

# SYMPOSIUM K

## Biological and Bio-Inspired Materials and Devices

March 29 - April 1, 2005

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\* Invited paper

**8:30 AM \*K1.1**

**Biogenic Nanostructured Silica Formation in Diatoms: Proteins, Genes, and Structure.** Mark Hildebrand, Scripps Institution of Oceanography, UCSD, La Jolla, California.

Diatoms are unicellular algae with cell walls made of nanostructured silica. The estimated tens-of-thousands of diatom species each have unique silica morphology, and these organisms are especially adept at making complex three-dimensional silica structures. The size of these structures, their reproducible formation, and the ease with which they can be obtained in large numbers, make diatoms attractive sources of nanostructured materials. Formation of these structures occurs in a membrane-bound intracellular compartment known as the silica deposition vesicle, which is expanded and molded during formation. Understanding the basic processes of structure formation by 1) characterizing the cellular components involved, and 2) developing the means to genetically manipulate structure, would facilitate possible applications of these organisms in materials science. We have been examining the process of cell wall formation in the diatom *Thalassiosira pseudonana* both structurally and genetically. Using light and electron microscopy, we identified intermediates in the formation of the cell wall, suggesting that there are at least two distinct steps in this process. Using a proteomic approach, we identified proteins associated with an enriched cell wall fraction, and monitored expression of genes encoding these proteins during cell wall synthesis. From this data, we have focused on two aspects of cellular metabolism related to silicification; polyamine synthesis and vesicle trafficking. Using known inhibitors of polyamine synthesis, we have been able to modify the resulting silica structure, in a manner consistent with the two step process seen by electron microscopy. These approaches are facilitating our understanding of the process of silica structure formation in diatoms, and constitute the first steps in manipulation of structure.

**9:00 AM \*K1.2**

**Biosilica Nanofabrication in Diatoms: The Structures and Properties of Regulatory Silaffins.** Nils Kroeger<sup>1</sup> and Nicole

Poulsen<sup>1,2</sup>; <sup>1</sup>Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, Georgia; <sup>2</sup>Biochemistry 1, University of Regensburg, Regensburg, Germany.

Diatoms are a large group of unicellular microalgae encased by silica cell walls that exhibit species-specific, mostly porous micro- and nanopatterns. Recently, unique proteins (silaffins) and unusually long polyamine chains (LCPA) have been identified and implicated in silica morphogenesis. Based on the data obtained from work on the diatom *Cylindrotheca fusiformis* it has been suggested that diatom biosilica morphogenesis may generally require at least two components: LCPA, which accelerate silicic acid polycondensation and regulatory silaffins that modulate the activity of LCPA. However, the architecture of *C. fusiformis* biosilica is rather unusual being mainly composed of long non-porous bands. Therefore, it has been unclear, if general conclusions about the mechanism of biosilica morphogenesis can be drawn from studies on *C. fusiformis*. Furthermore, elucidating the role of silaffins in biosilica morphogenesis has been hampered by the lack of structural and functional data of silaffins from other diatom species. Recently, we have characterized the silaffins from the diatom *Thalassiosira pseudonana*, a species exhibiting porous biosilica nanopatterns. It is demonstrated that this organism contains LCPA as well as regulatory silaffins, which drastically influenced silica formation in vitro. These results clearly support the hypothesis that silaffins play a general role in diatom biosilica nanofabrication. Chemical characterization of *T. pseudonana* silaffins and isolation of the corresponding genes enabled unprecedented insight into the structure of regulatory silaffins.

**9:30 AM K1.3**

**Control of Nanoparticle Assembly using DNA-Modified Diatom Templates.** Nathaniel Louis Rosi, Emma Kate Payne, Shad Thaxton and Chad A. Mirkin; Chemistry, Northwestern University, Evanston, Illinois.

Microorganisms have proven to be versatile templates for the organization of nanostructured materials into larger scale functional architectures. An ideal biological template would be one that could be chemically modified in a versatile manner using conventional bench-top methods so that the interaction between the template and the nanostructured materials could be understood and easily controlled. To this end, we have investigated using diatoms as templates for the assembly of pre-fabricated nanoparticles. Specifically, we show that diatom cell walls can be covalently

functionalized with DNA and then used as templates for the sequence-specific assembly of DNA-functionalized nanoparticles. We further demonstrate that the DNA can program the assembly of multiple layers of nanoparticles onto the template. This is a potentially powerful method for producing intricately ordered, hierarchically assembled macroscopic structures whose properties can be tuned at the nanoscale.

**9:45 AM K1.4**

**Blue Luminescent Biogenic Silicon-Germanium Oxide Nanocomposites.** Shuhong Liu<sup>1</sup>, Clayton Jeffries<sup>1</sup>, Gregory L. Rorrer<sup>1</sup>, Chih-hung Chang<sup>1</sup>, Jun Jiao<sup>2</sup> and James A. Hedberg<sup>2</sup>;

<sup>1</sup>Chemical Engineering Department, Oregon State University, Corvallis, Oregon; <sup>2</sup>Physics, Portland State University, Portland, Oregon.

Marine diatoms are a class of microalgae that possess cell walls composed of silica nanoparticles. These organisms actively assimilate silicic acid Si(OH)<sub>4</sub> from seawater, polymerize silicic acid to silica nanoparticles by a protein-mediated precipitation process, and then assemble the silica nanoparticles into intricate patterns that constitute the cell wall microarchitecture (consists of around 30nm of SiO<sub>2</sub> nanoparticles) of the diatom frustule. The biomineralization capacity of marine diatoms, *Nitzschia*, was harnessed to biologically manufacture silicon oxide / germanium oxide nanocomposite materials. Germanium was incorporated into living diatom cell mass by a two-stage cultivation process. The micro- and nanostructures of biogenic oxide nanocomposite before and after post processing were characterized by electron diffraction, HR-TEM with EDX, and XRD. Photoluminescence (PL) measurements were performed on these biogenic nanocomposites with different post processing for removing the organics. Strong blue photoluminescence was observed from samples treated with H<sub>2</sub>O<sub>2</sub> and oxygen plasma. A clear blueshift was observed from the biogenic oxides with the addition of germanium. It is believed that self-trapped exciton affected by quantum confinement effect is responsible for the PL from these biogenic oxide nanocomposites. This research is supported by National Science Foundations Bioengineering and Environmental Systems program under grant number BES-0400648.

**10:30 AM \*K1.5**

**The Role of Electrostatic Interactions, Hydrogen Bonding and the Hydrophobic Effect in the Regulation of Amorphous Silica Structures.** Carole Celia Perry, Siddharth V. Patwardhan, David Belton and Graham Tilburey; Biomedical and Natural Sciences, Nottingham Trent University, Nottingham, United Kingdom.

In nature, several classes of biosilicifying organisms process soluble silicon to generate hierarchically organised ornate biogenic silica structures under mild conditions of pH and temperature and exert precise control in shaping biosilica. In contrast, current synthetic procedures typically employ relatively harsh conditions for the preparation of silicas and exhibit relatively poor morphological control. In order to gain insights into biosilicification, several studies have been carried out on biosilicifying organisms wherein organic biomolecules have been isolated and identified. These bioextracts control in vitro silicification via catalysis, aggregation and/or scaffolding. This contribution looks at the effect of different strength bonding interactions between silicon (in a variety of forms from the simple molecule through to particles and aggregates) and biomolecules on all stages of silica formation in vitro. The behaviour of amino acids and other small nitrogen containing molecules, proteins (including biosilica extracts such as silicatein, collagen and recombinant proteins of specific secondary structure), monosaccharides, polysaccharides and other functionalised polymers will all be discussed. The effect of these interactions on the form of the silica and hence its properties (either as a biomaterial) or as a commercially relevant product will be discussed.

**11:00 AM K1.6**

**Self-Assembly of Proteins that Direct Biological Silicification.** Meredith Murr<sup>1</sup>, Hiro Tsuruta<sup>3</sup> and Daniel E. Morse<sup>2,1</sup>; <sup>1</sup>Department of Molecular, Cellular, and Developmental Biology, University of California at Santa Barbara, Santa Barbara, California; <sup>2</sup>Institute for Collaborative Biotechnologies, University of California at Santa Barbara, Santa Barbara, California; <sup>3</sup>Stanford Synchrotron Radiation Laboratory, Menlo Park, California.

Siliceous marine sponges have developed unique biomolecular machinery for the precisely controlled supramolecular assembly of silicon-based biomaterials. In particular, the needle-like glass structures, or spicules, of the marine sponge *Tethya aurantia* are composed of a central (axial) protein filament that directs the formation of the surrounding silica. The filament is 2 μm long and 1 μm in width, and is composed of three related proteins known as silicatein α, β, and γ. These serine hydrolases, which show significant homology to a family of cathepsin proteases, catalyze the

condensation of silicon alkoxide precursors into silica glass (SiO<sub>2</sub>) and spatially direct deposition of the silica around the axial filament. Fiber diffraction studies indicate that the silicatein filament possesses long-range order, with component proteins arranged in a helical assembly. However, it is unclear how silicatein proteins organize into a filament or how the filament structure might influence inorganic product formation. Recent studies demonstrate that silicatein subunits self-assemble in vitro into filamentous structures, thereby recapitulating the biological process. In vitro assembly is influenced by temperature, pH and ionic strength, and has been investigated using static and dynamic light scattering and transmission electron microscopy to characterize assembly intermediates and kinetics. These experiments suggest a mechanism in which silicatein subunits assemble into 15 nm spheres that align in a linear arrangement to form protofilaments; these further assemble into higher order filamentous structures. Sequence differences between the silicateins and cathepsins, a family of well-characterized soluble cysteine proteases, reveal the presence of unique hydrophobic regions on the surface of silicatein. This suggests that unlike soluble monomeric cathepsins, silicatein monomers associate via hydrophobic protein-protein interactions. A detailed understanding of the self-assembly mechanisms of the silicatein filament may provide new opportunities for nanofabrication through silicon biotechnology.

#### 11:15 AM K1.7

##### **Biomimetic Silicification of 3-D Polyelectrolyte Scaffolds Assembled by Direct Writing.** Mingjie Xu<sup>1</sup>, Eric Duoss<sup>2</sup> and

Jennifer A. Lewis<sup>2,1</sup>; <sup>1</sup>Chemical & Biomolecular Engineering, University of Illinois at Urbana-Champaign, Urbana, Illinois; <sup>2</sup>Materials Science and Engineering, University of Illinois at Urbana-Champaign, Urbana, Illinois.

We have developed a novel approach to patterning 3D polyelectrolyte scaffolds by direct-write assembly (DWA). Central to our approach is the creation of concentrated polyelectrolyte inks that flow through fine deposition nozzles and then "set" almost instantaneously to facilitate shape retention as they span gaps in underlying layers. By tailoring ink composition, we have patterned polyamine-rich scaffolds that, when introduced to silicic acid, will undergo silicification under ambient conditions. This mineralization process, which mimics diatom formation, yields high-resolution inorganic replicas of the polyelectrolyte scaffolds. Si and O element mapping by STEM and Auger revealed that these species were uniformly distributed throughout the cross-section of the replicated structure. By coupling DWA with biomimetic silicification, we have demonstrated the formation of artificial diatom-like structures. In ongoing efforts, we are exploring the mineralization of hydrogel- and other polyelectrolyte-based scaffolds patterned by DWA.

#### 11:30 AM K1.8

##### **Sol-Gel Syntheses on Single-Cell Scaffolds: Applying Complex Chemistries to Nature's 3-D Nanostructured Templates.**

Michael Weatherspoon, Christopher Gaddis, Shawn Allan, Ye Cai, Michael Haluska, Robert Snyder and Kenneth Sandhage; Materials Science and Engineering, Georgia Institute of Technology, Atlanta, Georgia.

Intense global activity to produce advanced micro-to-nanoscale devices has led to appreciable interest in biologically self-assembled nanoparticle structures. Certain micro-organisms are adept at assembling three-dimensional (3-D) biomineralized (bioclastic) micro-structures with precise shapes and fine (nanoscale) features. An exceptional variety of intricate 3-D structures are generated by aquatic micro-algae known as diatoms. Diatoms are single-celled organisms that assemble microshells (frustules) comprised of silica nanoparticles. While multifarious frustule shapes exist for potential devices, the range of potential device applications are limited by their natural silica-based chemistry. On the other hand, extensive work over the last half of the past century has yielded a variety of synthetic, solution-based routes to ceramic compounds with complex shapes and chemistries. We demonstrate here, for the first time, a sol-gel coating technique that enables such silica-based diatom frustules to be converted into new functional ceramic materials (i.e., ZrO<sub>2</sub> and BaTiO<sub>3</sub>) while preserving their intricate shapes and features. Nanocrystalline ZrO<sub>2</sub> replicas have been synthesized by firing continuous alkoxide-derived coatings on silica frustules, and then removing the silica by selective dissolution. BaTiO<sub>3</sub>-based replicas have been fabricated with a multi-step process: i) reactive conversion of the silica scaffold into a chemically-compatible substrate, and then ii) firing of an alkoxide-derived coating. The ability to control and manipulate the chemistry of such 3-D assemblies, without altering the shape and fine features of the original bio-scaffolds, endows these structures with a much broader range of properties than are displayed by silica. Potential applications for such chemically-tailored, biologically-assembled structures will be discussed.

#### 11:45 AM K1.9

##### **Small-angle X-ray Scattering, FTIR and SEM Characterization of Nanostructured PVA/TEOS Hybrids by Chemical Crosslinking.** Herman Sander Mansur and Alexandra Piscitelli Mansur; Metallurgical and Materials Engineering, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil.

In the present work, novel hybrid nanostructured composite materials were produced to be used in many potential applications such as biomedical, drug delivery systems, tri-dimensional scaffolds for biomaterials and tissue engineering, biomembranes and optical devices among others. Hybrids were synthesized by reacting poly (vinyl alcohol) (PVA) in aqueous acid solution with silicon alkoxide tetraethoxysilane (TEOS). PVA/TEOS hybrids were also modified in the nanometer-scale by crosslinking with bi-functional aldehyde, glutaraldehyde (GA) during the synthesis involving hydrolysis and polycondensation of PVA/TEOS. The characterization of hybrids was carried out by using Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR) and Small Angle X-ray Scattering (SAXS) techniques. FTIR spectra have showed major vibration bands associated with organic-inorganic chemical groups (-OH, C=O, C-H, Si-O, Si-OH) present in the hybrid composite PVA/TEOS. Also, typical absorption bands related to glutaraldehyde alkyl chain have indicated the crosslinking reaction of the hybrid network with glutaraldehyde (PVA/TEOS/GA). Small-angle X-ray scattering results have indicated different nano-ordered disperse phases for PVA, PVA/TEOS hybrid and PVA/TEOS/GA chemically crosslinked hybrid. SEM photomicrographs have clearly indicated quite different morphologies from chemically crosslinked polymer network compared to PVA hydrogel samples without glutaraldehyde reaction. The SAXS and FTIR spectroscopy characterizations have confirmed that hybrid materials were successfully obtained based on the combination of PVA and TEOS with glutaraldehyde crosslinked nanometer-scale network.

SESSION K2/L2: Joint Session: Functional Biomaterials and Biomimetics  
Chairs: Trevor Douglas and William J. Landis  
Tuesday Afternoon, March 29, 2005  
Room 3002 (Moscone West)

#### 1:30 PM \*K2.1/L2.1

##### **Lamellar Bone: Old and New Insights into Structure and Function.** Steve Weiner<sup>1</sup>, Eugenia Klein<sup>1</sup>, Meir Barak<sup>1</sup>, Paul Zaslansky<sup>1</sup> and Ron Shahar<sup>2</sup>; <sup>1</sup>Department of Structural Biology, Weizmann Institute, Rehovot, Israel; <sup>2</sup>Faculty of Agriculture, Hebrew University, Rehovot, Israel.

The lamellar structure of bone is widespread, especially among the mammals. It was first identified by van Leeuwenhoek in 1691, and is still not fully understood. The basic motif resembles that of plywood, with parallel arrays of mineralized collagen fibrils arranged in layers with different orientations in a two-dimensional plane. Within the collagen fibril, are layers of very small plate-shaped crystals of carbonated apatite. Adjacent fibrils tend to have their layers aligned, but there is a progressive rotation of the fibrils from one surface plane of an individual lamella to the next. Here we confirm and amplify aspects of this structure using a Schottky FEG SEM with in-lens SE detector, and also show that there is a third structural element with mineralized collagen fibrils aligning the canaliculi, and hence being aligned perpendicular to the main lamellar plane. These fibrils originate from the main lamellar structure, but describe a 90 degree rotation to align themselves orthogonally to the lamellar plane. They may well fulfill a "pinning" function, by firmly bonding adjacent lamellae. Lamellae are often initially deposited as parallel arrays, but as a result of remodeling, reform as cylindrical secondary osteons. The elastic properties of lamellar bone are for the most part due to the lamellar structure, whereas the fracture properties are profoundly influenced by the cylindrical osteonal structure. Careful comparisons of lamellar bone types measured in water under tension and compression using electronic speckle pattern interferometry (ESPI), reveal new insights into the structure-mechanical properties of lamellar bone. Supported by grant DE006954 from the NIDCR to SW.

#### 2:00 PM \*K2.2/L2.2

##### **Mechanisms Governing the Inelastic Deformation of Bone.** Anthony Evans, Materials, UCSB, Santa Barbara, California.

To understand the inelastic response of cortical and trabecular bone, a three-part investigation has been conducted. In the first, a flexural test protocol has been designed and implemented that monitors the axial and transverse strains on both the tensile and compressive surfaces of cortical bone. The results are used to assess the relative contributions of dilatation and shear to the inelastic deformation. Unload/reload tests have characterized the hysteresis and provided

insight about the mechanisms causing the strain. In the second part, a constitutive law representative of the deformation is selected. It is implemented to illustrate the coupled buckling and bending of ligaments that occurs in osteoarthritic trabecular bone loaded in compression. The third part devises a model for the intrinsic stress/strain response of bone, based on a recent assessment of the nano-scale organization of the collagen fibrils and mineral platelets. The model is used to rationalize the inelastic deformation in tension, as well as the permanent strain and the hysteresis.

#### 2:30 PM K2.3/L2.3

**Evidence for a Possible Mechanical Role of Bone Matrix Proteoglycans and Glycoproteins.** Paul Hansma, Georg Fantner, Johannes Kindt, Philipp Thurner, Leonid Pechenik, Marquesa Finch, Patricia Turner, Georg Schitter, Blake Erickson, Zachary Schriock, Laura Star Golde, Erik Strong and Simcha Frieda Udwin; Physics, University of California, Santa Barbara, Santa Barbara, California.

Evidence from Atomic Force Microscope indentation, pulling and imaging together with evidence from macroscopic testing and enzymatic digestion suggests that collagen fibrils and mineral plates are not the only components of bone with a mechanical role. There appears to be "glue" that binds mineralized collagen fibrils to other mineralized collagen fibrils. Order of magnitude calculations show that less than 1% by weight of this "glue" can have profound effects on the fracture resistance of bone, because it involves a remarkable natural toughening and strengthening system: sacrificial bonds and hidden length. The sacrificial bond-hidden length system can dissipate large amounts of work against entropic forces while stretching out the hidden length that is exposed when sacrificial bonds break. This appears to occur when mineralized collagen fibrils are torn apart or slid relative to each other during bone fracture. In bone, this system depends on the presence of multivalent positive ions such as calcium ions. This dependence allows us to follow the involvement of the sacrificial bond-hidden length system right up to macroscopic fracture testing. Many bone matrix proteoglycans and glycoproteins have negatively charged groups at physiological pHs that could be bound together into sacrificial bonds by multivalent positive ions, and are thus natural candidates for this "glue". We cannot, however, rule out a possible involvement of nonfibrillar collagen. Further research will be necessary to determine precisely which candidate or candidates are involved.

#### 2:45 PM K2.4/L2.4

**Contact-induced Deformation and Failure of Dental Multilayers: Effects of Loading Rate.** Xinrui Niu, Min Huang, Jikou Zhou and Winston O. Soboyejo; Mechanical and Aerospace of Engineering, Princeton University, Princeton, New Jersey.

This paper presents the results of a combined experimental, analytical and computational study of contact deformation and cracking in dental multi-layers. Dental structures are idealized as layered composites (real teeth and dental restorations). The mechanisms of contact-induced deformation and cracking are then studied at different loading rates. A combination of viscous deformation and fracture mechanics models is used to predict the effects of loading rate on the failure conditions in the dental multilayers deformed under monotonic and cyclic loading. These employ rate dependent constitutive relationships and visco-elastic material properties that are obtained from joint and foundation materials subjected to compressive loading in air and in water. Analytical and finite element predictions of loading rate effects are shown to be in good agreement with experimental measurements, when the combined effects of viscous deformation and sub-critical cracking are modeled within a mechanistically-based framework.

#### 3:30 PM \*K2.5/L2.5

**High-Efficiency Fiber-Optical Network in a Glass Sponge.** Joanna Aizenberg<sup>1</sup>, Andrew D. Yablon<sup>2</sup>, V. C. Sundar<sup>1</sup>, James C. Weaver<sup>3</sup> and Micha Ilan<sup>4</sup>; <sup>1</sup>Bell Labs/Lucent, Murray Hill, New Jersey; <sup>2</sup>OFS Laboratories, Murray Hill, New Jersey; <sup>3</sup>UCSB, Santa Barbara, California; <sup>4</sup>Tel-Aviv University, Tel-Aviv, Israel.

Even the most advanced optical designs made by humans are often primitive relative to the optical systems that have evolved in Nature. I will describe natural fiber-optical systems produced by a deep-sea sponge *Euplectella*, whose hierarchical architecture and hybrid character offer outstanding optical and mechanical properties. We demonstrate that the sponge forms glass fibers that are remarkably similar to commercial silica optical fibers. We show the spicules to have a characteristic design that encompasses a high refractive index core composed of Na-doped silica, with the refractive index higher than that of vitreous silica; and a low refractive index cladding composed of an organic-containing glass cylinder wrapped in organically glued, multiple layers of hydrated silica. The presence of the lens-like structures at the end of these biofibers that improves the

light-collecting efficiency, high fracture toughness arising from their composite structure, the presence of index-raising dopants and the absence of residual stress in these fibers suggest advantages of the ambient temperature synthesis favored in nature and provide new ideas for the fabrication of improved optical systems, constructed using a bottom-up approach.

#### 4:00 PM \*K2.6/L2.6

**Biomimetic Materials Chemistry.** Rajesh R. Naik, Ryan M. Kramer, Melanie M. Tomczak, Joseph M. Slocik, Laura A. Sowards, Sharon E. Jones and Morley O. Stone; US Air Force Research Laboratory, Dayton, Ohio.

The interface between biology, chemistry, and materials science has motivated biomimetic approaches to the formation of inorganic nanomaterials. Biomolecules (proteins, peptides) and biomolecular architectures are being used as templates for the synthesis of inorganic nanomaterials. Our research efforts have been directed at not only understanding how biological organisms control nucleation and growth of inorganic materials, but also how this activity can be controlled in vitro. Biomolecules or biomolecular architectures can be used as building blocks in the bottom-up fabrication of inorganic structures. I will cover our efforts using biomolecules for growing inorganic structures and to exploit self-assembling structures for material synthesis by engineering desired functionalities into the self-assembling biomolecules for bottom-up fabrication.

#### 4:30 PM K2.7/L2.7

**Chemically-Tailored Nanofibers Derived from Self-Assembled Natural Templates.** Samuel Shian<sup>1</sup>, Dori Landry<sup>2</sup>, Ye Cai<sup>1</sup>, Brian Palenik<sup>2</sup>, Mark Hildebrand<sup>2</sup> and Ken H. Sandhage<sup>1</sup>; <sup>1</sup>Materials Science and Engineering, Georgia Institute of Technology, Atlanta, Georgia; <sup>2</sup>Scripps Institute of Oceanography, University of California, San Diego, La Jolla, California.

Spectacular feats of nanoparticle self-assembly can be found in nature. Intricate three-dimensional (3-D) microshells (frustules) comprised of amorphous silica nanoparticles are constructed by diatoms (aquatic micro-algae). Each of the tens of thousands of extant diatom species assembles a frustule with a unique shape and pattern of fine (sub-micron) features. The diatom *CorethronCriophilum* forms a frustule containing spines with a high aspect ratio (a few hundred in diameter, tens of microns in length). These natural "nanofibers" would be attractive for a variety of applications, if the silica-based chemistry could be altered to expand the range of properties without loss of the fiber shape. In this work, we demonstrate how the silica-based spines of the *CorethronCriophilum* diatom can be converted into titanium dioxide nanofibers via use of a halide gas/silica displacement reaction. The chemical conversion process was performed in two steps: i) a displacement reaction of TiF<sub>4</sub>(g) with SiO<sub>2</sub>(s) to yield TiOF<sub>2</sub>(s) and then ii) conversion of the TiOF<sub>2</sub>(s) into TiO<sub>2</sub>(s) upon exposure to oxygen. The influence of processing parameters on the extent of reaction and on the resulting morphology will be discussed. With judicious choice of processing conditions, the *CorethronCriophilum* spines were successfully converted into nanocrystalline titania (anatase) nanofibers.

#### 4:45 PM K2.8/L2.8

**A Halogen Smile: Br and I in the Jaws of Nereis, a Marine Worm.** Henrik Birkedal<sup>1</sup>, Rashda Khan<sup>2</sup>, Nelle Slack<sup>3</sup>, Chris Broomell<sup>4</sup>, Helga C. Lichtenegger<sup>5</sup>, Frank W. Zok<sup>3</sup>, Galen D. Stucky<sup>2,3</sup> and Herbert Waite<sup>4</sup>; <sup>1</sup>Department of Chemistry, University of Aarhus, Aarhus, Denmark; <sup>2</sup>Department of Chemistry and Biochemistry, University of California, Santa Barbara, Santa Barbara, California; <sup>3</sup>Materials Department, University of California, Santa Barbara, Santa Barbara, California; <sup>4</sup>Department of Molecular, Cellular, and Developmental Biology, University of California, Santa Barbara, Santa Barbara, California; <sup>5</sup>Department of Materials Science and Testing, Vienna University of Technology, Vienna, Austria.

The marine worm *Nereis* has a pair of pincer-like jaws that it uses to collect food. They consist of a proteinaceous matrix reinforced by Zn, the concentration of which increases towards the tip of the jaw [1]. The jaws also contain three of the halogens: Cl, Br and I. Chlorine is in part co-localized with zinc [1] while the heavier halogens are not. Here we show that they are rather concentrated towards the jaw outer surface and that they are bound to the amino acids of the protein matrix by post translational modifications. Several of these modified amino acids have not previously been observed in Nature. We suggest that the exterior of the jaw is analogous to the sclerotized protein of insect cuticle and speculate on the possible roles of this outer coating. [1] H.C. Lichtenegger, T. Schöberl, J.T. Ruokolainen, J.O. Cross, S.M. Heald, H. Birkedal, J.H. Waite, and G.D. Stucky. Proc. Natl. Acad. Sci. USA, 2003, 100, 9144-9149.

SESSION K3: Poster Session: Biological and  
Bio-inspired Materials and Devices I  
Chairs: Elaine DiMasi, Trevor Douglas and Kenneth  
Sandhage  
Tuesday Evening, March 29, 2005  
8:00 PM  
Salons 8-15 (Marriott)

**K3.1**  
**Synthesis and Structural Characterization of Silica Gels Prepared with Amine and Polyamine Catalysts.** Katya Delak<sup>1</sup> and Nita Sahai<sup>2,1</sup>; <sup>1</sup>Chemistry, University of Wisconsin, Madison, Wisconsin; <sup>2</sup>Geology and Geophysics, University of Wisconsin, Madison, Wisconsin.

Diatom and sponge proteins implicated in biogenic silica formation contain amine and polyamine moieties that have been shown to be necessary for protein catalytic activity. Previously, we have shown that simple amines and polyamines, chosen for their similarity to the moieties found in silica-precipitating proteins, can catalyze organosilicate hydrolysis and condensation at near-neutral pH. In this study, we examine the influence of these same catalysts on the formation of silica gels from tetraethylorthosilicate. We characterize the influence of amine type and pH on gelation time. In addition, we use light scattering techniques coupled with electron microscopy to determine how the choice of catalyst and pH condition can be used to tailor the resulting gel and precipitate morphology.

**K3.2**  
**Development of a Biocompatible Ink for Thermal Inkjet Printing.** Helen E. Smith<sup>1,2</sup>, Lawrence L. Brott<sup>2</sup> and Rajesh R. Naik<sup>2</sup>; <sup>1</sup>Department of Chemistry, University of Dayton, Dayton, Ohio; <sup>2</sup>Materials and Manufacturing Directorate, Air Force Research Laboratory, Wright-Patterson Air Force Base, Ohio.

The increasing demand for patterned biomolecules requires a new method for such patterning which is less harsh than the current methods involving the utilization of photoresist, lithography, and UV processing. One possible alternative involves the use of ink jet printing technology, a field with vast potential and increasing application. The development of a water-based, biologically compatible ink formulation for use with thermal ink jet printing will be discussed. The deposited ink adheres to diverse substrates while retaining the bioactivity of the biomolecule. Additionally, a silica precipitating peptide dissolved in an aqueous solution can be patterned utilizing this technique, allowing for the formation of patterned silica nanoparticles on a flexible substrate.

**K3.3**  
**Polychaete Worm is an Expert Sand Mason.** Hua Zhao, Chengjun Sun and Herbert Waite; Dept. of Mol Cellu and Dev Biology, Marine Science Institute, Santa Barbara, California.

The marine polychaete *Phragmatopoma californica* cements together building materials in its habitat such as sand and shell to construct its dwelling tubes in a manner closely resembling stone masonry. The cement is intriguing, first because so little is used, and second, because it solidifies rapidly from a liquid emulsion reminiscent of a complex coacervate. Several lines of evidence strongly suggest that phosphoserine-rich proteins are the dominant proteins in this cement. In this paper, we present two serine-rich precursor protein cDNA sequences which were isolated from the cDNA library constructed from the cement gland in the thorax of *P. californica*. Both cDNA encoded sequences consist of repeated motif (S)nY, where n=3-12. Based on the deduced sequence, the amino acid composition of the two proteins is up to 60 mol% and 90 mol% serine, with almost 10 mol% tyrosine also present in both. These are the highest serine-containing proteins found so far in nature and, following phosphorylation, account for the high level of bound Mg and Ca in the cement.

