

## IMAGING OF MEMBRANE VESICLES

### BY TEM AND AFM

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The spontaneous formation of phospholipids in water, above their gel/liquid crystalline transition temperature, into globules formed by one lipid bilayer (unilamellar liposomes) or by several bilayers of lipid separated by aqueous layers (multilamellar liposomes) has led to their use as model systems for biomembranes. Ions as well as higher molecular weight particles like proteins can be trapped within these vesicles (1,2). Intramembrane proteins (IMP) can directly be incorporated into the membrane (3,4). These model systems, for example, are suitable for studying interactions of IMPs and the surrounding lipids or soluble proteins (4,5). Furthermore, the activity of incorporated pores or ion pumps respectively exchangers can be investigated (5).

With microscopic techniques, the morphology of the examined samples is imaged. For Transmission Electron Microscopy (TEM), several preparation methods are available: negative staining, freeze-fracture, embedding, cryo-sectioning and direct viewing with cryo-TEM. Among these techniques, only the freeze-fracture replication technique is able to image the membrane surface in TEM. Alternatively, Scanning Electron Microscopy (SEM) and the recently developed Scanning Probe Microscopy (SPM) techniques (6,7) are used. Among the SPM techniques Atomic Force Microscopy (AFM) is the most suitable one to examine membrane vesicles. With AFM interatomic forces between the probe and the sample surface are detected which reflect the surface morphology (7).

In this paper preliminary results of imaging of synthetic liposomes with incorporated rhodopsin molecules, the pigment of the vertebrate visual system, and membrane vesicles from rod photoreceptor cells of the bovine retina are presented. It could be shown that negative stained liposome and membrane vesicle samples can be used parallel for TEM and AFM investigations. Large scale images, i.e. imaging areas of some squaremicrons, show analogous structures in TEM and AFM. Due to the higher resolution capability of AFM ultrastructural details could be observed in the examined membranes whose origins are not yet clear. AFM of liposomes under native conditions, i.e. in a fluid cell, reveals also ultrastructural details, but up to now only in a poor and noisy quality.

#### Experimental:

Reconstitution of rhodopsin in liposomes prepared from egg-phosphatidylcholin (egg-PC, Fluka) was performed as described by Jackson & Litman (3). Membrane vesicles from rod cells of the bovine retina were prepared according to Smith (8). For negative staining, carbon coated TEM grids (200 mesh, Science Service) were hydrophilized by glow discharge. Subsequently, the grids were placed on a drop of the vesicle suspension allowing the vesicles to adhere to the carbon film. After 1 min the grids were transferred onto a drop of a 2 % (w/w) uranylacetate solution for staining, washed several times in aqua bidest and air dried.

For cryo-TEM, grids were prepared according to Akira & Adachi (9). The grids were covered by a Triafol film (Merck) containing holes of different sizes, picked up in a tweezer and transferred to a KF-80 rapid cooling device (Leica). A small drop of the vesicle suspension was placed on the filmed side of the grid and almost all liquid was removed by touching with a blotting paper. Subsequently, the sample was rapidly frozen by immersion into liquid ethane at 135 K.

For TEM the specimen were examined with a ZEISS CEM 902 operated at 80 kV and equipped with the Zeiss cryo-transfer system. All AFM investigations were performed with a Nanoscope II (Digital Instruments Inc.).

#### Results and discussion:

Fig.1 shows liposomes which were conventionally negative stained. The membranes appear as regions of electron transparency against the dark background of the glassy electron dense stain. Almost all liposomes are unilamellar and some form large agglomerations. The individual vesicles are always roundshaped and appear sometimes spherical. In fig.2 liposomes are shown which were cryofixed and examined at 105 K in the TEM. One multilamellar vesicle can be seen among the roundshaped unilamellar liposomes. The lipid bilayer is always clearly resolved. The poor contrast of vitrified samples in cryo-TEM images was improved by zero-loss filtering in the Zeiss CEM 902.

In fig.3, the TEM grid used for fig.1, was examined with AFM. The notion that liposomes which were negative stained with uranylacetate keep their spherical shape even in the air dried state was confirmed. It is known that uranyl ions interact with the phosphate groups of PC and stabilize them by crosslinking (10). The supporting carbon film as well as the membranes proved to be stable enough for AFM investigations. Higher resolution AFM images of PC liposomes reveal small bumps of approximately 2 nm diameter which appeared sometimes ordered (not shown). The origin of these bumps is not yet clear, but probably they were formed by reaction products of the uranyl ions with the components of the membrane, i.e. the phosphate groups of PC and the incorporated protein. A discrimination of protein and lipid was not possible. To avoid the effects of staining agents on the structure of the membranes, native liposomes were examined in the AFM in a fluid cell. Fig.4 shows some liposomes attached to a hydrophilized carbon film. The minor stability of the native membranes complicates higher resolution. First experiments nevertheless show similar structures in the membranes as the crosslinked and air dried samples (not shown).

