Biomaterial Interfaces
Room: K - Session BI+AS+NS-ThA

Micro and Nanoengineering of Biointerfaces I

Moderator: G.J. Leggett, University of Sheffield

2:00pm **BI+AS+NS-ThA1 Spatial Organization and the Mechanics of Signal Transduction in Cell Membranes**, ***J.T. Groves***, University of California, Berkeley **INVITED**

Signal transduction in living cells is carried out through cascades of chemical reactions, which generally begin on the cell membrane surface.

In recent years, there has been growing realization that the large-scale spatial arrangement of cell surface receptors can regulate the outcome of ensuing signal transduction process. Signaling through the T cell receptor (TCR) in the context of the immunological synapse provides a case in point. Spatial reorganization of TCRs occurs on multiple length-scales, and apparently with multiple purposes, during antigen recognition by T cells. The cell membrane and cytoskeleton, working as an inseparable unit in this case, create the mechanical framework within which TCR signaling processes occur. To better study these phenomena, a new experimental strategy, in which the spatial positions of cell membrane receptors are directly manipulated through mechanical means, has emerged. By physically inducing a 'spatial mutation' of the signali ng apparatus, the role of spatial organization in signal transduction as well as the mechanisms by which it arises can be illuminated. Specific applications of this strategy to TCR signaling and other cell-cell signaling systems will be discussed.

2:40pm **BI+AS+NS-ThA3 Investigation of Array Spotting of Polymer Supported Lipid Bilayers**, ***S. Kaufmann***, *M. Homenuke*, ETH Zurich, Switzerland, *J. Sobek*, University of Zurich, Switzerland, *E.O. Reimhult, M. Textor*, ETH Zurich, Switzerland

Supported lipid bilayers (SLB) constitute a simple model of cell membranes and are of particular interest as components of future generations of biosensors based on transmembrane proteins. Techniques which are able to produce arrays with small micrometer-sized sensor areas in a cheap and fast way are beneficial. A major challenge producing such arrays of SLBs is their need for an aqueous environment during formation and operation, which has so far prevented the wide-spread use of common techniques to produce arrays such as spotting.

Poly(ethylene glycol) (PEG) can be incorporated into the membrane of liposomes through lipid molecules end-functionalized with a PEG chain and these liposomes were shown to spontaneously fuse to PEG-SLB on glass surfaces with a highly hydrated PEG cushion on each side of the membrane allowing ample space and protection for incorporation of membrane proteins[1,2]. Since Cremer and coworkers [3] also showed that with increasing PEG concentration the air-stability of PEG-SLBs increases it is a very promising SLB system to use for spotting where membrane air exposure during processing is a severe constraint.

We present an investigation of the limits to spontaneous PEG-SLB in terms of PEG-lipid density, demonstrating that crossing the mushroom-to-brush regime of polymer concentration prevents the PEG-SLB formation due to steric effects and shielding of the interactions[2]. Furthermore, we present conditions under which formation of PEG-SLBs is facilitated and can proceed by liposome fusion also in the brush regime as well as characterization of the kinetics of formation and the structure of these PEG-SLBs. The use of such buffers and liposomes for production of membrane arrays on glass using a non-contact piezo-spotter was then explored in detail in order to find optimal conditions of buffer composition and PEG concentration.

---

[1] Kaufmann, S. et al., *Soft Matter*, **2009**, accepted

[2] Diaz, A.J. et al., *Langmuir*, **2008**, 24, 6820

[3] Albertorio, F. et al., *Langmuir*, **2005**, *21*, 7476-7482

3:00pm **BI+AS+NS-ThA4 Direct Laser Patterning of Soft Matter: Photothermal Processing of Supported Phospholipid Multilayers with Nanoscale Precision**, ***M. Mathieu***, *D. Schunk, S. Franzka, C. Mayer, E. Hasselbrink, N.O. Hartmann*, University of Duisburg-Essen, Germany