**K3.4**  
**Elasticity and Piezoelectricity in Biological Systems on the Nanoscale: From Bones to Butterflies.** Brian J. Rodriguez<sup>1</sup>, Alexei Gruverman<sup>2</sup> and Sergei V. Kalinin<sup>3</sup>; <sup>1</sup>Physics, North Carolina State University, Raleigh, North Carolina; <sup>2</sup>Materials Science and Engineering, North Carolina State University, Raleigh, North Carolina; <sup>3</sup>Condensed Matter Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

Functional properties of biological systems are determined by an intricate set of mechanical and electromechanical interactions on the length scales that span several orders of magnitude: from macro to nano. Electromechanical coupling is a universal property of biological systems that was first discovered when Luigi Galvani observed the effect of "animal electricity" in a frog leg. Understanding the inherently intertwined mechanical and electromechanical properties, such as elasticity and piezoelectricity, in living systems can provide an

insight into the functionality of biomaterials and understand the biological relevance of these properties. Here, we present a scanning probe microscopy based approach for elastic and electromechanical imaging and spectroscopy of biological systems - from imaging the elasticity map in butterfly wings to differentiation of elastic and piezoelectric properties in enamel and dentine layers of human tooth to measuring the electromechanical response of a collagen molecule bundle. This allows us to repeat Galvani's experiment on the nanoscale - more than two centuries later and with a million times higher resolution. Research performed as a Eugene P. Wigner Fellow (SVK). AG acknowledges financial support of the National Science Foundation (Grant No. DMR02-35632).

**K3.5**  
**Comparison of Piezoresistive and Optical Read-Out Methods for Microcantilever-Based Biosensor Fabricated by Surface Micromachining Technique.** Kwang-Ho Na<sup>1</sup>, Hyung Do Kim<sup>1</sup>, Kyung Ah Yoo<sup>1</sup>, C. J. Kang<sup>2</sup> and Yong-Sang Kim<sup>1</sup>; <sup>1</sup>Electrical Engineering, Myongji University, Yongin, South Korea; <sup>2</sup>Physics, Myongji University, Yongin, South Korea.

The widespread availability of inexpensive microfabricated cantilever in renewed interest in biosensor-based. Molecular recognition on the cantilever surface results in a mechanical response, that produces a microcantilever bending of few nm and a shift of the resonant frequency. The most common method is measuring the deflection of a cantilever because it is not sensitive to the damping in the liquid environment, in which only a single side is coated with receptor molecules. The surface stress change of the sensitized cantilever side with respect to the other side gives a cantilever bending owing to the expansion/contraction of a cantilever side with respect to the other. Commonly the most wide spread detection technique, used in most commercial AFMs, is the optical leverage method that had advantage of high resolution. However it has many disadvantages in biosensors. It is difficult to make it portable because external optical devices such as laser diode and photodiode are needed and measurements in opaque liquids are impossible. If the piezoresistive readout in biosensor is used to measure the deflection of the microcantilevers, the upper drawbacks will eliminate because of the direct measurement without optical devices. We investigated the characteristics of piezoresistive and optical read-out methods after measuring the binding of microcantilever-based biosensor at the same time. The microcantilever-based biosensor was fabricated by surface micromachining technique. The polysilicon piezoresistor was used for piezoresistive read-out and deposited by low pressure chemical vapor deposition (LPCVD) with a thickness of 300nm. The microcantilever is bending from the difference of the surface stress caused by the formation of a glutaraldehyde/cystamine dihydrochloride bilayer on the gold-coated microcantilever. The fluid cell for liquid flow was fabricated using PDMS and glass. The liquid flow was driven by gravity and the flow rate of 12~14 $\mu$ l/min was kept constant during the whole measurement. In order to evaluate the characteristics of the microcantilever, the cystamine terminated with thiol was covalently immobilized on the gold-coated side of the cantilever and glutaraldehyde that would be bonded with amine group in the cystamine was injected subsequently. This process was characterized by measuring the deflection of the cantilever in real time monitoring. The deflection of the cantilever was measured both by piezoresistive method and by optical read-out method at the same time for the analysis of the sensitivity and the resolution.

**K3.6**  
**Influence of the Crosslinked Chitosan Sphere and Films on the Calcium Carbonate Crystallization.** Andronico David Neira-Carrillo<sup>1,2,3</sup>, Francisco Martinez<sup>3</sup>, Jaime Retuert<sup>2,3</sup>, Maria Soledad Fernandez<sup>1,2</sup> and Jose Luis Arias<sup>1,2</sup>; <sup>1</sup>Cs Biological veterinary and Animal Science, University of Chile, Santiago, Province, Chile; <sup>2</sup>Center for Advanced Interdisciplinary Research in Materials, CIMAT, Santiago, Chile; <sup>3</sup>Faculty of Physics and Mathematics, University of Chile, Santiago, Chile.

Biom mineralization is the process by which living forms influence the precipitation of mineral materials and leads to the formation of precisely controlled inorganic-organic composites, in which the minute organic component exerts substantial control on the mineralization process, which results in the formation of particles of uniform size, novel crystal morphology, specific crystallographic orientation and interesting properties (1,2). Crystal growth is typically heterogeneous crystallization and occurs in association with surfaces and occurs in a constrained volume. In nature, the living forms produce a geometrically well defined microenvironment, controlling not only the addition of the functionalized organic macromolecules but also variables such as localization and velocity of ions flux within a constrained volume. In order to investigate the influence of the crosslinked chitosan sphere and films on the CaCO<sub>3</sub> crystallization in vitro in a constrained volume, we have prepared sphere of chitosan in NaOH solution and compared with films. The crystallization method

was based on the Sitting-drop method by Dominguez-Vera (3). We suspecting that crosslinked grade of chitosan sphere and films alter the diffusion of CO<sub>2</sub> gas during the crystallization essays and lead to defined crystals morphology. Furthermore, a functionalized sulfate polysiloxane polymer as a additive on the CaCO<sub>3</sub> crystallization was used. This polymer was incorporated in situ of the chitosan sphere formation and mechanically, too. The crosslinking agents: Formaldehyde, Glutaraldehyde, Epichlorhydrine, Poly(propylene glycol)diglycidyl ether were used. The swelling(%) of the crosslinked sphere and films chitosan in buffer TRIS at pH 9 were determined. Chitosan samples of high (Aldrich, 83% deacetylation) and low (Fluka, 75% deacetylation) molecular weight were used. The intra-chitosan crystals shows different morphologies and were related to the crosslinked grade of the chitosan sphere and films. The obtained crystals were observed in a Tesla BS 343 A scanning electron microscope (SEM)

### **K3.7**

**X-ray Absorption Spectroscopy Characterization of Crystal Growth on Organic Templates.** Jonathan Lee, Tony van Buuren, Robert W. Meulenberg, Trevor M. Willey, Louis J. Terminello and James J. De Yoreo; Lawrence Livermore National Laboratory, Livermore, California.

X-ray Absorption Spectroscopy (XAS) has been used to characterize the structural evolution of bio-inspired crystallization systems. We present an XAS investigation of calcite growth on carboxyl terminated alkanethiol self-assembled monolayers (SAMs) prepared on Au(111) substrates. In the natural world, bio-organisms utilize surface matrices of organic molecules to exert remarkable control over the mode of mineral crystallization from solution. Elaborate, hierarchical inorganic assemblies are often generated, which can exhibit architecture on the sub-micron scale. Such precise engineering of crystal structure and, therefore, material properties would find direct application in the fabrication of inorganic components for optical and electronic devices. Hence, an understanding of the underlying physical processes is required to aid development of new material growth technologies. Self-assembled monolayers of *w*-substituted alkanethiols serve as templates for patterned crystallization and, as such, mimic the natural processes of biomineralization. In addition, these systems offer a relative simplicity of structure. As a consequence, they represent suitable models from which to characterize the interaction between organic and inorganic phases during crystal nucleation and growth. This interaction resides at the heart of biomineralization processes. XAS provides ideal capabilities for the investigation of structural development at the organic/inorganic interface during crystallization. Due to the chemical specificity of the technique, atoms at the buried interface can be probed directly. Furthermore, the X-ray Absorption Near Edge Structure (XANES), the first component of XAS, can be used to characterize the geometric and electronic structure of organic monolayers. Moreover, the Extended X-ray Absorption Fine Structure (EXAFS), the second component of XAS, provides assignment of the local environment about a specific element and can yield the structure of the crystalline mineral. This work was supported by the Division of Chemical Sciences, Office of Basic Energy Science, and performed under the auspices of the U.S. DOE by LLNL under contract No. W-7405-ENG-48

### **K3.8**

**Biosynthesis of CaCO<sub>3</sub> Crystals.** Kaustav Sinha<sup>1</sup>, Debabrata Rautray<sup>2</sup>, Murali Sastry<sup>2</sup> and Absar Ahmad<sup>2</sup>; <sup>1</sup>Department of Materials & Metallurgical Engineering, University of Nevada, Reno, Reno, Nevada; <sup>2</sup>Department of Materials Chemistry and Biochemical Sciences, National Chemical Laboratory, Pune, Maharashtra, India.

The biogenic CaCO<sub>3</sub> crystals are grown by simple exposure of aqueous Ca<sup>2+</sup> ions to *Fusarium* sp. and *Rhodococcus* sp. The reaction of Ca<sup>2+</sup> ions with the *Fusarium* sp. produces cruciform-shaped calcite particles while, the highly unstable vaterite polymorph in a disklike morphology is obtained with *Rhodococcus* sp. The morphology and crystallography of CaCO<sub>3</sub> crystals in solution is modulated by proteins/biomolecules not normally associated with calcareous microorganisms. Many fungi and actinomycetes are known to produce reasonable amounts of CO<sub>2</sub> during growth. The CO<sub>2</sub> released from such microorganisms is used to react with Ca ions and synthesize truly biogenic CaCO<sub>3</sub> crystals.

### **K3.9**

**Surface Treatment of Polystyrene with Ozone / UV in Water and Aqueous Ammonia Solution and Enzymatic Activity of Surface-Immobilized Glucose Oxidase.** Ken Yanagisawa<sup>1</sup>, Takuro N. Murakami<sup>1</sup>, Yoshiaki Hirano<sup>1</sup>, Yoshikazu Tokuoka<sup>1</sup>, Mitsuo Takahashi<sup>2</sup> and Norimichi Kawashima<sup>1</sup>; <sup>1</sup>Biomedical Engineering, Toin University of Yokohama, Yokohama, Kanagawa, Japan; <sup>2</sup>Chemical Science and Engineering, Tokyo National College of Technology, Hachioji, Tokyo, Japan.

In recent years, the controlling of adsorption and adhesion of biomolecules, medicines, or cells on the polymer surface has attracted considerable attention in the application of biosensors, artificial blood vessels and cell culture scaffolds. There are various techniques using plasma treatment, chemical vapor deposition, and graft copolymerization for surface modification of polymers. In particular, ozone aeration with UV light irradiation treatment can easily be carried out in various gas, solvent and solution media at room temperature without vacuum system and is suitable for heat unstable polymers. We have investigated the surface modification of polystyrene (PS) film using ozone aeration and UV irradiation (O<sub>3</sub>/UV treatment) aiming at biomedical applications of the modified film. We confirmed that hydroxyl (OH) and carbonyl (C=O) groups are introduced on the PS film surface by the treatment in distilled water. In addition, the treatment in aqueous ammonia solution produced amino (NH<sub>2</sub>) and amide (CONH<sub>2</sub>) groups on the film surface. These additional groups on the polymer surface are needed for protein immobilization. O<sub>3</sub>/UV treatment for polymer surfaces can be applicable to generate functional groups on the surface necessary for modification with various biomolecules and living cells without any chemical treatment that causes contamination. In the present study, we have examined the immobilization of glucose oxidase (GOD; EC 1.1.3.4) as model enzyme on the PS film surface treated by O<sub>3</sub>/UV in water or aqueous ammonia solution and the relation between the enzyme activity and treatment media. Immobilization of GOD on PS films was carried out by the following method: PS films were placed in water or aqueous ammonia solution and aerated using ozone gas with irradiation of UV light. The films treated in water (W-PS) and in aqueous ammonia solution (A-PS) were immersed in an aqueous GOD solution (10 mg/ml) at room temperature for 24 hr. The total amount of GOD immobilized on the films was determined by the dye-binding method. The activity of GOD on the film was evaluated by decrease in the glucose concentration during the reaction between aqueous glucose solution and GOD-immobilized PS film. The presence of GOD on the film surface was confirmed for the W-PS and A-PS by film coloration. The amount of GOD on W-PS was approximately 2 times higher than that on A-PS, which indicates that W-PS is better than A-PS for GOD immobilization. The glucose concentration over A-PS was lower than that over W-PS. Thus, the concentrations over W-PS and A-PS reduced from 28mM to 25mM and 23mM, respectively. The activity of GOD on A-PS was higher than that on W-PS and a portion of GOD on W-PS became inactive. Hence, A-PS was concluded to be better than W-PS for making functional surface with GOD immobilized on it. The activity of GOD was found to depend on treating medium (water or aqueous ammonia solution) in O<sub>3</sub>/UV treatment.

### **K3.10**

**Enhanced Biocompatibility of GPC by MeV Ion Bombardment.** Robert Zimmerman<sup>1</sup>, I. Gurhan<sup>2</sup>, C. Muntele<sup>1</sup>, S. Sarkisov<sup>1</sup>, M. Rodrigues<sup>3</sup> and D. Ila<sup>1</sup>; <sup>1</sup>Alabama A&M University, Normal, Alabama; <sup>2</sup>Ege University Faculty of Engineering, Ismar, Turkey; <sup>3</sup>University of Sao Paulo, Ribeirao Preto SP, Brazil.

Glassy Polymeric Carbon (GPC) is completely biocompatible and is widely used as a material for artificial heart valves and in other biomedical applications. Although it is ideally suited for fluid flow in the blood stream, collagenous tissue that normally forms around the moving parts of a GPC heart valve loses adhesion and creates embolisms downstream. We have shown that moderate fluence of MeV ions, especially oxygen ions, increases the surface roughness of GPC on a scale appropriate for enhancing tissue adhesion. Ion bombardment also increases the surface hardness of GPC, already an extremely hard material. In vitro biocompatibility tests have been carried out with model cell lines to demonstrate that MeV ion bombardment can favorably influence the surface of GPC for biomedical applications.

### **K3.11**

**Hydroxyapatite Crystallization Kinetics in Amorphous Calcium Phosphate Films Coated on Titanium via Micro-Arc Process.** Yun-Mo Sung<sup>1</sup>, Yeong-Keun Shin<sup>1</sup>, Kyung-Woo Kim<sup>1</sup> and Sang-Ho Hwang<sup>2</sup>; <sup>1</sup>Materials Sci. & Eng., Daejin University, Pochun-si, Kyunggi-do, South Korea; <sup>2</sup>R&D Department, Nano-Tech Inc., Pochun-si, Kyunggi-do, South Korea.

Hydroxyapatite (HAP) coating has been studied to improve biocompatibility of Ti or Ti-alloy implants. Micro-arc process (MAP) is an electrochemical route applicable for the HAP coating and through it porous HAP films strongly bonded to Ti implants can be achieved. Also, it allows precision and easy coating on complicate implant parts. In this study amorphous calcium phosphate films with ~20 nm thickness were coated on Ti using MAP. The coated films were immersed into a buffer solution consisting of Ca<sup>2+</sup> and P<sup>5+</sup> ions. The temperature and time period of the solution treatments were controlled for the crystallization kinetics study. X-ray diffraction (XRD) analyses on the films showed crystallinity of ~92% after the solution treatment at 40°C for 12 h. The volume fraction values of

HAP crystal formation in the amorphous films were obtained using a quantitative XRD analyses. Johnson-Mehl-Avrami (JMA) analyses were performed using the volume fraction values and Avrami exponent ( $n$ ) value was determined from the slopes of JMA plots. From Arrhenius plots the activation energy value was determined, and the nucleation and growth mechanism was considered for the HAP crystallization. Transmission microscopy (TEM) analyses were performed on the partially crystallized calcium phosphate films and compared to the results from JMA kinetics analyses.

### **K3.12**

**Fabrication of Semiconductor Nano-particles in the Protein Cage of Apoferritin.** Kenji Iwahori<sup>2</sup>, Keiko Yoshizawa<sup>2</sup> and Ichiro Yamashita<sup>3,2,1</sup>; <sup>1</sup>Materials Science, Nara Institute of Science and Technology, Ikoma, Japan; <sup>2</sup>CREST, Japan Science and Technology Agency, Ikoma; <sup>3</sup>ATRL, Matsushita Electric Industrial. Co., Ltd., Seika, Japan.

Material researchers have been interested in Nano-particles (NPs) because of their potentials to be used as nanotechnology key component. Metal or semiconductor NPs are small enough for the electron energy level to become separated as quantum dots. Magnetic NPs make it possible to produce high density storage media. Therefore, a lot of methods to produce NPs have been studied including the chemical synthesis, Laser abrasion, and biological methods. We adopt a cage-shaped protein, apoferritin, to synthesize inorganic NPs in its internal cavity and propose a new process which utilizes the ferritin molecules to build nano-electronics device key-component(1). Apoferritin, the cellular iron-storage protein, is a spherical hollow shell composed of 24 polypeptide subunits and has the ability to store iron as ferrihydrite in the cavity. The inner and outer diameters of the protein shell are about 7 nm and 12 nm respectively. There were preceding reports of metal complex NPs synthesis in the apoferritin cavity. We also succeeded to synthesize the some metal NPs in the apoferritin cavity, for example, Co, Ni, Cr NPs (2). Besides these NPs, semiconductor NPs synthesis in the apoferritin cavity have been desired. However, there was only one report describing semiconductor CdS (3) and CdSe NPs synthesis (4) in the apoferritin cavity. To make compound semiconductor and ZnSe NPs in the apoferritin cavity, we designed a new chemical synthesis system which makes the chemical reaction of compound semiconductor element ions dramatically slow, so that the semiconductor NPs can be synthesized in the internal cavity of the apoferritin. By optimizing reaction parameters, the ZnSe NPs are efficiently produced. These NPs were characterized by the high resolution TEM, XRD, EDX and EELS analysis and they are proved to be ZnSe NPs. Furthermore, we employed some mutant apoferritins to study the difference of the core formation ratio (CFR) and to understand the mechanism of ZnSe NPs synthesis in the apoferritin cavity. From these results, three factors are proved to be important. (i) 3-fold channel; selective introduction of Zn ion into apoferritin cavity, (ii) apoferritin internal potential; Zn ion accumulation in the apoferritin cavity and (iii) the ferroxidase center; Zn ion binding and then making the ZnSe nuclei at internal apoferritin cavity. The application of these obtained NPs as the key component of nano-electronics devices is in progress. References (1) Yamashita I., Thin Solid Films., 393, 12 (2001). (2) Okuda, M., Iwahori, K., Yamashita, I., Yosimura, H., Biotech. Bioeng., 74(2),188 (2003) (3) Wong, K. K. W.; Mann, S. Adv. Mater. 8, 928 (1996) (4) Yamashita, I.; Hayashi, J.; Hara, M. Chem. Lett., 33, 1158 (2004)

### **K3.13**

**Fabrication of Indium Oxide Semiconductor Nano-particles using Ferritin.** Mitsuhiro Okuda<sup>1</sup>, Ichiro Yamashita<sup>1,3</sup>, Kenji Iwahori<sup>3</sup> and Hideyuki Yoshimura<sup>2</sup>; <sup>1</sup>ATRL, Matsushita Electric Industry Co., Ltd., Kyoto, Japan; <sup>2</sup>Meiji University, Kanagawa, Japan; <sup>3</sup>CREST, Japan Science and Technology Agency, Nara, Japan.

Inorganic materials of nanometer order attract attention from application point of view. Many methods for fabrication of nano-inorganic materials were developed including physical and chemical methods. It is also required for nano-inorganic materials to be ordered as two-dimensional crystal as a first step for making nanometric functional structures. We propose biological method to synthesize nano-inorganic materials and make two- or three-dimensional crystal of them. Ferritin is a spherical protein with a diameter of 12 nm. It has a cavity, 7 nm in diameter, surrounded by 24 polypeptide subunits. It is known that there are hydrophilic channels which penetrate the protein shell and are considered to be the pathways of Fe(II). Natural apoferritin consists of two types of polypeptide subunits, H and L chains, the relative ratio of which varies with the type of biological species and organ. The H-subunit has a ferroxidase catalytic site at the interior surface where Fe(II) is oxidized to Fe(III), whereas the L-subunit shows no ferroxidase activity. Under physiological conditions, apoferritin stores iron in the cavity as ferrihydrite. Artificial biomimetalization of some other materials in the cavity has been reported. Despite its lack of ferroxidase activity, recombinant L-apoferritin, which consists of only

L-subunits, can accumulate iron in the cavity. Recombinant L-apoferritin is known to crystallize two- or three-dimensionally by forming a salt bridge between the cadmium binding sites. Here we report that iron storage protein recombinant L-apoferritin can accumulate nanosize indium oxide in the apoferritin internal cavity. The indium cores are formed in the recombinant L-apoferritin cavities by incubating indium and recombinant L-apoferritin solution around pH 2.8 at room temperature for more than 24 h. The final concentration of each material were 0.1mg/ml recombinant L-apoferritin, 40mM HCl, 200 mM monobasic sodium phosphate, 16 mM ammonium and 1mM indium sulfate. The addition of the indium sulfate was the final step to start the core formation reaction. The samples were negatively stained by aurothioglucose and observed by TEM. Aurothioglucose dose not stain the cavity because steric hindrance prevents it from going through the narrow channels. Almost all incubated recombinant L-apoferritins formed indium oxide cores. The elements of obtained cores were determined by energy dispersive X-ray analysis (EDX). The EDX spectra show two indium peaks. High resolution TEM image showed the clear lattices which correspond Indium oxide crystal. These results indicate that recombinant L-apoferritin formed indium oxide core in the cavity. References (1) Hikono, T., Uraoka, Y., Fuyuki, T., Yamashita, I., Jpn.J. Appl. Phys, 42, 398 - 399. (2003) (2) Okuda, M., Iwahori, K., Yamashita, I., Yoshimura H., Biotech. Bioeng., 74(2) , 188-194 (2003)

### **K3.14**

**Metal-peptide Nanoassemblies: Combining the Principles of Supramolecular Coordination Chemistry with De Novo Protein Design.** Michael Y. Ogawa, Mihail Tsurkan and Fei Xie; Department of Chemistry and Center for Photochemical Sciences, Bowling Green State University, Bowling Green, Ohio.

Our group is developing methods to exploit the directional bonding properties of coordination compounds to orient synthetic  $\alpha$ -helical coiled-coil protein dimerization domains in ways that can produce new nanostructured materials. The current approach utilizes both non-covalent and disulfide crosslinked coiled-coils as bridging ligands to join together Pt(en) coordination complexes in geometries that are dictated by the steric demands of the metal center, where en = ethylene diamine. The peptide sequences employed in this study were based on the IEALEGK heptad repeat which has been extensively used by our group to prepare a variety of metal-substituted, two-stranded  $\alpha$ -helical coiled-coils for electron-transfer studies. However, in this work the non-natural amino acid 4-pyridyl alanine (Pa) was placed at position 14 of the sequence, which is the most solvent-exposed position of the second heptad repeat: AcK(IEALEGK)(IEALEPaK)(IEALEGK)<sub>2</sub>GNH<sub>2</sub> = PAL14. For some studies a cysteine residue was placed at position 19 which exists within the hydrophobic peptide-peptide interface of the coiled-coil: AcK(IEALEGK)(IEALEPaK)(IEACEGK)(IEALEGK)GNH<sub>2</sub> = PAL14C19. These modifications were made in order to incorporate a strong metal binding site into the peptide, and to engineer an inter-chain disulfide bond crosslink to stabilize the coiled-coil structure. When Pt(en)(NO<sub>3</sub>)<sub>2</sub> was treated with excess PAL14 in aqueous solution for several days at ambient temperature, a metal-peptide corner unit was prepared in which the centrally located pyridine sites of two peptide chains were coordinated to the cis positions of the square-planar Pt center. The circular dichroism spectrum of a sample of purified Pt corner units shows that multiple corner units do indeed self-assemble through the formation of non-covalent  $\alpha$ -helical coiled-coils. Of special interest are the high pressure size exclusion chromatography (HPSEC) results which indicate that the non-covalent association of Pt-peptide corner units produces assemblies of 1, 2, 4, and 6 corner units. Interestingly, the major product formed in this process corresponds to the assembly of 6 Pt-peptide corners. Related work involved the incubation of Pt(en)(NO<sub>3</sub>)<sub>2</sub> with the disulfide crosslinked PAL14C19 coiled-coil peptide. Here, SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) shows the existence of a progression of metal-peptide oligomers which correspond to the assembly of 1-5 metal-peptide units. The results show that formation of the lower molecular weight products was favored, in contrast to the behavior observed for the non-covalent assembly described above.

### **K3.15**

**Selective Deposition of DNA-functionalized Gold Nanospheres into Surface Nanopores.** Angelika B. Niemz<sup>1</sup>, Krisanu Bandyopadhyay<sup>1,2</sup>, Eric Tan<sup>1</sup>, Lin Ho<sup>1</sup>, Annie Tan<sup>2</sup> and Shenda M. Baker<sup>2</sup>; <sup>1</sup>Keck Graduate Institute, Claremont, California; <sup>2</sup>Chemistry, Harvey Mudd College, Claremont, California.

We report the optimization of a novel approach for depositing individual DNA-functionalized gold nanospheres into hexagonal arrays of surface nanopores generated from diblock copolymer thin films. These self-assembled DNA nanosphere arrays can be used as scaffold to direct the ordered and selective secondary self-assembly of other



Research Institute, University of New Orleans, New Orleans, Louisiana; <sup>2</sup>Research Center of the Ministry of Education for High Gravity Engineering and Technology, Beijing University of Chemical Technology, Beijing, China.

In this presentation, we report a successful synthesis of magnetic hollow silica nanostructures (MHSNS). The MHSNS were fabricated with one step coating of Fe<sub>3</sub>O<sub>4</sub> magnetic nanoparticles (NPs) and silica on nanosized spherical and tubular calcium carbonate (CaCO<sub>3</sub>) surface under alkaline conditions, in which the nanosized CaCO<sub>3</sub> were used as nano-templates and tetraethoxysilane and magnetic NPs as precursors. The as-synthesized nanostructures were immersed in an acidic solution to remove CaCO<sub>3</sub> NPs, forming MHSNS. The MHSNS were characterized by SEM, TEM, and SQUID. SEM and TEM results showed that a thin layer of silica (10 nm) embedded with the magnetic NPs was successfully formed and nanosized calcium carbonate nano-templates were completely removed. SQUID results demonstrated that magnetization of MHSNS was dependent of temperature, exhibiting superparamagnetism. In addition, the bio-applications of such MHSNS are also discussed.

### **K3.21**

**Synthesis and Characterization of Biomolecular Hybrids as Novel Energy Sources.** Dean Ho, Benjamin Chu, Hyeeseung Lee, Evan Brooks, Karen Kuo and Carlo D. Montemagno; Bioengineering, UCLA, Los Angeles, California.

The integration of energy-transducing proteins with polymeric biomembranes can be used to engineer novel energy sources. The Bacteriorhodopsin (BR) protein functions as a light-activated proton pump that can develop chemical gradients across artificial membranes. As it is stable in temperatures of up to 140 degrees Celsius and pH levels as low as 0, it serves as an ideal light-dependent switch for device engineering to induce partial Cytochrome C Oxidase (COX) reversal. COX has been shown to be partially reversible given a high electrochemical transmembrane proton gradient as well as high concentration of oxidized Cytochrome C. Under such circumstances, reaction energetics favors the oxidation of water to deliver electrons to the iron/copper center of COX. These electrons are in turn donated to the Cytochrome C acceptor which can be harvested for current measurement. Biocompatible membrane systems developed using ABA triblock copolymers were used to enhance the stability of the biomolecular hybrid devices. These membranes have been observed to preserve protein activity on the order of weeks compared with days for lipid-based membranes. In addition to possessing block configurations that can be tailored to possess varying lengths and chemistries, the polymer also contains endgroups that can be crosslinked via UV-induced free radical polymerization. This property was crucial to enhancing membrane impermeability to ion flux, which was needed to preserve the gradients necessary for protein coupling. By embedding both proteins into polymeric vesicles, we have demonstrated the use of BR as a light-dependent switch to induce electron release by COX. Successive cycles of light/dark conditions resulted in increases and decreases, respectively, of current generation from the hybrid system. This was accomplished using the detection of current vs. time from composite vesicles in the absence of an applied voltage. Cyclic voltammetry was also used to study light-dependent current increases of the hybrid system. The composite vesicle output efficiencies were determined to be as high as 8.1% from cyclic voltammetry using the visible spectrum of light, with current increases being greater than 13 microamperes. These key findings have demonstrated the feasibility of using proteins as core technology for devices based on inherent molecular function.

