CEITEC
MASARYK UNIVERSITY

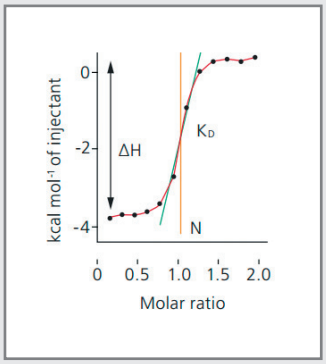
VP-ITC calorimeter

Isothermal Titration Calorimetry (ITC)

VP-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}).

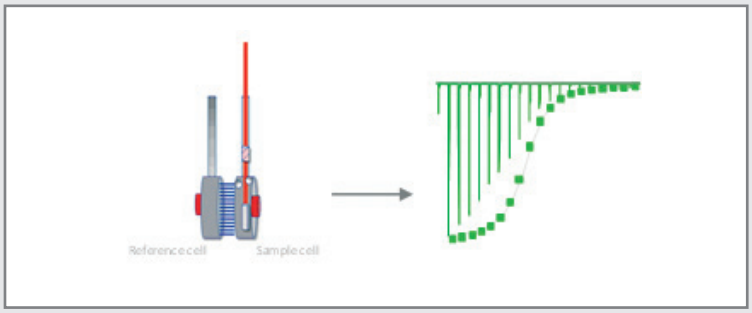
ITC method can be used for

- 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

Instruments: VP-ITC (Malvern)



Features:

- operating temperature range is of 2°C to 80°C
- calorimetric cell - volume 1400 ml, Hastelloy, coin-shaped

Assemblies:

- ThermoVac - device for thermostatting and degassing samples (0-80°C)

Operational mode:

VP-ITC measurement is performed manually by the user itself after training

Data evaluation software: Origin software, NITPIC (possibility to train people in data processing)

Provided services:

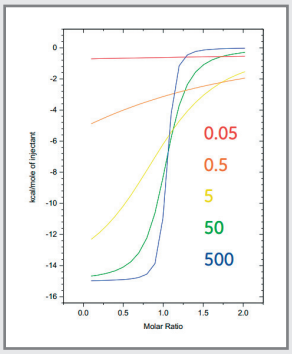
- instrument user training
- basic ITC data evaluation training
- consulting/assistance

Data collection:

- Standard titration method or Single injection method

Sample requirements - importance of sample preparation

- **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): 1800 µl
- The ligand solution (the sample placed in the injection syringe): 450 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; K_A …association constant; K_D … dissociation constant
- At least 10 ml of the used buffer must be sent for each measurement (for VP-ITC).
- If it is possible, choose a pH buffer with low heat ionization in order to minimize artifactual heats of buffer ionization.
- 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 tan DTT.



The shape of the binding isotherms depend on a unitless constant c

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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