

Troca H/D

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

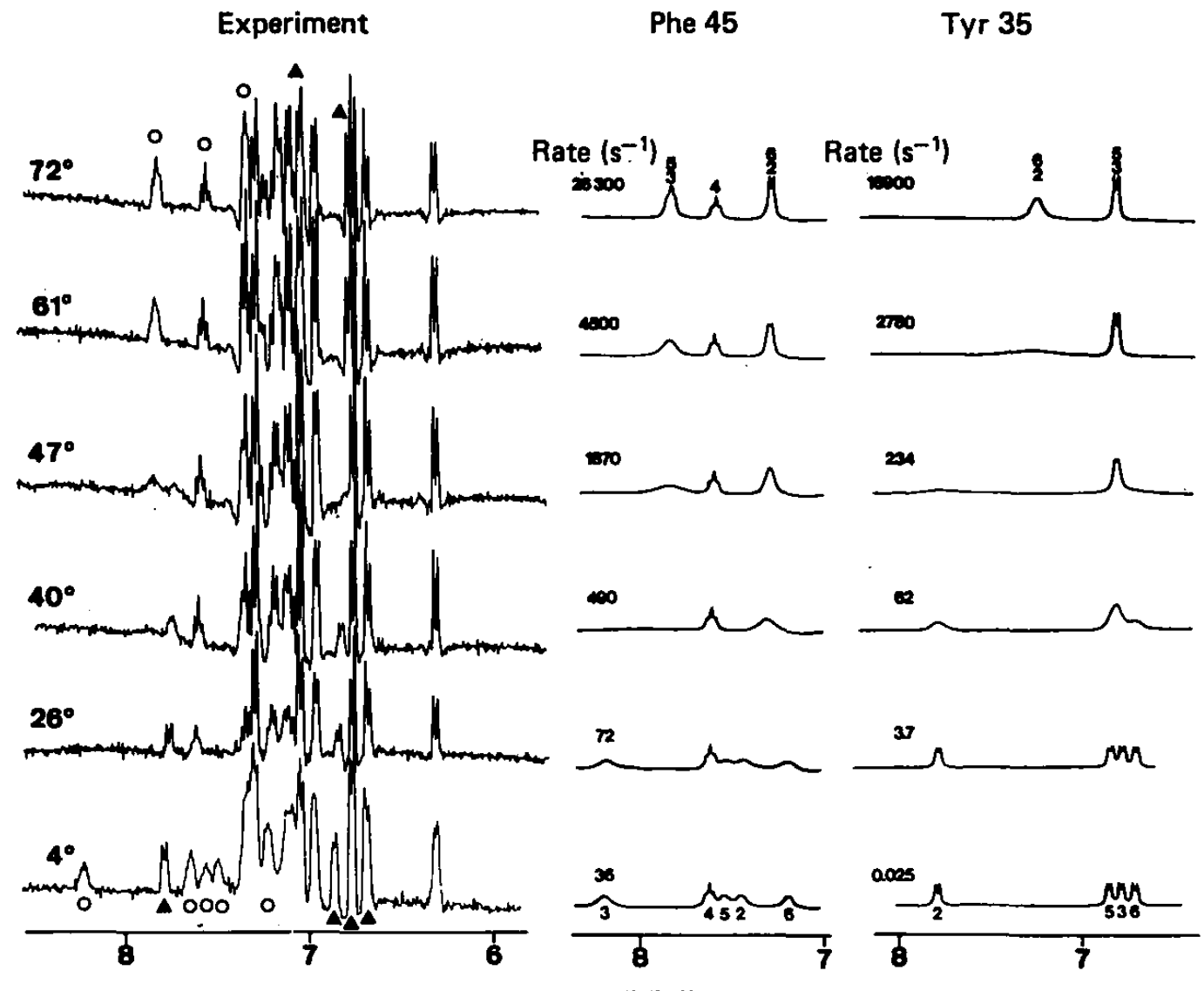
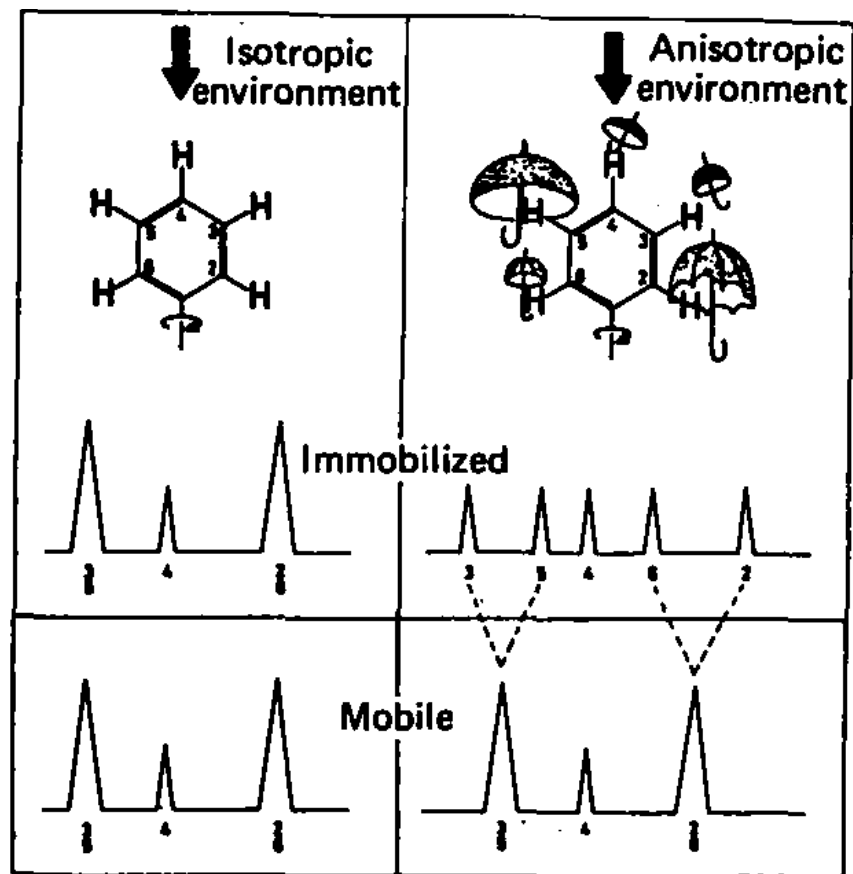
1978: Wüthrich e Wagner "Internal motion in globular proteins"

