Using *R* for Data Simulation and Regression of Isothermal Titration Calorimetry of Proteins with Alternative Conformations

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All proteins have alternative conformations. For some, the partition function is much concentrated on one of the conformations than others. When a ligand binds to a protein with alternative conformations, data analysis of isothermal titration calorimetry (ITC) experiments can become complicated. A model system of a ligand binding to only one of two alternative conformations of a protein was analyzed in the *R* language environment, with extensive use of the package **minpack.lm**. A multitude of symmetry is found for this reaction system between the total ligand concentration and the total protein concentration. Reverse ITC titrations with the same experimental parameters yielded exactly the same data for this system, according to the analysis and simulations. Regression of simulated ITC data for the system with the simple binary binding model all converged when binding could be observed, though there are situations where binding can not be observed even when there is significant binding between the ligand and the one binding-capable protein conformation. Depending on a defined ratio of reaction parameters between the two reactions, namely the transition reaction between the two protein conformations and the binding reaction, regression of the system to the binary binding model can appear normal or give abnormal stoichiometry. Changing concentrations of reactants in different ITC runs did not affect the regression behavior of the system with the binary binding model, meaning that the same observed reaction enthalpy and binding constant values were obtained. These simulations provide the reaction signatures of the modeled system, which can be helpful when determining the reaction mechanism of a reaction. The simulations also demonstrated that ITC alone can not deconvolute the two sets of reaction constants for the system, and that potential pitfalls need to be cautioned against when interpreting ITC data with assumed reaction mechanisms, especially in drug discovery campaigns.

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