domains because they are sufficient to affect actomyosin interactions *in vitro*. However, because the C'-terminal domains are required to anchor cMyBP-C to the thick filament, a limitation of such studies is that the precise spatial distribution of the N'-terminal domains is lost. To overcome this limitation we devised a novel protein engineering approach to remove and replace N'-terminal domains of cMyBP-C at their exact positions *in situ*. The approach uses cardiomyocytes from gene-edited ''Spy-C'' mice that express a modified cMyBP-C containing an internal TEV protease site followed by a short ''SpyTag'' sequence. Removal of N'-terminal domains is accomplished by incubating permeabilized Spy-C myocytes with TEV protease to cut cMyBP-C and expose the SpyTag on the remaining C'-terminal domains of cMyBP-C. New recombinant N'-terminal domains are then ligated via a covalent bond formed between SpyTag and its partner protein, SpyCatcher, which is encoded at the ends of the new recombinant N'-terminal domains (Zakeri et al., *PNAS*, 109:E690–7, 2012). Here we show proof-of-principle for the Spy-C method by demonstrating TEV cleavage of cMyBP-C and using immunofluorescence to show that added N'-terminal domains localize properly in sarcomeres from Spy-C mice. Force measurements in permeabilized myocytes further confirm the feasibility of using the Spy-C method to measure function of exchanged cMyBP-C N'-terminal domains *in situ*. The Spy-C method thus offers a new approach for introducing nearly any desired modification into the N'-terminal domains of cMyBP-C (e.g., mutations, PTMs, FRET sensors) to study their structure and function *in situ*. [NIH HL080367, AHA 17IRG33411051, and a UA Precision Mouse Modeling Program Award)].

2719-Plat

Engineered Heart Tissues Expressing Mutant Desmoplakin Exhibit Altered Twitch Kinetics

Ronald Ng[1], Xia Li[2], Heather Manring[3], Jinkyu Park[4], Jiesi Luo[4], Daniel Jacoby[4], Maegen A. Ackermann[5], Stuart Campbell[1].
[1]Department of Biomedical Engineering, Yale University, New Haven, CT, USA, [2]Yale University, New Haven, CT, USA, [3]Department of Physiology and Cell Biology, The Ohio State University, Columbus, OH, USA, [4]Cardiovascular Research Center, Section of Cardiovascular Medicine, Department of Internal Medicine, Yale University, New Haven, CT, USA, [5]Department of Biomedical Engineering, The Ohio State University, Columbus, OH, USA.

Arrhythmogenic cardiomyopathy (AC) is a disease characterized by electrical abnormalities and fibro-fatty infiltration of the myocardium. It is often linked to mutations in desmosomal genes, but molecular mechanisms whereby desmosomal mutations give rise to end-stage AC remain to be fully established. In order to probe phenotypic consequences of an AC-linked desmosomal mutation, we identified a candidate family with a history of sudden cardiac death and a variant of unknown significance (R451G) in the gene encoding desmoplakin (DSP). After establishing statistically significant linkage between the R451G variant and symptomatic family members (LOD score = 3.4), a heart autopsy specimen was obtained from a deceased DSP R451G-positive family member who had been diagnosed with AC according to the ARVC 2010 task force criteria. Immunofluorescence microscopy of this tissue showed significant reduction of desmoplakin and connexin 43 at the intercalated disks. We hypothesized that mutant desmoplakin is avidly degraded by the cell, resulting in haploinsufficiency, poor intercalated disk organization, and reduced electrical conduction velocity of the myocardium. In order to test these hypotheses, we created engineered heart tissues (EHTs) from an induced pluripotent stem cell line generated from a symptomatic, R451G-positive family member. EHTs were grown under electrical stimulus and isometric conditions for 2 weeks before contraction force and electrical conduction velocity through the tissues were measured. Surprisingly, we found that conduction velocities were unchanged between EHTs expressing mutant versus wild type desmoplakin. While there was no statistical difference in the peak contraction force, mutant tissues had a significantly slower rate of contraction: time from stimulus to peak twitch force was 17.6% longer in mutant EHTs. This early biomechanical deficiency in DSP R451G cells may indicate poor coupling between the sarcomere lattice and the intercalated disk as a fundamental disease mechanism in AC.

2720-Plat

Creatine-Kinase Shuttle and Rapid Mitochondrial Membrane Potential Conductivity are Needed Simultaneously to Maintain Uniform Metabolite Distributions in the Cardiac Cell Contraction Cycle

Shouryadipta Ghosh[1], Kenneth Tran[2], Edmund Crampin[1], Eric Hanssen[1], Vijay Rajagopal[1].
[1]University of Melbourne, Melbourne, Australia, [2]University of Auckland, Auckland, New Zealand.

