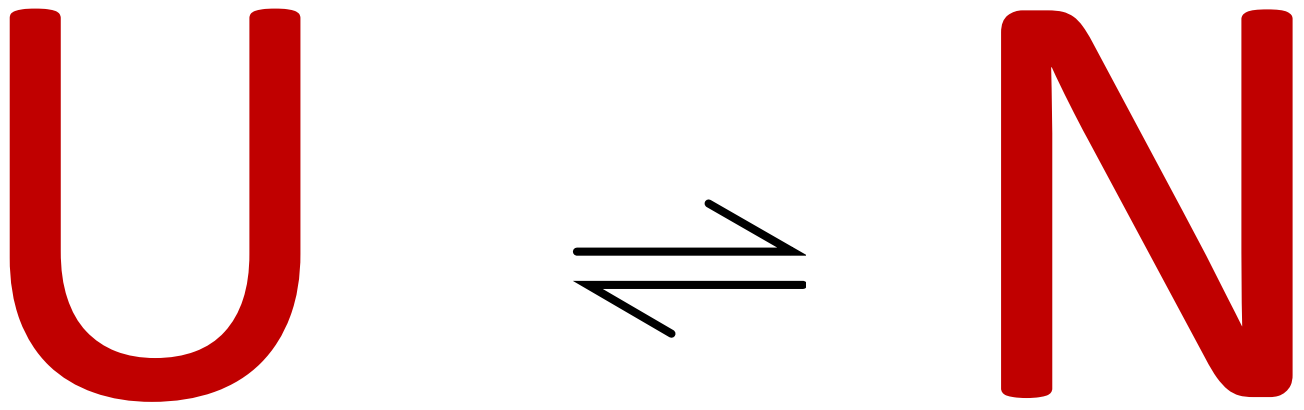
Protein Folding



(Unfolded) (Native)

- **Goal:** Understand relationship between Native (N) and Unfolded (U) state

Why Study Protein Folding?

- **Chemical interactions:** Folding involves the many chemical interactions and allows us to study how they work.

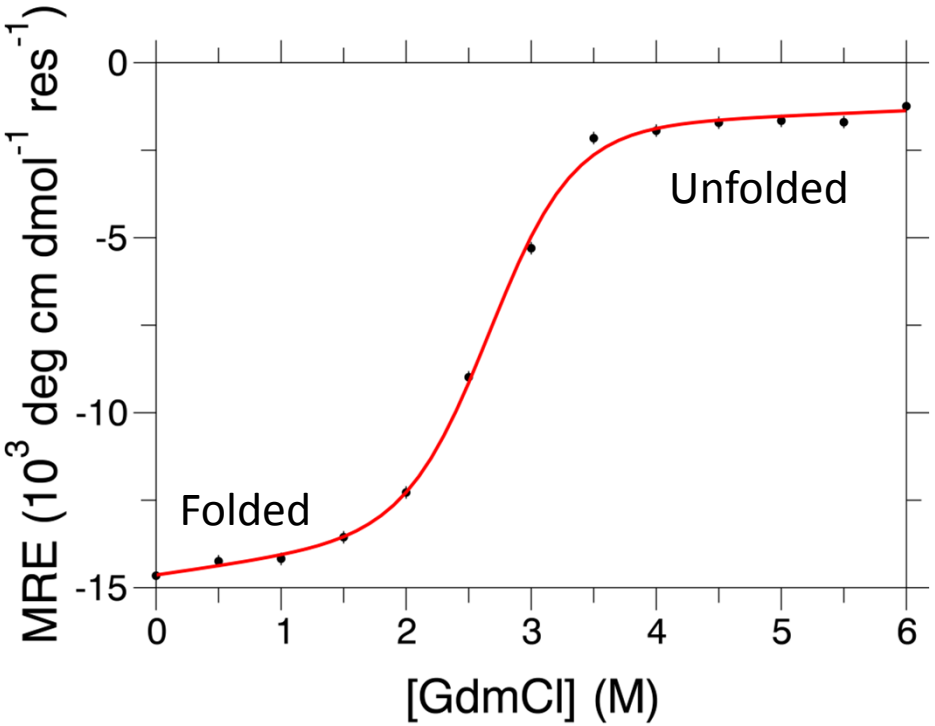
- **Applications to disease:** Some diseases (mad cow, cystic fibrosis, Alzheimer's) are caused when proteins don't fold correctly.