Internal motion in globular proteins

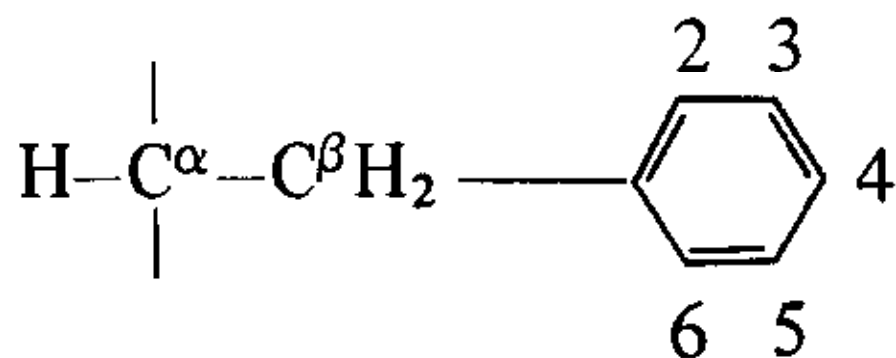
Kurt Wüthrich and Gerhard Wagner

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

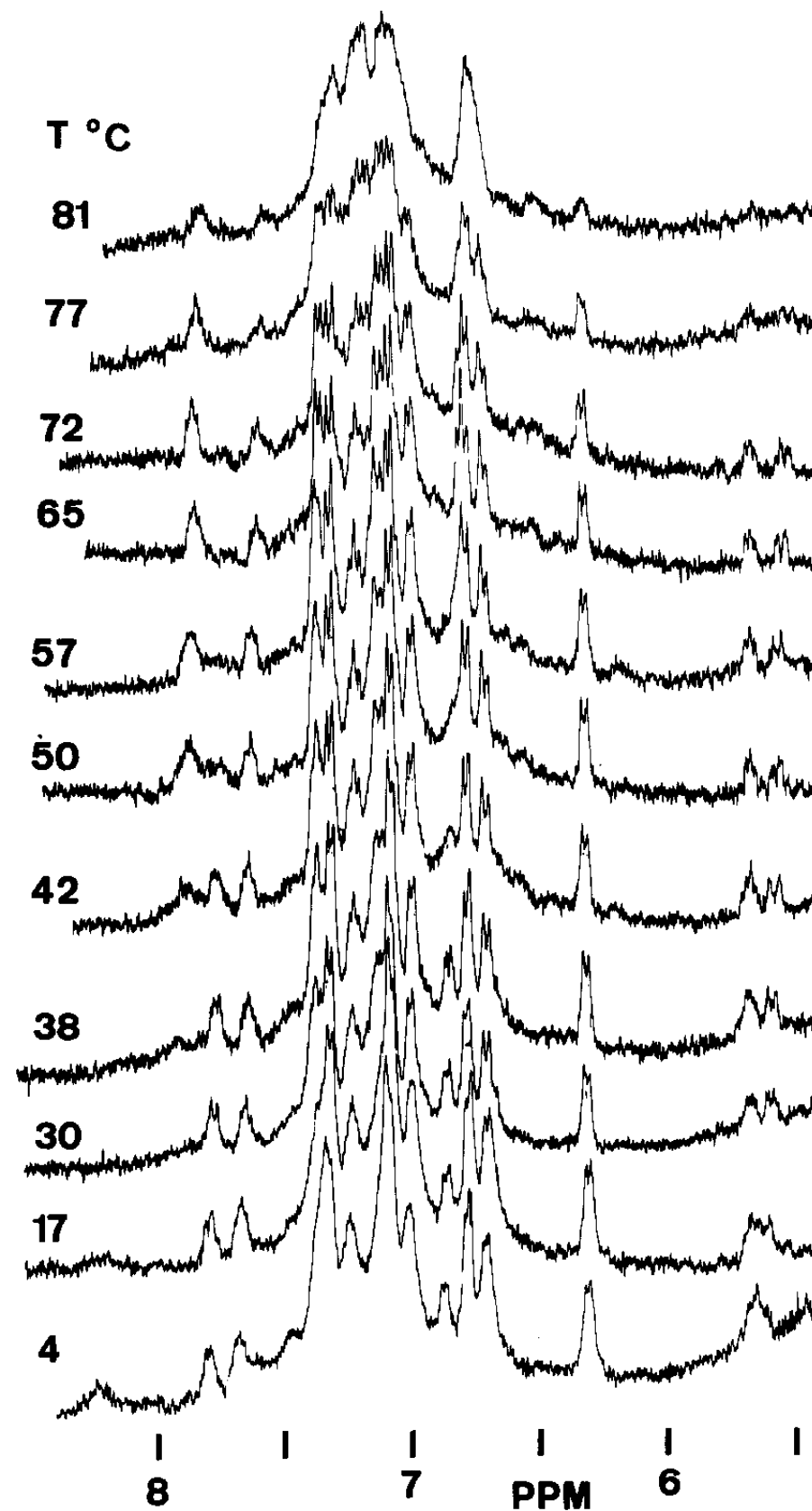
TIBS (1978) 227-230



