

Acúmulo de evidências consolidou a visão de que proteínas são flexíveis

Enzimas exibem dinâmica no sítio ativo

IDPs, IDRs, e condensados

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.





"Flipping" de anéis aromáticos





Wüthrich e Wagner (1975)

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



Hvidt e Nielsen (1966)



EX1: $k_{obs} = k_{op}$ EX2: $k_{obs} = k_{int} (k_{op}/k_{cl})$ $\Delta G_{op} = -RT ln(k_{op}/k_{cl}) = -RT ln(k_{obs}/k_{int})$

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





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Figure 3. Time course of the peak integrals of NOE cross-peaks between amide protons and nonexchangeable protons in lac repressor headpiece. The peak integrals were determined by 2D integration of the NOE cross-peaks in the region from Fig. 2. The lines drawn are least-square best-fits to singleexponential function. O, cross-peak between the amide proton of Ala 43 and its own CH₃; •, cross-peak between the amide proton of Glu 44 and the C⁰H proton of the same residue.

Figure 4. Plot of the exchange rates of the amide protons of lac repressor headpiece vs the amino acid sequence at pH = 4.2. The symbols \blacktriangle and \checkmark indicate that the exchange was faster or slower, respectively. than the indicated value. The three a-helices I (from residue 6 to 13), II (from residue 17 to 25) and III (from residue 34 to 45) are indicated, with marks at positions of hydrogen bonds,

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

Protein dynamics investigated by the neutron diffraction-hydrogen exchange technique

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Nature 296: 713 (1982)





- -Demanda um reator nuclear (fonte de neutrons)
- -Um cristal de tripsina foi mergulhado em D2O
- -1 ano a pH 7 e 20 °C

-Mecanismo de troca envolve flutuações locais, quebra de ligações de H Os NHs mais enterrados trocam por um mecanismo de desenovelamento global



 $k_{ch} = k_{int}$

Bai et al. (1994) Proteins 20: 4-14 Englander et al. (1997) Protein Sci. 6: 1101-1109

Experimentos de cinética de troca H/D (HDX) usando RMN log(P) P=kobs/kint

