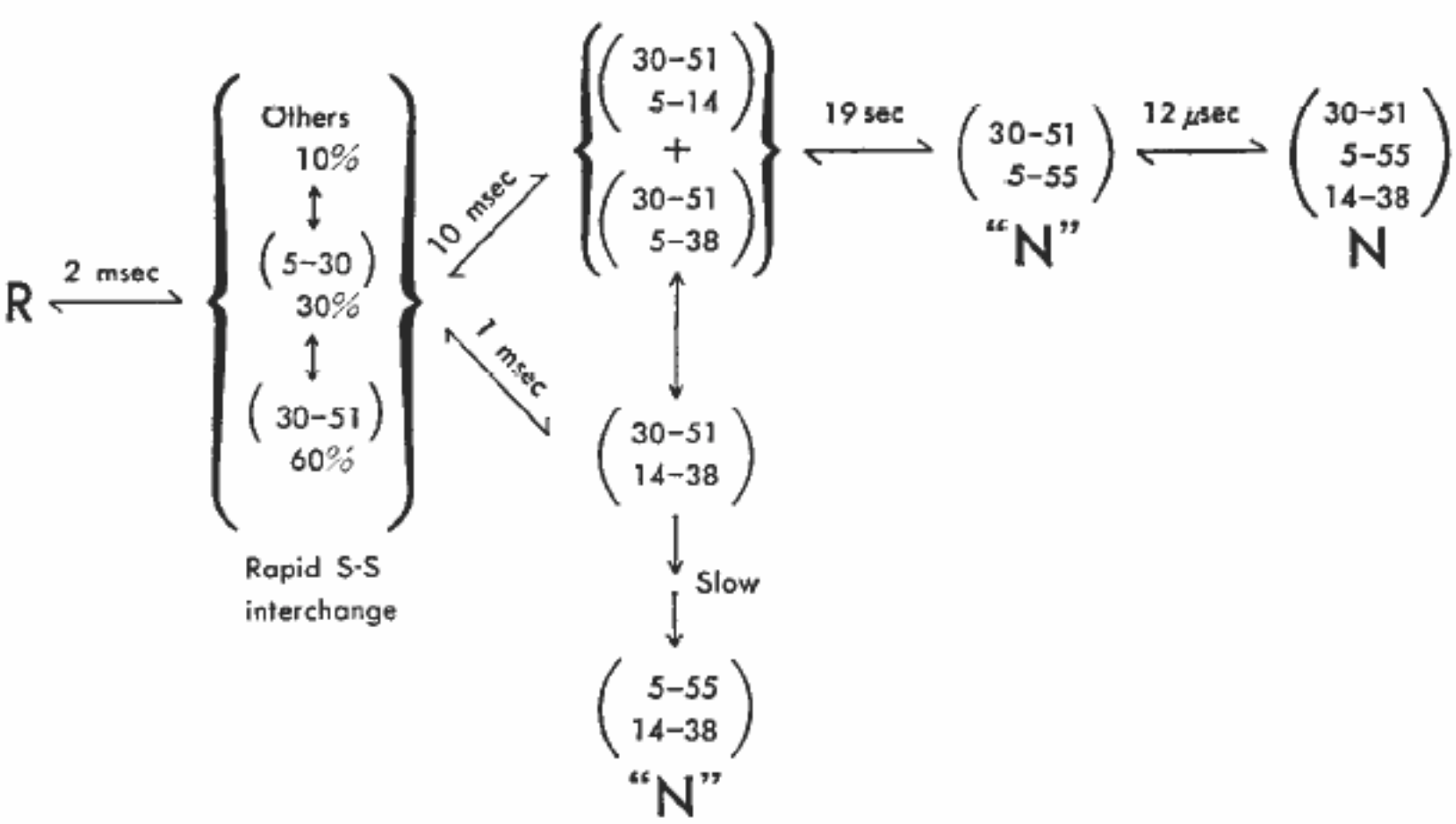
Protein Folding Pathways

MBIII, Jan 20, 2005

Native vs non-native pathways

- Review Chreighton's viewpoint: Sec 7.5.4 of his book Proteins
- Review P. Kim & JW viewpoint: Science 1991, 253,1386
- Review CJC & DT theory: PNAS 1995, 92, 1277
- Gray et al exp. on intrachain diffusion times.
- Role of denaturants on unf. & folding kinetic

Creighton's view: Non-native intermediates



Adv. in Biophysics, 1984, 18, 1; Proteins book Page 319

Creighton's method

Identification of disulfide intermediates. In the earlier studies, folding of reduced BPTI (R) was initiated by the addition of an oxidizing agent. At various time points, folding was stopped by addition of iodoacetate, a reagent that alkylates free thiols and thereby prevents further oxidation or thiol-disulfide exchange. After separation of the trapped intermediates by ion-exchange chromatography (IEC), the disulfide linkages of the intermediates were determined by two-dimensional paper electrophoresis (*6*).

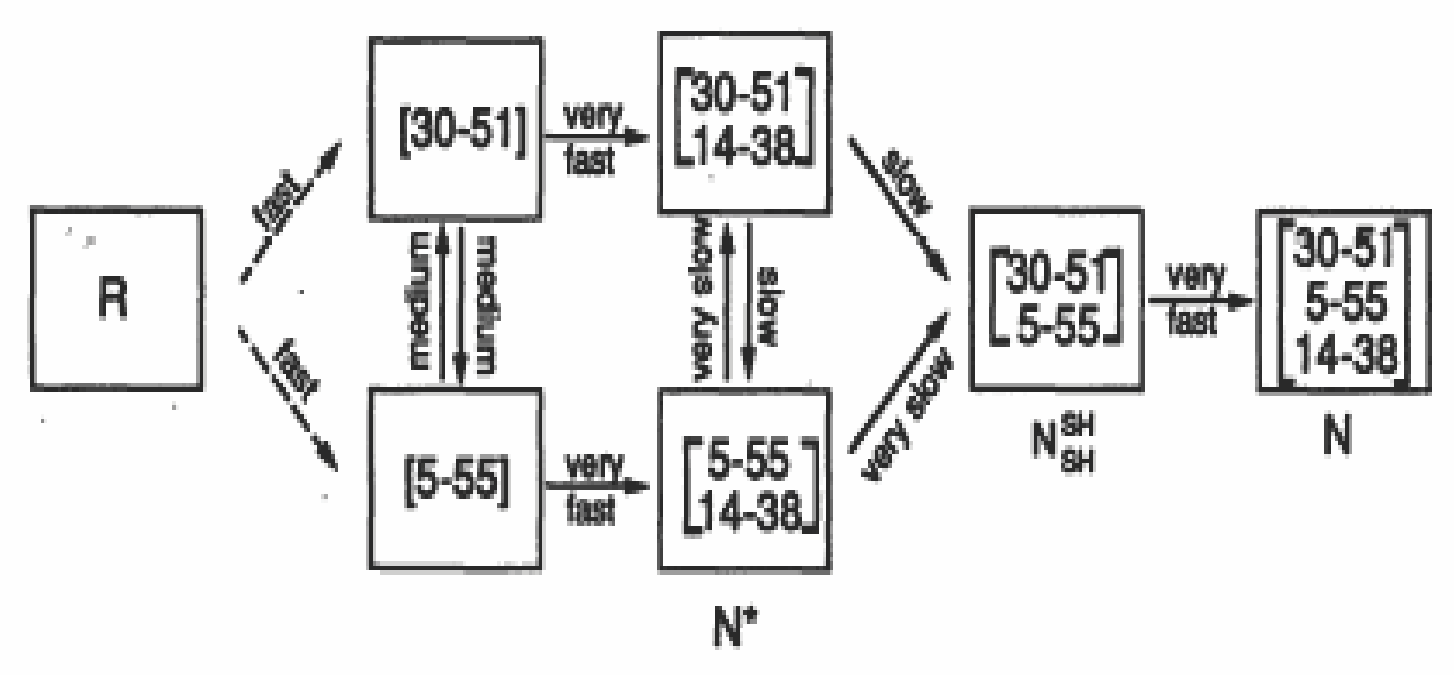
Adv. in Biophysics, 1984, 18, 1; Proteins book Page 319

