Amide Proton Exchange

NH + H* -> NH* + H

Forms of H

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

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* -> NH* + H

Hydrogen Bonds

Ligand-binding Sites

Ligand-induced Conformational Changes

- Allosteric Changes

Conformational Breathing

Energy Landscape

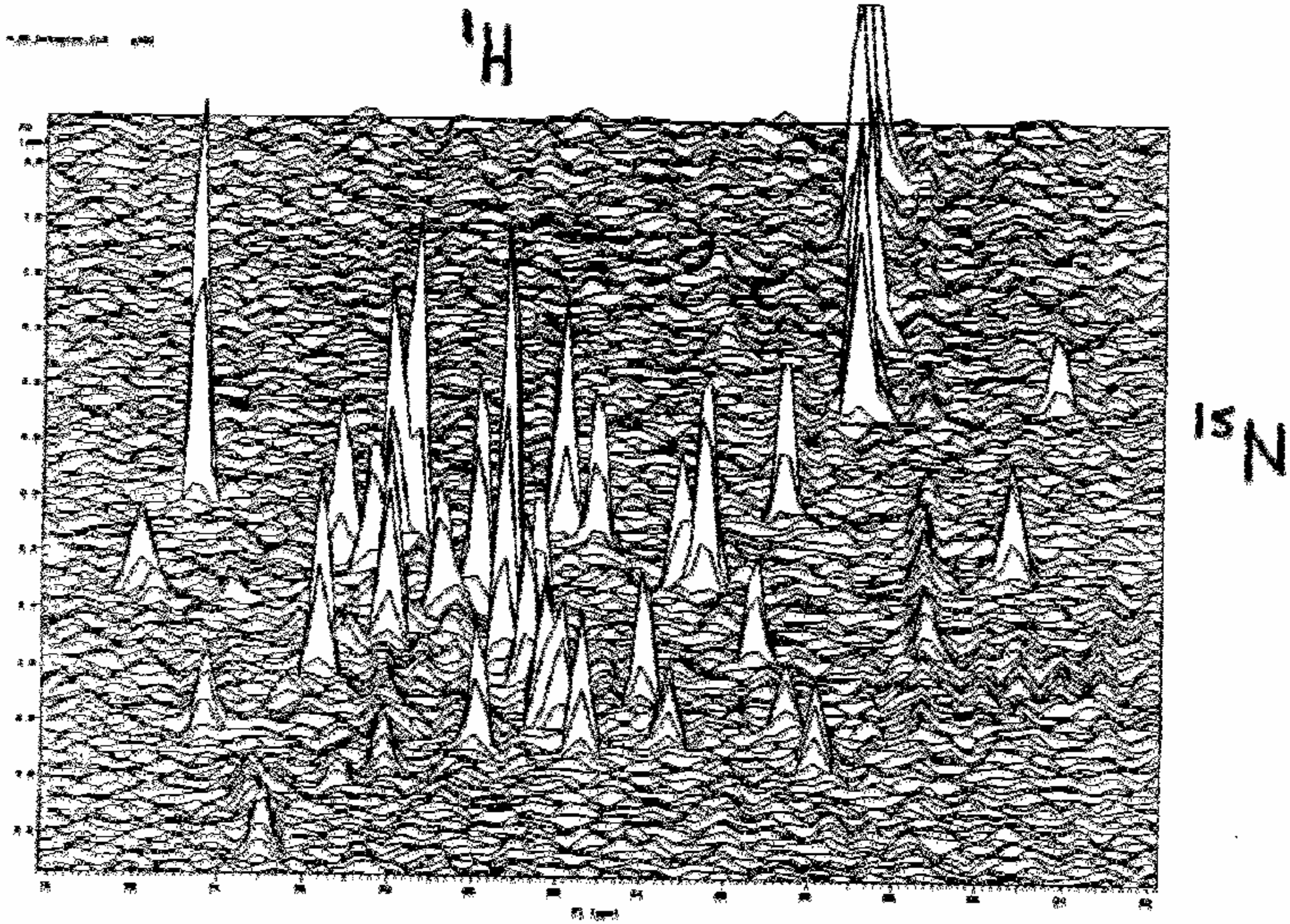
Identify Flexible Regions of Proteins

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-methylacetamide 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}	-



