Macromolecular Structure Refinement

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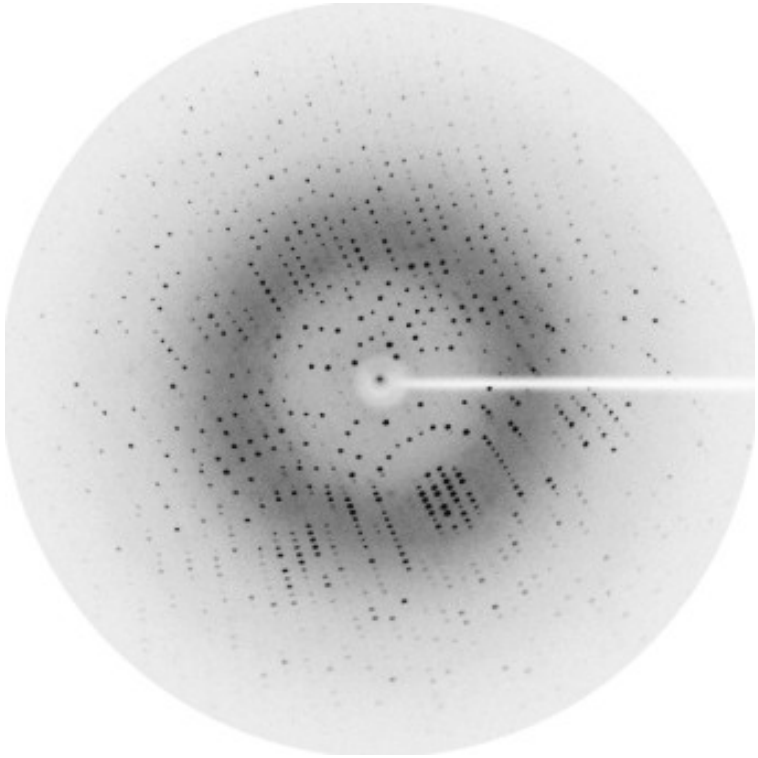
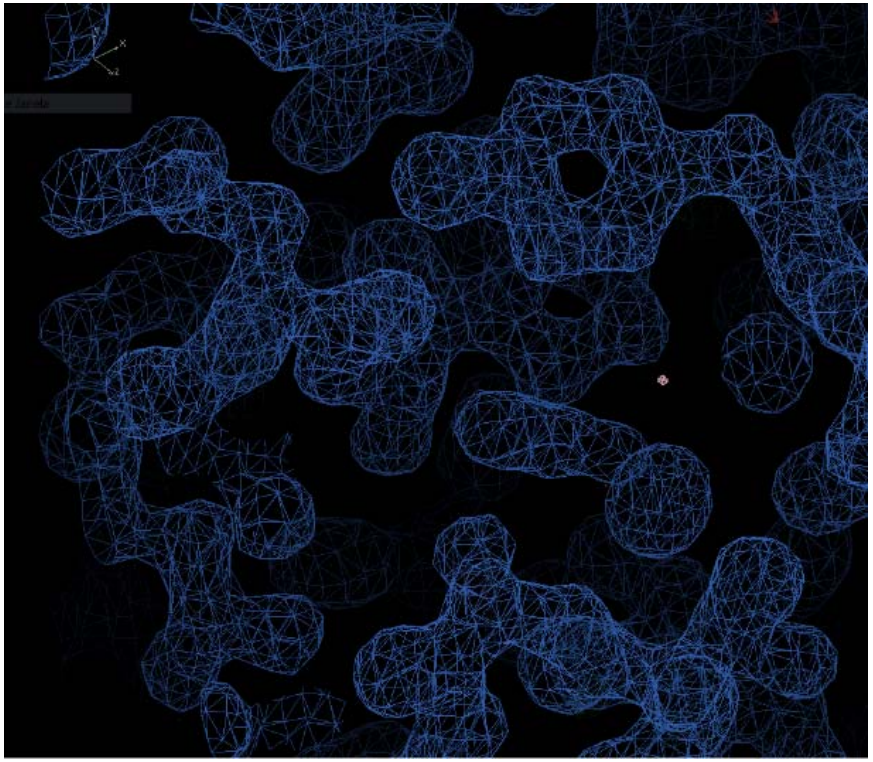
Industry and Medicine Applied Crystallography

From X-ray diffraction to the first map - recap





$$\mathbf{F}_{hkl} = \sum_{j=1}^{N} f_j \exp\left[2\pi i(hx_j + ky_j + lz_j)\right]$$

$\mathbf{F}_{hkl}$ is a function of the **contents** of the unit cell and is the **Structure Factor** of the reciprocal lattice point (reflection) with coordinates (indices) h,k,l.

$$\mathbf{F}_{hkl} = A_{hkl} + iB_{hkl}$$

with

$$A_{hkl} = \sum_{j=1}^{N} f_j \cos 2\pi(hx_j + ky_j + lz_j)$$

and

$$B_{hkl} = \sum_{j=1}^{N} f_j \sin 2\pi(hx_j + ky_j + lz_j)$$

The structure factor - recap

The structure factor is a complex number and is composed of an amplitude $|F_{hkl}|$ and a phase angle ϕ_{hkl}:

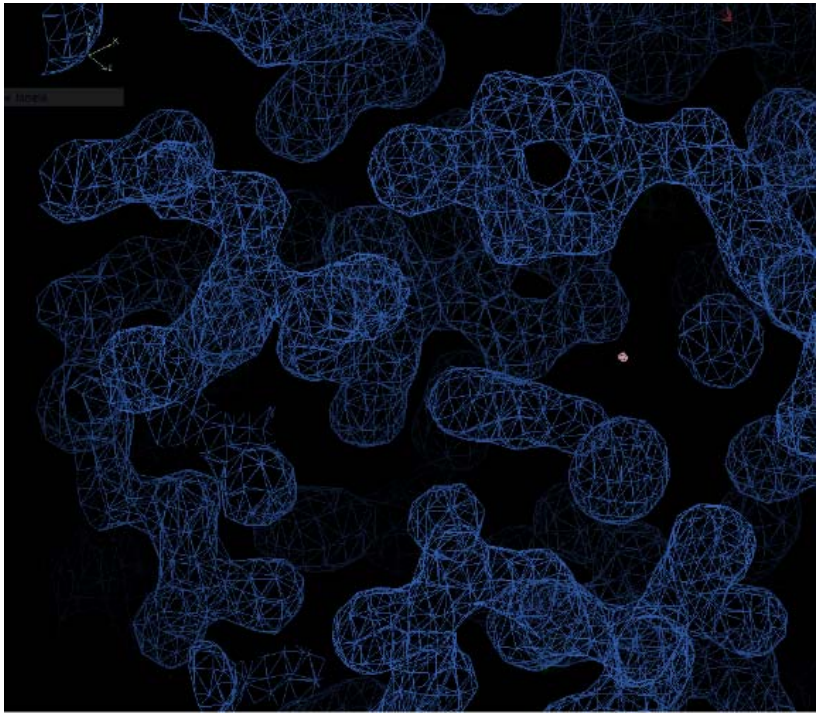
$$|F_{hkl}| = (A_{hkl}^2 + B_{hkl}^2)^{1/2} \qquad \phi_{hkl} = \arctan(B_{hkl}/A_{hkl})$$

Experimentally, only **diffracted intensities** are measured, and the structure factors cannot be obtained directly:

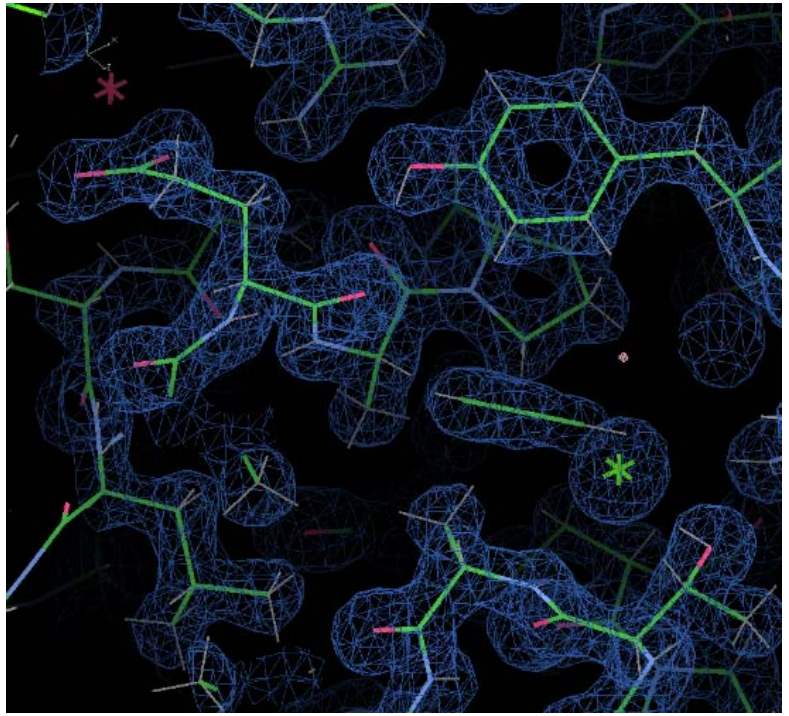
$$I_{hkl} \propto |\mathbf{F}_{hkl}|^2 = \mathbf{F}_{hkl} \cdot \mathbf{F}_{hkl}^*$$

Therefore, the **phase information is lost**.

