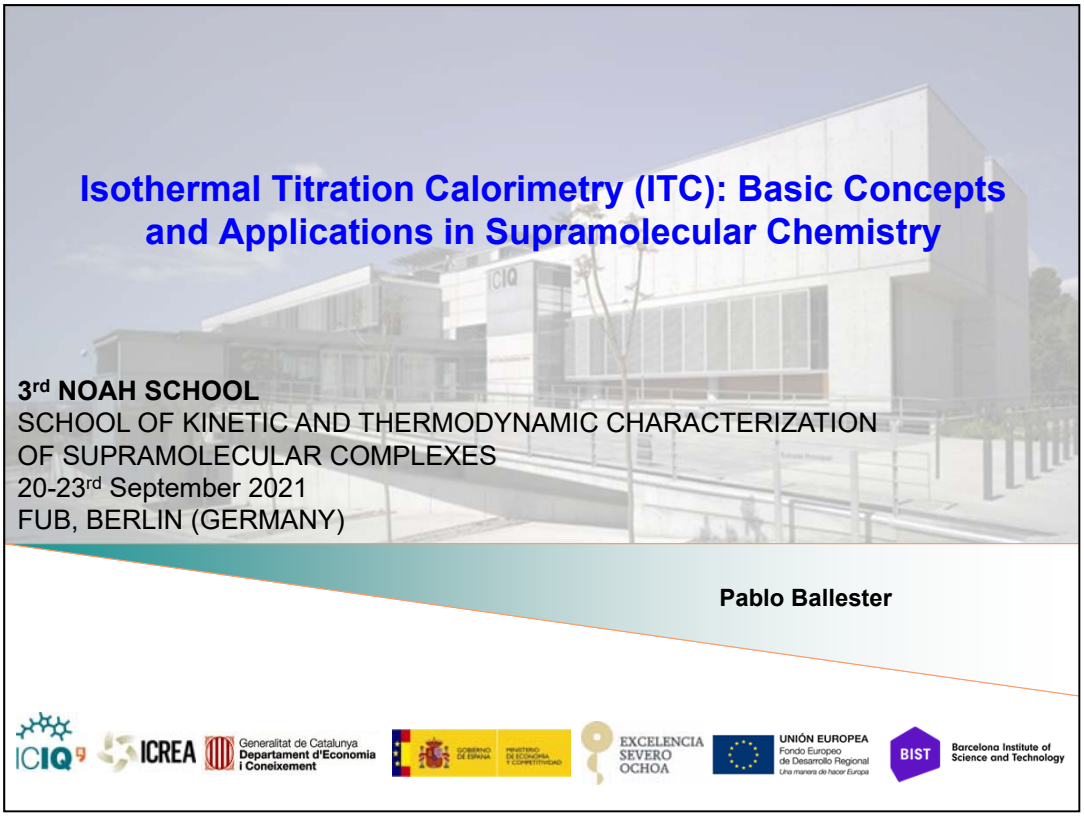

Isothermal Titration Calorimetry (ITC): Basic Concepts and Applications in Supramolecular Chemistry

3rd NOAH SCHOOL
SCHOOL OF KINETIC AND THERMODYNAMIC CHARACTERIZATION
OF SUPRAMOLECULAR COMPLEXES
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ITC Basic Concepts and Applications

What is ITC and how it works?.

Establishing titrations conditions.

Simulated thermograms for diverse binding models and different titrations conditions.

Kinetic information from ITC.

ITC experiments applied to supramolecular systems.

- What is ITC and how it works?

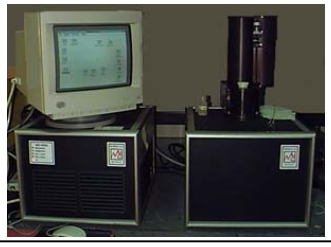
What is ITC?

Isothermal Titration Calorimetry (ITC) was coined in the late 1980s.

The method allows for simultaneous determination of n, K_a and ΔH.
More recently, kinetic measurements (k_{off}/k_{on}) have also been derived from ITC experiments (KinITC).

In 1990 ITC was introduced to biochemistry as a new method without citing previous work. Determination of K_a by titration calorimetry was first reported in 1965.

The introduction of power compensation calorimeters with small volumes and low detection limits by Microcal (Malvern) and Calorimetry Sciences, TA Instruments, (Waters) during the 1990/2000s made ITC experiments the accepted norm.



What is ITC?

An ITC experiment consists in a stepwise titration that collects 10 to 25 data points and requires 2 to 3 h.

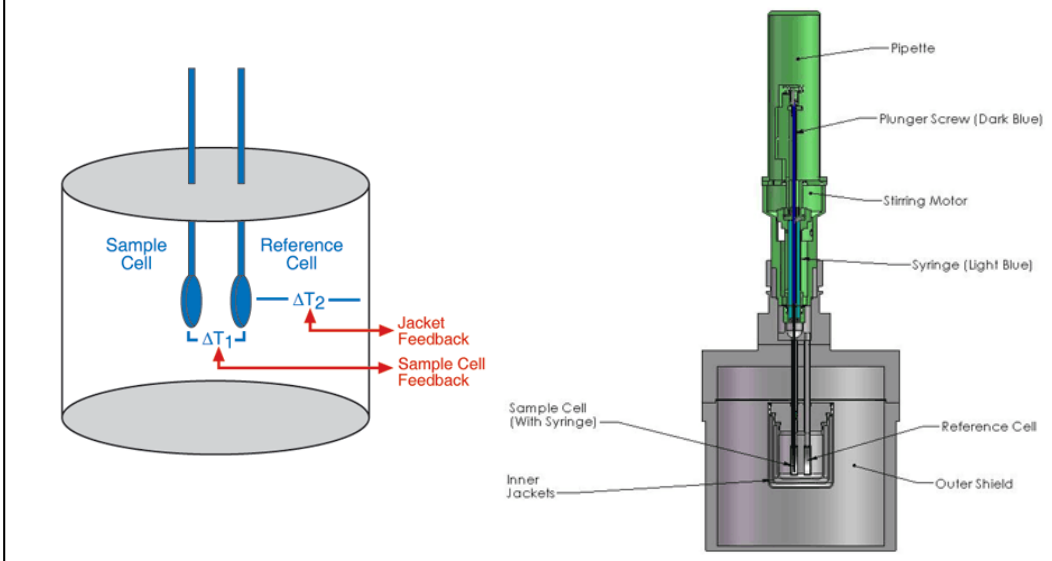
The usual method of heat measurement for this technique is power compensation.

The calorimeter is usually operated with an overfilled reaction vessel. The addition of titrant displaces reactant solution.

The titrant is added in increments which can vary in size and number.

The computation of K_f and ΔH is done on the increments of heat produced between data points (differential analysis)

How does it work?

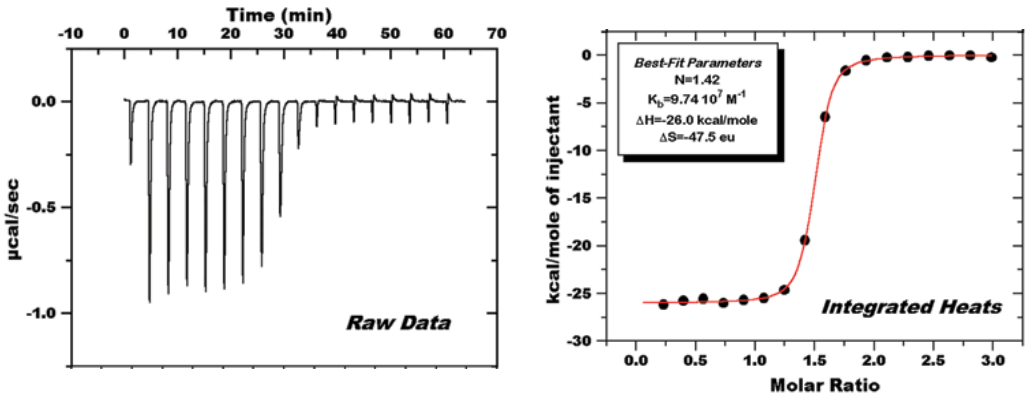


https://www.malvernpanalytical.com/en/products/technology/microcalorimetry/isothermal-titration-calorimetry

MicroCal's ultrasensitive ITC systems use a cell feedback network (CFB) to differentially measure and compensate for heat produced or absorbed between the sample and reference cell. Twin coin-shaped cells are mounted in a cylindrical adiabatic environment, and connect to the outside through narrow access tubes (Figure 1). A thermoelectric device measures the temperature difference between the two cells and a second device measures the temperature difference between the cells and the jacket. As chemical reactions occur in the sample cell, heat is generated or absorbed. The temperature difference between the sample and reference cells (ΔT1) is kept at a constant value (i.e. baseline) by the addition or removal of heat to the sample cell, as appropriate, using the CFB system. The integral of the power required to maintain ΔT1 = constant over time is a measure of total heat resulting from the process being studied. Figure 2 is a schematic drawing of the ITC cells and syringe.

Results of a run

The calorimetric method fundamentally depends on measuring the difference between the equilibrium amount of reaction and what would be measured if the reaction went to completion.



In an ITC experiment, a syringe containing a “ligand” solution is titrated into a cell containing a solution of the “macromolecule” at constant temperature. When the ligand is injected into the cell, the two materials interact, and heat is released or absorbed in direct proportion to the amount of binding. As the macromolecule in the cell becomes saturated with ligand, the heat signal diminishes until only the background heat of dilution is observed. A major advantage of the MicroCal ITC instruments is the availability of three user-selectable modes of operation: high gain, low gain, and passive (US Patent Number 5,967,659). The high gain mode is suggested for most ITC experiments, allowing the fastest re-equilibration between injections, thereby providing the shortest experimental times. The passive mode has the lowest noise, and is useful when examining very small signal changes in systems having slow transients.

- Establishing titrations conditions

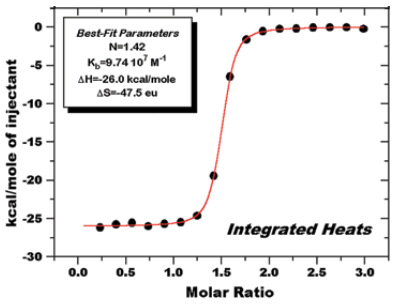
Accuracy of calculated K_f and ΔH°

Accuracy depends on how the data points are distributed and this depends on the product:
n × K_f × [A], *aka* as the Wiseman "*c*" value.

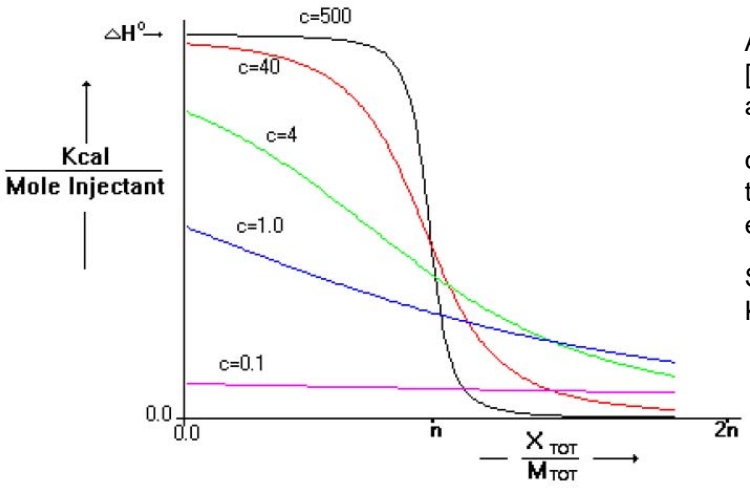
n number of binding events which is related to the stoichiometry/ies of the complex/es formed.
[A] is the concentration of the analyte in the reaction vessel (cell).
K_f is the equilibrium constant for the binding process.

K_f accuracy is affected significantly by the measurements around the equivalence point (stoichiometry of the complex.

Measurements at the beginning of the titration are most affecting to ΔH°.



Establishing titration conditions for ITC aplicability



For simple 1:1 binding:
$K_f = [HG] / [H][G]$ (1)

At the equivalence point:
$[H] = [G] = (1-\alpha_{ep})\,[H]_T$
and $[HG] = \alpha_{ep}\,[H]_T$

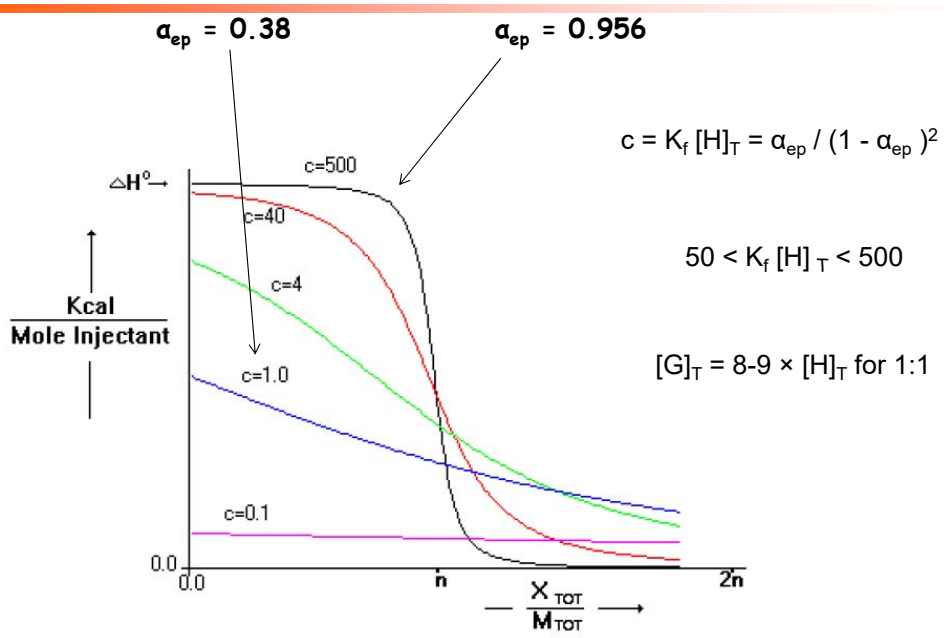
α_{ep} fraction of reactants that have reacted at the equivalence point.

