Bionanotech - proteins

- Engineering
- Design
- Catalysis
- Self-assemblies
- Templates & scaffolds
- Surface coatings
- Sensors
Tuan Vo-Dinh, Fitzpatrick Institute for Photonics

- Nanostructures and self-assemblies
- Drug delivery
- Biosensors
- Molecularly imprinted polymers

Humana Press
Protein Nanotech

- Engineered assemblies
- Organization of nanomaterials
- Biosensors
- Biomineralization

<table>
<thead>
<tr>
<th>Biological Species</th>
<th>Example</th>
<th>Typical size</th>
<th>MW (daltons)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small protein</td>
<td>Chymotrypsin</td>
<td>4 nm sphere</td>
<td>10⁴-10⁵</td>
</tr>
<tr>
<td>Large protein</td>
<td>Asp.transcarb.</td>
<td>7 nm sphere</td>
<td>10⁵-10⁷</td>
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<tr>
<td>Small assembly</td>
<td>Ribosome</td>
<td>20 nm sphere</td>
<td>10⁵-10⁷</td>
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<tr>
<td>Large assembly</td>
<td>Viruses</td>
<td>100 nm sphere</td>
<td>10⁷-10¹²</td>
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<tr>
<td>Nucleic acids</td>
<td>tRNA</td>
<td>10 nm rod</td>
<td>10⁴-10⁵</td>
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Fig. 1. A general strategy for designing fusion proteins that assemble into symmetric nanostructures. (a) The green semicircle represents a natural dimeric protein (i.e., a protein that associates with another copy of itself), whereas the red shape represents a trimeric protein. The symmetry axes of the natural oligomers are shown. (b) The two natural proteins are combined by genetic methods into a single fusion protein. Each of the original natural proteins serves as an "oligomerization domain" in the designed fusion protein. Two different hypothetical fusion proteins are shown to illustrate that the oligomerization domains can be joined rigidly in different geometries. (c) A ribbon diagram of a fusion protein showing one method for joining two oligomerization domains (red and green) in a relatively rigid fashion. One of the natural oligomerization domains must end in an α-helical conformation, and the other must begin in an α-helical conformation. The two are then linked by a short stretch of amino acids (blue) that have a strong tendency to adopt an α-helical conformation. Thus, the two oligomerization domains are joined physically in a predictable orientation. (d) A designed fusion protein self-assembles into a particular kind of nanostructure that depends on the geometry of the symmetry axes belonging to its component oligomerization domains (Table 1). A molecular layer arises from an arrangement like that in b (Left). (e) A cubic cage arises from an arrangement like the one in b (Right).
Table 1. Construction rules for designing nanohedral protein materials from dimeric and trimeric oligomerization domains

<table>
<thead>
<tr>
<th>Symmetry*</th>
<th>Construction</th>
<th>Geometry of symmetry elements$^*$</th>
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<tr>
<td>Cages and shells</td>
<td></td>
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<tr>
<td>T$^*$</td>
<td>Dimer-trimer</td>
<td>54.7°</td>
</tr>
<tr>
<td>O$^*$</td>
<td>Dimer-trimer</td>
<td>35.3°</td>
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<tr>
<td>I</td>
<td>Dimer-trimer</td>
<td>20.9°</td>
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<td>Double-layer rings</td>
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<tr>
<td>D$_{nn}$</td>
<td>Dimer-dimer</td>
<td>180°/n</td>
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<td>Two-dimensional layers</td>
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<td>p321</td>
<td>Dimer-trimer</td>
<td>90°</td>
</tr>
<tr>
<td>p3</td>
<td>Trimer-trimer</td>
<td>0°</td>
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<td>Three-dimensional crystals</td>
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<tr>
<td>I2,3</td>
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<td>54.7°</td>
</tr>
<tr>
<td>P4,32 or P4$_3$2</td>
<td>Dimer-trimer</td>
<td>35.3°</td>
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<tr>
<td>P23</td>
<td>Trimer-trimer</td>
<td>70.5°</td>
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<td>Helical filaments</td>
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<td></td>
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<tr>
<td>Helical$^1$</td>
<td>Dimer-dimer</td>
<td>Any angle,</td>
</tr>
</tbody>
</table>

This table gives only those symmetries that can be constructed by combining two oligomerization domains of the dimeric or trimeric type. Other kinds of oligomerization domains, such as tetramers, would give additional possibilities not listed here.

