SYMPOSIUM Z

Hybrid Biological-Inorganic Interfaces

April 13 - 15, 2004

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Symposium Support
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* Invited paper

SESSION Z1: Bio-Functionalization of Inorganics
Tuesday Morning, April 13, 2004
Room 3007 (Moscone West)

8:45 AM Z1.1
Immobilization of glucose oxidase for amperometric biosensor applications using sol-gel-derived titania-pyrrole nanohybrids.
Yun-Mo Sung, Yong-Ji Lee and Kyong-Soo Park; Materials Sci. & Eng., Daejin University, Kyunggi-do, South Korea.

Recently, much interest has been raised to the precise and simple analysis of glucose as the number of diabetes incidences increases rapidly worldwide. An electrochemical method using enzyme electrode has been extensively studied for this purpose and sol-gel method has been proved to be one of the most promising routes to immobilize enzyme on electrodes. Sol-gel-derived silica-polymer hybrids have been widely investigated as a matrix material for bioimmobilization. However, this system shows low reliability due to fragility and also, the enzymes are often degraded due to acidic environment during electrode fabrication process. In this study sol-gel-derived titania-pyrrole nanohybrids were developed as a host material for the immobilization of glucose oxidase and electropolymerization was employed to construct bioelectrochemical electrodes. Sol-gel-derived titnia-pyrrole was analyzed to form hydrogen bonds with enzyme molecules, which prevents the enzyme from leaking out of the enzyme electrode. Also, sol-gel-derived titania-pyrrole nanohybrid matrix was not fragile and this hybrid matrix was synthesized in near neutrality conditions to avoid degradation of the functionality of enzyme. The concentration of titania, pyrrole, and glucose oxidase in the electrodes was varied to obtain both optimum processing and operating conditions for the biosensor applications. Scanning electron microscopy (SEM) images of the electrodes show that the glucose oxidase was homogeniously dispersed in the titania-pyrrole matrix, which brings a high performance of the bioelectrocchemical electrode. Amperometric experiments were carried out for the electrodes with different compositions and the optimum value for biosensing was determined. Also, optimum operating conditions for temperature and pH were selected for this system. The electrodes processed and operated at optimum conditions showed much improved long-term stability, sensitivity, and reproducibility compared to silica-polymer systems. The application of this system to the next-generation microelectrodes for glucose sensing was discussed based upon the possibility of electropolymerization of titania-pyrrole hybrids.

9:00 AM Z1.2
Biomolecular Assemblies On Mesoporous Silica Beads - A Microfluidic Based Biosensing Approach.
Menake E Piyasena[2], G V Rama Rao[1], S Rathod[1], L K Ista[1], Timothy L Ward[1] and Gabriel P Lopez[1,2]; [1]Chemical and Nuclear Engineering, The University of New Mexico, Albuquerque, New Mexico; [2]Chemistry, The University of New Mexico, Albuquerque, New Mexico.

Bead-based molecular assemblies are finding widespread use in sensing and assays. Lipid bilayers supported on flat or spherical silica substrates continue to be the subject of research activity as models of cell membranes and as biomimetic membrane platforms for biotechnological applications. Mesoporous silica beads can be versatile hosts for supported lipid bilayer membranes in which the porous structure of the beads forms an isolated cytosol-like compartment that can be used to store ions, dyes, drugs, and biological molecules and thus increase the functional versatility of microbeads in biotechnological applications. We report on the biofunctionalization of mesoporous silica beads through the application of supported lipid bilayers and its plausible use in microfluidic applications. Monodisperse mesoporous silica microspheres were synthesized based on evaporation induced self assembly of surfactants in microdroplets produced by vibrating orifice aerosol generator. The mesoporous beads were characterized in terms of their capacity to absorb dye molecules and as robust supports for lipid bilayers. To obtain a reasonable estimate of the average dye-accessible volume of the void space of the porous beads, the beads were incubated in solutions of fluorescein dye(nanomolar-micromolar concentration range) for 72 h. The beads were centrifuged and washed twice in the 50 mM Tris buffer, after which they were immediately coated with unilamellar lipid vesicles of phosphatidylcholine (egg PC), thus trapping the dye inside. The beads were then analyzed with confocal microscopy. The lipid bilayer membranes were found to be uniform over the entire bead surface. Thus well characterized beads were packed into microchannels made of poly(dimethyl siloxane). The integrity of the membrane-encapsulated porous beads containing ion sensitive fluorescent dyes was monitored by spectrofluorometry by subjecting them to various flow conditions and ionic strengths. It was found that there was no leakage of fluorescein dye from the particles and remained stable for several days. These biomimetic bead-supported bilayer architectures may have variety of biotechnological applications including microimmunoassays and fluorescence-based high-throughput screening of biochemical recognition and protein function.

9:15 AM Z1.3
Preferential Binding of Peptides Selected by Phage Display to Single Wall Carbon Nanotubes.
Mark James Pender[1], Rajesh Naik[1], Sharon Jones[1], Jeffrey Hartgerink[2] and Morley Stone[1]; [1]MLPJ, Air Force Research Laboratory, WPAF B, Ohio; [2]Department of Chemistry and Department of Bioengineering, Rice University, Houston, Texas.

While carbon nanotubes can be synthesized in a variety of ways and on large scales, further ease and control in the manipulation and separation of these technologically important structures is desired. The range of single wall nanotube types and diameters produced in a single batch makes it difficult to use batch-produced nanotubes in many electronic applications. Recent work has demonstrated that peptides determined via phage display methodologies can differentiate between different crystal faces of the same material.(1) Such sensitivity and selectivity may be used to purify and select SWNTs with specific properties and localize them on substrates in predefined arrays. Here we demonstrate peptide sequences determined by phage display techniques that bind specifically to SWNTs and provide spectroscopic characterization of the nanotubes and the peptides to detail the interaction. Initial conclusions on using peptides for SWNT type selection and attempts to pattern these specific peptides on substrates and to localize SWNTs of a specific type on a substrate will be discussed. 1a) Naik, R.R.; Stringer, S.J.; Agarwal, G.; Jones, S.E.; Stone, M.O. Nat. Mater. 2002, 1, 169-172. b) Whaley, S.R.; English, D.S.; Hu, E.L.; Barbara, P.F.; Belcher, A.M. Nature 2000, 405, 626-627.

9:30 AM *Z1.4
Interfacing Carbon Nanotubes with Biological Systems.
Hongjie Dai, Stanford Univ, Stanford, California.

In this talk, I will present our latest research on the functionalization of carbon nanotubes aimed at interfacing novel nanomaterials with biological systems. I will present nanotube electronic devices functionalized with proteins for detecting protein-protein binding in solutions. The mechanism of protein detection with nanotube biosensors will be discussed. Further, I will present an investigation of how functionalized nanotubes interact with biological living cells, aimed at exploring the biocompatibility and toxicity of nanotubes.

10:30 AM Z1.5
Bio-Functionnalization of Microporous Nanoparticles.
Tristan Doussineau[1,2], Monique Smaihi[1], Jean-Olivier Durand[2] and Valentin Valtchev[3]; [1]IEM UMR 5635, CNRS, Montpellier, France; [2]CMOS UMR 5637, UM2, Montpellier, France; [3]LMM UMR 7016, ENSCMu, Mulhouse, France.

The demand to develop highly sensitive, nonisotopic analysis systems for biological applications, has driven nanomaterials towards biomedical fields and biotechnology(1). As a complement to the commonly used dense nanoparticles, nanosized microporous zeolite crystals(2) may provide additional interesting properties. Well defined pores and cavities of zeolite frameworks may be used as a host for small drug molecules or contrast agents. Functionalized colloidal zeolite nanoparticles, stable in most solvents, have been prepared by grafting organosilane or phosphonate reagents at their surface. Further complex functionalization with various bio-molecules can be accomplished by appropriate chemical reaction with these grafted functions (primary amino groups or more specific functions (semi-carbazide)). The present study reports immobilization of various proteins on these functionalized zeolite nanoparticles. Cytochrome C has been covalently attached to zeolite nanoparticles using the glutaraldehyde methodology, allowing chemical attachment of the terminal aldehyde to amine groups in the protein. The protein functionality was maintained after immobilization since a strong absorbance was still observed on the protein-functionalized zeolite nanoparticle. Biotin was also grafted using N-hydroxysuccinimide esters. Retaining of activity was attested by reaction with avidin which resulted in precipitation of the colloidal particles. Studies of the nanoparticles reactivity with functionalized peptides are also in progress. The obtained colloidal suspensions have been characterized by complementary techniques providing information on the size distribution, morphology and porosity of the particles. All these properties will be discussed and compared as a function of the nature of the immobilized biomolecule. These results showed that microporous zeolite-type nanoparticles are good candidates for biologically or pharmaceutically functionalized systems that could find applications, for example, in immunodiagnostics and drug delivery. References a- M. Bruchez Jr, M. Moronne, P. Gin, S.Weiss and A.P.Alivisatos, Science, 1998, 281, 2013-2016. b- W. Shenton, S.A. Davis and S. Mann, Adv. Mater., 1999, 11, 449-452. c- G.P. Mitchell, C.A. Mirkin and R.L.J. Letsinger, J. Am. Chem. Soc., 1999, 121, 8122-8123. 2 a- B. J. Schoeman and J.Sterte, Kona, 1997, 15, 150-158. b- M. Tsapatsis, M. Lovallo, T. Okubo and M. E. Davis,

Mater. Res. Soc. Symp. Proc., 1995, 371, 21-26. c- M. A. Camblor, A. Corma, A.Misud, J. Perez-Pariente and S. Valencia, Stud. Surf. Sci. Catal., 1996, 105 A, 341-348. d- J.-P. Dong, J.Zou and Y.-C. Long, Microporous Mesoporous Mater., 2003, 57, 9-19.

10:45 AM Z1.6
Conjugation of functionalized iron oxide nanoparticles to a protein patterned surface. Stephanie Grancharov[1,2], Stephen O'Brien[1], Chris Murray[2] and Glenn Held[2]; [1]Applied Physics and Applied Math, Columbia University, New York, New York; [2]Nanoscale Materials and Devices, IBM TJ Watson Research Center, Yorktown Heights, New York.

We demonstrate that highly crystalline, monodisperse maghemite nanoparticles, which are synthesized in organic solvents, can be effectively transferred into an aqueous medium and biotinylated. The nanocrystals remain monodisperse as characterized by TEM and XRD, as well as superparamagnetic as determined through SQUID measurements. We subsequently develop procedures to selectively deposit proteins, e.g. avidin, onto a silicon wafer employing a positive photoresist and silane chemistry. The functionalized silicon wafers ultimately provide a surface where biotin-avidin conjugation occurs to produce selective patterning of nanoparticles, as observed with SEM and florescence measurements. Harnessing biological selectivity using labeled magnetic nanoparticles in this way may lead to useful applications in sensor technology in the future.

11:00 AM Z1.7
Computational Studies of Functionalization of Diamond Surfaces. Peter Zapol[1,2,3], Paul C. Redfern[2], Larry A. Curtiss[1,2], Nicole M. Haralampus Grynaviski[1] and Millicent A. Firestone[1,3]; [1]Materials Science Division, Argonne National Laboratory, Argonne, Illinois; [2]Chemistry Division, Argonne National Laboratory, Argonne, Illinois; [3]Center for Nanoscale Materials, Argonne National Laboratory, Argonne, Illinois.

One approach to functionalization of diamond surfaces involves a photochemical modification. In this approach UV light excites the system resulting in covalent attachment of organic groups to the surface. The diamond surfaces have been previously functionalized by a number of chemicals containing vinyl groups. Here we report on quantum chemical studies of various pathways for the addition of 1-chlorohexane, 6-chloro-1-hexene, and 4-chlorostyrene to a hydrogen terminated diamond surface. The quantum chemical studies use density functional theory and cluster models for the surface. Reaction pathways based on excitation of the reactant molecules and of the diamond surface are investigated. Several mechanisms for photoattachment of reactants are proposed based on the calculated potential energy surfaces. The results from these calculations are used to provide a plausible explanation for recent experimental results in our laboratory obtained for functionalization of diamond by 1-chlorohexane, 6-chloro-1-hexene, and 4-chlorostyrene. This work is supported by the U.S. Department of Energy, BES-Materials Sciences, under Contract W-31-109-ENG-38.

