Two-state folding, Transition states, and Nucleation mechanisms

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Two-state folding

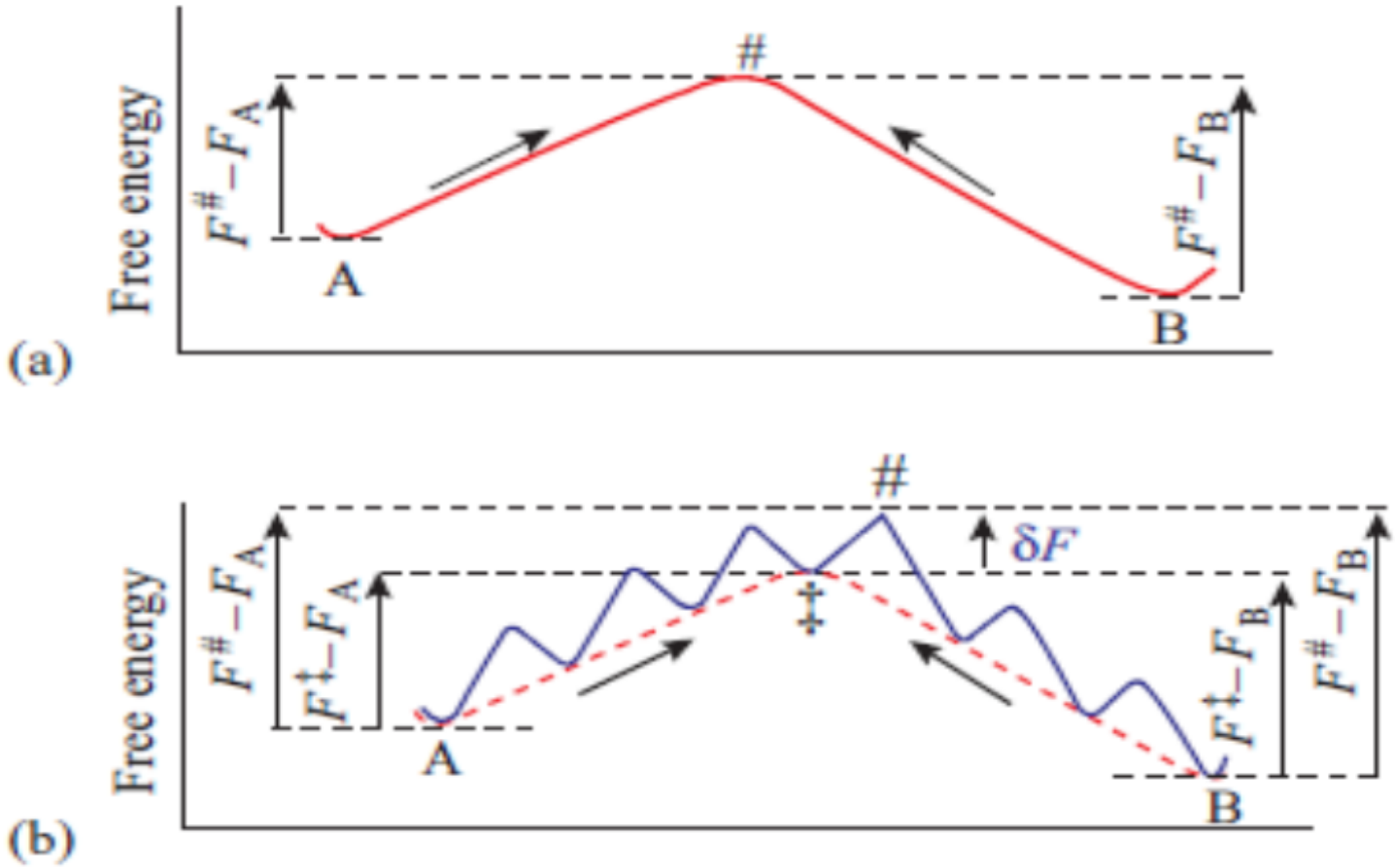
- Only the native and completely folded state are seen in a “two-state” transition.
- Two-state folding can occur under physiological conditions when the folding is most rapid, and when the majority of larger proteins display the accumulating folding intermediates like the molten globule.

