MOSBRI
Molecular-Scale Biophysics Research Infrastructure

Protein stabilisation: Design, Experiments and Assessment (ProteSta)

ALS1 (BIFI-LACRIMA) 3rd-7th July 2023, Zaragoza, Spain

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

DAY 1 (3 July, 2023) 9:00 Course Presentation

			MOSBRI ALS1 Zaragoza 2023 ALS1: Protein Stabilization: Design, Experiments and Assessment (ProteSta)
	DAY 1		
Institute BIFI		09:00	**Presentation**
		09:15	**Principles of Protein Stability (I)**
		10:30	*Break*
		11:00	**Principles of Protein Stability (II)**
		13:00	*Lunch*
School of Sciences		15:00	**Analysis of Spectroscopic Unfolding Assays**
		17:00	*Break*
		17:30	**Short Talks (I)**
		19:00	*End of first day*
	DAY 2		
Institute BIFI		09:00	**Differential Scanning Calorimetry (DSC)**
		10:30	*Break*
		11:00	**Differential Scanning Fluorimetry (DSF)**
		12:00	**Visit to Experimental Facilities / Experimental Guidelines**
		13:00	*Lunch*
School of Sciences		15:00	**Analysis of DSC & DSF Unfolding Assays**
		17:00	*Break*
		17:30	**Short Talks (II)**
		19:00	*End of Second Day*
	DAY 3		
Institute BIFI		09:00	**Stabilization through Formulation and Mutagenesis (I)**
		10:30	*Break*
		11:00	**Stabilization through Formulation and Mutagenesis (II)**
		12:00	**Visit to Experimental Facilities / Experimental Guidelines**
		13:00	*Lunch*
		15:00	**Molecular Dynamic Simulations for Stability Calculations**
		17:00	*Break*
		17:30	**Poster Session (I)**
		18:30	*End of Third Day*
	DAY 4		
Institute BIFI		09:00	**Computational Tools for Protein Stabilization (I)**
		10:30	*Break*
		11:00	**Computational Tools for Protein Stabilization (I)**
		13:00	*Lunch*
		15:00	**Protein Stability: Biotechnology and Biomedicine**
		17:00	*Break*
		17:30	**Poster Session (II)**
		18:30	*End of Fourth Day*
	DIA 5		
Institute BIFI		09:00	**Round Table and Discussion**
		10:30	*Break*
		11:00	**Group Discussion on the Course**
		12:00	**Survey**
		12:30	*End of Fifth Day*

Institute BIFI: Campus Rio Ebro (Tram Stop)

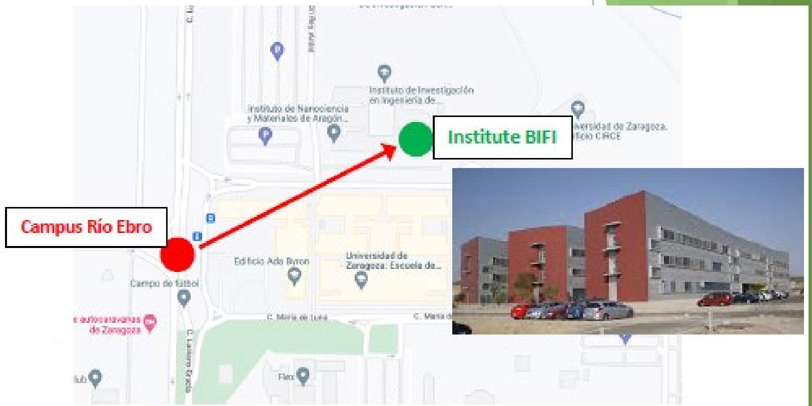
School of Sciences: Plaza de San Francisco (Tram Stop)

Important: You must buy a Tram Ticket (SINGLE TICKET, valid for a single trip and 1 hour) in the ticket machines located at the tram stops before getting into the tram

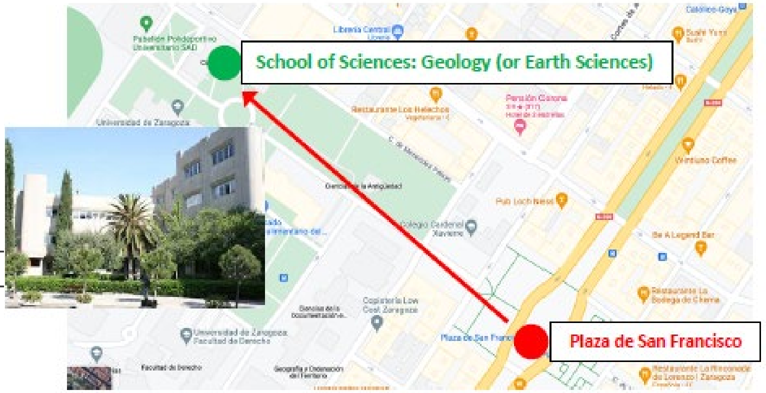
https://www.tranviasdezaragoza.es/en/

The tram has only one line with two directions:

