Biomaterial Interfaces Division
Room: 108 - Session BI-MoM

Biomolecules at Interfaces

Moderator: A. Rosenhahn, Karlsruhe Institute of Technology, Germany

8:20am **BI-MoM1 Cell Instructive Biomaterials by Non-Equilibrium Self-Assembly**, *R.V. Uljin, V. Jayawarna, S. Roy, M.J. Dalby,* The University of Strathclyde, UK, ***S.L. McArthur***, Swinburne University of Technology, Australia **INVITED**

Peptides are highly versatile building blocks for the production of supramolecular gels which mimick certain properties of biological systems but with minimal complexity. These gels may be ideally suited to interface synthetic systems with biology, and as such have attracted much interest in a range of areas including health care and energy related technologies. We have developed versatile gelator systems based on aromatic peptide amphiphiles, which self-assemble via a unique mechanism involving π-interlocked β-sheets. These systems display highly tunable supramolecular functionalities, giving rise to materials with controlled mechanical properties, nanotopography and bioactive properties. We will show that these properties can be controlled by exploiting non-equilibrium self-assembly which may involve the use of biocatalysts to control the self-assembly process. We will discuss progress in fundamental understanding of these systems as well as applications as matrices for stem cell differentiation.

References:

Nature Chemistry, 2010, 2, 1089-1094..; Chem. Soc. Rev. 2010, 39, 3351-3357.; Nanoscale, 2010, 2, 960-966.; Chemical Communications, 2010, 46, 3481-3483.; Nature Nanotechnology, 2009, 4, 19-24.; Langmuir, 2009, 25, 9447-9453.; Faraday Discussions, 2009, 143, 293-303.; Langmuir, 2009, 25, 7533–7539.; Acta Biomaterialia, 2009, 5, 934-943.; Biomaterials, 2009, 30, 2523-2530.; Soft Matter, 2009, 5, 1728-1734.; Small, 2009, 5, 587-590.; Adv. Mater., 2008, 20, 37-41.; Small, 2008, 4, 279-287.

9:00am **BI-MoM3 Lipid Vesicle Fusion for Studies of Cell Functions**, ***L. Simonsson***, *A. Gunnarsson, M. Kurczy, P. Jönsson, AS. Cans, F. Höök*, Chalmers University of Technology, Sweden

Fusion of lipid vesicles and cells is a natural process which takes place in eukaryotic cells. It is a vital process, since it enables cells' communication with the outside, both via vesicle content release and through delivery of e.g. membrane proteins to the outer cell membrane. Exocytosis is still not fully understood and although attempts have been done to deliver membrane constituents to supported lipid bilayers, improvement in e.g. efficiency remains. In order to gain a deeper understanding of the mechanism of membrane fusion as well as improve the delivery of arbitrary membrane constituents including complete cell membrane fragments, to supported lipid bilayers, we have in this work developed two novel and powerful techniques.

To mimic exocytosis, we use giant unilamellar vesicles (GUVs) as a model of the cell membrane, cholesterol-DNA[1-3] as a mimic of the SNARE-proteins and small unilamellar lipid vesicles filled with easily oxidized catechol to represent the cellular vesicles. We probe the fusion process using a carbon fiber electrode, detecting the released catechol. By building this advanced but yet controllable model system of exocytosis, we believe that a wide range of studies can be made in order to decipher the process of exocytosis. Future applications are delivery of e.g. membrane proteins to GUVs, as well as for vesicular drug delivery to cells.

In order to deliver membrane constituents to SLBs, we use a controlled bulk flow through a microfluidic channel to move the front edge of a supported lipid bilayer and fuse it with vesicles adsorbed in front of it. The membrane constituents of the adsorbed vesicles are efficiently incorporated into the supported lipid bilayer and can be manipulated in 2D (accumulated and separated) by again using the bulk flow. We show that this method is perfectly compatible with cell membrane fragments derived directly from 3T3 fibroblast cells[4]. The method enables studies of e.g. receptor-ligand interactions as well as membrane protein separation in a native environment.

