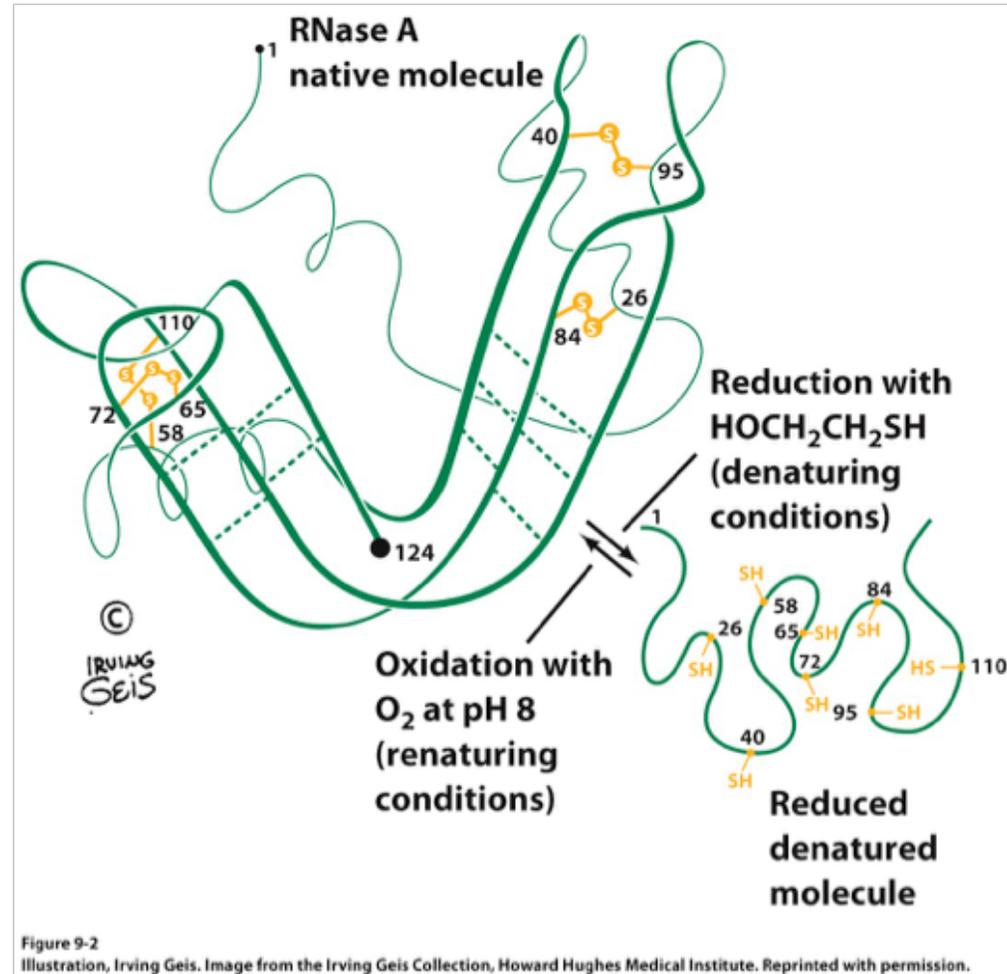


# Hipótese termodinâmica de Anfinsen

- Christian Anfinsen (1916 – 1995, Estados Unidos)
- Prêmio Nobel de Química em 1972 (em conjunto com Stanford Moore e William Stein)
- A informação que determina a estrutura terciária de proteínas reside na química da sua sequência de aminoácidos
- Descoberta de uma enzima microsomal que cataliza a troca de ligações disulfeto, acelerando a reação de enovelamento de proteínas desnaturadas que contêm pontes disulfeto
- Investigou uma nuclease extracelular de *Staphylococcus aureus*, determinou a sua sequência de aminoácidos e descreveu as características cinéticas, físicas e imunológicas em colaboração com cristalógrafos que resolveram a estrutura cristalográfica da enzima

“The genetic information of an organism is stored in DNA molecules which, via RNA molecules, are converted during the formation of proteins. Proteins consist of a long chain of amino acids that fold themselves in a special way. The chemical processes inside cells are controlled by a type of protein called enzymes. Christian Anfinsen studied the enzyme ribonuclease, which divides RNA into smaller components. In 1961, Anfinsen proved that the sequence of amino acids, in itself, determines the way the chain folds itself and that no additional genetic information is required in this process.”

# Christian Anfinsen (anos 50): conformação nativa da RNase A é a mais estável



“Scrambled” RNase A pode ser renaturada removendo-se uréia na presença de traços de BME o que indica que o estado nativo é termodinamicamente mais estável

# ● A hipótese termodinâmica de Anfinsen

A sequência de aminoácidos determina a estrutura tridimensional

The studies on the renaturation of fully denatured ribonuclease required many supporting investigations (6–8) to establish, finally, the generality which we have occasionally called (9) the “thermodynamic hypothesis.” This hypothesis states that the three-dimensional structure of a native protein in its normal physiological milieu (solvent, pH, ionic strength, presence of other components such as metal ions or prosthetic groups, temperature, and other) is the one in which the Gibbs free energy of the whole system is lowest; that is, that the native conformation is determined by the totality of interatomic interactions and hence by the amino acid sequence, in a given environment. In terms of natural selection

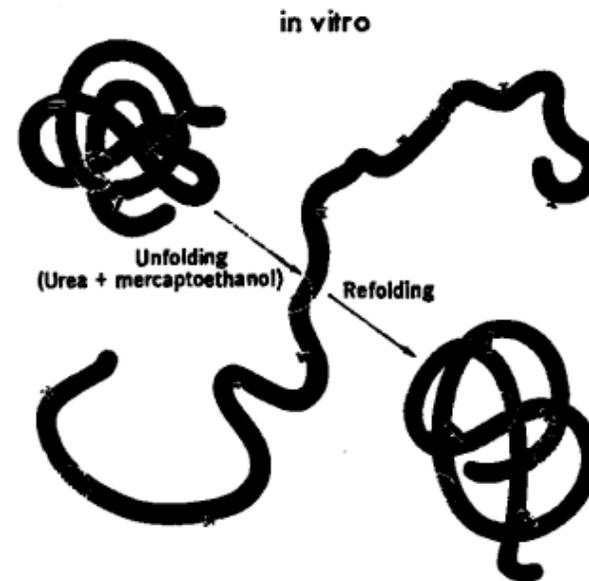


Fig. 2. Schematic representation of the reductive denaturation, in 8M urea solution containing 2-mercaptoethanol, of a disulfide-cross-linked protein. The conversion of the extended, denatured form to a randomly cross-linked, “scrambled” set of isomers is depicted at the lower right.

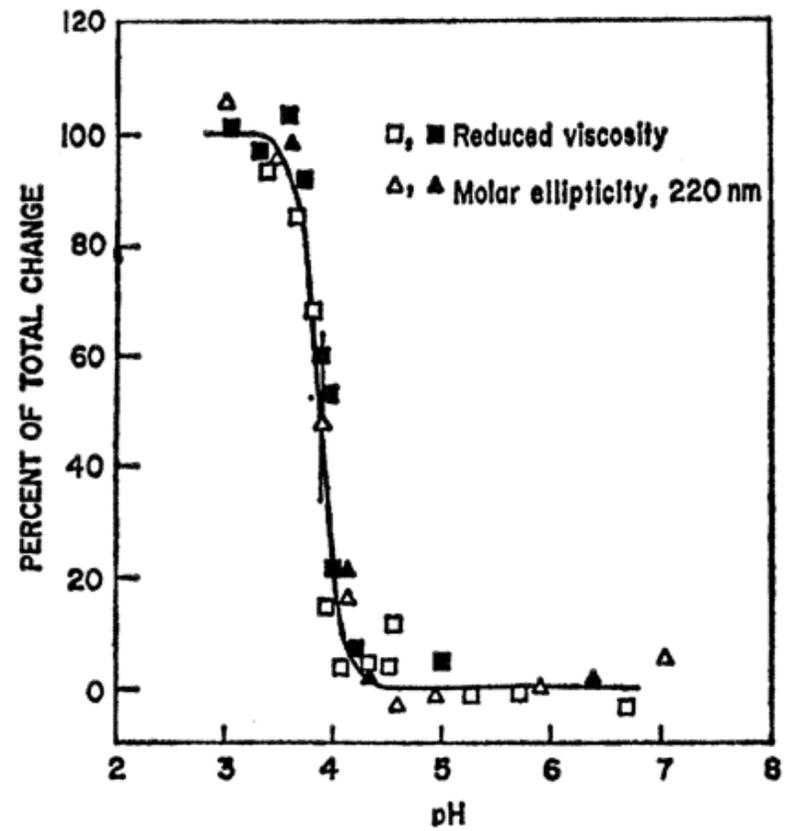
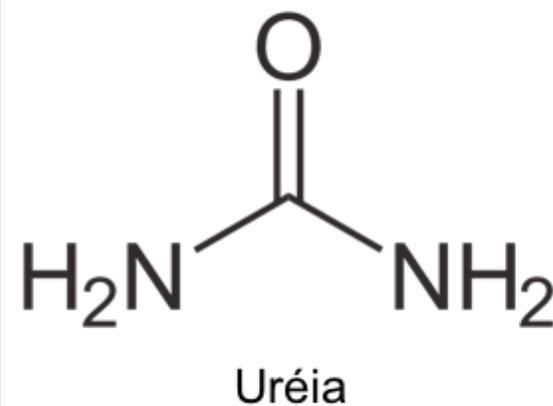
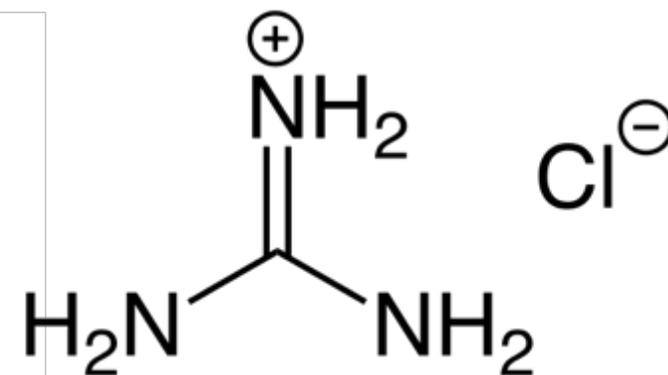
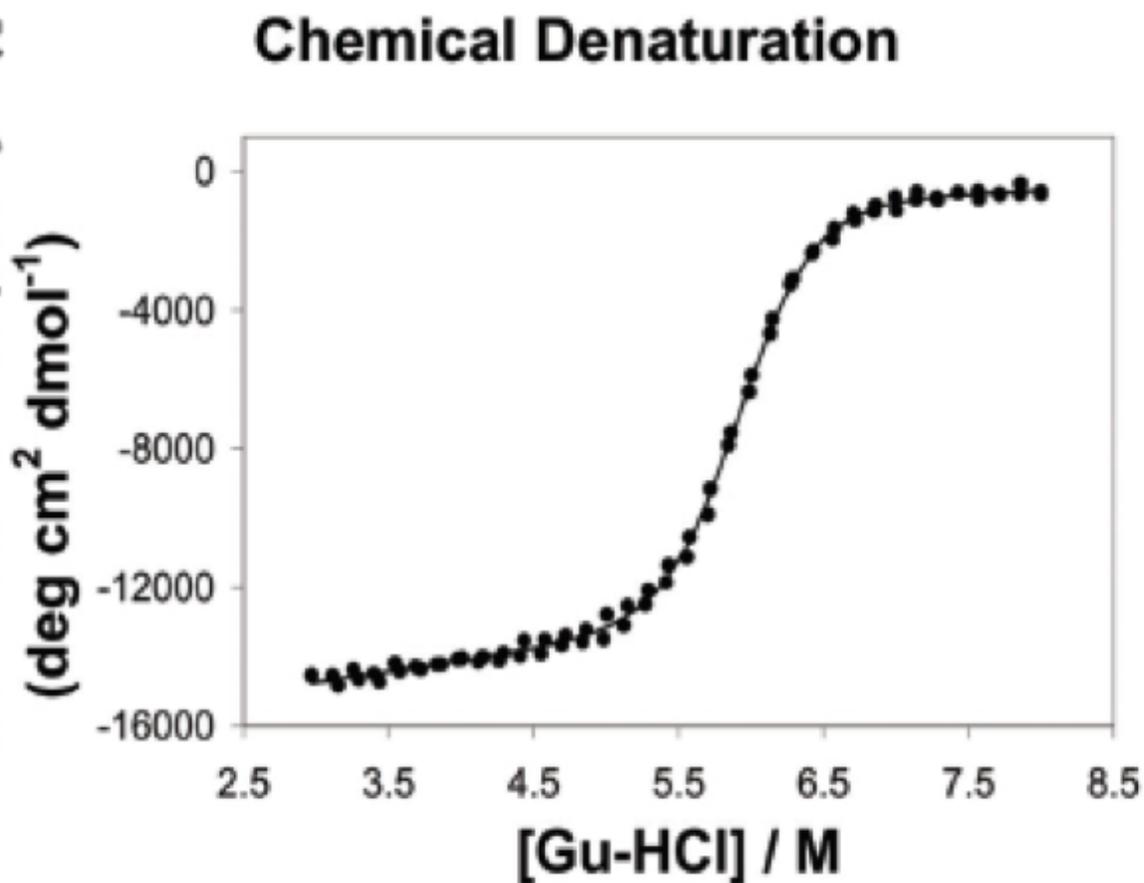


Fig. 9. Changes in reduced viscosity and molar ellipticity at 220 nm during the acid-induced transition from native to denatured nuclease. □ and ■, Reduced viscosity; △ and ▲, molar ellipticity at 220 nm; □ and △, measurements made during the addition of acid; ■ and ▲, measurements made during the addition of base (44).

- Como medir a constante de equilíbrio de enovelamento/desenovelamento?

