Amide Proton Exchange

$NH + H^* \rightarrow NH^* + H$

Forms of H

- ^1H, ^2H, ^3H, $^1H_{sat}$

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

$$NH + H^* \rightarrow NH^* + H$$

- 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)

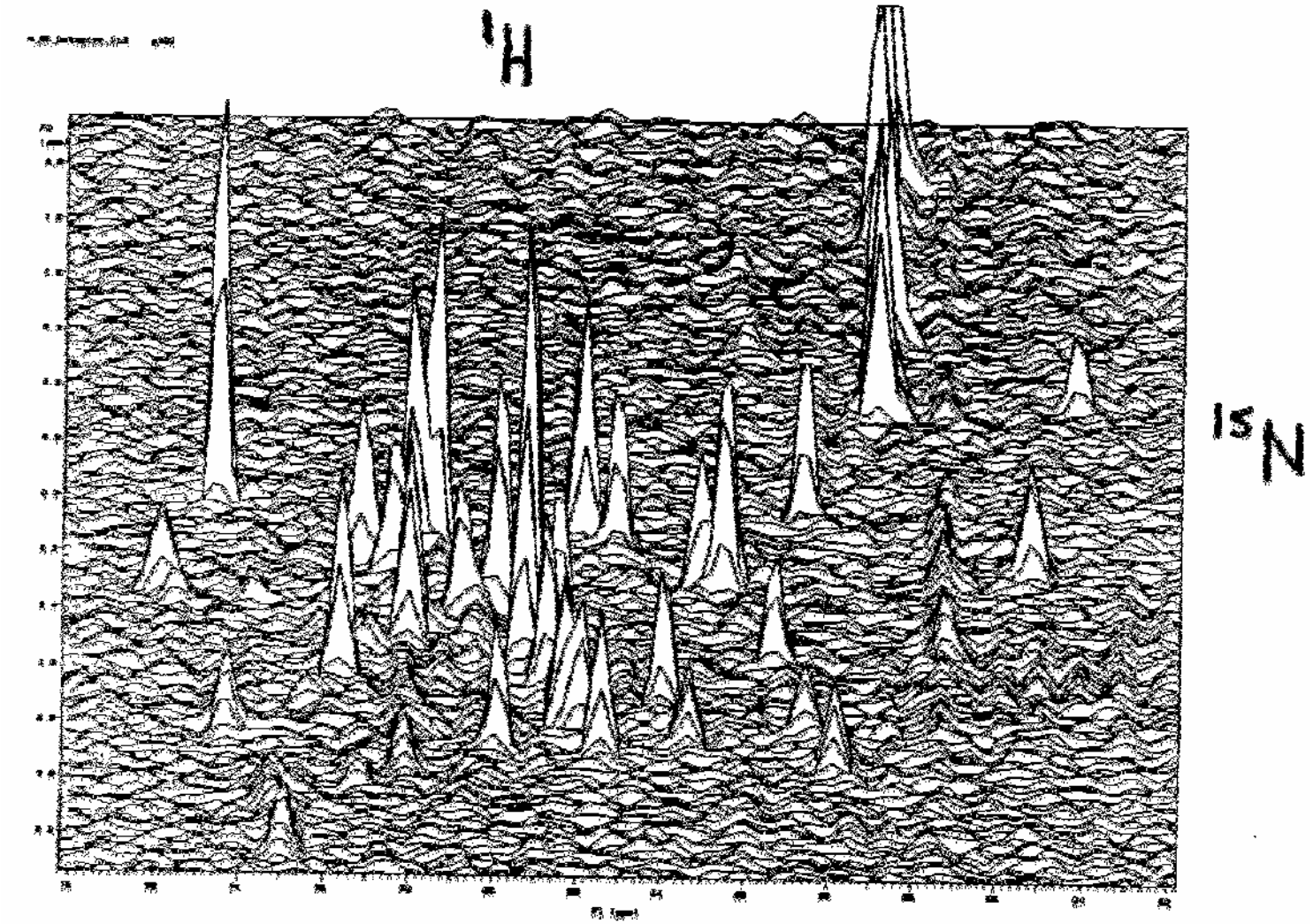
Neutron Diffraction Crystallography

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}	/ -


^{1}H
^{15}N

Amide Proton Exchange

Englander et al
Ann Rev Biochem 41 810 (1972)

Isotopes & Forms of Hydrogen

^1H, ^2H, ^3H, $^1H_{sat}$

Consider the following equilibrium:

$$NH^* + HOH \underset{k_1'}{\overset{k_1}{\rightleftharpoons}} NH + HOH^*$$

Then

$$\frac{d[NH^*]}{dt} = -k_1[NH^*][HOH] + k_1'[NH][HOH^*]$$

Special conditions

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

then Ignore reverse reaction

since $[NH^*][HOH] \gg [NH][HOH^*]$

and $[HOH] \approx$ constant.

