

6-1

Polymerized Colloidal Array Photonic Crystal Chemical Sensors

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We have developed a novel class of smart optical materials based on soft materials which are responsive to their environment and which can be actuated chemically or photonically. Highly charged, monodisperse colloidal particles will self assemble in water into crystalline colloidal arrays (CCA), which are either body centered or face centered cubic structures. We have developed smart materials from these self-assembled structures, which utilize the highly efficient Bragg diffraction of light from the CCA periodicity. We polymerized these CCA into acrylamide hydrogels. These CCA-embedded hydrogels show the rich volume phase transition phenomena characteristic of these soft materials. These materials act as frequency agile optical filters. We have functionalized these hydrogels with dyes and photochromic molecules, as well as with molecular recognition agents which cause the hydrogel to change volume in response to either photons, or the presence of specific analytes (eg Pb^{2+} , glucose etc). The resulting volume changes alter the array spacing, which causes the diffracted light wavelength to shift, or causes the diffraction efficiency to change. We will discuss the volume phase transition properties of these arrays and also describe the use of these arrays and also describe the use of these arrays as chemical sensors, novel ns optical switching materials as well as optical memory devices.

6-2

Lyotropic Liquid Crystal Templating of Mesoporous Hollow Spheres: A New Route to Materials for Controlled Release and Encapsulation?

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Hollow spheres (500 nm diameter) containing a periodic array of 3 nm pores in a hexagonal lattice in the shell wall were created through liquid crystal templating of the growth of ZnS on polystyrene and silica colloidal particles, followed by dissolution of the colloidal particle. The colloidal particles were first dispersed into a lyotropic liquid crystal formed from a nonionic surfactant and water that also contained thioacetamide and zinc acetate. Then, ZnS, formed from the reaction of thioacetamide and zinc acetate, heterogeneously deposited in a superlattice structure as defined by the liquid crystal on the surface of the colloidal particles. The mineralized colloidal particles were separated from the liquid crystal, and the colloidal particles were dissolved, resulting in a hollow sphere perforated with a periodic array of nanoholes. Both silica and polystyrene colloidal particles could be used as templates; silica particles are removed with fluoride ions, while polystyrene particles are removed with organic solvents. Initial experiments which demonstrate the sequestering of macromolecules within the mesoporous hollow spheres while permitting the passage of smaller molecules will be described.

6-3

Rippled Metal Nanoparticles: A New Protein Resistant Material

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Here we present a new family of mixed ligand nanoparticles that shows sub-nanometer patterns (e.g. ridges) on their ligand shell. This unique *sub*-nano-structuring of their ligand shell provides new properties to the particles. In particular, we focus on silver and gold particles that have ridges composed of hydrophilic valleys and hydrophobic peaks. We will show the ability to control the supramolecular ordering of the ligands on the nanoparticle surfaces. Indeed, by systematically varying the mixture of ligands introduced during nanoparticle synthesis, one can control the resulting surface properties of the nanoparticles. Scanning tunneling microscopy images show ridges 3 Å deep and 6 Å wide on the ligand shell of nanoparticles. Control of both these parameters is provided by the choice of the ligands and of their molar ratio respectively. We also demonstrate that the nanoparticle ligands interact so as to align the stripes of neighboring nanoparticles over large length scales. The synthetic mechanism that leads to the formation of this supramolecular ordering will be discussed. These particles show unique and unexpected wettability, solubility, self-assembly and surface chemistry properties. They, also, show a remarkable resistance to protein nonspecific adsorption, one order of magnitude better than state of the art antifouling materials.

6-4

Fluorescent Nanoparticles in Cellular Differentiation

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We encapsulate individual nanocrystals in phospholipid block-copolymer micelles, and demonstrate both in vitro and in vivo imaging. When conjugated to DNA, the nanocrystal-micelles act as in vitro fluorescent probes to hybridize to specific complementary sequences. More importantly, when injected into *Xenopus* embryos, the nanocrystal-micelles are stable, non-toxic ($<5 \times 10^9$ nanocrystals per cell), cell autonomous, and slow to photobleach. Nanocrystal fluorescence can be followed to the tadpole stage, allowing lineage tracing experiments in embryogenesis. We show that nanocrystal size is a crucial parameter that determines the particle localization during the embryo development.

6-5

Nano-Pumps in Hydrogels: Electroosmotic Mass Transport Control

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Gels are a common matrix for biosensors. Hindered transport through the polymeric matrix slows down the response rate in such sensors. As the number of diagnostic and analytical applications for gel-based sensor devices increases, so does the necessity of new pumping mechanisms for faster response. The network and mechanical properties of the gel make mechanical mixing

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schemes inappropriate. We are investigating novel internal pumping strategies based on electrically driven convection as a way to accelerate mass transfer in polyacrylamide gels. The gels are doped with charged silica colloids that drive local electroosmotic flow in response to externally applied electric fields. The uniformity of the particle dispersion throughout the gels is investigated by small angle neutron scattering. We use fluorescence spectroscopy to measure the mass transport of a fluorescent dye, amino-methylcoumarin, in these gels as a function of particle loading and applied field strength. Studies of silica particles with different sizes show that the electroosmotic mass transport enhancement is strongest when using nanoscale silica particles. We are also investigating the effects of electrolyte concentration on the electroosmotic pumping effect.

