Protein Unfolding Data Analysis

Spectroscopy

Adrian Velazquez-Campoy

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101004806

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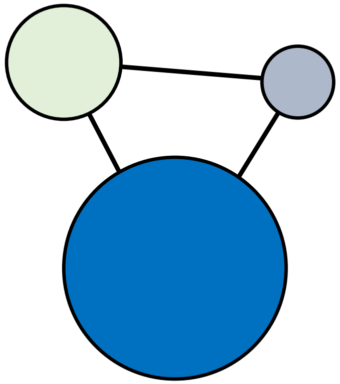
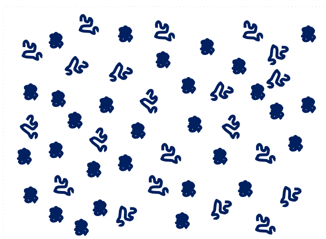
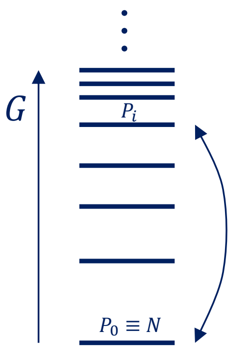
Stability

ΔG Gibbs energy of stabilization or unfolding

Difference in Gibbs energy between the native state and the unfolded state







$$\chi_i \propto e^{-(\Delta G_i/RT)}$$

$$\Delta G_i = G_i - G_0 = -RT \ln \frac{[P_i]}{[P_0]} = -RT \ln \beta_i$$

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$$\Delta G = \Delta G(T, P, pH, \mu, [D], [L])$$

$$\Delta G([D]) = \Delta G_0 - m[D]$$

$$\Delta G(T) = \Delta H(T_m)\left(1 - \frac{T}{T_m}\right) + \Delta C_P\left(T - T_m - T\ln\left(\frac{T}{T_m}\right)\right)$$

- **Goal:** **Increase environmental stress and displace the equilibrium in order to make populations of comparable size, exploring different population ratios between N and U**
  Calculate/estimate K

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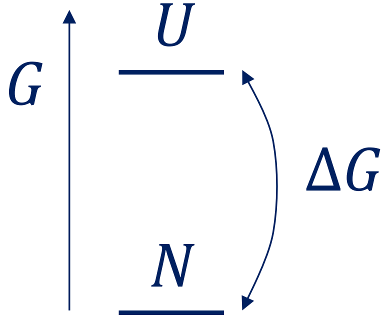

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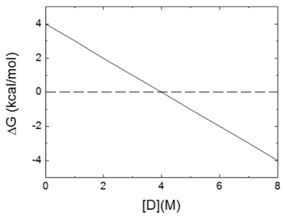
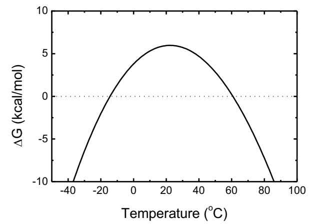


$$\Delta G(T) = \Delta H(T_m)\left(1 - \frac{T}{T_m}\right) + \Delta C_P\left(T - T_m - T\ln\left(\frac{T}{T_m}\right)\right)$$



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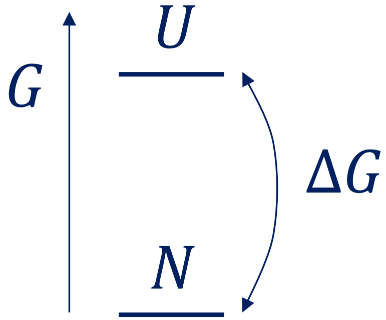

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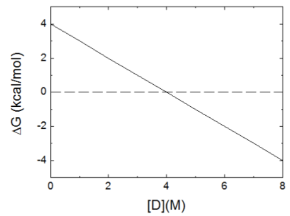
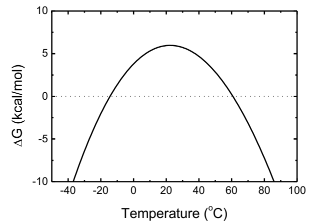


$$\Delta G(T) = \Delta H(T_m)\left(1 - \frac{T}{T_m}\right) + \Delta C_P\left(T - T_m - T\ln\left(\frac{T}{T_m}\right)\right)$$



ΔG_0	**Stability energy**
m	**Susceptibility against denaturant concentration**
$\Delta H(T_m)$	**Unfolding enthalpy**
T_m	**Unfolding temperature**
ΔC_P	**Unfolding heat capacity**

