P6-2

TIME-RESOLVED CRYSTALLOGRAPHY OF PROTEINS AT THE FEMTOSECOND X-RAY PLASMA SOURCE IN ELI BEAMLINES

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The advances in designing novel X-ray sources will allow for time resolved X-ray scattering and diffraction on a femtosecond to millisecond time scale. Unlike large scale facilities such as synchrotrons, intensive lasers can be used for the generation of short X-rays pulses in a setup similar in size to the commercially available laboratory X-ray sources. The femtosecond laser driven emission of X-ray pulses from plasma (XPS) offers higher time resolution for fast kinetic measurements than continuously emitting sources. The ELI beamlines facility is planned to start operation by the end of 2016 in Dolni Brezany, Czech Republic. It will give a unique advantage for time resolved crystallography and wide angle scattering for a crystalline samples, including proteins. The generated pulses will span approx. 100 fs with a repetition rate of 1 kHz. The scattered and diffracted by the protein crystal X-rays will be counted using a DECTRIS Eiger 1M area detector which operates at the same frame rate as the source, i.e. 1 kHz. Such setup can be combined with several pump probe lasers to study the fast kinetics for example in proteins relevant to plant photosystems or vision in animals. Because of the interdisciplinary nature of the fields and of the ELI beamlines facility regular discussions between experts in the field of high power laser-matter interaction and potential users, as well as young scientists, are organized.

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Posters - Chemistry of Crystallization

P7-1

A NEW DESIGN OF PLATE GEOMETRY FOR EFFICIENT PROTEIN CRYSTALLIZATION SCREENING

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We propose in this report a new design of geometry for protein crystallization plates. Each plate comprises 96 units corresponding to the 96 conditions of commercial crystallization screening kits. Each unit consists of 4 wells in which four different volume ratios of protein solution to precipitant solution can be set up. Based on the geometry we manufactured two types of crystallization plates: 1) Microbatch plate (M plate): the 96 units are separately sealed but the 4 wells in each unit are sealed to share the same common space; 2) Cross-diffusion microbatch [1] plate (CDM plate): all 96 4 wells are sealed to share the same common space, so that all volatile components in the droplets can freely diffuse in the common space. Figure 1 shows schematically the geometry of these two crystallization plates.

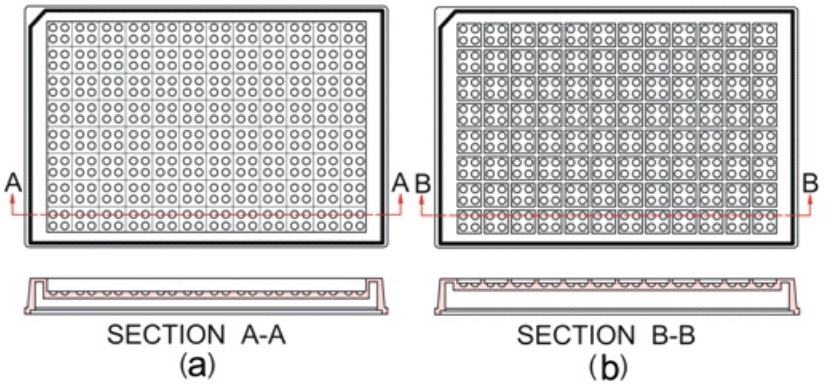

Figure 1. Two types of crystallization plates used in this study. (a) CDM plate; (b) M plate [1].

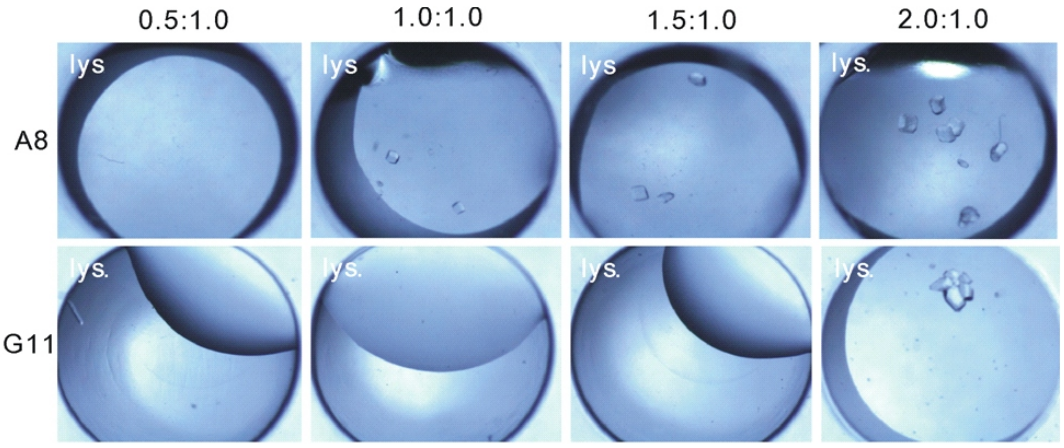

Figure 2. Crystallization examples at different initial volume ratios of protein solution to precipitant solution [2].