Kim’s view: Native intermediates

- “The striking and counter-intuitive result was that three of the well populated species contain disulfide bonds not present in the native protein”

J Weissman and P.S. Kim, Science 1991, 253, 1386

Kim’s view: Native intermediates



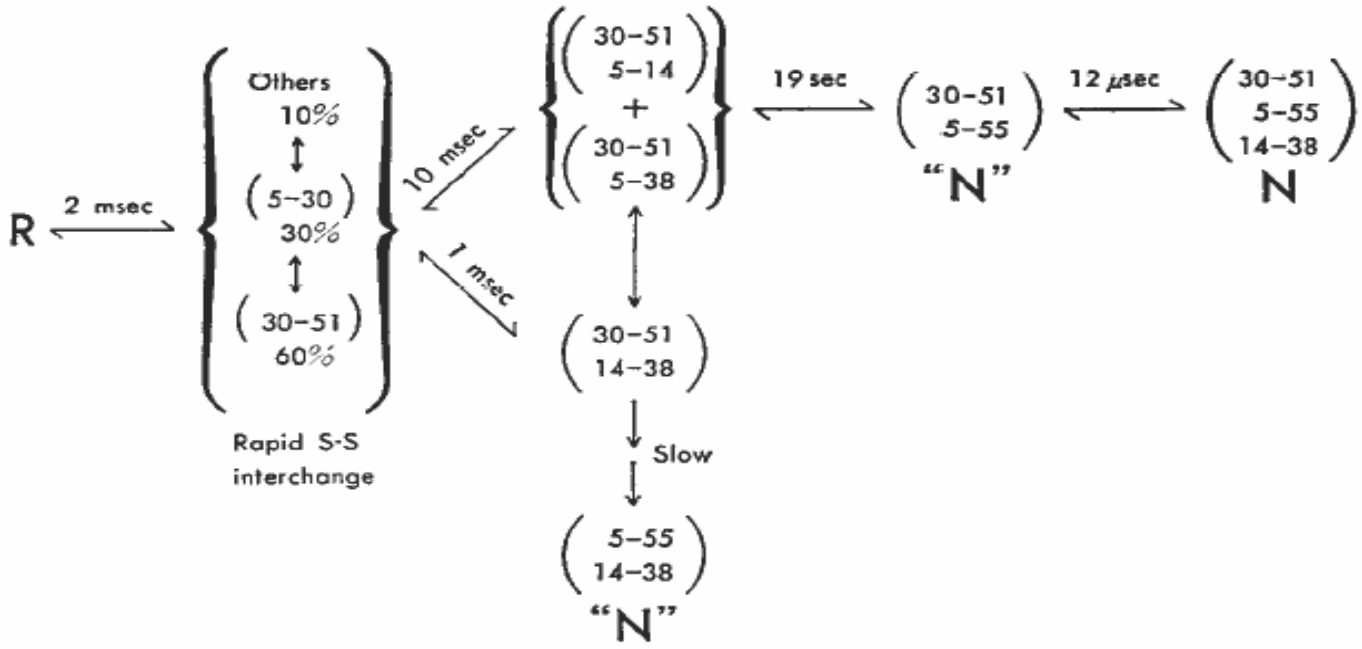
J Weissman and P.S. Kim, Science 1991, 253, 1386

Kim's method

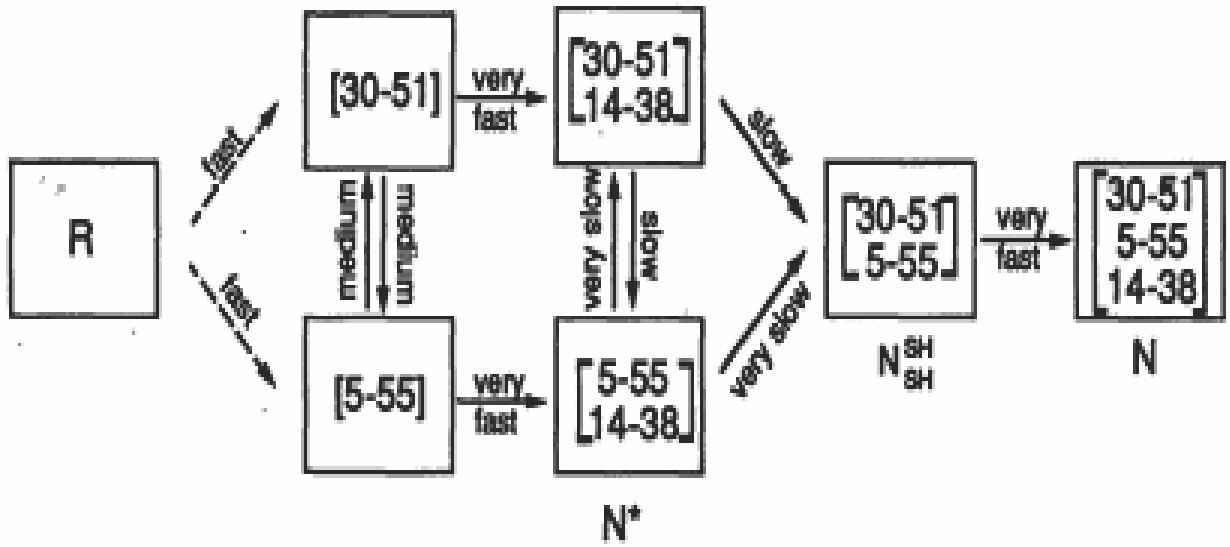
In our studies, a rapid and sensitive method for identifying disulfide bonds in an intermediate was developed. Starting with a purified intermediate, in which the thiols of cysteine residues that were not disulfide-bonded had been blocked previously with iodoacetate, we used the following method (7). (i) The disulfide bonds in the intermediate were reduced; (ii) the resultant thiols were labeled with a fluorescent iodoacetate derivative, IAEDANS; (iii) the protein was digested with thermolysin; (iv) labeled fragments (indicating Cys residues that were originally involved in disulfide bonds) were identified by reversed-phase

