MS04.03a.05 THE ROLE OF PROTEIN PURIFICATION IN THE STRUCTURE DETERMINATION OF AN INTEGRAL MEMBRANE PROTEIN. G. McDermott†, S.M. Prince, M.Z. Papiz*, A.M. Hawthornthwaite-Lawless*, A.A. Freer, N.W. Isaacs, R.J. Cogdell#. Dept.'s of Chemistry and #Biochemistry, University of Glasgow, Glasgow, G12 8QQ, UK. and; *CLRC Daresbury Laboratory, Daresbury, Warrington, WA4 4AD, UK. †Present address: Dept. of Chemistry, University of Crete, Iraklion, Greece

Structural elucidation of the Nonameric LH2 complex, an integral membrane protein from the photosynthetic purple bacterium Rps. acidophila, required more than ten years work. For a large proportion of this time progress was frustrated by the poor "quality" of diffraction exhibited by crystals of the complex. In the initial stages of the analysis this was manifest in low resolution and poor reproducibility of diffraction. Latterly, when this problem had been alleviated, it became apparent that the level of isomorphism between "native" crystals was low. Clearly, a deleterious factor which rendered the search for heavy atom derivatives somewhat ambiguous.

Optimisation of diffraction, both in terms of maximum observed resolution and degree of isomorphism between "native" crystals, was a dynamic and ongoing process. This presentation will describe the evolution of the purification and crystallisation protocols and relate protocol changes to enhancement of diffraction quality.

The essence of this presentation will be derived from the distillation of a large volume of empirical observation. Consequently, some tentative proposals on diffraction improvement stratagems, potentially applicable to other membrane protein systems, will also be presented

MS04.03a.06 CRYSTAL STRUCTURE OF THE LIGHT HARVESTING COMPLEX II (B800/850) FROM *Rhodospirillum molischianum*. Juergen Koepke[1], Xiche Hu[2], Klaus Schulten[2], Hartmut Michel[1], [1]Max-PlanckInstitut für Biophysik, Abteilung Molekulare Membranbiologie, Heinrich-HoffmannStr. 7, 60528 Frankfurt am Main, [2]Beckman Institute, University of Illinois at Urbana-Champaign, Urbana, IL 61801

The crystal structure of the LH-II from *Rhodospirillum molischianum* has been determined by molecular replacement at 2.4 Å resolution using X-ray diffraction. The search model for molecular replacement was an octamer of α,β heterodimers homologous to the nonameric LH-II from *Rps. acidophila.* It was generated by means of comparative modelling, energy minimization and molecular dynamics simulations. The crystal structure displays two concentric cylinders of membranespanning helical protein subunits with the α-apoprotein inside and the β-apoprotein outside. Sixteen B850 BCA molecules form a continuous overlapping ring with each BCA oriented perpendicular to the plane of the membrane and sandwiched between the helical apoproteins. The eight B800 BCA are nearly parallel to the membrane plane, and, situated between the outside β-apoproteins, form another concentric ring. Eight membrane spanning lycopene pigments intertwine between the tails of the B800 and B850 BCAs.

The Mg ligands for the B850 BCA are α-His34 and β-His35 as expected from comparison with the *Rps. acidiphila* structure, but the Mg of the B800 BCA is bound to α-Asp6 and not to a histidine or a methionine. The Q_y transition dipole moments of neighboring B850 and B800 BCA are nearly parallel to each other, which is optimal for efficient Förster exciton transfer. B800 BCA and one of the two B850 BCAs are involved in an edge to edge contact with lycopene, thus Dexter mechanism can be functional for energy transfer from lycopene to BCAs. The ring structure of the B850 BCAs is optimal for light energy transfer.

MS04.03a.07 PEPTITERGENTS: NOVEL PEPTIDES CAPABLE OF SOLUBILIZING MEMBRANE PROTEINS FOR CRYSTALLIZATION. Robert M. Stroud and Christian Schafmeister, S-964 Dept. of Biochemistry & Biophysics UCSF Box 0448 San Francisco, CA 94143-0448

Peptides that form α-helices with a strongly amphipathic nature are capable of solubilizing membrane proteins if they fulfill certain criteria. Adequate length, a flat hydrophobic surface, and a polar exterior are the main components of these peptides. Variations in the initial peptide structure have been made and resulted in many variations on the original theme. The crystal structure of the initial peptide used to solubilize membrane proteins is described and shows the nature of the interface between hydrophobic surfaces and laterally between adjacent membrane-spanning peptitergents. The crystal structure was solved entirely from α-helical models using molecular replacement.

MS04.03a.08 OVEREXPRESSION, REFOLDING, AND CRYSTALLIZATION OF AN 80 KD OUTER MEMBRANE PROTEIN. Susan Buchanan[1], Barbara Smith[1], Lalitha Venkatramani[2], Dick van der Helm[2], and Johann Deisenhofer[1], Howard Hughes Medical Institute, UT Southwestern Medical Center, 5323 Harry Hines Blvd. Dallas, TX 75235-9050[1]. Department of Chemistry and Biochemistry, University of Oklahoma, Norman, OK 73019[2]

Ferric enterobactin receptor (FepA), an outer membrane protein from Esherichia coli, has been overexpressed to produce large quantides of insoluble cytoplasmic inclusion bodies. The inclusion bodies have been solubilized in urea and refolded using a combination of sulfobetaine 3-14 and sodium dodecylsulfate. The refolded protein was subsequently purified by FPLC using anion exchange and gel filtration chromatography. Refolded FepA was crystallized according to methods developed for native (membrane-inserted) FepA; the resulting crystals have the identical space group and unit cell dimensions determined for native FepA crystals. A low temperature native data set has been collected to 2.9 Å resolution and a search for heavy atom derivatives is in progress, using crystals from both native and refolded sources. Current yields from the inclusion body expression system are approximately 10 mg/l, making this method suitable for structual studies of other outer membrane proteins.

Membrane Proteins II

MS04.03b.01 STRUCTURE-FUNCTION RELATIONSHIPS IN THE MEMBRANE CHANNEL PORIN. Georg E. Schulz, Institut fur Organische Chemie und Biochemie Albertstr. 21, 79104 Freiburg im Breisgau, Germany

Porins form channels in the protective outer membrane of Gram-negative bacteria that are permeable for polar molecules, but discriminate against nonpolar ones. The first crystal structure had been reported in 1990 for *Rhodobacter capsulatus* [1]. All structurally known porins have subunits with 16- or 18-stranded ß-barrels surrounding a pore with a diameter of about 1 nm. Three barrels associate along their axes to form a trimer [2-6]. All porins contain two girdles of aromatic residues facing the membrane at its two polar-nonpolar borderlines, which are likely to fulfill a shielding function. Moreover, all general pores are lined by ionogenic groups that segregate into negatively and positively charged rims. It is suggested that they constitute an electric separator testing solute polarity [6-8]. In two porins which had been classified as unspecific, we detected ligand binding sites.

Large amounts of the porin from *R.blastica* were expressed in inclusion bodies in *E. coli* and recovered to form crystals iden-