- Avenida de la Academia, to go to Institute BIFI
- Mago de Oz, to go to the city (hotels and School of Sciences)



I+D Building, Conference Room (floor 1.1, above Entrance Desk)



Geology Building, Computation Room (3rd floor)

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DAY 1 (3 July, 2023) 9:15/13:00 Principles of Protein Stability

Javier Sancho

- What do we mean by protein stability?
- The U⇔F equilibrium
- The unfolded state matters
- Effect of denaturants on protein stability
- Effect of temperature on protein stability
- Effect of ligands on protein stability
- Two-state or not two-state, that it the question

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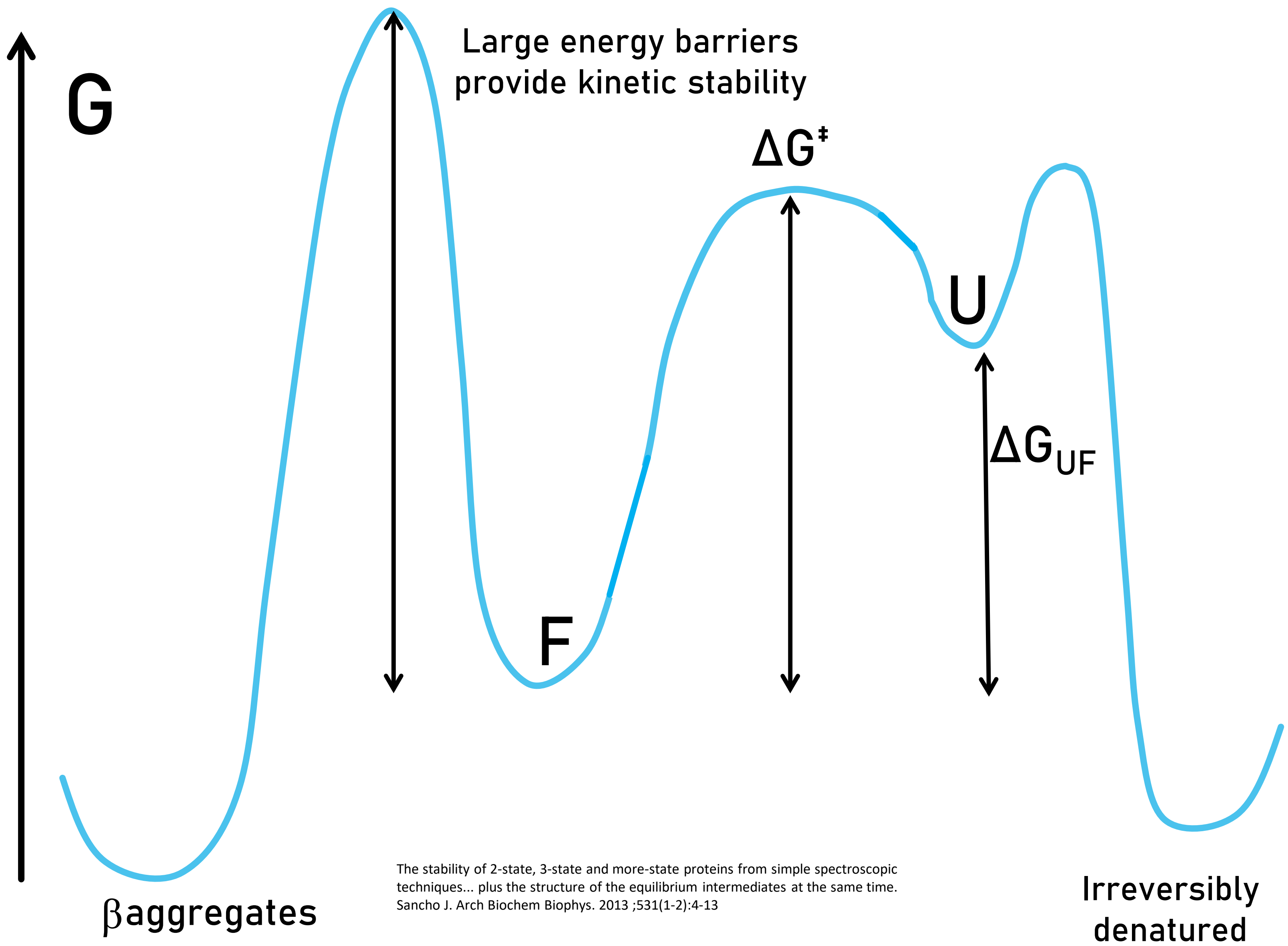
What do we mean by protein stability?



