Αρχές κρυσταλλογραφίας μακρομορίων

Ειρήνη Μαργιωλάκη

ESRF, Grenoble, France

ID31 beamline

Andy Fitch
Jon Wright
Yves Watier

<u>Former members of ID31:</u>

Lucy Saunders
Ines Collings
Sotonye Dagogo
Lisa Knight
Mark Jenner
Sebastian Basso

Biochemistry &
Structural biology - X-ray Crystallography group
Department of Biology, UPAT

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

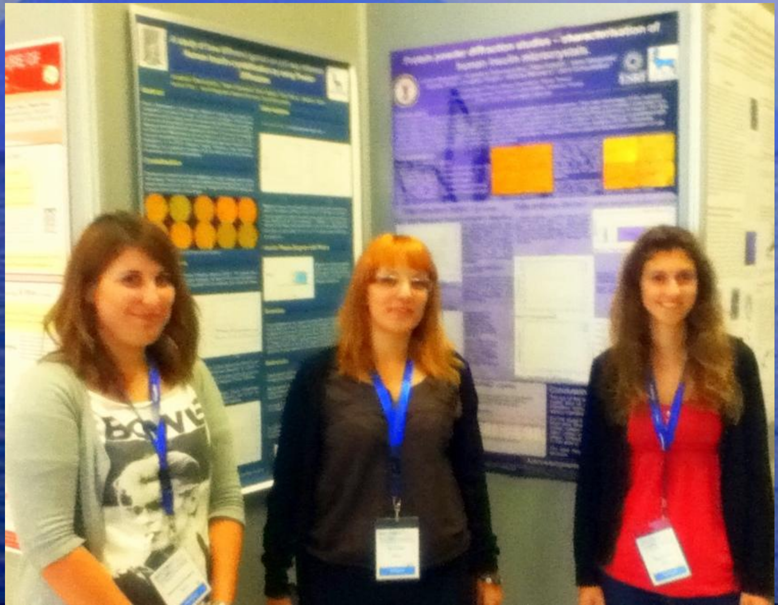
Members from left to right: Stefanos Saslis (BSc), Magda Christopoulou (MSc), Souzana Logotheti (BSc), Stavroula Fili (MSc), Fotini Karavassili (PostDoc), Irene Margiolaki (Group leader), Eleftheria Rosmaraki (Assist. Prof., Department of Biology, UPAT), Alexandros Valmas (PhD), Mary Spiliopoulou (PhD), Vivi Kontou (MSc), Nikos Nikolopoulos (MSc), Katerina Pappa (BSc).

More Members: Christos Kossinas (MSc), Dimitris Triandafillidis (BSc), Aikaterini Filopoulou (BSc), Anastasia Bazioti (BSc), Marianna Giannopoulou (BSc), Spyros Mihelakakis (BSc), Maria Athanasiadou, Frosso Drakouli, Giorgos Nikolaras.

Former Members: Athanasios Galanis (Post Doc), Partha Pratim Das (Post Doc), Andrew Stewart (Post Doc), Anastasia Giannopoulou (MSc), Elena Kotsiliti (MSc), Marigiota Kalatha (BSc), Konstantina Magiouf (BSc), Danae Lahana (BSc), Nickie Messini (BSc), Sofoklis Vlahos (BSc), Maria Balassi (BSc), Costas Bastalias (BSc), Nikos Parthenios (BSc), Mirto Douni (BSc).

UPAT team
Department of Biology

August 2011: IUCr Madrid



Natasha Giannopoulou
Elena Kotsiliti
Fotini Karavasili

Basic Principles of X-ray diffraction and Crystallography

- Introduction: Crystallisation, Diffraction, Crystallography

A Challenging Project 2003 - Present

- Protein Crystallography via powder diffraction
- Synchrotron radiation, powder diffraction

Developments

- Novel methods for data analysis: Case studies of small proteins

Projects @ UPAT

- Structural virology (proteins related to emerging viruses)
- Pharmaceutical Proteins

Crystals of Biological Macromolecules

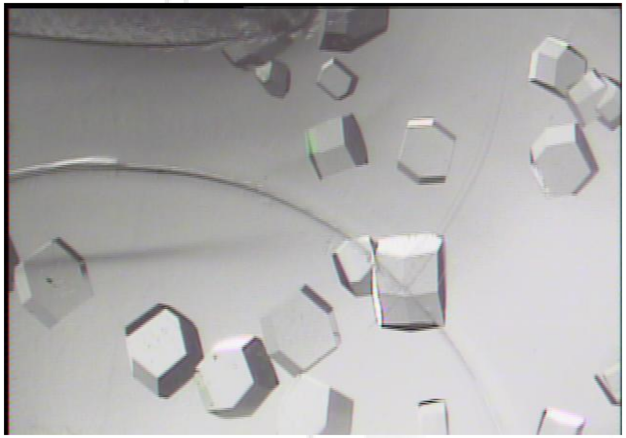
Κρύσταλλοι

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).

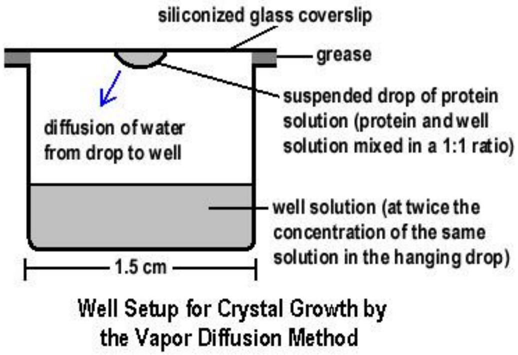
Πως κρυσταλλώνονται οι πρωτεϊνες

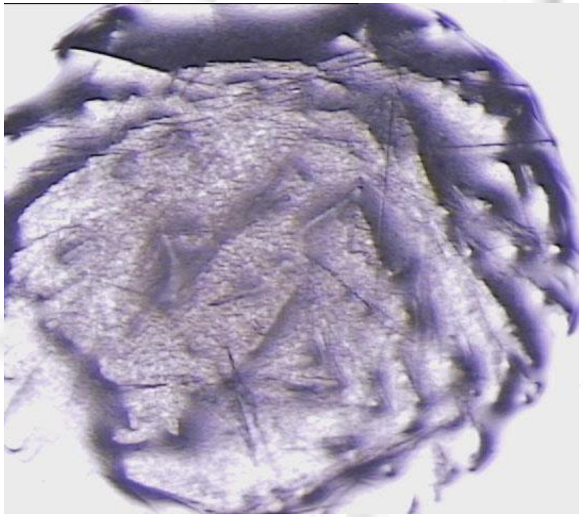
- Συνήθως με μεγάλη δυσκολία!
- Για διαλυτές πρωτεϊνες:
- Διάλυση και καθύζηση
- (dissolve and then precipitate)
- Κρίσιμες παράμετροι: pH, ionic strength, precipitant

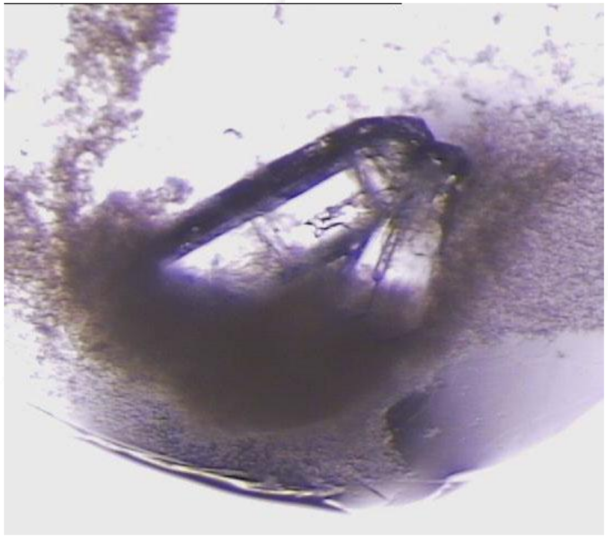


- Συνήθως κρυστάλλωση σε μικρές σταγόνες
- screens + robots

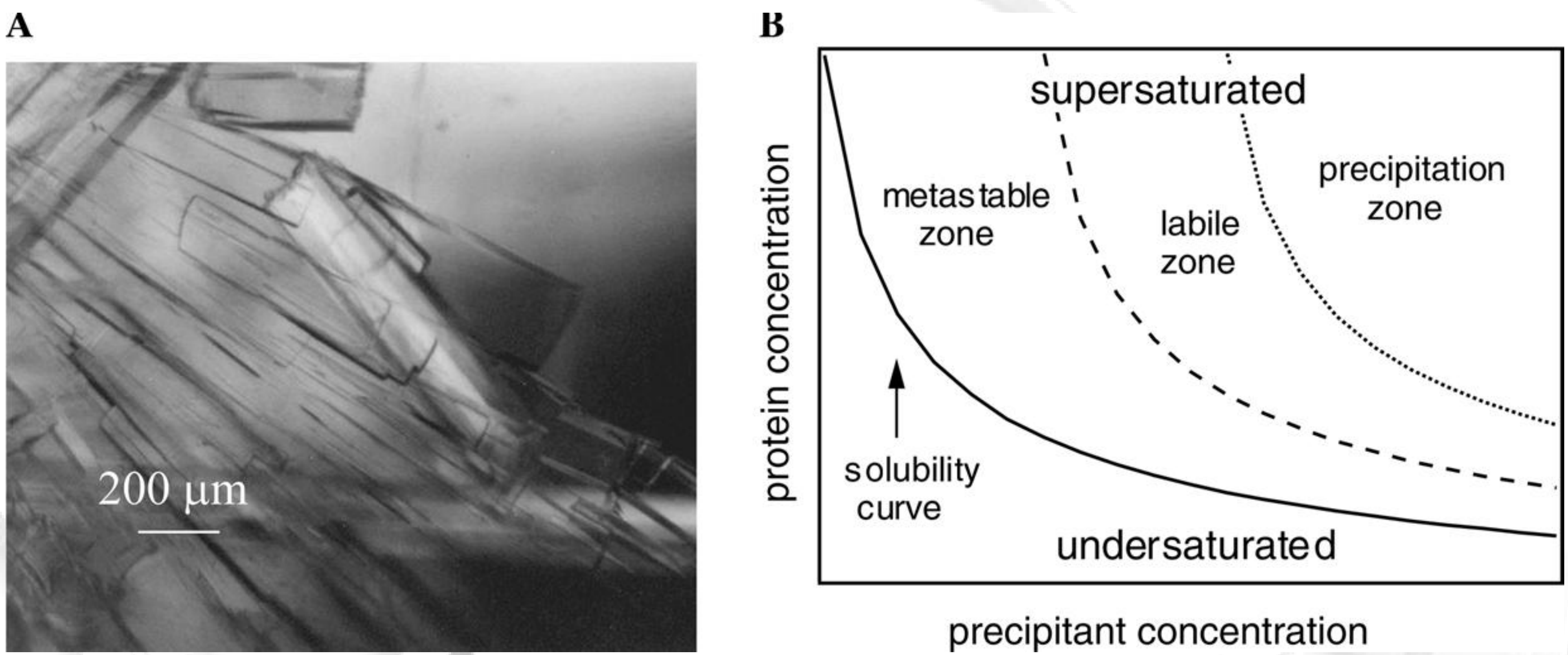
Κρυστάλλωση Πρωτεϊνών







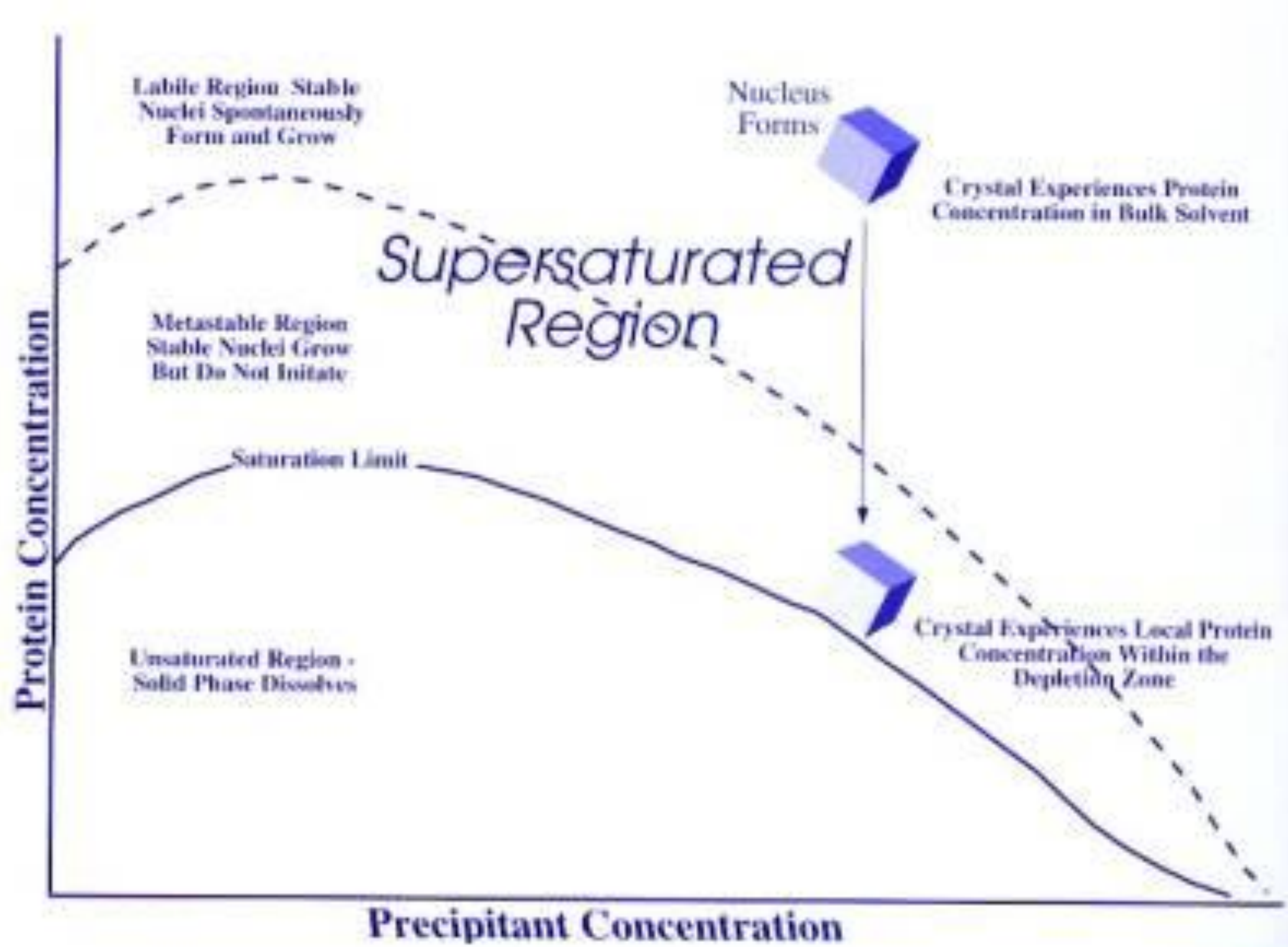
Protein crystallisation & phase diagrams



(A) Crystals of wild-type bovine B crystallin.

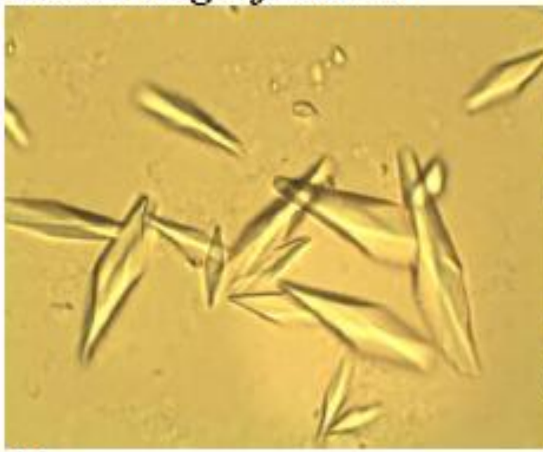
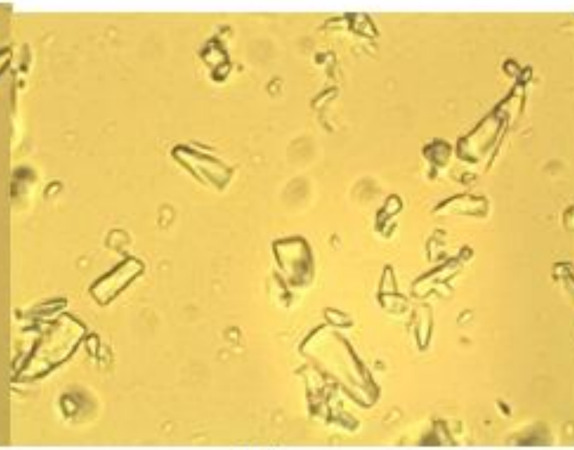
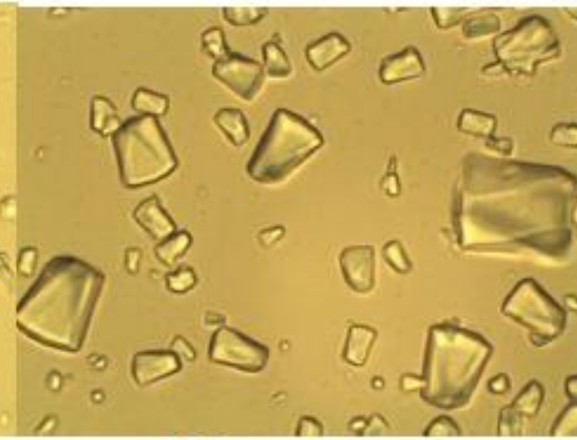
(B) A schematic phase diagram showing the solubility of a protein in solution as a function of the concentration of the precipitant present.

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

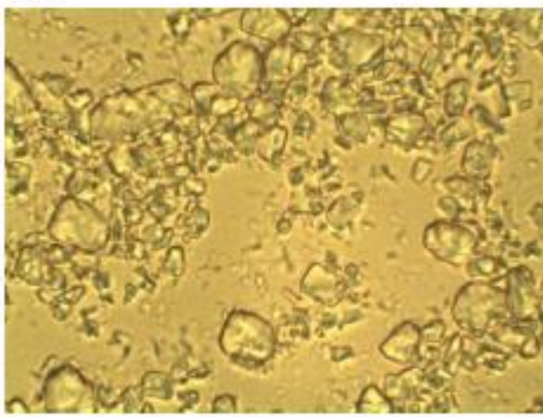
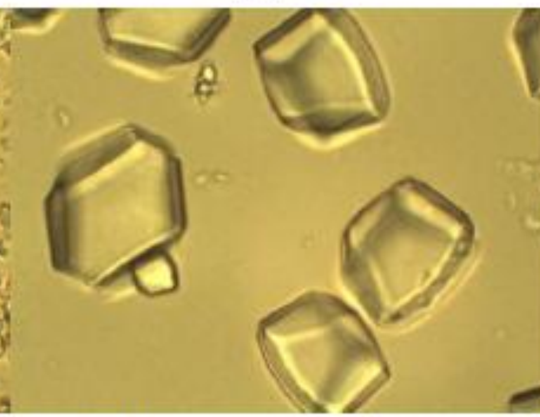
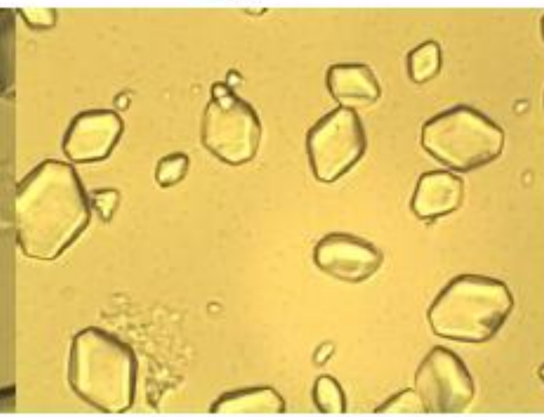

Labile Region Stable
Nuclei Spontaneously
Form and Grow
Nucleus
Forms
Crystal Experiences Protein
Concentration in Bulk Solvent
Supersaturated
Region
Metastable Region
Stable Nuclei Grow
But Do Not Initate
Saturation Limit
Crystal Experiences Local Protein
Concentration Within the
Depletion Zone
Unsaturated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

Protein Powder Samples

100 x magnification

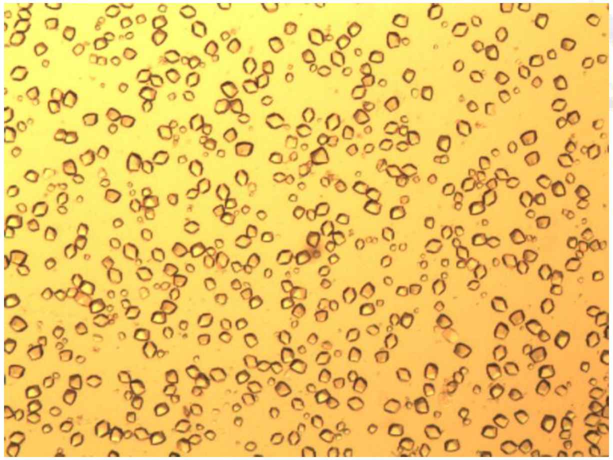




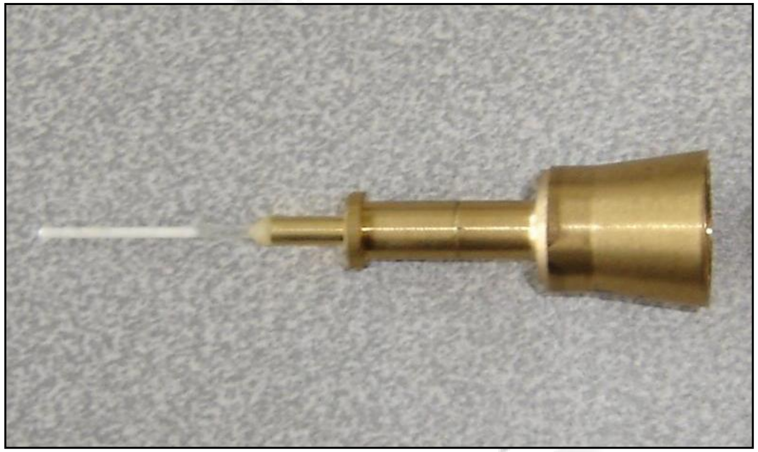
10 11 12

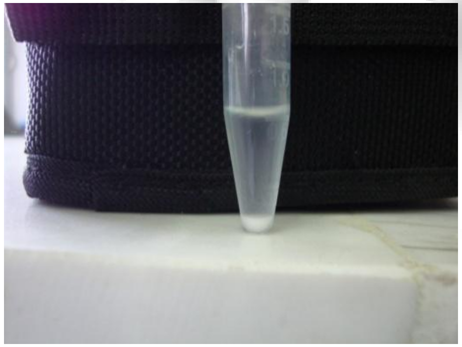




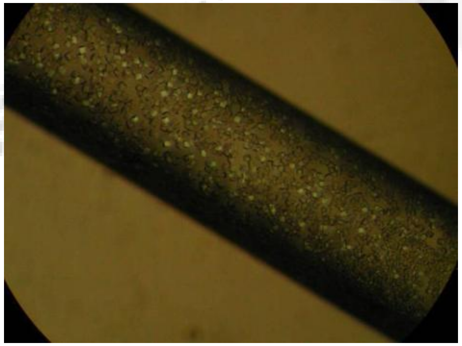
13 14 15

Protein Powder Samples







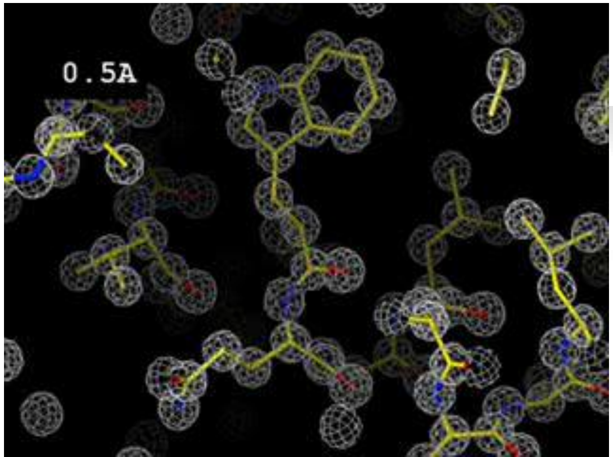


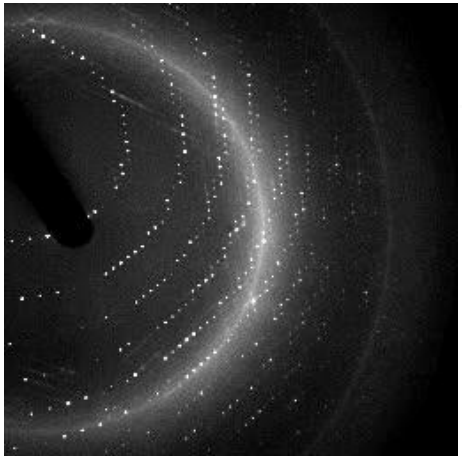
X-Ray Diffraction

We need to understand structure in terms of *particles* and *waves*

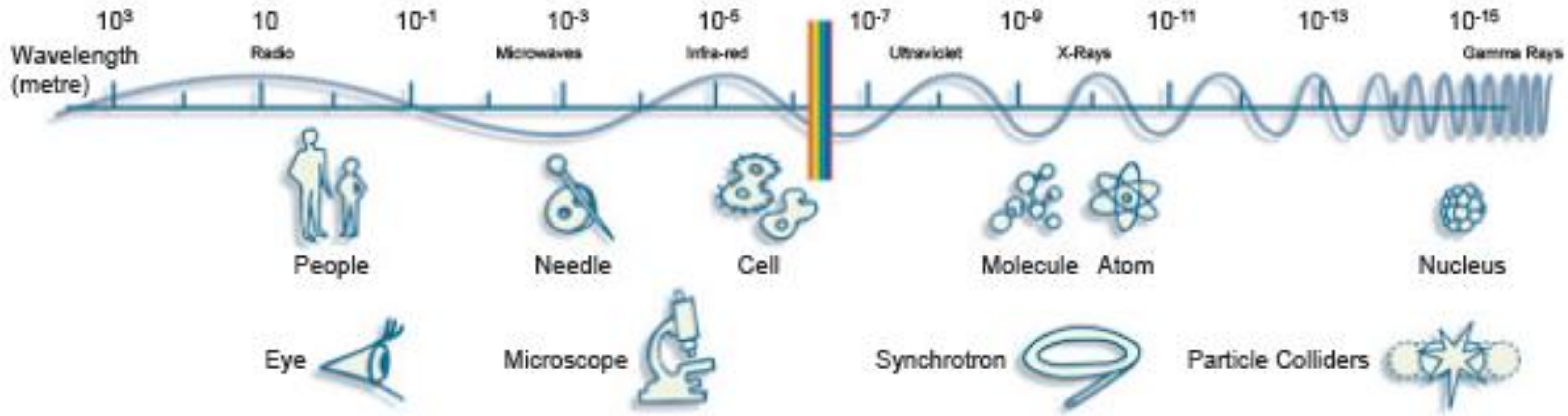
Density of matter at a given point in space
$\rho(\mathbf{r})$

