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

Supporting information

PRODAN differentially influences its local environment

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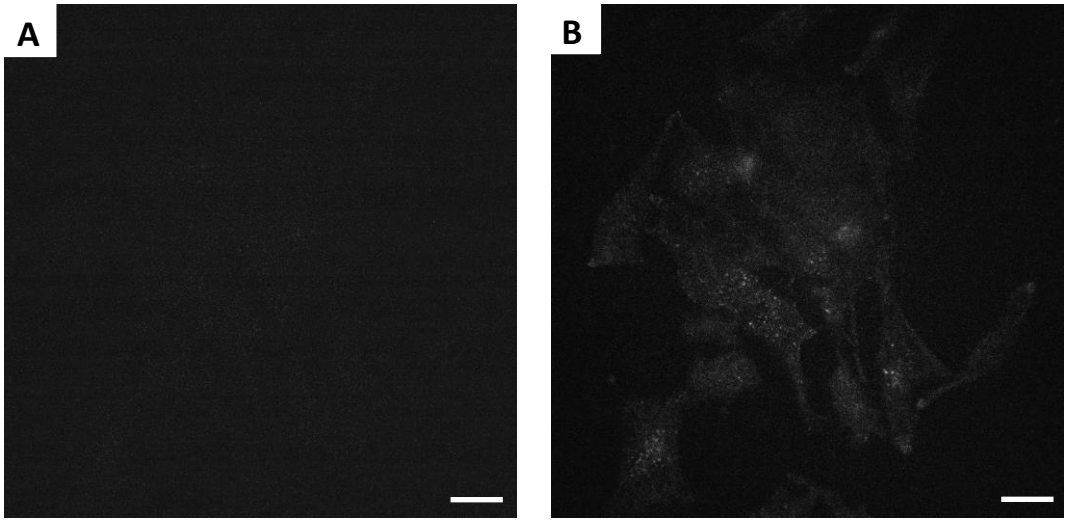

Fig. S1: Live HeLa cells imaged without the presence of PRODAN at experimental laser power (A) and at laser power 10 times higher than used in the main experiments (B). Scale bar 10 µm.

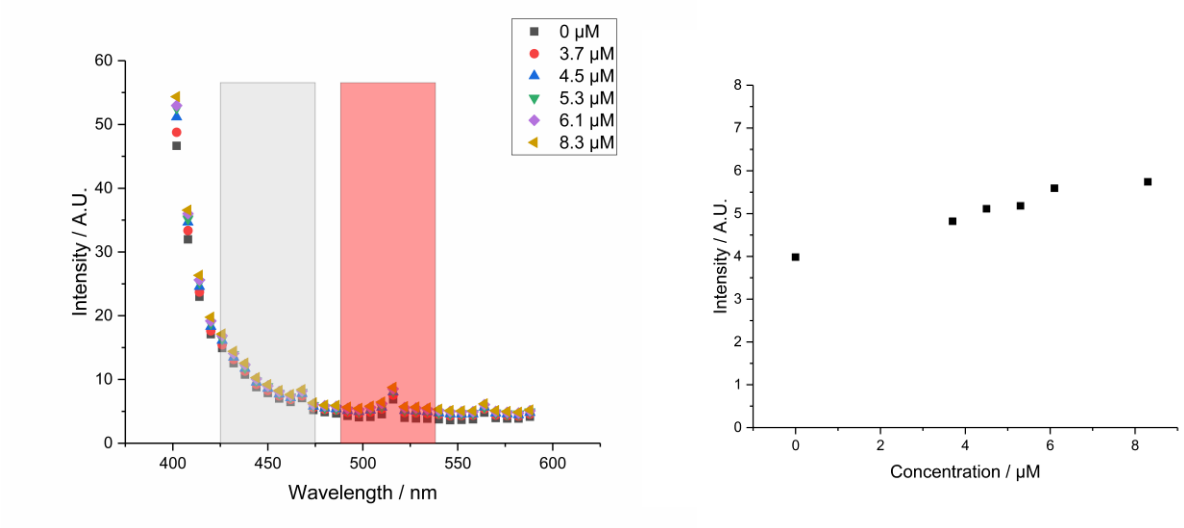

Fig. S2: Fluorescence intensity of imaging medium with different concentration of free PRODAN (left), grey and red areas show the intensity channels used in GP calculations. The fluorescence intensity of the imaging medium at 520 nm for different PRODAN concentrations (right). All imaging was carried out in the same conditions as the main experimental imaging.

