UNC CHARLOTTE

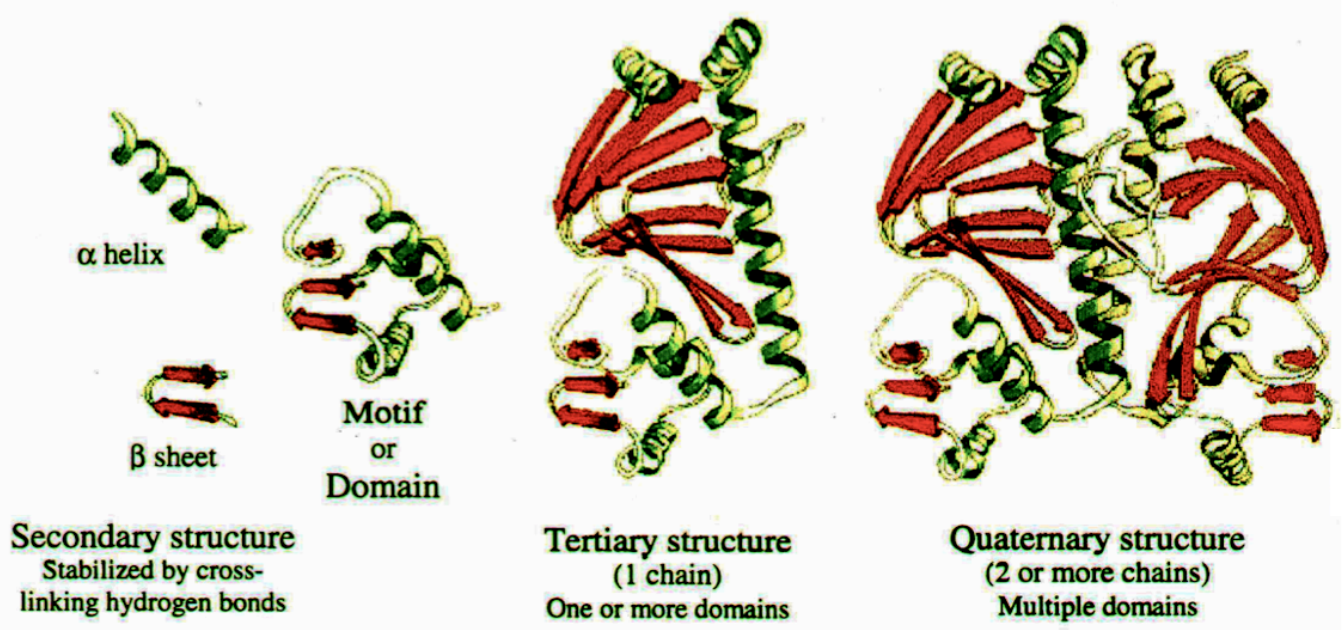
Interrelationships Between Protein Stability, Flexibility and Dynamics

Donald Jacobs, Associate Professor
Department of Physics and Optical Science

Depending on thermodynamic and solvent conditions, the interrelationships between thermodynamic stability of a protein its mechanical properties and how the protein responds dynamically are complex. The goal is to distill down the essential physics that can be leveraged to quantify the linkage between these different properties, and thereby tackling the complexity through a simple overarching principle.

Key idea: Correlations in the atomic motions of a protein must be uncovered.