From first map to structural model



Model building



$|F_{hkl}|, \phi_{hkl}$

Initial e.d. map

X,Y,Z, B

Atomic model

Structure refinement - overview

Once a good starting model is obtained, it is **refined**, i.e., the **structural parameters** describing it are **optimized**, in order to minimize a target function, based on a difference between $\mathbf{F_o}$, the observed structure factors, and $\mathbf{F_c}$, the structure factors calculated from the model.

The refined structural parameters usually consist of:

- **1 overall scale factor** between $|F_o|$ and $|F_c|$
- a small (1-10) number of parameters to account for some sources of **systematic deviations** between $|F_o|$ and $|F_c|$ (e.g., bulk solvent correction).
- **3 positional** and **1 isotropic** or 6 anisotropic **thermal motion parameters** per atom.

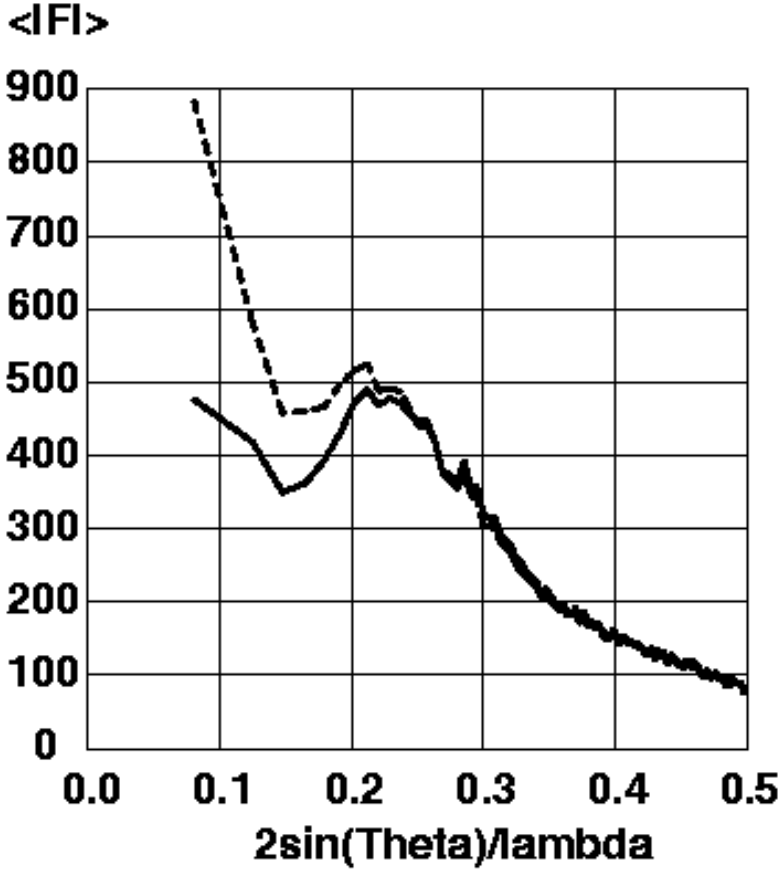
Structure refinement – the bulk solvent correction

Protein crystals contain between ~30% and ~70% solvent, most of which is disordered in the **solvent channels** between the protein molecules of the crystal lattice (this disordered solvent is denoted as **bulk solvent**).

If no model for this continuous bulk solvent electron density is taken into consideration, atomic protein models are artificially placed in a "vacuum" environment, leading to a **vast overestimation** of the electron density contrast at the protein surface. This in turn leads to **calculated structure factor amplitudes** which are **systematically much larger** than the observed structure factor amplitudes at resolutions below ~5Å.

http://legacy.ccp4.ac.uk/newsletters/newsletter34/bsdk_text.html

Structure refinement – the bulk solvent correction



Magnitude of observed structure factor amplitudes (solid line) and calculated structure factor amplitudes without a bulk solvent correction (dashed line) on an arbitrary scale against resolution.

http://legacy.ccp4.ac.uk/newsletters/newsletter34/bsdk_text.html

Structure refinement – the bulk solvent correction

This **systematic deviation** between observed and calculated structure factor amplitudes leads to severe problems in scaling, in least-squares refinement with its assumption of Gaussian error distributions, and in electron density difference map calculations. In the past, it was common practice to circumvent these problems by **cutting the data** at a lower resolution of, say, 6Å.

However, doing so creates **distortions** of the local electron density contrast in the protein region. A better solution is to include an **appropriate model** for the bulk solvent, thus allowing the use of all data during scaling, refinement, and electron density difference map calculations.

http://legacy.ccp4.ac.uk/newsletters/newsletter34/bsdk_text.html

Most commonly used **Target Functions** in X-ray Crystallography:

- Least-squares

$$E^{LSQ} = E_{\text{restraints}} + w_a \sum_{hkl} (|F_o| - k|F_c|)^2$$

- Maximum Likelihood

$$E^{ML} = E_{\text{restraints}} + w_a \sum_{hkl \in \text{working set}} \left(\frac{1}{\sigma^2_{ML^{cv}}} \right) (|F_o| - \langle |F_o| \rangle^{cv})^2$$

where F_c and $\langle |F_o| \rangle^{cv}$ are calculated from the model.

The structure factor is a **non-linear function** of the structural parameters; therefore, a solution is found **iteratively**.

The standard (full-matrix) least-squares method used in the refinement of small-molecule crystal structures allows the direct estimation of the standard deviations for the refined structural parameters.

But a macromolecule can have **hundreds or even thousands of atoms**, so the full-matrix method becomes computationally very expensive and alternative methods for calculating the parameter shifts have been devised; however, reliable error estimates are no longer available.

$$\mathbf{F}_{hkl} = \sum_{j=1}^{N} f_j \exp\left[2\pi i(hx_j + ky_j + lz_j)\right]$$

In the structure factor equation, x, y, and z are **fractional coordinates**, i.e., within the unit cell their value varies between 0 and 1.

x is the distance along unit cell edge ***a*** divided by its value, and the same applies to **y** and **z**.

Atomic coordinates for **small molecules** are usually stored as fractional coordinates but the atomic coordinates of macromolecules are normally stored in a Cartesian coordinate system, and denoted by X, Y and Z, according to some orthogonalization convention.

In a **real crystal structure** atoms are not static but **vibrate** about **equilibrium positions**.

Simplest approximation - **isotropic** vibrations:

- ✓ the vibration is the same in all directions
- ✓ a Gaussian probability distribution function describes motions

The **Temperature Factor** can be written as:

$$T_{hkl} = \exp\left[-2\pi^2(\langle u_j \rangle/d)^2\right] = \exp\left[-B_j(\sin\theta/\lambda)^2\right]$$

where $\langle u_j \rangle$ is the **mean displacement** of the atom about its equilibrium position and **d** is the **resolution** of the reflection **hkl**.

B_j is the atomic **Isotropic Thermal Motion Parameter**.

A more realistic approximation - **anisotropic** vibrations:

- ✓ the vibration is **NOT** the same in all directions
- ✓ a **triaxial** Gaussian probability distribution function describes motions – assumes that atoms move in a **harmonic potential.**

The Temperature Factor can be written as:

$$T_{hkl} = \exp\left[-2\pi^2(U_{11}h^2a^{*2}+U_{22}k^2b^{*2}+U_{33}l^2c^{*2} +U_{12}hka^*b^*+U_{13}hla^*c^*+U_{23}klb^*c^*)\right]$$

$$= \exp -(\beta_{11}h^2+\beta_{22}k^2+\beta_{33}l^2+\beta_{12}hk+\beta_{13}hl+\beta_{23}kl)$$

$$T_{hkl} = \exp\left[-2\pi^2(U_{11}h^2a^{*2}+U_{22}k^2b^{*2}+U_{33}l^2c^{*2} +U_{12}hka^*b^*+U_{13}hla^*c^*+U_{23}klb^*c^*)\right]$$

