One aspect of the present invention relates to a method of preparing a fibrous protein smectic hydrogel by way of a solvent templating process, comprising the steps of pouring an aqueous fibrous protein solution into a container comprising a solvent that is not miscible with water; sealing the container and allowing it to age at about room temperature; and collecting the resulting fibrous protein smectic hydrogel and allowing it to dry. Another aspect of the present invention relates to a method of obtaining predominantly one enantiomer from a racemic mixture, comprising the steps of pouring an aqueous fibrous protein solution into a container comprising a solvent that is not miscible with water; sealing the container and allowing it to age at about room temperature; allowing the enantiomers of racemic mixture to diffuse selectively into the smectic hydrogel in solution; removing the smectic hydrogel from the solution; rinsing predominantly one enantiomer from the surface of the smectic hydrogel; and extracting predominantly one enantiomer from the interior of the smectic hydrogel. The present invention also relates to a smectic hydrogel prepared according to an aforementioned method.

9 Claims, 33 Drawing Sheets
Figure 2
Figure 4
Figure 6

Lines approx 40 nm

2 μm
Figure 11

Raw IR transmission of tapes

- rolled edge and planar
- planar tape section RAW data
- single thin edge (+ empty space)

Protein Amide A  CO2 Stretch from air

Amide I, II, III, etc.
Figure 12
Figure 15

Reflection IR, polycrystal

Transmission IR, polycrystal
Figure 17
Figure 18
• Long homogeneous helical domains
• Not necessarily “folded”
• Polymorphic secondary structure
• Material formed by mesophase
• Structural role
• Extended fibers or fibrils
  – similar to synthetic polymers
  – can be polycrystalline, mineralized
Figure 21

- "Liquid Crystal"
  - Molecules anisotropic
  - Orientation, possibly some position

Chiral one dimensional liquid crystal

Two dimensional liquid crystals
1. Nanocomposite - nanodomains of one phase separated by nanoscale domains of a second phase

2. Simple Patterned Nanocomposite (from smectic layers)

3. "Cocrystal" - peptide phase crystallizes and/or inorganic phase crystallizes within layers
Figure 24

Silkworm
Figure 25

Peptide (GAGAGS) core

Met triggered Spider silk (biosynthetic)
Figure 26
Peptides

Native Proteins (silks)
Air water interface from EDTA-Na aqueous solution of (Glu)_2(Gly-Asp-Val-Gly-Gly-Ala-Gly-Ala-Thr-Gly-Gly-Ser)_2(Glu)_5

12 micron stripes

300 nm bands

5 nm layers, 1 nm sublayers

30 nm feature
Figure 31

Designed Amphiphilic Helix

Glycine

Hydrophilic

Hydrophobic

Glass or plastic

Hydrophobic Solvent

Interface — Molecules form Amphiphilic helix and orient

Protein or Peptide Molecules in Aqueous Solution
TEMPLATED NATIVE SILK SMECTIC GELS

RELATED APPLICATIONS


GOVERNMENT SUPPORT

The invention was made with support provided by NASA (grant NAGS-1699) and NSF (grant BES 9727401); therefore, the government has certain rights in the invention.

BACKGROUND OF THE INVENTION

There are broadly three different types of liquid crystalline material: nematic, cholesteric, and smectic. The types are distinguished by differences in molecular ordering. Such materials only show a liquid crystal phase over a limited temperature range between the solid and isotropic liquid phases. Within the liquid crystal phase temperature range, a material may exhibit one or more of the nematic, cholesteric or smectic phase types. Normally, a material is chosen such that it forms only one type of liquid crystal phase over its working temperature range.

Liquid crystalline elastomers combine the various broken symmetries of liquid crystalline phases with the elasticity of polymer networks. One obvious effect is that the single crystal elastomers can undergo spontaneous shape changes when they undergo a liquid crystalline transition. There are many more subtle effects in the interplay between the fluctuations of the familiar liquid crystalline and elastic degrees of freedom (for example, and perfect single crystal nematic elastomer can, in theory, exhibit elastic moduli of zero when deformed in certain directions. These dramatic effects are, of course, drastically influenced by the disorder, which makes them perfect for studying quenched disorder.

Biopolymer networks are found all over nature. For example, the cytoskeleton is supported by a network of actin, which is a semi-flexible polymer with globular proteins as a monomer unit. These networks are proving to be ideal model systems for understanding the physics of semi-flexible polymers, both in solution and cross-linked network states.

The fibrous proteins can be considered to be a special class of proteins that serve important structural functions in the extracellular environment. In living organisms some of these proteins, such as collagen, are found in thin layers, sandwiched between other extracellular biomaterials. When studying the physical chemistry of extracellular fibrous proteins in vitro the use of a two-dimensional thin film or interfacial environment will help the proteins self-assemble more efficiently by providing a restricted environment in comparison to three-dimensional bulk systems. It is thus beneficial to study the synergistic interaction between the behavior of fibrous proteins in dimensionally restricted environments (such as thin films or two-dimensional layers) and the generation of structure and long range order through self-assembly.


Silks, and their analogues, have recently been the focus of interest for applications in biomaterials because of the intriguing properties of the silk fiber. The simplicity of their sequences lends them to be used as model fibrous proteins.

Most of the studies on the properties of silks, as well as other fibrous proteins either examine gross materials properties such as mechanical properties, thermal stability and surface roughness or examine very localized chemical details in the molecule. Literature on long-range ordered “helicoids” is less abundant.


An aqueous-hexane interface was chosen as an initial probe of fibroin liquid-liquid interface behavior. This interface, in the absence of fibroin, is believed to be about 10 Å thick. Carpenter, I. L.; Hehre, W. J. *Journal of Physical Chemistry* 1990, 94, 531-536; Michael, D. B.; Benjamin, I. *Journal of Physical Chemistry* 1995, 99, 1530-1536. The silk at the aqueous-hexane interface forms a film as it ages, and this film can be picked up onto sample grids for observation in a transmission electron microscope (TEM). The hexane was expected to be a better solvent for the alanine residues in silk than the water, forcing them to the hexane side of the interface. The aqueous phase should be a better solvent for serine.

**SUMMARY OF INVENTION**

In one embodiment, the present invention relates to a method of preparing a fibrous protein smectic hydrogel by way of a solvent templating process comprising the steps of:

a. pouring an aqueous fibrous protein solution into a container comprising a solvent that is not miscible with water;

b. sealing the container and allowing it to sit at about room temperature overnight; and

c. collecting the resulting fibrous protein smectic hydrogel and allowing it to dry.

In a further embodiment, the present invention relates to the above solvent templating process, wherein the solvent is chloroform.

In a further embodiment, the present invention relates to the above solvent templating process, wherein the solvent is hexane.

In a further embodiment, the present invention relates to the above solvent templating process, wherein the fibrous protein is selected from the group consisting of silk, collagens, keratins, actins, chorions, and serovins.

In a further embodiment, the present invention relates to the above solvent templating process, wherein the fibrous protein solution is greater than about 4% by weight.

