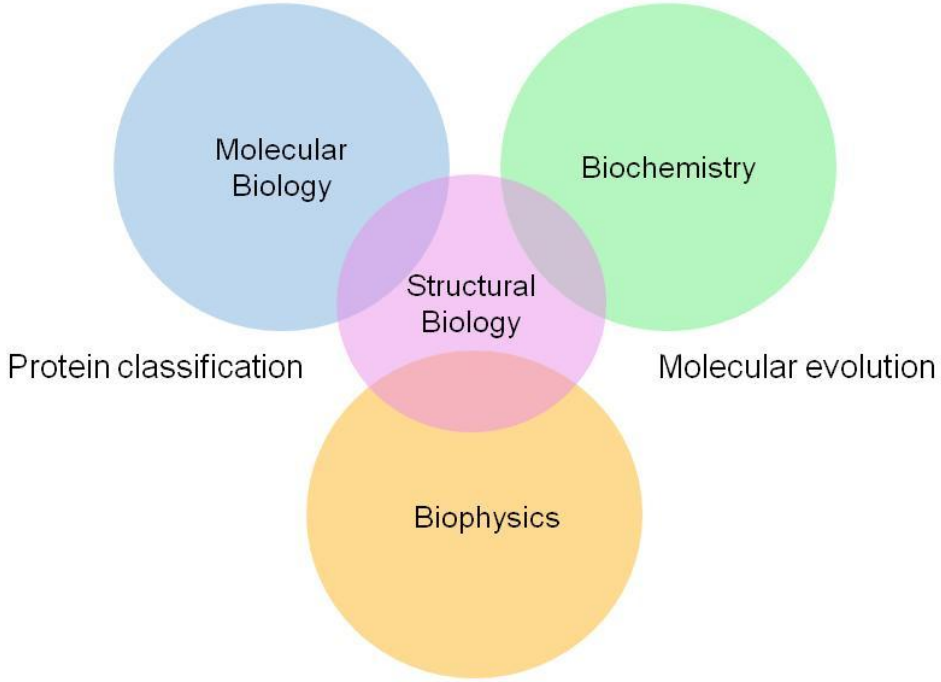
Αρχές κρυστάλλωσης μακρομορίων

Ειρήνη Μαργιωλάκη

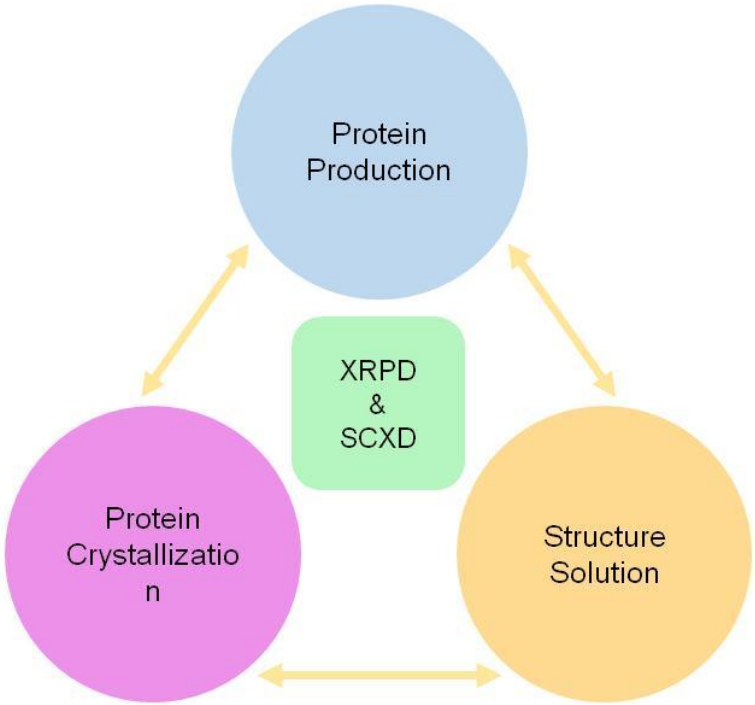
https://sites.google.com/view/margiolaki-biology-upat/home

Structural Biology

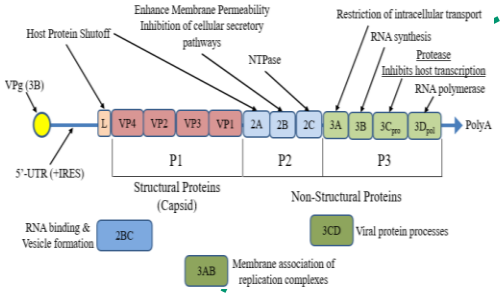
Understanding of cellular mechanisms
from enzymology to macromolecular machines

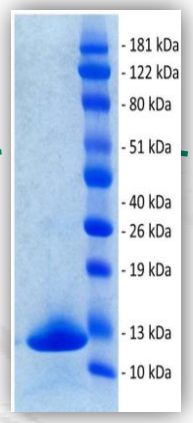


Main method of macromolecular structure studies:
X-ray Crystallography



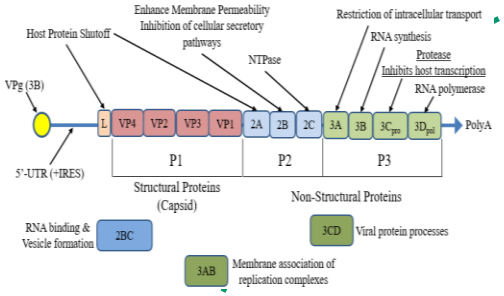
Workflow

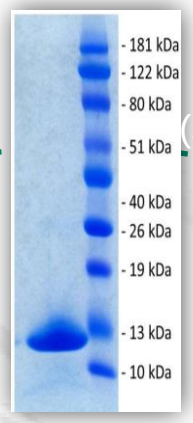

Host Protein Shutoff
Enhance Membrane Permeability
Inhibition of cellular secretory pathways
NTPase
Restriction of intracellular transport
RNA synthesis
Protease
Inhibits host transcription
RNA polymerase
VPg (3B)
L
VP4
VP2
VP3
VP1
2A
2B
2C
3A
3B
3C$_{pro}$
3D$_{pol}$
PolyA
5'-UTR (+IRES)
P1
P2
P3
Structural Proteins (Capsid)
Non-Structural Proteins
RNA binding & Vesicle formation
2BC
3CD
Viral protein processes
3AB
Membrane association of replication complexes

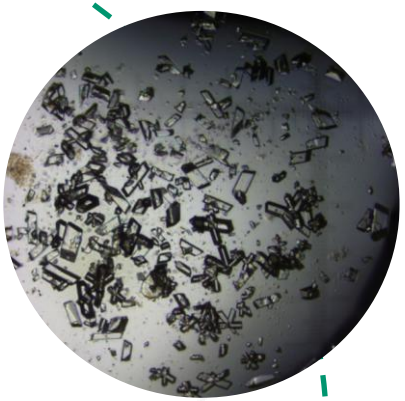

181 kDa
122 kDa
80 kDa
51 kDa
40 kDa
26 kDa
19 kDa
13 kDa
10 kDa

Protein Production
XRPD & SCXD
Structure Solution
Protein Crystal Production

Workflow







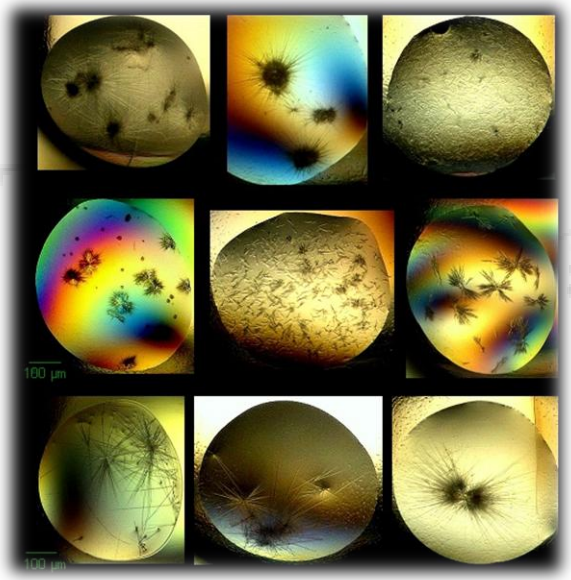
Protein Production

XRPD & SCXD

Structure Solution

Protein Crystal Production

Crystals of Biological Macromolecules



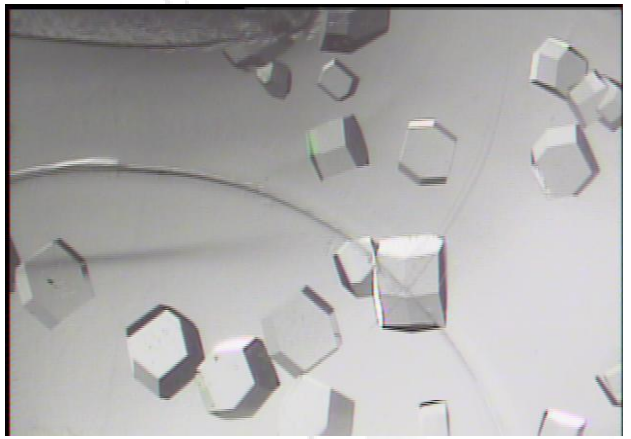
Κρύσταλλοι

Είναι μια φάση της στερεάς κατάστασης στην οποία τα μόρια (ή άτομα) που αποτελούν τον κρύσταλλο είναι περιοδικά διευθετημένα στον τρισδιάστατο χώρο.
Η περιοδική αυτή επανάληψη επιτυγχάνεται μέσω της απλής μετάθεσης (δηλαδή απλή μετακίνηση χωρίς περιστροφή) ενός επαναλαμβανόμενου μοτίβου.

Κάθε περιοδική διευθέτηση δεν είναι κρύσταλλος (π.χ. υγροί κρύσταλλοι, δομή του DNA).

Πως κρυσταλλώνονται οι πρωτεϊνες

- Συνήθως με μεγάλη δυσκολία!
- Για διαλυτές πρωτεϊνες:
- Διάλυση και καθύζηση
- (dissolve and then precipitate)
- Κρίσιμες παράμετροι: pH, ionic strength, precipitant



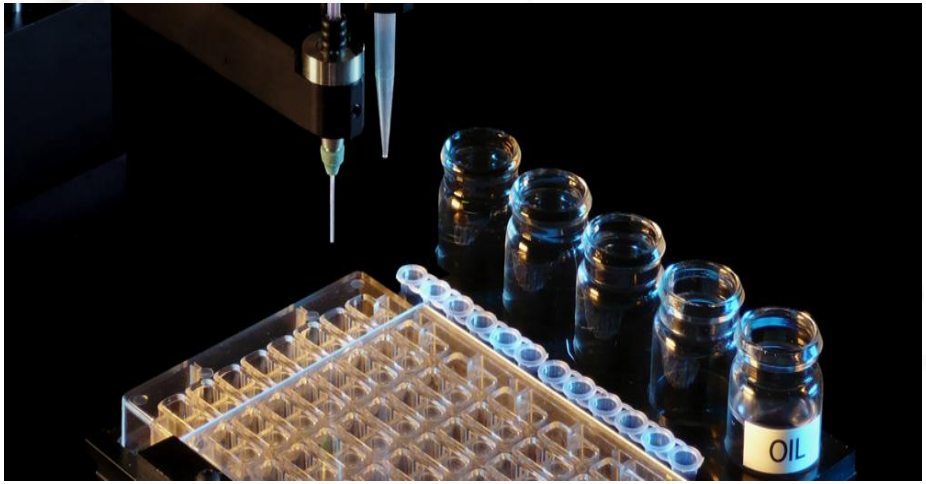
- Συνήθως κρυστάλλωση σε μικρές σταγόνες