Supported phospholipid bilayers and multilayers are widely recognized as model systems of biological membranes. Recently, these coatings have also gained significant attention as flexible biomolecular matrixes in various micro- and nanofabrication schemes [1]. Here, we report on direct laser patterning of supported phospholipid bilayer stacks. Direct laser patterning techniques are widely recognized as powerful tools in rapid prototyping and small volume fabrication. They offer a high flexibility in fabrication of complex 2D structures and patterning can be carried out at fast writing speeds over macroscopic length scales at ambient pressures or even in liquids [2]. For patterning multi-layered dioleyl-phosphatidic acid (DOPA) films were deposited on native silicon samples via spin coating. Then photothermal processing with a focused laser beam at $\lambda = 514$ nm is used for removal of the coating at predefined positions without causing any significant change in adjacent areas. Moreover, processing with nanoscale precision is feasible despite the soft and fluid nature of phospholipid films. In particular, holes with diameters from 1.8 µm down to 300 nm and below are fabricated using a laser spot diameter of about 2.5 µm [3]. Furthermore, partial removal can be carried out at incremental steps leaving a distinct number of bilayers behind. The underlying nonlinear dependence of the patterning process on the laser intensity is traced back to the interplay between the laser-induced transient local temperature rise and the thermally activated desorption of the phospholipid molecules. Generally, the lateral resolution in photothermal processing depends on the thermal and chemical stability of the coating. Phospholipid films, of course, are soft supramolecular assemblies. Despite their soft nature, however, the collective interactions are quite strong. This gives rise to a strong nonlinearity as observed here. Considering these features, photothermal laser processing constitutes a powerful tool for micro- and nanopatterning of phospholipid films.

1. A. Terheiden, C. Mayer, K. Moh, B. Stahlmecke, S. Stappert, M. Acet, B. Rellinghaus, Appl. Phys. Lett. 84 (2004) 3891.

2. D. Dahlhaus, S. Franzka, E. Hasselbrink, N. Hartmann, Nano Lett. 6 (2006) 2358.

3. M. Mathieu, D. Schunk, S. Franzka, C. Mayer, E. Hasselbrink, N. Hartmann, Small, accepted.

3:40pm **BI+AS+NS-ThA6 The Role of Liposomes in Fluorescent Based Microarrays: From Surface Immobilization of Membrane Proteins to Highly Fluorescent Labels**, ***M. Bally***, ETH and University Zurich, Switzerland, *K. Bailey*, CSIRO, Australia, *S. Syed, S. Buergel, J. Voeroes*, ETH and University Zurich, Switzerland

Technologies utilizing arrays of immobilized biomolecules on planar surfaces are emerging as powerful high throughput tools for bioanalytical measurements. Nowadays, optical sensors based on fluorescence detection are the most widespread. However, many applications especially in the area of protein sensing, rely on the availability of optimized sensing interfaces and signal amplification strategies. Liposomes, due to their hollow particle-like structure and their unique chemical and physical properties, have greatly contributed to the development of sensitive and accurate biological assays.

In this presentation, we highlight with results obtained recently, the contributions of phospholipid vesicles to the development of high performance fluorescence based biosensors.

First, liposomes are an optimal platform for the surface immobilization of membrane proteins since they provide the natural environment required for the functional surface immobilization of these fragile molecules. We demonstrate the creation of a functional, heterogeneous array of G-protein coupled receptors. Vesicles obtained from cellular membrane extracts containing either the H1R-histamine receptor or the M2R-muscarinic receptors were immobilized on a conventional oligonucleotide microarray via complementary tags. Fluorescent ligand binding assays were then performed illustrating that the receptors kept their native conformation. As an alternative platform, we introduce a novel approach for the creation of vesicle multilayers using zirconium phosphate chemistry. As demonstrated in a model biomolecular binding assay, such three dimensional constructs increase the protein loading capacity of a sensor surface.

Liposomes are also excellent candidates as labels for biological assays: phosphocholine-based vesicles are non-fouling and biomolecules or marker molecules (e.g. fluorophores or enzymes) can be easily attached to their surface or encapsulated in their inner cavity. We show that fluorescently labeled phospholipid vesicles provide simple and cheap means for signal amplification and sensitive protein detection on a microarray format. Using vesicles, up to 100 fold increase in sensitivity was observed in a model protein microarray with confocal read-out, compared to a conventional assay performed with fluorophore labeled antibodies.