### **K3.22**

**Biocompatible Thin Films by the Electrostatic Self-Assembly Process.** You-Xiong Wang<sup>1</sup>, Kit Cheung<sup>1</sup>, Nicholas P. Evans<sup>3</sup>, John L. Robertson<sup>3</sup>, Cristelle F. Jullian<sup>2</sup>, William B. Spillman<sup>2</sup> and Richard O. Claus<sup>1</sup>; <sup>1</sup>Fiber & Electro-Optics Research Center, Virginia Polytechnic Institute and State University, Blacksburg, Virginia; <sup>2</sup>Virginia Tech Applied Bioscience Center, Virginia Polytechnic Institute and State University, Blacksburg, Virginia; <sup>3</sup>Virginia-Maryland Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

The use of biomaterials to interface with living systems, such as fluids, cells, and tissues of the body, has played an increasingly important role in medicine and pharmaceuticals. In particular, the design of biocompatible synthetic surfaces to control the interaction between a living system and an implanted material remains the major theme for biomaterial applications in medicine. The novel and low-cost electrostatic self-assembly (ESA) technique provides an effective approach to fabricate various biomaterials on substrate surfaces, and gives great opportunity to develop unique biocompatible materials with well-organized interfaces. The incorporation of various ceramic biomaterials, water-soluble polymers and other materials such as heparin into self-assembled thin films can provide the opportunity

to develop unique biocompatible materials with well-controlled interfaces. We have been successfully fabricated such biocompatible thin films on various substrates, including polymeric catheters and tubing's by the ESA process. The protein adsorption and hemocompatibility tests, including LDH, cell adhesion and clot mass, of the thin films have provided many interesting results. The thin films fabricated with biocompatible materials by ESA processing will have broad applications in tissue engineering, such as bone implants, anti-restenosis coating on medical devices (stents), and scaffolds to restore damaged organ structure.

### **K3.23**

**Immobilization Of Thylakoid Membranes Via Self-Assembled Monolayers.** Kien Bang Lam<sup>1,2</sup>, Elizabeth F. Irwin<sup>3</sup>, Kevin E. Healy<sup>3,4</sup> and Liwei Lin<sup>1,2</sup>; <sup>1</sup>Department of Mechanical Engineering, University of California at Berkeley, Berkeley, California; <sup>2</sup>Berkeley Sensor and Actuator Center, University of California at Berkeley, Berkeley, California; <sup>3</sup>Department of Bioengineering, University of California at Berkeley, Berkeley, California; <sup>4</sup>Department of Materials Science and Engineering, University of California at Berkeley, Berkeley, California.

Photosynthetic thylakoid membranes were immobilized onto a gold substrate functionalized by self-assembled monolayers (SAMs) of cystamine and pyrroloquinoline quinone (PQQ) as part of our efforts to fabricate MEMS photosynthetic solar cells (PSC) and biosensors [1]. Thylakoid membranes are sub-cellular plant structures with embedded photosynthetic photosystems that capture the energy of incident photons to oxidize water and transfer the resulting electrons into the electron transport chain. A quartz crystal microbalance with dissipation (QCM-D) was used to verify and monitor the deposition kinetics of the SAMs and thylakoids. The Sauerbrey equation—which relates the change in resonant frequency of the crystal with the change in mass attached to the crystal surface, including bound water—was employed to calculate the surface densities of the added layers [2]. First, a monolayer of cystamine was chemisorbed to a clean Au surface and rinsed with water to remove physisorbed material. Most of the cystamine adsorbed within the first minute of being introduced to the Au surface, resulting in a surface density of 7.9 x 10<sup>-10</sup> mol/cm<sup>2</sup> including bound water. PQQ was then covalently bound to the cystamine layer using carbodiimide chemistry in HEPES buffer (pH 7.5), yielding a monolayer with a surface density of 3.2 x 10<sup>-10</sup> mol/cm<sup>2</sup> that was stable during rinsing. The cystamine and PQQ surface densities measured using QCM-D are comparable to but more accurate than values previously reported using cyclic voltammetry [3]. Finally, thylakoid membranes were isolated from baby spinach using a standard fractionation procedure and covalently bound to the PQQ monolayer, also using carbodiimide chemistry. The resulting thylakoid monolayer had a surface density of 370 ng/cm<sup>2</sup> and was also stable during rinsing. This thylakoid-PQQ-cystamine-Au monolayer stack allows the transfer of electrons generated by photosynthetic activity from the thylakoids directly to the underlying gold and provides a platform suitable for various MEMS applications. In PSCs, the immobilized thylakoids could be used to harness light to generate electrical current. For biosensor applications, the thylakoids could be used to detect biochemical agents such as herbicides that interrupt flow of electrons in the transport chain. [1] K. B. Lam, E. Johnson, and L. Lin, "A Bio-Solar Cell Powered By Sub-Cellular Plant Photosystems," in Proc. IEEE Conf. on Micro Electro Mechanical Syst.(MEMS 2004), Maastricht, The Netherlands, Jan. 25-29, 2004, pp.220-223. [2] M. Rodahl and B. Kasemo, "A simple setup to simultaneously measure the resonant frequency and the absolute dissipation factor of a quartz crystal microbalance," Review of Scientific Instruments, vol. 67, no. 9, pp.3238-3241, 1996. [3] A. Bardea, E. Katz, A. F. Buckmann, and I. Willner, "NAD<sup>+</sup>-dependent enzyme electrodes: electrical contact of cofactor-dependent enzymes and electrodes," J. Am. Chem. Soc., vol. 119, pp.9114-9119, 1997.

SESSION K4: Biomolecular Assembly and Bio-inspired Synthesis

Chairs: Rajesh Naik and Aida Takuzo  
Wednesday Morning, March 30, 2005  
Room 3002 (Moscone West)

### **8:00 AM K4.1**

**DNA-Assembled Nanocomponent Arrays with Hierarchically Controlled Intercomponent Spacing.** Yariy Y. Pinto<sup>1,2</sup>, John D. Le<sup>2</sup>, Nadrian C. Seeman<sup>3</sup>, Karin Musier-Forsyth<sup>2</sup>, T. Andrew Taton<sup>2</sup> and Richard A. Kiehl<sup>1</sup>; <sup>1</sup>Electrical and Computer Engineering, University of Minnesota, Minneapolis, Minnesota; <sup>2</sup>Chemistry, University of Minnesota, Minneapolis, Minnesota; <sup>3</sup>Chemistry, New York University, New York, New York.

The self-assembly of nanocomponents into arrays with controlled intercomponent spacing could enable nanoscale manufacturing of

novel materials and structures for a wide range of applications, including nanoelectronic and nanomechanical systems. Hierarchical structures that are assembled in steps and have dimensions that are independently controlled at each step are highly desirable since they offer flexibility in their design and versatility for applications. In this talk, we describe the self-assembly of such hierarchical structures using Watson-Crick hybridization. The process begins with the construction of a 2D scaffolding from a set of 21 synthetic oligonucleotides that is designed to assemble into four different double cross-over (DX) molecules, which in turn assemble into a 2D DNA crystal. One of the DX molecules includes an extended single-stranded DNA feature that is designed to hybridize to oligonucleotides bound to nanocomponents, resulting in rows of closely spaced hybridization sites. The extended feature used in this study is a 5'-(dA)<sub>15</sub> sequence. After its formation in solution, the 2D DNA scaffolding is attached to a mica surface, thereby providing a template for later nanocomponent assembly. The prototype nanocomponents used in this study are composed of 6-nm Au nanoparticles functionalized with multiple strands of 3'-thiolated (dT)<sub>N</sub>. The sequence of N thymine bases is complementary to a segment of the extended feature on the DNA scaffolding described above. In the second step of the process, a solution of the nanocomponents is deposited on the mica and allowed to hybridize in situ to the pre-assembled DNA scaffolding. This process results in the fabrication of rows of nanocomponents with an inter-row spacing of 64 nm. In order to investigate the relationship between N (the number of thymine bases in the strands forming the nanocomponent shell) and the spacing between the Au particles along the rows, the self-assembly was studied for N over the range of 2 to 15. Characterization of the arrays by atomic force microscopy and transmission electron microscopy shows that high yield 2D arrays are formed for N in the range of 7 to 15. Arrays did not form for N less than 7, possibly because of aggregation of the components under the hybridization conditions. The spacing between components within the rows scaled with N over the range of 7 to 15, with a corresponding gap between the Au particle cores of 2 to 15 nm. These results demonstrate the hierarchical self-assembly of nanocomponent arrays in which the inter-row spacing is controlled by the DNA scaffolding design and the spacing between components is controlled by the number of bases in the nanocomponent's DNA shell.

#### 8:15 AM **K4.2**

**Toward Single DNA Sequencing: Single Nucleobase Sensitivity of  $\alpha$ -Hemolysin ( $\alpha$ -HL) Transmembrane Protein Based Biosensor.** Nurit Ashkenasy<sup>1</sup>, Jorge Sanchez-Quesada<sup>1</sup>, M. Reza Ghadiri<sup>1</sup> and Hagan Bayley<sup>2</sup>; <sup>1</sup>Chemistry, The Scripps Research Institute, La Jolla, California; <sup>2</sup>Chemistry, University of Oxford, Oxford, England, United Kingdom.

There has been an intriguing suggestion that *Staphylococcus aureus*  $\alpha$ -hemolysin ( $\alpha$ -HL), a stable heptameric transmembrane protein pore, might be of use as the sensor element in a rapid pore-mediated single-molecule DNA sequencing process. This unnatural utilization of the protein has been the focus of intense work in the past few years. One fundamental requirement that had not been fully assessed is the pores nucleobase resolution. In this work we show that  $\alpha$ -HL can recognize ss-DNA with an apparent single nucleobase resolution. DNA strands that contain hairpin at one end are introduced at the cis side of a lipid bilayer containing a single oriented  $\alpha$ -HL pore. DNA threading process is initiated by applying positive transmembrane holding potentials, formation of an  $\alpha$ -HL-DNA pseudorotaxane is signified by the reduction in ion channel conductance caused by the presence of the ss-DNA inside the pore. Streptavidin binding to the biotinated strand-edge at the trans side complete Rotaxane formation. Homopurine based rotaxanes and pseudorotaxanes are shown to possess significantly smaller current than homopyrimidine based structures. Series of adenine (a purine based nucleotide) and cytosine (a pyrimidine based nucleotide) DNA block copolymers and cytosine homopolymeric strands with position-specific single-nucleotide adenine substitutions are used to discover and locate a specific nucleotide position responsible for the measured current, twenty nucleotides away from the edge of the hairpin. The pore location at which detection occurs is found to be near the trans entrance. The discovery that  $\alpha$ -HL can recognize ss-DNA with an apparent single nucleobase resolution strengthens the case for its utility in rapid single-molecule DNA sequencing.

#### 8:30 AM **\*K4.3**

**Building from Bottom up: Fabrication of Materials using Peptide Motifs.** Shuguang Zhang, Center for Biomedical Engineering NE47-379, Center for Bits & Atoms, Massachusetts Institute of Technology, Cambridge, Massachusetts.

Materials science has generally been associated with metallurgy, alloy, ceramics, composites, polymer science, fiber spinning, coating, thin film, industrial surfactants and block copolymer development. That is about to change. Materials science will also expand to discovery and fabrication of biological and molecular materials with diverse

structures, functionalities and utilities. The advent of nanobiotechnology and nanotechnology accelerated this trend. Similar as construction of an intricate architectural structure, diverse and numerous structural motifs are used to assemble a sophisticated complex. Nature has selected, produced and evolved numerous molecular architectural motifs over billions of years for particular functions. These molecular motifs can now be used to build materials from the bottom up. Materials science will begin to harness nature's enormous power to benefit other disciplines and society.

#### 9:00 AM **\*K4.4**

**Cell Biology and Biochemistry of Coccolithophore Biom mineralization.** Elma L. Gonzalez, Ecology and Evolutionary Biology, Univ of California-Los Angeles, Los Angeles, California.

The calcifying coccolithophore (Haptophyte) assembles a mineral and organic structure of exquisite design in a sub-cellular space while using a tool-kit comprised of only proteins, lipids and polysaccharides. The coccoliths are assembled in an 'assembly plant' enclosed by a membrane that defines this coccolith vesicle (cv). The cv maintains the conditions and carriers sufficient to import the raw materials (Ca<sup>2+</sup> and CO<sub>3</sub><sup>2-</sup>) for calcite formation and, furthermore, to initiate and sustain mineralization. The coccolith membrane, and its protein/enzyme complement, is derived from the trans-Golgi apparatus. The shape, size and structure of the organo-mineral complex are heritable. The mechanistic aspects of coccolith assembly are virtually unknown. How does calcium arrive at the cv? What is the identity of the carbon species taken up by the cv? How are Ca<sup>2+</sup> and carbon species taken into the cv? The calcification reaction is acidogenic. How is the pH of the cv maintained to permit mineralization? What is the role of polysaccharides in Ca<sup>2+</sup> transport?; how is the traffic of particles regulated?; and how is the calcium ion released from the coccolithosome particle? One direction of inquiry has led us to characterize a proton-pumping vacuolar ATPase, one of the protein complexes of the cv membrane. This calcium-stimulated proton pump is a multi-subunit enzyme. We have cloned and sequenced one of its components, the membrane-spanning subunit c, and found its amino acid sequence to be highly similar to that of the corresponding ATPase subunit from a wide range of taxa, including fruit flies and fish. We have proposed that the proton-pumping V-ATPase of the cv is involved in pH maintenance.

#### 9:30 AM **K4.5**

**Electroactive Luminescent Nanowires of Self-Assembled Oligoelectrolyte-Amyloid Fibrils.** Anna Herland<sup>1</sup>, Per Bjork<sup>1</sup>, Peter Nilsson<sup>1</sup>, Johan Olsson<sup>2</sup>, Peter Konradsson<sup>2</sup>, Per Hammarstrom<sup>2</sup> and Olle Inganas<sup>1</sup>; <sup>1</sup>Applied Physics, IFM, Linkoping, Sweden; <sup>2</sup>Chemistry, IFM, Linkoping, Sweden.

The development of self-assembled nanoscopic materials for controlled bottom-up fabrication of biomolecular devices is of current interest. In this regard, the self-assembly and the three-dimensional well-ordered structures of biomolecules could be used as excellent construction tools for the assembly of electronic devices. Among the geometries needed, wires are ubiquitous. These wires can be carrying current to devices, or can be formed into integrated devices. Amyloid fibrils have been used to create templates for metal nanowires, where metals were associated to the amyloid fibril structures post-self-assembly of the fibrils. We have taken a different route and generated self-assembling electroactive bio-organic nanowires with 10 nm (width) and lengths up to 10  $\mu$ m. The nanowires are based on protein amyloid fibril co-assembly with conjugated oligoelectrolytes with a thiophene backbone. The nanowires are formed in acidic environment (pH 1.6) and at moderately elevated temperature (65° C). The luminescent oligomers were integrated into the fibril, which was evident from intensity and spectral distribution of the photoluminescence and from the morphology of supramolecular structures in the form of bundled nanowires. The electro-optical properties of the wires are demonstrated with reversible electrochemical doping induced fluorescence quenching, thus demonstrating both electrical transport and electroactivity. We suggest that this self-assembly method can be used for several types of electroactive organic materials. The possibility to synthesize and design amyloid forming peptides and proteins can be used in the formation of wires or devices. The peptides can further be designed with address functions for anchoring of the wire to electrodes or other wires. Furthermore, changes in optical properties of the conjugated oligoelectrolytes can be used to probe amyloid fibril formation. As stated above the oligoelectrolytes can be present during amyloid fibril formation or be added to pre-formed fibrils, which results in a different spectral distribution. The conformation changes of the protein results in alterations in the geometry and the electronic structure of the oligoelectrolyte chains, which have been monitored with absorption and emission spectroscopy. This principle has lately been shown when conjugated polymers (CPs) were used as optical probes to monitor conformation changes of synthetic peptides [1,2] and calcium-induced conformation changes in calmodulin[3]. References: (1) Nilsson, K. P.; Rydberg, J.;

Baltzer, L.; Inganas, O. Proc Natl Acad Sci U S A 2003, 100, 10170-10174. (2) Nilsson, K. P.; Rydberg, J.; Baltzer, L.; Inganas, O. Proc. Natl. Acad. Sci. USA. 2004, 101, 11197-11202. (3) Nilsson, K. P.; Inganas, O. Macromolecules 2004, Web Release Date: 21-Oct-2004.

#### 10:15 AM K4.6

**Fabrication of Hierarchical Structures using Protein Cages as Building Blocks.** Michael T. Klem<sup>1,3,4</sup>, Eric Gillitzer<sup>2,3,4</sup>, Peter Suci<sup>2,3,4</sup>, Mark Allen<sup>1,3,4</sup>, Mark Young<sup>2,3,4</sup> and Trevor Douglas<sup>1,3,4</sup>; <sup>1</sup>Chemistry & Biochemistry, Montana State University, Bozeman, Montana; <sup>2</sup>Plant Sciences, Montana State University, Bozeman, Montana; <sup>3</sup>Center for BioInspired Nanomaterials, Montana State University, Bozeman, Montana; <sup>4</sup>Thermal Biology Institute, Montana State University, Bozeman, Montana.

Biomimetic approaches to materials chemistry have provided a new avenue for the synthesis and assembly of nanomaterials. There is growing interest in materials chemistry to take advantage of the physical and chemical properties of biomolecules for development of the next generation of nanoscale materials. Protein cages exist in a variety of sizes and shapes and the protein surfaces can be used as synthetic platforms for chemical modification. The ability of some protein cages to form self assembled arrays on a variety of substrates is of significant interest as possible precursors to interesting nanomaterials such as magnetic semiconductors. This work makes use of chemical and genetically modified spherical protein cages like the Cowpea Chlorotic Mottle Virus (CCMV) that self assemble into hierarchical structures by design on length scales approaching microns in 2 or 3 dimensions. A solid phase synthetic approach was adopted to generate protein cages with an asymmetric presentation of selected functional groups. Combination of protein cages with differing presentations of functional groups allow for the formation of larger structures through a "lock and key" mechanism. The incorporation of magnetic nanoparticles was also performed generating 2- and 3-dimensional magnetic arrays. The resulting structures were then characterized by a variety of techniques including TEM, AFM, IR, magnetometry, and dynamic light scattering. This work highlights three important concepts. First, through a combination of genetic and chemical modifications, we can engineer chemical functionality to symmetry related sites on the exterior protein cage surface. Second, through a solid phase synthesis approach, we can selectively block functional groups in a spatially controlled manner, thereby breaking the symmetry of the particle. Finally, protein cages with asymmetric functional groups can impart the ability to form hierarchical structures in 2- or 3-dimensions.

#### 10:30 AM K4.7

**Self-Assembled Material Nanostructure Defined By The Secondary Structure Of Amphiphilic Diblock Copolypeptides.** Lisa M. Pakstis<sup>1</sup>, Darrin J. Pochan<sup>1</sup>, Timothy Deming<sup>2</sup>, Eric Holowka<sup>2</sup> and Andrew Nowak<sup>2</sup>; <sup>1</sup>Materials Science and Engineering, University of Delaware, Newark, Delaware; <sup>2</sup>Bioengineering, University of California, Los Angeles, Los Angeles, California.

Diblock copolypeptides consisting of a hydrophilic lysine (K) block and a hydrophobic leucine (L) block were designed to self-assemble due to their amphiphilic nature and the defined secondary structure of the hydrophobic block. In aqueous solution, these copolypeptides assemble into stiff, porous hydrogels at low volume fractions of polymer (vol. fraction polypeptide  $\geq 0.5$  wt%). The micro and nanoscale morphology of the hydrogels has been well characterized using laser scanning confocal microscopy (LSCM), cryogenic transmission electron microscopy (cryo-TEM), and ultrasmall and small angle neutron scattering (USANS and SANS). The microscopy and scattering data revealed the formation of membranes on the nanoscale that interconnect to create an innately porous network on the nano- and microscale. Altering the molecular design, such as hydrophobic to hydrophilic block ratios and overall polypeptide chain length, effected the overall hydrogel rigidity, determined rheologically, with very weak hydrogels being formed from chains with  $< 10$  mol% hydrophobic block and with degrees of polymerization above 300 and below 120. Decreasing the polypeptide chain length to below a degree of polymerization  $\sim 100$  resulted in vesicle formation on the microscale without disrupting the nanoscale membrane formation. Alterations to the assembly pathway resulted in the formation of twisted fibrils or hexagonal single crystals. In all assemblies, regardless of the resulting structure, the secondary structure of the hydrophobic block remains an  $\alpha$  helix, as shown with circular dichroism (CD). These results indicate that the nanoscale assembly of these polypeptides into membranes is intrinsic to this class of molecules whereas any hierarchical, microscale assembly can be controlled through the assembly environment and molecular design.

#### 10:45 AM K4.8

**Biomimetic Nanotechnology: Exquisite Control Over Self-Assembly of Designed Peptide Multilayer Nanofilms.** Bingyun Li, Yang Zhong and Donald T. Haynie; Louisiana Tech

University, Ruston, Louisiana.

This work describes the development of a novel methodology for the fabrication and stabilization of multilayer peptide nanofilms. The specific approach involves the exceptionally versatile technique of nanomanufacturing known as electrostatic self-assembly (ESA) of oppositely-charged polyelectrolytes. The polyelectrolytes of greatest interest here are designed peptide chains. The amino acid cysteine is introduced, as it permits reversible disulfide bond formation (chemical cross-linking) between peptide chains. The role of solution pH, ionic strength, and adsorption cycle in ESA of designed 32mer peptides has been investigated in detail using a combination of physical techniques. Moreover, we have studied the role and importance of disulfide bond formations in stabilizing ESA multilayer nanofilms. Our results show that the ESA process can be exquisitely controlled, and substantially increased stability of ESA films is achieved by disulfide cross-linking. Unlike other cross-linking methods (e.g. glutaraldehyde treatment), disulfide bond formation is "peptide-inherent" and is reversible. The results form a basis for the production of biological multilayer thin films with desired properties and superior stability. This is expected to lead to broader applications of ESA nano-assembly in biotechnology and biomedicine.

#### 11:00 AM K4.9

**Bio-inspired Design of Modular Multi-domain Polymers for Advanced Biomaterials.** Zhibin Guan, Jason T. Roland, Dora Guzman and Jane Z. Bai; Chemistry, University of California, Irvine, California.

Native load-bearing proteins, such as the muscle protein titin, exhibit a remarkable degree of combined toughness, strength, and elasticity which have yet to be matched by synthetic materials. Single molecule nanomechanical studies on titin and other modular proteins suggest that these exceptional properties arise from a modular elongation mechanism. The sequential unfolding allows modular biopolymers to sustain a large force over the whole extension of the chain, which makes the polymer strong, along with a large area under the force-extension curve, making it tough as well. In addition, when the external force is removed, the unfolded domains of modular proteins will refold automatically, making them elastic. Inspired by nature, one research effort in my group is aimed at designing synthetic macromolecules that form high order structures by programming non-covalent interactions into polymer chain. The goal is to achieve synthetic biomaterials with combined strength, toughness and elasticity. Three classes of well-defined modular polymers have been synthesized in our laboratory: (1) using quadruple hydrogen-bonding motif 2-ureidon-4-pyrimidone (Upy) to direct the formation loops along a polymer chain (J. Am. Chem. Soc. 2004, 126, 2058); (2) using a peptidomimetic beta-sheet based double-closed loop (DCL) as module (J. Am. Chem. Soc. ASAP); and (3) an engineered protein G domain III as module. Single molecule force-extension experiments revealed the sequential unfolding of the loops or domains as these modular polymers are stretched, resulting in sawtooth-patterned curves similar to those seen in titin and other biopolymers. In this talk, we will discuss our designs, syntheses and single-molecule studies of polymers having modular domain structures.

#### 11:15 AM K4.10

**Synthesis of Transient Amorphous Calcium Carbonate, and Its Transformation to Oriented Calcite Crystals.** Yong-Jin Han<sup>2</sup> and Joanna Aizenberg<sup>1</sup>; <sup>1</sup>Materials Research Department, Bell Laboratories, Murrant Technologies, Murray Hill, New Jersey; <sup>2</sup>Lawrence Livermore National Laboratory, Livermore, California.

The ability of biological systems to exert precise control over the shape, size, orientation and hierarchical ordering of inorganic materials is of great interest to chemists and materials scientists, who are beginning to recognize its potential in the development of new synthetic pathways and in the improvement of existing materials. Amorphous calcium carbonate (ACC), one of many polymorphs of calcium carbonate but highly unstable under normal conditions, is often observed in biology, with somewhat enigmatic function, ranging from the structural support to the storage of calcium and carbonate ions for future precipitation in a more stable crystalline form. In this presentation, we report our experimental results on materials synthesis using the latter biological strategy. We form transient ACC film on a specially designed self-assembled monolayer (SAM) and use it as ion storage. The recrystallization of ACC into oriented calcite crystals is then induced by introducing the nucleation site into the system without additional calcium or carbonate ions. The mechanism of phase stabilization and recrystallization as well as its implication in biomineralization will be discussed.

#### 11:30 AM K4.11

**The Characterization of a Novel DNA Immobilization on Diamond by Carboxylic Aromatic Compounds.** Junghoon Yang<sup>1,2</sup>, Kwang-Soup Song<sup>1,2</sup>, Guo-Jun Zhang<sup>2</sup>, Hitoshi

Umezawa<sup>1,2</sup>, Iwao Ohdomari<sup>1,2</sup> and Hiroshi Kawarada<sup>1,2</sup>; <sup>1</sup>Waseda University, Tokyo, Japan; <sup>2</sup>Nanotechnology Research Center (NTRC), Tokyo, Japan.

Since diamond has excellent electrical and chemical properties such as wide potential window, chemical-physical stability, biocompatibility and so on, diamond is expected as a suitable material for electrochemical and biological applications. In case, H-terminated diamond surface are directly aminated for controlling the density of probe DNA. Then, partially aminated diamond surface was modified by carboxylic aromatic compound(CAC), terephthalic and trimesic acid., as linker. In addition, amino-terminated DNA oligonucleotides were immobilized on the micro-structurally patterned diamond surfaces. The novel immobilization method by the surface functionalization by CAC is more effective on diamond surface than on other materials. First, the space between the binding sites of probe DNA has to be considered for hybridization efficiency because the excess density of probe DNA decreases the probability of hybridization. The partially aminated diamond surface is not necessary to space due to control density of amino function, directly. In addition, amide bindings between aminated-diamond surface and CAC or probe DNA and CAC are more stable than van der Waals binding because carboxylic group have been included unshared electron more than aldehyde group. Therefore, direct immobilization method can overcome this disadvantage, weak interaction between probe DNA and other functional groups. For the surface-functionalized by DNA, aminated diamond surface was formed in ammonia gas on H-terminated diamond surface by UV. Then, all of aminated-diamond surface except for the masked micropatterns by gold has been fluorinated in order to improve the signal-to-background ratio. The immobilization specificity was evaluated by means of 5 amino-modified oligonucleotides labeled with Cy-5 at its 3' end attached onto microstructured patterns treated different CAC with NHS/EDC for activation of carboxyl group. We confirm that probe DNA oligonucleotides were immobilized on diamond substrate and hybridized with target DNA. Also, Fluorescence intensity increased when target DNA was hybridized on carboxylated diamond surface with both trimesic acid and terephthalated diamond surface because the different density of carboxylic function. The fluorescence intensity increased by a factor of 1.7, which reflected the fact that the carboxylated binding-site of terephthalic acid is twice as high as that of trimesic acid. The 0.3 difference is due to steric effect between immobilized probe DNA.

#### 11:45 AM **K4.12**

##### **Metallic Pd and Pt Nanoparticles on S-layer Proteins Studied by Small Angle X-ray and Neutron Scattering.**

Barbara Aichmayer<sup>1,2,3</sup>, Michael Mertig<sup>4</sup>, Alexander Kirchner<sup>4</sup>, Oskar Paris<sup>3</sup>, Ingomar Jaeger<sup>1,2</sup> and Peter Fratzl<sup>3</sup>; <sup>1</sup>Department of Material Physics, University of Leoben, Leoben, Austria; <sup>2</sup>Erich Schmid Institute of Materials Science, Austrian Academy of Sciences, Leoben, Austria; <sup>3</sup>Department of Biomaterials, Max Planck Institute of Colloids and Interfaces, Potsdam, Germany; <sup>4</sup>Max-Bergmann-Center of Biomaterials, University of Technology, Dresden, Germany.

Pd and Pt nanoparticles were produced using isolated S-layer proteins (crystalline bacterial surface layers which form regular two-dimensional arrays) as templates. After adsorption of the precursor material (dissolved K<sub>2</sub>PdCl<sub>4</sub> or K<sub>2</sub>PtCl<sub>4</sub>), either a chemical reducing agent or exposure to synchrotron radiation led to the formation of monodisperse metallic nanoparticles with radii from 1.5 to 8 nm depending on the reaction conditions. In order to study the metallization process and the interplay between the metal loading and the protein template, we characterized the S-layers as well as the size and arrangement of the metal particles by means of small angle x-ray and neutron scattering. In-situ investigations of the synchrotron radiation induced formation of Pd particles on the S-layers of *Sporosarcina ureae* showed that the protein templates were indeed capable of stabilizing particles with radii of 1.2-1.5 nm. We did not observe any coarsening during our investigations. The small angle scattering studies were complemented by UV/Vis spectroscopy and transmission electron microscopy investigations which showed that the metal particles were preferentially formed in the pores of the periodic protein structure. From our results we conclude that S-layer proteins are suitable templates to produce stable arrays of metallic Pd and Pt nanoparticles. We acknowledge the financial support of the EU project BIO-CAT (European Union grant number GRD1-2001-40424).

SESSION K5: Bio-inspired Material Synthesis  
Chairs: Trevor Douglas and Darrin Pochan  
Wednesday Afternoon, March 30, 2005  
Room 3002 (Moscone West)

#### 1:30 PM **\*K5.1**

##### **The Bio-Nano-Process: Making Semiconductor Devices using Protein Supramolecules.** Ichiro Yamashita, <sup>1</sup>ATRL, Matsushita Electric Industry Co., Ltd., Kyoto, Japan; <sup>2</sup>CREST, Japan Science and Technology Agency, Kyoto, Japan; <sup>3</sup>NAIST, Nara, Japan.

