apply droplets of the organic solvent onto a surface of the hydrophilic solvent
36
apply pressure on the droplets
38
end

Fig. 3

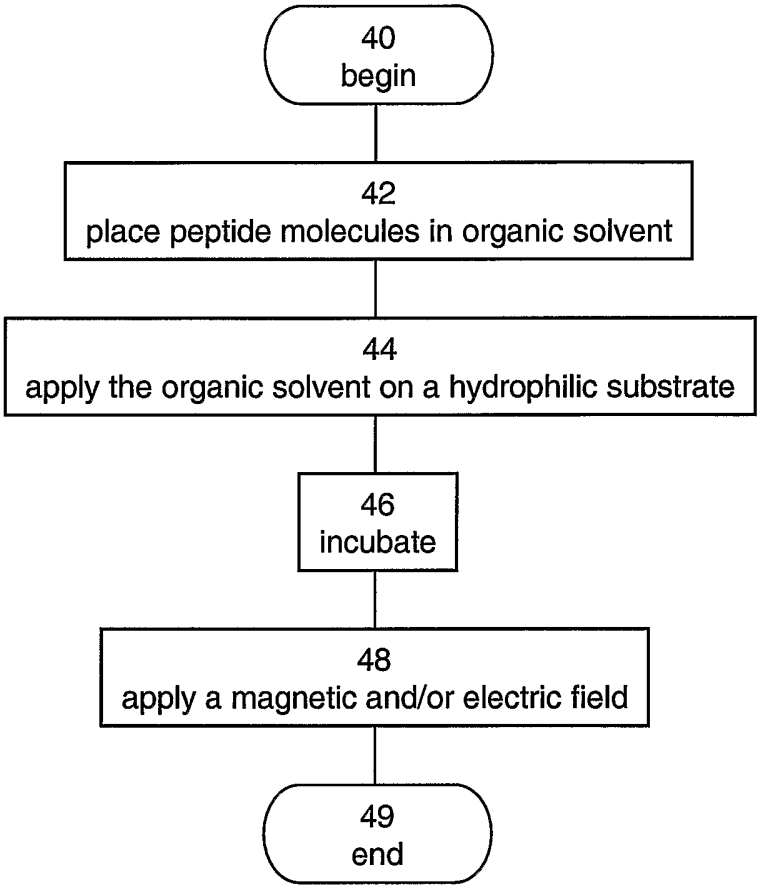

40
begin
42
place peptide molecules in organic solvent
44
apply the organic solvent on a hydrophilic substrate
46
incubate
48
apply a magnetic and/or electric field
49
end

