ITC Cell Selection

An Overview of our ITC Offerings:

	LV Affinity ITC	SV Affinity ITC	LV Nano ITC	SV Nano ITC
Hastelloy option	✓	✓		✓
Automated option	✓	✓		
low (0-10 %) organics	✓	✓	✓	✓
weak binding, mM	✓+	✓	✓	✓
Strong binding, nM	✓	✓+	✓	✓+
100% organic solvent assays	not recommended	not recommended	✓	✓
active cell volume (µL)	182	965	170	950
manual loading cell volume (µL)	300-350	1300-1400	300-350	1250-1400

✓+ = the best choice

For the 100% organic solvent assays, you must purchase the organic compatible buret handle with Kalrez o-rings.

"Not recommended" doesn't mean "not compatible".

FACTORS TO CONSIDER

1. Sample consumption
2. Cell Material
3. Solvent Compatibility
4. Relative Sensitivity
5. Binding Range
6. Cleaning/reproducibility
7. Time Consumption
8. Ease of use
9. Benefits of the new Affinity Design

The Nano and Affinity ITCs from TA Instruments can measure binding interactions from mM to nM.

1. SAMPLE CONSUMPTION

The low volume ITC, gold or Hastelloy is preferred if sample limited. Loading an Affinity or Nano low volume (LV) requires only 300 µL whereas the standard volume (SV) requires 1250-1400 µL. The syringe size for the LV is 50 µL versus 250 µL for the SV. The automated system has a larger sample requirement than the manual system, however, the automated system can be switched to manual mode by clicking a button in the software.

2. CELL MATERIAL

The gold cell is more sensitive than the Hastelloy cell and the peak shapes are more regular than the Hastelloy. An exception to suggesting gold is only if the users want to study acetonitrile, ammonia, hydrogen peroxide, mercury, or any of halides with an X_2 formula, not HX. Acid halides (HX) are compatible with either cell but at high concentrations they will etch the stainless steel syringe that is used by the Nano, Affinity, and competitor's injection syringe. Gold has not been shown to interact with cysteine, a sulfur containing amino acid common to proteins. If there is still a concern it has been mathematically calculated to be less than 1% at typical assay concentrations (MCTN-2017-01).

3. SOLVENT COMPATIABILITY

Any ITC offered from TA Instruments is compatible with low percentages of organic solvents like methanol, ethanol, or DMSO. If a user wants to collect data in 100% organic solvents, then the automated Affinity ITC is not an option. The Semi-automated affinity also contains FKM in the pumps and this material is not compatible with all organic solvents. The preferred instrument for 100% organic solvent work is either the Nano ITC, LV or SV with the organic resistant buret handle with Kalrez O-rings. For any

organic solvent experiment to work, the solvent must be dry and pressure-volume work minimized.

4 & 5. RELATIVE SENSITIVITY and BINDING RANGE

Appropriate concentrations for an ITC experiment depends on the magnitude of heat, which is related to the enthalpy and number of moles in the reaction cell, and the expected binding constant. An ideal assay is completed between 5*K_d to 500*K_d. Outside of this range the errors of the fitted increases.

Tight Binders, nM affinity

For the most accuarte data an SV ITC is recommended. A tight binding system requires a low concentration. Ultimately this means that less moles will be involved in the binding assay. For a standard volume instrument, at the same concentration as the low volume instrument, the moles involved would be 5x more in the cell and 4-5x more moles introduced from the syringe per injection. Ultimately an SV instrument will produce more heat and will have more data points through the inflection, the portion of the curve used to determine the K_d. However, the penality to the SV unit is that the consumption is 4x greater.

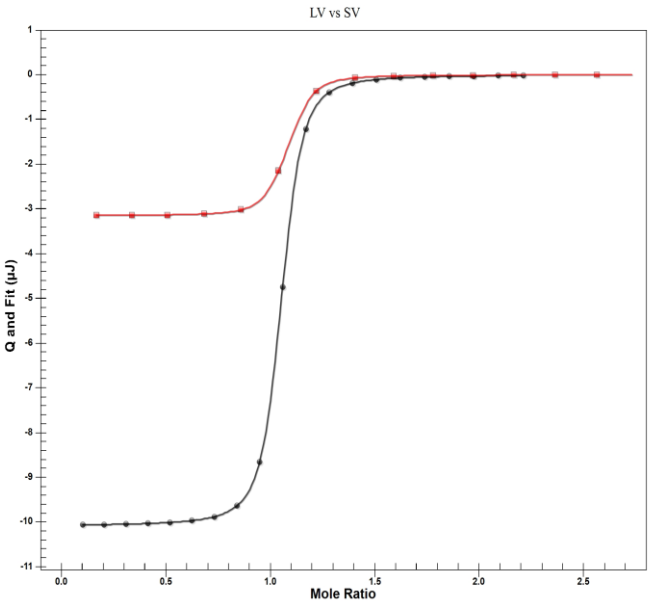


Figure 1. Simulated 5 nM binding system. [Cell] = 2.5 µM [syringe] = 30 µM. C-value = 500.

