Limitations of Electronic Energy Transfer in the Determination of Lipid Nanodomain Sizes

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Supplementary material

MC simulations (1)

The simulation started by redistribution of probes between the circular domains and the remaining bilayer according to the affinity of the probes to the particular phase. In order to obtain the generated decay the time a randomly excited D molecule spends in the excited state was calculated. This time depends on the overall energy transfer rate Ω_i according to $\Delta t_i = -\ln\alpha/\Omega_i$, where α is a randomly generated number between 0-1. New configurations were generated 3000 times, whereas each configuration step was used 100 times. Periodic boundary conditions were used and the size of the replicated box was 20 R_0 × 20 R_0. Input parameters of the simulation were: area the domains occupy in the bilayer, the domain radius, distribution constants of D:s and A:s, R_0, the D-lifetime and surface concentration of D:s/A:s. Simulations have been carried out under the dynamic limit conditions and among isotropically oriented DA.

Additional figures showing the limits of FRET

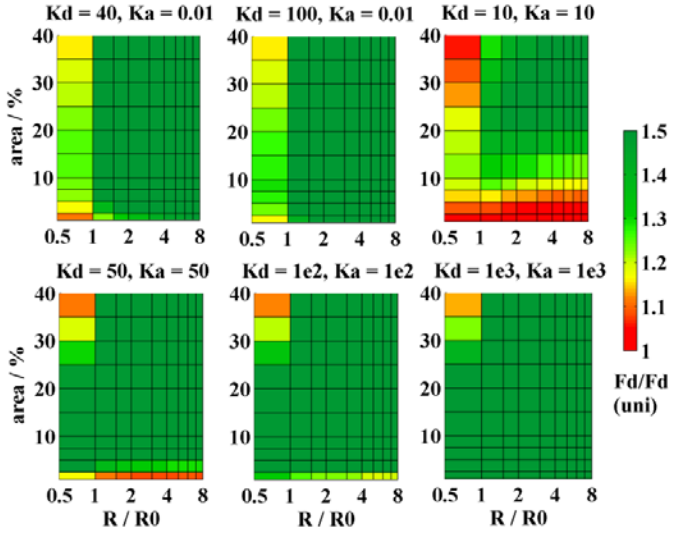

Fig. S1: Resolution of FRET represented by the ratio of the intensity of D (F_D) to the intensity of D when DA are distributed uniformly in the bilayer (F_D(uni)) as a function of the domain area and the domain radius divided by R_0. Lifetime of D was fixed at 6 ns for both L_o and L_d. The red colour corresponds to the classification (clas.) 1, orange colour to the clas. 2, yellow colour to the clas. 3 and green colour to the clas. 4. For [K_D = 10, K_A = 10], [K_D = 50, K_A = 50], [K_D = 100, K_A = 100] and [K_D = 1000, K_A = 1000] the ratio F_D(uni)/F_D is displayed instead. Values above 1.5 are displayed with the same colour at the limiting value.

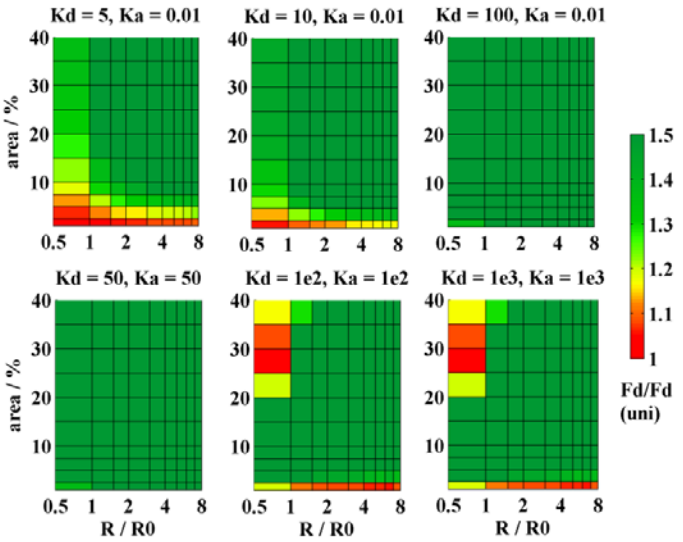

Fig.S2: Resolution of FRET represented by the ratio of the intensity of D (F_D) to the intensity of D when DA are distributed uniformly in the bilayer (F_D(uni)) as a function of the domain area and the domain radius divided by R_0. Lifetime of D was 6 ns the L_d and 8 ns in the L_o phase. For [K_D = 50, K_A = 50], [K_D = 100, K_A = 100] and [K_D = 1000, K_A = 1000] the ratio F_D(uni)/F_D is displayed instead. For more details see Fig. 1.