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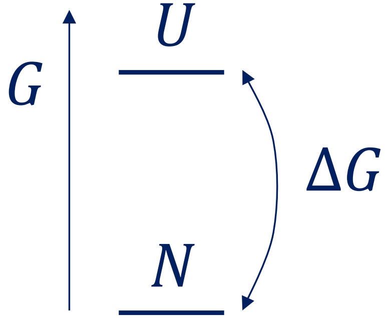

$$\Delta G = \Delta G(T, P, pH, \mu, [D], [L])$$

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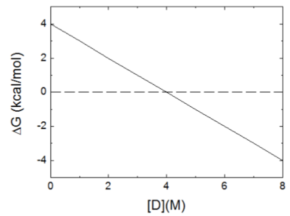
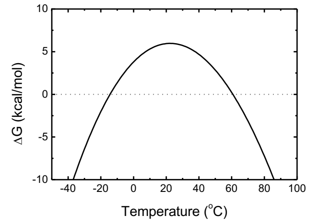


$$\Delta G(T) = \Delta H(T_m)\left(1 - \frac{T}{T_m}\right) + \Delta C_P\left(T - T_m - T\ln\left(\frac{T}{T_m}\right)\right)$$



Parameters define the stability profile or diagram: ΔG([D]) y ΔG(T)
Parameters can be determined experimentally

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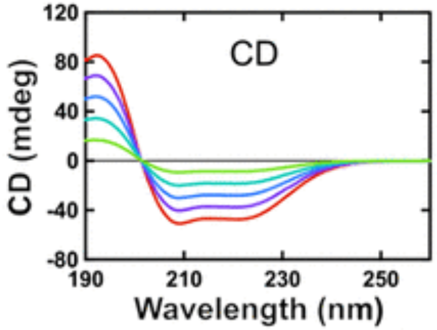
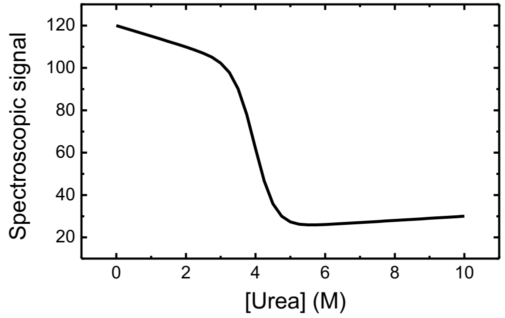
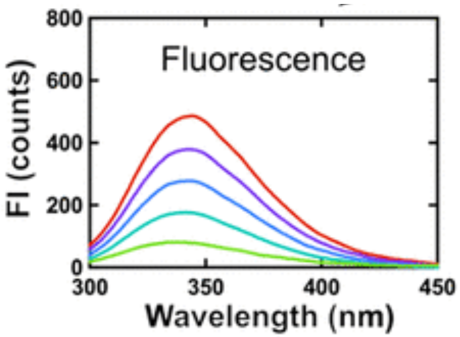
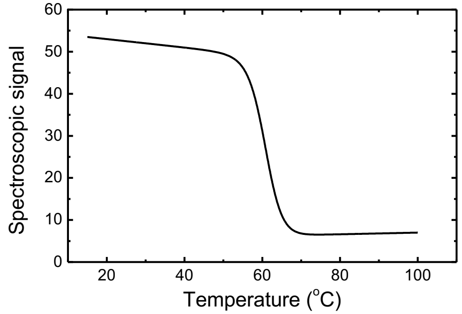
Unfolding experiment









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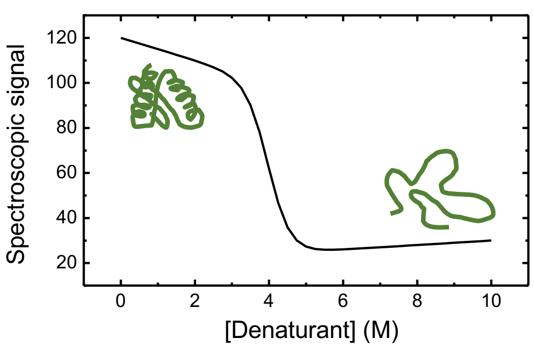

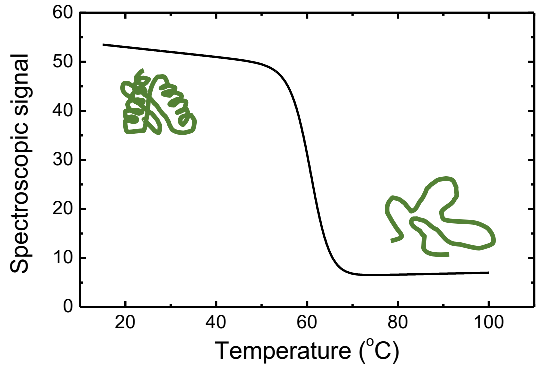

Cooperative unfolding → Reduction in the number of accessible states

Partially folded states are not significantly populated

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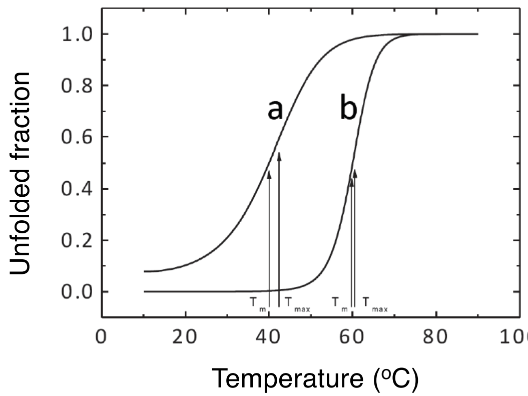
▶ Data analysis?

