






Difracción de Rayos X de Proteínas

1. **Refinamiento, modelado y validación estructural.**
2. Simetría. **estructural.**
3.  Mejora de las fases.
Cristales y su obtención.
4. Difracción de Rayos X.
 Mapa de densidad electrónica y resolución.
5. Aspectos experimentales: equipos de difracción y procesamiento de datos.
 Construcción del modelo.
6. El Problema de la Fase: métodos de **resolución estructural.**
 Refinamiento.
7. **Refinamiento, modelado y validación estructural.**
 Modelo estructural final y validación.
8. Aplicación: relaciones estructura-función.

SINTESIS DE FOURIER. CALCULO DE MAPAS



MR - MIR - MAD

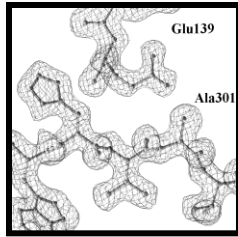
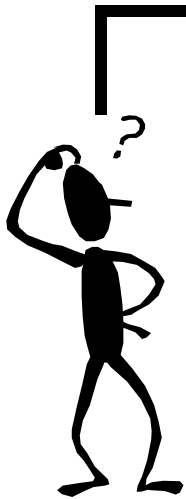
$\{(x \ y \ z)\}$

$$F = \sum f_j \exp\{2\pi i(hx + ky + lz)\}$$

$$|F_{CAL}| + \Phi_{CAL}$$

$$\rho(x \ y \ z) = 1/V \sum |F_{OBS}| \exp\{-2\pi i(hx + ky + lz) + i\Phi_{CAL}\}$$

$|F_{OBS}|$ MEDIDOS
EXPERIMENTALMENTE





MR - MIR - MAD

MODELO INICIAL

ERRORES GRAVES

MEJORA DE LAS FASES

MODELADO

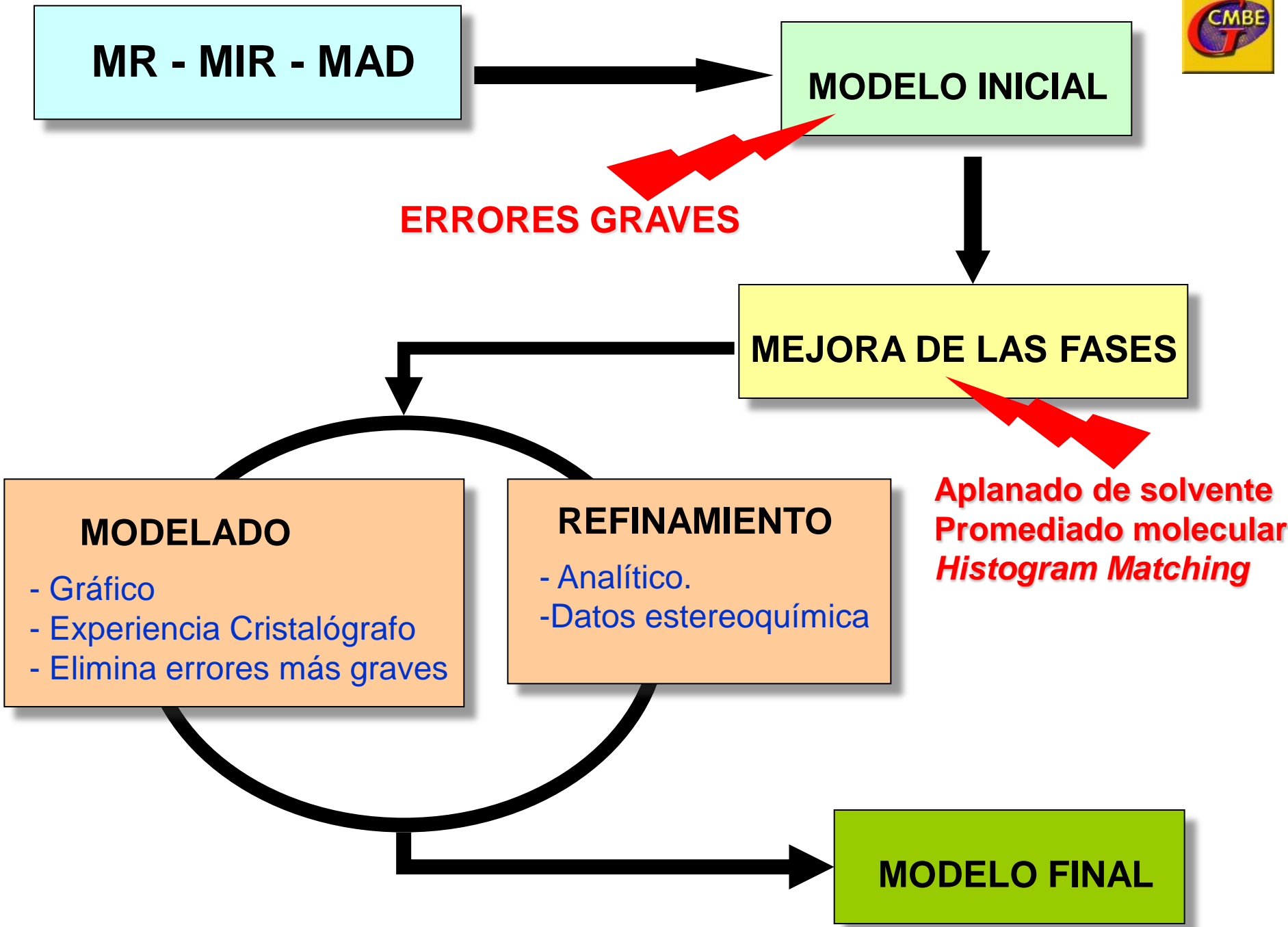
- Gráfico
- Experiencia Cristalógrafo
- Elimina errores más graves