Two-state folding

- Two-state folding transitions, which occur within a wide range of conditions, provides the best opportunity to study the *transition state* , or bottleneck, of the folding rate.
- The transition rate corresponds to the free energy *maximum* on the pathway from one stable state (the free energy *minimum*) to another.
- The rate of the process is limited by the *low occupancy* of the transition state.

- In other words, the kinetics of the transition is determined by the height of the free energy maximum along the reaction pathway.

- k(a->b) = k0 * exp(-(F#-Fa)/RT)



- **Figure 20.1. Overcoming of the free energy barrier on the pathway from the stable state** A to the stable state B (the left arrow in each scheme), and from B to A (the right arrow in each scheme). *FA, FB and F# are the free energies of the stable states A, B and the transition* state # (which is the "barrier", i.e., the free energy maximum on the pathway). (a) Elementary reaction; (b) multi-step processes consisting of several elementary transitions via free energy minima of a high free energy (compared with that of the A and B states). *δF, the free energy* barrier for one step; ‡, the free energy minimum of the highest free energy *F‡.*

Multi-step processes

- The rate of a multi-step process is similarly determined only by the highest free energy barrier on the pathway.
- The rate of a multi-step process can be expressed through the rates of one-step transitions between the intermediate free energy minima and the heights of these minima.

Folding nucleus

- The nucleation mechanism is typical of the first-order phase transitions in conventional physics, such as crystal freezing.
- It is an “all-or-none” transition, which implies that it is a first-order phase transition.

Folding nucleus

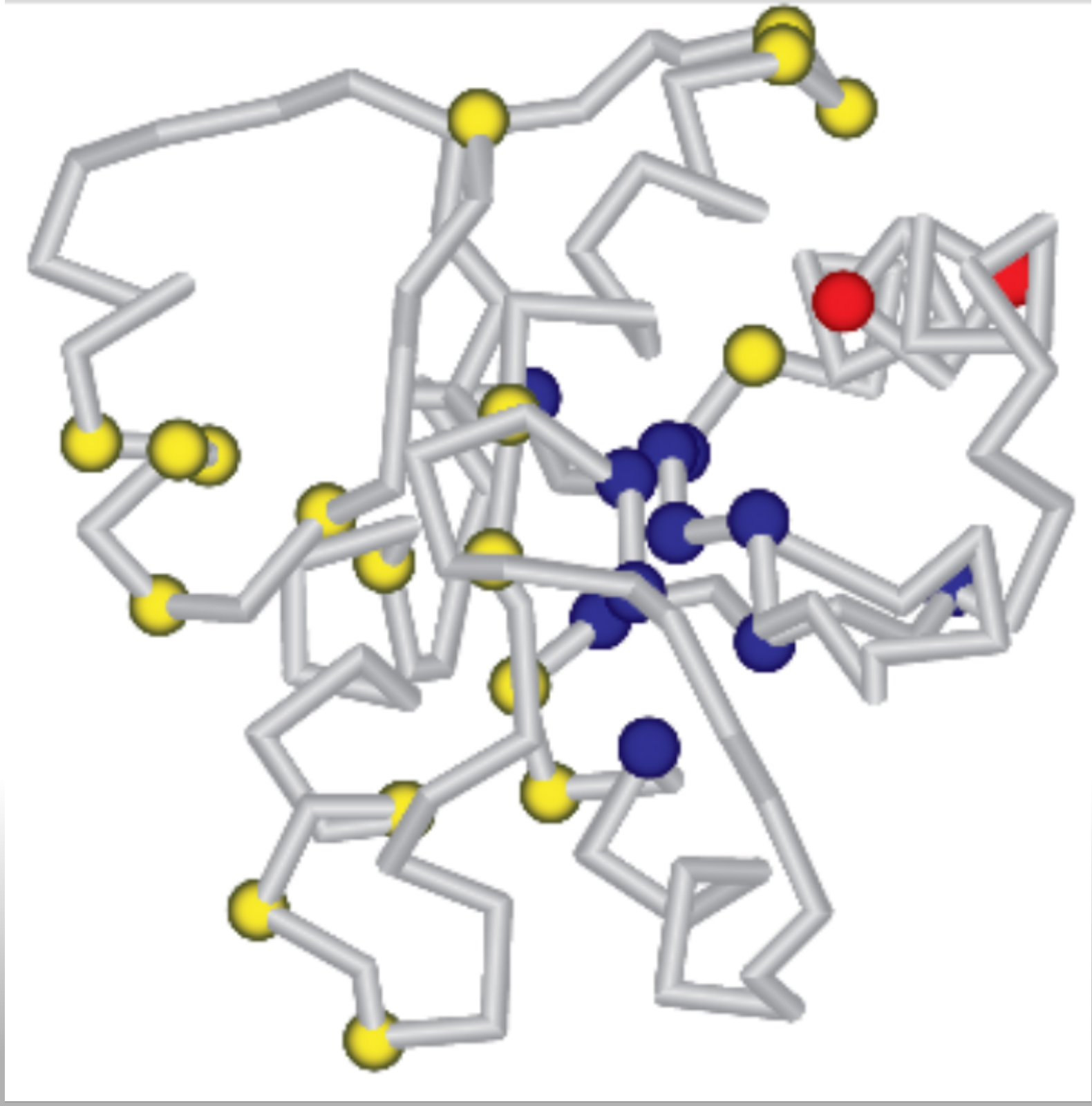
- If a mutation in a single residue changes the transition state stability value F#-Fu and the native protein stability Fn-Fu equally, this means that the residue in question is involved in the folding nucleus.
- This implies it has the same contacts and conformation as in the native protein.

