Electronic Supplementary Material (ESI) for Integrative Biology.

Supplementary Information

Integrin partitioning in cholesterol poor domains

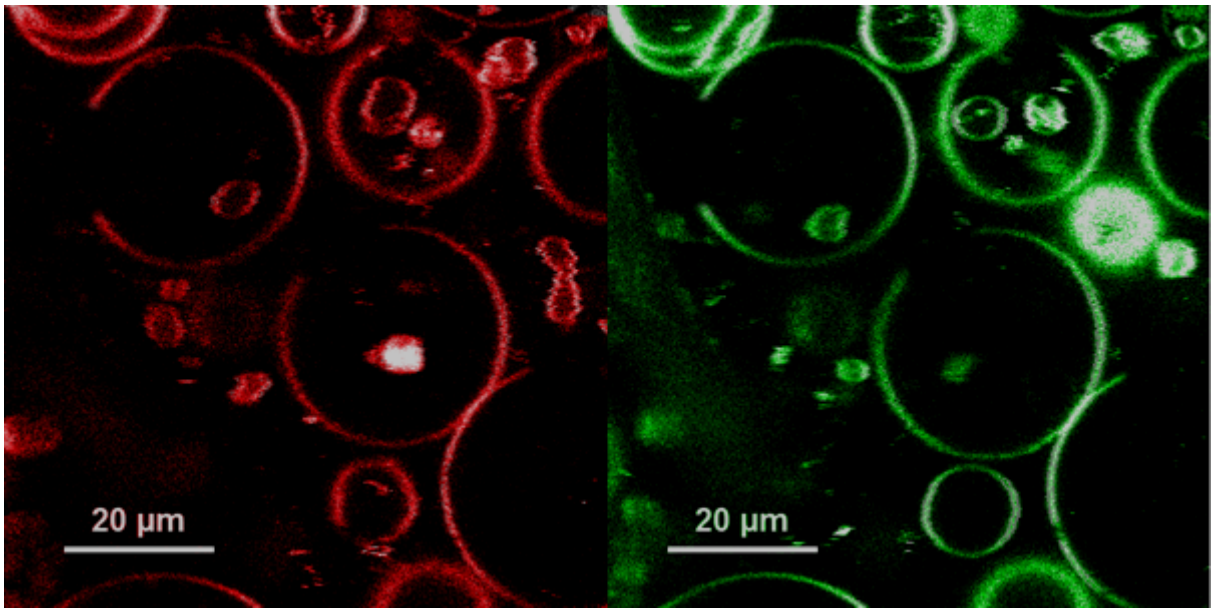

Figure 5: TAMRA labelled integrin $\alpha_{IIb}\beta_3$ (right) co-localises into the same phase as the liquid disordered phase tracer DiD (left). This shows that $\alpha_{IIb}\beta_3$ is excluded from cholesterol rich rafts.

