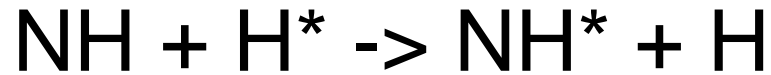


Amide Proton Exchange



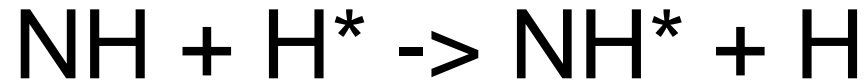
Forms of H

- ^1H , ^2H , ^3H , $^1\text{H}_{\text{sat}}$

Amide NH usually measured

- Thiol SH, Hydroxyl/Carboxyl OH, Amine NH_3^+
- CH do not exchange (except special His ring CH)

Information Obtained by Amide Proton Exchange



Hydrogen Bonds

Ligand-binding Sites

Ligand-induced Conformational Changes

- Allosteric Changes

Conformational Breathing

Energy Landscape

Identify Flexible Regions of Proteins

Protein Folding Mechanisms / Intermediates

Methods of Measurement

With 3H: Freeze Drying; Dialysis; Gel Filtration; HPLC
Mass Spectrometry (bulk or fragments)

Neutron Diffraction Crystallography

Nuclear Magnetic Resonance Spectroscopy

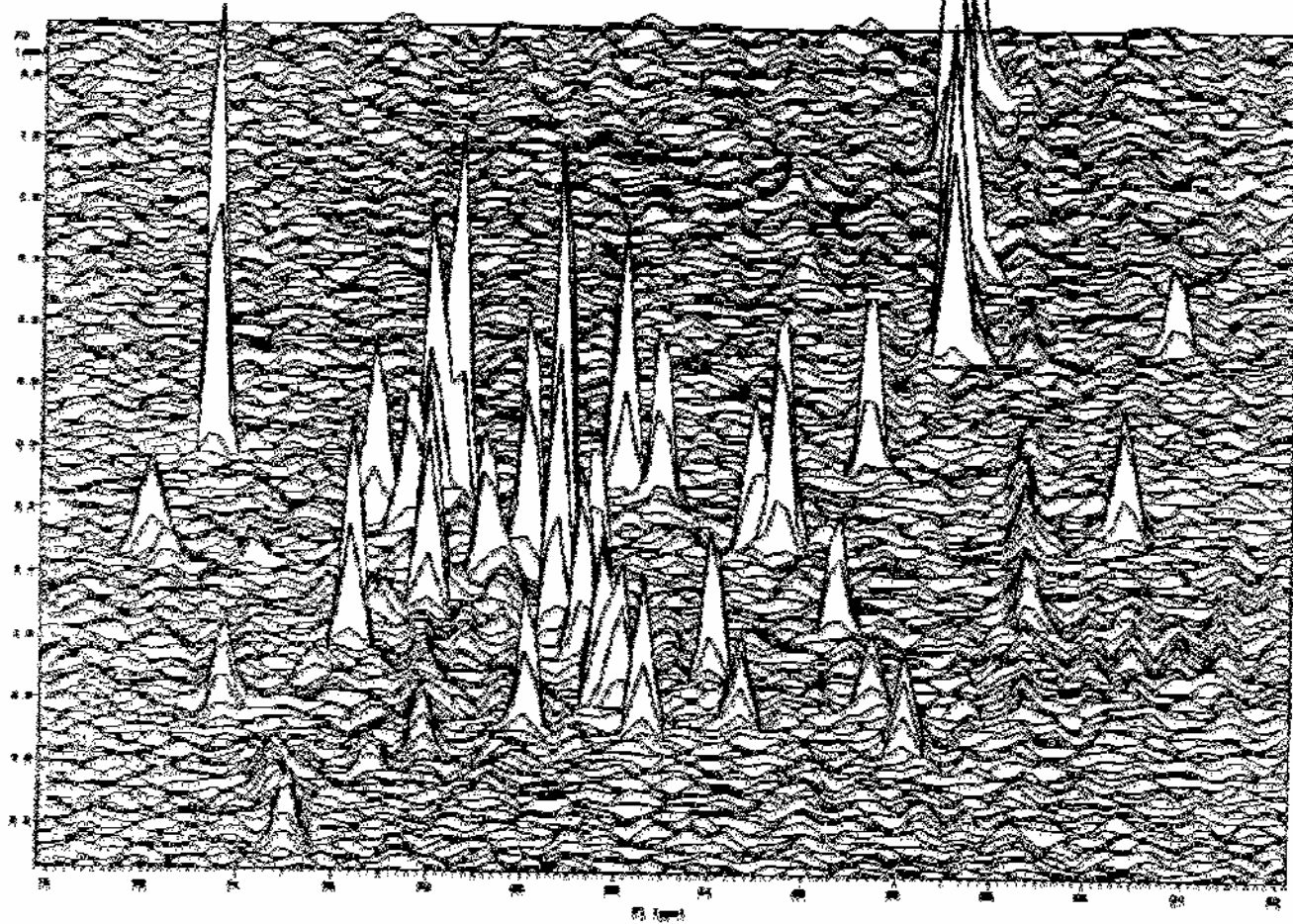
- ^1H / ^2H exchange
- Lineshape Analysis
- Saturation Transfer