Amplitude of a wave of given periodicity
$F(\mathbf{Q})$

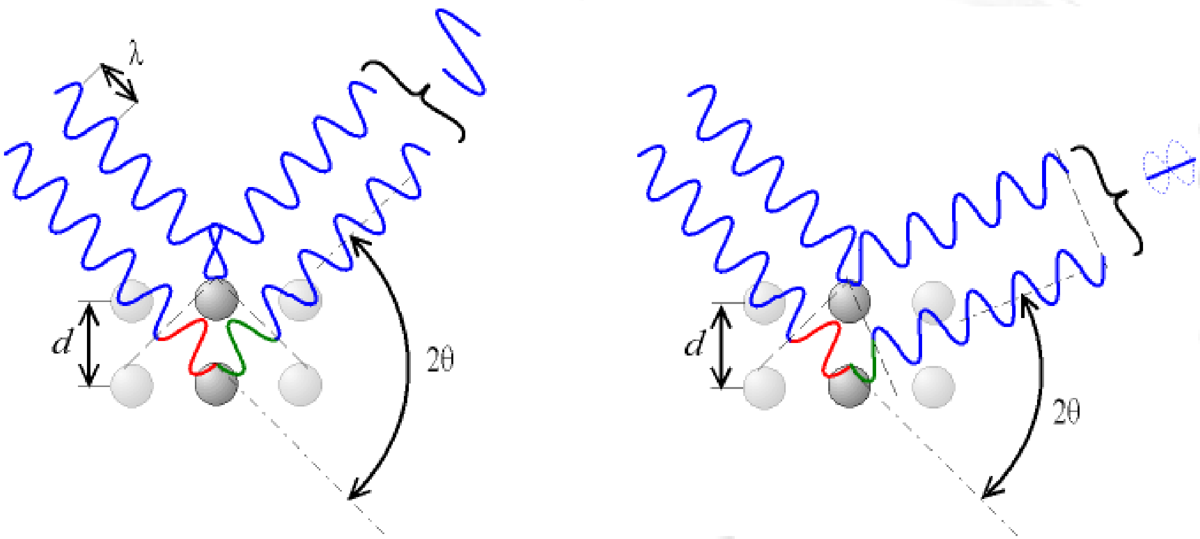




Γιατί χρειαζόμαστε ακτίνες Χ;

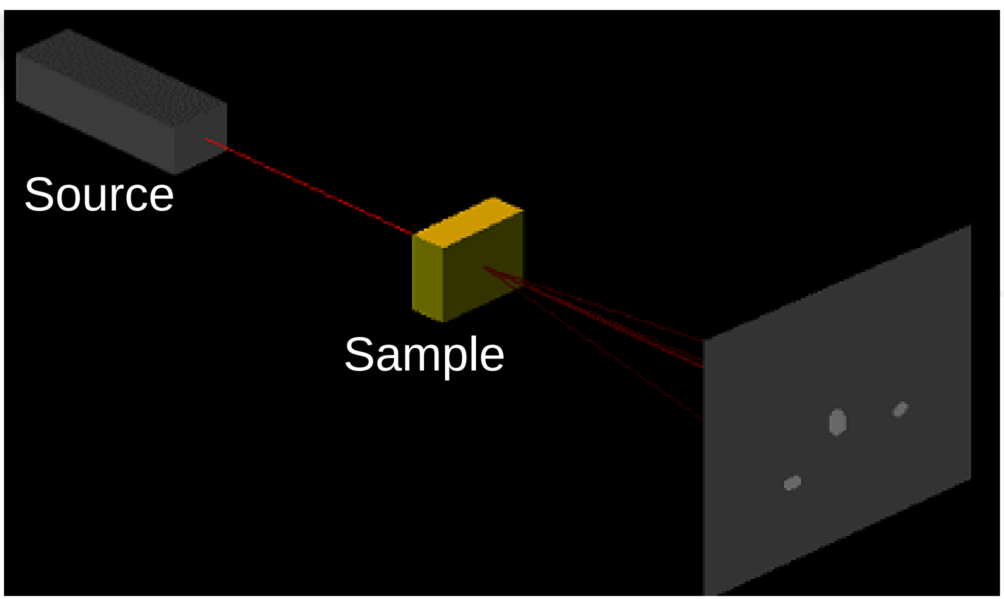


X-RAY DIFFRACTION

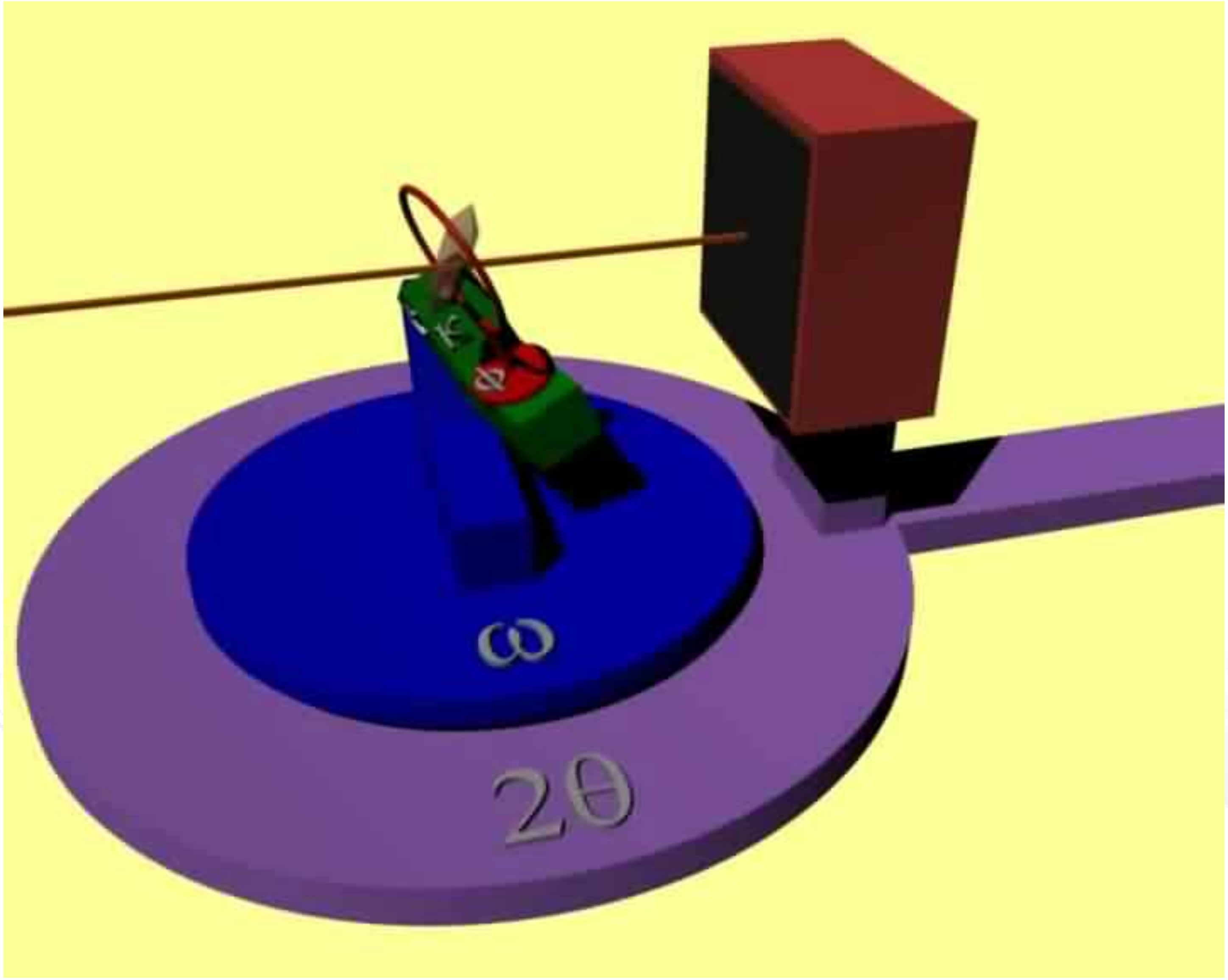


Bragg's Law:

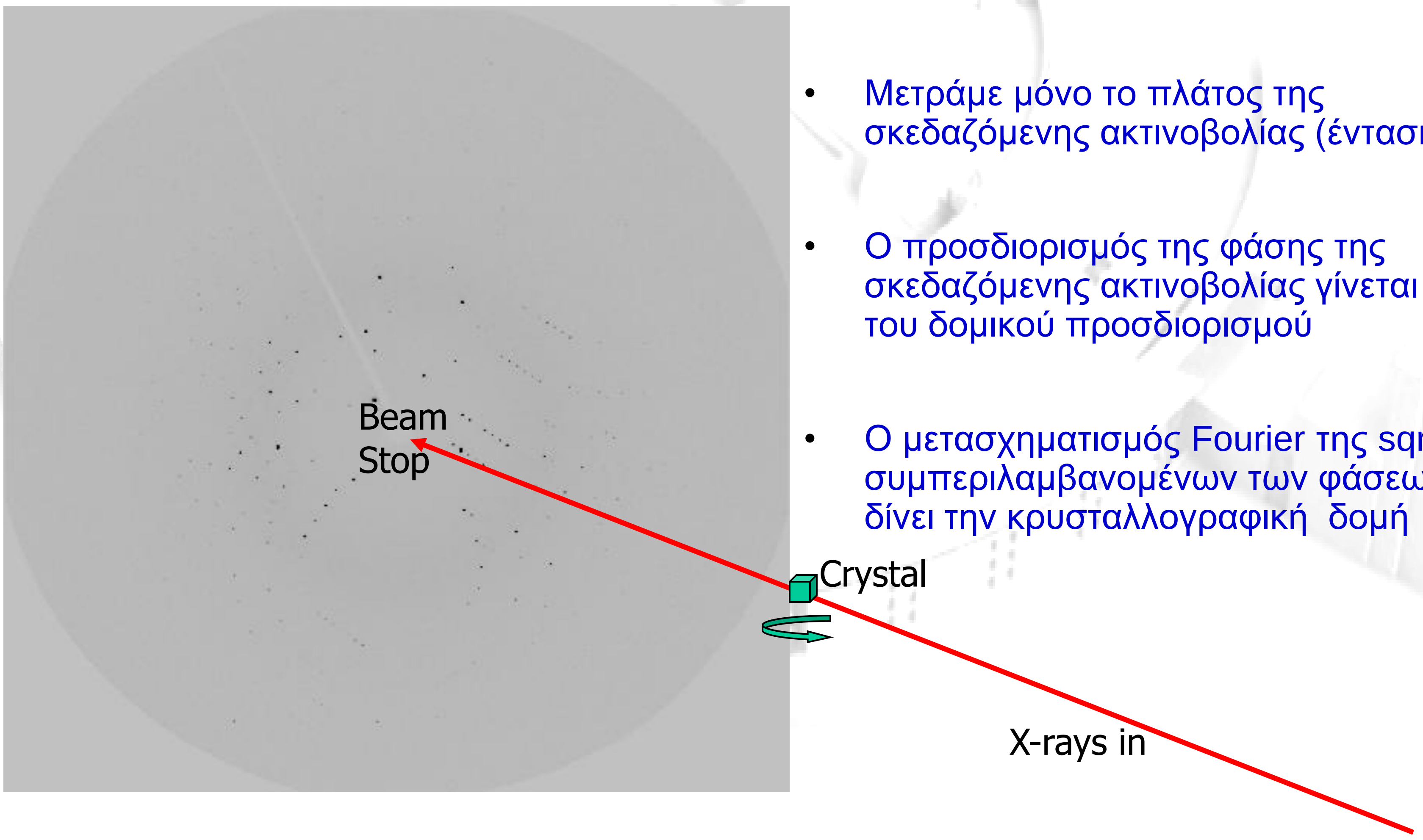
$$n\lambda = 2d\sin\theta$$



Max von Laue *discovered the diffraction of X-rays by crystals, a discovery for which he was awarded the* ***1914 Nobel Prize for Physics.*** The initial application of structure determination was developed first and foremost by the two English scientists, **Bragg** father and son, and as early as **1915 they were rewarded with the Nobel Prize for Physics**.


ω
2θ

Περίθλαση Ακτίνων Χ



- Οι ακτίνες Χ σκεδάζονται προς κατευθύνσεις οι οποίες σχετίζονται με το κρυσταλλογραφικό πλέγμα

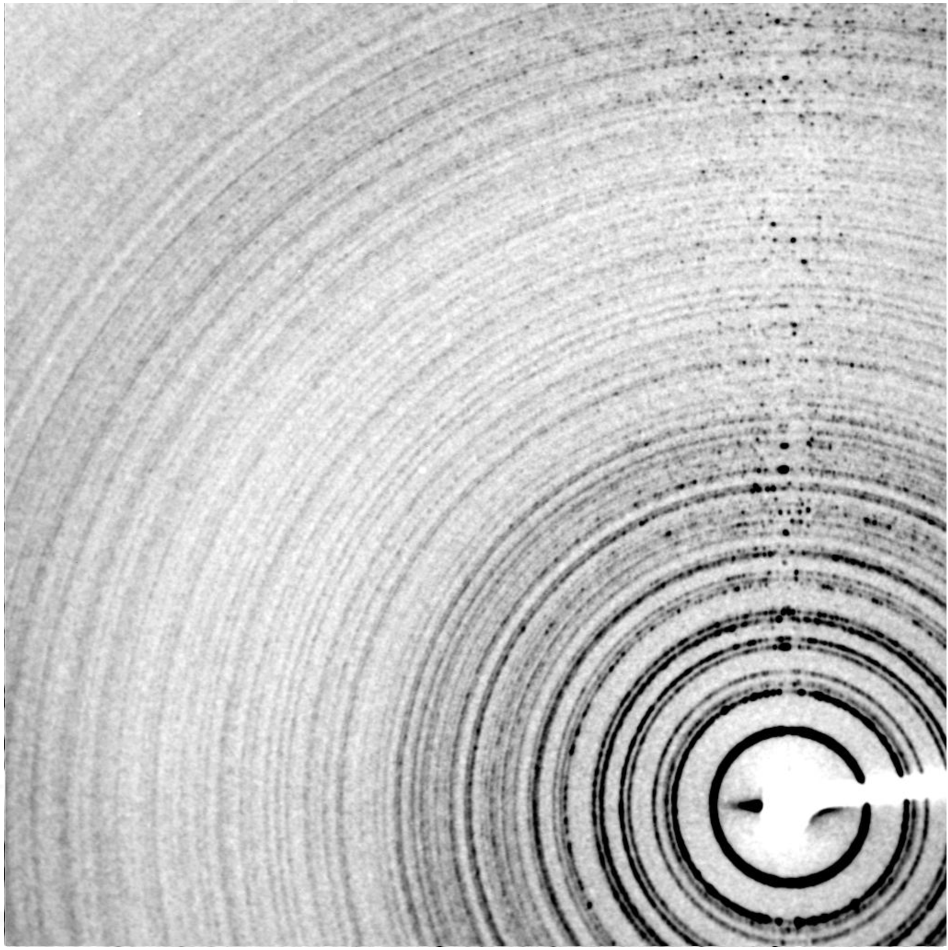
- Μετράμε μόνο το πλάτος της σκεδαζόμενης ακτινοβολίας (ένταση)

- Ο προσδιορισμός της φάσης της σκεδαζόμενης ακτινοβολίας γίνεται μέσω του δομικού προσδιορισμού

- Ο μετασχηματισμός Fourier της sqrt(I) συμπεριλαμβανομένων των φάσεων δίνει την κρυσταλλογραφική δομή

Many small single crystals make a powder

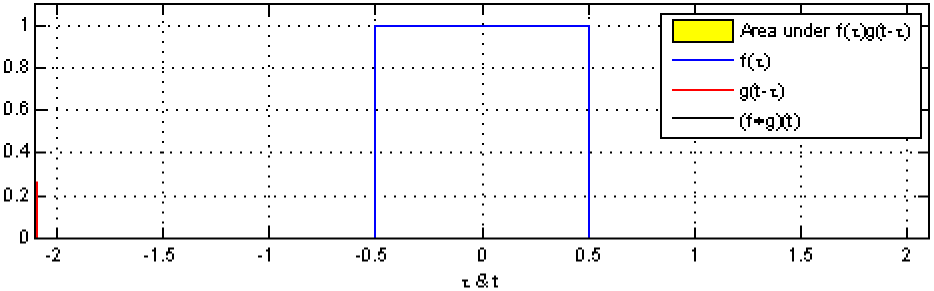
- » 1
- » 2
- » 3
- » 5
- » 10
- » 20
- » 50

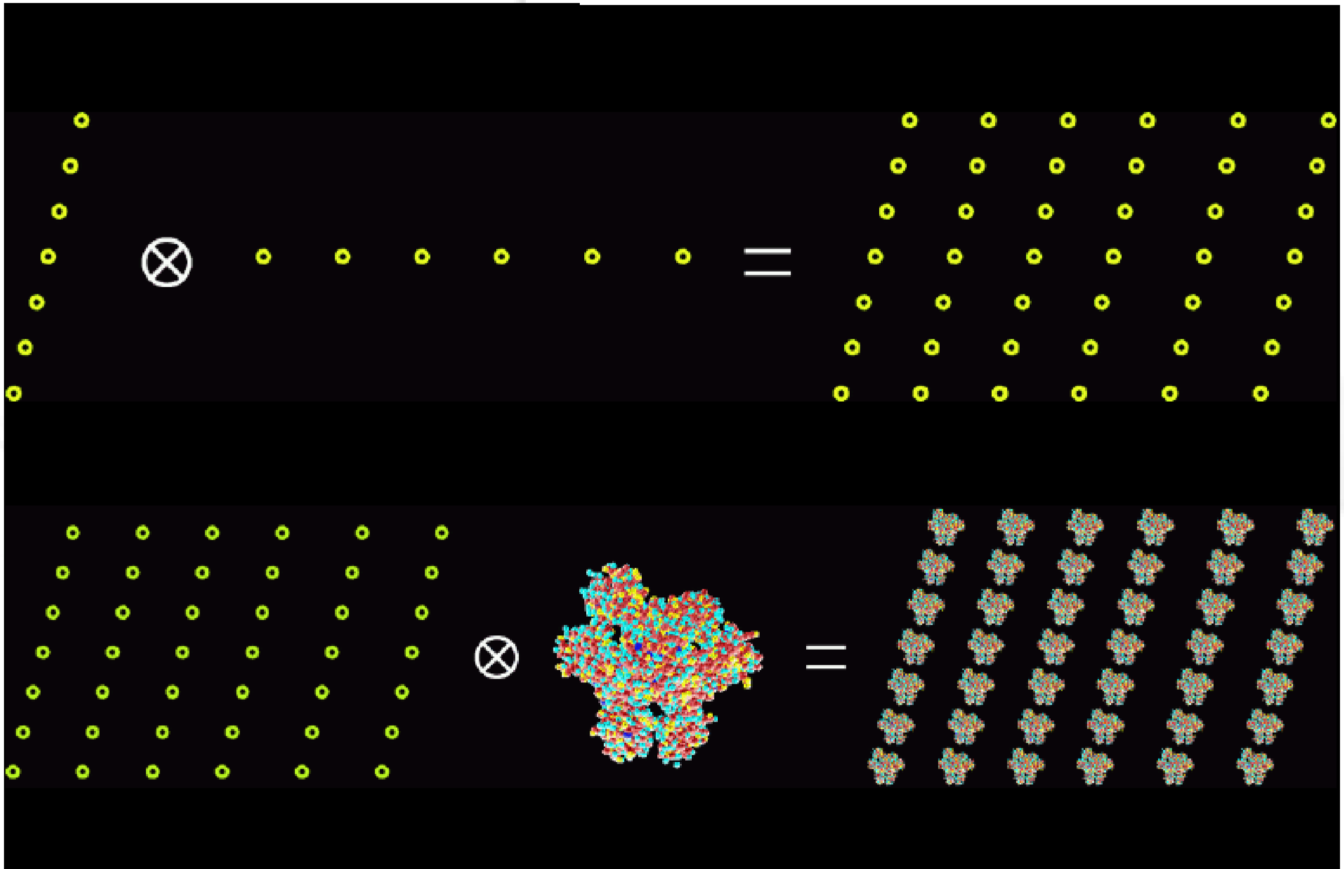


- Spots cover spheres in 3D reciprocal space
- 2D area detector takes a slice
- (on Ewald sphere)
- 1D powder scan measures distance from origin

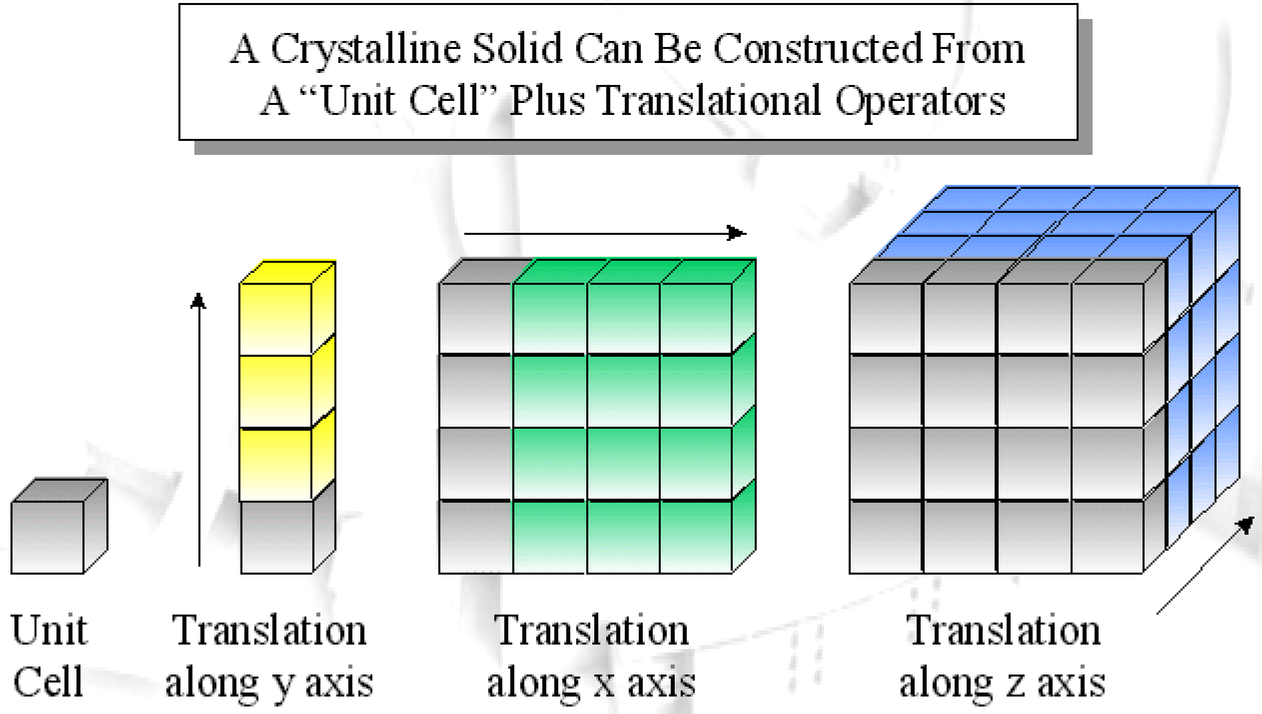
Το κρυσταλλογραφικό πλέγμα ως αποτέλεσμα συνέλιξης

Convolution of two square pulses: the resulting waveform is a triangular pulse. One of the functions (in this case *g*) is first reflected about τ = 0 and then offset by *t*, making it *g*(*t* − τ). The area under the resulting product gives the convolution at *t*. The horizontal axis is τ for *f* and *g*, and t for f*g .

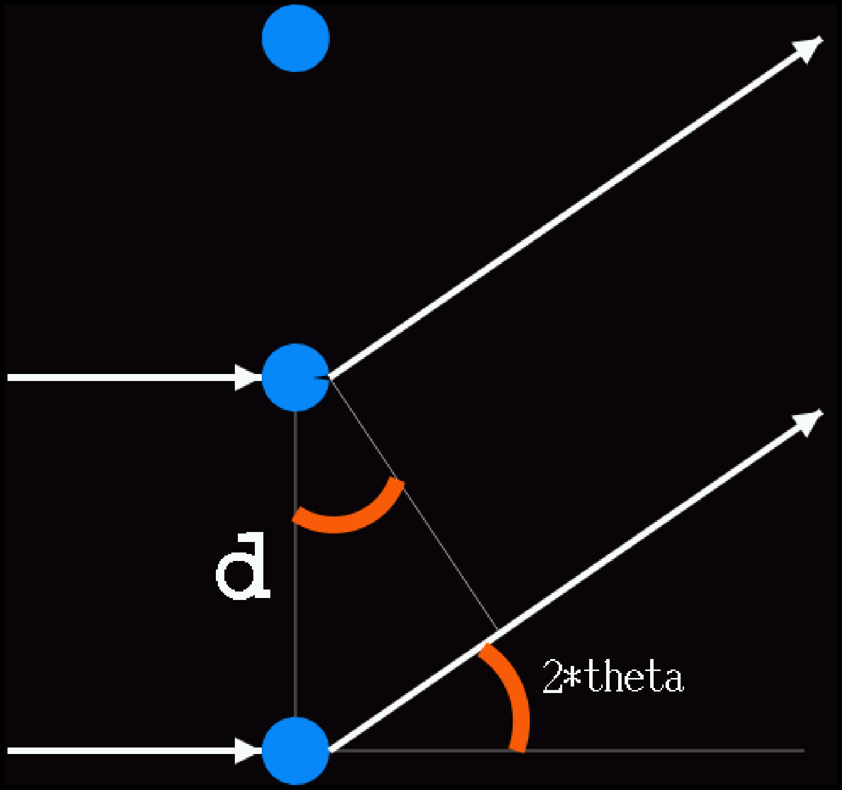




Crystal lattice (real space)



Το αντίστροφο πλέγμα



$$d \sin(2\theta) = n\lambda$$

(d) Crystal=lattice+basis

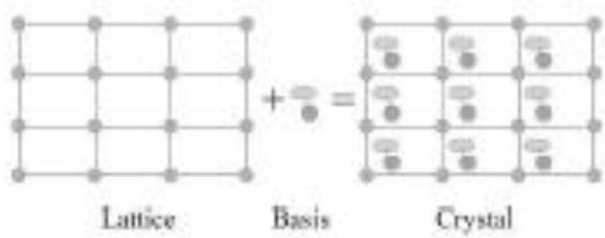
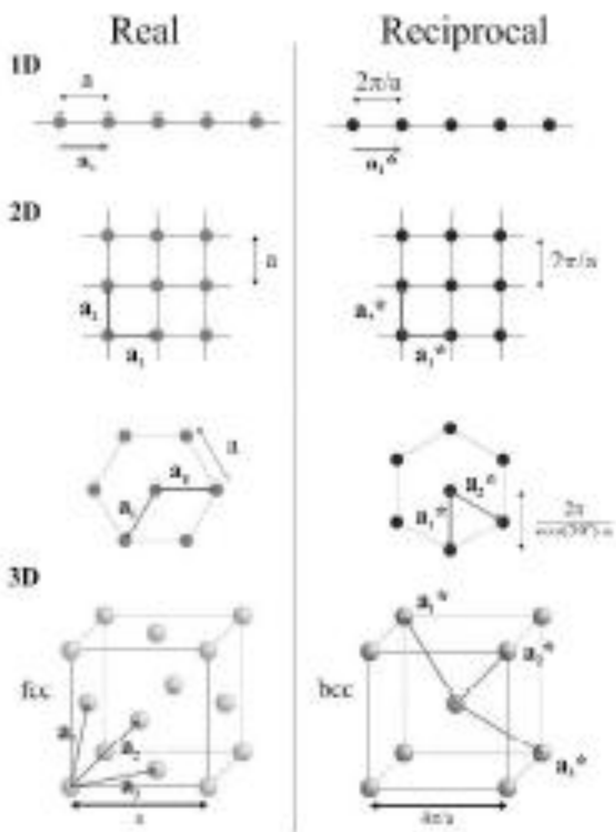


Fig. 7: Creation of a crystal structure using its lattice and basis



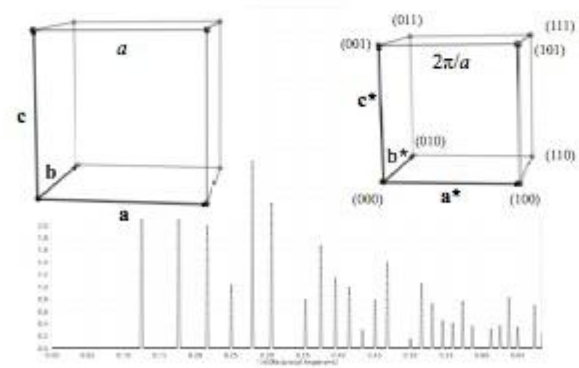
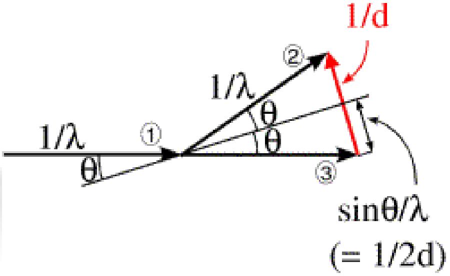
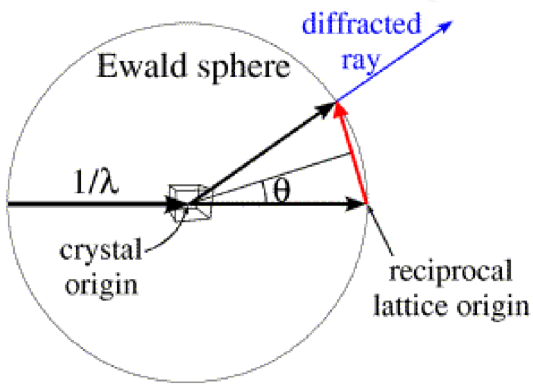
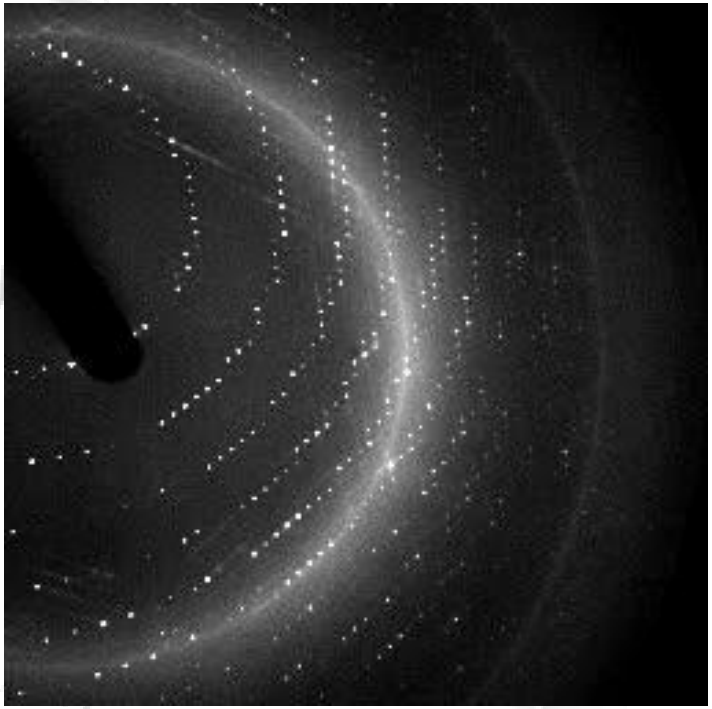


Fig. 8: Examples for direct and reciprocal lattice in one, two and three dimensions.

