Lezione 6

Andrea Ilari

IL PROBLEMA DELLA FASE

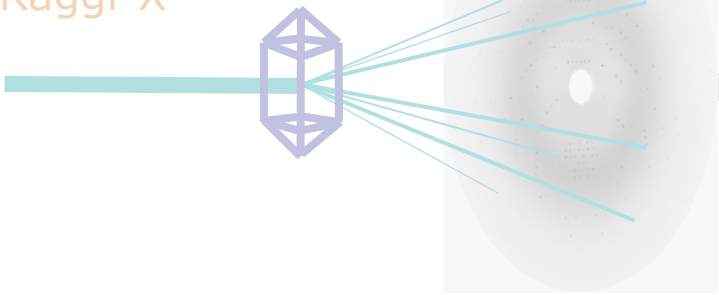
IBPM-CNR

L'esperimento di diffrazione

Cristallo proteico

Immagine di diffrazione

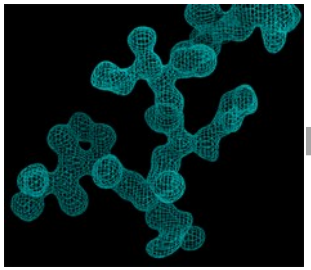
Raggi-X



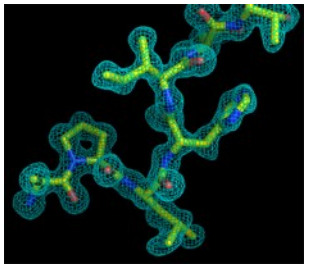
anti Trasformata

FT^{-1}

Mappa di densità elettronica



Costruzione del modello



Expression
Purification
Crystallization



X-ray diffraction (data collection)

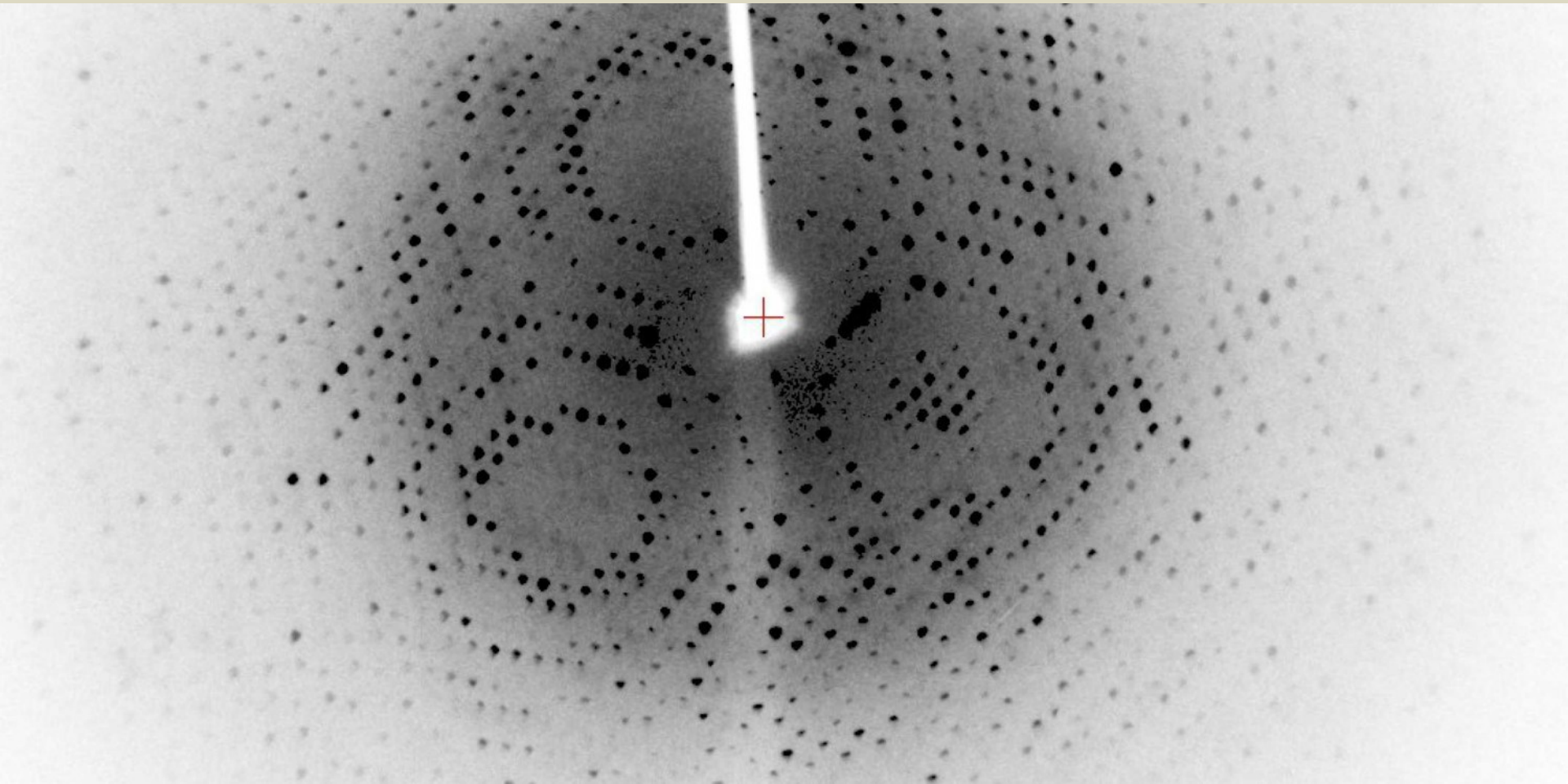


Phasing and model building



ANALISI DEI DATI

Indicizzazione – Integrazione - Scalaggio



INDICIZZAZIONE E INTEGRAZIONE: ANALISI DEI DATI

Con la RACCOLTA DATI i programmi di analisi fanno 3 cose:

INDICIZZAZIONE

Analizzo le prime immagini di diffrazione:

- misurano **le posizioni** dei picchi

⇓

- ricavano **il gruppo spaziale**

INTEGRAZIONE

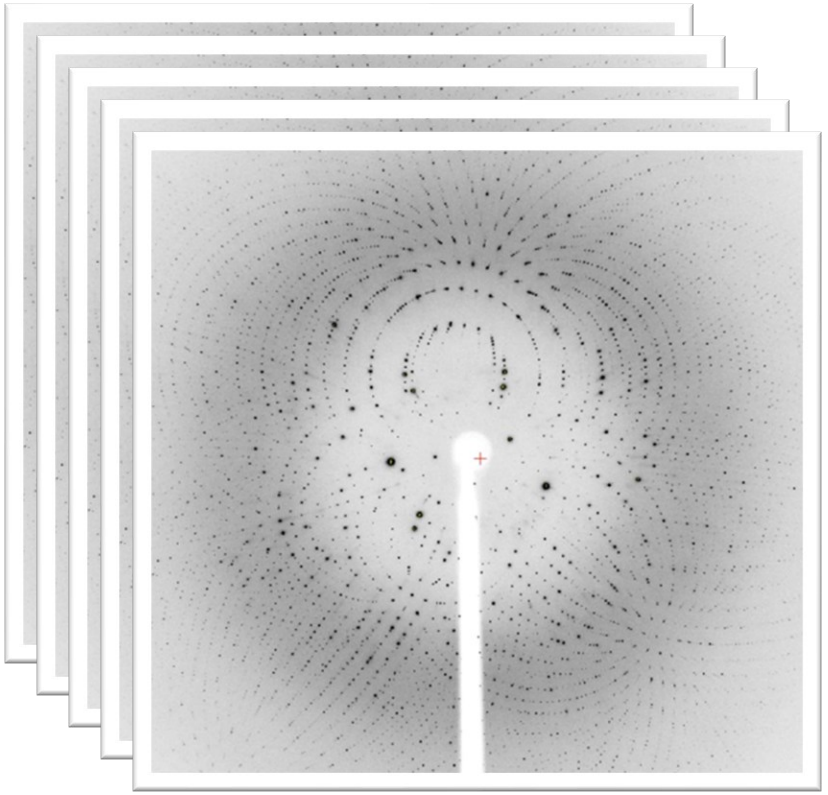
Analizzo tutte le immagini di diffrazione:

- misurano le **Intensità di ogni picco (spot)**

SCALAGGIO

Unisco tutti i dati ottenuti:

- scrivono in un **output le Intensità relative ad ogni posizione nello spazio reciproco (spot) a cui assegnano gli indici *h, k, l***
  - **R merge**
  - **mosaicità** (disordine intrinseco)
  - **completezza**, ridondanza
  - ***I/σ(I)*** (segnale/rumore)



RACCOLTA DATI:
Una serie di immagini di diffrazione dei raggi-X ottenute facendo ruotare il cristallo

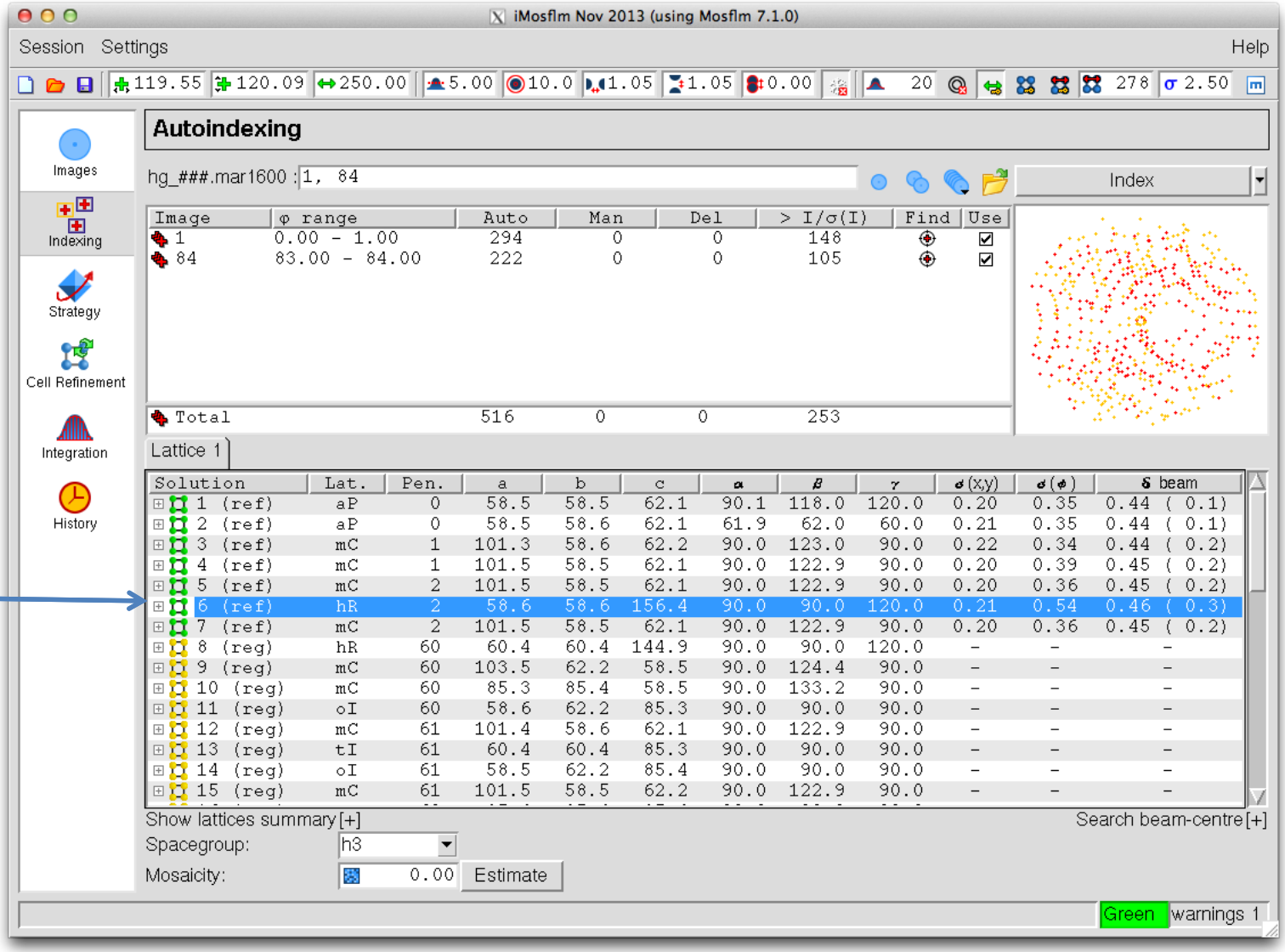
INDICIZZAZIONE (indexing)

1. INDICIZZAZIONE

Analizzo le prime immagini di diffrazione:

- misurano **le posizioni** dei picchi → ricavano **il gruppo spaziale e i parametri della cella**

Cella più probabile



INTEGRAZIONE (integration)

1. INTEGRAZIONE

Analizzo tutte le immagini di diffrazione:

- misurano le **INTENSITÀ di ogni picco (spot)**

Misuro l'intensità (annerimento) di ogni spot



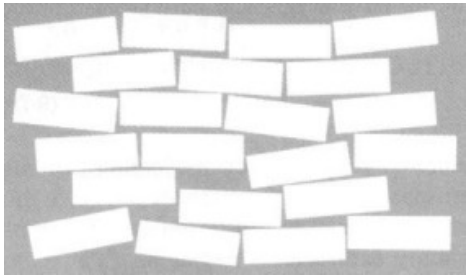
1. SCALAGGIO

Unisco e confronto tutti i dati ottenuti:

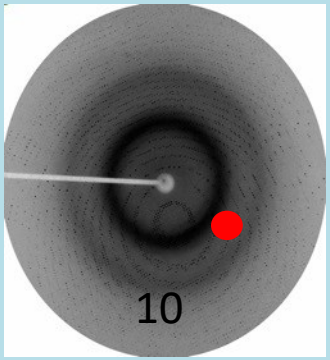
• scrivono in un **output le Intensità relative ad ogni posizione nello spazio reciproco (spot) a cui assegnano gli indici *h, k, l*** (indici di Miller)

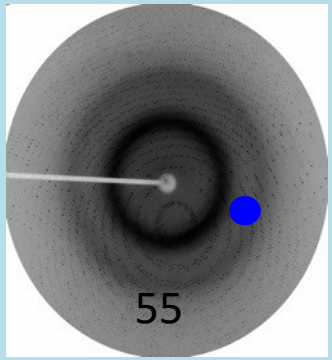
STATISTICHE
(indicano la bontà del dato sperimentale)

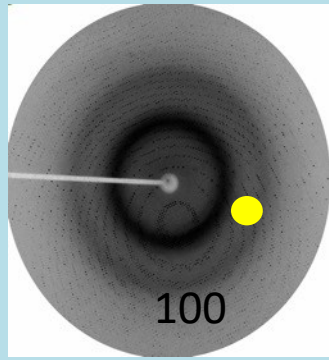
- **mosaicità** (disordine intrinseco del cristallo)



- **R merge**
- **completezza**, ridondanza
- ***I/σ(I)*** (segnale/rumore)
- Etc…



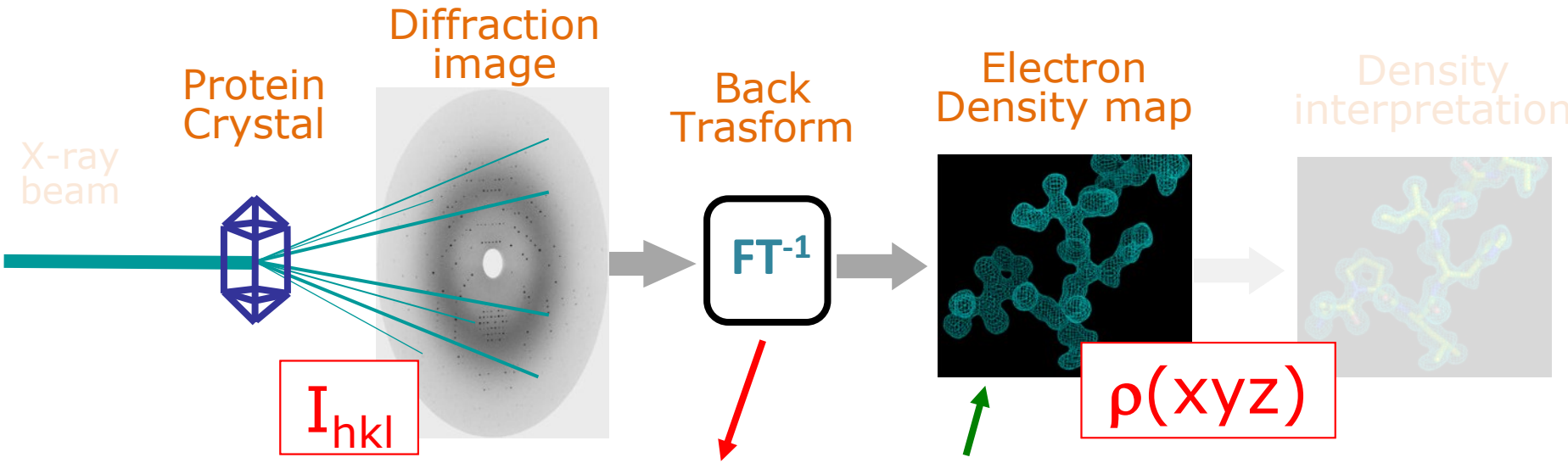




$I_1(234)$ $I_2(234)$ $I_3(234)$

$$R_{merge} = \frac{\sum_{hkl}\sum_{i=1}^{n}\left|I(hkl) - I_i(hkl)\right|}{\sum_{hkl} N \times I(hkl)}$$

L'ESPERIMENTO DI DIFFRAZIONE



$$\rho(xyz) = 1/V \sum_{hkl} |F_{hkl}| \exp(i\alpha_{hkl}) \exp(-2\pi i\, hx + ky + lz)$$

α_{hkl} is lost in the experiment - the phase problem!

PERSA

structure factor amplitude $|F_{hkl}| \propto (I_{hkl})^{1/2}$

OTTENUTI DAI DATI DI DIFFRAZIONE



DENSITÀ ELETTRONICA= SOMMA DI FATTORI DI STRUTTURA

$$F(hkl) = |F_{hkl}| \exp(i\alpha_{hkl})$$

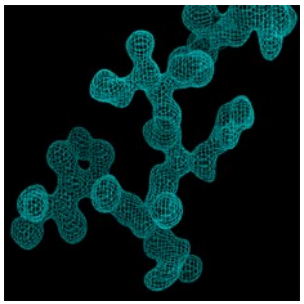
Eq DENSITÀ ELETTRONICA

$$\rho(xyz) = 1/V \sum_{hkl} |F_{hkl}| \exp(i\alpha_{hkl}) \exp(-2\pi i\, hx + ky + lz)$$

α_{hkl} is lost in the experiment - the phase problem!

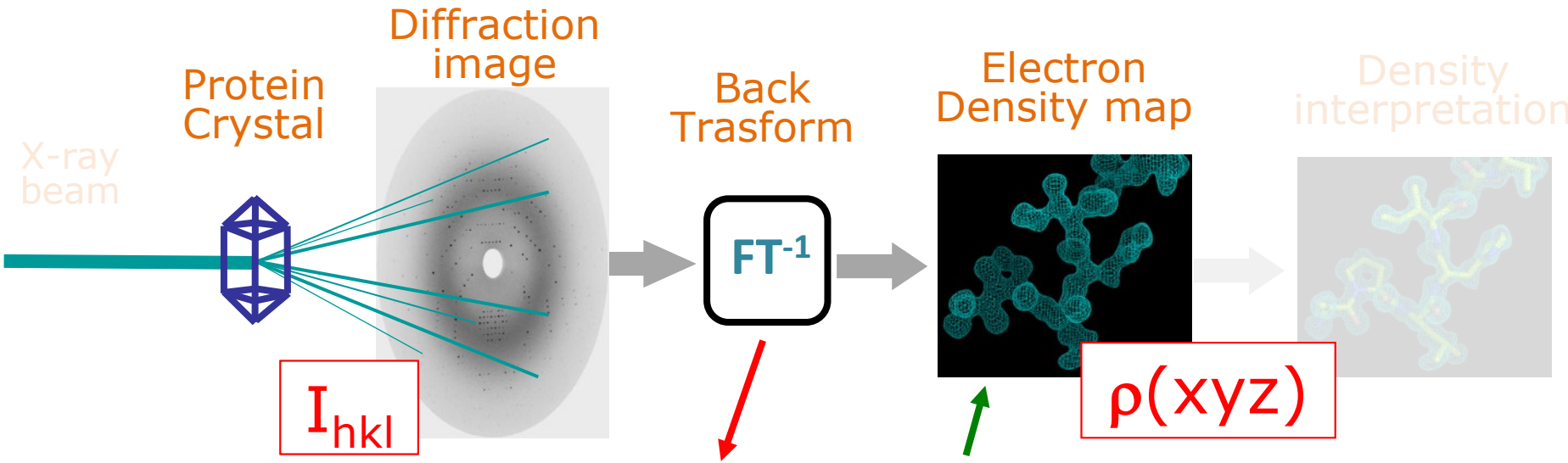
structure factor amplitude $|F_{hkl}| \propto (I_{hkl})^{1/2}$

SAPZIO REALE



$$\rho(\mathbf{x}) = \frac{1}{V} \sum_{\mathbf{h}} \mathbf{F(h)} \exp(-2\pi i \mathbf{h} \cdot \mathbf{x})$$

L'ESPERIMENTO DI DIFFRAZIONE



$$\rho(xyz) = 1/V \sum_{hkl} |F_{hkl}| \exp(i\alpha_{hkl}) \exp(-2\pi i\, hx + ky + lz)$$

FASE

α_{hkl} is lost in the experiment - the phase problem!

