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.

Physical	*Chemical*
Temperature Surfaces Methodology/approach to equlibrium Gravity Pressure Time Vibrations/sound/mechanical perturbations Electrostatic/magnetic fields Dielectric properties of the medium Viscosity of the mediurate of equlibrationm 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
Biochemical	*Biological*
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
	Purity of macromolecules
	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)

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?

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 (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).

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

E1

CONVENTIONAL CRYSTALLIZATION METHODS AND THEIR MODIFICATIONS: EXERCISES

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The most frequently used crystallization method is the **vapor diffusion technique.** In practice, sitting or hanging droplets (typically mixture of equal volumes of protein and crystallization solution) of up to a few microliters are placed over a reservoir containing a commercial screen solution. Can this method be optimized to boost the outcome?

Yes, it can! Once the solubility of the protein has been optimized with the help of the Hofmeister series and DLS measurements, simple but effective modifications of the classical hanging or sitting drop vapor diffusion experiments will help you to increase your chances of obtaining crystals.

During the hands-on practical exercises, several of the pre and post set-up alterations will be demonstrated and/or discussed in detail. We will use the Gryphon crystallization robot for setting up 96 well plates and show how to optimize the outcome of these kinds of methodologies and how to prepare it for later ease of analysis.

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.
7. http://www.iobcr.org

sulfate > phosphate > acetate > citrate > chloride > nitrate >> chlorate > thiocyanate
Li+ > Na+ > K+ > NH4+ > Mg2+

Most stabilizing	>>>	**Most destabilizing**
Salting out	>>>	**Salting in**
Lower solubility	>>>	**Higher solubility**

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 phenomena 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

Martin Caffrey

see L3

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.

D’Arcy, A., Bergfors, T., Cowan-Jacob, S.W. and Marsh, M. (2014) Microseed matrix screening for optimization in protein crystallization: what have we learned? *Acta Cryst* **F70**, 1117-1126.

Seeding Lab Exercises enclosed at the end of book of abstracts.

E5

CRYSTALLIZATION UNDER OIL - UNCONVENTIONAL CRYSTALLIZATION TECHNIQUES FOR SCREENING AND OPTIMISATION

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Dear 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.

E6

CONVENTIONAL TECHNIQUES AND CRYSTALLIZATION OF OWN CRYSTALS

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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 precipitate 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 traditional 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.

E7

EVALUATION OF CRYSTALLIZATION TRIALS WITH THE UVEX MICROSCOPE

James Gordon

see L11

E8

RANDOM MICROSEED MATRIX-SCREENING

Patrick Shaw Stewart

see L9

E9

PROTEIN CRYSTALLIZATION USING THE GCB

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In counter-diffusion 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 are obtained consecutively in a single experiment, screening a wide region of the phase diagram [1-4].

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. In this practical we will test the potential of the capillary counter-diffusion technique for initial crystallization screening in capillaries of 0.1 mm inner diameter (less than 300 nl for capillary of 40 mm length) 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. We will also discuss the used of gels in combination with the protein solution.

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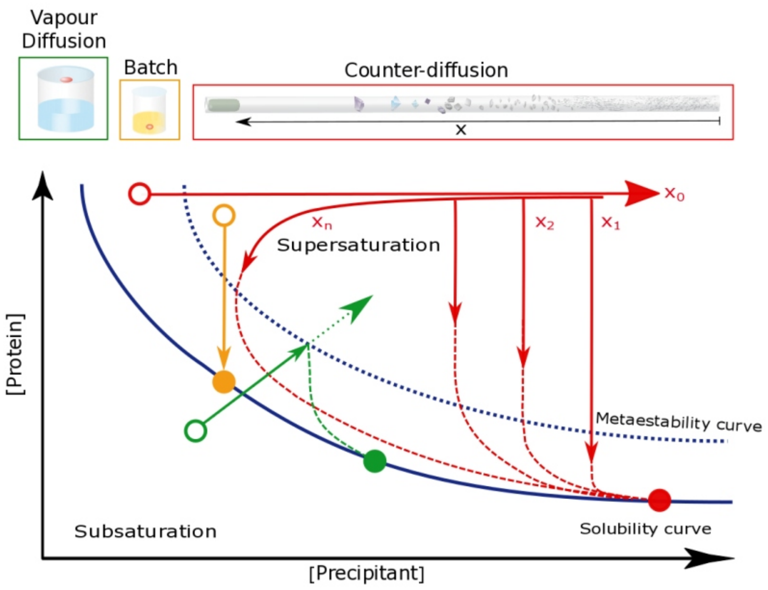
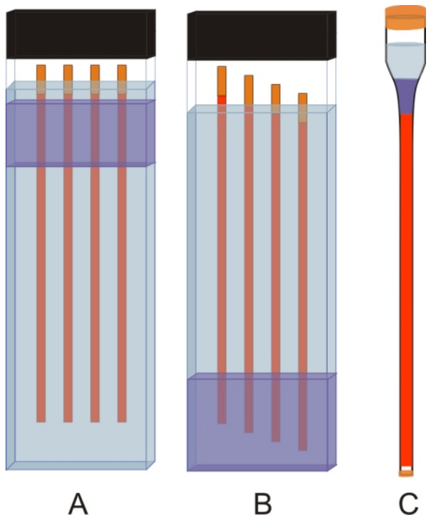
3. Otálora, F.; Gavira, J. A.; Ng, J. D.; García-Ruiz, J. M., *Progress in Biophysics and Molecular Biology,* 2009, 101, 26-37.
4. Gonzalez-Ramirez, et al., CG&D, 2017, 17(12), 6780-6.





