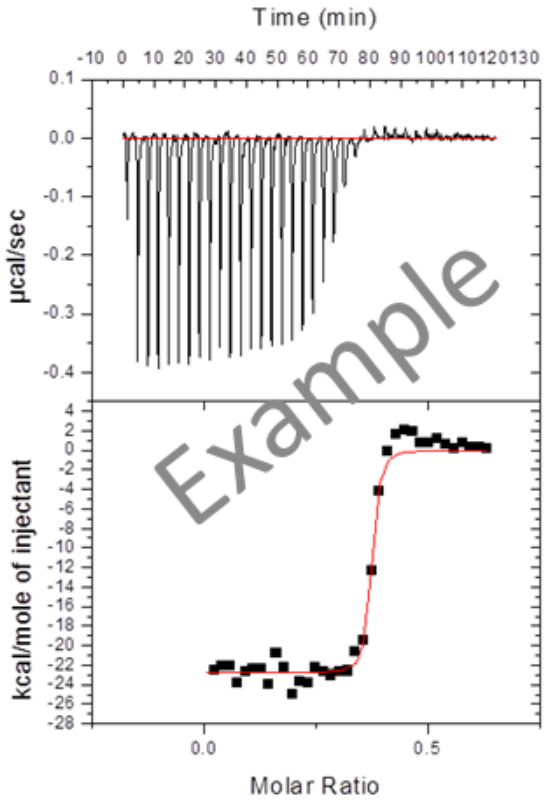
Isothermal Titration Calorimetry (ITC)

Isothermal titration calorimetry, or ITC, is a standard biophysical method for measuring molecular interactions. When a protein binds a ligand, heat is either released or absorbed and this heat is quantified in an ITC experiment. From analysis of this data, thermodynamic parameters of the interaction can be determined, notably the binding affinity.



XTAL maintains and operates a Microcal Auto-ITC in-house. The automated nature of this unit allows for hands-free loading, operation and cleaning as well as sequential unattended runs.

The ITC unit consists of two identical cells, a reference cell and a sample cell, contained in an adiabatic chamber. For a typical protein-ligand study, a known concentration and volume of protein solution is placed in the sample cell with water in the reference cell. The cells are then equilibrated to the same temperature, typically 20–30 °C, using a thermostat system. During an ITC run, small but precise volumes of ligand are periodically injected into the sample cell. The unit directly measures the amount of power required to maintain both cells at equal temperature for each injection. After the titration is complete, the resulting spectrogram is analyzed to quantify the heat changes upon binding. In addition to measuring the binding enthalpy (ΔH), the analysis also provides a measure of the binding stoichiometry (N) and affinity (K_a). By extension, the entropy (ΔS), free energy (ΔG) and dissociation constant (K_d) are also determined. In a single experiment, ITC can provide a complete thermodynamic profile of the interaction of molecules free in solution without modification, tags, or coupling to a surface.

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