Cardiac muscle cells are reported to contain a cell wide mitochondrial reticulum where mitochondria are interconnected in networks though intermitochondrial junctions. We have also observed that mitochondria form heterogeneous clusters within localised regions of the cell. Recent studies suggest that conduction of membrane potential through the mitochondrial networks can act as one of the dominant mechanisms to distribute energy across the cell. There are several other myofibril based metabolite diffusion systems present in cardiomyocytes which can also contribute to intracellular energy transport. What is the interplay between these different mechanisms and the heterogeneous distribution of mitochondria?

We are developing detailed finite element models of cardiac ultrastructure to investigate the role that mitochondrial membrane potential, oxygen and the creatine-kinase shuttle have in homogenizing ATP supply and demand within a heterogeneous cellular architecture. Our results show that diffusion of O2 is insufficient to reach interior of a cardiomyocyte in a high workload - leading to a regional hypoxia at the core of the cell. Our simulations also indicate that rapid diffusion of creatine and phosphocreatine may be still be required to ensure that metabolites like ATP and ADP are uniformly distributed in the midst of a heterogeneous distribution of mitochondria that we observe in the cell. However, our current model simulations suggest that conduction of membrane potential from the mitochondria at the cell periphery can provide sufficient protonomotive force to the mitochondria at the cell core to sustain a uniform distribution of metabolites and force dynamics across the cell cross section.

Platform: Membrane Dynamics and Fusion II

2721-Plat

Spatio-Temporal Dynamics and Turnover of Lipopolysaccharide in the Bacterial Outer Membrane

Sam Lenton[1], Rosalyn M. Leaman[1], Richard J. Spears[2], Martin A. Fascione[2], Dmitri O. Pushkin[3], Mark C. Coles[1,4], Christoph G. Baumann[1].
[1]Biology, University of York, York, United Kingdom, [2]Chemistry, University of York, York, United Kingdom, [3]Mathematics, University of York, York, United Kingdom, [4]Kennedy Institute of Rheumatology, University of Oxford, Oxford, United Kingdom.

Gram-negative bacteria have a cell envelope consisting of a peptidoglycan layer located between an inner cytoplasmic cell membrane and an outer-membrane (OM). The OM is an asymmetric bilayer consisting of an inner leaflet of phospholipid and an extra-cellular facing outer leaflet composed of lipopolysaccharide (LPS), in which outer membrane proteins (OMPs) are embedded. The mechanism whereby OMPs and LPS turnover during cell growth is not well understood. There is recent evidence that OMPs are not inserted uniformly across the cell surface, but at discrete spots. Once inserted, OMPs are not observed to diffuse far from these insertion sites, leading to patches termed OMP 'islands'. Whether LPS is also inserted in discrete locations is unknown. The interactions between LPS and OMPs are important for OM function and integrity. However, the extent to which LPS contributes to the confinement of OMPs is not well understood. Here, we have studied the spatio-temporal dynamics of LPS using metabolic incorporation of a sugar analogue and site-specific bio-orthogonal chemical labeling with a fluorescent dye. Using confocal FRAP and single-molecule fluorescence microscopy techniques, we observed that LPS does not diffuse freely in the OM and is inserted at discrete locations during cell growth. These characteristics were independent of LPS structural complexity (i.e. deep rough, rough and O-antigen restored strains). The results are discussed in the context of OM turnover during bacterial growth and adaptation to an environmental niche.

2722-Plat

The Biophysical Asymmetry of Mammalian Plasma Membranes

Joseph H. Lorent, Eric Malmberg, Ilya Levental.
Integrative Biology and Pharmacology, UT Health Science Center at Houston, Houston, TX, USA.

Compositional asymmetry between the two leaflets of a membrane bilayer is a fundamental feature of eukaryotic plasma membranes. Maintaining lipid asymmetry is energetically demanding, requiring active transport of phospholipids from one leaflet to the other. Phospholipid asymmetry has been described largely in the context of apoptosis, where translocation of the anionic lipid phosphatidylserine from the inner to the outer leaflet of the plasma membrane is a marker for macrophage engulfment. However, it has recently become evident that lipid asymmetry can also be reversible, and regulates many physiological processes, including immune cell signalling and cell-cell fusion. Owing to their distinct compositions, the two leaflets of the plasma membrane are believed to have distinct physical properties. Besides the commonly noted surface charge, the hypothesized differences in sphingolipid content and acyl chain composition suggest that other physical properties (lipid packing, lateral diffusion, permeability) would also be distinct between the two leaflets. Here,