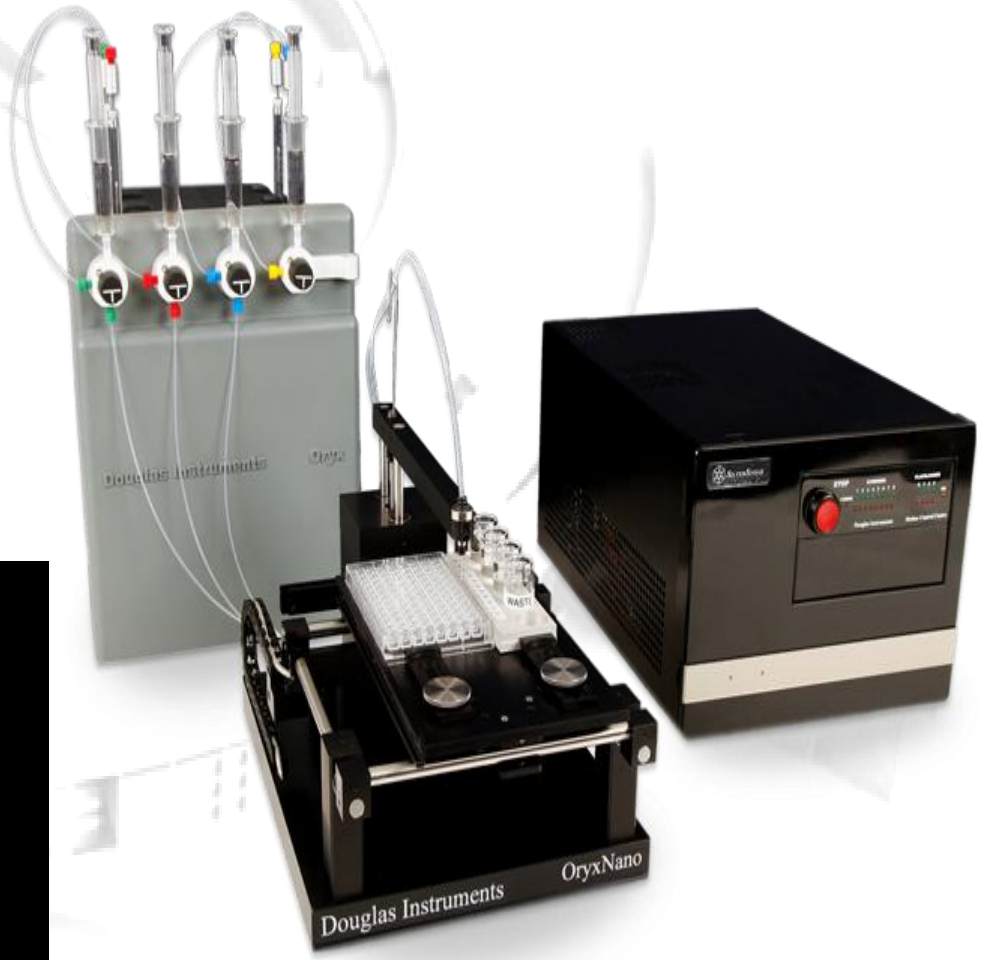
- screens + robots

Crystallization robot

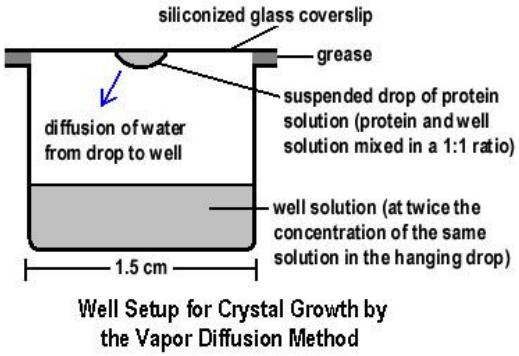
Douglas Instruments
OryxNano

- Minimal amount of sample used
- Accurate and reproducible pipetting
- Quicker experiments

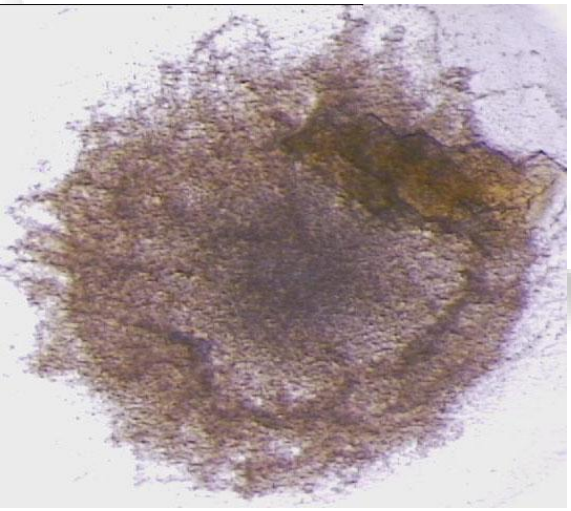


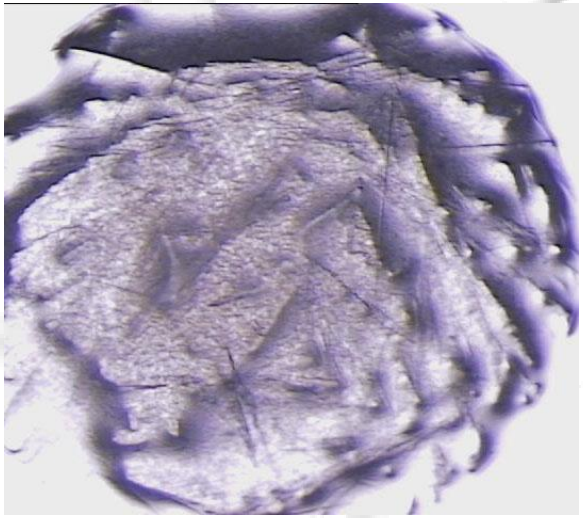


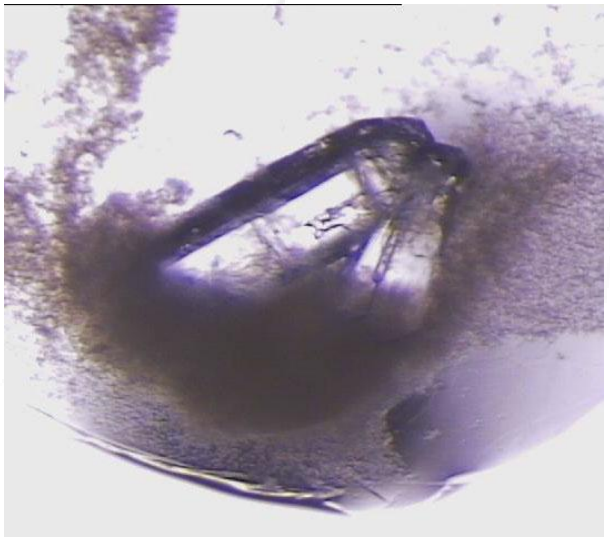
Κρυστάλλωση Πρωτεϊνών



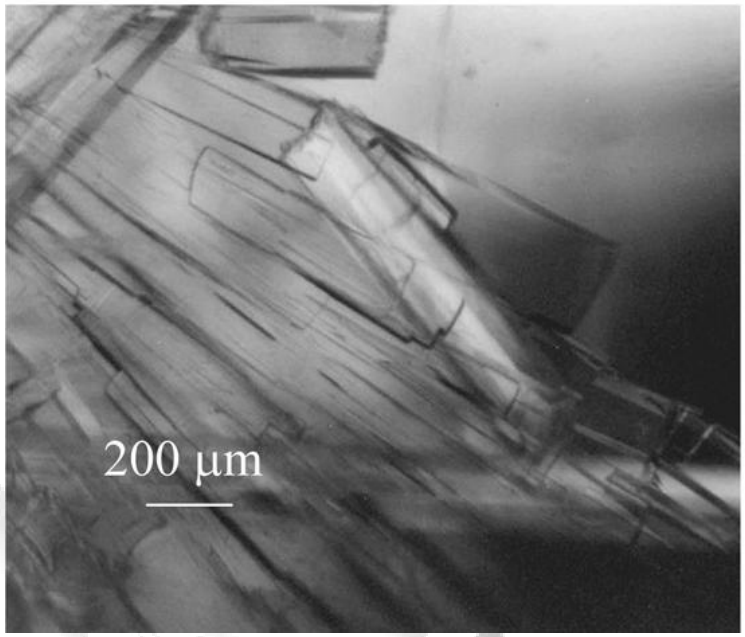
Well Setup for Crystal Growth by the Vapor Diffusion Method

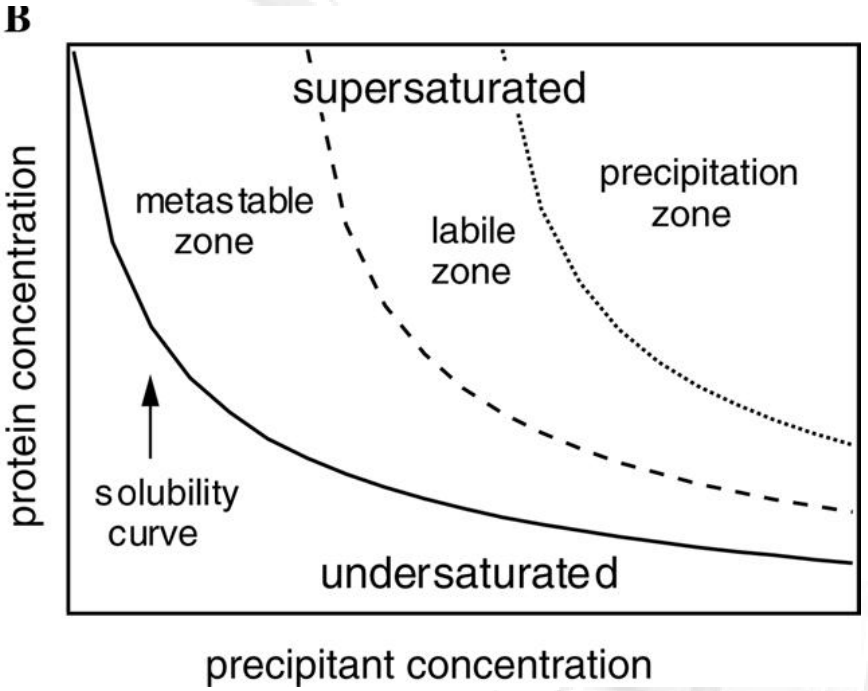






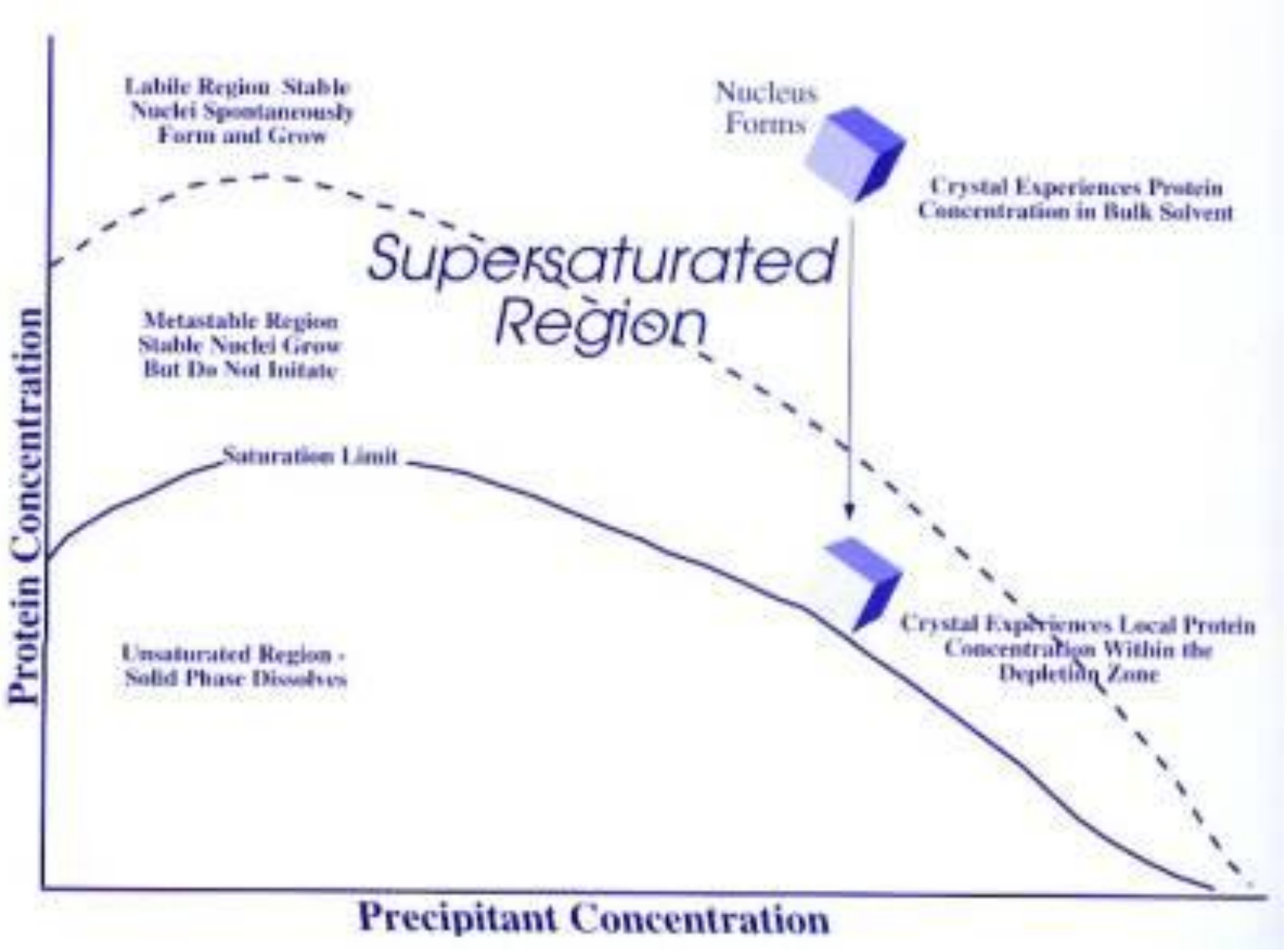
Protein crystallisation & phase diagrams