^1H
^{15}N

Amide Proton Exchange

Englander et al
Ann Rev Biochem 41 810 (1972)

Isotopes & Forms of Hydrogen

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

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

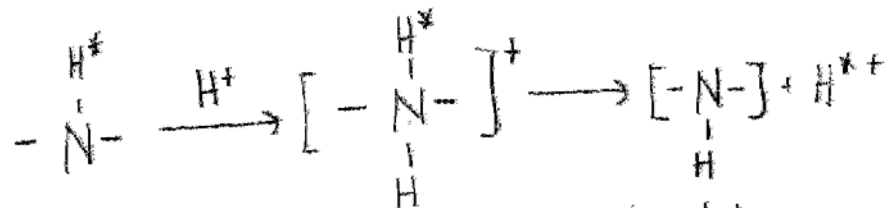
$$\frac{d[NH^*]}{dt} = -k_{ex}[NH^*]$$

Pseudo First Order Rate Constant

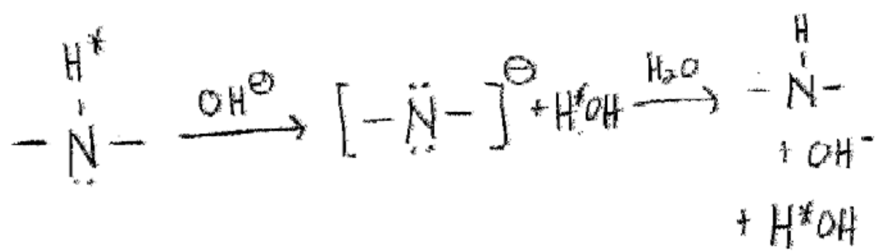
$$\boxed{[NH^*](t) = [NH^*]^0 e^{-k_{ex}t}}$$

Polypeptides
DNA, RNA

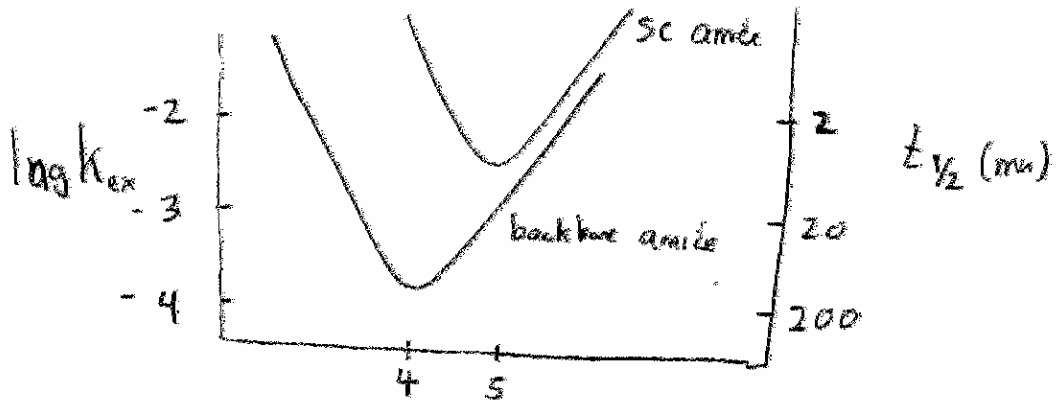
Mechanism of NH exchange:



