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Biophysics Lab Course

Phase Transition in Phospholipid Vesicles

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1 Introduction

In a large part, the knowledge about structure and dynamic of biological membranes is based on biophysical studies at artificial biomembranes. Hereby synthetic model membranes were utilized, formed by lipids in a defined composition. Depending on the method of preparation, various lipid layer systems can be made, depicted in figure 1.

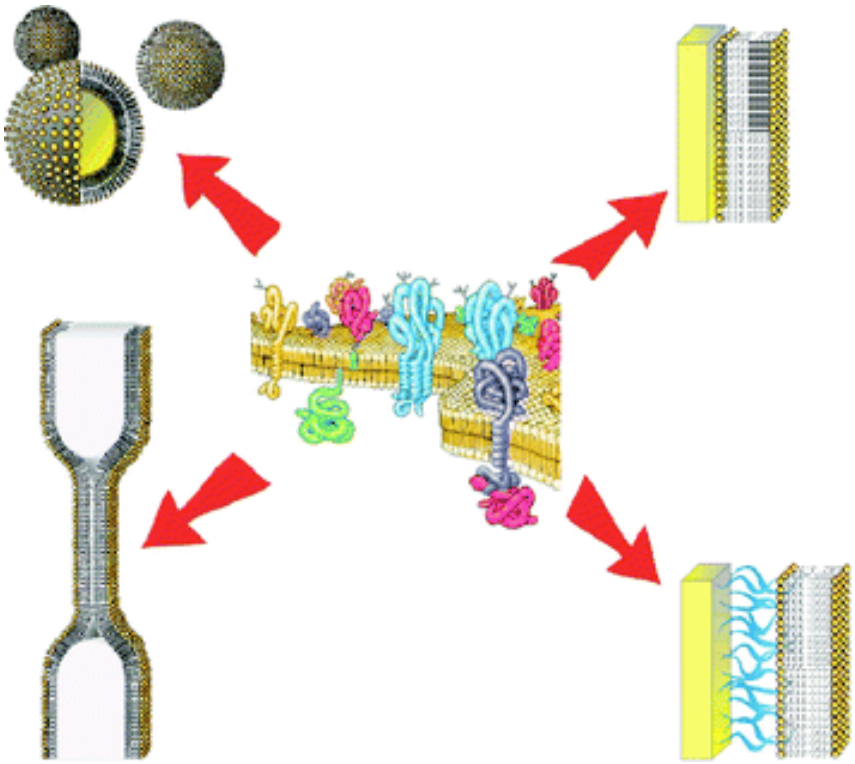


Figure 1: Schematic description of four different model systems for a biological membrane (center). Vesicles (upper left) are useful systems to study e.g. membrane fusion processes, while bilayer lipid membranes (BLM, lower left) allow for the electrical characterisation of membranes but suffer from instability. Solid supported membranes (upper right) provide increased stability, however the space between the membrane and substrate limits the ability to functionally incorporate membrane proteins. In a tethered bilayer lipid membrane (tBLM, lower right), the bilayer is separated from the substrate using a spacer that also covalently anchors the inner leaflet to the substrate [1].

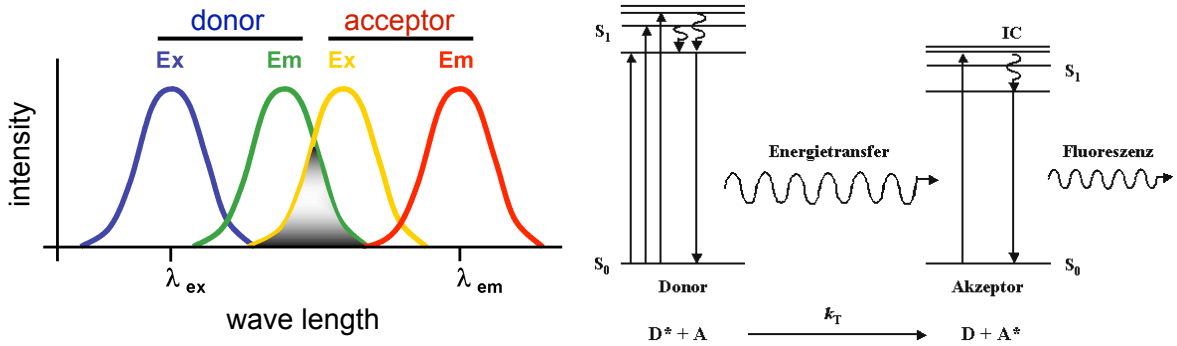
A widely used model system are vesicles - spherical lipid bi- or multilayers . They can be easily produced with a defined size and composition. Due to the dispersion of lipids in water, multilamellar vesicles are formed which can be transformed in unilamellar vesicles by ultrasonification or extrusion.