- **Evolution & protein origins:** Understanding folding allows us to understand the chemical basis of life's origins.

Protein Folding: Questions

- What are the important forces in protein folding?
  - Does our basic understanding of chemistry apply?
- Can we predict a fold from the amino acid sequence alone?
  - Can we predict binding and protein interactions?
- What is the mechanism of folding?
  - What are the relevant intermediates?

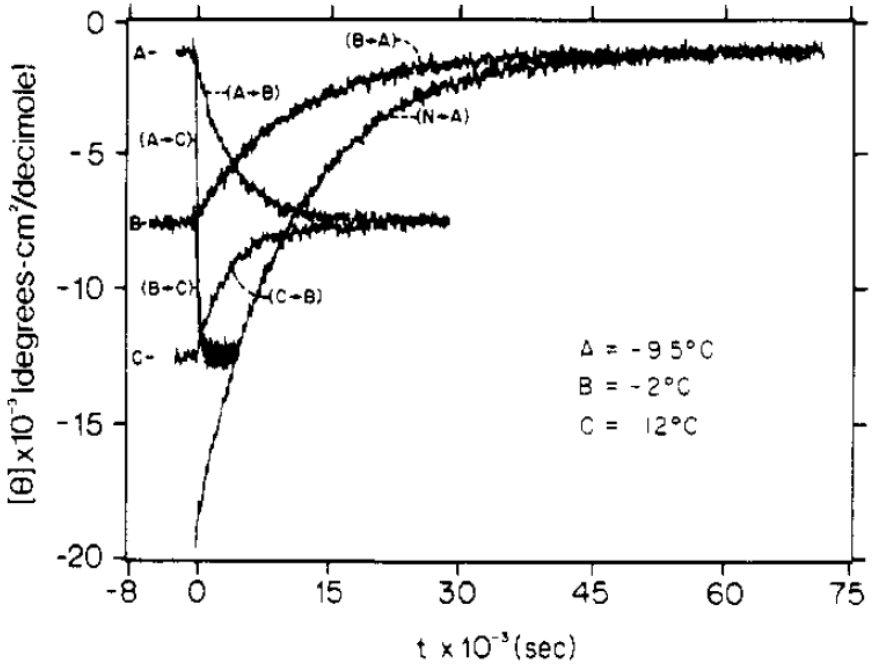
Equilibrium Folding Transitions



- Many folding transitions can be fit with a two-state thermodynamic model

Kinetic Measurements

FIGURE 1: Kinetics of the unfolding and refolding of I3C-C97/C54T T4 lysozyme in 3 M Gdn·HCl at pH 6.32, monitored by circular dichroism at 223 nm. Five curves are the results of temperature-jump experiments: B → A, −2 to −9.5 °C; C → B, 12 to −2 °C; A → B, −9.5 to −2 °C; A → C, −9.5 to 12 °C; B → C, −2 to 12 °C. The sixth curve, N → A, is the result of a concentration-jump experiment, mixing native protein with 3 M Gdn·HCl at −9.5 °C.



