1859-Wkshp
Advances in Atomic-Level Simulations of Large-Scale Functional Motions of Membrane Transporters
Emad Tajkhorshid, Mahmoud Moradi, Jing Li, Po-Chao Wen, Sundar Thangapandian, Josh Vermaas.
Department of Biochemistry, and Beckman Institute, University of Illinois, Urbana Champaign, Urbana, IL, USA.
Membrane transporters are specialized molecular devices that use various forms of cellular energy to drive active transport of their specific substrates across the membrane. Their fundamental role in diverse key biological processes has placed them among central drug targets, furthering widespread interest in their biophysical and mechanistic studies at a molecular level. Large-scale conformational changes are central to the function of membrane transporters. Description of these structural changes, however, requires sampling high-dimensional free energy landscapes that are inaccessible to conventional sampling techniques such as regular molecular dynamics (MD) simulations. We have recently developed a novel computational approach that, while being numerically expensive, has been the most efficient way to describe large-scale structural transitions in membrane transporters (as well as for any other macromolecular systems) using non-equilibrium methods employing system-specific collective variables, and a novel combination of several state-of-the-art sampling techniques [Moradi and Tajkhorshid, PNAS 110:18916-21 (2013); JCTC 10: 2866-80, 2014]. The approach is based on loosely coupled, multiple-copy MD simulations of large macromolecular systems preserving realistic representations of the systems in explicit membranes, and therefore relies on massive computing resources. Here we describe the application of the methodology to the study of several classes of membrane transporters, in order to characterize the inter-conversion of these molecular devices between the major conformational states necessary for their function, to characterize the free energy profiles associated with these transitions, and more importantly how chemical details such as ion/substrate binding drastically modulate the energy landscapes. The results of these simulations elucidate highly relevant mechanistic details of the function of membrane transporters providing a detailed structural basis for the experimentally observed phenomena.

Workshop: Microfluidics Tools for Studying Molecules and Cells

1860-Wkshp
Integrated Microfluidic Devices for Studying Aging and Adhesion of Individual Bacteria
Stephen C. Jacobson[1], Joshua D. Baker[1], David T. Kysela[2], Yves V. Brun[2].
[1]Department of Chemsitry, Indiana University, Bloomington, IN, USA,
[2]Department of Biology, Indiana University, Bloomington, IN, USA.
Analysis of single cells provides powerful insight into biological processes that are often missed when a population of cells is studied as an ensemble. We are developing microfluidic-based approaches coupled with optical microscopy to track individual bacteria and to improve the temporal and spatial resolution of single-cell measurements. The microfluidic devices automate the steps of cell culture, synchronization, reagent delivery, and analysis. To study cell growth and aging, we integrated nanochannel arrays into the microfluidic devices that physically trap bacteria. The bacteria grow and divide along the nanochannels in one-dimension, and parent cells and their progeny are easily tracked from generation to generation. With these devices, we are able to determine cell growth and division rates, accumulation of cellular damage over time, and inheritance from one generation to the next. For the adhesion studies, swarmer cells are synchronized in our ''baby machine'' and delivered to adjacent microchannels where we monitor adhesion of individual bacteria to channel surfaces. To better understand how bacteria attach to surfaces, rates of attachment of mutant strains that lack pili and motility are compared to the rate of attachment of wild-type cells.

1861-Wkshp
Democratization of Next-Generation Imaging, Diagnostics and Measurement Tools through Computational Photonics
Aydogan Ozcan.
Electrical Engineering & Bioengineering, University of California, Los Angeles, Los Angeles, CA, USA.
My research focuses on the use of computation/algorithms to create new optical microscopy, sensing, and diagnostic techniques, significantly improving existing tools for probing micro- and nano-objects while also simplifying the designs of these analysis tools. In this presentation, I will introduce a new set of computational microscopes which use lens-free on-chip imaging to replace traditional lenses with holographic reconstruction algorithms. Basically, 3D images of specimens are reconstructed from their ''shadows'' providing considerably improved field-of-view (FOV) and depth-of-field, thus enabling large sample volumes to be rapidly imaged, even at nanoscale. These new computational microscopes routinely generate >1-2 billion pixels (giga-pixels), where even single viruses can be detected with a FOV that is >100 fold wider than other techniques. At the heart of this leapfrog performance lie self-assembled liquid nano-lenses that are computationally imaged on a chip. The field-of-view of these computational microscopes is equal to the active-area of the sensor-array, easily reaching, for example, >20 mm$_\perp$2 or >10 cm$_\perp$2 by employing state-of-the-art CMOS or CCD imaging chips, respectively.
In addition to this remarkable increase in throughput, another major benefit of this technology is that it lends itself to field-portable and cost-effective designs which easily integrate with smartphones to conduct giga-pixel tele-pathology and microscopy even in resource-poor and remote settings where traditional techniques are difficult to implement and sustain, thus opening the door to various telemedicine applications in global health. Some other examples of these smartphone-based biomedical tools that I will describe include imaging flow cytometers, immunochromatographic diagnostic test readers, bacteria/pathogen sensors, blood analyzers for complete blood count, and allergen detectors. Through the development of similar computational imagers, I will also report the discovery of new 3D swimming patterns observed in human and animal sperm.

