Model of two dimensional diffusion of dye molecules in a membrane

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Supporting Material to a model performed for

Rapid SNARE-mediated fusion of liposomes and chromaffin granules with giant unilamellar vesicles
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Determination of number of dye molecules

This calculations were inspired by a description by Encapsula NanoSciences (see *References*).

Total number of particles in a liposome membrane (N) can be calculated from the following formula:

$$N = \frac{\left[4\pi\left(\frac{d}{2}\right)^2 + 4\pi\left(\frac{d}{2} - h\right)^2\right]}{a} \tag{1}$$

where d is the liposome diameter, h is the thickness of the bilayer, and a is the lipid headgroup area.

For performing simulation following parameters were taken:

- $d = 100\,\mathrm{nm}$, as the average diameter of an LUV (large unilamellar liposome) used in this study (prepared according to Hernandez et al., 2012);
- $h = 4.6\,\mathrm{nm}$, as the average membrane thickness (usually between 4–5 nm, see e.g. Träuble and Haynes, 1971; Gallová et al., 2004);
- $a = 0.582\,\mathrm{nm}^2$, as the average headgroup area, calculated as the weighted average (weight represents fraction of total lipids, concentrations and areas used are presented in table 1).

Table 1. Lipid headgroups area

Lipid	Concentration (mol%)	Area (nm^2)	Reference
PC[1]	50	0.71	Israelachvili and Mitchell, 1975
PE[2]	20	0.41	Israelachvili and Mitchell, 1975
PS[3]	20	0.63	Pan et al., 2014
cholesterol	10	0.19	Israelachvili and Mitchell, 1975

[1]phosphatidylcholine
[2]phosphatidylethanolamine
[3]phosphatidylserine

Assuming these values, LUV would be composed of $N \approx 98500$ molecules. Labelled lipid simulated here is Texas Red coupled PE, that was incorporated into membrane in a fraction of 1 mol%. Taken this together, it can be calculated that there will be ~ 985 dye molecules.

Radius of a membrane patch

Assuming $d = 100\,\text{nm}$ as the average diameter of an LUV (see *Determination of number of dye molecules*), membrane area (A) can be calculated from:

$$A = 4\pi \left(\frac{d}{2}\right)^2 \tag{2}$$

Taken this area value, a circle radius (R) of a corresponding area can be calculated from:

$$R = \sqrt{\frac{A}{\pi}} \tag{3}$$

Tthese simple calculations give $R \approx 100\,\text{nm}$.

Diffusion-mediated particle displacement

The diffusion of dye particles was simulated by using a random walk. According to Einstein (1905), in each time step dt (in simulation $dt = 1\,\text{ms}$) every molecule was displaced by a distance dx, in every direction (here in x and y):

$$dx = \sqrt{dt \times 2 \times D} \tag{4}$$

with D being a diffusion coefficient of a particle (here taken average value of $D = 2.83\,\mu\text{m}^2/\text{s}$ from Fig. 1 in Witkowska and Jahn, 2017).

Particles coming from an LUV after fusion to a GUV, form a round spot with randomly distributed dye molecules. Position of these spots at time $t = 0\,\mathrm{ms}$ can be easily determined coming from polar coordinates:

$$\varphi_N = 2\pi \times rand_N \tag{5}$$

$$r_N = R \times \sqrt{rand_N} \tag{6}$$

$$X_N = r_N \times cos\varphi_N \quad Y_N = r_N \times sin\varphi_N \tag{7}$$

where φ and r are the angle and radius of polar coordinates, respectively; N is the particle number (with a total number of 985 molecules, see *Determination of number of dye molecules*); *rand* is a random number from a uniformly distributed set of numbers between 0 and 1; and X_N and Y_N are the Cartesian coordinates of the N^{th} molecule, assuming patch centre at (0,0).

From the initial coordinates particles then diffuse in the GUV membrane. The molecule displacement in x and y after each time step dt can be obtained from:

$$X_N(t) = X_N(t - dt) + dx \times randn_N \tag{8}$$

$$Y_N(t) = Y_N(t - dt) + dx \times randn_N \tag{9}$$

where t is time, and *randn* is a is a random number from a normally distributed set of numbers having 0 mean and variance 1.

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