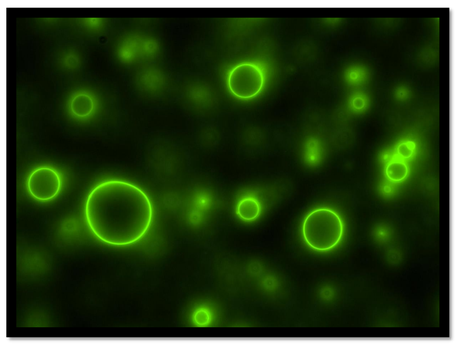
Lipid-Protein phase separation in giant unilamellar vesicles.

Juan Ureña, (Eli) Il-Hyung Lee
Assistant Professor, Department of Chemistry and Biochemistry, Montclair State University, NJ, USA
e-mail: leei@montclair.edu, website: http://www.lipidprotein.com

Abstract

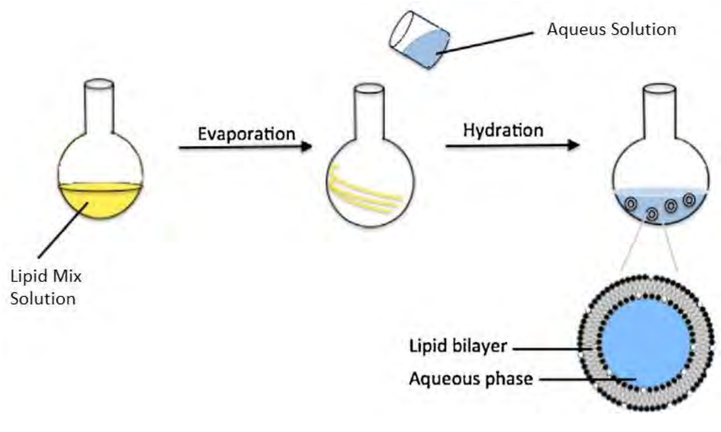
The cell membrane is the biological barrier that surrounds the cell and protects it from the outside environment; it is mainly composed of macromolecules that assemble and organize within itself. It has a well-documented property of demixing into composition domains. This phenomenon is known as lipid raft, in which lipids and protein organize within the cell membrane creating well-defined domains. Additionally, some multivalent binding proteins are known to form protein phase droplets or protein liquid condensates at a high enough concentration. Given that, this work intends to study how proteins anchored to the membrane organize themselves when lipid raft and protein domain coexist. For this purpose, we created giant unilamellar vesicles (GUVs) with various compositions to simulate the behavior of a typical cell membrane. We observed that homogeneous GUVs with 5% or lower Ni-DGS in their composition went from homogeneous to phase separation after being exposed to SUMO3-GFP, SUMO10His, and SIM10Cutproteins. Meanwhile, phase-separated GUVs with 10% or higher Ni-DGS in their composition went from phase separation to homogeneous under the same conditions. These results suggest cooperative phase separation in which the opposite driving force of protein crowding is competing with each other. These findings are certainly remarkable and may open the door to better understand the mechanisms in which biological macromolecules compartmentalize into microdomains in the cell membrane.

Epi-fluorescence microscope imaging



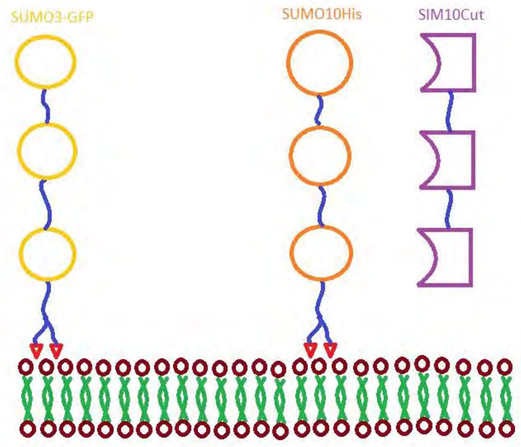
To analyze the Lipid-Protein interaction, a Nikon Eclipse Ti2 microscope system was utilized. Lasers in the range of 488 and 543 nm were used for excitation of GFP and TexasRed, respectively. This imaging technique allows to visualize biological process that is taking place in the membrane.

