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*Published in:*  
Biophysical Journal

*DOI:*  
[10.1016/j.bpj.2015.11.3101](https://doi.org/10.1016/j.bpj.2015.11.3101)

**IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.**

*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
2016

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Baoukina, S., Ingolfsson, H. I., Marrink, S. J., & Tieleman, D. P. (2016). Curvature-Induced Lipid Sorting in Plasma Membrane Tethers. *Biophysical Journal*, 110(3, Suppl. 1), 580A-580A.  
<https://doi.org/10.1016/j.bpj.2015.11.3101>

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membrane tubules upon protein binding is sensitive to membrane composition, phase separation and free energy of protein binding. Taken together with a predictive theoretical model that is currently under development, we believe that these results significantly advance our current understanding of the thermodynamics of membrane bending by protein crowding.

#### 2863-Pos Board B240

##### A Detergent-Free Approach to Membrane Protein Research: Polymer-Bounded “Native” Nanodiscs

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Styrene-Maleic acid (SMA) copolymers have emerged as a powerful alternative to detergents for applications in membrane research [1]. Most notably, these amphiphatic polymers can be used to directly extract and purify membrane proteins from intact cells of different organisms in the form of “native nanodiscs”. These particles stabilize the protein in a near native environment comprising conserved native lipids as well as other membrane components and they readily allow for structural and functional characterization of the protein [2,3].

To evaluate the general applicability of SMA-mediated membrane protein solubilization, we employed a combined imaging and biochemistry approach using HeLa cells as a model. The results indicate that SMA solubilization of (human) cells is an all-or-none process that is not specific for any (sub) cellular membrane, as seen by the solubilization of all intracellular organelles that were tested. These findings suggest that SMA isolation is applicable to any membrane protein irrespective of which cellular membrane it resides in.

Since lipid properties strongly influence the solubilization process [4], we then tested whether SMA exhibits selectivity for certain lipids within a given membrane. To this end, we studied the effect of the polymer on model membranes with different lipid compositions. The results revealed a promiscuity of SMA with respect to lipid headgroups in homogeneous lipid mixtures. However, in phase-separating systems of fluid phases with either gel-phase or liquid-ordered phases it showed a distinct preference for lipids in the fluid phase. Implications for the solubilization of proteins from such membranes will be discussed.

[1] Dörr JM, et al., 2015, Eur Biophys J, submitted.

[2] Swainsbury DJK, et al., 2014, Angew Chem Int Ed Engl 53(44):11803-11807.

[3] Dörr JM, et al., 2014, PNAS 111(52):18607-18612.

[4] Scheidelaar S, et al., 2015, Biophys J 108(2):279-290.

#### 2864-Pos Board B241

##### “Anionic H-Bonds” Structure Two Simple Bilayers, One Natural

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“Anionic H-Bonds” Structure Two Simple Bilayers, One Natural Living membranes consist of bilayers of primarily anionic polar lipids. We describe two simple, single-chain lipid bilayers: pure oleate C<sub>18Δ9</sub> carboxylate, and a chlorosulfolipid (CSL), a C<sub>24</sub>, hexachloro-1,14-disulfate, from the fresh water (pH 4.3) alga, *Ochromonas danica*. Oleate bilayers are formed either from Na-oleate or from micelles of oleic acid. Both monolayer headgroups, carboxylate and sulfate, trap a H<sup>+</sup> between their oxyanion pairs, (“anionic H-bond”). In an aprotic medium they have strong H-bonds (<=20 kcal/bond), analogous to the maleate anion (JACS 2015, 137, 5730). The maleate anionic H-bond is stabilized by ring strain; here van der Waals interactions between the thin methylene chains, together with the bulk of both headgroups, compacts oxyanions forming the anionic H-bonds. *O. danica*’s CSLs’ sulfates, at C<sub>1</sub> and C<sub>14</sub>, form two sulfate sheets, one at C<sub>1</sub> between headgroup pairs and the other at C<sub>14</sub> in the low dielectric domain. There aren’t other lipids nor proteins in the *O. danica* surface bilayers. Thus each bilayer has four sulfate sheets, two at C<sub>1</sub>, C<sub>14</sub>. Computations show that chloro groups bond hydronium ions at near covalent bond strength in a hydrophobic domain. Inside each monolayer, the C<sub>14</sub>-sulfate sheet creates a negative field deep in the bilayer. Two chloros are on C<sub>2</sub> adjacent to the C<sub>1</sub>-sulfate surface. They attract hydronium ions to the surface bilayer, each brings water and H<sup>+</sup> to the C<sub>14</sub> sulfate sheet. Water stabilizes the sulfate sheet and H<sup>+</sup> forms anionic H-bonds between sulfates. These four strong sheets protect *O. danica* from osmotic bursting as do walls in prokaryotes.

