Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Information for:

Phospholamban spontaneously reconstitutes and generates a cation channel in Giant Unilamellar Vesicles

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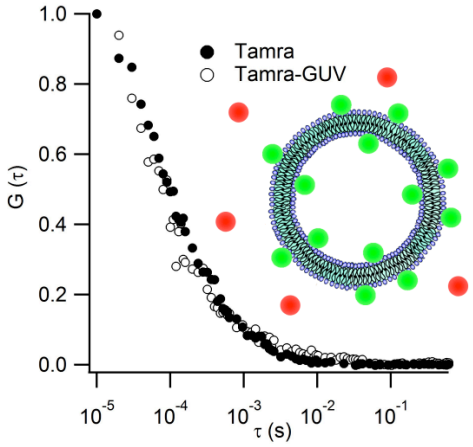
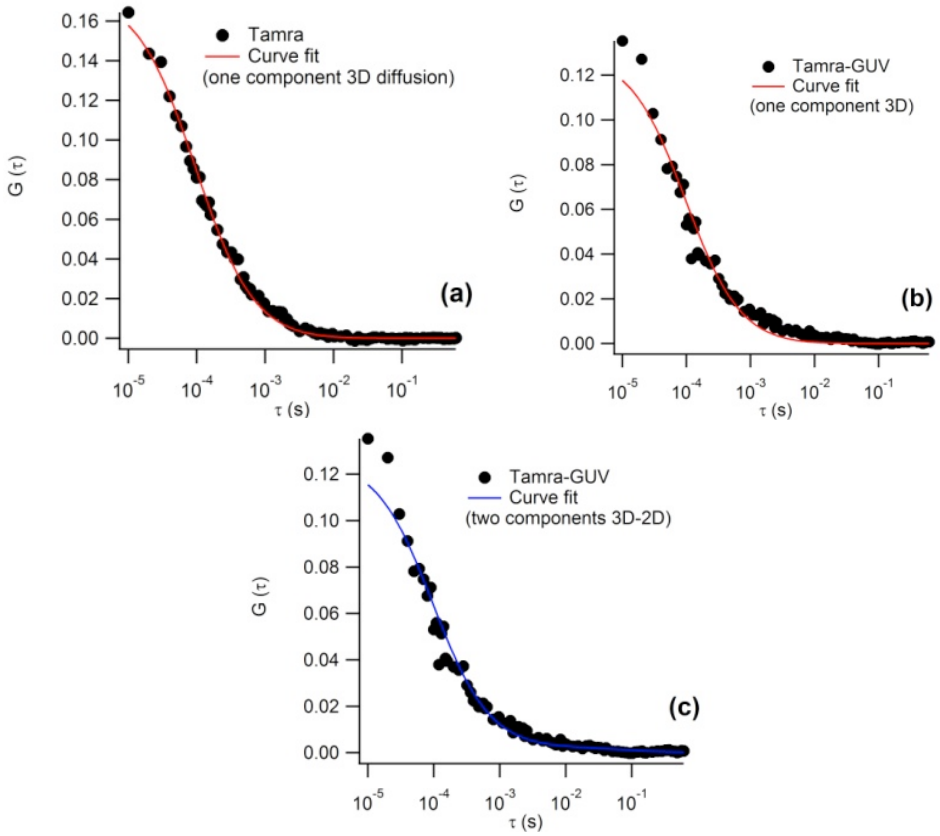


Figure S1 Representative normalized FCS curves acquired for free Tamra (filled circle) in comparison to those measured for free Tamra incubated with GUVs on top of the vesicles (empty circle). Clearly, the decay rate of the FCS curves are similar, highlighting that the incorporation of the probe in the lipid membrane is very poor.



	D (μm²s⁻¹) (1 comp fit)	D_2 (μm²s⁻¹) (2comp fit)	f_2
Tamra	280 ± 10	/	/
Tamra-GUV	280 ± 0	2 ± 5	2 %

(d)

Figure S2 Representative FCS curves acquired for (a) free Tamra and (b, c) Tamra incubated with GUVs (filled circle). Curve fitting according to a (a,b) one component 3D diffusion model (red continuous line, equation 2, main text); (c) 3D-2D two components diffusion model (blue continuous line, equation 3, main text). The overall curve fitting results are reported in the table (d): *D* is the value of the 3D diffusion coefficient of the fluorescent probe Tamra (either bare Tamra or Tamra incubated with GUVs) obtained through a one component 3D diffusion model (equation 2, main text); D_2 is the 2D diffusion coefficient of the fluorescent probe Tamra incubated with GUVs, obtained through a two components 3D-2D diffusion model, being the 3D

diffusion coefficient D_1 fixed at 280 $\mu m^2 s^{-1}$ to improve the robustness of the fitting and f_2 the weight factor of the population undergoing a 2D diffusion with diffusion coefficient D_2, with respect to the population undergoing a 3D diffusion with diffusion coefficient D_1 = 280 $\mu m^2 s^{-1}$.