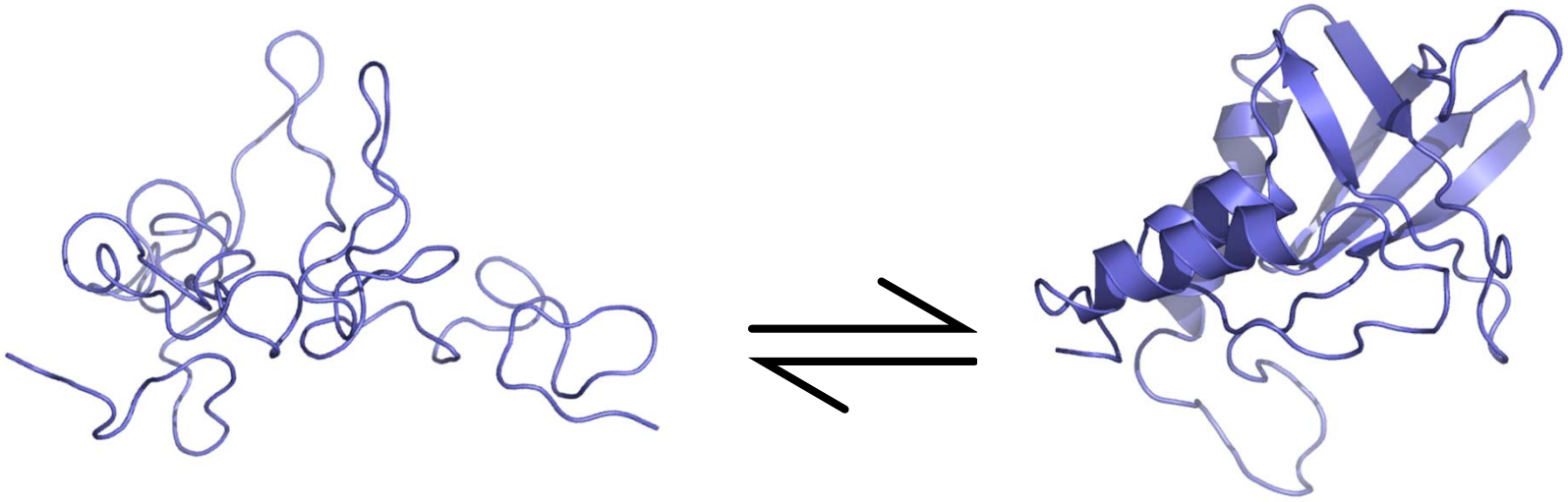
Temperature jump will *always* be exponential. (Why?)

- But, two state mechanism appears to apply:

$$K = \frac{k_1}{k_{-1}}$$

Chen, B. *et al.* (1989) *Biochemistry*. **28**: 691.

Two-State Folding

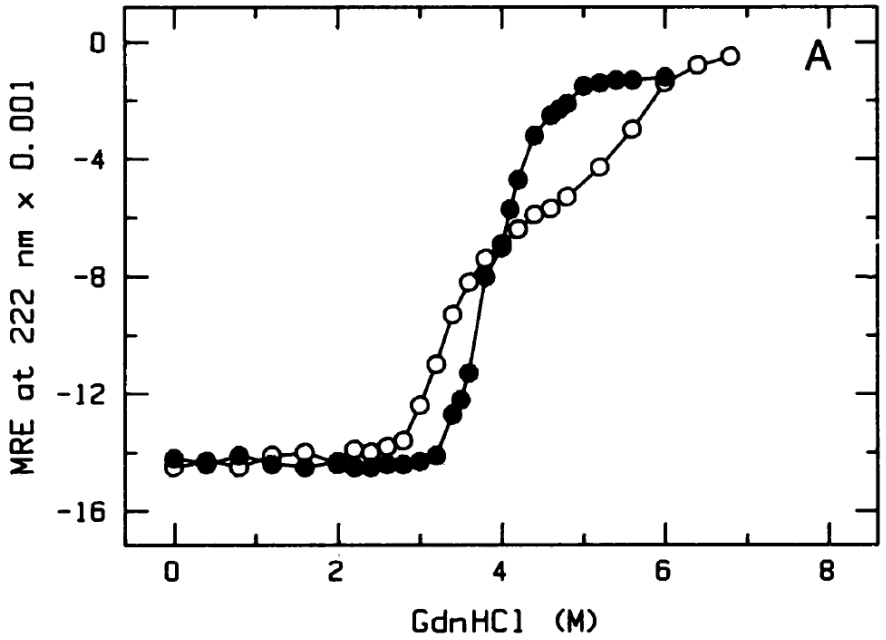


(Unfolded?) (Native)

- What are the implications?

When is Folding Not Two-State?

