# *GUEST EDITORIAL*

## Protein Folding

Protein folding was first recognized as a "problem" forty years ago when Anfinsen showed that denatured ribonuclease could be refolded to its native form in vitro.[1] The essence of the problem is understanding how a randomly configured heteropolymer navigates through configuration space to the unique structure of a functional protein. Levinthal argued that a random search of the configuration space for a 100-amino acid protein would require $10^{27}$ years,[2] leading him to conclude that proteins follow discrete folding pathways.[3] The "new view" of folding maintains that the energy surface for folding is shaped like a funnel, and that the dynamics of folding involve a large number of elementary steps occurring in parallel.[4]

Protein folding is a singular problem in chemical kinetics requiring the conversion of a heterogeneous ensemble of reactants into a homogeneous product. Theory has played a central role in defining key questions in this area.[4−6] Lattice-model simulations, in particular, have been effective in reproducing several aspects of folding in real proteins. This research is helping to define the nature of the energy surface and the origin of the barriers to folding.

We felt it was time to put together a special issue of *Accounts of Chemical Research* focused on experimental research in protein folding. Work in this area is undergoing explosive growth. Burst-phase dynamics observed in stopped-flow investigations of folding kinetics, as well as a veritable avalanche of stimulating theoretical papers, have provided the impetus for the development of faster methods (ultrafast mixers, laser temperature jumps, and laser-initiated photochemistry) for triggering folding reactions. These studies of the early events in protein folding are providing important new insights that will allow theoreticians to refine their models and make new predictions about the mechanisms of these complex transformations.

Several articles address the folding of a small single-domain heme protein, cytochrome *c*. That each of these has a different interpretation of the folding kinetics speaks to the complexity of the problem and the vibrancy of the field. An ultrafast mixer has been employed by Roder to characterize the burst-phase dynamics in ferricytochrome *c* folding. Rousseau has used ultrafast-mixing techniques and Raman spectroscopy to probe the ligand substitution kinetics. Englander has employed a combination of stopped-flow mixing and pulsed deuterium exchange NMR data to develop a detailed model of the reaction surface and folding mechanism in ferricytochrome *c*. Using a photochemical trigger, Eaton and co-workers have studied early ligand-binding events involved in ferrocytochrome *c* folding. In our own work, we have developed methods for triggering heme−protein folding by laser-initiated electron-transfer reactions.

Two articles describe laser-temperature-jump investigations of protein folding. Gruebele has examined the fast folding of cold-denatured apomyoglobin. Dyer and Woodruff have used laser temperature jumps and infrared probes to study helix formation in small peptides and proteins. Oliveberg's Account sounds a note of caution regarding the possibility of confusing transient protein aggregates with folding intermediates. Finally, Dobson and co-workers review recent efforts to use "real time" NMR spectroscopy to probe protein folding.

Protein folding was once considered an almost intractable problem; new experimental and theoretical efforts, however, are beginning to reveal the secrets of this prototypal spontaneous self-assembly process.

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