Supporting Material

Direct visualization of the action of Triton X-100 on giant vesicles of erythrocyte membrane lipids

Bruna R. Casadei, Cleyton C. Domingues, Eneida de Paula, Karin A. Riske

Partition of the fluorescent probe Rh-DPPE between the *Ld* and *Lo* phases

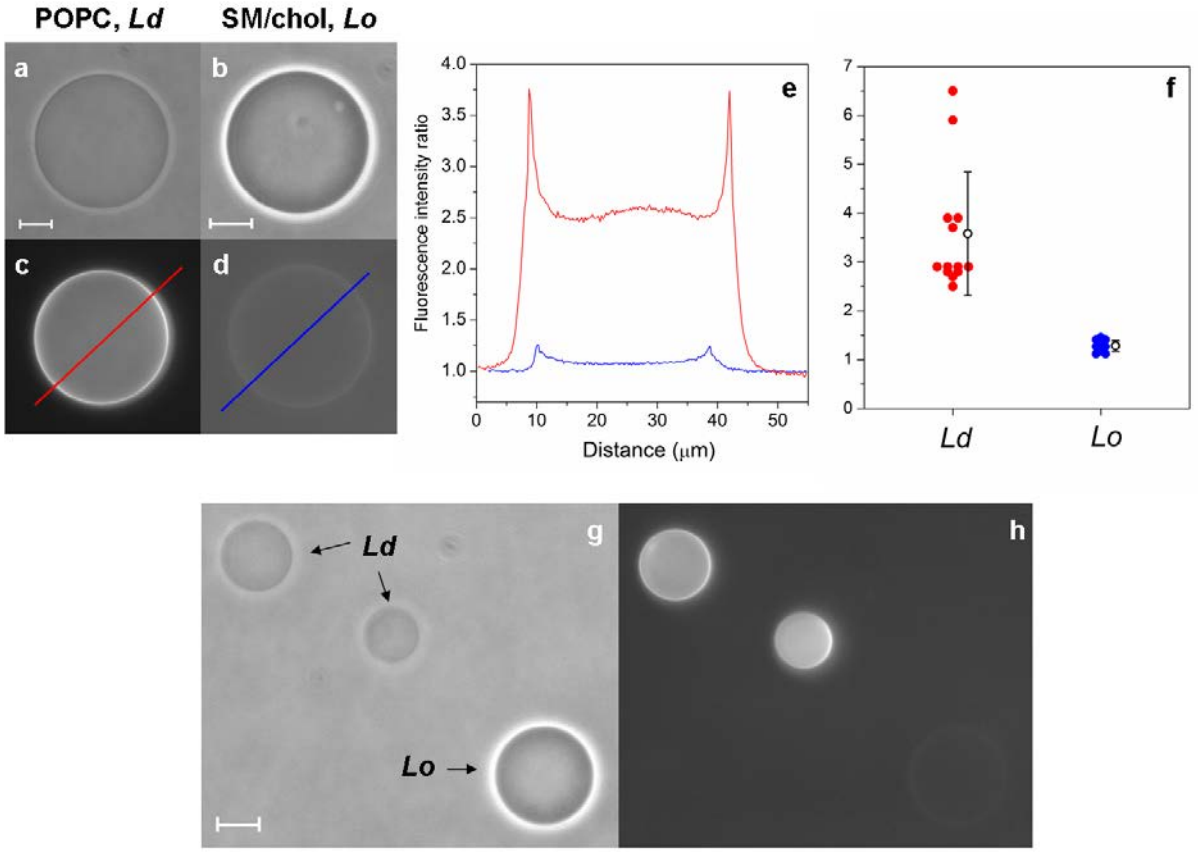

Figure S1 – GUVs of POPC (*Ld* phase) and SM:chol 7:3 (*Lo* phase) were grown in solutions of different sugar composition: 50 mM sucrose with 150 mM glucose (POPC) or 200 mM sucrose (SM:chol). Then, 70 μL of each vesicle suspension were added to 2 mL of a 200 mM glucose solution. A 7 μL aliquot of a concentrated solution of Rh-DPPE in ethanol were added to this solution. After 2 h to allow for equilibration of the fluorescent probe, the solution was placed in an observation chamber and observed with phase contrast and fluorescence microscopy. Under phase contrast, each vesicle population could be distinguished by its optical contrast: (a) low contrast for the *Ld* vesicles and (b) high contrast for the *Lo* vesicles. When the observation was switched to fluorescence mode, the partition of Rh-DPPE could be assessed by the fluorescence intensity. Clearly, all GUVs of POPC (low optical contrast) were highly fluorescent (c), whereas all GUVs of SM:chol (high optical contrast) were only faintly fluorescent (d). Representative intensity profiles measured across (red and blue lines in c and d) two vesicles in the *Ld* (red) and *Lo* (blue) phases are shown in e). The fluorescence intensity was normalized by the background fluorescence. The graph in f) shows the fluorescence intensity ratio of the membrane measured on several GUVs (each point corresponds to one vesicle). The mean values and standard deviation are shown in black. The snapshots g) phase contrast mode and h) fluorescence mode show a field where both *Ld* and *Lo* GUVs are present. The scale bars represent 10 μm.

