



Laboratory exercises

INTRODUCTION TO LAB EXERCISES - MACROMOLECULAR CRYSTALLIZATION

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Crystallization of macromolecules used to be rather empirical procedure, and because of its unpredictability and frequent irreproducibility, it has long been considered as an "art" rather than science. It is only in the last 20 years that a real need has emerged to better understand and rationalize the crystallization of biological macromolecules. As the molecules involved in crystallization exhibited such contrarious behavior and were poorly characterized, conducting serious research into their crystallization was conceded as hopeless. Only when the demand for crystals by crystallographers and later molecular biologists could no longer be ignored, researchers pursued in earnest the study of macromolecular crystal growth (McPherson 1999).

Researchers working in the protein X-ray crystallography laboratory understand that the first requirement for protein structure determination is to grow suitable crystals.

Without crystals there is no X-ray structure determination of a protein!

The characteristics of crystals and biological macromolecules crystallization data have been compiled in the Biological Macromolecule Crystallization Database (BMCD) (Gilliland *et al.* 1994, Gilliland 1998). The BMCD contains crystal data and the crystallization conditions, which have been collected from the literature. The current version of the BMCD contains 43406 crystal entries (Tung and Gallagher, 2009) from macromolecules for which diffraction quality crystals have been obtained. These include proteins, protein-protein complexes, nucleic acid, nucleic acid:nucleic acid complexes, protein:nucleic acid complexes, and viruses. All crystallographic information about particular macromolecule including X-ray diffraction data and statistics information are available online in the Protein DataBank (RCSB PDB) database (Berman *et al.* 2000). The RCSB PDB provides a variety of tools and resources for studying the structures of biological macromolecules and their relationships to sequence, function, and disease.

CRYSTALLOGENESIS KEYWORDS

Macromolecules

All of the macromolecules are polymers of one of the precursor classes that include the amino acids, the ribonucleotides and deoxyribonucleotides, sugars of various sorts, fatty acids, *etc.* These small molecules are linked together in a sequence by complicated series of chemical reactions in the cell to form the macromolecules such as proteins, nucleic acids (RNA and DNA), polysaccharides

and lipids. The structural complexity and physiological role of macromolecules are a function of the diversity of the precursors, the sequence in which they are joined together, the number of precursors in the polymer, and finally, the 3D form after polymer synthesis.

Macromolecules assume 3D structures that sequester and pack hydrophobic groups in their interior and leave hydrophilic groups exposed to solvent. Solvent molecules form solvent layers around macromolecules.

Targets of biological crystallogenesis

For biologists, studying crystal growth should be correlated with biological problems, and crystallization projects on macromolecular complexes, membrane proteins, and especially engineered proteins, are being developed.

For physicists, growing large monocrystals can be a goal in itself, and one might speculate that exploration of optical, electronical, mechanical, and other physical properties of crystalline arrays made from biomacromolecules or assemblies can lead to novel frontiers in the material science.

For chemists, usage of chemical and molecular biology tools could lead to designing molecular devices and other nanostructures mimicking macromolecular crystals in the future.

Crystals

Crystals are chemically well defined; often they are geometrical solids with regular faces and sharp edges. From the physical point of view, crystals are regular three-dimensional arrays of atoms, ions, molecules, or molecular assemblies. Ideal crystals can be pictured as infinite and perfect arrays in which the building blocks (the asymmetric units) are arranged according to well-defined symmetries into unit cells that are repeated in 3D by translations. Experimental (laboratory-grown) crystals have finite dimensions; their periodicity is never perfect, due to different kinds of local disorders or dislocations. The phenomenon underlying structural chemistry and biology is the ability of crystals to diffract X-rays, neutrons, or electrons.

Macromolecular crystals

Macromolecular crystals are rather small with poor mechanical properties and a high content of solvent filled channels that make up 20-80% of their volume. These crystals are always extremely fragile and sensitive to external conditions. For this reason macromolecular crystals have to be kept in the solvent-saturated environment, else dehydration will lead to crystal cracking and destruction.

