The Nature of Non-Arrhenius Kinetics in the Heat Denaturation of Proteins

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BCADD –2026 Symposium, October 20

Protein unfolding – unimolecular process

$$k = A \times e^{-\frac{E_a}{RT}}$$

Arrhenius Parameters in Denaturation of Proteins

E_a	Usually	$50 - 100$ kcal/mol,	Can be up to 200-300 kcal/mol
A	Usually	$> 10^{25}\ s^{-1}$	Can be up to 10^{218} s^{-1}

Nature of these *A*-values (Baklanov and Kiselev, BCADD-2025)

Are these *A*-values unphysical ? (Sarkar et al. *ACS Nano* **2019**, 13, 8669)

$$k = A \times e^{-\frac{E_a}{RT}} \xrightarrow[T\to\infty]{} A \text{ (can not be } \gtrsim 1 \cdot 10^{14}\ s^{-1}\text{)}$$

Non-Arrhenius kinetics of protein denaturation

Convex shape of Arrhenius plots

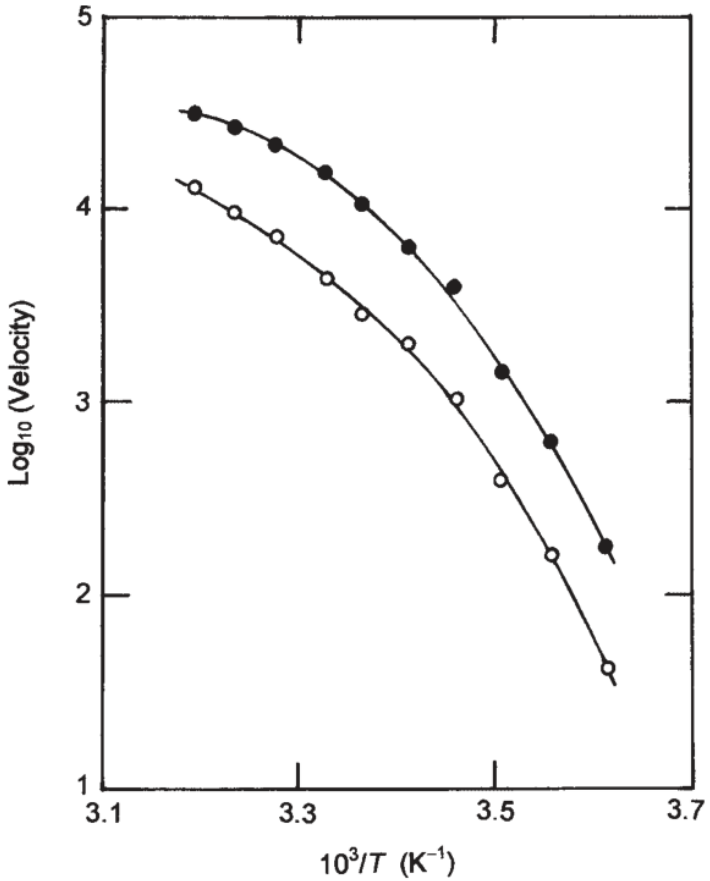


Figure 11. Curvilinear Arrhenius plots of the temperature dependence of reaction velocity for the low-temperature (○) and room-temperature (●) forms of phosphoribosyladenosine triphosphate synthetase. [Data taken from Bell *et al.* (1974).]

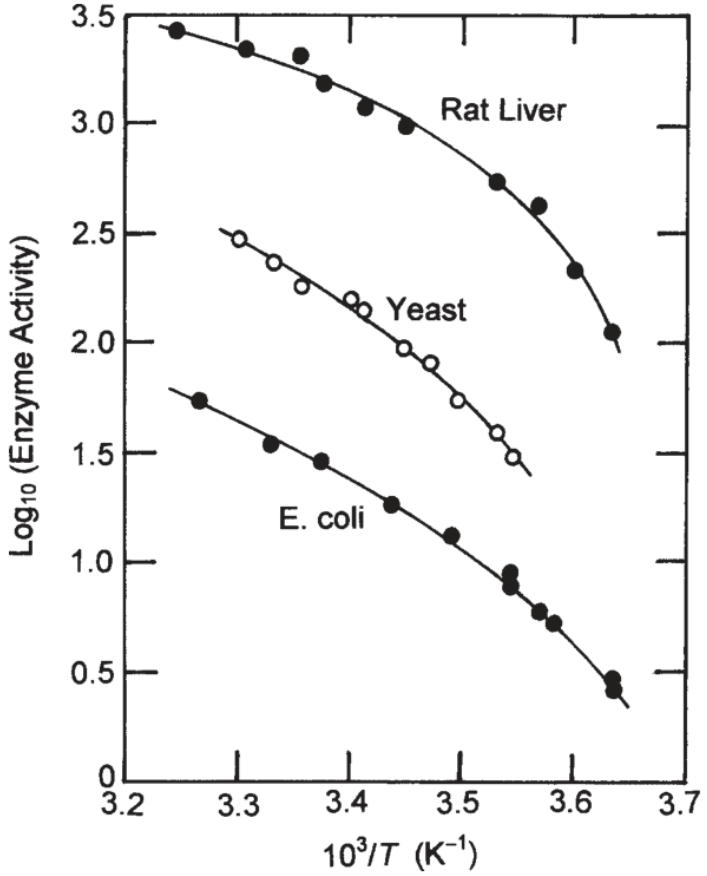
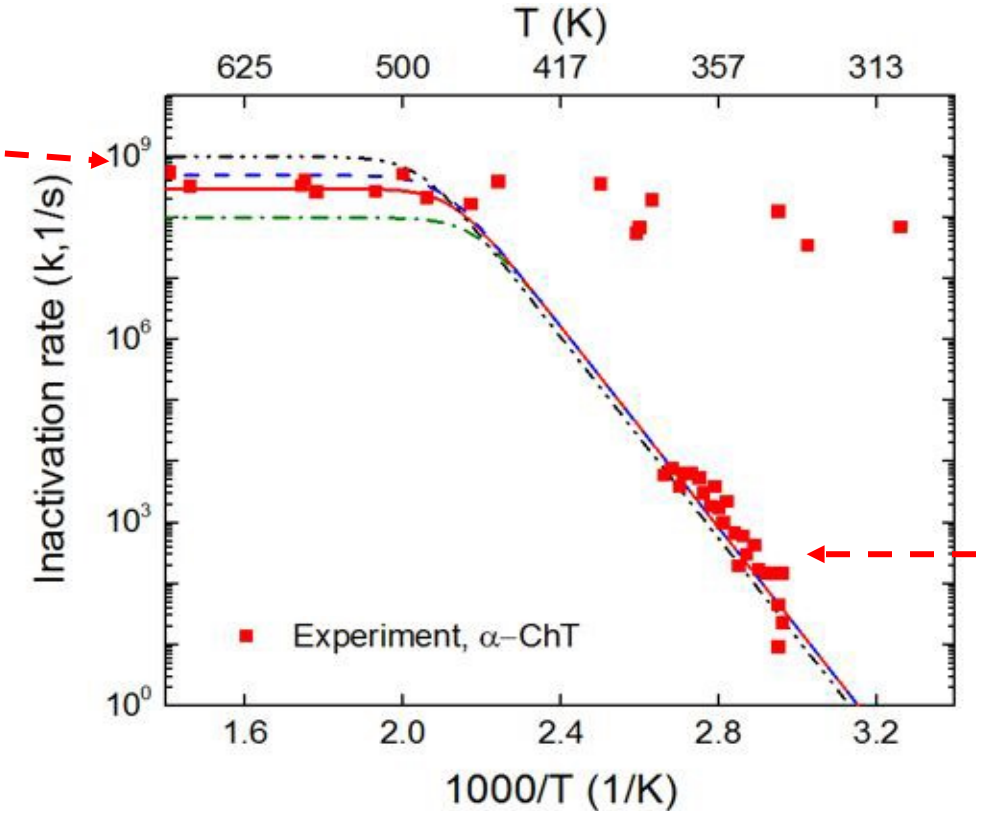


