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Singlet oxygen luminescence kinetics in heterogeneous environment – Identification of the photosensitizer localization in small unilamellar vesicles Supplementary information

The spherical symmetrical diffusion model - numerical calculation

The numerical calculation is based on Fick's first law of diffusion [1]:

$$\frac{dn}{dt} = -D \cdot A \cdot \left(\frac{dc}{dx}\right)_{p,T} \qquad (1)$$

dn/dt represents the flux, which is proportional to the diffusion constant D, the cross section of the transit area A and the concentration gradient dc/dx.

To make a numerical simulation according to our model (Fig. 1 in the paper), we first need to choose the thickness of the layers and a corresponding time step. While too few layers can't sufficiently reflect the heterogeneity of the area, a layer thickness too small dramatically increases the calculation effort. We chose a description of the liposomal bilayer with 4 model layers of each 1 nm thickness, since we estimate the liposomal bilayer thickness to be 4nm according to [2] and we handle the bilayer as almost homogeneous.

To estimate a corresponding time step, its influence on the spatial distribution of oxygen relative to the chosen layers has to be taken into account. In Fig. 1 the spatial distribution of oxygen in water is shown for different time steps after 1D diffusion starting with a delta distribution. It can be described using the well-known Gaussian distribution

$$c(x,t) = {}^{1}/_{\sqrt{2\pi}\cdot\sigma} \cdot e^{-\left(\frac{x^2}{2\sigma^2}\right)} \quad \text{with} \quad \sigma = \sqrt{2Dt} \qquad (2)$$

(2) solves the 1D diffusion equation (2nd Fick's law) for this starting condition. For illustration how many layers would be affected, distances that correspond to layers are indicated presuming the distribution to be centred in one layer.

As visible quite easily, shorter times would be more correct but would require longer calculation times, while for longer times more than just the directly neighbouring layer is affected. It is aspired to limit the interaction to neighbouring layers as this makes the simulation much easier. We chose a time step of 0.125 ns for our calculations. Though Fig. 1 gives the impression of 0.05 ns being a better choice for a time step, comparing the results of the simulation for different time steps no significant difference for all time steps up to 0.125 ns was discernible For the sake of reducing calculation time we therefor chose aforementioned time step. Also, keep in mind that Fig. A starts with a delta distribution, which causes a high concentration gradient.

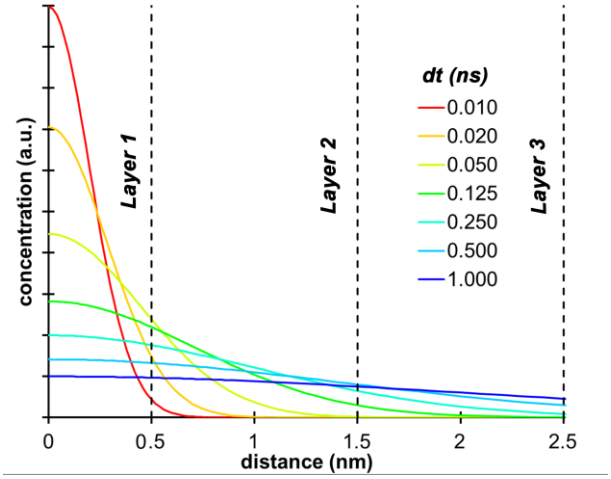

Fig. A: Spatial distribution of oxygen in water after 1D diffusion for the time indicated in the legend. It is assumed that the oxygen is delta distributed in the centre of layer 1 at time 0.

For a broader distribution with lower gradient as given in reality, the changes are much more moderate.

As our model works with layers we have to discretize (1):

$$\frac{\Delta n}{\Delta t} = -D \cdot A \cdot \left(\frac{\Delta c}{\Delta x}\right)_{p,T} \qquad (3)$$

Δc is then the difference of concentration between two layers and Δx the distance between them, hence the thickness of the layers. Exactly spoken, this formula is valid for Δc = const only. However, if the time interval is selected short enough, this limitation is sufficiently respected. Consequently, time steps that are too long cause the simulation to become unstable. If we assume the changes in concentrations to be sufficiently small during each time step, the change of the concentration in layer j (c_j) is:

$$\Delta c_j = \frac{\Delta n}{V_j} \qquad (4)$$

Δn is left without index, even though it is different for each pair of layers, but we limit the description here to one neighbouring pair of layers (j and j+1).