11:15 AM Z1.8
Immobilisation of Biomolecules on a Diamond Surface. Phillip John[1], Neil Polwart[1], Michael Anderson[1] and Jae-Kap Lee[2,1]; [1]Chemistry, Heriot-Watt University, Edinburgh, United Kingdom; [2]Thin Film Technology Center, Korea Institute of Science and Technology, Seoul, South Korea.

A third generation sensor has been fabricated for potential in-situ detection of glucose using diamond technology. The sensor was designed by isolating the active flavin adenine dinucleotide (FAD) and covalently binding a modified FAD to a (100) oriented diamond film. The FAD centers were chemically modified to yield 2-aminoethyl-FAD in which the amino group is more chemically reactive towards the carbonyl terminated diamond surface. The apo-glucose oxidase was reconstituted to form an enzyme terminated diamond surface. The ampometric response of the biosensor was linear with glucose concentration in the clinically important range. The kinetics and thermodynamics of the reactions have been measured using FTIR ATR with a diamond coated silicon trapezoid. For comparison, the kinetics of the reaction of 2-adamantanone, a molecular mimick of the (100) diamond surface, with aromatic amines have been measured. Work will also be described on the immobilisation of biomolecules onto high surface area diamond nanospheres with diameters in the range 150-650 nm and excellent dispersity.

11:30 AM Z1.9
Immobilization of Peptides on Electrochemically Functionalized Ultrananocrystalline Diamond Surfaces. Jian Wang, Orlando Auciello, John A. Carlisle and Millicent A. Firestone; Materials Science Division, Argonne National Laboratory, Argonne, Illinois.

The integration of well-organized mono- or multilayers of biomolecules with hard materials accompanied by retention of the biomolecule functionality is a frontier in science with significant potential applications in biomedical technology, biosensing, bioeletrocatalysis, and biomolecular electronics. Ultrananocrystalline diamond (UNCD) is an excellent host material because of its superb mechanical, chemical and electrical properties. Consequently, we develop a multistep electrochemical/wet chemical synthesis procedure to immobilize dye-labeled peptides onto electrochemically functionalized UNCD surfaces. Covalent modification of conductive (N-doped) UNCD films is accomplished by electrochemically reducing aryl diazonium cations in a nonaqueous medium. The electrochemical reduction of 4-nitrophenyl diazonium cations leads to the attachment of 4-nitrophenyl groups to UNCD surfaces. The nitro group (NO_2) can be subsequently reduced to an amine group (NH_2) when the modified UNCD surface is exposed to a protic medium. The primary amine can then be used for the covalent tethering of peptides and ultimately proteins via an accessible tyrosine amino acid residue. Electrochemical techniques, i.e., cyclic voltammetry and ac impedance measurements are used to perform the surface functionalization as well as to study the charge transfer mechanism. Core level photoemission spectroscopy is used to determine the surface coverage. The success and the stability of the peptide and protein on the electrochemically modified UNCD surface has been characterized by spectroscopy (UV/VIS, fluorescence), atomic force microscopy and biochemical assays. Potential applications of this hybrid inorganic-biomolecular composite in biosensing devices will be discussed. * This work was supported by the US Department of Energy, BES-Materials Sciences, under Contract W-13-109-ENG-38.

11:45 AM Z1.10
An Organic/Inorganic Modified Surface for Oligonucleotide Arrays. Daniel R. Talham[1], Isa O. Benitez[1], Guillaume Nonglaton[2], Muriel Pipelier[2], Bruno Bujoli[2] and Charles Tellier[3]; [1]Department of Chemistry, University of Florida, Gainesville, Florida; [2]Laboratoire de Synthese Organique, Universite de Nantes, Nantes, France; [3]Laboratoire de Biocatalyse, Universite de Nantes, Nantes, France.

A new process for preparing oligonucleotide arrays is described that uses surface grafting chemistry which is fundamentally different from the electrostatic adsorption and organic covalent binding methods normally employed. Solid supports are modified with a mixed organic/inorganic zirconium phosphonate monolayer film providing a stable, well-defined interface of zirconium phosphonate sites. Oligonucleotide probes terminated with phosphate are spotted directly to the zirconated surface forming a covalent linkage. Specific binding of terminal phosphate groups with minimal binding of the internal phosphate diesters has been demonstrated. Non-spotted areas are subsequently passivated with BSA to eliminate non-specific binding to the surface. Using 33-mer oligonucleotide probes, a signal to noise ratio of better than 1000 has been achieved detecting Cy3-labelled complement targets at 100 nM concentrations.

SESSION Z2: Biological Hybrids for Sensing
Tuesday Afternoon, April 13, 2004
Room 3007 (Moscone West)

1:30 PM Z2.1
Enhancing electron transfer at the protein-electrode interface. Siu-Tung Yau, Physics and Astronomy, Hunter College of City University of New York, New York, New York.

The redox reactions of cytochrome c immobilized on the bare surface of microelectrodes and of macroscopic electrodes (macroelectrodes) are investigated using cyclic voltammetry. The faradaic electron transfer due to cytochrome c adsorbed on microelectrodes that is composed of the edge plane of highly oriented pyrolytic graphite shows an anomalous enhancement compared to that for macroelectrodes composed of the basal plane. For both macroelectrodes and microelectrodes, the redox reaction of immobilized cytochrome c can be switched between an ON state and an OFF state by controlling the electrochemical potential extrema. In the ON state, the redox peak currents of the macroelectrode can be manipulated by varying the potential extrema. It is possible to enhance the redox reaction in a particular direction, shifting the electron transfer from a quasi-reversible process to an irreversible one. The difference in the chemical properties of the surface of the two kinds of electrodes results in different responses to changes in the potential extrema.

1:45 PM Z2.2
Electrical Detection of Biological Binding Events at Modified Group IV Semiconductors by Electrochemical Impedance Spectroscopy. Wensha Yang[1], Wei Cai[1], Tami L. Lasseter[1], John R Peck[3], James E. Butler[2], John N. Russell[2], Daniel W. van der

Weide[3], Lloyd M. Smith[1] and Robert J Hamers[1]; [1]Dept. of Chemistry, University of Wisconsin-Madison, Madison, Wisconsin; [2]Naval Research Laboratory, Washington, District of Columbia; [3]Dept. of Electrical and Computer Engineering, University of Wisconsin-Madison, Madison, Wisconsin.

The fabrication of interfaces between biological molecules and microelectronic materials has great potential for the development of new types of bioelectronic devices, such as biosensors and bioactuators. We have investigated the fabrication of direct interfaces of biomolecules with surfaces of covalent materials such as silicon and diamond, and we have been investigating the relationships between the interfacial structure and the resulting electrical response observed upon DNA hybridization and antibody-antigen binding. The biological sensitivity and selectivity can be optimized using classical fluorescence methods. By using electrochemical impedance spectroscopy, it is possible to directly detect DNA hybridization and antibody-antigen interactions in real time with high sensitivity. Because EIS can be performed at the open circuit potential (no net DC current flow) and with only small applied potentials on the order of kT, it is a very non-perturbative method for probing biological binding processes. By mapping the electrical response as a function of frequency and of potential, we have identified at least two distinct mechanisms that can be used as the basis for direct detection. At low frequencies binding can be detected by virtue of the fact that diffusion through the interfacial layers is modified. At higher frequencies binding can be detected through a field effect induced in the underlying semiconductors. Significant differences in electrochemical response are observed for DNA hybridization and antibody-antigen binding, which we attribute primarily to the differences in molecular charge. The field effect is also sensitive to the doping type and concentration. For applications such as homeland security and environmental sensing the stability of the interfaces becomes a critical factor. Stability is affected both by the substrate material and by the chemical structure of the interface. The proper choice of substrate material and optimization of the interface chemistry make it possible to significantly improve the stability of the interfaces. This talk will discuss the overall chemistry associated with directly interfacing biomolecules such as DNA and antibodies to silicon, diamond, and related covalent materials, as well as the factors controlling interface stability and electrical response.

2:00 PM *Z2.3
DNA:Nanowire Conjugates for Sensing and Assembly.
Christine D. Keating, Rebecca Stoermer, James Sioss, Marcus Helfrich, William Charette and Lisa Dillenback; Chemistry, Penn State University, University Park, Pennsylvania.

This presentation will describe striped metallic nanowires modified with DNA. These DNA:nanowire bioconjugates are interesting both for applications in multiplexed biosensing and as a route to deterministic assembly of electronic materials from the bottom up. The attachment chemistry and surface coverage of biomolecules on the particle surface is critically important for retention of bioactivity. We have attached DNA oligonucleotides to metal nanoparticles ranging from 12-nm diameter colloidal Au spheres to 6 micron long, 320 nm wide striped metal nanowires. Several attachment chemistries have been employed: thiol self assembly, neutravidin/biotin attachment, and glass coating followed by organosilane derivatization. These chemistries will be compared for the efficiencies of nanowire-bound DNA hybridization and enzymatic extension, and applications in multiplexed bioanalysis will be discussed.

2:30 PM Z2.4
Active Control of DNA-Conformation on Au: Persistent Switching by Electro-Modulation.
Ulrich Rant[1], Kenji Arinaga[1,2], Shozo Fujita[2], Naoki Yokoyama[2], Gerhard Abstreiter[1] and Marc Tornow[1]; [1]Walter Schottky Institute, E24, Technical University Munich, Garching, Germany; [2]Fujitsu Laboratories Ltd., Atsugi, Japan.

We present investigations on the electrical manipulation of single stranded (ss) DNA oligonucleotides, tethered to Au-electrodes at their 5' end in aqueous solution. Changes in the DNA's orientation are induced by applying low frequency AC potentials to the supporting Au-electrodes, by which the strands are switched between a 'lying' and a 'standing' conformation on the surface. Modulation of the DNA orientation is monitored by fluorescence measurements, utilizing distance dependent energy transfer of the fluorescent label at the 3' end to the metal surface. In order to attain persistent switching with negligible degradation or desorption of the tethered DNA layer, the electrochemical potentials applied to the Au-electrode have to be chosen with respect to the surface's potential of zero charge (pzc). We introduce a novel electro-optical method to determine this pzc for the DNA/Au system. By establishing the appropriate electrochemical parameters, we succeeded to demonstrate persistent, electrically driven orientation-switching of DNA-strands on Au surfaces, featuring formidable long-term stability. To study the collective molecular behaviour of the switching DNA layer, we systematically investigated the influence of the surface coverage density of DNA strands on their ability to freely gyrate with respect to their tethered end. Here, we correlate the absolute number density of DNA molecules, as measured electrochemically, to the fluorescence modulation amplitude. As expected, we find that increasing the DNA coverage leads to a diminished mobility of the strands on the surface. We are able to relate our results to a theoretical model involving steric obstruction of neighbouring strands and fluorescence energy transfer to the Au-surface, yielding good quantitative agreement. In fact, comparing the results for two ssDNA systems of different lengths (12mer and 24mer) exhibits different onsets for the observed increase in orientation-modulation when lowering the surface coverage, in support of our presented model. Finally, we propose how extracting characteristic time constants from sweeping the modulation frequency can allow the study of molecular dynamics of DNA or other mobile polyelectrolyte systems on surfaces in electrolyte environment.

2:45 PM Z2.5
Characterization of Bacteriorhodopsin-Based Bio-optoelectronic Device.
Tao He[1], David Cahen[1], Mordechai Sheves[2], Noga Friedman[2] and Sidney Cohen[3]; [1]Department of Materials and Interfaces, Weizmann Institute of Science, Rehovot, Israel; [2]Department of Organic Chemistry, Weizmann Institute of Science, Rehovot, Israel; [3]Chemical Research Support, Weizmann Institute of Science, Rehovot, Israel.

By green (560 nm) photon absorption, the purple membrane (PM) protein bacteriorhodopsin (bR) can pump protons from its cytoplasmic to extracellular side, accompanied by an immediate charge separation step. This accounts for the photoelectric properties of bR. Photoexcitation can also lead to the formation of a relatively long-lived intermediate that adsorbs at 412 nm (M412), resulting in a significant shift in the absorption maximum of ca. 150 nm. In addition, bR exhibits long-term stability over a wide range of pH values, temperature, humidity, and in a variety of (photo)chemical environments. All of these make it a promising biomaterial for optoelectronic applications. To generate the maximal voltage per absorbed photon, preparation of highly oriented PM patches, using simple and practical assembling methods is a key point. Since bR is more negatively charged on its cytoplasmic surface, a good orientation of PM patches is obtained through electrostatic adsorption on the surface of Si or quartz glass modified with positively charged 3-aminopropyltrimethoxysilane (APS) or poly-(diallyldimethylammonium)chloride. The results of AFM topography, FT-IR and UV-vis absorption highlight the presence of more or less dense net of patches of PM on the surface. The AFM images and ellipsometric data show the resulting thin film to be a monolayer with partially overlapping patches. A native bR monolayer can be converted completely to the M412 intermediate under steady state irradiation of green light and under a variety of environmental conditions, while with multilayers this occurs only under a very limited set of conditions. For Si/APS/PM heterostructures a steady-state surface photovoltage is observed, caused by a complicated sequence of events. This starts with the formation of an electric dipole moment that develops when a photon is adsorbed by the retinal located in bR protein. Current-voltage data, obtained using a Hg drop electrode, suggest that monolayer PM patches acts as nonlinear resistive elements. The results of scanning capacitance microscopy indicate that PM seems to act as a (wide band gap) semiconductor, p-type at negative and n-type at positive bias. Experiments to explore these findings are in progress.