Simonsson et al., ChemPhysChem, (2010)

Stengel et al., J. Am. Chem. Soc., (2007)

Stengel et al. J. Phys. Chem. B, (2008)

Simonsson et al., Submitted

9:20am **BI-MoM4 Watching Biomineralization at Work: The Specific Interactions of Statherin with Hydroxyapatite Surfaces Probed at the Molecular Level**, ***T. Weidner***, *M. Dubey, K. Li, 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

Biomineralization proteins act as nature's crystal engineers and adsorb onto crystal surfaces with high binding affinity and precision using specific substrate-surface binding motifs. Owing to the importance of the underlying physiological processes and a general interest in biomineralization mechanisms, the binding of regulatory proteins has attracted significant interest. We have studied statherin, which regulates the growth of hydroxyapatite (HAP) in bone and tooth enamel and prevents the buildup of excess HAP by inhibiting spontaneous calcium phosphate growth. A detailed understanding of the underlying molecular recognition mechanisms would help bioengineers and scientists to devise new biomimetic approaches for clinical applications and biomineral nanofabrication. 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 and solid state NMR (ssNMR) data.(1) We have combined both techniques with near edge X-ray absorption fine structure (NEXAFS) spectroscopy to characterize the structure of the binding domain of statherin, SN-15, on HAP. Protein adsorption was verified using XPS and ToF-SIMS. NEXAFS N *K*-edge spectra clearly show that hydrogen bonding is important for the binding of both peptides. SFG confirmed an α-helical secondary structure of SN-15 on HAP with the helix axis parallel to the surface. Deuteration was used to specifically probe the orientations of all hydrophobic side chains (leucine, isoleucine, phenylalanine) with SFG *in situ*. The leucine chain was tilted 120° from the surface normal (pointing towards the surface) and isoleucine was tilted 5° from the surface normal. We also employed fluorine labels to probe individual phenylalanine rings with NEXAFS spectroscopy. Measurements of ring orientations in combination with ssNMR surface distance and rotamer dynamics data allowed us to develop a clear picture of the side chain structure.

1. Weidner T, Breen NF, Li K, Drobny GP, & Castner DG (2010) A Sum Frequency Generation and Solid-State NMR Study of the Structure, Orientation and Dynamics of Polystyrene-Adsorbed Peptides. *Proc. Natl. Acad. Sci. U. S. A.* 107:13288–13293.

9:40am **BI-MoM5 ECM Ordering Effects as a Marker for Early Tissue Formation on Artificial Substrates - A Sum-Frequency-Generation Spectroscopy Study**, *M.-O. Diesner*, ***P. Koelsch***, Karlsruhe Institute of Technology (KIT), University of Heidelberg, Germany

The *in situ* monitoring of the interphase between a substrate and a cellular layer is of great interest as it allows determination of changes in surface properties and extracellular matrix (ECM) organization. The latter is an early indicator of major cellular processes like migration, adhesion, proliferation, metastasis, tissue formation, and gain or loss of differentiation occurring. Typically, ECM studies of adherent cells involve labeling and fixing cell samples, which may result in their disruption and in the loss of valuable information. In addition, the weak signal-to-noise ratio of fluorescent probes limits the probing capabilities at early stages of cell adhesion.

Recent work from our group has shown that sum-frequency-generation (SFG) spectroscopy can be used to interrogate the ordering of the ECM beneath adherent cells on an artificial substrate during these early stages.[1,2] It turns out that SFG spectroscopy is suitable to probe the layer in between a solid substrate and living cells and that the information which can be obtained on such systems are twofold: first, changes of the surface coating can be investigated in real-time and in vitro on a molecular scale while cells adhere to it. Secondly, SFG spectroscopy is suitable for the determination of ordering parameters within the ECM without the need for labeling and processing.

In this contribution we will report on ordering phenomena occurring at early stages of rat and mouse embryonic fibroblasts adhesion on Au-coated Si wafers. Several phases observed during the adhesion process will be discussed and the results obtained by nonlinear optical spectroscopy will be correlated to classical tools including Western blot analysis of ECM constituents, fluorescent probes, and genetic screens blocking the formation of fibrils.

References

[1] M.-O. Diesner, C. Howell, V. Kurz, D. Verreault, and P. Koelsch. In vitro characterization of surface properties through living cells. J Phys Chem Lett, 1:2339– 2342, 2010.

[2] C. Howell, M.-O. Diesner, M. Grunze, and P. Koelsch. Probing the extracellular matrix with sum-frequency-generation spectroscopy. Langmuir, 24:13819–13821, 2008.

