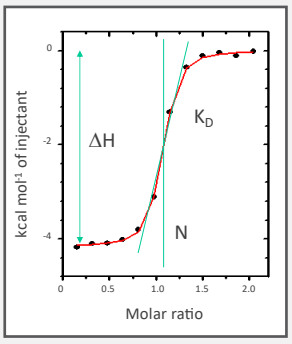
MicroCal PEAQ-ITC Automated

Isothermal Titration Calorimetry (ITC)

Calorimeter is designed to measure the **heat of binding**. In a typical arrangement, the titrant, also referred as the ligand, is injected into the sample cell containing the macromolecule sample solution. The calorimetric measurement can be done over a range of biologically relevant conditions (temperature, salt, pH, etc.). No labelling is necessary and the complete thermodynamic profile of the interaction can be obtained in a single measurement. ITC system directly measure submilimolar to nanomolar binding constants (10^3 - 10^9 M^{-1}). The interactions with nanomolar to picomolar binding constants (10^9-10^{12} M^{-1}) can be measured using the competitive binding technique, the same principle can be used for low affinity interactions (10^3-10^2 M^{-1}).

ITC Method can be used for:

- characterization of biomolecular interactions of **small molecules, proteins, antibodies, nucleic acids, lipids** and **others**
- **enzyme kinetics studies, biological activity** or the effect of molecular structure changes on binding mechanism determination
- determination of thermodynamic parameters **K_A, ΔH** and **ΔS** values, **stoichiometry** or kinetics parameters **K_m** and **k_{cat}**



Technical specifications:

Instrument: MicroCal PEAQ-ITC Automated (Malvern)

Features:

- temperature range for measurement: 2 °C – 80 °C
- controlled sample storage: 4 °C – 25 °C
- cell volume: 200 µl
- syringe volume: 40 µl
- cell specification: coin-shaped Hastelloy alloy

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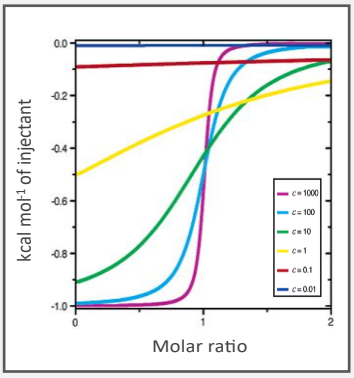
Sample requirements

- Proper sample preparation is **crucial** for the successful ITC measurement. The buffer solution, in which the macromolecule and the ligand are dissolved, **should be exactly the same** (dialysis or lyophilisation and dissolution in the buffer for ITC). The pH should be checked before the measurement.
- **The macromolecule sample (the sample placed in the cell): 370 µl**
- **The ligand solution (the sample placed in the injection syringe): 120 µl**
- **Sample concentrations must be determined precisely.**
- Generally, a concentration of ligand should be 10 times higher than the concentration of macromolecule otherwise the concentration should be optimized.
- High affinity interactions can be studied at low concentrations. In this case the minimum concentration of macromolecule sample which causes measurable heat is 10 µM. For low affinity interactions the macromolecule sample concentration should be 5 times of K_D or higher, but higher concentration may be limited by availability or solubility of samples.
- Calculating the cell sample concentration – $M = c / (n \times K_A) = c / n \times K_D$

  M … molar concentration of the cell sample;
  c-value … should lie between 10-500;
  n … binding stoichiometry;
  KA …association constant;
  KD … dissociation constant

- At least 10 ml of the used buffer must be sent for each measurement.
- If it is possible, choose a pH buffer with low heat ionization in order to minimize artifactual heats of buffer ionization (e.g. phosphate, citrate, acetate).



Shape of the curve dependency on the c-value

Sample preparation

- Both macromolecule and ligand must be in identical solutions, otherwise large heats of dilution will mask the desired observation. Solvent matching is best achieved by exhaustive dialysis of the macromolecule, using the final dialysis buffer to dissolve the ligand.
- Dialysis buffer should be used for running baseline controls, diluting samples, etc.
- Filter out any visible particulates (if possible).
- Check the pH carefully, after the solutions have been prepared. If the pH differ by more than 0.05 pH units, then adjust one of the solutions.
- Solution concentrations should be determined after final preparation. **Accurate determination of binding parameters is only possible if concentrations are known precisely.**
- The accuracy of stoichiometry, association constant and enthalpy determination is directly proportional to the accuracy with which the syringe reactant concentration is determined. This is in contrast to the accuracy of the cell reactant concentration which only affects N.
- Synthetic peptides or oligonucleotides should be desalted *prior* to suspension in ITC buffer. Residual chemicals from synthesis (e.g. TFA and salts) will cause a buffer mismatch and high heats of dilution.
- If the presence of reducing agent is required for a protein stability, then **ß-mercaptoethanol (less than 5 mM) or TCEP (less than 2 mM) should be used rather than DTT.**
- If you use DMSO to solubilize a ligand, you will need to add DMSO to the macromolecule solution to match the concentration in the ligand solution. Many proteins are stable in the short term in up to 2-5% DMSO. Add the DMSO to the protein solution immediately prior to running the ITC experiment.

Established methodologies and provided services:

- calorimetric measurement of **protein-ligand** interaction (Standard titration method, Single injection method) or **competitive-based** measurement - **low** (10^3 M^{-1}) or **high** (10^9 M^{-1}) **affinity interactions**
- data evaluation - thermodynamic parameters determination using curve fitting models: One set of binding sites, Two sets of binding sites
- eventuality of manual data evaluation using fitting models: Sequential binding sites, Competitive binding, Dissociation (data evaluation assistance)
- basic ITC data evaluation training

Instrument operation

Operational mode:

- calorimetric measurements are performed only by CF staff

Provided services:

- sample measurement (obtaining of raw data)
- standard sample analysis (One set of sites, Two set of sites, Dissociation model etc.)
- data evaluation user training
- consulting/assistance

Data evaluation SW:

- MicroCal PEAQ Analysis software v 1.41, Origin 7, NITPIC (advanced)

It is recommended to discuss the project and the details of the experiment (sample preparation, sample requirements) with the Core Facility members in advance.

Contacts

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Instrument Location:

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