3:30 PM Z2.6
Effects of Electrode Immobilization on Electron Transfer Across Neutravidin Bound with Biotin-Labeled Ruthenium Pentaamine.
Sulay Jhaveri, Leonard Tender, Scott Trammell and Daniel A Lowy; Center for Bio-Molecular Science and Engineering, Naval Research Laboratory, Washington DC, District of Columbia.

We have been investigating the electrochemistry of non-redox proteins rendered electroactive by conjugation with redox probes. Such proteins can serve as scaffolds for the attachment of redox active molecules, and when appended with binding elements, enable reagentless detection based on changes in voltammetry concomitant with analyte binding. Here we report voltammetry of the biotin-binding protein neutravidin bound with a novel biotin derivative, Ru(NH3)5(N-[(N-[(4-pyridyl)methyl]biotinamide] referred to here as Ru(NH3)5biotin. Varying the number of redox probes per protein and variations in the method of immobilization reveal interesting electrochemical features. Because neutravidin has 4 binding sites for biotin, it was incubated either with equimolar or saturating amounts of redox-labeled ligand to achieve either mostly mono-labeled or mostly tetra-labeled neutravidin. The isolated protein-redox conjugates were either electrostatically immobilized onto electrodes, or covalently coupled. The key observations described here include orientations that result in defined electron-transfer paths

across the immobilized protein by which oxidation/reduction of redox probes directed away from the electrode is mediated by redox probes directed toward the electrode. Furthermore, we observe negligible intra-conjugate electron-transfer on the electrode surface, suggesting that each conjugate is electrically isolated from its neighbors. The ability to engineer electron-conduction across proteins immobilized on electrodes such as those observed here will be an important component toward realization of protein-based approaches to high density molecular electronic devices.

3:45 PM *Z2.7

Nanotechnology for Cracking the Problems of Membrane Proteins: Functional Incorporation of Integral and Embedded Membrane Proteins into Soluble Nanodiscs. Stephen G. Sligar, Dept of Biochemisty, Chemistry and the College of Medicine, University of Illinois-Urbana, Urbana, Illinois.

Nanobiotechnology is the marriage of biology with the nanotechnological advances in materials, instrumentation and processing in order to realize a fundamentally new understanding of biological function as well as to visualize and manipulate hierarchical supramolecular assemblies. An important goal is the development and execution of methodologies for the determination of biological structure and function in the 5 nm - 500 nm 'mesoscale' size range, thus providing the important architectural information of specific aggregates of nucleic acids, lipids and proteins which constitute important cellular machinery. Integral membrane proteins represent a scientifically and commercially important class of macromolecules which have been consistently difficult to study due to poor solubility. Seven transmembrane proteins comprise a ubiquitous class of membrane receptors essential to a vast number of cellular signaling pathways making them the largest single family of current pharmaceutical targets. We have developed a simple system of self-assembly, Nanodiscs(tm), that functionally solubilize integral and embedded membrane proteins into a stoichiometrically controlled, monodisperse, biomimetic environment. These resultant supramolecular architectures allow the direct visualization of single membrane protein structures and measurement of physical properties on single molecules. The ability to directly probe the function of single membrane proteins incorporated into mimics of the natural cellular environment can have an enormous impact on the understanding and control of biological signaling, receptor mediated growth control processes and the high throughput screening for the elucidation of pharmacologically active agents.

4:15 PM Z2.8

Determination of Charge Injection Barriers at RNA Interfaces Using Photoemission Spectroscopy. Niels Dam, Brian Doran, Chris Braunagel and Rudy Schlaf; Electrical Engineering, University of South Florida, Tampa, Florida.

Self-assembly of molecular devices using bio-mimetic strategies has become an intense field of study. In particular, the use of custom synthesized deoxyribonucleic acid (DNA) polymers attached to macromolecular materials appears to be a promising route to self-assembly of complex 3D structures, such as molecular transistors or biological sensor applications. In our experiments we investigated the electronic structure of ribonucleic acid (RNA) interfaces with inorganic (electrode-) materials as model systems for charge transfer from DNA to inorganic materials. One of the important questions to be answered is, whether charges can be transported efficiently across RNA/DNA-electrode interfaces or not. In recent years, photoemission spectroscopy, in combination with in-situ thin film preparation, has demonstrated to be an excellent method for the direct measurement of charge injection barriers at organic heterointerfaces. In our experiments, we have successfully been able to deposit RNA polymers directly from solution in an UHV environment using the electrospray (ES) method. ES is a well-known method used in mass spectroscopy of large molecules, which we applied for thin film deposition in vacuum. The thin films prepared by us using this method proved to be largely contamination free as demonstrated by our measurements. Using this method, the charge injection barriers between RNA and Au, and between RNA and highly oriented pyrolytic graphite (HOPG) were determined by depositing RNA thin films in several steps in-situ without breaking the vacuum. The deposition series started at sub-monolayer coverage on the in-situ sputter-cleaned (Au) or cleaved (HOPG) substrate surfaces. Before growth and in-between deposition steps monochromatic x-ray and ultraviolet photoemission spectra (XPS, UPS) were measured, resulting in a series of spectra allowing to follow the interface electronic structure development as the deposited layer increases in thickness. This enabled the direct determination of the orbital line-up, and the nature of the chemical interaction at the interfaces.

SESSION Z3: Poster Session
Tuesday Evening, April 13, 2004
8:00 PM
Salons 8-9 (Marriott)

Z3.1

Interaction Between Calcite Crystals And Alpha-Chitin: A Molecular Modeling Study. Alejandro Heredia Barbero[1,2] and Vladimir Alexander Basiuk[1]; [1]Quimica de Radiaciones y Radioquimica, Instituto de Ciencias Nucleares, UNAM, Mexico DF, Mexico DF, Mexico; [2]Estado Solido, Instituto de Fisica, UNAM, Mexico DF, Mexico DF, Mexico.

White spots appear in the shrimp shells when frozen. They consist of calcium carbonate as highly symmetrical calcite spheres embedded into amorphous α-chitin matrix. α-Chitin macromolecules as organic ligands are capable of binding inorganic ions at certain sites, giving rise to nucleation and growth of highly ordered crystalline structures. By using molecular modeling (MM+ universal force field and PM3(tm) semi-empirical method), we were searching for functional groups and regions in the biopolymer, which could be responsible for the nucleation and growth of calcite crystals. The nucleation was modeled by minimizing energy of the systems composed of α-chitin oligomers (of up to 5 monomeric units), six Ca2+ and six CO32- ions. We have found two general trends: (1) the inorganic ions tend to gather (nucleate); (2) this process is energetically preferable at the oligomer sides, and not at its terminae. To model interactions between calcite crystals and α-chitin, fragments of three main crystalline planes of calcite ((100), (001) and (104)) were built (frozen geometry in all calculations). α-Chitin trimers were used to simulate the biopolymeric phase. According to experimental results published elsewhere, the (104) planes are inhibited when exposed to aqueous solutions of lysozyme and other biopolymers. We found theoretically a high affinity of the (104) plane to α-chitin trimers, which is in a good agreement with the experiment. The interactions accounting for this phenomenon apparently involve C=O and NH groups of the saccharide and resonant carbonate ions at the (104) crystalline faces. Specific arrangement of surface ions at the calcite faces matching spatial orientation of the functional groups in α-chitin molecules can be considered as a crucial factor stabilizing the naturally occurred biomineralized structure. The molecular modeling approach can be useful to give an insight into mechanisms of the local-scale crystal growth as well as into specific mineral-biopolymer interaction.

Z3.2

Lipid superposition and transmembrane movement in gel-liquid coexisting supported lipid bilayers, an AFM study. Wan-Chen Lin[1] and Marjorie Longo[2,1]; [1]Biophysics, University of California, Davis, Davis, California; [2]Chemical Engineering and Materials Science, University of California, Davis, Davis, California.

Using atomic force microscopy (AFM) imaging, we find that the inter-monolayer coupling of phase-separated supported bilayers formed by vesicle fusion can be controlled by the method of vesicle preparation. We also find that bilayers with partially asymmetric coupling are metastable and convert, through lipid flip-flop, to a state where all gel phase lipid domains occupy one monolayer. The supported bilayers (on a mica substrate) consisted of phase-separated dilauroylphosphotidylcholine (DLPC) and distearoylphosphotidylcholine (DSPC) and were formed by quenched vesicle fusion [Biophy. J. 83(6): 3380-3392 (2002)]. Coupled DSPC-rich domains were found to be about 1.8 nm higher than fluid phase while un-coupled DSPC-rich domains were only about 1.0 nm higher than fluid phase. We determined that when vesicles, formed through short ultrasonification, were used to make the supported bilayers, relatively soft domains were present, within the DLPC bilayer, that contained mainly DSPC, but also some DLPC. Within an hour, sub-domains of DLPC in one monolayer formed within and around the coupled DSPC regions. This is a metastable state because subsequently all of the DSPC moved to one monolayer, i.e. became completely uncoupled. The converting time constant is highly related to the interfacial line tension between different phases and also corresponds to the lipid flip-flop time constant. Other more vigorous vesicle formation and processing conditions resulted in supported bilayers which were always completely uncoupled. On the contrary, when vesicles formed under less vigorous conditions (e.g. extrusion) were used to form the supported bilayers, only coupled DSPC domains were formed and they did not convert to uncoupled domains at least for several days. These domains never appeared to contain sub-domains of DLPC. We relate these stable and metastable states to hydrophobic mismatch and the state and energy of mixing.

Z3.3

Preparation, Photo- and Cathodoluminescence of Polymer(PMA) or Biopolymer(protein)-Nanoporous Silicon Heterostructures. Liubomyr St. Monastyrskii and Y. Kosobutskii; electronics, Iv.Franko Lviv Nation Univ, Lviv, Ukraine.

Interfaces between living biomaterials and technical surfaces are in progress and will play a central role in the field of biosensors,neuronal networks and medical implants. Nanoporous Si based optoelectronics for biosensors and bioelectronic applications is a new direction of investigations.So biohybrids on the nanoporous silicon base are very interesting guestion of nanotechnology. Porosity of prepared Si layers was 20-80 percentage with thikness of porous layers about 0,1-100 μm.The process of PMA films preparation from the water solution on PS surface under athmosferic conditions have been studied. Precipitation was conducted by placing PS samples into PMA water solution for 5−24 h. PMA water solution with different molecular masses (10000−70000) and different ionization degrees (0 −1.0) have been studied. The real medium speed of precipitation was 0.8−1 μm/h for 5−10 μm PMA film thickness. The obtained heterostructures of porous silicon−PMA had photoluminescence properties. Photoluminescence curves had Gaussian shape with one wide maximum at room temperature which was moved by 70−80 nm towards the short wave band of the spectrum in connection with the PS spectrum without polymer film. The maximum of luminescence was located at about 600−625 nm owing to the ionization degree. With regard to polymer passivation of the PS surface, the peculiarities of film growth proper should be emphasized as well as their kinetics; the dimension of molecular clusters with molecular mass 120000 (PMA−120) is about 20 nm, and the dimension for molecular mass 50000 (PMA−50) is 40 nm. Hence, the number of macromolecular monolayers for the obtained films with 1−10 μm thickness is 50−500. Biopolymer layers on PS surfacees were prepared by PS loading in liquid protein. The thicness of biopolymer films was near 0,5 μm.It is known that protens consist near 100 organic acids with negative and positive active groups. Early we have shown by EPR and AES methods existing free bonds Si, hidrogen, nature Si-O-C thin film coating on the PS surfaces. So electrically charged surface of PS interact with charged groups of biopolymer and create surface film of protein. We have investigated photo- and cathodoluminescence of preparated heterostructures. It was one wide band of PL with maximun near 750 nm for PS and near 700 nm for heterostructures PMA-porSi.PL spectra of heterostructures biopolymer- porous silicon and PS were similar,only intensity heterostructurelighting was 1,5 time decreased. The cathodoluminescence spectra of PS surface and biopolymer −PS were similar.There were two wide bands with 380-400 μm and 520-550 μm maxima.Intensity of cathodoluminescence was three times higther at liquid nitrogen temperature.Also we have investigated decreasing evolution of CL spectra:fastly decreased shot-waves centers of lighting 380-400μm. So, we have shown a possibility of preparation polymer PMA and biopolymer (protein) on porous silicon surface light emitting heterostructures.It was investigated and optimized tachnological conditions heterostructures preparation. Under electron beam and UV nitrogen laser exiting polymer(PMA,protein) − nanoporous silicon have light emitting properties in different regions of spectra with maxima at 380 − 400 nm,520-550 nm(CL) and 700-750 nm(PL).Polymer coating both PMA or protein weacly changed luminescence spectra and only decreased luminescence intensity.