The stability of 2-state, 3-state and more-state proteins from simple spectroscopic techniques... plus the structure of the equilibrium intermediates at the same time. Sancho J. Arch Biochem Biophys. 2013 ;531(1-2):4-13

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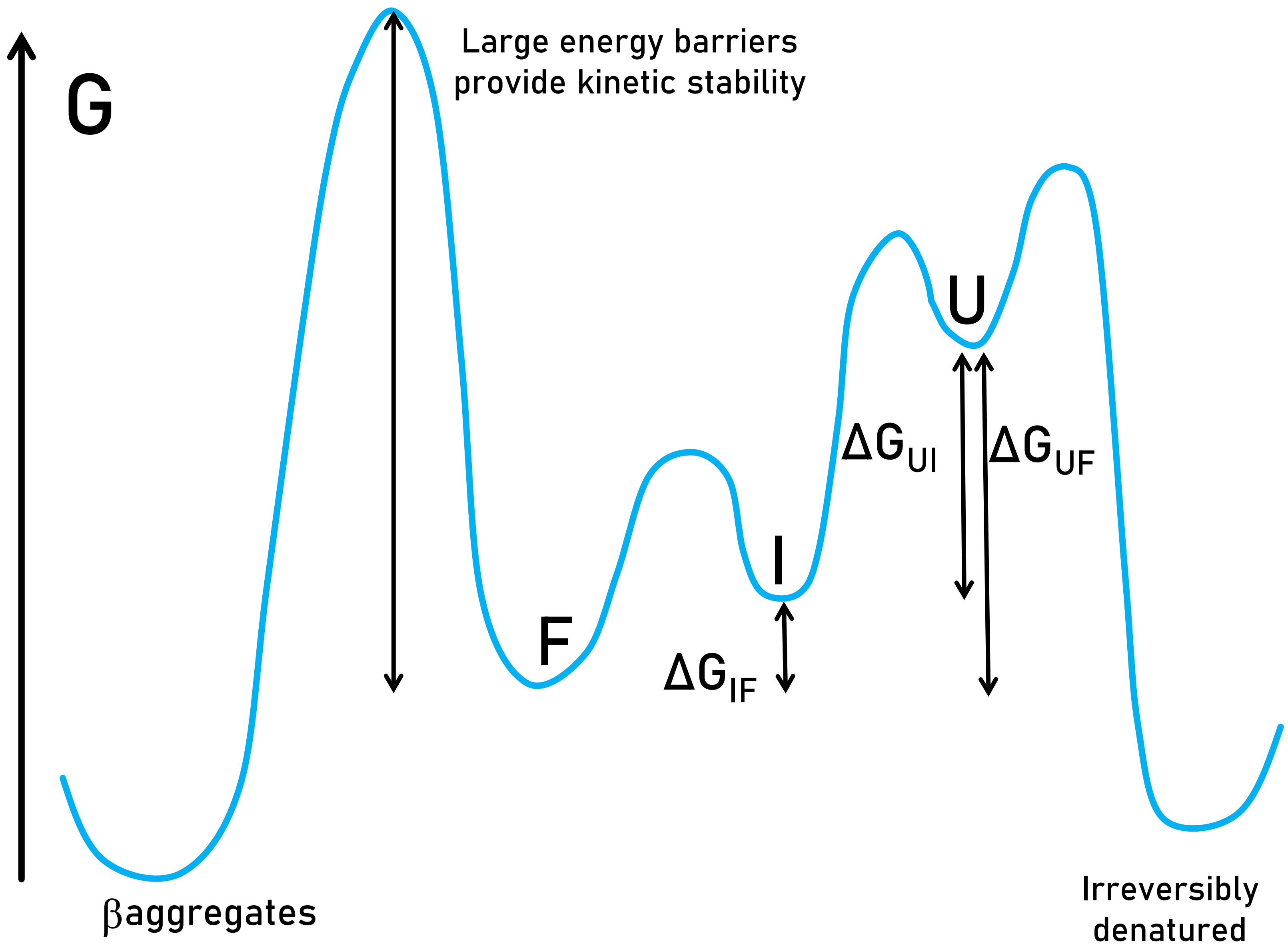
What do we mean by protein stability?



