MOSBRI
Molecular-Scale Biophysics
Research Infrastructure

Protein stabilisation: Design, Experiments and Assessment (ProteSta)

ALS1 (BIFI-LACRIMA) 3rd-7thJuly 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 3 (5 July, 2023) 9:00/12:00 Stabilization by formulation and mutagenesis

- Protein stabilization by formulation: some rational
- Examples
- Protein stabilization by mutagenesis: some rational
- Examples
- Three-state proteins

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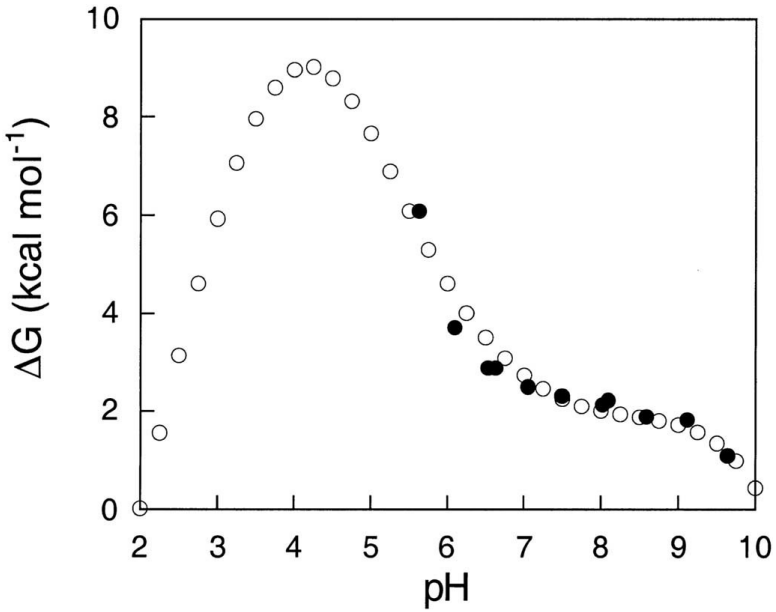
Protein stabilization by formulation: some rational

Some chemicals you may considered

- Buffers
- Salts (NaCl)
- Amino acids (histidine, glycine, arginine)
- Sugars (sucrose, manitol, sorbitol)
- Polymers (dextran, PEG)
- Specific binders to the native state: ligand binding



Salt-induced stabilization of apoflavodoxin at neutral pH is mediated through cation-specific effects. Maldonado et al. Protein Science 11: 1260-1273

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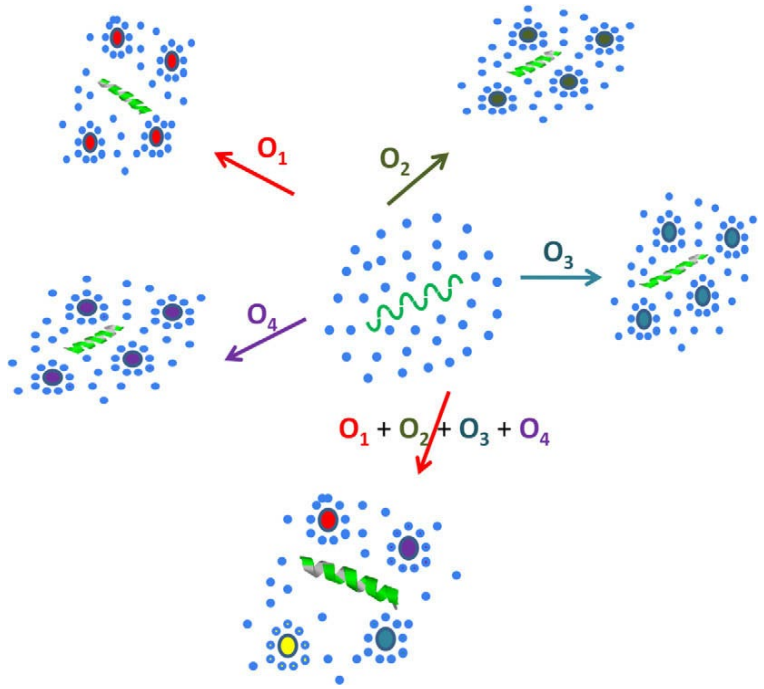
Protein stabilization by formulation: some rational

Protein stabilization by reducing water availability



Role of naturally occurring osmolytes in protein folding and stability
R. Kumar. Archives of Biochemistry and Biophysics 491 (2009) 1–6

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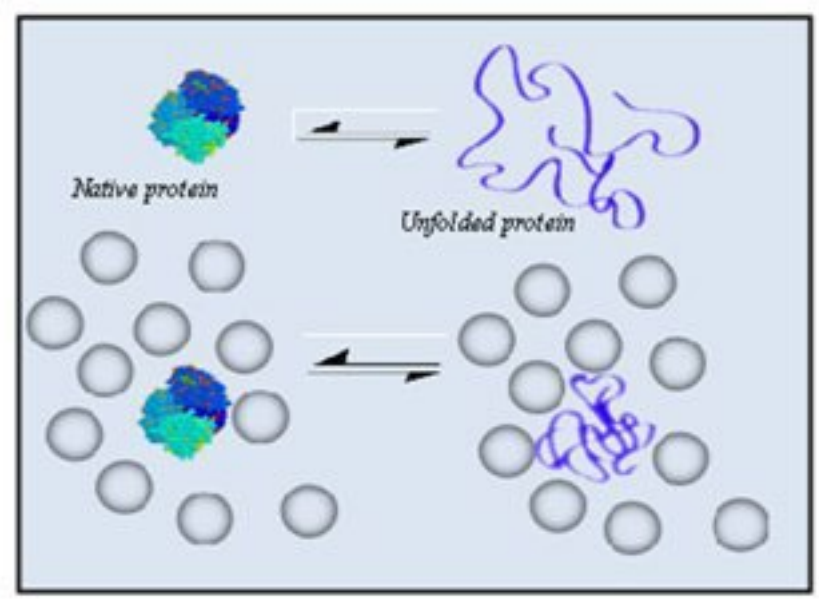
Protein stabilization by formulation: some rational

Protein stabilization by crowding the solution



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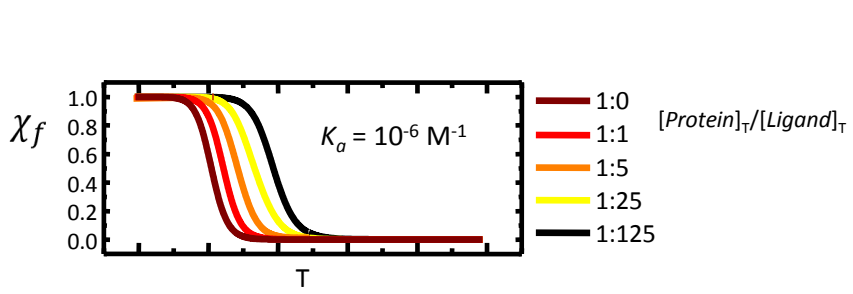
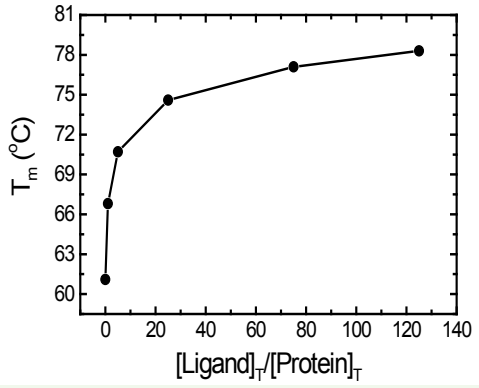
Protein stabilization by formulation: some rational

Protein stabilization by ligand binding

$$U \overset{K}{\leftrightarrow} F \qquad K = \frac{[F]}{[U]} \qquad \Delta G = -RT \times \ln K$$

$$U \overset{K}{\leftrightarrow} F + L \overset{K_a}{\leftrightarrow} FL \qquad K_{lig} = \frac{[F]+[FL]}{[U]} \qquad K_a = \frac{[FL]}{[F]\times[L]} \qquad \Delta G_{lig} = \Delta G - RT \times \ln(1+K_a \times [L])$$