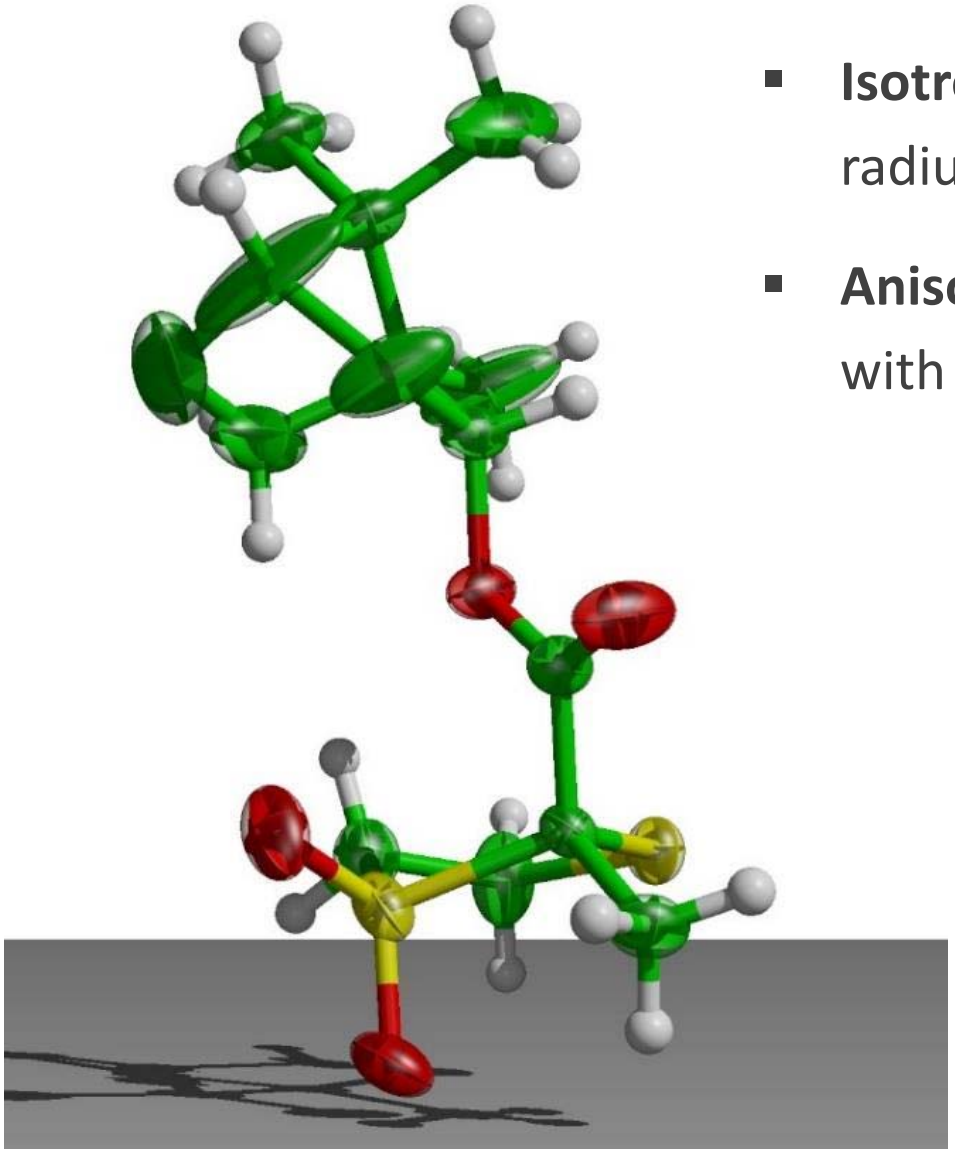
$$= \exp -(\beta_{11}h^2+\beta_{22}k^2+\beta_{33}l^2+\beta_{12}hk+\beta_{13}hl+\beta_{23}kl)$$

The $\mathbf{U_{ij}} = \langle u_i u_j \rangle$, represent the atomic displacement along the Cartesian i-axis multiplied by its displacement along the j-axis.

The $\boldsymbol{\beta_{ij}}$ are the atomic **Anisotropic Thermal Motion Parameters**.

The atomic **Structure Factor** equation then becomes:

$$\mathbf{F}_{hkl} = \sum_{j=1}^{N} f_j \exp\left[2\pi i(hx_j+ky_j+lz_j)\right] T_{hkl}(j)$$



In a crystal structure representation:

- **Isotropic** atoms are represented as **spheres** with radius proportional to $\mathbf{\langle u^2 \rangle^{1/2}}$ **or** $\mathbf{B^{1/2}}$
- **Anisotropic** atoms are represented as **ellipsoids** with axial lengths proportional to $\mathbf{U_{ij}^{1/2}}$ **or** $\mathbf{\beta_{ij}^{1/2}}$

Structure refinement – the temperature factor

In protein crystal structures it is often possible to consider that large regions of the molecules vibrate together as **rigid bodies**: a simplified treatment of the thermal motion is therefore possible:

The **TLS rigid body refinement** of **anisotropic** atomic thermal motion parameters:

Translation – translation of the fixed point

Libration – rigid body rotation about an axis through a fixed point

Screw – correlation terms between **T** and **L**

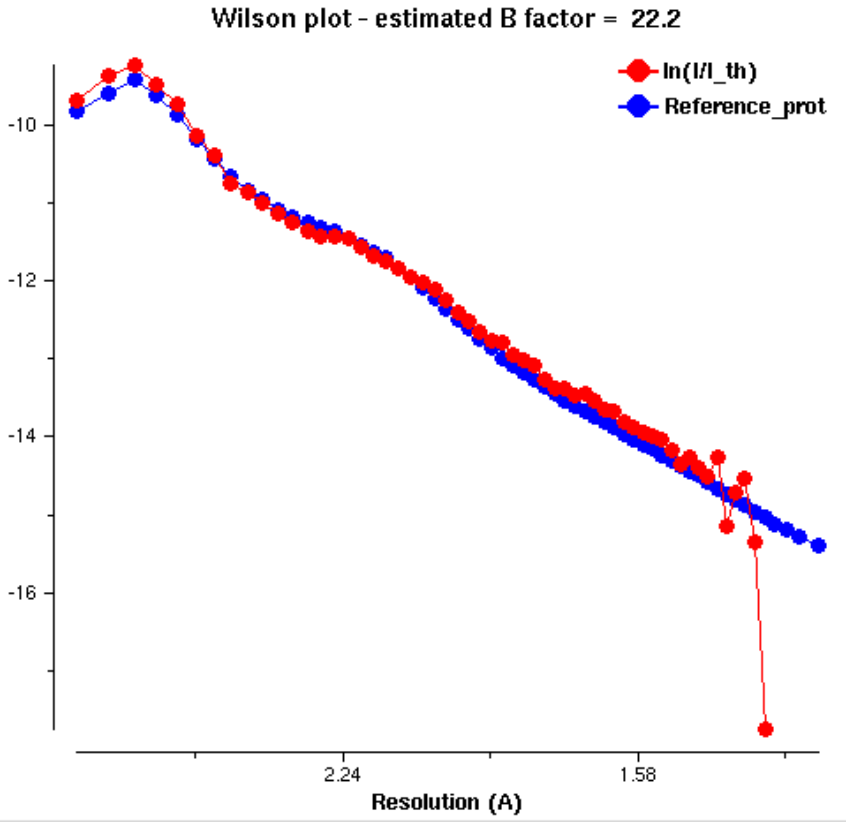
In this way, it is possible to describe a **local anisotropic thermal motion** using a **small number of parameters** (20) per rigid body group containing many atoms.

Schomaker & Trueblood (1968) Acta Crystallogr. B24, 63-76.

Structure refinement – the Wilson plot

The Wilson plot is used to calculate an approximate scale factor to place $|F_o|$ on an absolute scale and also to obtain an estimation of an overall B-value for the crystal structure.

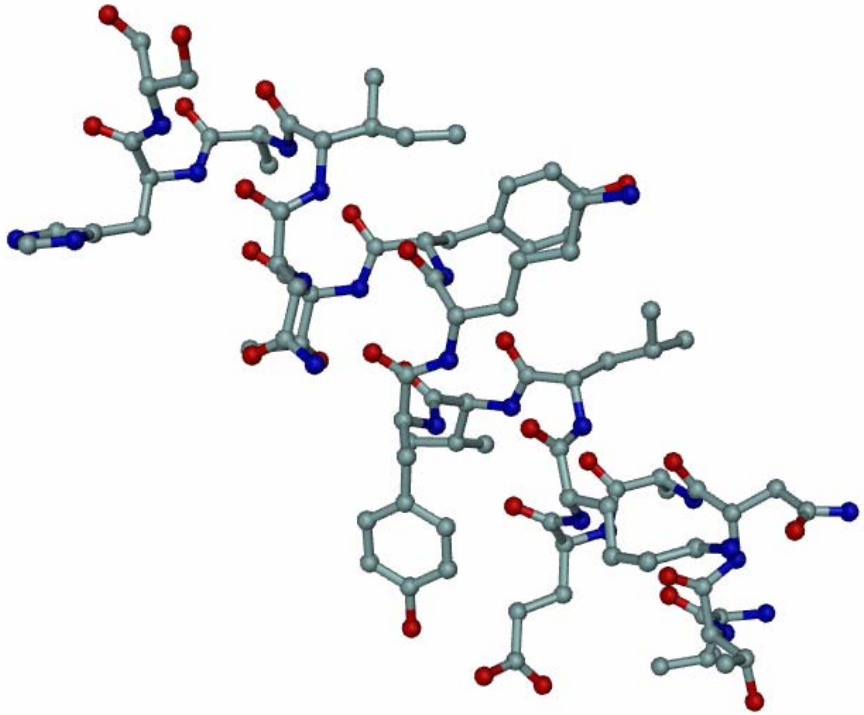
$$K_P \langle I_P \rangle = \Sigma f_j^2 \exp(-2B_P \sin^2\theta/\lambda^2)$$



Plotting ln ($\langle I_P \rangle$ / Σf_j^2) vs. $\sin^2\theta/\lambda^2$ we should obtain a straight line with slope $-2B_P$ and intercept $-\ln K_P$.

