

SUPPORTING INFORMATION

Effect of cytochrome *c* on the phase behavior of charged multicomponent lipid membranes

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*Equilibration of the adsorption of cyt *c* to the vesicle membrane*

To evaluate whether the adsorption of cyt *c* has reached equilibrium, we measured the fluorescence intensity of cyt *c* adsorbed on a selected vesicle throughout a period of 30 min after introducing 0.56 μM cyt *c* in the chamber. From the confocal images of vesicle cross sections we extracted the fluorescence intensity of cyt *c* adsorbed to the vesicle domains separately. The fluorescence intensity signal was acquired as described in the main text. Example of the time dependence of the fluorescence intensity on a vesicle with composition 20/60/20 DOPG/eSM/Chol is shown in Fig. S1. Error bars illustrate the standard deviation from analyzing the same image several times. Our studies suggest that between 15 and 20 minutes are sufficient for the adsorption of cyt *c* to reach equilibration.

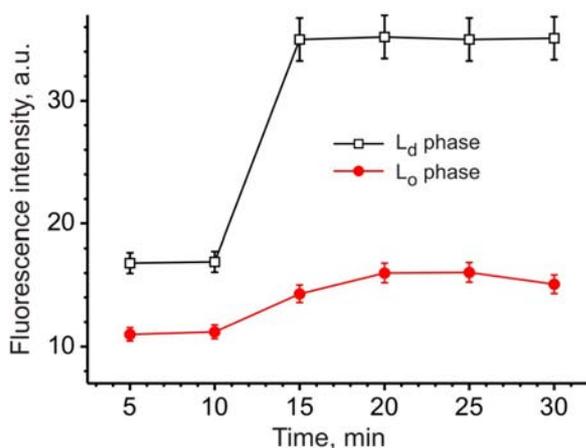


Figure S1. Time dependence of the fluorescence intensity of cyt *c* adsorbed to the L_d and L_o domains shown separately. The membrane composition was 20/60/20 DOPG/eSM/Chol. Time zero corresponds to the time at which the solution of cyt *c* was introduced in the vesicle chamber.

Bleaching of the used fluorescence dyes

We tested whether significant bleaching of the fluorescent label of *cyt c* by laser exposure occurs during the time required for capturing of consecutive images (altogether not longer than 3 minutes). The fluorescence intensity signal was acquired as described in the main text. Example of the fluorescence intensity measured on consecutive snapshots on a vesicle with composition 20/60/20 DOPG/eSM/Chol is shown in Fig. S2. Both the signal from the fluorescently labeled *cyt c* (Alexa Fluor 633) and one of the lipid dyes (DSPE-PEG200-CF) are shown. As demonstrated in Fig. S2, during the time necessary for image capturing, the signal from the fluorescently labeled *cyt c* does not change significantly. Thus, the intensity values measured for the estimate of the protein partitioning in the two phases are not influenced by bleaching of the dye. In contrast, the lipid dye bleaches significantly. However, this is not important in our case, as for the calculation of the partitioning ratios we use the fluorescence intensity of the labeled *cyt c* only.

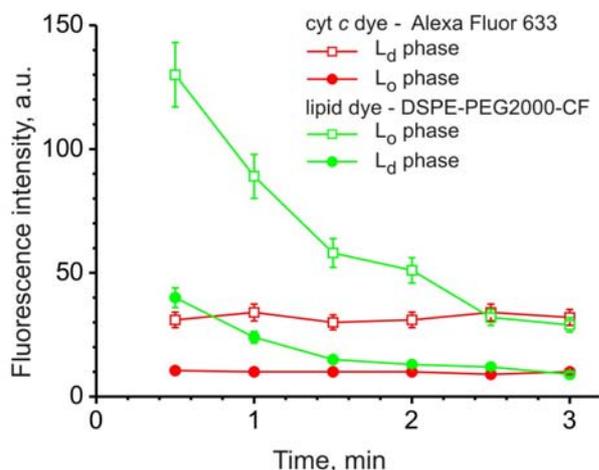


Figure S2. Bleaching of the fluorescent dyes during laser source exposure shown by intensity data for the labeled *cyt c* and lipids. The data was measured separately for the two phases. Error bars illustrate the standard deviation from analyzing the same image several times.