FIG. 2. Equilibrium denaturation by Gdn-HCl. ●, Wild-type bGH; ○, mutant bGH. All measurements were obtained at 230C. Denaturation was detected by CD at 222 nm and 0.04 mg/ml. All samples were at pH 8.5 and contained 50 mM ammonium bicarbonate, except the size-exclusion HPLC, which contained 10 mM Tris (pH 8.0).

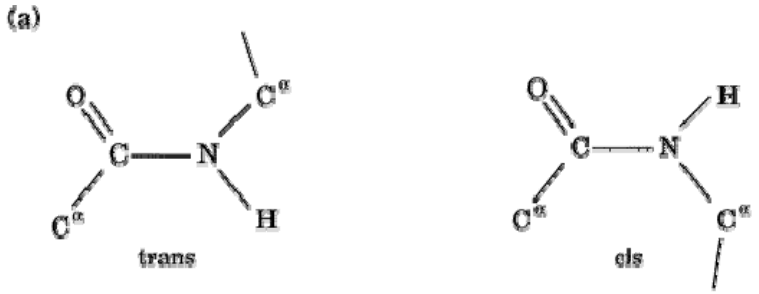


- **Case #1:** Intermediates
  - On pathway: $N \leftrightarrow I \leftrightarrow U$
  - Off pathway: $I \leftrightarrow N \leftrightarrow U$

Brems, D. N. *et al.* (1988) *PNAS.* **85:** 3367.

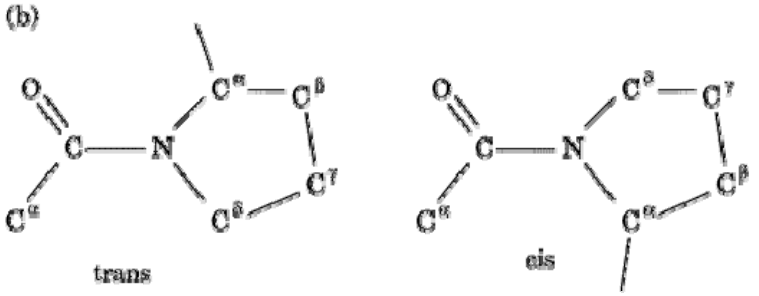
When is Folding Not Two-State?

Other Amino Acids: >99%



Other Amino Acids: <1%

Proline residues: 80-90%



Proline residues: 10-20%

- **Case #2:** Cis-Trans Proline Isomerization
  - Proteins with *cis*-Pro will generally have a second exponential phase for Pro isomerization (k ≈10-20 sec^{-1})

Wedemeyer, W. J., *et al.* (2002) *Biochemistry.* **41:** 14853.

When is Folding Not Two-State?

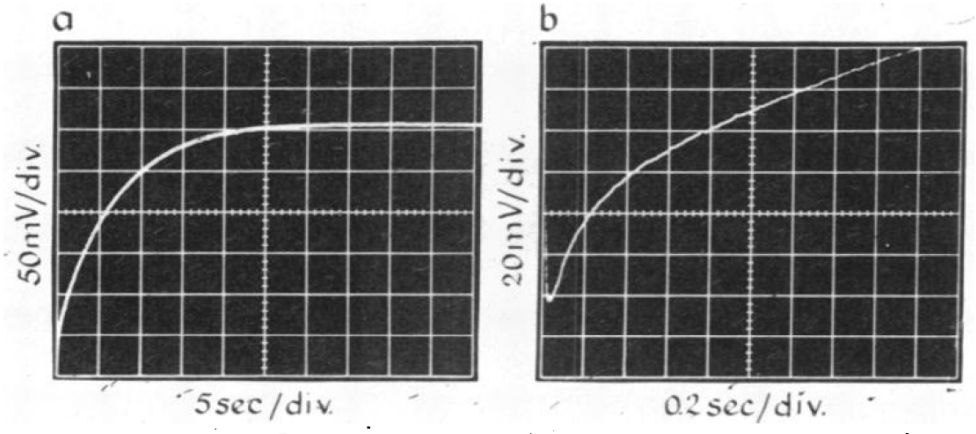


FIG. 3. Binding of 2′CMP occurs during both the fast and slow refolding reactions of RNase A (pH 2.0 → 5.8, 47°, 250 nm). Final concentrations: RNase A, 0.46×10^{-4}M; 2′CMP, 0.75×10^{-4}M. Total signal 2.2 V.

