Thursday Afternoon, November 2, 2017

Biomaterial Interfaces Division
Room: 12 - Session BI+AS-ThA

Biomolecules and Biophysics at Interfaces

Moderators: Stephanie Allen, The University of Nottingham, UK, Markus Valtiner, TU Bergakademie Freiberg

2:20pm **BI+AS-ThA1 Engineering and Imaging Excitons for Brain Imaging of Modulatory Neurotransmitters**, *M. Landry*, ***Abraham Beyene***, University of California at Berkeley **INVITED**

For over 60 years, drugs that alter, mimic, or block modulatory neurotransmitters have formed the core arsenal for the treatment of neurological disorders such as depression, addiction, schizophrenia, anxiety, and Parkinson's disease. However, methods to diagnose and validate drug efficacy have remained largely the same: questionnaires and behavioral observations. The archaic nature of neurological disorder diagnosis results from the lack of tools to detect the molecular 'key players' of neuronal communication – the three primary modulatory neurotransmitters dopamine, serotonin, and norepinephrine. In this talk, we describe the design, characterization, and implementation of near-infrared optical sensors to image neurotransmitter dopamine. We show direct visualization of endogenous dopamine release over multiple rounds of acute brain slice stimulation, for over 80 minutes. We next introduce a new form of fluorescence microscopy for deep-brain neurotransmitter imaging: double infrared excitation-emission imaging. We characterize our findings in the context of their utility for high spatial and temporal neurotransmitter imaging in the brain, describe nanosensor exciton behavior from a molecular dynamics (MD) perspective, validate nanosensor use in vitro, and for nanosensor use in vivo, to correlate external stimuli (experiences, behavior) to chemical output (neurotransmission).

3:00pm **BI+AS-ThA3 Neurotrophin-like Peptides at the Interface with Gold Nanoparticles As New Nanoplatform for CNS Disorders**, ***Cristina Satriano***, *P. Di Pietro, N. Caporarello, C.D. Anfuso, G. Lupo*, University of Catania, Italy, *A. Magrì*, National Council of Research (IBB-CNR), Italy, *D. La Mendola*, University of Pisa, Italy, *E. Rizzarelli*, University of Catania, Italy

Neurotrophins are vital proteins for neural developing and maintenance as well as promising drugs in several neurodegenerative disorders.

In the present work we propose a combined approach of peptidomimetic and nanomedicine to tackle their current limits in an effective clinical application. Specifically, neurotrophin-mimicking peptides may allow for reducing some adverse side effects shown by the whole protein [1]. Moreover, the immobilisation of these peptides on nanoparticles offers many advantages, such as the protection against degradation, an enhanced permeability of barrier membranes and, if any, intrinsic nanomaterial therapeutic properties (for example, the anti-angiogenic and plasmonic features of gold nanoparticles, AuNPs) [2].

The functionalisation of spherical AuNPs of 12 nm of diameter by peptides owing respectively to the N-terminal domains of nerve growth factor, NGF1-14, and brain derived neurotrophic factor, BDNF1-12, were scrutinised both in the direct physisorption and in the lipid bilayer-mediated adsorption processes. UV-visible and X-ray photoelectron spectroscopies, QCM-D, dynamic light scattering, zeta potential analyses and atomic force microscopy were used to investigate the hybrid nano-biointerface. Both peptide- and lipid-dependant features were identified, in order to have a modulation in the nanoparticles peptide coverage as well as in the cellular uptake of NGF and BDNF peptides, as investigated by confocal microscopy. The promising potentialities in the capability to cross the blood brain barrier (BBB) were demonstrated with Human Brain Microvascular Endothelial Cells, a cell model representative of human brain endothelium that exhibits barrier properties comparable to other BBB models.

[1] Pandini, G., Satriano, C., Pietropaolo, A., Gianì, F., Travaglia, A., La Mendola, D., Nicoletti, V.G. and Rizzarelli, E. (2016). The Inorganic Side of NGF: Copper (II) and Zinc (II) Affect the NGF Mimicking Signaling of the N-Terminus Peptides Encompassing the Recognition Domain of TrkA Receptor. *Frontiers in Neuroscience*, *10*.

