# Monday Morning, October 30, 2017

## Biomaterial Interfaces Division
## Room: 12 - Session BI-MoM

### Engineering a Paradigm Shift in Control of Microbes and Fouling
**Moderators:** Joe Baio, Oregon State University, Daniel Barlow, US Naval Research Laboratory

8:20am **BI-MoM1 Characterization of Adult Barnacle Adhesion Upon Reattachment to Hydrophobic Surfaces**, ***Manuel Figueroa***, *G. Dickinson*, The College of New Jersey

Although a wide range of environmentally friendly surface coatings can reduce biofouling on marine structures, there is still not a fundamental understanding of barnacle adhesion upon reattachment. This study assessed the effect of hydrophobicity on adhesion in the barnacle *Amphibalanus amphitrite*, an abundant and widespread biofouler. Self-assembled monolayers were made on glass slides from alkyl silanes with methylated and fluorinated terminal groups to produce hydrophobic surfaces. Coated and uncoated glass slides underwent a 2-week barnacle reattachment assay. Barnacles were removed using a force gauge and critical shear stress was calculated for each substrate. Following reattachment assays, a Coomassie Blue G250 protein stain was used to quantify the amount of glue remaining on substrates by measuring pixel density with ImageJ software on glue scans.

Critical shear stress was found to be significantly higher for both hydrophobic surfaces as compared to the hydrophilic uncoated glass, and correspondingly the density of residual glue was higher on hydrophobic surfaces. Given that hydrophobic substrates can exclude water from the surface, they may provide a protected environment for glue release that is favorable for adhesive bond formation with the substrate as well as inter and intramolecular bonding within the glue layer. Critical shear stress showed a strong positive correlation with residual glue density, suggesting barnacle release occurs primarily via cohesive failure. Scanning electron microscope micrographs depicted a diverse mixture of features in the glue remnants depending on the coating and its location under the base plate. These features, which included a sponge-like matrix, globular structures, viscous fingering and nanoscale fibers contribute to adhesion strength. The design of marine coatings must continue to consider the nanoscale topography as an essential attribute to reducing biofouling as well as the ability of a coating to exclude water from the surface.

8:40am **BI-MoM2 Constructing and Deconstructing the Barnacle Adhesive Interface**, *C.R. So, K.P. Fears,* US Naval Research Laboratory, *H. Ryou,* ASEE Research Fellow at US Naval Research Laboratory, *D.E. Barlow, D.H. Leary, J.A. Wollmershauser, C.M. Spillmann,* ***Kathryn Wahl***, US Naval Research Laboratory

Barnacles are sessile marine arthropods that live and reproduce on nearly any available surface in the ocean. They adhere via a thin adhesive layer developed through a multistep secretory process synchronized with growth via molting. Unlike other arthropods, the combination of expansion, molting and protein secretion within the narrow adhesion interface leads to a nanofibrillar protein layer manipulated by shear stresses, protected by calcite, and containing a cocktail of chemically active molecules and proteins. Here we use *in vivo* imaging, mechanics, and spectroscopy of barnacle growth and development, coupled with mass spectrometry and proteomics to reveal much about the biophysics and biochemistry of barnacle adhesion. We will discuss the role of interfacial processes, self-assembly, amino acid composition, and chemical manipulation in the construction and function of the adhesive.

9:00am **BI-MoM3 Live Confocal Microscopy of *Balanus Amphitrite* Reveals Anti-Fouling Strategy of a Marine Fouler**, ***Kenan Fears***, US Naval Research Laboratory, *B. Orihuela, D. Rittschof,* Duke University Marine Laboratory, *K.J. Wahl,* US Naval Research Laboratory

The adhesion of hard foulers (e.g., barnacles and tubeworms) has plagued the maritime community for as long as mankind has been setting sail. Since the biological processes responsible for adhesion occur at buried interfaces, elucidating the mechanisms by which foulers adhere is challenging. Through the use of multiple fluorescent probes, peptides, and antibodies, we have been able to discern an unprecedented level of detail about biological processes that occur at the interface between acorn barnacles (Balanus Amphitrite) and the underlying substratum during the barnacle growth cycle. Barnacles secrete a lipidaceous substance around the outside of their shell, prior to expansion that dislodges microorganisms and biofilms to present a cleaned surface. During molting, epithelia cells build a new interfacial cuticular layer, which becomes autofluorescent as it is sclerotized, above the existing cuticle whose degradation coincides with the exuviation of the main body's cuticle. Rather than being directly secreted onto the substrate, nanostructured barnacle cement accumulates in the space in between the new and old cuticle. As the barnacle expands, the cuticular layers are stretched and pulled around the outside of the side plate. The strain causes the old cuticle to randomly tear, allowing the new cuticle to deposit cement into the interface as it is dragged across the substrate. Furthermore, antibody staining allowed us to spatially and temporally identify where different cement proteins are presenet. These results illustrate that the methodologies we have developed to break down and analyze barnacle cement collection are yielding a more accurate representation of the proteins at the buried interface, and providing insight on their roles which will lead to improved strategies to both combat and mimic barnacle adhesives.