In a further embodiment, the present invention relates to the above solvent templating process, wherein the fibrous protein is silk.

In a further embodiment, the present invention relates to the above solvent templating process, wherein the fibrous protein solution is greater than about 8% by weight.

In a further embodiment, the present invention relates to the above solvent templating process, wherein the fibrous protein is silk, and the solvent is iso-amyl alcohol.

In a further embodiment, the present invention relates to the above solvent templating process, wherein the fibrous protein is selected from the group consisting of silk, collagens, keratins, actins, chorions, and serovins.

In a further embodiment, the present invention relates to the above solvent templating process, wherein the fibrous protein solution is greater than about 4% by weight.

In a further embodiment, the present invention relates to the above solvent templating process, wherein the fibrous protein is silk.

In a further embodiment, the present invention relates to the above solvent templating process, wherein the fibrous protein is silk, and the solvent is iso-amyl alcohol.

In a further embodiment, the present invention relates to the above solvent templating process, wherein the fibrous protein solution is greater than about 8% by weight.

In a further embodiment, the present invention relates to the above solvent templating process, wherein the fibrous protein is silk, and the solvent is iso-amyl alcohol.

In a further embodiment, the present invention relates to the above solvent templating process, wherein the fibrous protein is silk.
In a further embodiment, the present invention relates to the above method of obtaining predominantly one enantiomer, wherein the fibrous protein solution is greater than about 8% by weight and the fibrous protein is silk.

In another embodiment, the present invention relates to the fibrous protein smectic hydrogel prepared by the above solvent templating method.

In a further embodiment, the present invention relates to the fibrous protein smectic hydrogel prepared by the above solvent templating method, wherein the fibrous protein is selected from the group consisting of silk, collagens, keratins, actins, chorions, and sermins.

In a further embodiment, the present invention relates to the fibrous protein smectic hydrogel prepared by the above solvent templating method, wherein the fibrous protein is silk.

In a further embodiment, the present invention relates to the fibrous protein smectic hydrogel prepared by the above solvent templating method, wherein the fibrous protein smectic hydrogel is greater than or equal to about 38 nm thick.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts the surface (right) and fracture surface of chloroform templated silk. The wavy texture is everywhere on the solvent templated side of the materials surface.

FIG. 2 depicts the chloroform templated film. Waves reorienting and becoming terraces can be seen, behavior which is not expected for simple wrinkles due to contraction.

FIG. 3 depicts a regular pattern of nubby small structures comprising the waves.

FIG. 4 depicts an amyl alcohol film showing a surface that looks like a "nonwoven woven" fabric.

FIG. 5 depicts a surface texture seen at an angle showing a thin layer very different from the chloroform films.

FIG. 6 depicts amyl alcohol templated samples soaked in bipyridylic triRuiI chloride hexahydrate giving a high magnification image and a 40 nm layered feature.

FIG. 7 depicts films after soaking in a dysprosium chloride solution for added contrast. The wavy layered structure of the chloroform templated film is apparent here.

FIG. 8 depicts a film's texture that is even and regular.

FIG. 9 depicts self-fabricated textured "tapes" from a peptide with sequence (Glu)_{5}(Ser-Gly-Ala-Gly-Val-Gly-Arg-Gly-Ser-Gly-Val-Gly-Leu-Gly-Ser-Gly-Asn-Gly)_{2}(Glu)_{5} (SEQ ID NO:1). Optical micrograph shows a 10-15 micron texture which persists through the material thickness. The material is optically transparent. Polarizing optical microscopy reveals patterned birefringence, indicating that the topographic texture is due to a changing material orientation. SEM image shows the topographic structure of the tape. The difference in periodicity observed in SEM and optical microscopy is due to the fact that top surface and bottom surface ridges are both observed in the optical image (resulting in an apparently shorter period).

FIG. 10 depicts self-fabricated tapes of (Glu)_{5}(Ser-Gly-Ala-Gly-Val-Gly-Arg-Gly-Ser-Gly-Val-Gly-Leu-Gly-Ser-Gly-Asn-Gly)_{2}(Glu)_{5} (SEQ ID NO:1) have "patterned" or a long-range ordered structure consisting of hierarchical nanoscale to microscale patterns; 1: the self-limited width and thickness of the fibers (~120 microns, 50 microns respectively) form the largest length scale in the hierarchy; a 40 micron periodic texture is observed running along the tape; 2: within the ridges of the 40 micron texture a 3 micron subtexture is observed; 3: a submicron texture of inclined sheets or layers can be observed (~40 nm, but exact size is below the resolution of the scanning electron microscope); TEM studies indicate a layer spacing of ~5 nm.

FIG. 11 depicts an IR spectra of self-fabricated tapes of (Glu)_{5}(Ser-Gly-Ala-Gly-Val-Gly-Arg-Gly-Ser-Gly-Val-Gly-Leu-Gly-Ser-Gly-Asn-Gly)_{2}(Glu)_{5} (SEQ ID NO:1). Typically IR spectra for molecules are seen as very small differences in IR transmission relative to a large background, which must be subtracted out. Raw data (no background subtraction) is shown for transmission FTIR spectra through different regions (orientations) of the tape structure. Two orientations show very typical protein absorbance spectra over a high background. However in some orientations the IR radiation does not reach the detector.

FIG. 12 depicts an IR spectrum modified by tape with scale expanded to show spectral features instead of an absorption or transmission spectrum, a pattern of 2 overlaid sinusoids (one has a 50 cm period, the other a 25 cm period. The effect for this material appears strongest in the 1750-3500 cm^{-1}, or 5.7-2.9 micron range.

FIG. 13 depicts twisted polycrystals obtained by salt precipitation of an oligopeptide with Na-EDTA.

FIG. 14 depicts ordered "corkscREW" polycrystalline oligopeptide salt precipitate as a hierarchy of twisted ordered structures.

FIG. 15 depicts reflection and transmission FTIR spectra for ordered polycrystals. TOP: reflection infrared spectrum, Raw data. A glassy disordered material of the oligopeptide is more reflective than the background. An ordered periodic nanolayered material from the same peptide is shown, and clearly reflects far less of the infrared radiation. BOTTOM: transmission spectra for background, unordered peptide material and a chemically identical nanolayered ordered material of the peptide. Spectrum is greatly attenuated for the ordered material.

FIG. 16 depicts ordered textured surfaces and interiors from templated gels (a) chloroform templated gels have a wavy surface texture covering the surfaces which were in contact with water; (b) a fracture surface from the chloroform templated gel reveals a "skin" of the wavy pattern, which forms channels down into the interior; the interior has a different structure, which appears to be made of wavy plates; (c) templated surface of amyl alcohol templated material (in contact with water); (d) higher magnification image of the edge of the region in c, showing a "skin" core structure and a patterned texture throughout the material; (e,f) amyl alcohol dried film after swelling in an aqueous solution of ruthenium compound and extraction of ruthenium compound by swelling in water; (e) wavy lines indicate reorientation of ordered structures within the material; (f) at high magnification (20, 000x) lines 38 nm in width are observed.

