

MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

J. D. WATSON
F. H. C. CRICK

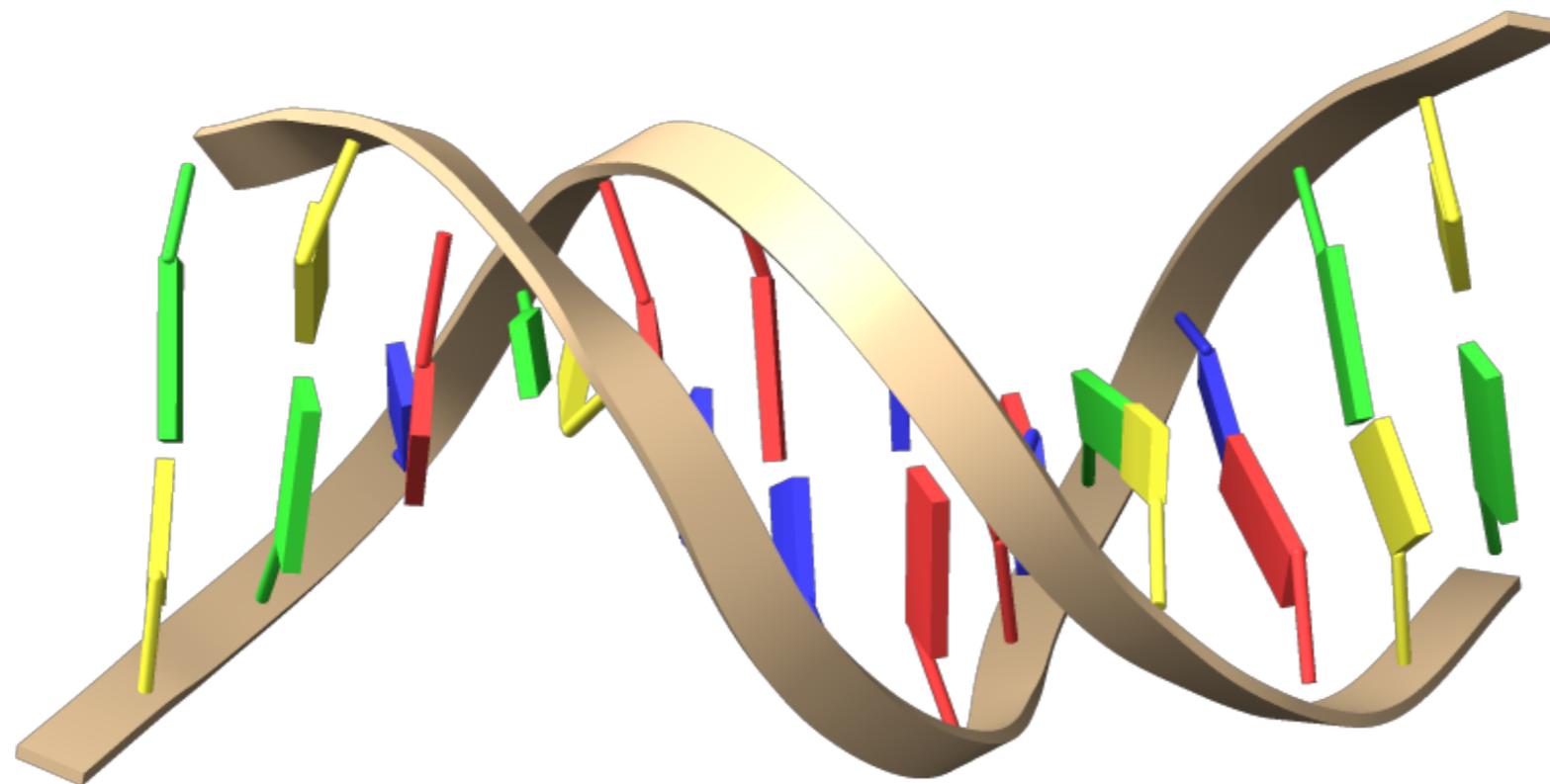
Medical Research Council Unit for the
Study of the Molecular Structure of
Biological Systems,
Cavendish Laboratory, Cambridge.
April 2.

GENETICAL IMPLICATIONS OF THE STRUCTURE OF DEOXYRIBONUCLEIC ACID

By J. D. WATSON and F. H. C. CRICK

Medical Research Council Unit for the Study of the
Molecular Structure of Biological Systems, Cavendish
Laboratory, Cambridge

Nature 1953



PDB 1D29

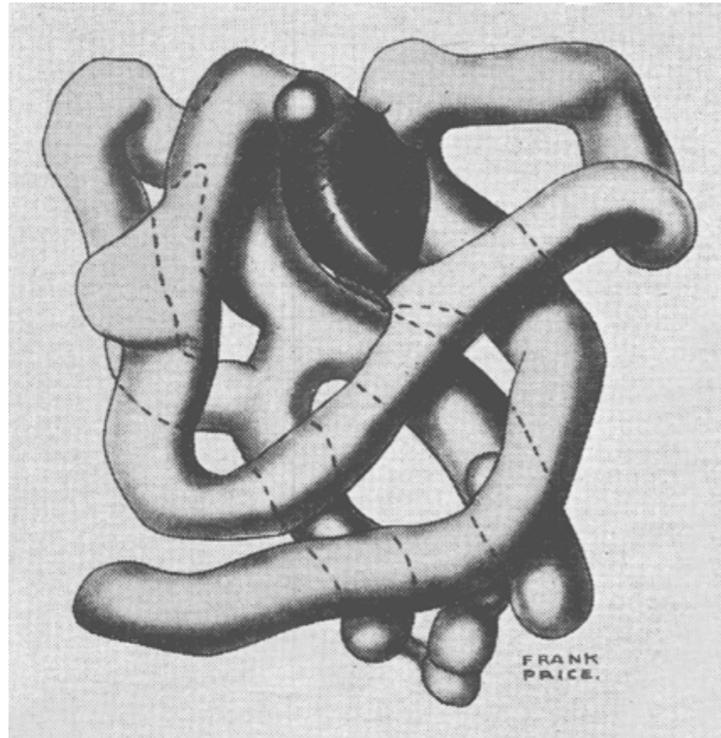
Crystal structure analysis of the B-DNA dodecamer CGTGAATTCACG.

[Larsen, T.A.](#), [Kopka, M.L.](#), [Dickerson, R.E.](#)

(1991) *Biochemistry* 30: 4443-4449

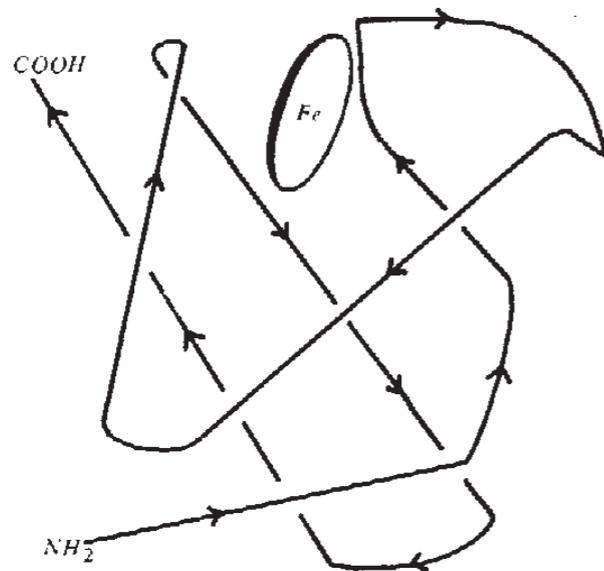
1960 - Estrutura cristalográfica da mioglobina a 2 Å de resolução

6 Å



(a)

2 Å



(b)

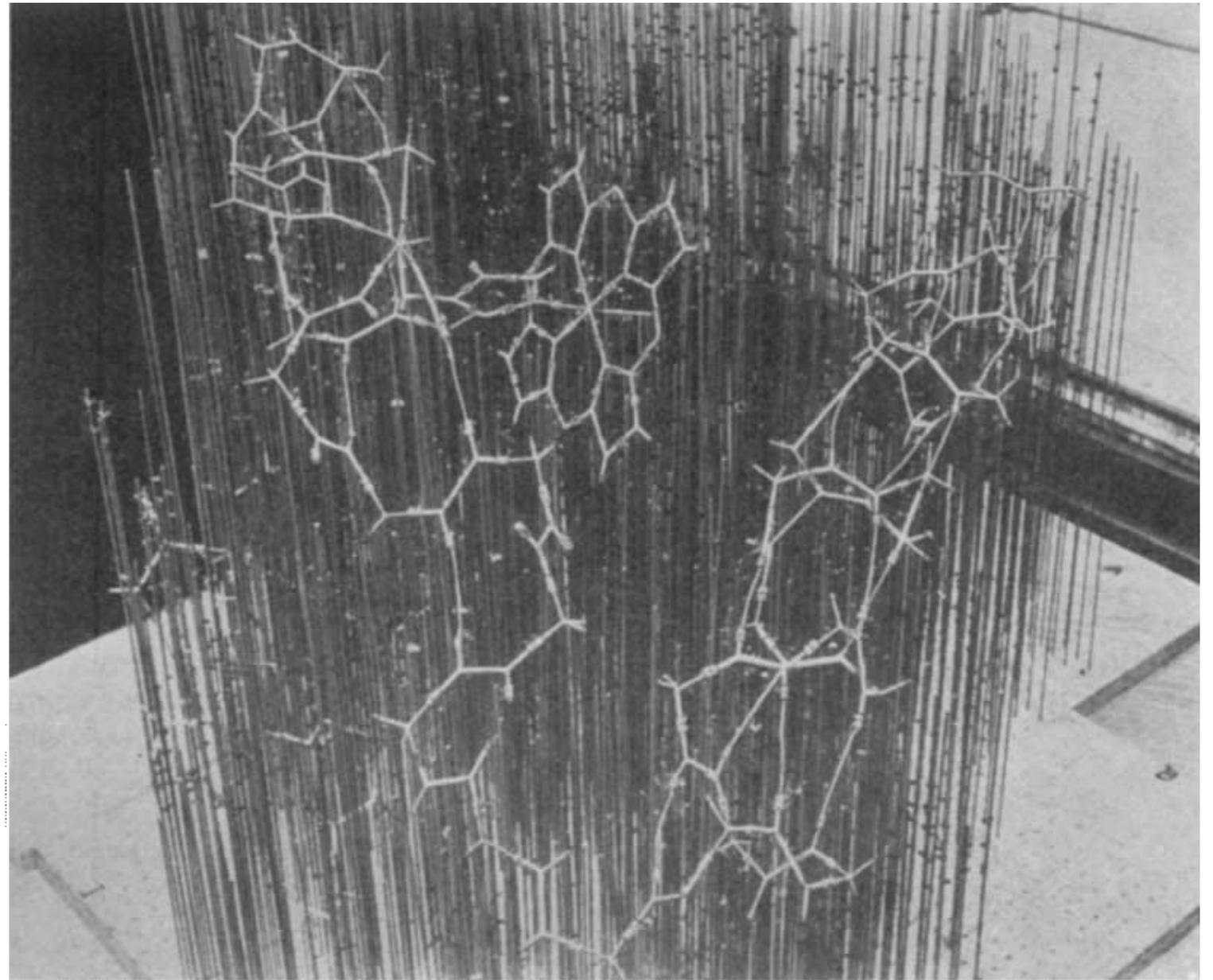


Fig. 4. Photograph of a model of part of the molecule near the h em group, showing the vertical rods and coloured clips which indicate the electron density at each point of the grid, and atomic models of the h em group and neighbouring helices. The terminal carboxyl end of the chain is on the extreme left

“...these results are the first to direct proof that α -helices are present and, indeed enable them to be seen directly for the first time.”

J. C. Kendrew et al. (1960) Nature

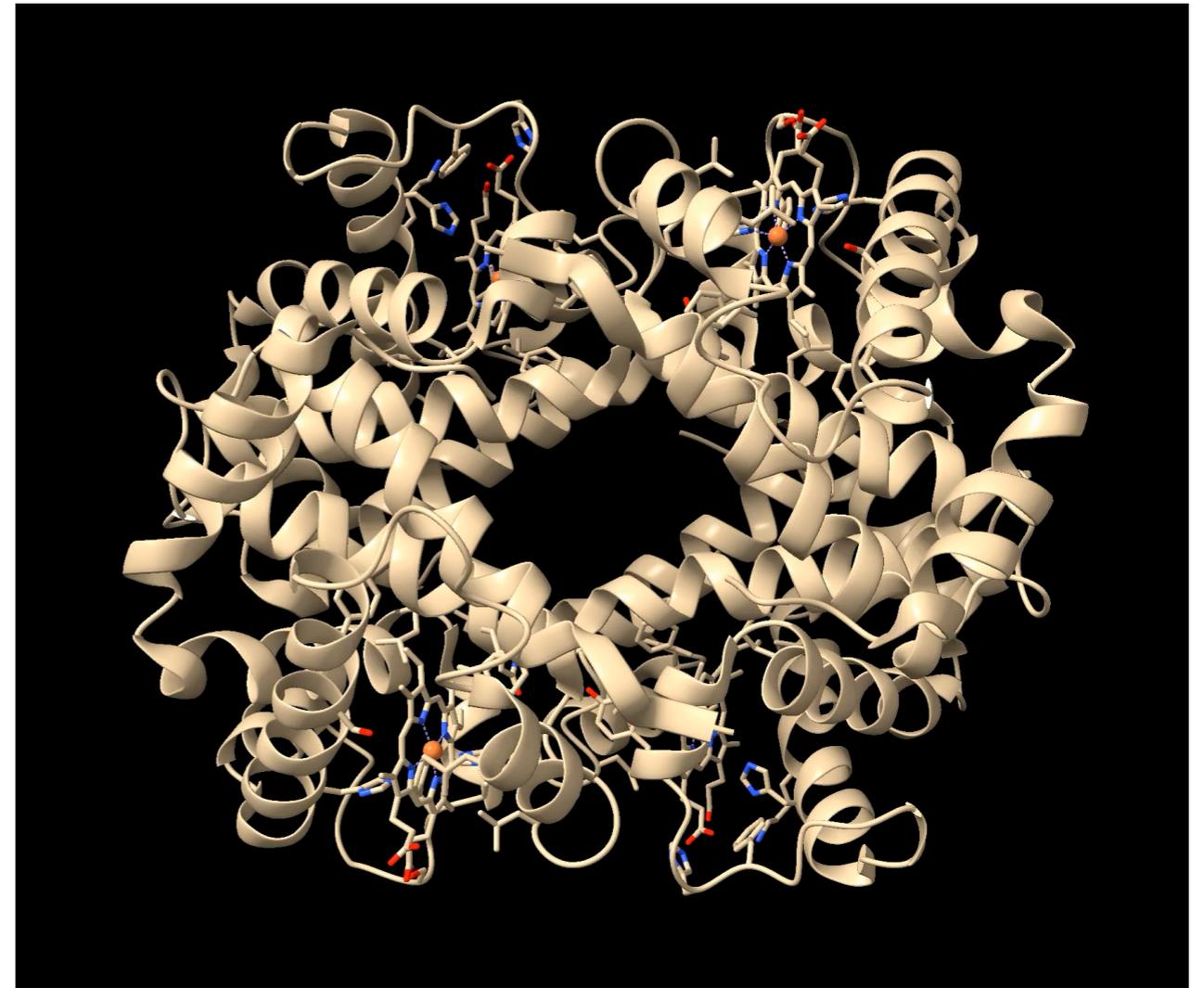


PDB 1MBN

The Stereochemistry of the Protein Myoglobin

[Watson, H.C.](#)

(1969) Prog Stereochem 4: 299

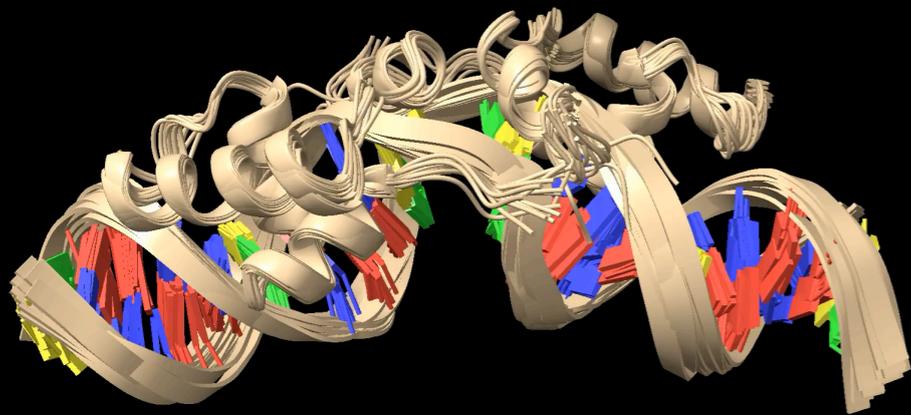


PDB 2DHB

Three dimensional fourier synthesis of horse deoxyhaemoglobin at 2.8 Angstrom units resolution.

