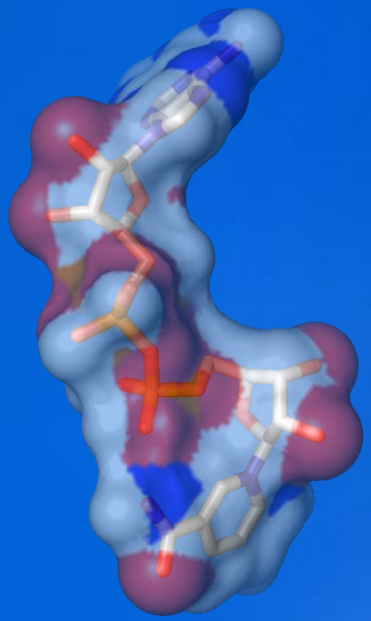
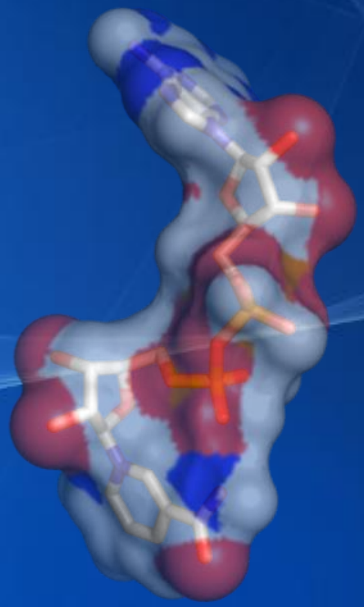
FFI0750 – Biologia Molecular Estrutural





Prof. Rafael V. C. Guido

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Aula 01

Bacharelado em Ciências Físicas e Biomoleculares

Instituto de Física de São Carlos - USP

Comprimentos de onda associados a diferentes regiões do espectro eletromagnético usados no estudo de estruturas de proteínas

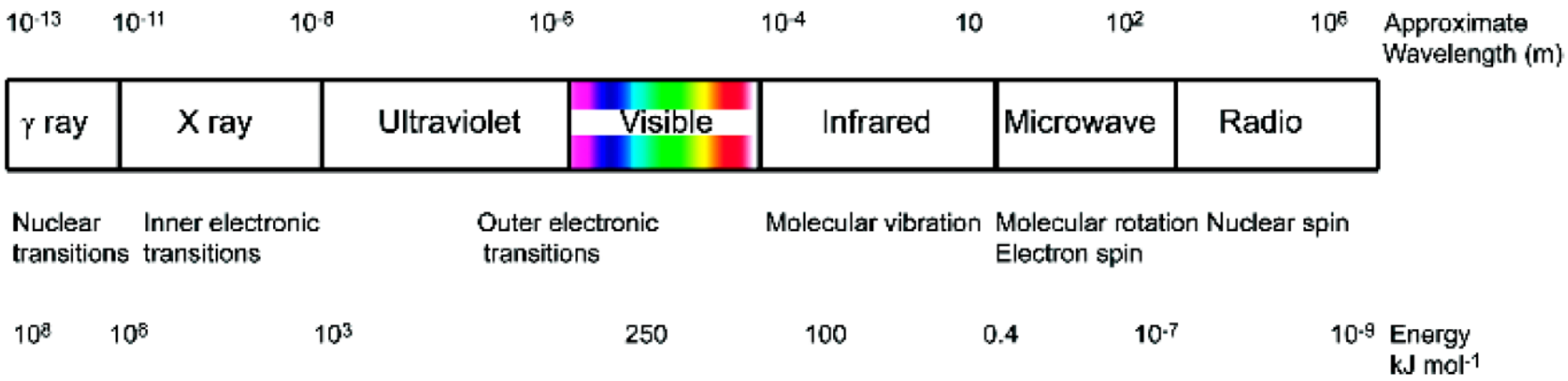
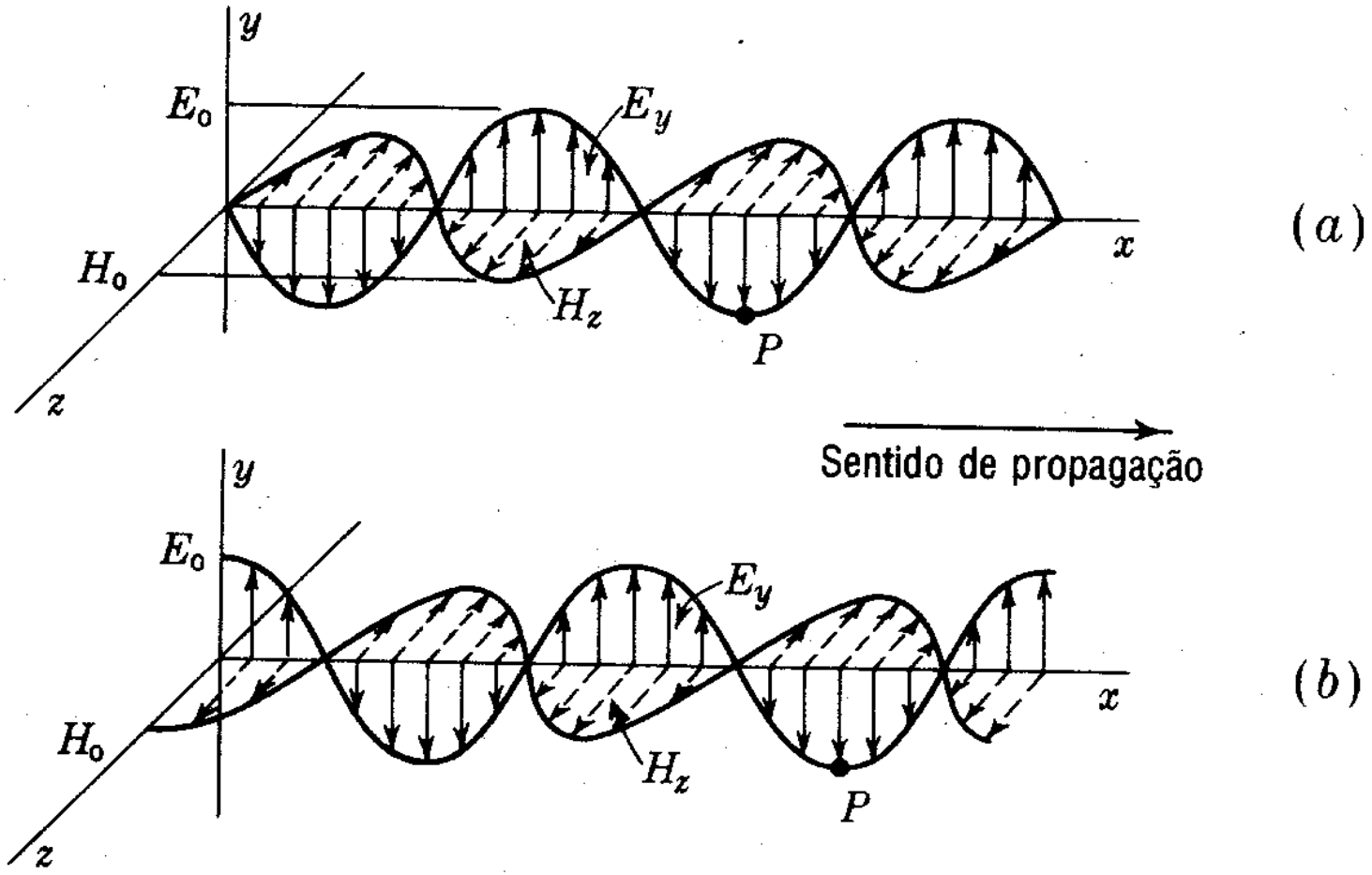


Table 10.1 The frequency range and atomic parameters central to physical techniques used to study protein structure

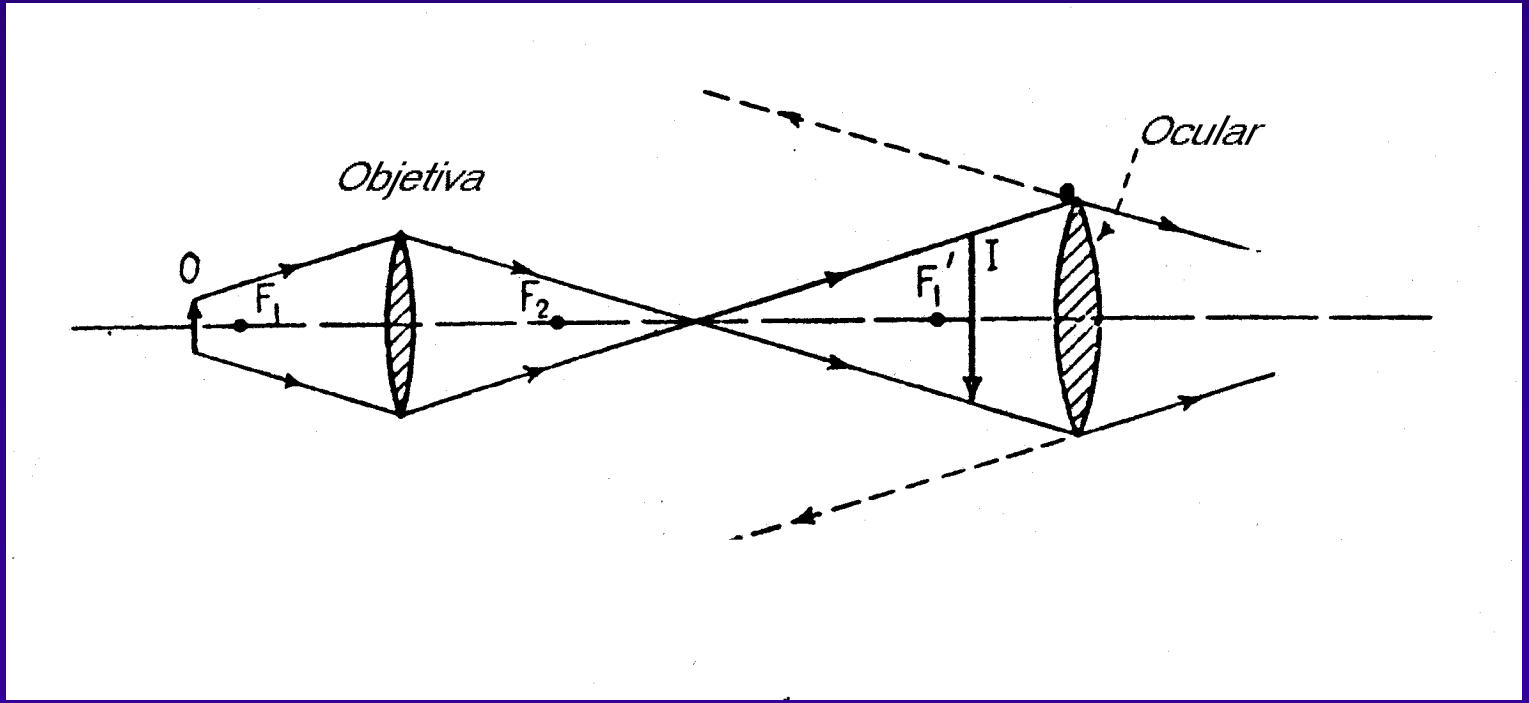
Technique	Frequency range (Hz)	Measurement
NMR	$\sim 0.6 - 60 \times 10^7$	Nucleus' magnetic field
ESR	$\sim 1-30 \times 10^9$	Electron's magnetic field
Microwave	$\sim 0.1-60 \times 10^{10}$	Molecular rotation
Infrared	$\sim 0.6-400 \times 10^{12}$	Bond vibrations and bending
Ultraviolet/visible	$\sim 7.5-300 \times 10^{14}$	Outer core electron transitions
Mossbauer	$\sim 3-300 \times 10^{16}$	Inner core electron transitions
X-ray	$\sim 1.5-15 \times 10^{18}$	Inner core electron transitions