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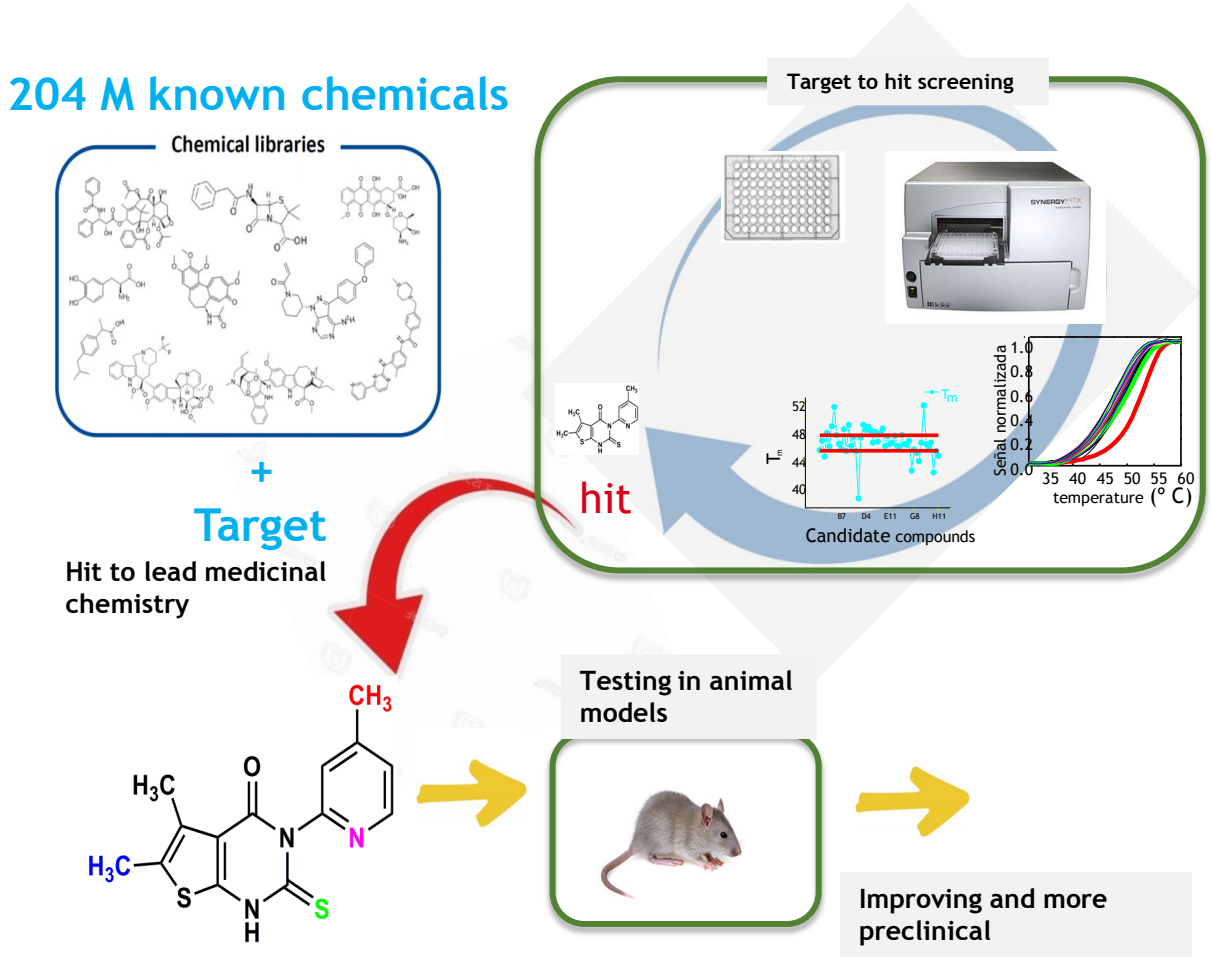
Examples

Protein stabilization by ligand binding: application in drug discovery



LACRIMA
Laboratorio Avanzado de Cribado e Interacciones Moleculares de Aragón

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Year	Target protein	Type of compound	Application	Patent
2008	PAH	pharmacological chaperons	Phnylketonuria (PKU)	X
2009	Fld	antimicrobials	*Helicobacter pylori*	X
2009	Stem cells	apoptotic cell death specific of ESC or EF	Cell therapy	
2012	Aβ(1-42)	aggregation inhibitors	Alzheimer disease	X
2013	NS3 protease	antivirals	Hepatitis C	
2016	hIAPP	aggregation inhibitors	Type II Diabetes	
2016	PEPCK-C	Enzyme inhibitors	Isoform phenotyping	
2017	IDP	antitumoral	Pancreatic cancer	
2017	FNR	antimicrobial	*Xanthomonas citri*	
2018	α-syn	aggregation inhibitors	Parkinson disease	X
2018	FAD synthetase	antimicrobials	*C. ammoniagenes* *M. Tuberculosis* *S. pneumoniae*	
2019	AIF	apoptosis regulation	Apoptosis regulation	

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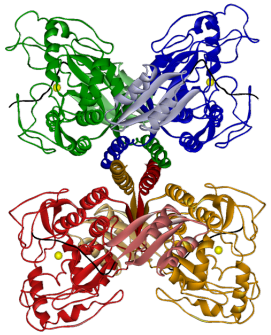
Examples

Pharmacological chaperons

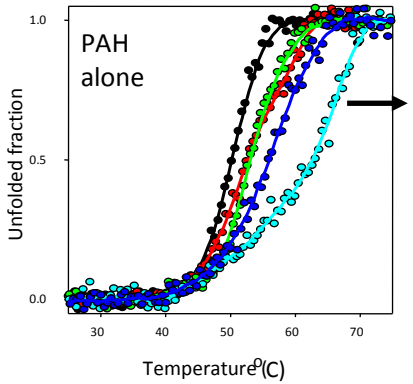
Phenylketonuria

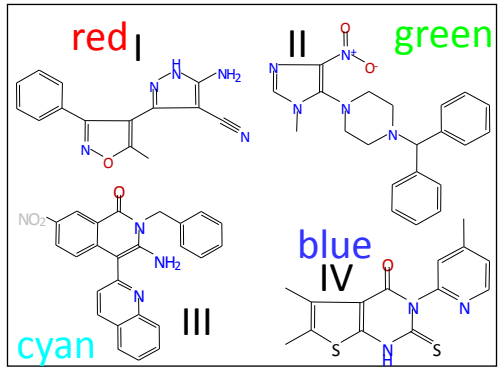
(1:15000 in Europe)



Destabilizing mutations in PAH
2208 variants known
(as of May 22, 2023)

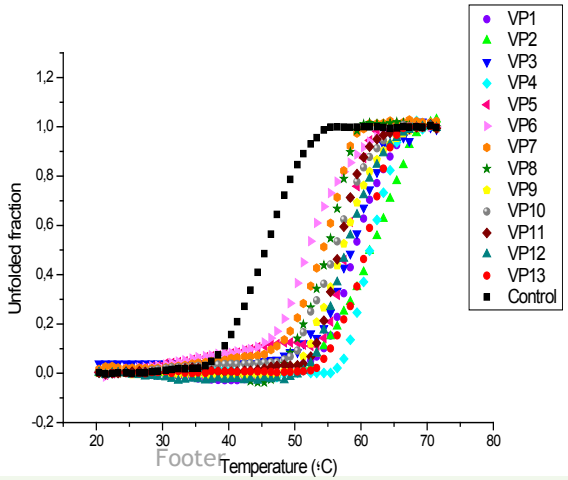
1500 compounds screened → 4 stabilizing compounds found





Identification of pharmacological chaperones as potential therapeutic agents to treat phenylketonuria
Pey et al. 2008. Journal of clinical investigation 118: 2858-2867.

10000 compounds screened → more stabilizing compounds found



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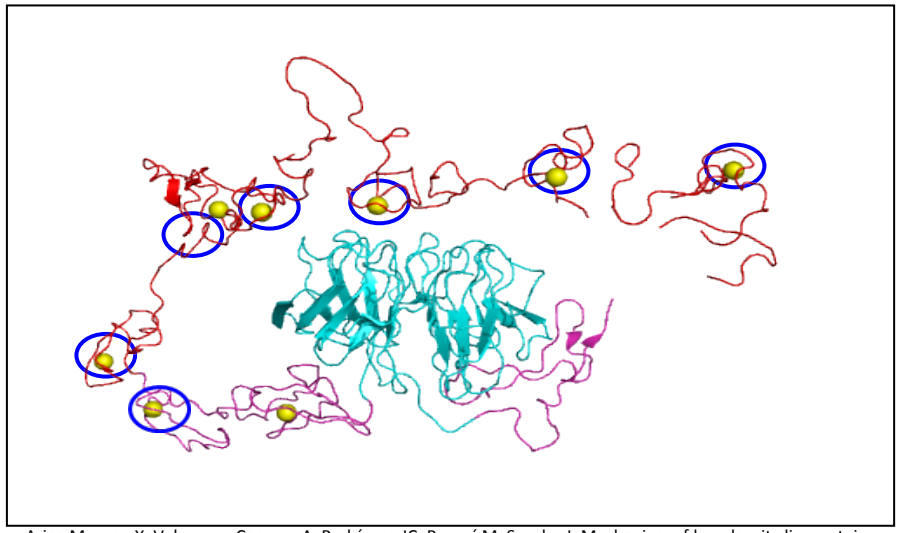
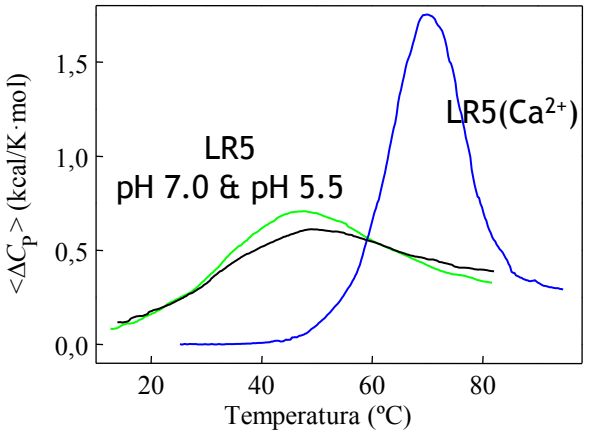
Examples

Protein stabilization by ligand binding: role in cellular mechanims

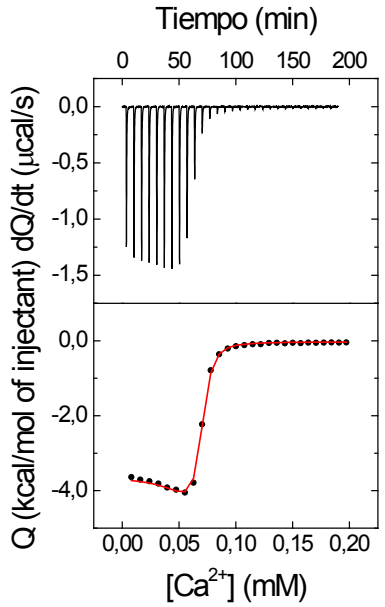
LDL delivery by LDL receptor



Arias-Moreno X, Velazquez-Campoy A, Rodríguez JC, Pocoví M, Sancho J. Mechanism of low density lipoprotein (LDL) release in the endosome: implications of the stability and Ca2+ affinity of the fifth binding module of the LDL receptor. J Biol Chem. 2008 Aug 15;283(33):22670-9.