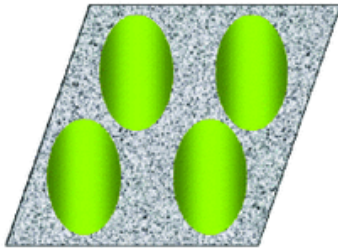
REFINAMIENTO

- Analítico.
- Datos estereoquímica

Aplanado de solvente
Promediado molecular
Histogram Matching

MODELO FINAL

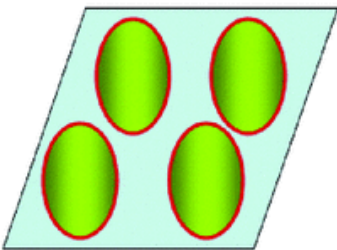




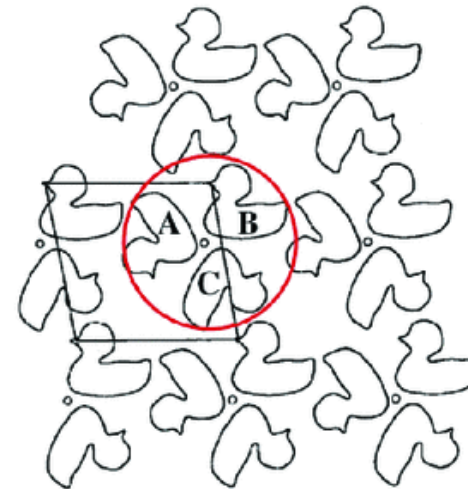
Aplanado de solvente

$$\langle \rho_{\text{protein}} \rangle \approx 0.43 \text{ e \AA}^{-3}$$

$$\langle \rho_{\text{solvent}} \rangle \approx 0.33 \text{ e \AA}^{-3}$$

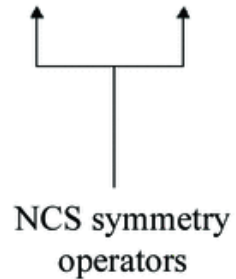


Promediado molecular

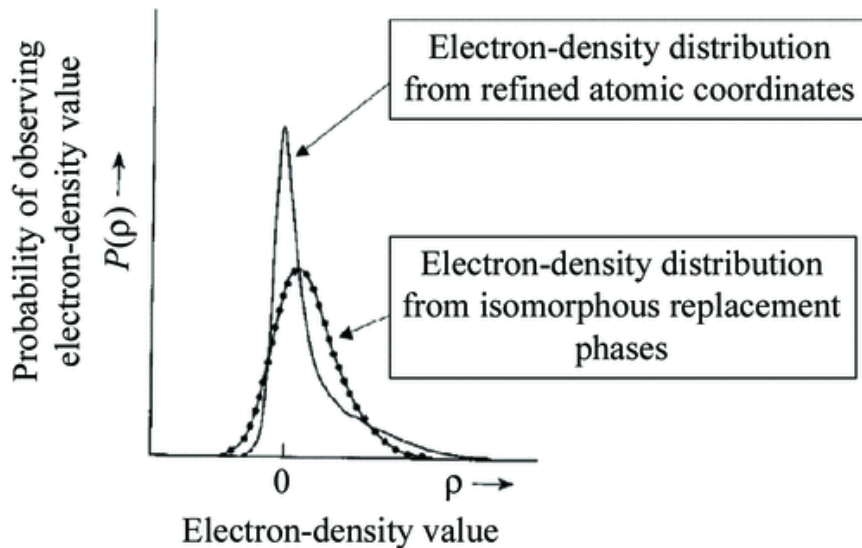


$$\rho(\mathbf{x}_B) = \mathbf{R}_B \rho(\mathbf{x}_A) + \mathbf{t}_B$$

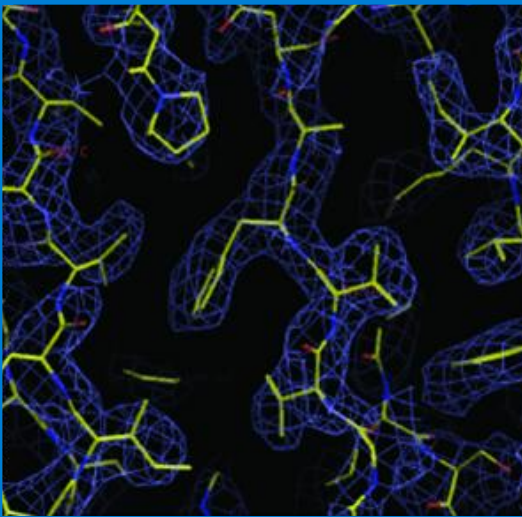
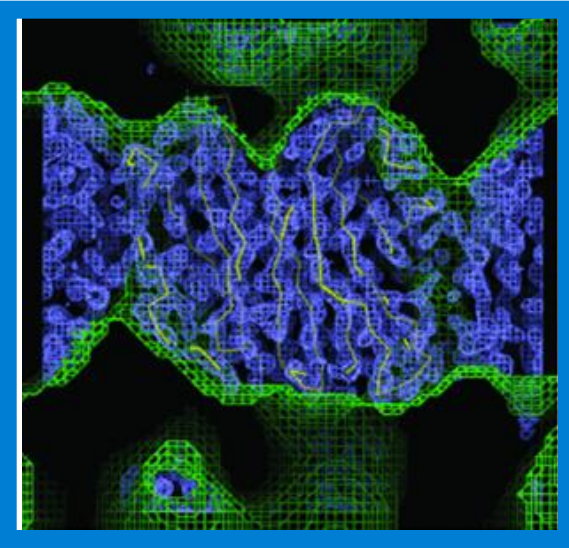
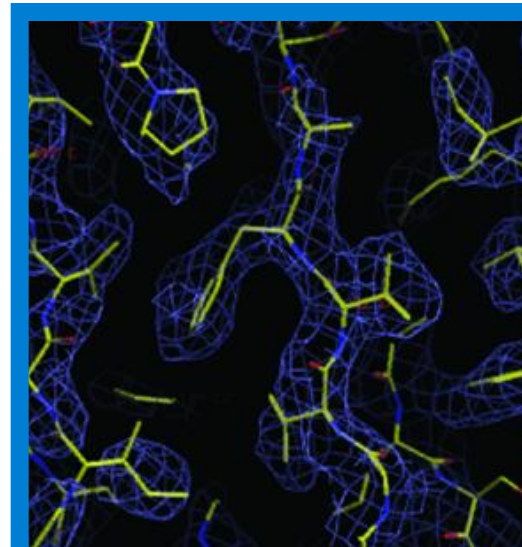
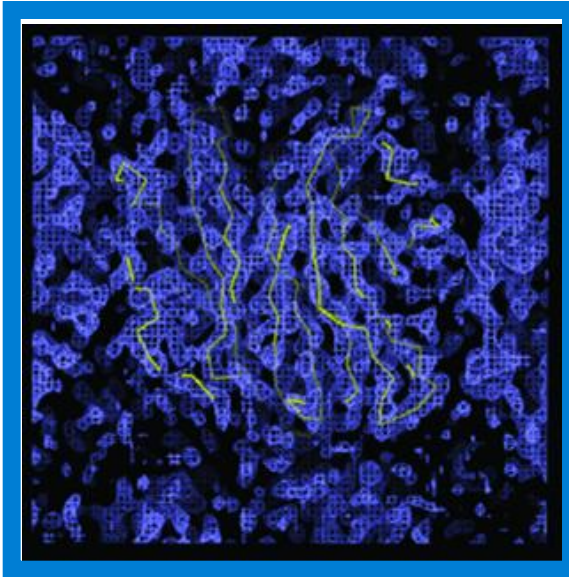
$$\rho(\mathbf{x}_C) = \mathbf{R}_C \rho(\mathbf{x}_A) + \mathbf{t}_C$$



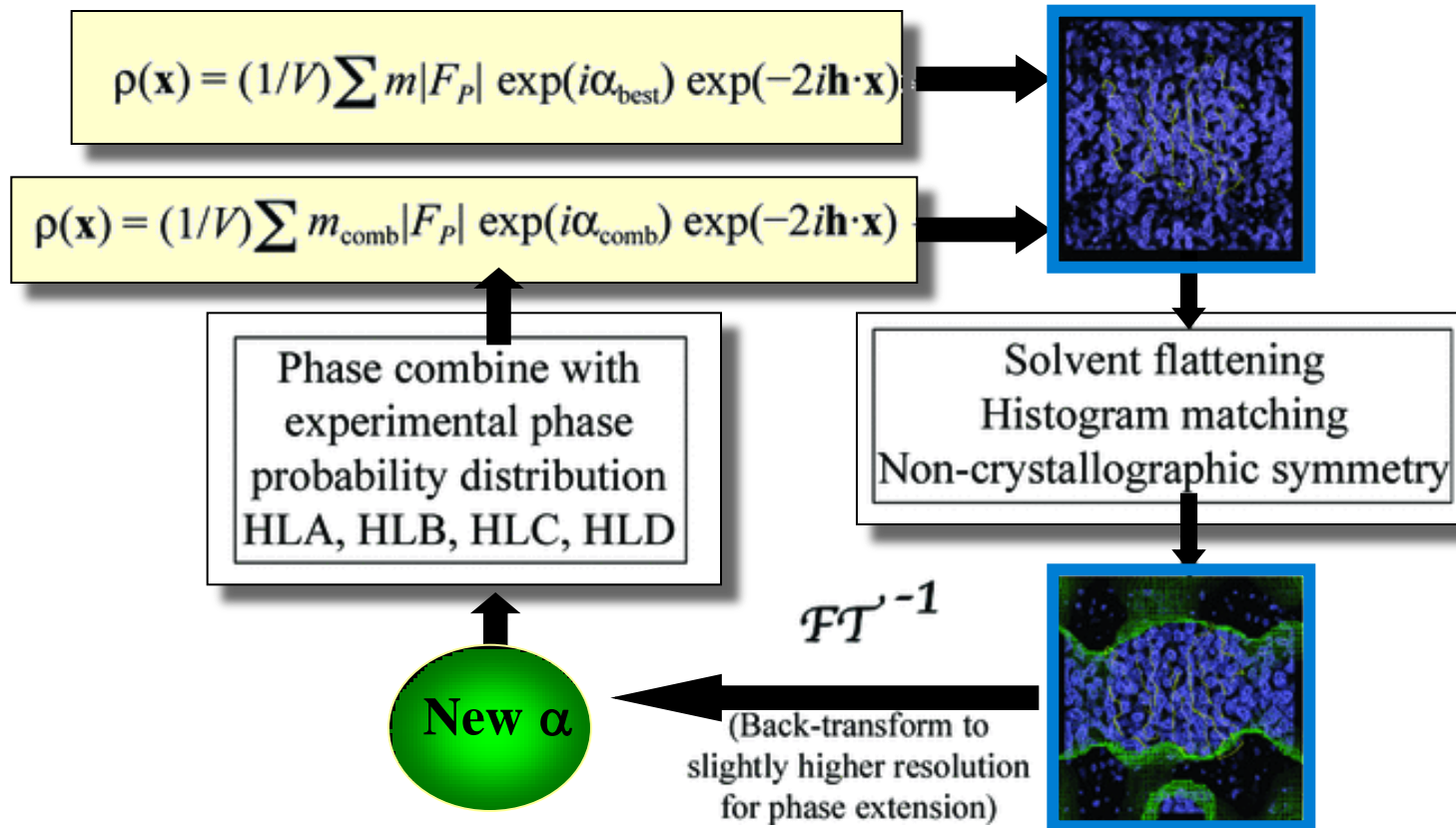
Histogram Matching



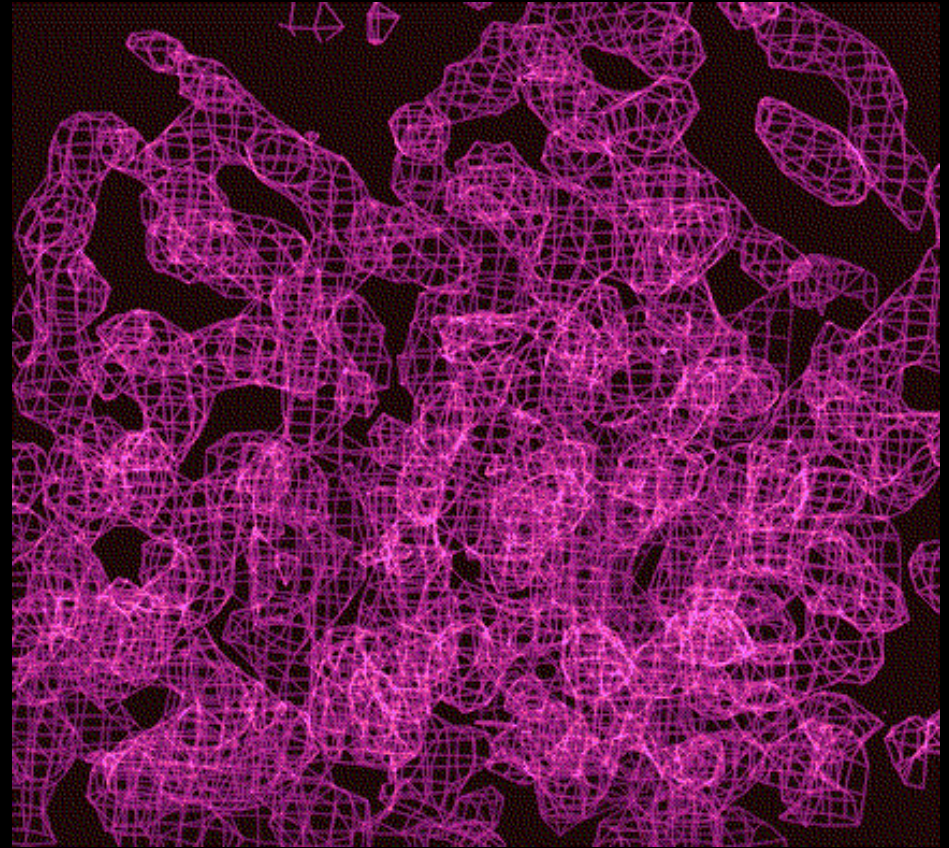
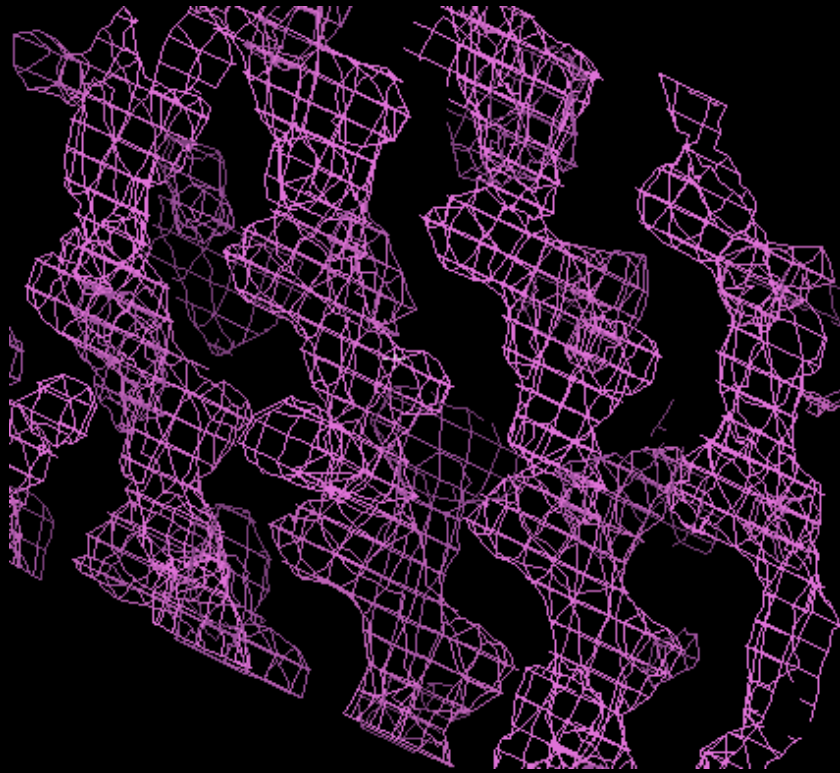
MEJORA DE LAS FASES: APLANADO DE SOLVENTE



