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Study of membrane thickness by energy transfer

REINER PETERS

Physikalisch-chemisches Institut der Universität Marburg, Marburg/Lahn and Max-Planck-Institut für Biophysikalische Chemie (Karl Bonhoeffer-Institut), Göttingen (Germany)

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SUMMARY

The monolayer assembling technique as used by Kuhn and coworkers to study the transfer of excitation energy is applied to determine membrane thickness. Membranes of human red blood cells are embedded in monolayer assemblies which contain chromophores in a known molecular arrangement. Energy transfer between chromophores on one side of the membrane and a thin gold layer on the other side is observed by fluorescence microscopy. The value for the thickness of the red cell membrane as determined by this technique agrees with values known from the literature.

Monomolecular layers of fatty acids can be spread at the air–water interface[1] and may be transferred from there to a solid support[2]. Using certain stearyl substituted dyes in addition to fatty acids monolayer assemblies have been built up which consist of dye layers and fatty acid layers[3]. Molecular properties of such assemblies have been studied by macroscopic measurements of electronic excitation energy transfer. Energy transfer at a microscopic level is demonstrated in Fig. 1.

Now it will be shown how cellular membranes can be embedded in monolayer assemblies. For this purpose a method for the preparation of dry ghosts described by Waugh and Schmitt[4] has been modified. Glass slides covered with a thin gold layer (transmission, 70%) by vacuum evaporation were placed horizontally into a small chamber. The chamber was filled with a diluted suspension of human erythrocytes, which had been freshly collected and washed 3 times. After a few minutes the chamber was continually perfused with isotonic saline until all erythrocytes not sedimented on the slide were washed out. The adhering erythrocytes were hemolysed by perfusing with 7 mM phosphate buffer, pH 7.6 [6] for 4 min. The slide was then transferred into a Langmuir trough containing a subphase of $2.5 \cdot 10^{-4}$ M $CdCl_2$, pH 5.5 (for details of the monolayer technique employed in this laboratory see ref. 5). After a monolayer had been spread and compressed by 30 dynes/cm the slide was withdrawn through that monolayer and dried. As has been

pointed out[4] the covering monolayer is an essential part of the preparation because it lowers the surface tension. Thus the delicate membranes dry uniformly without rupture. While drying, the ghosts become flat discs, the opposing parts of the cellular envelope adhering firmly. After this step the slides could be covered with additional monomolecular layers. Dye layers were spread from a solution of *N,N'*-distearyloxacyanine★ mixed with arachidic acid at a molar ratio of 1:5. This dye is excited by ultraviolet radiation and shows a blue fluorescence (for details see ref. 3). The preparations were inspected with a Zeiss Standart Universal fluorescence microscope employing a vertical illuminator. No fluorescence was detected in preparations without dye layer. Fluorescence microscopic pictures of assemblies covered with a dye layer are shown in Fig. 2.

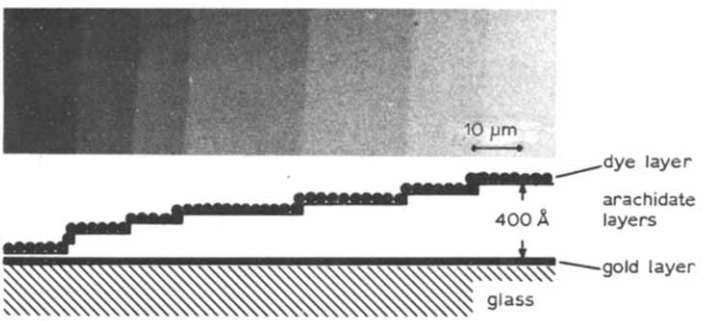

Fig. 1. Fluorescence microscopic picture of a monolayer assembly showing energy transfer between a fluorescent dye and gold. As indicated schematically below the photograph the distance between the dye layer and the thin gold layer is varied from 26.5 Å to 397.5 Å by a stepwise arrangement of arachidate layers. Left-hand side, small distance between dye and gold, much excitation energy of the dye transferred to gold, weak fluorescence. Right-hand side, large distance, little energy transfer, strong fluorescence.

This technique can be used to determine the thickness of the membrane in a very simple manner. In Fig. 3 a monolayer assembly is shown including a dry ghost and a stepwise arrangement of arachidate layers. Careful visual comparison of the fluorescence intensities already shows that the intensity above the ghost is very similar to that of part b of the photograph, whereas fluorescence in part a is markedly weaker, in part c markedly stronger. Since in part b seven arachidate layers are deposited between dye layer and gold layer and the ghost is covered by one arachidate layer, the ghost obviously corresponds to six arachidate layers. The thickness of one membrane is therefore approximately 80 Å (one arachidate layer is 26.5 Å thick[7]). This value, although only a preliminary estimate, agrees well with values known from the literature. Coleman *et al.*[8] for instance, obtain by X-ray diffraction a value of 80–85Å for the dry red cell membrane.

