Biomaterial Interfaces
Room: Taos A - Session BI2-WeM

Proteins & Peptides on Surfaces
Moderator: P. Kingshott, Aarhus University, Denmark

10:40am **BI2-WeM9 The Surface Mediated Assembly of Small Biomolecules Relevant to Prebiotic Chemistry**, ***D.J. Frankel***, *L. Donlon*, Newcastle University, UK

On prebiotic Earth where the absence of large molecules such as enzymes would be a barrier to the formation of complex biological structures, self assembly mediated by surfaces could have offered an alternative mechanism for polymerisation. In this work we examine the interactions between various monomers of biological relevance including nucleic acid bases and amino acids. Unlike related studies the assembly was conducted in an aqueous environment which is the most prebiotically relevant solvent. STM shows that chiral amino acids form two dimensional crystals completely independently of the underlying graphite surface. Moreover the chiral amino acids can interact with nucleic acid bases to produce unique crystal structures. Unlike UHV single crystal studies the surface/adsorbate interaction is less relevant and the structures assembled in essence forgets the surface. We propose a mechanism by which subsequent solution based molecules can interact with these ordered surfaces to produce selective interactions.

11:00am **BI2-WeM10 Stabilization of Proteins by Sol-Gel Encapsulation and Study of its Interactions with the Host Matrix**, ***S. Sammeta***, *G. Doroudian, G.P. Lopez, E.Y. Chi*, University of New Mexico

Utilization of biological molecules such as proteins in biosensing and biofuel cell applications is one of the most innovative research fields. A major challenge that needs to be overcome in these applications is the adequate stabilization of proteins. Porous silica material made by the sol-gel process provides a promising host matrix for the encapsulation of proteins to increase their stability. The main objective of this study is to elucidate protein-host matrix interactions for the stabilization of proteins. In our study, lysozyme and cytochrome c were used as model proteins. We have developed two novel vapor exposure sol-gel techniques to encapsulate proteins using tetra methyl orthosilicate (TMOS) as a precursor: (I) in-situ protein exposure method, where a buffer containing a protein is directly exposed to TMSO vapor, and (II) buffer exposure method, where a buffer was first exposed to TMOS vapor and then a protein was added. Additionally, organically-modified glasses are used to study the effect of host-matrix hydrophobicity on protein structure and stability. Circular dichroism and high resolution derivative UV spectroscopy are used to evaluate the structure and thermal stability of encapsulated proteins. The effect of protein concentration and sucrose (a model osmolyte) on the structure and stability of encapsulated proteins are characterized. Cytochrome c retained native-like structures while lysozyme becomes partly unfolded when encapsulated in the silica matrix. Entrapment generally increases the thermal stability of proteins. Proteins encapsulated via the in situ technique are found to have higher thermal stability compared to those encapsulated using method II. In general, method II gave rise to proteins with more secondary structure. However, proteins in this matrix are less resistant to thermal denaturation. In addition, there are concentration-dependent decreases in protein secondary structure when encapsulated by method II. We believe that the trends are due to protein adsorption onto silica which causes denaturation. Osmolytes shift the protein native state ensemble towards more compact conformations, thereby increasing the conformational stability of proteins. We observed enhanced secondary structure of cytochrome c with the addition of 0.5 M sucrose and this enhanced structure and stability is preserved when the protein is encapsulated in silica gel in the presence of sucrose. The effect of increasing the host-matrix hydrophobicity by incorporating alkyl group substituted alkoxysilanes into the silica matrix on protein structure and stability will be presented.

11:20am **BI2-WeM11 ToF-SIMS Imaging to Characterize DNA Microarray Surfaces**, ***L.J. Gamble***, *N. Vandencasteele, L. Arnadottir, D.G. Castner,* University of Washington, *D.W. Grainger*, University of Utah

Commercial DNA array slides are commonly made by microprinting techniques. These nanoliter doplets evaporate within seconds and this fast drying may contribute to heterogeneous spots and inconsistent results. Successful development and optimization of DNA-functionalized surfaces for microarray and biosensor applications requires a better characterization of immobilized DNA. In this work, time-of-flight secondary ion mass spectrometry (ToF-SIMS) is applied to the study of spotted DNA surfaces on commercial microarray slides. ToF- SIMS results are directly compared to fluorescence images. Maximum Autocorrelation Factor (MAF) image analysis, a technique independent of the scaling of the raw data, is used to analyze the ToF-SIMS images. An IONTOF TOF.SIMS 5-100 instrument using a Bi source is used to analyze the samples. Immobilized DNA probes with 10, 20 and 40μM DNA concentrations as well as different Cy3 label concentrations are spotted on a commercially available microarray polymer slide. The effect of the spotting solution concentration as well as the amount of Cy3 label on spot uniformity is studied. MAF analysis of the ToF-SIMS image for a 20μM DNA spot shows that the areas seen as having high fluorescence intensities are related to higher concentrations of phosphate groups (from the DNA backbone) as well as sulfates and peaks with masses corresponding to the DNA bases. A comparison of MAF analysis of ToF-SIMS images for different DNA spotting concentrations indicates that the concentration of the spotting solution has an effect on the uniformity of the spot.

11:40am **BI2-WeM12 Protein Nanopatterning for Studying Cell Adhesion**, ***S. Kristensen***, *J. Malmström, J. Lovmand, M. Duch, D.S. Sutherland*, Aarhus University, Denmark

Synthetic materials are often used for biomedical applications. Interaction of cells with the interfaces and tissue components determine the biological outcome of the device. Knowledge about the interaction between the cells and biointerfaces is hence of importance in area such as biomaterials, tissue engineering and cell culture. The interaction of the cells with its surroundings is mediated at the molecular and macromolecular level. Specific interaction with the extracellular matrix components or macromolecules in the outer membrane of adjacent cells provides signaling and communication pathways. Here patterns of extracellular matrix protein are used to study the development of cellular adhesion complexes.

Protein nanopatterns at the 100-3000 nm scale and with lateral ordering between independent ligands and controlled lateral mobility has been made by using a nanoscale chemical contrast of Au patches in a background of SiO_2 by colloidal lithography. The nanostructured surfaces are made by depositing a triple polyelectrolyte layer (PDDA/PSS/PAX) at Au substrates. Latex particles self assemble at the surface governed by electrostatic forces followed by SiO_2 evaporation and removal of the particles. The generated short range ordered arrays were further modified by octadecylmercaptane adsorption. The samples were subsequently treated with PLL-*g*-PEG for 30 min. followed by adsorption of fibronectin for 2 h. Myoblast cell(C2C12) or MDA-MB-435 cells were added to the samples and allowed to adhere to the surfaces for 6 h or 24 hours.

Fibronectin distribution at the nanopatterned surfaces was studied via liquid AFM showing that protein were adsorbed preferentially on the alkane thiol patches. SEM images showed that protein was patterned over large areas. Protein patterns of several other proteins such as Osteopontin, Vitronectin and Laminin were also demonstrated. Fluorescent microscopy showed that cells adhered to the patterns of size from 200nm and up. Small focal complexes were observed at the 200nm structures which were not linked to the actin cytoskeleton. For 500nm and 1000nm patchs cell showed small focal adhesions connected to thin actin fibers and the adhesions were limited to individual patches.

We utilize colloidal lithography to fabricate protein patterns of size from nano to micro scale and from different proteins. The patterned areas are of a sufficiently large area to carry out large scale cellular characterisation in terms of adhesion morphology and differentiation. The protein patterning makes it possible to limit the length of developing focal adhesions to single patches and hence alter the cells ability to generate forces, spread and move.

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