Lifetime of perylene

As is obvious from Fig. S3 lifetime of perylene depends on the physical state of the lipid bilayer, and possibly also on its chemical composition. For the L_o state the fitting routine yielded A_1 = 0.87, τ_1 = 8 ns A_2 = 0.13, τ_2 = 3.24 ns with the average lifetime of 7.8 ns whereas for the L_d state the optimization minimum was reached at A_1 = 0.93, τ_1 = 6.6 ns A_2 = 0.07, τ_2 = 3.00 ns with the average lifetime of 6.45 ns. A_i are preexponential factors and τ_i the fluorescence lifetimes. Since perylene has K around 1it could be used for detection of domains that occupy at least tens of percent of the entire bilayer surface.

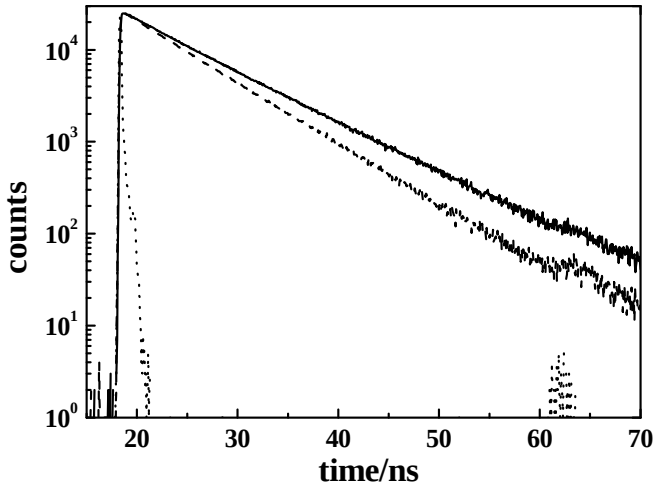

Fig. S3: Time-resolved fluorescence decay of perylene in Sph/Chol (70/30) bilayer, which was in the L_o state (solid), and DOPC/Chol bilayer, which was in the L_d state (dashed). Lamp profile is displayed as well (dotted).

Distribution constants of some probes with increased affinity to the L_o raft phase in bilayers containing cholesterol

Tab.S1: Distribution coefficients of some probes that have increased affinity to the L_o phase in bilayers with Chol.

Probe	Bilayer composition	*K*
Perylene	DOPC/Sph/Chol/DOPG/DPPE-biotin (29/39/25/5/2)	0.8 ± 0.2
Cholera toxin	DOPC/Sph/Chol/DOPG/DPPE-biotin (44/24/25/5/2)	6 ± 3
Cholera toxin	DOPC/Sph/Chol	11 ± 5 (2)
NBD-DPPE	POPC/Sph/Chol	4.3 ± 1.2 (3)

Determination of distribution coefficients

Distribution coefficients of Alexa488 labelled cholera toxin subunit B (CtxB-488) and perylene between L_o and L_d phase was determined in giant unilamellar vesicles (GUVs). GUVs were prepared by a gentle hydration approach. 1 mL of lipid mixture (*cf.* Table 1) in chloroform containing 1 mg of lipids was dried with a rotary evaporator and kept for additional two hours under vacuum. Thin lipid film was hydrated with 3 mL of pre-warmed buffer (10 mM HEPES, 150 mM NaCl, 1 mM $CaCl_2$, 0.1 M sucrose, pH = 7) saturated with nitrogen. The tube was then sealed, heated to 60 ºC, kept overnight at this temperature, and slowly cooled down. White cloud containing liposomes was gently vortexed before further use. The measurements on GUVs were performed in FCS2 chamber (Bioptechs, Butler, PA). The chamber with a BSA-biotin/streptavidin coated coverslip was filled with a buffer solution (10 mM HEPES, 150 mM NaCl, 1 mM $CaCl_2$, 0.1 M glucose, pH = 7), 20 µL of the solution containing GUVs was added and after 30 minutes when enough liposomes were attached to the coverslip, the chamber was carefully flushed with excess of the buffer solution.

In the case of perylene, the dye was present already in the lipid mixture in chloroform. The probe-to-lipid ratio was 1:200. In the case of CtxB-488, 20 µL of CTxB-488 (1 µg/mL) was added to the chamber containing already immobilized GUVs and after 15 minutes flushed with excess of the buffer solution.