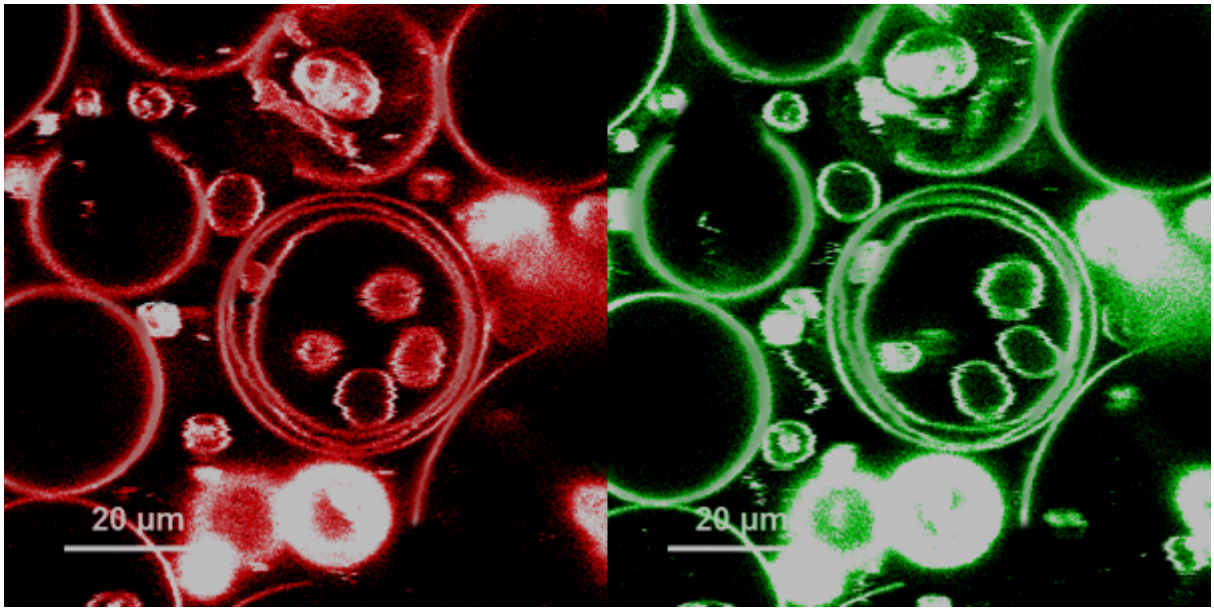

Figure 6: Raft forming $\alpha_{IIb}\beta_3$ reconstituted GUVs (as prepared, without dilution or vesicle size selection) were incubated with 3 mM DTT and $\alpha_{IIb}\beta_3$ partitioning was examined. Integrin activation did not change TAMRA labelled $\alpha_{IIb}\beta_3$'s (right) affinity for the liquid disordered phase (left).

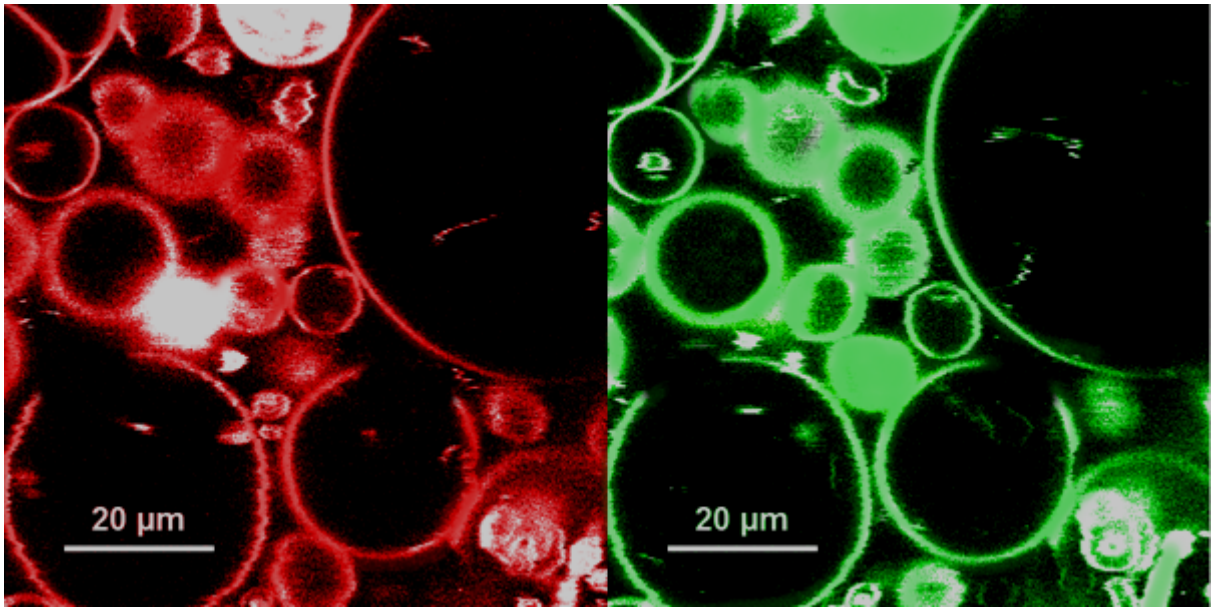

Figure 7: Raft forming $\alpha_{IIb}\beta_3$ reconstituted GUVs (as prepared, without dilution or vesicle size selection) were incubated with 3 mM DTT and 0.1 mg/ml fibrinogen before $\alpha_{IIb}\beta_3$ partitioning was examined. One again TAMRA labelled $\alpha_{IIb}\beta_3$'s (right) affinity for the liquid disordered phase (left) was unchanged.

Reconstitution of $\alpha_{IIb}\beta_3$ into electroformed "Nature's Own" GUVs

A number of starting concentrations of labelled $\alpha_{IIb}\beta_3$ were used, ranging from 1 integrin per 8000 lipid molecules, to 1 integrin molecule per 80,000 lipid molecules. In all cases the starting concentrations used had little effect on the final $\alpha_{IIb}\beta_3$ concentration of the GUVs. This was apparent from the average number of particles detected in the focal volume, N, as given in equation 1.

