Sample Preparation Guidelines (ITC)

(as suggested by *MicroCal*)

Proper sample preparation is essential for successful ITC testing. In particular, the **minimal guidelines below must be strictly followed** to insure an accurate estimate of stoichiometry (n), heat of binding (ΔH), and binding constant (K_b) (or dissociation constant $K_d = 1/K_b$).

1.) The *macromolecule solution* (the sample to be placed in the reaction cell) must have a volume of at least 2.1 ml. The lowest concentration which can be studied is 3 μM and this is adequate only for tight binding where K_d is smaller than 1 μM. For weaker interactions, the macromolecule concentration should be 5 times K_d, or higher if possible. Preferably, the macromolecule solution should be dialyzed exhaustively against buffer for final equilibration.

2.) The *ligand solution* (the sample to be placed in the injection syringe) must have a volume of at least 0.7 ml. Its concentration should be at least 10 times higher than the concentration of macromolecule (if the macromolecule has multiple binding sites for ligand, then the ligand concentration must be increased accordingly). The buffer solution in which the ligand is dissolved should be *exactly the same buffer* against which the macromolecule has been equilibrated.

3.) *After* both solutions have been prepared, the pH of each should be checked carefully. If they are different by more that 0.05 pH units, then one of the solutions must be back-titrated so they are within the limit of 0.05 pH units. If any particles are visible in either solution, they should be filtered out.

4.) If possible, the concentrations of both solutions should be accurately determined *after* final preparation. Accurate determination of binding parameters is only possible if concentrations of binding components are known precisely.

5.) At least 20 ml of buffer must be secured along with the two samples, since this is used for rinsing the cell and for dilution if necessary.

6.) If possible, DTT should be avoided as a disulfide reagent and replaced by β-mercaptoethanol or TCEP.

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In *general* the organic buffers should be avoided (like for example TRIS) since they usually have high enthalpy of ionization and if protons are taken up/released during titration, buffer ionization will contribute to the observed enthalpy change.