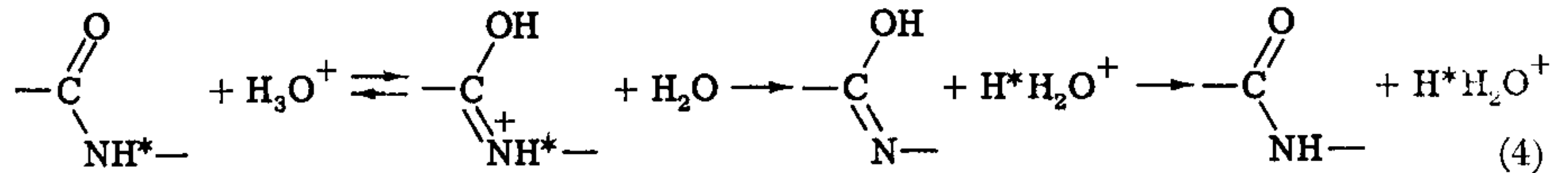
“Flipping” de anéis aromáticos



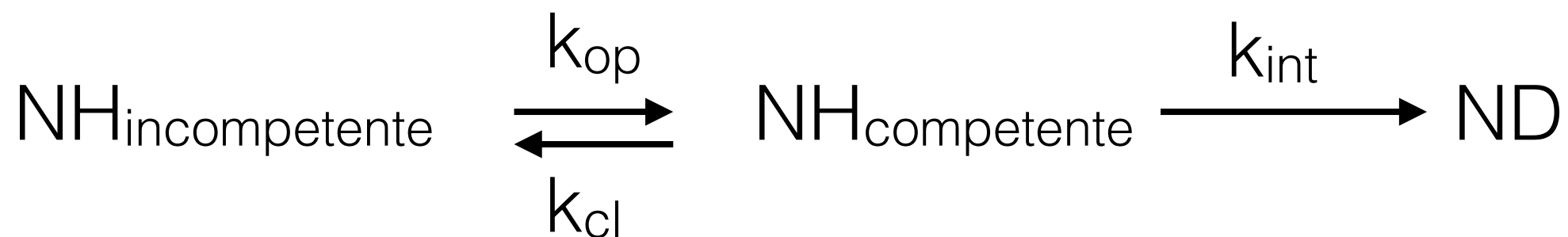
Wüthrich e Wagner (1975)



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



Hvidt e Nielsen (1966)



