New and Notable

Out and In: Simplifying Membrane Protein Studies by AFM

Daniel J. Muller
Center of Biotechnology, Technische Universität Dresden, 01307 Dresden, Germany

Characterizing the oligomerization, supramolecular assembly, and structure-function relationship of membrane proteins in their native environment builds an immediate need to answer many pertinent questions in modern molecular and cell biology and particularly in drug research. Although of all the proteins encoded by the human genome only ≈30% are membrane proteins, they constitute >50% of all drug targets. This is because membrane proteins are virtually involved in stimulating and controlling every process of life.

In this issue Milhiet and co-workers report a new and simple strategy to observe native membrane proteins reconstituted into supported lipid bilayers. Firstly, a lipid bilayer was deposited onto a supporting mica surface. The self-healing property of supported lipid membranes minimizes defects and ensures an almost complete coverage of the support. After this, the supported bilayer was destabilized using a sugar-based detergent and solubilized membrane proteins were added to the solution. Depending on the incubation parameters, the membrane proteins were inserted into the lipidic bilayer. The membrane proteins were then ready to be imaged using high-resolution atomic force microscopy (AFM). AFM topographs revealed the supramolecular assembly of light harvesting complexes and reaction centers from three different organisms. The spatial resolution of these topographs was sufficient to resolve the unidirectional orientation and stoichiometry of individual transmembrane proteins. The preparation method only requires picomolar amounts of proteins and is thus applicable to membrane proteins that cannot be expressed at levels required for other structural studies such as x-ray crystallography, electron crystallography, or NMR. The approach is particularly suitable to characterize how different lipid compositions influence the formation of supramolecular membrane protein assemblies. Such insights contribute to a detailed understanding of protein-lipid interactions. Similarly a systematic study on how environmental conditions change supramolecular membrane protein assemblies can be performed. Being of further importance to many biologists, the setup enables one to characterize the structure-function relationship of membrane proteins embedded into their native environment, the membrane. Several examples of AFM investigations providing such insights have been demonstrated on membrane proteins that have been first reconstituted and then adsorbed onto a supporting surface (1). The simple preparation method of Milhiet et al. makes characterizing these relationships easier, and it may be speculated that this approach will open a fast track toward investigating membrane proteins by AFM routinely. In the future this approach may be applied to characterize membrane proteins of bilayers supported by different surfaces such as those used for many biotechnological applications.

However, several bottlenecks must be encountered and overcome to reach these goals. It remains to be demonstrated that the method is applicable to membrane proteins other than the ones investigated by Milhiet et al. The success of this method for other membrane proteins may be expected since many laboratories already established procedures to reconstitute membrane proteins into bilayer systems. The challenge, though, would be to transfer and to apply this knowledge to supported lipid membranes. A major breakthrough would be to demonstrate that membrane proteins can be inserted into planar lipid membranes supported by other surfaces. Usually the separation of a membrane adsorbed onto a hydrophilic support ranges between 0.5 and 2 nm. Water molecules bridging this gap certainly have different properties compared to water in the bulk solution. The water structure is highly disturbed and its mobility significantly reduced. At the same time lipid molecules and membrane proteins establish nonnative and nonspecific interactions with the support (2). In case of mica these interactions are very weak. Nevertheless, they restrict the lateral mobility of membrane proteins. As a result, membrane proteins exhibiting large anchors or aqueous domains show significantly impaired behavior in supported bilayers. Depending on their chemical and physical surface properties, other supports can establish much stronger interactions with membrane proteins and lipid bilayer. There have been very few careful studies on the functional consequences of these interactions, but as we know from other studies on native membranes such interactions can enhance or even impair the membrane protein function (3,4). To suppress nonspecific and nonnative interactions of membrane proteins and lipids with the support has led to the idea of spatially separating both from the supporting surface (2). The final goal would be to show that the supported membrane bilayer has functional properties similar to that of the cell surface.

Polymer supported membranes are prepared by adsorbing a phospholipid bilayer onto a polymer cushion covering the support. This polymer cushion serves as a lubricating layer between the membrane and the support. It assists self-healing of local defects in the membrane, allows the insertion of even large transmembrane proteins, and eliminates unspecific interactions with the support.

Submitted July 20, 2006, and accepted for publication July 24, 2006.

Address reprint requests to Daniel J. Muller, E-mail: mueller@biotec.tu-dresden.de.

0006-3495/06/11/3133/02 $2.00

doi: 10.1529/biophysj.106.092999

However, instead of using a nonbiological polymer, components of the extracellular matrix may be used as a cushion. In this case the environment of the membrane would be the native one. An alternative strategy would be to separate the lipid bilayer and the support by replacing the headgroups of some lipids using lipopolymer tethers. Lipopolymer and protein tethers act as spacers typically ranging between 10 and 100 nm, thus controlling the distance between the membrane and the support. By adjusting the spacer length this distance can be controlled, whereas the spacer density would control the lateral mobility of lipids and membrane proteins.

Milhiet and co-workers have contributed an exciting work toward reconstituting membrane proteins into supported planar bilayers and for their characterization using AFM. This approach may open a new and easily accessible avenue to characterize the structure and function relationship of membrane proteins. The ultimate goal, however, will be to further develop this approach to be able to reconstitute any given membrane protein into a lipid bilayer supported by a carrier mimicking the native environment of a cell membrane. Then the power of AFM for resolving the structure and function relationship of membrane proteins can be fully exploited, tackling an almost uncountable number of pertinent questions in cell biology, biophysics, biotechnology, and pharmacology.

REFERENCES

1. Muller, D. J., K. T. Sapra, S. Scheuring, A. Kedrov, P. L. Frederix, D. Fotiadis, and A. Engel. 2006. Single-molecule studies of membrane proteins. *Curr. Opin. Struct. Biol.* In press.
2. Tanaka, M., and E. Sackmann. 2005. Polymer-supported membranes as models of the cell surface. *Nature.* 437:656–663.
3. Mackenzie, K. R. 2006. Folding and stability of alpha-helical integral membrane proteins. *Chem. Rev.* 106:1931–1977.
4. Tristram-Nagle, S., and J. F. Nagle. 2004. Lipid bilayers: thermodynamics, structure, fluctuations, and interactions. *Chem. Phys. Lipids.* 127:3–14.