Define $k_1[HOH] \equiv k_{ex}$

Then

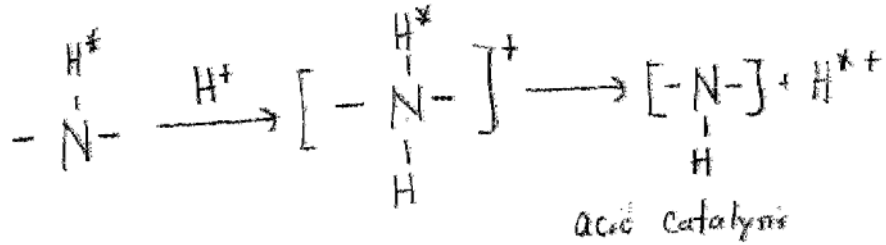
$$\frac{d[NH^*]}{dt} = -k_{ex}[NH^*]$$

Pseudo First Order Rate Constant

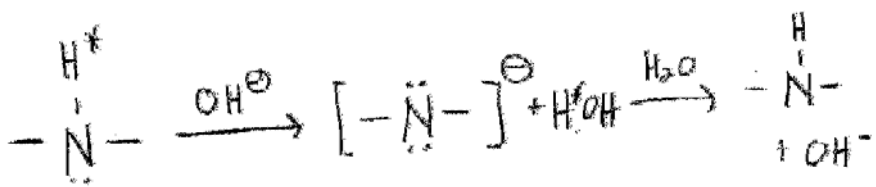
$$[NH^*](t) = [NH^*]^0 e^{-k_{ex}t}$$

Mechanism of NH exchange:

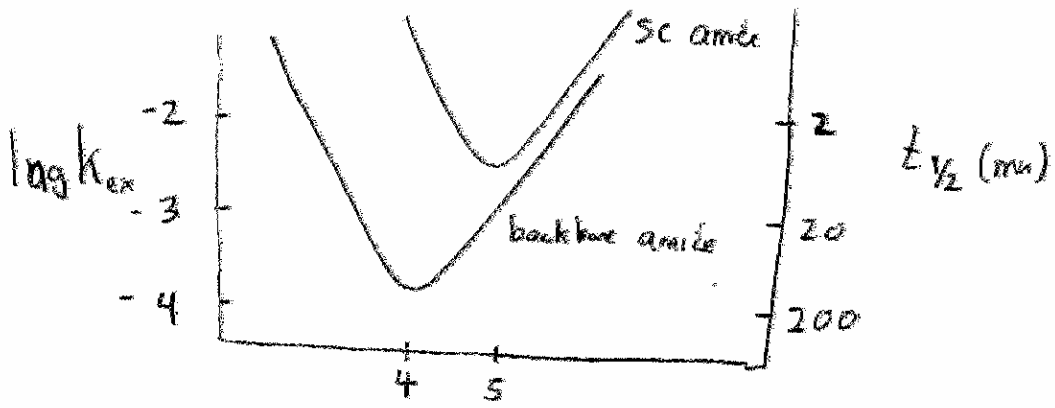
Polypeptides DNA, RNA



acid catalysis



base catalysis



273° K

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

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

For poly-L-Ala

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

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

273° K

Modulating Effects

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

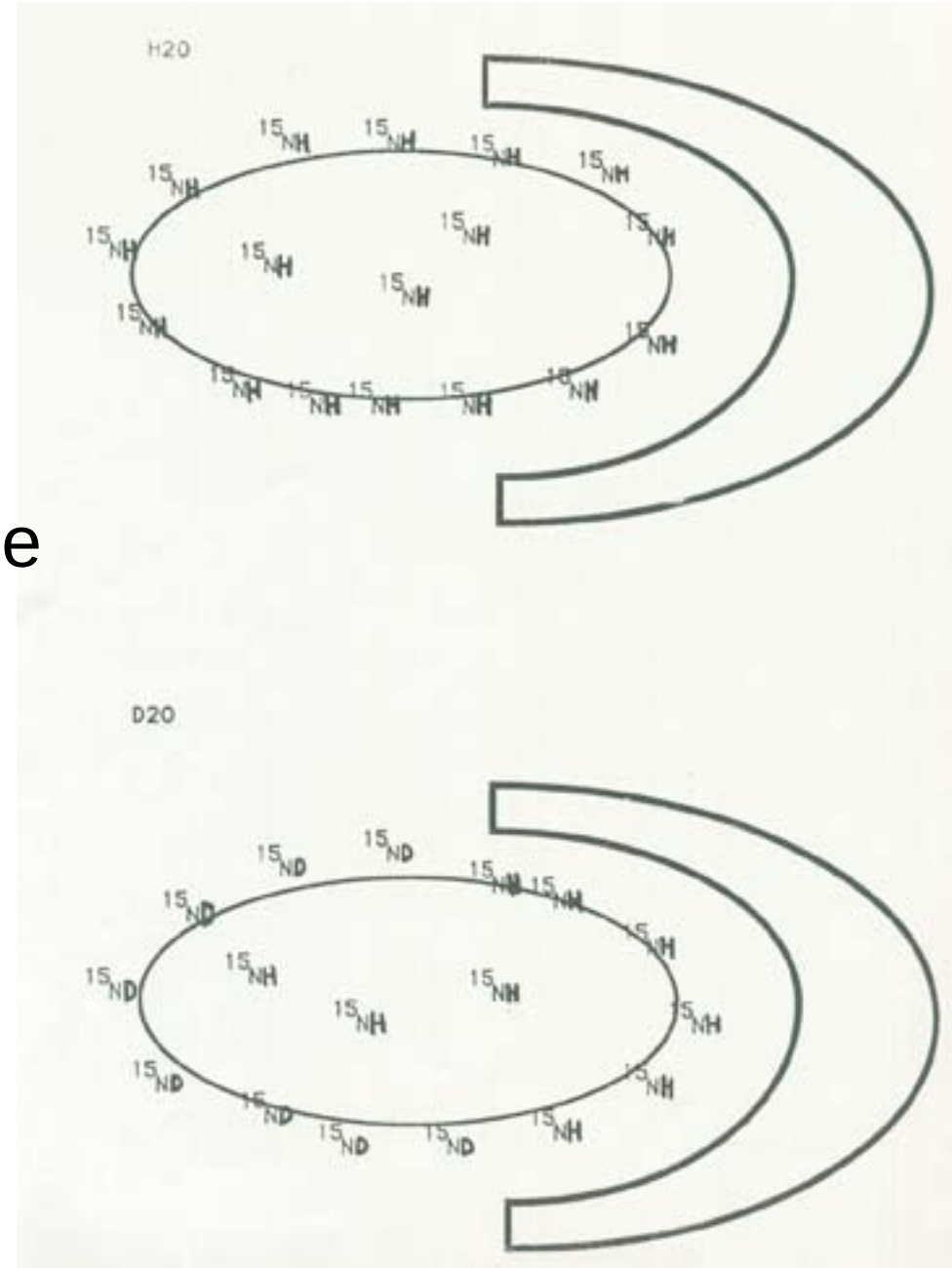
2. Solvent Effects
   - Urea, Guanidine have small effects
   - Salts (< 1 M) have very small effects
   - Dioxane Co-Solvent - little effect
   - Carboxylic Acids, Imidizole - general catalysis