*T, O, I, and D$_n$ refer to tetrahedral, octahedral, icosahedral, and dihedral symmetry, respectively. The remaining symbols are denoted by their Hermann-Mauguin symbols.

$^1$The angle formed between the two symmetry elements is given, followed by I or N to denote intersecting or nonintersecting axes.
Fig. 3. Electron microscopy and model of a designed protein filament. (a) A ribbon model of a single molecule of the designed fusion protein. (b) A ribbon model of the protein filament as it was intended to assemble, with separate protein molecules colored differently. (c) Negatively stained electron micrograph of a bundle of filaments formed by the designed fusion protein. The bundle is 15–20 filaments across and reveals details indicative of the individual dimeric oligomerization domains that make up the fusion protein. In addition to bundles, networks of filaments were also observed in other micrographs.
Self-Assembly of Proteins into Designed Networks
Philippe Ringler and Georg E. Schulz*
3 OCTOBER 2003 VOL 302 SCIENCE

A $C_4$-symmetric tetrameric aldolase was used to produce a quadratic network consisting of the enzyme as a rigid four-way connector and stiff streptavidin rods as spacers. Each aldolase subunit was furnished with a His$_6$ tag for oriented binding to a planar surface and two tethered biotins for binding streptavidin in an oriented manner. The networks were improved by starting with composite units and also by binding to nickel–nitrilotriacetic acid–lipid monolayers. The mesh was adjustable in 5-nanometer increments. The production of a net with switchable mesh was initiated with the use of a calcium ion–containing β-helix spacer that denatured on calcium ion depletion.

L-rhamnulose-1-phosphate aldolase

streptavidin

biotin

6x5x4 nm

7x7x5 nm
Protein arrays

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Fig. 1. Block construction and self-assembly in solution. All electron micrographs were negatively stained; all scale bars are 20 nm. The particles and nets were picked from numerous similar pictures. (A) View onto the flat 7-nm-by-7-nm top face (depth of 6 nm) of the C4-symmetric enzyme RhuA [14]. The point mutations for biotin labeling are stated for one subunit. The C termini (Ct) at the top face carry His6 tags [18]. Block bR is RhuA with eight tethered (dashed lines) biotins at the newly introduced cysteines, two of which are depicted as binding to a streptavidin (block S). The 6-nm-by-5-nm face (depth of 4 nm) of S is shown [15, 16]. The bR:S unit is in turn bound to block b6S, which is a streptavidin with (the four depicted) bis-biotin linkers. (B) Block bR:S4 produced on a Ni-NTA column [18]. Three samples and an average of 21 are given. (C) Block bR2:S7, which occasionally eluted from the Ni-NTA column, showing three samples and an average of 11. (D) Block bR4:S12 produced as a by-product during self-assembly of bR and bR:S4. Three samples and an average of six are shown. The shortest distances between bR units are around 13 nm, confirming the presence of one S as spacer. (E) Networks produced by self-assembly of bR and bR:S4, reaching sizes of 50 nm by 50 nm.
Protein arrays

3 OCTOBER 2003 VOL 302 SCIENCE

Fig. 2. Representative negatively stained example of the networks formed by self-assembly of blocks $^\alpha R$ and $^\beta R$/$S_4$ at a lipid monolayer. The lattices extend over more than 200 nm by 200 nm but contain irregularities. (Insert) A magnified sample.
Protein arrays