9:20am **BI-MoM4 Considering the Consequences of a Paradigm Shift in Biofouling Management**, ***Daniel Rittschof***, *B. Orihuela*, Duke University, *K. Efimenko, J. Genzer*, NC State University

Present Fouling Management Strategies that use long-lived, broad-spectrum biocides are not sustainable because they alter ecosystem services and threaten food security. As globalization continues, human populations increase and wild fisheries collapse there will be increasing pressure and genuine need for less environmentally damaging approaches. A question that should be asked up front for any new fouling management technology is what are the environmental, food security and human health consequences if a technology gains market share. Information on impacts of industrial grade components, acute and chronic toxicity, breakdown and non-toxic biological effects such as teratogenicity, carcinogenicity and environmental steroid activity should be evaluated. This presentation looks at a few of the details of basic silicone coatings which have had their components purified and then tested for acute toxicity, impacts on a hydrolytic enzyme and teratogenicity. Some components like catalysts and small cyclics are extremely toxic. Other components impact enzyme activity, some inhibit activity other potentiate activity. Terratogenicity assays are so sensitive that even effects of medical grade silicones can be demonstrated. This information needs to be taken as preliminary factual information that can be used by engineers in developing risk benefit analysis.

9:40am **BI-MoM5 Microbiological Fouling on Aircraft: Understanding the Mechanisms of Polyurethane Topcoat Deterioration by Fungal Isolates**, ***Daniel Barlow***, *J.C. Biffinger,* US Naval Research Laboratory, *C.-S. Hung*, Air Force Research Laboratory, *L.J. Nadeau,* Air Force Institute of Technology, *A.L. Crouch, T. Zicht,* Air Force Research Laboratory, *J.N. Russell, Jr.,* US Naval Research Laboratory, *W.J. Crookes-Goodson*, Air Force Research Laboratory

Fungal and bacterial fouling on military aircraft is a problem that can lead to polyurethane top coat deterioration and pose health hazards to personnel; the phasing out of hexavalent chromium in coatings is expected to worsen fouling problems. Thus, better understanding of the relevant microbiological interactions with polyurethanes is required to identify new ways to inhibit fouling and associated affects. We have screened over 400 aircraft isolates for polyurethane degradation, with Cryptococcus strains among the most aggressive polyurethane degraders. These strains were further characterized for their capability to metabolize and grow on expected hydrolysis products from polyester components of the polymers, showing that fungal growth occurs to varying degrees on the metabolites. Gas chromatography also showed that microbes metabolize polymers and hydrolysis products to $CO_2$. Polymer metabolization to $CO_2$ results in bulk polymer loss and optical profilometry confirmed that fungal cells steadily “eat” trenches into solid polyurethane films over time. Initial polyurethane film degradation processes at the micro and nano scales were analyzed by confocal Raman and AFM-IR (combined AFM and infrared spectroscopy). These results showed varying, non-uniform degradation events among cells, indicating that variations in single cell physiology play roles in early stage degradation. The spectroscopic results are consistent with lipase activity as the primary driver of degradation.

10:00am **BI-MoM6 Dynamic Accumulation Assays under Laminar Flow Conditions to Probe Attachment of Marine Biofilm Formers**, ***Kim Alexander Nolte***, *J. Schwarze, A. Rosenhahn*, Ruhr-University Bochum, Germany

Novel materials with environmentally benign fouling-release properties have been developed during the last years to substitute toxic coatings. Assessment of fouling-release coating's efficiency is of key relevance for the down selection of chemistries. Several techniques are accessible that quantify, how easy fouling organisms can be removed, including calibrated, turbulent flow channels, push-off tests, water jets, and microfluidic devices [1, 2]. We developed a laboratory assay based on a parallel plate flow chamber that allows testing of coating candidates against algal cell adhesion with precisely controlled flow rates and cell concentrations. Using self-assembled monolayers as model surfaces and diatoms as model organisms we were able to show that the adhesion strength [1] correlates with the accumulation dynamics if an appropriate wall shear stress is applied. Similar to the critical wall shear stress for removal assays, a range of wall shear stresses was identified within which the discrimination potential was maximized [3]. The setup has been parallelized to increase throughput and to become able to test a large number of coating chemistries per day. Due to the modular assembly of our setups, not only model surfaces and thin organic films, but also practical coatings can be tested.

