

have not been identified yet, ASHH1 and ASHH2 have been suggested to add monomethyl and trimethyl to H3 K36, respectively, [4]. In order to determine the protein structures, SET domain proteins (ASHH1, SUV4) were overexpressed in *E. coli* BL21 cells and purified by Maltose affinity chromatography. After the first step purification, the protein production was relatively high; ~ 50 mg of protein from 1.5 L induced cells. The proteins were further purified by using Ion-exchange chromatography and Size-exclusion chromatography and we are at the moment ready to start crystallographic process to determine the structures.

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Protein Crystallisation Screening by the Counter-diffusion Technique. Luis A. Gonzalez-Ramirez^a, Alfonso G. Caballero^a, D. Choquesillo-Lazarte^a, J. M. Garcia-Ruiz^a. ^aLaboratorio de Estudios Cristalograficos, IACT(CSIC-U. Granada), Granada, Spain.

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Crystallisation remains the rate-limiting step in protein structural determination due to the large number of variables that must be systematically adjusted for optimal crystal formation. Typical parameters which influence crystal growth include the temperature, the concentration, purity and nature of the protein in question as well as the concentration and nature of other chemical components normally used such as buffers, additives and precipitating agents [1].

Over the last 20 years, numerous efforts in order to make crystallization conditions more tractable have led to a number of novel crystallisation conditions screenings and crystallisation strategies [2, 3]. A particularly useful method to grow protein crystals is the counter-diffusion. This technique is based on the diffusion of both precipitating agent and protein in opposite directions thereby generating a supersaturation wave across the growth chamber. This supersaturation wave effectively screens different crystallisation conditions of progressively lower supersaturation values while travelling along the crystal growth chamber [4].

This work describes the screening of crystallisation conditions for 40 proteins using the counter-diffusion technique. The experimental set up involves the use of very thin capillaries ($\phi = 0.1$ mm) within the Granada Crystallisation Box (GCB) [5], commercially available from Triana Science & Technology. One of the many advantages of using such thin capillaries is that it greatly reduces the amount of protein solution needed for each single screening

experiment to as little as 300 nanolitres.

We have analysed the effect of the pH in combination with the most frequently used precipitating agents, namely, three polyethylene glycols of varying molecular weight (PEG-400, PEG-4000, PEG-8000) plus ammonium sulphate on the crystallisation of 40 proteins. Additionally, we have tested whether the use of a mixture of the three PEGs has the same effect on the crystallisation that any of them separately. The results of the different screened crystallisation conditions in terms of the success of crystallisation and crystal quality (as determined by X-ray diffraction) are discussed.

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Keywords: protein crystallisation; crystallisation techniques; screening; counter-diffusion

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Protein Crystallization: Robotics, Procedures and Developments. Fabrice Gorrec^a, Olga Perisic^a, Katharine Michie^a, Gebhard Schertler^a, Jan Löwe^a. ^aMRC Laboratory of Molecular Biology (LMB), Cambridge, UK.

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LMB scientists can undertake initial crystallization experiments and also crystal condition refinements for each new protein sample using automated protocols [1]. The LMB protein crystallization facility is high-throughput and includes various robots (at room temperature and at 4°C). The protocols are straightforward and setting up plates is easy. This enables LMB scientists to operate independently. This is crucial when targets are difficult to crystallize and many rounds of screens are required to test new constructs, like for the cell division protein FtsQ [2]. The standard initial screening protocol is comprised of 17 MRC sitting-drop plates pre-filled with a wide variety of commercially available screen kits using the Genesis workstation (Tecan). Process is fast and requires only small volume of protein. The 17 plates are set up within an hour using 272 μ l of sample (for 100 nl drops of the target protein and mother liquor) on the ScreenMaker (Innovadyne) or the Mosquito (TTP LabTech). About 10,000 plates a year are set up for initial screening alone. Refinement, custom matrices and scale-up screens are made in any type of plate. A refinement screen in MRC plate is made in three minutes on the Sciclone i1000 workstation (Caliper) with a custom-made in house SBS footprint reservoir (commercialized by Swissci) containing stock solutions.

New methods and tools are continuously developed and integrated into our crystallization facility. For example, The MRC multi-wavelength imaging system allows assessment of crystals regardless of clarity of the drops. The LMB screen database [3] is a Web based tool to perform basic data mining about the initial screens. To further aid users in the optimization of difficult crystallization conditions,

liquid handling advances, new micro-plates and specialized gas-tight and strong-binding plate seal developments have enabled the adoption of automated procedures for seeding, for experiments under oil (with the MRC micro-batch plate) and for crystallization at high temperature (up to 50°C). A novel crystallization screen called Morpheus (commercialized by Molecular Dimensions) as also been developed recently [4].

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Keywords: automation; screening; innovation

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X-ray Diffraction from Crystals in Crystallization Plates. Tadeusz Skarzynski. *Oxford Diffraction Ltd., Oxford Industrial Park, Yarnton, Oxfordshire, OX5 1QU, United Kingdom.*

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The PX Scanner is a unique instrument from Oxford Diffraction enabling the identification and characterization of protein crystals with X-rays, in-situ, in the crystallization plate. The PX Scanner addresses the bottleneck in high-throughput crystallization allowing a quantitative evaluation of the diffraction properties of crystals without having to manually extract crystals from the crystallization plate. Combining an optical imaging system with a powerful, micro-focus Cu X-ray source, highly sensitive CCD detector and intuitive software provides an essential laboratory resource for crystallographers involved in difficult structural biology projects.

The PX Scanner is now further enhanced with new software named CrystalEyes. CrystalEyes expands the functionality of the PX Scanner, utilizing new algorithms to improve the acquisition and quality of the optical and X-ray images. Furthermore, new database access capabilities allow the data to be easily output from the PX Scanner and integrated with a 'LIMS' style lab data management system.

We will show how the PX Scanner can be used as a powerful tool providing valuable feedback at all stages of macromolecule crystallization, including differentiating salt from protein crystals in initial screens, selecting the best crystals for synchrotron X-ray data collection, optimizing harvesting, cryo-protecting and soaking conditions, and determining crystal lattice parameters.

Keywords: crystallization of macromolecules; in-situ experiments; X-ray methods