Thursday Afternoon, October 21, 2010

Nanometer-scale Science and Technology
Room: La Cienega - Session NS+BI-ThA

Biomolecular Templates & Bioinspired Nanomaterials

Moderator: B. Borovsky, St. Olaf College

2:00pm **NS+BI-ThA1 Bio-functionalization of Nanopatterned Surfaces and their Integration with DNA Nanostructures**, ***M. Palma***, *J.J. Abramson, E. Penzo, A. Gorodetsky, R. Wang, M.P. Sheetz, C. Nuckolls, J. Hone, S.J. Wind*, Columbia University

The ability to control biomolecules on surfaces with nanometer resolution is of great interest in the field on nanoscience and nanotechnology. DNA nanoarrays, in particular, are of interest in the study of DNA-protein interactions, for biodiagnostic investigations and as a tool to drive self-organization of nanomoieties on surfaces. In this context, achieving a highly specific nanoscale assembly of oligonulceotides at surfaces is critical.

Here we describe different strategies to control the immobilization of single- and double-stranded DNA, as well as DNA nanostructures (DNA "origami"), on nanopatterned surfaces, with features down to the sub-10nm regime.

Using electron-beam and nanoimprint lithography we fabricated sub-10nm metal dots arranged in multiple configurations on Si or glass substrates. We have developed strategies for the selective bio-functionalization of these patterns, at the single nanodot level: each step of the biochemical functionalization has been monitored by Fluorescence Microscopy. The bio-functionalization approach used allowed for the formation of non-sterically hindered DNA nanodomains where the dsDNA attached to the dots maintains its native conformation, as confirmed by restriction enzymes studies. This allowed us, moreover, to follow the activity (at surfaces) of a restriction enzyme in real time and at the nanoscale: the monitoring of protein-DNA interactions with such biological nanoarrays will be discussed.

We will highlight the broader utility and application of such nanopatterned surfaces for the self-organization of DNA nanostructures. In-situ hybridization between the complementary strands on DNA nanostructures and on functionalized nanodots has been achieved, resulting in the ordered placement of the origami on the dot patterns, as demonstrated by Atomic Force Microscopy (AFM) imaging, both in liquid and in air.

Finally, we will discuss the application of DNA origami as functional scaffolds for the assembly of different nanomoieties (e.g Au nanoparticles and carbon annotubes): highly complex arrangements can be created with high resolution and high throughput, opening the possibility for the realization of electronic devices at the molecular scale.

2:20pm **NS+BI-ThA2 De Novo Nanostructure Design: from Protein Folding to Self-Assembly-Templated Nanomaterials**, ***M. Ryadnov***, National Physical Laboratory, UK

Rational design of self-assembled nano-to-micro scale structures offers an efficient tool for molecular nanotechnology improving our ability to engineer nanostructured materials at whim. Such attention is driven by the need for approaches leading to specialist nanostructures whose properties can relate to particular biological functions. Critical in this respect becomes the hierarchical nature of self-assembly rendering the process a "bottom-up" strategy in challenging different levels of nanostructural complexity [1].

Generic protein folding motifs are proving to be instrumental for prescriptive nanoscale engineering. Of particular demand are nanostructures which can be made functionally and architecturally amenable in cellular environments. In this report, bioinspired nanoscale designs based on peptide self-assembling systems possessing antimicrobial [2], cell-supporting [3], encapsulating[4] and tuneable morphological[5] properties will be discussed.

1. Ryadnov, M. G. (2009) *Bionanodesign: Following the Nature's touch.* RSC Publishing, 250 pp.

2. Ryadnov, M. G., Mukamolova, G. V., Hawrani, A. S., Spencer, J. & Platt, R. (2009) RE-coil: An antimicrobial peptide regulator. *Angew. Chem. Int. Ed. 48*, 9676-9679.

3. Ryadnov. M. G., Bella, A., Timson, S. & Woolfson, D. N. (2009) Modular design of peptide fibrillar nano- to microstructures. *J. Am. Chem. Soc.*, 131, 13240-13241.

4. Ryadnov, M.G. (2007) A self-assembling peptide polynanoreactor. *Angew Chem Int Ed Engl. 46*, 969-972 .

5. Ryadnov, M. G. & Woolfson, D. N. (2003) Engineering the morphology of a self-assembling protein fibre. *Nature Mater., 2*, 329-332.

2:40pm **NS+BI-ThA3 Rare Earth Nanoparticles to be used as Both Fluorescent Probes and MRI Contrast Agents**, ***L. Axelsson***, *M. Ahrén, L. Selegård, F. Söderlind*, Linköping University, Sweden, *P. Nordblad*, Uppsala University, Sweden, *M. Lindgren*, Norwegian University of Science and Technology, Norway, *K. Uvdal*, Linköping University, Sweden

Nanotechnology continuously explores new fields, and nanomedicine presents an entirely new research area with unlimited possibilities. For the last 20 years, gadolinium complexes have been used clinically as contrast enhancing agents for Magnetic Resonance Imaging (MRI). Simultaneously, Quantum Dots (QDs), with its excellent photostability and high quantum yield, are developed to replace organic fluorophores for medical diagnosis. The aim of this study is to develop nanoprobes that possess both the magnetic properties suitable for a contrast agent, and luminescent properties.