[Bolton, W., Perutz, M.F.](#)

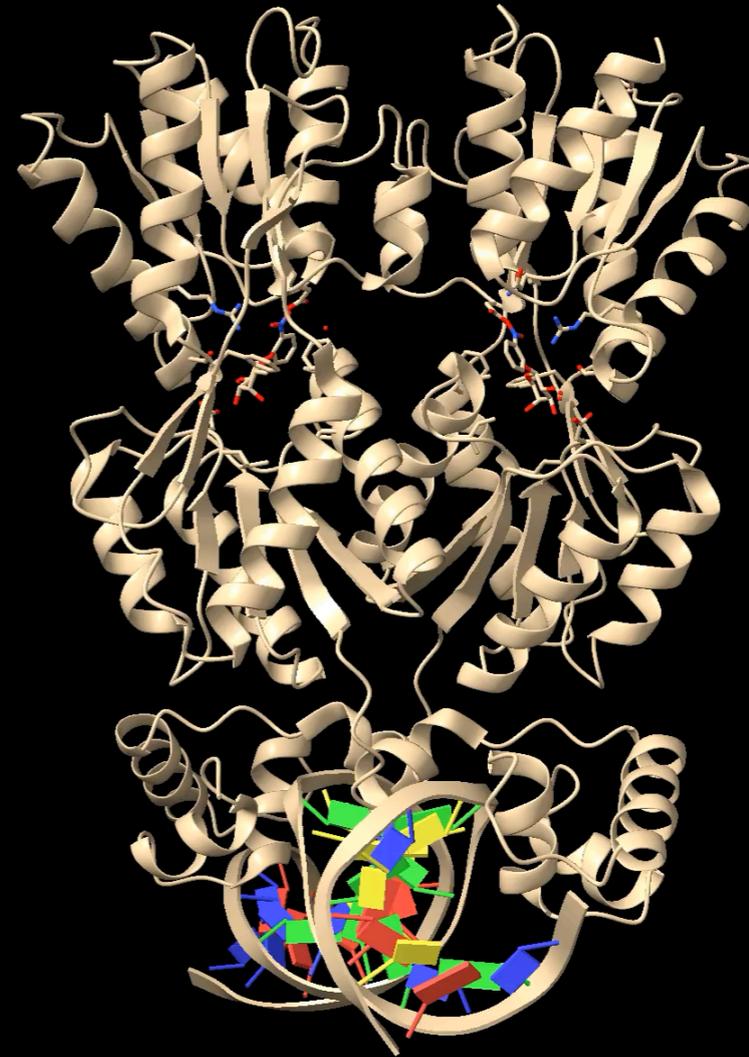
(1970) Nature 228: 551-552



PDB 2JBC

Altered Specificity in DNA Binding by the Lac Repressor: A Mutant Lac Headpiece that Mimics the Gal Repressor

[Salinas, R.K.](#), [Folkers, G.E.](#), [Bonvin, A.M.J.J.](#), [Das, D.](#), [Boelens, R.](#), [Kaptein, R.](#)
(2005) *ChemBiochem* 6: 1628

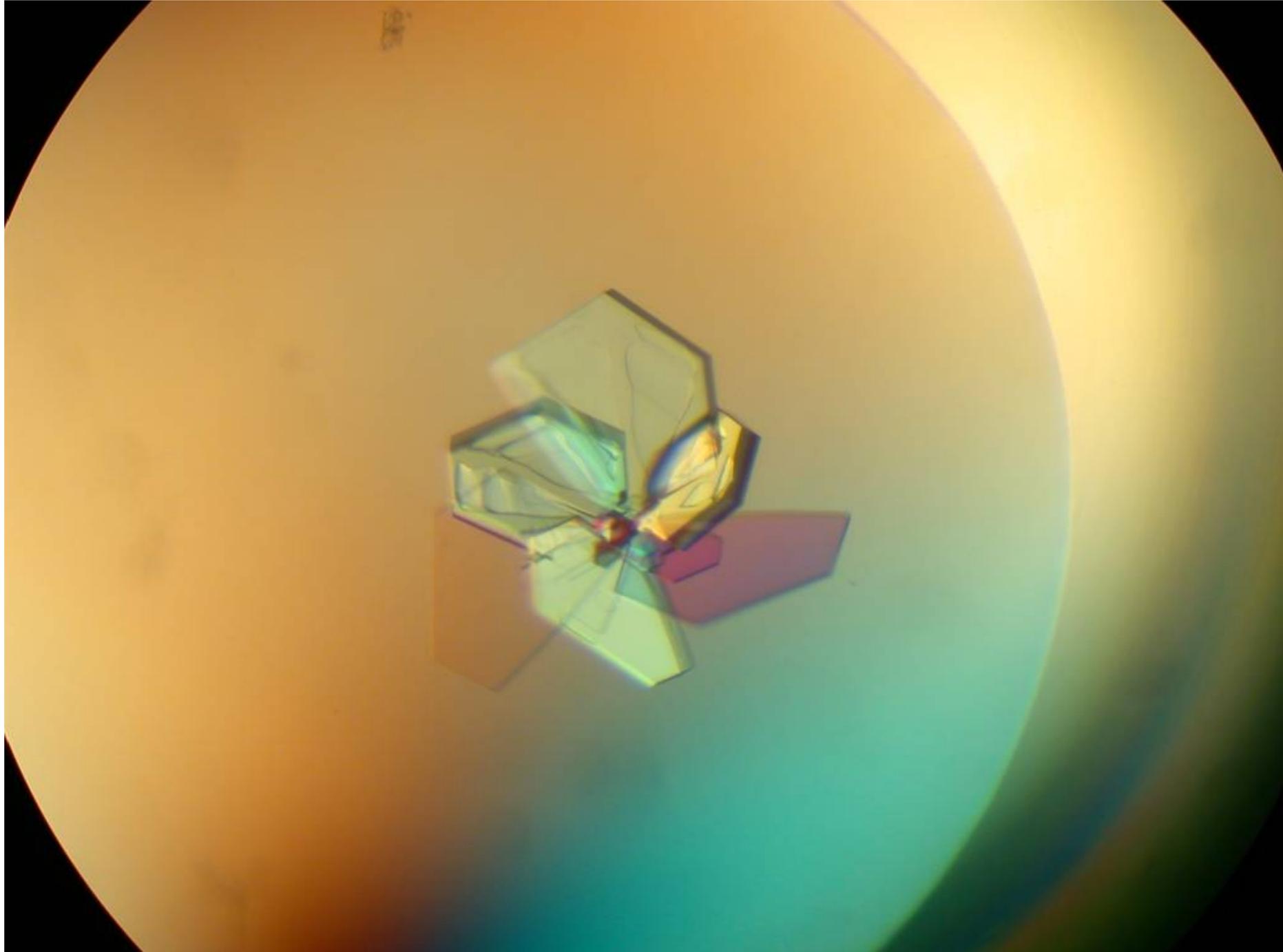


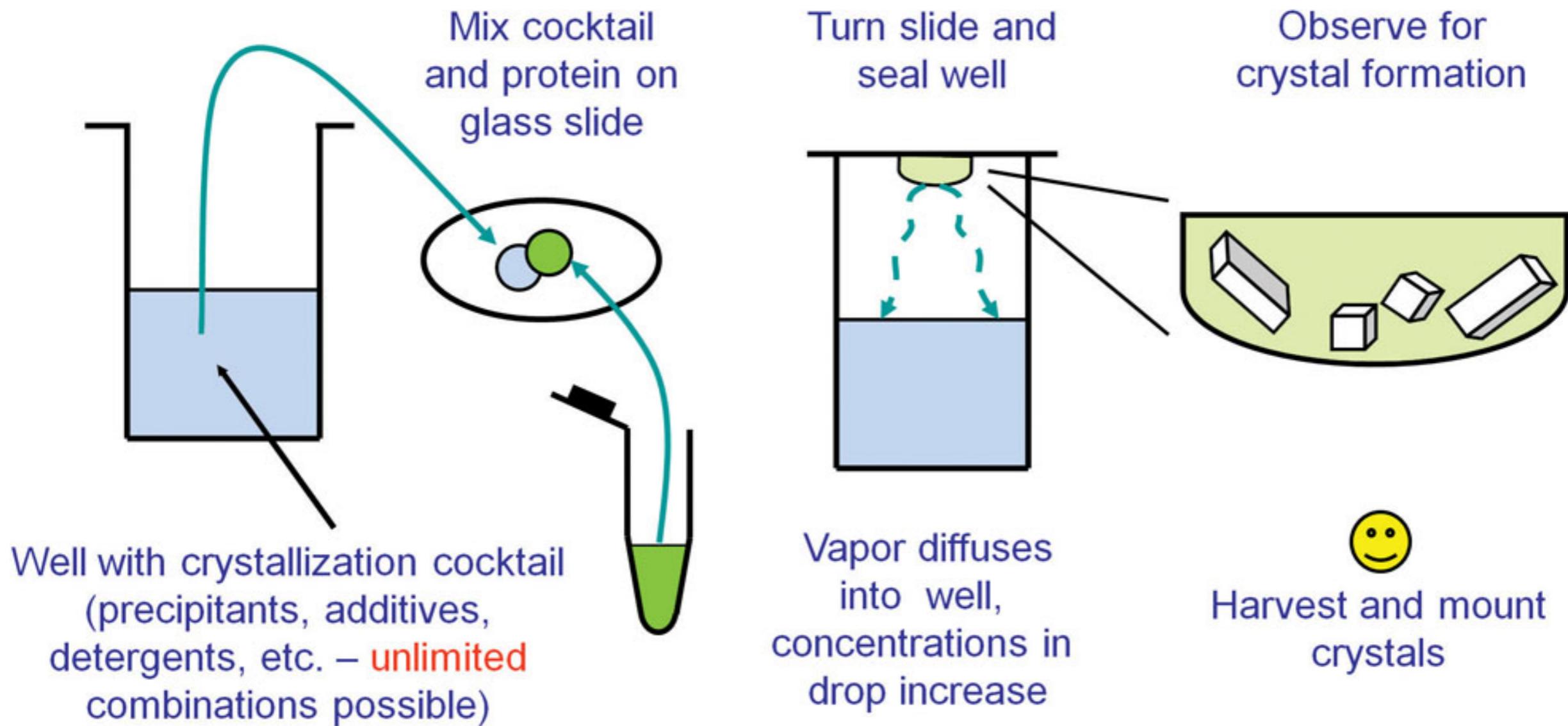
PDB 1EFA

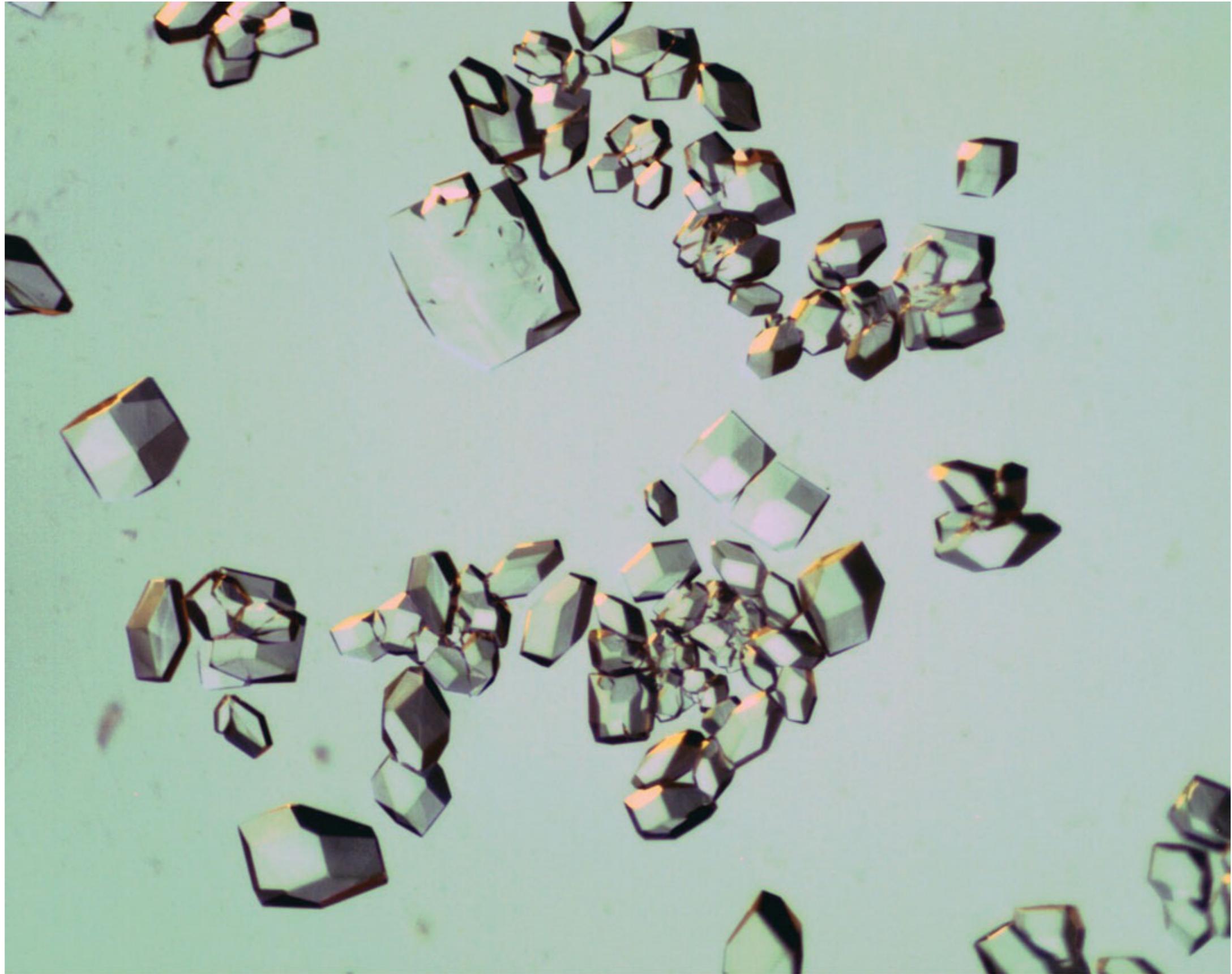
A closer view of the conformation of the Lac repressor bound to operator.

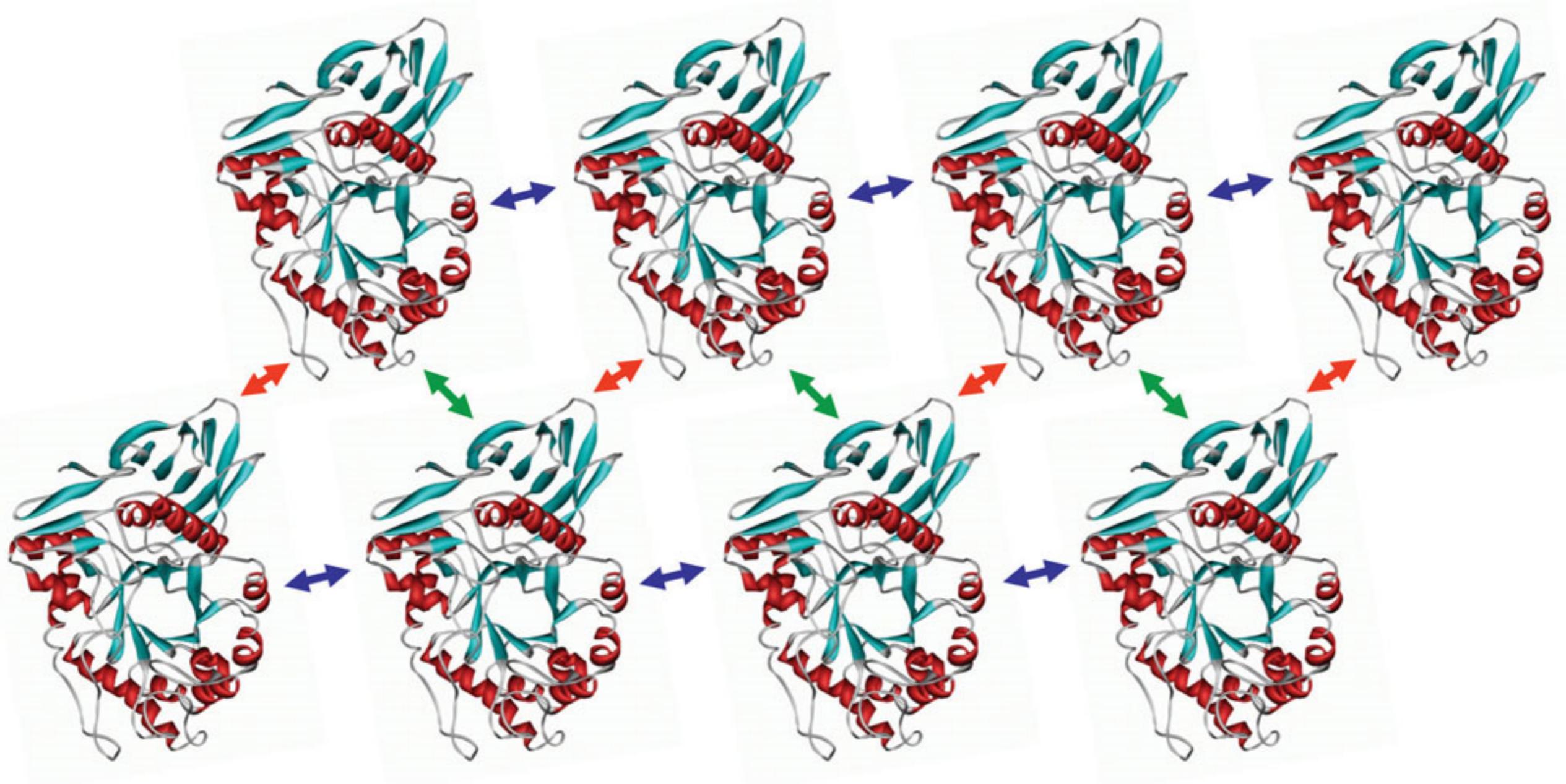
[Bell, C.E.](#), [Lewis, M.](#)
(2000) *Nat Struct Biol* 7: 209-214