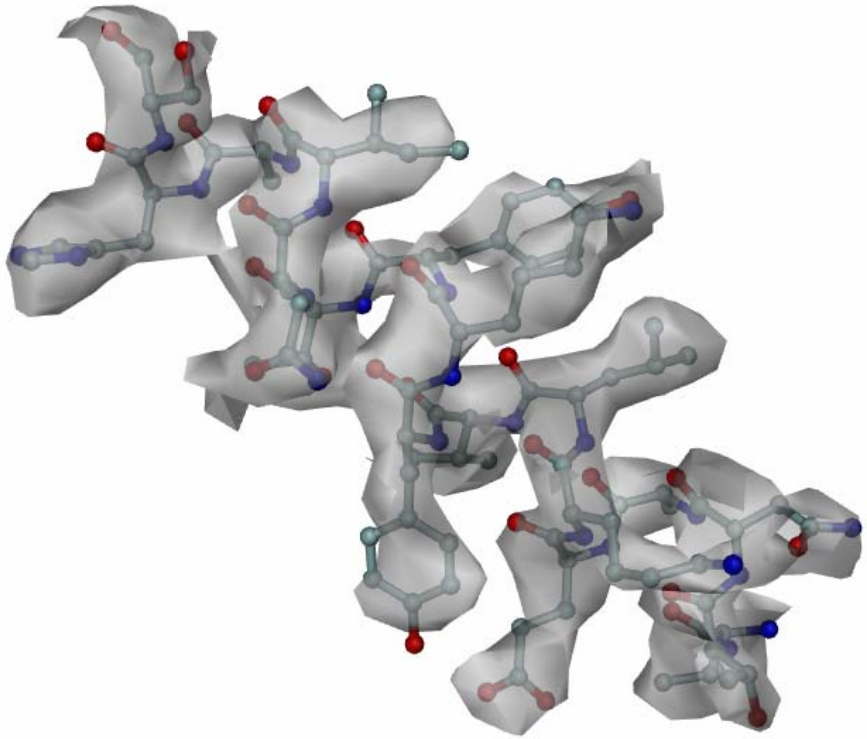
However, at low resolution the plot deviates significantly from linearity and only data from ~3Å resolution is used in practice.

The data resolution influences the level of detail of the electron density map

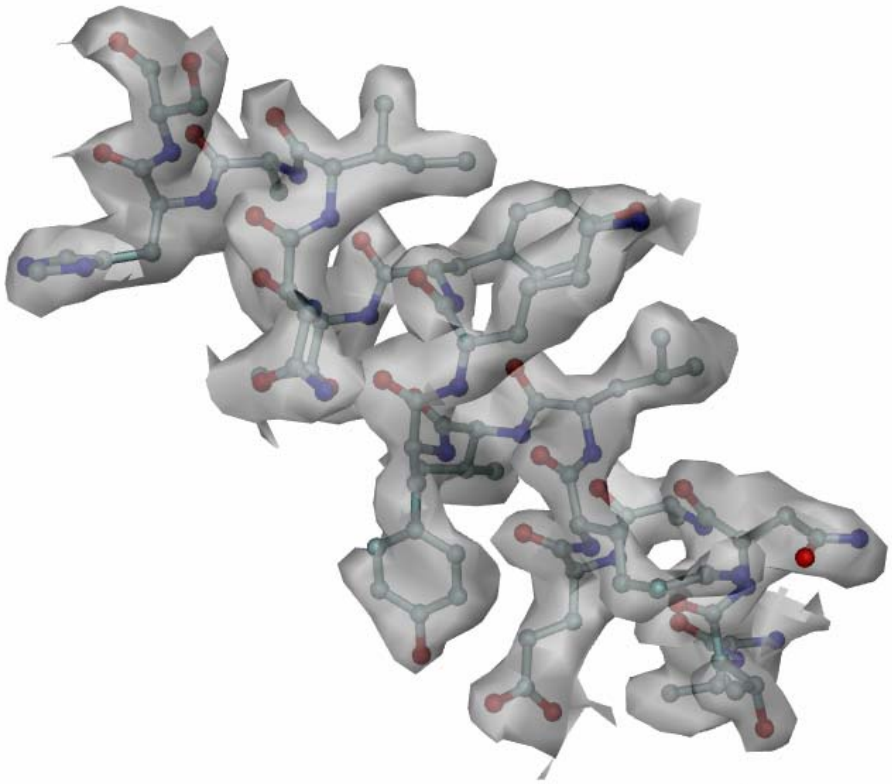


An α-helix in the structure of Cytochrome c" from *Methylophilus methylotrophus*
Sample from lab of Prof. Helena Santos; 3D Structure at 1.2 Å by Dr. Francisco Enguita

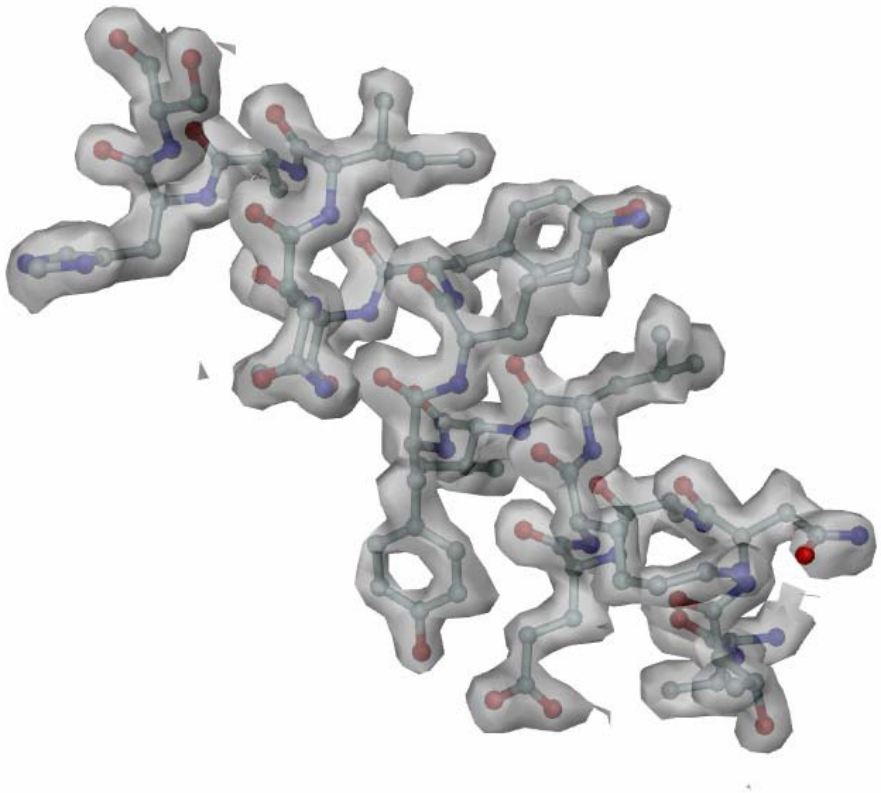
Low resolution: Final map calculated at 3.0 Å resolution and contoured at 1.0 σ



Medium resolution: Final map calculated at 2.5 Å resolution and contoured at 1.0 σ

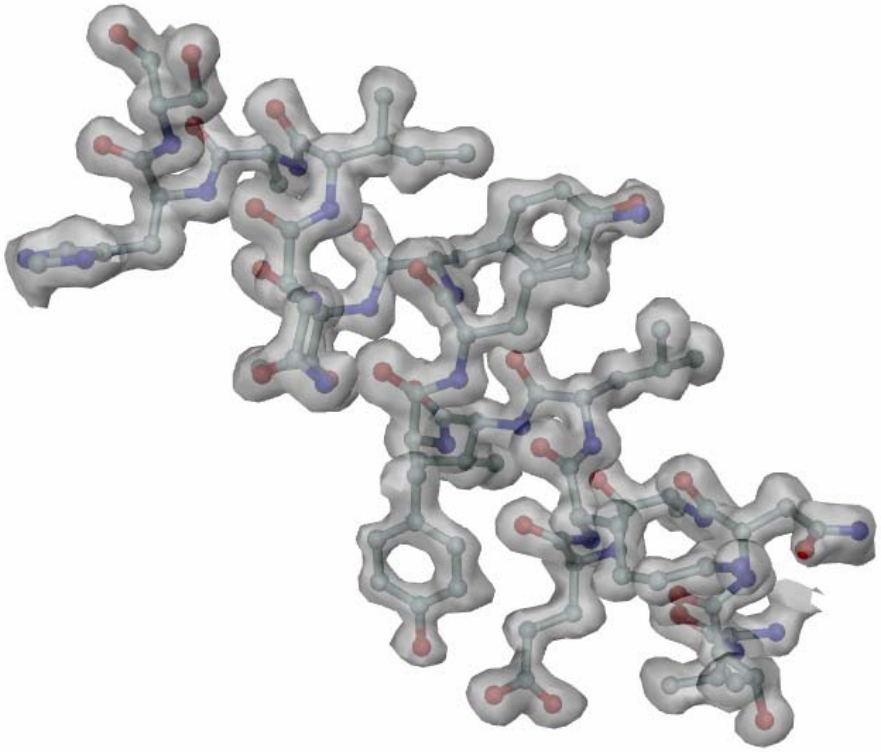


High resolution: Final map calculated at 1.8 Å resolution and contoured at 1.0 σ



Structure refinement – stereochemical restraints

Near atomic resolution: Final map calculated at 1.2 Å resolution and contoured at 1.25 σ



Which data resolution would be best for refinement? Why?

$$E^{LSQ} = E_{\text{restraints}} + w_a \sum_{hkl} (|F_o| - k|F_c|)^2$$

Unlike small molecules, protein crystals seldom diffract to **atomic resolution** (*i.e.*, the individual atoms cannot be clearly resolved in the electron density maps).

Data resolution is related with the number of diffraction intensities that have been measured.

