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Protein sequences derived from the over 4 million genes have the potential to produce an evolutionary tree that unequivocally and accurately traces the divergent course of evolution of all species. Evolutionary trees rely upon identifying an essential protein present in all species. The short chain oxidoreductase (SCOR) family is a family of such proteins. One subgroup of the SCOR family has 11,000 members in the gene bank including from 5 to 50 members in all species. There is not one fully conserved residue in the family and the enzymes vary in length from 240 to 350 residues. By combining structural information in the Protein Databank with sequence data we are able to align over 98% of all family members. From this alignment we can determine the mechanism of cofactor binding, probable function, preferred aggregation state and subtle variants in mechanism of action of each. We can accurately catalog 30% of the sequences as to their specific substrates and characterize the topology of highly specific substrate binding pockets for an additional 50% of the structures as they cluster in substrate sequence space. Analysis of the substrate specific subgroups permits the identification of residues responsible for protein/protein interactions. Analysis of insertions and deletions in the loops connecting the beta-sheets and alpha helices of the Rossmann fold reveals correlations between indels in the loops and speciation. By examining and sorting all 11,000 SCOR sequences, as Gregor Mendel sorted peas and Barbara McClintock sorted corn kernels, it is possible to determine the exact details of 3 billion years of divergent evolution of species, sequence, three-dimensional-fold, and substrate specificity.

Keywords: evolution, substrate, rossmann fold

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Structure of dengue virus - Implications for flaviviral assembly and opportunities for drug design

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Epidemic flaviviral diseases are widespread in tropical regions and are caused by infections due to viruses such as Dengue, Yellow Fever, Japanese Encephalitis and West Nile viruses. Various therapeutic targets have been identified from structural studies, including structural proteins such as envelope (E), membrane (M) and capsid (C) proteins, and non-structural proteins, e.g. viral protease, helicase, RNA polymerase and methyl transferase. Currently there is no commercial vaccine or antiviral drugs for dengue infection, many ongoing research programs are focused on developing potential drugs against dengue virus. Dengue envelope protein involves protein-cell membrane interaction which leads to viral cell entry. We have performed phylogenetic analysis of envelope protein of dengue viruses from Southeast Asia from 1990 - 2007, built the homology model of envelope protein of several emerging Singapore strains, and compared with available crystal structures of dengue envelope protein. A putative ligand-binding pocket was identified, its conformational change is crucial to dengue virus membrane fusion. Further docking studies on envelope protein inhibitors provide insights into the role of binding pocket and facilitate the design of novel potent inhibitors against evolving dengue diseases.

Keywords: homology modeling, structure-based drug design, virus structures

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Photochemical neutral radical induced nucleation of proteins

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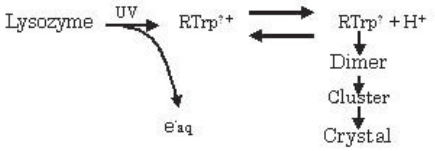
The crystallization is one of the bottlenecks for the protein X-ray crystallography. We reported that the number of crystals of hen egg-white lysozyme increased in metastable solution by UV-light irradiation and this phenomenon depends on irradiation light wavelength.1 Neutral radicals of tryptophan residue ($RTrp^{\bullet}$) of lysozyme were observed by transient absorption measurements. The photochemical dimerization of lysozyme was observed by SDS-PAGE for this solution. These results suggested that the dimer plays role of the smallest cluster. Scheme 1 shows the mechanism of photochemically induced nucleation of lysozyme. The photochemical reaction of tryptophan residue of lysozyme is photo-ionization leading to the generation of radical cation ($RTrp^{\bullet+}$) and hydrated electron. The RTrp. of lysozyme formed by deprotonation of $RTrp^{\bullet+}$. We, here, demonstrate the results of crystallization experiments of lysozyme at some dimer quantities. The pKa value of $RTrp^{\bullet+}$ was estimated by transient absorption measurements under various pH conditions.



Scheme 1 The mechanism of photochemically induced nucleation of lysozyme.

[References]
1. T. Okutsu et al., Cryst. Growth Des., 5 (2005) 1393.

Keywords: protein crystallization development, photochemistry, photodimerization

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The three dimensional structure of red, yellow and green fluorescent proteins from Zoanthus

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The three-dimensional structures of the wild type red (zRFP574), yellow (zYFP538) and green (zGFP506) fluorescent proteins (FP), from button polyp Zoanthus have been determined at 1.51 Å, 1.8 Å and 2.2 Å respectively and crystal structures of the zGFP506 mutant variant (zGFP506_N66D) with replacement of the chromophore first residue, Asn66Asp, in transition ‘green’ and matured ‘red’ states have been determined at 2.4 Å and 2.2 Å respectively. The novel posttranslational modification of the chromophore-forming sequence -Asp66-Tyr67-Gly68- in zRFP574 expands the protein maturation beyond the green-emitting form and results in decarboxylation of the Asp66 side chain. It was suggested that the electrostatic conflict between closely spaced, negatively charged side chains of the chromophore Asp66 and the proximal catalytic Glu221 is most likely the trigger of the reactions chain resulting in the observed

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