3 OCTOBER 2003  VOL 302  SCIENCE

Fig. 3. Networks with streptavidin spacer rods produced in solution. The electron micrographs are negatively stained. The depicted samples are representative. (A) Block $bR_4S_{16}^{bbS_{12}}$ produced by self-assembly of $bR\cdot S_4$ with $bbS$, showing three samples and an average of six. The distance between $bR$ blocks is 21 nm (scale bar, 20 nm), in agreement with the expected $S_{bbS\cdot S}$ spacer. (B) Streptavidin rods of around five units produced by mixing $bbS$ with a smaller amount of $S$ (left). Longer rods of around 20 units obtained by mixing equal particle numbers of the five-unit rods and $S$ (right). (C) Self-assembly of $bR\cdot S_4$ with $(bbS\cdot S)_x^{bbS}$ rods (18) of various lengths.
Fig. 4. The 998-residue construct PGAL-β-PGAL (17, 18). (A) Ribbon model of the polypeptide indicating N and C termini of the construct. On Ca$^{2+}$ removal, the β helix (scale bar, 2 nm) denatures, giving rise to a 20-nm peptide tether. (B) Electron micrographs of negatively stained solutions of PGAL-β-PGAL picked from numerous similar pictures (scale bars, 20 nm). The left side shows PGAL-β-PGAL after EDTA-free preparation (22), forming dumbbell-shaped particles (circled) corresponding to the model of (A). On the right side, the same preparation after treatment with 10 mM EDTA shows separated globular PGAL molecules (the denatured peptide is not visible).
Protein arrays

A strategy for creating regular protein arrays from a natural oligomeric protein and a bivalent ligand [42]. In the case illustrated, a tetrameric lectin (four yellow ellipses) was mixed with a synthetic, two-headed carbohydrate molecule (red). Each protein complex is polyvalent by virtue of its ability to contact four other complexes. The result was an ordered three-dimensional material with subunits arranged in a ‘diamond-like’ lattice. A ribbon model of the tetrameric protein is shown (in the inset), as is a transmission electron micrograph of the material. Dotan et al measured 6.9 ± 0.3 nm between the centers of neighboring molecules in the micrograph. Reprinted with permission from [42], copyright 1999 Wiley-VCH.

Bionanotech - proteins

- Engineering
- Design
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- Surface coatings
- Sensors
ordered nanoparticle arrays formed on engineered chaperonin protein templates

R. ANDREW MCMILLAN1, CHAD D. PAAYOLA1, JEANIE HOWARD2, SUZANNE L. CHAN2, NESTOR J. ZALUZEC2 AND JONATHAN D. TRENT*1

1NASA Ames Research Center, Center for Nanotechnology and Astrobiology Technology Branch, Mail Stop 239-15, Moffett Field, California 94035, USA
2SETI Institute, 2035 Landings Drive, Mountain View, California 94043, USA
3Argonne National Laboratory, Materials Science Division, 9700 South Cass Avenue, Argonne, Illinois 60439, USA
4e-mail: amcmillan@mail.arc.nasa.gov; jtrent@mail.arc.nasa.gov

- **Chaperonins**
  - Natural function: protein folding assistants or actually misfolding “inhibitor”.
  - Multimer assembles given ATP & Mg++; large internal cavity.
  - 15 - 20 nm assemblies.

- **Sulfolobus shibatae**
  - Lives in geothermal hot-springs (85°C, pH 2).
  - Thermostable protein easy to purify and engineer.
  - Sequence & structure available for modeling.
  - Assembles into higher order structures (2D crystals).
Ordered nanoparticle arrays formed on engineered chaperonin protein templates

R. ANDREW MCMILLAN*, CHAD D. PAAYOLA*, JEANIE HOWARD, SUZANNE L. CHAN, NESTOR J. ZALUZEC AND JONATHAN D. TRENT*

*NASA Ames Research Center, Center for Nanotechnology and Astrobiology Technology Branch, Mail Stop 239-15, Moffett Field, California 94035, USA
SETI Institute, 2035 Landings Drive, Mountain View, California 94043, USA
Argonne National Laboratory, Materials Science Division, 9700 South Cass Avenue, Argonne, Illinois 60439, USA
*E-mail: amcmillan@mail.arc.nasa.gov; jtrent@mail.arc.nasa.gov

HSP60 beta

Top view

3 nm pore

LaBean COMPSCI 296.5

4/25/06

9 nm pore
Chaperonin arrays

Figure 2 2D crystals of engineered chaperonins as quantum dot array templates. a, TEM image of a negatively stained 2D crystal of the beta chaperonin variant with cysteine substitutions at the apical pores. The two-sided-plane group p312 was assigned to the lattice through image analyses of micrographs of *S. shibatae* beta chaperonin 2D.