Infrared and IR Spectroscopy

	N-methylacetamide	
	N-H	N-D
Amide I (C=O stretch)	1653 cm^{-1}	1642 cm^{-1}
Amide II (N-H bend / C-N stretch)	1567 cm^{-1}	1475 cm^{-1}
Amide III (C-N stretch / N-H bend)	1299 cm^{-1}	960 cm^{-1}
Amide A N-H stretch (Fermi resonance)	3280 cm^{-1}	-
Amide B N-H stretch (Fermi resonance)	3090 cm^{-1}	-

0.00 0.000000 0.00 0.00

H

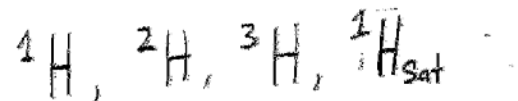


15 N

Amide Proton Exchange

Englander et al
Ann Rev Biochem 41: 810 (1972)

Isotopes & Forms of Hydrogen.



Consider the following equilibrium:



Then

$$\frac{d[\text{NH}^*]}{dt} = -k_f [\text{NH}^*][\text{HOH}] + k_i' [\text{NH}][\text{HOH}^*]$$

Special conditions

$$[\text{NH}^*]_{t=0} \ll [\text{HOH}]_{t=0}$$

then

Ignore reverse reaction.

$$\text{since } [\text{NH}^*][\text{HOH}] \gg [\text{NH}][\text{HOH}^*]$$

and

$$[\text{HOH}] \approx \text{constant.}$$

Define

$$k_f [\text{HOH}] \equiv k_{\text{ex}}$$

Then

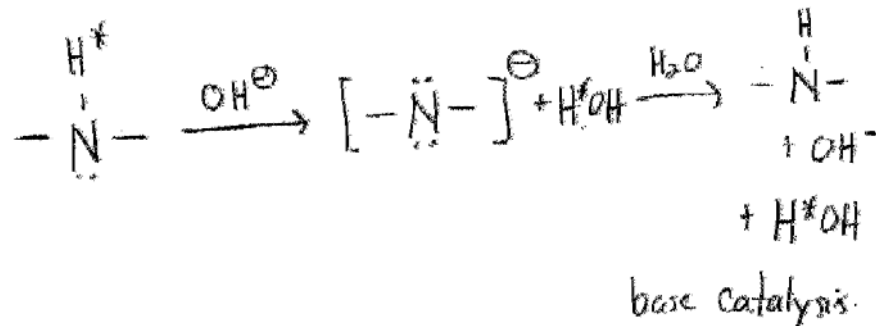
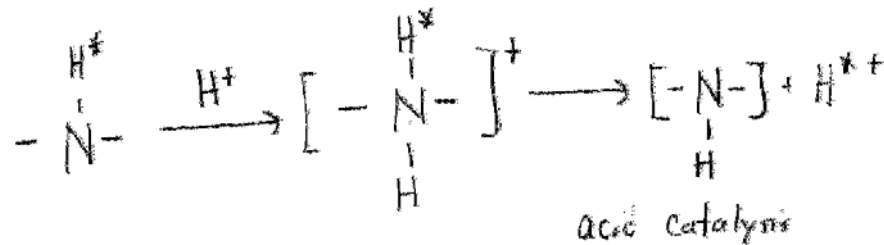
$$\frac{d[\text{NH}^*]}{dt} = -k_{ex} [\text{NH}^*]$$