Biology and semiconductor technology have progressed independently. The distance between them has been large and a substantial interdisciplinary research area has been left untouched. Now, bio- and semiconductor technology have come and met in the nanometer size region. In such context, the fusion of biology and semiconductor technology is about to start and a new interdisciplinary research area is emerging. There are two manners to fuse bio- and semiconductor technology. Biology uses the semiconductor technology and vice versa. The former type of fusion was already widely realized. On the other hand, the later has been rare and there has been no interdisciplinary research to fabricate nanostructures for semiconductor devices using bio-molecules. We propose a process to fabricate inorganic structure using protein supramolecules which is named BIO NANO -PROCESS(the BNP), and we demonstrated the fabrication of the semiconductor device key component by the BNP(1,2) Some proteins have the ability to sequester inorganic materials in their cavities, which is called biomineralization(3,4). We made 7nm semiconductor nanoparticles (NPs) using apoferritins. Proteins have another ability to self-assemble into functional nanostructure. We made 2D crystal of the ferritin with semiconductor core. Moreover, the protein shell of ferritin with semiconductor core can be selectively eliminated by heat-treatment or UV-ozone cleaning to leave only semiconductor NPs. Combining these three abilities of biomineralization, self-assembly and selective elimination, the monolayer of 7nm semiconductor NPs are made on the silicon wafer(1,2), which can be directly used as the key component of the floating nanodot-gate memory. It is also possible to make 2D iron oxide NPs layer on the silicon wafer. These NPs were used as a mask to ultimate nanosize etching by neutral beam and the silicon columns with 7nm in diameter and 50nm in height were realized.(5) The development of nanoelectronics devices using these nanostructures are in progress. I believe that the BNP will provide a breakthrough to the production of nanometer-size semiconductor devices and nanoelectronics devices for biological applications. This work is partially supported by MEXT Leading Project. References (1) Yamashita, I., Thin Solid Films. 393 12-18 (2001) (2) Hikono, T., Uraoka, Y., Fuyuki, T., Yamashita, I., Jpn.J. Appl. Phys. 42 L398-L399. (2003) (3) Okuda, M., Iwahori, K. Yamashita, I., Yoshimura H., , Biotech & Bioeng. 74(2) ,(2003) (4) Yamashita, I., Hayashi J., and Hara, M., Chem. Lett. 33, No. 9, 1158-1159, (2004) (5) Kubota, T., Baba, T., Samukawa, S., Kawashima, H., Uraoka, Y., Fuyuki, T., and Yamashita, I., Appl. Phys. Lett., 84, No. 9, 1555-1557 (2004)

#### 2:00 PM **K5.2**

##### **Positioning of DNA Nanowires Decorated with Luminescent Conjugated Polyelectrolyte.** Per Bjork, Anna Herland, Peter Asberg, Peter Nilsson and Olle Inganäs; Applied Physics, IFM, Linköping, Sweden.

The ability to control the preparation of ordered nanometer scale structures at certain predefined positions is a major challenge in the field of nanotechnology. In this regard, biological systems offer inspiration for device formation with macromolecular assemblers that possesses suitable geometries and functions at the nanoscale. DNA is one of the most commonly used biomolecular building block for high aspect ratio nanowires. Utilizing different molecular combing techniques, aligned 1D and 2D arrays of stretched DNA nanowires can easily be prepared on surfaces by deposition from liquids. However, controlling the position of these arrays is of vital importance. We have used surface energy patterning, by a soft lithography method, to direct the stretching of  $\lambda$ -DNA to predefined patterns. Here, we apply the bare PDMS stamp to a hydrophilic substrate. When the stamp is in close contact with the substrate diffusing low molecular weight species in the PDMS will be transferred, and a hydrophobic pattern is formed on the substrate. These domains can function as anchoring sites for DNA. By altering the pattern of the stamp,  $\lambda$ -DNA deposited from solution is stretched, located and aligned in confined areas, and between two regions of the surface. Apart from controlling the position, DNA needs to be functionalized with electronic materials in order to assemble functional electronic devices, as intrinsic conduction in DNA does not give the basis for electronics. The complexation of luminescent conjugated polyelectrolytes (CPE) with biological polyelectrolytes offers an approach for building materials and devices where the assembly of electronic polymers is controlled by the interactions with macromolecular assemblers, in this case  $\lambda$ -DNA. A complex of the conjugated polyelectrolyte, with a polythiophene backbone, and the  $\lambda$ -DNA is formed in solution and then stretched into aligned photoluminescent arrays by molecular combing techniques on energy patterned surfaces. Interaction studies of polyelectrolyte layers/solutions and DNA has been done using SPR and optical methods [1, 2]. Furthermore, targeting positions on the

DNA using the nucleotide sequence and hybridization as address tags and as recognition events may be possible for more advanced assembling. References [1] P. Björk et al., Dynamics of complex formation between biological and luminescent conjugated polyelectrolytes - a surface plasmon resonance study, Biosensor and Bioelectronics, In Press. [2] K. P. R. Nilsson and Olle Inganäs, Chip and solution detection of DNA hybridization using a luminescent zwitterionic polythiophene derivative, Nature Materials 2, 419-424.

### 2:15 PM K5.3

**Bio-Inspired Evolution of Zinc Oxide-based Materials Directed by Amino Acids and Peptides.** Joachim Bill, Peter Gerstel, Rudolf Hoffmann and Fritz Aldinger; Max-Planck-Institut fuer Metallforschung/INAM, University of Stuttgart, Stuttgart, Germany.

Zinc oxide represents a promising material for functional applications, e. g. as a phosphor or a transparent and conductive oxide. Due to these applications, miniaturization as well as nanostructuring of zinc oxide-based materials and devices is a challenging research item. For that purpose, the deposition from aqueous media basically provides an effective means. However, in the case of zinc oxide the deposition behavior is strongly controlled by the tendency to form elongated micron-sized crystals that make the formation of smooth homogeneous nanostructures impossible. Recently, the preparation of zinc oxide-based nanostructured films was reported [1]. This method involves macromolecular organic additives like graft copolymers or homopolymers, which are added to the aqueous deposition medium. Owing to the interaction of these polymers with zinc oxide in solution the growth of micron-sized crystals is suppressed and organic/inorganic hybrid nanoparticles are formed. The subsequent assembly of these particles can be controlled by organically modified surfaces and yields nanostructured films with luminescent properties. Living nature also applies a variety of organic molecules that interact with metal ions. One example is the zinc finger, which is made of amino acid units. These units built up a configuration of a DNA-binding protein that resembles a finger and contains usually histidine and cysteine, binding a zinc ion. Within this paper the suitability of amino acids and oligopeptides as structure-directing agents is discussed. According to that bioinspired approach these biomolecules were investigated in a combinatorial way with respect to the evolution of zinc oxide-based architectures. Whereas the before mentioned macromolecular organic additives mainly support film formation these small molecules are able to trigger the morphology in general, dependent on the kind and sequence of the amino acid units. It will be shown that this approach opens up pathways for nanopatterning as well as for the deposition of a variety of morphologies ranging from grain-like via two up to three dimensional features. Besides morphological aspects the structural characterization of these solids by means of X-ray diffraction, electron and atomic force microscopy as well as photoelectron and infrared spectroscopy will be discussed in order to extract the function of the biomolecules with regard to the formation of the inorganic phases. In addition, functional properties, like the wetting and optical behavior will be treated. [1] see e. g. R. C. Hoffmann, S. Jia, J. Bill, M. R. De Guire, F. Aldinger, "Influences of Additives on the Formation of ZnO Thin Films by Forced Hydrolysis", J. Ceram. Soc. Jpn, Supplement 112 (200)

### 2:30 PM K5.4

**Biomimetic Synthesis of Metal Oxides using Protein Cages as Reaction Vessels.** Mark Allen<sup>1,4</sup>, Debbie Willits<sup>2,4</sup>, Keith Gilmore<sup>3,4</sup>, Mark Young<sup>2,4</sup> and Trevor Douglas<sup>1,4</sup>; <sup>1</sup>Chemistry and Biochemistry, Montana State University, Bozeman, Montana; <sup>2</sup>Plant Sciences, Montana State University, Bozeman, Montana; <sup>3</sup>Physics, Montana State University, Bozeman, Montana; <sup>4</sup>Center for BioInspired Nanomaterials, Montana State University, Bozeman, Montana.

Supramolecular proteins that assemble into cage like architectures have been used for nanomaterials synthesis. Specifically ferritin and ferritin like proteins can be used as size constrained reaction vessels that encapsulate materials that have sizes that are determined by the internal dimensions of the protein cage. These range from 5 nm for the ferritin like protein from *Listeria innocua* to 24 nm for the interior of an engineered plant virus (Cowpea chlorotic mottle virus). Inorganic materials synthesized within these constrained reaction volumes are monodisperse in size. The crystallinity and phase of material prepared is determined by the reaction conditions, which are mild compared to other preparative methods. This presentation will focus on the synthesis and characterization of inorganic materials prepared inside a variety of protein cages that range in exterior diameters from 9 to 30 nm and interior diameters of 5 to 25 nm. Particularly, the size dependent magnetic behavior of these nanoparticles will be discussed. When 5 nm ferrimagnetic spinel ferrites (Fe<sub>3</sub>O<sub>4</sub>) are prepared inside the ferritin like protein from *Listeria innocua*, the resulting behavior is superparamagnetic at room

temperature. However, when the same material is prepared inside Cowpea chlorotic mottle virus, the resulting magnetic behavior is that of a room temperature ferromagnet as determined by AC magnetic susceptibility measurements and vibrating sample magnetometry. This illustrates the utility of using protein cage architectures for materials synthesis where size dependent magnetic properties can be tuned by choice of the protein cage.

### 2:45 PM K5.5

**Biotope-Based Nanostructuring and Metallization.**

Mato Knez<sup>1</sup>, Sinan Balci<sup>2</sup>, Anan Kadri<sup>3</sup>, Fabian Boes<sup>3</sup>, Alexander M. Bittner<sup>2</sup>, Christina Wege<sup>3</sup>, Holger Jeske<sup>3</sup> and Klaus Kern<sup>2</sup>; <sup>1</sup>Exp. II, Max-Planck-Institut MSP, Halle, Sachsen-Anhalt, Germany; <sup>2</sup>Nanoscale science, Max-Planck-Institut FKF, Stuttgart, Germany; <sup>3</sup>Molekularbiologie und Virologie der Pflanzen, University of Stuttgart, Stuttgart, Germany.

Ordered structures in the nanometer scale become more and more important in research and for future applications. One of the most promising approaches is the use of large supramolecular assemblies with intrinsic order, e.g. biomolecules. We present a biochemical approach to surface structuring and metallization on the nanometer scale. The templates we use are Tobacco mosaic virus (TMV) and Potato virus X (PVX), nanotubular plant viruses that consist of self-assembled RNA strands and proteins. The immobilization of the viruses is attained on well-defined inorganic surfaces. We work with bare and chemically modified substrates or with self-assembled monolayers. In this way we achieve a fine tuning of the chemical properties of the surface in order to address the chemical groups on the viral surface. For the approach to ordered structures of TMV on surfaces we use the method of micro contact printing, while imaging is carried out with scanning probe microscopy, especially non-contact AFM. For the metallization we employ the technique of electroless deposition of metals. By making use of the metal cation-binding properties of certain amino acid moieties we are able to deposit small clusters of gold, nickel, cobalt and copper either on the outer surface or inside the nanoscale virus channel. With this method nanowires of nickel and cobalt with 3 nm diameter and up to 600 nm lengths can be achieved. The metallization is investigated with a transmission electron microscope.

### 3:30 PM \*K5.6

**Molecular Chaperons for Stimuli-Responsive Nanomachines.**

Takuzo Aida and Kazushi Kinbara; Department of Chemistry and Biotechnology, The University of Tokyo, Tokyo, Japan.

Chaperonin proteins GroEL and T.th cpn assist folding of newly formed or denatured proteins by the action of ATP. These chaperonins have a nanoscopic cylindrical cavity, where denatured proteins are captured. The included proteins, after folding, are released by the action of ATP as the result of an induced conformational change of the cavity [Roseman, A.M.; Chen, S.; White, H.; Braig, K.; Saibil, H.R. Cell 1996, 87, 241]. We succeeded in the fabrication of the first ATP-responsive nano machine by integration of the unique biological mechanism involving chaperonin proteins into the chemistry of semiconductor nanoparticles [Ishii, D.; Kinbara, K.; Ishida Y.; Ishii, N.; Okochi, M.; Yohda, M.; Aida, T. Nature 2003, 423, 628]. CdS nanoparticles (2-4 nm) were prepared according to a method reported by Murakoshi and coworkers. For the complexation with chaperonins, a DMF solution of CdS nanoparticles was added to a Tris/HCl buffer solution of GroEL or T.th cpn. Complexes of T.th cpn and GroEL with CdS nanoparticles were isolated by size-exclusion chromatography (SEC). For T.th cpn complex, an analytical SEC trace of this solution with an UV/fluorescence dual detector showed single, sharp elution peaks, which were superimposable with one another at nearly the same elution volume as intact T.th cpn. Since intact T.th cpn is hardly fluorescent, the above results strongly indicate that CdS nanoparticle is colocalized with T.th cpn to form an inclusion complex [T.th cpn/CdS nanoparticle]. A TEM picture of [T.th cpn/CdS nanoparticle] showed that the central part of the cavity of T.th cpn is considerably dark, due to the presence of CdS nanoparticle within the protein cavity. [T.th cpn/CdS nanoparticle] is thermally stable and maintains its characteristic photoluminescence activity up to 80 C, while [GroEL/CdS nanoparticle] is stable only up to 60 C. When a Tris/HCl buffer solution of ATP containing magnesium chloride was added to a buffer solution of [T.th cpn/CdS nanoparticle] containing KCl, the mixture turned slightly cloudy within seconds to give colloidal substances, where the supernatant solution after centrifugation was no longer fluorescent. The release of CdS nanoparticles from [T.th cpn/CdS nanoparticle] by the action of ATP was clearly demonstrated by analytical SEC with an UV/fluorescence dual detector. After the addition of ATP, the UV response of the SEC trace of [T.th cpn/CdS nanoparticle] showed a sharp elution peak assignable to T.th cpn and an additional broad peak in the lower molecular weight region due to ATP and its hydrolyzed products, while no fluorescence responses were observed for these two peaks. The fraction corresponding to T.th cpn, isolated

by SEC from the reaction mixture, lost the majority (nearly 90%) of cadmium ion, as determined by ICP-MS. [GroEL/CdS nanoparticle] also showed a similar response to ATP.

#### 4:00 PM **K5.7**

**Structural Investigation of Bio-Directed Hierarchical Assembly of Multifunctional Materials from Proteins and Diblock Copolymers.** Linda Katherine Molnar<sup>1,2</sup>, Dongseok Shin<sup>3</sup>, Rebecca Breitenkamp<sup>3</sup>, Todd Emrick<sup>3</sup> and Thomas P. Russell<sup>3</sup>; <sup>1</sup>NASA Ames Center for Nanotechnology, NASA Ames Research Center, Moffet Field, California; <sup>2</sup>Eloret Corporation, Sunnyvale, California; <sup>3</sup>Polymer Science and Engineering, University of Massachusetts, Amherst, Massachusetts.

Our goal is to combine polymers and proteins to form a hierarchically structured multifunctional material that has both the highly ordered structure of the polymer and the order and biological function of the protein. By chemically linking or embedding self-assembling biological elements in structure-forming block copolymers, the self-organization of the biomolecules can affect the evolution of the block copolymers, and similarly, the structural evolution of the copolymers can affect self-assembly of the proteins. The materials utilized were an asymmetric diblock copolymer of polystyrene (PS) and polyethyleneoxide (PEO) denoted P(S-b-EO) and horse spleen ferritin (HSF). Solvent casting has been shown to be a viable and rapid route by which arrays of nanoscopic PEO domains oriented normal to the surface can be produced in a glassy PS matrix in films with thickness several times the period of the copolymer. HSF in modified (genetic or chemical) or unmodified forms has shown varying effects on the self-assembly and microphase separation of the P(S-b-EO) block copolymer. Ultimately, we aim to predict and control these outcomes for the fabrication of hybrid inorganic-organic materials. Ferritins are iron storage protein cages belonging to Class II diiron-carboxylate proteins composed of 24 subunits arranged in octahedral symmetry, which self-assemble to form a 12 nm diameter cage with a 7.5-8.0 nm diameter cavity. About 4000 iron atoms can be stored in the central core of ferritins as iron (III) oxyhydroxide, mainly ferrihydrite ( $5\text{Fe}_2\text{O}_3 \cdot 9\text{H}_2\text{O}$ ). Most ferritins are very stable particles, and can withstand 65 °C and tolerate a pH range between 4 and 9. Many studies have demonstrated that ferritins can be used as nanoreactors for the formation of inorganic nanocrystals. Combined with their ability to self-assemble into well-defined 2-D structures on a solid surface or at a liquid-air interface as well as the possibility of detecting direct electron transfer from ferritin to gold electrodes makes them attractive biomolecules for both structural studies and applications. The production of these new multifunctional materials with the order of polymers and the specificity of proteins are expected to form the functional component of devices where both organization and specific biological function are required, e.g., sensors, adaptable materials, biocompatible devices.

#### 4:15 PM **K5.8**

**Magnetite-PLGA Microparticles As Potential Oral Delivery Vehicles of Therapeutic Proteins.** Jianjun Cheng<sup>1</sup>, Dennis Ho<sup>1</sup>, Chris Yim<sup>1</sup>, Omid C. Farokhzad<sup>2,3</sup> and Robert S. Langer<sup>1</sup>; <sup>1</sup>Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts; <sup>2</sup>Department of Anesthesiology, Brigham and Women's Hospital, Harvard Medical School, Cambridge, Massachusetts; <sup>3</sup>Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, Massachusetts.

Oral delivery of peptides and protein therapeutics has been extensively studied in the past several decades. This route of administration is preferred because it increases patient compliance and comfort over parenteral route, which accounts for the administration of more than ninety percent of FDA approved protein drugs. Clinically effective oral delivery systems for protein therapeutics have not been established. Proteins administered orally result in extremely poor absorption into circulatory system due to the degradation of proteins in harsh acidic and enzymatic conditions in stomach and low permeation of proteins across the intestinal membranes. Polymeric nano- and micro-particles are found to effectively protect encapsulated proteins from acidic or enzymatic degradation in gastrointestinal tract, but majority of the particular protein delivery vehicles pass through small intestines without being absorbed. Retention of these oral delivery vehicles in the small intestine for an extended period of time may result in an increase of the delivery efficiency. Magnetically modulated particulate systems have been used in in-vivo imaging and targeted drug delivery, and can successfully localize imaging ligands or drugs in disease sites. In this study, we investigated magnetic-responsive particulate carriers for oral protein delivery. Magnetite (Fe<sub>3</sub>O<sub>4</sub>) nanoparticles was co-encapsulated with insulin into poly(lactide-co-glycolide) (PLGA) microparticles (1-10 nm size, visualized by SEM) through the double-emulsion method. After the magnetite-insulin-PLGA microparticles were orally administered to mice, a magnetic field was created externally to the

mouse abdomen around the mouse intestine area to retain or slow down the transit of these magnetite-Insulin-PLGA microparticles. A single administration of 50 unit/kg of these microparticles to fasted mice resulted in a gradual decrease of whole blood glucose concentration from 130 mg/dL at t = 0 to 50 mg/dL at t = 12 hours in the presence of external magnetic field. As a comparison, the whole blood glucose concentration of mice administered in the same way in the absence of an external magnet reached the lowest level of 70 mg/dL at t = 8 hours and recovered to above 80 mg/dL after t = 10 hours. Other data that show effective oral insulin delivery using this magnetic responsive particulate system will also be presented.

#### 4:30 PM **K5.9**

**Supramolecular Design: Synthesis of Complex Nanostructures and their Application as Drug- and DNA-delivery Systems.** Itzia Cruz-Campa, Juan C. Noveron, Lynn Santiago, Renato Aguilera and Armando Varela-Ramirez; University of Texas at El Paso, El Paso, Texas.

Living matter is organized with atomic precision at the nanometer scale via non-covalent interactions through self-assembly processes. Using these basic principles we designed molecules that combine the metal-ligand coordination and hydrogen bonding interactions with the properties that amphiphiles exhibit in water to create intricate nanostructures with dimensions between 40-500 nm. The analysis of these structures was carried out using single-crystal X-ray diffraction, atomic force microscopy and transmission electron microscopy. Spherical water-soluble nanostructures exhibit drug-delivery properties and were able to render inactive insoluble substances into pharmacologically potent drugs with potential anti-cancer properties. Similarly, they were able to encapsulate large DNA plasmids and transport them in serum to the nuclei of human cells. The transfection of a piece of DNA encoding for a fluorescent protein will be presented as a proof of concept demonstration.

#### 4:45 PM **K5.10**

**A New Generation of Multifunctional Nanoparticles Possessing Magnetic Motor Effect for Drug or Gene Delivery.** Tae-Jong Yoon<sup>1</sup>, Jun Sung Kim<sup>2</sup>, Byung Geol Kim<sup>1</sup>, Kyeong Nam Yu<sup>2</sup>, Myung-Haeng Cho<sup>2</sup> and Jin-Kyu Lee<sup>1</sup>; <sup>1</sup>School of Chemistry, Seoul National University, Seoul, South Korea; <sup>2</sup>College of Veterinary Medicine and School of Agricultural Biotechnology, Seoul National University, Seoul, South Korea.

Cobalt ferrite-silica (core-shell) magnetic nanoparticles labeled with organic dye were prepared by a modified polyvinylpyrrolidone method and sol-gel process. The thickness of the silica shell was controlled by varying the ratio between the magnetic nanoparticle and tetraethoxysilane plus organic dye-labeled silanes, rhodamine B isothiocyanate (RITC) or fluorescein isothiocyanate (FITC). The nanoparticle surface was also modified with biocompatible poly(ethylene glycol). These organic dye-labeled nanoparticles exhibit a unique combination of magnetic and optical properties and are innocuous to cells due to the silica coating. Incorporation of the fluorescent dyes into the nanoparticles enabled us to monitor the movement of cells doped with magnetic nanoparticles under an external magnetic field by optical microscopy, suggesting that our multifunctional magnetic nanoparticle could be easily realized on the in vivo target-of-interest and could be ultimately applied to non-invasive delivery systems such as those used in in vivo gene or drug delivery.

#### SESSION K6: Biophotonics and Biosensors

Chairs: Andrew Parker and Shu Yang

Thursday Morning, March 31, 2005

Room 3002 (Moscone West)

#### 8:00 AM **K6.1**

**Surface Plasmon Resonance Biosensor based on Phase Measurement.** Kotaro Kajikawa<sup>1,2</sup> and Ryo Naraoka<sup>1</sup>; <sup>1</sup>Information Processing, Tokyo Institute of Technology, Yokohama, Japan; <sup>2</sup>PRESTO, JST Japan Science and Technology Agency, Saitama, Japan.

Surface plasmon is a surface electromagnetic wave, which is originated from the association of charge-density oscillation of free electrons at a metal surface and a light wave. Since the surface plasmon resonance (SPR) provides a highly sensitive and label-free bio-sensing method, it is widely used in these days in the fields of biochemistry and genetic engineering. Recently the phase measurement of reflected field in the attenuated total reflection (ATR) upon the SPR condition is paid attention because the phase measurement allows us considerably high sensitivity. The shift of the phase is affected by the existence of the dielectric layer on the metal surface, so that we can detect the affinity of the biological molecules by monitoring the phase shift of the

reflected light. There are a few experimental reports on the phase detection. In most of the studies, the phase detection is based on the heterodyne detection because it provides real-time measurement that is important for biological affinity kinetic measurements. The rotating analyzer method is another way for phase detection. Although it does not provide the rapid detection, the advantages of the rotating analyzer method compared with the heterodyne technique, are (1) the optical geometry is simple (2) it can be applied to spectroscopic measurements (3) it is applicable not only to sensor applications such as affinity biosensors but also highly sensitive spectroscopy. However there are only few reports that adopted the rotating analyzer method for phase detection and little is known about the details in the analysis. In this paper we demonstrate the highly sensitive sensing of molecular adsorption on metal surface using the rotating analyzer method under the SPR condition. We found that the method yields the RI resolution of the ambient medium  $10^{-7}$ , which is almost compatible with that of the heterodyne method and is two orders of magnitude better than that of the conventional SPR method based on the angular detection. Real-time measurement using this method is also demonstrated.

**8:15 AM K6.2**  
**Single Aperture Functionalization for Bio-Organism Sensing Applications.** Joakim Nilsson and Sonia E. Letant; Lawrence Livermore National Laboratory, Livermore, California.

Chemically functionalized apertures with large aspect ratios constitute a platform of choice for bio-organism sensing. We fabricated apertures on pre-patterned silicon platforms with aspect ratios  $>10:1$  using focused ion beam (FIB) drilling, followed by dry/wet oxidation. Localized DNA functionalization of the nanopores was then achieved by self-assembled monolayer chemistry combined with a silicon nitride mask. Ionic flow measurements recorded through single nanopores at each stage of the device preparation allowed to demonstrate the open nature of the channels and to follow the aperture functionalization process step by step. The chemical selectivity of the DNA-functionalized devices will be investigated by measuring the duration and amplitude of the ion current blockade generated by single DNA-functionalized beads electrophoretically driven through the apertures. Future applications include the selective detection of bacteria and viruses. This work was performed under the auspices of the U.S. Department of Energy by University of California Lawrence Livermore National Laboratory under contract No. W-7405-ENG-48. UCRL-ABS-207152.

**8:30 AM \*K6.3**  
**Box Jellyfish: Visually Guided Animals with Four Parallel Brains.** Dan-Eric Nilsson, Cell and Organism Biology, Lund University, Lund, Sweden.

Box jellyfish are simple animals with an unusually impressive set of eyes. Each individual has four sensory clubs carrying two different imaging eyes and two different pairs of non-imaging eyes. This makes for a total number of 24 eyes of four different types. The information from the eyes is processed in one nervous centre in each sensory club, and the animal does not possess any other central processing units. The consequence is that the behaviour of the jellyfish is controlled by four parallel brains. This unusual design is sufficient for a remarkably complex visual behaviour. The animals display courtship behaviour and are very skilled at using visual cues to position themselves very close to the shore where food is abundant. The visual behaviours indicate that the animals can detect and adequately respond to a number of different visual cues. Electrophysiological measurements suggest the possibility of colour vision, and optical investigations have shown that the imaging eyes deliver information which is unlike that of all other eyes in the animal kingdom. In other eyes, the photoreceptors (raw pixels) generally display very narrow Gaussian receptive fields, which is the basis for high-resolution visual tasks. In box jellyfish, the receptive fields of individual photoreceptor cells are very wide and complex, somewhat resembling receptive fields of neurons in higher visual centres of vertebrates. It appears that box jellyfish eyes are specialised for particular visual tasks where much of the processing can be done already by the optics of the eye. The result is likely to relieve the simple nervous system from some processing tasks, and at the earliest possible stage reduce the information to a small bandwidth.

**9:00 AM \*K6.4**  
**Controlling the Flow of Color: Photonic Systems in Lepidoptera.** Pete Vukusic, School of Physics, University of Exeter, Exeter, Devon, United Kingdom.

Studies of structural color in terrestrial systems, such as those associated with brightly coloured insects and birds, have seen significant advances in recent years. Complex partial photonic bandgap structures in the elytra of Coleoptera and the scales of Lepidoptera suggest broad innovation in nature's use of materials and

manipulation of incident light. The existence of an analogue of optically active cholesteric liquid crystalline structure has been shown responsible for the circularly polarised reflection from certain beetles exocuticle. In other insects, principally certain butterflies, ultra-long-range visibility of up to one half-mile is attributed to photonic structures that are formed by discrete multilayers of cuticle and air. This contrasts, with other butterfly species for instance, in which photonic structures are designed much more for crypsis and not only produce strong polarisation effects but can also produce colour stimulus synthesis using a doubly periodic multilayered photonic structure. Optical systems also exist that employ remarkable 3D photonic crystals of cuticle to produce partial photonic band gaps, with the effect that bright colour is reflected over a broad angle range. From the perspective of modern optical technology, this indicates an evolutionary step further along the photonic road, since in principle, 3D periodicity potentially manipulates the flow of light in all directions. In certain butterflies the common 3D structure, referred to as inverse-opal, comprises hollow voids surrounded by a honeycomb of cuticle. The physical structure of this inverse-opal photonic crystal, while varying somewhat between examined species, appears consistently as a minor variation of tetrahedral. Interestingly, band gap calculations indicate that a perfect tetrahedral configuration offers the highest reflectivity over the broadest angle range for a given refractive index contrast between component media. Given constraints associated with cuticular morphology and the ecological and intraspecific selection pressures thought to exist, it appears the physical design of this photonic structure has converged towards one of the most optically efficient configurations. Numerous studies, many of them very recent, have sought to discover and characterise the photonics associated with a diverse range of natural specimens. Many of them have revealed system designs that have evolved and existed naturally for millennia and that were, until their discovery in nature, thought to have been the recent product of technological innovation. Principally, this talk will detail the advances made in the characterisation of Lepidopteran photonic systems, believed by many to be among the most diverse in the natural world.

**9:30 AM K6.5**  
**Tunable Bio-inspired Microlens Arrays with Integrated Pore Structures.** Shu Yang<sup>1</sup>, Kuang-sheng Hong<sup>1</sup>, Jing Wang<sup>2</sup>, Haim H. Bau<sup>2</sup> and Joanna Aizenberg<sup>3</sup>; <sup>1</sup>Materials Science and Engineering, University of Pennsylvania, Philadelphia, New Jersey; <sup>2</sup>Mechanical Engineering and Applied Mechanics, University of Pennsylvania, Philadelphia, Pennsylvania; <sup>3</sup>Bell Laboratories, Lucent Technologies, Murray Hill, New Jersey.

We are interested in learning from natural optical systems, whose hierarchical architecture and hybrid character offer outstanding optical properties and enable multi-faceted roles. It was found that in light-sensitive brittlestars, uniform microlens arrays with integrated pores are formed, which enable diurnal migration of pigment-filled chromatophore cells through the pores. Such biolens exhibit transmission tunability, diaphragm action, numerical aperture tunability, wavelength selectivity, minimization of the "cross-talk" between the lenses, and improved angular selectivity. Inspired by the unique lens structure and functionality, we have created porous hexagonal microlens arrays that are analogous to the biological structures. Using the porous network as a microfluidic system, we mimic the pigment movement in the brittlestar stereom. We study the possibility of actuating different liquids (e.g. with selective refractive index and/or including dyes that can absorb certain wavelength) in and out of pores, to achieve a wide range of tunability of the lens optical properties.