- **Case #2:** Cis-Trans Proline Isomerization
  - Proteins with *cis*-Pro will generally have a second exponential phase for Pro isomerization ($k \approx$10-20 sec^{-1})

Garel, J. and Baldwin, R. L. (1973) *PNAS.* **70**: 3347.

How to Perturb Folding

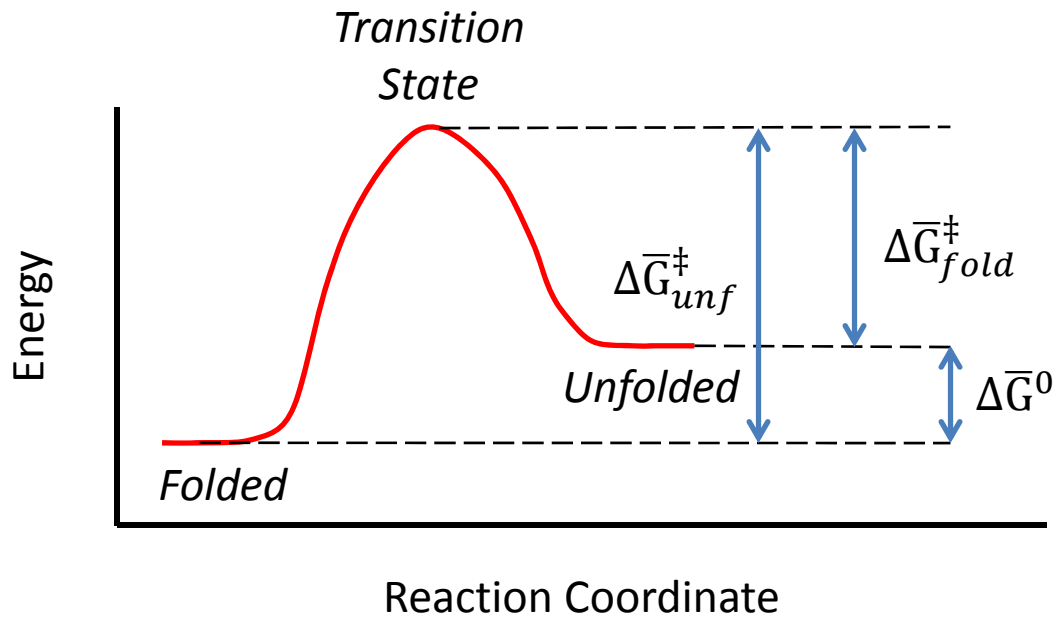
- **Chemical Environment:**
  - Chemical denaturants destabilize proteins
  - Salt concentration screens electrostatic forces

- **Temperature & Pressure:**
  - Predictable effects on $\Delta \bar{G}^0$

- **Vary the Sequence (Mutate the DNA):**
  - Introduce structural change in to the protein, e.g.