We have designed a novel nanomaterial of gadolinium oxide nanoparticles doped with europium ($Eu:Gd_2O_3$) or terbium ($Tb:Gd_2O_3$). Using nanoparticles, the local signal intensity in MRI can be increased compared to Gd complexes with only one Gd ion per complex. When introducing luminescent europium or terbium ions into the gadolinium oxide nanocrystal, fluorescent properties are added, creating a bifunctional nanocrystal. In addition to the favorable size for biomedical applications, nanoparticle contrast agents can bring advantages such as longer rotational correlation time to obtain increased relaxivity, and surface-coating possibilities for attaching targeting molecules. This will enable tailored design of a new generation of contrast agents. We present highly crystalline, 5 nm large nanoparticles, showing typical Eu^{3+} or Tb^{3+} fluorescence with a long luminescent lifetime. The strength of both europium and terbium ions is the suitable properties for excitation in an ordinary confocal microscope, which makes them promising as components when designing nanoprobes for cell studies. Relaxation measurements show relaxivity ratios in the same range as the pure Gd_2O_3 nanoparticles. The nanoparticles present a promising bifunctional core material, acting as a platform when developing advanced nanoprobes for future applications in biomedical imaging.

3:00pm **NS+BI-ThA4 Plasma Polymerized Amino Acids used for Bio-Assisted Fabrication of Nanostructures**, ***R. Jakubiak***, Air Force Research Laboratory, *K. Anderson*, Georgia Institute of Technology, *J. Slocik*, UES, Inc., *M. McConney*, Georgia Institute of Technology, *J. Enlow*, UES, Inc., *T. Bunning, R. Naik*, Air Force Research Laboratory, *V. Tsukruk*, Georgia Institute of Technology

Plasma-enhanced chemical vapor deposition (PECVD) allows deposition of conformal, ultrathin, and uniform polymer coatings from gaseous, liquid or solid precursors onto a variety of materials. Our process uses a modified afterglow plasma reactor operated at room temperature where plasma polymerization occurs downstream from plasma generation. This allows controllable retention of the precursor's functionality needed for surface-induced biomineralization on soft or delicate substrates that cannot withstand high temperature or multiple wet-chemistry treatments. Amine-functionalized substrates, derived from the plasma polymerization of L-tyrosine, enabled biomineralization of gold nanoparticles from a solution of gold chloride. Templated gold nanoparticle coatings were formed by the placement of a shadow mask on the substrate during plasma deposition creating a micropatterned plasma polymerized tyrosine film. Subsequent gold chloride exposure created a gold nanoparticle network replica of the initial micropattern. Similar processing conditions were used to biomineralize titania on highly order three-dimensional structures.

3:40pm **NS+BI-ThA6 Molecular Shuttles for 'Smart Dust' Biosensors, Active Self-Assembly, and Protein-Resistant Coatings**, ***H. Hess***, Columbia University **INVITED**

Biomolecular motors, such as the motor protein kinesin, can serve as biological components in engineered nanosystems. Initially, a nanoscale transport system termed molecular shuttle has been explored by others and us as a model system. The development of this system has revealed a number of challenges in engineering at the nanoscale, particularly in the guiding, activation, and loading of these shuttles. Overcoming these challenges requires the integration of a diverse set of technologies, and continues to illustrate the complexity of biophysical mechanisms.

A proof-of-principle application of the developed technologies is a "smart dust" biosensor for the remote detection of biological and chemical agents, which is enabled by the integration of recognition, transport and detection into a submillimeter-sized microfabricated device.

The application of nanoscale forces introduces an interesting element into self-assembly processes by accelerating transport, reducing unwanted connections, and enabling the formation of non-equilibrium structures. The formation of nanowires and nanospools from microtubules transported by kinesin motors strikingly illustrates these aspects of motor-driven self-assembly.

Finally, a critical aspect of the design of these hybrid systems is the controlled adsorption of proteins. In pursuit of this goal of controlled adsorption, we have utilized kinesin motors as probes of residual protein adsorption to non-fouling coatings and achieved the detection of a few adsorbed molecules per square micrometer (adsorbed mass on the order of pg/cm^2). Furthermore, we have developed a Random Sequential Adsorption model which successfully explains residual protein adsorption as the result of randomly occuring "bald" spots on a surface covered with PEG-chains.

