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Creating Free-Standing Lipid Bilayers on Fused Silica Substrates with Nanograting Structure

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Supported lipid bilayer membranes have been widely used to study protein-lipid membrane interactions. However, the membrane physical properties are often influenced by the solid support, which may affect the interested interactions. Some previous studies have created free-standing bilayers by depositing unilamellar vesicles on the pores with sizes smaller than the vesicles or by using shear-driven bilayers to span over nanopores at high pH. However, these methods may not form continuous bilayers in a large scale or may not apply to various types of lipids. Here, we developed a theory, based on extended-Derjaguin-Landau-Verwey-Overbeek (DLVO) theory and Helfrich bending theory, to estimate the appropriate conditions for creating free-standing lipid bilayers on solid supports with nanograting structure. We calculated how the buffer conditions, lipid types, and the solid support geometry can influence the affinity between the lipid membrane and the solid support, and the bending energy of the lipid membrane. When the bending energy dominates the entire energy of the system, we predicted that the membrane would form free standing bilayers above the trenched area of the nanograting solid support. On the other hand, if the support-membrane affinity dominates, the lipid bilayer can easily bend and follow the contour of the solid support. In order to tune the relative size of the support-membrane affinity, we prepared buffers with a series of ionic strength (from 5 mM to 100 mM NaCl) and fused silica substrates with periodic 100 nm and 200 nm nanograting structure. The characterization of membrane states by fluorescence recovery after photobleaching measurement shows that our experiment results well match the theoretical predictions and that the developed theory could be used to predict the suitable conditions for the free-standing bilayers to form on solid supports with various kinds of geometry.

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Microfluidic Fabrication of Giant Unilamellar Lipid Vesicles with Controlled Microdomain Formation

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Conventional methods for vesicle production lack the uniformity in size and composition required for many scientific and technological applications. To overcome these limitations, we present a microfluidic approach for the production of giant unilamellar vesicles (GUVs) using monodisperse water-in-oil-in-water (W/O/W) double emulsion drops as templates. The middle oil phase is a mixture of the lipids that ultimately form the GUV membranes, dissolved in a mixture of a highly-volatile good solvent and a less volatile poor solvent. The shell formed by the middle phase around the aqueous double emulsion core is less than a micron thick, much smaller than in typical double emulsion templates; this enables the fabrication of GUVs that contain minimal residual solvent within their membranes. The evaporation of the good solvent forces the bad solvent to dewet from the double emulsion drops, forming GUVs that stably encapsulate the material within their cores. By carefully controlling the lipid composition of the middle phase, we show that lipid microdomains form during the dewetting process. Our work thus demonstrates a straightforward approach to GUV fabrication that enables control over the GUV size, lipid composition, and the formation of microdomains within the GUV membrane.

Platform: Cell Mechanics and Motility I

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Cell Reorientation under Cyclic Stretching

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Mechanical cues from the extracellular microenvironment play a central role in regulating the structure, function and fate of living cells. Nevertheless, the precise nature of the mechanisms and processes underlying this crucial cellular mechanosensitivity remains a fundamental open problem. Here we provide a novel framework for addressing cellular sensitivity and response to external forces by experimentally and theoretically studying one of its most striking manifestations- cell reorientation to a uniform angle in response to cyclic stretching. We first show that existing approaches - which are focused on the cell cytoskeleton - are incompatible with our extensive measurements of cell reorientation. We then propose a fundamentally new theory, that shows that energy minimization, at the level of the cell-substrate focal adhesion sites, drives the reorientation process. The overall reorientation dynamics are entirely controlled by two constant cell parameters which can be independently measured; a focal adhesion anisotropic elastic parameter - controlling the final alignment angle - and molecular kinetic rates of focal adhesions - governing the reorientation timescale. We demonstrate that the theory is in excellent quantitative agreement with the complete temporal reorientation dynamics of individual cells, measured over a wide range of experimental conditions. It, moreover offers new venues for predicting and controlling cell behavior in response to mechanical cues from the microenvironment.

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Compression, Volume and Proliferation Arrest

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Mechanical cues can influence tumor growth and cell proliferation (Jain, 2012; Fletcher, 2012, Käs, 2010). In particular, we showed in previous studies that a constant mechanical stress applied onto multicellular spheroids inhibits their growth reversibly by inhibiting cell proliferation without an effect cell death. Although it seems clear that mechanical stress can affect cell proliferation, it remains unclear how the cells feel their mechanical environment. We propose that the cell volume could be such a sensor.
Macroscopically, we observed that when we apply a compressive stress onto spheroids, their volume decreases faster than the typical time required for protein synthesis or cell division. Interestingly, this decrease is strongly correlated with the drop of the spheroid growth rate observed on longer time scale.
We developed a novel approach that enables us to have access to the cell diameter inside the spheroid. We showed that on very short timescale, the volume reduction of the spheroid correlates with a cell volume reduction inside the spheroid, mostly in the center. Using immunofluorescence staining, we show that this reduction is followed by and correlated to an over expression of the cyclin-dependant kinase inhibitor p27(Kip1). On longer timescale, the over expression of p27(Kip1) correlates with the drop of cell proliferation inside the spheroid. We believe that this volume reduction triggers a biochemical sequence that arrests the cells in the cell cycle. By combining flow cytometry and Western blots, we observe that the cells are indeed blocked at the level of the restriction point.
We have obtained the same results using 5 different cancer cell lines suggesting that regulation of the cell cycle through mechanical stress is a general phenomenon. This phenomenon strongly supports a conserved mechanism by which cells can control their proliferation by controlling their volume.

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The Evolution of Mechanical Properties of Differentiating Stem Cells is Fate- and Function-Dependent

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Although cellular mechanical properties are known to alter during stem cell differentiation, understanding of the functional relevance of such alterations is incomplete. Here, we show that during the course of differentiation of human myeloid precursor cells into three different lineages, the cells alter their viscoelastic properties, measured using an optical stretcher, to suit their ultimate fate and function. Myeloid cells circulating in blood have to be advected through constrictions in blood vessels, engendering the need for compliance at short timescales ($<$ seconds). Intriguingly, only the two circulating myeloid cell types have increased short time scale compliance and flow better through microfluidic constrictions. Moreover, all three differentiated cell types reduce their steady-state viscosity by more than 50% and show over 140% relative increase in their ability to migrate through tissue-like pores at long time-scales ($>$ minutes), compared to undifferentiated cells. These findings suggest that reduction in steady-state viscosity is a physiological adaptation for enhanced migration through tissues. Our results indicate that the material properties of cells define their function, can be used as a cell differentiation marker and could serve as target for novel therapies.

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Viewing Nuclear Deformation with Sideways Microscopy

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Structural information related to rearrangement of the cytoskeletal and nucleoskeletal structure, induced strains, and biochemical distributions are metrics for understanding cell response. However, structural information during applied stress is limited by our ability to image the cells under load. In order