The accuracy of the measurement may be improved considerably. If conditions are chosen optimally for energy transfer (as possible by variation of the transmission of the

★The dye was synthesized by J. Sondermann in this institute.

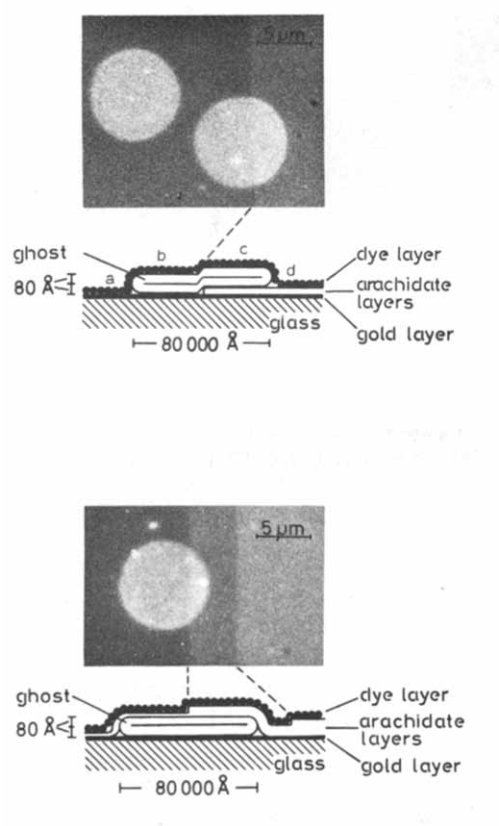

Fig. 2. Fluorescence microscopic picture of dry ghosts embedded in monolayer assemblies. The position of the ghosts within the assemblies is indicated schematically below the photograph. (Note the different scale for thickness and diameter of the ghosts.) a. The assembly was built up as follows: Slide covered with a thin gold layer, part of the gold layer covered with an arachidate double layer, deposition of ghosts and dye layer. As expected from Fig. 1 a larger distance between dye layer and gold layer corresponds to a larger fluorescence intensity: Fluorescence is strongest in part c (two arachidate layers *plus* ghost separating dye layer and gold layer), weaker in part b (only ghost between dye and gold), in part a (direct contact of dye and gold) very weak fluorescence, which increases markedly in part d (two arachidate layers separating dye and gold). b. Procedure different from that in a: Ghosts were deposited directly on the gold layer and covered with one arachidate layer. This was followed by the stepwise arrangement of arachidate double layers and deposition of the dye layer. It may be seen that exactly as in a, a larger distance between dye layer and gold layer corresponds to a larger fluorescence intensity.

gold layer) theoretical considerations[3] show that

$$\frac{d_0}{d_1} = \left[2 \frac{I_0}{I_1} - 1 \right]^{1/4}$$

where d_0 and d_1 are distances between dye layer and gold layer, I_0 and I_1 are the corresponding

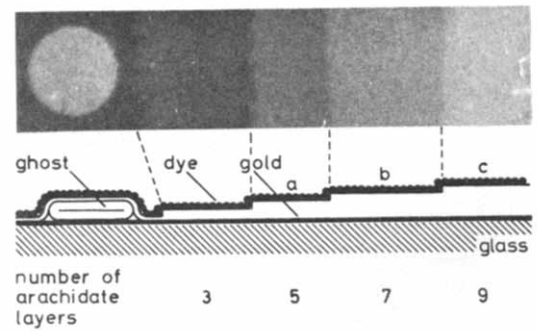

Fig. 3. Fluorescence microscopic picture of a dry ghost embedded in a monolayer assembly. The ghost was deposited on the gold layer and covered with one arachidate layer. This was followed by the stepwise arrangement of arachidate double layers and deposition of the dye layer. The assembly shown here may be used to determine the membrane thickness as described in the text.

fluorescence intensities. This relation holds for constant refractive index of the interlayers, which is given approximately: the refractive index of fatty acids is 1.522 (ref. 9), the refractive index of the ghosts is 1.504 (ref. 4). A difference in fluorescence intensity of 10% detected, for instance, by a microfluorometric device therefore corresponds to 5% or 4 Å of membrane thickness. In macroscopic measurements of energy transfer systems similar to the one shown in Fig. 1 a resolution as high as this has indeed been demonstrated[3].

This paper is limited so far to the determination of the thickness of dry cellular membranes. However, as will be discussed finally, the described technique can also be used to study the internal structure of membranes. Moreover, thickness and structure of membranes may be studied under physiological conditions. If for instance intact, suspended erythrocytes are adsorbed to a slide covered with a monomolecular dye layer, energy transfer between the dye layer and hemoglobin inside the adsorbed erythrocytes can be used to determine the membrane thickness *in vivo*. Membrane structure may be studied by measurements of energy transfer between a monomolecular dye layer and natural or artificially introduced chromophores within membranes adsorbed to that dye layer.

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