LR5 titration with Ca^{2+}



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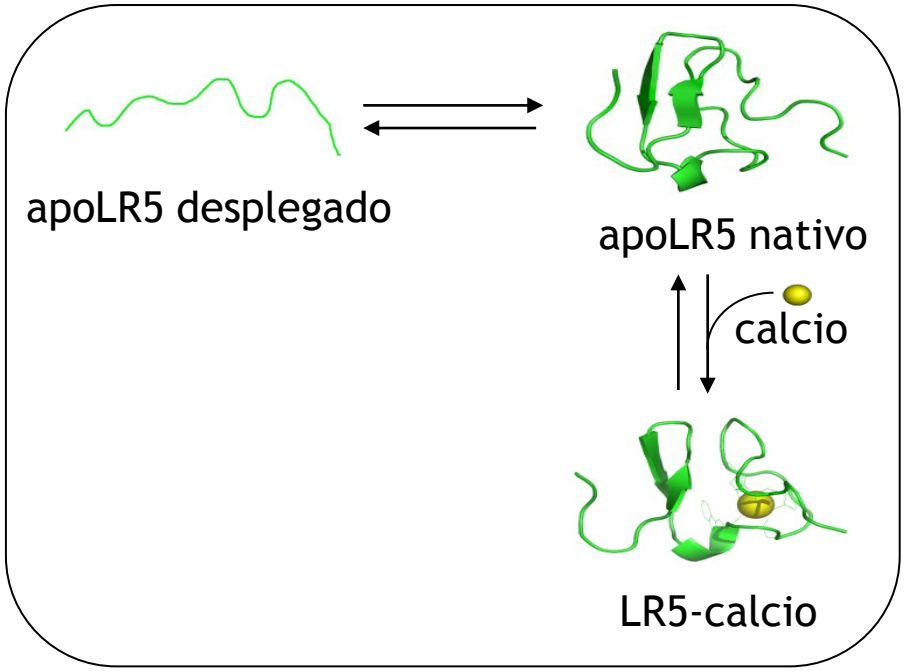
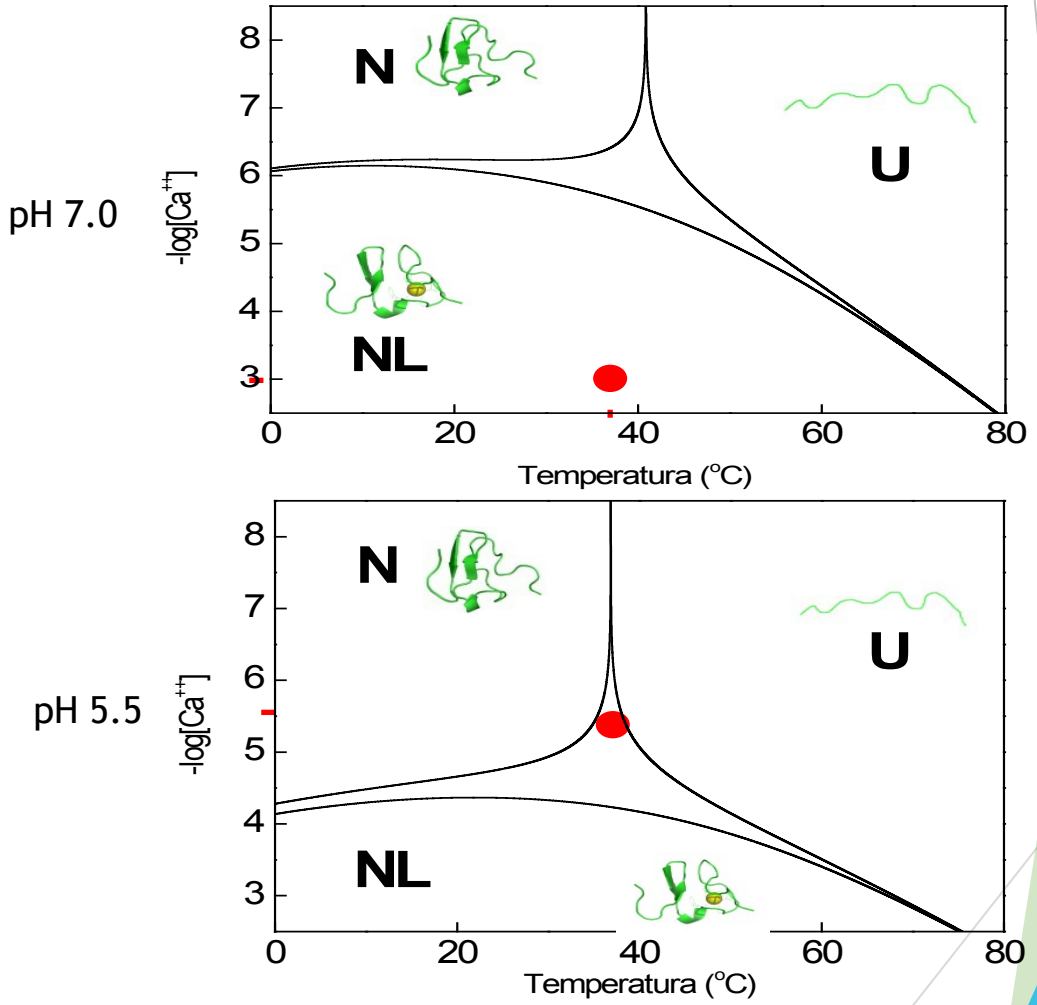
Examples





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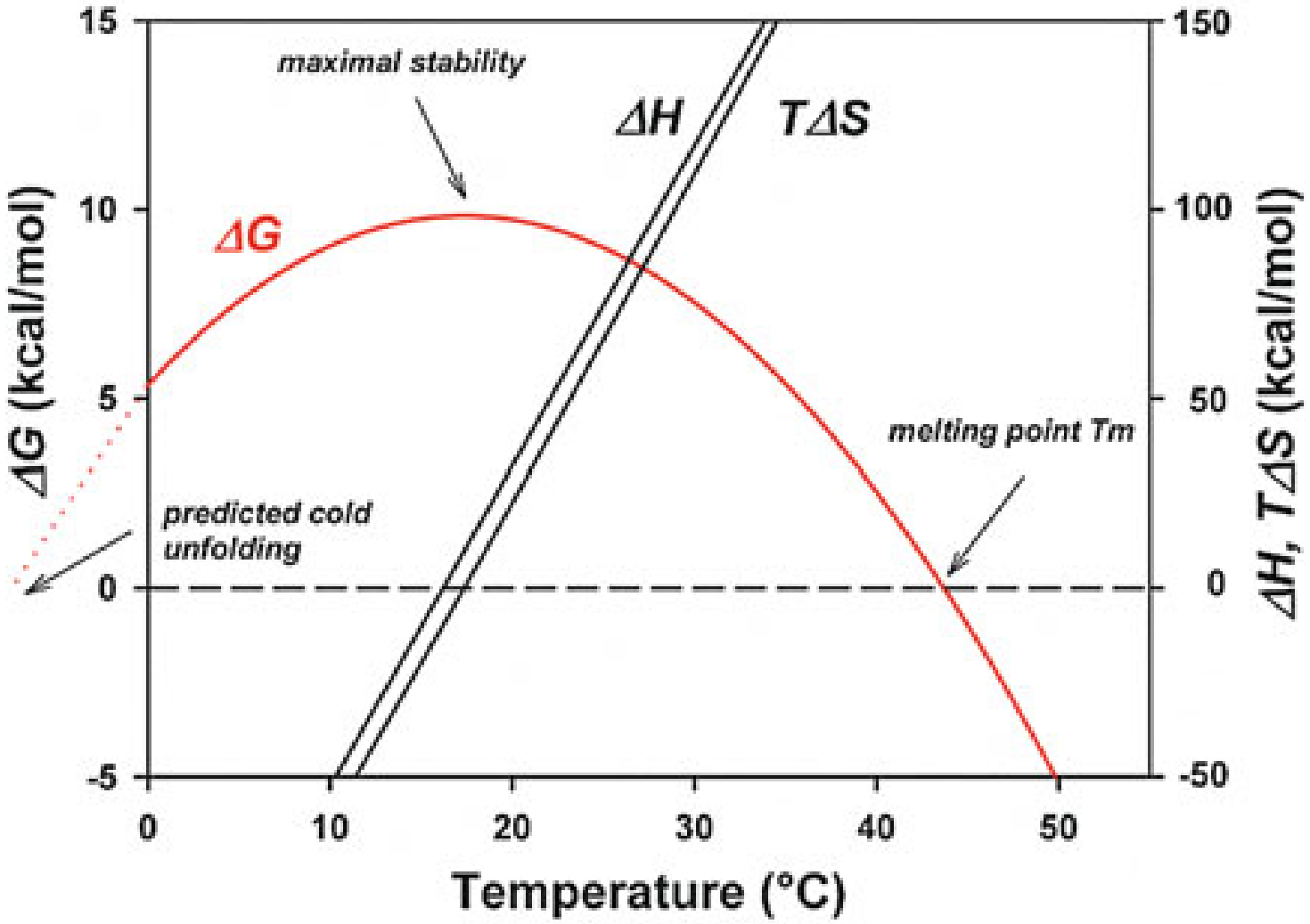
Protein stabilization by mutagenesis: some rational



