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New insights into the properly folded membrane protein production for structural studies

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Motivation and Aim: Membrane proteins are involved in many processes in living cells. Their malfunction is associated with diseases, including socially important ones. Despite the significance, the exact way of their function at the molecular level is poorly studied. Partially this takes place because of substantial difficulties in preparation of properly folded samples (i. e. in an active state or in native conformation) for structural and functional investigation. Despite all recent achievements, there is still no general approach to natively folded membrane protein production. To fill this gap in knowledge, here we present three independent strategies: helical membrane protein refolding from organic solvent mixtures, cell-free synthesis of full-length active membrane protein and protein ligation as well as discuss their scope of application.

Methods and Algorithms: Bacterial expression and continuous exchange cell-free synthesis were applied for protein production. Common chromatographic techniques such as immobilised metal affinity chromatography and gel filtration were used for protein purification. A wide set of membrane mimicking media was screened for incorporation of the transmembrane domains of membrane proteins. Protein ligation was performed in an enzyme-depended reactions.

Results: We considered three different approaches to prepare the properly folded membrane proteins. First approach is the refolding of the helical membrane proteins from organic solvent mixtures. A wide range of parameters was screened. Optimal composition of the mixture for lipid/detergent particle formation from the organic-water solutions was established and the effect of the protein on this process was investigated. The rational technique of protein incorporation was developed and approved for several membrane proteins from different classes. Second approach is cell-free synthesis of full-length active membrane protein. An extensive multi-parametric screening of co-translational incorporation of protein into the membrane mimetics (detergent micelles and bicelles, liposomes and lipid-protein nanodiscs of different size and composition) revealed that Facade-based bicelles inhibits translation machinery in much lower extent than other detergent-based mimetics did. The yield of an active membrane protein was comparable to one obtained in the presence of lipid-protein nanodiscs, making Facade-based bicelles a prospective tool to be used for an active membrane protein production. Third approach is the protein ligation. We showed that the hydrophobic transmembrane and hydrophilic intracellular parts of the protein could be produced separately in

different expression systems in properly folded states, and after that successfully ligated into a single molecule by special enzyme. A set of the reaction parameters were tested and discussed.

Conclusion: Three approaches presented here illustrate possible independent ways to obtain the membrane protein samples in native conformation (i.e. in a properly folded state). Despite that each of them on their own cannot be successfully applied to every membrane protein, altogether they significantly expand the spectra of the membrane proteins applicable for structural biology.

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