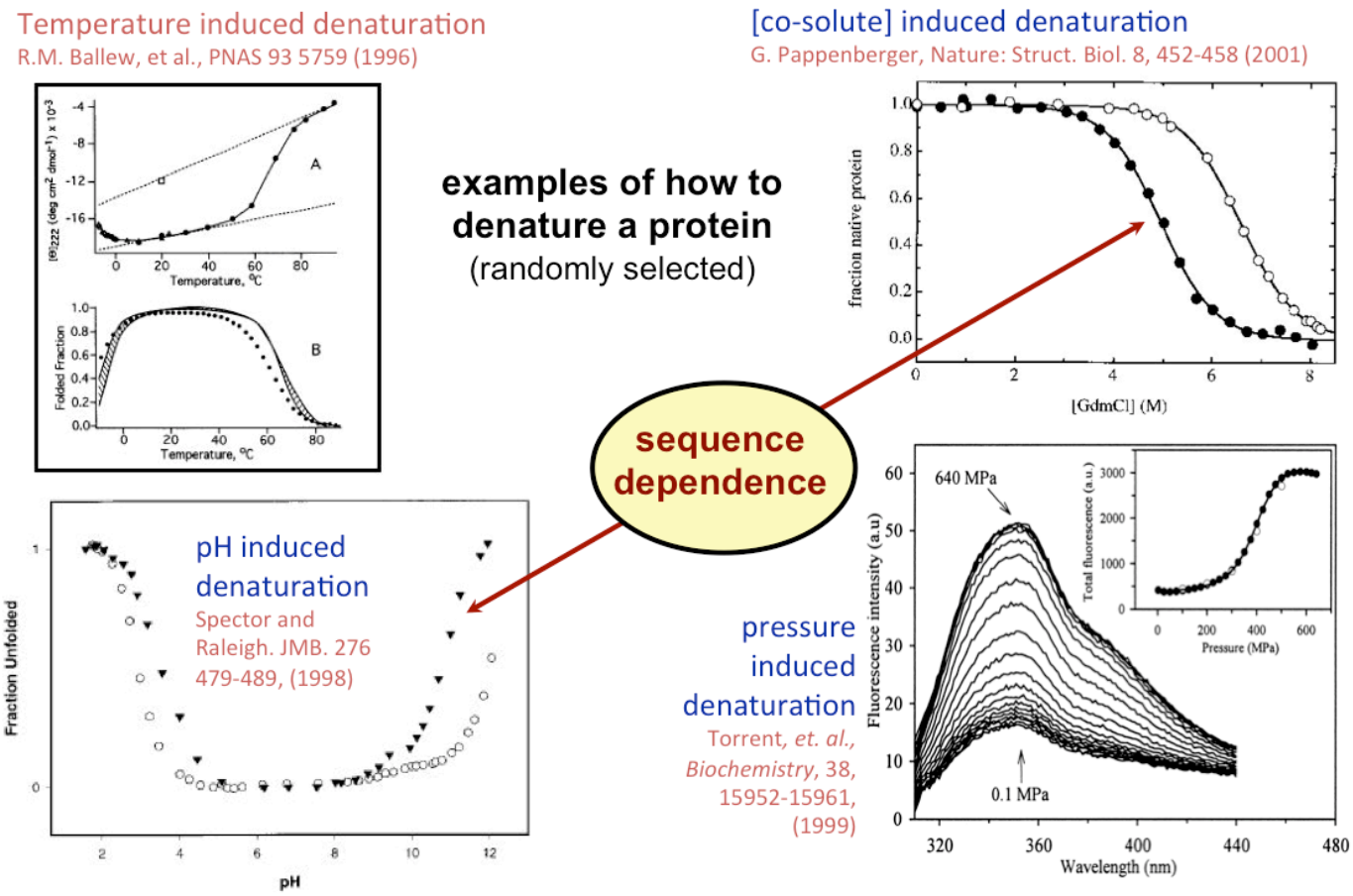
"One of the more astounding accomplishments of living cells is their ability to orchestrate and control specific chemical interactions between tens of thousands of proteins in the same reaction mixture" --- Richard Fine



Cartoon pictures are commonly employed to display protein structure. The majority of proteins have native states defined by folded structure, consisting of motifs or domains. These structures are stable both thermodynamically and mechanically due to many interactions. A key interaction is the hydrogen bond. Cross-linking hydrogen bonds play a critical role in maintaining stability. Quantitatively, it is not clear why cross-linking intramolecular hydrogen bonds will stabilize a protein. Indeed, because of the competition between hydrogen bond formation that occurs within a protein, compared to those interacting with solvent, the net effect of stabilization is not obvious. There are examples where intramolecular hydrogen bond formation destabilizes a region of a protein. In particular, the formation of intramolecular hydrogen bonds in globular proteins is thermodynamically destabilizing at very low and very high temperatures, but formation of a hydrogen bond is always mechanically stabilizing.