FIG. 17 depicts amyl alcohol templated gel after soaking in Aqueous Tris(2,2'-bipyridyl) dichloro ruthenium(II) hexahydrate ("Rubipy") solution for 1 day. Much of the Rubipy has migrated from the solution into the silk gel. Initial migration is rapid and chirally selective (occurs over roughly 1 hour). Additional migration occurs slowly after this for roughly 1 day and is less chirally selective. Chloroform templated gels do not exhibit complex diffusion behavior and are chirally selective throughout the swelling process.

FIG. 18 depicts a cross section of amyl alcohol templated gel after swelling in Rubipy for 1 hour. The Rubipy penetrated rapidly into the outer "skin" layers of the gel (bright orange), and more slowly into the interior (yellowish region).
FIG. 19 depicts an X-ray diffraction pattern from chloroform template gel. Dark arcs along the diffraction rings (arrow) indicate orientation.

FIG. 20 depicts the non-globular nature of fibrous proteins.

FIG. 21 depicts the long range order of liquid crystals.

FIG. 22 depicts “frustration” in nanolayered crystals.

FIG. 23 depicts nanocomposites.

FIG. 24 depicts banded structures from native silk.

FIG. 25 depicts banded structures from engineered protein designed peptide (SEQ ID NO: 4).

FIG. 26 depicts how hairpin structures allow silk liquid crystallinity.

FIG. 27 depicts spider silk modification. The images depict the air-water interface from an EDTA-Na aqueous solution of (Glu)₅(Gly-Asp-Val-Gly-Gly-Ala-Gly-Ala-Thr-Gly-Gly-Ser)₂(Glu)₅ (SEQ ID NO: 2).

FIG. 28 depicts amphiphilic spider silk motif.

FIG. 29 depicts silkworm silk peptide models.

FIG. 30 depicts film morphology and helix anchoring: (a) a designed helix with a stronger hydrophobic/hydrophilic difference will be more readily stabilized and anchored parallel to the interface; (b) helical axis is perpendicular to specific layer planes; and (c) helices which tend to be parallel to interface and film result in layers more often normal to film.

FIG. 31 depicts the templating-against-solvent technique.

FIG. 32 depicts patterned peptide films.

FIG. G3 depicts silk templated gels-surface “skin”.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

For convenience, before further description of the present invention, certain terms employed in the specification, examples and appended claims are collected here. These definitions should be read in light of the remainder of the disclosure and understood as by a person of skill in the art.Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by a person of ordinary skill in the art.

The articles “a” and “an” are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

The terms “comprise” and “comprising” are used in the inclusive, open sense, meaning that additional elements may be included.

The term “including” is used to mean “including but not limited to”. “Including” and “including but not limited to” are used interchangeably.

The term “mesogenic” is art-recognized and refers to the mesomorphous phase of a liquid crystal in which molecules are closely aligned in a distinct series of layers, with the axes of the molecules lying perpendicular to the plane of the layers.

The term “gel” is art-recognized and refers to a colloid in which the disperse phase has combined with the dispersion medium to produce a semisolid material.

The term “hydrogel” is art-recognized and refers to a colloid in which the particles are in the external or dispersion phase and water is in the internal or dispersed phase.

The term “alkyl” refers to the radical of saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (cyclic) groups, alkyl substituted cycloalkyl groups, and cycloalkyl substituted alkyl groups. In preferred embodiments, a straight chain or branched chain alkyl has 30 or fewer carbon atoms in its backbone (e.g., C₃₋C₅₀ for straight chain, C₃₋C₅₀ for branched chain), and more preferably 20 or fewer. Likewise, preferred cycloalkyls have from 3-10 carbon atoms in their ring structure, and more preferably have 5, 6 or 7 carbons in the ring structure.

Unless the number of carbons is otherwise specified, “lower alkyl” as used herein means an alkyl group, as defined above, but having from one to ten carbons, more preferably from one to six carbon atoms in its backbone structure. Likewise, “lower alkenyl” and “lower alkynyl” have similar chain lengths. Preferred alkyl groups are lower alcohols. In preferred embodiments, a substituent designated herein as alkyl is a lower alkyl.

The term “alkenyl” as used herein includes 5-, 6- and 7-membered single-ring aromatic groups that may include from zero to four heteroatoms, for example, benzene, naphthalene, anthracene, pyrene, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, triazole, pyrazole, pyridine, pyrazine, pyrdazine and pyrimidine, and the like. Those aryl groups having heteroatoms in the ring structure may also be referred to as “aryl heterocycles” or “heteroaromatics.” The aromatic ring can be substituted at one or more ring positions with such substituents as described above, for example, halogen, azide, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, alkoxy, amino, nitro, sulfonyl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkythio, sulfonyl, sulfonamido, ketone, aldehyde, ester, heterocyclic aromatic or heteroaromatic moieties, —CF₃, —CN, or the like. The term “aryl” also includes polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings (the rings are “fused rings”) wherein at least one of the rings is aromatic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocyclyls.

The terms ortho, meta and para apply to 1,2-, 1,3- and 1,4-disubstituted benzenes, respectively. For example, the names 1,2-dimethylbenzene and ortho-dimethylbenzene are synonymous.

The terms “heterocyclyl” or “heterocyclic group” refer to 3- to 10-membered ring structures, more preferably 3- to 7-membered rings, whose ring structures include one to four heteroatoms. Heterocycles can also be polycycles. Heterocyclyl groups include, for example, azetidine, azepine, thiophene, thianthrene, furan, pyran, isobenzofuran, chromene, -xanthene, phenoxythiazole, pyrrole, imidazole, pyrazole, isothiazole, isoxazole, pyridine, pyrimidine, pyridazine, indolizine, indole, indole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carbonile, phenanthridine, acridine, pyrimidine, phenanthroline, phenazine, phenarsazine, phenothiazine, furazan, phenoxazine, pyrroldione, oxazole, thiocarbazole, piperidine, morpholine, lactones, lactams such as azetidinones and pyrroldinones, sultams, sultones, and the like. The heterocyclic ring can be substituted at one or more positions with such substituents as described above, for example, halogen, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, amino, nitro, sulfonyl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl,
ether, alkylthio, sulfonyl, ketone, aldehyde, ester, a heterocyclic, an aromatic or heteroaromatic moiety, —CF₃, —CN, or the like.

The terms “polycyclic” or “polycyclic group” refer to two or more rings (e.g., cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocyclyls) in which two or more carbons are common to two adjoining rings, e.g., the rings are “fused rings”. Rings that are joined through non-adjacent atoms are termed “bridged” rings. Each of the rings of the polycycle can be substituted with such substituents as described above, as for example, halogen, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, hydroxyl, amino, nitro, sulfonyl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, ketone, aldehyde, ester, a heterocyclic, an aromatic or heteroaromatic moiety, —CF₃, —CN, or the like.

The term “carbocycle”, as used herein, refers to an aromatic or non-aromatic ring in which each atom of the ring is carbon.