Figure 10. Arrhenius plots of succinate dehydrogenase activity in mitochondrial preparations isolated from rat liver (Raison and McMurchie, 1974), *Escherichia coli* (Esfahani *et al.*, 1971) and yeast (Watson *et al.*, 1975). [Data taken from the indicated sources.]

J. Mol. Recognit. 2006; **19**: 389–407

Non-Arrhenius kinetics of protein denaturation

Molecular Hyperthermia

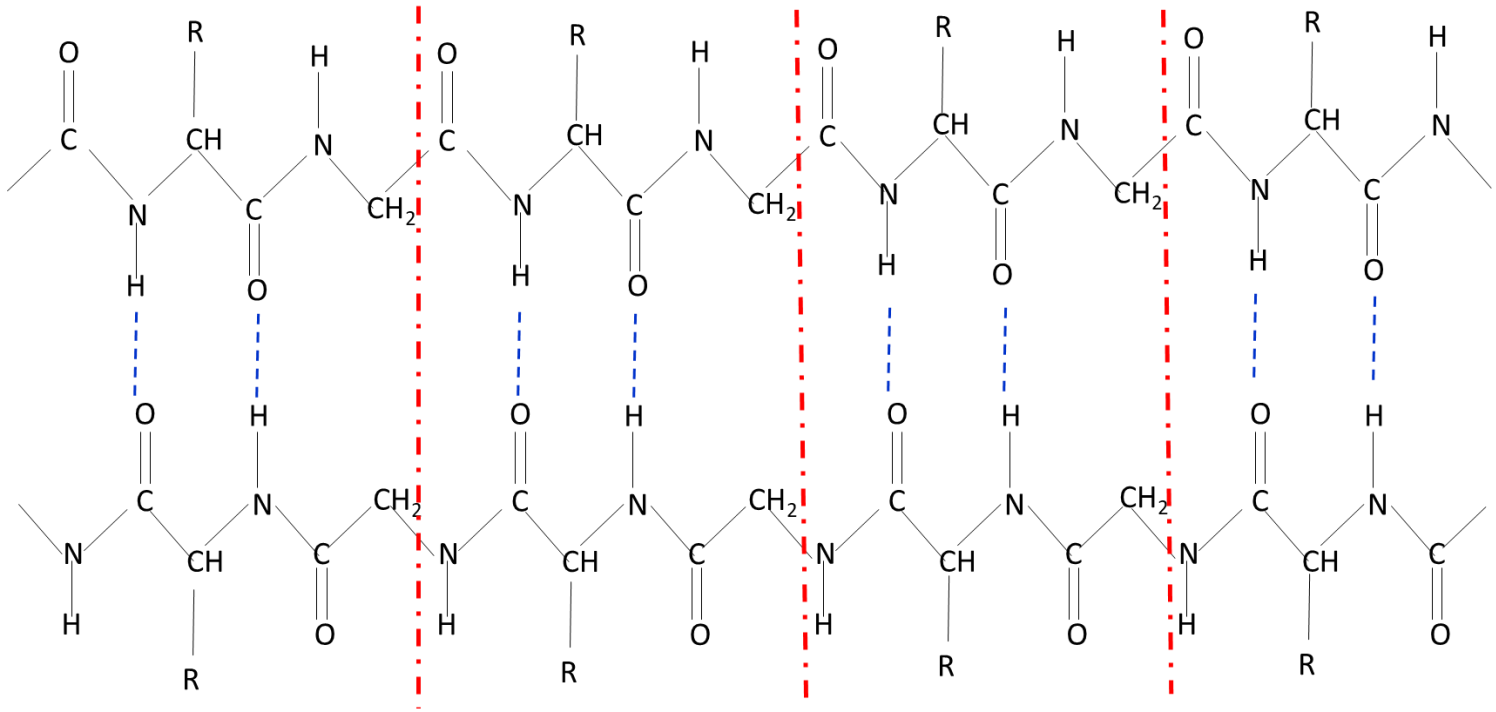


T-jump

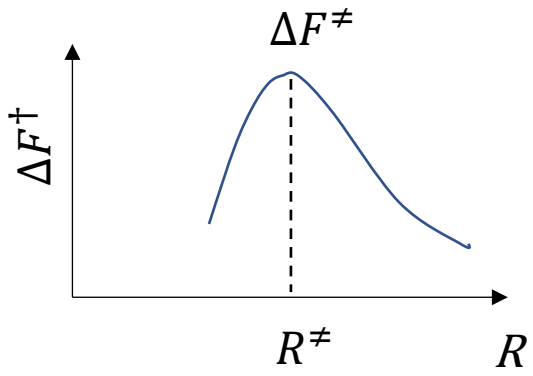
	Low T (300-370 K)	High T (450-600 K)
A, s^{-1}	10^{24}	$3 \cdot 10^9$
E, kcal/mol	43	2-3