Luz visível utilizada na descrição visual da natureza

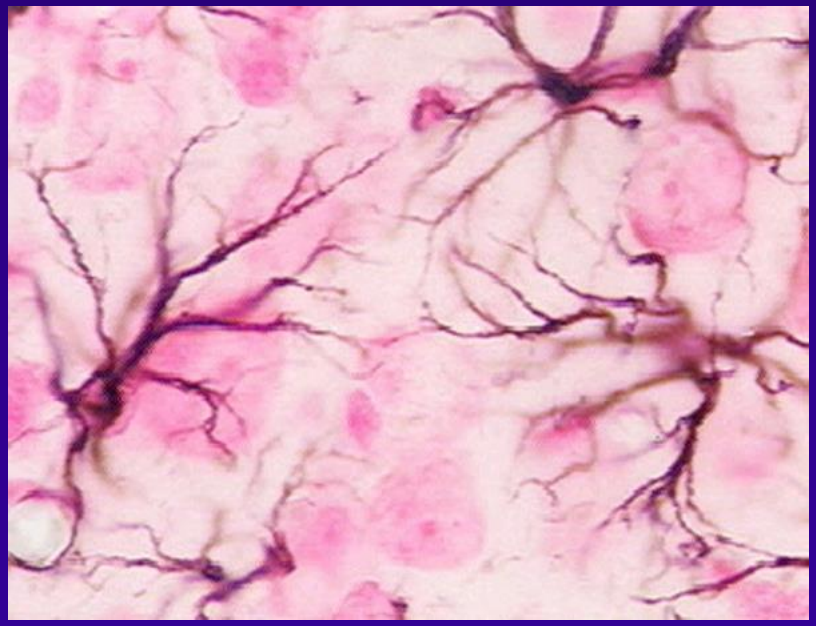

y
E_0
E_y
H_0
H_z
x
P
z
(a)
Sentido de propagação
y
E_0
E_y
H_0
H_z
x
P
z
(b)

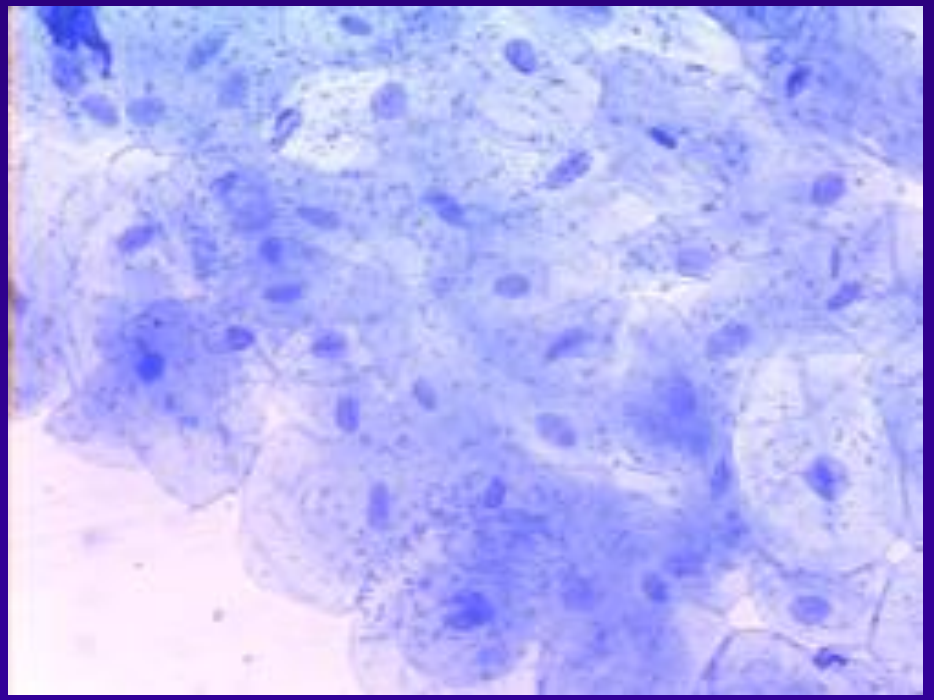
- Ondas de rádio e microondas causam alterações nas propriedades magnéticas dos átomos que são detectadas por várias técnicas, incluindo RMN e ressonância de spin eletrônico (ESR).
- Nas regiões de micro-ondas e infravermelho do espectro, a irradiação causa movimentos de ligação e, em particular, movimentos sobre ligações, como 'alongamento', 'curvatura' ou rotação.
- As regiões ultravioleta (UV) e visível do espectro eletromagnético são de maior energia e idenficam mudanças na estrutura eletrônica através de transições que ocorrem em elétrons nas camadas externas dos átomos.
- Os métodos de fluorescência e absorbância são amplamente utilizados em bioquímica de proteínas e são baseados nessas transições.
- Os raios X são usados para sondar mudanças nas camadas internas de elétrons dos átomos.

Microscópio Óptico

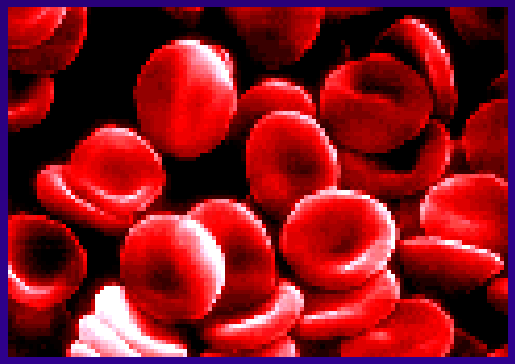


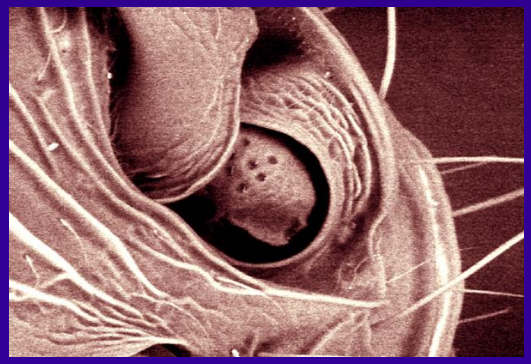
Com o microscópio ótico foi possível elucidar a estrutura celular dos organismos vivos