[2] Di Pietro, P., Strano, G., Zuccarello, L. and Satriano, C. (2016). Gold and silver nanoparticles for applications in theranostics. *Current Topics in Medicinal Chemistry*, *16*(27), 3069-3102.

4:00pm **BI+AS-ThA6 Controlling and Probing the Orientation of Immobilized Protein G B1 on Gold Nanoparticles Using Time of Flight Secondary Ion Mass Spectrometry and X-ray Photoelectron Spectroscopy**, ***Yung-Chen Wang***, *D.G. Castner*, University of Washington, Seattle

Nanoparticles (NPs) have been widely used in many fields of science due to their unique physical properties. While many applications of NPs such as imaging probes or drug carriers often require the conjugation of proteins or biomolecules, the surface interactions between NPs and biomolecules remains underexplored. For example, the immobilization of immunoglobulin G (IgG) onto nanoparticle surfaces is critical for the development of many immunosensors and drug delivery nanocarriers. Notably, the orientation of the immobilized IgG can have significant impact on the clinical outcomes of these carriers by impacting its biostability and efficacy.

In this work, Protein G B1, a protein that can selectively bind to the Fc tail of IgG, was immobilized onto gold NPs (AuNPs) functionalized with maleimide and oligo-(ethylene glycol)(OEG) self-assembled monolayers (SAMs). Protein G B1 was immobilized onto AuNPs through specific maleimide-cysteine interaction. As the wild type Protein G B1 does not contain a cysteine, we can strategically introduce cysteine mutants on Protein G B1 to control the location of the maleimide-cysteine bonding. We used the surface sensitive analysis techniques of x-ray photoelectron spectroscopy (XPS) and time of flight-secondary ion mass spectrometry (ToF-SIMS) to characterize the surface elemental composition, coverage, and orientation of the protein G B1 immobilization process.

XPS analysis confirmed the AuNP functionalization with the maleimide SAMs. After incubation with protein containing cysteine mutant, the immobilization of the protein was demonstrated by the increased nitrogen signal on the surface of the AuNP. Wild type Protein G B1 cannot form the maleimid-cysteine bond and was effectively removed through conventional centrifugation-resuspension washes and dialysis cleaning.

ToF-SIMS analysis also confirmed the successful functionalization and protein immobilization on the AuNPs by identifying signature secondary ions of the maleimide functional group and amino acids. Utilizing the small sampling depth (~2nm) of ToF-SIMS relative to the size of Protein G B1 (~3nm), the orientation of immobilized protein G B1 was determined by comparing the ratio of secondary ion intensity originating from the opposite regions of the protein. Overall, site-specific maleimide-cysteine interaction and systematic surface characterizations enabled us to both control and probe the orientation of immobilized proteins on AuNPs. The systematic characterization of this study provided detailed information about protein-NP interactions and a platform for controlled immobilization for IgGs on NPs.

4:20pm **BI+AS-ThA7 Angiogenin Peptides and Gold Nanoparticles for Modulated Angiogenesis Processes**, *L.M. Cucci, C. Satriano, E. Rizzarelli*, University of Catania, Italy, ***Diego La Mendola***, University of Pisa, Italy

Angiogenin (Ang) is a physiological constituent of the human plasma and is a protein overexpressed in different types of tumours [1]. Gold nanoparticles (AuNPs) exhibit anti-angiogenic activity [2] and inhibit growth factor-mediated signalling *in vitro* as well as vascular endothelial growth factor (VEGF)-induced angiogenesis *in vivo* [3].

Herein, the fragment Ang60-68, including the putative cellular binding site of the protein Ang, has been synthesized and used to functionalize spherical AuNPs of 12 nm of diameter. The Ang mimicking activity of the peptide was evaluated by the staining of actin, a key target of the entire Ang, in terms of cell cytoskeleton reorganisation.