PERSA

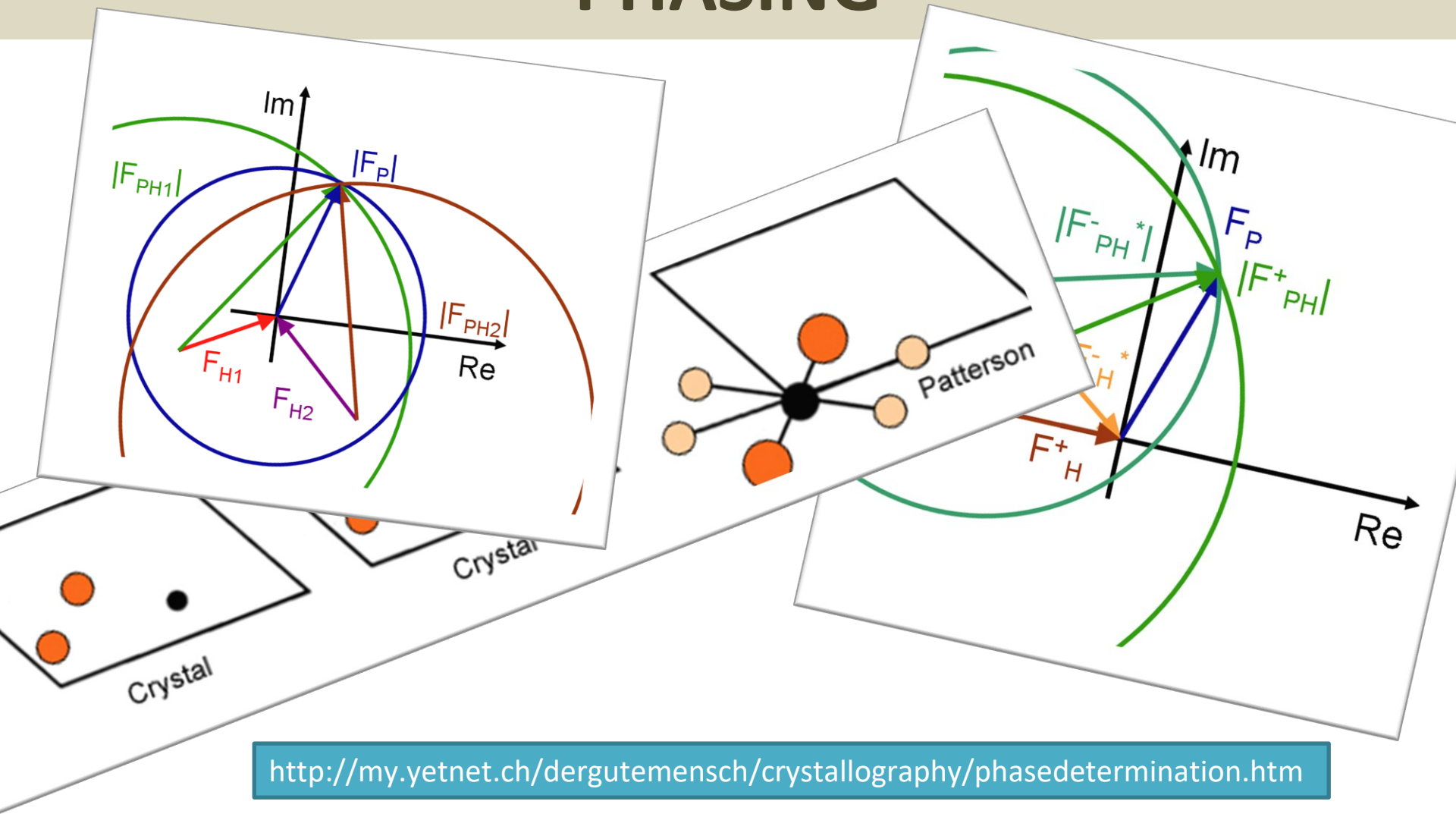
AMPIEZZA

structure factor amplitude $|F_{hkl}| \propto (I_{hkl})^{1/2}$

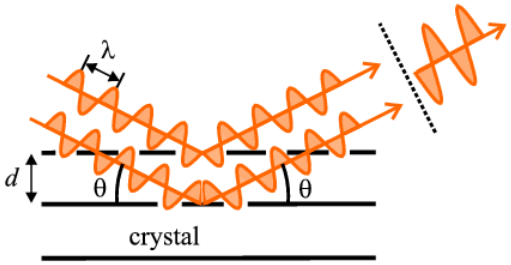
OTTENUTI DAI DATI DI DIFFRAZIONE



PHASING



http://my.yetnet.ch/dergutemensch/crystallography/phasedetermination.htm



SPOT = $F(hkl) = |F_{hkl}| \exp(i\alpha_{hkl})$

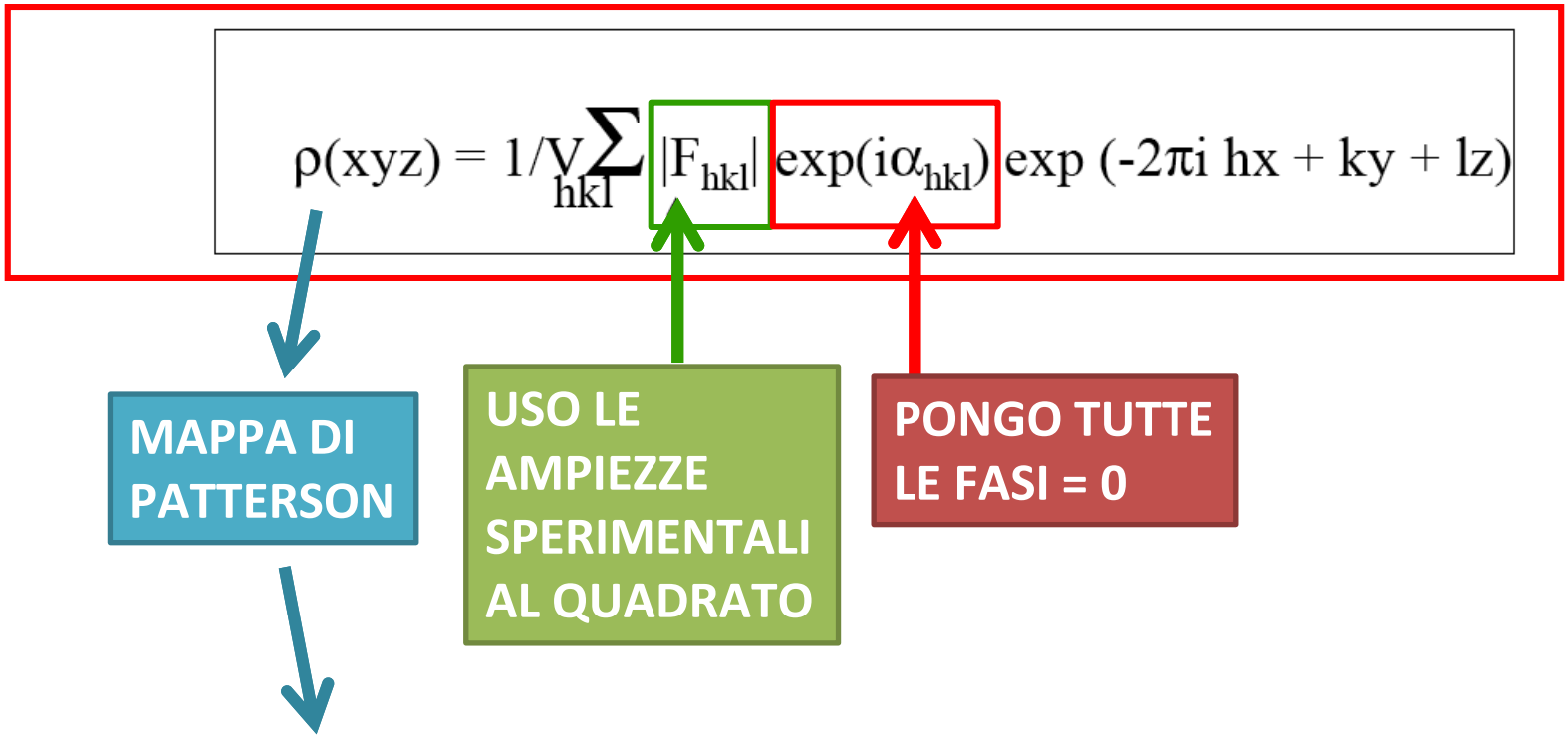
$|F_{hkl}|$ → **AMPIEZZA**

$\exp(i\alpha_{hkl})$ → **FASE**

Metodi per determinare la FASE

- Metodi Diretti (solo per piccole molecole)
- Molecular Replacement
- Metodi basati su atomi pesanti
  - Sostituzione isomorfa (SIR – MIR)
  - Diffrazione Anomala (SAD - MAD)
  - Metodi combinati (SIRAS – MIRAS)

PHASING: la funzione di PATTERSON



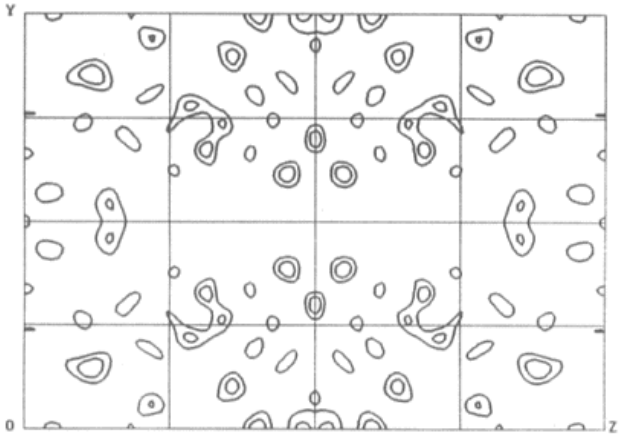
$$P(u,v,w) = 1/V \; \Sigma \; |F^2_{hkl}| \; \exp[-2\pi i(hu+kv+lw)]$$

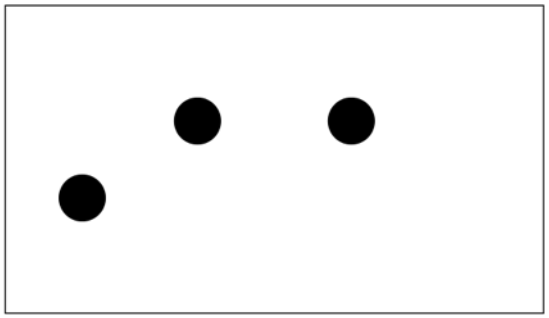
POSSO CALCOLARE LA MAPPA DI PATTERSON DAI DATI SPERIMENTALI, PERCHÉ NON DIPENDE PIÙ DALLA FASE

MAPPA DI PATTERSON

$$P(u,v,w) = 1/V \Sigma \left| F^2_{hkl} \right| \exp[-2\pi i(hu+kv+lz)]$$

LA **MAPPA DI PATTERSON**
MAPPA DI VETTORI INTERATOMICI:
CONTIENE **INFORMAZIONI SULLE DISTANZE INTERATOMICHE**
QUINDI INFORMAZIONI STRUTTURALI



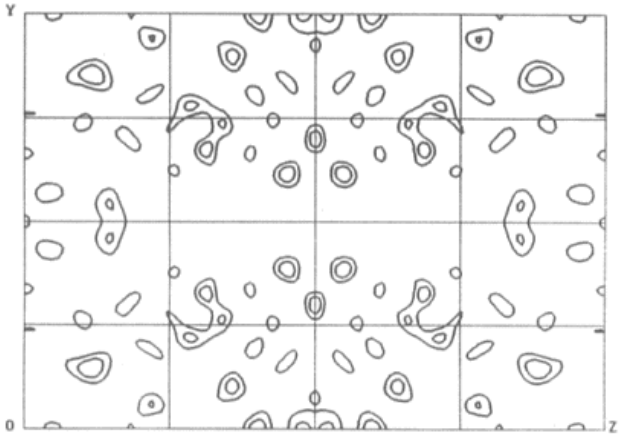


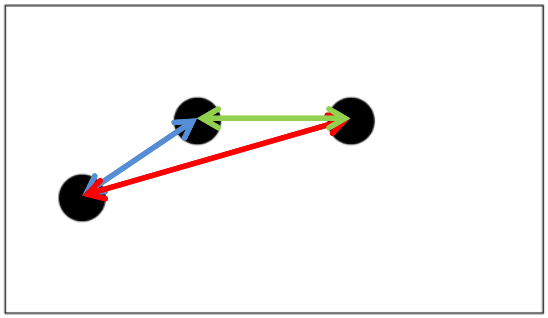
Disposizione di 3 Atomi nel cristallo

MAPPA DI PATTERSON

$$P(u,v,w) = 1/V \Sigma \left| F^2_{hkl} \right| \exp[-2\pi i(hu+kv+lz)]$$

LA **MAPPA DI PATTERSON**
MAPPA DI VETTORI INTERATOMICI:
CONTIENE **INFORMAZIONI SULLE DISTANZE INTERATOMICHE**
QUINDI INFORMAZIONI STRUTTURALI



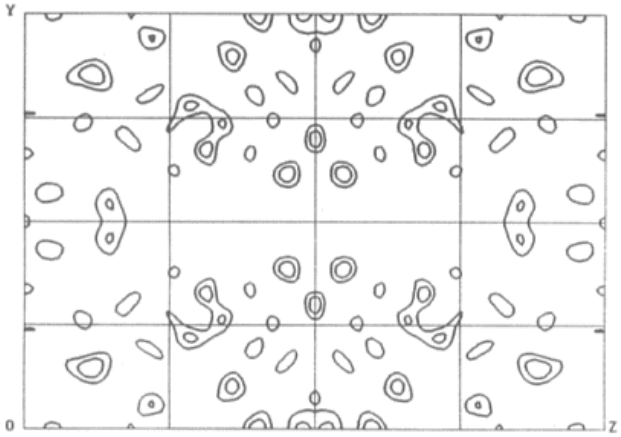


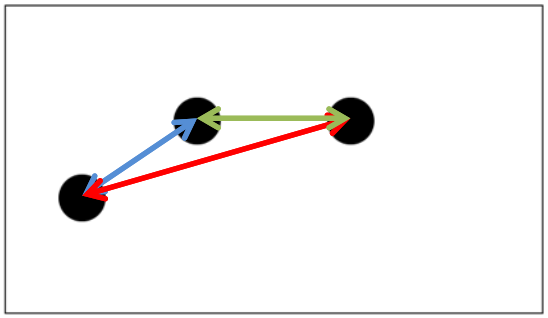
Disposizione di 3 Atomi nel cristallo

MAPPA DI PATTERSON

$$P(u,v,w) = 1/V \Sigma |F^2_{hkl}| \exp[-2\pi i(hu+kv+lz)]$$

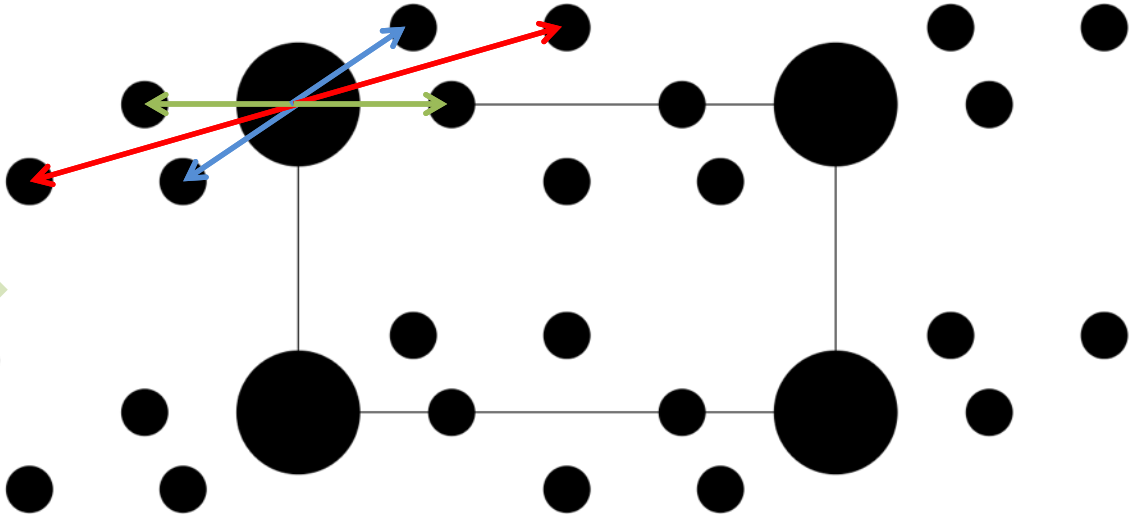
LA **MAPPA DI PATTERSON**
MAPPA DI VETTORI INTERATOMICI:
CONTIENE **INFORMAZIONI SULLE DISTANZE INTERATOMICHE**
QUINDI INFORMAZIONI STRUTTURALI





Disposizione di 3 Atomi nel cristallo

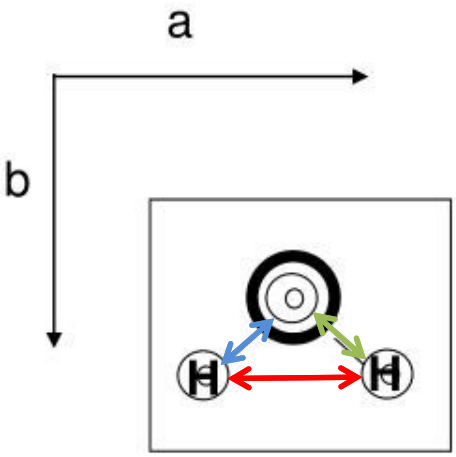
P(u,v,w)



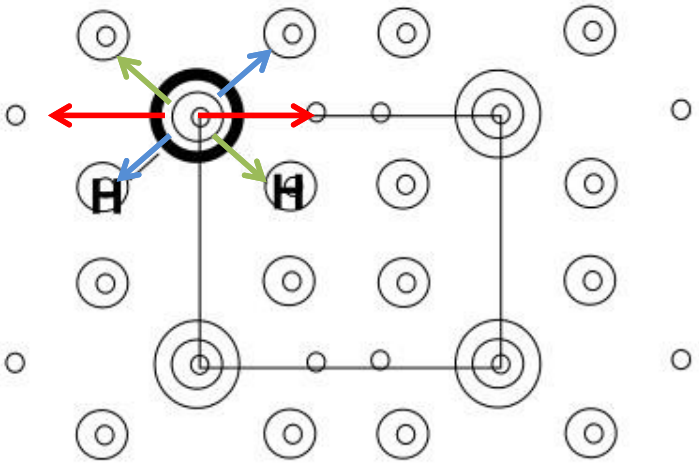
Picchi della mappa di Patterson corrispontente

Patterson maps are more complicated than electron density maps.

Imagine the complexity of a Patterson map of a protein



Unit cell repeats fill out rest of cell with peaks



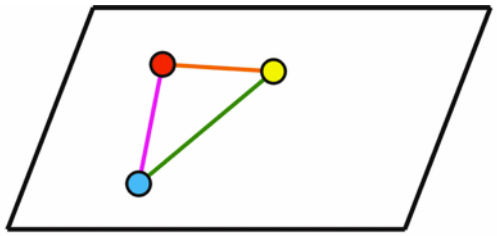
Electron Density Map
single water molecule in the unit cell

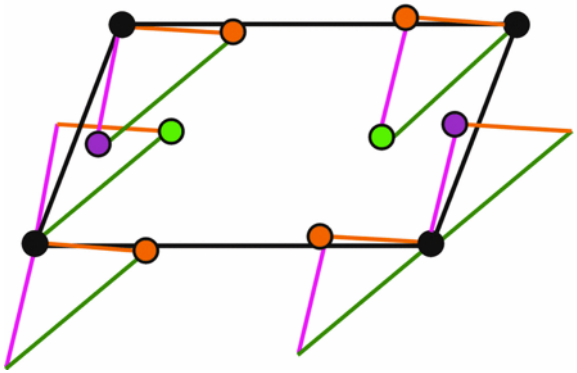
Patterson Density Map
single water molecule convoluted with its inverted image.

MAPPA DI PATTERSON

$$P(u,v,w) = 1/V \Sigma \left| F^2_{hkl} \right| \exp[-2\pi i(hu+kv+lz)]$$

METODI DIRETTI



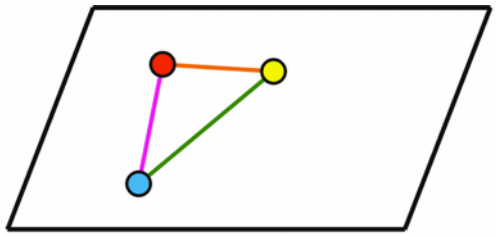


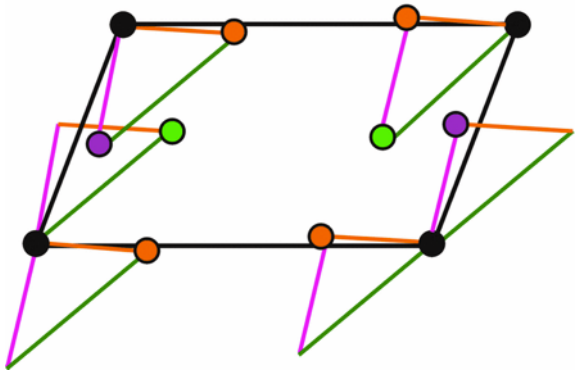
Dalla mappa di Patterson
per molecole con < 100 atomi
Posso provare tutte le posizioni atomiche possibili
E determinare le fasi

MAPPA DI PATTERSON

$$P(u,v,w) = 1/V \Sigma \left| F^2_{hkl} \right| \exp[-2\pi i(hu+kv+lz)]$$

METODI DIRETTI





Dalla mappa di Patterson
per molecole con < 100 atomi
Posso provare tutte le posizioni atomiche possibili
E determinare le fasi

SOLO
Piccole
molecole

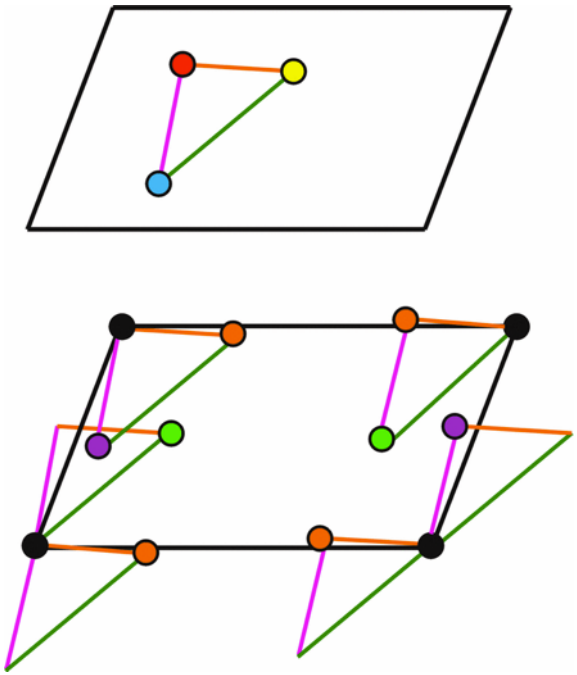
PHASING: MOLECULAR REPLACEMENT

$$\rho(xyz) = 1/V \sum_{hkl} |F_{hkl}| \exp(i\alpha_{hkl}) \exp(-2\pi i\, hx + ky + lz)$$

MAPPA DI PATTERSON

AMPIEZZE SPERIMENTALI

PONGO TUTTE LE FASI = 0



PER LE PROTEINE?

