decarboxylation. The chromophore structures of wild type zGFP506 and those of its mutant variant zGFP506_N66D in 'green' and 'red' states support this suggestion. The post-translational modification of the chromophore triad -Lys66-Tyr67-Gly68- in zYFP538 results in the unusual three ring structure consisting of a five-membered imidazolinone ring, the phenolic ring of Tyr67 and the additional six-membered tetrahydropyridine ring. The chromophore formation finalizes in cleavage of the protein backbone at CO-N bond of Lys66. It was suggested that the energy conflict produced by the buried positive charge of the intact Lys66 side chain in the hydrophobic pocket formed by the Ile44, Leu46, Phe65, Leu204 and Leu219 side chains is most likely the trigger expanding the posttranslational modification of zYFP538 beyond the green emitting form.

Keywords: chromophore structure, fluorescent proteins, Zoanthus sp.

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The role of protein methylation rescue method for protein crystallization

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Surface lysine methylation (SLM) is a technique for improving the rate of success of protein crystallization by chemically methylating lysine residues. The exact mechanism by which SLM enhances crystallization is still not clear. To study these mechanisms, and to determine the conditions where SLM will provide the optimal benefit in crystallization rescue, we compared methylated protein structures containing N,N-dimethyllysine (dmLys) to a non-redundant set od 11690 non-methylated structures from the PDB. By measuring the relative frequency of intermolecular contacts (where two residues are in proximity with a distans of 3.5 Angstroems or less) of basic residues in the methylated vs. non-methylated sets, dmLys-Glu contacts are seen more frequently than Lys-Glu contacts. By observing in the proteins with both native and methylated structures, the increased rate of contact for dmLys-Glu is due to both a slight increase in the number of H-bonds and to the formation of methyl C-H...O interactions. By comparing the relative contact frequencies of dmLys with other residues, the mechanism by which methylation of lysines improves the formation of crystal contacts appears to be similar to that of Lys to Arg mutation. An attempt to analyze methylated structures with the Surface Entropy Reduction prediction (SERp) server suggested that tools that analyze protein sequences for SER mutation may also be helpful in determining candidates for SLM.

Keywords: protein crystallography, protein crystallization, bioinformatics

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Progress in structure determination of the 18kDa TSPO and the outer matrix Matrilin 3 protein

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The translocator protein (TSPO), previously called the peripheral benzodiazepine receptor (PBR) is an 18kDa outer mitochondrial membrane protein, found complexed with VDAC and the adenine nucleotide translocator (ANT). The TSPO has been implicated in regulation of cholesterol transport into the mitochondria, respiration, apoptosis and cell proliferation. In order to understand the mode of ligand binding, we have embarked upon obtaining the 3D structure of the TSPO by X-ray crystallographic methods. We have cloned the gene encoding for full length R. norvegicus TSPO into the E.coli strain C41. Initial trials show that the membrane fraction of the cells binds the PBR ligand PK11195. The recombinant TSPO protein was extracted from the membrane fraction by using 1% SDS as a detergent. The protein was then efficiently purified by metal-chelation chromatography. Crystals were obtained within two weeks after crystallization trials. Matrilin-3 is an extracellular matrix protein found in cartilage. Mutations in the gene encoding for the von Willebrand factor A (vWFA) domain of the human Matrilin 3 lead to skeletal disorders, such as multiple epiphyseal dysplasias. The genes encoding for both the Matrilin 3 and the vWFA domain from M. musculus were cloned and over expressed as insoluble inclusion bodies in E.coli. We are currently working on the proteins purification process in order to obtain large amounts of pure and soluble proteins. This will then be used for crystallization and structure determination.

Keywords: membrane protein X-ray crystal structure determina, disease, membrane protein crystallization

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The role of chilectins in rhematoid arthritis

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Rheumatoid arthritis is a painful, debilitating disease of (in particular) the aged. Recent work has identified two proteins that are highly overexpressed/secreted by chondrocytes in arthritic cartilage, human cartilage gp39 (Hcgp39) and the chitinase 3-like protein 2 (YKL-39). These genes share surprising homology with enzymes (chitinases) that degrade the fungal cell wall, and are therefore termed chitinase-like lectins (chilectins). Since humans do not contain chitin (the target of the active, fungal, chitinases) a number of hypotheses have been presented as to the function of the chilectins. In humans, expression of chilectins have been associated with the pathogenesis

of arthritis, cancer and asthma and there are correlations between expression levels and disease prognosis. However, the function of these secreted proteins is completely unknown. This project is aimed at uncovering the physiological roles of the chilectins, using a combination of structural biology and cell biological studies.