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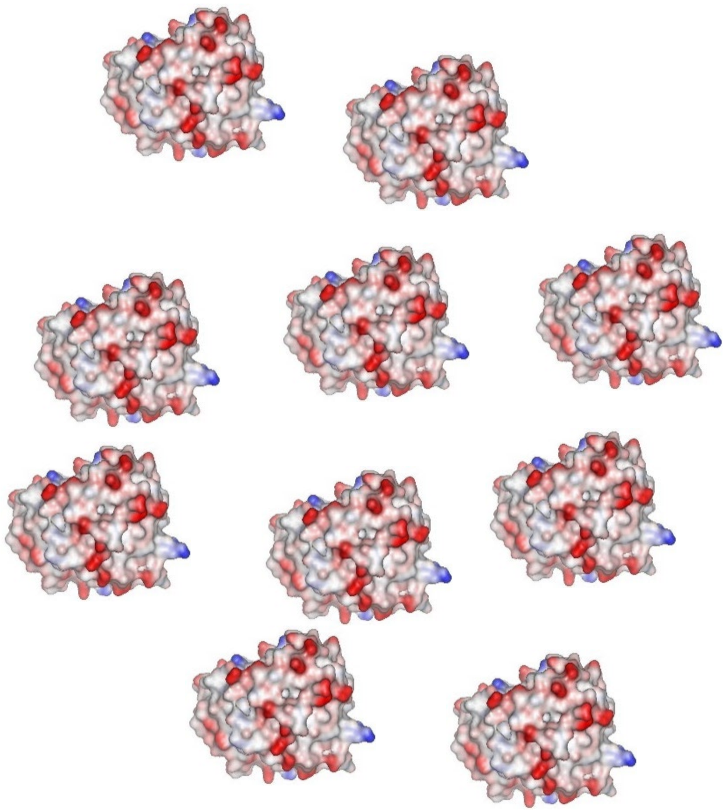
The U⇔F equilibrium

$$K=[U]/[F]$$

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

K ⟷ ΔG



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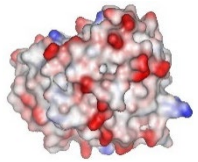
The unfolded state matters



ProtSA

PDB

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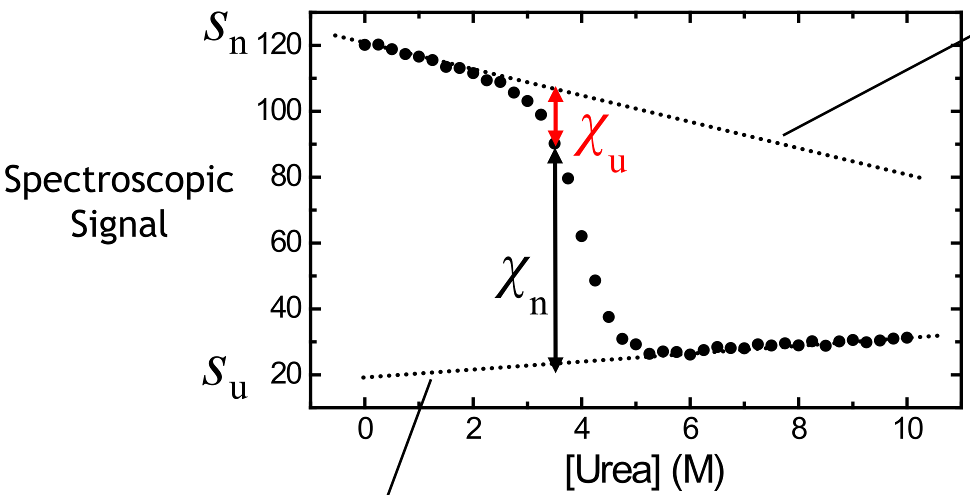
The effect of denaturants on protein stability



