FY2014 Annual Project Summary

Proposal Number: 6552

Title: Meso-scale Liquid Confinement Systems for Enhanced Bioseparations and Bioconversion Strategies
PI: Volker Urban
Co-PIs: Douglas Hayes, Hugh O'Neill, Sai Venkatesh Pingali, Paul Langan

Project Description
The project builds new capabilities and captures world leadership for neutron scattering analysis of fluid meso-structures that take inspiration from the functionality of biological organelles. We will advance understanding of these complex architectures by studying related man-made systems: bicontinuous microemulsions (BMEs). BMEs are less complex and not as highly-organized as organelles, but they share important characteristics, such as high interface area, compartmentalization and continuous channels for fluid transport. As such, BMEs have potential in many technological areas such as drug production and catalysis. Small-angle neutron scattering (SANS) has a unique ability to probe these structures *in situ*, and in response to changes in relevant parameters, for example solute concentrations. This project will develop and supply experimental capabilities that allow refined studies of BMEs that are not currently available anywhere; it further focuses on carefully selected questions that will demonstrate advancement of our understanding of BMEs and by extension fluid membrane systems in general. Finally, our selection of membrane proteins incorporated into BMEs will focus on functionality, thus emulating nature's approach of meso-scale "factories". The project will deliver new neutron scattering capabilities, publications in high impact journals, data for future proposals, and interdisciplinary postdoctoral training.

Mission Relevance
This proposal aligns neutron scattering capabilities at SNS and HFIR with bioenergy missions at BESC and other bioenergy research centers such as the Photosynthetic Antenna Research Center (EFRC). The work will position ORNL to effectively compete in any growth opportunities in this area led by DOE. The new capabilities generated in this proposal will provide opportunities for neutron scattering research that are not available elsewhere and will therefore contribute to sustaining the international leadership of the ORNL neutron scattering facilities. Studies of integral membrane protein structure remain a grand challenge and have a high potential for attracting funding from NIH. Bicontinous systems may prove to be efficient bio-reactors for enzymatic production of drugs, with high interest to NIH. Future collaborations between ORNL and UTK researchers will train students in neutron scattering techniques and contribute to the sustained growth of the neutron user community at ORNL.

Results and Accomplishments
Scientific/Technical: We have realized a 4-port flow-cell sample environment for SANS studies of multiphase microemulsion systems. The system includes a quartz flow cell, manufactured by a commercial leader of scattering cuvettes (Hellma), and two commercial Hamilton syringe pumps that are used to inject the oil and aqueous solvents into top and bottom sections of the cell in a well-controlled manner. We have added HERE student Ms. Rachel Dunlap in January 2014 to the LDRD team to support studies of membrane-proteins in BME. We have successfully competed for neutron beam time and have carried out neutron scattering experiments on the CG-3 Bio-SANS instrument. The results suggest that membrane proteins can influence synthetic surfactants in a BME to adopt architectures that are commensurate with the lipid bilayer thickness that normally accommodates such proteins in their natural biological environment. In addition, we have conducted circular dichroism and fluorescence studies that demonstrate that membrane proteins, in particular melittin, behave in microemulsions in a way that is similar to their interaction with natural lipid bilayer membranes; i.e. melittin folds into alpha-helical

secondary structure and inserts into the lipid-like non-polar environment that is provided by the microemulsion. *Program Development:* The bicontinuous microemulsion approach as a potential platform for studying photosynthetic antenna structure and function in membrane systems was included in the renewal proposal for the Photosynthetic Antenna Research Center (PARC); a DOE-BES funded Energy Frontier Research Center (EFRC), led by Robert Blankenship at Washington University in St. Louis. PARC has been successfully renewed for 4 more years. Results of our work were communicated in several presentations at scientific conferences.

Information Shared

Douglas G. Hayes, Ran Ye, Volker S. Urban, Sai V. Pingali, Hugh O'Neill, "Incorporation of Membrane Proteins in the Bicontinuous Microemulsion Phase of a Winsor-III System," invited oral presentation, American Oil Chemists' Society Annual Meeting, San Antonio, TX, 4-7 May 2014.

Volker Urban, Douglas Hayes, Ran Ye, Sai Venkatesh Pingali, Hugh O'Neill, Rachel Dunlap, "Microemulsions as a New Platform for Studying Membrane Proteins by SAS," oral presentation at the Annual Meeting of the American Crystallographic Associaiton (ACA), Albuquerque, NM, May 2014.

Sai Venkatesh Pingali, Volker S Urban, Hugh M O'Neill, Rachel N Dunlap, Paul Langan, Douglas Gordon Hayes, Ran Ye "Vertical Scan of the Middle, Bicontinuous Microemulsion Phase via SANS Through Use of a New Vertical Stage Sample Environment," poster presented at the American Conference of Neutron Scattering, Knoxville, TN, June 2014.

Douglas Gordon Hayes, Ran Ye, Volker S Urban, Sai Venkatesh Pingali, Hugh Michael O'Neill, Rachel N Dunlap, Paul Langan "SANS Analysis of Proteins Encapsulated into Bicontinuous Microemulsions Phase of Winsor-III Systems," oral presentation at the American Conference of Neutron Scattering, Knoxville, TN, June 2014.