(Sarkar et al. *ACS Nano* **2019**, 13, 8669)

Heat denaturation of protein (unfolding), cleavage of the interchain H-bonds network

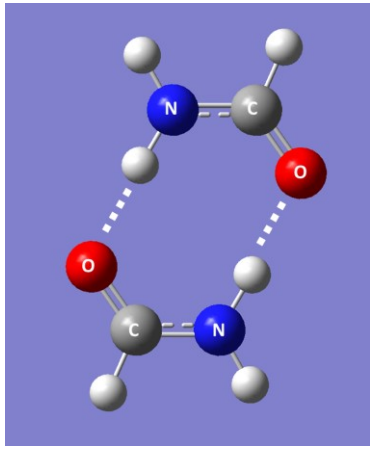


$$k(T) = \frac{k_B T}{h} \cdot e^{-\frac{\Delta F^{\neq}(T)}{RT}}$$

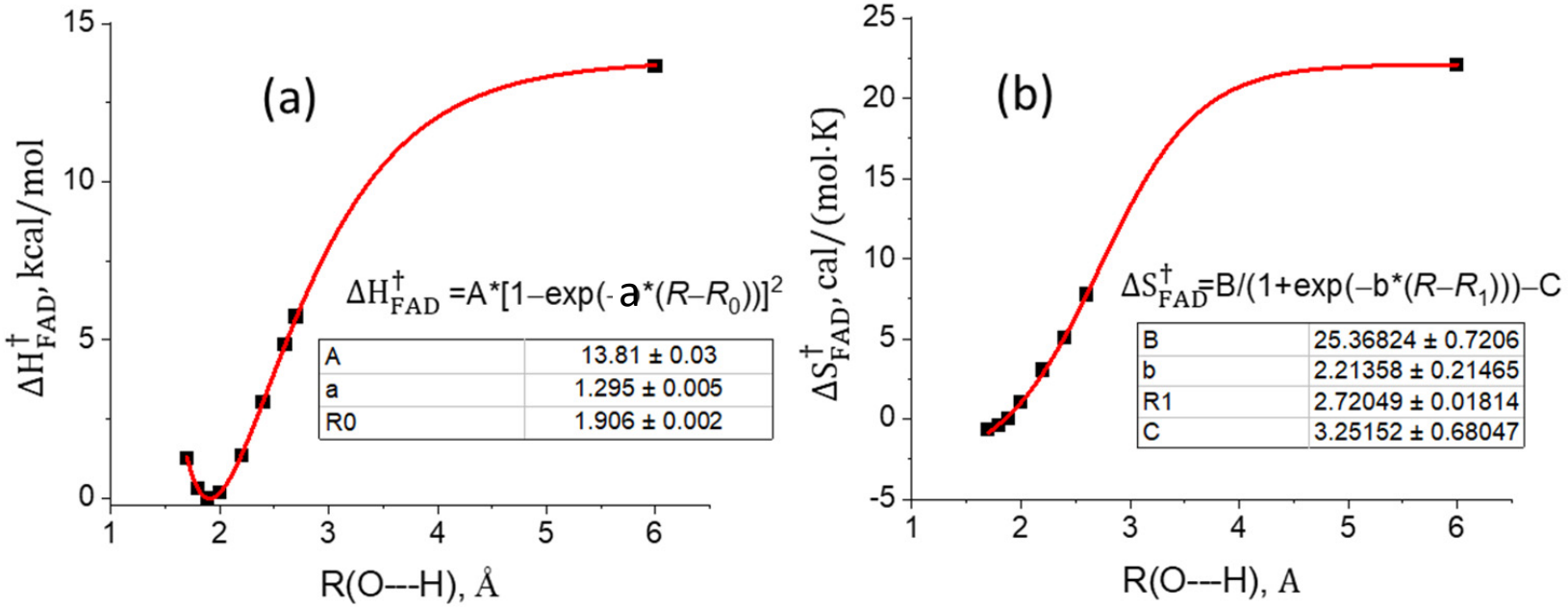


$$\Delta F^{\dagger}(R) = \Delta H^{\dagger}(R) - T \cdot \Delta S^{\dagger}(R)$$

Dimer of formamid as a model for $\Delta F^{\dagger}(R)$ calculations



1. MP2/6–31g(d,p) – structure, energy, vibrational wavenumbers as a function of *R*,
2. Rigid Rotor-Harmonic Oscillator - Enthalpy and entropy as a function of *R*