NON POSSO USARE I METODI DIRETTI perché ho troppi atomi, ma posso partire dalla disposizione atomica di proteine omologhe → **elevata identità di sequenza (> 30-50%) → simile struttura 3D**

Molecular Replacement (MR)

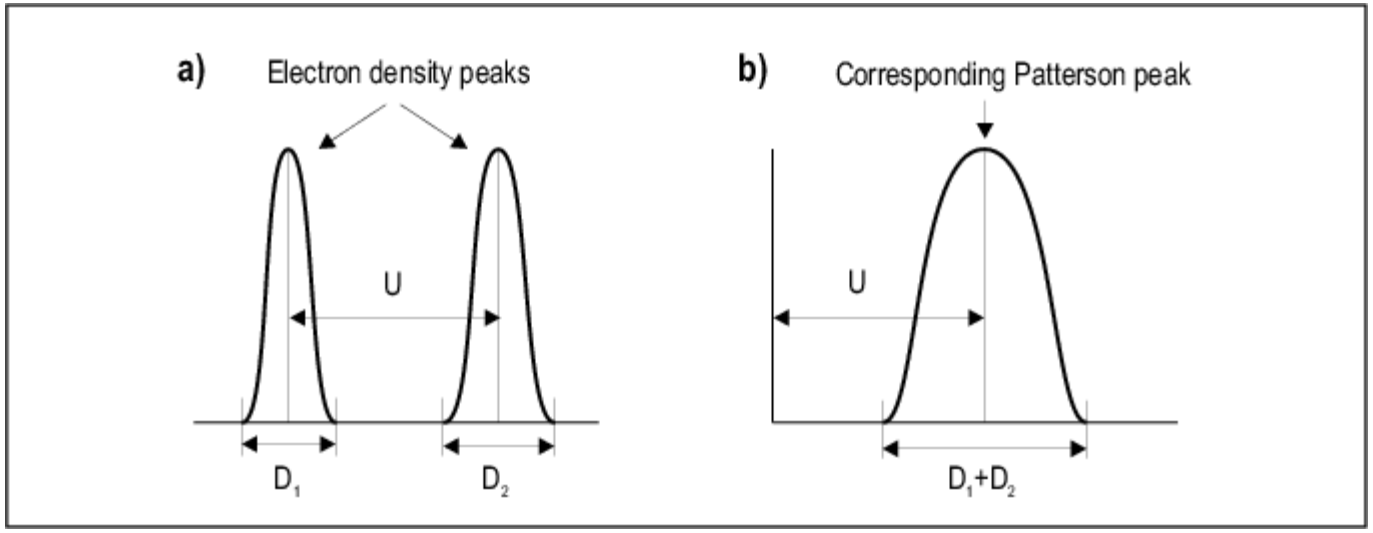
- La proteina di interesse deve avere una proteina omologa di cui sia presente la struttura nel PDB (**search model**)
- L'identità di sequenza deve essere maggiore del 30 %
- Si confrontano le due Patterson, quella ottenuta dal modello e quella ottenuda dai dati sperimentali

$$P(\vec{u}) = \int_V \rho(\vec{x})\rho(\vec{x}+\vec{u})dv$$

model

$$P(\vec{u}) = \frac{1}{V}\sum_{\vec{h}}\sum_{\vec{h}'} F(\vec{h})F(\vec{h}')\, e^{-2\pi i\vec{h}'\vec{u}} = \frac{1}{V}\sum_{\vec{h}} |F(\vec{h})|^2\, e^{-2\pi i\vec{h}\vec{u}}$$

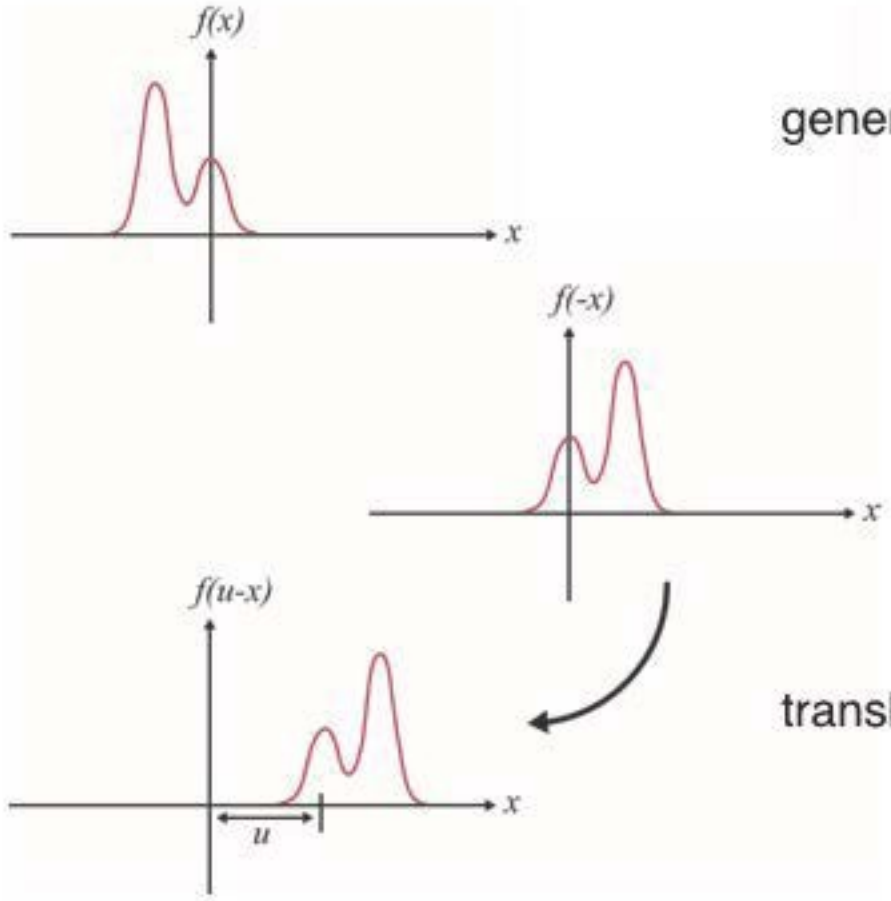
experimental



Convolution operation

La Patterson del modello Scaricato dal pdb è calcolata applicando l'autoconvoluzione alla funzone della densità elettronica

$$f(x) * g(x) = \int f(x)g(u-x)dx$$



PHASING: MOLECULAR REPLACEMENT

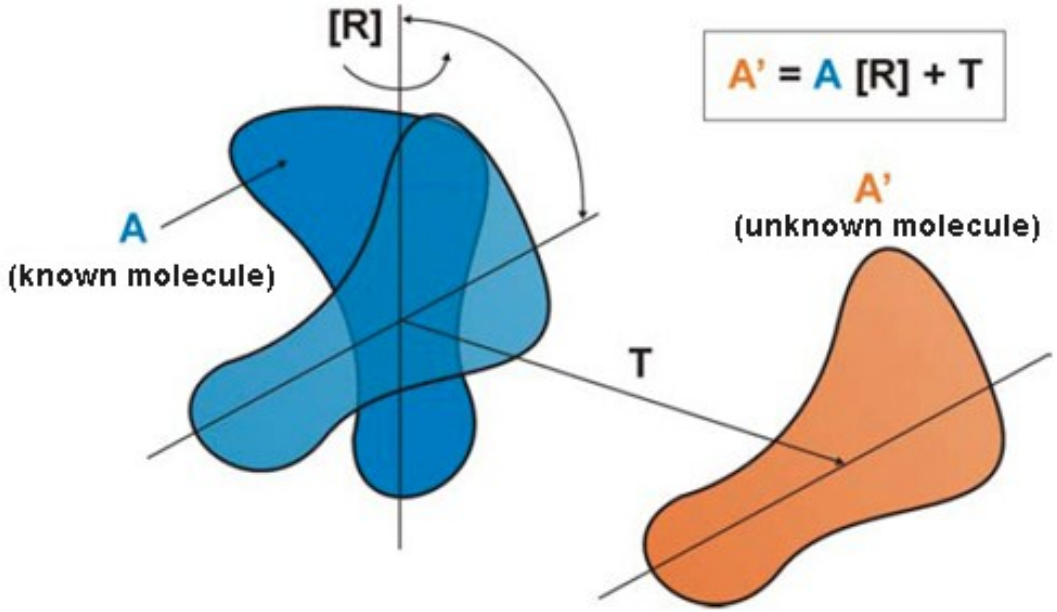




MAPPA DI PATTERSON MODELLO

Ruoto e Traslo la mappa del modello per farla coincidere con quella della proteina

MAPPA DI PATTERSON PROTEINA



PHASING: MOLECULAR REPLACEMENT



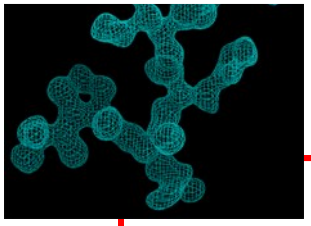


MAPPA DI PATTERSON MODELLO

Ruoto e Traslo la mappa del modello per farla coincidere con quella della proteina

MAPPA DI PATTERSON PROTEINA

MAPPA DI DENSITÀ ELETTRONICA



AMPIEZZE SPERIMENTALI

FASI DEL MODELLO orientato Come nel cristallo reale

$$\rho(xyz) = 1/V \sum_{hkl} |F_{hkl}| \exp(i\alpha_{hkl}) \exp(-2\pi i\, hx + ky + lz)$$

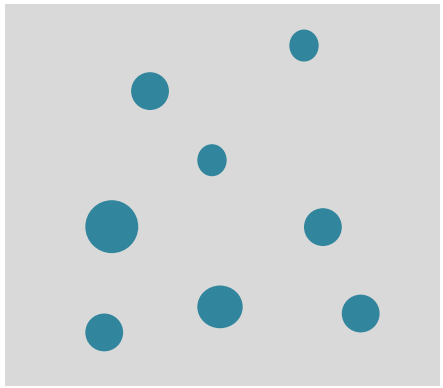
METODI BASATI SUGLI ATOMI PESANTI

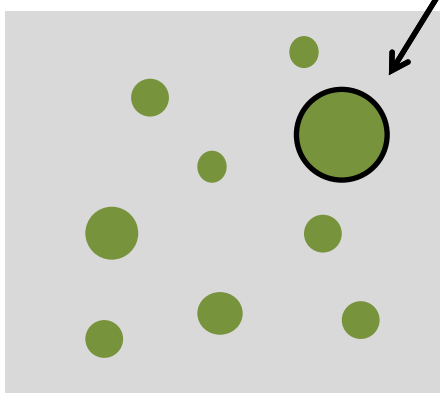
Principio di base:
se ad un'onda incognita aggiungo **un'onda nota**, l'onda risultante mi permetterà di risalire all'incognita

Mezzi sperimentali utilizzati:
- **Atomi pesanti** (alto numero di elettroni)
- **Atomi che a λ diverse diffrangono in modo diverso** perché assorbono una parte dei raggi X (diffusori anomali)

Metodologia:
- **Il cristallo viene messo in contatto con una soluzione di un sale di atomi pesanti oppure gli atomi pesanti vengono incorporati nelle proteine (Selenio-metionine)**
- Ottengo che pochi atomi si posizionano in punti discreti e uguali in tutte le molecole
- Questa aggiunta **non deve variare in alcun modo (<0.5%) i parametri della cella** o l'orientazione della molecola nella stessa (isomorfismo)
- **L'immagine di diffrazione non cambia, cambia solo l'intensità di alcuni riflessi**

SOSTITUZIONE ISOMORFA





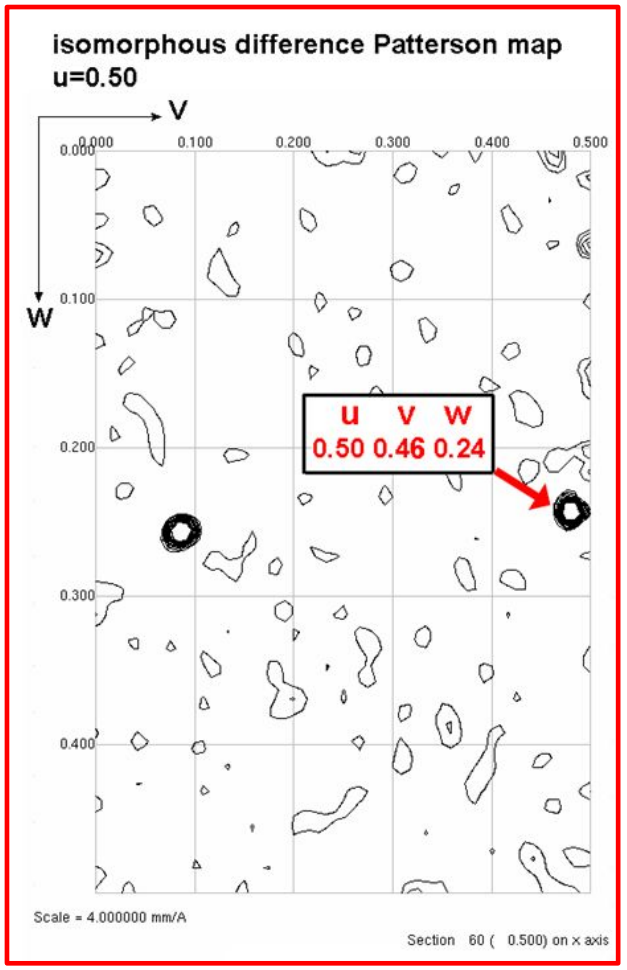
$|F_P|$ Proteina Nativa

$|F_{PH}|$ Proteina + Heavy Atom

$F_P = |F_P| \exp(i\alpha_P)$???

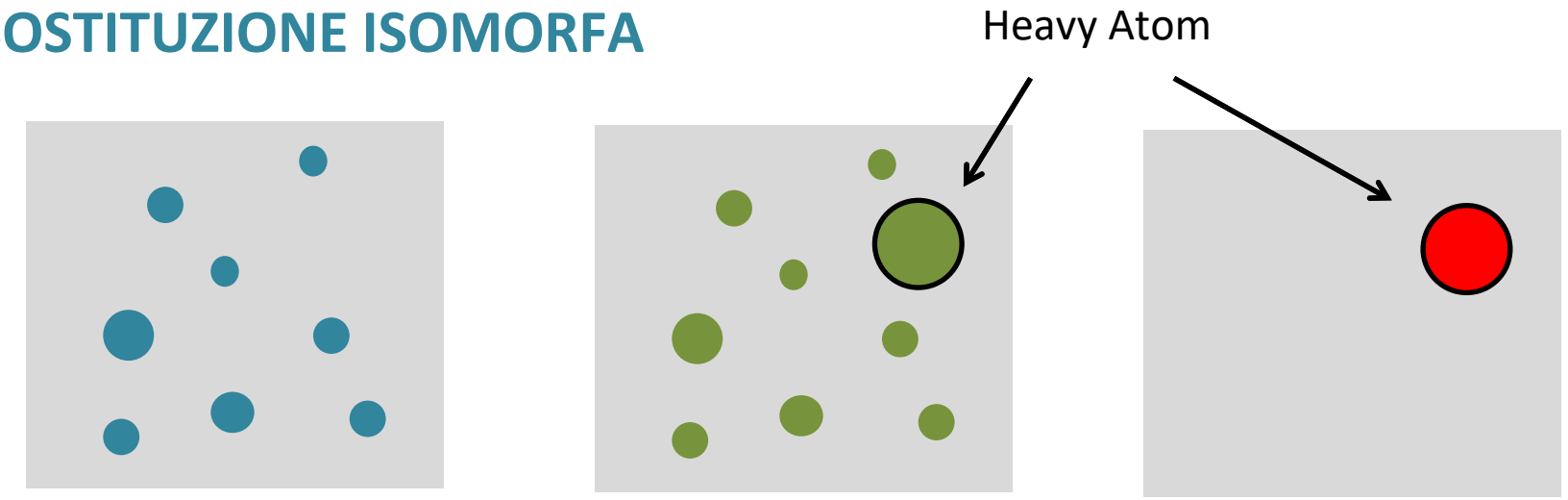
$F_{PH} = |F_{PH}| \exp(i\alpha_{PH})$???

SIR
Single
Isomorphous
Replacement



Posso fare la differenza delle mappe di Patterson

SOSTITUZIONE ISOMORFA



$|F_P|$ Proteina Nativa

$|F_{PH}|$ Proteina + Heavy Atom

F_H Heavy Atom

$F_P = |F_P| \exp(i\alpha_P)$???

$F_{PH} = |F_{PH}| \exp(i\alpha_{PH})$???

$F_H = |F_H| \exp(i\alpha_H)$

SIR
Single
Isomorphous
Replacement

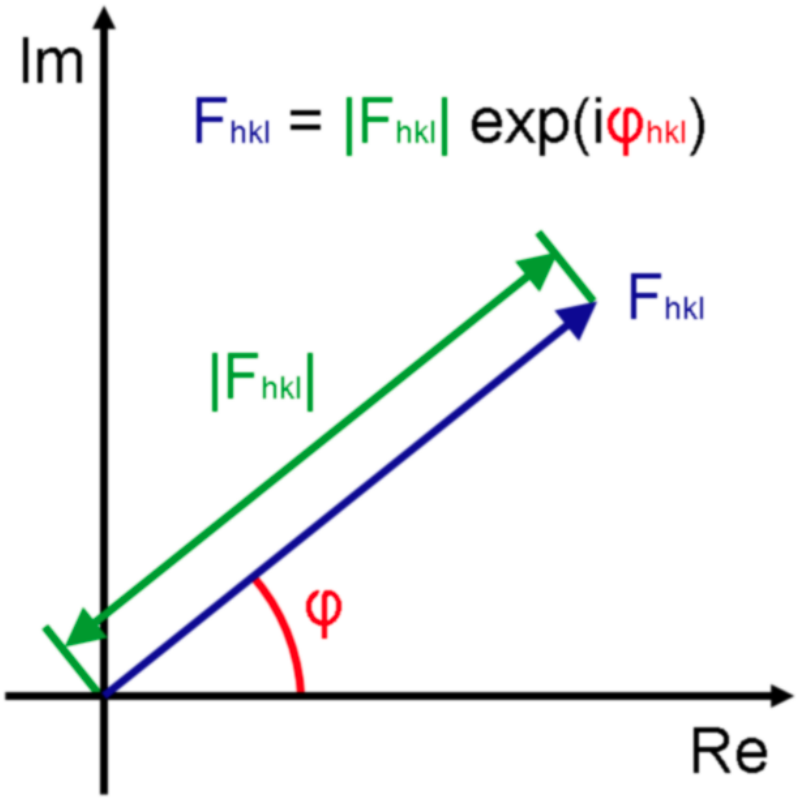
USO I METODI DIRETTI
Per calcolare la
Heavy Atom
sub-structure

METODI BASATI SUGLI ATOMI PESANTI

$$F(hkl) = |F_{hkl}| \exp(i\alpha_{hkl})$$

AMPIEZZA

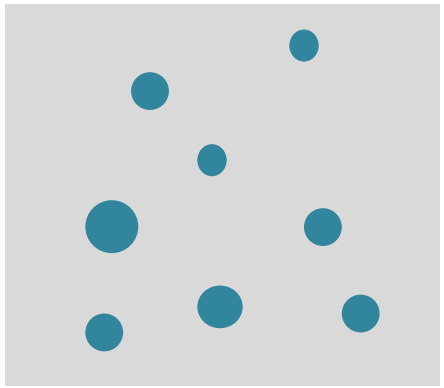
FASE



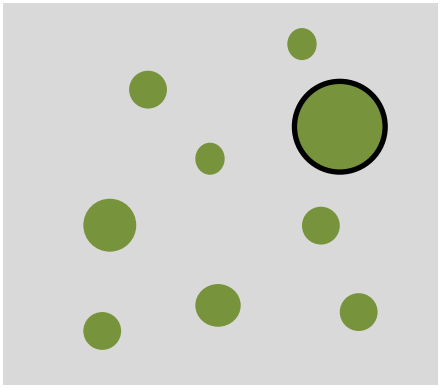
…UN ALTRO MODO DI RAPPRESENTARE I FATTORI DI STRUTTURA

SOSTITUZIONE ISOMORFA

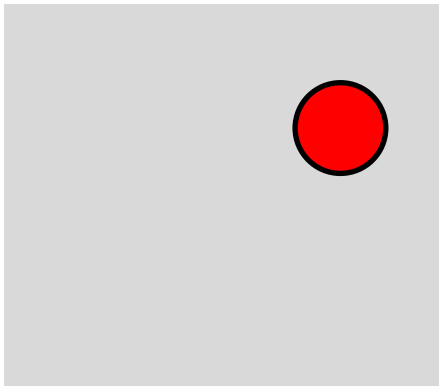
$$F_{hkl} = |F_{hkl}| \exp(i\alpha_{hkl})$$



$|F_P|$ Proteina Nativa



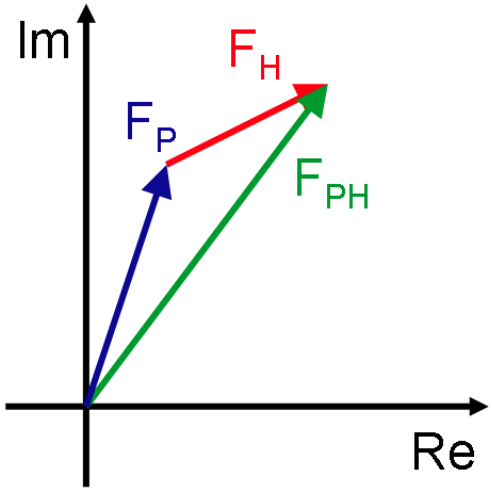
$|F_{PH}|$ Proteina + Heavy Atom

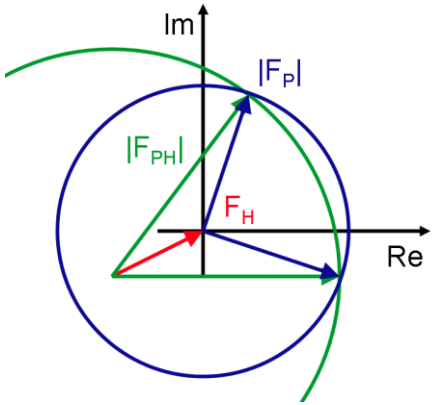


F_H Heavy Atom

SIR
Single
Isomorphous
Replacement

$$F_{PH} = F_P + F_H$$

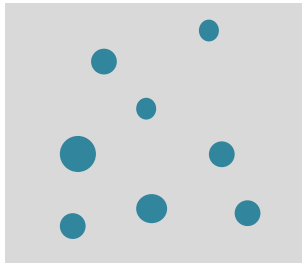




2 possibili soluzioni per la fase di F_p (phase ambiguity)
Controllo le mappe per capire quella corretta

