Applications of Solid-State Nuclear Magnetic Resonance (SSNMR) and Neutron Reflectivity (NR) to the Investigation of Protein/Surface Interfaces

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Introduction
Naturally occurring biominerals possess an impressive array of strength, order, and nanostructure, but the protein-mineral interactions that result in these properties are not well understood; as such, this is a primary goal. The secondary structure and orientation of biomineralization proteins are thought to play an important role in the formation of biominerals; however, there is little experimental evidence to explain the underlying mechanisms. SSNMR and NR have the potential to elucidate site-specific structure and orientation information of biomineralization proteins, inaccessible by other methods, and are highlighted in an investigation of the protein-crystal interaction of an enamel-forming protein. Methods to investigate crystal nucleation and growth also are presented.

Purpose
This research aims to probe the protein orientation and structure of an amelogenin protein, leucine-rich amelogenin peptide (LRAP) on the hydroxyapatite (HAP) surface.

Methods
SSNMR, NR, and computational methods were utilized to study the surface-immobilized LRAP protein. The structure of the N-terminus was studied, and the protein-surface interaction of the C-terminus was investigated.

Results
These experiments demonstrate that the C-terminus is oriented next to the HAP surface. NR measurements yielded a distance from the deuterated region to the COO^- terminated surface of 5-10Å, in good agreement with the SSNMR data, which yielded a $^{13}C_{ala46}$-^{31}P distance of 7-7.5Å from the labeled alanine to the hydroxyapatite surface. Modeling results are consistent with experimental measurements, resulting in an average $^{13}C_{ala46}$-^{31}P distance of 7.3Å for LRAP oriented onto the 100 face of the HAP. Structural measurements using SSNMR indicate a significant change in the structure of the N-terminus upon binding to the HAP surface.

Conclusions
These results demonstrate the unique capability of SSNMR, NR, and computational modeling to provide quantitative, site-specific, molecular-level detail of the protein-surface interface.

Potential for Utilizing Ion Channels in Nanotechnology

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Ion channel proteins act as elementary electrical components of biological cells. Our group develops structural and functional models of voltage-gated and mechanosensitive ion channels. The voltage-gated channels have electronic characteristics similar to those of transistors (especially field-effect transistors) and piezoelectrical devices. They also have additional properties, such as the ability to conduct current among a variety of inorganic ions, that conventional electronic devices do not possess. The mechanosensitive channels are nanoscale pressure valves that regulate intracellular hydrostatic pressure by forming large pores through the cell's membrane under specific physiological conditions. Nanoelectromechanical systems that utilize mechanisms similar to those of biological cells may be especially useful for biological implants that interact directly with cellular tissues and organs. Due to their special electronic and mechanical properties, ion channels constitute a natural pool of candidates to be nanoscale building blocks from which more complex machines can be built. To utilize ion channels in this way, it is vital to understand their structures and how they function. Unfortunately, structures of ion channels are very difficult to determine experimentally, and only a few are known. Even then, typically only one static conformation is determined, which is insufficient to understand how it functions. To address this problem, our group developed a unique and experimentally verified set of modeling principles and tools to predict the ion channel structures and conformational changes. These tools can reduce the number of costly in vitro experiments and optimize the design for manufacturing. We also identified several basic construction principles that are utilized by these ion channels for gating and ion conduction. Another problem is that proteins degrade, especially when placed in an organism. Fortunately, ion channels can be synthesized that are much more durable and will not be attacked by the body's immune system. Our methods, and the principles that we have identified, should be useful in designing such synthetic analogs.

Naturally Occurring Fluorescent Ceramic Bioimaging Probes

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We have begun developing the next generation of bioimaging probes that are tunable, highly fluorescent, nontoxic, and biocompatible. These new nanoprobes are not quantum dot or cadmium chalcogenide (CdE, where E=S, Se, Te) nanoparticles. Instead, these new fluorescent ceramic bionanoprobes are based on naturally occurring fluorescent compositions and lanthanide-doped luminescent ceramic materials. Our research is a cross-disciplinary venture between (1) a group of materials scientists and chemists from the SNL who have proven expertise in nanoparticle synthesis and biofunctionalization and (2) academic National Institutes of Health-funded researchers (UNM School of Medicine and the UND) who have expertise in imaging cell signaling and trafficking pathways and phospholipid recognition, respectively. The SNL chemistry group is working concurrently toward two goals: (1) the synthesis of nanoprobes from entirely new materials and (2) innovations in functionalization that will enable the selective delivery of these probes for cellular imaging. These results will set the stage for in vivo studies whose goals would be to (1) detect cancer sites, inflammation, and other disease processes and (2) study the delivery and specific release of drugs at disease sites by further manipulation of the chemistry.

Microfluidic Mixers for Measuring the Kinetics of Protein Folding

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We are developing a microfluidic mixer for studying protein folding. These devices enable us to access conformational changes under conditions far from equilibrium and at previously inaccessible time scales. We currently can achieve the fastest mixing time reported—t=8 μs—while reducing the sample consumption to femtomoles. In addition, in a single experiment, we can record time scales from under 10 μs to over 100 ms. I discuss the design and optimization of the mixer, using modeling of convective diffusion phenomena, and characterization of the mixer performance, using microparticle image velocimetry, dye quenching, and Förster resonance energy-transfer measurements of single-stranded DNA. We demonstrated the feasibility of measuring fast protein folding kinetics on acyl-CoA binding protein and are now moving to studying fast collapse in a variety of other proteins. In parallel, we are also improving the design to reduce the mixing time further through shape optimization and use of nanofabrication.

Unraveling the Architecture and Structural Dynamics of Pathogens by High-Resolution in vitro Atomic Force Microscopy (AFM)

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Purpose
The capability to image surfaces of single microbial cells and viruses at the nanometer scale under native conditions would profoundly impact mechanistic and structural studies of pathogenesis, immunobiology, environmental resistance, and biotransformation.

Methods
We have utilized high-resolution in vitro AFM for studies of the structure, assembly, and environmental dynamics of plant and human viruses, bacteria, and bacterial spores.

Results
The lateral resolutions of ~2.5 nm were achieved on pathogens in vitro. For icosahedral viruses, it was demonstrated that protein capsomeres on the virion surfaces could be clearly resolved (Malkin et al. 2004). Further dissection of viral particles with detergents and enzymes has revealed the internal subviral structures of several large human viruses (Malkin et al. 2004). This allowed the intriguing modeling of vaccinia virion based on the hierarchy of observed substructure (Malkin et al. 2003). We found that strikingly different species-dependent structures of the spore coat of the *Bacillus atrophaeus* (*B. atrophaeus*) species appear to be a consequence of nucleation and crystallization mechanisms that regulate the assembly of the outer spore coat and proposed a unifying mechanism for outer spore coat surface self-assembly (Plomp et al. 2005). We discuss the results of the direct visualization of the environmental response of individual *B. atrophaeus* spores and concomitant changes of the internal spore core/cortex and spore coat surface morphology (Plomp et al. 2005).

Conclusions
These studies establish in vitro AFM as a powerful new tool capable of revealing pathogen architecture, structural dynamics, and variability at nanometer-to-micrometer scales.

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Force Spectroscopy of Single-Molecule Adhesive Interactions in Three Polymer-Tethered Systems: Protein-Receptor, Thiol/Thiolate-Gold, and Lipid-Lipid

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Motivation

Adhesion is the initial step in a variety of biological functions and occurs predictably at interfaces, for example, at cell membranes (cell signaling/vesicle trafficking), between cells (sperm-egg fusion), and between cells and organisms such as bacteria and viruses. Although some molecules (e.g., intrinsic membrane proteins) are firmly embedded within the interface, many biological molecules are attached to flexible tethers that extend the molecules away from surfaces and limit their interaction volumes. Due to the utility of tethers, researchers have attempted to utilize polymers on surfaces to mimic or inhibit functions such as targeted delivery, inflammatory responses, and self-assembly. These approaches have met with varied success, in part because of a limited understanding of how the polymer tethers affect and are themselves affected by binding. We report on the use of end-functionalized tethers to examine polymer architecture and single-molecule adhesive interactions in three multivalent adhesive systems.

Methods

We use an atomic force microscope (AFM) in force spectroscopy mode to directly measure the adhesion force between molecules tethered with polymers to the AFM cantilever and molecules of interest on surfaces. This technique generates adhesion force versus tip-sample separation data that are analyzed to produce the forces required to rupture bond pairs and pull individual lipids out of a synthetic cell membrane.

Results and Conclusions

We have developed a methodology that permits high-fidelity measurements of the specific interactions between single molecules on surfaces. Furthermore, using an AFM to perform these measurements enables the position of the interactions to be measured with nanometer precision, offering the possibility of localizing interactions with great accuracy on biological structures (e.g., cells, organelles, or even smaller macromolecular assemblies). In addition to measuring adhesion, we use this technology to measure the forces involved in stretching polymers and demonstrate the utility with three biologically significant systems.

Confocal Microscope for Simultaneous Time- and Frequency-Resolved Photon Detection With Single-Molecule Detection Sensitivity

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We present initial studies demonstrating the capabilities of a new confocal fluorescence microscope. This microscope allows, for the first time, the wavelength and absolute emission time of each detected photon to be simultaneously measured with single-molecule sensitivity. We present single-molecule fluorophores and single-pair fluorescence resonance energy transfer, in particular fluorescence measurements of single rhodamine 6G, tetramethylrhodamine, and Cy3 molecules embedded in thin films of polymethylmethacrylate and of single-pair fluorescence resonance energy transfer between 2 Alexa (Invitrogen) fluorophores spaced apart by a short polyproline peptide. By simultaneously probing multiple parameters of the fluorescent sample, one can more completely characterize the single-molecule system. In particular, some of the motivations for developing single-molecule, multiparameter measurements are to (1) accurately identify multiple fluorophores within a single sample based on a range of fluorescence characteristics, (2) fully characterize the fluorescence of the molecule to obtain the most complete information on its local environment, and (3) gain insights into mechanisms that control the fluorescence properties by measuring auto- and cross-correlations of emission spectra, decay times, and intensities. The ability to measure these correlations is valuable for understanding both the fundamental photodynamics of the excited molecule and how the fluorescence reports on the local environment. This capability has many applications in biology and biophysics.

Kinesin- and Microtubule-Based Transport Systems: Nanoscale Science and Engineering Perspectives and Applications

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Energy-consuming transport systems play a key role in a wide array of biological processes, such as the segregation of chromosomes during mitosis and the reorganization of melanophores that enable fish to change color. One such transport system involves a protein dyad in which the biomolecular motor protein kinesin actively translocates molecular cargo along cytoskeletal fibers (i.e., microtubules, MTs). Although the biophysical mechanism of kinesin-MT transport has been extensively studied, the functional role of this system with respect to the phenotypic expression of complex, cellular processes has received much less attention. Our work specifically focuses on two aspects of the kinesin-MT transport system: (1) understanding the intricate and stochastic nature of this highly efficient transport system and (2) exploiting kinesin and MTs as functional components of nanoscale, integrated materials and devices. To this end, we have isolated a gene encoding a robust kinesin from the thermophilic fungus *Thermomyces*

lanuginosus and engineered the recombinant protein for integration at synthetic interfaces. Detailed biochemical and biophysical characterization of this kinesin has provided fundamental insights into both how structural domains impart thermostability and how protein structure and function may be manipulated at hybrid interfaces. Our team also demonstrated the ability to transport a wide array of synthetic nanoparticles (e.g., semiconductor nanocrystals) and described the design rules that control the assembly and transport of composite materials using kinesin and MTs. In addition, we engineered antigenic selectivity into this transport system and demonstrated the active capture, transport, and detection of individual virus particles in integrated microfluidic systems. Continued development of this technology, coupled with a more detailed understanding of the stochastic biological process, will enable the development of materials and devices with a wide range of biomimetic functionality.

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Manipulation and Monitoring of Cellular Matrices With Arrays of Nanostructured Electrophysiological Probes

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Purpose
We report progress in the application of vertically aligned carbon nanofibers (VACNFs) as parallel subcellular probes for biological manipulation and monitoring. VACNFs possess many attributes that make them very attractive for implementation as functional, nanoscale features of microfabricated devices. They can be synthesized at precise locations on a substrate, can be grown many microns long, and feature sharp, nanodimensioned tips. This and their needlelike, vertical orientation on a substrate make them particularly attractive as multielement cellular scale probes or as a parallel embodiment of traditional single-point microinjection or microelectrophysiological systems.