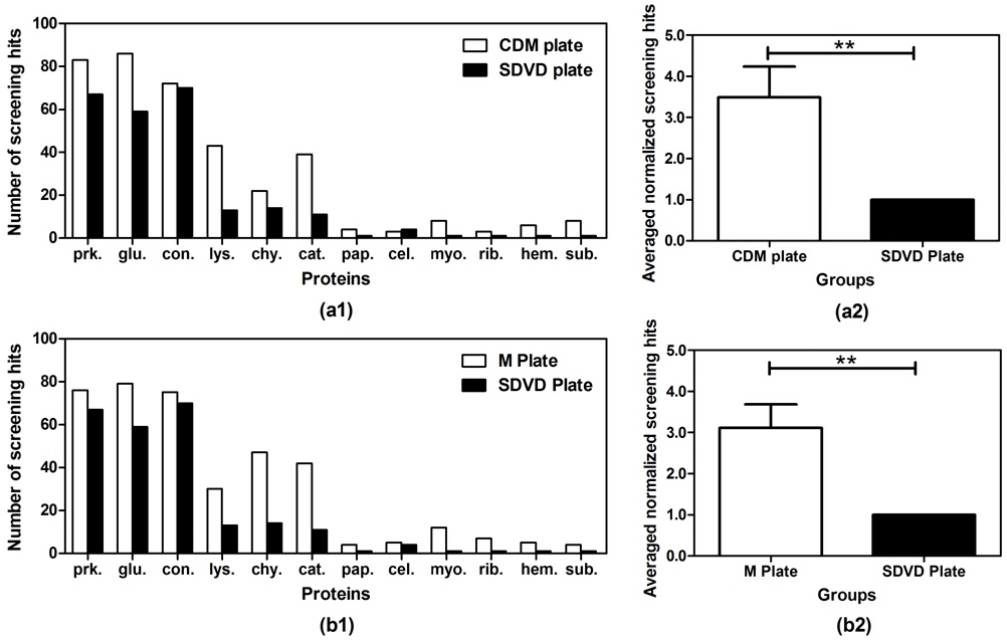

Figure 3. Comparisons of the number of crystallization screening hits using different crystallization plates (SDVD Plate, M Plate, and CDM Plate)[2]. The number of crystallization screening hits for the M and CDM plates is without redundancy, i.e., hits in one unit count as one hit. (a1) Comparisons of the number of screening hits between CDM and SDVD Plates. (a2) The averaged normalized screening hits on the CDM plate compared to the SDVD Plate, **P $<$ 0.01, error bar: mean ± SEM, n =12. (b1) Comparison of the number of screening hits between M and SDVD Plates. (b2) The averaged normalized screening hits on the M plate compared to the SDVD Plate, **P$<$ 0.01, error bar: mean ± SEM, n =12.

The crystallization plates exhibit strong flexibility to adopt different crystallization screening strategies. A very promising method is to vary the volume ratio of protein solution to precipitant solution among the 4 wells. Hence, 4 different initial concentrations can be simultaneously used for each precipitant solution, so that more chances of obtaining crystals may be achieved.

Figure 2 shows some examples that, different initial volume ratios can provide more chances to obtain crystals: when at one initial concentration, there may be no crystals at all (like in those clear drops), but at other initial concentrations using the same precipitant, crystals may appear (like in those drops with crystals).

Figure 3 shows the comparisons of crystallization results using CDM and M plates as compared against the traditional sitting drop vapour diffusion (SDVD) plate. It can be seen that both CDM and M plates are powerful to increase the efficiency to crystallize proteins.

AparApart from varying the volume ratios of protein solution to precipitant solution, another strategy is to vary the pH level in the droplet. By adding an extra buffer at different pH to the wells separately, we may adjust the final pH in the crystallization droplet, thus creating different crystallization conditions simultaneously.