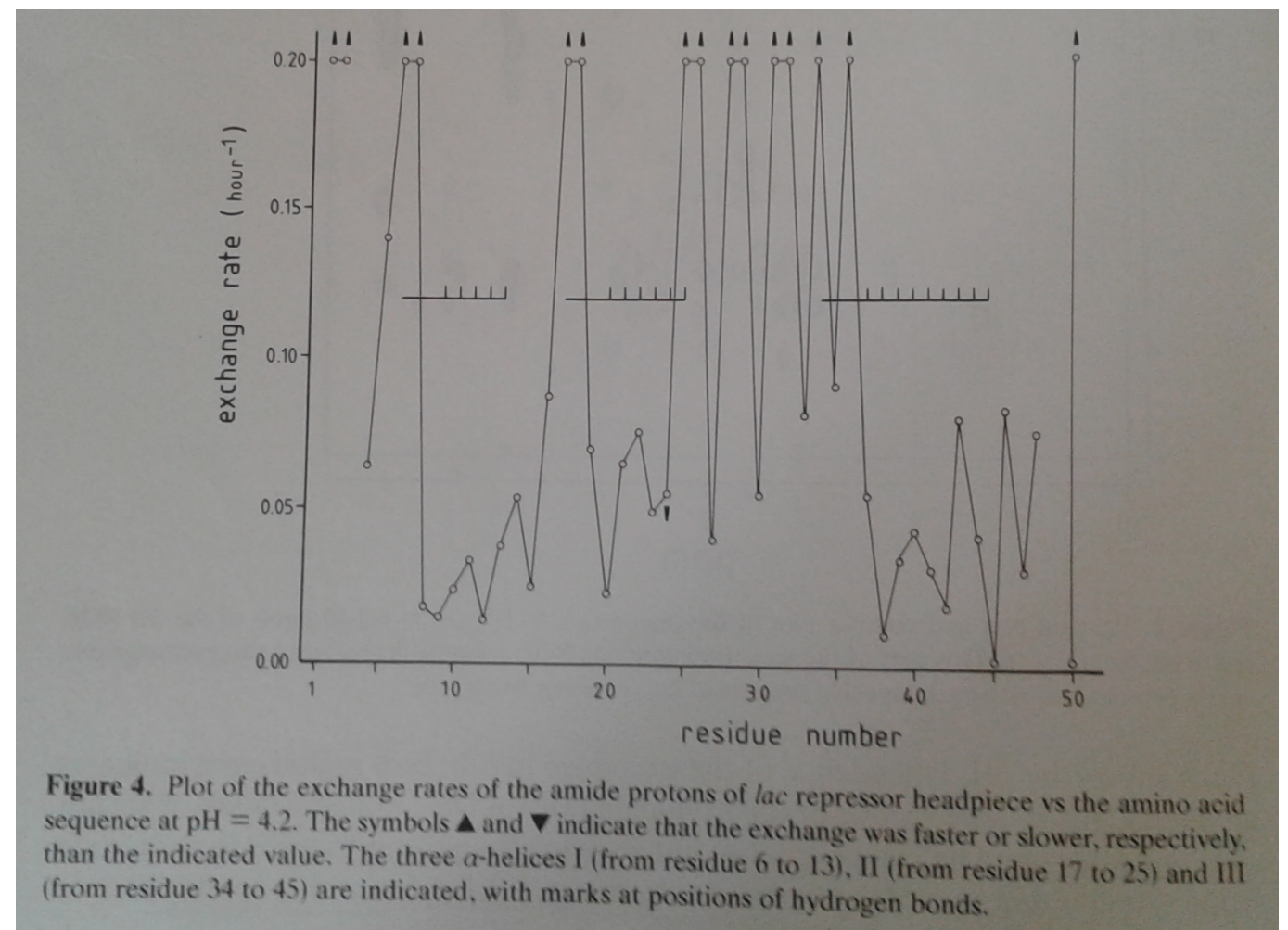
