Examining lipid- protein domain phase-separation and behavior at the inter-membrane junction

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Abstract

Cell membranes are a vital part of all cells, the semipermeable lipid bilayer structure providing a key defense for its cell against the environment, while also responsible for signaling responses, cell-cell interactions, and transportation. Our immune system relies on the membrane's identification tags to recognize our cells and avoid attacking them. In addition, many viruses take advantage of the receptor molecules that sit on the cell's surface by binding to it with their fusion proteins to induce fusion between the two membranes. Interactions facilitated by the inherent fluidity of the heterogenous mixture lipid membranes, phase separation promoting large scale rearrangements based on small environmental stimuli changes. By using a simple model system to artificially mirror the way lipids and proteins organize under different conditions, we can study the complex spatial organization and molecular composition between these molecules at the inter-membrane junction. Our model proteins used in this research are SUMO10-His domains and SIM10MBPHis-Fluo domains, incubated with SLB (Supported Lipid Bilayer) and Giant Unicellular Vesicles (GUVs) and explored under Fluorescence Imaging. So far, observations report a tendency for the model proteins to highly concentrate evenly on the interface, and this behavior appears to affect the lipid composition separation of the vesicles, forming heterogeneous phase separated domains.

Introduction to Fluorescence Imaging

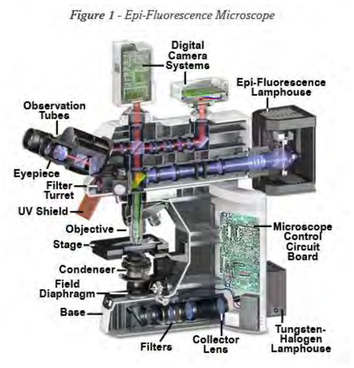
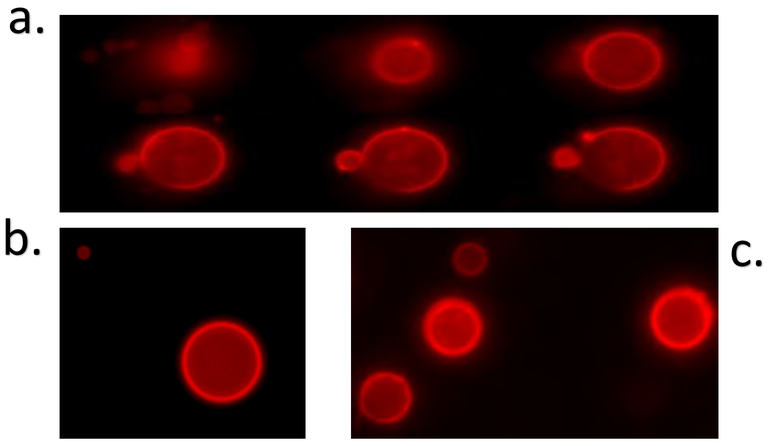


Fig.1.- Example of an Epi-Fluorescence Microscope. Reprinted from Introduction to Fluorescence Microscopy, by K.R. Spring and M.W.Davidson,(n.d.). https://www.microscopyu.com/techniques/fluorescence/introduction-to-fluorescence-microscopy. Copyright 2021 by Nikon Industries Inc.

According to Spring and Davidson (n.d.), using a fluorescence microscope (fig.1), excitation light of a "specific wavelength (or defined band of wavelengths)" is created by transmitting "multispectral light from an arc-discharge lamp" through an excitation filter; a filter selective towards certain wavelengths. This light is then reflected off the dichromatic mirror' surface through the objective lens to illuminate the specimen and excite the fluorochromes. Fluorochromes function as fluorescent dyes; staining a targeted structure with fluorescent molecules that- once exposed to light excitation- will emit light at a longer wavelength almost simultaneously as photons are being absorbed. This longer, weaker, wavelength of light is separated from the excitation light thanks to the objective; filtering the emission light from the excitation light using the emission filter (or barrier) after passing back through the dichromatic mirror. This filtration ensures the darkest background to contrast with the fluorescent light appearing in the final image. In our experiment, the two fluorophores used are GFP and Texas Red. GFP is exposed to a blue excitation light and emits a lime-green fluorescence; in our imaging this lime-green fluorescence depicts protein concentration and protein domains. Texas Red is exposed to a green excitation light and emits a bright red fluorescence; in our imaging the red fluorescence depicts lipids and lipid domains. Using the fluorescence emission of light from a fluorochrome stained substance, we can observe and identify the lipid membranes and protein domains with clear specificity in real-time, monitoring the phase changes of these vesicles.

