experiments with [U-15N,2H,13C]-BpFPK. The assignments allowed for the mapping of peaks representing isoleucine residues onto the crystal structure. This analysis has allowed specific regions of the enzyme involved in the binding of allosteric ligands and the propagation of the allosteric effect to be identified. Funding: NIH-GM32126, NIH-CBI, Welch-A1543.

148-Plat Microsecond-Resolution Recording of T4 Lysozyme Observes a Brownian Ratchet
Maxim V. Akhterov1, Yongki Choi2, Tivoli J. Olsen3, Patrick C. Sims1, Mariam Ifitkhar1, O. Tolga Gul1, Brad L. Corso1, Gregory A. Weiss3, Philip G. Collins1.
1Department of Physics and Astronomy, University of California, Irvine, Irvine, CA, USA, 2Department of Physics, North Dakota State University, Fargo, ND, USA, 3Department of Chemistry, University of California, Irvine, Irvine, CA, USA.

Single-molecule techniques can monitor the kinetics of transitions between enzyme open and closed conformations, but such methods usually lack the resolution to directly observe the underlying transition pathway or any intermediates. We have recently described a single-molecule electronic technique that breaks this barrier (1-3). Using a 1 MHz-bandwidth carbon nanotube transistor, the transition dynamics of T4 lysozyme have been recorded with microsecond resolution. We directly resolve a smooth, continuous transition with an average duration of 37 microseconds that suggests a concerted mechanism for glycoside hydrolysis. Unexpectedly, the mechanical closing and re-opening of the enzyme have identical distributions of transition durations, and the motions do not depend on whether the enzyme is in its catalytic or non-productive state. These results illustrate the principle of microscopic reversibility applied to a Brownian ratchet, with lysozyme tracing a single path for closing and the reverse pathway for enzyme opening, regardless of its instantaneous catalytic productivity.


149-Plat Human Neuraminidase Enzymes alter the Lateral Mobility and Function of Integral Receptors
Christopher W. Cairo.
Chemistry, University of Alberta, Edmonton, AB, Canada.

Glycolipids and glycoproteins are important components of membrane structure. Mechanisms which alter the structure of glycans at the membrane could influence cellular responses. For example, by removing an important binding epitope or else by unmasking a new one, protein-glycan interactions may be disrupted or reformed. Our group has been working to understand the role of human neuraminidase enzymes (hNEU) in regulating cellular adhesion and migration through integral receptors. Of the four hNEU isoforms, three have activity at the plasma membrane and lysosome; thus, these enzymes could regulate the composition of the plasma membrane by stripping neuraminic acid (Neu5Ac; also known as sialic acid) from membrane glycans. Using recombinant enzymes and selective hNEU inhibitors developed within our group, we can selectively probe increased or decreased activity of individual isoforms in vitro; allowing us to test the effect of specific enzymes. Previous studies have suggested that integrin-mediated adhesion may be altered through hNEU activity. We measured the lateral mobility of integrins in cells treated with NEU3 and NEU4 using single-fyne tracking (SDT) by total-internal-reflection fluorescence microscopy (TIRF). We find that hNEU can dramatically change the diffusion of integrin receptors, and that the effect is dependent on the cell type and the isoenzyme used. Adhesion and cell migration assays of cells treated with chemical inhibitors of the enzymes reveal that hNEU activity is intimately involved in the regulation of integrin adhesion to their native ligands. We will present lateral mobility and cell migration assays with enzyme and inhibitor conditions. Our results suggest an important role for hNEU as regulators of membrane composition and the activity of adhesion receptors.

Platform: Membrane Dynamics

150-Plat Nothing to Sneeze at: A Full-Scale Computational Model of the Human Influenza Virion
Tyler Reddy1, David Shorthouse1, Daniel Parton2, Elizabeth Jefferys2, Patrick O’Neill1, David Shorthouse1, Elizabeth Jefferys2, Philip W. Fowler1, Matthieu Chavent1, Marc Baaden1, Mark S.P. Sansom1.
1SBCB Unit, Biochemistry, University of Oxford, Oxford, United Kingdom, 2Memorial Sloan Kettering Cancer Center, New York, NY, USA, 3Institut de Biologie Physico-Chimique, Paris, France.

Tackling the ongoing challenge of influenza infectivity would benefit greatly from a molecular understanding of why the influenza A virion exhibits seasonal patterns of infectivity and has wide-ranging survival times in different environments. A computational approach to the study of the behaviour of the virion that focuses on the poorly-understood structural and dynamic role of the lipids is presented here. We have combined experimental data from X-ray crystallography, NMR spectroscopy, cryoelectron microscopy, and lipidomics to build a full-scale computational model of the influenza A virion. This represents the first set of microsecond-scale molecular dynamics simulations of an enveloped virion in explicit solvent that we are aware of. We report results for a set of simulations at different temperatures and with varying lipid compositions. The Forssman glycolipid, which is prevalent in the influenza A lipoprotein, influences several biophysical characteristics of the virion model, including diffusion and clustering of proteins and lipids. The distribution of peplomers on the virion surface is consistent with accessibility to bivalent antibodies. The distances of binding sites for host cell sialic acid-containing receptors have been analyzed in the virion model for a variety of planar host cell membrane attack orientations. The relatively rigid membrane of the influenza A virion model exhibits a number of biophysical properties consistent with experimental measurements, and may serve as a useful platform for in silico assessment of virion behaviour.

151-Plat Role Of Membrane-Bending Proteins as Membrane Tension Sensors in Cell Migration
Toshiki Itoh.
 Biosignal Research Center, Kobe University, Kobe, Japan.

The plasma membrane (PM) tension has emerged as a key regulator for fundamental cellular functions. However, a missing link between PM tension and biochemical reaction precludes our understanding of how PM tension is coupled to cellular events like directed migration. We found that FBP17, an F-BAR domain protein, acts as a membrane tension sensor that organizes cell polarity during cell migration. The mechanism is based on membrane-bending activity of the F-BAR domain that is counteracted by PM tension. Because FBP17 binds and activates WASP/N-WASP to promote actin polymerization, it corresponds to the local activator of actin polymerization in the feedback loop regulated by PM tension for self-organized formation of the leading edge. These findings provide an important mechanistic insight into cell migration underpinning a wide variety of physiological events such as development, immune response, and cancer metastasis.

152-Plat Experiment and Simulation Reveal the Bending Properties of Nanoscopic Lipid Domains
Jonathan D. Nickels1, Michael Ohi1, Xiaolin Cheng1, Christopher Stanley1, Frederick Heberle1, Robert Standaert2, John Katsaras1, 1Oak Ridge National Lab/Univ. of Tennessee, Knoxville, Oak Ridge, TN, USA, 2Oak Ridge National Lab/Univ. of Tennessee, Knoxville, Oak Ridge, TN, USA.

The plasma membrane (PM) tension has emerged as a key regulator for fundamental cellular functions. However, a missing link between PM tension and biochemical reaction precludes our understanding of how PM tension is coupled to cellular events like directed migration. We found that FBP17, an F-BAR domain protein, acts as a membrane tension sensor that organizes cell polarity during cell migration. The mechanism is based on membrane-bending activity of the F-BAR domain that is counteracted by PM tension. Because FBP17 binds and activates WASP/N-WASP to promote actin polymerization, it corresponds to the local activator of actin polymerization in the feedback loop regulated by PM tension for self-organized formation of the leading edge. These findings provide an important mechanistic insight into cell migration underpinning a wide variety of physiological events such as development, immune response, and cancer metastasis.

Platform: Membrane Dynamics