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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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Disclaimer

Abstract

This protocol details Surface Density Calculation.

Attachments

PDF

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 proteintag 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 (~0.7nm^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:

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

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

$$\phi_{prot,GUV} = \frac{AI_{prot,GUV}}{prot\ dye_{bulk}/mem\ dye_{bulk}}$$