6-6

Highly Luminescent Nanoparticles for in Vivo Cancer Imaging and Detection

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Deep-tissue optical imaging is of particular interest, as the equipment costs are lower than for competing technologies such as magnetic resonance imaging. For this purpose, the development of novel contrast agents with near-infrared (NIR) fluorescence is especially important. We report on the use of NIR and upconversion semiconductor nanoparticles in deep-tissue *in vivo* optical imaging. Semiconductor nanocrystals of CdMnTe/Hg were grown in aqueous solution and then coated with bovine serum albumin (BSA). The nanocrystals were approximately 5 nm in diameter and have a broad fluorescence peak in the NIR (770nm). Nanocrystals were injected either subcutaneously or intravenously into athymic mice and then excited with a spatially broad 633 nm source; the resulting fluorescence was captured with a sensitive CCD camera. We have demonstrated that the nanocrystals are a useful angiographic contrast agent for vessels surrounding and penetrating a murine squamous cell carcinoma in a C3H mouse. Preliminary assessment of the depth of penetration for excitation and emission was done by imaging a beating mouse heart, both through an intact thorax and after a thoracotomy. The temporal resolution associated with imaging the nanocrystals in circulation has been addressed, and the blood clearance for this contrast agent has also been measured. There was no significant photobleaching or degradation of the nanocrystals after an hour of continuous excitation. The stability of the nanocrystals together with the time resolution of the optical detection makes them particularly attractive candidates for pharmacokinetic imaging studies. The author would like to thank the supports by NIH and Army Medical for grants and his NIH partners for collaborations.

6-7

Harnessing Nanoparticles for Glycobiology

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In this paper, we would like to present a new method to stabilise and functionalise nanomaterials (Au nanoparticles, semiconductor Q-dots, and magnetic nanoparticles) using a "peptide

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toolbox". The peptides play three important roles during synthesis: (i) control of the nucleation and growth processes to produce the desired morphology and internal structure; (ii) protect the nanoparticle/cluster cores from chemical degradation and maintain their physical stability (dispersion) in aqueous and biological environments; (iii) to allow functionalisation. For ligand exchange only (ii) and (iii) apply. The ability to tune the properties of the peptides (by varying the length, and sequence of amino acids, makes them a unique class of ligands for combinatorial nanomaterial synthesis.

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Heparan sulfate proteoglycans (HSPGs), which are strategically located on the surface of mammalian cells, act as regulators of most aspects of cell behaviour and function are involved in the pathology of many disease. The regulatory properties of HSPGs are thought to depend upon the fine structure of the HS glycosaminoglycan chain. Analysis of the structure and function of HS is hampered by the fact that their synthesis is not template driven. There is no amplification step, which would allow analysis of single structures produced by a biologically defined unit such as a cell or group of cells. Consequently, only abundant sources of material representing an average of structures may be analysed.

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Nanoparticles offer the possibility of single particle detection that permits the analysis of polysaccharide chains at a hitherto unachievable sensitivity, which will open up new experimental approaches in glycobiology.

6-8

Semiconductor Nanocrystals and Their Practical Applications

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Semiconductor nanocrystals have been the subject of research for decades. Recently, we have witnessed the advent, prototyping and commercialization of quantum dot based products. Since quantum dots represent a tunable band gap semiconductor material, they have inherent advantages over traditional semiconductors with fixed bandgaps. Quantum confinement allows us to tune the electronic structure based on size of the crystal as long as the physical size is less than the exciton Bohr radius. Many people are capitalizing on this quantum mechanical feature and fashioning these quantum dots into products from in vivo diagnostics to thermoelectrics, night vision pigments to wear indicators, LEDs to sunscreens. The compelling features of tunable electronic and optical properties along with their colloiddally grown form factor makes them an enabling material for many new markets. For diagnostic applications in the life sciences, quantum dots are a natural as they give unlimited emission wavelengths, legendary photostability, broad excitation, and are "relatively" simple in that they are sold as a colloidal suspension. The tunable emission wavelengths allows thing like deep tissue imaging since NIR emission wavelengths are possible, which transport through tissue without scattering. The photostability is called upon in live and fixed cell imaging applications that require extended interrogation under illumination. Broad excitation and emission color variety enable color multiplexing for higher throughput screening. These are no longer research materials since they are commercially available today. The potential for these materials has yet to be fully realized. We are at the headwaters of the development of nanocrystal products.