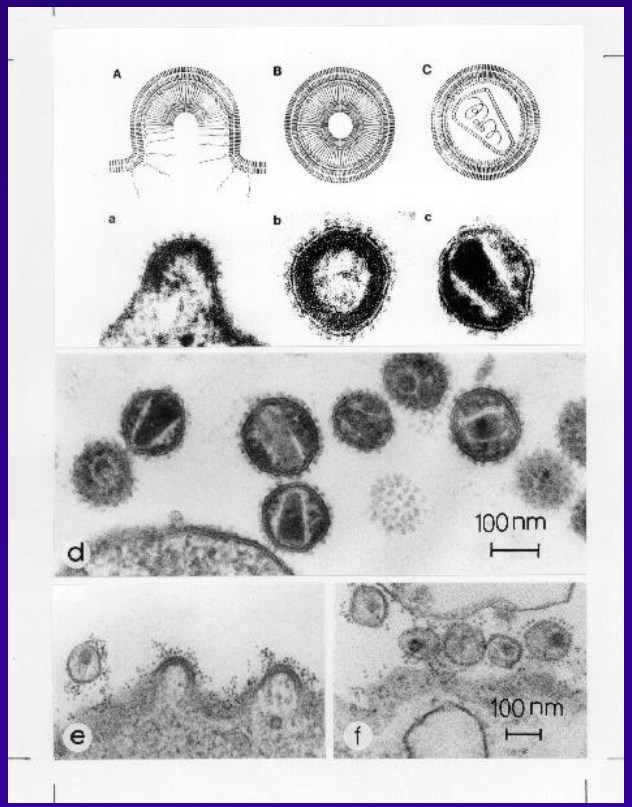


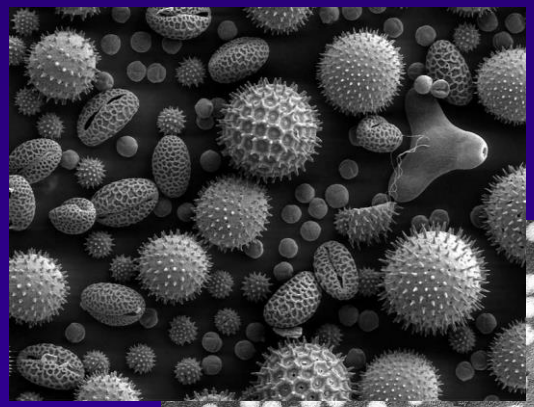


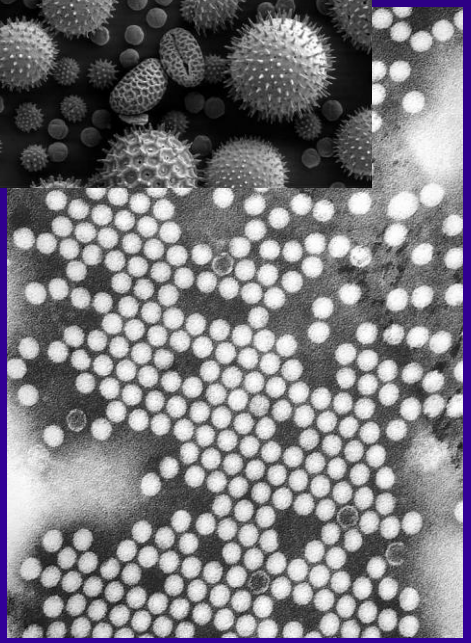
Microscópios Eletrônicos (de transmissão e varredura) permitiram visualizar as estruturas subcelulares e os vírus



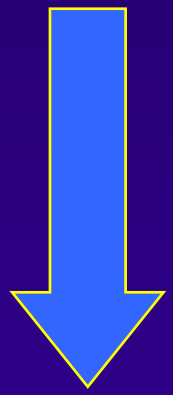








Biologia Moderna = Biologia Molecular



Todos os eventos associados à Vida ocorrem em nível molecular

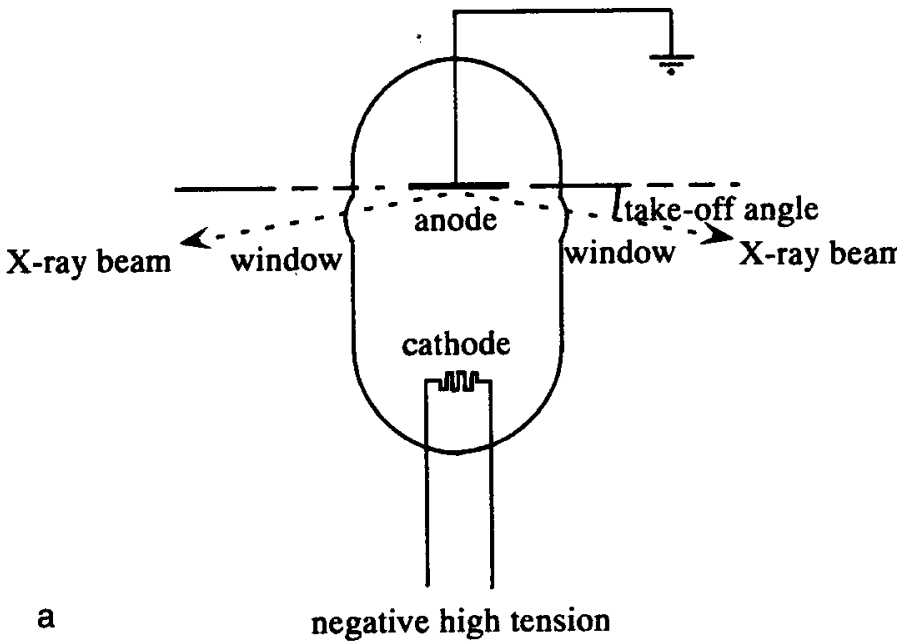
A Química da Vida:

"Praticamente todas as reações químicas presentes nos organismos vivos não seguem o seu curso energeticamente mais favorável"

As Moléculas da Vida:

- Ácidos Nucleicos : DNA e RNA
  - Guardar e transmitir as informações de como sintetizar proteinas
- Proteinas : são as ***operárias*** dos organismos vivos, realizando praticamente todas as funções essenciais.
- Carboidratos: fontes de energia, complementam as proteinas na comunicação entre as moléculas
- Fosfolipídeos: constituintes principais das membranas celulares

Raios-X em Tubos Selados



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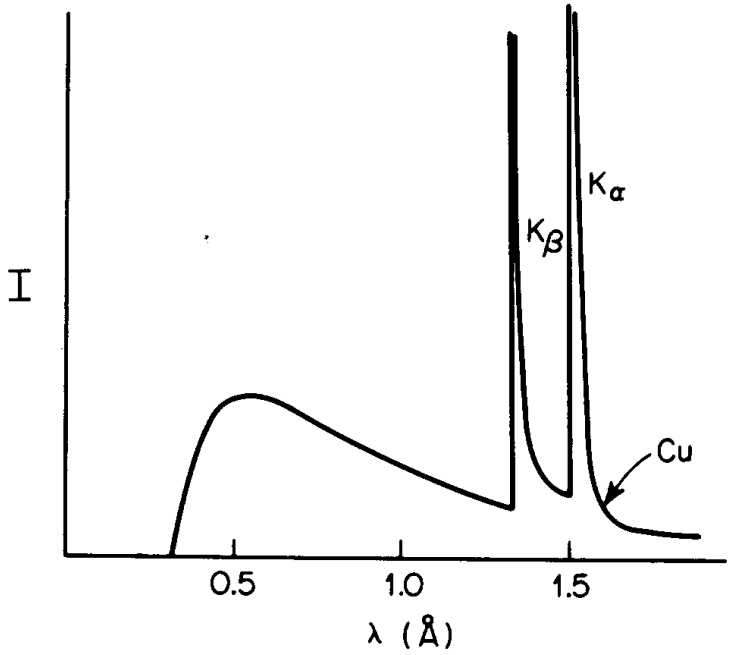
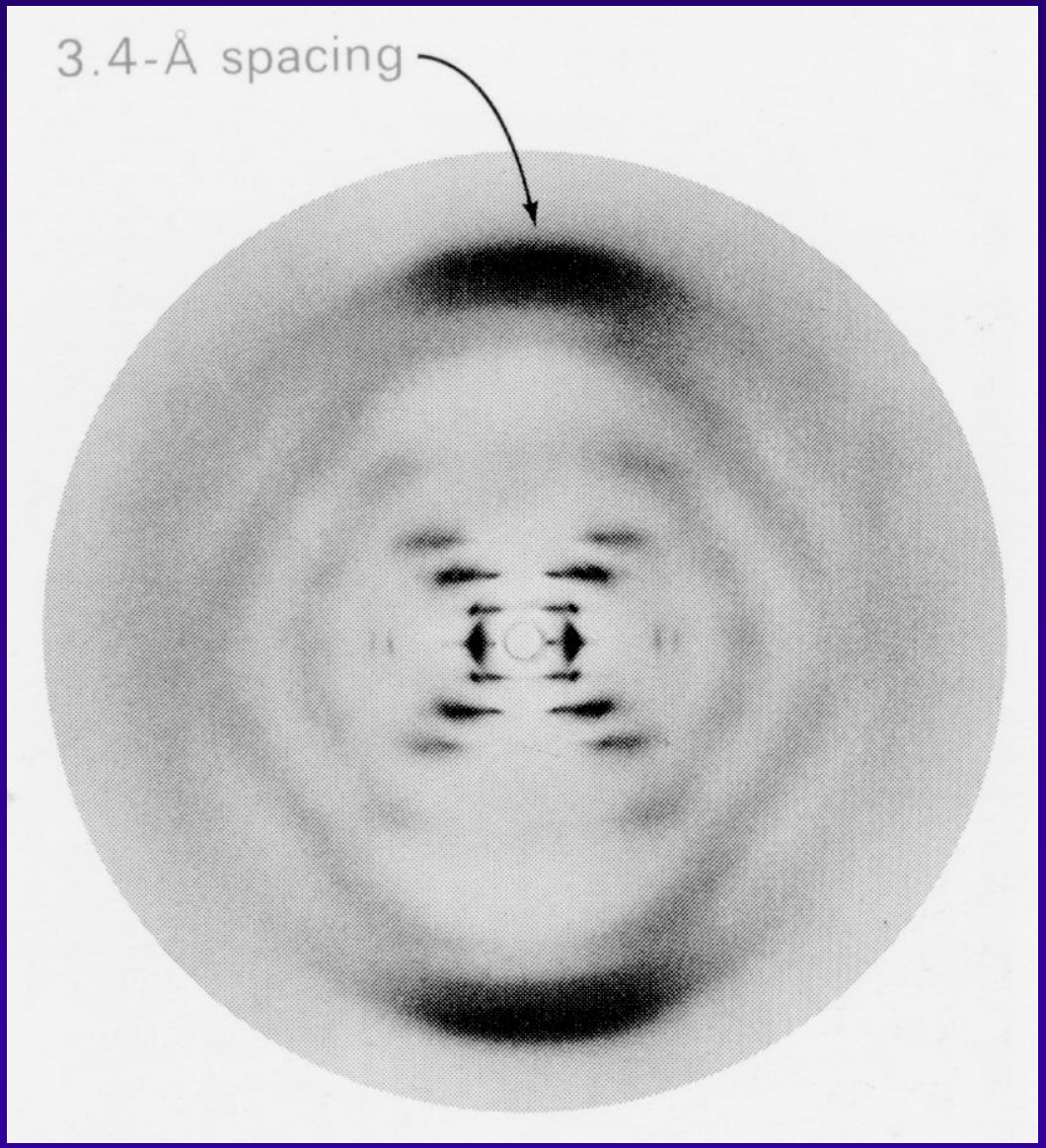


Difração de Raios-X por Fibra de DNA



DNA tipo “B”

(hidratado e pouco cristalino)

O diagrama com um “X” é a assinatura de estruturas em hélices

As reflexões do “X” indicam que a periodicidade da hélice é ~34Å

A reflexão axial indica diâmetro da hélice da ordem de 20 Å

(*Reprinted from Nature, Vol.* 171, *p.* 737, *April* 25, 1953)

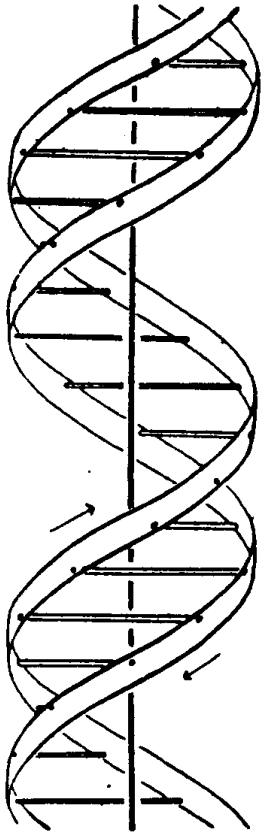
A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey[1]. They kindly made their manuscript available to us in advance of publication. Their model consists of three inter-twined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled



This figure is purely diagrammatic. The two ribbons symbolize the two phosphate—sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

equipment, and to Dr. G. E. R. Deacon and the captain and officers of R.R.S. *Discovery II* for their part in making the observations.