J Weissman and P.S. Kim, Science 1991, 253, 1386

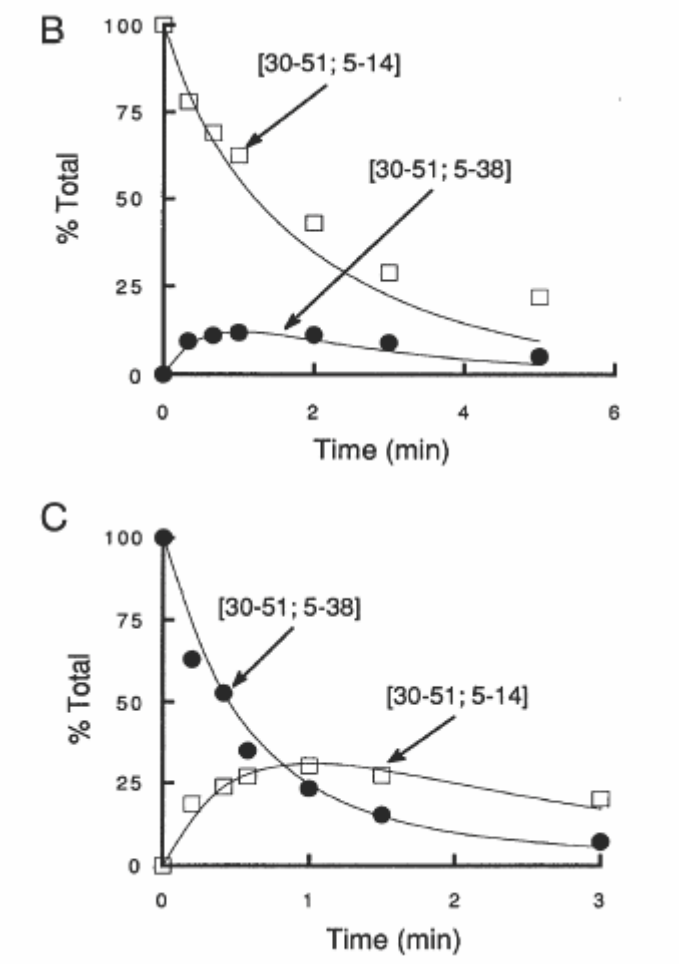
Creighton



KIM's



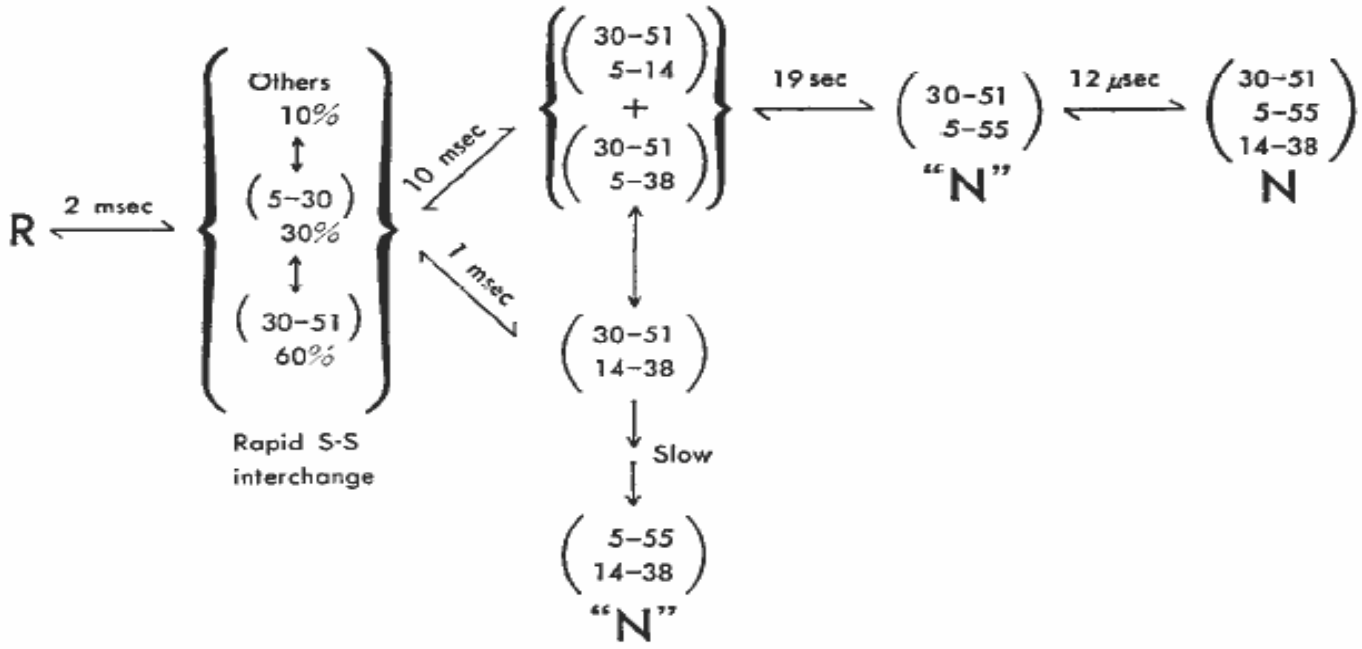
Rearrangement of non-native intermediates



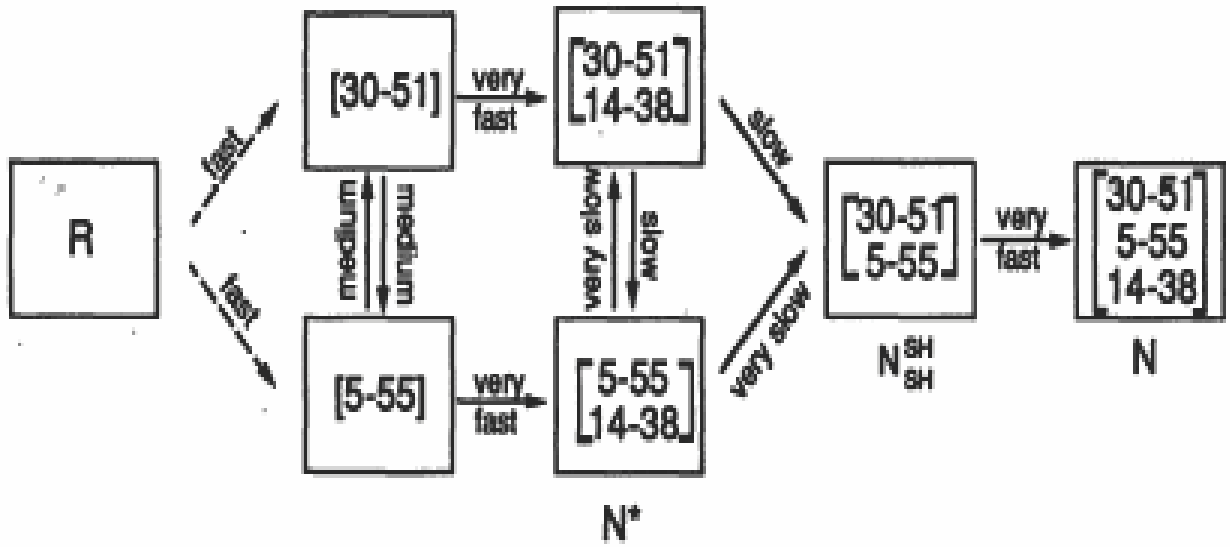
While non-native intermediates decrease in concentration,
N' [30-51; 14-38] → 80%
and
N_{SH} [30-51; 5-55] → 10%

J Weissman and P.S. Kim, PNAS 1992

Creighton



KIM's



Probability of loop formation

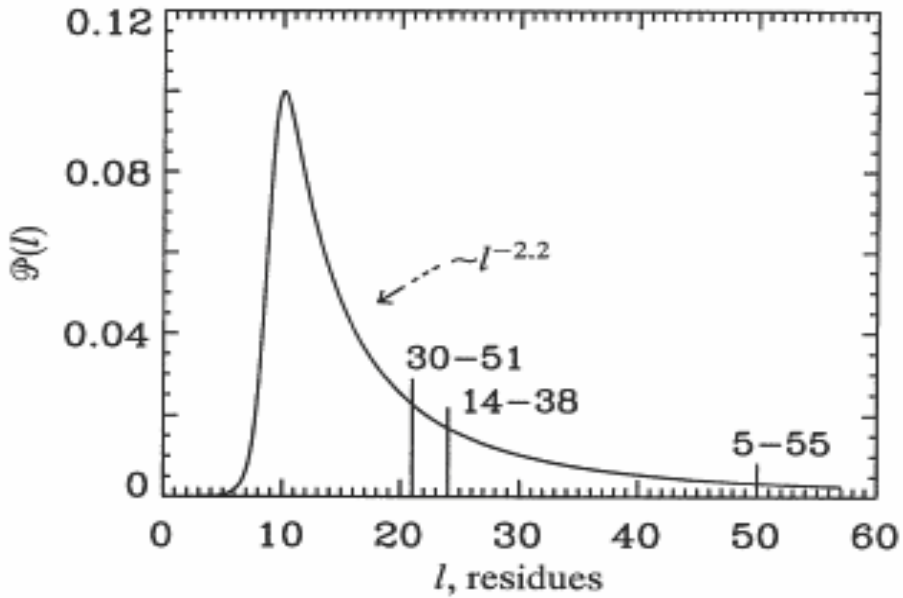


FIG. 1. Loop formation probability—$\mathcal{P}(l) = l^{-2.2}18.42/(\exp[1.8(9.0 - l)] + 1)$—in a polypeptide chain as a function of loop length $l = |i - j|$, where i and j are the positions of two residues along the chain. The probabilities of forming the native disulfide bonds of BPTI are also indicated.

$$\mathcal{P}(l) \sim l^{-\nu(d+\theta_2)}, \qquad [1]$$

Model disulfide bond formation based on $\mathcal{P}(l)$

- Assume for simplicity $\mathcal{P}(l1,l2) = \mathcal{P}(l1)\mathcal{P}(l2)$
- Kinetics:

$$\mathrm{R} \xrightarrow{\mathcal{P}(|i-j|)/\tau_c^0} \text{[i-j]}, \qquad \text{[2a]}$$

$$\text{[i-j]} \xrightarrow{\mathcal{P}(|k-l|)/\tau_c^1} \text{[i-j; k-l]}, \qquad \text{[2b]}$$