GP of Laurdan in erythro-GUVs in the presence of TX-100

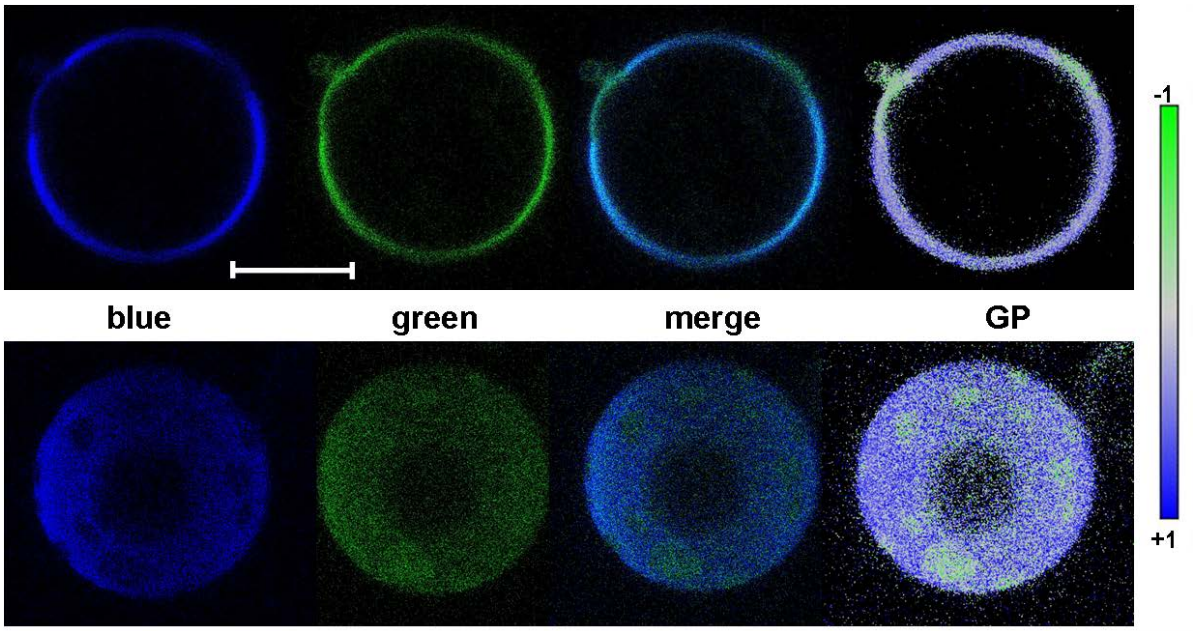

Figure S2 – Erythro-GUVs with 0.7 mol% Laurdan were grown in 0.2 M sucrose, diluted in 0.2 M glucose with 0.2 mM TX-100 and observed with a confocal microscope Leica SP8 equipped with a 63x oil objective (1.47 NA). Excitation was done with a 405 nm laser and two emission channels were recorded simultaneously: blue (446 ± 20 nm) and green (500 ± 20 nm). Images acquired for two representative erythro-GUVs are shown. The top row shows the equatorial plane of one vesicle and the bottom row shows the projection of part of half hemisphere of another vesicle (note that the black region in the center of the vesicle refers to non-scanned vesicle cap), obtained from superposition of 20 z-slices (~0.8 μm each). The blue, green and merged channels are shown for each vesicle. The last images on each row show the generalized polarization GP calculated from the images as $GP = (I_{blue} - I_{green}) / (I_{blue} + I_{green})$. The GP intensity scale is shown on the right. Higher GP values indicate more ordered regions and lower GP values more fluid regions. The experiment shows that the vesicle matrix is composed of the more ordered phase (*Lo*) and the round domains are more fluid (*Ld*). The scale bar represents 10 μm

Video sequences of Figures 1-3 are available online:

Movie S1. Sequences obtained during injection of a 5 mM TX-100 solution in 0.2 M glucose with a glass micropipette close to erythro-GUVs observed with A) phase contrast microscopy mode, and B) fluorescence microscopy mode of erythro-GUVs containing 1mol% Rh-DPPE. Total elapsed time is A) 60 s and B) 140 s.

Movie S2. Sequences obtained with fluorescence microscopy after addition of small aliquots of an erythro-GUVs (with 1 mol% Rh-DPPE) suspension A) 0.2 mM, B) 0.3 mM and C) 0.4 mM TX-100. Total elapsed time is A) 210 s B) 200 s and C) 100 s.

Movie S3. a) Fluorescence microscopy sequence of a GUV composed of 2:1:2 POPC:SM:chol (molar ratio) containing 1 mol% Rh-DPPE during injection of a 5 mM TX-100 solution in 0.2 M glucose with a glass micropipette. Total elapsed time is 160 s. b) a) Fluorescence microscopy sequence of an erythro-GUV containing 1 mol% Rh-DPPE during injection of a 5 mM TX-100 solution in 0.2 M glucose with a glass micropipette. Temperature: 37 ^{o}C. Total elapsed time is 100 s. c) a) Fluorescence and phase contrast microscopy sequence of a GUV composed of 2:1:2 POPC:SM:chol (molar ratio) containing 1 mol% Rh-DPPE during injection of a 5 mM TX-100 solution in 0.2 M glucose with a glass micropipette. Temperature: 37 ^{o}C. Total elapsed time is 80 s.