Ala-**Pro**-Glu-Glu → Ala-**Ser**-Glu-Glu

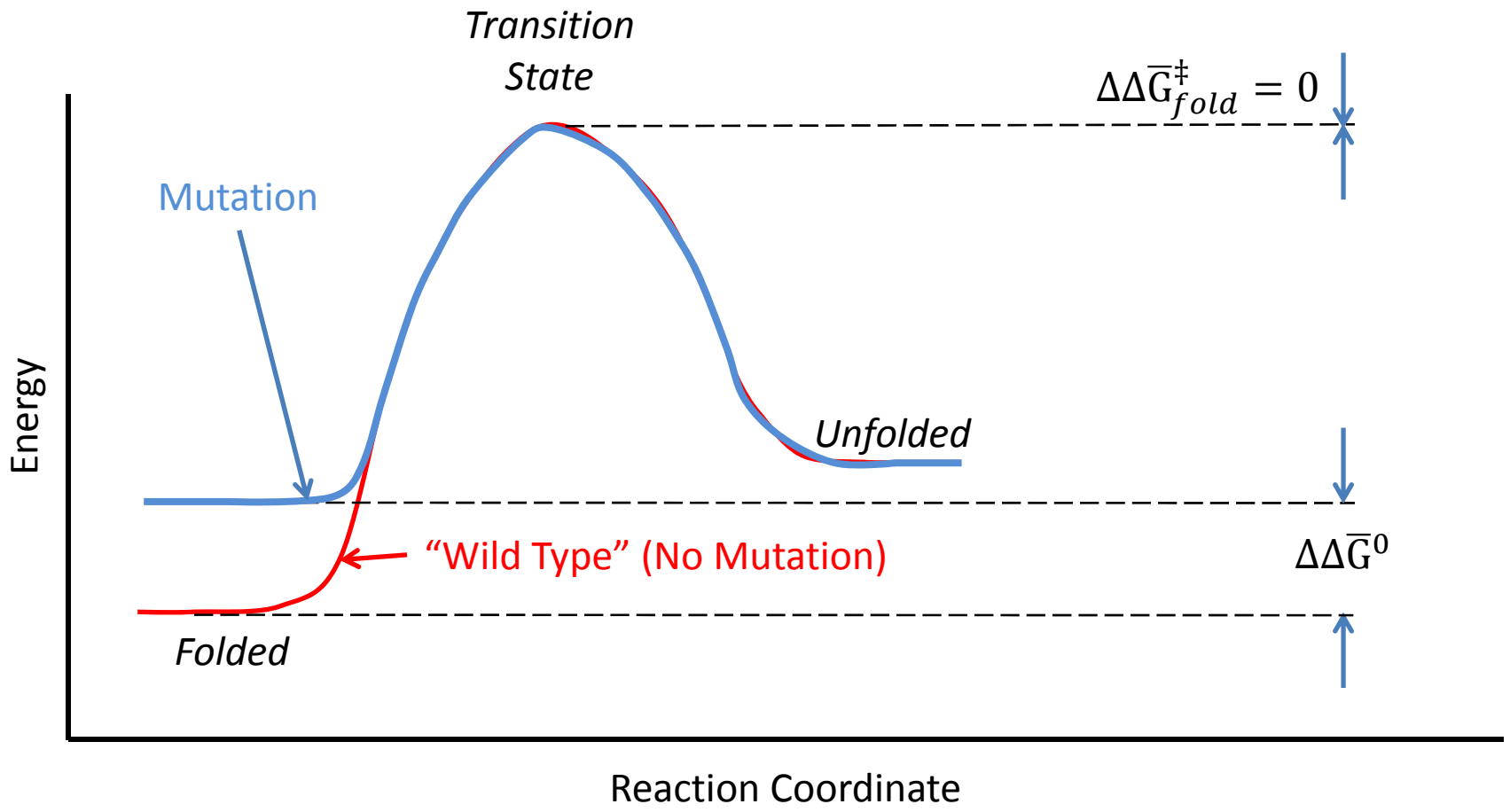
Phi Value Analysis



- **Idea:** Vary the sequence at one site; measure change in $\Delta\overline{G}^{\ddagger}_{fold}$ and $\Delta\overline{G}^{0}$