SESSION Z4: Nano-biological hybrids
Wednesday Morning, April 14, 2004
Room 3007 (Moscone West)

8:30 AM *Z4.1
Self-Assembly of Mesoscopic "Amphiphiles". Chad A. Mirkin, Sungho Park, Jung-Hyurk Lim and Sung-Wook Chung; Department of Chemistry and Institute for Nanotechnology, Northwestern University, Evanston, Illinois.

The assembly properties of two- and three-component rod-like building blocks consisting of gold and polymer block domains have been investigated. These structures behave like mesoscopic amphiphiles and form a series of single layer superstructures consisting of mushrooms, tubes, and sheets depending upon the compositional periodicity. Unlike molecular systems, the template used to initially synthesize them plays a critical role in the assembly process by pre-aligning them in a manner that facilitates their assembly by optimizing the correct collisional orientation and frequency to effect assembly. Tubular structures with tailorable diameters can be assembled in a predictable manner based upon an estimate of the hybrid rod packing parameters.

9:00 AM Z4.2
PH-controlled Dynamic Assembly of Gold Nanoparticles. Molly Stevens[2,1], Nolan Flynn[1], Chun Wang[1], David A Tirrell[3] and Robert Langer[1]; [1]MIT, cambridge, Massachusetts; [2]Materials, Imperial College London, London, United Kingdom; [3]Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California.

The ability to direct the assembly of inorganic nanoparticles has received growing interest in the creation of new nanotechnology devices. The development of new methods to control nanoparticle assembly may also impact on certain applications in medical science such as the generation of novel tunable and/or switchable materials. In particular, the ability to dynamically assemble and dis-assemble such structures under physiologically accessible environmental conditions, as triggered for example by changes in pH would be valuable for materials to be utilized for sensing in vivo and drug delivery. Here we report the coiled-coil peptide based assembly of gold nanoparticles and demonstrate that the system can be controlled under mild conditions (near-neutral pH and ambient temperature). PH-control over the self-assembly of gold nanoparticles was investigated utilizing 8.5 nm nanoparticles functionalized with an acidic leucine zipper-like peptide. The peptide adopts an alpha-helical conformation when it forms a homomer coiled-coil structure, the stability of which is modulated by electrostatic interactions across the interface of adjacent helices. Circular dichroism spectroscopy of the free peptide in solution revealed an increase in the helicity of the peptide, indicating the association of coiled-coil structures, as the pH was decreased from 11.5 to 4.5. A reversible pH-induced transition was observed between pH 8.5 and 7. Transmission electron microscopy images of the peptide-coated nanoparticles revealed that a well-dispersed population of nanoparticles could be caused to aggregate upon decreasing the pH with a similar reversible pH-induced transition observed. The peptide sequence was also varied to produce coiled-coils with different stabilities that were utilized to generate more stable binary nanoparticle systems with controlled spacing and architecture.

9:15 AM Z4.3
Isolation of Oxyethylene-Encapsulated Gold Nanoclusters Functionalized with a Single DNA Sequence. Sulay Jhaveri[1,2], Edward Foos[1], Daniel Lowy[1,2], Edward Chang[1], Mario Ancona[1] and Arthur Snow[1]; [1]Naval Research Laboratory, Washington DC, District of Columbia; [2]Nova Research Inc., Alexandria, Virginia.

Gold nanoclusters have received considerable attention as potential building blocks for a variety of applications at the nanoscale including chemical sensing, electronics, optics and biology. To fully exploit their potential, a major hurdle remains in the development of procedures for their rational organization at the nanoscale. One attractive approach, mostly hypothetical at present, is to employ DNA and its highly specific base-pairing to guide sophisticated self-assembly processes. Following the initial work1,2 that first developed procedures for attaching ssDNA strands to gold nanoclusters (starting from citrate-stabilized clusters), we employ a new type of cluster, encapsulated with an oxyethylene thiol. This type of cluster seems advantageous for self assembly in that it can resist the non-specific adsorption of bio-molecules. We report a new extraction-based method for isolating the ssDNA-functionalized product that does not rely on gel electrophoresis for product isolation, and demonstrate its efficacy with UV-Vis measurements. We have also attempted experiments to hybridize these ssDNA-tagged nanoclusters to ssDNA templates, which would in turn direct the coordination of arrays of nanoclusters, and these results will also be presented. 1. Zanchet et al., 2001, Nano Letters, 32-35. and references therein 2. Tanton, 2002, Current Protocols in Nucelic Acids Chemistry, Unit 12.2 and references therein

10:00 AM Z4.4
Recent Advances in the Preparation, Characterization and Application of Gold and Silver Nanoparticles. Janos H. Fendler, Chemistry, Clarkson University, Potsdam, New York.

Recent advances in the preparation, characterization and application of gold nanoparticles will be surveyed. Emphasis will be placed on the observed highly specific molecular interactions which have been found to depend on the nature, the size and the surface characteristics of the components and their environments. Two examples of our recent work will be used for illustration. In the first, details will be provided on the size dependent formation of gold encased silver nanoparticles in aqueous dispersions and in self-assembled films on gold substrates. In the second example, effects of the substrate on the formation of self-assembled monolayers (SAMs) and on the attachment of metallic and semiconducting nanoparticles onto these SAMs will be discussed.

10:15 AM *Z4.5
Biological Tagging Applications of Semiconductor Nanocrystals. Paul Alivisatos, Chemistry, UC Berkeley, Berkeley, California.

Semiconductor nanocrystals exhibit strongly size dependent emission spectra due to the quantum size effect. Further, the nanocrystals have nearly continous excitation spectra above the threshold for absorption. As a consequence, the nanocrystals can be used as luminescent probes in biological staining experiments. The nanocrystals are, in many

ways, superior to existing organic chromophores. Relevant applications and surface chemistry will be described in this talk.

10:45 AM Z4.6
Synthesis and Aggregation Mechanism of Nanostructures Comprised of Biotinylated CdS@SiO2 Nanoparticles.
Philip Joseph Costanzo[1], Timothy E. Patten[1] and Thomas Seery[2]; [1]Chemistry, University of California at Davis, Davis, California; [2]Institute of Materials Science, University of Connecticut, Storrs, Connecticut.

Novel micro- and nano-scale aggregates were prepared from inorganic building blocks using biological crosslinkers. Two difunctional, asymmetric PEG linkers were synthesized and utilized to prepared water-soluble, biotinylated CdS@SiO2 particles with various surface contents of the biotin groups. Aqueous dispersions of these particles were prepared and upon addition of avidin, aggregation was observed using DLS, TEM, and SEM analysis. The kinetic growth mechanism for nanoparticle aggregation was characterized by dynamic light scattering (DLS), scanning electron microscopy (SEM), and transmission electron microscopy (TEM) in conjunction with variation of particle functionality and avidin concentration. The aggregation process was also observed using fluorescent microscopy within a microchannel apparatus for the development of a new sensor mechanism. The aggregation rate for the formation of the aggregates was dependent upon the stoichiometric balance / imbalance of functional groups and by the competition between intra- and inter-particle binding.

11:00 AM Z4.7
Control of biomolecular function by heating of metal nanoparticle antennas. Kimberly Hamad-Schifferli, Mechanical Engineering, MIT, Cambridge, Massachusetts.

Gold nanoparticles can be linked to DNA and proteins for the purpose of controlling activity. The nanoparticles are inductively heated, which is achieved by placing the sample in an external magnetic field which induce oscillating currents in the nanoparticles. As a result, the nanoparticles are heated and transfer heat to the attached biomolecule. This slightly denatures the biomolecule, halting its activity. Once the alternating magnetic field is switched off, the biomolecule relaxes back to its normal structure and resumes activity. Because the nanocrystal can be incorporated into systems in vivo, this technique has the potential to allow external control of biomolecules in cells and organisms. Induction heating of nanocrystals linked to DNA oligonucleotides in solution has been shown to dehybridize the DNA in a manner that is both reversible and specific. Studies of the degree of the heat localization
and the conformation of the oligos on the nanoparticle will be described.

SESSION Z5: Biomimetics and Biomineralization
Wednesday Afternoon, April 14, 2004
Room 3007 (Moscone West)

1:30 PM Z5.1
Thermoresponsive, Biomimetic Nanostructures as Active Scaffolds for Inorganic Nanoparticles. Daniel N.T. Hay[1], Soenke Seifert[2] and Millicent A Firestone[1]; [1]Materials Science, Argonne National Laboratory, Argonne, Illinois; [2]Advanced Photon Source, Argonne National Laboratory, Argonne, Illinois.

We have recently reported the synthesis of a poly(*N*-isopropylacrylamide)-lipid conjugate and its use in the preparation of a new family of thermoresponsive, self-assembled nanostructures. These materials are quaternary phases, comprising the conjugate, a phosopholipid, and a co-surfactant dispersed in water, that self-assemble at room temperature to form liquid-crystalline gels, adopting an expanded lamellar structure. A modest increase in temperature triggers the reversible conversion of the aggregate to a collapsed lamellar structure, while a modest reduction in temperature results in its conversion to a non-lamellar structure. In this work, we explore the use of these materials as "active" scaffolding that allows for both the spatial organization of inorganic nanoparticles (e.g., Ag, Au, CdS) and, by exploiting the stimuli-responsiveness of these materials, the modulation of their packing arrangement and spatial proximity, thereby offering an elegant way to tune their collective optical and electronic properties. The preparation of these biomimetic "nanoscaffolds", formation of hybrid nanoscomposites, characterization of the organization of the encapsulated inorganic nanoparticles, and the use of temperature as a facile means to modulate their optical and electronic properties will be presented. This work was performed under the auspices of the Office of Basic Energy Sciences, Division of Materials Sciences, United States Department of Energy, under Contract No. W-31-109-ENG-38.
* Correspondence to firestone@anl.gov

1:45 PM Z5.2
Injectable Biomimetic Collagen/Hydroxyapatite Nanocomposites for Osseous Tissue Regeneration.
William J Znidarsic[1], Hoon Choi[1], I-Wei Chen[1,2] and V. Prasad Shastri[3,1]; [1]Materials Science and Engineering, University of Pennsylvania, Philadelphia, Pennsylvania; [2]Laboratory for Research on Structure of Matter, University of Pennsylvania, Philadelphia, Pennsylvania; [3]School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.