**Table 1.** Parameters effecting the crystallization of macromolecules.

<i>Physical</i>	<i>Chemical</i>
Temperature Surfaces Methodology/approach to equilibrium Gravity Pressure Time Vibrations/sound/mechanical perturbations Electrostatic/magnetic fields Dielectric properties of the medium Viscosity of the mediurate of equilibrationm Homogeneous or heterogenous nucleants Rate of equilibrium	pH Precipitant type Precipitant concentration Ionic strength Specific ions Degree of supersaturation Reductive/oxidative environment Concentration of the macromolecules Metal ions Crosslinkers/polyions Detergents/surfactants/amphophiles Non-macromolecular impurities
<i>Biochemical</i>	<i>Biological</i>
Purity of the macromolecule/impurities Ligands, inhibitors, effectors Aggregation state of the macromolecule Post-translation modifications Source of macromolecule Proteolysis/hydrolysis Chemical modifications Genetic modifications Inherent symmetry of the macromolecule Stability of the macromolecule Isoelectric point History of the sample (denaturation, degradation)	Rarity of most biomacromolecules Biological sources and physiological state of organisms or cells Bacterial contaminants
	<i>Purity of macromolecules</i> Macromolecular contaminants (odd macromolecules or small molecules) Sequence microheterogeneities (fragmentation by proteases or nucleases) Conformation microheterogeneities (flexible domains, oligomers, aggregation) Batch effects (two batches are not identical)

Crystallization and crystallization strategy

Crystallization is a multiparametric process involving the three classical steps of nucleation, growth, and termination of growth. The tactics to crystallize proteins can be separated into two parts assuming that a significant quantity of pure homogeneous protein is available. First the initial condition must be established where the protein molecule can be displaced into a state of supersaturation followed by an equilibration process that favors minimal nucleation and optimal crystal growth. The methods of crystallizing proteins usually have to be applied over a broad set of conditions adjusting chemical variations such as pH, ionic strength, metal ions or detergents. Physical factors including temperature, gravity, surfaces, viscosity, dielectric properties or vibrations must also be considered during the crystallization process. Biochemical issues also come into play where the purity, modification or aggregations determine the fate of successful crystal growth. In 1999 McPherson (1999) reviewed a comprehensive list of factors effecting protein crystal growth (Table 1). Even when initial crystallization conditions were successfully determined and crystals were produced, their quality may not be sufficient for X-ray diffraction and thus not adequate for subsequent structure determination. Here optimization of the initial screening conditions must be performed to improve the quality of the crystal to enable X-ray analysis. This entails fine ‘tuning’ of any of the variable parameters described above aimed at optimizing the supersaturation state to produce a crystal of the highest quality.

Because of the multiparametric nature of the crystallization process and the diversity of the individual properties

of proteins, it is strongly recommended to collect as much information about the protein of interest as possible. Table 2 lists examples of questions essential to consider prior to any crystallization experiments.

Crystallization experiments

Generally, the protein crystallization experiments proceed in two steps. First step is test screening of the protein solubility considering the precipitants (precipitating agents) and other solution components. Usually in this step, insoluble protein is observed as an amorphous precipitate, which means that the precipitation conditions were too severe to allow crystal growth. In the second optimization step conditions, which gave rise to precipitates in the first step, are modified systematically to allow the advance to insoluble state that is required for the formation of crystal nuclei.

Crystallization results

For the examination of the crystallization trials a stereomicroscope is used. Crystals are usually easy to distinguish from amorphous precipitate. Diffractable crystals are typically single, transparent, of a defined form characterized by planar faces and free of cracks and any defects. Crystals are often birefringent, *i.e.* they appear dark and bright as they rotate under crossed polarizers in the stereomicroscope. Several methods to test whether crystals are protein or salt are now available. These include crush test, dehydration test, and dye binding test, gel electrophoresis and X-ray diffraction.