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P7-2

CHANGE OF FRACTAL DIMENSION DURING THE NUCLEATION PHASE OF LYSOZYME CRYSTALLIZATION

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In this study we focused on the question how to grow crystals as large as possible in light of their use as samples for neutron protein crystallography. We concentrated on the early stages of the crystallization process where the directions are set whether many small crystals grow or few large ones. We used lysozyme as a model system since it has been studied well in the past and the phase diagram of its crystal growth is known. We used a combination of three scattering techniques since the involved size ranges require a large *q*-range. Small angle neutron scattering was used in combination with static light scattering on the same sample in order to obtain structural information on the growing crystal seeds. In situ dynamic light scattering at the neutron scattering sample cell was used to obtain an overview of all sizes present in the crystallization process by measuring their hydrodynamic radii. The small angle neutron scattering technique requires crystallization in heavy water instead of normal water. We found that the crystallization conditions did not differ too much from the ones mentioned in the literature for light water when using a corrected pD value of pD = pH+0.4. The crystallization is initiated by mixing a 60 mg/ml Lysozyme solution with a 6 wt% NaCl acetate buffer solution (both at pD=4.75 and at 298 K) in a 1 :1 ratio. Immediately after mixing, dimers of lysozyme molecules are formed and the structure factor seen in the lysozyme stock solution disappears. Under the chosen conditions we could observe a fractal growth of the cluster of monomers with a change of the fractal dimension from 1.0 to 1.7 in the first 90 minutes. This can be interpreted as clusters being formed first which grow more in a linear manner with little branching. Later, a swelling occurs corresponding to a growth in the dimension perpendicular to the previous linear growth. With these results theoretical models of crystal growth can be improved. Furthermore, the early detection of crystal seeds can be used to rapidly change the crystallization conditions (e. g. temperature) in order to avoid the production of more crystal seeds.

P7-3

CRYSTALLIZATION OF HALOALKANE DEHOLOGENASE DgaA ISOLATED FROM *GLACIECOLA AGARILYTICA NO2*

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A novel haloalkane dehalogenase DgaA, isolated from *Glaciecola Agarilytica NO2* was successfully crystallized.

Haloalkane dehalogenases represent an important class of microbial enzymes that catalyse an irreversible hydrolysis of halogenated compounds to the corresponding alcohol, halide ion and proton [1]. This enzymes are widely used for biodegradation and biosensing of environmental pollutants, decontamination of warfare agents, recycling by-products from chemical processes [2].

DgaA was isolated from psychrophilic and moderate halophillic organism *Glaciecola Agarilytica* NO2, found in the East Sea marine sediment in Korea [3].

In order to obtain the structure of DgaA x-ray diffraction analysis of enzyme's crystals was employed. Initial screening for crystallization conditions has been performed on Gryphon crystallization robot (Art Robbins Instruments, USA) using sitting drop vapour diffusion method [4]. After initial crystallization, microcrystals were found in several conditions of JCSG - *plus* and Structure screen 1&2 (Molecular Dimensions Ltd, UK) commercial

screens. Optimization procedures including protein and salt concentration variations resulted in appearance of crystals sufficient for x-ray diffraction experiments. Needle clusters and plate-like crystals with size about 1.3 × 0.059 mm and 0.2 × 0.065 mm, respectively, were obtained. The datasets were collected at resolution ranging from 1.8 to 2.4 Å.

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P7-4

EFFECTS OF POLYETHYLENE GLYCOL 4000 AND SODIUM CHLORIDE: 3-DIMENSIONAL PHASE DIAGRAM FOR A PROTEIN CRYSTALLIZATION

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Crystallization is associated with a decrease in the solvent accessible surface of a protein. For the crystallization of protein molecule, it is important to neutralize its charge with counterions to produce electroneutrality [1]. Previously, we have shown that the importance of co-existing sodium chloride in polyethylene glycol (PEG) 4000 - based crystallization solution, the most widely used precipitant [2]. However, the relation between PEG 4000 and sodium chloride could not be enough demonstrated. Therefore, we performed lysozyme crystallization in the concentration range of PEG 4000 (0-25 %) and sodium chloride (0.1-1.0 M) using batch method in a capillary of 0.3mm diameter to make a 3-dimensional phase diagram. In the course of experiments, we found that lysozyme crystals could grow in lower concentration (0.1~0.2 M) of sodium chloride if PEG 4000 was co-existed. In the solution of lower PEG 4000 concentration, a few but large crystals grew (Fig. 1-1). On the other hand, in the solution of higher PEG 4000 concentration, many but small crystals grew (Fig. 1-2), and if the PEG 4000 concentration is much higher, large but clustered crystals grew (Fig. 1-3). These results are consistent with the classical nucleation theory [3, 4, 5]. The X-ray diffraction data showed that the crystals grown in higher PEG 4000 solution diffracted to higher resolution. These results suggest that it may be better to use solution of lower concentration PEG with certain amount of sodium chloride to grow single crystals, but optimization of the concentration of PEG 4000 and sodium chloride may be a critical problem from a point of view of diffraction study.

