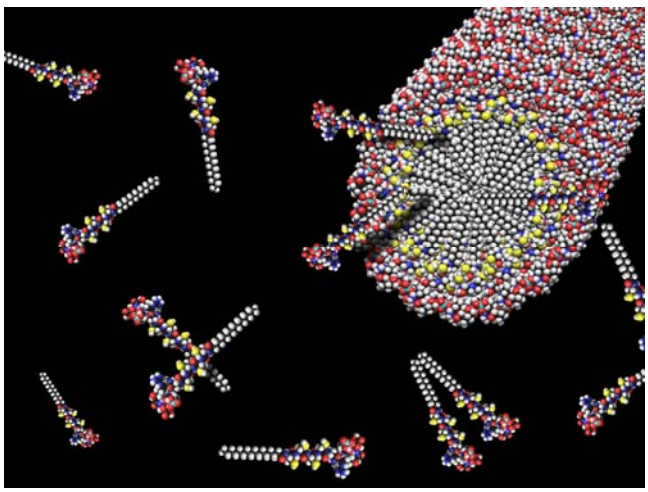
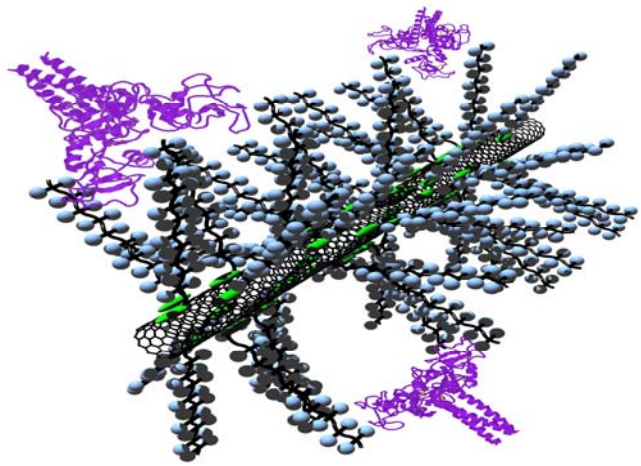
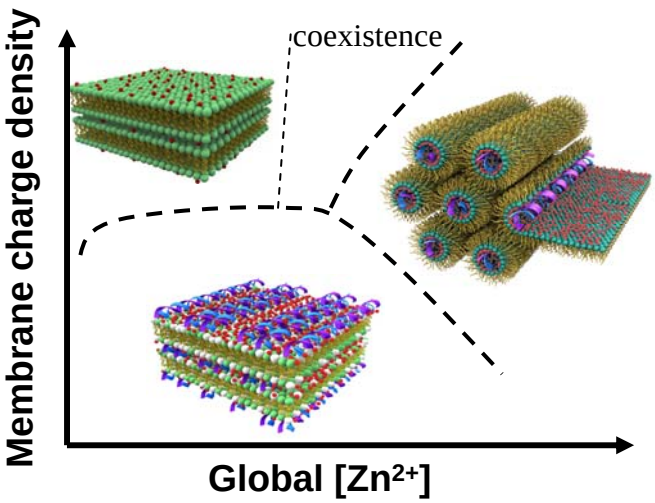
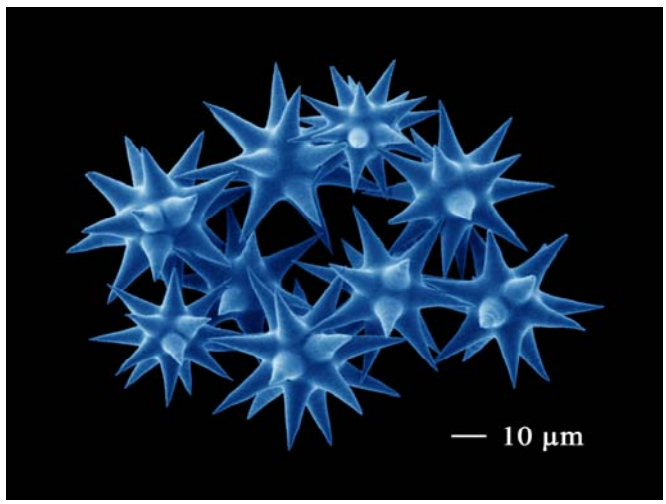
Biomolecular Materials Program Meeting - 2005

August 25 – 28, 2005, Airlie Conference Center, Warrenton, VA









Office of Basic Energy Sciences

Division of Materials Sciences and Engineering

Office of Science
U.S. DEPARTMENT OF ENERGY

Cover

Top Left: Self-assembly of peptide amphiphiles into bioactive nanofibers presenting biological signals at Van der Waals density.
***Courtesy**: Samuel Stupp (Northwestern University)*

Top Right: Carbohydrate-functionalized polymers designed to mimic the structures of mucin glycoproteins assembled on carbon nanotubes.
***Courtesy**: Carolyn Bertozzi and Alex Zettl (Lawrence Berkeley National Laboratory)*

Bottom Left: Hierarchy of ion-mediated interactions expressed in self-assembled structures of DNA-anionic membrane complexes.
***Courtesy**: Gerard Wong (The Frederick Seitz Materials Research Laboratory, University of Illinois-Urbana Champaign)*

Bottom Right: Proteins occluded within these star-burst structures of silica, made by a sponge, have revealed a novel route to the low-temperature catalytic nanofabrication of silica, silicones and semiconductors for energy applications.
***Courtesy**: Daniel Morse (University of California-Santa Barbara)*

This document describes activities performed under contract number DE-AC05-00OR22750 between the U.S. Department of Energy and Oak Ridge Associated Universities.

Foreword

This volume highlights the scientific content of the 2005 Biomolecular Materials Program Meeting sponsored by the Division of Materials Sciences and Engineering (DMS&E) in the Office of Basic Energy Sciences (BES) of the U. S. Department of Energy (DOE). This meeting is the first in a series of research theme based Contractors' Meetings and will focus on BES-funded research at the intersection of physical sciences and biology. The meeting will feature research that cuts across several DMS&E core research program areas. Biomolecular Materials is a relatively new but growing Core Research Activity (CRA) component in DMS&E. This activity formally came into existence following the recommendations of a workshop sponsored by the Basic Energy Sciences Advisory Committee to explore the potential impact of biology on the physical sciences including, in particular the materials and chemical sciences. The major programmatic emphasis is on exploring the molecules, structures, processes and concepts of the biological world that could be used or mimicked in designing novel materials, processes or devices with potential energy significance in support of the DOE missions.

The purpose of the Biomolecular Materials Program Meeting is to bring together researchers funded by BES in this new and emerging research area, to facilitate the exchange of new results and research highlights, to foster new ideas and collaborations among the participants, and to identify needs of the research community. The meeting will also help DMS&E in assessing the state of the program, charting future directions and identifying programmatic needs. The agenda reflects some of the major research themes covered within the broad, expanding field of biomolecular materials.

Many of the BES Contractors' Meetings are passing the quarter-century mark in longevity and are very highly regarded by their participants. We sincerely hope that the Biomolecular Materials Program Meeting will join the others in keeping with the long-standing BES tradition.

We thank all of the invited speakers and meeting attendees for their active participation in sharing their ideas and research results. The dedicated efforts of the Meeting Chairs, Mark Alper and Dan Morse, in organizing and coordinating the meeting are sincerely appreciated. Thanks also go to Christie Ashton from DMS&E and Brian Herndon and Kellye Sliger from Oak Ridge Institute of Science and Education for their fine work in taking care of logistical aspects of the meeting.

Arvind Kini and Tim Fitzsimmons
Division of Materials Sciences and Engineering
Office of Basic Energy Sciences
Office of Science
U.S. Department of Energy

U. S. Dept of Energy
Office of Basic Energy Sciences
Biomolecular Materials Program Meeting
August 25-28, 2005
Mark Alper (LBNL) and Dan Morse (UC-SB), Meeting Chairs

THURSDAY, AUGUST 25

3:00 – 6:00 pm	Registration
5:00 – 6:00 pm	Reception (No Host)
6:00 – 7:00 pm	***** Dinner *****
7:30 pm	*Introductory Remarks* Harriet Kung, Director, Division of Materials Science and Engineering Arvind Kini Program Manager, Materials Chemistry and Biomolecular Materials
Session I	**Self-assembly I – Complex Structures and Systems** Chair: Mark Alper, LBNL
8:00 – 8:30 pm	Samuel Stupp, Northwestern *The Expanding Field of Biomolecular Materials*
8:30 -- 9:00 pm	Atul Parikh, UC-Davis *Patterning and Structure Formation in Fluid Lipid Membranes*
9:00 – 9:30 pm	Hiroshi Matsui, Hunter College *Bionanotechnology Approach in Material Synthesis and Device Fabrication by Applying Peptide/Protein Assemblies*
9:30 – 10:00 pm	Joanna Aizenberg, Lucent (***Invited Talk***) *Controlling the inorganic crystallization at the organic interface: Lessons from biomineralization*

FRIDAY, AUGUST 26

7:00 – 8:00 am	Breakfast
Session II	**Self-assembly II – Understanding and Controlling Self-assembly** Chair: Dan Morse, UC-SB
8:30 – 9:00 am	George Whitesides, Harvard *Dynamic and Static Self-assembly of Meso-scale Objects*
9:00 – 9:30 am	Paul Alivisatos, LBNL *Programmable assembly of nanocrystals using DNA*
9:30 – 10:00 am	Jennifer Lewis, FS-MRL *Bio-Inspired Assembly of 3-D Micro-Periodic Structures*
10:00 – 10:30 am	***** Break *****
10:30 – 11:00 am	Zhigang Suo, Harvard *Electric field directed motion of molecules on solid surfaces*
11:00 – 11:30 am	Gordon Osbourn, SNL-NM *Programming Dynamic Self-Assembly: Theory and Simulations*
11:30 – 12:00 Noon	Brian Crane, Cornell ***(Invited Talk)*** *Structures and Activities of Protein Complexes that Mediate Bacterial Chemotaxis*
12:00 Noon –1:00 pm	***** Lunch *****
1:00 – 4:00 pm	**Time for Interactions & Discussions**
4:00 – 6:00 pm	***Poster Session I***
6:00 – 7:00 pm	***** Dinner *****
Session III	**Bioinspired/Biomimetic Materials and Assemblies** Chair: Yok Chen, DOE
7:00 – 7:30 pm	Kent Blasie, U-Penn *Design & Structural Characterization of Amphiphilic 4-Helix Bundle Peptides for Novel Electronic and Photonic Biomolecular Materials*

7:30 -- 8:00 pm	Andy Shreve, LANL *Control of Optical Properties of Materials Using Bioinspired Assemblies*
8:00 – 8:30 pm	Zhibin Guan, UC-Irvine *Biomimetic Polymer Design at the Interface of Chemistry and Biology*
8:30 – 9:00 pm	George Bachand, SNL-NM *Active Assembly of Dynamic and Adaptable Materials*
9:00 – 11:00 pm	***Continuation of Poster Session I***

SATURDAY, AUGUST 27

7:00 – 8:00 am	Breakfast
Session IV	**Topic: New Tools and Techniques** Chair: Richard Kelley
8:30 – 9:00 am	Sunil Sinha, UC-SD *Using X-rays and Neutrons to probe structual inhomogeneities in membranes and organic films*
9:00 – 9:30 am	Sow-Hsin Chen, MIT *Inelastic X-Ray Scattering Studies of Phonon Propagation and Damping in Biomolecular Assemblies*
9:30 – 10:00 am	Ben Ocko, BNL *X-ray scattering studies of soft matter and biomolecular materials*
10:00 – 10:30 am	***** Break *****
10:30 – 11:00 am	Steve Granick, FS-MRL *Integrated Platforms for Optical and Force Microscopy*
11:00 – 11:30 am	Jim DeYoreo, LLNL (***Invited Talk***) *The scanned probe microscope as a platform for in situ imaging, measurement, and manipulation of biomolecular systems*
11:30 – 12:00 Noon	Janos Kirz, ALS, LBNL (***Invited Talk***) *Toward Structural Biology using the LCLS*

12:00 Noon –1:00 pm	***** Lunch *****
1:00 – 4:00 pm	**Time for Interactions and Discussions**
4:00 – 6:00 pm	***Poster Session II***
6:00 – 7:00 pm	***** Dinner *****
Session V	**Biomolecular Functional Systems: Bio/Non-bio Integration** Chair: Tim Fitzsimmons, DOE
7:00 – 7:30 pm	Jeff Brinker, SNL-NM *Cell-Directed Assembly of the Bio-Nano Interface*
7:30 – 8:00 pm	Millie Firestone (ANL) and Mike Wasielewski (Northwestern) *Controlling the Photophysics of Protein Arrays in Thermoresponsive Soft Materials*
8:00 – 8:30 pm	Dirk Trauner, LBNL *Tinkering with Nature's Molecular Machines: Development of Light-Activated Ion Channels*
8:30 -- 9:00 pm	Jim Heath, Caltech *The nano/bio interface for in vitro and in vivo diagnostics of health and disease*
9:00 – 11:00 pm	***Continuation of Poster Session II***

SUNDAY, AUGUST 28

7:00 – 8:00 am	Breakfast
Session VI	**Topic: Biotemplated Materials Synthesis** Chair: Arvind Kini, DOE
8:00 – 8:30 am	Dan Morse, UC-SB *Biomolecular Mechanisms Reveal New Routes to Novel Nanostructured Materials for Energy Applications*
8:30 -- 9:00 am	Surya Mallapragada, Ames *Macromolecular templates for self-assembly of bioinspired nanocomposites*

9:00 – 9:30 am	Matt Francis, LBNL *Synthetically Modified Viral Capsids: Building Blocks for Nanoscale Materials*
9:30 -- 10:00 am	Yongsoon Shin, PNNL *Replication of Carbohydrates into Hierarchically-Structured Ceramics*
10:00 -- 10:30 am	***** Break *****
10:30 -- 11:00 am	Dan Feldheim, NCSU *RNA Mediated Synthesis of Catalysts for Hydrogen Production and Oxidation*
11:00 – 11:30 am	George Crabtree, ANL *Basic Research Needs for Solar Energy Utilization*
11:30 – 11:45 am	Closing Remarks Mark Alper and Dan Morse, Meeting Chairs Arvind Kini and Tim Fitzsimmons, Meeting Organizers
11:45 am	***** Lunch and Adjourn ***** (Optional Box Lunches Available)

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Session I:

Self-Assembly I – Complex Structures and Systems

Expanding Frontiers in Biomolecular Materials

Samuel I. Stupp

Department of Materials Science and Engineering, Department of Chemistry, Feinberg School of Medicine

Northwestern University

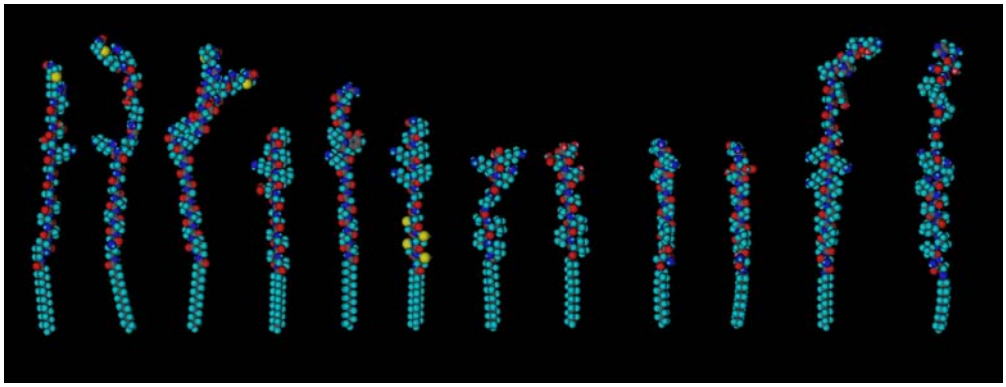
The interface between materials science and biology has its genesis more than half a century ago at the time when known materials were first used to repair human tissues. These biomaterials, many still in use today, are basically inert but can repair the structure of tissues or restore the function of failed organs. The field is now expanding into the exciting realm of bottom up molecular and supramolecular design of materials to interact directly with cells. Such "bioactive" materials could soon become the drivers of tissue and organ regeneration in humans. The new opportunities are emerging at the convergence of nanoscience and biology, and are opening other frontiers that will have broad impact in science. The use of designed materials to learn biology, the design of materials that imitate biology, or the use of biology to make abiotic materials are all part of this exciting new field.

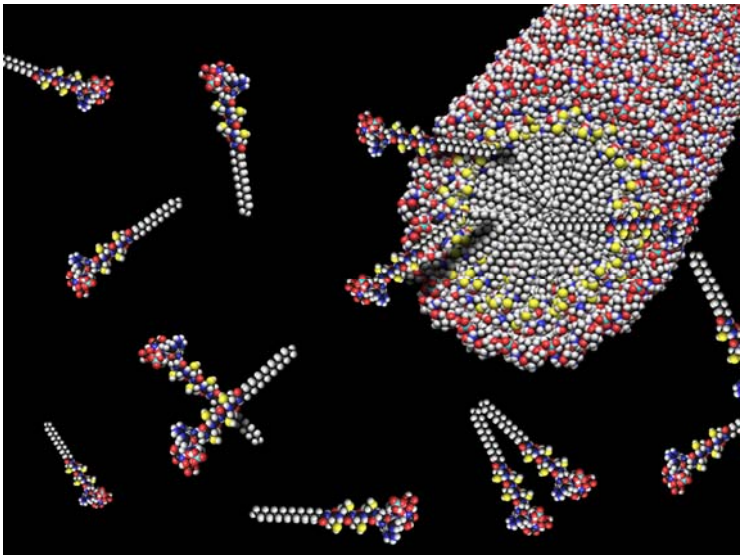
Bioactive Materials

The molecular design of materials to interact with cells needs to be guided by biomimetics of extracellular matrices. Cells in mammalian biology live surrounded by solid or fluid matrices from which they receive signals and mechanical support. Signalling to cells by the extracellular matrix is a marvel of engineering since the matrix is a scaffold that binds and releases signals with enormous spatial and temporal sophistication. The actual signals are either small molecules or macromolecules such as proteins and polysaccharides. Molecular dynamics and molecular recognition events as the signals approach the cell surface must be tightly controlled in the matrix environment for efficacy at regulating biological events. For example, a given protein signal (ligand) may bind to a signal protein on a cell surface (receptor), or to a dimer or higher complex of proteins resulting in completely different biological outcomes (1). An artificial matrix may have multiple functions, including the regulation of cell proliferation, the prevention of cell death (apoptosis), as well as the control of stem cell differentiation to get the desired functions and synthesis of natural matrix. By far the most common theme in these materials has been the incorporation of the tripeptide, arginine-glycine-aspartic acid (RGD) (2). This tripeptide is present in the extracellular protein known as fibronectin and it is the epitope that binds to dimers of the transmembrane proteins known as integrins. These ligand-receptor binding events trigger biological adhesion of cells to their matrix, an extraordinarily complex phenomenon involving many proteins within the cell and the cell’s cytoskeleton. Biological adhesion is key to the cell’s survival and to signal transduction events that can result in cell proliferation an d differentiation.

In order to create artificial bioactive materials, we designed molecules known as peptide amphiphiles to self-assemble into cylindrical nanofibers (3). These molecules consist of a peptide segment which is their hydrophilic block covalently bound to a very hydrophobic

segment which can be a simple alkyl tail found in ordinary lipid molecules or a more complex aromatic–aliphatic segment or even a steroid structure such as cholesterol. The peptide segment can take various covalent architectures such as that of branched structures (4). The figures below show a collection of molecules synthesized in our laboratory using both solid phase synthesis or solution phase chemistry, and also a molecular graphics representation of self-assembly of these peptide amphiphile molecules into nanofibers. We are able to construct peptides by solid phase techniques either from C- to N- or N- to C-terminus (5).





The supramolecular design of these materials is based on their strongly amphiphilic structure. This offers the possibility of self-assembly in water since their hydrophobic parts will want to avoid contact with an aqueous interface. Our peptide amphiphiles were specifically designed to form nanoscale “cylinders” not tubes or twisted fibrils, and this is made possible by their strongly amphiphilic nature and by the specific amino acids sequences used in the peptide segment. We believe molecular shape in peptide amphiphiles is not the main driving force for cylinder formation. Our view is that cylinder formation is driven by the tendency of fractions of our peptide sequences to form β sheets which terminate in one molecule-thick dense brushes of very hydrophobic segments. Peptide sequences such as A_4G_3 and SLSL are examples of segments in PA molecules close to the hydrophobic segment that can drive β sheet formation. The supramolecular sheets should have a strong tendency to hide their hydrophobic side from water and collapse to form solid cylinders. The nature of the cylindrical assembly in our

systems allows us to present high densities of biological signals perpendicular to the long axis of the nanofiber, an external presentation which is favorable for cell signaling. If peptides were assembling into twisted sheets or tubes as they do in other systems, ideal spatial orientation of bioactive signals would not be possible. The lecture will illustrate the use of these nanofibers to mediate biological cell adhesion, and also an unprecendented rapid and selective differentiation of neural stem cells into neurons (6).

An additional design element in our PAs is the presence of net charge in the peptide sequence. Net charge keeps molecules dissolved in water and suppresses their self-assembly as a result of coulombic repulsion. Neutralization of charge or Debye-Hückel screening of charge triggers nanofiber formation since the entropic penalty of bringing millions of molecules together to make the slender objects is well compensated by the large numbers of hydrogen bonds formed among peptide segments and the hydrophobic collapse of the sheets. Nanofibers can therefore grow by changing pH or simply raising the concentration of screening electrolytes in the water. As the fibers grow they bundle and form a network in the aqueous medium leading to gelation. Gelation through network formation among nanostructures becomes an obvious mechanism to immobilize an artificial bioactive matrix to signal cells.

Biomimetic Materials

Another frontier in biomolecular materials attempts to imitate biological structures in synthetic systems, particularly hierarchical ones, with the intent of understanding biology and exploring the possibility of materials with novel functions. A good example in the context of energy would be biomimetic structures that imitate the photosynthetic machinery. Among biological hierarchical structures, helical architectures are of particular interest because of their many functions in living organisms. Built from small chiral molecules such as amino acids or nucleotides, helices in biology include varieties of structures with different levels of complexity, such as α-helix peptides, DNA double helices, collagen filaments , microtubules , flagella and some helical viruses or bacteria. Our laboratory has recently developed a scheme to create helical supramolecular assemblies in which we can control pitch through the covalent structure of self-assembling monomers (7).

Our strategy for pitch control has been inspired by the supercoiling observed in the DNA double helix which responds to a torsional strain around its axis (13,14), by allowing its axis to relax into a secondary coil while maintaining the proper number of base pairs. This secondary coil is called a supercoil, in distinction from the helix defined by the sugar-phosphate backbones. In living cells, topoisomerases relax or introduce supercoiling to facilitate opening of DNA helices during replication and transcription processes (14), resulting in supercoils with variable pitch. This supercoiling process can be visualized as twisting a telephone cord (figure 1A). A secondary coil (or a superhelix) forms with the same handedness when overwound and a coil with opposite handedness when underwound. The pitch of the superhelix depends on the magnitude of the torque applied.

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(1) R. Derynck and Y.E. Zhang, Nature **425,** (2003), p. 577

(2) M.D. Pierschbacher and E. Ruoshlahti, Nature **309** (1984), p. 4385

(3) J.D. Hartgerink, E. Beniash, and S.I. Stupp, Science **294** (2001), p. 1684

(4) M. O. Guler, S. Soukasene, J.F. Hulvat, and S.I. Stupp, Nanoletters, **5** (2005), p. 249

(5) H.A. Behanna, J. Donners, A.C. Gordon, and S.I. Stupp, J. Amer. Chem. Soc. **127** (2005), p. 1193

(6) G.A. Silva, C. Czeisler, K.L. Niece, E. Beniash, D.A. Harrington, J.A. Kessler, and S.I. Stupp, Science **303** (2004) p. 1352

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Dynamic Self-Assembly: Functional Reorganizations in Biomembranes

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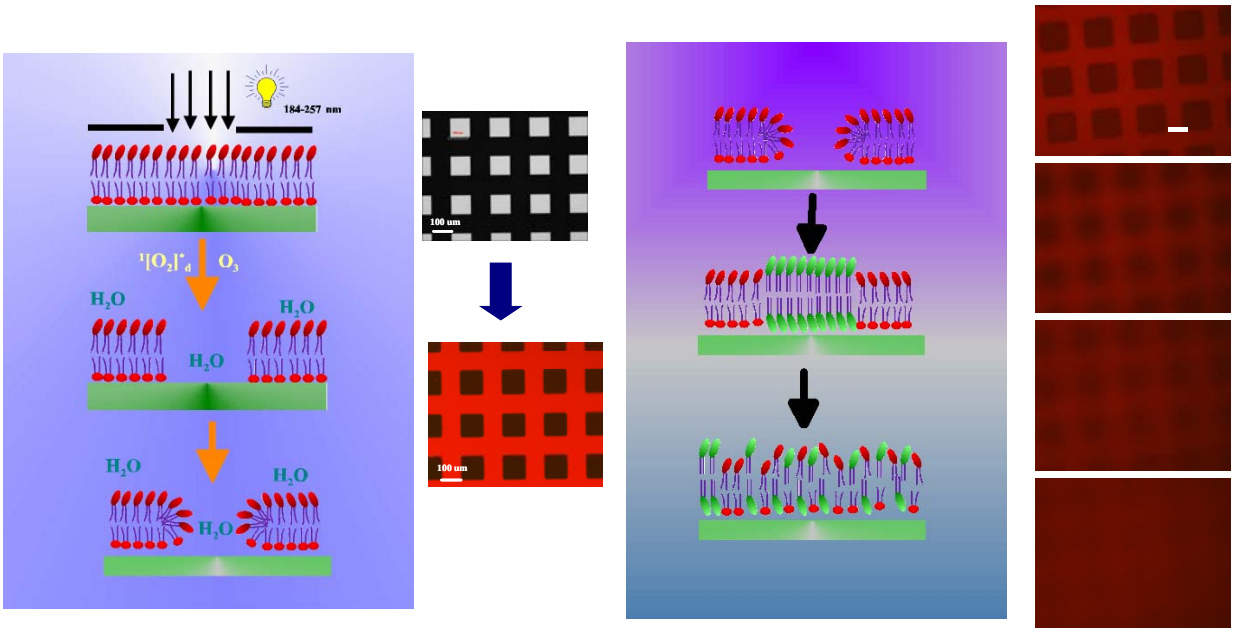
Program Scope This program seeks to explore dynamical self-assembly processes within the quasi-two dimensional, fluid phospholipid membrane media. Of particular interest to this program are biologically important reactive-diffusive processes including (1) lipid-lipid mixing and phase separation such as occurs in lipid rafts; (2) dynamic reorganizations of phase separated lipid mixtures following a biologically relevant chemistry (e.g., selective lipid oxidation); (3) membrane receptor-clustering and pattern formation following a protein recognition or a binding event; and (4) protein-protein clustering mediated by the inherent membrane dynamics. Our strategy involves the design of useful fluid biomembrane based platforms suitable for such studies and their implementation using biomimetic systems to generate fundamental design rules that can be used to synthesize novel materials based on biologically inspired principles of dynamic self-assembly.

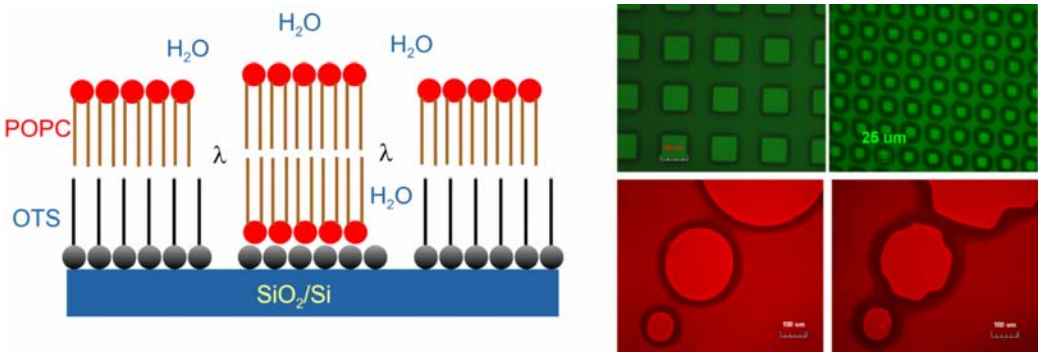
Recent Progress. In the 1st year of the grant, we have made some notable progress in the areas of platform developments. Specifically, we have developed new material constructs to display well-defined phospholipid patterns in bilayer geometries that share diffusive boundaries for study of in-plane lateral dynamics. To examine out-of-plane dynamics in membrane media, we have developed a novel membrane platform which displays lipid monolayers and bilayers on single samples. Applications of these constructs provide an understanding of how biological membranes handle their heterogeneity and dynamics to facilitate temporal emergence of membrane functions. Our key accomplishments are highlighted below.

1. Membrane Photolithography is a new technique to optically perforate single phospholipid bilayers to create lipid-free patches in otherwise contiguous membranes (Pub 1). Refunctionalization of optoporated membranes using secondary vesicle fusion provides a means to juxtapose chemically and structurally distinct lipidic phases which undergo diffusive dynamics to approach equilibrium. Applications of these construct provides a simple means to examine how domain structures and phase separation emerge in membrane media (pub2). When fluidity disparity between the juxtaposed phases is large, the structure kinetically traps. Using the kinetic arrest methods, we have developed a means to display patterns of lipid rafts (manuscript in preparation).

2. The applicability of the membrane photolithography approach in non-planar membrane configuration was established using colloidal patterning demonstrated in Pub 3. Here refunctionalization of membrane voids on colloidal substrates using proteins resulted in novel lipid-protein configurations. The use of these construct to examine membrane-membrane interaction dynamics is in progress.



3. Using patterned hydrophilicity surfaces, we have shown that vesicle fusion responds to local changes in the substrate energies: low energy, hydrophobic surfaces support vesicle fusion dynamics to give rise to lipid monolayers and high energy hydrophilic surfaces support membrane spreading to produce lipid bilayers. The use of patterned energy surfaces template these fusion dynamics in spatially defined manners to display lipid monolayers and bilayers on single substrates (Pub 4).



4. Applications of the construct developed above (pub 4) toward membrane dynamical reorganizations provide a novel means to examine how in-plane lateral self-assemblies are impacted by the coupling of out-of-plane dynamics. The work is illustrated using probe partitioning and raft formation (pub 5).

Future Plans.

In the second year of the grant, we will focus on how dynamics and heterogeneity couple within the bilayer media. Specifically, we expect to address the following five

outstanding challenges to advance our understandings of kinetic effects in dynamics and reorganizations within membrane media.

1. Engineering Phase separation Within Supported Phospholipid Membranes using System Size confinement and Substrate Topographies.
2. Dynamics of Raft Formation within Membrane Media
3. Engineering Diffusive Paths within reactive-diffusive bilayers to mimick cytoskeleton-induced membrane compartmentalization
4. Effects of Interlayer Coupling on Phase Separation dynamics and Protein Pattern formation in Membranes.
5. Explore the use of membranes derived directly from living cells so that proteins in their native environments (and hence presumably intact functionalities) can be incorporated into fluid biomimetic material structures.

A successful completion of these studies will provide design rules for dynamic reorganizations within the membrane media which will then be used to design active and dynamic biomimetic materials for energy applications in subsequent years.

Publications Resulting From The Grant (2004-2005)

1.1. Peer-Reviewed Papers.

1. C. K. Yee, M. L. Amweg, A. N. Parikh, Membrane Photolithography: Patterning Fluid, Supported Lipid Bilayer Membranes in the Aqueous Phase, **Advanced Materials**, 16, 1184-1189, **2004**
2. C. K. Yee, M. L. Amweg, A. N. Parikh, Direct Photochemical Patterning and Refunctionalization of Supported Phospholipid Bilayers, **J. Amer. Chem. Soc**. 126, 13962-72, **2004**
3. Yu, C.-H.**,** Parikh, A. N**.,** Groves, J. T**.** Direct Patterning of Membrane-Derivatized Colloids Using *in situ* UV-Ozone Photolithography, **Advanced Materials**, 17, 1477-1480, **2005**.
4. M. C. Howland, A. R. S. Butti, S. S. Dixit, A. M. Dattelbaum, A. P. Shreve, A. N. Parikh, Phospholipid Morphologies on photochemically patterned bilayers, **J. Amer. Chem. Soc**., 127, 6752-6765, **2005**.
5. Templated Phospholipid Morphologies for Discriminating Leaflet-Dependent Properties in Model Biomembranes, A. R. S. Butti, M. C. Howland, A. N. Parikh, Submitted (2005).
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Recent Published Abstracts.

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3. Smith AM, Huser T, Parikh AN**,** Multiphoton membrane photolithography Biophys. J. 88 (1): 232A-232A Part 2 Suppl. S JAN 2005

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Invited Presentations

1. **Conferences**, MRS Annual Meeting, San Francisco (2 talks), March. 2005; ACS National Meeting, Washington, D.C. , Aug. 2005; Pacifichem, Hawaii, 2005; ACS Mid-West Regional Meeting, Indianapolis, IN, 2004; **Others.** Biosecurity and Nanoscience Lab, Lawrence Livermore National Laboratory, 2004, Dynamic Energy Landscapes in Functional Systems Workshop, Santa Fe, NM, 2004, Next-Generation Neutron Source Workshop, San Diego, CA 2004, Membranes and Neutron Spin-Echo, Bloomington, IN, 2005.

Biological Bottom-up Assembly of Multi-Functional Protein Nanotubes and Their Applications in Room-Temperature Material Syntheses and Device Fabrications

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Program Scope

The future growth of the electronics industry requires development of faster integrated circuits with reduced production costs and lower power consumption. More efficient circuits can be achieved by increasing the packing density of nanometer-sized circuit elements. This "nano-approach" also leads to manufacture light-weighted electronics, which is especially important for development of portable devices. While the top-down approach such as lithography has systematically reduced the circuit dimension, the reduction of production cost is necessary for the mass production. In order to overcome this problem, various building blocks for nano-scale electronics have been developed from the bottom-up approach, whereas these building blocks are necessary to be addressed to the exact positions with high precision and reproducibility for the array construction of nanometer-scale integrated circuits. The bottom-up nano-fabrication can also be performed much more efficiently and economically compared to the exiting technology if the syntheses of electric materials and their assemblies can be conducted in milder experimental conditions, such as room temperature, ambient pressure, smaller lab space, and less manpower.

We propose the bionanotechnology approach for affordable and efficient fabrications of nanometer-scale electronics using biological functions. Our approach also has potential to produce material structures that are not observed in man-made materials in milder experimental conditions, which will be beneficial for future commercialization of those products. Previously, we developed the technique to create simple circuit geometries by anchoring bionanotubes, whose ends were functionalized by "linking proteins", onto well-defined positions on the complementary receptor-patterned substrates.[1-4] We also developed a novel fabrication method, to incorporate "mineralizing peptides" on the sidewalls of bionanotubes and grow nanocrystals on the bionanotubes in order to control their electric properties from semiconductor to metal.[5-10] In this proposed research, we combine these two technologies to fabricate multi-functional bionanotubes that contain both the "linking proteins" and the "mineralizing peptides" in order to build the bionanotube-based electric circuits.

The outcome of proposed researches for the part in room-temperature material syntheses itself will also have broad impacts in basic sciences and applied engineering because this developing technology has potential to create novel nanomaterials possessing physical, structural, and catalytic properties with no synthetic counterparts.

Recent Progress

• *Immobilization of sequenced "mineralizing peptides" on the sidewalls of bionanotubes and nanocrystal growth on the bionanotubes in controlled size, packing density, and shape*

To apply bionanotubes as building blocks in electronic devices, the bionanotubes are necessary to increase their conductivity. We reported a new biological approach to develop uniform and isotropic nanocrystal coatings on the sidewalls of bionanotubes by immobilizing sequenced "mineralizing peptides" on the bionanotubes.[6] The template nanotube was self-assembled from small bolaamphiphile peptide monomers in NaOH/citric acid solution via three-dimensional intermolecular hydrogen bonds among the monomers.[11] This template nanotube can immobilize the "mineralizing peptides" at free amide sites on the sidewall via hydrogen bonding.[5] After those nanotubes were centrifuged and run through size-separation columns to isolate the template nanotubes in the desired sizes,[12] these nanotubes were incubated with the "mineralizing peptides" in a pH7 buffer solution for the peptide immobilization. After the reduction of ions, highly crystalline nanocrystals were uniformly coated on the bionanotubes with the high-density coverage.

In addition, we discovered that the conformations and the charge distributions of the "mineralizing peptides", determined by pH and ion concentrations of the growth solutions, control the size, the packing density, and the shape of nanocrystals on the bionanotubes. For example, when a peptide, Ala-His-His-Ala-His-His-Ala-Ala-Asp (HRE), was immobilized on the bionanotubes and grew Au nanocrystals on the sidewalls, the packing density of Au nanocrystals in the average diameter of 8 nm was controllable as a function of pH.[5] The growth solutions with higher pHs increased the packing density due to the increase of the number of nucleation sites on the peptides.

When Cu nanocrystals were mineralized on the bionanotubes incorporating a peptide, His-Gly-Gly-Gly-His-Gly-His-Gly-Gly-Gly-His-Gly (HG12), the diameters of Cu nanocrystals were controlled between 10 and 30 nm on the bionanotubes by controlling the conformation of HG12 via pH change.[7] As the pH of the growth solution increased, folded HG12 peptides were aggregated on the bionanotube, which increased the space to grow Cu nanocrystals and induced larger Cu nanocrystal growth. The size change of Cu nanocrystals on the bionanotubes induced significant change in electronic structures, and this observation indicates that the band gaps of bionanotubes will be tunable by controlling the size, packing density, and shape of nanocrystals on the sidewalls. The HG12 peptide could also control the size of semiconducting Cu_2S nanocrystals on the bionanotubes.

The shape control of Ag nanocrystals on the bionanotubes was demonstrated using the AG4 peptide, Asn-Pro-Ser-Ser-Leu-Phe-Arg-Tyr-Leu-Pro-Ser-Asp, as the "mineralizing peptide".[8] Previously, this peptide sequence was found to recognize and affect the Ag nanocrystal growth kinetics on the (111) face via the combinatorial phage display peptide library.[13] When the AG4 peptide was sequenced and incorporated onto the bionanotube surfaces, the biomineralization of Ag ions on the bionanotubes led the isotropic hexagon-shaped Ag nanocrystal coating under pH control because the shape of the (111) face of Ag nanocrystal is hexagonal. The plasmon band of hexagon-shaped Ag nanocrystals on the bionanotubes red-shifted as the size of nanocrystals increased, and those spectra indicate the distinctive electronic structures of hexagon-shaped Ag nanocrystals compared to spherical Ag nanocrystals in the similar size domains, which will add another dimension to control the band gap and the conductivity of bionanotubes.

• *Simultaneous Targeted Immobilization of Anti-Human IgG-Coated Nanotubes and Anti-Mouse IgG-Coated Nanotubes on the Complementary Antigen-Patterned Surfaces via Biological Molecular Recognition*

Introduction of self-assembly in nanometer-sized building blocks is expected to accomplish bottom-up fabrications of electronics in a more reproducible, efficient, and economic manner; however, it is necessary to selectively place multiple types of nano-building blocks (e.g., metal nanotubes and semiconductor nanotubes) at specific locations on surfaces with high precision and reproducibility for more complex nanometer-scale device assemblies, as proposed in our proposal. Biological molecular recognition such as antibody-antigen bindings is suitable to use in the building-block assembly since nature always assembles materials with complex functions and structures at room temperature reproducibly.[1-3] Our proposed approach is to immobilize antibody-coated nanotubes at specific complementary binding positions patterned on surfaces. To demonstrate this hypothesis, two types of nanotubes coated with different antibodies (anti-mouse IgG and anti-human IgG) were anchored selectively onto their complementary antigen areas, patterned by tips of atomic force microscope (AFM).[4] Because those nanotubes can be coated by various metals and semiconductors with controlled morphologies, this outcome opens the possibility to accomplish the proposed unconventional device fabrication methodology that antibody nanotubes coated with different types of metals/semiconductors can be self-assembled on antigen-patterned surfaces via biological molecular recognitions.

Future Plans

• *Improvements in accuracy and yield of the nanotube-placement in electric circuits:*

Under corporation with a theory, we started finding that the accuracy and the yield of the antibody nanotube placement can be improved by diluting the concentration of antigens on surfaces. We will establish this understanding and control to accomplish complex circuit fabrications using protein nanotubes with the higher accuracy and the higher yield.

• *Conductivity measurements of functionalized protein nanotubes:*

We will establish conductivity studies of various functionalized nanotubes as proposed in our grant document in two ways; first, we will use a conductive AFM to determine the conductivities of nanotubes on Au substrates. Then, the conduct ivies will be determined more precisely at the NIST by bridging those nanotubes between two electrodes, patterned by photolithography.

• *Room temperature-material syntheses using the peptide nano-doughnuts as templates:*

We will synthesize important electronic components such as GaN, Cu_2S, ZnS, and TiO_2 at room temperature, which are normally synthesized at high temperatures with current technologies. We will apply the peptide nanotubes and nano-doughnuts as templates to fabricate the improved circuit components.

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Session II:

Self-Assembly II – Understanding and Controlling Self-Assembly

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DYNAMIC SELF-ASSEMBLY, EMERGENCE, AND COMPLEXITY

Complexity, Emergence, and Dynamic Self-Assembly. "Complexity"—in at least one definition—is the behavior of "components" that interact with one another, normally non-linearly. The components can be almost anything—packets of air (in weather), stock traders (in the stock market), drivers (in traffic), or parcels of electrical energy (in power grids). The prototypical complex systems are those in biology (for example, the cell, in which the components are the molecules that make it up; the organism, in which the components are cells; ecosystems, in which the components are organisms). Understanding the behavior of complex systems is both important and interesting. The broad, overarching question concerning them is whether there is a unifying "science" of complexity, or whether each complex system is complex in its own, distinct way. The narrower, more focused, issues in complexity dealing with the behaviors of individual systems.

Biological systems are of particular interest for their relevance to health and global stewardship. They are also particularly complicated. A new field—systems biology—promises to "explain" the cell, and life, in reductionist terms. This goal is an admirable and difficult one.

We are interested in several aspects of this field: i) **Complexity** itself is, as I have indicated, the study of the consequences of interactions between components, expressed in a very general sense. ii) **Emergence** is the name given to the unexpected phenomena that appear in complex systems. There is an argument in the community interested in this area as to whether emergent phenomena are (sometimes) genuinely new in some sense, or whether they are simply familiar chemistry and physics packaged in an unfamiliar way. This question is, in some senses, a semantic one, and although it is interesting, it is not one that we spend time in trying to answer. iii) Complex and emergent systems are often **dissipative** and **out-of-equilibrium**. Understanding these sorts of systems is one of the broad frontiers of modern science, since these systems are represented in almost every circumstance of real interest in technology. Recognizing interesting, diagnostic, and unexpected behaviors in complex systems is often most practically done by recognizing the appearance of patterns. This area—pattern formation in dissipative systems—we call **dynamic self-assembly**. Considering this type of pattern formation as a form of self-assembly enables us to make very profitable connections to equilibrium self-assembly—an area that has been one in which there has been both great focus and great progress in the last two decades.