Substitution in (1) yields:
$K_f\,[H]_T = \alpha_{ep} / (1 - \alpha_{ep})^2$

$c = n \times K_f \times [H]_T$

Simulated titration curves for complexation processes with differing equilibrium constants. The calorimetric method for determination of equilibrium constants depends on the reaction being significantly incomplete at the equivalence point. Quantitatively the degree of completion at the equivalence point determines how “rounded” the titration curve is and is related to the product Kf and [H].

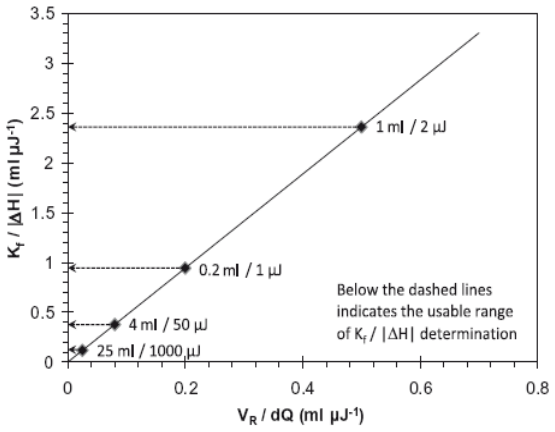
Establishing titration conditions for ITC aplicability



This equation is the basis for establishing titration conditions that allows application of the calorimetric method. Similar expressions have been derived and are the basis of the common statement that the product Kf[A] must be between 10 and 1000 or the calorimetric method cannot produce a valid Kf value. Too little reaction occurs if Kf[A]<10 and the reaction is too close to completion if Kf[A]>1000. c=1, alfaep=0.38; c=500, alfaep=0.956; c=1000, alfaep=0.969; c=10000, alfaep=0.990. The Kf[A] value needs to be between 50 and 500 to minimize uncertainty in the determined Kf.

If Kf[A] <50 correlation between DH and Kf makes it mathematically impossible to precisely separated these quantities. I Kf[A] > 500, the endpoint will be sharp, and it is normally not possible to obtain sufficient data points around the equivalence point to obtain a precise value of Kf. The curvature around the equivalence point is the determinant of Kf. At least 5 data points are required to define this curved portion of the titration curve. Note that Kf of the system is not a variable but a fundamental property. [A] must be changed to adjust the value Kf[A for a five reaction.

Upper limits for $K_f/\Delta H°$ ratio: calorimeters capabilities



Hansen et al Anal. Biochem. 409 (2011) 220-229

$K_f [H]_T = \alpha_{ep} / (1 - \alpha_{ep})^2$

can be extended to allow calculation of the range of conditions suitable for a given calorimeter :

$K_f / |\Delta H°| < 4.72\ V_R/\delta Q$;
for $\alpha_{ep} = 0.956$; c = 500

V_R = volume reaction vessel
δQ = detection limit

Assuming no change in detection limit, the latest innovation in ITC V_R = 200 μL, compared with V_R = 1000 μL, decreases the accessible upper limit of K_f by a factor of 5.

The figure shows the limit obtained from this inequality for typical calorimeters. To compare the capabilities of calorimeters for

equilibrium constant determinations is not the equilibrium constant per se but rather the ratio of the equilibrium constant to the absolute value of the enthalpy change (i.e., Kf/|DH|).

The upper limit of Kf/|DH| for a given calorimeter is fixed by the ratio VR/dQ (i.e., the ratio of the active volume of the reaction vessel to the detection limit for measurement of the heat per injection). The lines show the upper level of this value for commercially available calorimeters. The volume of the reaction vessel and heat detection limit are shown. For a given volume, the detection limit and the magnitude of DH determine the upper limit of Kf values that can be determined accurately. For a given detection limit, the upper limit on Kf values increases proportionally to an increase in volume.

The detection limit restrict the upper boundary for Kf determination because $[H]_T$ must be decreased to keep the product Kf[H] within the acceptable range, and as [H] decreases, the heat per injection becomes too small to measure accurately.

Similarly, the maximum measurable heat per injection (maximum of the dynamic range) is another characteristic of a calorimeter that must be taken into account when considering the limits of accessible KfCR values. When Kf is small, CR must be made large to stay within the range of acceptable KfCR values. Under these conditions,

especially if DH is large, the total heat per injection in an incremental titration or the heat rate in a continuous titration may exceed the dynamic range of the calorimeter

Exercise: Mentimeter

For a Microcal VP-ITC with 1.0 ml cell and δQ = 1 µJ and aiming at a Wiseman value of 40.

To accurately determine a 1:1 binding constant with a magnitude of 7×10^7 M^{-1} and $\Delta H = -21.7$ kJ/mol?

What are the best/optimum concentrations of H and G for the experiment?

Is the detection limit and cell volume of the calorimeter suitable for the experiment?

Example

For a Microcal VP-ITC with 1.0 ml cell

Is it possible to accurately determine a 1:1 binding constant with a magnitude of $7 \times 10^7\ M^{-1}$?

What host concentration should I use?
$c = n K_f [H]_T$; $40 = 1 \times (7 \times 10^7) \times [H]_T$; $[H]_T = 5.7 \times 10^{-7}$ M

What concentration should I use for the guest in the syringe?
$[G]_T = 8 \times 5.7 \times 10^{-7}$ M = 4.5×10^{-6} M

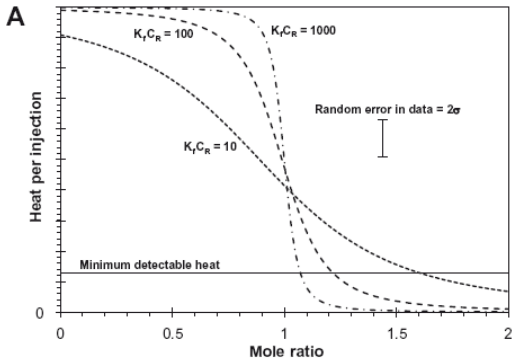
$K_f / | \Delta H^\circ | < 4.72\ V_R/\delta Q = 4.72 \times 1/ 1 = 4.72$ mL/μJ

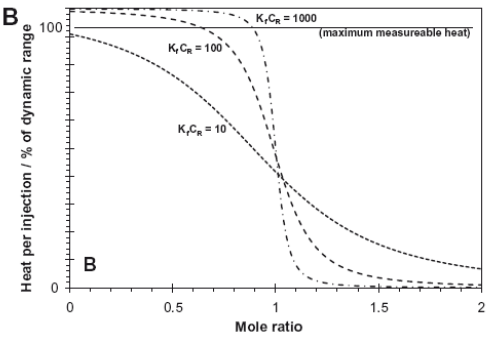
With and enthalpy of binding of ΔH = - 5.2 kcal/mol = - 21.74 kJ/mol

Upper K_f value to be accurately determined $K_f < 21740 \times 4.72 = 1.0 \times 10^5\ M^{-1}$
the enthalpy of binding should be two orders of magnitude
larger for optimal measurements

To produce optimal results, the uncertainty in the heat per data point in the titration curve, dQ, must be less than 1% of the total heat produced up to the equivalence point (i.e., Qep > 100dQ, where dQ may be defined as twice the standard deviation in the measured heat per data point).

Random errors in titration curves





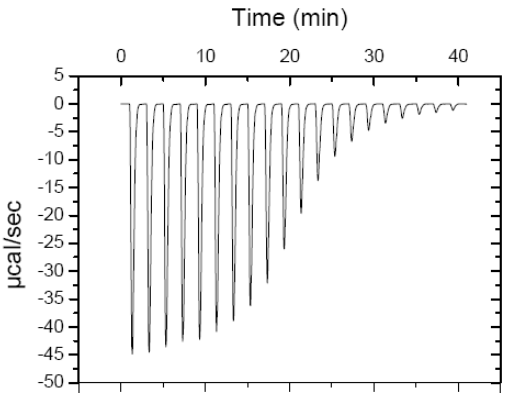
Large K_f, requires small $[H]_T$, random error from the detection limit occurs

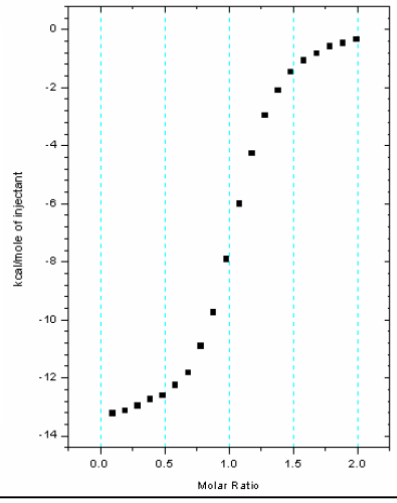
Small K_f, requires large $[H]_T$, the heat per injection may exceed the maximum measurable

Titration curves illustrating the effects of random error on titration curves under reaction conditions with large Kf that requires a small [A] value, a situation where significant random error from the detection limit occurs throughout the titration curve (A), and conditions with small Kf that requires a large [A] value, a situation where the heat per injection may exceed the maximum measurable heat and the detection limit makes an insignificant contribution to random error (B).

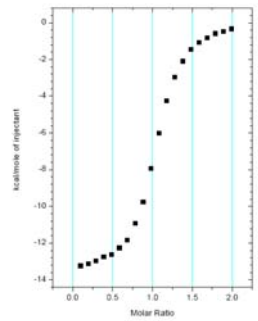
Analysis of calorimetric data

Once data have been collected (heat (μcal) *vs* time and integrated heat (kcal/mol injectant) *vs* molar ratio), a model that describes the chemistry of the system must be chosen and the thermogram analyzed mathematically using computer programs.

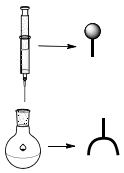




Subtracting unspecific heat effects: Heat of dilution

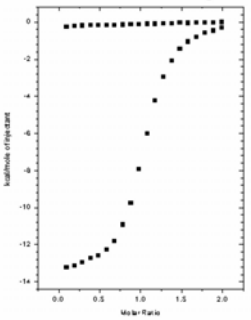


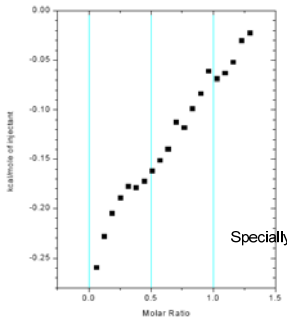
A



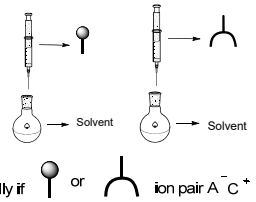
C

Simple subtraction
data point values to
zero offset





B

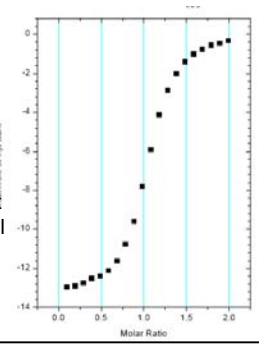


Specially if or ion pair $A^- C^+$

Competing or coupled equilibria include:
Heat of dilution, dimerization,
dissociation etc

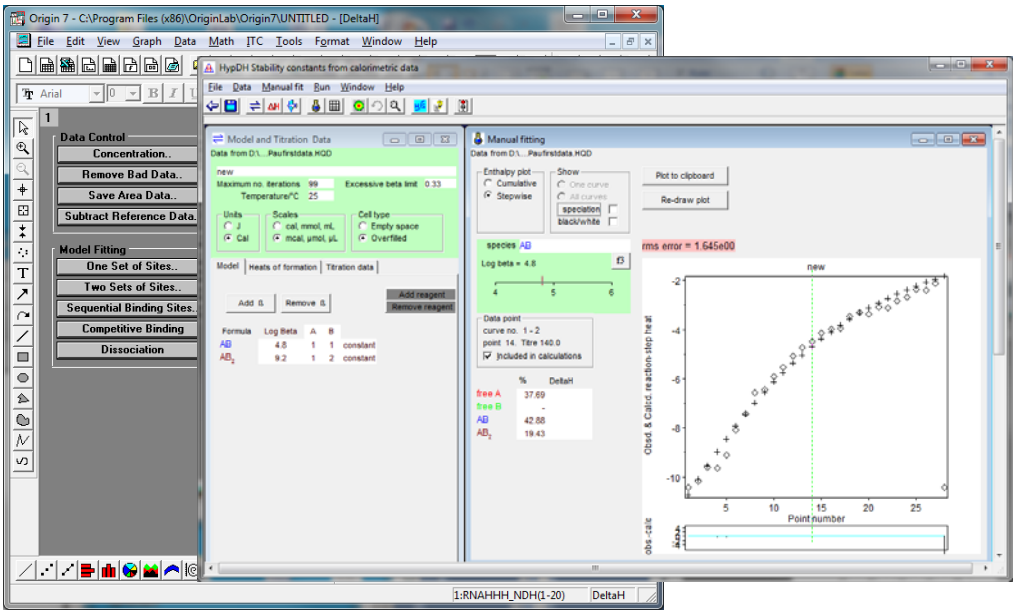
D

Idealistic data fit
to simple binding model
or inclusion of multiple
equilibria with explicit
consideration of coupled
reaction with fixed
thermodynamic constant
obtained form the control
experiments



Influences that the do not derive from the supramolecular interactions under investigation. Before analysis the data can be subtracted by using blank titrations one or the other binding partner. But synergistic effects from the solution or the instrument may not be taken into account with blank experiments. In the simple 1.1 idealistic binding model the a zero offset after blank subtraction must be obtained in the region where host-guest complexation reaches saturation. In these cases, the calculated fit function shows systematic deviations, and while error minimization may finally converge, the energetic parameters deduced can nonetheless be characterized by low accuracy and precision.