[1] M. Alles, A. Rosenhahn, *Biofouling.* **2015,** 31, 469–480.

[2] MP. Schultz, *et al*., *Biofouling.* **2000,** 15, 243-251.

[3] K. Nolte, J. Schwarze, A. Rosenhahn *Biofouling.* **2017,** in press

10:40am **BI-MoM8 Coatings with Amphiphilic Surfaces Via Self-Stratification for Marine Fouling-Release Applications**, ***Dean Webster***, *T. Galhenage, S. Stafslien, L. Vanderwal*, North Dakota State University

Due to the complexity of adhesion mechanisms of marine life to surfaces, it is becoming apparent that combating biofouling will require coatings having complex surfaces. Specifically, coatings having mixed hydrophobic and hydrophilic surface domains are being shown to be able to mitigate the adhesion of a broader variety of marine organisms than can the silicone elastomer fouling-release coatings. Since it is also desirable to have a coating that can adhere well to a variety of substrates and stand up to occasional cleaning, a tough coating system is needed.

The incorporation of a low surface energy polymer such as a siloxane into a robust coating system such as a polyurethane results in stratification of the low surface energy component to the surface. By chemically binding hydrophilic groups to the siloxane, both functional groups stratify leading to a polyurethane coating having amphiphilic character on the surface. By varying the molecular parameters of the hydrophobic and hydrophilic components, the surface composition can be tuned to achieve a range of fouling-release properties when characterized using a broad variety of marine organisms. Different architectures of the reactive amphiphilic component have been explored including block and graft copolymers as well as dual-functionalized prepolymers.

11:00am **BI-MoM9 Zero-Energy Flux Recovery in Biofouled Liquid Gated Membranes**, *J.C. Overton*, ***Caitlin Howell***, University of Maine

Membranes coated with antifouling immobilized liquid layers have been recently shown to permit filtration while reducing surface fouling. In this work, we test the ability of liquid gated membranes created with expanded polytetrafluoroethylene and perfluorinated liquids to reduce the buildup of internal pore fouling using whey protein, an extremely challenging biological foulant. We find no differences in the decrease of flux or rate of fouling between coated and uncoated membranes in a dead-end filtration setup. However, upon stopping flow for 15-30 minutes, up to 70% of the original flux can be recovered with no additional energy input. This cycle can be repeated multiple times, with approximately 5% decrease in flux recovery each time. We use standard fouling equations and light microscopy to demonstrate that this zero-energy recovery may occur within the pores of the membrane due to the refilling of the pore with lubricating liquid, pushing the proteins off the pore walls. This work could have important applications in filtration processes with high fouling rates, reducing costs associated with standard chemical or physical cleaning methods.

11:20am **BI-MoM10 Stimuli Responsive Polymers in Biofouling and Bioadhesion**, ***Gabriel Lopez***, University of New Mexico **INVITED**

This talk will review work by the Lopez lab and its collaborators on the role of stimuli-responsive polymers in processes associated with biopolymer adsorption, biofouling and bioadhesion. Wikipedia currently defines stimuli-responsive (or “smart”) polymers as those that change their shape or properties “according to the environment they are in.” From this perspective, almost any polymer in solution that adsorbs to an interface placed in its proximity can be thought of as a stimuli-responsive polymer. In the present context, a less trivial distinction includes polymeric systems that are sensitive to external fields (e.g., thermal, stress, optical, electric) in a way as to dramatically affect tendency for adsorption or adhesion. Such polymeric systems include synthetic polymers comprising engineered interfaces with biologically relevant aqueous phases (brushes, solution and vacuum deposited films, gels) and biopolymers (proteins) in aqueous phases. These systems and their ability to dramatically influence adsorption, attachment and adhesion are of potential use in a wide range of biotechnological, biomedical, aquatic, marine and food production applications including processes such as separations, assays, controlled delivery, cell culture, packaging, energy transfer and transportation.

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