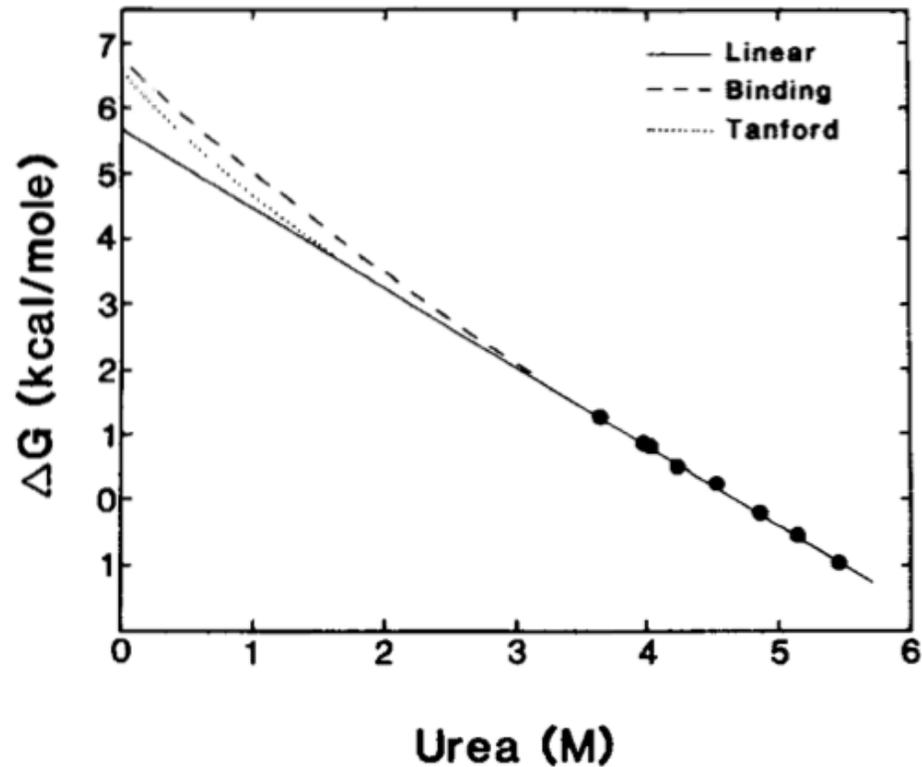
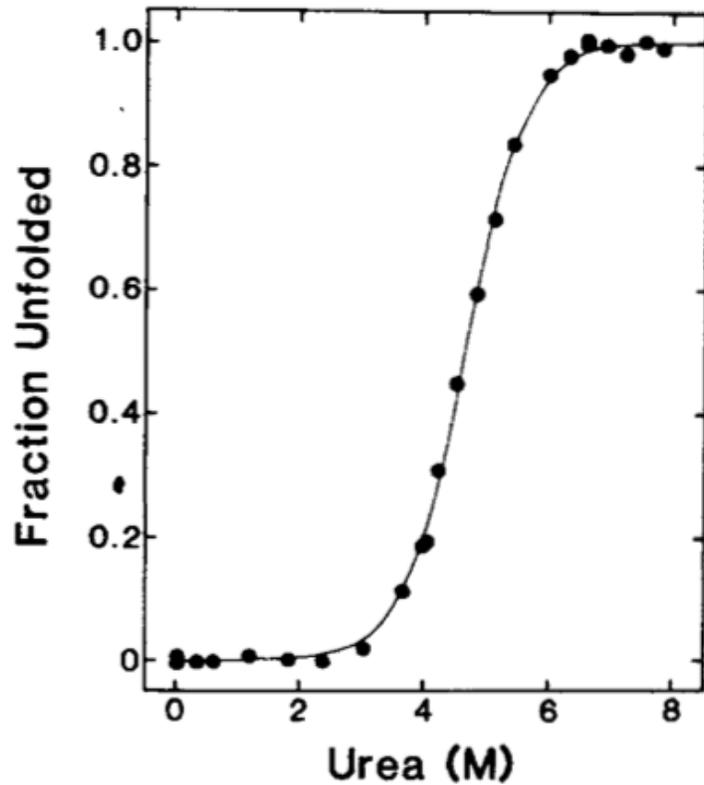
Mean Residue Ellipticity  
(deg cm<sup>2</sup> dmol<sup>-1</sup>)



- Como medir a constante de equilíbrio de enovelamento/desenovelamento?

$$K = f_u / f_N \quad N \longrightarrow U$$

$$\Delta G = \Delta G(\text{H}_2\text{O}) - m [\text{denaturant}]$$



## Termodinâmica de enovelamento de proteínas

Proteína	$\Delta G^0$ (kcal/mol)	$\Delta H^0$ (kcal/mol)	$T\Delta S^0$ (kcal/mol)
CI2	-6.62	-32.26	-25.64
EglinC	-8.82	-27.48	-18.66
RNAse T1	-8.96	-67.16	-58.22
Citocromo c	-8.87	-21.27	-12.40
Barnase	-11.69	-73.37	-61.71

Fonte: Lesk, A. *Introduction to protein science*. Oxford University Press, 2ª edição, p. 352 (2010)

## Efeito hidrofóbico

Propriedades termodinâmicas de transferência de hidrocarbonetos para a água, baseado nas solubilidades a 298K

	$\Delta G^0$ (kJ/mol)	$\Delta H^0$ (kJ/mol)	$\Delta S^0$ (kJ/Kmol)
benzeno	19,33	2,08	-57,8
pentano	28,62	-2	-102,7
hexano	32,54	0	-109,1
ciclohexano	28,13	-0,1	-94,7



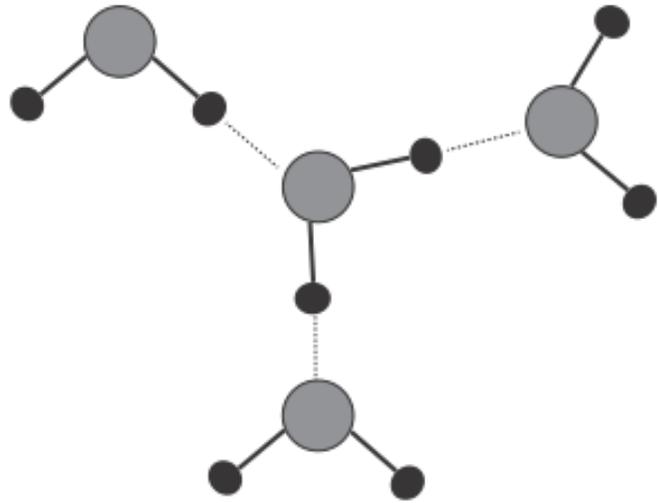
hidrofílico: dissolve em água  
hidrofóbico: não dissolve em água

Dill, K. et al. Molecular Driving Forces, Garland Science, 1ª. ed., 2003, Madison (pág. 579)

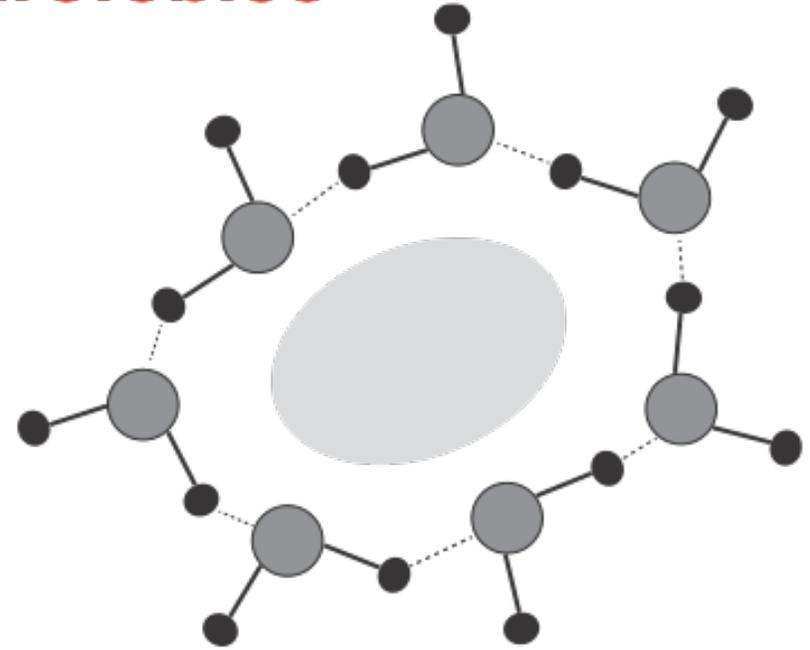
$$\Delta G = \Delta H - T\Delta S$$



# Moléculas de água mantêm a rede de ligações de hidrogênio ao redor do soluto hidrofóbico



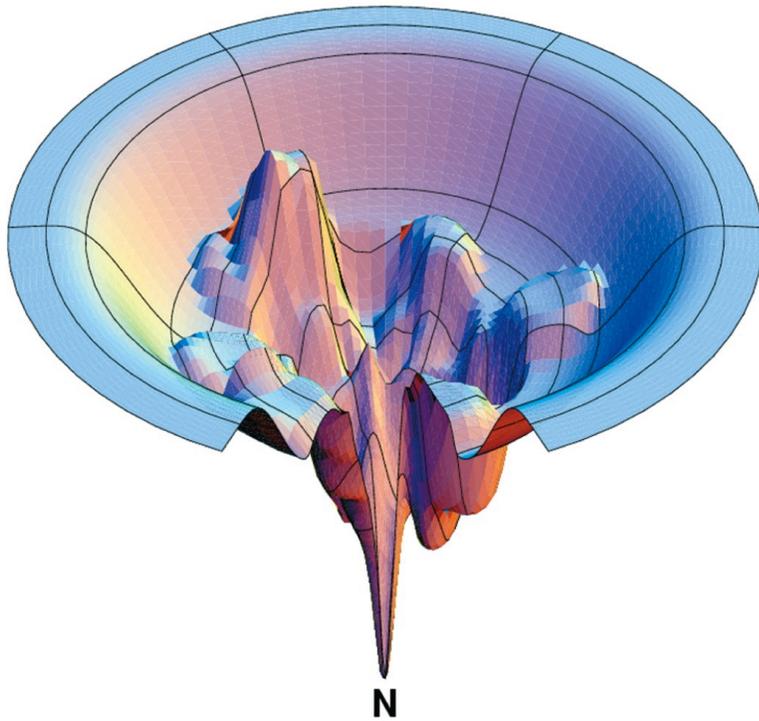
Ganho de entropia do solvente



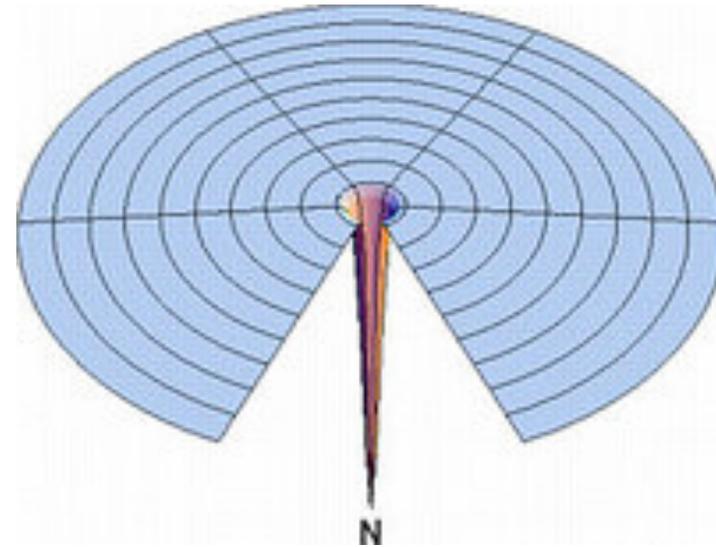
Perda de entropia do solvente

## Landscape theory of protein folding

Uma proteína supera o paradoxo de Levinthal ao “descer” uma superfície de energia livre multidimensional: *folding funnel*



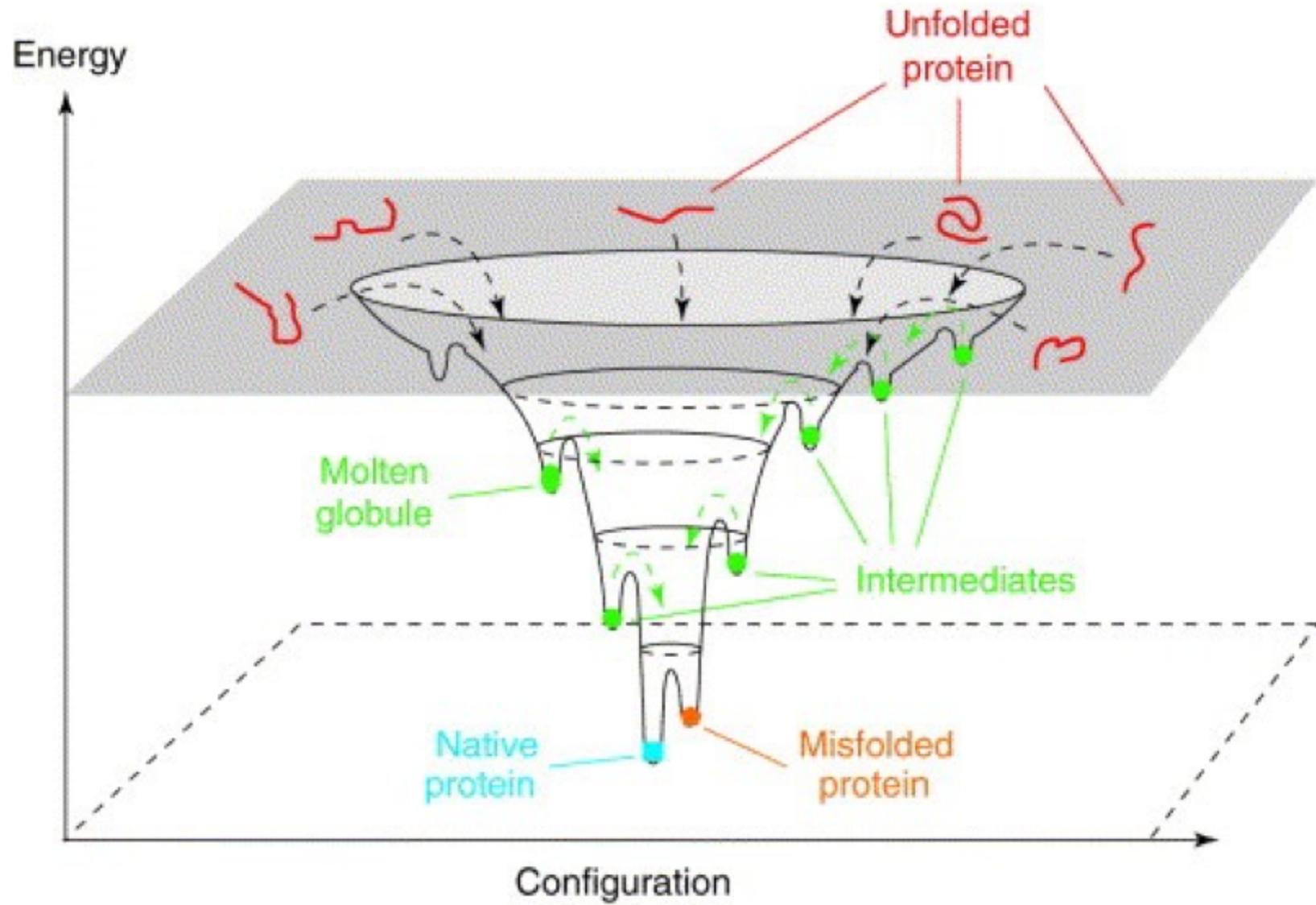
*Folding funnel*



*Golf funnel*: situação mencionada por Levinthal

# Proteínas enovelam-se rapidamente

Proteína	Número de aminoácidos	Tempo que demora o enovelamento ( $\mu\text{s}$ )
Domínio de ligação a Albumina	47	1
$\alpha$ 3D	73	3
Tryptophan cage	20	4,1
Villin headpiece subdomain	36	4,3