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

$$k_H = 12.9 \times 10^2 \, M^{-1} \, min^{-1}$$

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

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

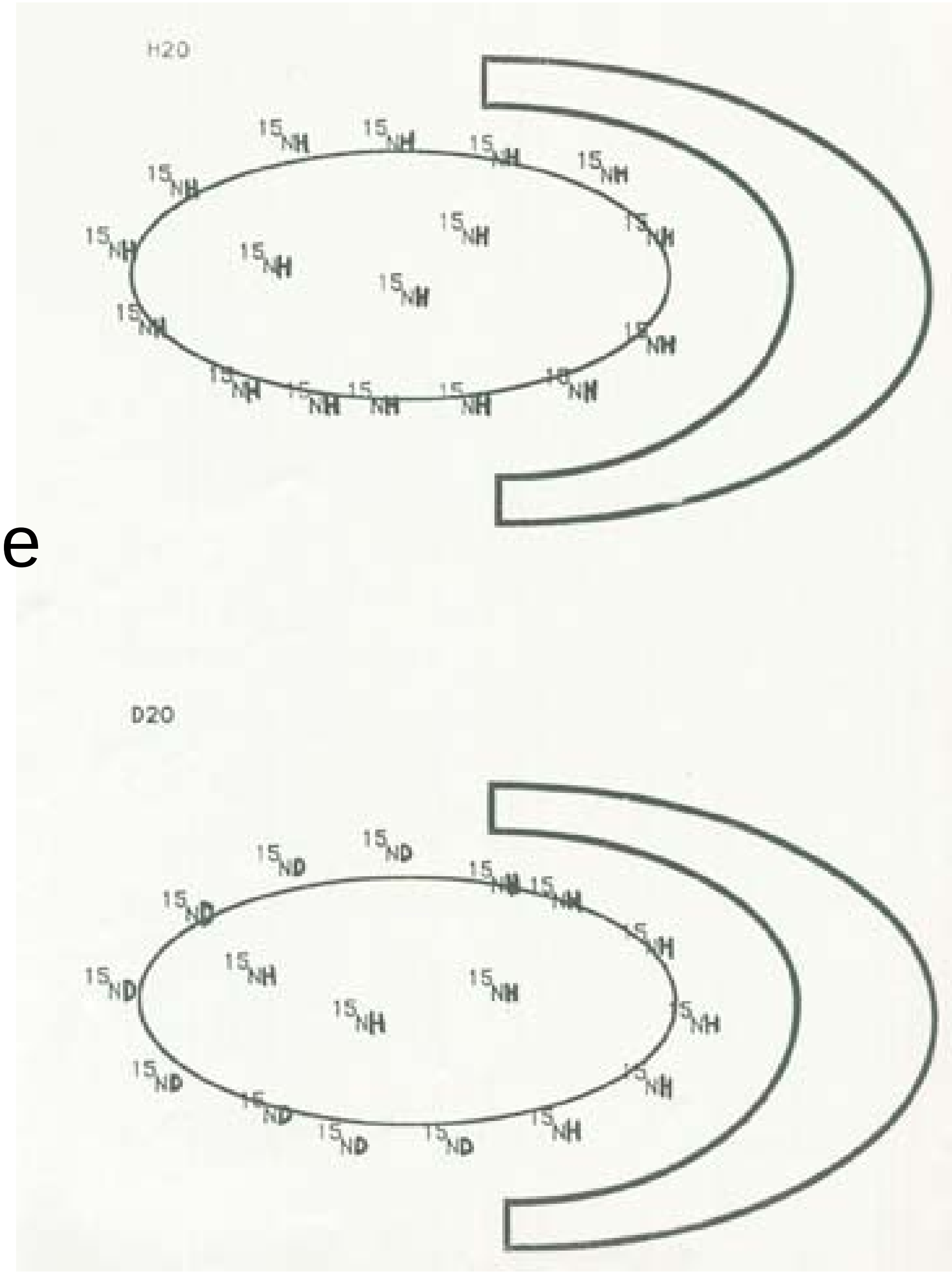
3. Isotope Effects

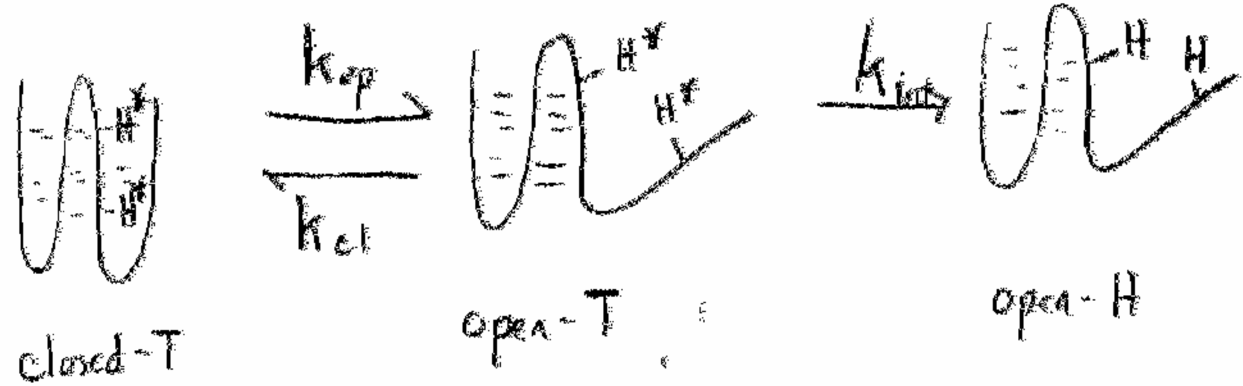
$$\frac{^3H}{^1H} = 1.21 \qquad \frac{^3H}{^2H} = 1.05$$

Amide Proton Exchange

Protection Factor

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





k_op
k_cl
k_int
H*
H
closed-T
open-T
open-H

$$DOD \longrightarrow DOH$$

$$NH_{cl} \underset{k_{cl}}{\overset{k_{op}}{\rightleftharpoons}} NH_{op} \xrightarrow[k_{int}]{} ND_{op}$$

