physiological pH range. In the presence of PI two opposing effects lead to a net charge that is similar to the charge found for PI(4,5)P$_2$ in the absence of PI. The enhanced negative charge in the membrane due to the presence of PI leads to an increased PI(4,5)P$_2$ protonation (reduced charge). This effect is opposed by PI/PI(4,5)P$_2$ hydrogen bond formation which results in increased deprotonation of the phosphomonoester groups. As a result, PI appears to have a minor effect on PI(4,5)P$_2$ ionization, however, fluorescence microscopy measurements of PC/PI/PI(4,5)P$_2$ GUVs show a pronounced effect on PI(4,5)P$_2$ morphology.

1120-Plat
Single Molecule Study of the Processive Ras/SOS Interaction
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Ras is a membrane-anchored small GTPase protein that plays an important role in regulating essential cellular functions such as proliferation, differentiation, and apoptosis. Its deregulation is a hallmark of many cancers and developmental defects. Son of Sevenless (SOS) is a guanine nucleotide exchange factor (GEF) enzyme that activates Ras by catalyzing the exchange of GDP to the GTP in Ras. Previously, we have shown that in addition to the catalytic site, SOS has a catalytically inactive distal Ras-binding site, which allows SOS to localize and up-concentrate at Ras containing membranes, dramatically increasing the Ras-GDP turnover rate. Together, the catalytic and allosteric sites form the catalytic core of SOS (SOc). The existence of the extra binding site for Ras also raises the question of whether SOS is processive, capable of generating remaining surface bound via the distal binding site while catalyzing the nucleotide exchange of multiple Ras. In this study we employ various fluorescence-based methods such as Fluorescence Correlation Spectroscopy (FCS) and Total Internal Reflection Fluorescence (TIRF) microscopy to study the interactions of SOS with lipid bilayers (SLBs) to demonstrate that SOc is processive. Single molecule tracking of SOc allows us to correlate the diffusion behavior between Ras and SOc and further confirms the specific interaction. By confining individual SOc enzymes to micron-scale two-dimensional Ras-functionalized SLB "reaction chambers" we can simultaneously monitor enzymatic activity from hundreds of single SOc, probing the variability in catalytic rate and processivity within the enzyme ensemble. Our data indicates that SOc has a broad range of processivities ranging from a few up to a thousand turnovers.

1121-Plat
Partitioning of Cholesterol and Ganglioside GM1 in Phase Separated Lipid Bilayers Imaged by Secondary Ion Mass Spectrometry
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An exquisite molecular machine, the mitotic spindle, organizes and separates chromosomes during cell division. To uncover how this machine operates we are reconstituting spindle functions and applying advanced biophysical tools for manipulating and tracking individual molecules. My talk will focus on kinetochores, the multi-protein organelles that link chromosomes to spindle microtubules, thereby driving chromosome movement. Kinetochores also perform vital regulatory activities that ensure the accuracy of mitosis. For example, a popular view is that tension selectively stabilizes proper kinetochore-microtubule attachments. Proper (‘bi-oriented’) attachments come under tension from opposing microtubules, and this tension is thought to cause their stabilization. Conversely, improper attachments lack tension, so they fail to become stabilized and detach quickly, giving another chance for proper attachments to form. We recently used laser trapping-based assays to show that tension stabilizes attachments between individual kinetochore particles purified from budding yeast and single microtubule tips. The tension in this case acts directly on the kinetochore–microtubule interface, causing it to adopt a more stable configuration in a manner similar to a Chinese ‘finger trap’ toy, or to the catch bonds that enhance cell-cell adhesion. Now we are investigating how this direct stabilization works in tandem with phosphoryregulation. Aurora B kinase is known to promote mitotic accuracy through phosphorylation of kinetochore subcomplexes. Current efforts toward understanding how phosphorylation affects kinetochore function will be discussed.