- Unless you are doing a screening evaluation, never use sigmoidal fittings (e.g., Boltzmann function)
- Never apply "naïve" normalization
- The inflection point in chemical denaturation provides $[D]_m$
- The inflection point in thermal denaturation DOES NOT provide T_m ($T_m \lesssim T_{max}$)



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▶ Standard approach based on the partition function *Q*

$$P_0 \leftrightarrow P_1 \leftrightarrow P_2 \leftrightarrow \cdots \leftrightarrow P_i \leftrightarrow \cdots \leftrightarrow P_n$$

$$[P_i] = \beta_i [P_0]$$

$$[P_i] = \left(\prod_{r=1}^{i} K_r \right) [P_0]$$

$$Q = \sum_{i=0}^{n} \frac{[P_i]}{[P_0]} = \sum_{i=0}^{n} \beta_i = \sum_{i=0}^{n} e^{-(\Delta G_i/RT)}$$

Wyman & Gill. "Binding and Linkage", University Science Books, 1990

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▶ Standard approach based on partition function *Q*

$$Q = \sum_{i=0}^{n} \frac{[P_i]}{[P_0]} = \sum_{i=0}^{n} \beta_i$$

$$\chi_i = \frac{[P_i]}{[P]_T} = \frac{\beta_i}{Q}$$

$$\beta_i = exp(-\Delta G_i/RT)$$

$$\Delta G_i = \Delta G_i(T, pH, \mu, [D], [L], a_w, \dots)$$

$$\Delta G_i([D]) = \Delta G_{0,i} - m_i[D]$$

$$\Delta G_i(T) = \Delta H_i(T_{m,i})\left(1 - \frac{T}{T_{m,i}}\right) + \Delta C_{P,i}\left(T - T_{m,i} - T\ln\frac{T}{T_{m,i}}\right)$$

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▶ Standard approach based on the partition function *Q*

$$Q = \sum_{i=0}^{n} \frac{[P_i]}{[P_0]} = \sum_{i=0}^{n} \beta_i$$

$$\chi_i = \frac{[P_i]}{[P]_T} = \frac{\beta_i}{Q}$$

$$\beta_i = exp(-\Delta G_i/RT)$$

$$\langle S \rangle = \sum_{i=0}^{n} \chi_i S_i = \frac{\sum_{i=0}^{n} \beta_i S_i}{\sum_{i=0}^{n} \beta_i}$$

Average of any system property (measurement)

$$NLSF \rightarrow \{T_{mi}, \Delta H_i, \Delta C_{Pi}; i = 1, \ldots, n\}$$

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$$N \leftrightarrow U$$

i	G	ΔG	$\exp(-\Delta G/RT)$
	G_N	0	1
	G_U	ΔG	K

$$Q = 1 + \beta = 1 + K$$

$$\chi_N = \frac{1}{1+K}$$

$$\chi_U = \frac{K}{1+K}$$

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Chemical Denaturation

$$\Delta G([D]) = \Delta G_0 - m[D]$$

$$\Delta G_0 = \Delta G([D])|\Big|_{[D]=0} \qquad m = -\left(\frac{\partial \Delta G([D])}{\partial [D]}\right)_{T,P}$$

$$\Delta G([D]) = -RT \ln K([D])$$

$$\Delta G([D]_m) = 0 \longrightarrow [D]_m = \frac{\Delta G_0}{m}$$

$$\Delta G([D]_m) = 0 \longrightarrow \begin{cases} K = 1 \\ \chi_N = \chi_U = 0.5 \end{cases}$$

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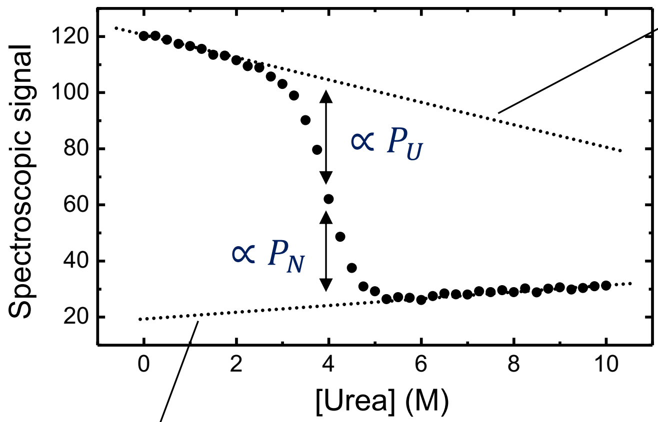


