High-Throughput Crystallization @ UZH

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Abstract:

- The high-throughput protein crystallization center at UZH (PCC) has the capacity to screen up to 20,000 crystallization conditions per day.
- Crystallization experiments are set up at 20°C with the Phoenix liquid handler (ARI).
- Crystallization experiments using temperature sensitive protein samples are routinely setup at 4°C using the Gryphon-LCP (ARI).
- The researcher can select any drop volumes between 50 nl and 800 nl for the vapor diffusion crystallization experiments.
- In addition to VD crystallization experiments, PCC offers LCP-, seeding-, counter diffusion- and under-oil crystallization techniques.
- Two new Rock Imager (Formulatrix) incubation and imaging systems will replace the two CrystalFarms (Brooks) at 4°C and 20°C.
- The researcher can monitor and analyze the crystallization experiments through the web.
- OptiMatrixMaker (Protein BioSolutions) does allow the researcher to rapidly device an optimization screen for crystal refinement.
- The UV/fluorescence microscope (JAN Scientific) allows the recognition of small protein crystals and distinguishes easily from inorganic crystals.
- The PX Scanner (Agilent) allows protein crystal testing for *in situ* diffraction.

Organization of the High-Throughput Crystallization Center

Screen preparation

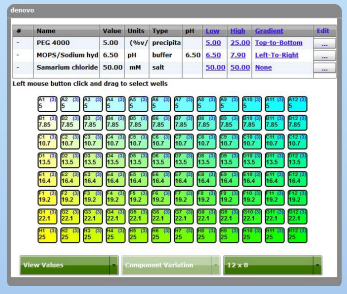
Aquarius + Lissy - Liquid Handler
- Reformatting commercial screens into a 96-well format
- Prepares gradients for grid screens
- Setting up crystallization plates
- Volume range: 20-1000µl

Crystallization screens

- Crystal Screen, Peg/Ion, SilverBullet, Silverbullet Bio, JBScreen Plus HTS. — Hampton Research
- Clear Strategy I&II, HELIX, Structure Screen I&II, MIDAS, MemStart, MemSys, MemGold. — Molecular Dimensions
- Low Ionic, Membrane, CryoScreen, JCSG+, PACT, MbClass II, ComPAS, Anion/Cation Suite. — Fluka / Qiagen
- PEG 400 – 20'000, 5 - 30% + salt additions $(NH_4)_2SO_4$, 15 - 80%.
- "Opti Matrix Maker" custom screen for refinement. — "In-house"

Optimization Screen

"Opti Matrix Maker" custom screen for crystal refinement using the online EZ-Screen program tool.



Opti Matrix Maker (Protein BioSolutions)

Crystallization techniques offered:

- Vapour diffusion
- Lipidic cubic phase + bicelle
- Matrix seeding
- Counter diffusion (CrystalHarp™)
- Under-oil crystallization

Plate setup

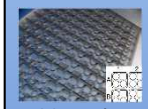
Phoenix + Gryphon-LCP (ARI)

- 96+3/4 channel dispenser
- 4°C or 20°C, sitting drop VD; LCP; Matrix seeding.
- drop volumes: 50 - 800nl
- 70 plates/day

Protein crystallization experiments are scheduled days in advance by the researcher. This allows PCC to setup the experiments immediately after sample delivery.
Experiments are tailored to the researchers specifications and the requirement of the protein such as dispensing temperature, drop ratios and plate type.

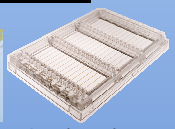
Crystallization plates

SWISSCI

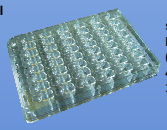


MRC-3 Lens plate (UVP and PS)
3x96 VD-experiments
5 µl round well

LCP-Plate
96 LCP-experiments
100 nl CP + 400nl ML



CrystalHarp-Plate
48 CD-experiments



scale-up:
MRC MAXI-Plate
(UVP and PS)
48 VD-experiments
10 µl round well

Required protein sample volume

Drop volume [nl] in 96 wells	Phoenix or Gryphon-LCP
50 nl	8 µl
100 nl	12 µl
200 nl	22 µl
800 nl	80 µl (dead volume 10 µl)

Research groups interested in this protein crystallization service can contact PCC directly by mail: **xtal@bioc.uzh.ch**

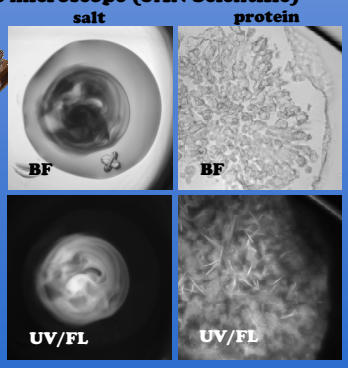
Incubation & Imaging

CrystalFarm (Brooks)
- Capacity of 400 SBS micro plates
- Multiple plate formats
- Incubation temperature 4°C + 20ºC
- 1 MP color CCD camera with an 11:1 zoom and 1µm optical resolution

Rock Imager (Formulatrix)
- Capacity of 1000 SBS micro plates
- UV-imaging for protein verification
- Peltier-controlled incubation temperature 4°C + 20ºC
- 5 MP color CCD camera with an 12:1 zoom and 0.83 µm optical resolution

Protein crystal verification

UV-Fluorescence microscope (JAN Scientific)



PX scanner for *in situ* diffraction

Conclusion:

- The high-throughput protein crystallization center at UZH (PCC) has the capacity for setting over 70,000 crystallization experiments per week.
- For standard drop volume ratios (100:100;100:200;200:100nl) 41µl protein solution is needed for one crystallization plate with 288 VD-experiments.
- Routine setup include vapor diffusion, lipidic cubic phase, bicelle, matrix seeding, free interface diffusion and "under-oil" crystallization techniques.
- Plates are routinely set up at 20°C or 4°C, independent of the incubation temperature of 20°C or 4°C.
- Scale-up experiments are successful and reproducible.
- The crystallization service is open to all academic research groups in- and outside UZH.
- The facility also extends its service to research groups in private industry.

Universität Zürich[UZH] | ETH Eidgenössische Technische Hochschule Zürich Swiss Federal Institute of Technology Zurich | Protein Production Platform P-CUBE | ZCMSM Zurich Center for Molecular Structure and Mechanism | STRUCTURAL BIOLOGY National Center of Competence in Research