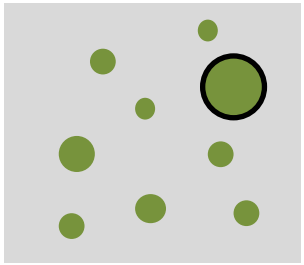
SOSTITUZIONE ISOMORFA

$$F_{hkl} = |F_{hkl}| \exp(i\alpha_{hkl})$$



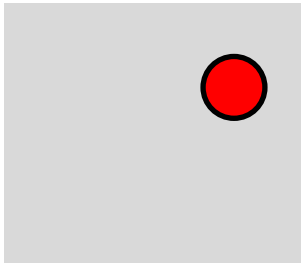
$|F_P|$

Diff. Proteina Nativa



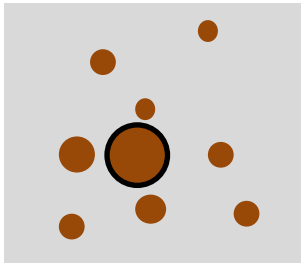
$|F_{PH1}|$

Diff. Prot + HA 1



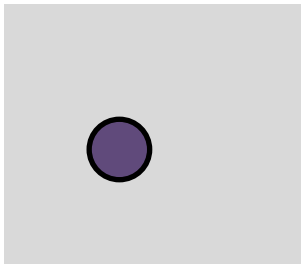
F_{H1}

Diff. HA 1



$|F_{PH2}|$

Diff. Prot + HA 1

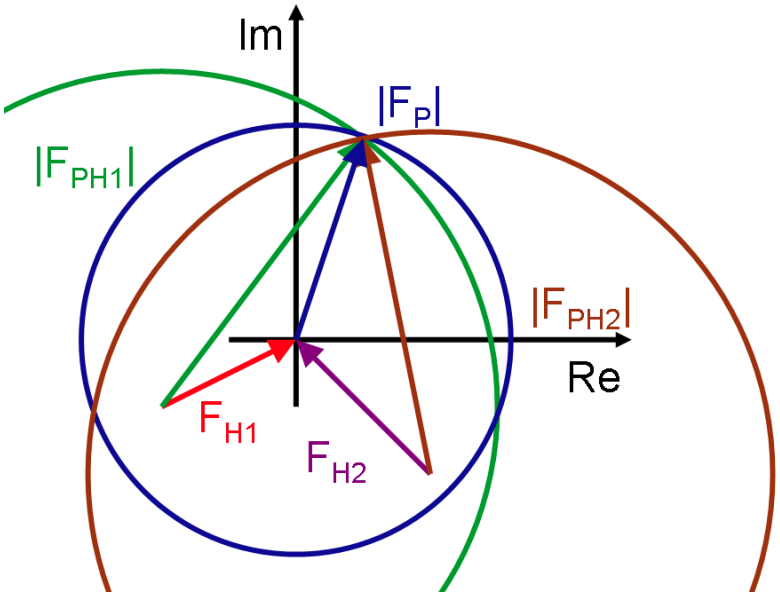


F_{H2}

Diff. HA 1

MIR
Multiple
Isomorphous
Replacement

AGGIUNGO UN ALTRO ATOMO PESANTE
Non ho più ambiguità



Classi di atomi pesanti (H, heavy atoms)

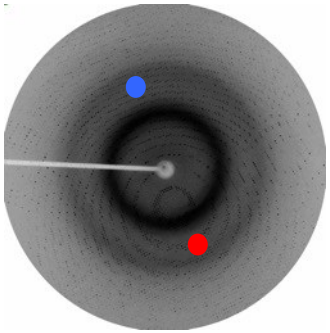
- Ioni metallici legati elettrostaticamente alla molecola
- Metalli endogeni che possono essere facilmente sostituiti (es. Sr^{2+} per Ca^{2+})
- **Selenio (es. Se-Met)**
- Composti metallici legati covalentemente (reazione chimica su cristallo)
- Agglomerati multimetallici per molecole > 500 Kda (es. cluster di oro o di tantalio)
- Gas nobili come Xe e Kr
- Alogenuri (es. tri-ioduri e basi brominate)

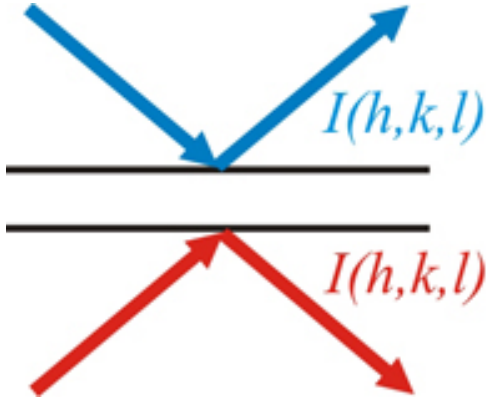
DIFFRAZIONE NORMALE

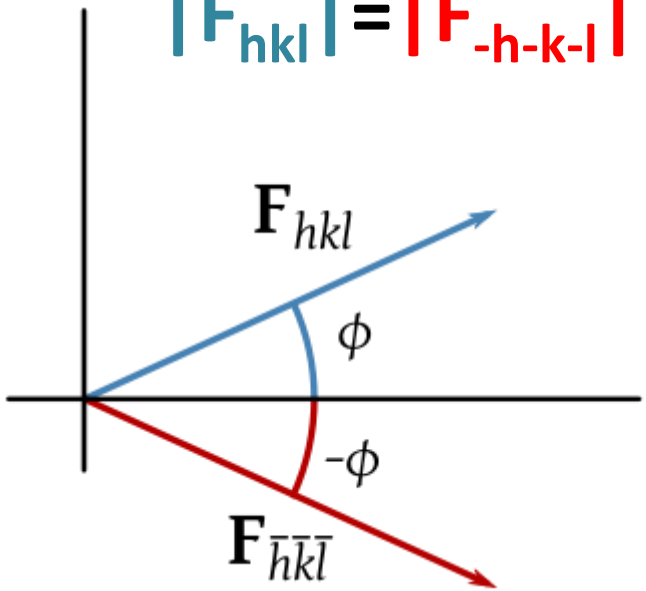
$$F_{hkl} = |F_{hkl}| \exp(i\alpha_{hkl})$$

LEGGE DI FRIEDEL

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





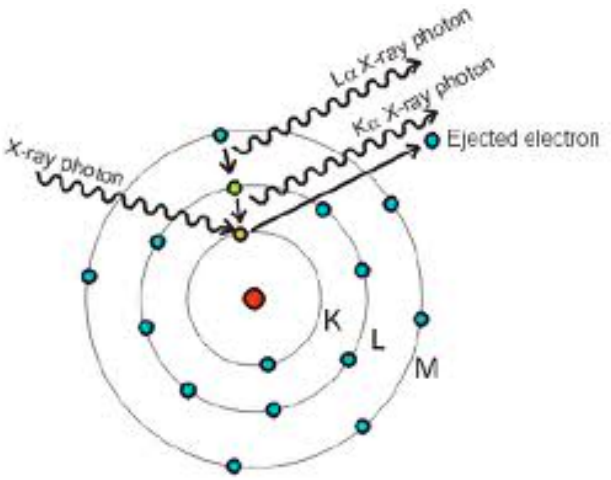


In condizioni di diffrazione non anomala 2 riflessi simmetrici (coppie di Friedel) hanno stessa ampiezza e fase opposta

DIFFRAZIONE ANOMALA

$$f(\lambda) = f^{\circ} + f'(\lambda) + if''(\lambda)$$

A DETERMINATE λ GLI ATOMI PESANTI POSSONO ASSORBIRE I RAGGI X
Estraendo un e^- da un orbitale più interno.
Il segnale dell'onda diffratta viene modificato.



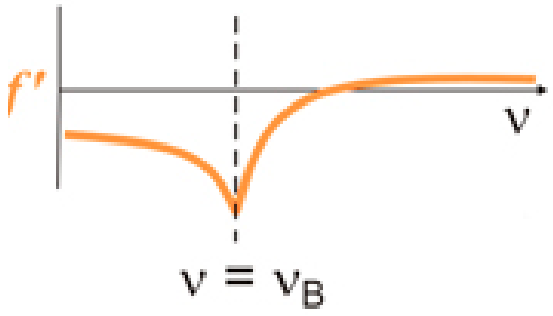
$$f = f_o + f' + i f''$$

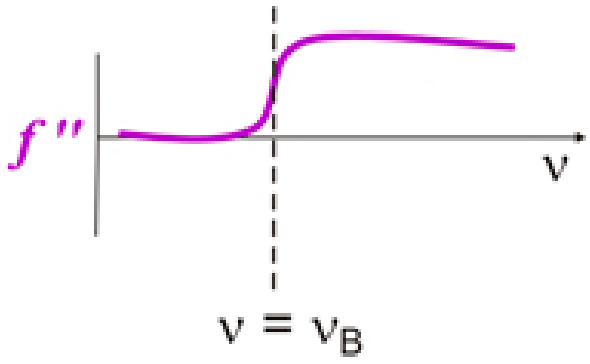
f_o = normal atomic scattering factor (real number)

f' and f'' = real and imaginary correction factors

$$f' = g\left(\frac{\nu_B}{\nu}\right)^2 \log_e \left|\left(\frac{\nu}{\nu_B}\right)^2 - 1\right|$$

$$f'' = g\frac{\pi\nu_B^2}{\nu^2}$$





DIFFRAZIONE ANOMALA

$$f(\lambda) = f^{\circ} + f'(\lambda) + if''(\lambda)$$

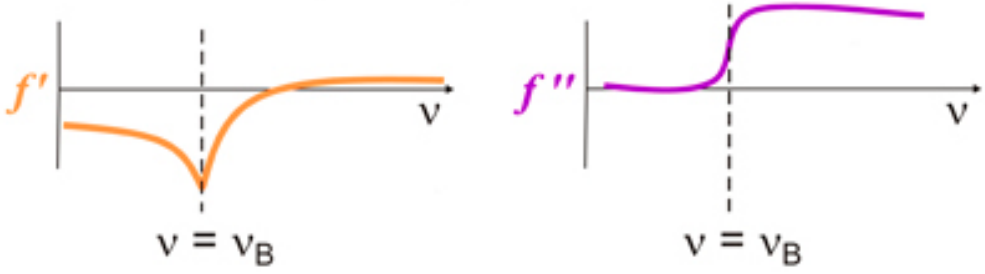
- **A particolari λ (edges, cuspidi)** i termini **f'(λ)** e **f''(λ)** mostrano, rispettivamente, un **minimo** ed un **massimo**
- **Il termine dispersivo [f'(λ)] diminuisce** il fattore di diffrazione normale perché parte dell'energia viene spesa per far avvenire la transizione
- **Il termine di assorbimento [f''(λ)] subisce una variazione di fase di π /2** perché l'energia di eccitazione viene riemessa (diffratta) con un certo ritardo

$$f = f_o + f' + i f''$$

f_o = normal atomic scattering factor (real number)

f' and f'' = real and imaginary correction factors

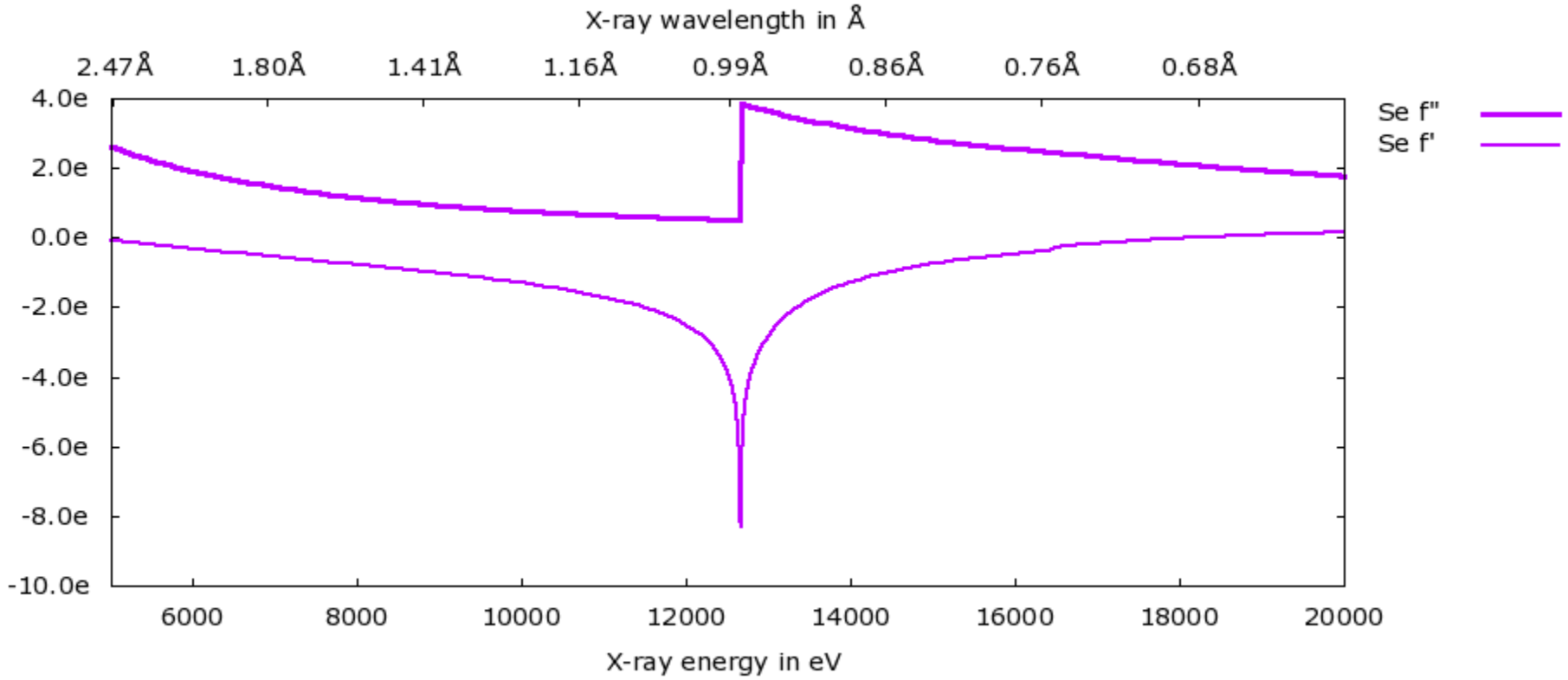
$$f' = g\left(\frac{\nu_B}{\nu}\right)^2 \log_e\left|\left(\frac{\nu}{\nu_B}\right)^2 - 1\right| \qquad f'' = g\frac{\pi\nu_B^2}{\nu^2}$$



DIFFRAZIONE ANOMALA

$$f(\lambda) = f^{\circ} + f'(\lambda) + if''(\lambda)$$

Solitamente si usano Proteine espresse in presenza di **Selenio-Metionine** (automazione)



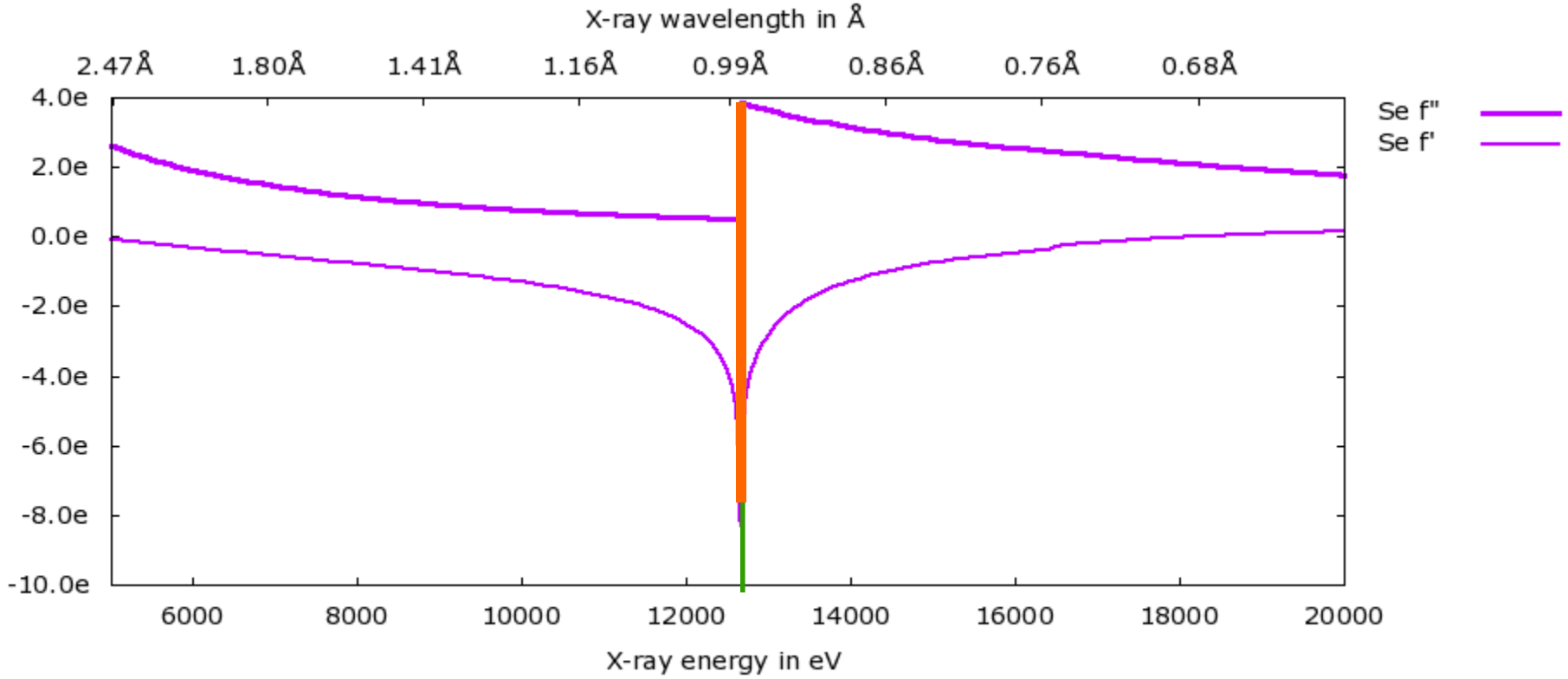
DIFFRAZIONE ANOMALA

$$f(\lambda) = f^{\circ} + f'(\lambda) + if''(\lambda)$$

Single Anomalous Dispersion

SAD

1 raccolte dati a 1 sola lunghezza d'onda (edge)



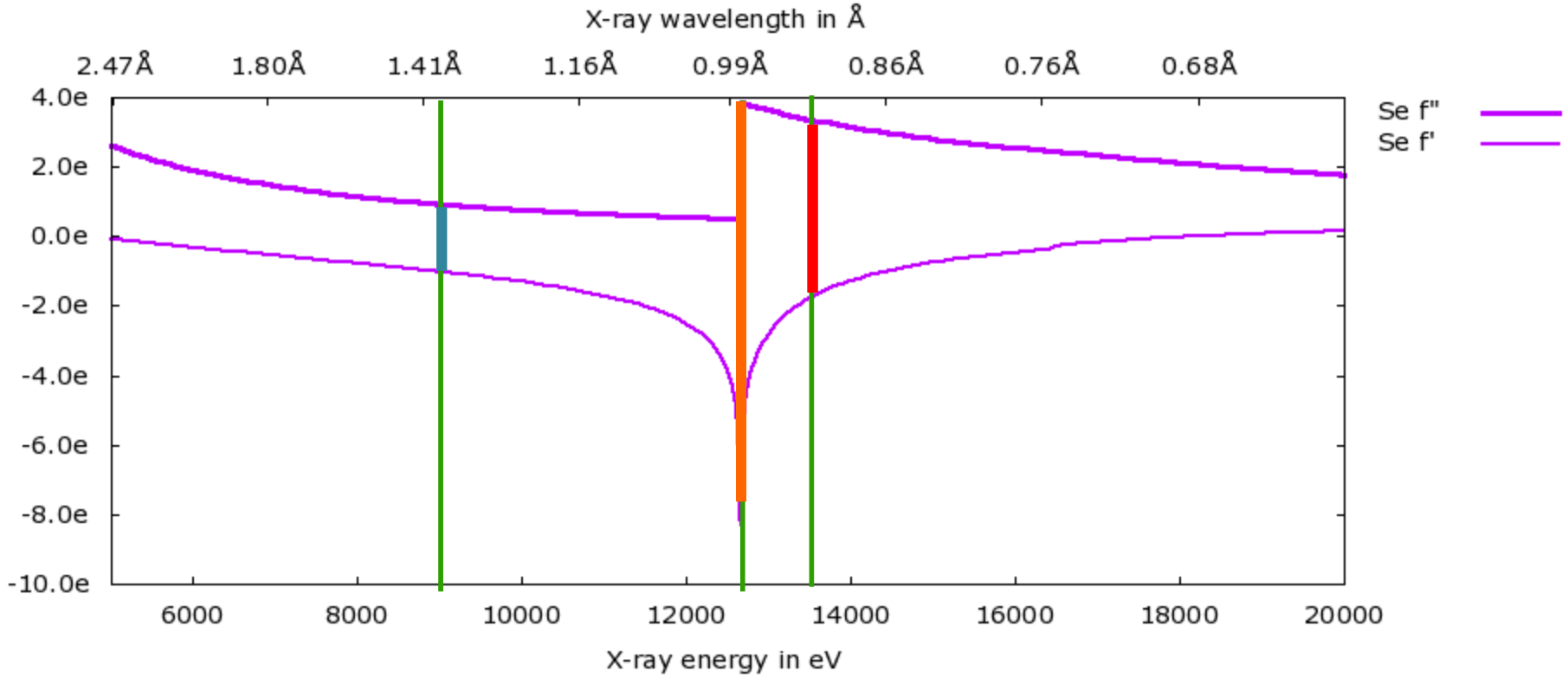
DIFFRAZIONE ANOMALA

$$f(\lambda) = f^{\circ} + f'(\lambda) + if''(\lambda)$$

Multiple Anomalous Dispersion

MAD

3 raccolte dati a 3 diverse lunghezze d'onda



DIFFRAZIONE ANOMALA

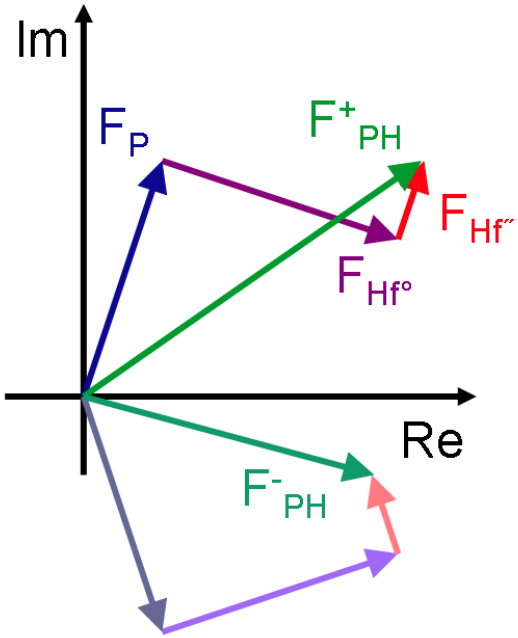
$$f(\lambda) = f^{\circ} + f'(\lambda) + if''(\lambda)$$