$$S_N = \left(s_n + p_n[D]\right)$$

$$S = \chi_n S_n + \chi_u S_u$$

$$1 = \chi_n + \chi_u$$

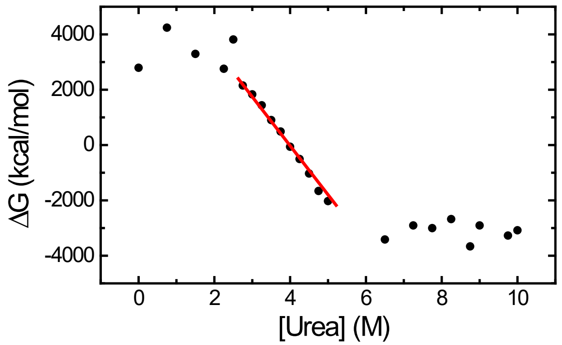
$$S_U = \left(s_u + p_u[D]\right)$$

$$\chi_u = \frac{S - S_n}{S_u - S_n}$$

$$\chi_n = \frac{S - S_u}{S_n - S_u}$$

$$K = \frac{S_n - S}{S - S_u}$$

$$\Delta G = -RT \ln \frac{S_n - S}{S - S_u}$$



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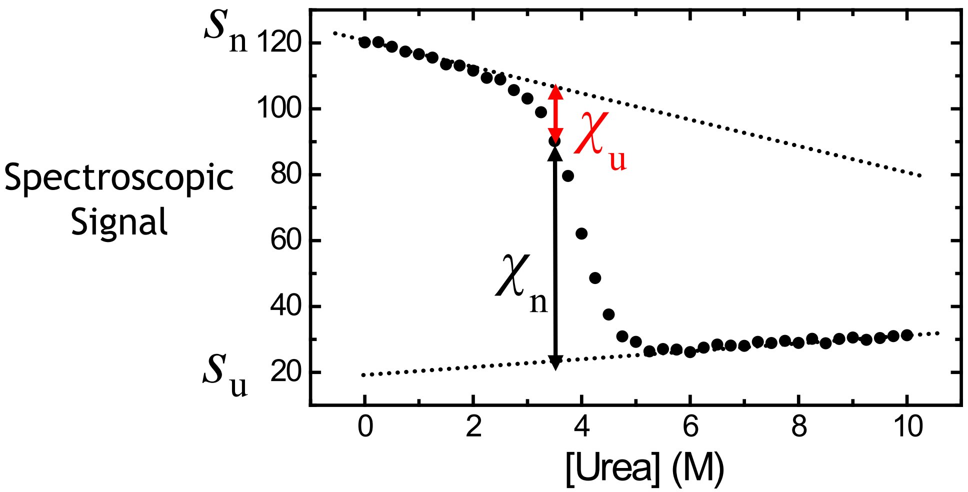
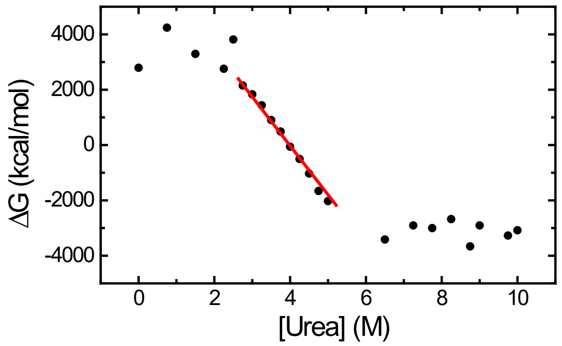
The effect of denaturants on protein stability



$$\Delta G([D]) = \Delta G^0 - m[D]$$

$$\Delta G([D]=0)$$

$$m = \left(\frac{\partial \Delta G([D])}{\partial [D]}\right)_{T,P}$$



$\Delta G = 7600 \pm 200$ cal/mol
$m = 1920 \pm 40$ cal/mol·M

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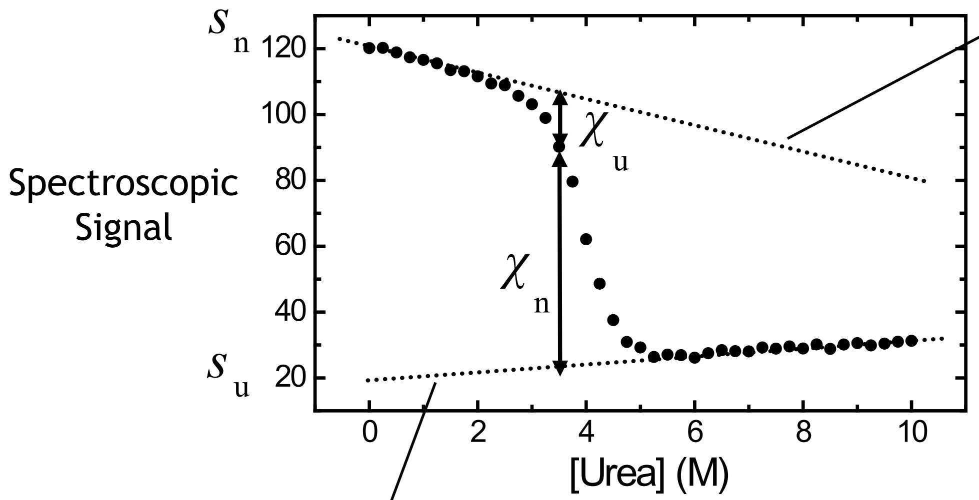
The effect of denaturants on protein stability



$$S_N = (s_n + p_n[D])$$

$$S_U = (s_u + p_u[D])$$

$$S = \chi_n S_n + \chi_u S_u$$

$$K = \frac{S_n - S}{S - S_u}$$

$$S = \frac{S_n - K \times S_u}{1 + K}$$

$$K = e^{\frac{-\Delta G}{RT}}$$

$$\Delta G([D]) = \Delta G^0 - m[D]$$

$$S = \frac{(s_n + p_n[D]) + e^{-(\Delta G/RT)} \times (s_u + p_u[D])}{1 + e^{-(\Delta G/RT)}}$$

