Protein Unfolding Data Analysis

DSC & DSF

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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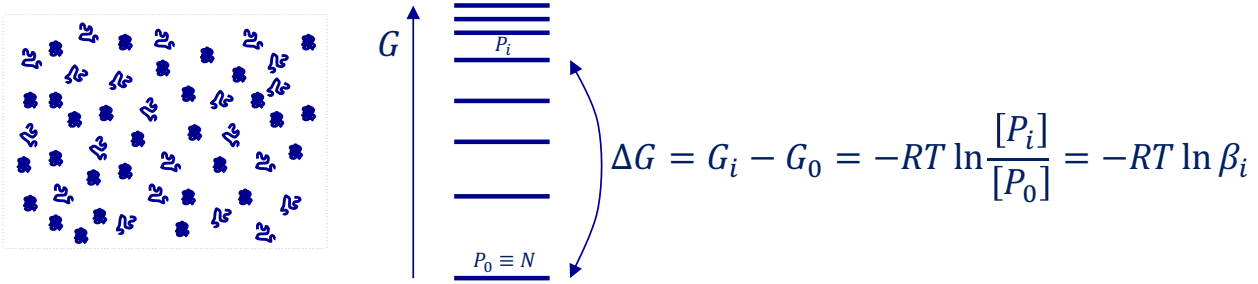
Stability

ΔG Gibbs energy of stabilization or unfolding
Difference in Gibbs energy between the native state and the unfolded state



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▶ Standard approach based on partition function *Q*

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

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

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

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

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

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▶ Standard approach based on partition function *Q*

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

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

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

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

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Standard approach based on partition function *Q*

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

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

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

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

Average of any system property (measurement)

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

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▶ Standard approach based on partition function *Q*

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

$$\langle \Delta H \rangle = RT^2 \frac{\partial \ln Q}{\partial T} = \frac{\sum_{i=0}^{n} \beta_i \Delta H_i}{\sum_{i=0}^{n} \beta_i} \qquad \beta_i = exp(-\Delta G_i / RT)$$

$$\langle \Delta C_P \rangle = \left(\frac{\partial \langle \Delta H \rangle}{\partial T} \right)_P = \langle \Delta C_P \rangle_{int} + \frac{\langle \Delta H^2 \rangle - \langle \Delta H \rangle^2}{RT^2} \cong \frac{\langle \Delta H \rangle (T + \Delta T) - \langle \Delta H \rangle (T)}{\Delta T}$$

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

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Single-transition unfolding

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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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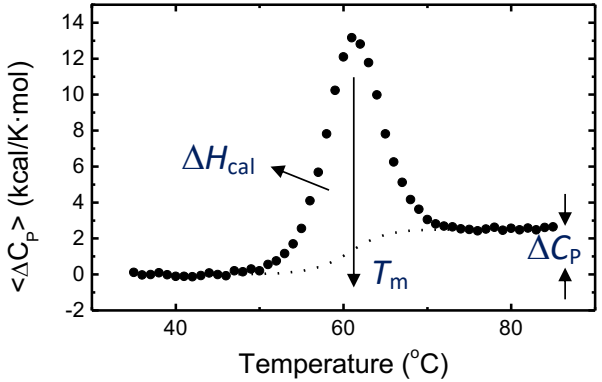
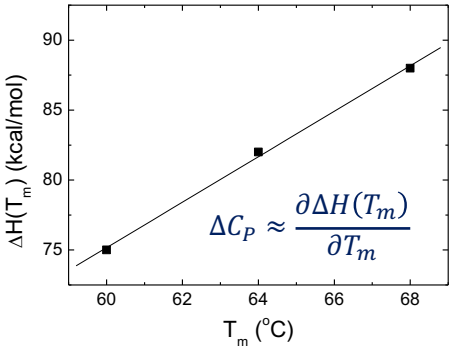
Single transition unfolding: model-free analysis