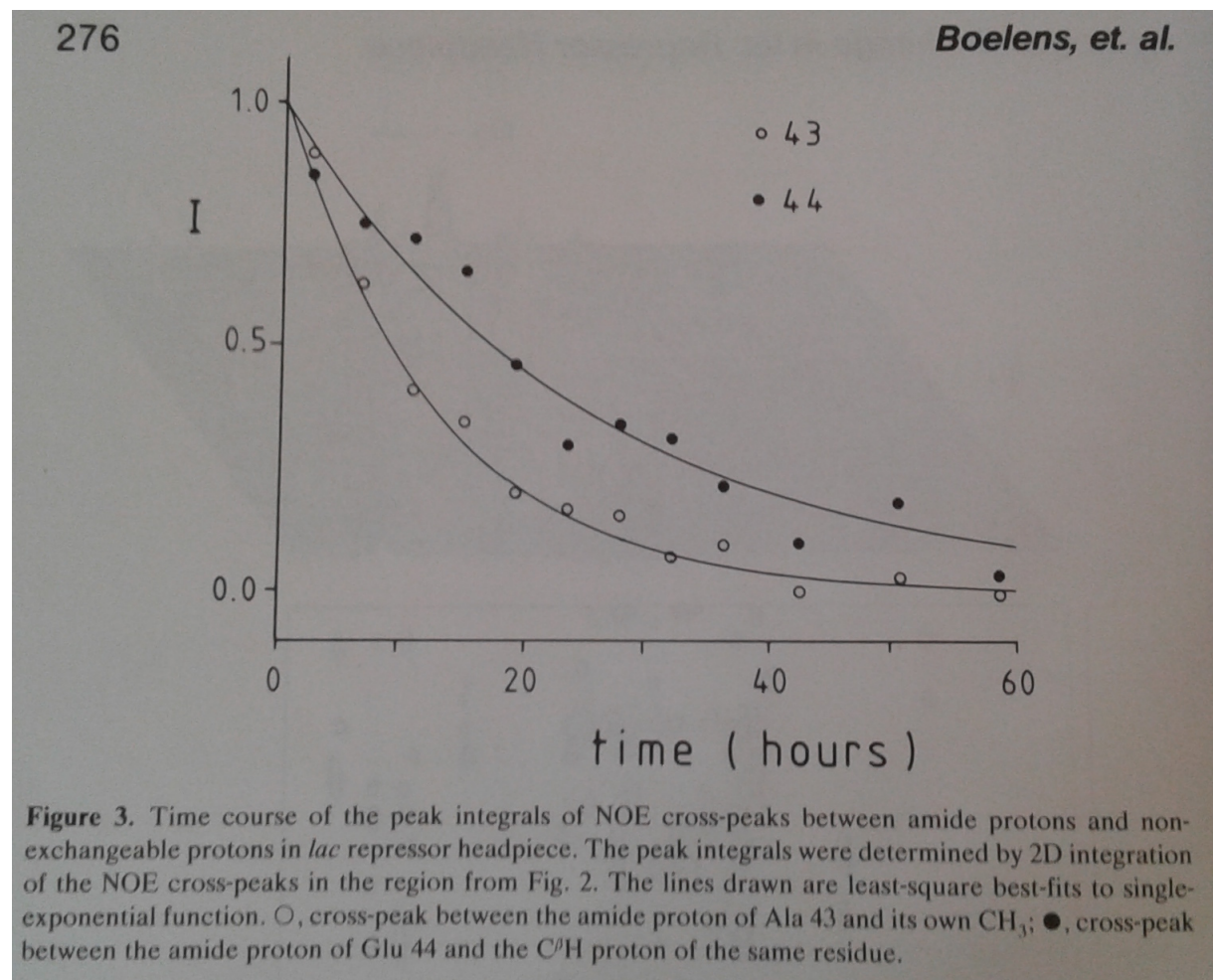
EX1: $k_{\text{obs}} = k_{\text{op}}$