Non-native

$$\text{[i-j; k-l]} \begin{cases} \xrightarrow{\mathcal{P}(|i-j|)/\tau_1} \text{[i-j]} \rightarrow \text{repeat 2b} \rightarrow \text{repeat 3 (or 4)}, \\ \xrightarrow{\mathcal{P}(|k-l|)/\tau_1} \text{[k-l]} \rightarrow \text{repeat 2b} \rightarrow \text{repeat 3 (or 4)}, \end{cases} \qquad \text{[3a]}$$

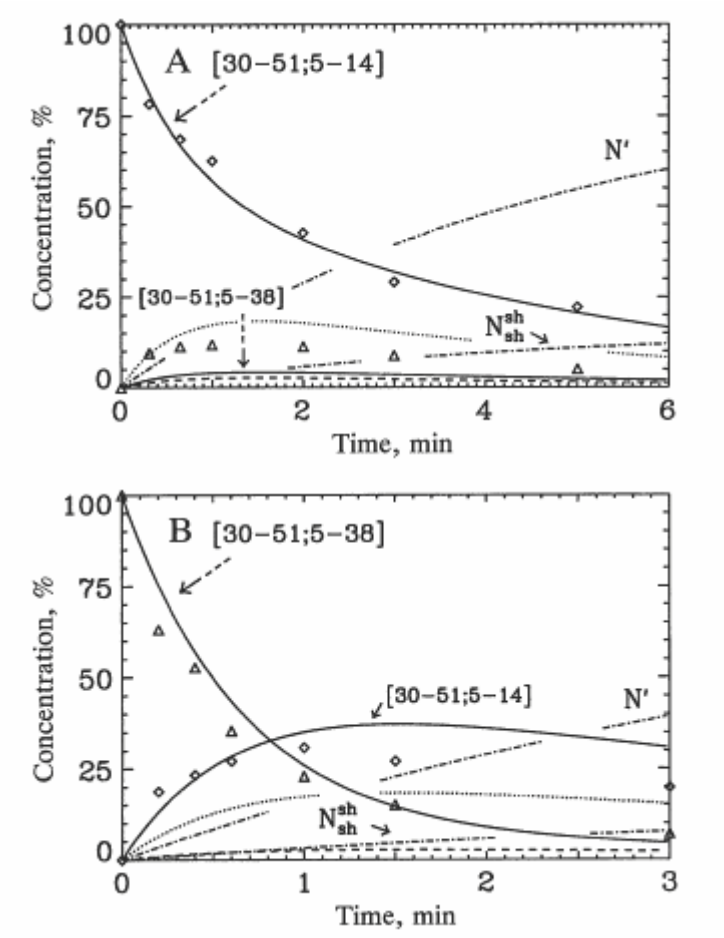
Semi-native

$$\text{[i-j; k-l]} \xrightarrow{1/\tau_1} \text{[i-j]} \rightarrow \text{repeat 2b} \rightarrow \text{repeat 3 (or 4)}. \qquad \text{[3b]}$$

Native

$$\text{[i-j; k-l]} \begin{cases} \xrightarrow{\mathcal{P}(|i-j|)/\tau_1} \text{[i-j]} \rightarrow \text{repeat 2b} \rightarrow \text{repeat 3b (or 4)}, \\ \xrightarrow{\mathcal{P}(|k-l|)/\tau_1} \text{[k-l]} \rightarrow \text{repeat 2b} \rightarrow \text{repeat 3b (or 4)}, \end{cases} \qquad \text{[4]}$$

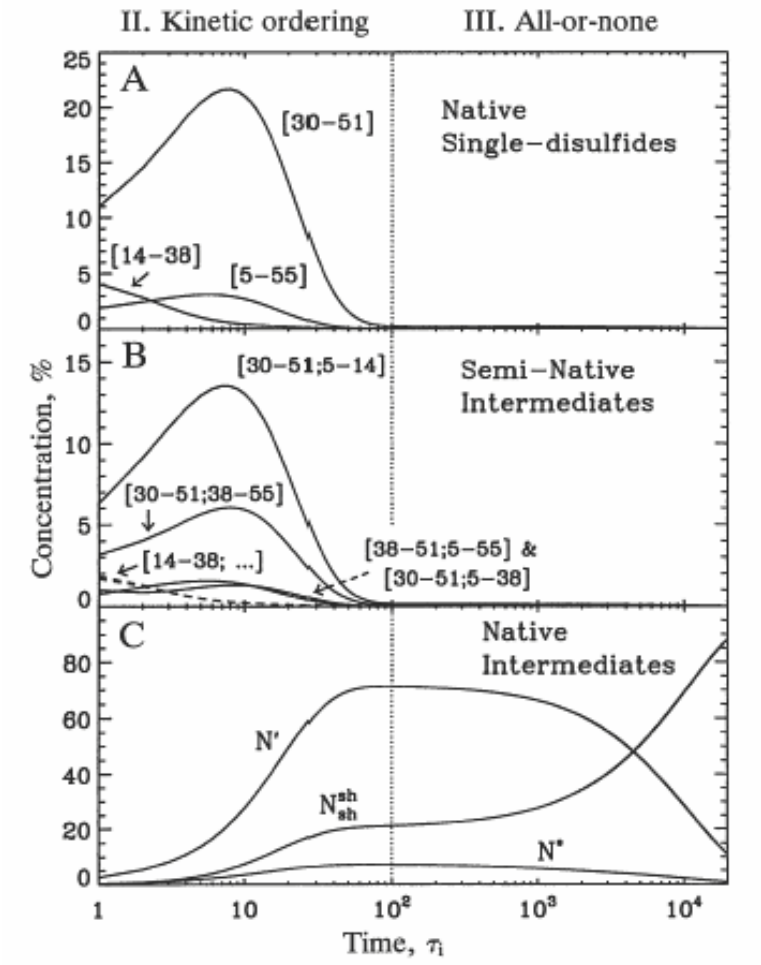
Rearrangement of non-native intermediates



While non-native intermediates decrease in concentration,
N' [30-51; 14-38] → 80% (WK 80%)
and
N_{SH} [30-51; 5-55] → 16% (WK 10%)

CJC & D. Thirumalai, PNAS 1995

Predictions of the concentration of intermediates in BPTI refolding



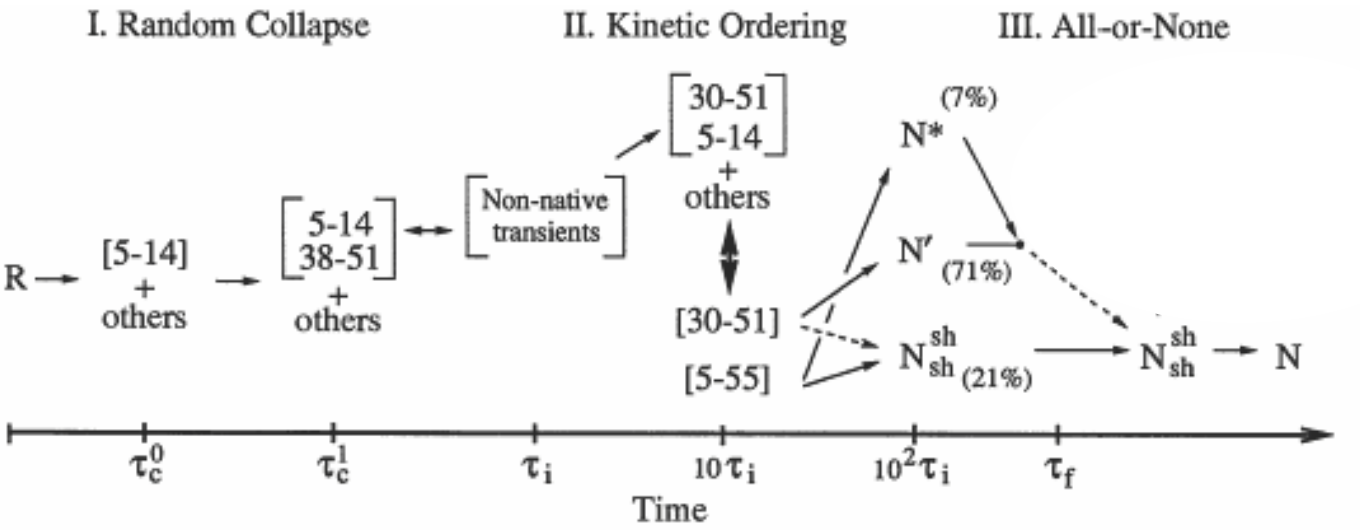
[30-51]/[5-55] = 7; WK = 6; Creighton 20.
→ These are not early fast-folding events!

