Biomaterial Interfaces
Room 101A - Session BI+AS+SA-TuA
Biophysics and Characterization of Biological and Biomaterial Surfaces

Moderators: Eva Chi, University of New Mexico, Axel Rosenhahn, Ruhr-University Bochum, Germany

2:20pm **BI+AS+SA-TuA1 Resolving Non-specific and Specific Adhesive Interactions of Catechols at Solid/Liquid Interfaces at the Single Molecular Scale**, *T. Utzig,* Max-Planck Institut für Eisenforschung GmbH, Germany*; P. Stock,* Max Planck Institut fur Eisenforschung GmbH, Germany*;* ***Markus Valtiner****,* Technische Universität Freiberg, Germany

The adhesive system of mussels evolved into a powerful and adaptive system with affinity to a wide range of surfaces. It is widely known that thereby 3,4-dihydroxyphenylalanine (Dopa) plays a central role. However underlying binding energies remain unknown at the single molecular scale. Here, we use single molecule force spectroscopy to estimate binding energies and binding mechanism of single catechols with a large range of opposing chemical functionalities. Our data demonstrates significant interactions of Dopa with all functionalities, yet most interactions fall within the medium-strong range of 10-20 k_BT. Specifically, Dopa-molecules interact with surfaces exposing different functionalities via different types of interactions ranging from bidentate H-bonding plus metal coordination (titania), monodentate H-bonding (SAMs exposing H-donor or H-acceptor headgroups), the hydrophobic interaction (alkyl SAM) or interactions involving the p-electron system of Dopa's catechol ring (gold). Only bi-dentate binding to TiO_2 surfaces exhibits a higher binding energy of 29 k_BT. Our data also demonstrates at the single molecule level that oxidized Dopa and amines exhibit interaction energies in the range of covalent bonds, confirming the important role of Dopa for cross-linking in the bulk mussel adhesive. We anticipate that our approach and data will further advance the understanding of biologic and technologic adhesives.

2:40pm **BI+AS+SA-TuA2 Protein-Nanoparticles Interactions: Surface Chemistry, Protein Corona and Secondary Structural Changes**, *I. Ojea, R. Capomaccio, L. Calzolai, D. Gilliland, P. Colpo,* ***Giacomo Ceccone****,* EC-JRC-IHCP, Italy*; G. Siligardi, R. Hussein,* Diamond Light Source, Oxfordshire, UK

The characterisation of protein corona formed around nanoparticles is a very important and challenging issue in the investigation of nanomaterials behaviour in biological environment and has been studied by many authors [1, 2, 3,4].

On the other hand, it is recognized that detailed physico-chemical characterization of nanomaterials is becoming increasingly important both from the technological and from health and safety point of view. Moreover, an incomplete characterisation may inhibit or delay the scientific and technological impact of nanoscience and nanotechnology [5]. In this respect, surface chemical analysis methods, such as X-ray Photoelectron Spectroscopy (XPS) and Time of Flight Secondary Ion Mass Spectrometry, can provide an important contribution to more fully characterizing nanomaterials [6].

In this work, we have investigated the interaction of human serum albumin (HSA) with gold nanoparticles (AuNPs) functionalized with thiols. In particular, 15 nm AuNPs functionalized with PEG thiols have been studied before and after interaction with HSA.

The different steps of sample preparation have been characterised by DLS, CPS and TEM, whilst the surface chemistry has been mainly assessed by XPS. Finally, the interaction between nanoparticles and HSA has been studied by Synchrotron Radiation Circular Dichroism (SRCD) to gather information on the protein structure [7]. In particular, XPS and ToF-SIMS data revealed the presence of HSA on pegylated nanoparticles, whilst the use of SRCD in combination with separation techniques allowed the determination of the structure and morphology of HSA-AuNPs complexes [8]. Moreover, SRCD experiments indicate that AuNPs increase the UV and thermal stability of HSA.

[1] Bigdeli A., et al., ACSNano, **2016**, DOI: 10.1021/acsnano.6b00261

[2] Huang R., et al. Nanoscale, **2013**, 5, 6928–6935

[3] Winzen S., et al., Nanoscale**, 2015**, 7, 2992–3001

[4] Lynch I. and Dawson K.A., NanoToday, **2008**, 3(1-2) 42-47

[5] Baer D, et al., Anal. Bioanal. Chem., **2010**, 396(3), 983–1002

[6] Grainger D and Castner D, Adv. Mater., **2008**, 20, 867–877

[7] Laera S., et al., Nanoletters, **2011**, 11, 4480–4484.

[8] Capomaccio R., et al., Nanoscale, **2015,** 7, 17653–17657.

