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Growth of high-quality and large crystals of HIV protease for neutron crystallography

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The biological structure and function of proteins are dominated by hydrogen atoms. Neutron protein crystallography provides a powerful complement to X-ray analysis by enabling the visualization of hydrogen atoms, which allows rational drug design and the understanding of enzymatic processes. A major hurdle to neutron protein crystallography is that unusual large crystals (> 1 mm^3) are required to compensate the weak flux of available neutron beam. Although recent advances in protein expression and purification techniques permit large amounts of proteins to crystallize, the bottleneck of protein crystallography still remains the growth of single crystals with adequate quality and sufficient crystal size. To overcome the difficulties, we have developed a couple of techniques such as a two-liquid system (Adachi *et al.*, 2003) and stirring technique (Adachi *et al.*, 2004), which realized the growth of large single crystals for various proteins. Here, we have assessed available techniques to grow large crystals of HIV protease-inhibitor complex. The two-liquid system and stirring technique as well as slow cooling and conventional seeding methods have been adopted. The combination of two-liquid system and slow-cooling method was especially effective for growth of large single crystals (2 x 2 x 0.5mm) of HIV protease-inhibitor complex.

Keywords: protein crystal growth, HIV, neutron crystallography

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X-ray crystallographic study of the C-terminal domain of Tic110 protein from *Cyanidioschyzon merolae*

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Tic110 (translocan of the inner envelope membrane) is an intergral membrane protein containing a short N-terminal membrane anchor and a hydrophilic region (~98 kDa) that extends into the plastid stroma. Here, the crystallization and preliminary analysis of the C-terminal domain (659-1007) of Tic110 protein hydrophilic region from *Cyanidioschyzon merolae* Tic110 (cmTic10C) are reported. The cmTic110C has been crystallized at 293 K using PEG 400 as precipitant. These crystals belong to the hexagonal space group $P6_122$ (or $P6_522$), with unit-cell parameters $a = b = 123.2$, $c = 246.4$ Å. A 99.3% complete native data set from a frozen crystal has been collected to 4.5 Å resolution at 100 K with an overall R_{merge} of 7.9%. The presence of two subunit of cmTic110C per asymmetric unit gives a crystal volume per protein weight (V_M) of 3.46 $Å^3$ Da^{-1} and a solvent content of 64.5%.

Keywords: crystal growth, X-ray diffraction data, transport

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Protein crystallization through screening of pH and precipitants using counterdiffusion technique

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Protein crystallization has gained a new strategic and commercial relevance in the post-genomic era due to its pivotal role in Structural Genomics: producing high-quality crystals has always been the rate-limiting step in protein structure determination. Novel crystallization screens and strategies [1] have been developed to make the search for initial crystallization conditions more manageable. Among them, counterdiffusion (CD) technique has proved to be very suitable to grow protein crystals. Since it starts far from the equilibrium, the result of a CD experiment evolves along the length of the growth chamber in time. This means that it is possible to obtain sequentially amorphous precipitation, microcrystals and crystals of the highest quality in a single experiment [2]. Here we present the results of the crystallization screening carried out for several proteins by means of capillary CD technique, using the new version of the Granada Crystallization Box, provided by Triana S&T [3, 4]. Because of the use of short and thin capillaries (0.1 mm diameter), the required volume for experiment is reduced to less than 300 nL. The effect of pH (4 to 9) and precipitants (three different polyethylene glycols, from low to high molecular weight, a mixture of them and ammonium sulphate) related to the isoelectric point (pI) will be discussed in terms of crystallizability and also X-ray diffraction crystal quality using a home lab source.

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Keywords: crystallization of proteins, crystallization methods, counterdiffusion

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Snapshots in the reaction pathway of bilin reductase PcyA

