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Characterization and Analysis of Biomimetic Membranes

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Characterization and analysis of biomimetic membranes represent a challenge to the relatively young field of nanotechnology. Performing measurements on these few nanometers thick, soft, viscoelastic and moderately dynamic systems is close to the limits of the available tools and methods. It is thus important to understand the physics involved in the characterization process to be able to ask the right questions and deduce a correct interpretation of the answers. In this chapter, we provide an overview of the physical properties of biomimetic membrane systems, describe the tools that can measure these properties, and identify a few common errors and artifacts. At the end, we briefly discuss the possibilities and potentials in the emerging methods.

4.1. Important Properties of Biomimetic Membranes

Before engaging ourselves in the discussion of the characterization of the biomimetic membranes, let us consider of what respect do we need to perform such measurements. The first and most important question one can ask about a biomimetic membrane system is why do we resort to use it. A biomimetic membrane is, by its nature, a necessary compromise; a testbed of selected processes, conveniently reducing the complexity of living organisms. Accordingly, it is not, and it cannot be, the artificial version of a piece of a cell wall; it is less, but what makes it less, that makes it also more. Since the surface of the membrane can be easily accessed, the mechanism of selected membrane processes, related to signaling, regulation and metastasis of the cells can be studied in controlled conditions with high resolution. Changes in the chemical environment, membrane potential, mechanical stress as well as other perturbations can be precisely and systematically applied. The membrane can serve as an anchor bed for functionalised biomolecules used in biosensor applications. Accordingly, the target of characterization is not so much the membrane itself, but rather the membrane-inserted structures (practically, both) where the
requirements towards the method are set by the dimensions and properties of the membrane inserted structures under investigation (Figure 4.1). This requirement leads to the preferred selection of imaging or analytical methods of subnanometer resolution. Whereas it is disputed if studies of processes on artificial membranes in vitro have any relevance to the same processes in vivo, a number of well planned, comprehensive works prove the sceptics wrong. The key of performing such studies is a good understanding of the limits of a biomimetic system; the carefully drawn line between the general and the circumstantial characteristics of the observed phenomena.

It is crucial therefore to know and understand and well characterize our system. When identifying the parameters that we need to know, the most obvious basis of comparison is the membrane involved in the biological process we aim to study. Composition of the membrane and the buffer solutions are key control parameters, which are however purposefully selected and thus known from the beginning; their relevance was discussed in other chapters. The properties of the membrane not known from the start, however, are just as important. First of all, the general mechanical descriptors of the system: the amount of lipid forming the membrane, that is, mass and thickness; the coverage of the surface, and the nature of the discontinuities in this coverage, simply said, morphology. Equally important are the properties describing the membrane in interactions: the stability against mechanical intrusions, shear and penetration, the rate of regeneration, the potential for incorporation of e.g. aminoacid structures: elasticity, viscosity and surface energetics. May be less obvious are the dielectric properties, which however closely relate to the electrical field around the membrane, held responsible for many aspects of protein-membrane interactions.

Last but not least, we have to mention that most biomimetic systems are actually supported membranes: phospholipids deposited to a convenient—usually

**Figure 4.1.** AFM morphology image of A) a phospholipid membrane patch without any proteins inserted, inset: a zoom into the membrane, B) a membrane surface with connexin hexamers (gap junctional hemichannels) fused into the membrane. Examples of open hemichannels are encircled. C) shows gel electrophoresis results of the analysis of the protein; the 43 kDalton band confirms the presence of the connexins. Reprinted from Thimm et al.\textsuperscript{7}. Copyright (2005) by the American Society for Biochemistry and Molecular Biology.