3:00pm **BI+AS+SA-TuA3 Measuring the Impact of the Surface of Protein Stability using Single Molecule Experiments with the AFM**, ***Phil Williams****, S. Allen, A. Oyefeso, G. Milson, E. Fornari,* University of Nottingham, UK **INVITED**

Seven out of the top eight top-selling medicines of 2014 were biological in origin (so-called biopharmaceuticals or biologics). Successful formulation of such biopharmaceuticals has created new challenges to the pharmaceutical industry since the physical and chemical properties of the biological molecule (protein, peptide, RNA, DNA) differ from those of small 'classical drug' molecules. Whilst single molecule force spectroscopy has given new insight to many ligand/receptor interactions, the requirement to chemically functionalize the surfaces of both the substrate and the tip render the technique of little interest to the pharmaceutical industry since such functionalization, by definition, changes the chemistry of the ligand and receptor. Furthermore, this experimental methodology precludes effective screening of agents binding to a target receptor.

I will highlight our development of a fragment screening methodology using the AFM for single molecule force measurements without chemical modification of the ligands. I will introduce the method validating its approach using the streptavidin/biotin system that is often used as a model. I will then demonstrate the potential of the methodology to find fragments that interact with thrombin, a target for cardiovascular disease therapy.

In developing the above approach, it became apparent that actually neither the tip nor the substrate needs to be functionalized. I will conclude by discussing a promising method to screen for excipients that may stabilize protein structure in formulation and storage, where no chemical functionalization is necessary. The technique permits the measurement of the stability of proteins to be measured through their susceptibility to denaturants, such as urea and guanidinium chloride, and the effect of excipients on the measured stability to be assessed. For some proteins, the stability measured through traditional bulk methods, such as fluorescence, match those measured using the AFM, whereas for others there appears to be a significant difference. I propose, therefore, that this AFM method offers an interesting way to study protein denaturation at an interface.

4:20pm **BI+AS+SA-TuA7 In Vitro Characterization of Interfaces for the Development of Antibacterial and Biocompatible Surfaces**, ***Katharina Maniura****,* Empa, Swiss Federal Laboratories for Materials Science and Technology, Switzerland **INVITED**

Cell culture and bacterial studies of novel materials and new functional surfaces often show very poor correlation with clinical outcomes. This fact not only poses a major challenge for basic and industrial researchers, it is also associated with high costs.

Generally, the majority of biomaterials are tested using *in vitro* cell monocultures, however, this approach neglects possible synergistic interactions between different cell types and paracrine signalling mediating the tissue-specific response to a material.

Immediately upon implantation, medical implants get exposed to the patient's blood and this initiates the first phase of wound healing and subsequent cell recruitment and response deciding about material integration or non-integration.

We have established that blood pre-incubation of implant surfaces mimics a more physiological situation, providing a more predictive *in vitro* model for the evaluation of novel implant surfaces.

Similarly, many promising antimicrobial materials failed to make the translation from bench to bedside, partially due to insufficient *in vitro* biofilm models used for predicting the long-term *in vivo* antimicrobial and anti-biofilm activity. For the evaluation of novel surfaces the actual forseen implantation location and its biological environment need be considered to design a more predictive bacterial study with conditions mimicking the *in vivo* situation.

5:00pm **BI+AS+SA-TuA9 Vibrational Sum-Frequency Scattering Spectroscopy for Characterization of Biomaterial Interfaces in Biological Environments**, ***Patrik Johansson****, C. McDonald, Y.-C. Wang, P. Koelsch, D.G. Castner,* University of Washington

Most biomaterials have a 3-dimensional structure, of which the interfacial properties play an essential role in their interactions with biomolecules in the surrounding environment. The dynamics of protein adsorption onto biomaterials, and the induced conformational changes or selective

orientations following such interactions, are phenomena that to a large extent govern the biocompatibility of such materials. However, direct measurement of these interactions in biological environments are challenging as most techniques often (1) lack interfacial specificity, (2) require model samples with inherent limitations, or (3) lack specificity for the chemistry, orientation, and conformation of the probed species. In this work, we demonstrate how vibrational sum-frequency scattering (SFS) can be used to provide all this information, without the use of labels, from biomolecules specifically at the surface of biomaterials in biological environments.

We first show that SFS can yield chemical information via vibrational spectra selectively from molecules used to functionalize the surface of nanoparticles. Spectral changes upon addition of proteins to the samples do not only confirm adsorption onto the nanoparticles, but also provide information about the secondary conformation for the adsorbed proteins. It is likely that continuous development of SFS will make it an essential tool for evaluating the biocompatibility and other properties of nanoparticles for use in biomedical applications.

We have also applied SFS on protein fibers, for which a detailed understanding of the structure, function, interactions, conformation, and dynamics is critical for refining strategies in tissue engineering, as well as for the development of treatments for progressive diseases involving protein fibers, such as Alzheimer's disease (AD). In our studies, we have found that collagen fibers assembled *in vitro* exhibit a very large SFS cross-section, and that the spectral signatures are dependent on the scattering angle, implying that this parameter can be adjusted to selectively study specific features of the fibers. Data analysis routines, including maximum entropy method calculations, reveal the relative phase of various chemical groups in the fibers, which can be utilized for determining their relative orientations.