Methods
Progress in fabricating, characterizing, and biologically integrating several embodiments of VACNF cell-probing systems is discussed. Detailed methods will be presented that enable the integration of VACNFs into microfabricated devices. Postsynthesis chemical and physical derivitization schemes are outlined that promote the biological utility of VACNF devices. Finally, methods to interface these structures with biological matrices are discussed.

Results
The results of both intracellular and extracellular interfacing of VACNF architectures are overviewed from the vantages of cell interaction and proliferation on nanofiber-based devices, electrophysiological probing and stimulation of excitable cell matrices, including quail neuroretina and rat pheochromocytoma cells, and long-term biochemical manipulation of the intracellular domains of various cell types via transcription from cell-penetrant, DNA-modified nanofiber scaffolds.

Conclusions
The merging of recent advances in the synthesis of nanostructured materials with the mature technology of microfabrication provides opportunities to interface with biological systems at the subcellular and even the molecular scale. Cellular integration of VACNF-based devices has been demonstrated to provide the ability to monitor and manipulate cell and tissue matrices both chemically and electrochemically at high spatial resolutions and on a massively parallel basis. We anticipate that these architectures will provide an effective platform to promote the understanding and influence of molecular-scale phenomena in cell and tissue matrices.

Lithographically Directed Nanofluidic Assembly

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Techniques for assembling nanostructures are essential to developing useful systems. Fabrication processes suitable for the nanoscale have been developed to a high level of sophistication in the semiconductor industry; however, because of the high temperatures or aggressive chemistries employed, they are typically not suitable for patterning biological nanostructures or objects such as nanocrystals, nanotubes, and nanorods.

This research focuses on the use of standard nanolithography, together with fluidics, which enables the placement of nanostructures at precise locations and in precise quantities. It has long been observed that the retreating contact line of a liquid containing particles, such as coffee, leaves a concentrated, ring-shaped deposit. The nonuniform evaporation from a liquid droplet with a pinned contact line leads to a flow within the liquid that sweeps the particles to the contact line. Pinning a liquid contact line at a lithographically defined feature concentrates and deposits particles at that feature.

This presentation details results from a set of experiments using features defined in photoresist that have been used to control the assembly of Au nanocrystals from 2 to 50 nanometers in size and of CdSe tetrapods. Precise control of the number of nanocrystals is achieved by adjusting the ratio of the lithographic feature size to that of the nanocrystals.

This technique operates at room temperature, is compatible with aqueous liquids, and can be used on substrates *after* they have been patterned with, for example, conventional electronic devices. Therefore, it could be used for the precise placement of biomolecules within an integrated device. The limitations on large-area nanoscale patterning typically imposed by the use of electron-beam lithography can be overcome by means of nanoimprint techniques; preliminary results will be presented.

Multiplex Immunoassays Using Biofunctionalized Nanorod Particles and Automated Optical Readout

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Purpose

Medical diagnostics, biothreat sensing, and environmental monitoring all demand the simultaneous detection of multiple targets with high sensitivity and specificity. Particle-based assays offer the additional advantages of flexibility and customization, but complex sample processing requirements limit their use in small, deployable automated systems for the field or clinic. By implementing the particle assay concept with a new class of engineered nanorod particles (Nanobarcodes® particles), we enable the construction of automated multiplex assay systems with high performance and compact sample control within a microfluidic format.

Methods

Nanobarcodes® particles are fabricated by sequential electrodeposition of metals within porous alumina templates, yielding optically encoded striping patterns that can be read automatically. The addition of self-assembled monolayer coatings and target-specific antibodies allows each encoded class of nanorod particles to be directed against a different antigen target, with fluorescent readout of binding events also carried out automatically. Studies were performed to characterize the colloidal properties (zeta potential) of suspended nanorod particles as a function of pH, the ionic strength of the suspending solution, and the surface functionalization state. Additional studies have produced the means for noncontact manipulation of the particles, including insertion of magnetic nickel stripes within the encoding pattern and control via externally applied electromagnetic fields.

Results

A prototype assay panel directed against bacterial, viral, and soluble protein targets demonstrates simultaneous detection at sensitivities comparable to state-of-the-art immunoassays, with minimal cross-reactivity. The zeta potential studies define conditions for optimal transport of the functionalized particles in aqueous suspension, and the particle manipulation techniques provide for the effective control of particle movement within an enclosed assay system.

Conclusions

A multiplex immunoassay system has successfully been implemented on an engineered nanorod solution array platform, demonstrating required levels of sensitivity and specificity. The unique physical properties of the nanorod particles enable the ongoing development of an integrated microfluidic system for sample preparation, assay completion, and optical readout.

Controlled Synthesis and Processing of Multifunctional Nanostructures for Biological Sensing and Delivery

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Purpose
To probe the nanobio interface, nanoparticles, nanotubes, and nanorods must be synthesized and characterized with tailored diameters, lengths, and crystal structures. For in vitro delivery and sensing, nanostructures must be purified, processed, and functionalized into functional forms such as fibers, membranes, and porous polymer nanocomposites.

Method
The controllable growth of single-wall carbon nanotubes (SWNTs), single-wall carbon nanohorns (SWNHs), nanoparticles, nanowires, and nanobelts are described using in situ, time-resolved diagnostics during growth by two methods: laser vaporization and chemical vapor deposition. Through coordinated chemical processing and dispersion, nanomaterials are purified and dispersed into functional forms. Interactions between SWNTs and SWNHs and living cells are being explored for toxicological effects.

Results
Loose SWNTs have been produced by laser vaporization and purified to extremely high levels (<0.02 wt. % metal impurities). The purified SWNTs were formed into a variety of transparent, conducting membranes and sensors that are described. The purity of the nanotubes has a high impact on the time response of the sensors. Alignment techniques are described that result in the incorporation of SWNTs along the axis of micron-size polymer fibers, as characterized by polarized Raman microscopy. Polymer films containing aligned nanotubes were developed for sensor applications, and scanning electron microscopy-based imaging techniques were developed to characterize the network of nanotubes within the polymer film understand the current-voltage characteristics of the sensors. These same techniques are being applied to a variety of oxide nanowires, nanobelts, and other nanostructures. In addition, we describe the synthesis and application of vertically aligned nanotube arrays (VANTAs) on catalyst-patterned substrates, which provide high thermal diffusivity pathways at the nanoscale through polymers to provide thermally and electrically conductive vertical interconnects in porous media. In situ, time-resolved reflectivity is described to precisely control the length of VANTAs and produce VANTAs with predescribed optical properties.

Conclusions
We demonstrate the controlled synthesis and characterization of carbon nanotubes, carbon nanohorns, and a variety of oxide nanostructures necessary for biological sensing as well as nanotoxicological and therapeutic applications. We also show processing techniques for their incorporation into polymeric media suitable for biological introduction and applications.

Characterization of Nuclear Impalement Event During Gene Delivery Using Arrays of Vertically Aligned Carbon Nanofibers

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Purpose

Parallel gene delivery using arrays of vertically aligned carbon nanofibers (VACNFs) is a recently emerged nonviral technique for genetic transformation. This approach exploits a universal delivery mechanism—microinjection—that has been demonstrated as effective for all cell types. However, unlike conventional microinjection, which is a labor-intensive and slow serial method for single-cell delivery, vast arrays of VACNFs can provide for massively parallel gene delivery to cellular matrices. The impalefection efficiency depends on many factors, such as plasmid loading and its adhesion to nanofibers, the efficiency of cell membrane penetration, and the viability of impaled cells. The efficiency of plasmid delivery into a nuclear domain is believed to be one of the crucial factors.

Methods and Results

Catalytically synthesized nanofibers are deterministically grown in a vertical orientation and feature nanoscale tip geometries and lengths of up to several tens of microns. The synthesis is performed by catalytically controlled, plasma-enhanced chemical vapor deposition from patterned Ni catalyst on Si 100 mm diameter wafers. Chinese hamster ovary cells are impaled onto arrays of VACNFs by centrifuging from suspension and successive pressing. Prior to impalement, VACNFs are functionalized by attachment of plasmid coding for GFP or YFP. Following penetration, we observe gene expression of reporter proteins from nanofiber-scaffolded DNA. Laser scanning confocal microscopy (LSCM) is used to evaluate nuclear impalement events. In addition to LSCM, scanning electron microscopy (SEM) is used to image-punctured and -fractured nuclei. Butler nuclei isolation protocol is used to isolate impaled nuclei, followed by fixing and drying. To observe impalement, nuclei are flipped by adhesion to a carbon tape, and some are fractured by this process.

Conclusions

We have conclusively demonstrated that VACNFs penetrate into the nuclear domains of live cells during impalefection using SEM and LSCM.

Functional One-Dimensional (1-D) Lipid Bilayers on Carbon Nanotube Templates

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The integration of modern 1-D inorganic materials with biological machines and environments is critical for the development of new types of biosensors, bionanoelectromechanical system devices, and nanostructured bioinorganic composite materials. Yet, robust strategies for the integration of 1-D materials with key biological environments, such as lipid membranes, are still missing. We present a new way of modifying 1-D materials that is based on polyelectrolyte, layer-by-layer self-assembly followed by lipid bilayer formation. We demonstrate our strategy by synthesizing 1-D lipid bilayers wrapped around carbon nanotubes modified with a hydrophilic polymer "cushion." We also show that the lipid molecules maintain their mobility even in these highly strained structures and present a diffusion model for the quantitative description of their behavior. We found that the lipid mobility in 1-D bilayers is slower than the mobility typically observed for flat supported bilayers, which we attribute to strong electrostatic interaction with underlying polymer support. We also present the first evidence of membrane protein association with 1-D lipid bilayers.

Hybrid Nanoparticles Enable Intracellular Manipulation

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A method to control and initiate the chemical reactions of biomolecules such as DNA and antibodies using novel hybrid nanometer-size metal oxide semiconductors has been developed. DNA oligonucleotides and antibodies are linked to specially designed nanoparticles equipped with "conductive leads" that, similar to natural photosynthesis, establish efficient light-induced crosstalk across the biomolecule and metal oxide interface. Linking biomolecules to titanium dioxide (TiO_2) nanoparticles imparts a high degree of specificity to the metal oxide nanoparticles, targeting them to biological moieties of interest such as genomic DNA and/or T lymphocytes. The biomolecules build recognition properties into the hybrid system, resulting in site-selective reactivities; metal oxide nanoparticles are capable of electronically responding to biomolecule interactions through the mechanism of light-induced charge separation**.** Light-induced charge separation in nanoparticles extends to the biomolecules and alters their structural and thermodynamic properties, affecting their functioning. Electronic coupling of biomolecules (DNA, antibodies, peptide recognition sequences) to nanocrystalline semiconductors creates light-activated composites for spatially and temporally controlled in vivo gene surgery and targeted cell metabolic intervention. Unique characterization tools at the CNM such as hard x-ray nanoprobe will be utilized for real-time monitoring of light-induced chemistries within the cell framework. Light-activated chemistries of modified TiO_2 nanoparticles will be harnessed for the prevention, control, and cure of a variety of diseases.

Culturing Neural Cells for Microfluidic Networks

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Growing cells in microfluidic networks provides several advantages over traditional cell cultures: Nutrient supply and waste removal are continuous processes, growth geometry is three-dimensionally constrained, electrical and chemical gradients can be readily achieved, and transient chemical stimuli can be delivered to the cells. In short, culturing cells in a microfluidic network can better approximate the in vivo environment. Neuronal cultures in particular can benefit from the microfluidic environment. Synaptic connections between neurons within the microfluidic system are more easily studied because of the geometric constraints on growth direction. In addition, the delivery of chemical stimulants or toxins is localized to the cell body or toward neuronal projections.

We consider the culture of the pluripotent embryonal carcinoma (P19) cell line, which can be induced at a given time to differentiate into cells representative of the central nervous system, including neurons, glia, and fibroblast cells. Inorganic polyelectrolyte multilayers (PEMs) are used to encourage attachment of the P19 cells to various substrates. We found that neuronal projections are particularly amenable to the PEM surface treatment. Using microfluidic networks, the PEMs can be micropatterned on a surface, allowing the direction of neurite outgrowth to be constrained, simplifying the neural networks formed. The cells can be introduced into microfluidic networks, where viability is sustained for several days.

Nanoscience at the Argonne National Laboratory (ANL) and the Center for Nanoscale Materials (CNM)

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Future breakthroughs in nanoscience and nanotechnology are anticipated to be founded on the creation of new classes of functionally and physically integrated hybrid materials that incorporate nanoparticles, three dimensionally tuned nanoscale architectures, and biologically active molecules and will offer opportunities for impact in diverse applications ranging from quantum computation, energy, and advanced medical therapies. Advances in the creation of hybrid nanomaterials will require new fabrication techniques; new probes capable of characterizing the electronic, magnetic, structural, and chemical properties of nanostructured materials; and significant innovation in theory and modeling.