Generally, natural membranes consist of various lipid components coexisting in different physical states. In the experiment, there are various strategies to induce a phase separation in model membranes. One method is based on the separation in ordered and disordered domains due to the mixture of lipids with different structural features. Another methode to induce a phase separation consist in the variation of temperature. By means of fluorescence microscopy, DPPC (1,2-dihexadecanoyl-sn-glycero-3-phosphocholine) containing domains could be visualized which are formed below the phase transition temperature [2]. This phase separation can be also examined by fluorescence resonance energy transfer (FRET). Hereby the fluorescence

resonance energy transfer between two fluorescent probes is investigated which have a different affinity for the ordered and the disordered phase respectively [3].

2 Fluorescence resonance energy transfer

The mechanism of the energy transfer is a physical resonance phenomenon. Hereby the electron system of the donor is considered as a mechanical oscillator which transfers its exitation energy - analogous to the example of two coupled pendulums - to a second oscillator. Indeed this coupling is only possible if the electronic transition of the acceptor is corresponding to the frequence of the donor. Thus, the condition for an energy transfer between the molecules is an overlap between the absorption spectra of the acceptor and the emission spectra of the donor (see figure 2 a [4]).



(a) Schematic spectra of a donor-acceptor pair (b) Jablonski diagram of the Förster energy transfer

Figure 2: FRET

The energy transfer occurs due to a weak coupling between the orbitals of a donor and an acceptor molecule up to a distance of 8 nm. Hereby, a donor molecule will be exited and gets in the first electronically exited state S_1. At an appropriate spatial proximity to an acceptor molecule, the deactivation of the donor does not take place by fluorescence but rather by the transfer of the exitation energy to an acceptor. The acceptor is raised in the first electronically exited state S_1 and for his part able to fluoresce ($S_1 \rightarrow S_0$). The appropriate energy diagram is depicted in figure 2 b [5].

3 Detection of phase transition by FRET

The detection of the lipid domain formation will be carry out by means of a two-probe donor-acceptor fluorescence spectroscopy. The two probes have a relatively different affinity for the lipid domains. If dynamic domains are formed, resulting in phase coexistence, the two probes will demix and separate into the coexisting gel or fluid domains where they have in each case a higher relative solubility. The separation of the two probes into the coexisting gel and fluid domains increases the average separation between the donor and the acceptor. Consequently, the fluorescence resonance energy transfer from the donor to the acceptor decreases and leads to an increase in the fluorescence intensity of the donor.

4 Tasks

1. 7.34 mg DPPC (M=734 g/mol) was dissolved in 10 ml Chloroform. Three samples containing 150 ul DPPC solution were prepared. 1 ul NBD solution (c=1 g/l, M=956.25 g/mol) was added to the first, 1 ul Rhodamine (c=0.5 mg/ml, M=1333.81 g/mol) to the second and 1 ul from each fluorescent dye to the third sample. The chloroform was subsequently removed by using a stream of N_2 and dried over night at vacuum.

   Calculate the molare ratio of DPPC and the dyes as well as the average molar mass of the mixtures (at home!).

2. Disperse the dried lipid/dye mixture in a 50 mM KCl and 1 mM NaN_3 buffer solution to get a final lipid concentration of 0.11 g/l. Keep the lipid/dye suspension for at least one hour at a temperature of 10 °C above the main phase transition temperature of DPPC (T_c=41 °C). During this time period vortex the suspension several times.

3. Measure the fluorescence spectra of the three samples at a temperature of 32 °C and determine the wavelength of the emission maxima (see also exp. 5).

4. Measure the temperature dependance of the fluorescence emission intensity of the samples at the maximum wavelength (Use the maxima wavelength of the donor for the DPPC / Rhodamine / NBD mixture). Use a temperature interval from 32 °C to 50 °C (steps of 2 K).

5. Plot the measured data, determine the phase transition temperature of DPPC and interpret the resulting curves.

5 Focus on preparation

- structure and functions of cell membranes
- model membranes advantages, disadvantages
- function and properties of lipids
- phase transitions and pattern formation in model membranes
- fluorescence fundamentals
- spectroscopy
- fluorescence quenching and Förster resonance energy transfer

References

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[2] Shoemaker, S. D. and Vanderlick, T. K.: Material studies of lipid vesicles in the L_α and L_α - gel coexistence regimes *Biophys. J.* **84** (2003) 998-1009

[3] de Almeida, R. F., Loura, L. M., Fedorov, A., and Prieto, M.: Nonequilibrium phenomena in the phase separation of a two-component lipid bilayer *Biophys. J.* **82** (2002) 823-834

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