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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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