We have approached the problem of complexity in a fashion that might seem, at first, to be internally inconsistent. Our program is based on developing systems of components

that are as *simple* as possible, and then studying their behavior in the belief that they will form a *complex* system. Our strategy is to use components are as simple as we can make them, in which (unlike biological systems, at present) we understand very well how the components interact, and in which we can limit the number of components, and the types of components, to small numbers. We believe that by studying these very "simple" systems, we can begin to define how many components we can hope to understand in a complex system, and to what level we can rationalize and simulate the behavior of our model complex systems based on fundamental principles. We find that we lose our ability to rationalize the behavior of these systems at alarmingly low levels of complexity and small numbers of components. We infer from the simplicity of the systems that begin to show unexpected behavior that the scientific community simply does not now have the mathematical/analytical/conceptual tools to understand multicomponent, complex systems involving many components and very non-linear interactions.

This state of affairs is, of course, both a challenge and an opportunity: the field of "complexity" in both biological and non-biological contexts is still there waiting to be created. While the development of the ideas that will eventually form the spine of this field is in progress, there are, fortunately, other reasons with shorter-term objectives that make the study of complexity worthwhile. i) The activity of designing and studying these systems provides—in every case that we have examined—a route leading to the discovery of new phenomena in materials- and condensed-matter science; ii) considering biology as a specific type of complex system stimulates the transfer of ideas from biological systems into non-biological (biomimetic) systems; iii) the new phenomena that are emerging unexpectedly from these studies offer opportunities to develop new kinds of devices and sensors.

We have examined four major types of systems in this program. We have described the first two—pattern formation in mm-scale magnetic disks spinning while suspended at a fluid-fluid interface—and crystallization of electrostatically charged polymer beads in published papers in several papers (leading references are appended below); we will only sketch results coming from them here. The last two—formation of bubbles (vapor in liquid) or drops (liquid in liquid) and the behavior of foams formed from them, and the behavior of microorganisms (in our work, *E. coli*, and *Chlamydomonas reinhardii*)—are under active exploration now.

Systems.

1. **Spinning Disks.** These systems of spinning, magnetic disks interact through two forces: i) a central, attractive force between the disks and an external magnetic field; this force tends to decrease the spacing between the disks; ii) a repulsive force reflecting the interaction of fluid vortices generated by the disks as they spin. The disks form a number of remarkable patterns; these patterns can be rationalized for small numbers (in some instances up to 10-15) of beads. This system provides one excellent model system with which to study dynamic self-assembly.

2. **Electrostatically Charged Beads.** In these systems, tribocharging leads to the formation of Coulombic crystals, including crystals with net charge.

3. **Bubbles and Drops.** Bubbles are proving an astonishingly rich field in terms of the diversity of the structures that can be generated, and in the complexity of the behaviors that even very simple systems demonstrate. This work has focused on understanding the fundamental fluid physics of generation of bubbles and drops in new types of bubble- and foam-generators, and in understanding the interactions between bubbles in dynamic, flowing foams.

4. **Biological Systems.** The work in biological systems had concentrated on examining the behaviors of motile organisms (*E. coli* in a motile mutant; "*Chlamy*" as an example of an intrinsically motile, phototropic organism; here an alga). Our work has focused on understanding some of the behaviors of individual organisms, as a preliminary to building more complex, multi-organism systems.

Other Studies in Biomimetic Systems. Part of this work has been extended, in work stimulated by DoE but supported in substantial part by NSF to functional, biomimetic self-assembly—especially of relatively primitive microelectronic circuits.

Inferences. This program is the beginning of our efforts in a very large and complicated area. We have confirmed that: i) it is practical to design and assembly "simple" complex systems, that the dynamic behavior of these systems—including dynamic self-assembly—yields a rich variety of new behaviors, and that these behaviors can—to a substantial extent—be modeled and rationalized based on fundamental principles ; ii) these systems begin to show emergent (defined here as unexpected and new) behaviors with astonishingly small numbers of components defining the system; iii) these behaviors have properties that point to interesting potentials for applications; iv) the most complex of the systems—microorganisms—already show remarkable and potentially useful behavior at the level of single organisms, when explored from the vantage of research intended to define their characteristics as components of complex systems; v) consideration of the behavior of biological systems through the filter of self-assembly suggests a number of biomimetic strategies for fabricating functional systems.

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DNA-Templated Nanoparticle Assembly:
Novel Physical Properties and Structural Motifs.

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Program Scope

Although many synthetic routes to nanocrystals of controlled size and morphology are known, directed assembly of such building blocks into functional materials remains a challenge. Biomolecules offer unique advantages for nanoscale assembly, and DNA in particular is a versatile scaffold due to its ease of synthesis and programmability. This goal of this program is to develop new materials that can be accessed through DNA-templated self-assembly of inorganic nanoparticles, and to explore the physical properties of these assemblies, both in solution and on surfaces. In particular, our methodology ensures formation of discrete, well-characterized assemblies that may eventually provide building blocks for self-assembled nanoscale plasmonic or electronic device applications.

Recent Progress

Recent efforts have focused on two primary targets: directed assembly of and enzymatic manipulation of discrete gold nanocrystal (AuNC) and quantum dot groupings, and characterization of the unique physical properties of discrete nanoparticle assemblies in solution and on surfaces.

Semiconductor quantum dots (QD) have distinctive physical properties which make them useful in a broad range of applications[1] from biological labels to solar cells.[2] These properties are strongly influenced by the immediate environment of the QD. In particular, the interactions between QDs with nearby metal nanoparticles appear to affect blinking and other photophysical properties which are key to many QD applications.[3] However, the dynamics of this interaction are not well understood. In our studies, DNA has been used to create assembles of nanoparticles comprising a single CdSe/ZnS core-shell QD and precise numbers of AuNC (from 1 to 7) distributed radially around the QD (Figure 1).[4] Investigations of the photoluminescent behavior of these assemblies are now being conducted.

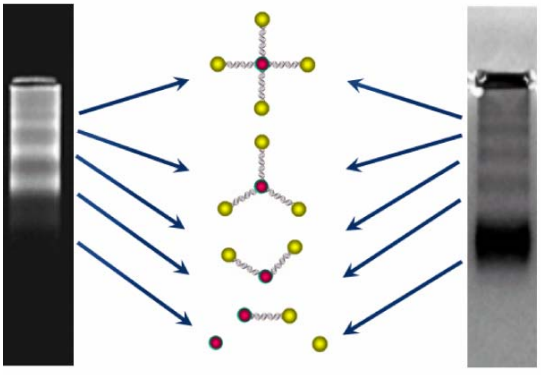

Figure 1. Gel electrophoresis migration pattern of QD/Au nanostructures. The same gel is illuminated under UV (to see QDs by fluorescence, left panel), and under white light (to see Au absorption, right panel). In each structure, a single QD (red) is surrounded by one or more AuNC (yellow) attached by DNA.

Nanostructure assembly on branched DNA scaffolds is an attractive route to fabrication of junctions for plasmonic devices in which functionally distinct inputs and outputs are required. Here, multiple branched molecules have been used within a single structure, providing a polybranched scaffold, as shown in Figure 2.[5] For plasmonic applications, interparticle distance is a key factor in determining the strength of plasmon coupling.[6] Three architectures have been explored for the hybridization of conjugate to scaffold, allowing modulation of interparticle distance in the resulting assemblies. Additionally, one of the architectures incorporates a hairpin loop in the DNA conjugate sequence, which in the future may allow assembly morphology to be controlled based on temperature, by hybridizing and dehybridizing the hairpin.

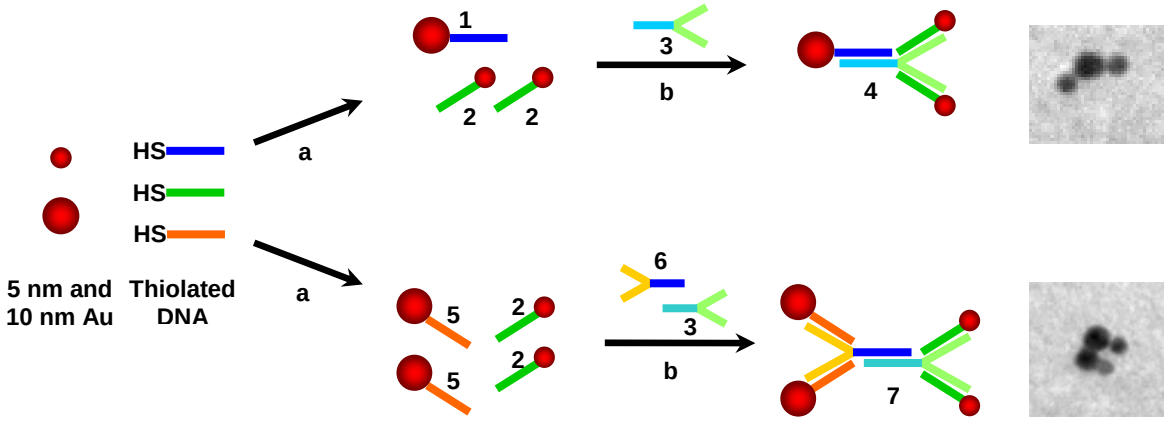

Figure 2. Nanoparticle assemblies using branched DNA scaffolds. **a**) Conjugation and purification of single conjugates **1**, **2**, and **5**. **b**) Hybridization with branched scaffolds **3** and **6** to produce asymmetric trimer **4** and tetramer **7**. Transmission electron micrographs of asymmetric trimer and tetramer are shown to the far right.

Enzymatic manipulation of conjugates offers the possibility of amplifying complex nanomaterials, utilizing a nanoparticle assembly to direct the formation of further assemblies. Initial enzymatic studies have focused on ligation of two DNA-Au monoconjugates to create a covalently bound dimer of AuNCs on a single strand of DNA. Such a procedure must address several concerns, including a robust passivation of the nanoparticle surface to make the particles compatible with the buffer conditions required for enzyme activity. Characterization by gel electrophoresis and TEM indicates that ligation has been carried out successfully, and current experiments aim to use sequential ligation and melting steps to achieve amplification of the dimer structures, in a process similar to the polymerase chain reaction (PCR) used for DNA fingerprinting.

Plasmon coupling between pairs of metal nanoparticles has long been known to exist;[7] here, it has been developed as a molecular ruler for monitoring biomolecular conformational changes.[8] This technique offers several advantages over Förster resonance energy transfer (FRET), the standard for such applications: detection over longer distances (tens of nanometers versus a few nanometers for FRET), detection through long timeperiods without signal loss due to photobleaching (hours or days versus minutes for FRET), and the possibility of concurrently visualizing the nanoparticle probes using electron microscopy. In our experiments, a strong spectral shift was observed due to plasmon coupling in particle dimers bound together by a single strand of DNA in comparison to the individual particles (Figure 3). Further, changes in

environmental conditions such as ionic strength resulted in small, reproducible shifts in the dimer plasmon corresponding to a change in interparticle distance due to ionic shielding effects. This technique also allowed observation of single DNA hybridization events which caused changes in interparticle distance of only a few nanometers.

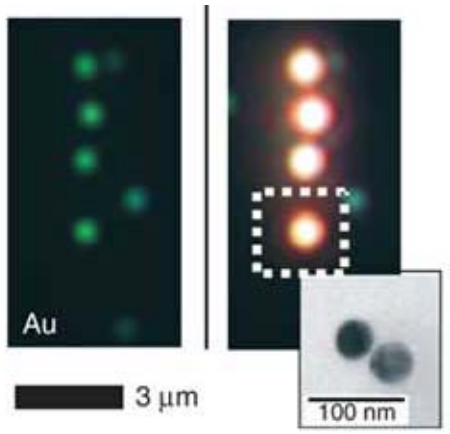

Figure 3. Plasmon coupling between pairs of 40-nm Au nanoparticles results in a pronounced spectral shift. The left panel shows the plasmons of single particles bound to a surface. The right panel demonstrates the change in plasmon intensity and wavelength when a single strand of difunctionalized DNA causes binding of a second nanoparticle to some of the surface-bound particles, creating dimers as shown in the inset.

Future Plans

Building on the library of DNA-based nanostructures developed to date, we plan to expand both the range of physical characterization techniques and the nanostructure complexity available through DNA-templated self-assembly. Techniques such as dark-field microscopy, small-angle X-ray scattering, and dynamic light scattering allow the solution-phase characteristics of nanoparticle assemblies to be monitored non-invasively and in real time. Studies of nanoparticle assemblies deposited on surfaces will also be continued. Complex assembly motifs such as branched structures may be combined with longer linear arrays to form extended structures suitable for device studies.

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DOE Sponsored Publications in 2003-2005

A Molecular Ruler Based on Plasmon Coupling of Single Gold and Silver Nanoparticles, C. Sönnichsen, B. M. Reinhard, J. Liphardt, and A. P. Alivisatos, *Nature Biotech.* **2005**, *23*, 741-745.

Directed Assembly of Discrete Gold Nanoparticle Groupings Using Branched DNA Scaffolds, S.A. Claridge, S.L. Goh, J.M.J. Fréchet, S.C. Williams, C.M. Micheel, and A.P. Alivisatos, *Chem. Mater.*, **2005**, *17*, 1628-1635.

Discrete Nanostructures of Quantum Dots/Au with DNA, A. Fu, C.M. Micheel, J. Cha, H. Chang, H. Yang, and A.P. Alivisatos, *J. Am. Chem. Soc.*, **2004**, *126*, 10832-10833.

Electric field directed motion of molecules on solid surfaces

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Adsorbed on a solid surface, molecules are electric dipoles (**Fig. 1**). They diffuse on the surface. An external electrode can direct their motion. For example, a tip of scanning probe, once charged electrically, can guide the motion of the molecules. As another example, one can embed an array of individually addressable electrodes near the surface of a dielectric substrate. Varying the electrode voltages individually, one can program molecular assembly, direct an island to move in a desired direction, split an island into several smaller ones, or merge several islands into a larger one. The dexterity may lead to new technologies such as reconfigurable assembly and molecular car. This work develops a model to simulate the motion of the molecules under the combined actions of the dipoles, intermolecular forces, entropy, and electrode voltages. The work also compares with the experimental evidence in the literature, and maps possible ways for practical implementation.

Fig. 1

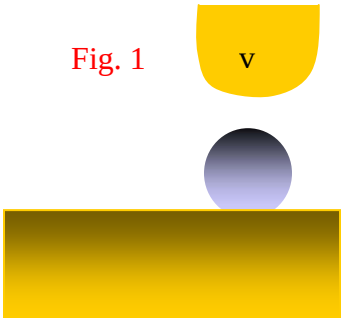


Fig. 2 shows a time sequence from the simulation [1]. The time is given in units of a characteristic time in the model. The initial concentration fields randomly fluctuate around the average ($C_{av} = 0.4$ in this simulation). At around $t = 10^2$, the monolayer already separates into two phases. Further annealing does not change the size of the islands appreciably, but improves the spatial ordering of the islands somewhat. At $t = 8 \times 10^6$, the pattern consists of grains, each grain being a triangular lattice consisting of fewer than ten islands across.

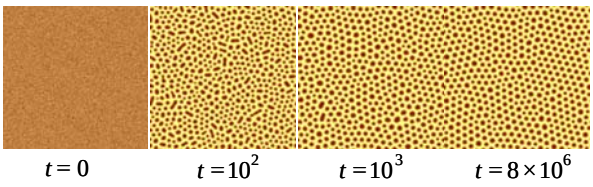
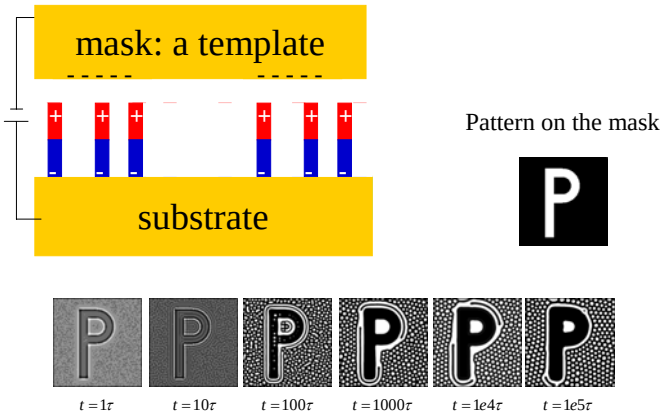


Fig. 2 *A simulated time sequence. The molecules are randomly distributed on a solid surface initially, and self-assemble into an array of nanoscale islands.*

Fig. 3 shows an example of field-directed assembly (FDA) [2]. A submonolayer of dipoles adsorb on a solid substrate surface. At a suitable temperature, the molecular dipoles are mobile on the surface. Without an external electric field, the dipoles spontaneously assemble into an array of nanoscale islands. Now above the molecules is placed a metallic mask, which is pre-patterned with electric charge. Apply a voltage between the mask

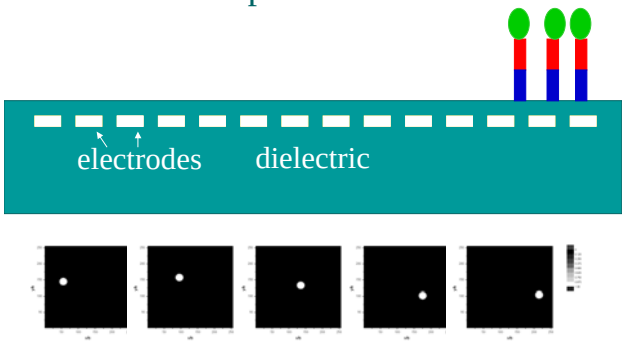
Fig. 3 Field-Directed Assembly



and the substrate. The protruding parts of the mask cause stronger electric fields between the mask and the substrate. Depending on the polarity, the dipoles are either attracted toward, or repelled from, the spots on the substrate under the protruding mask. Consequently, the patterned mask serves as a template to guide the molecular dipoles to diffuse on the substrate surface. The process transfers the topographic pattern on the mask to a molecular pattern on the substrate. The electrostatic interaction facilitates the pattern transfer. In the figure, the mask pattern is the letter P. The bottom panel shows the time sequence of the molecular distribution on the substrate surface. The letter P is transferred, but small islands form in the background, above which the mask is unpatterned.

Consider a short-chain molecule with three characteristics: its one end adsorbs to a solid surface, its mid-chain has a group with an electric dipole moment normal to the solid surface, and its other end is a passenger receptor. The molecule has a *modular* structure. The division of labor offers the flexibility to design separate modules, at the molecular level, to fulfill distinct functions. On the solid surface, a collection of such molecules aggregates into a monolayer island by the van der Waals interaction between the chains. The diameter of the island is of the nanoscale. Under no external force, the island undergoes Brownian motion on the solid surface. However, the motion can be directed by external electrodes. **Fig. 4** illustrates the programmable motion of a monolayer island. An array of individually addressable electrode pixels are embedded in a dielectric. Mobile molecular dipoles adsorb on the dielectric surface, and aggregate into a monolayer island. Charge the electrode pixels sequentially, and the interaction between the electrodes and the dipolar molecules propel the island to move. The bottom panel shows a simulated time sequence [3]. A single island is programmed to move on a sinusoidal path.

Fig. 4 Molecular car and its on-chip infrastructure



The dipole-dipole interactions mediate through the electrostatic field in the space. If the space is shaped, the electrostatic field will be affected, and so will the molecular pattern. To illustrate this idea, we have developed a model to evolve molecular pattern on the surface of a wire, or the inner surface of a tube [4]. Molecules assemble into parallel rings on the wire, and parallel stripes on the internal surface of a tube. When the tube radius is comparable to, or smaller than, the island size, the stripes switch to the rings; occasionally, the stripes form spirals.

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Session III:

Bioinspired/Biomimetic Materials and Assemblies

DOE Biomolecular Materials Program Meeting: Extended Abstract (August 2005)

Biomolecular Materials Group Project: DOE DE-FG02-04ER46156

"Design, Synthesis & Characterization of Novel Electronic & Photonic BioMolecular Materials"

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Program Scope:

Cofactors, are compounds that confer function to many biological proteins. Artificial protein models (or "maquettes"), based on α-helical bundle structural motifs, can now be designed to incorporate synthetic non-biological cofactors allowing for the possibility of peptide-based systems with novel properties not exhibited by biological systems. For example, extended π-electron systems can now be designed and tailored, with appropriate donors, acceptors and constituents, to exhibit selected nonlinear optical responses and light-induced electron transport and/or proton translocation over large distances. Importantly, the interior of the artificial protein scaffolding can be used to control the solubility, position, orientation, and indeed the properties of the cofactor within the peptide. However, an ensemble of such artificial proteins with these designed novel properties cannot form a material unless they can be ordered in one, two or three dimensions on macroscopic length scales. Gaining control over such intermolecular ordering would then result in a material whose macroscopic properties derive at a minimum from the incoherent superposition of the designed molecular properties of the ensemble, with the additional possibility of the ensemble generating coherent phenomena based on these properties. As importantly, the exterior of the artificial protein scaffolding can be used to control the peptide's supra-molecular assembly into sufficiently ordered nano-phase materials whose macroscopic behavior arises from such novel properties. In addition, the protein scaffold can be employed to control the degree of interaction/non-interaction between neighboring cofactors in the ordered nano-phase material. The latter may be especially important to ensure that the designed microscopic properties at the molecular level of the peptide-cofactor complex translate into the desired macroscopic phenomena in the ordered material.

The structural motifs chosen for the artificial peptides can be dramatically more stable than natural proteins. However, the interior of the scaffold may not be so stable to the supra-molecular assembly process required to form a sufficiently ordered material. Thus, it is important to monitor structurally the various stages of the self-assembly process starting from the designed peptide, e.g. a particular α-helical bundle, to the incorporation of the non-biological cofactor, through to the supra-molecular assembly of the peptide-cofactor complexes to a material ordered on a macroscopic scale. Since the desired material properties need not require long-range periodic order, as opposed to orientational order, in one, two or three dimensions, structural determination in the absence of such "crystallinity" is essential.

Progress Year-01:

Project 1 (Therien):

- A primary focus involved the fabrication of a highly conjugated (polypyridyl)ruthenium-(porphinato)zinc(II) cofactor and its incorporation into a computationally designed

(Saven/DeGrado) ligand-binding 4-helix bundle peptide. Supermolecules of the type, which bear terpyridyl 4' functionality, were prepared by metal-catalyzed cross-coupling reactions. These species are closely related to Ru-PZn, a chromophore that possesses the benchmark β_λ value at 1300 nm. Replacement of the 4' carboxylate functionality with phosphate provided a chromophore (PO_3^{2-}-RuPZn) that was water-soluble in presence of the surfactant. Like the carboxylate derivative, computational studies indicated that this zwitterionic chromophore had a favorable H-bonding interaction with a Lys side chain, which was engineered at an appropriate location in the tetra-α-helical peptide interior. Hyper-Rayleigh light scattering experiments show that for PO_3^{2-}-RuPZn, a β_{1300} value similar in magnitude to that previously observed for Ru-PZn was measured, indicating that these types of synthetic modifications do not impact the extraordinarily large telecommunication-relevant-wavelength β_λ values characteristic of this chromophoric platform.

- Titration studies involving PO_3^{2-}-RuPZn utilized electronic absorption spectroscopy to assess the changes in cofactor optical transitions that occurred concomitant with binding to the designed peptide. A clear shift in the Q-band region is observed upon the addition of peptide, while further analysis shows that the stoichiometric ratio of cofactor to histidine-containing peptide (P1) is 1:1, rather than 1:2, as suggested by the design of this a_2b_2 tetra-α-helical protein. This may be due to the fact the original design was done for iron porphyrin with has a higher affinity for six-coordinate binding sites than analogous zinc porphyrin, which is generally five-coordinate

Project 2 (Saven & DeGrado):

- De novo design of three-dimensional array (crystal structure) of three-helix bundles, wherein computational design methods are used to identify the relative orientation and mutations of previously designed helical bundle. This will serve as a first step in the design of ordered two- and three-dimensional crystals. The computational design of structure and sequence has been completed (Saven). The peptide has been synthesized and is presently being characterized and screened for crystallization conditions (DeGrado).

- Computational methods Saven, DeGrado) were developed to construct D_2-symmetrical bundles that encapsulate two non-biological metallo-porphyrin cofactors (Therien) per bundle. Experimental characterization is in progress and to date, has demonstrated formation of the bundle/cofactor structure consistent with the design (DeGrado).

- The backbone structure and porphyrin-binding geometry of the porphyrin conjugate is integrally periodic, repeating every 28 residues. As a result, the core of the basic unit described above can be repeated to create proteins with multiple porphyrin-binding sites, such as the 4-porphyrin complex, which was also synthesized in the previous period and is currently being characterized in detail (DeGrado). Preliminary experiments indicate that it assembles in the desired manner with DPP-Fe.

Project 3 (Blasie):

- Design and characterization in isotropic detergent solution of an two different amphiphilic 4-helix bundle peptides AP0 & AP2 (Blasie) capable of binding three different non-biological cofactors possessing extended π-electron systems DPP-Zn, Zn-3-3-Zn & Ru-Zn (Therien).
- Structural characterization of two different amphiphilic 4-helix bundles (Blasie) incorporating the Zn-3-3-Zn non-biological cofactor (Therien) vectorially-oriented at the liquid-vapor interface. The characterization utilized polarized electronic spectroscopy and synchrotron radiation-based x-ray reflectivity and grazing-incidence x-ray diffraction.

Publications Year-01:

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2. Tronin, A., Xu, T. and Blasie, J.K. (2005) *In situ* Determination of Orientational Distributions in Langmuir Monolayers by Total Internal Reflection Fluorescence. Accepted for publication in *Langmuir*.
3. Xu, T., Wu, S.P., Therien, M.J. and Blasie, J.K. (2005) Characterization of the Interaction of the Amphiphilic 4-Helix Bundle Peptides AP0 & AP2 with Non-Biological Cofactors Containing Extended π-Electron Systems. To be submitted to *Advanced Materials.*
4. Strzalka, J.W., Tronin, A., Xu, T., Wu, S.P., Therien, M.J. and Blasie, J.K. (2005) Structures of the Amphiphilic 4-Helix Bundle Peptides AP0 & AP2 Incorporating Non-Biological Cofactors Containing Extended π-Electron Systems and Vectorially-Oriented at a Soft Interface. To be submitted to *Advanced Materials.*
5. X. Fu Stowell, B. North, H. Kono, V. Nanda, W. F. DeGrado, and J. G. Saven, Computational methods for scanning the sequence-energy landscape of related protein structures. To be submitted to *Structure*.

Future Work Year–02:

Project 1 (Therien):

a) We are transitioning our work with PO_3^{2-}RuPZn and related amphiphilic NLO chromophores to focus on their incorporation into peptide bundles that are designed (Saven/DeGrado) with a 5-coordinate binding site suitable for a Zn-cofactor.
b) FPLC, CD, NMR, and sedimentation studies of NLO chromohore binding will be carried out in parallel with tetra-α-helical proteins that are designed to fold from a single-peptide sequence, and those that are based on an a_2b_2 structure comprised of four separate peptides/protein, in order to determine the minimum set of requirements necessary for high affinity binding of chromophores that contain a 5-coordinate (porphinato)zinc(II) unit.

Project 2 (Saven & DeGrado):

In the coming year we will continue to hone the design process, both from the perspective of designing backbones that support the binding of cofactors, as well as designing sequences that stabilize the fold of these backbones.

a) In the area of sequence design, improved potential functions will be developed that are applicable to non-natural cofactors and implemented a variety of Monte Carlo methods for searching sequence space. A key target of the computational design will be peptide complexes that selectively bind Therien group Zn-cofactors.
b) We will continue to characterize the optical (Therien) and structural (DeGrado) properties of the designed constructs.
c) Experimentally, the length of the proteins that are being designed has exceeded the limit for peptide synthesis, so new constructs are being prepared by recombinant methods.
d) We will continue to work on the computational design and characterization of two- and three-dimensional arrays of helical bundle proteins.
e) We will initiate work in which the designed proteins are tethered in monolayers and multilayers (with Blasie).

Project 3 (Blasie):

Using the accomplishments from year-01 as a critical basis, we will

a) Undertake molecular dynamics computer simulation of the amphiphilic 4-helix bundle peptides AP0 & AP2, with incorporated Zn-3-3-Zn or Ru-Zn cofactors, in ensembles vectorially oriented at a soft interface between polar & non-polar media;
b) Undertake neutron interferometry studies of the same amphiphilic 4-helix bundle peptides AP0 & AP2, with incorporated Zn-3-3-Zn or Ru-Zn cofactors (Therien), vectorially-oriented on the soft surface on an alkylated multilayer substrate, the peptide containing perdeuterated residues at selected positions along the bundle axis.
c) Utilizing the key experimental constraints provided by b)-above, the molecular dynamics simulations from a)-above will be employed to provide the full 3-dimensional structure of the 4-helix bundle/cofactor complex at atomic resolution.

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Molecularly Engineered Biomimetic Nanoassemblies

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Program Scope

The program aims to develop an increased understanding of fundamental interactions that govern complex molecular and biomolecular assemblies, and to explore how structure, dynamics and function are linked in such assemblies. Areas of emphasis include the development of bio-inspired assemblies with energy transduction functions, control of optical or electronic responses in complex assemblies, and exploration of the role of dynamics in controlling assembly structures and functions. In a broader sense, the scientific directions explored also provide a foundation for exploration of other biologically inspired nanoscale assemblies with diverse functions. The approach used includes a combination of materials synthesis and fabrication, static and time-resolved spectroscopic characterization, optical and scanning probe microscopies, neutron reflectivity, and theory and modeling of electronic responses and assembly structures. Our research team includes personnel with expertise in synthesis, self-assembly, materials characterization, molecular biology, biochemistry, modeling and theory, and we also work extensively with investigators at the Los Alamos Neutron Science Center.

Recent Progress

We have studied the structure and properties of several different types of bio-inspired thin-film assemblies with electronic, optical or biological responses. These have included Langmuir-Blodgett (LB) assemblies of amphiphilic electronically active species.[1,2] Here, for example, we have prepared multi-layered assemblies consisting of a thin-film of a water-soluble conjugated polyelectrolyte (MPS-PPV) on which amphiphilic electron-transfer intermediate and acceptor species are deposited via LB methods. These assemblies demonstrate greater than 95% charge-transfer efficiency as measured by luminescence quenching. Studies of dynamics of charge separation and recombination are in progress, and structural studies of these materials using neutron reflectivity is planned. We have also developed electronically active thin films based on amphiphilic quaterthiophene derivatives. We have shown that these compounds, which are made amphiphilic through an oligomeric ethylene glycol head group, will self-assemble into vesicle architectures in aqueous solutions. Further, these species can be spin cast into multi-lamellar assemblies on surfaces. The assemblies are photoconductive, stable in air, and their layered structure has been characterized using x-ray diffraction. We have also studied the electronic and optical properties of amphiphilic conjugated oligomers using both experimental and theoretical approaches in order to understand the size dependence of electronic state energies and localization in both organic and organometallic phenylene acetylene species.[3-5] In addition, we have studied the energetics of derivatized fullerenes that will be used in assemblies, and have found that excitation energies are strongly influenced by the overall symmetry of substituents on

the cage and that symmetric fullerene derivatives are more readily reduced than monosubstituted fullerenes.[6]

In addition to LB and spin-casting methods, we have explored the use of phospholipid assembly strategies to create functional material architectures. We have shown that patterned hybrid and supported bilayer structures can be formed by phospholipid assembly on UV-patterned self-assembled monolayers, and have characterized the lateral mobility and spatial confinement of such structures.[7] In addition, we have shown that phospholipid bilayers and monolayers can be formed on electronically functional surfaces consisting of fullerenes and modified fullerenes. These assemblies have been characterized by optical microscopy, and neutron reflectivity experiments are in progress. We are currently exploring the use of co-assembly of phospholipids and amphiphilic phenylacetylenes or quaterthiophenes in order to create membrane-based assemblies with embedded electronically active charge-transfer species. Finally, we have begun to develop controlled multi-layer phospholipid assemblies through the use of biotinylated lipids. An initial substrate-supported phospholipid bilayer containing biotinylated lipids is exposed to streptavidin, and then incubated with biotinylated-lipid containing vesicles. At sufficient surface coverage, vesicle interactions lead to subsequent bilayer formation, and repetition of this cycle can generate controlled multi-layered assemblies. These systems have been characterized with atomic force microscopy, and future neutron reflectivity experiments are planned. In addition, we are currently exploring the development and use of amphiphilic recognition peptides to control the interaction of phospholipid membranes with electronic nanomaterials including conjugated polymers and fullerenes.

Biological assembly also often involves the control of polyelectrolyte interactions in complex solutions, and we have been studying the use of such interactions to control the optical properties of conjugated polyelectrolytes. We have shown that the luminescence of a water-soluble poly(phenylene vinylene) derivative can be substantially enhanced through interactions with added dendrimers.[8] Characterization of the luminescence using static and time-resolved spectroscopy in combination with density-based material separation techniques has demonstrated that the enhancement is likely associated with the breaking apart of aggregated structures in solution. Surfactants are also effective at reducing aggregation and increasing luminescence, and incorporation of conjugated polymers into surfactant-templated mesostructured silica materials has been shown to lead to robust thin films in which the emissive polymer chains are non-aggregated. We have also studied how interactions in complex solutions can determine quenching behavior when charged or neutral quenchers are added to conjugated polyelectrolytes. In addition, with the porous thin-film materials, we have shown that the polymer remains accessible to small molecule quenchers, and reversible quenching has also been demonstrated in these systems.

Future Plans

Future work will progress in several directions, many of which have already been mentioned. In the area of thin-film assemblies, further characterization of already prepared assemblies will involve the use of time-resolved optical spectroscopies and x-ray or neutron reflectivity. We will also continue combined experimental and theoretical studies of electronic structures of nanoscale building blocks in order to optimize the properties of assembly components. The development of

phospholipid-based assemblies will progress with the incorporation of electronically active components into the phospholipid matrix and further characterization of phospholipid architectures formed on active substrates. The amphiphilic electronically active components will include organic phenylacetylene oligomers, platinum-based organometallic conjugated species, and thiophene oligomers. The development of biological recognition approaches for controlling membrane-material interactions and defined multi-layered membrane assemblies will be further explored, and these structures will be characterized using neutron reflectivity. Further characterization of quenching dynamics in multi-component assemblies using additional time-resolved spectroscopic experiments will also be a topic of future work. In general, our future efforts will continue to rely upon an integrated suite of experimental and theoretical approaches, including spectroscopy, optical and scanning probe microscopy, electrochemistry, molecular biology, chemical synthesis, molecular biology, x-ray and neutron scattering, mesoscale material modeling and electronic structure calculations, with the overall goal of increasing understanding of how to manipulate structure and dynamics to generate complex functions in self-assembled and bio-inspired material architectures.

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Biommimetic Polymer Design at the Interface of Chemistry and Biology

Award #: DE-FG02-04ER46162

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I. Research Progress

Toward the major goal of developing inorganic-polymer nanocomposite materials with advanced mechancial properties following nature's strategy, we have made progresses both in design of modular polymer binders and self-assembly model studies for nanocomposite materials in the past five months.

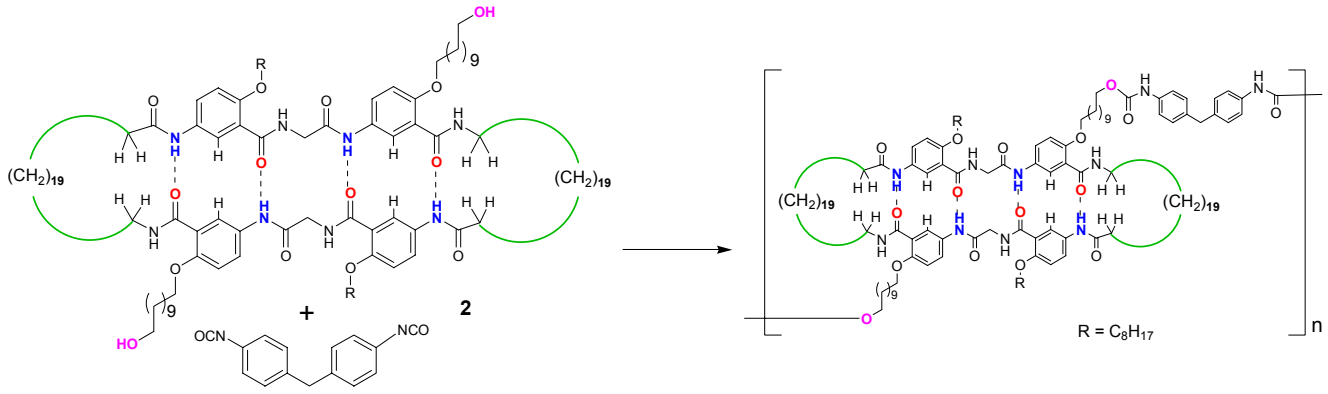
A. Modular Polymer Binders for Nanocomposites. Modular polymers are under investigation in our group for the purpose to combine mechanical strength, fracture toughness, and elasticity into one system following modular polymer design observed in biopolymers such as titin and other connective proteins.[1-5] Many binder biopolymers in biominerals are also found to have a modular structure. We plan to use our synthetic modular polymers as binders for the synthesis of inorganic-polymer nanocomposite materials. We have previously reported the design of polymers containing folded loops held by the strong hydrogen-bonding motif, 2-ureido-4-pyrimidone (UPy).[6] While demonstrating our biomimetic concept, the UPy system had a few limitations. The structure of the polymer had non-uniformity that arose from the polydispersed poly(tetramethylene oxide) loop and the different enchainment of the UPy units (head-to-head, head-to-tail and tail-to-tail).[6] The UPy units could also randomly bind to each other within a chain or between different chains. Finally, the binding strength of UPy is not tunable. For further exploration of modular biomimetic materials with high strength and toughness, more uniform and higher ordered polymer systems would be desirable.

For this purpose, a peptidomimetic β-sheet motif was designed to construct a modular polymer that has a better-defined structure. The module in this system is composed of a β-sheet like duplex that is connected at both ends with hydrocarbon loops. As a module is stretched, the force will shear the hydrogen bonds in the duplex and the loops will be extended. After releasing the force, the double-closed loop topology should ensure the strands rebind to their original pairs. We envision that this system should overcome the limitations of our previously reported UPy system. First, the double-closed loop (DCL) topology will enhance the possibility for each hydrogen-bonding unit to bind to its original counterpart, therefore, minimizing dimerization of nonadjacent units on the same chain or from different chains. Second, by choosing a monodispersed alkyl linker in the monomer synthesis, the loop size is

mono-dispersed. Lastly, by choosing the sequence and adjusting the length, the binding strength between β-sheets can be easily tuned.

A new modular polymer having the structure shown in Scheme 1 has been synthesized by conensation of DCL monomers and 4,4′-methylenebis(phenyl-isocyanate) (MDI). GPC analysis showed number average molecular weights for the DCL polymers as high as 89,000 g/mol (Scheme 1).

Scheme 1. Synthesis of the modular polymer containing β-sheet peptidomimetic modules



The modular polymers were subjected to single molecule force-extension experiments using atomic force microscopy (AFM) following the literature protocols.[6,7] The saw-tooth patterns were observed in the force-extension curves, and are similar to those seen in both natural and synthetic modular polymers (Figure 1). The patterns in the force-extension curves were more uniform for the DCL modular polymer than for the UPy system, which was attributed to its more uniform structure. The number of peaks in stretching curves ranges from 2 - 7 with the most probable range being 3 - 5. The chain detaches from the surface typically after 60 – 120 nm stretching. The most probable peak force for unfolding each module is ~ 50 pN. This force value is lower than that of our UPy modular polymers which had an unfolding force of ~100-200 pN. This is consistent with the binding strengths of the two modules: the dimerization constant (K_{dim}) measured in chloroform for the UPy and the current peptidomimetic β-sheet units are ~ 10^7 and 10^4,respectively.[8,9] The stretching curves can be fitted by the classical worm-like-chain (WLC) model for single polymer chain.

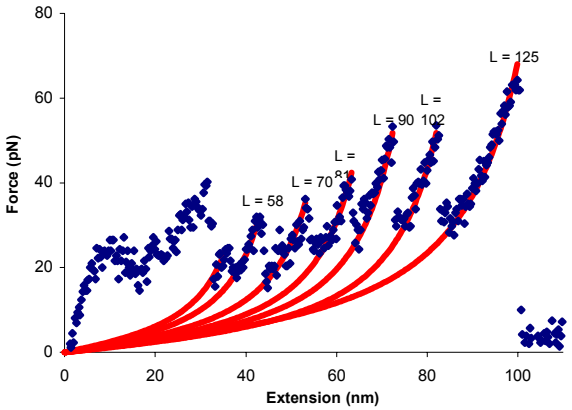


Figure 1. An AFM single molecule force-extension curve for DCL modular polymer. The red solid line is the fitting with Worm-Like-Chain (WLC) model at a 0.55 nm persistence length (L is the contour length during stretching).

B. Model Studies on Self-Assembly for Nanocomposites Formation. In parallel to our synthesis and studies of modular polymer binders, we also started investigation of self-assembled synthesis of nanocomposites. We plan to investigate the following two routes: (1) formation of a self-assembled polymer matrix which induces in situ mineralization to form nanocomposites; and (2) formation of well-defined nano-crystals followed by directed self-assembly to form 3-D nanocomposites. We started with the second approach in our initial studies. For this purpose we synthesized both zeolite and silver nanocrystals as model systems for self-assembly studies. Following literature procedures, we have prepared a s eries of zeolite and silver nanocrystals having different sizes. We will continue optimizing the reaction conditions to obtain nanocrystals with uniform sizes and shapes. Following that we will derivatize the surfaces of the nanocrystals for directed self-assembly.

II. Future Research Plans:

For the coming fiscal year, we will continue our efforts in developing nanocomposite materials with the following specific goals:

1. *Developing more feasible chemical designs of modular polymer binders for nanocomposites*: Built upon our basic understanding of molecular mechanisms for advanced materials properties, we plan to simplify our current modular designs to make more feasible binders polymers for nanocomposite synthesis. We will investigate a number of alternative designs including multi-block copolymers having hydrogen-bonding blocks.

2. *Self-assembly for inorganic-polymer nanocomposites*: We will continue our model studies on self-assembly formation 3-D inorganic-organic nanocomposites. We will begin with investigating self-assembling strategies for formation of hierarchically ordered inorganic-organic nanocomposites. After identifying and optimizing self-assembling conditions, we will incorporate modular polymer binder

3. *Computational studies*: We plan to carry out computational modeling studies to simulate the unfolding events at molecular level and to build the connection between the molecular events and the bulk materials properties for our modular multi-domain polymers. To fully understand the molecular mechanisms for materials properties, it is important to complement our current experimental studies with computational modeling. As the modular systems become more complex, this need becomes more critical. We just started collaboration with Professors Xi Chen at Columbia University

(http://www.civil.columbia.edu/faculty/chen.html) and Qiang Cui at the University of Wisconsin (http://kandinsky.chem.wisc.edu/~qiang) on computational studies at molecular scale using molecular dynamic simulation and macroscopic scale using continuum mechanics. Although we did not specifically propose computational modeling studies in our original proposal, the research developments now require a substantial effort in this direction.

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Active Assembly of Dynamic and Adaptable Materials

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Program Scope

The objective of this project is to learn how to exploit key strategies used by living systems to develop materials whose assembly, reconfiguration, and disassembly can be programmed or "self-directed" in controlled environments. Our initial focus involves adapting energy-consuming proteins that organisms use for processes ranging from macromolecule transport to cell division to materials manipulations that are not limited by constraints imposed by diffusion or equilibrium processes. Key components include microtubules, which are dynamic nano-fibers that can be assembled and disassembled in response to environmental stimuli,[1,2] and motor proteins such as kinesin, which "walk" along the microtubules to transport materials to desired locations.[3,4] The concerted interactions between motor proteins, microtubules, membranes, and target building blocks such as nanoparticles will facilitate the assembly of hierarchical materials that can adapt or be programmed to respond to stimuli provided by artificial microfluidic environments. Program components include: *Dynamic Assembly* (polymerization and depolymerization of microtubules), *Active Transport* (adapting motor proteins to transport nanomaterials), *Multi-component Materials* (examining the behavior of microtubules and motor proteins in composites that also contain nanoparticles and membranes) and *Component Modeling and Simulations* (development of new stochastic models to predict the adaptable behavior of complex biological assembly processes).