In recognition of these challenges, the U.S. Department of Energy's Office of Basic Energy Sciences, in partnership with the State of Illinois, is establishing the CNM at the ANL, just 20 miles outside of Chicago. The CNM's mission is to provide the scientific community with the full complement of capabilities and expertise for the design, synthesis, characterization, and understanding of materials at the nanoscale. The CNM is developing special expertise in the creation of new classes of functional materials that are based on nanoscale structural hierarchies

and the combinatorial integration of chemically diverse inorganic, organic, and biological building blocks. This scientific thrust is supported by parallel developments of unique fabrication and physical characterization tools at the CNM, such as electron beam lithography and other nanopatterning tools, and the hard x-ray nanoprobe for characterization, together with the ANL's world-class user facilities for x-ray (Advanced Photon Source), electron (Electron Microscopy Center), and neutron (Intense Pulsed Neutron Source) research.

We highlight research and capabilities at the CNM and the ANL that focus on fusing connections at the biological-inorganic interface. This research involves the design, synthesis, and characterization of structural hierarchies that integrate "soft" biological and organic molecular assemblies with "hard" inorganic nanoarchitectures to create functional amalgams that combine normally disparate chemical and physical properties within a single system. Targets include integrating the dynamic catalytic, dielectric, and electromagnetic properties of nanoscale inorganic materials with the molecular selectivity, reactivity, and electronic properties of biological molecules. Such nanostructured, bioinorganic amalgams have applications in a broad range of areas. Specific examples include the integration of biomolecules with nanostructured piezoelectric materials to create electronically activated assemblies for applications in sensing, diagnostics, and nanofluidics. A second example includes electronic coupling of biomolecules (DNA, antibodies, peptide recognition sequences) to nanocrystalline semiconductors to create light-activated composites for spatially and temporally controlled in vivo gene surgery, targeted cell killing, metabolic intervention, and in vitro molecular electronic devise and sensing application. These examples illustrate a range of enabling new nanoscience and nanotechnology capabilities at the CNM that have potential for impact in medical imaging, diagnostics, drug delivery, and therapeutics.

Apolipoprotein E4 (apoE4) Conformational Changes: Relationship to the Alcohols

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The objective of this work is to study the plasma-free, fatty-acid-dependent conformational changes of apoE4 by using site-directed spin-labeling followed by electron paramagnetic resonance spectroscopy (SDSL-EPR), a sensitive and powerful method for observing conformational changes in proteins. ApoE is one of the important components of the human lipoproteins. Preprandial (fasting) and postprandial (3.5 h and 6 h) plasma samples were isolated from blood obtained from human volunteers given a 40% fat meal. An enzyme lipoprotein lipase acts on triglyceride-rich lipoproteins that are synthesized on ingestion of a fatty meal and releases free fatty acids into the bloodstream. We extracted plasma samples with a mixture of isopropanol and heptane to extract free fatty acids. We used apoE4 double mutants containing Cys residues at 76-239 or 77-241. The targeted Cys sites subsequently were labeled with nitroxide spin label for the SDSL-EPR study. We investigated the effect of plasma-free fatty acid extracts and also tested the effect of buffer extract as a control on apoE4 76C-239C or 77C-

241C. Buffer extract, which had remnant levels of isopropanol and heptane, showed a big effect of apoE4 with Cys substituted at the '*aa'* positions, 76 and 239, which are thought to be located near the interface where the C- and N-terminal domains interact. The increase in EPR signal amplitude of apoE4 (76 C-239 C) on treating with the buffer extract showed a reduced interaction between the spin labels, implying a movement of the labels away from one another. These results indicate that alcohol changes apoE4 conformation; it opens the protein to a greater extent. SDSL-EPR study can provide potential information at the molecular level on how alcohol affects the conformational state of apoE.

Toward Understanding the Molecular Modulation of Renal Stone Formation

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Purpose
The advent of scanned probe microscopy (SPM) has provided a tool for investigating the molecular mechanisms of tissue mineralization and pathogenesis. We are using SPM to determine the underlying physical principles by which important inhibitory constituents such as citrate and osteopontin (OPN) control the crystallization of calcium oxalate monohydrate (COM), a major component of human renal stones.

Method
We combined molecular modeling and in situ SPM to understand the thermodynamic and kinetic impacts of these modulators on the growth of COM and determine the stereochemical relationship between the growth modifier and atomic features on the crystal surface.

Results
We found that COM grows on atomic steps at dislocations like other solution-grown crystals such as calcium carbonate. By monitoring the evolution of the nanoscale growth structures in the presence of the citrate or OPN, we found that both additives inhibit step growth on distinct crystal faces. Citrate predominantly inhibits the growth on the so-called (-101) face by forming a stereochemical match between the citrate carboxylic groups and the Ca ions at the step edges. Molecular modeling calculations show that the highest binding energy on the (-101) face is about -170 $kJ \bullet mol^{-1}$, whereas it is only -102 $kJ \bullet mol^{-1}$ on the (010) face. The morphological change in step structure is consistent with that of the bulk crystal shape. In contrast, OPN has a much stronger effect on the growth of the (010) face, where the step height is similar to the size of the protein.

Conclusion
Both OPN and citrate inhibit the growth and modify the shape of COM through step-specific interactions, but they do so on different faces. Results also suggest the potential for synergistic effects of simultaneous action by both modulators to inhibit the overall growth of COM.

This work was performed under the auspices of the U.S. Department of Energy by the University of California, Lawrence Livermore National Laboratory, under Contract No. W-7405-Eng-48.

Carbon Nanotube Drug Delivery Immunocarriers Identify and Block gClq-R Expression on Human Colon During Bacterial Attack

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Human gClq-R (p33, p32, TAP) is an invasion protein known to facilitate *Listeria monocytogenes* (*L. monocytogenes*) attachment and entry into Caco-2 and Vero cells. In addition, it has been implicated in the pathogenesis of HIV, hepatitis C and other viral pathogens. We designed a small carbon nanotube loop (18-44 nm in diameter) to deliver clusters of monoclonal antibody (mAb) against gClq-R to (1) block gClq-R on the surface of living cells during bacterial attack and (2) allow ultrastructural visualization (time of appearance, distribution, and fate) of gClq-R expressed on the apical surface of colon cells. Carbon "nanoloops" were functionalized with mAb and incubated with Caco-2 colon cell monolayers for 20 minutes prior to inoculation with *L. monocytogenes* bacteria or *Bacillus cereus* endospores. Following incubation (30-270 min at 36^{o}C), the cell monolayers were rinsed with PBS, and the supernatant was analyzed for the presence of nanoloops, cells, debris and bacteria. Washed monolayers were prepared for histochemical and ultrastructural analysis by light microscopy (LM), transmission electron microscopy (TEM), and field emission scanning electron (FESEM) microscopy. Control nanoloops were functionalized with isotype/serotype-matched IgG or recombinant purified gClq-R and similarly incubated with cells during bacterial attack. We found that control nanoloops did not attach to the cultured cells or the bacteria. However, following bacterial interaction with the Caco-2 apical surfaces, mAb-functionalized nanoloops selectively and profusely bound to microvilli adjacent to attached microbes. When mAb-functionalized nanoloops were incubated with Caco-2 cells exposed to *L. monocytogenes,* bacterial entry was blocked, ultimately sparing the cells from the destruction seen in the *L. monocytogenes* control preparations.

These functionalized carbon nanotube immunocarriers represent a new biocompatible, directed drug delivery vehicle capable of transporting bioactive molecules to specific biological targets, suggesting possible therapeutic uses. Of equal importance is the ability to ultrastructurally visualize where and how these immunocarriers react on, and within, treated cells.

Human Cell Cytotoxicity Model Shows Cell Processing of Functionalized Nanoparticles

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To study the immediate response of human cells (ultrastructurally and histochemically) to fabricated nanoparticles functionalized with different proteins, an in vitro model was developed whereby monolayers of confluent human cells grown on 12 mm coverslips (in individual shell vials) were incubated and inoculated with different functionalized nanoparticles under controlled experimental conditions (i.e., exposure duration and dose in the presence of bacteria). Functionalized nanoparticles were incubated with the two types of human cells (Caco-2 and NCI-292 cells) under normal cell culture conditions and during bacterial attack. Following

incubation, the cells were carefully washed with buffered saline (PBS) to remove unattached material, and the supernatants were screened to identify nanoparticles, bacteria, contaminants, or cellular debris by light microscopy (LM) and field emission scanning electron microscopy (FESEM). Cell monolayers were examined by LM to identify damage, overgrowth, or cell death (apoptosis, necrosis). Glutaraldehyde-fixed monolayers were prepared for FESEM and transmission electron microscopy to evaluate high-resolution changes in cell morphology, nanoparticle distribution, binding, and nanoparticle intracellular processing (association with vacuoles, nuclei, and organelles; nanoparticle degradation; and possible bioaccumulation). We found that nanoparticles, which were originally bound to the colon and lung cell apical structures, were not subsequently observed within the cytoplasm, nucleus, or organelles of these epithelial cells. Upon membrane recycling, the nanoparticles were seen at the intercellular space and ultimately at the basal free space (3-4.5 hr), showing no nanoparticle incorporation within the cytoplasm and no signs of damaged tight junctions, altered cell function, toxicity, or cell death.

This in vitro human cell cytotoxicity model provided a rapid way to evaluate initial (30 min-7 hr), cell responses to newly developed nanomaterials using routine histochemical and ultrastructural methods to identify nanoparticles that, at the cellular level, were biocompatible with the specific cells used and to distinguish those nanomaterials that produced signs of cell toxicity and needed further design modification.

A Nanolithography-Based Platform for Investigating Protein Aggregation

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Purpose
Assembly of macromolecules is an important process in both structural biology and medicine. For example, controlled growth of well-ordered macromolecular crystals is a critical step in structure determination, whereas uncontrolled aggregation of proteins is the source of devastating pathologies such as Creutzfeldt-Jakob syndrome. The purpose of our work is to create a platform for investigating macromolecular aggregation and ordered assembly using scanned probe nanolithography.

Method
Target macromolecules are expressed with cystine residues or histidine tags. Atomically flat gold substrates first are coated with self-assembled monolayers of polyethylene glycol (PEG)-terminated alkane thiols, and then nanometric patterns of alkane thiol-based chemical linkers are made by scanned probe nanolithography. Linkers presenting maleimide groups are used to bind covalently to Cys residues, and nickel-chelating nitrilotriacetic acid groups are used to attach reversibly to His tags through a metal coordination complex. In the latter case, to explore the effect of macromolecular mobility on assembly, we modulated the strength of binding by adding competing metal coordinating ligands such as imidazoles. Aggregation and ordering then are observed directly using in situ atomic force microscopy (AFM) following introduction of a solution containing the target macromolecules into the AFM fluid cell. To investigate the role of macromolecular flux and interaction potentials, we varied the solution concentration and added varying amounts of PEG to the solution, respectively.

Results
Results obtained using site-mutated cow pea mosaic virus as a model system show the dependence of cluster size, spacing, and order on virus concentration, PEG concentration, and deposition time. Both attachment and detachment events are directly observable and show enhanced stability of large clusters.

Conclusion
We have developed a generic platform for investigating macromolecular aggregation and ordering in vitro that enables the elucidation of the physical controls over aggregation by directly measuring aggregation dynamics and assessing the role of solution composition.

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Nanostructured, Stimuli-Responsive Hydrogels for Biomedical Applications

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Purpose
The design, synthesis, and biomedical applications of nanostructured, stimuli-responsive hydrogels are described. Applications include drug delivery, tissue engineering, and magnetic resonance imaging (MRI).

Methods
Two families of thermogelling, biodegradable polymer formulations based on poly(DL-lactic acid-*co*-glycolic acid)/(poly(ethylene glycol) graft copolymers were synthesized: poly(ethylene glycol) grafted with poly(lactic acid-*co*-glycolic acid) (PEG-g-PLGA) and poly(lactic acid-*co*-glycolic acid) grafted with poly(ethylene glycol) (PLGA-g-PEG). Polymer composition and stimuli-responsive gelation behavior were investigated using ^{1}H and ^{13}C nuclear magnetic resonance and dynamic rheometry. Gel nanostructure was studied using cryotransmission electron microscopy (cryo-TEM). The feasibility of insulin delivery with injectable polymer depot was investigated using the diabetic rat model. The repair of cartilage defects was studied using the osteochondral rabbit knee model. In vivo MRI of gels was studied in a mouse model.

