**85-Plat**

**Synthesis and Biophysical Characterization of the Chlorosulfolipids of *Ochramonas danica***

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The freshwater algae *Ochramonas danica* produces a range of polychlorinated single-chain amphiphiles. Danicalipin A, a hexachlorosulfolipid makes up 90% of the polar lipid content of the flagellar membrane of *O. danica*. Its exotic structure presents both synthetic challenges and raises questions about its function within the membrane. To address these questions, we have combined total synthesis and membrane biophysics to investigate the effects of structural elements of chlorosulfolipids on their behavior in monolayers and bilayers. The discovery of a titanium-based catalytic, enantioselective dichlorination of allylic alcohols enabled the eight-step synthesis of (+)-Danicalipin A as a single stereoisomer in sufficient quantities for *in vitro* analysis. Nanoscale secondary ion mass spectrometry (NanoSIMS) confirmed that Danicalipin A is localized within plasma membrane of *O. danica* cells. Preliminary biophysical characterization of Danicalipin A has revealed that it alters the phase behavior and lateral organization in monolayers and bilayers of other lipids present in the membrane of *O. danica*. Danicalipin A incorporates into monolayers of phospho- and glycolipids at the air-water interface and increases the surface pressure at which the liquid-expanded to liquid-compact phase transition occurs, as well as increasing monolayer compressibility. Similarly, in giant unilamellar vesicles, Danicalipin A lowers the transition temperature of saturated phospho- and glycerolipids and causes phase separation. Natural and unnatural analogs to Danicalipin A are being synthesized to examine the effects of the stereochemistry, chlorination pattern, and sulfation on these biophysical properties. These results may reveal how the membrane of *O. danica* accommodates high concentrations of chlorosulfolipids, which are toxic to other organisms.

**86-Plat**

**Using Hyscore Spectroscopy of Nitroxides to Profile Water Content of Lipid Bilayers with 2 Å Spatial Resolution**

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Mapping water molecules across lipid bilayers at high spatial resolution is important for both understanding membrane biophysics and membrane protein biological function. Current biophysical methods to determine water concentration in biological systems have several limitations, the main one being insufficient spatial resolution. Water penetration profiles of lipid bilayers have been measured using electron spin echo envelope modulation (ESEEM) of nitroxide spin labels based on magnetic interactions of nitroxides with the matrix water molecules. However, the matrix ESEEM effect is caused by through-space dipolar interactions, restricting spatial sensitivity to ~10 Å, a distance which spans a significant portion of the lipid bilayer. Overhauser DNP is a less direct method to determine local water concentration, via measuring bulk water polarization induced by microwave irradiation of a spin label, and depends on complex spin dynamics inside the lipid bilayer. Hyperfine sublevel correlation (HYSCORE) spectroscopy is a sensitive technique to detect hydrogen bonds formed with paramagnetic centers. Here we demonstrate the use of HYSCORE spectroscopy to directly and accurately measure the fraction of water molecules hydrogen bonded to the nitroxide oxygen atom. To convert HYSCORE data into local water concentration we employed a normalization factor for the H-bonded deuteron signal that was taken as intensity of the ESEEM signal measured under identical experimental conditions and spectrometer tuning parameters. We demonstrate that the water molecules hydrogen-bonded to transmembrane WALP peptides, which were spin-labeled at various positions with MTSL, can be accurately determined even in the hydrophobic region of the lipid membrane. A correlation between the observed H-bonded signals and local water concentration has been established using model systems containing mixtures of diglyme and $CH_3OD$, as well as diglyme and deuterated water, with Tempol as the spin probe.

**87-Plat**

**Characterization of Phases and Interactions Among Lipids Involved in Drug Delivery: An NMR and Small-Angle X-Ray Scattering Study**

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Small interfering RNA (siRNA) which silences genes in gene therapies can be delivered into target cells using specialized lipid nanoparticles (LNPs). LNPs designed to encapsulate siRNA are composed of several components: a phospholipid, a cationic lipid, cholesterol, and a polyethylene glycol-lipid. Each of these constituents contributes to the successful delivery of the siRNA. The LNP enters the target cell and delivers its contents via endocytosis. As the endosome matures, the environment becomes more acidic and the cationic lipid (DLin-KC2-DMA) of the LNP becomes protonated. This facilitates the release of the siRNA into the cytoplasm via attractive electrostatic interactions between the cationic lipid and an anionic lipid found in the endosomal membrane (lysobisphosphatidic acid (LBPA)). These interactions are thought to disrupt the membrane by inducing a phase change from bilayer to non-bilayer phases, such as the inverted hexagonal or bicontinuous cubic phases. The inefficiency of this process is one of the barriers to the potency of siRNA drugs. Computer simulations can provide valuable insights for optimizing the LNP composition to improve drug delivery efficiency. For the predictions to be successful it is critical that the modelled lipids behave properly. In this work, we characterize lipids found in LNPs and endosomal lipids using nuclear magnetic resonance (NMR) and small angle X-ray scattering (SAXS). NMR allows us to determine the lipid phase(s) present and the order (mobility) of the lipid chains. SAXS provides complementary information about the phases via long-range correlations within the sample. These data are used to validate computer simulations of LNP component lipids and endosomal lipids leading to a stronger understanding of the behaviour of more complex systems including complete LNPs and ultimately enhancing the potency of these drug delivery systems.

