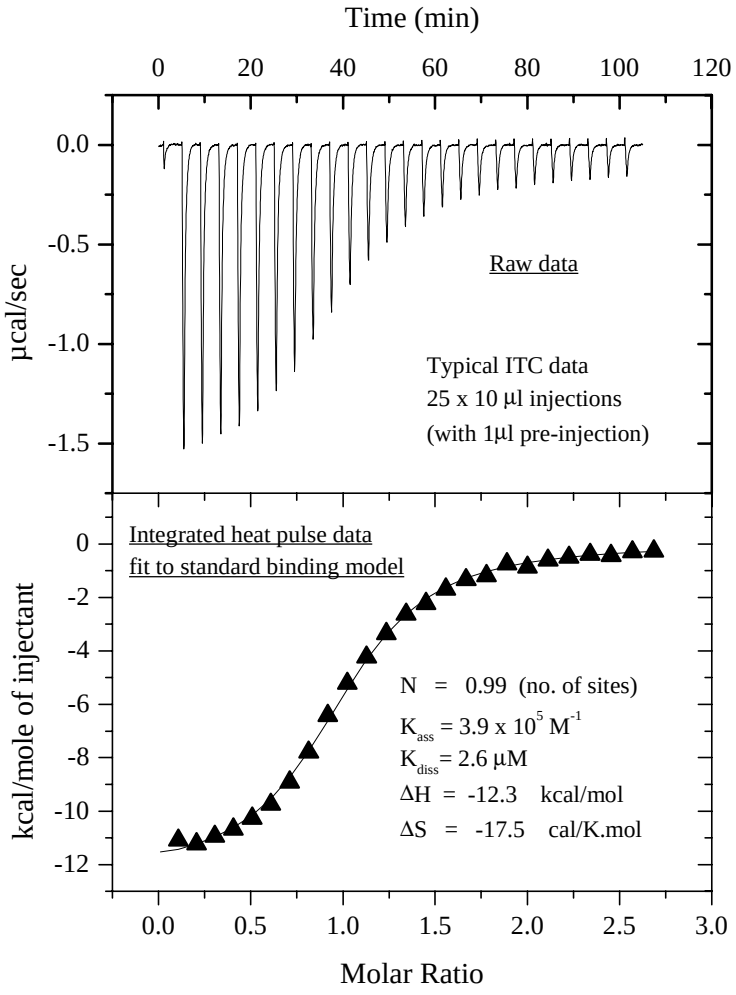
ITC Notes

Typical ITC data for straightforward, uncomplicated binding (Lysozyme/tri-NAG):-



Microcal VP-ITC

ITC Cell: hen egg white lysozyme, 36 µM (ca. 0.5 mg/ml)

Injection syringe: 0.45mM tri-NAG (tri-N-acetyl-glucosamine)

Buffer: 0.1M acetate, pH 5

25 x 10µl injections (with 1µl pre-injection), three minutes apart

Each exothermic heat pulse (upper panel) corresponds to injection of 10 µl of tri-NAG (0.45mM) into the protein solution (36µM). Integrated heat data (lower panel) constitute a differential binding curve that may be fit to a standard single-site binding model to give, in this instance, the stoichiometry of binding, N = 0.99, binding affinity, K_{ass} = 3.9 x 10^5 M^{-1} (K_{diss} = 2.6 µM), and enthalpy of binding, ΔH = -51.7 kJ mol^{-1} .

The integrated heat data have been corrected for heats of ligand dilution, which are relatively small in this case. Poor solvent/buffer matching between protein and ligand

solutions will give rise to large heat of mixing/dilution effects that can obscure the binding heats. Take care to use a buffer with sufficient buffering capacity to minimise pH changes when making up ligand solutions.

Baseline drifts and/or slow return to baseline of heat pulses after each injection usually indicate slow reactions (not usually binding). This can occur due to slow oxidation of DTT or other reducing agents in the system, or due to enzyme-catalysed reactions, for example.

Notes on interpretation:

Why "N" might not turn out as you expect…

"N" is the average number of binding sites per mole of protein in your solution, assuming:

a. that all binding sites are identical and independent
b. that you have pure protein (and ligand)
c. that you have given the correct protein and ligand concentrations
d. that all your protein is correctly folded and active…

This is rarely true in practice! Protein (and ligand) concentration determinations depend on the accuracy of the methods used. Protein extinction coefficients, for example, are rarely known better than ±5%, and are usually worse. Poor measurement techniques, incorrect UV baseline corrections, attempts to conserve material using "micro" cuvettes for example can lead to serious errors. Even if all your measurements are dead accurate, not all the protein may be correctly folded (a common experience with recombinant proteins).

Possible cases:-

$N < 1$	the protein concentration is lower than you think, or… the protein is impure, or… the protein (polypeptide) is pure but not all correctly folded, or… the ligand concentration is higher than you think, or… the simple non-cooperative binding model is inappropriate, or… …all of the above
$N > 1$	your protein has multiple binding sites, or… the ligand concentration is lower than you think, or… the simple non-cooperative binding model is inappropriate, or… …all of the above
$N = 1$	probably just a lucky day!

In cases of **weak binding** ($K_{diss} >> C_E$) the value of "N" is not well determined by the shape of the titration curve and may "float" unrealistically during fitting. In such cases it is usually best to fix N = 1 and draw no conclusions about actual stoichiometries. The K values are usually reasonable in such cases (at least as an order of magnitude).

The simple way to visualise K_{diss} is as the free ligand concentration, [L], when 50% of the sites are saturated. For weak binding (as defined above), $[L] \approx C_L$, and the rough K value can often be guessed straight from the graph.

More complex systems, with multiple binding sites, cooperativity, etc., can be difficult to interpret unambiguously. In such cases, multiple experiments over a range of concentrations will usually be necessary, and reversing the ITC cell/syringe contents can be helpful.

Don't expect to make sense of absolute ΔH and ΔS values !

Alan Cooper
Glasgow – June 2001