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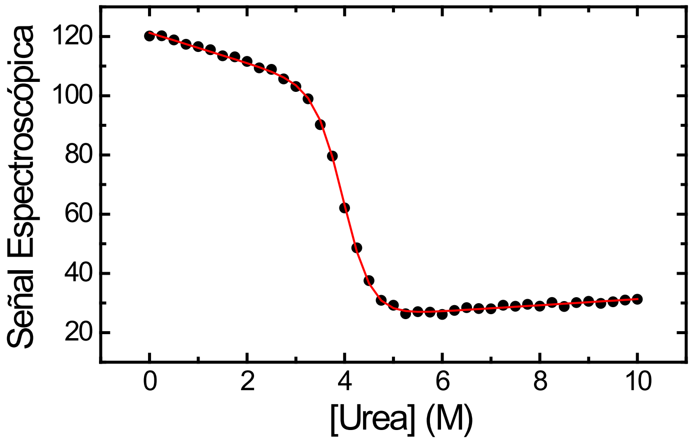
The effect of denaturants on protein stability

$$S = \frac{\left(s_n + p_n[D]\right) + e^{-(\Delta G^0 - m[D])/RT}\left(s_u + p_u[D]\right)}{1 + e^{-(\Delta G^0 - m[D])/RT}}$$



ΔG^0	8200 ± 50 cal/mol
m	2050 ± 10 cal/mol·M

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The effect of temperature on protein stability

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

$$\Delta G = \Delta H - T\Delta S$$

$$\Delta S(T_m) = \Delta H(T_m)/T_m$$

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

$$\Delta S = \Delta S(T_m) + \Delta Cp\ (ln\frac{T}{T_m})$$

$$\Delta G(T) = \Delta H_m\left(1 - \frac{T}{T_m}\right) + \Delta Cp\left[T - T_m - T ln\frac{T}{T_m}\right]$$

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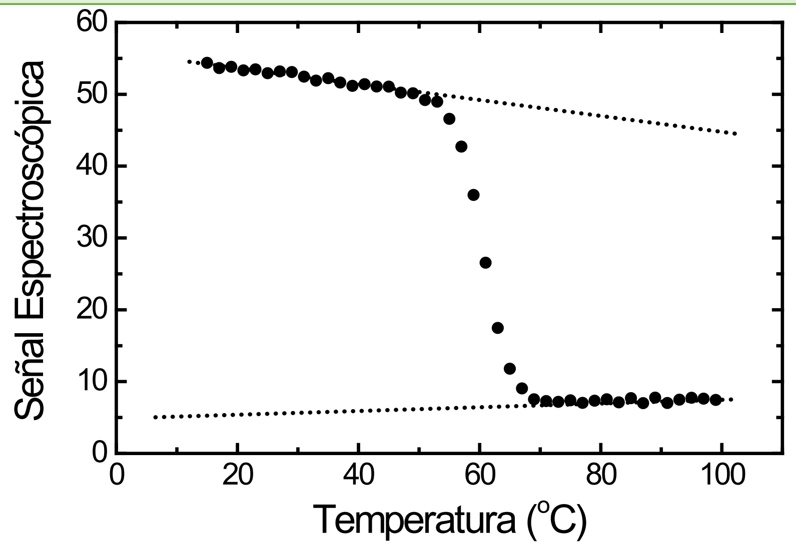
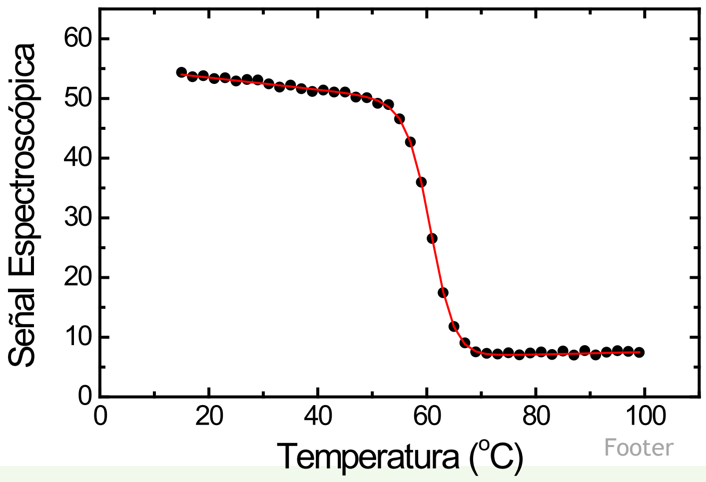
The effect of temperature on protein stability



$$S_N = (s_n + p_n \; T \;)$$

$$S_U = (s_u + p_u \; T \;)$$

$$S = \frac{(s_n + p_n T) + e^{-\left(\Delta H_m\left(1-\frac{T}{T_m}\right) + \Delta Cp\left[T - T_m - T\ln\frac{T}{T_m}\right]\right)/RT}\,(s_u + p_u T)}{1 + e^{-\left(\Delta H_m\left(1-\frac{T}{T_m}\right) + \Delta Cp\left[T - T_m - T\ln\frac{T}{T_m}\right]\right)/RT}}$$