$$\Delta H(T_m) = \int_{T_0}^{T_1} \langle \Delta C_P \rangle dT$$

$$\Delta S(T_m) = \int_{T_0}^{T_1} \frac{\langle \Delta C_P \rangle}{T} dT$$

- T_m and ΔH: well determined
- ΔC_P: poorly determined



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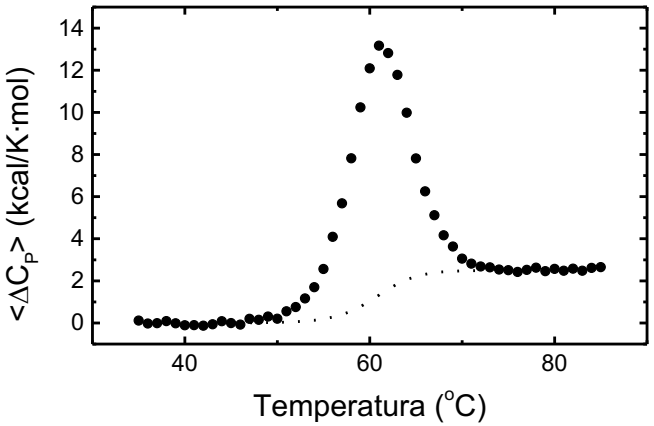

$$\langle \Delta H \rangle = \chi_N \Delta H_N + \chi_U \Delta H_U = \chi_U \Delta H$$

$$\Delta H(T) = \Delta H(T_m) + \Delta C_P (T - T_m)$$

$$\Delta G(T) = \Delta H(T_m)\left(1 - \frac{T}{T_m}\right) + \Delta C_P \left(T - T_m - T \ln \frac{T}{T_m}\right) \qquad K = e^{-(\Delta G(T)/RT)}$$

$$\chi_N = \frac{[P_0]}{[P]_T} = \frac{1}{1+K} \qquad \chi_U = \frac{[P_1]}{[P]_T} = \frac{K}{1+K}$$

$$\langle \Delta C_P \rangle = \frac{K}{(1+K)^2}\frac{\Delta H^2}{RT^2} + \frac{K}{1+K}\Delta C_P \cong \frac{\langle \Delta H \rangle (T+\Delta T) - \langle \Delta H \rangle (T)}{\Delta T}$$

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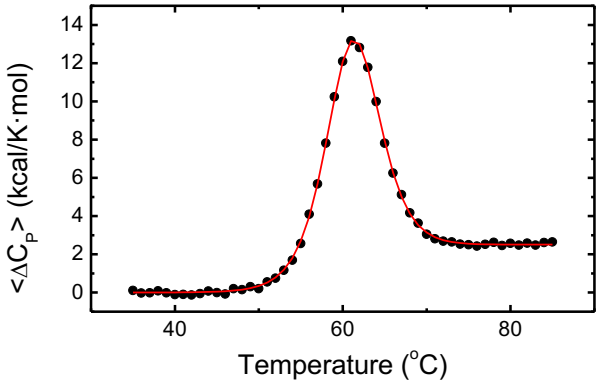
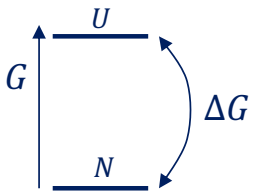
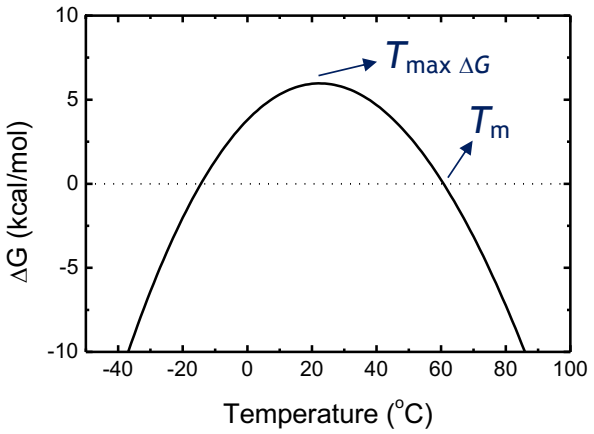
Single transition unfolding: NLSF analysis



T_m	60.75 ± 0.03 °C
$\Delta H(T_m)$	102 ± 0.3 kcal/mol
ΔC_P	2.51 ± 0.02 kcal/K·mol



Stability profile ΔG(T)



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Two-transition unfolding

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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_1K_2

$$Q = 1 + \beta_1 + \beta_2 + \beta_3 = 1 + K_1 + K_2 + K_1K_2$$

$$\chi_N = \frac{1}{1 + K_1 + K_2 + K_1K_2} \qquad \chi_{I_2} = \frac{K_2}{1 + K_1 + K_2 + K_1K_2}$$

$$\chi_{I_1} = \frac{K_1}{1 + K_1 + K_2 + K_1K_2} \qquad \chi_U = \frac{K_1K_2}{1 + K_1 + K_2 + K_1K_2}$$