Write differential rate equations

$$[ND]\ (t) = \exp(-k_{ex}t)$$

$$k_{ex} = \frac{k_{op}\ k_{int}\ [DOD]}{k_{op} + k_{cl} + k_{int}\ [DOD]}$$

Limiting Cases

EX1 Limit

$$k_{int}\,[DOD] \gg k_{cl}$$

$$k_{int}\,[DOD] \gg k_{op}$$

$$k_{ex} = k_{op}$$

EX2 Limit

$$k_{cl} \gg k_{int}\,[DOD]$$

$$k_{cl} \gg k_{op}$$

$$k_{ex} = \frac{k_{op}\; k_{int}\;[DOD]}{k_{cl}} = K_{op}\, k_{int}\;[DOD]$$

$$k_{ex} = \frac{k_{op}\; k_{int}\;[DOD]}{k_{op} + k_{cl} + k_{int}\,[DOD]}$$

$$K_{op} = \frac{k_{op}}{k_{cl}}$$

Equilibrium constant

EX2 Limit

$$k_{ex} = K_{op} \; k_{int} \; [DOD]$$

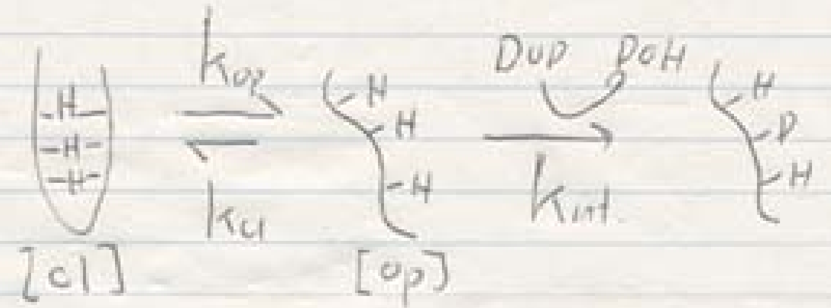
$$k'_{int} = k_{int} \; [DOD]$$

Pseudo FirstOrder
Rate Constant