4:20pm **NS+BI-ThA8 Probing Biomineralization Protein Interactions with Hydroxyapatite Using SFG and NEXAFS Spectroscopy**, ***T.M. Weidner***, *M. Dubey, N.F. Breen, J. Ash, J.E. Baio,* University of Washington, *C. Jaye, D.A. Fischer,* National Institute of Standards and Technology, *G.P. Drobny, D.G. Castner*, University of Washington

The structural integrity of hydroxyapatite (HAP) in tooth enamel is maintained through the saliva environment that is supersaturated with calcium and phosphate salts. The biomineralization protein statherin adsorbs onto HAP surfaces with high binding affinity. It regulates HAP growth and prevents the buildup of excess HAP on the tooth surface by inhibiting spontaneous calcium phosphate growth. Owing to the importance of the underlying physiological processes and a general interest in biomineralization mechanisms, the binding of statherin to HAP has attracted significant interest in the biomaterials community. Sum frequency generation (SFG) spectroscopy can probe protein orientation and secondary structure at the solid-liquid interface and we have recently shown it can address specific protein regions with atomic resolution when combined with isotopic labeling.[1] Near edge X-ray absorption fine structure (NEXAFS) spectroscopy can give valuable information about the structure and binding chemistry of proteins on surface. We have combined both techniques to characterize the structure of the binding domain of statherin, SN15, a short peptide with 15 residues (Ac-DSSEENKFLRRIGRFG-OH) adsorbed onto a model HAP surface. Protein adsorption was verified using X-ray photoelectron spectroscopy and time-of-flight secondary ion mass spectrometry. SFG confirmed a loosely helical secondary structure of SN15 on HAP. Deuteration was used to specifically probe the orientations of the hydrophobic leucine and isoleucine side chains with SFG in situ. Side-chain orientations were determined using ratios of the symmetric and asymmetric CD_3 stretching modes. The leucine chain was tilted 120° from the surface normal (pointing towards the surface) and the isoleucine was tilted 5° from the surface normal. For the first time, element labels were employed to probe individual side chain orientations with NEXAFS spectroscopy. Para- and perfluorination of the phenylalanine rings F7 and F14 allowed us to precisely measure their orientations using angle dependent NEXAFS data. The tilt angles from the surface normal were determined to be 26° for F7 and 35° for F14.

[1] T. Weidner, N. F. Breen, K. Li, G. P. Drobny, D G. Castner, submitted.

4:40pm **NS+BI-ThA9 Assembly of Nanoparticles for Patterning and Functional Materials in Nature's Way at Liquid-Liquid Interfaces**, *L. Isa, E. Amstad, M.H. Textor,* ***E.O. Reimhult***, ETH Zurich, Switzerland

An interesting aspect of the self-organization in Nature resulting in precise patterning of hierarchically structured materials is that the “synthesis” and patterning of the materials occur at liquid amphiphilic interfaces such as membranes. That particles can be organized and change the properties of liquid interfaces has long been known and explored as e.g. Pickering emulsions in foams, food processing and other large-scale, bulk materials applications. However, self-assembly of nanometer-sized colloids with defined surface properties at liquid-liquid interfaces is also a process with huge potential for the fabrication of controlled two-dimensional nanoscale structures and patterns as well as “nanomaterials”. This is due to three key factors: a) the particles are trapped at the interface, but b) retain lateral mobility and c) exhibit specific interactions, which when properly understood and controlled lead to assembly of controlled structures. We have recently explored both how the oil-water interface can be used for unprecedented control of the assembly of nanoparticle patterns and transferred to substrates for low-cost nanolithography, and how tailored core-shell nanoparticles with functional cores can be assembled at such interfaces.

I will describe how self-assembly at the liquid-liquid interface (SALI) can be used for the deposition of non-close-packed crystalline arrays of NPs for lithographic masks and the physical control parameters for the successful application of this method. Our approach allows us to control the spacing of particles in a wide range; we have demonstrated reproducible and homogeneous patterns with spacing between 3 to 20 particle diameters using colloids from 40 to 500 nm over chip-sized areas. The use of bimodal size distributions at controlled ratios also allows for induced phase separation and thus hierarchically ordered patterns to emerge.

By optimization of a simple Schäfer-type deposition setup and the choice of the proper oil phase, the particle patterns can be transferred to a substrate with few limitations. We will demonstrate use of the deposited particle patterns to fabricate a range of nanostructures for electrochemical and nanoplasmonic biosensing which previously could not be fabricated by particle lithography, and this at a fraction of cost to other available patterning techniques.