In the case of growing microcrystals, the seeding techniques can be used to grow the crystal. The seeds

Table 2. Essential questions to consider prior to successful crystallization.

Biology and production	Biochemistry
What is the biological origin of the protein of your interest? Has the gene encoding the protein been sequenced? Has the protein been cloned? How many mg/l of culture can you produce? How many mg of the protein can you obtain in standard purification?	How long does it take to purify one batch of the protein? How do you assess purity of the protein? (SDS-PAGE, HPLC, MASS) What are the principal characteristics of the protein? (Mr, isoelectric point, disulfide bridges, hydrophobicity, etc.) What are the friendly solvents? Is the protein monomeric or oligomeric? What is the stability of the protein vs time, T, or pH?

(microcrystals) are transferred to a new protein-precipitant drop using a streak seeding wand or a crystal transfer syringe, respectively. Seeds provide a template on which further molecules can assemble, and given the proper environment, time, and patience, the seed will enlarge into a crystal.

Experience and reproducibility are guides in making crystallization experiments. Don't forget this and welcome in PX!

Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE: *The Protein Data Bank*. Nucleic Acids Research 28, 235-242 (2000).

Gilliland GL: *A biological macromolecule crystallization database: A basis for a crystallization strategy*. J. Cryst. Growth 90, 50-51 (1998).

Gilliland, GL, Tung M, Blakeslee DM and Ladner J: *The Biological Macromolecule Crystallization Database, Version 3.0: New Features, Data, and the NASA Archive for Protein Crystal Growth Data*. Acta Cryst. D50, 408-413 (1994).

McPherson A: *Crystallization of biological macromolecules*. Cold Spring Harbor Laboratory Press, New York (1999).

E1

CONVENTIONAL CRYSTALLIZATION METHODS AND THEIR MODIFICATIONS: EXERCISES

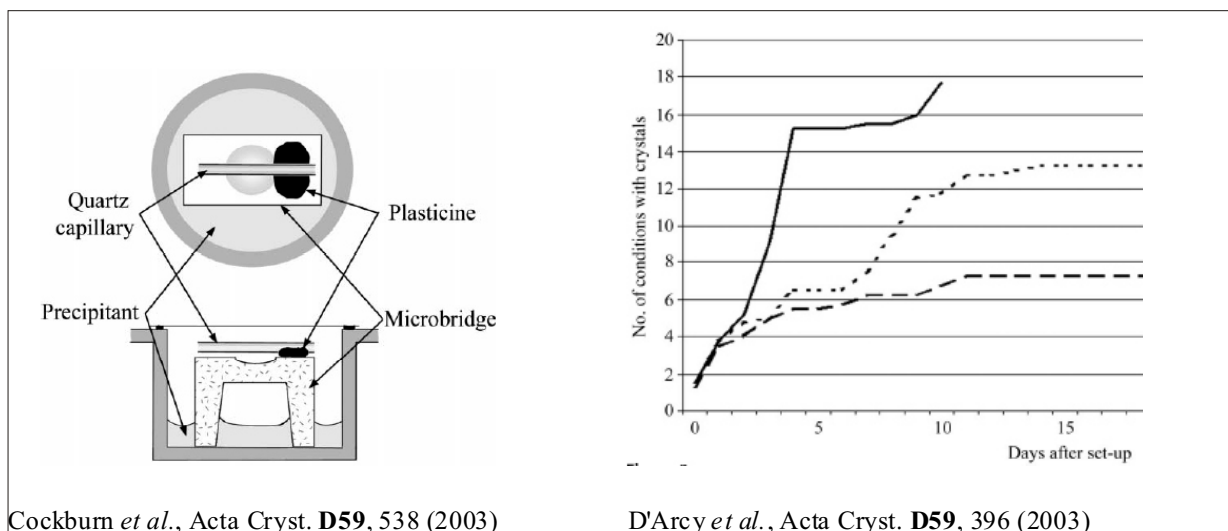
Jeroen R. Mesters

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The most frequently used crystallization method is the vapor diffusion technique. In practice, sitting or hanging droplets (mixture of equal volumes of protein and crystallization solution) of up to a few microliters are placed over a reservoir solution. But also the (micro-)batch method is still being used.

