S12-L6

TRANSMISSION ELECTRON MICROSCOPY FOR THE EVALUATION AND OPTIMIZATION OF CRYSTAL GROWTH

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Crystallization of protein samples remains the most significant challenge in structure determination by X-ray crystallography. Here we demonstrate the effectiveness of transmission electron microscopy (TEM) analysis to aid in the crystallization of biological macromolecules. We found the presence of well-order lattices with higher order Bragg spots, revealed by Fourier analysis of TEM images, as a good predictor of diffraction-quality crystals. Moreover use of TEM allowed 1) comparison of lattice quality among crystals from different conditions in crystallization screens; 2) detection of crystal pathologies that could contribute to poor X-ray diffraction, including crystal lattice defects, anisotropic diffraction and crystal contamination by heavy protein aggregates and nanocrystal nuclei; 3) qualitative estimation of crystal solvent content to explore the effect of lattice dehydration on diffraction; and 4) selection of high quality crystal fragments for microseeding experiments to generate reproducibly larger size crystals. Applications for X-ray free electron laser (XFEL) and micro electron diffraction (MicroED) experiments are also discussed.

Session XIII - Crystallization in Industry and Biomedicine

Thursday, July 7 - morning

S13-L1

A RATIONAL APPROACH TO CRYSTALLISING PROTEINS IN THE PHARMACEUTICAL INDUSTRY, THE IMPACT OF MICRO SEED MATRIX SEEDING

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Crystallization is often referred to as a bottleneck in protein structure determination. However applying rational, knowledge based strategies and attention to detail, has proved to be very successful in obtaining crystallization systems for studying protein/inhibitor complexes in drug discovery.

This seminar will describe the rationale and the methods that have been successfully used to crystallize medically relevant proteins [1] in four different pharmaceutical companies (Hoffmann-la Roche, Morphochem, Novartis and Actelion). The first part will deal with protein characterization prior to initiating crystallization trials [2], the different methods and strategies of screening and examples of protein modification for crystallization.

The second part will focus on seeding and describe general seeding methods as an introduction to the most important subject of the talk, Microseed Matrix Seeding (MMS) [3-4]. One of the most powerful methods introduced into protein crystallization in the past 10 years in particular for establishing suitable cocrystallisation or soaking systems for obtaining X-ray structures of inhibitors complexes.

Examples will be given of increased hit rates, elimination of twinning, improved diffraction and promoting different space groups.

1. Crystallizing proteins - a rational approach? D'Arcy A. *Acta Crystallogr D* Biol Crystallogr. 1994 July.
2. The protein as a variable in protein crystallization. D'Arcy A, Mac Sweeney A, Haber A. *Acta Crystallogr D* Biol Crystallogr. 2003 Jul;59(Pt 7):1343-6. Dale GE, Oefner C, D'Arcy A. *J. Struct Biol. 2003* Apr;142(1):88-97. Review.1;50(Pt 4):469-71.1.
3. An automated microseed matrix-screening method for protein crystallization. D'Arcy A, Villard F, Marsh M. *Acta Crystallogr D* Biol Crystallogr. 2007 Apr;63(Pt 4):550-4.
4. Microseed matrix screening for optimization in protein crystallization: what have we learned? D'Arcy A, Bergfors T, Cowan-Jacob SW, Marsh M. *Acta Crystallogr F* Struct Biol Commun. 2014 Sep;70(Pt 9):1117-26.

The following are acknowledged for their significant contributios over many years, Paulus Erbel, Sandra Cowan-Jacob, May Marsh, Aengus Mac Sweeney, Frederic Villard.

S13-L2

SUCCESSFUL GENERATION OF STRUCTURAL INFORMATION FOR FRAGMENT-BASED DRUG DISCOVERY

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Fragment based drug discovery has evolved to a mature hit finding strategy and there are today numerous successful examples in the literature. For a fragment based campaign to be successful the ability to obtain a large number of structures of diverse chemical fragments is unquestionable, yet it is often challenging to generate structures with bound fragments. A summary of recent literature reveals that a wide repertoire of experimental procedures is employed to generate ligand-bound crystal structures [1], illustrating that each protein and project needs specific attention.