6-9

Quartz Crystal Microbalance Detection of Peptide Epitope Protected Nanoclusters Using Antibody Recognition

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A quartz crystal microbalance (QCM) sensor was developed for the quantitative detection of peptide epitope-protected nanoclusters. We have addressed challenges in the area of QCM mass sensing through experimental correlation between damping resistance and frequency change for a reliable mass measurement. Electrode functionalization was optimized with the use of Protein A to immobilize and present polyclonal IgG for antigen binding. This method was developed for the detection of glutathione (antigen) protected clusters of nanometer size with high surface area and thiolate valency. Quantitation of glutathione-nanocluster binding to immobilized polyclonal antibody provides equilibrium constants ($K_a = 3.6 \pm 0.2 \times 10^5 \text{ M}^{-1}$) and kinetic rate constants ($k_f = 5.4 \pm 0.7 \times 10^1 \text{ M}^{-1}\text{s}^{-1}$ and $k_r = 1.5 \pm 0.4 \times 10^{-4} \text{ s}^{-1}$) comparable to literature reports. Additional studies using conformational or linear peptide epitopes from the protective antigen (PA) of *B. anthracis* presented on the surface of monolayer protected clusters to produce functional nanostructures identified one monoclonal anti-PA antibodies to be specific for the conformational loop structure PA680B. Quantitative studies using a quartz crystal microbalance immunosensor confirmed specificity. These results demonstrate an ability to map monoclonal antibody recognition to specific epitope structures on nanoparticles.

6-10

Tumor-targeted Gadolinium Nanoparticles for Neutron Capture Therapy and for MRI Contrast Enhancement

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Gadolinium can be used in neutron capture therapy (NCT) in which Gd, if directed to tumors in sufficiently high concentrations, can capture thermal neutrons resulting in the emission of tumor-destructive electrons. Gd is also an effective contrast agent in Magnetic Resonance Imaging. Nanoparticles containing Gd in the core or bound to the surface were engineered from oil-in-water microemulsion templates. A folic acid ligand chemically linked to distearoylphosphatidylethanolamine via a PEG spacer was used to obtain tumor-targeted folate-coated nanoparticles. These were characterized based on size distribution, morphology, biocompatibility and tumor cell uptake. The Gd nanoparticles did not aggregate platelets or activate neutrophils. These nanoparticles were shown to have enhanced retention in the circulation as well as increased tumor uptake in tumor-bearing athymic mice, thus exhibiting potential of enhancing the therapeutic success of NCT. Nanoparticles in which Gd was bound to the surface via DTPA moieties were shown to greatly enhance the contrast of T2-weighted, spin echo images. These particles have potential for use in MRI blood pool imaging as well as in the imaging of tumors via the Enhanced Permeation and Retention mechanism.

6-11

Strategies for On-Chip Assembly of Sensors and Biomaterials from Live Cells

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New techniques of assembly of biosensors, nanostructures and nanodevices from live cells will be presented. They are based on principles for nanoparticle assembly into well defined 2D and 3D structures that we have developed earlier. We demonstrate how on-chip dielectrophoresis can be used to co-assemble yeast cells and synthetic micro- and nanoparticles. Depending on the frequency of the field and relative polarizability of the cells and particles, one and two dimensional arrays can be obtained. These arrays can be bound into permanent biocomposites by using molecular recognition. Such cell-nanoparticle chains and membranes can form the basis of sensors, microscopic bioreactors and artificial tissue. We also present a method for assembling and immobilizing large-scale coatings from yeast cells. The coating method is based on convective assembly and deposition in a moving meniscus to make dense two-dimensional arrays. A robust technique for rapid deposition of monolayer cell coatings was designed on the basis of this method. One immediate application of these structures is in biosensors and test beds for toxicity and drug action. The coassembly of live cells and synthetic nanoparticles also yields new biomaterials, in which the functionality of the cells is coupled to the functionality of the nanoparticles.

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Custom BeadChip Technology for Molecular Diagnostics

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BioArray Solutions (BAS) has developed unique, proprietary technologies for the rapid and flexible analysis of DNA, proteins and cells on semiconductor chips. Our diagnostic platform combines semiconductor physics, extensive bead chemistry and molecular biology to bring unparalleled flexibility and performance to quantitative DNA, protein and cellular analysis. BAS' proprietary array manufacturing process includes an evolutionary, two-track manufacturing process that combines wafer-scale assembly with custom bead array production to provide "last-second" customization for maximum flexibility at the lowest possible cost. Thousands of arrays can be produced simultaneously. By arranging large numbers of particles into small areas of the substrate, hundreds to thousands of binding events may be monitored simultaneously. In one standard assay format, a standard CCD camera, in conjunction with an optical microscope, in a single snapshot records 4,000 binding events. With its BeadChip™ assays for genomics and proteomics, BioArray Solutions addresses the challenge of high performance accuracy in multiplexed assays, high patient sample volume and rapid response time.