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**Spectral STED Imaging of Cell Membranes**

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The lateral organization of molecules in the cellular plasma membrane plays an important role in cellular signaling. A critical parameter for membrane molecular organization is how the membrane lipids are packed. Polarity sensitive dyes are powerful tools to characterize such lipid membrane order. These dyes change their emission spectrum depending on the polarity of the environment which can be used to quantify the molecular ordering and to visualize lateral heterogeneity in membrane order of cellular membranes. These probes have been used in combination with confocal or multi-photon microscopy; however, the diffraction-limited spatial resolution of these techniques does not allow observation and full characterization of nanodomains/clusters in the plasma membrane. The investigation of potential lipid nanodomains, however, requires the use of super resolution microscopy. Here, we apply the polarity sensitive membrane dyes in super-resolution STED microscopy. Measurements on cell-derived membrane vesicles, in the plasma membrane of live cells, and on single virus particles show the high potential of these dyes for probing nanoscale membrane heterogeneity [Sezgin et al, Biophysical Journal, 2017].

## Platform: Cell Mechanics and Motility I

**89-Plat**

**Determination of 3D Amoeboid Migration Force through Utilization of Actuated Surface Attached Posts**

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Cell migration is essential for multicellular organization and survival but the forces that govern 3D migration are still poorly understood. Methods that measure force in 2D environments such as traction force microscopy and atomic force microscopy are not, in general, suited to measure forces during 3D migration, which comprises a majority of cell movement in complex tissues. To address this need, an easily visualized, reproducible, method of analyzing forces during cell migration through a 3D collagen matrix is being developed using actuated surface-attached posts (ASAP). ASAPs are arrays

of polydimethylsiloxane (PDMS) posts that can be calibrated to give force measurements based on deflection. The posts are embedded within a collagen matrix to simulate a physiologically relevant environment and cells are then induced to migrate through the collagen matrix, deflecting the posts. This method allows for measurement of forces based on the Young's modulus of the post in question and the points of contact. The cell lines used were U937 leukocytes and Ref52 fibroblasts which both gave strikingly different results. U937 cells migrated through the collagen matrix through an amoeboid mechanism which, combined with their small size, exerted little force on the environment and prevented said force from being measured with the current properties of ASAP. By contrast, mesenchymal Ref52 cells were able to bend the posts significantly which involved forming direct attachments with the post itself, consistent with the role of fibroblasts in remodelling the extracellular matrix. The maximum force measured was nearly 90 nN, consistent with traction analysis for fibroblasts migrating on 2D flexible substrates.

**90-Plat**

**Structure and Constriction Mechanism of the Actomyosin Ring**

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Cytokinesis is orchestrated by a contractile actomyosin ring, but its structure and mechanism remain elusive. We visualized the 3D structure of the ring in frozen-hydrated dividing yeast cells by electron cryotomography (ECT). Detailed arrangements of actin filaments within the ring and with respect to the membrane were seen for the first time, providing a crucial spatial constraint for the constriction mechanism of the ring. Using the ECT data and input from the literature we then explored sixteen mechanistic models by coarse-grained simulations at the 3D molecular details, revealing plausible mechanisms for preventing membrane distortion and protein aggregation. We found that, in the model that best fits experimental data, both bipolar and membrane-attached unipolar myosins exist in the ring, reconciling two different views in the field regarding the myosin configuration. In this model, ring tension is generated primarily by interactions between bipolar myosins and actin, and transmitted to the membrane via unipolar myosins. This model recapitulates a broad distribution of distances from actin filaments to the membrane observed in our tomograms and separation of two different myosin isoforms into the outer and inner subdomains of the ring reported in a previous fluorescence microscopy study. Further, it rationalizes how bundles of actomyosin were able to separate from the membrane in fluorescence microscopy experiments of the same previous study.