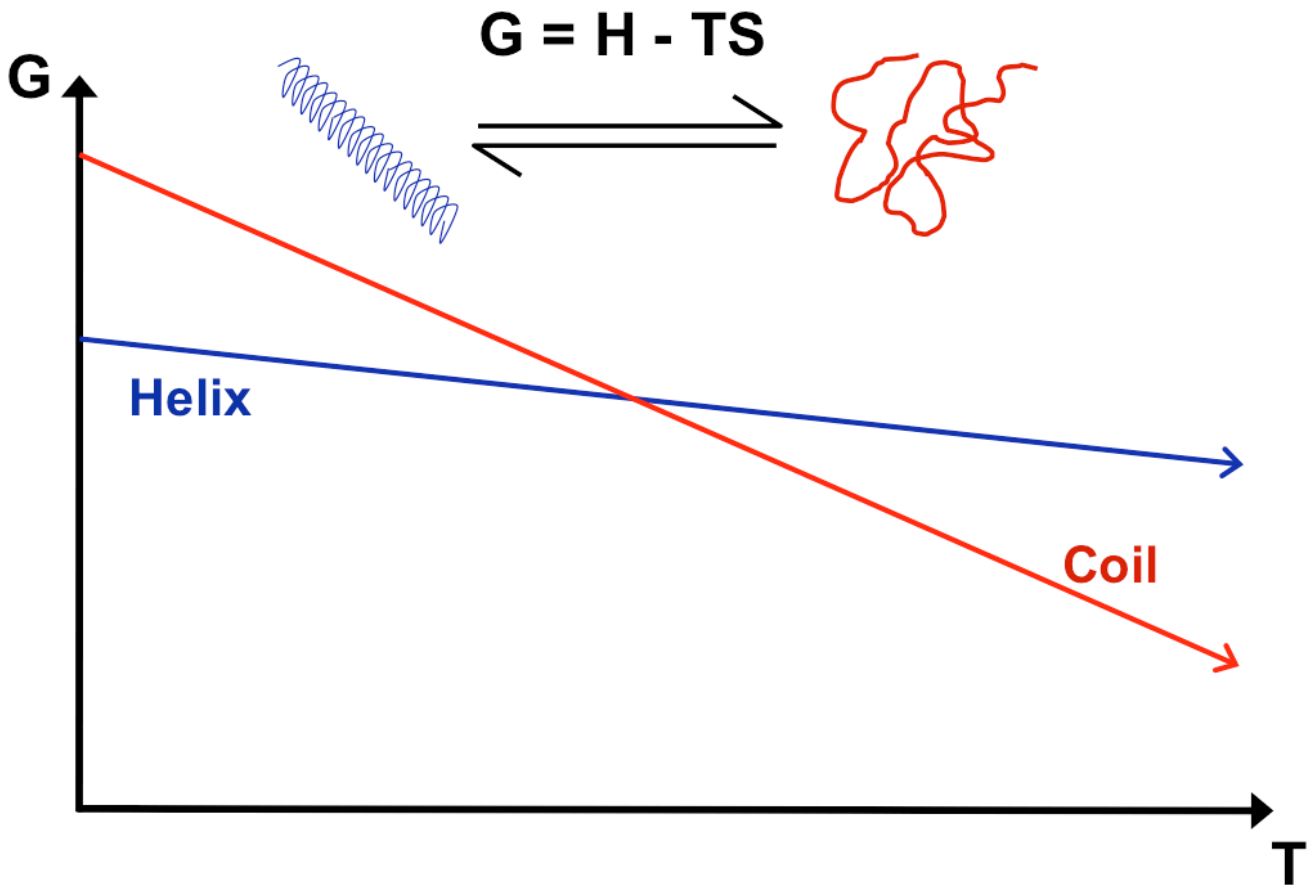
In general, thermal stabilization due to hydrogen bonds depends strongly on the nature of the solvent and thermodynamic conditions. To add to the complexity of the problem, the detailed nature of the effect of a hydrogen bond strongly depends on its local environment. To make matters much worse, besides hydrogen bonding, many other interactions affect protein stability. An accurate model must account for all types of interactions, including very weak interactions. However, to obtain an efficient model, it is common practice to drop unessential information. We know that even weak interactions are important to protein stability, which means there is no justifiable way to neglect any interaction. This creates a dilemma for developing a model that is both fast and accurate.

Key idea: Rather than trying to drop information, the dilemma is solved by striving to keep all of the information by bundling local information into packets, and then assembling the packets together.