Cristalografia



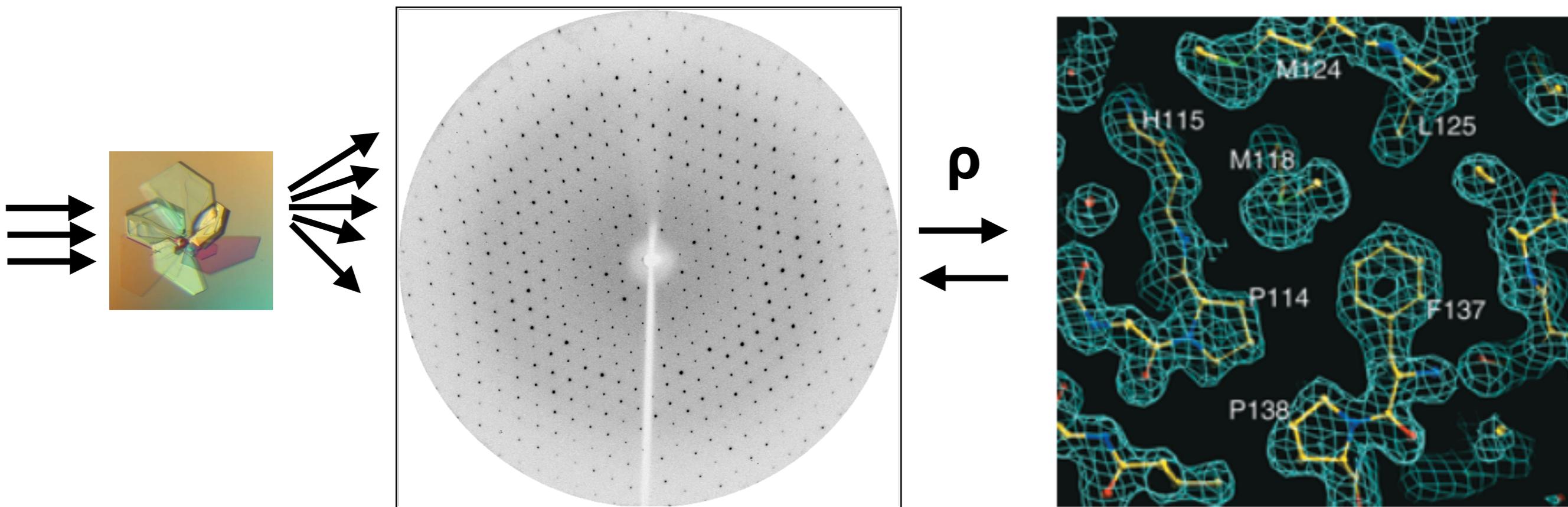




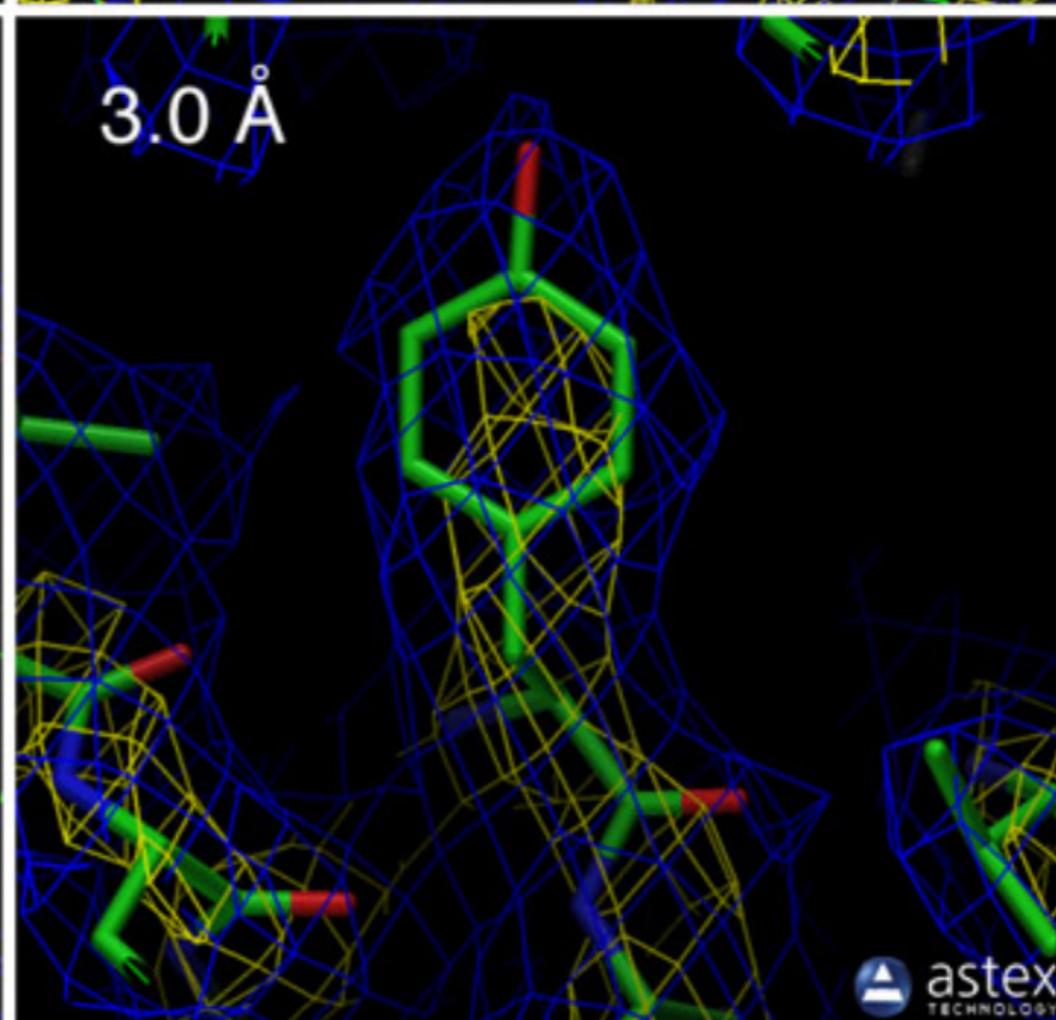
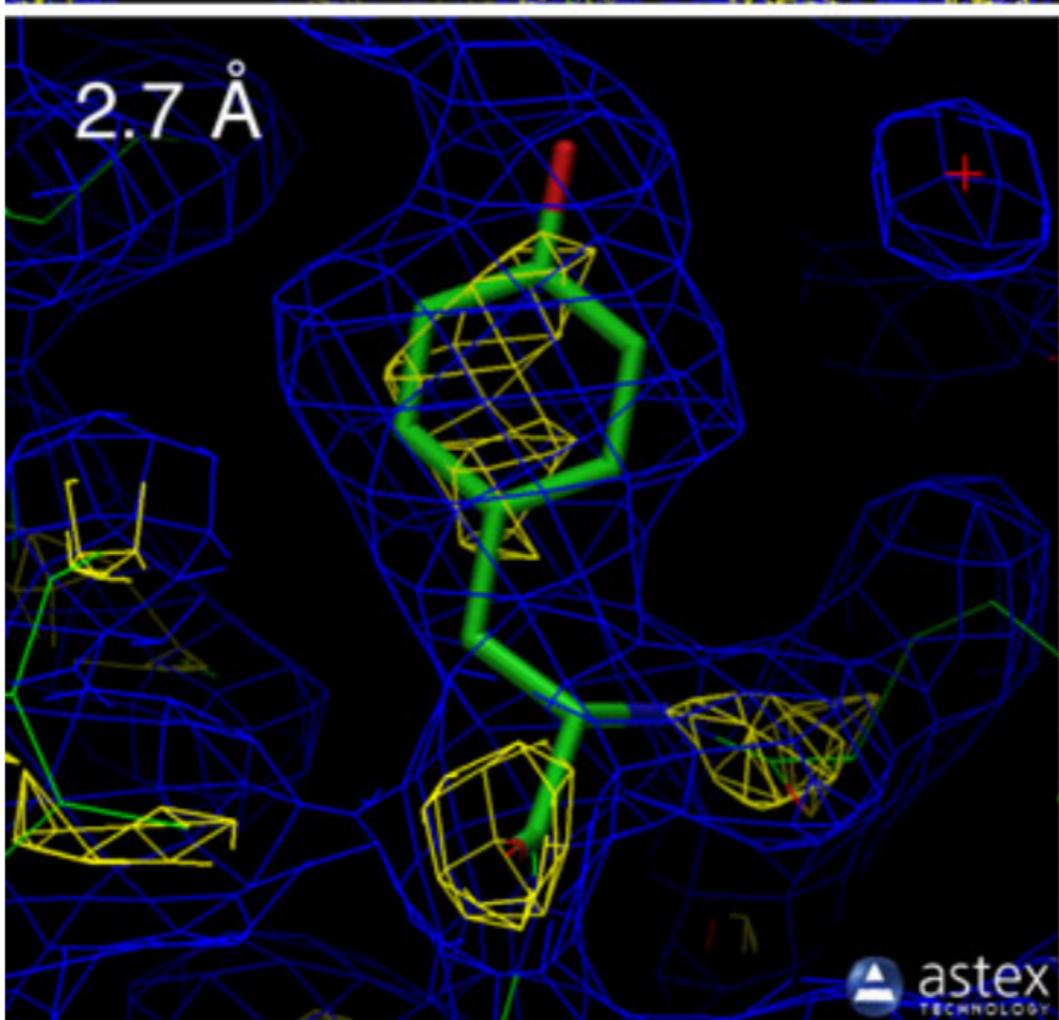
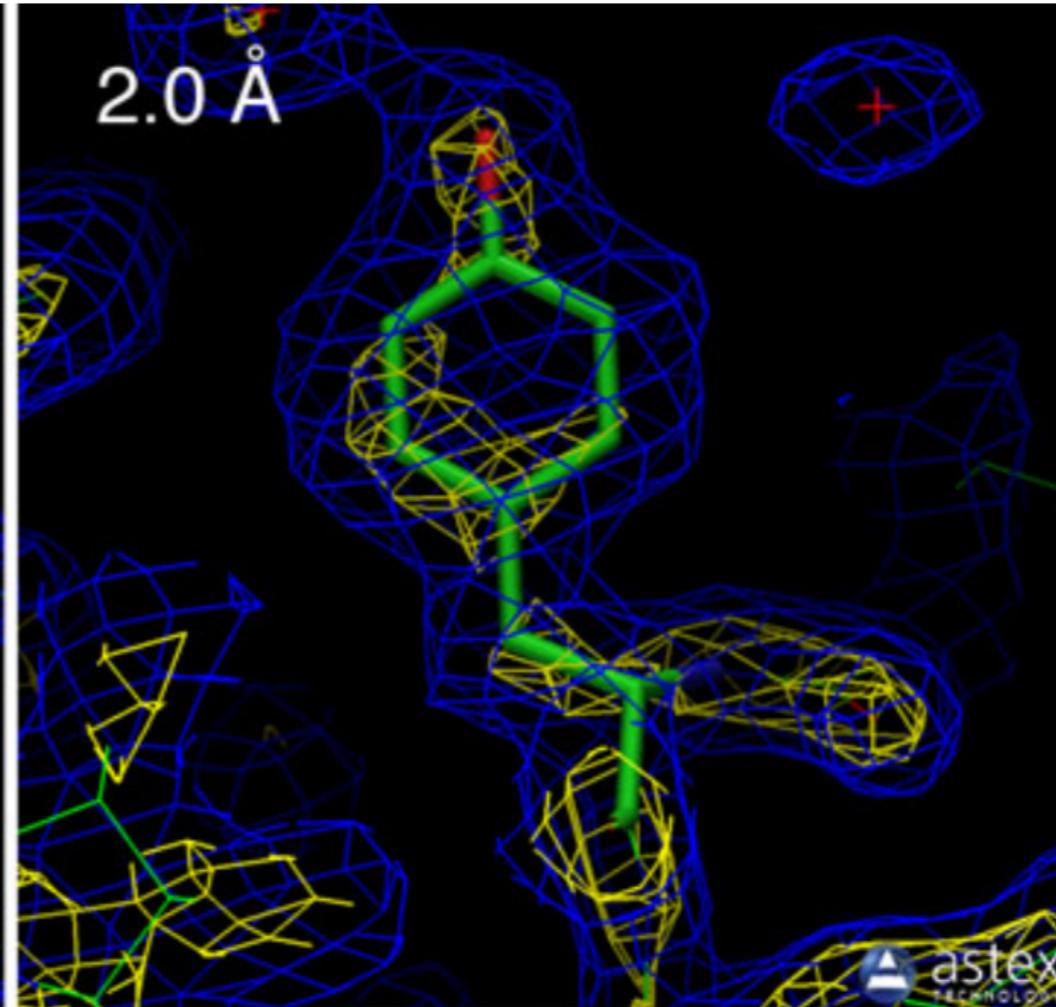
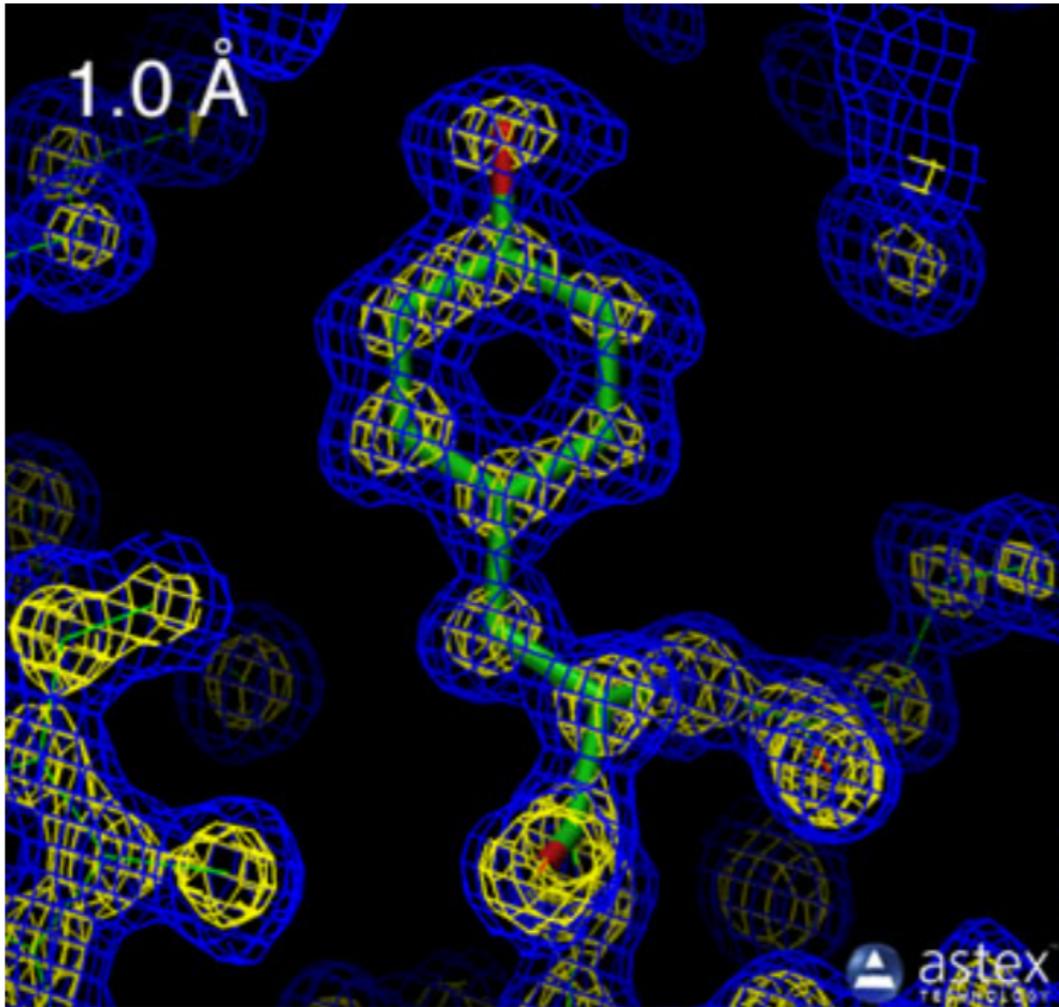


Retículo cristalino

$$\rho(\mathbf{x},\mathbf{y},\mathbf{z}) = (1/V) \sum_h \sum_k \sum_l |\mathbf{F}_{hkl}| e^{-2\pi i(hx+ky+lz + \phi_{hkl})}$$



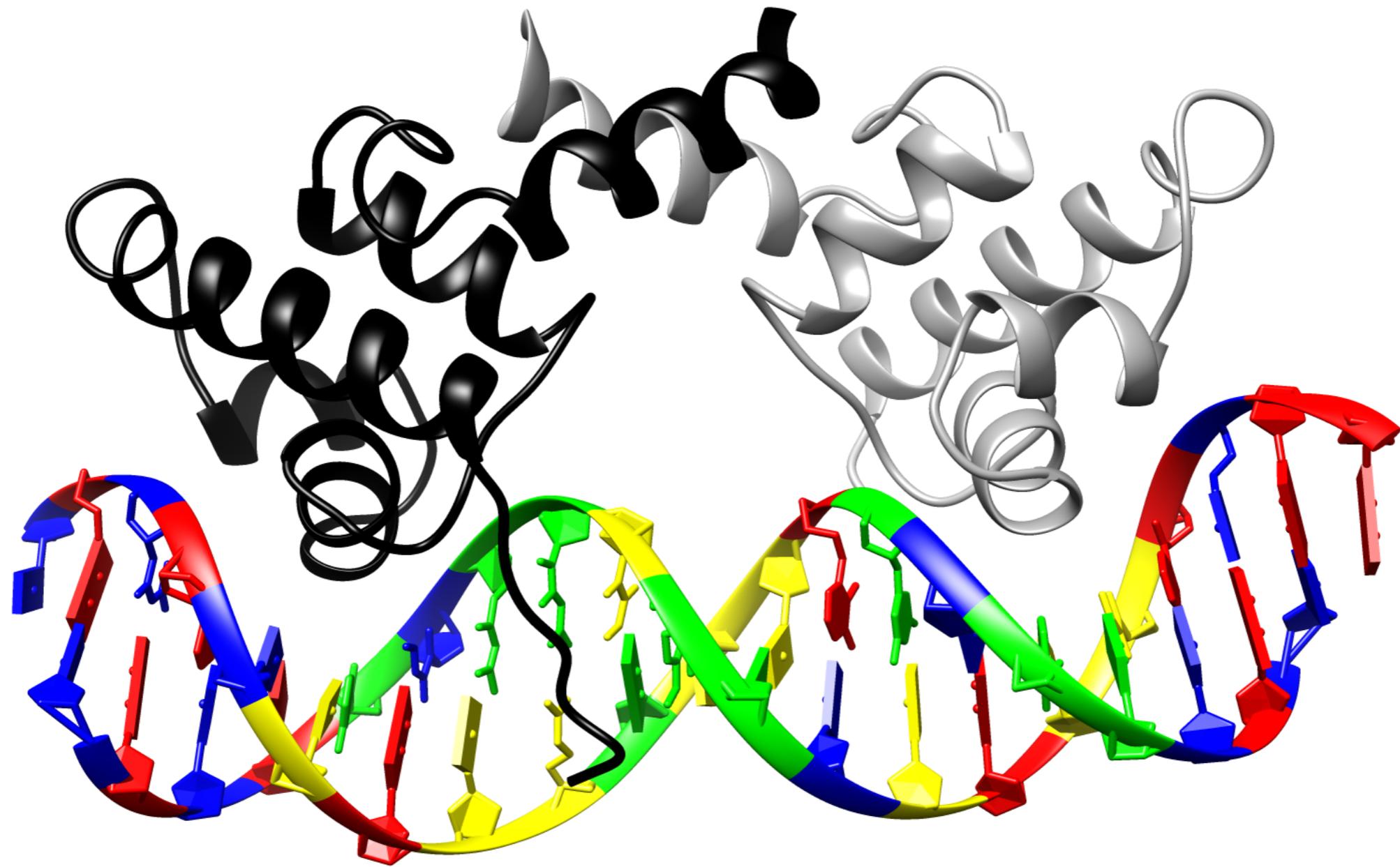
O padrão de difração e o mapa de densidade eletrônica estão relacionados por uma **transformada de Fourier**



Fatores importantes para avaliar uma estrutura cristalográfica

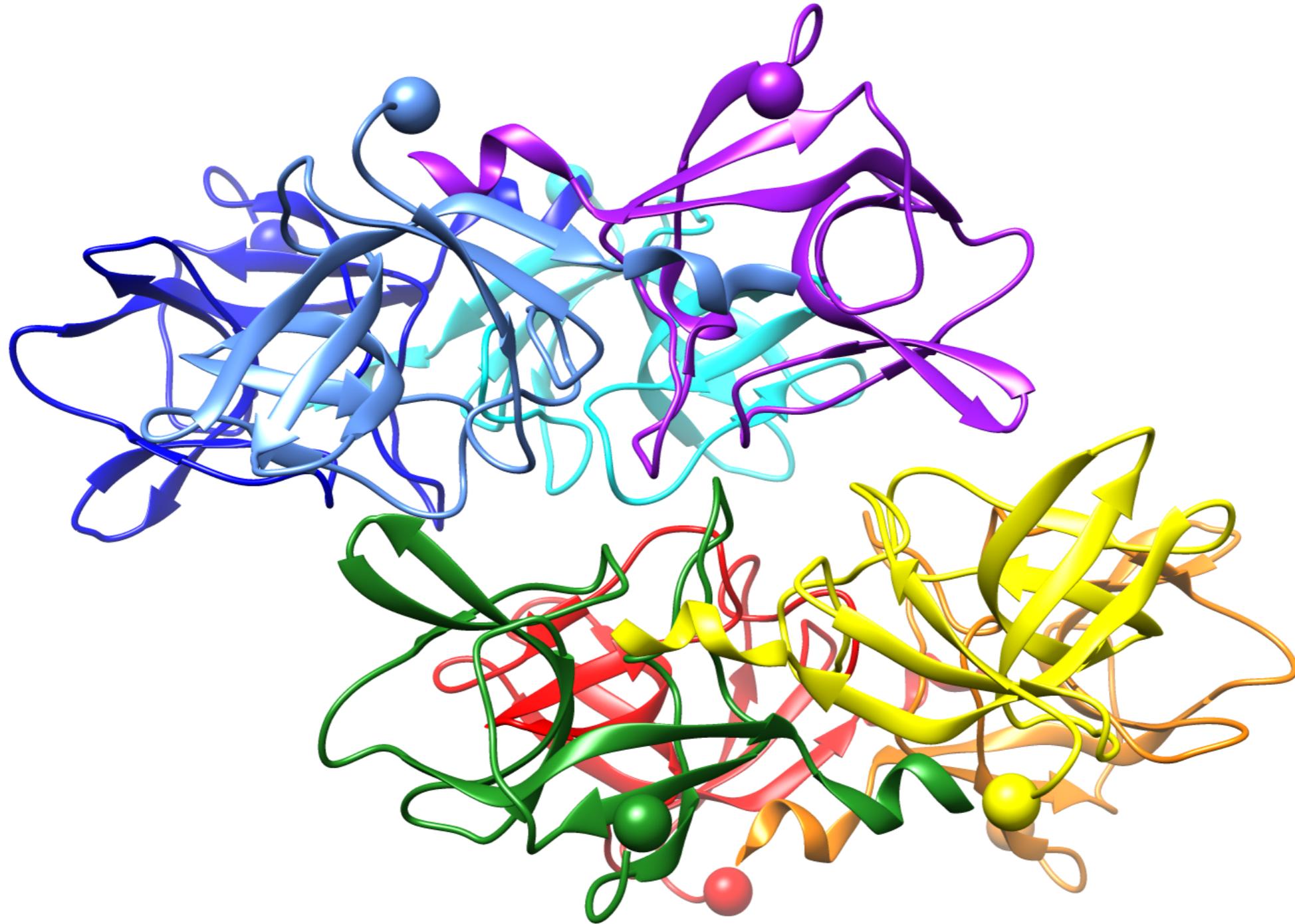
- Resolução do mapa
- R-factor (R work): mede a concordância do modelo com o mapa. Boas estruturas R-factor < 20%
- R-free: mede a capacidade do modelo de explicar um pequeno conjunto de reflexões não utilizadas no refinamento
- Estatísticas do Ramachandran
- RMSD de ligações de ângulos
- Clash score