Analysis of calorimetric data



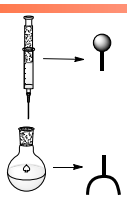
- Simulated thermograms for diverse binding models and different titrations conditions.

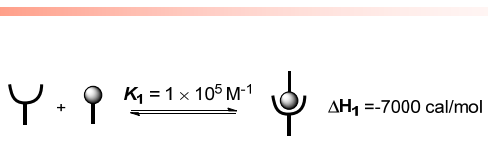
One to One Stoichiometry

$c = n \times K \times [A] = 1 \times 1 \times 10^5 \times 1.4 \times 10^{-5} = 1.4$

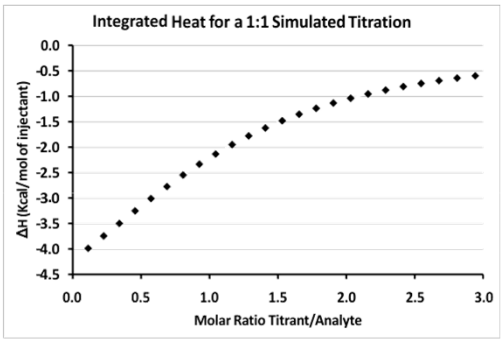
[T] = 2.24×10^{-4} M

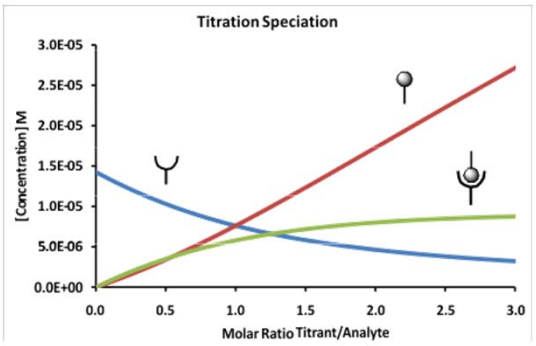
28 × 10µL injections



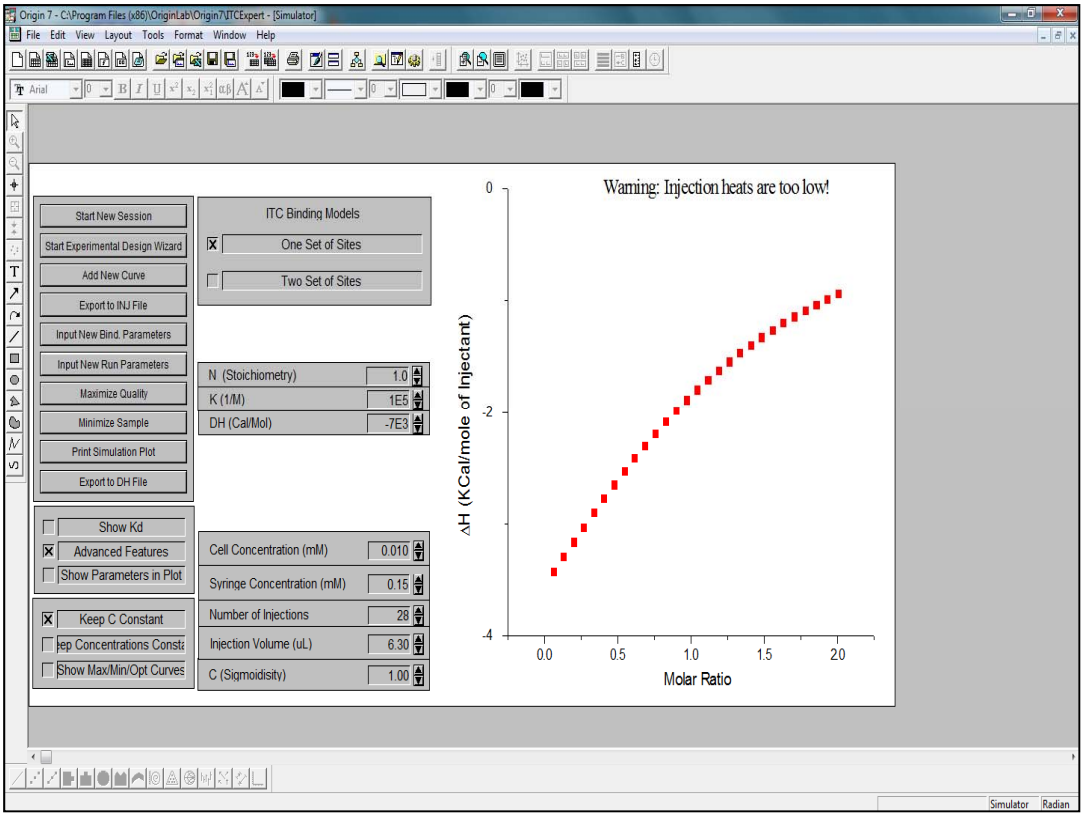


[A] = 1.4×10^{-5} M





$1 \times 10^5\ M^{-1} / 7000 = 3.4$ mL/µJ ; $K_f / |\Delta H^\circ| < 4.72\ V_R/\delta Q = 4.72 \times 1/2 = 2.3$ mL/µJ

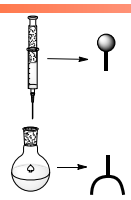

Origin 7 - C:\Program Files (x86)\OriginLab\Origin7\ITCExpert - [Simulator]
File Edit View Layout Tools Format Window Help
Start New Session
Start Experimental Design Wizard
Add New Curve
Export to INJ File
Input New Bind. Parameters
Input New Run Parameters
Maximize Quality
Minimize Sample
Print Simulation Plot
Export to DH File
ITC Binding Models
One Set of Sites
Two Set of Sites
N (Stoichiometry) 1.0
K (1/M) 1E5
DH (Cal/Mol) -7E3
Show Kd
Advanced Features
Show Parameters in Plot
Keep C Constant
Show Max/Min/Opt Curves
Cell Concentration (mM) 0.010
Syringe Concentration (mM) 0.15
Number of Injections 26
Injection Volume (uL) 6.30
C (Sigmoidisity) 1.00
Warning: Injection heats are too low!
ΔH (KCal/mole of Injectant)
Molar Ratio
0
-2
-4
0.0
0.5
1.0
1.5
2.0
Simulator
Radian

One to One Stoichiometry

$c = n \times K \times [A] = 1 \times 1 \times 10^5 \times 1.4 \times 10^{-4} = 14$

$[T] = 1.12 \times 10^{-3}$ M

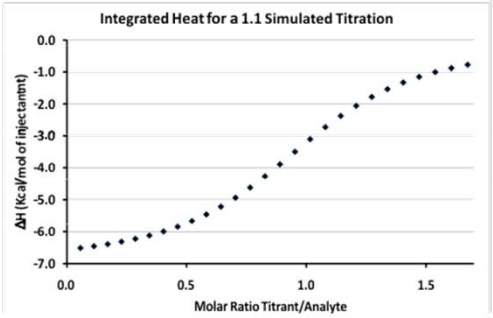
28 × 10µL injections

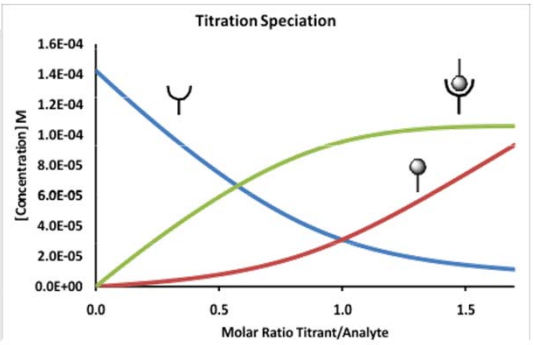


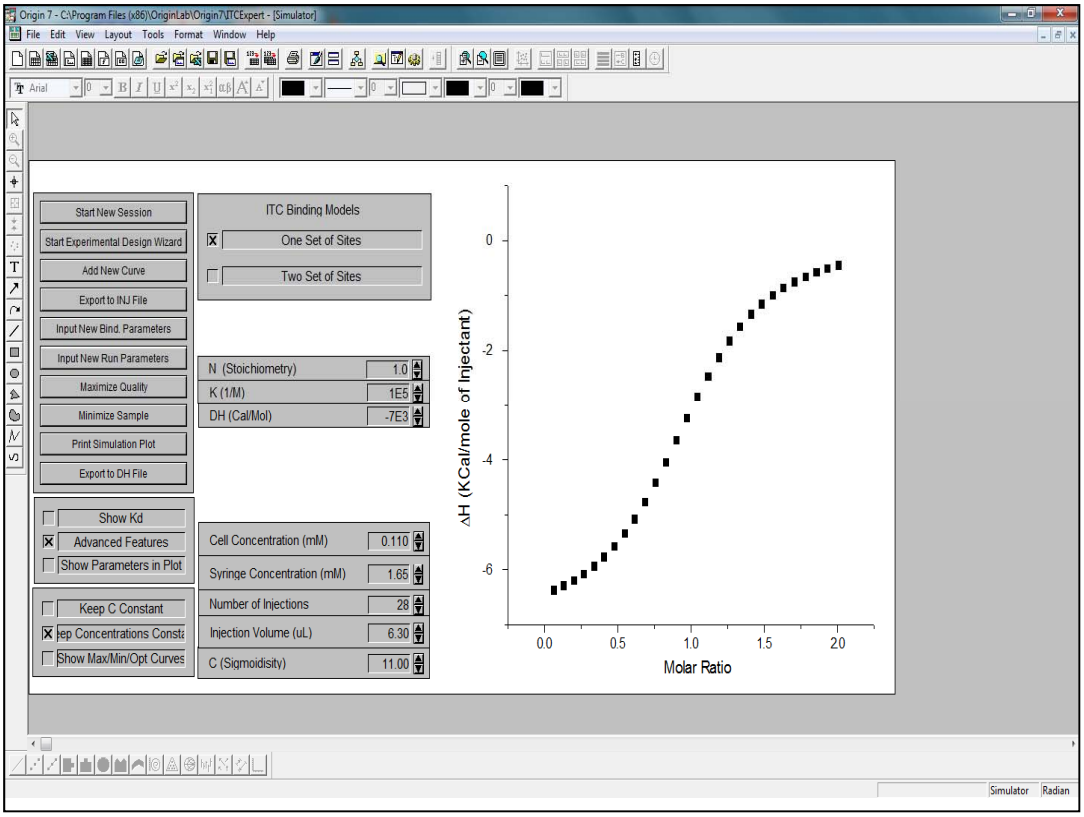
$K_1 = 1 \times 10^5$ M^{-1}

ΔH_1 =-7000 cal/mol

$[A] = 1.4 \times 10^{-4}$M





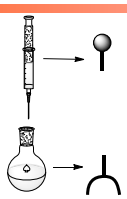

Origin 7 - C:\Program Files (x86)\OriginLab\Origin7\ITCExpert - [Simulator]
File Edit View Layout Tools Format Window Help
Start New Session
Start Experimental Design Wizard
Add New Curve
Export to INJ File
Input New Bind. Parameters
Input New Run Parameters
Maximize Quality
Minimize Sample
Print Simulation Plot
Export to DH File
ITC Binding Models
One Set of Sites
Two Set of Sites
N (Stoichiometry) 1.0
K (1/M) 1E6
DH (Cal/Mol) -7E3
Show Kd
Advanced Features
Show Parameters in Plot
Keep C Constant
Show Max/Min/Opt Curves
Cell Concentration (mM) 0.110
Syringe Concentration (mM) 1.65
Number of Injections 26
Injection Volume (uL) 6.30
C (Sigmoidisity) 11.00
ΔH (KCal/mole of Injectant)
Molar Ratio
0
-2
-4
-6
0.0
0.5
1.0
1.5
2.0
Simulator
Radian

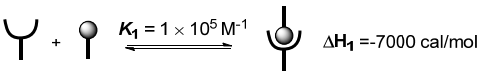
One to One Stoichiometry

$c = n \times K \times [A] = 1 \times 1 \times 10^{5} \times 1.4 \times 10^{-3} = 140$