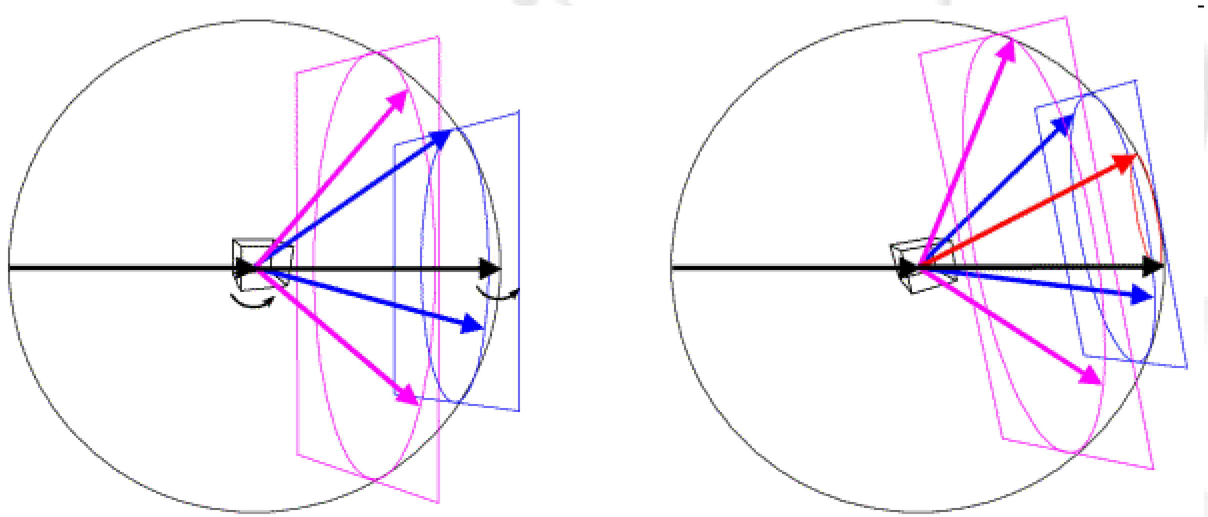
The Ewald sphere



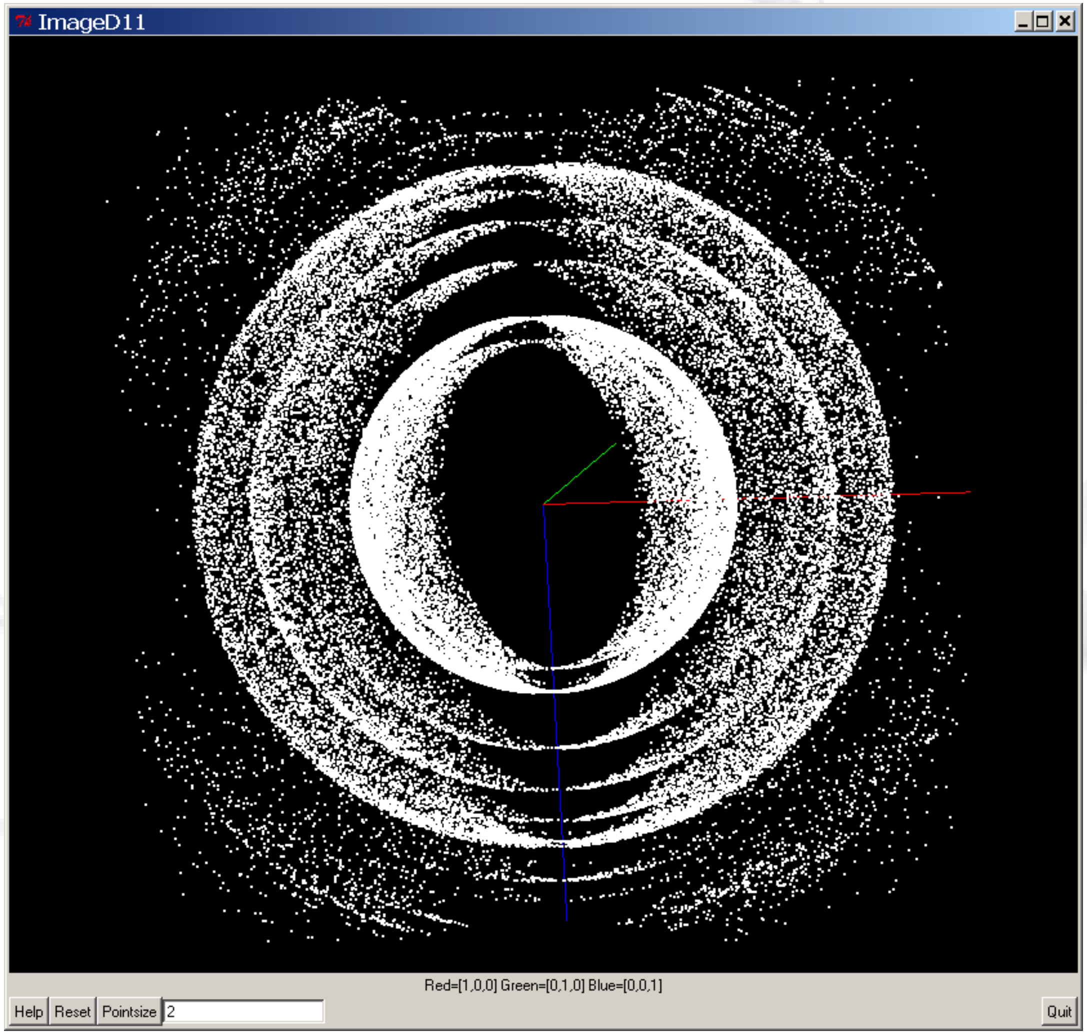




The Ewald sphere

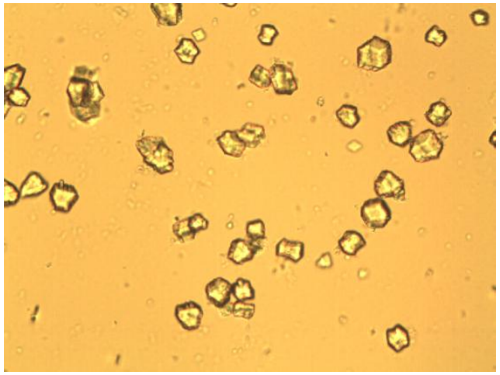


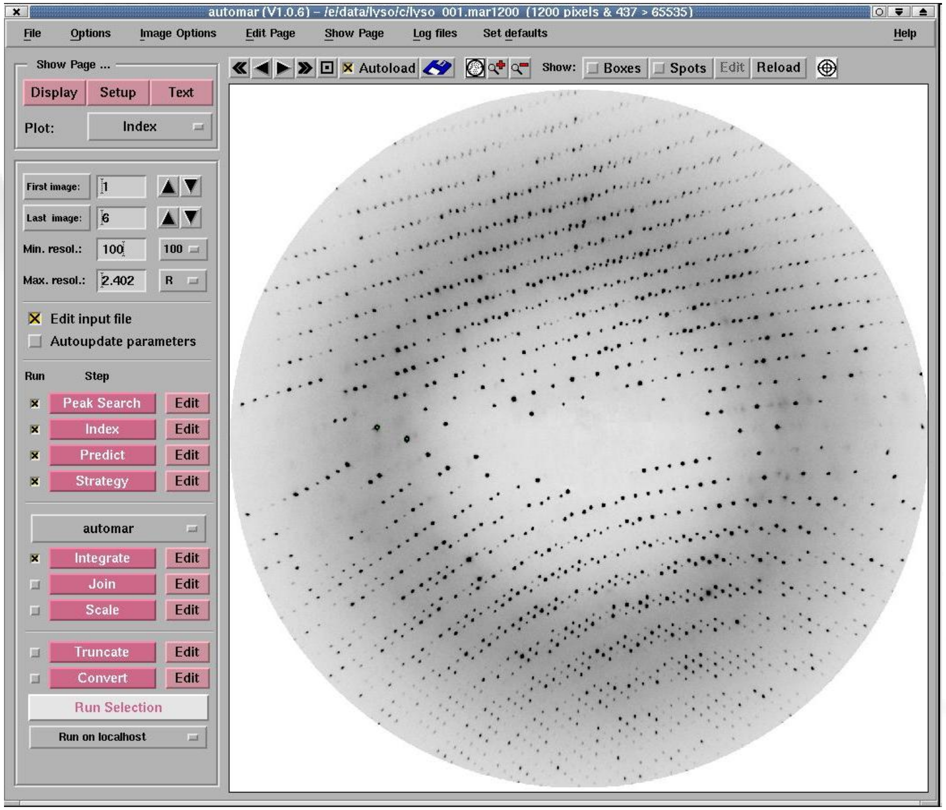
3D αναπαράσταση περίθλασης ακτίνων Χ από μονοκρύσταλλο

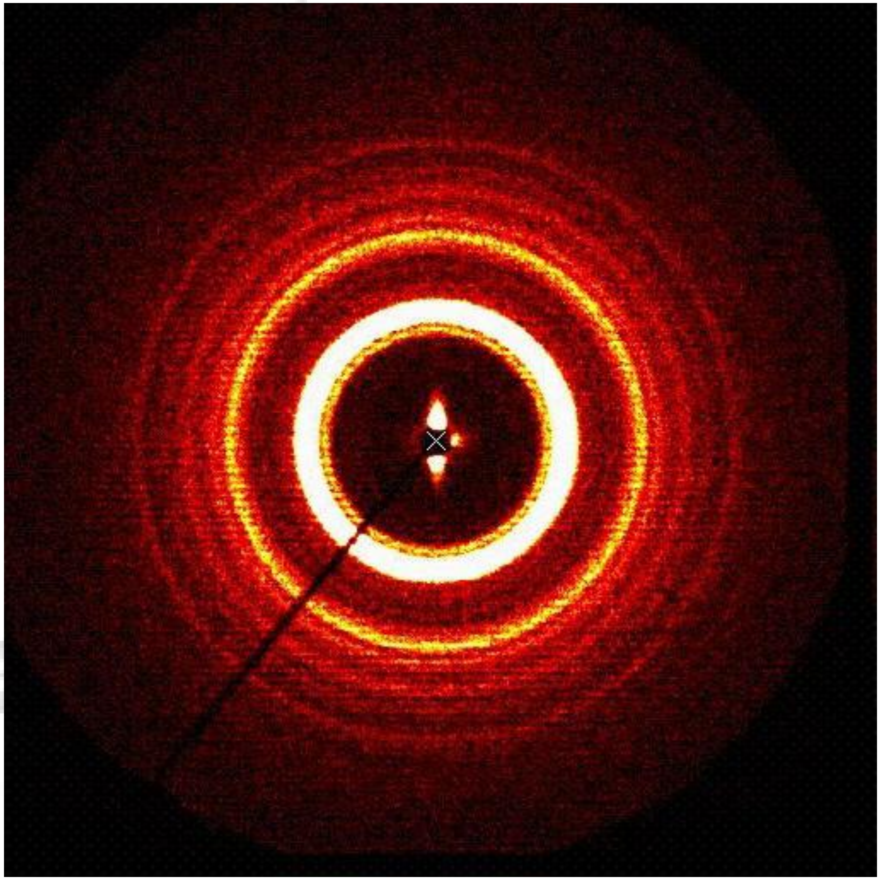


Περίθλαση από κρύσταλλο

$$\vec{\mathrm{F}}_{hkl} = \sum_i f_{i,\mathrm{h}} e^{2\pi i(hx+ky+lz)}$$







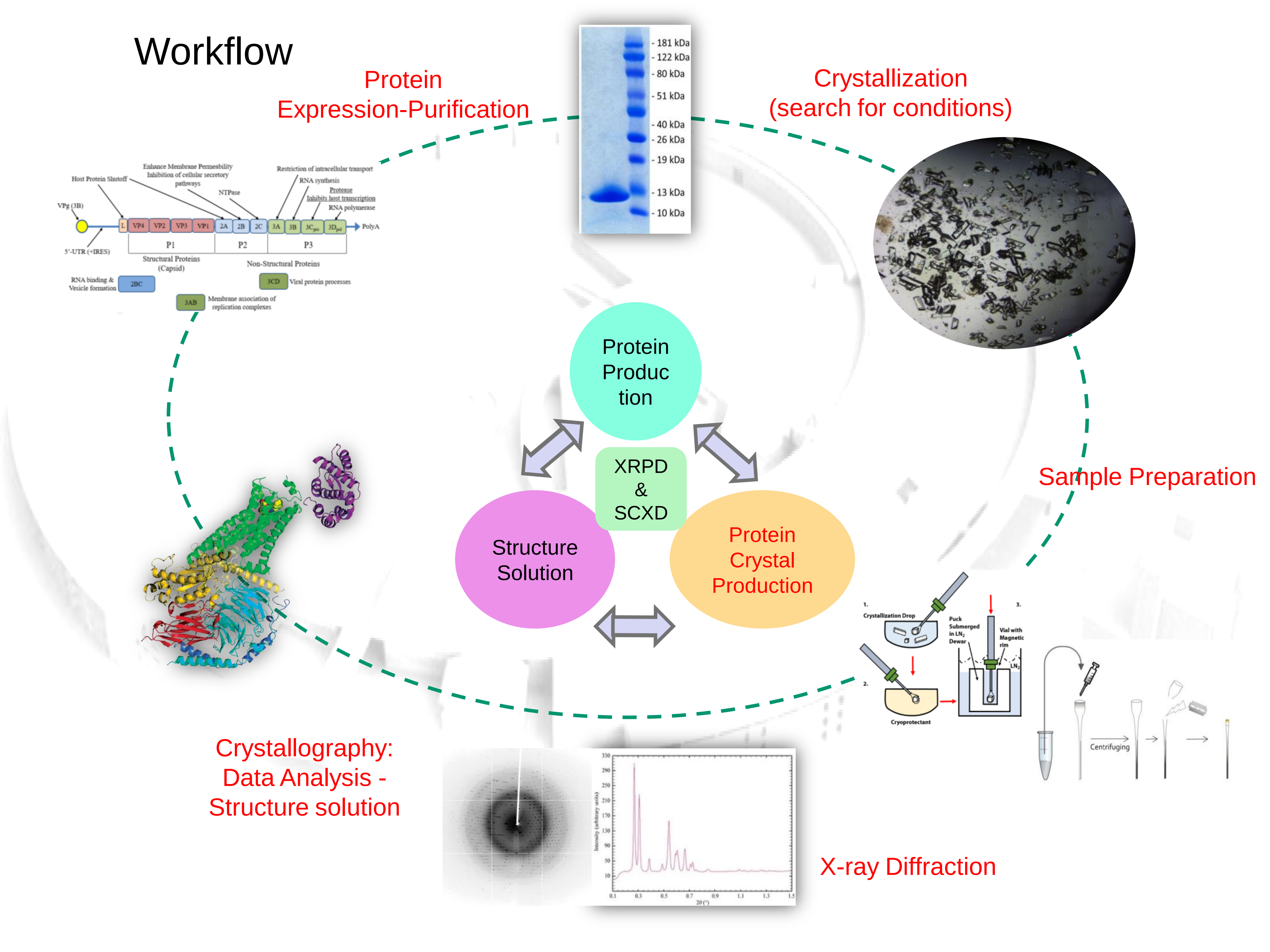
F(**Q**) is a complex number with a *magnitude* and a *phase*

$$F(\mathbf{Q}) = |F(\mathbf{Q})| \times \exp(i\phi)$$

The Phase Problem

Crystallography

Crystallography


Workflow
Protein
Expression-Purification
- 181 kDa
- 122 kDa
- 80 kDa
- 51 kDa
- 40 kDa
- 26 kDa
- 19 kDa
- 13 kDa
- 10 kDa
Crystallization
(search for conditions)
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)
5'-UTR (+IRES)
PolyA
L
VP4
VP2
VP3
VP1
2A
2B
2C
3A
3B
P1
P2
P3
Structural Proteins
(Capsid)
Non-Structural Proteins
RNA binding &
Vesicle formation
2BC
3CD
Viral protein processes
3AB
Membrane association of
replication complexes
Protein
Produc
tion
XRPD
&
SCXD
Structure
Solution
Protein
Crystal
Production
Sample Preparation
1.
Crystallization Drop
Puck
Submerged
in LN2
Dewar
3.
Vial with
Magnetic
rim
2.
Cryoprotectant
Centrifuging
Crystallography:
Data Analysis -
Structure solution
X-ray Diffraction

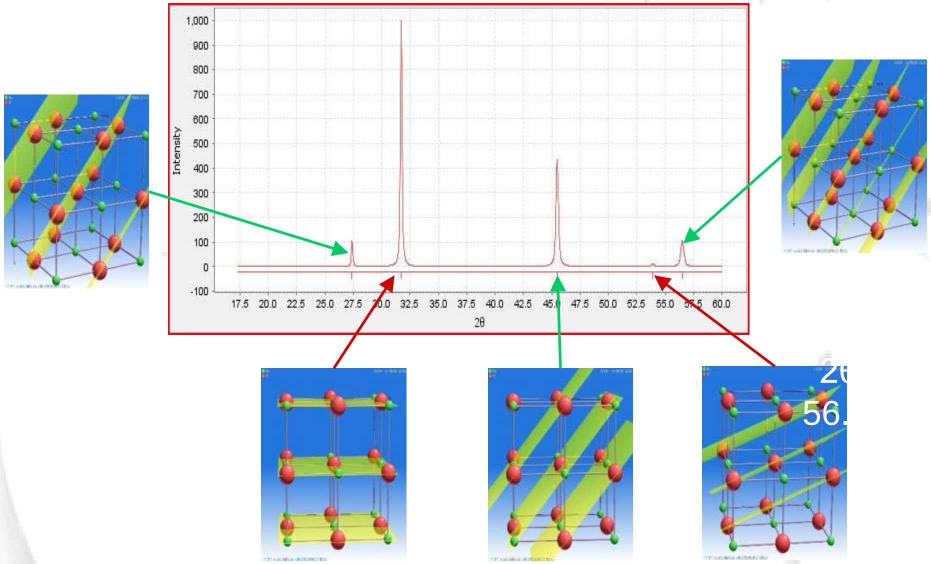
Κρυσταλλογραφία

The space groups in three dimensions are made from combinations of the **32 crystallographic point groups** with the **14 Bravais lattices** which belong to one of **7 crystal systems**. This results in a space group being some combination of the translational symmetry of a unit cell including lattice centering, and the point group symmetry operations of reflection, rotation and improper rotation (also called rotoinversion). Furthermore one must consider the screw axis and glide plane symmetry operations. These are called compound symmetry operations and are combinations of a rotation or reflection with a translation less than the unit cell size. The combination of all these symmetry operations results in a total of **230 unique space groups describing all possible crystal symmetries.**

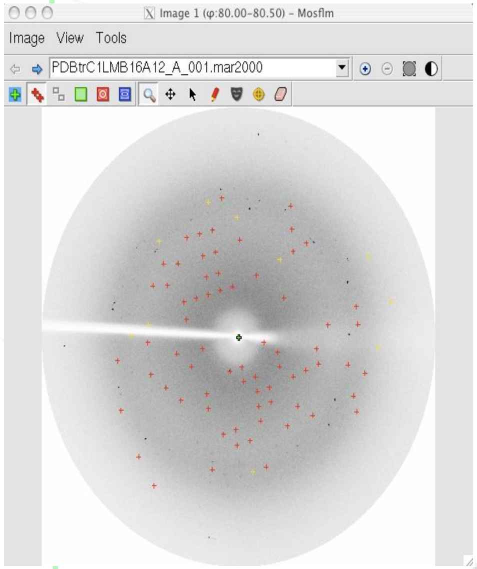
Διεθνής Ένωση Κρυσταλλογράφων – International Union of Crystallography (IUCr)
http://www.iucr.org/

Data Analysis-Indexing

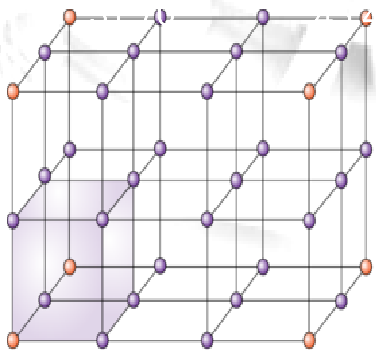
XRPD



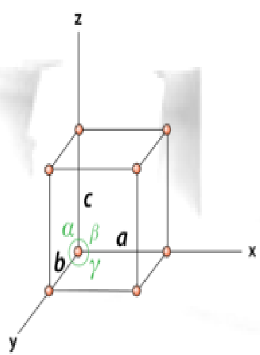
SCXD



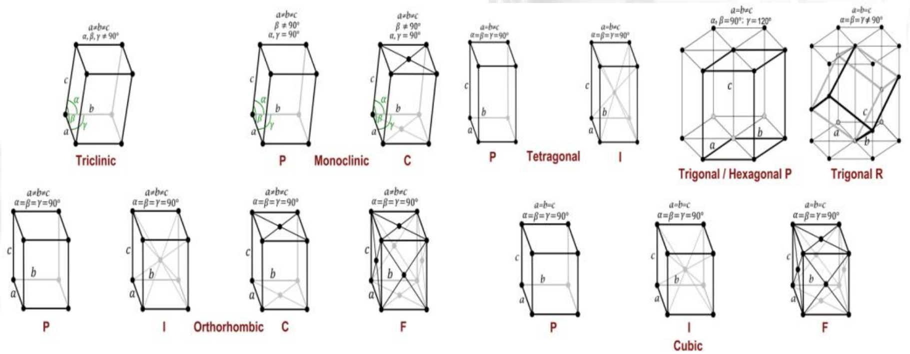
1. Spot finding and autoindexing
2. Parameter refinement
3. Integration
4. Scaling and merging.



Crystal Lattice

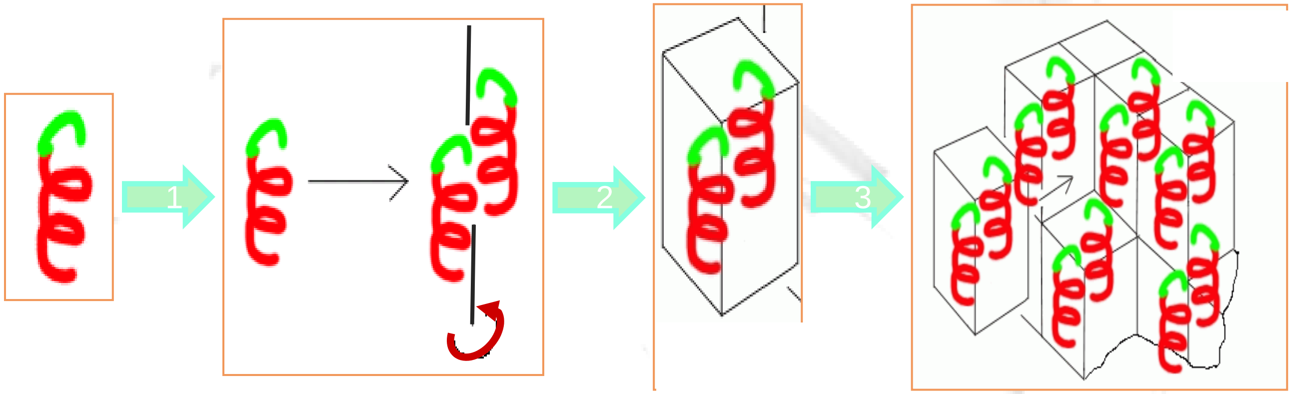


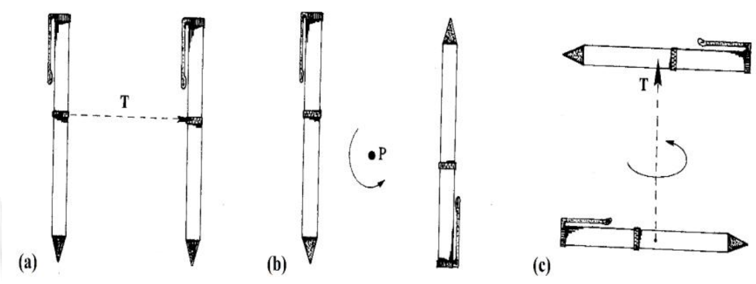
Unit Cell



Data Analysis

www.ruppweb.org/Xray/tutorial

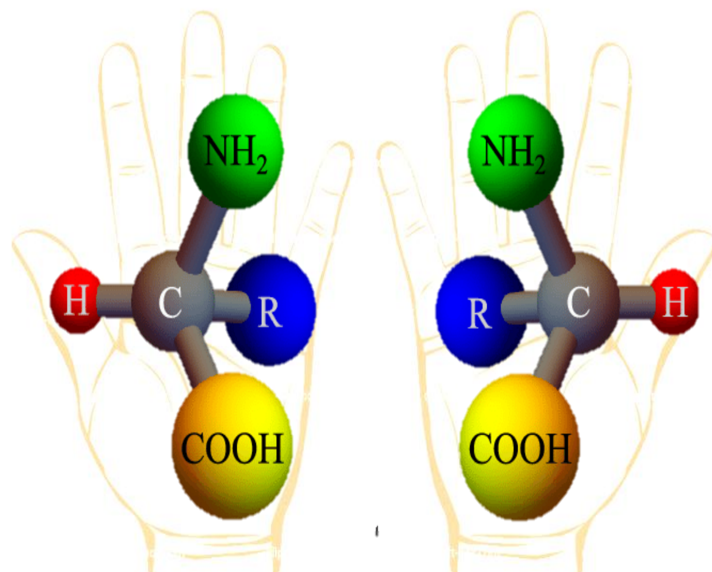




Space group

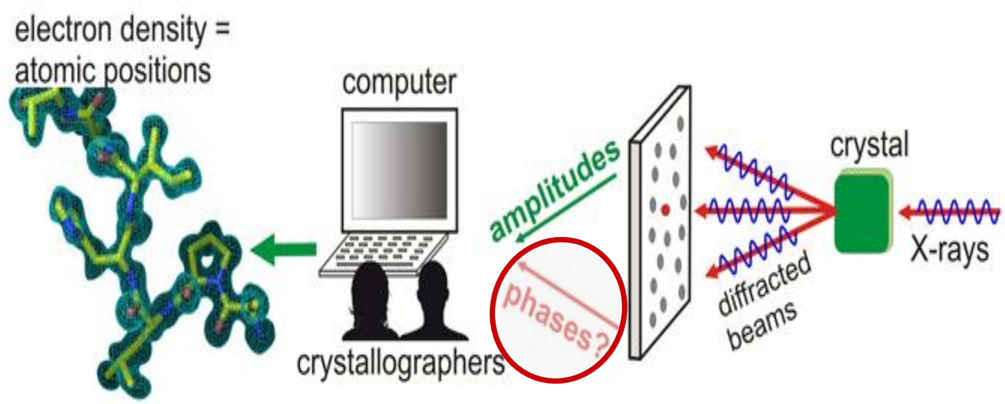
$P\,1\;2_1\;1$

Symmetry in terms of operators

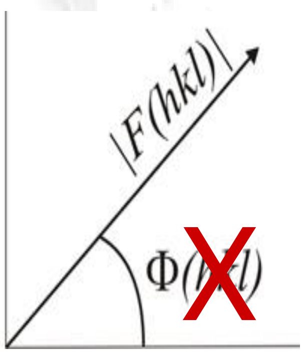


Data Analysis – Molecular Replacement

www.dictionary.iucr.org/Molecular_replacement | www.xtal.iqfr.csic.es

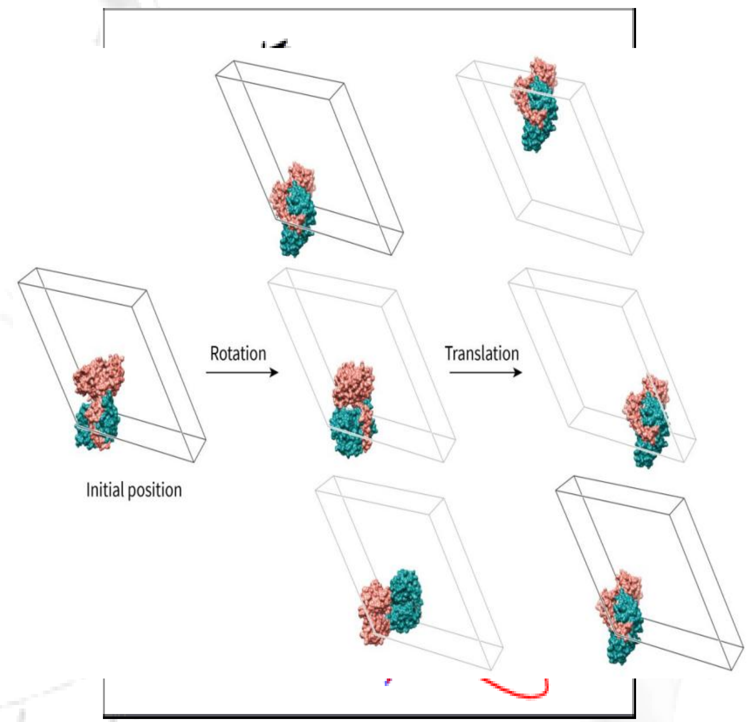


$$I_{hkl} \propto |F_{hkl}|^2$$



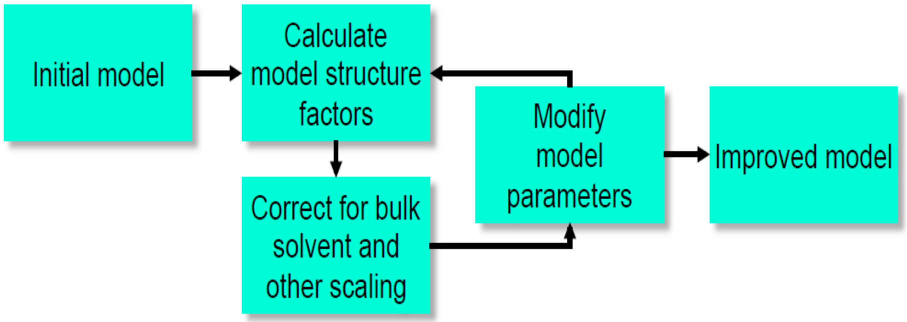
"Structure factor, F(hkl), is the resultant of all waves scattered in the direction of the hkl reflection by the n atoms contained in the unit cell."

