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 such that some conformations are much more probable than others. A compound ligand can also have significant alternative conformations. When isothermal titration calorimetry (ITC) experiments are carried out with such proteins and ligands, data analysis 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. In typical cases, the apparent enthalpy and binding constant obtained from regression agree with the values calculated with the Gibbs-Helmholtz equation using the simulation parameters. In cases where the binding curve is not typical, the apparent enthalpy and binding constant from regression can be off from calculated values with accompanying abnormal stoichiometry. In such cases, changing concentrations of reactants in different ITC runs can affect the regression behavior, and can help in obtaining more accurate thermodynamic reaction parameters. These simulations provide the reaction signatures of the modeled system, which can be helpful when deciphering a reaction mechanism or interpreting ITC data. The simulations also demonstrated the validity and usefulness of the Gibbs-Helmholtz equation in a situation where there is no temperature change.

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