Characterizing Binding Using Continuous Isothermal Titration Calorimetry

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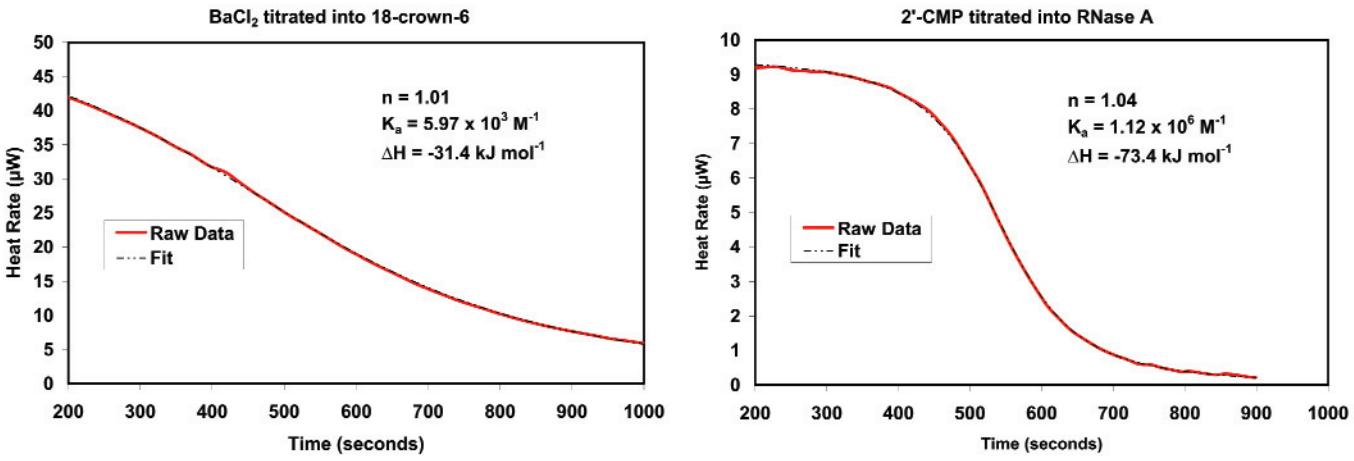

Both weak and strong binding reactions can be quickly characterized using cITC.

Continuous isothermal titration calorimetry (cITC) is ideal for studying rapid binding events typical of many biological recognition and binding reactions. The binding constant, stoichiometry and enthalpy of the reaction are accurately determined in significantly less time than that required using incremental isothermal titration calorimetry.

Structural biology and proteomics have provided an architectural framework for understanding how the three dimensional structure of a protein, controlled by an intricate network of numerous weak non-covalent interactions, directs the recognition, binding, catalysis and transport reactions characteristic of living systems. As the number and diversity of high resolution structures increases it is becoming apparent that understanding the molecular mechanisms regulating these dynamic intermolecular events requires not only structural knowledge of the participating species, but also an understanding of the underlying thermodynamics and kinetics controlling each process.

Isothermal titration calorimetry (ITC) is a rapid, sensitive and universally-applicable technique for studying the thermodynamics controlling binding equilibria. By investigating the thermodynamic properties of a system as a function of temperature, pH, ionic strength or ligand concentration in a series of ITC experiments, the contributions of specific intermolecular interactions to the overall free energy of a process can be revealed. Specific recognition and binding reactions between a ligand (*e.g.*, a drug) and receptor (target macromolecule) are fundamental to the development of efficacious pharmaceuticals, so methodological developments which enhance the sensitivity or versatility of ITC measurements are of considerable practical interest.

This Application Note describes the utility of one such development, continuous isothermal titration calorimetry (cITC). For a general description of the principles behind isothermal titration calorimetry and the types of biological problems that can be addressed, please see CSC's Overview Note entitled 'Life Science Applications of ITC'.

The principle behind cITC:

As described in CSC's Application Note entitled 'Characterizing Binding Interactions by ITC', ITC allows the binding constant (K_a), enthalpy (ΔH) and stoichiometry (n) of a reaction to be calculated from a single experiment provided the concentration of both the macromolecule and ligand are accurately known and fall within the range:

$$10 < K_a[M]_T < 1000$$

where $[M_T]$ is the total concentration of macromolecule in the sample cell titrated by ligand (Wiseman *et al.*, 1989). In an ITC experiment, 20-30 small aliquots of titrant are added in discrete incremental steps over a period of 50-80 minutes. In contrast, in a cITC experiment, binding between a ligand and receptor molecule is studied by slowly and continuously titrating one reactant into the other over a period of 15-20 minutes. Although traditional ITC remains the method of choice for characterizing binding events occurring over a period of seconds, cITC can be advantageous for studying very rapid binding reactions typical of many biological interactions. The primary advantage of cITC is that approximately a thousand data points are obtained from a single titration experiment, compared to the 20-30 data points obtained from an ITC experiment. Since the binding constant has a profound effect on the shape of the titration curve (see Fig. 2 in the CSC application note entitled 'Characterizing Binding Interactions by ITC'), sufficient data points are necessary to allow precise description of the curve and accurate determination of K_a and n. Using incremental titration, a single data point lying above or below the curve could be regarded as an anomaly and discarded, whereas the multiple data points obtained by cITC would clearly show any deviation from the main trend of the curve. Thus, cITC can be invaluable for discriminating between various binding models, or identifying the correct multi-component model.

