SYMPOSIUM V

Proteins as Materials

April 15 - 16, 2004

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* Invited paper

SESSION V1: Self-Assembly
Thursday Morning, April 15, 2004
Room 3000 (Moscone West)

9:00 AM *V1.1
Self-Assembling Symmetric Protein Complexes By Design: Shells, Filaments, Layers and Crystals. Jennifer E. Padilla, Janel Laidman and Todd O. Yeates; Chemistry & Biochemistry, University of California, Los Angeles, Los Angeles, California.

We present a general strategy for creating proteins that can self-assemble into symmetric protein complexes. The method makes use of naturally occurring oligomeric proteins whose structures are known. Two or more such proteins can be fused into a chimera whose oligomeric interfaces drive the assembly of a shell, filament, layer, or crystal according to design. Using proteins to create these new materials confers several advantages: 1) complexes are assembled from identical building blocks, 2) complexes make use of the high affinity and high specificity of protein-protein interfaces, 3) these self-assembled materials will have features such as pore sizes on the nanometer scale. This class of materials may find a wide range of uses due to the wide range of protein functionalities that can potentially be incorporated. Organizing proteins into layers or cages may be of utility in creating self-assembled monolayers or sequestering and delivering other molecules. At the heart of the method are the rules of symmetry that govern crystals and symmetric complexes. We have demonstrated the method through the production of protein cages and filaments and are currently pursuing layers and crystals. We will discuss the challenges that arise at this higher level of assembly and present our latest results towards the production of self-assembling protein layers and crystals.

9:30 AM V1.2
Designing Ligand-Induced Reversible Protein Polymers. Jonathan C Carlson[1] and Carston R Wagner[1]; [1]Medicinal Chemistry, University of Minnesota, Minneapolis, Minnesota; [2]Hauptman-Woodward Medical Research Institute, Buffalo, New York.

Chemically induced protein dimerization exploits the specificity of ligand recognition to regulate biological assembly, and in so doing provides a building block for the engineering of intelligent self-assembling systems. In an effort to extend the template of induced dimerization to the assembly of larger scale architecture, we have demonstrated the ability of a bivalent methotrexate ligand (bis-MTX) to selectively dimerize *E.coli* dihydrofolate reductase (ecDHFR) in solution, and determined the crystal structure of the complex. Ongoing efforts have probed the principles governing molecular recognition in this complex and explored the engineering of novel complementary homodimeric and heterodimeric interfaces. To expand these experiments from dimerization to oligomerization, we constructed a fusion protein containing two complete ecDHFR units in a single polypeptide chain ($ecDHFR_2$). Current efforts are focused on the analysis of $ecDHFR_2$–bis-MTX complexes and the characterization of reversible chemically induced protein polymerization. The pharmaceutical control intrinsic to this system represents a potentially novel class of synthetically regulated biological nanomaterials.

9:45 AM V1.3
Designing Protein Interfaces for the Chemical Induction of Protein Supramolecular Assemblies. Carston R. Wagner, Jonathan C. T. Carlson and Jessie L. Kerns; Medicinal Chemistry, University of Minnesota, Minneapolis, Minnesota.

By integrating protein assembly and ligand recognition, chemically induced protein dimerization provides a unique tool to explore regulated nanoscale biorecognition. We have demonstrated the ability of a bivalent methotrexate ligand (bisMTX) to selectively dimerize E. coli dihydrofolate reductase (ecDHFR), determined the crystal structure of the complex, and evaluated the factors regulating its ability to induce cooperative dimerization in solution. Significant findings included the strength of favorable protein-protein interactions in bisMTX/ecDHFR dimers, and the selectivity of dimerization for ecDHFR relative to mouse DHFR (>10e7). The present research has probed the role of specific amino acid residues in the energetics of the protein dimer interface. To this end, we have produced point mutations at key interfacial residues Ala 19, Asn 23, and Ser 148 to assay the balance between steric and electrostatic factors in the defined molecular context of the dimer interface. Molecular dynamics simulations have been conducted in parallel in an effort to mechanistically dissect the interactions mediating protein-protein complementarity. Current efforts are focused on the application of these results to the engineering of novel homodimeric and heterodimeric interfaces that can be used for the self-assembly of protein based supramolecular structures.

10:30 AM *V1.4
Rational design and bottom-up assembly of supramolecular fibrous protein structures. Derek Neil Woolfson, School of Life Sciences, University of Sussex, Falmer, East Sussex, United Kingdom.

