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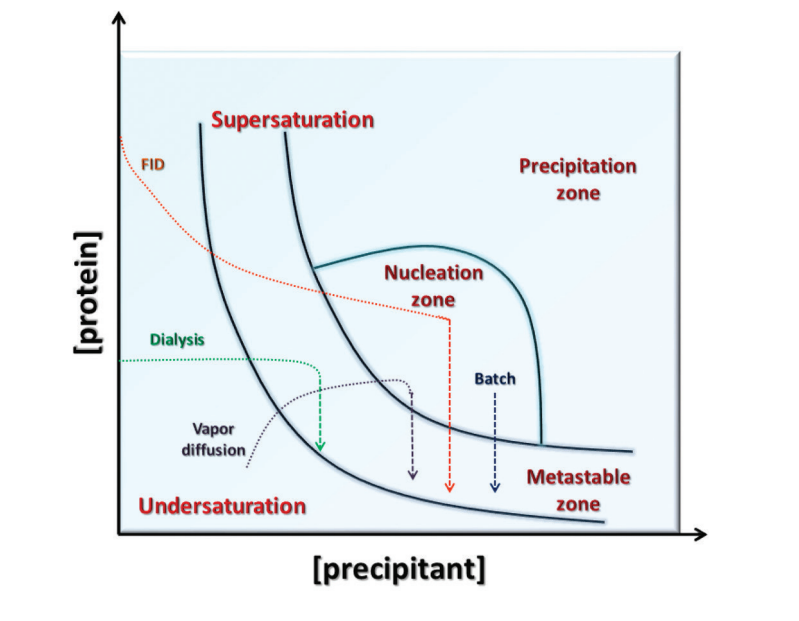
Biomacromolecular Crystallization

Crystallization of biomacromolecules (proteins, nucleic acids) and their complexes is a well-established method being employed for almost 50 years in 3D structure determination. Regularly ordered molecules (crystal) eradiated by X-ray beam give a diffraction pattern that can be further processed in order to determine electron density map of the molecule and subsequently the position of individual atoms.

Unlike inorganic and small organic molecules, biomacromolecules are generally less stable, what lead to development of various gentle methods of their crystallization. The common sense of all methods is to follow a trajectory in protein-precipitant phase diagram that results in formation of small number of "macroscopic" crystals (typical size of 20-500 um). These methods include batch, free interface diffusion, dialysis and the most widely used vapor diffusion technique. Recently, the methods for crystallization of membrane proteins were also developed.

Macromolecular crystallization is a complex process that can be affected (in both positive and negative way) by macromolecule concentration, composition and concentration of precipitant solution, technique applied, size/volume of the mixed drop, temperature, impurities, handling, etc.



Protein-precipitant phase diagram (Krauss, 2013 Int. J. Mol. Sci.)

Biomacromolecular Crystallization can be used for (mainly together with X-ray diffraction):

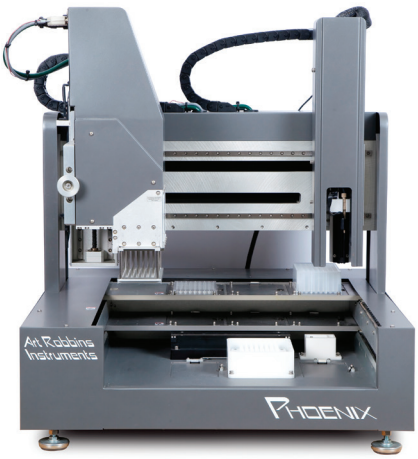
- Determination of 3D structure – no principal size limit is applied (both NaCl and ribosome were crystallized and their structure solved).
- Identification of conserved and flexible regions.
- Analysis of structural changes caused by mutations.
- Identification of ligand interaction, architecture and number of binding sites.
- Determination of oligomeric state of the protein.
- Protein identification and purification (not commonly used).

Technical equipment

Crystallization screening – 96well plates set-up: **Phoenix** (Art Robins)

Features:

- 96channel syringe head for contact pipetting into 96 or 384well plates
- Non-contact single-channel nano-dispenser
- Thermostated sample rack for sensitive samples



Advanced crystallization screening – 96well plates set-up: **Mosquito** (ttp labtech)

Features:

- Single-use micropipettes for contact dispensing
- 25 nl – 1.2 ul pipetting volume
- Suitable for microseeding
- LCP module for Liquid cubic phase set-up

Optimization of crystallization – 96well plates preparation: **Dragonfly** (ttp labtech)

Features:

- 1D-3D gradient preparation for 96well plates mixing up to 5 different solutions
- Non-contact pipettes – no risk of cross-contamination
- Minimal volume of single droplet addition – 0.5 ul

Temperature optimization of crystallization: **TG-40** (Centeo)

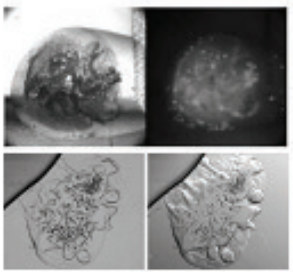
Features:

- Theoretical temperature control range: 4° - 50°C (± 0.05°C)
- Up to 40 separate experiments at once (5 temperatures x 8 wells)
- For detailed information, see the separate instrument profile card

Plate storage and inspection: **Minstrel HT-UV + Gallery HT** (Rigaku)

Features:

- Standard SBS, low-profile SBS and Linbro plates storage at 4°C and 20°C
- Visible light and UV imaging
- For detailed information, see the separate instrument profile card



Operational mode:

Standard services (screening plate set-up, optimization plate set-up, plate storage and imaging) are performed by Core Facility staff only. Images are available online via authorized website (http://147.251.155.180/). Advanced techniques (24well plate optimization, dialysis and counter diffusion techniques, membrane protein crystallization, etc.) are performed by Core Facility staf and/or users themselves after training (depends on specific demands of each technique).