N’ 71% (same as WK)
N* 7%
N_{SH} 21%

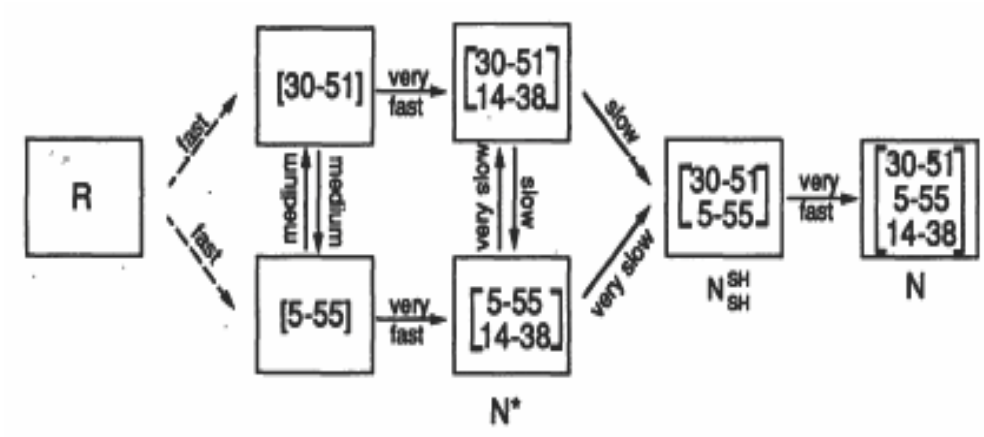
Question:
How do you go from N’ to N_{SH} ?

CJC & D. Thirumalai, PNAS 1995

Predicted folding pathways of BPTI



KIM's



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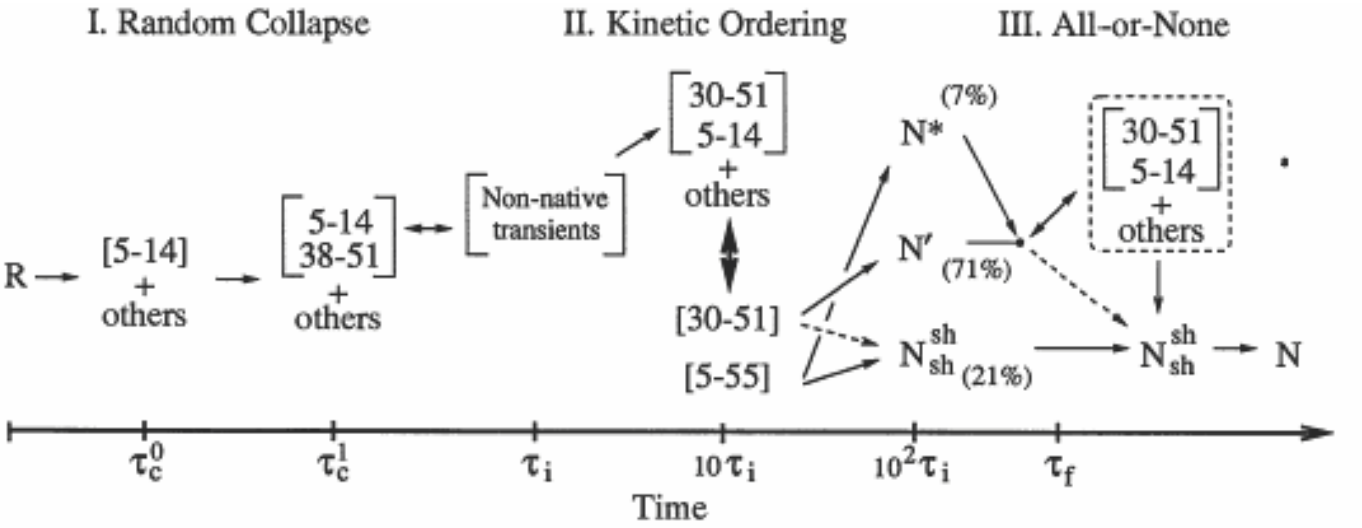

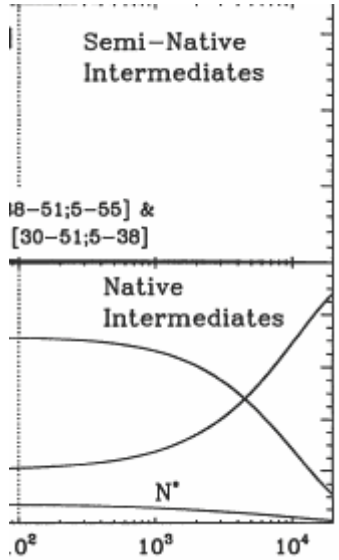

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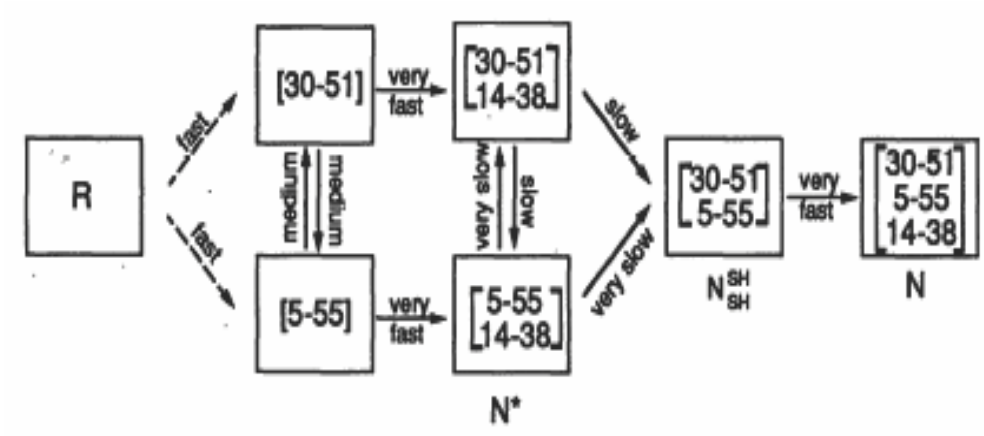
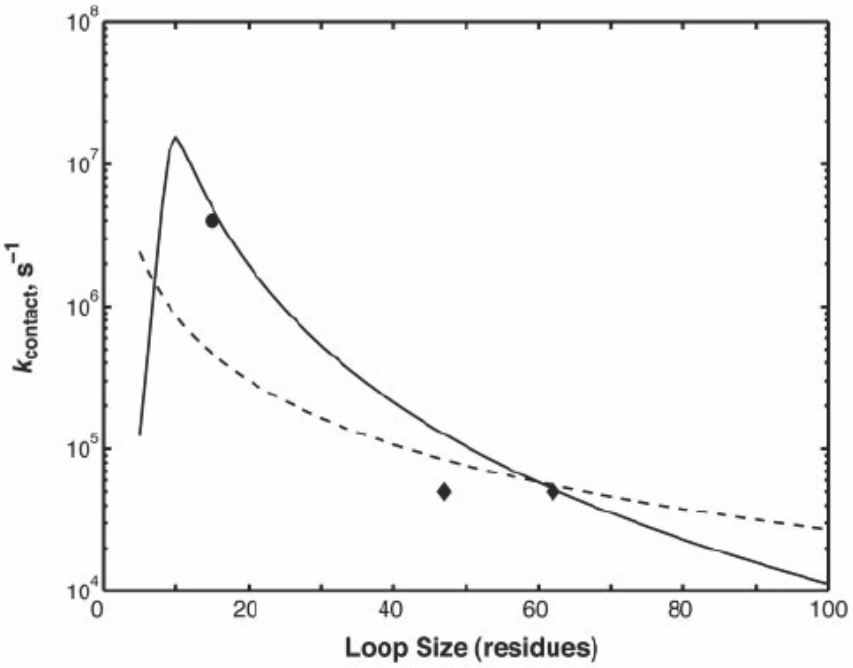