Images of the GUV cross-sections were acquired with MicroTime 200 inverted confocal microscope (PicoQuant, Germany) with a water immersion objective (1.2 NA, 60x) (Olympus, Hamburg, Germany). Pulsed diode lasers (LDH-P-C-470, 470 nm, and LDH-D-C-440, 440 nm PicoQuant, Berlin, Germany) with 40 MHz repetition rate were used. The signal was detected on the single photon avalanche diode (SPCM-AQR-14, PerkinElmer, Waltham, MA) using 515/50 (CtxB-488) and 480/40 (perylene) band pass filters (Chroma Rockingham, VT), respectively.

In the images, pixels corresponding to L_o and L_d phase were identified and the mean pixel intensity of the two phases (I_{Lo} and I_{Ld}) calculated. The distribution coefficient was obtained as:

$$K = \frac{I_{L_o}}{I_{L_d}} \cdot \frac{\tau_{L_d}}{\tau_{L_o}},$$

where τ stands for fluorescent lifetime of the label in the given phase (important for perylene).

FRET efficiency versus fluorescence lifetime

The lifetime of perylene changes with the lipid bilayer composition (*i.e.* 6 ns and 8 ns in L_d and L_o phase, respectively; *cf.* SI). This property improves FRET resolution. This is because the decay becomes slower due to the slower FRET (as compared to the uniform distribution of D/A) as well as the fact that donors residing in the rafts emit on a longer timescale. The FRET rate remains, however, nearly constant with respect to the case when D-lifetime is not influenced by the phase composition. This happens because of the 1/6 power dependence of the FRET efficiency on the lifetime and is demonstrated in Fig. S4.

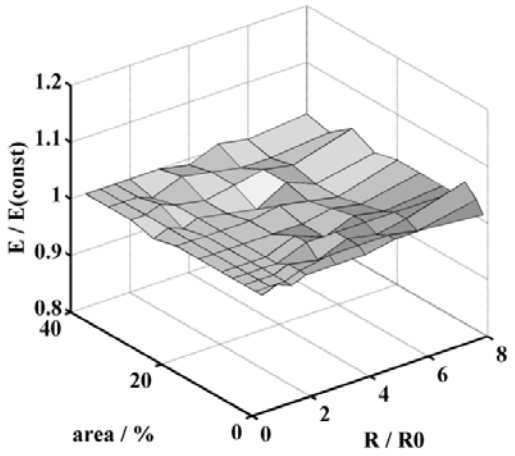

Fig. S4: Ratio of the FRET efficiencies with the lifetime of 6 ns in L_o and L_d , E, and the lifetime of 6 ns in L_d and 8 ns in L_o, E(const) as a function of the domain radius R/R_0 and the domain area.

Influence of the z-positions on the results

As is shown in Fig. S5 vertical z-position of the donors in the bilayer has only a modest impact on the generated curves. The conclusions drawn in the paper are thus general.

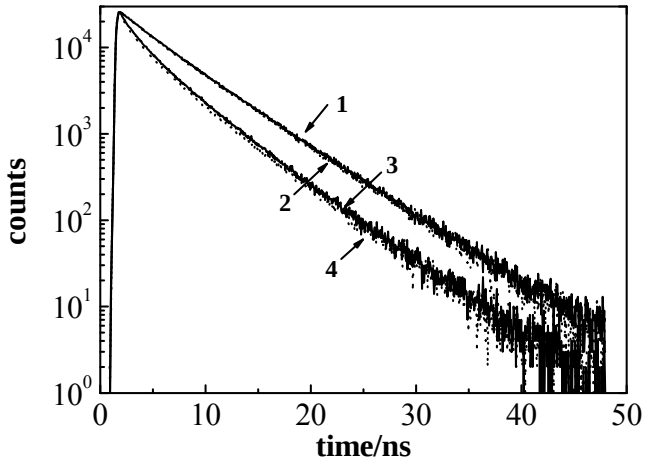

Fig. S5: Generated decays for D with $K_D = 100$, $K_A = 100$ localized at the interface (solid, curve 1) or inside the bilayer (dotted, curve 2) and with $K_D = 1$, $K_A = 1$ localized at the interface (solid, curve 3) or inside the bilayer (dotted, curve 4).

Supporting references

1. Johansson, L. B. A., S. Engstrom, and M. Lindberg. 1992. Electronic Energy Transfer in Anisotropic Systems. 3. Monte Carlo Simulations of Energy Migration in Membranes. J. Chem. Phys. 96:3844-3856.
2. Chiantia, S., J. Ries, N. Kahya, and P. Schwille. 2006. Combined AFM and two-focus SFCS study of raft-exhibiting model membranes. ChemPhysChem 7:2409-2418.
3. de Almeida, R. F. M., L. M. S. Loura, A. Fedorov, and M. Prieto. 2005. Lipid rafts have different sizes depending on membrane composition: A time-resolved fluorescence resonance energy transfer study. J. Mol. Biol. 346:1109-1120.