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Multi-Responsive Microgels: Morphology Control for Optimized Applications

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Carboxylic acid-functionalized poly(N-isopropylacrylamide) (PNIPAM) microgels exhibit “smart”, rapid, and reversible responses to changes in temperature, pH and ionic strength. To achieve a targeted set of environmental responses and optimize microgel morphologies for particular applications, one must control not only the bulk content but also the radial and intrachain distributions of functional groups within the microgel matrix, distributions which we have shown to play integral roles in regulating the properties of functionalized microgels. We have developed methods of controlling functional group distributions in carboxylic acid-functionalized PNIPAM-based microgels by tuning the hydrophobicity and copolymerization kinetics of the functional comonomer. The resulting functionalized microgels are extensively characterized, both directly via electron microscopy and indirectly using electrophoresis, titration, calorimetry, light scattering, and rheological techniques. Novel dimensionless plotting strategies allow us to directly compare the microscale and macroscale development of both the thermal and pH-induced transitions, giving insight into both the functional group distributions within the microgels and the underlying mechanisms of microgel swelling. The key influence of the radial and intrachain functional group distributions in the application performance of carboxylic acid-containing microgels is also specifically illustrated by testing the utility of the microgels as drug uptake/delivery vehicles and bioactive molecular conjugation supports.

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Design of Quantum Dot-Protein Bioconjugates for Use in FRET-Based Assays

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The unique spectroscopic properties of luminescent quantum dots (QDs), including broad absorption and size-tunable photoluminescence (PL) spectra ranging from the UV to IR and exceptional resistance to chemical and photo-degradation, are appealing for use in developing a variety of bio-inspired applications, ranging from molecular assays to *in vivo* cellular imaging. We have developed several approaches based on non-covalent self-assembly to conjugate biomolecules to CdSe-ZnS core-shell QDs that were rendered water-soluble using multidentate surface capping ligands. Antibodies were conjugated to these QDs either directly or via a bridging adaptor protein. QDs conjugated to proteins and antibodies prepared using our approaches were found to exhibit high specificity and stability in solution-based Förster resonance energy transfer (FRET) assays. In addition, we found that the readily tunable QD emission permitted effective tuning of the spectral overlap between the QD donor and dye acceptor, thus allowing excellent control over the FRET efficiency in these complexes. These findings were further exploited to design FRET-based nanoscale sensing assemblies for the specific detection of target molecules in solution. Combined with the advantages of CdSe-ZnS QDs, these hybrid bioinorganic conjugates represent a very promising tool for use in several biotechnological applications.

6-15

Up-Converting Phosphorescent Probes for Rapid Diagnostic Assays

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Recent applications of Up-Converting Phosphor Technology (UPTTM) have demonstrated that up-converting phosphors conjugated to biological-recognizing molecules (such as nucleic acids, peptides, or antibodies) can be used as alternatives to conventional fluorescent probes for high-sensitivity bioassays. In contrast to fluorescent dyes, these phosphorescent probes are excited by infrared lasers and emit intense phosphorescence in the visible range. Because no biological matrix possesses this unusual property, autofluorescence is completely absent in the up-converting phosphorescent assays, which makes UPTTM an ideal choice for detecting biomolecules from complex matrices. A systematic approach for the construction and characterization of Y₂O₂S up-converting phosphorescent probes for rapid diagnostic assays will be presented. Uniform 200 nm Y₂O₂S particles are synthesized using the homogenous precipitation method followed by a fluidized bed sulfurization technique. The prepared Y₂O₂S powders are chemically stable in the dried form, but degrade slowly in common buffers. The surface chemistry of Y₂O₂S can be controlled by encapsulation of particles with a sol-gel silica coating. Functionalized phosphors particles are made by grafting carboxyl silanes or polyelectrolytes on the silica surface and subsequently characterized using XPS, zeta-potential, and spectroscopic methods. Stability and reproducibility of bioconjugated up-converting phosphorescent probes prepared using carbodiimide chemistry are evaluated in bioassays.

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Block Ionomer Complexes as Novel Nanomaterials

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The block ionomer complexes are spontaneously formed by reacting the block (or graft) copolymers containing non-ionic and ionic polymeric segments (“block ionomers”) with oppositely charged species, such as polyions, proteins, surfactant ions, or metal ions. These complexes belong to the special classes of nanostructured materials combining the properties of cooperative polyelectrolyte complexes and amphiphilic block copolymers. These materials exhibit unique self-assembly behavior: they can spontaneously form either colloidal dispersions (vesicles, micelles, nanoparticles) or nanocomposite bulk materials. If the nonionic block in the block ionomer is hydrophilic (e.g. poly(ethylene oxide)), the resulting complexes are water-dispersed. A variety of polymer and surfactant components can be used in these composites allowing adjustment of the materials to respond to environmental changes in broad ranges, including pH, ionic strength, solvents and temperature variations. If the nonionic block is hydrophobic (e.g. polystyrene), the self-assembly with oppositely charged polyions leads to formation of multilayer polyion complex micelles in aqueous dispersion while interaction with surfactant ions results in formation of block ionomers complexes dispersed in organic solvents. These materials are promising in addressing various theoretical and practical problems, particularly, in pharmaceuticals, where block copolymers and polyelectrolyte complexes are already being intensively investigated as drug and gene delivery systems.