Fig.5 and 6 illustrate another example for parallel investigations of biomembranes in the TEM and AFM. Disk membranes of rods of the bovine retina show round membrane sheet structures in negative stained samples (fig.5). The image was taken in the ESI mode (Electron Spectroscopic Imaging) of the Zeiss CEM 902 with an electron energyloss of 250 eV. The background granularity has its origin in the uranylacetate solution and is superimposed in the TEM image. In the AFM image (fig.6) the same morphology can be seen. The background structure is also present and its influence on the membrane surface can be observed. AFM images with a higher resolution show again a clumpy structure with the same dimensions as observed in the examined liposomes (not shown). Experiments in buffer solution in the fluid cell are still in progress.

These first results show that samples prepared for TEM are suitable for AFM investigations as well. Cryo-TEM in combination with AFM in a fluid cell offer new possibilities in the field of biomembrane research.

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References: 1. Brandl M., Bachmann D., Drechsler M., Bauer K.H.; J. Biomat. Art. Cells, Art. Organs 1989,17/5,677-678; 2. Gregoriades G.; Liposomes as drug carriers, John Wiley, New York, 1988; 3. Jackson M.L., Litman B.J.; Biochem. Biophys. Acta 1984,812,369-376; 4. Dencher N.A.; in: NATO ASI Series Vol. H40, Dynamics and Biogenesis of Membranes, ed. J.A.F. Op den Kamp, Springer, Berlin Heidelberg, 1990,343-360; 5. Van Deenen L.L.M. et al., Ann. NY Acad. Sci. 1975,264,124-141; 6. Binnig G., Rohrer H., Gerber Ch., Weibel E.; Phys. Rev. Lett. 1982,49,57; 7. Binnig G., Quate C.F., Gerber Ch.; Phys. Rev. Lett. 1986,56,930; 8. Smith H.G., Stubbs G.W., Litman B.J., Exp. Eye Res. 1975,20,211-217; 9. Akira F., Adachi K.; J. Electr. Microsc. 1965,14/2,112-118; 10. Shah D.O.; J. Colloid & Interf. Sci. 1969,29,210.

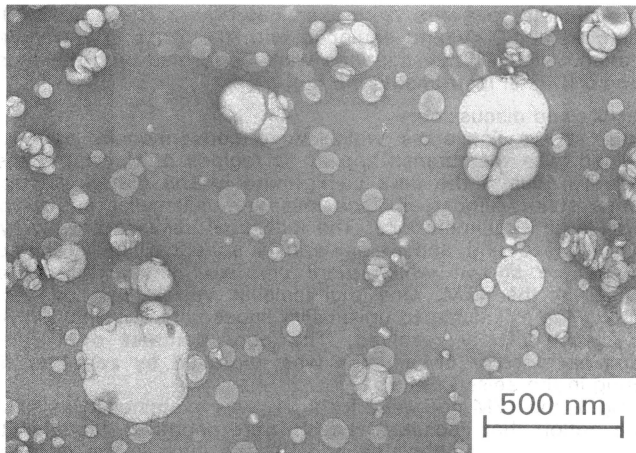


Fig.1 Global brightfield TEM image of negative stained and air dried egg-PC liposomes.

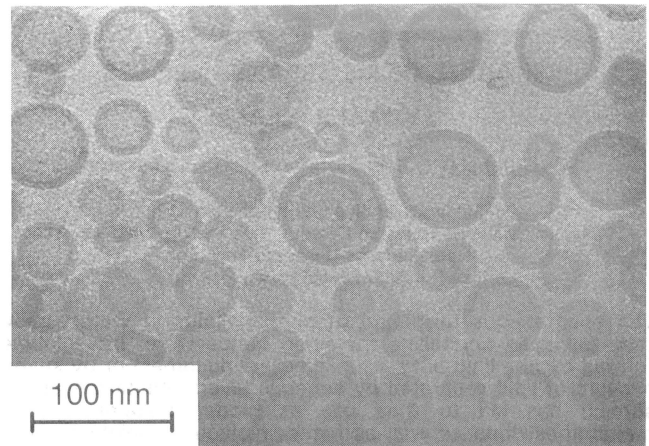


Fig.2 Zero-loss filtered cryo-TEM image of egg-PC liposomes.