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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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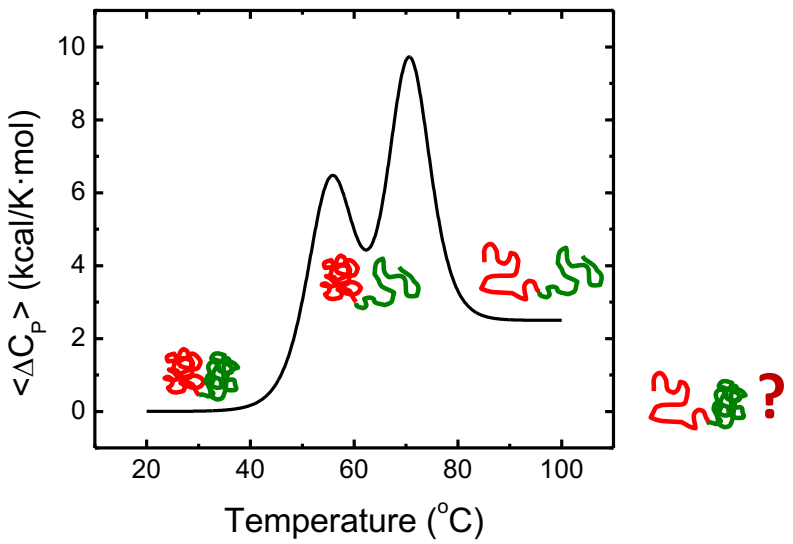
Two-transition unfolding (three-state unfolding?)

$$N \leftrightarrow \cdots \leftrightarrow U$$



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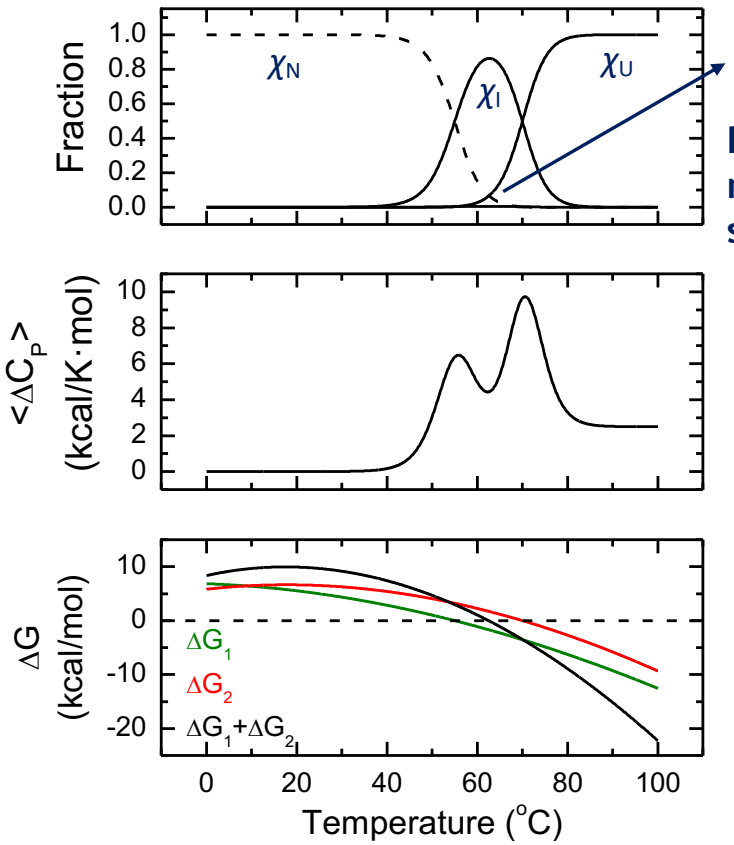

$P_{2_2} \sim 0$

Reduction in the number of accessible states

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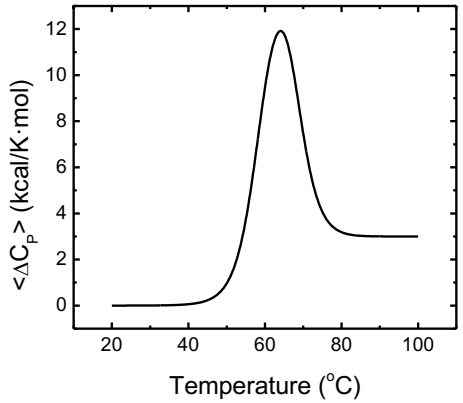
How many transitions?



