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Do It Fast: Immediate Functional Testing of Membrane Pumps Expressed into Nanodiscs

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Functional analysis of a large number of ion pumps and their variants is a time-consuming task and often retards research progress of this important class of proteins. The modus operandi is to heterologously overexpress the proteins into single-celled bacteria, yeast, or cell cultures and to purify by extraction from the membrane environment with the help of detergents and subsequent purification steps. The proteins are then subjected to biophysical investigations. In their recent work, Henrich et al. (1) elegantly show how this cumbersome procedure of expression, solubilization, purification, and analysis can be cut short and handled within a few days.

Because, at least by the invention of the new research field of optogenetics, which was fast-paced after the groundbreaking work of Nagel et al. (2), tuning of the photoinduced proteins to switch cell functions became more and more important. Before introducing them into a complex organism to make them react upon light, these proteins need to be carefully analyzed by biophysical methods.

The nanodisc technology, offering a cozy environment for membrane proteins, provides the opportunity to test them with many kinds of biophysical investigations, exemplified in Henrich et al. and Volz et al. (3,4), and recently reviewed by Denisov and Silver (5). Henrich et al. (1) take this approach a step forward by investigating the ion pump KR2 (rhodopsin-2 from *Krokinobacter eikastus*) (6,7) after having expressed the protein in vitro directly into nanodiscs. The authors could analyze the identical sample via electrophysiology, taking electrical recordings in a solid supported membrane setup (8), studying the photocycle with time-resolved UV/Vis spectroscopy and performing mass spectrometric investigations. In their approach, the protein cotranslationally finds its way into the synthetic bilayer of the disk and folds in the heterogeneous dielectric medium of the bilayer. This course of action offers distinct advantages as long as the protein of interest behaves well in the cell-free expression system being supplemented with nanodiscs. Not only is the purification cut down to a simple size exclusion chromatography step of the whole expression mixture, but even this step can be omitted for some biophysical experiments such as applying difference spectroscopy. Conventional detergent solubilization might destroy the integrity of the protein, which is elegantly omitted in this new approach.

Some pumps like KR2 and bacteriorhodopsin and channels like the small viral potassium channel (9) are accessible to cell-free expression but many others are not. Insertion and proper folding of the nascent polypeptide into the biomembrane are based on mechanisms that are hardly understood when it comes to membrane proteins. The lipid composition plays a crucial role for folding in nanodiscs. The need of assistance by a translocon for individual proteins has to be tested but is dispensable for other helical membrane proteins (10). This holds also for other helper factors, which might be absent in the cell-free extract but necessary for the insertion and folding of some proteins.

It was recently shown that insertion and folding into nanodiscs can be monitored during expression from the ribosome in a cell-free approach (11). This will not only help to understand the principles of membrane protein folding but also to find the optimal conditions for the successful expression of the protein of interest in nanodiscs which subsequently can be analyzed with a sequence of biophysical investigations as described in Henrich et al. (1). An additional aspect would be to develop assays, which concurrently track functionality of the nanodisc-embedded membrane proteins during production in the expression mix.

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