#### 2865-Pos Board B242

##### Curvature-Induced Lipid Sorting in Plasma Membrane Tethers

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Membrane tethers are nanotubes formed by lipid bilayers. They are efficient structures for cellular transport and communication, and for storage of excess membrane area. Previous tether pulling experiments provided insights on membrane mechanical properties, and the curvature effects on phase behavior and distribution of coexisting phases. However, detailed information on tether properties and variations in composition is challenging to obtain experimentally due to the small diameters and dynamic nature of tethers.

Here we provide a molecular view on curvature-induced lipid sorting in plasma membrane tethers. We pulled tethers from an idealized plasma membrane model using molecular dynamics simulations with the coarse-grained Martini model. The membrane consists of 63 lipid types with an asymmetric distribution of components between the leaflets [JACS, 2014, 136, 14554]. The tethers are formed by applying an external constant force to a lipid patch in the direction normal to the bilayer plane [Biophys J, 1012, 102, 1866]. Pulling is performed both from the inner and outer leaflets, corresponding to the direction in and out of the cell, respectively. As a result of pulling, we observe redistribution of different lipid types along the regions of different curvature without macroscopic phase separation. Depending on the direction of pulling, the distribution of lipids and the tether properties differ.

#### 2866-Pos Board B243

##### Direct Observation of Ordered and Disordered Membrane Domains in B Cell Plasma Membranes using Multi-Color Super-Resolution Fluorescence Microscopy and Application to B Cell Receptor Signaling

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Many immune receptors are hypothesized to be correlated with domains of unique membrane composition, sometimes termed “lipid rafts,” which modulate activity of the receptor during the immune response. This compositional heterogeneity is hypothesized to be analogous to liquid ordered/ liquid disordered (lo/l<sub>d</sub>) phase separation observed in giant unilamellar vesicles and in vesicles harvested from the plasma membrane. However, their existence and behavior has been difficult to measure directly due to the small size of these domains and the small difference between the composition of the domain and the rest of the plasma membrane. Here, we utilize multi-color super-resolution microscopy (STORM and PALM) to quantitate the local density of various l<sub>d</sub> or l<sub>o</sub> preferring membrane probes around B cell receptor (BCR) clusters. We show in control measurements that l<sub>d</sub> or l<sub>o</sub> preferring membrane probes have differential partitioning around clusters of proteins having strong phase preference. Clusters of l<sub>o</sub> preferring cholera toxin subunit B are enriched in l<sub>o</sub> probe and depleted of l<sub>d</sub> probe. Inversely, clusters of an l<sub>d</sub> preferring transmembrane peptide are depleted of l<sub>o</sub> probe and enriched in l<sub>d</sub> probe. We apply this technique to BCR, which is thought to anchor a raft domain during antigen binding. We find that BCR clusters in chemically fixed and live cells are enriched in l<sub>o</sub> probe and depleted of l<sub>d</sub> probe, indicating a l<sub>o</sub>-like composition is anchored around BCR clusters. We find that this anchored domain is sensitive to the ambient temperature and receptor phosphorylation. These experiments also quantitate the contribution of membrane composition on the interaction between Lyn and the BCR. These results show that lipid-mediated forces can play important roles in organizing proteins during signaling processes.

#### 2867-Pos Board B244

##### High Resolution Imaging Atomic Force Microscope Study of Interactions at the Membrane-Fluid Interface

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The cell membrane is essential for all living systems, serving as a barrier between cells and their environment. It is typically composed of a lipid bilayer, containing embedded and/or anchored proteins that mediate different biological function such as energy conversion, signal transduction and solute transport [1]. To elucidate the basic structure of biological membranes it is