Here we share our experience from setting up and executing fragment crystallography in the Soluble Epoxide Hydrolase project that altogether resulted in 55 complex structures, where 38 are fragments. The size of this dataset has allowed us to make retrospective analysis of ligand properties such as potency, ligand solubility, clogP and ligand efficiency to identify success factors for structure generation and ask the question if any of these could be used to guide crystallization efforts. Our results reveals that potency, ligand efficiency and, to some degree, clogP influence the success of complex structure generation whereas the measured aqueous ligand solubility did not.

1. L. Öster, S. Tapani, Y. Xue, H. Käck, Drug Discov Today., 9, (2015), 1104.

S13-L3

XRayLab: AN X-RAY DIFFRACTION FACILITY FOR THE INTERNATIONAL SPACE STATION DEDICATED TO THE STUDY OF SPACE-GROWN CRYSTALS

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The International Space Station (ISS) is a great technical achievement, designed as a flexible laboratory able to support science in a wide range of disciplines. Unfortunately, to make use of the ISS is still unappealing to a large number of potential users, due to the burden of complex rules and long procedures associated with developing and operating equipment on board the Station. The policy of the European Space Agency (ESA) about the utilization of the Columbus module changed recently, opening to the possibility of accessing the ISS on a commercial basis. This will allow for the establishment of new commercial services supporting the performance of additional science and technological research and development.

One of the most interesting aspects is related to the possible biological advancements coming from the study of space-grown protein crystals. One of the initiatives under preparation, with the support[1] of the European Union (EU), is centred on the XRayLab facility and aims at boosting the research in various fields, thanks to the singular effect of the microgravity on crystals' quality, associated with the unprecedented capability of performing in situ X-ray diffraction (XRD) measurements.

A promising application of this crystallography technique targets protein-pharmaceutical compounds molecular complexes crystallized in orbit, with the objective of:

- elucidating rigid protein atomic structures and configurations;
- identifying protein-ligand docking relationships (structure-activity relationships)

High quality crystals will grow in the microgravity environment guaranteed by the ISS, in a dedicated experimental microfluidic setting called the PharmaCard (Figure 1). The XRD measurements will be performed in situ, by means of this novel XRD instrument, to avoid deterioration of the protein crystals when exposed to the re-entry and ground transport stress environment. The acquired data, consisting of the XRD pattern images, will be either downlinked or stored in data storage media that will then be downloaded to ground for computational analysis and structural determination.

The PharmaLab facility will target applications in the sector of structure-based drug design and development. In addition, the use of an X-ray measurement facility and of microfluidics in space may have other interesting applications not only for proteins but also for pharmacological

compounds and other small molecules compounds. Crystallization is such a universal process that we cannot safely explore the space without a deep knowledge on how molecules join themselves under microgravity to form crystals, from nano- to metre-size scale[2].

1. This project has received funding from the European Commission's H2020 Framework Program for research, technological development and demonstration under grant agreement No. 666815.

2. J.M. Garcia Ruiz, F. Otálora, in *Answers to a questionnaire on the PharmaLab experimental facility for Space Applications Services* (Dec. 2015).