Continuous titration calorimetry has been an established technique since the 1960's (Izatt *et al.*, 1966) but until recently (Markova and Hallen, 2004) was rarely applied to biological samples despite the physiologically-relevant range of equilibrium constants accessible by the technique. Two data sets illustrating applications of the cITC technique are presented in Figs. 1 and 2. All experiments were performed on a CSC Nano ITC III at 25 °C and a

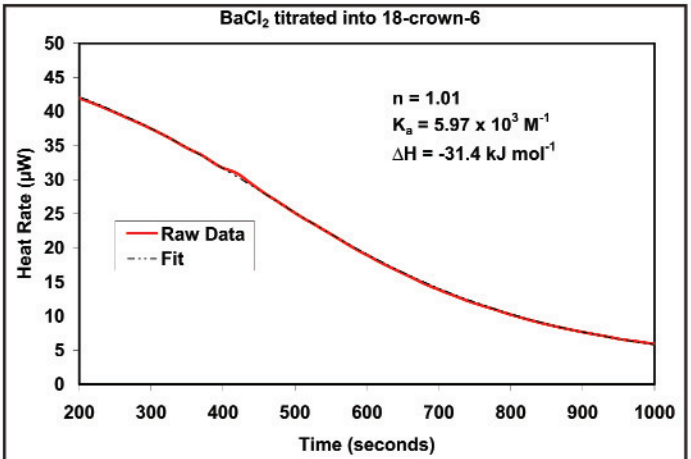

Fig. 1. Barium chloride titrated into 18-crown-6. Samples were prepared in deionized water. Injection rate: 0.101 µL/sec. Total injected volume: 100 µL. Concentration of 18-crown-6 in the 1.0 mL reaction cell: 1.00 mM. Concentration $BaCl_2$ in the syringe: 17.6 mM.

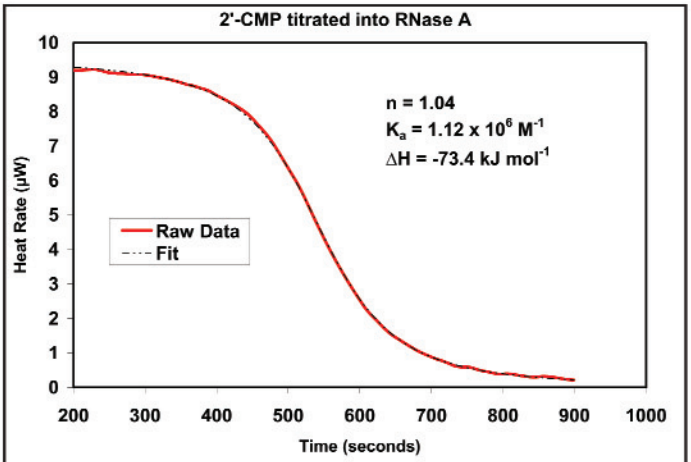

Fig. 2. 2'-CMP titrated into RNase A. Injection rate: 0.101 µL/sec. Total injected volume: 100 µL. Concentration RNase A in the 1.0 mL cell: 0.07 mM. Concentration 2'-CMP in the syringe: 1.30 mM. Solutions were prepared in 15 mM sodium acetate buffer, pH 5.5.

stir rate of 200 rpm. Note that cITC measurements require *no hardware modifications* to the Nano ITC III. Precise controlled delivery of titrant (from 0.05 to 0.15 µL/sec) allows a range of binding constants (millimolar, barium chloride binding to 18-crown-6; micromolar, 2'CMP binding to RNase A) to be determined, together with the stoichiometry and enthalpy of the reaction. All three values for each system can be determined using just nanograms of material in two, 20 minute experiments (titrant into receptor, and a blank of titrant into solvent to measure the heat of diulution). The results obtained are in agreement with literature values (barium chloride titration: Ziemer *et al.*, 2005; 2'CMP titration: Wiseman *et al.*, 1989).

Summary:

Continuous isothermal titration calorimetry is an attractive alternative to traditional ITC for rapid binding reactions. The experiments are faster (generating a full data set in the time normally required for just several incremental injections by ITC), provide a large number of data points allowing accurate determinations of a range of binding constants, and also furnish the enthalpy and stoichiometry of the reaction. In addition, the high density of data points makes it possible to differentiate between different binding models (Markova and Hallen, 2004). Importantly, the experiments can be preformed on a CSC Nano ITC III with no alterations to the hardware, and only minor changes to the data analysis software supplied with the instrument. Please contact CSC for further information regarding cITC protocols, and for the necessary software upgrades.

References:

(Preference has been given to current references. Citation does not imply that a paper is necessarily the original reference to a study.)

Izatt, R. M., J. H. Rytting, L. D. Hansen and J. J. Christensen. (1966) Thermodynamics of proton dissociation in dilute aqueous solution. V. An entropy study of adenosine, pentoses, hexoses and related compounds. *J. Am. Chem. Soc.* **88**, 2641-2645.

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Ziemer, S. P., T. L. Niederhauser, E. M. Woolley. (2005) Thermodynamics of complexation of aqueous 18-crown-6 with barium ion; apparent molar volumes and apparent molar heat capacities of aqueous (18-crown-6 + barium nitrate) at temperatures (298.15 to 393.15) K, at molalities (0.02 to 0.33) mol · kg^{-1}, and at the pressure 0.35 MPa, *J. Chem. Thermodynamics* **37**, 984-995.