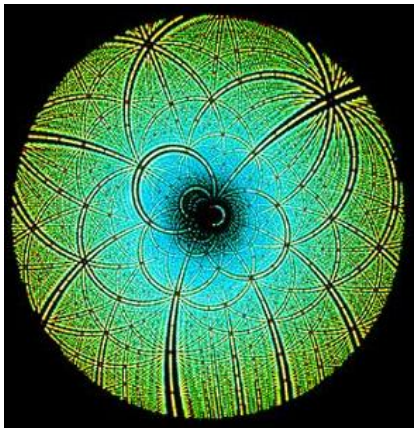
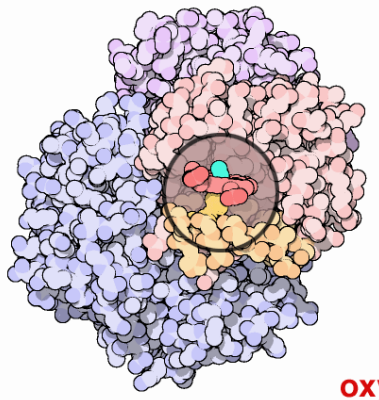


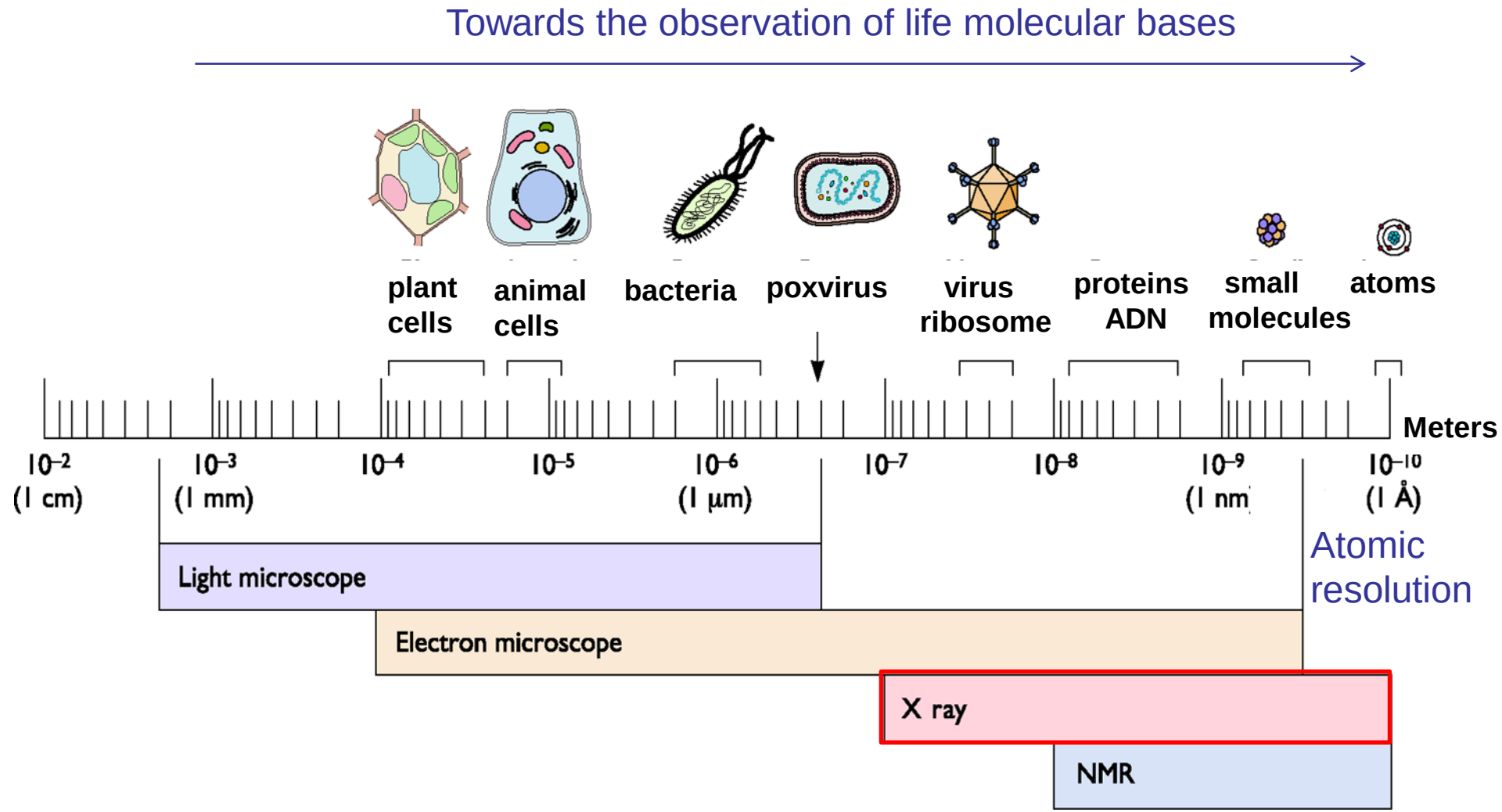
Some practical aspects of bio-crystallography

(preparing the afternoon tutorial)

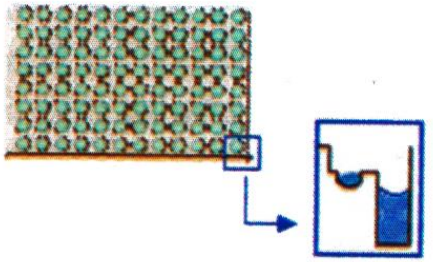
Prof. Patrice Gouet, Université Lyon 1

2014 année internationale de la cristallographie

Biocrystallography in Structural Biology

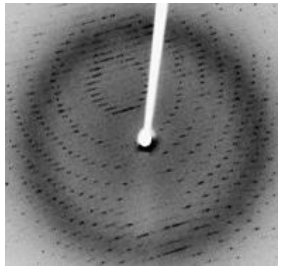


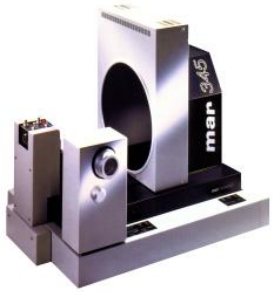
Structural studies by X-ray bio-crystallography



1. Crystallogenesis

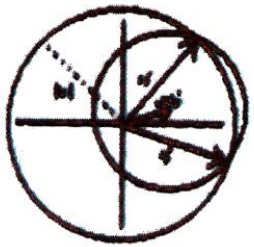
2. Mounting crystal





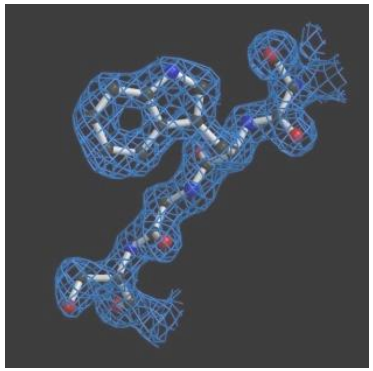
3. X-ray data collection

4. Data treatment

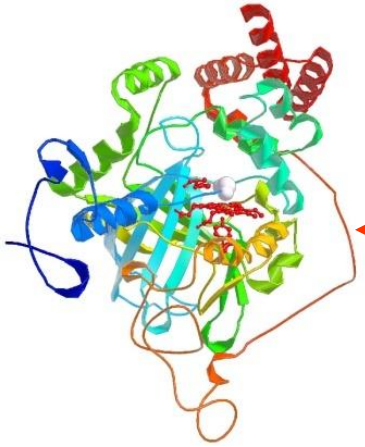


$$\rho(x,y,z) = TF^{-1}(|F_{hkl}| \times e^{i\alpha_{hkl}})$$

5. Data phasing



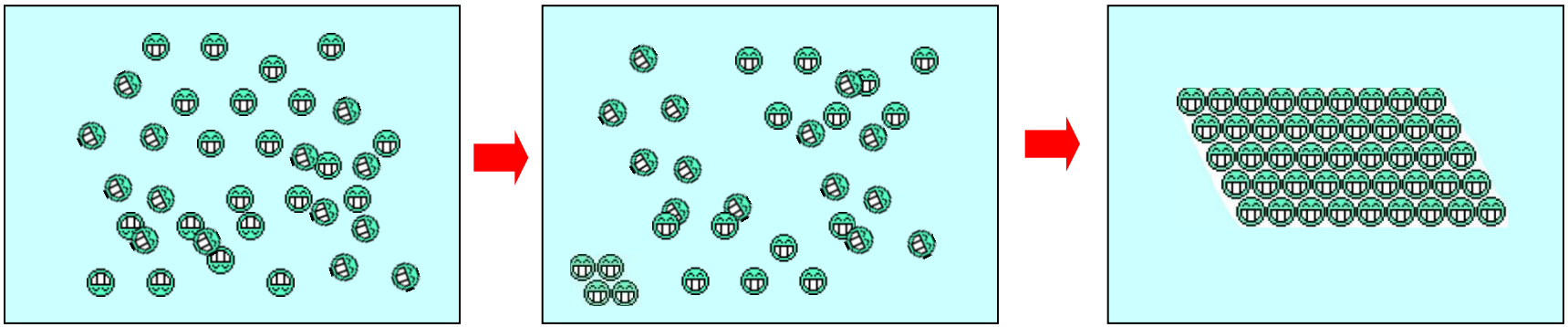
6. Electron density maps and structural refinement



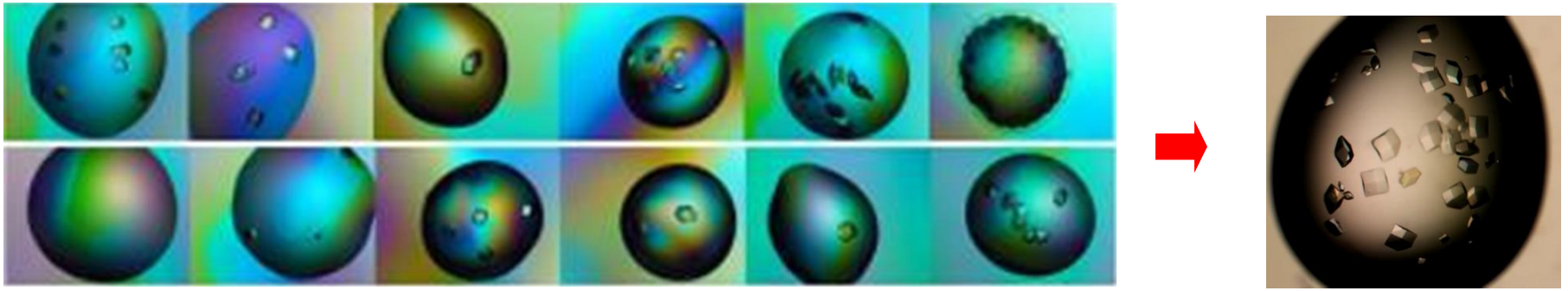
7. Structure analysis

1. Crystallogenesis

Purified protein in solution | Nucleation | Crystal growth

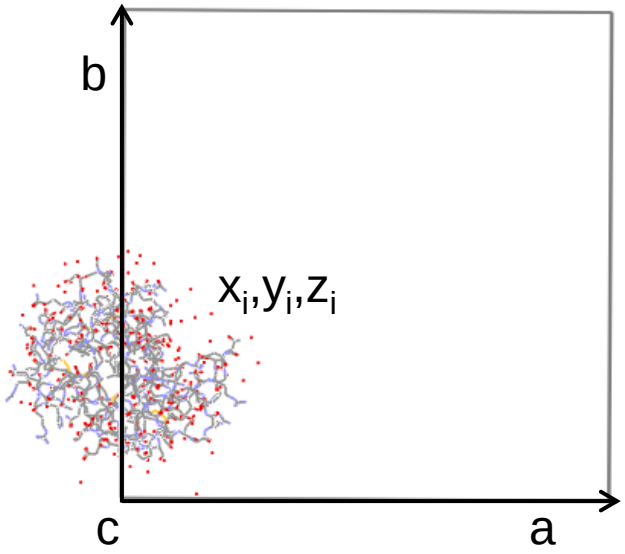


Crystals generally obtained by an evaporation method (**slow concentration**) in an aqueous medium that promotes crystallization (salt, pH ...)



Protein crystals are often **fragile** and **difficult** to obtain

Thousands of trials may be required to obtain crystals of sufficient size 1mm to 10µm that grow in 1h to 1year



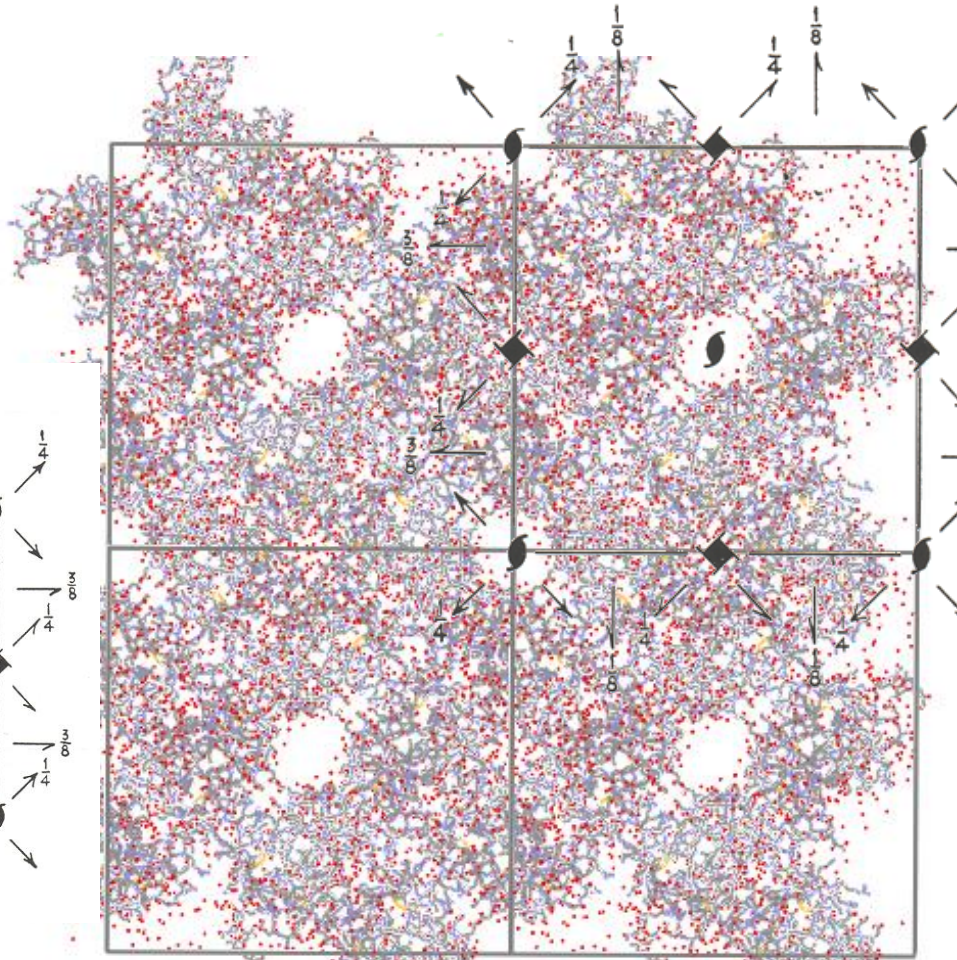
PDBid 1hew (lysozyme)