1862-Wkshp
A Microfluidic Rapid Freeze Quench Apparatus for High Field EPR Measurements
Alberto Collauto, Royi Kaufmann, **Daniella Goldfarb**.
Chemical Physics, Weizmann Institute of Science, Rehovot, Israel.
Rapid freeze quench (RFQ) EPR is a well-established technique for trapping reaction intermediates. A major difficulty in using commercial RFQ-EPR combined with standard X-band EPR spectroscopy is the relatively large amount of sample needed for each time point, and the associated amount that is wasted in the dead volume of the tubes and mixer. This is particularly prohibitive when one would like to couple RFQ with high resolution EPR techniques such as ENDOR (electron-nuclear double resonance) and DEER (electron-electron double resonance) that provide electron-nuclear and electron-electron distances and are less sensitive than standard continuous wave EPR. We have developed a dedicated microfluidic RFQ (*RFQ*) apparatus for W-band measurements, optimized for the small W-band sample size and a minimal sample amount for a series of ~7 time points collected in triplicates (~200 l of 0.03-0.1 mM labeled protein). The mixer is based on a recent published design[1] with a modified sample ejection system and cold trap. It current time window is 5-90 msec and its performance has been demonstrated on the reduction of nitroxide with dithionite[2]. The current state of the *RFQ* apparatus is demonstrated on : (i) the ATPase activity of an RNA helicase. Here Mg^{2+} was substituted with Mn^{2+} and the ^{31}P ENDOR spectrum was recorded. The ADP and ATP spectra have significantly different lineshapes and can therefore be used to probe the hydrolysis state. (ii) Conformational changes induced in a protein through ligand binding as detected by DEER.
1. Egawa T, Durand JL, Hayden EY, Rousseau DL, Yeh S-R. Anal Chem. 2009;81(4):1622-7.
2. R. Kaufmann, D. Goldfarb.. J Magn Reson. 2013;230:220-6.

1863-Wkshp
Cell and Vesicle Analysis in Microchambers
Petra S. Dittrich.
Biosystems Science and Engineering, ETH Zurich, Zurich, Switzerland.
Microfluidics is nowadays an established technology and provides as a huge toolbox for analytical and bioanalytical methods. Microfluidic platforms facilitate precise handling and positioning of cells, creating of chemically defined liquid environments, and tailoring mechanical or physical conditions. In recent years, we have developed several microfluidic platforms for single cell analysis. Combinations of cell trapping and encapsulation in microchambers accommodating volumes of tens to hundreds of picoliters facilitated the analyses of living cells and the chemical analysis of cell lysates. Moreover, we integrated these techniques with immunological methods, which allowed the quantification of proteins and other biomolecules with unprecedented high sensitivity.
The microfluidic platforms proved highly useful for analysis of giant unilamellar vesicles (GUVs), which we create in order to elucidate processes at the membrane. We could address questions of membrane permeability and membrane fusion. In addition, we could gain new insights into the properties of membranes, when exposed to mechanical forces. More precisely, we used vesicles with phase-separated domains and deformed them while they are trapped in the microfluidic device. We use confocal laser scanning microscopy to image the GUVs and could show that lipid sorting occurs, i.e., domains fuse, upon the increase of tension. Occasionally, we even observe budding of the liquid disordered phase. In further experiments, we exposed the GUVs to shear stress of defined strength and were able to visualize the changes of the domain shape and their relaxation after stopping the shear stress. Together, these studies may reveal in more detail the role of the membrane in the cellular response to mechanical strains.