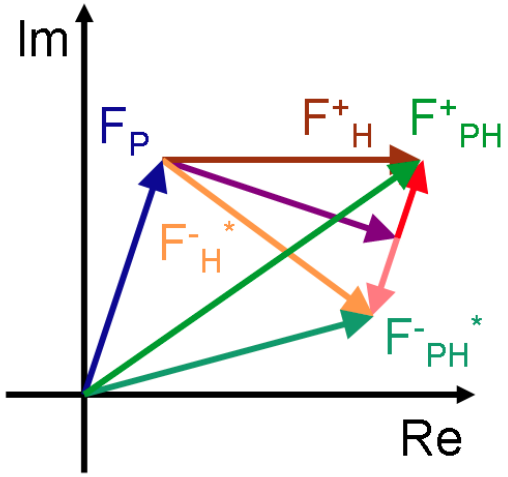
In presenza di diffrazione anomala

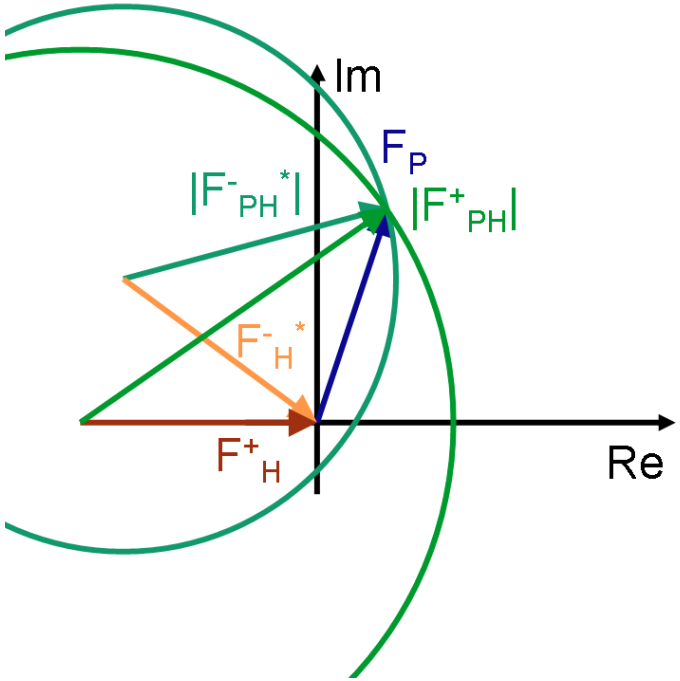
non vale più la legge di Friedel

$$|F^{+}_{PH}| \neq |F^{-}_{PH}|$$

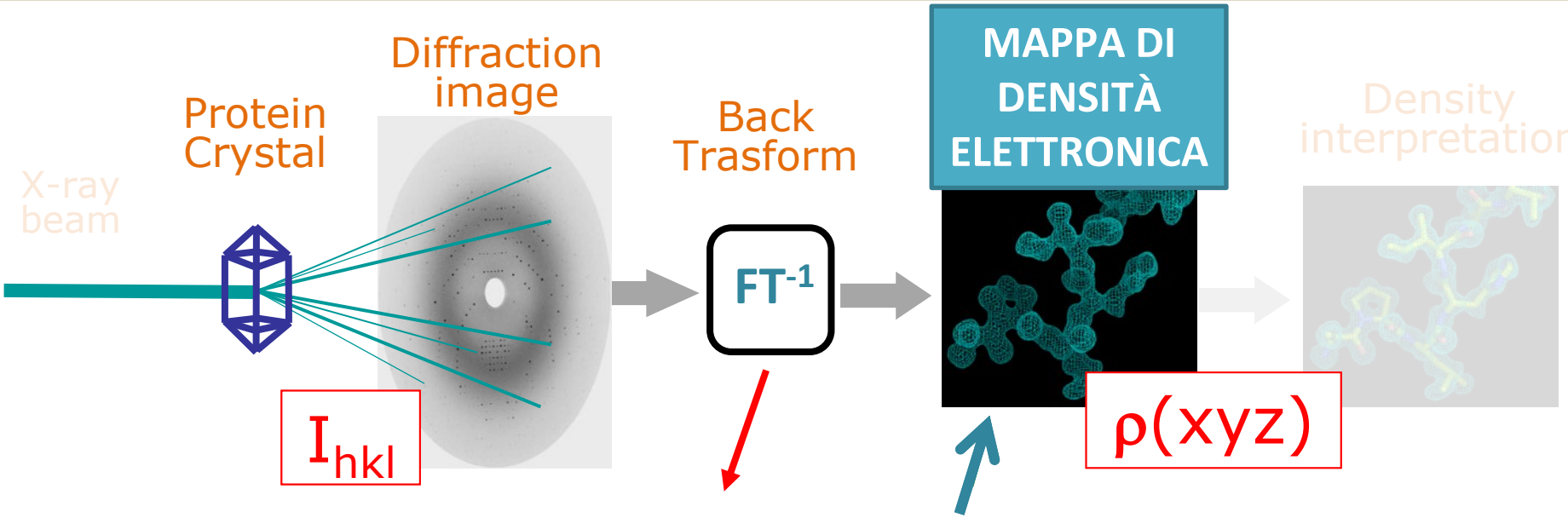
Questa amonalia consente di calcolare la posizione degli atomi pesanti mediante metodi diretti e poi di determinare la fase di F_p







L'ESPERIMENTO DI DIFFRAZIONE



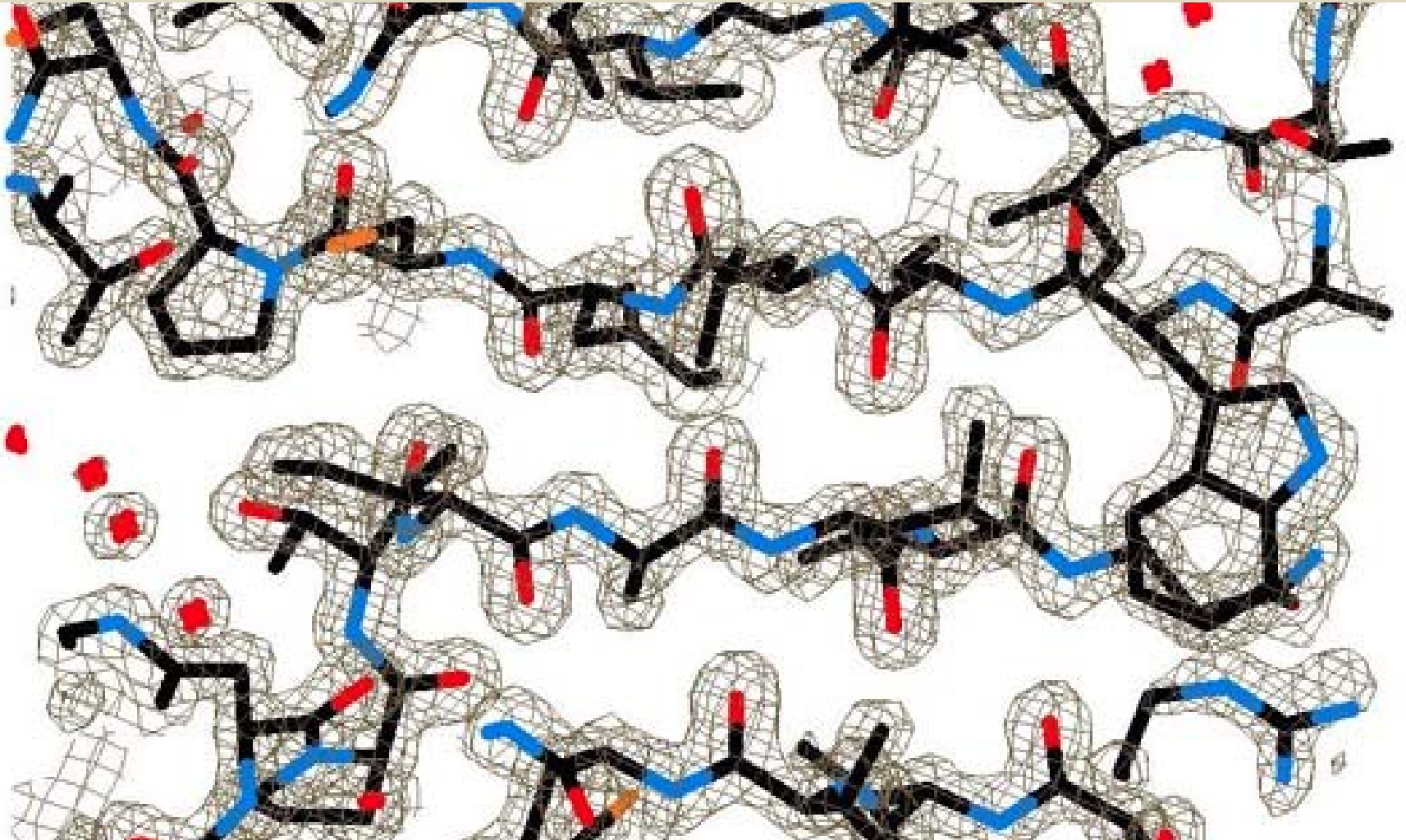
$$\rho(xyz) = 1/V \sum_{hkl} |F_{hkl}| \exp(i\alpha_{hkl}) \exp(-2\pi i\, hx + ky + lz)$$

USO LE AMPIEZZE SPERIMENTALI

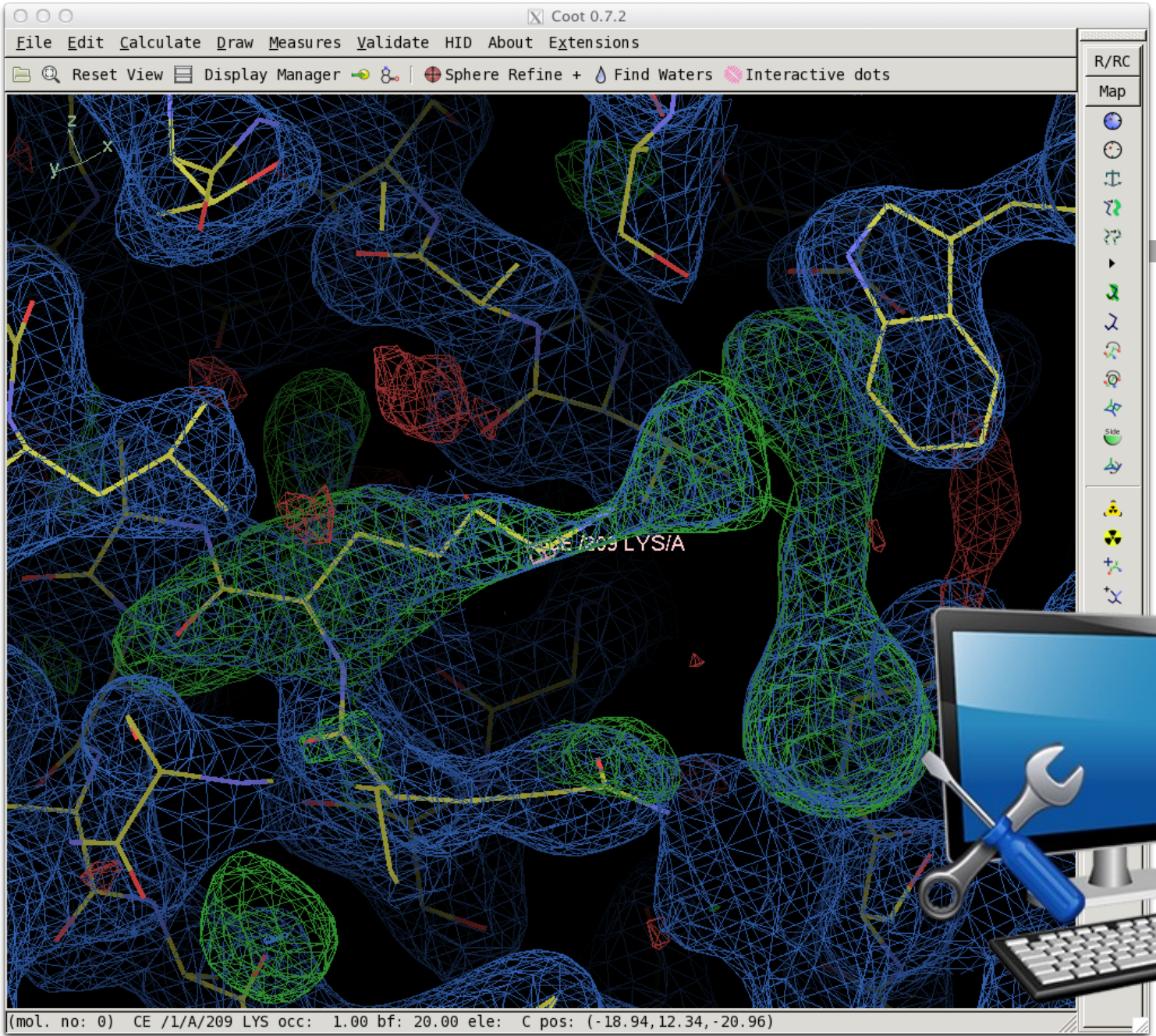
OTTENUTO CON UNO DEI METODI DESCRITTI



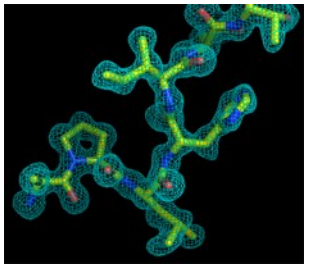
MODEL BUILDING AND REFINEMENT



MODEL BUILDING



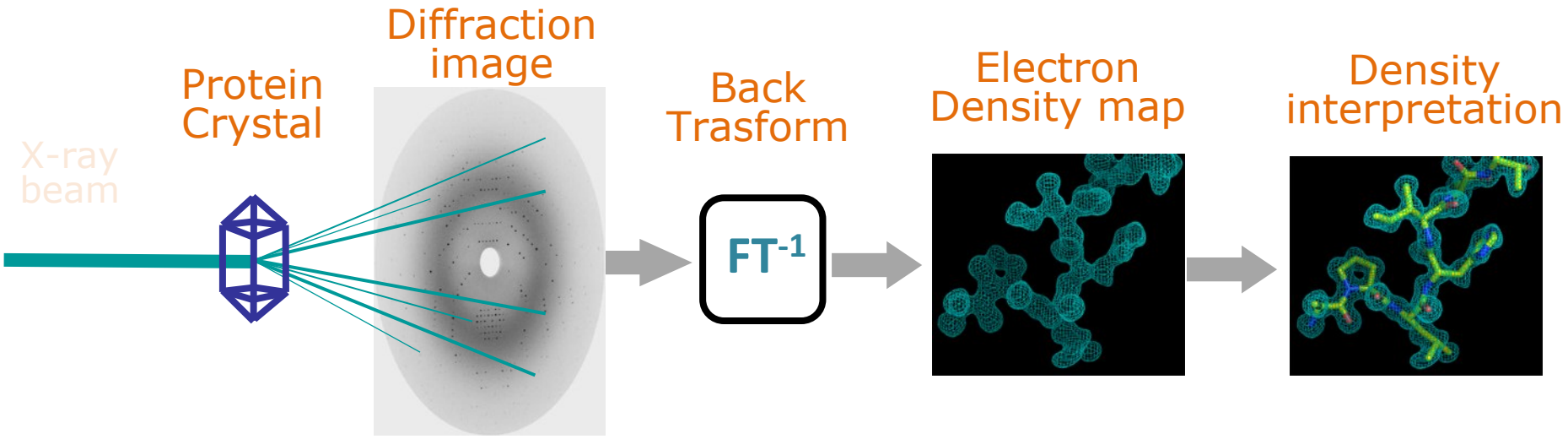
Density interpretation



IS FUN



MODEL BUILDING AND REFINEMENT CYCLE



$$\rho(xyz) = 1/V \sum_{hkl} |F_{hkl}| \exp(i\alpha_{hkl}) \exp(-2\pi i\, hx + ky + lz)$$

$|F_{obs}|$

Amplitudes from experimental data

$|F_{cal}|$

α_{cal}

Phases from model

1. **Build a model and Calculate new maps**
2. **Refine the model and get better maps**



Refinement Cycle

Refinement: Improving the agreement between the model and the experimental density.

Compare F_{obs} (From reflection Intensities) to F_{calc} (Calculated from the model)

Least squares minimization

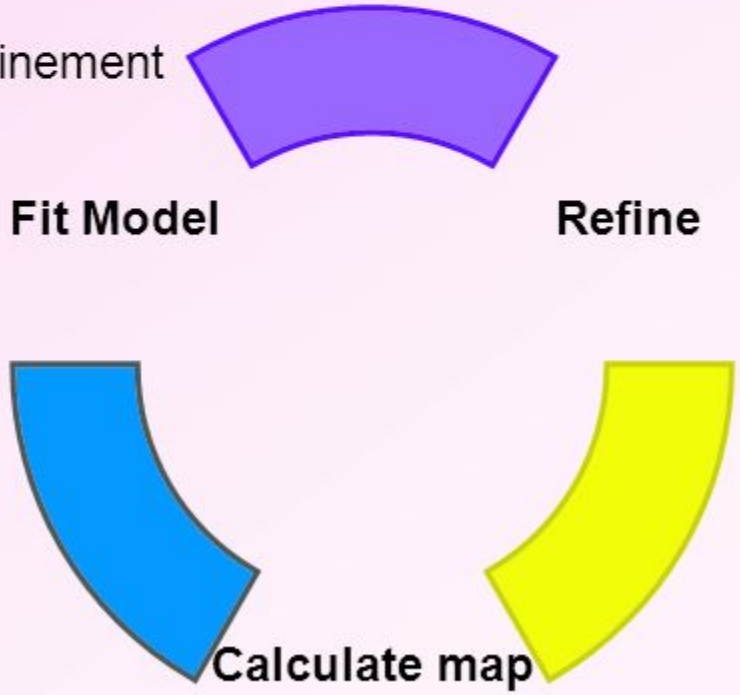
Simulated Annealing / Molecular dynamics

R_{factor} = numerical indicator to follow progress of refinement
agreement between data and model

data model

$$R = \frac{\sum \left| |F_{obs}| - |F_{calc}| \right|}{\sum |F_{obs}|}$$

data



In realtà io calcolo 2 tipi di mappe in cui al posto di $|F_{hkl}|$ metto:

$2|F_{obs}|-|F_{cal}|$ = mappa di densità elettronica (solitamente visualizzata in **blu**)

Oppure

$|F_{obs}|-|F_{cal}|$ = mappa DIFFERENZA (visualizzata in **verde** se > 0, in **rosso** se < 0)

$$\rho(xyz) = 1/V \sum_{hkl} |F_{hkl}| \exp(i\alpha_{hkl}) \exp(-2\pi i\, hx + ky + lz)$$

$|F_{obs}|$

Amplitudes from experimental data

$|F_{cal}|$

α_{cal}

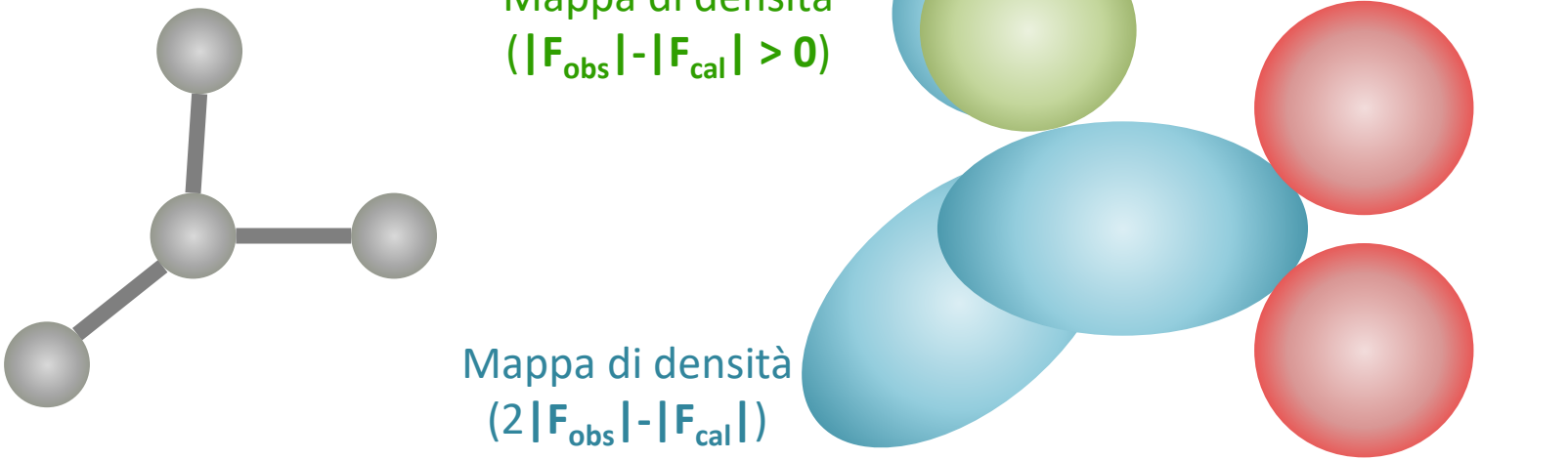
Phases from model

In realtà io calcolo 2 tipi di mappe in cui al posto di $|F_{hkl}|$ metto:

$2|F_{obs}|-|F_{cal}|$ = mappa di densità elettronica (solitamente visualizzata in **blu**)