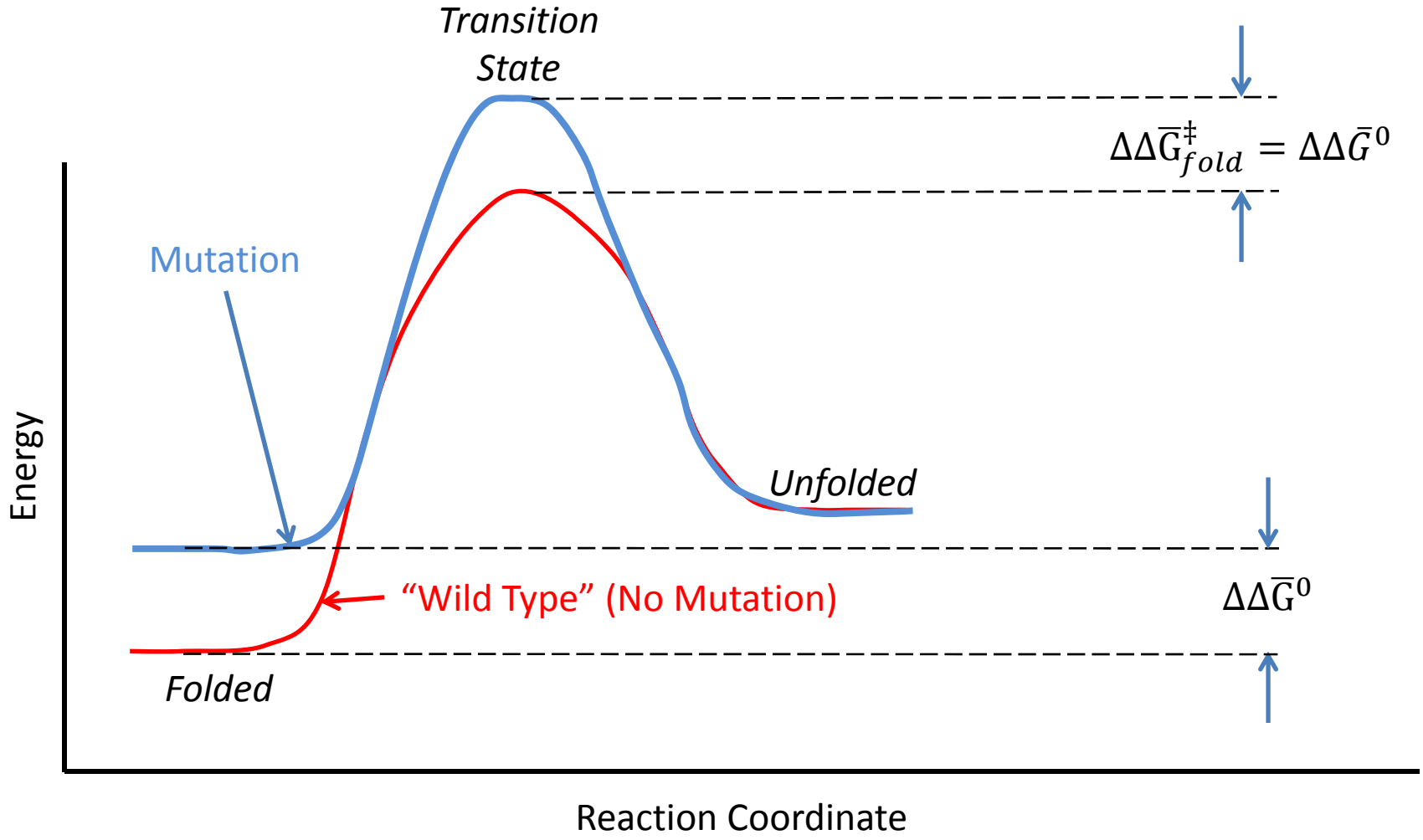
Phi Value Analysis

Extreme #1: Mutation only affects folded state



Phi Value Analysis

Extreme #2: Mutation affects both folded and transition states



Phi Value: Definition

- First proposed by Sir Alan Fersht:

$$\Phi = \frac{\Delta\Delta\bar{G}^{\ddagger}}{\Delta\Delta\bar{G}^{0}}$$

- Extreme cases:
  - $\boldsymbol{\Phi = 1}$: Residue in question is fully folded in transition state (both energies affected equally)
  - $\boldsymbol{\Phi = 0}$: Residue in question is not folded in transition state ($\Delta\Delta\bar{G}^{\ddagger}$ unaffected)
  - In between: ???