Two-state test (single transition test):

- **Overlapping of unfolding curves obtained with different techniques (spectroscopy)**
- **van't Hoff-calorimetric enthalpies ratio (calorimetry)** $\frac{\Delta H_{vH}}{\Delta H_{cal}}$

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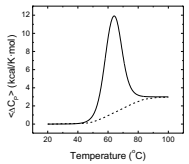
van't Hoff enthalpy

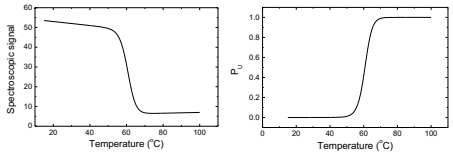
$$\Delta H_{vH} = RT^2 \frac{\partial \ln K}{\partial T}$$

$$\Delta H_{vH} = \frac{4RT_m^2 C_{P,max}}{\Delta H_{cal}}$$

$$\Delta H_{vH} = 4RT_m^2 \left(\frac{\partial \chi_U}{\partial T}\right)_{T_m}$$

model-free analysis





$\frac{\Delta H_{vH}}{\Delta H_{cal}} = 1$ **cooperative unit ≡ molecule (single transition)**

$$N \leftrightarrow U$$

$\frac{\Delta H_{vH}}{\Delta H_{cal}} < 1$ **cooperative unit < molecule (more than one transition)**

$$N \leftrightarrow I \leftrightarrow \cdots \leftrightarrow U \qquad \frac{\Delta H_{vH}}{\Delta H_{cal}} \neq \frac{1}{n_D}$$

$\frac{\Delta H_{vH}}{\Delta H_{cal}} > 1$ **cooperative unit > molecule (self-associated native state)**

$$N_n \leftrightarrow nU \qquad \frac{\Delta H_{vH}}{\Delta H_{cal}} \neq n \qquad \frac{\Delta H_{vH}}{\Delta H_{cal}} = 4\left(\frac{n-\sqrt{n}}{n-1}\right)^2$$

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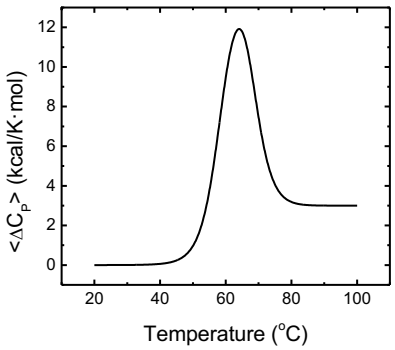
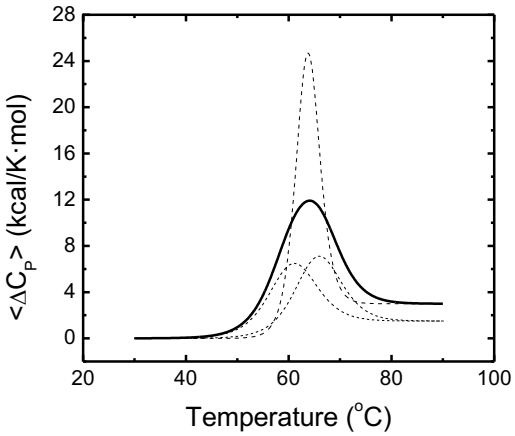
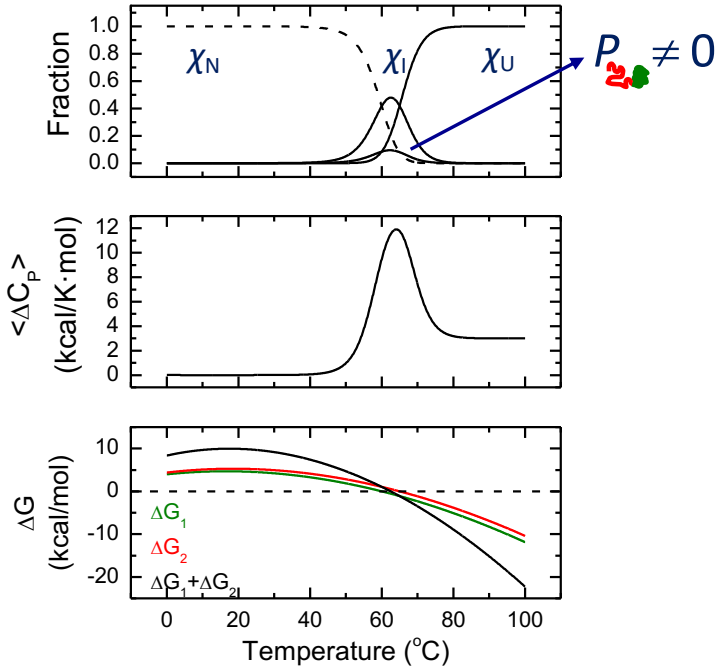