Oppure

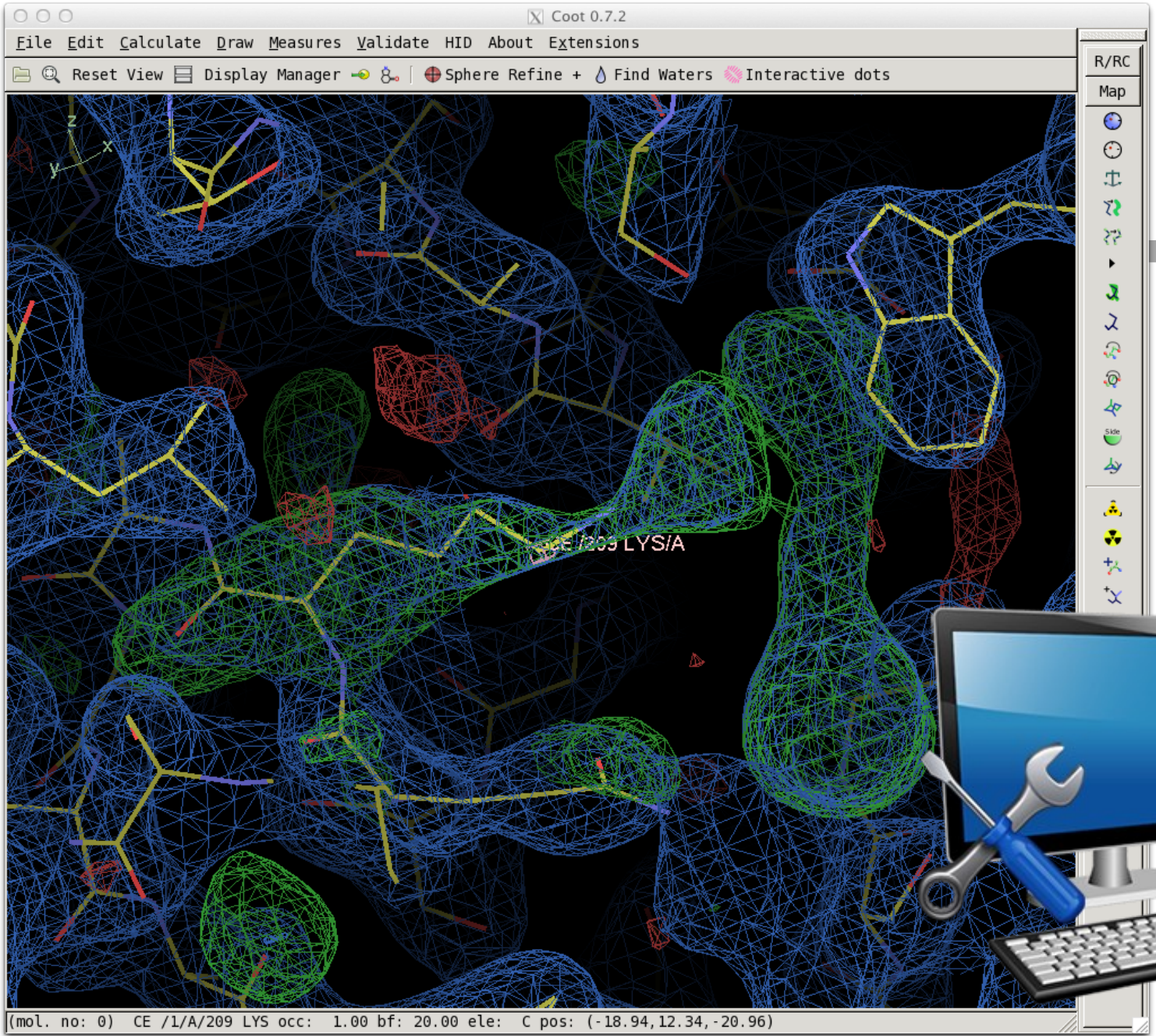
$|F_{obs}|-|F_{cal}|$ = mappa DIFFERENZA (visualizzata in **verde** se > 0, in **rosso** se < 0)



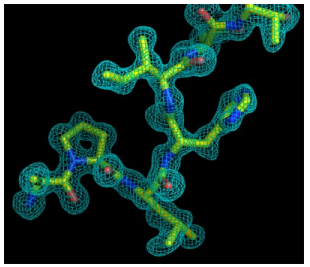
Proteina **$|F_{obs}|$**

Modello **$|F_{cal}|$**

MODEL BUILDING



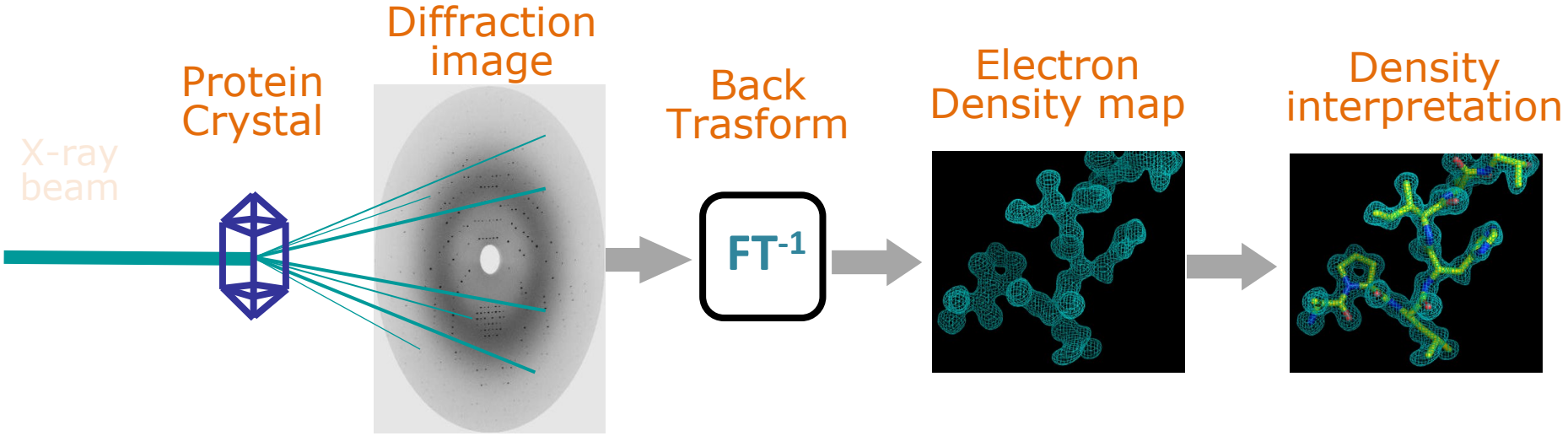
Density interpretation



IS FUN



MODEL BUILDING AND MAPS REFINEMENT



MODEL BUILDING may be done automatically but in most cases is **done by HUMANS.**

1. **Build a model and Calculate new maps**
2. **Refine the model and get better maps**

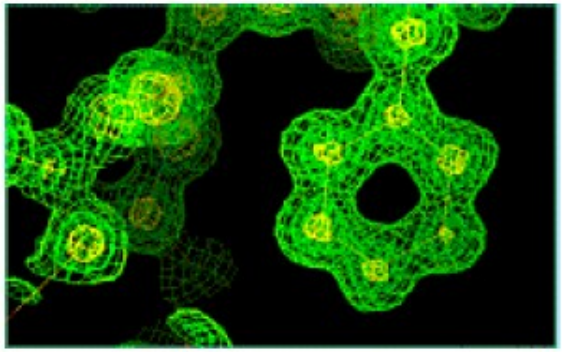




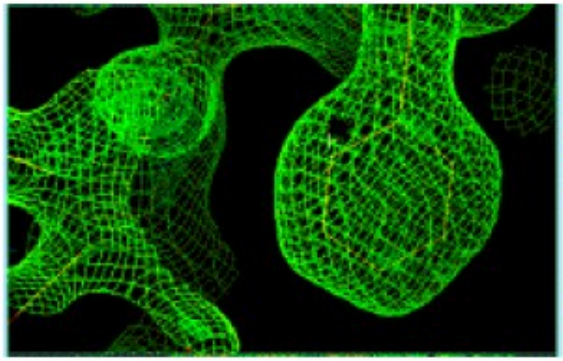
Errors in map interpretation depend on data **resolution** and **quality**

ALTA RISOLUZIONE → MAPPE MIGLIORI

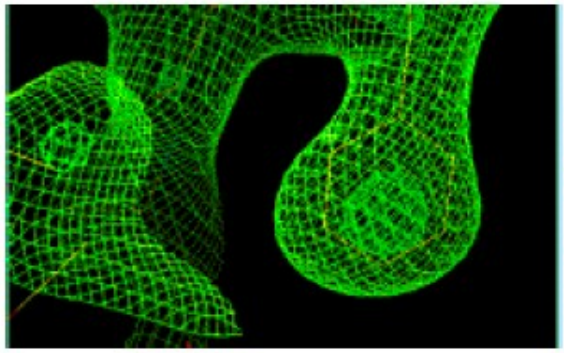
PROTEIN FEATURES	RES. (Å)
Alpha-helix	9
Beta-sheet	4
‘random’ main-chain	3.7
Aromatic side-chains	3.5
Interpretable conformation of side-chains	2.9
Ordered water molecules	2.7
Correct stereochemistry at Cbeta of isoleucine	2.2
Individual atoms	1.5



1.2 Å



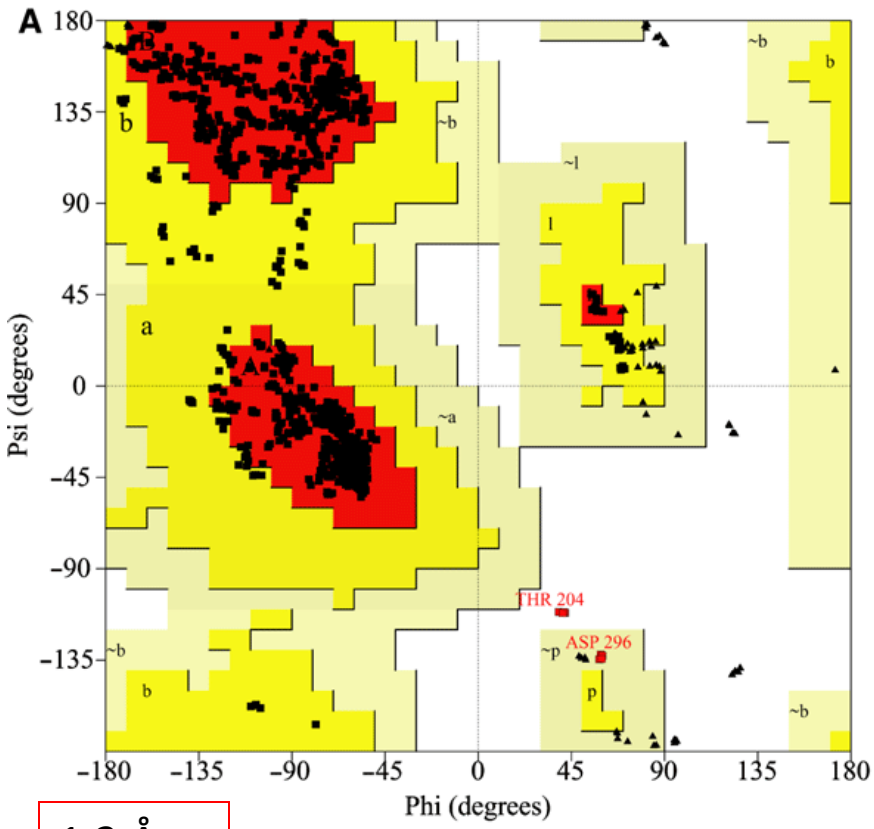
2.0 Å



3.0 Å

VALIDAZIONE DELLA STRUTTURA

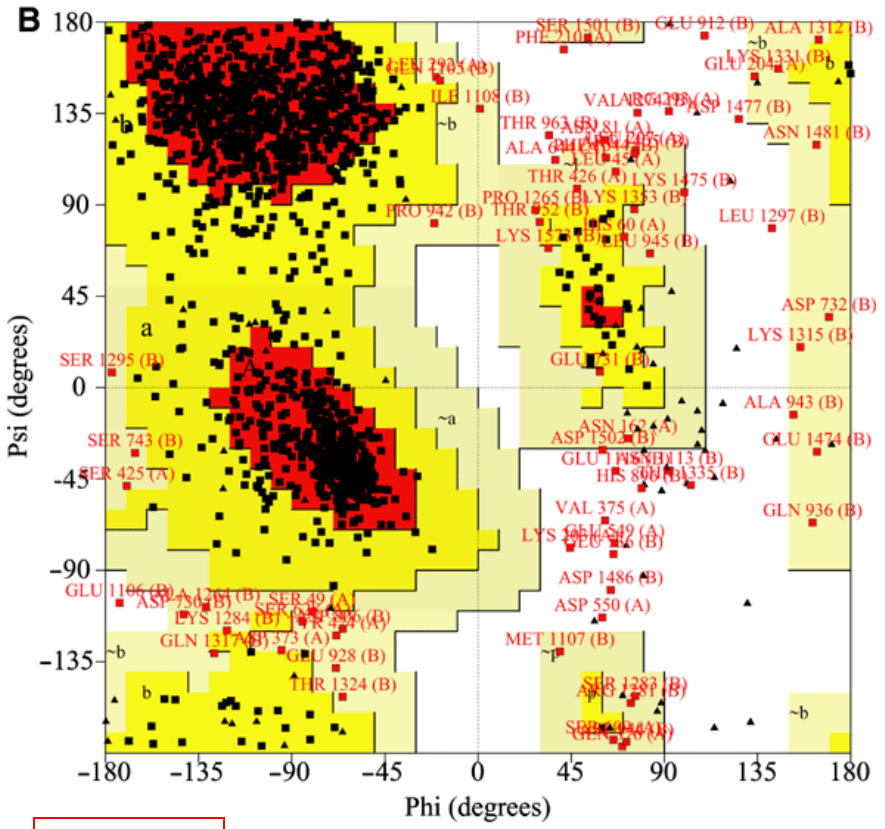
RAMACHANDRAN PLOT



1.2 Å

Plot statistics

Residues in most favoured regions [A,B,L]	1013	90.8%
Residues in additional allowed regions [a,b,l,p]	95	8.5%
Residues in generously allowed regions [~a,~b,~l,~p]	4	0.4%
Residues in disallowed regions	4	0.4%
Number of non-glycine and non-proline residues	1116	100.0%
Number of end-residues (excl. Gly and Pro)	8	
Number of glycine residues (shown as triangles)	128	
Number of proline residues	48	
Total number of residues	1300	



2.2 Å

Plot statistics

Residues in most favoured regions [A,B,L]	1091	79.8%
Residues in additional allowed regions [a,b,l,p]	209	15.3%
Residues in generously allowed regions [~a,~b,~l,~p]	46	3.4%
Residues in disallowed regions	21	1.5%
Number of non-glycine and non-proline residues	1367	100.0%
Number of end-residues (excl. Gly and Pro)	14	
Number of glycine residues (shown as triangles)	90	
Number of proline residues	77	
Total number of residues	1548	

CRISTALLOGRAFIA: THE SUPER SUMMARY

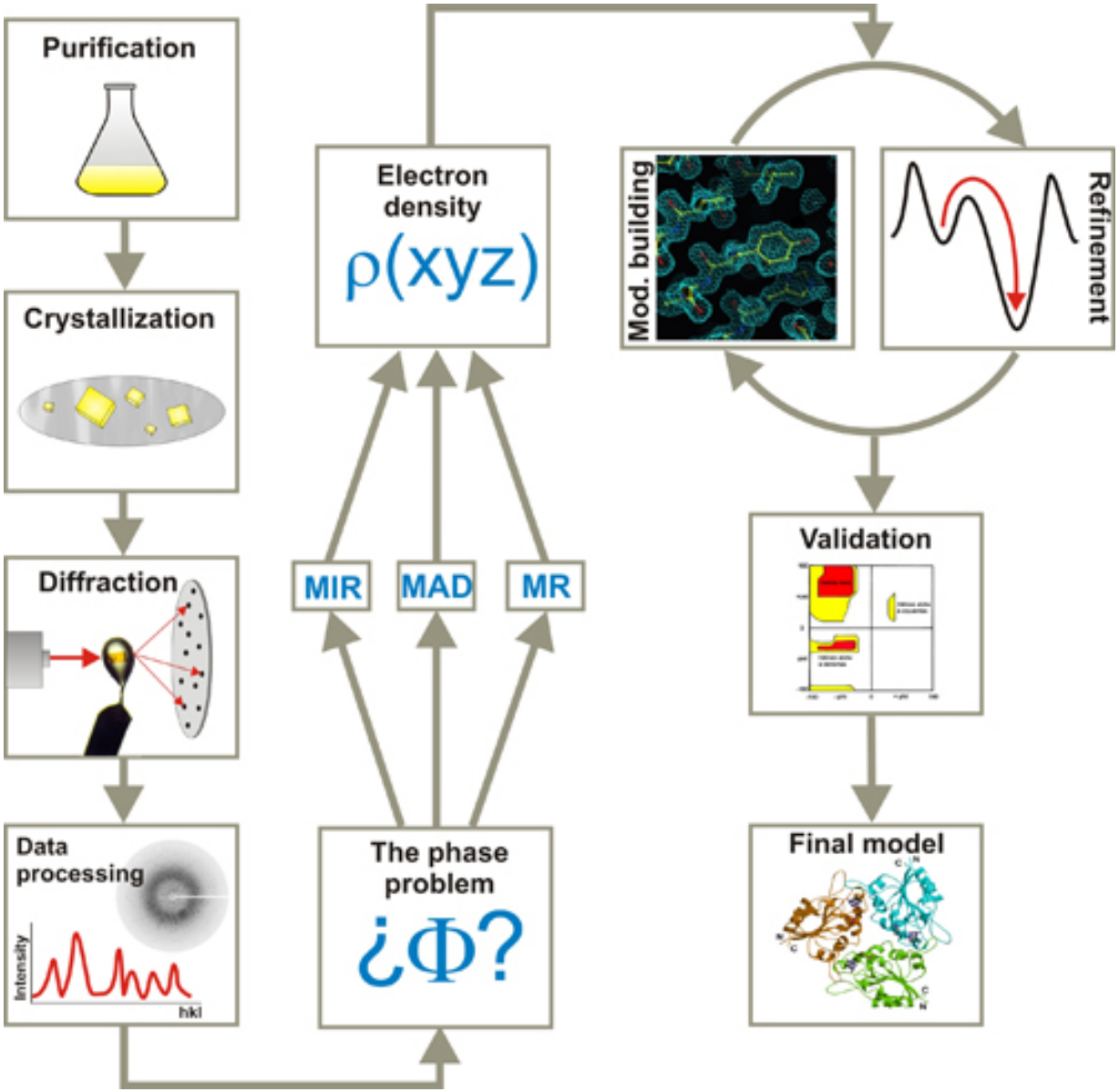


Table 1. Data Collection and Refinement Statistics of Activated PleD in Complex with c-di-GMP and GTPαS

Data Collection	
Space group	$P2_12_12$
Cell dimensions	
a, b, c (Å)	128.9, 132.6, 88.4
Resolution (Å)	30–2.71 (2.85–2.71)
R_{merge} (%)	9.8 (43.6)
$I/\sigma(I)$	10.2 (1.9)
Completeness (%)	95.0 (78.0)
Redundancy	2.9 (2.4)
Refinement	
Number of reflections	39,043
R_{work}/R_{free}	21.7/25.4
Number of atoms	
Protein	7,012
Ligands	272
Water	14
B factors (Å^2)	
Protein	41.0
Ligands	43.7
Water	29.2
Rmsds	
Bond lengths (Å)	0.011
Bond angles (°)	1.5

Data in parentheses belong to the outer resolution shell.

QUALITÁ
DATI SPERIMENTALI


Structure
Article
Cell
Structure of BeF_3^--Modified Response Regulator PleD: Implications for Diguanylate Cyclase Activation, Catalysis, and Feedback Inhibition
PAPER

QUALITÁ
AFFIDABILITÁ
MODELLO

QUALITÁ **DATI SPERIMENTALI**

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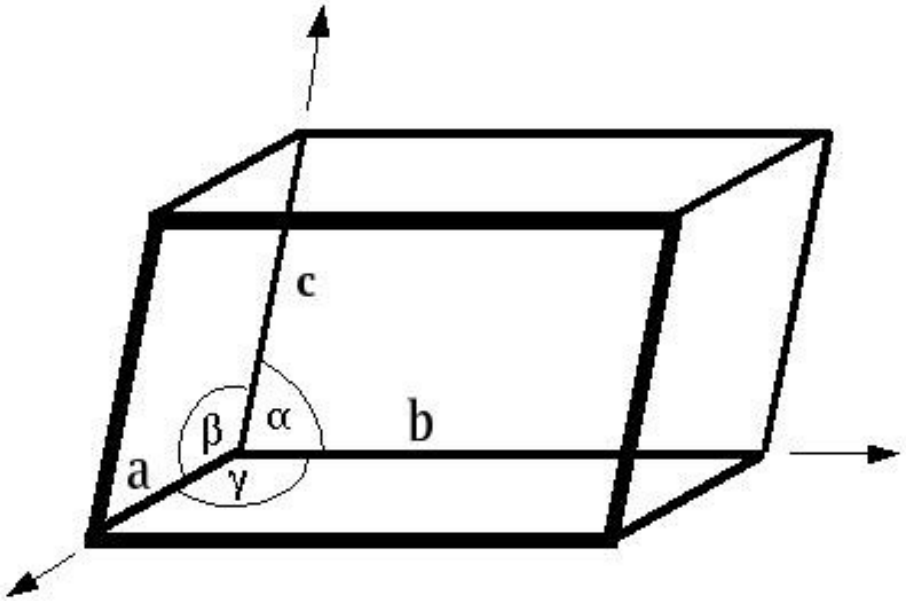


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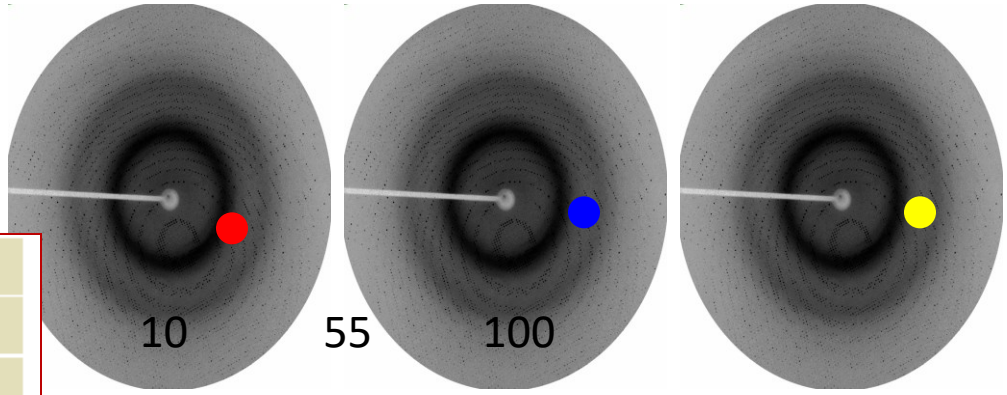
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Data in parentheses belong to the outer resolution shell.