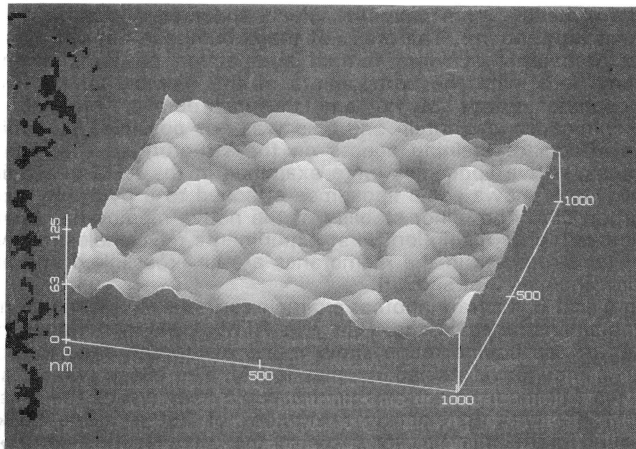


Fig.3 AFM surface plot of egg-PC liposomes. A different place of the same sample as used for figure 1 is shown.

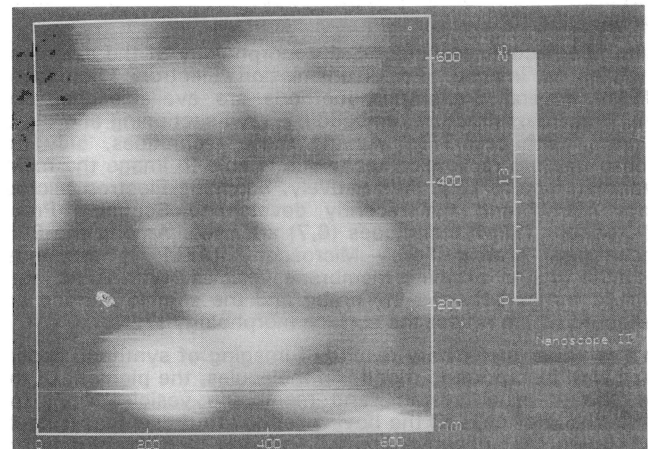


Fig.4 AFM topview image of egg-PC liposomes. The sample was examined in an AFM combined with a fluid cell.

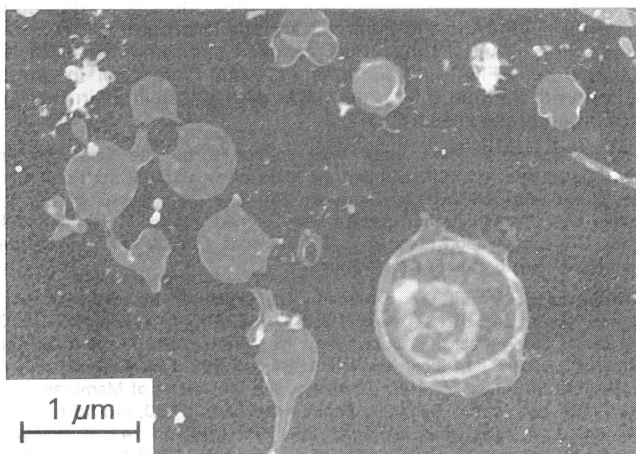


Fig.5 TEM image of negative stained and air dried membrane vesicles prepared from outer segments of bovine retinal photoreceptor rod cells. The image was taken in the ESI mode (Electron Spectroscopic Imaging) of the Zeiss CEM902 at 250 eV energy loss ("structure sensitive contrast").

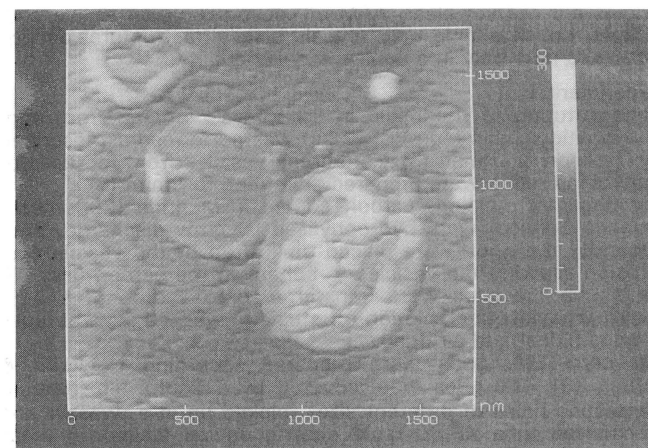


Fig.6 AFM topview image of membrane vesicles prepared from outer segments of bovine retinal photoreceptor rod cells. A different place of the same sample as used for figure 5 is shown.