Most important, the experimentation does not stop after the cover slide is placed over the reservoir, so to speak. The classical vapour diffusion experiment can be modified by several pre and post set-up techniques:

During the hands-on practical exercises, the pre and post set-up alterations will be demonstrated and discussed in



Cockburn *et al.*, Acta Cryst. **D59**, 538 (2003)

D'Arcy *et al.*, Acta Cryst. **D59**, 396 (2003)



more detail. Also the standard microbatch trials in Terasaki plates (droplets under paraffin oil) can be manipulated by choosing different oils (mixture of silicon and paraffin oil).
Primers and Links

1. J. Drenth, Principles of Protein X-ray Crystallography (Third Edition, Chapter 16), Springer Science+Business Media LLC.
2. T.M. Bergfors, *protein crystallization strategies, techniques, and tips*, IUL Biotechnology series.
3. A. Ducruix and R. Giegé, *crystallization of nucleic acids and proteins*, Oxford University Press.
4. A. McPherson, *crystallization of biological macromolecules*, Cold Spring Harbor Laboratory Press.
5. S. Iwata, *methods and results in crystallization of membrane proteins*, International University Line Biotechnology series.
6. N. Chayen, Protein Crystallization Strategies for Structural Genomics, IUL Biotechnology Series.

E2

THE SECRET LIFE OF YOUR CRYSTALLIZATION DROP: DO YOU KNOW WHAT REALLY HAPPENS IN YOUR DROPS?

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The chemical composition of a crystallization cocktail determines whether a protein solution can reach a thermodynamically metastable state of supersaturation from which it can return to an equilibrium in which the separating protein-rich phase may be a ordered protein crystal. Whether this state can be actually reached, depends on the kinetics which are much harder to control. Many method-dependent parameters such as drop size, protein-precipitant ratio, or mixing rates, are not known and affect kinetic phenom-

ena such as nucleation and convection. With a digital microscope camera we will observe instant nucleation and crystallization in a protein drop showing convection and diffusion patterns with microcrystals floating and moving through the crystal while they grow. During the growth phase of the crystals we will review again selected problems in crystallization space using the phase diagrams.

E3

CRYSTALLIZATION OF MEMBRANE PROTEINS IN LIPIDIC SYSTEMS

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One of the primary impasses on the route that eventually leads to membrane protein structure through to activity and function is found at the crystal production stage. Diffraction quality crystals, with which an atomic resolution structure is sought, are particularly difficult to prepare currently when a membrane source is used. The reason for this lies partly in our limited ability to manipulate proteins with hydrophobic/amphipathic surfaces that are usually enveloped with membrane lipid. More often than not, the protein gets trapped as an intractable aggregate in its watery course from membrane to crystal. As a result, access to the structure and thus function of tens of thousands of membrane proteins is limited. The health consequences of this are great given the role membrane proteins play in disease; blindness and cystic fibrosis are examples. In contrast, a veritable cornucopia of soluble proteins have offered up

their structure and valuable insight into function, reflecting the relative ease with which they are crystallized. There exists therefore a pressing need for new ways of producing crystals of membrane proteins. In this presentation, I will review the field of membrane protein crystallogenesis with reference to the latest update of the Membrane Protein Data Bank (<http://www.mpdb.tcd.ie>). Emphasis will be placed on crystallization approaches which make use of the lipidic systems. In my talk I will describe these methods and our progress in understanding how they work at a molecular level. The practicalities of implementing these methods in low- and high-throughput modes will be examined. A practical demonstration of the lipidic cubic phase or *in meso* method will be given at the *FEBS Lab Exercises* on Saturday, June 21.