$I_1(234)$ $I_2(234)$ $I_3(234)$

$$R_{merge} = \frac{\sum_{hkl}\sum_{i=1}^{n}\left|I(hkl) - I_i(hkl)\right|}{\sum_{hkl} N \times I(hkl)}$$

QUALITÁ e AFFIDABILITÁ del **MODELLO**

Table 1. Data Collection and Refinement Statistics of Activated PleD in Complex with c-di-GMP and GTPαS

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a, b, c (Å)	
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Ligands	43.7
Water	29.2
Rmsds	
Bond lengths (Å)	0.011
Bond angles (°)	1.5

Data in parentheses belong to the outer resolution shell.

$$\rho(xyz) = 1/V \sum_{hkl} |F_{hkl}| \exp(i\alpha_{hkl}) \exp(-2\pi i\, hx + ky + lz)$$

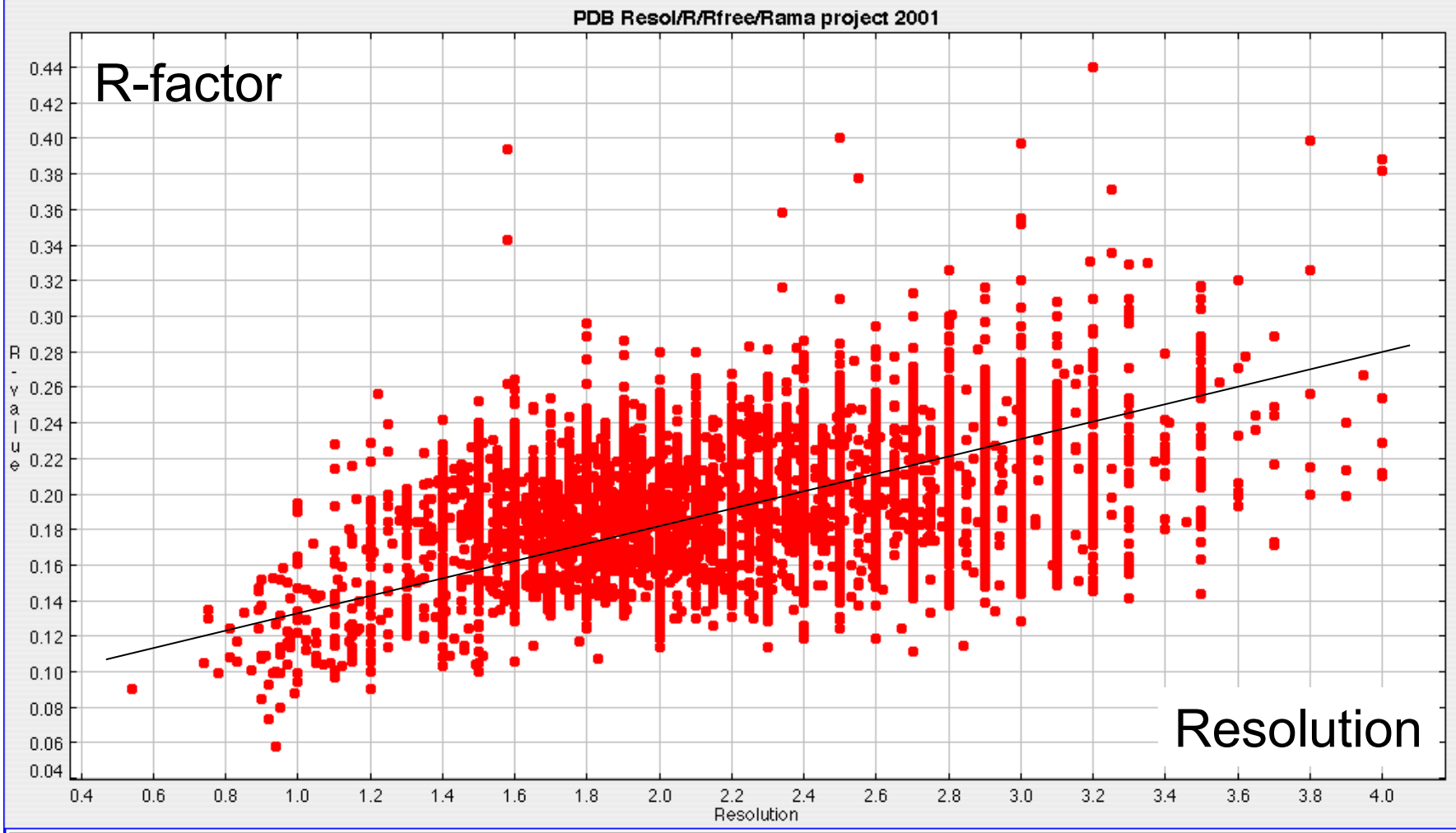
$|F_{obs}|$ DATI sperimentali

$|F_{cal}|$ α_{cal} MODELLO

$$R_F = \frac{\sum_{hkl} \left| |F_o(hkl)| - |F_c(hkl)| \right|}{\sum_{hkl} |F_o(hkl)|}$$

Harry Plotter

File Plotter Special



X-parameter: Resolution

Y-parameter: R-value

Plottype: Scatter-plot

Version:

Marks:

Enable links:

Harry Plotter v.1.0

dots

Plotter

Reset

Clear

Help Edit Print

Current Mousepointer Position:

Resolution: 1,8759

R-value: 0,0535

Changed marks to: dots

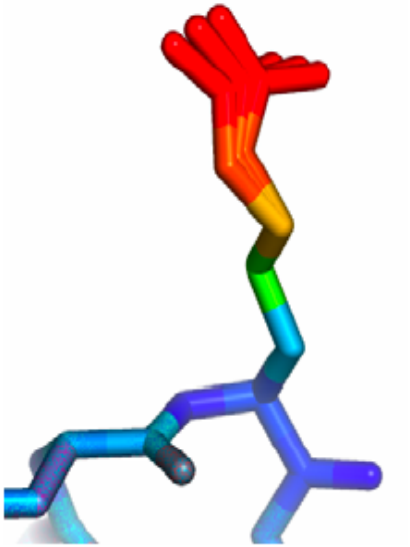
QUALITÁ e AFFIDABILITÁ del **MODELLO**

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Temperature Factor is a measure of motion or disorder



Colour atoms by B-factor

low amount of motion
high amount of order
low B-factor (5-10)



high amount of motion
low amount of order
high B-factor (50-100)

PDB ENTRY

RCSB PDB PROTEIN DATA BANK An Information Portal to 124286 Biological Macromolecular Structures

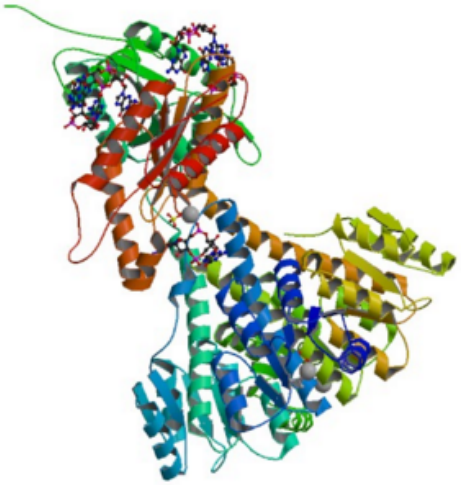
Search by PDB ID, author, macromolecule, sequence, or ligands | Go

Advanced Search | Browse by Annotations | Search History (1) | Previous Results (4)

PDB-101 | Worldwide PDB | EMDataBank | NDB Nucleic Acid Database | StructuralBiology Knowledgebase | Worldwide Protein Data Bank Foundation

Structure Summary | 3D View | Annotations | Sequence | Sequence Similarity | Structure Similarity | Experiment | Literature

Biological Assembly 1



View in 3D: NGL or JSmol or PV (in Browser)

Standalone Viewers

Simple Viewer Protein Workshop
Ligand Explorer Kiosk Viewer

Display Files | Download Files

2WB4

ACTIVATED DIGUANYLATE CYCLASE PLED IN COMPLEX WITH C-DI-GMP

DOI: 10.2210/pdb2wb4/pdb

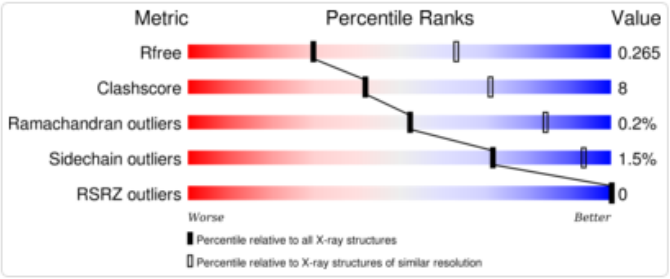
Classification: TRANSFERASE
Deposited: 2009-02-20 **Released**: 2010-04-21
Deposition author(s): Wassmann, P., Schirmer, T.
Organism: Caulobacter vibrioides
Expression System: ESCHERICHIA COLI

Structural Biology Knowledgebase: 2WB4 (52 models >21 annotations) SBKB.org

Experimental Data Snapshot

Method: X-RAY DIFFRACTION
Resolution: 2.8 Å
R-Value Free: 0.268
R-Value Work: 0.239

wwPDB Validation 3D Report | Full Report



PDB ENTRY

Sequence | Sequence Similarity | Structure Similarity | Experiment | Literature

Display Files | Download Files

2WB4

ACTIVATED DIGUANYLATE CYCLASE PLED IN COMPLEX WITH

DOI: 10.2210/pdb2wb4/pdb

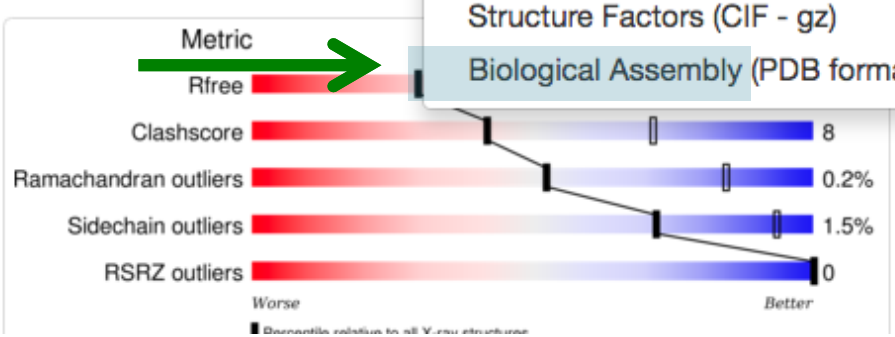
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wwPDB Validation



FASTA Sequence

PDB Format
PDB Format (gz)

PDBx/mmCIF Format
PDBx/mmCIF Format (gz)

PDBML/XML Format (gz)

Structure Factors (CIF)
Structure Factors (CIF - gz)
Biological Assembly (PDB format - gz) (A+S)

PDB ENTRY

STRUCTURE QUALITY AT A GLANCE

2WB4

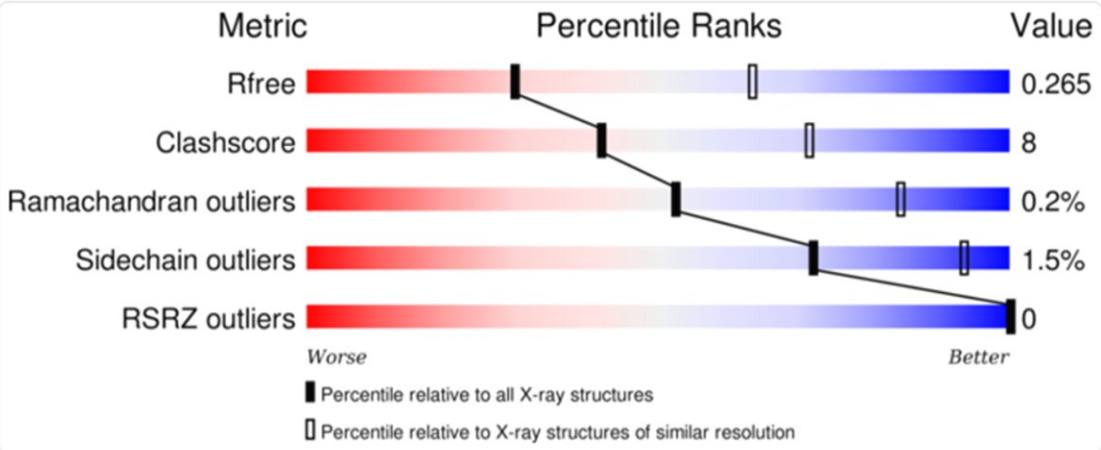
ACTIVATED DIGUANYLATE CYCLASE PLED IN COMPLEX WITH C-DI-GMP

Experimental Data Snapshot

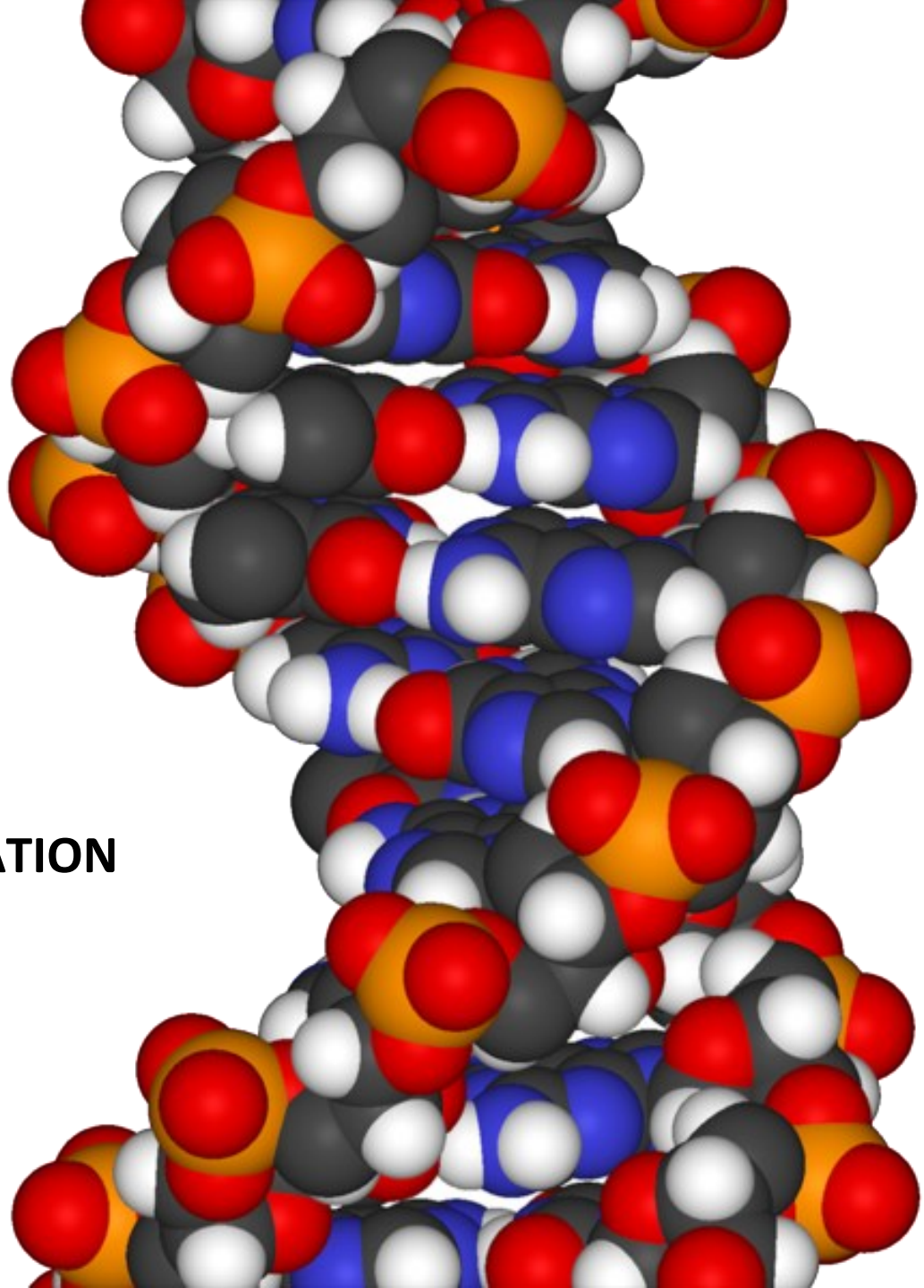
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wwPDB Validation

3D Report | Full Report

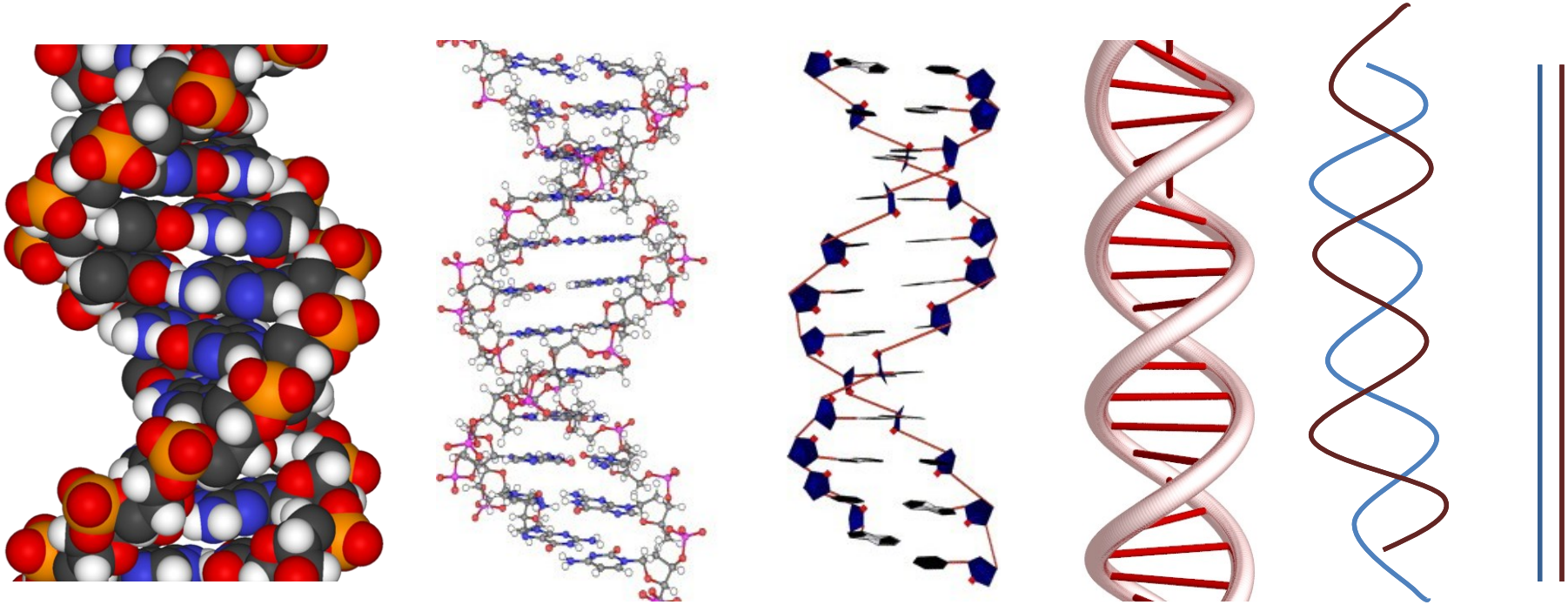


STRUCTURE VISUALIZATION



Structure Visualization

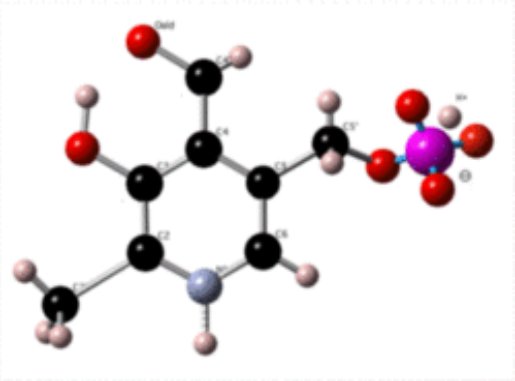
Models and Conventions



STRUCTURE VISUALIZATION

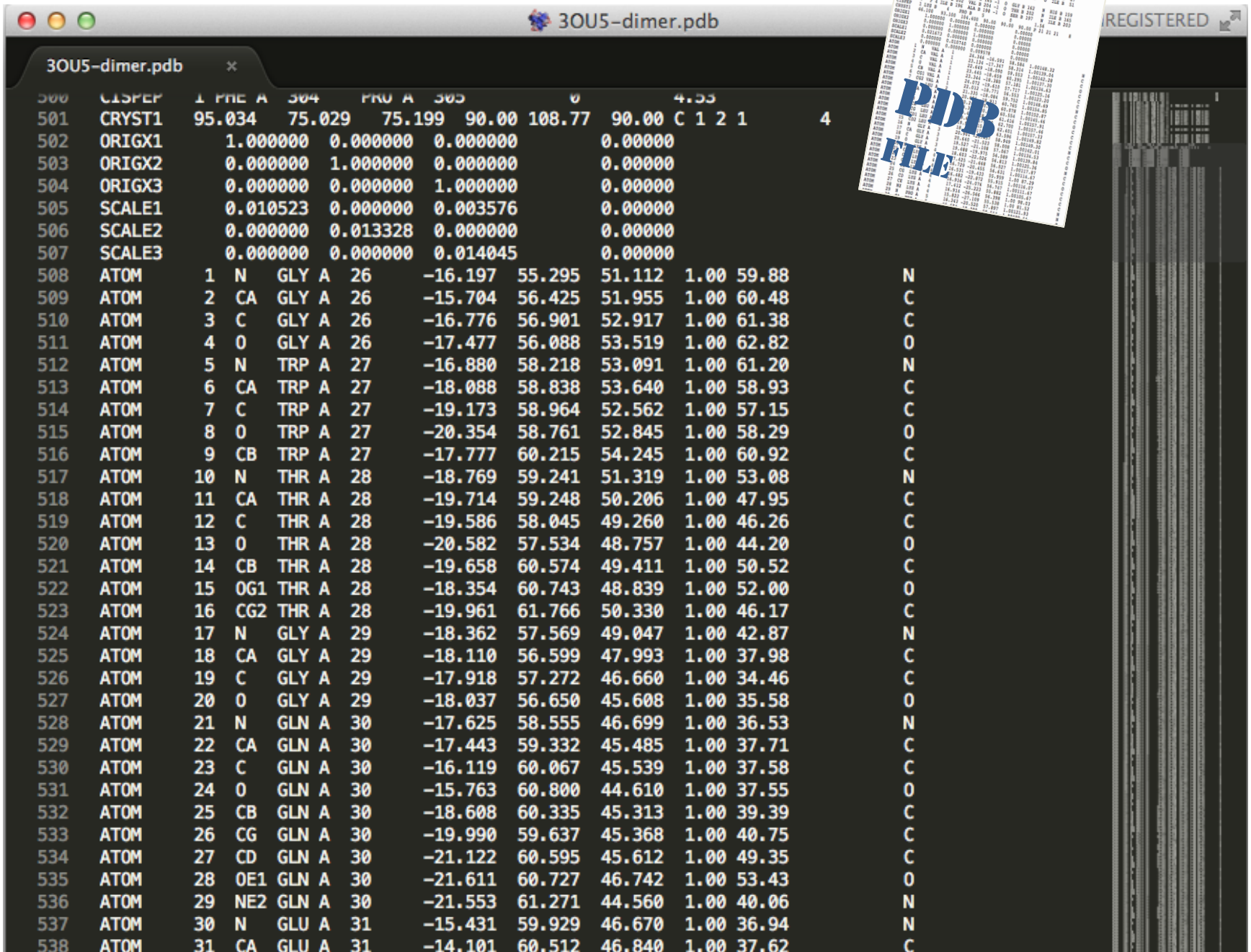
.