Results
Aqueous solutions of PEG-g-PLGA and PLGA-g-PEG graft copolymers flow freely at room temperature and form gels at higher temperatures. The existence of nanosize (9 nm diameter) polymer micelles in water was confirmed by cryo-TEM and dye solubilization studies. Using an injectable polymeric depot, we demonstrated the control of blood glucose level by sustained insulin delivery and cartilage repair by chondrocyte cell delivery in nonhuman animal models. With one injection of the graft copolymer/insulin formulation, the blood glucose level was controlled from 5 to 16 days in diabetic rats by varying the polymer composition. Polymer composition determines the degradation profile, which in turn controls the rate and duration of insulin delivery. The cartilage defect was notably repaired using chondrocyte suspension in the thermogelling PLGA-g-PEG copolymer compared with a control. In addition to insulin delivery

and cartilage repair, in vivo MRI studies showed the distinct boundaries of a gel depot after intramuscular injection in mice.

Conclusions
This research demonstrates that nanostructured biodegradable hydrogels are promising materials for protein delivery and cell-based therapies that could be monitored by MRI.

Micro-Nuclear Magnetic Resonance (NMR) Imaging and Magnetic Resonance Imaging (MRI) With Lithographically Fabricated Radiofrequency Coils (RFCs)

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Purpose
We are developing high-sensitivity NMR detectors for spectroscopic applications. These devices are also being developed to provide high-sensitivity MRI sensors with spatial resolutions on the order of microns.

Methods
Our detectors are based on three-dimensional (3-D), lithographically fabricated RFCs with feature sizes on the order of microns and coil diameters of ~200 μm. These RFCs provide greater than three orders of magnitude increase in mass sensitivity.

Results
These detectors are being developed to provide high-sensitivity screening of molecular-binding events of natural and synthetic ligands, including nanometer molecular therapeutics. The use of microcoil radiofrequency receivers, fabricated by our unique LLNL 3-D lithographic process, addresses the sensitivity issue common to NMR and provides new options for high-throughput characterization. These detectors can readily be integrated with complementary separation and concentration techniques (e.g., high-pressure liquid chromatography) for multidimensional analysis. We are also employing these systems for metabolite studies of biofilms of the microbe *Pseudomonas aeruginosa* (*P. aeruginosa*). Resonators based on our coils also are being developed as well for MRI tools. The high sensitivity of these coils will provide unique 3-D molecular structural, dynamic, and functional information in three dimensions, noninvasively and nondestructively, on the length scale of microns for such applications as the characterization of biofilm growth and numerous intracellular and intercellular processes.

Conclusions
We present initial NMR results obtained with both 1 mm and 360 μm outer diameter receiver coils along with ongoing methods of resolution enhancement and the future integration of a capillary liquid chromatography separation-detection system. We present both preliminary NMR and MRI studies of the biofilms *P. aeruginosa*. Current and future applications of laboratory-based microcoil NMR and MRI techniques also are discussed.

Accelerated Band 3 Clustering in *Plasmodium falciparum*-Infected Homozygous Hemoglobin C Erythrocytes Assayed by Single-Cell Autocorrelation Analysis Using Quantum Dots

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The molecular stability of hemoglobin is critical for normal erythrocyte oxygen transport. Hemoglobin C (HbC), a mutant trait hemoglobin, has an increased susceptibility to oxidative damage due to an amino acid substitution ($\alpha_2\beta_2^{6Glu\rightarrow Lys}$) in the beta chain. The growth of *Plasmodium falciparum* (*P. falciparum*) is abnormal in homozygous CC cells, and CC individuals show innate protection against severe (cerebral) malaria. However, the exact mechanism of this innate protection is unknown. We investigated one possible mechanism of innate protection by comparing the distribution of host membrane band 3 molecules in both AA and CC erythrocytes using quantum dots. The high photostability of quantum dots facilitated the construction of three-dimensional (3-D) cell images and quantification of total fluorescence. Deconvolved 3-D images of CC cells revealed irregular erythrocyte shapes, and both biochemical and total fluorescence analyses suggested a decreased band 3 epitope reactivity in all malaria-infected erythrocytes. However, the reduction in epitope reactivity was more pronounced in CC erythrocytes. Power spectra and one-dimensional autocorrelation analyses of fluorescence from individual CC erythrocytes showed a marked increase in band 3 clustering. In addition, the degree of band 3 clustering in uninfected CC erythrocytes was essentially indistinguishable from infected AA erythrocytes. However, there were marked differences in band 3 cluster sizes between infected AA and CC erythrocytes. Band 3 clusters averaged ~500 nm in infected AA erythrocytes but averaged ~1 μm in infected CC erythrocytes. This increase in band 3 cluster size in infected CC cells may enhance recognition sites for autoantibodies, which may be involved in the innate protection mechanisms of CC individuals against severe *P. falciparum* malaria.

Host Genotype Modulates the Surface Topography of *Plasmodium falciparum*-Infected Erythrocytes

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In human *Plasmodium falciparum* (*P. falciparum*) malaria, there is a well-established clinical association between innate protection and hemoglobin genotype. In contrast to normal hemoglobin AA erythrocytes, the hemoglobin C (HbC) genotype is associated with a 29% reduction in the risk of severe malaria in AC (HbC-trait) individuals and a 93% risk reduction in CC (homozygous HbC) individuals. Irrespective of host-cell genotype, parasite-induced, knob-like projections are produced on the erythrocyte surface. The knobs play a major role in the

adherence of malaria-infected erythrocytes to capillary endothelia. As the intraerythrocytic cycle of malaria progresses in AA erythrocytes, the knobs are uniformly distributed over the surface of the erythrocyte and the total number of knobs per unit area increases linearly as the parasite matures. To evaluate the influence of host-cell genotype on knob formation, we studied AC and CC erythrocytes using a combination of atomic force and light microscopy for concomitant wide-field fluorescence and topographic imaging. A detailed analysis of knob width showed a major peak, with a width of ~70 nm in all erythrocytes infected with *P. falciparum*. However, in both AC- and CC-infected erythrocytes, a second knob population with a peak of ~120 nm was found. The population size increased as the parasites matured. Furthermore, spatial knob distribution analyses demonstrated that the knobs on CC erythrocytes are more aggregated. These data support the concept that molecules on all HbC-carrying erythrocyte membranes involved in cytoadherence produce anomalous knob-related membrane clusters. These clusters may inhibit the adhesion of malaria-infected erythrocytes to cerebral vessels, resulting in the increased survival potential of malaria-infected HbC individuals.

Nano-Traincars Moving Down DNA Tracks: Single-Molecule Fluorescence Resonance Energy Transfer (FRET) and Simulations of a DNA Sliding Clamp Moving Along DNA

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Like nano-traincars on tracks, DNA sliding clamp proteins can "ride" more than 10,000 bases along a DNA double helix (>3 μm linear distance). The clamps can also be driven along by protein motors and can carry other proteins along. The clamps accomplish this by encircling the DNA, a unique topology in biology. In the absence of other proteins, DNA sliding clamps apparently “freely slide” along the DNA; yet, analysis of the protein structures reveals many positive charges along the inner ring that should create strong salt bridges with the DNA. Why then do the clamps not remain stationary on the DNA? And how can clamps proceed past sizable distortions in the DNA double helix? Using a combined experimental and computational approach, we have investigated the interactions between DNA and a DNA sliding clamp protein, the beta subunit of pol III. We used molecular dynamics simulations to illuminate the details of the DNA-protein interactions and are using single-molecule FRET measurements to infer the speed and diffusional character of the clamp's translocation.

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Nanosensors and Nanoprobes for Molecular Diagnostics and Imaging

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We have developed a new generation of nanosensors and nanoprobes that combines biorecognition and nanotechnology for in vivo monitoring of biochemical processes in a living cell. This technique could provide unprecedented insights into intact cell function, allowing for first-time studies of molecular functions in the context of the functional cell architecture in an integrated system approach. This presentation describes two areas of research related to the development of nanoprobes and nanosensors for single-cell analysis and imaging: (1) plasmonics nanoprobes for surface-enhanced Raman scattering (SERS) molecular diagnostics and imaging and (2) nanosensors for in vivo analysis of a single cell.

Plasmonics refers to research investigating the enhanced electromagnetic properties of metallic nanostructures. The term is derived from *plasmons,* the quanta associated with longitudinal waves propagating in matter through the collective motion of large numbers of electrons. According to classical electromagnetic theory, molecules on or near metal nanostructures will experience enhanced fields relative to that of the incident radiation. These fields can be quite large (10^6-10^7, even up to 10^{15} enhancement at "hot spots"). The first research approach involves the development of metallic nanostructures that can produce the SERS effect for ultrasensitive biochemical analysis. The intensity of the normally weak SERS process is increased by factors as large as 10^6 to 10^{11} for compounds adsorbed onto a SERS substrate, allowing for ultra trace-level detection. These substrates generally can be fabricated as silver-coated nanoprobes (300 nm in diameter) that are capable of enhancing the Raman signal of adsorbed compounds. The development of a SERS gene probe technology based on the solid nanostructures is described. Sensitive and selective detection of HIV DNA and BRCA1 breast cancer gene using the SERS technology is discussed.

Recent advances in nanotechnology leading to the development of optical fibers with nanoscale dimensions have opened new horizons for intracellular measurements in living cells. For example, an antibody-based nanosensor was developed to monitor benzopyrene tetrol (BPT), a DNA-adduct biomarker of human exposure to the carcinogen benzo(a)pyrene. Interrogation of single cells for the presence of BPT was carried out using antibody nanoprobes for excitation and a photometric system for fluorescence signal detection. We recently demonstrated the application and utility of nanosensors for monitoring onset of the mitochondrial pathway of apoptosis in a single living cell by detecting enzymatic activities of caspase-9. The tetrapeptide leucine-glutamic acid-histidine-aspartic acid (LEHD) is a caspase-9 substrate. The fluorescent molecule 7-amino-4-methyl coumarin (AMC) has an emission maximum at 460 nanometers. Minimally invasive analysis of single live MCF-7 cells for caspase-9 activity was demonstrated using the optical nanosensor, which employed a modification of an immunochemical assay format for the immobilization of LEHD-AMC. The substrate LEHD-AMC was cleaved by caspase-9, and the released AMC molecules were excited and emitted a fluorescence signal. By monitoring the changes in fluorescence signals, we detected caspase-9 activity triggered by apoptosis within a single living MCF-7 cell.

These studies demonstrate the first applications of nanobiosensors for measurements of molecular processes inside a single cell. These nanodevices also could be used to develop advanced biosensing and bioimaging systems to study in situ intracellular signaling processes and gene expression and molecular processes inside individual living cells. Such nanoprobes open new horizons to a host of applications in molecular imaging, biology research, medical diagnostics, and investigations of the therapeutic action of pharmaceutical agents in single living cells.

Multimodality Nanoparticles as Imaging Reagents for Antibody-Based Therapeutics

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One of the major challenges for antibody-based therapeutics is the lack of sensitive and convenient methods for in vivo imaging that tracks the distribution, metabolism, and movement of the drug delivery system and is an effective means to monitor the treatment efficacy of the drugs. The lack of sensitivity also made early detection unrealistic. Currently, radiolabels are most sensitive; however, radioactivity labels are undesirable for large-scale use due to the harmful effects of ionizing radiation to both the operators and the patients. Current-generation magnetic resonance imaging (MRI) contrasting reagents work in a very high concentration range of several millimolars, and there is a high false positive rate. To solve this problem, we constructed a novel class of imaging reagent that uses near-infrared CdSe nanocrystals. The nanocrystals are clustered with either Gd-based MRI contrasting reagents for regular MRI imaging or a novel zero- to low-field MRI agent. This dual modality nanoparticle composite would be detectable with both deep tissue, near-infrared in vivo imaging and MRI/zero-field MRI. To target it to breast cancer, the nanoparticle also uses single-chain antibody against ErbB2, which is a protein in the EGFR family overexpressed in 15% to 50% and higher of breast cancers, depending on the stage of the disease. The nanoparticle is highly fluorescent with a high quantum yield, and the clustering of the Gd chelating compound or zero-field MRI agent is demonstrated to be at least 500 per nanoparticle. This new class of nanoparticle-based imaging solution also can be applied to the diagnostic and monitory imaging of other cancers and other diseases.