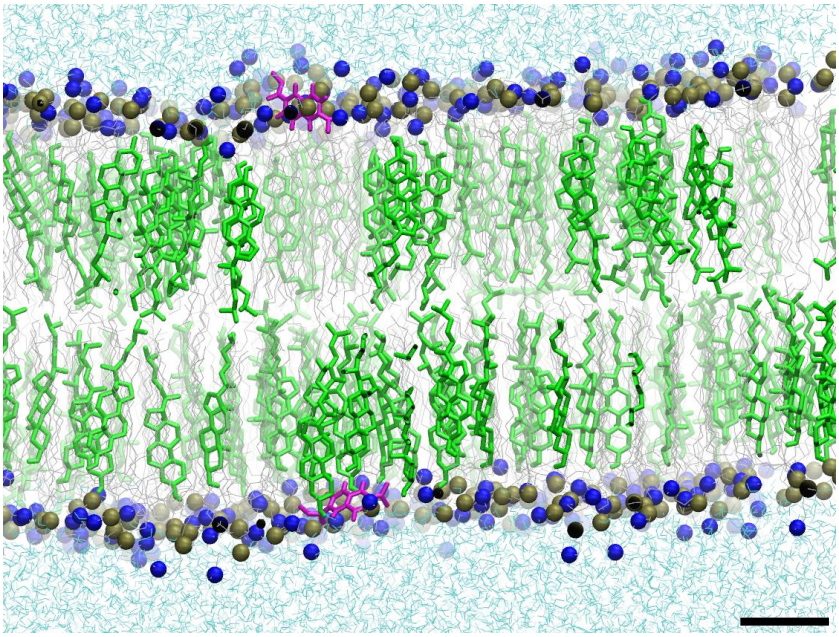

Fig. S3: The final resting positions of PRODAN molecules (thick magenta lines) in the DPPC: Cholesterol system after PRODAN molecules were inserted into the bilayer. Water molecules are represented by thin cyan lines, cholesterol with bright green, nitrogen and phosphorus with blue and beige sphere respectively and the phospholipid tails with grey lines. Scale bar 1 nm.

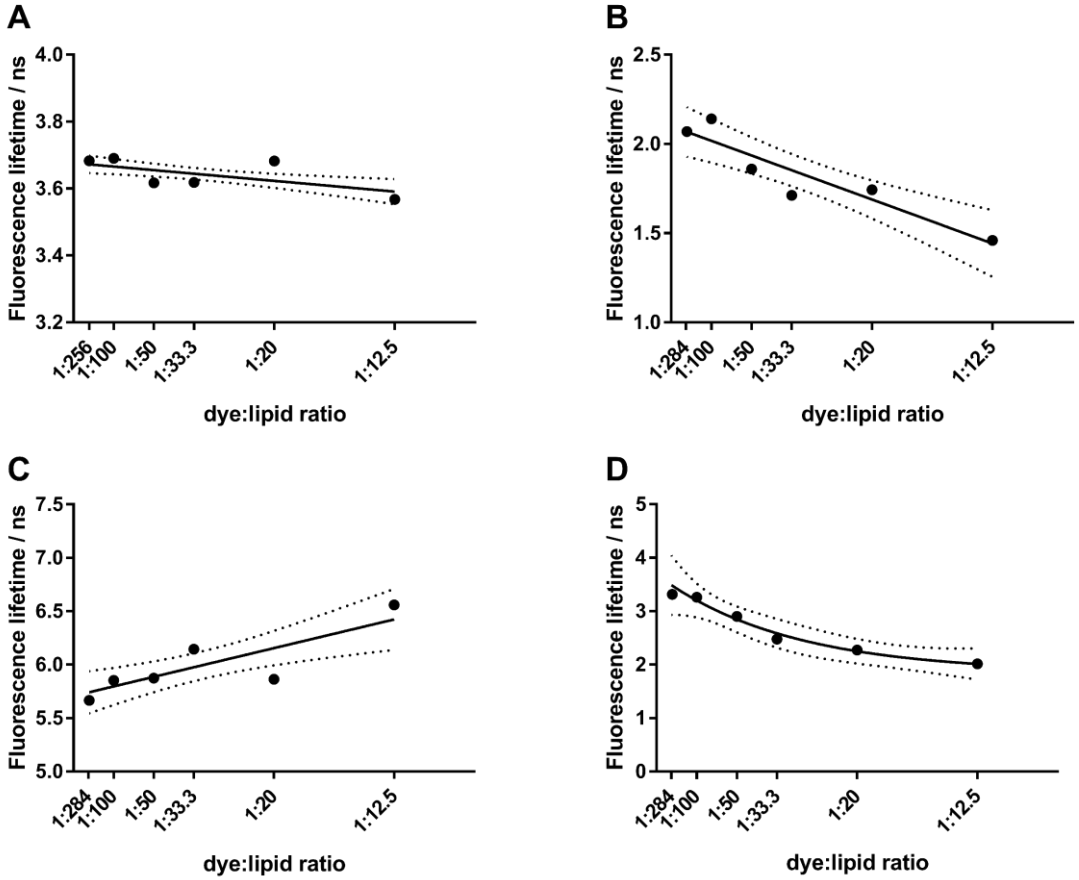

Fig. S4: Fluorescence lifetime of PRODAN in vesicles at different concentration. PRODAN in DOPC (Ld) vesicles exhibits a single fluorescence lifetime (A). In the DPPC:Chol (Lo) vesicles PRODAN exhibits two distinct fluorescence lifetimes (B and C) obtained by a double exponential fit to the decay curve. The average fluorescence lifetime of PRODAN in DPPC:Chol vesicles (D) was calculated using equation S1.

$$\tau_{av} = \frac{A_1\tau_1^2 + A_2\tau_2^2}{A_1\tau_1 + A_2\tau_2} \quad \text{(S1)}$$

where τ_{av} is the average fluorescence lifetime, τ_1 the first fluorescence lifetime with an associated amplitude A_1 and τ_2 is the second fluorescence lifetime with an associated amplitude A_2.