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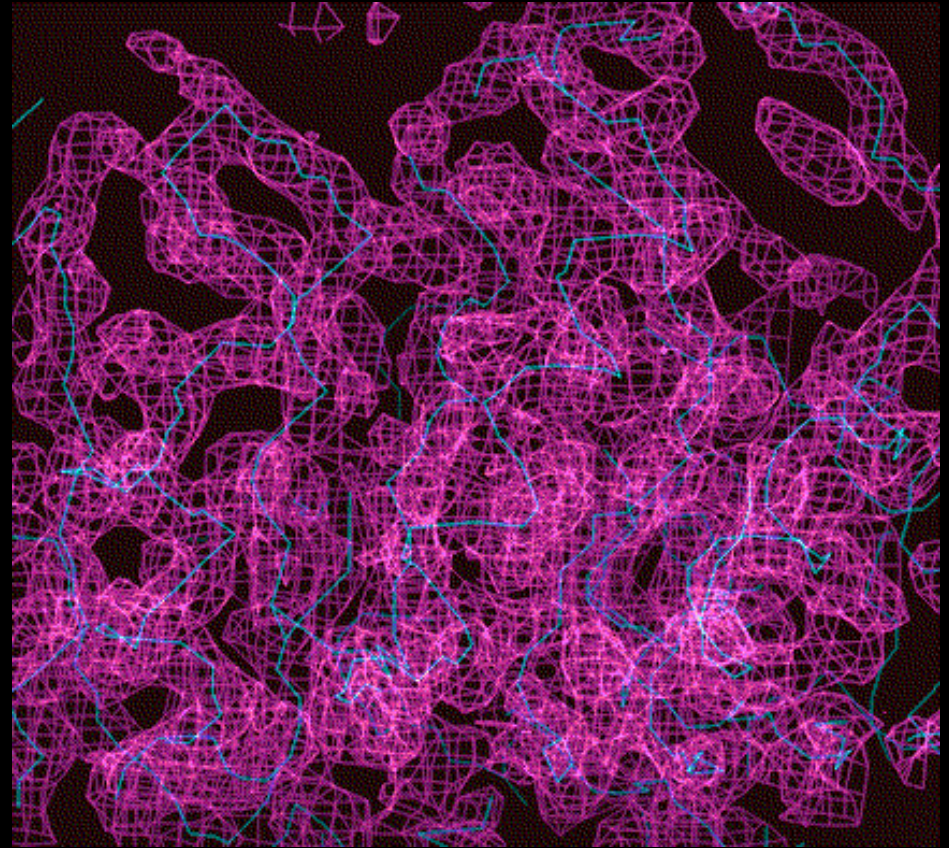
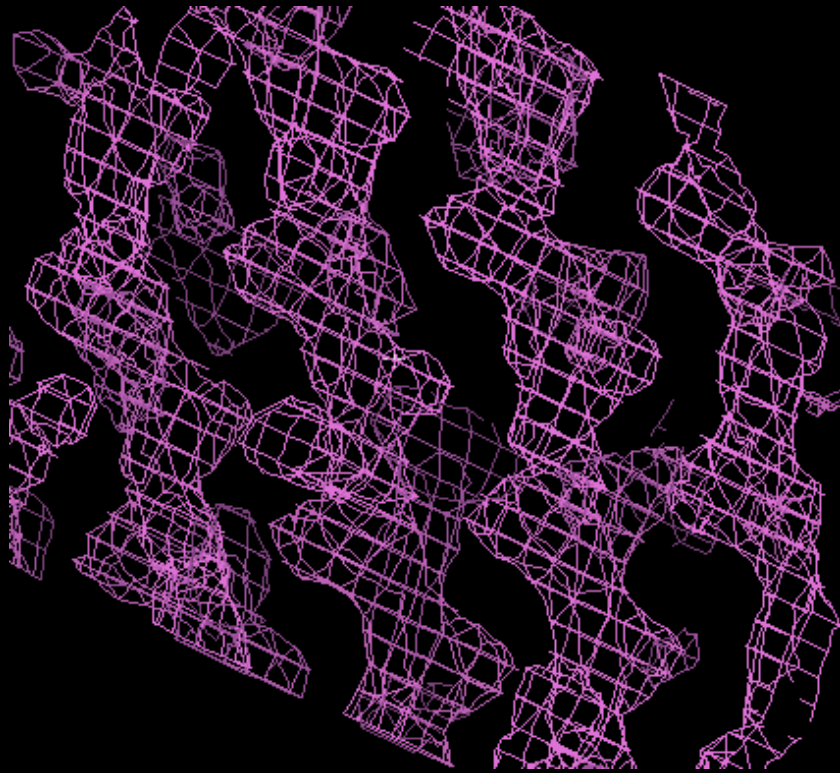


Obtención del modelo inicial



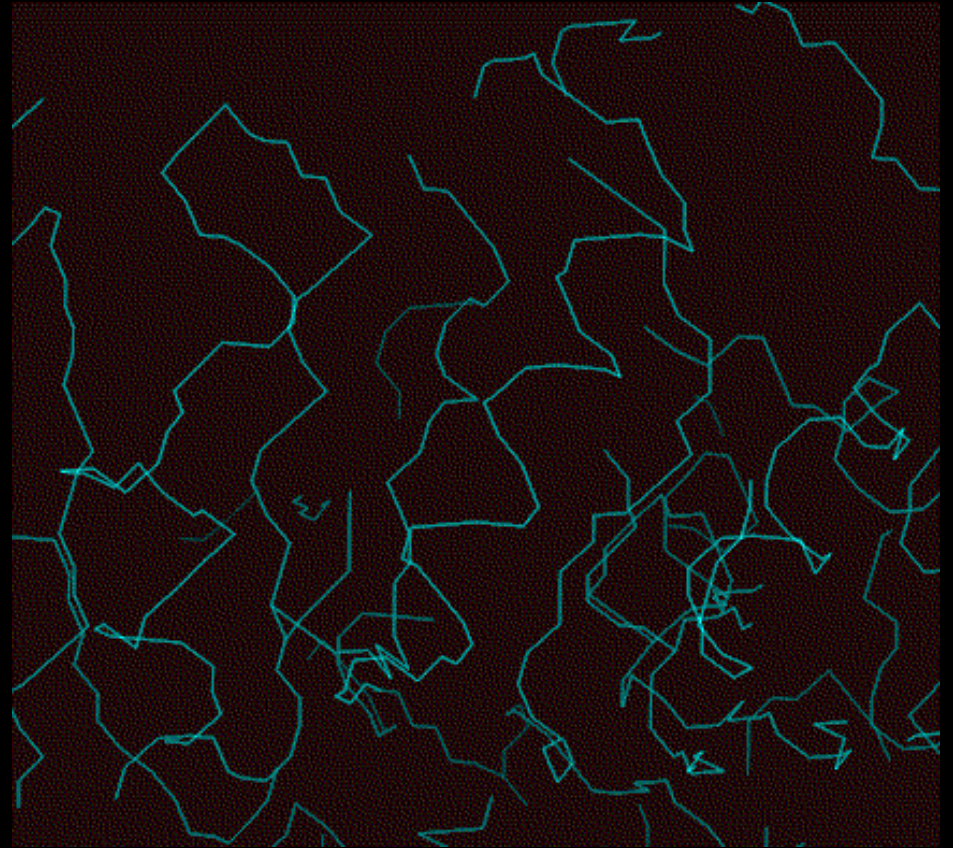
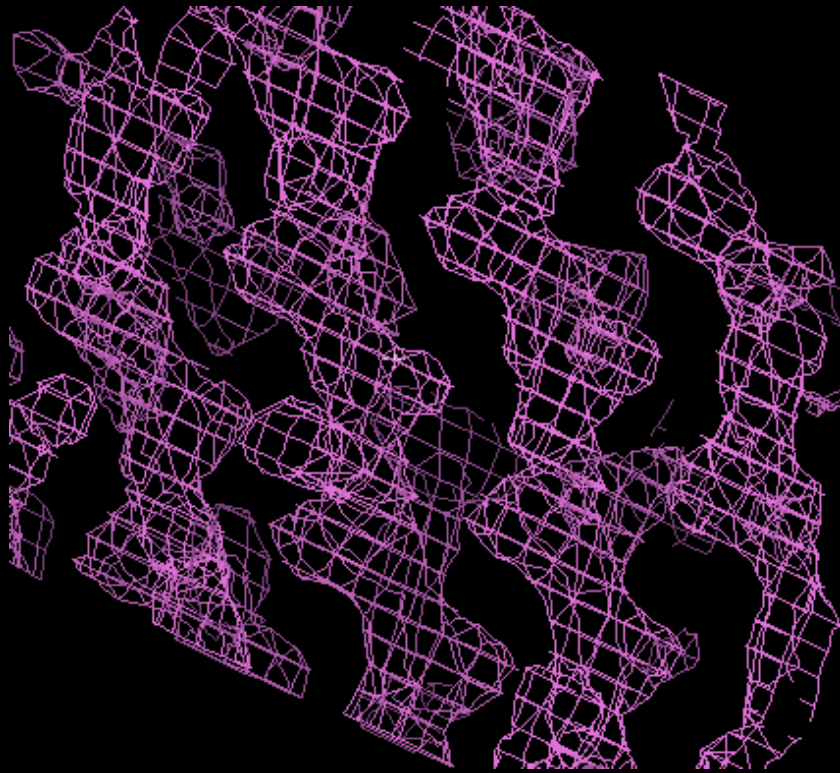
$$\rho(x, y, z) = 1/V \sum |F(h, k, l)| \exp \{-2\pi i (hx + ky + lz) + i\Phi(h, k, l)\} = f(\text{Intensidades, fase})$$