Recent Progress

Dynamic Assembly

In cells, microtubules have lifetimes that are typically limited to minutes due to a process called dynamic instability, in which individual microtubules are constantly cycling between polymerizing and depolymerizing states.[2] While dynamic instability is utilized in several critical cellular functions (such as cell division), we have applications for microtubules in active transport and assembly that require the use of stable microtubule shuttles or fiber networks. We have now evaluated a wide range of chemical crosslinking agents that form covalent bridges between the tubulin units comprising the microtubule. We find that the most effective crosslinkers and crosslink concentrations can extend microtubule lifetimes from minutes to almost one week without interfering with microtubule functionality. However, as these crosslinkers decorate the exterior surfaces of microtubules, we also find that improper crosslinking can block motor protein binding sites, impede motors as they "walk" along the microtubule, transform microtubules into tubulin sheets, and even prevent the polymerization of tubulin into microtubules. All of these behaviors have been rationalized based on the known crystal structure of tubulin, which allows us to predict where crosslinks will go as a function of how long the crosslinker is and what amino acid groups on the tubulin surface it can react with. The structural analysis enables us to develop optimized crosslinking strategies.

Active Transport

In FY04, we succeeded in producing microtubule shuttles that were functionalized to carry particulate cargo. In the presence of self-assembled monolayers of the motor protein kinesin, the microtubule shuttles and their associated cargo can be propelled along specific trajectories within lithographically-defined microfluidic channels. Schemes have been developed for transporting particles ranging in size from 10 μm polystyrene beads down to 15 nm nanocrystal quantum dots. This year, we have expanded our research to understanding how to control all aspects of cargo handling, including particle harvesting, transport, and ultimate delivery and assembly. The model system we have investigated involves studying the collisions between biotinylated microtubule shuttles carrying single streptavidin-coated polystyrene beads. Video images obtained via fluorescence microscopy reveal that collisions between shuttles result in six distinct behaviors: bypass events (where crossing microtubules ignore each other), microtubule bending, particle dislodgement, particle transfers between microtubules, co-joining of microtubules to a single particle, and particle-induced microtubule severing. We can now predict and manipulate the distribution of such events based on physical parameters including the force and velocity of the mobile microtubules, the collision geometry, component concentrations, and the number and strength of inter-component linkages.

Multi-component Materials

The most common structure used by living systems to deploy microtubules and motor proteins is called a microtubule-organizing center (MOC) (or a centrosome in living cells).[1,5] The MOC consists of a central particle to which an array of microtubules is anchored. Materials are manipulated by the MOC by controlling both the dynamic instability of the microtubule network and the transport of nanomaterials by motor proteins that walk within the network. This year, we have developed several successful strategies for producing artificial microtubule organizing centers (AMOCs). Functionalized silica and polystyrene particles have been used to produce AMOCs with high concentrations of randomly oriented microtubules, as well as AMOCs in which the microtubules are all oriented with either their (+) or (-) ends radiating out from the central particle. Oriented microtubules will be important for directing the manipulations of particles and other nanomaterials using both active transport and dynamic instability processes. In addition to MOCs, team members at both Sandia and the University of Washington simultaneously discovered ways use active, energy-driven assembly processes to construct mobile microtubule rings using microtubules, motor proteins, and nanoparticles. Under specific conditions, particle-laden microtubule shuttles can curve back on themselves. If particles such as quantum dots on one end of a shuttle bind to the other end, a circular ring structure forms. The ring continues to interact with tethered kinesin motors, causing it to rotate around its center. To our knowledge, these mobile ring composites are an artificial construct that has never been observed before in either living or artificial systems.

Component Modeling and Simulations

The versatility and power of microtubule organizing centers in manipulating nanomaterials has been explored using new stochastic simulation tools developed at Sandia in FY04. In the simulations, component behaviors including dynamic instability, microtubule stabilization, motor protein directionality, and nanoparticle harvesting can all be programmed to explore different strategies for assembling and reconfiguring nanomaterials. For example, a simulation

has been performed showing how a MOC can be used to collect nanoparticles to build a nano "rock wall". In the simulation, a centrosome is placed in the center of a random array of particles functionalized with motor proteins. Using dynamic instability, growing and shrinking microtubules are able to explore a large area surrounding the MOC (with all + ends radiating outward from the MOC). If a microtubule encounters a particle, particles are harvested via binding to associated motor proteins. All particles are then actively transported and concentrated at the MOC by the active motors (such as dynein) that are programmed to walk from the + to the – ends of the microtubules. A specific region is then programmed with microtubule stabilizers. As dynamic instability proceeds, all tubulin monomers are eventually consumed in microtubules that have contacted the stabilizers, forming a fiber network between the stabilizers and the MOC. Once the fiber network is stabilized, - end to + end-walking motors such as kinesin are activated, transporting all particles from the MOC out to the stabilizer zone to build a continuous particulate array. Such simulations are providing us with insights regarding the factors that must be controlled to assemble and reconfigure materials using our newly developed MOCs.

Future Plans

Now that we have learned how to create artificial microtubule organizing centers, we have targeted two model systems for demonstrating the use of MOCs for active transport and assembly processes: 1) Artificial Diatoms – Diatoms assemble their skeletons by first creating a microtubule network having the desired configuration using a combination of dynamic instability and stabilizers.[6] Then, motor proteins are used to carry silica nanoparticles along the network, eventually replicating the network with a continuous particulate coating. We plan to replicate such an assembly process by learning how to selectively stabilize microtubules, and learning how to control motor protein functions including particle harvesting, active transport, and cargo delivery within microtubule organizing centers. 2) Artificial Chameleons – Organisms such as chameleons change their color by using MOC arrays to change the spacings between pigment crystals that are carried by motor proteins along the microtubules.[7,8] We plan to replicate this system using quantum dots. Simulations are already helping us to develop the design rules for a programmable optical array based on supported MOC microsystems.

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Session IV:

Topic: New Tools and Techniques

A Combined X-Ray and Neutron Scattering Study of Nanoscale Heterogeneity in Lipid Bilayers.*

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Program Scope:

Lipid rafts are pervasive nanometer scale (50-500 nm), dynamic ordered assemblies floating freely in the surrounding fluid membranes of living cells. They are emerging as a definite structural motif with a broad functional significance [1-3]. Processes as diverse as cell polarity, signal transduction, protein trafficking, and membrane sorting appear to proceed within raft microstructures. Individual rafts are small entities containing thousands of lipids but only a few proteins. By clustering small rafts into larger functional platforms, proteins are brought together, e.g., during signaling (e.g., coalescence and capping). Understanding the mechanisms by which small rafts are stabilized in cellular membranes and how lipidic physical-chemical processes contribute to the functionalities of the raft associated proteins will address the emerging hypothesis in membrane biology that lipidic microenvironments (and their changes) regulate broad classes of membrane-associated functions. It has been further suggested that such an understanding will provide a basis for new means to regulate cell surface signaling by exogenous manipulation of membrane lipid compositions. From the vantage of materials science, they exemplify an important class of nanoscale structures whose dynamic reorganization is critical to their function. Grazing Incidence Wide Angle X-ray Diffraction has been carried out on monolayers of sphingomyelin and its synthetic derivatives at the air-water interface by Vaknin and coworkers[4-6].

Preliminary Results:

Because rafts are nanometer scale fluid devices (in the vicinity of the optical diffraction limit) exhibiting complex dynamics, their visualization in live cells using conventional light microscopies has been difficult. Model systems (e.g., monolayers, bilayers, and giant vesicles) designed using purportedly raft-forming lipidic mixtures, in this regard, have proved very valuable in establishing the physical-chemical characteristics of lipid rafts. A striking feature from the model system studies is the observation of large variations in the sizes of these raft microdomains observed from nominally comparable lipid mixtures and at comparable temperatures. For instance, in giant unilamellar vesicles, the use of confocal microscopy and other fluorescence imaging methods, have revealed micrometer scale (2-10 um) domains. By contrast, raft-like microdomains in supported bilayers appear to be in the nanoscale dimensions. These discrepancies led us to hypothesize possible incompleteness in the phase separation and

the preponderance of metastable or kinetically arrested phase separation in supported bilayers, presumably due to substrate-induced pinning and drag. Addressing this question is particularly important because the presence of diffusional barriers (e.g., membrane proteins anchored to cytoskeletons) in true biological membranes may be providing natural means to limit their apporach to thermodynamic equilibrium (with large unfavorable raft domains) and strengthen the domain boundaries. We reason that if the observed phase separation in supported membranes is a result of the kinetic arrest, then the phase separation characteristics would depend on the initial vesicle sizes and the thermal annealing may lead to near-thermodynamic phase separation such as occurs in giant unilamellar vesicles.

The occurrence of rafts with short-range order in lipid bilayers can be expected to produce diffuse (i.e. off-specular) surface X-ray or neutron scattering provided there is some electron or nuclear scattering density contrast between the chains assembled into the rafts and the remaining liquid-like lipid layers. This can be studied with Grazing Incidence Small angle X-ray or Neutron Scattering (GISAXS or GISANS, respectively). Our preliminary experiments suggest that using high-intensity synchrotron radiation in conjunction with 2D area detectors, longer counting times, and appropriate analyses (background subtraction) it may be possible to quantitatively determine raft structures within fluid membranes at sizes and separations ranging from the nanometer to the micron range. Combined with specular reflectivity measurements and wide angle grazing incidence diffraction measurements, one should be able to characterize the morphology of the chain layering and association of the various lipid chains as a function of composition and initial conditions. Wide angle Grazing Incidence X-Ray Diffraction on similar systems have been carried out recently by Vaknin and coworkers and Lee and coworkers

In a recent preliminary study, we conducted specular and off-specular X-ray scattering studies using single supported phospholipid bilayers. The bilayer mixtures employed an equimolar mixture of sphingomyelin and a fluid phospholipid, DOPC. Concentration of cholesterol was systematically varied from 0-50 %. Our initial analysis of the specular data, which reports on the variations in electron density perpendicular to the substrate, suggests the presence of a topographic heterogeneity consistent with the formation of lipid rafts in mixed lipid samples.

In off-specular studies of cholesterol-containing samples at above 20% cholesterol concentration, we see the appearance of off-specular peaks in the vicinity of Qx of 0.02 nm-1 . (Fig. 1) We estimate that the presence of these peaks, if reproducible, will verify the presence of nanoscale aggregates (~ 250 nm) within the membrane medium. While we emphasize that these results are preliminary, they validate the usefulness of the approach. We are also planning both specular neutron reflectivity and GISANS studies on similar systems at the Los Alamos Neutron Scattering facility.

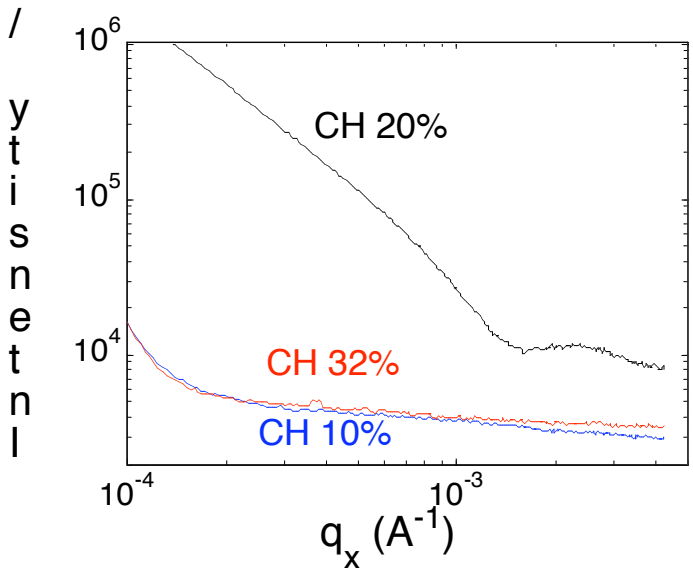

Fig. 1 Off-specular scattering intensity vs. in-plane wavevector transfer for different cholesterol concentrations.

Future Plans:

Raft Characterizatiton. Continue to develop a detailed quantitative characterization of cluster sizes and their distributions using canonical raft mixtures such as used in our preliminary study. Deliberate departures from raft-forming compositions provide a useful reference for X-ray scattering and neutron reflectivity measurements.

Non-equilibrium Phase Separation. To determine if the phase separation in raft forming supported lipid bilayers is an incomplete, non-equilibrium processs, we will implement a two-step experimental approach. We will compare the off-specular x-ray reflectivity of supported bilayers prepared by following now well-established fusion and rupture of small unilamellar vesicles (30-80 nm), large unilamellar vesicles (100-200 nm), and giant unilamellar vesicles (500 nm- 3 um) on oxidized silicon or quartz substrates at room temperature (24 C) and 37 C. Next, the as-prepared samples will be thermally annealed by heating the samples to above the transition temperatures of the lipid constituents (typically below 60 C) and cooling back down to 37 C and room temperature conditions. Subsequently**,** we will design a system of diffusional barriers to restrict system sizes and probe its influence on raft dimensions using our combined X-ray and neutron scattering methods.

Our typical lipid mixtures will comprise an equimolar mixture of purified DOPC (Dioleoyl-sn-Glycero-3-Phosphocholine), cholesterol, and sphingomyelin or 2:1:1 and 1:1:1 mixture of POPC (1-Palmitoyl-2-Oleoyl-sn-Glycero-3-Phosphocholine): cholesterol: sphingomyelin. To provide contrast in electron densities in-plane, phospholipids will contain Bromine. These lipids are commercially available from Avanti Polar Lipids, Inc. As a further option, gold nanoparticles could be covalently linked to lipids to provide additional contrast. Our initial choice of the lipid compositions above provides direct comparison with literature and our own recent fluorescence microscopy investigations. Both mixtures are known to result in the formation of co-existing cholesterol-rich raft-like microdomains embedded in fluid phospholipid phase. Vesicles in the

desired size range will be produced by vesicle extrusion and probe sonication procedures. Vesicle spreading and bilayer formation will occur in a 75 mM phosphate buffer saline (PBS, pH 7.4) at room temperature. For all samples, parallel epifluorescence imaging will be performed at UC Davis. To enable fluorescence contrast, the samples will be doped with a low concentration (well below 1 mol %) of BODIPY-labeled Gm1 which partitions preferentially within the raft-like liquid-ordered phases.

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Inelastic X-Ray Scattering Studies of Phonon Propagation and Damping in Biomolecular Assemblies

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Program Scope

We have formulated a theory for analyzing a spectrum taken from biomaterials with a newly developed high-resolution inelastic x-ray scattering (IXS) technique. We call this a Generalized Three Eigen-Mode (GTEE) theory [1]. We have made a series of IXS measurements of fully hydrated lipid bilayers without [2-3] and with [4-5] cholesterol added, in their gel and liquid crystalline phases, using a spectrometer located at 3XC beam line of the Advanced Photon Source (APS) in Argonne National Laboratory. Our GTEE theory was used to analyze the spectra taken. We succeeded in obtaining from the analyses the dispersion relations of the in-plane density oscillation waves propagating on the bilayers with cholesterol for the first time [4]. It was shown that an addition of cholesterol to a bilayer is dynamically equivalent to lowering the temperature of the bilayer [4]. Recently, we also succeeded in measuring phonons propagating along the cylindrical axis of liquid crystalline B-DNA. In particular, we discover a strong damping effect of the multi-valent counterions on the propagation of phonons. This new finding has a significant relevance on biological function of DNA in cells [5-7].

Recent Progress [7]

The phonon propagation and damping along the axial direction of films of aligned 40*wt*% calf-thymus DNA rods is studied by IXS. The IXS spectra are analyzed with GTEE theory, from which we extract the dynamic structure factors $S(Q,E)$, as a function of transferred energy $E = \hbar\omega$; and the magnitude of the transferred wave vector Q. $S(Q,E)$ of a DNA sample typically consists of three peaks, one central Rayleigh scattering peak, and two symmetric Stokes and anti-Stokes Brillouin side peaks. By analyzing the Brillouin peaks, the phonon excitation energy and damping can be extracted at different Q values from about 4 nm^{-1} to 30 nm^{-1}. A high frequency sound

speed is obtained from the initial slope of the linear portion of the dispersion relation below $Q = 4\ nm^{-1}$. The high frequency sound speed obtained in this Q range is 3100 *m/s*, which is about twice faster than the ultra sound speed 1800 *m/s*, measured by Brillouin light scattering at $Q \sim 0.01\ nm^{-1}$ at the similar hydration level. Our observations provide further evidence for the strong coupling between the internal dynamics of a DNA molecule and the dynamics of the solvent. The effect on damping and propagation of phonons along the axial direction of DNA rods due to divalent and trivalent counterions has been studied. It is found that the added multivalent counterions introduce stronger phonon damping. The phonons at the range between ~ 12.5 nm^{-1} and ~ 22.5 nm^{-1} are overdamped by the added counterions according to our model analyses. The intermediate scattering function is then extracted. And it shows a clear two-step relaxation with the fast relaxation time ranging from 0.1 to 4 *ps*, and the slow relaxation time ranging from 2 to 800 *ps*.

Future Plans

We have demonstrated [1-7] that the measurements of bio-molecular materials with IXS can provide rich information about the collective motions of the bio-molecular systems, which are very difficult to obtain with other techniques. We have also shown that the GTEE theory developed by our group is a very powerful tool for analyses of the IXS spectra [1].

With our preliminary measurements of liquid crystalline DNA samples, we have concluded that: 1) water molecules in the vicinity of DNA molecules have strong influence on the internal dynamics of the DNA molecules; 2) different counterions in the solvent have different degree of damping effects on the collective motions of the DNA. These observations are biologically very significant [7].

Our results are the first observation which shows coupling of the solvent dynamics with the internal dynamics of DNA molecules through propagation of the phonon-like collective excitations. This provides information of the coupling of the solvent dynamics to the internal dynamics of biological molecules from observing the dynamics of high Z atoms of the system, such as C, P, and O. This is because x-ray cross section is proportional to the square of the atomic number Z. So x-ray is more sensitive to large Z atoms rather than the protons in the bio-molecule. On the other hand, neutrons are very sensitive to motions of the protons. Therefore, compared to neutrons, IXS directly observes the dynamics of backbone of a

bio-molecule, which is more straightforwardly linked to the systems' intrinsic dynamics.

It has been shown that with the measurements of the inelastic neutron scattering (INS), the mean square displacement of protons in hydrated protein and DNA samples undergoes a sharp transition at temperature about 200~220 K [8-12]. This kind of transition has been linked with some biological functions of protein molecules. Since our measurements can provide different information by observing the dynamics of large Z atoms, it will be very appealing to study whether there is a similar transition by observing the collective dynamics of the system at low temperatures.

It also has been shown that the transition around 200~220 K of protein molecules observed with INS is strongly dependent on different type of solvents [11-12]. Therefore, we would like to study the effect of different type of solvents to the internal dynamics of DNA molecules by changing solvent molecules.

Due to the fact that x-ray cross section is proportional to the square of atomic number Z, we would also like to directly measure the so-called counter-ion fluid in the system by adding counterions with very large atomic number, such as cesium, or barium. By comparing the results from the sample with counterions having much smaller atomic number, it could provide a direct measurement of the dynamics of counterions around DNA molecules. With this method, it will greatly facilitate understanding the dynamics of counterions around DNA molecules. And this kind of measurement becomes now feasible only with the availability of the high resolution IXS technique.

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Directed Self-Assembly of Soft-Matter and Biomolecular Materials

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Program Scope

The central objective of the BNL soft-matter and biomolecular materials program is to understand the fundamental interactions in these complex materials that give rise to their nanolength scale properties. A major aspect of the program is to investigate the structure, phase behavior and growth of liquids, liquid crystals, and biomolecular materials on physically and chemically nano-templated surfaces. These nano-templated surfaces are being generated using a wide range of techniques including diblock copolymer nanostructures, AFM-scribing, AFM-based chemical patterning, e-beam lithography and self-assembled masks. A second aspect of the program is to develop a fundamental understanding of how monolayers of organic molecules and protein macromolecules and other biomolecular materials self-assemble on fluid or solid surfaces, at buried interfaces, and under applied electric fields. The research relies on a variety of synchrotron x-ray probes including microscopy, resonant scattering, microbeam scattering, small angle scattering, and grazing-incidence surface diffraction and scanning probe microscopies.

Recent Progress

A major aspect of the program is to develop a better understanding how liquids wet nano-patterned surfaces with topological or chemical features. In addition, these same surfaces are used to promote the self-assembly of soft-matter and biomaterials. The macroscopic description of wetting and non-wetting liquid films is expected to break down in the nm scale region. Many theoretical predictions related to these nano-properties have not been tested due to the technical difficulties of measuring the structure of nanoliquids. Our research aims to investigate these nano-liquids by using in-situ synchrotron surface x-ray scattering (SXS) and non-contact atomic force (AFM). Nanostructured surfaces are being developed which are suitable for these studies. In addition, the effect of these on macroscopic wetting is also being investigated. Preliminary studies show that the contact angle of water can be significantly enhanced by coating arrays of nanocavities with silane surfactants compared to flat surfaces enhanced with the same surfactants.

By using specially prepared block copolymer films as an etch mask, hexagonally patterned, nm sized cavities have been created over the large areas required for SXS measurements. The parabolic shaped cavities, 20 nm wide and separated by ~40 nm, were prepared in collaboration with Chuck Black (IBM). The wetting behavior on these surfaces differs from the predicted thickness dependence for wetting films on flat surfaces calculated using Van der Waals interactions. This prediction gives rise to a $\Delta\mu^{-1/3}$ thickness dependence. The SXS results obtained with x-ray reflectivity (XR) and grazing incident diffraction (GID) show a transition from the filling to the growing regimes as a function of ΔT, the temperature difference between the substrate and reservoir which is a a measure of $\Delta\mu$. In the filling regime, $\Delta T>0.5K$, the wetting behavior was dominated by surface topology, and resulted in a $\Delta\mu^{-0.76}$ like behavior.

Another important class of nanowetting problems is on chemically patterned surfaces with two different surface chemical terminations. The liquid is chosen such that the vapor only wets one of the two regions. The morphology of nanoliquids is investigated with non-contact AFM, in part to see where the macroscopic description of capillarity breaks down. One system

under investigation is the condensation of ethanol on surfaces with both methyl and carboxylic acid terminations where the ethanol only wets the acid region. We have prepared these surfaces by selectively electro-oxidizing the terminal methyl group using a biased, conducting AFM tip in a humid environment using a technique invented by J. Sagiv and coworkers. An important characteristic of the pattern is the absence of geometrical features on the 0.2 nm scale. Controlled amounts of ethanol are condensed on the "chemical stripes" from the vapor and their shape is imaged in-situ versus their width ($40 < w < 300$ nm). At the bulk liquid/vapor coexistence, the profile shapes and the measured width (w) dependence of the height follows a $w^{1/2}$ behavior and agrees well with the predictions of Density Functional Theory. To better understand the mechanism of the electro-oxidation chemical conversion process (see above) we have carried out systematic studies as a function of the exposure time with the biased AFM tip. Our results show that the "water bridge" which is generated between the AFM tip and the surface limits the feature sizes. At long times (t = 1-1000 s), the diameter follows a log(t) dependence.

Further progress in the study of soft-matter, biomaterials and liquids on nanopatterned surfaces requires the development of improved chemical patterning methods. To this end, we are developing processes that utilize silane chemistry. A new nanoscale chemical patterning technique that combines the electro-oxidation patterning method described above and Dip Pen Nanolithography has been developed. In a single sweep of an ink-coated, biased conducting AFM probe-tip, an underlying thin organic film is oxidized and the ink molecules are transferred directly to the oxidized regions. This Electro Pen Nanolithography technique provides very fast - exceeding 10μm/sec - writing speeds with line-widths as small as 50 nm. Controlled multi-write operations permit the growth of an integer number of molecular layers. We have also used self-assembled, polystyrene nanospheres as masks to create sub 100 nm chemically patterned disks and demonstrated the selective adsorption of proteins on these disk regions Finally, block copolymers methodologies offer an additional opportunity for making, chemically and topologically patterned surfaces over large areas. The rate of solvent evaporation and the evaporation-induced flow have been used to highly orient cylindrical domains of a diblock copolymer parallel to the surface over very large surface regions (> cm^2). In collaboration with the Russell Group (Univ. Mass.), Grazing Incidence Small Angle X-ray Scattering (GISAXS) studies have played a key role in defining the mechanism by which ordering propagates through the film during the solvent evaporation.

One aspect of our research on liquid crystal (LC) and biomolecular phases involves understanding how they self-assemble and interact with nanopatterned surfaces. Concerning novel self-assembled phases, (i) indirect evidence was found supporting the proposal that tilted layered LC phases could develop in-plane orientational order with nanoscale features; (ii) a novel lipid bilayer phase with in-plane square modulations was discovered in the phospholipid DTPC; (iii) by deuterating one lipid in a mixed lipid system, neutron scattering revealed lipid curvature-induced phase separation as the mechanism underlying observed distortions in the hexagonal lipid phases. Concerning interactions with patterned surfaces, we developed and tested a sample cell comprised of an AFM-scribed polymer surface and an opposing planar degenerate alignment surface that could be used to direct the nanoscale alignment of layered LC smectic phases. This opens the door to novel device applications and fundamental studies of LCs under orientational strains previously restricted to *fluid nematic* LCs.

Our research on preparing ordered monolayer organic and biomolecular films showed progress in several directions. Using XR and transport measurements, it was shown that tetracene could be functionalized to bond to a sapphire substrate and form a dense monolayer with high-mobility. Also using high-energy XR (16 keV), we demonstrated that we could deposit alternating lipid and protein layers at the silicon substrate/aqueous buffer interface. Our work on imaging and understanding epitaxial interactions revealed unexpected single domain growth in ultrathin, ferroelectric films. Additional XR structural studies, carried out under applied electric

fields using 32 kev x-rays, showed that the standing-up phase of alkane-thiol self-assembled monolayers (SAMS) are densely packed at the Hg/Si buried interface.

We are also exploring a *biomimetic* approach to nanoscience inspired by biominerals, complex structures dictated by biomacromolecule assembly processes. A fundamental question that we probed with synchrotron techniques concerned structural templating, as between a protein and a crystal face. Our studies of calcium carbonate at monolayers proved, instead, that kinetics dominates mineralization. Further work showed how biopolymers and Mg affect these kinetics. Mg-calcite grown on SAMs has a critical difference in texture from the underlying substrate. We discovered that two routes to Mg-calcite formation coexist: oriented nucleation dependent on SAM functionality, and an amorphous precursor route independent of the SAM. Finally, we recently demonstrated that microbeam diffraction could be used to probe structural correlations across the nacre-prismatic interface of abalone shell.

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Future Plans

We will extend our studies of wetting phenomena, currently focused on parabolic shaped nanocavities in silicon, to other patterned surfaces. SXS is uniquely suited for studying wetting behavior on nanopatterned surfaces since it provides detailed information on the amount of adsorbed liquid, the distribution within the cavities, and the liquid film thickness. These wetting studies will take advantage of our extensive expertise in using x-ray scattering to investigate nanoscale wetting phenomena. Experiments will be extended to cylindrical and pyramidal geometries and to UV prepared gratings with sub 60 nm spacings. An important goal is to develop nm scale, chemically patterned surfaces, suitable for our SXS studies.

In the case of small drops (< 50nm), their shape may also be influenced by the line tension in addition to the surface tension. We propose to measure the contact angles of ultra-small drops which form on chemically patterned symmetric discs and unusually shaped patterned surfaces, both of which are particularly sensitive to the effects of the line tension. Preliminary results on closely spaced patterned lines support this approach. In addition to the static wetting studies describe above, we will investigate the spreading of liquids onto nanosized hydrophilic channels. This will be achieved by connecting chemically patterned lines (nanochannels) to a large reservoir that can be filled with a nonvolatile liquid such as silicon oil. Existing measurements on alcohol adsorption on patterned surfaces will be extended to ionic and polar liquids. Finally, the unusual phenomenon of surface freezing in chain molecules will be extended to nanodrops to see how this novel phenomenon is modified at very high curvature.

An important aspect of our program is the development of new techniques for preparing chemically patterned surfaces. We intend to further our studies of Electro Pen Nanolithography by investigating different inks and by investigating what factors determine the line-widths. Patterning methods will also be developed which utilize block copolymer masks and silane chemistry. Our preliminary studies have shown that the AFM patterning (discussed above) can be used to orient and align the standing-up phase of block copolymer films and further work will be carried out in this direction.

One of our long-term goals is to prepare and characterize arrays of membrane proteins. Currently we are incorporating proteins into single mixed lipid bilayer assemblies. We are also advancing our capabilities to measure 2D protein arrays by studying whether microfocusing can reduce x-ray radiation damage and extending our coherent diffraction technique, which provides model-independent atomic-resolution electron density maps of free-surfaces and buried interfaces, to bio-systems. As an intermediate step, we are currently refining our approach on the surface structure of semiconducting organic crystals.

Having succeeded at producing cells that induce nanoscale distortions in layered LCs, we will use these cells to apply bend distortions that are incompatible with layer ordering.

Theoretical work (Petschek, CWRU) will be combined with microbeam diffraction to probe whether periodic defect arrays or phase melting results. We will continue our studies of LC and biomolecular self-assembly. Our goals are to understand what interactions are responsible for the rich variety of tilted layered phases with different inter-layer and intra-layer ordering, to understand how chain-packing patterns and film thickness affects the modulated lipid bilayer phases, and how molecules of unusual shape (bent-core, T-shaped, bowl-shaped) self-assemble into thin films with unique electro-optical properties.

Biological and biomedical applications of inorganic nanoparticles (NPs) are being developed by others due to their unique size-dependent properties which are relevant to biological processes and drug delivery systems. Our goal is to investigate (i) the structures of the lipid membrane coatings around NPs and (ii) the interactions of the lipid-coated NPs with various lipid membrane surfaces. We will start with simplest model systems and gradually increase the system complexity. The initial studies will be conducted without proteins; instead they will focus on the characterization of lipid membranes on curved surfaces and their role in membrane fusion process. Further studies will focus on the reconstitution of membrane fusion proteins and their effects on fusion process.

DOE Sponsored Publications (2002-2005)

A complete list of publications can be found at http://www.solids.bnl.gov/~scmg/publications.htm

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Liquid Materials Behavior Unique to Nanochannels and Confinement

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Program Scope

This research aims to understand experimentally at the molecular level fluids in intimate contact with surfaces, the main innovation being the combination of modern methods of single-molecule sensitivity with parallel force microscopy. This family of scientific and technological problems concerns not only the interface of a single surface against a sea of homogeneous bulk liquid but also alternatively two surfaces where the surface-surface spacing is comparable to the correlation length of the fluids themselves. The specific goals are: (1) To determine the structure of buried fluid films by direct experiment; (2) To determine the dynamic structure by direct experiment (the methods here are mainly spectroscopic), not just in the quiescent state, but also under the influence of external fields such as shear; (3) To provide data needed for a firm theoretical analysis of a wide range of interfacial thickness, from nanometers to micrometers, involving fluids whose complexity ranges from simple (alkanes) to complex (polymers, lipids, their supramolecular assemblies, and proteins).

Recent Progress

In a breakthrough, this year has seen the success of our long-standing project to combine methods of few-molecule fluorescence spectroscopy, and of vibrational (confocal Raman) spectroscopy, with friction measurement. The resulting combination of information obtained at the molecular level from spectroscopy, and at the ensemble-average force level using a surface forces apparatus, open unprecedented windows towards explaining origins of friction at the molecular level. This constitutes an exciting outgrowth from this laboratory's prior emphasis on friction. Successful experiments were performed using several complementary techniques, all devoted to understanding the lubricant nanoenvironment that underlies friction. First, translational diffusion was studied, using the few-molecule technique of fluorescence correlation spectroscopy (FCS). Second, rotational diffusion was studied, using time-correlated single photon counting on the nanosecond time scale. Third, to address the important ancillary questions of surface-induced lubricant structure, confocal Raman spectroscopy was used to study confinement-induced ordering of various types of lubricant molecules.

Separately, we have considered phospholipid bilayers supported on planar substrates and in free-standing vesicles, which are fundamental for fundamental biomolecular materials studies as well as applied problems such as their use as biosensors and nanoreactors. We are interested in what determines the lateral mobility of the individual molecules that comprise these fluid yet two-dimensional systems – a problem that is fundamental to their function. To the best of our knowledge, all prior studies of lipid mobility have considered area-averaged quantities, leaving open the possibility that

the area-average might mask an interesting distribution. We found that the translational diffusion of phospholipids in supported fluid bilayers splits into two populations when polyelectrolytes and proteins adsorb at incomplete surface coverage. Spatially-resolved measurements using fluorescence correlation spectroscopy (FCS) show that a slow mode, whose magnitude scales inversely with the degree of polymerization of the adsorbate, coexists with a fast mode characteristic of naked lipid diffusion. Inner and outer leaflets of the bilayer are affected nearly equally. This offers a new mechanism to explain how nano-sized domains with reduced mobility arise in lipid membranes.

Other recent accomplishments involve encapsulated proteins and pattern formation. In our newly-developed method for encapsulation of proteins in nanoporous glasses, external catalysts are not needed; the guest proteins themselves catalyze the polymerization of silica and formation of the glass matrix. Because the growing matrix constricts the protein conformation, enzymatic activity of the proteins can be altered in a controlled way. Reactions of proteins and small molecules confined in nanoporous solids depends on the reactants' interactions with each other and the solid matrix. We learned how to detect and, if necessary, eliminate these interactions and thus simplify the reaction kinetics. We showed that protein-doped silica has several advantages as a stationary phase in capillary columns for liquid chromatography and identified several factors that determine the usefulness of immunoaffinity columns for detection of bioanalytes.

The spontaneous emergence of patterns at surfaces is a recurring theme in modern materials chemistry. In a model system of polymer, solvent, and atomically-smooth surface that hundreds of concentric rings, each approximately nanometers high and microns wide, form when a drop is allowed to evaporate from a restricted geometry leaving behind a dry nonvolatile polymer solute. The evaporating drops were placed between a sphere and a flat surface (implemented by using cylinders of equal radius of curvature at right angles to one another). This method of dynamic self-assembly offers a new method to produce gradient nanoscale structures, as well as a simple, versatile, generalizable approach to produce yet more complex patterns.

Future Plans

Most studies in this emerging area of modern chemistry and materials science involve measurements of the ensemble-averaged response of the system, but this is no longer necessarily the case when one considers the possibility of single-molecule imaging. We will build on recent progress showing that quantitative analysis of single-molecule images of fluorescent-labeled lipid molecules as they diffuse in confined films and within supported lipid bilayers in aqueous solution. The trajectories of dozens of individual molecules can be followed as a function of elapsed time and from comparison of their responses, giving valuable insight into heterogeneity that these studies have already shown to be intrinsic to soft lubrication.

In the area of nanofluidics, the path is now clear to employ single-molecule imagine for the quantitative elucidation of how fluid flows at solid boundaries, and there are exciting prospects to measure the actual velocity of fluid at the wall, with an improvement in experimental resolution 1-2 orders of magnitude above all previous studies of which we are aware. In the area of phospholipid bilayers, the path is clear to extend the existing experiments to protein systems, and with the fluorescence correlation

spectroscopy approach, in combination with single-molecule imaging, to explore the chemistry of how proteins associate with lipids, modifying their diffusion. Furthermore we anticipate that during the next year it will become possible to directly measure the cross-correlation between motion of lipid molecules and of the proteins that associate with the phospholipid membrane, thereby quantifying on-off association times in these important materials.

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The scanned probe microscope as a platform for *in situ* imaging, measurement, and manipulation of biomolecular systems

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Motivation

The scanned probe microscope (SPM) has become an indispensable tool for investigating surfaces at nanometer length scales. It is especially suited to biomolecular materials because it can operate in fluids, resolve individual macromolecules and give dynamic information on a time scale commensurate with biomolecular events. Moreover, operation of the scanned probe is not limited to imaging; rather it enables measurement of intermolecular forces, simultaneous collection of single molecule spectroscopic data, and even manipulation of macromolecules either through direct contact or by creation of nanoscale chemical templates. In this talk we illustrate each of these capabilities through examples of research taken from studies of biomineralization, membrane dynamics, and directed assembly of macromolecular complexes.

Biomineralization

Biomineralization is a process by which living organisms use biomolecular controls to direct the formation of mineralized structures, either via a surrounding matrix or by introduction of macromolecules into the mineralizing precursor phase. Amongst the many unanswered questions about this process are those that probe the stereochemical relationship between the control molecules and the mineral phase and the resulting impact on crystallization pathways. Over the past decade, the SPM has been used to directly observe the impact of small organic molecules, simple amino acids, peptide chains and full proteins on the growth of crystal surfaces. Figure 1 shows two examples from the calcium carbonate system. The first shows is the impact of aspartic acid (Asp) and poly-Asp on the growth morphology and kinetics of calcite. The second ia the effect of AP8-α, the most acidic protein fraction extracted from the calcium carbonate shell of Abalone. The data reveal a number of significant features: 1) The overall crystal morphology mimics changes to the elementary steps expressed on the naturally occurring crystal faces. 2) Individual Asp enantiomers break the crystal symmetry and induce chirality in the step and crystal morphology. 3) The ability of Asp_n to inhibit calcite growth scales exponentially with peptide chain length. 4) The full protein behaves very differently from the peptide chains and produces an *acceleration* of growth, rather than inhibition.

These results along with others obtained on various crystal systems, combined with molecular dynamics simulations, have revealed two important principles. The first is that bio-molecular modifiers alter the shape of crystals through step-specific interactions that modify the step edge energetics to create new shapes.[1] Through the self-replicating nature of crystal growth, the elementary step shape then extends itself to the facet shape, and finally, the crystal shape. The second is that inhibition by peptide chains is governed simply by the number of sites along a step edge that are blocked by the amino acid residues.[2] However, the behavior of full proteins, while still reflecting a step specific interaction, is not a simple extrapolation of that seen for the peptide chains, presumably because of the structure that arises through protein folding.

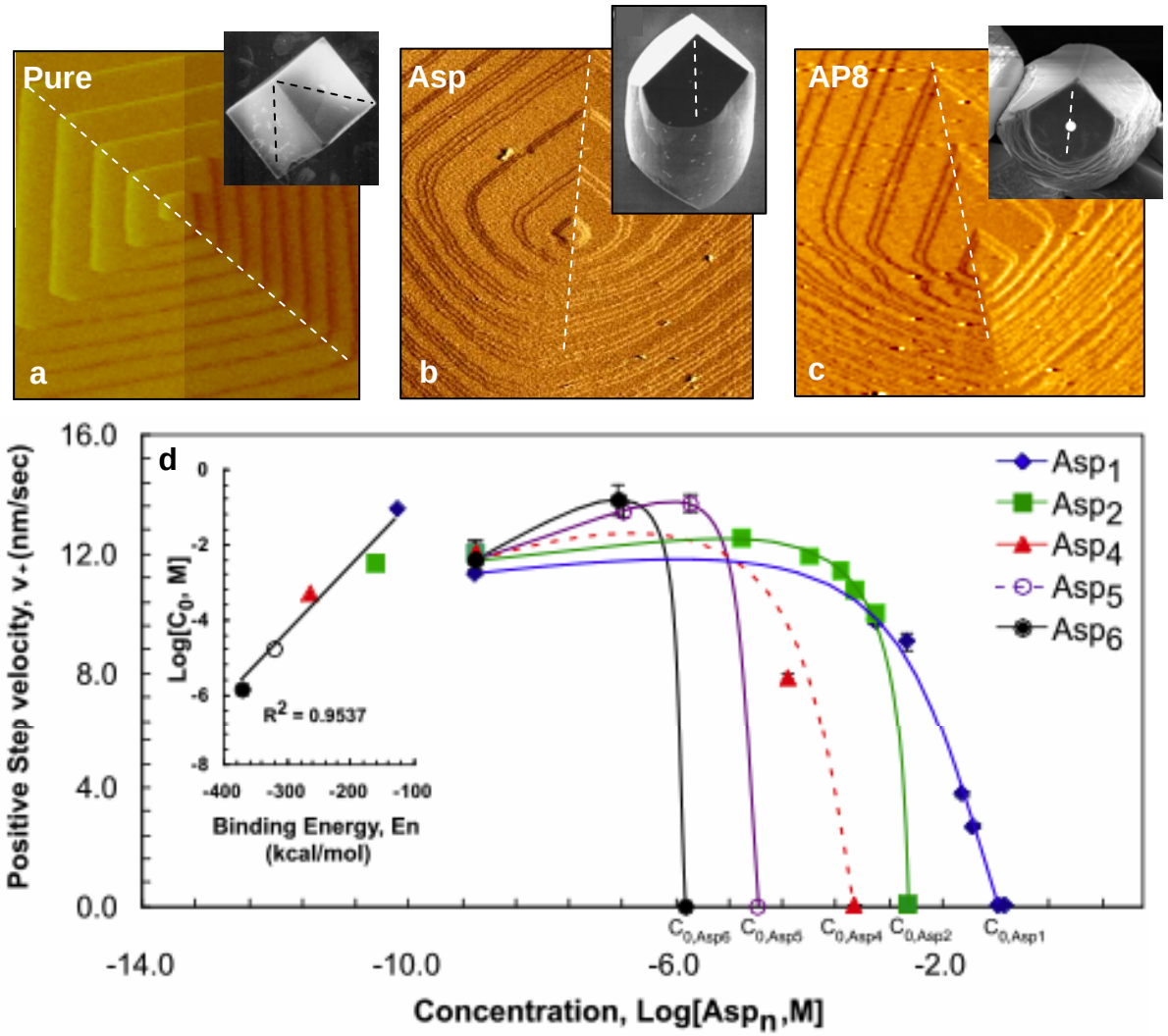

Figure 1 – (a) - (c) AFM images of growth hillocks and SEM images of crystal habits showing how change in crystal habit is directly related to modification of step morphology on natural crystal faces for a wide range of modifiers.[1] (a) pure calcite; (b) calcite plus D-aspartic acid; (c) calcite plus 8kD protein extracted from Abalone. Dashed lines show glide and mirror planes on calcite and COM respectively. (d) Step speed data from AFM measurements showing that concentration C_0, of Asp_n needed to stop growth of calcite steps increases exponentially with chain length.[2] Inset shows plot of measured Log[C_0] vs. calculated step-binding energy. Linear dependence implies that simple Langmuir adsorption model can account for results provided step coverage needed to stop step motion remains constant with chain length.

Organization and transformation of bacterial membranes

In many systems, macromolecules themselves assemble to produce functional materials. In the case of membranes, the architecture of assembly changes in response to external conditions, often leading to spontaneous structural transformation. Figure 2 shows an example from the investigation of *Bacillus* spore germination that illustrates how the SPM can be used to explore this phenomena and understand the mechanisms of transformation.[3] The results show that the spore coat is comprised of a crystalline array of protein complexes that forms in the same way that a protein crystal itself forms: by the addition of protein complexes to molecular steps on the spore surface. Upon an increase in temperature of the nutrient solution to conditions in

which the spores germinate, two processes take place. First, the step edges retreat as protein complexes desorb. Second, pits open and spread to form cracks in the spore coat that allows nutrient to penetrate inside the spore. The coat then breaks open and new bacteria emerge. In other words, in unfavorable conditions for bacterial growth and survival, the spore coat acts as an impervious crystalline barrier. When the temperature is increased to be favorable for the bacteria to growth, germination is initiated not merely by an internal response to the temperature increase, but rather by dissolution of the spore coat.

