Reliable structural and energetic model of the "unfolded state" of proteins

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The traditional structural and functional representation of proteins, limited to their native folded state has been challenged by a new description where partially or completely unfolded conformations are increasingly important. The poor knowledge about the non-native conformations, such as the partially unfolded intermediate states, the intrinsically unfolded domains, and the ensemble of conformations known as the "unfolded state", constitutes a pressing problem. In this work we aimed at developing a realistic structural and energetic model for the "unfolded state" of proteins that allows to calculate one thermodynamic property, the unfolding enthalpy (ΔH_u), as a first step towards predicting stability (ΔG_u). From one model protein we performed MD simulations both of the solved wild type structure (the folded state) and of a randomly, sufficient selected subset from ~2200 unfolded structures released from the ProtSa server (an 'a priori' representation of the "unfolded state") for each of these two proteins. We take into account the solvent effect on the calculation of the theoretical ΔH_u by differentiating between the contributions of the first water shells ("biological waters") and that the bulk. Despite huge absolute enthalpies are obtained from these simulated states, *i.e.* folded and unfolded, the estimation of ΔH_u ($\Delta H_u = H_u - H_f - \Delta H_{solv\text{-}eff}$) is accurate and reproducible. A second related estimation, the calculation of the unfolding specific heat variation (ΔCp_u) is also carried out. Quantum corrections and rmsd/system-size effects are assessed. ΔCp_u estimation also match well with experimental results. Statistical analyses to warranty convergence of results and to establish the minimal number of unfolded structures required to reliably represent the "unfolded state" are performed. We propose the present methodology to reliably establish the "unfolded state" (a "minimum unfolded state") in proteins with similar characteristics to that here modelled.