Folding nucleus

- On the other hand, if the residue's mutation changes *only* the native protein stability value Fn-Fu, but does not changes the folding rate, this means that the residue in question is not involved in the nucleus and comes to the native structure only after the rate-limiting step.

Folding nucleus

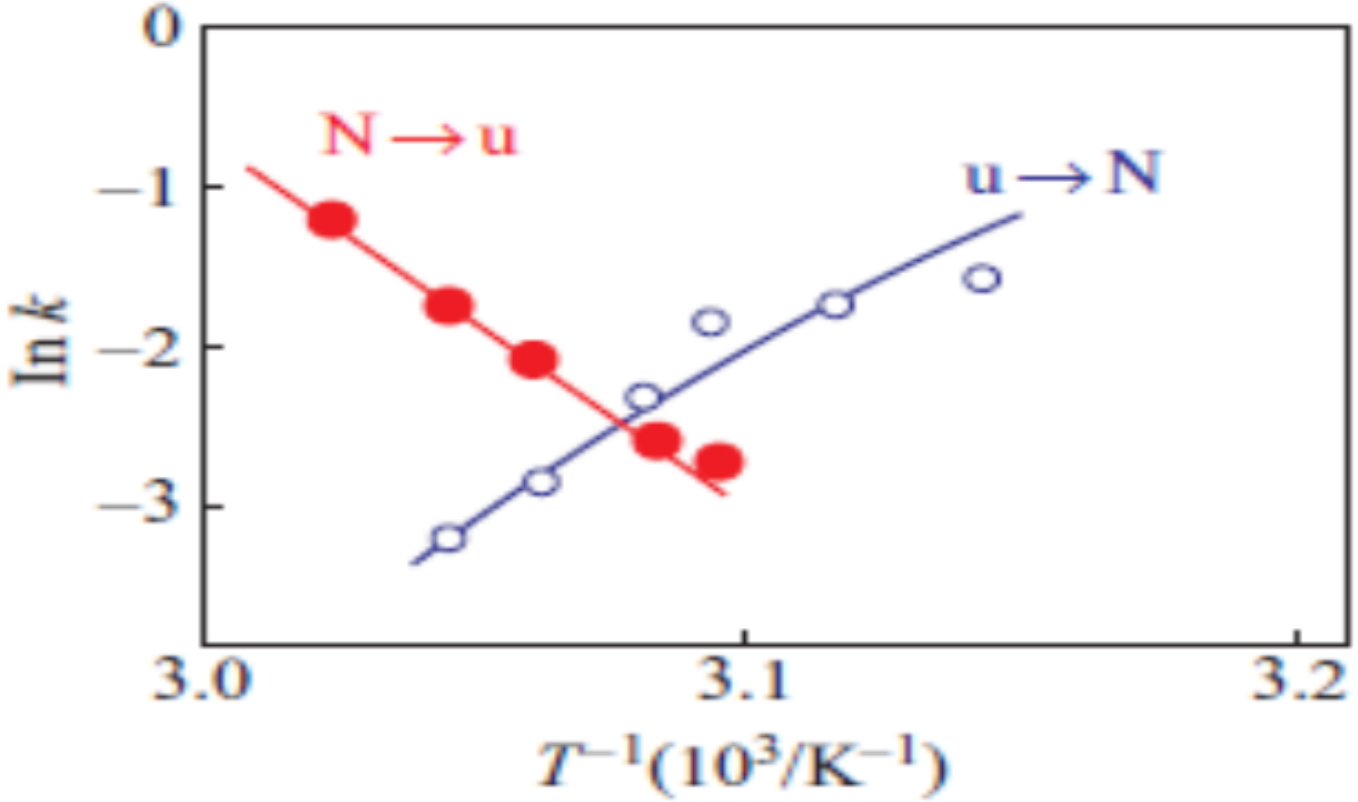
- If the residue's mutation affects the transition state stability to a lesser degree than the native protein stability, this implies that the residue in question either belongs to one of the few alternative folding nuclei, or forms only a part of its native contacts within the nucleus (i.e. resides at surface of nucleus).
- Usually the folding nucleus is compact and does not coincide with the protein's hydrophobic core.



