

Sample Preparation:

Isothermal titration calorimetry (ITC) is a technique that can measure binding affinity, enthalpy, and stoichiometry (η) in one experiment. The signal that is measured is the heat that is released or absorbed upon binding of two reactants.

In general, in ITC experiments you have one macromolecule in a cell and titrate it with the second macromolecule. It is important to spend time setting up experiment and picking macromolecule concentrations, such that a titration curve is obtained from which the binding parameters are well determined. The concentration ranges needed for ITC can be determined using the following equation.

$$c = \eta * K_a * [M]_T$$

Where K_a is the binding constant, and $[M]_T$ is the total macromolecule concentration in the cell. Ideally the concentration of $[M]_T$ used should be chosen, such that c is between 1 and 1000. In general you want the macromolecule in the cell to be 10- 30 times the K_d . The ligand or macromolecule that is titrated into the cell, should be about 10 - 20 fold higher in concentration than the macromolecule in the cell. If you have solubility constraints, the macromolecule with solubility issues should be in the cell, and the other used as the titrant. The volume of the individual injections should be chosen such that the molar ratio of $[M]_T$ to ligand is 1:1 after 10-15 injections. For easiest loading, it is best to have about 2.2 ml of macromolecule and 2 ml of the titrant at the desired concentration.

It is important to extensively dialyze macromolecules into the appropriate buffers. In order to get rid of as much noise as possible, after dialysis it is important to filter samples with a 22 uM filter. The macromolecule concentration should be measured as accurately as possible. Before using samples it is also critical that they be degassed. This can be done at the ITC, and should be done for about one hour.

An excellent review by Velazquez-Campy, Leavitt, and Freire, *Characterization of Protein-Protein Interactions by Isothermal Titration Calorimetry*, can be used for more information.