6-17

Generation of Functionalized Colloidal Gold Nanoparticles Using Bi-Functional Reducing Agents

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Our laboratory is using colloidal gold nanoparticles to develop tumor-targeting nanotherapeutics. Our first drug uses thiol groups present on the drug's key components to bind them to the nanoparticle surface. Yet, we recognize the thiol chemistry alone may limit the types of therapeutics that can be developed on the platform. To address this question we generated functionalized colloidal gold nanoparticles that contain a variety of functional groups present on their surface. Our approach uses bi-functional reducing agents (BFRA) that generate the gold particle, by reducing gold chloride under reflux, and embed/add functional group(s) on the particles' surface. The BFRAs consist of core polymer containing both free thiol groups and secondary reactive groups. The free thiol groups serve to reduce chloroauric acid into gold nanoparticles, while the secondary reactive groups present on the polymer are used to bind drugs. Two chemically distinct classes of BFRAs were used to manufacture the gold particles. The first type consisted of a 10kD branched-chain polymer of PEG having four thiol groups. The second reducing agent was synthesized on a polylysine core polymer which was thiolated using 2-iminothiolane. The particle size, shape, and drug binding characteristics for various preparations are described.

6-18

Integration of Biocompatible Surface Chemistries in the BARC Biosensor System

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We are developing a highly sensitive and selective biosensor system that uses giant magnetoresistive sensors arrayed in a Bead ARray Counter (BARC™) microchip to directly detect magnetic microbead labels. The beads are used both to label biorecognition events and as transduction elements to reduce background through a patent-pending process known as fluidic force discrimination (FFD). FFD is a controlled bead removal procedure that leverages the strength of biomolecular recognition against fluidic forces to selectively remove non-specifically bound bead labels.

A number of surface chemistries have been explored to functionalize the BARC chips, with the best results achieved on Si₃N₄ terminated chips. Neutravidin is covalently attached to Si₃N₄ via glutaraldehyde, and biotinylated capture probes are then employed for biosensing. Highly sensitive DNA hybridization assays (10 fM) have been performed and the magnetic sensor signal confirmed with optical bead counting. Application of the BARC platform for the multiplexed detection of four biowarfare agents without PCR, in 30 minutes, and at room temperature, will be described, as will preliminary results using similar chemistry for sandwich immunoassays.

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Quantification of the Field Interaction Parameter and the Binding Constants of Several Antibody-magnetic Nanoparticle Conjugates

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It has been previously reported that the magnetophoretic mobility of labeled cells show a saturation type phenomenon as a function of the concentration of the free antibody-tag conjugate. Starting with the standard antibody-antigen relationship, a model was developed which takes into consideration multi-valence interactions and various attributes of flow cytometry and cell tracking velocimetry, CTV, measurements to determine both the apparent dissociation constant and the antibody binding capacity of a cell. This model, combined with experimentally obtained data of the field interaction parameter of specific types of magnetic nanoparticles, was then evaluated on peripheral blood lymphocytes labeled with anti CD3 antibodies conjugated to FITC, PE, or DM (magnetic beads). Reasonable agreements between the model and the experiments were obtained. In addition, estimates of the limitation of the number of magnetic nanoparticles that can bind to a cell as a result of steric hinderance was consistent with measured values of magnetophoretic mobility. Finally, a scale up model was proposed and tested which predicts the amount of antibody conjugates needed to achieve a given level of saturation as the total number of cells reaches 10^{10} , the number of cells needed for certain clinical applications, such as T-cell depletions for mismatched bone marrow transplants.

6-20

Preparation of Organic Nanoparticles Using Microemulsions. – Their Potential Use in Transdermal Delivery

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Organic nanoparticles of cholesterol and retinol have been synthesized in various microemulsions (AOT/heptane/water; CTABr/hexanol/water; Triton X-100/decanol/water) by direct precipitation of the active principle in the aqueous cores of the microemulsion. The nanoparticles are observed by transmission electron microscope using the adsorption of contrasting agents such as iodine vapor, osmium tetroxide or uranyl acetate. The size of the nanoparticles can be influenced, in principle, by the concentration of the organic molecules and the diameter of the water cores which is related to the ratio $R=[H_2O]/[Surfactant]$. The particles remain stable for several months. The average diameter of cholesterol nanoparticles is comprised between 5.0 and 7.0 nm, while that of retinol is smaller, being ca. 2.5 nm. The average size of the cholesterol nanoparticles does not change much either as a function of the ratio R or of the concentration of cholesterol. The constant size of the nanoparticles can be explained by the thermodynamic stabilization of a preferential size of the particles. Different solvents are used to carry the active principle into the aqueous cores and they do not influence the precipitation reaction in a significant way.

6-21

Fluorescent Silica Beads for Detection of Cervical Cancer

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We present the use of self-assembled fluorescent silica (glass) beads for detection of cervical cancer. The cells from three different individuals (3 normal and 3 tumor) were tested for affinity by using the beads, a few microns glass nanoporous particles which contain fluorescent dyes sealed inside the pores. Using atomic force microscopy, we studied forces acting between the silica particles and the cells in-vitro. Using those data, we developed two different methods for detecting the affinity between silica and cells in-vitro. In one method we use a simple precipitation of the beads onto the cells, and with subsequent washing, the unbounded beads are removed. The second method involves using centrifugation for the removal of the unbounded beads. Both methods show unambiguous identification between the normal and tumor cells.