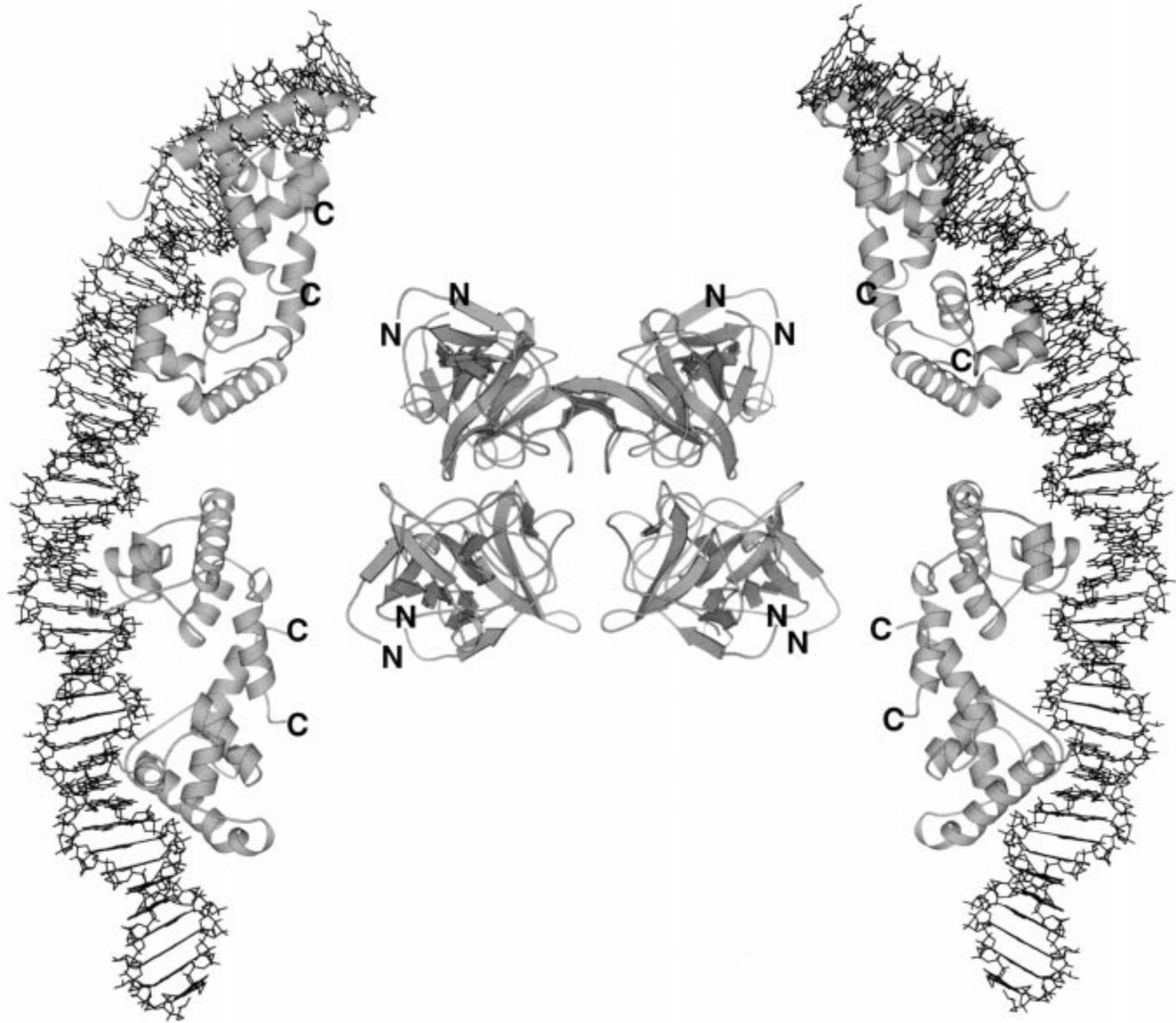
Fatores de transcrição em bactéria: LamdaR em complexo com o operador



Beamer & Pabo (1992)

LambdaR forma um octômero





Bell & Lewis, 2001

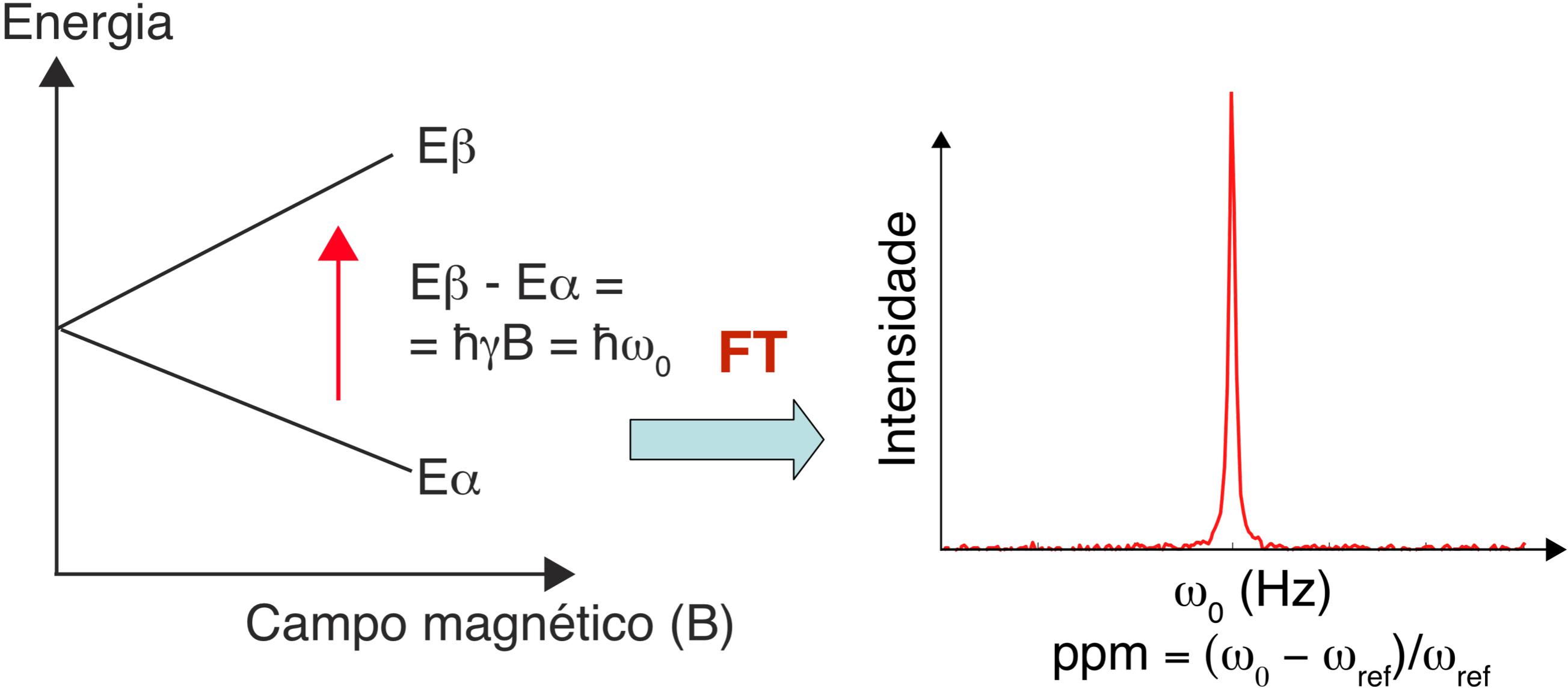
Ressonância Magnética Nuclear

Solution NMR sample

- pure, stable and high concentration
- 500 μL of 0.5 mM solution \rightarrow \sim 5 mg per sample
- preferably low salt, low pH
- no additives



RMN detecta transições entre estados do spin nuclear na presença de um campo magnético estático

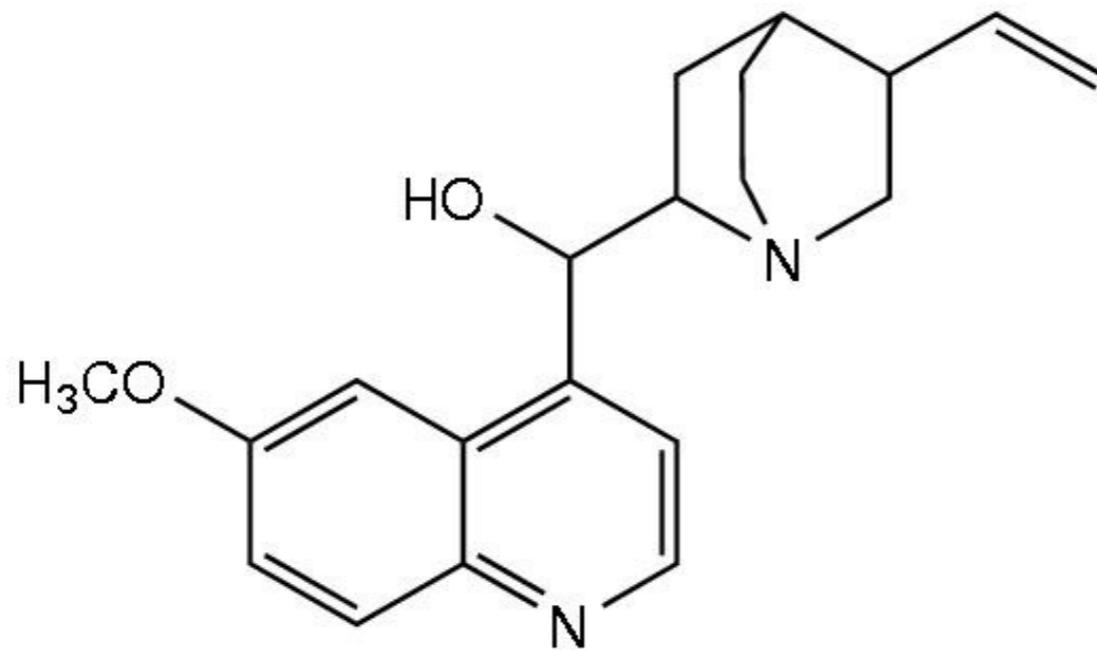


Efeito Zeeman; Exemplo para spin 1/2

Núcleos frequentemente observados em moléculas biológicas

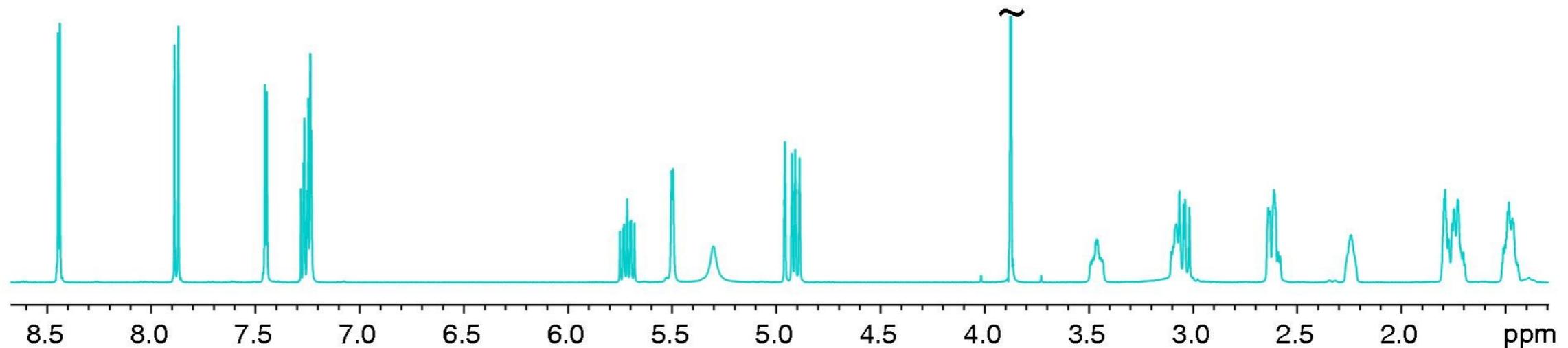
Núcleo	I	γ (T s ⁻¹)	Abundância natural
¹ H	1/2	26.75 x 10 ⁷	99.99
² H	1	4.107 x 10 ⁷	0.012
¹³ C	1/2	6.728 x 10 ⁷	1.07
¹⁵ N	1/2	-2.713 x 10 ⁷	0.37
³¹ P	1/2	10.839 x 10 ⁷	100.00

Deslocamento químico: a frequência de ressonância do spin nuclear depende da densidade eletrônica ao redor do núcleo



More conveniently expressed as **parts per million (ppm)** by comparison to a reference frequency:

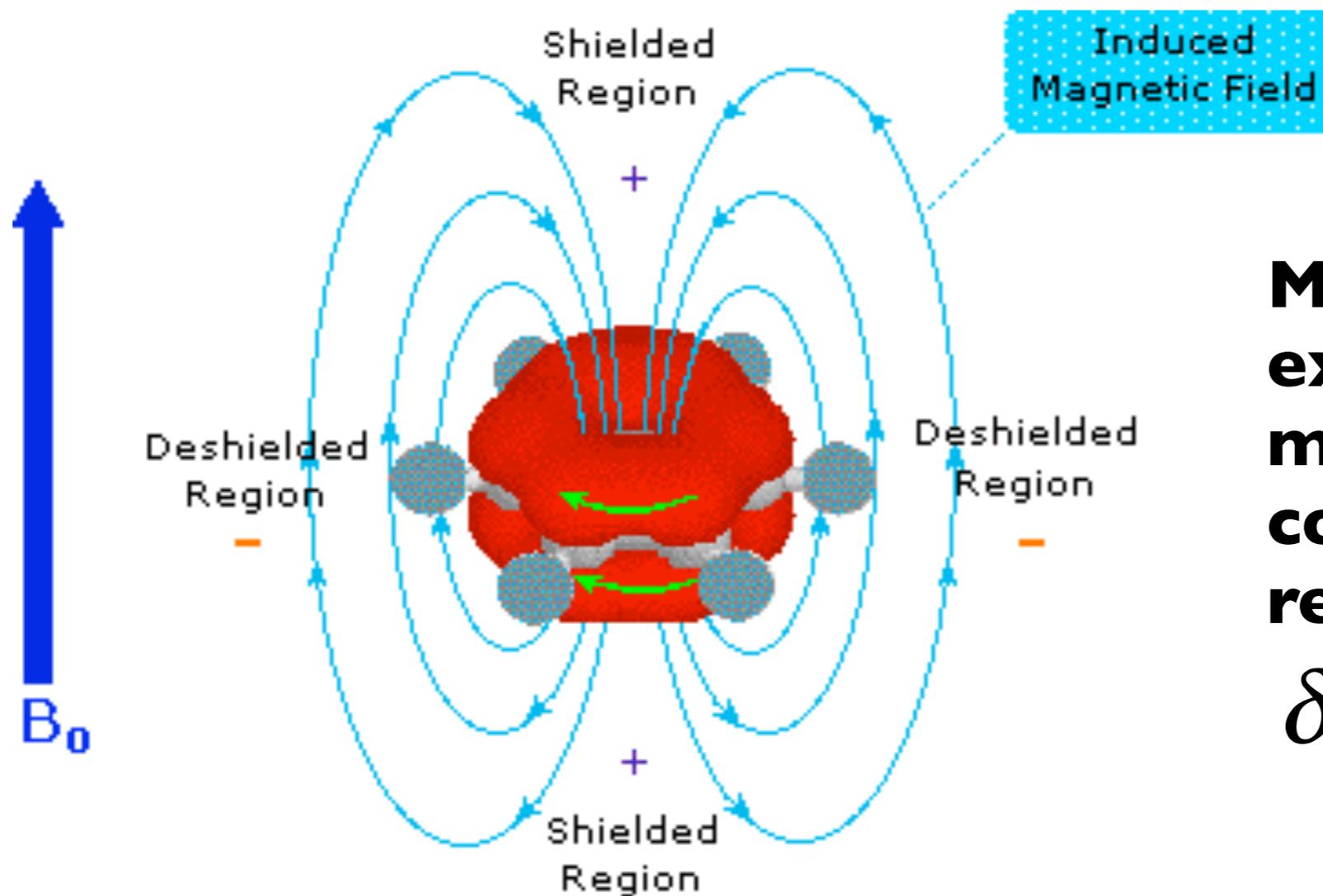
$$\delta = 10^6 \frac{\nu - \nu_{ref}}{\nu_{ref}}$$



