Biomaterial Interfaces Division
Room B117-119 - Session BI+AS+PS-TuM

Biomolecules and Biophysics at Interfaces

Moderators: Christopher So, Naval Research Laboratory, **Markus Valtiner**, Vienna University of Technology, Austria

8:00am **BI+AS+PS-TuM-1 Probing Protein Structure on Nanoplastic Surface by Sum Frequency Scattering**, ***Akriti Mishra***, *T. Weidner,* Aarhus University, Denmark

The safe use of nanoparticle protein conjugates in biomedical applications like disease diagnosis, drug delivery, biosensing, etc. depends on the efficacy and stability of these conjugates in body fluids. To date, several analytical techniques like UV-Vis, dynamic light scattering, Fourier transform infrared spectroscopy, circular dichroism, nuclear magnetic resonance, etc. have been used to study the interaction of proteins on nanoparticle surface. Since most of the techniques can not differentiate between the surface bound and the free proteins in solution, it becomes impossible to gather any information about the interfacial proteins. The confirmation of a protein after adsorption on nanoparticle surface can be drastically different from that in solution, which may hamper or amend the activity and function of proteins. Surface sensitive sum frequency scattering (SFS) stands out best in this case since it selectively probes the vibrational modes of the adsorbed analytes on any interface. Sum frequency generation from flat interfaces has been successfully shown to provide rich information about the structure, order, and composition of molecules at the interface. Recently, our group has shown that SFS can effectively probe the structure and orientation of model peptides at nanoscopic oil particle surfaces.1 We will here discuss how also complex human corona proteins can be probed on particle surfaces. We focus on alpha synuclein (aS) interactions with nanoparticles relevant for medical applications and environmental nanoplastics. aS is a 14 kDa intrinsically disordered protein known to form amyloids called Lewy bodies, which can propagate across the neurons to induce Parkinson's disease (PD). Using SFS we follow how aS binds and folds on polymer nanoparticle surfaces. SFS spectra in the amide I region strongly suggest that aS folds into beta sheet and fibrillated structures at the nanointerfaces This is in contrast with flat surfaces, where monomers and helical folds dominate based on reflection SFG experiments.2 We believe, aS binding to the nanoparticles leads to close packing of aS monomers, which leads to the formation of beta sheet and fibrillar type structures.

Fig 1. Schematic of the SFS experiments to follow the binding of aS to polymer nanoparticles particles and the corresponding SFS spectrum

References:

1.) Thaddeus W. Golbeck, Kris Strunge, Adam S. Chatterly, and Tobias Weidner* J. Phys. Chem. Lett. 2022, 13, 10858-62.

2.) Kris Strunge, Tucker Burgin, Thaddeus W. Golbek, Steven J. Roeters, Jim Pfaendtner and Tobias Weidner* Umbrella-like helical structure of alpha-synuclein at the air-water interface observed with experimental and theoretical sum frequency generation spectroscopy, in preprint.

8:20am **BI+AS+PS-TuM-2 The Structure of Alpha-Synuclein at Lipid Interfaces Determined by Experimental and Theoretical Sum Frequency Generation Spectroscopy**, *K. Strunge, K. Pedersen, T. Golbek, M. Brgenhøj, D. Otzen, B. Schiøtt,* ***Tobias Weidner***, Aarhus University, Denmark

The aberrant folding of α-synuclein (αS) into amyloid aggregates is associated with Parkinson's disease. It has been shown that the refolding into oliogomers and harmful fibrils can be catalyzed by lipid-membrane surfaces. Despite the importance of lipid interactions, the 3D-structure of lipid-membrane bound αS, and thereby, the mechanism of the catalysis process, is still not known at the molecular level. Here, we report interface-specific sum-frequency generation (SFG) experiments revealing how monomeric αS binds, folds and orients at anionic lipid membranes. Since SFG is inherently surface specific and unbond proteins are not detected, the experiments can be performed at high αS concentrations, far beyond previous structural studies. To interpret the experimental SFG data and develop a high fidelity structural model of the aS binding motif, we developed an analysis method in which out-of-equilibrium molecular-dynamics (MD) simulations are linked to excitonic amide-I SFG spectra calculations. 10s of thousands of theoretical spectra calculated for frames of extensive MD simulations are evaluated pooled for their experimental fitness to determine the structure of aS binding at low, physiological and pathological aS concentrations. We find that at low and physiological αS concentrations, the protein binds in a flat geometry, while at elevated, pathological concentrations, a transition to an upright αS binding pose occurs. This upright conformation promotes lateral interactions and likely explains how protein concentrations can catalyze the formation of αS amyloids.

