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-

tical to those of the wild-type. This opens the possibility of protein engineering for changing channel properties [9]. The report includes the porins from *R. capsulatus* and *R. blastica*, as well as the maltoporin from *Salmonella typhimurium*. Purification for crystallization of these porins and other membrane proteins will be described.

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MS04.03b.02 STRUCTURAL BASIS OF SUGAR TRANSLOCATION THROUGH MALTOPORIN CHANNELS. T. Schirmer, R. Dutzler, Y.-F. Wang*, J.P. Rosenbusch* Departments of Structural Biology and *Microbiology, Biozentrum, University of Basel, CH-4056 Basel, Switzerland.

Maltoporin (LamB), an integral membrane protein from *E. coli*, facilitates the diffusion of maltooligosaccharides across the outer membrane. The structure exhibits a trimer of 18-stranded antiparallel β-barrels. Each barrel contains a channel with an exposed hydrophobic patch formed by six aromatic residues ('greasy slide') that are linearly arranged. Apart from this the channel is lined exclusively by ionizable residues.

Crystal structures of maltoporin in complex with maltooligosaccharides of various lengths reveal that the sugars are bound to the channel constriction and are in apolar contact with the 'greasy slide'. A multitude of H-bonds that are formed between the sugar hydroxyl groups and residues from the channel lining explain affinity and specificity of this interaction. A complex structure with sucrose reveals non-productive binding above the channel constriction. The structural data will be discussed with respect to function and a detailled path for sugar translocation will be proposed.

Schirmer, T., Keller, T. A., Wang, Y.-F. & Rosenbusch, J. P. (1995). Structural basis for sugar translocation through maltoporin channels at 3.1Å resolution. *Science*, **267**, 512-514.

Dutzler, R., Wang, Y.-F., Rosenbusch, J. P. & Schirmer, T. (1996). Crystal structures of various maltooligosaccharides bound to maltoporin reveal a specific sugar translocation pathway. *Structure*, **4**, 127-134.

MS04.03b.03 THE STRUCTURE OF PROSTAGLANDIN SYNTHASE: A MEMBRANE-BOUND ENZYME. R. Michael Garavito, Department of Biochemistry, Michigan State University, East Lansing, MI 48824-1319.

Prostaglandin H synthase (PGHS) is an integral membrane enzyme which converts arachidonic acid, an essential fatty acid, into the prostaglandin precursors PGG_2 and PGH_2 by means of a free radical mechanism. Nonsteroidal anti-inflammatory drugs (NSAIDs), which inhibit prostanoid biosynthesis by targeting the cyclooxygenase activity of PGHS, are used to treat certain symptoms of inflammatory and cardiovascular diseases as well as cancer; in the latter, aspirin is now a proven anti-cancer prophylaxis. Two isoforms of PGHS have been discovered: PGHS-1 is involved in homeostatic or "house-keeping" prostaglandin biosynthesis while PGHS-2 induced by cytokines during inflammatory events.

We have refined the structure of ovine PGHS-1 to 3.1 Å resolution and have characterized PGHS-1 complexed with four NSAIDs: bromoaspirin, flurbiprofen, iodosuprofen and iodoindomethacin. I will also discuss the nature of enzyme-drug interactions and structure-function relationships, with particular focus on the mechanisms of substrate and NSAID interactions as well as the functional differences in NSAID binding between PGHS-1 and PGHS-2.

An unexpected conclusion from the crystal structure of PGHS-1 is that it is a monotopic membrane protein: a symmetric dimer of PGHS apparently integrates only into one leaflet of the lipid bilayer. Moreover, there is increasing evidence that specific sites on the PGHS molecule interact with other proteins in the lumen of the endoplasmic reticulum (ER), particularly those involved with membrane protein targeting. The nature of integration of PGHS into the membrane bilayer and the mode of targeting the enzyme to the ER membrane and nuclear envelope will be major points of discussion.

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MS04.03b.04 THE STRUCTURE OF AN INTEGRAL MEMBRANE PROTEIN LIGHT-HARVESTING COMPLEX N.W. Isaacs, R.J. Cogdell#, A.A. Freer, A.M. Hawthornthwaite-Lawless*, M.Z. Papiz*, S.M. Prince, and G. McDermott. Depts of Chemistry and #Biochemistry, University of Glasgow, Glasgow, G12 8QQ, UK. and *CCLRC Daresbury Laboratory, Daresbury, Warrington, WA4 4AD, UK.

The crystal structure of the integral membrane light-harvesting complex from *Rhodopseudomonas acidophila* strain 10050 showed that the active assembly consists of two concentric cylinders of single helical protein subunits which enclose the pigment molecules. Eighteen bacteriochlorophyll *a* (Bchl *a*) molecules are sandwiched between these membrane-spanning helices to form a continuous overlapping ring with their bacteriochlorin planes perpendicular to the plane of the membrane surface. A further nine Bchl *a* are positioned between the outer helices with their bacteriochlorin ring planes parallel to the membrane plane. Carotenoid molecules span the assembly making van der Waals contacts to both types of Bchl *a* pigments as well as the protein helices. A close analysis of the structure shows a beautiful interlocking of the protein and pigment molecules to allow for the optimisation of energy transfer between pigments in a single complex and between adjacent complexes, connecting to the reaction centre where charge separation takes place.

This work has been supported by the BBSRC.

MS04.03b.05 8.5 Å PROJECTION MAP OF THE LIGHT HARVESTING COMPLEX I FROM RHODO SPIRILLUM RUBRUM REVEALS A RING COMPOSED OF 16 SUBUNITS. Simone Karrasch, Per A. Bullough, Robin Ghosh, MRC Laboratory of Molecular Biology Hills Road Cambridge CB2 2QH, U.K

Two-dimensional crystals from light-harvesting complex I (LHC I) of the purple non-sulphur bacterium Rhodospirillum rubrum have been reconstituted from detergent-solubilized protein complexes. Frozen-hydrated samples have been analyzed by electron microscopy. The crystals diffract beyond 8 Å and a projection map was calculated to 8.5 Å. The projection map shows 16 subunits in a 116 Å diameter ring with a 68 Å hole in the centre. These dimensions are sufficient to incorporate a reaction centre in vivo. Within each subunit, density for the α- and the β-polypeptide chains is clearly resolved and the density for the bacteriochlorophylls can be assigned. The experimentally determined structure contradicts models of the LHC I presented so far.