$$F \rightleftharpoons U \qquad \Delta G_{unf}$$

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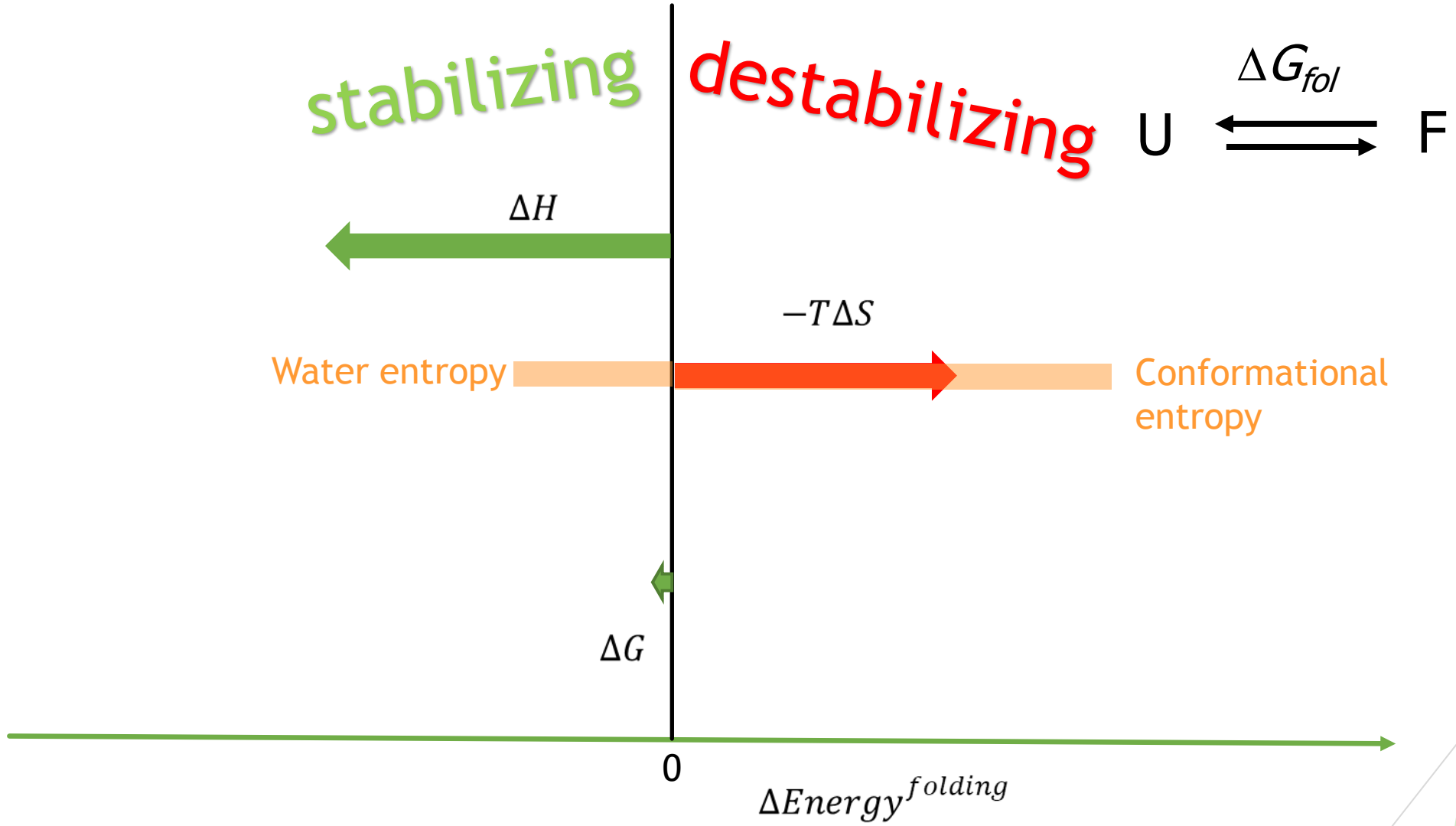
Protein stabilization by mutagenesis: some rational



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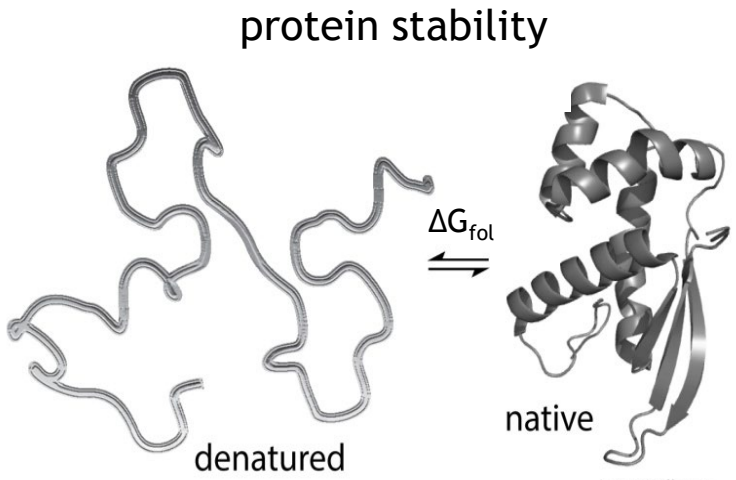
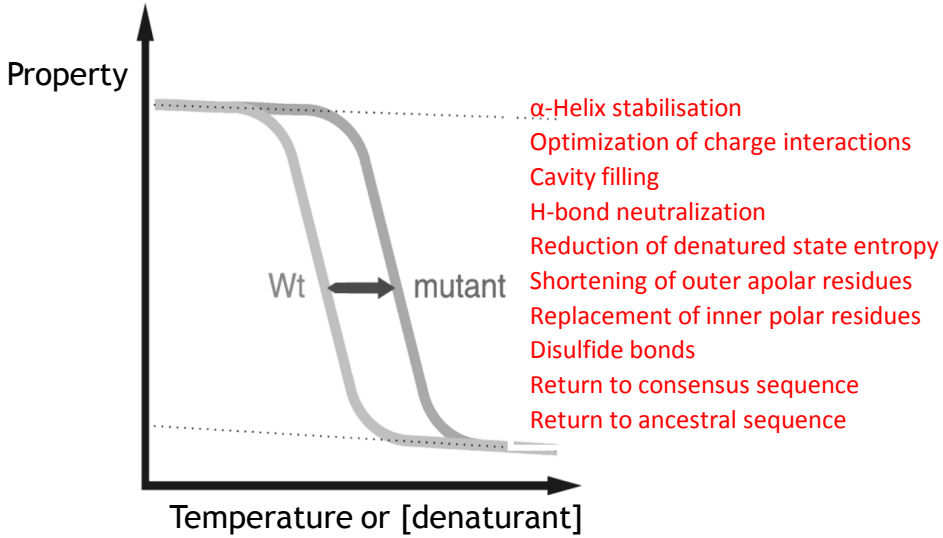
Protein stabilization by mutagenesis: some rational

To get inspiration you may think of ΔH, ΔS or evolution





$\Delta\Delta G_{mut\text{-}wt} = \Delta G_{mut} - \Delta G_{wt} > 0.5$ kcal/mol

- Random substitution: 2.5 % stabilizing mutations
- Directed evolution; deep sequencing
- **Designed substitutions**
- **AI??**

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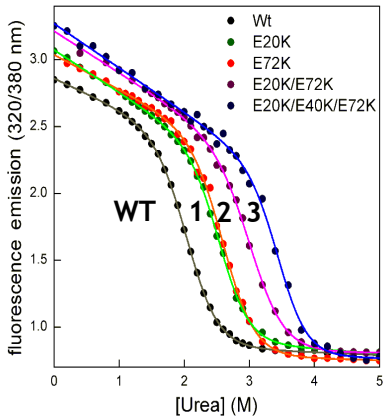
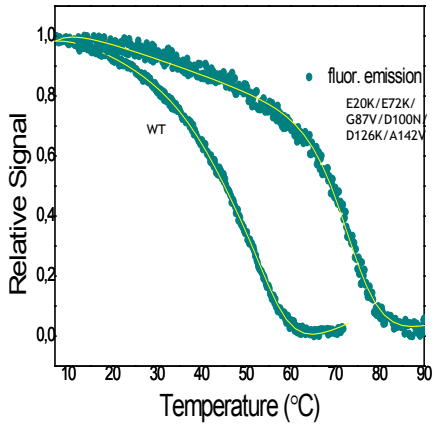
Examples