Obtención del modelo inicial



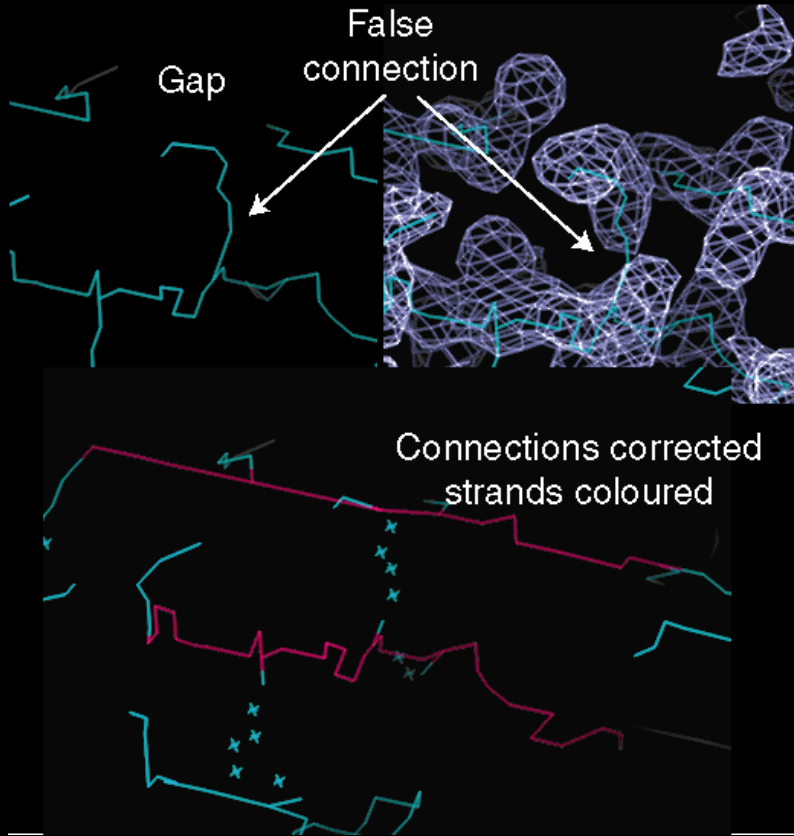
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Obtención del modelo inicial



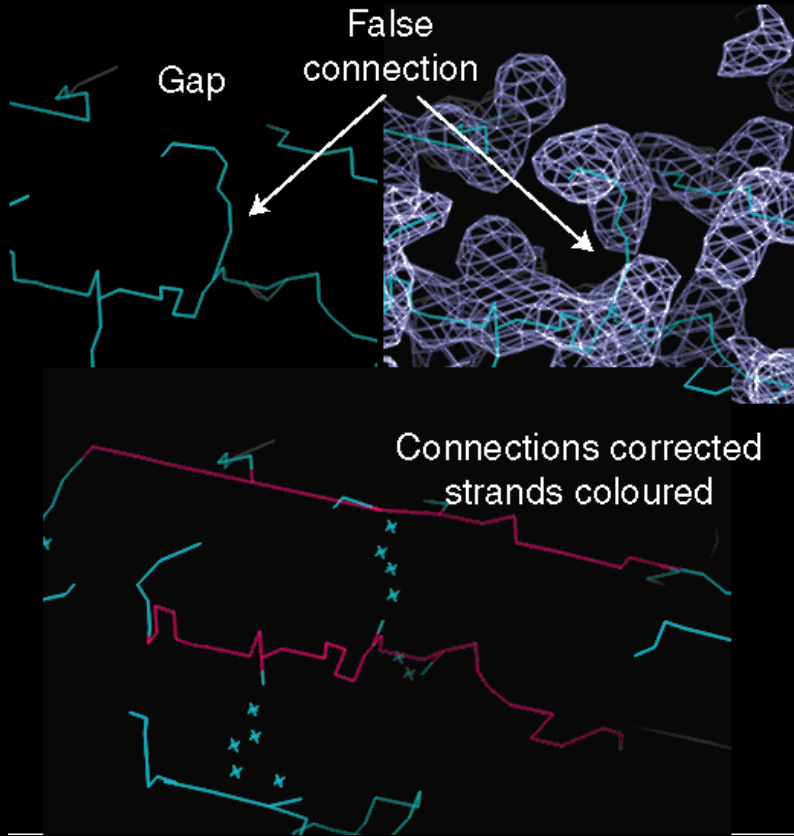
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Obtención del modelo inicial



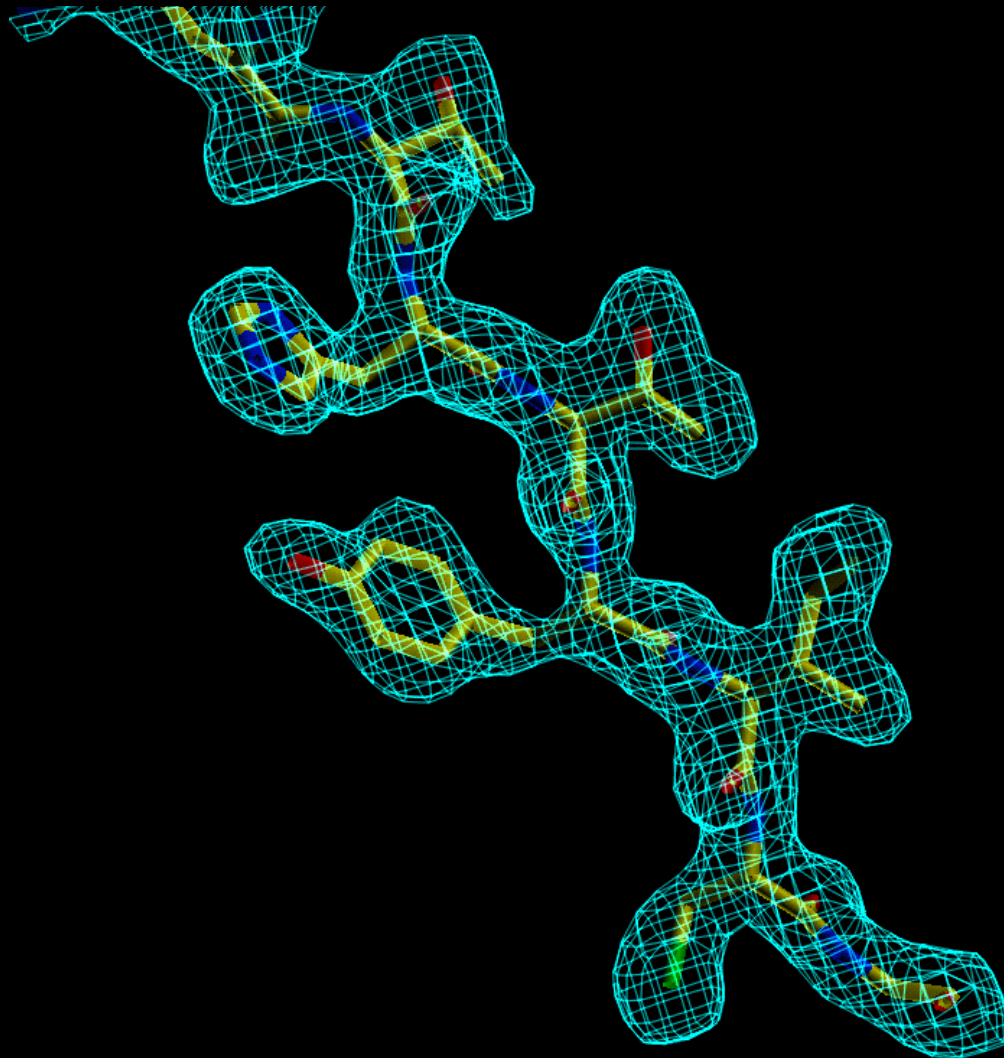
$$\rho(x, y, z) = \frac{1}{V} \sum |F(h, k, l)| \exp \{-2\pi i (hx + ky + lz) + i\Phi(h, k, l)\} = f \text{ (Intensidades, fase)}$$

Obtención del modelo inicial

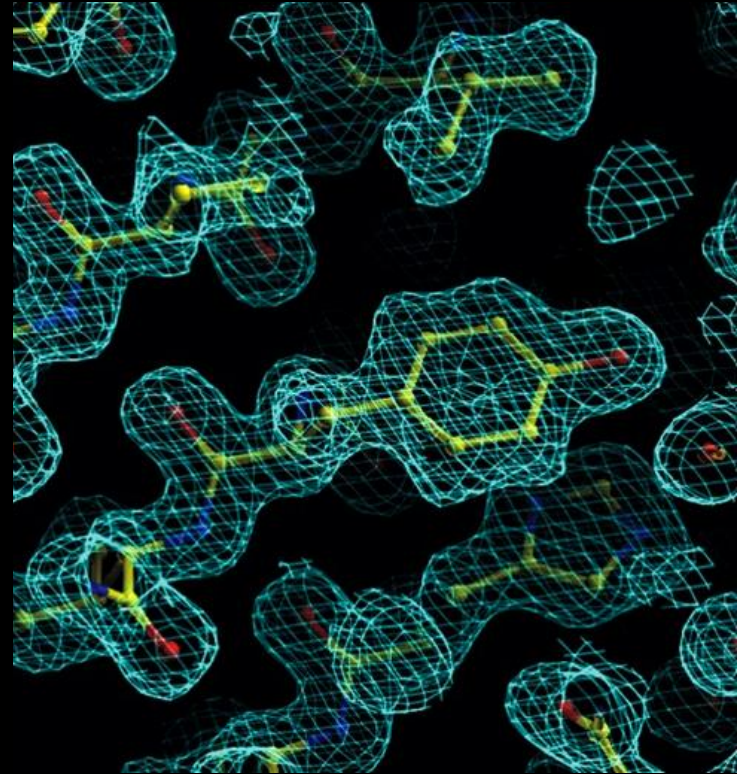
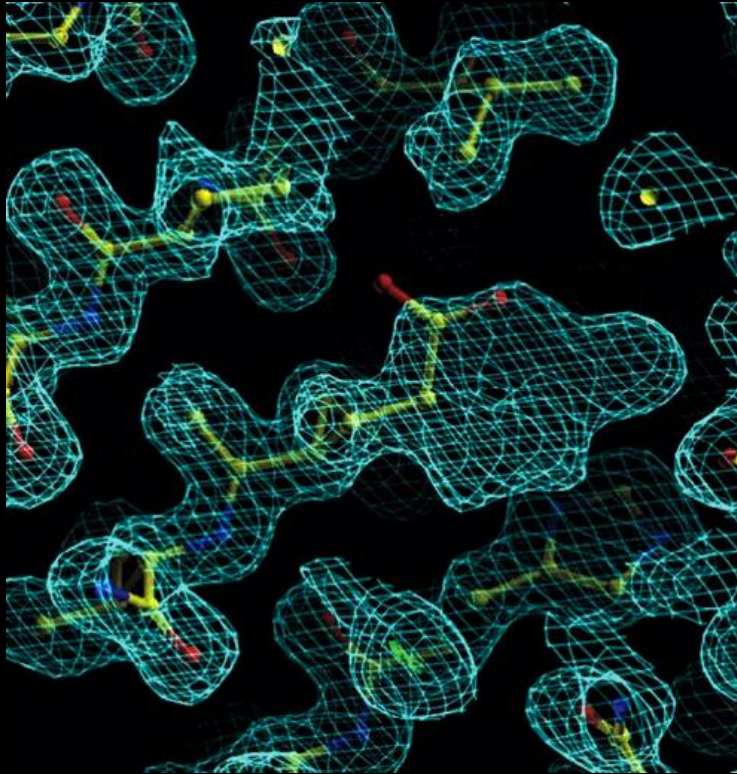