In any refinement or parameter optimization procedure, it is essential that the **number of observations** N_{obs} largely exceed the **number of parameters** N_p being optimized.

In small molecule crystal structures, N_{obs}/N_p is usually close to 10.

In protein crystal structures it is usually much lower, and even below 1 at low (e.g., 3 Å) resolution.

$$E^{LSQ} = E_{\text{restraints}} + w_a \sum_{hkl} (|F_o| - k|F_c|)^2$$

For example, the molecule of Hen Egg White Lysozyme contains about **1000 protein non-hydrogen atoms** and a **1.68 Å resolution dataset** contains about **13600 reflections.** The N_{obs}/N_p ratio is therefore 3.4.

To ensure that the refinement procedure is **well-behaved** (i.e., leads to a result that has chemical significance) it is necessary to include the **prior stereochemical knowledge** of **amino acids** or **ligands** in the refinement as **additional observations**. This prior stereochemical data is given as **restraint dictionaries.** For example:

- ✓ The typical length of a carbon-carbon single bond is *ca.* 1.5 Å.
- ✓ The C_n-N_n-C_n^{α}-C_{n+1} dihedral angle must usually be 180° (**trans** peptide bond)

The dictionary information is usually derived from small molecule structures. For example, this is the chemical bond information for the aminoacid **Alanine**:

			type	length	e.s.d.
ALA	N	H	single	0.860	0.020
ALA	N	CA	single	1.458	0.019
ALA	CA	HA	single	0.980	0.020
ALA	CA	CB	single	1.521	0.020
ALA	CB	HB1	single	0.960	0.020
ALA	CB	HB2	single	0.960	0.020
ALA	CB	HB3	single	0.960	0.020
ALA	CA	C	single	1.525	0.021
ALA	C	O	deloc	1.231	0.020

$$E^{LSQ} = E_{\text{restraints}} + w_a \sum_{hkl} (|F_o| - k|F_c|)^2$$

w_a is a **weighting term** between the **purely crystallographic** and the **stereochemical terms** in the target function.

***w_a* too high** – the crystallographic term dominates: **loose stereochemical restraints** - a better agreement is reached between $|F_o|$ and $|F_c|$ at the expense of a poorer model stereochemistry.

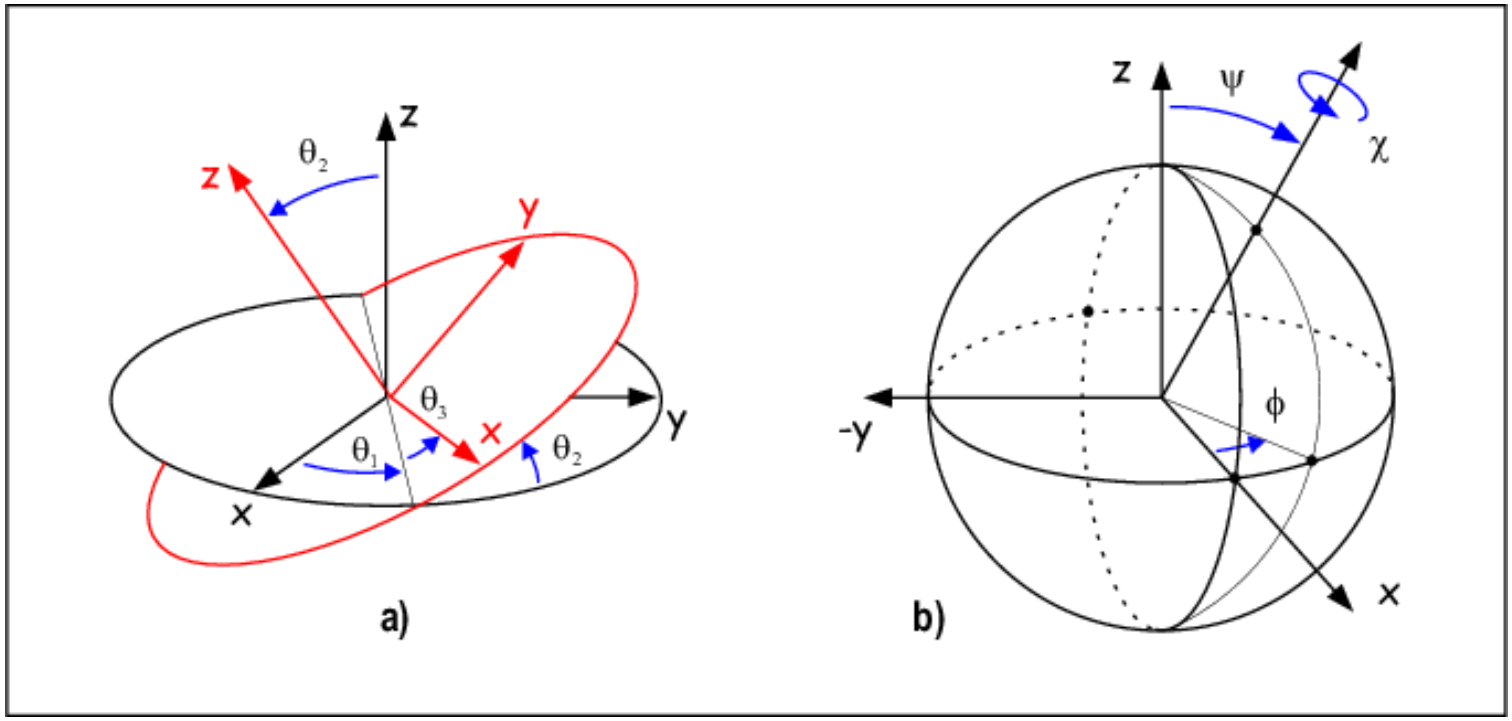
***w_a* too low** – the stereochemical term dominates: **tight stereochemical restraints** – the model stereochemistry is in better agreement with the dictionary values at the expense of a poorer agreement between $|F_o|$ and $|F_c|$. Real discrepancies may be obscured.

Often, more than one copy of the molecule is present in the asymmetric unit of the crystal structure and the **biological unit** may be composed of more than one monomer.

These monomers may be related by **non-crystallographic symmetry** (**NCS**) operators (usually pure rotation axes).

In these cases, especially at low resolution [below *ca.* **2.0 Å**] it is advantageous to restrain the molecular geometries to be reasonably similar, using **non-crystallographic symmetry restraints**.

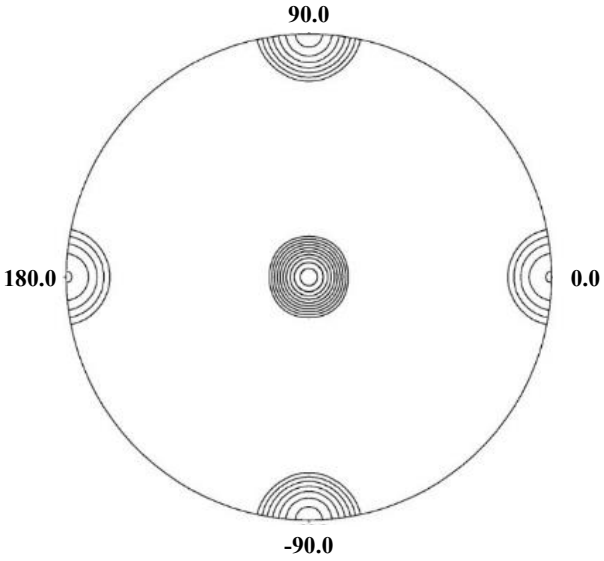
NCS may be **detected** via a **self-rotation Patterson map** in **spherical polar coordinates** ($\phi\omega\kappa$ or $\phi\psi\chi$)



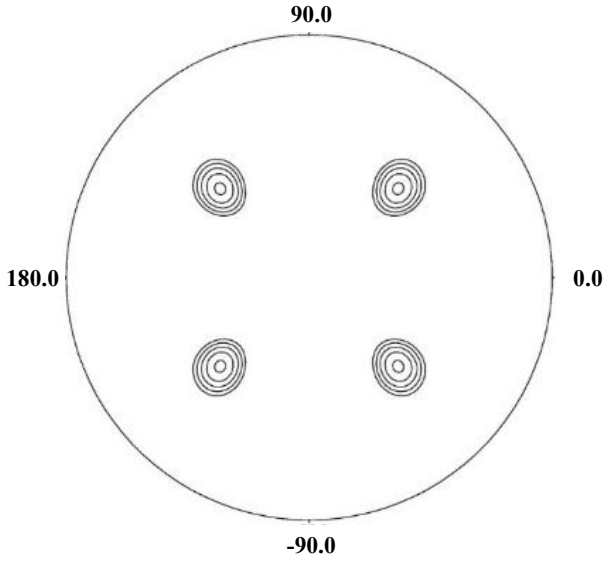
http://www.xray.bioc.cam.ac.uk/xray_resources/whitepapers/mr-in-action/node4.html

For example, **ferritin** from *Pyrococcus furiosus* crystallizes in the orthorhombic space group $C222_1$

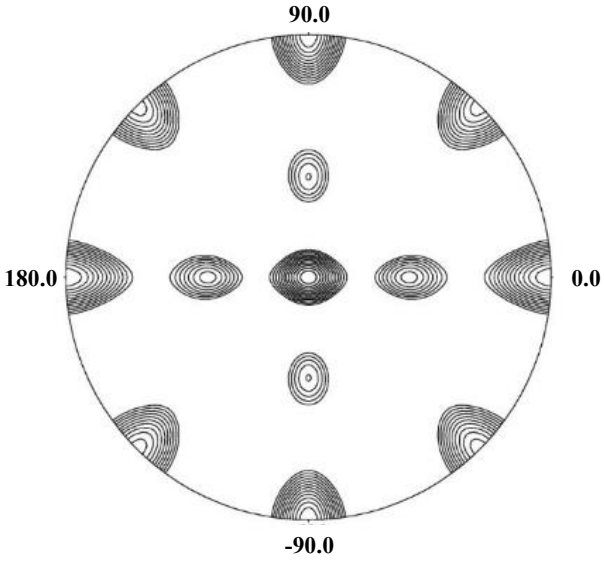
However, the self-rotation Patterson Map shows evidence of NCS:



κ=90



κ=120



κ=180

Matias *et al.*, *Acta Crystallogr.* (2005) *F*61, 503-506.

