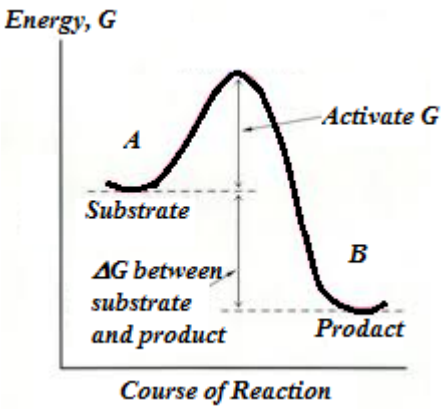
Two-state folding

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.

$$\mathbf{k}(T) = \mathbf{k}_0 * \mathbf{exp(-(G-Ga)/RT)}, \text{ where}$$

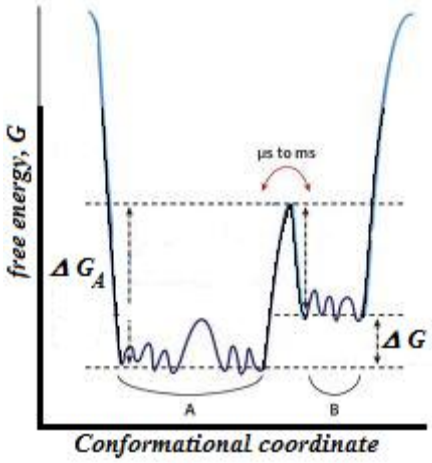
k- reaction constant; **R**- gas universally constant; **T** – Kelvin temperature; $\mathbf{G}_A$ and **G** are the free energies of the stable states *A* and the transition state (which is the “barrier”, .e., the free energy maximum on the pathway).



Overcoming of the free energy barrier on the pathway from the stable state A to the stable state B. On the figure shown activate energy.

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 (show figure) can be expressed through the rates of one-step transitions etween 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 changes the transition state stability value **ΔG** and the native protein stability **ΔG** 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 changes only the native protein stability **ΔG**, 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.
- If the residue's changes 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.

Molten Globule

The term molten globule (MG) was first coined by A. Wada and M Ohgushi in 1983. It was first found in cytochrome c, which conserves a native-like secondary structure content but without the tightly packed protein interior, under low pH and high salt concentration. For cytochrome c and

some other proteins, it has been shown that the molten globule state is a "thermodynamic state" clearly different both from the native and the denatured state, demonstrating for the first time the existence of a third equilibirum (i.e., intermediate) state.

The term "molten globule" is extended to include various types of partially folded protein states found in mildly denaturing conditions such as low pH (generally pH = 2), mild denaturant, or high temperature. Molten globules are collapsed and generally have some native-like secondary structure but a dynamic tertiary structure as seen by far and near circular dichroism (CD) spectroscopy, respectively. These traits are similar to those observed in the transient intermediate states found during the folding of certain proteins, especially globular proteins that undergo hydrophobic collapse, and therefore the term "molten globule" is also used to refer to certain protein folding intermediates corresponding to the narrowing region of the folding funnel higher in energy than the native state but lower than the denatured state. The molten globule ensembles sampled during protein folding and unfolding are thought to be roughly similar.

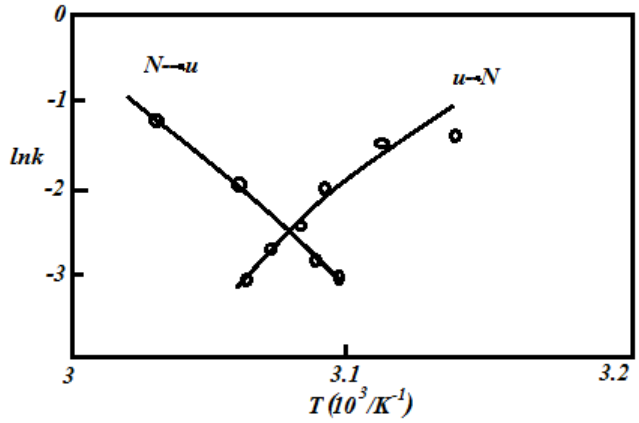
The MG structure is believed to lack the close packing of amino acid side chains that characterize the native state (N) of a protein. The transition from a denatured (U) state to a molten globule may be a two state process

$$U \leftrightarrow MG$$

Or it may be a continuous transition, with no cooperativity and no apparent "switch" from one form to the other. The folding of some proteins can be modeled as a three-state kinetic process:

$$U \leftrightarrow MG \leftrightarrow N$$

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 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" 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.