The hybrid peptide-nanoparticle assembly was obtained by physical adsorption of the peptides at the surface of AuNPs and was analysed by UV-visible spectroscopy, in order to characterise, with titration experiments, the variations of the plasmonic properties of AuNPs as well as the peptide spectral features. Another hybrid nanosystem was prepared by the immobilisation on AuNPs of the fluorescent analogous, Fam-Ang59-68, synthetized through an amidic bond which involved the N-terminal residue with the carboxyfluorescein (Fam) moiety.

The hydrodynamic size of the peptide-Au nanosystems was determined by dynamic light scattering (DLS) analysis.

Proof-of-work experiments with human neuroblastoma cells line were carried out to prove the non-toxicity of Ang-mimicking peptide functionalised gold nanoparticles. Furthermore, laser scanning confocal microscopy (LSM) images showed the localization of the peptide-nanoparticles at the cell membrane and their sub-cellular distribution. These data reveal an auspicious new platform for imaging and therapeutic activities in angiogenesis-involved diseases.

[1] D.J. Strydom, *Cellular and Molecular Life Science*, **1998**, 54:811–824.

[2] P. Mukherjee, R. Bhattacharya, P. Wang, L. Wang, S. Basu, J.A. Nagy, A. Atala, D. Mukhopadhyay, S. Soker, *Clinical Cancer Research*, **2005**,11(9), 3530-4.

[3] S. Basu, J.A. Nagy, S. Pal, E. Vasile, I.A Eckelhoefer, V.S. Bliss, E.J. Manseau, P.S. Dasgupta, H.F. Dvorak, D. Mukhopadhyay, *Nature Medicine*, **2001**, 7, 569-574.

5:00pm **BI+AS-ThA9 Exploiting Protein-Polyelectrolyte Interactions to Control and Tune Protein Immobilization at Interfaces. Applications in Biocatalysis and Separation Technology**, *C. Dupont-Gillain, A. Bratek-Skicki*, ***Aurélien vander Straeten***, UC Louvain, Belgium

INTRODUCTION: For many applications in biomedical science and biotechnology, it is challenging to control and tune the nature, amount, and activity of proteins at interfaces. Since proteins are polyampholytes, they do interact with polyelectrolytes (PE), in a way which strongly depends on the pH and ionic strength of the medium. It is usually considered that PE provide a mild environment to proteins, which may help keeping their activity unaffected by surface immobilization. Here, we explore two different approaches to take advantage of the PE-protein interactions for the controlled and tunable surface immobilization of proteins.

STRATEGY: In a first approach, mixed brushes of poly(ethylene oxide) (PEO), a protein-repellent polymer, and of a negatively- or positively-charged PE, respectively poly(acrylic acid) (PAA) and poly(2-(dimethylamine)ethylmethacrylate) (PDMAEMA), were prepared by the "grafting to" approach. These stimuli-responsive mixed brushes were used to selectively adsorb/desorb a given protein from a mixture of several proteins. In a second approach, PE-protein complexes were prepared then immobilized at interfaces within layer-by-layer (LbL) assemblies. This was in particular performed for PE-enzyme complexes, including PE-lysozyme and PE-glucose oxidase complexes. Systems including several enzymes were designed, with a view to further enable enzymatic cascades. Polymer brush formation and protein immobilization were monitored using quartz crystal microbalance, X-ray photoelectron spectroscopy and time-of-flight secondary ions mass spectrometry. Gel electrophoresis was used to determine the nature of proteins collected from the interface. PE-protein complex formation was assessed based on turbidimetry and dynamic light scattering measurements. Enzyme activity was measured based on standard assays.

RESULTS: (i) *Mixed polymer brushes-protein interactions*: From adsorption experiments with single and mixed solutions of albumin, lysozyme and fibrinogen on PAA/PEO and PDMAEMA/PEO brushes, it was demonstrated that the selective adsorption of one protein could be achieved, as well as the sequential desorption of these proteins when the three of them were adsorbed initially, by means of appropriate pH and I triggers. (ii) *PE-enzyme complexes as building blocks for LbL assembly*: PE-enzyme complexes were successfully built and characterized, then incorporated into LbL assemblies. The specific activity of lysozyme was higher when immobilized as a complex rather than in its native form.