Chemical shift

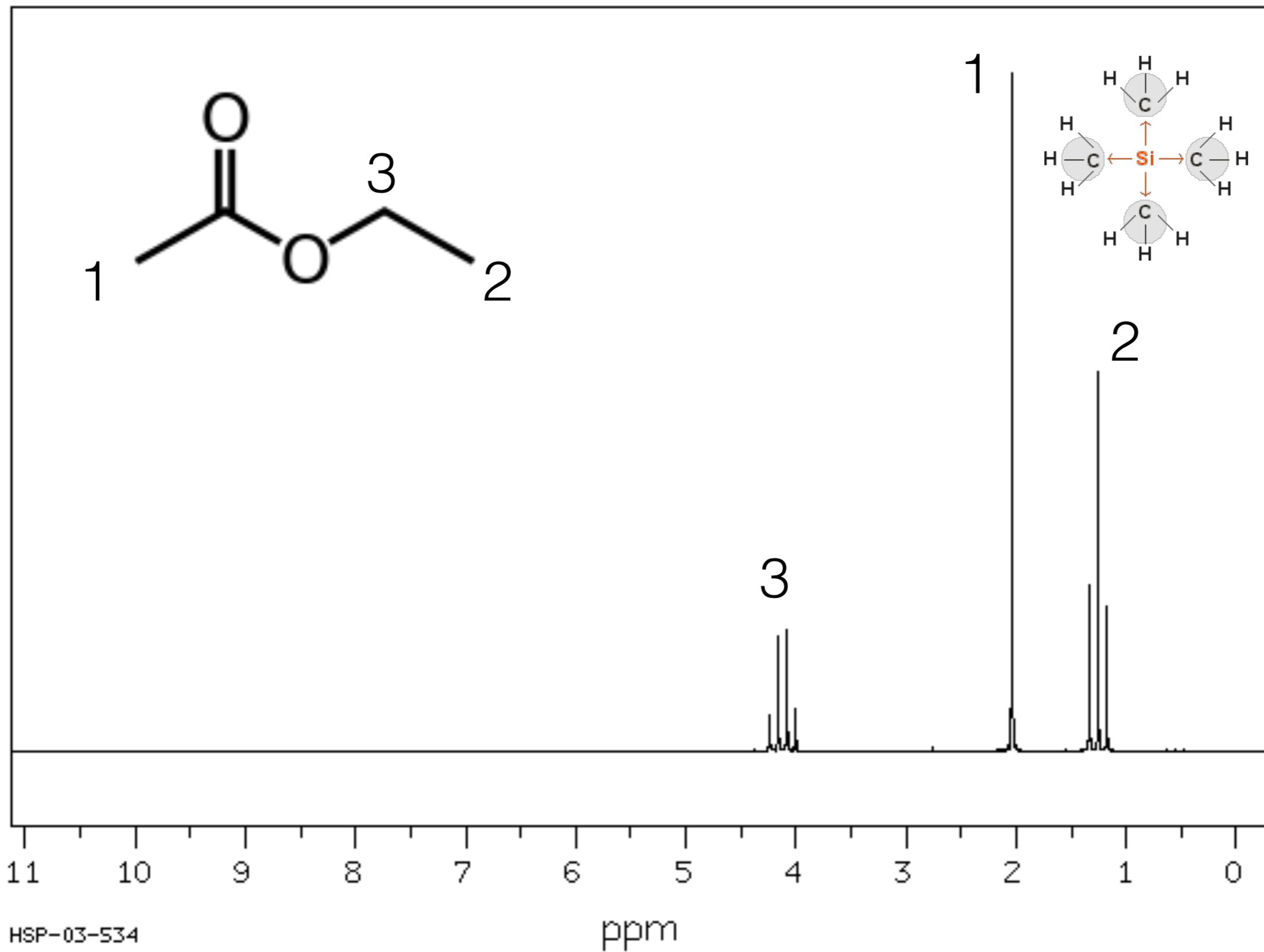
$$\nu = \frac{\gamma B_0}{2\pi} (1 - \sigma)$$

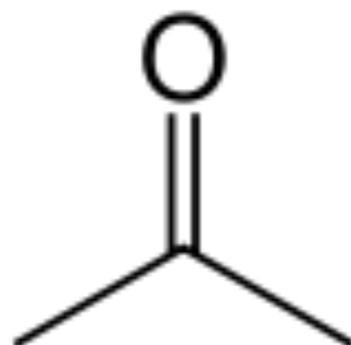
shielding constant



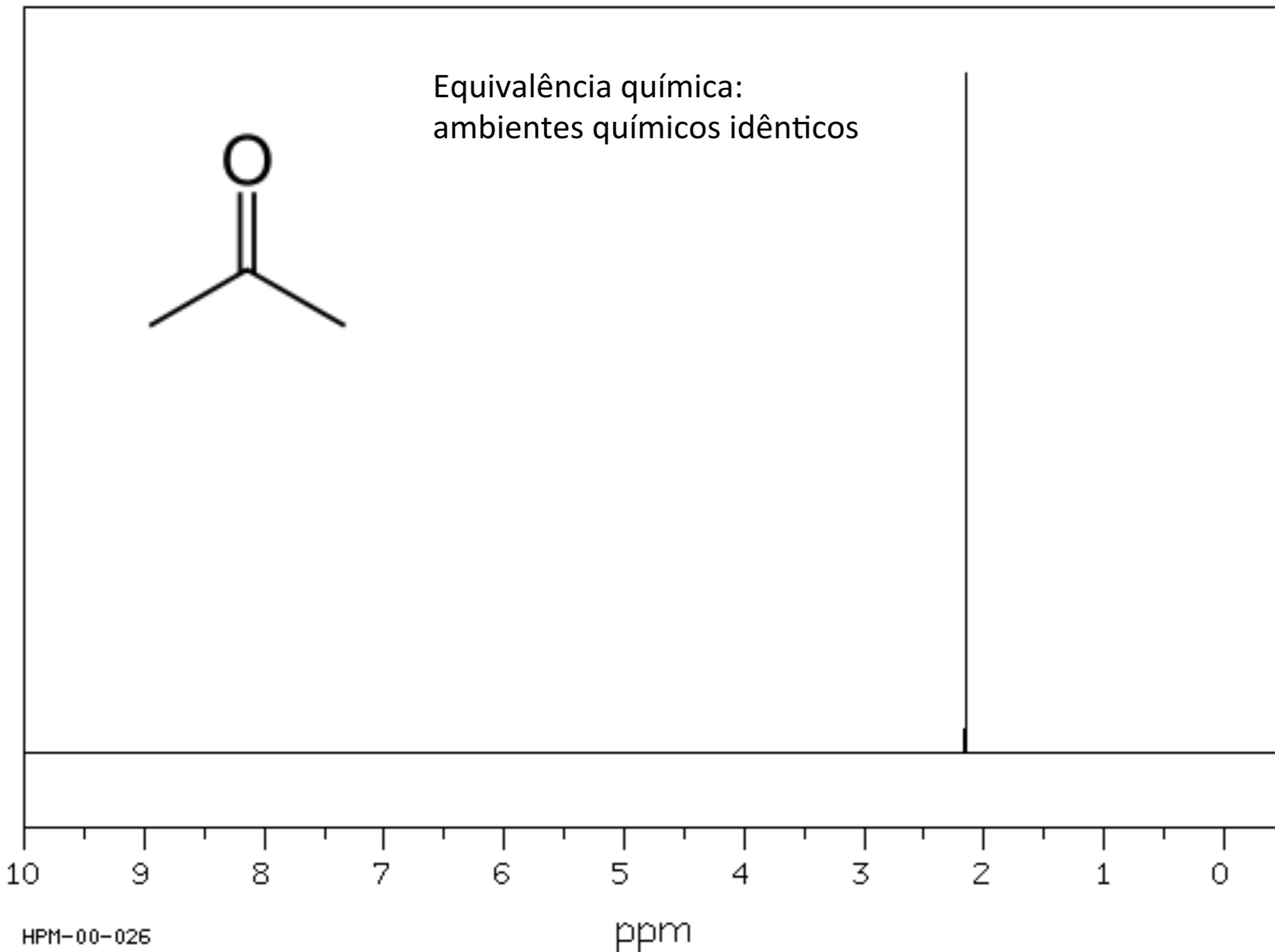
More conveniently expressed as part per million by comparison to a reference frequency:

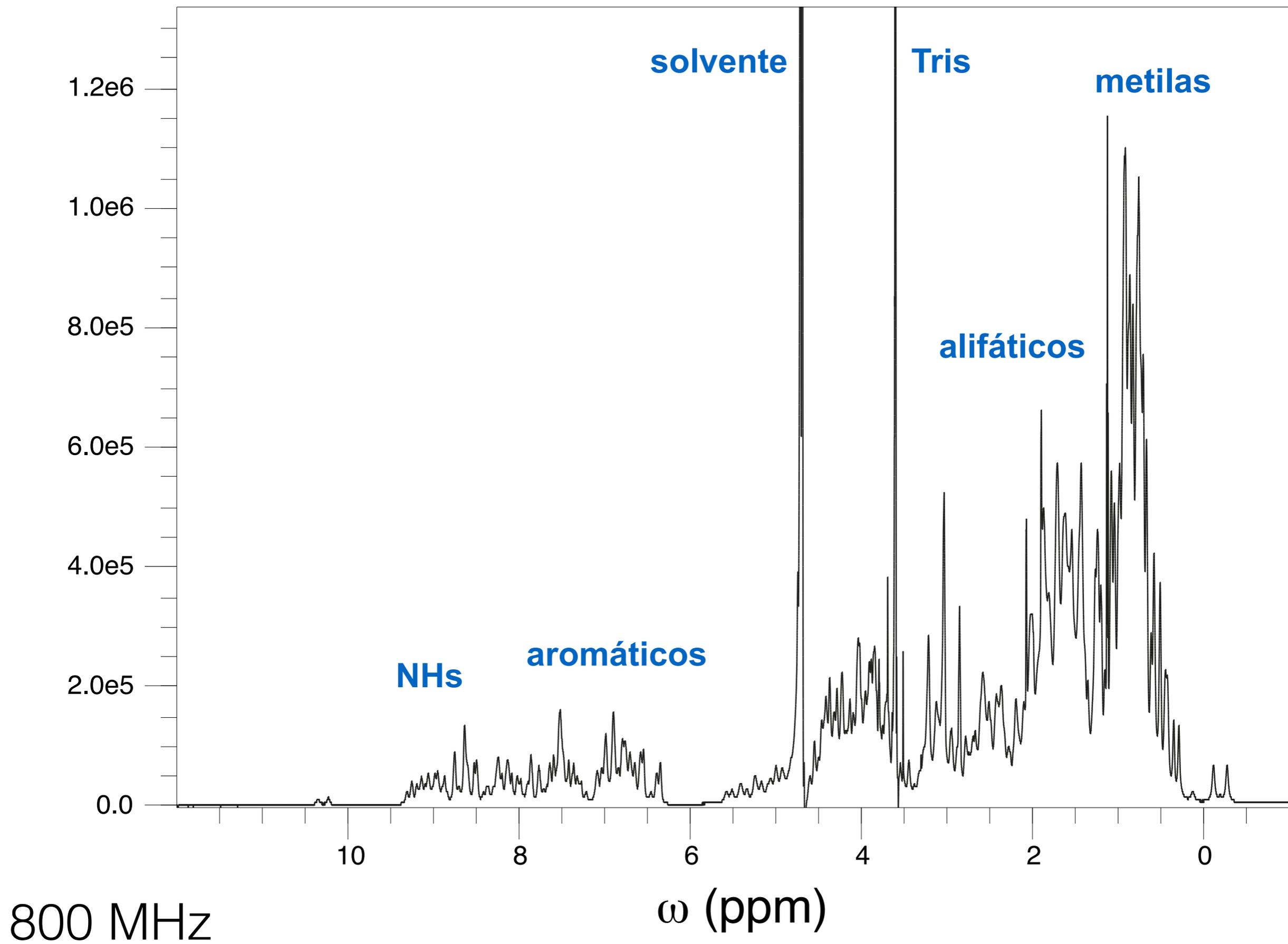
$$\delta = 10^6 \frac{\nu - \nu_{ref}}{\nu_{ref}}$$