Quadratic space group $P4_32_12$

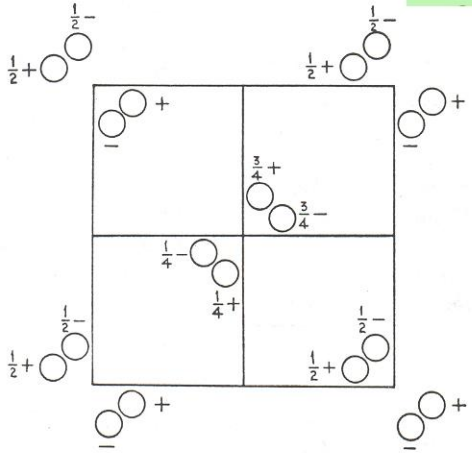
a=**b**=79 Å **c**=37 Å $\alpha=\beta=\gamma=90°$

Asymmetric unit = 1/8 cell

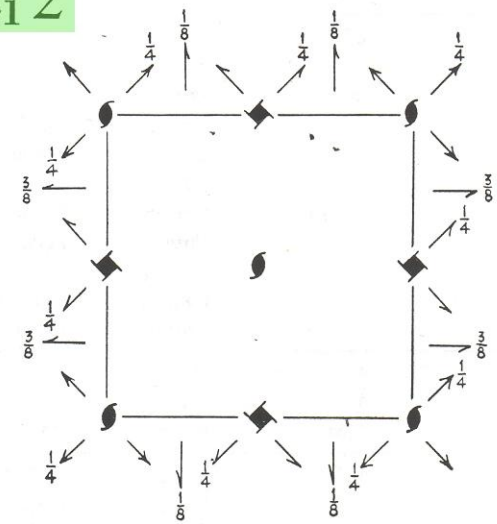
1 monomer per asymmetric unit



$P\,4_3\,2_1\,2$



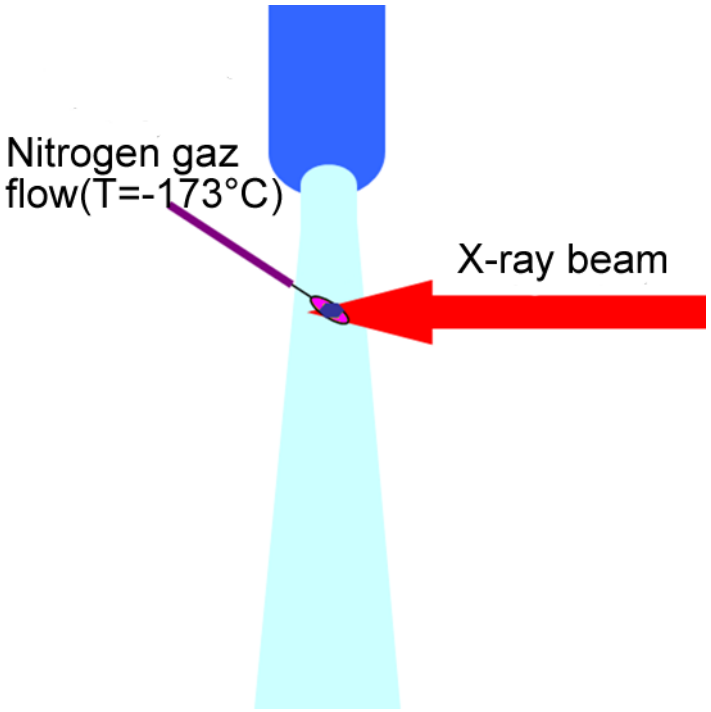
8 equivalent per cell



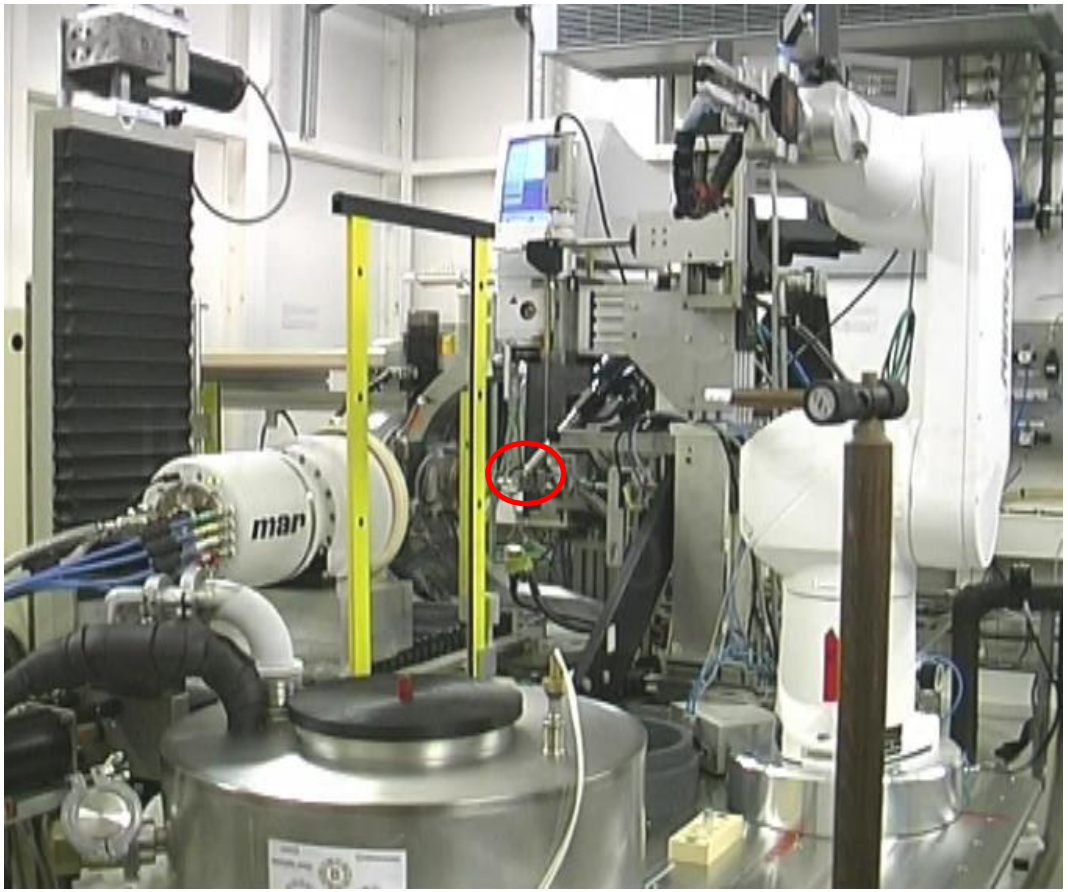
4_3 //**c**, 2_1 //**a**,**b**, 2 //diagonals

Crystal packing for **1hew**
62% protein and 38% solvent

2. Crystal fishing and mounting on a goniometer head



Crystal **fishing** with a cryo-loop and **freezing** to preserve the crystal (dehydration, radiation damage)

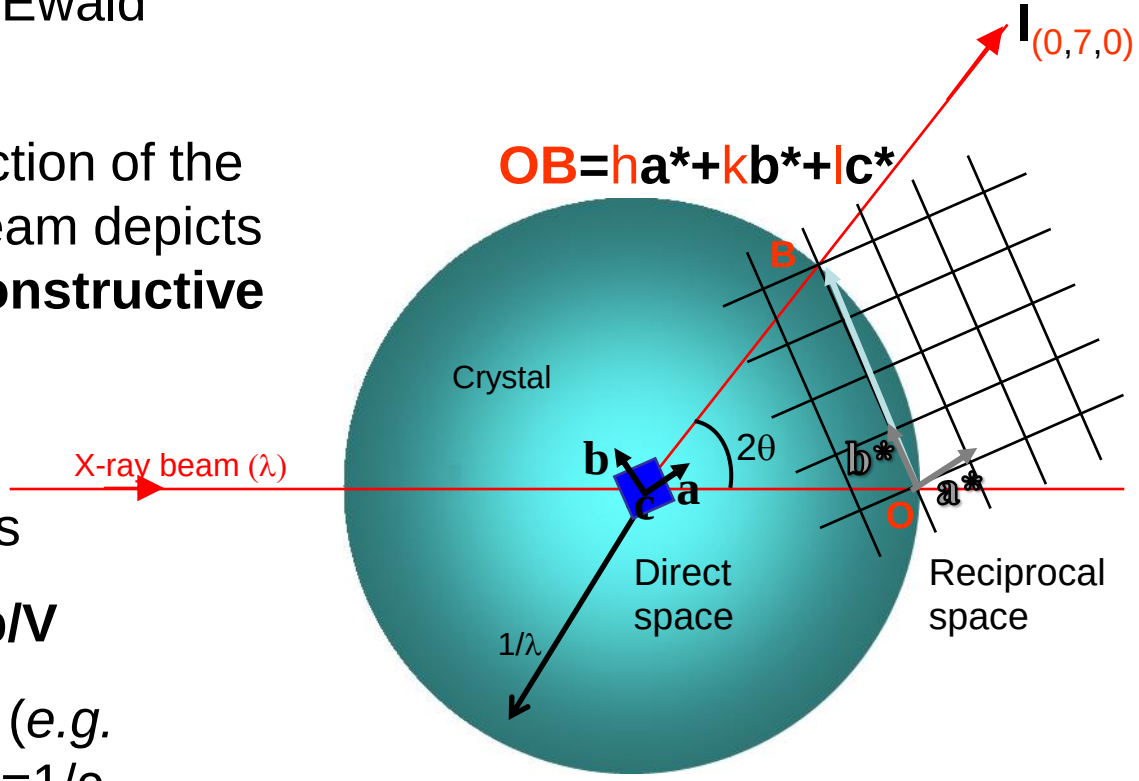


Automated crystal mounting at the ESRF beamline **FIP BM30A**, Grenoble

3. Ewald representation for the diffraction of a 3D crystal

The crystal is centered on the Ewald sphere of radius **1 / λ**

A grid centered on the intersection of the sphere and the RX incident beam depicts X-ray diffraction conditions (**constructive interferences**)



Reciprocal unit cell parameters

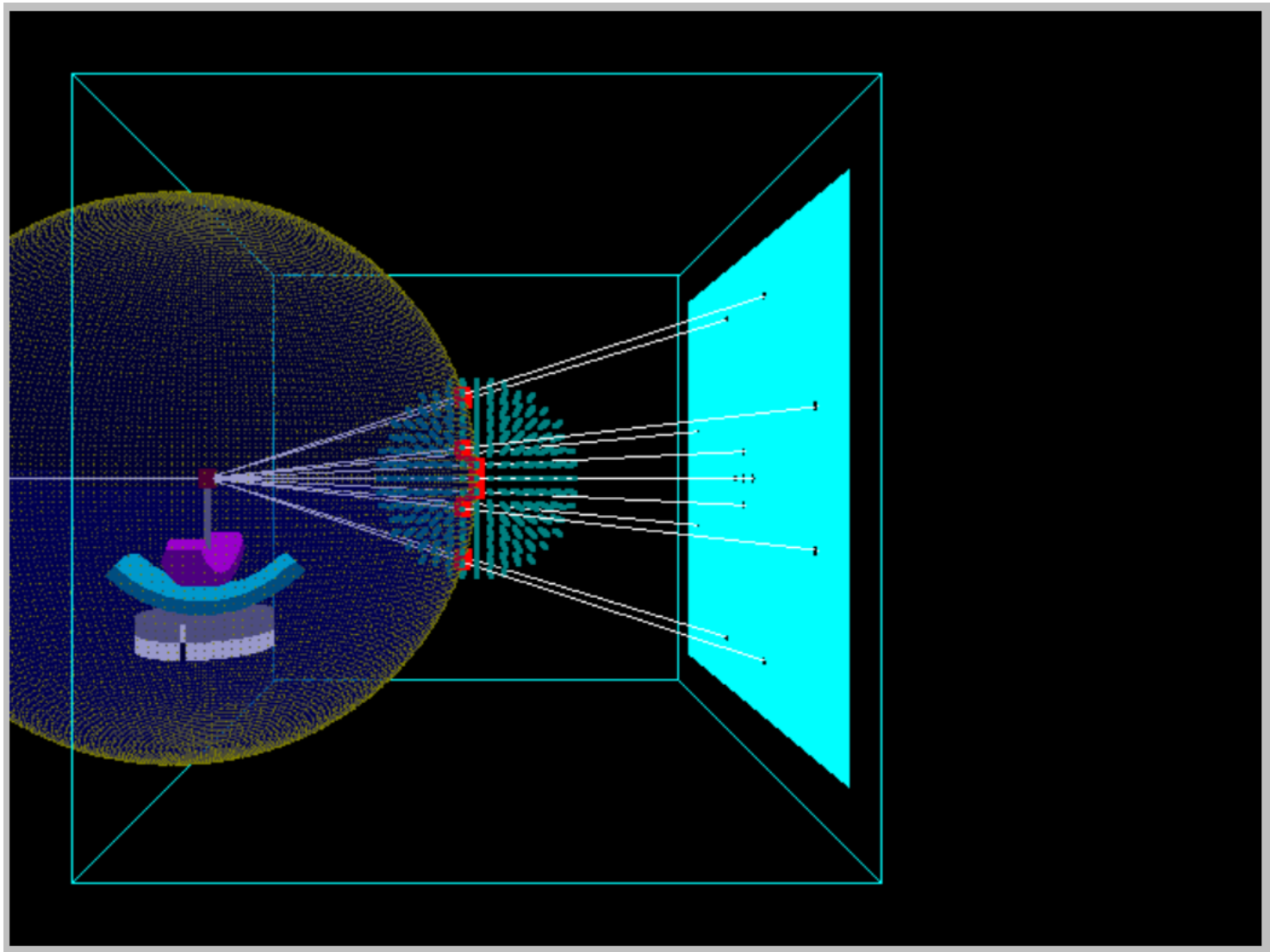
a*=b^c/V ; b*=c^a/V ; c*=a^b/V

For a crystal with α= β= γ=90° (*e.g.* quadratic) a*=1/a, b*=1/b et c*=1/c

A wave is scattered in a direction **OB** and with a 2θ angle when a node of the **hkl** grid intersects with the Ewald sphere.

