Determining the Gaussian Curvature Modulus of Lipid Membranes in Simulations: A Comparative Study via Global Shape Transformations and Local Stress Distributions

Mingyang Hu1, Djurre H. de Jong2, Siewert J. Marrink3, Markus Deserno1.
1Carnegie Mellon University, Pittsburgh, PA, USA, 2Groningen Biomolecular Sciences and Biotechnology Institute & Zernike Institute for Advanced Materials, University of Groningen, Groningen, Netherlands.

The Gaussian curvature modulus $k$-bar matters for many important biological processes that involve topological and/or boundary changes of cell membrane, e.g. endo- and exo-cytosis. However, only sparse experimental measurements of have been reported, and even fewer accurate results obtained via computer simulations exist. Here, we propose a novel approach to determine $k$-bar in silico by monitoring patch-closure processes of pre-curved circular bilayers.

Applying this method to two different coarse-grained (CG) membrane models, namely the generic Cooke model [1] and the more finely-resolved and systematically parameterized MARTINI model [2], we find elastic ratios between the two curvature moduli, $k$-bar/$k$, in the range between $-0.85$ and $-1.05$, in line with previous estimates in literature. Yet, for the same systems studied, another well known method, which derives the material parameters from moments of the lateral stress profile, produces results that are neither in accordance with the patch-closure method nor, in fact, physically plausible. One noted exception is the MARTINI model that takes disproportionately long to equilibrate. It has been suggested to instead simulate curved membrane tethers and measure their axial force [2], but this method has technical difficulties for models that are not strongly coarse grained. Here we consider an alternative strategy recently proposed by Noguchi [3], namely, measuring the response of a membrane to buckling. We provide highly accurate analytical expressions to analyze parallel and perpendicular stresses, valid far into the highly nonlinear regime, and we derive fluctuation correction terms. Using a variety of membrane models, ranging from strongly coarse grained to atomistic, we show that highly accurate values of the mean curvature modulus can be obtained with remarkable computational ease. The technique also permits to check whether deviations from quadratic curvature elasticity are important, and it offers insights into the thermodynamics of the bending energetics itself.

References:

Opening Barrier Renormalization by Membrane Local Curvature Fluctuations around the Mechanosensitive Channel: Analytical Expression

Anna A. Drozdova, Sergei I. Mukhin.

We calculate renormalization of the opening energy barrier of mechanosensitive channel by local curvature fluctuations: $U = U_0 + U_4 + U_6$, Eq. (1). $P_i(z,H)$ and $P_2(z,H)$ are lateral pressure profiles in the flat and curved bilayer respectively, $H$ and $A(z)$ are local mean curvature and the difference between open/closed section cross-section profiles. Function $P_i(z,H)$ was derived in [1] analytically using flexible strings model of lipid chains [2]. The average over fluctuations of the membrane’s shape $h(x,y)$ in the Monge representation [3] uses Boltzmann factor of a curved conformation $exp(-F(h,T))$ with elastic free energy functional $F(h)$ taken in the Helfrich form, Eq. (3); $k$ and $\tau$ are bending rigidity and surface tension. Comparing our results with molecular dynamics data [4] for curved bilayers, we estimate relative importance of the contributions of the lipid tails and phospholipid headgroups to the energy barrier $U$. References:
protein coat. The assembly of the COPI complex is initiated by the GTPase Arf1 in a nucleotide-dependent manner. After GDP/GTP exchange, soluble Arf1 binds to the COPI coat. To visualize the formation of its myristoylated N-terminal amphipathic helix (myrAH) into the proximal leaflet of the Golgi membrane. The subsequent liberation of transport vesicles requires the full COPI complex and has been observed in vivo and in vitro. However, the role of Arf1 in the process of curvature induction has not been fully elucidated. To study the effects of Arf1 on membrane morphology we have evaluated binding and incorporation of recombinant S. cerevisiae Arf1p into lipid mono- and bilayers. Using a Langmuir film balance setup and binding assays with artificial liposomes, we observe a myristoylation-dependent increase in membrane surface area upon addition of Arf1p. Confocal laser scanning microscopy and cryo electron microscopy reveal highly curved membrane structures upon incorporation of myristoylated Arf1p. Our results support a mechanism of positive curvature induction based on the bilayer couple theory.