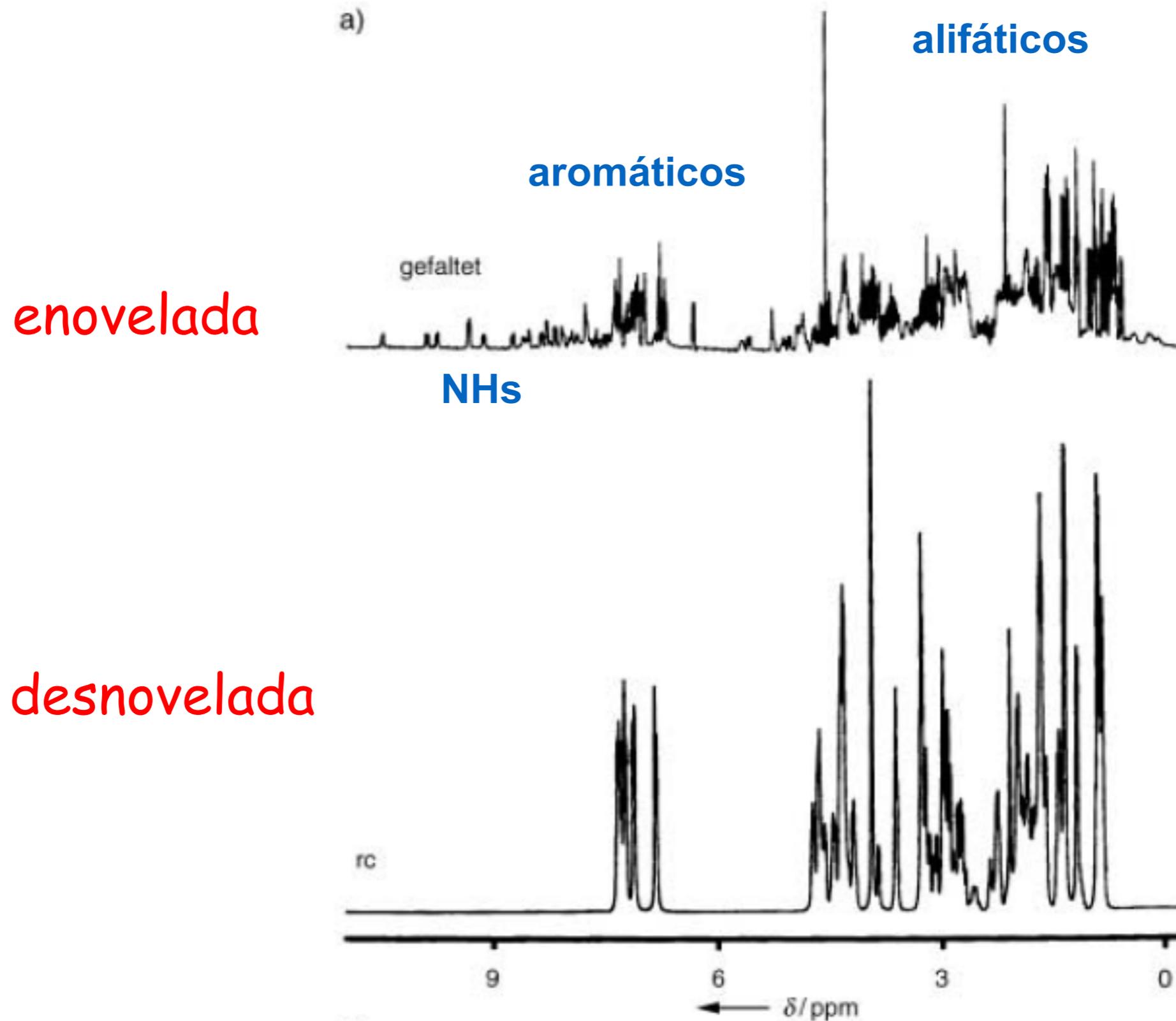


Equivalência química:
ambientes químicos idênticos





RMN de proteínas

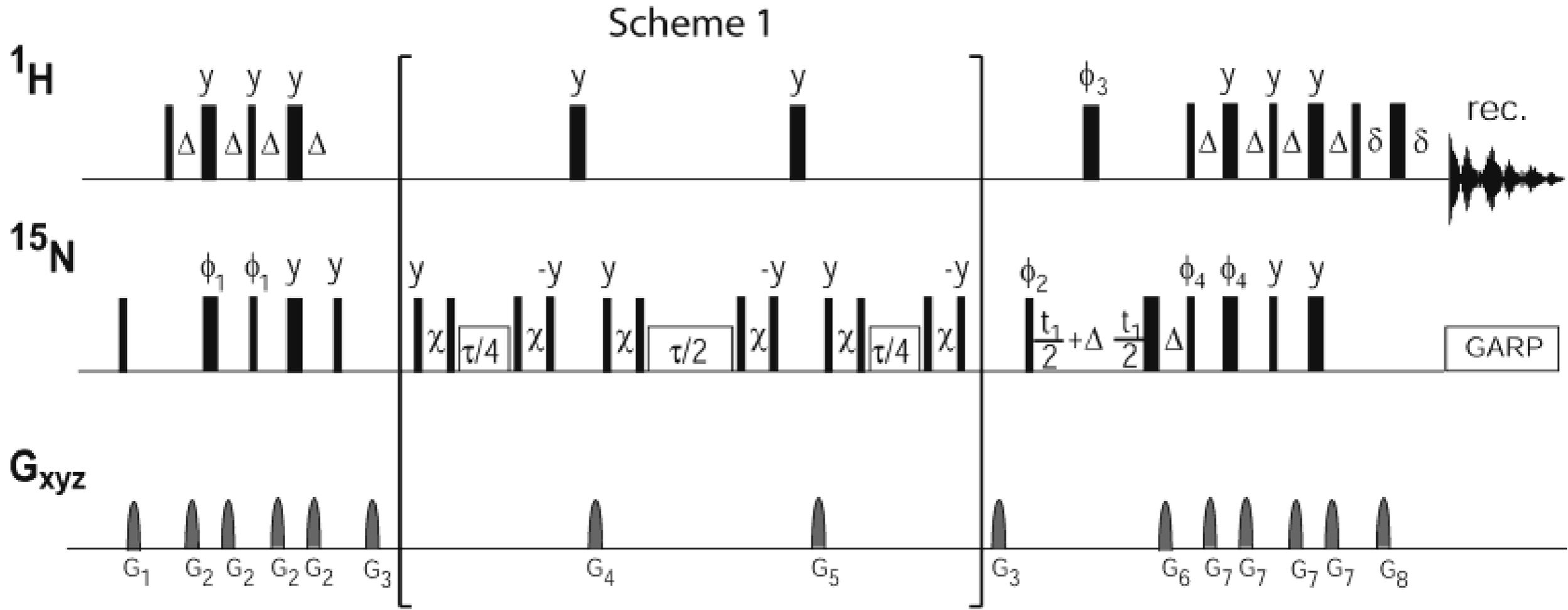


Enriquecimento isotópico

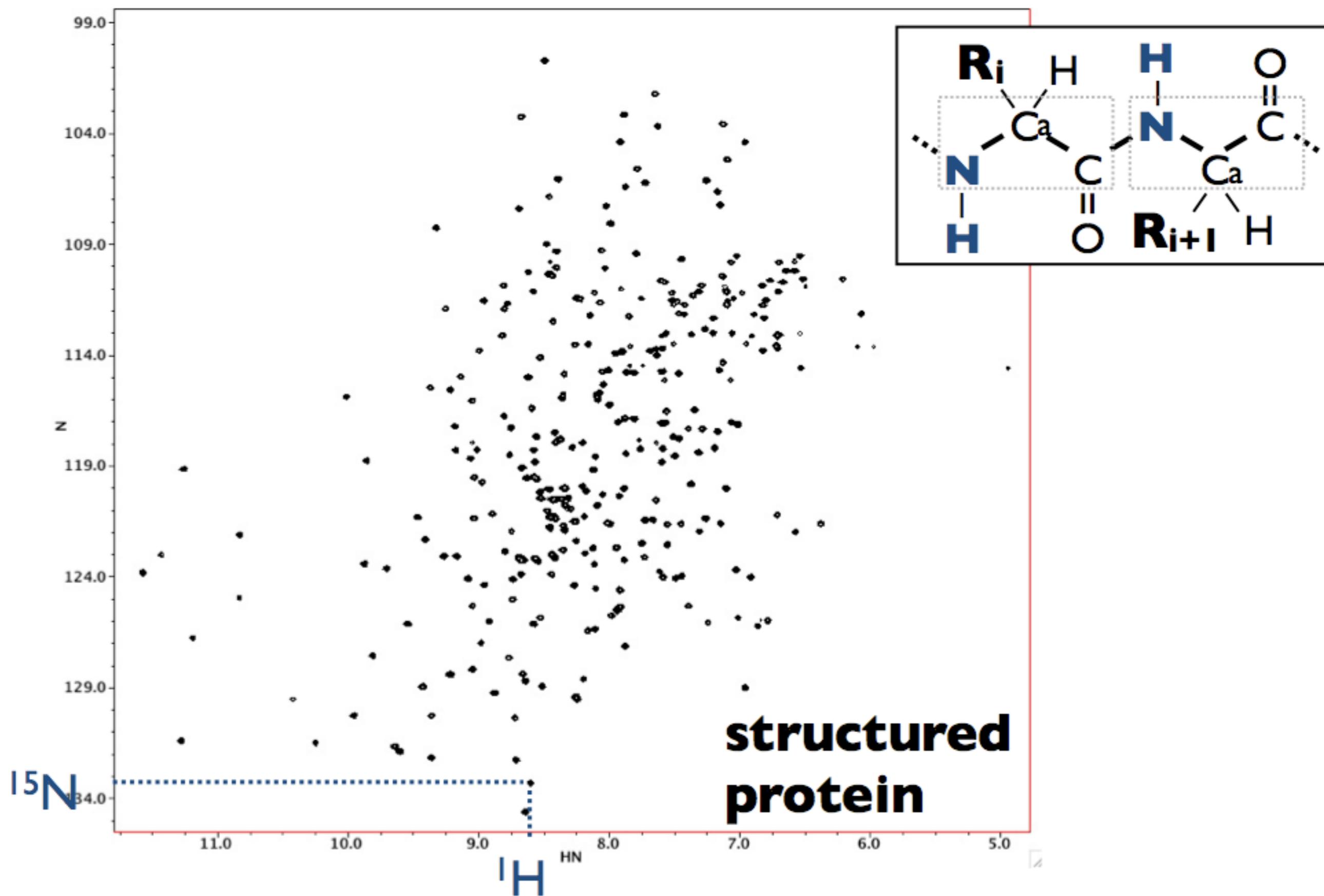
Núcleo	I	γ (T s ⁻¹)	Abundância natural
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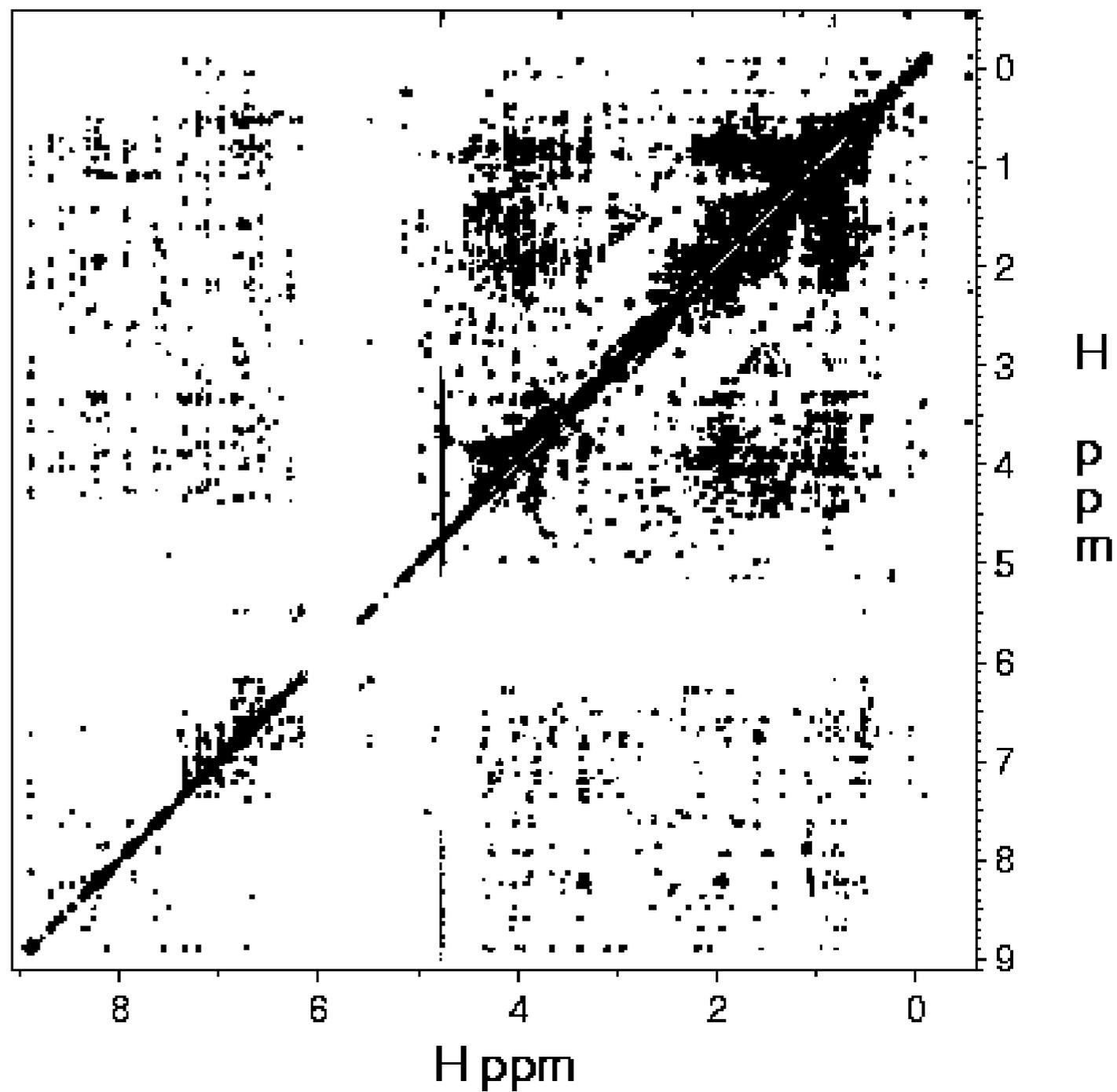
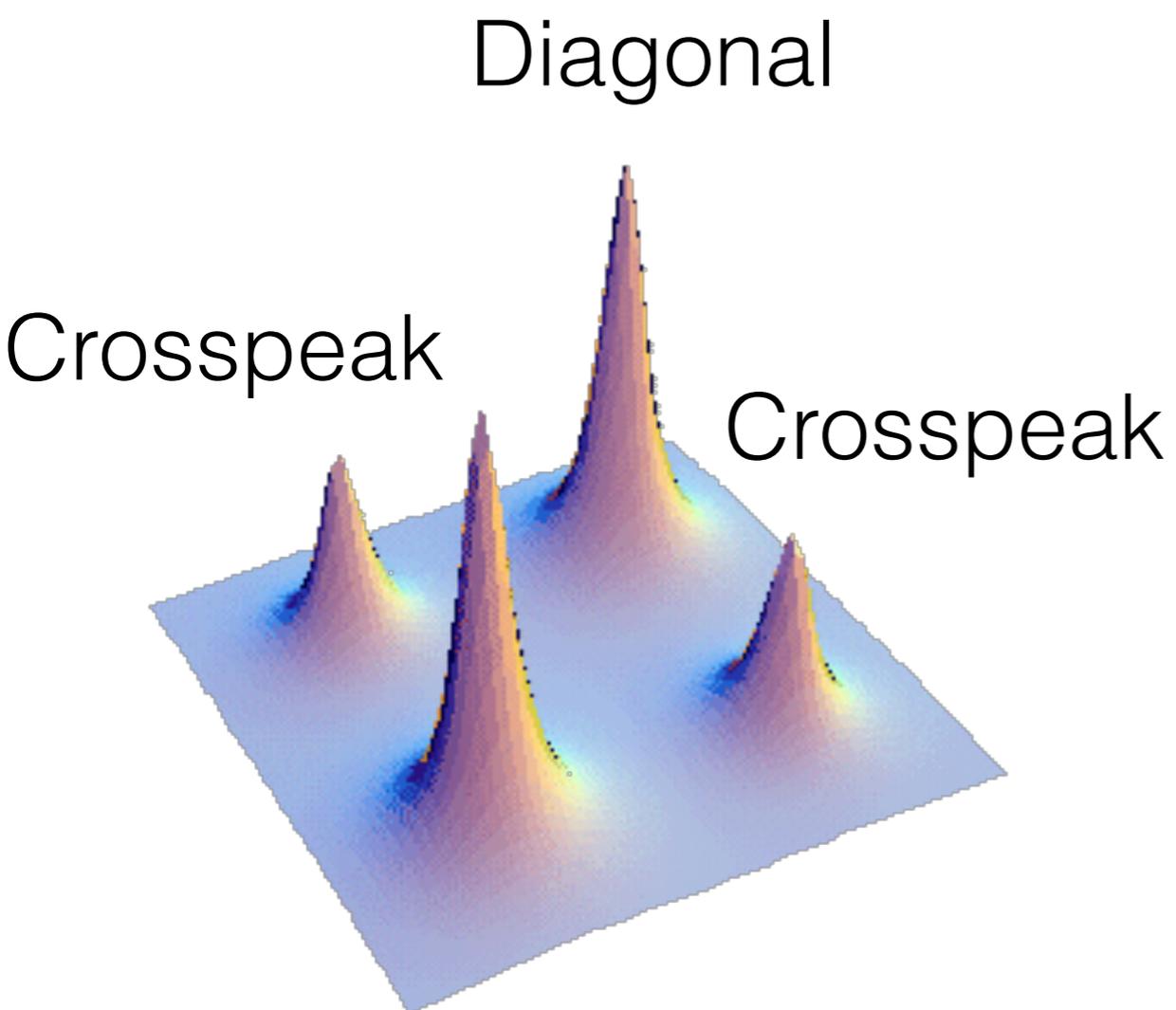
Sequências de pulsos modernas



^{15}N -HSQC: fingerprint



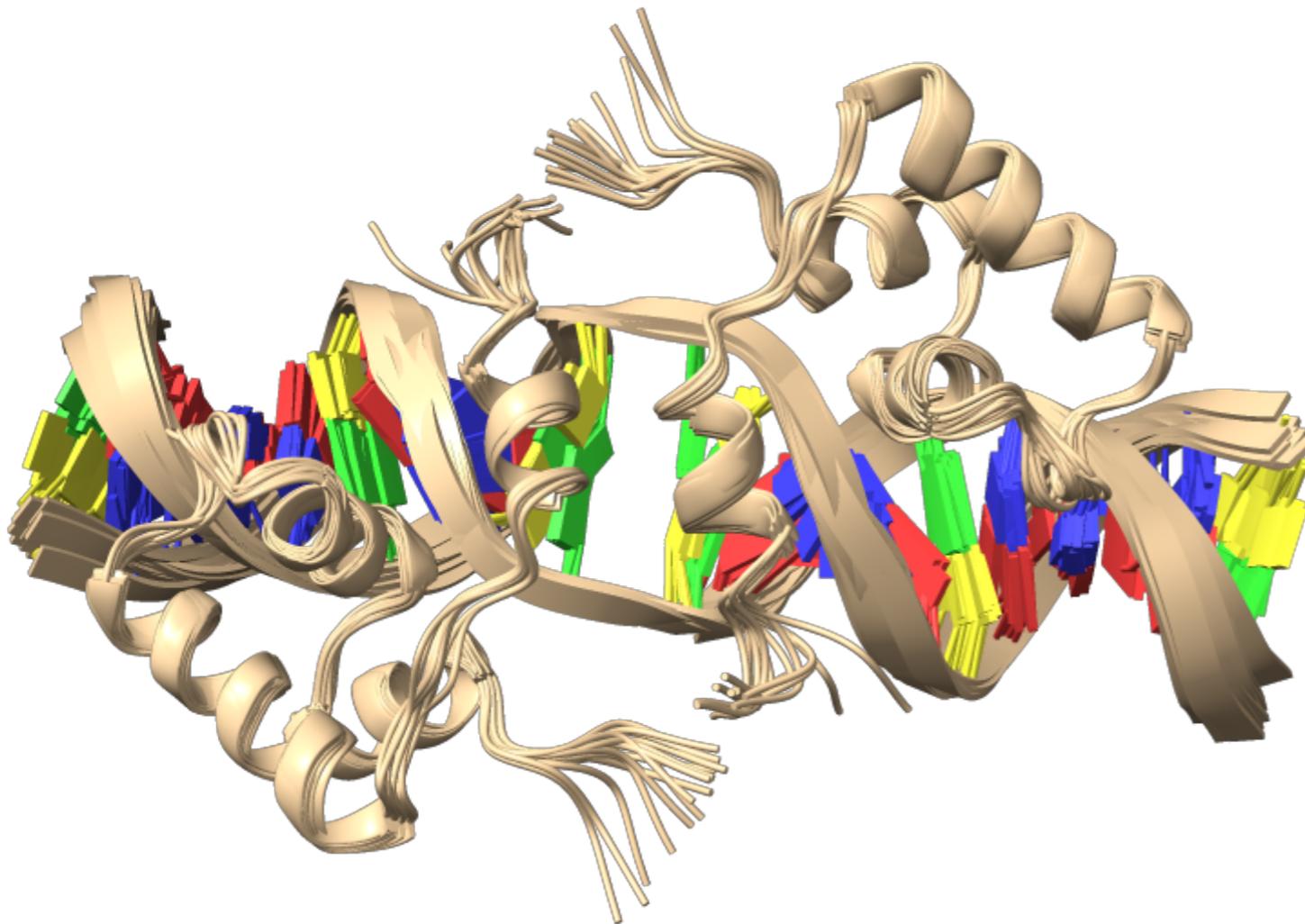
2D NOE



NMR & Structural biology

Structure determination

- **NMR enables the calculation of high-resolution protein 3D structures in solution**



1. Unambiguous restraints

Intraresidual	535
Sequential	675
Medium range	239
Long range	767
Intermolecular	87

2. Talos dihedral angles

140

3. Consistent violations

Distances > 0.5 Å	0
Torsion angles > 5°	0

4. RMS deviation from the lowest energy structure

All core residues (30-38, 163-252)	
Backbone atoms (N,CA,CO)	0.58 ± 0.06
Heavy atoms	1.64 ± 0.07

5. Ramachandran plot (30-38, 163-252)

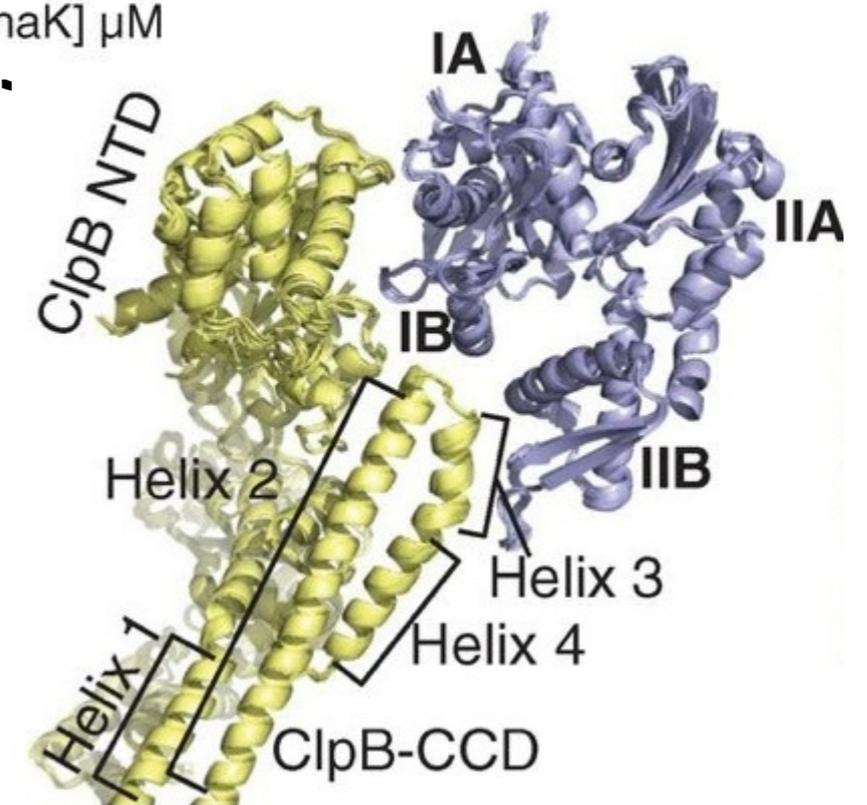
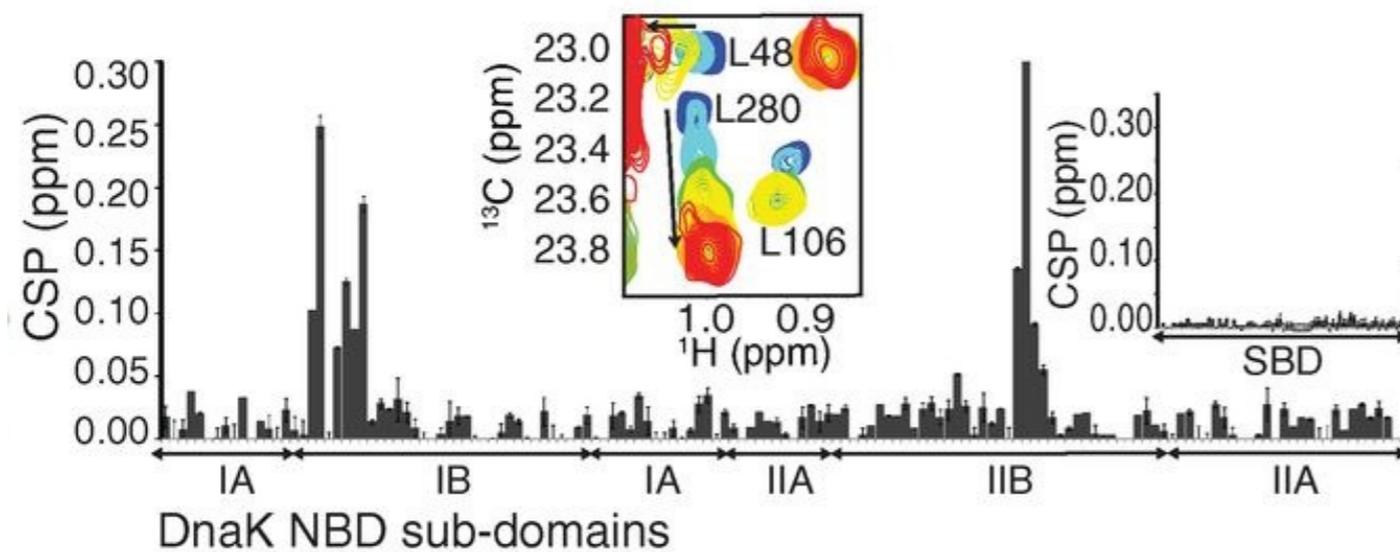
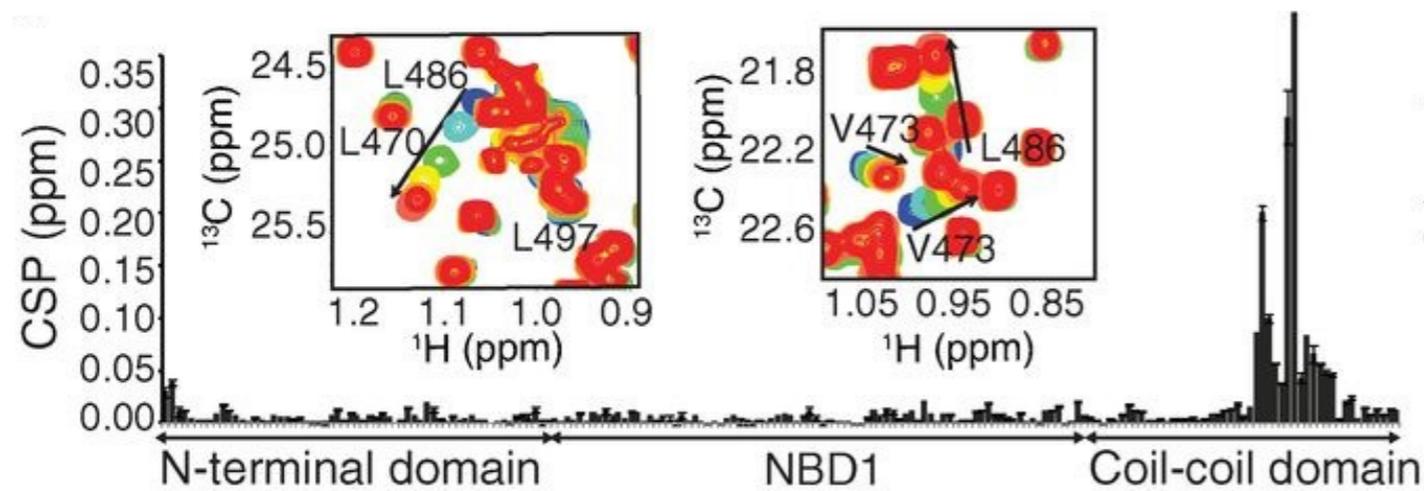
Most favored region (%)	86.31 ± 2.51
Additionally allowed region (%)	12.36 ± 2.59
Generously allowed region (%)	1.32 ± 0.37
Disallowed region (%)	0.00