The structure factor (**amplitude** F and **phase** ϕ) of the scattered wave is:

$$F_{hkl} \exp i\phi_{hkl} = \sum_{j=1}^{N} f_j [\exp i2\pi(hx_j + ky_j + lz_j)]$$ ⟵ (Fourier Transform)



Goniometer Controls

0.0 0.0 0.0

small center | large center | phi center

0 center

Y Eye Point Control

0 center

X Eye Point Control

0 center

Z Eye Point Control

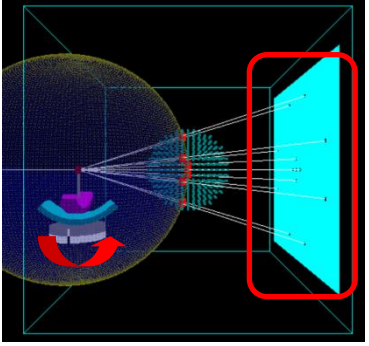
Auto Rotation

Laue Photography

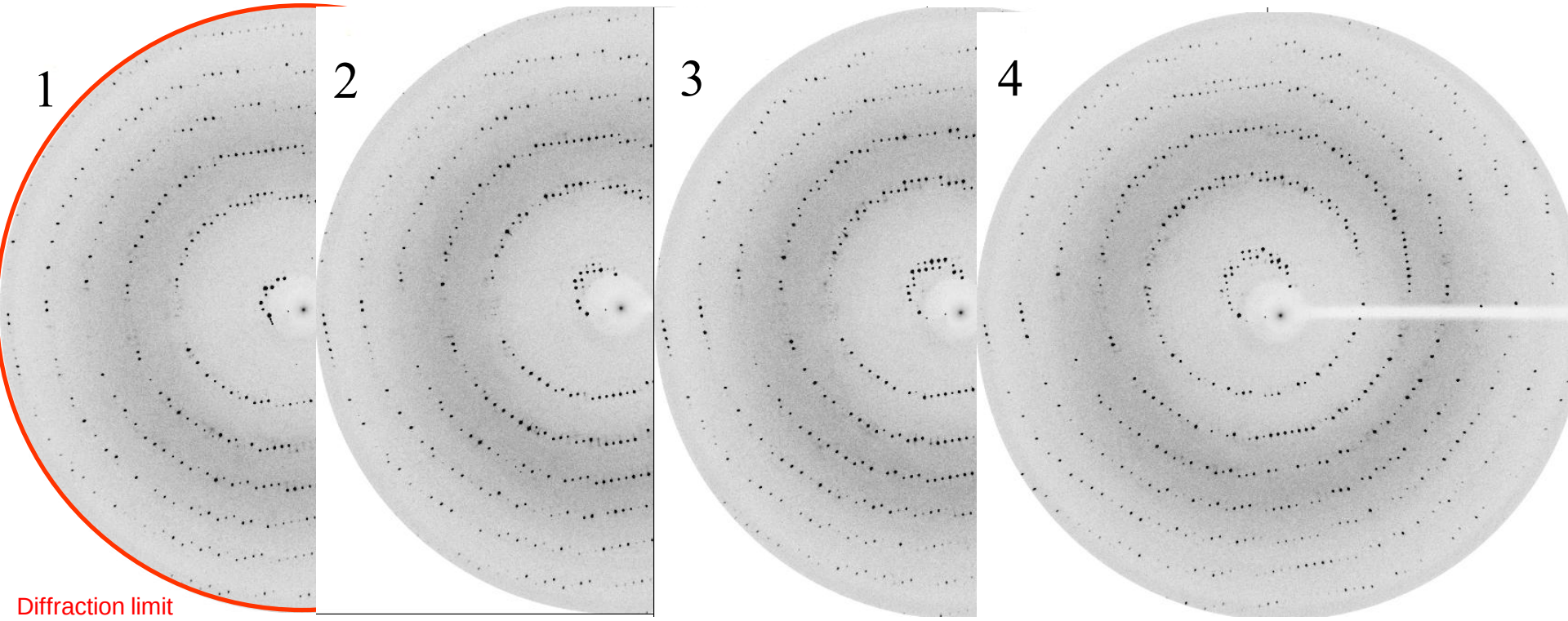
Lattice Type P

1.0 center

Crystal to Detector Distance



Data collection by oscillation steps



0° → 1°
`lyso_001.mar2000`

1° → 2°
`lyso_002.mar2000`

1° → 2°
`lyso_003.mar2000`

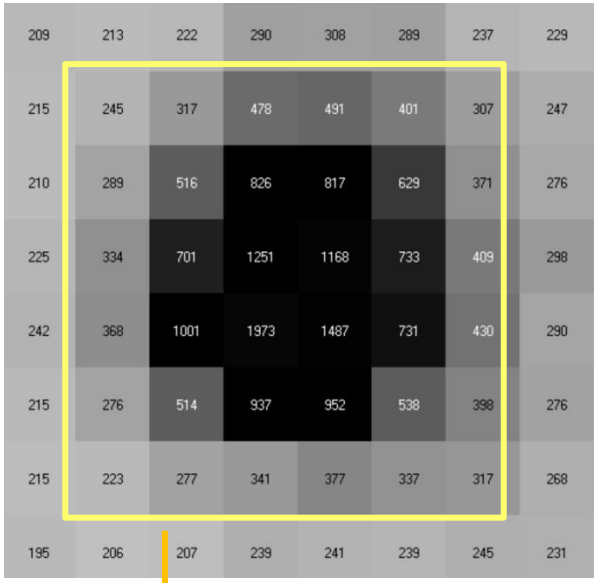
2° → 3°
`lyso_004.mar2000`

. . . . . .

180

179° → 180°
`lyso_180.mar2000`

4. Data processing (iMosflm/XDS)

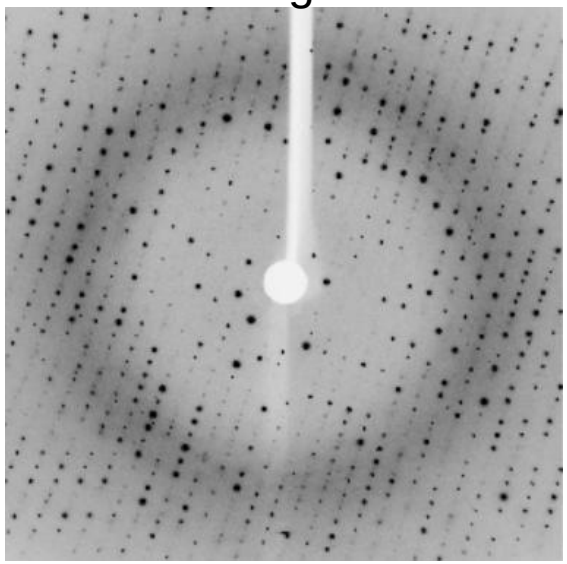


1/ Indexing (calculating crystal unit cell & orientation with one or more images)

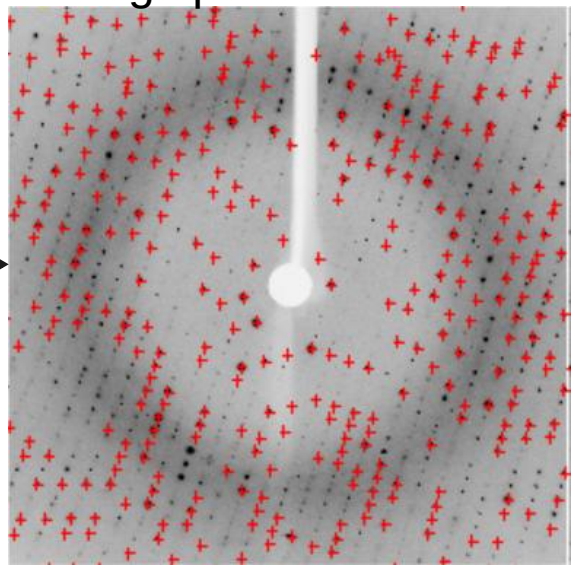
Autoindexing

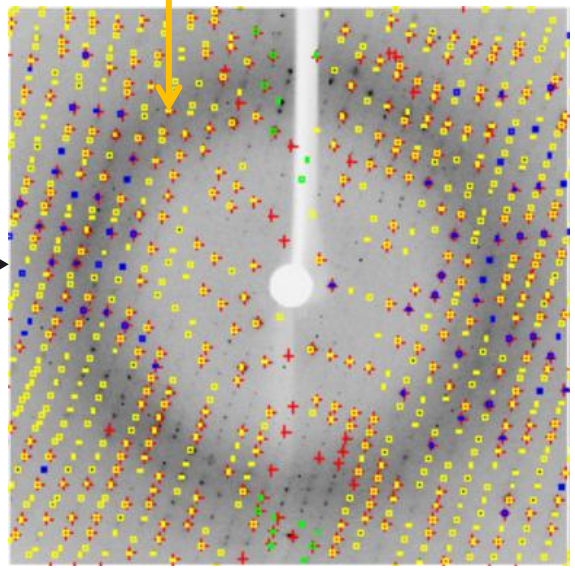
a=b=79 Å c=38 Å $\alpha=\beta=\gamma=90°$

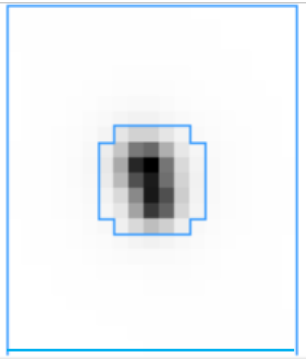
Image 0-1°



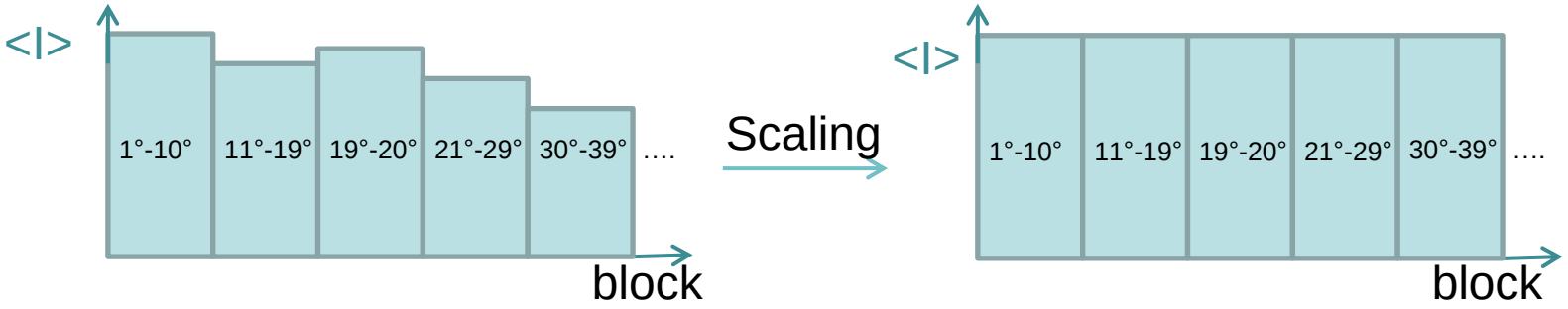
Finding spots with iMosflm





2/ Integrating (measuring intensities  from first to last image)

3/ Scaling intensities by blocks of images (*10° in iMosflm for the lysozyme crystal*). Conserved <I> between blocks



4/ Merging symmetry-related I(hkl) (*Laue class 422 for the lysozyme crystal*) with assessment of data quality

$$R_{sym} = \frac{\sum_{hkl}\sum_{i=1}^{n}\left|I_i(hkl) - I_i(\bar{h}\bar{k}\bar{l})\right|}{\sum_{hkl}\sum_{i=1}^{n} I_i(hkl)}$$

Good data set usually means overall R_{svm} between 2% and 7%

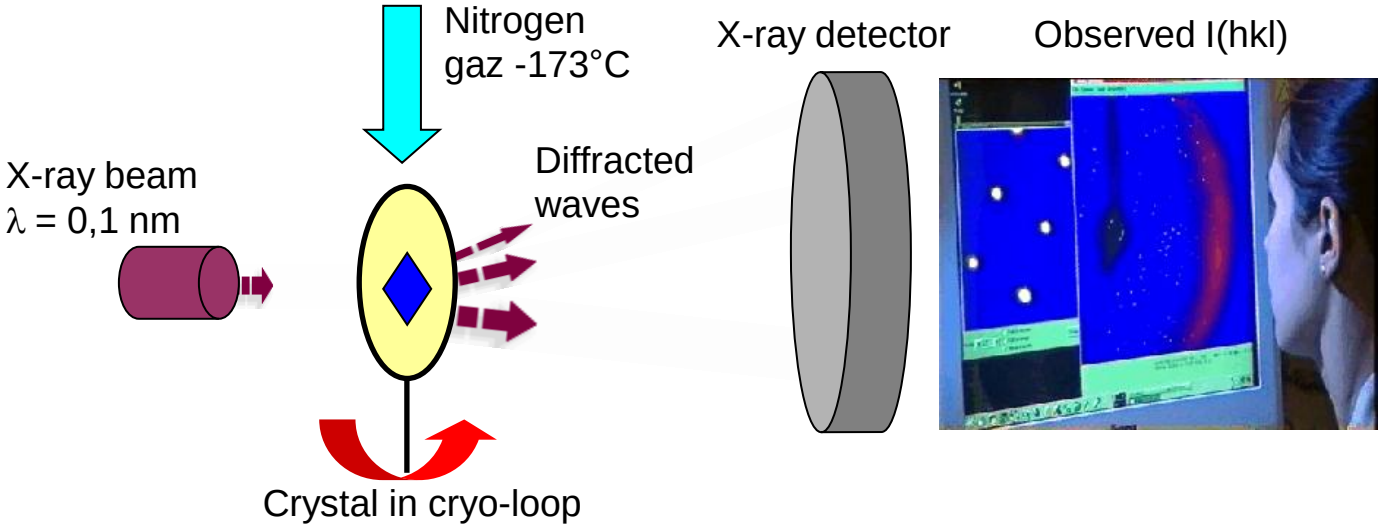
5/ Amplitudes from merged intensities $I=F^2$ (*resulting file*)

h	k	l	F	SIGF	DANO	SIGDANO	F(+)	SIGF(+)	F(-)	SIGF(-)
0	0	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0	0	2	-1.00	0.00	-1.00	0.00	-1.00	0.00	0.00	0.00
0	0	3	-1.00	0.00	-1.00	0.00	-1.00	0.00	0.00	0.00
0	0	4	101.12	6.29	0.00	0.00	100.92	9.00	100.05	9.11
0	0	5	5087.18	868.91	5087.18	868.91	5087.18	868.91	5004.75	871.44
0	0	6	-1.00	868.91	-1.00	868.91	-1.00	868.91	5004.75	871.44
0	0	7	-1.00	868.91	-1.00	868.91	-1.00	868.91	5004.75	871.44
0	0	8	712.77	26.26	0.00	0.00	713.90	35.18	706.38	40.04
0	0	9	251303.12	24365.59	251303.12	24365.59	251303.12	24365.59	246856.75	27390.66
0	0	10	-1.00	24365.59	-1.00	24365.59	-1.00	24365.59	246856.75	27390.66
0	0	11	-1.00	24365.59	-1.00	24365.59	-1.00	24365.59	246856.75	27390.66
0	0	12	374.42	11.63	0.00	0.00	377.39	14.45	367.19	19.85
.....										
.....										
36	20	1	239.06	4.01	-32.37	8.15	221.41	6.19	253.78	5.30

resolution limit

5. Difference between X-ray diffraction and optical microscopy

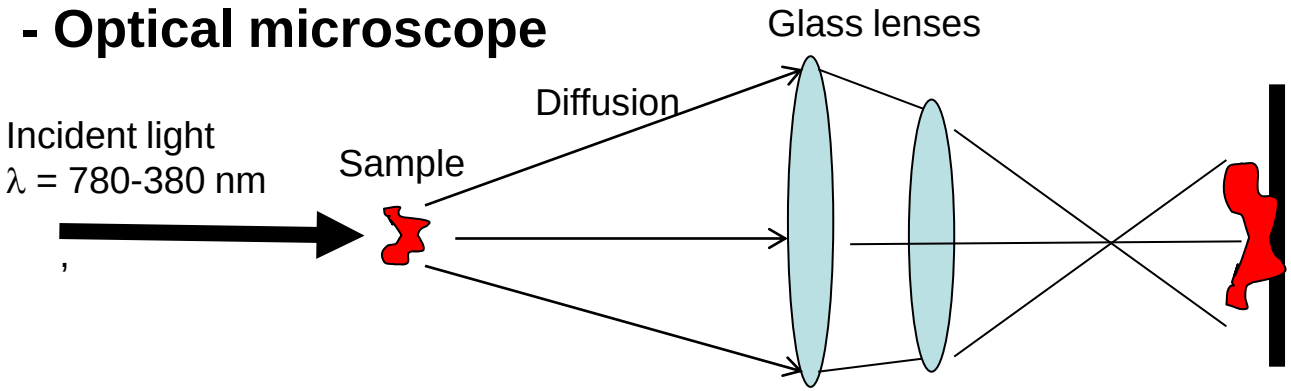
- X-ray diffractometer



Fourier Transform → Inverse Fourier Transform →

Magnified sample
(paramecium, ~0,2mm)