The development, maintenance, and remodeling of tissue in vivo is influenced by the biochemical and mechanical environment. Surface-active ceramics such as Bioglass, which is primarily composed of calcium and silica; and calcium phosphates like hydroxyapatite (HAp) and tricalcium phosphate (TCP) have been shown to stimulate bone formation (osteogenic) and enhance the integration of implants. Therefore, other synthetic biomaterials maybe rendered osteogenic by coating with these ceramics. Currently, both HAp and TCP are used as coating on metal hip prosthesis with improved results. These ceramics have potential as injectable synthetic bone graft substitutes, but their poor processibility and brittle nature have been limiting factors. These deficiencies can be overcome by dispersion in a more compliant medium such as a polymer. Such composites have mechanical properties comparable to cortical bone. However, their osteogenic potential is diminished due to an inaccessibility of the HAp or TCP phase to cells. We have developed nanoscale assemblies of HAp, by solution phase deposition onto functionalized silica colloids, with the intention of dispersing them in biocompatible gels to yield injectable systems for bone repair. Such a system would address the processibility and accessibility of HAp in composites. Since in vivo HAp is deposited on collagen fibril templates, we recreated these conditions by coating the silica colloid with type-I collagen using a layer-by-layer assembly process. We first prepared mono-dispersed silica colloids, 600 nm in diameter using a modified Stober process. To enable electrostatic deposition of collagen, the silica surface was first functionalized with amine groups, followed by polyelectrolyte adsorption to render the surface highly negatively charged. The changes in surface charge through the various modification steps were determined by measuring the z potential. SEM analyses revealed no discernable change in the particle size following these modification steps indicating that these layers are on the order of a few nm. The collagen content on the nanoparticle surface was determined using BCA protein assay and was found to be 0.09+/-0.01μg/cm2, which corresponds to 80% of monolayer coverage per particle. Precipitation from calcium phosphate solution resulted in 20-100 nm thick coatings as estimated by light scattering and SEM data. X-ray diffraction analysis revealed the presence of (200) and (112) peaks characteristic of HAp. We have shown that these HAp nanocomposites support the proliferation of MC-3T3-E1, murine osteogenic cells in vitro. Currently, the differentiation of murine and human mesenchymal progenitor cells on these composites is being studied. These HAp biomimetic nanostructures have also been dispersed successfully in calcium alginate gels to yield injectable systems that may be suitable for bone regeneration applications especially in the maxillo-facial area.

2:00 PM *Z5.3
Biomineralization of Synthetic Nanofiber Networks in Biological Media. Samuel I. Stupp[1,2,3] and Erik D. Spoerke[1]; [1]Materials Science & Engineering, Northwestern University, Evanston, Illinois; [2]Chemistry, Northwestern University, Evanston, Illinois; [3]Feinberg School of Medicine, Northwestern University, Chicago, Illinois.

Bone and other mineralized tissues in biology form through very complex and poorly understood mechanisms involving organic templates for mineral crystals, precursor molecules, ion channels, and multiple proteins including enzymes. Distilling the chemistry and the materials science from the complex processes of biomineralization using model systems will yield important information to create biomimetic materials and develop advanced bone, tooth, and joint medicine. We reported earlier (*Science* **2001**, *294*, 1684-1688) on self-assembling nanofibers formed by peptide amphiphiles that have the capacity to recreate some aspects of the nanoscale structure of bone. In this work we report the use of similar systems to trigger massive mineralization of three dimensional macroscopic networks formed in vitro using cell culture media and calcium ion mediated self-assembly. The artificially designed systems induce massive mineralization under conditions where biopolymer networks fail to trigger any mineralization. We demonstrate that template design, hydrolytic enzymes, and precursor molecules are all necessary to drive biomineralization of the organic network.

3:00 PM Z5.4
PCR-Based Panning for the Identification of Biological Templates for Inorganic Material Synthesis. Rajesh R Naik[1], Matthew B Dickerson[2,1], Christopher J Murray[3], Joseph C

McAuliffe[3], Kenneth H Sandhage[3] and Morley O Stone[1]; [1]MLPJ, Bldg 651, Air Force Research Laboratory, Dayton, Ohio; [2]School of Materials Science and Engineering, Georgia Institute of Technology, Atlanta, Georgia; [3]Genencor International Inc., Palo Alto, California.

Combinatorial phage display is a common technique used to select for ligands that are capable of interacting with specific targets. Phage peptide display libraries are commonly used to select for peptides that bind to inorganic surfaces (metals, metal oxides and semiconductors). These binding peptides can serve as templates to control the nucleation and growth of inorganic nanoparticles in vitro. Here we describe the identification of peptide sequences that bind to inorganic nanoparticles from a phage peptide display library using a Polymerase Chain Reaction (PCR)-driven method. The amino acid sequences obtained by the PCR method are a distinct set of sequences that would otherwise be missed using the regular panning method. Peptides identified by the method described here are also shown to function as templates for the synthesis of inorganic nanoparticles.

3:15 PM Z5.5
Application of MCM-41 Coated Hydroxyapatite to Drug Delivery System. Younghee Kim[1], Kyoungja Kim[2], Soo Ryong Kim[1] and Yoon Ju Lee[1]; [1]Ceramic-Building materials Division, Korea Institute of Ceramic Engineering and Technology, Seoul, South Korea; [2]Nano-materials application Division, Korea Institute of Ceramic Engineering and Technology, Seoul, South Korea.

Porous hydroxyapatite has attracted the interest of many researchers in bioengineering area since it has high biocompatibility and similarity in the pore structure with natural bone. There is high possibility of applications of porous hydroxyapatite in the field of drug delivery for hard tissue as a implant material, for example, deliveries of growth factor like beta-TGF or an anti-inflammatory medicine or medicine for bone marrow cancer, etc. Also, mesoporous silica like MCM-41 or silica xerogel has been utilized as the matrix for controlled drug delivery. Silanol group on the surface of the mesoporous silica interacts with the organic functional groups of the drug molecule. From this fact, it can be deduced that mesoporous silica plays a role as a matrix for controlled adsorption and liberation. In this study, mesoporous silica was coated on the surface of hydroxyapatite to improve controlled drug delivery property. Sol-gel derived mesoporous silica coating is applied to cover hydroxyapatite block, whilst maintaining the three-dimensionally interconnected pores. After surfactant extraction from the mesoporous silica, sample drug, ibuprofen was loaded into the pore of the mesoporous silica. Releasing profiles was investigated by measuring the drug concentration in the fluid using a UV-VIS spectrophotometer. By optimizing the methods of loading, releasing of the drug can last for more than 3days. This preliminary study confirms that mesoporous silica coated hydroxyapatite possess the possibility to apply controlled drug delivery systems for hard tissue.

3:30 PM Z5.6
The Study of a Natural Interphase Between Bulk Cementum and Dentin Hard Tissues. Sunita Ho, Rosalyn Sulyanto, Mehdi Balooch, Sally Marshall and Grayson Marshall; Preventive and Restorative Dental Sciences, University of California San Francisco, San Francisco, California.

The study of natural interfaces may provide information necessary to engineer functionally graded biomaterials, which can be used for bioengineering applications. In this study it is hypothesized that a chemically and structurally varying 80 to 200 μm interface brackets a narrower 10 to 50 μm proteoglycan (PG) rich cartilaginous cementum dentin junction (CDJ) between bulk lamellar cementum and tubular dentin. 3 mm thick samples of human molars (N = 5) were ultrasectioned. The structure of the samples was characterized using an AFM under dry and wet conditions. The hardness was determined using a nanoindenter and a microindenter. The chemical composition was studied using Raman microspectroscopy. 300 nm thick ultrasections were used for structure characterization using transmission light microscopy. The presence of PGs within the interface was studied using Chondroitinase ABC enzyme digestion. Dry AFM scans of untreated samples revealed a 10 to 50 μm wide valley adjacent to cementum, followed by a 50 to 100 μm wide area with no characteristic pattern before reaching dentin. When wet, the valley was transformed into a peak. A gradual transition in hardness from cementum to dentin over a width of 100 to 200 μm was observed. The difference in hardness for cementum (C) and dentin (D) at a nanoscale was larger (C: 0.62 $\pm$ 0.21GPa; D: 0.77 $\pm$ 0.14GPa) than at a microscale (C: 0.49 $\pm$ 0.03, D: 0.69 $\pm$ 0.07), because of the masking of inhomogeneities at a microscale due to a larger sampling area. Light microscopy revealed a 50 to 100 μm transparent region with no characteristic pattern adjacent to dentin. A variation in organic and inorganic composition over 80 to 140 μm was observed using spectroscopy. The height differences relative to cementum and dentin of enzyme digested CDJ and control were determined by measuring the CDJ profile using the AFM. When dry the relative height of the treated CDJ was twice as deep as the control. When wet the same regions yielded relative heights twice higher than the control. The trend was the same for 3 and 5 hour enzyme digestions. Wet nanoindentation yielded significantly higher (t-test, $p < 0.05$) elastic modulus (E) and hardness (H) for controls (E: 3.33 $\pm$ 1.2 GPa; H: 0.08 $\pm$ 0.03 GPa) than enzyme digested CDJ (E: 0.69 $\pm$ 0.15 GPa; H: 0.02 $\pm$ 0.004 GPa). The existence of PGs could cause swelling of the CDJ. However, the removal of PGs by enzyme digestion may cause breakdown of the collagen-PG network, allowing free-floating fibrils within the CDJ and decreasing the biomechanical resistance of the region. The observed gradual variation in mechanical properties between the two hard tissues could be caused by changes in structure and chemistry. Based on these results it appears that a hydrophilic PG rich 10 to 50 μm CDJ lies within a biomechanically and structurally different 80 to 200 μm interphase between lamella cementum and bulk dentin. Supported by NIH/NIDCR grants P01DE09859 and T32DE07306.

3:45 PM Z5.7
Synthesis of mesoporous silica by sol-gel mineralization of cellulose nanorod nematic suspensions. Erik Dujardin[1,2], Matthew Blaseby[1] and Stephen Mann[1]; [1]School of Chemistry, University of Bristol, Bristol, United Kingdom; [2]NanoScience Group, CNRS - CEMES UPR 8011, TOULOUSE, France.

The recent emphasis in material science on nanometer-scale structural and organizational design has triggered an unprecedented input of biological concepts into the synthesis of materials with advanced properties [1]. One technologically relevant example is that of mesoporous materials. Mesoporous silicas have been prepared since 1991 by templated mineralization of natural or synthetic organic substrates which resulted in a large variety of pore sizes and structures. However, industrial applications are still impeded by the high cost of the sacrificial templates. We will show that mesoporous silica can be prepared by sol-gel mineralization of inexpensive templates, namely cellulose nanorods nematic suspensions [2]. Indeed, the acid hydrolysis of renewable sources such as cotton yields colloidal suspensions of cellulose crystals having nanoscale dimensions (e.g. 15 x 200 nm). Above a threshold concentration, those nanorods self-organize into a chiral nematic liquid ("cholesteric") crystal phase, the chirality of which is set by the choice of the cellulose-containing raw material and the ionic strength of the suspension. Silica replicas of the colloidal suspensions were obtained by the addition of an alkaline solution of alkoxysilane followed by thermal removal of the cellulose template. The silica replicas are birefringent, which opens possible applications in colour information technology. Structural characterization by optical and electron microscopy also showed that the replica exhibited a cylindrical mesoporosity of about 15 nm in diameter, consistent with the cellulose template. Moreover, the chirality of the native cellulose colloidal substrate appeared to be imprinted in the glass replica, which would make it possible for this material to be used as a chiral stationary phase in enantiomeric separation or as a chiral supporting solid for asymmetric catalysts. [1] E. Dujardin, S. Mann, Adv. Mater., 2002, 14, 775-788. S. A. Davis, E. Dujardin, S. Mann, Cur. Opin. Sol. State Mater. Sci., 2003, in press. [2] E. Dujardin, M. Blaseby, S. Mann, J. Mater. Chem., 2003, 13, 696-699.

4:00 PM Z5.8
An Approach to the Encapsulation of Membrane Proteins in Ordered Mesoporous Hosts. Millicent A Firestone[1], Anna Bhattacharyya[2], Anthony Crisci[3] and Lennox E Iton[1]; [1]Materials Science Division, Argonne National Laboratory, Argonne, Illinois; [2]Illinois Institute of Technology, Chicago, Illinois; [3]University of Illinois, Urbana, Illinois.

Water soluble proteins can be encapsulated in the mesopores of ordered mesoporous silicas and immobilized with their biomolecular activities preserved. The surface charge density in the unmodified mesoporous silica determines its affinity for water soluble proteins bound via electrostatic interactions. Thus, the retention of cytochrome c (horse heart) in the pores of a mesoporous silica (MCM-41) synthesized using a cationic surfactant template is found to be significantly higher than the retention in a structurally equivalent material (SBA-15) synthesized using a neutral triblock copolymer as template. This is attributed to the different chemical modes of assembly of the silica framework in the two cases. The surface of the mesoporous silica can also be modified with covalently bound groups to enhance the protein interaction. For example, aminoalkyl or mercaptoalkyl groups can be attached to the pore surfaces either by using a co-condensation technique during the synthesis of the mesoporous silica, or by using methods of post-synthesis modification. The binding of the protein to these surface-modified mesoporous silicas can be effected either by electrostatic interactions or by the use of covalent grafting techniques. Protein denaturation is avoided, but we have observed perturbations of the encapsulated cyt. c that vary

with the nature of the mesopore surface. Membrane proteins, on the other hand, cannot be directly immobilized in inorganic mesoporous hosts if their biomolecular activity is to be preserved. These proteins require that their native environments be very closely mimicked. Polymer-grafted lipid-based complex fluids, comprised of a phospholipid, an amphoteric surfactant, and a hydrophilic polymer (polyethylene oxide) dispersed in water, self-assemble into ordered mesophases that contain the desired contiguous hydrophilic and hydrophobic components. It has been previously demonstrated that membrane proteins (e.g., bacteriorhodopsin) can be confined in these fluids with their biochemical activity preserved. One conception of a robust host matrix for active membrane proteins involves integrating the mixed environment of the complex fluid into the rigid framework of a mesoporous silica to produce an ordered hybrid nanostructure. We have demonstrated the synthesis of an ordered composite material using a lamellar complex fluid as the template for growth of a silica framework. This opens the possibility of creating robust biocomposite materials wherein the specialized natural function of the encapsulated membrane proteins can be exploited for energy transduction, e.g., in photoinduced electron transfer reactions. This work was supported by the U.S. Department of Energy, Division of Materials Science, Office of Basic Energy Sciences, under Contract W-31-109-Eng-38.