6-22

Fluorescence Analysis of Polymersome and Filomicelle Delivery

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PEG-polyester block copolymers can be made of the right proportion to assemble into controlled release vesicles and cylinder micelles, or polymersomes and 'filomicelles', respectively. Comparisons of these two morphologies, in terms of how they interact with cells and how they behave in vivo, bring new meaning to 'bio-nano'. We make extensive use of fluorescence microscopy to characterize the degradation and release processes as well as cell entry pathways in vitro. We also use such methods in vivo to track their fates, allowing us to identify super-long circulating filomicelles. Emerging tumor studies will be described.

6-23

Superparamagnetic Nanoparticle-bound Chlorotoxin for Brain Tumor Imaging

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A multifunctional nanoprobe capable of targeting glioma cells, detectable by both magnetic resonance imaging and fluorescence microscopy was developed. The nanoprobe was synthesized by coating iron oxide nanoparticles with covalently-bound bifunctional polyethylene glycol (PEG) which were subsequently functionalized with chlorotoxin, a glioma tumor targeting peptide, and the near infrared fluorescing molecule, Cy5.5. Both MR imaging and fluorescence microscopy showed significantly preferential uptake of the nanoparticle conjugates by glioma cells. Such a nanoprobe can potentially be used to image resections of glioma brain tumors in real time and to correlate preoperative diagnostic images with intraoperative pathology at cellular-level resolution.

6-24

Novel Magnetic Nanosensors to Probe for Molecular Interactions in High Throughput using NMR and MRI

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Designing activatable imaging agents to sense molecular markers and molecular interactions associated with disease would result in the development of more sensitive diagnostic agents and the development of target-specific probes for in vivo molecular imaging applications. Toward this goal, we have developed an assay to sense molecular targets using magnetic nanosensors and nuclear magnetic resonance (NMR) in high-throughput. These magnetic nanosensors consists of biocompatible magnetic nanoparticles capable of detecting a molecular target by changes in the NMR signal of the solution as the nanoparticles self-assemble in the presence of the target. Using four different types of molecular interactions (DNA-DNA, protein-protein, protein-small molecule, and enzymatic reactions) as model systems, we have shown that these magnetic nanosensors can detect these molecular interactions with high sensitivity and selectivity using standard NMR or MRI instrumentation. The target-induced change in NMR signal is detectable in turbid media or whole-cell lysate and is proportional to the amount of target present. The assay is performed in solution, does not require isolation or purification of the samples and could potentially be used for in vivo imaging. We will present data showing the utility of the technique to detect molecular targets related to cancer, atherosclerosis, inflammation and infection. The technology is versatile enough to sense the mRNA, protein and enzymatic activity of a molecular target. Finally, we have been able to detect specific viruses in solution, allowing for sensitive and selective detection of low numbers of viral particles.

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Selectively Moving Biopolymers Through Functionalized Nanotube Membranes

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The ability to regulate transport across cellular boundaries is essential to the cell's existence as an open system. There is a steady traffic of ions, molecules, polymers and other species across the plasma membrane. For example, sugars, amino acids, and other nutrients enter the cell; waste products of metabolism leave. The cell takes in oxygen for cellular respiration and expels carbon dioxide. It also regulates its concentrations of inorganic ions, such as Na⁺, K⁺, Ca²⁺, and Cl⁻, by shuttling them one way or the other across the plasma membrane. Mother Nature has created natural channels that are highly selective; they allow certain molecules and ions to pass more easily than others or they reject them altogether. Understanding and mimicking of the transport processes in cells is both challenging and rewarding from scientific and technological points of views. We have prepared highly selective template-synthesized abiotic nanotube membranes that can be used as model systems for better understanding of transport processes in natural systems and also mimicking natural ion- and protein-channels. Our nanotubes have diameters of the same order (1-100 nm) as those found in the natural protein channels. We have designed these nanotube membranes to selectively recognize and transport nucleic acid oligomers by modifying the inner surface of nanotubes with complementary nucleic acid "transporters". We show that

these membranes can facilitate the transport of the complementary DNA strands relative to DNA strands that are not complementary to the transporters. Under optimum conditions, single-base mismatch transport selectivity is obtained. The second part of my talk consists of fabrication of single-conical gold nanotube membranes functionalized with DNA that mimics voltage-gated potassium channel. These DNA-immobilized single-conical nanotubes exhibit “on-off” characteristics with external applied potential. These bio-functionalized nanotube membranes may find many potential applications in bioseparations, bio- and chemical-sensing, drug-detoxifications, and other biomedical and biotechnological applications.