- Optical microscope

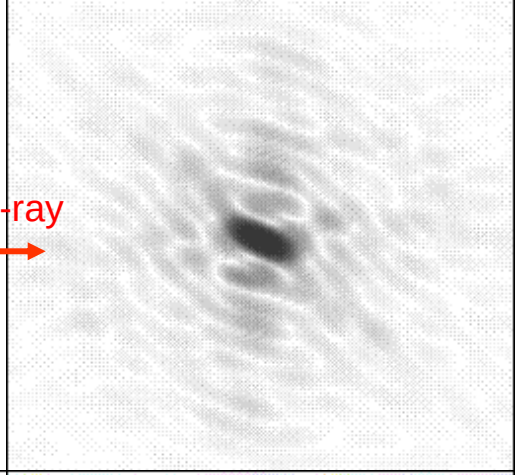


X-rays are high energy waves. There is no physical way to change their trajectories to directly observe a magnified image → **Phasing problem** in X-ray crystallography

A. Phasing with molecular replacement

your crystal

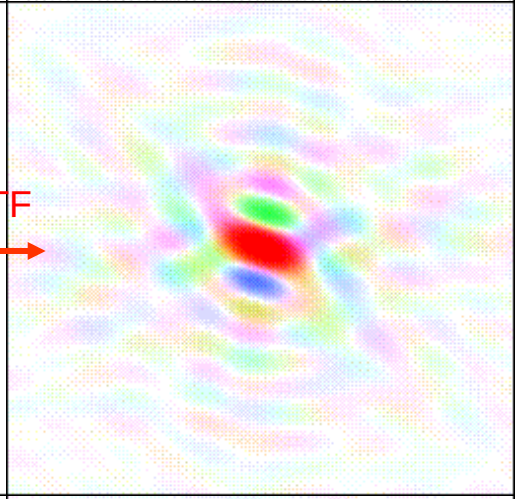
X-ray



Experimental Fobs

your PDB model
atoms x_i,y_i,z_i

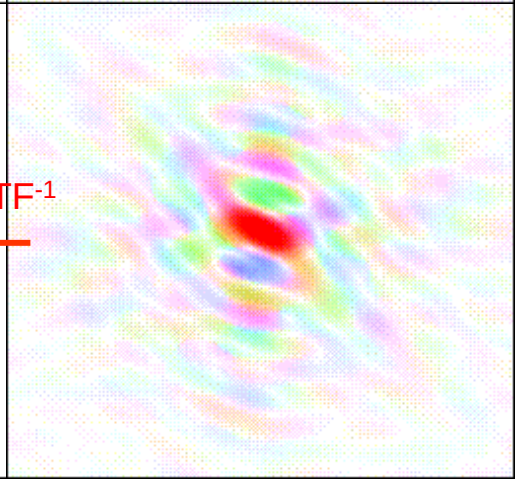
TF



Calculated Fcalc, ϕcalc

resulting electron density maps

TF^{-1}



Calcul of TF^{-1}(Fobs ϕcalc)

Assessment with R_{factor} (<50%)

$$R = \frac{\sum_{hkl} \left| |F_{obs}| - k|F_{calc}| \right|}{\sum_{hkl} |F_{obs}|}$$

Use of the Patterson function in Molecular Replacement

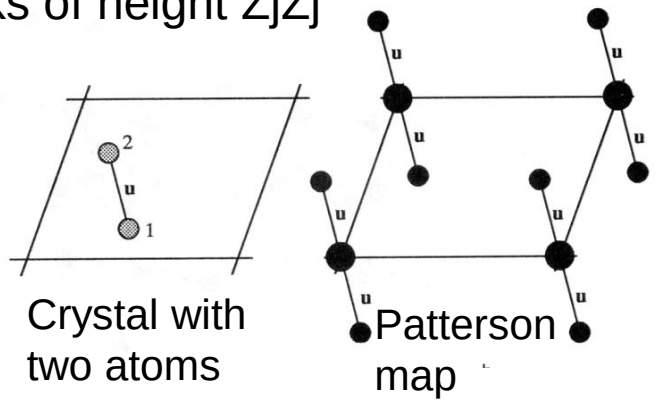
1. The Patterson function is defined as the TF^{-1} from intensities

$$P(\mathbf{u}) = \frac{1}{V_c}\sum_{\mathbf{h}} |F(\mathbf{h})|^2 \exp(-2\pi i \mathbf{h}\cdot\mathbf{u}) \quad \text{with} \quad \mathbf{u} = u\mathbf{a} + v\mathbf{b} + w\mathbf{c}$$

2. It is also defined as a unit cell with N^2-N+1peaks of height ZjZj' positioned at the end of interatomic vectors **r**j-**r**j'

$$P(\mathbf{u}) = \sum_j \sum_{j'} Z_j Z_{j'} \delta(\mathbf{u} + \mathbf{r}_j - \mathbf{r}_{j'})$$

where Z_j is the number of electrons of the atom j positioned at $\mathbf{r}_j$

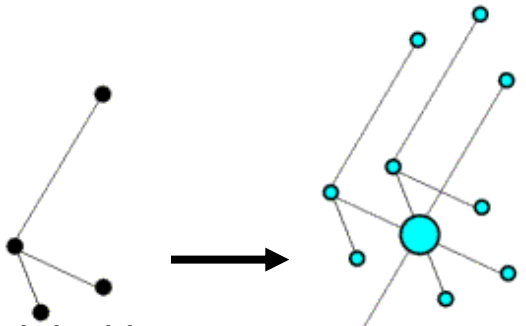


Crystal with two atoms

Patterson map

3. Suppose a **model** consisting of four atoms similar to the crystallized molecule

→ the Patterson map of the model is calculated in a crystal with arbitrary large unit cell parameters



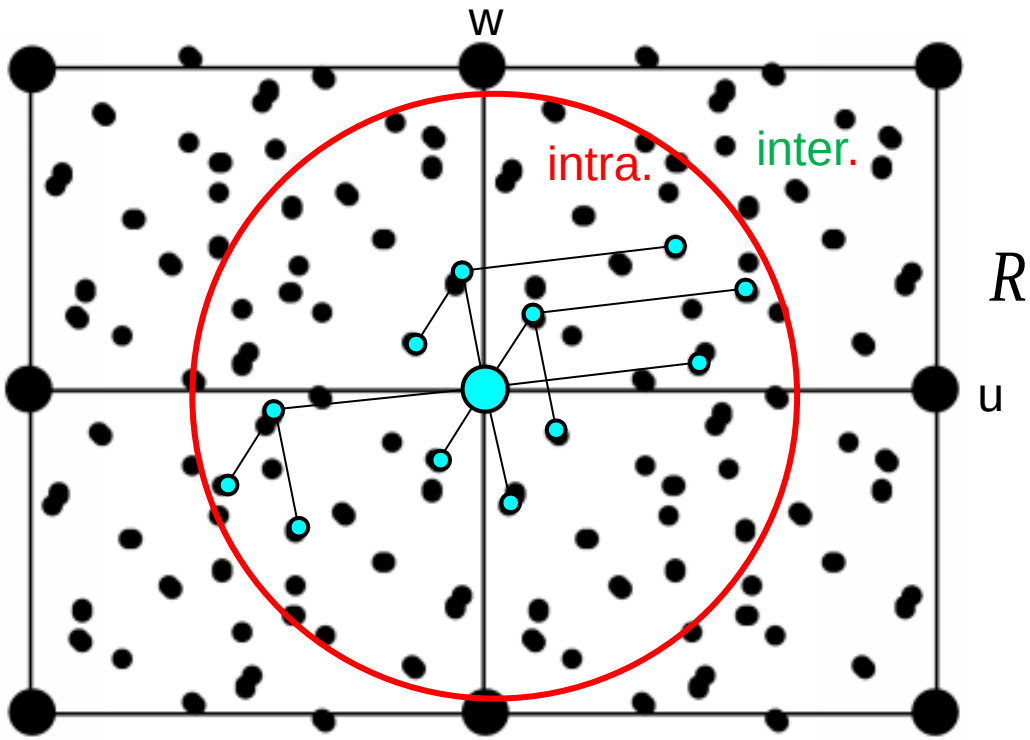
Model with coordinates x_i, y_i, z_i

Intramolecular vectors

I. the rotation function

Overlay between $\mathbf{P_A}$**native** and $\mathbf{P_B}$**model** is calculated between the 2 cards within a region of radius R and centered in u=0,v=0,w=0

Intramolecular vectors are kept and intermolecular vectors are excluded



$$R([\mathrm{R}]) = \int_U P_A(u)\, P_B([C]u)\, du$$

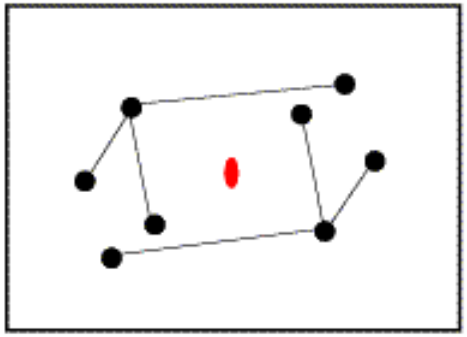
native model

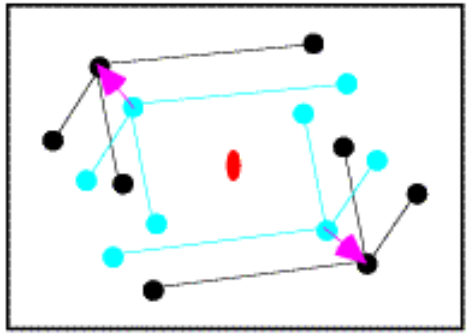
$TF^{-1}(I(hkl))$

The orientation of the four atom model in the experimental unit cell is determined at the **maximum overlay** (maximum of the rotation function *R*([R]))

II. the translation function

Superimposition between **the observed intermolecular vectors** and the **calculated intermolecular vectors** by moving the oriented model along the axes u, v and w:





$$T(\mathbf{t}) = \int_V P_{1,2}(\mathbf{u}, \mathbf{t}) \times P(\mathbf{u})\, d\mathbf{u}$$

calculated Patterson observed Patterson

The solution will correspond to the maximum of the Translation function *T*(t)

→ The four atom model is now **oriented** and **positioned** in the experimental cell.

III. Crystallographic assessment and refinement

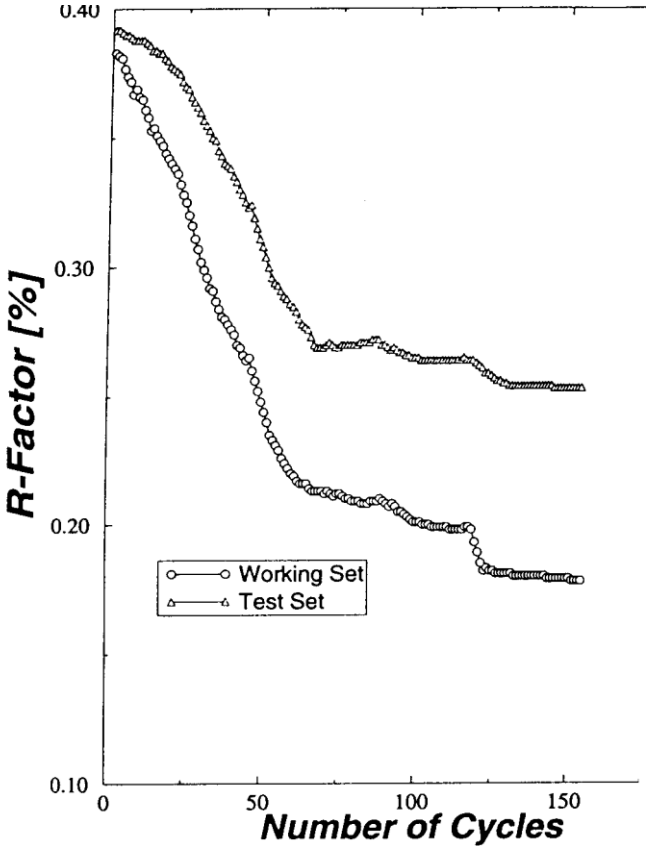
The first validation of the structure is the measure of the confidence factor R_{factor}

$$R = \frac{\sum_{hkl} \left| |F_{obs}| - k|F_{calc}| \right|}{\sum_{hkl} |F_{obs}|}$$

A second confidence factor, R_{free}, is calculated on 5% of the data, which are not included in the restrained positional refinement

$$R_T^{free} = \frac{\sum_{hkl \subset T} \left| |F_{obs}| - k|F_{calc}| \right|}{\sum_{hkl \subset T} |F_{obs}|}$$

If the restrained refinement is correct (*performed with Refmac5*), the R_{free} must decrease with the R_{factor}



B. Phasing with anomalous signal

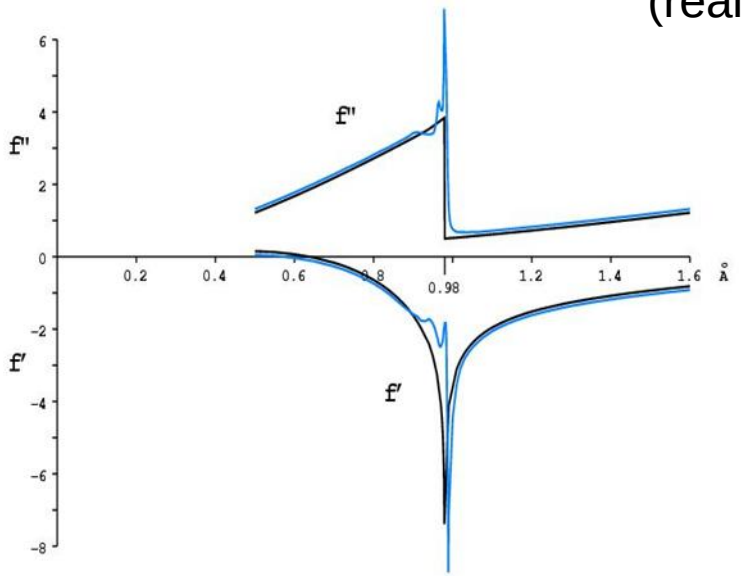
Correction to the scattering factor of an atom in case of anomalous scattering

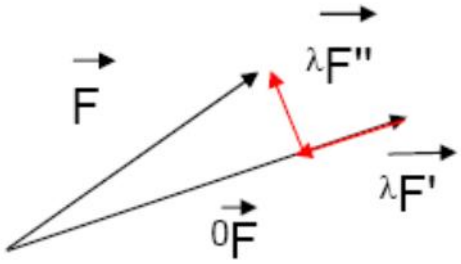
$$f = {}^{0}f + {}^{\lambda}f' + i\,{}^{\lambda}f''$$

