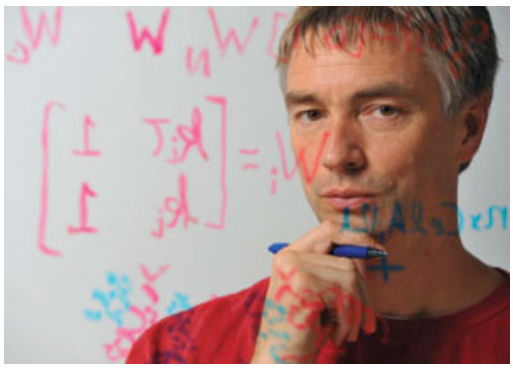
Physical Chemistry Seminar

Tuesday, March 5, 2013 | **11:00 am** | **Room 1315 Chemistry Building**

Using repeat proteins to learn about cooperatively, folding pathways, and sequence determinants of stability



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Host: Professor Silvia Cavagnero

One of the most striking features of the protein folding process is cooperativity. Although the structures of the native states of proteins comprise many short- and long-range elements that might appear to be independent (in a thermodynamic sense), these elements are in fact highly coupled, populating not as independent subsets but as a whole. Cooperativity in folding is likely to use the same underlying interactions, and thus, have the same thermodynamic features as functional properties such as allostery and assembly. Despite the importance of cooperativity in folding, our analysis of it remains largely at the "yes" or "no" (i.e., two-state versus multistate) level. We have been using linear repeat proteins to extend our description of cooperativity in folding to a quantitative (thermodynamic) level. By constructing an array of 33-residue ankyrin repeats that have varying length but identical (consensus) sequence, we have resolved the high cooperativity of unfolding into local versus nearest-neighbor energy, entropy, and heat capacity. This approach has revealed an extremely strong coupling free energy between repeats. We find this coupling to be entropically driven at low temperatures, consistent with hydrophobic desolvation, and to be highly sensitive to histidine protonation state and to salt concentration, suggesting an electrostatic contribution to interface formation. This high level of coupling is considerably stronger than that seen in a set of consensus TPR repeats. A high level of cooperativity can also be achieved with consensus LRR β-sheet repeat proteins, although we find that design well-behaved consensus LRRs is a considerably greater challenge than for helical arrays. Linear repeat proteins are also well-suited for kinetic studies of folding. Due to the high level of structural symmetry of naturally-occurring (sequence-variable) repeat proteins, and the additional sequence symmetry of consensus repeat counterparts, we can examine the effects of chain-length on folding rates, the presence of parallel pathways, and the relationship between stability variation and cooperativity in determining rates and pathways. In kinetic studies with consensus ankyrin repeat folding, we find multiple parallel pathways with a transition state that appears to have around 1.5-2 repeats folded but not yet paired (i.e., coupled). In contrast, we find that most naturally occurring repeat proteins fold through a single dominant pathway that corresponds to a region of lowest stability, and will illustrate this with folding and HX studies on a LRR protein. From the recurrent observation that the local free energy variation (1-2 kcal/mol) of naturally occurring repeat proteins is enough to collapse parallel folding into a single dominant pathway, it is tempting to generalize that folding by parallel pathways may be the exception rather than the rule, perhaps even more for globular proteins than for repeat proteins.

Refreshments will be available prior to the seminar at 10:45 a.m. outside room 1315

Graduate Students may meet with the speaker at 1:00 p.m. in Room 8335