Protein-Ligand ITC in the Presence of Co-Solvents

Attempting to quantify the binding of a small-molecule ligand to a protein in the presence of a co-solvent (e.g. DMSO used to dissolve the compound at high concentration) often leads to artifactual high heats of dilution from mismatched co-solvent concentration between the syringe and the cell. You can't rely on a control titration (compound into buffer) to correct for the mismatch, as the heats of dilution for the co-solvent are frequently large and will dwarf any binding signal. Here is the only reliable protocol for performing titrations in the presence of a co-solvent (DMSO is used as an example):

1. Work out the DMSO tolerance of your protein and / or the minimum concentration you need to keep compounds soluble.
2. Dialyse your protein into your assay buffer
3. Make a compound stock in fresh pure DMSO by weighing. Choose a stock concentration such that you obtain your desired syringe concentration after diluting the stock to give the DMSO concentration determined in (1). i.e. if you can work at 5 % DMSO, use a stock compound concentration 20 fold higher than your desired syringe concentration (20 fold dilution = 100% stock / 5% final).
4. Make exactly the same volume of syringe solution and cell solution. This is vital, and the only way to ensure DMSO matching. So, for a final syringe concentration of 5 mM compound 5 % DMSO, you might do the following (the measurements here are for the ITC200, scale up the volumes for the VP-ITC):
   (a) pipette 380 μl of assay buffer (no DMSO) into one eppendorf, and 380 μl of protein in assay buffer (no DMSO) into another eppendorf.
   (b) add 20 μl of 100 mM compound (in pure DMSO) to the eppendorf containing the buffer only. This will give you the syringe solution of 5 mM compound in buffer + 5 % DMSO.
   (c) using the same pipette, with the same setting (ideally lock the volume setting), add 20 μl of pure DMSO to the eppendorf containing the protein in buffer (ideally the same DMSO that you used to make the compound stock solution). Mix immediately with the pipette to avoid high local [DMSO]. This will give you your cell solution of protein in buffer + 5 % DMSO. Make sure you account for the slight concentration change of the protein (a factor of 0.95).

(d) make a further 6 * 400 μl of 380 μl buffer + 20 μl pure DMSO. Use 3-400 μl to fill the reference cell (degas the solution) for the best signal to noise and so that the DP value gives a good indication of whether the cell is correctly filled (if it is correctly filled, the measured DP value after equilibration will approach the value set in the software). Use the remaining solution to wash out the sample cell and then the syringe, finishing by emptying the sample cell and syringe before loading them with the experimental solutions.

(e) You can run the control titration in the same way, omitting the protein from the cell solution. If you have a post-saturation plateau in your binding curve, you can also approximate the heat of dilution for the compound by using the "simple math" function to manually add or subtract small heat values to the measured enthalpy until you minimise the χ^2 function of the fit. The total amount that you should add or subtract should be approximately the same as the height of the post-saturation plateau in the titration curve. This works because the fit (1:1 binding model) forces the post- saturation baseline to be zero, which is where it should be after the correct heat of dilution has been subtracted.

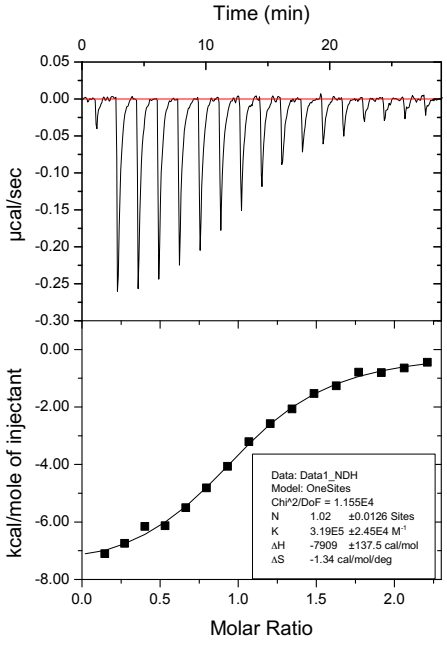

Figure 1: A titration of a small molecule against a protein in 5 % DMSO. Note the very small heats of dilution, evincing the close match of DMSO concentrations between the cell and the syringe solutions.