$\Delta H(T_m)$	101 ± 2 kcal/mol
T_m	60.69 ± 0.04 °C
ΔC_P	2.2 ± 0.7 kcal/K·mol

The stability of 2-state, 3-state and more-state proteins from simple spectroscopic techniques... plus the structure of the equilibrium intermediates at the same time. Sancho J. Arch Biochem Biophys. 2013 ;531(1-2):4-13

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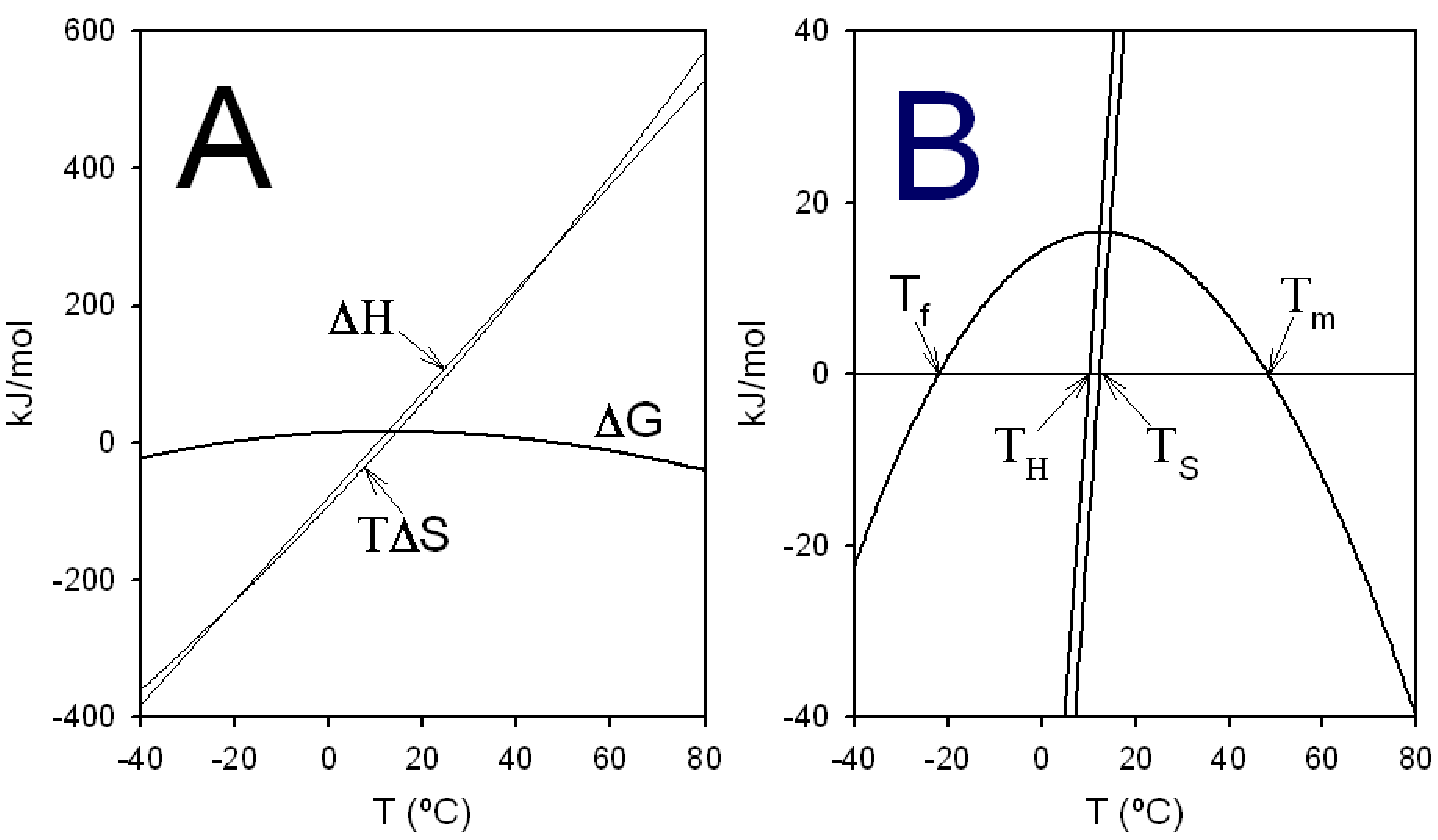
The effect of temperature on protein stability



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The effect of ligands on protein stability

$$
\begin{array}{lcl}
N & \leftrightarrow & U \\
+ & K^0 & \\
L & & \\
\updownarrow K_a & & \\
NL & &
\end{array}
$$

$$K = \frac{[U]}{[N]+[NL]} = \frac{[U]}{[N]}\frac{1}{1+K_a[L]} = \frac{K^0}{1+K_a[L]}$$

$$\Delta G = \Delta G^0 + RT\ln\left(1+K_a[L]\right)$$

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$$\Delta G(T, pH, [D], [L], \ldots)$$

