

The Lateral Diffusion of Integrin $\alpha_{IIb}\beta_3$ Reconstituted into physiologically relevant GUVs

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Abstract

Platelet integrin $\alpha_{IIb}\beta_3$ has been previously inserted into a number of artificial systems, but these systems have been limited by non-specific protein interactions or their lipid compositions. Here, a novel method of $\alpha_{IIb}\beta_3$ insertion has been developed that allows for proper protein reconstitution, unrestricted by lipid composition. This method has been utilised to reconstitute $\alpha_{IIb}\beta_3$ into two lipid compositions that seek to better mimic the cell membrane. The first of these compositions, “nature’s own”, is a complex lipid composition that seeks to more accurately model the platelet cell membrane. Using this composition, the diffusion of $\alpha_{IIb}\beta_3$ is investigated via FLCS. The various structural conformations induced by integrin activators do not appear, within the limits of the experiment, to influence $\alpha_{IIb}\beta_3$ ’s rate of diffusion. However, use of the same integrin activators in the presence of $\alpha_{IIb}\beta_3$ ’s primary ligand fibrinogen does indeed lead to a change in integrin diffusion owing to the increased molecular weight of the receptor-ligand interaction. The second lipid composition utilised is a “raft forming” mixture that forms well defined liquid disordered and liquid ordered phases. Using confocal microscopy and well characterised lipid dyes, integrin $\alpha_{IIb}\beta_3$ was discovered to have particular affinity for the DOPC rich liquid disordered phase of raft forming GUVs, and was effectively excluded from the cholesterol and sphingomyelin rich liquid ordered phase.

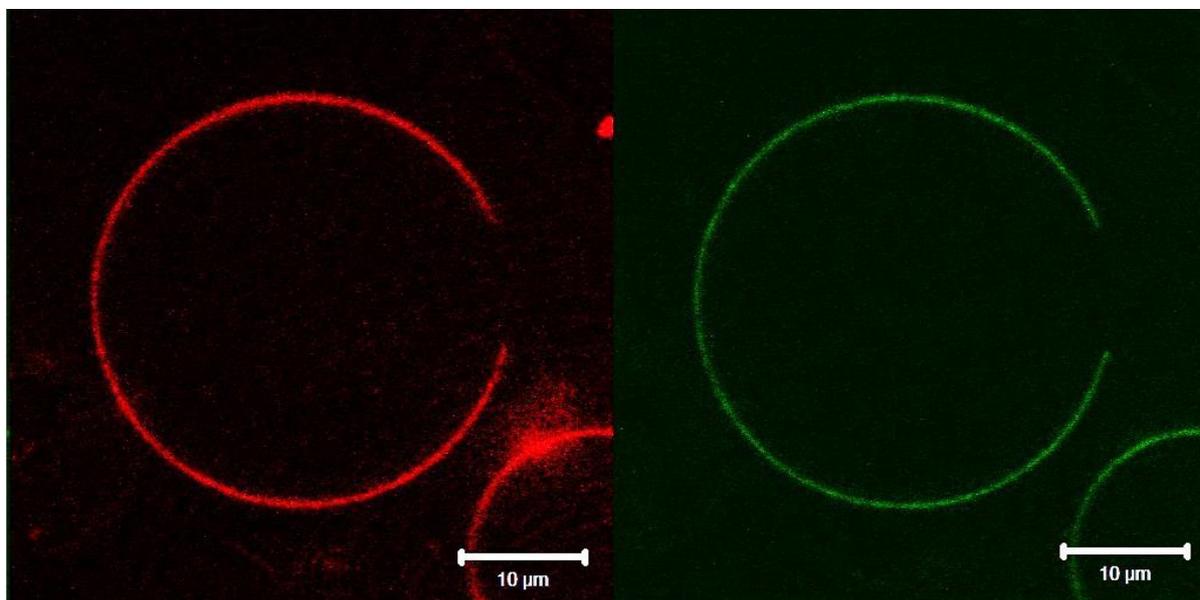


Figure 1: Left) The liquid disordered probe DiD partitioning into the DOPC rich phase of raft forming GUVs composed of 50 % DOPC, 25 % CH and 25 % SM. Right) TAMRA labelled $\alpha_{IIb}\beta_3$ co-localises into the same regions as DiD showing that $\alpha_{IIb}\beta_3$ also has affinity for the liquid disordered phase in raft forming GUVs.