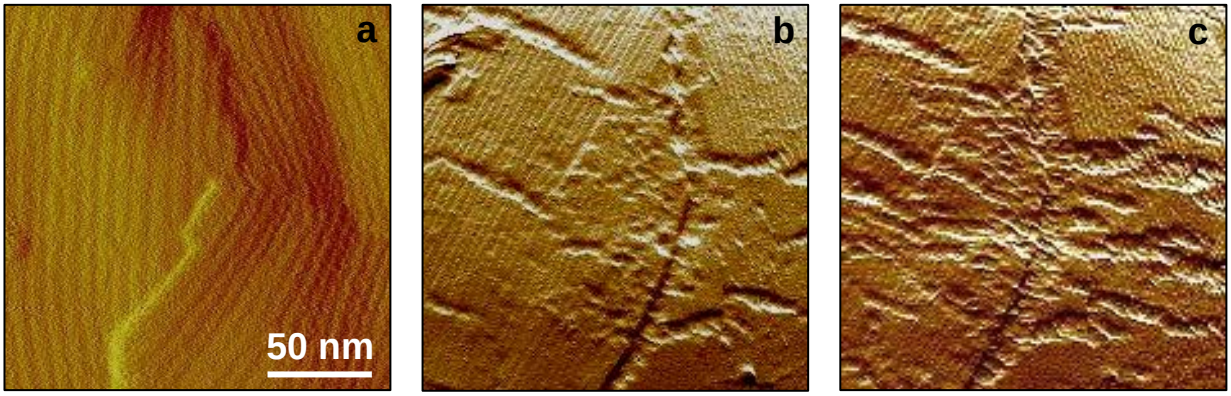

Figure 2 – AFM images showing (a) highly organized structure of outer spore coat on *Bacillus Atrophaeus* and, (b) and (c), onset of germination process characterized by spore coat dissolution through pit formation and step retreat.[3]

Directed assembly of macromolecular complexes and viruses

The organization of macromolecular complexes into 2D and 3D architectures, allows organisms to achieve a high density of functionality as well as to create templates for further organization of hierarchical structures, as in the case of biomineralization. Taking a cue from biological systems, we can consider organizing nanostructures and functional molecules into deterministic patterns at surfaces by directing self-assembly. Indeed, one approach to achieving higher density of functionality and shorter range order that has been a recent topic of research is to use macromolecular complexes — such as viruses — as programmable building blocks, either for synthesis of nanoparticles and nanowires or for precise placement of molecular moieties exhibiting optical and electronic functions. Because these building blocks are typically many tens of nanometers in size, they offer the possibility of organization using existing templating methods that operate at the length scale of 50-100 nm. Moreover, because their surface chemistry can be manipulated in a site-selective manner, they naturally lend themselves to templated assembly of heterogeneous structures with controlled placement and orientation.

The SPM is also well suited to this application. Either through dip-pen nanolithography or nanografting, the SPM can be used to chemically pattern surfaces with features that range in size from 10 nm to 10 microns. Moreover, by making these patterns out of self-assembled monolayers with chemoselective head groups, one can template the deposition of viruses or other macromolecules engineered to present specific functional groups that are reactive with those headgroups. Figure 4 shows an example of directed assembly of Cowpea Mosaic Virus (CPMV) genetically engineered to present either histidine (His) tags or cysteine (Cys) residues at specific sites on the capsid surface.[4] Atomically-flat gold substrates were prepared by first coating them with self assembled monolayers (SAMs) of polyethylene glycol (PEG) terminated alkane thiols.

Nanometric patterns of alkane thiol-based chemical linkers were then made by scanned probe nanolithography, such that attachment to the Cys-residues and His-tags was through covalent bonding with maleimides groups or metal coordination complex linkage with nickel-chelating nitrilotriacetic acid (Ni-NTA) groups, respectively. In the latter case, one can modulate the virus-surface binding strength by addition of competing metal coordinating ligands such as imidazoles, allowing exploration of the effect of virus mobility on assembly. To investigate the role of virus flux and inter-viral interactions, we varied the virus and PEG concentration of the virus solution, respectively. SPM was then used to investigate the degree of ordering, packing geometry, assembly kinetics, and cluster-size distribution both on the regions patterned to bind chemoselectively with the virus, as well as the surrounding PEG-terminated region.

The results show that the degree of ordering depends on all parameters chosen: the surface chemistry, the virus concentration, the PEG concentration, and the feature size of the patterns. For example, as the PEG concentration is increased, 2D arrays of viruses evolve from poorly-ordered to well-ordered rhombehedral to hexagonally close packed assemblies. For the 1D patterns and the His-tag:Ni-NTA linkage, as virus flux is increased, the density of viruses on lines grows until they are fully covered. With the addition of PEG, the width of those lines of viruses grows, reaching six rows wide and tending towards hexagonal close packing. Disordered clusters also form on the PEG terminated regions and as the virus concentration or PEG concentration is increased, the density and size of the clusters increases. The rate of assembly, both on and off the lines, follows a $t^{0.5}$ dependence, but the on-line growth rate is more rapid due the to higher sticking coefficient and 1D nature of the diffusion field. These results can be understood within the framework of assembly principles borrowed from small molecule systems, where the ratio of flux to mobility determines the density and morphology of island formation, combined with assembly principles governing colloidal systems, where entropic factors drive condensation.

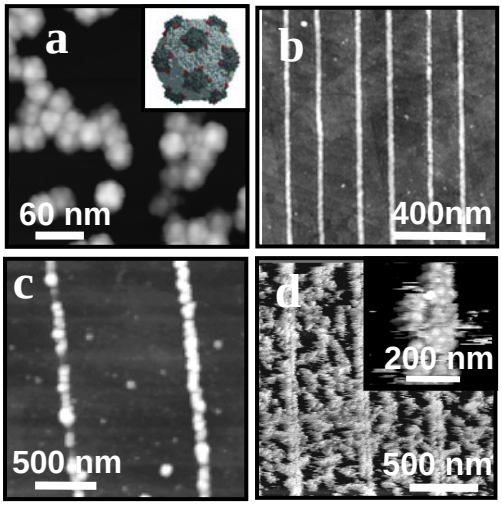

Figure 4 – AFM images collected in height mode showing: (a) CPMV viruses. Inset shows model of molecular structure. (b) Pattern of 30nm wide lines formed by nanografting maleimide terminated alkane thiols into a PEG terminated SAM. (c) Deposition of His-tag modified viruses onto Ni-NTA lines from solution with low virus concentration and no PEG in solution. Resulting arrays are one virus wide. (d) Same as (c) but with high concentration virus solution and moderate concentration of PEG. Inset is close-up of (d) showing rows are multiple viruses in width.[4]

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Session V:

Biomolecular Functional Systems: Bio/Non-Bio Integration

Cooperative Phenomena in Molecular Nanocomposites

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Program Scope

Future advances in nanoscale technology will rely heavily on multi-component materials that have unique properties at various length scales. Multi-component materials have an overall greater "functionality" than the individual components.[1] The purpose of this project is to establish key scientific principles needed to design and fabricate new composite materials that integrate functional, responsive aspects of molecular assemblies into multidimensional architectures. Molecular nanocomposites should possess all the attributes of flexible, sensitive molecular functions usually restricted to thin films with the robustness and ordering of three-dimensionally structured solid materials. Critical aspects of this project are divided into three tasks: (1) understanding the cooperative behavior of molecular assemblies, (2) developing defined nanostructural architectures, and (3) assembling and characterizing cooperative behavior in nanocomposite materials. To address these materials issues, which span a size scale of five orders of magnitude, we apply expertise in organic synthesis, soft materials assemblies, sol-gel/metal oxide formation, and templated systems. Unique function, such as gated nanopores,[2] reversible polymer π-conjugation,[3] and molecular corrals in structured lipid membranes,[4] is derived from the nanoscale architecture and specific assembly of and actuation within the structure. Much of the inspiration for this work comes from biological composite systems that have evolved to protect, amplify, or optimize molecular responses. Novel materials functionality brought forth through this program could enable the growth of new classes of materials that provide efficient energy and molecular transport, sensing and protection from toxic agents, and production of green fuels for future transportation.

Recent Progress

Molecular assemblies

New lipids were synthesized in our effort to further the understanding of chemical recognition in molecular assemblies. These lipids, functionalized with headgroups of 2,2'-bipyridine (PSBiPy) and N-methylamide (PSMA) and a pyrene fluorophore attached to the hydrophobic tail, were incorporated into phosphocholine lipid membranes. Recognition of metal ions at the headgroup position causes the lipids to respond by

changing their aggregational state due to the development of electrostatic charge leading to a concomitant change in the membrane's fluorescence. The bipyridine functionalized membranes exhibited excellent selectivity for Cu^{2+}, as was designed, at low and neutral pH resulting from the high affinity of bipyridine for Cu^{2+} (log K_{M-L} = 8.0). What was unusual, however, was that the poor metal chelating N-methylamide of PSMA afforded a bilayer with ppb sensitivity for the metal ions Fe^{3+}, Cr^{3+}, and Fe^{2+}. These results have motivated a reassessment of the assumptions regarding chemical recognition on molecular membranes.

Nanostructural architecture

A method to synthesize D-Phe-D-Phe peptide nanostructures[5] in aqueous solution has been developed along with its use as a template to produce nanoscale platinum tubes. The platinized nanotubes were about 5 to 60 μm in length, 380 to 2000 nm for outer diameter, 350 to 500 nm for inner diameter, and 100 to 200 nm for the wall thickness. High-resolution TEM (HRTEM) image showed that about 2-3 nm Pt nanoparticles are embedded in the walls of the peptide nanotubes. For insights into the self-assembly of diphenylalanine, Monte Carlo simulations were performed using a lattice model. The molecular structure was constructed to reflect the conformation of diphenylalanine and its interaction with other diphenylalanine molecules. At increasing concentrations, the assembled structure alters from a vesicle to tubule and finally into a multi-lamellar assembly. The results suggested that the phenyl groups stack together to form a hydrophobic region with the charged groups oriented through the electrostatic interaction in a tail-to-head arrangement in the peptide nanostructures. These materials have potential uses in hydrogen storage, catalysis, and structural materials.

Nanocomposites

Introducing a living cell into an inorganic/surfactant system capable of self-assembly adds both a metabolically active entity and a heterogeneous, dynamically controlled surface to the system. We very recently adapted evaporation induced self-assembly (EISA), using biocompatible phospholipid templates, to immobilize living cells within periodic, porous, silica nanostructures. We find that the interface plays an important role in both promoting cell viability and dictating the structure of the inorganic phase, with the cell actively influencing its microenvironment. In the presence of cells, a multi-layered,

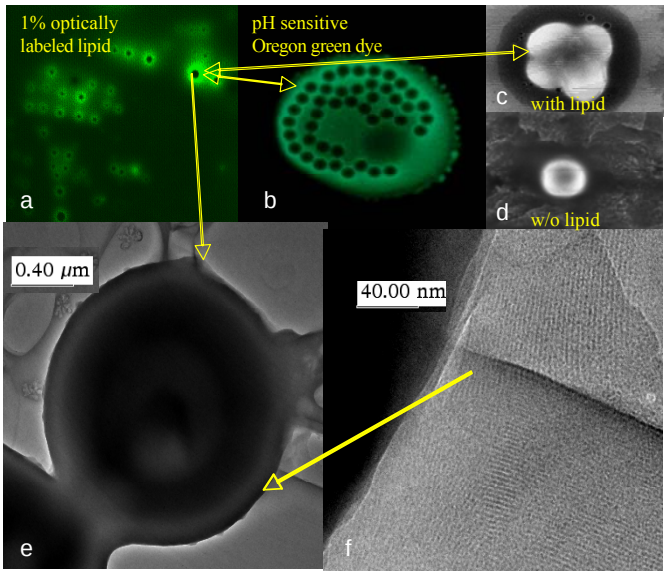

Figure 1. Fluorescence, TEM, and SEM images of the cell-directed bio-nano interface. a) confocal fluorescence image showing preferential (optically-labeled) lipid accumulation around yeast cells, b) Oregon green dye shows local change in pH in lipid enriched area, c,d) SEM images of systems prepared with and without lipid showing the coherent cell/lipid/silica interface with lipid and non-coherent interface w/o lipid, e,f) TEM images showing the smooth lipid/silica interface.

organized lipid interface develops, that mediates the local fluidity and chemistry of the cell surroundings as well as the global organization of the silica mesophase (Figure 1). We plan to further characterize the structural development of these unique bio-nanocomposite materials *in situ*, and then begin to explore how cells may serve as tools in the organization of biomolecules (e.g., proteins and lipids) in hierarchical architectures and how that organization could produce cooperative behavior.

Future Plans

Molecular assemblies

Work has begun to develop a new electrochemically-activated monolayer system that can be addressed in water. With this system, we hope to be able to reversibly program surfaces with desired functional groups with a high degree of lateral resolution (a few microns). The monolayer system is based on the molecule cyclodextrin. Neutral aromatic molecules such as ferrocene are readily adsorbed into the cyclodextrin cavity. However, when the ferrocene is oxidized to the cationic ferrocenium ion, attractive interactions between the ferrocene and the cavity are turned off, promoting desorption. We have produced cyclodextrin monolayers and are currently investigating the adsorption and desorption of functionalized ferrocenes in the cavity using cyclic voltammetry, infrared spectroscopy, and secondary ion mass spectrometry. For pattern resolution studies, we plan to write patterns of ferrocene into cyclodextrin surfaces using an AFM tip as an electrode and follow the changes in the lines we write vs. time using z-contrast AFM imaging techniques. We also plan to use programmable surfaces such as cyclodextrin to grab nanoparticles having controlled sizes and shapes, allowing us to synthesize and then release asymmetric particles that are functionalized on specific faces (i.e. those facing the solution rather than the substrate).

Nanostructural architectures

Very recently, we have found that the diameters of the peptide nanotubes can be controlled by a seeding/ templating procedure using nanospheres. Using 50-nm Latex beads to influence the growth diphenylalanine nanotubes are obtained with diameters ranging between 50-200 nm. We will optimize growth conditions to enhance uniformity of the tube diameters. Addtionally, we shall incorporate active chemical species, such as photocatalytic porphyrins, into the nanotubes and study the optical and chemical behavior of these composites in their unique long cylindrical architectures. Further molecular simulations shall be pursued to determine the factors that control the self-assembly and how these factors may be altered to gain more control over the dimensions of the nanotubes and allow incorporation of other components to add additional functionality.

Nanocomposites

We remain interested in understanding the cell-directed assembly process and its extension to the fabrication of unique, functional bio/nano interfaces. To this end we will continue *in situ* GISAXS experiments allowing the real time monitoring of the self-assembly process. New directions here will be to extend our studies from yeast to other organisms like bacteria and to perform cell-directed assembly with added liposomes

containing trans-membrane proteins. Knowing that the cells mediate their local pH as a response to hyperosmotic stress, the incorporation of an optically-driven proton pump like BR within the multi-lamellar bio/nano interface surrounding the cells could be used to manipulate optically the local pH. This in turn may be important in controlling how cells organize nano-objects at their surfaces during cell-directed-assembly, allowing the formation of novel nano/bio interfaces – like the integration of cells onto arrays of nanowire electrodes.

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Controlling the Photophysics of Protein Arrays in Thermoresponsive Soft Materials

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Program Scope

The *Nanostructured Biocomposite Materials for Energy Transduction* research program has as its overarching goal the design, synthesis, and characterization of functional nanoscale materials that exploit the native capabilities of biological molecules to store and transduce energy. Membrane proteins, for example, facilitate many key cellular processes, including signal recognition, ion transport, and energy transduction. In effect, they possess all of the basic properties necessary for the construction of nanoscale devices appropriate for any of a variety of tasks, including optical/electronic signal amplification, switching, gating, data storage, sensing, and energy storage and conversion.[1] The use of membrane proteins as the basis for functional nanoscale devices, however, has received considerably less attention than have water-soluble proteins, a result of the lack of materials allowing for their structural and functional stabilization outside of their native environment. To address this challenge, we have developed a platform to organize a variety of biomolecules comprising a series of polymer-grafted, lipid-based complex fluids whose structure and function mimic those of natural cell membranes. These synthetic/biological molecule composites (proteo-complex fluids) will ultimately be stabilized by integrating them with selected hard (inorganic), nanostructured materials, among them mesoporous oxides and metals, and polarized ferroelectrics. Integration of the soft and hard components enables connection of the nanoscopically-organized biomolecule arrays to the macroscopic world, an important requirement if the resultant composites are to find application.

Recent Progress

Our recent work has focused on the development of soft materials employing host-mediated energy transduction to regulate the collective properties of encapsulated protein arrays. This portion of the program has involved the synthesis of thermoresponsive soft nanostructures based upon our original polymer-lipid-based complex fluid [2] composition (a mixture of a phospholipid (dimyristoylphosphatidycholine, DMPC), a PEG grafted-lipid conjugate (PEGylated-dimyristoylphosphatidylethanolamine, DMPE-EO_n) and a zwitterionic co-surfactant (e.g., *N,N*-dimethyldodecylamine-*N*-oxide)) and their characterization. These molecular components have been shown to spontaneously self-assemble when dispersed into water, forming non-covalent aggregates that are both uniform and optically transparent, and to undergo a thermoreversible phase transition, converting between an optically-birefringent, elastic solid (gel) lamellar phase ($L\alpha g$) and a non-birefringent, low-viscosity, 2D hexagonally-ordered array of prolate micelles (H_1). Both phases feature lattice dimensions on the order of hundreds of nanometers that are tunable over a wide range by simply changing either the water content or the number of repeat units on the appended PEG chains. The inverted phase transition allows for the introduction of delicate biomolecules in the low viscosity (cold) state; subsequent warming to room temperature promotes organization of the biomolecules into either the water or alkyl chain bilayer domains.[3] To date, we have designed and prepared three new soft nanostructured materials (complex fluids), two of which are derivatives of the original

composition in which the PEGylated lipid component is replaced with either a triblock or a diblock copolymer of general formula, PEO_n-PPO_m-PEO_n or PEO_n-PPO_m. A third formulation exploits the temperature-dependent properties of poly(N-isopropylacrylamide).[4] These new materials permit external control of both the dimensions of the water channel in the lamellar structure or the aggregate structure (between 1D, 2D or 3D). This control of scaffold architecture/symmetry is significant because it may provide a facile means to regulate the internal packing arrangement and ordering of encapsulated guests (*e.g.*, proteins) and thus, their collective properties. Thus, the thermoresponsive soft materials developed under this program represent an advance in biomimetic scaffolds in that they are not passive, but rather "active" in the sense that the arrangement and ordering of the encapsulated proteins can be host-modulated.

Of particular interest in our initial evaluation of these novel materials has been their influence on the properties of a model photoactive membrane protein, the bacterial photosynthetic reaction center (RC). The RC is a multisubunit, trans-membrane protein complex whose native function is to convert light into electrochemical energy through generation of a stabilized, transmembrane charge separation (of ca. 30 Å). This charge separation is extremely efficient, with quantum yields approaching 100%. In addition, RCs can perform a cascade of light-driven electron transfer reactions whose rates span nearly 14 orders of magnitude (from fs to s). Harnessing this optimized functionality outside of the cell is critically dependent upon stabilizing and vectorially orienting the protein in a synthetic matrix. If this can be accomplished, RCs may potentially provide the basis for the development of a wide variety of light-transducing materials such as solar cells, photodetectors, and photodiodes.

Recently, we have demonstrated the stable reconstitution of RCs into the "first generation" thermoresponsive, soft, nanostructures (PEGylated-lipid based compositions).[5] Introduction of photosynthetic RCs into the complex fluids was found to have little influence on the lamellar gel phase architecture, but did significantly alter the cold phase, converting it from a 2-D hexagonal to a discrete 3-D cubic structure. More importantly, the RCs were found to remain structurally and functionally intact upon reconstitution into the complex fluids. Specifically in the cold phase of the complex fluid, primary electron transfer kinetics were found to be identical to those of detergent-micelle-stabilized RCs under all conditions studied. At a threshold concentration, RCs in the warm, lamellar gel phase of the complex fluids show an extended lifetime. In addition, prolonged equilibration (hours) of the RCs in the lamellar gel phase results in non-monoexponential kinetics of primary electron transfer, with a subpopulation of RCs displaying extended excited state lifetimes. We have tentatively attributed this behavior to reversible, in-plane protein aggregation. These results show that stimulus-responsive, complex fluids may offer an opportunity to employ dynamic architectures as a means to externally control the organization (i.e., internal packing arrangement) and, hence, the physical properties of the intercalated guest protein. This work represents an important step towards the fabrication of organized arrays of membrane proteins and the control of their collective properties – materials parameters that are critical to the development of functional, protein-based, nanostructured devices.

Future Plans

Because the complex fluids are non-covalent aggregates that form *via* self-assembly of pre-synthesized molecular components, there is tremendous flexibility in creating new systems simply by the omission or inclusion of various molecular constituents. One way of achieving greater tunability over architecture is the introduction of an additional stimulus-responsive feature, such as pH or ionic strength control, in

addition to temperature control. Our future studies will thus center on the synthesis and characterization of new stimuli-responsive soft materials, particularly pH responsive materials, with the goal of achieving significantly greater control of their supramolecular architecture. Whereas temperature control was found to produce two or three distinct structural states, we believe that careful control of the ionization state of tethered polyelectrolytes may offer much finer control over nanospace architecture, a key step in controlling the encapsulated proteins and regulating energy transfer and conversion.

Work in the development of stimuli-responsive soft materials has demonstrated there is a significant need for developing design principles to guide our synthetic efforts. This defines an second important avenue of investigation: the development of mathematical models that can aid in *a priori* predictions of the conformation of appended or intercalated polymers under nanoscale confinement, their association with a charged biomembrane, and their response to external stimulus changes (temperature and pH), eliminating the time-consuming synthesis of bioconjugates that may not possess the desired physical properties.

Next, the integration and modulation of the photophysical properties of RCs will be further studied by enhancing the RC functionality through co-integration of the associated light harvesting proteins which, in nature, serve to collect sunlight and efficiently transfer its energy to the RC. This will be an important step in practical use of RCs, allowing it to function as a true solar-element. The detectable changes observed in electron transfer events in the thermally-triggered structural states in the complex fluids clearly delineate yet another area of study: devising approaches to defining the relationship between architecture and lateral (in-plane) membrane segregation and the collective response of an array of proteins.

Finally, controlling the orientation of encapsulated proteins is essential if a macroscopic effect is to be observed. Thus, characterization and processing the soft materials so as to achieve vectorially-ordered arrays of encapsulated RCs represents an essential step on the path to useful protein-based materials. Our prior work suggests one means of achieving the needed directional alignment of proteins is exploitation of the inherent differences in the diamagnetic and electric susceptibilities between the matrix and the encapsulated proteins. This alignment may be achieved by combining magnetic field processing of the matrix (previously demonstrated to produce near mono-lamellar domain lamellar ordering)[6] with the application of an external electric field, which would serve to promote unidirectional alignment of the encapsulated proteins in ordered lamellar domains. Towards this end, we will initially use time-resolved electron paramagnetic resonance spectroscopy to study the orientational and directional charge separation properties of the RCs in proteo-complex fluids as a means to deduce the distribution of guest molecule orientation within the lamellar domains. Orientation will be easily tracked as photoinduced charge separation results in the formation of a radical ion pair whose orientation is well defined relative to the protein structure. In contrast, we plan to use mutant RCs lacking cofactors where charge recombination reactions dominate and results in a high yield of the triplet state of the dimeric special pair electron donor within the RC. The principal magnetic axes of this triplet state are also well defined relative to the protein structure, and thus provide another tool for determining orientation of the protein relative to that of the lamellar domains.

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Manipulating Nature's Molecular Machines: Development of Light-Gated Ion Channels

Dirk Trauner

Many proteins function like molecular machines that undergo mechanical movements in response to input signals. These signals can consist of changes in voltage, membrane tension, temperature or, most commonly, ligand concentration. A major benefit for biology and technology could be achieved if these nanoscopic protein machines could be reengineered to possess artificial control elements. Optical switches would be especially powerful, since they could be activated remotely with precise temporal and spatial control.

We have introduced a modified voltage-gated potassium channel that can be conditionally activated with an azobenzene photoswitch.[1] This device, termed SPARK (for synthetic photoisomerizable azobenzene regulated K-channel), can be incorporated in biological membranes and used to control the firing pattern of neurons. More recently, we have succeeded in manipulating a second channel of central importance in neurobiology. Using structure-based design, we have created an ionotropic glutamate receptor (iGluR) that can be turned on and off by irradiation with different wavelengths of light.[2] Our new device, termed LiGluR, thus functions as a light- and ligand-gated ion channel and can be used to rapidly depolarize biological membranes with light.

While the development of light-activated ion channels could have a major impact on neurobiology, the reengineering of Nature's molecular machines could also provide valuable devices for bioelectronics and nanotechnology. Our SPARK channel functions, in essence, as a nanoscale photo- and field-effect transistor. LiGluR, on the other hand, can be activated by light and soluble agonists. Since the SPARK and LiGluR channels are each regulated by two different input signals, they – literally - function as logic gates (AND and OR gates, respectively). Due to the spectral sensitivity of the azobenzene moiety, however, they also provide an analog output. Since modified ion channels can be readily incorporated in natural and synthetic membranes, the integration of these nanoscale devices into macroscopic systems should be straightforward.

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Title: The nano/bio interface for in vitro and in vivo diagnostics of health and disease.

Abstract A number of technology platforms that rely on combinations of chemistry, nanotechnologies and microfluidics are emerging for both *in vitro* and *in vivo* diagnostics of health and disease. In this talk, I will discuss some of these approaches, and I will focus on fundamental and enabling issues that must be addressed for their successful implementation.

Many proposed *in vitro* diagnostics technologies are based upon the label-free, electronic-based detection of genes and/or proteins, meaning that there is one protein or gene capture agent for each protein or gene to be detected. One such method is based upon an AC-impedance measurement. For this technique, the biological medium (serum, for example) and the sensor element (a silicon electrode, for example) are two conductors, and changes in the dielectric interface between these two conductors, such as a target/probe binding event, are recorded by measuring the frequency-dependent complex impedance. A second method, based upon various types of inorganic or organic semiconductor nanowires, relies on the probe/target binding event to modulate the local chemical potential near the nanowire surface, and thus gate the DC conductivity of the nanosensor. For both sensor types, I will discuss how the fundamental sensor platforms, and even large-scale sensor libraries, may be fabricated using existing methods. However, the issues that are limiting even the modest implementation of either of these technologies involves the chemical interface between the biosensor element and the biomolecular capture agent, and the biomolecular capture agents themselves. I will discuss possible solutions, including preliminary data, for both of these limiting issues.

In vivo diagnostics of disease is also a rapidly advancing field, and one of the most developed technologies for this is positron-emission-tomography (PET)-based molecular imaging. PET-based molecular imaging is distinct from other modalities, such as CT and MRI, in that PET images dynamic metabolic processes, rather than static physiological structure. Over the past several years, increased demands have been placed upon in vitro molecular imaging as disease pathways have become better understood. For many diseases, clinical presentations that were once diagnosed as a single disease are now viewed as multiple diseases, each with a (potentially) different treatment. However an accurate diagnosis is essential for both developing and directing such specialized treatments. The result is that new technologies for expanding and diversifying the capabilities of molecular imaging are needed. I will discuss two such approaches. The first relates to the chemistry behind the preparation of highly targeted molecular imaging radiopharmaceuticals. The second will focus on miniaturized chip-based chemical synthesis laboratories for the rapid and efficient preparation of those compounds. Both of these approaches are framing fundamental materials and chemical issues that will need to be solved for the technologies to move forward.

Many of the basic scientific issues surrounding both *in vitro* and *in vivo* diagnostics involve a common set of chemical and materials challenges. Identifying these common challenges will be a theme of this presentation.

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Session VI:

Topic: Biotemplated Materials Synthesis

Biomolecular Mechanisms Reveal New Routes to Novel Nanostructured Materials for Energy Applications

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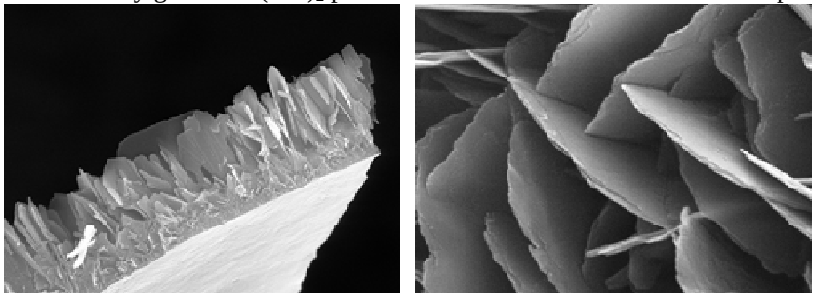
Program Scope

Biological systems fabricate multifunctional, high-performance materials at low temperatures and near-neutral pH with a precision of three-dimensional nanostructural control that exceeds the capabilities of present human engineering. Our analyses of the proteins, genes and molecular mechanisms governing the formation of silica in a marine sponge have revealed a unique mechanism of synthesis with industrial applicability for the low-temperature manufacture of nanostructured materials with compositions and structures that were not previously attainable. Advantages of these novel materials for applications in energy generation, transduction and storage are suggested, and are under investigation.

Recent Progress

We discovered the mechanism governing the nanofabrication of silica in a biological system, and translated this mechanism to develop a new low-temperature route for the synthesis of a wide range of nanostructured metal-oxide, -hydroxide and -phosphate semiconductor thin films without the use of organic templates. As a first proof of principle, we have used this process for the low-temperature synthesis of a strongly photoconductive cobalt hydroxide-based thin film material never before attainable through conventional or high-temperature methods. This material exhibits high dopant density, high surface area of single-crystal domains, exceptionally long minority carrier lifetime and strong absorption in the visible, making it potentially attractive for photovoltaic applications. Doping with high levels of lithium ions has been achieved, making this material potentially useful for improvements in lightweight batteries. Because no organics are used, this new biologically inspired synthesis method yields exceptionally pure inorganic semiconductors, and thus is potentially integrable with conventional manufacturing methods such as MOCVD.

Biomimetically grown $Co(OH)_2$ plates connected to a flat conductive backplane



In research leading to these developments, we discovered that the silicateins, a family of enzyme proteins we found occluded within the silica needles made by a marine sponge, can

catalyze and structurally direct the polymerization of silica, silsesquioxanes, organometallics and a wide range of metal oxide semiconductors from the corresponding molecular precursors at neutral pH and low temperature. These were the first reported examples of enzyme-catalyzed, nanostructure-directed synthesis of semiconductors. Interaction with the template-like protein surface stabilizes polymorphs of these materials (e.g., the anatase form of titanium dioxide and the spinel polymorph of gallium oxide) otherwise not formed at low temperatures. This observation and the preferential alignment of the Ga_2O_3 nanocrystallites suggested a pseudo-epitaxial relationship between the mineral crystallites and specific functional groups on the templating protein surface. Genetic engineering of the protein, in conjunction with diffraction studies of the semiconductor products and the templating surface, confirmed the mechanism of action and identified the functional groups responsible for catalysis and templating. This mechanistic understanding was confirmed and extended through the synthesis of a series of "biomimetic" peptides, polymers, small bifunctional organics and multifunctional self-assembled monolayers, yielding new structure-directing catalysts of siloxane and metallo-oxane polycondensation from the corresponding molecular precursors at low temperature and neutral pH.

The success of these results permitted us subsequently to translate the fundamental mechanisms underlying silicatein-mediated catalysis and templating to a process wholly controlled by chemistry and physics, without the use of any biochemical or organic molecules. As described above, we have used this biologically inspired process for the low-temperature synthesis of more than 27 different metal-oxide, -hydroxide and -phosphate semiconductors - many in forms that could not be attained by conventional syntheses. The electronic properties of these novel materials suggest strong advantages for energy applications.

Future Plans

We plan to extend this approach to the biologically inspired low-temperature nanofabrication of unique III-V semiconductor thin-films, as these materials offer unique advantages for energy applications. Our aim is to predictively translate our findings made in our continuing investigations of the molecular mechanisms governing silica nanofabrication in biological systems to develop a robust new methodology capable of producing nanostructurally controlled metalloid and metal oxide semiconductors are related materials for improved efficiency of energy generation, transduction, storage and use. We aim to test the utility of the bio-inspired synthesis method we are developing through the low-temperature nanofabrication of materials for improved performance of *lithium-ion electrical storage batteries.* Our studies will continue to build on our ongoing collaborations with research leaders at two DOE facilities, the Stanford Synchrotron Radiation Laboratory (SSRL) and the Center for Nanophase Materials Science (CNMS) at the Oak Ridge National Laboratory (ORNL

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Self-assembling Templated Polymer-Hydroxyapatite Bioinspired Nanocomposites

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Program Scope

Nature provides elegant examples of hierarchical structures that are hybrid materials formed by mineralization of organic templates that facilitate and direct the structure and morphology of the inorganic phase. The synthesis and design of nanocomposite materials will greatly benefit from a better understanding of how the organic template controls the structure and morphology of the inorganic materials. In contrast to work done by other groups in biomineralization, [1-3] our aim is to build hierarchical macroscale organic-inorganic structures by self-assembly *all the way from the nanoscale to the macroscale* using self-assembling multiblock copolymer templates. This work can be broadly divided into four parts: (i) synthesis and characterization of the multi-block polyelectrolyte copolymer templates with unique hierarchically self-assembling properties, (ii) understanding the self-assembly behavior of these multi-block polyelectrolytes using theoretical methods, (iii) controlled mineralization of these polymeric templates at multiple length scales, and (iv) development and utilization of novel solid-state NMR techniques to elucidate the structure of these organic-inorganic nanocomposite materials. Because of its biological importance, adaptability for templated growth, availability of ample data along with a wealth of structure and compositional variability synthesis of hydroxyapatite using pentablock copolymers as templates was chosen as the system of interest.

Recent Progress

We have synthesized new multi-block copolymers that show very interesting and unique self-assembly behavior that serve as templates for growth of the inorganic phase. We are investigating the fundamentals of phase behavior of these pH- and temperature-sensitive block copolymers in aqueous solutions. As the pH is increased, small angle X-ray scattering (SAXS) and neutron scattering (SANS) studies at Argonne National Laboratory show evidence of transitions from spherical to cylindrical micelles in solution (Fig.1). These micelles in solution can act as templates for growth of the hydroxyapatite phase. At higher concentrations, these copolymers self-assemble in solution, at elevated temperatures and pH values, to form gels (Fig. 2) as well as organized, crystalline, elastic, macroscale solids.[4] To our knowledge, this is the first report of the self-assembly of synthetic polymers that form such macroscale solids in solution.

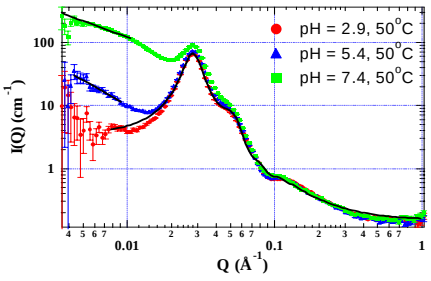

Fig. 1 SANS of pentablock solutions showing a transition from spherical to cylindrical micelles with increasing pH

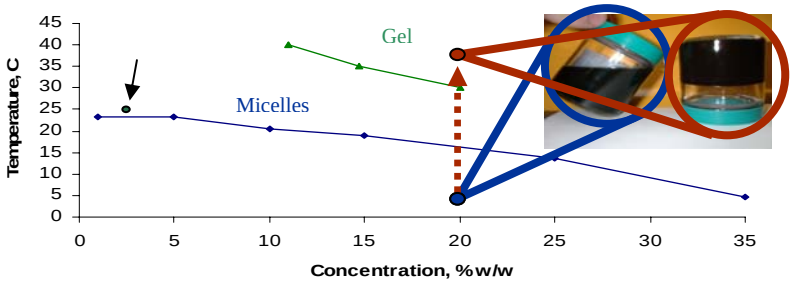

Fig. 2. Phase transitions of pentablock copolymers at pH 7.0

We have developed molecular dynamics (MD) simulations and modeled the pH and temperature transitions based on the changing relative hydrophobicities of the various blocks in the copolymers that provide the driving force for the self-assembly. By modeling the solvent explicitly, the systems that can be simulated are quite small. But we see that typical micelles become more cylindrical (Fig. 3) as either the temperature or the pH is increased, which is the same results seen in experimental scattering studies above (Fig. 2). Implicit solvent allows for simulations of much bigger systems, where many micelles are present.[5] Different phases are shown as a function of both concentration and solvent quality, and several micelle superstructures are found. A typical snapshot of the simulation is shown for a concentrated system, where an ordered structure of micelles is found (Fig. 4).

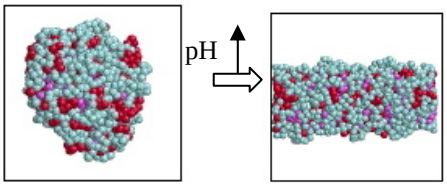

Fig. 3 MD simulations of transition from spherical to cylindrical micelles with pH

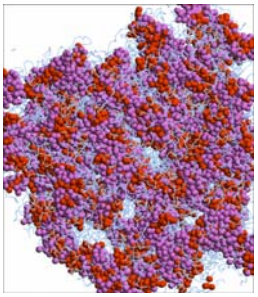

Fig. 4 Micelle superstructures

Growth of three-dimensional hydroxyapatite on these polymer micelle templates was investigated. Initially a carboxylated Pluronic that forms spherical micelles and gels was used as a template, but further studies were conducted with the pentablock copolymers. Saturated calcium phosphate solution was obtained by mixing calcium nitrate and ammonium dihydrogen phosphate, in a copolymer micelle solution. Hydroxyapatite formation was observed within 1 hour and the growth of the inorganic phase was completed within 24 hours. Transmission electron microscopy observations indicate that spherical particles of roughly 40 nm in diameter were formed, twice the size of the micelles of the polymer alone (Fig. 5). At long aging times, agglomeration of the inorganic particles to larger fractal units of 300-400 nm was observed.

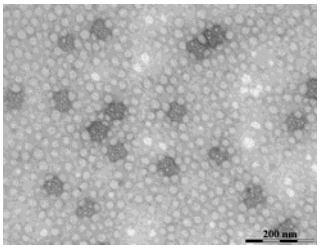

Fig. 5 TEM of coated micelles

Composite particles containing polymer core were separated from un-coated micelles by dialysis of polymer molecules at low temperature. Electron microscopic study of the dialyzed sample confirmed the presence of calcium phosphate containing agglomerated particles of about 30-40 nm in diameter. Radius of gyration measurements of polymer micelle and hydroxyapatite coated micelle solutions using light scattering confirmed existence of inorganic phase coating of the micelles. Energy dispersive spectroscopy also showed presence of Ca, P and O indicative of calcium phosphate mineralization of the micelles and the ratio of Ca to P was similar to that of stoichiometric hydroxyapatite (HAp).

Since the interactions between the inorganic phase and the polymers are ionic in nature, while the driving force for the self-assembly of the polymers is due to hydrophobic interactions, we hypothesized that the self-assembly of the polymer will proceed even in the presence of the inorganic phase. The coated micelles, up on increasing the polymer concentration, formed gels by self-assembly similar to the polymer itself, even in the presence of the inorganic component. This provides a unique bottom-up approach to materials design and fabrication, since the nanostructure is preserved in the macroscale solids formed.

Particular emphasis is on the structural and morphological characteristics of the inorganic phase in these solids as controlled by the assembly of the organic template. Solid-state NMR is a very powerful technique to investigate polymer-apatite nanocomposites. Novel solid-state NMR techniques were developed[6] to investigate these nanocomposite structures, and they were tested on actual bone samples, to validate the techniques and to compare the nanostructure of these synthetic polymer-hydroxyapatite composites with those of natural bone. In order to determine the true shape of the nanocrystals in bone, three methods have been applied to the same samples: (i) The crystallite thickness was determined by ^{1}H-^{31}P NMR using the distant-dependent dipolar dephasing of ^{31}P by the protons in the collagen matrix (but not by the hydroxide protons). (ii) SAXS I(q) curves were measured at Argonne National Lab, and simulated for densely packed particles of various geometries, including long boards of 3.2 nm thickness and 9 nm width, using a newly developed numerical method. (iii) The WAXD I(2θ) profiles were measured without distortions by "baseline correction" and fully simulated using the Debye formula. The analyses of the NMR, WAXD, and SAXS data show consistently that the crystallites in bone are >20-nm long "boards" with thicknesses of three and four apatite unit cells, i.e. 2.4 nm and 3.2 nm, and widths varying between 6 and ~12 nm.

Future Plans

One of the difficulties in synthesis of inorganic materials on self-assembled templates is the necessity to maintain the saturated concentration to allow growth of the solid phase on the template but below the critical super saturation level to prevent homogeneous precipitation in the solution. Similarly, maintaining saturation level throughout the solution uniformly is a formidable challenge as it is necessary to continuously supply reactants at a same rate the reactants are consumed. A number of elegant but quite elaborate experimental set ups have been employed,[2] yet in many cases, the homogeneous nucleation in the bulk, in addition to growth at the organic sub-phase, have been observed. Our experience in homogeneous precipitation by urea (or similar analogs) provides a simple solution to super saturation and non-uniformity in the concentration of reactants in the solution. Upon heating the aqueous solution, urea slowly decomposes to release OH^- and HCO_3^- uniformly allowing precipitation of inorganic phase on the template functional groups without leading to nucleation in the bulk solution.[7] Adequate urea decomposition may be realized at ambient temperatures by addition of chemical catalysts such as hydrazine hydrate, or enzyme urease.

High concentrations of pentablock copolymer solutions self-assemble to hexagonal close-packed cylinders at elevated temperature and pH. Hexagonal close-packing of polymer cylinders at the nanoscale is analogous to collagen formation and morphology found in the bone structure. Mineralization of close-packed polymer cylinders will closely mimic bone formation except for the temperature and pH employed in laboratory experiments. Higher concentration polymer solutions will be heated and pH of the solution will be increased to form hexagonal close packed polymer fibrils. Once the polymer system is characterized for the morphology by AFM and TEM, mineralization with HAp will be studied at various pH and temperature combinations. Stability of polymer structure at the mineralization conditions (T, pH, concentration of ions) will be assessed by in situ measurements. Scattering and electron microscopy techniques will be used to characterize the nanocomposites. MD simulations of the self-assembly process will be crucial in guiding this work.

In parallel, conditions leading to appropriate level of mineralization will be predicted by determining the concentration of all species in the solution computationally using equilibrium constants at the temperature and pH under consideration. The system is rather complicated involving dissociation of tri-protonic phosphoric acid, multitude of calcium phosphate species in the solution, hydrolysis of other constituents including ammonia, carbonates (from urea) and possibly others at the temperature of concern. Fundamental understanding of mineralization of pentablock polymers requires careful analysis of polymer-HAp interface by NMR and other techniques sensitive to short-range interactions. Based on the successful calibration using bone apatite described above, NMR and quantitative scattering analyses will be applied to the biomimetic materials, to determine the composition and size of the inorganic particles formed.