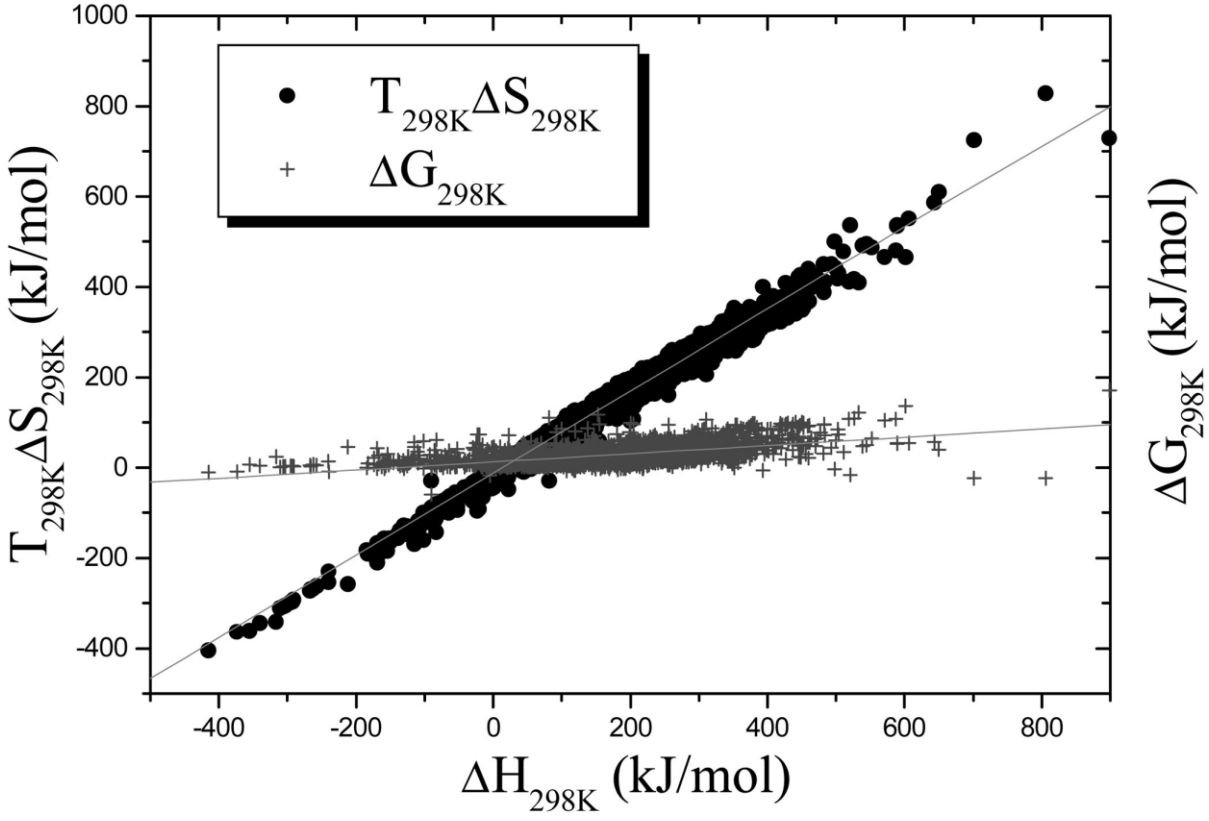


$O=(HCNH_2)_2$

$$\Delta F^{\dagger}(R) = \Delta H^{\dagger}(R) - T \cdot \Delta S^{\dagger}(R) =$$

$$\Delta H_{\infty} \cdot [1 - \exp(-a \cdot (R - R_0))]2 \qquad T \cdot \Delta S_{\infty} \cdot [1/(1 + \exp(-b(R - R_1))]$$

Enthalpy and entropy of proteins denaturation (L. Liu et al. *Biophysical Chemistry* ***84,*** 239 (2000))



