## Dilution ITC:  analysis for monomer-dimer equilibrium

*Taken from ref.[1]*

Dilution ITC experiments involve sequential injections of concentrated solution (2-3 mM) into the stirred calorimeter cell (1.4ml) initially containing buffer alone, with a typical injection sequence of 12×20μl at 3-5 minute intervals. This gives rise to a series of endothermic heat pulses which, after correction for appropriate buffer mixing control experiments under identical conditions, may be analysed using Microcal Origin and purpose-made software in terms of a simple dimer dissociation model to give $K_{dim}$ and $\Delta H_{dim}$.

Typical ITC dilution data for the dissociation of insulin dimers in solution. (A) Endothermic heat pulses for 10 μl injections of insulin solution into buffer. (B) Integrated heat data fit to a dimer dissociation model with $K_{diss} = 12$ μM and $\Delta H_{diss} = 41$ kJ mol$^{-1}$
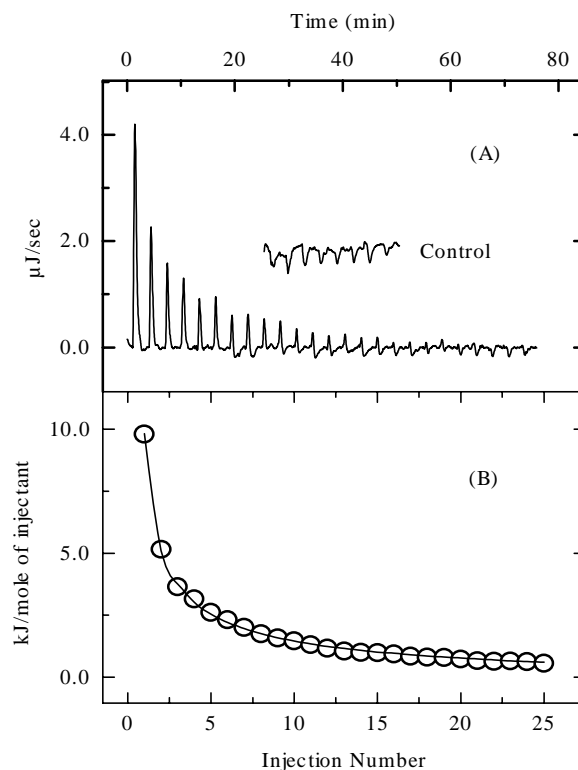


M.Lovatt, A.Cooper & P.Camilleri (1996). *Energetics of cyclodextrin-induced dissociation of insulin.* Eur.Biophys.J. 24, 354-357.

Heats of dilution data for a simple monomer-dimer system are analysed as follows. If only monomer or dimer states of (macro)molecule P are possible:

$$P \; + \; P \; \rightleftharpoons \; P_2 \quad ; \quad \Delta H_{dim} \quad ; \quad K_{dim} \; = \; [P_2]/[P]^2$$

the equilibrium concentration of monomers is given by:

$$[P] \ = \ \{(1 + 8.K_{dim}.C)^{1/2} \ - \ 1\}/4.K_{dim} \qquad .....equ.(A.1)$$

where C is the total concentration of P, expressed as monomer:

$$C \ = \ [P] \ + \ 2[P_2] \qquad .....equ.(A.2)$$

In an ITC dilution experiment we measure the heat change ($\delta q$) when a small volume ($\delta V$) of concentrated solution (concentration $C_0$) is injected into the calorimeter cell (volume $V_0$) containing initially buffer but, for later injections, more dilute solution. The heat arises from dimers present in the higher concentration solution that dissociate upon entering the lower concentration environment.

For the *i* th injection of a series the observed heat is given by:

$$\delta q_i \ = \ \Delta H_{dim}\{V_0([P_2]_i \ - \ [P_2]_{i-1}) \ - \ \delta V([P_2]_0 \ - \ [P_2]_{i-1})\} \qquad .....equ.(A.3)$$

where $[P_2]_0$ , $[P_2]_i$ and $[P_2]_{i-1}$ are the dimer concentrations in the original (syringe) solution and in the calorimeter cell after the *i* th and *(i-1)* th injections: total concentrations $C_0$ , $C_i$ and $C_{i-1}$, respectively. [The last term in this expression is a small correction factor to allow for the quantity of solution displaced from the constant-volume calorimeter cell during each $\delta V$ addition.]

Equations (A.1) to (A.3) are used in standard non-linear regression (least-squares) procedures to fit experimental dilution data and obtain estimates of $K_{dim}$ and $\Delta H_{dim}$. Similar, though more algebraically complex expressions may be derived for dissociation processes involving higher oligomers or other mechanisms. Such mechanisms frequently give sigmoidal dilution thermograms, in contrast to the hyperbolic shapes for the dimer dissociation shown here, and this might give empirical indications that the process under investigation is more complex than simple dimers can model.

Interestingly, Equ.(A.1) is algebraically identical (apart from a factor 2) to that giving free monomer concentrations in a simple infinite-polymerization model [2]. Consequently, calorimetric dilution data alone might be insufficient to discriminate between dimer or polymer interaction models, and other experimental approaches might be needed to resolve possible ambiguities.

References:

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3. M.Lovatt, A.Cooper & P.Camilleri (1996). *Energetics of cyclodextrin-induced dissociation of insulin.* Eur.Biophys.J. 24, 354-357.
4. A.Cooper (1997). *Microcalorimetry of protein-protein interactions*. Methods in Molecular Biology Vol.88: Protein Targeting Protocols, pp.11-22 (ed. Roger A. Clegg, Humana Press 1997).

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