Robust rules that link protein sequence and structure are required for rational, de novo protein design. With such rules and a little imagination it should be possible to create new proteins that expand upon natures repertoire of structures and functions and, so, produce potentially useful new molecules including biomaterials. In the last decade our understanding of one type of protein structure, the alpha-helical coiled coil, advanced sufficiently to allow confident rational designs of prescribed motifs. Coiled coils are oligomeric proteins in which each polypeptide chain forms an alpha-helix, two or more such chains wrap around each other to form rope-like structures. In particular, we have good rules for the construction of homo- and hetero-dimeric motifs known as leucine zippers. Leucine zippers carry an advantage for experimental design studies in that they are chemically accessible peptides and are relatively straightforward to characterise. All natural leucine zippers, and indeed coiled coils in general, are blunt-ended structures; i.e. the ends of alpha helices match up precisely. Despite this apparent natural constraint, we were intrigued to know if sticky ended structures could be engineered and, if so, whether such structures could be used as building blocks for the assembly of large fibrous structures. In short, we achieved this with the first-generation design of what we call the Self-Assembling Fibre peptides (SAFs), see Pandya et al. (2000) Biochemistry 39 8728. The SAF system comprises two complementary peptides that assemble to form a sticky ended heterodimer. The overlapping ends of this building block contain features that promote longitudinal assembly, rather like the assembly of lego blocks. Fibre formation by the SAF peptides was confirmed by circular dichroism spectroscopy, electron microscopy and X-ray fibre diffraction. As desired, the fibres are long (up to tens of microns), straight and without kinks or branches. Interestingly, however, the fibres are thicker (approximately 50 nm) than we expected from our original design concept. In redesign experiments to explore thickening, we have discovered that the fibres can be stabilised and thickened further. Moreover, these redesigned fibres display an intriguing degree of internal and external order on the nanoscale. Furthermore, addition of fluorescently SAF peptides to the matured fibres indicates that the fibres are polar; that is, new peptides are selectively recruited to just one end of the fibres. In addition, we have designed special peptides that incorporate into the SAFs and alter their morphology; specifically, we have designed branched (Ryadnov & Woolfson (2003) Angew Chem Int Ed 42 3021) and kinked fibres (Ryadnov & Woolfson (2003) Nature Mater 2 5 329). We aim to build on these discoveries and develop applications for the SAFs in surface and tissue engineering.

11:00 AM V1.5
Studies of self-assembling peptide systems with short patterned sequences. Yuri Zimenkov and Vincent Conticello; Chemistry, Emory University, ATLANTA, Georgia.

Several series of short self assembling peptides based on repeating coiled-coil motifs were designed, synthesized and studied by different methods. Engineering of interactions between neighboring peptide chains led to systems' ability to self-organize into long fibers with thickness of tens of nanometers, as confirmed by electron microscopy. All the systems were shown to be essentially α-helical. We were able to process some of the peptides with relatively low melting temperature to yield macroscopic fibers, up to several centimeters long.

11:15 AM V1.6
Novel Nanotube Construction through Amyloid-β peptide Congener Self-Assembly. Kun Lu[1], Jaby Jacob[2], Pappannan Thiyagarajan[2], Vince P Conticello[1] and David G Lynn[1]; [1]Chemistry, Emory University, Atlanta, Georgia; [2]Intense Pulsed Neutron Source, Argonne National Laboratory, Argonne.

Fibrillar structures are central to virtually all facets of movement in biology. Here we report the self-assembly of amyloid fibril components into homogeneous peptide hollow nanotubes. In our attempts to better understand and increase the energies of β-sheet lamination, the central segment of the amyloid-β peptide of Alzheimer's disease was placed under acidic 40% acetonitrile/water conditions, and allowed to self-assemble. A pronounced β-sheet signature developed following typical nucleation dependent kinetics. An initially formed peptide bilayer sheet appeared to coil into helical ribbons, coiling sufficiently over time to form the final nanotube. Small angle neutron (SANS) and X-ray scattering (SAXS) defined the outer and inner radius of the formed nanotubes in solution to contain a 44 nm inner cavity with 4 nm thick walls. Atomic force microscopy (AFM) and transmission electron microscopy (TEM) images further confirmed the homogenous arrays of solvent filled nanotubes arising from a flat rectangular bilayer sheet, 130 nm wide by 4 nm thick, with each bilayer leaflet composed of laminated β-sheets. Such robust and persistent

self-assembling nanotubes with positively charged surfaces of very different inner and outer curvature offer a unique, robust, and easily accessible scaffold for nanotechnology and have potential applications in research, industry and medicine.

11:30 AM *V1.7
Responsive Hydrogels from De Novo Designed Peptides: Linking Peptide Folding to Self-Assembly.
Joel Patrick Schneider, Chemistry and Biochemistry, University of Delaware, Newark, Delaware.

Peptide design has been used to construct chemically and mechanically responsive materials. A general peptide design is presented that links the intramolecular folding of beta-hairpin peptides to their propensity to self-assemble affording hydrogels rich in beta-sheet. Environmental responsiveness has been specifically engineered into the material by linking intramolecular folding to changes in solution pH or temperature, mechanical responsiveness by linking hydrogelation to self-assembly. CD and IR spectroscopies show that at low pH or low temperature, individual peptides are unstructured affording a low viscosity aqueous solution. Under basic conditions or elevated temperatures, intramolecular folding takes place affording amphiphilic beta-hairpins that intermolecularly self-assemble. The folding and self-assembly events are totally reversible affording a "smart" material responsive to its environment. Rheology shows that the hydrogel is strong but is shear thinning. However, quick mechanical strength recovery after cessation of shear is observed due to the inherent self-assembled nature of the scaffold. Characterization of the gelation process, from the molecular level up through the macroscopic properties of the material, suggests that by linking the intramolecular folding of small designed peptides to their ability to self-assemble, responsive materials can be prepared. Microscopic characterization of the hydrogel reveals a water filled porous scaffold on both the nano- and microscale making this material a suitable candidate for use in tissue engineering. The possibility of controlling bulk material properties via peptide design at the molecular level will be discussed.

SESSION V2: Device Applications
Thursday Afternoon, April 15, 2004
Room 3000 (Moscone West)

1:30 PM *V2.1
Developing New Selective Methods for the Ordered Attachment of Proteins to Surfaces. Julio A. Camarero[1], James J. de Yoreo[1], Chin Li Cheung[1], Mathew A. Coleman[1], Tinawei Lin[2] and John E. Johnson[2]; [1]Lawrence Livermore National Laboratory, Livermore, California; [2]The Scripps Research Institute, La Jolla, California.