Experimental verification of $\mathcal{P}(l)$

Chang, Lee, Winkler & Gray, PNAS 2003. Electron transfer rates in Cyt-C

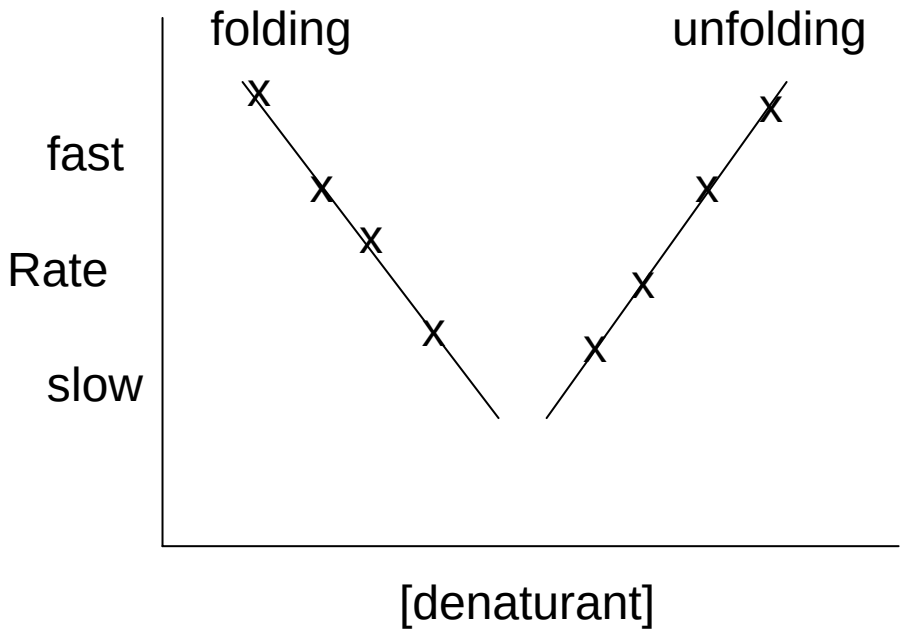
Ru complex into contact. The 250-ns contact time for formation of a 15-residue loop in denatured cytochrome *c* is in accord with a statistical model developed by Camacho and Thirumalai [Camacho, C. J. & Thirumalai, D. (1995) *Proc. Natl. Acad. Sci. USA* 92, 1277–1281] that predicts that the most probable transient loops formed in denatured proteins are comprised of 10 amino acids. Ex-



Effects of denaturant on unfolding and folding kinetics

- $\ln k_{ufold} = \ln k_0 + m[\text{denaturant}]$
- $\ln k_{fold} = \ln k_0 - m[\text{denaturant}]$

- Chevron plot



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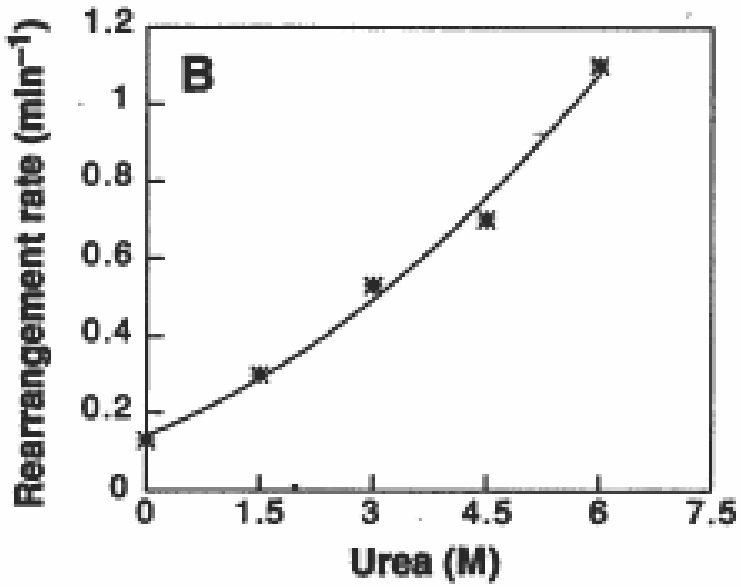
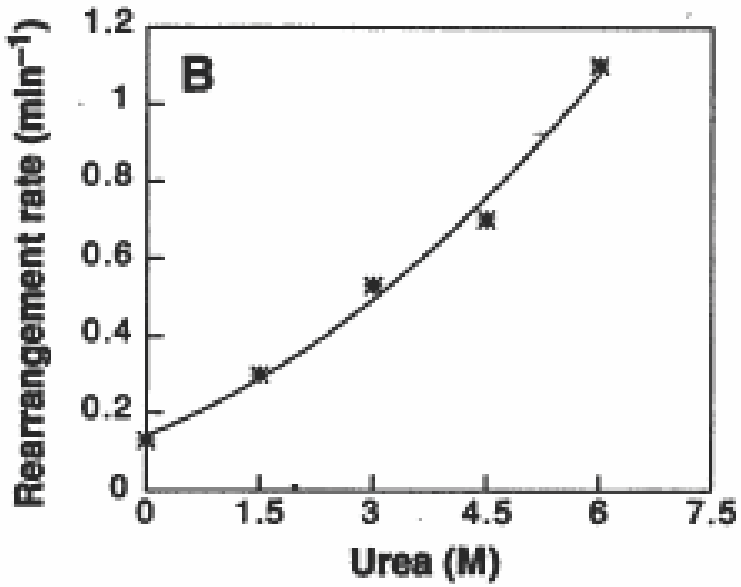


Rearrangement rate of Native intermediate [30-51; 14-38] → N_{SH} increases the folding rate

WK, Science 1991

Effects of denaturant on unfolding and folding kinetics



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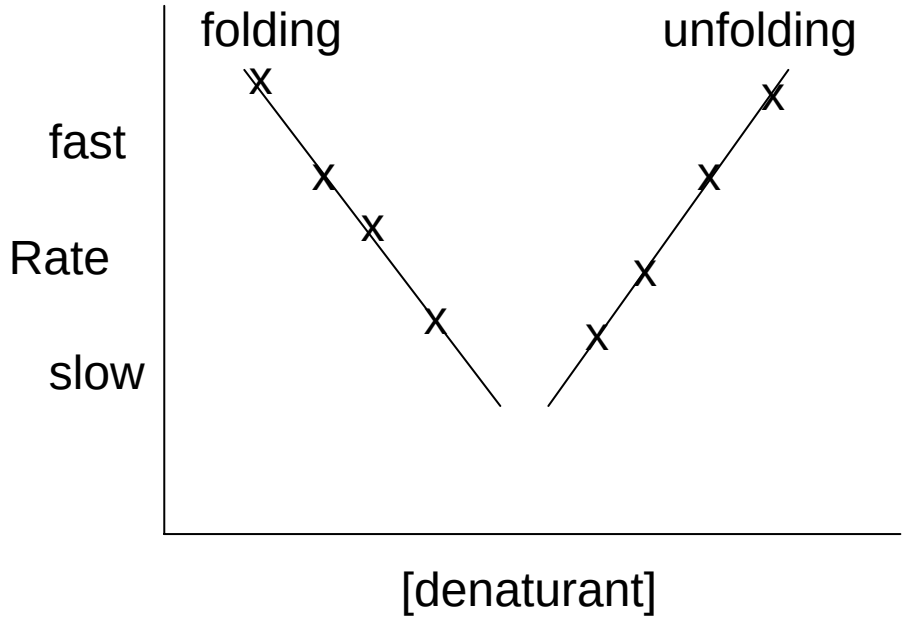
Chevron plot



What is wrong with this figure?