Finally, we have demonstrated that amyloid fibers and spherulites, which are structures found in the brain tissue of patients with AD, exhibit strong nonlinear optical properties. We believe that SFS can reveal new details about the development and interactions of these structures, which can provide clues about AD pathology and help finding new biomarkers for the disease.

5:20pm **BI+AS+SA-TuA10 Imaging ToF-SIMS of Human Breast Cancer Tissues: Connecting Chemical Images to Biology***, **Blake Bluestein,*** University of Washington*; F. Morrish, D. Hockenbery,* Fred Hutchinson Cancer Research Center*; L.J. Gamble,* University of Washington

Breast cancer, the most common cancer among women, is known to vary in responsiveness to chemotherapy. Therefore, the role of changes in tumor metabolism affecting the response to chemotherapy is under scrutiny. Time-of-flight secondary ion mass spectrometry (ToF-SIMS) provides a powerful approach to attain spatially-resolved molecular data from cancerous tissues. We use imaging ToF-SIMS and principal components analysis (PCA) to study human biopsy tissue samples to clarify links between fatty acid composition within and around tumors and the potential drug resistance of these tumors. An important component of this project is ToF-SIMS analysis of pre and post neoadjuvant frozen patient specimens. Since treatment occurs with the tumor in place, analysis of biopsies taken pre- and post-treatment allows characterization of molecular changes in tumors as a response to treatment. Two sets of pre and post chemotherapeutic treated tissue have been studied. Additionally, 11 triple negative (TN) pre-treatment tissues have been studied using PCA to determine if molecular differences within tumor tissues can be correlated with patient response to treatment.

Data were acquired with an IONTOF TOF.SIMS V using a Bi_3^+ analysis beam. Multiple 1mm^2 areas per tissue section were analyzed by stitching together 25 200µm^2 raster area scans. Data was acquired in both positive and negative polarities. Scores images generated by imaging PCA correlated with cellular and stromal areas were then used as masks to select regions of interest (ROI) that were reconstructed with ToF-SIMS software. Reconstructed spectral data of cellular and stromal areas was subsequently analyzed using PCA to ascertain molecular differences between tumor tissues.

Utilizing ROIs to select specific regions within analysis areas followed by spectral PCA for two different sets of pre and post treatment tumor biopsies showed a near distinctive chemical separation between pre and post. Chemical differences observed between the pre and post treatment tissue biopsies were related to changes in fatty acids, monoacylglycerols, diacylglycerols and cholesterol. Pretreatment samples showed higher loadings for vitamin E and C18:1 while post treatment samples had higher loadings for sphingomyelin and saturated fatty acids (stearic acid and palmitic acid). Spectral PCA of cellular and stromal region data from the 11 TN tissues separates patients that respond to chemotherapy and those that do not. Patients that respond to chemotherapy show higher loadings of sphingomyelin and saturated fatty acids, while nonresponding patients correlate with loadings of cholesterol, C18:1 and C18:2.

5:40pm **BI+AS+SA-TuA11 Some of These Images are Just Like the Others: Finding Similar Images in Imaging Mass Spectrometry Data Sets***, **Daniel Graham,*** *L.J. Gamble,* University of Washington

Mass spectrometry imaging (MSI) has been applied to many areas of research due to the rich chemical information it can provide. However, MSI also brings a set of challenges due to the enormous size of the data sets. Most modern imaging mass spectrometers produce data that consists of a full mass spectrum at every pixel of each image. This data set can be analyzed either as a series of spectra from a given area of the image, or as a series of images from a given set of peak masses. When looking at a series images, it is of interest to find all masses that have the same spatial distribution since this could provide information about the chemical differences seen throughout a sample, and identify fragments that originate from the same molecules or that co-localize within the analyzed area. In this presentation we demonstrate a simple, useful tool we have developed to process mass spectrometry images and identify which peaks show similar spatial patterns. For this we have created the 'Correlated Image Finder' as part of our NBtoolbox for multivariate analysis of mass spectrometry imaging data. This tool uses one of two methods to find similar images. The first method calculates the correlation coefficient between the pixels of each image and sorts the images according to a user chosen correlation cutoff. The second method uses a simple image subtraction method to find images that match within a user chosen cutoff. For either method, the images are first down binned to reduce image noise and then thresholded and scaled in order to compare all peak images on an equal scale.

The Correlated Image Finder has been tested on a wide variety of images. Examples will be shown from ToF-SIMS and MALDI imaging data. It was seen that the Correlated Image Finder is able to find images showing similar spatial distributions. The Correlated Image Finder can be used on any set of image data and examples will be shown from both 2D and 3D image data sets from tissues, cells and polymers. The results from the Correlated Image Finder can help simplify MSI data interpretation and can also help understand trends seen using other analysis methods such as principal component analysis.

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