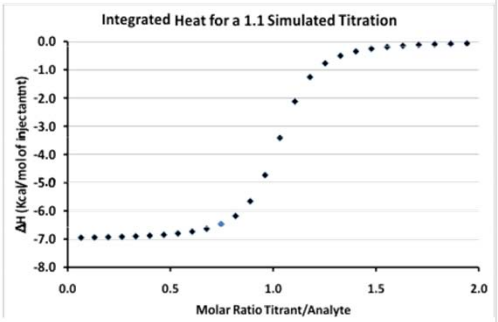
[T] = 1.3×10^{-2} M

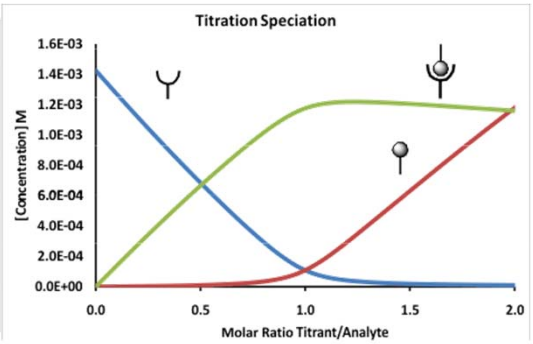
28 × 10µL injections

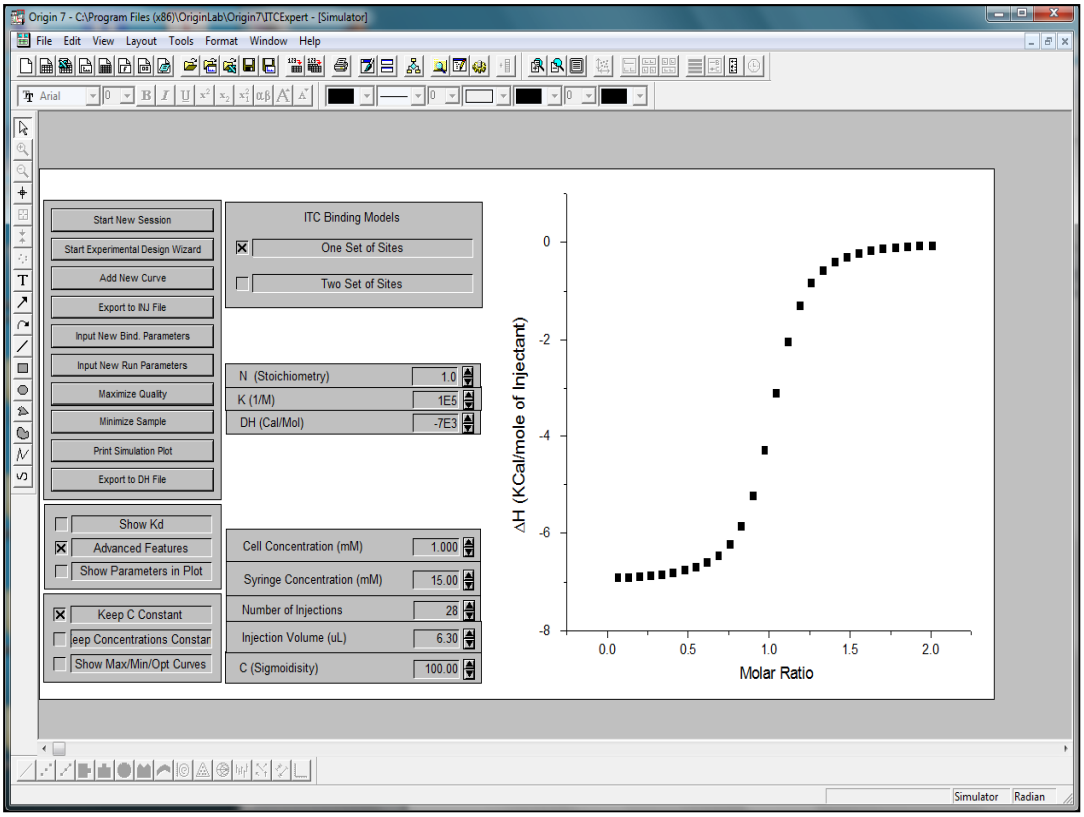




[A] = 1.4×10^{-3}M





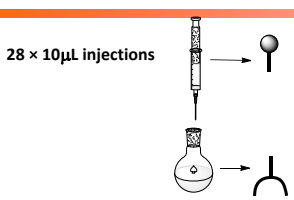

Origin 7 - C:\Program Files (x86)\OriginLab\Origin7\ITCExpert - [Simulator]
File Edit View Layout Tools Format Window Help
Start New Session
Start Experimental Design Wizard
Add New Curve
Export to INJ File
Input New Bind. Parameters
Input New Run Parameters
Maximize Quality
Minimize Sample
Print Simulation Plot
Export to DH File
ITC Binding Models
One Set of Sites
Two Set of Sites
N (Stoichiometry) 1.0
K (1/M) 1E5
DH (Cal/Mol) -7E3
Show Kd
Advanced Features
Show Parameters in Plot
Keep C Constant
eep Concentrations Constar
Show Max/Min/Opt Curves
Cell Concentration (mM) 1.000
Syringe Concentration (mM) 15.00
Number of Injections 28
Injection Volume (uL) 6.30
C (Sigmoidisity) 100.00
ΔH (KCal/mole of Injectant)
0
-2
-4
-6
-8
0.0
0.5
1.0
1.5
2.0
Molar Ratio
Simulator
Radian

One to One Stoichiometry

$c = n \times K \times [A] = 1 \times 1 \times 10^{5} \times 1.4 \times 10^{-3} = 1400$

[T] = 1.3×10^{-1} M

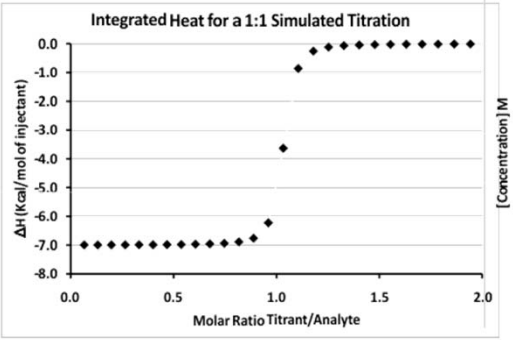
28 × 10µL injections

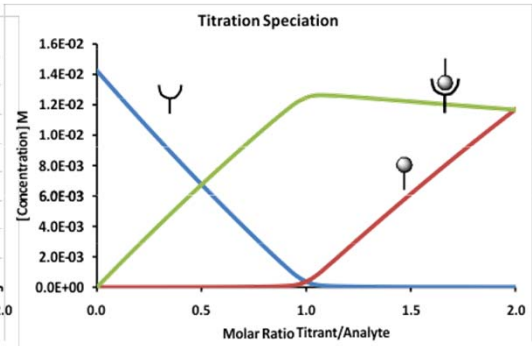


$K_1 = 1 \times 10^{5}\ M^{-1}$

ΔH_1 =-7000 cal/mol

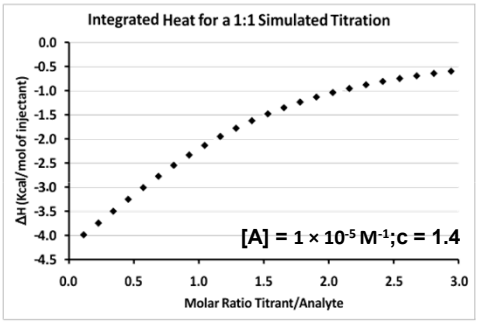
[A] = 1.4×10^{-2} M

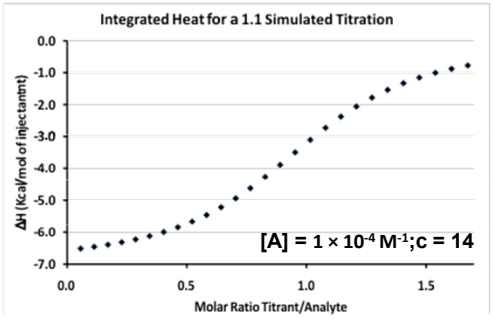


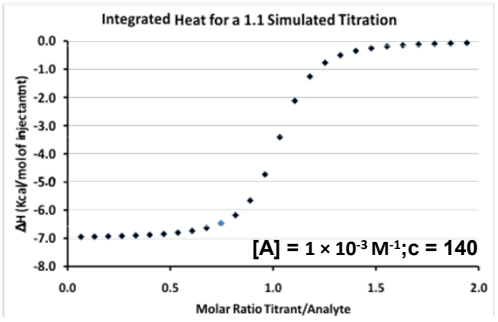


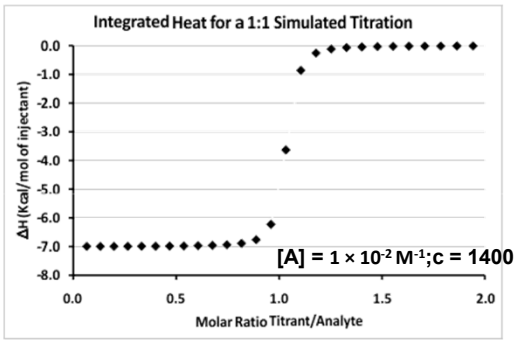
Effect of the Concentration of the Analyte

$c = n \times K \times [A]$; $K = 1 \times 10^5\ M^{-1}$

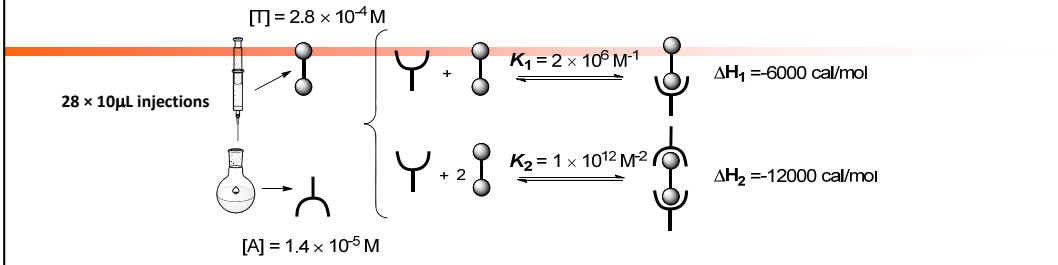


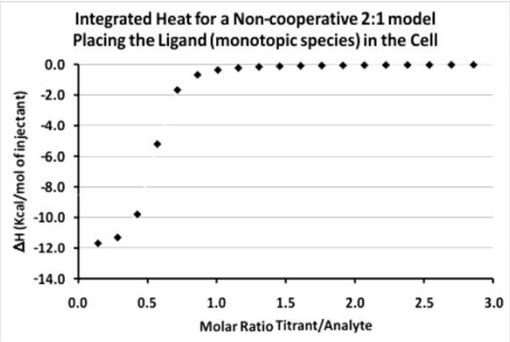


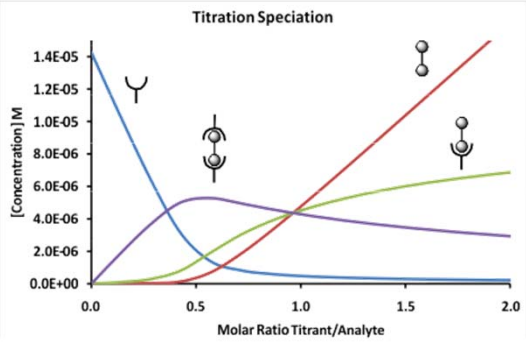




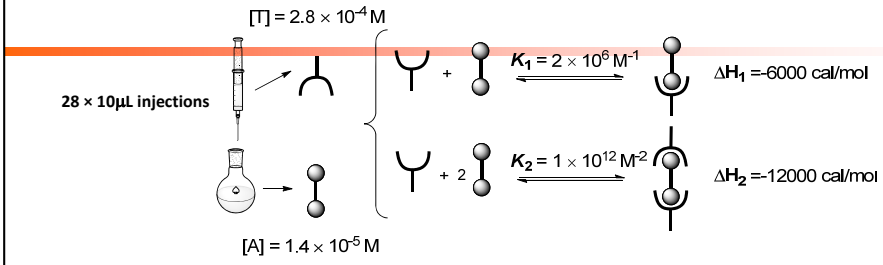
Two to One Stoichiometry (Ligand in the Cell) Non cooperative