T/BS

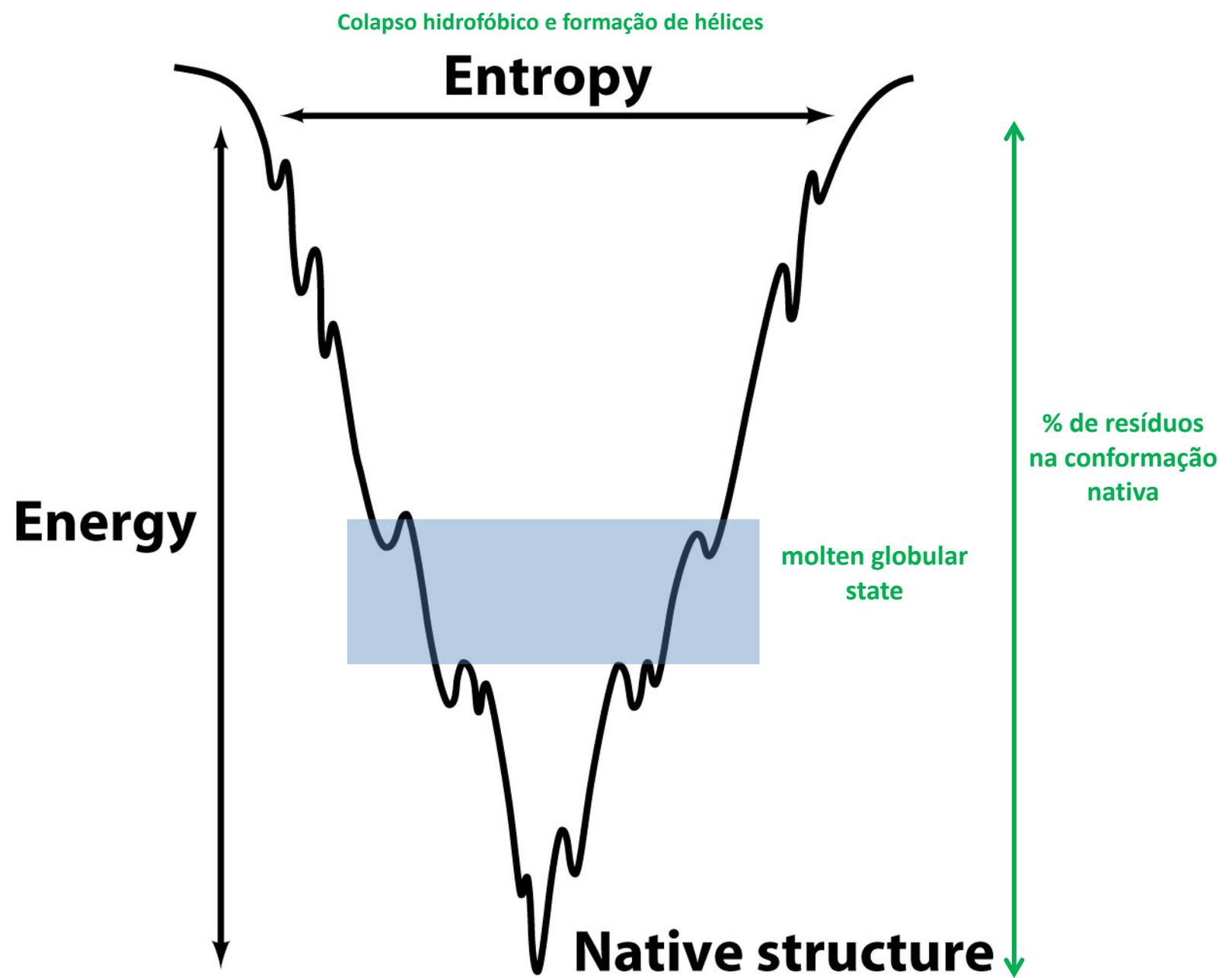


Figure 6-41 Fundamentals of Biochemistry, 2/e  
© 2006 John Wiley & Sons

- **Como proteínas enovelam-se *in vivo*?**

Enquanto que a maior parte dos princípios discutidos aplicam-se ao enovelamento *in-vivo*, dentro das células o enovelamento é mais complicado devido a:

- Proteínas são sintetizadas a uma velocidade (4-20aa/segundo) muito mais lentamente do que velocidades de enovelamento típicas ( $\sim < 1s$ )
- Superpopulação de partículas dentro da célula (densidade de *E. coli*  $\sim 340$  mg/ml de proteína) aumenta a chance de superfícies hidrofóbicas expostas interagirem com outras moléculas

*In-vivo*, o enovelamento de proteínas é auxiliado por **Chaperonas: um conjunto de proteínas auxiliares que ajudam outras proteínas a enovelarem-se corretamente**

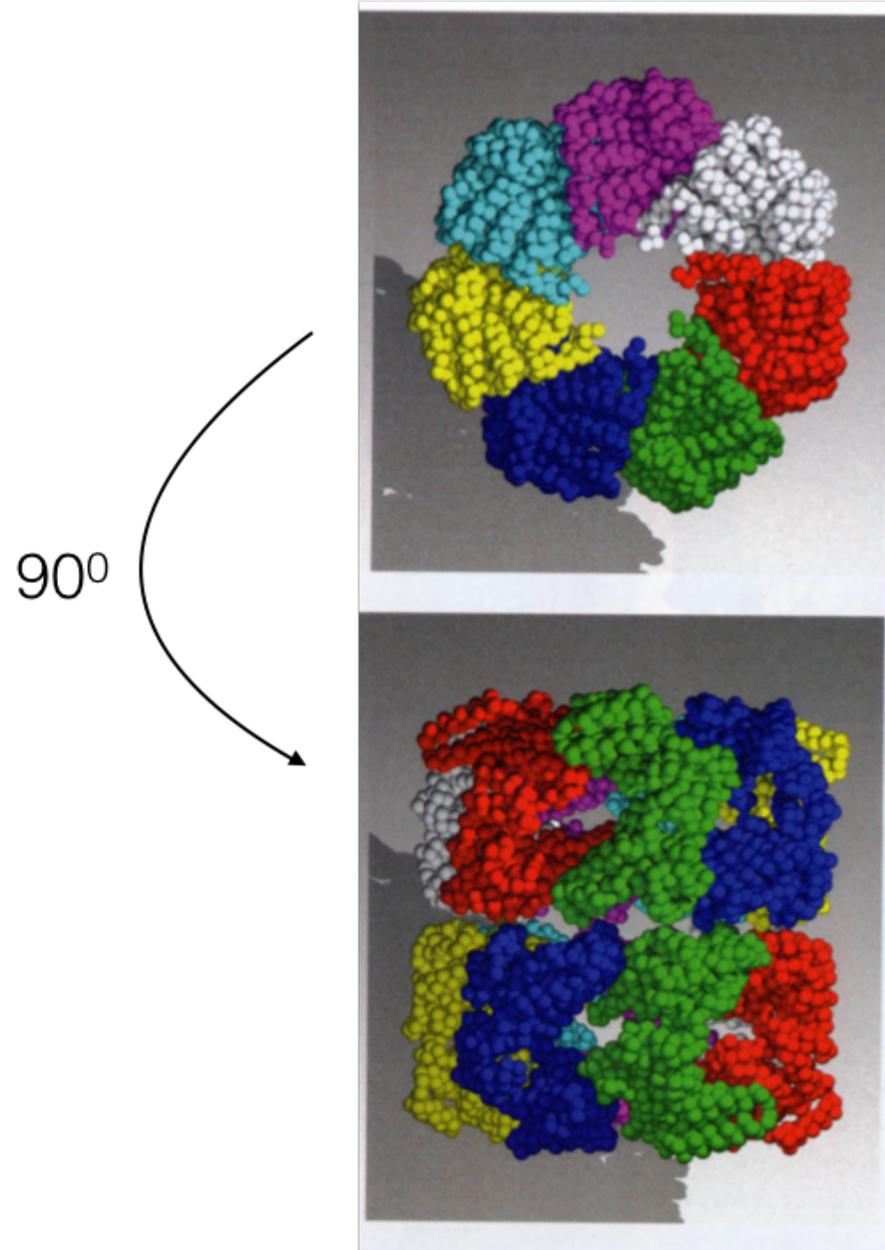
-*protein disulfide isomerases* - catalizam a troca de pontes SS

-*peptidyl-prolyl isomerases* - aceleram a isomerização cis-trans de prolina

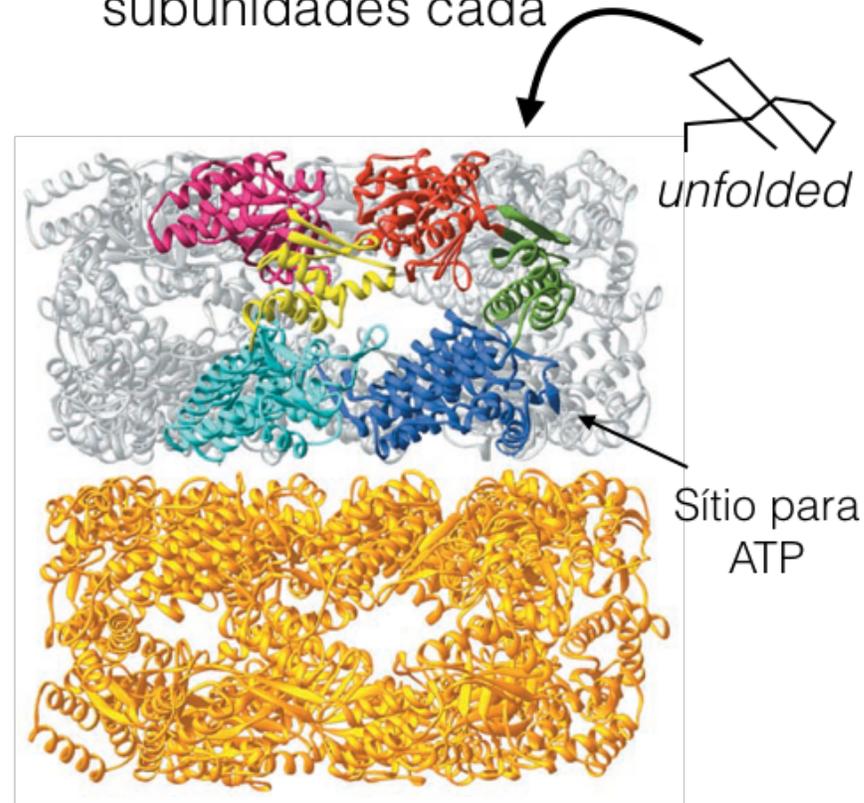
-*Hsp (Heat Shock Proteins)* - ligam-se a superfícies hidrofóbicas

