1015-Plat Heart Pre-crosslinking Prior to Cell Dissociation Stabilizes Fine Cardiomyocyte Cytoarchitecture

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The visualization and subcellular distribution of proteins in the heart is commonly achieved by specific protein immunolabeling of heart isolated cells with the Langendorff perfusion system using proteolytic enzymes. Once the cells are isolated, they are typically fixed with paraformaldehyde, permeabilized with detergents and exposed to the desired antibody. However, whether isolated cells maintain the same protein organization as at the moment of the animal death and heart dissection is unknown. This is probably true for many cellular components but as the cell isolation can last 1–2 hours in solutions without hormones and active substances, it is very likely that some proteins may change during the dissociation procedure. Here, we performed the cell dissociation after an initial pre-crosslinking with 0.5% paraformaldehyde or 1 mM disuccinimidyl suberate (DSS) to fix the proteins close to the time of heart dissection. This procedure should maintain local protein arrangements while leaving sites exposed to collagenase action to achieve cell dissociation. We tested in murine cardiomyocytes anti-estrogen receptor alpha (ERα) and caveolin-3 antibodies. We found two major differences by comparing no pre-crosslinking with pre-crosslinking with DSS or PFA. ERα labeling intensity of the cells (T-tubules and nucleus) was much weaker in non pre-crosslinked conditions with a great variability among different cells. In contrast, with PFA or DSS pre-crosslinking, ERα labeling was much sharper and intense with a more uniform labeling intensity. In both pre-crosslinking conditions, caveolin-3 showed a clear clustered labeling at the surface and T-tubular membranes with similar intensities. Without pre-crosslinking T-tubular caveolin-3 labeling was weaker. Thus, the pre-crosslinking seems to stabilize the heart protein arrangements at the beginning of the cell dissociation which should maintain the subcellular structure closer to the heart structure in the intact animal.

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1016-Plat The *Drosophila* Heart Shows Distinct Responses to Depressed or Enhanced Myosin Motor Activity and Serves as a System for Identifying Genetic Suppressors of Cardiac Pathologies

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Myosin is a multi-domain ATP-dependent molecular motor of cardiac muscle. Mutations that diminish or augment myosin's molecular performance are associated with distinct cardiomyopathies. We studied the effects of two *Drosophila melanogaster* myosin mutations on cardiac structure and function. The mutations affect the myosin transducer, which lies near the nucleotide-binding site and is intimately involved in determining the motor's biomechanical properties. We found that *D45* flies express myosin with depressed ATPase and *in vitro* actin motility while Mhc^5 flies express myosin with enhanced molecular properties. Beating heart tubes of live flies were imaged using direct immersion DIC optics in conjunction with a high-speed digital camera. Cardiac movements were monitored via edge tracings obtained from processed movies. Microscopic and computational analyses revealed altered cardiac dimensions and contractility or distorted rhythmicity in the mutants. Interestingly, depressed motor function in *D45* flies produced a dilatory cardiac response, as found in vertebrates expressing cardiac myosin with specific dilated cardiomyopathy mutations. This suggests an apparently conserved pathological response to impaired motor function. Mhc^5 hearts with enhanced myosin function showed phenotypes analogous to those seen in restrictive cardiomyopathy (RCM), suggesting the human disease could have similar origins (to date, inherited RCM in humans has only been linked to troponin mutations). Thus, *Drosophila* may be an effective high-throughput system for exploring basic conserved mechanisms of cardiomyopathies and for identifying novel mutations in protein targets that lead to specific cardiac disorders in the human population. We currently are using the power of *Drosophila* genetics to manipulate contractile protein stoichiometry and genetic interactions between sarcomeric components. Our goal is to suppress defects in hearts expressing mutations in myosin or in the inhibitory troponin subunit.

Platform AF: Membrane Engineering

1017-Plat Dissecting the nanomechanical response of supported single phospholipid bilayers with Force Spectroscopy

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Understanding the effect of mechanical stress on biological membranes is of fundamental importance in biology since cells are known to naturally perform their function under the effect of a complex combination of forces. The chemical composition of such membranes is the ultimate responsible for determining the cellular scaffold, closely related to its function. Micro-scale assays have revealed a wealth of information regarding the overall membrane mechanical resistance. Nonetheless, the diversity in the chemical composition of such membranes makes it difficult to individually probe the mechanical contribution of every particular membrane component. Here we use force spectroscopy to quantitatively characterize the nanomechanical resistance of supported lipid bilayers as a function of their chemical composition thanks to a reliable molecular fingerprint that reveals itself as a repetitive jump in the approaching force curve, hallmark of bilayer rupture. By