E4

MANUAL SEEDING LAB EXERCISES

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One of the most common questions in macromolecular crystallization is: "I have some small (or ugly, or bad) crystals now, what can I do to improve them?" One powerful optimization tool is seeding. In this technique, crystals grown previously are introduced into new drops. The "seed crystals" can be microcrystals, spherulites, or even other solid phases found by the initial screening experiments. In the new drops, these "seed crystals" become ready-made templates for the protein molecules to accumulate on. This eliminates the need for spontaneous nucleation, which is a kinetically limiting step in crystallogenesis.

There are many ways of performing seeding and this lab exercise will demonstrate two of them, streak-seeding (Stura, 1999) and jab-seeding (Mac Sweeney & D'Arcy, 2009). The exercises demonstrate seeding by manual methods, but seeding can also be done robotically, as will be demonstrated by Patrick Shaw Stewart. The purposes of the experiments are:

- To learn how to transfer seeds by easy, fast, and technically simple manual methods.

- To observe the effects of decreasing protein concentration on the seed growth.
- To observe the effects of diluting the number of seeds
- To compare streak-seeding and jab-seeding as methods for microseed transfer

Stura, E. Chapter 14: Seeding (1999) in *Protein Crystallization*, Ed. T. Bergfors, International University Line, La Jolla, California.

Bergfors, T. (2003) *Seeds to Crystals*. *J. Structural Biol.* Vol. 142, 66-76.

Mac Sweeney, A. and D'Arcy, A. Chapter 6: Seeding (2009) in *Protein Crystallization*, 2nd Edition, T. Bergfors, Ed., International University Line, La Jolla, California.

Seeding Lab Exercises enclosed at the end of book of abstracts on page c49.

E5

PROTEIN CRYSTALLIZATION USING THE GCB

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The Granada Crystallization Box Domino (GCBD®) will help us to implement several experiments using the counterdiffusion technique (Protocols 1 & 2). In this technique the solutions of protein and precipitant agent are set to diffuse against each other resulting in a spatial-temporal gradient of supersaturation along the length of the capillary. Unlike conventional techniques, different supersaturation conditions leading to protein precipitation, nucleation and crystal growth can be obtained consecutively in a single experiment [1-3].

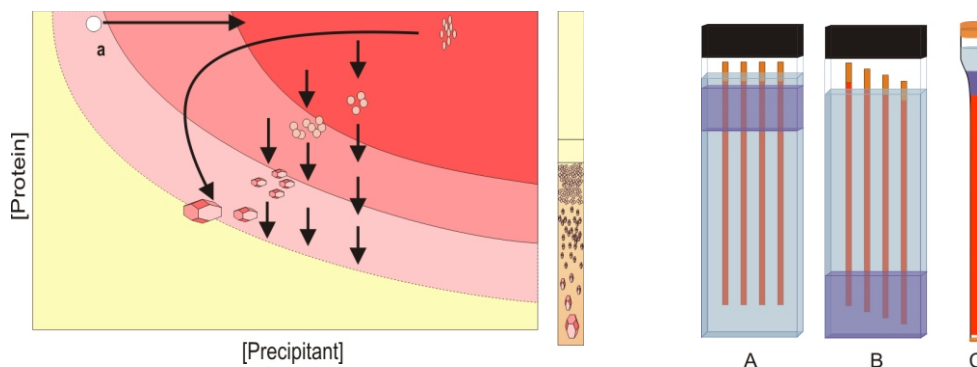
Counter-diffusion has proved its ability to increase protein crystal quality and it is regularly used for optimization after initial crystallization conditions have been found with other techniques. Using the pre-filled GCB Domino® we will test the potential of the counter-diffusion technique for initial crystallization screening in capillaries of 0.1 mm inner diameter (less than 400 nl for capillary length of 50

mm) and optimization in capillaries of 0.2 mm. We will do optimization of lysozyme or thaumatin crystals varying only protein concentration. The effect of protein concentration on crystal density and size should be observed.