[1] Young, F. B., Gerrard, H., and Jevons, W., *Phil. Mag.*, **40**, 149 (1920).
[2] Longuet-Higgins, M. S., *Mon. Not. Roy. Astro. Soc., Geophys. Supp.*, **5**, 285 (1949).
[3] Von Arx, W. S., Woods Hole Papers in Phys. Oceanog. Meteor., **11** (3) (1950).
[4] Ekman, V. W., *Arkiv. Mat. Astron. Fysik.* (*Stockholm*), **2** (11) (1905).

MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

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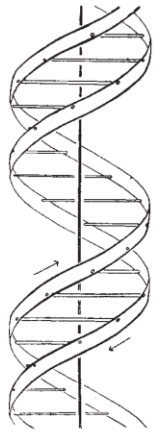


This figure is purely diagrammatic. The two ribbons symbolize the two phosphate—sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate di-ester groups joining β-D-deoxy-ribofuranose residues with 3′,5′ linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. Each chain loosely resembles Furberg's[2] model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furberg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There is a residue on each chain every 3·4 A. in the z-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 A. The distance of a phosphorus atom from the fibre axis is 10 A. As the phosphates are on the outside, cations have easy access to them.

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical z-co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine. The sequence of bases on a single chain does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other chain is automatically determined.

It has been found experimentally[3,4] that the ratio of the amounts of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribose nucleic acid.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data[5,6] on deoxy-ribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereo-chemical arguments.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

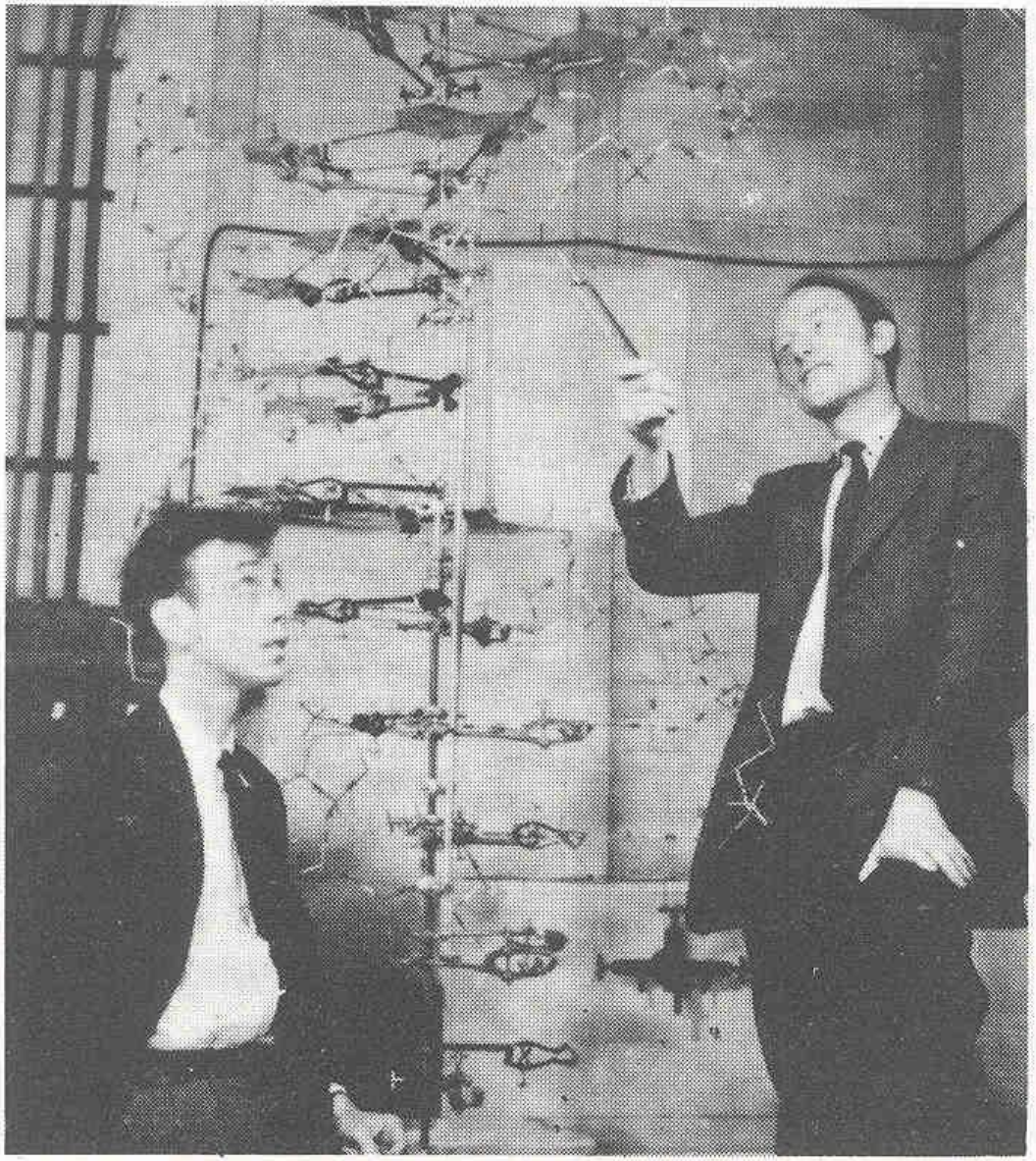
We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on inter-atomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at King's College, London. One of us (J. D. W.) has been aided by a fellowship from the National Foundation for Infantile Paralysis.

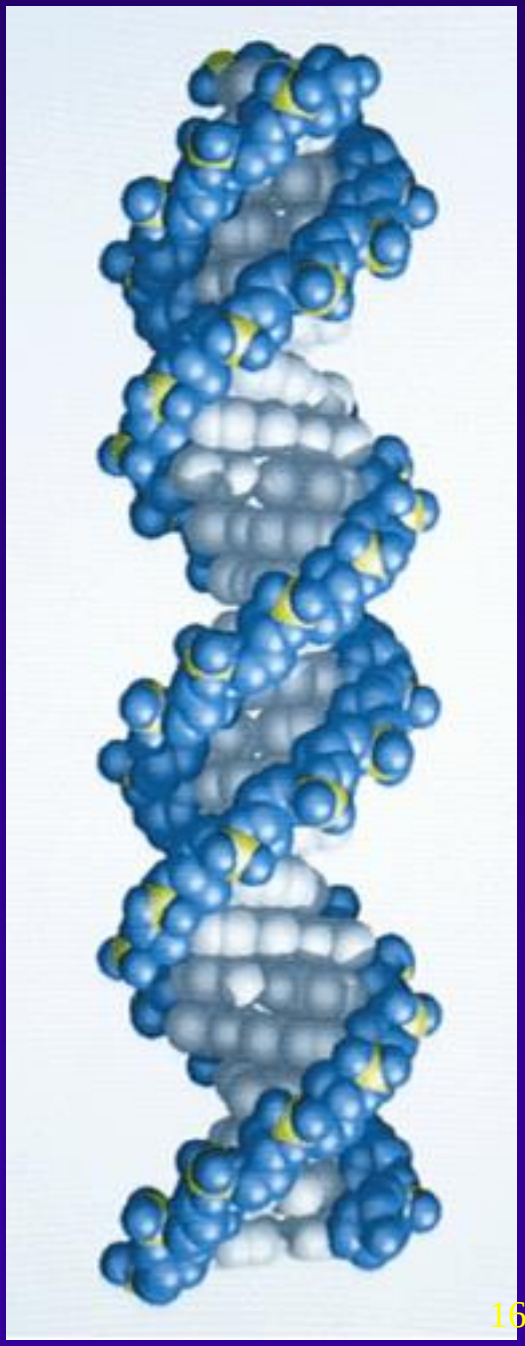
J. D. WATSON
F. H. C. CRICK

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

[1] Pauling, L., and Corey, R. B., *Nature*, **171**, 346 (1953); *Proc. U.S. Nat. Acad. Sci.*, **39**, 84 (1953).
[2] Furberg, S., *Acta Chem. Scand.*, **6**, 634 (1952).
[3] Chargaff, E., for references see Zamenhof, S., Brawerman, G., and Chargaff, E., *Biochim. et Biophys. Acta*, **9**, 402 (1952).
[4] Wyatt, G. R., *J. Gen. Physiol.*, **36**, 201 (1952).
[5] Astbury, W. T., Symp. Soc. Exp. Biol. 1, Nucleic Acid, 66 (Camb. Univ. Press, 1947).
[6] Wilkins, M. H. F., and Randall, J. T., *Biochim. et Biophys. Acta*, **10**, 192 (1953).