systematically testing a set of bilayers composed of phospholipids with different chemical composition, we first show that both the hydrophilic headgroup and the hydrophobic tail have an independent important contribution to the overall bilayer mechanical stability. While the mechanical resistance dramatically changes for different phospholipids composed of a 18:0 chain with varying headgroups (PC, PE, PG, PS), the chain length increases the mechanical stability in ~ 6 nN for every extra pair of $-CH_2$ groups present in the chain along the series DMPC-DSPC. Furthermore, each chain instauration readily descreases the mechanical stability of the bilayer in $\sim$1.5 nN. The effect of different sterols on a solid (DPPC) membrane has been also addressed. This work opens up avenues for characterizing the membrane (nano)mechanical stability, suggesting that it can be regarded as the 'sum of its parts'.

1018-Plat Rapid Prototyping Of Multicomponent Phospholipid Nanostructure Arrays Using Dip-pen Nanolithography

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Dip-pen nanolithography, developed extensively for the deposition of molecular nanostructures on solid surfaces, is shown to allow for a massively parallel deposition of lipidic nanostructures on a variety of solid surfaces. Lipid "inks" deposited onto the tip of an atomic force microscope are constructively transferred when aided by humidity controlled hydration and capillary interactions, onto a variety of substrates. Careful selection of environmental conditions, substrate pre-treatment, and contact times allow the DPN-mediated molecular transfers to routinely produce controlled deposition of lipid multilayers between 0.5 and 20 bilayers at nanometer scale lateral resolution ($\sim$ 100 nm feature sizes) with a throughput on the order of 5cm̂2/min.(Lenhert et al., 2007) Using this approach, parallel deposition of different phospholipid ink combinations in arbitrarily nanostructured patterns in close proximity or even in direct contact can be conveniently achieved using tip micro-arrays. Subsequent immersion of these nano-arrays into aqueous solution provide controlled means to spread single lipid bilayers and/or produce tethered vesicles from pre-determined arrays of nanometric lipid sources. Biophysical applications of these lipid nanoarrays in determination of

1. effects of ambient aqueous phase properties (pH and temperature) on lipid spreading;
2. emergence of co-existing phases via lipid-lipid interdiffusion and demixing; and
3. rapid prototyping of lipid nanoarrays will be discussed.

1019-Plat Curvature and Composition Coupling In Lipid Bilayer Membrane Tubes

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Membrane curvature and composition are expected to couple in many fundamental cell-biological processes, such as lipid and protein sorting, trafficking, and protein coat functions, which are important in many intracellular trafficking pathways. The mechanisms of these processes are not well understood. It is therefore important to quantitatively investigate the biophysics of curvature/composition coupling and test existing biochemical and mechanical models. In this study, we focus on curvature sorting of membrane components, including lipids, peptides and trans-membrane proteins.

In order to vary the degree of membrane curvature, we pull membrane tubes (tethers) from lipid model membranes (giant vesicles) and cell blebs (giant plasma membrane vesicles) via micropipette aspiration and a membrane-attached bead. Lipid dye fluorescence intensities were measured by confocal fluorescence imaging in a plane orthogonal to the membrane tether as a function of membrane curvature. This allowed obtaining information about curvature-dependent partitioning of lipid dyes. Using the same method, we furthermore examined biologically relevant lipid binding proteins for their curvature dependent partitioning. Moreover, we found evidence for a curvature induced mixing-demixing phase transition coupling to a curvature gradient in giant plasma membrane vesicles.

1020-Plat One-Dimensional Lipid Bilayers on Carbon Nanotube Templates: Structure, properties and device integration

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One-dimensional nanomaterials present an exciting opportunity for creating functional biologically inspired structures because they have unique materials properties, dimensions comparable with the typical size of biological assemblies or individual molecules, and geometry suitable for integration into functional devices and assemblies. We have integrated carbon nanotubes with phospholipid bilayers in a "one-dimensional lipid bilayer" assembly that consists of a continuous lipid membrane wrapped around a carbon nanotube. We will discuss the structure and properties of this bio-nanomaterial assembly as well as its applications for studies of lipid mobility in highly-curved geometries and biological ion transport

1021-Plat Synergy of Membrane Curvature-Stabilization and Electrostatic Interaction leads to Formation of Block Liposomes by Colossal Charged Lipids

