such membrane reorganization alters the effects of PIP2 on the target proteins gelsolin and DrrA.

Comparison between experimental and numerical phase diagrams suggests that a simplified electrostatic model can predict Ca<sup>2+</sup>-driven formation of PIP2 clusters, but cannot account for the difference between Ca<sup>2+</sup> and Mg<sup>2+</sup> in condensing PIP2-containing membranes. Differences among Ca<sup>2+</sup>, Mg<sup>2+</sup> and multivalent polyamines in membrane condensing were revealed experimentally and related to differences in their dehydration enthalpies. Ca<sup>2+</sup>-induced perturbation of PPI-protein interactions was assayed by monolayer insertion studies using a PI4P-binding protein, DrrA. Taking advantage of its unique biphasic effect on monolayer surface pressure, in which specific insertion can be isolated from non-specific adsorption, we show that Ca<sup>2+</sup> suppresses the specific insertion of DrrA in a concentration-dependent manner. The perturbation of PIP2protein interactions induced by cholesterol-mediated phase segregation was probed by measuring the inhibition of gelsolin's actin filament severing activity by PIP2-containing vesicles. Cholesterol-mediated phase segregation enhances the inhibition of gelsolin by PIP2, and this effect correlates with changes in membrane ordering. This result suggests that PIP2-protein interaction depends not only on global PIP2 concentrations but also on PIP2 lateral distribution without changes in lipid synthesis or degradation. The results of this work shed light on the links between PIP2 signaling and dynamic local response at the cell membrane/cytoskeletal interface.

## 3614-Pos Board B342

Dynamic Association and Dissociation of the Tumor Supressor PTEN to Model Membranes

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The tumor suppressor gene, *Phosphatase and tensin homolog deleted on chromosome 10* (PTEN) encodes a 403-amino acid protein that is mutated or deleted in a variety of human cancers. It binds to the plasma membrane to dephosphorylate the 3-position of phosphatidylinositol-3,4,5-trisphosphate producing phosphatidylinositol-4,5-bisphosphate (PI[4,5]P<sub>2</sub>). PTEN is an agonist to PI 3-Kinase, thereby inhibiting the PI3K/Akt signaling pathway and controlling cell proliferation and survival. PTEN membrane association depends strongly on the composition and lateral distribution of the lipids in the membrane. Using kinetic methods, we aim to examine this multi-step process. Stopped-flow and steady state fluorescence experiments support our hypothesis that the association of PTEN to the plasma membrane is synergistic; depending on both electrostatic interactions between the protein and anionic lipids like phosphatidylserine (PS) as well as specific binding to PI(4,5)P<sub>2</sub> to form a stable protein-membrane complex. In addition, our results suggest that other lipids found in the inner leaflet of the plasma membrane might participate in PTEN binding.

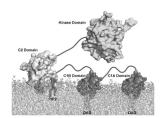
Furthermore, we use single molecule TIRF microscopy to determine the membrane binding and lateral diffusion constant of wtPTEN on supported lipid bilayers with differing lipid compositions. The information gained from these two techniques describes the dynamic and equilibrium behavior of wtPTEN binding to various binary and tertiary lipid compositions.

# 3615-Pos Board B343

# A Tale of Two Domains: Different Roles of C1A and C1B Domains in PKC Interactions with Membranes Jianing Li

Department of Chemistry, The University of Chicago, Chicago, IL, USA. Protein Kinase C (PKC) is an important kinase family that controls major signaling pathways and has been associated with many diseases. Although conventional PKCs (for example,  $\alpha PKC$ ) are known to be activated upon two regulatory domains, C1A and C1B, binding to lipid activators in the membrane, key interactions and dynamics involved in the binding process are hard to study solely with experiments. With large-scale molecular dynamics simulations, we have studied the  $\alpha PKC$  C1A and C1B domains in water and in various membranes, to understand their roles in PKC activation. Our

simulations, for the first time, reveal the specific and non-specific interactions between the C1A/C1B domain and the membranes. Our results show that these tandem domains, although similar in structure, are different in terms of affinity to anionic lipids and response to lipid activators. Additionally, membranes are found to play a particular role to stabilize the C1A and C1B conformations. In gen-



eral, our study suggests a PKC working model supported by further experimental evidence, which provides molecular-level understanding as a foundation for future investigations to modulate PKC activation through the C1A and C1B domains.