The structure factors F(hkl) are waves and therefore can be represented as vectors by their amplitudes, [F(hkl)], and phas es Φ(hkl) measured on a common origin of phases.



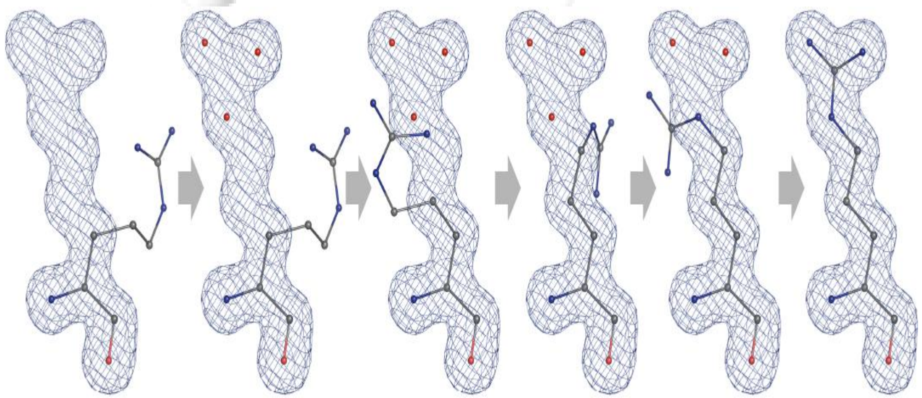
Spiliopoulou, Triandafillidis *et al.*, 2020, *Crystal Growth & Design*

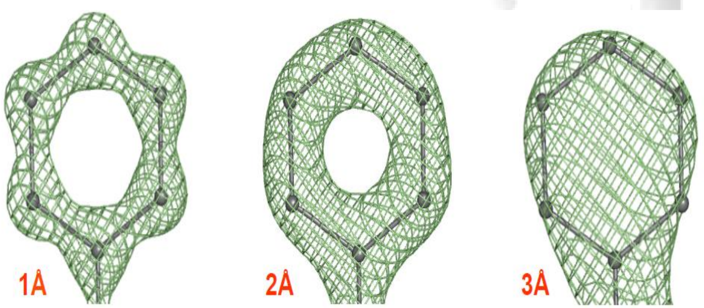
Data Analysis – Structure Refinement



- MR is the most frequently used method for structure solution.
- Coordinates are not always quite accurate

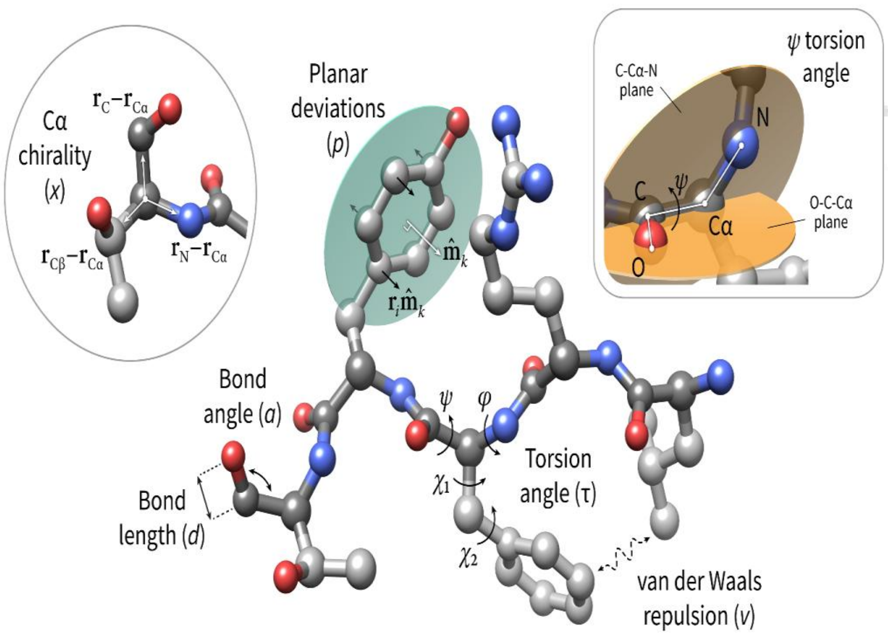
Better phases can be calculated from the atomic positions, which allow re-determining of the electron density function with a higher precision. From the new electron density map, more accurate atomic positions can be derived, which lead to even better phase angles, and so forth.

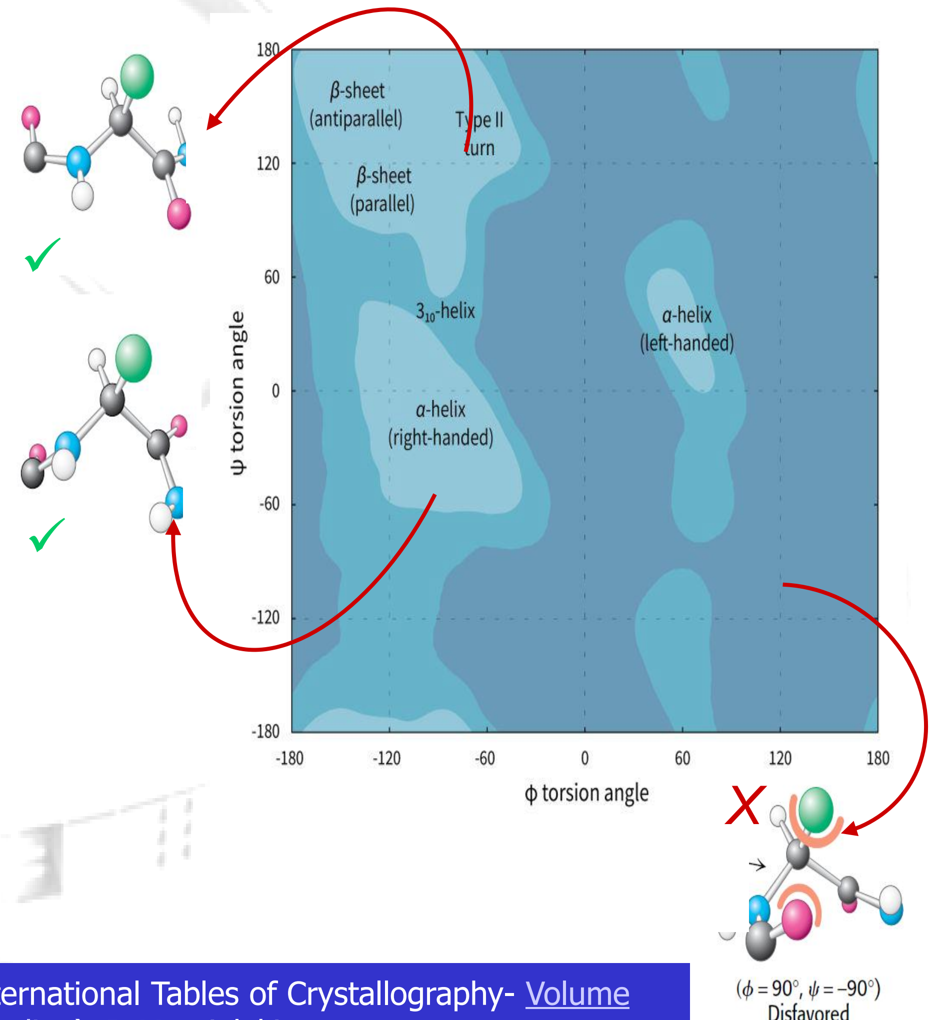




https://ocw.mit.edu/courses/chemistry | https://www.phenix-online.org/presentations/

Data Analysis – Structure Refinement





- **Phi (φ)** C-Ca-N-C torsion angle.
- **Psi (ψ)** O-C-Ca-N torsion angle.

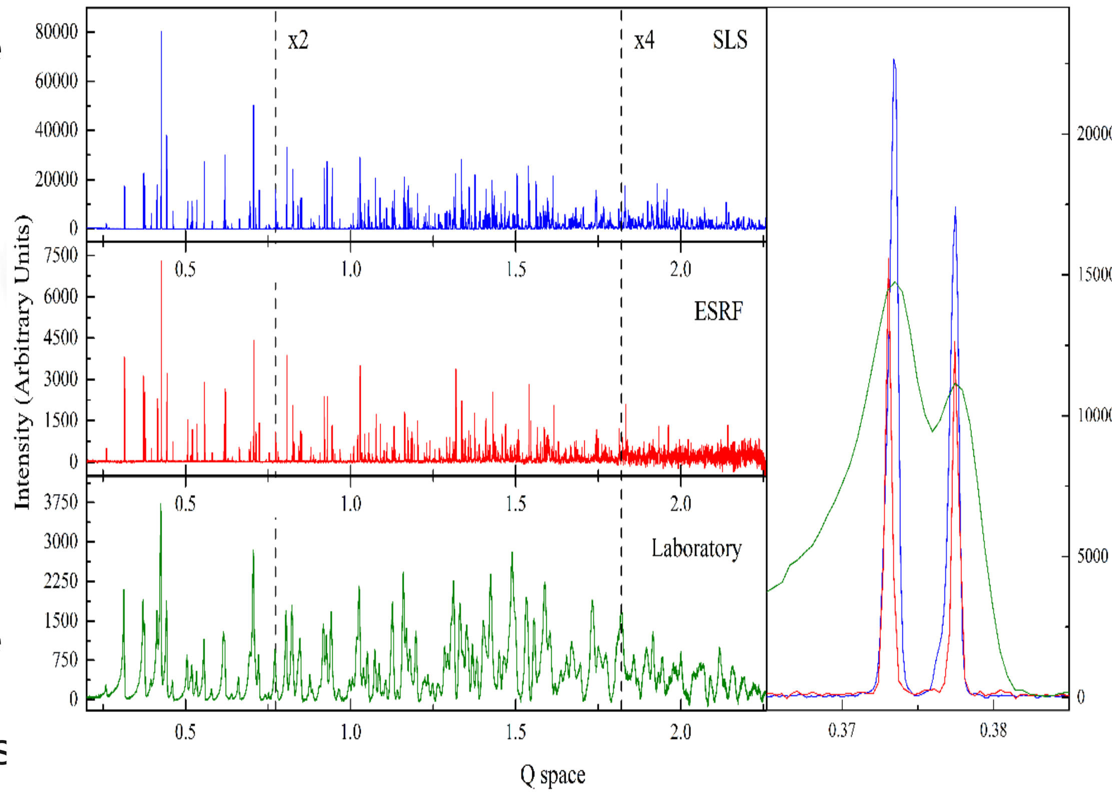
"Macromolecular Powder Diffraction", Book Chapter for the International Tables of Crystallography- Volume H: Powder Diffraction, chapter 7.1, 718-736, 2019, (available online), I. Margiolaki

Spiliopoulou, Triandafillidis *et al*., 2020, *Crystal Growth & Design*

Synchrotron Vs Laboratory X-ray sources

✓ Pros
- Available at the lab
- Quick crystal screening
- Low levels of radiation damage
- Longer experiments monitoring time resolve phenomena

X Cons
- Low brilliance/Low energy
- Fix wavelength
- Low data quality



✓ Pros
- Extremely brilliant source
- High energy beams - penetrate deeper into matter
- Small and tunable wavelengths
- Incomparable data resolution

X Cons
- Limited access - High cost/hour
-

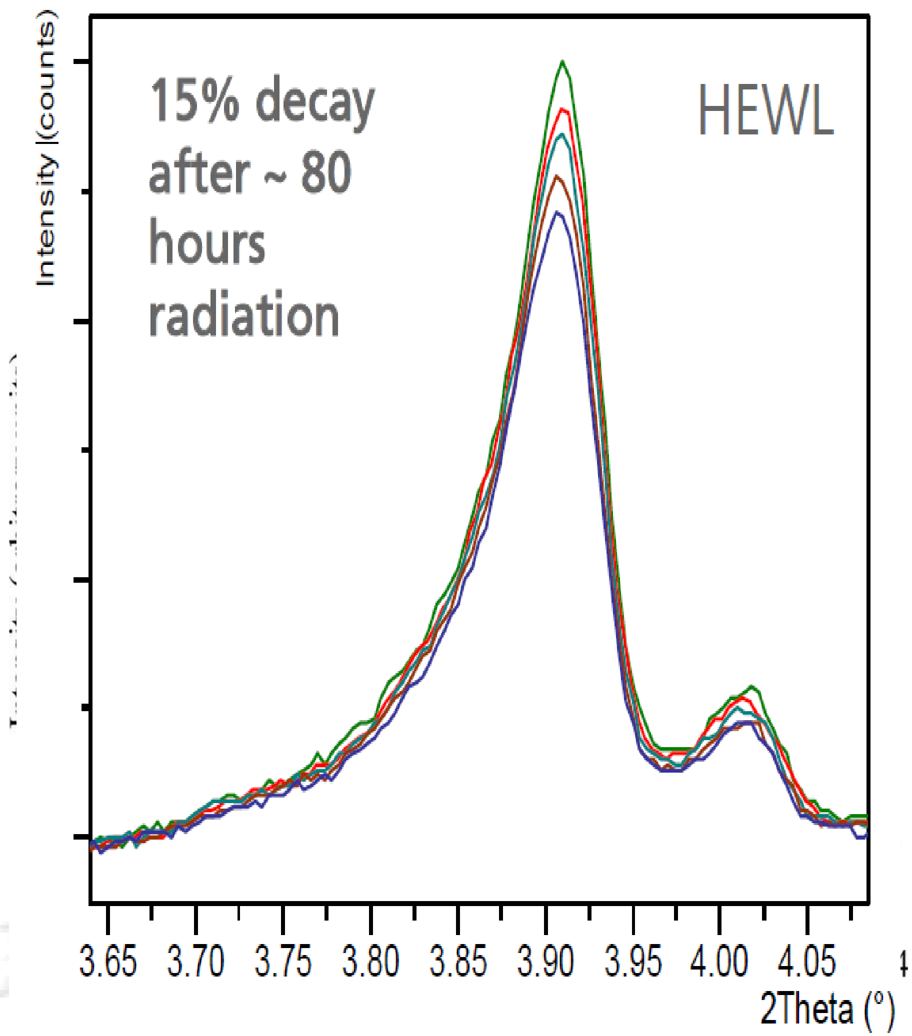
Laboratory XRPD - the challenge

Proteins are challenging samples:

- Weak scatter
  - ➢ *High intensity required (& low background)*
  - ➢ *Linear detector / area detector (with high resolution)*

- Large molecules / cells
  - ➢ *Good low angle performance (peak position & asymmetry/ resolution)*
  - ➢ *High angular resolution*

- Often not stable under radiation



ESRF **Vs SLS**

Angular resolution (ESRF > SLS)

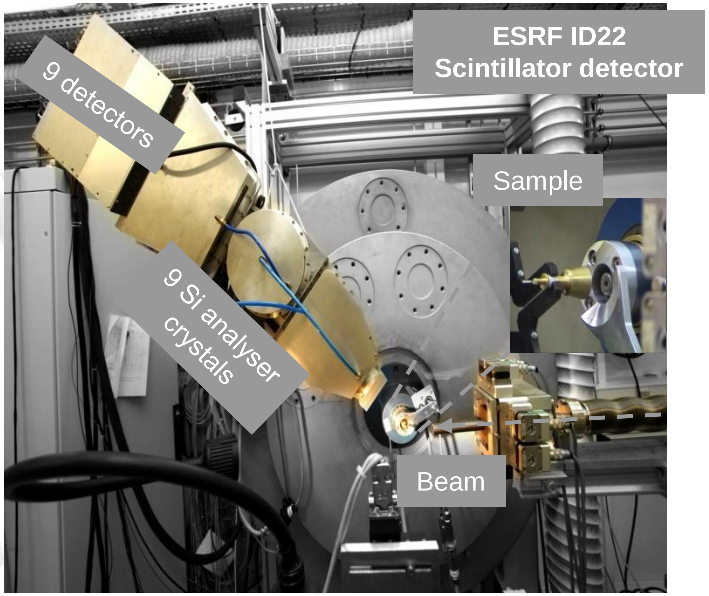
How sharp are the peaks?

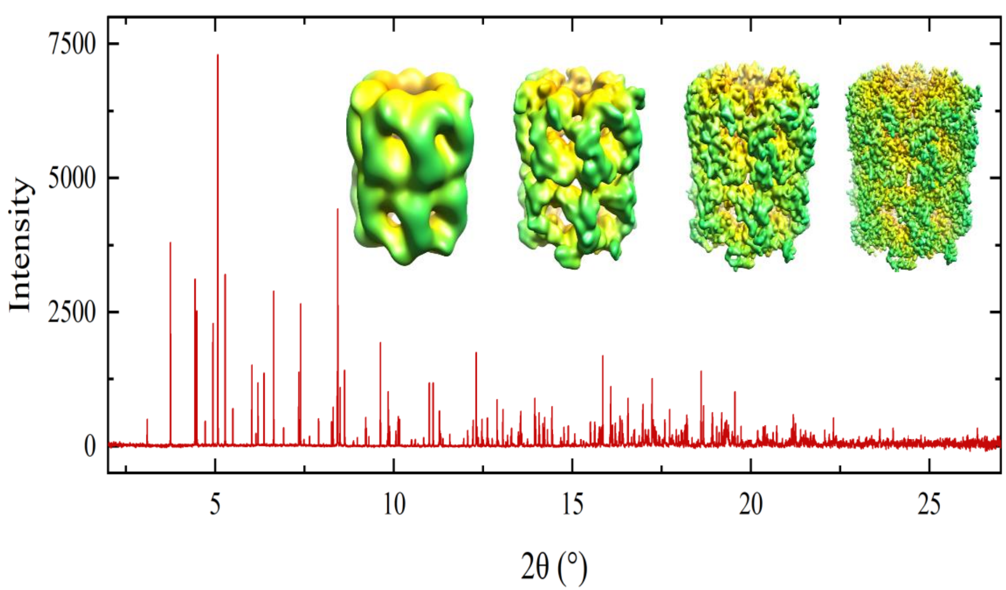
- Accurate extraction of overlapping peak intensity

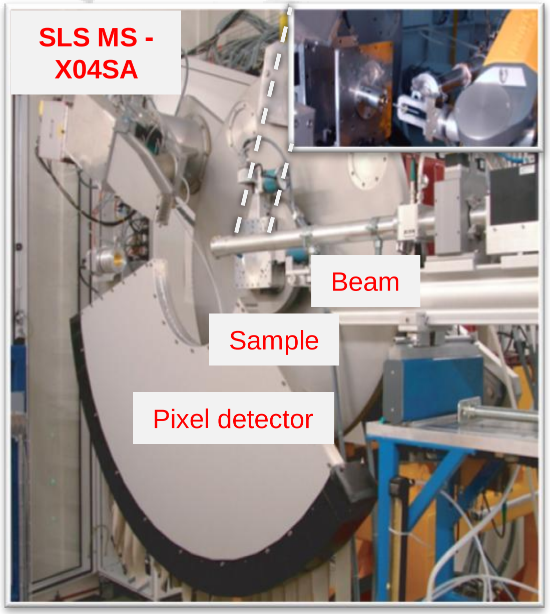
d-spacing resolution (ESRF < SLS)

How many peaks can be observed?

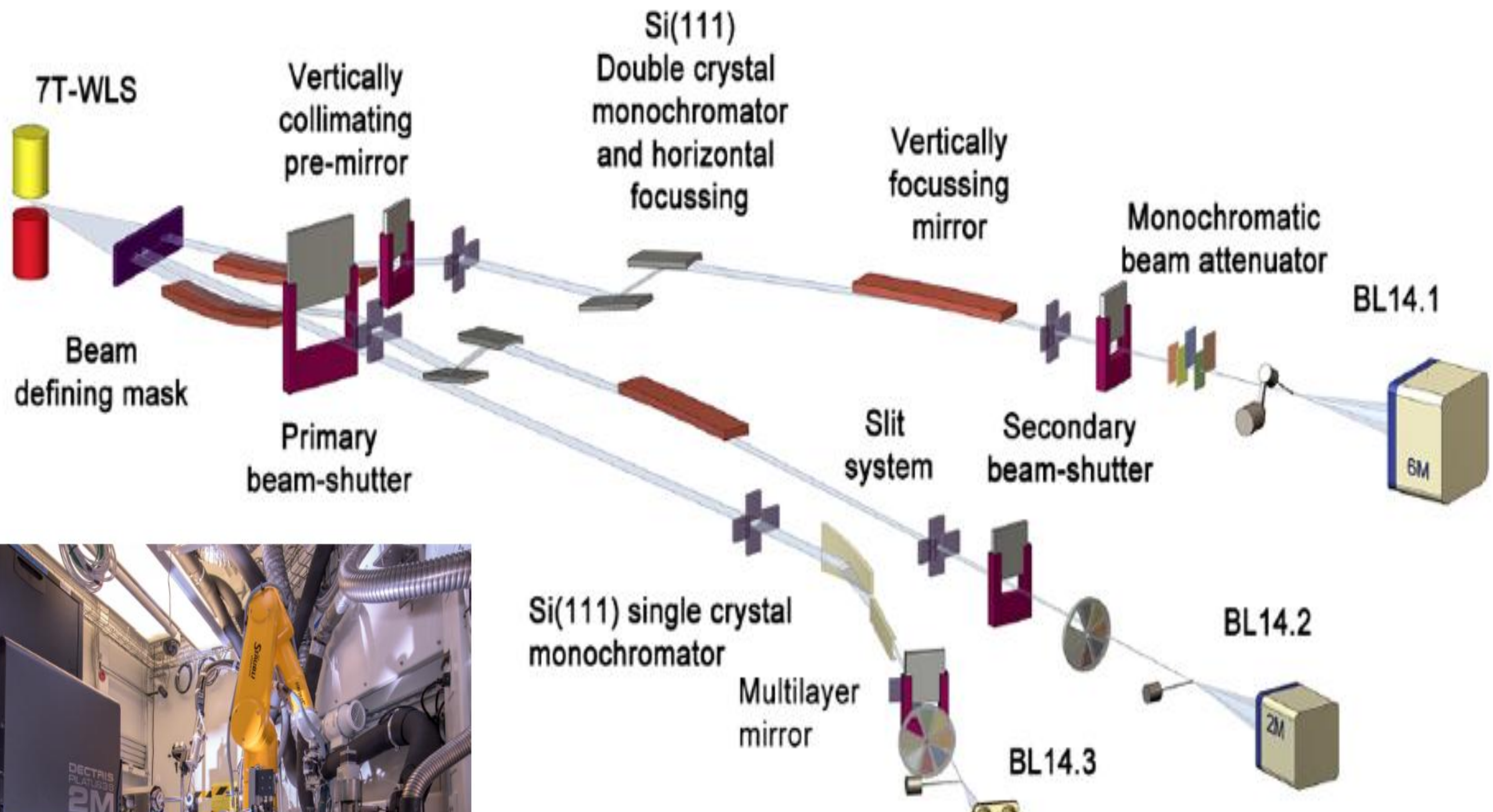
- More detailed electron density maps



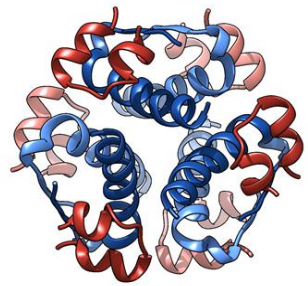


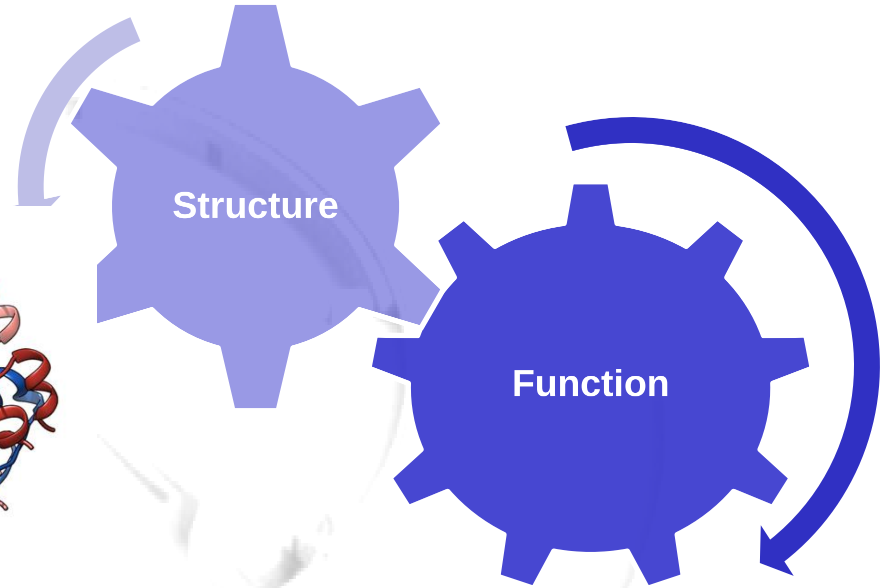


Experimental Hutch - SCXD



Aim



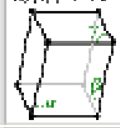
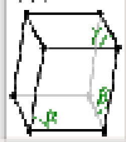
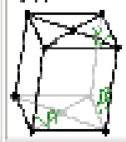
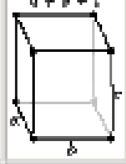
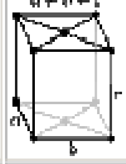
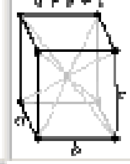
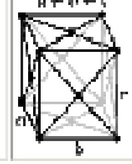
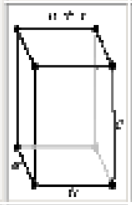
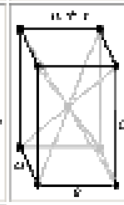
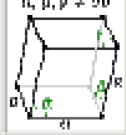
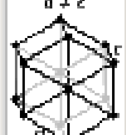
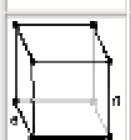
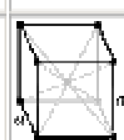
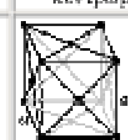