Figure 3 Gold QD binding to engineered chaperonins and chaperonin-templates. a, Low-magnification TEM image of 10 μm diameter 2D crystal of the 9-nm-pore chaperonin variant with bound 10 nm gold QDs. The image contrast is from gold QDs bound to the crystalline lattice of the underlying protein template. Drying causes significant sample cracking and contributes to distortions and separation of regions of order within the array. b, Higher-magnification stained TEM image of side views of 5 nm gold QDs (arrowed) tethered at the apical pores of the 3-nm-pore mutant chaperonins. Inset: Slab-view cutaway diagrams of postulated orientation of 5 and 10 nm gold QDs bound at the apical pores of the two chaperonin variants. c, Stained image of 5 nm gold QDs bound within the pores of the 3-nm-pore crystalline template. Occupied rings show the QDs (dark areas) are surrounded and held in place by the chaperonin pores. Empty rings have a brighter, less electron-dense appearance. We are currently investigating why some sites are unoccupied. We suspect that this is related to the solvent accessibility of thiols on the apical loops, or to the size variation of the 5 nm QDs. d, Ordered region of 10 nm gold bound to a 9-nm-pore template with similar area coverage as in c. The chaperonins are occluded by the 10 nm QDs.
Chaperonin arrays

Figure 4: Semiconductor QD arrays. a, DIC light micrograph of an 8 μm crystalline disc of the 3-nm-pore template with bound 4.5 nm luminescent CdSe-ZnS QDs. b, Both dry and rehydrated arrays fluoresced indicating the QDs bound to the template. 2D crystals of a beta variant without added cysteines showed minimal QD binding (see Supplementary Information). c, Low-magnification TEM of an unstained array of CdSe-ZnS QDs. Image contrast is due to the bound semiconductor QDs. d, Higher-magnification image of the same crystal showing an ordered region of QDs bound to the chaperonin lattice.

Figure 5: Chaperonin subunit mediated self-assembled Nanogold arrays. a, Covalent attachment of 1.4 nm monomaleimido Nanogold to subunits of the 9-nm-pore beta chaperonin variant through Michael addition of cysteine thiol to QD surface maleimide groups. Right: The possible arrangement of nine 1.4 nm covalently attached Nanogold QDs.
S-layer arrays

- Crystalline bacterial cell surface layers.
  - ~$5 \times 10^5$ monomers/cell
  - Biosynth ~500 copies/sec
- Purified S-layer proteins self-assemble into arrays.
- Pores and functional groups available for engineering.
Crystalline Bacterial Cell Surface Layers (S Layers): From Supramolecular Cell Structure to Biomimetics and Nanotechnology

Uwe B. Sleytr,* Paul Messner, Dietmar Pum, and Margit Sára


Figure 1. Schematic illustration of the supramolecular architecture of the three major classes of prokaryotic cell envelopes containing crystalline bacterial cell surface layers (S layers). a) Cell envelope structure of Gram-negative archaea with S layers as the only cell-wall component external to the cytoplasmic membrane. b) Cell envelope as observed in Gram-positive archaea and bacteria. In bacteria the rigid wall component is primarily composed of peptidoglycan. In archaea other wall polymers (e.g. pseudomurein or methanochondroitin) are found. c) Cell envelope profile of Gram-negative bacteria composed of a thin peptidoglycan layer and an outer membrane. If present the S layer is closely associated with the lipopolysaccharide of the outer membrane (modified based on reference [13]).

Figure 6. Schematic illustration of the recrystallization of isolated S layer subunits into crystalline arrays (a). The self-assembly process can occur in suspension (b), at the air/water interface (c), on solid supports (d), and on Langmuir lipid films (e).
Figure 2. Electron micrograph of freeze-etched preparations of intact cells from a) Aneurinibacillus thermoacidophilus DSM 10155 showing a square (p4) S-layer lattice and b) archaean Methanomicrobium mobile DSM 1539 covered with a hexagonal (p6) array. F: flagella. The bar corresponds to 100 nm.