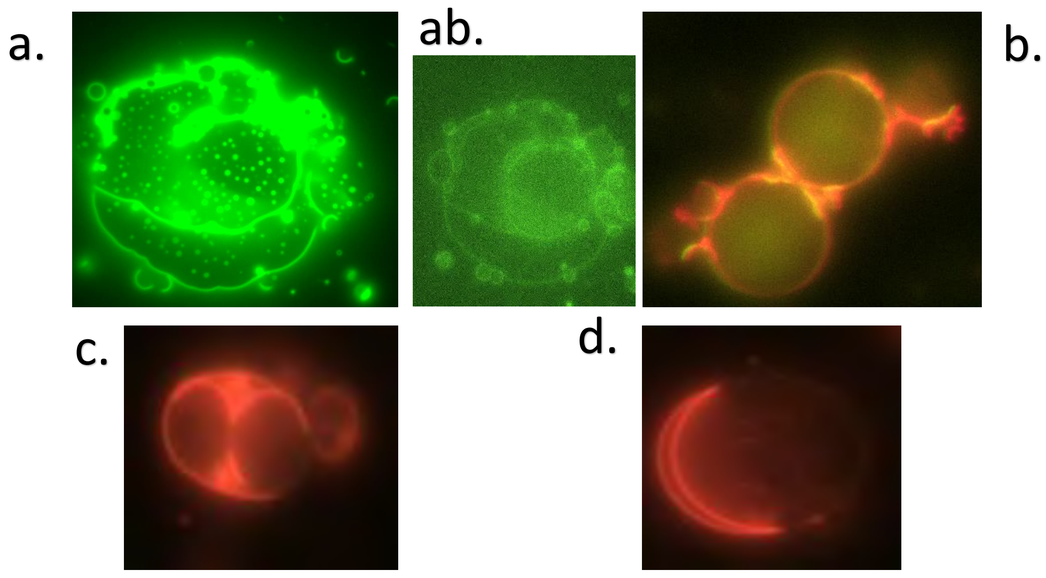
In our research, SIM10fluo contains the fluorescent tag and incubated with the GUV solution, while SUMO10His is incubated with the SLB solution. The compositions of these solutions may change in order to document their effects.