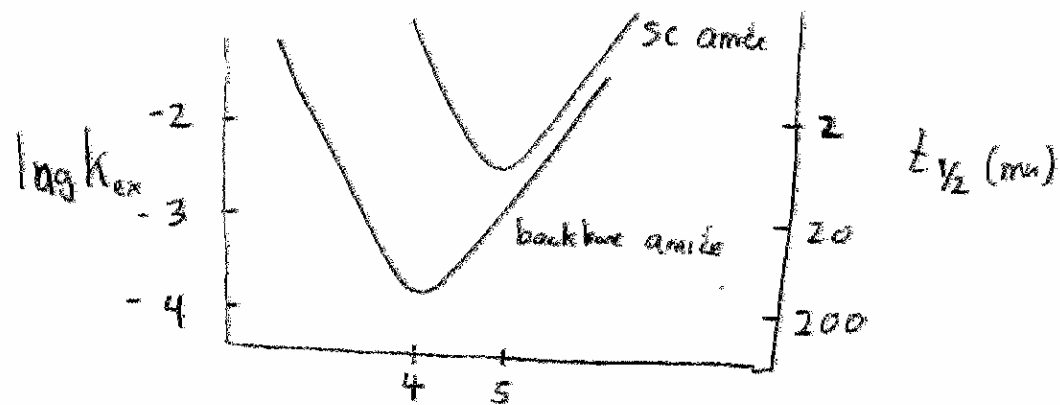
Pseudo First
Order Rate
Constant.

$$[\text{NH}^*](t) = [\text{NH}^*]^0 e^{-k_{ex}t}$$

Mechanism of NH exchange:

Polypeptides
DNA, RNA





273°K

$$t_{1/2} = \frac{0.69}{k_{ex}}$$

$$k_{ex} = k_H [H^+] + k_{OH} [OH^-]$$

For poly-L-Ala.

