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NMR study of lipid/protein interactions in trifluoroethanol-water mixtures proposes the strategy for the refolding of helical membrane proteins

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Motivation and Aim: Membrane proteins are a common target for drugs, and they need thorough structural studies to further design the specific medicines. However, prior to structural investigation a synthesized membrane protein should be brought into its native state, and this process is usually hard and tricky. To date, no universal protocol for membrane protein refolding is established, and many structural studies of membrane proteins start from an empirical and costly search of working refolding conditions and an appropriate membrane-like environment. In this work we aimed to develop a rational approach to refolding recombinant helical membrane proteins from the trifluoroethanol (TFE)-water mixtures, subsequently embedding them into lipid bicelles.

Methods and Algorithms: Mixtures of lipid, detergent and model protein were solubilized in TFE-water mixtures of different ratios, the radii of aggregates and molecule interactions were examined by high-resolution nuclear magnetic resonance (NMR) spectroscopy and dynamic light scattering (DLS). DMPC lipid, DHPC and CHAPS detergents were used to form membrane-like environments; single-helical transmembrane domains of TrkA; TLR9 receptors and the voltage-sensing domain of KvAP channel played the role of model proteins.

Results: We studied the behavior of lipid-protein-detergent associates in TFE-water mixtures with contents, gradually altering from 100 % TFE to 100 % water and established the key TFE/water ratios interesting for the refolding. Thus, the points of lipid aggregation, lipid-protein and lipid-detergent-protein complex formation were determined. Based on this data we came up with the possible protocols of refolding, tested their efficiency on the model proteins and proposed two protocols which are a promising starting point for newly studied proteins. The folding of tested proteins was controlled by NMR.

Conclusion: Upon adding water to the TFE solution of lipid and detergent the membrane protein interacts with lipids and then gets incorporated into lipid particles; at high water contents the detergent also enters the particles. Nevertheless, bicelles are formed only at 100 % water and even 10 % of TFE alters their morphology. TFE-water ratio as well as the moment of adding the detergent to the mixture can strongly affect the efficiency of refolding; solubilization of a membrane protein in a lipid-detergent mixture at 20–30 % or 50 % water appears to be a preferable refolding protocol.

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