8:40am **BI+AS+PS-TuM-3 Lubricant Viscosity Affects the Antifouling Activity of PFPE Based SLIPS Coatings**, ***Onur Özcan***, *J. Karthäuser, R. Kopecz, A. Gelhar, A. Rosenhahn,* Ruhr-Universitat Bochum, Germany

Settlement of organisms on submerged surfaces can enhance the spread of life-threatening infections.[1] Therefore it is desired to identify methods for the prevention of biofilm formation. The omniphobic properties of slippery liquid infused porous surfaces (SLIPS) have been shown to provide outstanding protection against biofouling, icing, corrosion and to be repellent against complex liquids like blood.[2] In this study, we examine the fouling behavior of E. coli, P. fluorescence, and B. subtilis on seven different superhydrophobic perfluoropolyether (PFPE) urethane methacrylate-based SLIPS with varying lubricant viscosities. The polymers were fabricated following our previously published grafting-through protocolby which superhydrophobic micro-structured porous PFPE matrices could be obtained by adding cychlohexanol as pore forming agent to the monomer mixture.[3,4] The coatings were incubated in an excess of seven different lubricants of varying viscosities to obtain SLIPS. In dynamic attachment assays we were able to show the antifouling capabilities of these SLIPS with organism reductions of up to 90% compared to the dry, smooth, and hydrophobic butyl methacrylate references. Our results further revealed critical species-specific settlement on the coatings that depended on the viscosity of the incorporated liquid, highlighting the relevance of the choice of the lubricant in the design of low-fouling SLIPS.

[1] M.V. Horton, J. E. Nett, Curr. Clin. Microbiol. Rep. 2020, 7, 51-56. [2] T.-S. Wong, S. H. Kang, S. K. Y. Tang, E. J. Smythe, B. D. Hatton, A. Grinthal, J. Aizenberg, Nature 2011, 477, 443-447. [3] F. Koschitzki, R. Wanka, L. Sobota, J. Koc, H. Gardner, K. Z. Hunsucker, G. W. Swain, A. Rosenhahn, ACS Appl. Mater. Interfaces. 2020, 12, 34148-34160. [4] N. Keller, J. Bruchmann, T. Sollich, C. Richter, R. Thelen, F. Kotz, T. Schwartz, D. Helmer, B. E. Rapp, ACS Appl. Mater. Interfaces, 2019, 11, 4480-4487.

9:00am **BI+AS+PS-TuM-4 Orientation of the Dysferlin C2A Domain is Responsive to the Composition of Lipid Membranes**, *A. Carpenter,* Oregon State University*; S. Roeters, T. Weidner,* Aarhus University, Denmark*;* ***Joe Baio***, Oregon State University

Dysferlin is a 230 kD protein that plays a critical function in the active resealing of micron-sized injuries to the muscle sarcolemma by recruiting vesicles to patch the injured site via vesicle fusion. Muscular dystrophy is observed in humans when mutations disrupt this repair process or dysferlin is absent. While lipid binding by dysferlin's C2A domain (dysC2A) is considered fundamental to the membrane resealing process, the molecular mechanism of this interaction is not fully understood. By applying nonlinear surface-specific vibrational spectroscopy, we have successfully demonstrated that dysferlin's N-terminal C2A domain (dysC2A) alters its binding orientation in response to a membrane's lipid composition. These experiments reveal that dysC2A utilizes a generic electrostatic binding interaction to bind to most anionic lipid surfaces, inserting its calcium binding loops into the lipid surface while orienting its β-sheets 30–40° from surface normal. However, at lipid surfaces, where PI(4,5)P2 is present, dysC2A tilts its β-sheets more than 60° from surface normal to expose a polybasic face, while it binds to the PI(4,5)P2 surface. Both lipid binding mechanisms are shown to occur alongside dysC2A-induced lipid clustering. These different binding mechanisms suggest that dysC2A could provide a molecular cue to the larger dysferlin protein as to signal whether it is bound to the sarcolemma or another lipid surface.

