Membrane Proteins I-Workshop on Methods

MS04.03a.01 DESIGN AND APPLICATION OF MATRICES FOR THE CRYSTALLIZATION OF MEMBRANE PROTEINS. J.Eric Gouaux, Langzhou Song, Melissa Weissling, and Jeff Johnson, Department of Biochemistry & Molecular Biology, University of Chicago, 920 East 58th Street, Chicago IL 60637.

In this laboratory we use both full factorial and sampling (sparse matrix) approaches for the crystallization of membrane proteins. We typically vary salt, PEG, or PEG/NaCl concentrations in the first dimension and pH in the second dimension of full factorial searches. The first sparse matrix strategy has been designed for the detergent monooctyl tetrathyleneglycol (C8E4). An important element of this design involves selecting crystallization conditions that are close to the phase separation or consolute boundary of the detergent.

We have determined the phase separation behavior of C8E4 in the presence of 19 ionic salts and 3 PEGs at both 20°C and 4°C. In general, we examined salt concentrations in steps of 0.2 M and PEG concentrations in steps of 2.5%. Phase separation of the solution was monitored by inspection of the solutions under a stereo microscope.

Crystallization solutions were formulated by sampling the space defined by the axes of salt, PEG and pH in a relatively coarse but uniform manner. The salts were arranged by anion according to the Hofmeister series and the PEGs were arranged by molecular weight. To select the concentrations of salt and PEG, the phase separation studies of C8E4 were consulted. The concentrations of salt and PEG were chosen so that the resulting solution would be close to the phase separation boundary. Using the αHL heptamer solubilized in 15 mM C8E4, 10 mM Tris, pH 8.0 and the sparse matrix solutions, hanging drop crystallizations were set up at 20°C and 4°C. After 2 weeks microcrystals were present in 11 of the 60 drops at both temperatures. Using full factorial designs, the 20°C conditions were optimized with respect to PEG and salt concentrations and also to temperature in a few cases. X-ray diffraction analysis of these crystals indicated that 2 forms were suitable for high resolution structure analysis. In addition to the results on αHL using the C8E4 matrices, we will present data obtained from crystallizations currently in progress.

MS04.03a.02 CRYSTALLIZATION OF MEMBRANE PROTEINS WITH THE HELP OF ANTIBODY FRAGMENTS. Christian Ostermeier, So Iwata, Hartmut Michel, Max-Planck-Institut fuer Biophysik, Heinrich-Hoffmann-Str. 7, 60528 Frankfurt, Germany.

For X-ray structure determination of proteins, well-diffracting three-dimensional crystals are required. However, crystallization of membrane proteins remains difficult. Known crystal packing structures indicate that exclusively the polar surfaces of the membrane proteins are responsible for establishing the ordered three-dimensional crystal lattice. Therefore it should be possible to increase the chances for growing well-ordered crystals of a membrane protein through the enlargement of its polar surfaces. Here, we show that the crystallization of the multisubunit membrane protein cytochrome c oxidase from Paracoccus denitrificans became possible due to the complexation with an antibody Fv fragment. The structure was solved to a resolution of 2.8 Å. This reveals the secret of the structure of this redox driven proton pump, which plays an essential role in the respiratory chain of many organisms. The approach of co-crystallizing membrane proteins with antibody fragments should be useful in obtaining well-ordered crystals of membrane proteins in general.


MS04.03a.03 TWO-DIMENSIONAL CRYSTALLISATION MEMBRANE PROTEINS. Werner Kühbrandt, European Molecular Biology Laboratory, Meyerhofstr. 1, 69117 Heidelberg, Germany.

Two-dimensional (2D) crystals are used increasingly successfully for determining the structure of membrane proteins at high and medium resolution of electron cryo-microscopy. Membrane proteins are often not available in quantities sufficient for 3D crystallisation trials, and not many have yielded highly ordered 3D crystals for X-ray crystallography. 2D crystallisation of membrane proteins requires less material and tends to occur more readily than 3D crystallisation, due to the special nature of these proteins which favours in-plane hydrophobic interactions.

Different mechanisms of 2D crystal formation will be discussed, and recent examples will be presented. The structure of the plant light-harvesting complex (LHC-II) which was determined by electron crystallography of 2D crystals at 3.4 Å resolution will be described briefly.

MS04.03a.04 DETERGENT LOCALIZATION IN MEMBRANE PROTEIN CRYSTALS USING NEUTRON DIFFRACTION. P.A. Timmins, Institut Laue-Langevin, BP156, 38042 Grenoble Cedex 9, France. E. Pebay-Peyroula, Institut de Biologie Structurale et Université Joseph Fourier, 41 avenue des Martyrs, 38027 Grenoble Cedex, France. S. Penel, Institut Laue-Langevin, BP156, 38042 Grenoble Cedex 9, France.

The organization of detergent in single crystals of membrane proteins can only be visualized by neutron diffraction. Due to the similarity of electron density between detergent and water these two disordered phases are indistinguishable by X-ray diffraction. By soaking crystals in mixtures of H₂O and D₂O it is possible to enhance the relative contrasts for neutrons of any of the components in the crystals. The linearity of the complex structure factor as a function of contrast allows phase information to be derived as well as efficient scaling of data containing different amounts of D₂O. Contrast may also be varied by substituting components of a macromolecular complex with their perdeuterated analogues. This technique has recently been used to study the detergent organization in a number of different proteins including three different crystal forms of two different porins. In addition to varying the contrast through the deuterium content of the water, detergents deuterated in their acyl chains have been used to investigate in more detail the interactions between detergent and protein. The detergent in crystals of the porin from Rhodobacter capsulatus forms what appears to be a layer structure but which in fact consists of rather flat micelles interacting through their head groups. In the case of the Ompf porin from E. coli the protein does not on its own form a continuous three-dimensional lattice. The interactions between proteins are in fact mediated in part by detergent-detergent interactions.