The LV Affinity ITC with gold cell can provide good data for the right binding systems at nM affintiy and shouldn't be discarded as an option. It requires less sample and a user could run 3 assays using the same volume required in the SV unit. Below is actual data collected using an Affinity ITC LV evaluating the binding of a nM system.

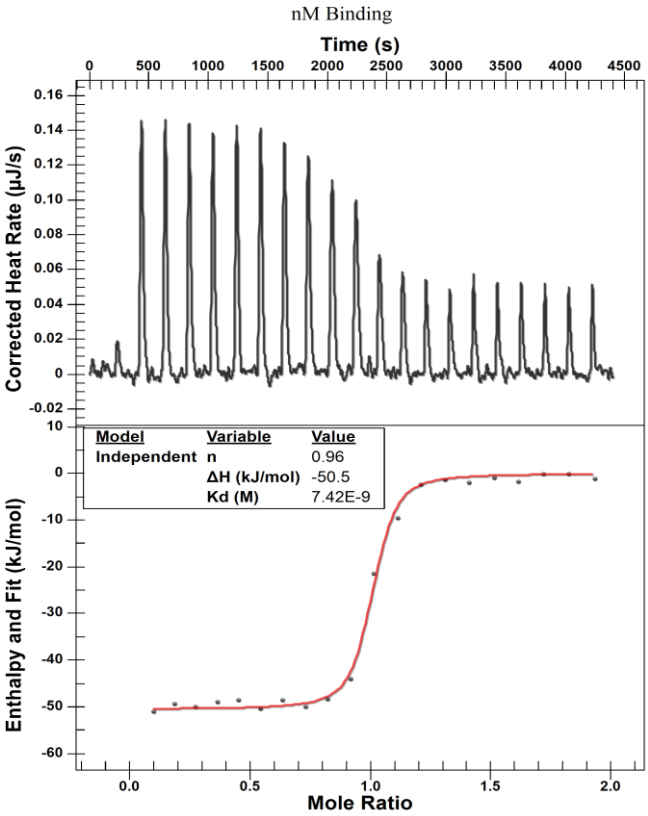


Figure 2. Actual nM binding data collected on a LV Affinity ITC, 30 µM ligand into 3.8 µM protein.

Weak Binders, mM affinity

An LV Affinity ITC is recommended for weaker binding chemistry. With the larger injection syringe, 250 µL instead of 50 µL, it enables users to collect well past saturation into an area where the injection heat has plateaued. This constant heat is required for the most accuarate fit of the data.

6. CLEANING AND REPRODUCIBILITY

The semi-auto affinity ITC and auto-affinity ITC can perform thorough cleaning scans. Manual cleaning is still the fastest way to thoroughly clean and automated systems require more cleaning time because of the additional parts that encounter the solutions.

7. TIME CONSUMPTION

An LV Affinity ITC has the lowest time consumption - the assays and equilibration times are shorter. It also has the added benefit of loading the 250 µL injection syringe with

sufficient volume to run several assays and a control without cleaning or re-loading between the runs.

8. EASE OF USE

The automated system is the easiest to load. It only requires a pipetting into a tray. The Semi-Auto Affinity and Nano ITC load the cells in the same way, a single motion into the cell without pushing the solution up and down. This simple loading is attributed to the ideal cell geometry, a cylindrical cell. The affinity injection syringe has been reported as easier to use than past designs.

9. BENEFITS OF THE AFFINITY ITC

Overall the Affinity ITC has more flexibility in the run software. A user can change the differential power required and extend the range of the instrument, they can easily tune the injection rate if more viscous solutions are used. Speaking of viscosity, because the affinity is injection on top of the paddle and not on the bottom, it mixes more thoroughly and efficiently leading to sharper peaks at slower stir rates. A typical affinity titration is competed at 125 rpm, thereby minimizing any possible shearing of samples. It also can stir in both forward and reverse, an important feature for users concerned with settling of materials. One direction pushes down while the other pulls material up. Overall the affinity is the best option from TA. These features are explained in detail in the white paper: MCTN -2017-01.

FINAL COMMENTS

Ultimately the most diverse instrument is the LV Semi-Auto Affinity ITC. Exceptions to this suggestion include when working with organic solvents or looking for the least amount of error in a low nM binding system. Overall the ease of use, sample consumption, time consumption, and instrument range places the LV affinity ITC instrument as the best instrument in meeting the most needs.