9:20am **BI+AS+PS-TuM-5 Probing the Interfacial Action of *Thermomyces Lanuginosus* Lipase at Lipid Surfaces with Vibrational Sum Frequency Spectroscopy – from Monolayers to Emulsions**, ***Khezar Saeed***, *K. Strunge, T. Golbek, T. Weidner,* Aarhus University, Denmark

Lipases are a diverse class of biologically important enzymes with a key role in the digestion of dietary fats. The general ability to catalyse triacyl glyceride hydrolysis also enables their application to a wide variety of systems outside of the digestive tract, including transesterification, enantioselective synthesis and as an additive to laundry detergents. Key to their efficacy is the phenomenon of interfacial activation. For lipases this almost universally involves the "opening" of a lid domain upon interaction with a lipid surface, revealing a hydrophobic region containing the active site. The lipase derived from the *Thermomyces lanuginosus* fungus (TLL) is

used extensively on an industrial scale as an additive to laundry detergents. As such significant effort has been expended to genetically engineer improvements to the lipase function, with particular attention paid to this lid region. Gaining a deeper understanding of the interfacial activation mechanisms of such lipases could inform the design of improved enzymes in the future.

The inherent surface sensitivity of vibrational sum frequency generation (VSFG) spectroscopy can provide the required molecular level information to further our understanding of the interfacial activation of TLL. VSFG spectroscopy relies on the selection rules associated with frequency mixing of high power visible and infrared laser beams, resulting in a vibrational spectrum of solely the interfacial region. Three key results are presented here:

(i)The TLL-catalysed reaction at the air/triglyceride/water interface can be monitored by reflection VSFG spectroscopy, showing loss of ester carbonyl modes and appearance of carboxylate stretching modes of the fatty acid products.

(ii)Comparison of experimental and predicted VSFG spectra of the amide I band are used to interpret structural changes in the lid domain of TLL upon interaction with a hydrophobic surface.

(iii)Specially formulated emulsions allow further analysis using our new angle-resolved sum frequency scattering spectrometer, showing the first example of reaction dynamics at a particle surface probed by vibrational sum frequency scattering spectroscopy.

This work highlights the utility of VSFG spectroscopy for studying interfacial reactions. Not only does it offer a label-free method of following surface reactions, but it also provides structural and orientational information on interfacial species when combined with appropriate simulations. Furthermore, the results from the sum frequency scattering spectrometer open the door to studying a whole new class of chemical systems at particle surfaces with as yet unseen levels of molecular detail for such systems.

11:00am **BI+AS+PS-TuM-10 An *in Situ* Look at Interfacial Controls on Nucleation, Self-Assembly, and Crystal Growth in Biomolecular and Biomimetic Systems***, Jim De Yoreo,* Pacific Northwest National Laboratory **INVITED**

From harvesting solar energy to capturing CO_2 to purifying water, living organisms have solved some of the most vexing challenges now faced by humanity. They have done so by creating a vast library of proteins and other macromolecules that can assemble into complex architectures and direct the mineralization of inorganic components to produce materials characterized by a hierarchy of structure. While the high information content contained within the intricate sequences of the proteins is crucial for accomplishing these tasks, self-assembly and mineralization are nonetheless constrained to proceed according to the physical laws that govern all such processes, even in synthetic systems. An understanding of the mechanisms by which biological systems successfully manipulate those laws to create hierarchical materials would usher in an era of materials design to address our most pressing technological challenges. In this talk, I will present the results of recent research using in situ atomic force microscopy and in situ transmission electron microscopy to directly observe interfacial structure, protein self-assembly, and nanocrystal formation in biomolecular and biomimetic systems, including protein-directed nucleation of calcium carbonate and calcium phosphate and mineral-directed nucleation of two-dimensional protein assemblies. The results elucidate the mechanisms by which the interface between biomolecules and materials directs nucleation, self-assembly and crystal growth, leading to unique materials and morphologies. The results reveal the importance of surface charge, facet-specific binding, solvent organization near interfaces, and, more generally, the balance of protein-substrate-solvent interactions in determining how ordered materials emerge in these systems.