1259-Pos Board B151
Plasmamembrane Organization in Signaling
Rolf Harkes, Thomas Schmidt.
Leiden universiteit, Leiden, Netherlands.
The composition of the plasma membrane has long been modeled as a fluid mosaic. Singer and Nicolson, 1972) Studies in the last few years have identified microdomains like lipid rafts and caveolae that constrain membrane proteins within a small region of the cellular plasma membrane. These domains facilitate anchoring of different signaling proteins, like H-Ras, that has been shown to co-localize with nano domains upon activation (Lommerse et al. 2005; Roblth et al. 2004). It is believed that these nanodomains function as important platforms for a multitude of signaling cascades that are initiated at the plasma membrane. Given that many of the transmembrane signals will need a coordinated domain organization, it is of importance to investigate properties like size, shape, stability and their mutual interaction.

Here we transfected 3T3-cells to express the membrane anchor of H-Ras linked to Dendra2 or mEos2. Photo Activated Localization Microscopy (PALM) and Stimulated Emission Depletion Microscopy (STED) were used to make high resolution images of H-Ras distribution on the membrane as a probe for inner membrane domains. The GPI-anchored protein CD59 is used as a probe for the outer membrane domains to investigate colocalization of internal and external membrane domains. Using a SNAP-tag we covalently link Alexa647n to CD59 and image with direct stochastic optical microscopy (dSTORM).

1260-Pos Board B152
Polyunsaturated Free Fatty Acids Inhibit Coupling Between the Er Ca++ Sensor STIM1 and the Ca++ Channel Protein Orai1 Stimulated by Ige Receptor Crosslinking or Thapsigargin in a Process that Correlates with Disruption of Lipid Order
David Holowka, Marek Korzeniowski, Barbara Baird. Cornell University, Ithaca, NY, USA.
Polyunsaturated fatty acids (PUFAs) have been found to be effective inhibitors of cell signaling in numerous contexts. We find that acute addition of these PUFAs in micromolar concentrations, including linoleic acid, substantially inhibits Ca++ responses in mast cells stimulated by antigen-mediated crosslinking of FceRI or by the SERCA inhibitor, thapsigargin. In addition to inhibiting store-operated Ca++ entry, linoleic acid inhibits antigen-stimulated release of Ca++ from intracellular stores and granule exocytosis. Using AcGFP-Orai1 and STIM1-mRFP to monitor stimulated coupling in COS7 cells by FRET in a fluorescence assay, we find effective inhibition of this association by linoleic acid added either before or after stimulation by thapsigargin and ATP. Store-operated Ca++ entry is inhibited by linoleic acid, does not inhibit FRET or Ca++ signaling when added at the same concentrations. We showed previously that giant plasma membrane vesicles exhibit liquid order/liquid disorder phase separation at low temperatures, and we find that stearic acid enhances whereas linoleic acid prevents this phase separation. Our results suggest that PUFAs interfere with STIM1-Orai1 coupling by a mechanism that correlates with inhibition of membrane order heterogeneity in cells.

1261-Pos Board B153
Techniques for Direct Imaging of Nanoparticles in the Live Cell Plasma Membrane
Mario Brambrueher, Christoph Salzelechner, Julian Weghuber, Rene Platzner, Johannes B. Huppa, Gerhard J. Schutz.
1Vienna University of Technology, Vienna, Austria, 2Upper Austria University of Applied Sciences, Wels, Austria, 3Institute for Hygiene and Applied Immunology of the Center for Pathophysiology, Infectionology and Immunology, Vienna, Austria, 4Institute for Hygiene and Applied Immunology of the Center for Pathophysiology, Infectioniology and Immunology, Vienna, Austria, 5Institute for Hygiene and Applied Immunology of the Center for Pathophysiology, Infectioniology and Immunology, Vienna, Austria.
We recently developed a method termed TOCSSL (‘Thinning Out Clusters while Conserving Stoichiometry of Labeling’) which allows for the first time the direct imaging of nanoscopic stable platforms with raft-like properties differing in the live cell plasma membrane. Our method senses these platforms by their property to assemble a characteristic set of fluorescent marker/proteins/lipids on a time-scale of seconds. A special photobleaching protocol was used to reduce the surface density of labeled mobile platforms down to the level of well-isolated diffraction-limited spots, without altering the single spot