## Supplementary Information

# Equilibrium fluctuation analysis of single liposome binding events reveals how cholesterol and $Ca^{2+}$-ions modulate glycosphingolipid *trans*-interactions

Angelika Kunze, Marta Bally, Fredrik Höök and Göran Larson

### FRAP

FRAP experiments were carried out employing the Nikon Eclipse Ti-E inverted microscope using a 60X magnification oil immersion objective. Three different lipid compositions were studied: (i) 99 wt% POPC and 1 wt% NBD-PC (**SLB 1**), (ii) 94 wt% POPC, 5 wt% $Le^x$ and 1 wt% NBD-PC (**SLB 2**), and (iii) 92 wt% POPC, 5 wt% $Le^x$, 1 wt% NBD-PC, and 2 wt% cholesterol (**SLB 3**). SLBs were prepared on glass-bottom microtiter wells as described above. After formation bilayers were rinsed thoroughly with TRIS with 0 or 10 mM $CaCl_2$, respectively. Prior to bleaching 10 images of the SLBs were taken to compensate for uneven illumination over the sample. Thereafter a spot (~25 μm in diameter) was bleached for 2 s with a diode pumped solid state laser at 475 nm (BWB-475-20E; B&W Tek Inc., Newark DE, USA), followed by a series of 100 images taken at 1 s intervals. Diffusion was determined from five discrete spots each SLB. Image analysis was performed using the Matlab script "frap-analysis" described in detail by Jonsson et al..[1]

Cholesterol-free and cholesterol containing SLBs were found to be mobile in TRIS with and without $Ca^{2+}$-ions. Table S1 summarizes the diffusions coefficients *D* found for SLBs containing $Le^x$ and cholesterol in buffer with 0 or 10 mM $CaCl_2$.

**Table S1: Diffusion coefficient of the different SLBs in TRIS with 0 or 10 mM $CaCl_2$.**

| | | $D$ / $\mu m^2 s^{-1}$ |
|---|---|---|
| **POPC** | 0 mM $CaCl_2$ | 1.96 ± 0.15 |
| | 10 mM $CaCl_2$ | 1.72 ± 0.08 |
| **POPC/$Le^x$** | 0 mM $CaCl_2$ | 2.14 ± 0.09 |
| | 10 mM $CaCl_2$ | 1.66 ± 0.05 |
| **POPC/$Le^x$/cholesterol** | 0 mM $CaCl_2$ | 1.86 ± 0.08 |
| | 10 mM $CaCl_2$ | 1.46 ± 0.05 |

## References


1 Jönsson, P., Jonsson, M. P., Tegenfeldt, J. O. & Höök, F. A method improving the accuracy of fluorescence recovery after photobleaching analysis. *Biophys. J.* **95**, 5334-5348 (2008).