The various approaches presented here will contribute to the development of sensitive and high performance microarrays for a variety of applications including the investigation of membrane proteins.

4:00pm **BI+AS+NS-ThA7 Fusion of Biomimetic 'Stealth' Probes into Lipid Bilayer Cores**, ***B. Almquist***, *N. Melosh*, Stanford University

The ability to specifically and non-destructively incorporate inorganic structures into or through biological membranes is essential to realizing full bio-inorganic integration, such as arrayed on-chip patch-clamps, drug delivery, and biosensors. However, molecular delivery and interfaces to inorganic objects, such as patch-clamp pipettes, generally rely upon destructive formation of membrane holes and serendipitous adhesion, rather than selective penetration and attachment to the bilayer. In fact, materials greater than a few nanometers in size have not been shown to penetrate lipid bilayers without disrupting the continuity of the membrane. In this talk, I will discuss the development of nanofabricated probes that spontaneously insert into the hydrophobic membrane core by mimicking the hydrophobic banding of transmembrane proteins, forming a well-defined bio-inorganic lateral junction. These biomimetic 'stealth' probes consist of hydrophilic posts with 2-10 nm hydrophobic bands formed by molecular self-assembly, and are easily fabricated onto a variety of substrates including silicon wafers, nanoparticles, and atomic force microscope (AFM) tips.

By fabricating this architecture onto AFM probes, we have directly measured the penetration behavior and adhesion force of different molecular functionalities within the bilayer. It has been found that following insertion, the stealth probes remain anchored in the center of the bilayer, while purely hydrophilic probes have no preferred location. The strength of the stealth probe adhesion varies greatly between short and long chain alkane functionalizations, indicating that chain mobility, orientation, and hydrophobicity all contribute to molecular stability within the bilayer. In addition, the consequences of geometric factors such as band thickness and the presence of multiple bands on interface stability have been established. By selectively choosing the desired properties of the hydrophobic band, it will be shown that it is possible to tune the failure tension of the interface from values comparable to that of pristine lipid vesicles to only a fraction of the strength. Finally, the ability to transfer the stealth probe behavior to other platforms (e.g. nanoparticles for drug delivery) will be discussed.

4:20pm **BI+AS+NS-ThA8 Seeing Nanopore-spanning Supported Lipid Bilayers**, ***K. Kumar***, *S. Kaufmann, M. Textor, E.O. Reimhult*, ETH Zurich, Switzerland

Supported lipid bilayers (SLBs) present a highly interesting cell-membrane-like format to study sensitive ion channels or other membrane proteins. If formed by the rupture of liposomes, they then have the major advantage over other planar membrane architectures for biosensing in that they can be formed completely bereft of organic solvents by self-assembly, enabling the further incorporation of the aforementioned sensitive membrane proteins.[1] Porous structures allow the use of fusogenic surfaces which enhance the formation of SLBs from liposomes, while accommodating the incorporation of larger membrane proteins by decoupling them from the surface. We have developed a particle lithography process to produce high aspect ratio pores with a diameter tunable between 40 nm and 200 nm into silicon nitride, silicon oxide or titanium oxide to take advantage of a range of different surface chemistries. SLBs were formed on these porous substrates and characterized by fluorescence and force microscopy. These results suggest that the underlying nanotopography of the substrate plays a major role in both the formation and characterisation of nanopore-SLBs. The outcome of liposome adsorption is strongly influenced by roughness features in the same size range as the liposomes, which has important implications for the reproducible formation and characterization of nanopore-spanning planar lipid membranes necessary for future applications in integrated membrane-based sensing.