we test this hypothesis directly by using a combination of fluorescence lifetime imaging of order sensitive probes with microinjection and selective quenching. Our results show that in live cell plasma membranes, inner leaflet order is slightly, but significantly, lower compared to the outer leaflet. The magnitude of the difference is smaller than expected from compositional asymmetry, suggesting that strong coupling between leaflets in asymmetric membranes prevents high lipid order differentials. We also find that a loss of membrane asymmetry during phospholipid scrambling or apoptosis drastically reduces the order of the plasma membrane and eliminates the order differences between leaflets. Finally, we observe that these biophysical differences are important for cell physiology, as inhibition of lipid scrambling suppresses the functional activation of immune cells. These findings document the biophysical asymmetry of the plasma membrane and suggest an important role for this asymmetry in cell function.

2723-Plat
Formation and Stability of Membrane Necks from Molecular Simulation
Rikhia Ghosh, Andrea Grafmüller, Reinhard Lipowsky.
Theory and Biosystems, Max Planck Institute of Colloids and Interfaces, Potsdam, Germany.
In their fluid state, lipid membranes are able to execute a variety of shape transformations. One intriguing example is provided by vesicle budding, i.e., by the expulsion of a small, spherical bud connected to the mother vesicle by a narrow membrane neck. The formation of such necks provides an essential step of many biological processes (cellular uptake, secretion as well as cell division or cytokinesis). In the framework of curvature elasticity, formation and stability of such a narrow neck are determined by local stability conditions, which depend both on the local mean curvature and on the spontaneous curvature of the membrane. However, these conditions ignore the molecular structure of the membrane, which may affect the shape of the neck. Furthermore, because of their locality, the stability conditions cannot describe the overall response of a neck to mechanical perturbations. In order to address these questions, we use molecular simulations to study the neck formation. Budding and neck formation are induced by small solutes such as monosaccharides that adsorb onto the bilayer membranes. If the two leaflets of a membrane are exposed to different solute concentrations, the membrane becomes asymmetric and acquires a certain spontaneous curvature. We start from a spherical vesicle that is exposed to a certain solute concentration in the exterior solution. When we reduce the volume of this vesicle, we observe formation of narrow membrane necks provided the concentration exceeds a certain threshold value. We induce mechanical perturbation using a geometry that resembles micropipette aspiration on the micrometer scale to observe neck opening for which we calculate the associated free energy landscape.

2724-Plat
Diffusion of Proteins and Lipids in Protein-Rich Membranes
Matti Javanainen[1,2], Hector Martinez-Seara[3], Ralf Metzler[4], **Ilpo Vattulainen**[1,2].
[1]Department of Physics, Tampere University of Technology, Tampere, Finland, [2]Department of Physics, University of Helsinki, Helsinki, Finland, [3]Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic, [4]Institute for Physics and Astronomy, University of Potsdam, Potsdam, Germany.
Cell membranes are complex structures that act as hosts for membrane-associated proteins to form dynamical complexes with lipids and other proteins. The process driving the formation of functional protein-lipid complexes is lateral diffusion where lipids and proteins migrate in the membrane plane in a stochastic but concerted fashion [1,2]. In protein-poor membranes the diffusion of lipids and proteins is fairly well understood. Experimental studies have provided evidence that for (trans)membrane proteins of radius R, their diffusion coefficient D scales logarithmically as $D \sim \log(1/R)$. The well-known Saffman-Delbrück (SD) theory [3] is in line with this picture. However, the challenge is to understand the diffusion of proteins and lipids in membranes of living cells that are extremely rich in proteins. Based on extensive computer simulations we here discuss how crowding with proteins affects lateral diffusion [4-6]. We show that protein crowding leads to deviations from the SD relation and to the emergence of a stronger Stokes-like dependence $D \sim 1/R$, highlighting that the lateral dynamics in crowded membranes is significantly different from protein-poor conditions. We also demonstrate how protein crowding gives rise to dynamical correlations observed as anomalous diffusion. Finally, we discuss biological consequences of protein crowding and anomalous diffusion in, e.g., formation of functional multiprotein complexes. [1] Falck E, et al. J Am Chem Soc 130, 44 (2008). [2] Niemela PS, et al. J Am Chem Soc 132, 7574 (2010). [3] Saffman P, Delbrück, M. Proc Natl Acad Sci 72, 3111 (1975). [4] Jeon, JH et al. Phys Rev X 6, 021006 (2016). [5] Javanainen M, et al. Faraday Discuss 161, 397 (2013). [6] Javanainen M, et al. J Phys Chem Lett 8, 4308 (2017).