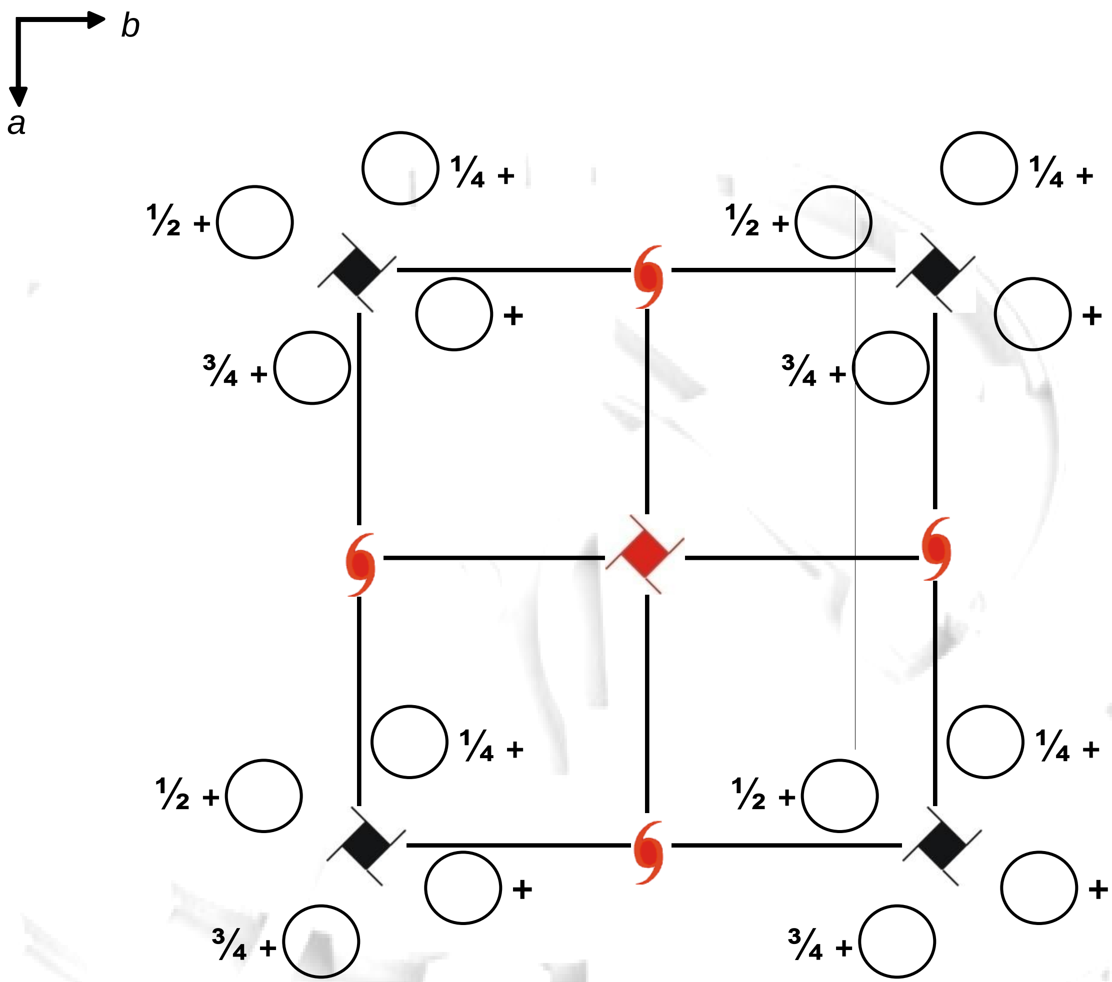
Lead Finding and Optimization → Pre -Clinical Development → Clinical Development Phase 1-3 → Manufacturing

- Selectivity, potency and efficacy evaluation
- Developability

- Phase selection and identification
- Monitoring Crystallization Processes

- Amelioration of production
- Product Quality

Κρυσταλλικό σύστημα	Κρυσταλλικά πλέγματα			
Τρικλινές	$\alpha, \beta, \gamma \neq 90°$ 			
Μονοκλινές	απλό	κεντρωμένο		
	$\alpha \neq 90°$ $\beta, \gamma = 90°$ 	$\alpha \neq 90°$ $\beta, \gamma = 90°$ 		
Ορθορομβικό	απλό	μονοεδρικά κεντρωμένα	ενδοκεντρωμένο	ολοεδρικά κεντρωμένο
	$a \neq b \neq c$ 	$a \neq b \neq c$ 	$a \neq b \neq c$ 	$a \neq b \neq c$ 
Τετραγωνικό	απλό	ενδοκεντρωμένο		
	$a \neq c$ 	$a \neq c$ 		
Ρομβοεδρικό	$\alpha, \beta, \gamma \neq 90°$ 			
Εξαγωνικό	$a \neq c$ 			
Κυβικό	απλό	ενδοκεντρωμένο	ολοεδρικά κεντρωμένο	
				



$P4_1$ No.76

Z=4; (x,y,z) $(\bar{x},\bar{y},1/2+z)$ $(\bar{y},x,1/4+z)$ $(y,\bar{x},3/4+z)$

International Tables of Crystallography – Volume A

http://it.iucr.org/

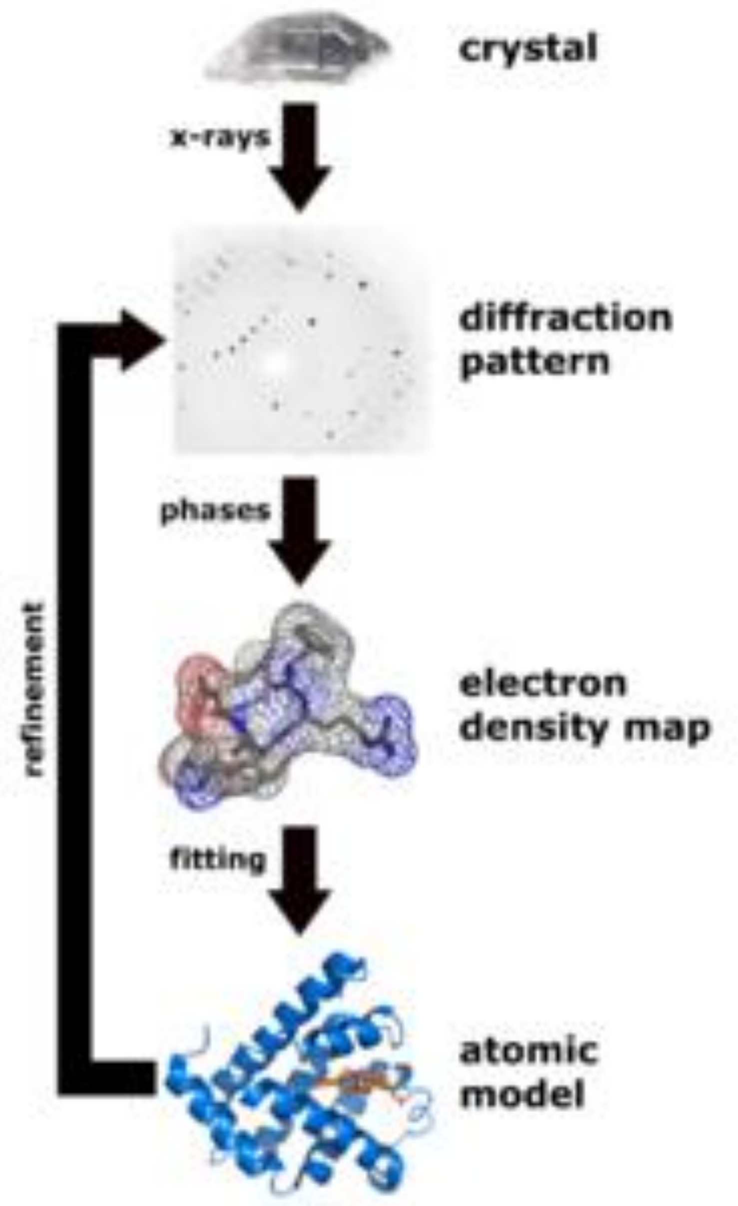
Το κρυσταλλογραφικό ζητούμενο

Αυτό που επιδιώκουμε είναι να μπορούμε να δούμε κάποιο βιομακρομόριο σε ατομική διακριτικότητα.

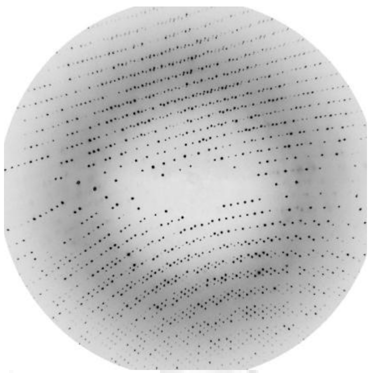
Το "δούμε" χρησιμοποιείται με την σημασία της οπτικής, δηλαδή αυτό που επιδιώκουμε να επιτύχουμε είναι την δημιουργία ενός ειδώλου του μορίου στο οποίο (είδωλο) να είναι διακριτά τα άτομα που αποτελούν το μόριο.

Κρυσταλλογραφία με Ακτίνες Χ

X-ray crystallography is the science of determining the arrangement of atoms within a crystal from the manner in which a beam of X-rays is scattered from the electrons within the crystal. The method produces a three-dimensional picture of the density of electrons within the crystal, from which the mean atomic positions, their chemical bonds, their disorder and sundry other information can be derived.



Παράγοντας δομής



$$\vec{\mathrm{F}}_{hkl} = \sum_i f_{i,\mathrm{h}} e^{2\pi i(hx+ky+lz)}$$

$$I_{hkl} \propto \left|F_{hkl}\right|^2$$

Υπολογιστική κρυσταλλογραφία

$$\vec{\mathrm{F}}_{hkl} = \int_x \int_y \int_z \rho(xyz) e^{2\pi i(hx+ky+lz)} dV$$

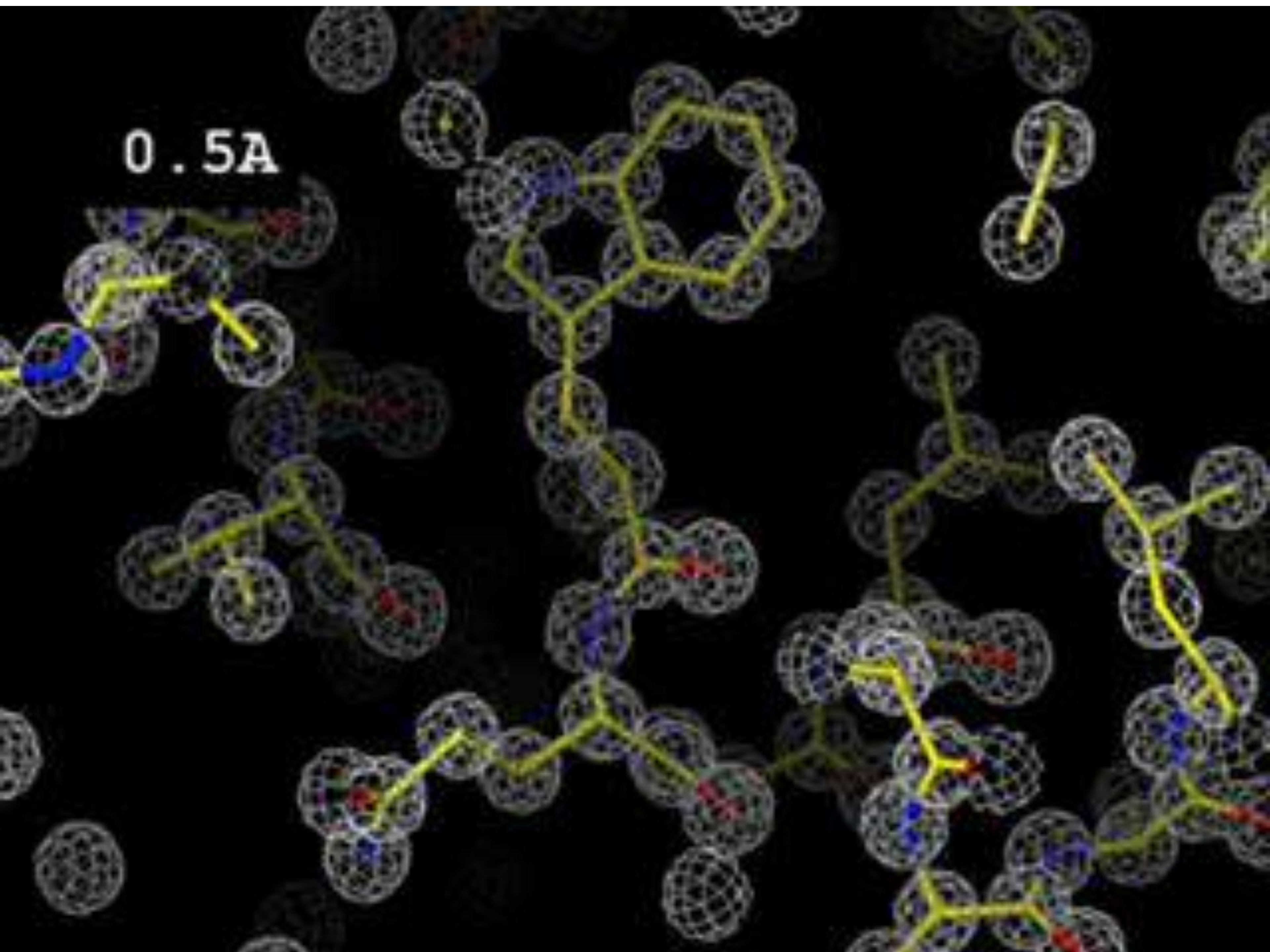
$$\vec{\mathrm{F}}_{hkl} = \sum_i f_{i,\mathrm{h}} e^{2\pi i(hx+ky+lz)}$$

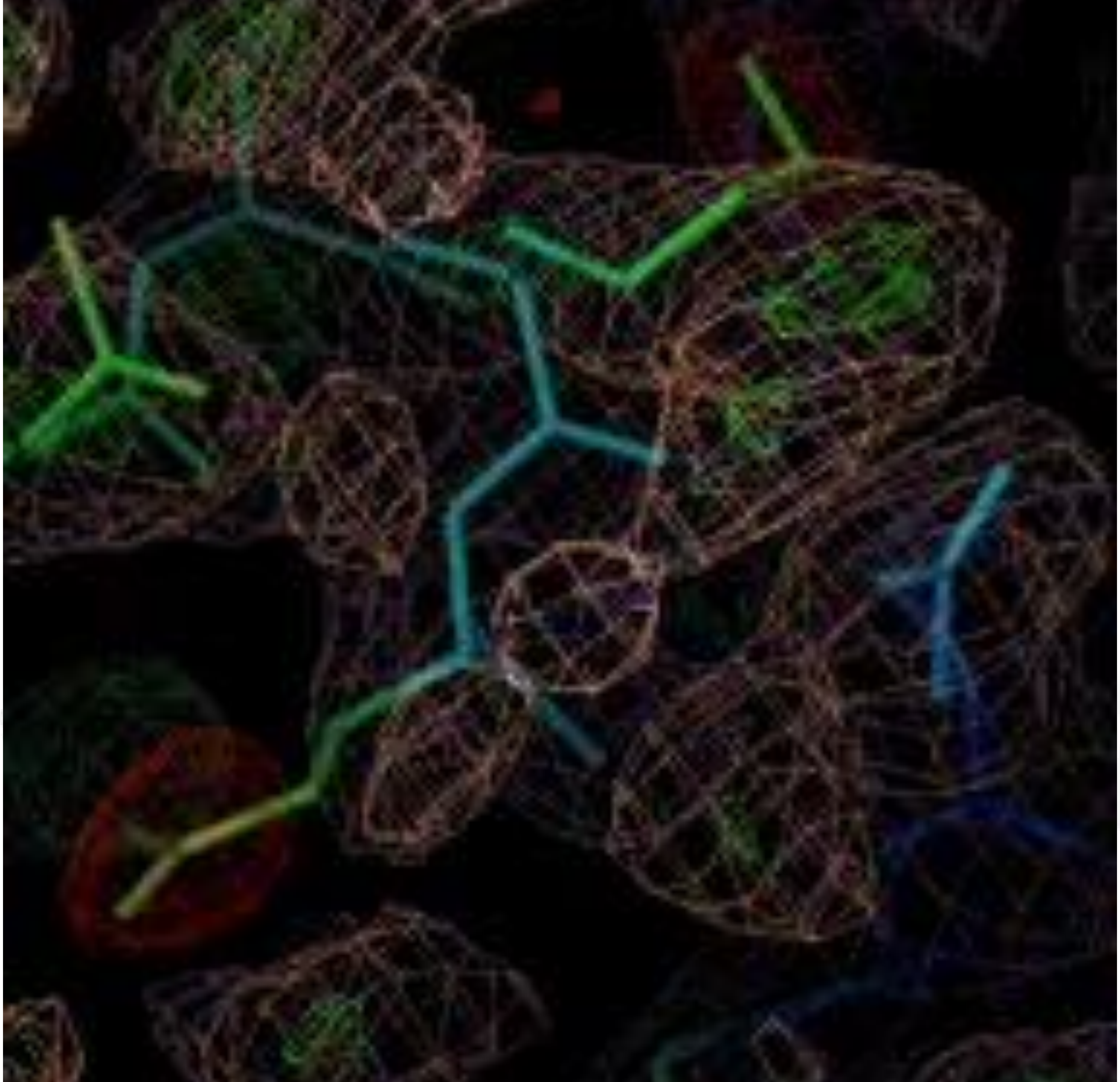
$$\rho(xyz) = \sum_{hkl} \vec{\mathrm{F}}_{hkl} e^{-2\pi i(hx+ky+lz)}$$

$$\vec{F}_{\vec{h}} = \int_V \rho(\vec{r}) e^{2\pi i \vec{r}\cdot\vec{h}} dV$$

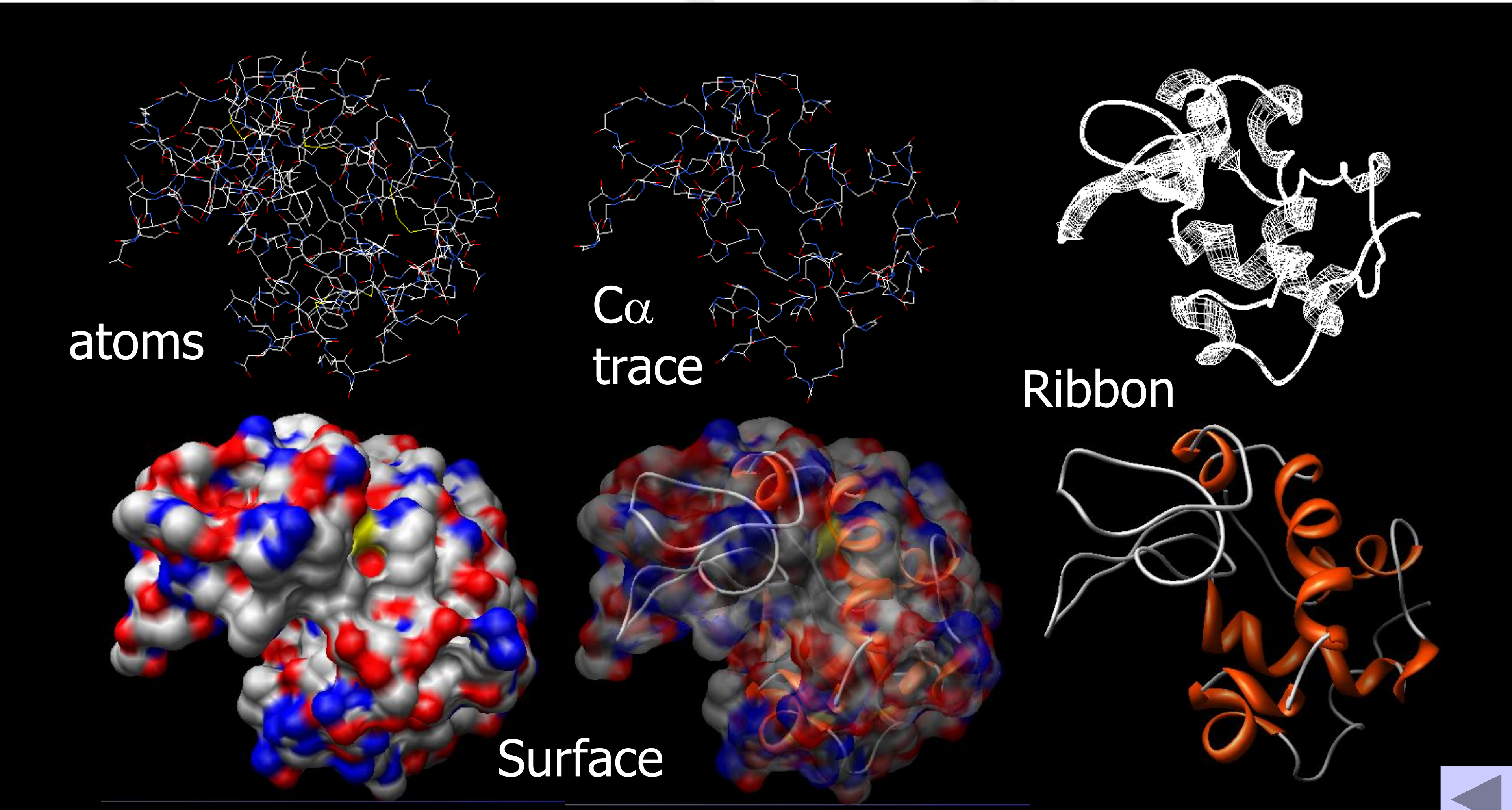
Αυτή η εξίσωση είναι μέλος μιας πολύ γνωστής οικογένειας εξισώσεων, των εξισώσεων των μετασχηματισμών Fourier.

το φάσμα σκέδασης από ένα αντικείμενο είναι ο μετασχηματισμός Fourier του αντικειμένου.

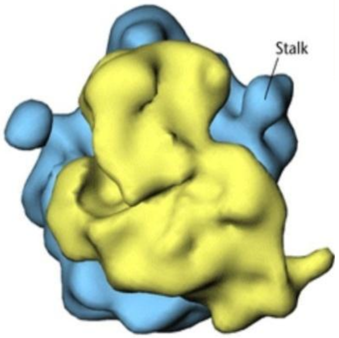

0.5A

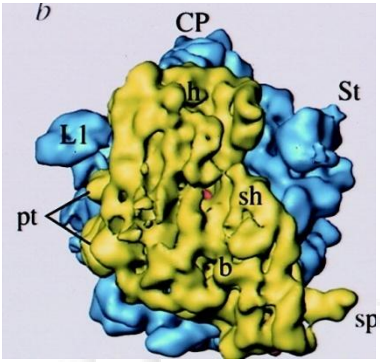


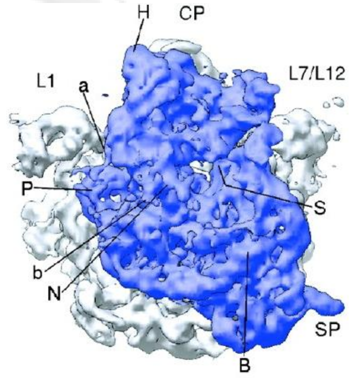
Αναπαράσταση Μοριακής Δομής Πρωτεϊνών

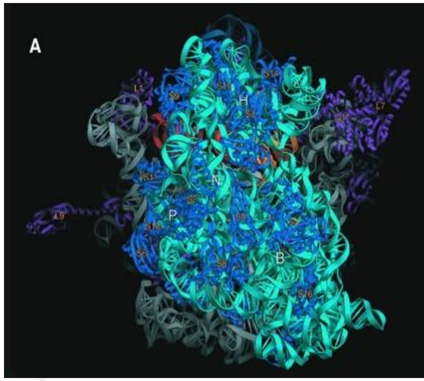


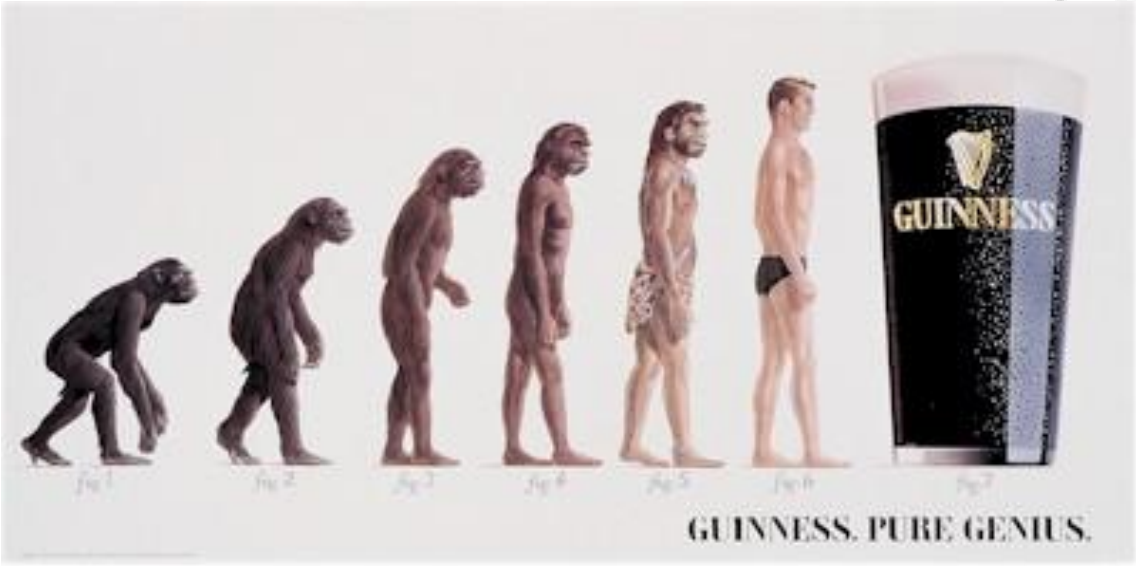
Evolution of Ribosome Structural Studies











Protein Crystallography

TRADITIONAL METHOD:
Single Crystal Diffraction

•Gives 3D information about crystal structure

•Very small amount of protein required

HOWEVER

•Can be very difficult to grow large enough single crystals of some proteins

•Very specific crystallisation conditions may not represent the natural environment

COMPLEMENTARY METHOD:
Powder Diffraction

•Three dimensional information is collapsed down into 1D – loss of information!