$$k_{ex} = K_{op} \; k_{int}$$

$$K_{op} = \frac{k_{ex}}{k_{int}}$$

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



$$\rightarrow K_{op} = \frac{[op]}{[cl]} = \frac{k_{op}}{k_{cl}}$$

equilibrium constant

k_{op}, k_{cl}: rate constants

$$K_{op} = \frac{[op]}{[cl]} \qquad K_{cl} = \frac{[cl]}{[op]}$$

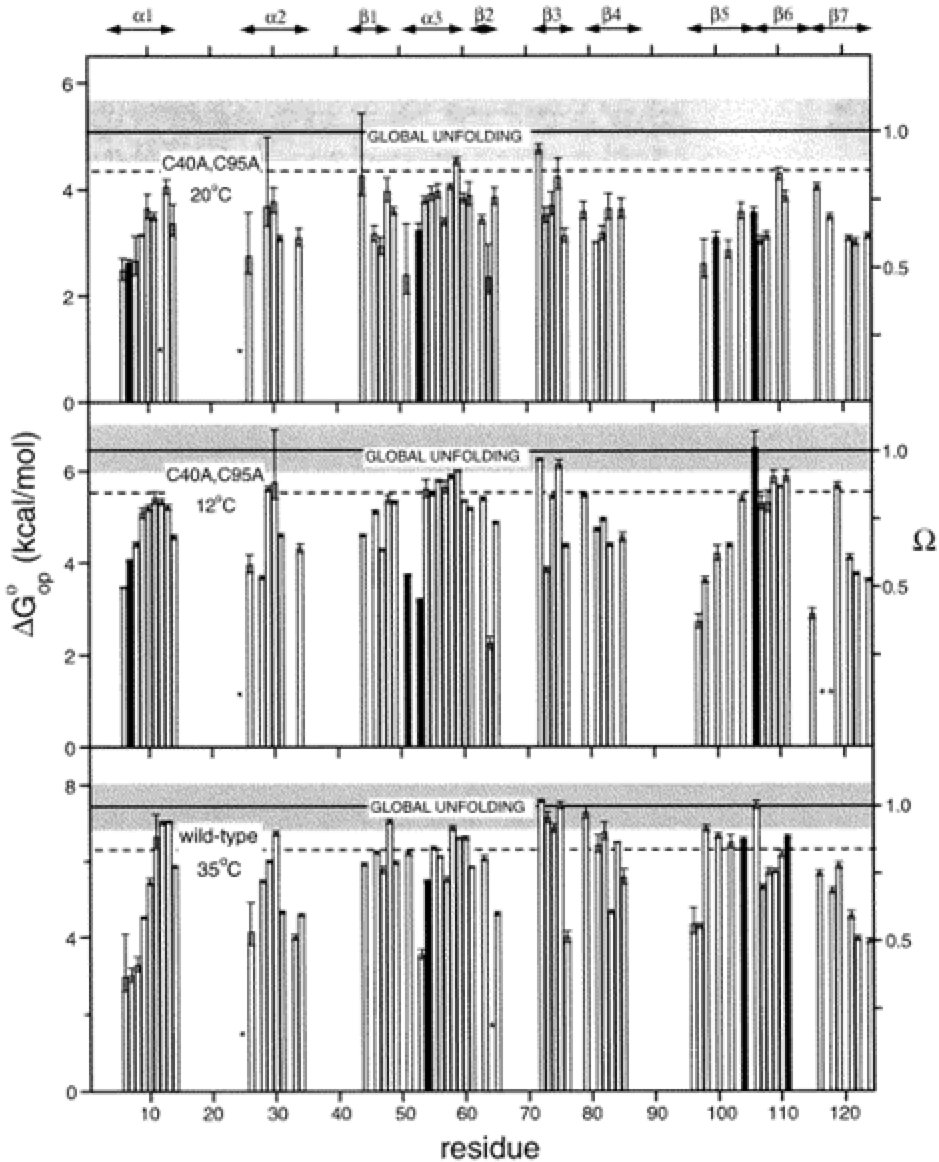
$$K_{op} = \frac{1}{K_{cl}}$$

From H/D Exchange Data; EX2 Case.

$$K_{op} = \frac{k_{ex}}{k_{int}[D]}$$

k_{ex} ← measured

$k_{int}[D]$ ← calculated from amino acid sequence.



α1
α2
β1
α3
β2
β3
β4
β5
β6
β7
GLOBAL UNFOLDING
C40A,C95A
20°C
C40A,C95A
12°C
wild-type
35°C
ΔG°op (kcal/mol)
Ω
residue

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