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://publbio.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

FROM THE BIOMOLECULE SOLUTION TO ITS 3D STRUCTURE IN A MICROFLUIDIC CHIP

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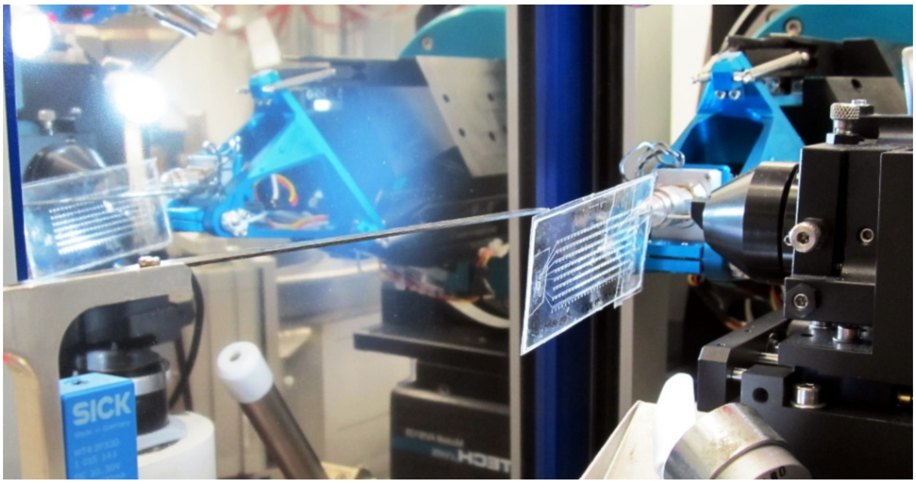
Microfluidic devices offer many advantages for crystal growth: they can easily handle nano-volumes of solutions and have been used to miniaturize crystallization methods such as batch, free interface diffusion, counter-diffusion or dialysis. Thus, they provide a very efficient parallelization of crystallization 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 have been designed to be compatible with the *in situ* crystal analysis by X-ray diffraction (Pinker et al. 2013).

Different microsystems, their manufacturing processes and function will be presented during practical work. The participants will have the opportunity to crystallize a model protein in a ChipX microsystem, which implements the method of counter-diffusion. In this experiment, the protein sample (3 µl) is first loaded in the chip inlets to fill eight microchannels (V=300 nl). Then, 5 µl of crystallization cocktail (crystallant solution) are loaded in the reservoirs at the other extremity. The crystallants diffuse into the channels to trigger crystallization.

Pinker F, Brun M, Morin P, Deman AL, Chateaux JF, Oliéric V, Stirnimann C, Lorber B, Terrier N, Ferrigno R & Sauter C. ChipX: a novel microfluidic chip for counter-diffusion crystallization of biomolecules and in situ crystal analysis at room temperature. Crystal Growth & Design (2013), 13: 3333–3340.



E12

PRACTICAL CONSIDERATIONS FOR THE CRYSTALLIZATION OF PROTEIN-NUCLEIC ACID COMPLEXES

Christian Biertümpfel

see L22

E13

DYNAMIC LIGHT SCATTERING

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After purification, a biological macromolecular might not be in the desired aggregation state or a biologically relevant complex of molecules might not have formed. Some buffer conditions might support complex formation apart from its natural environment but often such conditions are unknown. With the *in situ* Dynamic light scattering (*in situ* DLS) [1,2] method, smallest sample volumes and a huge number of samples become accessible. It is a non-invasive technique that can provide detailed information about the state of aggregation and the absolute size of a target protein. Application of this advanced DLS method provides a time, material and manpower efficient way to qualify samples for subsequent structure determination methods e.g. single particle cryo-EM, SAXS, NMR and crystallization.