Figure 3. Schematic drawing of different S layer lattice types. The regular arrays exhibit either oblique (p1, p2), square (p4), or hexagonal lattice symmetry (p3, p6). The morphological units are composed of one, two, three, four, or six identical subunits.
S-layer arrays


Figure 4. a) Three-dimensional model of the protein mass distribution of the S-layer of *Bacillus stearothermophilus* NRS 2004/3a/V2 (outer face). The S layer is about 8 nm thick and exhibits a center-to-center spacing of the morphological units of 13.5 nm. The protein meshwork shows one square-shaped, two elongated, and four small pores per morphological unit. The model was obtained after recording several tilt series of a negatively stained preparation in a transmission electron microscope, performing Fourier-domain computer image reconstructions over each individual tilted view, and combining all processed views to a three-dimensional data volume. b), c) Computer image reconstruction of the scanning force microscopic images of the topography of the inner face of the S layer lattice from *B. sphaericus* CCM 2177 (b) and *Bacillus coagulans* E38–66/V1 (c). The images were taken under water. The surface corrugation corresponding to a gray scale from black to white is 1.8 nm. The center-to-center spacing of the morphological unit of the square lattice (b) is 14.5 nm. The S layer lattice in (c) shows an oblique unit cell (a = 9.4 nm, b = 7.5 nm, base angle = 80°). The bars correspond to 10 nm.
S-layer arrays


Table 1. Properties of S layers.[3, 7, 14, 26, 43, 44, 54, 55]
The S layer lattices can have oblique (p1, p2), square (p4), or hexagonal (p3, p6) symmetry.
The center-to-center spacing of the morphological unit can be between 3 and 35 nm.
The lattices are generally 5 to 20 nm thick (in archaeeae up to approximately 70 nm).
The outer surface is generally less corrugated than the inner surface.
The S layer lattices exhibit pores of identical size and morphology.
In many S layers two or even more distinct classes of pores are present.
The pore sizes range from approximately 2 to 8 nm.
The pores can occupy 30 to 70 % of the surface area.
The relative molecular mass of constituent subunits is in the range of 40000 to 200000.
These are weakly acidic proteins (pI ≈ 4 – 6), except for Methanothermus fervidus (pI = 8.4) and lactobacilli (pI > 9.5).
Large amounts of glutamic and aspartic acid (about 15 mol %) are present.
There is a high lysine content (about 10 mol %).
There are large amounts of hydrophobic amino acids (about 40 – 60 mol %).
Hydrophilic and hydrophobic amino acids do not form extended clusters.
In most S layer proteins about 20 % of the amino acids are organized as α helices and about 40 % occur as β sheets.
Aperiodic foldings and β-turn content may vary between 5 and 45 %.
Posttranslational modifications of S layer proteins include cleavage of N- or C-terminal fragments, glycosylation, and phosphorylation of amino acid residues.
S-layer arrays

Diversity of:
- Geometry
- Pore size
- pI
- Glycosylation sites
- Etc.

Figure 8. Schematic drawing of the cell envelope profile of Gram-positive (a) and Gram-negative bacteria (b) underlining the structural similarities between S layer glycoproteins and lipopolysaccharides. Abbreviations: C: core region; CM: cytoplasmic membrane; CW: cell wall; G: glycan chain; O: O-antigen; OM: outer membrane; PG: peptidoglycan (taken from reference [43] with permission of Landes/Academic Press).
S-layer arrays

Figure 9. Schematic drawing showing a) S layer ultrafiltration membranes (SUMs) and b) S layer microparticles (SMPs) as matrix for the immobilization of functional macromolecules. c, d) Electron micrographs of freeze-etched preparations of S layers with hexagonal lattice symmetry. In (c) polyvalent ferritin (PCF) was used for labeling negatively charged domains on the surface of the archaeon *Thermoproteus tenax.* In (d) ferritin was covalently linked to the carbodiimide-activated carboxy groups of the S layer lattice of *Thermotoga maritima.* The bars correspond to 100 nm.
S-layer arrays

Figure 11. Schematic illustration of the "semifluid" membrane model composed of a phospholipid bilayer or tetraetherlipid monolayer Langmuir film supported by an S layer. The proportion of lipid molecules which can covalently be linked to the porous S layer lattice or which interact by noncovalent forces with domains of the S layer protein subunits significantly modulates the lateral diffusion of the free lipid molecules and consequently the fluidity of the membrane (modified based on reference [200]).