As used herein, the term “nitro” means —NO₂; the term “halogen” designates —F, —Cl, —Br or —I; the term “sulfhydryl” means —SH; the term “hydroxyl” means —OH; and the term “sulfonyl” means —SO₂—.

The terms “amine” and “amino” are art-recognized and refer to both unsubstituted and substituted amines, e.g., a moiety that can be represented by the general formula:

```
R₉ — R₁₀
```

wherein R₉ and R₁₀ represent a group permitted by the rules of valence.

The term “acylamino” is art-recognized and refers to a moiety that can be represented by the general formula:

```
R₉ \text{N—O} \text{—R'₁₁}
```

wherein R₉ is as defined above, and R’₁₁ represents a hydrogen, an alkyl, an alkenyl or —(CH₂)ₘ—Rₙ, where m and n are defined above.

The term “amido” is art-recognized as an amino-substituted carbonyl and includes a moiety that can be represented by the general formula:

```
R₉ \text{—N—O—} \text{—R'₁₁}
```

wherein R₉, R₁₁, are as defined above. Preferred embodiments of the amide will not include imides which may be unstable.

The term “alkylthio” refers to an alkyl group, as defined above, having a sulfur radical attached thereto. In preferred embodiments, the “alkylthio” moiety is represented by one of —S-alkyl, —S-alkenyl, —S-alkynyl, and —S—(CH₂)ₘ—Rₙ, wherein m and Rₙ are defined above. Representative alkylthio groups include methylthio, ethylthio, and the like.

The term “carbonyl” is art-recognized and includes such moieties as can be represented by the general formula:

```
O — X — R'₁₁,
```

wherein X is a bond or represents an oxygen or a sulfur, and R'₁₁ represents a hydrogen, an alkyl, an alkenyl, —(CH₂)ₘ—Rₙ or a pharmaceutically acceptable salt, R'₁₁ represents a hydrogen, an alkyl, an alkenyl or —(CH₂)ₘ—Rₙ, where m and n are as defined above. Where X is an oxygen and R'₁₁ is not hydrogen, the formula represents an “ester”. Where X is an oxygen, and R'₁₁ is as defined above, the moiety is referred to herein as a carboxyl group, and particularly when R'₁₁ is a hydrogen, the formula represents a “carboxylic acid”.

Where X is an oxygen, and R’₁₁ is hydrogen, the formula represents a “formate”. In general, where the oxygen atom of the above formula is replaced by sulfur, the formula represents a “thioformate” group. Where X is a sulfur and R’₁₁ is not hydrogen, the formula represents a “thiol ester.” Where X is a sulfur and R’₁₁ is hydrogen, the formula represents a “thioformate.” On the other hand, where X is a bond, and R’₁₁ is not hydrogen, the above formula represents a “ketone” group. Where X is a bond, and R’₁₁ is hydrogen, the above formula represents an “aldehyde” group.

The terms “alkoxy” or “alkox” as used herein refers to an alkoxyl, as defined above, having an oxygen radical attached thereto. Representative alkoxy groups include methoxy, ethoxy, propoxy, tert-butoxy and the like. An ether is or resembles an alkoxyl, such as can be represented by one of —O-alkyl, —O—alkenyl, —O—alkynyl, —O—(CH₂)ₘ—Rₙ, where m and n are as defined above.

The abbreviations Me, Et, Ph, Tf, Nf, Ts, and Ms represent methyl, ethyl, phenyl, trifluoromethanesulfonyl, nonafluorobutanesulfonyl, p-toluenesulfonyl, and methanesulfonyl, respectively. A more comprehensive list of the abbreviations utilized by organic chemists of ordinary skill in the art appears in the first issue of each volume of the Journal of Organic Chemistry; this list is typically presented in a table entitled Standard List of Abbreviations. The abbreviations contained in said list, and all abbreviations utilized by organic chemists of ordinary skill in the art are hereby incorporated by reference.

Analogous substitutions can be made to alkenyl and alkynyl groups to produce, for example, aminooalkenyls, aminoalkynyls, amidooalkenyls, amidooalkynyls, iminooalkenyls, iminooalkynyls, thioalkenyls, thioalkynyls, carbonyl-substituted alkenyls or alkynyls.

As used herein, the definition of each expression, e.g., alkyl, m, n, etc., when it occurs more than once in any structure, is intended to be independent of its definition elsewhere in the same structure.

It will be understood that “substitution” or “substituted with” includes the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and the substituent, and that the substitution results in a stable compound, e.g., which does not spontaneously undergo undesired transformation, such as by rearrangement, cyclization, elimination, etc.
As used herein, the term "substituted" is contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carboxylic and heterocyclic, aromatic and nonaromatic substituents of organic compounds. Illustrative substituents include, for example, those described herein above. The permissible substituents can be one or more and the same or different for appropriate organic compounds. For purposes of this invention, the heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valences of the heteroatoms. This invention is not intended to be limited in any manner by the permissible substituents of organic compounds.

The phrase "protecting group" as used herein means temporary substituents which protect a potentially reactive functional group from undesired chemical transformations. Examples of such protecting groups include esters of carboxylic acids, silyl ethers of alcohols, and acetics and ketals of aldehydes and ketones, respectively. The field of protecting group chemistry has been reviewed (Greene, T. W.; Wuts, P. G. M., Protective Groups in Organic Synthesis, 2nd ed.; Wiley: New York, 1991). 

Certain compounds of the present invention may exist in particular geometric or stereoisomeric forms. The present invention contemplates all such compounds, including cis- and trans-isomers, R- and S-enantiomers, diastereomers, (+)-isomers, (-)-isomers, racemic mixtures thereof, and other mixtures thereof, as falling within the scope of the invention. Additional asymmetric carbon atoms may be present in a substituent such as an alkyl group. All such isomers, as well as mixtures thereof, are intended to be included in this invention.

If, for instance, a particular enantiomer of a compound of the present invention is desired, it may be prepared by asymmetric synthesis, it may be isolated using chiral chromatography methods, or by derivation with a chiral auxiliary, where the resulting diastereomeric mixture is separated and the auxiliary group cleaved to provide the pure desired enantiomers. Alternatively, where the molecule contains a basic functional group, such as amino, or an acidic functional group, such as carboxyl, diastereomeric salts are formed with an appropriate optically-active acid or base, followed by resolution of the diastereomers thus formed by fractional crystallization or chromatographic means well known in the art, and subsequent recovery of the pure enantiomers.

For purposes of this invention, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 74th Ed., 1988-89, inside cover.

Fibrous-Protein Smectic Hydrogels

We have demonstrated control over helicoid structure—the material superstructure generated by an array of twisting molecules (like a chiral liquid crystal) in a model fibrous protein (silk). The processes used are very simple and can be applied easily elsewhere to create nanostructured “designer” biomaterials for studies in areas ranging from cell biology and surface interactions to surface nanofluidics. These simple processes were carefully designed using what we know about fibrous proteins (as different from synthetic polymers and globular proteins), and their success underscores the possibilities for manipulating these molecules with processes that are tailored for fibrous proteins (rather than using polymer techniques that destroy their structure, or trying to get them to behave like globular proteins). The characterization results highlight a few key features that distinguish the nature of these proteins.