Other implementation of the counterdiffusion techniques (crystallization in gel) and its applications will be commented.

1. Garcia-Ruiz, J., M. *Method. Enzymol.* 2003, 368, 130-154.
2. Ng, J. D.; Gavira, J. A.; García-Ruiz, J. M., *Journal of Structural Biology*, 2003, 142, 218-231.
3. Otálora, F.; Gavira, J. A.; Ng, J. D.; García-Ruiz, J. M., *Progress in Biophysics and Molecular Biology*, 2009, 101, 26-37.

Crystallization protocols are enclosed at the end of book of abstracts on page 65.



E6

CRYSTALLIZATION UNDER OIL - UNCONVENTIONAL CRYSTALLIZATION TECHNIQUES FOR SCREENING AND OPTIMISATION

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Dear Course Participants,

I have written down some of the methods that I will talk about in the workshop in the form of exercises, to give you step by step protocols of setting up the experiments.

The methods are mostly for optimization – in cases that you get poor crystals that need improvement. Some can also be applied for screening as mentioned in the specific protocols.

These methods have been successful in yielding diffraction quality crystals of a variety of proteins in cases where conventional methodology failed. I have used lysozyme and trypsin as models in some of the protocols in

order to have examples and results that you can see within a short time, but of course, the aim is to use these techniques with your problem proteins.

There is no magic bullet to solve all the crystallization problems however having a portfolio of different techniques is very helpful.

GOOD LUCK!

Naomi

Exercises 1-7 enclosed at the end of book of abstracts on page c53.

E7

"RANDOM" MICROSEEDING

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One of the lab exercises will be microseeding with random crystallization screens. This method will often give additional hits and reduce the need for crystal optimization. To find out more please see the references and link below.

If you have proteins that you would like to crystallize by microseeding, please bring them. An appropriate amount to bring would be around 60 μ l. We normally set up drops using 0.3 (protein) + 0.2 (reservoir) + 0.1 (seed stock). So 60 μ l of protein would allow you to set up two 96-well plates.

If you're short of protein, however, we can have a go with just 10 μ l.

Please also bring crystals if you have any, to make the seed stock. If your crystals were grown by vapor diffusion, remove the reservoir solutions, cover the drops with

pure paraffin oil, and reseal with tape. This will preserve them during your trip.

If your protein crystallizes quickly, we can set up crystals on the first day (with the robot) and harvest them to make the seed stock on the second day.

We don't recommend transporting the seed stocks at room temperature because the seed crystals may dissolve.

We are providing several commercially available crystallization screens. However, if you know the condition your protein crystallizes in, we recommend to bring your crystallization buffer as well.

Allan D'Arcy, Frederic Villarda, May Marsh. 'An automated microseed matrix-screening method for protein crystallization'. Acta Crystallographica section D63 (2007) 550–554. On-line at <http://scripts.iucr.org/cgi-bin/paper?S0907444907007652>.



Patrick D. Shaw Stewart, Stefan A. Kolek, Richard A. Briggs, Naomi E. Chayen and Peter F.M. Baldock. 'Random Microseeding: A Theoretical and Practical Exploration of Seed Stability and Seeding Techniques for

Successful Protein Crystallization'. Cryst. Growth Des., 2011, 11 (8), pp 3432–3441. On-line at <http://pubs.acs.org/doi/abs/10.1021/cg2001442>
<http://www.douglas.co.uk/mms.htm>.

E8

DYNAMIC LIGHT SCATTERING

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Dynamic Light Scattering (DLS) is an application to determine particle sizes in solution. It can be understood as a method to measure the velocity of the Brownian motion. This velocity is related to the particle size by the Stokes-Einstein equation, which links diffusion velocity, viscosity and temperature of the solvent with particle size (radius). In order to obtain correct values, therefore it is necessary to know these parameters.