The stabilizing effect tends to be accumulative

Anabaena PCC 7119 flavodoxin



Bueno et al.
J. Mol. Biol. (2006) 358:701-712



Lamazares et al.,
Sci Rep. 5:9129 (2015)

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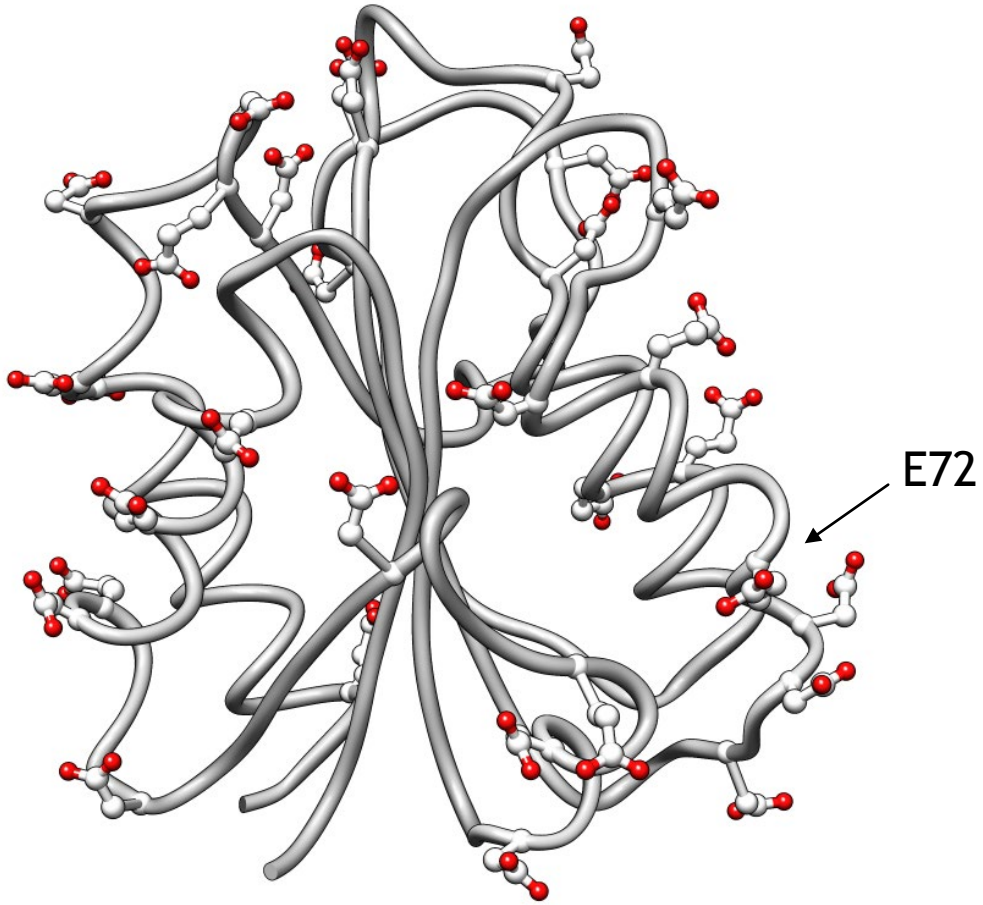
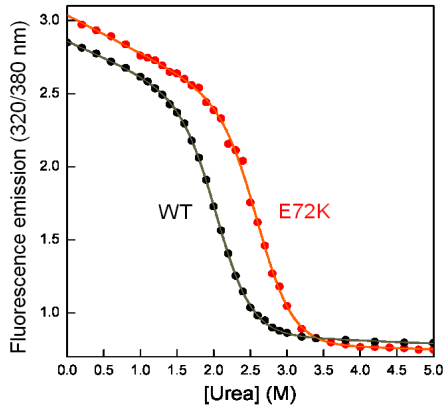
Three-state proteins



2-state chemical unfolding

$$N \xleftrightarrow{\Delta G_{NU}} U$$

global stability



$\Delta\Delta G_{NU} = -1.4$ Kcal mol^{-1}

Campos et al., J. Mol. Biol (2004) 344:223-237

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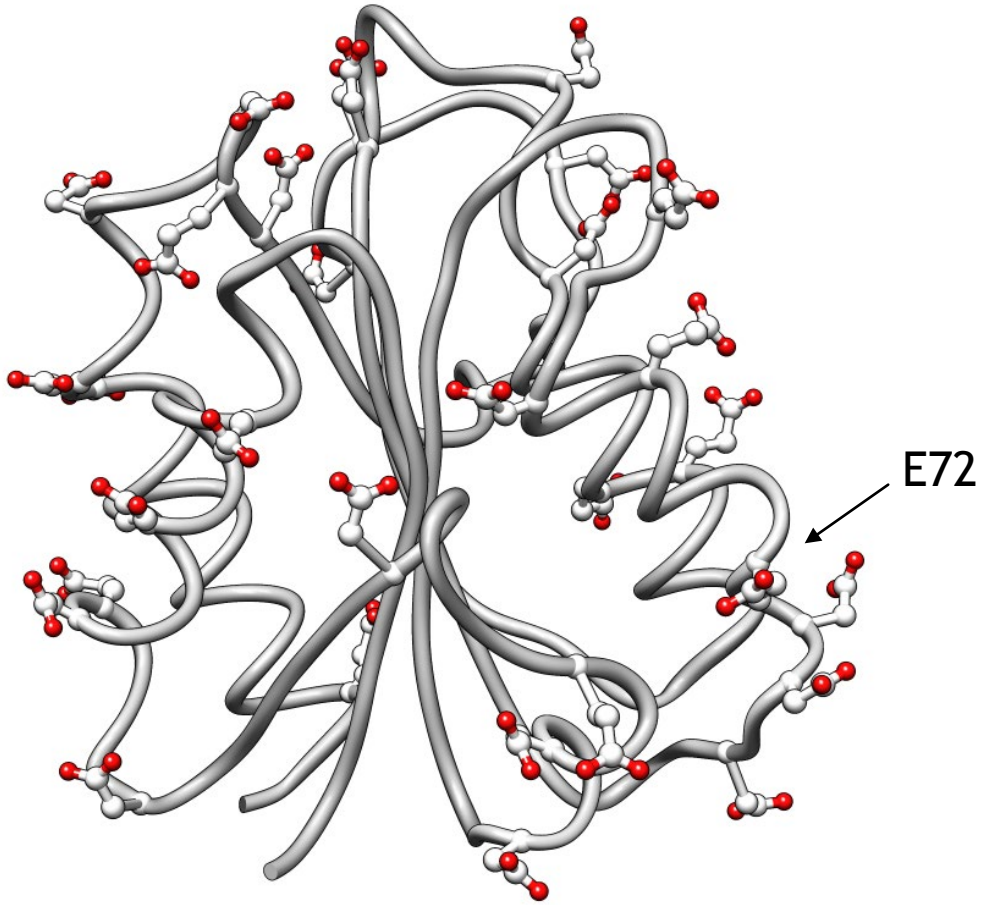
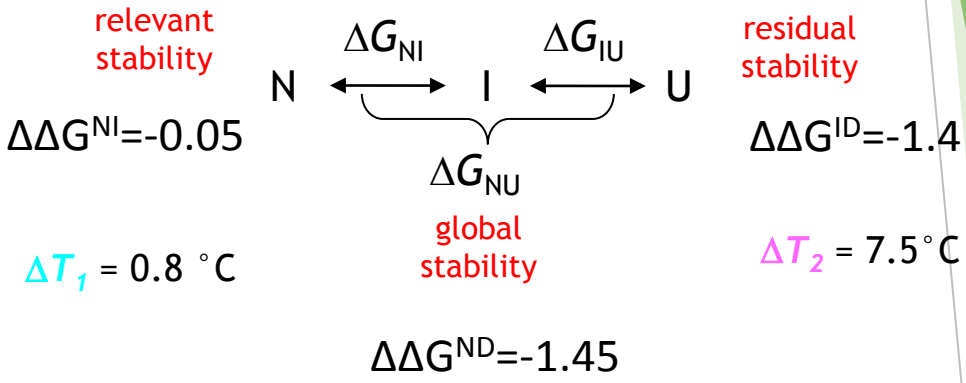
Three-state proteins