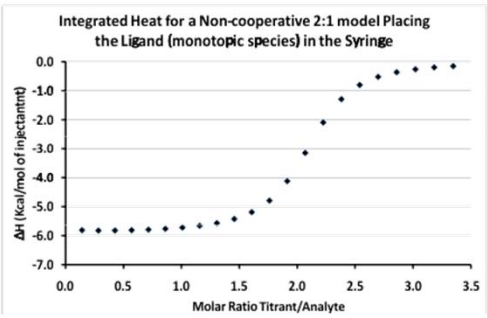


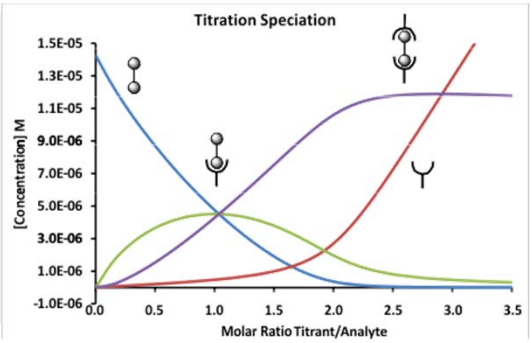


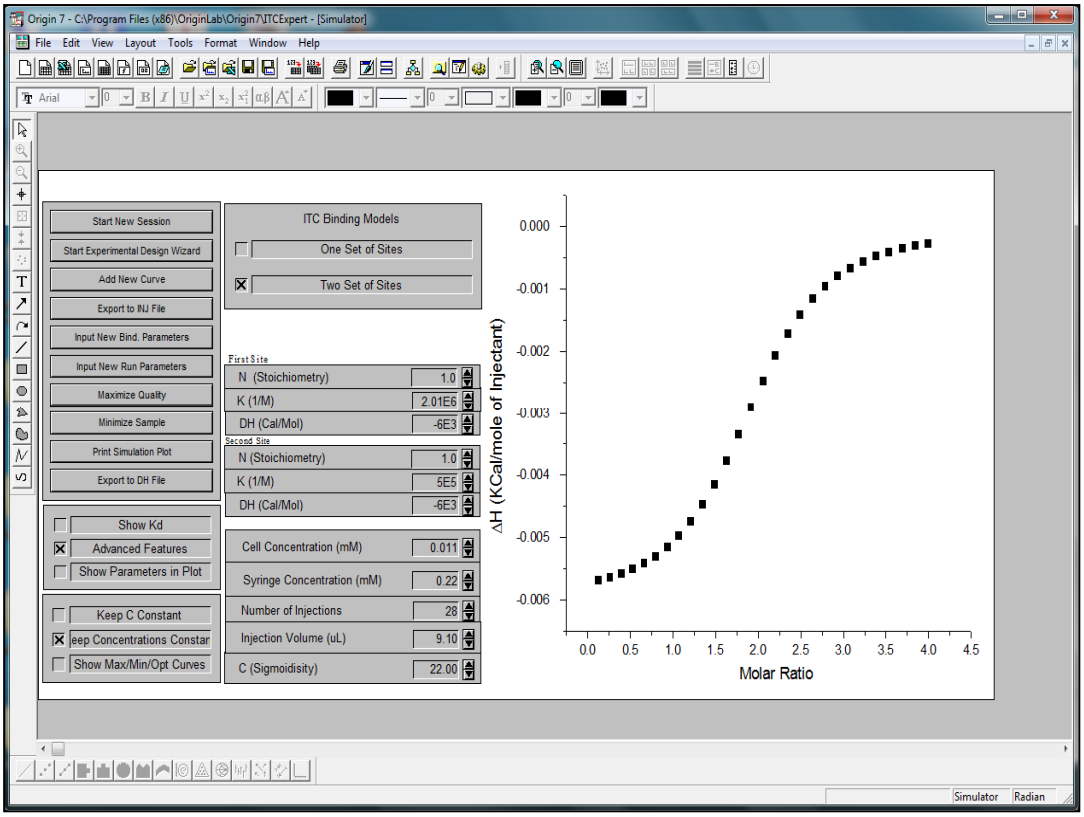


Two to One Stoichiometry (Ligand in the Syringe) Non cooperative

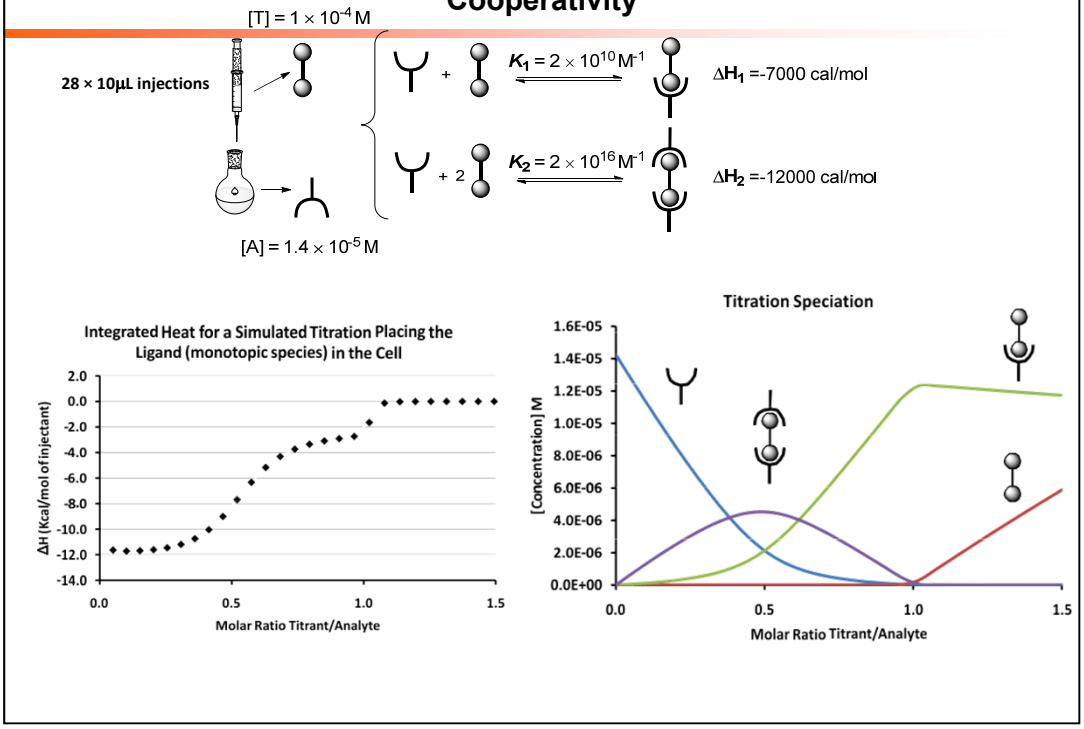




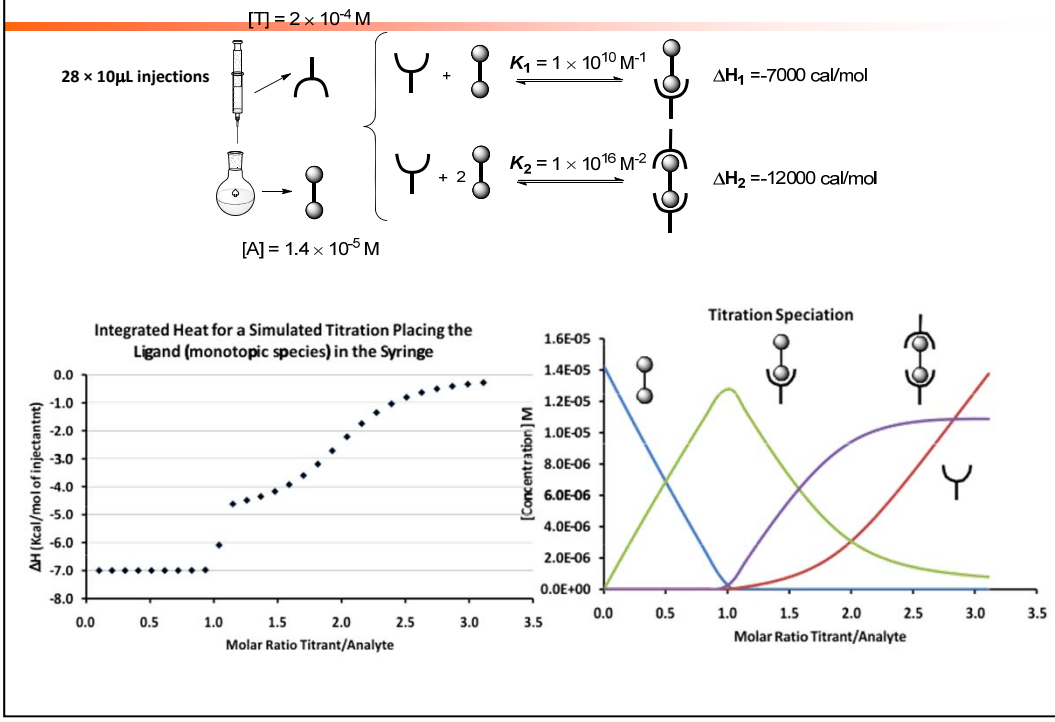


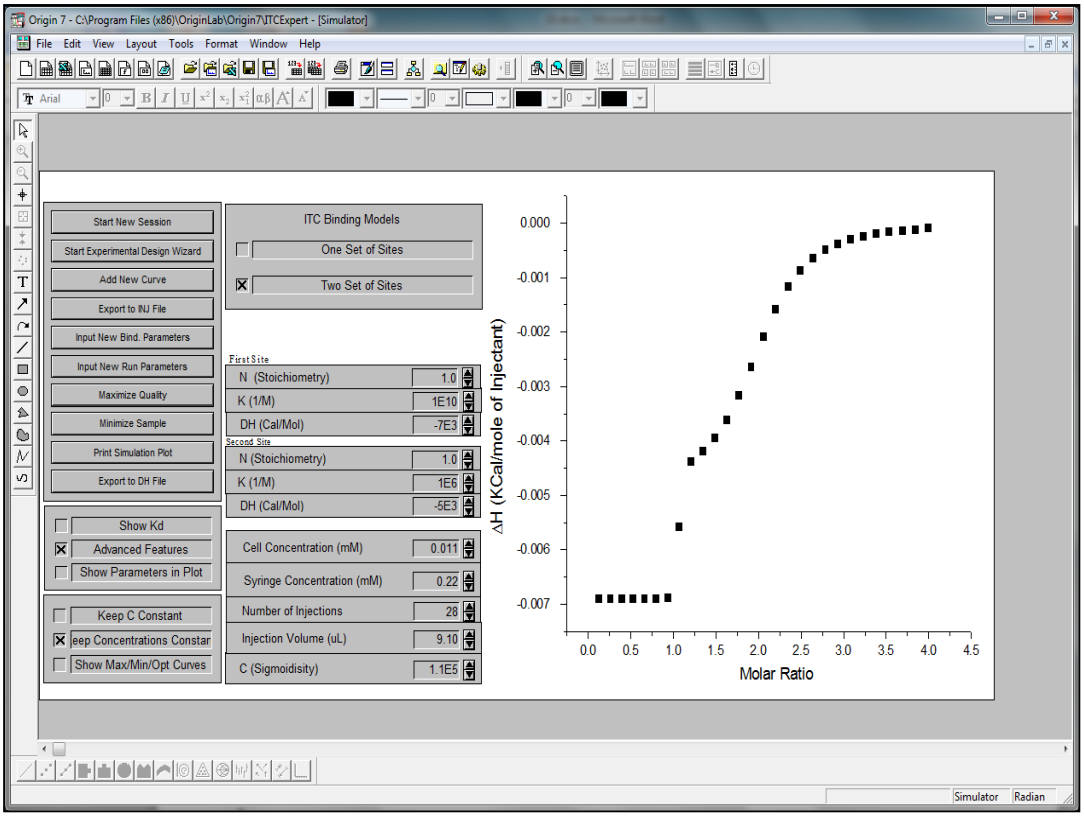

Origin 7 - C:\Program Files (x86)\OriginLab\Origin7\ITCExpert - [Simulator]
File Edit View Layout Tools Format Window Help
Start New Session
Start Experimental Design Wizard
Add New Curve
Export to INJ File
Input New Bind. Parameters
Input New Run Parameters
Maximize Quality
Minimize Sample
Print Simulation Plot
Export to DH File
Show Kd
Advanced Features
Show Parameters in Plot
Keep C Constant
eep Concentrations Constar
Show Max/Min/Opt Curves
ITC Binding Models
One Set of Sites
Two Set of Sites
First Site
N (Stoichiometry) 1.0
K (1/M) 2.01E6
DH (Cal/Mol) -6E3
Second Site
N (Stoichiometry) 1.0
K (1/M) 5E5
DH (Cal/Mol) -6E3
Cell Concentration (mM) 0.011
Syringe Concentration (mM) 0.22
Number of Injections 28
Injection Volume (uL) 9.10
C (Sigmoidisity) 22.00
ΔH (KCal/mole of Injectant)
0.000
-0.001
-0.002
-0.003
-0.004
-0.005
-0.006
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5
Molar Ratio
Simulator
Radian

Two to One Stoichiometry (Ligand in the Cell) Negative Cooperativity



Two to One Stoichiometry (Ligand in the Syringe)

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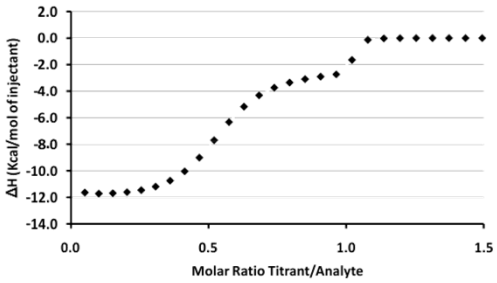
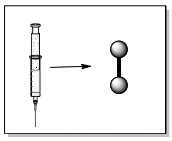
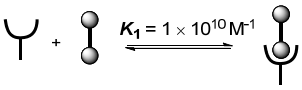
File Edit View Layout Tools Format Window Help
Start New Session
Start Experimental Design Wizard
Add New Curve
Export to INJ File
Input New Bind. Parameters
Input New Run Parameters
Maximize Quality
Minimize Sample
Print Simulation Plot
Export to DH File
Show Kd
Advanced Features
Show Parameters in Plot
Keep C Constant
eep Concentrations Constar
Show Max/Min/Opt Curves
ITC Binding Models
One Set of Sites
Two Set of Sites
First Site
N (Stoichiometry) 1.0
K (1/M) 1E10
DH (Cal/Mol) -7E3
Second Site
N (Stoichiometry) 1.0
K (1/M) 1E6
DH (Cal/Mol) -5E3
Cell Concentration (mM) 0.011
Syringe Concentration (mM) 0.22
Number of Injections 28
Injection Volume (uL) 9.10
C (Sigmoidisity) 1.1E5
ΔH (KCal/mole of Injectant)
0.000
-0.001
-0.002
-0.003
-0.004
-0.005
-0.006
-0.007
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5
Molar Ratio
Simulator
Radian

Direct and Inverse Titrations for Two to One Stoichiometry

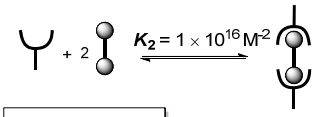
Integrated Heat for a Simulated Titration Placing the Ligand (monotopic species) in the Cell





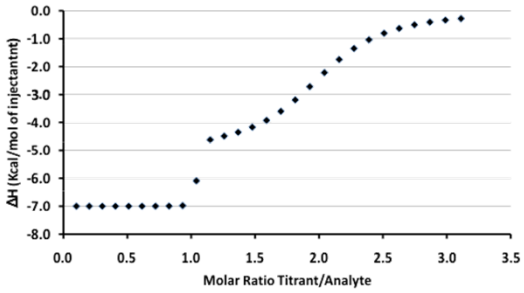


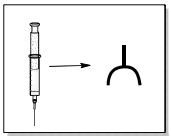
ΔH_1 =-7000 cal/mol



ΔH_2 =-12000 cal/mol

Integrated Heat for a Simulated Titration Placing the Ligand (monotopic species) in the Syringe





- Kinetic information from ITC.