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Synthetically Modified Viral Capsids: Building Blocks for Nanoscale Materials

Matthew B. Francis DOE Contractors Meeting Abstract - Summer 2005

Introduction. Research in the Francis group has focused on the development of robust and practical biomolecular scaffolds for the ordering of nanoscale materials. By using the inherent periodic spacing of protein assemblies as a template, highly ordered arrays of nanocrystals and other material components can be prepared in an efficient parallel manner. Furthermore, as multiple sites on each biomolecule can be modified, complex, multicomponent assemblies could be accessible that would be extremely difficult to synthesize using other methods. Current efforts are also directed toward the attachment of the scaffolds to the surface of electrodes using additional bioconjugation reactions, or the "growth" of these materials through self-assembly processes nucleated by surface-bound initiators.

Covalent Modification of the Tobacco Mosaic Virus. In order to explore these possibilities, new chemical methods were required to attach nanoscale components to the surfaces of self-assembling proteins. This possibility was first explored in the context of the tobacco mosaic virus (TMV) capsid, a 300 nm hollow tube that is assembled from 2,100 identical protein monomers, Figure 1. The full protein structure possesses a central cavity that is 4 nm in diameter, through which small molecules can diffuse readily. By using previously described culturing and isolation protocols, 1-2 g of highly pure virus can be obtained from each kilogram of infected tobacco leaves; thus, this target could provide a highly practical building block for the preparation of self-assembling nanoscale materials. However, despite the large volume of research that has been carried out on TMV, the covalent functionalization of the wild-type capsid has never been reported.

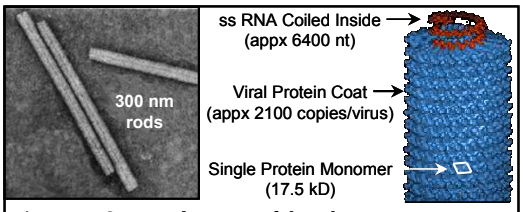


Figure 1. Structural aspects of the tobacco mosaic virus.

To address this challenge, two powerful modification reactions have been developed for the attachment of organic molecules to TMV capsids. It has been found that the exterior surface of the virus can be modified using diazonium salts, Figure 2a. Under the proper conditions, these reagents can achieve full conversion, and extensive trypsin digest and MS/MS experiments have revealed that the modification occurs exclusively at tyrosine 139. This strategy has worked particularly well for the installation of a ketone functional group (shown in red), which has been coupled to animo-oxy derivatives (blue) through oxime formation. As a result, a modular strategy has been developed for the introduction of 2,100 new functional groups on the outside surface of the tube (shown in yellow in Figure 2c). These positions are spaced evenly at 3.3 nm intervals.

A highly selective method has also been developed for the interior functionalization of the capsid. Through the use of carbodiimide coupling agents, a wide range of amines (shown in green in Figure 2b) can be attached to Glu 97 and Glu 106. These residues line the interior cavity of the capsid, thus providing an opportunity to control the transport properties of the 4 nm interior channel, Figure 2c. In additional experiments, these interior sites have been linked with difunctional amines, resulting in the crosslinking of adjacent capsid

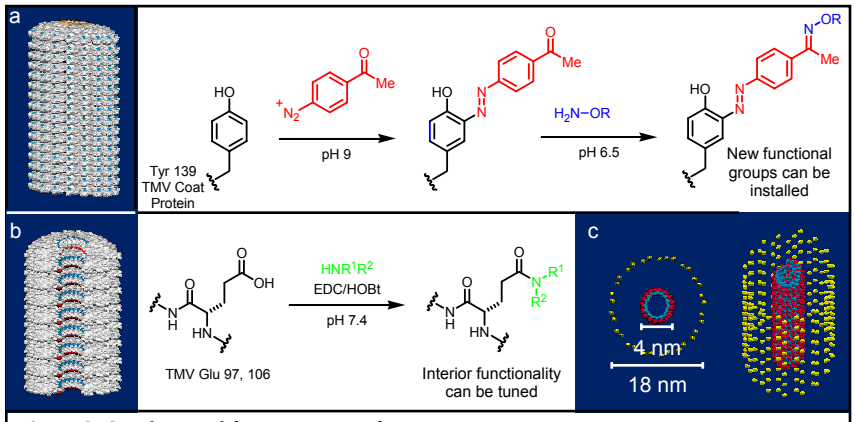


Figure 2. Covalent modification strategy for TMV

monomers. It is anticipated that this will dramatically stabilize the protein assembly, and thus will allow an expanded range of conditions for materials synthesis.

With this scaffold in hand, current experiments are focusing on the construction of highly regular arrays of nanocrystals and chromophores. To prepare inorganic particles with surface functionality that is complementary to the TMV ketones, gold nanocrystals that are surface passivated with thioanilines have been synthesized, Figure 3. Fluorescence and TEM measurements have indicated that these particles are 1.8 nm in diameter and have narrow polydispersity. Through an additional coupling reaction, some of the pendent anilines have been functionalized with amino-oxy derivatives through amide bond formation. The ability of the resulting nanocrystals to couple to ketone-labeled TMV is currently being evaluated. Once prepared, the collective photophysical properties of annular and tube-like assemblies of nanocrystals will be determined. The full spectroscopic evaluation of these aspects, as well as the theoretical interpretation of their behavior will be carried out in collaboration with other members of the LNBL faculty.

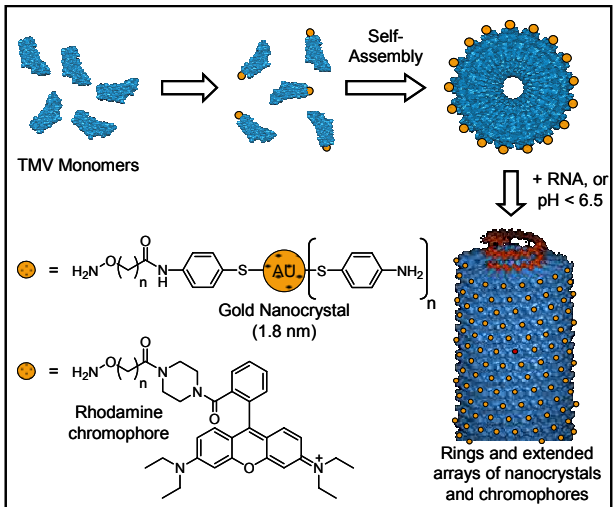


Figure 3. Self-assembling arrays of chromophores and nanocrystals templated by modified TMV capsids.

In terms of chromophore arrays, a series of amino-oxy-functionalized rhodamine derivatives have been synthesized and coupled to the periphery of the TMV rods, Figure 3. The close association between the exterior coupling sites (3.3 nm) is well within the Förster radius for energy transfer; however, the rigidity of the protein scaffold prevents non-radiative quenching arising from interchromophore contact. These systems could offer significant advantages over existing light harvesting systems, as assemblies possessing hundreds or thousands of donor chromophores could be accessed through efficient self-assembly processes. Furthermore, the site-isolation allows the use of donor chromophores with overlapping excitation and emission spectra.

Additional experiments are exploring the ability of TMV rods coated with hydrophobic polymers to form Langmuir-Blodgett films. As the proteins will no longer be soluble in aqueous solution, thin capsid films should assemble at the air/water interface. It is expected the polymers will form microcrystalline domains (much like those in isotactic polypropylene), resulting in the formation of highly ordered and robust membrane-like materials. After preparation, these films can be "lifted" from the surface of the water by dipping substrates into the solution, and atomic force microscopy (AFM) and other methods will be used to relate the type and length of the polymer chains to the packing arrangement and the macroscopic properties of the materials.

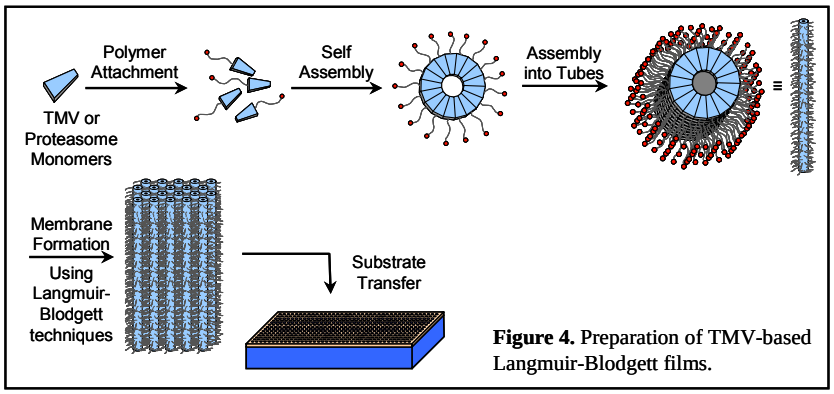


Figure 4. Preparation of TMV-based Langmuir-Blodgett films.

Covalent Modification Bacteriophage MS2. Recent efforts have also focused on the dual surface modification of icosahedral viral capsids, ultimately resulting in a versatile, self-assembling protein scaffold for the construction of core/shell materials. For these studies, we have chosen the bacteriophage

MS2 as our target. This virus comprises a single strand of RNA (3800 nucleotides) encased in a 27 nm hollow shell assembled from multiple copies of a single protein, Figure 5. Of particular importance to applications that require modification of the capsid interior, the protein shell of MS2 possesses 32 holes that are 1.8 nm in diameter, thus allowing small molecules to access the internal volume of the capsid. MS2 infects specific strains of *E. coli* as its host, and is completely harmless to humans. It can be propagated using routine broth culture techniques and purified using a precipitation procedure, typically yielding 30 mg of highly pure virus per liter of broth.

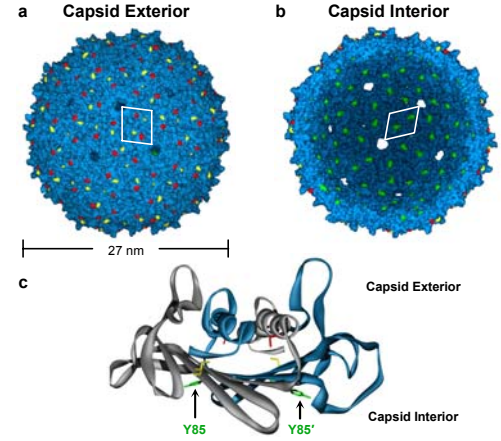


Figure 5. Bacteriophage MS2. **a)** Access to the capsid interior is provided by 32 pores that are 1.8 nm in diameter. The position of two modifiable cys residues are indicated in red and yellow. **b)** A cut-away view of the capsid reveals tyrosine 85 (green) as a surface-accessible residue on the interior surface of each capsid monomer. **c)** The location of this residue is indicated in a "side-on view" of two coat protein monomers. The position of this structural unit is indicated in white in **a** and **b**.

Following isolation of the viral capsids, a highly efficient method has been developed for the removal of the RNA genome from the core of the particles. This can be accomplished by exposing native MS2 to pH 11.7 for a period of two hours. It is believed that the highly alkaline conditions degrade the RNA selectively and reduce its affinity for the capsid interior by deprotonating the associated lysine residues, although the method by which the RNA fragments escape the capsid shell is currently under investigation. Following this exposure, the empty capsids can be isolated through precipitation with over 80% protein recovery. As shown in Figure 6a, the RNA core found in native MS2 normally prevents the staining agent ($UO_2(OAc)_2$) from entering the capsid interior. However, after removal of the genome, the center of the particles appears dark due to penetration by the stain, Figure 6b. No capsid fragments or altered assemblies have been observed to date. UV spectral analysis of empty capsid shells isolated using gel filtration indicates that the RNA absorbance at 260 nm is completely absent, Figure 6c. Subsequent empty capsid stability studies have indicated that the shells do not disassemble in the pH range of 3-9 over a 12 hour period, with only minor losses occuring at pH < 3 or pH > 10, Figure 6d.

The exterior surface of each of the 180 identical subunits comprising the capsid shell possesses two cysteine residues (Figure 5a), thus providing a convenient handle for modification through disulfide exchange. Through MALDI-MS-based quantification, it has been determined that >90% of these sites can be modified by some small molecules, resulting in the installation of up to 360 functional groups on the outer surface of the sphere. Using this technique, polymer chains and initiators for atom transfer radical polymerization (ATRP) have been coupled to the capsid, and the attachment of inorganic nanocrystals, actin polymerization terminators, and chromophores is underway.

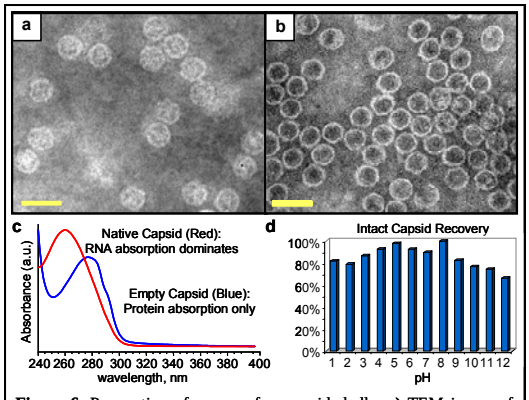
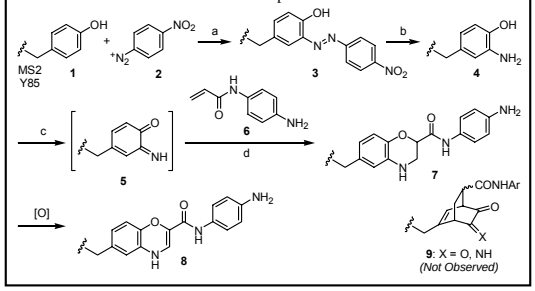


Figure 6. Preparation of genome-free capsid shells. **a)** TEM image of native MS2 capsids stained with uranyl acetate. The RNA-packed interior prevents the staining agent from entering the protein shell. **b)** after exposure to pH 11.8 for 2 h, the RNA genome is cleanly removed, allowing the stain to reach the center of the capsid. **c)** The loss of RNA can be confirmed by the absence of absorption at 260 nm in samples isolated using gel filtration. **d)** The resulting capsids have are stable over a wide pH range. Each sample represents a quantitative gel filtration analysis after a 12 h exposure period. The scale bars in **a** and **b** indicate 50 nm.

In order to modify the interior surface of the capsid, a new bioconjugation reaction has been developed. A native residue, tyrosine 85, was targeted for this purpose, providing 180 modification sites on the interior surface, Figure 5b,c. Using diazonium coupling reactions, tyrosine 85 was modified with high efficiency and selectivity. In particular, virtually complete coupling was achieved in 15 minutes through exposure to 5 equivalents of diazonium salt **2**, Scheme 1. Analysis of modified capsids isolated using size exclusion chromatography indicated the presence of a new absorption band at 355 nm, and MALDI-TOF MS analysis of the protein monomers confirmed single modification. Both

methods verify that >95% conversion was achieved with excellent protein recovery. The selectivity of this reaction for tyrosine 85 was confirmed through trypsin digest analysis.

Scheme 1. Modification of the MS2 capsid interior.



Unfortunately, diazonium salts lacking the nitro substituent attained lower levels of conversion, thus limiting the range of functionality that can be installed using this method. To overcome this problem, the azo bond of **3** was reduced with sodium dithionite to afford *ortho*-amino tyrosine derivative **4**. This reaction reaches full conversion in 2 h at room temperature, and can be followed by the absorbance loss at 355 nm. This functional group can then be oxidized to *o*-iminoquinone **5** with $NaIO_4$, affording a highly reactive functional group for further elaboration.

The reactivity of this functional handle was tested in the context of a Diels-Alder reaction. By screening a series of dienophiles under a variety of reaction conditions, it was found that acrylamide **6** gave particularly efficient conjugation, with conversion levels exceeding 90% in 2 h at rt, as determined by MALDI-TOF MS analysis. No other modification sites were identified after trypsin digest analysis, and exposure of "empty" capsid **1** to **6** and $NaIO_4$ yielded no reaction. Although two Diels-Alder products (**7** and **9**) can be expected, current data are more consistent with initial adduct **7**. High resolution MS analysis of the modified fragment after trypsin digestion indicates that the nitrogen atom remains in the product, and that a subsequent oxidation occurs to afford benzoxazine **8** as the final reaction product. Although $BnONH_2$ reacts with **5** to form oximes, no such adducts are formed for the Diels-Alder product, further suggesting that **9** is not observed. Assignment of the regiochemistry of the reaction product is underway. As indicated by SEC and TEM analysis, no morphological changes were observed for the capsids after this modification procedure.

Current efforts are focused on the construction of site-isolated chromophore arrays on MS2 capsid surfaces, the isolation of reactive catalysts on the capsid interior, and the attachment of hydrophobic polymers to the capsid exterior. It is anticipated that these materials could serve as nanoscale reactors and packing materials for microfluidic chromatography devices. In collaboration with the Groves lab, we will also evaluate the capsids as scaffolds for supported lipid bilayers. Finally, the dual surface modification strategy will be explored as a means to desymmetrize the capsids shells by linking monomers with rigid tethers before capsid assembly.

As a result of these studies, two new functionalizable scaffolds for nanoscale materials construction have emerged. While the initial efforts have been focused on the chemical modification of the protein building blocks, we are now in an excellent position to prepare hybrid inorganic/organic material components for a wide range of energy-related applications.

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Replication of Carbohydrates into Hierarchically-Structured Ceramics

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Project Scope

One of two project goals is focused on understanding the underlying solution chemistry that drives replication of plant tissue into structured ceramic materials. Current research activity in this task invokes hierarchical cellulose structures that serve as highly anisotropic templates from which such ceramic replicas can be prepared. Processing parameters, such as solution temperature, pH, concentration, and post-replication thermal processing are optimized to yield positive replicas of the original biological templates.

In related work, treatment of simple sugars under moderate isothermal conditions and elevated pressures in a closed system was found to produce uniform, porous carbon nanospheres that form by an Aldol condensation route. The dehydroxylation reaction can be probed in real time during the course of the reaction using optical methods. The use of glucose derivatives as precursors to the formation of porous carbon spheres has direct application to DOE core missions associated with separations, catalysis, and sensing.

Recent Progress

Synthesis of hierarchically ordered silica materials with wood cellular structures (poplar or pine) has been realized through *in-situ* mineralization of ordered cellular structures by means of surfactant-directed mineralization as a function of pH.[1,2] At low pH, silicic acid deposits onto the buried interfaces of the wood cellular structure without clogging the pores to subsequently form a positive replica following calcination. At high pH, silica particles rapidly condense and fill the open cells and pits within the structure to form a negative replica of the structure. The silica monoliths so-derived contain intact wood cellular structures (multi-layered cell walls, pits, and well-preserved vessels) with positive and negative structural contrast. Surfactant-templated mineralization using an ionic surfactant (CTAB) not only preserves structural integrity, but also integrates hexagonally ordered nanoporosity (pore diameter: 2nm) into the structure of the cell walls following thermal treatment in air.

Rather than oxidizing the residual organic components by pyrolysis in air, the organic can be carbonized under N_2. As the result, multi-wall carbon nanotubes (MWNT) have been produced on surfactant-templated wood cellular surfaces along with the formation of mesostructured carbon networks inside of the cell walls upon pyrolysis in N_2.[3] The surfactant-templated silica mineralized wood was heated at 150ºC in air for 18 hours to promote surfactant polymerization. The black composite material following graphitization in N_2 at 800ºC (heating rate: 1K/min) was next washed with a 10 wt% HF solution to remove unreacted silica in the structure leaving behind the carbon scaffold containing the carbon MWNTs.

Only the basal spacing (d_{100}) could be observed in X-ray diffraction traces; higher order diffraction features could not be seen due to the highly porous nature of the wood network. Following treatment at 150ºC in nitrogen, the *d*-spacing associated with the mesostructured silica-poplar composite was found to be 3.49 nm when CTAB was used as the surfactant, which is very similar to that found for bulk MCM-41 also prepared under acidic conditions. Such low temperature polymerization of surfactants like CTAB and Pluronic P-123 at temperatures between 150ºC and 200ºC for 10–15 hours was necessary to maintain structural integrity of the network so that it could withstand the higher temperature thermal treatment without collapsing.[4,5]

The observed decrease in d-spacing of 2.45 nm following graphitization at 800°C results from loss of low molecular weight materials as well as shrinkage of the intrinsic wood pore structure.

An HF etch was used as a means to highlight carbon MWNTs from the composite material. Following the HF etch, the dark colored product was found to contain less than 0.1 wt% silica co-mingled with the intact carbon replicas of the intact wood structures. The majority of MWNTs were randomly distributed on the carbonized wood cellular surface and throughout the cellular networks due to the loss of their anchoring sites. However, even though MWNT bundles were mainly observed on the surface, ordered mesostructured carbon structures were also observed inside of the replicated wood cell walls. This suggests that mineralized mesostructured silica formed inside of the cell walls indeed leads to formation of ordered mesostructured carbon that persists even after residual silica is removed by the HF wash. The wood substrate appears to retain carbon mesostructures that are present inside of the graphitized wood cell walls.

It should be noted that the acid catalyzed mineralization process does in fact preserve all the complex wood cellular structures (cells, pits, lumens) throughout the matrix reformulation in carbon. Along with the decrease in basal plane spacing after graphitization at 800ºC, the somewhat low BET surface area (117 m^2/g) and pore volume (0.06 cm^3/g) were significantly increased to 362 m^2/g and 0.27 cm^3/g following the HF wash. This suggests that residual silica was removed from the interstices within the mesostructured carbon region and also between the MWNT bundles. The diameter of the MWNTs formed in this study is quite small (6–7 nm) compared with carbon nanotubes prepared by chemical vapor deposition (CVD) methods. The basal spacing of mesostructured carbon which comprises the cell walls is of the order of 15 – 20 nm, considerably larger than that observed in bulk MCM-41 samples. This explains why the CTAB-templated silica walls coalesce during the template replication process.[6] The large amount of surfactant and high silica precursor concentration used in the synthesis procedure promote formation of a stable cellulose network that changes little upon graphitization. MWNTs had greater lengths (up to 500 nm) than those produced by other procedures (200 nm) that did not invoke a porous wood structure scaffold. Similar results were obtained when a different wood substrate (pine) was used instead of poplar. When a different surfactant was used in the synthesis (Pluronic P-123), carbon nanotubes having somewhat larger diameters (10-13 nm) were observed, but ordered mesoporous carbon structures within the cell walls were not seen.

The carbothermal reduction of mineralized cellulose with metal oxides produces metal carbides (MC).[7,8] The biomorphic cellular MC ceramics were prepared by controlling the amount of metal oxides, and the size of MC particles. The cell, lumen, and pits were mainly retained through agglomeration of nanoparticles. XRD patterns of as-synthesized SiC from a pine/silica composite prepared at 1400°C in Ar revealed mainly the cubic type (β-SiC, $2\theta = 35.562$, $d_{111} = 2.522$Å) phase. The *d* values of four peaks are 2.522, 2.189, 1.542, and 1.314 Å, respectively. At 1200°C a feature ascribed to crystalline silica (cristobalite) was observed at $2\theta = 21.898^o$. At 1400°C this feature was not seen, indicating that the entire amount of mineralized silica quantitatively reacted with the matrix during pyrolysis. After treatment at 700°C for 2h in air to oxidize residual carbon, a greenish yellow SiC was obtained. For TiC synthesis, XRD traces were obtained of the material derived from the filter paper/Tyzor-LA reaction following carbothermal reduction at various temperatures for 2h in Ar. The rutile form of TiO_2 was dominant with only a trace amount of anatase seen following heating at 900°C. At 1000°C, five new diffraction peaks emerge at 2θ values of 36.29, 42.13, 61.13, 73.25, and 77.05^o and the features ascribed to rutile are absent. These peaks originate from titanium oxycarbide (TiC_xO_y).[9] Thus, the formation of the TiC phase is thought to begin between 900 and 1000°C in this experiment. The XRD patterns of the composite heated between 1100-1500°C were similar to those seen at 1000°C. Subsequent heating at higher temperature sharpens the diffraction pattern and removes spurious features thereby confirming the presence of crystalline face-centered cubic TiC with no other phases.

Whole cellular structures such as cells, pits, and lumens were primarily retained following thermal treatment.[10] SEM observation revealed the presence of whiskers (~10%) randomly deposited on the sample surface as well as nano-sized crystalline SiC powders (~90%). The TEM images show the typical morphology of SiC nanoparticles and whiskers. Micrographs of the TiC that is formed at 1500^{o}C are in a highly crystalline cubic form of TiC nanoparticles that replicate the initial cellulose structures present in the wood. The TEM images of the TiC product obtained at 1500^{o}C show fine TiC nanoparticles with particle sizes on the order of 10-50nm (BET surface area, 50 m^2/g) that are loosely agglomerated. The BET surface area of MC was dependent on the reaction temperature used during processing. The TiC products synthesized at temperatures lower than 1200^{o}C maintained a high surface area (>200m^2/g), but yielded a lower surface area at higher temperature due to the increase of crystallinity and the collapse of microporous structures even under conditions where the hierarchical structures should be maintained. SiC materials prepared at 1400^{o}C showed 100-150m^2/g surface area after purification.

The lattice parameter increases with reaction temperature while oxygen content decreases, indicating that the TiO component in TiC is lowered thereby reducing the oxygen content at higher temperature. The lattice parameter in TiC synthesized at 1500^{o}C was 4.327Å, which was consistent with that of pure TiC.[11] In fact, the lattice parameter of the composite heated above 1300^{o}C is also very close to that of TiC. The oxygen content in the TiC_xO_y samples was estimated by XPS measurements of the fine powders. Based upon measured spectra, the sample obtained following heating at 1200^{o}C contained 3.27wt% oxygen. In contrast, the oxygen content for samples heated at 1300, 1400, 1500^{o}C was 0.93, 0.46, and 0.24wt%, respectively.

Future Plans

Use of mesoporous structured materials as templates can be extended to form metal carbides. For example, two possible approaches are available in the preparation of SiC: the mesoporous silica-based approach and the mesoporous carbon-based approach. First, in the mesoporous silica-based approach, carbon precursors are infiltrated, polymerized, subjected to pyrolysis in Ar to form SiC, reacted with HF to remove unreacted silica, and calcined in air to remove carbon leaving SiC. The infiltration of extra carbon and careful control the reaction temperature would result in mesoporous SiC. Second, in the mesoporous carbon-based approach, silica precursors are infiltrated, and processed using a similar approach. In this approach, uniform coating of silica precursors through the carbon network is a key to maintaining the mesostructure after reaction.

To understand the strength of mesoporous carbon networks (graphitic walls) we will use a cellulose network, which is semi-crystalline and readily available in nature. Our previous work provides important information regarding the ability of semi-crystalline cellulose to maintain its structure during mineralization and pyrolysis processing to form metal carbides (SiC and TiC). This is rationalized by understanding the function of lignin in the wood structure which acts as a glue to fasten cell walls together. Lignin is easily leached from the structure under acidic conditions (slower leaching is observed in base). However, the cellulose network is very stable under harsh chemical conditions (both acidic and basic condition) and at temperatures up to 250^{o}C. We will investigate the function of both carbon precursors, cellulose and lignin, in the formation of metal carbides. Cellulose fibers from wood and bleached fibers (no lignin) can be used for comparison as carbon precursors. Lignin-bleached fibers are anticipated to form uniform and ordered metal carbides, while unbleached fibers are expected to form amorphous metal carbides with nonuniform SiO_2 layers due to a non-uniform distribution of lignin over the cellulose structure. Semi-crystalline cellulose, alkyl (or aryl) cellulose, and lignin would also form different metal carbides upon carbothermal heating in Ar following addition of metal preceramic precursors. Biological templates with different cellulose/lignin ratios, such as bamboo, corn stalk, and pollen, also can be used to understand the crystalline form of metal carbides, cubic or hexagonal, that result. In the case of TiC, it is important to minimize the content of oxygen

during the carbothermal reduction of TiO_2/C composites to increase product quality. Uniform distribution of TiO_2 over carbon precursors and control of Ti/C loading (Ti/C=1.0) are important to achieve high quality TiC (lattice parameter (4.327A). We will intend to produce nanocrystalline TiC powders (10-30nm) with high surface area.

It is critical to control surface morphology and functionality in the synthesis of porous carbonaceous material due to the presence of a highly hydrophobic surface. In this regard, an effective method to prepare uniform carbon spheres involves hydrothermal aromatization and carbonization of glucose at 160-180^{o}C in an autoclave.[12] The narrow-size distribution of the isolated carbon spheres was demonstrated by SEM and TEM (50-1000nm) depending on reaction time, reaction temperature, and sugar concentration in the starting solution. Some particle spheres also were seen to form hexagonal contacts with one another. The low thermal treatment method keeps hydrophilic surface hydroxyl groups (-OH) on the carbon spheres, which originate from the precursor sugar. Various chemical reactions of glucose can take place under hydrothermal conditions and result in a complex mixture of organic compounds, and it is rather difficult to characterize the reaction intermediate species in the sealed pressure vessel. The growth of carbon spheres seems to follow the LaMer model, shown schematically in Figure 1.[13]

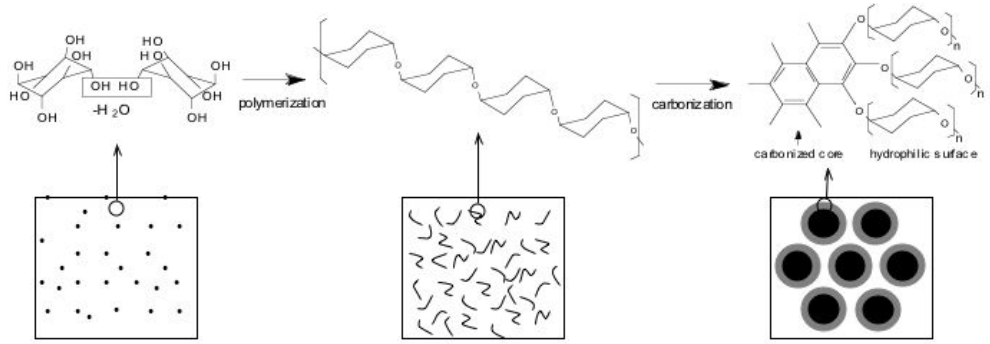

Figure 1. Schematic growth model of carbon spheres.

The dynamics of the carbonization reaction during growth of the carbon spheres can be understood on a molecular level in terms of condensation/carbonization of the glucose precursor molecule. We will use glucose derivatives including sorbitol to investigate that Aldol condensation is key to the polymerization process. Glucose is expected to undergo Aldol condensation, and polymerize further upon heating, while sorbitol is expected to be stable under these conditions due to the absence of aldehyde or ketone groups. We will also investigate the ring strain effect on the reaction if Aldol condensation process does indeed occur. For example, fructose (5-membered ring) is expected to exhibit faster condensation kinetics due to ring strain in contrast to glucose (6-membered ring). To examine water-mediated hydrogen bonding effects, glucoronic acid, glucosamine, and glucose-6-phosphate will be tested at the same condition. Such glucose derivatives would form functional carbon spheres faster than normal glucose, and would result in the formation of useful surface functionalities for further applications in sensors and adsorption. To characterize transient species in real-time during the condensation process, an *in situ* Raman scattering approach is under development.

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Research Summaries

Biomineralization and the Organic-Inorganic Interface

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Our *biomimetic* approach to nanoscience is inspired by biominerals, complex structures dictated by biomacromolecular assemblies. Using in-situ synchrotron x-ray scattering methods, we probe the organic-mineral interface to obtain time-resolved structural information on mechanisms of mineralization at organized organic matrices. One fundamental question concerns structural templating, as between an ordered organic film and a crystal face. Our studies of calcium carbonate at Langmuir monolayers on liquid surfaces proved, instead, that kinetics dominates mineralization [DiMasi 2003]. Further work has shown how biopolymers and cations affect these kinetics. At Langmuir films, poly(acrylic acid) and poly(aspartic acid) additives extend the lifetime of biologically relevant amorphous calcium carbonate precursor phases, while Mg^{2+} affects induction times. The presence of Mg^{2+} when calcite is grown on self-assembled monolayers on solid templates, by contrast, creates two coexisting routes to Mg-calcite formation: oriented nucleation dependent on SAM functionality, and an amorphous precursor phase independent of the SAM [Kwak 2005]. Since our studies have shown that molecular recognition of mineral faces is generally not a dominant mechanism, our current efforts focus on spatial templating at slightly larger length scales. Here, local fluctuations in cation and counterion concentrations, created by the patterned templates, allow us to design local environments which can tune the kinetics of mineralization. Especially promising is the mineralization of protein networks similar to the extracellular organic matrix. Preliminary work shows that elastin and keratan sulfate networks nucleate crystals, similar to avian eggshell membrane. Study of this system will reveal which properties of glycoprotein assemblies are important for mineralization.

New structural studies of biominerals accompany our in-vitro investigations. Recently we used microbeam diffraction to probe structural correlations across the nacre-prismatic interface of abalone shell [DiMasi 2004]. In fact, biominerals, with their hierarchical organization, remain at the cutting edge of synchrotron imaging capabilities. Synchrotron sources now enable diffraction-enhanced imaging, x-ray microbeam analysis, computed tomography and phase radiography to probe heterogeneous microstructures. Chemical information is obtained from soft x-ray photoemission and infrared spectromicroscopy techniques. High resolution diffraction, small-angle scattering, and x-ray absorption methods all contribute to the picture of crystalline and amorphous phases formed. Finally, many of these experiments are sensitive to the crucial organic components that help give biominerals their special properties. We highlighted new results and challenges in a workshop devoted to Synchrotron Imaging of Biominerals at the NSLS 2005 Annual Users' Meeting. This workshop is archived as streaming video and can be accessed from Brookhaven National Laboratory webserver http://www.solids.bnl.gov/~dimasi/nsls05ws2/.

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Ultrafast nonlinear vibrational spectroscopy of water and water at biological interfaces

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Program Scope

Water is an enormously complicated liquid. A great deal of new information about water dynamics and the dynamic evolution of hydrogen-bonding structures has been obtained recently by ultrafast nonlinear vibrational spectroscopy experiments performed on the OH-stretching transitions of water and its isotopomers[2,3]. Water in confinement is found everywhere in nature. In biomaterials confined water exists in hydrophobic pockets, on the surfaces of biomolecules and in thin shells solvating ions. Confined water evidences radically different properties that are no less complicated than liquid water but are much less understood. Essentially all the nonequilibrium properties of biological systems are strongly affected by the dynamical behavior of confined water. Following many successful studies of water itself[4,5], ultrafast nonlinear spectroscopy methods have recently been extended to water confined in the interior of reverse micelles[1] and in the solvent shells of anions[6]. Our program seeks to use new techniques in vibrational spectroscopy to study the structure and nonequilibrium properties of water in confinement. It is well known that although the OH-stretch transition of water is a super broad band, there is a strong association between the frequency redshift in this band and the strength of hydrogen bonding. Therefore ultrafast vibrational spectroscopy probes hydrogen-bond dynamics of water[2,7]. Dynamical properties are studied by perturbing the water using femtosecond resonant infrared (IR) pulses that pump water OH-stretch excitations or femtosecond laser-driven shock waves that produce large-amplitude disruptions of the hydrogen bonding network.

Recent progress

We have developed two instruments based on amplified femtosecond Ti:sapphire laser systems and IR optical parametric amplifiers that can be used to study water dynamics. One system perturbs water with OH-stretch excitation and then monitors the flow of vibrational energy through the water and into the confining medium. This system uses an IR pump and Raman probe method (IR-Raman) that is a kind of three-dimensional vibrational spectroscopy[5,8]. The second system perturbs molecular layers with a laser-driven shock wave and probes the response using vibrational sum-frequency generation spectroscopy (SFG)[9]. SFG is a selective and sensitive probe of the structure of water at surfaces and interfaces[10]. Our laser utilizes a broadband multiplex SFG technique[11] that probes vibrational transitions in a given spectral range on a single-shot basis, which is vital for shock-compression diagnostics.

The structure of a reverse micelle is depicted in Fig. 1. The micelles used in our initial experiments[1] were suspended in CCl_4. They consisted of a nanodroplet of about 35 water molecules confined by a sphere consisting of about 18 molecules of the surfactant AOT. AOT is strongly associated with the nanodroplet surface through the anionic sulfonate head groups. A

femtosecond IR pulse was used to excite the OH stretch of the confined water. A time series of anti-Stokes Raman spectra were obtained subsequent to this excitation. In anti-Stokes Raman, the intensity of each transition is proportional to the number of vibrational excitations in the mode being probed. We saw that one effect of confinement was to increase the water OH stretch lifetime from its usual value[12] of 0.25 ps in the bulk to 1 ps in confinement. We were also able to watch vibrational energy flow from the water, through the sulfonate head groups and the carbon-carbon stretches of the AOT surfactant out into the CCl_4 over an ~10 ps time interval[1].

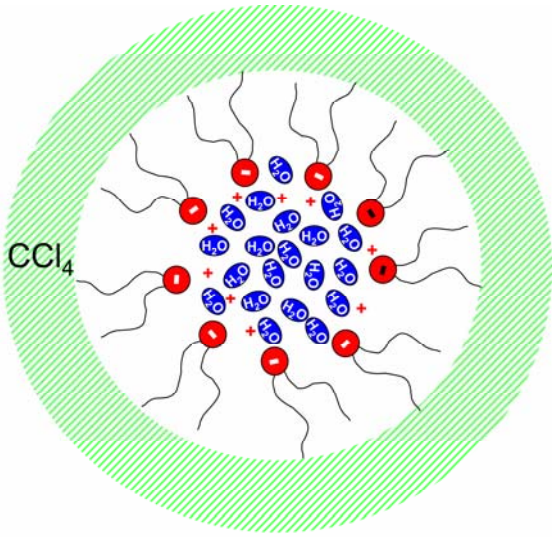

Figure 1. Schematic of a reverse micelle. Femtosecond IR pulses pump OH stretch of the confined water. Raman probing provides a time-series of spectra[1] showing the path of vibrational excitation from water to the surfactant anionic head groups, through the surfactant tails and out to the CCl_4.

This work established that the IR-Raman technique is a powerful method to study the properties of water in confinement and that the effects of confinement on the water can be dramatic. In addition we can study the interactions of the water with the confining media with exquisite precision, even down to identifying the atomic groups of the confineming media that interact most strongly with the water. The ultrafast vibrational energy transport phenomena studied here are precisely analogous to what happens when a point within a biomolecular structure is driven out of equilibrium.

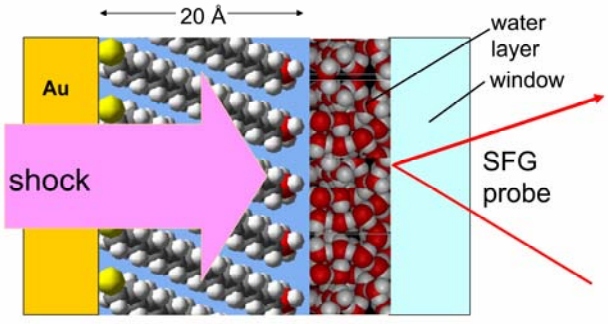

Figure 2. Schematic of arrangement to perturb a self-assembled monolayer using a femtosecond laser-driven shock wave. The monolayer will disrupt the hydrogen bonding structure of the confined water layer, which can be sandwiched between hydrophobic or hydrophilic layers.

Femtosecond laser-driven shock waves subject materials to sudden (<3 ps) large-amplitude compressively-driven motions[9,13]. We have done a great deal of work to develop this technology. In our experiments, shocks are generated in a multilayer microfabricated target array that includes a shock generation layer and a thin sample layer. Typical shock pressures are 1-10 GPa (10-100 kilobars), which produce typical compression amplitudes of 5-25%. For instance myoglobin, a 40Å diameter protein, can be compressed[14] by 4-8Å. Thus shock compression provides a method to study the responses of biomolecular systems to fast large-amplitude motions.

The arrangement used for shock-SFG measurements is shown in Fig. 2. A femtosecond shock drive pulse passes through a glass substrate to be absorbed in the skin of a Cr layer. The skin temperature rises rapidly, creating a dense plasma whose expansion drives a shock through the other layers and into the molecular sample. In Fig. 2, the sample is a self-assembled

monolayer (SAM) of a long-chain hydrocarbon with a thin layer of water confined between the SAM surface and a glass window. In a series of experiments, we have characterized the effects of a 1.6 GPa shock front on SAMs having an even or an odd number of carbon atoms[9,15]. The SFG technique sees only the methyl head groups. Using polarization measurements in the CH-stretch region, we were able to measure the instantaneous tilt angle of this methyl group. We found that depending on the molecular structure and shock strength, the shock might simply bend the chains elastically or it might induce *trans*-to-*gauche* isomerization by rotation around carbon-carbon bonds. Chains with gauche defects are shorter and fatter. SFG has been used extensively to study water surfaces and water interfaces confined at hydrophilic and hydrophobic interfaces[10]. With the addition of a water layer, as in Fig. 2, we will be able to simultaneously probe the SAM head groups and the water layer, in order to watch the disruption and subsequent reformation of hydrogen bonding in the water layer. This process is precisely analogous to what happens when a biomolecule covered with a thin layer of confined water undergoes a fast large-amplitude conformational change.

Future plans

With the IR-Raman measurements of vibrational energy transfer, the long-range goal is to develop the ability to study the structure and dynamics of water confined at the surfaces of biomolecules. For instance it is well known that many enzymes function just as well, often even better, in organic solvents than in water. This is believed to result from a thin layer of water confined at the enzyme surface. We need to develop the practical ability to make laser-compatible suspensions of enzymes in organic solvents. Then we can use femtosecond pulses to pump vibrational excitations of the confined water. Studies of the water itself, especially the rates and mechanisms of vibrational relaxation, provide information about the confined water structure and dynamics. When vibrational energy flows out of the water into the confining biomolecular surface, it will create a local hot spot that can be observed with anti-Stokes Raman scattering. We will thus be able to identify the functional groups of the biomolecule surface most strongly associated with the confined water.

With the shock experiments, we need to fabricate stable samples with confined water layers and characterize the interfaces by static SFG measurements. It will then be possible to shock water confined under a variety of well-controlled conditions. The SAM head group and the glass surface can be made either hydrophilic or hydrophobic. By changing the SAM chains, and by using laser pulse shaping methods, we can deliver different kinds of large-amplitude perturbations to the water.

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Bioinspired assembly of inorganic materials at soft-hard interfaces

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Program Scope

Inorganic thin films and nanostructures are typically grown on inorganic substrates, often under ultra-high vacuum. In nature, however, precisely-controlled assemblies of inorganic crystals are grown in ambient and relatively dirty conditions, using organic 'substrates'. This program seeks to learn more about inorganic self-assembly at soft surfaces, primarily through the use of in situ grazing incidence X-ray scattering. Langmuir monolayers floating on water or other liquids are of particular interest because of the ease with which the organic structure ('substrate') can be controlled, and also because the subphase can be a supersaturated solution of the inorganic material being grown. While nucleation under floating monolayers is a model of biomineralization, this project is primarily materials- and surface-science-oriented: the goal is to find new ways to make inorganic films (whether biologically relevant or not); to learn to control the structure and morphology as easily and as well as biological systems do; and to ultimately use this knowledge to develop facile methods of assembling inorganic heterostructures at a variety of length scales.

Recent Progress

Mann [1-3] and others [4] have shown in past years that, when the subphase of a Langmuir monolayer is a supersaturated solution, crystals will nucleate preferentially under the organic monolayer, and have particular crystal structures (in materials where more than one structure is possible) and specific crystal orientations that depend on the monolayer material and on parameters such as temperature, pressure and pH. However, these studies are of the final products (often 'harvested' crystals), which means that much information is lost. In particular, the relationship between the organic and inorganic structures can only be guessed at. This project has focused on looking at the soft-hard interface during the crystal growth process.

