**Use MicroCal Auto-iTC200 to Study Molecular Interactions**

**Technical Notes**

*Sample volume:* For a routine experimental set up, 400 µl macromolecule are required to be loaded into the 96 well plate and of this volume 200 µl are loaded into the sample cell. For the ligand 120 µl need to be loaded into the 96 well plate and then 40 µl will be loaded by a syringe. Up to four 96 well trays can be loaded and programed to run without intervention. Your samples should be degassed and free of any particulates.

*Materials available in our core*: EGMIC staff refill the consumable supplies during weekly inspection including degassed water, methanol, cleaning detergent and compressed nitrogen gas. Only use the deep well plate and the plate seal provided by our core.

*Instrument maintenance:*EGMIC staff conduct routine maintenance on the instrument and oversee regular preventative maintenance. In addition to the routine maintenance, we ask that all users incorporate water runs as the first and last run. Only your actual sample run time will be charged. Water runs and maintenance related runs will not be charged.

*How to get trained:* contact EGMIC with your interest. We will communicate with you about your experimental design and setting up your account/project in the PPMS system. You need to have appropriate training before you operate the instrument. EGMIC can provide in-person and group training on instrument operation and experimental preparation.

*Data analysis:* Data analysis will be done by Origin 7.0 software. We can help you on basic data analysis and assay development trouble shooting. Please feel free to contact the technical support from Malvern Panalytical (support.us@malvern.com) for technical assistance and data analysis tips.

**General Protocol**

Here is an example of an auto-iTC assay using the MicroCal auto-iTC 200. Both ganglioside GM1 and Cholera Toxin Subunit B (CTXB) are dissolved in PBS buffer (pH 7.4). GM1 (2 µM) is added to the sample cell and CTXB (50µM) is picked up by the syringe and injected into the cell with 19 aliquots of 2 µl for 4 s (the first injection is 0.4 µl for 0.8 s) with a delay interval between the injections of 120 s. The syringe stirring speed is set at 750 rpm. Experiments are performed at 25oC. A reference titration of the ligand in PBS buffer is used to correct for heat of dilution. The thermodynamic data is processed with Origin 7.0 software.

1. ***Sample preparation***

Samples must be pure and free of degradation. Both the syringe extracted ligand and the sample cell macromolecule must be very precisely buffer-matched, including all solvents and additives. A slight mismatch in buffer components between macromolecule and ligand can contribute substantial heat and mask the binding signals. Choose a buffer that has a low heat of ionization, such as phosphate, and avoid DTT. Initial experiments could be performed by using the sample concentration at 10 times of estimated KD and ligand concentration in the syringe at 10-20 times the molar concentration of the sample cell.

1. ***Instrument setup***

Experimental parameters can be customized for each individual experiment specified in the ITC methods including total number of injections, cell temperature, reference power, injection volume, spacing, stir speed, automation methods define automation procedures for workflow and cleaning.

1. ***Data analysis***

The raw data obtained is heat released or absorbed as a function of injection volume. Data analysis is performed with the Origin 7.0 software using several options of fitting models to determine the binding constants (KD), reaction stoichiometry (n), enthalpy (ΔH) and entropy (ΔS). This provides a complete thermodynamic profile of molecular interaction.