Part of the instrument is a laser diode. The laser light is focused in the sample cell. The particles scatter the laser light in all directions. Because the particles are in constant motion, the intensity of the scattered light, as viewed from a certain direction, changes accordingly by alternating con-

structive and destructive interference. The velocity of the resulting intensity fluctuations depends on the Brownian motion and it is correlated with the hydrodynamic radius of the particle. By analyzing these intensity fluctuations, it is possible to determine the particle velocity and hence, via above equation, the desired particle radius. The scattered light is consequently collected and registered by a photo multiplier tube, counting single photons. The output signal of this tube is directed to an autocorrelator, which finally calculates the autocorrelation function (ACF).

User guide for the DLS System and Measurements is enclosed in course materials on pages c61-c64 and c68-c70.

E9

TRACE FLUORESCENT LABELING FOR PROTEIN CRYSTALLIZATION SCREENING

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Fluorescence can be a powerful tool to aid in the crystallization of proteins. Fluorescent labeling methods require removal of unbound probe prior to use of the protein in the screening experiment. For the trace labeling approach the protein is covalently derivatized with a high quantum yield visible wavelength fluorescent probe. The final probe concentrations are typically < 0.25 % of the protein molecules labeled, which has been shown to not affect the crystal nucleation or diffraction quality (Forsythe et al., 2006). The labeled protein is then used in a plate screening experiment in the usual manner. As the most densely packed state for the protein is the crystalline form, then crystals show under fluorescent illumination as the brightest objects in the well. Crystals are clearly seen even when buried in precipitate.

This approach also finds 'hidden' leads, in the form of bright spots, with ~30% of those lead found being optimized to crystals in a single pass optimization trial. Visible fluorescence also enables selection of conditions that bypass interfering substances and greatly reduces materials considerations.

Forsythe, E. L., Achari, A., and Pusey, M. (2006), *Trace Fluorescent Labeling for High-throughput Crystallography*. Acta Cryst. D62:339-346.

Hands-on Practical Outline enclosed at the end of book of abstracts on page c67.



E10

PUBLICATION OF SCIENTIFIC RESULTS WITH EMPHASIS ON CRYSTALLIZATION COMMUNICATIONS

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The demonstrator will be available (1) to consult on all matters related to drafting and publishing of crystallography papers and (2) to demonstrate new web pages designed to help authors. This can include actual editing of draft manuscripts brought by participants. Participants who

would like help with drafts should visit the new web pages for authors at <http://publio.iucr.org>. If you have not registered with the World Directory of Crystallographers, which is required for access, you may do so at <http://www.iucr.org/people/wdc/help/online-services>.

E11

CRYSTALLIZATION IN MICROFLUIDIC CHIPS

Claude Sauter

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Microfluidic devices offer many advantages for crystal growth: they can easily handle nano-volumes of solutions and have been used to miniaturize crystallization methods such as batch, free interface diffusion, counter-diffusion or dialysis. Thus, they provide a very efficient parallelization of crystallization assays for high throughput screening applications. In addition, due to their small cross-section, microfluidic channels and chambers constitute convection-less environments that are *a priori* favorable to the growth of high quality crystals. Finally, some of these microfluidic chips are compatible with the *in situ* crystal analysis by X-ray diffraction. Different microsystems, their function and manufacturing process will be presented during the workshop. Participants will have the opportunity to perform crystallization assays and to grow crystals of model proteins in such devices.

User manual for XZTM crystallization plates enclosed at the end of book of abstracts on page c61.