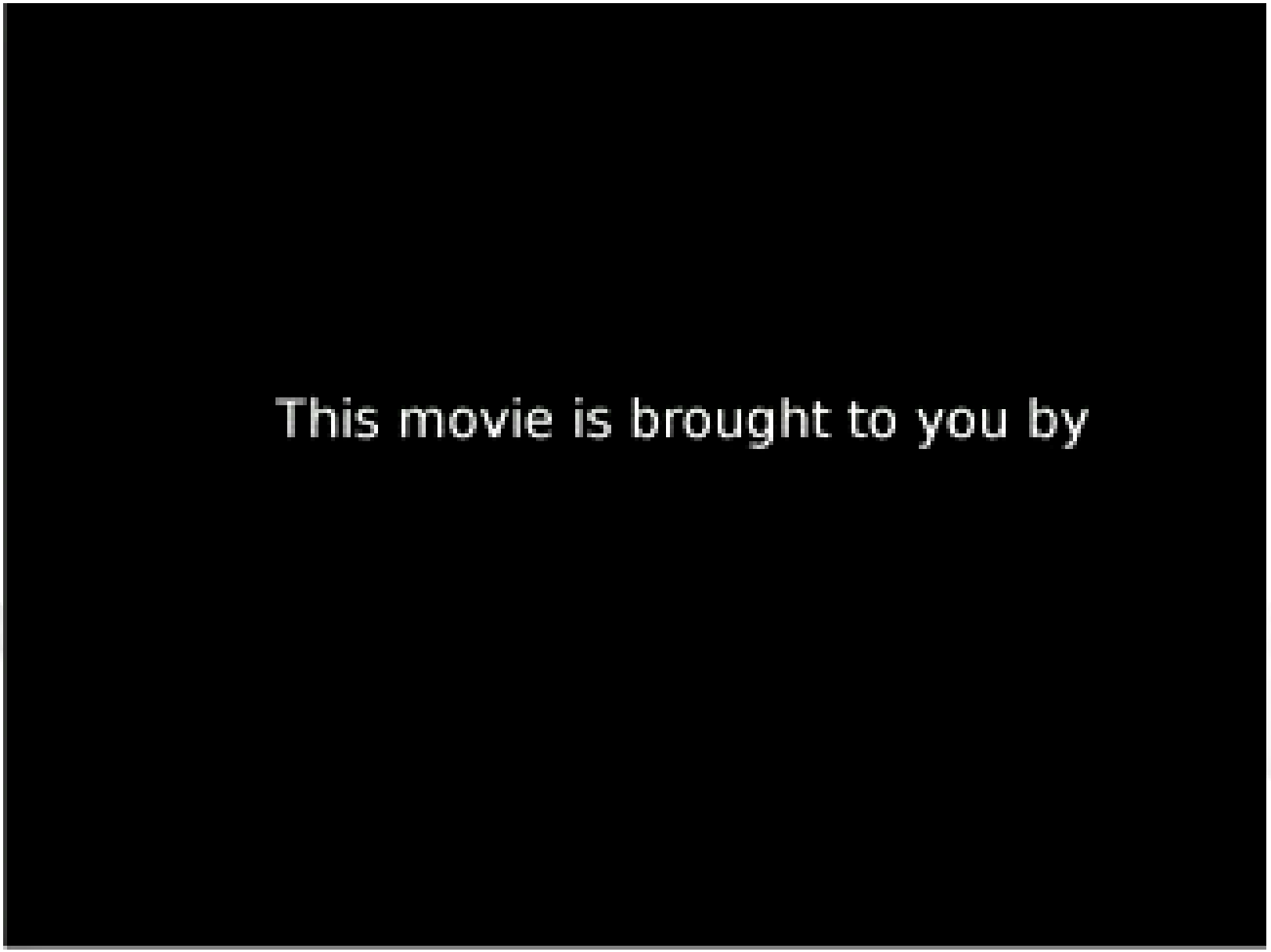




(A) Crystals of wild-type bovine B crystallin.
(B) A schematic phase diagram showing the solubility of a protein in solution as a function of the concentration of the precipitant present.

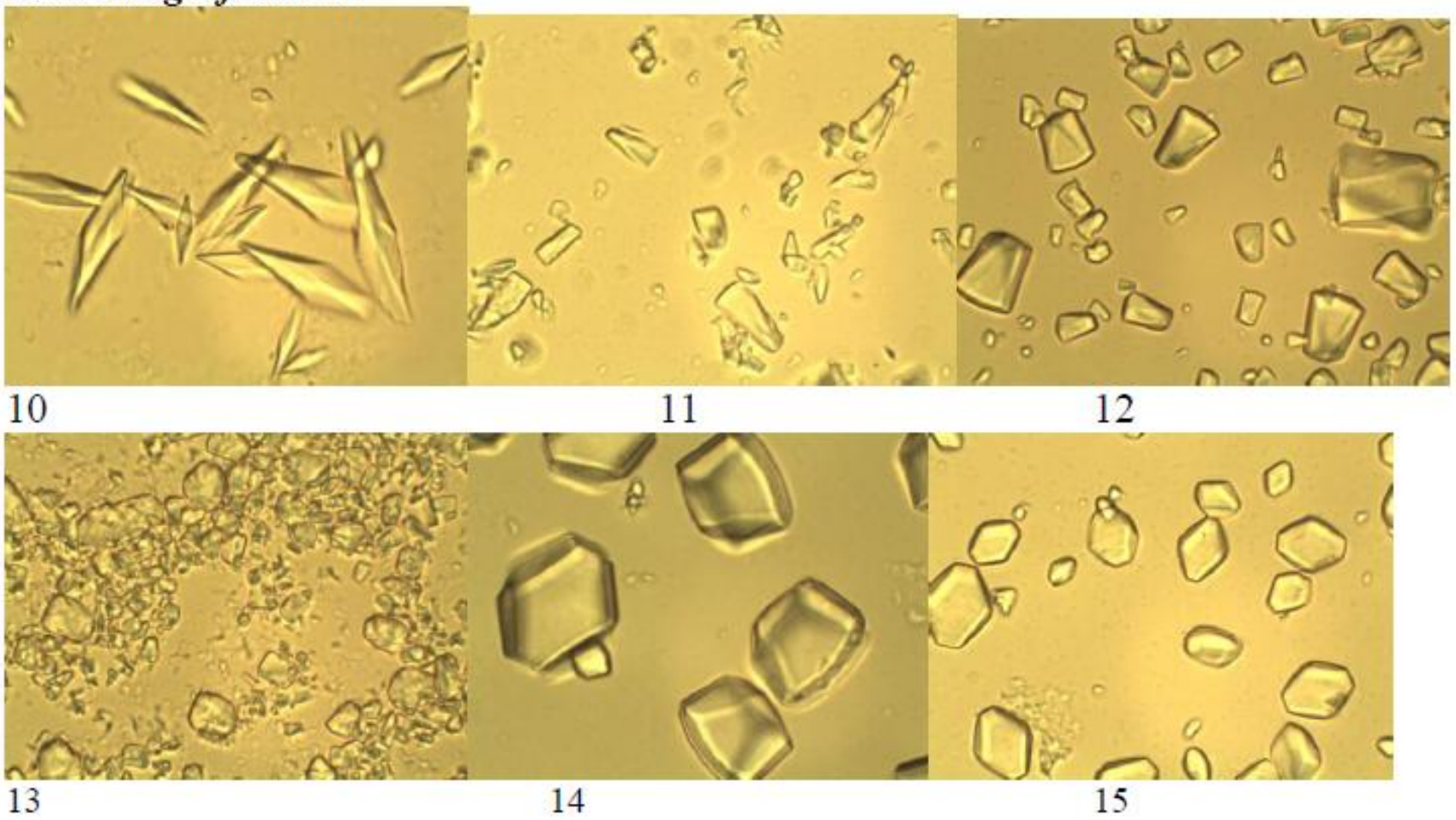
Protein crystallization and phase diagrams
Neer Asherie, Methods 34 (2004) 266–272


Labile Region Stable Nuclei Spontaneously Form and Grow
Nucleus Forms
Crystal Experiences Protein Concentration in Bulk Solvent
Supersaturated Region
Metastable Region Stable Nuclei Grow But Do Not Initate
Saturation Limit
Crystal Experiences Local Protein Concentration Within the Depletion Zone
Unsaturated Region - Solid Phase Dissolves
Protein Concentration
Precipitant Concentration