$$k_H = 12.7 \times 10^2 \text{ M}^{-1} \text{ min}^{-1}$$

$$k_{OH} = 4.2 \times 10^9 \text{ M}^{-1} \text{ min}^{-1}$$

273°K

Modulating Effects

1. Temperature $\Delta H^\ddagger \approx 17$ kcal/mol.
(tripling of rate for every 10°).

2. Solvent Effects

- Urea, Guanidine have small effects

- Salts ($< 1M$) have very small effects

- Dioxane Co-Solvent - little effect

- Carboxylic Acids, Imidazole - general catalysis

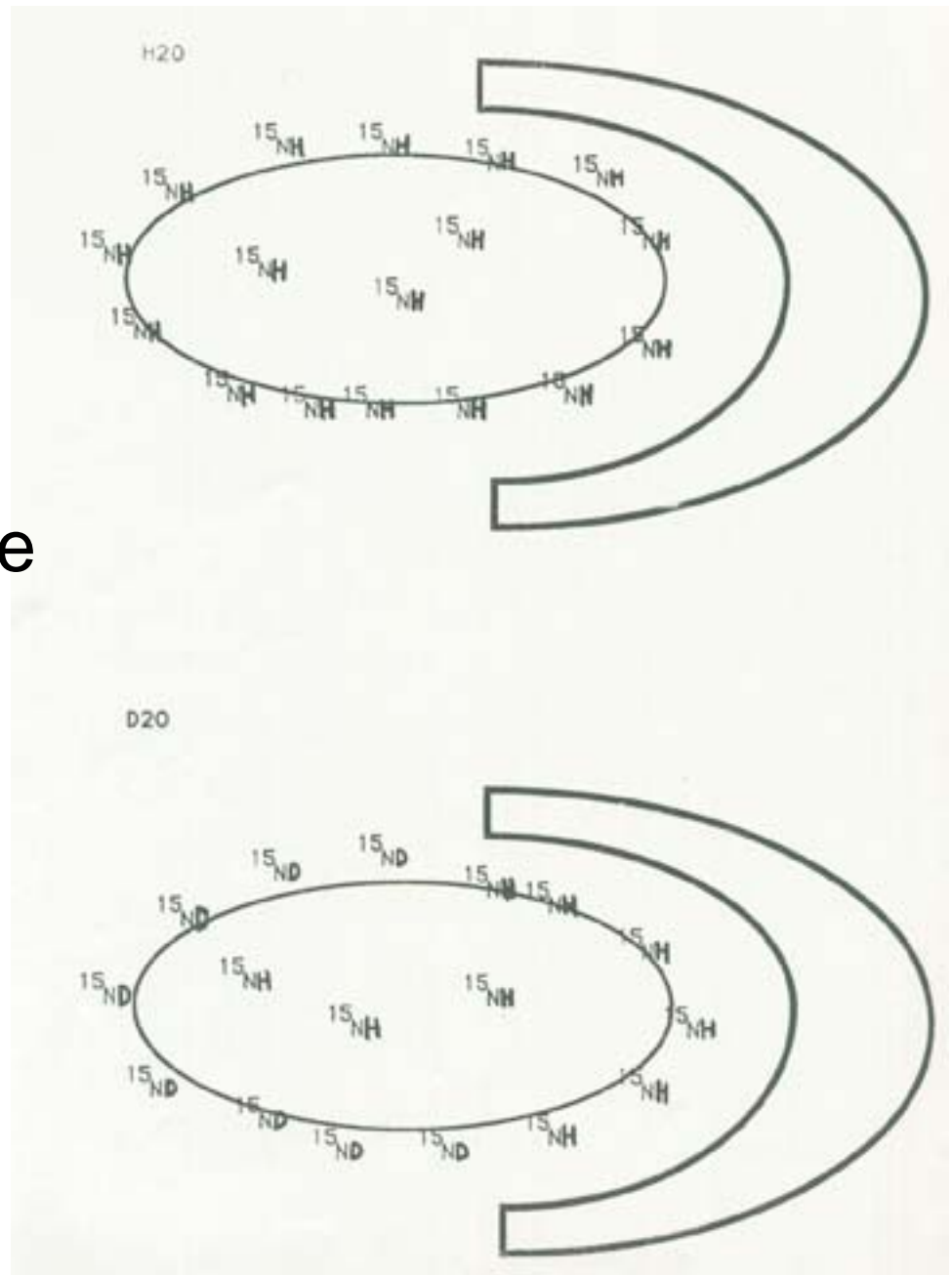
3. Isotope Effects.