Measuring the Kinetics of Molecular Association by Isothermal Titration Calorimetry (reversible reaction)

$$A + B \leftrightharpoons C \quad \text{with parameters } k_{on}, k_{off}$$

$$\frac{d[C]}{dt} = k_{on}[A][B] - k_{off}[C]$$

$$K_d = k_{off}/k_{on} \qquad K_a = \frac{k_{on}}{k_{off}}$$

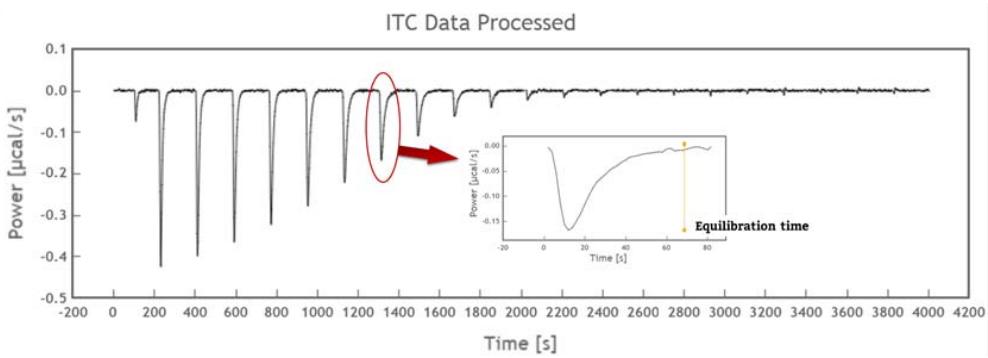
Dumas P, Ennifar E, Da Veiga C, et al. Methods Enzymol. 2016;567:157-180.

The real-time power response inherent in an isothermal titration calorimetry (ITC) experiment provides an opportunity to directly analyze association kinetics, Interactions occurring with relaxation times ranging from slightly below the instrument response time constant (VP-ITC 12.5 s in this case) to as large as 600 s. For Microcal ITC 3.5s.

In a binding titration scenario, in the most general case an injection can reveal an association rate constant (kon).

Equilibration Time Curve

Determining the “effective end” of each injection



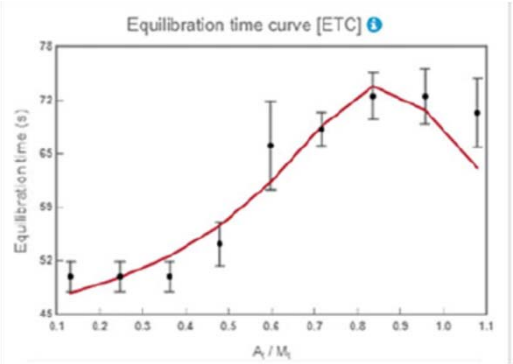
Piñeiro A, Munoz E, Sabin J, et al. AFFINImeter: a software to analyze molecular recognition processes from experimental data. Anal. Biochem. 2019;577:117-134

Under more restrictive conditions (reversibility throughout the full titration) , the instrument time constant-corrected power decay following each injection is simply an exponential decay described by a composite rate constant (kobs), from which both kon and the dissociation rate constant (koff) can be extracted.

The output from a power compensation microcalorimeter can be viewed as a convolution of the reaction “impulse” heat evolution function with the calorimeter “response” function, the latter being effectively modelled as first order with rate constant kITC.

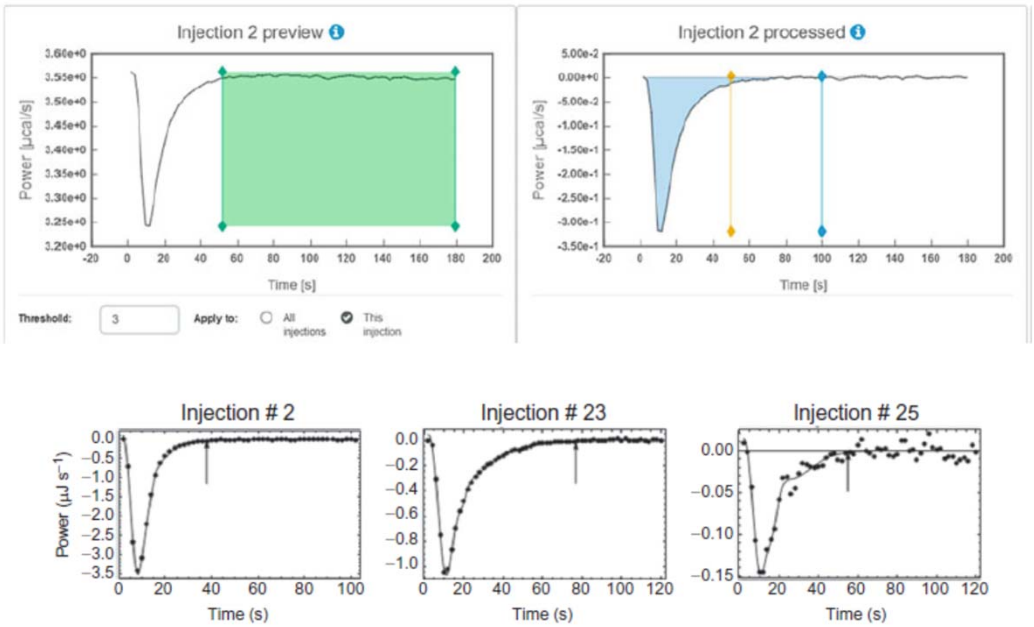
Determining the “effective end” of each injection

$$t_{\mathrm{end}}\left(N_{\mathrm{inj}}\right) = \alpha\left[\tau\left(N_{\mathrm{inj}}\right) + \tau_{\mathrm{ITC}}\right] + \tau_{\mathrm{inj}} + \tau_{\mathrm{mix}}$$



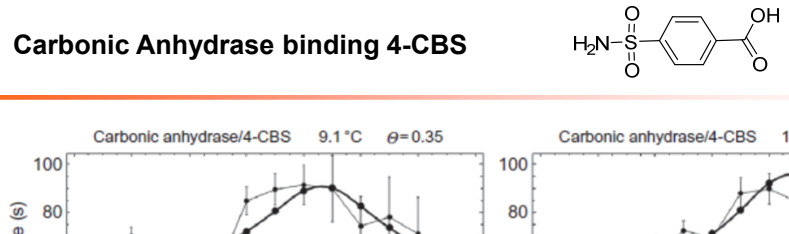
In this way, the best possible kinetic parameters were available for comparison with the results from kinITC–ETC.

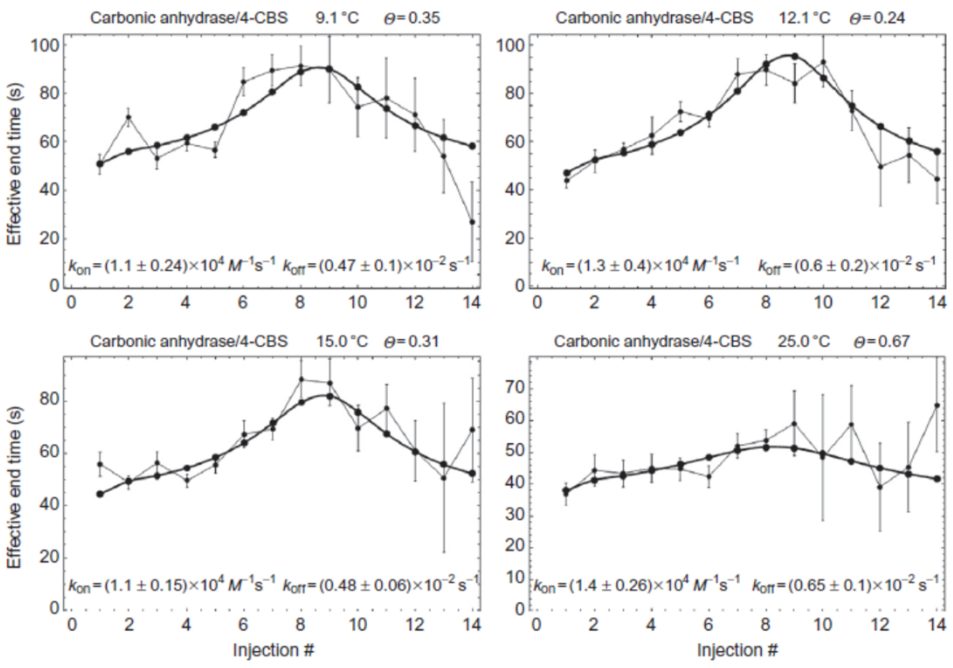
KinITC- Determining the “effective end” of each injection



The simplified kinITC method, therefore, is based upon the determination of the effective end time or, in other words, of the equilibration time of each injection, which yields a more or less bell-shaped ETC. In this way, the best possible kinetic parameters were available for comparison with the results from kinITC–ETC.

Carbonic Anhydrase binding 4-CBS

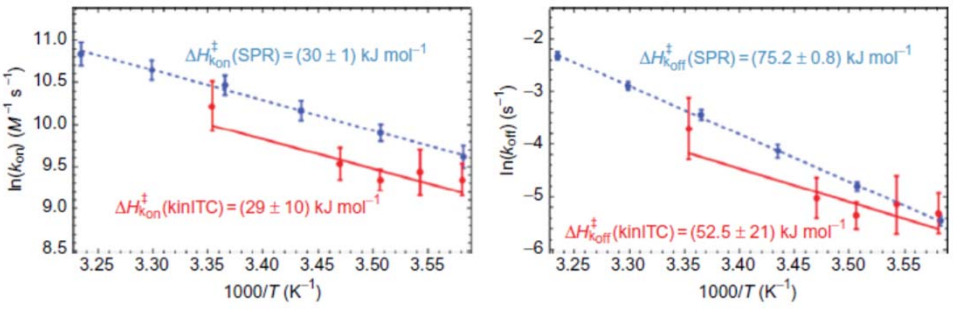




The initial concentrations of carbonic anhydrase (compound A) were [A] = 24:4, 24:2, 24:5, 24:6, and 17:6μM at, respectively, 6.1 °C (not shown), 9.1, 12.1, 15, and 25 °C. The concentration

of 4-CBS was [B] = 315μM at all temperatures and the injected volumes were 1.9 μL up to 15 °C and 1.4 μL at 25 °C.

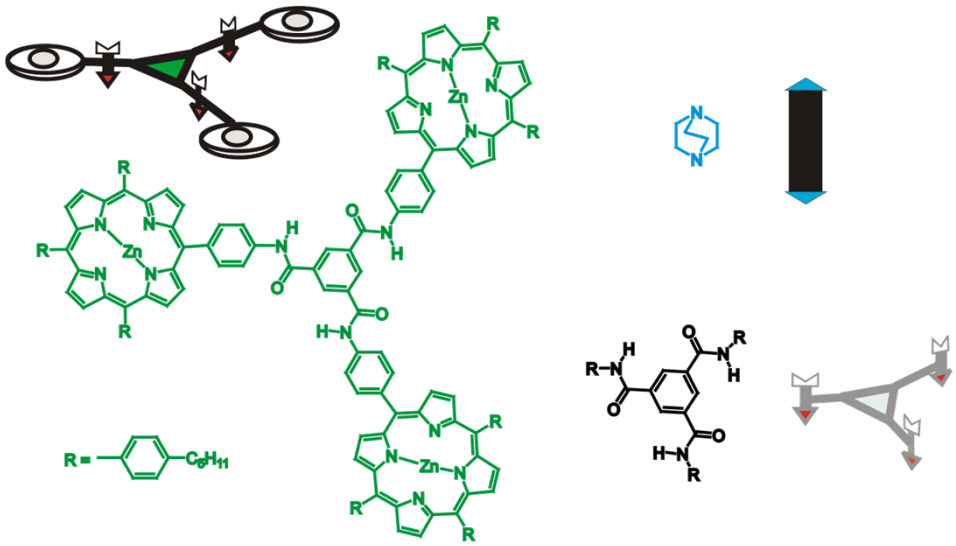
Arrhenius Plots : KinITC *vs* SPR



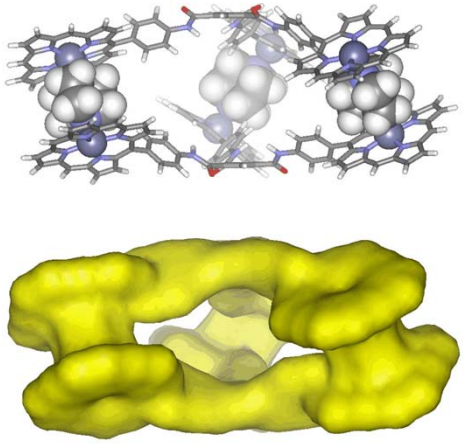
This experimental case, therefore, appears to be representative of a favorable situation leading to kinetic parameters comparing well with those from SPR results of the best possible quality due to the unusually great number of independent replicate experiments

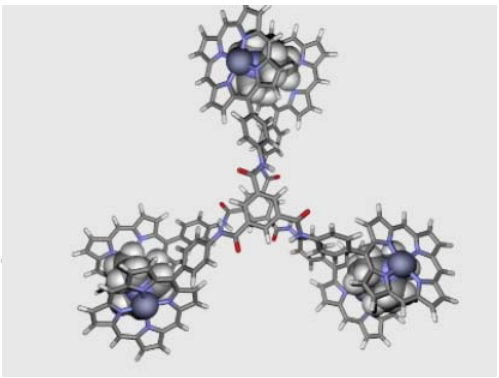
- ITC experiments applied to supramolecular systems.

