Crystallization of integral membrane proteins (Susan Buchanan)

X-ray crystallography has become a very powerful tool for determining the structures of integral membrane proteins, with almost 200 unique membrane protein structures solved as of December 2007 (for a complete list, see Stephen White's summary at http://blanco.biomol.uci.edu/Membrane_Proteins_xtal.html). However, membrane protein structures still represent less than 1% of all structures in the PDB. The major bottlenecks in the field are the expression of sufficient quantities of functional membrane proteins and the growth of well ordered crystals for X-ray analysis. This short review covers only aspects pertaining to crystallization. Our approach to membrane protein crystallization was recently written up for Current Protocols in Protein Science (Unit 17.9, available from the NIH library website). An interesting review has been written by Patrick Loll[1].

1. Sample preparation.
Assuming you have expressed your membrane protein target at reasonable levels in the appropriate membrane environment, you will need to use a detergent or mixture of detergents to solubilize the protein out of the membrane, essentially replacing lipid molecules with detergent molecules. Two useful guides to detergents are available from Calbiochem (EMD Biosicences) and Anatrace. Aim to maximize the quantity of protein solubilized from membranes while retaining the highest possible activity; optimize for functionality rather than overall yield. It is fairly common to use one detergent for solubilizaton, a different one for chromatographic purification, and a third for crystallization. In all cases the detergent concentration should be above the critical micelle concentration (cmc) to insure that micelles are present. For example, we often solubilize membranes in 5% Elugent (a glucoside mixture from Calbiochem) or 1% dodecyl maltoside (DDM), we purify almost everything in 0.1% DDM, and then we exchange the DDM into other detergents for crystallization. The greatest variety and highest purity detergents are available from Anatrace.

2. Evaluation of detergents for crystallization.
The detergents that maintain function and stability are specific for individual membrane proteins. If you have an activity assay, screen each candidate detergent after complete detergent exchange (done using a Ni column, anion exchange, or size exclusion column). If no activity assay is available, Da Neng Wang has come up with an evaluation based on peak profile from a size exclusion column[2].

3. Crystallization using detergent(s).
For each successful detergent-exchanged sample (of 5 mg or more), you should screen for crystallization as you would for soluble proteins. We use commercially available screens for soluble proteins, and also some screens specific for membrane proteins from Qiagen (Nextal) and Molecular Dimensions. At this stage we also begin including other detergents and additives in our screens – this is described in the Current Protocols chapter. Both additives and detergents can have large effects on crystals obtained in various primary detergents. In several cases, the additives and secondary detergents modulate the size and shape of the detergent micelle, compacting it and sometimes

making it more suitable for crystallization. It may also be necessary to add lipids during crystallization[3,4] or during both purification and crystallization[5].

Crystallization using lipids.
Another way to form membrane protein crystals substitutes lipids for the detergent molecules using bicelles or lipidic cubic phase methods[6]. These approaches have yielded several structures not obtained using the traditional detergent approach, most recently of the β2 adrenergic G-protein coupled receptor[7,8].

Expanding the hydrophilic surface.
One factor hindering the ability to obtain well ordered crystals for membrane proteins is that some targets have very little hydrophilic surface (sometimes only short loops connecting transmembrane α-helices or β-strands). It is generally the hydrophilic regions that are used for crystal contacts, although a number of structures also use (less specific) hydrophobic contacts for this purpose. One way to improve crystals is therefore to expand the available hydrophilic surface. This has been done successfully using monoclonal antibodies (several structures, but the most recent example is for β2 adrenergic receptor[8]). Another method employs binding a hydrophilic protein partner to the membrane protein[9,10] or inserting a hydrophilic fusion protein such as T4 lysozyme[7].

Further information.
Perhaps the best source of local information on membrane protein crystallization can be found by joining the membrane protein interest group (http://tango01.cit.nih.gov/sig/home.taf?_function=main&SIGInfo_SIGID=117) and attending the monthly seminars. This group brings together diverse research groups studying a wide variety of membrane proteins through structure/function analyses. Lots of people are always available to answer questions or advise on new projects.

References


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