Keywords: chilectin, chitinase, arthritis

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A structural investigation in to the basis of Celiac disease

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Celiac disease (CD) is a common T cell mediated disease in which the body develops an inappropriate immune response to dietary gluten. Gluten ingestion by CD affected individuals' result in symptoms such as bloating, diarrhoea, and malabsorption. The only available treatment against these symptoms is strict adherence to a lifelong gluten free diet. Major Histocompatibility Complex (MHC) class II proteins HLA-DQ2 and/or HLA-DQ8, are critical for the development of a CD4+ T cell response towards gluten. The structures of both HLA-DQ2 and HLA-DQ8 were previously solved in complex with an antigenic gluten peptide. HLA-DQ2 was solved in complex with an a-gliadin-I gluten peptide, one of two strongly antigenic registers that overlap within an optimal peptide. The 2nd register, a-gliadin II, has been shown by functional studies to be a stronger antigen. Crystallization studies are underway to assess the functional and structural properties of this immunodominant epitope. The structure of HLA-DQ2/a-gliadin II gluten peptide complex will aid in our understanding of CD4+ T cell recognition of gluten epitopes in CD. No structures of a TcR specific for any HLA-DQ2/DQ8-gliadin peptide have been reported. Crystals have been optimised for solution of an apo gluten specific TcR, and I am attempting to crystallize a HLA-DQ2/DQ8-peptide-TcR complex. This project aims to structurally characterise the interactions between HLA-DQ8-gluten peptide complex, and an HLA-DQ8/peptide specific TCR. The ternary complex will identify important contact residues in the peptide that allow TcR recognition and hence may aid in the development of new treatments (e.g. peptide vaccine), and provide significant insight into the structural mechanism that results in the activation of CD4+ T cells in CD.

Keywords: MHC proteins, T cell receptor, X-ray crystallography of proteins

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Purification, crystallization and preliminary X-ray analysis of photosystem II dimer from a red alga

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Photosystem II (PSII) is a membrane protein complex performing light-induced electron transfer and water-splitting reactions, leading to the evolution of molecular oxygen which is required for all oxygenic life on the earth. The central part of photosystem II is highly conserved from prokaryotic cyanobacteria to eukaryotes; however, there are some apparent differences in the extrinsic proteins involved in oxygen evolution among different organisms. So far, the crystal structure of PSII from cyanobacteria has been reported, whereas no reports have been published on the structure of any eukaryotic PSII. Red alga is one of the eukaryotic algae, and its PSII differs from that of cyanobacteria in that the former contains a 20 kDa protein, a unique, fourth extrinsic protein. In order to elucidate the structure of red algal PSII and its differences with cyanobacterial PSII, we purified and crystallized PSII from an acidophilic, thermophilic red alga *Cyanidium caldarium*. In order to obtain pure PSII dimer suitable for crystallization from the red alga, we improved the purification procedure published previously, which yielded a highly purified PSII dimer preparation with high oxygen-evolving activities comparable with that of thermophilic cyanobacterial PSII. We succeeded in the crystallization of red algal PSII dimer and obtained two types of crystals with different space group crystals. One type of the crystal had a space group of $P222_1$ with unit cell dimensions of a = 146.8Å, b = 176.9Å, c = 353.7Å. Another type of the crystal had a space group $P2_12_12_1$ with unit cell dimensions of a = 209.2Å, b = 237.5Å, c = 299.8Å. Multiple data sets of native crystals have been collected and processed to 3.7 Å, which may enable us to resolve the structure of red algal PSII.

Keywords: photosynthesis, membrane proteins, crystallization

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Structural analysis of ATP:Cob(I)alamin adenosyltransferase

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B12 is an important nutrient for maintaining life in all animals, lower eukaryotes, and prokaryotes, but is only synthesized by a prokaryotes. In mammalian, B12 should be converted to two biologically active cofactors, methylcobalamin (MeCbl) and adenosylcobalamin (AdoCbl), and these cofactors are used by enzymes, the functions of which are needed for the Acetyl-CoA synthesis, methyl transfer, ribonucleotide reduction, fermentation process, and methionine synthesis. Because B12 synthesis is limited within the micro-organisms, the adenosyltransferases that are able to transfer the 5'-deoxyadenosyl moiety from ATP to the cobalt atom of cob(I)alamin is needed to construct the complete biosynthetic pathway of AdoCbl in higher order organisms. Until now, two crystal structures of PduO enzyme with Mg-ATP are available in the RCSB protein data bank (2nt8, 2idx), and these structures indicates that the enzyme is a trimer and each subunit consists of five helix-bundle. Further, co-crystal structure with Mg-ATP reveals that unseen ATP binding motif at the N-terminal of the protein in native crystal structure is visible in substrate-protein complex crystal