Examples of crystallization chips with standard SBS footprint

E12

CRYSTALLIZATION OF OWN PROTEINS USING COMMERCIAL SCREENING KITS

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Crystallization of salts and proteins is based on the same physico-chemical principles: Supersaturated solutions are thermodynamically unstable and aim to reach equilibrium by the phase transition and exclusion of the excess of the substance by forming precip-

itate or crystals. The main difference between the crystallization of salts and proteins is based on protein properties, namely their limited stability, high flexibility and tendency to aggregate and denature. Formation of protein crystals is influenced by a large number of parameters which should

be optimized to allow forming and stabilisation of specific intermolecular interactions. Moreover, the limited amounts of highly purified proteins and necessity of large number of crystallization experiments led to designing of special micro-methods. Vapour diffusion using the hanging or sitting drop, batch method using oils and microdialysis are the tra-

ditional and most popular techniques used in protein crystallization. These methods will be used for crystallization of model and own proteins and their advantages will be discussed.

E14**CRYSTAL OBSERVATION, TESTING, HANDLING, MOUNTING AND CRYOCOOLING****Jiří Brynda, Petr Pachi**

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For the observation of crystallization experiments and crystal handling a stereomicroscope usually with a polarizing filter is used. Crystal can usually be distinguished from other matters in the droplet by its edges. Birefringence is also a good sign as it indicates an anisotropy in the material along the viewing axis. Birefringence also helps to distinguish small microcrystals or spherulites from amorphous precipitates. Absence of birefringence in protein crystals is a result of a case when a crystal is viewed along an axis, and in case of cubic space groups.

Protein crystals can be easily distinguished from salt crystals by staining with methylene blue or based on the difference in mechanical properties. Protein crystals are very sensitive and can be easily crushed with a needle. The protein content of the crystal can be analyzed by electrophoresis, but the final test obviously is the diffraction pattern.

Crystals of size and quality (monocrystal with size of at least 0.05mm) suitable for diffraction measurements need to be moved from the crystallization solution and mounted on the diffractometer. Protein crystals must always stay in mother liquor during the whole mounting process and the data collection. For X-ray measurement at room temperature the crystal is placed in the thin wall glass or quartz capillary surrounded with liquid vapors. In most cases, however, the cryocooling is the method preferred for crystal handling, diffraction data collection and storage. In addition to the benefits of eliminated radiation damage and increased resolution, the mounting of crystals is greatly simplified compared to the capillary mounting procedure.

Depending on the mechanical properties of the crystal fibers, spatulas or, most widely fiber loops are used. Using the nylon fiber loop, protein crystal is picked up by swiftly moving the loop alongside the crystal from the crystallization mother liquor.

The crystal is held within the loop by a surface tension and after equilibration in cryoprotective buffer must be cooled to cryogenic conditions as soon as possible. A simple and often effective approach is to flash cool the crystal in a goniostat nitrogen stream right on the X-ray device.

This technique has the added advantage in leaving the crystal in position for immediate analysis and data collection. An alternative method, rapidly plunging the crystal into a liquid cryogen, also offers several advantages. It reduces the time between mounting the crystal and flash cooling, it produces higher cooling rate and results in more even cooling of both sides of loop-mounted sample. Crystal flash cooled in liquid nitrogen must be placed for data collection in the cold gas stream on a goniostat without any warming.

Once a crystal has been successfully cooled to cryogenic temperature it can be in principle stored for indefinite time. This allows cooling and characterizing crystals on a conventional source in the home laboratory and then storing them until synchrotron time becomes available. Dewars that can be used for transport, including shipment by airplane, are available.

Today you can buy the Crystal Handling Kit from Jena Bioscience (CO-150) which can help you to acquire skills in protein crystallization, crystal mounting and data collection.

H. Hope, *Annu. Rev. Biophys. Chem.* 19 (1990) 107.

K. D. Watenpaugh, *Curr. Opin. Str. Biol.* 1 (1991) 1012.

D W. Rodgers, *Structure* 2 (1994) 1135.

Warkentin et al. (2006) *J. Appl. Cryst.* 39: 805.



Crystal Mounting using MiTeGen's MicroMounts™.