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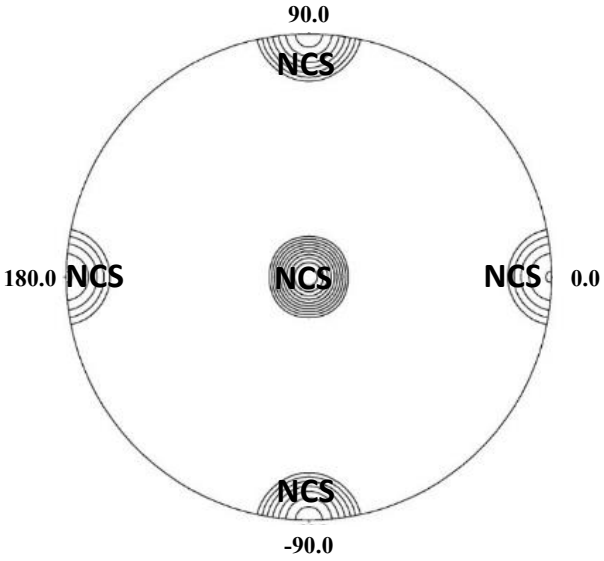
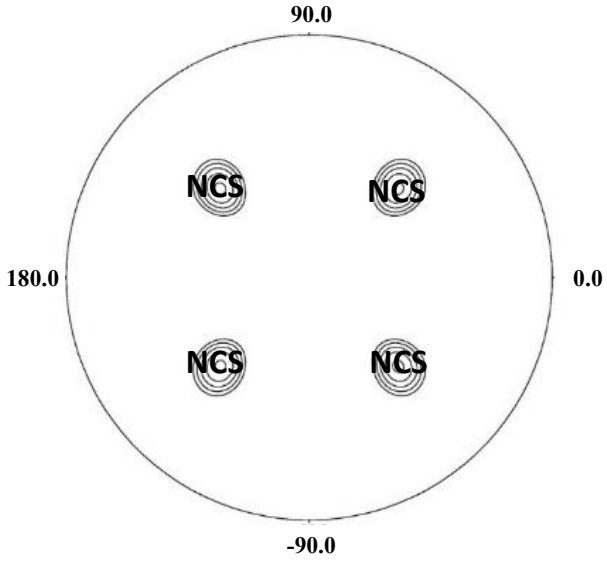
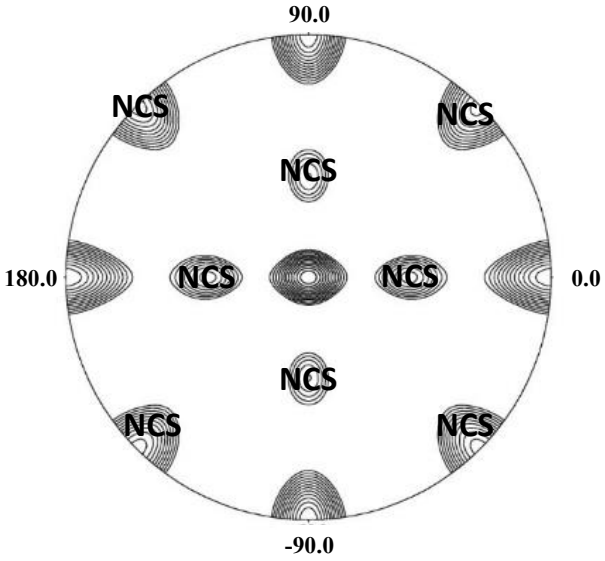


κ=90



κ=120



κ=180

432 NCS of the ferritin 24-mer

Matias *et al.*, *Acta Crystallogr.* (2005) *F*61, 503-506.

NCS may also be **detected** via a **least-squares superposition** between the atomic coordinates of the **different independent monomers** in the **asymmetric unit** of the crystal structure:

For example, the two monomers in the crystal structure of the dimeric di-hemic split-Soret cytochrome *c* are related by a non-crystallographic 2-fold symmetry axis.

```
INFO:: coordinates transformed by orthogonal matrix:
,    0.3573,    0.3407,    0.3573|
|    0.3409,   -0.1091,    0.9338|
|    0.3571,    0.9338,  -0.02125|
(     91.28,    -35.95,     1.004)
  Rotation - polar (omega,phi,kappa)  45.6087 69.0672 179.9932
  Rotation - euler (alpha,beta,gamma) 69.0623 91.2175 110.9280
  Translation - Angstroms             91.2824 -35.9457 1.0037
INFO: core rmsd achieved: 0.2937 Angstroems
```

Low to medium [1.8 Å] resolution:

- ✓ **Non-hydrogen atoms** are usually refined with **3 positional** and 1 **isotropic thermal motion** parameters (4 parameters per atom).
- ✓ Hydrogen atoms are **not refined** but may be included in the model in **calculated positions**.
- ✓ The most common source of systematic deviation between F_{obs} and F_{calc} is the **bulk solvent correction**.
- ✓ **Limited data resolution** forces the inclusion of **stereochemical restraints** to avoid unreasonable results - the **individual atom positions are not fully resolved** in the electron density maps. Use of NCS restraints is also advantageous.
- ✓ At most, **isotropic atomic displacement parameters** may be refined, and the **TLS approximation** may be useful.

Medium [1.8 Å] to high [1.2 Å] resolution:

- ✓ Many non-hydrogen atoms may be refined with 3 positional and 6 anisotropic thermal motion parameters (9 parameters per atom).
- ✓ Hydrogen atoms may be refined from calculated positions using the **riding model**.
- ✓ The most common source of systematic deviation between F_{obs} and F_{calc} is the bulk solvent correction.
- ✓ Inclusion of stereochemical restraints is still necessary to avoid unreasonable results.
- ✓ The use of NCS restraints and the TLS refinement of anisotropic thermal motion parameters may still be useful, especially in the earlier stages.

Very high [1.2 Å or higher] resolution:

- ✓ Non-hydrogen atoms are usually refined with **3 positional** and **6 anisotropic thermal motion** parameters (**9 parameters per atom**).
- ✓ Hydrogen atoms may be refined with **3 positional** and **1 isotropic thermal motion** parameters (**4 parameters per atom**). Sometimes it is still necessary or convenient to refine the structural parameters of the hydrogen atoms using restraints.
- ✓ The most common source of systematic deviation between F_{obs} and F_{calc} is the bulk solvent correction.
- ✓ Stereochemical restraints may still be necessary in some parts of the structure.

Use of Fourier Maps:

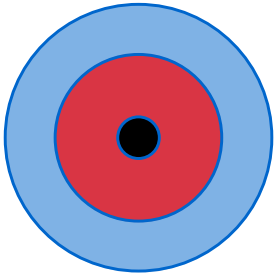
The $\mathbf{F_o}$ **map**: $|F_{hkl}|_{obs}$ coefficients and phases derived from experimental data is used to build the initial model.

The $\mathbf{F_o\text{-}F_c}$ **map**: $|F_{hkl}|_{obs} - |F_{hkl}|_{calc}$ coefficients and phases from current refined model is used to find missing atoms from the model during refinement.

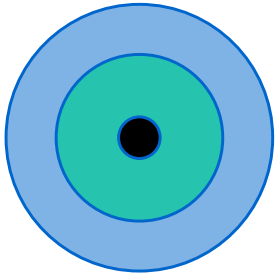
The $\mathbf{2F_o\text{-}F_c}$ **map**: $2|F_{hkl}|_{obs} - |F_{hkl}|_{calc}$ coefficients and phases from current refined model is used to inspect electron density of current model.