Various methods are available for attaching proteins to solid surfaces. Most rely on non-specific adsorption, or on the random cross-linking of proteins to chemically reactive surfaces. In both cases the protein is attached to the surface in random orientations. The use of recombinant affinity tags addresses the orientation issue. However, in most cases the interactions of the tags are reversible and therefore not stable over the course of subsequent assays or require large mediator proteins. Covalent attachment and orientation of a protein to a solid support requires two unique and mutually reactive groups on the protein and the support surface. The reaction between these two groups should be highly chemoselective, thus behaving like a molecular "velcro". Many experimental techniques in biology and biophysics, and applications in diagnosis and drug discovery, require proteins immobilized on solid substrates. In fact, the concept of arrays of proteins attached to a solid support has attracted increasing attention over the last three years due to the sequencing of several genomes, including the human genome. Protein arrays can be used easily for the parallel analysis of whole proteomes. Another powerful application employs ordered nanometric arrays of proteins as nucleation templates for protein crystallization.. The present work describes our ongoing efforts towards the creation of micro and nano-scaled ordered arrays of protein/virus covalently attached to site-specific chemical linkers patterned by different nano- and microlithograpic techniques. We will present a new and efficient solid-phase approach for the synthesis of chemically modified long alkyl-thiols. These compounds can be used to introduce chemoselective reacting groups onto gold and silicon-based surfaces. We will show that these modified thiols can be used for creating nano- and micrometric chemical patterns by using different lithographic techniques. This patterns can react chemoselectively with proteins and virus which have been chemically or recombinantly modified to contain complementary chemical groups at specific positions thus resulting in the oriented attachment of the protein or virus to the surface. Also a total novel and generic approach for the chemoenzymatic and photo-switchable attachment of proteins to surfaces will be also presented.

2:00 PM V2.2
Biomimetic surfaces via dextran immobilization; characterization and analysis. Davide Miksa[2,1], Elizabeth Irish[1], Dwayne Chen[2], Russell J. Composto[1] and David M. Eckmann[2]; [1]Material Science and Engineering, Uni. of Pennsylvania, Philadelphia, Pennsylvania; [2]Anesthesia, Uni. of Pennsylvania Health System, Philadelphia, Pennsylvania.

Biomimetic surfaces were prepared by chemisorption of oxidized dextran (1, 2, 4 mg/mL) onto SiO2 substrates that were previously modified with aminopropyl-tri-ethoxy silane (APTES). The kinetics of dextran oxidation by sodium metaperiodate (NaIO4) were quantified by 1H NMR and pH measurements. The extent of oxidation was then used to control the properties of the biomimetic surface. Oxidation times of 0.5, 1, and 2 hours resulted in <20, 30, and 60% oxidation, respectively. When the extent of oxidation is low, fewer attachment points between the amine terminated surface (SiO2 + APTES) and the oxidized dextran are expected and, therefore, the chains form a brushy layer. Conversely, longer oxidation times were used to achieve a more densely packed dextranized surface. The ellipsometric thickness of the dry dextran did not change significantly (ca. 5.26 ± 0.78 Å) as the concentration varied from 1 to 4 mg/mL. Also, the thickness was observed to remain relatively constant upon varying oxidation times from 0.5 to 2 hours. Nevertheless, the water contact angle increased by $10°$ as the oxidation time increased. This result may be attributed to the molecular relations underlying a brushy and densely packed dextran layer. Varying degrees of protein adsorption were observed when the biomimetic surfaces were exposed to solutions of bovine serum albumin (BSA). The data suggests that a combination of electrostatic as well as steric-entropic interactions control protein adsorption. Finally, atomic force microscopy (AFM) was used to characterize all the surfaces at the μm scale.

2:15 PM V2.3
Development of a Miniaturized Protein/Conductive Polymer Thermal Sensor Array. Lawrence L Brott, Rajesh R Naik and Morley O Stone; US Air Force Research Laboratory, WPAFB, Ohio.

The use of thermal sensors and imaging devices has become so widespread that numerous applications ranging from military sensors to firefighting equipment rely heavily upon this technology. There is a desire, however, to improve upon the sensitivity of uncooled sensors while reducing the complexity of their fabrication. Our research takes a biomimetic approach through the incorporation of thermosensitive proteins to enhance the properties of the infrared sensing device. By integrating these proteins into a matrix of poly(vinyl alcohol) doped with conducting carbon black and plasticizer, a relatively simple and reliable thermally sensitive chip can be fabricated. Here, we describe our work on the miniaturization of our array from the millimeter- to micron-size scale and the challenges it presents. The effect processing has on micron-scale protein/polymer films will be discussed along with the altered sensor dynamics at these reduced size scales.

2:30 PM *V2.4
Design and production of protein polymer drag-tags for application in free-solution, microchannel DNA sequencing. Annelise E. Barron, Jong-In Won and Robert Meagher; Chemical Engineering, Northwestern University, Evanston, Illinois.