•Larger protein sample required

HOWEVER

•Polycrystalline powder can often be obtained when a good single crystal cannot

•More crystallisation conditions possible

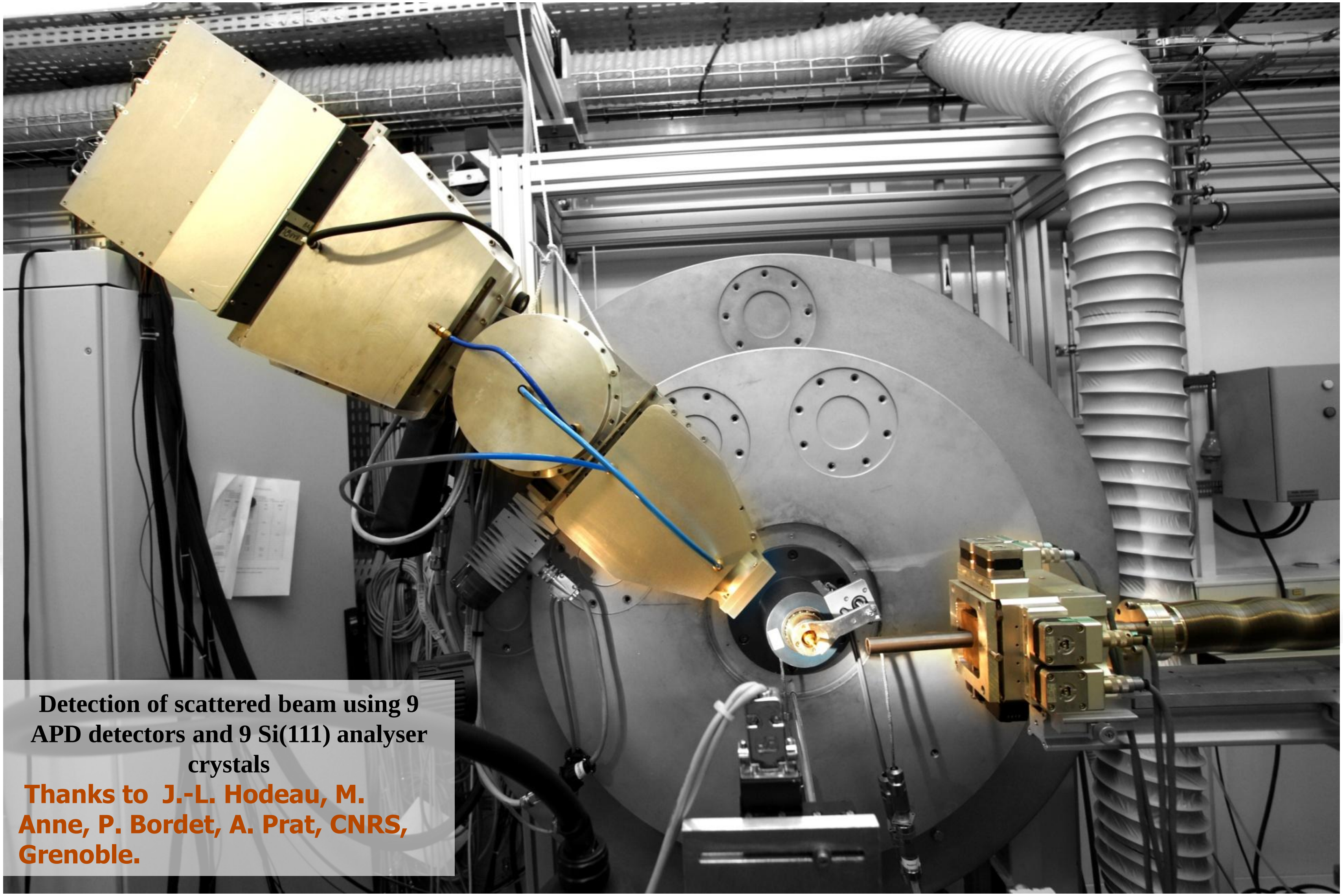
•Phase mixtures and phase transitions can be observed in-situ

Proteins Characterised successfully using Powder Data

15 proteins characterised using synchrotron data

41 structural models deposited in the Protein Data Bank (PDB) employing powder diffraction
http://www.rcsb.org/pdb/home/home.do

ID31: High Resolution Powder diffraction Beamline

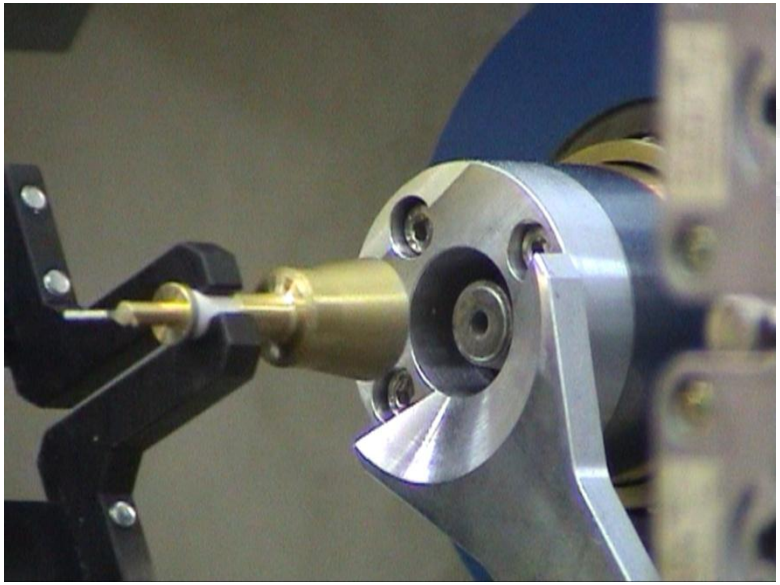


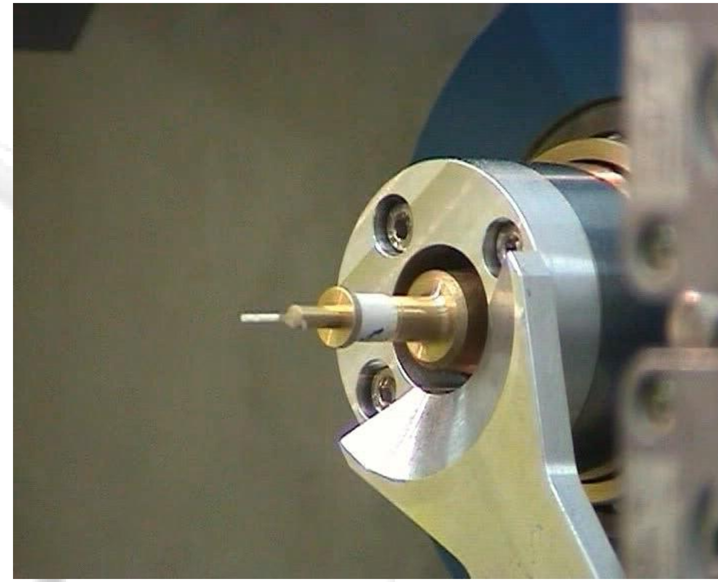
Detection of scattered beam using 9 APD detectors and 9 Si(111) analyser crystals

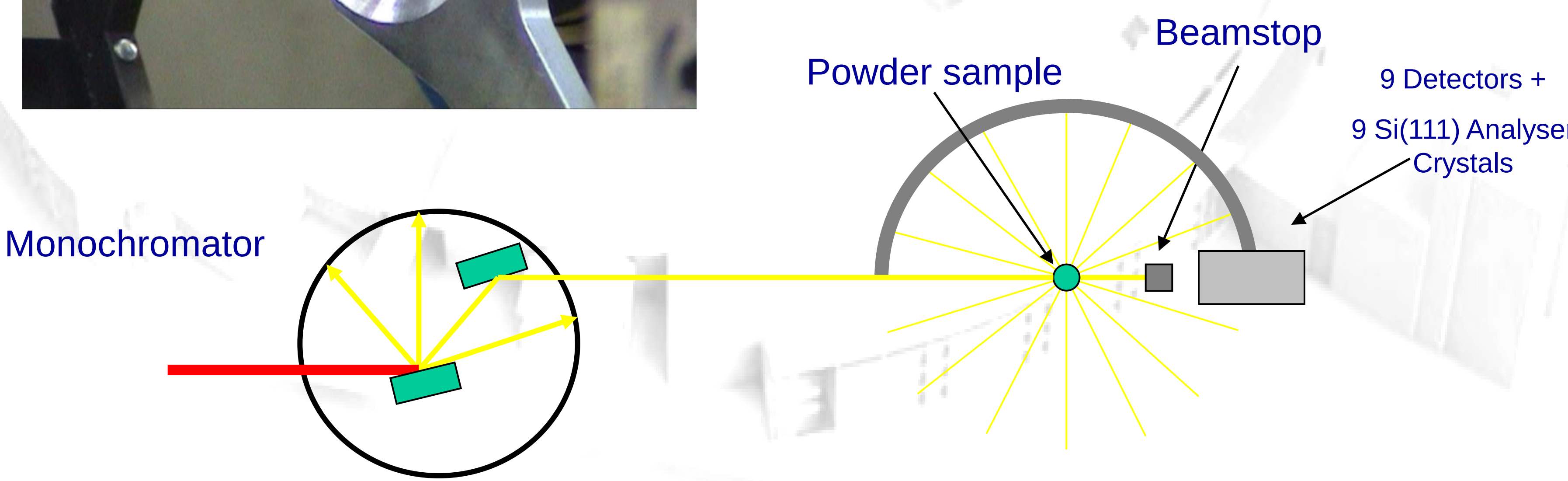
Thanks to J.-L. Hodeau, M. Anne, P. Bordet, A. Prat, CNRS, Grenoble.

Fitch, J. Res. Natl. Inst. Stand. Technol. 109, 133-142 (2004)

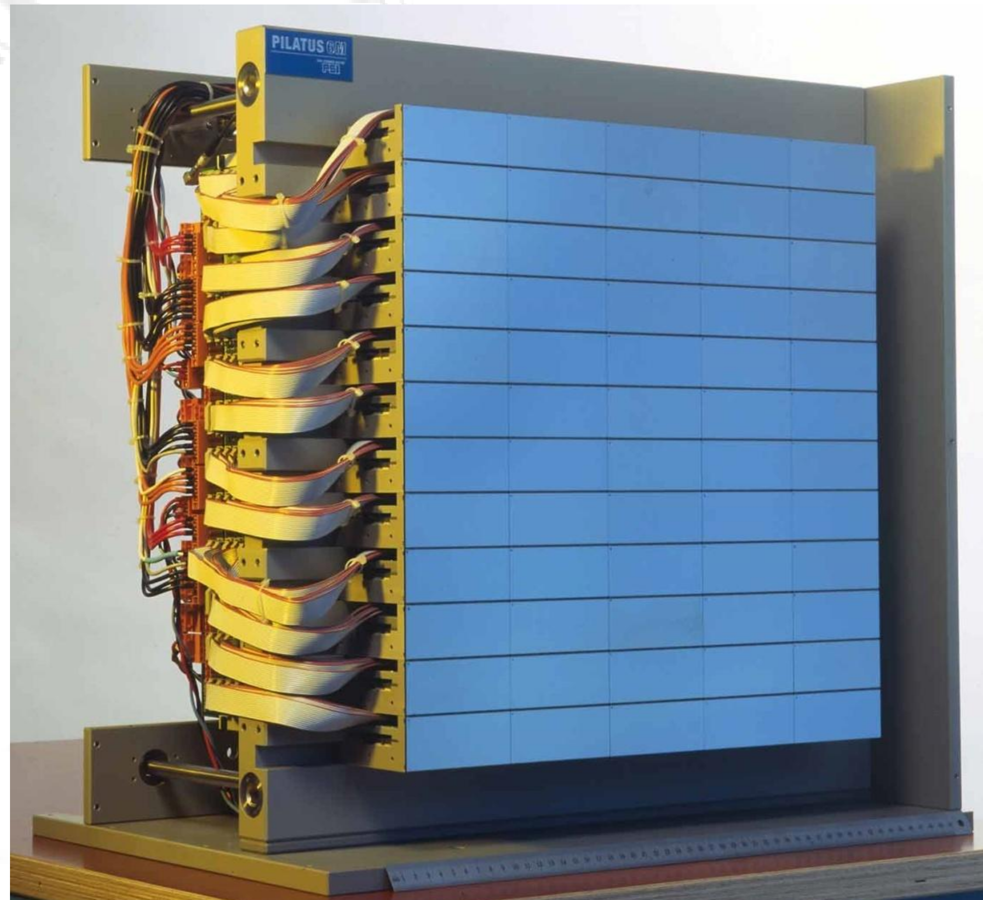
Sample translation







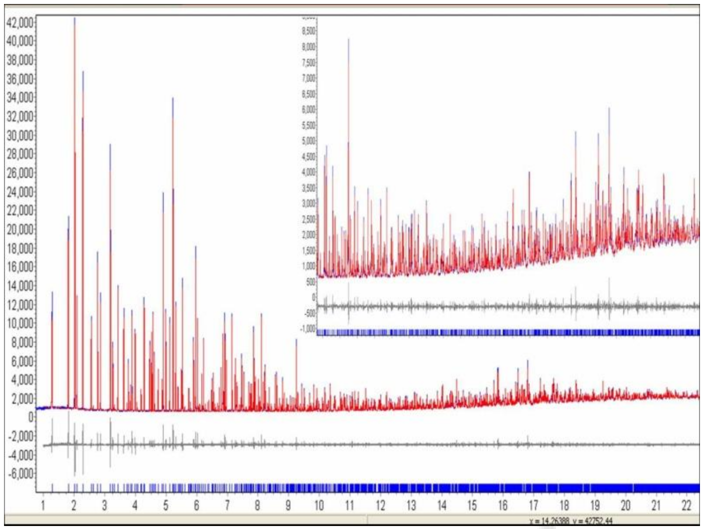
Novel Ultra fast detectors (MYTHEN & PILATUS) available at SLS



http://pilatus.web.psi.ch/mythen.htm

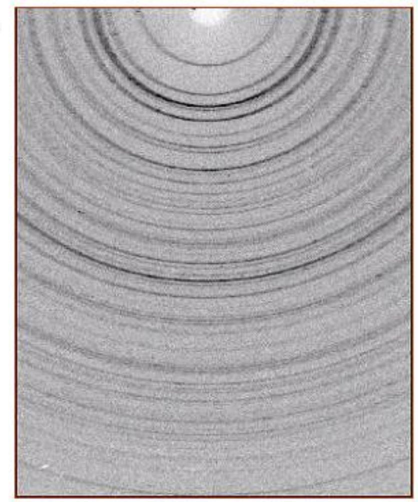
http://pilatus.web.psi.ch/pilatus.htm

Exploring different detection systems



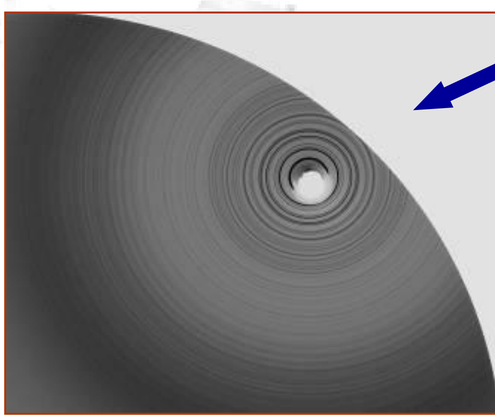
HEWL data collected at the analyzer crystal beam line- ID31.

ID11



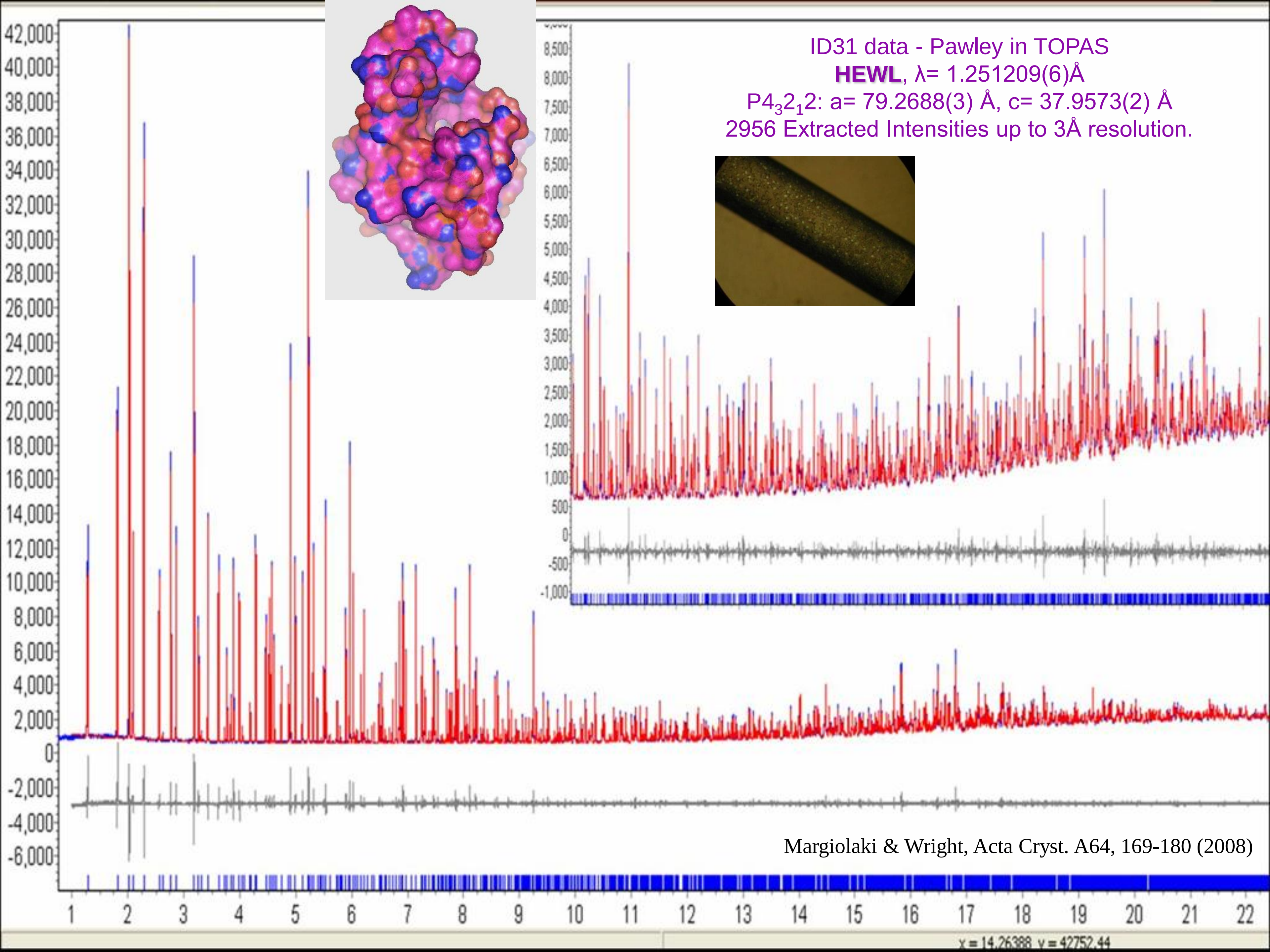
ID11 using a FReLon camera

SNBL-BM01A



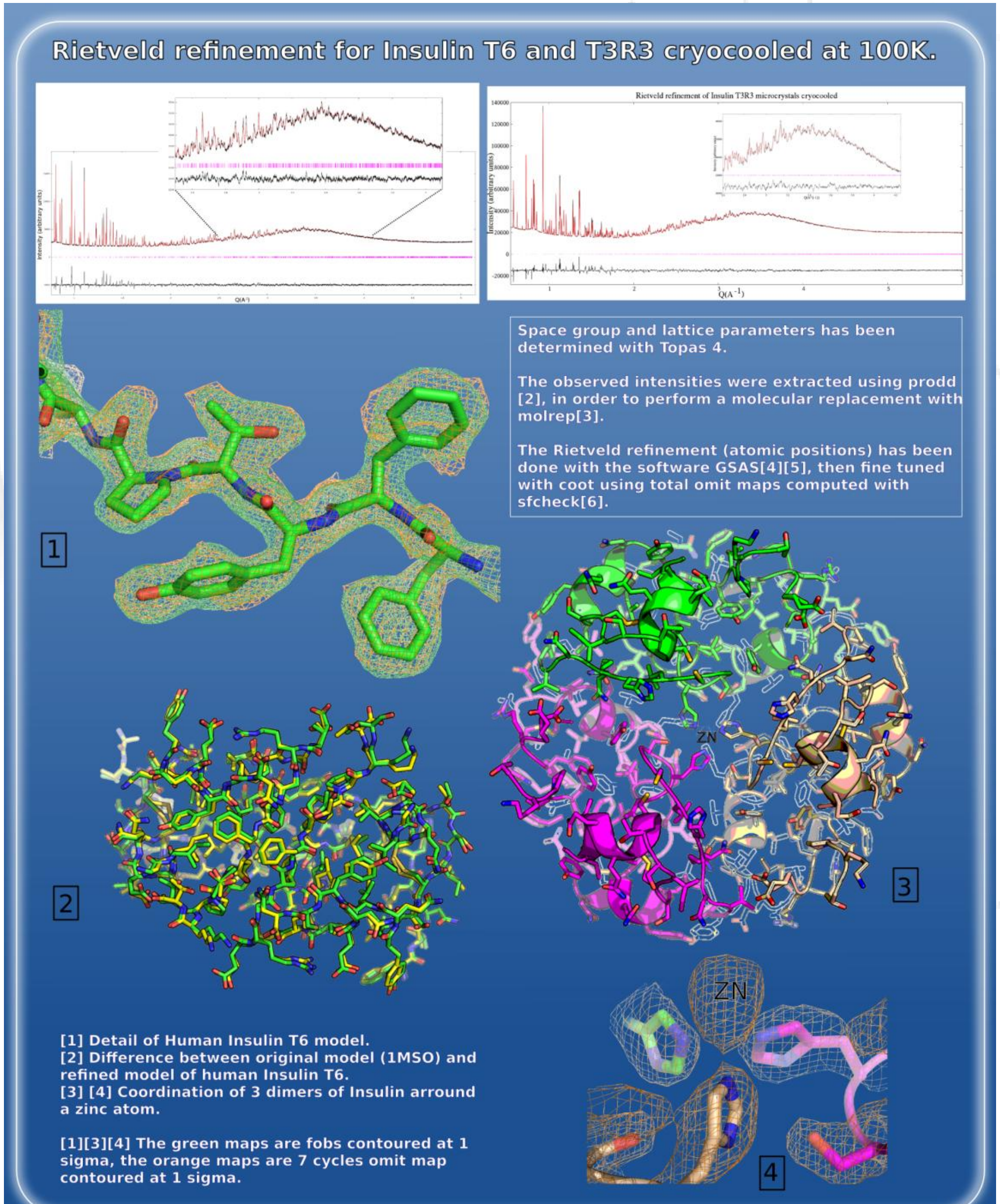
The area detector station of SNBL- BM01A.

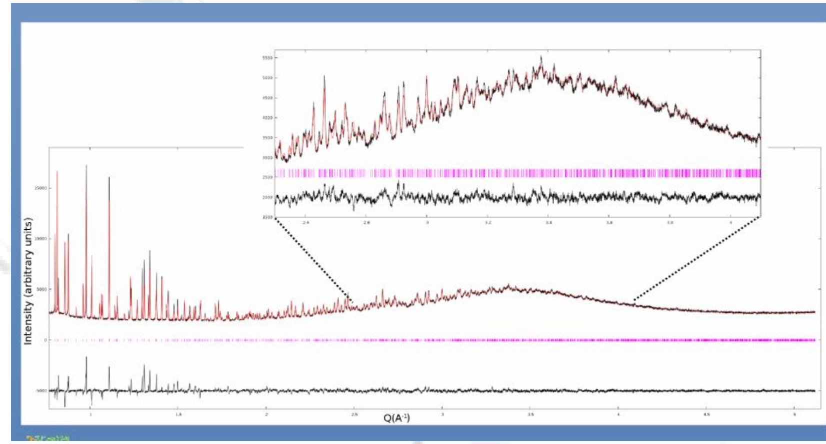
I. Margiolaki, J. P. Wright, A. N. Fitch, G. C. Fox, A. Labrador, R. B. Von Dreele, K. Miura, F. Gozzo, M. Schiltz, C. Besnard, F. Camus, P. Pattison, D. Beckers, T. Degen, Z. Kristallogr. Suppl. 26 (2007) 1-13

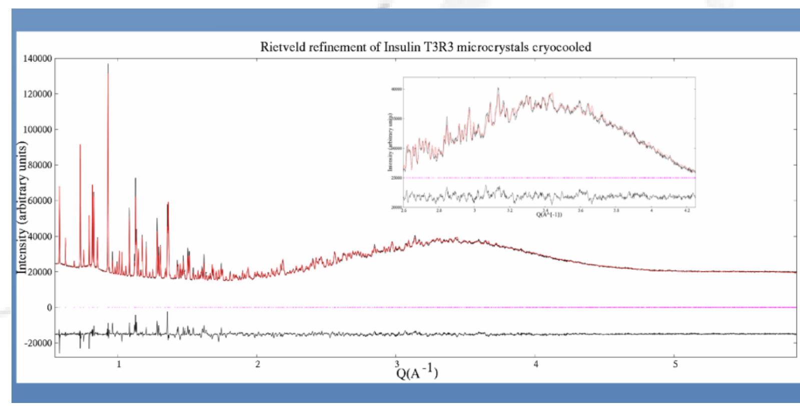


Margiolaki & Wright, Acta Cryst. A64, 169-180 (2008)

Successful cryocooling of Insulin







Y. Watier et al.
Acta Cryst. (2008). A64, C312

Improved methods for Intensity extraction and refinement via the use of multiple profiles

- PRODD, Wright et al. Z. Kristallogr. Suppl. 26 (2007) 27-32
- GSAS, Von Dreele, R. B. (2007). J. Appl. Cryst. 40, 133–143 & Basso et al., Acta Cryst. D61, 1612-1625 (2005)

Combination of Software designed for single crystal and powder diffraction data

- CCP4, http://www.ccp4.ac.uk/
- CCP14, http://www.ccp14.ac.uk/

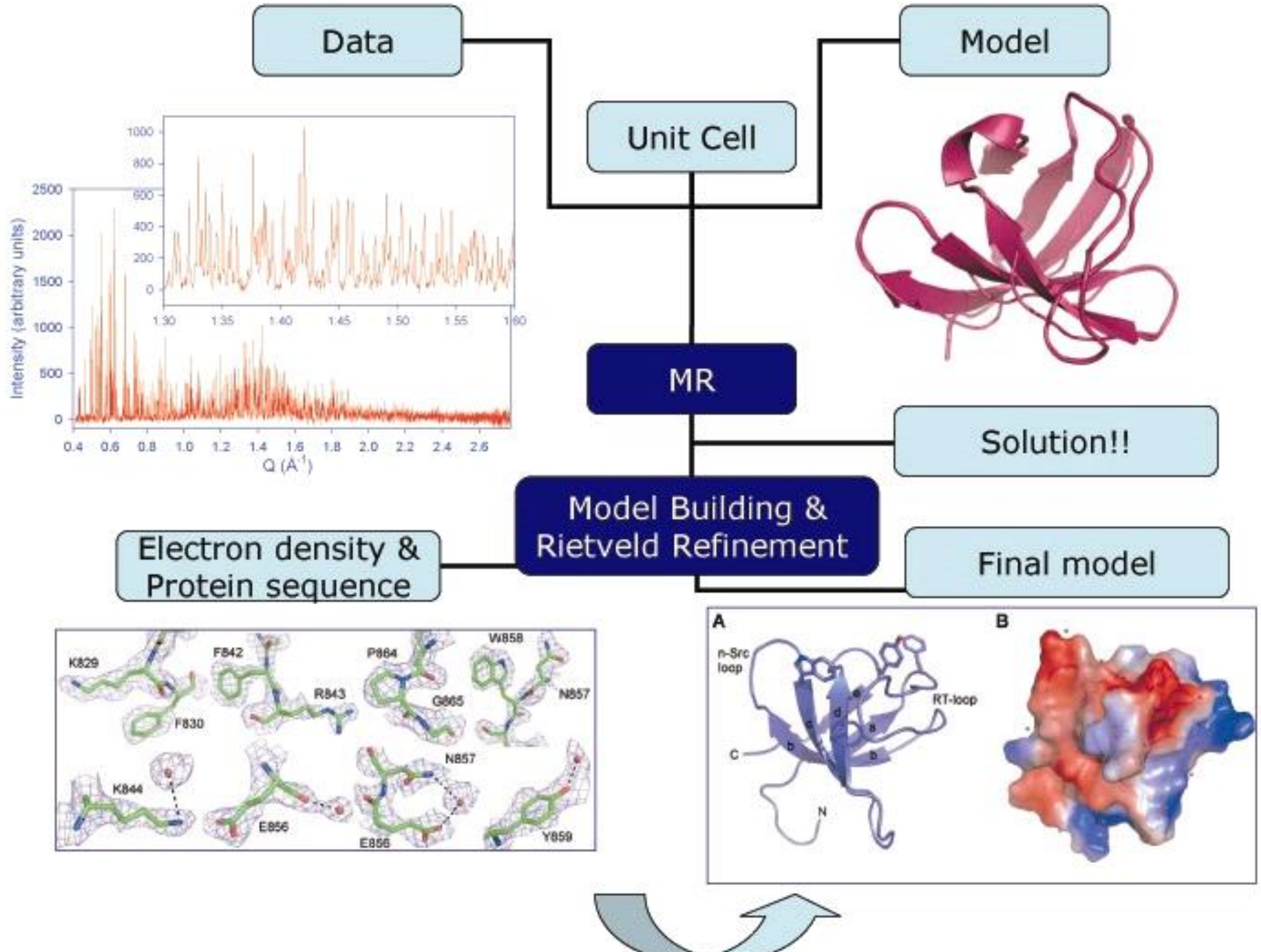
Traditional Methods in protein crystallography