Figure 12. Schematic drawing illustrating the concept of solid-supported lipid membranes stabilized by S layers. In (a) the generation of the lipid bilayer makes use of the strong chemisorption of thiollipids to gold. The second leaflet and the S layer which had recrystallized at the lipid film before in a Langmuir trough were transferred onto the thiolipid-coated solid support by the Langmuir–Schaefer technique. Integrated functional molecules allow the investigation of transmembrane functions. b) As an alternative to soft polymer cushions an S layer is located between the solid support and the lipid layer. Optionally, the external leaflet of the lipid bilayer can be stabilized by the attachment of an S layer (modified based on...
S-layer arrays


Figure 15. a) Electron micrograph of a gold “superlattice” consisting of monodisperse gold nanoparticles with mean diameters of 4 to 5 nm. b) Schematic drawing of a cross-section through a support coated with S layers, where the gold nanoparticles had formed in the pore region of the protein layer. The bar corresponds to 50 nm.
Bionanotech - proteins

- Engineering
- Design
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- Templates & scaffolds
- Surface coatings
- Sensors
Self-assembling peptide templates

Various types of self-assembling peptide systems. (a) Amphiphilic peptides in β-strand conformation are chiral objects. As a consequence, they self-assemble into twisted tapes. (b) Helical dipolar peptides can undergo a conformational change between α-helix and β-sheet, much like a molecular switch [44*]. (c) Surface-binding peptides can form monolayers covalently bound to a surface [45*]. (d) Surfactant-like peptides can form vesicles and nanotubes [10, 10*].

Current Opinion in Chemical Biology 2002, 6:865–871
Self-assembling peptide templates

(a) Axis view of a peptide nanotube
(b) Side view

Current Opinion in Chemical Biology 2002, 6:865–871
Casting Metal Nanowires Within Discrete Self-Assembled Peptide Nanotubes

Meital Reches and Ehud Gazit*

Tubular nanostructures are suggested to have a wide range of applications in nanotechnology. We report our observation of the self-assembly of a very short peptide, the Alzheimer’s β-amyloid diphenylalanine structural motif, into discrete and stiff nanotubes. Reduction of ionic silver within the nanotubes, followed by enzymatic degradation of the peptide backbone, resulted in the production of discrete nanowires with a long persistence length. The same dipeptide building block, made of D-phenylalanine, resulted in the production of enzymatically stable nanotubes.
Fig. 1. Self-assembly of well-ordered and elongated peptide nanotubes by a molecular recognition motif derived from the β-amyloid polypeptide. (A) The central aromatic core of the β-amyloid polypeptide is involved in the molecular recognition process that leads to the formation of amyloid fibrils. Various fragments of the core form amyloid fibrils or inhibit their formation. (B) TEM images of the negatively stained nanotubes formed by the diphenylalanine peptide. (C) HR-TEM images of negatively stained peptide nanotubes, visualized by field emission gun microscope.
Metal nanowires from peptide templates

Fig. 2. Structural analysis of the tubular nanoparticles. (A) Low-magnification SEM images of a field of discrete nanotubes that are present as individual entities. (B) High-magnification SEM image of an individual nanotube. (C) A statistical distribution of nanotube diameters.
Metal nanowires from peptide templates

Fig. 3. Casting of silver nanowires with the peptide nanotubes. (A) The nanowires were formed by the reduction of silver ions within the tubes, followed by enzymatic degradation of the peptide mold. (B) TEM analysis (without staining) of peptide tubes filled with silver nanowires. (C and D) TEM images of silver nanowires that were obtained after the addition of the proteinase K enzyme to the nanotube solution.
Metal nanowires from peptide templates

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Fig. 4. Formation of peptide nanotubes by different aromatic peptides. (A) TEM image of the stable nanotubes formed by the D-amino acid building-block analog (B) TEM image of a tubular structure formed by the NH$_2$-Phe-Trp-COOH dipeptide. Amorphous aggregates can be observed in the background.