$$S_N = a_N + b_N[D]$$

$$S_U = a_U + b_U[D]$$

$$S = \chi_N S_N + \chi_U S_U$$

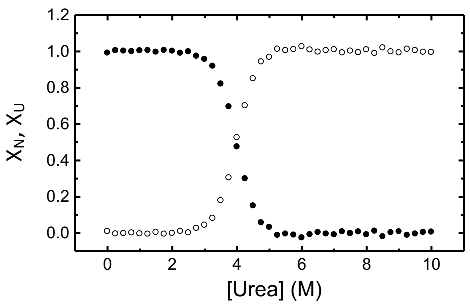
$$\left.\begin{aligned} \chi_N &= \frac{S - S_U}{S_N - S_U} \\ \chi_U &= \frac{S_N - S}{S_N - S_U} \end{aligned}\right\}$$

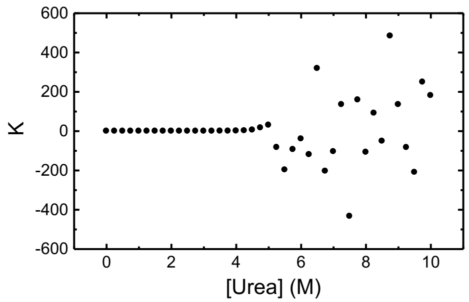
$$K = \frac{\chi_U}{\chi_N}$$

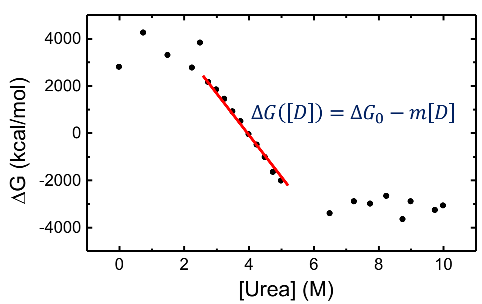
$$\Delta G = -RT \ln K$$

ΔG_0 7600 ± 200 cal mol^{-1}

m 1920 ± 40 cal mol^{-1} M^{-1}







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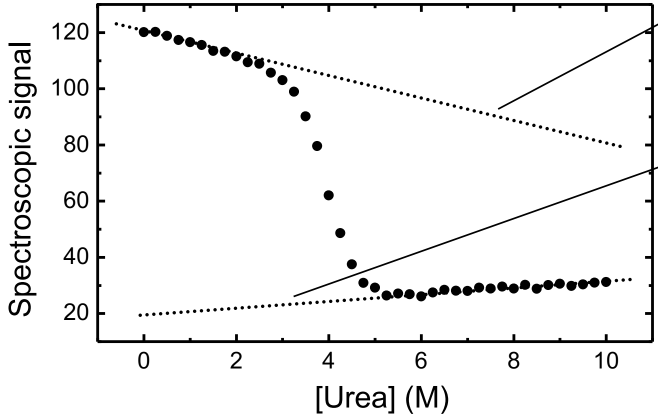
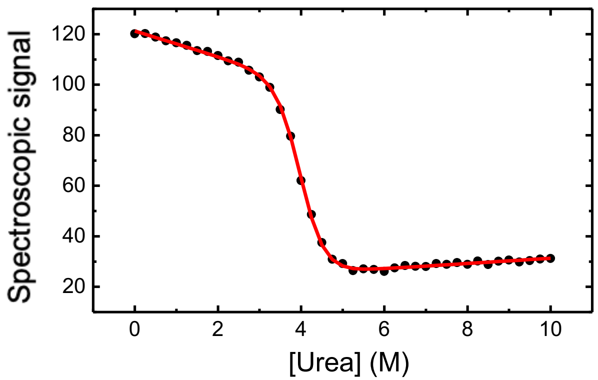


$$S_N = a_N + b_N[D]$$

$$S_U = a_U + b_U[D]$$

$$S = \chi_N S_N + \chi_U S_U$$

$$S = \frac{(a_N + b_N[D]) + e^{-(\Delta G_0 - m[D])/RT}(a_U + b_U[D])}{1 + e^{-(\Delta G_0 - m[D])/RT}}$$



ΔG_0 8200 ± 50 cal mol^{-1}
m 2050 ± 10 cal mol^{-1} M^{-1}

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Thermal Denaturation

$$\Delta G(T) = \Delta H(T_m)\left(1 - \frac{T}{T_m}\right) + \Delta C_P\left(T - T_m - T\ln\left(\frac{T}{T_m}\right)\right)$$

$$\Delta H(T_m) = \Delta H(T)|\Big|_{T=T_m}$$

$$\Delta C_P = \left(\frac{\partial \Delta H(T)}{\partial T}\right)_P = T\left(\frac{\partial \Delta S(T)}{\partial T}\right)_P$$

$$\Delta G(T) = -RT\ln K(T)$$

$$\Delta G(T_m) = 0 \longrightarrow \begin{cases} K = 1 \\ \chi_N = \chi_U = 0.5 \\ \Delta S(T_m) = \dfrac{\Delta H(T_m)}{T_m} \end{cases}$$