3-state thermal unfolding



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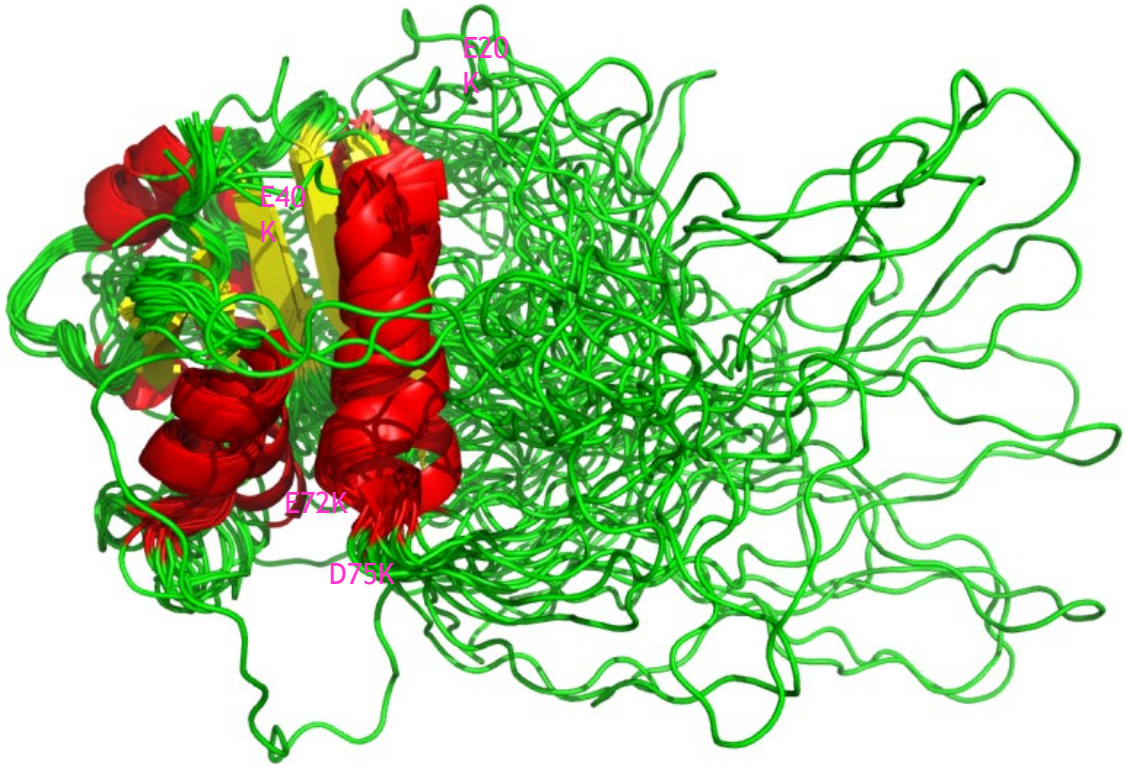
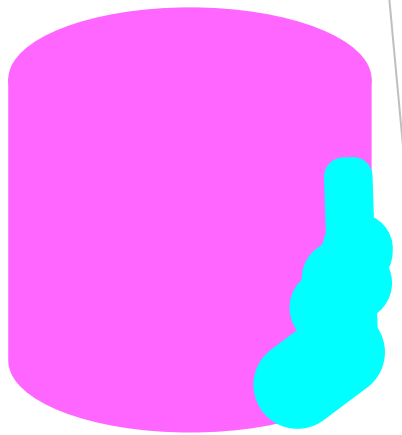
Three-state proteins





Ayuso-Tejedor et al., J. Mol. Biol. 400:922-934 (2010)

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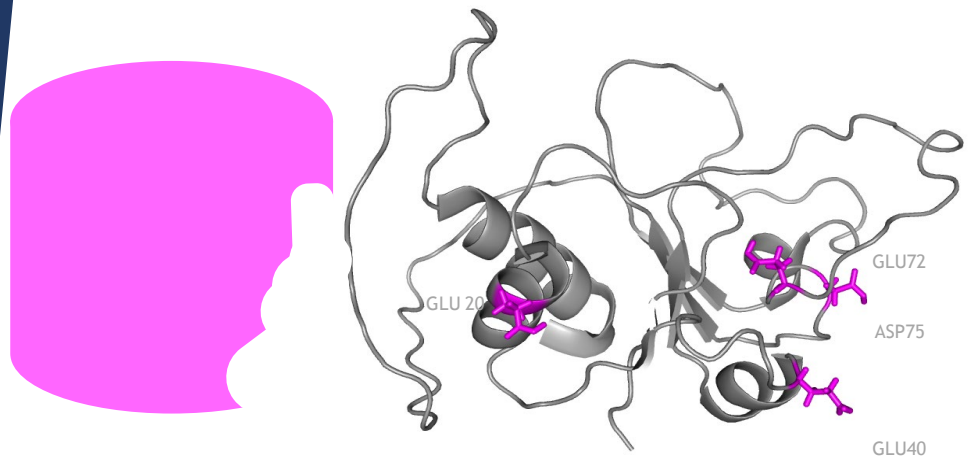
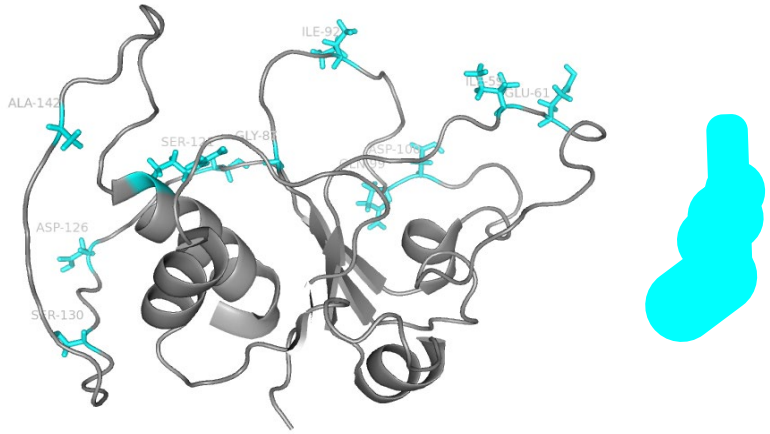
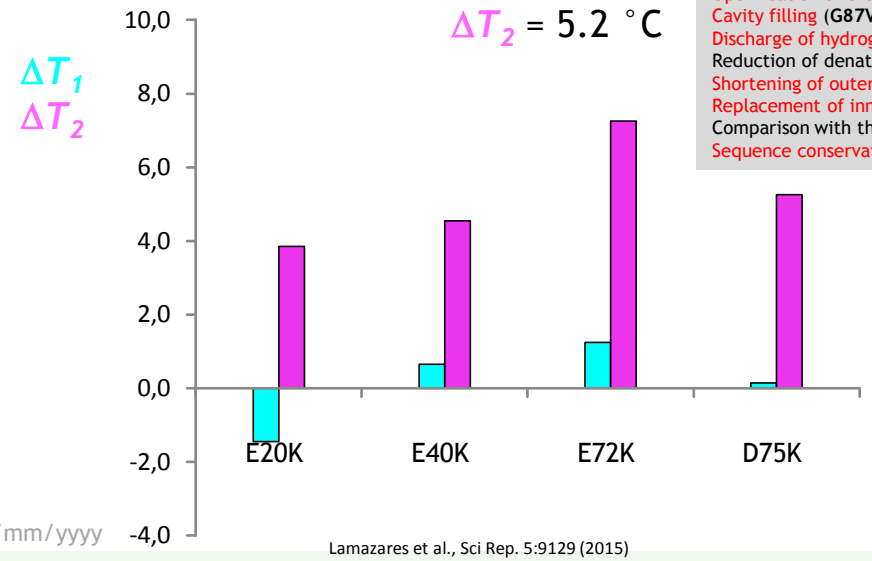
Three-state proteins





$\overline{\Delta T_1}$ = 0.1 °C

$\overline{\Delta T_2}$ = 5.2 °C

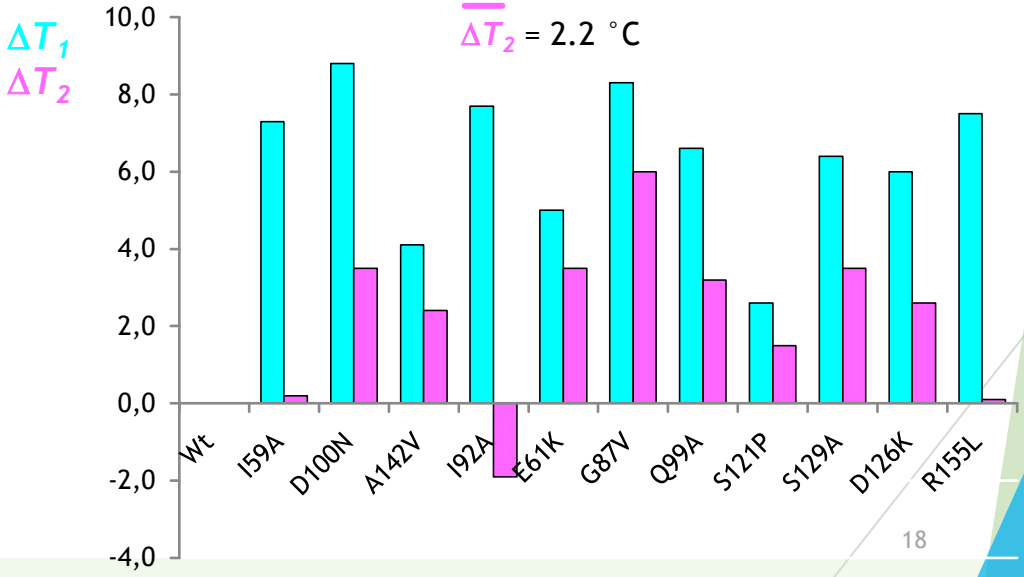


Lamazares et al., Sci Rep. 5:9129 (2015)