Shown above are some examples of how changing the thermodynamic conditions (temperature, T) or (pressure, p) or solvent conditions (affecting pH) using chemical additives (possibly denaturants) can thermodynamically drive a protein to fold into a native state, or unfold into a denatured state. It is very interesting that a small change in protein sequence (also called its primary structure) can lead to a dramatic difference in thermodynamic, mechanical and dynamic properties, which explains why there are changes in function.

Key idea: Protein stability is dependent on both sequence and thermodynamic/solvent conditions.

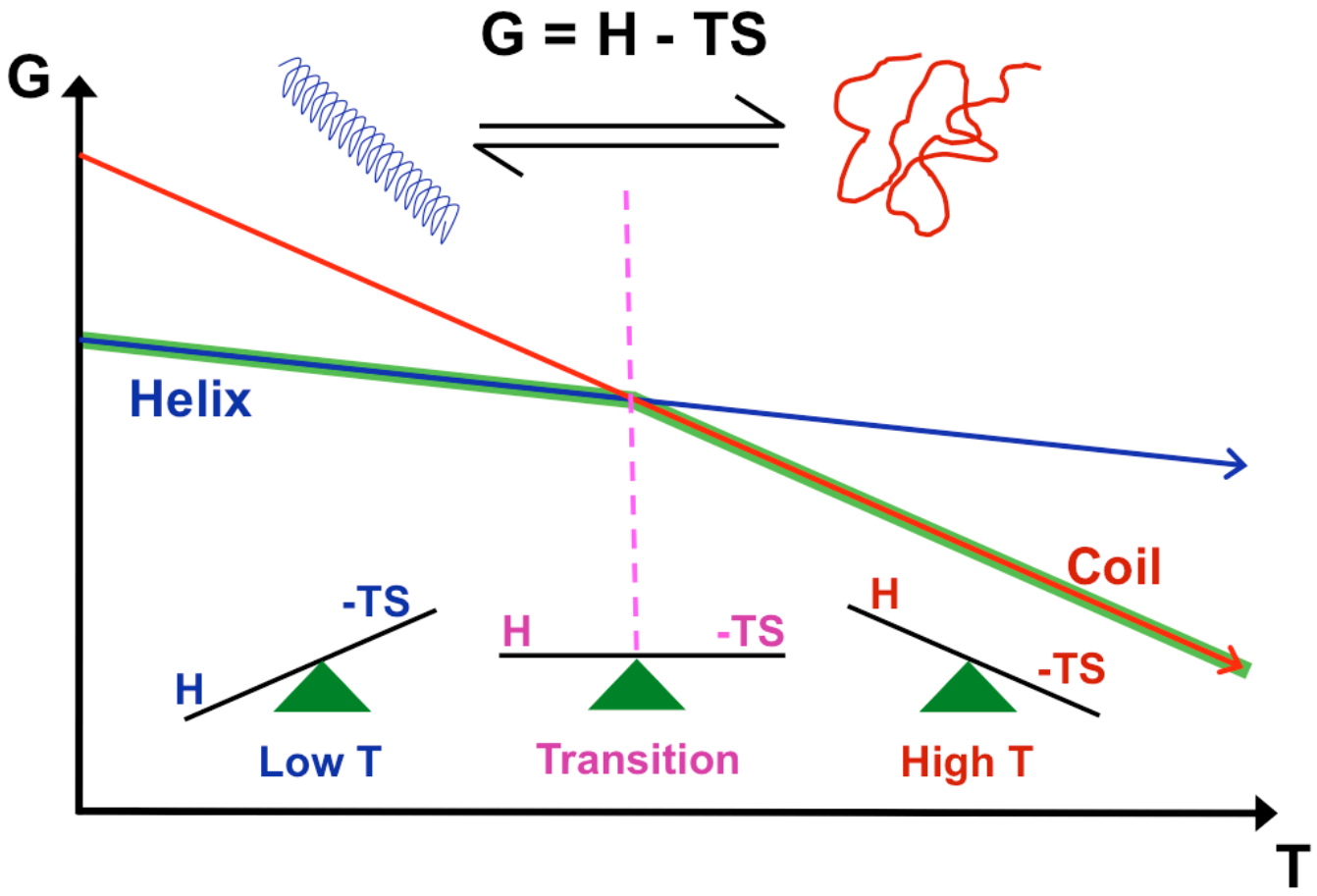


J.A. Schellmann, J. Phys. Chem. 62, 1485-1492 (1958)

For some time, the basic idea of protein folding (illustrated as an alpha-helix to coil transition) can be explained by accounting for energy and entropy contributions. The helix structure represents one thermodynamic state defined by an ensemble of microstates, while the coil represents another thermodynamic state also defined by an ensemble of microstates. An ensemble of microstates lists all structural variations. Different structures are often called configurations or conformations. While there is a difference in meaning between the words configuration versus conformation, the meanings are usually different for different people, and usually used interchangeably outside of rigorous definitions.