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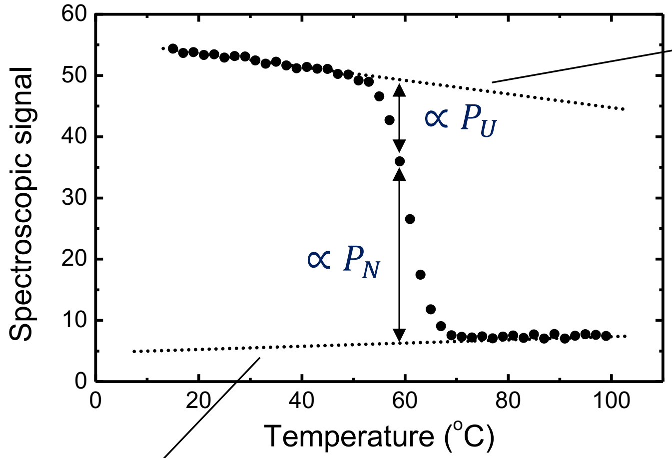


$$S_N = a_N + b_N T$$

$$S = \chi_N S_N + \chi_U S_U$$

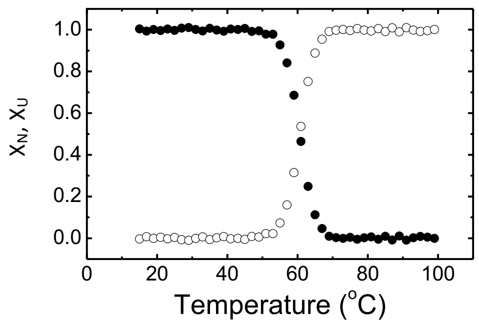
$$S_U = a_U + b_U T$$

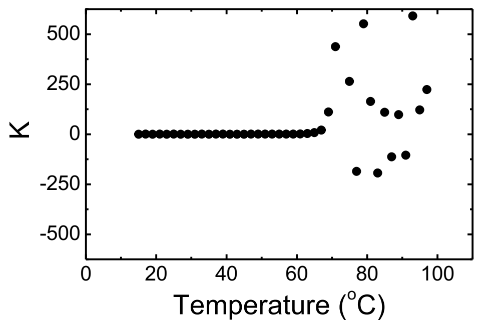
$$\left.\begin{aligned}\chi_N &= \frac{S - S_U}{S_N - S_U}\\ \chi_U &= \frac{S_N - S}{S_N - S_U}\end{aligned}\right\}$$

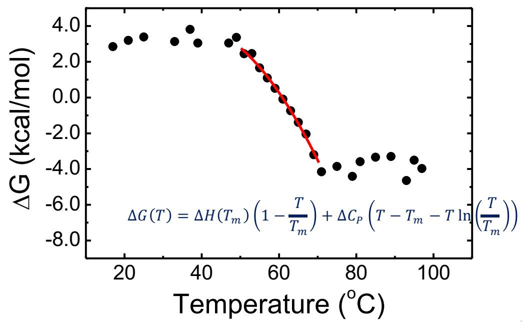
$$K = \frac{\chi_U}{\chi_N}$$

$$\Delta G = -RT \ln K$$

$\Delta H(T_m)$	109 ± 2 kcal mol^{-1}
T_m	60.9 ± 0.2 °C
ΔC_p	4 ± 1 kcal K^{-1} mol^{-1}







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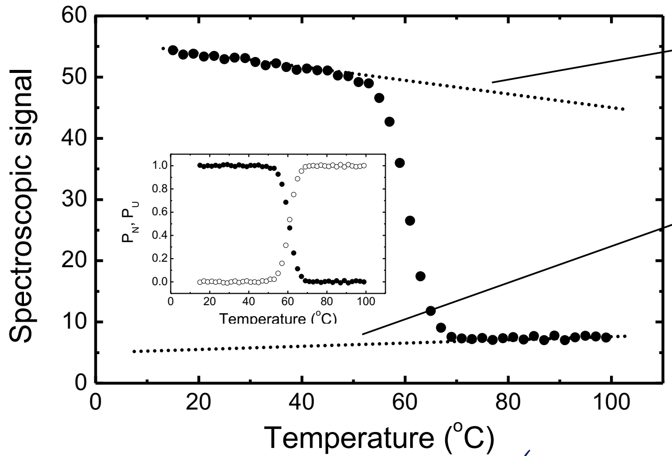