4:15 PM Z5.9
Cultivation of primary hepatocytes on nanoporous aluminum oxid membranes. Andreas Heilmann[1], Nico Teuscher[1] and Hendryk Aurich[2]; [1]Fraunhofer Institute for Mechanics of Materials, Halle (Saale), Germany; [2]Department of Medicine, Martin Luther University Halle Wittenberg, Halle (Saale), Germany.

Primary hepatocytes were cultivated on both sides of porous aluminum oxide membranes using a new developed novel two chamber bioreactor for cell cultivation. Both reactor chambers are only separated by the porous membrane and can be perfused independently. The nanoporous aluminum oxide membranes were prepared by anodic oxidation and had adjustable pore diameter between 0.01 and 0.35 micrometers. The regular open pores are oriented vertically to the membrane surface and go through from upper to back side. The aluminum membrane films are biocompatible and deliver excellent growth conditions for primary hepatocytes isolated from normal adult rat liver. It was found that the cell growth rates depend strongly on the membrane roughness and on the membrane surface. In a permanently running perfusion system supporting the two cultivation chambers, the cells grow on porous membranes as an integral part of the bioreactor system. The primary hepatocytes were cultured under chemically defined serum-free conditions. The cells were able to proliferate and to maintain there differentiated hepatic functions (urea production, CYP450 activity) for a period of at least 2 weeks. The system may serve as a model system for metabolic, pharmacologic-toxicologic studies, and studies on pathogens as well as a bioartificial liver system under defined chemical conditions.

SESSION Z6: Assembly of Biological Monolayers and Films
Chair: Robert J. Hamers
Thursday Morning, April 15, 2004
Room 3007 (Moscone West)

8:30 AM Z6.1
Characterization of amino acid thin films and interfaces using photoemission spectroscopy. Martin M Beerbom, Roy Gargagliano, Niels Dam and Rudy Schlaf; Electrical Engineering, USF, Tampa, Florida.

Protein/inorganic materials interfaces are interesting for many bio-engineering applications such as bio-sensors or molecular electronic devices. L-cysteine is particularly interesting since it can form self-assembled monolayers on gold due to its sulfur containing thiol-group. It therefore offers promising opportunities to form self-assembled device structures on Au surfaces. In our experiments we investigated the L-cysteine/Au interface using x-ray and ultraviolet photoelectron spectroscopy (XPS, UPS) in combination with in-vacuum and ex-vacuum deposition methods. Film morphology was investigated using atomic force microscopy (AFM). Commercially available L-cysteine was deposited by evaporation in ultra high vacuum (UHV), electrospray (ES) injection into UHV, and by dip-coating in N_2 environment. The dip depositions were carried out in a glove box directly attached to the UHV system enabling direct sample transfer without significant environmental contamination. Electrospray injection was performed using a specially designated deposition chamber allowing the clean deposition of L-cysteine thin films directly from solution in the UHV environment through a differentially pumped injection head. All depositions were carried out in multiple steps starting at ultra-low coverages below one monolayer nominal thickness to investigate the interface chemistry. Evaluation of the UP-spectra measured after each deposition step allowed the determination of the electronic structure of the L-cysteine/Au interface. In particular, the charge injection barriers at the interface were determined. Correspondingly measured core level XP-spectra enabled the characterization of the growth modes depending on the deposition technique. They support the conclusion that the deposition process is governed by different growth modes before and after completion of the first monolayer, due to the specific thiol-Au interaction. In our presentation we will compare the results from the three different coating processes. Particular attention will be given to the Au/L-cysteine interaction at the interface, the film morphology, and the electronic structure of the interfaces.

8:45 AM Z6.2
Recognition and Binding of Engineered Polypeptides on Functional Inorganics. Mehmet Sarikaya[1,2], Candan Tamerler[1], Memed Duman[1], Eswar Venkatasubramanian[1,4], Sevil Dincer[1], Emre E. Oren[1], Haixia Dai[2], Corrine Nguyen[2], Daniel T. Schwartz[2] and Francois Baneyx[2,3]; [1]Materials Science and Engineering, University of Washington, Seattle, Washington; [2]Chemical Enginering, University of Washington, Seattle, Washington; [3]Bioengineering, University of Washington, Seattle, Washington; [4]Chemistry, University of Washington, Seattle, Washington.

In biological hard tissues, proteins control inorganic materials assembly, morphogenesis, and formation through molecular recognition and specific binding. Instead of natural proteins, which may be large and complex, hard to isolate and purify, and difficult to use in reconstraction of the hybrid structures, one can use molecular biology techniques, such as directed or forced evolution, to obtain inorganic-binding polypeptides and achieve the same ability to control materials formation using polypeptides at the molecular level. Adapting both cell surface display and phage display protocols, we have selected and isolated short polypeptide seqeunces (7-15 amino acids long) that bind to noble metals (e.g., Au, Pt, and Pd), and oxide semiconductors (e.g., Al2O3, ZnO, and Cu2O) and used them in assembly and formation of inorganics with controlled structures. Here we focus on the understanding of the nature of molecular recognition, binding and kinetics of these polypeptides that were selected for their specific affinity to given inorganic compounds. Considering that molecular recognition of an inorganic surface by a molecule may involve both from chemical (e.g., polarity, H-bonding, charge effects) and physical interactions (structural size, crystallography, and morphology), we have developed/modified an array of techniques (spectroscopic, imaging, and modeling) to quantitatively characterize binding characteristics of these polypeptides. In this presentation, we focus on several techniques. For example, we show that fluorescence microscopy is a routine tool not only as part of the polypeptide selection protocol but it also allows identification of cross-specificity of a given selected polypeptide among several micropatterened inorganics simultaneously (e.g., between a metal and an oxide, or among several metals). Both quartz crystal microbalance (QCM) and surface plasmon resonance spectroscopy (SPR) techniques provide quantitative binding information under controlled solution environments (such as composition, pH, and temperature) and, therefore, they are heavily used in obtaining thermodynamic parameters such as binding/unbinding and equilibrium constants, and free energy of binding. QCM and SPR, combined with AFM, also provide detailed kinetics of binding by iteration. Post-selection genetic engineering techniques can be applied to these polypeptides not only to investigate their mechanisms of binding through identification of core amino acids but also to create second generation libraries, similar to the evolution processes where recursive cycle of mutations and selection, resulting in the progeny of improved features. The research was supported by US-ARO through the DURINT Program.

9:00 AM *Z6.3
Molecular assembly of multifunctional polymers and micro-/nanopatterning techniques: Directing biological response to metal oxide surfaces. Marcus H Textor, Department of Materials, Laboratory for Surface Science and Technology, BioInterfaceGroup, ETH Zurich, Schlieren, Switzerland.

The spontaneous assembly of multifunctional molecules at surfaces has become an important technique to design hybrid inorganic-polymeric-biological interfaces for biosensor applications and model surfaces for cell-biological studies. While alkanethiol self-assembled monolayers on gold surfaces are routinely used today, there is a need for a wider range of reliable assembly systems that are compatible with oxide-based substrate surfaces. The general objective is to produce interfaces via cost-effective, robust techniques that allow the elimination of non-specific protein adsorption and the addition of bioligands in controlled density and conformation in order to direct the biological response to biomaterials and biosensor chips. Poly(ethylene glycol)-grafted polyionic copolymers assemble spontaneously from aqueous solutions at charged interfaces resulting

in well-defined, immobilized monolayers. The degree of interactiveness can be controlled quantitatively through the design of the polymer architecture. If the polymer is functionalized with bioligands such as peptides (to mimic cell-interactive proteins), biotin (link to avidin) or NTA-Nickel (link to histagged biomolecules), biomaterial and biosensor interfaces with quantitative control over ligand density can be efficiently produced. Chemical patterning of surfaces into (bio)adhesive and non-adhesive areas in the micrometer to nanometer range has become an important tool to organize biological entities such as cells and biomolecules at interfaces in a highly controlled manner. Two novel surface modification techniques are presented that combine conventional microfabrication (top-down) with molecular self-organization (bottom-up approach). Biologically meaningful patterns of protein-adhesive and non-adhesive areas in a size range from micrometers to as small as 50 nm could be produced. Fluorescence microscopy, XPS, ToF-SIMS and AFM were used to control ex situ each surface modification step, while the kinetics of the surface reactions including the interaction with biological media were monitored in situ with an optical, evanescent field based sensor (OWLS) and the quartz crystal microbalance (QCM-D) technique.

10:00 AM *Z6.4
Self organized complex organic structures and their characterization by dynamic scanning probes. Harald Fuchs[1,2], Steven Lenhert[1,2], Xiaochun Wu[1,2] and Lifeng Chi[1,2]; [1]Physikalisches Institut, Westfaelische Wilhelms-Universitaet, Muenster, NRW, Germany; [2]Center for Nanotechnology, CeNTech, Muenster, NRW, Germany.

Organic layered structured can be produced by conventional techniques such as the Langmuir Blodgett (LB) technique, the Self Assembly (SA) technique and by Organic Molecular Beam Epitaxy (OMBE) /1/. While a large number of studies was done in the past targeting towards the generation of extended defect free monolayer and multilayer structures, much less is done with respect to the control of structures in low dimensional organic film systems. Optical projection lithography as well as e-beam writing can be applied to generate complex patterns. These steps require development procedures possibly deteriorating the unexposed film areas. A more gentle and versatile way is to use Constructive Lithography /2/ allowing us to perform local multistep processes on the same OTS-film using sequentially different deposition materials such as ordered organic molecules, and metals. A novel approach applying LB-transfer is to make use of dissipative structures which can form complex patterns over a macroscopic area on a solid support, with the local structures exhibiting a characteristic length scale in the nanometer regime /3/. These structures can then be used as templates for the deposition of inorganic systems such as metals, nanoclusters as well as biological cells. The generation and applications of these bio-inspired systems /4/, their properties as well as their characterization with improved scanning probe techniques will be discussed /5, 6/. The problem of self organized interconnection wiring will be addressed /7/. References /1/ R. Nowakowski, C. Seidel, H. Fuchs Phys. Rev. B 63, 195418 (2001) /2/ St. Hoeppener, R. Maoz, S.R. Cohen, L.F. Chi, H. Fuchs, J. Sagiv, Adv. Mat. 14 (No. 15), 1036-1041 (2002) /3/ M. Gleiche, L.F. Chi, H. Fuchs Nature 403, 173-175 (2000) /4/ Ch. M. Niemeyer, M. Adler, B. Pignataro, St. Lenhert, S. Gao, L.F. Chi, H. Fuchs, D. Blohm, Nucleic Acids Research 27, No. 23, 4553-4561 (1999) /5/ B. Anczykowski, B. Gotsmann, H. Fuchs, J.P. Cleveland, V.B. Elings Appl. Surface Science 140, 376-382 (1999) /6/ A. Naber, D. Molenda, U.C. Fischer, H.-J. Maas, C. Hoeppener, N. Lu, H. Fuchs Phys. Rev. Lett. 89, 210801-(1-4) (2002) /7/ N. Lu, J. Zheng, M. Gleiche, H. Fuchs, L.F. Chi, O. Vidoni, T. Reuter, G. Schmid Nano Letters 2 (No. 10), 1097-1099 (2002)

10:30 AM Z6.5
Structure and electrochemical properties of sol-gel-derived V_2O_5-Pyrrole nanohybrid glucose sensors. Yun-Mo Sung, Kyong-Soo Park and Yong-Ji Lee; Materials Sci. & Eng., Daejin University, Kyunggi-do, South Korea.