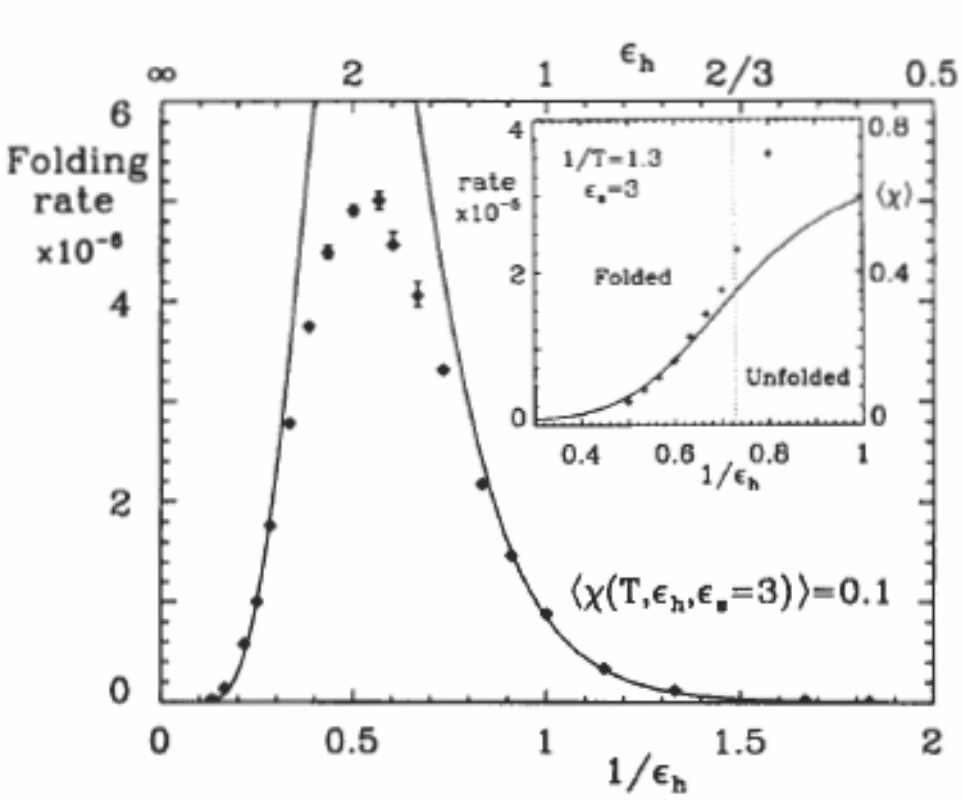
Role of denaturants

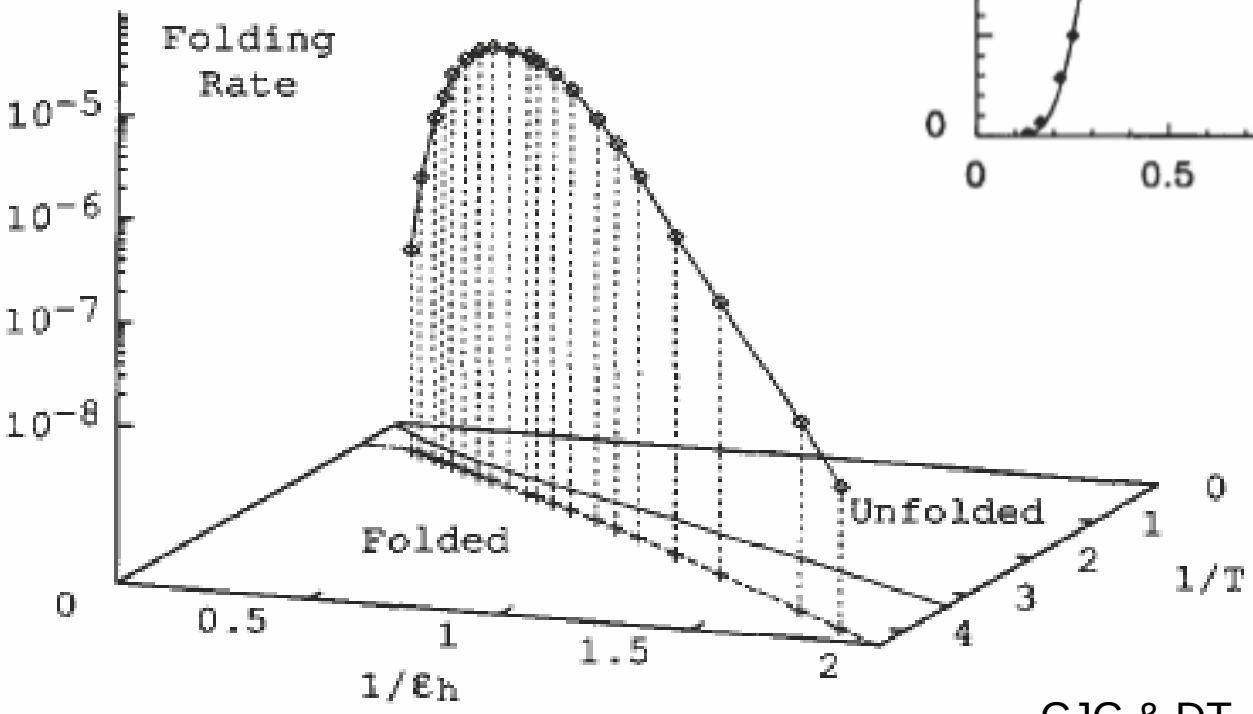
- Urea or Guanidinium hydrochloride

- It is likely that denaturants associate preferably with hydrophobes, acting as bumpers effectively screening the hydrophobic interactions