The coil state has relatively much more conformations in its ensemble compared to the number of conformations that make up the ensemble of the alpha-helix state. As such, the coil state has high entropy, while the alpha-helix has low entropy. On the other hand, the alpha-helix has more compact structure, which gives more intramolecular interactions that lowers its overall energy compared to the coil state. Through a back of the envelope estimate, one can quickly understand that a transition can occur, because at low temperatures, the alpha-helix will have lower free energy, while at high temperature, the coil will have lower free energy. The state with the lowest free energy is the stable one. When the free energies of the two states are equal, one can expect 50% of the peptides to be in a alpha-helical form, and 50% of the peptides to be in coil form. This is a two state model.

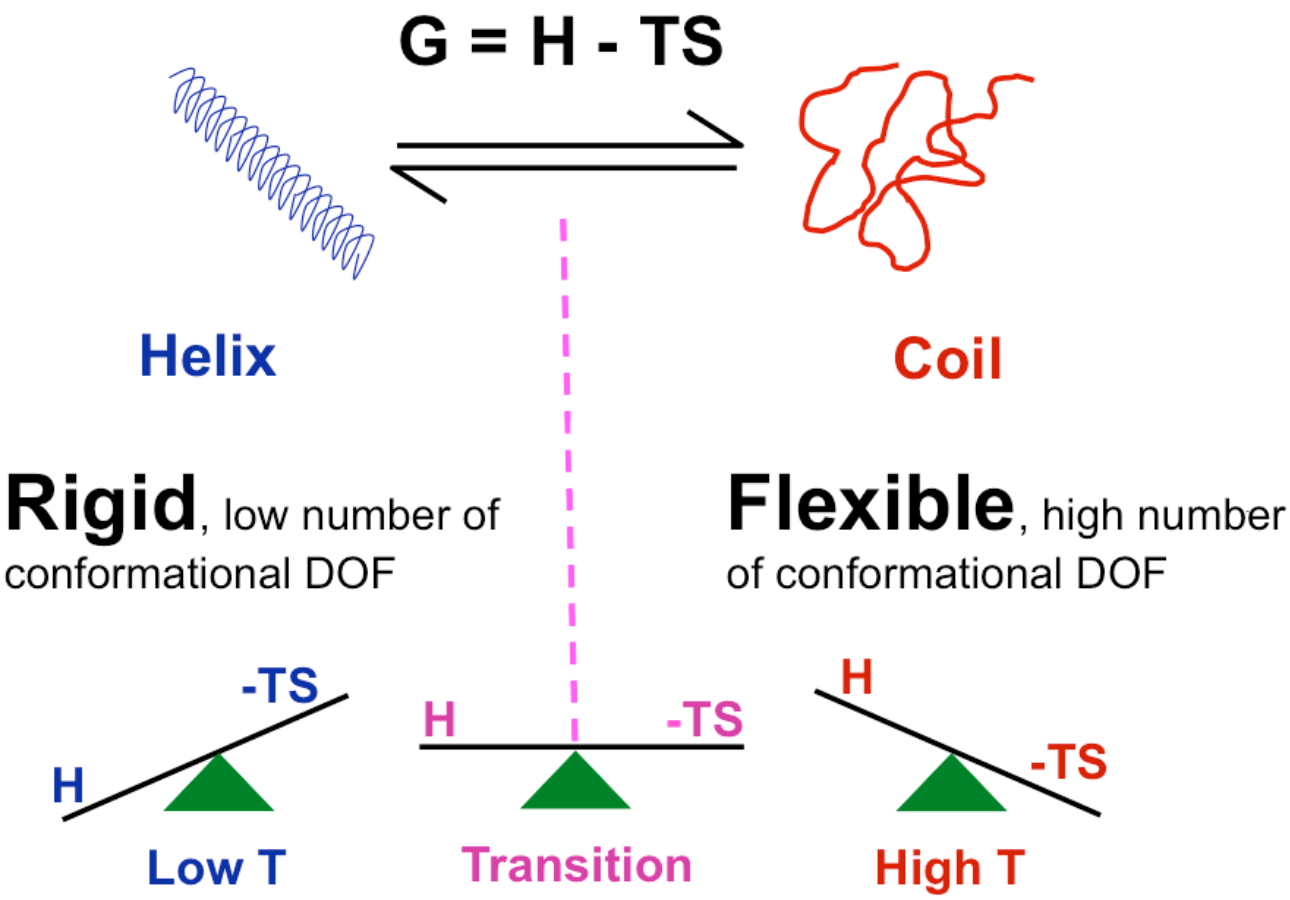
Key idea: Thermodynamic states represent conformational ensembles characterized by energy and entropy contributions.