Remarkably, two processes have been developed that allow the creation of highly structured biomaterials from regenerated silks. A silk solubilization process has been modified to obtain concentrations in excess of 8 wt % silk in aqueous solution. An earlier process yields purified silk solutions of up to only about 4-5 wt %, depending on the purity of the solution and freshness of the raw silk used. A solvent templating process yields nanostructured permeactive materials from aqueous silk solutions with concentrations of ≥about 4 wt %.

In general the solvent templating process yields thicker, bulk solids with the thin film feature (many layers stacked up) when using about an 8 wt % aqueous silk solution as opposed to a 4 wt % solution which yields a thin film. In tandem, these two discoveries allow the creation of protein membranes, films, and gels which are made of discrete stacks of protein layers which can be about 38 nm or thicker. Wrinkling and perforation of these layers, combined with chiral stacking interactions (a tendency to twist) result in a number of different very regular microscale patterned surface textures. Many of the films selectively absorb small molecules and ions from solution, and many are chirally selective as well. Thus they may find application as therapeutic agent delivery materials, components in a chiral separations process, matrices for chiral enzymes and catalysts, and as chiral templates. The morphology and microstructure of the films can be controlled by choice of solvent, starting concentration of protein, and environmental factors such as temperature, humidity, addition of ether and/or alcohol to the protein solution, addition of acid to the protein solution, or addition of divalent ionic salts to the protein solution. Altering these parameters results in different permeation properties for the protein materials, different molecular orientations observed within the films, and different surface topographies. The length scale of the topographic features and the protein nature of the films also suggest applications in tissue engineering and cell biology, where microscale and nanoscale patterns have been shown to strongly influence cell growth, differentiation, and tumorigenesis. The data gathered to date suggest that the materials may be chemically patterned as well, allowing bioactive sites and molecules to be precisely placed on the material surface and throughout the material. This arrangement would result in extremely predictable and reproducible diffusion rates out of the material for therapeutic agent delivery applications, as well as suggesting novel surgical materials patterned to address cellular processes involved in healing. The material fabrication process is based on chemical and physical features common to many fibrous protein molecules such as collagens, keratins, actins, chorions, seroins, and other silks. Many of these features are also found in non-protein biopolymers such as cellulose, many polysaccharides, and nucleic acids. We would thus expect the process to be useful in making patterned bio compatible nanomaterials from a large number of natural molecules in addition to silks.

Silk-Based Smectic Gels

Concentrated solutions (in general about 4 wt % for thin films, about 8 wt % for bulk solids comprising several layers stacked up) of silk can be used to grow hydrogels from an aqueous organic, liquid-liquid interface. For example, a silk solution is placed into a vessel, a solvent such as chloroform, hexane, or amyl alcohol is carefully layered on top of the silk solution (underneath in the case of chloroform, which is denser than aqueous solutions). The layered liquid is covered to prevent evaporation and excessive competing interactions with air, and a film forms at the interface. In the case of bulk solid hydrogels, the film grows into the aqueous silk phase. Solvent templated processing of natural silks results in the formation of a nanolayered structure, where the layer thick-
Highly structured templated solid materials cannot be obtained for silks below concentrations of about 4 wt % protein, and the most organized and oriented structured materials are obtained from solutions with protein concentrations of greater than about 5 wt %. Furthermore, standard film casting techniques do not yield ordered solids, even when the starting solutions contain about 5-8 wt % protein. The choice of solvent used in templating is also important. Solvents which are not at all miscible in water, such as hexane, do not template hydrogels, but instead form viscous liquid crystalline films which are localized in a very thin region at the interface. Solvents which do not have a greater affinity than water for some of the side chains in silk (or other protein used) do not result in templating behavior. An example is dichloroethane, which has a low affinity for both the polar and non-polar side chains in a typical protein and exhibits no surface templating behavior. Solvents which are somewhat miscible in water, or which make a low energy interface with water are weakly templating. For example, using propanol and butyl alcohol with a silk solution results in a loose, poorly localized gel, due to the large thickness and weak chemical gradient in the interfacial region. Dried material from these gels is poorly oriented, not well-ordered, and either does exhibit the pronounced permselective properties and microstructure observed in templated films, although weak versions of these properties are sometimes observed.

The choice of salt in the aqueous silk solution is also important. A switch from LiSCN to LiBr enabled the preparation of the higher concentrated silk aqueous solution used for templating ordered bulk solids.

The result of a molecular design which consists of a large self-fabricating unit and smaller solubilizing/functional ends, is that the thermodynamically favorable state for the entire molecule will be similar to the thermodynamically favorable state for the self-fabricating block. There may be a structural compromise due to the presence of the end blocks, but since the fabricating block dominates the mass and volume of the molecule these compromises are expected to be minor. However, the situation for the solubilizing/functional end blocks is quite different. In a molecular packing geometry dominated by interaction between self-fabricating blocks the local packing in regions containing chain ends will often be highly strained due to thermodynamic frustration. If the ideal thermodynamically favorable geometry for the end blocks is not compatible with the packing favored by the self-fabricating blocks (which are the smallest and perhaps most hydrophobic part of the molecule) the end blocks will be forced into a state that is far from their (local) thermodynamic ideal, and will be “frustrated”. By designing multiblock “miniblock” oligomers with block to block disparities in residue size, volume, preferred conformation, etc. we can design frustrated semeiotically ordered solids, where the density and interaction behavior in the interlayer region is strongly perturbed with respect to bulk material or non-frustrated surfaces with the same composition.

The use of smectic forming self-fabricating blocks, oligomeric molecular weight and associated liquid to solid transitions, and a nanoscopic designed frustrated interlayer region (from end blocks) allows us to construct molecularly designed materials with nanoscale fluid channels. These channels are essentially the endblock-rich regions in the multilayered smectic generated structures. Through engineered mismatching of the properties of monomers used to specify the end blocks vs. the self-fabricating blocks, different properties in these channels can be designed in. The regions in question contain chain ends and are thus somewhat less constrained than the regions comprised of self-fabricating blocks. The chain ends protruding into the interlayer region create a brush at the molecular scale. Molecules absorbed into the miniblock derived material will migrate preferentially into the interlayer regions because:

1. Space exists or can be made to accommodate additional molecules (through localized swelling in the interlayer region).
2. Thermodynamic frustration can be alleviated by adding molecules, changing the overall chemistry and preferred state of the region.
3. Strong interactions between self-fabricating blocks preclude incorporation of additional molecules.
4. Interactions and properties designed into the endblocks promote localization of an added molecule (solute) into the interlayer region.

Designated interactions can include acid endblocks to attract and localize basic solutes, low amino acid volumes in the endblocks to attract solute molecules that balance the interlayer volume and density, matched endblock-solute hydrophobic/hydrophilic interactions. It is important to note (and a key feature) that the “solute localizing” properties designed for the end blocks need not be entirely enthalpic (chemical interactions) in nature, but can include entropy-based design ideas as well (volume, molecule shape, flexibility).