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Functionalizing FePt Nanoparticle Surfaces with Silane Chemistry

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The preparation of monodisperse FePt nanoparticles was first reported by Sun et al. Though the initial motivation for developing these particles was for ultra high density hard drives, these have also found their way into biological applications owing to their high magnetization values. There are reports in the literature where FePt has been used for cell separation and protein tagging applications. The FePt prepared by the procedure developed by Sun et al. uses oleic acid and oleyl amine ligands for stabilizing FePt in non-polar solvents. To make these particles suitable for biological applications 1) they should be made hydrophilic and 2) they should have functional groups on their surface to bind to biological entities. Towards this aim, our XPS studies consistently show that a layer of iron oxide exists on the FePt nanoparticles. This motivated us to study alkoxy silane based ligands as a means to introduce the desired functionality. Studies with magnetite nanoparticles have shown the affinity of alkoxy silane ligands for the iron oxide surface. The present work studies the binding of various silanes on the FePt surface by conducting FTIR and XPS studies.

6-27

Biomaterials from Nanocolloids: Applications for Neurons

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The presentation will review the recent advances in the use of nanocolloids to add new functionalities to biomaterials. Layer-by-layer assembly (LBL) affords preparation of ordered layered structures from virtually unlimited palette of nanocolloids. Various functionalities of nanocolloids afford preparation of targeted composites for evaluation of different neuronal functions. Four examples will be discussed. Multilayers from TiO₂ nanoshells afford selective determination of neurotransmitters due to ion-sieving effect. Strong, flexible and electroconductive implants can be made from SWNT LBL multilayers. Stringent testing of biocompatibility of these composites was undertaken and it was demonstrated that they are suitable for long-term contacts with tissues. Stimulations of neurons through these films was

demonstrated. Nanoparticles with silver nanocolloids can be used to suppress inflammation processes due to infection – one of the most important problems with implantable devices. Photoactive multilayer from semiconductor particles were used to NG108*15 neuron precursor cells on them. It was found that light adsorbed in the nanoparticle layers results in the electrical excitation of the neurons making this system a functional analog of retina. Assemblies of clay-polymer systems demonstrated exceptional toughness similar to that observed in bones. Layered nanocomposites represent an exceptionally versatile tool for production of biomaterials with novel applications derived from unique properties of nanostructured matter.

6-28

NanoCrystal® Technology: Medical and Diagnostic Applications

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NanoCrystal® Technology is a proprietary platform technology that is helping to resolve one of the long-standing issues in the pharmaceutical industry, i.e. the effective delivery of poorly water soluble compounds for therapeutic and/or diagnostic applications. NanoCrystal® technology is an enabling drug delivery technology that offers an opportunity to formulate problematic compounds. The approach is a media milling process that is water-based, wherein, drug crystals are shear fractured into a nanometer-sized particle. NanoCrystal® dispersions are stable and have a mean diameter less than 200nm with 90% of the particles being less than 400nm. These colloidal dispersions can be processed into tablets or capsules that readily re-disperse in simulated biological fluids. NanoCrystal® Technology has been applied to poorly water-soluble compounds from discovery through development to enhance the performance of these problematic compounds. Case studies will be presented to demonstrate the various medical and diagnostic applications that have benefited from this formulation approach and future opportunities in the area of targeted drug delivery will be addressed.

6-29

Design of Multidentate Surface Ligands for Biocompatible Quantum Dots

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The utility of stable water-soluble luminescent quantum dots (QDs) has been demonstrated in biosensing and cellular imaging applications. However, due to the nature of the inorganic QD core, the native surface properties limit their compatibility with aqueous environments. We have designed a series of organic poly-ethylene glycol based surface capping ligands that allow for QD manipulation in aqueous media over a wide pH range. We utilized readily available thioctic acid and various oligo- and poly-ethylene glycols (PEG) in simple esterification schemes, followed by reduction of the dithiolane to produce multi-gram quantities of capping ligand (DHLA-PEG). This strategy was further applied to prepare biotin-terminated DHLA-PEG capping ligands. To form water-soluble QD assemblies, native trioctylphosphine and trioctylphosphine oxide (TOP/TOPO) capped nanocrystals were mixed with an excess of the desired surface ligand and incubated for a few hours to displace the TOP/TOPO molecules. This permitted us to obtain

homogeneous dispersions of QDs that were stable over wide pH ranges and extended periods of time. We also prepared QDs having mixed surfaces of dihydrolipoic acid (DHLLA) and DHLLA-PEG (or DHLLA-PEG-biotin). This design and conjugation strategy may facilitate the development of a new generation of QD-bioconjugates for use in a variety of biological applications.

6-30

Strategies for the Design and Readout of Ultrahigh Density Immunodiagnostic Platforms

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The drive for early disease detection, the growing threat of bioterrorism, and a vast range of challenges more generally in biotechnology has markedly amplified the demand for ultrasensitive, high-speed diagnostic tests. This presentation describes efforts to develop platforms and readout methodologies that potentially address demands in this arena through a coupling of nanometric labeling with surface enhanced Raman spectroscopic, micromagnetic, and scanning probe microscopic and readout concepts. Strategies will be described for both the fabrication and readout of chip-scale platforms that can be used with each novel readout modality. Examples will focus on the use of protein arrays as platforms targeted for immunoassays in early disease diagnosis and the rapid, ultralow level detection of cancer markers and viral pathogens. Each example will also discuss challenges related to sensitivity and nonspecific adsorption and to fluid manipulation at micrometer length scales.