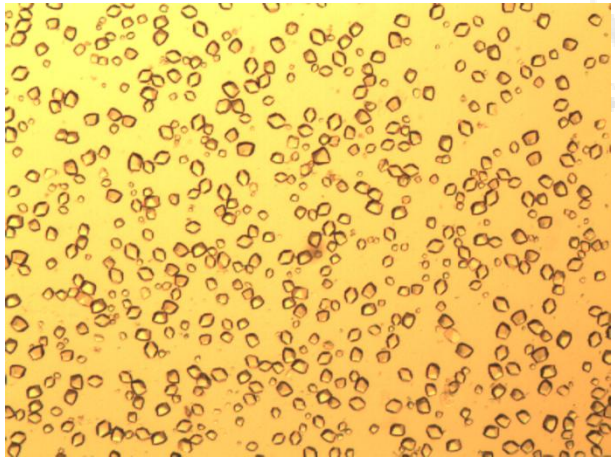

This movie is brought to you by

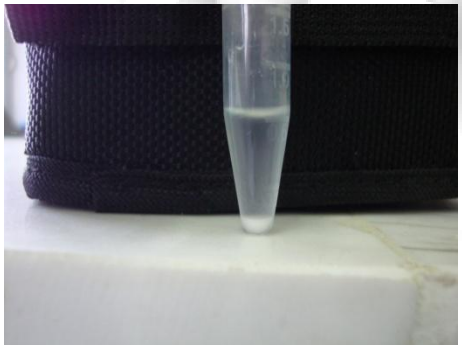
Protein Polycrystalline Samples

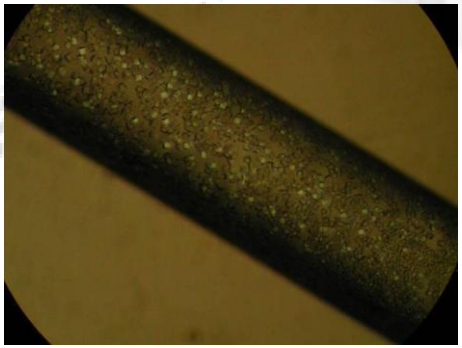
100 x magnification



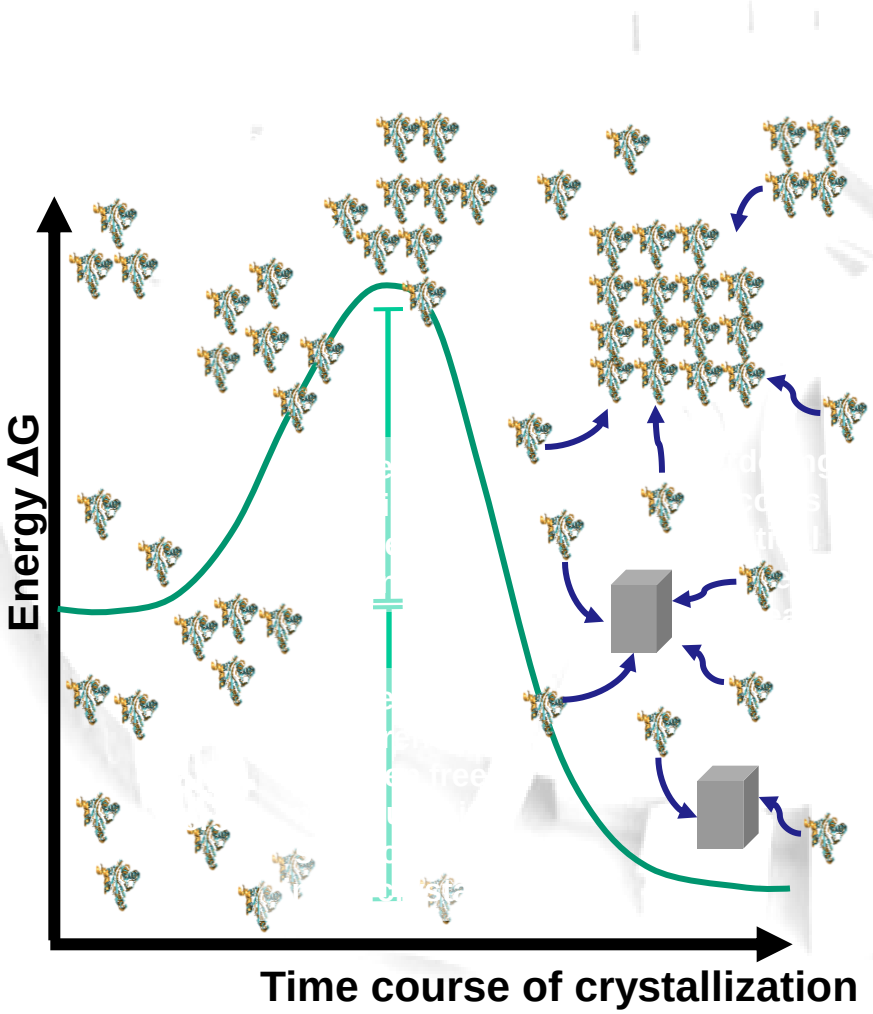
Protein Polycrystalline Samples

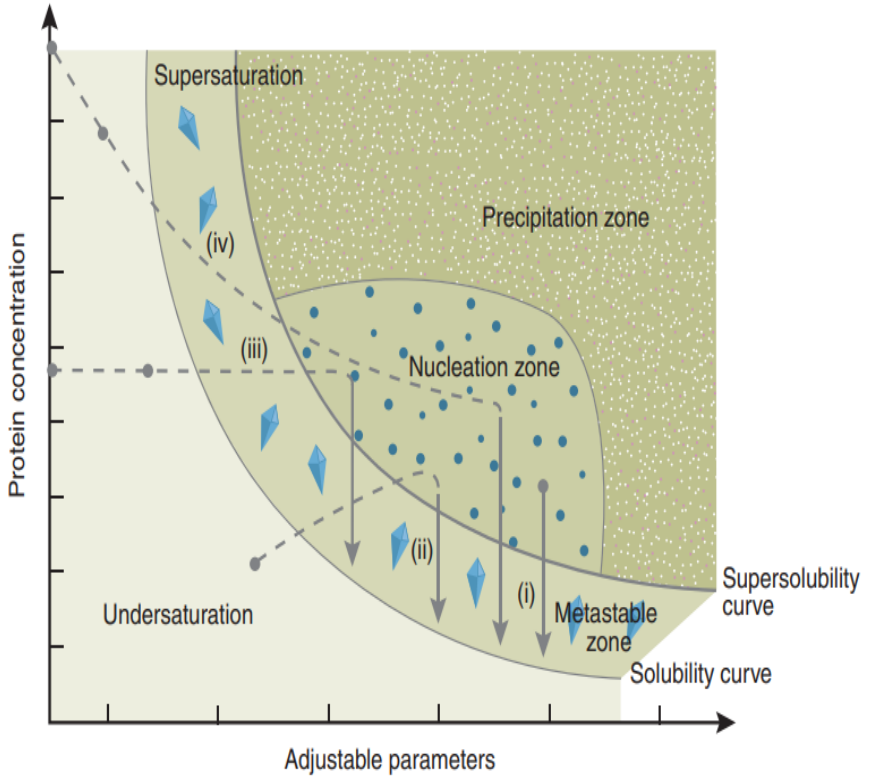






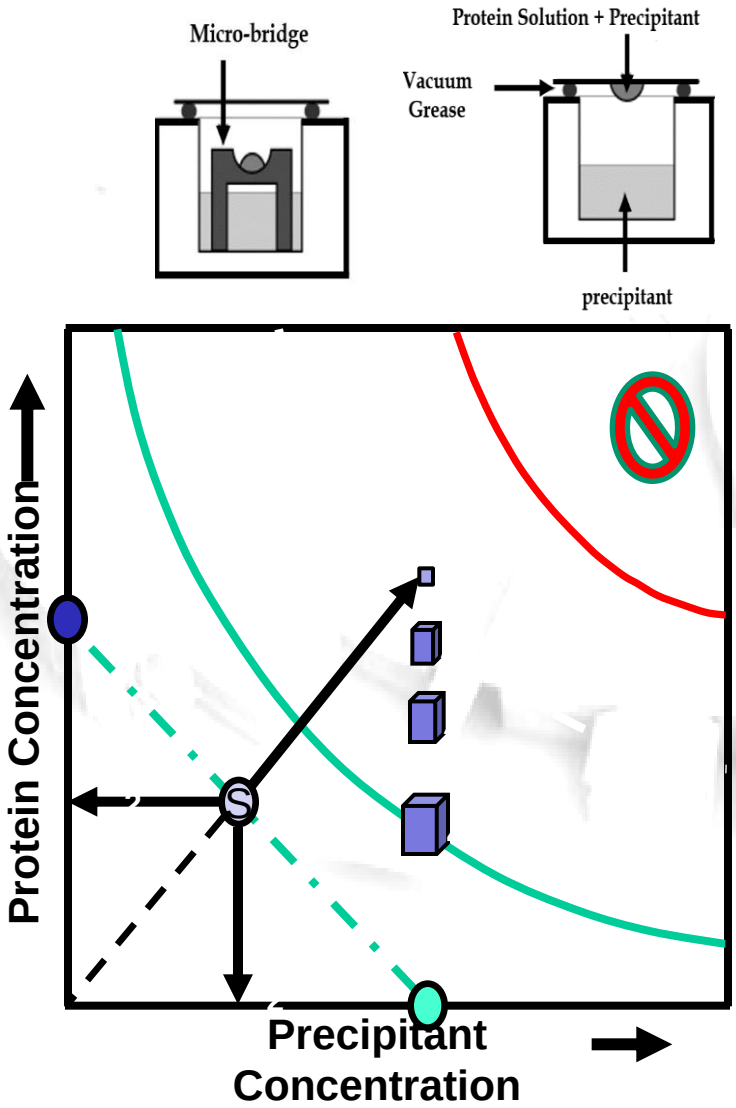
Crystallization

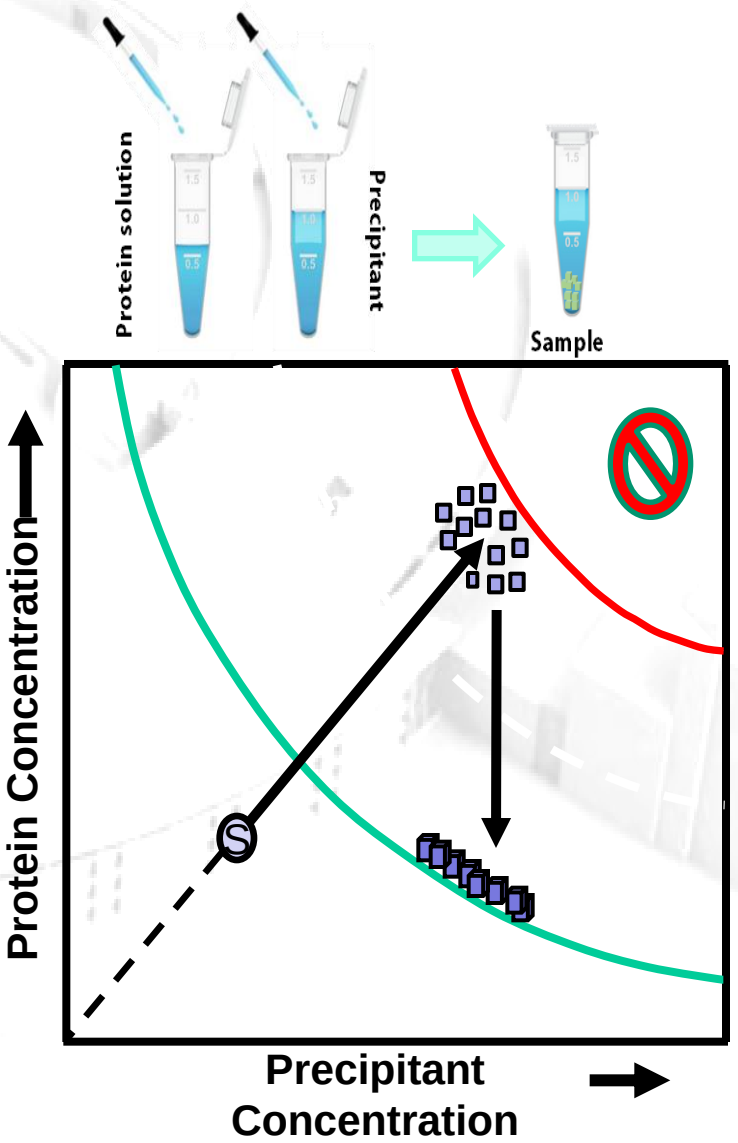




ionic strength	pH	polymers
temperature	ligands	

Crystallization

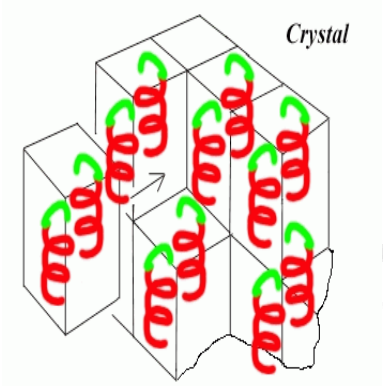




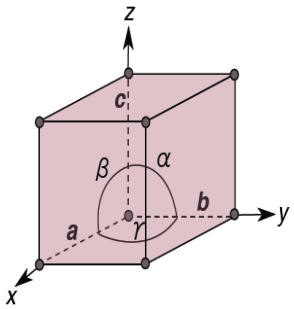
Rupp, B., 2010. Biomolecular crystallography: principles, practice, and

Protein Crystals

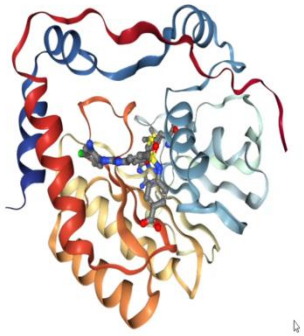
20 – 80 % s

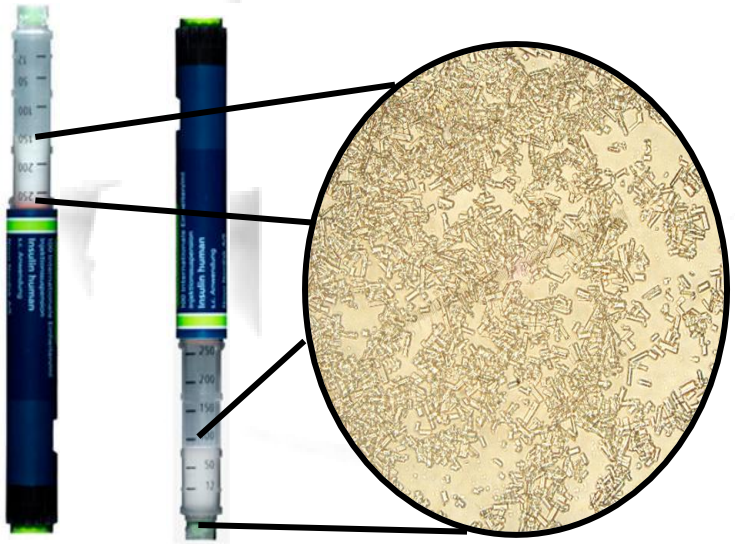


http://www.ruppweb.org



www.doitpoms.ac.uk





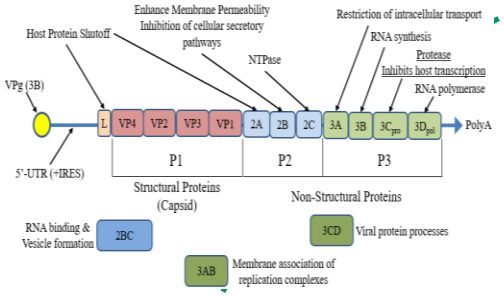
"NPH remains a popular basal insulin in type 2 diabetes mellitus (T2DM)." Lucidi *et al*., 2015

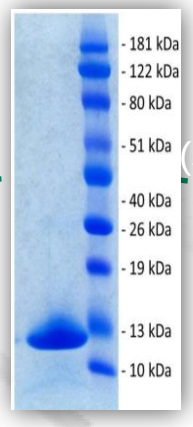
"Small rod shaped microcrystals less than 20µm × 5µm × 5µm in size" Norrman *et al*. 2007

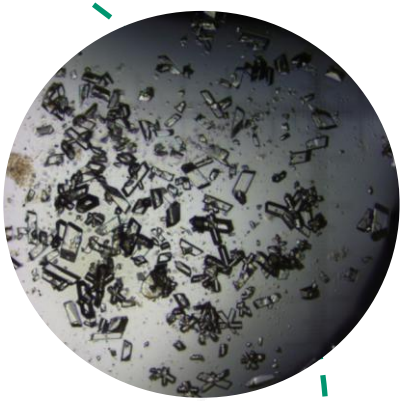
"The pharmacokinetics of NPH vary considerably due to the formulation of NPH insulin per se and/or lack of iappropriate resuspension before injection." Lucidi *et al*., 2015

"Direct characterization: quality control and regulatory reasons. Extraction of information towards improvement of the existing formulations." Norrman *et al*. 2007

Workflow





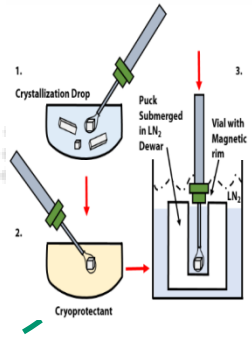


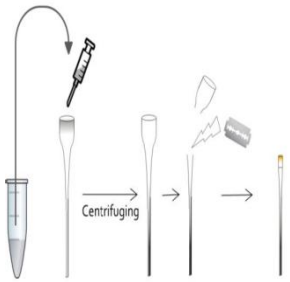
Protein Production

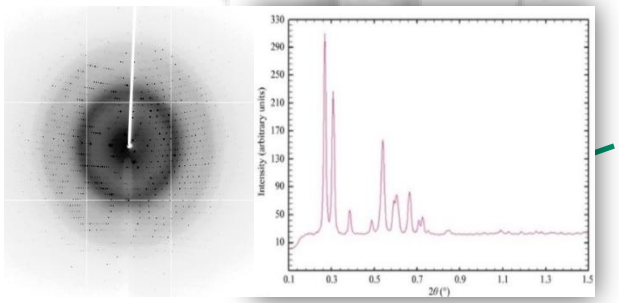
XRPD & SCXD

Protein Crystal Production

Structure Solution

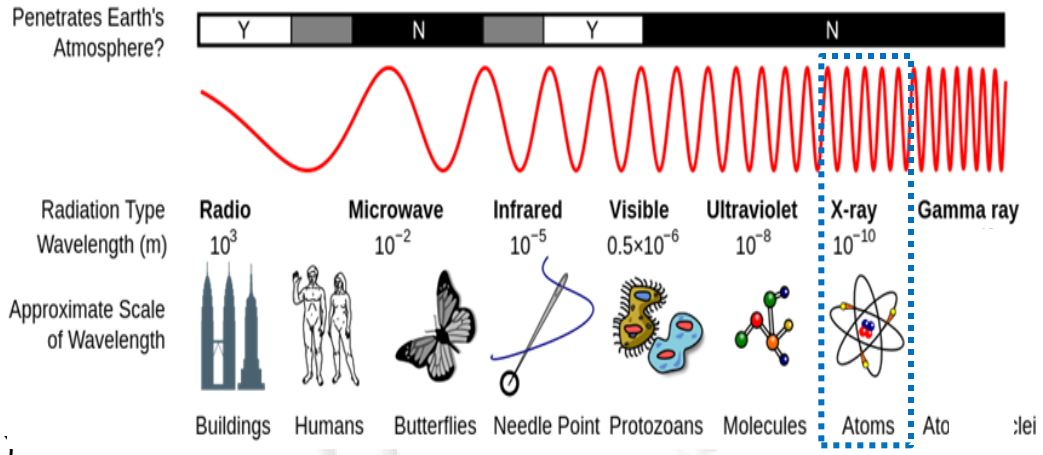


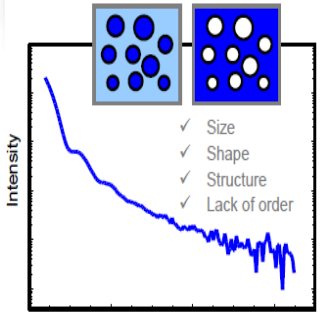




Electromagnetic Spectrum / X-rays

Wilhelm Conrad Röntgen
Physics & X-ray Astronomy
Discovery of X-rays (1895)
Nobel Prize in Physics (1901)



