

Percolation Behavior of Structure Networks from Thermostable Proteins: Implications for Thermal Adaptation, Substrate Binding and Enzyme Activity

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Protein stability can be expressed in terms of the free energy difference associated with macro- and micro-unfolding of the native structure.^[1] Macro-stability (ΔG_{mac}) is characterized by the work required to transfer the protein from the folded to the unfolded macroscopic state. Micro-stability, on the other hand, is characteristic of the rigidity of the structure, with ΔG_{mic} as the free energy associated with the local, reversible unfolding reactions within the folded state. Due to very large contributions of stabilizing and destabilizing interactions involved in the formation of the folded state, protein structures can be described as molecular networks.^[2] The network concept is widely used to analyze and predict the stability and dynamics of complex systems.^[3]

In the present work, we simulate the dilution of the noncovalent bonds during thermal unfolding of a protein structure, and identify the emergence of flexible regions as unfolding proceeds. The method is applied to a dataset of thermostable proteins and their homologues from mesophilic organisms. The structure networks at different temperatures are decomposed into rigid clusters and flexible regions using the FIRST software.^[4] The percolation behavior and other structural properties obtained from network analysis are compared and related to the thermodynamics of protein stability.

We show that the general percolation behavior of protein structure networks is complex. Nevertheless, the percolation behavior of structure networks from thermophilic proteins and their mesophilic homologues allows us to characterize key events in the thermal adaptation of proteins. Many thermophilic proteins in our dataset appear to be more stable than their mesophilic homologues. The comparison of structure networks from thermophilic and mesophilic enzymes further allows investigating the determinants of substrate binding and activity.

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