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Recently, we have reported block liposomes (BLs), a new vesicle phase formed in mixtures of MVLBG2, DOPC and water (A. Zidovska et al., *Submitted*, 2007). MVLBG2 is a newly synthesized highly charged (16+) lipid (K. Ewert et al., *JACS,* 2006) with giant dendrimer-like headgroup therefore having a headgroup area several times larger than DOPC. The nature of the headgroup leads to a conical shape of the molecule resulting into high spontaneous membrane curvature, when incorporated into lipid bilayer. Therefore, in combination with zero-curvature DOPC, the system exhibits a rich phase diagram revealing novel vesicle morphologies. We carried out structural studies of this phase with differential contrast microscopy (DIC) and cryo transmission electron microscopy (cryo-TEM). At the micron scale DIC reveals a new phase consisting of uniform bone-like vesicles which is present in a narrow composition range. This novel morphology persists down to the nanometer scale. Cryo-TEM reveals a population of nanotubes which are capped by spherical unilammelar vesicles with diameters of few hundred nm and a surprising new morphology of lipid nanorods (fibers) resulting from a spontaneous topological transition from tubes to nanorods. In this work we investigate the contribution of spontaneous curvature and membrane charge to the formation of BLs. By comparing with a system of matching membrane charge density but zero spontaneous curvature and by screening the charge of MVLBG2 but keeping the curvature constant, we were able to identify both, spontaneous curvature and membrane charge, as critical parameters for BLs-formation. The effect of salt and pH on the shape evolution of the BLs was also carefully studied.

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1022-Plat Tethered Bilayer Lipid Membranes: a versatile Model Membrane Platform

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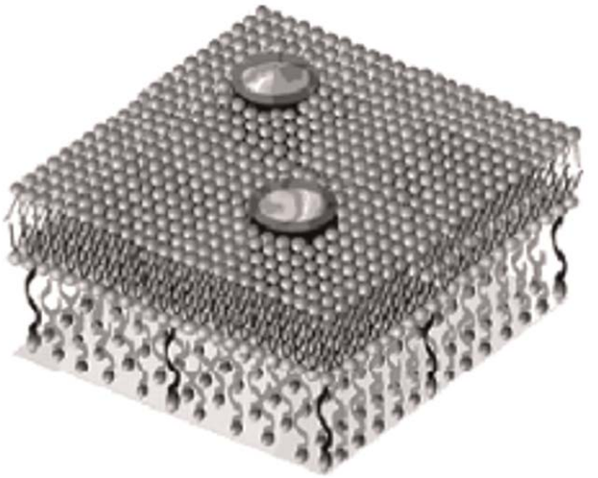
Tethered layer lipid membranes (tBLM) have been develloped in the past years to a versatile platform that can mimick essential features of a biological membrane. A tBLM consists of a lipid bilayer, where the inner leaflet is covalentely attatched to a solid support via a spacer unit. Thus, they provide a stable and insulating system, that can be used to study various membrane related processes. We have used tBLMs to study the functional incorporation of ion channels as well as to monitor the interaction of antimicrobial peptides and other proteins with the lipid bilayer. The stuctural properties of the membrane have been analzed in detail by Neutron Scattering. The results allow for an optimization of the molecular architecture.

Experimental results obtained by Surface Plasmon Resonance and Impedance Spectroscopy on the function of the ion channels and the interaction pathways of the peptides will be presented.



1023-Plat Molecular Dynamics Study Of Vesicle Deformation Mechanisms

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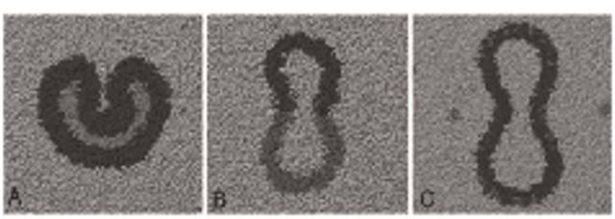
Lipid bilayer membranes are known to form various structures like large sheets or vesicles. When both bilayer leaflets have equal composition, membranes preferentially form flat sheets or spherical vesicles. However, vesicles with a wide variety of shapes, including ellipsoids, discoids, pear-shaped, cup-shaped and budded vesicles, have been shown experimentally. Such shapes were predicted theoretically from energy minimization of continuous sheets as well. We show, using coarse-grained molecular dynamics simulations, how relatively small asymmetry in composition between the two leaflets may result in spontaneously curved bilayers and all these vesicle shapes. Three types of bilayer asymmetry are considered. Firstly, the situation where the headgroup-solvent interaction and thus the lipid packing alters due to a change in pH or ion-concentration of the vesicle interior/exterior (A). Secondly, where asymmetry arises from phase separation of two lipid types (B). And thirdly, where asymmetry arises from growth of one of the bilayer leaflets by incorporation of additional lipids from the solvent (C).



