Session X - Teaching Macromolecular Crystallization

Tuesday, July 5 - late afternoon

S10-L1

BIOLOGICAL CRYSTALLIZATION: FROM THE CLASSROOM TO THE BENCH

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Growing crystals is a fascinating and visual process. It is also an excellent way to discover the scientific process and to grasp the challenges of modern biology. In 2014 in the frame of the international year of crystallography, we used simple crystallization experiments to raise interest in major advances in crystallography and structural biology among members of the general public and particularly secondary school students. Activities were undertaken in a variety of contexts: through participation in the national science festival, crystallization contests, exhibitions, talks at schools, interactive webcasts and participation in our university's science outreach program.

This enriching experience could be easily implemented by any laboratory or university. This presentation will illustrate our approach and the educational materials we have developed.

S10-L2

A LABORATORY COURSE ON CRYSTALLIZING MEMBRANE AND SOLUBLE PROTEINS AND COMPLEXES BY THE LIPID CUBIC PHASE (*IN MESO*) METHOD

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The lipid cubic phase or *in meso* method is, by now, a proven and robust method for crystallizing membrane proteins. It also works with soluble proteins. Already, it is responsible for over 300 entries in the Protein Data Bank, some corresponding to high profile targets such as the $_2$-adrenoreceptor-G protein and the rhodopsin-arrestin complexes [1-3]. Because crystallization takes place in a bicontinuous mesophase, the method in its execution requires manual dexterity to properly prepare, dispense, screen and harvest from this characteristically sticky and viscous hosting liquid crystal. Over the past decade, we have been active in running workshops worldwide with a view to passing along this specialized technique. In support of this outreach activity, several papers that include instructional videos have been made available online and open-access [4]. Recently, the method was included as part of a laboratory module in a third year undergraduate biochemistry course at Trinity College Dublin. Given that the method works with soluble proteins, for reasons of ease and cost, lysozyme was used as the test protein following a protocol specifically designed to produce recognizable crystals *in meso* within an hour at room temperature [5]. Eighteen students successfully completed the module that came in three parts. The first involved preparing the protein solution, forming and dispensing the protein-laden mesophase, setting up screens and monitoring crystal growth. A one-hour lecture on the theory and practice of the method was given the week preceding the laboratory exercise and students were advised to view the online video that describes the method well in advance of the lab. The second part began with a one-hour lecture on principles and practices of macromolecular crystallography. This was followed by laboratory work that involved evaluating the crystallization screening results, recording crystal characteristics (size, shape, density, distribution), discussing protein solubility and lipid/water temperature-composition phase diagrams, and viewing *in meso* robots, an imager/incubator and a rotating anode X-ray diffractometer in action. The final part consisted of a computer lab where the students were introduced to and trained in the rudiments of molecular modelling/graphics using Coot and PyMol. Because the students enrolled in the course were specializing in Molecular Medicine, insulin was used for the computer lab work. In this presentation, our experiences teaching the course, how it might be improved, and feedback from the students will be described.

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1. Caffrey. M. 2015. A comprehensive review of the lipid cubic phase or in meso method for crystallizing membrane and soluble proteins and complexes. Acta Cryst. F71, 3-18.

2. Rasmussen *et al.* 2011. Crystal structure of the 2 adrenergic receptor–Gs protein complex. Nature 477:549-555.

3. Kang *et al.* 2015. Crystal structure of rhodopsin bound to arrestin determined by femtosecond X-ray laser. Nature 523, 561-567.

4. Caffrey, M., Porter, C. 2010. Crystallizing membrane proteins for structure determination using lipidic mesophases. J. Vis. Exp. 45: www.jove.com/index/details.stp?id=1712

5. Aherne, M., Lyons, J.A., Caffrey, M. 2012. A fast, simple and robust protocol for growing crystals in the lipidic cubic phase. J. Appl. Cryst. 45:1330-1333.

S10-L3

TEACHING (MACROMOLECULAR) CRYSTALLIZATION WITH MOVIES

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The aim of a crystallization lecture is to provide biocrystallographs, who intend to tackle protein-crystallization, with theory and practical examples.

We introduce the fundamental phenomena in protein crystallization: supersaturation, nucleation, growth and transformation of crystals. Moreover, from a physical point of view proteins serve as macromolecular model systems because biological macromolecules and small organic or mineral molecules follow the same rules, that is to say crystal growth mechanisms are the same [1].

Based on data obtained using in-situ investigation of crystallization [2], we illustrate physico-chemical properties of crystallization with movies: How supersaturation is achieved? How do nucleation, growth, polymorphism, demixion and kinetic ripening proceed? We show that these movies are perfect tools to teach crystal growth. Thus in our lecture, we give concrete examples illustrating protein crystallization [3.