When growing BaF_2 under fatty acid monolayers [5], the thickness of the deposited film is found to depend on solution concentration. The inorganic film always grows with the (001) direction vertical. At the highest concentrations studied the lattice spacings are those of the bulk crystal, but at lower concentrations, when the film is very thin (~10Å), the lattice is strained ~5% (comparable to strains seen in UHV thin film growth). In the low-concentration limit, the organic and inorganic structures are *commensurate*, with the ratio of the two unit cell areas being 1.50 (see Fig. 1). While such commensurate relationships have been proposed as an explanation for the oriented growth seen under Langmuir monolayers, this is the first time it has been observed directly. What was not

expected—and could not have been observed without an in situ structural probe--is that both the organic and inorganic lattices distort in order to achieve this epitaxy (the BaF_2 lattice is compressed, the monolayer lattice is expanded). In other words, the organic template serves as a 'compliant substrate' to facilitate epitaxial growth.

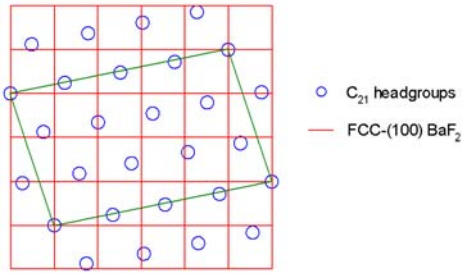

Fig. 1: Relationship between floating monolayer structure (circles) and inorganic BaF2 lattice (lines). The large tilted rectangle shows the common supercell.

BaF_2 is not unique: very similar results are seen with SrF_2 [6]. There is oriented growth, and the lattice is strained at the early stages of growth. Lateral domain sizes and non-uniform strain magnitudes (lattice spacing distributions) have been extracted from peak width data: the lateral domain size increases as the film grows thicker , and the non-uniform strain decreases along with a decrease in the average (macroscopic) strain.

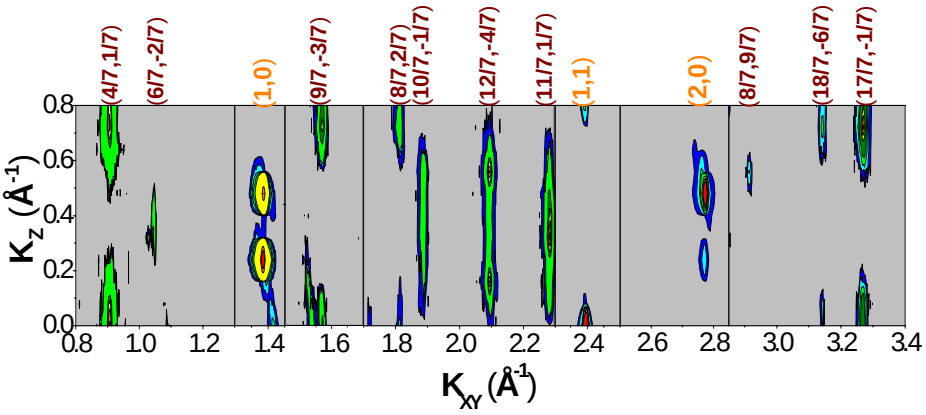

Fig. 2. Intensity contours, reconstructed from diffraction scans, showing the (101), (102), (110), (112), (201) and (202) peaks of hydrocerussite nucleated under a Langmuir monolayer, plus a number of fractional-index peaks (elongated in the z-direction) corresponding to a $\sqrt{7}\times\sqrt{7}$ supercell of the mineral's (100) surface lattice. From [7]

Another phenomenon familiar in inorganic surface science, but never before reported in ambient conditions, is seen during nucleation from supersaturated lead carbonate solutions [7]. The diffraction peaks correspond not to pure lead carbonate but to the mineral hydrocerussite, $2PbCO_3.Pb(OH)_2$. In other words, the floating monolayer controls what crystal will be grown. Further, in addition to the monolayer peaks and the bulk mineral peaks, fractional-order diffraction peaks are seen corresponding to a $\sqrt{7}\times\sqrt{7}$ supercell of the hydrocerussite (001) surface lattice (Fig. 2). This surface supercell

obviously has an area of 7.0 times the mineral lattice unit cell area; but it also has 9.0 times the area of the floating organic monolayer. It appears that the role of this reconstructed layer is to "match" the organic and inorganic structures, with the bulk crystal being unstrained.

Future Plans

Grazing-incidence X-ray scattering studies of floating acid monolayers on dilute (not supersaturated) solutions, starting with the work of Böhm et al. [8] and followed by our own work [9, 10], have revealed the existence of ultrathin layers just under the organic monolayer, with very large unit cells. The unit cell size has been difficult to explain on the basis of the chemical composition of the dissolved material. Based on the very recent observation of surface reconstruction [7], a possible explanation may be that there is a reconstructed layer even when an observable bulk crystal has not developed under it. To explore this possibility, and thus solve this long-standing puzzle, studies as a function of concentration and time will be performed. The evolution of the inorganic structures will be followed from ultrathin layers (seen with dilute solutions) to bulk crystals (seen with supersaturated solutions).

In Langmuir monolayers, a variety of ordered phases can be formed by changing pressure and temperature [11]. In principle, therefore, one should be able to determine the optimum soft structure for deposition of a given hard material. In our studies so far, it has turned out that the monolayer is a 'compliant' substrate: its structure changes in the presence of a dilute or supersaturated solution. This does not mean that pressure or temperature are irrelevant. It is possible and even likely that different compounds will be deposited if one starts with a Langmuir monolayer in a different phase. (For example, hydrocerussite is only one of a number of crystalline lead hydroxide carbonate compounds that are known.) Other parameters to be changed include organic head group---in addition to acids, we can spread alcohols, amines, acids, etc. Past studies by Mann's group and others have reported various effects (some molecules work better than others when nucleating a given compound [12], or a particular structure or orientation is achieved with one molecule but a different one with another molecule [2], etc.). Systematic in situ studies are planned to learn more about the underlying principles.

Even though this proposal is not about biomineralization as such, the growth of calcium carbonate and phosphate are of obvious interest. It is likely that calcium carbonate nucleation is not controlled by the ordering of the soft surface (in fact there is evidence that initial growth is amorphous [13]) but by its functionality. There are many reports [14-17] of calcium carbonate or calcium phosphate (hydroxyapatite) nucleation, at functionalized organic surfaces that are less ordered and/or less well-defined than Langmuir monolayers. These studies (and others like them) are very important, but from the point of view of this project they are ad hoc : what happens at the soft/hard interface is not known. In situ X-ray studies of such nucleation processes will be performed.

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Highly Branched and Dendritic Polymers Inspired from Enzymes in Catalysis

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Program Scope.

As part of a fundamental study of bioinspired catalysts this program explores the design and function of synthetic macromolecules that mimic the shape and function of natural enzymes. The fundamental design of the macromolecules is based on highly branched structures that allow them to adopt a globular shape a few nanometers in diameter (just like many enzymes) in which the active catalytic center is surrounded by molecular components that contribute to mass transport in and out of the active center. Therefore, the macromolecules are expected to behave as nanoreactors[1] providing not only for catalytic transformations but also for free-energy driven mass transport.

In the early pat of this program we demonstrated that these concepts can be realized with appropriately functionalized dendrimers[2] Our current targets explore alternative macromolecular architectures that are more readily accessed such as star[3] and dendronized[4] polymers.

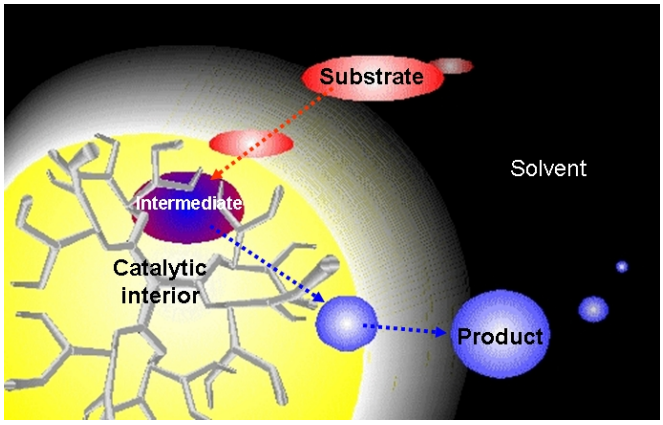

Figure 1. Schematic representation of a synthetic bioinspired catalytic nanosystem.

Design features that are important include an encapsulated catalytic core protected from environmental deactivation and peripheral encapsulating moieties that provide a polarity gradient aimed toward the catalytic center in order to drive the substrate into the catalytic "cavity" to minimize its free energy. Following its transformation, the product must then be able to travel in the reverse direction through the polarity gradient to the external solvent for which it has affinity (Fig. 1)

A long term goal of this project is to design multicenter nanoscale catalytic systems capable of effecting a sequential series of transformations, much as would be achieved with a series of enzymes where the product of one reaction becomes the substrate for the next.

Recent Progress.

After a successful demonstration of the validity of our concept of free-energy driven catalytic nanosystem using dendrimer catalysts[1,2], we have focused on the demonstration of the feasibility of a reaction cascade using two nanoscale catalysts in a single medium. Since it is important to demonstrate that each catalytic site can function even in the presence of the other, we chose two catalytic systems that are normally incompatible in solution. In this case one catalyst is an acid, and the other is a base; as a result, unless some form of protection of the catalytic site is achieved, the two catalysts will undergo a mutual reaction resulting in their deactivation. To

avoid such mutual deactivation and achieve preservation of both structure and activity, the catalytic sites must be site-isolated as is the case for the reactive sites of enzymes and of some dendrimers.

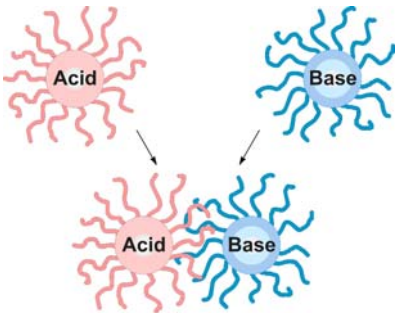

Figure 2. Acid and base containing stars with non-interpenetrating cores resist mutual deactivation

We have designed two different star polymers in which multiple copies of a catalytic moiety – acidic in one case, and basic in the other - are confined to the core of each star. We have then used these stars mixed in a single reaction environment to catalyze successively two steps of a reaction sequence.[5]

Our results clearly show that encapsulation of both acidic and basic groups within highly branched, yet fully soluble, multi-arm star polymers suppresses their mutual deactivation (Fig.2) allowing for the sequence of acid and base reactions to be performed in succession.

The star polymers, each of a size comparable to that of many proteins, were prepared using the living polymerization methods optimized with the high throughput combinatorial synthetic methods we have developed recently.[6-7] These stars bear some resemblance to dendrimers in that they also adopt a globular shape in solution but resist interpenetration due to the highly crosslinked nature of their core. The preparation of the stars involves an arm-first polymerization approach that affords a very low polydispersity linear product. This linear pre-polymer is then subjected to copolymerization with a mixture of a crosslinking monomer and a species that carries the catalytic function (in this case an acidic or a basic moiety) to form the final stars. Despite their highly crosslinked cores, the stars are fully soluble and small molecules can easily penetrate their core thus enabling the catalytic sites to perform their function.

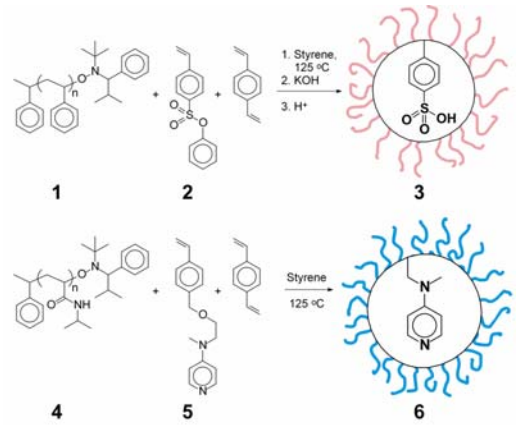

Figure 3. Preparation of star polymer with site-isolated catalytic cores

Figure 3 shows the preparation of the acid- and base-cored stars. The pre-polymer arms **1** and **4** are prepared by living polymerization using a nitroxide mediated living free radical polymerization method. The catalytic functionalities **2** and **5** are then introduced in the next step together with divinylbenzene used as a core-forming crosslinking monomer. Typically the stars we prepare using this method have approximately upwards of 100 arms with a total molecular weight of approximately up to about 600,000 Da depending on specific reaction conditions. In all cases a multiplicity of catalytic centers is introduced in the core of each star to ensure high activity.

The novel nanoscale catalysts were then tested in a one-pot, two-step reaction in which the first step requires and acid catalyst while the second requires a base catalyst as shown in Figure 4.

The overall yield in this process carried out with a mixture of the two catalysts reached 65% while no product was observed in control experiments in which one or both of the site

isolated catalysts were replaced by small molecule analogs due to mutual deactivation of the catalytic species.

Thus, we have shown that core-confined groups in these high molecular weight star polymers appear to be site-isolated as is observed with moieties at the core of dendrimers. As a result of this site-isolation sequential catalysis of a two-step reaction using catalytic species that are normally incompatible with each other is possible. This ability to generate a pathway by which a cascade of reactions is enabled is reminiscent of biological systems where a series of discreet reactions, performed by different enzymes, is used to create a wide range of chemical functionality and diversity through the combination of a few simple steps.

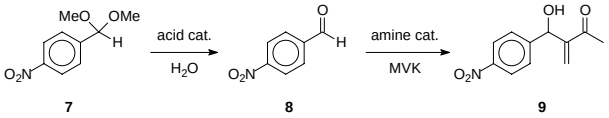

Figure 4. the two-step reaction sequence carried out in one-pot with two site-isolated stars as the catalysts

A variety of other nanoscale catalytic systems based on enzyme mimicry are currently under development. In addition, as will be described briefly below, we are exploring novel nanoscale architecture such as organic based dendronized nanotubes for the attachment of multiple catalytic sites within a single molecule.

In another related part of our study of synthetic enzyme mimics, we have explored the role of architecture and nanoenvironment in the catalytic properties of dendritic acylation catalysts containing 4-(dialkylamino)pyridines as active moieties. The study was done using benzyl ether and aliphatic ester dendrimers as well as comparable dendronized linear polymers. Catalysis experiments involving acylation reactions with sterically demanding tertiary alcohols as substrates clearly indicated that, just as in enzymes, it is the nanoenvironment that plays the dominant role in determining catalytic activity while polymer architecture appears to play little or no role in affecting catalysis. With respect to molecular transport and catalysis, this represents the first comparative study of the effect of architecture and nanoenvironment using structurally similar dendritic materials.

Future Plans

We plan to continue our fundamental exploration of nanometer size globular catalysts based on dendritic structures with several designs of oxidation catalysts. In addition we must develop novel synthetic methods for the construction of single molecules capable of accommodating several different catalytic sites, each site-isolated from its neighbors. To this effect we are looking a novel ways to prepare dendronized structures, including for example the use of click chemistry.[9-10]

In addition we have started to explore the preparation of shaped tubular macromolecules for use in both transport (mimics of ion channels) and catalysis. Initial studies have been directed towards living metathesis polymerizations on dendronized functional norbornene monomers.

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Biology-inspired programmable assembly of functional nano-structures.

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Program scope

A diversity of nanoscale objects, ranging from nanoparticles to supramolecular complexes, became recently available as the result of rapid developments in nanosciences. Nevertheless, to employ the unique functionality of nano objects into devices, novel methods of material assembly are required. Our approach to achieve this goal utilizes a strategy of bio-programmable assembly, which is based on the high degree of selectivity and addressability specific to biomolecules such as nucleic acids and proteins. However, there are a number of fundamental issues, related to the energetics of self-assembly, kinetic effects, and collective effects, which need to be addressed. An understanding of the interplay between selective biological factors and non-selective physical factors in hybrid systems is the key question of bio-programmable assembling, and has become the central goal of our research program. In addition, underlying all work in bioassembly is the need for a broader library of biological tools that provide the required selectivity, and methods for linking the biological elements to the inorganic materials we wish to assemble. Our research strategy combines an exploration of the microscopic structure of nano scale objects with a range of methods for the assembly of such systems, including both biochemical and physiochemical approaches.

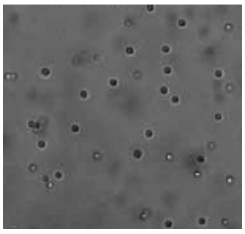
The proposed approach for programmable assembly of hybrid materials can be generalized to a broad variety of inorganic and organic nano- and microscale objects, where specific functionality can be preserved in the hybrid environment through an appropriate choice of ligands. Hybrid composites with their tunable structure, biocompatibility and processing advantages offer a wide range of prospects for applications in nano-electronics, novel materials, and biosensors by combining the functionality (electronic, magnetic, optical, etc.) of inorganic materials with the selectivity and specificity of biological entities (DNA, enzymes, antibodies, etc). The proposed approach will address the most imposing challenges of self-assembly, which compete with conventional "top-bottom" fabrication methods: *arbitrary, non-periodic* assembly at the nanoscale. That can potentially open the route for routine fabrication on scales not assessable by any other methods of modern technology.

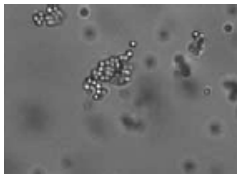
Recent progress

A variety of different biological systems has been suggested for the realization of biomimetic nano-assembly including viruses[1], DNAs[2, 3], peptides and proteins[4]. The validity of this approach has recently been demonstrated for the assembly of semiconductor and metallic nanowires[5], based on the hybridization of DNA oligomers and the assembly of the DNA functionalized microspheres[6, 7]. However, an understanding of the complex system behavior resulting from biological and physical factors is missing. This is especially important for hybrid systems which include biological, organic and inorganic components having a diversity of interactions, such as van der Waals and electrostatic forces, hydrogen bonds, and entropic effects. Such interplay results in a particularly complicated morphology and rich phase behavior for hybrid systems[8]. Although there are many possibilities for biologically inspired selectivity, we have chosen to focus on a DNA based approach as a model system to address the interplay between physical and biological factors in assembly. DNA offers unique recognition capabilities, mechanical

and physicochemical stability, and synthetic accessibility of practically any desired nucleotide sequences. Moreover, the unique selectivity of Watson-Crick base pairing allows high efficiency, reversibility and specificity of the interactions of the DNA-tagged components.

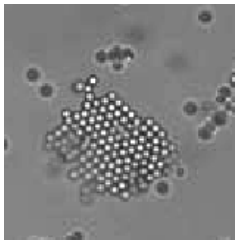
DNA-mediated assembling of micron sized particles. In our first experiments we studied how specific DNA-DNA interactions affect the behavior of the model system that consists of micron sized polymer beads, functionalized with single strand oligonucleotides (32 nucleotides long). Due to the large particle size relatively to the single stranded DNA strands, the interaction is mostly short-range, and it is provided by the hybridization of the complimentary DNA strands when particles are in nearly touching proximity. In the control experiment where we used particles functionalized with similar or non-complimentary oligonucleotides short-time collisions are occuring due the Brownian motion in aqueous solution (upper figure on the right). No organized particle assembly was observed for this type of system. In contrast, when the system consisted of equal parts of particles functionalized with complimentary DNA, aggregation of the particles occurs into clusters with sizes ranging from a few to tens of particles (lower figure on the right). The aggregates are mostly 3D and do not exhibit any particular order.

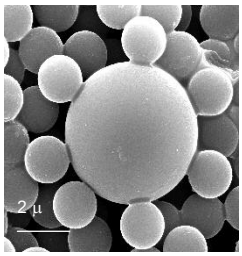




It has been demonstrated by Chaikin's group[6] that the addition of block copolymers to the particle surface introduces additional repulsive interactions due to steric and/or electrostatic effects. This inclusion affects the inter-particle potential strongly enough such that the aggregation becomes reversible when the system is incubated at a temperature close to the DNA-melting temperature of the double stranded helixes.

The first results of our study demonstrate that stabilization of the particle suspension can be achieved through different approaches: (i) decreasing ionic strength of the solution that increases the electrostatic repulsion between negatively charged phosphor backbones of oligonucleotides; and (ii) through balancing the number of hybridizations per pair of particles. Our preliminary results show that the dynamic range of interactions can be tuned in such a way that the phase of the system can be varied from a nearly dispersed particle state or disordered particle aggregates to ordered particle assemblies. The latter state was observed when the attraction between particles was carefully tuned (top figure on the right, optical image in aqueous solution). This ordered phase for DNA functionalized particles was observed[7] only recently in the very narrow temperature range (1^{o}C) near the DNA melting temperature. Our results suggest that by fine-tuning the inter-particle potential the ordered phase can be obtained at room temperature and can exist over a much wider range of temperatures (tens of degrees). Additional control on the richness of the assembled structures can be achieved by incorporating particles with different sizes into the system. An example of the observed ordered structure, based on the principle of a limited number of hybridizations, is shown on the lower right SEM image.





Utilization of protein-DNA interaction. Protein-DNA interactions can be exploited to functionalize nano-particles and carbon nano-tubes, which can be used for several applications, including biosensors. In these nanosensors, the selectivity and specificity is provided by the biological entity, which upon interaction with its target compound transfers a detectable signal to the nano object to which it is couples. In addition DNA binding proteins, such as proteins belonging to the MerR family of regulatory proteins, can be used for programmable bending of DNA helixes.

In our first experiments we concentrated on the interaction between ArsR, the regulator of arsenite resistance in *Ralstonia metallidurans* CH34, and its DNA recognition sequence. A major difficulty of DNA binding regulatory proteins is their solubility during protein overproduction. We

successfully cloned, over-expressed and purified this ArsR protein in *E. coli*. We subsequently showed that the protein was functional and had a reversible interaction with its DNA recognition sequence: in the absence of arsenite compounds, the ArsR protein bound specifically to its DNA recognition sequence, while in the presence of arsenite the protein dissociated from the DNA. This is shown in the figure on the right. We will further exploit this system as a model to develop metal specific biosensors.

We also propose the DNA binding domain of the PbrR regulator of *Ralstonia metallidurans* CH34 as a model system for double stranded DNA-protein scaffold construction. The PbrR protein is the regulator for the *pbr* lead resistance operon in *R. metallidurans*, whose DNA binding domain recognizes the 28 base pair long palindrome sequence TT**GACCCTGTAG**C*G*A**CTAAAGGGTC**TTC. We have cloned and over expressed the PbrR DNA binding domain in a pET-expression vector, and will use the purified domain in binding studies with double stranded DNA scaffolds that contain the PbrR recognition sequence at defined locations to test if the PbrR-DNA binding domain can be successfully applied as a tool and a model for the binding of a protein to ds-DNA. Imaging techniques such as AFM and TEM will be used to study the kinetics and topology of these self-assemblies. The PbrR protein belongs to the family of the so-called MerR-like regulators, from which many different members are available that all recognize different palindromic DNA sequences[9]. The basic knowledge obtained during our study on the application of the PbrR DNA binding domain in self assembly studies will be exploited to broaden this concept to the DNA binding domains of other MerR like regulators and their palindromic DNA recognition sequences, thus creating a toolbox of DNA binding domains and their DNA recognition sequences for a la carte assembly of hybrid nanoscale protein DNA complexes.

Future plans

1. Study of assembly phase behavior as a function of potential modulation via DNA tuning.

Addressable interactions will be introduced into nanoparticle systems through DNA strands, complementary to the oligonucleotides that are attached to the particles. In this system DNA will provides specific recognition functionality while the nanoparticles will provide non-specific interactions through van der Waals and depletion forces, steric and electrostatic interactions. Unlike the micron-sized particles described above, the energetic landscape on the nanoscale is quite different due to the elevated contribution of long-range interactions. The main focus is to understand how the interplay between selective DNA binding and non-selective, isotropic inter-particle interactions will result in the formation of various self-assembled structures. The interaction potential will be further tuned with the addition of designed DNA to the assembly solution. DNA libraries will be developed allowing both single end (buffer) and double end (linker) affinity to the complementary oligonucleotide.

In addition to studies on 3D systems we will investigate 2D systems at liquid-air interfaces and liquid-solid interfaces. For liquid-air interfaces, the particle adsorption can be controlled by the particle's hydrophobicity through the relative concentration of oligonucleotides and hydrophobic ligands. Large scale (>micron) structures at liquid-air interface will be probed using Brewster Angle Microscopy. Information about the surface structure, both normal and lateral, will be probed in-situ by a combination of x-ray reflectivity and grazing incidence diffraction. The initial experiments on this system will "tune" the interparticle interaction via variation of the oligonucleotides/hydrophobic ligand ratio, and allow observing their effects on 2D assembly via scanning probe and synchrotron techniques. For liquid-solid interface, the particle-substrate interactions will be controlled using the interactions between complimentary DNA strands on the particle and on the functionalized surface. The behavior of DNA strands in confined geometries, the formation of particle mono layers and pre-

designed multilayers will be studied under environmental conditions using scanning probe and surface x-ray scattering methods.

2. Developing a custom designed suite of tools for biomimetic self-assembly using specific affinity tags for inorganic nanostructures, connection elements, and biochemical set of reactions.

Complementary to the ordered structures described above we will use the highly selective biological properties of DNA based systems to create unique structures that are organized in arbitrary, non-periodic architectures. Such structures will then be used as scaffolds for the spatially defined deposition of functional entities (such as nanoparticles), thus extending this concept to create predefined 3D nanoscale objects. We will use a stepwise approach to invest in different concepts of bio-programmable self-assembly. The design of our molecular toolbox will consist of DNA for the scaffold construction, and protein domains and peptide sequences to further functionalize the DNA scaffold. Our research toward the biomolecular toolbox will be based on the following interactions and biological structures, presenting an increasing degree of complexity:

1. Nucleic acid interactions (DNA) based on hybridization of DNA oligomers: use of repetitive DNA sequences to build unique two and three dimensional structures via a process of self assembly. This will be the first step in this part of our research and will provide the necessary basic knowledge to understand the possibilities and limitations of simple nucleic interactions to create well-defined scaffolds that can be used for more complex self-assembly systems. Our first aim will be to assemble double stranded DNA rods, which is a logical extension of our proposed research to create 2D NxM matrixes from ssDNA. In a later stage, the work will be extended to the design of 2D network scaffolds based on dsDNA.
2. Once we are successful in self-assembling DNA rods in a predefined manner, we will create the necessary tools for functionalizing the DNA matrix. Based on our knowledge of regulatory proteins and their unique DNA recognition sequences we will create a toolbox of DNA binding domains of these regulatory proteins. As each of these proteins will recognize and bind to a very specific DNA sequence, this research will provide us the tools for the a la carte binding via self assembly of protein domains at sequence defined locations in the DNA matrix. This will be used for the defined deposition of proteins on the DNA rods and, a later stage, 2D DNA scaffolds.
3. The toolbox will be further optimized by linking affinity tags with high specificity to different nanoparticles and to the DNA binding domains. This will allow further functionalizing via self-assembly of the DNA scaffold with nanoparticles in a predefined manner.

The final outcome of this work will be a validated toolbox for the creation of predefined 3D nanoscale objects.

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Synthetic Biofunctional Interfaces

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Program Scope

Living organisms have unanimously adopted the phospholipid membrane as the material of choice for the organization and coordination of nearly all biochemical processes. Composed of a self assembling bilayer of phospholipids, cholesterol, and other amphiphilic molecules, biomembranes exhibit a collection of unusual physical properties not observed in other materials. Characteristics such as the coexistence of liquid crystalline and disordered fluid phases, thermodynamic critical points near physiological growth temperatures, and the mechanical coupling of distant biomolecular reactions (via membrane bending effects) conspire within cell membranes to enable and regulate life processes. Phospholipid membranes can be assembled on solid substrates such that both the structure and fluid nature of the membrane is preserved. A critical and unique feature of the supported membrane interface is that the membrane fluidity permits macroscopic rearrangements of the surface. Fluid rearrangements of the cell membrane are emerging as a broadly significant theme among cell surface interactions, and may be a definitive strategy of information exchange within multicellular organisms, including humans[1]. The unique interface between a solid material and a fluid membrane offered by supported membranes provides an ideal mechanism to functionalize inanimate substrates with life-like properties.

At the present time, only a handful of specific biological interactions have been functionally deployed between living cells and synthetic solid surfaces. It is our hypothesis that development of fluid membrane surface coatings will vastly expand the functional biochemical repertoire that can be exploited to mediate interactions between material substrates and living cells.

The overarching theme of this project is to develop design rules and synthetic strategies to construct biomembrane derivitized surfaces that interact with living cells in functional and prescribed manners. Such capabilities, once achieved, will constitute a major step towards the domestication of life at the cellular level. The ability to tell cells precisely what to do and where to do it will have substantial impact in areas of chemical and material synthesis, energy transduction, and molecular detection.

Recent Progress

We have previously demonstrated that supported membranes can be used to modulate the adhesion and growth of mammalian cells in culture. This initial work employed an adhesion promoting lipid, phosphatidylserine, to direct the cellular behavior[2]. We established that supported membranes function under general cell culture conditions and are suitable mediators of interactions between living cells and inanimate materials. In the next phase of this work, we are emphasizing the use of specific signaling proteins to elicit more specialized cellular responses. Initial work along these lines, employing a glycosylphosphatidylinositol (GPI) - linked form of the neuronal synapse protein neuroligin, is yielding promising results[3].

Specific interaction between cognate surface molecules is thought to play a role in recognition by axons of appropriate targets with which to form synapses, in triggering the assembly of the synaptic transmitter secretion machinery, and in producing and maintaining a tight cell-cell adhesion to stabilize the connection and structure the synaptic cleft. Interaction between pre and postsynaptic cells can be mediated by homophilic adhesion molecules, such as NCAM, cadherins, integrins and Syncam, or by heterophilic adhesion molecules, such as postsynaptic neuroligin (Nlg) and presynaptic beta-neurexin (Nrx). In addition to the common property of adhesion, each of these proteins interacts at its intracellular end with scaffolding proteins, enabling it to play a part in the organization of the protein machinery of the synapse.

Despite their similarities, these adhesion proteins appear to be specialized for distinct functions. Cadherins and integrins are found at both neuronal and non-neuronal cell-cell junctions, while NCAM,

Syncam and Nlg/Nrx are specifically localized to neurons. Moreover, Syncam and Nlg-Nrx interactions have the special ability to trigger presynaptic differentiation. Non-neuronal Nlg-expressing cells are sufficient to induce presynaptic differentiation in a contacting Nrx-bearing axon. Recombinant GPI-anchored Nlg (GPI-Nlg) incorporated into an artificial lipid bilayer formed on glass beads also triggers presynaptic differentiation in a Nrx containing axons, indicating that a postsynaptic cellular response is not necessary for presynaptic reorganization and that the Nlg ectodomain presented in a bilayer serves as a sufficient surrogate for the postsynaptic cell (Figure 1).

We have created a system to study these relationships, adapting the Nlg-bilayer approach to a format that is supported on a flat, optically clear glass coverslip surface onto which Nrx-expressing cells are dropped. Supported bilayers containing GPI-linked protein ectodomains are ideal substrates to mimic cell-cell binding. The lipid bilayer composition can be chosen to minimize non-specific binding that could obscure the binding assay and the cellular response, and it provides the correct protein orientation and lateral mobility necessary for membrane protein function. Most significantly, this hybrid live cell-supported membrane configuration is ideally suited for the functional integration of living cells into solid-state devices. Our studies of this system are revealing the necessary design rules that will ultimately govern such constructions.

In parallel work, we have identified specific needs for novel detection and analysis geared towards membrane surfaces. To address these needs, we have recently developed a colloid-based platform that enables label- and power-free detection of molecular events on membrane surfaces with near single-molecule sensitivity[4]. This assay strategy is expected to have great utility for portable analytical and sensing systems. Two-dimensional dispersions of lipid membrane-derivatized silica beads exhibit colloidal phase transitions that are governed by membrane surface interactions. The collective phase behavior serves as a cooperative amplifier that produces a readily detectable response from a small number of molecular events on the membrane surface. Using direct optical imaging, we observe multiple near-equilibrium phases and find that protein binding to membrane – associated ligand at densities as low as 10^{-4} monolayer can trigger a phase transition. Statistical analysis of bead pair distribution functions enables quantitative comparison among different membrane systems and reveals subtle, pre-transition, effects (Figure 2). This system is being developed as a

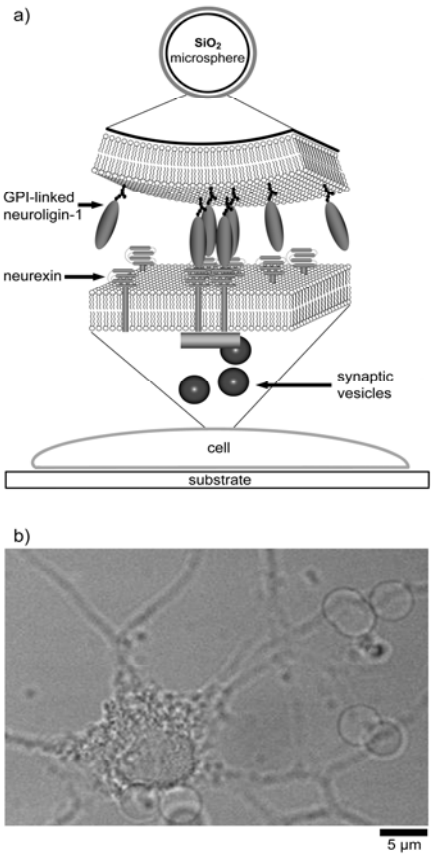

Figure 1: Supported membrane displaying signaling proteins (neuroligin) interacting with a living cell: schematic (a) and actual experiment (b). **M. Baksh**, C. Dean, **S. Pautot**, S DeMaria, **E. Isacoff**, and **J.T. Groves**, *submitted to Langmuir*.

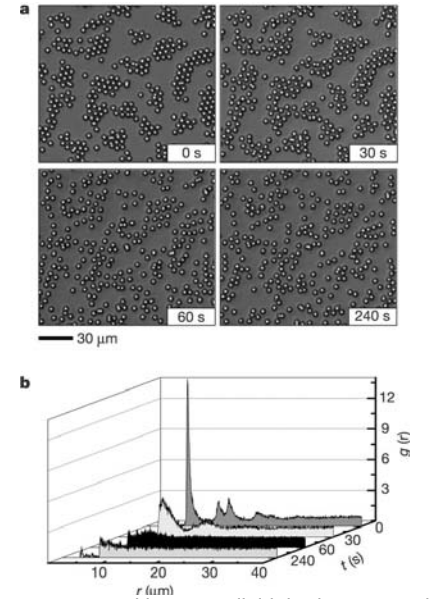

Figure 2: Tracking a colloidal phase transition triggered by protein binding. **M. Baksh**, M. Jaros, & **J.T. Groves** *Nature* 2004

bioanalytical technique for studying protein interactions on membrane surfaces for both research and industrial applications.

It is worth noting that the membrane-coated particles used for the live cell triggering experiments are identical to the particles used in the colloid assay. Thus possibilities for integration of the two techniques exist. Along these lines, we have also been developing photolithographic patterning technology suitable for patterning the surfaces of membrane-coated particles[5]. Our technique utilizes *in-situ* UV-ozone photolithography in conjunction with a micro-photomask array to align and pattern approximately one million beads per exposure. An example of a patterned particle is illustrated in Figure 3. This particle contains membrane (red) on one side and a protein (green) on the other hemisphere. A wide variety of patterns can be generated with the system. These are useful for presentation of multiple, and spatially juxtaposed signals to cells such as occurs in natural living environments.

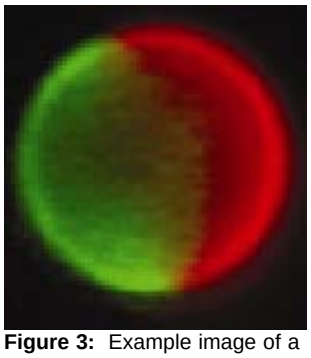

Figure 3: Example image of a patterned membrane-coated colloidal particle (6.8 μm diameter). **C. Yu**, A.N. Parikh, **J.T. Groves**.

Future Plans

The overarching goal of this project is to develop design rules and synthetic strategies to construct biomembrane derivitized surfaces that interact with living cells in functional and prescribed manners. Seedling results, from a variety of different experimental systems, indicate that this prospect will be realizable. For example, naturally and unnaturally membrane - linked proteins from the immune system have proven to be remarkably functional in the supported membrane configuration and have been able to elicit specific responses from living T cells. Detailed investigations of the immune synapse have demonstrated that a subtle balance of free movements and geometrical constraints of these membrane - linked proteins regulate the signal that a cell perceives when encountering another cell surface. Furthermore, recent evidence has revealed common mechanisms of function between the nervous and immune system, suggesting the existence of universal themes. Observations of clustering - mediated behavior in unrelated bacterial systems (chemoreceptors) underscores the recurring role of the spatial arrangement of cell surface receptors as a functional aspect of the ensuing signal. We seek to decode this language and deploy it intentionally on artificial surfaces to exert a desired reaction from living cells.

The immediate concern of our efforts over the next couple years will be the construction of surfaces displaying signal proteins in a functional and well - defined configuration. We will continue using the GPI - linked form of the neuronal synapse protein, neuroligin (Nlg) as the paradigmatic test case. A key realization emerging from this work is the necessity of a fluid membrane environment: Nlg deposited onto polystyrene beads exhibits little to no activity. This result, which is the first of its kind in a neuronal system, will be developed further in the coming years. Special emphasis will be directed towards the role of membrane fluidity and phase separated (raft) structures in the reconstitution of biological functionality with non-living supported membrane surfaces.

Another future direction, on which work has already begun, involves development of alternative synthetic strategies for the association of proteins with membranes. The GPI linkage system described above is highly functional, but the proteins are difficult to produce. They must be purified from the membranes of the host expression cells. As an alternative to this, we are exploring synthetic strategies for the attachment of membrane linkages to soluble portions of membrane proteins after purification. Our initial work with lapidated GFP (in collaboration with **C. Bertozzi**) is now in press in *JACS*. In collaboration with **M. Francis**, other potentially more general linkage strategies are under investigation. These synthetic strategies sidestep some of the inherent difficulties presented by the extraction and purification of large transmembrane signaling proteins and will be important for widespread application of this type of technology.

DOE Sponsored Publications in 2002 – 2004

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VIRUS ASSEMBLIES AS TEMPLATES FOR NANOCIRCUITS

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Program Scope

The assembly of nano-scale materials into functional devices represents one of the most pressing obstacles to the development of this technology. Interestingly, many biological molecules function on this scale and possess unique properties that impart the ability to assume defined conformations and assembles, as well as interact with specific chemical or biological substrates. These traits are ideally suited for developing new models and methods for the production of novel materials at the nanoscale level. The primary goal of this project is to develop biologically based fabrication strategies for the assembly of nanoscale devices. This project brings together expertise in materials chemistry/colloids and interfacial engineering (Dr. Harris) and protein engineering/biology (Dr. Culver) to address the application of biologically derived molecules for the production of nanoparticles. Specific efforts focus on the use of a well-defined plant virus, *Tobacco mosaic virus* (TMV), to investigate the parameters required to efficiently pattern biotemplated nanowires and tubes. In the long term, the parameters and processes develop in this program should have broad application for the use and engineering of a diverse array of biologically derived templates

Recent Progress

Improved viral templates for the deposition of inorganic compounds. The ability to coat the wild-type virus with various metals is mainly driven by electrostatic interactions. However, the relatively weak nature of these types of interactions generally results in incomplete and uneven coatings. In addition, attempts to purify coated wild-type virus, via centrifugation and dialysis, result in the removal of coated metals from the viral template. To strengthen the interaction between the virus template and inorganic material we engineered its coat protein to display metal attractive amino acids. Specifically, we added sulfur-containing cysteine residues to the inner and outer virion surfaces. Exposed sulfhdryl groups provided a strong binding site for the attachment of metals such as gold (Figure 1). Furthermore, by placing cysteine residues at specific positions along the virion surface we were able to selectively target the deposition of inorganic molecules to specific locations on the virion.

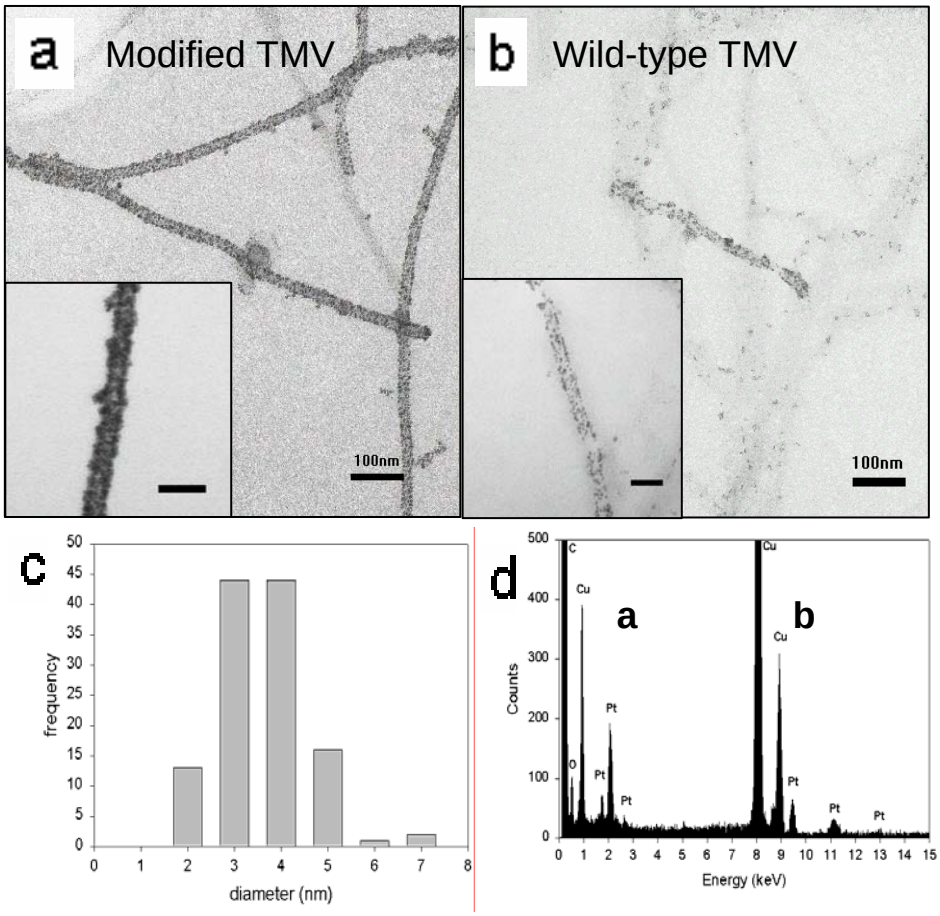

Fig. 1. Comparison of platinum coatings using either wild-type TMV or 2cys TMV as template. A and B, electron microscopy. C, average particle size of Pt clusters on the 2cys virus. D, electron dispersive spectroscopy (EDS) showing the presence of Pt in coated samples.

Improved viral template-coating methods. Previous studies had indicated that TMV could be coated with various inorganic materials including PbS and SiO_2.[1,2,3] To improve our coating efficiencies, the concentration of the precursor metal ion (the critical coagulation concentration or CCC) that resulted in TMV aggregation was determined as a reference concentration for our coating experiments. It was found that the best coatings of Pt, Ag, Au and Pd were obtained when the precursor metal ion concentration was approximately an order of magnitude or 10X the CCC.