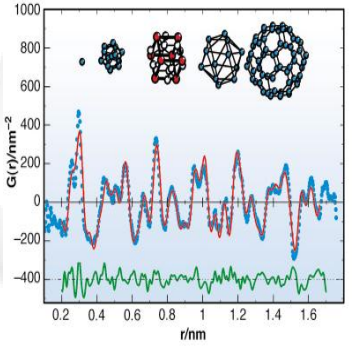


USAXS

SAXS (1D & 2D), BioSAXS

WAXS (1D & 2D)

Total Scattering (PDF)

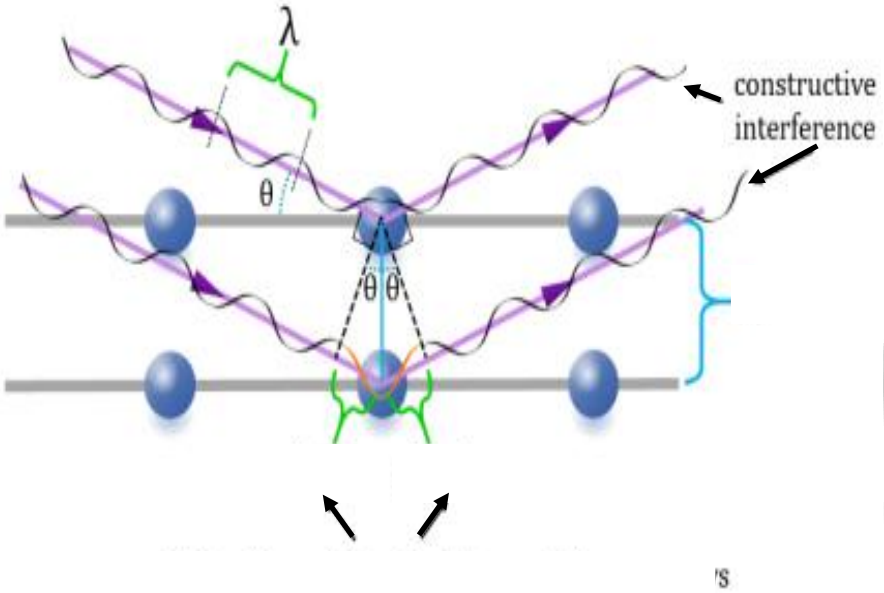


X-Ray Diffraction (XRD)

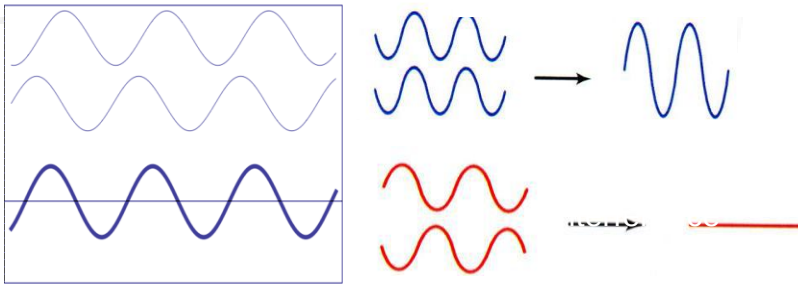
ESRF Highlights, 2006

X-ray scattering / diffraction

Bragg's Law: $n\lambda = 2d \sin\theta$



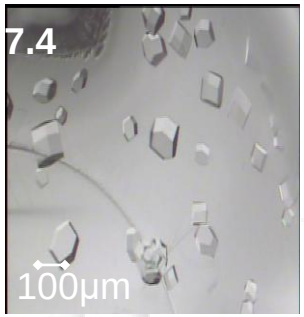
- n a positive integer
- λ is the wavelength of the incident X-rays,
- d is the interplanar spacing of the crystal and
- θ is the angle of incidence.



X-ray diffraction
Single Crystal (SCXD) & Powder (XRPD)

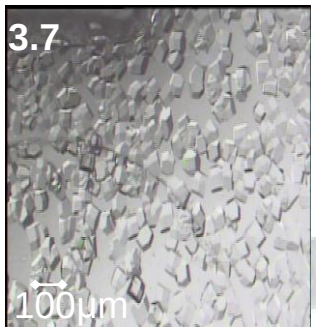
SCXD

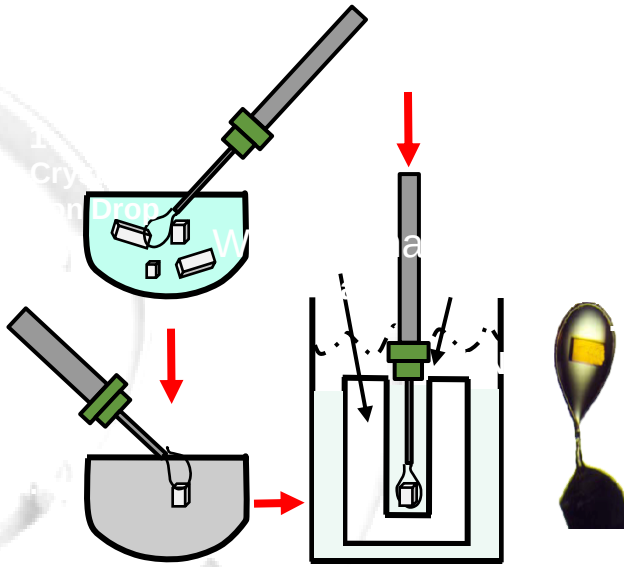
- Very small amount of molecule required
- Easy data handling/data processing
- Can be very difficult to grow large enough single crystals of some molecules



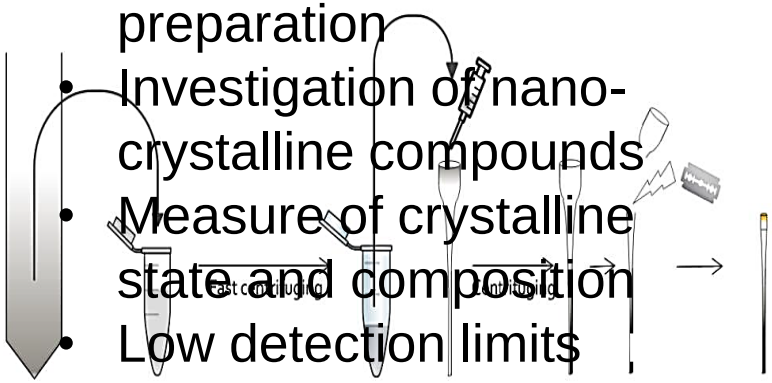
XRPD

- Polycrystalline powder can often be obtained when a good single crystal cannot
- Study of sub-micron or twinned samples
- Complexed systems have hundreds diffraction peaks which are usually highly overlapped- loss of information!





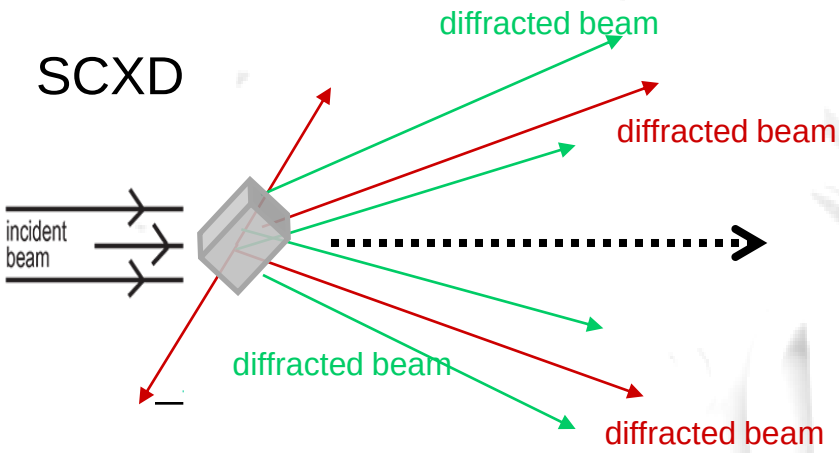
- Ease of use
- Minimum sample preparation
- Investigation of nano-crystalline compounds
- Measure of crystalline state and composition
- Low detection limits

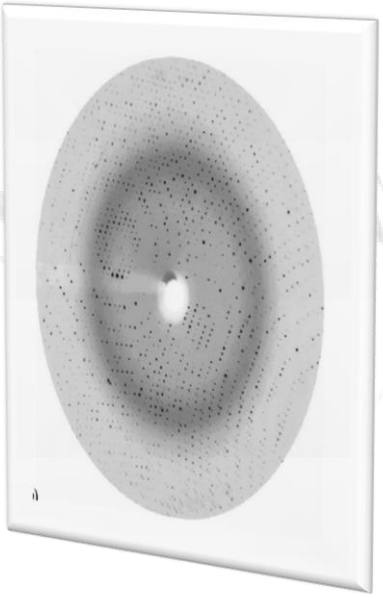


Basso *et al*.,2005, *Acta Cryst*. D61, 1612–1625.

X-ray diffraction
Single Crystal (SCXD) & Powder (XRPD)

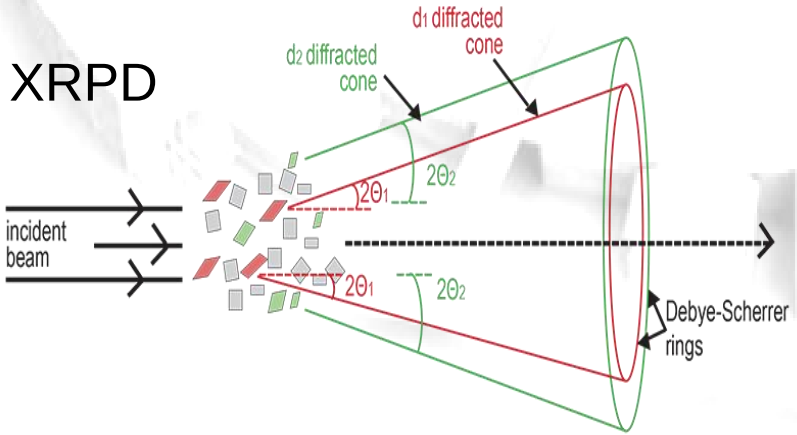
SCXD

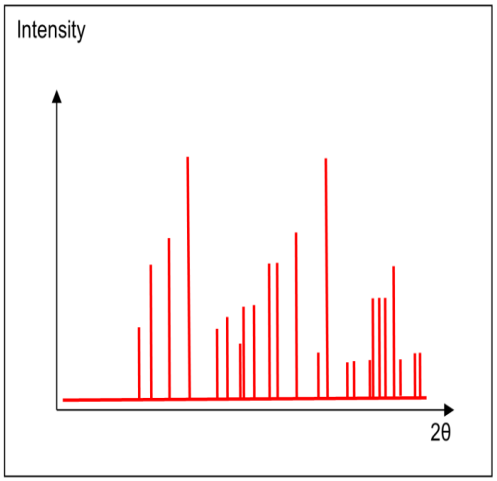




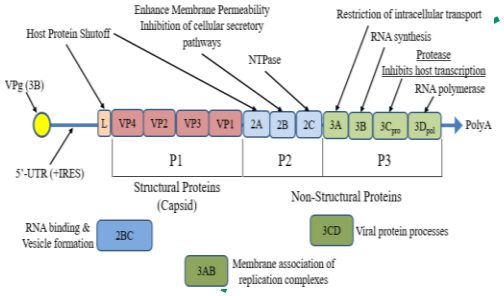
A common full dataset:
360° cryst. rotation/1800
(1 image per 0.2°)

