Chapter 1

Thesis Overview

It is not known how unfolded proteins acquire their functional shapes, and how they do so on biologically relevant timescales. A simple calculation conducted by Cyrus Levinthal in 1966 showed that if proteins were to fold by randomly searching through all possible conformations, the time to fold even a small protein of 100 amino acids would be on the order of the age of the universe.[1,2] A *sequential folding* model was proposed; its simplest form, folding would proceed along a defined pathway with discrete intermediates while nucleation being the slowest step. The "new view" of protein folding kinetics[3] replaced the concept of a single "folding pathway" by suggesting presence of multiple folding routes on an energy landscape shaped like a funnel. Each unfolded protein molecule may fold along its own path on this energy surface but eventually most of them will reach the so-called native state. The molecules that collapse to native topologies fold fast whereas the structures that collapse to non-native topologies fold slowly.

The material presented in this thesis describes development of a new, fluorescence-based methodology for mapping the complex energy landscapes of proteins. Cytochrome *c* (yeast variant) was chosen as a model protein for the studies, as it is one of the most thoroughly investigated electron-transfer proteins.[4-6] Chapter 2 describes probing the structural features of the polypeptide ensemble at equilibrium. This chapter details preparation and characterization of cytochrome *c* variants with a fluorophore placed at different points along the polypeptide structure and the compound 1,5 NAC-AEDANS used to model the photophysical properties of the completely unfolded proteins. Chapter 3 and Chapter 4 present a study of the structural characteristics of the polypeptide ensemble as it evolves to the native state. Chapter 5 explores the relationship of the salt-induced partially folded state, known as the molten globule, to the states populated transiently

during refolding. In Appendix, an unfinished work on folding of reduced cytochrome *c* in presence of imidazole followed by fluorescence and a promising reductive flash-quench-scavenge system for a delivery of reductive pulse to generate reduced cytochrome *c* are described.

REFERENCES

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