Absorption component (imaginary part)

Number of electrons

Dispersion component (real part)

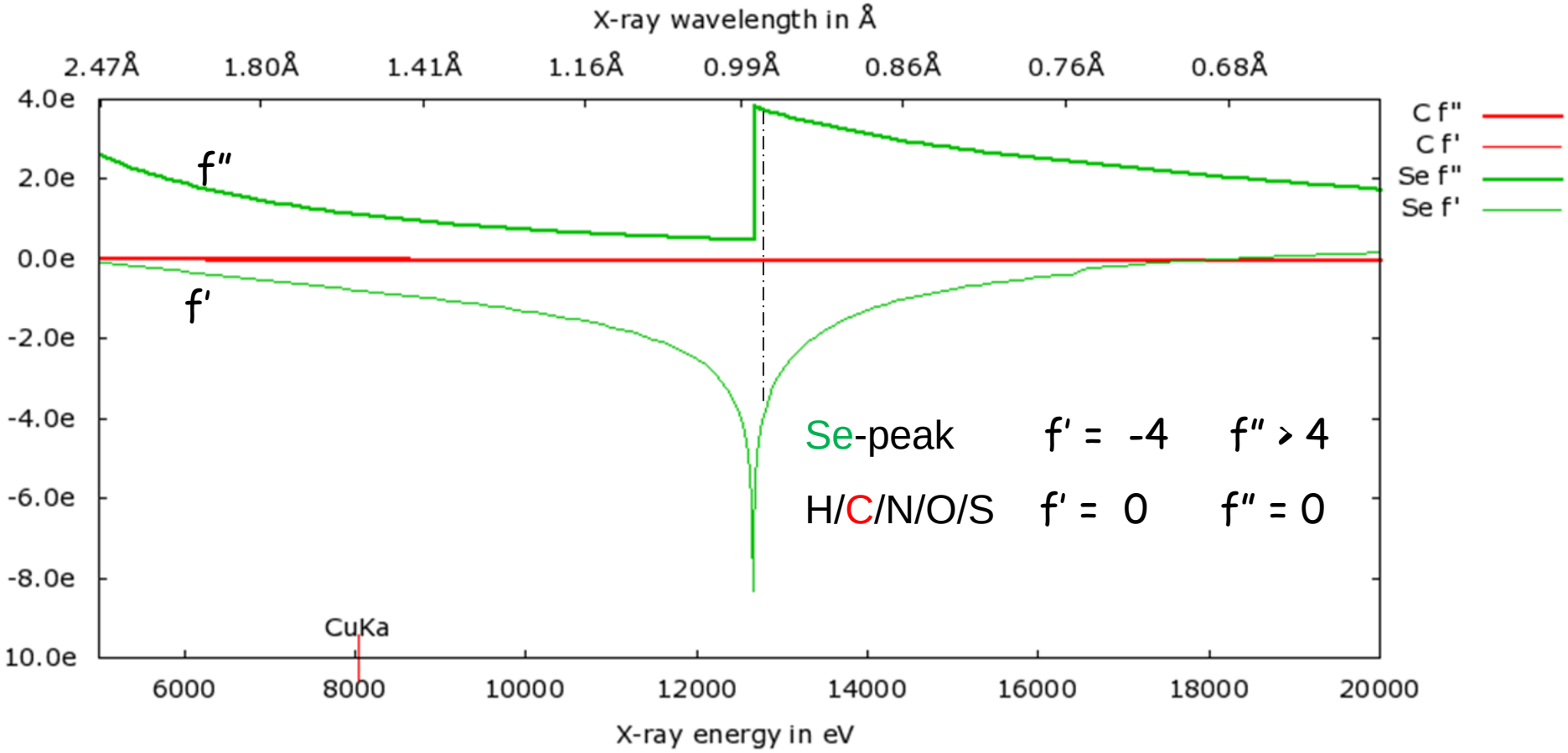




Change in scattering factor, which can vary with λ

Anomalous correction f” is proportional to absorption and fluorescence and f’ is its derivative

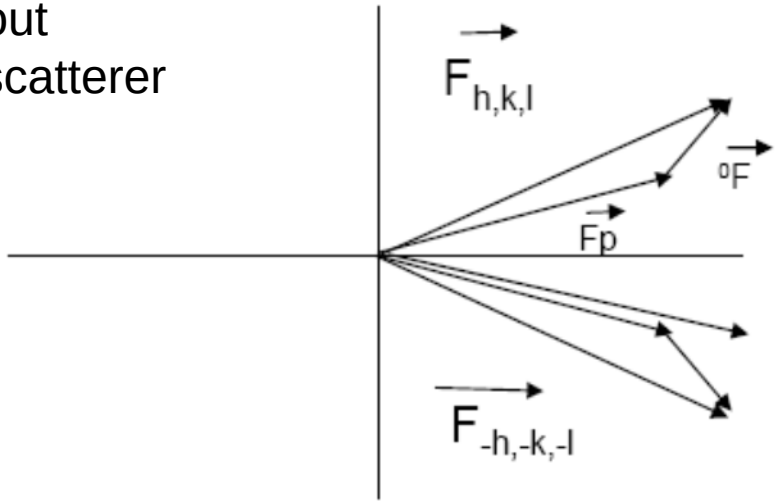
Anomalous signal of Selenium near K-edge (λ=0.98 Å)



$$f = {}^{0}f + {}^{\lambda}f' + i\,{}^{\lambda}f''$$

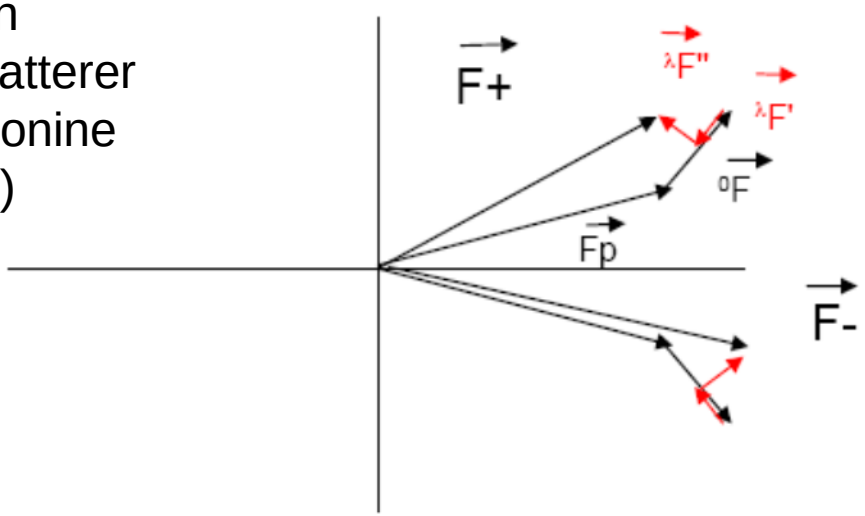
The breakdown of Friedel's law

Protein without anomalous scatterer



$|F_{h,k,l}|=|F_{-h,-k,-l}|$

Protein with an anomalous scatterer (Seleno-Methionine and λ =0.98 Å)



$|F_{h,k,l}| \neq |F_{-h,-k,-l}|$

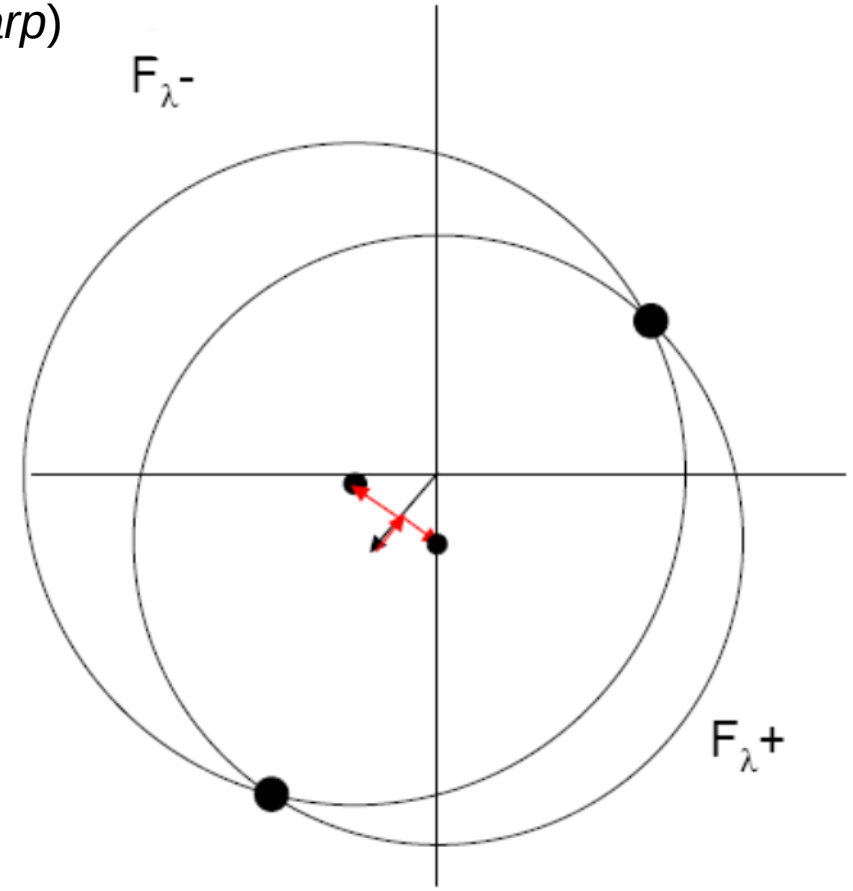
The difference between F(h,k,l) and F(-h,-k,-l) is DANO

$$\vec{F}_\lambda = \vec{Fp} + \overrightarrow{F_0 - F_\lambda' + iF_\lambda''} \qquad \vec{Fp} = \vec{F}_\lambda - \overrightarrow{(F_0 - F_\lambda' + iF_\lambda'')}$$

protein

anomalous scatterer

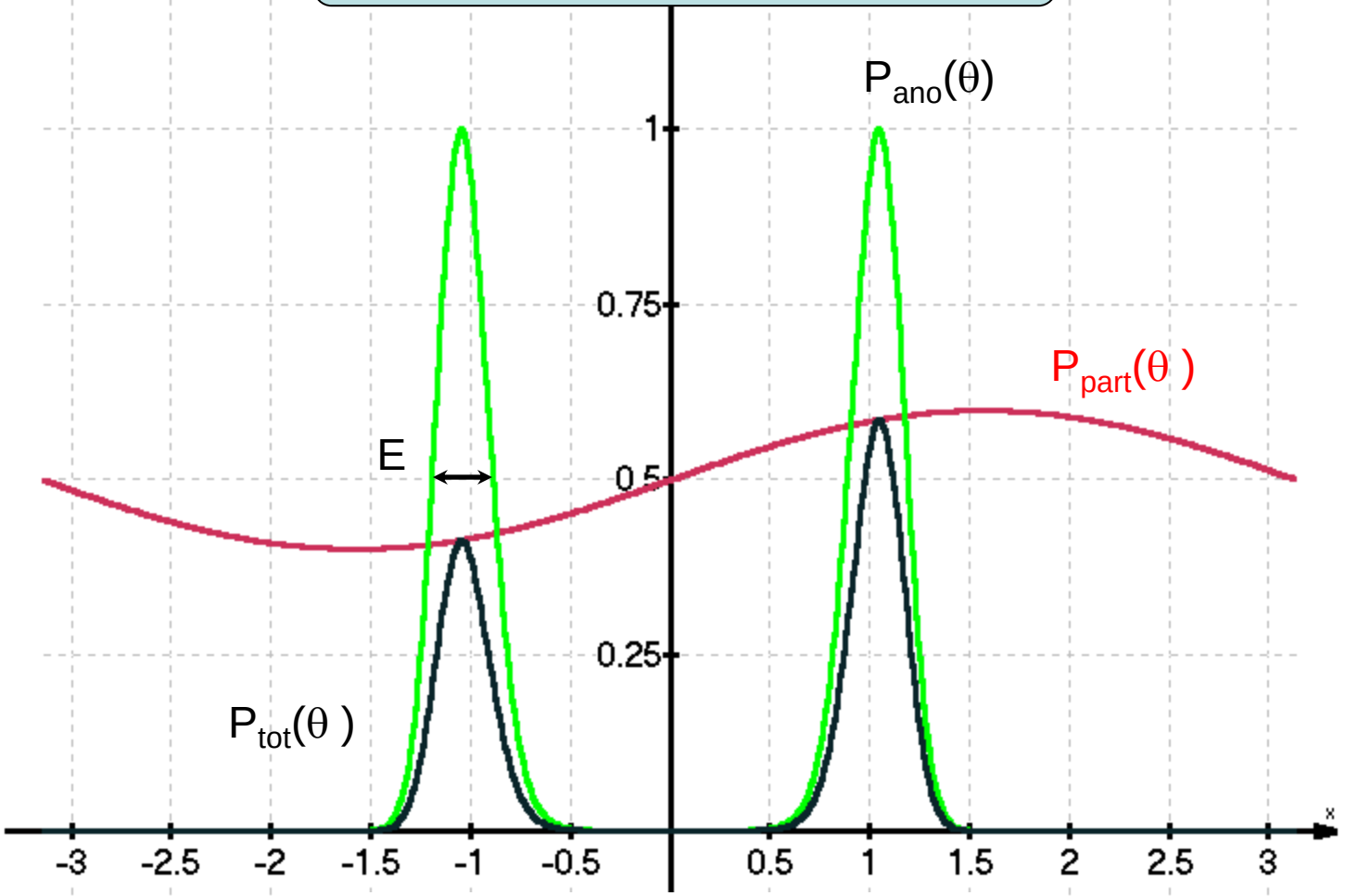
positions of the anomalous scatterer in the unit cell estimated with an anomalous Patterson (*Sharp*)



Two possible phases for the protein **Fp ->** be **MAD** or **SAD**

Phases probabilities in SAD

$P_{tot}(\theta) = P_{ano}(\theta) * P_{part}(\theta)$



$\theta = \varphi_T - \varphi_A + \pi/2$

The crystallographer may take the **mean** of the two possible phases and use this along with a weight: $\boldsymbol{F} = w\,|F_P|\,\exp(i\phi_{mean})$ and then use solvent flattening

Improving phases by solvent flattening (*Solomon*)