**9:45 AM K6.6**  
**Conjugated Polyelectrolytes: Conformation Sensitive Optical Probes for the Recording of Biological Processes.** Peter Nilsson<sup>1</sup>, Anna Herland<sup>1</sup>, Johan Olsson<sup>3</sup>, Johan Rydberg<sup>2</sup>, Lars Baltzer<sup>2</sup>, Peter Konradsson<sup>3</sup>, Per Hammarstrom<sup>3</sup> and Olle Inganäs<sup>1</sup>; <sup>1</sup>IFM, Biomolecular and Organic Electronics, Linköping, Sweden; <sup>2</sup>IFM, Organic Chemistry, Linköping, Sweden; <sup>3</sup>IFM, Chemistry, Linköping, Sweden.

Conjugated polyelectrolytes with ionic side chains have been used for the detection of single nucleotide polymorphism (SNP) in DNA [1], conformational alterations of synthetic peptides [2, 3], conformational alterations of Calmodulin and binding of Ca<sup>2+</sup>-activated Calmodulin (CaM) to Calcineurin (a part of the intra-cellular signal pathway) [4], and amyloid fibril formation of amyloidogenic proteins. The conformational flexibility of polymers, also found in conjugated polyelectrolytes, allows direct connection between the geometry of chains and the resulting electronic structure and processes. If conformational changes of biomolecules could lead to different conformations of the polyelectrolyte backbone, an alteration of the absorption and emission properties from the polyelectrolyte would be observed. The detection method is based on non-covalent assembly of the conjugated polyelectrolyte and the receptor of interest. Upon

exposure to the analyte of interest or a conformational change of the receptor, a conformational alteration of the polymer backbone and a change in the electronic properties of the polymer occurs, and these alterations can be detected by absorption or fluorescence from the polymer. Introduction of the receptor molecules will induce aggregation of the polymer chains and planarization of the polymer backbone, detected as a decrease of the intensity and a red shift of the fluorescence. Upon addition of the desired analyte the intensity of the emitted light is increased and blue shifted. This phenomenon is due to a separation of the polymer chains and twisting of the polymer backbone, induced by the receptor-analyte interaction. Conjugated polyelectrolyte can be used as novel conformation sensitive optical probes for the detection of several biomolecular processes. The biomolecular interaction or the conformational alteration of the biomolecule are reflected as an alteration of the geometry and the electronic structure of the bound polyelectrolyte chains and has so far been detected by absorption and emission, but electrical detection of these transitions will most likely be possible. We foresee that the present mechanism may be used for detection of a variety of biomolecular processes, and that the simplicity and the diversity of this methodology make it suitable for making inexpensive protein- and DNA-chips for rapid detection of biomolecular recognition. [1] Nilsson, K. P. R., & Inganas, O. *Nature Materials*, 2, 419-424. (2003) [2] Nilsson, K. P. R., Rydberg, J., Baltzer, L., & Inganas, O. *Proc. Natl. Acad. Sci. USA*, 100, 10170-10174. (2003) [3] Nilsson, K. P. R., Rydberg, J., Baltzer, L., & Inganas, O. *Proc. Natl. Acad. Sci. USA*, 101, 11197- 11202 (2004) [4] Nilsson, K. P. R. & Inganas, O. *Macromolecules Article in press* (2004) DOI: 10.1021/ma048605t

#### 10:30 AM \*K6.7

**Smart Dust: Self-Assembling, Self-Orienting Porous Silicon Photonic Crystals in Sensor and Microfluidics Applications.** Michael J. Sailor, Jamie R. Link and Jason R. Dorvee; Department of Chemistry and Biochemistry, University of California - San Diego, La Jolla, California.

The development of small particles of microporous Si as components of an autonomous system that can sense, perform rudimentary signal processing, communicate, and move about will be described. Each particle contains in its nanostructure the necessary components to allow self-assembly, spectroscopic identification, chemical sensing, and motion. The particles are generated by electrochemically etching discrete porous one-dimensional dielectric stack (rugate) mirrors into silicon. The complex multilayered structure produces a distinctive reflectivity spectrum that serves as a robust code, allowing positive identification and discrimination of many different types of particles. The intensity and wavelength of reflected light is determined in part by the refractive index of the porous nanostructure, which is modified by adsorption of vapors or by specific chemical reactions within the chemically modified porous Si matrix. Sensing is accomplished when liquid or vapor infuses into the porous mirrors, inducing predictable shifts in the optical spectra. Chemically asymmetric particles are also described, which can spontaneously align at an organic liquid/water interface. Finally, the synthesis of photonic crystals with superparamagnetic nanoparticles of Fe<sub>3</sub>O<sub>4</sub> incorporated into the porous nanostructure will be described. The addition of magnetic properties allows the materials to chaperone microliter-scale liquid droplets by application of an external magnetic field.

#### 11:00 AM K6.8

**Biofunctionalization of Magnetic Nanoparticles and Incorporation of Same into a Magnetic Tunnel Junction Based Biosensor.** Stephanie Grancharov<sup>2,1</sup>, Hao Zeng<sup>1</sup>, Shouheng Sun<sup>1</sup>, Stephen O'Brien<sup>2</sup>, Chris Murray<sup>1</sup>, John Kirtley<sup>1</sup> and Glenn Held<sup>1</sup>; <sup>1</sup>Nanoscale Materials and Devices, IBM, Yorktown Heights, New York; <sup>2</sup>Applied Physics and Applied Math, Columbia University, New York, New York.

Methods of synthesizing monodisperse, strongly magnetic ferrite nanoparticles (NPs) have been well documented. However, encapsulation of these particles within an overlayer of biologically active molecules and their subsequent stabilization in a physiological medium has not yet been reported. Such particles could be used to bind and transport proteins. Following introduction into a living organism, they could also provide a means of monitoring and influencing cellular processes. Perhaps most importantly, these bio-functionalized magnetic NPs would provide a crucial component in the ultra-sensitive magnetic detection of both proteins and nucleic acids. We report the successful bio-functionalization of 12 nm manganese ferrite (MnFe<sub>2</sub>O<sub>4</sub>) NPs. We demonstrate the site-specific binding of biotin and DNA functionalized NPs onto protein and complementary DNA patterned silicon oxide substrates, respectively. Imaging these substrates with scanning squid microscopy, we show that these particles retain their magnetic properties. Finally, we demonstrate a novel method of detecting the hybridization of these magnetic NPs to a substrate at room temperature using a biosensor comprised of a protein patterned magnetic tunnel junction<sup>4</sup> situated

in orthogonal magnetic fields.

#### 11:15 AM K6.9

**Electropermeabilization of Mammalian Cells Visualized with Fluorescent Semiconductor Nanocrystals (Quantum Dots).** Yinghua Sun<sup>1</sup>, P. Thomas Vernier<sup>3</sup>, Jingjing Wang<sup>5</sup>, Andras Kuthi<sup>2</sup>, Laura Marcu<sup>4,5</sup> and Martin A. Gundersen<sup>2,1</sup>; <sup>1</sup>Department of Materials Science, University of Southern California, Los Angeles, California; <sup>2</sup>Department of Electrical Engineering-Electrophysics, University of Southern California, Los Angeles, California; <sup>3</sup>MOSIS, Information Sciences Institute, University of Southern California, Los Angeles, California; <sup>4</sup>Cedars-Sinai Medical Center, University of Southern California, Los Angeles, California; <sup>5</sup>Department of Biomedical Engineering, University of Southern California, Los Angeles, California.

As a bright and stable inorganic fluorescence probe quantum dots have great advantages for the long-term cell observation and in vivo tracking. Electroporation or electropermeabilization has been insensitively studied recently as an effective technology for gene transfection and drug delivery. Pulsed electric fields can induce reversible membrane breakdown and result in dynamic pores in the cell membrane. This makes the cell permeable to specific molecules in short time but these pores can spontaneously reseal without lethal consequences for the cell. It is found the transfer of small molecules with electroporation is very rapid and efficiency but the big challenge is the transfer of macromolecules, such as plasmids. According to the experiment and simulation results macromolecules could not enter cells freely by diffusion like small molecules or ions. An electropermeabilization model has been proposed and discussed by Zimmermann and Teissie that electric field can trap large particles on the cell membrane and then cells take them inside by some uncertain kinetics in a relative long time. In this work the behavior and processes of electroporation were tracked in 48 hours with quantum dots based on their special properties. First it is the fluorescent nanometer-scale particles with the similar size as DNA molecules. Secondly quantum dots can keep photostability and chemical stability inside cells for over days or even weeks without strong decay in fluorescence and obvious effect on the cell viability. In addition, their high brightness is very helpful to track the motion of small amount of particles in a dynamic live system. The long-term observations revealed that nanometer particles were trapped on the cell membrane after pulsing and the trapping time depended on the cell types and particle sizes. Most quantum dots taken by cells aggregated in lysosomes in the cytoplasm without entering nuclei. And the motion of quantum dots inside cells and the behavior of myeloma and ovarian cells were tracked in 48 hours after electroporation.

#### 11:30 AM K6.10

**Biologically-Compatible Gd@(Carbon Nanostructures) as Advance Contrast Agents for Magnetic Resonance Imaging.** Balaji Sitharaman<sup>1,2,3</sup>, Keith Hartman<sup>1,2,3</sup>, Kyle Kissell<sup>1,2,3</sup>, Lesa Ann Tran<sup>1,2,3</sup>, Lon J. Wilson<sup>1,2,3</sup>, Irene Rusakova<sup>4</sup>, Robert D. Bolskar<sup>5</sup>, Sabrina Laus<sup>6</sup>, Eva Toth<sup>6</sup>, Alain Borel<sup>6</sup>, Gabriel Gonzalez<sup>7</sup>, Lothar Helm<sup>6</sup> and Andre E. Merbach<sup>6</sup>; <sup>1</sup>Chemistry Dept, Rice University, Houston, Texas; <sup>2</sup>Center for Nanoscale Science and Technology, Rice University, Houston, Texas; <sup>3</sup>Center for Biological and Environmental Nanotechnology, Rice University, Houston, Texas; <sup>4</sup>Texas Center for Superconductivity, University of Houston, Houston, Texas; <sup>5</sup>TDA Research Inc., Wheat Ridge, Colorado; <sup>6</sup>Institut de Chimie Moléculaire et Biologique, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Vaud, Switzerland; <sup>7</sup>Departament de Química Inorgànica, Universitat de Barcelona, Barcelona, Barcelona, Spain.

Paramagnetic gadolinium-containing carbon nanostructures are currently being pursued as a new paradigm in magnetic resonance imaging (MRI) contrast agent (CA) design. (1) These compounds offer fundamental advantages over commercially-available Gd<sup>3+</sup>-chelated compounds, the most important being the complete lack of metal-ion dissociation under physiological conditions. Additionally, these systems exhibit unusually large proton relaxivities (efficacies) and offer the potential for intracellular imaging. Our recent work with derivatized Gd@C60-based nanomaterials, Gd@C60[C(COOH)<sub>2</sub>]10 and Gd@C60(OH)<sub>x</sub>, have shown them to exhibit exceptionally large proton relaxivities approaching 100 mM<sup>-1</sup>s<sup>-1</sup>, approximately twenty times larger than current clinically-used MRI agents. Water-proton relaxivities have been measured in aqueous solution at variable temperature (278-335 K) and, for the first time, as a function of magnetic field (5x10<sup>-4</sup> - 9.4 T; NMRD profiles). The proton relaxivities display a remarkable pH-dependency, increasing dramatically with decreasing pH (pH: 3-12). Water-soluble fullerene materials (such as the neuroprotective fullerene drug, C3) readily cross cell membranes, suggesting an application for the gadofullerenes as the first intracellular, as well as pH-responsive, MRI CAs. (2,3) In addition to these gadofullerene-based CAs, we have also recently prepared the first carbon nanotube-based MRI CAs. These CAs are derived from ultra-short (20-50 nm) carbon nanotubes (US-tubes)

which have been internally loaded with aqueous Gd<sup>3+</sup> ions to yield Gd<sup>3+</sup>@US-tube species with relaxivities 50-100 times greater than any Gd<sup>3+</sup>-based MRI CA in current clinical use. References (1) Bolskar, R. D.; Benedetto, A. F.; Husebo, L. O.; Price, R. E.; Jackson, E. F.; Wallace, S.; Wilson, L. J.; Alford, J. M. J. *Am. Chem. Soc.* 2003, 125, 5471. (2) Toth, E.; Bolskar, R. D.; Borel, A.; Gonzalez, G.; Helm, L.; Merbach, A. E.; Sitharaman, B.; Wilson, L. J. *J. Am. Chem. Soc.*, 2004, (in press). (3) Sitharaman, B.; Bolskar, B.; Rusakova, I.; Wilson, L. J. *Nano Letters*; 2004; (in press). This research is sponsored by the Robert A. Welch Foundation (C-0627) and the NIH (Grant 1-R01-EB000703).

#### 11:45 AM **K6.11**

**Bio-functionalization of Monodisperse Magnetic Nanoparticles and their use as Biomolecular Labels in a Magnetic Tunnel Junction based Sensor.** Glenn Held<sup>1</sup>, Stephanie G. Grancharov<sup>1,2</sup>, Hao Zeng<sup>1</sup>, Shouheng Sun<sup>1</sup>, Shan X. Wang<sup>3</sup>, Stephen O'Brien<sup>2</sup>, C. B. Murray<sup>1</sup> and J. R. Kirtley<sup>1</sup>; <sup>1</sup>IBM Research, Yorktown Heights, New York; <sup>2</sup>Dept. of Applied Physics and Applied Mathematics, Columbia University, Columbia, New York; <sup>3</sup>Dept. of Materials Science and Engineering, Stanford University, Stanford, California.

Bio-functionalized monodisperse magnetic nanoparticles (NPs) with small size would enable the ultra-sensitive magnetic detection of both proteins and nucleic acids. When introduced into a living organism, they could also provide a means of monitoring and influencing cellular processes. Methods of synthesizing monodisperse and magnetically stable ferrite NPs with size smaller than 20 nm have been developed. However, encapsulation of these particles within an overlayer of biologically active molecules and their subsequent stabilization in a physiological medium has been a challenge. In this letter, we report the bio-functionalization and detection of 12 nm manganese ferrite NPs. We have achieved the site-specific binding of biotin functionalized NPs onto avidin patterned silicon oxide substrates and DNA functionalized NPs onto complementary DNA patterned silicon oxide substrates. Scanning SQUID microscopy images of these substrate bound NPs show that they retain their magnetic properties and thus can be detected by a magnetic tunnel junction (MTJ) field sensor. Finally, we demonstrate a novel method of detecting either protein binding or DNA hybridization at room temperature using the NPs and a MTJ biosensor situated in orthogonal magnetic fields.

SESSION K7: Bio-inspired Devices  
Chair: Michael Sailor  
Thursday Afternoon, March 31, 2005  
Room 3002 (Moscone West)

#### 1:30 PM **\*K7.1**

**Active Biological Transport Systems as Functional Components of Nanoscale Materials and Devices.** George D. Bachand, Susan B. Rivera, Andrew K. Boal, Marlene Bachand, Jun Liu and Bruce C. Bunker; Biomolecular Materials and Interfaces, Sandia National Laboratories, Albuquerque, New Mexico.

Energy-consuming transport systems play a key role in a wide array of biological processes such as chromosomal segregation, organelle positioning, and melanophore reorganization. The exploitation of such non-equilibrium processes in nanomaterial architectures may enable the development of new devices and materials in which the assembly, disassembly, and organization may be programmed or self-regulated. Our work has specifically focused on a biological active transport system consisting of kinesin biomolecular motors and microtubule filaments as a means for organizing and transporting nanocomposite materials at synthetic interfaces. For this work, surface-tethered kinesin motor proteins are used in the gliding motility geometry to propel functionalized microtubule "shuttles" across a surface. A number of critical technical issues have been addressed to date and include: (1) engineering robust biological components, (2) developing interfacial chemistries for attachment of synthetic nanoparticles, and (3) characterizing factors affecting nanoparticle transport. The prerequisite and enabling technologies necessary to utilize kinesin and microtubules to develop integrated nanomaterials and devices will be discussed. In addition, several key demonstrations will be presented to illustrate the application of this transport technology in hybrid systems. \*Sandia is a multiprogram laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the United States Department of Energy under contract DE-AC04-94AL85000.

#### 2:00 PM **\*K7.2**

**Nanomachines Made from DNA.** Andrew Turberfield, Department of Physics, Univ of Oxford, Oxford, United Kingdom.

DNA is a wonderful material for nanoscale construction. It is a structural material whose assembly can be programmed by making

use of its information-carrying capability; its hybridization can also be used as an energy source for molecular devices. I shall describe our recent work on three-dimensional nanofabrication, DNA-templated protein crystals and molecular machinery. I shall review research on nanomachines made from DNA and describe our own progress towards the construction of a free-running synthetic molecular motor.

#### 2:30 PM **K7.3**

**Biomimetic Actuating Surfaces.** R. Lloyd Carroll<sup>1</sup>, Daniel B. Blum<sup>1</sup>, Brandon R. Lunk<sup>1</sup>, Jing Hao<sup>1</sup>, Ben Wilde<sup>2</sup> and Rich Superfine<sup>1</sup>; <sup>1</sup>Physics and Astronomy, University of North Carolina - Chapel Hill, Chapel Hill, North Carolina; <sup>2</sup>Computer Science, University of North Carolina - Chapel Hill, Chapel Hill, North Carolina.

Biology has had millennia to perfect the mechanisms it uses to move fluids over surfaces. One of the most ubiquitous of these mechanisms is through the use of cilia, hairlike projections on cellular surfaces that actively beat in fluid to impart momentum. Cilia are present at all stages of development, in vastly different species, suggesting the motif is useful and efficient for the purpose of fluid transport in living systems. These systems are complex and important, both from a fundamental science perspective and from a clinical aspect. Understanding how cilia work and how they fail has implications for Cystic Fibrosis treatment, for instance. In our group, we are studying living systems, while at the same time fabricating artificial cilia systems for modeling and applications. We have approached the problem of actuation of nano- and micro-scale structures by the use of magnetic actuation, and shown success in emulating some of the behaviors of biological cilia. We have prepared magnetic nanorod-based surfaces and shown exciting actuation and fluid flow in biologically relevant systems. Flexible magentolastomeric cilia structures have also been developed using templating techniques, and show promise for applications in microfluidics.

#### 2:45 PM **K7.4**

**Carbon Nanotube Bio-Complexes for Bio-Molecular Recognition.** Xiao-Wu Tang, Sarunya Bangsaruntip, Nadine Wang Shi Kam, Nozomi Nakayama, Qian Wang, Erhan Yenilmez and Hongjie Dai; Chemistry, Stanford University, Stanford, California.

Novel nanomaterials for bioassay applications represent a rapidly progressing field of nanotechnology and nanobiotechnology. Here, we report the advancements in developing 1) electrochemical sensors based on single-walled carbon nanotube (SWNT) field effect transistors (FET) and 2) a generic approach for patterning bio-molecules on SiO<sub>2</sub> or quartz surface using SWNT thin film as the anchor. A SWNT is a quasi-one dimensional wire with only surface atoms. The electronic properties of a SWNT are extremely sensitive to its surrounding chemical and electrostatic environment, thus allows direct electrical detection of biological events on tube surface [1]. In addition, SWNT-FET sensors (nm to  $\mu$ m in size) can be integrated into massive arrays in lab-on-chip fashions for analyzing and detecting [2,3] large numbers of bio-molecules with high throughput. Carbon nanotube synthesis by catalyzed Chemical Vapor Deposition (CVD) combined with microfabrication is our basic approach to produce arrayed nanotube devices for sensing applications. Real-time monitoring of 15mer and 30mer DNA hybridization at  $\sim$ nM concentration in phosphate buffered saline (PBS) has been demonstrated. Single-molecule detection is anticipated with short channel ( $\sim$ 10nm) SWNT-FET with a single semiconducting tube across the source and drain electrodes. SWNT-FETs passivated with hydroxythiol molecules exhibit excellent sensitivity to pH and are proven to be stable in a wide pH range. pH-sensitive SWNT-FETs will serve as a platform for building Enzyme-Modified FETs (EnFET) where hydrogen ions are produced or consumed by the enzymatic reaction. Recent progress has been made in synthesis and purification of high density SWNT thin film on both SiO<sub>2</sub> and quartz surfaces, which are easily patterned by photolithography techniques. Bio-molecules such as protein and nucleic acid oligomers can then be linked to the patterned SWNT thin film and the immobilization chemistry can be investigated by optical fluorescence measurements. Polymer chains irreversibly adsorb onto nanotubes to form a monolayer via van der Waals and hydrophobic interactions in aqueous solutions[4]. Synthesis of SWNT bio-complexes directly on substrate provides an alternative to current immobilization chemistries in DNA micro-array and proteomics. Confocal images of fluorescence labeled DNA oligos and biotin immobilized on patterned nanotube arrays will be presented. References: 1. R. Chen, H. Choi, S. Bangsaruntip, E. Yenilmez, X. Tang, Q. Wang, Y. Chang, and H. Dai, *JACS* 126, p. 1563. 2. R. Chen, S. Bangsaruntip, K. A. Drouvalakis, N. Wang Shi Kam, M. Shim, Y. Li, W. Kim, P. J. Utz, and H. Dai, *PNAS* 100 (2003) p. 4984. 3. G. Dovbeshko, O. Reptytska, E. Obraztsova, Y. Shtogun, *Chem. Phys. Lett.* 372, p. 432. 4. M. O'Connell, P. Boul, L. Ericson, C. Huffman, Y. Wang, E. Haroz, C. Kuper, J. Tour, K. Ausman, R. Smalley, *Chem. Phys. Lett.*, 342, p. 265.

**3:30 PM K8.1**

**Peptide-Modified Hydrogels For Neural Stem Cell Control.**  
Krishanu Saha, David Schaffer and Kevin Healy; University of California-Berkeley, Berkeley, California.

Controlling the behavior of viable, multipotent stem cells is a major challenge in regenerative medicine. By tuning specific properties of a biomaterial, we have made significant progress towards precise in vitro control of neural stem cells. Such stem cells of the nervous system have very recently been shown to grow and develop into neurons and other types of cells, making them attractive for Parkinson's and Alzheimer's therapy. Our materials design has focused on creating synthetic equivalents of endogenous regulatory signaling molecules by developing biomimetic polymer surfaces. In this work, we proliferated and differentiated rat hippocampal stem cells seeded on peptide-modified hydrogel surfaces, consisting of a biocompatible, non-fouling interpenetrating polymer network (IPN) of poly(acrylamide-co-ethylene glycol/acrylic acid) [P(AAm-co-EG/AAC)]. The bioactive component of the hydrogel was a 15 amino acid oligopeptide containing the Arg-Gly-Asp (RGD) sequence, which is present in many active domains of adhesion proteins found within extracellular matrices (e.g. laminin in the brain) and binds to integrin receptors. Both proliferation and differentiation of neural stem cells were supported and influenced by the biomaterial. In proliferating culture conditions, the RGD-containing peptides stimulated cells to proliferate and fostered adoption of cell monolayer morphology consistent with increased proliferation. Proliferation on RGE-negative control surfaces was severely reduced, and different cell morphologies including large spherical cell aggregates were observed on surfaces without the bioactive peptide. The number of spherical cell aggregates increased as the surface bioactive peptide concentration decreased. Similar spherical aggregates have been seen in suspension for mammalian neural stem cells and have influenced the proliferative potential of such cells before differentiation into mature neuronal, oligodendrocytic, and astrocytic phenotypes. In differentiating culture conditions, the high RGD density surface supported differentiation at high cell densities for 1-2 weeks. However, immunostaining revealed that these cells contained altered levels of mature cell markers as compared to cells on natural laminin matrices. Our results suggest that synthetic polymeric surfaces containing only the RGD-integrin binding domain can promote in vitro expansion of specific phenotypes. This is a major step in engineering a purely synthetic environment, completely free of animal-derived products, to precisely control the signals presented to stem cells. Further work with this biomaterial can be used to investigate various material property effects, including that of mechanical stiffness, on stem cell behavior.

**3:45 PM K8.2**

**Chitosan-alginate Hybrid Scaffolds for Bone Tissue Engineering.** Zhensheng Li<sup>1,2</sup>, Miqin Zhang<sup>1</sup>, Kip D. Hauch<sup>1</sup>, Demin Xiao<sup>2</sup> and Hassna Ramay<sup>1</sup>; <sup>1</sup>Material Sciences and Engineering, University of Washington, Seattle, Washington; <sup>2</sup>Department of Orthopedics, Shenzhen People's Hospital, Shenzhen, China.

Here we report on the development of a biodegradable porous scaffold made from naturally derived chitosan and alginate polymers with significantly improved mechanical and biological properties as compared to its chitosan counterpart, and it is structurally stable due to the strong ionic bonding between the amine groups of chitosan and the carboxyl groups of alginate. The chitosan-alginate scaffold with porosity of ~92% attained compressive modulus of 8.16 MPa and yield strength of 0.46 MPa, respectively, which are about three times of the values for the pure chitosan scaffold. The cell-material interaction study indicated that osteoblast cells seeded on the chitosan-alginate scaffold and cultured without osteogenic medium appeared to attach and proliferate well and promoted the deposition of minerals in a very short time. Unlike chitosan scaffolds which can only be fabricated from acidic solutions, the chitosan-alginate scaffold developed in this study can be prepared in either acidic, basic or neutral solution. This unique attribute provides a favorable environment for incorporating proteins with less risk of denaturation for sustained release in vivo. The in vivo study showed that the chitosan-alginate scaffolds promoted rapid vascularization and deposited connective tissue and calcified matrix within the entire scaffold structure. These encouraging results support the potential applications of the chitosan-alginate scaffolds as an improved alternative to other natural polymer-based scaffolds for tissue engineering applications. This work was supported by the grant NIH-NHLB (HL64387-03) and University of Washington Engineered Biomaterials Research Center (NSF-EEC 9529161).

**4:00 PM K8.3**

**Biodegradable and Thermo-responsive Poly(N-isopropylacrylamide) Hydrogels for Bone Tissue Engineering.** Eugene H. Chung<sup>1</sup>, Dale R. Sumner<sup>3</sup> and Kevin E. Healy<sup>2,1</sup>; <sup>1</sup>Department of Bioengineering, University of California at Berkeley, Berkeley, California; <sup>2</sup>Department of Materials Science and Engineering, University of California at Berkeley, Berkeley, California; <sup>3</sup>Department of Anatomy and Cell Biology, Rush Medical College, Chicago, Illinois.

Scaffolds for bone repair have in the past generally consisted of bone grafts, which are inherently limited in supply and variable in quality. More consistent supply and quality may be achieved with synthetic biomimetic scaffolds. Additionally, as matrix viscoelastic properties have emerged as important factors in cell-biomaterial interactions, the synthesis of hydrogels with tunable viscoelastic properties has become essential. Scaffolds for bone tissue engineering composed of semi-interpenetrating polymer networks (sIPNs) of poly(N-isopropylacrylamide-co-acrylic acid) crosslinked with an acrylated collagenase-labile peptide (QPQGLAK-NH<sub>2</sub>) were synthesized by redox free radical addition polymerization in the presence of polyacrylic acid-graft-RGD. With the QPQGLAK-NH<sub>2</sub> crosslinker providing biodegradability, and the RGD peptide providing cell-binding domains, the sIPNs constituted biomimetic hydrogels with tunable viscoelastic properties. The hydrogels were designed to systematically study the effect of hydrogel complex shear modulus (G\*) and cell-adhesiveness on bone cell proliferation and as such, hydrogels with various G\* and RGD concentrations were synthesized. G\* of the hydrogel was determined by rheology at 22°C and 37°C. The thermo-responsive properties of the hydrogels were manifested as significant increases in G\* with temperature. For a particular sIPN, G\* increased from 60Pa at 22°C to 170Pa at 37°C. Hydrogel degradation was monitored over several days in various protease solutions. sIPNs completely degraded in 24h in 25µg/ml collagenase; however, the hydrogels were not susceptible to nonspecific degradation by other proteases and thus were stable in the absence of collagenase. *In vitro* measurements of osteoblast proliferation on sIPNs with various G\* and RGD concentrations were performed with the WST-1 metabolic assay. Both adhesion and proliferation of bone cells were dependent upon G\* and RGD concentration; hydrogels with high G\* (170Pa) and high RGD concentration (100µM) correlated with the greatest proliferation (increase from 6000 cells/cm<sup>2</sup> to 28,000 cells/cm<sup>2</sup> after 5 days), whereas those with low G\* (10Pa) and without RGD did not support proliferation. Implantation of degradable hydrogels (G\* = 130Pa; 60µM RGD) for 4 weeks in the femoral marrow spaces of rats stimulated new bone formation. Analysis of the rat femur by µCT indicated a significant amount of mineralized bone where degradable hydrogels had been implanted, while femurs implanted with control hydrogels with non-degradable crosslinks produced significantly less new bone. These results have demonstrated the potential of a synthetic biomimetic hydrogel to serve as a scaffold for bone tissue formation and the importance of complex modulus on tissue engineering scaffold design. This work was supported by a Whitaker Foundation Graduate Fellowship to EHC and an NIH NIAMS Grant.

**4:15 PM K8.4**

**Blending Polymer of Polysulfone/ Polycaprolactone for Improvement of the Hemocompatibility and for Drug Controlled Release.** Yen-Yu Liu<sup>1</sup>, Chia-Hui Tsai<sup>1</sup>, Dean-Mo Liu<sup>2</sup> and San-Yuan Chen<sup>1</sup>; <sup>1</sup>Material Science and Engineering, National Chiao Tung University, Hsinchu, Taiwan; <sup>2</sup>ApaMatrix Technologies Inc., Moffatt Road, Richmond, British Columbia, Canada.