With rapid increase in the need of accurate and simple analysis method of glucose much effort has been poured to the development of high-performance and high-stability biosensors. An electrochemical sensing using an enzyme electrode is a promising method for this purpose and also, sol-gel route has been known as an attractive way for this biosensor fabrication. Although sol-gel chemistry for the biosensor fabrication has been mostly focused on the traditional silica-polymer hybrids they show some critical drawbacks such as low reliability due to brittleness and low functionality of enzyme due to the acidic condition for the electrode fabrication. In this study glucose oxidase (GOD) was entrapped in V_2O_5-Pyrrole hybrid as a conducting matrix and electropolymerization was performed to construct enzyme electrodes. The concentration of V_2O_5, Pyrrole, and GOD was varied to obtain optimum processing and operating conditions for the biosensors. Also, the molecular weight of pyrrole was changed to control the interlamellar distance of V_2O_5 and thus the overall molecular structure of V_2O_5-Pyrrole-GOD. The operating variables such as temperature and pH were also controlled to obtain optimum operating conditions as biosensors. Scanning electron microscopy (SEM) analyses on the biosensors revealed that GOD was well distributed in the V_2O_5-Pyrrole matrix. Also, transmission electron microscopy (TEM) presented the lamellar and intercalated structures of V_2O_5-Pyrrole-GOD systems. The V_2O_5-Pyrrole-GOD sensors showed improved amperometric properties including fast response rate, high sensitivity, and long-term stability compared to silica-polymer systems. Another major advantage of using this V_2O_5-Pyrrole-GOD sensor lies in the possibility of the fabrication of microelectrodes using electropolymerization due to the conductivity of V_2O_5-Pyrrole matrix.

10:45 AM Z6.6
Characterization and Control of gel-fluid phase transitions in supported phospholipid monolayer films. Lee J. Richter, Clayton S.-C. Yang, John C. Stephenson and Kimberly A. Briggman; National Institute of Standards and Technology, Gaithersburg, Maryland.

Hydrated supported phospholipid structures (Langmuir-Blodgett films, supported bilayers, etc.) have been widely studied as model systems for biological membranes. Supported monolayers and bilayers may also be used as platforms for novel biosensors and biomaterials. The physical structure (gel or fluid phase) of lipid domains in membranes influences cellular signaling and other biological function, and hence must be characterized for both model and biological systems. Until now, it has been difficult to characterize the thermal phase of supported films. We have used vibrationally resonant sum frequency generation to probe the thermal phase transitions of fully hydrated hybrid bilayer membranes. It is discovered that the gel-fluid phase transition temperatures of the lipid layer in supported hybrid bilayers for a series of saturated phospholipids are 10 °C higher than those in corresponding vesicles. The gel-fluid phase transition can be controlled by selective modification of the hybrid bilayer support. Optical characterization of the lipid phase, combined with chemical control of the phase transition, should enable unique studies of the influence of lipid structure on membrane protein structure and function.

11:00 AM Z6.7
Utilizing Self-Assembly of Biological Macromolecules for the Development of Hierarchal Bio-Inorganic Architectures. Ryan M. Kramer[1], Rajesh R. Naik[1], Carina Huber[2], Dietmar Pum[2], Uwe Sleytr[2] and Morley O. Stone[1]; [1]Materials and Manufacturing Directorate, Air Force Research Laboratory, Wright-Patterson Air Force Base, Ohio; [2]Zentrum fur Ultrastrukturforschung und Ludwig Boltzmann Institut fur Molekulare Nanotechnologie, Universitat fur Bodenkultur, Vienna, Austria.

The transition from top-down fabrication routes, to ones that utilize the innate self-assembly of biological systems, has allowed for the construction of hierarchal architectures initiated on the molecular level. Driven by the identification of peptide sequences that are able to both nucleate and grow metals and semiconductors, fusion proteins incorporating these peptide sequences have the ability to control precipitation in two- and three-dimensional orientations. We have found that both silver and cobalt can be precipitated within the inner core of a ferritin light chain fusion protein, constraining the precipitation within the three-dimensional limitations of the fully assembled shell. Additionally, we have fused these inorganic recognition peptide sequences to bacterial cell surface layer (S-layer) proteins and shown that both gold and silver can be precipitated on the surface of these crystalline surface layers within a hierarchal protein lattice. Utilizing the metal recognition motifs of these chimeric proteins, the crystalline self-assembly of S-layer proteins can also be preferentially formed on complementary metallic surfaces. Ideally, we envision that these protein matrices can be utilized for the three-dimensional scaffolding of inorganic-organic hybrid assemblies.

11:15 AM Z6.8
Active Interfaces and Functional Nanostructures through Cell-Directed Self-Assembly. Helen K Baca[1], Carlee Ashley[1], DeAnna Lopez[1], Darren Dunphy[2] and C Jeffrey Brinker[1,2]; [1]Chemical Engineering, University of New Mexico, Albuquerque, New Mexico; [2]Sandia National Laboratories, Albuquerque, New Mexico.

The design of active interfaces, where biological materials can exchange information with their environment through a variety of specific and non-specific interaction forces, will facilitate the development of responsive, functional biocomposite materials needed for a variety of medical and sensing applications. Living cells can give physiologically relevant information about an analyte, providing signal transduction and noninvasive detection through the production of reporter proteins. Central to the development of functional cytosensors is the localization and confinement of the cell on a sensor platform while maintaining cell viability, sustainability, and

functionality. Immobilizing whole cells in a porous, nanostructured silica host matrix through biocompatible template-directed self-assembly allows the cells to be protected and confined in a buffered environment while remaining accessible for nutrient, analyte and waste transport requirements. Templated self-assembly of inorganic mesophases has been well investigated, and can be adapted for whole-cell biocompatibility. However, introducing a living cell into an inorganic/surfactant system capable of self-assembly adds both a metabolically active entity and a heterogeneous, dynamically controlled surface to the system. We used evaporation induced self-assembly (EISA) and biocompatible phospholipid templates to immobilize cells within periodic, porous, silica nanostructures. We find that the interface plays an important role in both promoting cell viability and dictating the structure of the inorganic phase, with the cell actively influencing its microenvironment. We used time-resolved grazing incidence small angle x-ray scattering (GISAXS) and confocal microscopy to follow in-situ structure development at both the abiotic/biotic interface and in the surrounding silica mesophase. Phosphocholines, with zwitterionic headgroups and short double hydrocarbon tails allow the predictable development of specific inorganic mesophases, depending on both the structure and concentration of the lipid. In the presence of cells, however, a multi-layered, organized interface develops, that determines the organization of the silica mesophase. The living cell's ability to manipulate its surroundings through a well-defined cell-phospholipid-silica interface suggests that cells may serve as tools in the construction of hierarchical architectures that incorporate the directed organization of biomolecules of interest.

SESSION Z7: Bio-inspired Materials
Chair: Sonia Letant
Thursday Afternoon, April 15, 2004
Room 3007 (Moscone West)

1:30 PM Z7.1
An investigation of di-peptide modulation of calcite growth. N Han[2], M C Grantham[3], Jim Jon De Yoreo[1] and P M Dove[2]; [1]Chemistry and Materials Science, Lawrence Livermore National Laboratory, Livermore, California; [2]Geosciences, Virginia Polytechnic Institute, Blacksburg, Virginia; [3]Earth and Atmospheric Sciences, Georgia Institute of Technology, Atlanta, Georgia.

The central role(s) of the organic component in biologically controlled mineralization is widely recognized. These proteins are characterized by a high proportion of acidic amino acids, especially aspartate, Asp. Knowledge of the relationship between the structure of the organic components that give specialized functionality and their effect on crystallization is essential for understanding mineralization mechanisms. However, our current understanding of mechanisms by which these complex compounds promote, direct, and inhibit growth is very limited. As a step toward building a comprehensive model for protein controls on the directed growth of calcite, we are investigating the interactions of five di-peptides with the calcite surface during near-equilibrium growth. Using in situ Fluid Cell AFM, the effects of these Asp-Amino acid peptides on growth rate and hillock morphology were determined at near equilibrium conditions and 23.5C. Calcium and carbonate activity ratios are held to approximately 1.0 for all solutions, pH is 8.5, and ionic strength is 0.10 molal. Each of the peptides induces strong changes in growth morphology by changing the step edge morphology but each has a structure-specific effect on hillock morphology. Their impacts on the growth kinetics fall into two groups determined by functional group properties. Our findings for these simple compounds suggest predictable relations between the functional group chemistry of different peptides and indicate the cooperative nature of multiple groups in causing a suite of specialized effects on growth. This reiterates the site-specific nature of organic interactions with the kink-sites at calcite step edges.

1:45 PM Z7.2
Nucleation of calcite on self-assembled monolayers.
Dorothy M Duffy and John H. Harding; Physics and Astronomy, University College London, London, United Kingdom.

Recent experimental work has shown that self-assembled monolayers of long-chain carboxylic acids on gold substrates can control the orientation of calcite crystals nucleated on them. The monolayer favours the (01.n) interface of calcite where n is a small integer greater than unity. Previous simulations have suggested that the nucleation of calcite crystals on organic substrates is controlled by competition between the interactions of the crystal and water with the substrate. Fully ionised substrates have stronger adhesion to the crystal surfaces than neutral substrates and are therefore better at promoting nucleation. The pH of the solution both promotes nucleation and controls crystal shape since the ionised and neutral monolayers stabilise different surfaces. However, our previous work based on classical nucleation theory has suggested that the (00.1) interface should be more stable than the (01.2) interface. This is because the (00.1) interface offers a good fit between mineral and monolayer along both directions of the surface unit cell, whereas the (01.2) interface has significant mismatch in one direction. New simulations, where we explicitly consider small clusters of various sizes, have shown that the (01.2) interface is favoured for small clusters provided that they have the right shape - extended along the well-matched direction and sufficiently thin along the mismatched direction that the mismatch can be taken up by straining the organic monolayer. This suggests that the (01.n) faces are favoured at the nucleation stage and hence that the more stable (00.1) face is kinetically hindered.

2:00 PM Z7.3
Glycosaminoglycans as Templates for Calcite Nucleation and Growth. Jose Luis Arias, Marcela David, Andronico Neira and Maria S. Fernandez; Faculty of Veterinary Sciences, Universidad de Chile and Center for Advanced Interdisciplinary Research in Materials Science, Santiago, Chile.

Mineralization of egg and seashells is controlled by an intimate association of inorganic materials with organic macromolecules. Among them, particular polyanionic sulfated macromolecules referred to as proteoglycans have been involved in the calcification of these biominerals. The sulfated moieties of the proteoglycans are part of polymer chains constituted of building-blocks of disaccharide units, referred to as sulfated glycosaminoglycans (GAGs), which are covalently attached to a protein core. By using a sitting drop crystallization assay under controlled conditions of time, pH and reactants concentration, we have bested several sulfated and non-sulfated GAGs differing in their sulfate and carboxylate degree and pattern, on their ability to modify calcium carbonate crystal morphology as observed under scanning electron microscopy. Without the adddition of GAGs, regular rhombohedral calcite crystals are formed. When hyaluronic acid (HA), a non-sulfated but carboxylated GAG, was added, long piles of unmodified calcite crystals were observed. By adding desulfated dermatan, which is an epimeric form of 11A but shorter polymer, having their carboxylate groups in an inverted configuration, isolated rhombohedral calcite crystals showing rounded corners with planes oriented parallel to the c axis were observed. When dermatan sulfate was added, isolated calcite crystals exhibit a columnar morphology as a {hk0} cylinder with three {104} faces forming a cap at both ends. Heparin effects depend on the fraction used. Fast-moving heparin fraction (FM), is an undersulfated, low-molecular-weight heterogeneous polymer, while slow-moving heparin fraction (SM) is a high-molecular-weight homogeneous polymer rich in trisulfated disaccharide unit. FM addition has variable effects on calcite crystal morphology which can be related to the polymer heterogeneity. The addition of the hypersulfated heparin SM induced the formation of large rosette-like aggregated calcite crystals, where the majority of the {104} faces appeared not to be lost, but where the calcite crystals aggregate in a helical structure wth a clockwise spiral morphology. It is concluded that, the variation of the sulfate and carboxylate content and configuration of these polymers drastically changed the morphology of the calcite crystals. The production of calcite particles with defined morphologies could be interesting for the design of novel materials with desirable shape-and texture-depending properties. Granted by FONDAP 11980002.