(3) and (4) gives:

$$\Delta c_j = -\frac{D \cdot A}{V_j \cdot \Delta x} \Delta c \cdot \Delta t \qquad (5)$$

In our model the volume V_j of the layers grows, dependent on their position in the model (see Fig. 1 in the paper), which we approximate as:

$$V_j = \Delta x \cdot 4\pi(j \cdot \Delta x)^2 = j^2 \cdot V_1 \quad (6)$$

This approximation is good only for j>15. However, since the contribution of the innermost layers to the overall signal is not very important (as these layers represent a very small fraction of the overall volume) and the inner volume of the liposome is overestimated only by 1.6% relative to the volume of the liposomal bilayer, the error originating from this approximation is negligible considering the error margin of the DLS size determination.

For estimation of the transit cross section between layer j and j+1 we calculate the area of a sphere with a radius which is the geometric average of the radii of the involved layers, hence:

$$A_{j,j+1} = 4\pi(\Delta x)^2 \cdot j \cdot (j+1) \quad (7)$$

Then the amount of oxygen passing this interface is:

$$\Delta n = D \cdot \frac{A_{j,j+1}}{\Delta x} \Delta c \cdot \Delta t \quad (8)$$

The changes in concentrations c_j and c_{j+1} are then:

$$\Delta c_j = \frac{\Delta n}{V_j} = \frac{j+1}{j} \cdot \frac{D \cdot \Delta c \cdot \Delta t}{\Delta x^2}$$

$$\Delta c_{j+1} = \frac{\Delta n}{V_{j+1}} = \frac{j}{j+1} \cdot \frac{D \cdot \Delta c \cdot \Delta t}{\Delta x^2} \quad (9)$$

For each layer the change in concentration due to interaction with both neighbouring layers is calculated. Layer 1 and 1000 interact only on one side. The diffusion constant for water and lipid layers is chosen accordingly. At phase borders (neighbouring layers with different internal conditions) diffusion is calculated based on the parameters of lipid.

Singlet oxygen generation and decay are calculated for each time step. Generation of singlet oxygen (only in layer 37 and 38 following the triplet decay) is considered before diffusion of the respective cycle and decay of singlet oxygen (in all layers according to its local decay time) is considered thereafter.

Our experimental measurements are done with a time resolution of 20 ns. To enable simple fitting the calculated values of the simulation are recorded accordingly. Every 160 cycles the overall amount of singlet oxygen in water layers (n_W) and in lipid layers (n_L) is calculated separately by summing up the product of concentration and volume for each layer using (6):

$$n_W(t_i) = \sum_{j=1}^{34} c_j(t_i) \cdot j^2 \cdot V_1 + \sum_{j=40}^{1000} c_j(t_i) \cdot j^2 \cdot V_1$$

$$n_L(t_i) = \sum_{j=35}^{39} c_j(t_i) \cdot j^2 \cdot V_1 \quad (10)$$

t_i represents the time corresponding to channel i in our TCMPC measurements.

The final fitting procedure is done then by varying the parameter set (singlet oxygen decay outside the lipids and PS triplet decay time) and optimizing χ^2_{red} by changing A, B and C for the fit function $f(t_i)$:

$$f(t_i) = A \cdot n_L(t_i) + B \cdot n_W(t_i) + C \quad (11)$$

The ratio of the amplitudes A/B represents the ratio of radiative rate constants and C factors in the dark counts of the setup, which are equally distributed over time. As outcome of this fitting procedure we get the best achievable χ^2_{red} and the according rate ratio as a function of the parameter set.

The minimum of this function indicates the most probable parameter set (Fig. 4 in the paper).

Strictly spoken, the equations above are valid only for a flat free energy profile for translocation of $^1O_2$, which means that the solubility of $^1O_2$ is constant all over the sample. However, recent calculations of the free energy across a very similar bilayer (made from POPC) report anything but a flat profile [3]. The solubility of $^1O_2$ inside the bilayer was found to be much higher than outside. This was also found experimentally for DMPC before [4].

If we simplify the free energy profile to a step function, flat inside and outside the membrane and just unsteady at the interface, the differences in solubility just affect diffusion between two pairs of layers, namely those that represent phase borders.

In terms of diffusion, a layer with twice the solubility behaves like a normal layer with just half the concentration.

So if $^1O_2$ is S times better soluble in the membrane than in water, for calculating Δc at phase borders in (8) the concentration of $^1O_2$ in the lipid layer has to be divided by S.

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