$$\rho(x, y, z) = \frac{1}{V} \sum |F(h, k, l)| \exp \{-2\pi i (hx + ky + lz) + i\Phi(h, k, l)\} = f \text{ (Intensidades, fase)}$$

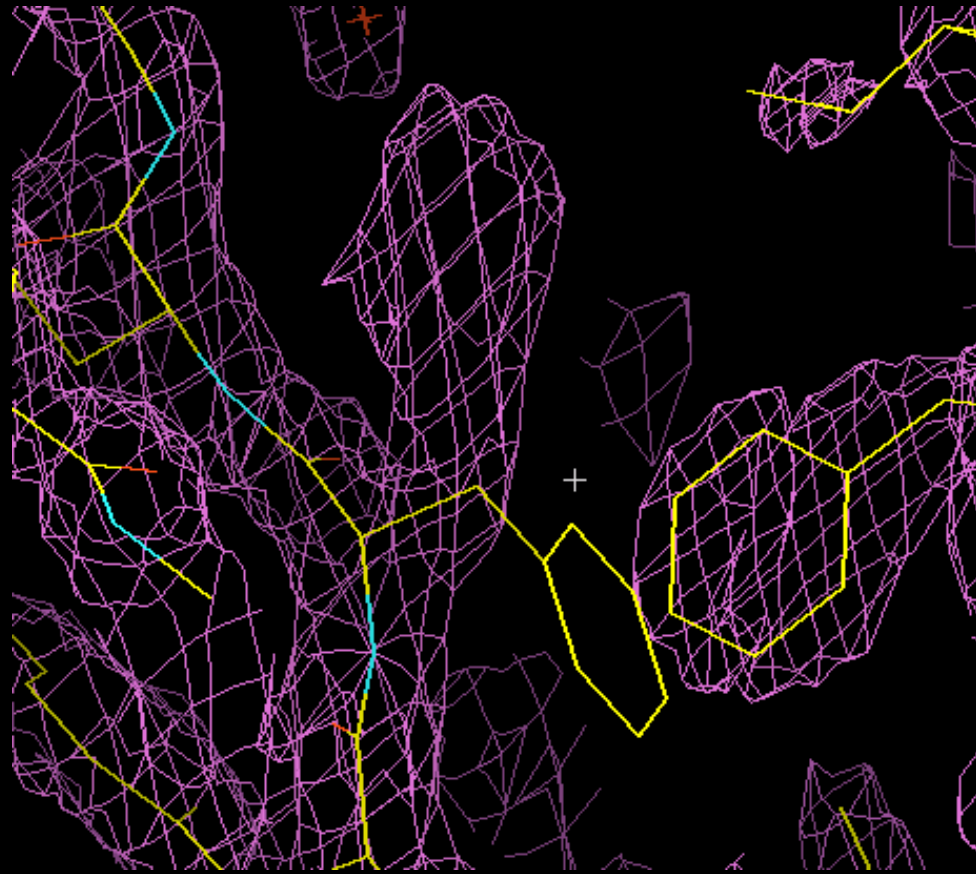
TRAZADO DE LA CADENA POLIPEPTÍDICA



→ Se corrigen errores en secuencia o a nivel de confórmeros.



Se corrigen errores en secuencia o a nivel de confórmeros



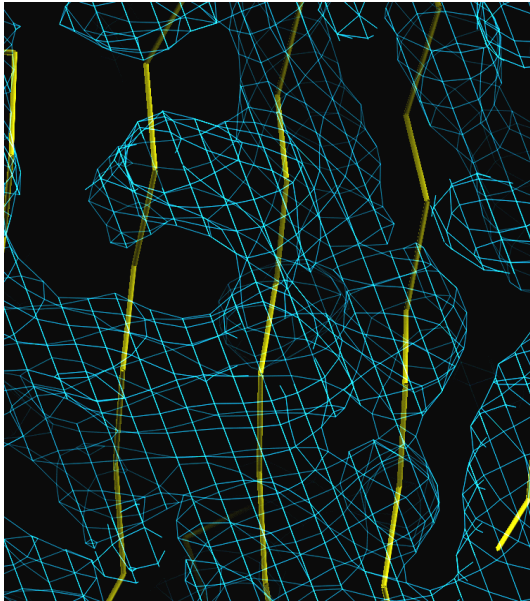
$$\rho(x, y, z) = 1/V \sum |F(h, k, l)| \exp \{-2\pi i (hx + ky + lz) + i\Phi(h, k, l)\} = f \text{ (Intensidades, fase)}$$

MAPA DE DENSIDAD ELECTRÓNICA Y RESOLUCIÓN

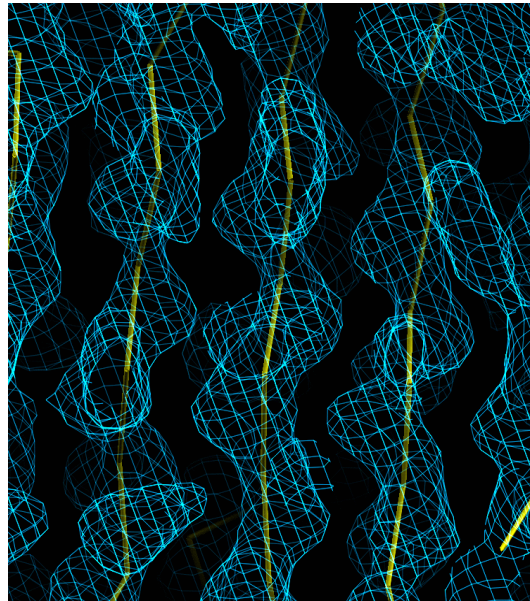


→ La calidad del mapa de densidad electrónica depende de la resolución.