Fig. 4

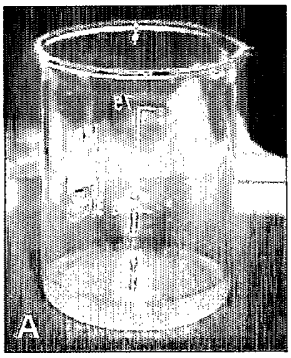


Fig. 5A

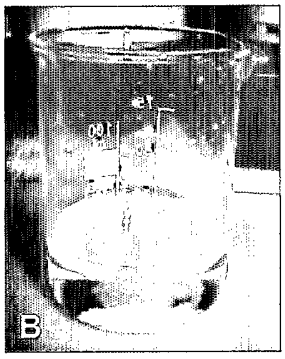


Fig. 5B

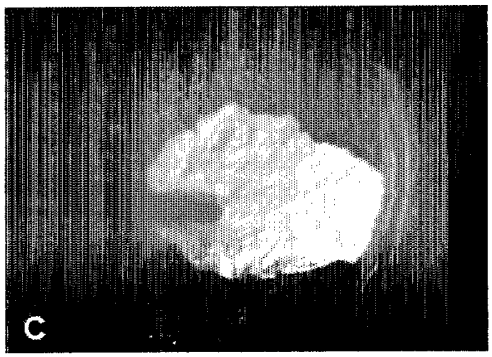


Fig. 5C

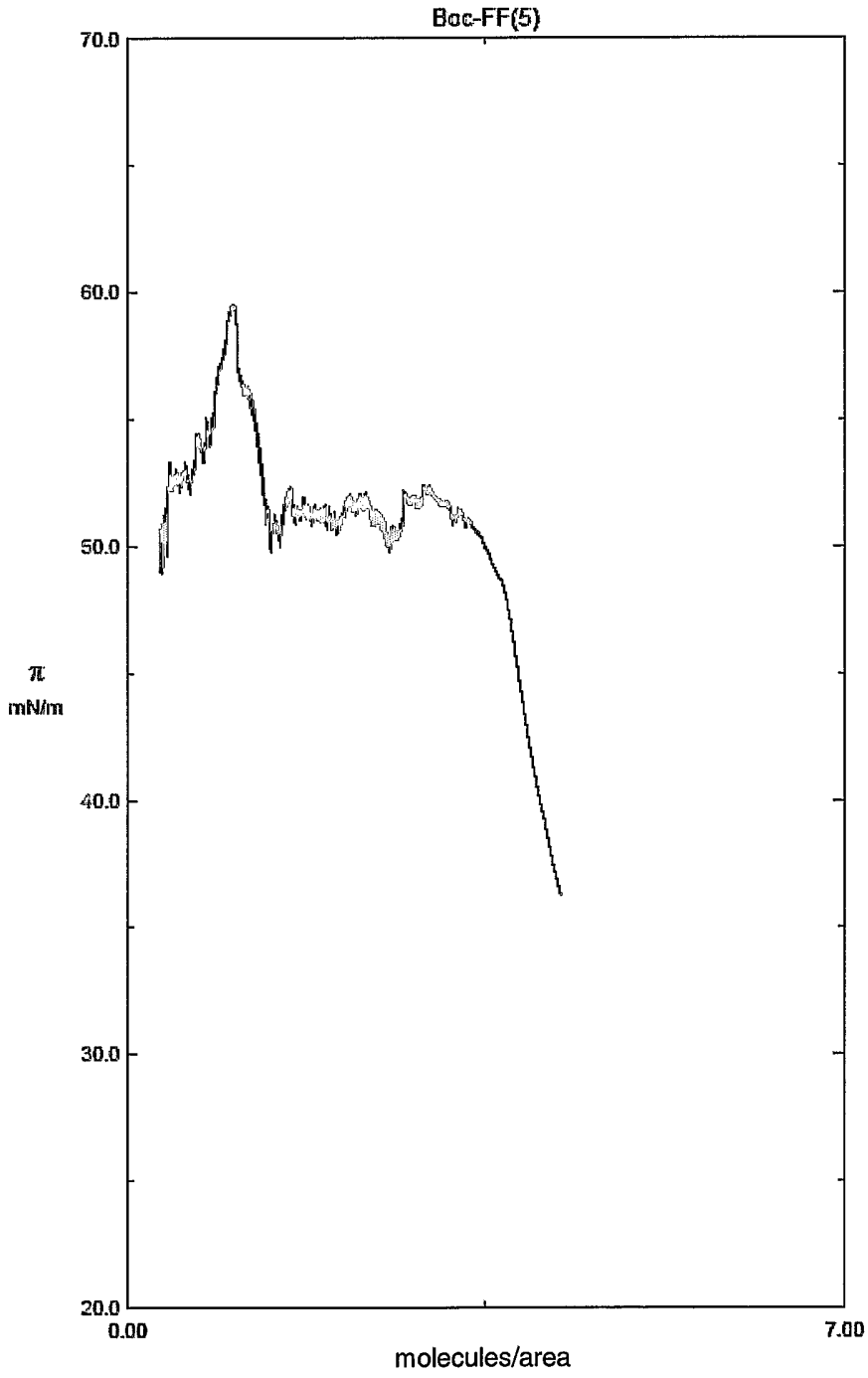


Fig. 6

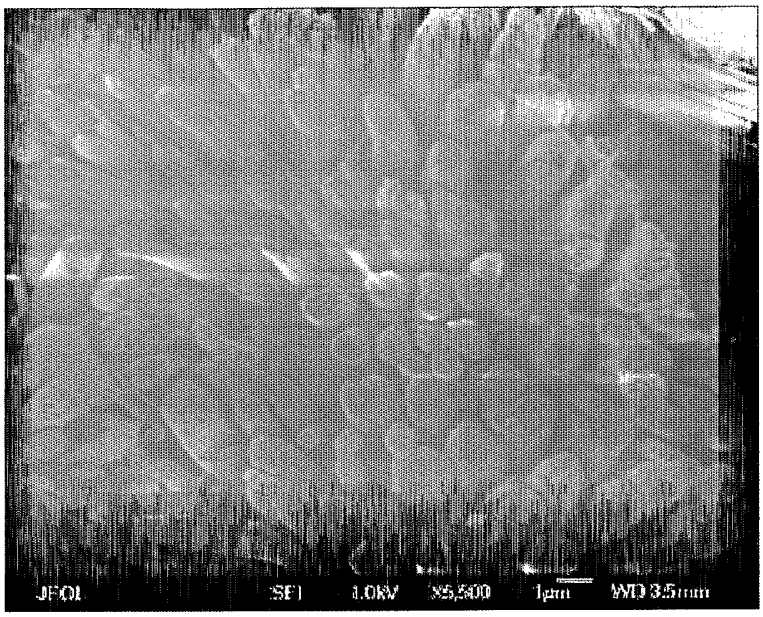

JEOL
SEI
1.0kV
X5,500
1μm
WD 3.5mm

Fig. 7A