High-Throughput, Small-Animal Single Photon Emission Computed Tomography (SPECT) for "In Vivo" Kinetic Studies of Novel Radiolabeled Nanostructures

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The National Institutes of Health's Molecular Imaging Roadmap proposed as a goal the development of high-specificity/high-sensitivity molecular imaging probes within 5 years. One promising platform that may accomplish this goal is the nanoparticle. For nanoconstructs to be useful as "in vivo" imaging probes, they must be functionalized and possibly targeted to make them biocompatible and specific. The behavior of nanoconstructs "in vivo" will require validation in relevant animal models. In addition, minor modifications of the nanoconstruct could significantly alter their biodistribution. In principle, imaging can provide the ability to rapidly evaluate the changes "in vivo" and the associated critical kinetic data. Magnetic resonance imaging can image probe feasibility; unfortunately, it cannot provide quantitative kinetic data. Positron emission tomography (PET) provides quantitative kinetic data, but the positron labeling of nanoconstructs is complicated, and throughput on existing microPET systems is limited. Therefore, the capability to detect single-photo-emitting radionuclides with high efficiency and high resolution and evaluate the entire animal kinetically is important. This would allow access to more robust labeling techniques, including neutron activation.

To address this need, we propose the development of a high-efficiency, fast-dynamic SPECT system capable of high-quality dynamic tomographic imaging of the rodent with time bins as short at 5 to 10 seconds. A set of continuously rotating compact detector heads equipped with optimized collimators will rotate closely to the animal placed in a cylindrical tube. Recent developments in on-board data digitization and wireless data transfer will be implemented to produce a reliable, compact, fast-rotating tomographic system. The system first will be employed to study the biodistribution and pharmacokinetics of trimetaspheres labeled with ^{123}I via iodination of a functional group or ^{177}Lu secondary to neutron activation of the metallofullerene in a rodent model of brain cancer. These data will be critical in determining the optimal functionalization and targeting for an intended therapeutic application.

Functionalization of Silica Surfaces and Nanoparticles With Receptor-Binding Proteins of Adenovirus

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The versatile biological functions and highly specific interactions of proteins make them an attractive component for functional self-assembled systems. Functionalized nanoparticles can interact specifically with cell receptors and potentially can be used for the directed assembly of biological and inorganic objects, drug delivery, and labeling. We investigated the functionalization of silica surfaces and nanoparticles, with the receptor-binding protein of

adenovirus, through the use of physical adsorption. This approach allows attachment of the viral protein to the inorganic surface with retention of its biological function. Atomic force microscopy, fluorescent microscopy, and x-ray reflectivity were used to characterize the morphology and structure on the adsorbed layer. The effect of adsorption density and interaction strength between the substrate and the protein on its biological activity has been investigated. Different mutants of the adenovirus protein were used to pinpoint the adsorption driving force, predominantly a hydrophobic interaction.

Novel Self-Assembling Systems for Drug Delivery and Gene Therapy

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Purpose
Our group at the Ames Laboratory has synthesized and characterized a family of novel polycationic block copolymers that exhibit unique self-assembly in aqueous solutions across several orders of magnitude from the nanoscale to the macroscale. The assembly of block copolymer chains into nanoscale micelles and gels and the pH sensitivity are crucial for these polymers to be used as self-regulating, glucose-responsive systems for insulin release. Since the polymers show reversible self-assembly into macroscale gels and solids at physiological temperatures, these nanodevices are ideal for delivering suicide genes to cancer cells over extended periods by injecting these polymer-DNA solutions intratumorally where they can form solid depots.

Methods
We have developed atom transfer radical polymerization techniques to synthesize these novel self-assembling pH- and temperature-sensitive multiblock cationic copolymers with a variety of nanoarchitectures. We have characterized the phase behavior of these materials in aqueous solutions and the structure of polymer-DNA complexes using dynamic light scattering, small-angle x-ray, and neutron scattering.

Results
These copolymers self-assemble to form nanoscale micelles in aqueous solutions. Above a critical gelation temperature and polymer concentration, the micelles entangle and self-assemble to form macroscale thermoreversible physical gels and also, more uniquely, elastic solids. Since the critical gelation temperatures are close to room temperature, these polymers can be used as injectable delivery devices and have significant advantages over the cross-linked, stimuli-sensitive hydrogels that have been investigated. These cationic polymers also exhibit complexation with DNA at physiological pH values and serve as excellent gene therapy vectors.

Conclusions
Novel polymeric nanodevices have been synthesized and characterized and show great potential for stimuli-sensitive drug delivery and gene therapy.

Simultaneous Force and Fluorescence Measurements of a Protein That Forms a Bond Between a Living Bacterium and a Solid Surface (Lower et al. 2005)

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All microbial biofilms are initiated through direct physical contact between a bacterium and a solid surface. This universal step of biofilm formation is expected to be controlled by intermolecular and intramolecular forces present at the bacterium-substrate interface. Atomic force microscopy (AFM) and confocal laser scanning microscopy were used simultaneously to observe the formation of a bond between a fluorescent chimeric protein on the outer membrane of a living *Escherichia coli* (*E. coli*) bacterium and a solid substrate in situ. The chimera was composed of a portion of outer membrane protein A (OmpA) fused to the cyan-fluorescent protein AmCyan. Sucrose gradient centrifugation and fluorescent, confocal slices through single bacterial cells demonstrated that the chimeric protein was targeted and anchored to the external cell surface of *E. coli*. The worm-like chain (WLC) model was used to predict the "force-signature" of the chimera bonded to another surface. In essence, this model predicted that each domain within the OmpA-AmCyan fusion should unravel in series, producing a force-extension profile defined by the persistence length (0.38 nm) and the contour length of the individual domains (i.e., OmpA or AmCyan). AFM force measurements between *E. coli* expressing the OmpA-AmCyan protein and a silicon nitride substrate (i.e., AFM tip) revealed a unique pair of "sawtooth"-like features that were present when a bond was formed between *E. coli* and the AFM tip. The observed sawtooth pair matched precisely the WLC model prediction for the mechanical extension of the OmpA and AmCyan domains in series. These sawteeth disappeared from the retraction curves when the cells were treated with protease. Furthermore, these unique sawteeth were absent for a mutant stain of *E. coli* incapable of expressing the AmCyan protein on its outer surface. Together, these data show that specific proteins exhibit unique force signatures characteristic of the bond that is formed between a living bacterium and another surface.

Reference

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Bioelectromechanical Imaging by Scanning Probe Microscopy (SPM): Repeating Galvani's Experiment on the Nanoscale

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Purpose
Since the discovery in the late 18th century of electrically induced mechanical response in muscle tissue, coupling between electrical and mechanical phenomena has been shown to be a near-universal feature of biological systems. The functional properties of biological systems are universally determined by an intricate set of mechanical and electromechanical interactions on the length scales that span several orders of magnitude: from macro to nano. The main motivation of studying electromechanical properties in biosystems is to understand the relationship between physiologically generated electric fields and mechanical properties on the molecular, cellular, and tissue levels.

Methods
We use vector piezoresponse force microscopy, a recently developed variant of SPM, to image piezoelectric properties in calcified and connective tissues such as tooth enamel and dentin and cartilage. This technique is complemented by acoustic and ultrasonic force microscopies to probe complementary information on local mechanical properties.

Results
We demonstrate real-space imaging of a spiral, single-collagen fibril in tooth enamel with ~5 nm resolution. In dentin and cartilage, the nanostructure of collagen network is visualized based on local electromechanical properties. We also illustrate an approach for imaging local molecular orientation from vector electromechanical data, a task inaccessible by other techniques. Imaging of elastic properties difference between enamel and dentin is also demonstrated.

Conclusions
By measuring the sub-angstrom mechanical response of a biological system induced by an electric bias applied to a conductive SPM tip, we visualize the spatial shape and molecular orientation of collagen molecules with ~5 nm resolution. This repeats the Galvani experiment on a nanometer scale—more than 200 years later and with a million times higher resolution. The potential of this approach for further studies of electromechanical coupling in functional tissues on the cellular level is discussed.

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Magnetic Virus

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Fabricating monodispersed, magnetic nanoparticles with uniform, selective biofunctionality is the key to nanoscience and technology. We report the creation of a novel magnetic particle, hybridized T7 phage, which leads to the generation of uniform and biofunctional magnetic particles. It was synthesized by filling a T7 ghost phage with cobalt or iron oxide. Such a hybridized phage, which contains a magnetic inorganic core surrounded by a capsid shell, was confirmed by bioassays, electron microscopy, energy dispersive x-ray analysis, and magnetic characterization. Benefiting from phage display technology, the hybridized phage has the capability to integrate an affinity reagent against virtually any target molecule. The approach provides an original method to magnetically "tag" a biosample and "biofunctionalize" a magnetic particle. More importantly, due to the nature of the phage, the resulting magnetic particles are monodispersed. Due to its uniformity in size and its versatility for functionalization, this "magnetic virus" is ideally suited for many biological and medical applications; we are investigating its prospect for biological sensing in particular.

A High-Flux Carbon Nanotube-Based Synthetic Membrane for Dialysis Applications

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The past few years have seen increased interest among scientists and physicians in the applications of nanotechnology in medicine, including dialysis. Some recent advances in dialysis have occurred in the area of porosity-controlled, high-flux synthetic membranes. Porosity control is necessary to achieve the desired separation/sieving profile, with maximal permeability for solutes of <40 kD molecular weight and minimal or no permeation of albumin (48 kD molecular weight). High-flux membranes offer the obvious advantage of reducing treatment time. We have developed a method (Holt et al. 2004) of membrane fabrication based on carbon nanotubes that has the potential to offer both the requisite porosity control as well as a high flux that is potentially orders of magnitude greater than that offered by current nanoengineered dialysis membranes. Carbon nanotubes are ideally suited for use in a nanoporous membrane, given their molecular size (with tunable internal diameters of 1-10 nm), high packing density (10^{10}-10^{13} cm^{-2}, depending on their outer diameter), and atomic smoothness, with the latter two characteristics important for achieving high flux. Thus far, we have demonstrated high-strength, void-free, multiwall carbon nanotube membranes (internal pore sizes ranging from 6-10 nm) using silicon nitride as the matrix material. Tracer studies using organic dyes, CdSe quantum dots, and fluorescently labeled polystyrene beads have been carried out that verify that the as-fabricated membranes are void free and have shown that the membrane exhibits a size cutoff. Porosimetry

and gas permeation studies also have been used in determining the pore-size distribution and permeability of the membranes. More recently, we demonstrated the fabrication of nanotube membranes (both single-wall, 1-2 nm pores, and multiwall, 6-10 nm pores) using the biocompatible polymer parylene. Porosity characterization studies are currently under way on these membranes, which may ultimately serve as our prototype dialysis membrane.

Reference

Holt JK, Noy A, Huser T, Eaglesham D, Bakajin O. Fabrication of a carbon nanotube-embedded silicon nitride membrane for studies of nanometer-scale mass transport. *Nanoletters* 2004 4(11):2245-2250.

Synthesis and Self-Assembly of Amphiphilic Block Copolymers as Potential Drug Delivery Agents

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There is a continuous need to develop new drug delivery systems that maximize a drug's bioavailability and therapeutic activity, while minimizing negative side effects and drug degradation. In the past two decades, significant research has been conducted on micelles formed by the self-assembly of amphiphilic block copolymers in aqueous solution as carriers for hydrophobic drugs, because the properties of the micelles can be controlled by the chemical composition, block length, and morphology of the amphiphilic block copolymer. Our studies have focused on gaining a better understanding of the physical and chemical parameters that control the capture and release of hydrophobic molecules in amphiphilic block copolymers and the synthesis of new biocompatible materials that could be used for drug delivery applications. This presentation highlights research on the synthesis and properties of two different amphiphilic block copolymers. In the first example, "crew-cut" block copolymers, in which the insoluble polystyrene block is larger than the soluble acrylic acid block, was synthesized by atom transfer radical polymerization methods (ATRP) to determine how the structure of the core influences the formation and stability of the polymer aggregates in solution and how it impacts the encapsulation and release of hydrophobic substrates. Since the physical state and properties of the core are largely determined by the glass transition temperature (T_g) of the core-forming block, poly(butyl-substituted styrenes) were investigated since their T_g is below room temperature (i.e., a liquid-like core), whereas polystyrene has a T_g =100 °C (i.e., a glassy core). In the second example, we have investigated the polymerization of acryloyl amino acid derivatives by ATRP methods to determine whether the controllable properties of synthetic polymers could be combined with the exceptional functionality, activity, and specificity of biological macromolecules. Block copolymers were also synthesized with poly(*N*-isopropyl acrylamide), a thermosensitive polymer, and their solution properties were studied as a function of temperature and pH.