Muitas chaperonas são ATPases

# Chaperones: GroEL/GroES

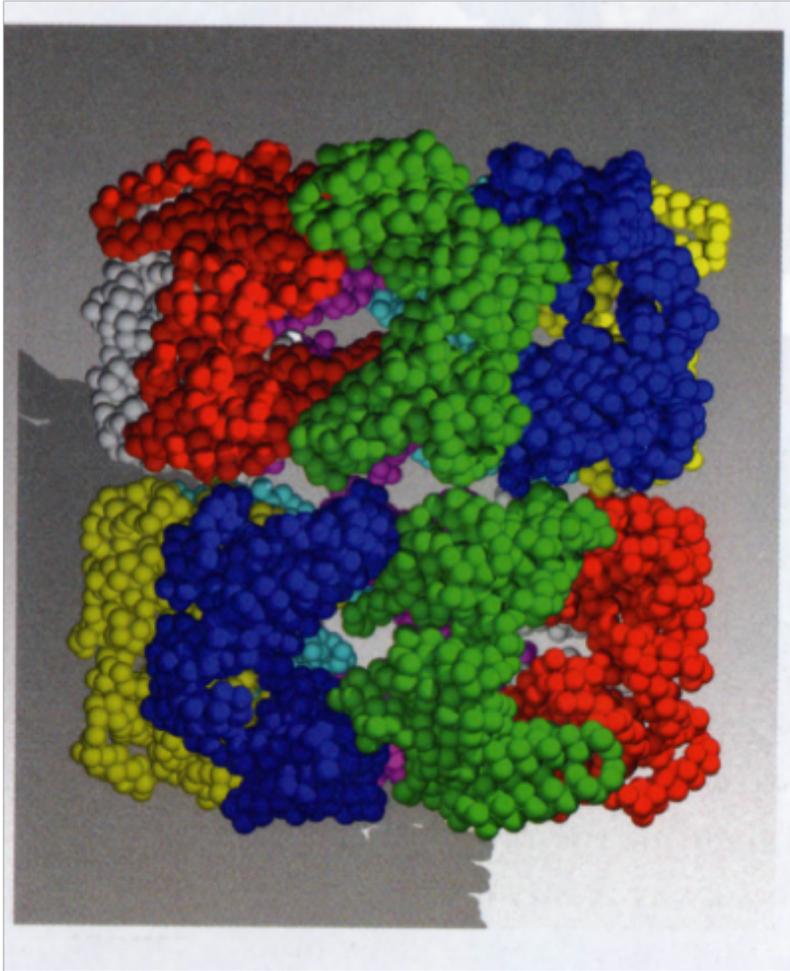


- GroEL (Hsp60)
- Dois anéis de 7 subunidades cada

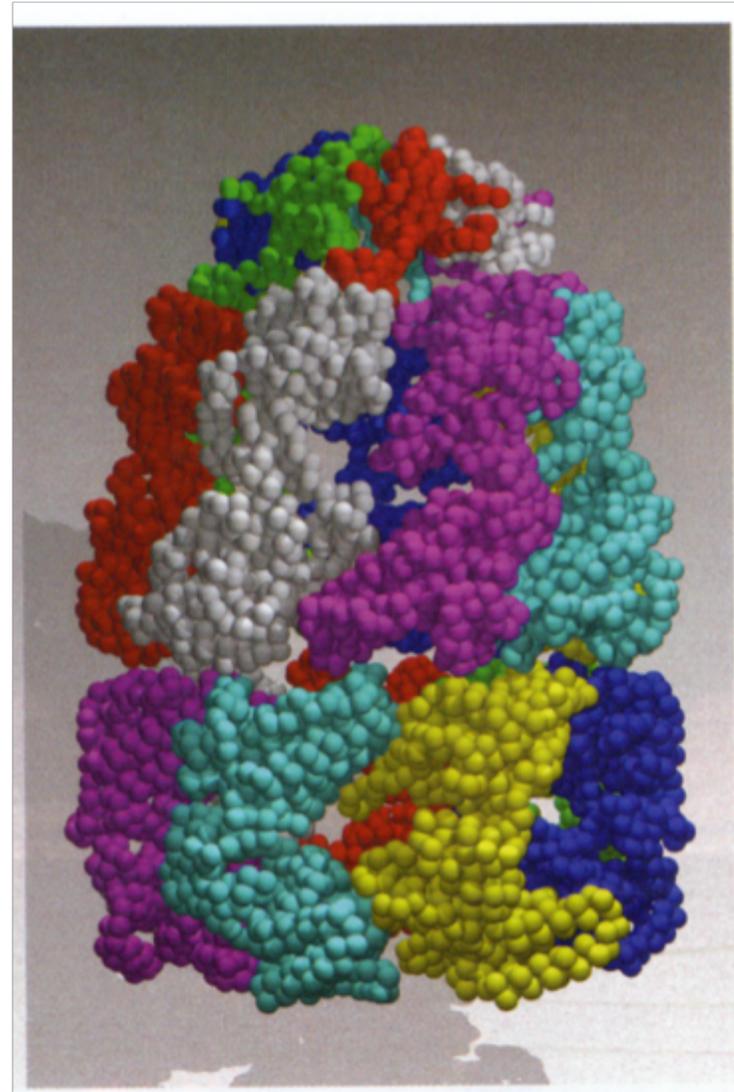


Fonte: Arthur Lesk, *Introduction to Protein Science*, Oxford University Press, 2a edição (2010) e Voet&Voet 4a edição

GroEL



GroEL-7ADP-GroES (Hsp10)



*cis*

*trans*

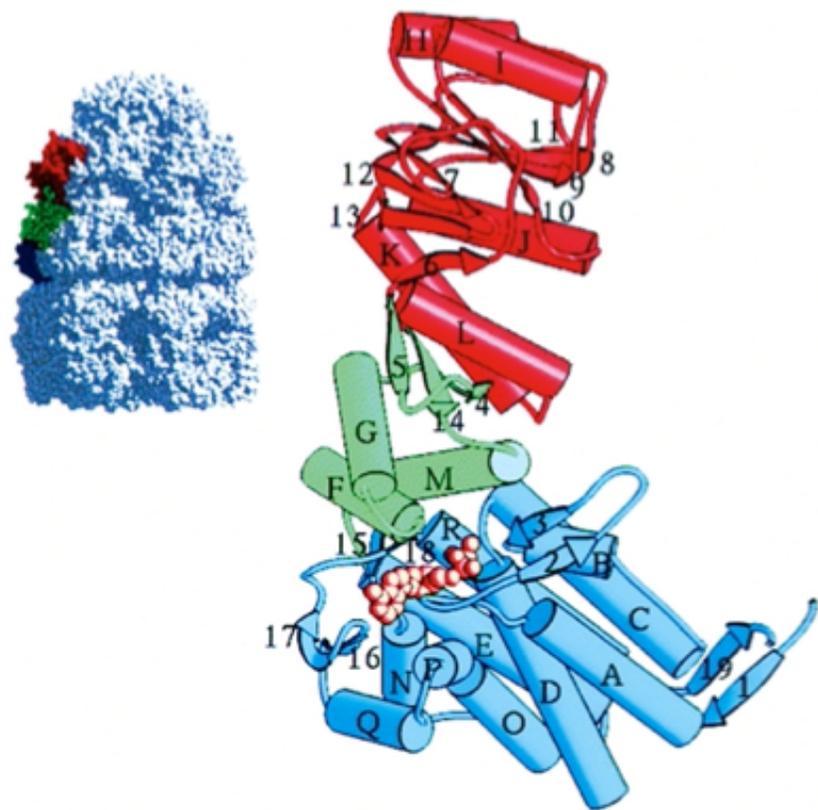


Figure 9-22b  
Courtesy of Arthur Horwich, Yale University

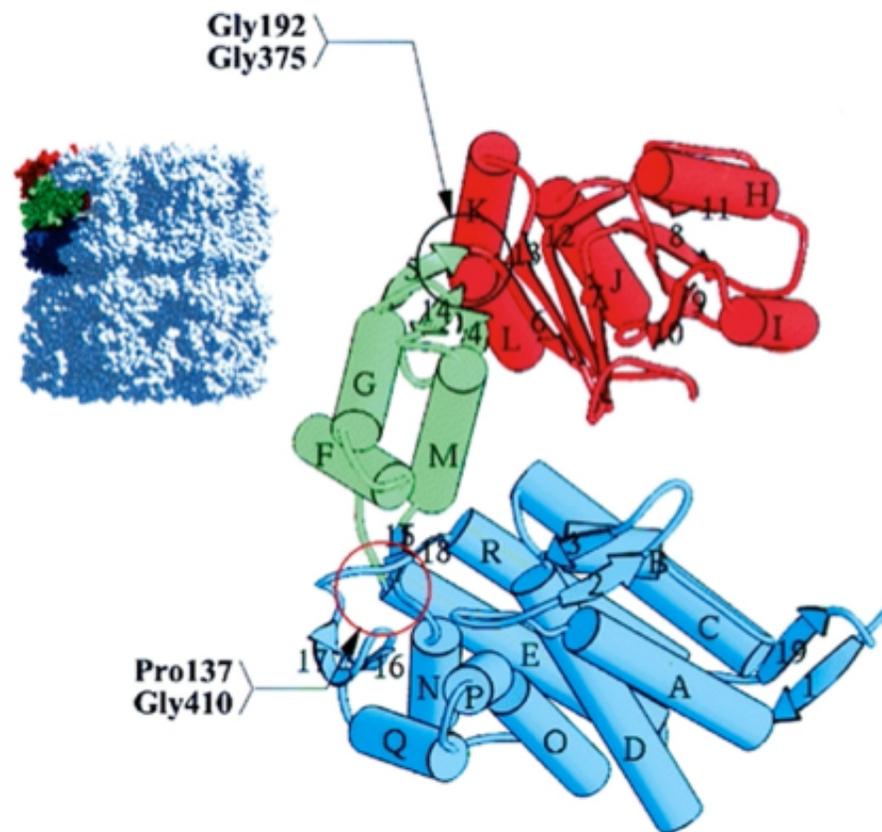
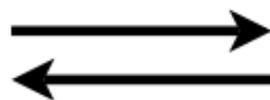
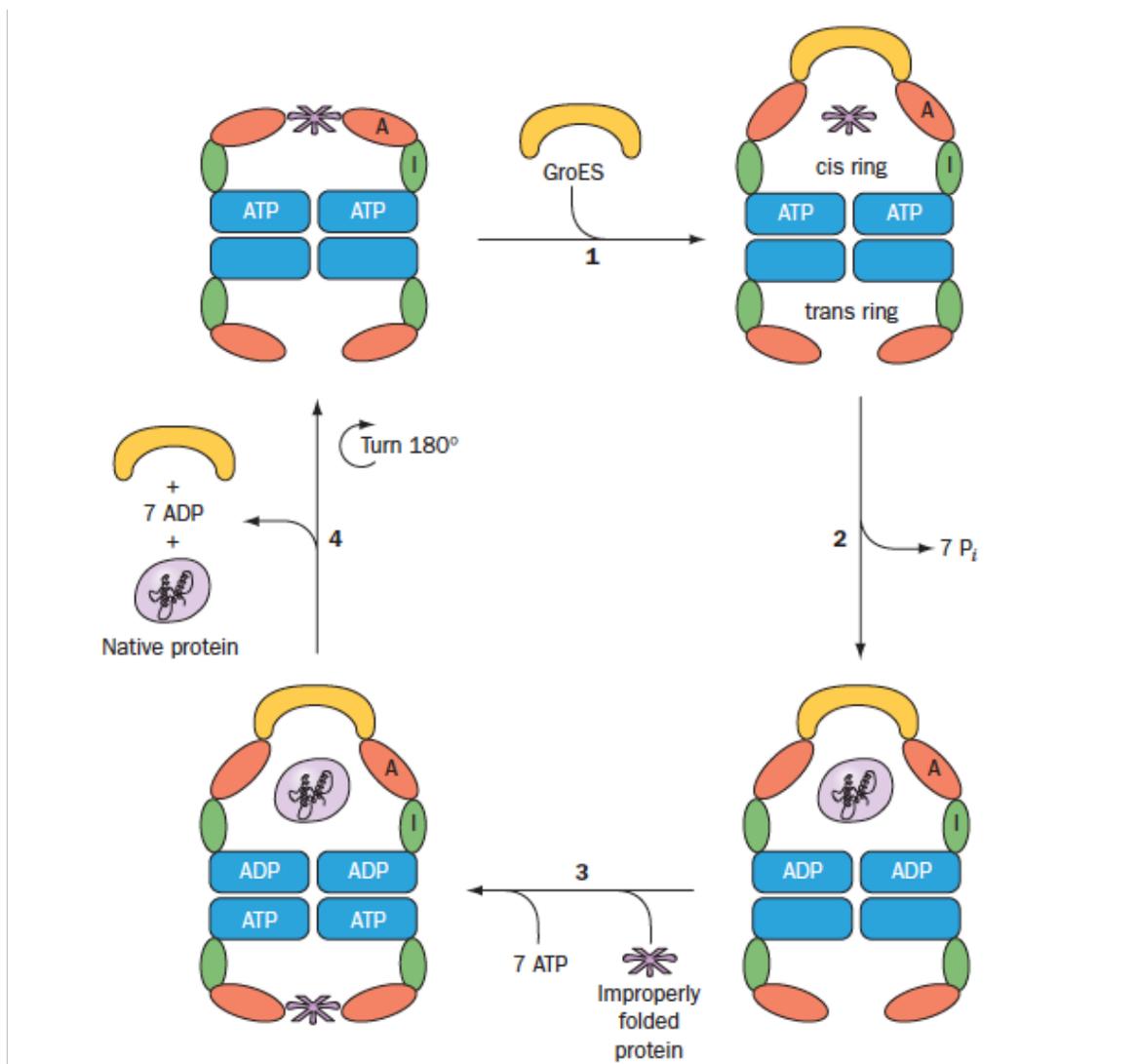


Figure 9-22a  
Courtesy of Arthur Horwich, Yale University



GroEL liga-se a superfícies hidrofóbicas com muito maior afinidade do que GroEL-GroES-ATP

*Annealing mechanism:* ao ligar superfícies hidrofóbicas GroEL impede a agregação de intermediários não produtivos de enovelamento, permitindo que estes intermediários desenovelem-se e reenovelem-se formando um intermediário produtivo

## 1977: McCammon, Gelin, e Karplus "Dynamics of folded proteins"

Nature (1977) 267 585-590

"The dynamics of a folded globular protein (BPTI) have been studied by solving the equations of motion for the atoms with an empirical potential energy function. The results provide the magnitude, correlations, and decay of fluctuations about the average structure. These suggest that the protein interior is **fluid-like** in that the local atom motions have a diffusional character"