11:40am **BI+AS+PS-TuM-12 the Surface Chemistry of Gecko Toe Pads***, **Mette Heidemann Rasmussen**, K. Holler,* Department of Chemistry, Aarhus University, Denmark*; J. Baio,* School of Chemical, Biological and Environmental Engineering, Oregon State University*; C. Jaye, D. Fischer,* National Institute of Standards and Technology, Gaithersburg*; S. Gorb,* Functional Morphology and Biomechanics, Zoological Institute, Kiel University, Germany*; T. Weidner,* Department of Chemistry, Aarhus University, Denmark

Geckos can climb nearly all surface and are able to cling to walls and ceilings using their toe pads. The gecko adhesion mechanism has been debated over the past years. Current models include van der Walls, hydrophobic and acid-base interactions. Even though the adhesion mechanism of the spatulas has been studied in detail, the surface chemistry involved in the gecko adhesion mechanism is unclear. What is the structure of the supporting proteins within the spatula at the very tips of the setae within the gecko toe pad? What is the role of lipids in the adhesion process? Understanding the surface chemistry of the adhesion of the gecko toe pads gives insight into this highly specialized biological interface, and give clues for materials scientists aiming at mimicking the gecko adhesion mechanisms. Using near edge X-ray absorption fine structure (NEXAFS) imaging and spectroscopy we have studied the structure and order of the molecules at the outermost surface layer of gecko toe pads. We show that the keratin molecules within the spatulas are highly organized and adopt a flat, strand-like geometry, which may support the stability and adaptability of gecko setae (1). We will also discuss evidence showing that a nanometer-thin ordered lipid layer is covering the beta proteins (2).

1. Structure of Keratins in Adhesive Gecko Setae Determined by Near-Edge X-ray Absorption Fine Structure Spectromicroscopy. J Phys Chem Lett. 2022 Mar 10;13(9):2193–6.

1. Evidence that gecko setae are coated with an ordered nanometre-thin lipid film. Biology Letters. 18(7):20220093.

12:00pm **BI+AS+PS-TuM-13 All-Atom Simulations of Peptide Aggregation: Understanding and Predicting Biopolymeric Morphologies***, A. Kwansa, A. Cannon,* North Carolina State University*; **Yaroslava Yingling**,* 911 Partners Way, Engineering Building I, Campus Box 7907

The self-assembly and aggregation of partly or completely disordered peptides have emerged as crucial areas of research with broad implications in therapeutics, supramolecular assembly, and functional biomaterials. Understanding the intricate processes underlying the self-assembly and aggregation of these proteins is essential for harnessing their functional properties and expanding their applications. Simulations can be used to isolate the importance of the interplay between aggregate morphology and secondary structure formation. However, most of the simulation's studies investigate either single peptide in solution or several short peptide analogues. We used large-scale all-atom MD simulations to investigate the structure of hydrated peptide aggregates in detail. Two example systems were investigated, reflectin and elastin-like peptides (ELP). Reflectin proteins, found in cephalopods, play a pivotal role in dynamic coloration for camouflage and communication. On the other hand, ELP proteins possess unique thermoresponsive properties, making them attractive for drug delivery systems, tissue engineering, and biomaterial design. We found significant differences between the structure of a single polypeptide in water and the structure of peptide within the aggregate. Overall, the aggregation process is driven by the formation of peptide–peptide interactions whereas the average hydration of peptides remains almost the same between dissolved and aggregated states. Even though the aggregation is driven by hydrophobic interactions, aggregate has no hydrophobic core and contains many water molecules. Overall, our findings provide an insight into the sequence-dependent structure of aggregates and molecular behavior of individual peptides during aggregation.

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