We describe research efforts towards the creation of a novel series of non-natural, repetitive polypeptides (protein polymers) expressed in bacteria, which are designed to serve as "drag-tags" for microchannel DNA separation by End-Labeled Free-Solution Electrophoresis (ELFSE). ELFSE is a promising bioconjugate method for DNA sequencing and genotyping by both capillary and microfluidic device electrophoresis, which will eliminate the need for loading viscous polymer networks into electrophoresis microchannels. To accomplish microchannel DNA separations with high performance, ELFSE requires totally monodisperse perturbing entities (i.e. drag-tags), such as proteins, which will create a large amount of frictional drag when pulled behind DNA during free-solution electrophoresis, and which have other properties suitable for microchannel electrophoresis. Natural proteins are unsuitable drag-tags due to their compact folded shapes, surface charges, and strong interaction with the walls of the electrophoresis microchannel. However, non-natural, repetitive polypeptides hold substantial promise as ELFSE drag-tags because their sequences can be designed for desired properties, such as water-solubility and charge-neutrality. A novel cloning method, developed in our laboratory, has enabled us to produce a series of long protein polymers having non-natural sequences of interest. This novel method allows production of long synthetic genes in a controlled and reproducible fashion, without intra-molecular cyclization. After the proteins were expressed and purified, the resulting proteins were conjugated to ssDNA oligomers using a bifunctional coupling reagent, and tested as drag-tags for DNA separation by free-solution capillary electrophoresis. Based on the results of these analyses, we have

demonstrated that high-resolution separation of DNA molecules can be accomplished in free solution, by conjugating these polypeptide drag-tags to DNA.

3:30 PM V2.5
Fluorophore Conformation in the Green Fluorescent Protein Biological Chromophore: A Quantum Mechanics/Molecular Mechanics Study. Steven Trohalaki, Soumya S. Patnaik and Ruth Pachter; Materials & Manufacturing Directorate, Air Force Research Lab, Wright-Patterson AFB, Ohio.

Green Fluorescent Protein (GFP) is a widely used fluorescent marker exhibiting two excitation peaks - a strong peak at 398 nm and a second at 475 nm, with the fluorescence at ca. 510 nm. Its molecular structure consists of a β-barrel composed of 11 β-strands and a central helix containing the fluorophore. Two different forms of the fluorophore - a protonated/neutral fluorophore and a de-protonated/anionic fluorophore - are thought to be responsible for the two distinct spectroscopic states, which are followed by a significant conformational change. Notably, the isolated fluorophore in solution is efficiently quenched. Conformational flexibility within the protein cavity therefore appears to be an important factor governing the photochemistry of GFP. In this work, we calculate the torsional potential of the two exocyclic bonds that connect the two rings in the fluorophore, taking into account its immediate environment by applying a quantum mechanics/molecular mechanics method, with the ultimate aim of evaluating the protein-environment effects on the fluorescence.

3:45 PM V2.6
Photoactive Protein Conformational Switching observed by Terahertz Time Domain Spectroscopy. Jing-Yin Chen[1], Joseph Knab[1], Jason R. Hillebrecht[2], Robert R. Birge[2] and Andrea Markelz[1]; [1]Physics, University at Buffalo, Buffalo, New York; [2]Chemistry and Molecular Biology, University of Connecticut, Storrs, Connecticut.

Devices based on protein systems are motivated by taking advantage of evolutionarily optimized response and self-assembly. Material optimization of relevant device parameters is accomplished through mutatgenesis. The development of materials based on biomolecular systems demands characterization of the physical properties relevant to the function of the biomolecules. Critical to biomolecular function is conformational flexibility. For example transmembrane signal transduction proteins, G protein coupled receptors, convey the stimulus reception to the interior of the cell by large-scale conformational change. To realize a materials science of protein-based systems requires a rapid characterization of the protein flexibility as a function of sequence mutation, environment, and sample preparation. Large-scale vibrational modes associated with conformational change in proteins lay in the far infrared or terahertz frequency range. Previously we have demonstrated that the terahertz absorbance strongly reflects the normal mode density of states suggesting that THz absorance may be sensitive to protein flexibility, (Whitmire et al. Phys. Med. Biol.2002 21:3797). Here we demonstrate the use of terahertz time domain spectroscopy as a method to detect conformational change and flexibility for a mutant of the photoactive protein bacteriorhodopsin, D96N. The photocycle of bacteriorhodopsin (BR) serves to pump protons across the cell membrane for energy storage. BR has been pursued as a possible optical memory storage medium, with engineering of the lifetimes of intermediate states and frequencies of activation through mutagenisis. For example the mutant D96N has a three order of magnitude increase in the photocycling time over wild type (WT). Previously we compared the change in THz absorbance for wild type (WT) and D96N mutant as a function of temperature and conformation, (Whitmire et al., Biophys. J. 2003 85: 1269). In that work WT BR was found to have a large increase in THz absorbance as the protein is excited from the resting state to the M intermediate state whereas no such absorption increase was observed for D96N, a result that could be indicative of a smaller conformational flexibility of D96N in the M state than WT. Here we more fully map the evolution of the THz dielectric response for D96N as a function of intermediate state. Measurements were made of hydrated films of D96N prepared from solution. The intermediate state content was controlled by illumination of the film by a filtered CW source and monitored using UV/Vis absorbance. We find that the terahertz dielectric response, while showing no change for the M intermediate, shows a decrease in both the real and imaginary part of the index for the P intermediate state. We compare these measurements with calculations of the absorbance using CHARMm. These measurements demonstrate the sensitivity of TTDS to protein conformation and flexibility.

4:00 PM V2.7
Engineered Inorganic-Binding Proteins as Molecular Erectors. M. Hadi Zareie[1], Bryan W. Reed[1] and Mehmet Sarikaya[1,2]; [1]Materials Science and Engineering, University of Washington, Seattle, Washington; [2]Chemical Engineering, University of Washington, Seattle, Washington.

