BUFFER PREPARATION GUIDELINES FOR NANO-ITC

- Buffer with a low ionization enthalpy is best such as **PHOSPHATE, ACETATE or HEPES**.

- Buffers such as **TRIS** and **TRICENE** are not recommended if only one experiment is performed to ensure an accurate enthalpy.

- Usage of **SALTS** is not restricted, but higher salt concentrations may inhibit binding events if the ligand and macromolecule form ion pairs

- Any **pH above 4.0** is OK, however the pH of the cell and syringe must match within **0.1 pH** units

- **Reducing agents are OK**. **TCEP** is preferred to **DTT and B-mercaptoethanol** , but any of the three can be used if concentrations in the syringe and cell are matched **ABSOLUTELY**. The cell and syringe sample must be dialyzed at the same time in the exact same buffer, and the samples **MUST NOT** be degassed

- **DMSO** and **ETHANOL** are **OK** to use to solubilize ligands, however the DMSO or Ethanol concentration must be matched absolutely in the ITC cell as well, down to 0.01%. Extreme care must be taken when pipetting to ensure a matched system as the heat of dilution of these compounds is very large.

- **GLYCEROL, SUCROSE, UREA and DETERGENTS** at concentrations where they will not easily foam are **acceptable**.

Please remember that ITC is sensitive enough to measure the heat of dilution of most mismatched compounds at even very low concentrations. Concentrations in the cell and injecting syringe MUST be the same (IMIDAZOLE for example)

Compounds that are **NOT OK**, are **IODIDE** compounds and certain **CYANIDE** compounds as they leach gold, also acids at **pH lower than 4.0 CANNOT BE USED** as they are not compatible with the stainless steel syringe needle.