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



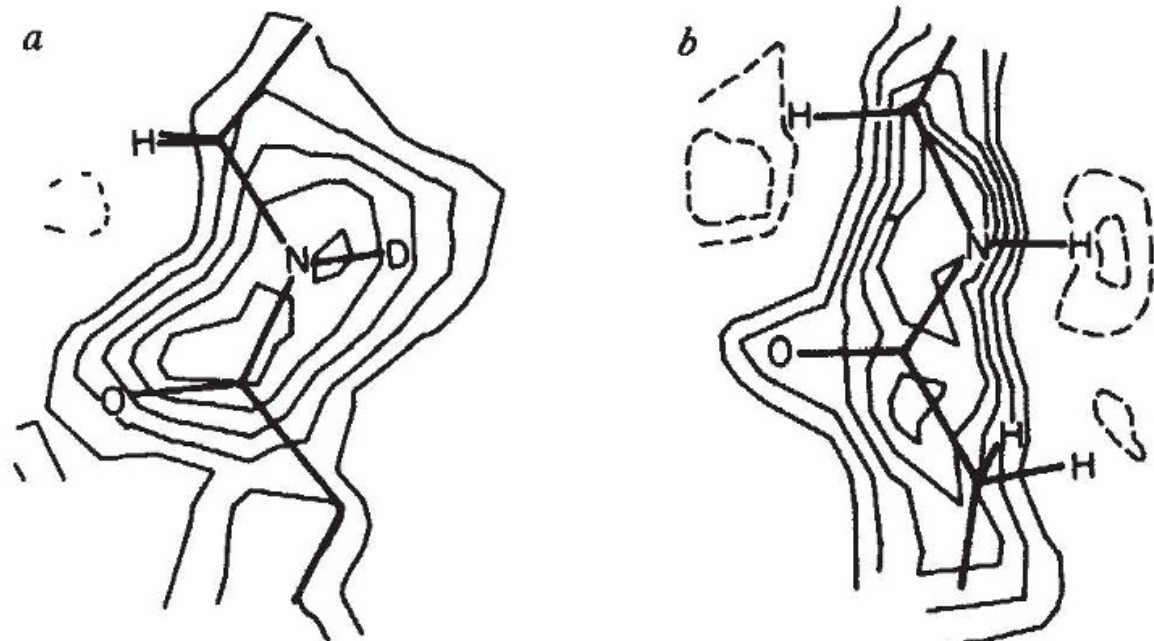
Qual é o mecanismo das flutuações conformacionais que levam à troca?