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In higher plants, algae, and cyanobacteria, phytobilins are utilized for photosensing and/or light harvesting. Phytobilins are synthesized by ferredoxin-dependent bilin reductases (FDBRs) from biliverdin IXα (BV) derived from heme by hemeoxygenase. Phycocyanobilin:ferredoxin oxidoreductase (PcyA), one of FDBRs, sequentially reduces the vinyl group of D-ring and A-ring of BV to produce 18^1,18^2-dihydrobiliverdin (18EtBV) and phycocyanobilin. We reported the crystal structures of PcyA from *Synechocystis* sp. PCC 6803 and its complex with BV at 2.5 Å and 1.51 Å resolutions, respectively. These structures revealed that Glu76, His88 and Asp105 are located near the U-shaped BV [1] and that upon BV binding induced-fit conformational changes occur in such a way that the substrate entrance is narrowed [2]. Focusing on the structural changes in PcyA during the sequential reduction of BV, we prepared the crystals of PcyA in complex with 18EtBV and BV13, an analog of 18EtBV. These pigments were chemically synthesized. The crystal structures of PcyA-18EtBV and PcyA-BV13 were determined at 1.48 Å and 1.04 Å resolutions, respectively, revealing PcyA conformation after the reduction of D-ring vinyl group in BV. The side chain of Glu76 rotates away from D-ring to form hydrogen-bonds with both Asn62 and Tyr238. On the basis of these structures, we discuss the sequential reduction mechanism of PcyA.

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Keywords: photosynthesis-related proteins, redox enzymes, reactive intermediates

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The crystal structure of lipase a from *Candida Antarctica*

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Lipases are widely used as catalysts in industrial applications due to their thermostability and enantioselectivity. *Candida antarctica* lipase A (CAL-A) is highly thermostable in organic solvents and has therefore become a frequently used catalyst in chemical and pharmaceutical industry. CAL-A shows some unusual properties, which makes it a highly attractive enzyme. CAL-A is the only known lipase to have Sn2-preference towards triglycerides. It is able to hydrolyze sterically hindered alcohols, both secondary and tertiary alcohols. In addition, it shows a high chemoselectivity for the N-acylation of beta-amino esters, which makes CAL-A an important catalyst in the production of enantiopure amino acids. We have determined the crystal structure of CAL-A at 2.1 Å resolution. CAL-A exhibits a typical alpha/beta hydrolase fold, consisting of a central beta-sheet and surrounding alpha-helices. The active site pocket is formed like a deep L-shaped tunnel covered by a lid that regulates the interfacial activation. Residues Ser184, Asp334 and His366 form the catalytic triad at the bottom of the pocket.

Keywords: lipases, enzyme structure, crystallography

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Novel approaches in protein crystallization

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Structure genomics (SG) studies usually include target selection, gene cloning, protein expression, purification, crystallization and structure determination. Among them, protein production and crystallization are the rate-limiting steps. We have developed novel methods in facilitating protein crystallization on our SG platform at Peking Univ. One example is that during the human chloride intracellular channel protein 2 (CLIC2) study, we have found that 5,5'-Dithio-bis(2-nitrobenzoic acid) (DTNB) modification of the surface cysteines significantly improved the crystal quality and crystals diffracted to 2 Å were obtained. We have further explored the application of DTNB modification for other protein crystallization trials. We have also made statistical analyses of crystallization condition parameters which impact protein crystallization processes from hundreds of different proteins grown in our lab for the SG projects. During the efforts, we optimize and further develop strategies of protein crystallization and are trying to change it from arts to science.

Keywords: DTNB modification, protein crystallization strategy, protein crystallization method

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A preliminary crystallographic study of CDCP2 from *Arabidopsis thaliana*

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CBS (cystathionine-beta-synthase) domain is a small intracellular module, mostly found in two or four copies within a protein, which has been identified in many divergent proteins in all kingdoms of life. Tandem pairs of CBS domains can act as binding domains for adenosine derivatives and may regulate the activity of attached enzymes or other domains. In some cases, CBS domains may act as sensors of cellular energy status by being activated by AMP and inhibited by ATP. Many proteins with CBS domain are easily detected in plant genome. However, their exact physiological functions need to be resolved. One of these, CDCP2 (CBS Domain Containing Protein 2) from *Arabidopsis thaliana* has been cloned and analyzed. It encodes 246 amino acid residues which contain two tandem CBS domains. CDCP2 protein was overexpressed heterologously in E. coli and purified it as homogeneity. As an initial step toward three-dimensional structure determination, crystals of recombinant CDCP2 protein have been obtained using hanging drop vapor diffusion methods. The crystals diffract to 2.4 Å resolution using Synchrotron sources and belong to trigonal space group, $P3(1)21$ or $P3(2)21$ with unit cell parameters of $a=b=56.12$ Å, $c=82.44$ Å, $\alpha=\beta=90^\circ$ and $\gamma=120^\circ$. To obtain more high-quality crystals of CDCP2, high-entropy side chains were removed from the surface of the protein. The new crystal form has been obtained using entropy-reduced CDCP2 protein and subsequent experiments for solving phase problems are underway.