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1024-Plat Isolating Native Vesicles from Cells and Patterning Them on Fabricated Silane Surfaces

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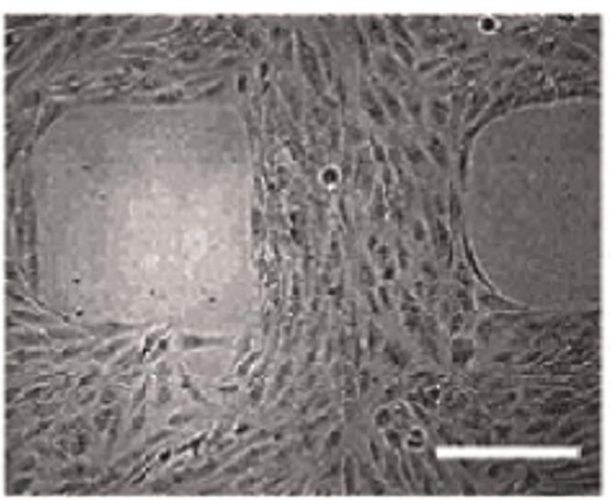
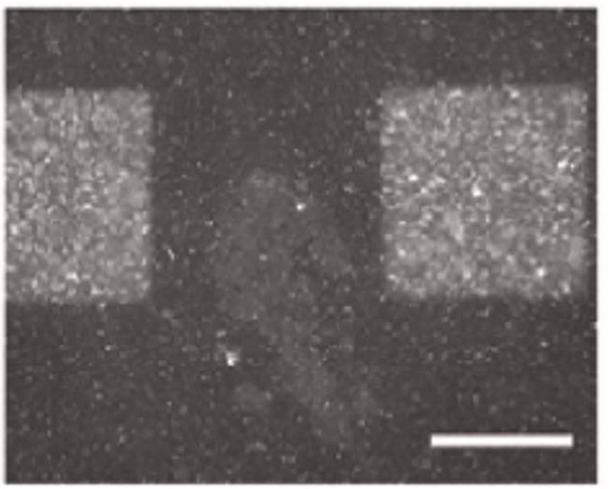
Vesicularlized fragments of plasma membranes (native vesicles) are easier to study, manipulate and store without losing the microstructural integrity and functions of important signaling proteins. Therefore, in lieu of complex whole cells, they may be used as convenient bio-analytical reagents to investigate cellular signaling processes. Here, we describe a one-step and less-invasive technique to isolate native vesicles from human retinal pigment epithelium (RPE) cells by extrusion though polycarbonate filters. Light scattering and fluorescence measurements demonstrate that these post-extrusion cell fragments form closed-shaped spherical vesicles. Fluorescence co-localization studies and single-vesicle Raman spectroscopy establish that they are enriched in glycosphingolipids and cholesterol, typically associated with lipid rafts. Furthermore, they can be deposited into spatial patterns when exposed to amphiphilic surfaces such as patterned n-octadecylsiloxane (OTS) monolayers. Surprisingly, unlike whole cells which avoid highly hydrophobic OTS surfaces, native vesicles display a higher affinity for hydrophobic surfaces, possibly because of their raft-enriched surface chemical compositions. We anticipate that these micropatterned vesicle arrays will open new routes in fabrication of membrane biochips containing functional proteolipidic, supramolecular structures derived directly from cells and possible interesting parallel invesigations of native/synthetic membrane and protein dynamics.





Protein-Ligand Interactions - I

1025-Pos Effect of Force Transducer Stiffness on Unbinding Kinetics Inferred from Molecular Force Spectroscopy

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Forced unbinding of complementary macromolecules such as ligand-receptor pairs can reveal energetic and kinetic details governing the mechanisms of extracellular load-transfer and cell-substrate adhesion, as well as physiological processes such as drug metabolism. Molecular-level experiments as well as atomic-level simulations have allowed access to individual dissociation events, and yet even with this detailed information, disparities in measured unbinding force lead to marked variation in inferred binding energetics and kinetics at equilibrium. We investigated these disparities through examination of atomic-level unbinding trajectories generated by steered molecular dynamics simulations as well as through molecular force spectroscopy experiments on the biotin-streptavidin system. We have identified one reason for the experimental discrepancies discussed above. In mathematical analysis of forced ligand-receptor unbinding, the force applied to the ligand-receptor complex is always considered; however, the effect of the force transducer on the energy landscape of the complex has been overlooked. Here, we demonstrate through simulation and experiment that the stiffness of the force transducer can have an appreciable effect on measured ligand-receptor unbinding force. To extrapolate equilibrium kinetic information from forced unbinding experiments, therefore, a range of loading rates as well as force transducer stiffnesses must be considered.