1. Reimhult, E. and K. Kumar, Trends in Biotechnology, 2008. 26(2): p. 82-89.

4:40pm **BI+AS+NS-ThA9 Formation of Protein Surface Patterns by Ligand Self-Selection *from Mixed Protein Solutions***, *M. Dubey*, University of Washington, *K. Emoto*, Great Basin Scientific, *H. Takahashi, D.W. Grainger*, University of Utah, ***D.G. Castner***, University of Washington

Time of Flight Secondary Ion Mass Spectrometry (ToF-SIMS) has been shown to be very sensitive for surface analysis and has been very useful for trace element detection. With the advent of improved analyzers, imaging ToF-SIMS provides spatial distribution of different species and helps in plotting the surface reactivity maps. Use of multivariate analysis, especially Principal Component Analysis (PCA) makes this technique even more powerful by differentiating regions with different chemistries. ToF-SIMS and PCA has been used in this work to study a very important two component patterned chemistry, which can have applications in bio-chips and cell-based biosensors. The chemistry is based on n-hydroxysuccinimide (NHS) esters; these molecules are widely used as leaving groups to activate covalent coupling of amine-containing biomolecules onto surfaces. The present work utilizes our knowledge of a previously studied model system, where NHS molecules were self-assembled on a gold substrate, and XPS and ToF-SIMS was used to characterize and understand the effect of hydrolysis and regeneration. We have demonstrated the extension of this chemistry to a commercial poly(ethylene glycol) (PEG)-based polymer films coated on glass slides. NHS and methoxy-capped regions were co-patterned onto these slides using photolithographic methods; then imaged with ToF-SIMS/PCA. NHS surface reactive zones are clearly resolved at high sensitivity despite the complexity of the matrix chemistry. Surface-specific protein coupling was observed by surface-selective reaction of streptavidin with the NHS patterns. The next step involved the preparation of photolithographic patterns of two affinity ligands (biotin and chloroalkane) for the specific immobilization of two different proteins (Streptavidin and HaloTag®). Spontaneous formation of high-fidelity surface patterns of the two proteins from their mixed solution was observed and characterized. In addition to Streptavidin and HaloTag®, ToF-SIMS detected the presence of non-specific BSA adsorption, a masking protein present in excess in the protein solutions, onto the patterned surfaces. ToF-SIMS amino acid-derived ion fragment yields summed to produce surface images can reliably determine which patterned surface regions contain bound proteins, but do not readily discriminate between different co-planar protein regions. However PCA of the ToF-SIMS data, improves discrimination of ions specific to each protein, facilitating surface pattern discrimination based on protein type. Also, ToF-SIMS imaging detected regions where residue from incompletely removed UV-exposed photoresist was present and its influence on protein adsorption.

5:00pm **BI+AS+NS-ThA10 Fabrication of Protein Patterns by Direct Electron-Beam Writing in a Protein-Repelling Template**, *N. Ballav*, Universität Heidelberg, Germany, *H. Thomas, T. Winkler, A. Terfort*, Goethe-Universität Frankfurt, Germany, ***M. Zharnikov***, Universität Heidelberg, Germany

One of the challenges of modern nanotechnology is the development of reliable, efficient, and flexible methods for the fabrication of ordered and complex patterns comprised of different proteins. An essential element of almost all available approaches is a protein-repelling "background" matrix, surrounding the active protein-adsorbing areas – the matrix prevents adsorption of proteins beyond these areas. Such a matrix is usually comprised of oligo- or poly(ethylene glycol)-based materials and is generally prepared by a backfilling procedure after the fabrication of the protein-attracting patterns. We present an alternative approach, showing that the protein-repelling matrix, both SAM- and polymer-like, can be used as a primary template for *direct* electron-beam writing of both *non-specific* and *specific* protein patterns of *any desirable shape*, including gradient ones, on a flexible length scale. The above factors make the approach quite versatile, which is additionally strengthened by intrinsic flexibility of electron-beam lithography, a wide range of suitable electron energies, broad availability of commercial oligoethylene glycol compounds, variable substrate material, and flexible choice of the target proteins. Complex gradient patterns fabricated by the suggested approach can become an important tool for mimicking natural biological interfaces which frequently possess gradient character – a typical way of encoding and displaying directional biological information.

Authors Index

Bold page numbers indicate the presenter