Use of Fourier Maps:

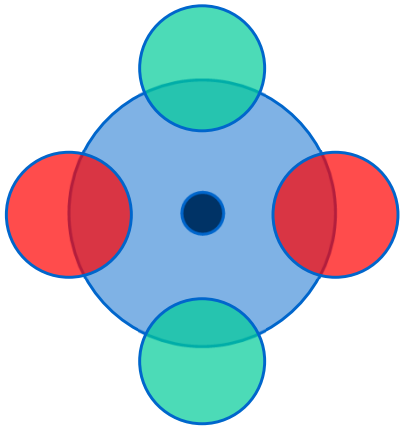
Unlike for small molecules structures, in MX we cannot usually distinguish between C, N and O atoms based on their electron density, but for heavier species (metals or ions) The $\mathbf{F_o}$-$\mathbf{F_c}$ map can give us some clues**:**



Atom with large B
Strong **negative** peak in $\mathbf{F_o}$-$\mathbf{F_c}$ map
Wrong Z – too many electrons



Atom with **small B**
Strong **positive** peak in $\mathbf{F_o}$-$\mathbf{F_c}$ map
Wrong Z – too few electrons



Atom surrounded by
weak **positive** and
negative peaks in $\mathbf{F_o}$-$\mathbf{F_c}$ map
Anisotropic termal motion

Available software:

REFMAC5 in the CCP4 suite
(https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3069751/)

phenix.refine in the PHENIX suite
(https://phenix-online.org/documentation/reference/refinement.html)

SHELXL (https://journals.iucr.org/c/issues/2015/01/00/fa3356/)

BUSTER (http://www.globalphasing.com/buster/)

All are free for academic users

Completing the model

- $2|F_o|-|F_c|$ maps are used to inspect the electron density of the current model.
- $|F_o|-|F_c|$ maps are used to locate any non-hydrogen protein atoms missing from the current model. **N.B.** It may not be possible to locate **all** of the non-hydrogen protein atoms in the crystal structure due to disorder effects.
- When the non-hydrogen protein atom model is as **complete** as possible, $2|F_o|-|F_c|$ and $|F_o|-|F_c|$ maps are used to locate **non-hydrogen solvent atoms**. There should be usually one ordered water molecule per protein residue. Although this process can be carried out automatically, at resolutions below 2.2 Å most water molecules are **not fully resolved** in the electron density maps, and greater care has to be applied in their assignment.

The crystallographic R-factor:

$$R_{cryst} = \Sigma \left| \left| F_{hkl} \right|_o - \left| F_{hkl} \right|_c \right| / \Sigma \left| F_{hkl} \right|_o$$

- For a **well-refined structure** from **good quality data**, the final value of R should be below 20%. Lower resolution data [below 2.0 Å] may give R values near 25% while higher resolution data [above 1.5 Å] may give R values near 10%.
- Unless the data extend to atomic resolution, the **ratio** between the **number of refined parameters** and the number of **measured structure factor amplitudes** is fairly small (less than 5) and does not ensure sufficient **over-determination** of the structural parameters.

To guard against the risk of **over-fitting** the experimental data, the **free R-factor** is used as well. **Prior to the start of refinement**, the data are divided into two sets:

- a **WORKING** set, containing about 95% of the total number of measured structure factor amplitudes, which will be used in the refinement and to calculate the Crystallographic R-factor.
- a **TEST** set, containing about 5% of the total number of measured structure factor amplitudes, which will **NOT** be used in the refinement but **WILL** be used to calculate the R_{free}, **free R-factor**:

$$\Sigma \left| \left| F_{hkl} \right|_o^{TEST} - \left| F_{hkl} \right|_c^{TEST} \right| / \Sigma \left| F_{hkl} \right|_o^{TEST}$$

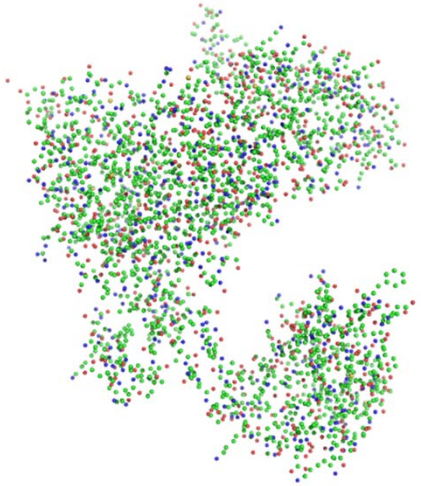
Following refinement progress

- Since the TEST set is **excluded** from the refinement process, the free R-factor is **higher** than the crystallographic R-factor, by as much as 10%, especially for lower [below 2.5 Å] resolution data.
- Differences larger than 10%, especially for **higher resolution** data [above 2.0 Å] should be regarded with **suspicion** and may be an indication that something is wrong with the model.
- There is even an example in the literature where it was possible to refine a protein structure traced backwards into an electron density map to R_{cryst}≈25%. However, the R_{free} for this structure was above 60% !

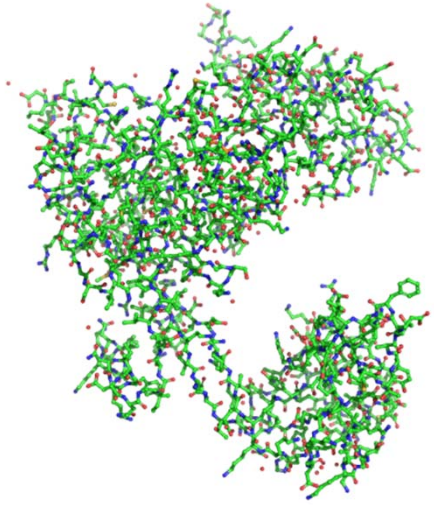
MX refinement is never finished, only abandoned – George Sheldrick