CONCLUSION:The developed systems may find direct applications in separation technology, on the one hand, and in biocatalysis, on the other hand.

5:20pm **BI+AS-ThA10 Determination of Confined Molecular Structure by using X-ray-Surface Force Apparatus (XSFA) Study in Bio-interface Application**, ***Hsiu-Wei Cheng***, *M. Valtiner*, Technical University Freiberg, Germany, *C. Merola*, Max-Planck Institute for Iron Research, Germany, *K. Schwenzfeier*, Technical University Freiberg, Germany, *M. Mezger*, *H. Weiss*, Max-Planck Institute for Polymer Research, Germany

In biology system, understanding of molecular dynamics at confined interface such as medicine diffusion across inter-cellular channel, lubrication at joints and electric signal transmission from nerves to nerves is boosting the modern medical and biomaterial study. To study the behavior of confined molecules in detail, a home-build X-ray surface force apparatus (XSFA) which combines a synchrotron X-ray with white light interferometry is used. In our first step, an imidazolium chloride based ionic liquid, which consists of a clear water induced phase change, was used as a modeling system to test the detection limit of XSFA. The result shows that the liquid phase change from liquid to liquid crystal can be clearly distinguished within a 50 to 100 nm confinement. Meanwhile, the application of X-ray reflectivity (XRR) reveal furthermore in-plane ordering information of the liquid crystal structure. Secondly, shear force were applied to study how confined liquids react to the friction to mimic the motion of joint. We found that friction behavior and molecular dynamics are strongly related to the gap size of the confinement, which is a useful information for artificial joint design. The combination of SFA and synchrotron X-ray has shown a great analytical potential to solve the interfacial molecular dynamic, which provides scientists another powerful tool to peer the world of molecule.

6:00pm **BI+AS-ThA12 Direct Quantification of the Hydrophobic-to-Hydrophilic Transition of Interaction Forces**, ***Laila Moreno Ostertag***, *T. Utzig*, *P. Stock*, Max Planck Institute for Iron Research, Germany, *M. Valtiner*, TU Bergakademie Freiberg, Germany

When two surfaces come in close contact, several forces arise and, depending on the nature of the surfaces, these forces will show different magnitude. This principle may also be applied to diverse biological systems. Van der Waals forces have been on the radar for a century or so, and the identification of electrostatic interactions can be traced back to ancient times. It has also been clear that the behavior of such surfaces in terms of their polarity is associated to another type of force, called hydrophobic interactions.[1,2] The combination of these contributions leads to a better understanding of the interactions as the surfaces get closer together.

In this regard, we have revisited the hydrophobic interactions theory by studying the interaction forces between apposing symmetric surfaces of varying hydrophobicity via Atomic Force Microscopy and correlating them to the behavior of water at the interface. Short hydrophobic chains ending in either non-polar, hydrophobic groups or in charged heads and combinations of them were attached to smooth surfaces and tested under constant ionic force conditions. Mathematical modeling of the interactions was applied to the experimental results in order to obtain numerical parameters that are associated to the surface properties. Interesting results that are in apparent contradiction with the expected trend of the hydration parameters were found but can be explained by what we suggest is a breakdown of the water structure at the interface, which in turn can contribute to the understanding of attraction or repulsion between certain biological systems in aqueous media.

1. Hammer, M. U.; Anderson, T. H.; Chaimovich, A.; Shell, M. S.; Israelachvili, J., The search for the hydrophobic force law. *Faraday Discussions* **2010,***146* (0), 299-308.

2. Donaldson, S. H.; Røyne, A.; Kristiansen, K.; Rapp, M. V.; Das, S.; Gebbie, M. A.; Lee, D. W.; Stock, P.; Valtiner, M.; Israelachvili, J., Developing a General Interaction Potential for Hydrophobic and Hydrophilic Interactions. *Langmuir* **2015,***31* (7), 2051-2064.

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