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Single-Molecule Detection of Unamplified Nucleic Acid Sequences

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New genome sequence information is rapidly increasing the number of nucleic acid (NA) targets of use for characterizing and treating diseases. Detection of these targets by fluorescence-based assays is often limited by fluorescence background from unincorporated or unbound probes that are present in large excess over target. To solve this problem, energy transfer-based probes have been developed and used to reduce the fluorescence from unbound probes. Although these probes have revolutionized NA target detection, their use requires scrupulous attention to design constraints, extensive probe quality control, and individually optimized experimental conditions. We describe a simpler background reduction approach using singly labeled quencher oligomers to suppress excess unbound probe fluorescence following probe-target hybridization. A second limitation of most fluorescence-based NA target detection and quantification assays is the requirement for enzymatic amplification of target or signal for sensitivity. Amplification steps make quantification of the original target copy number problematic due to variations in amplification efficiencies between sequence targets and experimental conditions. To avoid amplification, we coupled our quenching approach to a two-color NA assay with correlated, two-color, single-molecule fluorescence detection. We demonstrate greater than a hundredfold background reduction and detection of targets present at concentrations as low as 100 femtometers using the two-color assay. The application of this technique to the detection and quantification of specific mRNA sequences enabled us to estimate β-actin copy numbers in cell-derived total RNA without an amplification step.

A Cell Mimic Structure with Tailored Pore Sizes: Synthesis and Modeling

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Purpose
A nanobiotechnology-based device has been fabricated using standard microlithography techniques combined with the controlled synthesis of carbon nanofibers. The device is intended for biomedical sensing and actuation applications and operates by mimicking the structural and functional aspects of natural, biological cells. Biological structures span multilength scales, from the microscale to the nanoscale, presenting a significant challenge when creating structures that intend to emulate their properties. The lipid bilayer that provides the structural framework and controlled interface to the cell is only a few nanometers thick and specifically functionalized. Mimicking such a membrane structure requires the fabrication of a microdimensional artificial membrane barrier that allows for the specific transport of molecular species via molecular-scale pores while containing other molecular species. The cell mimic structure makes use of vertically aligned carbon nanofiber (VACNF) arrays as the structural foundation for mimicking the membrane architecture. Molecular-scale pore evolution and control, in the intrafiber space, was facilitated by the growth of a conformal, silicon dioxide sheath on the individual nanofibers to reduce the space between nanofibers to nanoscale and molecular-scale dimensions.

Methods
The method of creating and sealing three-dimensional (3-D) cell mimic structures based on standard integrated circuit microfabrication techniques is discussed. The cell mimic structures were sealed with an established concentration gradient across the membrane mimics to initiate diffusive transport. The membrane pore size was tailored by the conformal growth of a SiO_2 sheath around the VACNFs that constitutes the membrane using a plasma-enhanced, chemical-vapor deposition growth process; increased growth time translated into a thicker oxide sheath. The average pore size and number of pores were characterized by generating a 3-D, Monte Carlo simulation of both the stochastic membrane structure and the random walk process of diffusion across the spatially stochastic membrane. Field emission scanning electron microscopy analysis was used to characterize the shape and morphology of the outer surfaces of the membrane structure, whereas the simulation was used to generate the pore spatial network in the intrafiber space of the membrane.

Results and Conclusions
The SiO_2 oxide sheath proved a successful means to alter pore size and distribution in VACNF membrane structures. Specifically, the transport of nanosize beads, based on their diameter, could be controlled by tailoring the pore dimensions through control of the oxide sheath thickness. Moreover, the Monte Carlo simulation proved essential in predicting the average pore size and pore spatial distribution based on initial conditions derived from features of the experimentally fabricated membrane structure, including average fiber density and morphology. Nanoscale membrane characterization was effectively improved by the use of the Monte Carlo-based modeling scheme.

In vivo SERS Sensors of Local Chemical Environment in Live THP-1 Human Macrophages

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Purpose
Many disease states are caused by or result from abnormal localized chemical environments at the cellular and subcellular levels. To gain a more fundamental understanding of the underlying biochemistry of disease, there is a need for the ability to measure these environments inside live cells in real time.

Methods
Molecular imaging has the potential to monitor both normal and abnormal biochemical and physiological parameters. This presentation details the initial results in a program to develop a suite of advanced molecular imaging probes based on metallic colloids and nanoshells and ultrasensitive Raman imaging techniques. The Raman spectra of reporter species on the surface of the nanoparticles change with the binding of the target metabolite. The ratio of peaks of free and coordinated reporter species gives quantitative measurement of metabolite concentrations. We believe these particles will enable the unprecedented measurement of key metabolomic species in live cells.

Results
In vitro measurement of reporter species' surfaced-enhanced Raman scattering (SERS) spectra shows clear shifts, in the 1600 cm^{-1} range, with acidity. We have been able to deliver these Ag colloids into live THP-1 human macrophages. Our initial experiments to measure pH inside these live cells are promising.

Conclusions
Use of SERS probes may be a useful method for the measurement of small molecule metabolites in live cells. We will follow the development of probes for more difficult species to detect, such as Na^{+}, Ca^{2+}, and NO.

Bioinspired Organic-Inorganic Composites and Their Potential Applications in Bone Tissue Engineering

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Purpose
The controlled integration of organic and inorganic components that gives rise to tough, strong, resilient, and lightweight composites is emulated using natural bone as a guide. The resulting composites are explored as structural materials for both nonbiological and bone tissue engineering applications.

Methods
Bone biogenesis is thought to occur by templated mineralization of hard apatite crystals by an elastic protein scaffold, a process we sought to emulate with synthetic biomimetic hydrogel polymers. Cross-linked polymethacrylamide and polymethacrylate hydrogels were functionalized with either hydroxyl or anionic mineral-binding ligands and used to template the formation of hydroxyapatite. The cytocompatibility of these hydrogel copolymers was evaluated in cell culture. The hydroxylated hydrogel also was used to prepare high-mineral-content, flexible three-dimensional composites.

Results
Strong adhesion between the organic and inorganic materials was achieved for hydrogels functionalized with either carboxylate or hydroxy ligands. A fast and convenient process for preparing flexible hydrogel-hydroxyapatite composites with mineral-to-organic matrix ratios approximating that of human bone was also realized. Human osteoblastic cells were found to attach, spread, and proliferate on all synthetic hydrogel copolymers with no apparent cytotoxicity.

Conclusions
The mineral-nucleating potential of hydroxyl groups identified broadens the design parameters for the design of synthetic structural composites and suggests a potential role for the direct participation of hydroxylated collagen proteins in nucleating bone minerals. The bulk hydrogel-hydroxyapatite composites generated are being explored for bone tissue engineering applications.

Xenon-Enhanced Nuclear Magnetic Resonance (NMR) and Microfluidics for Applications in Biomedical Diagnostics

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Purpose
NMR spectroscopy and imaging are proven tools for the analysis of biological compounds, yielding information that in many cases is unrivaled by any other method. Their use as routine diagnostic tools often has been limited by the large quantity of sample that is needed to obtain detectable signal levels. We used hyperpolarized, xenon-enhanced NMR for obtaining sensitive assays and images of samples that are nanoliters in size or that contain picomoles of spins.

Methods
Xenon is nuclear-spin-polarized by optical pumping to levels that are at least four orders of magnitude above the thermal polarization even in the highest magnetic fields available for NMR. Due to its long relaxation time, it can easily be introduced into a sample, where its interaction with another substance or its image is either detected by NMR in situ or, in many cases more sensitively, using remote detection.

Results
We show in a first step that xenon can be used with a targeted biosensor to sensitively detect trace amounts of substance. The biosensor contains an engineered binding site for xenon, as well as an affinity site for the target molecule. A specific chemical shift change enables detection of a binding event. In a second step, we obtained detailed flow images and spectra of xenon in microfluidic channels. Using remote detection, we sensitively acquired signal from small segments even when they were enclosed in large volumes.

Conclusions
Due to its spin properties, hyperpolarized xenon is an ideal carrier of signal both for NMR spectroscopy and imaging. The use of xenon-enhanced NMR in the ways described enables the detection of dilute species, screening of microscopic samples, and imaging of small amounts of spins. Combinations of the tools we have developed should open an avenue for new applications in biomedical diagnostics both in vivo and in vitro.

Inorganic Nanocrystals: Synthesis, Surface Modification, and Biomedical Applications

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Inorganic nanocrystals with well-controlled sizes, shapes, and surface properties are important in a wide range of applications. We first describe a general strategy for synthesizing inorganic nanocrystals with tailored shapes. This method involves the pyrolysis of organometallic precursors in a solution of mixtures of hot organic surfactants. The surfactant mixtures are used to control the growth rates of different facets of the nanocrystals, allowing for wide tunability of

shape. This is illustrated with CdSe, CdTe, and Co nanocrystals. Then, the surface properties of the nanocrystals are modified to make them soluble in water for bioconjugation. Finally, we discuss the use of these nanocrystals in biomedical applications such as cellular imaging, tagging, and labeling.

Biomolecular Imaging and Probing of Intermolecular and Intramolecular Interactions Using Atomic Force Microscopy

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Atomic force microscopy (AFM) is a powerful tool for label-free detection and high-resolution imaging of large biomolecular complexes. High-resolution images provide crucial information about the structural biology of protein machinery that is unattainable using current nuclear magnetic resonance or crystallographic techniques. Current methods for achieving high-resolution images use contact mode and tune the electrostatic repulsion to minimize tip-sample contact forces. Nevertheless, rigid two-dimensional protein crystals are required to achieve this resolution. We discuss methodologies for achieving similar resolution of soft-protein complexes. These methodologies include using tapping mode to reduce shear forces, reducing tip-sample forces by electronically decreasing the cantilever damping, and relying on repulsive forces to more closely render the sample shape.

AFM is also a force probe for studying intermolecular and intramolecular interaction energies. Energy dissipation chemical force microscopy (EDCFM) is the most sensitive imaging technique for quantitatively detecting subtle differences in biological and chemical functionality through energy dissipated during tip-sample interaction. Tapping mode is used, and the tip-sample energy dissipation is calculated from the energy used to drive the oscillation and the energy lost to the surrounding through viscous damping. The ultra-high-sensitivity of EDCFM could be used to map out specific receptors on a cell surface or as a binding affinity assay.

AFM force curves measure the forces between molecules connected to the tip and surface. Brownian force profile reconstruction (BFPR) is a force curve technique that facilitates accurate measurement of the force as a function of distance for stiff molecular interactions without instabilities. BFPR uses the thermal motion of the cantilever to probe the energy landscape, and the interaction is reconstructed from these data. Due to its high accuracy at short distances, BFPR is ideal for measuring equilibrium protein-folding landscapes and elucidating the intramolecular forces that drive protein folding.

Single-Nanoparticle Molecular Ruler-Based Nanoplasmonic Detection of Enzymatic Activity With Subnanometer Precision

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One of the major challenges of quantitative biochemistry and molecular biology is to monitor enzymatic activity within a femtoliter volume in real time. We constructed a novel nanoscale plasmonic probe-based molecular ruler, which can perform label free, in real time, with sensitive monitoring of DNA or peptide length during enzymatic reactions. The bionanoplasmonic molecular ruler was fabricated by tethering specifically designed double-stranded DNA or peptide to single Au nanoparticles. Enzymatic activity was tracked via the evolution of the plasmon signal of a single Au-DNA or Au peptide nanoconjugate, which reflects DNA/peptide size changes introduced through site-specific degradation by enzyme. For the Au-DNA nanoconjugate, the scattering spectra of individual Au-DNA nanoconjugates are measured continuously in real time during nuclease incubation, with a temporal resolution of 1 spectrum per minute for more than 30 minutes. The scattering spectra of Au-DNA nanoconjugates show a blue-shift of the plasmon resonance wavelength, as well as a decrease in intensity and a time-resolved dependence on the reaction dynamics. With a series of enzymes that generate DNA incisions at different sites, the shifts of the plasmon resonance wavelength are observed to correlate closely with the positioning of the nuclease-targeted sites on the DNA, demonstrating DNA axial resolution in nanometer precision (1.4 nm wavelength shift per 0.3 nm difference in DNA length). This work promises a novel molecular ruler that can monitor enzymatic reactions with single-particle sensitivity in real time. We also developed an ultra-high-density nanoarray using x-ray lithography for parallel enzyme activity measurement for functional proteomic studies.

Optical and Magnetic Resonance Imaging for Targeted Nanoparticle Development

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At the PNL, several state-of-the-art imaging tools are available for visualizing superparamagnetic nanoparticles in biological systems and for monitoring metabolic response. Examples considered include (1) gas-phase ^{3}He magnetic resonance (MR) imaging for quantitative dosimetry of inhaled particulates, (2) combined confocal and MR microscopy for in vitro testing of targeted nanoparticle performance, (3) slow-magic-angle-spinning MR spectroscopy for monitoring metabolic response with enhanced spectral resolution in heterogeneous environments, (4) planned small-animal imaging at 21 Tesla for enhanced

magnetic particle detection, and (5) single-molecule fluorescence microscopy for gaining insights into the spatial behavior of individual nanoparticles and their interactions in the living cell. This presentation describes recent progress in each of these five areas and highlights possible opportunities for National Institutes of Health/U.D. Department of Energy collaboration. Special emphasis is placed on the utilization of described methods for developing targeted nanoparticles that can then be employed in conjunction with in vivo MR imaging and spectroscopy for visualizing cell trafficking, monitoring drug delivery, and/or assessing therapeutic response.

