

Structural consequences of the ionization of internal groups in proteins

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Abstract. Internal ionizable groups in proteins play essential functional roles in biochemical processes related to bioenergetics, including catalysis, H⁺ transport, and e⁻ transfer reactions. To elucidate the mechanisms and the structural basis of function in these proteins, it is necessary to understand how the ionization of internal groups is coupled to structural reorganization of the protein. Backbone relaxation is one of the ways in which proteins respond to the ionization of internal groups. We have studied proteins from a family of variants of staphylococcal nuclease in which internal positions have been substituted with polar or ionizable residues. Crystallographic structures as well as CD, steady state fluorescence, and NMR spectroscopy have identified cases where the ionization of the internal group leads to local or global unfolding of the protein. We have performed an extensive study with Self-Guided Langevin Dynamics (SGLD) simulations to model these backbone relaxation processes. In general, the simulations are in agreement with experimental observations. For example, a crystallographic structure of the V66R variant shows that a β strand is released from a β -barrel upon charging of the internal Arg-66. Breaking of hydrogen bonds between these β -strands are recorded occasionally in the SGLD simulations, sometimes leading to the irreversible, partial separation of the strands. Another set of SGLD simulations records the separation of several strands in the β -barrel and extensive hydration of the core in the I92D variant, which is known to unfold when the internal Asp-92 is ionized. The data suggest that SGLD simulations will be useful to reproduce long time-scale events such as complex structural reorganization induced by ligand binding, which are not reproduced well with standard molecular dynamics simulations.