UV/VIS spectroscopy measurements and protein fluorescence

To resolve the preferential adsorption of *cyt c*, we considered using the fluorescence of the protein. Under the conditions used in this work, we detected weak fluorescence signal from *cyt c* in the wavelength region between 540 nm to 620 nm. However, because of the weak emission and the necessity to work at relatively high concentration of the protein (in the millimolar range), as well as the overlap of the spectrum with that of the lipid dye DSPE-PEG2000-CF, using the fluorescence of *cyt c* turned inefficient for quantitative characterization of the protein adsorption.

We measured the absorption spectra of the unlabeled yeast *cyt c* under the conditions used in this work in the wavelength range of 300 – 600 nm. On the basis of the spectra we built a calibration curve, see Fig. S3, which was used subsequently to estimate the concentration of the protein after performing the procedures for fluorescent labeling. The absorbance spectra were measured at concentrations between 1 and 5 μM in a 2 mM HEPES/1 mM EDTA buffer (pH 7.5). The absorption spectra were recorded. The molar extinction coefficient measured at 410 nm was found to be $\epsilon_{410} = 74000 \text{ M}^{-1}\text{cm}^{-1}$.

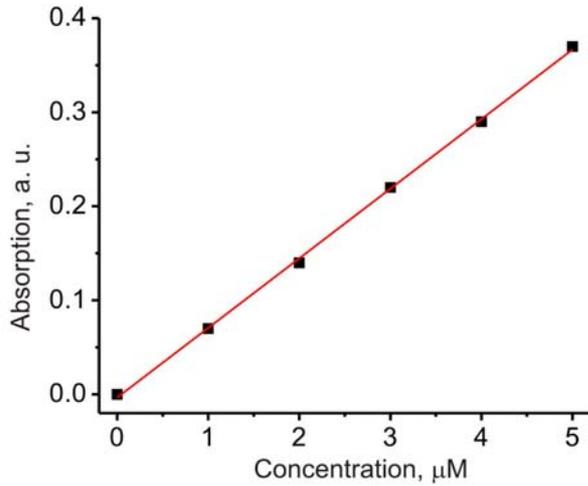


Figure S3. Absorption of yeast cyt *c* at 410 nm in 2 mM HEPES/1mM EDTA (pH 7.5) buffer at various concentrations between 1 and 5 μM . The solid line is a linear fit.