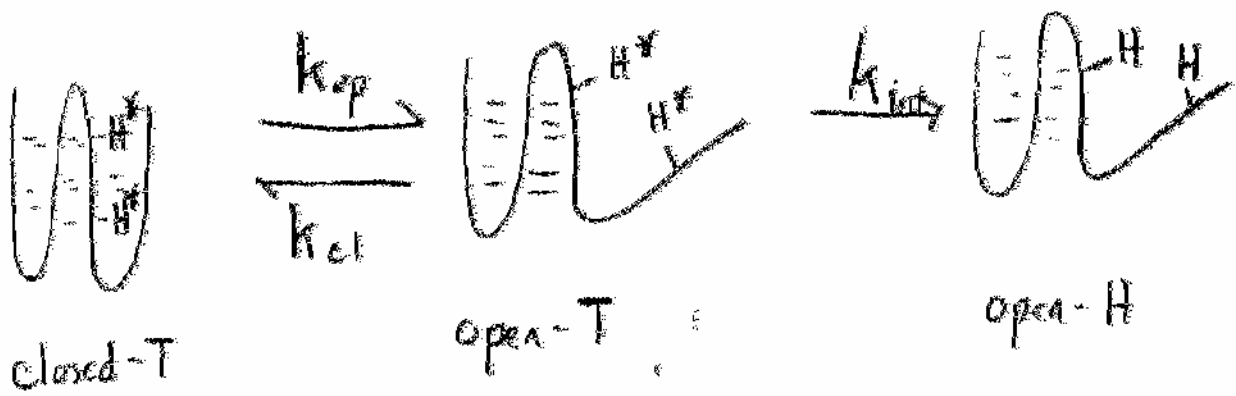
$$\frac{^3H}{^1H} = 1.21$$

$$\frac{^3H}{^2H} = 1.05$$

Amide Proton Exchange Protection Factor

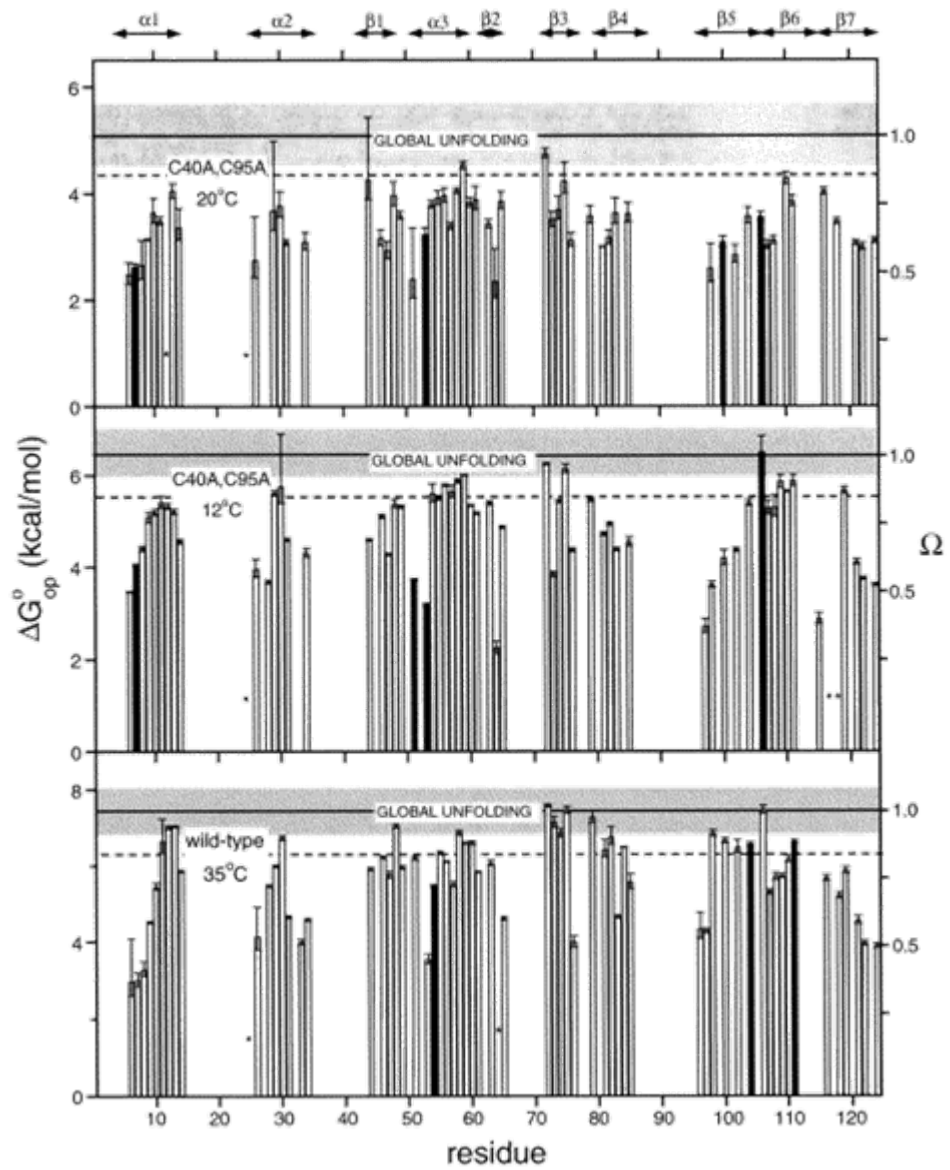
$$F = k_{\text{ex,bound}} / k_{\text{ex,free}}$$





$$k_{obs} = \left(\frac{k_{op}}{k_{cl}} \right) k_{int} \quad \text{EX}_2 \text{ mechanism}$$

$$K_{eq} = \frac{k_{obs}}{k_{int}} \quad \Delta G_u = -RT \ln \frac{k_{ex}}{k_{int}}$$



SUPREX

Ghaemmaghani et al. PNAS 97: 8296 (2000)