Metal Mediated Assemblies

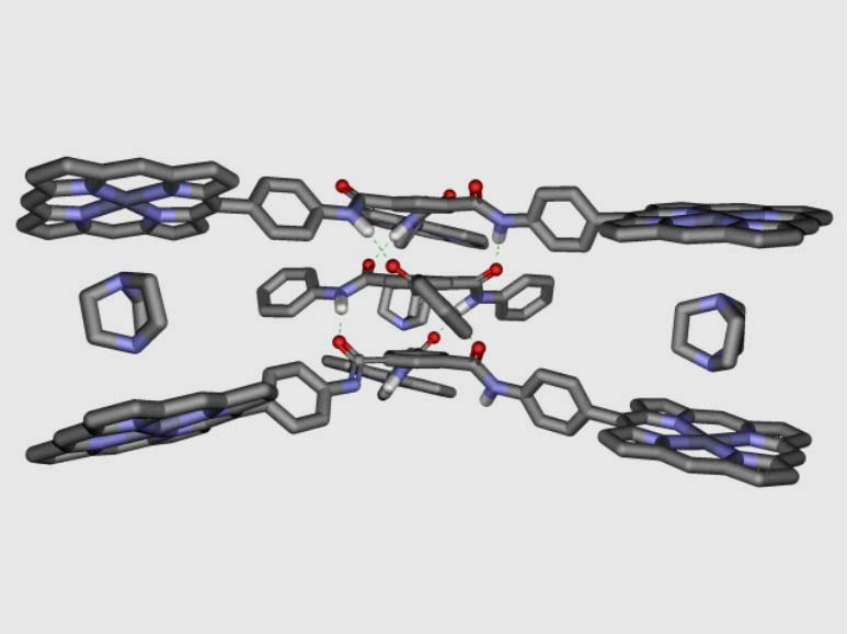


Multimolecular Coordination Cage

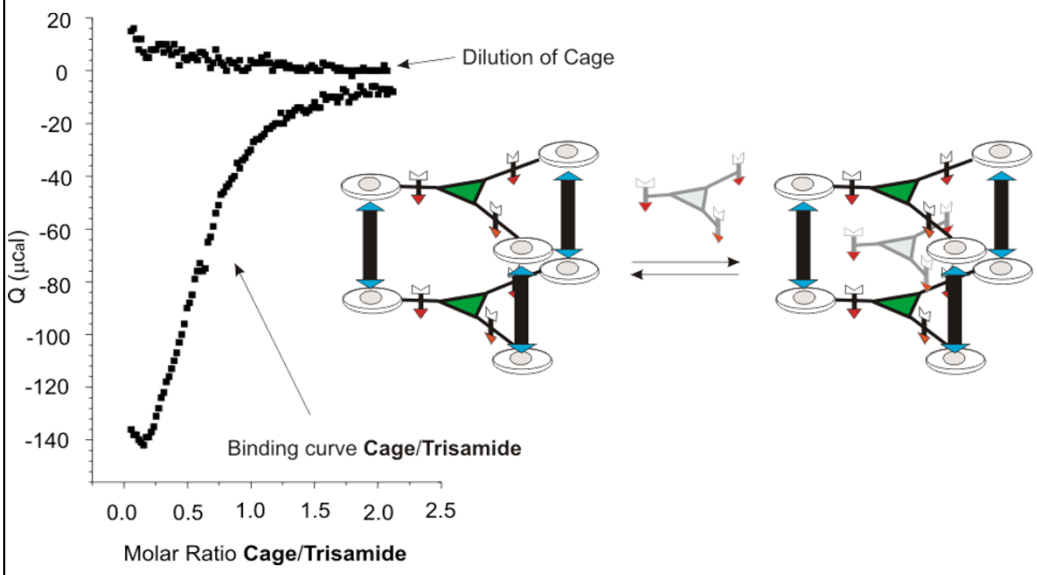




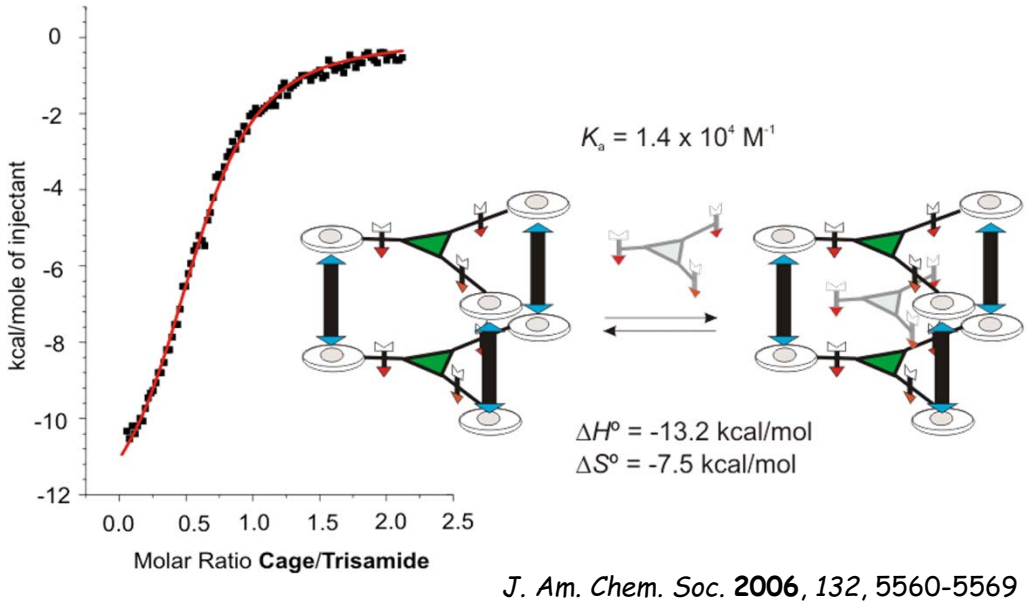
Binding a Guest inside the Cage



ITC Titrations with Coordination Cage

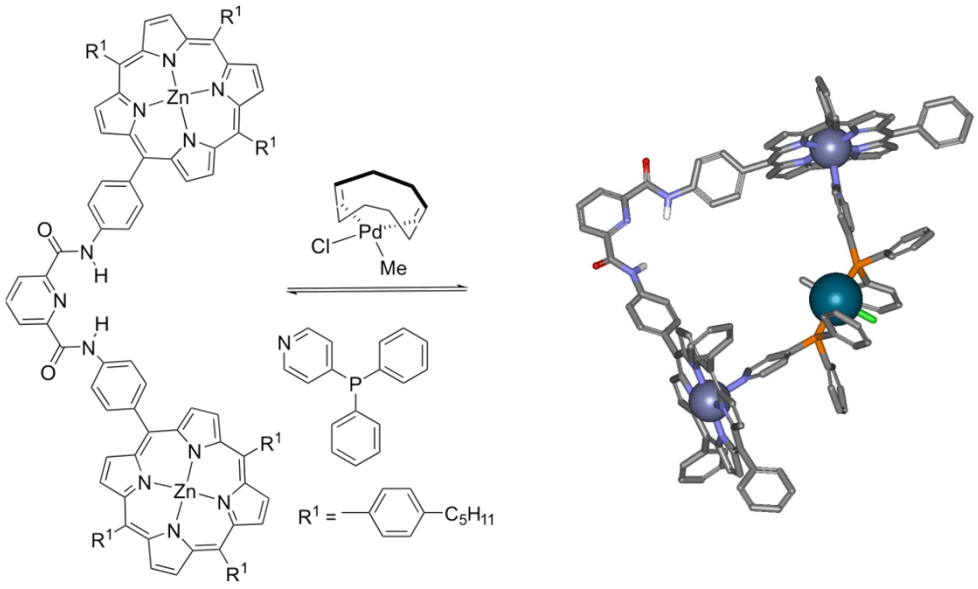


Metal Mediated Assemblies. Molecular Inclusion



J. Am. Chem. Soc. **2006**, *132*, 5560-5569

Heterobimetallic Bisporphyrin Macrocycle



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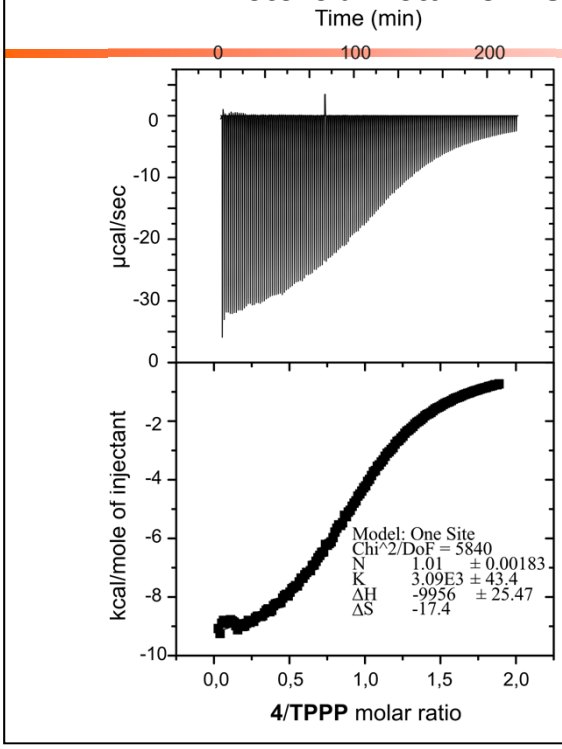

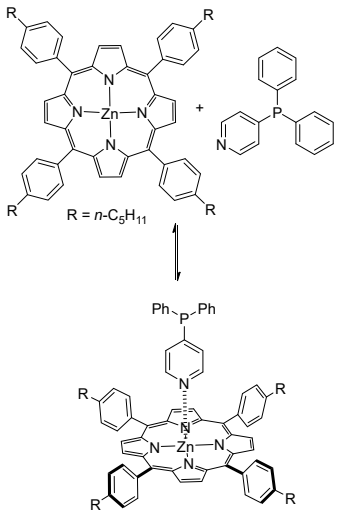

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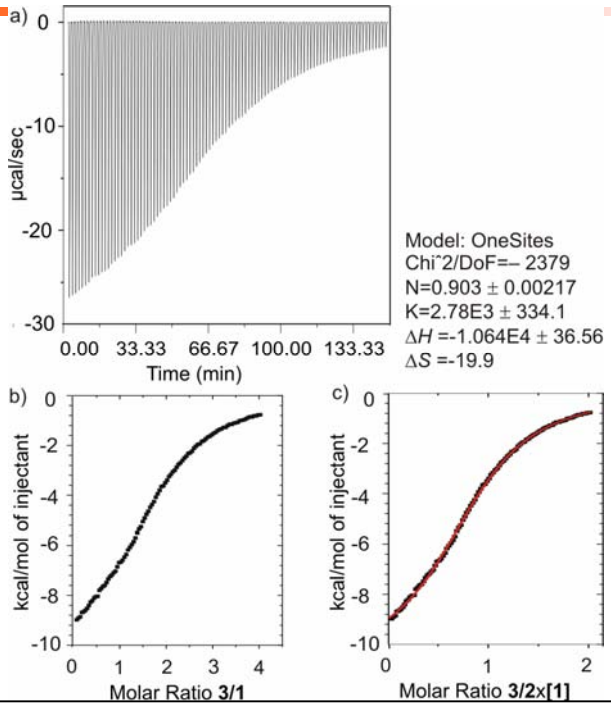

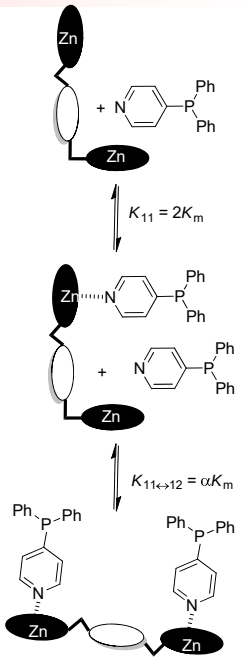

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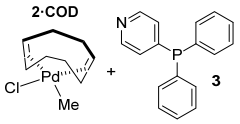

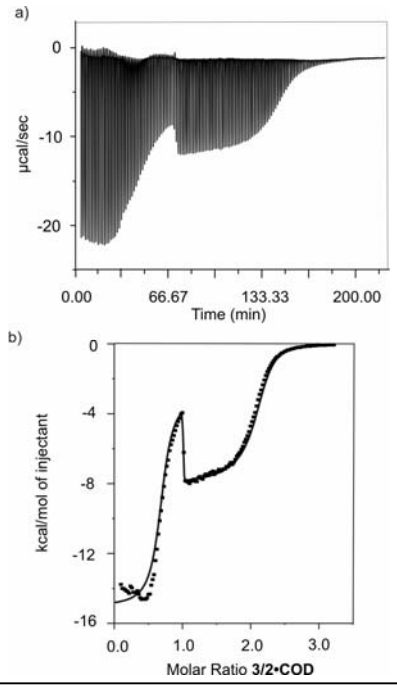

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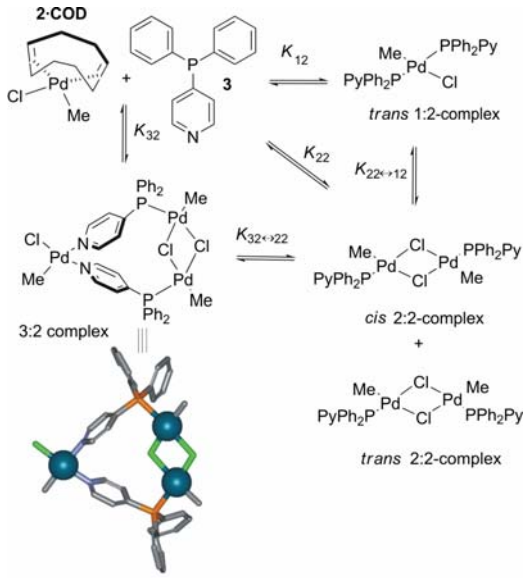

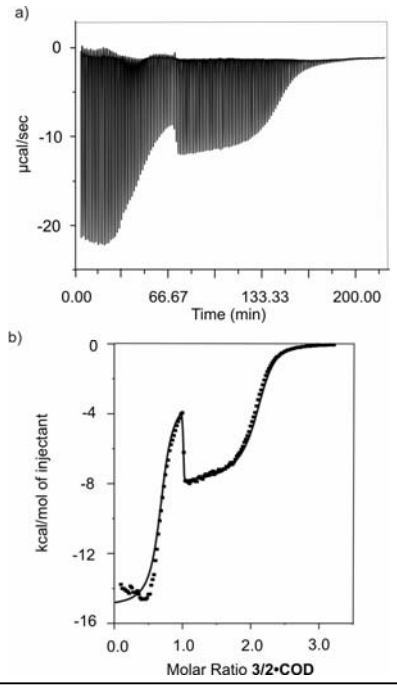

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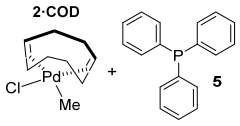