Controlled binding and assembly of proteins on inorganics are at the core of biological materials science and engineering with wide ranging applications. The attachment of biomolecules, in particular, proteins, on solid supports is fundamental in the development of advanced biosensors and bioreactors. This also forms the basis of creating biocompatible surfaces using polypeptides, their use in biomineralization and tissue engineering, and as heterofunctional linkers in drug delivery. Using combinatorial biology protocols (such as cell surface and phage display), it is now possible to select inorganic-binding short polypeptides with high affinity to specififc materials, e.g., noble metals, oxides and semiconductors. As a demonstration of the use of combinatorially selected polypeptides, here we present a case study of the procedures of selecting, quantitative binding and ordered assembly of a genetically-engineered inorganic binding polypeptide on an atomically flat solid surface and its utility as a molecular erector set in practical applications. We used a 3-repeat, 14-amino-acid long gold-binding protein (GBP-1) that forms a monolayer-thick film with nanostructured domains on gold surface. The protein film conforms into 6-fold symmetry, commensurate with the Au(111) lattice, suggesting crystallographic recognition. The single repeat GBP-1 was selected for its specificity to gold surface using cell surface display technology. The engineered proteins could have significant potential impact by providing self-assembled functional molecular erectors in nano- and nanobio-technology, e.g., as molecular platforms in proteomics and genomics. We demonstrate this potential by directed assembly of ferritin (target) onto streptavidin (probe) conjugated to biotinylated GBP-1, and quantitatively analyze the results using atomic force microscopy and surface plasmon resonance spectroscopy. This research was supported by US-ARO through the DURINT Program.

SESSION V3: Poster Session
Chair: Vince Conticello
Thursday Evening, April 15, 2004
8:00 PM
Salons 8-9 (Marriott)

V3.1
Stability of Chemically Crosslinked Microtubules. Andrew K. Boal, Susan B. Rivera, Nicholas E. Miller, George D. Bachand and Bruce C. Bunker; Biomolecular Materials and Interfaces, Sandia National Labs, Albuquerque, New Mexico.

Microtubules (MTs) are protein filaments that are formed by the polymerization of the protein tubulin. MTs are inherently unstable species, partaking in a variety of structural attenuation processes collectively known as dynamic instability. Currently, our research is involved in the application of MTs in the assembly of nanomaterials by utilization of MTs in tandem with the motor protein kinesin, which binds to MTs and moves along their length in a step-like fashion, for the active delivery and assembly of other nanomaterials. On of the key aspects of this research is finding ways by which the MT dynamics can be controlled, either by suppressing or selectively encouraging MT dynamic instability. To accomplish this goal, we have investigated the use of chemical crosslinking agents to form covalent linkages within the MT. Crosslinking results in the formation of higher molecular weight protein components, as evidenced by SDS-PAGE analysis and confirming the creation of protein-protein links. While heavy amounts of crosslinking was found to destabilize MTs in inactivate them with regards to kinesin binding, moderate amounts lead to MTs that had functional lifetimes of at least twice that of uncrosslinked MTs. Further studies demonstrated that crosslinked MTs exhibited a wider thermal stability window and were far more resistant to metal-ion induced depolymerization than uncrosslinked MTs. Applications of the differential stability between uncrosslinked and crosslinked MTs for the reversible formation of MT-based materials will also be discussed.

V3.2
Use of a self-assembling viral protein as a scaffold for inorganic material growth. Sophie M Rozenzhak, Rajesh R. Naik and Morley O. Stone; Materials and Manufacturing Directorate, Air Force Research Laboratory, Wright-Patterson Air Force Base, Ohio.

Bacteriophage T4 injects its host with DNA by the irreversible contraction of its tail sheath. The outer contractile protein, gp18, assembles into a 144-subunit tubular structure, which upon contraction decreases in length from 98 nm to 36 nm, and increases in diameter from 21 nm to 27 nm. Recombinant gp18 forms polysheaths that resemble the contracted tail, while deletion of certain sequences from the C-terminal end of the protein results in mutants that lack the ability to contract and instead form thin filaments. In this study, gp18 and its mutants were expressed in bacteria and used as a

template for binding and assembly of inorganic materials. Characterization via transmission electron microscopy and atomic force microscopy revealed self-assembled tubular structures of various sizes, similar to those reported previously. We are currently exploring the use of these self-assembling protein nanotubes as scaffolds for nucleating and growing inorganic materials.

V3.3
Design and Synthesis of Bioengineered Self-assembling Repetitive Polypeptides for Molecular Device Assembly.
Seiichiro Higashiya[1], Silvana C. Ngo[1], Ken S. Bousman[1], Chris C. Wells[1], John T Welch[1,2], Narendar Rana[2], Autumn Carlsen[2], Christopher Kossow[2], Eric T. Eisenbraun[2], Robert E. Geer[2] and Alain E. Kaloyeros[2]; [1]Chemistry, University at Albany, Albany, New York; [2]School of Nanosciences and Nanoengineering, University at Albany, Albany, New York.

