Antonio Trovato

Title: Calorimetric cooperativity revisited and generalized: the role of intermediate states

Abstract: The calorimetric criterion is one of the experimental approaches used in the last decades, in particular in the context of protein folding, for assessing the cooperativity of many biophysical processes, modelled as transitions between a high temperature to a low temperature state. In a maximally cooperative two-state transition, no intermediate states are present at equilibrium, implying that the effective two-state van't Hoff enthalpy change equals the experimentally measured enthalpy change. The ratio of the former quantities is then defined as the calorimetric cooperativity index, which is typically evaluated at the transition temperature. Here we prove a quantitative connection, valid at all temperatures, between the deviation of the cooperativity index from unity and the properties of the ensemble of intermediate states. This result appears to be surprisingly absent in the literature so far, and it allows us to show how deviations from two-state behaviour do not simply depend on how much intermediate states are populated, but rather on the distribution of their energy values. Moreover, it shows that the calorimetric criterion is in principle a necessary and sufficient condition for a two-state process, contrary to previous claims. We discuss how the latter ones are due to a non correct characterization of the high temperature state, highlighting how the definition of the low temperature and high temperature states as ”macroscopic” ensembles of microscopic states is taken into account within our result. In fact, experimental data provide ”per se” only a little knowledge about the two ensembles, with the arbitrariness of ensemble definition leading to a corresponding arbitrariness in the value of the cooperativity index. Finally, we discuss the above ideas, and how the generalized cooperativity index can be used to characterize dynamical transitions, from cooperative to noncooperative behaviour, within a simple exactly solvable model of sequential folding.