Denaturant

$$\Delta G_{\mathrm{i}}([D]) = \Delta G_{\mathrm{i}}^{0} - m[D]$$

Temperature

$$\Delta G_{\mathrm{i}}(T) = \Delta H_{\mathrm{i}}(T_{\mathrm{m,i}}) + \Delta C_{\mathrm{P,i}}(T - T_{\mathrm{m,i}}) - T\left(\Delta S_{\mathrm{i}}(T_{\mathrm{m,i}}) + \Delta C_{\mathrm{P,i}} \ln(T / T_{\mathrm{m,i}})\right)$$

pH

$$\Delta G_{\mathrm{i}}(pH) = \Delta G_{\mathrm{i}}^{0} + \sum_{\mathrm{j=1}}^{\mathrm{j=m_i}} n_{\mathrm{j}} RT \ln\left(\frac{1 + 10^{pK_{a,1} - pH}}{1 + 10^{pK_{a,\mathrm{i}} - pH}}\right)$$

Ligand

$$\Delta G_{\mathrm{i}}([L]) = \Delta G_{\mathrm{i}}^{0} + RT \ln\left(\frac{1 + K_{\mathrm{B,1}}[L]}{1 + K_{\mathrm{B,i}}[L]}\right)$$

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Two-state or not two-state, that it the question

2-state thermal unfolding

N ⟷ U

ΔG_{NU}

global stability

3-state thermal unfolding

relevant stability

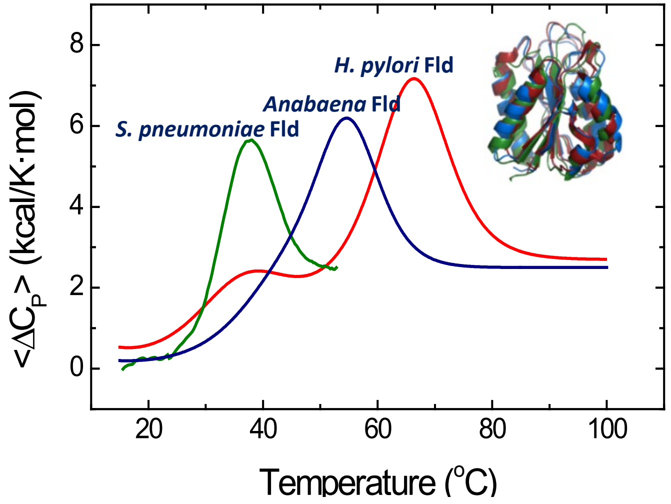
N ⟷ I ⟷ U

ΔG_{NI} ΔG_{IU}

residual stability

ΔG_{NU}

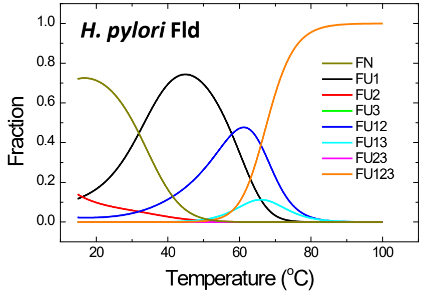
global stability

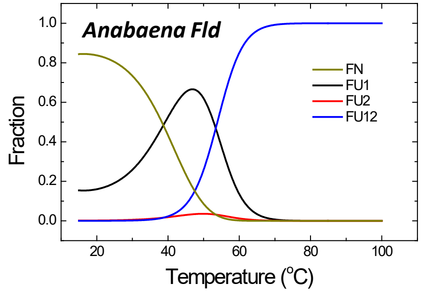


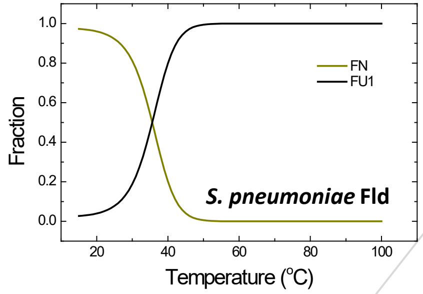
•Rodriguez-Cardenas et al. *PLoS ONE* 2016 11 e0161020

•Cremades et al. *Biochemistry* 2008 47 627-639

•Irun et al. *Journal of Molecular Biology* 2001 306 877-888







Do proteins always benefit from a stability increase? **Relevant** and **residual** stabilisation in a three-state protein by charge optimisation. Campos LA, Garcia-Mira MM, Godoy-Ruiz R, Sanchez-Ruiz JM, **Sancho J.** J Mol Biol. 2004 344:223-37.

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Principles of Protein Stability

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- More questions?

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