topography imaging with high resolution. Here we quantitatively compare the imaging capabilities of SICM with those of atomic force microscopy (AFM). By imaging microvilli on live epithelial cells, we found that sample stiffness limits the lateral resolution in the case of AFM but not in the case of SICM. Time-lapse investigations of microvilli dynamics were possible with both AFM and SICM, but SICM showed a better long-term stability. We conclude that SICM has advantages over AFM in terms of image quality, noninvasiveness, and insensitivity to the cells' elastic properties.

1611-Plat
Correlative AFM-FLIM Measurements in Living Cells, Tissues and in Solar Cell Materials
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We demonstrate the correlation of structural and mechanical information from atomic force microscopy (AFM) with functional information from fluorescence lifetime imaging (FLIM), in the same field-of-view and applied to living cells, tissues and even to solar cell materials. Some AFM systems can be directly aligned with FLIM systems on inverted microscope frames to perform AFM from above and FLIM from below the coverslip. This technique is applied on living cells to probe for stiffness changes (measured by AFM) introduced by the aggregation of certain proteins (measured by FLIM). Similarly, viscoelastic properties of biological tissues can be quantitatively resolved into their viscous component (by FLIM) and elastic component (by AFM) using this technique. In the field of perovskite solar cell research, this correlative information can reveal how structural defects on a perovskite grain relates to the photoluminescence lifetime properties. We demonstrate how correlative AFM-FLIM can inform materials scientists both the structural and functional impact of different sample preparations (changing grain size, chemical composition, atmosphere, sample illumination). We thus establish the versatile potential of correlative AFM-FLIM in biological and materials science research and discuss its limitations and caveats.

Platform: Membrane Dynamics and Curvature

1612-Plat
Transmembrane Beta-Barrel Proteins Rigidify the Bacterial Outer Membrane
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Bacteria must resist an onslaught of mechanical stresses originating from the native environment, such as those from turgor pressure and cellular adhesion. Recent studies suggest that an important load-bearing component of the cell envelope in Gram negative bacteria is its outer membrane. Despite this important role, the relationship between membrane composition and mechanical stability in the outer membrane has not been established. An additional complication arises from the spatial heterogeneity of the outer membrane, which is an emergent property resulting from a time dependence on the expression of outer membrane proteins (OMPs) and the confined diffusion of these proteins in the outer membrane. Because local variations in outer membrane composition will impact bacterial mechanobiology, we have developed a theoretical and computational framework for understanding how these proteins react to membrane stress and for asking directed questions into their role in altering membrane rigidity. Our analysis indicates that OMPs are the most rigid material component of the bacterial cell envelope by several orders of magnitude. These findings suggest that OMPs are essential for rigidifying the outer membrane, enabling it to serve as a mechanical barrier for the cell. Additionally, we have determined that OMPs change both their radius and height in response to mechanical stress. Altogether these results demonstrate that the outer membrane is inadequately described by the fluid mosaic model, and instead should be visualized as a wall consisting of OMP bricks connected by lipid mortar. This study has provided substantial insight into understanding how composition changes the ability of the outer membrane to serve as a load-bearing structure in the cell and reveals fundamental insights into molecules essential for bacterial physiology.

1613-Plat
***In Vivo* Dynamics and Phase State of Natural Lipid Droplets**
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All organisms store lipids as energy resource for metabolism. Such lipids are accumulated in the form of intracellular droplets. The structure of the lipid droplet is complex. The hydrophobic core represents a mix of un- and saturated triglycerides, enclosed by an amphiphilic shell of phospholipids with embedded proteins. Uncovering intrinsic biophysical properties of lipid droplets in living cells of various organisms is crucial for metabolism manipulation or reducing lipid storage. The type and organization of lipids affect their phase state and microscopic dynamics in the droplet. We characterized lipid droplets in the cells of fresh human and porcine subcutaneous fat tissues, as well as yeast cells using quasi-elastic neutron scattering, probing the molecular motions in a time scale of 6-400 picoseconds and a length scale of 3-20 Angstroms. The detected two-component dynamics in the droplet is associated with lipid unrestricted diffusion ($D \sim 0.006$ A^2/ps) and motions of its hydrocarbon chains ($D \sim 0.2$ A^2/ps) in a restricted volume (5-12 A). The dynamics of lipids is significantly reduced below 305 K and 266 K in porcine and human tissues, respectively, due to a first-order phase transition of lipids from fluid to gel (similar to synthetic lipid bilayer). The lipid phase change in human tissue is confirmed and enhanced at applied pressure of 90 bars. However, lipid droplets in the yeast cells remain in a fluid-like state within range of 280-310 K. We believe that phase behavior of lipid droplets is different in the cells of tissues and microorganism due to lipid composition, which was investigated by mass spectrometry. Lipid packing in the droplet is tight in the tissues, having mostly saturated lipids, and loose in the yeast droplet, having equal proportions of un- and saturated lipids.

1614-Plat
Membrane Curvature Generation through Asymmetric Desorption of Pi(4,5)P_2
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Phosphatidylinositol-4,5-bisphosphate [PIP_2] is an important signaling lipid in the eukaryotic cell plasma membrane, playing an important role in diverse signaling processes. The headgroup of PIP_2 is highly negatively charged with a high estimated critical micellar concentration compared to housekeeping phospholipid analogs. Given the crucial role played by PIP_2 in cellular processes, it is imperative to study its localization, interaction with proteins, and membrane shaping properties. Biomimetic membranes have served extensively to elucidate aspects including protein-lipid interactions, lipid lipid interactions, and membrane mechanics. Incorporation of PIP_2 in the biomimetic membranes, however, has at times resulted in discrepant findings described in the current literature. With the goal to elucidate the mechanical consequences of PIP_2 incorporation, we have studied the desorption of PIP_2 from biomimetic giant unilamellar vesicles [GUVs] by means of a fluorescent marker. The decrease in fluorescence intensity with the age of the vesicles suggested that PIP_2 lipids were being desorbed from the outer leaflet of the membrane. To ask the question whether this desorption was symmetric, the vesicles were systematically diluted. This resulted in an increase in the number of internally tubulated vesicles suggesting that the desorption was asymmetric. In order to quantify this effect, we obtained the spontaneous curvature that was being generated due to this asymmetric desorption. The deduction was made by pulling narrow membrane tethers from aspirated vesicles using optically trapped beads. The effect on the tether pulling force resulting from a variable membrane tension allowed us to quantify the generated spontaneous curvature. Given that the local concentration of PIP_2 in biological membranes is variable, their spontaneous desorption can result in generating highly curved structures which can serve as an initiator for signaling events.

1615-Plat
Measuring Hindered Diffusion Dynamics in Live Cell Plasma Membranes with Confocal and Super-Resolution Imaging
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The cellular plasma membrane is a highly heterogeneous structure organised on nano-scales and displays a crucial interaction platform for proteins, lipids and soluble ligands. Investigating the molecular membrane organisation by measuring diffusion dynamics offers a better understanding of its biological function.