6. WHAT CHECK structure Z-scores

Ramachandran plot appearance	-2.42 ± 0.26
2nd generation packing quality	0.25 ± 0.28
χ1 - χ2 rotamer normality	-1.00 ± 0.35
Backbone conformation	-4.23 ± 0.29

NMR & Structural biology

Biomolecular interactions



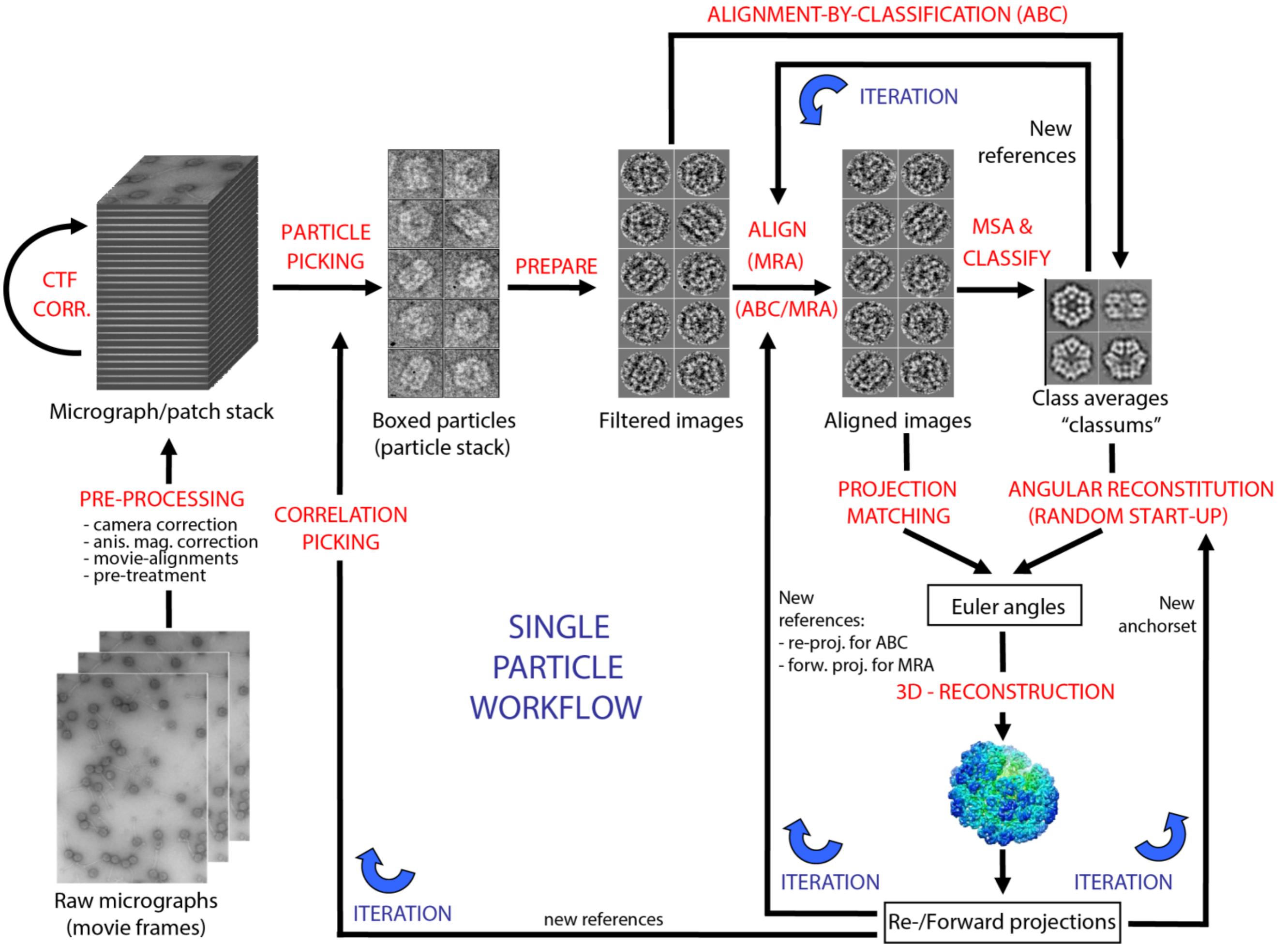
Criomicroscopia eletrônica

Transmission Cryo-Electron Microscopy

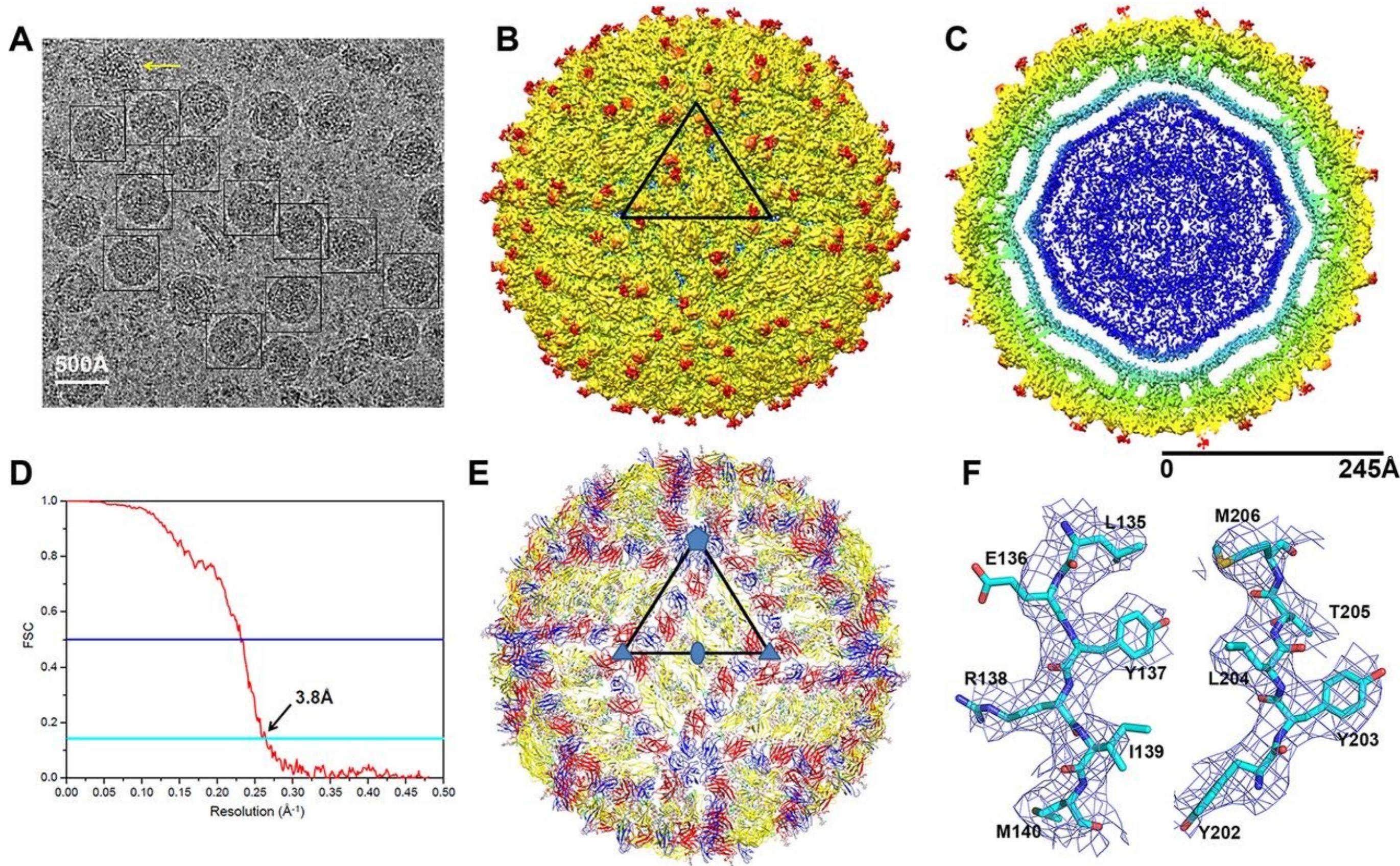
A tool used by structural biologists to study
molecular nanomachines

Gabriel Lander, Thesis Defense 2009

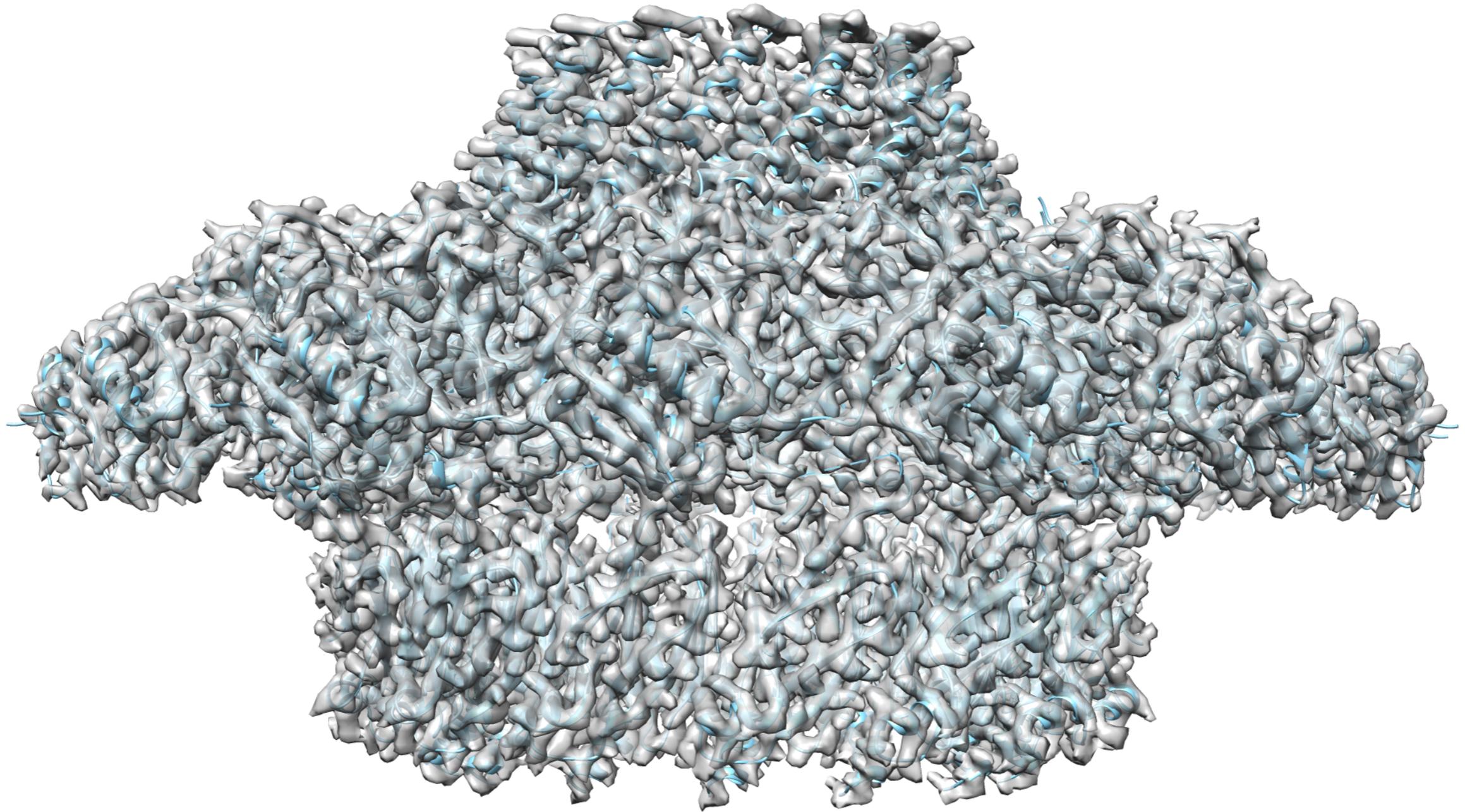
<https://www.youtube.com/watch?v=BJKkC0W-6Qk>



Cryo-Electron Microscopy

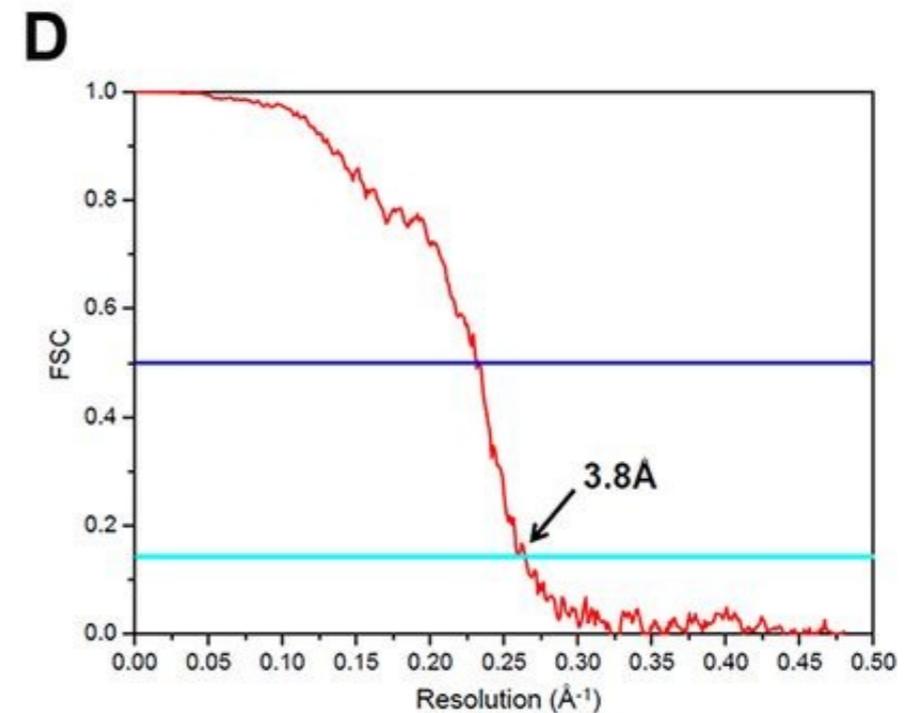


Cryo-EM structure of the Core Complex of the Type IV Secretion System



Como avaliar a qualidade de uma estrutura de Cryo-EM?

- Resolução global (dado pela FSC)
- Variação da resolução local
- Concordância entre o mapa final e o modelo
- Estatísticas do Ramachandran
- RMSD de ligações de ângulos
- Clash score



Cristalografia

RMN

Cryo-EM

Vantagens

- Não há limite de tamanho
- Maior resolução

- Aplicável quando não se obtém cristal
- É possível variar as condições da solução (pH, tampão etc)
- Dinâmica
- Interações
- IDPs

- Demanda pouca amostra (0.1 mg/ml)
- Aplicada em solução
- Ideal para proteínas de membrana
- Heterogeneidade conformacional

Desvantagens

- É preciso obter um cristal que difrate
- Demanda altas concentrações (> 5 - 10 mg/ml)

- Não aplicável a proteínas maiores que 20 - 30 kDa
- Demanda altas concentrações (> 5 - 10 mg/ml)
- Menor resolução

- Exige muito poder computacional
- Não aplicável a moléculas < 100 kDa

Banco de dados de estruturas de proteínas e ácidos nucleicos

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3D Structures

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PDB-101

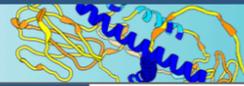
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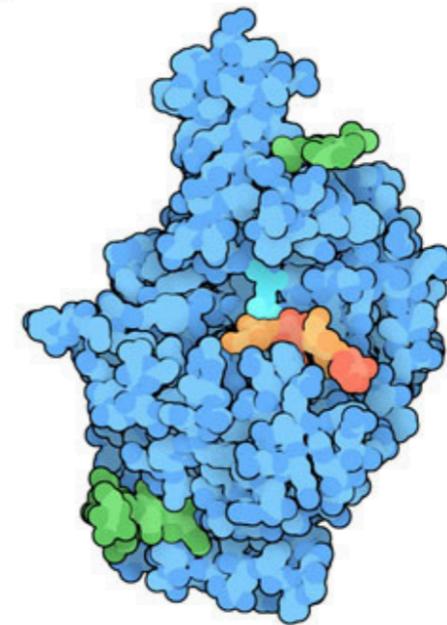
Experimentally-determined 3D structures from the **Protein Data Bank (PDB)** archive

Computed Structure Models (CSM) from AlphaFold DB and ModelArchive

These data can be explored in context of external annotations providing a structural view of biology.

