Sample Preparation for iTC200

NOTE: The guidelines below are based on MicroCal guidelines.

Sample Preparation Guidelines (ITC). Proper sample preparation is essential for successful ITC testing. In particular, **the minimal guidelines below must be strictly followed** to ensure 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 300 µl**. 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. In reality, good data were obtained for concentrations above 20 µM.
2. The *ligand solution* (the sample to be placed in the injection syringe) must have a volume of **at least 110 µl**. 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 than 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 100 ml of buffer must be submitted along with the two samples, since this is used for rinsing the cell and for dilution if necessary.
6. If possible, dithiothreitol (DTT) should be avoided as a disulfide reagent and replaced by β-mercaptoethanol or tris(2-carboxyethyl)phosphine (TCEP).