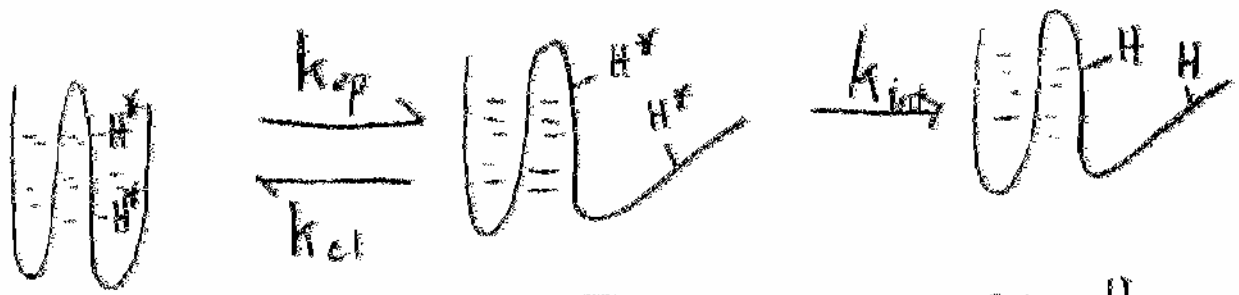
3. Isotope Effects

$$\frac{^3H}{^1H} = 1.21 \qquad \frac{^3H}{^2H} = 1.05$$

Amide Proton Exchange Protection Factor

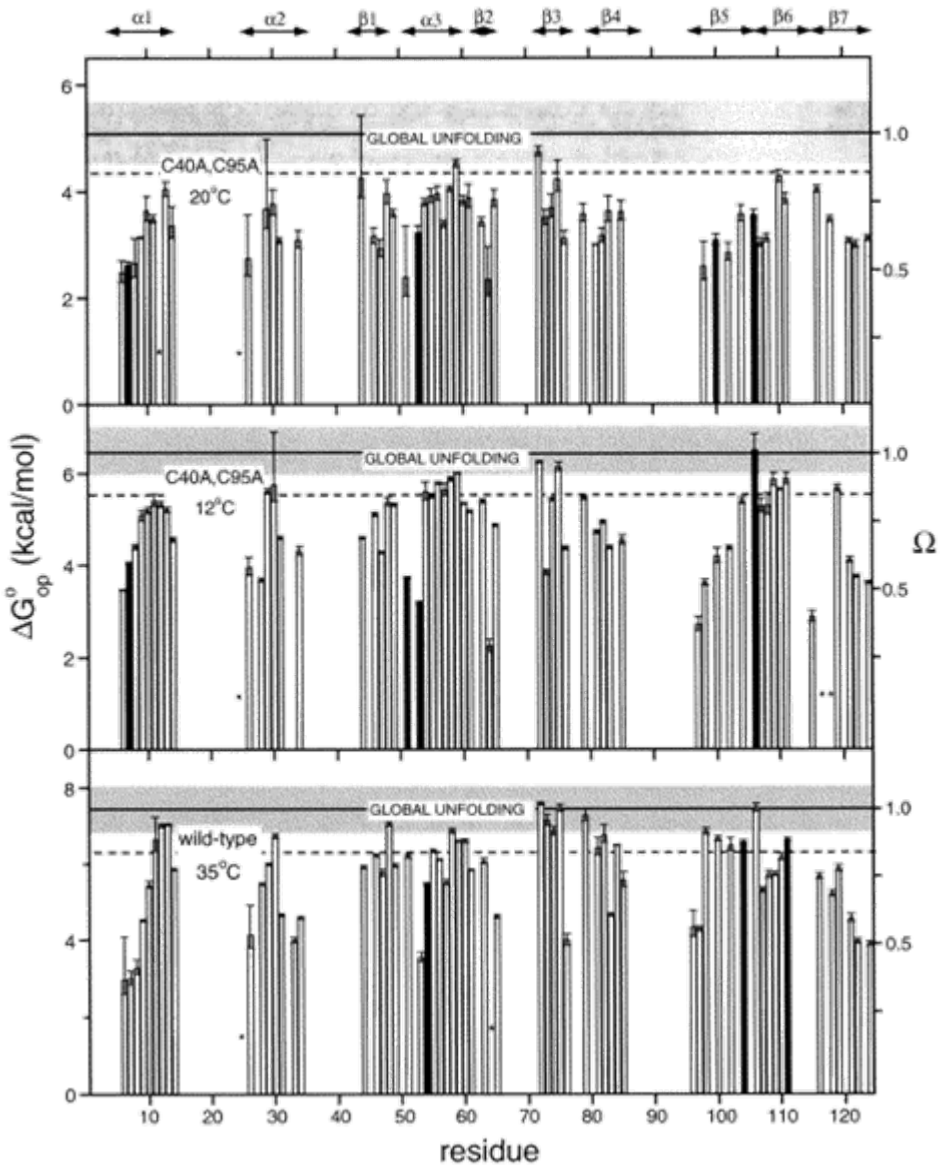
$F = k_{ex,bound} / k_{ex,free}$





$$k_{obs} = \left(\frac{k_{op}}{k_{cl}}\right) k_{int}$$ — EX_2 mechanism

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


α1
α2
β1
α3
β2
β3
β4
β5
β6
β7
GLOBAL UNFOLDING
C40A,C95A
20°C
GLOBAL UNFOLDING
C40A,C95A
12°C
GLOBAL UNFOLDING
wild-type
35°C
ΔG°op (kcal/mol)
Ω
residue
10
20
30
40
50
60
70
80
90
100
110
120

SUPREX
Ghaemmaghami et al. PNAS 97: 8296 (2000)