$$\frac{300 \cdot \Delta S_{\infty}}{\Delta H_{\infty}} \approx 0.9$$

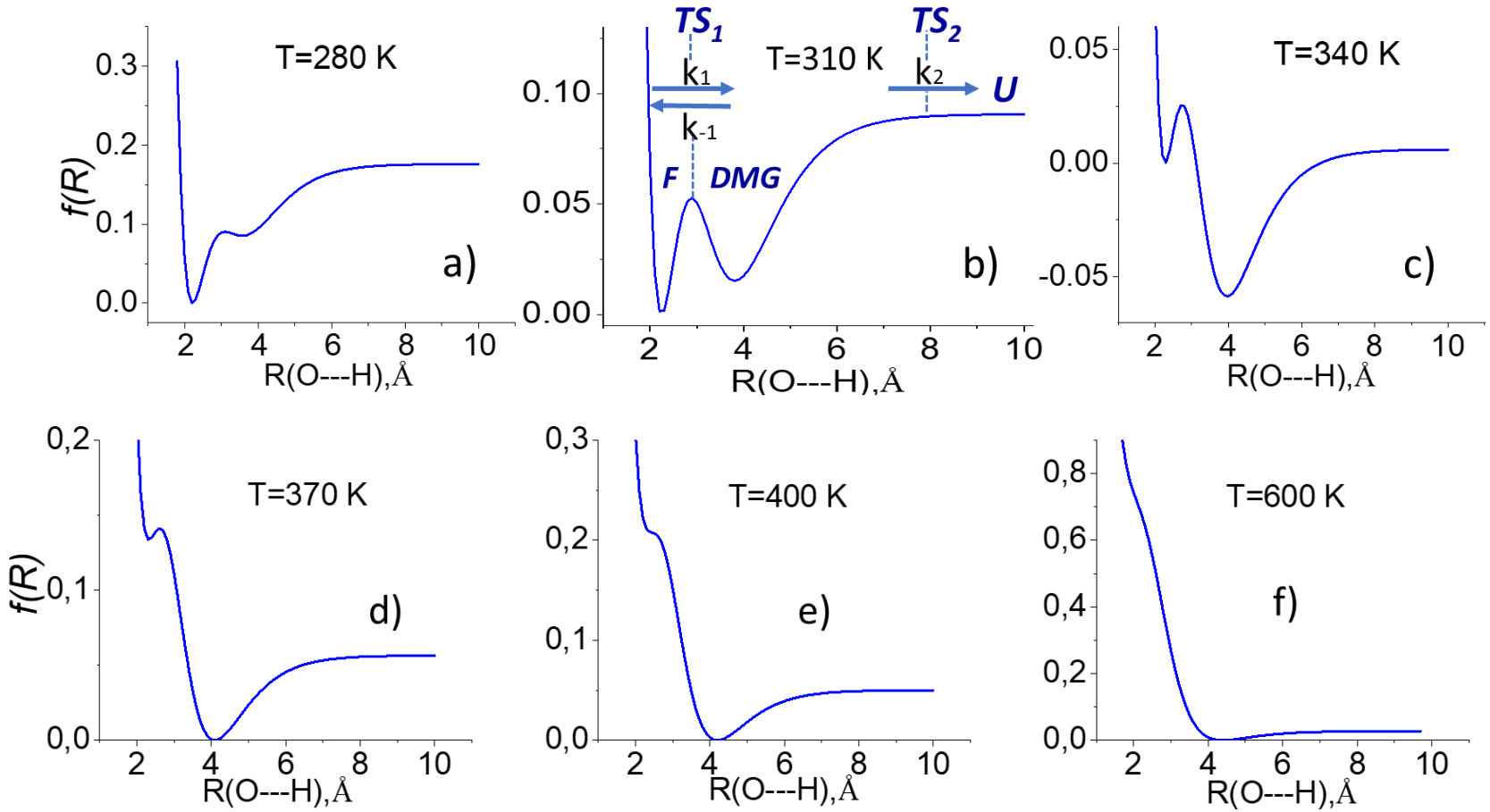
$$\Delta F^{\dagger}(R) = \Delta H_{\infty} \cdot [1 - \exp(-a \cdot (R - R_0))]2 - T \cdot \Delta S_{\infty} \cdot [1/(1 + \exp(-b(R - R_1)))] =$$

$$= \Delta H_{\infty} \cdot \left\{ [1 - \exp(-a \cdot (R - R_0))]2 - \frac{T \cdot \Delta S_{\infty}}{\Delta H_{\infty}} \cdot [1/(1 + \exp(-b(R - R_1)))] \right\}$$

$$\boldsymbol{\Delta F^{\dagger}(R) = \Delta H_{\infty} \cdot f(R)}$$

where $f(R) = [1 - \exp(-a \cdot (R - R_0))]2 - T/300 \cdot 0.9 \cdot 1/(1 + \exp(-b(R - R_1)))$

Free-energy profiles along unfolding reaction coordinate *f*(*R*)

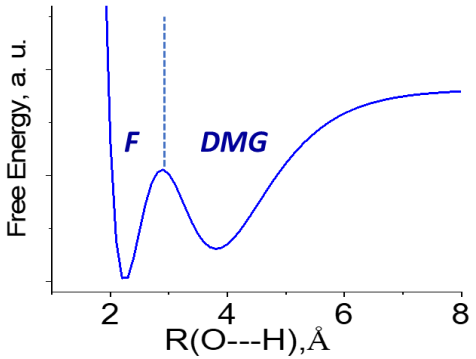


F-folded structure;

DMG-dry molten globule; expanded secondary structure, stabilized by entropy rise due to loosening of the H-bonds network