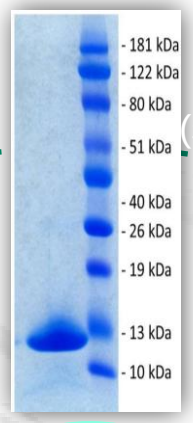
XRPD

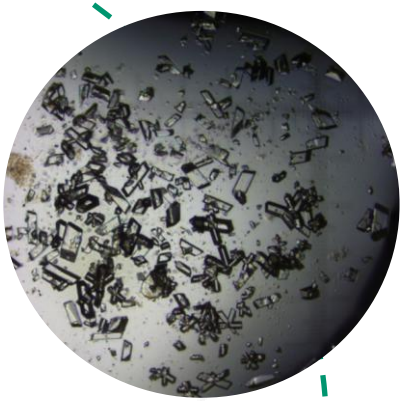




Workflow





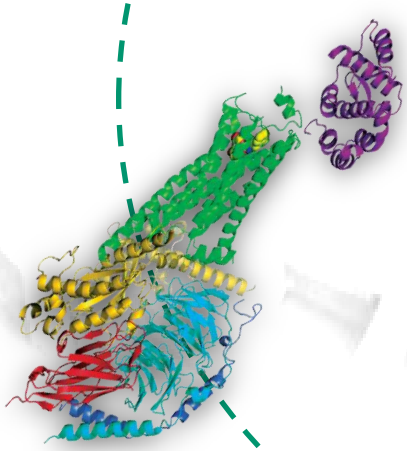


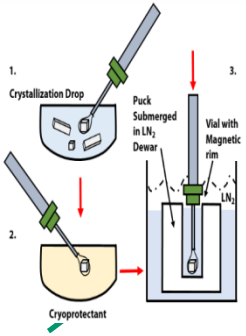
Protein Production

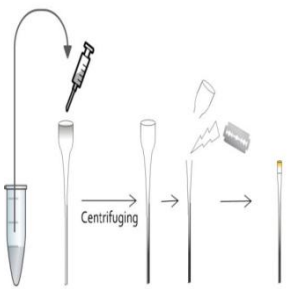
XRPD & SCXD

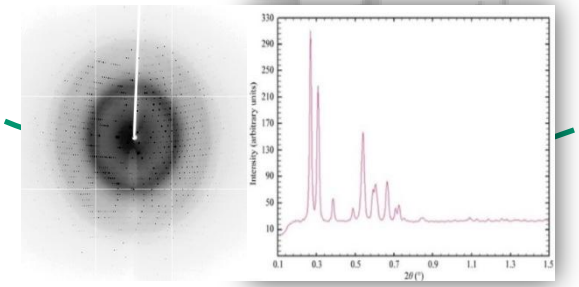
Protein Crystal Production

Structure Solution









A case study of a virus protein domain

576 Z. Kristallogr. **225** (2010) 576–580 / **DOI** 10.1524/zkri.2010.1348

Preliminary insights into the non structural protein 3 *macro* domain of the Mayaro virus by powder diffraction

Nicolas Papageorgiou[*, I], Yves Watier[II], Lucy Saunders[II], Bruno Coutard[I], Violaine Lantez[I], Ernest A. Gould[III], Andrew N. Fitch[II], Jonathan P. Wright[II], Bruno Canard[I] and Irene Margiolaki[II, IV]

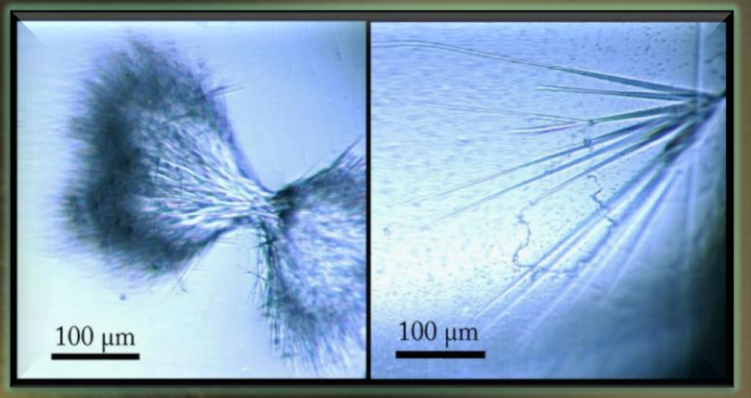
[I] Architecture et Fonction des Macromolécules Biologiques, CNRS and Universités d'Aix-Marseille I et II, UMR 6098, ESIL Case 925, 13288 Marseille, France
[II] European Synchrotron Radiation Facility, ESRF, BP-220, 38043 Grenoble, France
[III] CEH Oxford Mansfield Road Oxford OX1 3SR UK and Université des Virus Emergents Faculté de Médecine Timone 5eme étage Aile Bleue 27, Bd Jean Moulin 13385 Marseille Cedex 05, France
[IV] Department of Biology, Section of Genetics, Cell Biology and Development, University of Patras, Greece

Received September 10, 2010; accepted October 5, 2010

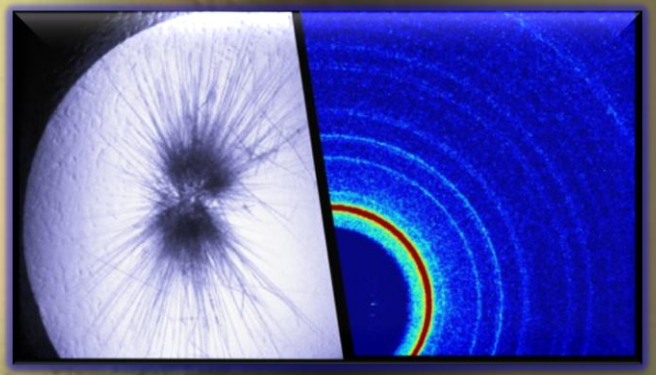
Structural virology / Powder diffraction / Mayaro virus / Non structural protein

Abstract. We present preliminary structural results of the non-structural protein 3 (nsP3) *macro* domain from the virus is responsible for a dengue-like acute febrile illness in tropical South America where it is endemically enzootic [9, 10]. The main vector is the Haemagogus mosquito. Recent demographic changes in South America as well as the possibility of transmission and infection of humans via

Emerging Viruses

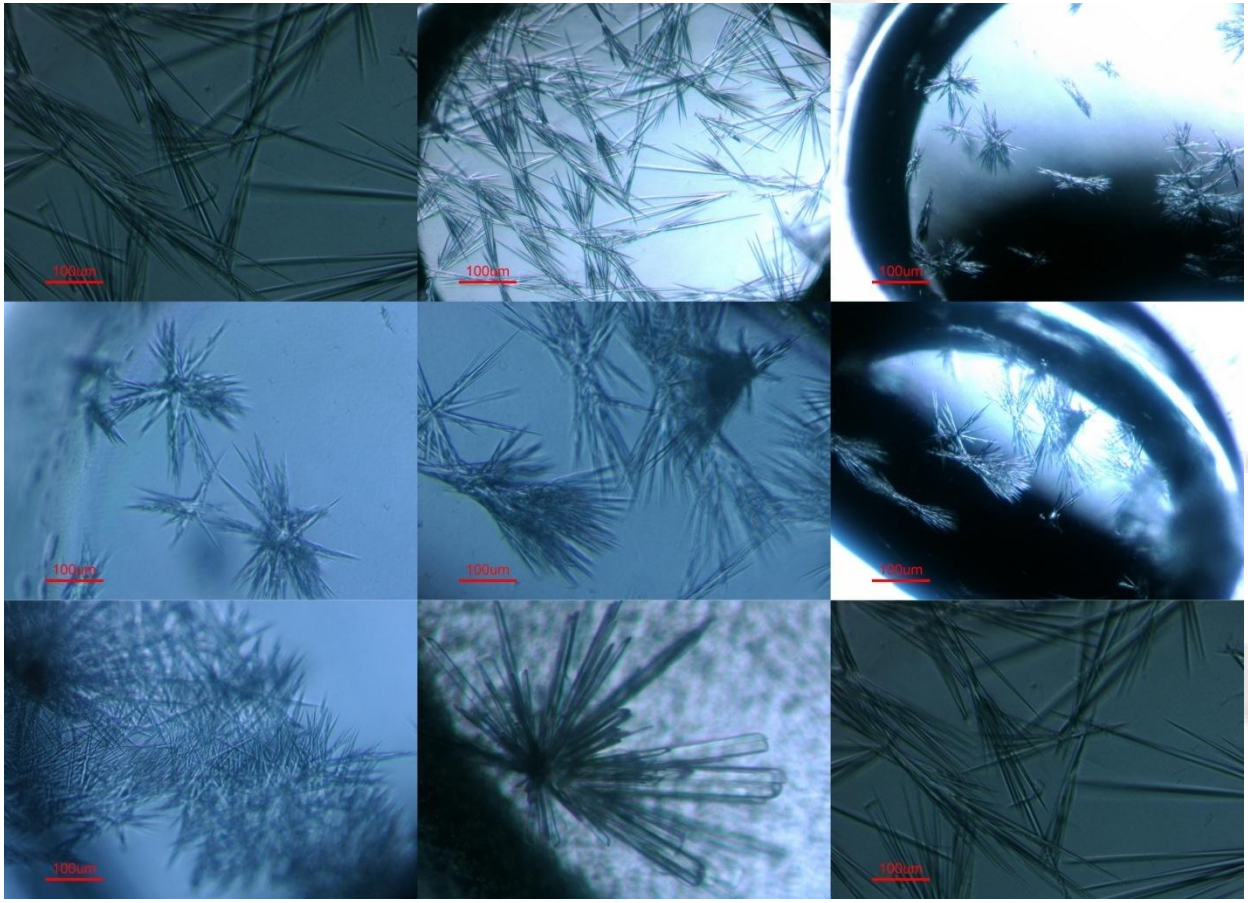


nsP3 macro domain of Mayaro virus



Mayaro virus disease: an emerging mosquito-borne zoonosis in tropical South America.
Clin Infect Dis. 1999 Jan;28(1):67-73