Disulfide bonds
α-Helix stabilisation
Optimisation of charge interactions (E61K, D126K)
Cavity filling (G87V, A142V))
Discharge of hydrogen bonds (D100N)
Reduction of denatured state entropy
Shortening of outer apolar residues (I59A, I92A)
Replacement of inner polar residues (Q99A,R155L)
Comparison with thermostable homologues
Sequence conservation (S121P, D129A)

$\overline{\Delta T_1}$ = 6.2 °C

$\overline{\Delta T_2}$ = 2.2 °C



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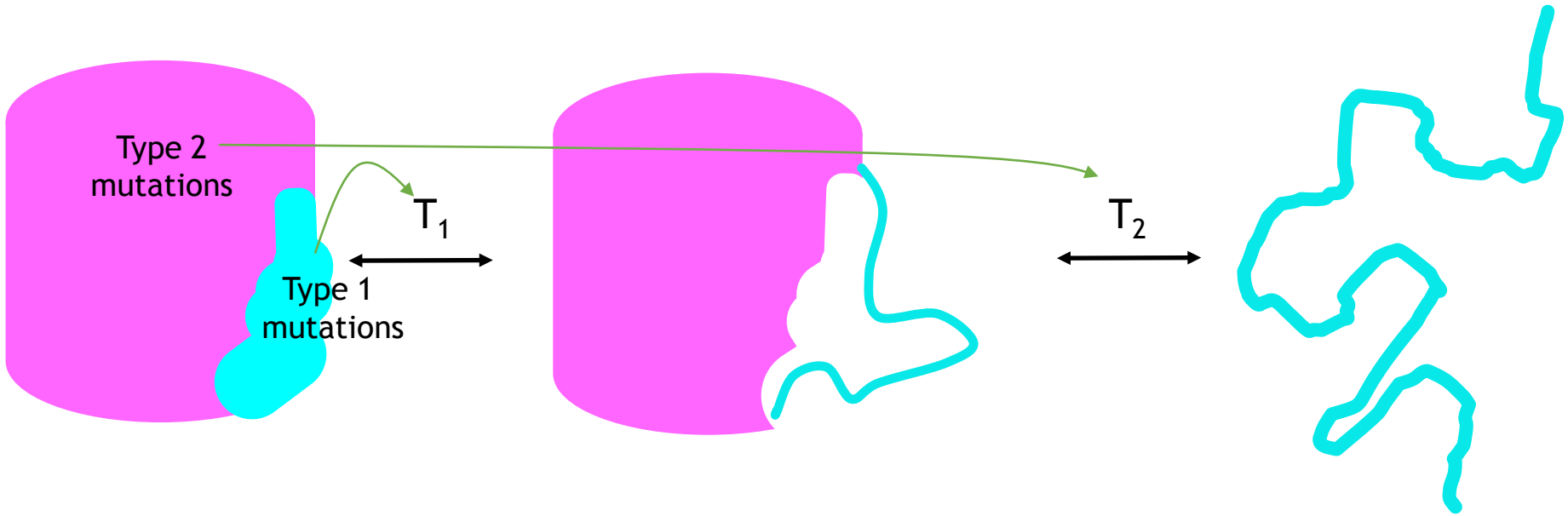
Three-state proteins



Type 1 mutations: located in the weakest region or at the interface with the rest, they increase the relevant stability

Type 2 mutations: they only increase the residual stability of the intermediate

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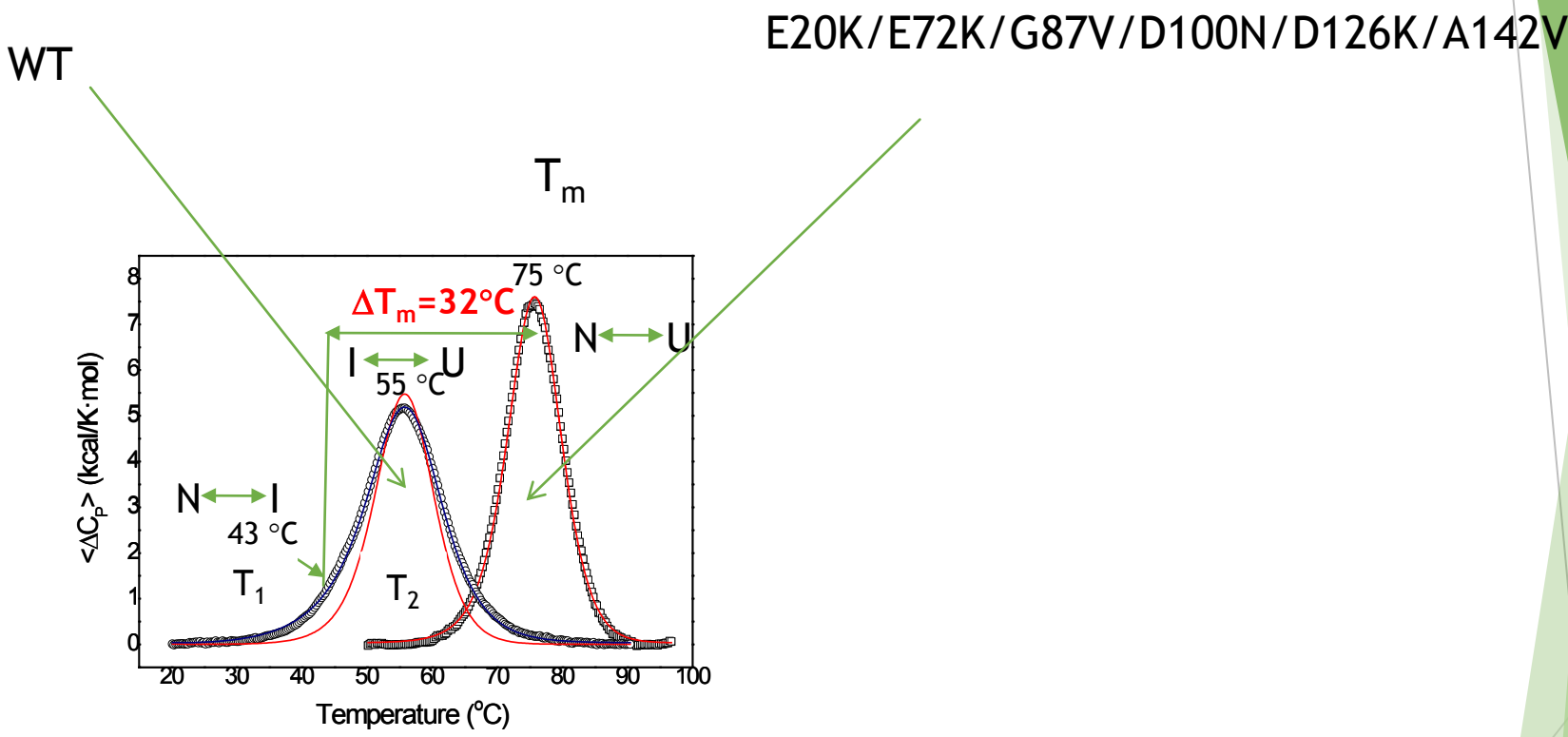
Three-state proteins



Lamazares et al., Sci Rep. 5:9129 (2015)

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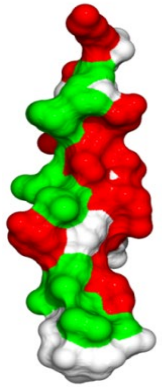
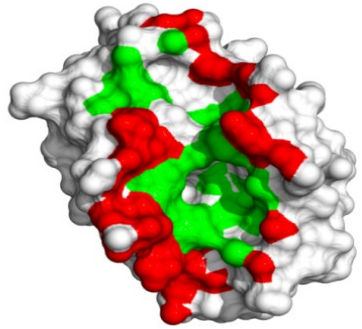
Three-state proteins

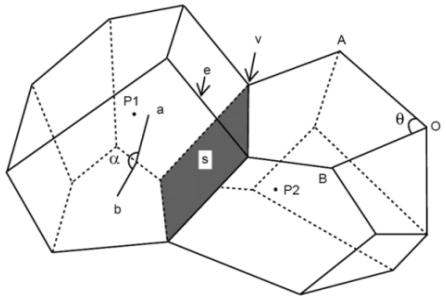
Polarity ratio





$$PR_{intraface} = \frac{\sum_{i=1}^{m} SASA_{(polar)_i}}{\sum_{j=1}^{n} SASA_{(hydrophobic)_j}}$$

Packing density



$$\rho_{intraface} = \frac{\sum_{i=1}^{N} V_i^{\circ}}{\sum_{i=1}^{N} V_i}$$

Angarica & Sancho *PLoS ONE 7(10):e48212. doi:10.1371/journal.pone.0048212* (2012)

