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Surface Density Calculation

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol details Surface Density Calculation.

Attachments



[416-900.pdf](#)

55KB

Materials

Materials: -

- Multichannel confocal fluorescence images; fluorophores in solution and on GUV at defined densities
- FIJI
- Image segmentation and quantification script (Python)

Surface Density Calculation

- 1 Quantify the fluorescence of a serial dilution of membrane fluorophore and of protein tag fluorophore in 1% SDS-containing buffer ([M] 10 micromolar (μM) , [M] 6 micromolar (μM) , [M] 2 micromolar (μM) , [M] 1 micromolar (μM) , [M] 0.5 micromolar (μM) of each fluor).
- 2 Compare the slopes of a plot of fluorescence intensity vs. concentration for the membrane dye and the protein dye to account for differences in optical properties.
- 3 Quantify the fluorescence of a series of GUV preparations containing varying mole percentage of membrane fluorophore (0.01%, 0.05%, 0.1%, 0.5%, 1%), using a rectangular selection of an approximately horizontal segment of each GUV.
- 4 Calculate the surface density of membrane dye from known surface area of lipids ($\sim 0.7 \text{ nm}^2$ /lipid).
- 5 Calculate the slope of a plot of membrane dye surface density vs. fluorescence signal, accounting for intensity from both lipid bilayer leaflets:

$$\phi_{\text{mem dye, leaflet}} = \frac{AI_{\text{mem dye, GUV}}}{2}$$

- 6 Relate surface density of lipid to surface density of protein label by normalizing to signal from bulk solution (from step 2):

$$\phi_{\text{prot, GUV}} = \frac{AI_{\text{prot, GUV}}}{\text{prot dye}_{\text{bulk}} / \text{mem dye}_{\text{bulk}}}$$