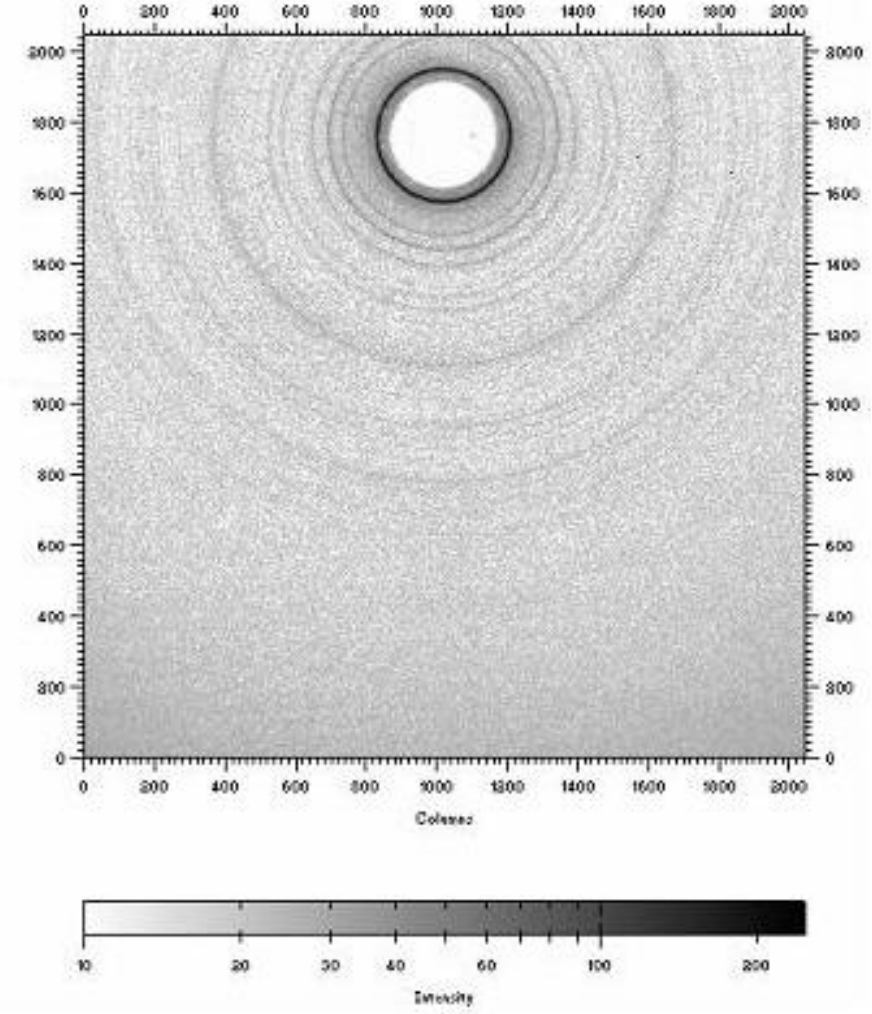
“Sea Urchin” crystals of MAYV

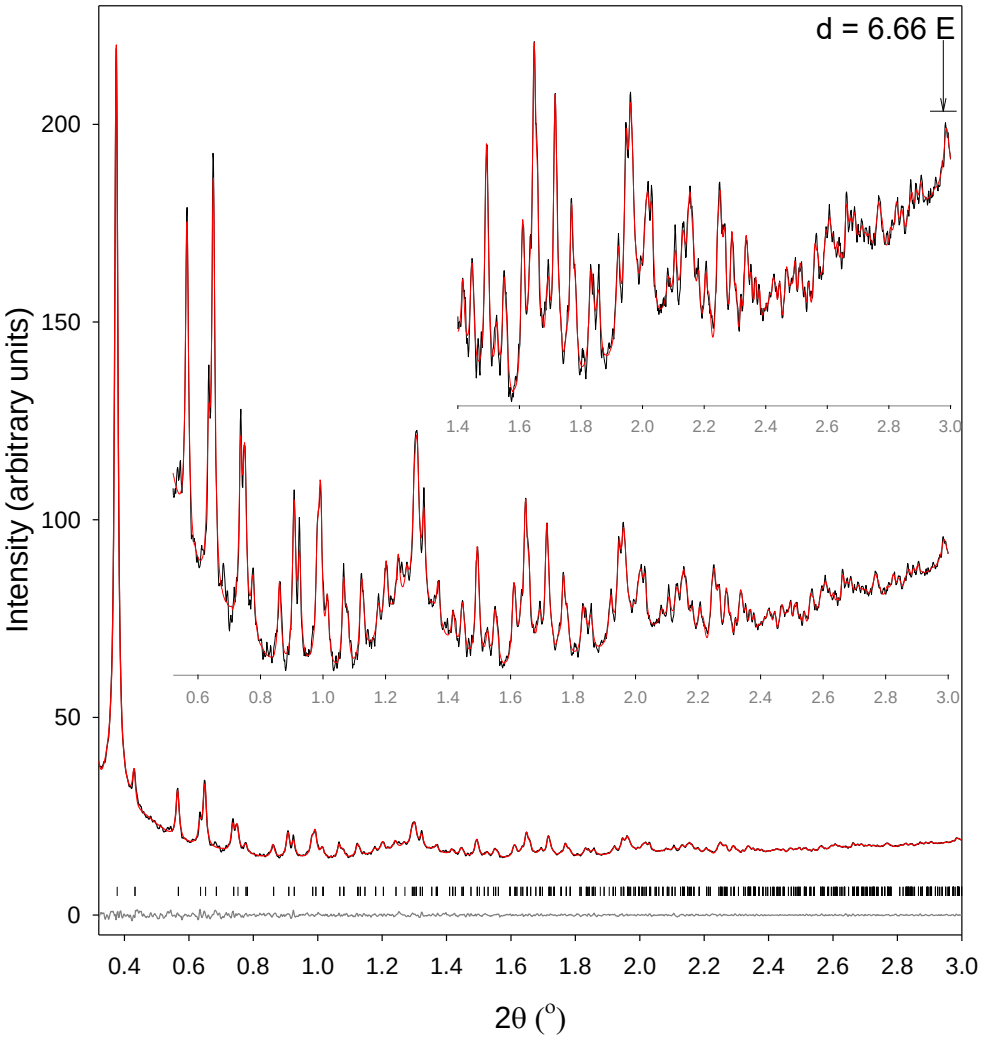


AFMB: Nicolas Papageorgiou, Bruno Canard, Bruno Coutard & Violaine Lantez

UPAT: Elena Kotsiliti, Alexandros Valmas

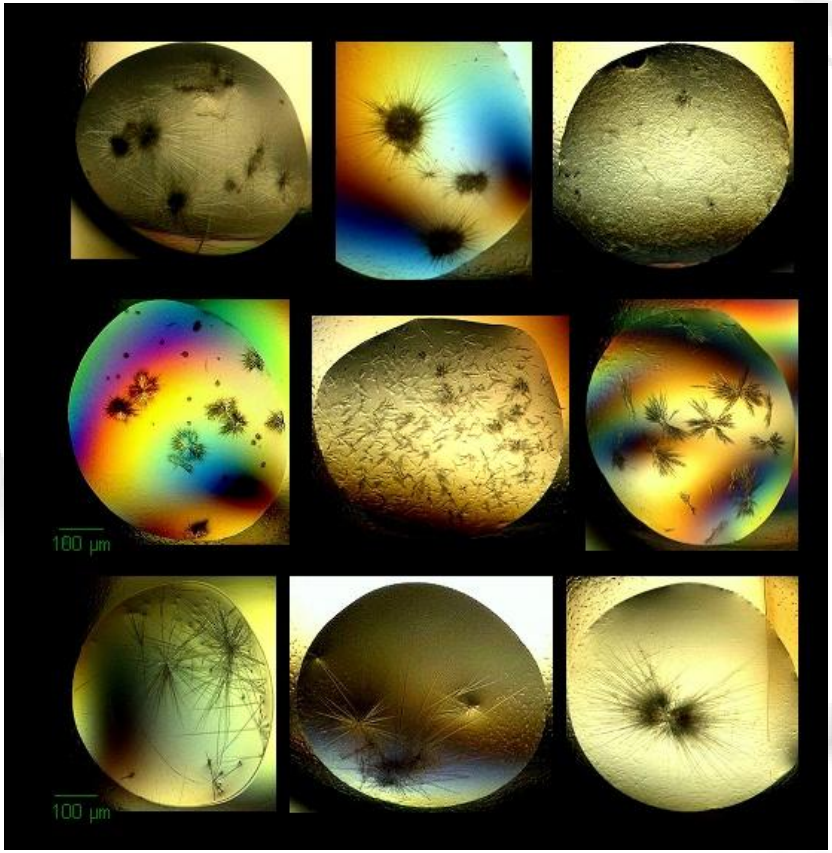
Data collected at ID11- ESRF

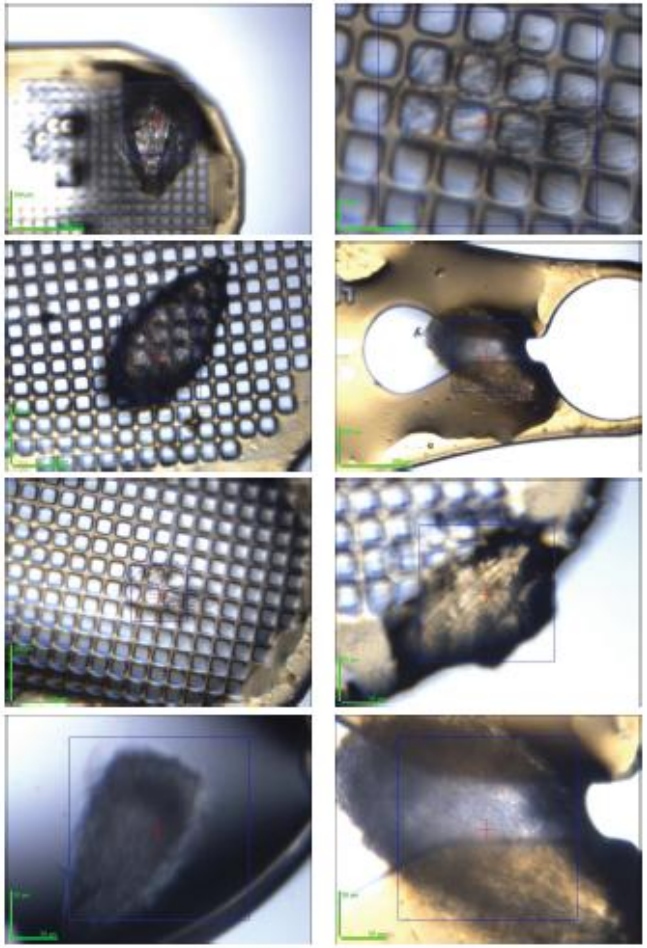


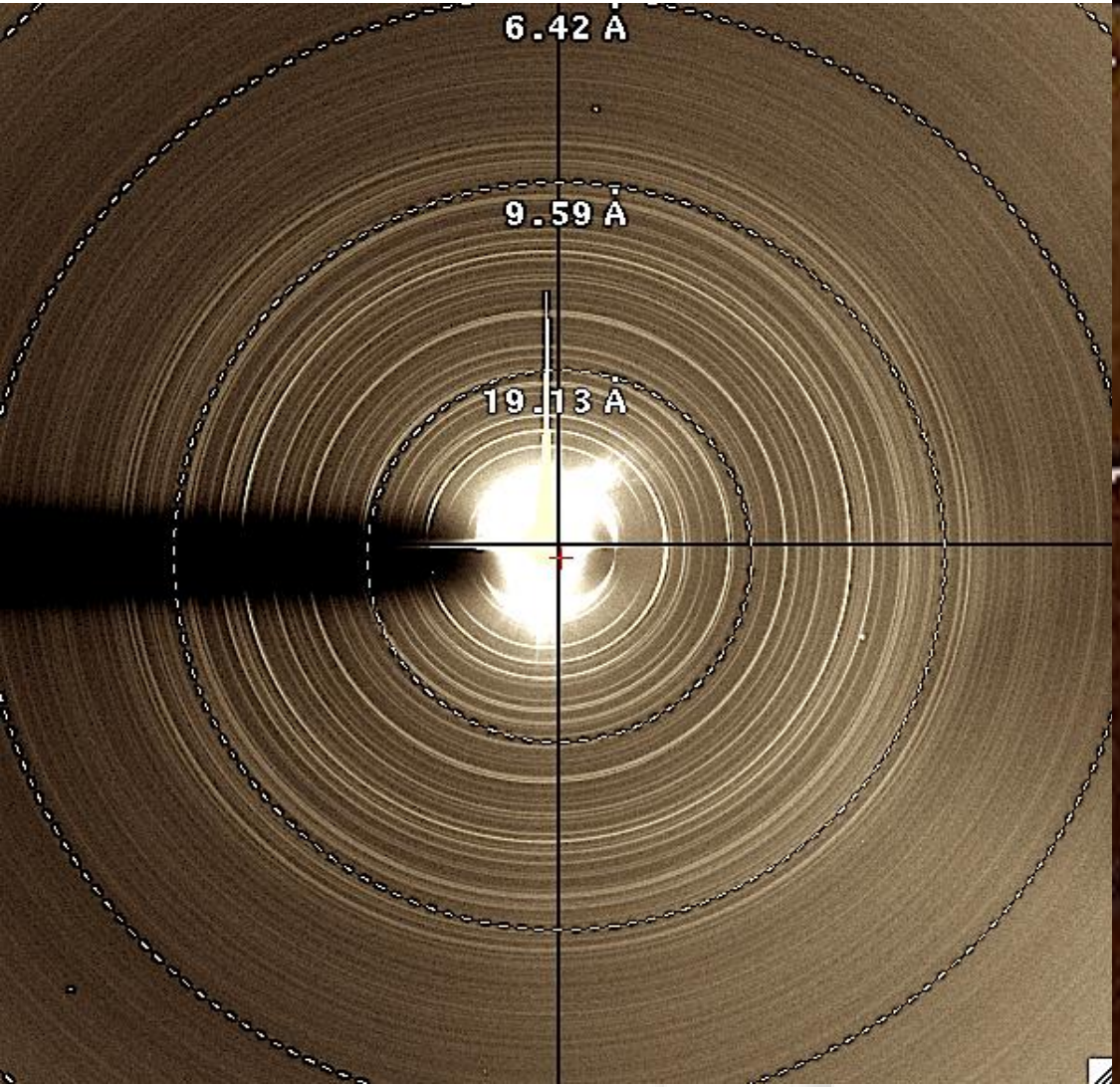


$P3_1$, a = 61.603(3) Å, c = 94.619(5) Å
2 molecules/ asu, 58% solvent content

Open air single urchin measurements

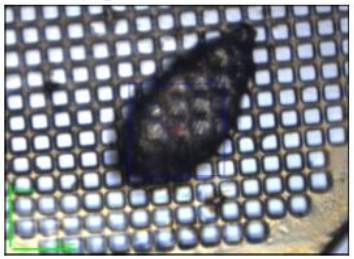


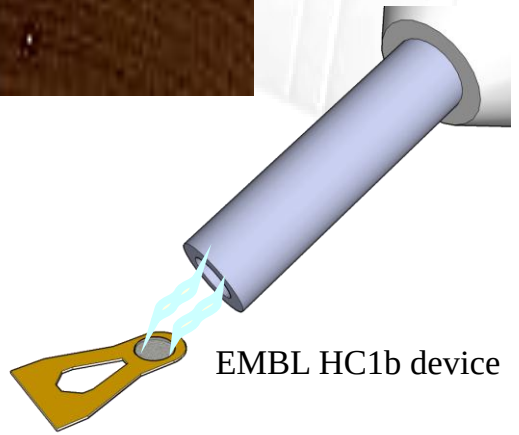




ID14- 2, area detector
λ=0.9934 Å

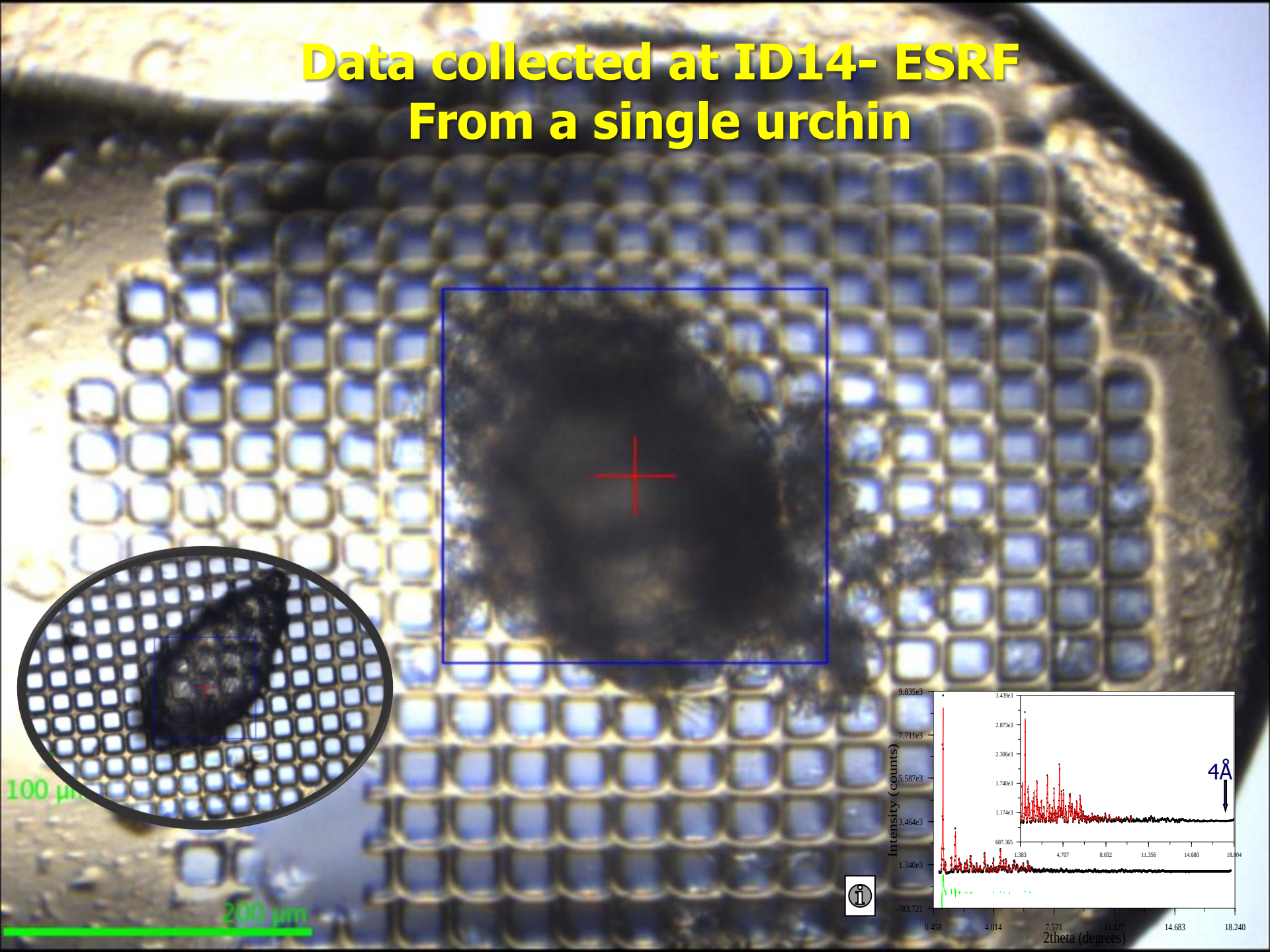
Matthias Bowler, Yves Watier,
Nicolas Papageorgiou, Irene Margiolaki



