

# Molecular Dynamics Simulations of Unfolded Protein States

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The field of protein folding has expanded in recent years, and it now encompasses the more complex, but biologically important relationship between protein misfolding, aggregation and disease. [1] In this respect, an important goal is to develop a model that can make quantitative predictions about the effect of mutations in the amino acid sequence. To develop such a model, detailed knowledge of the structural and thermodynamic properties of the different species involved in folding is necessary. The structure, dynamics, energetics and mechanistic relevance of the unfolded ensemble need to be understood. A number of recent experimental studies has highlighted that many proteins, when unfolded, are not random coils. Instead, residual structure, in some cases of a native-like nature, has been found in the unfolded state.

Site directed mutagenesis and nuclear magnetic resonance (NMR) spin relaxation experiments have been used to show that in unfolded lysozyme, different hydrophobic residues stabilize a network of native-like hydrophobic clusters with long-range non-native interactions. [2] Characterizing the unfolded state of lysozyme in detail experimentally remains challenging due to the rapid interconversion between different members of the configurational ensemble. In the present study, unconstrained molecular dynamics (MD) simulations are used to gain insight into the unfolded state of wild-type and mutated lysozyme in atomic detail. These simulations provide a complementary view to experimental results. Starting from an extended state, the systems reach the unfolded state in approximately 8 ns. An implicit solvent model was used. To prevent non-specific collapse of the chain, and to enhance sampling in the unfolded state, 16 interchanging replicas of the system were simulated simultaneously at different temperatures. The simulated ensembles are heterogeneous at 300 K, and average properties, such as the radius of gyration, are in accordance with experimental data. In addition, just as in the experiment, significant interaction between hydrophobic clusters can only be found in case of wild-type lysozyme. It is concluded that unfolded protein states can be simulated without the introduction of biasing forces or restraints using molecular dynamics.

[1] C.M. Dobson, *Nature*, **2003**, 426, 884-890.

[2] J. Wirmer, C. Schlörb, J. Klein-Seetharaman, R. Hirano, T. Ueda, T. Imoto, H. Schwalbe, *Angew Chem Int Ed*, **2004**, 43, 5780-5785.