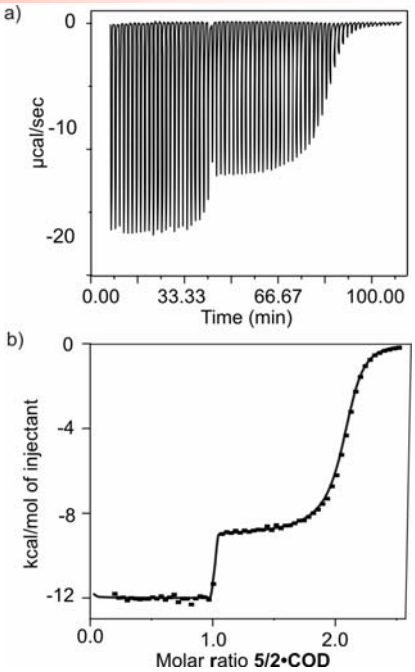

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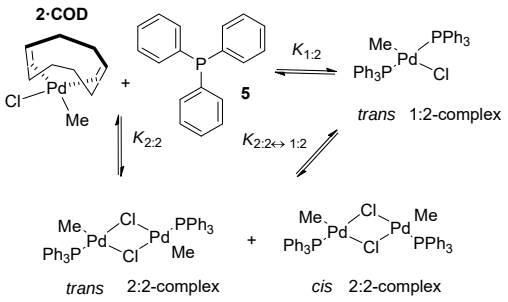
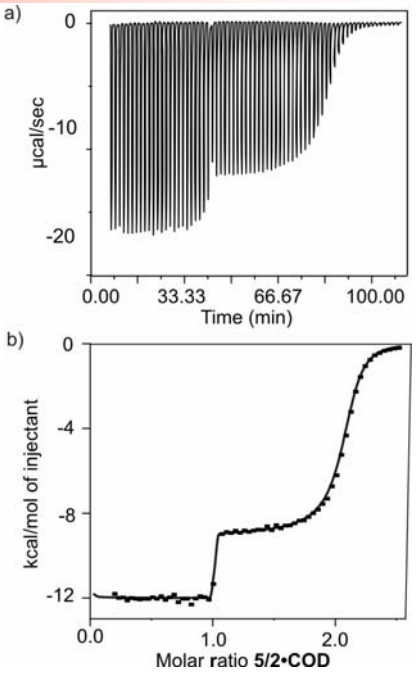
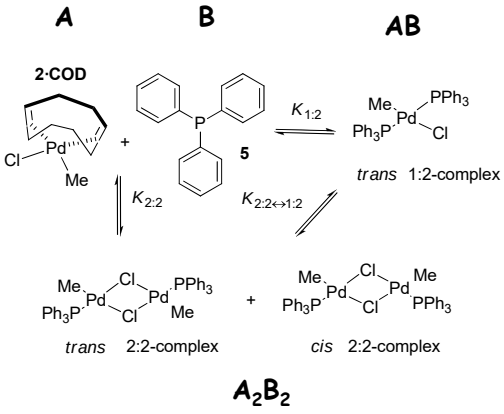
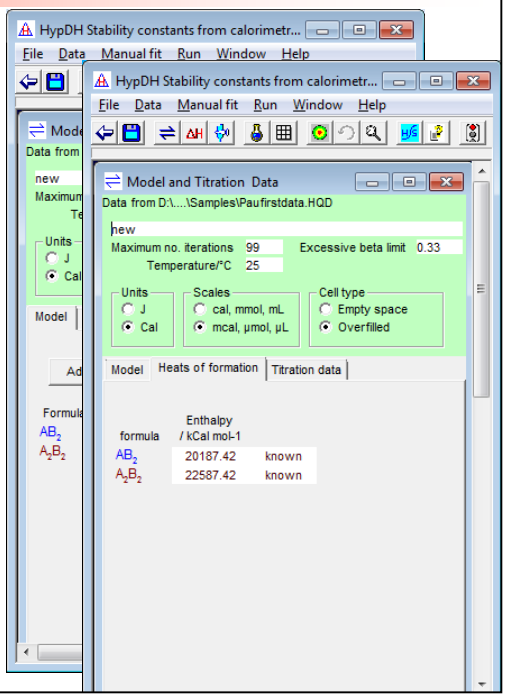




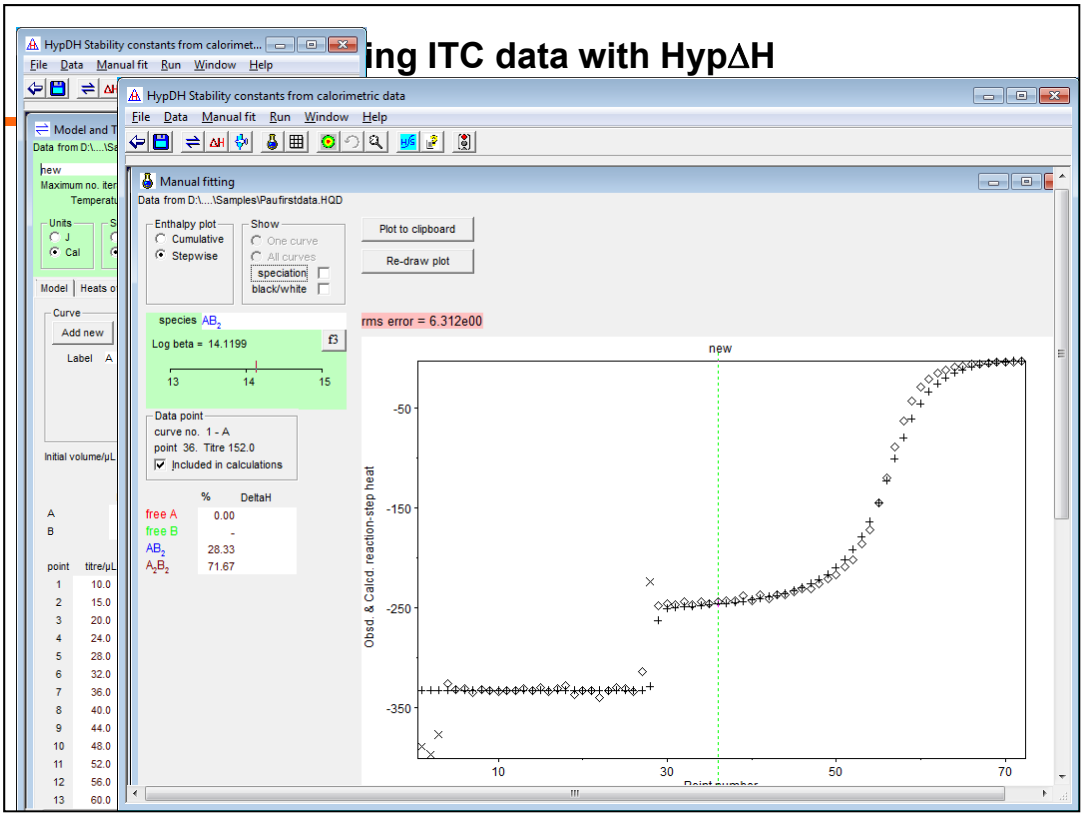
Fitting ITC data with HypΔH





Peter Gans, Protonic Software
Antonio Sabatini and Alberto Vacca (U. Firenze)

ing ITC data with HypΔH



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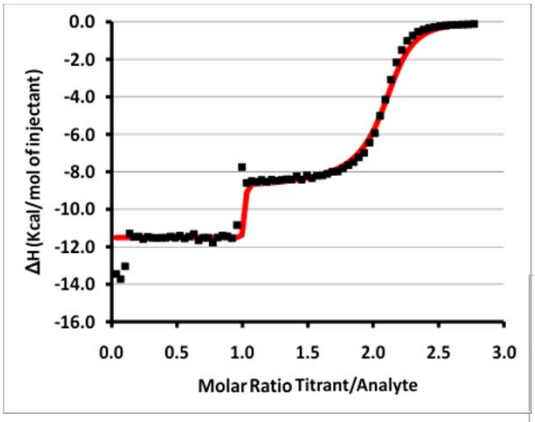
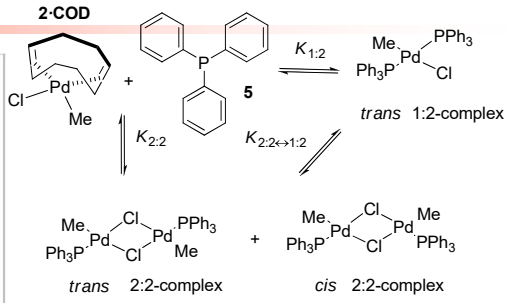
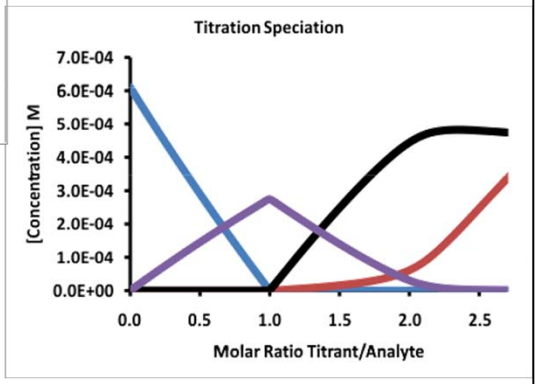
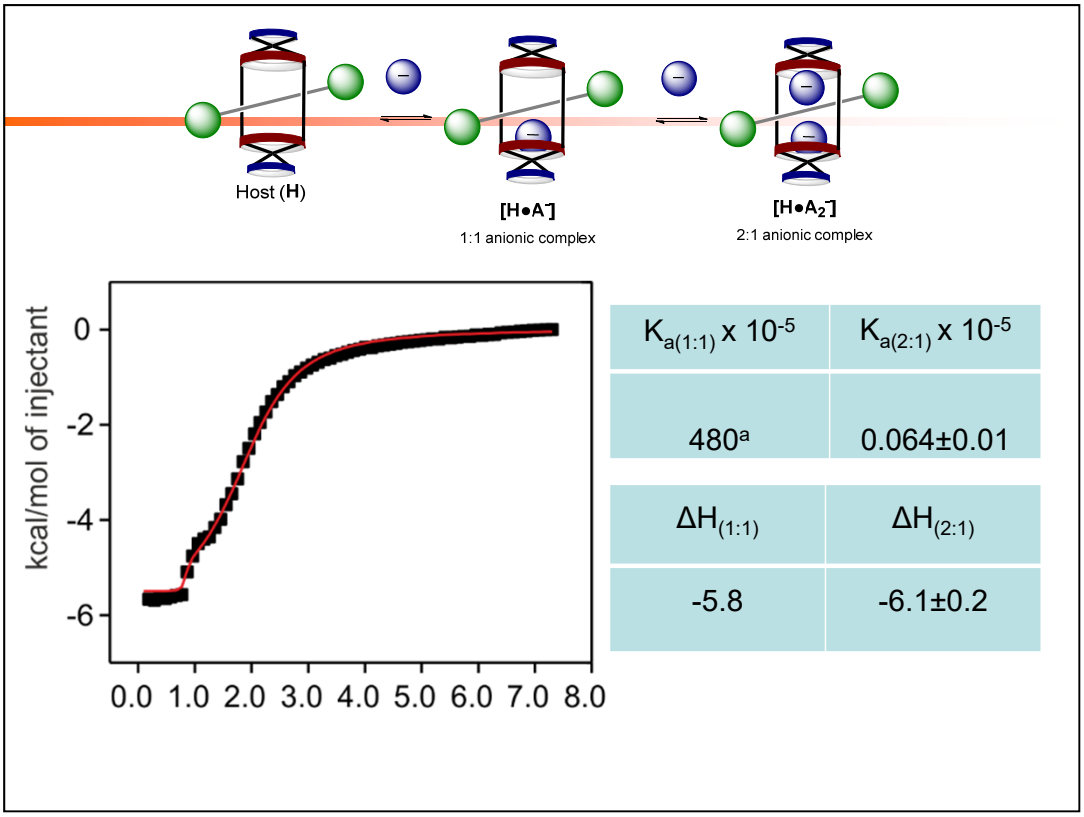






HypΔH

complex ($\mathbf{2}_m\cdot\mathbf{5}_l$)	log$K$($\mathbf{2}_m\cdot\mathbf{5}_l$)	ΔH° (kcal/mol)
$\mathbf{2}_2\cdot\mathbf{5}_2$	22.0	-22.6
$\mathbf{2}\cdot\mathbf{5}_2$	14.1	-20.2



$K_{a(1:1)}$ x 10^{-5}	$K_{a(2:1)}$ x 10^{-5}
480[a]	0.064±0.01
$\Delta H_{(1:1)}$	$\Delta H_{(2:1)}$
-5.8	-6.1±0.2

Conclusions

- In favorable circumstances logβ and ΔH^o for single and multiple equilibria can be determined from calorimetric data alone. Titration calorimeters, however, measure the sum of heat associated with all processes occurring upon addition of T.
- The selection of concentrations for [H] and [G] used in the titration are of utmost importance. Experimental conditions must be selected to avoid or try to minimize competing or coupled equilibria involving the interacting species. Subsequent data analysis must take into account the contributions of coupled equilibria to the overall measured heat.

Conclusions

- The precision of logβ values can be less than generally achieved with other titration techniques, but ΔH^o values have comparable precision.
- Titration data corresponding to multiple binding equilibria can be analyzed mathematically with appropriate software (HypΔH) and the condition-independent values for the supramolecular binding process or processes of interest can be determined.
- It is also possible to extract kinetic information for ITC experiments originally planned for the thermodynamic characterization of a binding system.

Acknowledgments