Table 2: $\alpha_{IIb}\beta_3$ saturation of GUVs. The use of varying starting concentrations of $\alpha_{IIb}\beta_3$ appeared to have little influence on the average number of molecules in the focal volume during FLCS point measurements, indicating that nature's own GUVs are easily saturated with properly reconstituted $\alpha_{IIb}\beta_3$. These concentrations are well suited to performing FLCS measurements.

Starting lipid:protein ratio	Average number of molecules in the focal volume (N)
8000:1	1.55 ± 0.17
20000:1	1.67 ± 0.20

40000:1	1.60 ± 0.22
80000:1	1.34 ± 0.25

Integrin orientation

In order to estimate the orientation of $\alpha_{IIb}\beta_3$ in GUVs, integrin reconstituted nature's own GUVs were constructed as normal. GUVs were then incubated with the serine protease proteinase k for 2 hours at 37 °C. This enzyme only had access to outwardly orientated atto655 labelled $\alpha_{IIb}\beta_3$ and thus inwardly orientated atto655 labelled proteins could not be digested. Confocal images were taken before and after proteinase k incubation and using a tracing tool in imageJ the average count per pixel at the GUV membrane was calculated based on 30 different GUVs. Before incubation the average count per pixel was found to be 46 ± 18 counts, while after incubation it was 21 ± 12 counts. This approximate halving of the intensity suggests that $\alpha_{IIb}\beta_3$ is orientated symmetrically within the lipid membrane.

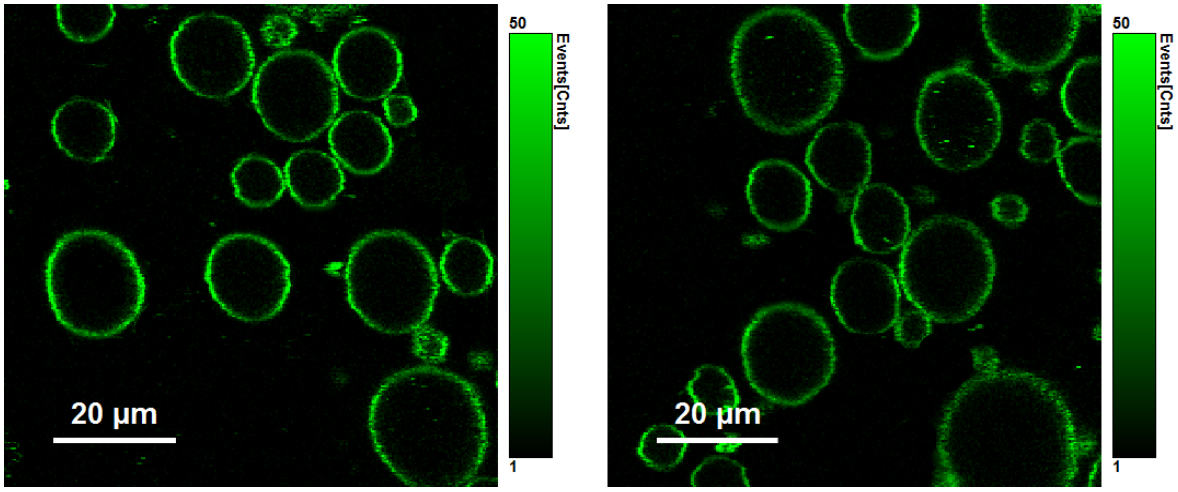

Figure S8: A representative FLIM image of atto655 labelled $\alpha_{IIb}\beta_3$ reconstituted GUVs before (left) and after (right) incubation with proteinase k. A reduction in intensity at the GUV membrane is clearly visible following enzyme digestion. A line tool in imageJ was used to trace around the GUV membrane in order to find the average intensity per pixel at the lipid membrane.