Molecules absorbed into these designed materials (from designed molecules) will interact with a densely packed “brush” of end blocks, and the strength and nature of this interaction will determine whether a solute molecule can enter the material and diffuse into the material interior. If the endblocks are chiral, a chiral interaction occurs between solute and the nanobrush within the material for every few Angstroms of diffusion. Even non-specific interactions are expected to be chirally selective for diffusion of enantiomers through the brush. The extremely large surface area provided by the brush for interactions provides high selectivity, the possibility of a largely entropy—driven designed diffusion and interaction process ensures that separation is not specific to a particular well matched solute-endblock pair.

Separation has been observed for a test chiral molecule in silk-like and collagen-like designed oligopeptides. Acid base interactions were used to localize the test molecules in the chain end regions. Two processes were used to absorb the test molecule into the material: co-self-assembly from solution and swelling of an assembled miniblock oligopeptide nano-material with a solution of the test molecule. Both processes result in chiral separation, but smectic or higher level order is required in the oligopeptide nanomaterial to achieve good results. Thus we can elucidate some key design features for chiral separation using these materials:

1. Robust smectic layer formation.
2. Functional blocks used to localize solute in interlayer region (entropy or entropically).
3. Chiral functional blocks forming nanoscale chiral pores or interlayer brushes to provide a high surface area of interaction.
4. Sufficient structure and density in the nanomaterial to prevent non-specific diffusion (smectic or other order, density comparable to homopolymer or greater).
5. Chemical compatibility with solute and solvent for solute. Ideally the nanostructured material should swell in the solvent to promote solvent diffusion, but not dissolve. Swell-
pattern are also observed. The layer spacing in synchrotron purity or the product polymer or other reaction product.

reactants and different conformational states of a chiral catalysis. Different activated states of beyond chiral separations to include chiral catalysis, enzyme

to chirally differentiated constraints on the diffusion and reaction processes. For example a chiral enzyme might experience enhanced chiral selectivity in a chiral environment due to chiral activated states of reactants and different conformational states of a chiral catalysis would be expected to be preferentially stabilized in an environment with chiral physical features on the length scale of a molecule when compared to a more symmetric environment. At the surface of a chiral nanopatterned material, a loosely bound enzyme (for example tethered to a swollen hydrophilic nanolayer by a single covalent bond) would experience an environment which has features of homogeneous catalysis. The enzyme would be surrounded by an essentially fluid "gel" where a sharp symmetry breaking solid surface is not defined. sorption to a solid surface) nor constrains the geometry of reactant approach. However, the enzyme would nevertheless be bound to the material and recoverable. Furthermore the fluid (of solubilizing ends or blocks) surrounding the enzyme would be densely packed and chiral, encouraging chiral interactions to stabilize different enzyme conformations (when compared to a symmetric environment).

Along similar lines a chiral catalyst for a polymerization could be embedded in the chiral nanomaterial membrane. Chirally biased transport of monomers and stabilization of a preferred chirality (for monomers that readily racemize) could be used to direct catalysis and subsequent regularity/purity or the product polymer or other reaction product.

Samples

A set of samples have been prepared from concentrated solutions of natural silk using a solvent templating technique. These samples initially formed as hydrogels which grew from a solvent interface into the aqueous silk phase. These hydrogels lose more than 90% of their volume on drying. Orders of a 100-110 dimensional detector at the beam line. Small features (very regular) of the same size are observed in FESEM.

FTIR data on either film was not able to be obtained at this time. In transmission and reflection IR experiments, most of the raw signal required to obtain a spectrum (a difference spectrum between raw signal and background) is lost. This spectral dropout occurs in the wavelength region from 2 and 8 microns. In ATR one does not see any spectrum or any significant raw signal using ZnSe as the ATR crystal. Since ATR should allow one to see any material that is really there, and ATR from similarly problematic films have been obtained when a Si cell was used, we speculate that the films strongly polarize infrared radiation in this wavelength region. The ZnSe crystal used is a hexagonal, randomly oriented material and is thus optically anisotropic—it induces a polarization state in the incident infrared radiation. Silicon is cubic and is optically isotropic. In addition to polarizing the infrared radiation, the samples have no spectrum in diffuse reflectance IR (thus ruling out Bragg diffraction), indicating that they behave as completely IR absorptive little black bodies even when ground into a fine powder. The grain size of the powder (a few microns) can be used to place an upper limit on the smallest film thickness required to obtain a protein black body in the infrared. This may correspond to one full cycle of some chiral feature in the films, but it is also possible that a partial feature has the same effect. This is similar to a related phenomenon where dense clusters in an inhomogeneous material preferentially absorb infrared radiation, resulting in a lower total signal for the material in the clusters, and spectral information weighted towards the less dense material. This phenomenon is relevant to the difference spectra; we are seeing anomalies in the raw spectra.

Since the structures are all reasonable in terms of chiral liquid crystalline and polymer phase behavior and microstructure, the observed phenomena are not specific to proteins but can be generalized to any molecule type, provided that it can be designed to form the appropriate shapes. So as discotic liquid crystalline phases and order are common to all disc shapes molecules, the structures may be general to all polymers which can form chiral hairpins and folded structures. Different molecule types may give better materials properties and also allow one to pump up the strength of the relevant chiral interactions and produce even twistier materials (with analogous morphologies).

The native silk films are comprised of wavy, probably interconnected, layers. FIGS. 1-8 depict SEM images of differentially oriented surface fractures, where the edges of the wavy layers can be seen. In other orientations the morphology looks like a honeycomb of ~75 nm features. Some ~11 nm pores in a very regular honeycomb inside the 75 nm features can be made out. These may be responsible for the bizarre infrared behavior observed. The materials show marked differences in surface morphology (as well as orientation) depending on templating solvent. There may also be small differences in the phantom layer spacing. The less ordered amyl alcohol film is a very good ion scavenger. The chloroform templated film scavenges chiral ions. Both films will soak up enough rare earth salt to become refractive and shiny.

All of the native silk materials will swell very slightly and soften in water or weak acid, and will scavenge bases and become hard. They are insoluble in alcohol. They are thermally stable to 290° C. at which point they degrade rapidly.
Persist to 170° C. Variants have been designed with different plastics, having properties similar to nylons. Preliminary combined with inorganics and salts to create nanocomposites (to thus far. In one of the classes of materials optical clarity and is retained to approximately 200° C. for the materials tested mal stability, toughness, and strength. Qualitative tests of like synthetic nylons (proteins are very fancy nylons in their they are not traditional "folded proteins", but act much more then notropic liquid crystalline behavior. Our ability to combined with a folding or aggregate transition which allows the molecule to change its thermodynamic behavior from flexible soap-like lyotropic liquid crystallinity to chiral oriented rigid rod liquid crystalline behavior. Our ability to induce a molecule to change its shape and chemical properties allows one to engineer irreversible solubility changes into the molecules, making stable materials from processable (under mild conditions) molecules.