Molecular self-assembly, central to the success of nanoscale molecular device construction and engineering, is ultimately dependent not only upon the development of architectures which promote spontaneous organization but also the ability to reproducibly control such organization at an engineered surface. Repetitive and block-copolymerized polypeptides have been prepared for molecular interconnect applications employing these principles. A generalized strategy for the library construction of artificial repetitive DNA coding sequences for the preparation of repetitive β-sheet polypeptides by head-to-tail polymerization has been developed. Multimerization/block copolymerization in the presence of adapters containing appropriate recognition sites of type II and IIs restriction endonucleases for respectively, cloning and regeneration of assembled DNA units was utilized. Repetitive coding sequences were successfully constructed in a reproducible and predictive manner without the need for special cloning vectors while suppressing the intramolecular cyclization of multimers that is problematic when longer sequences are constructed. Amphiphilic repetitive polypeptides containing Lys and Glu residues were prepared by these methods. These materials benefit not only from enhanced stabilization of the β-sheet by salt bridge formation but also from improved solubility. In the peptides prepared, β-turns were decorated alternately with electron-rich (Tyr) or electron poor (His-H+) to enhance π-π* interaction and coherent charge carrier states. Replacements of His with Tyr are suggested increase the hydrophobicity of these turns and promote head-to-head interstrand aggregation. Results for several key peptides and the structures with customized length and functionality have been shown to form highly ordered structures on nickel and graphite surfaces.

V3.4
Solubility and Regeneration of *Bombyxmori* Silk Films Cast from Ionic Liquids.
David M. Phillips[1], Lawrence F. Drummy[1], Richard A. Vaia[1], Barry L. Farmer[1], Rajesh R. Naik[1], Ashley E. Tan[1], Deborah G. Conrady[1], Paul C. Trulove[2], Hugh C. De Long[2], Morley O. Stone[1] and Robert A. Mantz[1]; [1]Materials and Research Directorate, Air Force Research Laboratory, Wright-Patterson AFB, Ohio; [2]Air Force Office of Scientific Research, Arlington, Virginia.

Silk is a natural fiber that has shown potential use beyond its 5000-year textile history. While the silkworm (*Bombyxmori*) has mastered the production of silk fibers, recent studies have focused on regeneration methods for recovering broken or damaged fiber that is unusable for the textile industry and processing the silk fibroin for applications beyond thread. As silk is insoluble in most solvents, typically regeneration begins with dissolving the silk fibroin in a highly concentrated, aqueous lithium salt solution to disrupt the Silk II crystal structure. Ionic liquids have the ability to directly dissolve crystalline, organic molecules. Swatloski *etal.* [1] have successfully used ionic liquids to effectively dissolve and regenerate cellulose in the form of a film. In this research, we focus on the solubility of silk fibroin as a function of the ionic liquid, as well as the suitability of ionic liquids to dissolve and regenerate silk fibroin for the purpose of casting films. Combinations of several ionic liquids and wash solvents are examined for their effect on the secondary structure and properties of the resulting silk fibroin films. [1] Swatloski, R.P.; Spear, S.K.; Holbrey, J.D.; Rogers, R.D., *J.Am.Chem.Soc.* **124**(18) 4974-4975 (2002).

V3.5
Fast simulation protocol of protein structural transitions: Modelling the of the relationship of structure and function.
Arun Kumar Setty and Daniel Zuckerman; CCBB, University of Pittsburgh, Pittsburgh, Pennsylvania.

Configurational fluctuation of a protein between two or more native, folded states is of fundamental importance as it is central to the biological functioning of the protein. While methods for determining a single, plausible pathway between native states have been proposed, the entire ensemble of transitions is necessary for a complete understanding of the dynamics. As conventional models are restricted to nanoseconds while the required timescales are in microseconds, we have developed a simplified model, which nevertheless gives extraordinary agreement with NMR solution data for a variety of proteins. We have studied the case of Calmodulin, an important 148 residue protein involved with calcium signalling pathways, in detail. The model, which now incorporates the chemically relevant hydrophobic and calcium coordinating interactions, provides (for the first time ever, to our knowledge) a complete ensemble of transitions. Structures of intermediate states and details of each pathway have been obtained. The transitional region indicates a complex dynamics and suggests the existence of long-lived intermediates. Due to the speed of the approach (about one transition per day of single desktop processor time), it is readily extensible to significantly larger systems. The technique is capable, with little modification, of predicting the effects of non-native amino acids on the dynamics. Furthermore, this protocol is well suited to the tailoring of biosystems for various applications, such as molecular level switching (due to the two state dynamics of calmodulin, for example), and for biosensing of calcium. Results for Calmodulin, and progress in adapting the technique of transitional path sampling to this problem will be presented.

SESSION V4: Bioengineering
Friday Morning, April 16, 2004
Room 2000 (Moscone West)

9:00 AM *V4.1
Silk Proteins – Processing and Assembly Rules from Nature.
David L. Kaplan, Biomedical Engineering, Tufts University, Medford, Massachusetts.

Silk protein designs, processing and assembly by spiders and insects provide a useful guide for the field of polymer science and engineering in general. Silks represent some of the most hydrophobic biopolymers generated in Nature. Yet these proteins are solubilized to 30 weight percent in water in the storage glands of these organisms prior to being spun into fibers. This process is able to avoid premature crystallization of the protein chains into beta-sheet structures that would result in premature precipitation, yet chain folding and supramolecular assembly proceed within the context of sequence chemistry and processing environment. The proteins are formed into fibers with remarkable mechanical properties, and post spinning, the proteins are no longer soluble in water. The novel mechanisms utilized by these organisms to solve this puzzle of aqueous processability is the subject of much of our research. These insights have led to new ways to think about how to marry polymer chemistry designs with processing environments, how to control block and sequence variations in the polymer chains, how to utilize relatively simple strategies to regulate protein folding and the assembly of these proteins into hierarchically ordered structures to achieve important mechanical properties, and how to control the crystallization process through physiologically relevant factors. The key to the process is control of water within the context of appropriate sequence chemistry. Water serves as plasticizer, processing medium and control point for protein-protein interactions. These "rules" of processing in Nature are already yielding important insight in how to emulate this process in vitro to achieve new and novel material properties with importance to the materials science and engineering community, at both fundamental and applied levels.