- Figure 20.6. Folding nucleus for CheY protein according to L´opez-Hern´andes E., Serrano L., Folding Design (1996) 1: 43–55. The residues studied experimentally are shown as beads against the background of the native chain fold. Dark blue beads show the residues involved in the nucleus, i.e., in the folded part of the transition state (they have f > 0.3, i.e., each of them forms more then 30% of its native contacts there). Yellow beads show residues having f < 0.3; they are not involved in the folded part of the transition state. Red beads show two residues that are difficult to interpret experimentally. They have such low (F# − Fu) and (FN − Fu) values (the latter is more important, since this value is the denominator in eq. (20.11), that the errors in their determination exceed the measured values themselves.

Molten Globule

- A transition state is far less uniform than the molten globule.
- As far as the “coil -> native state” transition is concerned, the transition state is similar to a piece of the native protein, while the rest of the chain remains in the unfolded state.
- It is plausible that other transition states could include a piece of the more structured state, while the rest of the chain remains in in a less structured state.

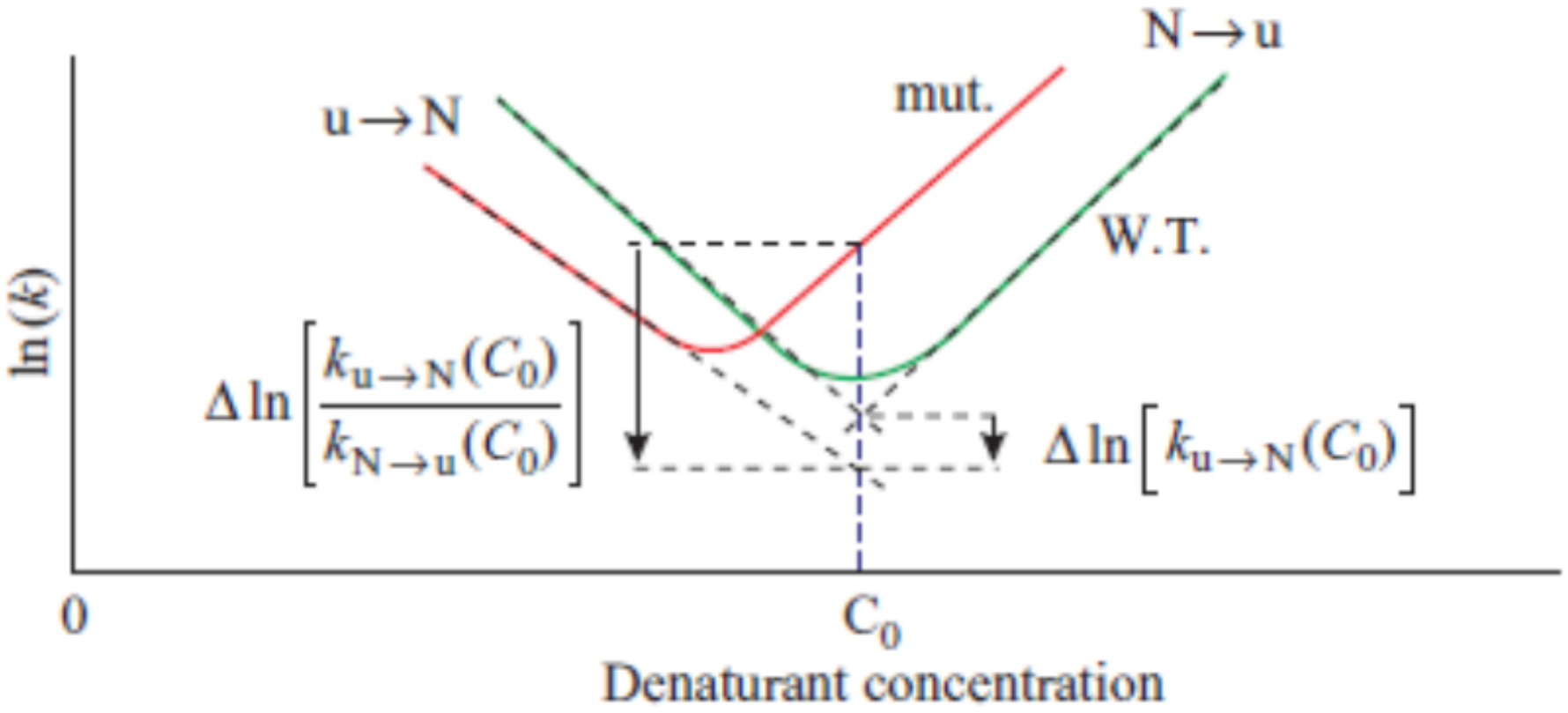


Arrhenius plots for the rates of lysozyme de- and renaturation vs. the reciprocal temperature value (*T−1).* The rate constants (k) are measured in sec−1. Renaturation: rate ku→N (experimental points ◦ and the thin dark blue interpolation curve); denaturation: rate kN→u (experimental points • and the bold red interpolation line). The mid-transition is the temperature point where ku→N = kN→u, i.e., where the curves intersect (at about 1000/3.08 = 325 K). Folding prevails in the “renaturation region” at low temperatures, unfolding prevails in the “denaturation region”