- Molecular Replacement
- Isomorphous Replacement

Second SH3 domain of Ponsin: SH3.2

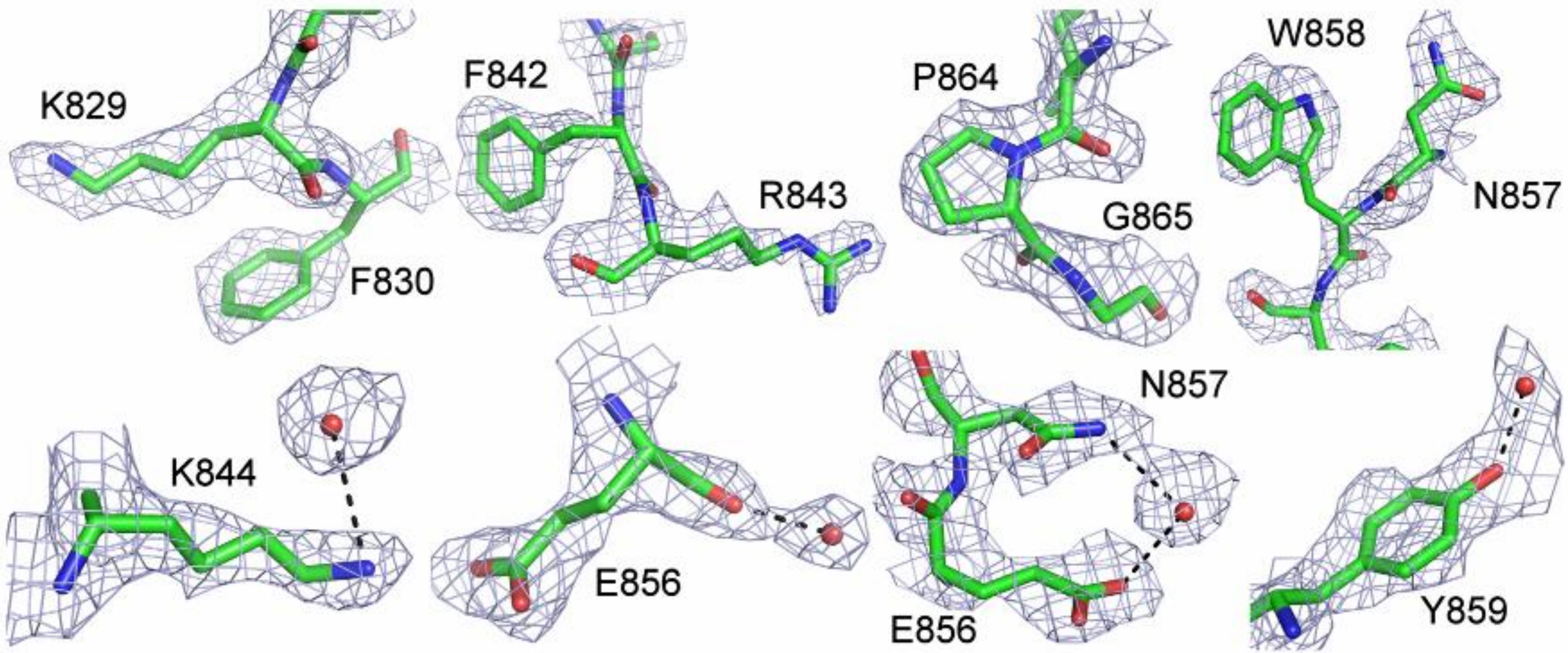
After purification the SH3.2 domain spontaneously formed a microcrystalline material suitable only for powder diffraction measurements

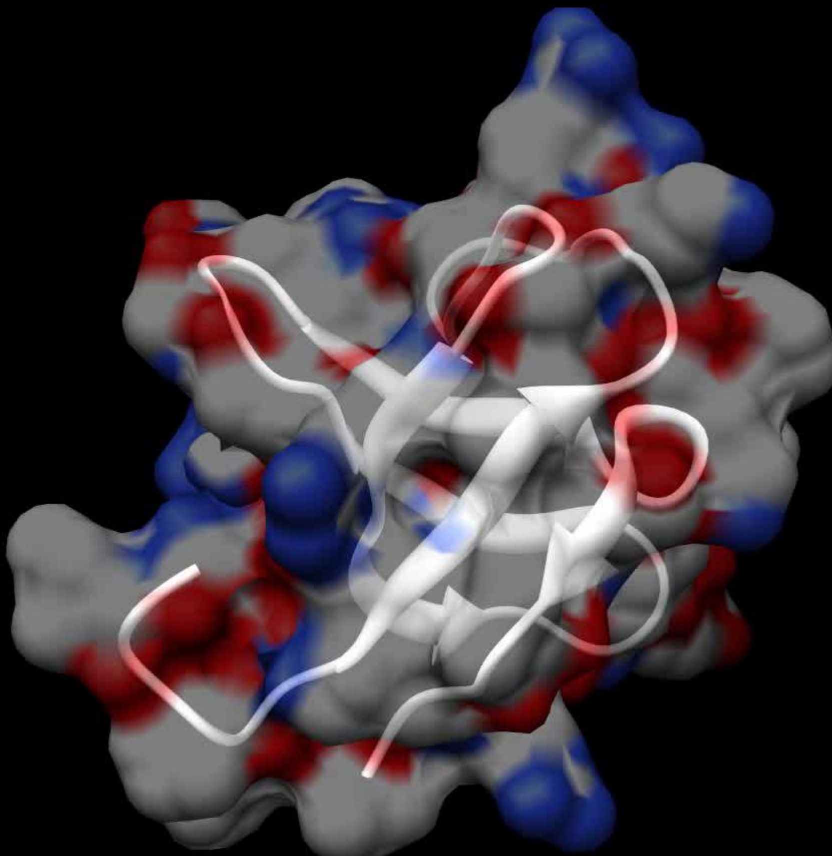
The first “real” protein structure resolved from ID31: Ponsin- SH3.2



Selected regions of the final refined structural model in stick representation and the corresponding total omit map contoured at 1σ.

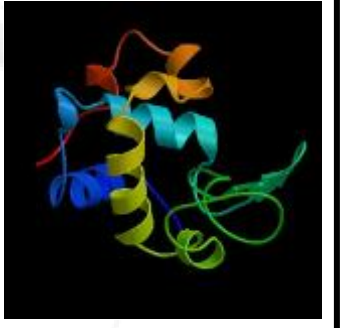
544 protein atoms and 36 water molecules were identified in total OMIT and difference electron density maps.





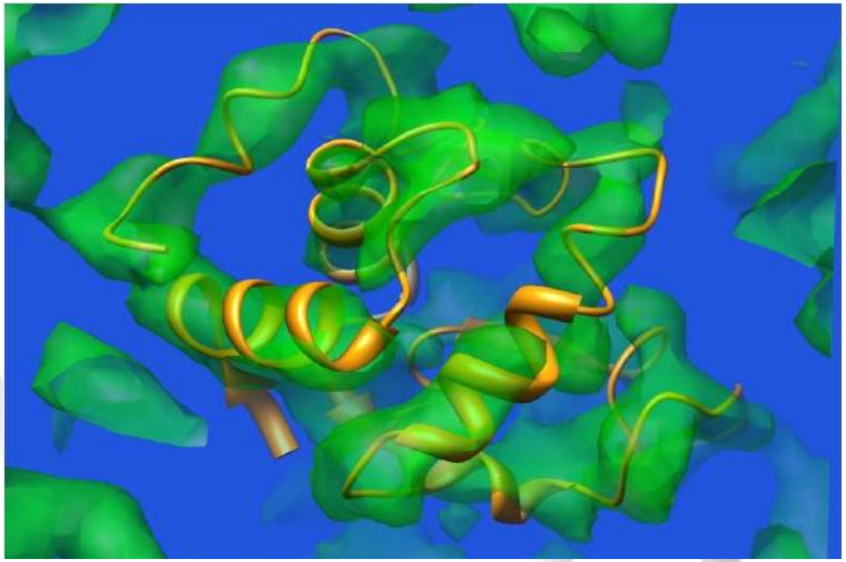
I. Margiolaki, J. P. Wright, M. Wilmanns, A. N. Fitch & N. Pinotsis
J. Am. Chem. Soc. 129, 11865-11871 (2007).

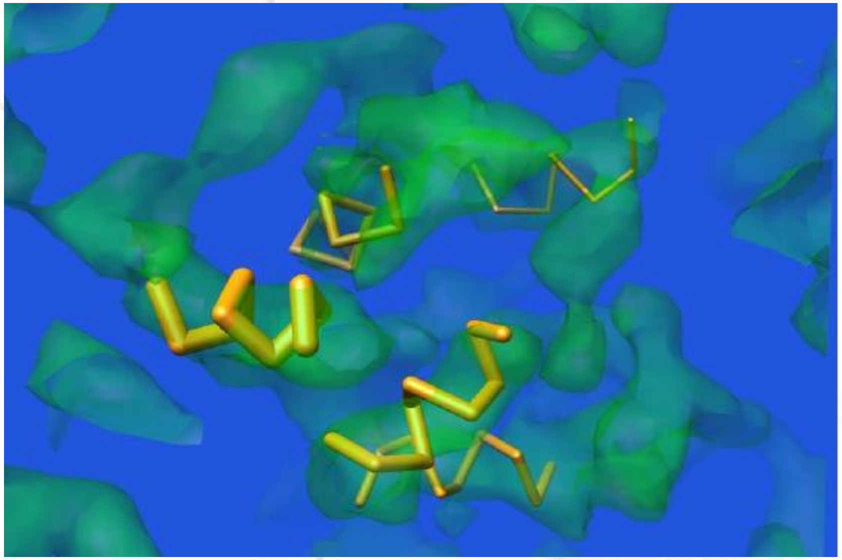
Test system

protein	Hen egg-white Lysozyme (HEWL)	
Molecular weight	14.4 kDa	
Unit-cell (Å)	a=b=79.2	c=38.0
Space-group	$P4_32_12$	
Heavy atoms	Gd (Z=64) Ho (Z= 67)	

Low resolution phasing in Gd and Ho derivatives of lysozyme

METHOD: Multiple Isomorphous Replacement (MIR)





Features of the Secondary Structure of the Protein Molecule from Powder Diffraction data

Acta Cryst. D66, 756-761 (2010)- Cover article

S. Basso, C. Besnard, J. P. Wright, I. Margiolaki, A. N. Fitch, P. Pattison, M. Sciltz

Software used

Powder Diffraction & CCP14

Fit2D
DASH
TOPAS
FULLPROF
GSAS

Single Crystal & CCP4

CCP4 software package
MOLREP
PHASER
CNS
REFMAC
PHOENIX
WINCOOT
PYMOL
CHIMERA

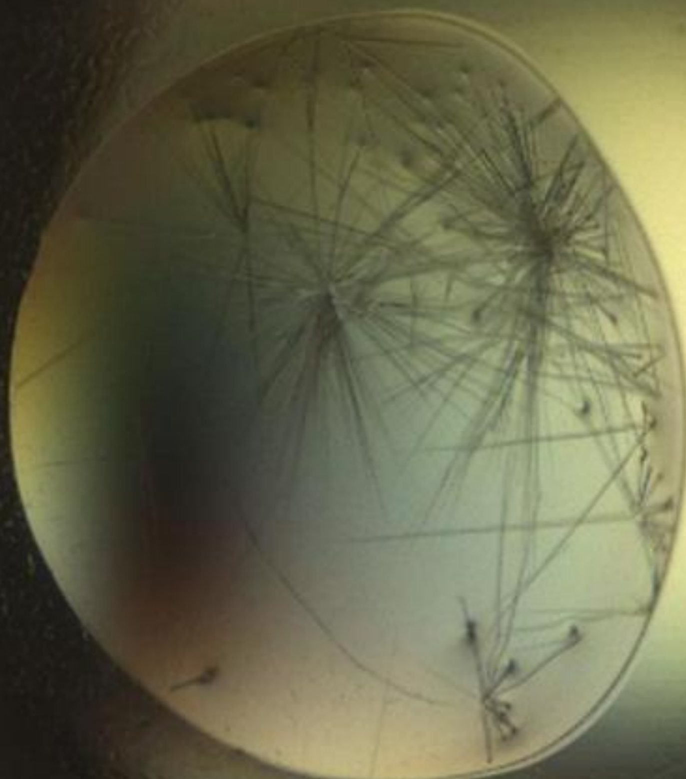
Home made

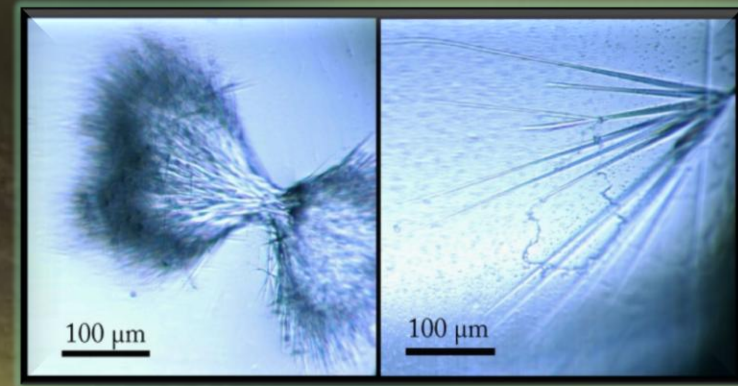
PRODD
SFCHECK
(modified version)
Short routines in PYTHON
Pycluster
ID31sum

Other useful software

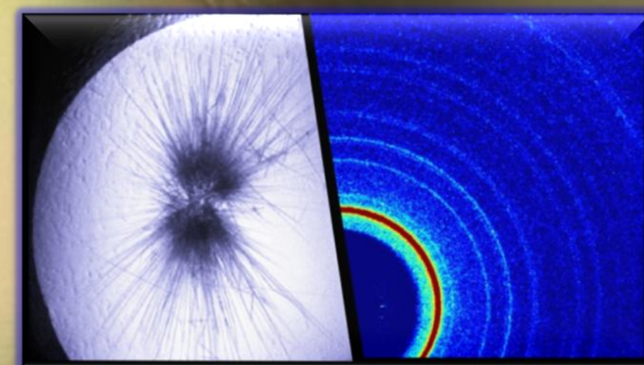
El nemo server
http://www.igs.cnrs-mrs.fr/elnemo/