2725-Plat
General Non-axisymmetric Shapes of Biological Membranes and their Importance in Understanding Endocytosis
Kranthi K. Mandadapu[1], Yannick Omar[2], Amaresh Sahu[1], Roger Sauer[2].
[1]Chemical and Biomolecular Engineering, University of California Berkeley, Berkeley, CA, USA, [2]RWTH Aachen, Aachen, Germany.
Many important biological phenomena are mediated by cell membranes and the shapes accommodated by these membranes. For example, endocytosis involves bending to form a bud or a tube from a flat shaped membrane under the action of proteins such as clathrin or BAR and their coupling to actin pulling forces. Various morphological shapes of membranes are governed by thermodynamic parameters such as osmotic pressure drop across the membrane or membrane tension. Most theoretical studies focusing on understanding membrane bending involve either small deformations about a given configuration or axi-symmetric shape transitions. In this talk, using the recent advancements in theoretical and computational modeling of membranes, we show that axi-symmetric or small deformations are inadequate to understanding the morphological phase-diagrams of membranes governed by protein induced spontaneous curvature and membrane tension. In particular, we discuss how axi-symmetric analysis of membranes misguide our understanding and demonstrate that non-axisymmetric shape transitions are almost always energetically favorable to axi-symmetric ones. We apply these methods to understand the classical endocytosis phenomena in mammalian and yeast cells. With the newly found non-axisymmetric shapes, we discuss the physical principles governing endocytosis at varying levels of membrane tension.

2726-Plat
Molecular Mechanism of Microdomain Dependent Protein Trafficking
Blanca B. Diaz-Rohrer, Kandice R. Levental, Ilya Levental.
Integrative Biology and Pharmacology, UT Health Science Center, Houston, TX, USA.
Eukaryotic cells are organized into spatially and functionally distinct membrane-bound organelles, whose functions are defined by their composition. Accurate sorting of membrane components between these compartments is necessary for maintaining organelle identity. For most membrane proteins, the determinants of their steady-state subcellular localization remain unknown. Lateral membrane domains (lipid rafts) provide an ideal platform for membrane sorting processes. However, the structural determinants of protein association with such domains are almost entirely unknown. We developed and characterized a robust experimental system for quantitative measurements of raft affinity in intact plasma membranes and used it to explore the determinants of transmembrane protein recruitment into raft domains. We identified several structural features associated with raft affinity, and established a quantitative and functional relationship between raft association and subcellular protein localization. Specifically, we observed that raft association is fully sufficient for PM recycling of certain proteins, and that abrogation of raft partitioning for these proteins led to their degradation in the lysosomes. These findings support the conclusion that ordered membrane domains mediate recycling of specific membrane components from the endosomal compartments to the PM. We have proceeded to define the molecular machinery that mediates raft lipid and protein sorting and recycling to the PM. Using a set of orthogonal transmembrane proteins as probes of raft and non-raft domains, we developed a high throughput siRNA screen to dissect the molecular machinery and dynamics for raft-mediated sorting. We identified a number of validated hits including known players of the early endocytic traffic (Rab5 and EEA1), but also novel players that appear to define a distinct class of trafficking mediators specific for raft-associated proteins. This pathway is not dependent on the classical recycling pathways defined by Rab4 and Rab11, rather defining a novel route for PM recycling of raft-preferring cargo.

2727-Plat
Capacitive Detection of Low-Enthalpy, Higher-Order Phase Transitions in Synthetic and Natural Lipid Membranes
Graham J. Taylor[1], Frederick A. Heberle[2], John Katsaras[2], C. Patrick Collier[3], Stephen A. Sarles[4].
[1]The Bredesen Center for Interdisciplinary Research, UTK-ORNL, Knoxville, TN, USA, [2]SWC Joint Center for Neutron Sciences, Oak Ridge National Laboratory, Oak Ridge, TN, USA, [3]Center for Nanophase Materials Sciences, Oak Ridge National Laboratory, Knoxville, TN, USA, [4]Mechanical, Aerospace, and Biomedical Engineering, The University of Tennessee, Knoxville, Knoxville, TN, USA.
Lateral organization and phase separation of lipids in natural membranes play key roles in regulating cellular processes. First-order phase transitions in model membranes consisting of few lipid components are well understood and readily detectable via calorimetry, densitometry, and fluorescence. However, far less is