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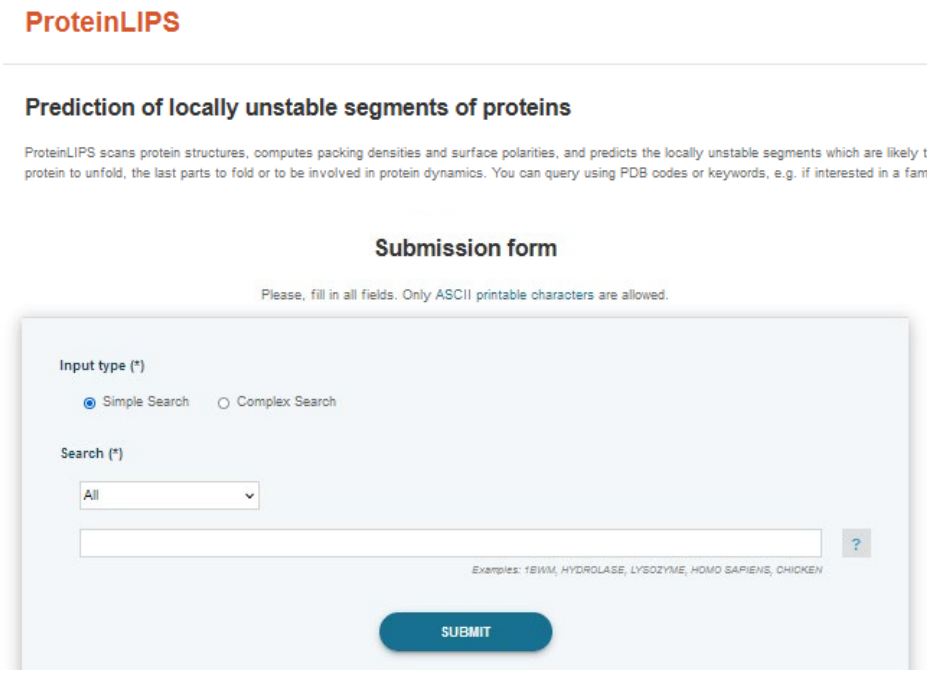
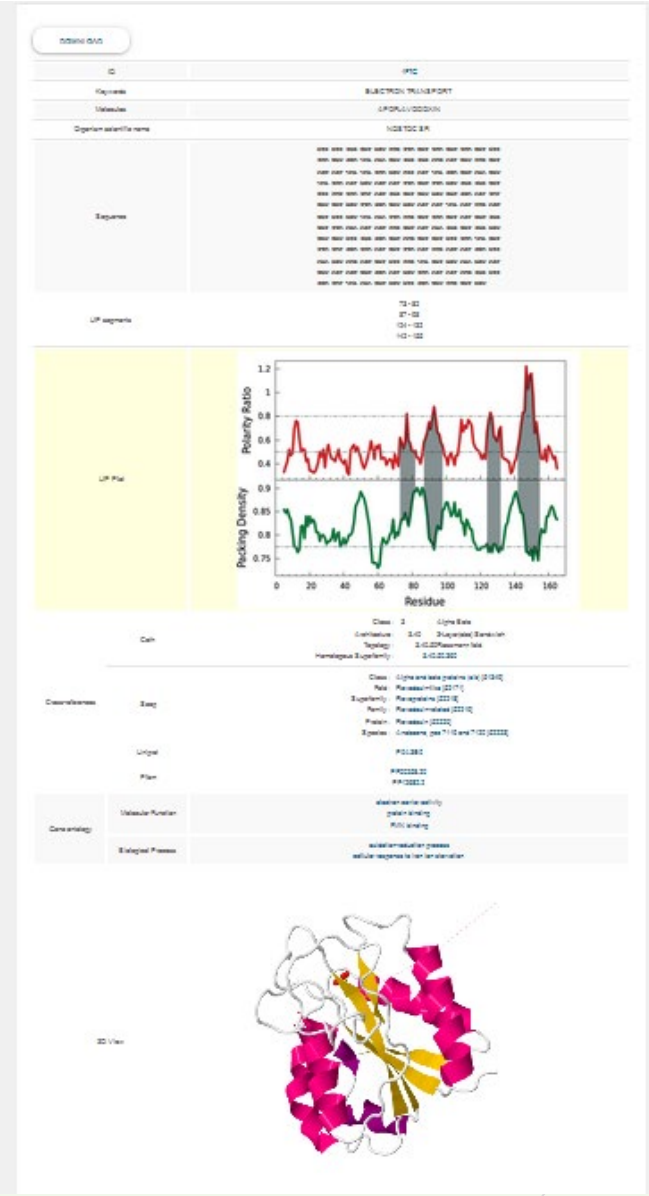
Three-state proteins

Old LIPS web

(only precalculated proteins) monomers)





Angarica VE, Sancho J. Protein dynamics governed by interfaces of high polarity and low packing density. PLoS One. 2012;7(10):e48212. doi: 10.1371/journal.pone.0048212. Epub 2012 Oct 26.

http://webapps.bifi.es/lips

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Three-state proteins

New LIPS web (any monomer or oligomer). Under construction.

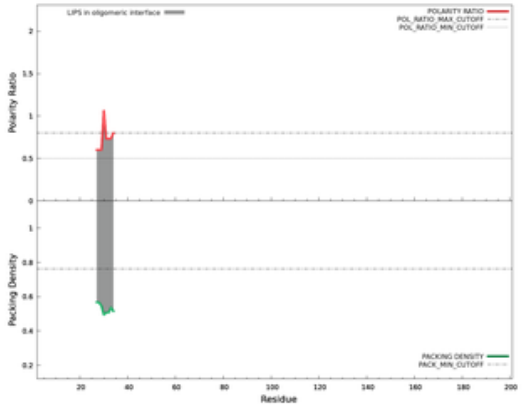
LIPS test

PDB: 7W6W

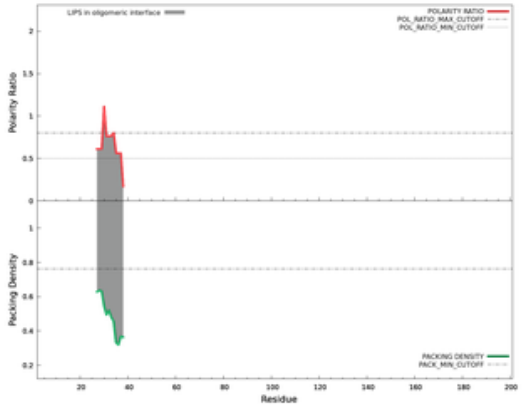
Estructura: 2-homo

Cadenas elegidas: AB

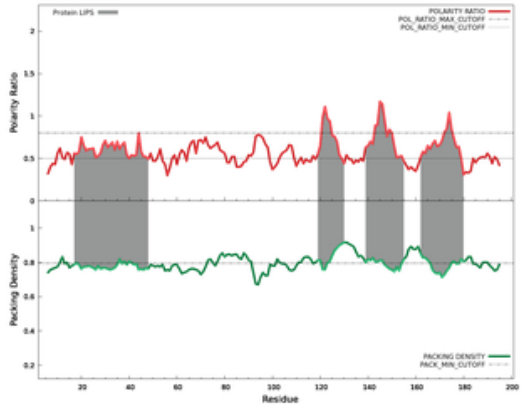
Preprocesado del PDB: Downloaded PDB from RCSB. No important issues found in the PDB analysed! Any minor issue resting in the PDB file will be translated to the generated LIPS profiles.



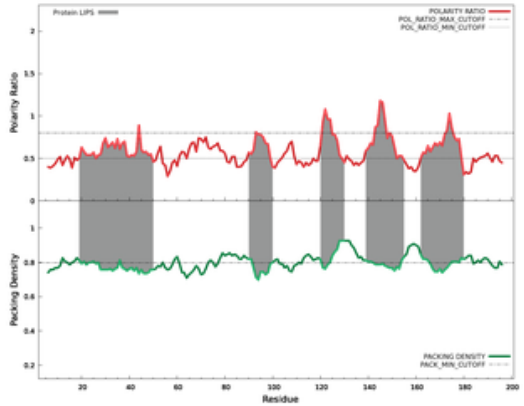
7w6w_dim_A.png



7w6w_dim_B.png



7w6w_mon_A.png



7w6w_mon_B.png

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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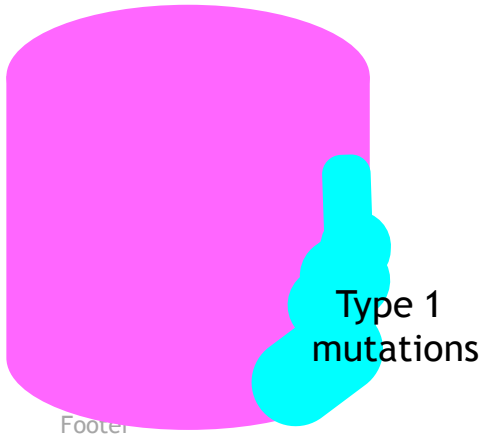
Three-state proteins

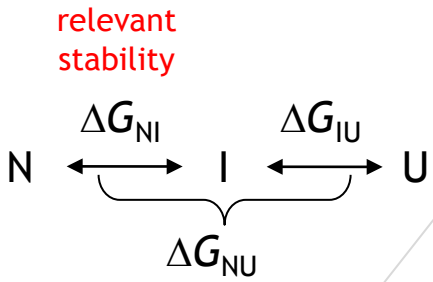
TO ENGINEER A MORE STABLE PROTEIN YOU WOULD LIKE:

1- To get a list of promising stabilizing mutations

and, unless the protein is 2-state

2-To identify the likely unstable regions of the protein





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DAY 3 (5 July, 2023) 9:00/12:00 Stabilization by formulation and mutagenesis

- Protein stabilization by formulation: some rational
- Examples
- Protein stabilization by mutagenesis: some rational
- Examples
- Three-state proteins

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