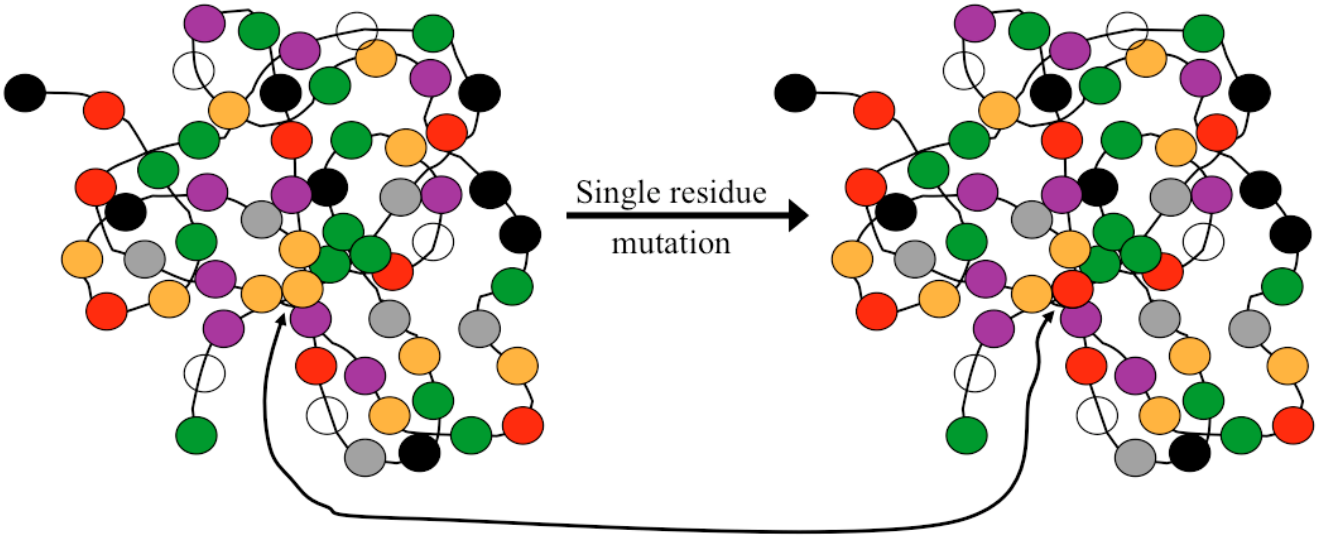
J.A. Schellmann, J. Phys. Chem. 62, 1485-1492 (1958)

Formation of a collection of intramolecular interactions that yield a net lower energy for the system when the structure is compact will be stabilizing at low temperature and for the same reason, will be destabilizing at high temperature because the gain in conformational entropy can compensate for an increase in energy, provided the temperature is high enough.

Key idea: Each interaction affects the delicate energy-entropy balance in a protein.



Key Idea: As more intramolecular interactions form to help keep the protein compact, the number of degrees of freedom (DOF) for describing conformational motions decreases relative to the number of DOF describing conformational motions when these intramolecular interactions are not present.