Controlled release of drugs of polysulfone (PSF), polycaprolactone (PCL) and a combination of both components are therapeutically important for enhanced medication efficacy after administration with a lowest dosage, where some clinically-observed side effects for certain of toxic drugs can be minimized. Property modification as a result of suitable blending may not only manipulate the drug release behavior but also influence the biocompatibility and the hemocompatibility of the resulting blended polymers, the latter has been recognized as an important factor for a number of blood-contacting implant devices. In this study, the permeation of ibuprofen through polysulfone (PSF) and polycaprolactone (PCL) blend membrane was investigated. Controlled release could be completely manipulated by altering the composition of polysulfone and polycaprolactone in the membranes. The permeability [DH] of the blend membrane was measured widely spreading in the order of 10<sup>-5</sup>~10<sup>-7</sup>cm<sup>2</sup>/hr. The surface morphology of the blend polymers appeared to be smooth and flat before diffusion test. The PSF/PCL blend polymer had a widely permeating performance and could maintain a stable release behavior and characteristic. It also functioned well as a blood compatibility material that is platelet adhesion was effectively reduced when the composition of PCL was 20% and 50% relative to the blend system. These blending polymers could be further used as blood contact devices.

#### 4:30 PM **K8.5**

##### **ABO Blood Group Typing with Artificial Receptors.**

Oliver Hayden, Karl-Juergen Mann and Franz Ludwig Dickert;  
University of Vienna, Institute of Analytical Chemistry, Vienna,  
Austria.

Molecular imprinting of synthetic polymers is a cost-effective and versatile method to synthesize robust man-made receptors. Here, we describe the first attempt to imprint polymers with mammalian cells. Polyurethane thin films were surface imprinted with whole erythrocytes and erythrocyte ghosts using a rational imprint-lithography technique. ABO blood group determination was achieved with piezoelectric devices coated with the bioimprinted polyurethanes. The imprinted polymers show a high selectivity for the adsorption of intact red blood cells rather than for mechanically ruptured cells. Red blood cells are selectively adhered on pits imprinted with the same templating erythrocytes. The selectivity pattern of ABO imprinted polymers reflect the antigen composition of the erythrocyte membrane. Furthermore, blood group typing was performed in whole blood samples. The implications of these results for biotechnological applications will be discussed.

#### 4:45 PM **K8.6**

##### **Active and Adaptive Photochromic and Thermochromic Electrospun Fibers, Fabrics and Membranes for Biosensors and Tissue Engineering.** John F. Rabolt<sup>1</sup>, Simon Frisk<sup>2,1</sup>,

Giuseppe Zerbi<sup>2</sup>, Chiara Bertarelli<sup>2</sup> and Andrea Bianco<sup>2</sup>; <sup>1</sup>Materials Science and Engineering, University of Delaware, Newark, Delaware; <sup>2</sup>Dip. Chimica, Materiali e Ing. Chimica, Politecnico di Milano, Milano, Italy.

Electrospun micro and nanofibers, fabrics and membranes with photo- and thermo-switchable color have been produced. A mixture of polymeric materials and diarylethene dyes with photochromic and/or thermochromic properties was electrospun from a common solvent at room temperature. The resulting fibers exhibit a uniform distribution of dye molecules as shown by laser scanning confocal fluorescence measurements. After electrospinning the photochromic and thermochromic properties are preserved in the fibers, fabrics and membranes. The very high surface-to-volume ratio of the fibers and non-woven fabrics increases the efficiency of the color switching process. The application of this reversible color changing to the detection of angiogenesis and the sensing degradation of tissue engineered constructs will be discussed.

SESSION K9: Poster Session: Biological and  
Bio-inspired Materials and Devices II  
Chairs: Elaine DiMasi, Andrew Parker and Shu Yang  
Thursday Evening, March 31, 2005  
8:00 PM  
Salons 8-15 (Marriott)

#### **K9.1**

##### **Novel Nanoscale Biosensor for Lactate Analysis in Sweat.**

Arun Kumar<sup>1</sup>, Jessica Otto<sup>1,2</sup>, Ashok Kumar<sup>1,2</sup> and Shekhar Bhansali<sup>1,3</sup>; <sup>1</sup>Nanomaterial and Nanomanufacturing Research Center, University of South Florida, Tampa, Florida; <sup>2</sup>Department of Mechanical Engineering, University of South Florida, Tampa, Florida; <sup>3</sup>Department of Electrical Engineering, University of South Florida, Tampa, Florida.

The sweat glands are coiled, tubelike structures located in the dermis and subcutaneous layer of the skin. Each tube extends to the skin surface and opens at a pore. These glands function to regulate body temperature through the evaporation of sweat from the body surface. Sweat consists of water and a small amount of electrolytes, minerals, and other wastes. Lactate and potassium were the only components found to correlate significantly with the state of hydration, stiffness, and pH of the stratum corneum (SC). Moreover, sweat lactate is a function of sweat gland energy metabolism. Because lactate is a product of the sweat gland itself, a decrease in oxygen supply induces a rise in sweat lactate concentration and a decrease in sweat gland activity. The increase in pressure to soft tissue will cause a rise in sweat lactate concentration with respect to the rest of the body's sweat lactate concentration. The development of pressure sores, or bedsores, has implications on the health of a bedridden individual as well as requirements for health care professionals. A novel noninvasive sweat lactate biosensor that could continuously monitor a patient's sweat lactate level would allow the health care professionals to know when to turn a bedridden individual and thus relieve pressure sores. In the present approach attempts have been made to develop an ion selective electrode at nanoscale which is capable of detecting sweat lactate concentration in very low amounts (micro liter) with high specificity and selectivity. The nanoscale ion selective electrode is

prepared by modifying nanoparticles with a biological recognition layer containing lactate oxidase and ferricyanide as an electron mediator. The nanoscale electrode is capable of selectively detecting lactate in the skin sweat using cyclic voltammetry. The values monitored were sweat lactate concentration simultaneously with the electrical potential difference at the duct orifice. Modified nanoparticles are characterized with SEM, FTIR, and UV visible. This research is supported from NSF IGERT grant.

#### **K9.2**

##### **Functionalized Nanoparticles for the Treatment of Osteoporosis.** Ganesan Balasundaram and Thomas Jay Webster; Biomedical Engineering, Purdue University, West Lafayette, Indiana.

The U.S. Bureau of Census has clearly demonstrated that we are an aging demographic. For example, the percentage of the population greater than 65 years of age is expected to increase from 12.4 to 23% between the years 2000 and 2100. Not surprisingly, a similar upward trend is projected for the debilitating age-related disease: osteoporosis. Specifically, osteoporosis cases will rise from 10.1 million in 2002 to 13.9 million in 2020. More importantly, nationwide, approximately 1.5 million bone fractures per year are attributed to osteoporosis. None of the current methods used to treat osteoporosis have experienced overwhelming success. For example, several major barriers exist for the use of any pharmaceutical agents to stimulate new bone formation. First, the agents can cause non-specific bone formation in areas not desirable. This is because these agents are often delivered in non-specific ways (such as through the mouth, directly into the blood stream, etc.). Second, if delivered locally to the tissue around the area of low bone density, they rapidly diffuse to adjacent tissues which limit their potential to promote prolonged bone formation in targeted areas of weak osteoporotic bone. It is because of these limitations that even the best strategies to sufficiently increase bone mass (although, to date, still unproven) require at least one year to see any change; a time period not acceptable especially for the elderly. For these reasons, in this study we used nanotechnology (or the design of materials with 10-9 m dimensions) to develop novel drug-carrying systems that will specifically attach to osteoporotic (not healthy) bone and distribute pharmaceutical agents locally to quickly increase bone mass. Specifically, inorganic and organic biodegradable nanomaterials (including polymers like poly-lactic-co-glycolic acid and ceramics like hydroxyapatite) were functionalized with bioactive chemicals such as bone morphogenetic protein-2 (BMP-2) that bond to bone of low mass. Such bioactive groups were placed on the outer surface of the nanoparticle systems using various techniques (such as aminophase silane chemistry). After bonding specifically to osteoporotic bone and not healthy bone, nanoparticle systems are designed to deliver bioactive compounds to locally increase bone mass. Lastly, the outer coating of the embedded nanoparticle systems were created to have different biodegradation rates for the release of bone-building agents over various time spans; this will allow for not only quick bone formation but also long-term sustained bone regeneration. In this manner, this study took a new approach to fight osteoporosis through the design of intelligent, nanoparticle systems that can attach to areas of low bone mass and then release bioactive agents to subsequently increase bone mass; such efforts represent a new direction to reverse osteoporosis.

#### **K9.3**

##### **Aminopropyl Embedded Silica Films as Potent Substrates in DNA Microarray Applications.** Kristjan Saal<sup>1,2</sup>, Tanel Tatt<sup>1,2</sup>,

Ilmar Kink<sup>1</sup>, Ants Kurg<sup>3</sup>, Rynno Lohmus<sup>1</sup>, Uno Maeorg<sup>2</sup>, Ago Rinke<sup>2</sup> and Ants Lohmus<sup>1</sup>; <sup>1</sup>Lab. Of Low Temperatures, Institute of Physics Univ. of Tartu, Tartu, Estonia; <sup>2</sup>Institute of Organic and Bioorganic Chemistry, University of Tartu, Tartu, Estonia; <sup>3</sup>Institute of Molecular and Cell Biology, Estonian Biocentre, Tartu, Estonia.

Sol-gel derived silane hybrid films ((3-aminopropyl)trimethoxysilane-tetramethoxysilane) will be shown to have properties that make the films suitable for DNA microarray applications. The detailed surface characteristics of the films were studied using aminated 25-mer oligonucleotide DNA and 1,4-phenylenediisothiocyanate linker. The binding of DNA onto the films was shown to depend on films' composition having an optimum where the binding was substantially superior compared to commercial analogues. The arrayed primer extension (APEX) analysis showed strong fluorescent signals of bound probes, and low background noise. The essential properties of the films were characterized by AFM, FTIR and wettability measurements.

#### **K9.4**

##### **A Fluorescence Biosensor Constructed from a Maltose Binding Protein Immobilized in Poly(ethylene glycol) Hydrogel.** Wensheng Cai, J. Bruce Pitner and Glenn Vonk; BD Technologies, RTP, North Carolina.

Fluorescent dye-labeled periplasmic binding proteins constitute a rapidly advancing area of biosensor development. Such proteins can

be modified to bind to their ligand (the analyte) and provide a direct fluorescence signal proportional to ligand concentration. Corresponding biosensors are often referred to as "reagentless" as they do not use competitive binding partners or other additional reagents. A change in fluorescence of the fluorescent label occurs due to protein conformational change upon binding of the protein to the corresponding ligand. Binding proteins must be immobilized within a biosensor matrix in a manner that allows analyte-induced conformational change of the binding molecules. Here, we describe methods of chemically immobilizing dye (IANBD) labeled maltose binding protein (MBP) in a poly(ethylene glycol) (PEG) hydrogel. The results showed that upon adding free maltose solution, the fluorescence intensity of immobilized MBP increased ~2 fold over that of ligand-free MBP, a fluorescence change close to that of MBP in solution. Determination of the immobilized protein's equilibrium dissociation constant was performed by titrating immobilized MBP with different maltose concentrations; the dissociation constant (K<sub>d</sub>) obtained was ~ 2.8 mM maltose. We also demonstrated that the hydrogel maltose sensor showed continuous response to maltose concentration. These findings suggest that immobilized engineered binding proteins in PEG hydrogels could enable development of implantable, continuous-reading biosensor.

### **K9.5**

**Bioinspired Sensors.** Nikolaos Chalkias<sup>1</sup> and Emmanuel Giannelis<sup>2</sup>; <sup>1</sup>Chemical and Biomolecular Engineering, Cornell University, Ithaca, New York; <sup>2</sup>Materials Science and Engineering, Cornell University, Ithaca, New York.

Nanohybrid artificial membranes made by intercalation of amphiphilic molecules into the galleries of a layered host exhibit characteristics similar to biological membranes and they can be used as sensors. Specifically the nanohybrid membranes can be used as sensors for different analytes including saccharin. Different responses have been observed even for molecules that have similar features for example, saccharin and its sodium salt suggesting that the nanohybrid might be useful in developing an electronic nose. The dynamic range of the saccharin sensor is 20 - 500 μM. In this paper we will present our results on sensor fabrication and testing and discuss possible sensing mechanisms.

### **K9.6**

**Physically Tunable Amphiphilic Diblock Copolyptide Vesicles.** Eric Peter Holowka<sup>1</sup>, Lisa Pakstis<sup>2</sup>, Darrin Pochan<sup>2</sup> and Timothy J. Deming<sup>3</sup>; <sup>1</sup>Materials Engineering, University of California Santa Barbara, Goleta, California; <sup>2</sup>Materials Engineering, University of Delaware, Wilmington, Delaware; <sup>3</sup>Bioengineering, University of California Los Angeles, Los Angeles, California.

Recently, the transition metal mediated living polymerization of block copolypeptides from α-amino-N-carboxyanhydrides (NCA) has allowed the synthesis of copolypeptides having a high degree of chain length control as well as the ability to incorporate a wide range of amino acids and amino acid analogues. An attractive benefit of this system is the ability to tailor polymer structure with amino acids that form known secondary structures, which can then be used to drive self-assembly of complex supramolecular structures. Spontaneous formation of copolypeptide unilamellar vesicles, which are stable upon formation, has been previously shown in our group with a (diethylene glycol modified lysine)-block-(leucine) copolypeptide. In this system, vesicle size was modified through changes made to the block segment lengths within the copolypeptide amphiphile. This is in contrast to liposomes, which can be physically processed to yield vesicles of different sizes. The cost of physical processability in liposomes is illustrated in the lack of stability of these vesicles over useful timescales. A valuable alternative would be a polymeric system capable of being physically processed into stable vesicles of different sizes. We report a lysine-block-leucine amphiphilic diblock copolypeptide vesicle forming system where vesicle size can be modified by physical methods. These vesicles have been found to be stable and do not change size for 3 weeks after formation. The assemblies are also stable in the presence of sucrose, which can be encapsulated within the vesicle shells.

### **K9.7**

**Preparation and Characterization of Porous Material from Self-Organized Hydroxyapatite/Collagen Nanocomposite.** Shunji Yunoki, Toshiyuki Ikoma, Akira Monkawa, Masanori Kikuchi and Junzo Tanaka; National Institute for Materials Science, Tsukuba, Japan.

Hydroxyapatite/collagen (HAp/Col) composites have been studied as bone filling materials that will substitute for autogenous bone implants. We have developed a novel HAp/Col nanocomposite with similar nanostructure to natural bone tissues through a self-organization mechanism. The consolidated HAp/Col nanocomposite showed excellent biocompatibility and biointegrative

activity for the bone tissues. The control of pore structure in the composite will improve cell migrations and mechanical strengths, and regenerate neo-vascularizations. In this study, porous HAp/Col nanocomposite with one-directional connective micropores was prepared by unidirectional solidification and subsequent freeze-dry processes. We elucidate the effects of pore structure and porosity on mechanical properties. The HAp/Col nanocomposite fibers (80/20 weight ratio) were synthesized by a co-precipitation method using Ca(OH)<sub>2</sub>, H<sub>3</sub>PO<sub>4</sub>, and Col as starting substances. The temperature and pH were kept at 40°C and 8-9, where Col fibril formation and HAp crystallization are simultaneously occurred. The slurry of HAp/Col nanocomposite fibers was washed with H<sub>2</sub>O and freeze-dried. The freeze-dried composite was thoroughly stirred with 200 mM-Na phosphate buffer of pH 6.8 at a concentration of 1.25 g/10.0ml. The paste-like HAp/Col nanocomposite was poured into a plastic cylindrical container with a brass bottom. Only the bottom face was immersed in liquid N<sub>2</sub> as a refrigerant and the ice crystals grew in the vertical direction from the bottom face. The frozen paste was freeze-dried, cut into 10-mm cubes, and dehydrothermally treated in vacuo at 140°C for 12 h to crosslink among the Col molecules. The pore structure was observed by a scanning electron microscopy (SEM). The mechanical properties were investigated by a compressing test using a texture analyzer. Unique pore structures were formed by the unidirectional solidification and freeze-drying. It was observed that unidirectional open pores (about 20 mm in diameter) were formed along the ice growth direction over the entire sample, except for the interfacial region (1 mm in thickness) between the paste and the brass bottom, where only random nanopores were observed. The diameter of the unidirectional pore was increased with increasing the freezing temperatures. The HAp/Col nanocomposite cubes showed anisotropic mechanical strengths and viscoelastic properties; the compressive strength parallel to the pore-axis was twice higher than that perpendicular to the axis, and the stress relaxation against pressure parallel to the pore-axis was much faster than that perpendicular to the axis. These results clearly indicate that the pore structure is of great importance for controlling the mechanical properties.

### **K9.8**

**Conjugated Polyelectrolytes on Solid Supports Report Conformation Changes in Docking Proteins by Optical Emission.** Peter Asberg, Peter Nilsson and Olle Inganas; Applied Physics, Physics and Measurement Technology, Linköping, Sweden.

Conjugated polyelectrolytes (CPs), conjugated polymers with ionic functionalities, can be utilized to study many kinds of biomolecular events, thus enabling different biosensor devices. CPs offer possibilities for very sensitive measurements, and may become ubiquitous for genomics and proteomics in the future, as the optical or electronic processes in these materials can be used to track biospecific interactions. One such CP that has demonstrated many useful interactions together with biomolecules is the polythiophene POWT and related polythiophene derivatives. Examples of biomolecular events that can be studied using this class of CPs are analyte/receptor interactions, DNA hybridization [1] or conformational changes in proteins [2]. The development of biochip devices capable of selectively these types of biomolecular interactions is highly topical, for parallel and high throughput detection. One condition to be able to use CPs for the detection of molecules in biological samples is that they are compatible with an aqueous environment. We have shown that POWT is active and capable of changing its conformation on a solid support using SPR [3] and QCM-D [Asberg, manuscript in preparation]. Therefore, POWT has good characteristics to follow biomolecular events in a biochip format. In this work we have focused on how to make biochips capable to distinguish between correctly folded and misfolded proteins. Detection of misfolded proteins or peptides is of special interest with the rising problem of prion diseases, and large scale production of protein pharmaceuticals. The present method is based on the modification of a surface using soft lithographic methods, microcontact printing (μCP) with PDMS stamps. Patterned PDMS stamps are used to create a hydrophobic pattern on a hydrophilic substrate [4]. Surface modification using μCP on selected areas is a process that minimizes the waste material. Sensitive molecules, such as biomolecules, should preferably be applied to the chip surface in their optimal solution. Depending on the wetting of the solution on the patterned substrate, the solute/surface interactions adsorbed molecules appear in a negative or positive pattern on the hydrophobic/hydrophilic pattern. This may depend on the degree and presence of hydrophobic/hydrophilic exposure, the charge and charge distribution on the biomolecule or conformation of the biomolecule. Using these detector materials anchored onto solid surfaces, we have been able to distinguish between native and fibrillar insulin as well as a synthetic peptide in random coil or helix formation. 1. Nilsson, K.P.R.; Inganas, O. *Nat Mater* 2003, 2, 419-410. 2. Nilsson K.P.R.; Inganas O *Macromolecules* 2004, DOI: 10.1021/ma048605t. 3. Bjork P.; Persson N-K.; Nilsson K.P.R.; Asberg P.; Inganas O. *Biosens and Bioelec* 2004, doi:10.1016/j.bios.2004.07.001. 4. Wang, X.J.; Ostblom, M.; Johansson, T.; Inganas, O. *Thin Solid Films* 2004, 449, 125-132.

#### **K9.9**

**Electrodeposition of Biotin-doped Polypyrrole on Microfabricated Electrodes.** Paul M. George<sup>1</sup>, David A. LaVan<sup>3</sup>, Ching-Yuan Chen<sup>2</sup> and Robert Langer<sup>2</sup>; <sup>1</sup>Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, Massachusetts; <sup>2</sup>Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts; <sup>3</sup>Department of Mechanical Engineering, Yale University, New Haven, Connecticut.

Polypyrrole (PPy) is a conducting polymer with growing applications in the biomedical field. Various dopants have been incorporated into PPy to improve properties ranging from conductivity to biocompatibility. Recently groups have modified the pyrrole monomer by the addition of a biotin molecule to create immunosensor applications. Biotin is a small molecule that is bound tightly by the protein, streptavidin. The biotin/streptavidin complex has been utilized to create sensors and anchor biotinylated molecules to various surfaces. The ability to incorporate biotin as a dopant during the electrodeposition step would eliminate the need for the complicated process of chemically altering the pyrrole monomer. We have used biotin in combination with sodium dodecylbenzenesulfonate (NaDBS) as dopants for the electrodeposition of PPy. NaDBS provides the desired conductive and structural properties while the biotin creates the interactive polymer surface. Because the biotin-doped PPy is formed in one electrodeposition process, the production complexity and times are greatly reduced from currently available methods. Drug delivery applications are also possible because the biotin is not part of the polymer backbone. The incorporation and functionality of the biotin in the PPy has been demonstrated through the addition of a fluorescently-tagged streptavidin after polymer deposition. Fluorescent intensity was measured to determine the biotin/streptavidin binding levels. The stability of the biotin in the polymer is important for future biomedical applications. This stability of the biotin in the polymer matrix was shown by fluorescent intensity measurements over a three week period. The ability to incorporate biotin into PPy through the electrodeposition process provides a unique platform for future sensor and drug delivery applications.

#### **K9.10**

**Development of Bioactive Polymers for use in Cardiac Tissue Engineering.** Samuel Thomas Wall<sup>1</sup>, Kris Saha<sup>2</sup>, David V. Schaffer<sup>2,1</sup> and Kevin E. Healy<sup>1,3</sup>; <sup>1</sup>Bioengineering, UC Berkeley, Berkeley, California; <sup>2</sup>Chemical Engineering, UC Berkeley, Berkeley, California; <sup>3</sup>Material Science and Engineering, UC Berkeley, Berkeley, California.

Tissue engineering to replace diseased or damaged tissue is a potentially effective method for treating medical conditions that currently have poor prognosis. This investigation focuses on the development of synthetic constructs for such treatment of ischemic cardiac injury as a result of myocardial infarction. To address this issue, we focus on matrix-assisted myocardium regeneration (MAMR), or the use of engineered materials to foster the restoration of functional tissue in damaged myocardium. Our lab has created artificial extracellular matrices (ECMs) that are environmentally responsive and orthogonally tunable with respect to mechanical properties (e.g. G\*), biological ligands, tissue adhesion, and protease degradation. We have synthesized and characterized the physical properties of semi-interpenetrating polymer networks (SIPNs) consisting of linear polyacrylic acid (pAAc) chains within a thermo-responsive N-isopropylacrylamide-co-acrylic acid network [p(NIPAAm-co-AAc)]. To impart bioactivity into the hydrogels, the pAAc linear chains have been functionalized with peptides containing the cellular binding domain RGD and a potent angiogenic growth factor. These modified p(NIPAAm-co-AAc) serve as useful tools for studying cell-material interactions within three dimensional structures and have the potential to be used as injectable scaffolds for MAMR cardiac engineering applications. The work presented here focuses on the development of the angiogenic aspect of this modular system. The active N-terminal region of the growth factor sonic hedgehog (Shh) was recombinantly produced with specific amino acid modifications to allow for maximal potency and direct conjugation to desired materials. Shh was targeted as it is a potent upstream regulator of numerous cellular growth and differentiation cascades and has been shown to promote the growth of robust vasculature when used in animal models. In this study, Shh was covalently linked to linear pAAc through the addition of maleimide functionality to the pAAc chain with EDC, Sulfo-NHS, and EMCH, which can then react with an added cysteine sulfhydryl on the protein surface. Reaction conjugation efficiency is ~ 80% as evaluated by gel electrophoresis. The engineered protein and the conjugated polymer were tested using a cellular differentiation assay. The model embryonic mouse fibroblast line C3H10T1/2 was used which differentiates from a multipotent state into osteoblasts when exposed to soluble active Shh. This differentiation leads to an increase in alkaline phosphatase (AP) expression in a dose-dependent manner in the 1 to 100 nM range;

however, the conjugates did not show expected activity, and subsequent tests demonstrate linear pAAc inhibits the Shh induced differentiation in a dose dependent manner. Additional testing is required to determine the nature of the differentiation inhibition by pAAc and if this in vitro reading has any in vivo significance.

#### **K9.11**

**Disposable Microchip-Based Electrochemical Detector using Prussian Blue-Modified Indium Tin Oxide Electrode.** Ju-Ho Kim<sup>1</sup>, In-Je Yi<sup>1</sup>, Chi-Jung Kang<sup>2</sup> and Yong-Sang Kim<sup>1</sup>; <sup>1</sup>Electrical Engineering, Myongji University, Yongin, Kyunggi-do, South Korea; <sup>2</sup>Physics, Myonji University, Yongin, Kyunggi-do, South Korea.

The amperometric detection of dopamine and catechol on a Prussian blue (PB) thin film, which is electrodeposited on indium tin oxide (ITO) glass substrate, was studied in this work. The PB thin film, or ferric hexacyanoferrate, can improve the electrocatalytic rate of the analysis. A capillary electrophoresis and a three-electrode system were used in our experiment. The system was realized with polydimethylsiloxane (PDMS)-glass chip and indium tin oxide (ITO) electrode. The injection and separation channels (80  $\mu$ m wide X 40  $\mu$ m deep) were produced by moulding a PDMS against a micro fabricated master with relatively simple and inexpensive methods. ITO electrode was fabricated by patterning the ITO film deposited on a fusion glass. A PB thin film was galvanostatically deposited onto an ITO electrode in a plating solution consisting of 20 mM FeCl<sub>3</sub>, 20 mM K<sub>3</sub>[Fe(CN)<sub>6</sub>], 0.2 M KCl, and 0.1 M HCl. A capillary electrophoresis and a three-electrode electrochemical detector were fabricated on the same chip. For comparing the performance of the PB-modified ITO electrodes with the bare electrodes, bare ITO electrode microchip was fabricated with the same dimension. The running buffer was prepared by 10 mM 2-(N-morpholino) ethanesulfonic acid (MES) titrated to pH 6.5 using 0.1 N NaOH. The testing analytes are consisted of 1 mM catechol and 1 mM dopamine. Separation of catechol and dopamine was performed using an electric field strength of 60 V/cm after applying an injection electric field of 60 V/cm. The electrochemical detection circuit could mostly decouple the interference of a separation electric field. Electrophoretic measurement of dopamine and catechol mixture is successful in the ITO electrode microchip. Results are indicated convenient and rapid separation and detection of two compounds, with a total time of around 80 sec applying a separation electric field of 60 V/cm. The measured current peaks of dopamine and catechol are proportional to their concentrations. For comparing the performance of the PB-modified ITO electrodes with the bare ITO electrodes, electrochromograms was measured for CE-ECD device with PB-modified electrodes under the same conditions. The performances including sensitivity and resolution of CE-ECD microchip with PB-modified ITO electrode are improved compared with bare ITO electrode. When we are using an ITO glass modified by an electrodeposited PB thin film can efficiently catalyze the oxidation of dopamine and catechol. In addition, we believe that the PB-modified ITO electrode can be a viable candidate for the fabrication of a portable, disposable, and digital biosensor.

#### **K9.12**

Transferred to K11.1

#### **K9.13**

**Single Walled Carbon Nanotube as an Efficient Platform for Biosensor: Electrical Biosensing and Fluorescence-Microarray based Protein Chip Applications.** Hee Cheul Choi and Hye Ryung Byun; Department of Chemistry, Pohang University of Science and Technology, Pohang, South Korea.

Recent observations of spontaneous adsorption of protein molecules as well as covalent coupling of specific protein molecules on the sidewalls of SWNT have inspired development of various types of biosensors. Carbon nanotube-field effect transistor (CNT-FET) composed of pure single walled carbon nanotubes (SWNT) are successfully demonstrated as a highly selective electrical biosensor. Further systematic studies for the elucidation of the mechanism of the conductance changes have been performed by fabricating CNT-FET devices having metal contact electrodes (Pd/Au) passivated with self-assembled monolayers of mPEG-SH. Metal-nanotube contact area of the CNT-FET is evicted to be highly responsible for the generation of the electrical signals while direct charge injection from biomolecules into carbon nanotube is negligible. Parallel to the electrical sensing approach, fluorescence-microarray based technique which is one of the most conventional methods for protein chip application is also attempted using high yield SWNT film as an efficient protein immobilization surface. Contrast to the typical flat surfaces used for protein chip, high yield SWNT forms a pseudo-3D network structures on which incoming protein molecules occupy minimum contact area. This increases the stability of the original protein structures after immobilization and eventually improves selectivity and sensitivity of the chip. In this presentation, successful demonstration of SWNT film

as a platform for the protein chip will be introduced. Typical values based on fluorescence intensity counts for specific and non-specific binding are 60,000 and 100, respectively.

#### **K9.14**

**Fabrication and Characterization of Polycaprolactone Foam Scaffolds for Stem Cell Studies.** Michael H. Tollon<sup>1</sup>, Bradley Jay Willenberg<sup>3</sup>, Christopher Batich<sup>1,3</sup>, Takashi Hamazaki<sup>2</sup> and Naohiro Terada<sup>2</sup>; <sup>1</sup>Materials Science and Engineering, University of Florida, Gainesville, Florida; <sup>2</sup>Pathology, Immunology and Laboratory Medicine, University of Florida, Gainesville, Florida; <sup>3</sup>Biomedical Engineering, University of Florida, Gainesville, Florida.

Tissue engineering has become a great interest in materials science research in the past decade. Porous biopolymers have played a vital role by creating a scaffold structure for tissue regeneration. Structural integrity is a key attribute during the course of tissue formation. Scaffolds supply the necessary foundation for cell attachment, proliferation and maintaining differentiated functions. Other important factors for cell scaffolding success are the addition of growth factors and/or other protein coatings. Our lab has chosen a synthetic biodegradable polymer that can create an interconnected foam structure. We have fabricated porous polycaprolactone (PCL) 3-D scaffolds and later seeded them with mouse embryonic stem cells (ES cells). *In vitro* degradation studies were conducted at 37°C while submerged in a phosphate buffered solution. The scaffolds were analyzed for mass loss, morphology, and molecular weight. Our PCL scaffolds lost less than 4% of their mass during the 9 week degradation study. It was also found that this tough and elastic material remains in its originally designed morphology much longer than other widely used biopolymers (e.g. poly (lactic-co-glycolic acid)). ES cells were seeded onto the scaffold after various protein coatings were applied. We explored various coating techniques to modify cellular results using these scaffolds. When PCL foams were coated with an assortment of proteins, ES cells attached, spread, and differentiated within the scaffolds. We feel these robust and durable scaffolds will be useful in future tissue engineering research.