Emerging Viruses



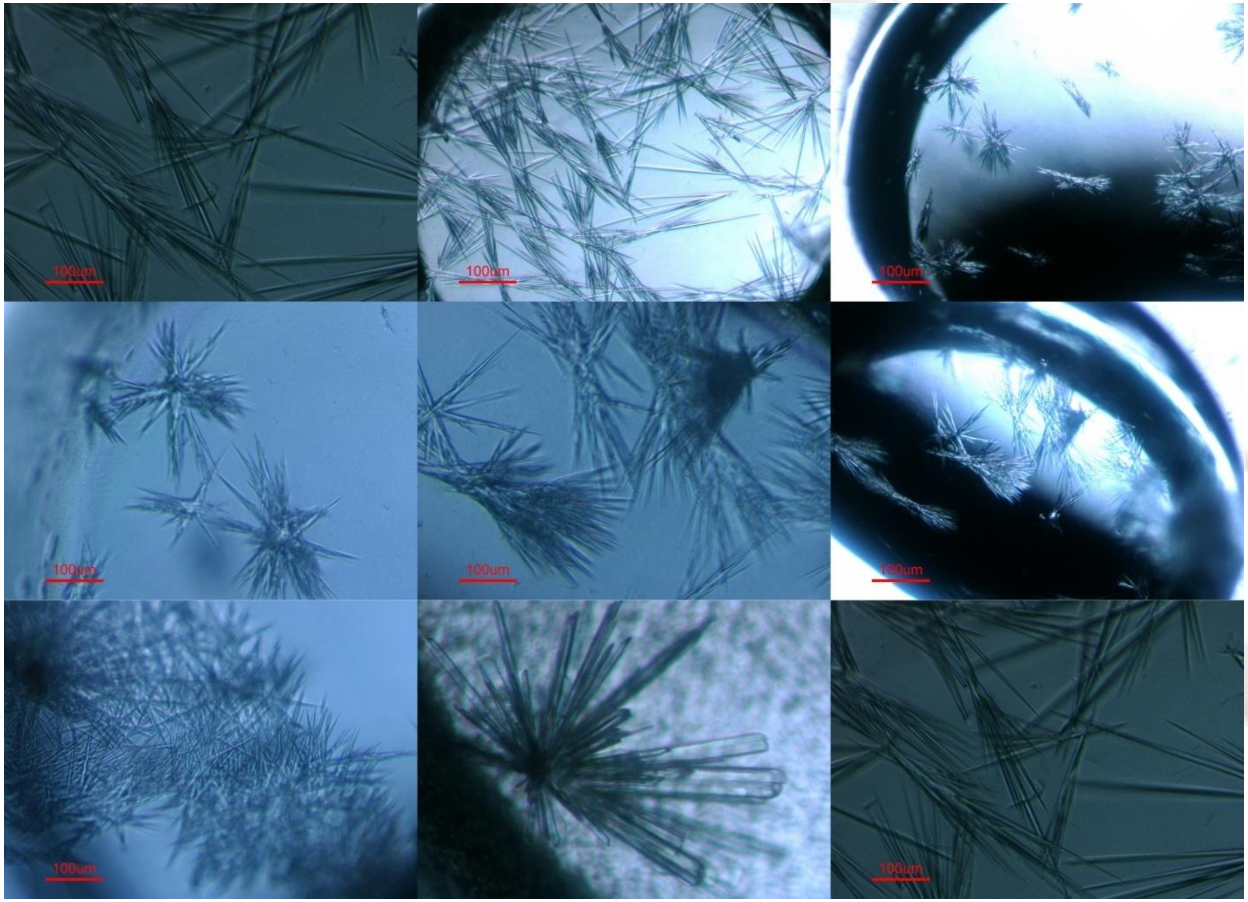


nsP3 macro domain of Mayaro virus



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

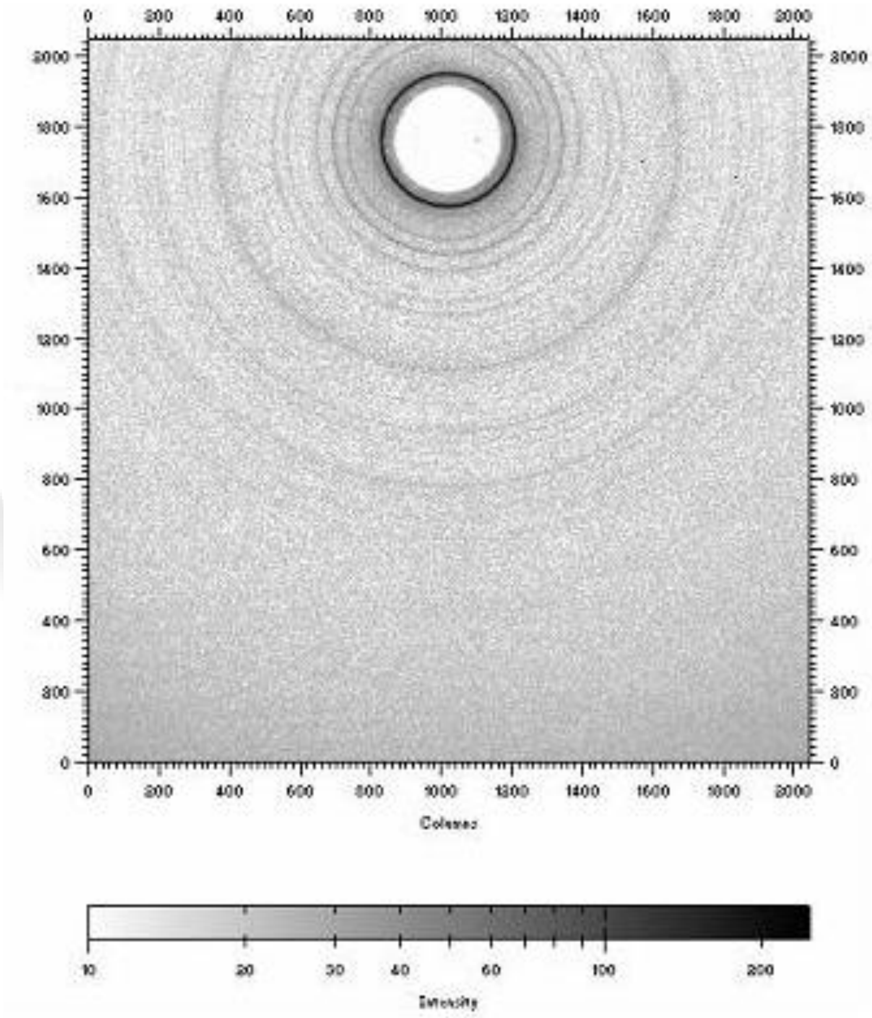
“Sea Urchin” crystals of MAYV

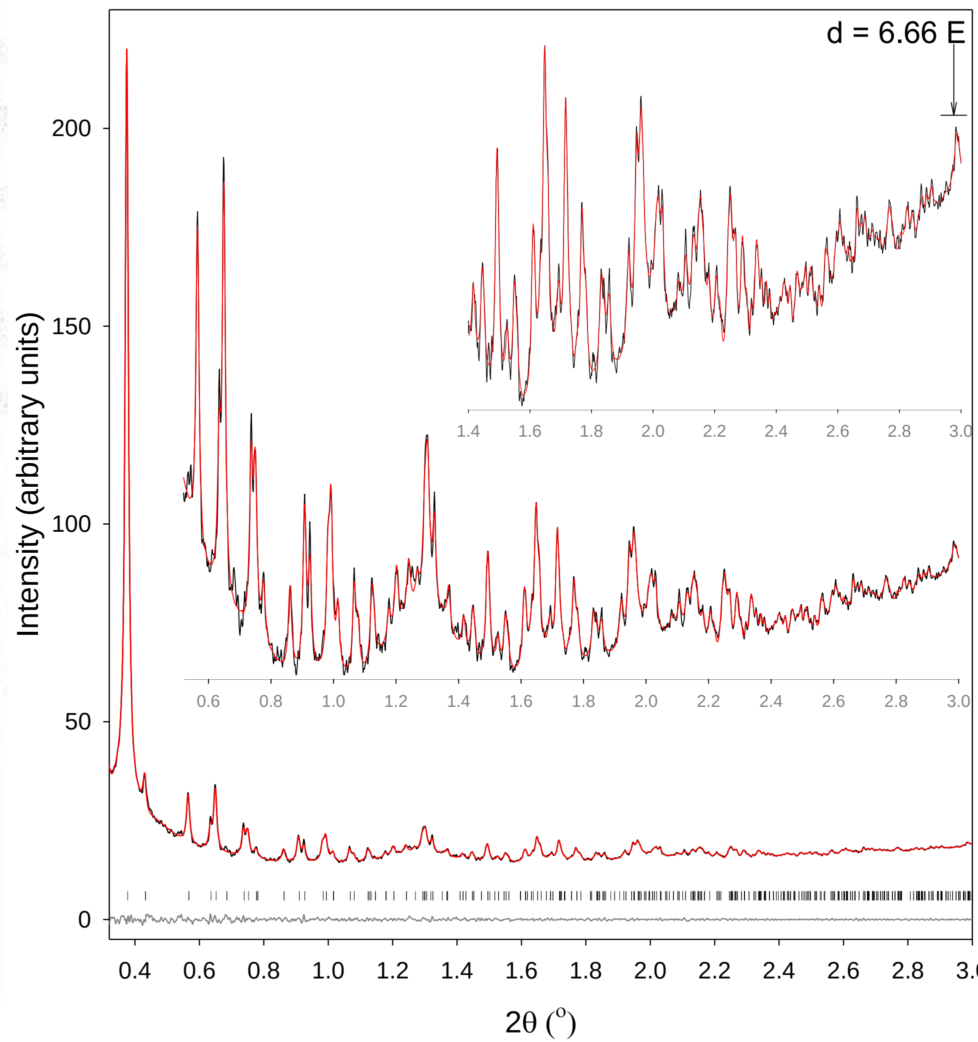


AFMB: Nicolas Papageorgiou, Bruno Canard, Bruno Coutard & Violaine Lantez

UPAT: Elena Kotsiliti, Alexandros Valmas

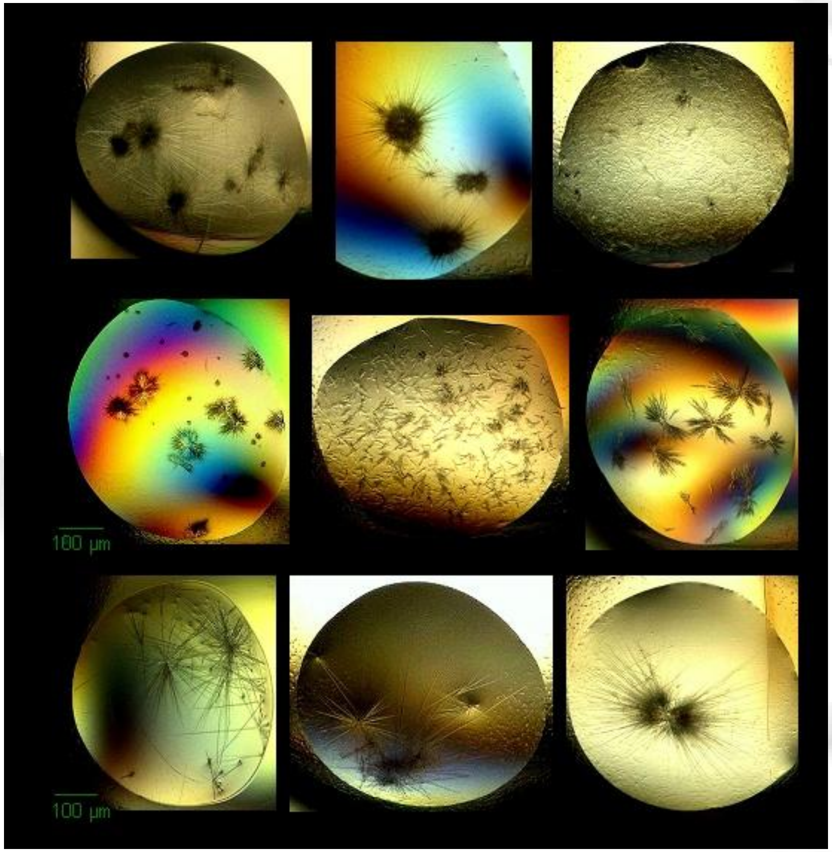
Data collected at ID11- ESRF

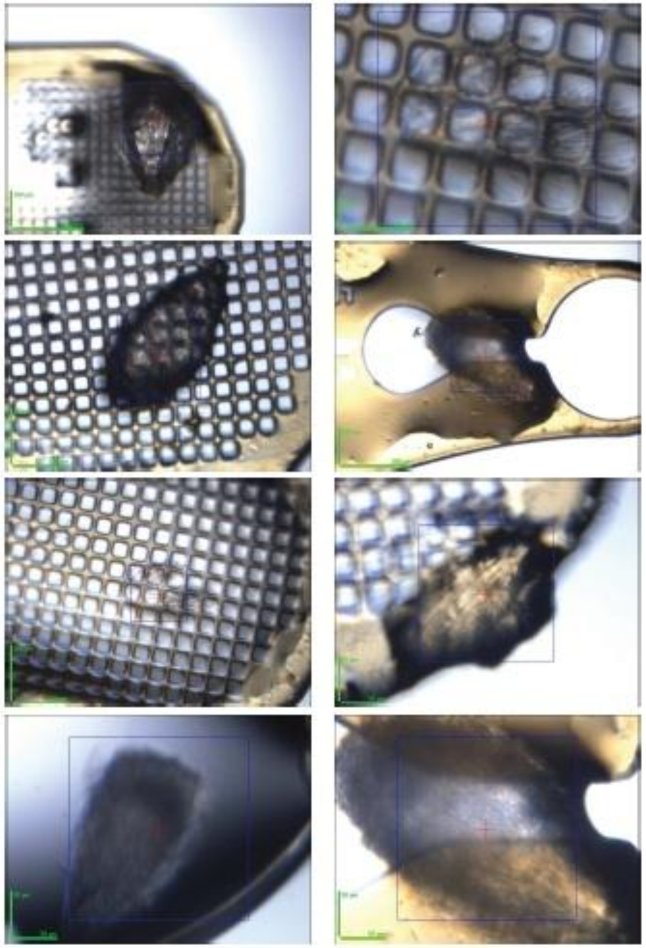


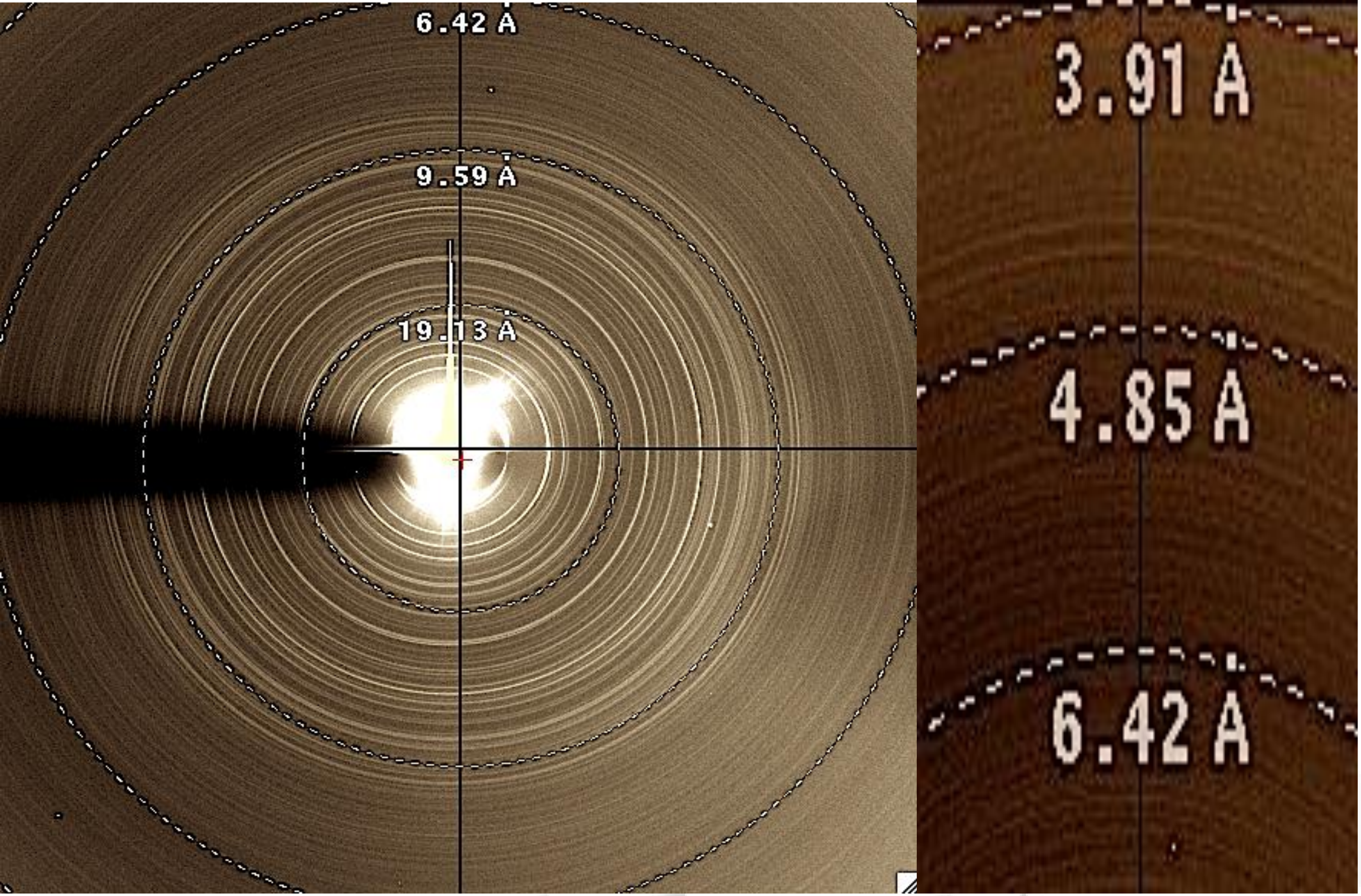


$P3_1$, a = 61.603(3) Å, c = 94.619(5) Å
2 molecules/ asu, 58% solvent content

Open air single urchin measurements

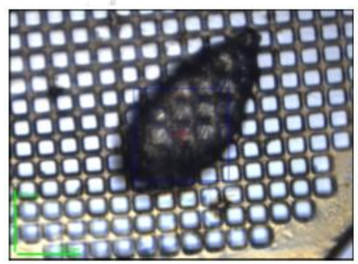


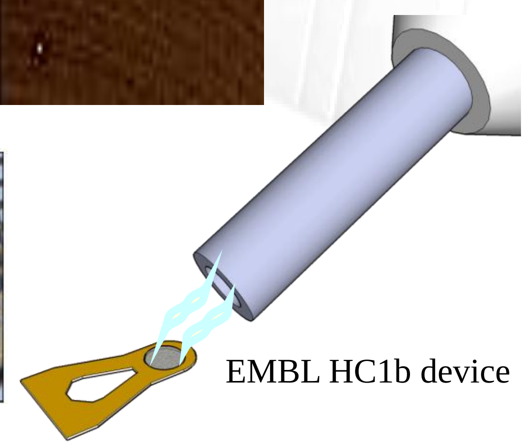




ID14- 2, area detector
λ=0.9934 Å

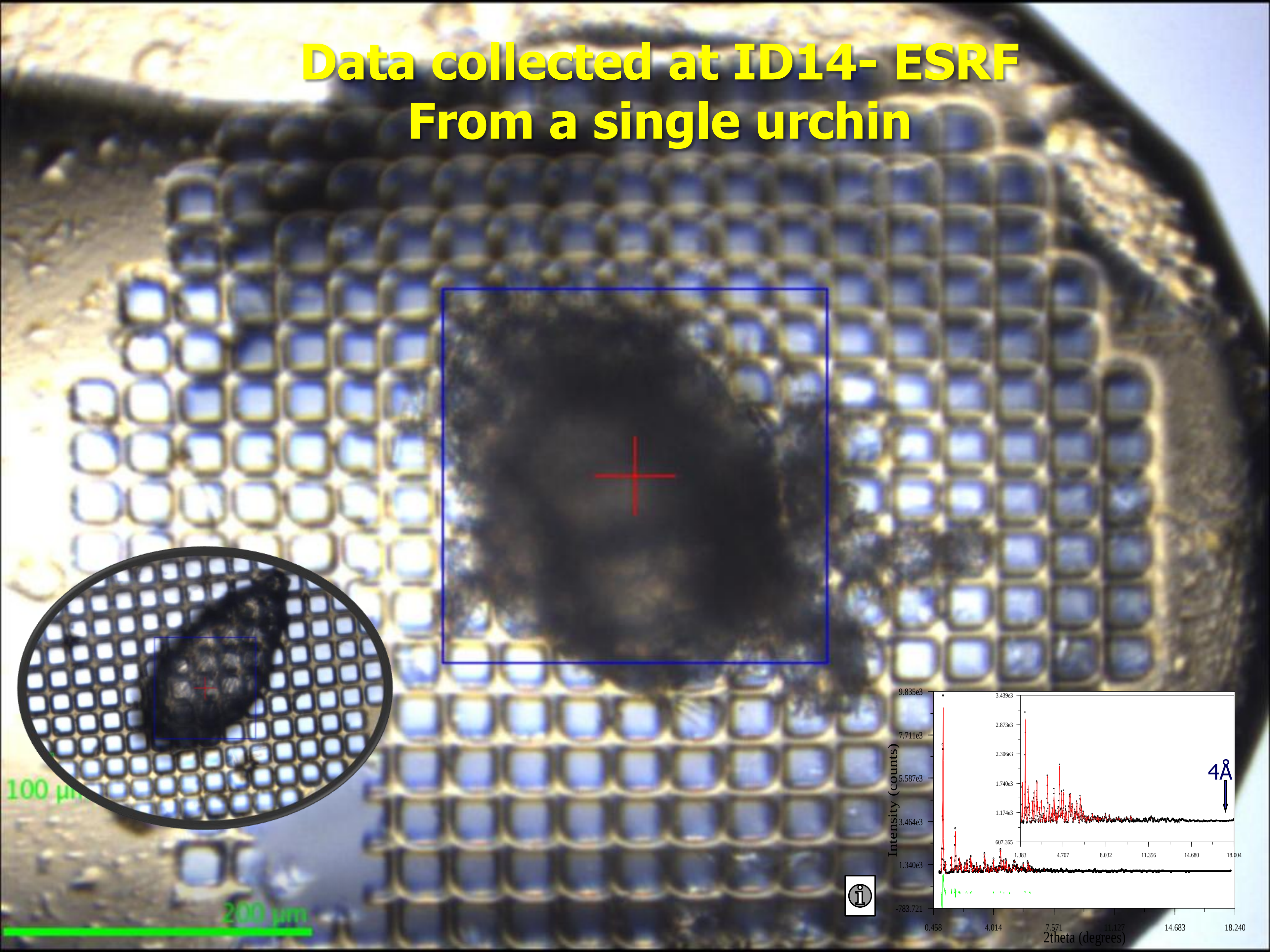
Matthias Bowler, Yves Watier,
Nicolas Papageorgiou, Irene Margiolaki



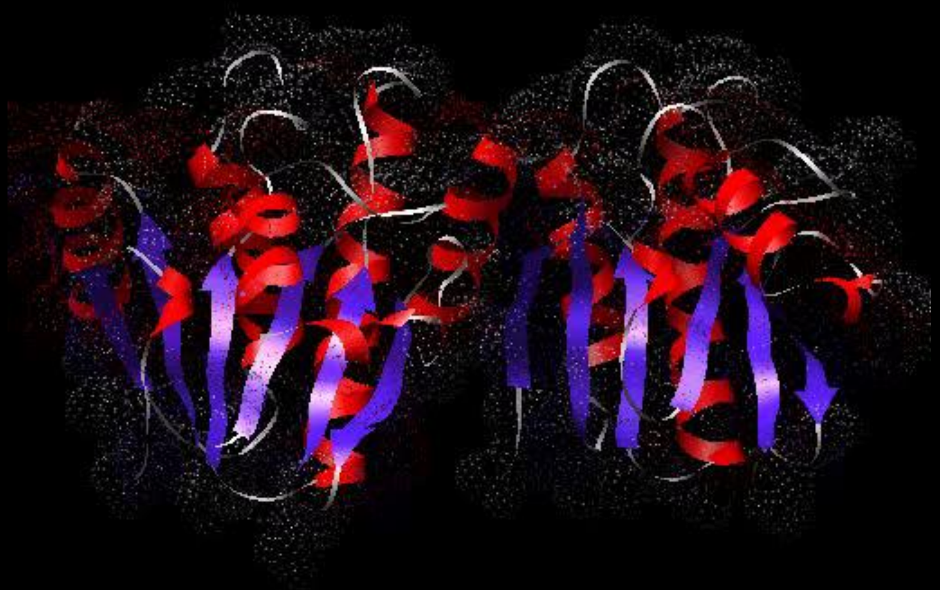


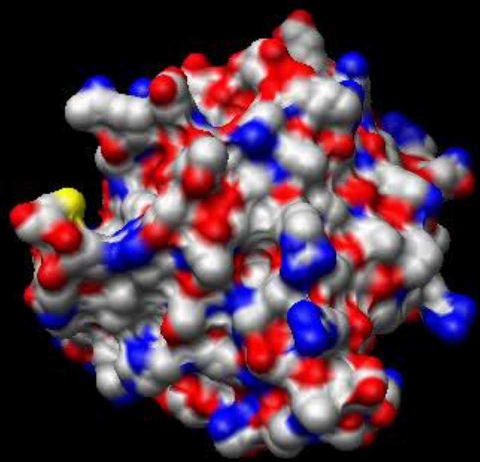
EMBL HC1b device

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)

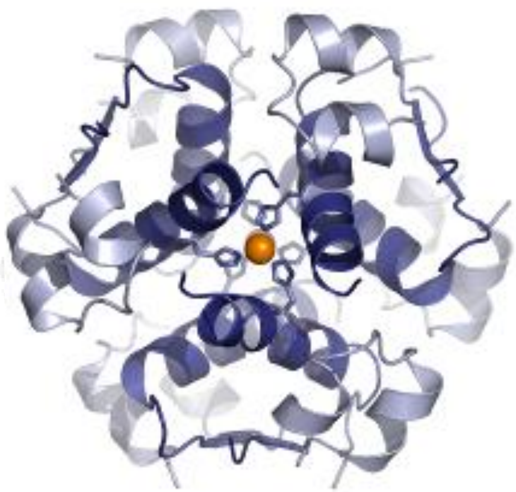
Nicolas Papageorgiou, Yves Watier, Lucy Saunders, Bruno Coutard , Violaine Lantez, Ernest A. Gould, Andrew N. Fitch, Jonathan P. Wright, Bruno Canard & Irene Margiolaki

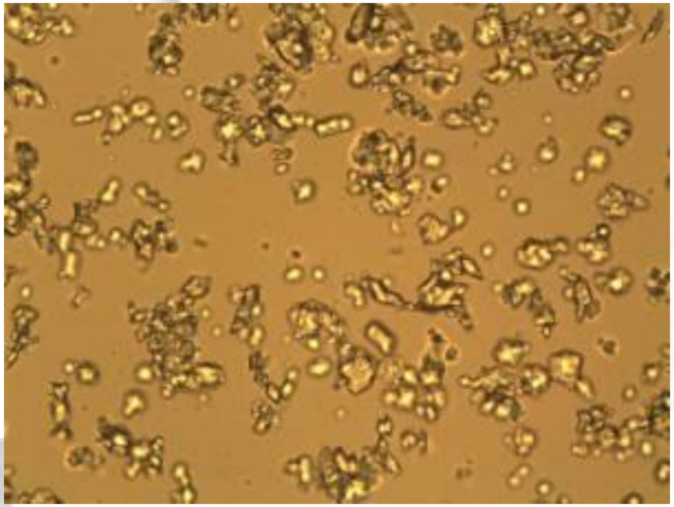
Industry

- Novo Nordisk – Copenhagen

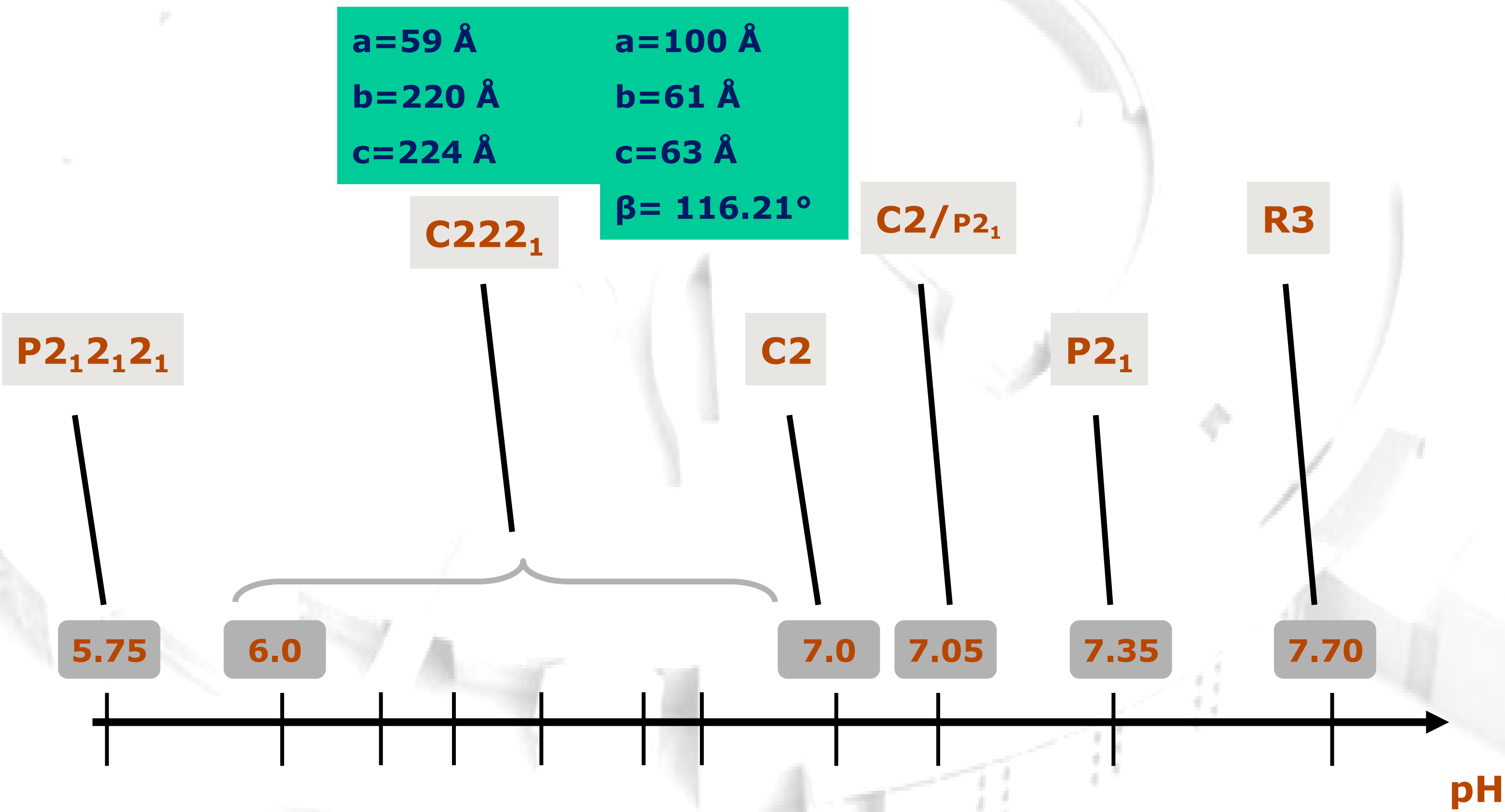
(Human Insulin)

Gerd Schluckebier
Mathias Norrman





Phase transitions as a function of pH



- Norrman, M. et al., (2006) J. Appl. Cryst 39 391-400
- Norrman, M. & Schluckebier, G. (2007) Bmc Struct.Biol. 7: 83-83
- Margiolaki , I. & Wright, J. P. Acta Cryst. A64, 169-180 (2008)
- Knight, L. et al. (In Preparation)

Foundation of an X-ray lab at UPAT

Kappa CCD - BRUKER

X-Pert1 and XPert Pro - PaNalytical

Thanks to Prof. Henk Schenk and his group at the UvA, Dr. Radovan Cerny, UvG, BRUKER, PaNalytical, UPAT

Application of the precession electron diffraction method at UPAT

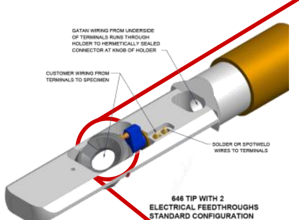
- ΕΡΓΑΣΤΗΡΙΟ ΗΛΕΚΤΡΟΝΙΚΗΣ ΜΙΚΡΟΣΚΟΠΙΑΣ
- & ΜΙΚΡΟΑΝΑΛΥΣΗΣ

- Equipment: TEM JEM-2100

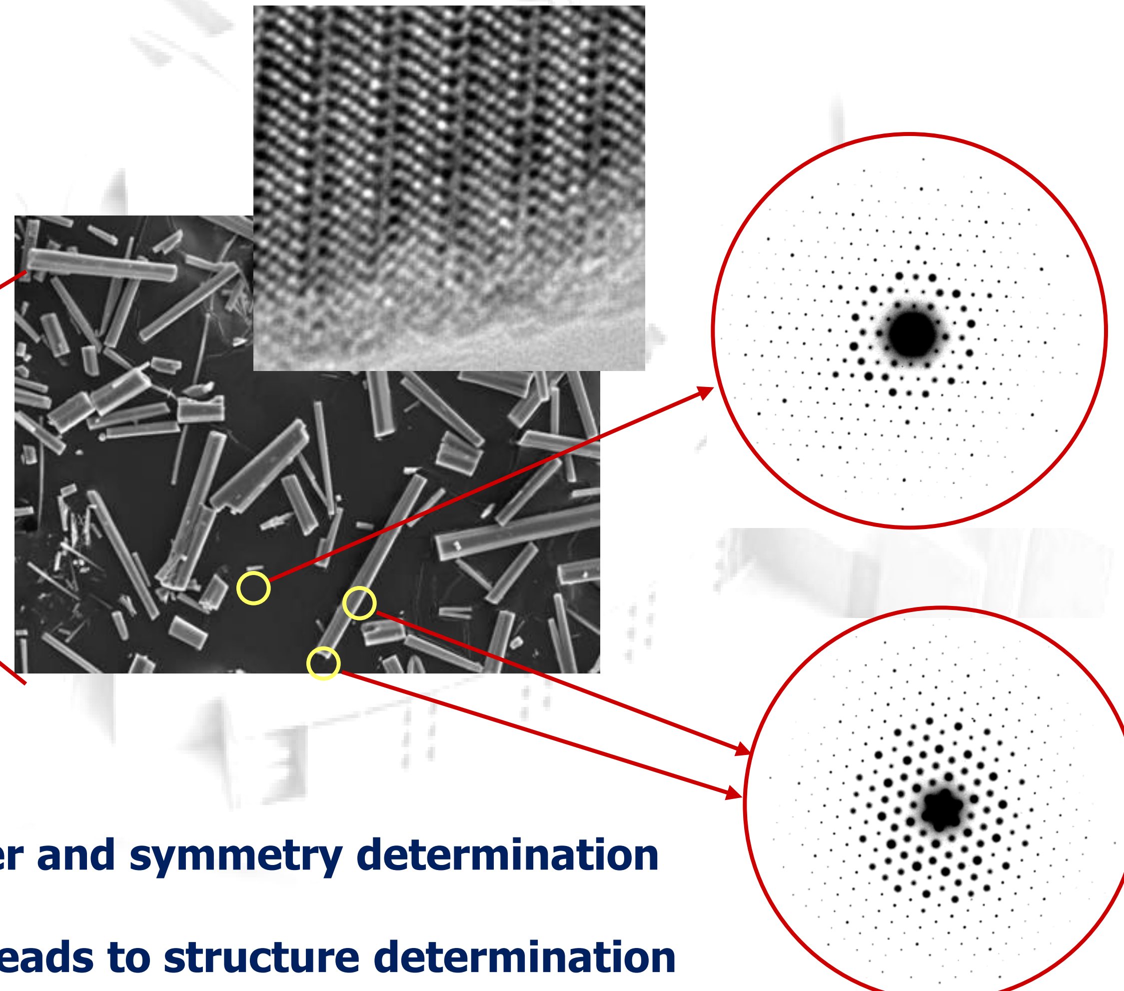
http://www.electronmicroscopylab.upatras.gr/index.files/Page821.htm
http://www.nanomegas.com/

Electron diffraction: advantages

Every TEM (electron microscope) may produce ED patterns and HREM from individual single nanocrystals



TEM goniometer



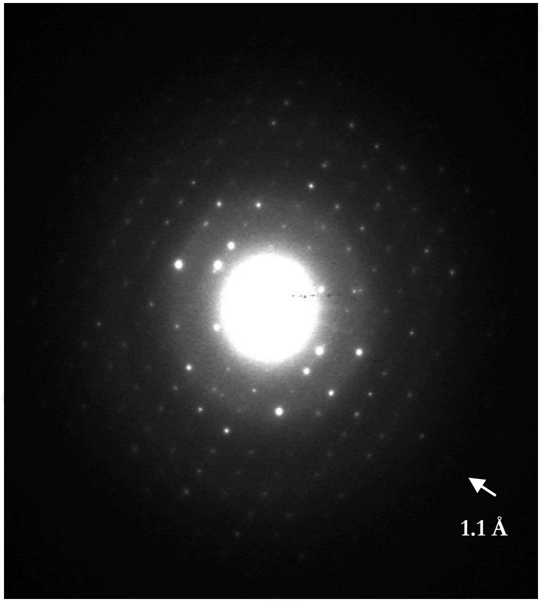
ED information: Cell parameter and symmetry determination

Measuring intensity values leads to structure determination

Precession from pharmaceutical nanocrystals

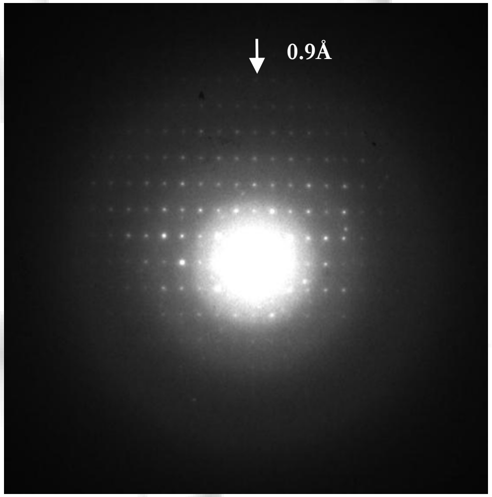
PED patterns in pharmaceutical crystals allow to work with close or with ZA oriented patterns , revealing true crystal symmetry and kinematical intensities good for structure determinations

amoxycillin

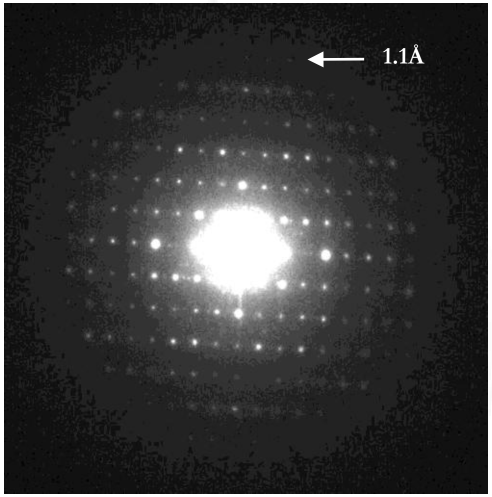


without precession

penicillin G-potassium



without precession



with precession

NanoMEGAS
Advanced Tools for electron diffraction

Samples C.Giacovazzo CNR Bari

Courtesy JP Abrahams, D.Georguieva Univ Leidenc

Resources

- http://www-structmed.cimr.cam.ac.uk/Course/Fourier/Fourier.html
- http://www-structmed.cimr.cam.ac.uk/Course/Adv_diff2/Diffraction2.html#crystal_diffraction
- http://www.ruppweb.org/Xray/101index.html
- http://img.chem.ucl.ac.uk/sgp/mainmenu.htm
- https://eclass.duth.gr/eclass/modules/document/document.php
- http://www.cryst.ehu.es/
- http://fds.oup.com/www.oup.com/pdf/13/9780199569045.pdf
- http://users.encs.concordia.ca/~woodadam/MECH221/Course_Notes/Crystal%20directions%20and%20planes.pdf
- http://journals.iucr.org/j/issues/2010/05/02/kk5061/kk5061.pdf

<u>Books</u>

- 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.

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.