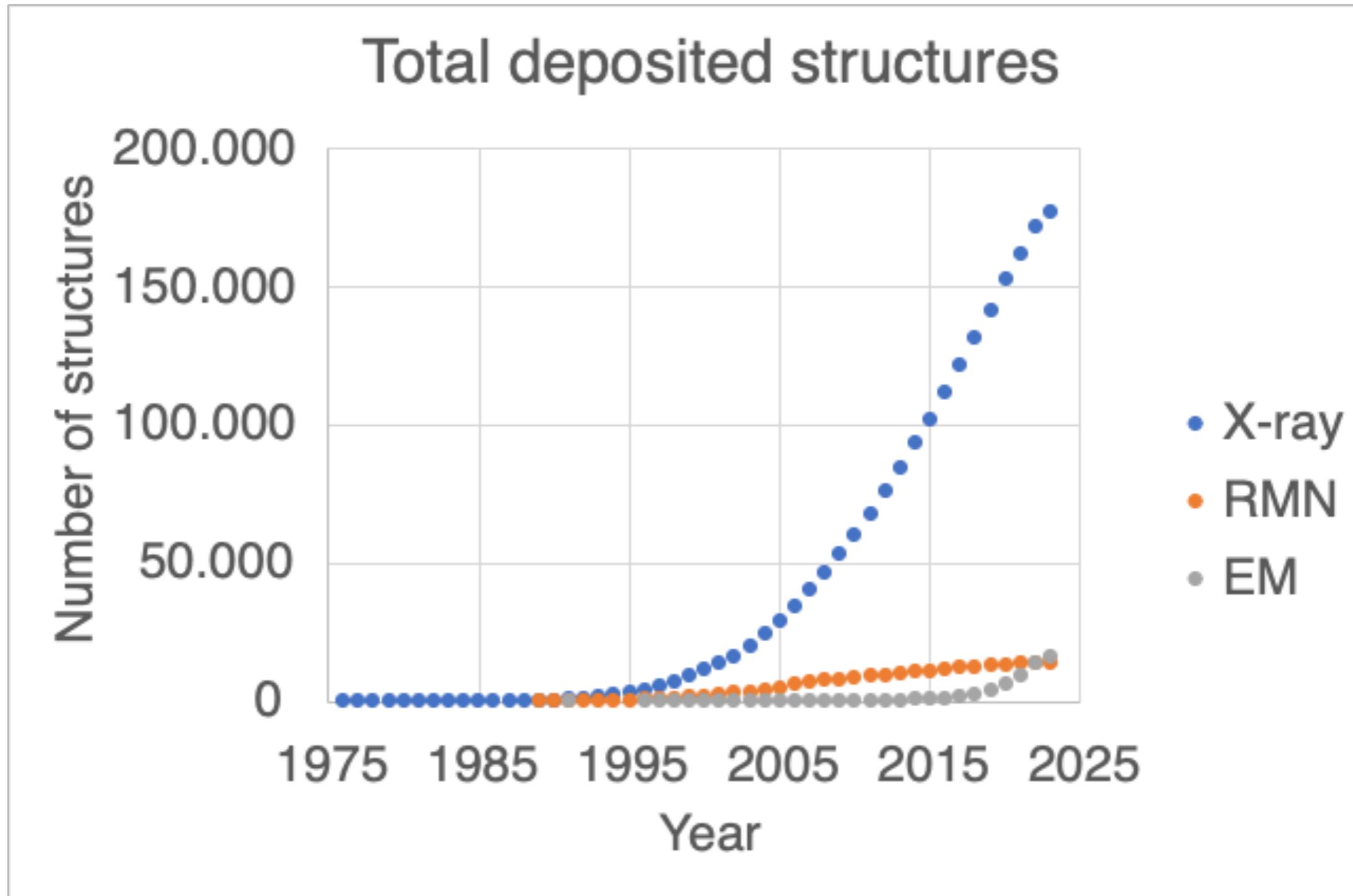


March Molecule of the Month

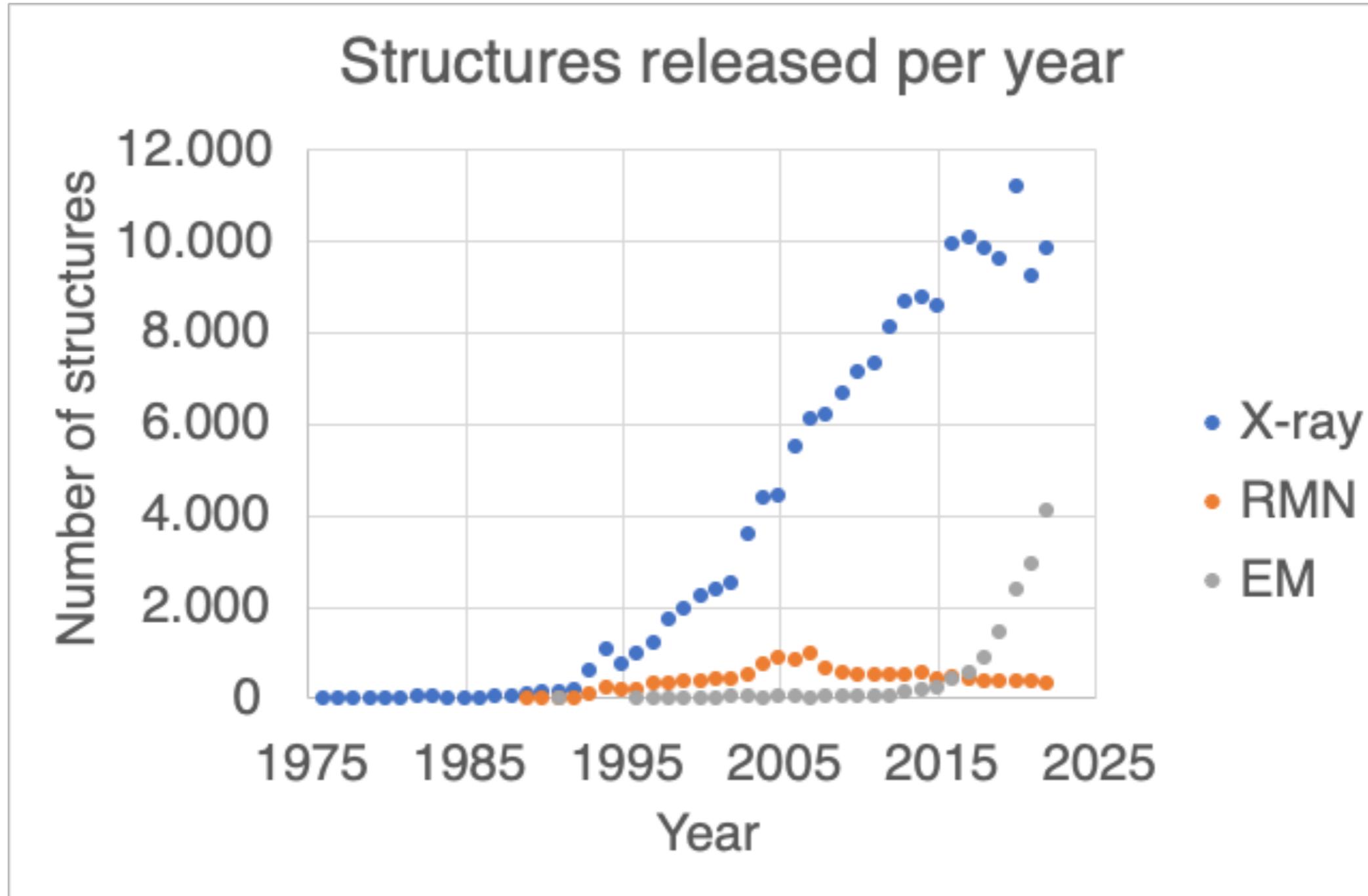


Hyaluronidases

Estatísticas do PDB

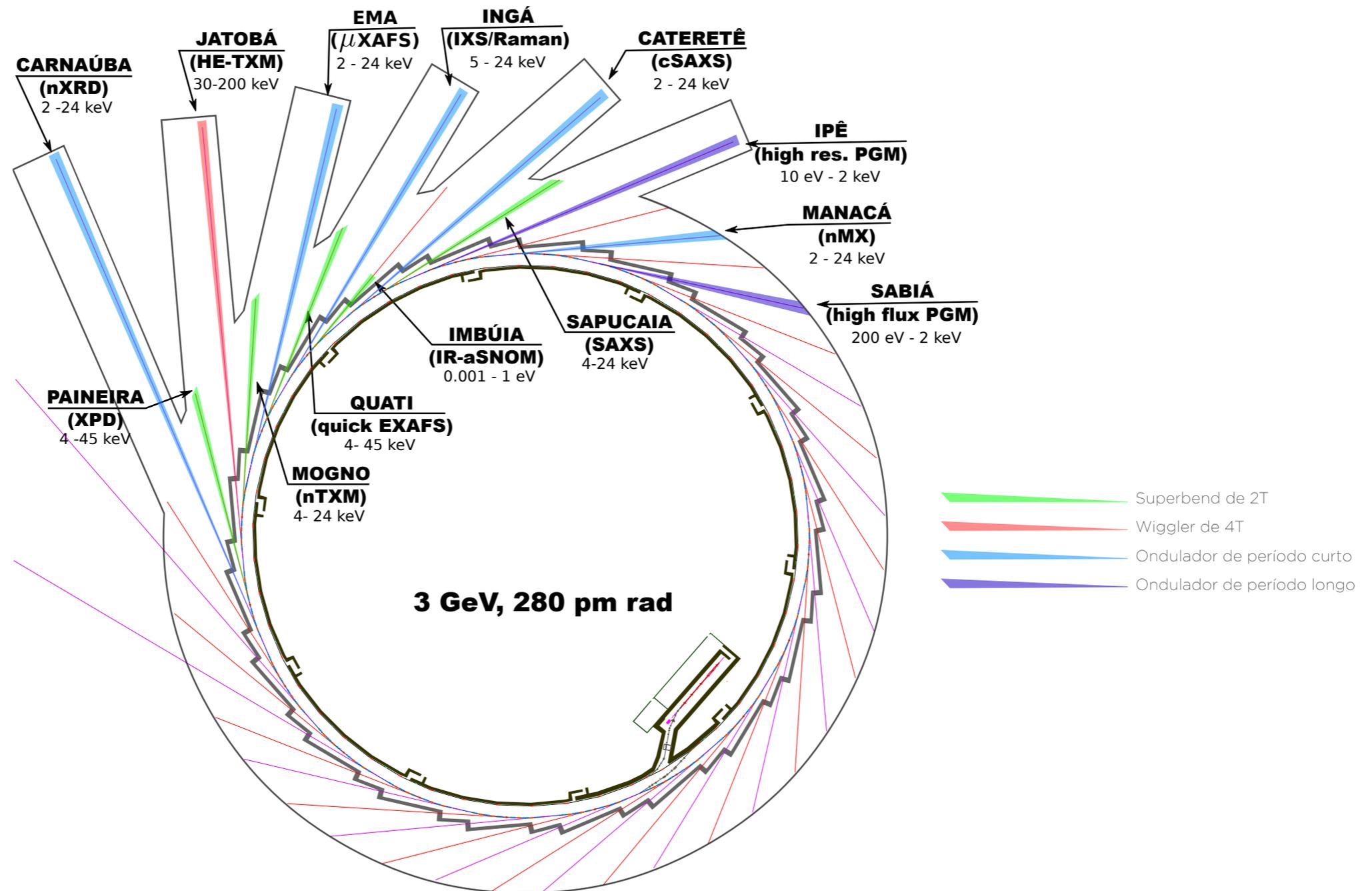


Estatísticas do PDB



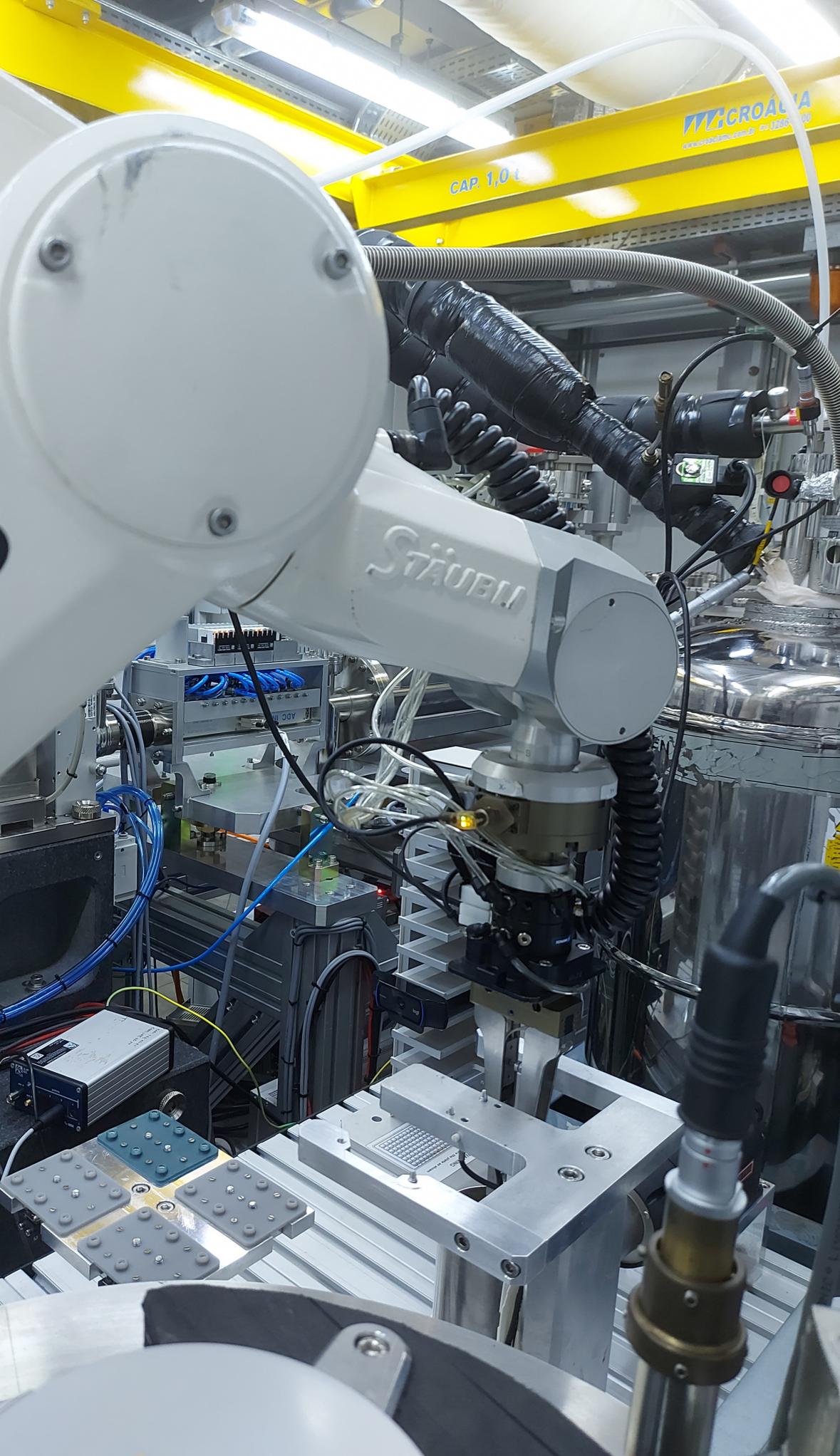
Qual é a infraestrutura disponível em São Paulo?

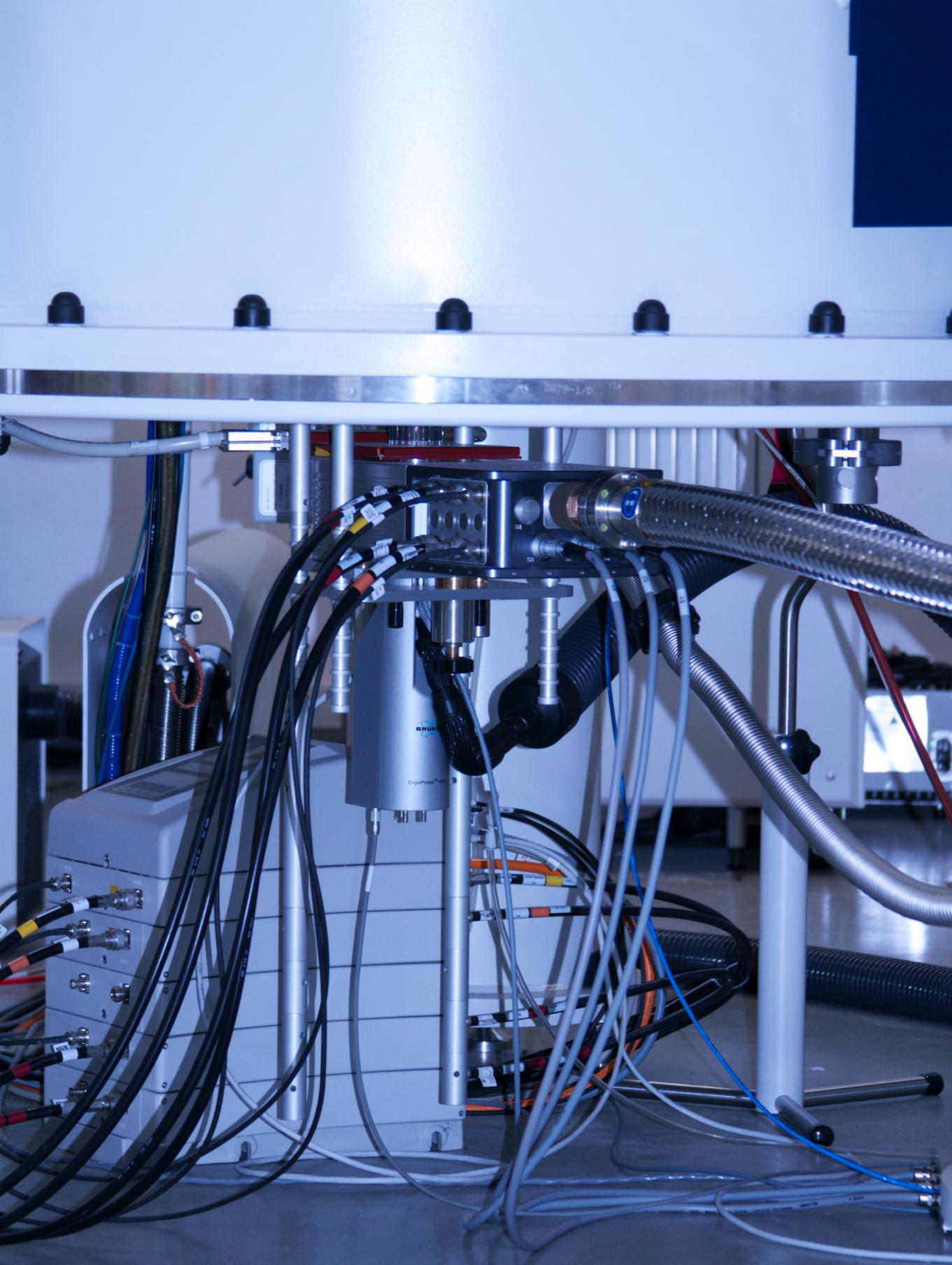
Laboratório Nacional de Luz Síncrotron (LNLS)



Acelerador de elétrons







IQUSP



IQUSP

LNNano

Titan Krios
Talos Arctica

IFSC - USP

Tundra Cryo-TEM
Aquilos 2 Cryo-FIB-SEM-iFLM