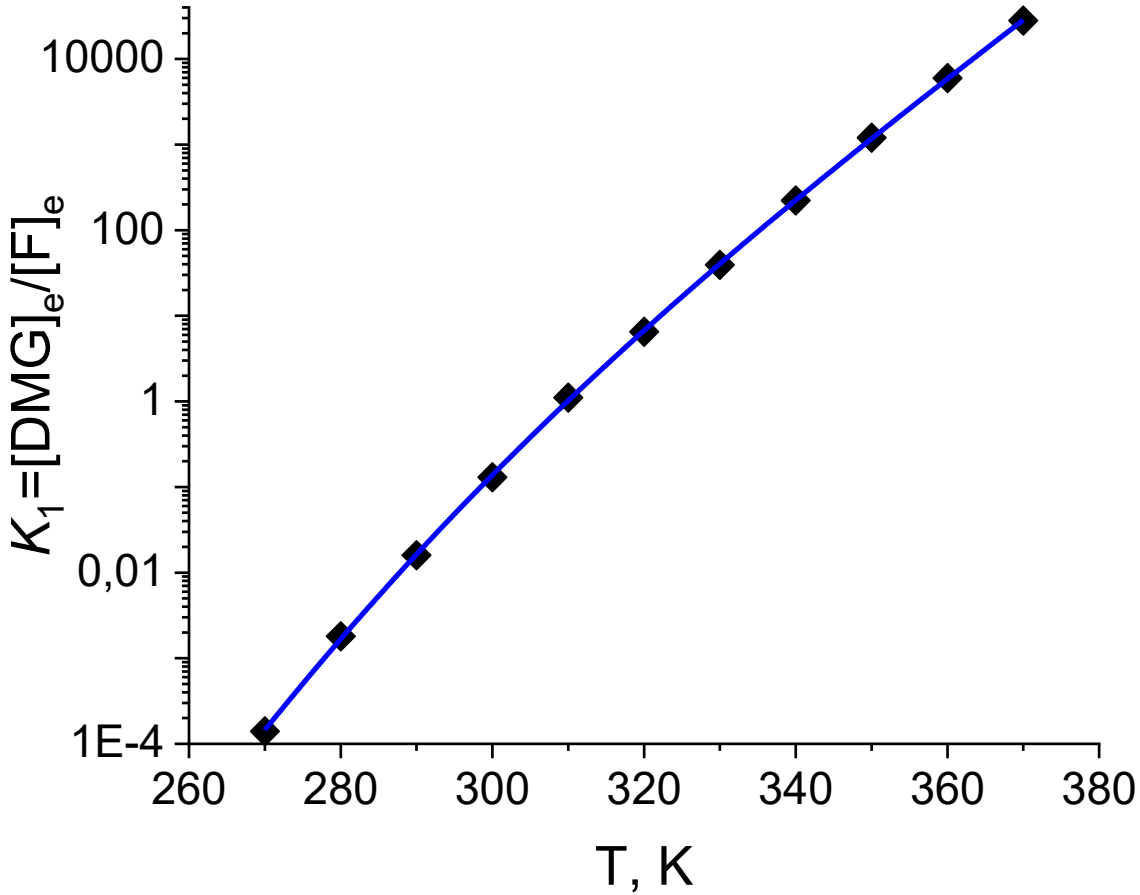
TS – transition state

Equilibrium constant



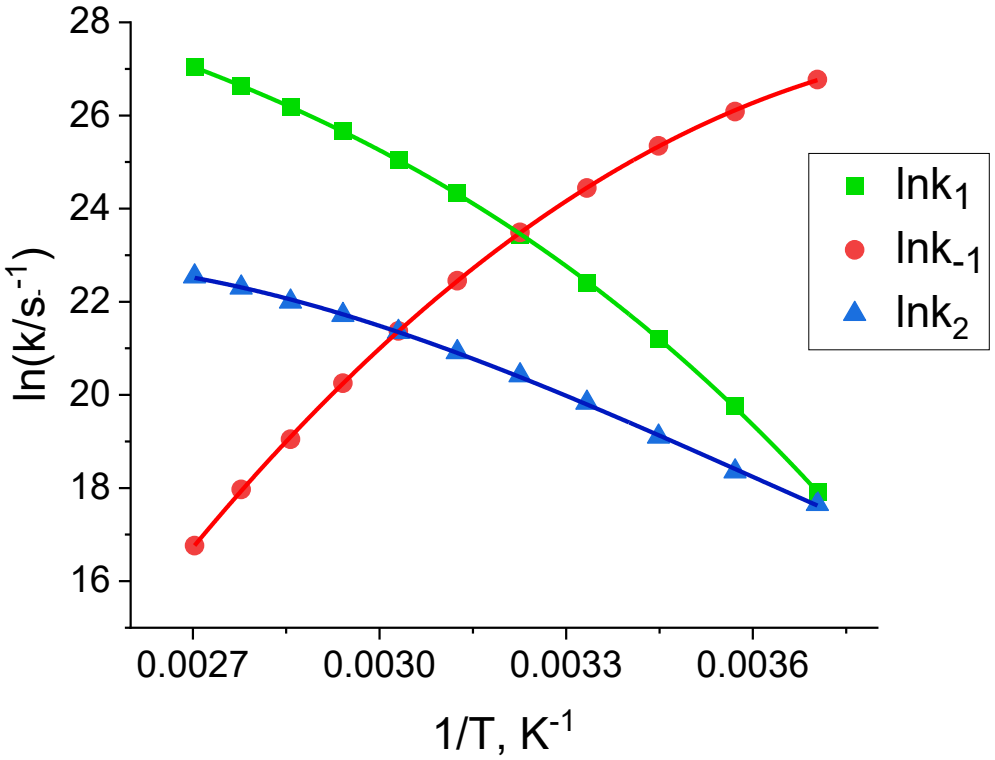
$$\textit{Folded state} \underset{}{\overset{K_1}{\rightleftharpoons}} \textit{Dry Molten Globule}$$

$$\Delta H_\infty = 50\ kcal/mol$$



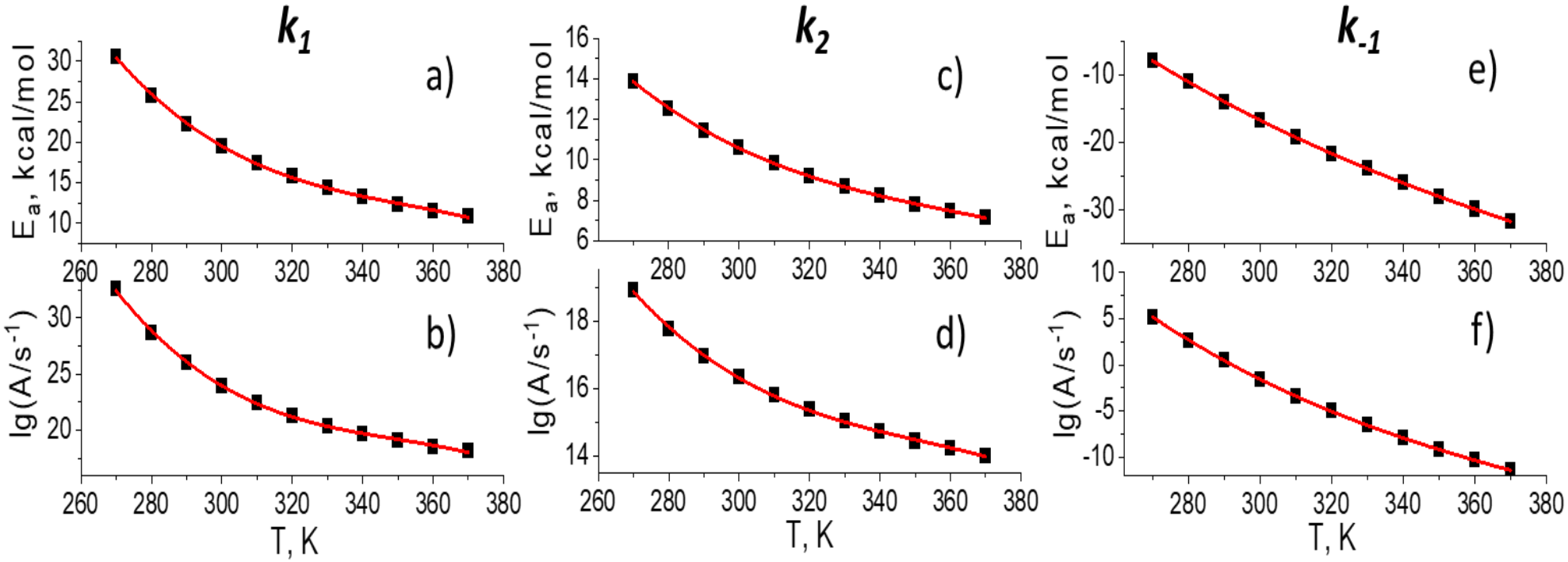
Rate constants of the elementary steps of the protein unfolding $k_i(T)$