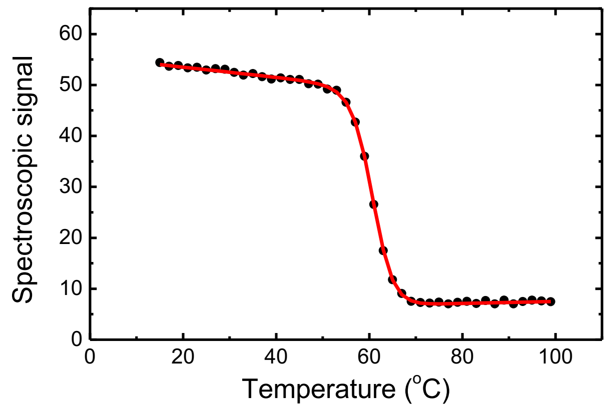
$$S_N = a_N + b_N T$$

$$S_U = a_U + b_U T$$

$$S = \chi_N S_N + \chi_U S_U$$

$$S = \frac{(a_N + b_N T) + e^{-\left(\Delta H(T_m)\left(1-\frac{T}{T_m}\right)+\Delta C_P\left(T-T_m-T\ln\left(\frac{T}{T_m}\right)\right)\right)/RT}(a_U + b_U T)}{1 + e^{-\left(\Delta H(T_m)\left(1-\frac{T}{T_m}\right)+\Delta C_P\left(T-T_m-T\ln\left(\frac{T}{T_m}\right)\right)\right)/RT}}$$

$\Delta H(T_m)$	101 ± 2 kcal mol^{-1}
T_m	60.69 ± 0.04 °C
ΔC_P	2.2 ± 0.7 kcal K^{-1} mol^{-1}



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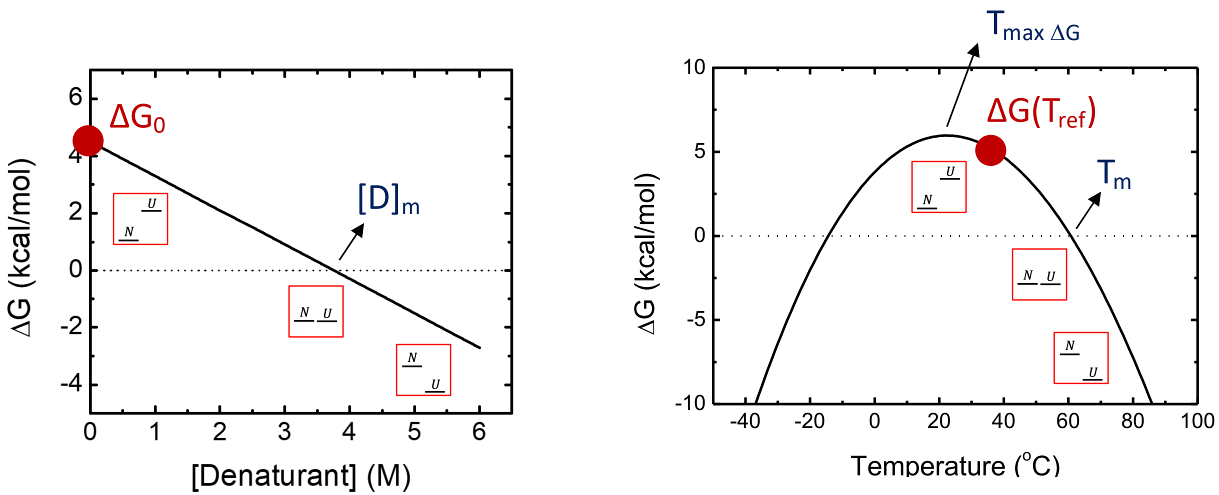
▶ Stability profile



$$\Delta G([D]) = \Delta G_0 - m[D]$$

$$\Delta G(T) = \Delta H(T_m)\left(1 - \frac{T}{T_m}\right) + \Delta C_P\left(T - T_m - T\ln\frac{T}{T_m}\right)$$