#### **K9.15**

**Viscoelastic Properties of Ultrathin Films of Hyaluronic Acid as Measured using a Quartz Crystal Microbalance with Dissipation.** James Eric Ho and Kevin E. Healy; Department of Bioengineering, UC Berkeley, Berkeley, California.

Hyaluronic acid (HA) has been proposed as an implant coating due to its anticoagulant and nonfouling nature. Its performance may relate to its viscoelastic properties, which have been shown to modulate cell response in other systems.<sup>1</sup> To elucidate these properties, the shear modulus, shear viscosity and thickness of HA thin films on quartz substrates were determined using a surface-sensitive technique termed quartz crystal microbalance with dissipation monitoring (QCM-D). The technique is an expanded variant of the linearized Sauerbrey approach for rigid thin films, which allows for incorporation of lossy effects that are frequency dependent. SiO<sub>2</sub>-coated quartz crystals (Qsense) were grafted with HA (1.3MDa, Genzyme) using a standard carbodiimide chemistry protocol.<sup>2</sup> The surface chemistry was characterized with XPS and contact angle goniometry, where the values were comparable to literature.<sup>2</sup> The crystals were measured under N<sub>2</sub> gas in a Qsense D300 to characterize the dried HA film thickness using the Sauerbrey equation. Modeling software by Qsense was used to delineate the viscoelastic properties of HA when swelled in PBS (Gibco) by using a Kelvin-Voigt (KV) approach. Humidity and buffer swelling experiments were performed to highlight the propensity of HA to adsorb large amounts of water. Ex situ measurements showed a dried HA layer thickness of 4 nm (according to Sauerbrey) and a PBS-swelled thickness of 29 nm (according to KV modeling), giving a swelling ratio over 7. The maximum shear modulus in buffer was 41kPa, while the shear viscosity in buffer ranged from 0.3 - 2.5 cP. Fibrinogen (Fgn) adsorption and hyaluronidase (Hase) activity was tested with QCM-D, while short-term osteoblast cell adhesion was measured in order to compare to the known nonfouling nature of HA. Fgn adsorption was performed at 10% serum concentration in PBS, while Hase activity experiments were done at 150 units/ml in PBS. Fgn adsorption to HA was inhibited by 80% compared to control, similar to radiolabeled experiments.<sup>3</sup> MC3T3-E1 cells were seeded at 1E5 cells/well (12 well TC plate) onto quartz crystals grafted with identical chemistry protocol and pretreated with 1% FBS. After 24 hours, control and HA-treated surfaces were rinsed and cell numbers were counted. The HA chemistry resisted cell adhesion, even though measurable serum protein adhesion to HA-grafted surfaces was observed. Hase appeared to have no effect on the ability of HA to prevent short-term cell adhesion, but QCM-D, XPS and contact angle studies demonstrated detectable Hase activity towards the grafted HA film, contrary to previous research.<sup>4</sup> References: 1. Wang H-B, Dembo M, et al. (2000) *Am J Physiol Cell Physiol* 279: C1345 - C1350 2. Stile RA, Barber TA, et al. (2002) *J Biomed Mater Res* 61: 391-398 3. Deffe KM,

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#### **K9.16**

**A Rapid Antigen Detection Assay using Photografted Whole Antibodies.** Robert Sebra<sup>1</sup>, Kristyn Masters<sup>2</sup>, Christopher Bowman<sup>1,3</sup> and Kristi Anseth<sup>1,2</sup>; <sup>1</sup>Chemical and Biological Engineering, University of Colorado-Boulder, Boulder, Colorado; <sup>2</sup>Howard Hughes Medical Institute, Chevy Chase, Maryland; <sup>3</sup>University of Colorado Health Sciences Center, Biomaterials Research Center, Denver, Colorado.

Antibody surface immobilization techniques have had a significant impact on the detection of specific antigens for clinical diagnostics. To date, most antigen detection assays (e.g., standard enzyme-linked immunosorbent assays) rely on monolayer formation or physisorption methods to immobilize antibodies to surfaces. However, that approach exhibits drawbacks associated with antibody coating stability, desorption, and non-specific protein interactions that lead to limited sensitivity (nM), and time-consuming assay procedures (1-10 hours). Further, these issues lead to an inability to detect antigens such as those that have a short half-life in biologically complex fluids. Thus, recent research has focused on methods to bind antibodies covalently to surfaces through conventional protein functional sites, such as amine and carboxy terminal groups, as well as antibody-specific thiol groups. While these approaches reduce the possibility of antibody desorption, surface-bound antibodies and biomolecules often lose their activity and/or selectivity due to conformational and mobility restrictions or mass transfer limitations. Furthermore, antibody activity is often lost due to a reduction of antigen binding sites resulting from the coupling process. In this work, acrylated whole antibodies, termed antimers, were synthesized with the goal of establishing a controlled polymerization method to immobilize antibodies to polymer surfaces in a manner that leads to increased accessibility and high mobility. Further, this approach enables covalent binding of the antibodies to grafted PEG-containing tethers to help prevent non-specific protein interactions while providing independent control over tether density, composition, and location. When integrated, these contributions greatly improve detection sensitivity (< pM) and response time (< 15 min). In this research we demonstrate three significant milestones: the ability to polymerize whole antibodies as polymer grafts, which is integral in maintaining antibody activity and selectivity, the retention of antimer-antigen selectivity in a variety of biologically relevant analyte environments, and the application of a novel photopolymerization method, which allows patterning and fabrication of polymeric microfluidic assays based on antibody-antigen detection. These milestones have been demonstrated with a variety of antigens with varying molecular weight, biological stability and function. Also, current work is being investigated to facilitate the detection of microbial antigens.

#### **K9.17**

**Mossbauer and Raman Spectroscopy of the Iron (III) -Porphyrin Biomaterial for Potential Application as a Spin Based Electronic Device.** Aboubaker Chedikh Beye<sup>1,4</sup>, Sosse Ndiaye<sup>1</sup>, Bassirou Lo<sup>1</sup>, Oumar Sakho<sup>1,4</sup>, Papa Douta Douta Tall<sup>1,4</sup>, N. Pearson<sup>2</sup>, O. Munro<sup>2</sup>, R. M. Erasmus<sup>3</sup>, Vittoria Pischedda<sup>3</sup>, Giovanni Hearne<sup>3</sup> and Wole W. Soboyejo<sup>4</sup>; <sup>1</sup>Groupe de Physique des Solides et Science des Materiaux, Universite Cheikh Anta Diop de Dakar, Dakar, Senegal; <sup>2</sup>School of Chemical and Physical Sciences, University of Natal, Pietermaritzburg, Natal, South Africa; <sup>3</sup>School of Physics, University of the Witwatersrand, Johannesburg, Gauteng, South Africa; <sup>4</sup>International Material Institute, Princeton University, Princeton, New Jersey.

Resonant Raman and 57Fe Mossbauer Spectroscopy (MS) under hydrostatic pressure in a diamond-anvil cell are used to investigate the spin-structure response of the 3d manifold of Fe in the ferric haem-porphyrin [Fe(TPP)(NCS)]. Extreme pressure conditions using extended Mossbauer system have enabled the magnetic-electronic investigations of samples in a volume of pico-litres in the sample cavity of a Diamond Anvil Cell. The study of the pressure response of such organo-metallic systems is fundamentally important because iron porphyrins are found in biology as haem proteins whose activity is sensitively dependent on the structural configuration which may in turn be influenced by the iron spin-state configuration. The results of 57Fe Mossbauer Spectroscopy (MS) under hydrostatic pressure show a pressure-induced spin-switching from the high-moment (nominal atomic spin S = 5/2) to the lowest moment (nominally S = 1/2) electronic state in [Fe(TPP)(NCS)] compound. Such a spin-switching is shown to occur at moderate pressure conditions of 5-10 GPa at room-temperature. Raman spectra of both spin states are compared and associated with different structural configurations of the molecule. Resonant Raman Spectroscopy are used to get additional informations on the vibrational modes. The change of the magnetic moment of such paramagnetic compound from ca. 5.9 BM at low

pressure to ca. 1.8 BM at high pressure is discussed in view of potential applications in molecular electronics.

#### **K9.18**

**Immobilization of Proteins on the Surface of Silanized Hydroxyapatite.** Akira Monkawa, Toshiyuki Ikoma, Syunji Yunoki, Yuri Kumagai and Junzo Tanaka; Biomaterials Center, National Institute for Materials Science, Tsukuba.

Hydroxyapatite (HAp) is widely used as bioceramics for bone and dental tissue reconstructions due to its excellent biocompatibility with hard tissues and high osteoconductivity. HAp has an interesting property as an adsorbent for biopolymers such as protein. The absorption ability of HAp is useful for the preparation of HAp/protein composites. However, the proteins were easily removed from the HAp surface *in vitro* and *in vivo* studies, which depend on the ionic strengths and pH values in aqueous systems. To overcome these problems, it is necessary to bond protein onto HAp surface firmly by using a binding material. Silane coupling agent is one of binding materials with the ability to bond inorganic materials such as glass, mineral fillers, metals and metallic oxides to organic resins. Furthermore, previous studies showed that the silane coupling agent is useful material for coating on the HAp surface. This study focused on the coupling agent as a binding material between HAp and four different proteins such as acidic proteins of fibrinogen, fibronectin and vitronectin, and basic proteins of collagen. These proteins were important factors for the cell attachment onto the materials surfaces in cell culture. We immobilized these proteins onto the surface of HAp sintered body with 20mm in diameter and 2mm in thickness by introducing covalent bonding via aminopropyltriethoxysilane (APS). The existence of APS on the HAp surface was confirmed by zeta-potential measurements. The proteins immobilized on the pure HAp and APS/HAp were observed by atomic force microscope (AFM, Simadzu; SPM 9500) analyses and zeta potential measurements. The stability of protein/APS/HAp and protein/HAp composites was evaluated after soaking in PBS and NaCl solutions with various concentrations. AFM analyses and zeta-potential measurements revealed that proteins are firmly bonded on the APS/HAp surface in the solutions. However, the Col/HAp composite is unstable in the high NaCl concentrations. The fibrinogen, fibronectin and vitronectin are unstable and removed from the pure HAp surface in the PBS solution, however, the APS/HAp was firmly bonded to these proteins in the PBS solution. We concluded that proteins immobilized on the APS/HAp are more stable than those immobilized on the normal HAp in high ionic strength solutions (PBS and NaCl).

#### **K9.19**

Transferred to K10.1

#### **K9.20**

**Hydroxyapatite Coatings Deposited by KrF-Laser Ablation and its Adhesion to Metallic and Ceramic Implants.** Won-Jun Lee<sup>1</sup>, Sang-Wook Lee<sup>1</sup>, Hyelee Kim<sup>1</sup>, Dae-Joon Kim<sup>1</sup> and Jung-Suk Han<sup>2</sup>; <sup>1</sup>Department of Advanced Materials Engineering and Bioengineering Research Center, Sejong University, Seoul, South Korea; <sup>2</sup>Department of Prosthodontics and Dental Research Center, College of Dentistry, Seoul National University, Seoul, South Korea.

Hydroxyapatite (HA),  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ , is currently used as a biomaterial for many applications in both dentistry and orthopedics, because it is the main chemical component of bone. Nevertheless, due to the poor mechanical properties of bulk HA, it cannot be used as implant device materials for load-bearing applications. The solution is to apply HA as a coating on Ti or Ti-based alloy implants. In this way, the mechanical properties of the implants are supported by the metallic structure, while the osteointegration is promoted by the bioactive surface of HA. Plasma-spray (PS) was the first method used for coating implants with HA, and the PS coatings exhibited faster bone healing than uncoated implants; however, there were some issues affecting the long-term stability of the implants. The main problems of PS coatings are related with the presence of other calcium phosphate phases, the porosity and the poor coating-substrate adhesion. In this study, the HA coatings with a high degree of crystallinity and density were prepared by pulsed laser deposition (PLD) method using a KrF excimer laser (at 248 nm). Crystalline HA films could be obtained at elevated temperatures under oxidizing atmosphere containing water vapor. Under such conditions, however, the surface of Ti-base implant is very susceptible to oxidation and the presence of titanium oxide layer at the HA/substrate interface dramatically decreases the adhesion force between the PLD HA film and Ti-base implant. In an attempt to resolve this issue, zirconia/alumina composites were applied as the implant materials. Optimum mechanical properties of implants were obtained by adding alumina into tetragonal zirconia solid solution which consists of  $\text{ZrO}_2$ ,  $\text{Y}_2\text{O}_3$  and  $\text{Nb}_2\text{O}_5$ . The crystallinity and stoichiometry of the PLD HA films deposited on zirconia/alumina were examined at various substrate temperatures and water vapor pressures. The adhesion

forces between the PLD HA film and zirconia/alumina substrate were also measured with varying PLD processing parameters, and the reactions at the HA/substrate interface were examined.

#### **K9.21**

**Fabrication and Characterization of Active Matrix Array for Cell Probing and Screening.** Seung-Ik Jun<sup>1</sup>, Timothy E. McKnight<sup>2</sup>, Anatoli V. Melechko<sup>2,1</sup>, Michael L. Simpson<sup>1,2</sup> and Philip D. Rack<sup>1</sup>; <sup>1</sup>Materials Science and Engineering, The University of Tennessee, Knoxville, Tennessee; <sup>2</sup>Molecular Scale Engineering and Nanoscale Technologies Research Group, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

In order to achieve multiple cell stimulation and recording with high electrode density and low manufacturing cost, we have fabricated and characterized an active matrix array for cell probing and screening that provides great potential to execute direct cell sensing/probing and recording with high electrode density. Each unit pixel in the array is individually addressed by a thin film transistor (TFT) and the vertically aligned carbon nanofibers (CNF) for probing cells are fabricated on the drain electrode of TFT. The CNF is grown by direct current plasma enhanced chemical vapor deposition (DC-CVD) using a nickel catalyst. The impedance difference between cell probing site and reference electrode is recorded by semiconductor analyzer connected with output terminals. Also, the impedance change with frequency of cell probing can be measured by applying frame signal input and recording the signal stored in storage capacitors after frame scanning in the TFTs. Consequently, actively addressed nanofiber arrays enable bidirectional interfacing with tissue matrices in a format that provides intercellular positioning of electrode elements as well as the potential for intracellular residence of probes within individual cells. In our research, we exploit these non-planar electrode systems for efficient coupling with excitable cell matrices as well as for intracellular biochemical manipulation of single cells. In this presentation, we will discuss the fabrication sequence of the inverted metal-oxide-semiconductor (MOS) TFT, and will elaborate the materials issues related to integrating the carbon nanofibers with the TFT.

#### **K9.22**

**SPM and Charge Transport Measurements Through DNA Molecules of Complex Sequence.** Hezy Cohen<sup>1</sup>, Claude Nogues<sup>1,2</sup>, Ron Naaman<sup>2</sup> and Danny Porath<sup>1</sup>; <sup>1</sup>Physical Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel; <sup>2</sup>Department of Chemical Physics, The Weizmann Institute, Rehovot, Israel.

The ability of DNA to transport charge carriers and the possible mechanisms for this transport were debated over the past few years. Part of the debate originated from the variety of measurement approaches, samples preparation, experimental set-ups and environmental conditions. The two main factors that limited charge transport in measurements previously reported were the attachment to the surface along the molecule and the non-chemical bonding of the molecules to the contact electrodes. Indeed, current was measured in short molecules and blocked in long molecules attached to the surface. Inspired by Cui et al.,<sup>1</sup> we have adopted an experimental approach that enables to overcome these difficulties by measuring current through DNA molecules chemically connected on both sides to a metal substrate and a gold particle,<sup>2</sup> which is contacted *inacontrolledway* by a metal atomic force microscope (AFM) tip.<sup>3</sup> Here, we report on experiments in which we measure currents higher than 220 nA at 2 V, indicating charge transport through 26 base-pairs long DNA molecules of complex sequence. Moreover, we observe a non-zero gap and a peak structure in the current-voltage curves that is reproduced for many molecules and by two different measurement methods. We present a comprehensive set of control experiments that verify our findings. These include: simultaneous topography-current maps, measurements on non-complementary strands, current-distance upon stretching the molecules, 3D-mode measurements performed on the insulating surrounding layer and checking the effect of humidity. 1. Cui, X. D.; Primak, A.; Zarate, X.; Tomfohr, J.; Sankey, O. F.; Moore, A. L.; Moore, A. T.; Gust, D.; Harris, G.; Lindsay, S. M. "Reproducible Measurement of Single-Molecule Conductivity" *Science* 2001, 294, 571. 2. Claude Nogues, Sidney R. Cohen, Shirley S. Daube, and Ron Naaman, "Electrical properties of short DNA oligomers characterized by conducting atomic force microscopy", *PCCP*, 2004, 18. 3. Hezy Cohen, Claude Nogues Ron Naaman and Danny Porath, "Direct Measurement of Electrical Transport Through Single DNA Molecules", submitted.

#### **K9.23**

**Self-assembled Cell-MEMS Devices for Correlation of Molecular and Mechanic-Electro Performance of Muscle Cells.** Jianzhong Xi, Eric Dy and Carlo Montemagno; Bioengineering Dept., UCLA, Los Angeles, California.

To combat the deleterious effects of radiation and other stresses on long-term human space exploration, it is critical to better understand cardiovascular cell function. The correlation of molecular activities and mechanic-electro performance of muscle cells will provide us with complete dynamic data to develop adequate countermeasures to combat these adverse effects. Unlike the conventional measurements techniques, we have created a novel method capable of in situ characterization of the mechanical properties of muscle at both tissue and single-cell levels using a self-assembly system. This system has shown the capability of spatially and selectively directed growth and differentiation of myocytes into single muscle bundles in situ, attachment of these functional bundles to MEMS structures, and the controlled release of the resultant hybrid devices without any manual assistance. The mechanical properties of the neonatal ventricular myocytes 1-3-day-old Sprague-Dawley rats (NRVMs), such as substrate-induced stress and Young's modulus, have been measured using this force transducer and were found to be 2-2.5 kPa and 40 kPa respectively. Here we expand this system to dynamically monitor the cellular activities in response to the external applied stresses. It has been noted that intracellular calcium concentrations of cardiac myocytes fluctuate between 10-5M - 10-7M upon contraction and relaxation respectively. Using an intracellular fluorescent calcium indicator we can correlate fluorescent density with cellular function. By monitoring the fluorescent signal, we can visualize calcium fluxes and in turn determine what state a particular cell is in. In addition to detecting cellular activity, the effect of UV radiation and mechanic stresses on the cells are also under investigation. Further study of genetically engineered cells will enable us to in situ monitor the molecular and genetic information and simultaneously investigate the correlation of their activities with the external applied stresses. The self-assembly system established is not only suitable for studying dynamic mechanics of muscle cells, including the correlation of intracellular activities with external factors, but also applied to the studies of other cells.

SESSION K10: Biomaterials: Theory and Experiment  
 Chair: Elaine DiMasi  
 Friday Morning, April 1, 2005  
 Room 2007 (Moscone West)

#### 8:30 AM K10.1

**Simulations and Design of a New Green Fluorescence Protein Mutant.** Murat Cetinkaya<sup>1</sup>, Ahmet Zeytun<sup>2</sup>, Andrew Bradbury<sup>2</sup> and Melik C. Demirel<sup>1</sup>; <sup>1</sup>Engineering Science and Mechanics, The Pennsylvania State University, University Park, Pennsylvania; <sup>2</sup>Bioscience Division, Los Alamos National Laboratory, Los Alamos, New Mexico.

A set of new green fluorescence protein (GFP) mutants are experimentally created by modifying four loop regions of GFP. The excitation / emission spectra of a number of different GFP mutants were determined and some GFPs showed a reduction in both the absorption and emission at each wavelength. We have performed molecular dynamics simulations of these mutants using the AMBER force field. We especially concentrated on loop3 (residues 171-175) modifications since new residue insertions to this region cause drastic changes in fluorescence intensity properties. A possible explanation is the quenching of the chromophore located at the center of the structure due to local unfolding. A 10 ns molecular dynamics simulation showed that insertion in the loop3 region creates local unfolding of the "beta-can" structure of GFP, confirming our hypothesis for the change in fluorescence intensity of the protein. We have also performed simulations regarding other loop regions and compared with the corresponding experimental data.

#### 8:45 AM K10.2

**Nano-Phononics in Biological Systems.** Alexander A. Balandin and Vladimir A. Fonoberov; Nano-Device Laboratory, Department of Electrical Engineering, University of California, Riverside, California.

Viruses have recently attracted attention as biological templates for assembly of nanostructures and nanoelectronic circuits [1]. They can be coated with metals, silica or semiconductor materials and form end-to-end nanorod assemblies. Such viruses as tobacco mosaic virus (TMV) and M13 bacteriophage have appropriate cylindrical shape and particularly suitable dimensions: M13 is 860 nm long and 6.5 nm in diameter, while TMV is 300 nm long, 18 nm in diameter and with a 4 nm in diameter axial channel. The knowledge of the phonon, i.e. vibrational, modes of these viruses is important for material and structural characterization of the virus-based nano-templates, for in-situ monitoring of the nanostructure self-assembly, and for understanding properties of the biological-inorganic interfaces. In this paper we review our recent theoretical and experimental results on phonon spectra of TMV and M13 bacteriophage immersed in air and water. The low-frequency phonon dispersion has been rigorously

calculated using the complex-frequency approach. The radial breathing modes of TMV and M13 viruses in air are found to be 1.85 cm<sup>-1</sup> and 6.42 cm<sup>-1</sup>, respectively. If the viruses are in water, the above frequencies become 2.10 cm<sup>-1</sup> and 6.12 cm<sup>-1</sup>, respectively [2]. The quality factor Re(w)/Im(w) for radial vibrations of TMV in water is about 3.6 for the radial breathing mode and about 10 for the second radial mode. Structurally informative bands of the vibrational spectrum have been studied experimentally by means of the non-resonant micro-Raman spectroscopy. We analysed the damping of vibrations in water and discussed the application of the micro-Raman spectroscopy for monitoring of the virus-based self-assembly processes [3]. The authors acknowledge the financial and program support of the Microelectronics Advanced Research Corporation (MARCO) and its Focus Center on Functional Engineered Nano Architectonics (FENA). [1]. W. Shenton, et al., Adv. Mater. 11, 253-256 (1999); C.E. Flynn, et al., Acta Materialia 51, 5867-5880 (2003). [2]. V.A. Fonoberov and A.A. Balandin, Phys. Stat. Sol. B 241, R67-R69 (2004); A.A. Balandin and A.V. Fonoberov, Vibrational modes of the nano-template viruses, J. Biomedical Nanotechnology, in press, 2005. [3] see details at <http://ndl.ee.ucr.edu/>

#### 9:00 AM K10.3

**Guanine Quartet Networks Stabilized by Cooperative Hydrogen Bonds.** Roberto Otero Martin, Maya Schoeck, Luis M. Molina, Erik Laegsgaard, Ivan Stensgaard, Bjork Hammer and Flemming Besenbacher; Department of Physics and Astronomy, University of Aarhus, Aarhus, Denmark.

Hydrogen bonding between DNA or RNA bases is one of the main interactions that determine the conformation and biochemical interactions of nucleic acid (NA) molecules. Apart from the Watson-Crick model for base pairing, NA bases can form other hydrogen-bonded aggregates that lead to different DNA structures, like G-quadruplexes or i-motifs. In spite of the increasing evidence for the in vivo existence and function of these structures, the exact physico-chemical nature of the hydrogen bonds and the importance of charge transfer contribution to the stabilization energy associated to hydrogen bonding in these structures is still under debate. Here we show, by high-resolution, variable-temperature Scanning Tunneling Microscopy (STM), that the NA base guanine (G), deposited under ultra-clean conditions onto the inert Au(111) substrate, self-assembles into a hydrogen-bonded network of G-quartets with the same structure as that found in quadruplex telomeric DNA. Comparison with our Density Functional Theory (DFT) calculations shows that the strong preference of G molecules to form quartets arises from a cooperative effect that strengthens the hydrogen bonds within the G-quartet network relative to those in isolated G dimers.

#### 9:15 AM K10.4

**Molecular Recognition in 2D Binary Mixtures of DNA Bases Studied by STM.** Maya Schoeck, Eva Rauls, Roberto Otero Martin, Wei Xu, Erik Laegsgaard, Ivan Stensgaard, Bjork Hammer and Flemming Besenbacher; Department of Physics and Astronomy, University of Aarhus, Aarhus, Denmark.

Molecular recognition events between complementary nucleic acid bases are fundamental for many biological processes, like DNA replication. These processes have found an application in the field of Nanotechnology, and strands of complementary DNA sequences have been used to direct the self-assembly of nanostructures. In principle, the complementarity in hydrogen-donors and acceptors groups in single DNA bases might as well lead to molecular recognition processes, that could be used to control 2D molecular assemblies as well. However, the existence of "wobble" or "deviant" base pairs, and a possible disturbing effect of the substrate on the hydrogen bonds made this possibility more difficult to explore. In this contribution we compare the 2D molecular networks formed on Au(111) upon deposition of the binary mixtures G-C (purine-pyrimidine pair of complementary bases) and A-C (purine-pyrimidine pair of non-complementary bases) by means of a combination of STM experiments and DFT calculations. We show that, after a gentle annealing to 80 C the non-complementary bases segregate into islands of pure A and a network of pure C, whereas the complementary bases G and C form a network that cannot be separated by annealing up to the desorption temperature for C. High-resolution STM images allow us to identify structures that contain G-C bonds, possibly with the a structure similar to the Watson-Crick pairs in DNA molecules. The stronger bond between G and C molecules with respect to G-G or G-C pairs explain the enhanced thermal stability of the combined G-C mixture. This result shows that the hydrogen-bonding interaction alone can steer the processes necessary for molecular recognition to take place in 2D networks, thereby opening new avenues to design molecular self-assemblies with desired geometries.

#### 9:30 AM K10.5

**A Solution to the Streptavidin-Biotin Paradox?** Frederic Pincet and Julien Husson; Laboratoire de Physique Statistique, Ecole

In the past decade, there has been an increasing number of experiments dedicated to the measurement of the strength of a single bond. The streptavidin-biotin complex has been more extensively studied than any other. However, the results of the various types of approach seem contradictory. Biomembrane Force Probe [1], DNA stretching [2] and flow chamber [3] experiments lead to very different conclusions. Besides, the energy landscapes obtained with these experimental measurements are not compatible with the one predicted by Molecular Dynamics Simulations [4]. Therefore, the streptavidin-biotin complex seems technique sensitive. All these discrepancies form an apparent paradox in the observed behavior of this complex. In this presentation, all these results will be conciliated by experiments performed with a Biomembrane Force Probe in which parameters of formation and detachment of the bond will be varied in an unusual manner in order to systematically probe the energy landscape. The results will be confirmed by completely applying Kramers equations on metastable systems. References: 1. Merkel, R., Nassoy, P., Leung, A., Ritchie, K., & Evans, E. (1999) *Nature* 397, 50-53. 2. Smith, S.B., Cui, Y., & Bustamante, C. (1996) *Science* 271, 795-799. 3. Pierres, A., Touchard, D., Benoliel, A-M, and Bongrand, P. (2002) *Biophys. J.* 82 3214-3223. 4. Izrailev, S., Stepaniants, S., Balsara, M., Oono, Y., & Schulten, K. (1997) *Biophys. J.* 72, 1568-1581.

#### 9:45 AM K10.6

**Quantum Chemistry Approach to Modeling of Molecular Adsorption at Crystal Interfaces.** Andrzej Wierzbicki<sup>1</sup>, Edward A. Salter<sup>1</sup>, Selim Elhadj<sup>2</sup> and Patricia M. Dove<sup>2</sup>; <sup>1</sup>Department of Chemistry, University of South Alabama, Mobile, Alabama; <sup>2</sup>Department of Geosciences, Virginia Tech, Blacksburg, Virginia.

Recent rapid progress in the development of quantum chemistry software and the constant improvement of computer performance have made it possible to start implementing more realistic models of molecular adsorption at crystal interfaces. It is widely believed now that interfacial adsorption models based on the molecular mechanics approach, which uses a continuum dielectric model to represent solvent, could provide an inadequate description of adsorption, especially in those cases where solvent molecules many significantly impact the adsorption process. We will address the complex issue of polypeptides adsorption at crystal interfaces in the presence of explicit water molecules. We will use the semiempirical and ab initio methods of quantum mechanics to address the role of the solvent for the binding energy of adsorption and the conformation of adsorbed polypeptides at crystal interfaces.

SESSION K11: Advances in Scanning Probes and  
In-Vitro Mineralization  
Chair: Elaine DiMasi  
Friday Morning, April 1, 2005  
Room 2007 (Moscone West)

#### 10:30 AM K11.1

**Optical Spectroscopies for Biological Structure at Interfaces.** Neil Anderson, Clayton Yang, John Stephenson, Lee Richter and Kimberly Briggman; NIST, Gaithersburg, Maryland.

*In situ* linear and novel nonlinear optical spectroscopies are being developed to probe biologically relevant, surface- and membrane-bound molecules. New interface-specific techniques based on infrared, Raman and vibrationally-resonant sum frequency generation (VR-SFG) spectroscopies are being used to study biological interfaces such as biological membrane mimics and incorporated membrane protein structures. These optical techniques can uniquely probe the structure of specific native functional groups of molecules without the need for molecular tagging. Moreover, the use of multiple techniques allows unambiguous determination of molecular structure and orientation of molecules at the interface. Presented results will include determination of the gel-fluid phase transition temperature of lipid layers in supported bilayer membranes, and the influence of membrane fluidity on the incorporation and structure of transmembrane polypeptides within the membrane.