Gentle hydration GUV creation



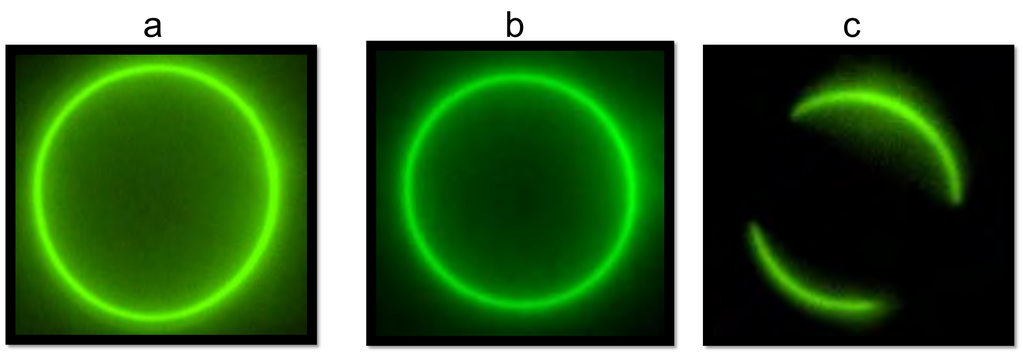
A mixture of lipids in a solution of chloroform was dried on a round bottom flask in a vacuum chamber for an hour. A lipid film was form on the bottom of the flask. 1ml of Sucrose solution was added and incubated overnight at around 40 degrees Celsius.

Schematic of protein organization on the lipid membrane.



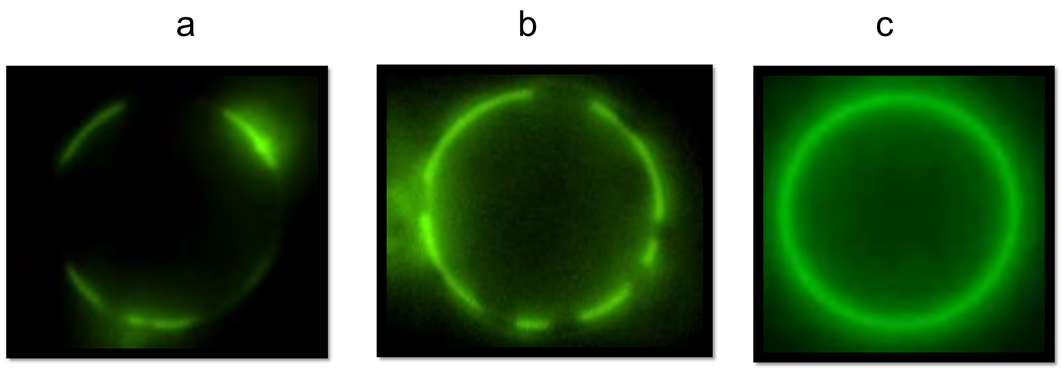
Schematic of a lipid membrane anchored protein system, using SUMO3-GFP, SUMO10His, and SIM10Cut proteins.

Homogeneous to Phase separated GUV



a. Homogeneous GUV.
b. Homogenous GUV after SUMO3-GFP protein exposure.
c. Phase Separated GUV after SUMO10His and SIMCut protein addition.

Phase Separated to Homogeneous GUV



a. Phase separated GUV.
b. Phase separated GUV after SUMO3-GFP protein exposure.
c. Homogeneous GUV after SUMO10His and SIMCut protein addition.

References:

1. Lee, I., Imanaka, M.Y., Modahl, E.H., & Torres-Ocampo, A.P. (2019). Lipid Raft Phase Modulation by Membrane-Anchored Proteins with Inherent Phase Separation Properties. ACS Omega, 4, 6551 - 6559.
2. Hyman AA, Simons K. Cell biology. Beyond oil and water--phase transitions in cells. Science. 2012 Aug 31;337(6098):1047-9. doi: 10.1126/science.1223728. PMID: 22936764.
3. Huang, William Y. C., Yan, Qingrong, Lin, Wan-Chen, Chung, Jean K., Hansen, Scott D., Christensen, Sune M., Tu, Hsiung-Lin, Kuriyan, John, and Groves, Jay T. Phosphotyrosine-mediated LAT assembly on membranes drives kinetic bifurcation in recruitment dynamics of the Ras activator SOS. United States: N. p., 2016. Web. doi:10.1073/pnas.1602602113.
4. Riham Gharib, Hélène Greige-Gerges, Sophie Fourmentin, Catherine Charcosset, Lizette Auezova, Liposomes incorporating cyclodextrin–drug inclusion complexes: Current state of knowledge, Carbohydrate Polymers, Volume 129, 2015, Pages 175-186, ISSN 0144-8617, https://doi.org/10.1016/j.carbpol.2015.04.048.