Many of the molecules under study exhibit a number of chiral smectic (nano-layered) liquid crystalline phases, which can be dried under controlled conditions to create nanolayered materials. An example is the textured oriented “tape” shown in FIG. 9 (silk-like). These materials also possess a hierarchy of patterned features at different length-scales, which may be responsible for some of their optical behavior. This hierarchical order or patterning is shown in FIG. 10 for the textured tape. A number of these nanolayered materials have been studied, and a common feature for both collagen-like and silk-like materials is the loss of part of the raw mid-IR spectrum when FTIR spectra are obtained from the materials in a spectrometer. The effect is orientation dependent for many of the materials. In FIG. 11 a set of transmission spectra are shown. The raw background has the highest intensity. Very thin tape regions in an orientation that does not have an abnormal affect on IR spectra produce very ordinary protein or peptide IR spectra. In certain orientations we see spectra such as the one represented in FIG. 11.

The regions of the infrared which are strongly affected (the wavelengths) have been correlated to the periodicity of materials morphology patterns for one of the classes of oligopeptide materials (collagen-like). Different processing conditions used to make the (silk-like) tapes, used as an example here, result in different periodicities in their morphological texture and in differences in the infrared wavelength regions affected. There is not enough data on all samples producing the effect to develop clear correlations between processing, morphological texture, and infrared behavior for all of the materials which possess unusual infrared behavior. However, it is believed that these correlations exist. Because very normal protein absorption spectra can also be obtained from very specific orientations of the nanolayered materials, or through grinding of the material to reduce the presence and persistence of long range order, the infrared absorption behavior of these substances is unremarkable at the molecular level. However the presence of a long-range ordered pattern of molecular orientations in the self-assembled materials is causing the infrared radiation to miss the spectrometer detector and perhaps go somewhere else. Because all of the observable materials textures are due to changes in the local molecu-
lar orientation or the orientation of nanoscale layers in the structure, periodic physical features should also correspond to periodic modulations in the refractive index of the material. The raw spectra in the strongly affected regions have a strong sinuousoidal character, as can be seen in the rescaled data in FIG. 12 (unfortunately, due to rescaling the Y-axis intensities are no longer physically meaningful). In many instances these sinuousoidal patterns are attenuated or “chirped” in the small wavelength/long wavelength part of the spectrum. If these materials can redirect or guide radiation in the mid-far infrared wavelength region, they may be quite useful in redirecting infrared signatures either to a heat sink, or a detector, or a spectrometer for in-line chemical analysis.

This effect is also observed in layered polycrystals of the same oligopeptides (collagen-like and silk-like classes) cocrystallized with an organic salt (FIG. 13). These layered polycrystals of materials affect the 3-10 micron region of the infrared. These local phase changes occur at fairly low temperatures (<100° C.). These local changes can be attributed to extension of the crystal faces and variations and their correlation to infrared spectral features. In this case the infrared effect appears most pronounced for the most highly organized and regular twisted polycrystalline materials. Attenuation is observed in both reflection infrared spectroscopy and transmission infrared spectroscopy (FIG. 15). The ordered material is thus both less transmissive and less reflective than the ZnS background in the affected region of the infrared. SANS data indicate a strong nanoscale layered periodicity (preliminary and unprocessed, not shown). Thermal studies using WAXS and SANS indicate a slightly lower thermal stability for these salted polycrystalline materials, around 160° C., when compared to pure oligopeptide materials. Very local phase changes occur at fairly low temperatures (<100° C.). These local changes can be attributed to extension of the molecules in the nanoscale layers, resulting in a change in layer spacing. Such local phase changes may provide useful avenues for manipulating the properties of the materials, but have not been studied in detail yet. The polycrystals are qualitatively “hard” (as compared to other polymeric crystals) and difficult to grind (tough). The relatively low thermal stability of these polycrystals may be due to the choice of salt—in this case a low melting organic molecule. Other salts have produced nanocomposites which retain the nanolayered structure imposed by the oligopeptides, but which appear to be more thermally stable than pure peptide, although this data is very preliminary.

The oligopeptide materials can be processed into tough precipitated tapes, polycrystalline aggregates, or thin films (poorer mechanical properties, but we’re addressing this). All of these materials manipulate the infrared spectra although the silk-like class of materials appear to affect a broader band of the spectrum than the collagen-like materials. The collagen-like materials affect the 3-10 micron region of the infrared most strongly, and have material periodicities in the same size range which appear correlated to the spectral band affected. These materials features can be manipulated through selection of sequence patterns in the chemical structure of the molecules, through tuning of the anchoring behavior of the molecules in the liquid crystalline state during processing, and are expected to also respond to a low voltage electrical field applied during formation of the liquid crystal and drying to form a solid material. Control of material texture variations and their correlation to infrared spectral behavior has not yet been systematically addressed for the silk-like class of biopolymer materials (tapes and polycrystals in the examples). However differences are observed depending on the solvent conditions used to create the tapes, and differences in the region of the infrared affected are also observed.

Preparation of Silk

Materials

Cocoons of B. mori silkworm silk were kindly supplied by M Tsukada, Institute of Sericulture, Tsukuba, Japan. Chloroform, hexane and iso-amyl alcohol were purchased from Aldrich and Fisher Scientific and used without further purification. Tris(2,2'-bipyridyl)dichlororuthenium(II) hexahydrate (“Rubipy”) was purchased from Aldrich. Preparation of Regenerated B. mori Silk Fibroin Solutions—The silk fibroin solutions were prepared by either one of two methods: Method A—for preparation of bulk solids — B. mori silk fibroin solutions were prepared as follows. Cocoons were boiled for 30 min in an aqueous solution of 0.02 M Na2CO3, then rinsed thoroughly with water to extract the glue-like sericin proteins. The extracted silk was then dissolved in 9.5 M LiBr solution at room temperature yielding a 20 wt % solution. This solution was dialyzed in water using Slide-a-Lyzer dialysis cassettes (Pierce, MWCO 2000) for 48 hrs. The final concentration of aqueous silk solution was 8.0 wt %, which was determined by weighing the remaining solid after drying. Millipore purified water, 17 MΩ, was used throughout all processing. No buffers, acids, or salts were added in final solution; Method B—for preparation of thin films— Bombyx mori silk cocoons were degummed using repeated washings in boiling water, sodium dodecyl sulfate (SDS) and Na2CO3 to remove the sericin, leaving pure fibroin. For the first washing 6.5% SDS and 1.0% Na2CO3 were used in boiling water. The cocoons were then rinsed with 0.4% Na2CO3 in boiling water, and subsequently rinsed with boiling water alone. Other cocoons were degummed without SDS, using only Na2CO3 and boiling water. Amino acid analysis has been used to assess the protein composition of fibroin prepared in this manner and no sericin was detected. Valluzzi, R.; Gido, S. P. Biopolymers 1997, 42, 705-717; Valluzzi, R.; Gido, S.; Zhang, W.; Muller, W.; Kaplan, D. Macromolecules 1996, 29, 8606-8614. The degummed fibroin was rinsed thoroughly with distilled water and dissolved in a 9.1 M solution of LiSCN in water. In order to remove the salt, the fibroin and LiSCN solution was then dialyzed against frequent changes of distilled water for several days. The dialyzed fibroin solutions were filtered using a 100 µm syringe filter to remove dust and any protein precipitate.

