proteins assembly, but the previous experiments did not include Syt1. In our set up, both surfaces are coated with a lipid bilayer. One of them mimics the synaptic vesicle membrane, on which we bind Syt1. The opposing bilayer mimics the inner leaflet of the plasma membrane and contains PIP2 and PS lipids. We will present results obtained with various lipid compositions and relevant mutations of the protein and how these interactions are impacted by the presence of calcium. Ultimately, we plan to provide a complete mapping of the energetics of the critical membrane interaction sites of Syt1.

175-Plat
Study of Insertion of Dengue E into Lipid Bilayers by Neutron Reflectivity and Molecular Dynamics Simulations
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The envelope (E) protein of Dengue virus rearranges to a trimeric hairpin to mediate fusion of the viral and host membranes. Insertion of E into host membranes is essential to the process, serving to anchor E into the target membrane and possibly also to disrupt local order within the membrane. Both aspects are likely to be affected by the depth of insertion, the orientation of the trimer with respect to the membrane normal, and the interactions that form between the trimer and the membrane. In the present work, we resolved the depth of insertion, the tilt angle, and the fundamental interactions for the soluble portion of Dengue E trimers (sE) associated with planar lipid bilayer membranes of various combinations of POPC with POPG, POPE, and cholesterol by neutron reflectivity (NR) and by molecular dynamics (MD) simulations. The tip of E containing the fusion loop (FL) is located at the interface of the headgroups and acyl chains of the outer leaflet of the lipid bilayers, in good agreement with prior predictions. The NR measurements and the MD simulations both indicate that E tilts with respect to the membrane normal upon insertion, promoted by either the anionic lipid POPG or cholesterol. The simulations show that tilting of the protein correlates with hydrogen bond formation between lysines located on the sides of the trimer close to the tip (K246 and K247) and nearby lipid headgroups. These hydrogen bonds provide the majority of the interaction energy whereas interactions involving the FL are a minor contribution. POPG promotes formation of these hydrogen bonds through direct interactions with K246, K247, and other polar residues whereas cholesterol indirectly facilitates formation of these hydrogen bonds as a result of a greater hydrated volume in the headgroups. Simulations in which the protein was held in a vertical orientation with respect to the membrane show that these strong hydrogen bonding interactions of K246 and K247 with lipid headgroups causes significant local membrane deformation of a 70:30 POPC:POPG bilayer as the lipids wrap around the periphery of the E trimer. We propose that these interactions play the dominant role in membrane anchoring and may also play a role in initiating mixing of the outer leaflets during the fusion process.

176-Plat
The Great Nuclear Escape: Structure-Based Mechanism of Membrane Budding during Nuclear Egress of Herpesviruses
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Herpesviruses are unusual among enveloped viruses because they bud twice yet acquire a single envelope. They are also the only known mammalian viruses that bud into the nuclear envelope. Recently, we discovered that the herpesvirus nuclear egress complex (NEC) could bud membranes without the help of other proteins and that it formed a coat-like hexagonal scaffold inside the budding membrane. This discovery established the NEC as the first virally encoded budding machine that operates at the nuclear, as opposed to cytoplasmic, membrane but left unknown the structure of the NEC coat and its role in the budding process. To bridge this gap in our knowledge, we determined the 2.8-Å crystal structure of the NEC from Herpes Simplex virus (HSV). In crystals, NEC packs into a hexagonal lattice that mimics the hexagonal NEC coats within budded vesicles. The crystal structure of the NEC lattice thus reveals molecular interactions that generate the hexagonal coat. To determine the role of the hexagonal NEC lattice in budding, we mutated residues at the oligomeric interfaces observed in the crystals with mutagenesis. Perturbation of the oligomeric interfaces through mutagenesis blocked NEC-mediated budding *in vitro* confirming that formation of the hexagonal NEC lattice drives budding. The NEC structure provide a three-dimensional blueprint for further dissection of its unique budding mechanism. Moreover, the structure represents the first atomic-level view of an oligomeric array formed by a membrane-deforming protein.

Platform: Cardiac, Smooth, and Skeletal Muscle Electrophysiology

177-Plat
Action Potential Heterogeneity in Murine Sinoatrial Node Myocytes
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Sinoatrial node myocytes (SAMs) act as cardiac pacemakers by generating spontaneous action potentials (APs). SAMs are characterized by the expression of the ''funny current'' (I_f) that contributes to the spontaneous diastolic depolarization phase of the sinoatrial AP. SAMs exhibit a large heterogeneity of morphological and electrophysiological properties. However, limited information is available about the intrinsic variability in AP waveforms in SAMs. In this study, we co-recorded APs and I_f from acutely isolated SAMs from mice using voltage and current clamp recordings in the same cells. We refined the definitions of commonly used AP waveform parameters to document the heterogeneity of APs in both perforated patch (PP) and whole-cell (WC) recordings. These definitions were then implemented in an open-source data analysis program (''paramAP'') written in Python 3 using NumPy, SciPy, and Matplotlib. ParamAP was utilized to characterize APs from more than 100 SAMs. Correlation analysis was used to evaluate relationships among AP waveform parameters and to identify parameters that are strongly correlated with I_f and AP firing rate. Furthermore, we document time-dependent changes in AP waveform parameters during both PP and WC recordings. In summary, paramAP is a powerful tool to help standardize AP waveform analysis and parameter definitions in murine SAMs. Our data suggest that commonly-held assumptions about sinoatrial node APs should be extended to accommodate a wider range of AP waveforms. In addition, the time-dependent changes in AP waveform parameters should be considered when comparing data from different studies.

178-Plat
SK4 Ca^{2+}-Activated K^+ Channels Regulate Sinoatrial Node Firing Rate and Cardiac Pacing *In Vivo*
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The sinoatrial node (SAN) controls the heart rhythm under physiological conditions. We recently showed that SK4 calcium-activated potassium channels (SK4) are important for automaticity of cardiomyocytes derived from human embryonic stem cells. Here we tested whether SK4 are expressed in adult mouse SAN and play a role in pacemaker function. TRAM-34, a selective blocker of SK4, significantly reduced the firing rate and depolarized the maximal diastolic potential in SAN cells. Western blots revealed the presence of an SK4 protein in mouse SAN. *In vivo* ECG recording in mice showed that intraperitoneal injection of SK4 blockers produced bradycardia and prolonged the PR interval. Mathematical modeling predicted that addition of SK4 to the model increases SAN firing rate, while its removal decreases pacemaker frequency. This work shows that SK4 play a role in SAN pacemaker function by acting at late repolarization and that they are potential therapeutic targets for treating cardiac arrhythmias.

179-Plat
Characteristics of Ivabradine-Sensitive Currents in Mouse Sinoatrial Node Myocytes
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Cardiac pacemaking is driven by spontaneous action potentials (APs) in sinoatrial node myocytes (SAMs). The funny current (I_f) is thought play a role in the generation of pacemaker activity in SAMs, but the degree to which it contributes is incompletely understood. In this study, we used AP clamp