Established methodologies and provided services:

- **Standard screen set-up** – 96well sitting-drop plate with 35 ul reservoir and protein:precipitant drops in three ratios (200+100 nl, 100+100 nl, 100+200 nl). Number of crystallization screens available (See the table below. For up to date list, please contact the CF staff.)
- **Non-standard screen set-up** – 96well sitting-drop plate with modified parameters. 1-96 wells with 1-3 drops each may be used with decreased/increased protein:precipitant ratio and volume (minimal volume of 25+25 nl, maximal total volume of 1 ul may be used).
- **Additive screen set-up** – 96well sitting drop plate with user-supplied precipitant enriched by available 96 additives (one per well).
- **Detergent screen set-up** – 96well sitting drop plate with user-supplied precipitant enriched by available 96 detergents (one per well).
- **96well optimization of crystallization** – 1D-3D gradient of precipitants in 96 well plate, typically followed by (non-)standard set-up. Users are kindly asked to provide individual stock solutions.
- **Advanced screening techniques** – available 96well screens may be set up for other than sitting-drop vapor diffusion method as well. This includes: hanging drop vapor diffusion, sitting or hanging drop vapor diffusion combined with under-oil approach, "batch method", dialysis and liquid cubic phase crystallization.
- **Advanced techniques** – Core Facility offer consumables and equipment for various other crystallization experiments including: pre-crystallization concentration test, 24well plate (Linbro®) crystallization using hanging drop and sitting drop vapor diffusion or dialysis techniques, crystallization in capillaries (free interface diffusion), micro- and macro-seeding, application of Naomi nucleants, Izit dye for discrimination of protein and salt crystals, heavy atom derivatization (almost 80 compounds available)
- Crystallization **plate storage** at stable temperature.
- Crystallization drop **imaging** in visible and UV following regular time scheme.
- CrystalTrak software interface and all images related to user samples are available for the users through secured online access (http://147.251.155.180/) 24/7.

Crystallization kits available at BIC Core Facility

Qiagen-Nextal	
Classics Suite	B
Classics II Suite	B
Classics Lite Suite	B
PACT Suite	B
AmSO4 Suite	
ComPAS Suite	
MPD Suite	
PEGs Suite	
PEGs II Suite	
pH Clear Suite	
pH Clear II Suite	
Protein Complex Suite	
Cryos Suite	S

Molecular Dimensions	
Clear Strategy I	B
Clear Strategy II	B
JCSG-plus	B
SG1	B
Structure I+II	B
Midas	
Morpheus	
Morpheus II	
PGA	
Helix	N
MemMeso	M
MemStart + MemSys	M
MemAdvantage	M, S
Stura Footprint Screen + MacroSol	S
The Calixar 2.0 additive kit	M, S, (24)
MemMagic Bicelle Screen kit	M, S, (4)

Hampton Research	
Natrix 1+2	N
Additive Screen	S
Detergent Screen	S
Nucleic Acid Mini Screen	N, (24)
Ionic Liquid Screen	S, (24)

Jena Biosciences	
JBScreen Classic HT II	B

B – basic screen, M – designed for membrane proteins, N – designed for nucleic acids, S – screen for special purposes, (XX) – non-96well screen (number of conditions)

Heavy atom kits available at BIC Core Facility (+ number of compounds)

Hampton Research	
Heavy atom screen M1	19
Heavy atom screen M2	19
Heavy atom screen Hg	15
Heavy atom screen Pt	12

Jena	
JBS Magic triangle	1
Lanthanide phasing kit	10

Sample requirements - importance of sample preparation

- Generally protein concentrations of 5-20 mg/ml are considered as suitable for crystallization trial. Cases with successful crystallization of samples far below and above this concentration range are reported.
- For standard 96well set-up (3 drops each well), at least 50 ul of the sample is required per plate plus at least 20 ul excess for liquid handling reasons per sample. For non-standard set-ups the volume may differ markedly – discuss these cases with CF staff.
- Additive screen, detergent screen and all types of optimization set ups require basic precipitant solution (individual component stock solutions for optimization) to be supplied by users themselves. At least 4 ml of the precipitant solution is needed per plate.
- UV imaging is used to differentiate protein (shiny) vs. salt (dark). Proteins that lack aromatic residues (especially Trp) give poor (or none) UV signal.
- Should your protein be colourful, colour-imaging could be performed (on demand only).
- All standard crystallization plates set-up by Core Facility BIC are suitable for storage and inspection in Rigaku Minstrel HT-UV. If you want to store/image your own plates, please discuss the compatibility with the CF staff.

Less experienced users are strongly advised to discuss the details of their experiment with Core Facility staff prior to booking the service. Any non-standard applications, special settings and other issues need to be discussed with the Core Facility members in every case.

Contacts

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Instrument Location:
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