ΔH_{cal} 144.3 kcal mol^{-1}
T_m 63.5 °C
C_{Pmax} 10.2 kcal K^{-1} mol^{-1}

ΔH_{vH} 63.4 kcal mol^{-1}
$\Delta H_{vH}/\Delta H_{cal}$ 0.44





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Unfolding-Binding Coupling

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i	ΔG_i	$\exp(-\Delta G_i/RT)$
	0	1
	ΔG_{bind}	$K_a[L]$
	ΔG^0	K^0

$$Q = 1 + K_a[L] + K^0$$

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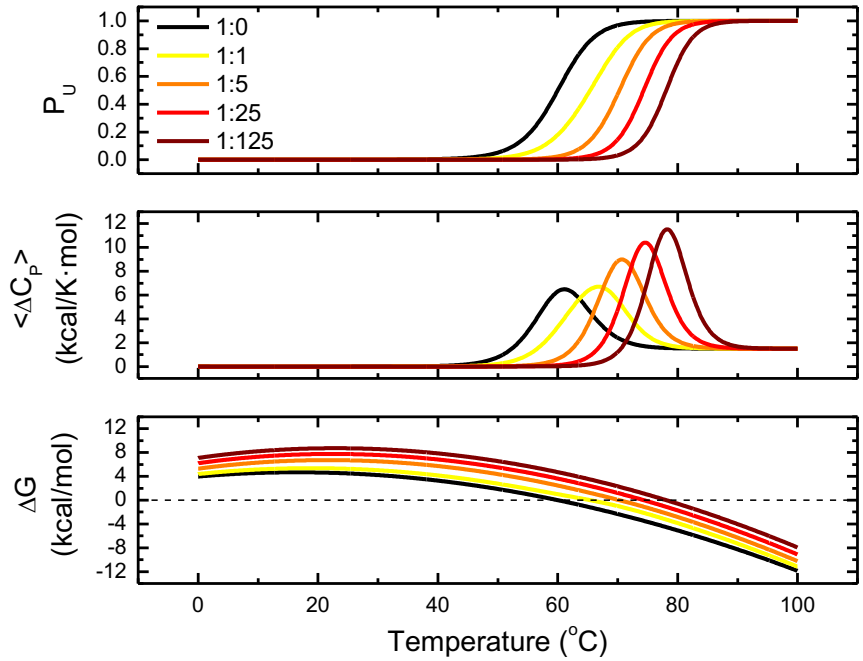

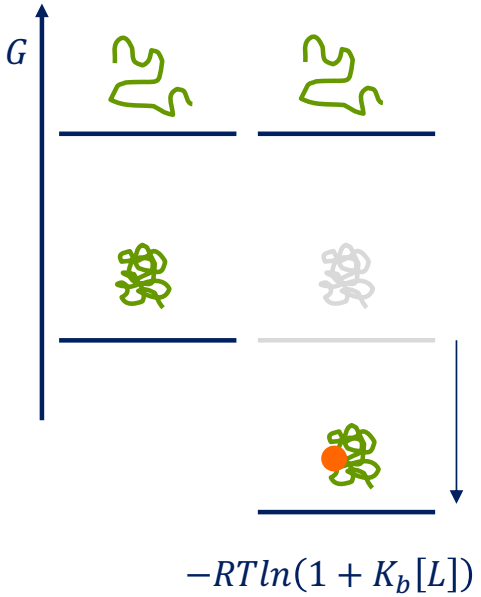

- **Indirect evidence for interaction**
- **Determination of binding affinity (?)**