Where freedom is given, liberties are taken - Gerard Kleywegt

Representing your structure

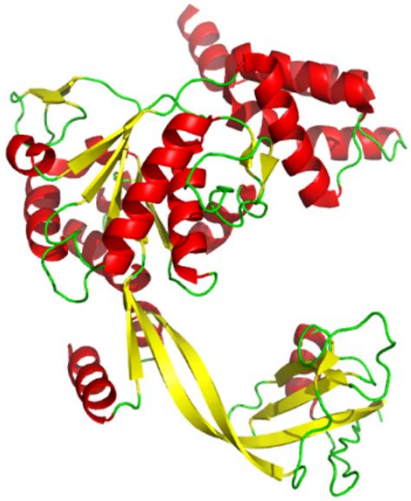


Atoms

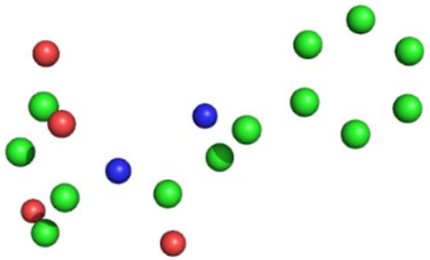


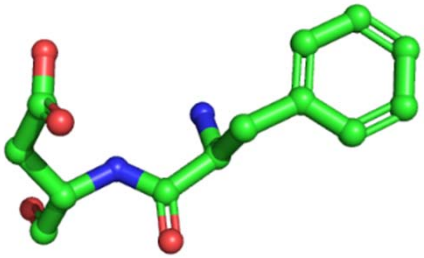
Chemical bonds

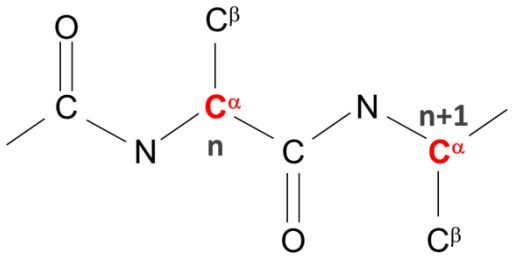
Cα chain



Secondary structure







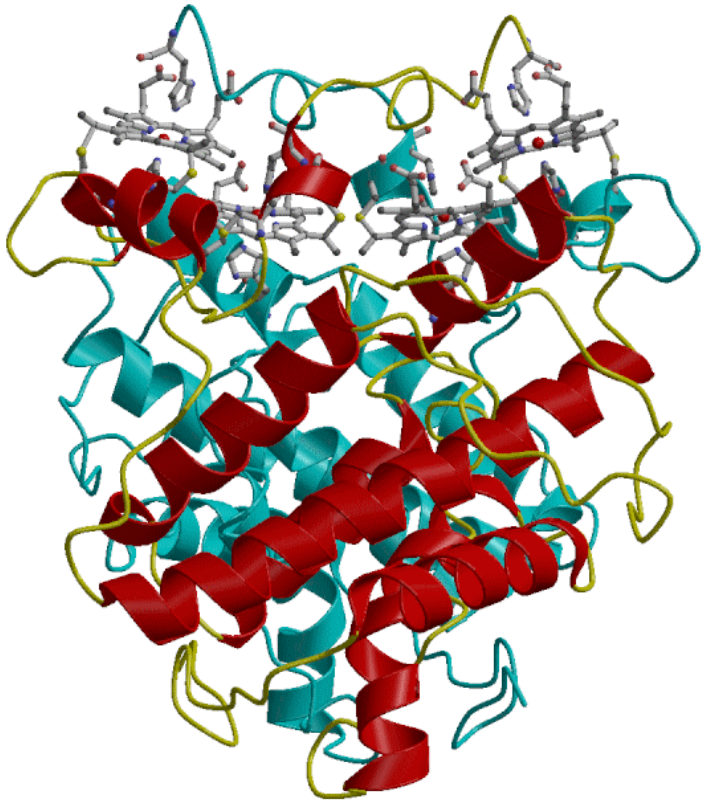
α-helices

β-sheets

loops

The final model

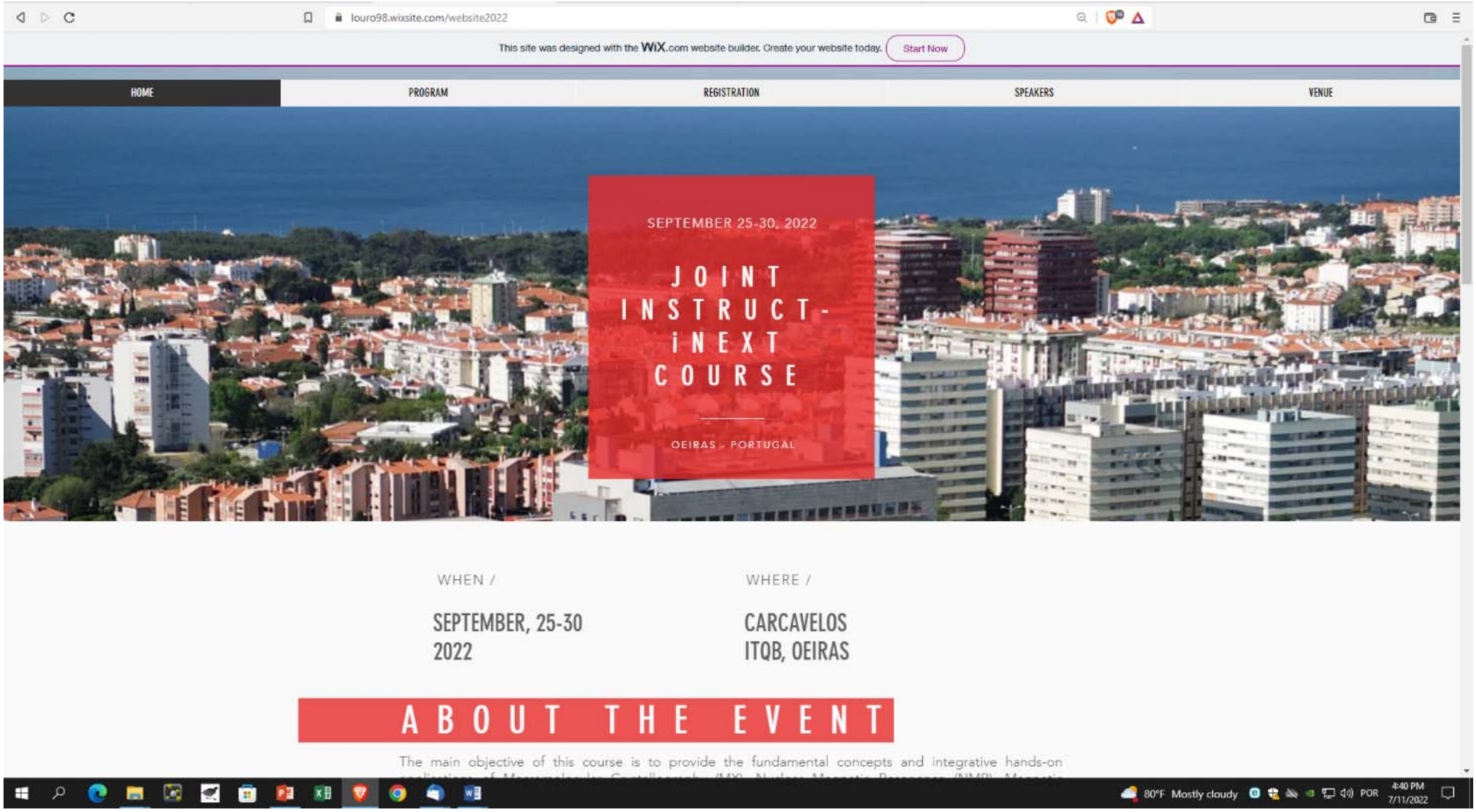
```
ATOM      1  C   GLY  1008      68.442  -1.211  31.641  1.00 45.23
ATOM      2  O   GLY  1008      67.791  -0.256  31.225  1.00 43.67
ATOM      3  N   GLY  1008      68.595  -1.937  29.290  1.00 52.32
ATOM      4  CA  GLY  1008      69.025  -2.247  30.676  1.00 47.67
ATOM      5  N   ARG  1009      68.694  -1.422  32.922  1.00 43.29
ATOM      6  CA  ARG  1009      68.196  -0.526  33.984  1.00 38.58
ATOM      7  CB  ARG  1009      68.598  -1.079  35.346  1.00 31.60
ATOM      8  CG  ARG  1009      68.620  -0.028  36.443  1.00 27.47
ATOM      9  CD  ARG  1009      68.827  -0.642  37.807  1.00 26.82
ATOM     10  NE  ARG  1009      67.744  -1.543  38.138  1.00 25.67
ATOM     11  CZ  ARG  1009      67.740  -2.372  39.159  1.00 26.08
ATOM     12  NH1 ARG  1009      68.793  -2.427  39.994  1.00 22.35
ATOM     13  NH2 ARG  1009      66.710  -3.180  39.396  1.00 25.89
ATOM     14  C   ARG  1009      68.764   0.903  33.823  1.00 39.00
ATOM     15  O   ARG  1009      69.908   1.103  33.389  1.00 38.11
ATOM     16  N   PHE  1010      67.917   1.896  34.164  1.00 36.70
ATOM     17  CA  PHE  1010      68.213   3.336  34.109  1.00 33.68
ATOM     18  CB  PHE  1010      69.593   3.693  34.700  1.00 30.22
ATOM     19  CG  PHE  1010      69.754   3.327  36.130  1.00 26.78
ATOM     20  CD1 PHE  1010      70.960   2.828  36.591  1.00 26.47
ATOM     21  CD2 PHE  1010      68.678   3.434  37.014  1.00 25.81
ATOM     22  CE1 PHE  1010      71.097   2.419  37.923  1.00 26.74
ATOM     23  CE2 PHE  1010      68.800   3.035  38.340  1.00 26.36
ATOM     24  CZ  PHE  1010      70.018   2.523  38.800  1.00 25.10
ATOM     25  C   PHE  1010      68.112   3.943  32.727  1.00 34.78
ATOM     26  O   PHE  1010      68.130   5.165  32.595  1.00 38.56
```



Model Repository: The Protein Data Bank

http://www.rcsb.org/pdb/ or http://www.pdbe.org

To be covered in the next class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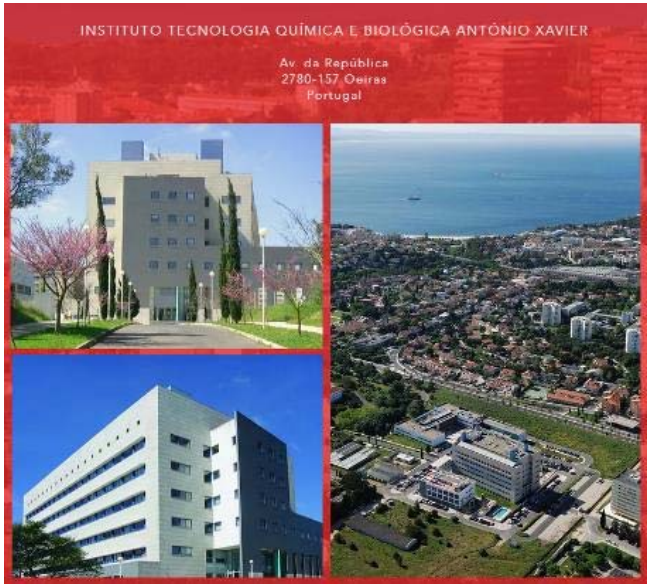
WHEN /
SEPTEMBER, 25-30 2022
WHERE /
CARCAVELOS ITQB, OEIRAS
ABOUT THE EVENT
The main objective of this course is to provide the fundamental concepts and integrative hands-on
80°F Mostly cloudy
POR
4:40 PM
7/11/2022



Joint Instruct iNext course on Integrative Structural Biology

APPLICATION DEADLINE: 23rd of JULY

Email: oeiras.instruct.course2022@itqb.unl.pt

	Monday 26 **MX day**	**Tuesday 27** **NMR day**	**Wednesday 28** **MRMS day**	**Thursday 29** **EM day**	**Friday 30**
9.00-10.00	NKI AlphaFold, PDB Redo A.Perrakis	Spin choreography R. Louro	Basics of MRMS J Jäenis	Basics of EM JM Carazo	Presentation/ discussion access proposals
10.00-10.20	Coffee break	Coffee break	Coffee break	Coffee break	Coffee break
10.20-11.20	Introduction to Crystallography M. Archer	Structure and motion R. Louro?	Native MS P Novak	From data acquisition to reconstruction M Gragera	Presentation/ discussion access proposals
11.20-12.20	Check A.Perrakis microtubules detyrosination	In-cell research Fe-S clusters maturation M. Piccioli	Protein surface mapping, crosslinking, HDX C Wootton	The resolution revolution and breakthroughs in EM JM Carazo	Presentation/ discussion access proposals
12.30	Lunch	Lunch	Lunch	Lunch	Lunch
14.00-15.30	Crystallization, Data collection /processing M. Archer, J. Brito, P. Matias	Data collection, 1D 2D 3D R. Louro	Sample prep/ data acquisition C. Cordeiro	Sample prep, data acquis/ processing M Gragera, J Brito	Social event
15.30-17.00	Model building COOT, search PDB M. Archer, J. Brito, P. Matias	Biomolecular signal assignment R. Louro	Data analysis/ interpretation C. Cordeiro	2D/3D classific, resol, and quality assessment M Gragera, J Brito	
17.00-17.15	Break	Break	Break	Break	
17.15-19.15	Poster session (with snacks)	Webpages Instruct/iNEXT-D Group+case study	Group work - prepare access proposals	Group work - prepare access proposals	
20.00				Social Dinner	