Molecular structure of nucleic acids: A structure for deoxyribose nucleic acid J. D. Watson & F. H. C. Crick *Nature* 171, 737-738 (1953)





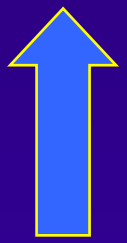
DNA → RNA → PROTEINAS

↑ ↑

Transcrição **Tradução**

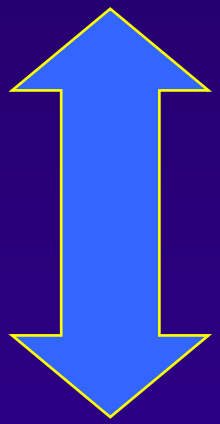
Função Biológica

Estrutura Macromolecular



- **Radiação Elétromagnética (Raios-X, radio-frequências, UV-Vis, infravermelho)**
- **Partículas (elétrons, neutrons)**
- **Microscopias (elétrons, força atômica)**

Biologia Molecular Estrutural



Estudos estruturais de moléculas biológicas utilizando-se de métodos físicos, químicos e de biologia molecular.

Estrutura, dinâmica, estabilidade

Elucidating Protein Structures

- High resolution experimental structures:
  - Protein Crystallography
  - NMR studies in solution
  - High Resolution Cryo Electron Microscopy
- Theoretical approaches:
  - Molecular modeling techniques
  - Molecular dynamics simulations
- Other complementary techniques
  - CD, Fluorescence, FTIR, Raman, XAFS, SAXS, MS, EPR,...

Interação da Radiação Eletromagnética com a Matéria

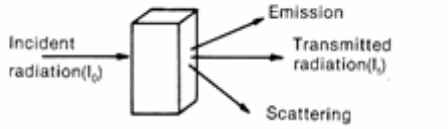


Figure 2.9 Electromagnetic radiation incident on a sample can give rise to absorption, emission, and scattering.

A informação biológica que pode ser obtida pode ser de natureza:

- Estrutural
- Dinâmica
- Energética
- Analítica

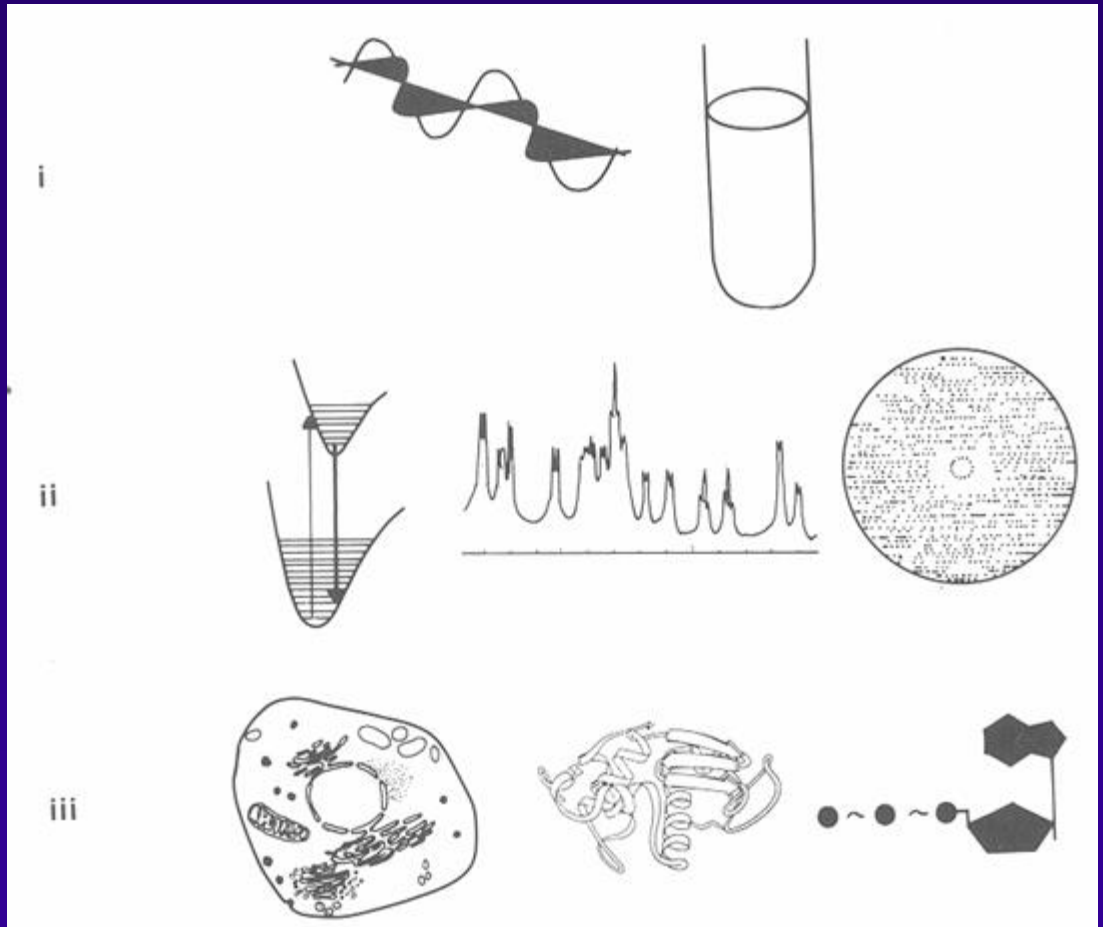


Figure 1.1 Spectroscopy involves (i) irradiation of the sample with some form of electromagnetic radiation. This results in scattering, absorption, and emission. In (ii) are shown the basic process of fluorescence (*emission*), a section of an NMR (*absorption*) spectrum of a protein, and a diffraction pattern from a crystal of a macromolecule, which arises from *scattering*. (iii) The interpretation of measurements of absorption, emission, and scattering leads to biological information on a wide range of systems, from cells to small molecules.

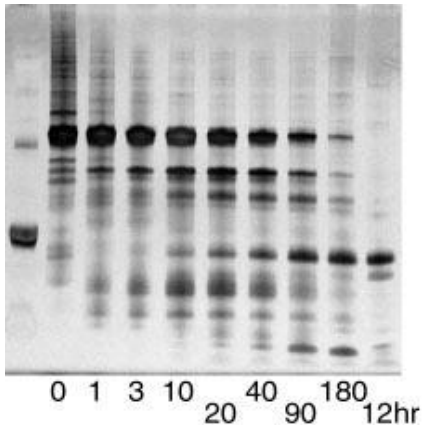
A informação disponível através das Técnicas Espectroscópicas em Biologia Estrutural

Table 1.1 Information available from various spectroscopic techniques

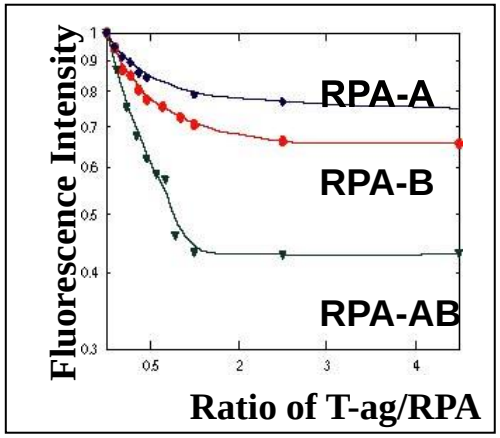
Technique	Chapter		Structure		Dynamics		Energetics
IR	3	*	Fingerprint	*	H–D exchange	*	Ionization states
UV/visible	4	*	Qualitative, DNA conformation	*	Follow reactions	*	Ligand binding
Fluorescence	5	**	Pairwise 2–5 nm (solution)	***	Molecular motion $\sim 10^{-9}$ s	**	Environmental probe, pH
NMR	6	***	Pairwise 0.2–1 nm (solution)	***	10^{-3}–10^{-9} s	**	Ionization states, ligand binding
EPR	7	*	Fingerprint around one center	**	Diffusion of spin label	*	Environmental probe
Scattering	8	**	Overall shape (solution)	**	Net movement of cells and molecules	*	Association/dissociation
Raman	9	*	Fingerprint	*	H–D exchange	*	Ionization states
Optical Activity	10	**	Secondary structure (solution)	*	Conformational changes	*	Ligand binding
Microscopy	11	****	~2 nm (solid); 1 μm (solution)	*	Photobleaching recovery		
Diffraction	12	****	0.2 nm (crystals)	*	Temperature factors	*	Energy calculations from coordinates, "strain"