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i	ΔG_i	$\exp(-\Delta G_i/RT)$
	0	1
	ΔG^0	K^0
	$\Delta G^0+\Delta G_{bind}$	$K^0K_a[L]$

$$Q = 1 + K^0(1 + K_a[L])$$

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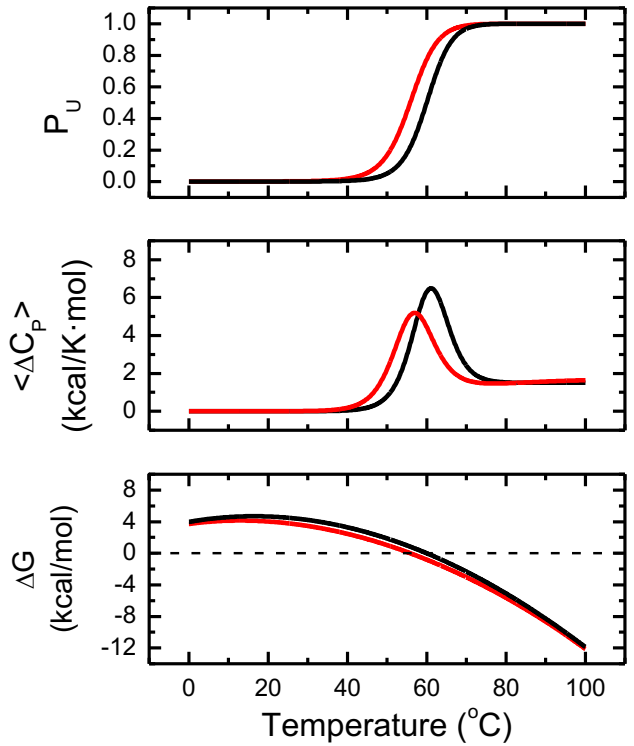

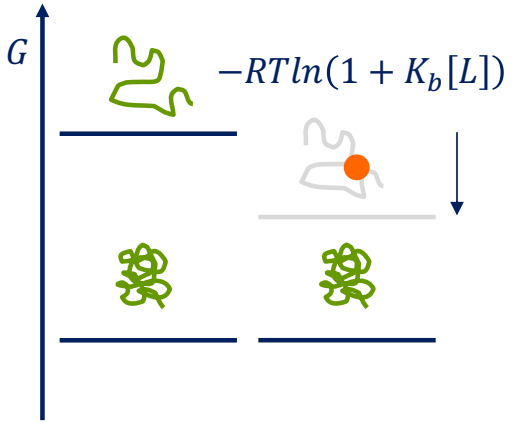

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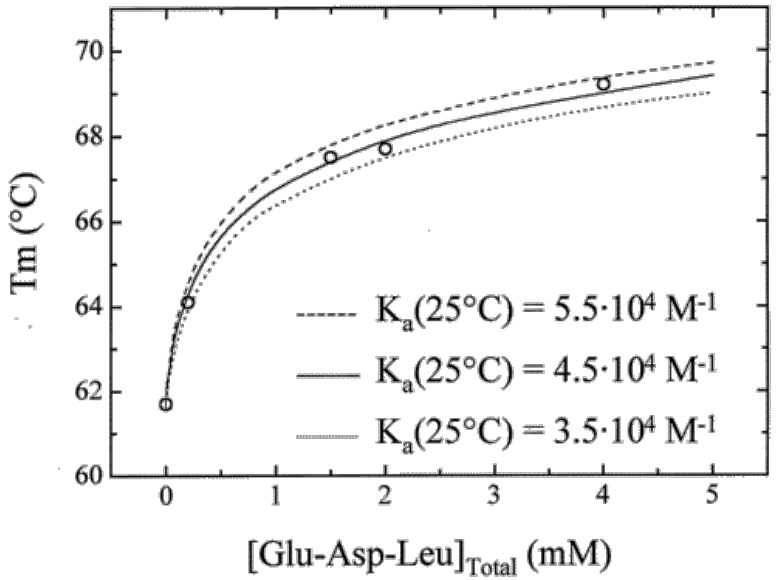

$$\frac{\partial \frac{1}{T_m}}{\partial \ln[X]} \approx \frac{R}{\Delta H(T_m)} \Delta n_X$$

$$\frac{\Delta T_m}{T_m} \approx \pm \frac{n_X R T_m^0}{\Delta H(T_m)} \ln\left(1 + \frac{[X]}{K_d}\right)$$

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DSF

Adrian Velazquez-Campoy

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DSF Monitoring protein unfolding using an extrinsic fluorescent reporter