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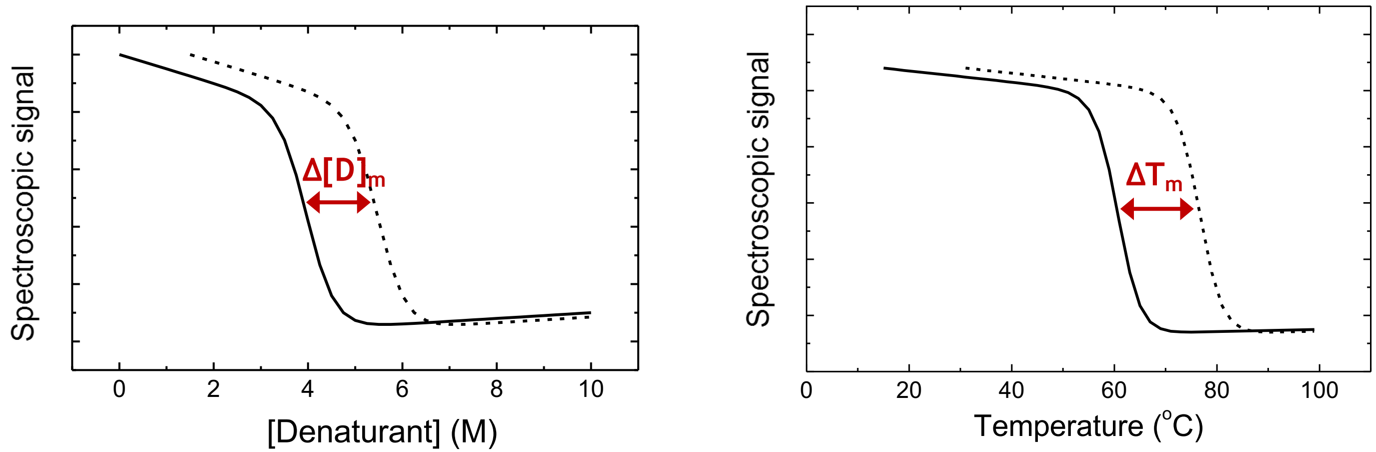
▶ Stability differences?



Changes in T_m and $[D]_m$ are easily to observe quantify, but they are NOT reliable indexes for stability or stability changes

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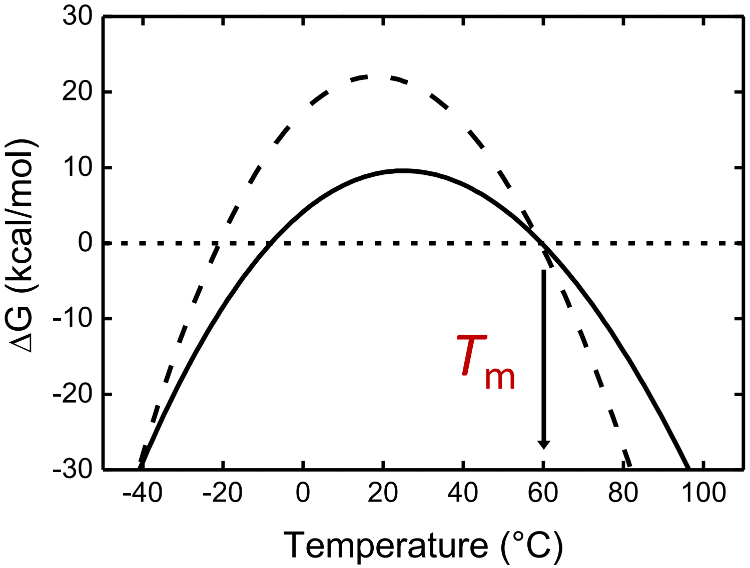
High and low temperature stability



$\Delta H(T_m)$	180 kcal mol^{-1}
$\Delta S(T_m)$	0.54 kcal K^{-1} mol^{-1}
ΔC_P	5.0 kcal K^{-1} mol^{-1}
$\Delta H(T_m)$	350 kcal mol^{-1}
$DS(T_m)$	1.05 kcal K^{-1} mol^{-1}
ΔC_p	8.0 kcal K^{-1} mol^{-1}

Which protein is more stable?

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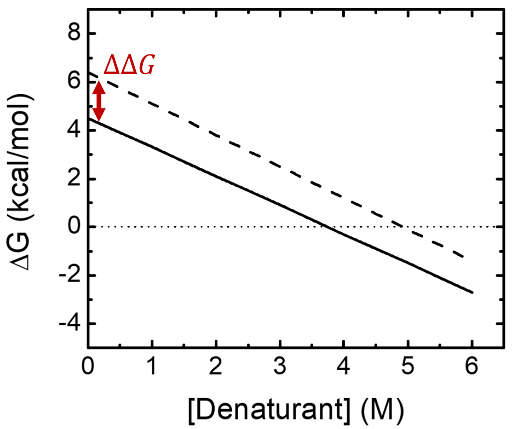
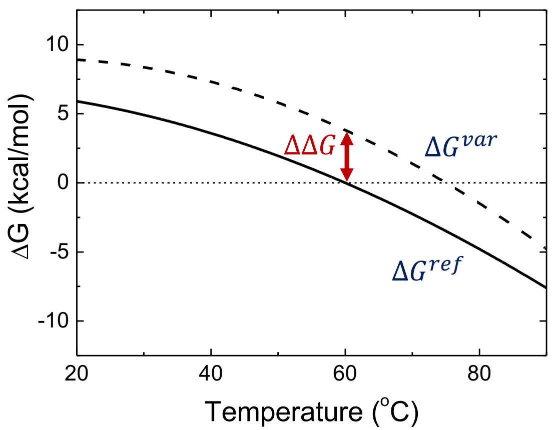
▶ Stability differences?