Protein dynamics investigated by the neutron diffraction–hydrogen exchange technique

A. A. Kossiakoff

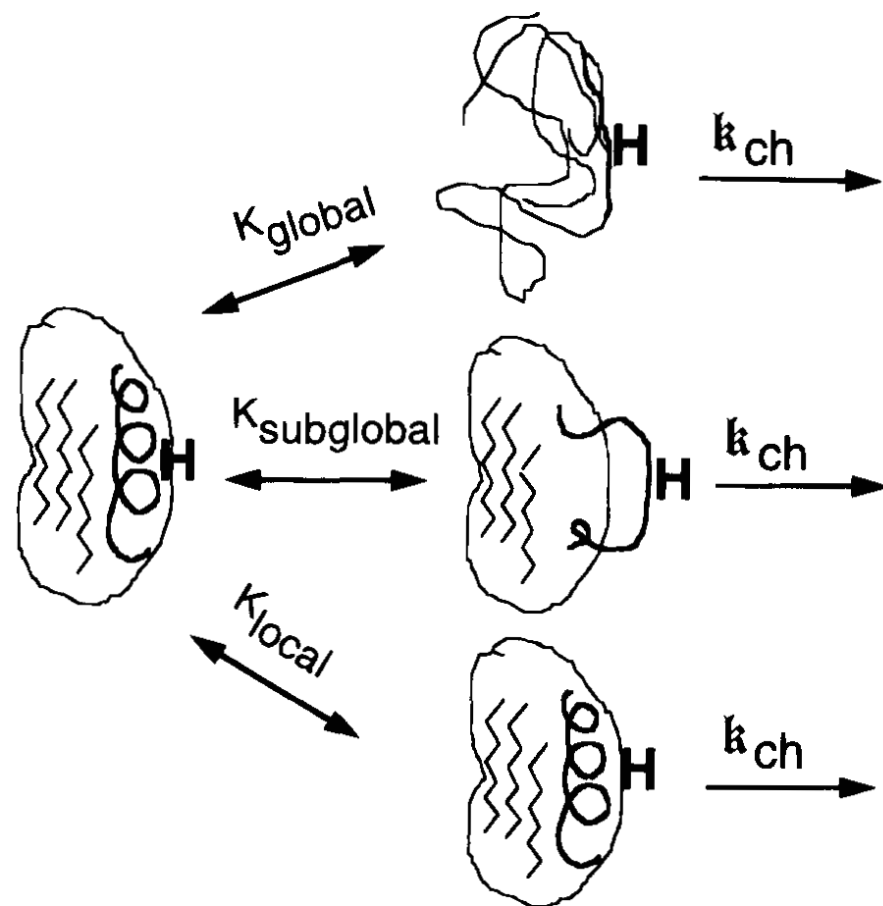
Department of Biology, Brookhaven National Laboratory, Upton, New York 11973, USA

Nature 296: 713 (1982)



- Demanda um reator nuclear (fonte de neutrons)
- Um cristal de tripsina foi mergulhado em 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