10:00am **BI-MoM6 Structure and Function of von Willebrand Factor on Synthetic Surfaces and Collagen**, ***E. Hillenmeyer***, *O. Yakovenko, R. Penkala, W. Thomas, D.G. Castner*, University of Washington

von Willebrand Factor (VWF) is a soluble clotting protein responsible for binding platelets through the glycoprotein 1b platelet receptor. VWF can become activated and bind platelets when bound to collagen in an injured blood vessel or under increased shear.

VWF can also bind platelets when adsorbed to synthetic surfaces, specifically biomaterials. There is evidence that surface characteristics influence VWF adsorption. Previous AFM studies of VWF adsorbed to hydrophilic (mica) and hydrophobic (octadecyltrichlorosilane modified glass) surfaces showed differences in adsorbed topography[1]. However, studies were not performed to relate adsorption differences to VWF function.

Previous studies in our lab have shown differences in function and structure of the platelet binding domain of VWF (A1 domain) when adsorbed to three surfaces: polystyrene (PS), tissue culture polystyrene (TCPS), and glass, with A1 most active when adsorbed onto PS. A1 function was tested by measuring platelet binding under flow. A1 surface structure was investigated using time of flight secondary ion mass spectrometry (ToF-SIMS) and binding of conformation-dependent antibodies in ELISA assays.

In the work presented here, we have used surface analysis techniques to obtain greater detail about structural differences of the VWF A1 domain adsorbed onto synthetic surfaces. We used near-edge x-ray adsorption fine structure (NEXAFS) to examine the amide backbone, corresponding to the pi* feature of the nitrogen edge NEXAFS spectrum. Differences were observed in the angle dependence of the spectra when A1 was adsorbed onto PS, TCPS, and glass, indicating significant structural differences in the protein when adsorbed onto different surfaces. Sum Frequency Generation (SFG) was also used to probe the structure of the amide backbone using amide I spectra.

In addition to examining VWF on synthetic surfaces, we have used ToF-SIMS to obtain structural information about A1 bound to collagen, as occurs during *in vivo* injury. Principal component analysis of ToF-SIMS data showed differences between A1 bound to collagen and A1 adsorbed directly onto polystyrene. This suggests that A1 adopts different conformations on the natural versus synthetic substrates, potentially leading to different mechanisms of activation.

In this work, we use surface analysis tools to increase our understanding of VWF behavior, both on synthetic surfaces and in complex, layered protein systems. Increasing our fundamental knowledge of VWF can improve our understanding of VWF interactions with biomaterial surfaces, as well as thrombosis during injury.

1. M. Raghavachari, et al. *Colloids Surf B* (2000) 19:315.

10:40am **BI-MoM8 ToF-SIMS Study of Orientation of $FnIII_{9-10}$ Fibronectin Fragment on Self-Assembled Monolayers**, ***L. Árnadóttir***, *L.J. Gamble*, University of Washington

Fibronectin (FN) is an extra cellular matrix protein that is involved in many cell processes such as adhesion, migration and growth. The orientation and conformation of FN adsorbed onto surfaces can therefore have a critical effect on cell-surface interactions. Experimental orientation studies of model systems also provide an important benchmark for molecular simulations and are of great value for further development of force fields used in many theoretical studies of protein adsorption. In this study the adsorbed orientation of the 9-10 fragment of FNIII was studied on three different model surfaces (self-assembled monolayers (SAM) of C_{11} alkanethiols on Au, $-CH_3$, $-NH_2$, and -COOH terminated SAMs.) The $FNIII_{9-10}$ fragment includes the two binding sites for the FN protein, a RGD site on segment 10 (seg10) and a synergy site on segment 9 (seg9). This fragment also has a significant asymmetry in its amino acid distribution with His found exclusively on seg9, Lys only on seg10 and three times more Tyr on seg10 then seg9. Taking advantage of this asymmetry, we use time of flight secondary ion mass spectrometry (ToF-SIMS) to study the different orientation on different surfaces and X-ray photoelectron spectroscopy (XPS) to determine the difference in surface coverage. While CH_3 and NH_2 have similar full coverage the highest COOH coverage is about half the maximal coverage observed for the two other surfaces. A ToF-SIMS comparison of the $FNIII_{9-10}$ on COOH and NH_2 at similar coverages shows significantly more His on the COOH and more Tyr on the NH_2 surfaces suggesting an opposite orientation of the fragment on these two surfaces. Results indicate that on the COOH surface the fragment is oriented with seg10 down while on the NH_2 surface seg9 is closer to the surface. Comparing similar coverages of the $FNIII_{9-10}$ on NH_2 and CH_3, the later has less Tyr signal (from seg10) suggesting that the seg10 is closer to the surface on CH_3 than on NH_2. In contrast, when $FNIII_{9-10}$ on CH_3 and COOH results were compared, the fragment on the CH_3 sample has less His signal suggesting that seg9 is closer to the surface compared to COOH.