5:00pm **NS+BI-ThA10 Characterization of Folate Receptor Targeting Drug Loaded PLGA-Lipid Hybrid Nanoparticles**, ***S. Sandoval***, *A. Liberman, J. Yang, S. Aschemeyer, L. Zhang, W.C. Trogler, A.C. Kummel*, University of California, San Diego

The response rate of breast cancer to first line chemotherapies is encouraging, but 20-30% of patients develop chemoresistance to these drugs, and consequently, have a cancer reoccurrence 7-10 months after their last treatment. Chemoresistance is believed to be due to drug efflux proteins responsible for the removal of many commonly used anti-neoplastic agents. One possible way to overcome these drug efflux pumps is to give higher doses of chemotherapy, but high doses of such agents commonly lead to chemocytotoxicity. Targeted PLGA-Lipid hybrid nanoparticle (NP) drug delivery systems have been developed that can deliver high doses of chemotherapy agents specifically to breast cancer cells. A practical cancer targeting drug delivery system will reduce the overall amount of chemotherapy agents given to patients for a given amount of targeted NPs endocytosed by cancer cells. Biodegradable NPs were synthesized using a novel nano-precipitation lipid-polymer hybrid platform which also allows for the encapsulation of hydrophobic chemotherapy drugs within the NPs. Using this method, drug free NPs have been shown to have an average diameter size of 81.78 nm (PDI: 0.25), while single loaded NPs, with Paclitaxel or Doxorubicin, show an average size between 72.33 to 89.64 nm (PDI: 0.242 to 0.339), signifying that the synthesis technique creates consistent sub 100nm particles. The majority of all NPs show a zeta-potential value of > -30 mV consistent with the NPs having sufficient repulsive interaction to be mono-dispersed in solution under physiological conditions. Folate receptors are often over expressed on the surfaces of cancer cells; therefore, folic acid was incorporated to the surface of these NPs as a cancer targeting ligand. Previous studies have shown that HeLa cells, a cervical cancer cell line, over expresses folate receptors. Immunofluorescence studies show that folic acid coated PLGA-Lipid hybrid NPs are readily endocytosed by HeLa cells compared to non-targeted NPs. Cytoxicity studies will determine the increased effectiveness of drug delivery with targeted PLGA-Lipid hybrid NPs vs. untargeted PLGA-Lipid hybrid NP in cell lines and in animal models.

5:20pm **NS+BI-ThA11 Controlled Surface Modification of Ultra-stable Superparamagnetic Iron Oxide Nanoparticles**, ***E. Amstad***, *M.H. Textor, E.O. Reimhult,* ETH Zurich, Switzerland

Biocompatibility, magnetic properties and ease of synthesis renders iron oxide nanoparticles (NPs) attractive for many especially biomedical applications such as magnetic resonance (MR) contrast agents, triggered drug release and cell separation. Good NP stability under physiologic conditions and controlled surface chemistry are key to successful application not only in the biomedical field but also for assembly into various materials.

NPs with close control over the interfacial chemistry and good stability at high salt concentrations and elevated temperatures can only be achieved if dispersants are irreversibly bound to the NP surface. The dispersant binding affinity is determined by its anchor group. Low molecular weight dispersants which consist of one high affinity anchor covalently linked to poly(ethylene glycol) (PEG) spacers have been proven well suited to sterically stabilize Fe_3O_4 NPs. However, we found that electronegatively substituted catechols such as nitrocatechols vastly outperform the well-known and often used catechol anchors such as DOPA and dopamine. Because of the optimized binding affinity of nitrocatechols, PEG-nitrocatechol coated Fe_3O_4 NPs remained stable under physiologic conditions up to 90 °C whereas e.g. PEG-dopamine stabilized Fe_3O_4 NPs started to agglomerate below body temperature.[1] Further investigations showed that the optimal binding affinity of nitrocatechols to Fe_3O_4 is closely related to a redox reaction between Fe^{2+} located at the Fe_3O_4 NP surface and nitrocatechols, which leads to electron delocalization in the adsorbed catechol ring, and a close to covalent bond of nitrocatechols to Fe_3O_4 surfaces. Irreversible binding of PEG-nitrocatechols to Fe_3O_4 NPs allowed us to closely control and investigate the influence of dispersant layer thickness by varying the nitrocatechol-PEG molecular weight. Furthermore, NPs could easily be functionalized by co-adsorbing differently end-functionalized dispersants on the Fe_3O_4 NP surface.[2]

In summary, nitrocatechols have a close to optimal binding affinity to Fe_3O_4 surfaces. This optimized binding affinity not only leads to ultra-stable PEG-nitrocatechol coated superparamagnetic Fe_3O_4 NPs but also allows for close control over the hydrodynamic diameter and interfacial chemistry, factors which crucially determine NP performance especially in biomedical applications.

[1] E. Amstad, T. Gillich, I. Bilecka, M. Textor, E. Reimhult, *Nano Letters* **2009**, *9*, 4042.

[2] E. Amstad, S. Zurcher, A. Mashaghi, J. Y. Wong, M. Textor, E. Reimhult, *Small* **2009**, *5*, 1334.

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