Métodos da Biologia Estrutural

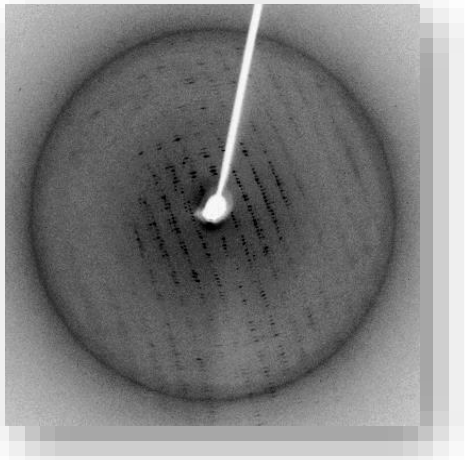
Expressão/Mutações



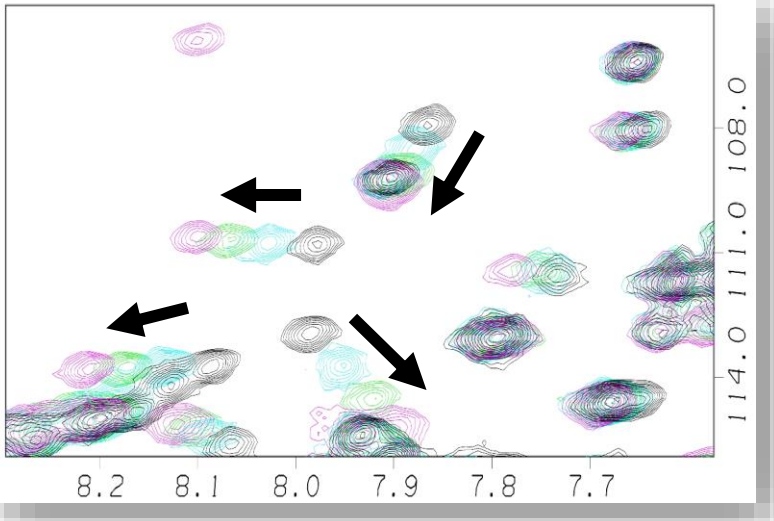
Análises Biofísicas



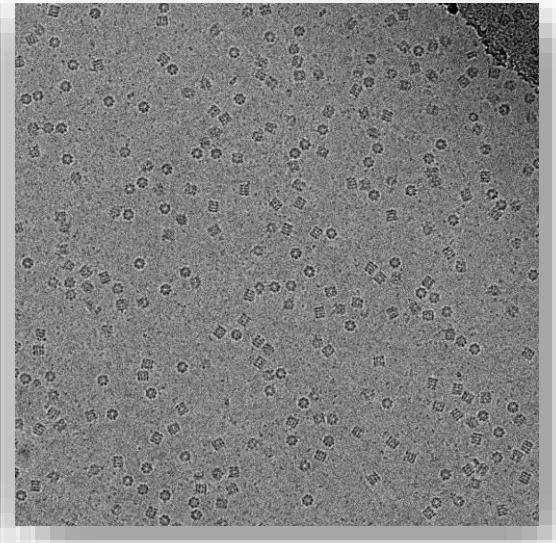
Cristalografia



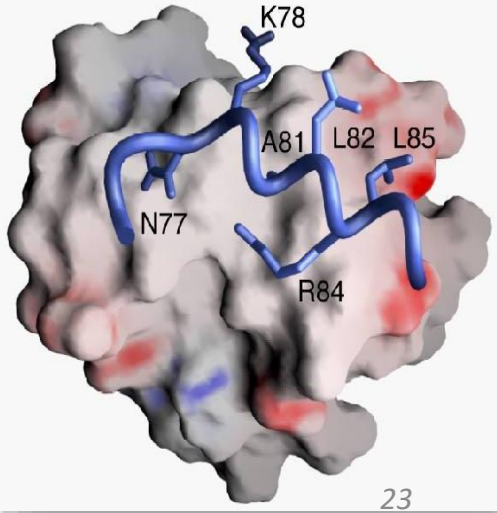
NMR



Cryo-EM



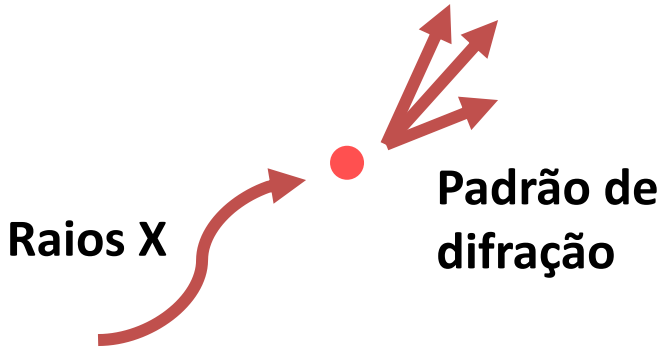
Computação



Biologia Estrutural de alta resolução – técnicas clássicas

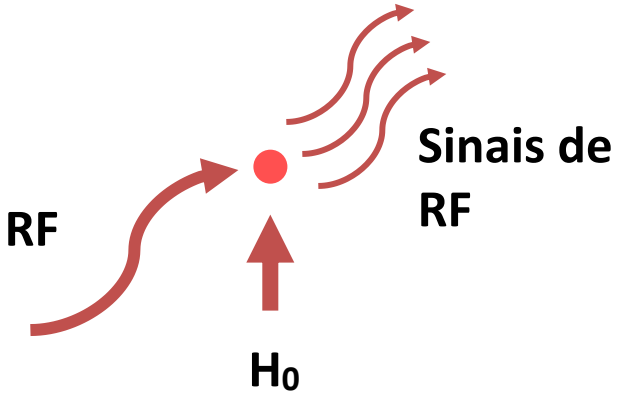
Cristalografia de raios X e RMN – técnicas experimentais de alta resolução determinação de posições atômicas

Cristalografia de Raios X



- **Detecção direta das posições atômicas**
- **Cristais**

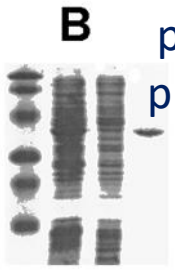
NMR



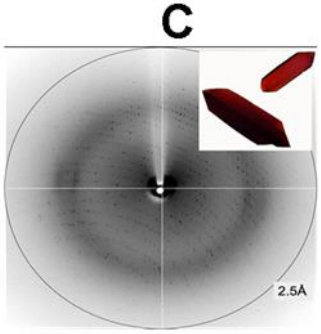
- **Detecção indireta das distâncias H-H; C-H; C-N; N-H**
- **Em solução**

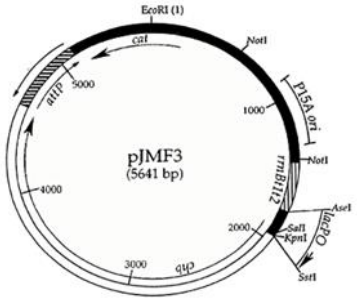
Biologia Estrutural de alta resolução – técnicas clássicas

Expressão e purificação do produto gênico



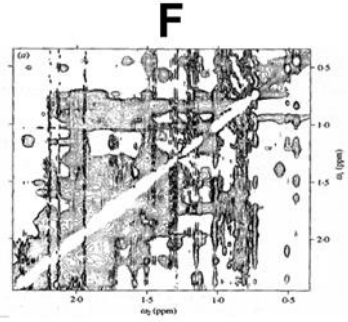
Cristalização e difração de raios X



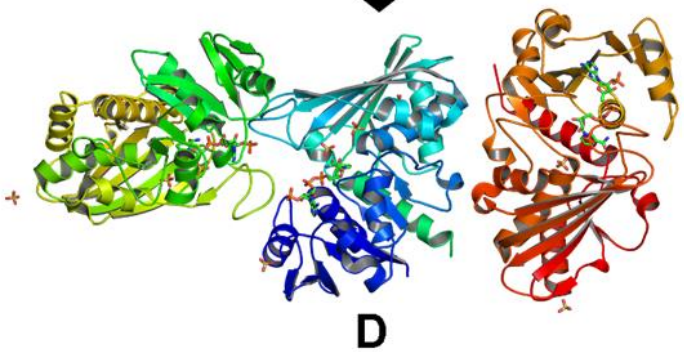


A

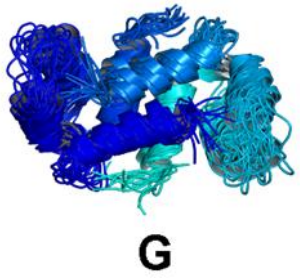
Clonagem do gene



Aquisição de dados de RMN



Determinação estrutural e refinamento do modelo



refinamento do modelo por restrições espaciais.

Cristalografia de raios X vs. RMN: competidores ou complementares?

Cristalografia de raios X	NMR
Vantagens	**Desvantagens**
- Sem limite de tamanho	- Proteínas menores que aproximadamente 40 kDa
- Estruturas podem ser muito precisas	- Estruturas tipicamente menos precisas que as de cristal
-Rápido	- Demorado
Desvantagens	**Vantagens**
- Necessário cristais	- Proteína em solução
- Densidade eletrônica é uma média de todas as moléculas no cristal – imagem estática	- Possibilidade de acompanhar processos dinâmicos
- Possível influência estrutural devido à rede cristalina	- Estrutura determinada da proteína nativa em solução – possibilidade de detectar diferentes conformações

Juntamente com métodos computacionais, cristalografia e RMN são métodos complementares