Dinâmica molecular de BPTI por 8.8 ps

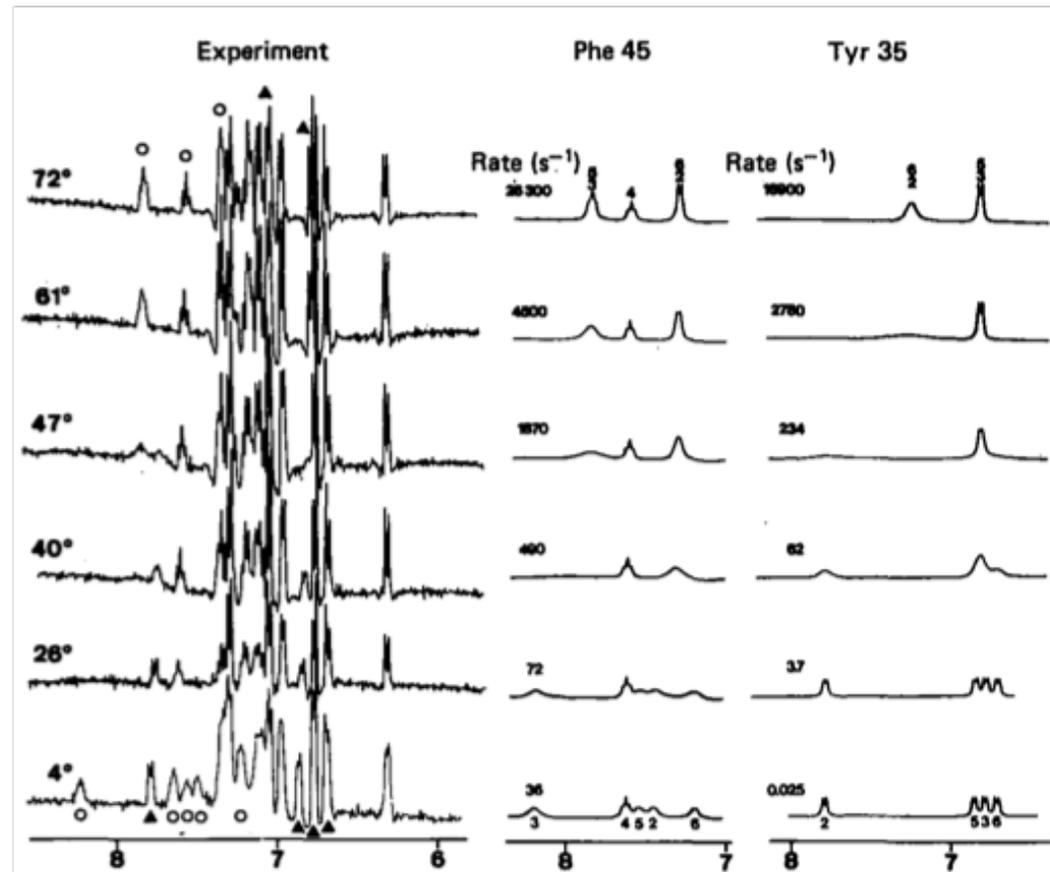
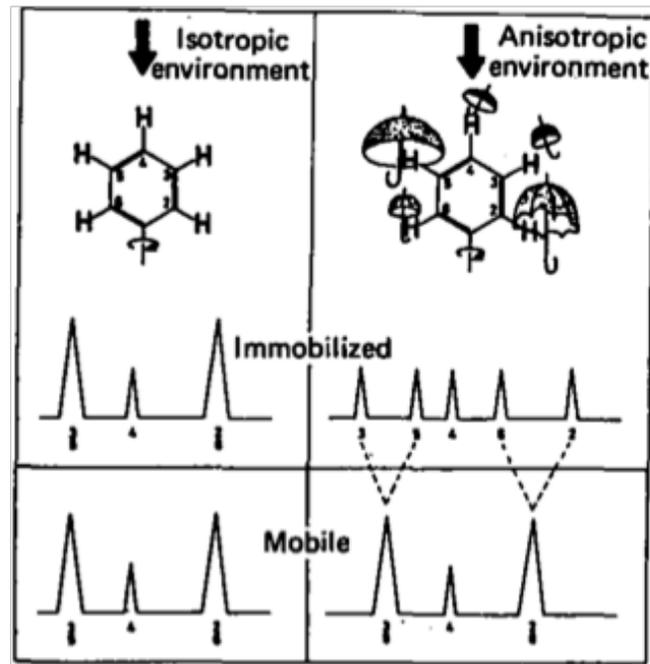
# 1978: Wüthrich e Wagner "Internal motion in globular proteins"

## Internal motion in globular proteins

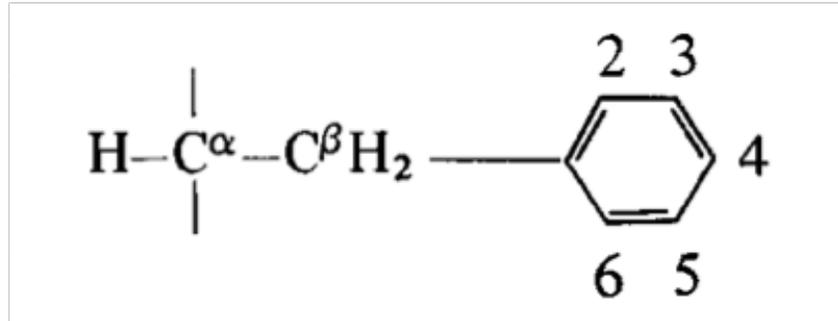
Kurt Wüthrich and Gerhard Wagner

*High resolution nuclear magnetic resonance provides insight into the dynamic ensembles which constitute the conformations of globular proteins in solution.*

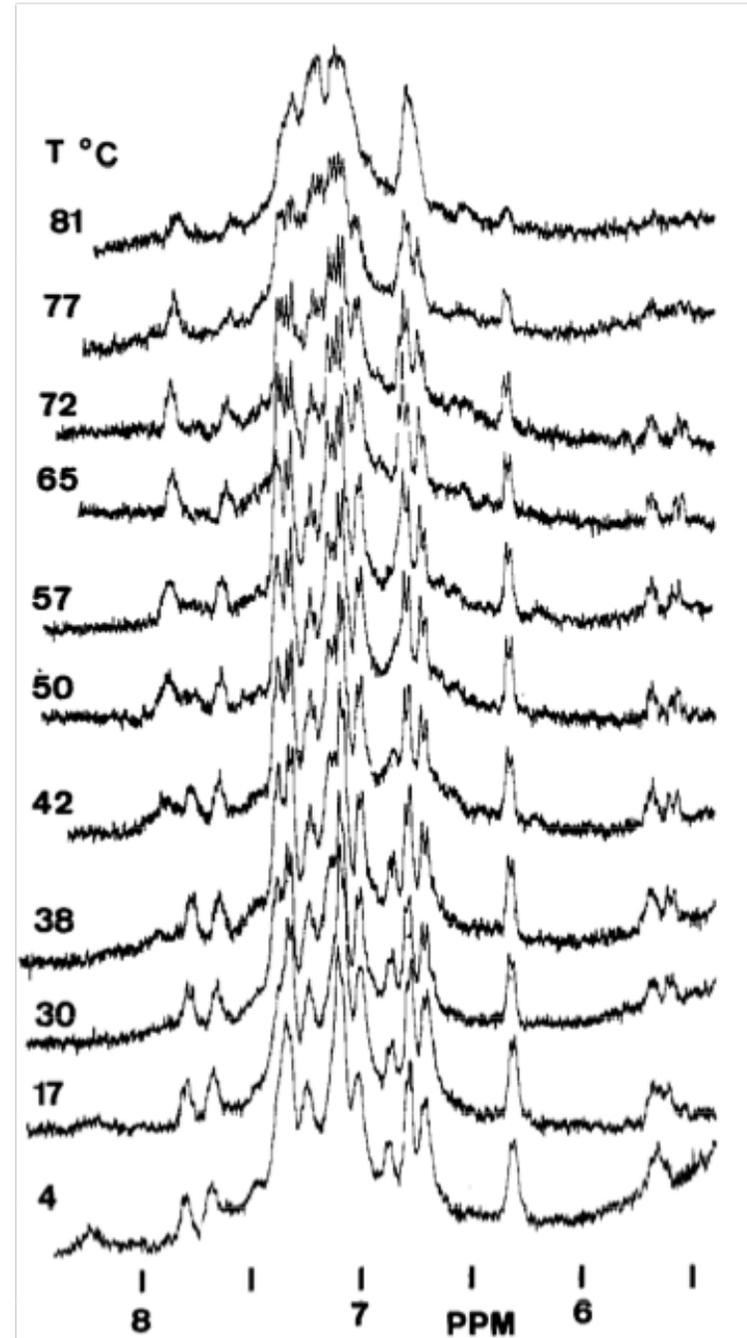
TIBS (1978) 227-230



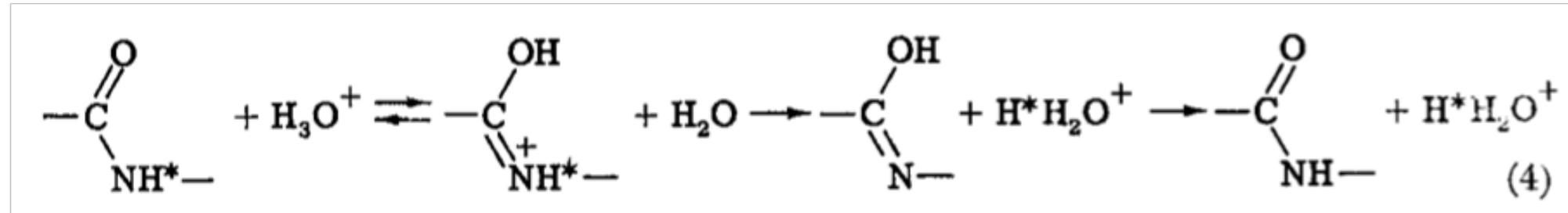
# “Flipping” de anéis aromáticos



Wüthrich e Wagner (1975)



# Medidas da cinética de troca H/D (HDX) em proteínas



Hvidt e Nielsen (1966)



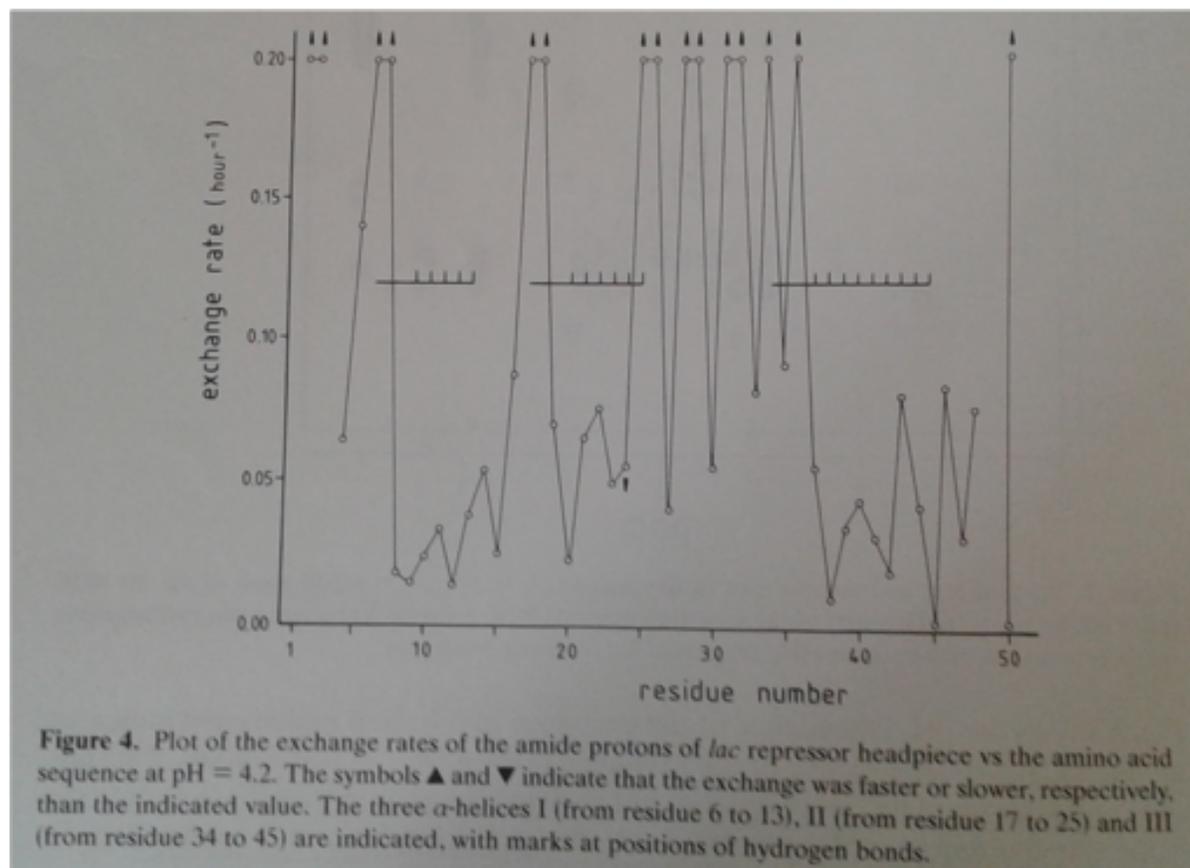
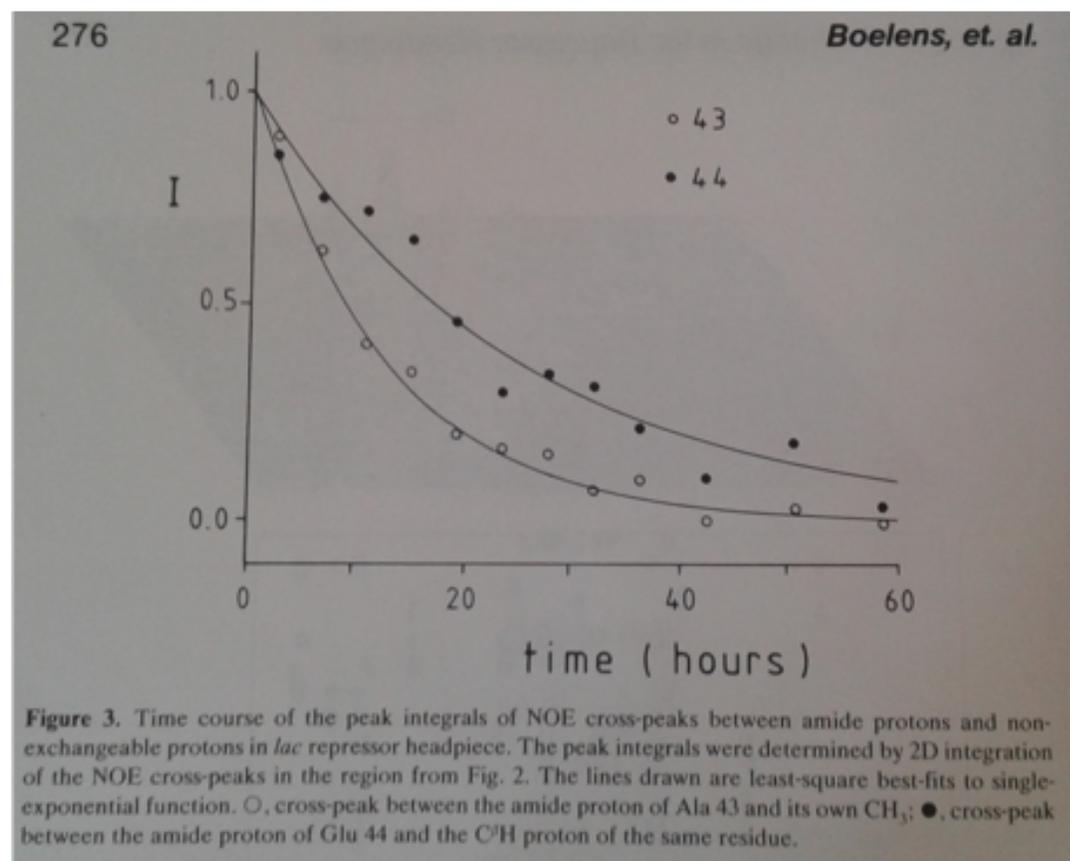
$$\text{EX1: } k_{\text{obs}} = k_{\text{op}}$$