Example 2

Preparation of Smectic Gels

Aqueous-Chloroform, Hexane and Iso-Amyl Alcohol interfaces were prepared by adding silk peptide solution into glass vials containing each solvent. The vials were then capped to prevent evaporation and left at room temperature overnight. The resulting interfacial gels were collected and dried at room temperature overnight.

Desalted, HPLC purified, and lyophilized collagen-like peptide was obtained from the Protein Chemistry Core Facility at the Tufts Medical School. The sequence was (Glu)5 (Gly-Val-Pro-Gly-Pro-Pro)6 (Glu)5 (SEQ ID NO: 3). The glutamic acid blocks were added to the ends of the peptides to promote solubility in water so that contaminant salts would not complicate analysis. Similar peptide design strategies have been used by Rotwarf et. al. to examine the solution
behavior of β-sheet forming peptides. Rotwarf, D. M.; Dav-  

genport, V. G.; Shi, P.-T.; Peng, J.-L.; Sheraga, H. A. Biopoly-  

mers 1996, 39, 531-536. The collagen-like peptide was dis-  

solved in 18 MQ Millipore filtered water at a concentration of  

1 mg/ml peptide in water. No salt or acid or extra reagent was  

required to aid dissolution. The solution was allowed to stand  

in an air-tight capped vial overnight, and then a gold mesh  

TEM grid (no substrate film) was dipped through the air-  

water interface.

Example 3

Characterization

Characterization

Gels treated with ruthenium compound were cut to obtain  

cross sections. The amyl alcohol gel in Rubipy is shown in  

FIG. 16. The ruthenium compound (Rubipy) has an orange  

color, and is in the silk gel in a higher concentration (bright  

red orange) than in the surrounding solution (light yellow  

orange). Gels treated with Rubipy for 1 hour were sliced open  

to obtain cross sections (FIG. 17). These cross sections allow  

us to compare the structure of the gel with a lot of Rubipy  

absorbed to the structure of the interior, which has a lower  

concentration of Rubipy. A cross section is shown in FIG. 18,  

and a dark red-orange Rubipy-rich skin can be seen surround-  

ing a clear yellowish silk core. Understanding and engineer-  

ing the skin core morphology formed through the templating  

process is important for controlling the characteristics and  

function of the gels.

SEM

Images of dried gels were obtained with a LEO Gemini 982  

Field Emission Gun SEM. Working distance was 7 mm and  

applied voltage was 1 to 2 kV. All images of gels were taken  

without any conductive coating. (FIG. 18).

XRD

WAXD experiments were done using Bruker D8 Discover  

X-ray diffractometer with GADDS multiwire area detector.  

40 kV and 20 mA and 0.5 mm collimator was used. The  

distance between the detector and the sample for WAXD was  

60 mm. CuKa. Layered structures (6-12 nm layers) with Silk  

I secondary structures (a non integer helix between the silk II  

β-strand and the silk III three fold helix) were observed. The  

chloroform gels had high orientation in WAXS (FIG. 19).

TEM

Interfacial films of silk fibroin and the peptide were char-  

acterized using a JEOL 2000 FX-II TEM operating at 200 kV  

accelerating voltage. Samples were maintained at below  

-150°C during the TEM characterization, utilizing a cryo-  

genic sample holder. Working at cryogenic temperatures was  

necessary in order to reduce beam damage and to prevent the  

loss of water from hydrated crystal structures in the high  

vacuum of the microscope. Electron diffraction and TEM  

bright field imaging were used to assess the structures in the  

films. An internal gold standard was used to determine lattice  

 spacings. Defocused diffraction imaging was used to deter-  

mine the relative orientations of the diffraction patterns and  

banding or crystallite facets in the morphology images.

Detection of salt contamination and the characteristics  

described in previous  

papers. Valluzzi, R.; Gido, S. P. Biopolymers 1997, 42, 705-  

717; Valluzzi, R.; Gido, S.; Zhang, W.; Muller, W.; Kaplan, D.  

Macromolecules 1996, 29, 8606-8614. No salt artifacts were  

observed in the structures obtained from the water-hexane  

interface.

INCORPORATION BY REFERENCE

All of the patents, patent applications, and publications  
cited herein are hereby incorporated by reference.

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascer-  
tain using no more than routine experimentation, many  
equivalents to the specific embodiments of the invention  
described herein. Such equivalents are intended to be encom-  
passed by the following claims.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 4
<210> SEQ ID NO 1
<211> LENGTH: 50
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide
<400> SEQUENCE: 1
Glu Glu Glu Glu Glu Ser Gly Ala Gly Val Gly Arg Gly Asp Gly Ser 1 5 10 15
Gly Asp Gly Ser Gly Val Gly Leu Gly Ser Gly Asn Gly Glu Glu Glu 35 40 45
Glu Glu
50

<210> SEQ ID NO 2
<211> LENGTH: 34
<212> TYPE: PRT
What is claimed is:
1. A structured templated fibrous protein smectic hydrogel prepared according to a method comprising:
   a. contacting an aqueous fibrous protein solution with a solvent that is not miscible with water,
   b. allowing the solution in contact with the solvent to age at about room temperature or under conditions preventing evaporation or both; and
   c. collecting the resulting fibrous protein smectic hydrogel, and optionally allowing the hydrogel to dry;
wherein the fibrous protein solution is present in greater than 4% by weight.
2. The structured templated fibrous protein smectic hydrogel of claim 1, wherein the fibrous protein is selected from the group consisting of silk, collagens, keratins, actins, chorions, and sero- 
sins.
3. The structured templated fibrous protein smectic hydrogel of claim 1, wherein the fibrous protein is silk.
4. The structured templated fibrous protein smectic hydrogel of claim 1, wherein the fibrous protein smectic hydrogel is a bulk solid comprising several ordered layers of the fibrous protein.
5. The structured templated fibrous protein smectic hydrogel of claim 1, wherein the fibrous protein solution is present in greater than or equal to 8% by weight.
6. A structured templated fibrous protein smectic hydrogel, wherein the fibrous protein is selected from the group consisting of silk, collagens, keratins, actins, chorions, and sero- 
sins.
7. The structured templated fibrous protein smectic hydrogel of claim 6, wherein the fibrous protein is silk.
8. The structured templated fibrous protein smectic hydrogel of claim 6, wherein the fibrous protein smectic hydrogel is a bulk solid comprising several ordered layers of the fibrous protein.
9. The structured templated fibrous protein smectic hydrogel of claim 7, wherein the fibrous protein smectic hydrogel is a bulk solid comprising several ordered layers of the fibrous protein.

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