**91-Plat**

**The Arp2/3 Complex is Necessary for Migration of Glioblastoma Cells on Compliant Substrates due to a Lamellipodia-Provided Mechanical Advantage**

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Glioblastomas (GBMs) are the most common and most lethal central nervous system tumors, with a median survival time of approximately 15 months. This dismal prognosis has been attributed to the wide spread dissemination of GBM tumor cells throughout the brain, which makes repeated recurrence likely. The brain is one of the most compliant tissues in the body, with an elastic modulus of around 1 kPa. Migration and invasion on such soft substrates in 2D requires the wide, ruffled leading edge of the lamellipodia, cytoskeletal structures formed through branched actin filament assembly, which is mediated via nucleation by the Arp2/3 complex. While this behavior has been well-documented, the reasons for the phenomena have yet to be shown. Here, we show that Arp2/3-inhibited GBM cells transmit forces that result in substrate deformation instead of locomotion. In contrast, Arp2/3-positive cells form lamellipodia, which transmit forces over a wider area than the parallel actin architecture that forms in the absence of Arp2/3, and thus successfully transmit forces that lead to locomotion. On-going work will investigate this mechanism of locomotion in a computational elastic-stochastic model of lamellipodia versus filopodia force transmission. The model will incorporate focal adhesions formed along a surface with a defined curvature and contour length that corresponds to the appropriate cytoskeletal structure. We expect the model to illustrate the mechanical advantage of lamellipodia on compliant substrates via development of sustainable forces, while filopodia exhibit load-fail dynamics. In summary, our work provides insight into mechanisms of the migratory behavior of GBM tumor cells in the brain parenchyma and demonstrates a possible therapeutic target for GBM tumors.

**92-Plat**

**Vinculin Forms a Directionally Asymmetric Catch Bond with F-Actin**

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Vinculin is an actin-binding protein thought to reinforce cell-cell and cell-matrix adhesions. However, how mechanical load affects the vinculin-F-actin bond is unclear. Using a single-molecule optical trap assay, we found that vinculin forms a force-dependent catch bond with F-actin through its tail domain, but with lifetimes that depend strongly on the direction of the applied force. Force toward the pointed (-) end of the actin filament resulted in a bond that was maximally stable at 8 pN, with a mean lifetime (12 sec) 10 times as long as the mean lifetime when force was applied toward the barbed (+) end. A computational model of actin dynamics near adhesions suggests that the directionality of the vinculin-F-actin bond can potentially establish long-range order in the actin cytoskeleton through a polarity-sorting effect, one that may reinforce persistent cell migration. We suggest that the recruitment of vinculin to cell-cell adherens junctions (AJs) may help the cell establish an actin organization conducive to AJ stability. This view is supported by recent results from the optical trap assay, which indicate that both the load- and direction-dependence of the bond between the cadherin-catenin complex and F-actin are dramatically altered by the presence of vinculin.

**93-Plat**

**3D Matrix Architecture Regulates Cell Migration through Degradability**

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Cells rely on four core biophysical processes to interact with and migrate through 3D extracellular matrix: adhesion, contractility, matrix remodeling, and cytoskeletal dynamics. Yet, the role of local matrix structure in modulating these biophysical processes and migration phenotype is incompletely understood. Here, we used novel matrix engineering techniques to vary matrix architecture independently of stiffness and density and then characterized the coordination of the four key biophysical processes and resulting migration outcomes. Processes were quantified in HT-1080 cells using: i) fluorescence recovery after photobleaching (FRAP) to study actin dynamics in cellular protrusions labeled with LifeAct, ii) traction force microscopy (TFM) of matrix-embedded beads to study adhesion and contractility, and iii) fluorescent dye quenched (DQ) collagen to study MMP activity. Cells embedded in matrices with small pores and short fibers produced smaller and shorter lived protrusions compared to cells embedded in matrices with longer, more bundled fibers. Actin FRAP studies revealed that this difference in protrusion lifetime was not caused by differences in actin polymerization rates. TFM and protrusion-ECM displacement correlation showed that in short fiber architectures, cells fail to properly bind and pull on the surrounding matrix. Interestingly, DQ collagen degradation assays showed that cells in the short fiber architecture locally degrade collagen more than cells in longer fiber architectures. Preliminary results suggest that this increased collagen degradation is accompanied by increased collagen internalization. Gene expression analysis revealed that cells in this condition upregulate uPARAP, a mannose receptor responsible for internalization of collagen fragments. Subsequently, these cells switch into a collective migration phenotype and form multicellular structures, whereas cells in long fiber matrices migrate as single cells. Our results suggest that collagen architectures with short fibers promote the upregulation of a collagen degradation and internalization pathway that triggers changes in migration phenotype.

**94-Plat**

**Tumor Invasion through Hyaluronic Acid Matrices is Mediated by CD44-Dependent Microtentacles**

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Glioblastoma (GBM) is a highly malignant primary brain cancer that is challenging to treat due to resistant and diffusely invasive tumor cells. The transmembrane receptor CD44 directly facilitates tumor cell invasion by engaging hyaluronic acid (HA) in brain matrix. Despite the acknowledged importance of CD44 as a mechanistic driver and potential therapeutic target in GBM and other tumors, CD44-based motility is poorly understood. As a whole, little is known about how cells navigate nonfibrillar 3D matrices such as the