The promoted approach ensures an appropriate particle size and size distribution on a sample carrier e.g. cryo-EM grid or NMR-tube, based on radius distribution measurements in 500 nl aliquots shortly before loading the sample on such a sample carrier. The droplet is stored in a standard “microbatch” plate under paraffin oil to prevent evaporation. Particle size and aggregation state determination is conducted directly in wells. The determined radius distribution in this aliquot represents what you’ll find on the grid or in your NMR tube if the sample transfer and freezing procedure worked as intended. If the particles determined by DLS show sizes of oligomers or aggregation, this is what later will be find on the grid when applying e.g. cryo-EM.

For identification of conditions supporting complex formation and preventing aggregation, a screening of various buffer conditions might be applied. This is achieved by mixing a sample with a specially designed set of buffers and additives systematically on sample aliquots as small as 100 nl. This approach results in many conditions (usually 96) far exceeding the capacities of standard DLS and most sample carriers. The aim is to identify the (few) conditions that stabilize the desired macromolecular complex. Most of the applied conditions have a negative effect on the sample, leading to a very inefficient usage of cryo-EM, NMR, SAXS and crystallization when all samples would be investigated. Here *in situ* DLS is a key technology to select conditions as well.

1. SpectroLight 600 an *in situ* DLS system, see www.xtal-concepts.de/.
2. J. Birch, D. Axford, J. Foadi, A. Meyer, A. Eckhardt, Y. Thielmann, I. Moraes, in press (2018).

User guides for the DLS System and SPL600 sytem are enclosed at the end of book of abstracts.

E14

TRACE FLUORESCENT LABELING FOR PROTEIN CRYSTALLIZATION SCREENING

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The use of trace fluorescent labeling can be a powerful diagnostic when evaluating the results from one’s crystallization screening plates. To use the method, the protein must first be labeled with a reactive fluorescent probe. A procedure has been developed that limits the amount of protein that is derivatized to the desired ~0.1 to 0.5 % levels and that can be comfortably carried out within 15 minutes. In essence, the process it to prepare a solution having a slightly higher concentration of your protein than will be used for the crystallization experiments. An aliquot of this, typically 10% of the volume, is removed and buffer exchanged to put the protein into a suitable reaction buffer. This is reacted with a small volume of the fluorescent dye, then buffer exchanged back to the original crystallization buffer conditions. The derivatized protein is then added back to the original solution and the volume adjusted to give the labeled protein at the final desired total protein crystallization concentration. Once the plate has been set up, one needs a method for fluorescently viewing the results. We have developed a low-cost imaging method based on using the camera on a smart-phone that can be implemented for as little as $25 - $35 [1]. The demonstration will show cover how to carry out the fluorescent labeling procedure and how to do the fluorescent imaging, as well as how and where to obtain and/or prepare the materials for both.

1. Crissy L. Tarver and Marc Pusey (2017). *A low-cost method for visible fluorescence imaging*. Acta Cryst. F 73:657-663.

E15

HOW TO PERFORM DIFFRACTION EXPERIMENT?

Vernon Smith

see L28

E16

TUTORIAL FOR SINGLE PARTICLE CRYO-EM DATA PROCESSING

Eva Cunha

Structural Biology and Drug Discovery Group, Norwegian Centre for Molecular Medicine (NCMM), Nordic EMBL Partnership, University of Oslo

We will cover the recently revolutionized technique of Cryo-EM whose leading developers were awarded the Nobel prize in Chemistry in 2017. The recent “resolution revolution” in cryo-EM, driven by developments in instrumentation such as direct detectors and the Volta phase plate, coupled with major improvements in data analysis, has put Cryo-EM at the forefront of structural biology, a novel method for attaining high-resolution models of difficult targets (low yield, moderately flexible). Since 2015, several records have been achieved with the highest resolution structure reported so far for glutamate dehydrogenase (soluble protein, 1.8 Å), anthrax toxin (membrane protein, 2.9 Å) and hemoglobin (only 64 kDa, 3 Å). Another advantage is the possibility of studying glycosylated proteins as well as the option of reconstituting membrane proteins into nanodiscs, providing a protein-enclosed lipid bilayer and thus a more native-like environment as opposed to detergents.

We will cover the practical aspects of data processing for single particle cryo-EM. Using the Relion pipeline, we will go over CTF estimation; manual and automated particle picking, particle extraction, 2D classification, initial model generation and 3D classification.

E17

ADVANCED AND NON-CONVENTIONAL METHODS FOR CONTROLLING THE SIZE AND THE SHAPE OF PROTEIN CRYSTALS

Abel Moreno

see L20

E18

CRYSTAL OBSERVATION, TESTING, HANDLING, MOUNTING AND CRYOCOOLING

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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.

Crystal Mounting using MiTeGen's MicroMountsTM.