$$F \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} DMG \xrightarrow{k_2} U$$

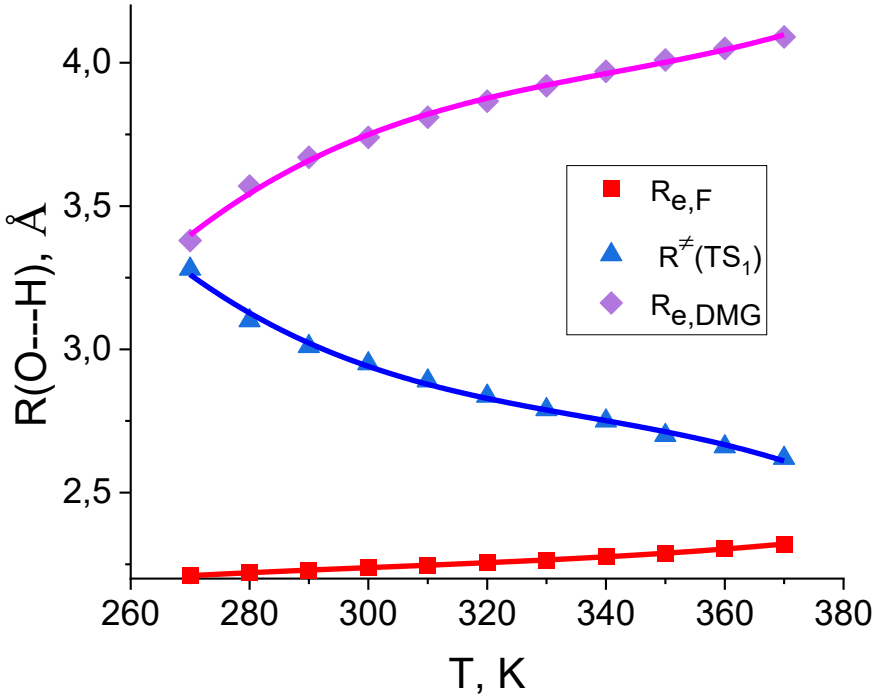


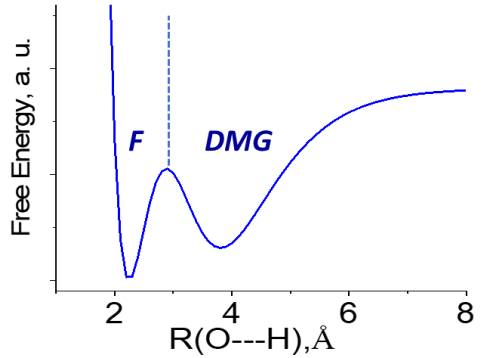
Arrhenius parameters of the elementary rate constants

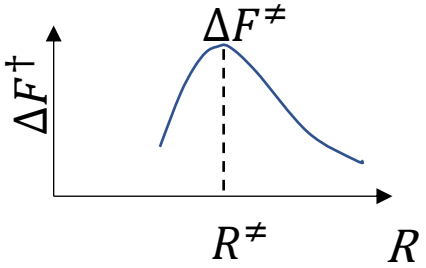
$$F \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} DMG \xrightarrow{k_2} U$$



Shift of the location of *F, DMG and TS_1* states *along reaction coordinate*







$$\Delta F^{\dagger}(R) = \Delta H^{\dagger}(R) - T \cdot \Delta S^{\dagger}(R)$$

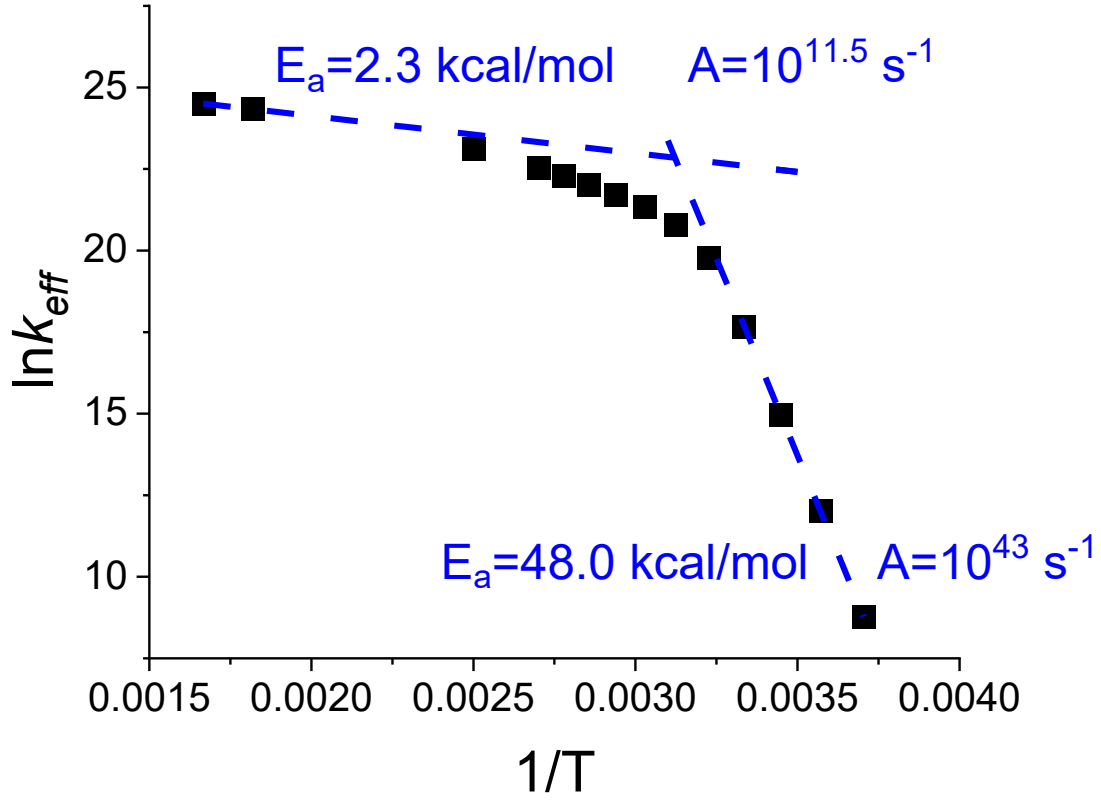
Arrhenius plot for calculated rate constant of protein unfolding