NMR

Cristalografia

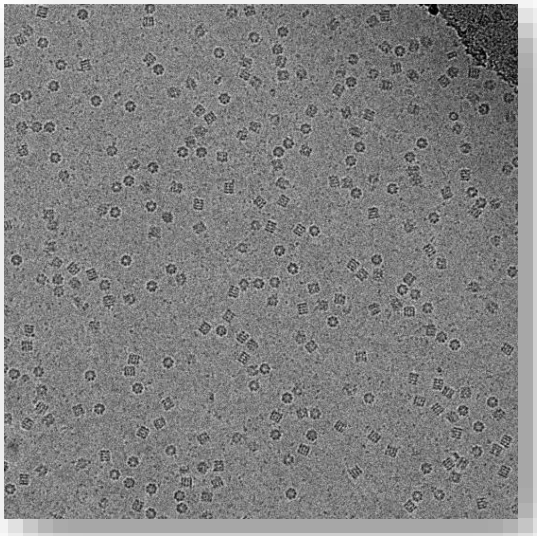
Computação

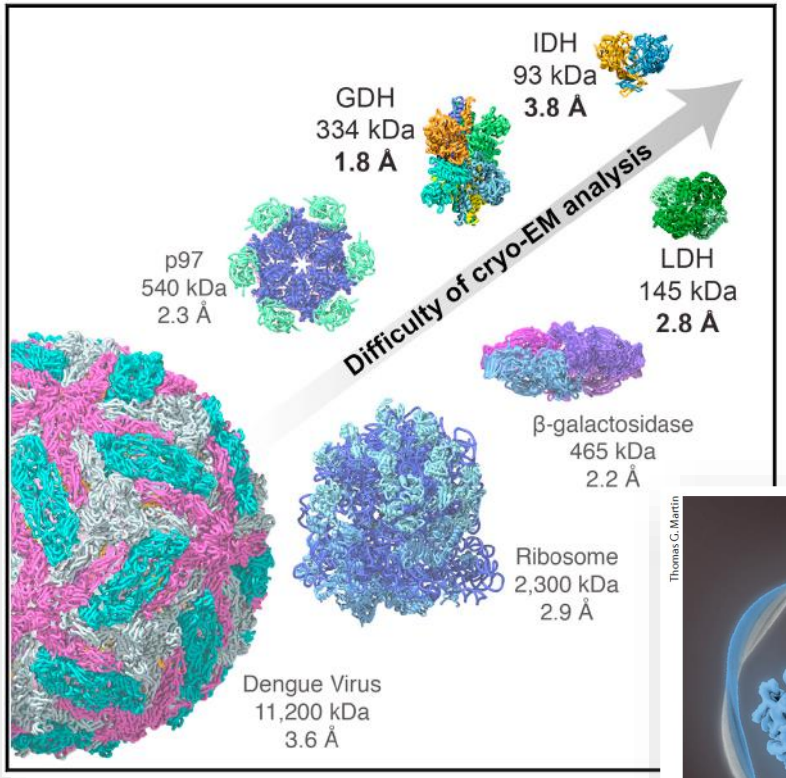
Cryo-EM

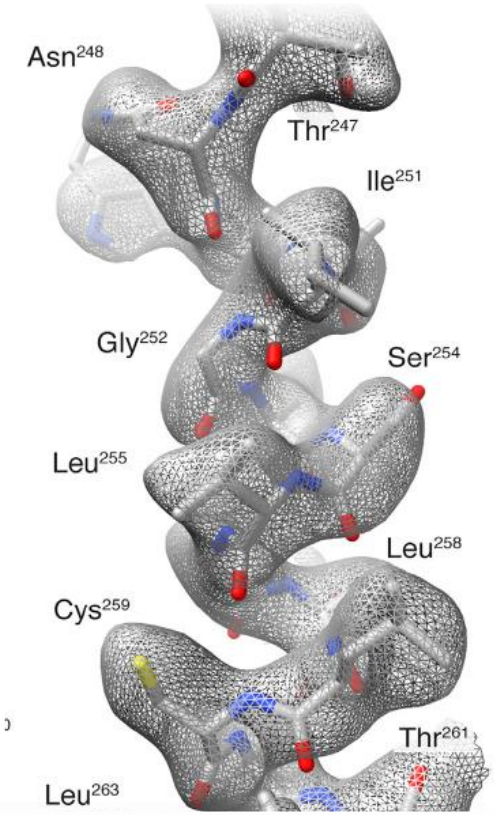
Cada técnica fornece uma contribuição única e complementares para a compreensão dos fenômenos biológicos estudados

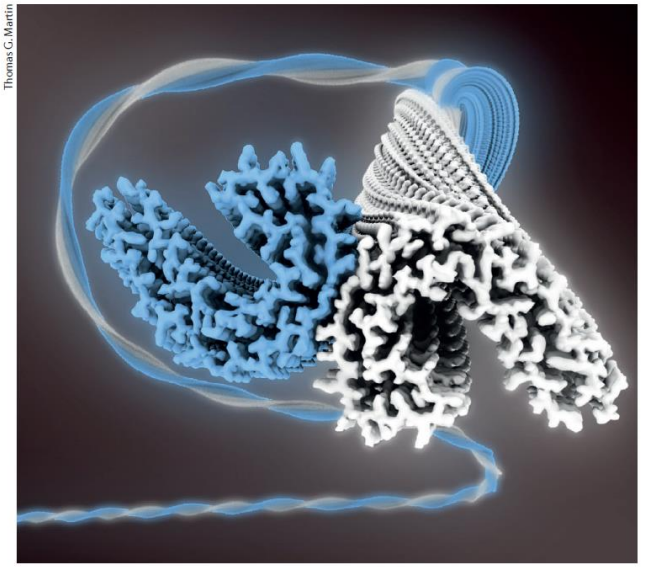
Outras técnicas biofísicas fornecem importantes informações complementares

Criomicroscopia eletrônica (Cryo-EM)



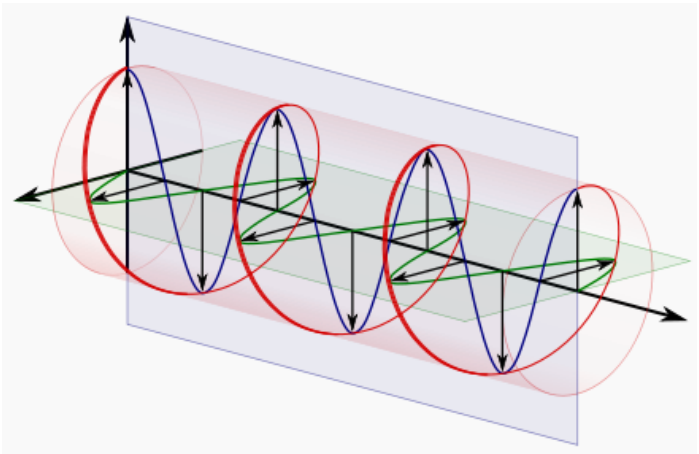




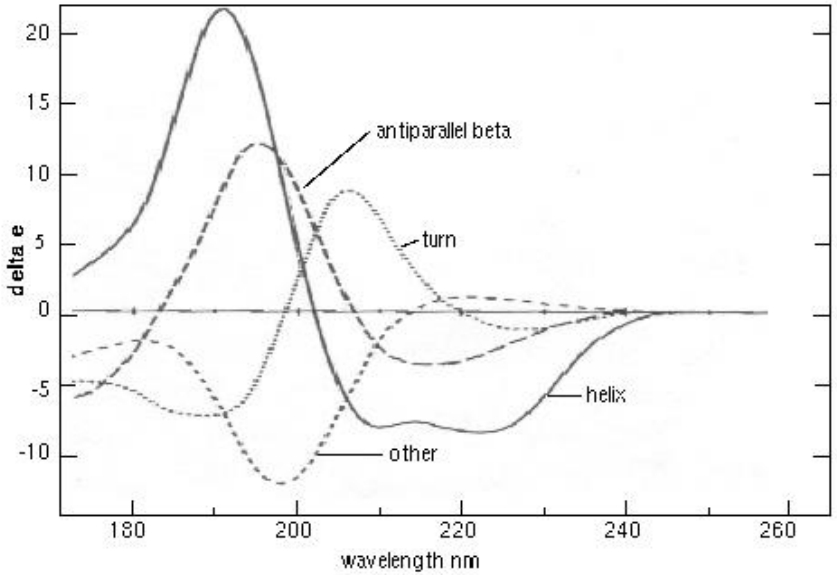


Outras técnicas biofísicas fornecem importantes informações complementares

Dicroísmo Circular (CD)

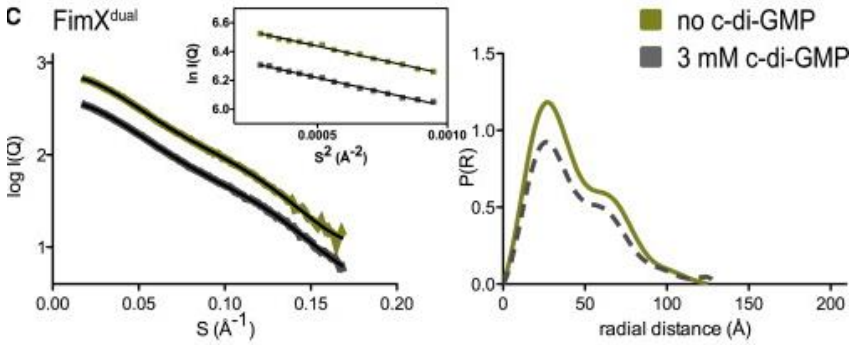


- Identificação de elementos de estrutura secundária – enovelamento
- Mudanças conformacionais
- Estabilidade protéica
- etc...

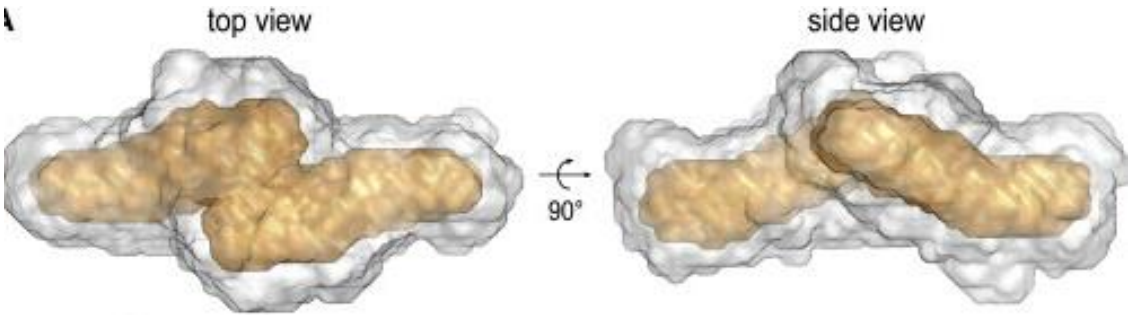


Outras técnicas biofísicas fornecem importantes informações complementares

Espalhamento de raios X a baixo ângulo (SAXS)



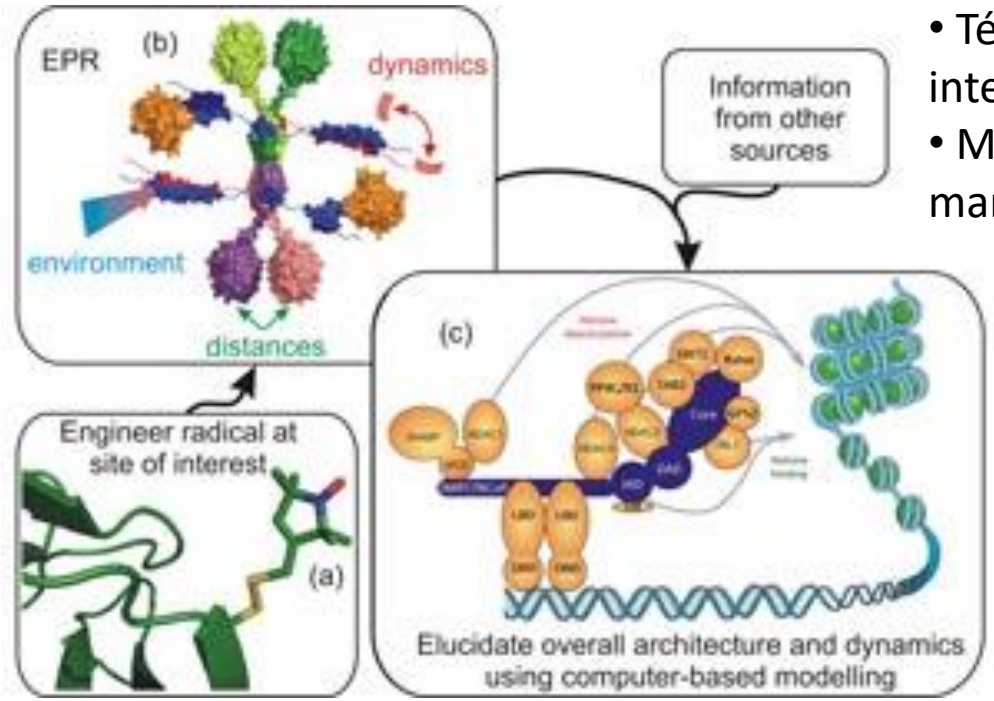
- Proteína em solução – testar diferentes condições
- Distribuição de distâncias inter-moleculares
- Modelo 3D *ab initio* de baixa resolução – envelope molecular
- Mudanças conformacionais



