

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 of 11690 non-methylated structures from the PDB. By measuring the relative frequency of intermolecular contacts (where two residues are in proximity with a distance of 3.5 Angstroms 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 determination, disease, membrane protein crystallization

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The role of chilectins in rheumatoid 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