NH$_2$-D-Phe-D-Phe-D-COOH + Proteinase K

NH$_2$-L-Phe-L-Trp-COOH
Bionanotech - proteins

- Self-assemblies
- Templates & scaffolds
  - Nanoparticles
  - Metals
  - Silica, minerals, ceramics, semiconductor
- Surface coatings
Most Materials are “Biohostile”

AFFINERGY

Site-Specific Biological Delivery Systems

Daniel Kenan

www.affinergy.com
Objective:

Attach a specific biological material (cell, antibody, protein, etc.) to...

A synthetic material (plastics, metals, glass, etc.)

1. Identify peptide that binds to biological material with high affinity and selectivity
2. Identify peptide that binds to synthetic material with high affinity
3. Use a small linker to tie the two peptides together

Interfacial Biomaterials (IFBM) Coating
Polystyrene Binding Peptide Synthesized with RGD

**FFPSSWYSHLGVLSSSGRGD**
Titanium orthopedic screw with Stem Cells

IFBM Attachment of preadipocytes

IFBM Blocking
IFBM’s Mediate Rapid & Efficient Cell Adhesion on Ti
Mineralization of IFBM-Supported Stem Cells at 45d
IFBM Platform

- Growth Factors
- Cell Receptors
- Cell Matrix Proteins
- Collagen
- Enzymes
- Antibodies

- Titanium
- Stainless Steel
- Teflon
- PET, PETG, PGA
- Polystyrene
- Polycarbonate
- Glass, Nylon, Latex

IFBM Discovery Platform:

- > 4 billion peptides in libraries
- 3 to 6 month cycle to find binders
- Binders found for every target tried
- Biases can be built into selection
- Range of affinities and specificities can be exploited
**Rapid Solutions Due to Modularity of Affinergy’s Platform**

<table>
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<th>Tie 2 Modulators</th>
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<td>Collagen Synthetic Scaffolds</td>
<td>Resorbables</td>
<td>Damaged Heart Tissue</td>
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Ang1 and Ang2 are used as modulators in this context.
Bionanotech - proteins

- Engineering & design
  - Custom catalysts
- Self-assemblies
  - Nano organizers
- Templates & scaffolds
  - Nanoimprint or DPN inks
  - Tissue regeneration
- Sensors
  - Sensitivity & selectivity
Molecularly Imprinted Polymers

- “Artificial proteins”
- Mimic protein functions
  - Molecular recognition (binding)
  - Catalysis
- Higher stability
- Manufacture greater quantities
Molecularly Imprinted Polymers

Imprinted polymers—Tailor-made mimics of antibodies and receptors

Karsten Haupt†
University of Paris 12, Science Faculty, Créteil, France. E-mail: karsten.haupt@biokem.lth.se

Fig. 3 Molecular imprinting of theophylline immobilized onto a solid support: immobilized template with monomers (1), composite material after polymerization (2), imprinted polymer after dissolution of the support (3).26

Fig. 2 A dendrimer with cross-linkable double bonds at the outer shell, and the covalently attached porphyrin template (in red) in the core.24
Molecularly Imprinted Polymers

*CHEM. COMMUN.*, 2003, 171–178

Fig. 6 Schematic representation of a competitive binding assay format based on proximity scintillation.\(^{44}\)

Fig. 8 (a) Screen-printed electrode coated with MIP-microparticles. The particles are covered with a thin agarose membrane. (b) Structure of the electroactive probe 2,5-dihydroxyphenylacetic acid, and differential pulse voltammetric scans obtained in the presence of different concentrations of the analyte 2,4-D.\(^{36}\)