$$F \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} DMG \xrightarrow{k_2} U$$

$$F \xrightarrow{k_{eff}}$$

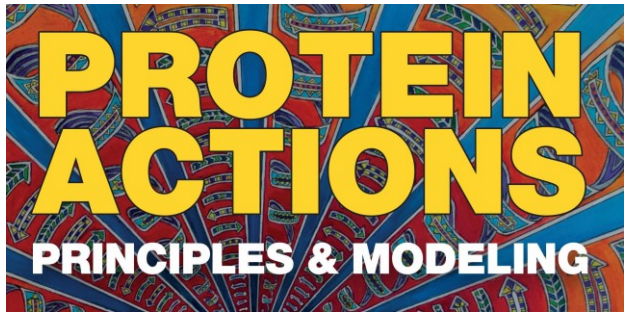
$$k_{eff} = \frac{K_1}{1+K_1} \cdot k_2$$

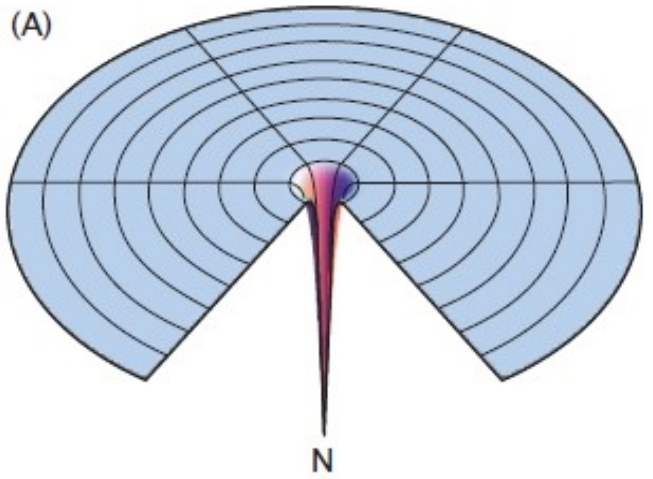
$$K_1 = \frac{k_1}{k_{-1}}$$

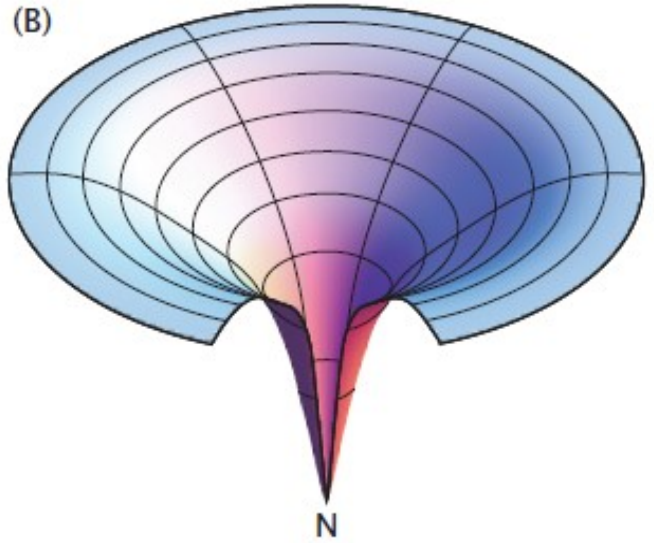


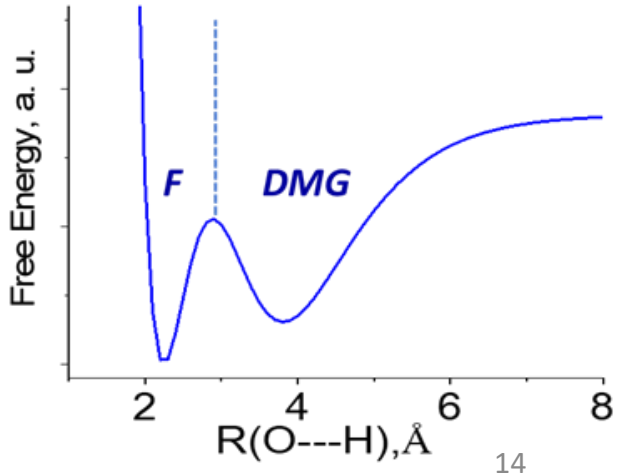
Levinthal paradox. DMG contribution to a “funnel directing a folding process”.

I. Bahar, R.L. Jernigan, K. Dill, 2017.









Thank you very much for your attention