Structure file:
Is a text file containing
3 coordinates (x,y,z) per atom,
And information on chemical nature and connectivity



-4.4457	-1.0032	0.4113	O
-2.4005	-1.9114	0.0531	O
3.9024	-0.4411	0.0362	C
3.1486	0.8194	-0.4313	C
1.6528	0.7393	-0.2472	C
-1.0946	0.5935	0.0901	C
-2.5728	0.5152	0.2719	C
3.6076	-0.7760	1.5005	C
3.5239	-1.6299	-0.8505	C
0.8690	0.2281	-1.2695	C
1.0897	1.1791	0.9405	C
-0.5135	0.1549	-1.0998	C
-0.2929	1.1057	1.1102	C
-3.3736	1.3495	-0.7486	C
-3.1004	-0.9233	0.2332	C
4.9775	-0.2527	-0.0735	H
3.3789	1.0242	-1.4854	H
3.5298	1.6907	0.1182	H

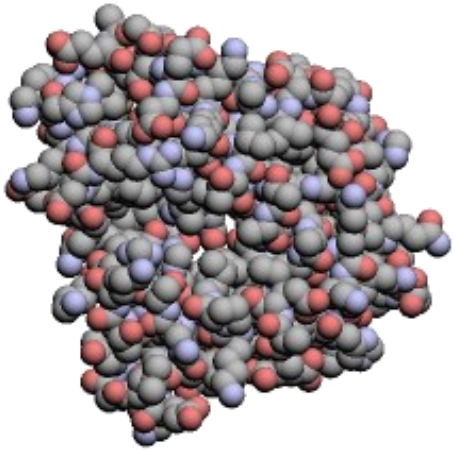
STRUCTURE VISUALIZATION



STRUCTURE VISUALIZATION = MOLECULAR GRAPHICS SOFTWARES

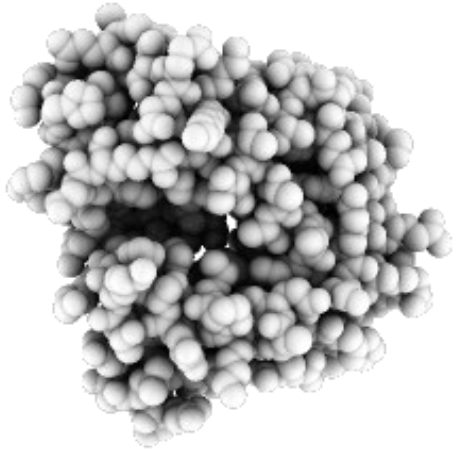
We can use the same RENDERING of non scientific 3D softwares to VISUALIZE our structure

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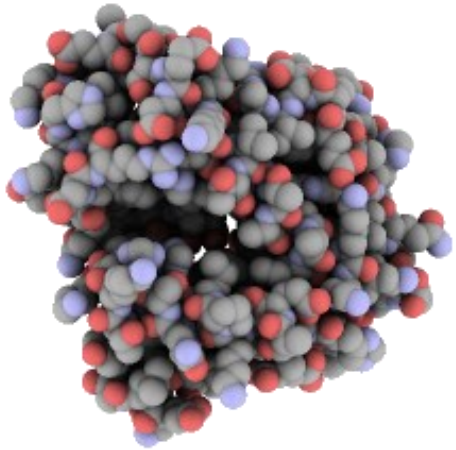
Illuminazione classica

+



Ambient occlusion

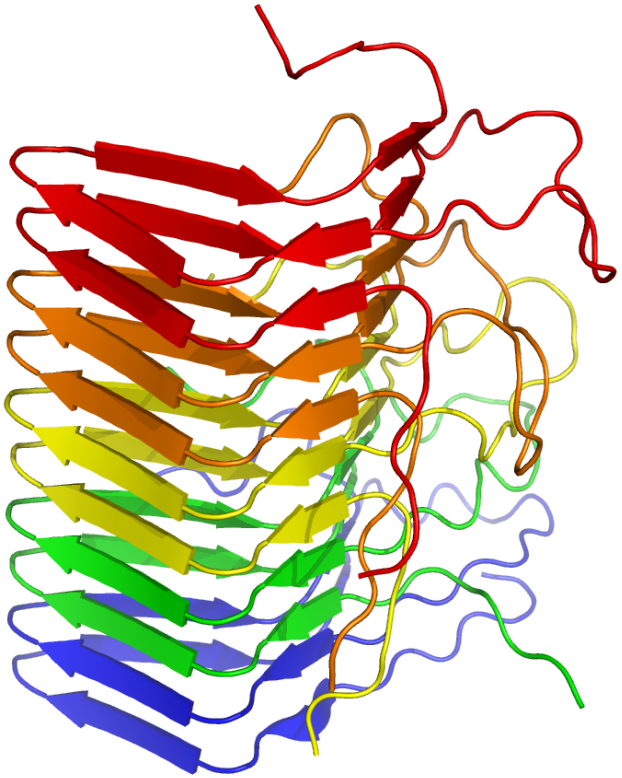
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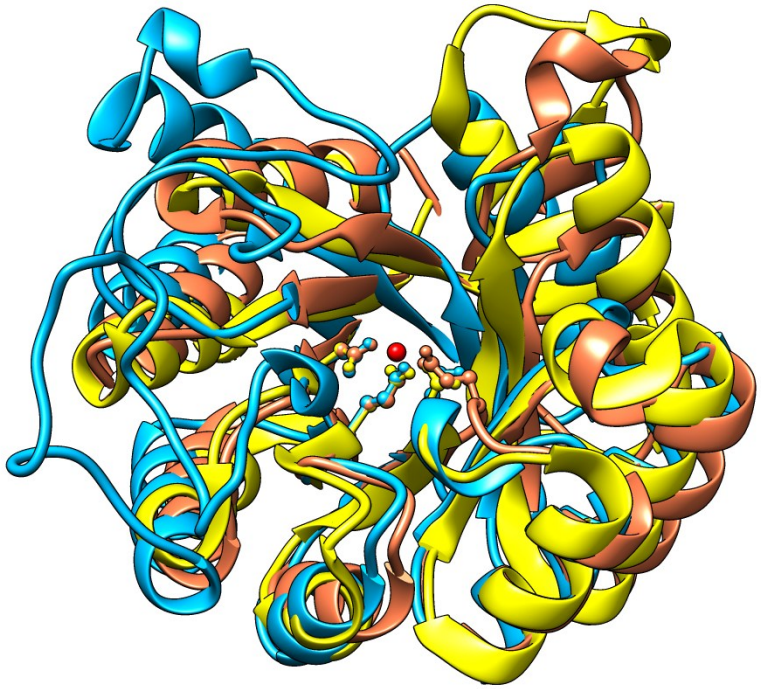
Migliore percezione della forma

STRUCTURE VISUALIZATION = MOLECULAR GRAPHIC SOFTWARES

PyMol



CHIMERA



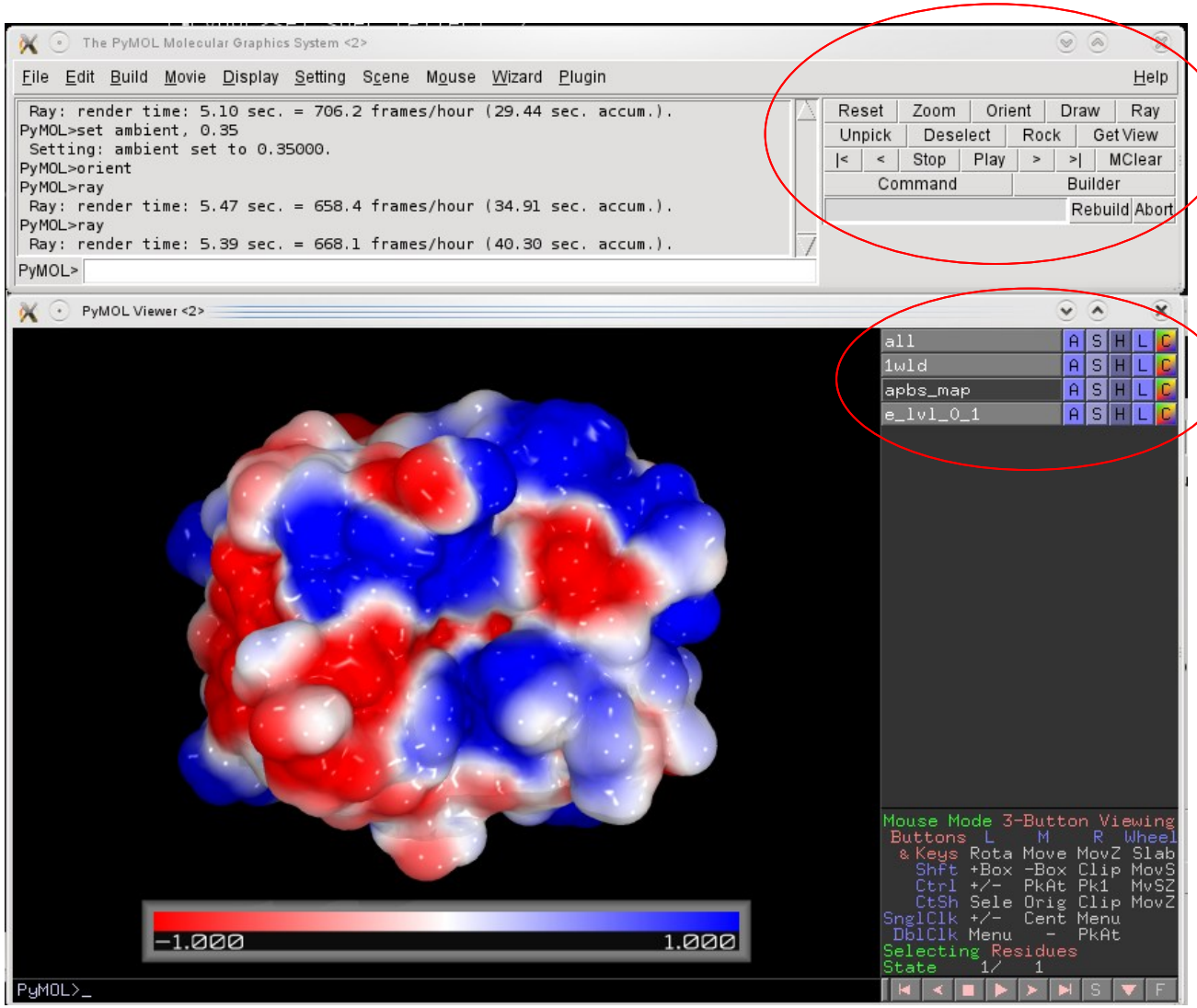
But also CueMol VMD QuteMol

STRUCTURE VISUALIZATION = MOLECULAR GRAPHIC SOFTWARES

PyMol

codice

Viewport



Fast keys

Molecole Caricate

A= *actions*
S=*Show*
H=*hide*
L=*label*
C=*color*

STRUCTURE VISUALIZATION = MOLECULAR GRAPHIC SOFTWARES

CHIMERA

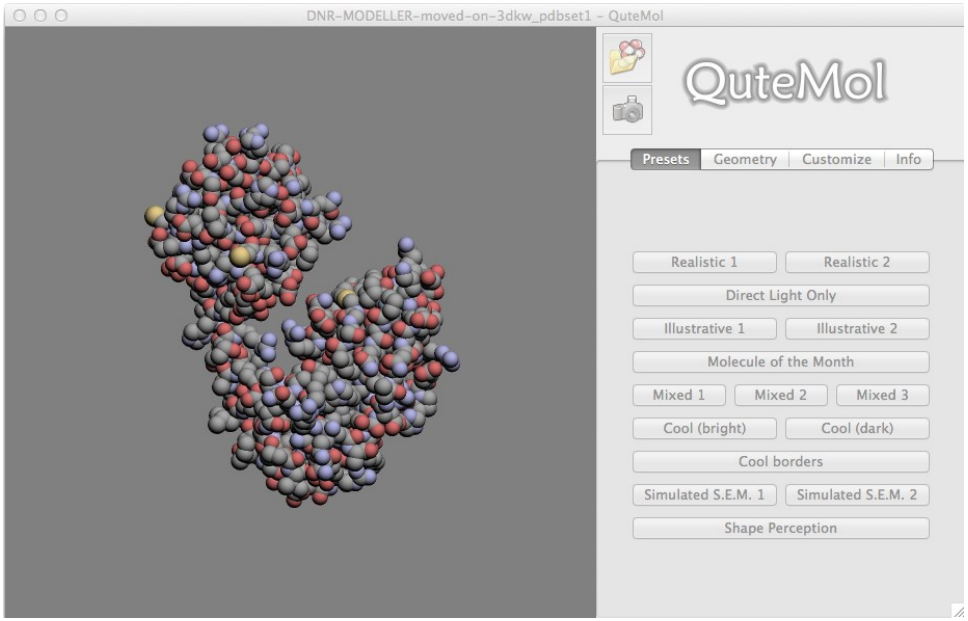
FREE

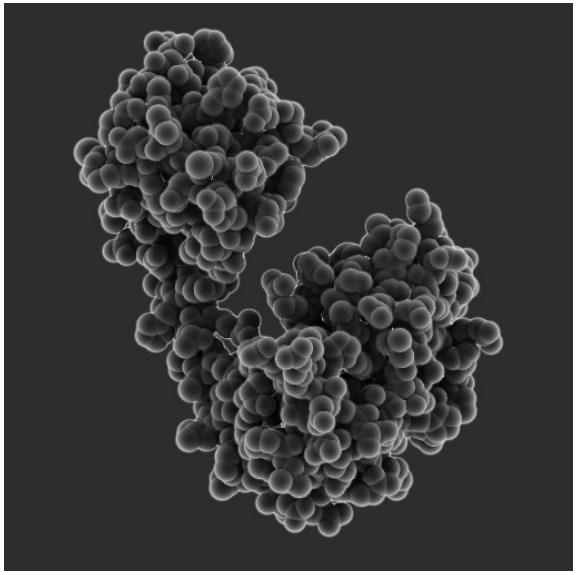


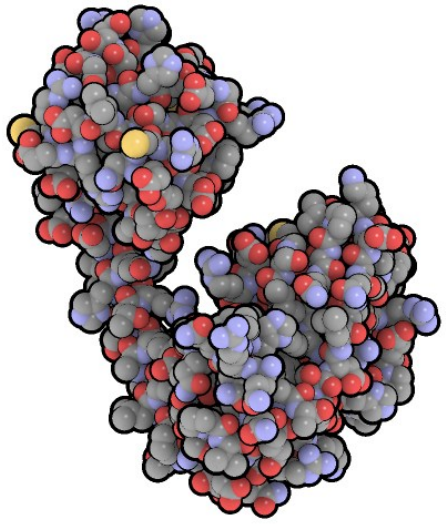
http://www.cgl.ucsf.edu/chimera/download.html

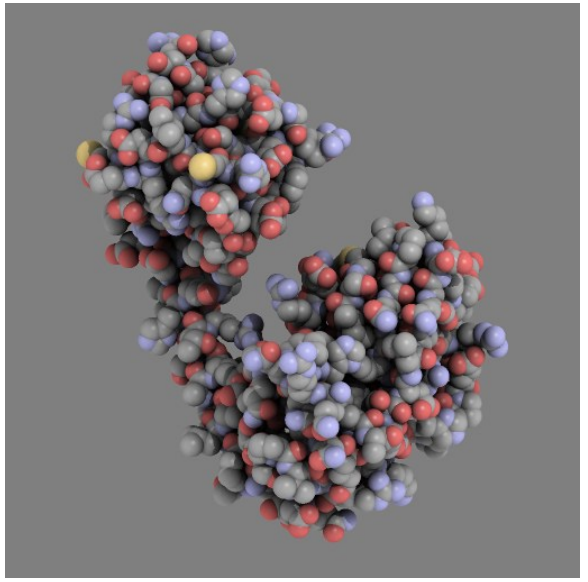
STRUCTURE VISUALIZATION = MOLECULAR GRAPHIC SOFTWARES

QuteMol

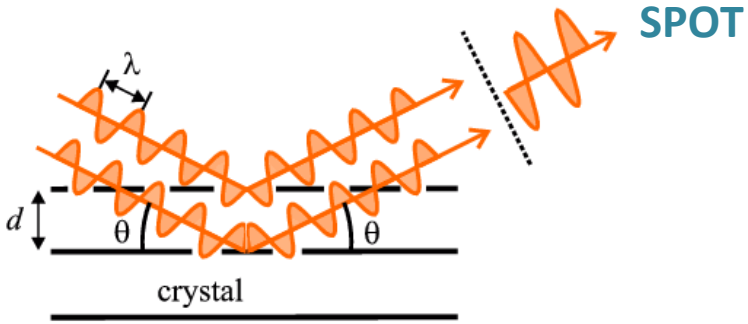






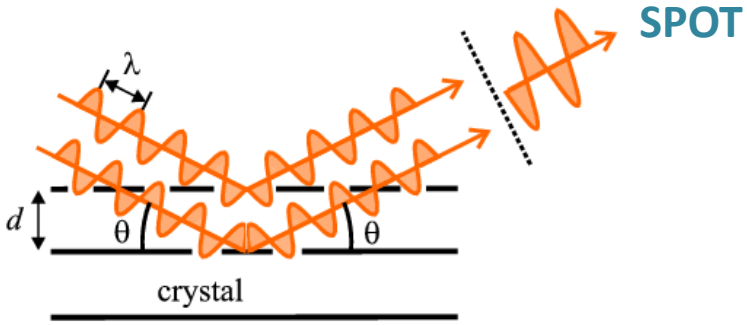


ORDER VS DISORDER





Order Vs Disorder



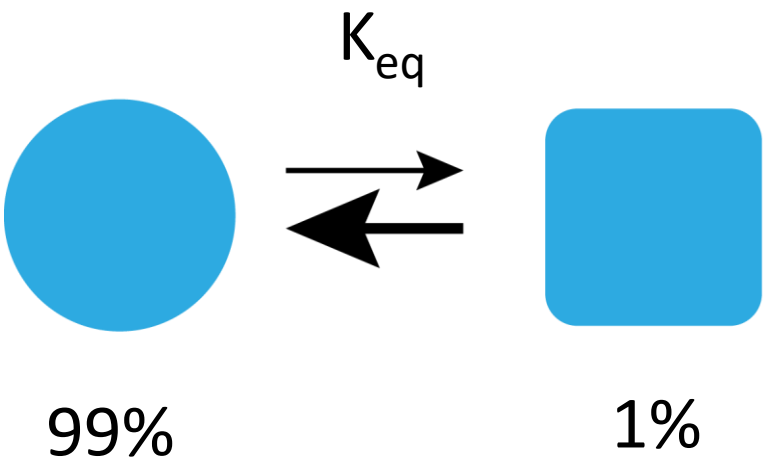
No Order, No Density!



We miss Dynamic parts!

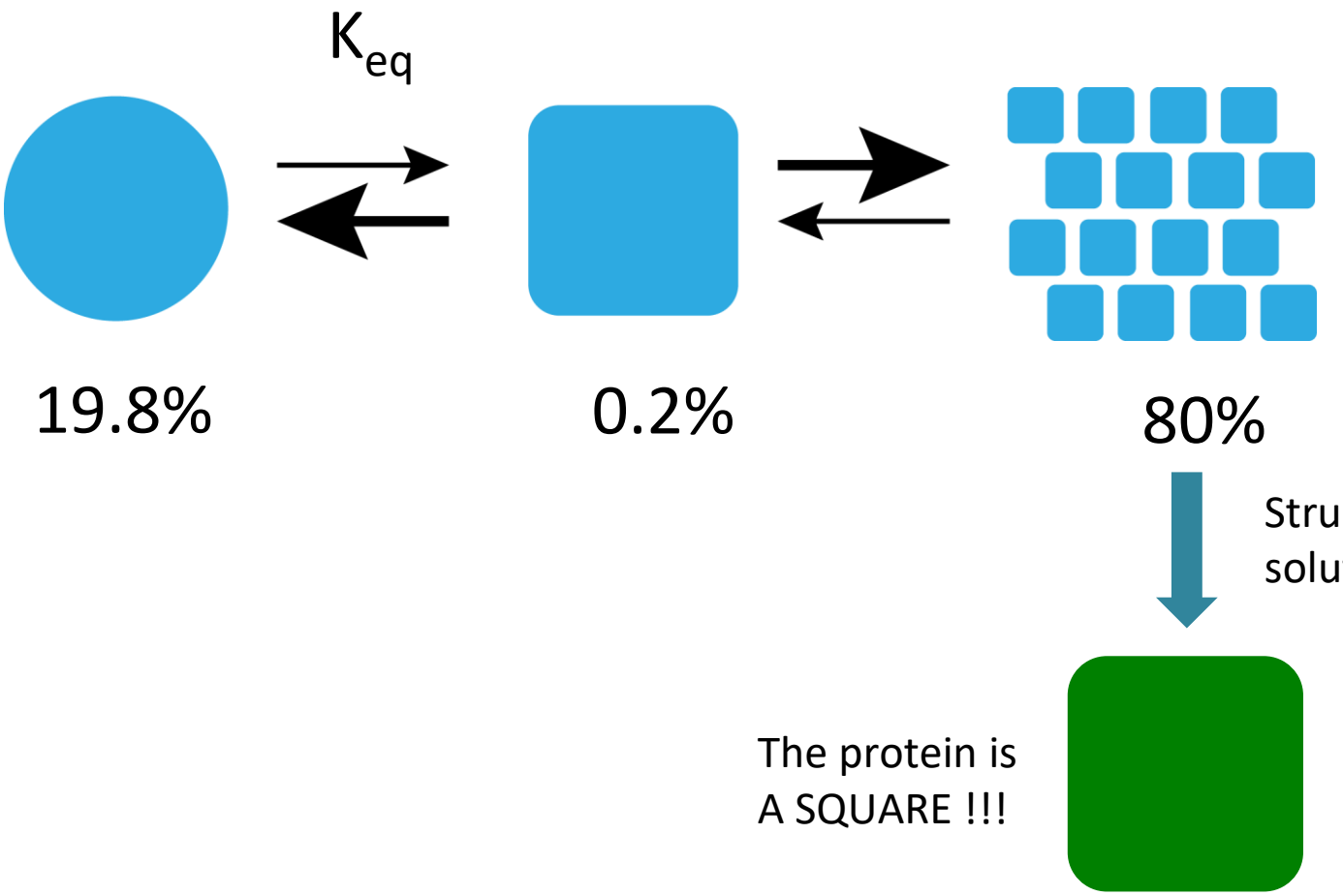
Structure Analysis – common errors

The conformation observed in the crystal structure is
ONE POSSIBLE conformation and not THE conformation of the protein



STRUCTURE ANALYSIS – COMMON ERRORS

The conformation observed in the crystal structure is
ONE POSSIBLE conformation and not THE conformation of the protein



VANTAGGI



- **Risoluzione atomica**
- Non ci sono limitazioni nelle dimensioni delle macromolecole

SVANTAGGI



- **Cristalli singoli**
- Altamente ordinati
- ragionevolmente grandi (30-100 µm)