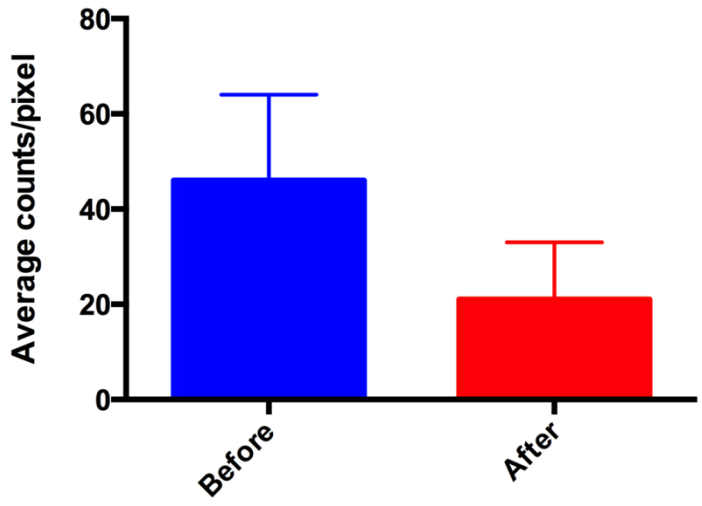

Figure 9: After the imageJ line tool was used to trace around the GUV membranes, the average counts per pixel before and after proteinase k incubation was calculated. Before incubation the average count per pixel was found to be 46 ± 18 counts, while after incubation it was 21 ± 12 counts. This suggests that the enzyme digested approximately half of the labelled $\alpha_{IIb}\beta_3$ proteins, and thus half the labelled $\alpha_{IIb}\beta_3$ proteins were orientated outwardly. Therefore it can be said that $\alpha_{IIb}\beta_3$ is orientated symmetrically throughout the GUV membrane.

Integrin Density:

R_0, the effective lateral focal radius at the cross-sectional area, is approximately 0.34 μm. Assuming the cross sectional area is planar within this radius, πr^2 can be used to determine the cross sectional area giving a value of approximately 36 μm^2. Given that the average number of fluorescent molecules within the focal volume (N) was approximately 1.5 throughout all experiments, we can say that at any given time there were 1.5 integrin proteins within this 36 μm^2 cross-section. This meant that there was 1 integrin protein per 24 μm^2 of the GUV membrane. Alternatively we can say that the protein density was 0.042 integrins per square μm.

Significance of diffusion coefficients

In order to analyse the data a one-way ANOVA with a Bonferroni multiple comparison post- test was performed (GraphPad Prism). Un-activated integrin versus DTT activated integrin with Fg, as well as un-activated integrin versus Mn^{2+} activated integrin with Fg were the only comparisons found to be statistically significant $P \leq 0.01$.

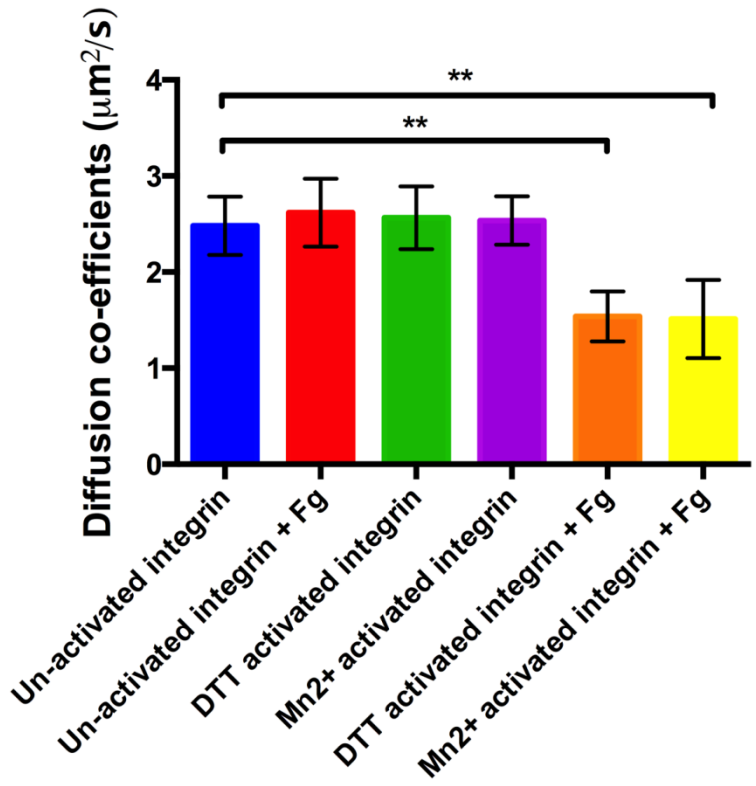

Figure 10: Statistical significance of $\alpha_{IIb}\beta_3$ diffusion co-efficients under various conditions. A one-way ANOVA with a Bonferroni multiple comparison post-test was performed to determine the statistical significance of the change in αIIbβ3–atto655 diffusion. Un-activated integrin versus DTT activated integrin with Fg, as well as un-activated integrin versus $Mn2^+$ activated integrin with Fg were the only comparisons found to be statistically significant ($P \leq 0.01$).