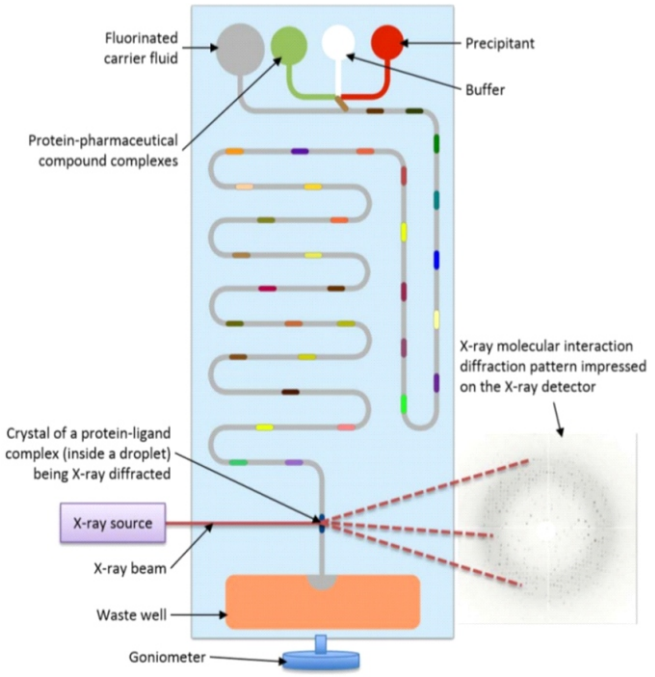

Figure 1. PharmaLab Card and XRD (concept)

S13-L4

A COMPARATIVE STUDY ON THE DIFFRACTION QUALITY OF PROTEIN CRYSTALS OBTAINED USING THE CROSS-DIFFUSION MICROBATCH AND SITTING-DROP VAPOR DIFFUSION METHODS

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Improving the crystal quality of a protein is always an important target for structure determination using X-ray diffraction. In this project, we present a systematic quality comparison of protein crystals grown using the cross-diffusion microbatch (CDM) method and the standard sitting-drop vapor-diffusion method. Crystallization conditions for eleven different proteins were screened using these two methods, and the crystals of all conditions were checked in terms of the resolution limit and mosaicity. It was found that crystals grown in the plate using the CDM method exhibit better morphology and higher crystal quality than crystals obtained using the standard sitting-drop vapor-diffusion method. X-ray diffraction tests show that the CDM method is indeed a practical and useful method for obtaining high-quality protein crystals to reduce the workload associated with both protein crystallization screening and optimization.

A crystallization plate using the CDM method is shown in Fig. 1 [1]. (a) Image of crystallization plate using the traditional sitting drop vapor-diffusion (SDVD) method. (b) Image of crystallization plate using the cross-diffusion microbatch (CDM) method. The size of this plate is made to be compatible with the SBS standard plate. This crystallization plate is similar to the traditional SDVD plate, ex-

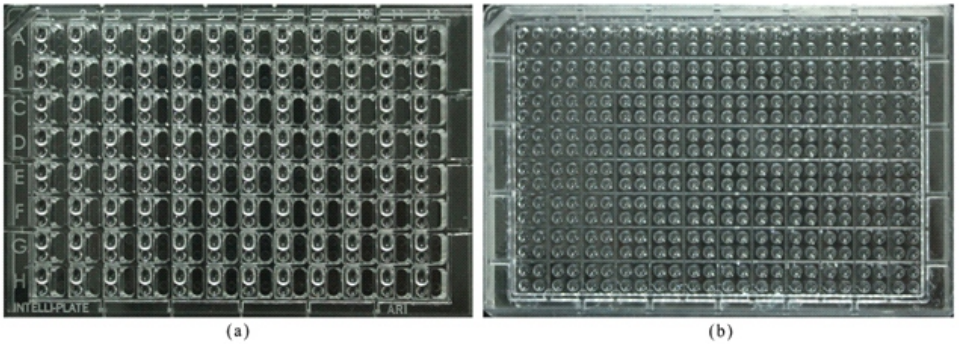

Figure 1. Comparison of images of the crystallization plate using the CDM method and the SDVD method.