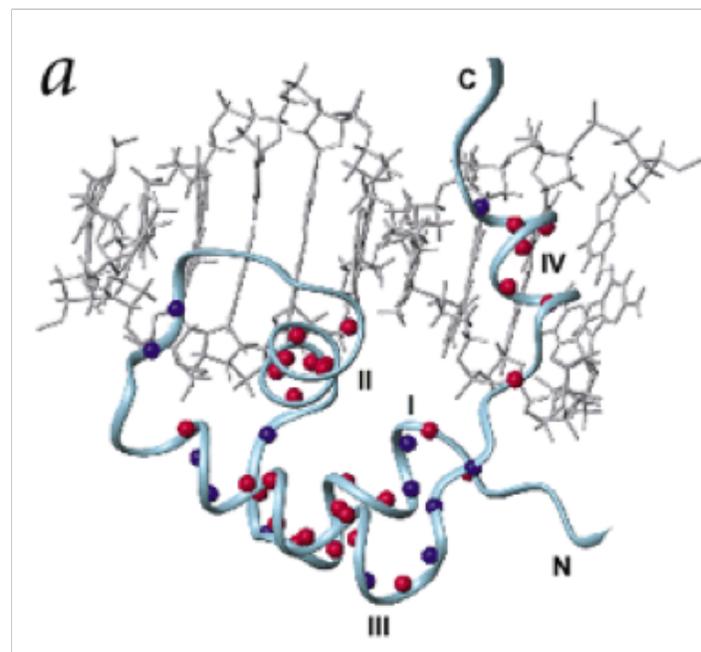
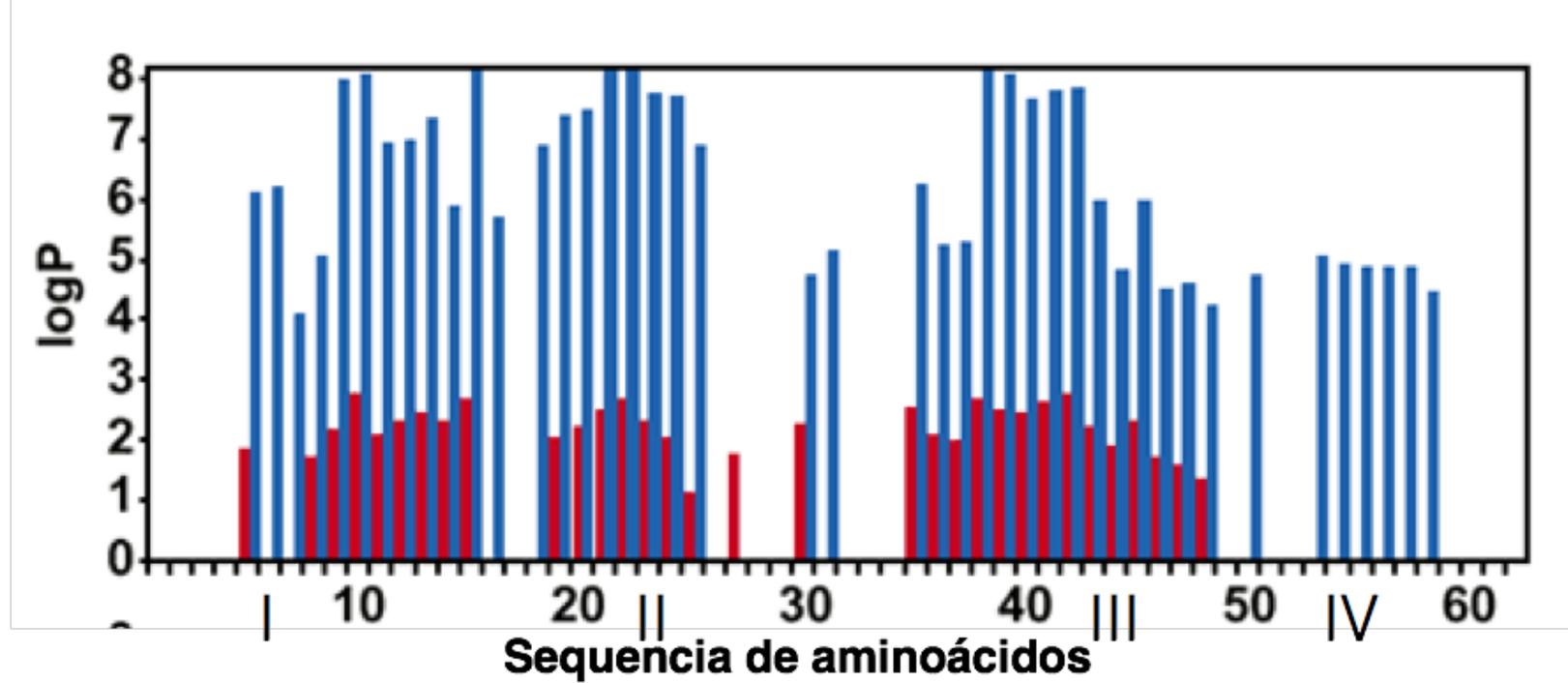
$$\text{EX2: } k_{\text{obs}} = k_{\text{int}} (k_{\text{op}}/k_{\text{cl}})$$

$$\Delta G_{\text{op}} = -RT \ln(k_{\text{op}}/k_{\text{cl}}) = -RT \ln(k_{\text{obs}}/k_{\text{int}})$$

# Boelens, Gros, Scheek, Verpoorte e Kaptein: "Hydrogen exchange of individual amide protons in the E. coli lac repressor DNA binding domain"

J Biomol Struct Dyn (1985) 3:269-80





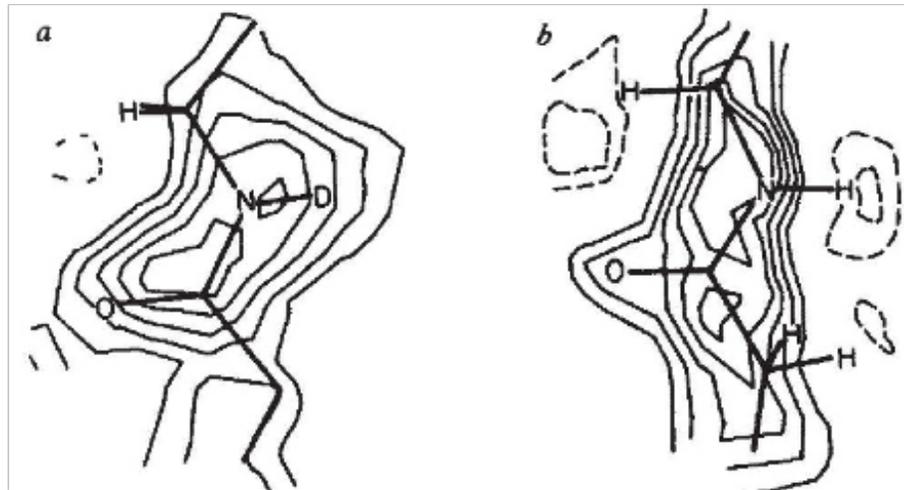
# Qual é o mecanismo das flutuações conformacionais que levam à troca?

## Protein dynamics investigated by the neutron diffraction–hydrogen exchange technique

A. A. Kossiakoff

Department of Biology, Brookhaven National Laboratory, Upton, New York 11973, USA

Nature 296: 713 (1982)



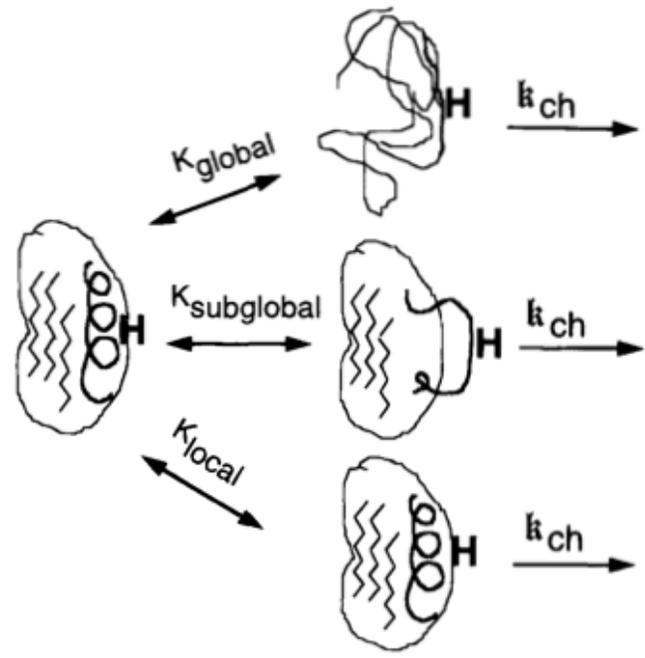
-Demanda um reator nuclear  
(fonte de neutrons)

-Um cristal de tripsina foi  
mergulhado em  $D_2O$

-1 ano a pH 7 e  $20\text{ }^\circ\text{C}$

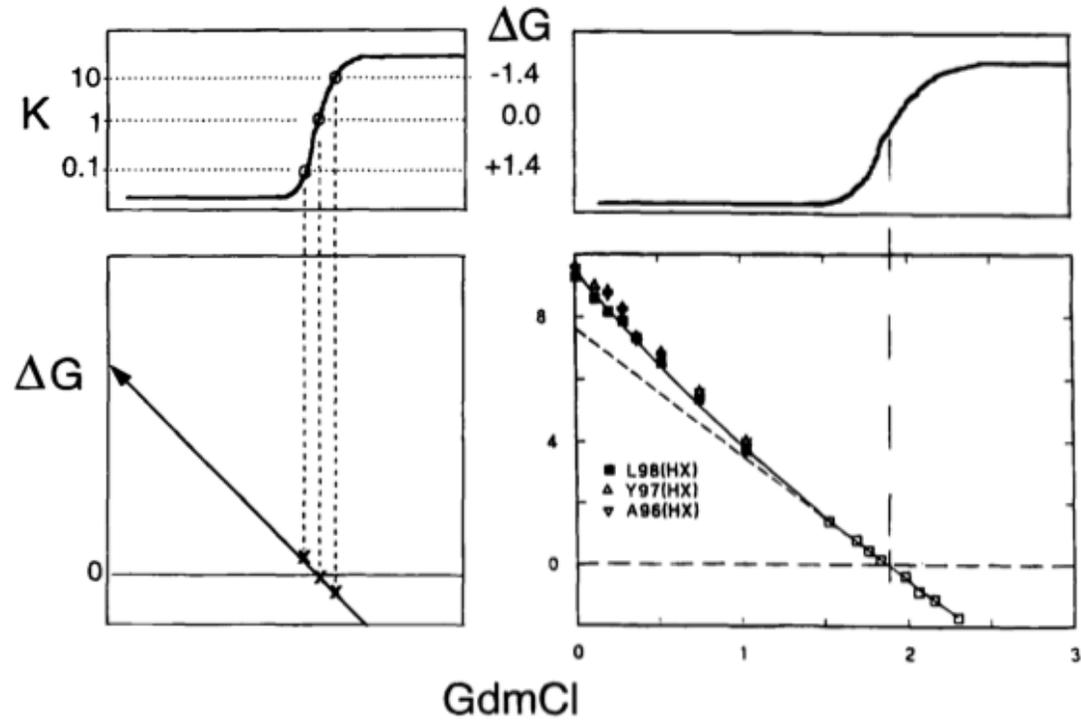
-Mecanismo de troca envolve  
flutuações locais, quebra de  
ligações de H