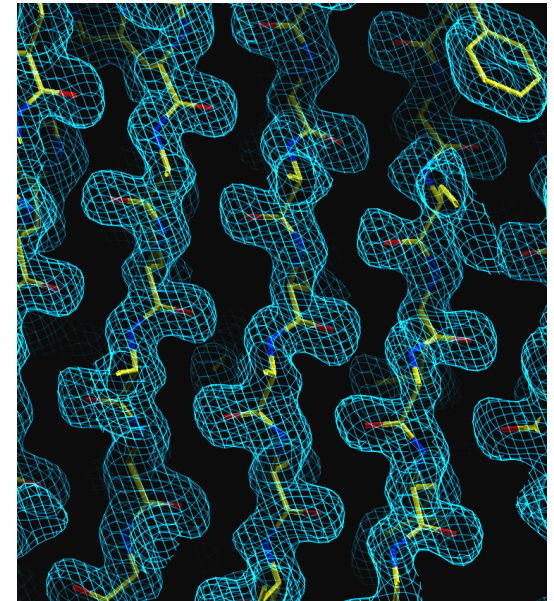
BAJA: 5Å



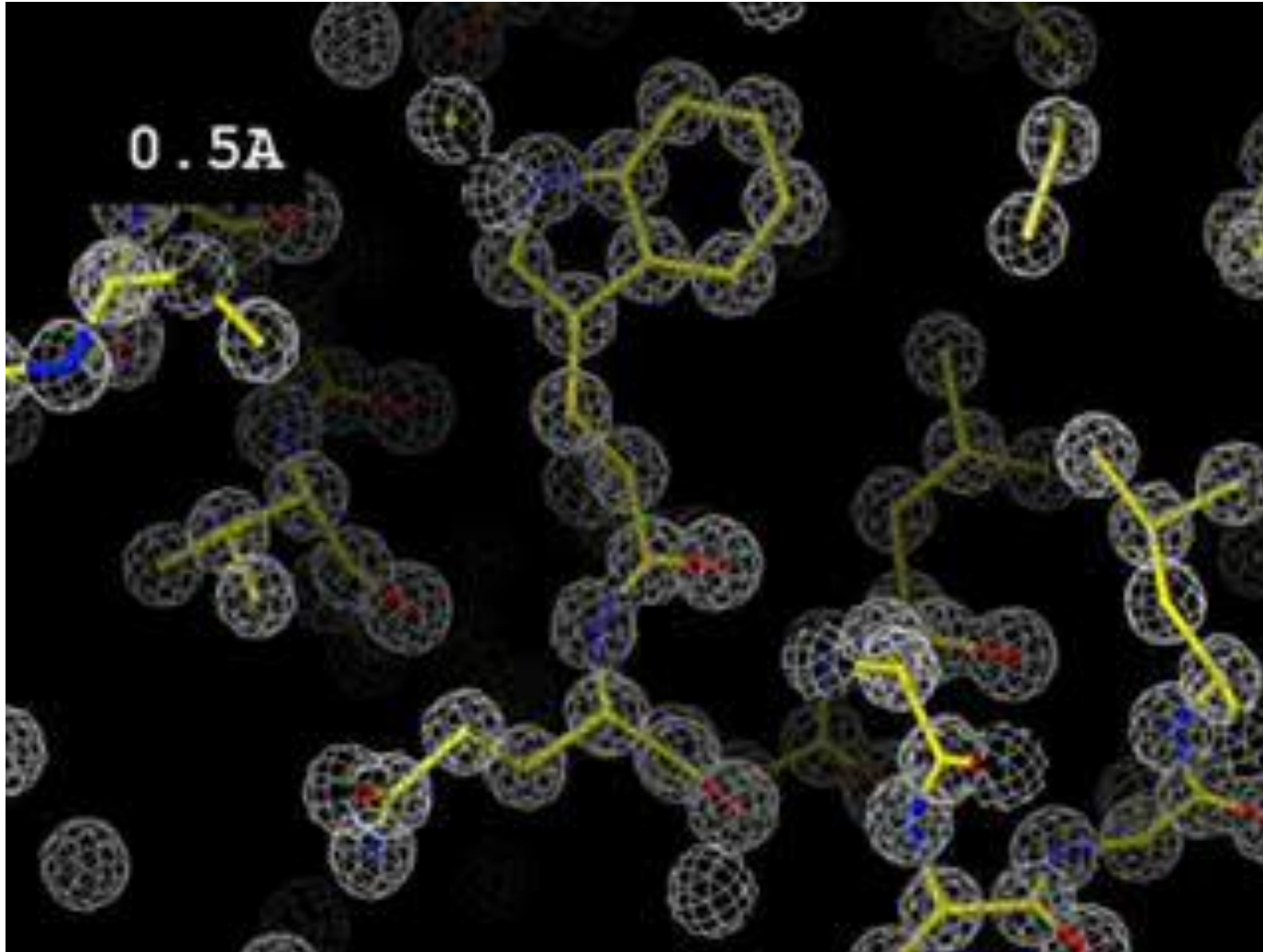
MEDIA: 3Å



ALTA: 1.7Å



MAPA DE DENSIDAD ELECTRÓNICA Y RESOLUCIÓN





Available refinement programs:

- SHELXL
- CNS
- REFMAC5
- TNT
- BUSTER/TNT
- RESTRAINT
- MOPRO



Purpose of Refinement:

- 1- Optimal fit of the model to the experimental data while retaining its chemical integrity.**
- 2- Partial models: Bootstrap missing parts.**
- 3- Improvement of phases to facilitate model building (automatic e.g. ARP/WARP or manual).**
- 4- Give deviation from chemistry and experiment to aid analysis of the model.**
- 5- Estimation of quality of the fitted model.**



Source of atomic models:

1- Molecular Replacement.

Problems:

- Wrong loops.
- Wrong side chains.
- Missing Domains.

2- Experimental Phasing.

Problems:

- Incomplete model.
- Misfitted fragments.

Advantage:

- Phase information is available.



X-ray Data:

1- Intensities or amplitudes of Structure factors.

Amplitudes are usually used in refinement. When errors from model and amplitudes are comparable then it may be better to switch to intensities.

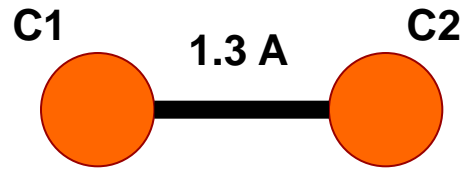
2- Experimental Phases if available.

Phase information gives very good results at the early and medium stages of refinement. When errors in phases are comparable with errors from the model then phase information can be dropped.



➔ Available Information and Data:

- 1- Bonded interactions: e.g. Bond lengths, angles..
- 2- Non-bonded interactions: vdw, hbond
- 3- Similarity of different copies of the same molecule (NCS)
- 4- Similarity of the molecule to already solved structures.
- 5- Similarity of the part of structure to already known fragments (helices, strands, loops).
- 6- Behavior of electron density.



B1= 20
B2= 50

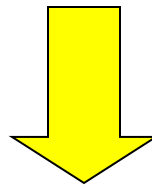
→ Role of restraints:

- 1- When atoms have high B values and experiment is at low resolution then electron density may not show separate peaks.
- 2- If restraint would not be used then atoms would collapse and become one.
- 3- Role of restraints is that to retain chemistry of atoms and at the same time describe electron density optimally.
- 4- If atoms are bonded it is unlikely that they will have hugely different B values.

→ Prior knowledge in Macromolecular Crystallography

There are two main sources of prior knowledge available for macromolecular crystallography: 1) Chemical and 2) structural

1- Chemical knowledge is valence bond properties of the structural units of macromolecules. Chemical units are: amino acids, DNA/RNA, sugars and many , many different ligands and small compounds. This type of knowledge is used routinely in refinement and model building.



This information is included in Topology/parameters dictionary

2- Structural knowledge is the wealth of information accumulated in the PDB: different types of helices, beta strands, turns, information about domain structures and many others.

REFINAMIENTO



El modelo se modifica para minimizar las diferencias entre los datos experimentales y los obtenidos a partir del modelo.

$$E_T = w E_{\text{xray}} + E_{\text{empirical}}$$

$$E_{\text{xray}} = \sum \{ |F_{\text{OBS}}| - k |F_{\text{CAL}}| \}^2$$

$$E_{\text{empirical}} = \sum_{\text{bonds}} K_b (b - b_o)^2 + \sum_{\text{angles}} K_\theta (\theta - \theta_o)^2 + \sum_{\text{torsional}} K_f (1 + \cos(n\phi - \delta)) + \sum_{\text{VDV}} + \dots$$



CONTROL:

- Estereoquímica.
- Factor de desacuerdo, R.

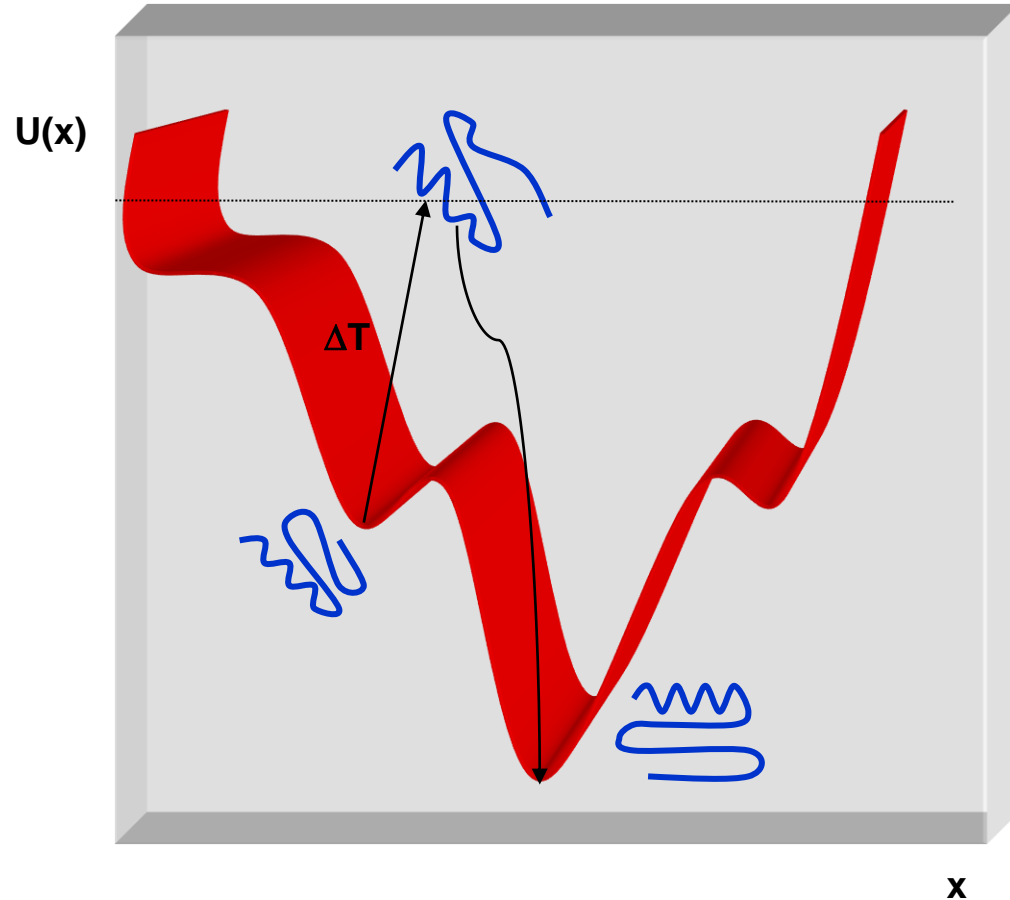
$$R = \frac{\sum \{ |F_{\text{OBS}}| - k |F_{\text{CAL}}| \}}{\sum \{ |F_{\text{OBS}}| \}}$$

→ MAIN TYPES:

- Least squares.
- Conjugate Gradient.
- Molecular Dynamics.

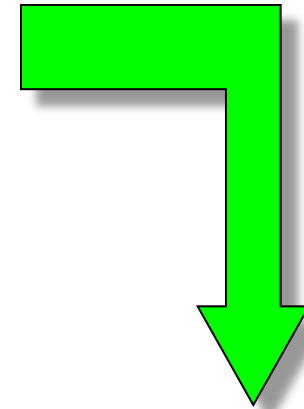
→ CONSIDERATIONS:

- Least squares
 - high number of experimental data.
 - convergence radius small ($\approx 1\text{\AA}$)
 - High accuracy.
- Conjugate Gradient.
 - Big convergence radius.
 - Less accuracy.
- Molecular Dynamics.
 - Largest convergence radius.
 - Least accuracy.



El tipo de refinamiento depende de:

- 1 - Resolución de los datos experimentales.
- 2 - Desviación del modelo inicial al final.



Resolución (Å)	Observaciones/parámetro (x,y,z,B)
3.5	0.5
3.0	0.8
2.5	1.4
2.0	2.8
1.5	6.2



→ Parameters:

- Usual parameters:

- 1- **Positions x,y,z**
- 2- **B values-isotropic or anisotropic.**
- 3- **Occupancy.**

(Atomic displacements are likely anisotropic but rarely have the luxury of refining individual anisotropic Us. Instead isotropic Bs.