6-31

Nucleic Acid Sequence and Protein Identification using Gold Nanoparticle Probes and the Verigene™ System

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Nucleic acid sequence identification is widely used for detection of viruses, bacterial pathogens, and genetic disease predispositions. Most current detection platforms require nucleic acid target amplification necessitating complex assay procedures and instrumentation. Nanosphere, Inc. has developed a novel gold nanoparticle probe-based platform to detect nucleic acids without target amplification. Two key factors have enabled this transformation. First, gold nanoparticles coated with oligonucleotides exhibit sharper melting transitions which increase sequence specificity in detection as says. Second, simple optical instrumentation that detects scattered light from gold nanoparticle probes provides a 1000 fold improvement in detection sensitivity over current fluorescence scanners. The combined sensitivity and specificity of this detection platform has enabled the multiplex detection of SNPs in total human genomic DNA, as well as PCR-less identification of bacterial pathogens. The sensitivity of this platform is enhanced by capturing the nucleic acid target (or protein target) with a magnetic bead and labeling the complex with a gold

nanoparticle functionalized with barcode DNA sequences. The barcode DNA sequences are used to identify the target sequence present and to amplify the signal since a single nanoparticle can carry thousands of barcodes. Following magnetic separation, the barcode DNA sequences are released and detected using the Verigene™ System.

6-32

Surface Functionalization of Semiconductor Quantum Dots for Applications in Biological Sensing

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Having diameters of only a few nanometers, colloidal CdSe semiconductor quantum dots (QDs) are highly emissive, spherical, inorganic particles that exhibit size-tunable physical properties due to the effects of quantum confinement. Typically, these materials are synthesized with relatively inert hydrocarbon surface-groups that must first be modified if they are to be compatible with biological systems. To that end, a variety of strategies have been established to make these hydrophobic surfaces of CdSe QDs applicable for use in biology. We have demonstrated that CdSe/ZnS core/shell QDs functionalized with streptavidin bind specifically to pathogenic *Escherichia coli* O157:H7 cells labeled with biotinylated antibodies. Using fluorescence microscopy of individual bacterial cells and standard fluorimetry of bacterial cell solutions, we will also present results comparing *E. coli* O157:H7 cells labeled with QDs to cells similarly labeled with a standard dye, fluorescein isothiocyanate (FITC). The particular biochemical interactions and surface functionalization incorporated in these methods can easily be generalized to allow for the rapid and selective detection of other common pathogens.

6-33

Combining Gold Nanoparticles with the Quartz Crystal Microbalance to Improve the Sensitivity of DNA Detection

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Investigation indicates that the appearance of the malignant tumor is highly correlative with the DNA mutation. It has become an important topic of the cancer study to detect the gene mutation and look for the relation between mutation and pathological change at the molecular level. Rapid detection for trace gene mutation can provide basic data for diagnose disease. Therefore, looking for a rapid and simple method used for detecting trace mutation is important and pressing. QCM is a simple, rapid and real-time measurement of DNA binding and hybridization at the sub-nanogram level. The nanogold particle has many special properties, for example high density, simple operation, and easy size-controlled. Combining these two techniques, i. e., nanoparticle modification of QCM surface and the application of gold nanoparticle amplifier, we improved the detection limit of DNA. This method makes it possible to detect single base mutation less than 10^{-16} mol / L.

Effects of Surfactants and Surface Charge on the Performance of Latex Immunoagglutination Assays in Vitro and in a Microfluidic Device

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The latex immunoagglutination assay is a relatively easy, rapid technique for detecting biomolecules. However, improvements in reliability and sensitivity are still required. The main issues involve colloidal stability of the antibody-latex complex and non-specific binding of antigens. To address these issues, we investigated the influence of surfactants and surface charge of particles toward its performance. To examine the effects of surfactants, we used the ionic SDS and the non-ionic Tween 80 with submicron polystyrene (PS) particles. To examine the effects of surface charge we compared plain PS with highly carboxylated PS. All latex particles were conjugated with anti-mouse IgG (developed in goat). The antigen was mouse IgG (positive control) or rabbit IgG (negative control). Immunoagglutination was monitored turbidimetrically using a spectrophotometer. We found that plain PS with either no surfactant or ionic SDS produced many false-positives and false-negatives. Results were substantially improved when non-ionic Tween 80 was mixed with plain PS. When carboxylated PS was used with no surfactant, the results were also very accurate and reliable. We also demonstrated these effects in a Y-channel microfluidic device. Immunoagglutination occurred faster and in greater quantity near the Y-junction, when Tween 80 surfactant was used rather than no surfactant.