1. Chernov A.A. , Journal of Materials Science: Materials in Electronics, 12 (2001) 437-449.

2. Astier J. P., Veesler S., Cryst. Growth Des., 8 (2008) 4215-4219.

3. Candoni N., Grossier R., Hammadi Z., Morin R., Veesler S., Protein & Peptide Letters, 19 (2012) 714-724.

S10-L4

OPTIMIZATION OF CRYSTALLIZATION USING DIALYSIS COMBINED WITH TEMPERATURE CONTROL

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The crystallization process includes both thermodynamic and kinetic features in multidimensional phase spaces, and our understanding is based on a crystallization phase diagram, represented in a simplified form in Figure 1. Thermodynamic data are the solubility curves, the presence of metastable phases, polymorphs, liquid-liquid separation… They depend on multiple parameters such as temperature, pH, solvent, impurities, etc. In addition, kinetic trajectories in the phase diagram are relevant to control most of the final properties of the synthesized crystals. The path followed in the diagram controls the nucleation and growth of the crystals, and thus their number, size, and morphology.

Two new and emerging uses result in specific challenges for crystallization of proteins. In both, precise control of crystal size is essential. New approaches to serial (time-resolved) crystallography, where crystals in the 1-20 m size are used to solve structures including those/structures of short-lived intermediates with reactions initiated by light or rapid mixing. Serial crystallographic methods are being increasingly used at synchrotron sources (serial synchrotron crystallography) due to advances in micro- and nano-focus beamlines, as well as at rapidly developing ultra-bright free-electron laser sources (serial femtosecond

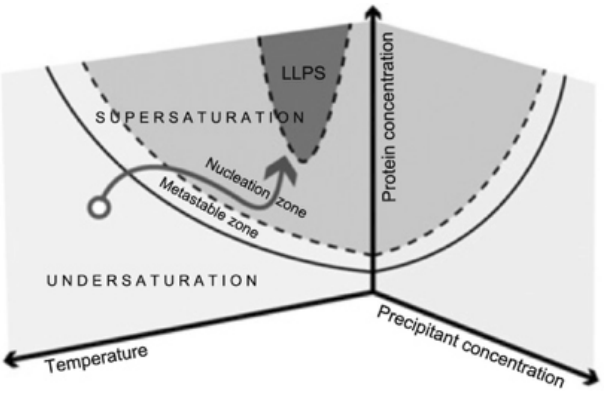

Figure 1: Schematic view of a multidimensional phase diagram. The arrow illustrates a specific pathway taken during crystallization.

crystallography), enabling structure determination of previously intractable proteins. At the other extreme are the requirements of the next-generation flagship neutron sources, such as the ESS (European Spallation Source, Lund). Because neutrons interact very weakly with matter, much larger, and ideally cubic crystals are needed with volumes of > 0.01 mm^3 (i.e. 200 m on a side) for neutron crystallography in the future.

We have developed an apparatus and a method for the optimization of crystal growth using precise temperature control and dialysis combined with real-time visualization. Several neutron crystallography targets are being investigated on the developed system and the positive results already obtained indicating that the control of crystal growth does not compromise the diffraction quality, and rather improves it. The goal of this lecture is to provide the audience with related protocols using a thorough knowledge of the phase diagram and demonstrate how to select the starting position and kinetic pathway in optimizing the crystallization experiments.

S10-L5

TEACHING PROTEIN CRYSTALLIZATION AT THE LABORATORY FOR CRYSTALLOGRAPHIC STUDIES (GRANADA, SPAIN)

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The Laboratory for Crystallographic Studies (LEC) has shown since its creation a clear commitment with the promotion of crystallography and crystal growth at all the levels of education, from primary schools to graduate University level. The "Krystalla Project" comprises a series of coordinated activities specifically designed to promote the importance of crystallography and crystallization, namely:

1 The itinerant exhibition "CRISTALES: A world to discover" which will exemplify the applications of crystallography on our daily life and the fundamentals behind our science.
2 The book-guide of the exhibition "CRISTALES: A world to discover"[1, 2].
3 A didactic edition of the documentary "The Mystery of the Giant Crystals" including the making-off of the movie, short videos explaining the fundamentals and applications of crystallization and scientific notes for teachers.
4 A series of workshops on "popular crystallography" and "crystallography" for kids.
5 The National Crystallization Competition in the School
6 A series of didactic guides to use well-known movies as crystallographic teaching and popularization materials.
7 A series of crystallization graduate and postgraduate courses as part of the International Master on Crystallography and Crystallization of the UIMP/CSIC.

We will present in this communication an overview o most od these projects.

1. J. M. García-Ruiz, F. Otálora, A. García-Caballero, L. A. González-Ramirez and C.Verdugo-Escamilla, *CRISTALES: a world to discover. An exhibition for schools and universities Journal of Applied Crystallography* **48** (2015) 1264.

2. J.M. García-Ruiz and F. Otálora (2016) *Cristales: Un mundo por descubrir. Bilingual edition Spanish/English. Triana S&T, Granada, 120pp. ISBN: 978-84-942454-1-1.*