- Derived parameters:

- 1- **Rigid body positional:** - After molecular replacement.
 - Isomorphous crystal (liganded, unliganded, different data..)
- 2- **Rigid body of B values- TLS:** -useful at the medium and final stages.
 - at low resolution when full anisotropy is imposible
- 3- **Torsion angles.**

REFINEMENT PROTOCOL



- Bulk-solvent & anisotropic scaling parameters



- Target weights calculation



- TLS refinement calculation



- Rigid body refinement



- Simulated annealing refinement



- Coordinate & B individual refinement



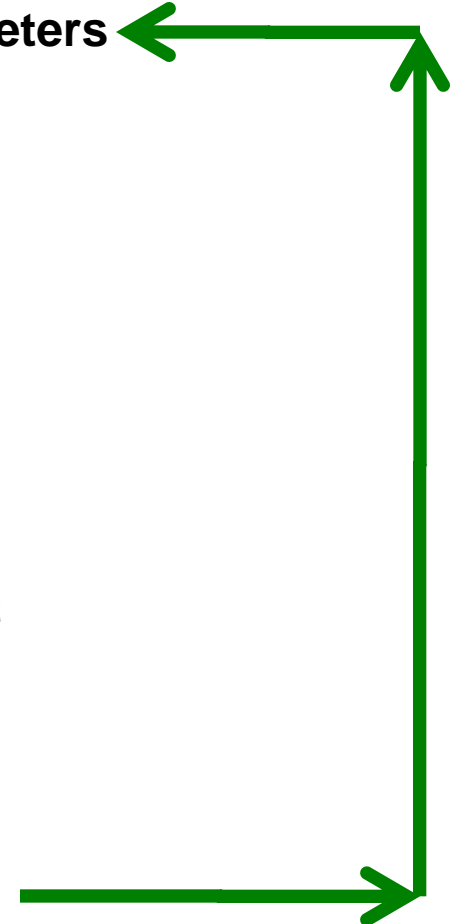
- Map calculation (2Fo-Fc, Fo-Fc)



- Protein & ordered solvent modeling



- Final Model



REFINAMIENTO



El modelo se modifica para minimizar las diferencias entre los datos experimentales y los obtenidos a partir del modelo.

$$E_{xray} = \sum \{ |F_{OBS}| - k |F_{CAL}| \}^2$$

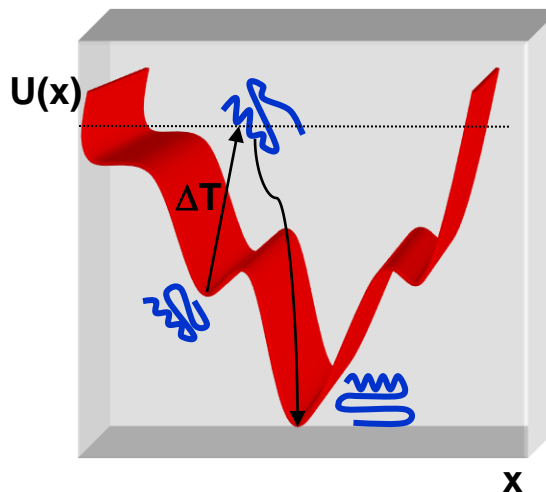
$$E_T = E_{xray} + E_{empirical}$$

$$E_{empirical} = \sum_{bonds} K_b (b - b_o)^2 + \sum_{angles} K_\theta (\theta - \theta_o)^2 + \sum_{torsional} K_f (1 + \cos(n\phi - \delta)) + \sum_{VDW} + \dots$$



TIPOS:

- Mínimos cuadrados.
- Gradiente conjugado.
- Dinámica Molecular.



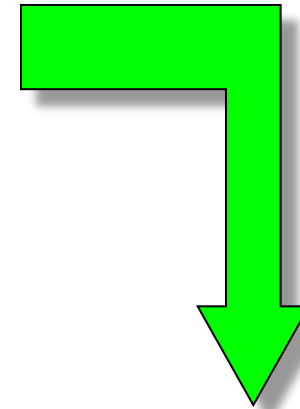
CONTROL:

- Estereoquímica.
- Factor de desacuerdo, R.

$$R = \frac{\sum \{ |F_{OBS}| - k |F_{CAL}| \}}{\sum \{ |F_{OBS}| \}}$$

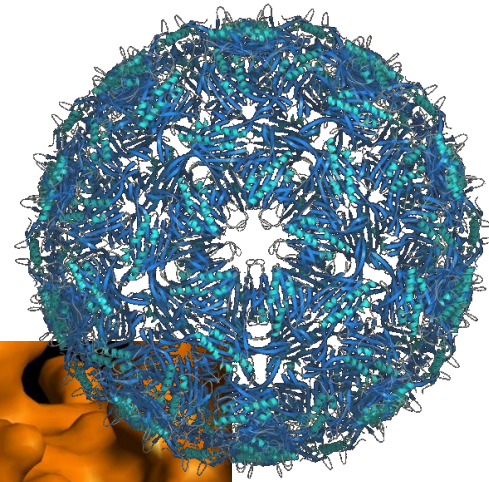
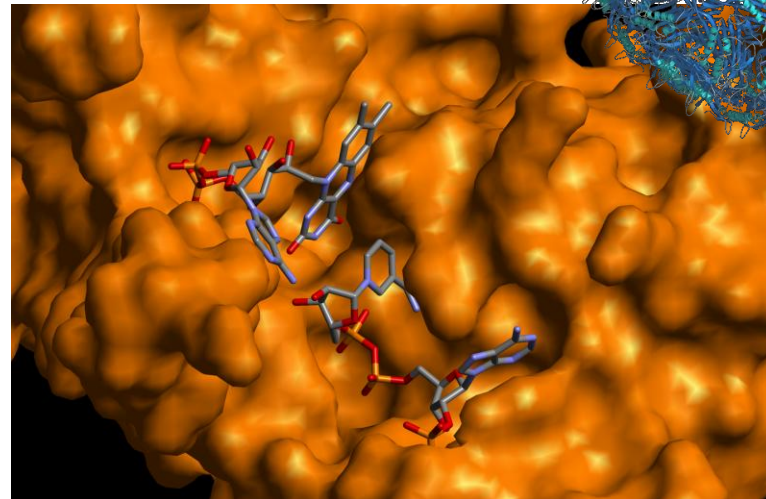
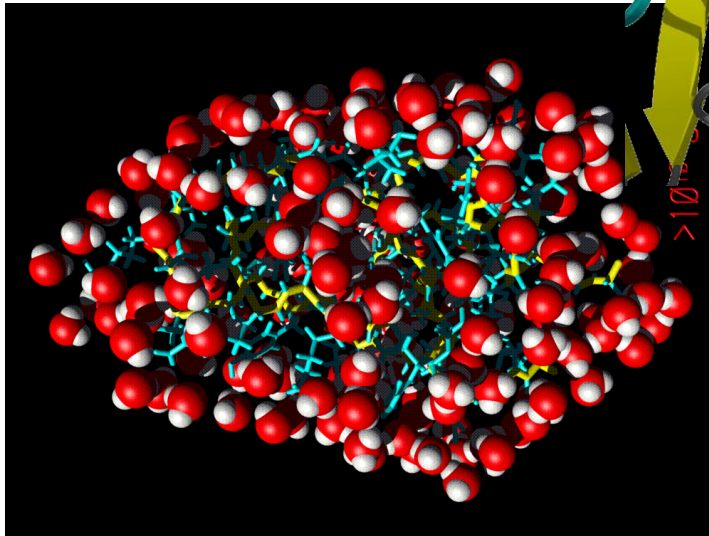
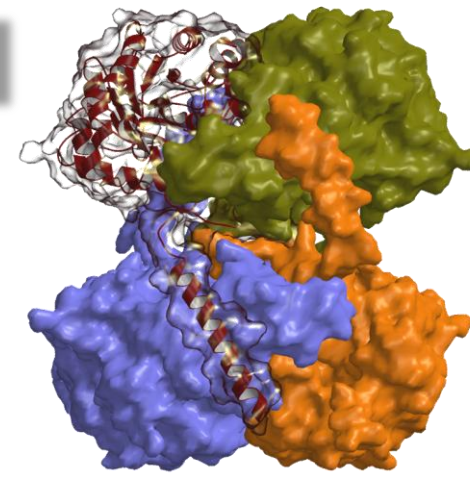
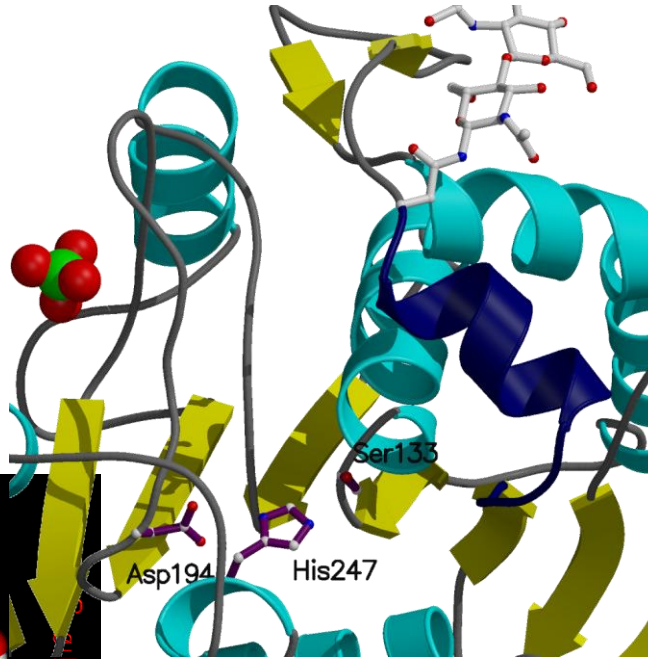
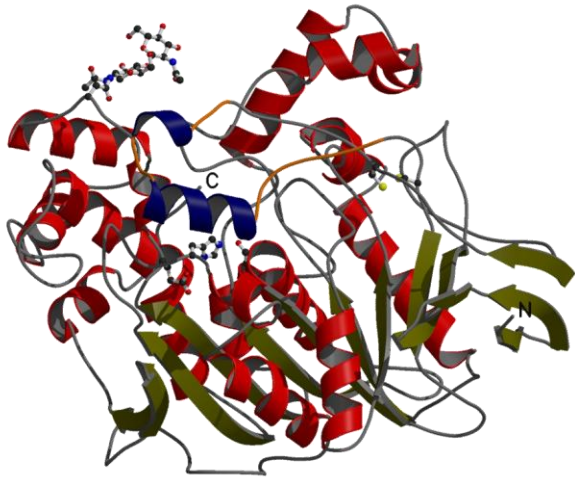
El tipo de refinamiento depende de:

- 1 - Resolución de los datos experimentales.
- 2 - Desviación del modelo inicial al final.