#### 3616-Pos Board B344

Defining the Roles of Various Lysines and Arginines in Amot Lipid Binding

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One of the defining traits of cancerous cells is proliferation. We are focusing on the proliferation of mammary cells. As an adaptor protein, the Amot membrane binding event is key to the localization and sorting of proteins responsible for cellular differentiation, proliferation, and migration. The Amot coiled-coil homology domain (ACCH) is a lipid-binding domain responsible for affinity and binding to endothelial membranes. Our working hypothesis is that the ability to modulate Amot lipid-binding will lead to means to prevent ductal cell hyperplasia progression into breast cancer tumors. We will determine which residues are responsible for lipid-binding by changing positively charged lysine and arginine into uncharged or negatively charged amino acids. Those mutations which show a significant decrease in lipid-binding will then be used to determine their down-stream effects in human cells. The laboratory has screened approximately 40 of these mutations using a liposome binding assay. This assay mimics how the protein binds with the cell membrane by using an in vitro mixture of lipids similar to that seen in endothelial cells. Forster resonance energy transfer (FRET) was used to confirm significant decreases in lipid binding of ACCH mutants selected from the liposome binding assay, as energy transfer only occurs when the tyrosines in the protein and the Dansylated liposome are in close proximity to each other. Based on these two screens we have narrowed the list to seven mutants that have a significant decrease in lipid binding. Currently, FRET is being used to determine the lipid binding coefficient for each mutant of interest. Mutants deemed important from this study will then be transformed into human cells to study their effects on cell polarity, signal transduction, cell shape, and cellular proliferation.

# 3617-Pos Board B345

How Membrane Curvature Drives the Up-Concentration of N-Ras Proteins to Ordered Lipid Domains: Correlation of In Vivo and In Vitro Experiments with Mean Field Theory Calculations and Coarse Grain Simulations Nikos S. Hatzakis¹, Jannik Larsen¹, Martin B. Jensen¹, Vikram K. Bhatia¹, Søren L. Pedersen², Heidi Koldsoe³, Philip Fowler³, Mark J. Uline⁴, Igal G. Szleifer⁵, Thomas Bjornholm¹, Mark Sansom³, Knud J. Jensen², Dimitrios Stamou¹.

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Sorting and trafficking of membrane-anchored Ras GTPases is critical for signaling and is believed to rely on their preferential portioning in ordered lipid-protein membrane domains (1). However studies in vitro have failed to quantify the preferential partitioning of full length Ras proteins into the liquid ordered phase(2), indicating that a physical principle underlying sorting of Ras is missing. We recently showed that lipidated proteins localize to highly curved membranes in vitro(3, 4). Here we provide a mechanistic insight on how membrane curvature can drive N-Ras sorting.

Combining the results of our in vitro assays, measurements on single vesicles, with in vivo studies, hypo-osmotic swelling of cells that flattens curved membrane regions, revealed that: a) N-Ras is preferentially recruited in areas of high membrane curvature and b) membrane curvature is the enabling factor underlying the selective partitioning of NRas in ordered domains. The combined readout of mean field theory calculations and coarse grain simulations provided a mechanistic insight on preferential partitioning in highly curved areas, via the changes in lateral pressure of the outer monolayer when curving an ordered versus a disordered membrane. In addition to providing the first biophysical sorting mechanism for Ras validated by both in vitro and in vivo measurements, our data indicate that membrane curvature may act as a generic cue underlying trafficking and sorting of multiple lipidated proteins.

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