Outras técnicas biofísicas fornecem importantes informações complementares

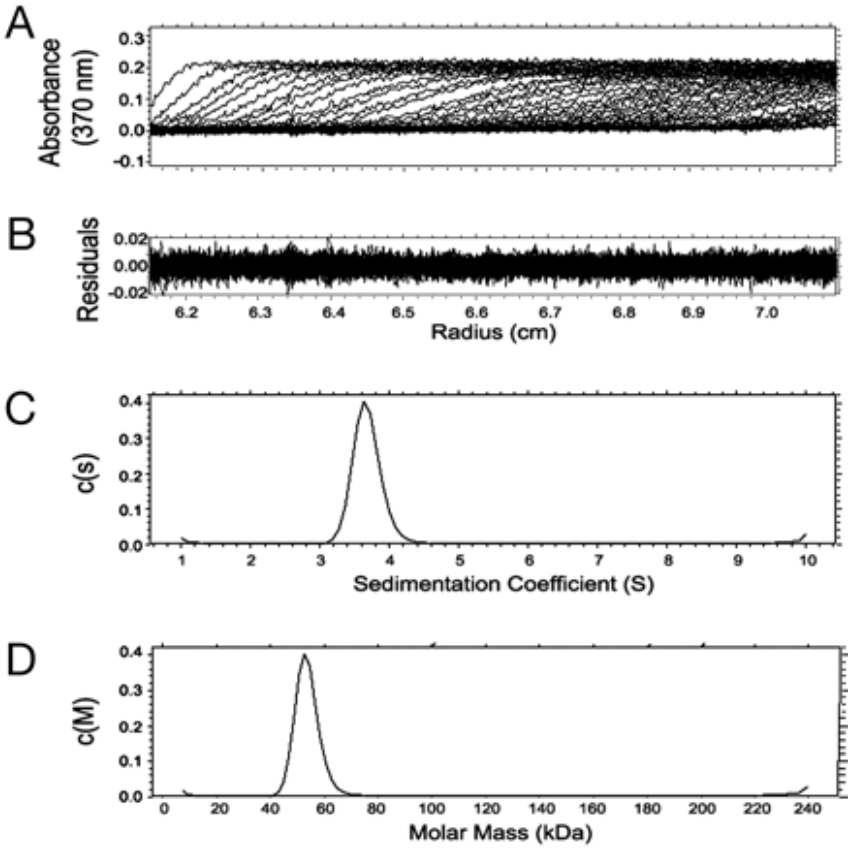
Ressonância Paramagnética eletrônica (EPR)

- Mesmo princípio do NMR – elétrons desemparelhados
- Muito poderosa para estudos de centros metálicos em proteínas
- Técnica de marcação com spin label – interação com membranas, dinâmica...
- Medidas de distâncias entre dois marcadores - DEER



Outras técnicas biofísicas fornecem importantes informações complementares

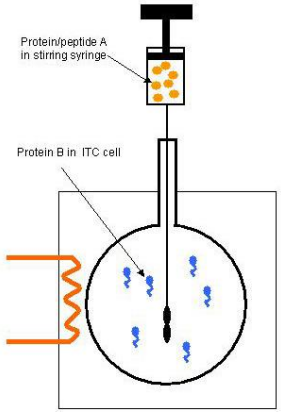
Ultracentrifugação analítica (AUC)



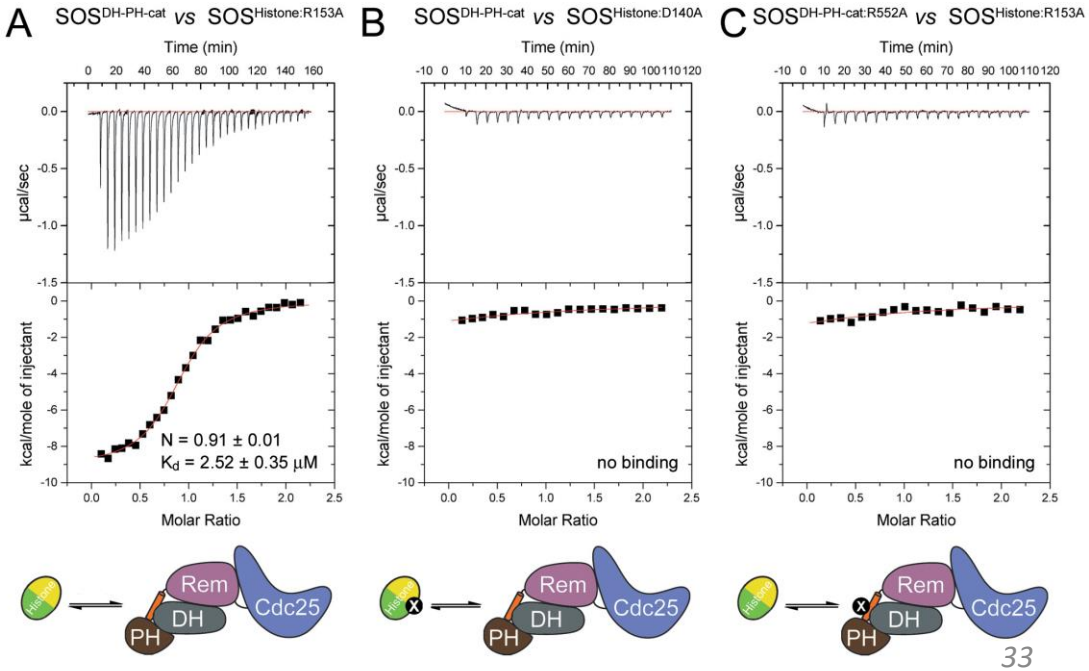
- Medidas de estados oligoméricos de proteínas em solução
- Constantes de dissociação
- Parâmetros energéticos

Outras técnicas biofísicas fornecem importantes informações complementares

Calorimetria de Titulação Isotérmica (ITC)

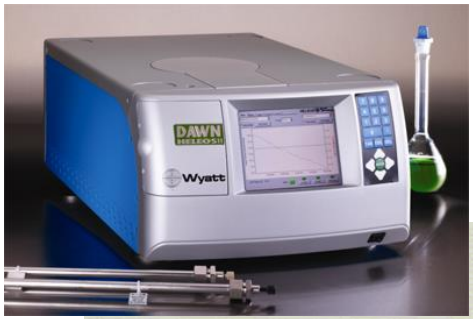


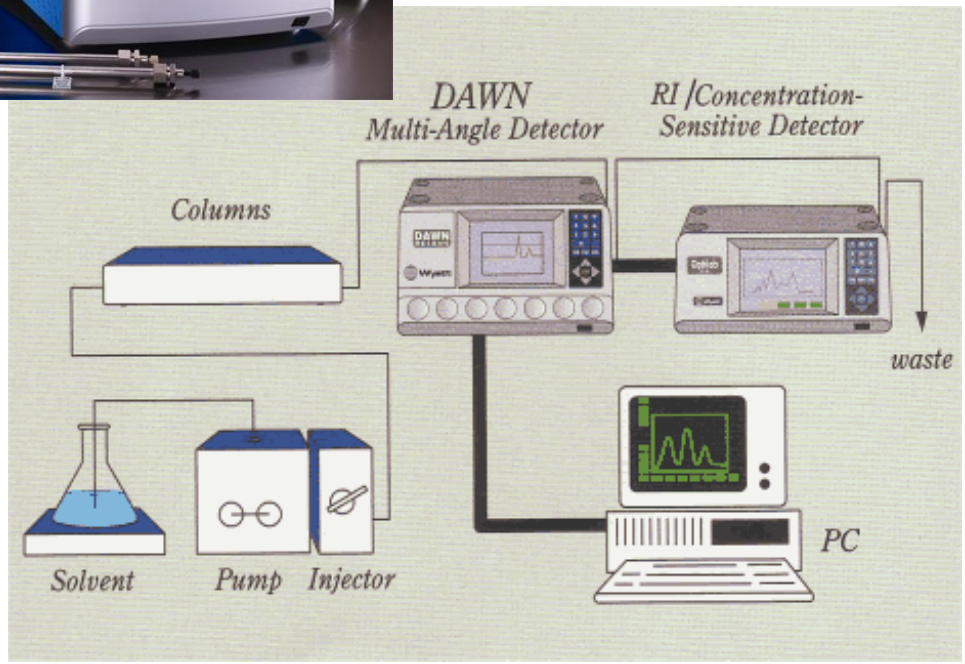
- Medidas de parâmetros energéticos e constantes de afinidade entre proteína-ligante e proteína-proteína
- Ensaio enzimático
- Número de sítios de ligantes



Outras técnicas biofísicas fornecem importantes informações complementares

Espalhamento de luz a múltiplos ângulos acoplado a cromatografia de exclusão molecular (SEC-MALS)





- Medidas de massa absoluta de partículas em solução
- Estado oligomérico
- Rápida e fácil