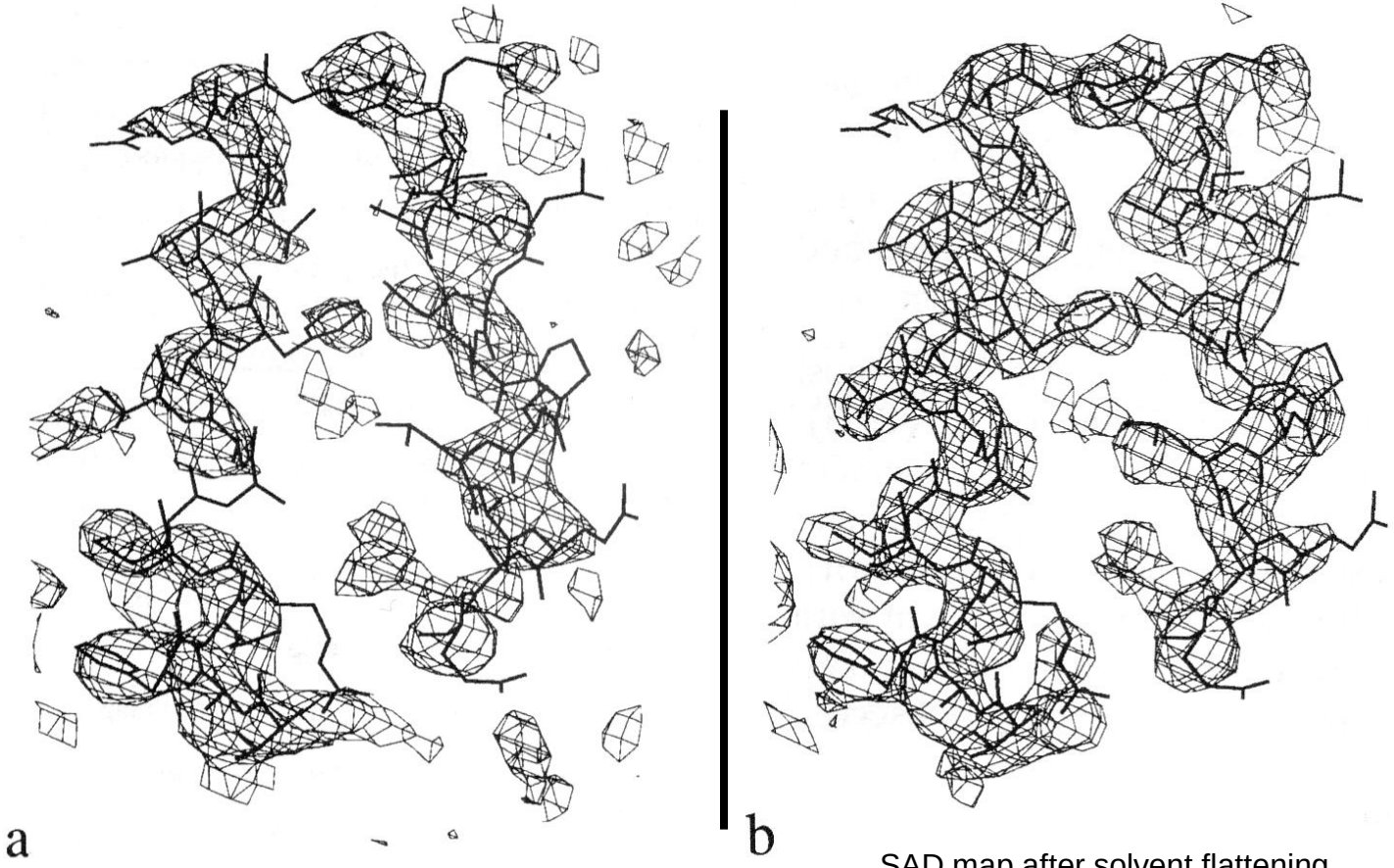
Calculating a mask where the protein zone is set to 1 and the solvent to 0

mask	Electron density maps
0000000000000000	1121232111221211 2
000**1111**0000**11**000	212**45475**122**56**232
00**111111111111**00	12**367867784567**31
00**111111111111**000	32**867559877685**432
0000**111111111**0000	1232**567798775**4322
0000000000000000	1223422122113214 1

Determination of the average density in the solvent mask region set to 0 ($\rho_s = 2$).

2222222222222222
222**45475**222**56**222
22**367867784567**22
22**867559877685**222
2222**567798775**2222
2222222222222222

Better phases will then be obtained by Fourier Transform and cycling



SAD map at 3.3 Å

SAD map after solvent flattening

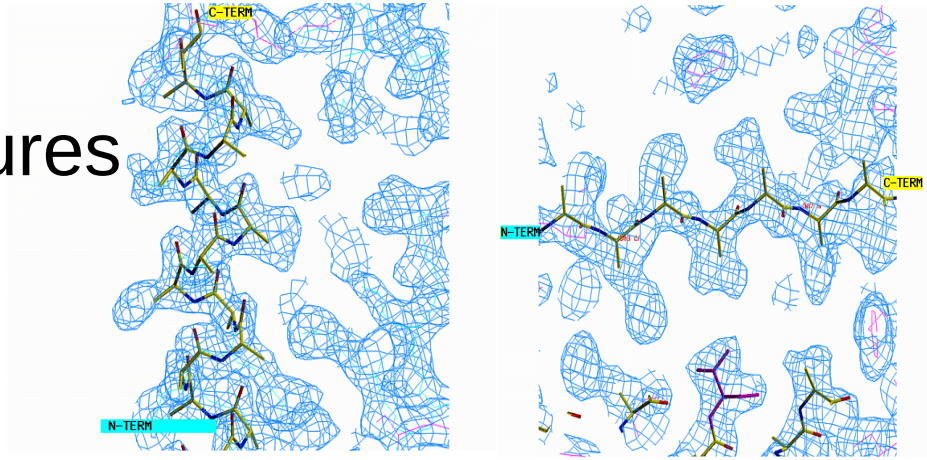
The procedure of solvent flattening can be improved in case of **non-crystallographic** symmetry.

The density of the protein is considered to be identical between regions linked by non-crystallographic symmetry and is **averaged** within masks (powerful for **icosahedral viruses**)

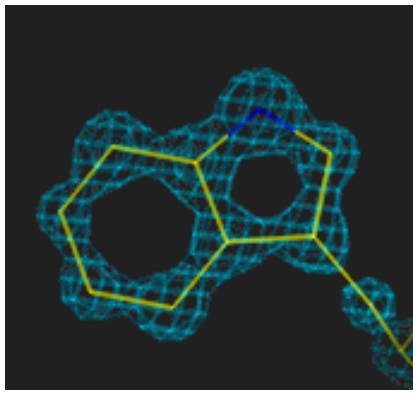
$$\rho_{avg}(x) = \sum_{i=1}^{N} \frac{1}{N} M_i(x) \sum_{j=1}^{N} \rho(x_{ij})$$

Model Building: Steps in making the first trace in electron density map

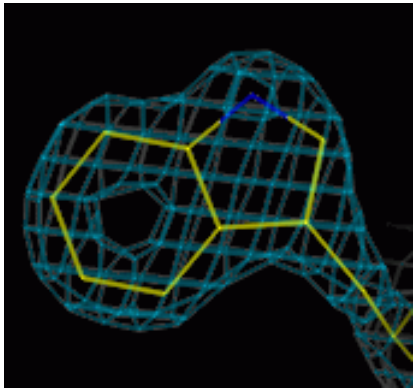
- Generating Cα chain trace
- recognize secondary structures
- Identifying chain direction
- sequence assignment
- add water molecules, etc...



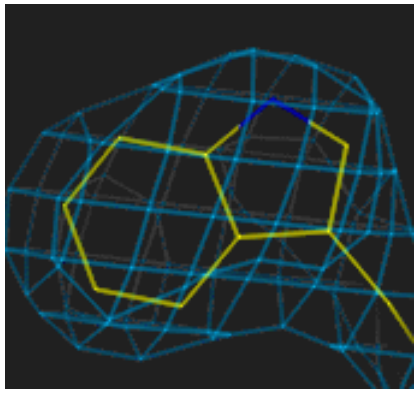
- The sulfur or Se-methionines are the perfect starting point for the sequence fitting if the map is from sulfur-SAD or Se-MAD phases.
- The large tryptophan may well be recognized in electron density maps.



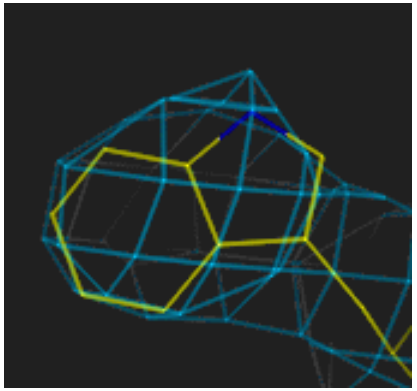
1.0 Å



1.8 Å



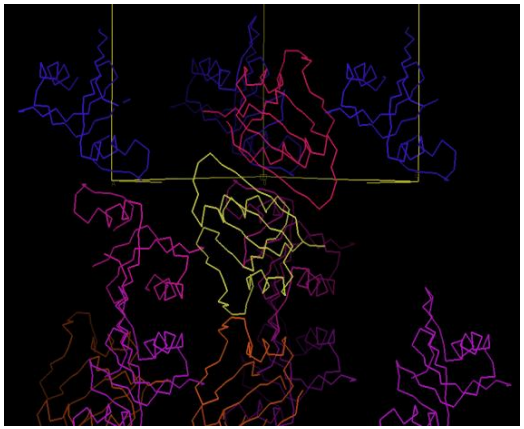
3.0 Å



4.0 Å

Structure Validation and Deposition

Generate symmetry related molecules and check contacts

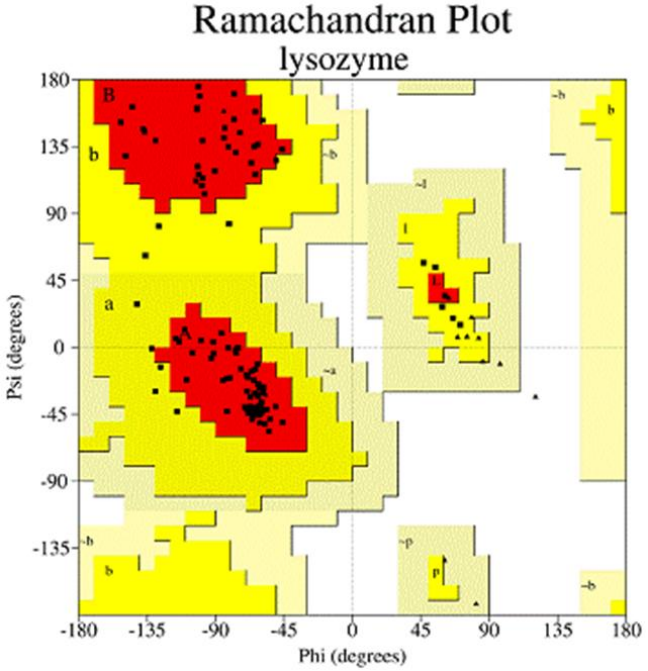


Missing density is much better than extra density

The model should make chemical sense

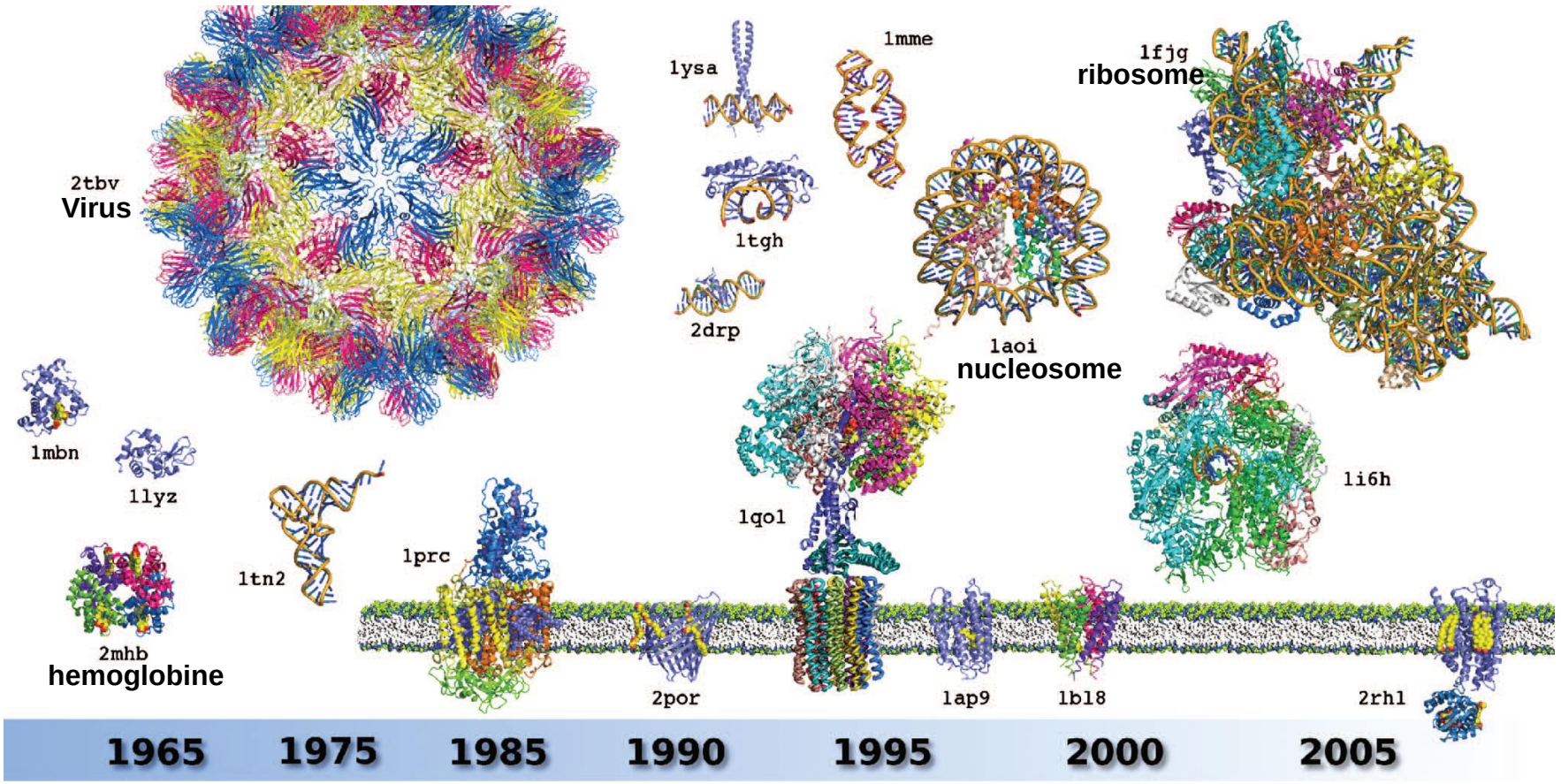
The stereochemical parameters such as bond length, bond angle etc, should be within the standard deviation from their ideal values

The Ramachandran Plot should be normal



==> WHATCHECK, MOLPROBITY, ...

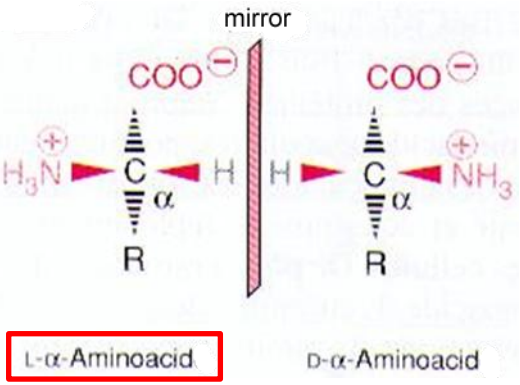
Protein Data Bank, > 100 000 atomic structures

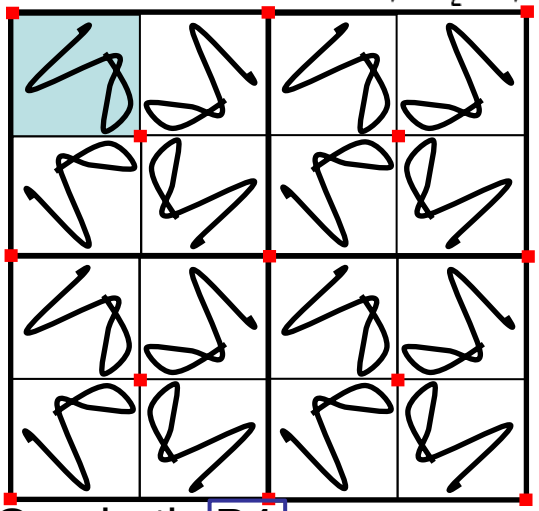


Why water boils at 100ºC and methane at -161ºC, why blood is red and grass is green, why diamond is hard and wax is soft… The answers to all these problems have come from structural analysis.