# Os NHs mais enterrados trocam por um mecanismo de desenovelamento global



$$k_{ex} = K_{ap} k_{ch}$$

$$\Delta G = -RT \ln K_{ap}$$



citocromo c

$$K_{ex} = k_{obs}$$

$$K_{ap} = K_{eq}$$

$$k_{ch} = k_{int}$$

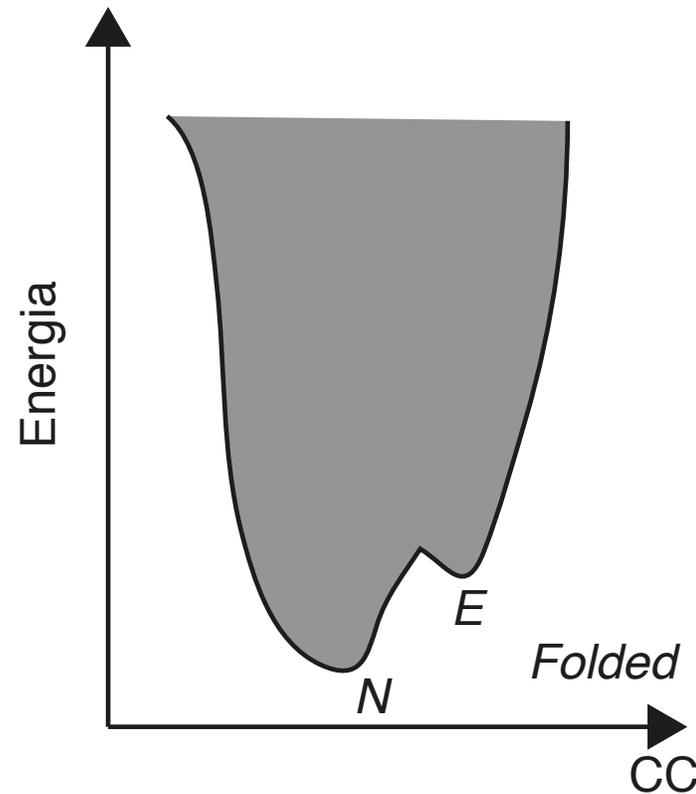
Bai et al. (1995) Science 269: 192-197

Bai et al. (1994) Proteins 20: 4-14

Englander et al. (1997) Protein Sci. 6: 1101-1109

## "Conformational energy landscape"

Frauenfelder, Sligar, e Wolynes (1991) The energy landscapes and motions of proteins  
Science (1991) 254 1598-603

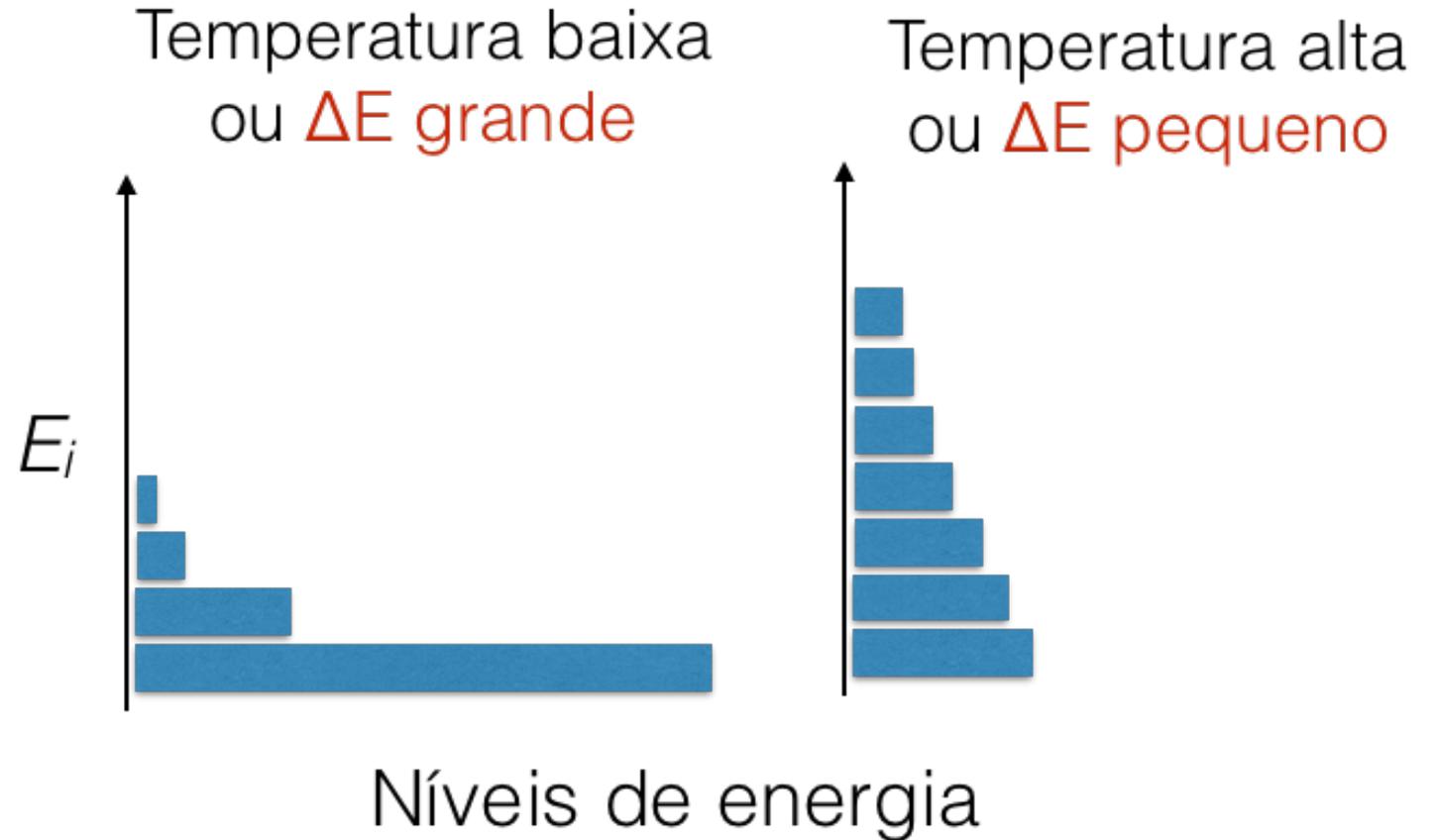


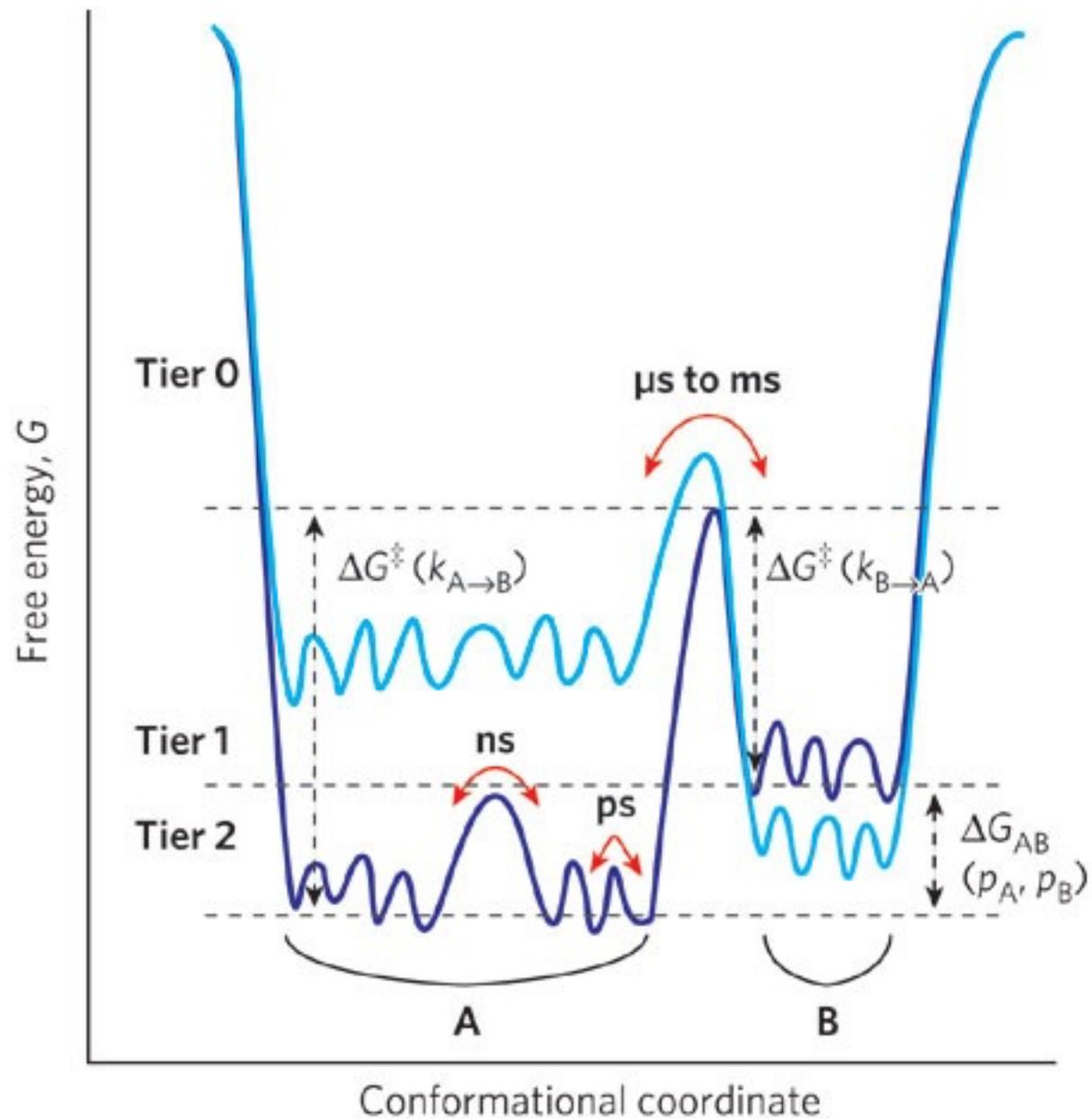
**Dinâmica:** Proteínas visitam diferentes conformações em torno da conformação média de acordo com a energia térmica. Todas as possíveis conformações de uma proteína são populadas de acordo com a sua energia em relação a  $kT$

# Lei de distribuição de Boltzmann

$$p_i = \frac{\exp\left(-\frac{E_i}{kT}\right)}{Z}$$

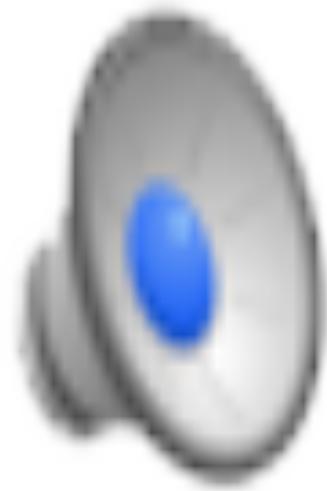
$$Z = \sum_{i=1}^N \exp\left(-\frac{E_i}{kT}\right)$$





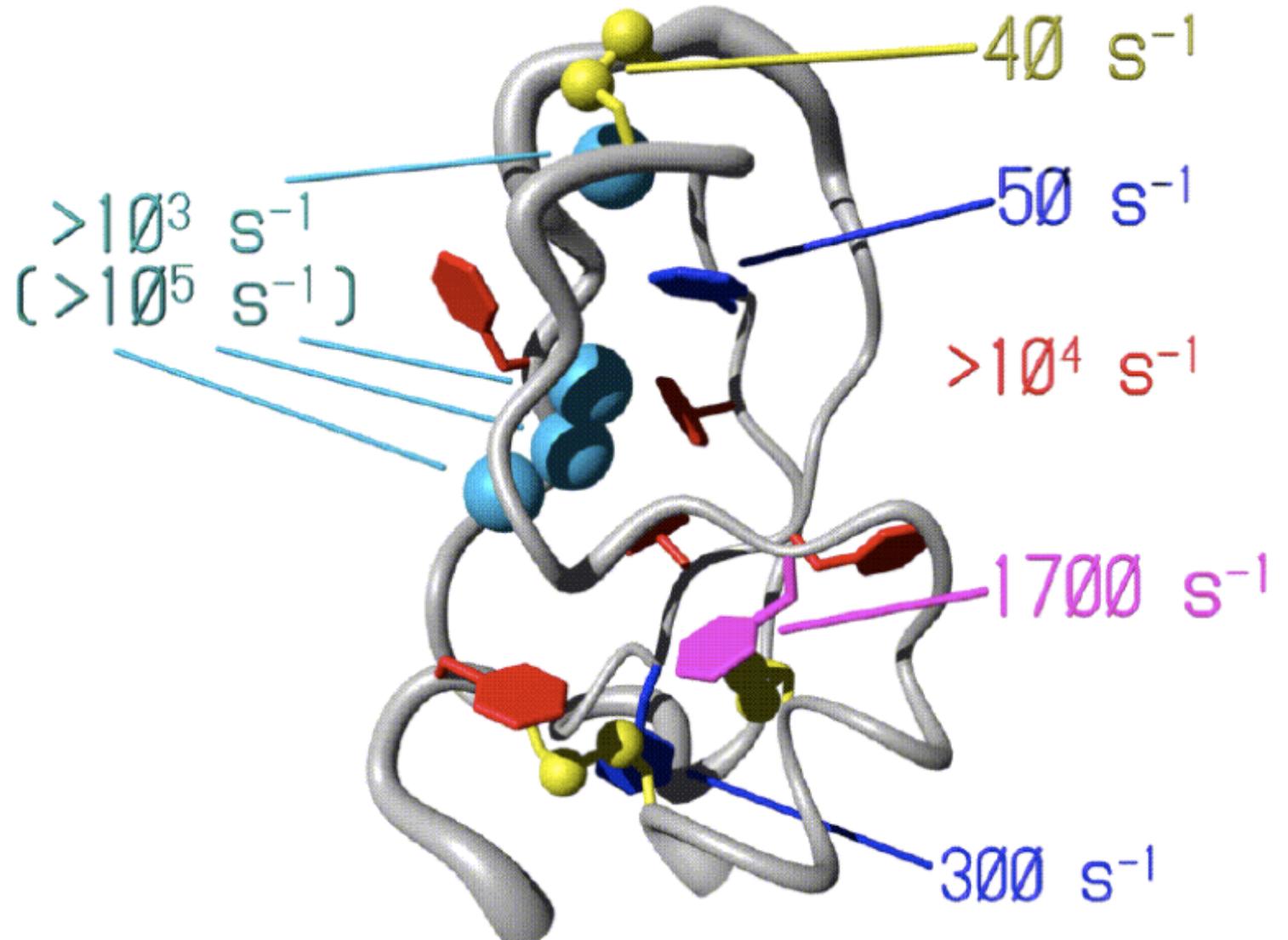
Henzler-Wildman, K. and Kern, D. (2007) *Nature* **450**: 964-972