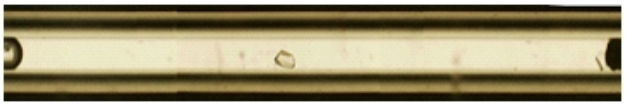

Figure 1-1. Lysozyme crystals grown in 5% PEG 4000 and 0.2 M NaCl.

Figure 1-2. Lysozyme crystals grown in 15% PEG 4000 and 0.2 M NaCl.

Figure 1-3. Lysozyme crystals grown in 20% PEG 4000 and 0.1 M NaCl**.**

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P7-5

STRUCTURE INVESTIGATION OF NATURE POLYETHER PRODUCT BIOSYNTHESIS

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Polyether nature products have been of special attention because of their fascinating structure and biological effects. They are widely used as anticancer, antibiotics, and antivirus therapeutics. Lasalocid A is an ionophore polyether that can be synthesized by a series of enzymes Lsd11 to Lsd19 in *Streptomyces lasaliensis*. It contains a polycyclic ether skeleton constructed by enantioselective epoxidation and epoxide-opening cyclization. Our group has investigated the epoxide hydrolysis step in lasalocid A biosynthesis. Now our interest is in the epoxidation step where the ether ring stereoconfiguration is determined by the epoxidase Lsd18. Solving Lsd18 atomic structure using X-ray crystallography would help us understand the mechanism of ether ring stereocontrol.

P7-6

METALLACARBORANE INHIBITORS OF CARBONIC ANHYDRASE IX, PROMISING COMPOUNDS FOR THERAPY AND DIAGNOSTIC

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We have previously identified carboranes and metallacarboranes modified by sulfamido or sulfonamido goup as a promising class of specific inhibitors of Carbonic Anhydrase IX (CA IX) [1].

Here we report on recent advances in the molecular design of carborane and metallacarborane inhibitors targeting CA IX isoenzyme. This enzyme, which is associated with solid hypoxic tumors, belongs to newly identified targets for cancer therapy and diagnostics.

The scope of currently available site-directed modifications on various boron cages is overviewed, with an emphasis on the progress in the synthesis of carboranes and metallacarboranes substituted by sulfamide, sulfonamide and other similar groups, i.e. functions known to bind tightly to the zinc atom in the active site of CA-IX. The new generations of polyhedral inhibitors of CA-IX, based on the careful selection of boron cages and optimized substitutions, exhibit significantly enhanced in vitro activities with corresponding Ki values in the range of tenths of pM to several nM. The structure-activity relationship (SAR) observed within a small library of ca. 60 substituted carboranes and metallacarboranes is discussed.

These results are complemented by synchrotron structures of enzyme-inhibitor complexes and by a short overview of pharmacologically relevant factors such as plasma protein binding, cell membrane penetration, and basic results from toxicology and pharmacokinetic studies (mouse model) performed on a panel of the selected inhibitors of CA IX enzymes. Due to promising inhibitory properties, these compounds are thus primarily considered as candidates for drugs applicable in cancer treatment.

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P7-7

TAILORED SUITS FIT BETTER: CUSTOMIZED CRYSTALLIZATION SCREENS – THE KEY TO SUCCESS?

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Producing well diffracting is one of the bottlenecks in macromolecular crystallography. Commercial sparse matrix are nowadays vastly used for the initial screening of crystallization conditions [1] . The design of these screens is based on conditions that have been successful in the past with other proteins [2]. However, these screens are have their weaknesses firstly because a substantial amount of structures were solved by high-throughput consortia. These aimed at the beginning to develop methods and solve the structures of procaryotically expressed, secreted and highly soluble proteins [3]. Secondly, repeated use of sparse matrix screens without adding new conditions just oversamples the pre-selected conditions but will not change the parameter space to a maybe more efficient and comprehensive one.

Crystallization is a thermodynamically driven process, which depends on a multitude of parameters that are not yet completely understood. However there is evidence that the propensity of a protein to crystallize in a certain condition is correlated to its melting temperature (T_m) [4].

With the aim to maximize the probability of success, we developed a crystallization strategy, where underlying concept is to mainly employ compounds, which increase the melting temperature of the protein. In the first step, the protein is subjected to thermofluor assays, in order to determine which compounds and which pH have the major impact on its stability. In the second step, the protein is then subjected to a customized crystallization screen designed to comprise compounds that were identified to increase the T_m.

The customized strategy yielded diffraction-quality crystals of a wide range of model as well as not-crystallised in-house proteins, where the standard approaches failed. Furthermore, the inherently simple design, modular and flexible nature of the platform makes it easy to modify and optimize certain steps. Information gained through the approach can be also used to improve the sample monodispersity, stability for storage, and even derive a possible function of not yet annotated proteins. Herein we will report on the results of our customized crystallization strategy, and give an outlook on future experiments and applications.

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