Patterned assembly of TMV nanotemplates. Another objective of this project involves the attachment of viral-derived nanowire templates onto conductive supports. To accomplish this goal we utilized nucleic acid hybridization to selectively attach viral templates to patterned supports. Specifically, we genetically engineered a cysteine residue onto the surface of the TMV coat protein to function in the attachment of metal or fluorescent markers.

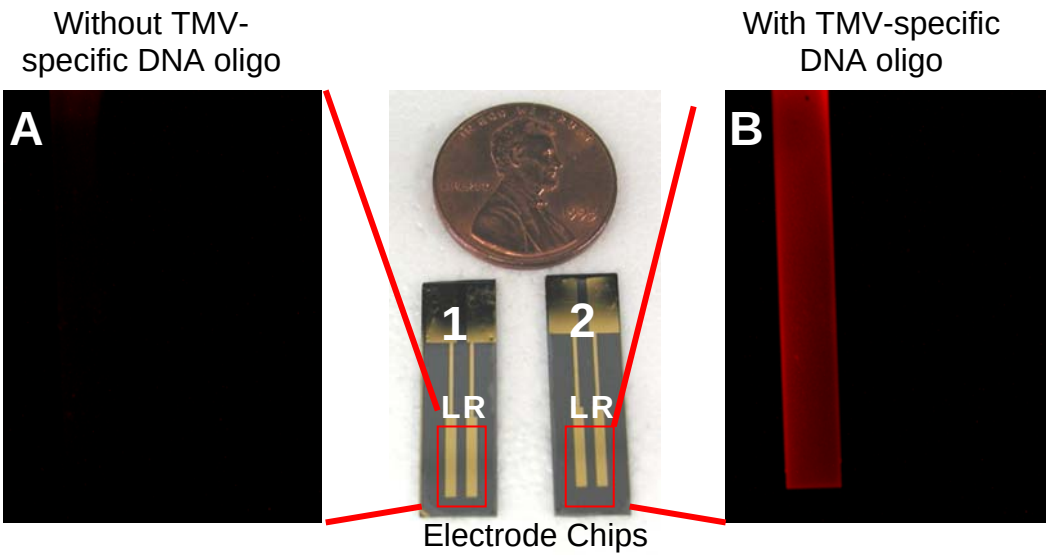

Fig. 2. Attachment of rhodamine-labeled TMV virions onto a gold patterned silicon chip using virus specific DNA oligo-hybridization. Each chip contains a left (L) and right (R) electrode. Chitosan is electrodeposited only on the left electrode of each chip. The right electrode carries no chitosan and functions as an internal control for non-specific binding. A, Chip 1 also functions as a negative control with chitosan electrodeposited onto the left electrode but without the TMV-specific DNA oligomer. B, Chip 2 carries electrodeposited chitosan plus the TMV-specific DNA oligomer on the left electrode. Both chips were immersed in a solution of the labeled virus for 1 hr and rinsed for 15min prior to examination by fluorescence microscopy. Only the left electrode on chip 2, which carries the chitosan deposited TMV-specific oligomer bound rhodamine-labeled TMV.

Functionalized TMV particles were partially disassembled to expose the 5' end of the viral genome and hybridized to complementary probe DNA linked to electropatterned chitosan coated silicon chips.[4] Results demonstrated that functionalized TMV templates could be specifically addressed as well as oriented using this hybridization-based assembly approach (Figure 2). Taken together these findings demonstrate the usefulness of an integrated approach that combines "top-down" silicon-based lithography with "bottom-up" biologically assembled nanoparticle templates for the fabrication of novel devices.

Future Plans

Assembling patterned and layered nanowire templates. The self-assembly properties of TMV provide a unique system in which to investigate the production of nanowires that contain alternating or layered inorganic material. An *in vitro* assembly system previously developed will be used to assess nanowire patterning.[5] Our goal is to use purified coat proteins and assembly intermediates as building blocks for the assembly of patterned nanowires. One approach is to purify modified coat proteins prior to the addition of metal coatings. Monomeric coat proteins could then be coated and allowed to self-

assemble. In this manner coat protein monomers linked to different inorganics could be mixed or layered to produce novel composites. An alternative approach would be to use modified coat proteins that carry specificities for different inorganic compounds. Purified coat proteins that specifically bind different inorganics could be used to assemble a layered or mixed template that could then be coated simultaneously with different inorganic materials. A third approach to enhance existing nanowire coatings involves the addition of a second metallic layer onto an already coated nanowire template. In preliminary studies we have experimented with the layering of different metallic coatings as a means to produce more continuous wires. At present we are working to identify the conditions needed to create layered coatings.

Establish a combinatorial display system for the selection of novel nanowire templates. In our current studies we have focused on the addition/substitution of specific amino acids as a means to improve TMV's ability to function as a template for nanowire synthesis. This approach has allowed us to identify regions within the viral coat protein that are structurally and biologically receptive to alterations. We plan to use this information to develop a combinatorial selection approach for the identification of virus templates with enhanced or novel coating specificities. Combinatorial methods have been extensively used to identify peptide sequences with unique binding or biological affinities, including the ability to bind inorganic compounds.[6,7,8] To create a TMV based peptide library we developed a TMV coat protein-cloning vector that enables us to add random peptide sequences to the surface exposed amino terminus of the virus coat protein. Infectious RNA transcripts generated from this library will be used to infect leaves of *Nicotiana benthamiana*. Virus replication will function to amplify the library for subsequent selection/panning experiments.

Develop additional patterning strategies for the self-assembly of viral templated nanowires. The uniformed and controlled assembly of nanoparticles is a potentially useful method to produce surface-confined architectures at a nanoscale level. In this objective we will take advantage of the natural ability of TMV to self assemble as a means to pattern templated nanowires. For these experiments gold micropatterned supports will be functionalized to create nucleation sites for the assembly of nanowire templates. Our long-term goal is identify the conditions needed to grow a viral rod between two conductive supports. Two schemes are currently being investigated for use in the surface arraying of TMV templates. The first scheme will surface mount purified viral coat proteins directly onto the conductive support using reactive sulfur groups, amine cross-linking, or coat protein specific antibodies. Purified protein will then be added under assembly conditions (low pH, increased temperature, etc.) to promote the assembly of virus rods. A second scheme for the assembly of

surface-confined virus templates will employ the use of sequence specific nucleic acid/DNA oligomers. RNA encoding a sequence complementary to the surface-mounted oligomers and the TMV assembly sequence will be generated *in vitro* and allowed to hybridize to the anchored oligomers. Purified coat protein will then be added to promote assembly on the exposed RNA. The advantage of using nucleic acid hybridization to localize/pattern viral templates comes from the specificity of the nucleic acid sequence, allowing a large number of different addresses to be used for nanowire patterning.

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DOE Sponsored Publications in 2004-2005

N. Dingle and M. T. Harris, 2005. "A Spherical Coordinate-Based Algorithm for Determining the Interfacial Tension and Contact Angle from Sessile Drop Profiles," *J. Colloid Interface Sci.*, 286, 647-660.

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S.Y. Lee, E. Royston, J. N. Culver and M. T. Harris, 2005. "Improved Metal Cluster Deposition on Genetically Engineered Tobacco Mosaic Virus Template," *Nanotechnology*, 16, S435-S441.

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Self-Assembling Biological Springs: Force Transducers on the Micron and Nanoscales.

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Program Scope

Helical ribbons with characteristic pitch angles (11 or 54°) form spontaneously in a variety of quaternary surfactant-lipid-sterol-water solutions. These helical ribbons form in a variety of axial lengths, widths and radii. Remarkably, however, they all have pitch angles of either 11 or 54° [1].

The first objective of our research program is to determine the molecular structure of the helical ribbons. We suspect that the remarkable stability of each of the two pitch angles maybe due to a crystalline structure of the ribbons and that the two different pitch angles correspond to two different crystal structures of the ribbons. Our working hypothesis is that the cholesterol molecules order in two dimensions, forming crystalline planes, which resemble the in-plane order of different forms of cholesterol crystals. The planes are stacked together forming a layered structure, which may have only short-range out-of-plane order.

Next, we have been able to tether fine glass capillaries to these ribbons and subject them to well defined axial forces [2]. We are preparing now to use the helical ribbons as mesoscopic springs to measure quantitatively the forces acting in nano-scale biological systems.

Recent progress

Previously, a number of powder X-ray scattering studies were performed on the structures filtered from the solution. These measurements showed a number of Bragg peaks, suggesting the presence of crystalline structures in the solution. However, the solutions usually contain the ribbons of both pitch angles, as well as small crystalline platelets, presumably of cholesterol, which are extremely difficult to separate. Moreover, since helical ribbons exist in a particular region of the quaternary solution phase diagram, concentrating the ribbons for powder diffraction can lead to rapid crystallization of cholesterol, which make powder diffraction measurements of the ribbons inconclusive. Therefore, in order to probe the structure of the helical ribbons, it is desirable to measure X-ray diffraction from individual ribbons. Moreover, these measurements should help understanding large shape anisotropy of the ribbons. In these ribbons the length and width differ by an order of magnitude. This is in contrast to cholesterol crystals, which grow in the form of platelets rather than oblong rectangles, which coil into helical ribbons.

With this motivation in mind, we have concentrated our efforts on isolating single ribbons from their solution, mounting them for X-rays and determining whether a diffraction pattern could be obtained from one ribbon. The ribbons are of 1000 μm long, 1 to 100 μm wide and 100 nm thick. Such small samples are extremely difficult to manipulate and mount for X-ray diffraction. Also, the signal from a 100 nm-thick sample is extremely weak. Nevertheless, we have succeeded in performing diffraction measurements on the largest single ribbons. Although these ribbons are too thick to fully twist into helices, we believe that there are close similarities in molecular structure between them and smaller helical ribbons. Therefore, this is a valuable first step towards determining the structure of the smaller helical ribbons themselves, which will be measured next.

In the case of large thick ribbons, we have found well-defined Bragg peaks, which

indicate the presence of crystalline order. These diffraction patterns were taken recently and their analysis is under way.

Fig. 1. Nylon loop filled with a thin layer of cryo-protecting oil with the ribbon attached to it. The ribbon is clearly seen as the vertical bend; X-ray beam hits at the intersection of the two hair-lines. The ribbon width is of 50 microns, similar to the cross-section of the X-ray beam.

X-ray diffraction measurements were made at the Advanced Photon Source (APS) at Argonne National Lab. We utilized the facilities of the SGX beamline, which is equipped with a CCD camera, oscillation stage and a cryostat for crystallography of proteins and small organic molecules. Helical ribbons of the two pitch angles form in Chemically Defined Lipid Concentrate (CDLC), which is commercially available and give a high yield of the structures. The ribbons are attached to nylon loops of 100 to 500 μm diameter, filled with a thin layer of oil. They are then flash freezed in liquid nitrogen to protect them from structural changes and from radiation damage from intense X-ray beam. X-ray diffraction is performed while the sample is kept at 100 K in a stream of cold nitrogen gas, which is used in modern protein and small-molecules crystallography.

Utilizing the high flux of APS proved to be essential for the success of these measurements since the samples are so thin. The small size of the samples also creates some difficulties for the analysis, due to the small signal-to-background ratio. However, we have performed several sets of measurements on many similar ribbons and we found that the diffraction patterns are reproducible between different samples, which gives us confidence in the crystalline structure of the ribbons in general.

Future Plans

The focus of our current research is the data analysis of the measured diffraction patterns. The small thickness and quasi-two-dimensional nature of the ribbons lead to a significant mosaic spread, weak and overlapping Bragg peaks and small number of spots. Hence, existing crystallographic software packages are not suitable for analyzing the diffraction from the ribbons. Therefore, we have developed our own software for interpretation of the diffraction data and

finding lattice parameters. It is clear so far, that the in-plane order strongly resembles that of cholesterol monohydrate.

So far we have only measured large single ribbons, which are too thick to fully twist into helices. The signal from the more interesting smaller helical ribbons will be weaker and the curvature will be greater. Micro-focusing will address both problems. Therefore, efforts are under way to measure the ribbons using micro-diffraction X-rays beamline. Our first measurements at the micro-diffraction beamlines (Sectors 2 and 34) at APS showed a clear Laue pattern from one of the structures when a focused white beam is used. As a result of these preliminary studies, it has become clear that qualitatively different approach is required in order to properly orient and locate individual ribbons in the X-ray micro-beam. Tethering of the ribbons to a substrate is necessary to achieve this goal. Although we have demonstrated earlier that the helical ribbons in the solution can be tethered successfully to a glass capillary [2], significant improvements in tethering technology are required for the X-ray micro-diffraction experiments.

Once all the experimental procedures are optimized, helices of both low and high-pitch will be measured in an effort to understand the differences in structure for each type. Next, we will use these techniques to measure helices formed in a variety of quaternary surfactant-lipid-sterol-water solutions to determine the structural basis of this universality in pitch angles. Moreover, we will consider further application of the X-ray diffraction methods, such as reflectometry or small-angular scattering in order to probe other aspects of the ribbons structure, such as distribution of the surfactants on the surface and between the layers.

Our experience with manipulation and tethering the ribbons for the X-ray micro-diffraction will benefit the next objective of this research program, namely using the helical ribbons as force transducers to measure quantitatively the forces acting in nano-scale biological systems.

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"Structure of helical ribbons self-assembled in quaternary water-cholesterol-surfactants solution." In preparation (2005)

Sugar-Substituted Poly(*para*phenyleneethynylene)s as Sensory Materials for Recognition of Bacteria and Lectins

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Program Scope

Our program looks into two areas of chromophoric systems useful for biological and biomaterials applications. The two platforms that are examined are sugar-appended and carboxylate substituted poly(*para*phenyleneethynylene)s. These materials are made by a Pd-catalyzed coupling of suitably halogenated and alkynylated aromatic precursors. These fluorescent polymers interact specifically with proteins and with bacteriae on the one hand and rotaxanated phenylene-ethynylene trimers on the other hand. The fluorescence and absorption of the rotaxanated materials are sensitive towards metal cations in water. We are expanding upon these two topics.

Recent Progress

*E. Coli Sensing with Mannosylated PPE **1**:* We have made **1** and **2**. The PPE **2** interacts with mannose-binding proteins, lectins, under agglutination and therefore serves as a sensor for lectins. Lectins are heavily involved in pathogen-cell interactions via multivalent binding of pathogenic host proteins to sugar-containing epitopes. Some plant lectins such as Ricin could be and were used in terrorist attacks. We have successfully monitored the interaction of PPE **1** with *E. coli* (from Prof. Orndorff, ORN 178, a mannose-binding strain, ORN 208, a non-mannose binding strain). We took SEM and fluorescence micrographs of ORN 208 and ORN 178 after incubation with an aqueous solution of **1**. Scanning electron micrographs of ORN 208 cells show a surface structure that can be attributed to residual pili, i.e. filaments that are attached to the body of the cell. PPE **1** does *not* bind to the cell surface of ORN 208 but forms a thick layer. On the other hand, mannose-binding ORN 178 does interact with **1**; all of the *individual* E.coli cells are coated with a layer of polymer and the cells are agglutinated. No surface details of the cell structure are visible anymore. Preparations of ORN 208 ORN 178 are incubated with PPE **1**, washed and then placed under a fluorescence microscope (Figure 1, left, middle). The non-mannose binding strain ORN 208 shows small scattererd colonies of bacteria, while in the case of ORN 178 the bacteria are agglutinated and form one large colony that is held together by the multivalently binding PPE chains. Both strains of E. coli are *stained* by PPE to be fluorescent and we are examining if the interaction of PPEs with other (non-toxic!) microorganisms might lead to differential cell staining and microstructure formation. When placed in Eppendorf caps, the differences between

ORN 208 and ORN 178 are even more prevalent upon exposure to **1**. While ORN 178 forms a brightly fluorescent pellet, visible with the naked eye, ORN 208 stays suspended. The model compound **2** did not show any appreciable binding to either of the strains. We have now synthesized the polymer **3** and we will investigate its binding and agglutination properties and compare those to the properties of **1.**

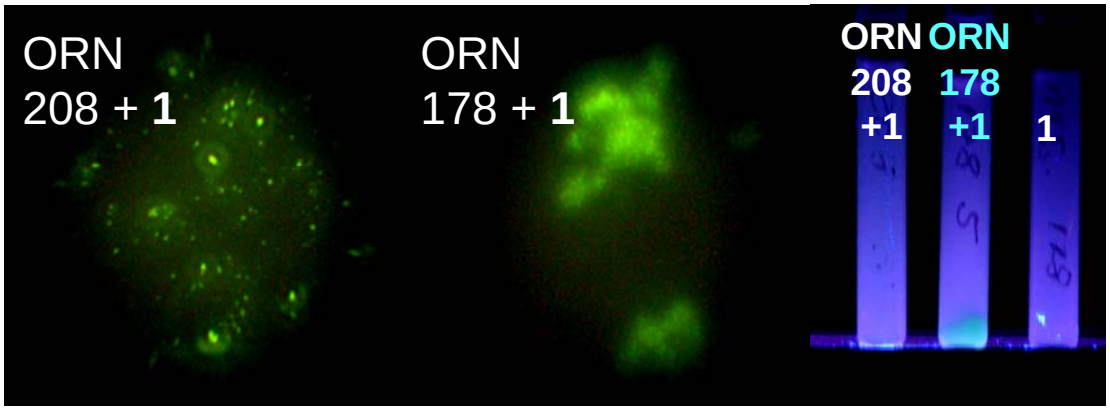
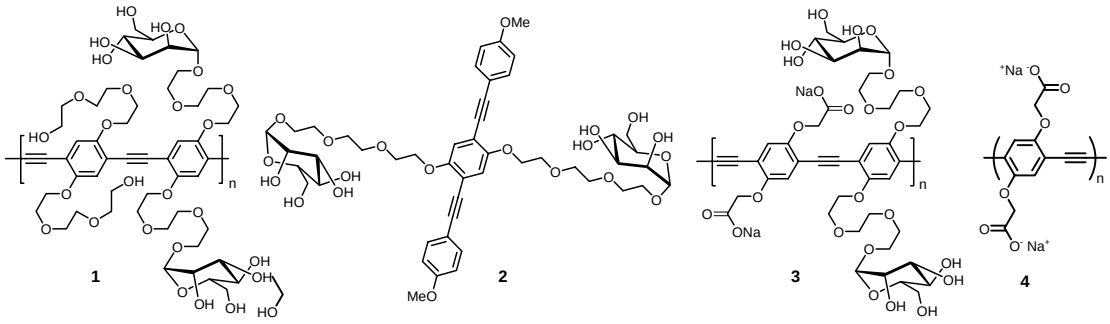


Figure 1. Fluorescence micrographs of non-mannose binding strains (left) and mannose binding strains (right) upon binding to PPE **1**. (40xmagnification). The mannose deficient strain does not aggregate upon exposure to **1** (left). The mannose binding E. coli strain (ORN 178) forms large aggregate plaques that are held together by the polymer (middle). Upon exposure of ORN 178 to **1** visible flocculation/ agglutination occurs. Only the mannose binding strain shows a fluorescent pellet. Neither control nor ORN 208 show flocculation (right).

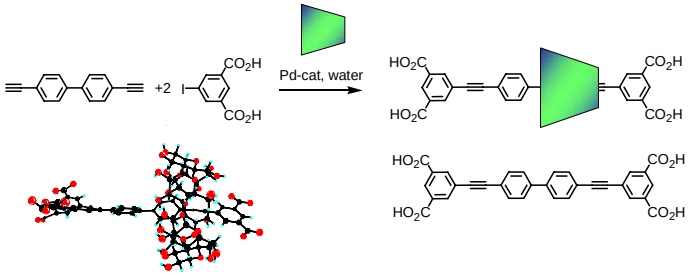
Synthesis of a Water Soluble Fluorescent PPE as Protein Specific Fluorescent Probes: We had observed a significant dependence of the fluorescence of the PPE **1** upon addition of metal cations. Because we are interested in mannosylated PPEs car-



rying anionic charges for lectin-polymer and cell polymer interactions, we made carboxylate-substituted **4**; the fluorescence of **4** is severely quenched by aqueous solutions of lead salts in PIPES buffer. Stern-Volmer constants in excess of 5×10^5 were observed. That is a significant result, because detection of lead in environmental and biological samples is an attractive proposition for soil remediation etc. Other

metal cations, such as copper(II) and zinc(II) do likewise quench the fluorescence of **4**, but with Stern-Volmer constants that are an order of magnitude smaller than those observed for the quenching of **4** by lead ions.

To investigate the use of PPEs in further biosensory applications we would have to exclude non specific interactions and binding of **4**. Consequently, we investigated the quenching of **4**'s fluorescence by proteins and enzymes and found that histone and hemoglobine give very higy (K_{SV} = 6 x 10^7 M^{-1}, 4 x 10^7 M^{-1}) Stern-Volmer constants, while cytochrome C, lysozyme and myoglobin give considerably lower, yet significant K_{SV} values. The very large quenching of **4**'s fluorescence by histone is understandable, because histone is a highly positively charged protein that will bind tightly to the negatively charged PPE chains. An interesting case is bovine serum albumine (BSA) where we find a formally negative Stern-Volmer constant, i.e. fluorescence enhancement. We attribute that to the surfactant character of BSA and invoke complex formation of BSA to **4** for the increased fluorescence. The effect is under investigation.



Acetylene-Dye Rotaxanes as biologically active and metal sensing probes: An extension of interactions of phenylene-ethynylene chromophores with biological systems and metal cations, we have rotaxanated a phenyleneethynylene-containing fluorophore by β-cyclodextrin in the Pd-catalyzed reaction of diethynylbiphenyl with 5-iodophthalic acid in water. The rotaxane **RD** is isolated in 11-14% yield besides the unrotaxanated fluorophore **FD** (40-60%). We observe that the emission quantum yield of **RD** is higher than that of **FD**. A crystal structure of **RD** was obtained and we have exposed **RD** and **FD** towards metal cations to find differential reactions of their fluorescence towards different metal cations.

Future Plans

We intend to expose the PPE **1** toward other bacteria and study the interaction of bacteriae with sugar-substitued PPEs more broadly to examine if we can differentially stain microbes. In addition we will make PPEs that are substituted by galactose and by sialic acid to access further lectins that we can detect. The sialic acid and galactose functionalized PPEs should be attractive sensors for the determination of ricin and ricin-like species. We will as well investigate the interaction with these

novel PPEs with bacteriae, particularly because sialic acid receptors are widely found on cellular epitopes.

To make these agglutination-type assays more sensitive, we will attach biotin at the ends of the sugar-decorated PPEs via an amine linker strategy and perform a pre-agglutination with streptavidin. The formed aggregates should be sensitized towards the agglutination process with lectins. We will attempt to increase the sensitivity so that approx. 1 femtomole of lectin can be detected.

In the area of the rotaxanated fluorophores we propose to make larger rotaxanated phenyleneethynylene and phenylenevinylene derivatives and investigate their photobleaching and sensing of metal cations.

DOE Sponsored Publications 2003-2005

Click Chemistry as a Powerful Tool for the Construction of Functional Poly(p-phenyleneethynylene)s: Comparison of Pre- and Postfunctionalization Schemes
Englert, B. C.; Bakbak, S.; Bunz, U. H. F.; *Macromolecules*, **2005**, 38, 5868-5877.

Nonspecific Interactions of a Carboxylate-Substituted PPE with Proteins. A Cautionary Tale for Biosensor Applications
Kim, I.-B.; Dunkhorst, A.; Bunz, U. H. F.; *Langmuir* **2005**, ASAP Article, DOI: 10.1021/la051152g

Permanent Bubble Arrays from a Cross-Linked Poly(para-phenyleneethynylene): Picoliter Holes without Microfabrication
Erdogan, B.; Song, L.; Wilson, J. N.; Park, J. O.; Srinivasarao, M.; Bunz, U. H. F.; *J. Am. Chem. Soc.* **2004**, 126, 3678-3679.

"Surfactochromic" Conjugated Polymers: Surfactant Effects on Sugar-Substituted PPEs
Lavigne, J. J.; Broughton, D. L.; Wilson, J. N.; Erdogan, B.; Bunz, U. H. F.; *Macromolecules* **2003**, 36, 7409-7412.

A biosensing model system: selective interaction of biotinylated PPEs with streptavidin-coated polystyrene microspheres
J. N. Wilson, Y. Q. Wang, J. J. Lavigne, U. H. F. Bunz, *Chemical Communications* **2003**, 1626-1627.

Molecular tuning of inorganic crystal growth by polypeptides.

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Program Scope: In the mollusk shell, there exist two layers, nacre and prismatic, which are manufactured by the mollusk using calcium carbonate minerals.[1-6] The intriguing feature of the mollusk shell is that it is a laminate structure comprised of two calcium carbonate polymorphs, calcite (prismatic layer) and aragonite (nacre layer) which exist side-by-side.[1] As it turns out, the presence of two crystallline polymorphs arise from the action of proteins within the shell layers.[1-6] Moreover, the proteins which are involved in polymorph selection also contribute to the fracture-resistance of the shell via intercalation within the mineral phase.[1] By understanding these protein-mediated processes at a molecular level, we will be able to devise new ways of constructing inorganic/organic composite materials which are polymorph-specific and offer fracture-resistant properties.

Recent Progress: We have pursued the identification and characterization of calcium carbonate mineral recognition polypeptide sequences derived from three mollusk shell nacre layer proteins (AP7, AP24, n16) and one prismatic layer protein (asprich-g). In particular, we have focused our attention on the effect that each mineral interaction domain has on *in vitro* calcium carbonate crystal growth, and, the primary and secondary structure of these sequences. In this manner, we can correlate morphological changes with potential molecular features of a given polypeptide, thereby bringing us a step closer to understanding the molecular mechanism(s) by which polypeptides control polymorph selection.

In vitro calcium carbonate crystal growth assays and SEM analyses reveal that the N-terminal mineral binding domains, AP7N, AP24N, and n16N, derived from the nacre biomineralization proteins, AP7, AP24, and n16, respectively, affect the morphology of calcium carbonate crystals in specific ways at the micron level. Both AP7N and AP24N, which adopt open, unfolded secondary structures, frustrate/inhibit the growth of rhombohedral calcite

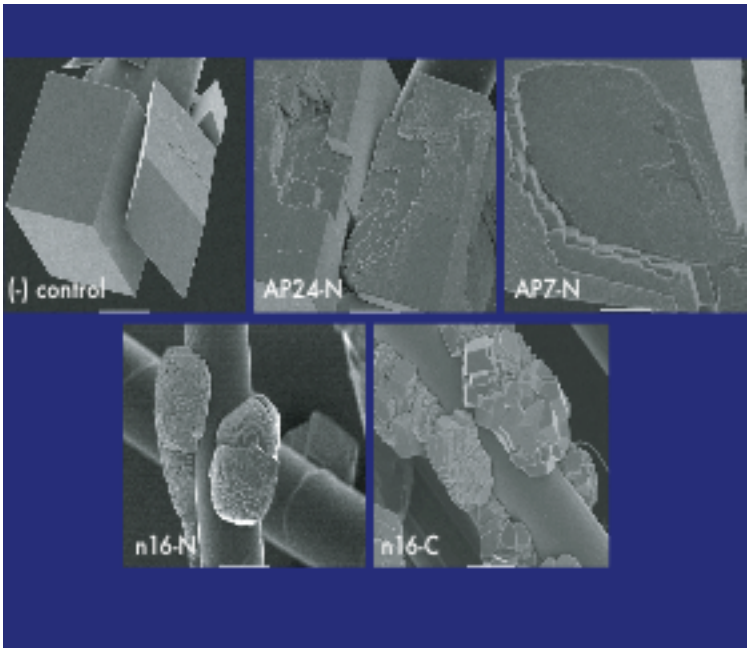

Figure 1

crystals[1,3], whereas n16N, which adopts an intermolecular β-strand structure, induces roundening of crystals and the introduction of staircase-like structures (**Figure 1**).[4] In all cases, these effects suggest that the nacre-specific polypeptides pin crystal growth via direct or indirect interactions at mineral surfaces. In the case of the unfolded, random-coil-like DEAD17 and acidic-2 polypeptide sequences derived from the prismatic layer protein, Asprich-g, we observe that both sequences introduce uneven crystal growth patterns on the surface of calcite crystals (**Figure 2**) which differ signficantly from the morphological changes observed in the presence of nacre polypeptides.

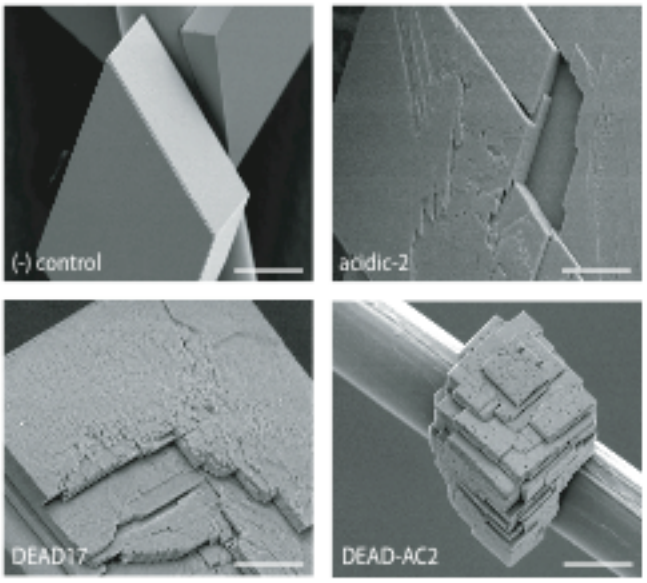

Figure 2

Using AFM microscopy, we investigated nanometer-level changes in morphology and crystal growth kinetics, and uncovered interesting, sequence-distinct effects on calcite hillock growth and morphology (**Figure 3**).[2] We observe that AP7N (**Figure 3B**) and AP24N (**Figure 3C**) are multifunctional, i.e, they pin acute steps and induce step bunching, they accelerate obtuse step velocities, and, induce the formation of rounded deposits on step terraces. However, the mineralization activity of AP24N is significantly greater than that observed for AP7N. Conversely, the effects induced by n16N on hillock

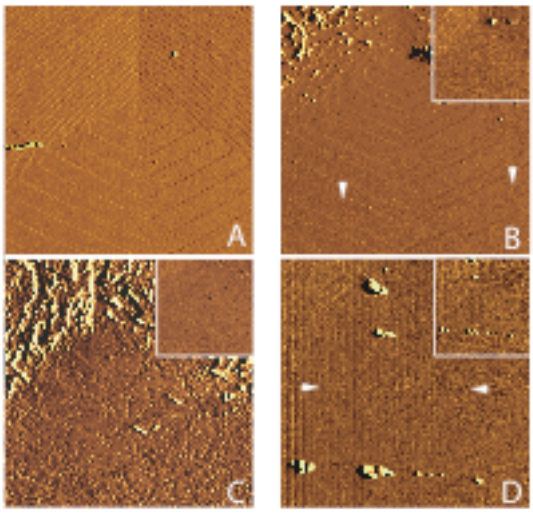

Figure 3

growth are very different; here, the polypeptide apparently induces the emergence of new step directions, perhaps by pinning of hillock corner sites (**Figure 3**), but does not exhibit the multifunctional features of AP7N or AP24N. For the prismatic - specific Asprich-g mineral interaction domains, we observe that DEAD17 is induces rounded acute steps, whereas acidic-2 pins acute steps and induces uneven obtuse step progression (**Figure 4**). In either instance, the morphological changes were more significant than that observed for the nacre-associated seqeunces.

From these studies, we conclude that the nacre-specific mineral interaction polypeptide sequences, which possess anionic, cationic, and hydrogen-bonding donor/acceptor amino acids and exhibit conformational lability, exhibit very different effects on calcium carbonate crystal growth compared to the primarily "acidic" prismatic-associated polypeptide sequences. In particular, nacre-associated polypeptide sequences such as AP7N and AP24N act in a catalytic fashion, whereas prismatic - associated sequences like DEAD17 and acidic-2 are inhibitory, but affect morphology more profoundly.

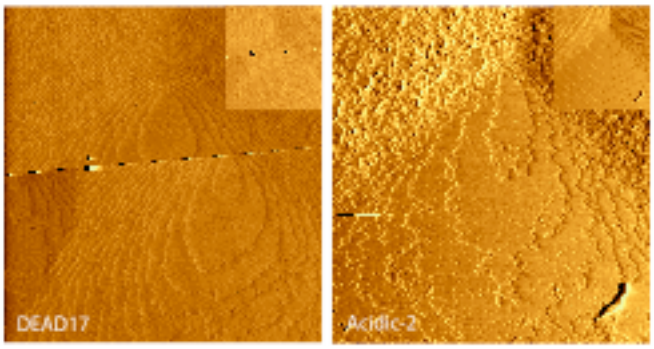

Figure 4

Future Plans:

We intend to perform amino acid substitutions within the nacre- and prismatic-

associated sequences in order to determine critical amino acids responsible for the observed morphological effects *in vitro*. We are also examining the structure and function of the parent proteins from which these mineral interaction sequences arise.

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Computer Simulations of Electrostatic Effects in Complex Fluids and Biomaterials

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Program Scope

Electrostatic interactions play a central role in many self-assembly processes, governing both the stability and the structure of complexes that arise in multicomponent fluids. Examples include the aggregation of polyelectrolytes (including biomolecules such as DNA and actin) under the influence of multivalent counterions and the phase behavior of suspensions of charged colloids. This program aims to address these classes of systems by means of computer simulations. In addition to molecular dynamics and Brownian dynamics methods, we employ newly developed Monte Carlo schemes of very high efficiency, which permit the study of systems that were hitherto inaccessible by computational means. Our numerical investigations are pursued in direct collaboration with several experimental groups, exploiting the strengths of simulations to access quantities and scenarios that cannot be addressed with current experimental techniques.

Recent Progress

1. Electrostatic complexation (Phys. Rev. Lett. 2005, in press)

It is well know that anionic biological polyelectrolytes can overcome their electrostatic repulsion and exhibit a mutual attraction in the presence of multivalent cations. These "like-charge attractions" result from ion correlations that cannot be understood within mean-field theories such as the commonly employed Poisson-Boltzmann formalism. When the multivalent cations are replaced by *macroions* as the mediating charge carriers, a more intricate situation arises. The formation of such macroion-polyelectrolyte complexes, which occur in many physical systems, is affected by a variety of competing factors: (i) The presence of salt can lead to an attraction driven by osmotic pressure. (ii) Differential screening of positive and negative charges distributed on the surface of a macroion may significantly modify interactions at the macroion-polyelectrolyte interface. (iii) Entropic gain due to mutual neutralization and consequent counterion release upon macroion-polyelectrolyte "adhesion" is expected to be important, but may in turn be modulated by the steric commensurability between the charge pattern on the polyelectrolyte and the macroion size. The relative importance of all these interactions, and how they modify each other in their combined effect on the structural evolution of macroion-polyelectrolyte complexes, is generally unknown. We have addressed this by means of a combination of small-angle x-ray scattering experiments (G. Wong, UIUC) and computer simulations of a prototypical system, consisting of a solution of actin and oppositely-charged lysozyme proteins, in the presence of a variable concentration of monovalent salt. The experiments have shown that the self-assembled complexes are comprised of hexagonally-coordinated columnar arrangements of actin held together by

one-dimensional arrays of lysozyme macroions located within the three-fold interstitial "tunnels" of the columnar actin sublattice. Molecular dynamics simulations using a realistic model of the actin helix have provided a detailed confirmation of this picture, and revealed structural reconstructions and corresponding salt redistribution within an actin--lysozyme bundle as the inter-actin separation was varied. Both experiment and simulation showed that the lysozyme is arranged in a close-packed manner, incommensurate with the actin periodicity. Most interestingly, the self-assembly of columnar actin-lysozyme complexes was found to be *enhanced* for higher concentrations of monovalent ions. By means of grand-canonical simulations we have been able to show that these results can be explained by significant repartitioning of salt between the condensed and the aqueous phases, which leads to a strong external osmotic pressure on the complex and, in addition, strongly modifies screening and counterion release effects. We expect these findings to be applicable to a considerable range of complexation phenomena in aqueous environments.

2. New simulation approaches to complex fluids (Phys. Rev. Lett. 2004, 92, 035504 and 93, 247802)

In a separate branch of this program, we aim to improve the efficiency of simulation techniques for complex fluids, focusing on the Monte Carlo method. One of the central aspects of this method is the possibility to introduce non-physical dynamics, permitting the study of systems that evolve over otherwise prohibitively large time scales. A well-known example is the cluster algorithm for lattice spin models introduced by Swendsen and Wang, which suppresses dynamic slowing down near a critical point. Since the conception of this method, its generalization to off-lattice fluids of interacting particles has been an elusive goal. Also away from the critical point the existence of *several different time and length scales* constitutes a major obstacle in the simulation of complex fluids. This situation commonly arises in multi-component systems, such as binary mixtures, colloidal suspensions and colloid-polymer mixtures, and has essentially precluded the computational study of many such systems. We have developed a novel, rejection-free cluster Monte Carlo method of considerable generality that alleviates this problem. It greatly facilitates the canonical simulation of large classes of continuum systems, such as complex fluids, by generating particle configurations according to the Boltzmann distribution, without suffering from severe slowing down in the presence of large size differences. For example, binary mixtures of small and large species with a size asymmetry of several order of magnitude can now be simulated efficiently.

Future Plans

The above-described research areas are currently being continued and extended into various directions. Our overarching goal is an improved understanding of complex fluids by means of advanced simulation methods, with a strong emphasis on phenomena dominated by electrostatic effects.
Specifically, the following projects are under way:

- The geometric cluster Monte Carlo algorithm is extended to incorporate Coulomb interactions as well as density fluctuations. This will permit us to address the

longstanding problem of phase separation in solutions of charged colloids, which are out of reach for conventional numerical approaches owing to the necessity to explicitly incorporate counterions and added salt.

- Aggregation and redissolution of polyelectrolytes in the solutions of multivalent salts. Here we also perform simulations that mimic electrophoresis processes, in order to clarify the issue over *charge reversal* or overcharging.
- Investigation of the role of polyampholytes in complexation and aggregation phenomena. This is an area of great practical relevance (many charged macromolecules are polyampholytes rather than polyelectrolytes), which however has been virtually ignored by all theoretical approaches
- The above-described work on actin-lysozyme complexes is extended to investigate the effect of charge variation on the protein, which experimentally can be achieved by means of mutations.

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Bioinspired Materials: Aptamer-Mediated Templates for Hybrid Elastic Nanomagnets

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Program Scope

This cross-disciplinary, multi-investigator project focuses on the creation of a new class of biomimetic materials involving magnetite nanocrystals grown in hierarchically self-assembled polymers using aptamers and mineralization proteins. This approach aims to recreate the structure of magnetite nanocrystals embedded in organic tissue seen in many different living species, that confers super-paramagnetic properties. Magnetobacteria have the capability of controlling and organizing the crystallization of iron oxide, magnetite (Fe_3O_4), and iron sulfide, (Fe_3S_4) to form nanosized magnetic particles of regular dimensions. [1] Consistent with the genetic influence on magnetic nanoparticle structure, it was recently shown that the mms6 protein produced by M.magneticumAMB-1 will direct the production of magnetic particles *in vitro* to form the same structure as is formed in the bacteria.[2] To mimic the bacterial systems, we are using the mms6 protein to design hierarchically self-assembled synthetic polymeric materials that serve as templates for directed growth of magnetite (Fe_3O_4) nanocrystals in a process mediated by aptamers (single stranded nucleic acids capable of molecular recognition) and mineralization proteins.

This novel approach involving DNA/RNA strands for achieving specificity of non-covalent binding to facilitate magnetite nucleation provides a very robust and modular method for the developing these and many other classes of self-assembling materials that would not be possible by conventional covalent linkage approaches. Ferrogels, fabricated by dispersing colloidal magnetite particles in polymer hydrogels, have been shown to have magneto-elastic properties.[3-5] However, in these materials, it is very difficult to uniformly disperse these particles in the polymer matrix, thereby affecting precise control of the magnetic properties. The multi-scale assembling polymers developed in our laboratory provide unprecedented opportunities for combining polymer self-assembly with aptamer-mediated mineralization to facilitate a bottom-up approach to form novel macroscale functional elastic materials with ordered magnetite nanocrystals.

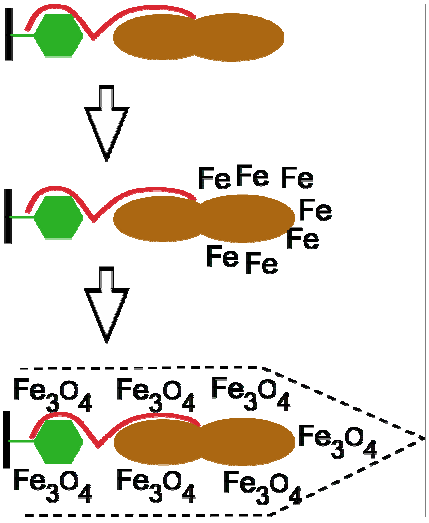

Fig. 1 Schematic of templated mineralization. denotes the aptamer, denotes the polymer with a suitable chemical functionality and denotes the mineralization -thrombin fusion protein

The combination of the "soft" mechanical properties of the polymer with the strong magnetic response of the magnetite offers new materials properties like giant shape memory effects, field induced deformations, or a magnetic field tuned sound spectrum. A variety of scattering, microscopy and other characterization techniques will be utilized to investigate the structure and magneto-mechanical properties of these hybrid materials. A key component of this

highly interdisciplinary and integrated work is a theoretical approach combining modern polymer theory and the theory of micro-magnetism. The strategy is demonstrated in Figure 1 where it is represented that the mms6 protein will be prepared as fusion proteins with thrombin or streptavidin and aptamer CLAMPs will be used to link the fusion proteins to a polymer at positions defined by the placement of a chemical functionality.

Recent Progress

To initiate these studies we obtained genomic DNA from *M. magneticum* AMB-1. The DNA was amplified using primers that are complementary to internal sites on the mms6 gene coding region. The primers were designed to amplify the region of the gene that corresponds to the mature (cleaved) mms6 protein. The PCR amplicon was cloned into the plasmid pTrcHis TOPO (Invitrogen). Clones were isolated and evaluated by restriction digestion, which verified the size of the expected insert. One clone was chosen for sequencing and found to have the correct sequence with an mms6 sequence in frame with an N-terminal poly-histidine tag. This expression vector can be used to prepare recombinant mature mms6 protein.

TOP10 *E. coli* cells were transformed with the mms6 expression vector and the recombinant mature mms6 protein was expressed from the expression. Most of the recombinant mms6 protein was insoluble and found in inclusion particles. Therefore, the protein was first solubilized in 8M urea and renatured by dialysis against successive buffers each with incremental decreases in urea. The renatured protein was then separated from the remaining proteins in the inclusion body by affinity chromatography through a Talon resin, a cobalt chelate that binds the polyhistidine tag. The purified protein was evaluated by gel electrophoresis and the Bradford protein assay to determine the yield and its level of purity. To establish the ability of the protein to bind iron, a blotting assay is being developed by which the recombinant protein is separated by gel electrophoresis, transferred to a nitrocellulose membrane and then incubated in a buffer containing Fe.[2] A positive control, transferrin, is being used to validate the assay. Using this assay, the mms6 protein is being tested for its iron-binding ability.