11:00am **BI-MoM9 Single Protein Manipulation with STM**, ***S. Khan***, *K. Clark, C. Henneken, E. Rauh, S.-W. Hla*, Ohio University

Scanning tunneling microscope (STM) is not only an instrument to image atomic landscape of material surfaces but also is a tool to manipulate individual atoms and molecules. If STM manipulation and spectroscopy can be applied to individual biomolecules, it will be advantageous for multiple research areas. Here we use a low temperature STM in an ultrahigh environment to image individual protein molecules with molecular resolution on Ag (111) surface at 4.6 K. A-b type amyloid precursor proteins molecules and various single chain proteins were deposited on atomically clean Ag (111) surface. STM images and tunneling spectroscopy enables direct sequencing of amino acid groups in these molecules. Moreover, using an STM manipulation procedure employing an attractive tip-molecule interaction, individual molecules were relocated on the surface; thereby their mechanical integrity can be tested. Protein folding is a major issue in biological processes. Here, we will demonstrate that folding of carbon back-bone in these proteins is possible using an STM manipulation procedure. This experiment presents a novel avenue of biological research where sequencing and manipulation may be performed one molecule-at-a-time. We acknowledge the financial support provided by US-DOE-DE-FG02-02ER46012.

11:20am **BI-MoM10 DNA Origami from Inkjet Synthesis Produced Strands**, ***I. Saaem***, *A. Marchi, J. Tian, T. LaBean*, Duke University

High-throughput synthesis of quality mixed oligonucleotides (oligos) allows for exhaustive studies of DNA nanostructured material formation. In situ DNA synthesis was achieved with a custom piezoelectric inkjet system using phosphoramidite chemistry, on functionalized cyclic olefin copolymer (COC) as a chip substrate. When amplified off of the chip via strand displacement amplification (SDA), the oligos were eluted and used directly for constructing multiple DNA origami nanostructures in a one-pot assembly. By performing SDA, each chip may be reused multiple times to produce pools of staple strands for repeated experiments. Two separate DNA origami structures were formed from a double-stranded scaffold strand. By redesigning specific staple strands, these structures can be connected to provide a larger nanostructured template.

11:40am **BI-MoM11 Solid State Electron Transport across Proteins**, ***D. Cahen***, *M. Sheves, I. Pecht, L. Sepunaru*, Weizmann Institute of Science, Israel

We found that solid-state electron transport (Etp) studies are possible across non-modified 'dry' proteins, between two solid electrodes, using the light-induced proton pumping protein, Bacteriorhodopsin (bR), the electron transfer (ET) protein, Azurin (Az), and Bovine Serum Albumin (BSA). Clear differences between these proteins, where for bR and Az we can show that they preserve their structure in the solid state measurement configuration, were observed, with small tunneling decay constants for all three proteins, suggesting that incoherent transport is the dominant mechanism. Putting our data in perspective by comparing them to all known protein Etp data in the literature, we concluded that, in general, proteins behave more akin to molecular wires than to insulators. An important part of these studies was the modification of the proteins by, e.g., removing or disconnecting the retinal in bR and removing the Cu redox centre in Az.

We now report that, notwithstanding the above-noted earlier conclusion on hopping, which was based on the Etp efficiency, Az shows 9-360K temperature independent Etp, until its denaturation temperature. Removal of the Cu changes this behavior to Arrhenius-like thermally activated Etp, which becomes temperature independent below ~ 200K, a behavior that apo-Az shares, qualitatively with bR, its variants and with BSA.

This difference between bR and Az leads to the truly remarkable situation that above room temperature the non-ET protein bR shows significantly better Etp than ET protein Az.

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