***Max Perutz**, July 1996, Churchill College, Cambridge*

Class	Laue Class	Space group (65 without mirror symmetry out of 230)
Triclinic	1	P1
Monoclinic	2	P2, $P2_1$, C2
Orthorhombic	222	P222, $P222_1$, $P2_12_12$, $P2_12_12_1$, $C222_1$, C222, F222, I222, $I2_12_12_1$
Quadratic	4 422	P4, $P4_1$, $P4_2$, $P4_3$, I4, $I4_1$, P422, $P42_12$, $P4_122$, $P4_12_12$, $P4_222$, $P4_22_12$, $P4_322$, $P4_32_12$, I422, $I4_122$
Trigonal	3 32	P3, $P3_1$, $P3_2$, P312, P321, $P3_112$, $P3_121$, $P3_212$, $P3_221$, R32
Hexagonal	6 622	P6, $P6_1$, $P6_5$, $P6_2$, $P6_4$, $P6_3$, P622, $P6_122$, $P6_522$, P622, $P6_422$, $P6_322$
Cubic	23 432	P23, F23, I23, $P2_13$, $I2_13$, P432, $P4_232$, F432, $F4_132$, I432, $P4_332$, $P4_132$, $I4_132$

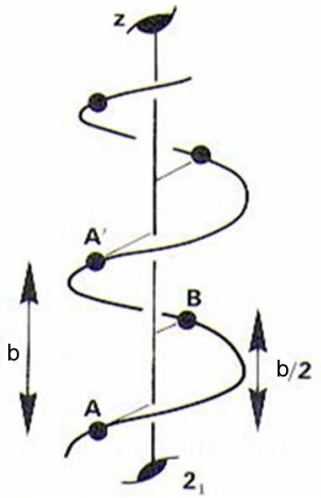




Quadratic P4

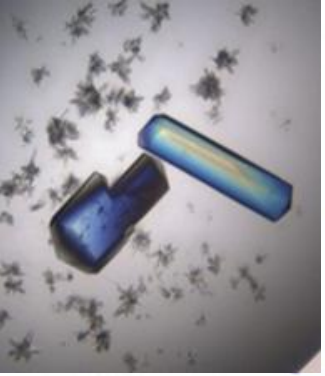
Primitive lattice + rotation 360°/4=90°

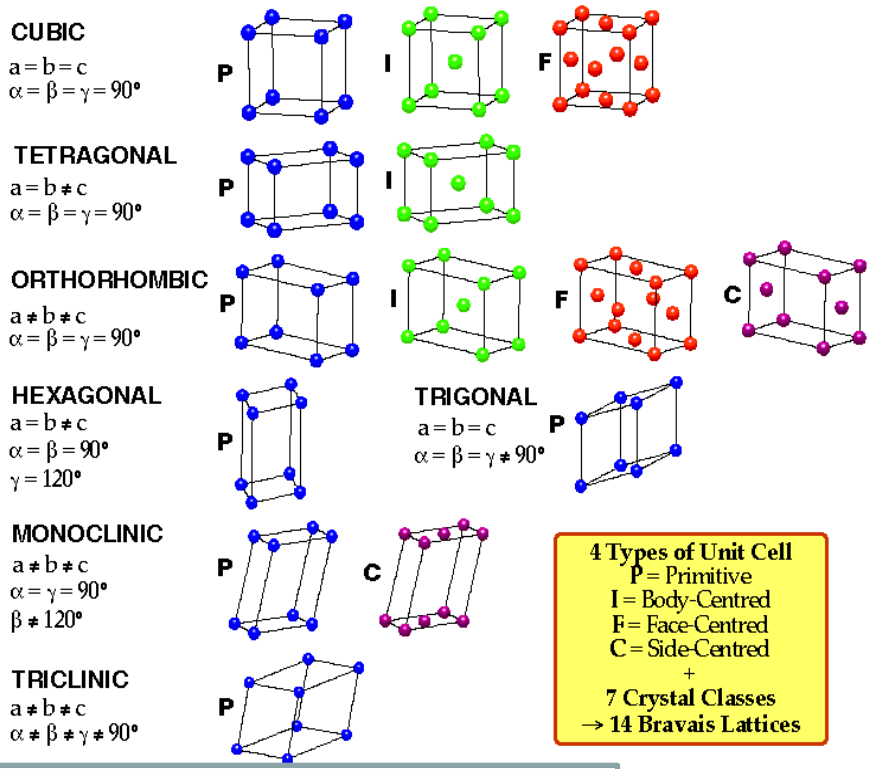
asymetric unit in ¼ cell



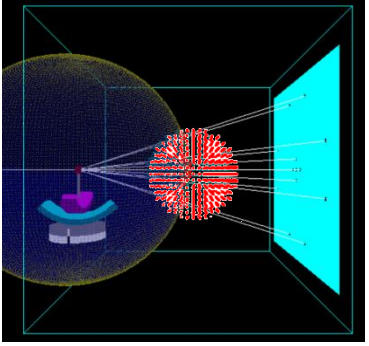
Helicoidal 2_1 along **b**

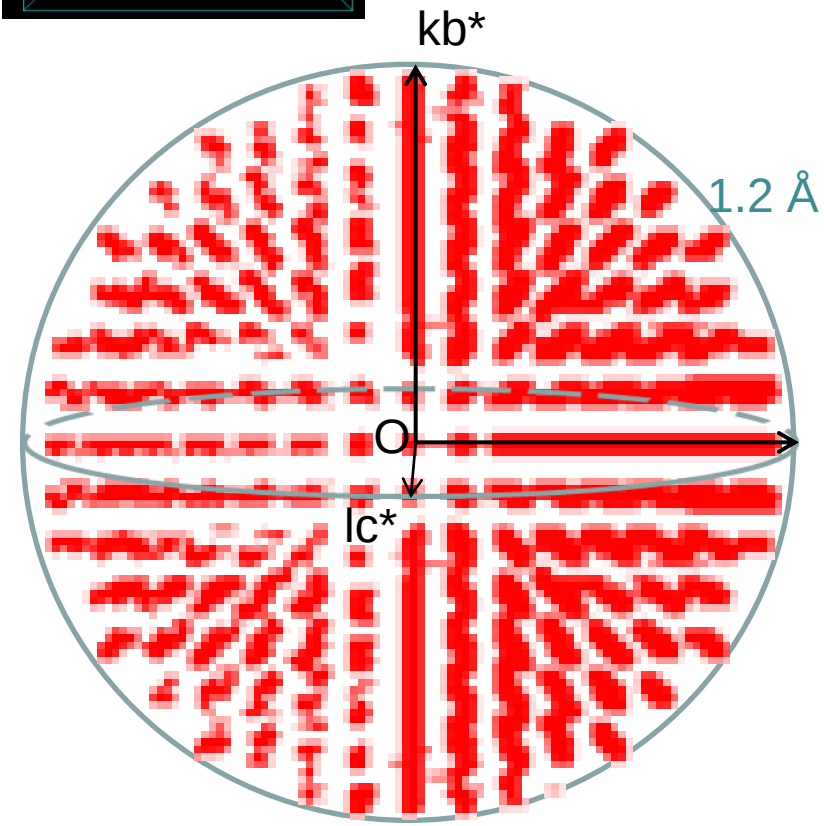
Rotation 180° and translation ½ along **b**





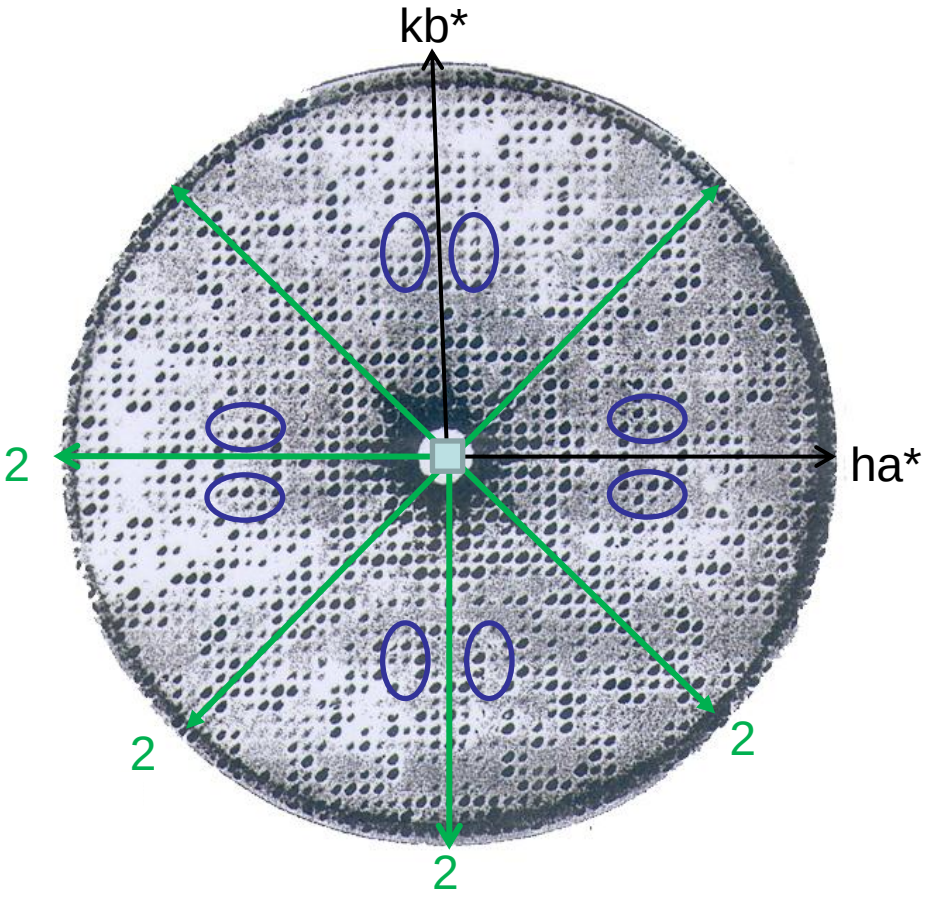
Class	Bravais lattice	Symmetry axis	Cell parameters
Triclinic	P	none	a≠b≠c α≠β≠γ
Monoclinic	P, C	one 2-fold (//b)	a≠b≠c α=γ=90°≠β
Orthorhombic	P, C, I, F	three 2-fold (//a, //b, //c)	a≠b≠c α=β=γ=90°
Quadratic	P, I	one 4-fold (//c)	a=b≠c α=β=γ=90°
Trigonal	P (or R)	one 3-fold (//c)	a=b≠c α=β=90° ; γ=120° a=b=c α=β=γ<120°≠90°
Hexagonal	P	one 6-fold (//c)	a=b≠c α=β=90°, γ=120°
Cubic	P, I, F	four 3-fold (along cube diagonals)	a=b=c α=β=γ=90°





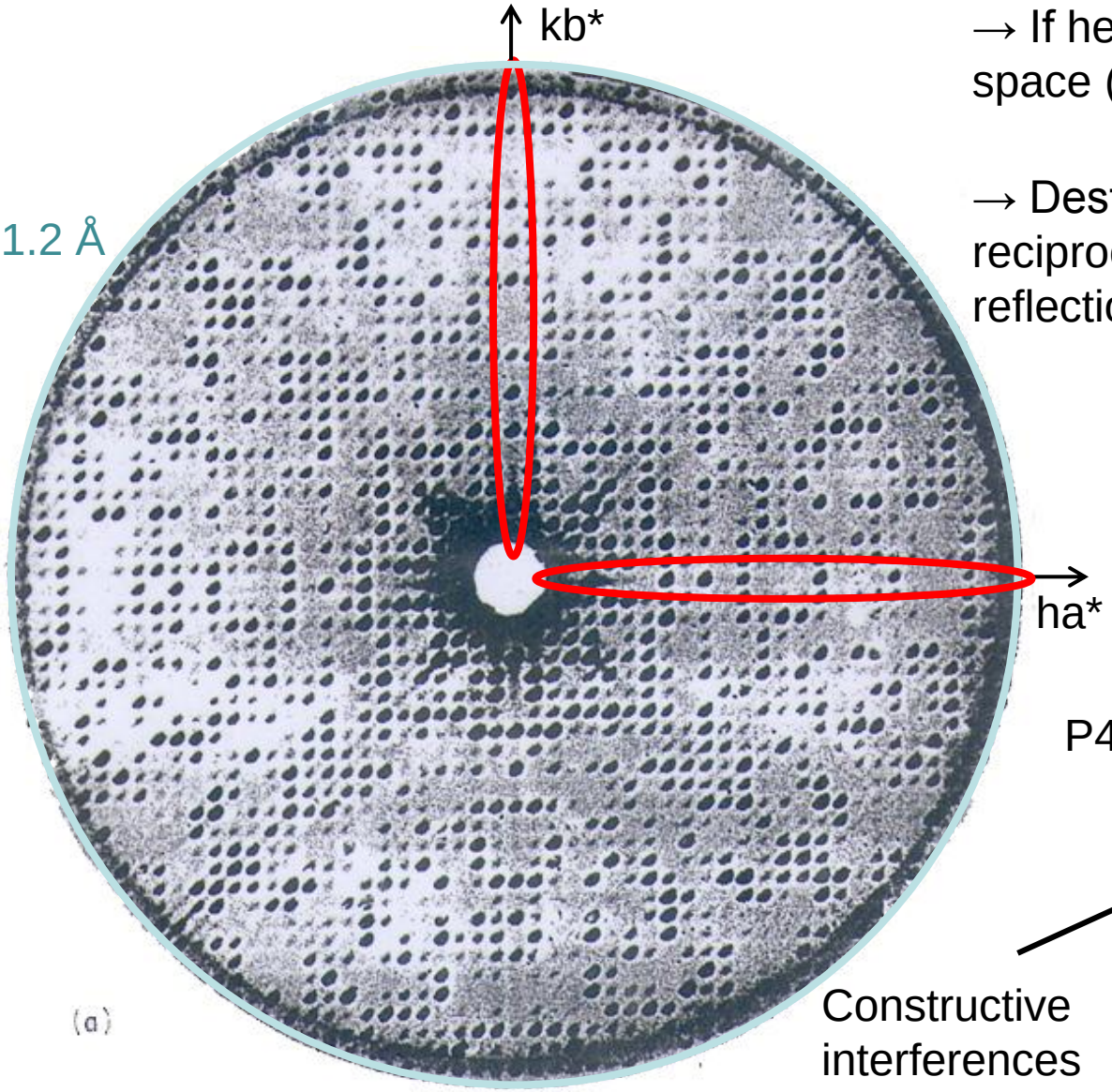
~100% of the I(hkl) collected (0°-1°, 1°-2°, …,179°-180°) to the resolution limit (1.2 Å)