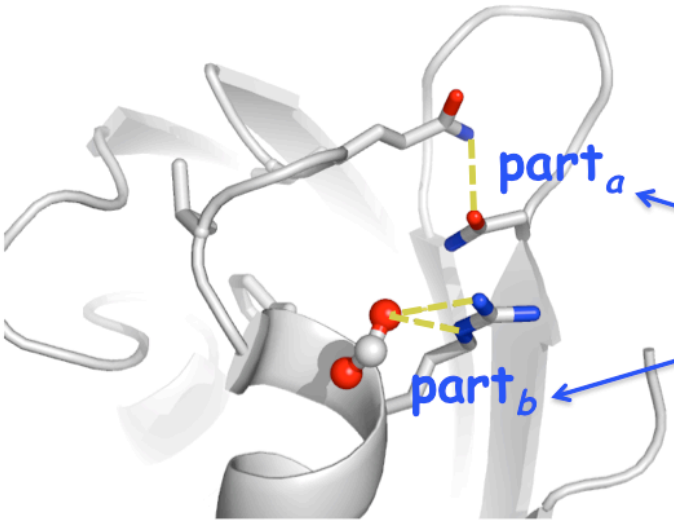
Linearity assumption of total free energy

$$G_{protein} - G_{ref\text{-}state} = \Sigma \Delta G = \Sigma (\Delta H - T\Delta S)$$

Calorimetry Measurements give: ΔG, ΔH, ΔS from model compound transfer

Many thermodynamic models and Ising-like models including the Zimm-Bragg & Lifson-Roig models for the helix-coil transition that interpret interactions in terms of free energies, not energies, are based on the assumption of additivity. This approach has the advantage that it is very fast and is wonderfully simple to work with. The only disadvantage of this approach is that it is wrong whenever there are any correlations within the interaction network. Models using the additivity assumption will have limited utility when component free energy contributions are expressed in terms of dynamic variables that are decoupled to one another. For example, normal modes of vibration or solvent accessible surface area might be such type of variables to some level of approximation. In general, the change in energy and entropy will depend on the local environment of the constituent being mutated, such as a residue.

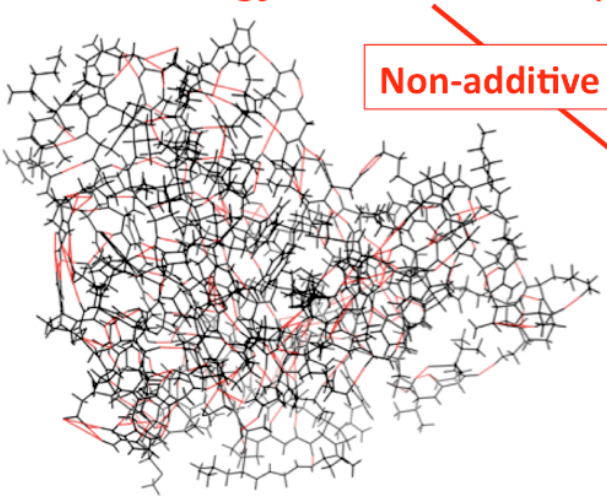
Key idea: Change in free energy due to a perturbation will strongly depend on the local environment of that perturbation, and, for additivity principles to hold, the atomic structure must be well described by uncoupled variables in order to maintain complete independence.



Ledger of free energy contributions from specific interactions

$$G^{FED}_{part}$$

Free Energy Reconstitution (FER)



Non-additive parts

$$G_{total} \equiv \sum_{parts} G^{FED}_{part}$$

Hidden Thermodynamics
Prevents additive rules from reproducing macromolecular free energies.

Hidden Thermodynamics of Mutant Proteins, Gao, Kuczera, Tidor and Karplus, Science 244, 1069 (1989).

Additivity Principles in Biochemistry, Dill, J Biol Chem 272, 701-704 (1997).

Decomposition of the Free Energy of a System in Terms of Specific Interactions, Mark and van Gunsteren, *J Mol Biol* 240, 167 (1994).

"In regard to the detailed separation of free energy components, we must acknowledge that the hidden thermodynamics of a protein will, unfortunately, remain hidden."

Molecular cooperativity appears when there is coupling between subsystems that represent individual molecular interactions. In proteins, a dense interaction network makes the additivity assumption flawed when local variables are used to describe component parts of the system because of the correlations that exist between the variables.

Key idea: Non-additivity is caused by molecular cooperativity, and hidden thermodynamics is related to not knowing what correlations are present that give rise to the observed molecular cooperativity.