2:15 PM Z7.4
Investigating the physical controls of Mg and Sr on calcite biomineral formation using AFM measurements of growth kinetics. Laura E. Wayslenki[2], Patricia M. Dove[2], Darren S. Wilson[2] and Jim Jon De Yoreo[1]; [1]Chemistry and Materials Science, Lawrence Livermore National Laboratory, Livermore, California; [2]Geosciences, Virginia Polytechnic Institute, Blacksburg, Virginia.

Sr and Mg are widely recognized as important modulators of biological calcite production in natural waters, producing inhibition and shape modification. Until recently, the common understanding of those effects has been based on observations in bulk systems. Here we report the results of AFM experiments aimed at providing a molecular-scale picture of calcite modification. Although Sr and Mg have similar chemical behavior in some systems, we show here that they have very different mechanisms of interaction with growing calcite crystals. We measured step velocities and step morphologies on calcite in flowing supersaturated solutions containing varying amounts of Mg or Sr. In the case of Mg, we also varied the temperature over a range of 15 to 30C. From our measurements we conclude that Mg inhibits calcite growth by raising the equilibrium solubility without altering the step kinetics at fixed supersaturation. The result is a monotonic decrease in step speed with increasing Mg content at fixed Ca and CO_3 concentrations. In contrast, at low concentrations, Sr actually accelerates step speeds, while, at higher concentrations it causes a steep fall in step speed due to step pinning. Analysis using classic step-pinning models fails to predict the detailed dependence of inhibition on Sr content. Morphologically, addition of Mg alters

calcite by preferentially slowing steps moving along the boundary between the two non-equivalent step types, which incorporate Mg at rates that differ by an order of magnitude. We present a plausible argument for stress due to this differential Mg incorporation as the source for the anisotropic effect. In contrast, the addition of Sr preferentially slows growth along the calcite glide plane, which does not separate distinct step-types. The data argue for stronger Sr binding at kinks moving along the steps towards this plane as opposed to those moving towards the boundaries between distinct step types. Finally, the temperature dependence measurements give an activation energy for growth of 0.5eV per molecule, a number which is is somewhat larger than that estimated for other solution-based crystals.

2:30 PM Z7.5
Physical chemical parameters for the protein-guided growth of enamel crystals. Stefan Habelitz[1], Alexander Kullar[1], Mehdi Balooch[1], Sally J Marshall[1], Pamela K DenBesten[2], Grayson W Marshall[1] and Wu Li[2]; [1]Dental Sciences, University of California, San Francisco, California; [2]Growth and Development, University of California, San Francisco, California.

During tissue mineralization, proteins of the extracellular matrix control morphology, growth rate and texture of crystals on a molecular level, while cellular involvement occurs predominantly through regulation of the ionic environment, e.g. concentration of Ca2+ and PO43-. It is hypothesized that in an artificial environment, the degree of saturation (DS) needs to be adjusted to allow specialized proteins to interact with the forming mineral. OBJECTIVE: To compare how mineralizing solutions with increasing DS and pH affect the growth of apatite in the presence of amelogenin proteins. METHODS: Full-length recombinant human amelogenin proteins were used at 1.5 mg/ml and mixed with mineralizing solutions at pH between 6.5 and 8.8 and DS between 4.8 and 15.5 at 37 °C. Highly polished bioactive fluoroapatite (FAP) glass-ceramics were used as templates and immersed into 400 μl of solution. Substrates were removed after 24 h, rinsed and gently dried for tapping-mode imaging by AFM. RESULTS: In protein-free solutions, heterogeneous nucleation in form of spherical precipitates (r < 10 nm) adhering non-specifically to glass matrix and FAP crystals formed at low DS (< 10). At DS > 10, both heterogeneous precipitation and homogeneous growth on FAP crystals were observed. The height of growth on the FAP crystals increased with DS to about 15 nm at the highest DS. Substrates that were immersed in mineralizing solutions with amelogenin protein did not show random precipitation of crystallites originating from heterogeneous nucleation. Moreover, these substrates showed homogeneous crystal growth on FAP only. The height of these layers increased by up to 20 times compared to protein-free solutions. The density and microstructure of the layers varied with DS. At DS < 11, the layers were a porous mesh of 150 nm needle-like aggregates. At DS > 11, dense layers composed of 100 to 200 nm high elongated and aligned particles formed. Layer height, however, was strongly decreased (15 nm) if the pH was 8.8. CONCLUSION: Amelogenin proteins promote homogeneous nucleation of apatite. DS and pH affect the ability of amelogenin to interact with mineral and to form dense mineralized layers. Supported by NIH/NIDCR Grants DE P01DE09859 and R21DE015416

3:15 PM *Z7.6
Kinetics of Amine-Catalyzed Silanol Condensation: Implications for Pathways to Biogenic Silica Synthesis.
Katya Delak[1,2] and Nita Sahai[2]; [1]Chemistry, University of Wisconsin - Madison, Madison, Wisconsin; [2]Geology and Geophysics, University of Wisconsin - Madison, Madison, Wisconsin.

Biogenic silica production in freshwater and seawater environments is of great interest because the pathways involved are much milder and more controlled than typical industrial sol-gel methods. The mechanisms behind biogenic silica synthesis may therefore provide inspiration for potential alternative, controlled and economical routes to current methods for silica production. The chemical mechanisms responsible for silica formation *invivo* are, however, not fully understood. Researchers have implicated amine and polyamine moieties of proteins in the catalysis of controlled silica precipitation, where the starting compounds were assumed to be silicic acid or organosilicates. Drawing on these findings, we have examined the influence of both mono and polyamines on the rates of hydrolysis and condensation of a model organosilicate compound. We have chosen trimethylethoxysilane as the starting compound because it has a single organosilicate bond, so the reaction can be followed with little ambiguity. Hydrolysis and condensattion lead to the products trimethylsilanol and hexamethyldisiloxane according to: (1) $(Me)_3SiOEt + H_2O \rightarrow (Me)_3SiOH + EtOH$ (Hydrolysis) (2) $(Me)_3SiOH + (Me)_3SiOH \rightarrow (Me)_3SiOSi(Me)_3 + H_2O$ (Condensation) Using quantitative ^{29}Si NMR spectroscopy we have followed the formation rates of the hydrolysis and condensation products. The results of these experiments provide information on the influence of such factors as amine nucleophilicity, polymer chain length and amine number on rates of catalysis. Finally, we propose mechanistic pathways for hydrolysis and condensation based on our kinetic findings.

3:45 PM Z7.7
Genetically Modified Biomolecules for the Synthesis of Metal Alloy Nanoparticles. Michael T Klem[1,3], Debbie Willits[2,3], Mark Young[2,3] and Trevor Douglas[1,3]; [1]Chemistry & Biochemistry, Montana State University, Bozeman, Montana; [2]Plant Sciences, Montana State University, Bozeman, Montana; [3]Center for Bioinspired Nanomaterials, Montana State University, Bozeman, Montana.

One of the major driving forces in nanomaterials synthesis is the establishment of precisely defined molecular (or extended) structures of well defined morphology. The use of biomimetic methods for materials synthesis allows one to leverage nature's ability to control size, morphology, and composition on a fine level. In the present work, we have used a small spherical heat shock protein (MjHsp) from *Methanococcusjannaschii* as a scaffold for the for the synthesis of $L1_0$ CoPt nanoparticles. This small protein assembles into an empty 24 subunit cage of octahedral symmetry whose interior and exterior surfaces are amenable to genetic and chemical modifications. Since metallic alloys are unknown in biological systems, a small peptide sequence was incorporated into the interior surface of the assembled protein cage. The incorporation of this peptide sequence has made possible to obtain the ordered tetragonal CoPt phase at physiological conditions compared with the thermodynamic minimum of approximately 500 °C. The resultant material was characterized by various magnetometry methods, transmission electron microscopy, electron diffraction, gel electrophoresis, dynamic light scattering, and UV-Vis spectroscopy. This approach opens up a new realm of exploration for obtaining unprecedented materials at the biological level and these methods offer a more benign approach toward materials synthesis over other traditional solution approaches.

4:00 PM Z7.8
Biomimetic Mineralization of Spinels in Engineered Viral Protein Cages. Mark A Allen[1,4], Trevor Douglas[1,4], Michael Klem[1,4], Debbie Willits[2,4], Mark Young[2,4] and Yves Idzerda[3,4]; [1]Chemistry and Biochemistry, Montana State University, Bozeman, Montana; [2]Department of Plant Sciences, Montana State University, Bozeman, Montana; [3]Department of Physics, Montana State University, Bozeman, Montana; [4]Center for BioInspired Nanomaterials, Montana State University, Bozeman, Montana.

The coat protein of Cowpea chlorotic mottle virus (CCMV) has been genetically engineered to act as a mimic for the iron storage protein ferritin. The modified viral coat protein assembles into a cage-like structure which catalyzes the oxidative hydrolysis and mineralization of nanoparticles of iron oxide in a spatially selective manner. In the wild type virus, RNA is packaged through complementary electrostatic interactions present on the interior surface of the protein shell. We have altered the electrostatics of the protein cage interior by site-directed mutagenesis of the coat protein subunit. Nine positively charged residues (lysine and arginine) on each subunit were replaced by acid residues (glutamic acid) yielding an assembled protein cage, which no longer packages RNA. The negatively charged interior interface of the protein cage acts like ferritin to catalyze the oxidative hydrolysis of Fe(II) to form a ferric oxyhydroxide nanoparticle. Manipulation of reaction conditions as well as chemical stabilization of the protein allows the use of the viral protein cage for the synthesis of transition metal oxide spinels (Fe_3O_4, Co_3O_4) constrained in size by the dimensions of the viral cage.

4:15 PM Z7.9
Fibroblastic Attachment to Surface-Modified Titanium Alloy Foam. Serene Cheung[1], Maxine Langman[2], Jonathan Blay[3], Maxime Gauthier[4], Michael Dunbar[5] and Mark Filiaggi[2,1]; [1]School of Biomedical Engineering, Dalhousie University, Halifax, Nova Scotia, Canada; [2]Applied Oral Sciences, Dalhousie University, Halifax, Nova Scotia, Canada; [3]Department of Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada; [4]Industrial Materials Institute, National Research Council, Boucherville, Quebec, Canada; [5]Department of Orthopaedic Surgery, Queen Elizabeth II Health Sciences Center, Halifax, Nova Scotia, Canada.

Purpose : Titanium and its alloys have been regarded as one of the few metals suitable for in vivo implantation and is considered to be biocompatible. In biomedicine, titanium implants are most often found in orthopaedic and dental applications, but with the advent of porous reticular metal foams, this class of materials can potentially act as scaffolds for soft tissue attachment via fibrous tissue ingrowth. The present work has examined short-term fibroblast attachment to such a structure, produced by the National Research Council of Canada using a proprietary process that combines powder metallurgy with polymer foam technology. Because cell attachment may be

modulated by surface chemistry and topography, this study also analyzed the effects of surface treatment using several aqueous reagents.

Methods : Porous Ti-6Al-4V discs measuring 10 mm in diameter and 1 mm in thickness were subjected to one of the following modifications: (a) 30 minutes of ultrasonic immersion in Kroll's reagent (2 mL HF, 4 mL HNO_3, 994 mL H_2O); (b) 30 minute passivation in 30% HNO_3; (c) 30 minutes in 6 N HCl/H_2SO_4; and (d) 60 minutes in 30% H_2O_2. Autoclaved discs served as experimental controls. For the cell culture, 3T3 fibroblasts were seeded at 5×10^5 cells per well in a 24-well ultra-low attachment polystyrene plate and incubated for 4 hours at 37°C. Attachment was quantified by the colorimetric MTT assay in conjunction with manual cell counts using a hemacytometer. The latter method determined the amount of unattached cells remaining in the medium, those weakly detachable following PBS rinses, and cells released only after aggressive trypsinization.

ResultsandConclusions : Cell attachment to this scaffold was reasonably favourable, with approximately 20% of seeded cells adhered to control discs. Three of the surface modification regimes in this preliminary work showed slightly higher cellular activity according to the MTT assay, but differences were not statistically significant (Student's t-test, $p > 0.05$). Results from the cell counts also did not unequivocally indicate any such benefit over untreated Ti-6Al-4V controls. The HF-based treatment however, may have been detrimental to short-term cell attachment, as indicated by lower MTT absorbance values, more unattached fibroblasts, and fewer cells released upon trypsinization. Further work is ongoing to clarify cellular behaviour pertaining to chemical surface manipulation, particularly the nature of fibroblast infiltration at longer timepoints.