In parallel, the self-assembly of the polymeric templates that will be used for controlling the placement of mineralization proteins is being investigated. pH and temperature-sensitive pentablock copolymers have been synthesized and characterized through funding from a different subtask and aqueous solutions of these pentablock copolymers were found to self-assemble into micelles and further into macroscale solids.[6-8] Small angle neutron scattering studies (SANS) at Argonne National Laboratory were conducted to investigate evolution of micellar structure in the pentablock copolymer solutions with increasing pH. SANS data indicate that micelles formed from the pentablocks at low pH exhibit scattering typical of spherical micelles with repulsive interactions due to the charged state of the coronal DEAEM units. Upon increasing pH above 7.0 the scattering pattern shifts to that of spherical micelles with hard sphere interactions. Upon further increasing the pH to 10.5, the scattering intensity in the low Q region exhibited a power law of Q^{-1}. This indicates the presences of rod-like or cylindrical micelles. These experimental results are consistent with molecular dynamics simulation results that predict the switch from spherical to cylindrical micelles up on increasing pH or temperature.[9]

Fig. 2 Self-assembled solid

Upon heating the basic solution of copolymer above 70^0C, it precipitated from solutions and the precipitate organized to form an elastic solids completely by self-assembly. The self-

assembled solid was found to be relatively dehydrated. The shape of the macroscopic solids was dependent on the shape of the containers they were formed in, and the dimensions of the solids were smaller than those of the container; a short cylinder is formed in Fig. 2. After self-assembly the solid was stable in solution down to room temperature. The solid could easily be manipulated out of solution with tweezers. Moreover, the self-assembly behavior was found to be completely reversible with pH. By lowering the pH below 7.0, the solid dissolved again and could be reformed by increasing pH and temperature.

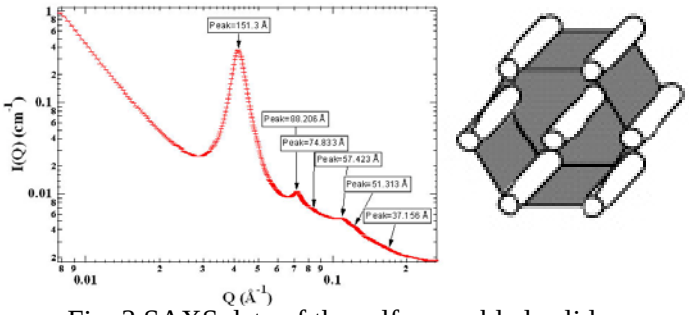

Fig. 3 SAXS data of the self-assembled solid showing a hexagonal structure, possibly formed by organization of cylindrical micelles

Small angle X-ray scattering (SAXS) studies at Argonne were used to examine the microstructure of the macroscopic self-assembled solid. SAXS data from the self-assembled solid (Fig. 3), exhibits distinct diffraction peaks indicative of a hexagonally packed crystal structure, probably formed by organization of the cylindrical micelles. The bulk copolymer, however, does not exhibit this hexagonal structure. The bulk copolymer sample melts at 40^{o}C, while the self assembled hydrogel exhibits a loss of mechanical stability near 90^{o}C. This represents a remarkable shift in thermomechanical properties through an entirely self-directed self-assembly process. These reversibly self-assembling polymers serve as ideal templates for non-covalent attachment of the mineralization proteins.

Future Plans

Figure 4 shows the variety of thrombin, streptavidin and mms6 fusion proteins that are planned for this project in conjunction with the polymer templates, after the fusion proteins are obtained using molecular biology techniques. In parallel, the mineralization protein will be used in conjunction with the pentablock copolymer preformed gels to investigate the size and shape of the magnetic nanoparticles formed using transmission electron microscopy. In addition, and for comparison, we are now using these copolymers to chemically synthesize magnetic nanocomposites containing magnetite nanoparticles. Various synthetic approaches are being used to incorporate magnetite nanoparticles into these amphiphilic pentablock copolymers. These include ultrasonic irradiation of iron pentacarbonyl in toluene in the presence of oleic acid as stabilizer; controlled decomposition of iron pentacarbonyl in mesitylene in the presence of the copolymer; and chemical co-precipitation of divalent and trivalent iron salts in high pH (pH > 9) aqueous solutions with controlled amounts of copolymer. We will probe the thermodynamic interactions, nanostructure and self-assembly of these novel hybrid materials using SAXS/SANS.

DOE Sponsored Publications in 2003-2005

This is a new project and no DOE sponsored publications have resulted from this work yet. However, DOE funding resulted in publications relating to the polymer templates used in this work, and those publications have been listed under the sub-task on self-assembled polymer templates for polymer-hydroxyapatite bioinspired nanocomposites

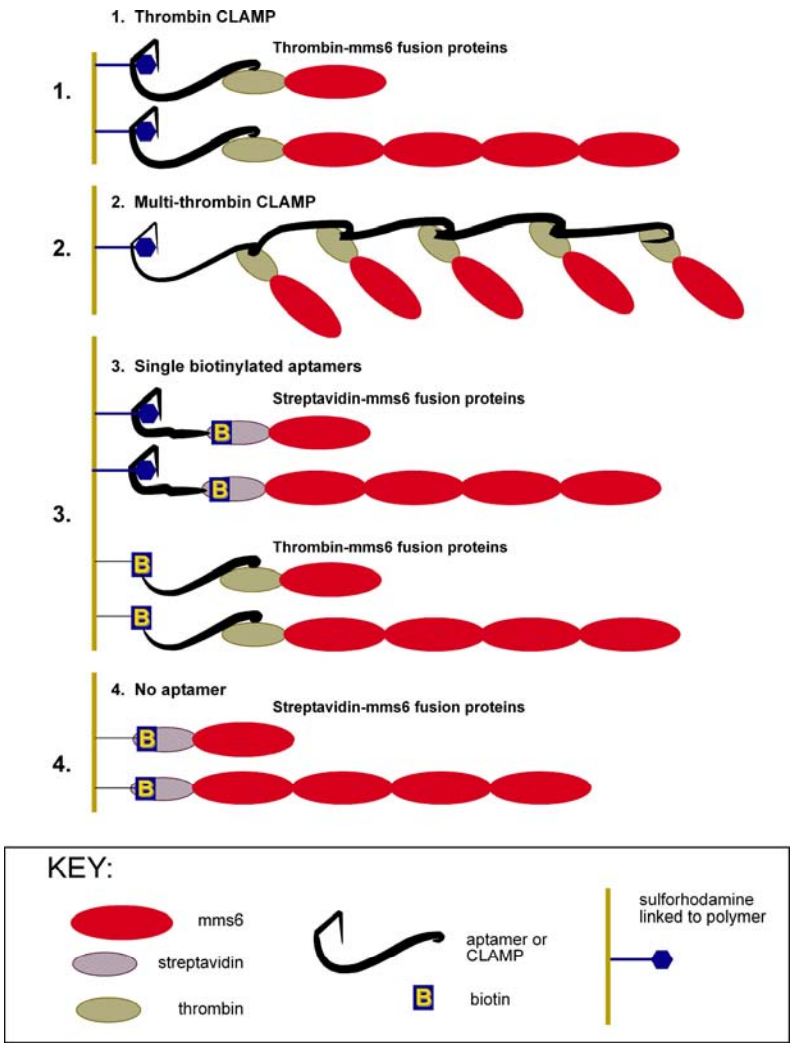

Fig. 4 Some options for mms6 fusion protein aptamer or CLAMP configurations that will be tested for their ability to promote assembly of magnetic particles. Fusion proteins are represented by oval domains linked in series. In some fusion proteins there are several linked mms6 protein domains. Each mms6 domain is about 12 kDa. Depending on the domain (streptavidin or thrombin) to which the mms6 domain is linked, different options are available for linking the protein to the polymer. Aptamers and CLAMPs will provide readily reversible linkages. The streptavidin interaction with biotin is much tighter but can be reversed with high temperatures.

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Synchrotron X-Ray Studies of Soft Matter and Bimolecular Materials

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The high brightness and energy tunability of synchrotron x-ray sources enable the structural analysis of the orientational and positional ordering that occurs over length scales from nanometers to microns when soft matter and biomolecular materials self-assembly into complex assemblies. We summarize our structural studies that utilize the x-ray techniques of coherent diffraction, microbeam diffraction, resonant scattering, and small angle scattering to probe structure. The measurements allowed us to study a wide range of problems including the discovery of novel types of modulated lipid bilayer phases, unambiguous evidence for the preparation of a single substrate-supported lipid bilayer, measurement of protein dimerization in solution, determination of molecular packing in semiconducting organic monolayers, identification of the rich variety of liquid crystal phases with different types of inter-layer and intra-layer orientational ordering, measurement of how atoms rearrange under the strains intrinsic to epitaxial films, and the interactions of layered liquid crystal phases with nanopatterned surfaces. The poster includes research by Ron Pindak, Lin Yang, Masa Fukuto and Brandon Chapman (BNL) in collaboration with Charles Rosenblatt and Isthi Syed (CWRU); Suntao Wang, and Cheng-Cher Huang (Univ. Minnesota); Huey Huang (Rice Univ.); Yizhak Yacoby (Hebrew Univ.).

Nanotube-Supported Phospholipid Bilayers

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Program Scope

The main scope of our research program is to attain fundamental understanding of effects of nanoscale confinement on the structure and dynamics of biomolecules and their assemblies including phospholipid bilayers and membrane proteins. We have discovered that under certain conditions many phospholipids self-assemble into a nanotube when placed inside a nanopore.[1,2] For nanopores that are macroscopically homogeneous and uniformly packed as in Anodic Aluminum Oxide membranes (AAO), such lipid nanotubes form arrays that are suitable for combinatorial assay applications. These structures – which we call lipid nanotube arrays – have a high density of the nanoporous channels providing at least a 500-gain in the bilayer surface area for the same size as the planar substrate chips. Moreover, we have found that despite being of a nanoscale size, many biophysical properties of the lipid nanotubes are remarkably similar to those of unsupported bilayers. Thus, these new nanotubular structures would be very advantageous substitutes to biological membranes and superior to the planar substrate-supported bilayers currently in use. In order to develop substrate-supported lipid nanotubes as a biotechological tool for membrane and membrane protein studies, the physical properties of the bilayers formed inside the nanopores have to be characterized and understood. It is also important to investigate whether the nanoscale confinement affects the bilayers properties and how these effects scale with the diameter of the nanopores. Currently, we are answering these questions through systematic studies of lipid nanotube arrays by complimentary spectroscopic techniques including EPR, NMR, two-photon fluorescence microscopy, and differential scanning calorimetry. The focus of the next phase of this program is to gain control of the lipid self-assembly and nanoscale confinement effects through developing uniform substrates as well as through chemical modification of substrate surfaces. This project addresses one of the critical research areas identified in the DOE-BOE reports on Nanoscale Science, Engineering, and Technology Research Directions as the design and synthesis of fully integrated hybrid materials combining unique properties of biomolecules with robustness and addressability of solid-state devices.[3]

Recent Progress

In order to determine structural organization of lipids confined in AAO nanotubes we have utilized spin labeling EPR at conventional (0.3 T) and high (3.5 T) magnetic fields. The EPR method is based on labeling a small fraction (such as 1:100) of lipid molecules with a nitroxide, the spectrum of which reports on local dynamics, magnetic interactions, and molecular orientations. These experiments led us to an unambiguous conclusion that the lipids are organized in bilayers but not the monolayers. Moreover,

local dynamics of the phospholipids appeared to be remarkably similar to that of the lipids in unsupported bilayers. These and related findings are described in more details in refs. 1, 2, and 4.

While thermodynamics and the mechanism of phase transitions in unsupported phospholipid bilayers are well documented, the phase properties of substrate-supported bylayers are studied in less details because of extremely small amount of lipids deposited on surfaces. Recently, we reported on the phase transition properties of nanopore-confined bilayers composed from a zwitterionic phospholipids DMPC (1,2-dimyristoyl-sn-glycero-3-phosphocholine) as studied by DSC and spin-labeling EPR.[5,6] The pore diameter varied from 40 to 200 nm. Both DCS and EPR demonstrated that the main phase transition temperatures for all lipid nanotubes studied and unsupported bilayers were essentially the same. Lipids' rotational dynamics was also unaffected according spin-labeling EPR. However, we observed an effect of pore diameter on the van't Hoff enthalpy. It appears that that while the dynamic properties (rotational correlation time and order parameter) of individual lipid molecules in the lipid nanotubes remain mainly unaffected by the nanoscale confinement down to ca. 40-45 nm pore diameter, the cooperative properties are affected to a larger degree. Specifically, with decrease of the nanopore diameter a decrease in the cooperative unit was observed. We concluded that the nanoporous substrate limits the growth of ordered domains that are characteristic of liquid crystalline phase.

In order to achieve adequate spectral resolution for multidimensional NMR the membrane protein samples should be uniformly aligned with respect to the magnetic field axis. Typically, such samples of membrane proteins are prepared using aligned lipid bilayers formed on planar solid substrates or by using magnetic forces to align bicelle discs in the external magnetic field. Recently, we described the use of nanopore-supported cylindrical lipid bilayers formed inside anodic aluminum oxide (AAO) substrates to align the transmembrane peptides for magnetic resonance studies.[7] Specifically, we reported on the first example of a high resolution solid-state ^{15}N 2D PISEMA NMR spectrum of a transmembrane peptide aligned using fully hydrated cylindrical lipid bilayers formed inside nanoporous anodic aluminum oxide (AAO) substrates. The transmembrane domain SSDPLVVA(A-*^{15}N*) SIIGILHLILWIL DRL of M2 protein from influenza A virus was reconstituted in hydrated DMPC bilayers that were macroscopically aligned by a conventional micro slide glass support or by the AAO nanoporous substrate. ^{15}N and ^{31}P NMR spectra demonstrate that both the phospholipids and the protein transmembrane domain are uniformly aligned in the nanopores.

Future Plans

Experimental studies of lipid nanotube arrays will be directed to the following specific aims:

1) *Effects of nanoscale confinement on physical properties of nanotube-supported bilayers.* Over the years, a wealth of information on physical properties of unsupported lipid bilayers has been acquired with the help of differential scanning calorimetry (DSC), fluorescence, NMR, spin labeling EPR and other spectroscopic methods. However, for phospholipid bilayers assembled on planar substrates DSC, EPR, and NMR were rarely employed because the amount of lipid material deposited onto substrates is insufficient

for spectroscopic studies unless the substrate coverage is increased by using multiple lipid layers or by studying dispersed materials. Because lipid nanotube arrays have a high density of the nanoporous channels providing large gains for the bilayer surface area, these structures could be studied by DSC and other methods. Thus, our current plans are to use a combination of solid state NMR, spin-labeling EPR, differential scanning calorimetry, and fluorescence to study effects of nanoscale confinement on cooperative properties of lipids, translational and rotational lipid dynamics, and macroscopic ordering of lipids in the nanotubes formed.

2) *Taking control of the nanoscale confinement effects*.

Once the effects of nanoscale confinement on physical properties of lipid bilayers and lipid-protein assemblies are understood, the properties of the system could be manipulated by properly choosing the nanoporous substrate. This could be achieved through fabrication of nanoporous AAO substrates with uniform pores and tunable pore diameter. In order to control the pore sizes and periodicity, we will be utilizing a two-step anodizing process using a high purity aluminum foil (99.998%) with thickness of 250-500 μm. Further fine-tuning of the nanoporous surfaces will be achieved through surface chemical modification. Chemical modifications of the polar surface of anodic aluminum oxide surfaces by silane chemistry resulting in covalent attachment of monolayers with interesting mechanical, chemical and electrical properties are well established. A wide variety of commercially available silanes would allow us to modify the initially hydrophobic properties of the alumina surface to a rather large degree.

(3) *Nanopore-supported phospholipids to align membrane proteins and peptides for structure functional studies with magnetic resonance.* Current NMR approaches for obtaining high-resolution structures of membrane proteins and peptides involve macroscopic alignment of functional biological membranes on glass plates. Such an alignment eliminates/reduces excessive paramagnetic shifts and line broadening that are typically observed for randomly dispersed samples. Similarly, some great improvement in spectral resolution and signal-to-noise ratio are observed in EPR for macroscopically-aligned spin-labeled membrane samples. For this purpose, several alignment techniques including pressure annealing, rehydrating and temperature annealing, smearing of membrane suspension on plates with partial dehydration, or magnetic field alignment of phospholipid bicelles have been developed. One of the recently improved approaches includes the isopotential spin-dry centrifugation of membrane fragments onto a planar support with simultaneous, or subsequent, partial evaporation of the solvent which aids alignment. However, all these methods have several limitation related to relatively short life time of the sample, difficulties of handling especially when ultrathin glass plates have to be stacked on top of each other to increase sensitivity in NMR experiments, and generally low bilayer to substrate volume ration. Our research will be focused on developing substrate-supported lipid nanotube method for aligning membrane proteins and peptides for structural studies with magnetic resonance. Preliminary 300 MHz NMR experiments carried out in collaboration with the group of Prof. Cross (NHMFL, Tallahassee, FL) demonstrated the feasibility of such approach by achieving good ^{31}P signal narrowing using just a few 60 μm AAO strips with deposited DMPC as well as the first two-dimensional solid state NMR experiment with a transmembrane peptide aligned by a nanoporous support.[7] In the next phase of this project we will be utilizing unique capabilities of the high resolution high field 900 MHz NMR spectrometer at NHMFL

(Tallahassee) to carry out structure-function studies of the ion channels. This part of the project will be carried in collaboration with the NHMFL.

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Biomimetic Synthesis of Bonelike Composites

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Program Scope:
We are interested in the biomimetic synthesis of bonelike composites as new generations of structural materials with controlled integration of organic and inorganic components across all length scales. Bone biogenesis is thought to occur by templated mineralization of hard apatite crystals by an elastic protein scaffold, a process we sought to emulate with synthetic biomimetic hydrogel polymers.

Recent Progress:
Cross-linked polymethacrylamide and polymethacrylate hydrogels were functionalized with mineral-binding ligands and used to template the formation of hydroxyapatite. A urea-mediated HA-mineralization method was developed to integrate HA with the hydrogel scaffold with excellent gel-mineral interfacial adhesion strength. No delamination of the mineral layer was observed when a Vickers indentation with 15 N load was performed on the surface of the composite. Templated growth of robust mineral layer was achieved for hydrogels functionalized with both anionic and hydroxylated ligands. The mineral-nucleating potential of hydroxyl groups identified here broaden the design parameters for synthetic bonelike composites.

Bulk pHEMA-HA composites with high HA content approximating that of human bone have also been developed. Despite the high mineral content, these composites, named as FlexBone, display remarkable resilience to high stress and strain. These materials can be formed in a syringe within minutes to hours, and cut into desired shapes and sizes, bent and compressed without fracturing. A wide range of hydrogel formulation and processing conditions, along with the variation in the crystallinity, particle size and aggregation properties of HA, are used to tailor the mechanical and micro-structural properties of FlexBone. Most FlexBone composites exhibit strong organic-inorganic interface throughout the 3-dimensional network, and can reach > 80% strain without fracturing. In addition, we have shown that the compressive behavior of as-prepared Flexbone composites are reversible at strains < 40%, making these materials suitable for moderate loading bear applications.

Future Plans:
We will further investigate the molecular mechanism of template-driven mineralization by synthetic model systems, particularly focusing on the elucidation of how the structure and density of mineral binding ligands of a 3-D scaffold dictate the morphology, crystallinity and interfacial adhesion strength of the templated mineral growth on a nanoscopic level.

The unique mechanical properties of FlexBone will be expanded to the synthesis of a broad range of composites for structural, dielectric, magnetic and electronic applications by substituting HA with other ceramics, semiconductor or metal particles of interest.

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Designed Materials via Inverse and Optimization Techniques

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"Self-assembly" typically describes processes in which entities (atoms, molecules, aggregates of molecules, etc.) spontaneously arrange themselves into a larger ordered and functioning structure. Biology offers wonderful examples, including the spontaneous formation of the DNA double helix from two complementary oligonucleotide chains, the formation of lipid bilayers to produce membranes, and the folding of proteins into a biologically active state. On the synthetic side, molecular self-assembly is a potentially powerful method to fabricate atomically precise materials and devices. For example, Whitesides [1] has shown intricate two-dimensional patterns can emerge in self-assembly of organic molecules on an inorganic surface. Jenekhe and Chen [2] have devised 'smart plastics' that assemble into photonic crystals. Stellacci et. al. [3] have shown how gold nanoparticles can be tagged with organic molecules in such a way that they assemble chains, or 'nanowires'. These examples provide glimpses into the materials science of the future, i.e., devising building blocks with specific interactions that can self-organize on a set of larger length scales. Theoretical work has primarily focused on finding the structure and macroscopic properties of many-body systems given the interactions – what we refer to as the "forward" problem of statistical mechanics. The forward problem has been extensively studied in the context of the freezing transition both analytically and numerically.

We introduce an *inverse statistical-mechanical* methodology to find optimized interaction potentials that lead spontaneously to a target many-particle configuration. The idea of *tailoring* potentials to generate targeted structures is motivated by the rich array of fundamental issues and questions offered by this fascinating inverse statistical-mechanical problem as well as our recent ability to identify the structures that have optimal or desirable bulk properties. The latter includes novel crystal structures for photonic band-gap applications [4], materials with negative or vanishing thermal expansion coefficients [5], materials with negative Poisson ratios [6], materials with optimal transport and mechanical properties [7], mesoporous solids for applications in catalysis, separations, sensors and electronics [8], and systems characterized by entropically driven inverse freezing [9]. We envision target structures possessing varying degrees of disorder, which enables us to extend the traditional idea of self-assembly to incorporate both amorphous and crystalline structures as well as quasicrystals. Colloids are the ideal system to test our optimized potentials, since both repulsive and attractive interactions can be manipulated (e.g., via depletion forces, dipole-dipole inter-

* Principal Investigator

actions, electrostatic interactions, etc.) and therefore offer a panoply of possible potentials that far extends the range offered by molecular systems.

Because there is a vast class of many-body potentials, we will focus on spherically symmetric (i.e. isotropic) pairwise additive interactions for simplicity here. There are many open questions even for this simple class of potentials. For example, it is not known what are the limitations of isotropic pairwise additivity for producing target structures. We know that such interactions cannot produce thermodynamically stable chiral structures with a specified handedness; equal amounts of left-handed and right-handed structures would result. When is anisotropy in the potential required? An answer based on intuition from molecular systems would fail here. For instance, the diamond lattice is thought to require directional interactions because such structures found in Nature result from covalent bonding. In fact, it is not known whether a diamond lattice could be created from an isotropic pair potential. This structure has a special status in photonics research because it had been shown [4] that a diamond lattice of dielectric spheres exhibits a photonic band gap across the Brillouin zone.

The two-dimensional analog of this open three-dimensional crystal is the three-coordinated honeycomb lattice. Accordingly, our general optimization procedure (described below) will be illustrated by applying it to produce an optimized circularly symmetric pair potential $V(r)$ that spontaneously yields the honeycomb lattice as the ground state (zero-temperature) structure in a positive density range. In contrast to previous approaches that have claimed to produce open lattice structures, our procedure incorporates the phonon spectra, which is a crucial ingredient. Because the honeycomb is an open lattice that is a subset of the triangular lattice, it is inherently challenging to assemble using isotropic potentials. Indeed, such a potential has never been found before.

We formulate statistical-mechanical inverse methods in order to determine optimized interparticle interactions that spontaneously produce target many-particle configurations. Motivated by advances that give experimentalists greater and greater control over colloidal interaction potentials, we propose and discuss two computational algorithms that search for optimal potentials for self-assembly of a given target configuration. The first optimizes the potential near the ground state and the second near the melting point. We begin by applying these techniques to assembling open structures in two dimensions (square and honeycomb lattices) using only circularly symmetric pair interaction potentials ; we demonstrate that the algorithms do indeed cause self-assembly of the target lattice. Our approach is distinguished from previous work in that we consider (i) lattice sums, (ii) mechanical stability (phonon spectra), and (iii) annealed Monte Carlo simulations. We also devise circularly symmetric potentials that yield chain-like structures as well as systems of clusters.

We have also investigated two other optimization problems. The first involves the suppression of long-wavelength density fluctuations through the control of collective density variables [10] and the other involves the construction of many-particle configurations for a targeted pair-correlation function [11].

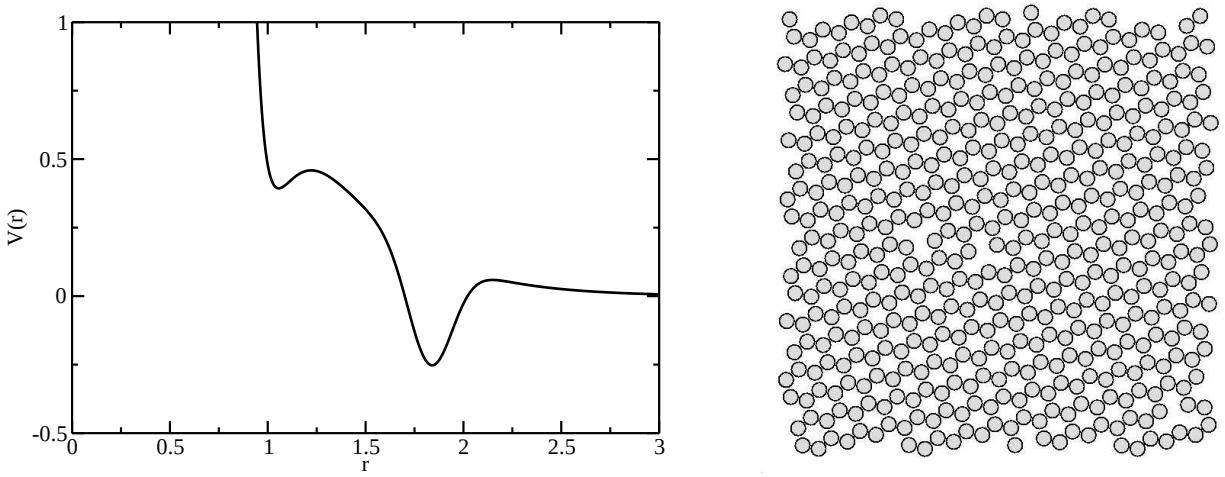

FIG. 1: Pair potential, $V(r)$, optimized for honeycomb self-assembly, and 500-particle annealed MC results for this potential.

Collective density variables $\rho(\boldsymbol{k})$ have proved to be useful tools in the study of many-body problems in a variety of fields that are concerned with structural and kinematic phenomena. In spite of their broad applicability, mathematical understanding of collective density variables remains an underexplored subject. In this work, we examine features associated with collective density variables in two dimensions using numerical exploration techniques to generate particle patterns in the classical ground state [10]. Particle pair interactions are governed by a continuous, bounded potential. Our approach involves constraining related collective parameters $C(\boldsymbol{k})$, with wave vector $\boldsymbol{k}$ magnitudes at or below a chosen cutoff, to their absolute minimum values. Density fluctuations for those $\boldsymbol{k}$'s thus are suppressed. The resulting investigation distinguishes three structural regimes as the number of constrained wave vectors is increased - disordered, wavy crystalline, and crystalline regimes - each with characteristic distinguishing features. It should be noted that our choice of pair potential can lead to pair correlation functions that exhibit an effective hard core and thus signal the formation of a hard-disk-like equilibrium fluid. In addition, our method creates particle patterns that are *hyperuniform*, thus supporting the notion that structural glasses can be hyperuniform as the temperature $T \to 0$.

The pair correlation function $g_2(r)$ provides a basic geometric descriptor for many-particle systems. It must obey two necessary conditions: (i) non-negativity for all distances r, and (ii) non-negativity of its associated structure factor $S(k)$ for all k. Here we utilize an improved stochastic construction algorithm for particle configurations to establish conditions in which (i) and (ii) are also sufficient, *i.e.* $g_2(r)$ is in fact realizable [11]. Two types of target pair correlation functions have been investigated in one, two, and three dimensions for hard-core particles, specifically a unit step function, and a contact δ plus step pair correlation function. Results indicate that the former target function is realizable up to a terminal density set by necessary condition (ii), at which the particle core packing fraction equals

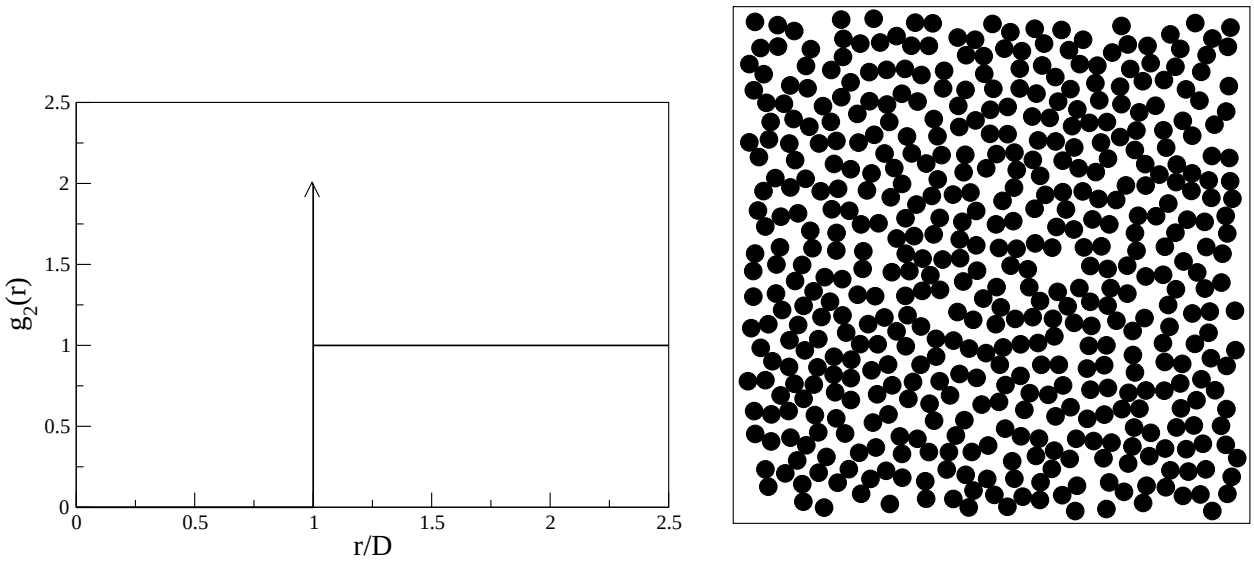

FIG. 2: Inverse construction of the contact δ plus step pair correlation function. Left panel: Graphical representation of the contact δ plus step pair correlation function. Right panel: A two-dimensional configuration of 500 particles that has been constructed for the contact δ plus step pair correlation function. The configuration consists of only dimers at the terminal density $\phi_c = 0.5$ with an average contact value $Z = 1.0$.

2^{-d} in d dimensions. Figure 2 shows a case in two dimensions. Furthermore, results are consistent with the proposition that for $d > 1$ the contact δ plus step function is realizable up to a terminal density due to condition (ii) at which the packing fraction of cores is $(d+2)/2^{d+1}$ [11].

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Biopolymers Containing Unnatural Building Blocks

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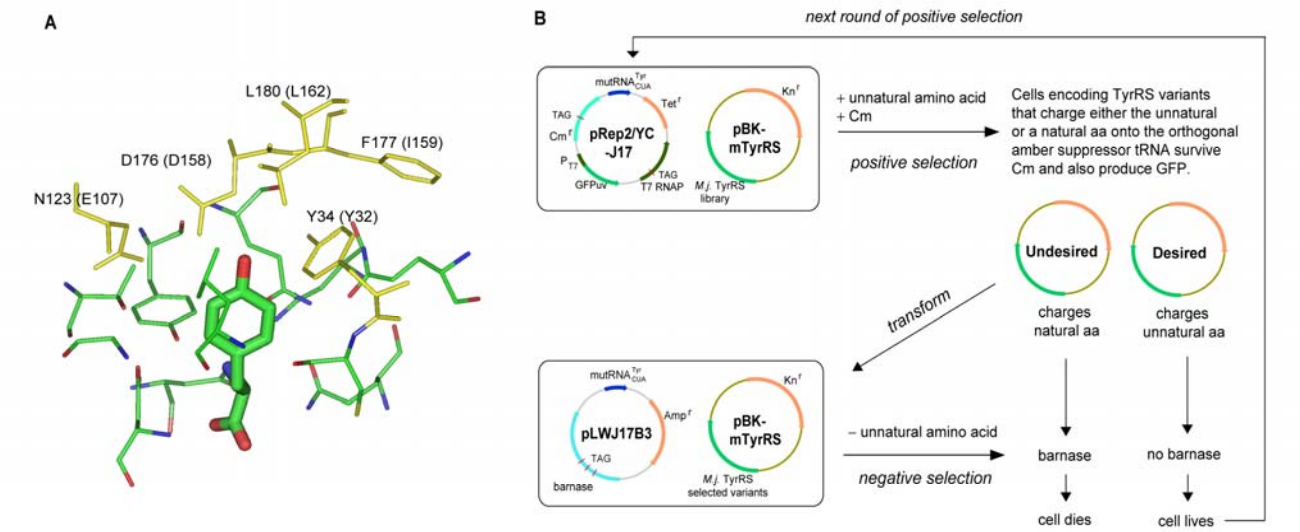
Program Scope

Although chemists can synthesize virtually any small organic molecule, our ability to rationally manipulate the structures of proteins is quite limited, despite their involvement in virtually every life process. For most proteins, modifications are largely restricted to substitutions among the common 20 amino acids. We have developed a methodology that makes it possible to add new building blocks to the genetic codes of both prokaryotic and eukaryotic organisms. Over 30 novel amino acids have been genetically encoded in response to unique triplet and quadruplet codons including fluorescent, photoreactive, redox-active amino acids, glycosylated amino acids, and amino acids with keto, azido, acetylenic, and heavy-atom-containing side chains. By removing the limitations imposed by the existing 20 amino acid code, it should be possible to generate proteins and perhaps entire organisms with new or enhanced properties.

Recent Progress

A general method has been developed to incorporate unnatural amino acids at defined sites in proteins in *E. coli*, yeast, and mammalian cells. In this method, new components are added to the protein biosynthetic machinery of the host organism. These included a codon that uniquely designates the unnatural amino acid and an orthogonal tRNA – aminoacyl-tRNA synthetase (aaRS) pair that can specifically incorporate the unnatural amino acid into proteins in response to the cognate codon. We initially used the amber nonsense codon as a unique codon since it is known that this codon can be efficiently suppressed without significantly affecting *E. coli* growth. An orthogonal tRNA – synthetase pair in *E. coli* was derived from a tyrosyl-tRNA synthetase (TyrRS) – $tRNA^{Tyr}$ pair from the archaea *M. jannaschii* (Mj). The orthogonal synthetase was engineered to acylate the cognate tRNA with the unnatural amino acid, and not any endogenous amino acids. This was accomplished by generating large libraries of synthetase variants by randomizing several residues in the amino acid binding site of the Mj TyrRS. The libraries were then passed through positive selections to select for synthetases that efficiently acylate the unnatural amino acid, and negative selections to remove synthetases that charge a natural amino acid to the cognate tRNA (Scheme 1) . This selection scheme has been used to incorporate over 20 unnatural amino acids into proteins in *E. coli* in good yield and with high fidelity. Several additional orthogonal *E. coli* pairs have also been generated from archaea. These and other new orthogonal pairs will likely increase the structural diversity and number of unnatural amino acids that can be incorporated into proteins by this method. Orthogonal pairs have also been generated for use in yeast, including an *E. coli* $tRNA^{Tyr}$ – TyrRS pair and $tRNA^{Leu}$ – LeuRS pair, which allow unnatural amino acids to be genetically encoded in yeast. In addition we have shown that four base codons and frameshift suppressors can be used to uniquely specify unnatural amino acid in *E. coli*. Finally, we have shown that one can not only genetically specify an unnatural amino acid in *E. coli*, but also biosynthesize it as well to create a completely autonomous 21 amino acid bacterium.



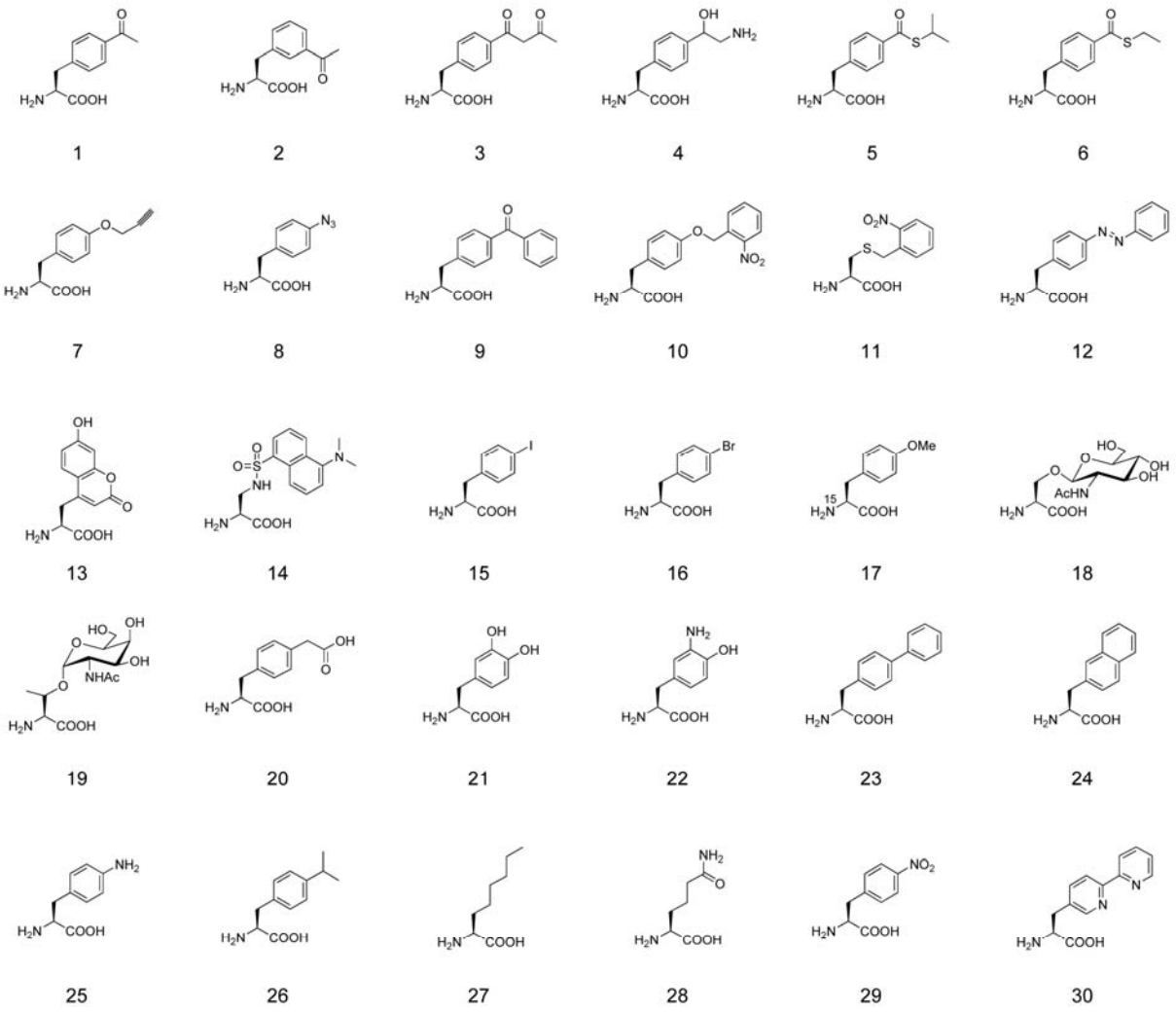
Scheme 1. Modification of the amino acid specificity of an orthogonal TyrRS in *E. coli*. a) A library of *M. jannaschii* TyrRS mutants was generated by randomizing 5 residues (in yellow) in the amino acid binding site; b) directed evolution of *M. jannaschii* TyrRS variants in *E. coli*.

Using this methodology, more than 30 unnatural amino acids have been incorporated into proteins with high fidelity in response to unique triplet or quadruplet codons in *E. coli*, yeast, or mammalian cells. For example, unnatural amino acids with uniquely reactive functional groups **1**-**8** have been genetically encoded in *E. coli* or yeast. These amino acids can be used to selectively modify proteins under mild conditions with a variety of reagents such as fluorophores, biotin, sugar analogues, and polyethylene glycols (PEGs), or to selectively immobilize protein on two dimensional surfaces in defined orientations for sensor approaches. Photoactive amino acids have also been selectively incorporated into proteins in *E. coli* or yeast, including the photo-crosslinking amino acids **8** and **9** which can be used to map bio-macromolecular interactions *in vitro* or *in vivo*, and the photocaged amino acids **10** and **11** which can be used to photomodulate protein activity. The heavy-atom containing amino acid **15** has been genetically encoded both in *E. coli* and in yeast, and can be used for SAD phasing in structure determination. In addition, ^{15}N-labeled O-methyltyrosine **17** has been selectively incorporated into proteins as an NMR probe. Unnatural amino acids corresponding to posttranslational modifications, including glycosylated amino acids **18** and **19**, have also been cotranslationally incorporated into proteins, thus providing a method for the synthesis of homogenous glycoproteins. The redox-active amino acids **21** and **22** can act as radical traps in electron transfer process. Finally, the bipyridyl-containing amino acid **30** has been added to the genetic code of *E. coli*. This amino acid chelates transition metal ions (e.g., Zn^{2+}, Cu^{2+}, Fe^{2+}, and Ru^{2+}), and may facilitate the generation of metalloproteins with novel properties (e.g., metal-dependent protein dimerization, metalloproteases, or *in vivo* imaging agents). Clearly a large number of structurally diverse building blocks have been added to the genetic code using this approach (Scheme 2). Most recently, we have also used this methodology in conjunction with phage display as a general approach to generate polypeptide libraries containing unnatural amino acids. This should significantly increase the scope of phage display technology. Other display formats such as ribosome and yeast display may also be extended by unnatural amino acid incorporation.

Future Plans

Given the structural diversity and number of unnatural amino acids that have been selectively incorporated into proteins to date, it is likely a large number of additional amino acids can be genetically encoded. We are currently evolving orthogonal tRNA – synthetase systems to incorporate other unnatural amino acids such as spin labels and IR probes, lanthanide chelators, and α-hydroxyl or N-methyl amino

acids (in order to make structurally defined, folded biopolymers with novel backbone, e.g., polyesters). To do so, it is necessary to incorporate two or more different unnatural amino acids simultaneously into the same proteins at distinct sites. In one case, we used a combination of amber and frameshift codons (TAG and AGGA, respectively) to incorporate two different unnatural amino acids in one protein. By using codons made up of more than 3 nucleotides or systematically reassigning degenerate triplet codons, it should be possible to further expand the code. We will also extend our studies aimed at using this methodology to generate structurely defined glycoproteins as well as using reactive amino acids to orient proteins on two dimensional surfaces. This methodology should provide a powerful new approach toward the study of proteins (both *in vivo* and *in vitro*) in which amino acids with novel physical, chemical, or biological properties can be tailored to address a specific structural/functional question at hand. Finally, the ability to add new building blocks to the genetic code may allow the rational design or evolution of proteins with new or enhanced properties.



Scheme 2. Representative unnatural amino acids that have been genetically encoded in *E. coli* and/or yeast.

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Slaved Diffusion in Phospholipid Bilayers

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Abstract

The translational diffusion of phospholipids in supported fluid bilayers splits into two populations when proteins and polyelectrolytes adsorb at incomplete surface coverage. Spatially-resolved measurements using fluorescence correlation spectroscopy (FCS) show that a slow mode, whose magnitude scales inversely with the degree of polymerization of the adsorbate, coexists with a fast mode characteristic of naked lipid diffusion. Inner and outer leaflets of the bilayer are affected nearly equally. Mobility may vary from spot to spot on the membrane surface, in spite of the lipid composition being the same. Confirmed by two-color fluorescence correlation spectroscopy, this offers a new mechanism to explain how nano-sized domains with reduced mobility arise in lipid membranes.

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