Molten globule

- The plot shows that the denaturation accelerates as it gets deeper into the “denaturation region”, and the renaturation accelerates as it gets deeper into the “renaturation region”.
- The unfolding rate grows with temperature T, which is typical of physicochemical reactions.
- However, the folding rate on the contrary wanes with increasing T.

“Chevron plot”



Chevron Plot Interpretation

- To estimate the involvement of a residue in the native-like part of the transition state (“folding nucleus”), one estimates:
  - (a) the mutation-induced shift of the folding rate and
  - (b) the mutation-induced shift of the native protein stability.

- **Figure 20.5.** A scheme illustrating a mutation-induced change of the chevron plot: k, apparent rate of transition. Green line, the initial, “wild type” protein (W.T.); red line, the same protein with one residue mutated (mut.). C0, the denaturant concentration at the mid-transition for the W.T. protein. The dotted lines show extrapolation of the folding and unfolding rates, $k_{u\rightarrow N}$ and $k_{N\rightarrow u}$, to the W.T. chevron bend region. Two measured mutation- induced shifts are shown. The first is the change in the height of the free energy barrier on the pathway from the unfolded to the native state: $(F^{\#} - F_u) = -RT\ln[k_{u\rightarrow N}]$. The other is the change in protein stability, i.e., in the free energy difference between the native and unfolded states: $(F_N - F_u) = -RT\ln[k_{u\rightarrow N}/k_{N\rightarrow u}]$. The ratio $(F^{\#} - F_u)/(F_N - F_u)$ is called f for the mutated residue. In the shown case $f \approx 1/4$, i.e., when in the transition state, the mutated residue has only a quarter of its native interactions. (A mnemonic rule. If the left branches of the W.T. and mut. chevrons are closer to each other than the right