9:30 AM V4.2
A Combinatorial Approach to Processing Regenerated Silk Films from *Bombyxmori*.
David M. Phillips, Lawrence F. Drummy, Robert A. Mantz, Richard A. Vaia, Barry L. Farmer, Rajesh R. Naik, Ashley E. Tan and Morley O. Stone; Materials and Manufacturing Directorate, Air Force Research Laboratory, Wright-Patterson AFB, Ohio.

Silk is a natural fiber that has shown great potential throughout its 5000-year textile history. Recent interest has focused on the processing conditions and resulting microstructure that allow for silk fibroin to achieve its remarkable properties upon spinning into fibers. The silkworm (*Bombyxmori*) has mastered these processing conditions, and attempts have been made to mimic the silkworm spinning process through electrospinning and extrusion of regenerated silk fibroin. Although the mechanical properties of the synthetically spun silk are inferior to the natural silk benchmark, properties such as fiber denier are now controllable. Regenerated silk fibroin has also been analyzed for potential use as films or membranes. Previous research on regenerated silk films focused on the microstructure, such as surface morphology [1] and crystal structure [2,3]. While these properties are important to understanding the basis for the macroscopic properties, they do not necessarily imply an application for these films. In this work, we examine the effect of processing conditions on the macroscopic properties, as well as the microstructure, of regenerated silk films. We efficiently explore the

process parameter space for these films with a combinatorial flow coating method. Unlike solution casting methods, our flow coating technique allows for the fibroin to preferentially align with the shear field, leading to films with controllable anisotropy. [1] Putthanarat, S.; Zarkoob, S.; Magoshi, J.; Chen, J.A.; Eby, R.K.; Stone, M.O.; Adams, W.W., *Polymer* **43**(12) 3405-3413 (2002). [2] Asakura, T.; Kuzuhara, A.; Tabeta, R.; Saito H., *Macromolecules* **18**(10) 1841-1845 (1985). [3] Muller, W.S.; Samuelson, L.A.; Fossey, S.A.; Kaplan, D.A., *Langmuir* **9**(7) 1857-1861 (1993).

9:45 AM V4.3
Self-assembled Protein Matrix Serves as a Novel Scaffold for In Vitro Mineralization and Cellular Modulation. Gen He and Anne George; Oral Biology, Univ. Illinois at Chicago, Chicago, Illinois.

Mechanical mismatch and the lack of interactions between implants and the natural tissue environment are the major drawbacks in bone tissue engineering. Biomaterials mimicking the self-assembly process and the composition of the bone extracellular matrix should provide new route for fabricating biomaterials possessing novel osteoconductive (as a scaffold for cell proliferation) and osteoinductive (as a template for de novo bone-like material synthesis) properties for bone repair. Self-assembled peptide amphiphiles have emerged as a new route for biomaterial synthesis. Tri-block copolymers of leucine zipper motif oligomerization form strong and reversible hydrogels. In the current study, tri-block chimeric protein was constructed by genetic engineering methods and purified as recombinant proteins. The protein comprises of leucine zipper motifs at both ends and an RGD-containing fragment of dentin matrix protein 1 (DMP1) in the centre, which is a bone/dentin specific protein identified as a hydroxyapatite nucleator. Circular dichroism spectroscopy data demonstrated that a chimeric protein was folded appropriately and self-assembled into a hydrogel. Atomic force microscopy and transmission electron microscopy analyses demonstrated that the material was highly ordered from nano- to micro-scale with a uniform porosity of 20-30 nm. In-vitro nucleation studies demonstrated that the hydrogel template could be mineralized with highly-oriented apatites in pseudophysiological buffer (165 mM NaCl, 10 mM HEPES, 2.5 mM CaCl2, 1 mM KH2PO4, pH 7.4). Cell culture experiments demonstrated that a monolayer of hydrogel coated on a glass plate could greatly facilitate osteoblastic cell adhesion. Further, immunoblotting and immuno-fluorescence analyses demonstrated that the cell surface expression level and the clustering of $\beta 1$ integrin in the osteoblasts cultured on the hydrogel were enhanced in a concentration dependent manner with respect to the substratum. Thus, self-assembled matrices could display RGD domains as functional nanoclusters and therefore efficiently enhance integrin signalling, potentially resulting in osteoblast differentiation and mineralization. In summary, the data demonstrate that a functional biomimetic protein matrix could be constructed that recapitulates the self-assembly process of bone formation. Further, mineralization and cell culture studies demonstrated its dual osteoconductive and osteoinductive properties. Although previous reports have demonstrated the feasibility of in vitro mineralization by self-assembled amphiphile peptides, no scaffold has yet been developed performing both mineralization template and cell substratum functions. The biomaterial presented here should open new avenues for bone tissue engineering.

10:30 AM *V4.4
De novo Proteins from Designed Combinatorial Libraries: native-like structures, active catalysts and novel biomaterials. Michael Hecht, Chemistry, Princeton University, Princeton, New Jersey.