Phi Values: Example

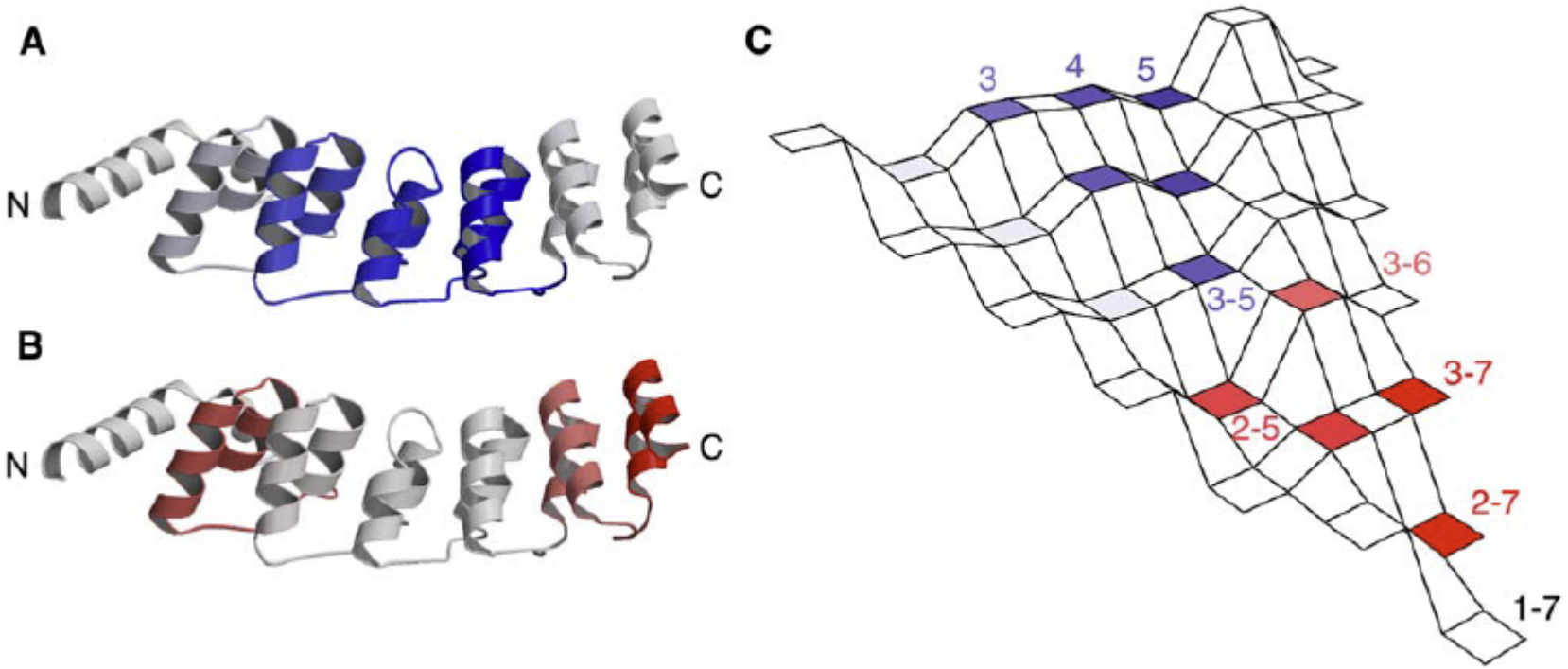


Figure 5. Transition State Ensembles and Folding Pathway of the Notch Ankyrin Domain

Ribbon diagram of the Notch ankyrin domain (1o8t.pdb) (Zweifel et al., 2003) with repeats shaded blue (A) and red (B) to match Φ-values for the $D \rightarrow I$ and $I \rightarrow N$ reactions, respectively. Shading for repeats with negative Φ-values was set to zero, as was shading for the first repeat. Shading for the seventh repeat was based on the stabilizing effect of adding a C-terminal extension (Nank1-7*) (Table 2). (C) Experimental energy landscape, determined from terminal deletion studies (Mello and Barrick, 2004), showing approximate locations of the $D \rightarrow I$ and $I \rightarrow N$ transition states (blue and red, respectively). The vertical position indicates free energy, whereas the horizontal axes indicate the number of repeats folded (back to front) and the center of mass of structure along the polypeptide chain (left to right) (Mello and Barrick, 2004). The figure was prepared by using Molscript (Kraulis, 1991), Raster3D (Merritt and Bacon, 1997), and MATHEMATICA (Wolfram Research, http://www.wolfram.com/).

Bradley, C. M. and Barrick, D. (2006) *Structure*. **14**: 1303.