I(hkl) respect the Laue class = keep crystal rotation symmetry



zone hk0

Crystal in $P4_32_12$ —X-ray→ I(hkl) in 422



zone hk0 of I(hkl)

→ If helicoidal symmetry in direct space (*e.g.* 2_1 along **b**)

→ Destructive interferences occur in reciprocal space (*e.g.* extinct odd reflections along **b* = 0k0**):

$P4_32_12$ or enantiomorph $P4_12_12$

Constructive interferences

Reflection conditions

General:

$00l : \; l = 4n$
$h00 : \; h = 2n$

and 0k0: k=2n

Data merging and reducing in asymmetric unit (Scala/Xscale)

- Merging data (I(hkl)averaging with Laue class 422, assessing data quality with R_{sym})

$$R_{sym} = \frac{\sum_{hkl}\sum_{i=1}^{n}\left|I_i(hkl) - I_i(\bar{h}\bar{k}\bar{l})\right|}{\sum_{hkl}\sum_{i=1}^{n} I_i(hkl)}$$

RESOLUTION LIMIT	NUMBER OF REFLECTIONS **OBSERVED**	**UNIQUE**	POSSIBLE	COMPLETENESS OF DATA	**R-SYM** observed	**I/SIGMA**
10.00	3042	1247	1650	75.6%	**2.7**%	31.09
6.00	22426	6027	6034	99.9%	2.8%	37.73
5.00	21552	5615	5615	100.0%	2.8%	37.37
4.00	48765	12658	12659	100.0%	3.0%	35.23
3.00	137230	35606	35610	100.0%	5.4%	19.25
2.70	88181	22953	22955	100.0%	19.4%	6.66
2.60	38834	10124	10124	100.0%	**26.4**%	3.88
total	360030	94230	94647	99.6%	4.1%	19.08

La fonction de Patterson

La fonction de Patterson est définie comme une TF-1 sur les intensités

$$P(\mathbf{u}) = \frac{1}{V_c}\sum_{\mathbf{h}} F(\mathbf{h})^2 \exp(-2\pi i \mathbf{h}\cdot\mathbf{u})$$

$$\text{avec } \mathbf{u} = u\mathbf{a} + v\mathbf{b} + w\mathbf{c}$$

Cette fonction est également définie comme étant la fonction de convolution de la densité électronique $\rho(\mathbf{r})$:

$$P(\mathbf{u}) = \rho(\mathbf{r}) * \rho(-\mathbf{r}) = \int_V \rho(\mathbf{r})\rho(\mathbf{u}+\mathbf{r})\mathrm{d}^3\mathbf{r}$$

En décrivant la densité électronique comme ponctuelle, on trouve:

$$\rho(\mathbf{r}) = \sum_j Z_j \delta(\mathbf{r} - \mathbf{r}_j)$$

où Zj est le nombre d'électrons de l'atome j situé en **r**j. La fonction de Patterson devient :

$$P(\mathbf{u}) = \int_V \sum_j Z_j \delta(\mathbf{r} - \mathbf{r}_j) \sum_{j'} Z_{j'} \delta(\mathbf{u} + \mathbf{r} - \mathbf{r}_{j'}) \mathrm{d}^3\mathbf{r} = \sum_j \sum_{j'} Z_j Z_{j'} \int_V \delta(\mathbf{r} - \mathbf{r}_j)\delta(\mathbf{u} + \mathbf{r} - \mathbf{r}_{j'})\mathrm{d}^3\mathbf{r}$$

$$= \sum_j \sum_{j'} Z_j Z_{j'} \delta(\mathbf{u} + \mathbf{r}_j - \mathbf{r}_{j'})$$

La fonction de Patterson est réduite à des pics de hauteur ZjZj'situés aux extrémités des vecteurs interatomiques **r**j-**r**j'

Le nombre de pics de hauteur ZjZj'est égal à N^2, dont N pics à l'origine et
Le nombre de pics en dehors de l'origine est donc de **N(N-1)**

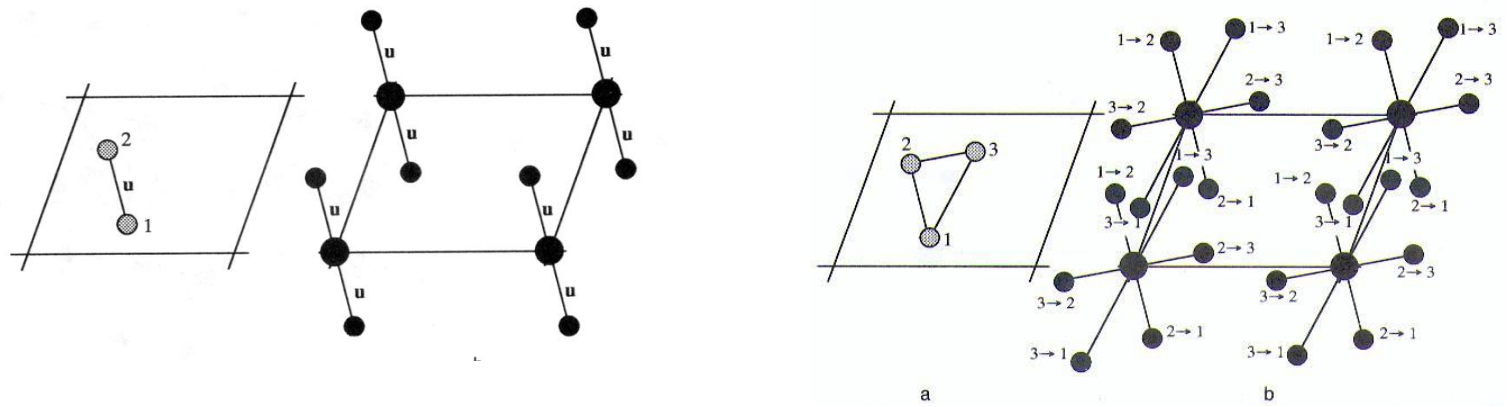
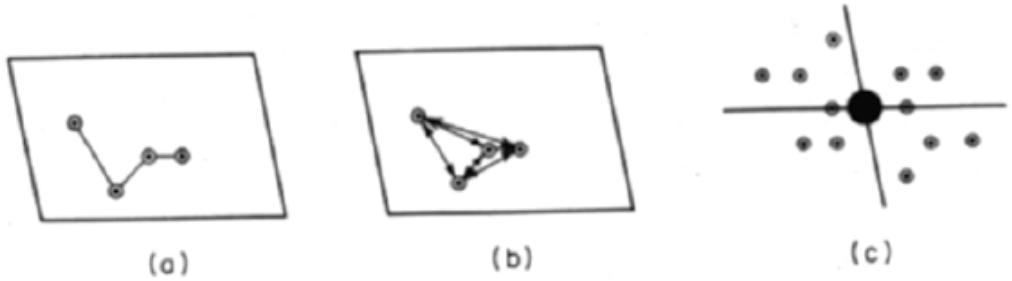
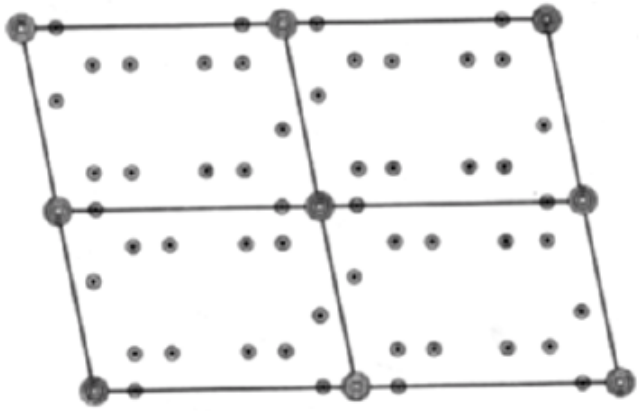


Figure : cristaux 2D avec 2 puis 3 atomes par maille et correspondance dans la maille de Patterson

-Le groupe de symétrie de la carte de Patterson est différent de celui du cristal. Il implique la création d'un centre de symétrie et la disparition de la symétrie hélicoïdale.

- Les atomes les plus lourds vont produire **les pics les plus intenses** sur les cartes de Patterson





Il est possible de résoudre informatiquement la structure d'une petite molécule (**cinquantaine** d'atomes) après inspection d'une carte de Patterson.

La fonction de Patterson est également employée dans la méthode de **Remplacement Moléculaire** comme utilisé ci-après

Energy minimisation

Calcul of potential energy from all atoms :

$$E_{\text{bond}} = \tfrac{1}{2}K_{\text{bond}}(b - b_0)^2$$

$$E_{\text{bond angle}} = \tfrac{1}{2}K_{\tau}(\tau - \tau_0)^2$$

$$E_{\text{torsion}} = \tfrac{1}{2}K_{\xi}(\xi - \xi_0)^2$$

$$E_{\text{dihedral}} = K_{\theta}\{1 + \cos(m\theta + \delta)\}$$

$$E_{\text{van der Waals}} = A \times r^{-12} + B \times r^{-6}$$

Plus weighted crystallographic energy from merged hkl : $\mathbf{E_{xray}=|Fobs-Fcalc|^2}$

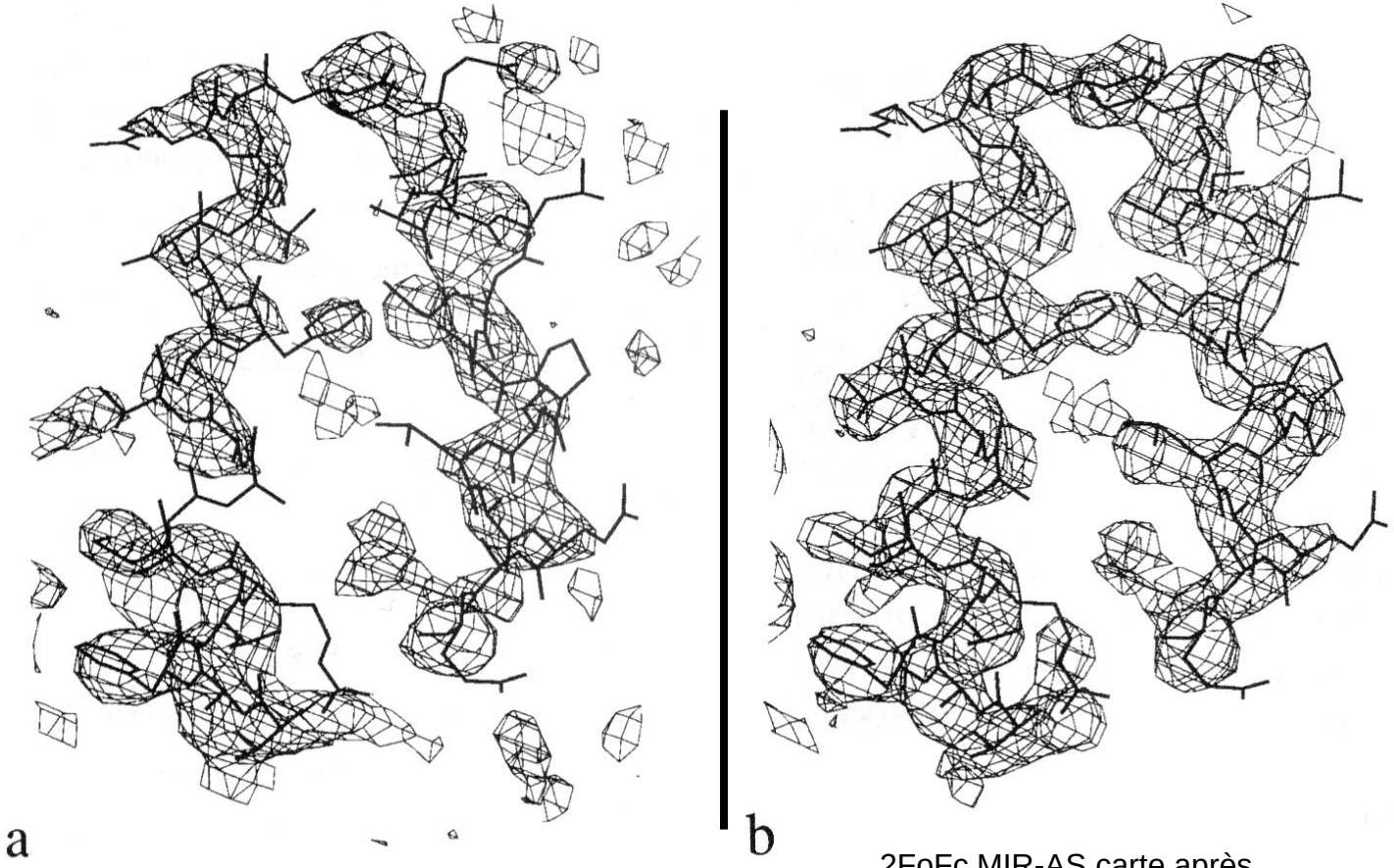
$$E_{\text{pot}} = E_{\text{covalent}} + E_{\text{non-covalent}} + \text{w}\, E_{\text{cryst}}$$

Energy minimization

Potential energy from all atoms :

Covalent energy	$E_{liaison} = \sum k_b (r - r_0)^2$ r_0 = Longueur idéale de liaison (1.53 Å pour C -C) r = Longueur réelle de la liaison k_b constante de force (1000 kcal/mol.A²) + $E_{angle} = \sum k_\theta (\theta - \theta_0)^2$ θ_0 = Angle de valence idéal (109 °C -C -C) θ = Angle de valence mesuré (sur la structure) k_θ constante de force (500 kcal/mol.rad²) + $E_{angle} = \sum k_\delta (1+\cos(n\delta+\phi_0))$ n=1,2,3,4,6 δ= Angle de torsion n = Périodicité k_δ constante de force + $E_{impropre} = \sum k_\omega (\omega - \omega_0)^2$ ω_0 = Angle de planarité idéale (180 °N -Cα -CO) ω = Angle de planarité mesurée k_ω constante de force
Non-covalent energy	+ $\sum 4\varepsilon_{ij}\left[\left(\frac{r_o}{r_{ij}}\right)^{12} - \left(\frac{r_o}{r_{ij}}\right)^{6}\right]$ r o distance de Van der Waals, r ij distance entre les atomes i et j + $\sum \frac{332 \cdot q_i \cdot q_j}{D \cdot r_{ij}}$ D constante diélectrique (80 dans l'eau, 1 dans le vide), qi,qj charges portées par les atomes i et j + $\sum \left[\left(\frac{A}{r_{ad}}\right)^{12} - \left(\frac{B}{r_{ad}}\right)^{10}\right]$ r ad distance donneur-accepteur, A et B sont des constantes

Plus weighted experimental crystallographic energy : $\mathbf{E_{xray} = (|Fobs| - k\,|Fcalc|)^2}$



2FoFc MIR-AS carte à 3.3 Å

2FoFc MIR-AS carte après « solvent flattening »

Cette procédure peut être améliorée en cas de **symétrie non-cristallographique**

La densité de la protéine est considérée comme devant être identique entre zones reliées par une **symétrie non-cristallographique** et est moyennée à l'intérieur de masques délimitant ces zones (**molecular averaging, important pour virus**)

$$\rho_{avg}(x) = \sum_{i=1}^{N} \frac{1}{N} M_i(x) \sum_{j=1}^{N} \rho(x_{ij})$$