MS06 O1

Déjà vu all over again. Why duplication matters in crystallisation Janet Newman *CSIRO-MHT 343 Royal Parade, Parkville, VIC 3052 Australia*

We have started looking at the effects of replication on outcomes of a crystallisation experiment. Initially experiments were set up to probe the effects of changing drop size on the reliability of the crystallisation, and the results from these experiments suggested that that replication is a powerful tool in the arsenal of the protein crystallographer.

MS06 O2

New Insights into Biomolecule Crystallization Processes using Dynamic Light Scattering and Imaging in Multi-well Plates Karsten Dierks[a], Arne Meyer[c], Howard Einspahr[a,b] , Christian Betzel[c], [a]*PLS Design, Hamburg, Eichenstrasse 42, 20255 Hamburg, Germany.* [2]*67 Green Avenue, Lawrenceville, NJ 08648, USA.* [3]*University of Hamburg, Department of Biochemistry and Molecular Biology c/o DESY, 22603 Hamburg, Germany.*E-mail: Betzel@unisgi1.desy.de

Keywords: Crystallization droplets, Dynamic light scattering, Imaging

Automated methods to crystallize macromolecules are widely used and can easily generate thousands of crystallization droplets. Nevertheless, evaluation of crystallization experiments to find optimal growth conditions remains a bottleneck. We have investigated methods to improve the process of finding crystal growth conditions. Besides imaging drops, two methods have emerged as most promising. One is dynamic light scattering (DLS), which has already many applications, but which we have found useful for detection of aggregation and nucleation in droplets. The other is the use of combined white/UV illumination for microscopic determination of whether crystal-like objects are biomolecular and identification of crystals in crystallization set ups. Prior to the crystallization experiment, DLS is a widely accepted method to determine the size and mode of aggregation (e.g. polydispersity) of proteins and other biomolecules in solution, but its use has been limited because the need to make measurements in cuvettes required rather large sample volumes. Protein crystallization experiments are usually carried out in multi-well plates with much lower droplet volumes in the range of 0.5 to 10 µl. We will describe a new method to image, measure and analyze the protein particle size directly in drops and in particular to investigate the stage of nucleation and the progress of crystal growth by *in-situ* DLS, i.e. directly in the droplets. This has several advantages: no additional pipetting is necessary to perform measurements; the crystallizations process can be monitored online *in situ*, without interruption; measurements can be taken from even small volumes. This new DLS technique has been adapted to an automated CCD-camera-based plate-screening system (Spectro-Imager 501, PLS-Design/RiNA GmbH) allowing automated monitoring and evaluation of the entire process of crystallization in one instrument. The data obtained provide information to understand in detail the process of crystal growth. We will also describe a method to identify protein crystals, exploiting the fact that most proteins and many biomolecules fluoresce when illuminated with UV light. We found that crystallization reactions can be categorized into a few different scenarios, mainly differing in the way the aggregates or nuclei grow until the onset of crystallization. Some substances crystallize e.g. in a diffusion-limited process. These show a characteristic behaviour of the growth rate of the initial crystal nuclei. Other scenarios and latest results will be presented from standard proteins as well as proteins which are of pharmaceutical interest.

MS06 O3

A nanoscale crystallization example: purification, crystallization and structural determination of human CD5 domain III Inés G. Muñoz[1], Bernardo Rodamilans1, Francisco J. Blanco2 & Guillermo Montoya1. *Structural Biology and Biocomputing Program, 1Macromolecular Crystallography Group and 2NMR Group, Spanish National Cancer Center (CNIO). Madrid. Spain.*
E-mail: imunoz@cnio.es

Keywords: CD5, SRCR

Scavenger Receptor Cysteine-Rich (SRCR) domains are ancient protein modules widely found among cell surface and secreted proteins of the innate and adaptive immune system, where they mediate ligand binding. The human lymphocyte receptor CD5, a key regulator of immune responses, is involved in the modulation of antigen specific receptor mediated T cell activation and differentiation signals. CD5 is a membrane glycoprotein which belongs to the group B scavenger receptor cysteine-rich (SRCR) superfamily. Although the yield of the purified protein was at the level of micrograms, well diffracting crystals of CD5 domain III have been obtained and allowed us to solve the crystal structure at 2.2 Å resolution. Previous attempts to purify CD5 proteins in heterologous expression systems such as Pichia have not produced proteins of sufficient quality. Our strategy therefore was to express the protein in mammalian cells and the development of a monoclonal antibody (Mab) to permit a single-step purification. Nanocrystallization facilities eased the screening. The first structure of a member of a group B SRCR domain reveals the fold of this protein module into a central core formed by two antiparallel β-sheets and one α-helix, illustrating the conserved core at the protein level of genes coding for group A and B members of the SRCR superfamily.
Our strategy to produce, isolate and crystallize CD5 domain III can be used for other mammalian proteins difficult to produce for structural or other biophysical studies.

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