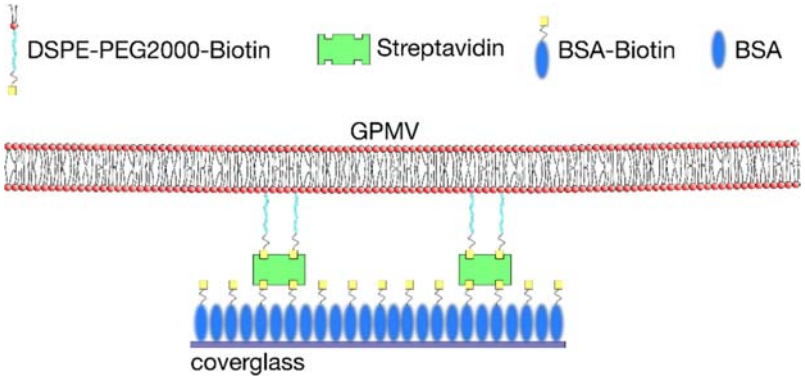
Supplementary Figures



Supplementary Figure S1. Immobilisation protocol of GPMVs. Microscope cover glass was coated with BSA and BSA-biotin and Streptavidin were added. GPMVs stained with DSPE-PEG200-Biotin lipids bound to the cover glass through the Streptavidin-biotin interaction.

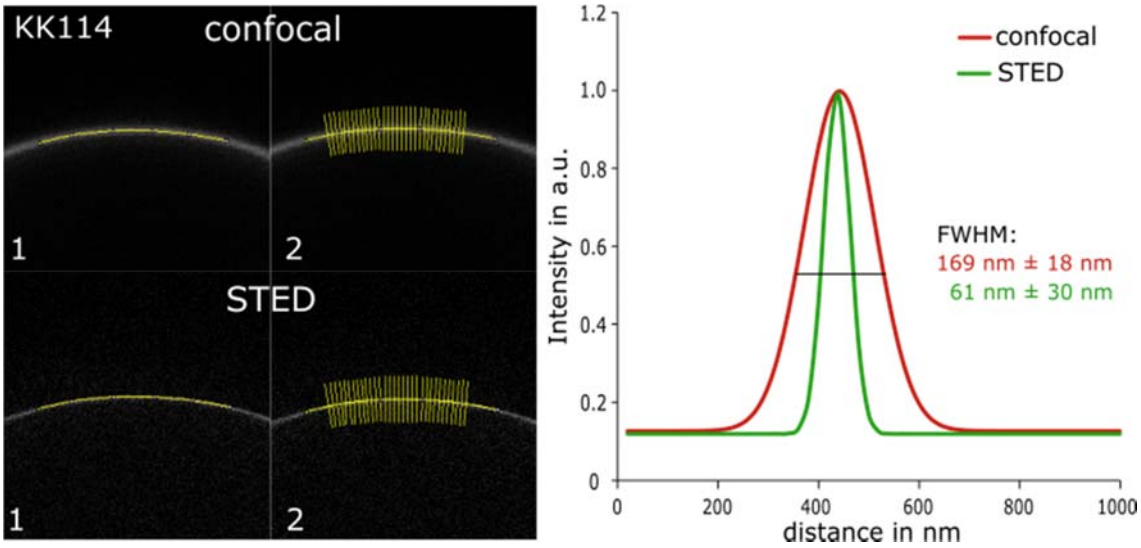
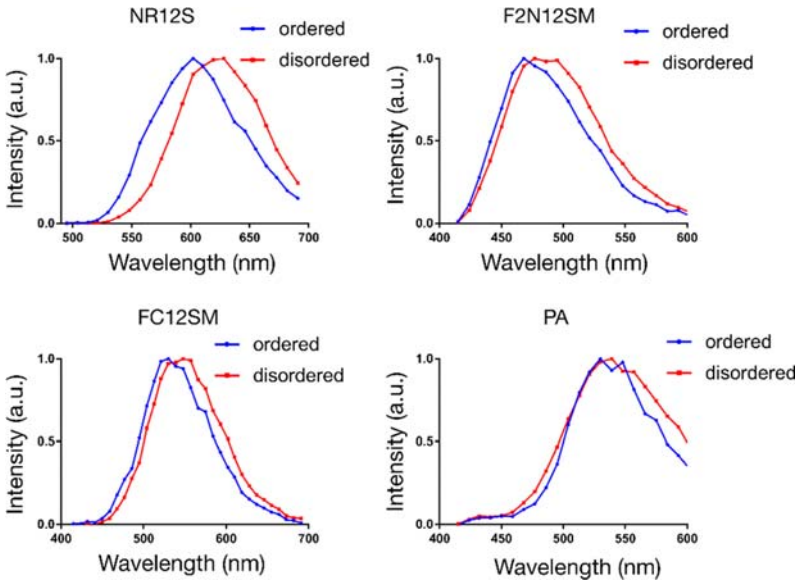


Figure S2. FiJi plugin for the determination of the width (FWHM) of the membranes of GPMVs as imaged with confocal or STED microscopy. (Left) Representative section of a confocal (upper) and STED (lower) microscopy image of the equatorial plane of a GUV (100 % DOPC) stained with KK114-DPPE (0.1 mol% KK114-DPPE). First (panel 1) a segmented line (yellow) is drawn along the membrane, followed by (panel 2) the determination of an intensity profile along the line (yellow) perpendicular to the previously segmented line for every third pixel. (Right) Schematic line profiles obtained in this way, allowing for the determination of FWHM values.



Supplementary Figure 3. Fluorescence emission spectra of B) NR12S C) F2N12SM, D) FC12SM and E) PA as averaged over the Ld (red) and Lo (blue) phases of the confocal images of the equatorial plane of accordingly-stained phase separated GPMVs.