Motivation:

- Low throughput of conventional techniques (sample amount, number of assays
- Conventional fluorescence plate readers do not allow broad temperature ramp
- Real-time qPCR allow broad temperature ramp, but do not record in the tryptophane fluorescence range

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DSF	**Thermofluor® or Thermal Shift Assay (TSA)**
	Stabilization effect induced by ligand binding or by solvent components on the thermal stability of a protein
“Differential”	**Usually, differences between a “reference” sample and “test” samples are measured**

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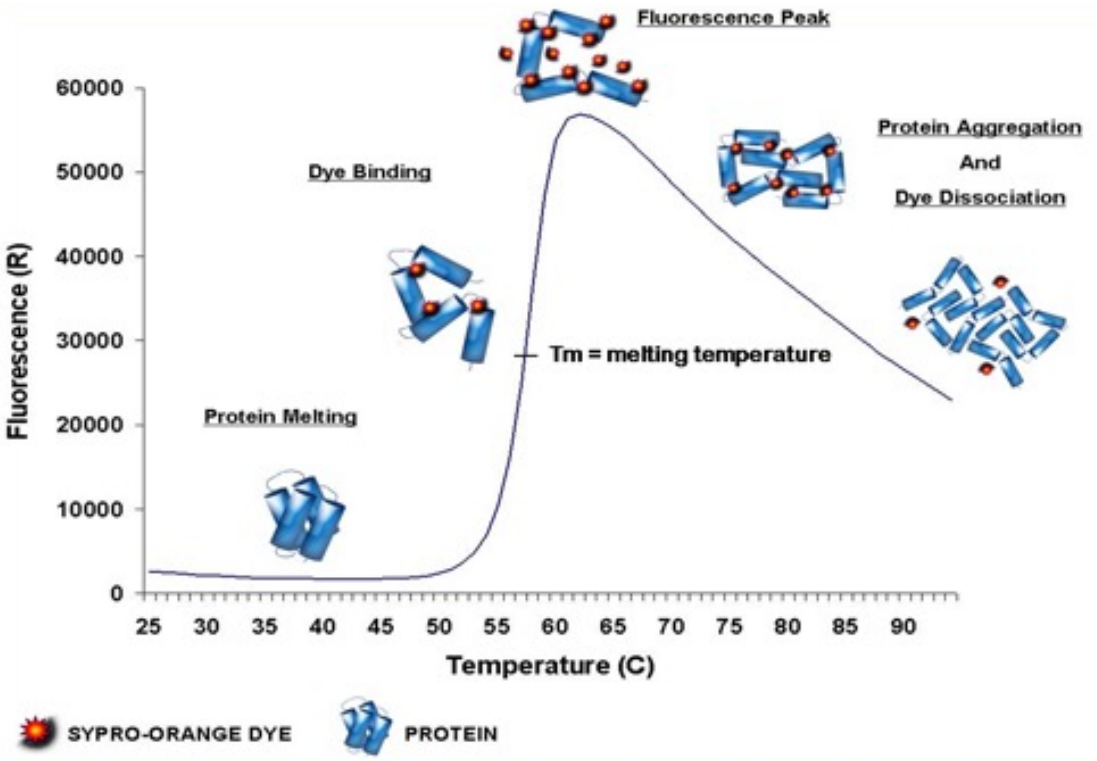

By Argonne National Laboratory - http://www.bio.anl.gov/molecular_and_systems_biology/Sensor/sensor_images/

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DSF

Now, also using intrinsic tryptophan or cofactors fluorescence

Applications:

- Drug screening
- Drug lead optimization **(Extent of stabilization does not correlate with binding affinity!)**
- Studies of enzyme mechanism
- Protein stabilization for optimized isolation
- Characterization of engineered proteins
- Optimization of protein crystallization conditions
- Screening for inhibitors of protein-protein interactions of modulators of protein conformational changes
- Membrane proteins
- Decrypting proteins of unknown biological function
- CETSA, for cellular thermal shift assay

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Because DSF is usually employed for comparing T_m's of samples under different conditions (e.g., with & without compound, at different pHs, with different excipients…), it is allowed to perform a minimal data analysis based on identifying the inflection point (T_{max} as a proxy for T_m).

Still, a thorough analysis can be done similar to that explained for thermal denaturations performed by conventional spectroscopy (i.e., estimation of T_m and $\Delta H(T_m)$).

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