Combinatorial libraries of de novo amino acid sequences can provide a rich source of diversity for the discovery of novel protein-based functions. Randomly generated sequences, however, rarely fold into well-ordered protein-like structures. To enhance the quality of a library, diversity must be focused into regions of sequence space that are consistent with well-folded structures. To produce focused libraries of de novo sequences, we design the binary pattern of polar and nonpolar amino acids to favor structures containing abundant secondary structure, while simultaneously burying hydrophobic side chains in the protein interior and exposing hydrophilic side chains to solvent. Recently, we determined the high-resolution solution structure of a 102-residue de novo protein from a binary patterned library, and found the experimentally determined structure is a well-ordered four-helix bundle, as specified by the initial design. This finding demonstrates that amino acid sequences that have neither been selected by evolution (in vivo or in vitro), nor designed by computer, can form native-like protein structures. The lecture will describe how binary patterning of polar and nonpolar amino acids has been used to design focused libraries of either alpha-helical or beta-sheet proteins. These libraries have successfully produced well-ordered native-like structures, cofactor binding proteins, enzyme-like catalysts, self-assembled monolayers, amyloid-like nanofibrils, prototype biosensors, and novel protein-based biomaterials.

11:00 AM V4.5
Force Measurements on P-protein Aggregates from Vicia Faba. Andreas Heilmann, Stefan Schwan, Andreas Cismak and Uwe Spohn; Fraunhofer Institute for Mechanics of Materials, Halle (Saale), Germany.

Micromechanically interesting protein aggregates, the so-called Forisomes [1] were recently isolated from selected phloem cells of vicia faba. The Forisoms can transform the chemical free enthalpy of their reaction with calcium ions into mechanical work. The thermodynamic cycle process can be closed by the extraction of these calcium ions with ethylenediamine-tetraacetic acid (EDTA). By means of scanning electron microscopy, an ordered fibre like structure of the Forisoms in the calcium free state was observed. After their reaction with caclium ions the Forisoms contract by 10 to 40% of its original length inceasing their cross sectional area by 125 to 300%. By reacting with 10 mM of calcium ions the Forisoms can generate a force between 20 and 100 nN. These forces were measured by microscopically observing the bending of thin glass fibres with a diameter from 9 to 20 m. More precise methods were applied based on bending of various cantilevers. The Forisom was coupled covalently to a stator and the corresponding bending sensor. The displacement x of the of the cantilever were correlated with the measured force. The displacement x was measured by a position sensitive detector (PSD) with a resolution of 2 nm. Also a cantilever with an int egrated piezoresistor was tested. The relative change of the resistance R/R was determined as a function of the bending force with a precision voltmeter measuring the output voltage of a Wheatstone measuring bridge. The dependencies of the measured forces on the calcium(II) concentration, the pH value and the temperature were investigated. The results open up a new way to miniaturized bio-actuators, e.g. artificial muscles. [1] M. Knoblauch, G.N. Noll, T. Mueller, D. Pruefer, I. Schneider-Huether, D. Scharner, A.J.E. van Bel, W. S. Peters; Nature Materials 2 (2003), 600

11:15 AM V4.6
Nanostructured, Protein-Based Materials for Energy Transduction. Millicent Anne Firestone[1], Soenke Seifert[1], Deborah Hanson[2] and Philip Laible[2]; [1]Materials Sciences, Argonne National Laboratory, Argonne, Illinois; [2]Biosciences, Argonne National Laboratory, Argonne, Illinois.

We are exploring the potential of soft-condensed matter phases as nanoscale "building" blocks", templates, and scaffolds for the fabrication of functional, protein-based materials that may ultimately form the basis of nanoscale devices. Polymer/lipid-based complex fluids comprise a class of soft materials exhibiting a number of properties that make them well-suited for use as either nanoscale scaffolding for the fabrication of functional materials, in particular, a high degree of anisotropy, segregated aqueous and organic domains, tunable lattice dimensions (14-55 nm), the ability to adopt a variety of structural motifs (lamellar, 2-D hexagonl, 3-D cubic), responsiveness to external stimuli, and readily adjustable physical properties (e.g., viscosity). Furthermore, because these materials are biomimetic, (i.e., the supramolecular architecture and physicochemical properties are reminiscent of natural cell membranes), both soluble (Cytochrome c) and membrane proteins (photosynthetic reaction centers, LH1, LH2) can be organized within them. In this presentation, the preparation of such protein arrays and the characterization (via small angle X-ray and neutron scattering) of guest (protein) organization and host matrix (complex fluid) are detailed. In addition, use of post-self-assembly processing (e.g., external electromagnetic fields) to produce anisotropically- and vectorially-ordered proteins is described. This work was performed under the auspices of the Office of Basic Energy Sciences, Divisionj of Materials Sciences, United States Department of Energy, under Contract No. W-31-109-ENG-38,

11:30 AM *V4.7
Reprogramming Protein Synthesis. David Tirrell, Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California.

Synthetic macromolecular materials are constructed from constituents far more diverse than those that make up proteins and nucleic acids. On the other hand, proteins and nucleic acids are made with architectural control that cannot be realized through chemical polymerization processes. This lecture will examine the possibilities for reprogramming protein synthesis to accommodate the diverse building blocks needed for materials design. Special emphasis will be placed on methods for engineering the aminoacyl-tRNA synthetases and for encoding novel amino acids at the genetic level.