Chip-Scale Instruments for Single-Molecule Separators and Sorters and High-Resolution Magnetic Resonance Imaging (MRI) Applications Based on Magnetic Particles

John Moreland

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We have developed patterned magnetic thin-film traps for capturing superparamagnetic beads in microfluidic wells. The traps are integral to a novel concept of using a magnetic force microscope (MFM) cantilever for transporting magnetic beads from one trap to another along the surface of a thin silicon nitride membrane. The MFM cantilever can be thought of as the arm of a nanometer-scale robot for moving single molecules attached to magnetic beads along the surface of an artificial membrane. Traps patterned on the membrane act as local binding sites for a given bead with a given molecule. Conceptually, the system can be thought of as an artificial cell wall with the potential for nanoengineered functionality at various points along its surface. In principle, magnetic binding sites not only would be used as traps but also would be associated with single-molecule functionality relating to optical, force, and electrical measurements.

We are also developing methods for chip-scale integration of DC and RF magnetic field sources and microelectromechanical cantilever oscillators to perform MRI and spectroscopy on small samples mounted onto the oscillators. Our goal is to push the technology to measure magnetic resonance phenomena at the nanometer scale. Shrinking the subsystems of a magnetic resonance instrument into a chip-scale system provides benefits in terms of reduced size and fabrication cost and better performance via an increased detector bandwidth and sensitivity.

Computer-Aided Nanodesign™ (CAN): Innovative Solutions for Nanobiology and Engineering

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The application of CAN software tools for tackling nanobiology issues is explored. CAN software tools incorporate multiscale modeling (quantum to mesoscale) techniques that can effectively predict nanoscale phenomena within the 1 to 100 nm length scale. As a result, these software tools are ideal for modeling nanomaterials that are used as imaging agents, drug delivery substrates, components of medical devices, or nanosensors/arrays. The use of CAN in the rational design of nanomaterials and subsequent fundamental analysis of the material-biomolecule interface also is explored. Specific case studies pertaining to the use of CAN in biosensor and drug delivery are provided along with an overview of how modeling techniques can accelerate the introduction of these innovative technologies within the marketplace.

Elucidating the Structure-Function Relationships of Transmembrane Nanopore Toxins: Mechanisms of Action and New Applications

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Different bacteria secrete specialized proteins to mount preemptive strikes against other organisms. For example, *Staphylococcus aureus* alpha-hemolysin and *Bacillus anthracis*-protective antigen destroy other cells and tissues in part by forming relatively large nanopores in target membranes. We use a combination of measurement tools (e.g., single-channel ionic conductance, electrochemical impedance, neutron scattering, computer modeling) to better understand the structure-function relationship of these proteins and the membranes that they bind to. In addition, we are developing methods for using nanopores for the sensitive and selective detection of a wide range of analytes.

This research was supported in part by the NIST, the NIST Advanced Technology Program, National Science Foundation, and Defense Advanced Research Projects Agency.

Surface-Enhanced Raman Spectroscopy (SERS) of Biomolecules Encapsulated in Liposomes

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With the ultimate goal of quantitative SERS of biomolecules, we have coupled a microfluidic lab-on-a chip device, manufactured by NIST scientists, to our Raman microscope. Inside the device, we are developing techniques to control liposome formation such that a known number of both gold nanoparticles as well as biomolecules can be encapsulated during vesicle formation. This engineered environment will allow the elucidation of structural details of an attached biomolecule through vibrational spectroscopy. To this end, we are using benchtop-formed liposomes to study encapsulation and separation of the nanoparticle vesicle system. Due to the inherently low Raman cross-section of the biomolecule, SERS has been used to identify the liposomes and has shown sensitivity to composition. Through ongoing imaging studies, particle location in relation to the liposomes will be determined. The experimental results are complemented by a computational effort carried out on an in-house computer cluster.

Magnetocarcinotherapy: A Nanotechnology Method for the Treatment of Cancers

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Magnetocarcinotherapy (MCT) represents an innovative approach combining cancer detection *and treatment* into a single modality that is unique both in concept and application and utilizing strong (high-remnant magnetization and coercivity) magnetic nanoparticles. The MCT technique consists of four primary stages: (1) binding a cancer-seeking agent to magnetic nanoparticles; (2) delivering the magnetic particles to the tumor site by introducing the cancer-seeking magnetic particles into the subject bloodstream (or by direct injection to the tumor site); (3) localizing the nanoparticle concentration by magnetic resonance imaging (MRI); and (4) inducing tumor necrosis by rapid and local thermal deposition with minimal collateral damage. This method uses no ionizing radiation or chemotherapy.

We have been pursuing demonstration of the feasibility of critical aspects of this novel molecular targeting concept for the treatment of tumors. We have developed methods to coat magnetic nanoparticles with gold that both increases biological half-life and provides a surface onto which targeting molecules or fragments (e.g., mAb) are bound. Numerous groups have demonstrated contrast-enhanced imaging using magnetic domains using MRI. Once a tumor is localized and imaged, an external time-varying magnetic field is applied to rapidly deposit (<100 sec) thermal energy into the tumor tissue, raising the local temperature by 20 to 30 ºC, sufficient to induce immediate cell necrosis. The external magnetic field couples to the localized concentration of magnetic nanoparticles and deposits thermal energy through viscous, hysteretic, and ohmic heating. Rapid deposition of thermal energy, primarily contributed through the viscous (friction)

mechanism, minimizes thermal diffusion and reduces collateral damage to healthy tissue. A significant modeling effort for thermal energy deposition has been conducted, and results are presented. A prototype radiofrequency system has been built, and preliminary results are in line with computer models.

Bioassay with Magnetic Microspheres in Flow: A Method for Highly Parallel Molecular Separations of Complex Biological Systems

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We present preliminary results for an instrument that employs flow-based analytical instrumentation and magnetic nanoparticle technology: a magnetic flow spectrometer for separation and a magnetic flow cytometer for identification. The flow spectrometer system will enable highly parallel continuous flow biomolecular separations on a preparative scale. The magnetic flow cytometer will combine a novel SQUID-based magnetic target-molecule-tagging concept with fluorescence-based analyte detection. The instrumentation proposed will contribute significantly to applications that include drug discovery, molecular targeting, DNA analysis, proteomics, and understanding the pathways of cell cycle regulation. Combining SQUIDs for target identification with laser diagnostics to assess binding provides an efficient, high-throughput multiplexed bioassay method based on traditional flow cytometry.

Operation of the proposed instrument involves three steps. (1) Magnetically encoded microspheres are prepared by encapsulating nanoparticles of strong ferromagnetic material with high-remnant magnetization and coercivity within polymer spheres. The distribution of microspheres can be sorted into different bins, depending on their intrinsic magnetic moment, by flowing through a chamber where a magnetic field gradient induces a force so that they are collected in different bins with narrow distributions of magnetic moment. This will require manufacturing a flow chamber with very small bin sizes, and our approach is to use microfabrication technology. Microspheres from each bin are chemically bound to target molecules so that each species of magnetic moment is bound to one unique kind of molecule. The collection of microspheres and associated target molecules then are mixed together and incubated with analytes. (2) The incubated collection of microspheres are flowed through a SQUID detector system, which identifies the target molecule by measuring the magnetic moment of the microsphere to which it is attached. (3) The analytes will be chemically prepared with molecular groups that fluoresce when illuminated by a laser beam, indicating the target-analyte binding.

Chemical Imaging of Biological Materials by Nano-Secondary Ion Mass Spectrometry (SIMS)

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The NanoSIMS 50 is the state of the art for in situ microanalysis by SIMS, combining unprecedented spatial resolution (as good as 50 nm) with ultra-high sensitivity (minimum detection limit of ~200 atoms). The NanoSIMS incorporates an array of detectors, enabling simultaneous collection of five species originating from the same sputtered volume of a sample. The primary ion beam (Cs^+ or O^-) can be scanned across the sample to produce quantitative secondary ion images. This capability for multiple isotope imaging with high spatial resolution is unique to the NanoSIMS and provides a novel new approach to the study of biological materials. Studies can be made of subregions of tissues, mammalian cells, and bacteria. Major, minor, and trace element distributions can be mapped on a submicron scale; growth and metabolism can be tracked using stable isotope labels; and biogenic origin can be determined based on composition. We have applied this technique extensively to mammalian and prokaryotic cells and bacterial spores. NanoSIMS technology enables the researcher to interrogate the fate of molecules of interest within cells and organs through elemental and isotopic labeling.

Quantitative Analysis of Membrane Composition by Secondary Ion Mass Spectroscopy

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Investigations of the lateral organization within membranes hinge on the ability to differentiate one component of interest from another. Typically, fluorophores are conjugated to specific components, and the organization is probed with fluorescence microscopy. However, bulky labels may change the physical properties of the components they are attached to, and only the labeled component can be visualized. We developed an approach to explore the lateral composition of supported lipid bilayers that employs an isotopic-labeling strategy and high-resolution secondary ion mass spectroscopy (SIMS), which is performed with a NanoSIMS 50 (Cameca). Lateral resolution as high as 50 nm is possible with very high sensitivity. We present a method to quantify isotopically labeled components within membranes. Homogeneous supported lipid bilayers that systematically varied in their deuterium-enriched lipid (1-palmitoyl-D_{31}-2-oleoyl-*sn*-glycero-3-phosphocholine) content were freeze-dried and examined with the NanoSIMS 50. The normalized $^{12}C^2H^-$ secondary ion signal intensity ($^{12}C^2H^-/^{12}C^-$) had an excellent linear correlation with the amount of deuterium-enriched lipid within the sample. This relationship may be exploited to obtain quantitative information on microdomains within a membrane by creating similar calibration curves for multiple, unique, isotopically labeled components within the sample.

Flow Cell for in vitro Studies of Nanoscale Structures and Assemblies by Ultrasmall-Angle X-Ray Scattering (USAXS)

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An isothermal capillary flow-cell (CFC) has been developed by the NIST in conjunction with the synchrotron-based, world-class USAXS facility, previously developed by the NIST and its collaborators at UNICAT Sector 33 of the Advanced Photon Source near Chicago. This new apparatus offers unparalleled capabilities for quantitative in vitro studies of complex solution-mediated nanoscale structures in applications such as drug delivery, diagnostics, contrast enhancement, and biomaterials development. Nominal setup for the apparatus includes a 1.5 mm diameter quartz capillary x-ray cell enclosed in an isothermal compartment, an isothermal reaction vessel for sample mixing and titration, a dual-syringe microlab for quantitative dosing, and a pump capable of maintaining uniform flow rates for extended periods. Temperature, flow rate, and chemical dosing are remotely controlled and programmable through the USAXS instrument control system. Remote sensing of pH (or other ion-selective electrode) and temperature allows precise control of experimental conditions. The CFC is compatible with a wide range of liquid media, including biorelevant aqueous solutions and many common organic solvents. The CFC metrology can measure, in situ and in real time, nanoscale-to-microscale structural characteristics as a function of changing physical and chemical conditions. It is therefore suitable for the study of, for example, solution-phase, soft-chemical routes to nanostructured materials based on templating and self-assembly, bundling and morphology in carbon nanotube dispersions, and the characterization of nanoscopic particles and higher order assemblies in their natural state. Furthermore, by flowing liquid samples, x-ray-induced damage to soft materials and the production of air bubbles can be reduced or eliminated. We present preliminary results that illustrate these capabilities and indicate how ongoing development will continue to improve the application of this metrology for problems relevant to biomedical research.