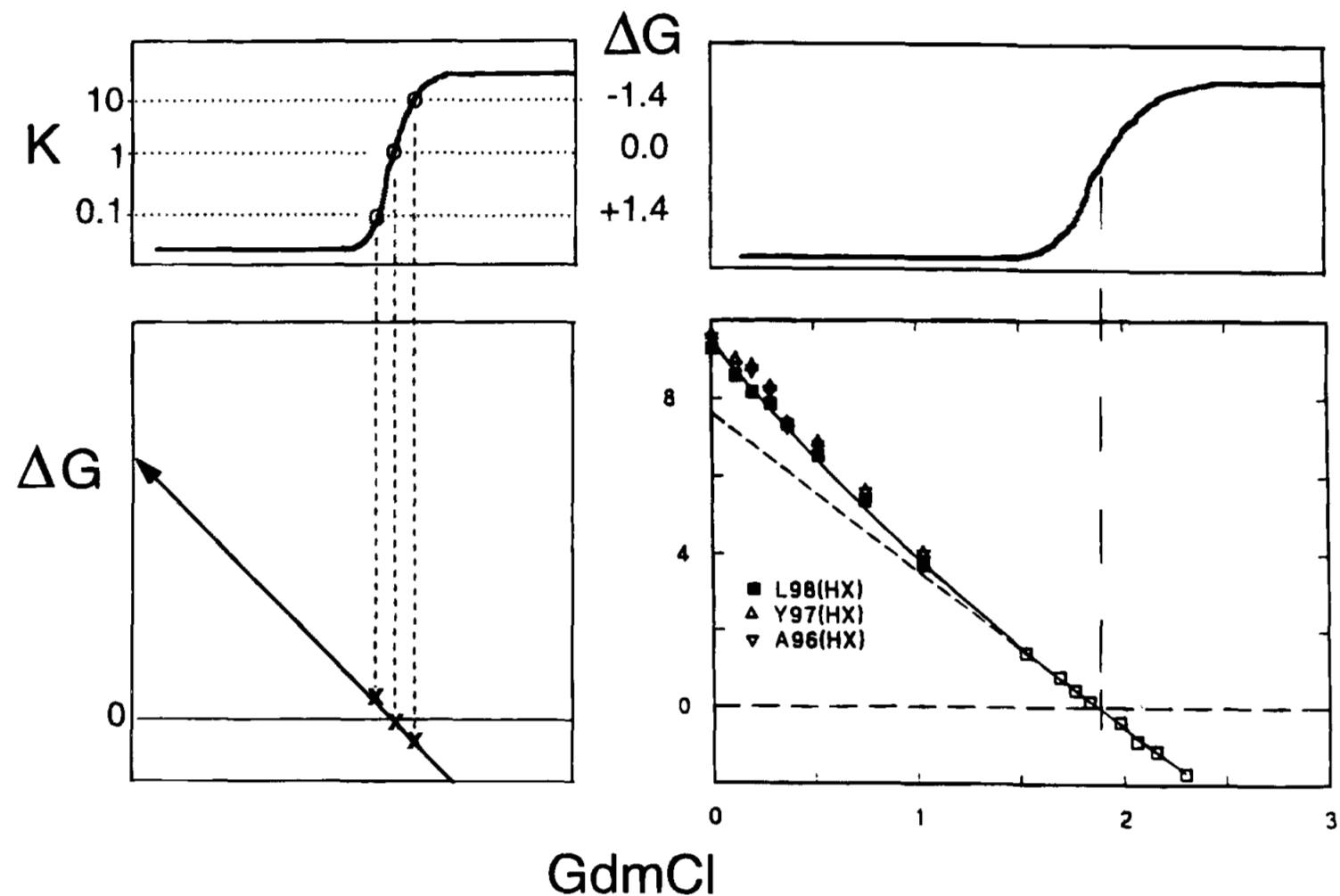
$$k_{\text{ex}} = K_{\text{ap}} k_{\text{ch}}$$

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

$$k_{\text{ex}} = k_{\text{obs}}$$

$$K_{\text{ap}} = K_{\text{eq}}$$

$$k_{\text{ch}} = k_{\text{int}}$$



citocromo c

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

Experimentos de cinética de troca H/D (HDX) usando RMN

$$\log(P) \quad P = k_{\text{obs}}/k_{\text{int}}$$

$$k_{\text{obs}} = K k_{\text{int}}$$