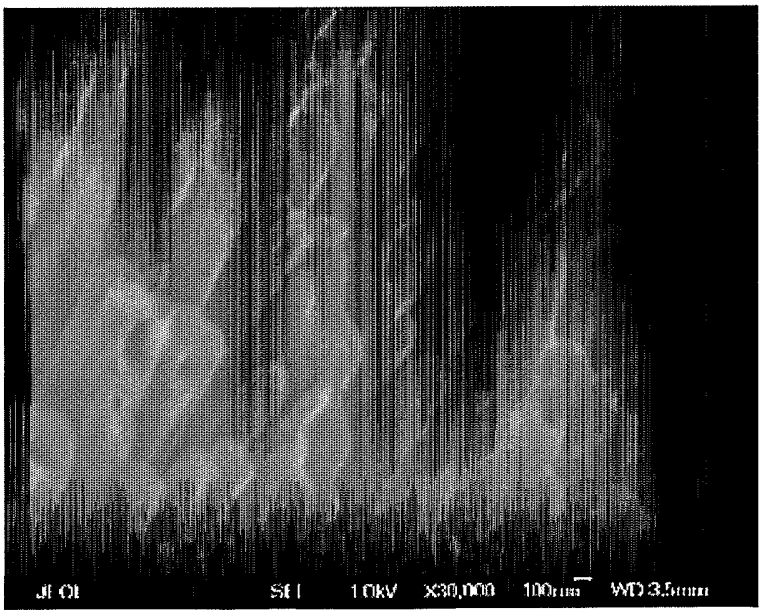

JEOL
SEI
1.0kV
X30,000
100nm
WD 3.5mm

Fig. 7B

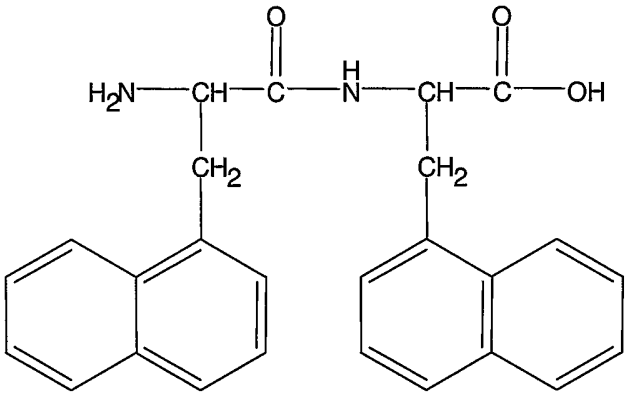


Fig. 8

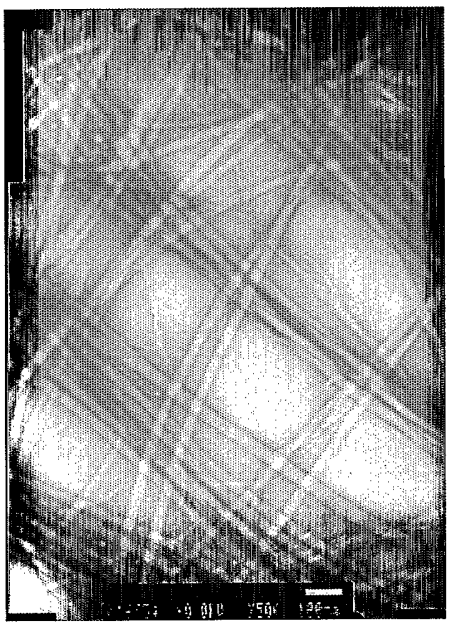


Fig. 9



Fig. 10A

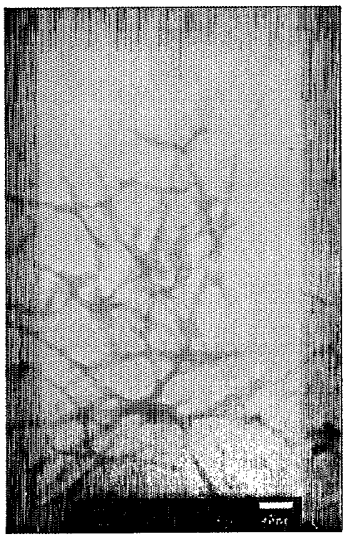


Fig. 10B



Fig. 10C

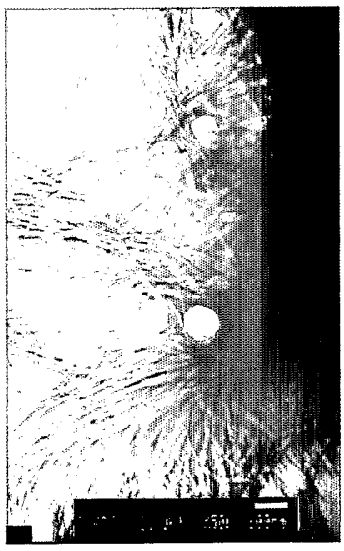


Fig. 10D