No adsorption of cyt c on DOPC/eSM/Chol membranes

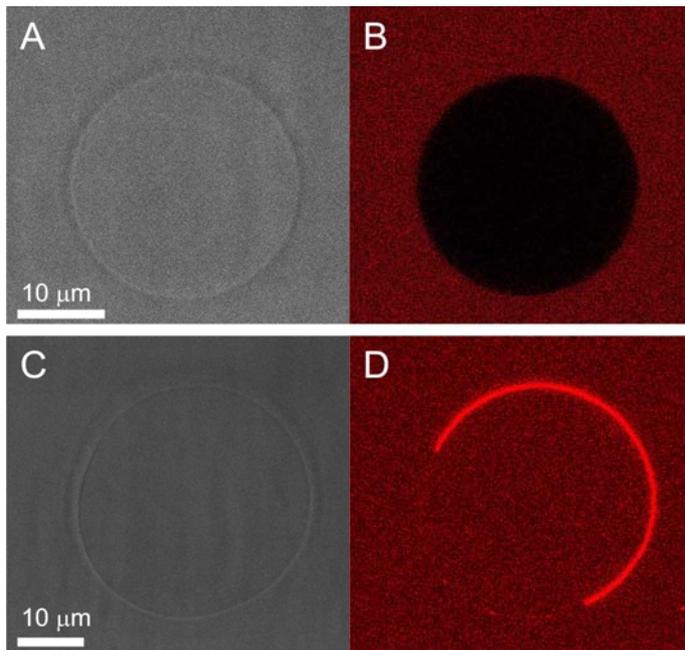


Figure S4. Evidence for no adsorption of cyt *c* to the membrane of DOPC/eSM/Chol 30/50/20 vesicles (A, B) compared to DOPG/eSM/Chol 30/50/20 vesicles (C, D): phase contrast micrographs (A, C) and confocal microscopy cross sections (B, D). Both vesicle types were incubated in 0.56 μM cyt *c* in buffer and the images were acquired at identical settings of the microscope. No protein fluorescence (red) is detected on the DOPC/eSM/Chol vesicle membrane (B). Furthermore, while the protein permeates the DOPG/eSM/Chol membranes, it does not

seem to cross the DOPC/eSM/Chol membrane as evidenced by the lack of protein fluorescence in the vesicle interior (compare the interior fluorescence with the fluorescence in the external media in B and in D).

Evaluating the relative amount of protein adsorbed to the vesicle membrane

To evaluate the amount of bound protein from the confocal images, we first measured the background intensities of samples containing the buffer or a solution of the labeled protein in buffer only. These intensities were compared with the intensity in a sample containing the vesicles and the protein in buffer. For this purpose, we made vertical (xz) scans of the samples right above the bottom glass of the observation chamber. For the vesicle sample, a scan of a vesicle-free area was taken. The microscope settings were adjusted to be exactly the same for all scans. The averaged (over x) intensities as a function from the distance from the glass (z) are displayed in Fig. S5. The peak close to the glass surface observed for the samples containing the protein corresponds to the adsorbed cyt c . At distances well above the glass ($z > 8 \mu\text{m}$), all intensities attain constant values of $I_b = 8.26 (\pm 0.10)$ for the sample with the buffer, $I_{\text{cyt}} = 35.61 (\pm 0.93)$ for the sample containing the protein solution, and $I_{\text{cyt, GUV}} = 19.94 (\pm 0.59)$ for the sample with the vesicles incubated in the protein solution. The concentration of free (not adsorbed) protein is proportional to $(I_{\text{cyt}} - I_b)$ and $(I_{\text{cyt, GUV}} - I_b)$, for the samples without and with vesicles, respectively. From the ratio of these two values, we estimate that around 57% of the total cyt c is adsorbed to 30/50/20 DOPG/eSM/Chol vesicles (Fig. S5). Similar experiments indicated that 41 % and 50 % of the total cyt c is adsorbed to 20/60/20 and 40/40/20 DOPG/eSM/Chol vesicles, respectively.

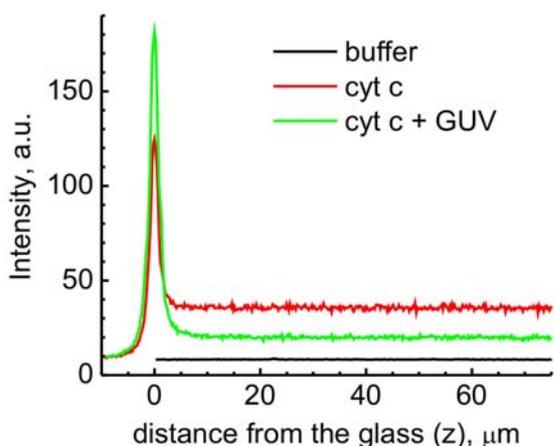


Figure S5. Averaged intensity in z directions for samples containing buffer (black curve), protein in buffer (red curve) and 30/50/20 DOPG/eSM/Chol vesicles incubated with protein in the buffer (green curve).