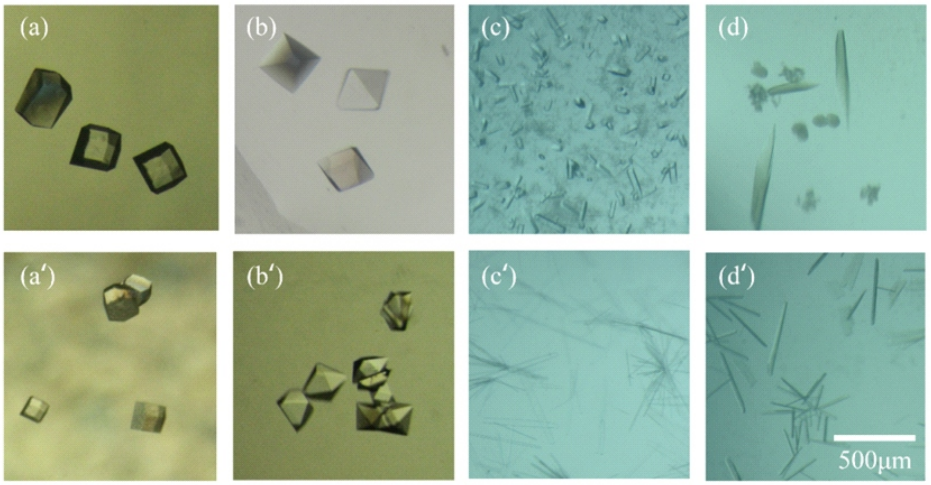

Figure 2. Typical morphology of crystals grown in the CDM and the SDVD crystallization plate.

cept for the material and the reservoir, which was replaced by more pits to increase the number of protein concentrations or pH gradients.

Fig. 2 shows some typical images [(a)-(d), crystals grown in a CDM crystallization plate; (a')-(d'), crystals grown in a SDVD crystallization plate]. The crystals grown in the CDM crystallization plate looked better than the crystals grown in the SDVD crystallization plates [2]. Some defective crystals were obtained from the SDVD crystallization plate. The crystal morphological comparison showed that the crystals grown in CDM crystallization plates displayed relatively better morphology than crystals grown in SDVD crystallization plates. (a), (a') Lysozyme; (b), (b') Proteinase K; (c), (c') -Chymotrypsinogen A II; (d), (d') Catalase.

Fig. 3 exhibits a comparison of the resolution limits of all the "Diffraction data" quality the crystals obtained from the CDM and the SDVD crystallization plate [2]. (a) In terms of the resolution limit of the proteinase K crystals, the results demonstrated a significant difference between the two groups ($P < 0.05$). (b) A comparison of the mosaicity of proteinase K. The results demonstrated significant difference between the two groups ($P < 0.05$). (c) A comparison of the resolution lysozyme crystals obtained in the CDM and the SDVD crystallization plate. The difference between two groups was significant ($P < 0.05$). (d) A comparison of the mosaicity of lysozyme crystals. The results showed an extremely significant difference between the two groups ($P < 0.01$). Therefore, the CDM crystallization plate clearly demonstrated an improvement in both resolution and mosaicity compared with the SDVD method.

In conclusion, it was found that the CDM method can improve the quality of protein crystals compared with the conventional SDVD method. This crystallization screening method could be suitable for routine protein crystallization.

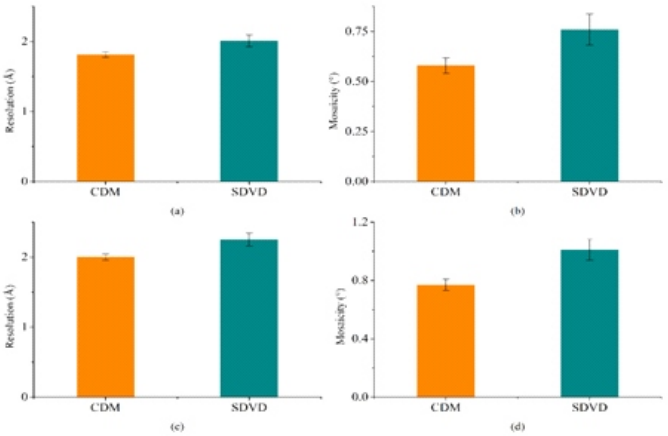

Figure 3. A comparison of the resolution limits of all the "Diffraction data" quality crystals obtained from the CDM crystallization plate and the SDVD

1. R. Q. Chen, D. C. Yin, Y. M. Liu, Q. Q. Lu, J. He, Y. Liu, *Acta Cryst.*, **D70**, (2014), 647.
2. H. Hou, B. Wang, S. Y. Hu, J. Z. Wang, P. F. Zhu, Y. Liu, M. Y. Wang, D. C. Yin, *CrystEngComm.*, **17**, (2015), 5365.

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