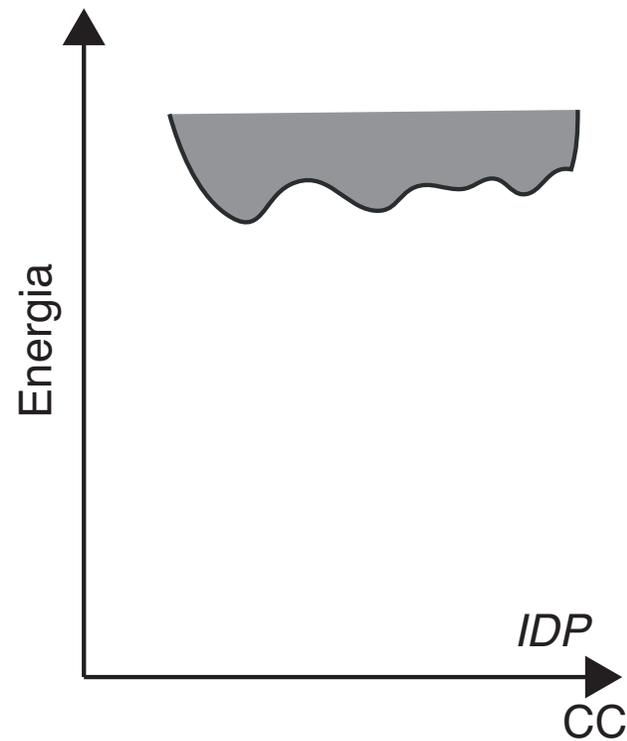
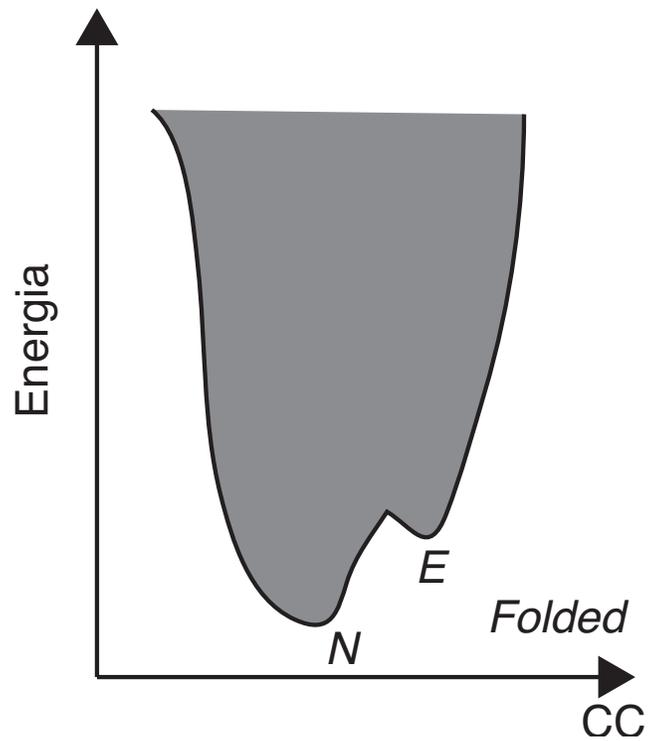
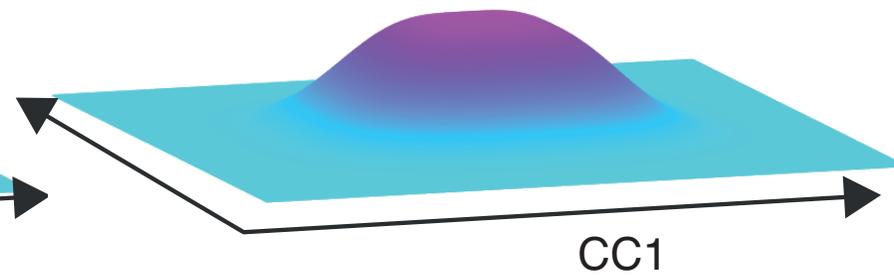
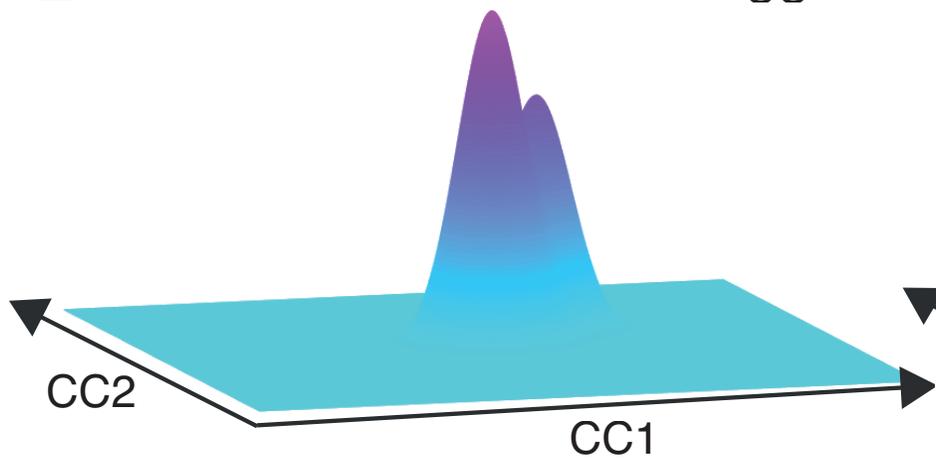
# Simulações computacionais de sistemas químicos



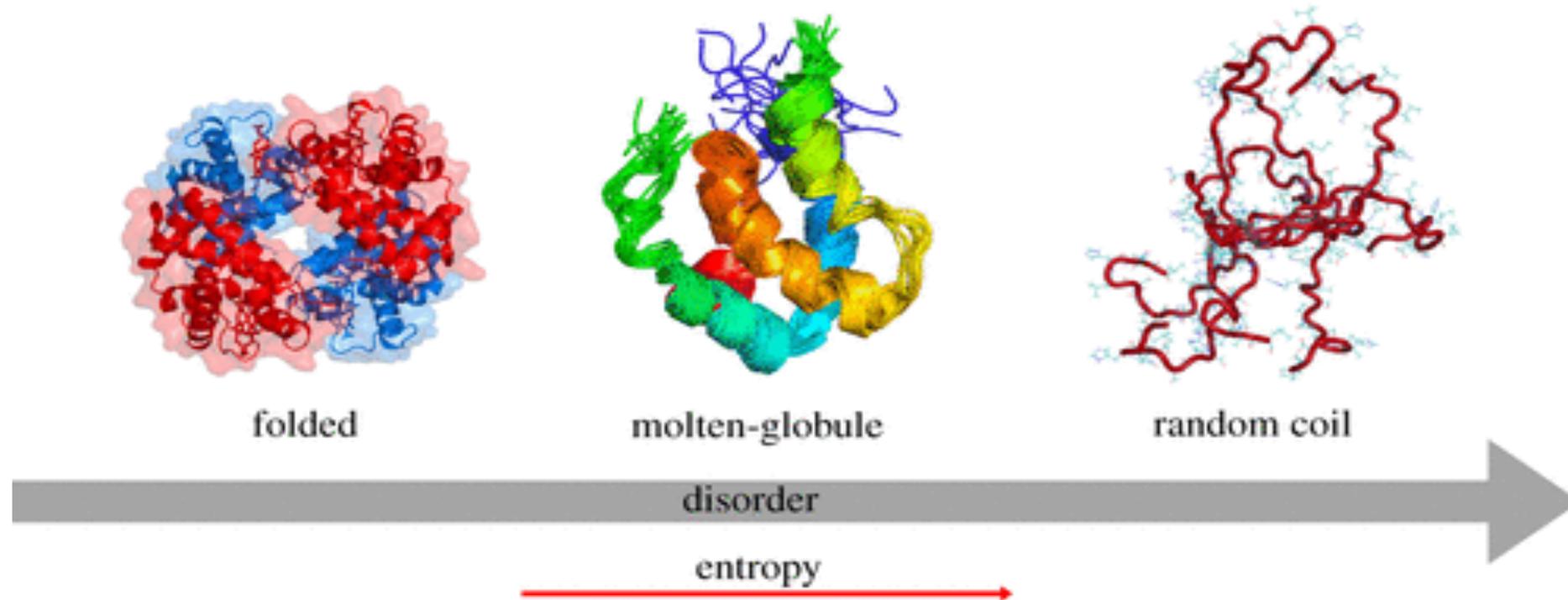
## Múltiplos tipos de movimento

- Bond vibration (fs)
- Loop motions (ns -  $\mu$ s)
- CH<sub>3</sub> motion (ps - ns)
- Aromatic ring flipping (ps - ms)
- Domain motions ( $\mu$ s - ms)



**A****B**

# Intrinsically Disordered Proteins (IDPs) e Intrinsically Disordered Regions (IDRs) apresentam diferentes graus de desordem



# Diferentes classes de IDPs/IDRs

- *Molecular recognition features (MoRFs)*

Sítios de 10 - 70 aminoácidos que adquirem estrutura ao se ligar a outra proteína ( $\alpha$ -MoRF,  $\beta$ -MoRF,  $i$ -MoRF). Exemplos: p53 contém múltiplos MoRFs, o domínio C-terminal da RNase E, regiões básicas de zippers de leucina, etc

- *Short linear motifs (SLiMs)*

Sítios de modificação pós-traducional, sítios de proteólise, sítio de fosfotirosina para reconhecimento de SH2, motivos PxxP de interação com SH3, sinal de localização nuclear (NLS), etc

- *Low complexity regions (LCRs)*

Regiões com baixa complexidade de sequência, isto é, muitas repetições de alguns aminoácidos. Exemplo: poliglutamina.

# IDPs frequentemente se enovelam ao interagir com outras proteínas

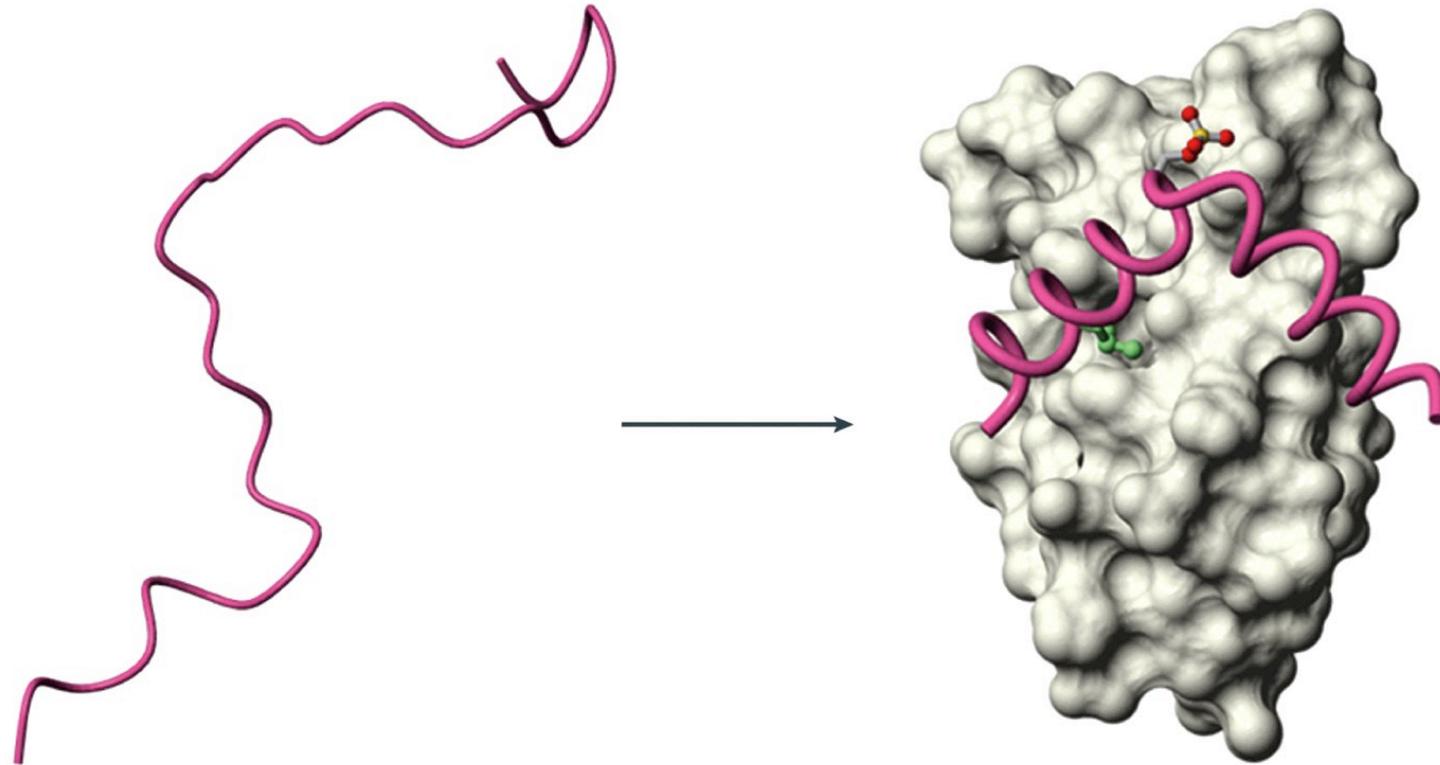


Figure 9-8  
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IDPs/IDRs se enovelam antes ou após a ligação?

# IPDs podem eventualmente formar condensados por separação de fase líquido-líquido...