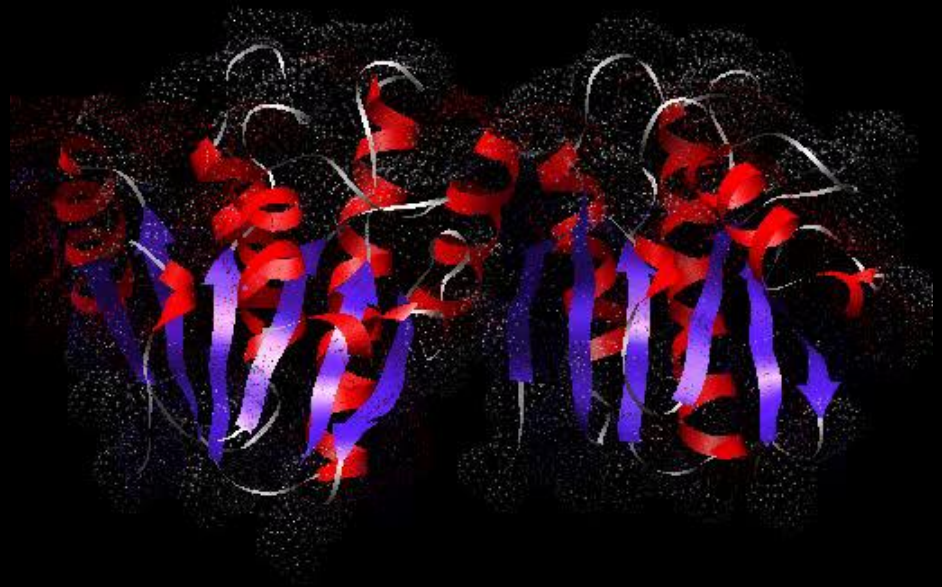


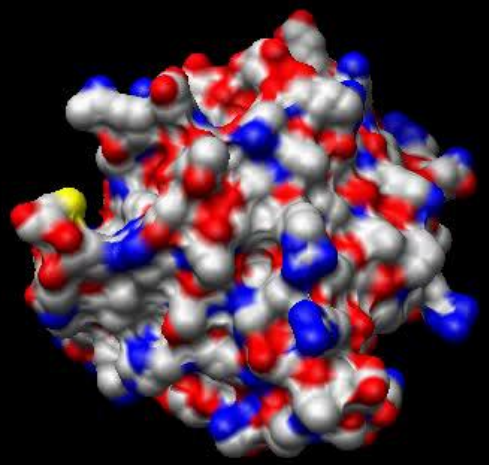
EMBL HC1b device

Data collected at ID14- ESRF
From a single urchin



Preliminary results after MR and RB





$P3_1$, a = 61.603(3) Å, c = 94.619(5) Å
2 molecules/ asu, 58% solvent content

Preliminary insights into the non structural protein 3 macro domain of the Mayaro virus by powder diffraction.
Z. Kristallogr. 225 (2010) 576–580 (EPDIC12 proceedings - Invited)

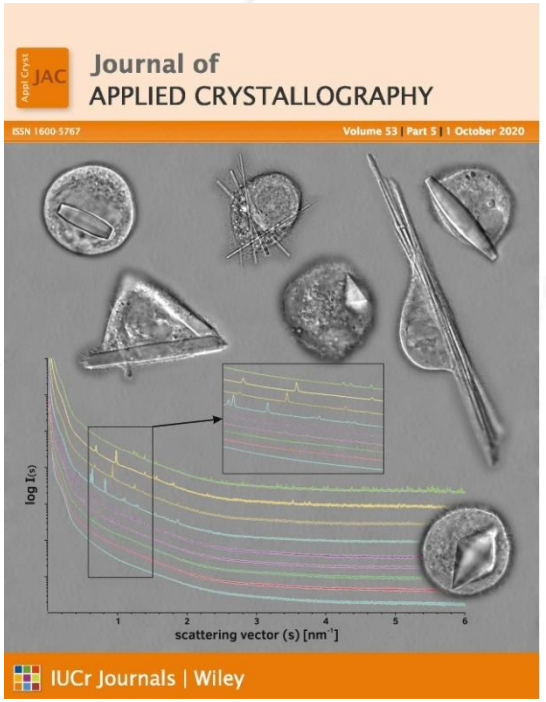
Nicolas Papageorgiou, Yves Watier, Lucy Saunders, Bruno Coutard , Violaine Lantez, Ernest A. Gould, Andrew N. Fitch, Jonathan P. Wright, Bruno Canard & Irene Margiolaki

A case study of protein crystals grown “in cell”

Rapid screening of *in cellulo* grown protein crystals via a small-angle X-ray scattering/X-ray powder diffraction synergistic approach

Janine Mia Lahey-Rudolph, Robert Schönherr, Cy M. Jeffries, Clément E. Blanchet, Juliane Boger, Ana Sofia Ferreira Ramos, Winnie Maria Riekehr, Dimitris-Panagiotis Triandafillidis, Alexandros Valmas, Irene Margiolaki, Dmitri Svergun and Lars Redecke

J. Appl. Cryst. (2020). **53**, 1169–1180



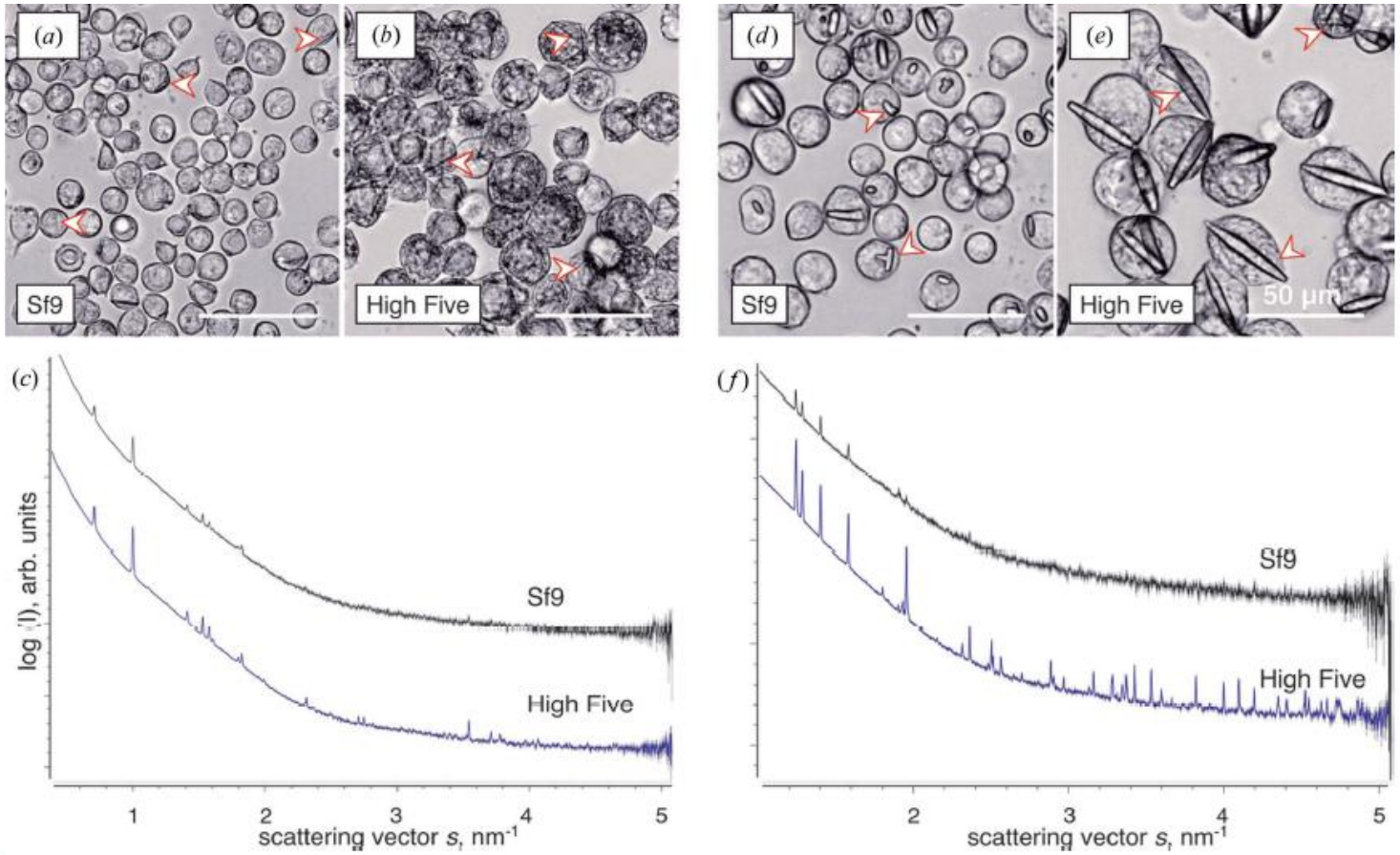


Figure 7
The unit-cell parameters of intracellular crystals do not depend on the insect cell line used for protein crystallization. Differential interference contrast light microscopy of intracellular CatB (*a*), (*b*) and HEX-1 (*d*), (*e*) crystals grown in Sf9 insect cells shows a highly comparable morphology compared with the growth in High Five cells. Red arrowheads highlight selected intracellular crystals. Corresponding X-ray scattering data show identical positions of the Bragg diffraction peaks in both cell lines for either CatB (SASBDB: SASDH96, SASDH86) (*c*) or HEX-1 (SASBDB: SASDHB6, SASDHA6) (*f*), indicating identical unit-cell parameters independent of the cell line used for crystal growth. The peak intensity is reduced in Sf9 cells owing to the observable drop in the crystallization efficiency. The standard deviation of each data point is presented as grey bars.

A very nice website regarding protein crystallization

https://xray.teresebergfors.com/

Resources

- https://sites.google.com/view/margiolaki-biology-upat/educational-material
- https://xray.teresebergfors.com/
- https://www.mdpi.com/journal/crystals/special_issues/special_physical_environments?fbclid=IwAR0cSJTOUb21RXlqEiOn4goTBJPYeh238jhcdPAw-Hen5Lni2c207gyV0lo
- https://www.researchgate.net/publication/7733332_Protein_crystals_for_the_delivery_of_biopharmaceuticals
- https://drive.google.com/file/d/10APQ2i0B7JbHRMdGtFPmMTFFxPBnuIoG/view

Books

- T.L. Blundell & L.N. Johnson (1976), "Protein Crystallography", Academic Press: London.
- Jan Drenth (1994), "Principles of Protein X-ray Crystallography", Springer-Verlag: New York.
- D. Sherwood (1976), "Crystals, X-rays and Proteins", Longman: London.
- Rupp, B. (2009). Biomolecular Crystallography. Hamden: Garland Science.
- https://utopia.duth.gr/glykos/pdf/Protein_crystallography.pdf
- A. Valmas, PhD thesis, University of Patras (2020).

Further reading

- Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N.,
- Weissig, H., Shindyalov, I. N. & Bourne, P. E. (2000). Nucleic Acids Res. 28, 235–242.
- Burns, G. & Glazer, A. M. (1990). Space Groups for Solid State
- Scientists, 2nd ed. New York: Academic Press.
- Clare, D. K., Bakkes, P. J., Van Heerikhuizen, H., Van Der Vies, S. M. & Saibil, H. R. (2006). J. Mol. Biol. 358, 905–911.
- Giacovazzo, C. (2002). Fundamentals of Crystallography. Oxford University Press.
- International Tables for Crystallography (2005). Vol. A, edited by Th. Hahn. Heidelberg: Springer.
- International Tables for X-ray Crystallography (1952). Vol. I, edited by N. F. M. Henry & K. Lonsdale. Birmingham: Kynoch Press.
- Rupp, B. (2009). Biomolecular Crystallography. Hamden: Garland Science.
- Watanabe, M., Mishima, Y., Yamashita, I., Park, S. Y., Tame, J. R. & Heddle, J. G. (2008). Protein Sci. 17, 518–526.