Giant Unicellular Vesicles (GUVs) phase domains



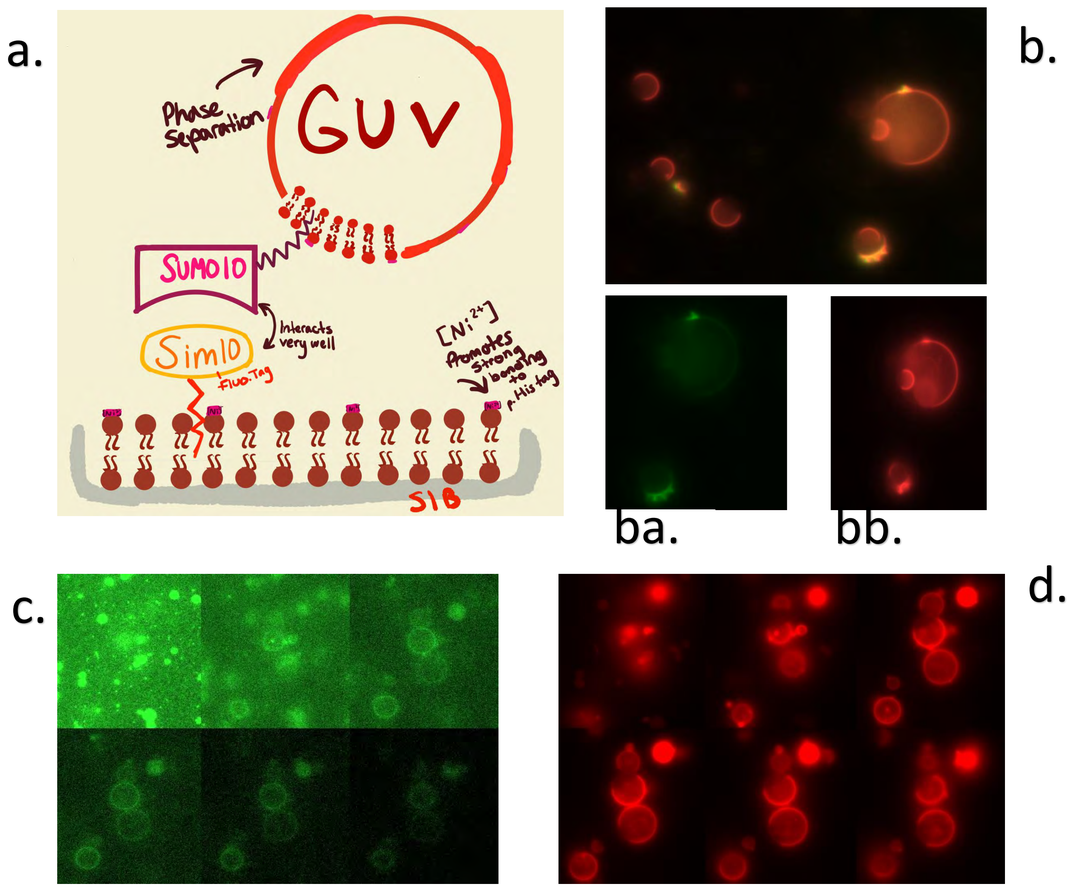
a. Z-stack image montage of GUV undergoing phase separation after interacting with a smaller vesicle.
b. Single channel image example of a uniform vesicle.
c. Single channel image example of multiple uniform vesicles. Bottom left vesicle's membrane has dimmer spots, possible indication of phase-separation.

Interesting and/or Irregular Domain Formation



a. Multichannel image of an odd GUV; Fig.ab displays the protein channel; unequal concentration distribution.
b. Multichannel image of two interacting GUVs with even model protein concentration.
c. Multichannel image of a GUV with two large phase-separated domains.
d. Multichannel image of a GUV with strong phase-separation, but odd domain shape.

Protein-Protein Interaction-led Phase Seperation



a. Schematic of a lipid membrane + anchored protein system with phase domain formation. Thicker regions of GUV depict phase separation.
b. Multi channel image examples of heterogenous phase separated domains with equal protein distribution. Fig.ba and Fig.bb are the protein channel and lipid channel, respectively.
c. GFP channel Z-step montage for phase separation of protein domain, anchored to Fig.d's lipid domains. The fluorescence intensity is lower due to the 1% Ni-DGS GUV mixture composition (changed from 4% Ni-DGS), so a lower concentration of SIM10Fluo proteins anchored onto the lipid membrane.
d. TR channel Z-step montage for phase separation of lipid domain after interaction with another GUV.

References and Adknowledgements

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References listed as follows:

Lipid Raft Phase Modulation by Membrane-Anchored Proteins with Inherent Phase Separation Properties. Il-Hyung Lee, Matthew Y. Imanaka, Emmi H. Modahl, and Ana P. Torres-Ocampo. ACS Omega **2019** *4* (4), 6551-6559, DOI: 10.1021/acsomega.9b00327

Introduction to Fluorescence Microscopy. Spring, K. R., Davidson, M. W. MicroscopyU **(n.d.),** https://www.microscopyu.com/techniques/fluorescence/introduction-to-fluorescence-microscopy.