Kinetic ITC methods in the field of biology

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Classical ITC experiments involves multiple injections of a molecule B into a cell containing a molecule A. Upon interaction of A and B following A + B ↔ C (characterized by the equilibrium constant $K_a = k_{on} / k_{off}$), there is emission or absorption of heat and the calorimeter measures in real time the corresponding heat power (in µcal / s). We have developed a method to recover k_{on} and k_{off} from the shape of each injection curve of a classical ITC titration experiment [1]. By using ITC in a classical way, this kind of information is systematically lost by the integration of the injection power curves. However, by using kinetic equations one can relate the kinetics of the reaction to the heat power produced or absorbed during the reaction. Several technical problems need to be solved to take into account the finite injection and mixing times of compound B into the measurement cell, as well as the finite response time of the instrument (see illustration at http://www-ibmc.u-strasbg.fr:8080/webMathematica/kinITCdemo/).

Ideally, ***kinITC*** experiments are performed at different temperatures. Using of the van 't Hoff equation allows to link the measured ΔH to the temperature variation of the corresponding equilibrium constant K_a. This is also a link to the kinetic parameters to be determined since $K_a = k_{on} / k_{off}$.

We also developed a simplified, and yet very efficient version of this method using data at a single temperature [2]. It is based on the variation from injection to injection of the time needed to return to baseline. The resulting 'Equilibration Time Curve' (ETC) allows deriving k_{on} and k_{off} as soon as K_a is known from the classical processing of the ITC experiment. The method is now available in the software AFFINImeter (http://www.affinimeter.com).

Importantly, the full ***kinITC*** technique can cope with two-step kinetic schemes. This will be illustrated with the binding of a ligand to an RNA followed by complete RNA folding. Both thermodynamic and kinetic information could be derived for each individual step [3].

References

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3. Guedich S., Puffer-Enders B., Baltzinger M. *et al.* (2016) RNA Biology, **13**, 373-390