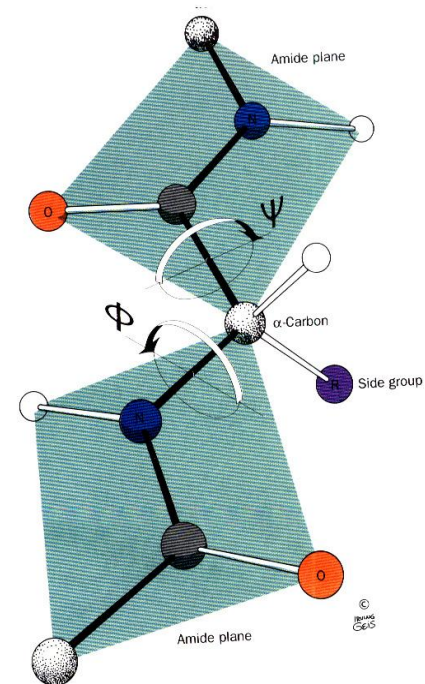
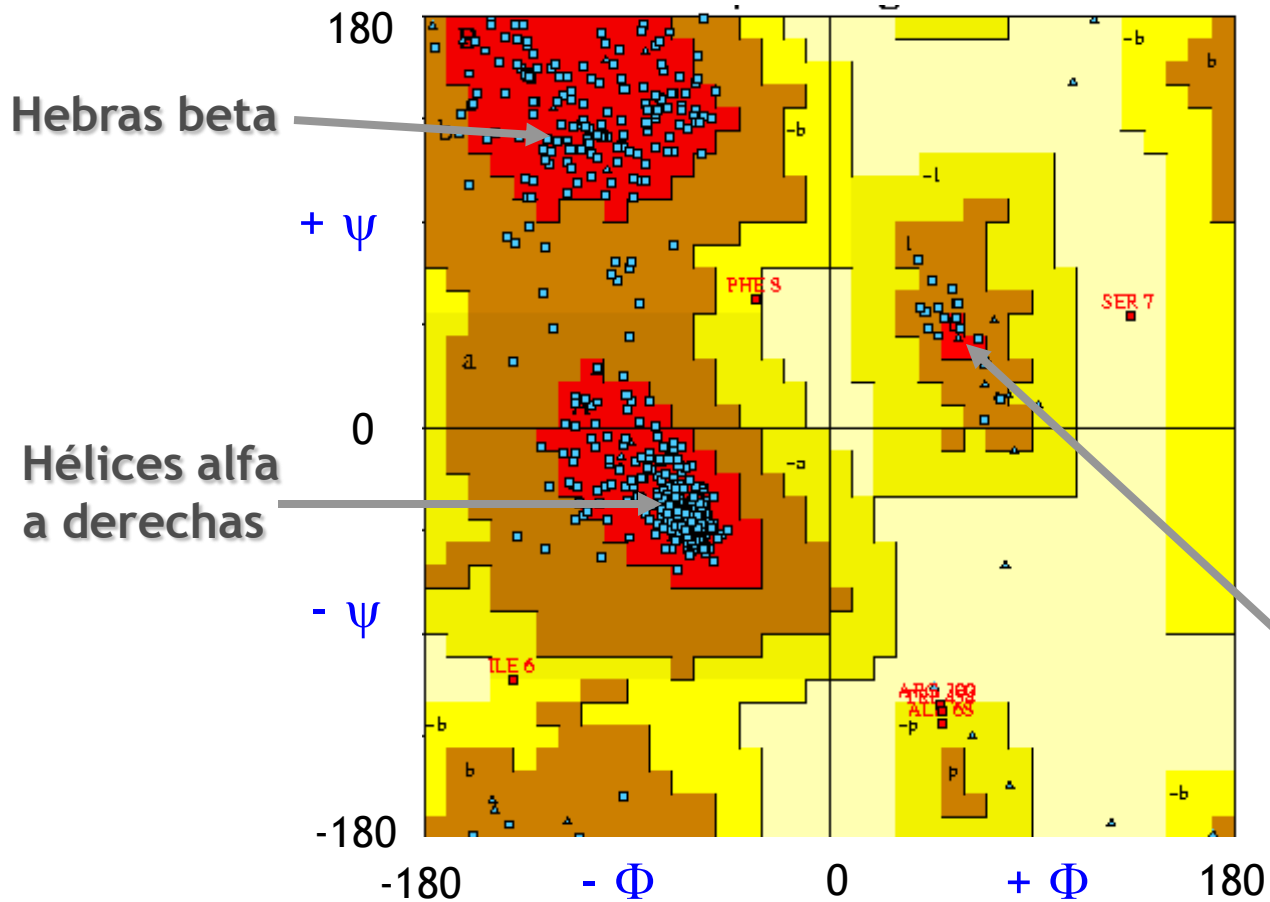


Resolución (Å)	Observaciones/parámetro (x,y,z,B)
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MODELO ESTRUCTURAL FINAL



Plot de Ramachandran

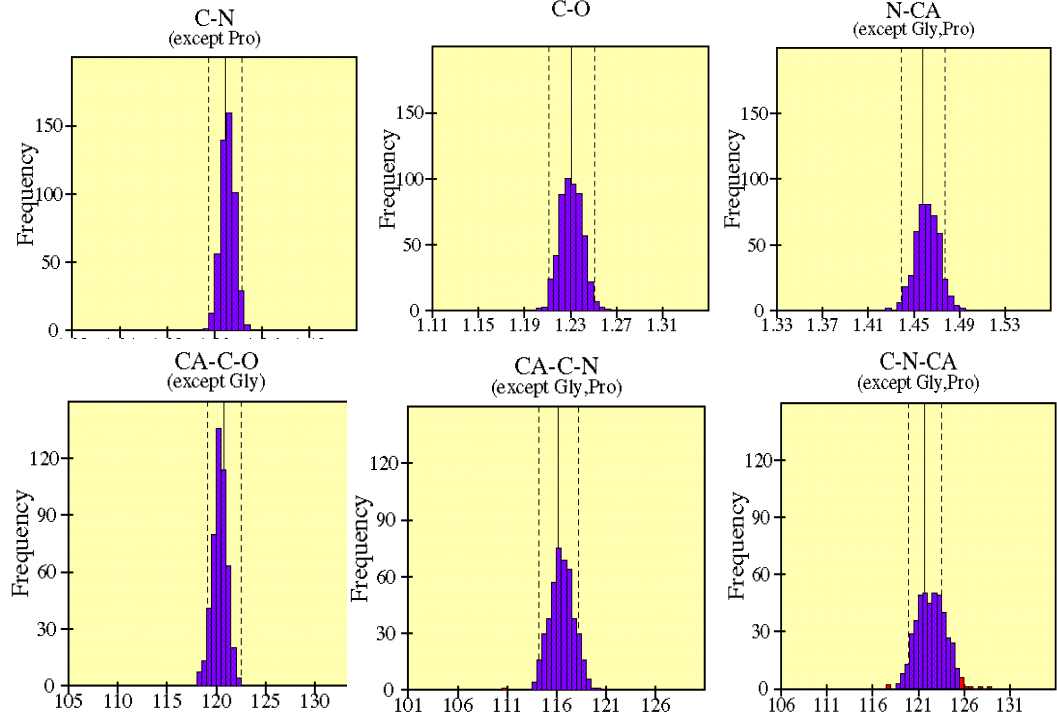
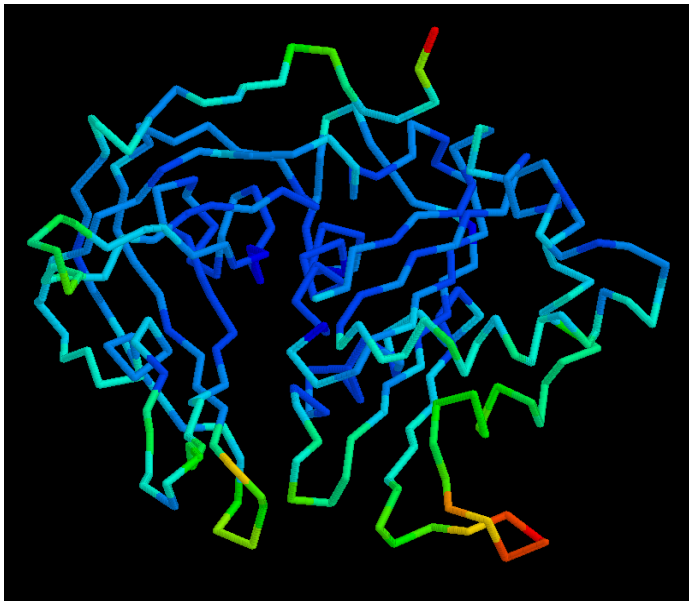


Hélices alfa a izquierdas

1. R y R_{free} bajos ($R < 0.2$, $R_{\text{free}} = R + (5-10)\%R$)

2. Estereoquímica del modelo frente a la ideal



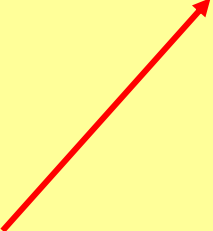
3. Distribución de factores térmicos.



El fichero de coordenadas PDB

Factor de ocupación

Coordenadas:(x, y, z)

.....
ATOM	1	CB	ASP	9	-31.877	64.364	9.711	1.00	37.33	6
ATOM	2	CG	ASP	9	-31.408	65.701	9.162	1.00	41.83	6
ATOM	3	OD1	ASP	9	-32.254	66.612	8.998	1.00	44.35	8
ATOM	4	OD2	ASP	9	-30.189	65.848	8.918	1.00	43.38	8
ATOM	5	C	ASP	9	-32.205	62.836	7.764	1.00	28.21	6
ATOM	6	O	ASP	9	-31.876	63.319	6.683	1.00	30.25	8
ATOM	9	N	ASP	9	-33.860	62.883	9.631	1.00	33.61	7
ATOM	11	CA	ASP	9	-32.913	63.691	8.811	1.00	31.98	6
.....

Factor térmico (B)

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1. Introducción.
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DE LAS COORDENADAS EN ADELANTE ...

Lo más atractivo comienza aquí ...
para descubrir y comprender:

- El plegamiento ...
- Los aspectos dinámicos ... (B)
- Los centros activos de los enzimas ...
- La función biológica ...
- Las interacciones con otras moléculas ...