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Two-transition unfolding

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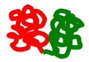
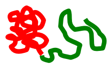
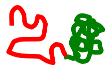
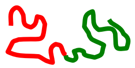
$$N \leftrightarrow I_1 \leftrightarrow I_2 \leftrightarrow U$$

i	ΔG_i	$\exp(-\Delta G_i/RT)$
	0	1
	ΔG_1	K_1
	ΔG_2	K_2
	$\Delta G_1+\Delta G_2$	$K_1 K_2$

$$Q = 1 + \beta_1 + \beta_2 + \beta_3 = 1 + K_1 + K_2 + K_1 K_2$$

$$\chi_N = \frac{1}{1 + K_1 + K_2 + K_1 K_2} \qquad \chi_{I_2} = \frac{K_2}{1 + K_1 + K_2 + K_1 K_2}$$

$$\chi_{I_1} = \frac{K_1}{1 + K_1 + K_2 + K_1 K_2} \qquad \chi_U = \frac{K_1 K_2}{1 + K_1 + K_2 + K_1 K_2}$$

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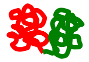
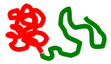
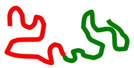
$$N \leftrightarrow I \leftrightarrow U$$

i	ΔG_i	$\exp(-\Delta G_i/RT)$
	0	1
	ΔG_1	K_1
	$\Delta G_1 + \Delta G_2$	$K_1 K_2$

$$Q = 1 + \beta_1 + \beta_2 = 1 + K_1 + K_1 K_2$$

$$\chi_N = \frac{1}{1 + K_1 + K_1 K_2} \qquad \chi_{I_2} \approx 0$$

$$\chi_{I_1} = \frac{K_1}{1 + K_1 + K_1 K_2} \qquad \chi_U = \frac{K_1 K_2}{1 + K_1 + K_1 K_2}$$

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▶ Selecting an appropriate unfolding model

- Understanding "names" of models, as well as physical idealization and mathematical formalism
- Make sense of estimated parameter values
- Make sense of parameters uncertainties

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▶ Best model for analysis?

- Employ structural/functional data, if available
- Revise carefully experimental data
- Identify unusual features
- Start with simplest compatible model (Ockham's razor)
- Increase complexity until unnecessary (no statistical improvement or no convergence)
- Understand assumptions/constraints of model
- Judge critically the estimated parameters

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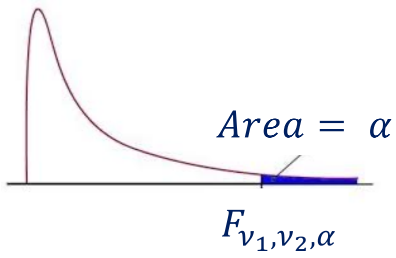
▶ When to stop increasing model complexity?

- **Select the (statistically) best model**

$$\chi^2 \equiv RSS = \sum_{i=1}^{n} \left(y(x_i) - f(\theta; x_i) \right)^2$$



Model 1: $n - p_1, \chi_1^2$

$(p_2 > p_1)$ **Model 2:** $n - p_2, \chi_2^2$

$$F = \frac{(\chi_1^2 - \chi_2^2)/(p_2 - p_1)}{\chi_2^2/(n - p_2)} \sim F_{p_2-p_1, p_2-p_1}$$

$F \leq F_{p_2-p_1, p_2-p_1, \alpha}$ **select Model 1 (H_0)**

$F > F_{p_2-p_1, p_2-p_1, \alpha}$ **select Model 2 (H_1)**

$$AIC = n \ln \left(\frac{\chi^2}{n - p} \right) + 2p$$

$$BIC = n \ln \left(\frac{\chi^2}{n - p} \right) + p \ln n$$

Select model with minimum AIC or BIC

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Global fitting

- **Strengthen model selection**
- **Strengthen parameter estimation**
- **Reduce uncertainties of estimated parameters**
- **Overcome experimental limitations**

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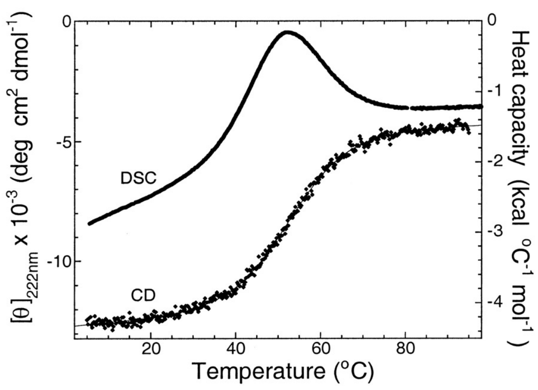
▶ Global fitting?

- Replicate experiments
- Concentration ranges
- Together with other biophysical techniques

...



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