#### 10:45 AM K11.2

**Bioelectromechanical Imaging by Scanning Probe Microscopy.** Galvani's Experiment on the Nanoscale. Sergei V. Kalinin<sup>1</sup>, Brian J. Rodriguez<sup>2</sup>, Junsoo Shin<sup>3,1</sup>, Arthur P. Baddorf<sup>1</sup> and Alexei Gruverman<sup>2</sup>; <sup>1</sup>Condensed Matter Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee; <sup>2</sup>Department of Materials Science and Engineering, North Carolina State University, Raleigh, North Carolina; <sup>3</sup>Department of Physics and Astronomy, The University of Tennessee, Knoxville, Tennessee.

Strong coupling between electrical and mechanical behavior is a universal feature of biological systems. Indeed, the effect of electric current on muscle contraction in a frog leg was discovered as early as the 18th century by Galvani. In the last 50 years, it was shown that nearly all biological tissues based on polymers such as collagen, cellulose, or keratin, possess strong piezoelectric properties. It has been postulated that piezoelectricity is intrinsically related to the fundamental mechanisms of tissue development including bone growth and remodeling. However, macroscopic studies of the elastic and electromechanical properties in biological systems are inherently limited by the complex structure of these materials, thus necessitating studies of electromechanical and elastic properties of biological systems from mesoscopic to molecular levels. Here, we demonstrate a scanning probe microscopy (SPM) based approach for electromechanical imaging and spectroscopy of biological systems based on the combination of 3-dimensional Piezoresponse Force Microscopy and Ultrasonic Force Microscopy that allows local electromechanical and elastic imaging with nanometer resolution. This technique is used to address several biological systems, including hair, tooth and butterfly wings. 3D piezoelectric activity in butterfly wings is interpreted in terms of the relative orientation of chitin molecules and is correlated with the surface topography and elasticity map. In the human tooth, we observed a vector electromechanical response of a single collagen molecule bundle that allows the local molecule orientation to be reconstructed. This allows us to repeat Galvani's experiment on the nanoscale, 230 years later and with a million times higher resolution. The future opportunities of electromechanical SPM for characterization of complex biological systems are discussed. Research performed as a Eugene P. Wigner Fellow (SVK) at ORNL, managed by UT-Battelle, LLC under DOE contract DCE-AC05-00OR22725. AG acknowledges financial support of the National Science Foundation (Grant No. DMR02-35632).

#### 11:00 AM K11.3

**Aspartate Chain Length Controls Calcite Step Morphology by Differential Step Recognition.** Selim Elhadj<sup>1</sup>, Edward Salter<sup>2</sup>, Andrzej Wierzbicki<sup>2</sup>, Patricia Dove<sup>1</sup>, Nizhou Han<sup>1</sup> and James De Yoreo<sup>3</sup>; <sup>1</sup>Geosciences, Virginia Tech, Blacksburg, Virginia; <sup>2</sup>Chemistry, University of South Alabama, Mobile, Alabama; <sup>3</sup>Chemistry and Material Sciences, Lawrence Livermore National Labs, Livermore, California.

Most controlled crystallization is believed to be achieved under the direction of macromolecular protein-based templates with specific affinities for accumulating and assembling the desired growth units. This reliance upon cellular proteins with specific sequences and structures suggests that the chemistry and stereochemistry of the amino acids forming the proteins are essential in conferring targeted activity in controlled biosynthesis. Calcium carbonate is a key biogenic mineral and model system to study the molecular mechanisms by which amino acids and polypeptides interact with individual steps. Investigations of molecularly resolved crystal-peptide interactions can reveal how biomolecules achieve regulated growth during biomineralization. Our previous studies have focused on the role of specific single and dipeptide species known to constitute a significant fraction of the proteins involved in biomineralization processes, in particular, acidic amino acids and aspartate. In this study, we extend our investigation to the role of polyaspartate chain length because they represent an integral part of the active site of these biomolecular sequences. In particular, the effect of polyaspartates (Aspn, n=1,2,4,5,6) on the kinetics and thermodynamics of calcite growth were investigated and compared with models of calcite-Aspn interactions. Using in situ Atomic Force Microscopy and precisely characterized solutions, our experimental measurements of growth kinetics and observations of the calcite hillock morphology at the nanoscale show that step directions with acute and obtuse geometries are affected differently for all aspartate derivatives. Short chain aspartates (n<=2) roughen acute steps more strongly than obtuse steps. This situation is exactly reversed for longer chain aspartates (n>3). Further, the Aspn concentration required to roughen the steps decreases with increasing length of the Asp peptide. Circular Dichroism measurements were performed to confirm the aspartate conformations assumed in the modeling. We examine the molecular origin for these experimental findings using molecular modeling. Not unexpectedly, due to cooperative binding, the concentration required to affect step edge morphology is predicted to decrease exponentially with increasing aspartate chain length. This trend correlates with the measured inhibition of step motion and can be explained by relating chain length to calcite step binding and dehydration energy. Our findings suggest that there is a qualitative change in the nature of aspartate-calcite step interactions with increasing aspartate chain length. This change is accompanied by an exponential increase in the degree of aspartate binding. This chain length dependent differential binding to opposing calcite steps may be an additional mechanism by which biomineralizing proteins can achieve regulation of calcite growth.

#### 11:15 AM **K11.4**

**Probing the Effect of Different Polypeptide Biomineral Recognition Sequences on the Growth of Calcite.** Il Won Kim<sup>1</sup>, Molly R. Darragh<sup>2</sup>, Christine A. Orme<sup>2</sup> and John Spencer Evans<sup>1</sup>; <sup>1</sup>Laboratory for Chemical Physics & Center for Biomolecular Materials Spectroscopy, New York University, New York, New York; <sup>2</sup>Department of Chemistry and Materials Science, Lawrence Livermore National Laboratory, Livermore, California.

Recent studies have established that the nacre layer of mollusks possess a number of proteins that assist in the formation and stabilization of the aragonite polymorph during shell development. However, very little is known regarding the mechanism of protein manipulation of calcium carbonate biomineral formation. In particular, the relationship between sequence, structure, and recognition of mineral surface features is not clearly understood. Previously, we identified 30 AA N-terminal binding domains that originate from three different nacre-specific proteins (AP7, AP24, n16). These three domains, termed AP7-N, AP24-N, and n16-N, induce morphological changes in calcium carbonates *in vitro* and are similar in amino acid composition, but differ significantly from each other with regard to their primary sequences. Using a flow cell-equipped AFM instrument, we investigated the sites of adsorption of each mineral-binding domain onto calcite dislocation hillocks. We find that AP7-N and AP24-N both preferentially interact with the acute step edges of dislocation hillocks, leading to bunching of these step edges. Moreover, AP7-N and AP24-N both induce the formation of deposits on terrace surfaces. Comparatively, AP24-N exhibited higher mineral modulation activity over AP7-N with regard to step edge inhibition and deposit formation. In contrast, n16-N exhibits an interaction preference for terrace surfaces, which leads to pinning effects that block the advance of acute and obtuse hillock steps and the emergence of new non-parallel steps. Random scrambling of the n16-N and AP7-N sequences resulted in substantially reduced mineral modification activities, indicating that the primary sequence of both polypeptides is crucial for recognition of surface features.

#### 11:30 AM **K11.5**

***InSitu* Characterization of Surface Evolution on Titanium in Hydrogen Peroxide Containing Solutions.** Julie J. Muyco<sup>1,2</sup>, Jeremy J. Gray<sup>1</sup>, Timothy V. Ratto<sup>1</sup>, Christine A. Orme<sup>1</sup>, Joanna McKittrick<sup>2</sup> and John Frangos<sup>3</sup>; <sup>1</sup>Lawrence Livermore National Laboratory, Livermore, California; <sup>2</sup>University of California, San Diego, La Jolla, California; <sup>3</sup>La Jolla Bioengineering Institute, La Jolla, California.

Titanium implants have been used for decades with success in various applications. The characteristic of titanium that allows acceptance in the body is not well defined. It is hypothesized that the interaction of titanium implants with inorganic components of bodily fluid is key to the acceptance of titanium in the body. The component of bodily fluid that is emphasized in these studies is hydrogen peroxide, a chemical species produced during the inflammatory response. When titanium is exposed to hydrogen peroxide, a Ti-peroxy gel (TiOOH) is formed. Three possible functions of Ti-peroxy gel are: reduction of the inflammatory response through the reduction of hydrogen peroxide and other reactive oxygen species; creation of a favorable surface for calcium phosphate nucleation; and as a transitional layer between the soft tissue and the stiff titanium. Traditional surface characterization techniques often require high vacuum environments that alter the actual sample-solution interface. Techniques that allow samples to remain in solution and be observed in real-time are used in our studies. Atomic force microscopy (AFM) force spectroscopy, electrochemical techniques, Raman spectroscopy, and ellipsometry are each used *insitu* to define kinetic and mechanical properties of Ti-peroxy gel as it forms over time on titanium during exposure to hydrogen peroxide. We observe real-time changes in the native oxide layer on titanium in hydrogen peroxide containing solution, including the formation of a Ti-peroxy gel layer. Peaks attributed to Ti-peroxy gel were seen to emerge over the course of several hours using *insitu* Raman spectroscopy. Force-distance curves suggest a layer that thickens with time on the titanium sample surface. Data from electrochemical impedance spectroscopy (EIS) and polarization resistance measurements showed that changes in the surface layers could be monitored in solution over time. This work was performed under the auspices of the U.S. Department of Energy by University of California Lawrence Livermore National Laboratory under contract no. W-7405-Eng-48. UCRL-ABS-207618

#### 11:45 AM **K11.6**

**An in-situ AFM Study of Crystal Growth on Brushite (010): The Influence of Modifiers.** Jennifer L. Giocondi<sup>1</sup>, Molly Darragh<sup>1</sup>, Roger Qui<sup>1</sup>, James DeYoreo<sup>1</sup>, Christine A. Orme<sup>1</sup>, Ruikang Tang<sup>2</sup>, George Nancollas<sup>2</sup> and John R. Hoyer<sup>3</sup>; <sup>1</sup>Directorate of Chemistry and Materials Science, Lawrence Livermore National Laboratory, Livermore, California; <sup>2</sup>Department of Chemistry, University at Buffalo, SUNY, Buffalo, New York; <sup>3</sup>The Children's

Hospital of Philadelphia, University of Pennsylvania, Philadelphia, Pennsylvania.

Understanding the mechanisms utilized in the biological construction of mineralized structures is important both for disease prevention and treatment as well as the synthesis of biomimetic materials. Brushite, dicalcium phosphate dihydrate, is an important biomineral found in such diverse matter as guano, human kidney stones, and dental calculi. It is also believed to be a precursor in the precipitation of hydroxyapatite to form bones and teeth. The goal of our research is to understand brushite growth on the microscopic level. In the work presented here we use in-situ AFM to investigate the crystal growth from dislocation hillocks on the (010) face of brushite in the presence of two macromolecules: citrate and osteopontin. Citrate was chosen to isolate the affect of carboxylic acid groups on the growth morphology and it has been shown that citrate inhibits brushite growth in bulk experiments. Osteopontin is an important protein involved with bone formation and is known to affect the growth of both hydroxyapatite and calcium oxalate. We found that osteopontin slows the kinetics of step motion and alters the step directions. Citrate on the other hand, has little effect on the step kinetics but changes the step-edge free energy leaving the step directions unchanged. These changes to the step-edge free energy affect the overall growth rate of the crystal by changing the density of steps on the surface. It is interesting to note that it may be much more efficient for organisms to control growth rate by adding small quantities of surfactant than to change the local solution supersaturation and that this may represent a new pathway for controlling mineralization. This work was performed under the auspices of the US Department of Energy under contract #W-7405-Eng-48.

SESSION K12: Biomimetic Minerals and High Resolution Probes  
Chair: Elaine DiMasi  
Friday Afternoon, April 1, 2005  
Room 2007 (Moscone West)

#### 1:30 PM **\*K12.1**

**Nanocrystalline Calcium Phosphates and Plaster of Paris from Deep-Sea Medusae - What can be Learned from Biomineralisation?** Matthias Epple, Inorganic Chemistry, Univ Duisburg-Essen, Essen, Germany.

Biomineralisation is the utilisation of inorganic materials by living organisms. Bone, teeth, and mollusk shells are prominent examples. By investigating and mimicking these biological processes and the structure of biominerals, new and better biomaterials for clinical use can be prepared. This is demonstrated first on nanocrystalline calcium phosphates that have chemical and crystallographic properties close to bone mineral. In combination with biodegradable polyesters, individual implants for the regeneration of skull defects can be prepared. However, nature's ability to crystallize and structure biominerals is still not achieved by materials scientists. This is shown on the example of deep-sea medusae that use statoliths of calcium sulphate hemihydrate (Plaster of Paris) for orientation. It is impossible to crystallise this hygroscopic material in the chemical laboratory.

#### 2:00 PM **K12.2**

**Synchrotron X-ray Studies on the Effect of Mg Ions on Oriented Growth of Calcite on Alkanethiol Functionalized Self-Assembled Monolayer.** Seo-Young Kwak<sup>1</sup>, Elaine DiMasi<sup>1</sup>, Yong-Jin Han<sup>2</sup> and Joanna Aizenberg<sup>2</sup>; <sup>1</sup>National Synchrotron Light Source, Brookhaven National Laboratory, Upton, New York; <sup>2</sup>Bell Laboratories, Lucent Technologies, Murray Hill, New Jersey.

The effects of Mg<sup>2+</sup> ions upon the nucleation of oriented calcite crystals in the presence of self-assembled monolayers (SAMs) of  $\omega$ -terminated alkanethiols (HS(CH<sub>2</sub>)<sub>n</sub>X; X = CO<sub>2</sub><sup>-</sup> and SO<sub>3</sub><sup>-</sup>, n = 10 and 15) supported on gold films were studied with Synchrotron X-ray diffraction. Mg<sup>2+</sup> ions were added to the crystallizing solution in various mole ratios of Mg<sup>2+</sup>/Ca<sup>2+</sup> (m) ranging from 0 to 4.0. Mg<sup>2+</sup> ions are of particular interest since they are found in biological environments in high concentrations and are believed to play a critical role in CaCO<sub>3</sub> formation. This study revealed new details about the underlying substrate texture; preferred orientations of calcite crystals nucleated from different SAMs were of different crystallographic planes depending on the functional groups. For low Mg<sup>2+</sup> content, SO<sub>3</sub><sup>-</sup> functionalized films nucleated primarily the (106) calcite face, C15 films nucleated the (012) face, and C10 films showed a greater number of surface-normal [104] peaks. However, Mg<sup>2+</sup>/Ca<sup>2+</sup> ratio of 2 and greater defeated this preferred orientation and created a powder pattern. In-plane diffraction from the coarse calcite powders indicated a shift to higher two-theta and a corresponding peak broadening with increasing Mg<sup>2+</sup>/Ca<sup>2+</sup> ratio. According to the refinement of lattice

parameters,  $Mg^{2+}$  incorporation shrinks the calcite lattice by a few percent ( $\Delta a \sim 1.3\%$  and  $\Delta c \sim 2.0\%$ ). We discuss these SAM-nucleated crystals in the contexts of geological and biogenic magnesian calcites.

### 2:15 PM K12.3

**Modification of Calcium Carbonate Crystal Morphology Induced by Macromolecules Extracted from Natural Bioceramics.** Maria Soledad Fernandez<sup>1</sup>, Andronico Neira-Carrillo<sup>1</sup>, Patricio Spencer<sup>1</sup>, Jose Ignacio Arias<sup>1</sup>, Maria Jose Navarrete<sup>1</sup>, Marcos Farina<sup>2</sup> and Jose Luis Arias<sup>1</sup>; <sup>1</sup>Veterinary Sciences, Universidad de Chile and CIMAT, Santiago, Chile; <sup>2</sup>Histology and Embryology, Universidade Federal Rio de Janeiro, Rio de Janeiro, Brazil.

Biom mineralization leads to the formation of inorganic crystals with unique, ordered, refined shapes that are regulated by specific macromolecules. Bioceramics are composite materials, composed of an intimately association of organic macromolecules and inorganic crystals organized on a scale from angstrom to millimetres, where the minute amount of organic material not only increases the mechanical properties of the bioceramic, but also contributes to the nucleation, growth, shape and final organization of the inorganic phase. In order to know how the organic phase regulates the formation of hard tissues, we evaluated the effect of soluble matrices extracted from different bioceramics on crystal morphology. Soluble matrices obtained after decalcification of the bioceramics were biochemically characterized by electrophoresis and dot blot using monoclonal antibodies against some proteoglycans, and the effect on crystal morphology using in vitro crystallization assays was analyzed by SEM. Crystal morphology modifications are closely related with the structure and charging of the organic macromolecules involved.

### 2:30 PM K12.4

**Lipidated Peptides as Templates for CaCO<sub>3</sub> Mineralization.** Kros Alexander<sup>1</sup>, Silvia Cavalli<sup>1</sup>, Daniela C. Popescu<sup>2</sup>, Emily E. Tellers<sup>1</sup>, Mark Overhand<sup>1</sup> and Nico A. J. M. Sommerdijk<sup>2</sup>; <sup>1</sup>Leiden Institute of Chemistry, Leiden University, Leiden, Netherlands; <sup>2</sup>Laboratory of Macromolecular and Organic Chemistry, Eindhoven University of Technology, Eindhoven, Netherlands.

The formation of crystals in nature is a very sophisticated process. In order to gain a better understanding of this fascinating event biomimetics are chosen as templates. In particular, well-defined Langmuir monolayers can be employed as molecular blueprints for the oriented nucleation of inorganic materials. In our study a series of amphiphilic peptides were designed incorporating a peptide head group of variable length: two, three and four repeated units of alternating hydrophilic and hydrophobic amino acid residues. This alternation plays a primary role in the generation of beta-sheet secondary structure at the air/water interface. Furthermore a phospholipid tail was introduced to stabilize the monolayer. This approach should provide a network of carboxylic acid groups positioned at specific distances in order to direct CaCO<sub>3</sub> calcite crystal growth. In the work described here self-organizing beta-sheet monolayers acted as template in the crystallization of CaCO<sub>3</sub>, resulting in habit-modified calcite crystals with two main kinds of morphologies.

### 2:45 PM K12.5

**Investigating Protein-Mineral Interactions in a Marine Invertebrate System.** Germaine Fu<sup>1</sup>, Suresh Valiyaveetil<sup>3</sup>, Brigitte Wopenka<sup>4</sup>, Siping Roger Qiu<sup>5</sup>, James J. De Yoreo<sup>5</sup> and Daniel E. Morse<sup>2,1</sup>; <sup>1</sup>Biomolecular Science and Engineering Graduate Program, University of California, Santa Barbara, Santa Barbara, California; <sup>2</sup>Institute for Collaborative Biotechnologies, University of California, Santa Barbara, Santa Barbara, California; <sup>3</sup>Department of Chemistry, National University of Singapore, Singapore, Singapore; <sup>4</sup>Department of Earth and Planetary Sciences, Washington University, St. Louis, Missouri; <sup>5</sup>Department of Chemistry and Materials Science, Lawrence Livermore National Laboratory, Livermore, California.

The predominance of acidic proteins and polysaccharides detected in biom mineralized CaCO<sub>3</sub> structures suggests that mineralization is controlled through interactions between charged macromolecules and mineral ions and surfaces. In this study, the most acidic proteins were selectively isolated from abalone shell nacre. These proteins are two variants of 8.7 and 7.8 kDa designated AP8 (for aragonite proteins of approximately 8 kDa). The AP8 proteins have compositions dominated by Asx (~35 mol%) and Gly (~40 mol%) residues, indicating that their structures have high Ca<sup>2+</sup> binding capacity and backbone flexibility. In vitro mineralization of CaCO<sub>3</sub> in the presence of the purified AP8 proteins results in elongated calcite crystals asymmetrically rounded at the acute edges of the rhombohedral faces. In contrast, crystals grown with nacre proteins depleted of AP8 retain the morphology of unmodified calcite rhombohedra, demonstrating that the AP8 proteins are more effective crystal-modulators than

other proteins from the same biom mineralized material. AFM analyses reveal that stereochemical recognition by the AP8 proteins occurs at the step edges of calcite hillocks to modify both the kinetics and morphology of crystal growth. By combining molecular with conventional macroscopic crystal analyses, we provide direct evidence to support the hypothesis that protein-mineral interactions at the step edges of crystal surfaces can be responsible for the corresponding morphologies of macroscopic crystals. These observations thus help resolve the competing theories of face- versus step-specific interaction between proteins and crystal surfaces proposed for related biom mineralization systems, and provide insight into the complex interactions that take place between newly forming biogenic minerals and the macromolecules that control their crystallization.

### SESSION K13: Biomimetic Bone and Implant Materials

Chair: Elaine DiMasi

Friday Afternoon, April 1, 2005

Room 2007 (Moscone West)

### 3:30 PM K13.1

**Well Dispersed Nanophase Titania in Poly-lactic-co-glycolic Acid (PLGA) Scaffolds for Bone Tissue Engineering Applications.** Huinan Liu<sup>1</sup>, Elliott B. Slavovich<sup>1</sup> and Thomas J. Webster<sup>2,1</sup>; <sup>1</sup>Materials Engineering, Purdue University, West Lafayette, Indiana; <sup>2</sup>Biomedical Engineering, Purdue University, West Lafayette, Indiana.

Bone substitutes are often required to replace damaged tissue due to injuries, diseases and genetic malformations. Traditional bone substitutes, such as autografts, allografts, xenografts and metal implants, are far from ideal and each have their own specific problems and limitations. Bone tissue engineering offers a promising opportunity for bone regeneration in a natural way. However, currently the scientific challenges of bone tissue engineering lie in the development of suitable scaffold materials that can improve cell adhesion, proliferation and differentiation. The design of nanophase titania/PLGA composites offers an exciting approach to combine the advantages of a degradable polymer with nano-size ceramic grains to optimize physical and biological properties for bone regeneration. Importantly, nanophase titania mimics the size scale of constituent components of bone since bone itself is a nanostructured composite composed of nano hydroxyapatite well-dispersed in a mostly collagen matrix. Previous studies have shown significant improvement in protein adsorption, osteoblast (bone-forming cell) adhesion and long-term functions on nano-grain ceramic materials compared to traditional micron-grain ceramic materials. For this reason, nanometer grain size titania was dispersed in a PLGA matrix by various sonication powers from 0 W to 332.5 W. The surface characteristics of the composites, such as topographies, surface area of titania coverage and surface roughness, were studied by scanning electron microscopy and atomic force microscopy. Results demonstrated that the dispersion of titania in PLGA was enhanced by increasing the intensity of sonication and that greater osteoblast adhesion correlated with improved nanophase titania dispersion in PLGA. Moreover, for the first time, results correlated better osteoblast long-term functions, such as alkaline phosphatase activity and calcium-containing mineral deposition, with improved nanophase titania dispersion in PLGA. In this manner, the present study demonstrates that PLGA composites with well-dispersed nanophase titania can improve osteoblast functions necessary for improved bone tissue engineering applications.

### 3:45 PM K13.2

**Dentin Matrix Protein 1 (DMP1) Actively Participates in Initiating and Modifying Calcium Phosphate Morphology during Crystal Growth.** Sivakumar Gajjerman, Karthikeyan Narayanan, Sankalp Jain, Amsaveni Ramachandran and Anne George; Oral Biology, University of Illinois at Chicago, Chicago, Illinois.

Mineralization of bones and teeth occur by the nucleation of hydroxyapatite crystals in an extracellular matrix consisting of predominantly type 1 collagen and a variety of noncollagenous proteins. Among the noncollagenous proteins, dentin matrix protein1(DMP-1) an acidic protein found in the mineralized matrix of bones and teeth has been postulated to play an important role in interacting with calcium ions and mineral surfaces. To investigate on the mineral initiating role of DMP1 we have devised a methodology to study its properties in a semi solid medium mimicking the extracellular environment in mineralized tissues. In this procedure the crystals were grown by controlled chemical reaction between calcium and phosphate ions at physiological pH and temperature with and without recombinant DMP1 (r-DMP1). After 45 days of growth the calcium phosphate deposits were analyzed by scanning electron microscopy (SEM). Results demonstrate the presence of spherulitic and platy crystals in the absence of r-DMP1, whereas only spherulitic crystals were seen in the presence of DMP1. These crystals were

further characterized by powder x-ray diffraction analysis (XRD), Raman spectroscopic analysis, Scanning electron microscopic analysis and energy dispersive spectroscopic (EDX) analysis. Results from these analysis showed that the spherulitic and platy crystals grown in the absence of r-DMP1 were predominantly monetite and brushite respectively. Crystals grown in the presence of r-DMP1 were identified as hydroxyapatite. Further, the calcium and phosphorous content were confirmed by EDX analysis and the Ca/P ratio of the crystals grown in the presence of r-DMP1 was found to be between 1.64-1.67. Thus, DMP1 can control the calcium phosphate crystal morphology during crystal nucleation and growth. This research was supported by NIH grant 16533

#### 4:00 PM **K13.3**

##### **Probing In Vitro Interactions of Immortalized Human Bone Marrow Stromal Cells with Novel Bioactive Glass Coatings.**

Jie Song<sup>1,2</sup>, Eduardo Saiz<sup>1</sup>, Vincent Eng<sup>1</sup>, Carolyn R. Bertozzi<sup>1,2,3</sup> and Antoni P. Tomsia<sup>1</sup>; <sup>1</sup>Materials Sciences Division, Lawrence Berkeley National Lab, Berkeley, California; <sup>2</sup>Molecular Foundry, Lawrence Berkeley National Lab, Berkeley, California; <sup>3</sup>Departments of Chemistry and Molecular and Cell Biology, University of California, Berkeley, California.

Metals have been widely used for fracture fixation, joint replacement and dental applications. To improve the ability of bioinert metallic implants to bond to osseous and dental tissues, bioactive surface coatings such as hydroxyapatite (HA) are often applied via plasma spray. Although these bioactive ceramics have been shown to facilitate the implant-tissue integration and lead to faster healing, in vivo studies have demonstrated that the unreliable metal-ceramic adhesion contributes to the long-term failure of these implants. A novel glass family in the Si-Na-K-Mg-Ca-P-O system has been developed to prepare graded glass-HA coatings on metallic implants using a simple enameling technique. The coatings consist of a high silica layer in contact with the alloy and a surface layer that is a mixture of a low silica glass and HA particles. The coatings form apatite in simulated body fluid while maintaining excellent adhesion with the alloy. To probe the physiological relevance of these novel coatings for potential implant applications, the proliferation and expression of osteoblastic marker proteins of normal human osteoblastic cells on these glasses should be examined. Normal human osteoblasts (NHOst) and bone marrow stromal cells (BMC) have limited proliferative life span in culture and gradually lose their osteogenic potential in culture, making the use of these cell lines for screening biocompatible implant coatings inconvenient. Immortalized human bone marrow stromal cells (hTERT-BMCs), established via ectopic expression of human telomerase in normal human BMCs, have extended life span in culture and do not exhibit growth deregulation. They were seeded onto a number of novel glasses, alloy substrates with graded coatings, and Ti6Al4V and tissue culture polystyrene controls for in vitro cell culture studies. These cells were cultured in the presence of 1,25-dihydroxyvitamin D3, which is known to induce differentiation of BMCs into osteoblasts lineage. Cell lysates were collected at various time points, and cell proliferation was examined using a dsDNA quantification assay. The expression of osteogenic marker proteins including osteocalcin and osteopontin, as well as important extracellular matrix proteins such as collagen I and cytoskeletal protein -tubulin, was examined by Western blotting and microtiter plate immunoassays. The expression of alkaline phosphatase activity and the extent of mineralization in culture were also quantified.

#### 4:15 PM **K13.4**

##### **Hydroxyapatite Thin Films Produced by Radio Frequency Magnetron Sputtering from Two Facing Targets.**

Donald E. Ellis<sup>1,2</sup>, Z. Hong<sup>1</sup>, L. Luan<sup>1</sup>, Alexandre Rossi<sup>3</sup>, Alexandre Mello<sup>3</sup>, J. G. Eon<sup>4</sup>, John Ketterson<sup>1</sup> and Joice Terra<sup>3</sup>; <sup>1</sup>Physics and Astronomy, Northwestern University, Evanston, Illinois; <sup>2</sup>Chemistry, Northwestern University, Evanston, Illinois; <sup>3</sup>Centro Brasileiro de Pesquisas Fisicas, Rio de Janeiro, RJ, Brazil; <sup>4</sup>Instituto de Quimica, Fed. University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil.

We have produced hydroxyapatite (HA) thin films on fused silica using R.F. magnetron sputtering from two facing targets in a right angle geometry. This design greatly reduces negative-ion resputtering effects caused by oxyanions, and thus preserves the stoichiometry of the targets. We studied both as-sputtered and annealed films (800C in Ar) Water vapor was introduced during either the deposition or annealing process. Films were characterized by AFM, stylus profilometry, XRD, XPS, FTIR and Raman spectroscopy. Our XPS measurements showed that the as-sputtered films retain the same Ca/P ratio as that of the targets over a wide range of Ar pressures. Our XRD results show that the films are highly-textured along the HA(x00) direction. A comparison was also made for growth on several different substrates (SiO<sub>2</sub>, Si(100), Si(110), Si(111) and Ti. Structural and bonding characteristics of the films are discussed in the light of atomistic simulations and Density Functional electronic structure calculations

#### 4:30 PM **K13.5**

##### **Template-free Routes to Hierarchically Porous Inorganic Monoliths.** Eric Toberer and Ram Seshadri; Materials, University of California, Santa Barbara, Santa Barbara, California.

Mammalian lungs possess a fractal pore morphology that combines large active surface areas with high flow-through. In a similar manner, we have developed novel template-free routes that permit the formation of hierarchically porous structures in functional inorganic materials. Our strategy involves starting with a monolithic, single-phase material, which we convert to a two-phase composite, and then successively leach out components of the material such that a hierarchically porous material is left behind. Porous monoliths of various transition metal oxides have been prepared that possess a continuous porosity on the order of one micron, and a finer scale of porosity on the order of tens of nanometers.