Methods for Evaluating Biological Responses to Engineered Nanophase Materials

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Novel nanophase materials are being developed at a rapid pace in research laboratories as a result of the National Nanotechnology Initiative and in commercial sectors because of potentially significant payoff of the materials. The engineered nanophase materials must have customized functionalities; these include, for example, well-defined sizes, shapes, textures, surface properties, and dimensional structures, which are critical to their developers and designated uses. Since biological systems (e.g., microbial, human tissues, and/or mice) are interfaced with the materials and there are few data on bioresponses, the study of biological interaction with engineered nanophase materials has become a challenging scientific problem at the beginning of

the 21st century. As the materials become adaptive and smarter and are able to network and perform self-regulation, the interaction of such materials will become even harder to study. The retrieval of information from the interactions to improve understanding of the nano-bio-info link will require new measurement and computing technologies. An issue lying at the heart of these problems is the lack of certified materials and methods to perform precision biological studies on engineered nanophase materials. A certified method permits experiments to be conducted with reliability and comparable in a scientific community. The U.S. Department of Energy's (DOE) Oak Ridge National Laboratory (ORNL) has been conducting research in the production and characterization of precision nanomaterials for biological research and has established a research program to investigate the biological functions of nanophase materials. This presentation introduces individual components of the ongoing nano-bio-info research program at DOE/ORNL, which encompasses materials science, chemistry, physics, microbiology, life science, and engineering. Previously published results are presented as examples of the products of this research program. New data also are presented to highlight the direction this research program is taking and to encourage collaboration among academia, industry, and government agencies.

Unexpected Energetic Controls During Mineralization of Biominerals

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Overview
Determining the methods that nature uses to control the growth and dissolution of biominerals such as teeth, bones, and kidney stones has important implications for health and disease. Although traditional crystal growth parameters such as supersaturation, pH, and temperature have important roles in biological mineralization processes, it recently has been appreciated that biological systems take advantage of other factors such as the influence of organic modulators and crystal size to change the energetics of crystallization.

Methods
We use scanning probe microscopy to image the motion of the atomic steps in real time. These topographic images taken in sequence form a movie that is used to monitor growth (or dissolution) dynamics as solutions are flowed over the mineral surface. The atomic step morphology and rates are correlated with bulk crystallization rates as measured by constant composition experiments. In both experiments, supersaturation, pH, temperature, and organic modifier concentration are controlled.

Results/Discussion
Generally, dissolution of minerals is regarded as a spontaneous reaction in which all the solid phase dissolves in undersaturated solutions. However, we show that the dissolution of calcium phosphate minerals can be suppressed when crystals reach a critical size related to their step-

edge free energies. This has important implications for nanostructured biomaterials such as bones and teeth and may act as a self-preservation mechanism that slows dissolution of these materials. Similarly, the interaction of citrate with calcium phosphate has an unexpected effect on step energetics that tunes the growth rate and that may represent a more economical way for an organism to mediate crystallization rate. Finally, we investigated the role of brushite in initiating kidney stone formation (calcium oxalate). In all examples, we show that surface energetics, rather than kinetics, determines the fate of the crystal.

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Intracellular Sensors Based on Surface-Enhanced Raman Scattering

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Understanding cellular processes at the molecular level requires the development of novel probes with high spatial resolution and sensitivity. We have begun developing optical probes that are capable of monitoring cellular metabolites in vivo. These probes are based on functionalized metal nanoparticles that generate a specific response upon interacting with the target analyte. This binding event is monitored optically using the surface-enhanced Raman scattering (SERS) signal from the functional group and the functional group/analyte complex. The SERS signal provides a unique, molecularly specific spectrum that allows us to identify and quantify the analyte molecule of interest. The nanosensors can be incorporated into individual cells, and their position can be measured to within 10 nanometers. In addition to the high spatial resolution afforded by these probes, they are also capable of achieving detection limits that approach the single-molecule level. In addition, the sensors are extremely robust, allowing measurements to extend over long durations without signal degradation. We present steps toward utilizing functionalized metal nanoparticles combined with SERS as chemical sensors. We also present the results from pH measurements using the functionalized nanoparticle sensors as well as progress toward the development of sensors for other analytes of interest. Finally, we present progress toward incorporating these nanoparticle sensors into living cells for localized measurement.

This work was performed under the auspices of the U.S. Department of Energy by the University of California, Lawrence Livermore National Laboratory, under Contract No. W-7405-Eng-48.

Direct Determination of Affinity in Individual Protein-Protein Complexes in Monovalent and Multivalent Configurations Using Dynamic Force Spectroscopy

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Recent advances in single-biological-molecule manipulation and measurement enable direct measurements of interaction forces in protein complexes. We used atomic force microscopy (AFM) to measure the binding forces between the single Mucin1 (Muc1) peptide and monoclonal antibodies screened against Muc1. Muc1 is overexpressed on cell surfaces in a number of human cancers and is a popular target for radioimmunotherapy agents. Our measurements utilized the proteins linked to the surfaces of the AFM tip and sample by flexible tethers that spatially separated specific interactions and allowed quick rejection of nonspecific binding events. We confirmed measurement of specific interactions by blocking it in a competition assay. Measurements of the binding strength as the function of the bond-loading rate (dynamic force spectra) allowed us to determine energy barriers, kinetic off-rates, and the distance to the transition state for simultaneous dissociation of one-, two-, and three-protein pairs. Dynamic force spectra for single and multiple bonds show very similar slopes corresponding to the bond width for individual protein complex, in full agreement with a theoretical prediction for unbinding of molecular bonds in parallel configuration. We also show that although our measured bond strength scales linearly with the number of molecule pairs, multivalency leads to a precipitous decrease in the kinetic off-rates for the complex dissociation.

Molecular Patterning via Photocatalytic Lithography

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Introduction

We have developed a novel method for patterning surface chemistry: photocatalytic lithography. This technique relies on inexpensive stamp materials and light and does not necessitate mass transport or specified substrates; the wavelength of light should not limit feature resolution (mm to nm). We have demonstrated the utility of this technique through the patterning of proteins, single cells, and bacteria.

Materials and Methods

Silicon, glass, and gold substrates were modified with nonfouling coatings. Controlled patterning and selective removal of the nonfouling coatings by local oxidation were achieved via photocatalytic activation (660 nm LED). Freshly patterned (bare regions) of substrates then were modified with adhesive chemistry.

Materials and substrates were characterized by microdroplet experiments, ultraviolet and visible absorption spectroscopy (UV-VIS), time-of-flight secondary ion mass spectrometry (Tof-SIMS),

and atomic force microscopy (AFM). The surfaces subsequently were exposed to fluorescently tagged protein, NeutrAvidin-FITC, and eukaryotic or prokaryotic cells. Adhesion of proteins, single cells, and bacteria was limited to regions of the adhesive chemistry, confirmed by fluorescence or optical microscopy.

Results and Discussion
Results indicate that photocatalytic lithography is a robust method of chemical patterning for biomolecules. Tof-SIMS imaging reveals good contrast between regions of adhesive and nonfouling chemistries. Fluorescently labeled protein adsorption experiments show consistent, localized protein adsorption only on adhesive islands. Cell and bacterial patterning was also successful.

The initial data suggest that photocatalytic lithography may overcome resolution limitations inherent in traditional photolithography and may allow rapid lithographic processing on the nanoscale with inexpensive optic systems and substrates. The technique also avoids pinholes that may form when patterning via mass transport bonding of chemistry to a surface. Furthermore, photocatalytic lithography is not substrate or chemistry dependent and is applicable to the study of biological functions interfacing with synthetic materials.

Conclusions
Photocatalytic lithography is a new, inexpensive, fast, and robust method of chemical patterning for biomolecules.

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The Physical Biosciences Institute (PBI) at the Lawrence Livermore National Laboratory (LLNL): Linking U.S. Department of Energy (DOE) Technology With Single-Cell Physiology

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Biology is widely believed to be at the threshold of a great transformation into a quantitative and predictive science that will depend heavily on new technologies from the physical sciences and advanced computations. The LLNL has many scientific and technical capabilities that position it to have leadership roles in aspects of this “new biology” that are important to basic biosciences, national security, and other DOE missions. The PBI was created to act as an incubator for multidisciplinary postdoctoral projects that link LLNL experimental and simulation capabilities to research projects in quantitative biology. Current PBI projects are as follows:

Force spectroscopy of multivalent protein-antibody interactions for optimization of radioimmunotherapeutics (T. Sulchek). We have developed techniques to directly determine the energy landscapes for biological molecule interactions using atomic force spectroscopy AFS). In particular, we used AFS to measure the binding forces between a single-molecule mucin1

(Muc1) protein and an antibody screened against Muc1. Muc1 is overexpressed on cell surfaces in a number of human cancers.

Single-molecule studies of chromatin (C. Jeans). In eukaryotic cells, DNA is packaged as chromatin, a highly ordered structure formed through the wrapping of DNA around histone proteins and further packed through interactions with a number of other proteins. For processes such as DNA replication, DNA repair, and transcription to occur, the structure of chromatin must be remodeled so that the necessary enzymes can access the DNA. This work uses AFS to determine the effects of modifications such as acetylation and phosphorylation on single-molecule binding forces in chromatin.

Bioengineered tools for analysis of cellular response to chemical signals (A. Hiddessen). Bioengineered cell holders based on micropatterning techniques, as well as cellular labeling and gene-silencing techniques, are being developed and investigated as a method for systematically determining how stem cell fates are regulated at the single-cell level. Ultimately, this work aims not only to map pathways controlling the proliferation and differentiation of individual stem cells but also to establish a high-throughput, bioengineered approach for analyzing the behavior of single cells as they are exposed to chemical cues derived from their environment.

Investigation of cellular functions by directly probing structures inside living cells using femtosecond laser pulses (N. Shen). We present a technique for the study of specific cellular functions that involve spatially distinctive subcellular structures by targeted ablation of these structures with femtosecond laser pulses. This technique enables noninvasive manipulation of an individual organelle without compromising cell viability. We probe mitochondria directly in human fibroblast and bovine endothelial cells with pEYFP-transfected mitochondria to investigate the functional and structural organization of the organelle.

Application of surface-enhanced Raman spectroscopy (SERS) nanoparticles to intracellular pH measurements (T. Laurence). The objective of this research is to develop methods to measure concentrations in chemical microenvironments in cells and tissues using recently developed, functionalized metal nanoparticles (50-100 nm in diameter). SERS allows sensitive detection of changes in the state of chemical groups attached to single nanoparticles. A nanoscale pH meter has already been tested in a cell-free medium, measuring the pH of the solution immediately surrounding the nanoparticles.

Small interfering RNA:DNA hybrids target mRNA to induce gene silencing in HeLa cells (L. Dugan). Small interfering RNA (siRNA) molecules are double-stranded RNA molecules that induce eukaryotic gene silencing. SiRNA:DNA hybrids (siHybrids) also reduce protein expression in mammalian cells. To determine whether siHybrids function similarly to siRNA, we quantified delivery using proton-induced x-ray emission and analyzed expression of lamin A/C in transfected HeLa cells. We show via Western blot, quantitative RT-PCR, and Northern blot analyses that siHybrids constructed with an RNA antisense sequence reduce gene expression by targeting mRNA for degradation, leading to reduced protein levels, similar to siRNA. These results indicate that the RNA interference pathway in HeLa cells is capable of recognizing and utilizing an RNA:DNA hybrid to induce gene silencing.

Using Micropatterned Polyelectrolyte Multilayers (PEMs) on Polydimethylsiloxane (PDMS) To Direct Neuronal Cell Adhesion and Growth

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Purpose

This work presents selective adhesion, viability and morphology assessments of neuronal (retinal) cells on micropatterned PEMs, with PDMS as the substrate.

Methods

PEMs were patterned on flat oxidized PDMS surfaces by sequentially flowing polyions through a microchannel network that was placed in contact with the PDMS oxidized surface. Silicon microfabrication procedures were used to produce the masters for molding the PDMS microfluidic channels. Polyallylamine hydrochloride (PAH) and polyethyleneimine (PEI) were used as the top-layer cellular adhesion material. The traditional practice of using blocking agents to prevent the adhesion of cells on unpatterned areas was avoided by allowing the PDMS surface to return to its uncharged state after the patterning was completed.

Results

The adhesion of neuronal cells on the PEM areas was observed within 5 hours after seeding, and proliferation was observed within 24 hours. Cell viability was evaluated 24 hours after seeding on the polyelectrolytes patterned on PDMS. These materials proved to be nontoxic to the cells used in this study regardless of the number of stacked PEM layers. Cell viability on a number of PEM layers, ranging from 1 to 11 layers, also was assayed. Cells were shown to be viable on all stacked layers where they were seeded. Cell morphology on the patterned PEMs also was studied. Phalloidin staining of the cytoskeleton revealed no apparent morphological differences in neuronal (retinal) cells plated on polystyrene or on the larger regions of PEI and PAH; however, cells were relatively more elongated when cultured on the PEM lines. Cell-to-cell communication between cells on adjacent PEM lines was observed as interconnecting tubes containing actin that were a few hundred nanometers in diameter and up to 55 μm in length.

Conclusions

This approach provides a simple, fast, and inexpensive method of patterning cells onto micrometer-scale features and will allow further exploration of cell growth and adhesion as well as cellular communication.