$$\epsilon_H(c_D) \simeq \epsilon_H(0) - k_C c_D,$$

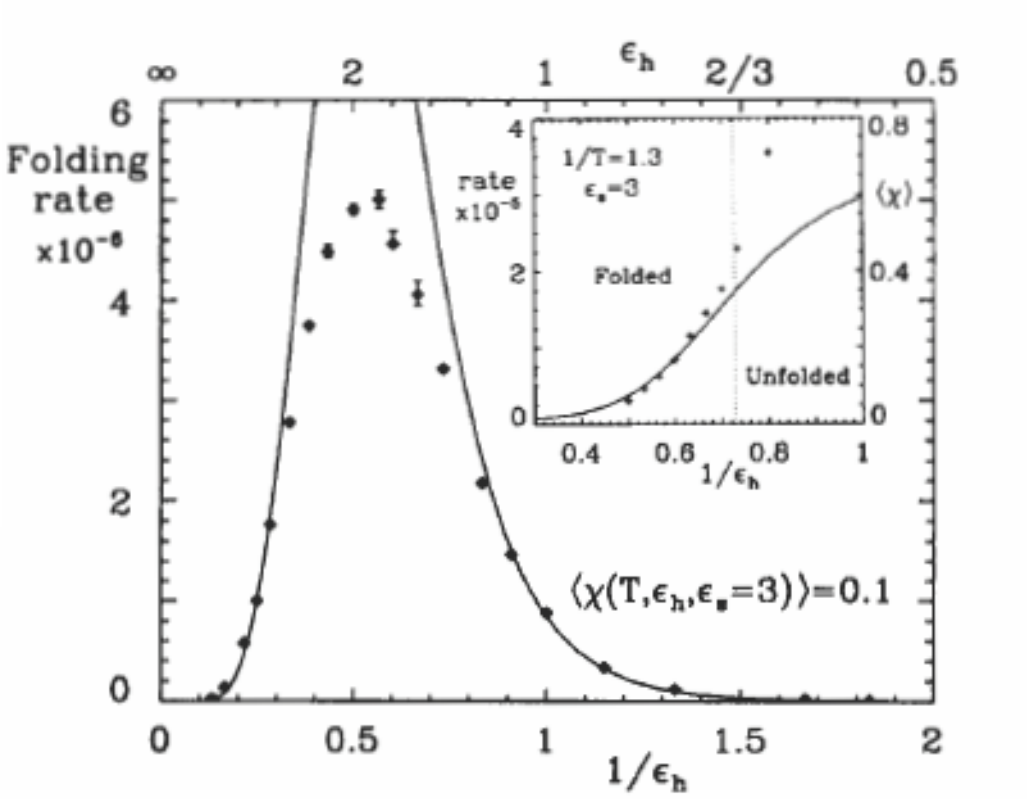
Kinetics

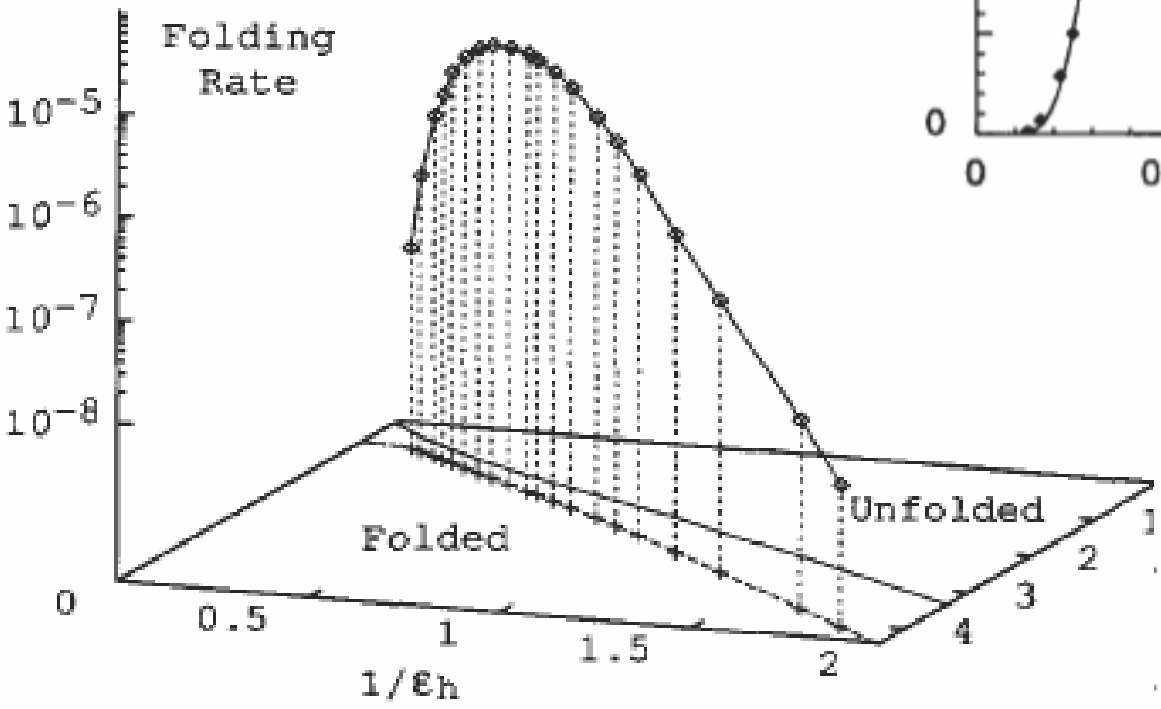


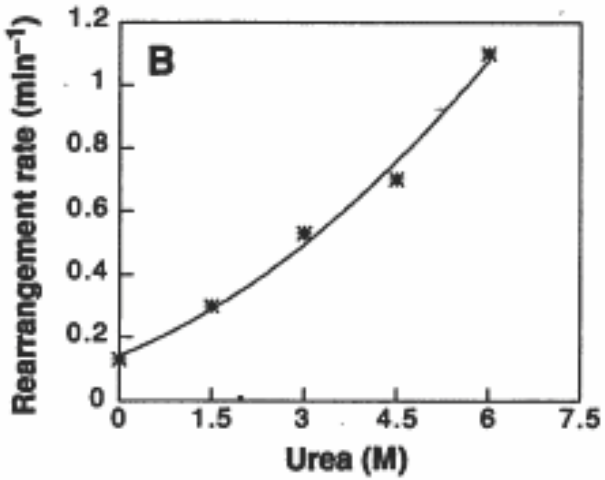


CJC & DT, Protein Science, 1996, 5, 1826

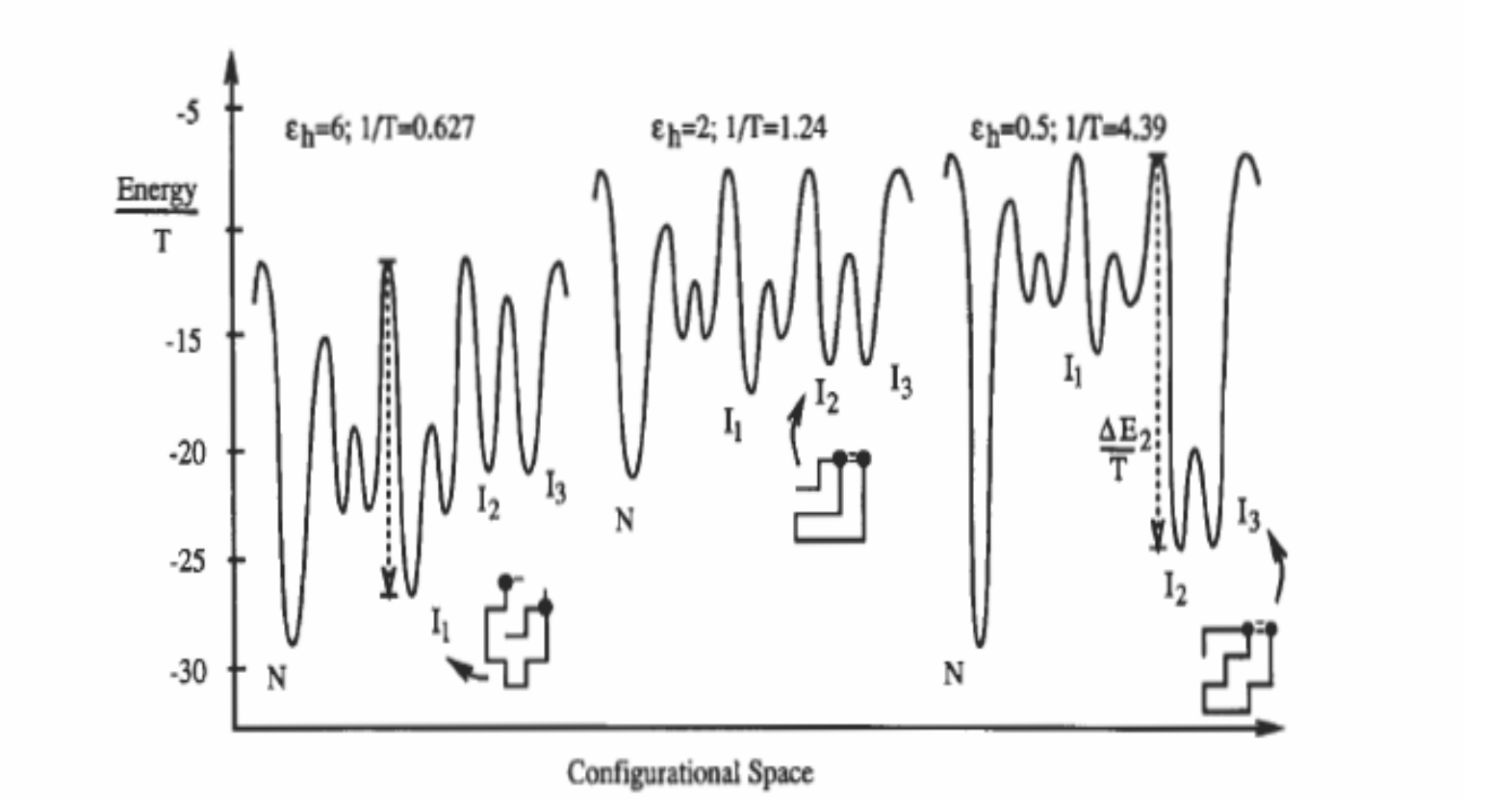
Kinetics


Folding rate x10⁻⁶
ϵh
∞
2
1
2/3
0.5
6
4
2
0
0
0.5
1
1.5
2
1/ϵh
⟨χ(T,ϵh,ϵs=3)⟩=0.1
rate x10⁻⁶
1/T=1.3
ϵs=3
Folded
Unfolded
⟨χ⟩
0.8
0.4
0
0.4
0.6
0.8
1
1/ϵh


Folding Rate
10⁻⁵
10⁻⁶
10⁻⁷
10⁻⁸
0
0.5
1
1.5
2
1/εh
Folded
Unfolded
1
2
3
4


B
Rearrangement rate (min⁻¹)
1.2
1
0.8
0.6
0.4
0.2
0
0
1.5
3
4.5
6
7.5
Urea (M)

Free energy landscape



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