Microcalorimetry for Life Science Research
MicroCalorimetry
The Universal Detector

Heat is either generated or absorbed in every chemical process

Capable of thermal measurements over a wide variety of solution conditions and temperatures

Measures reactions with time scales ranging from a few seconds to several hours

ITC Determines affinities from $10^2$ to $10^{12} \text{ M}^{-1}$
Differential Scanning and Isothermal Titration Calorimetry

ITC

DSC
Measuring Temperature Changes in Calorimetry
ITC

Temperature Range: 2\degree-80\degree C

Cell Volume: 1.3 or 0.2 ml
Representative ITC Data
ITC
Theoretical Basis

$\Delta G = -RT \ln K_B$

$\Delta G = \Delta H - T \Delta S$
Primary Causes of Enthalpy and Entropy

**Enthalpy (-ΔH)**
- Hydrogen bonds
- Ionic interactions

**Entropy (+ΔS)**
- Hydrophobic interactions
- Conformational freedom
ITC: A method for characterizing binding of molecules

In a single experiment, ITC can measure
- binding affinity \((K_a)\),
- heat of binding and Entropy \((\Delta H, \Delta S)\)
- number of binding sites \((n)\)

Multiple binding sites determinable in the same experiment.
Typical ITC Applications

Molecular Interactions:
Proteins, receptors, drugs, nucleic acids, lipids, metals, surfaces, etc. Native Molecules, in solution

Quality and process control

Enzyme Kinetics

Cell metabolism, inhibitors

Protein Stability
Protein-ligand interactions

Tobramycin binding to aminoglycoside nucleotidyltransferase (2”)

**Without MgAMPCPP**
- $K_D = 0.64 \mu$M
- $\Delta H = -18.2$ kcal/mole
- $\Delta S = -34$ cal/mole/°K

**With MgAMPCPP**
- $K_D = 0.21 \mu$M
- $\Delta H = -12.6$ kcal/mole
- $\Delta S = -12.3$ cal/mole/°K

Wright and Serpersu, Biochemistry 44, 11581-11591 (2005)
Protein-Protein interactions

C-terminal domain of nuclear RNA auxiliary factor (U2AF$^{65}$-UHM) binding to spliceosomal component mutant SF3b155-W7 (shown) or wild-type SF3b155

<table>
<thead>
<tr>
<th></th>
<th>SF3b155-W7</th>
<th>Wild-type SF3b155</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_D$ (µM)</td>
<td>2.50</td>
<td>2.83</td>
</tr>
<tr>
<td>$\Delta G$ (kcal/mol)</td>
<td>-7.8</td>
<td>-7.7</td>
</tr>
<tr>
<td>$\Delta H$ (kcal/mol)</td>
<td>-14.9</td>
<td>-9.4</td>
</tr>
<tr>
<td>$\Delta S$ (cal/mole/°K)</td>
<td>-23.4</td>
<td>-5.6</td>
</tr>
</tbody>
</table>

ITC Applications

DNA triplex formation

A 120 μM solution of 15-mer single stranded DNA was titrated into a 5 μM solution of 23-mer double stranded DNA at 25°C in 10 mM Sodium Acetate, pH 4.8

\[ K_D = 1.1 \times 10^{-8} \]
\[ \Delta G = -10.8 \text{ kcal/mole} \]
\[ \Delta H = -83.8 \text{ kcal/mole} \]
\[ \Delta S = -24.5 \text{ e.u} \]

Kamiya et al., JACS 118, 4532-4538
Thermodynamic Signatures for Drug Binding

MicroCal Application Note: ITC and Drug Design
The Evolution of HIV-1 Protease Inhibitors

-20000
-15000
-10000
-5000
0
5000
-10000
-15000
-20000
Indinavir
Nelfinavir
Saquinavir
Ritonavir
Amprenavir
Lopinavir
Atazanavir
KNI-577
KNI-272
KNI-764
TMC-126
TMC-114
ΔG
ΔH
-TΔS
Optimizing Diaminopyrimidine Renin Inhibitors

Aided by ITC Data

The Starting Point – A “Weak” Binder

![Chemical Structure of Compound 1]

- \( IC_{50} = 6.6 \mu M \)
- \( K_d = 3.6 \mu M \)
- \( \Delta H = -9.50 \text{ kcal/M} \)
- \( \Delta T = -2.00 \text{ kcal/M} \)

The \( IC_{50} \) and \( K_d \) are not very exciting, but the \( \Delta H \) is highly favorable. Why is that? Can this information help us design a better molecule?
The Binding Orientation for Cmpd 1 to Renin was Determined by X-ray Crystallography

Compound 1

The favorable $\text{H}$ is consistent with the strong network of H-bonds.

The low $\text{IC}_{50}$ and $K_d$ are consistent with unoccupied S2 and S3 pockets.

So what do we do now?
Dramatic Improvement in Enzyme Activity, Binding Affinity and Enthalpy

**Compound 1**

- **IC$_{50}$** = 6,560 nM
- **K$_d$** = 3,571 nM
- ?H = -9.50 kcal/M
- T? S = -2.00 kcal/M

**Compound 2**

- **IC$_{50}$** = 691 nM
- **K$_d$** = 535 nM
- ?H = -14.50 kcal/M
- T? S = -5.87 kcal/M
What Was the Result?

**Compound 2**
- \( \text{IC}_{50} = 691 \text{ nM} \)
- \( K_d = 535 \text{ nM} \)
- \( \Delta H = -14.50 \text{ kcal/M} \)
- \( \Delta T \Delta S = -5.87 \text{ kcal/M} \)

**Compound 3**
- \( \text{IC}_{50} = 58 \text{ nM} \)
- \( K_d = 79 \text{ nM} \)
- \( \Delta H = -10.00 \text{ kcal/M} \)
- \( \Delta T \Delta S = -0.23 \text{ kcal/M} \)

*Another 10-fold improvement!*
## ITC Applications

### Antibody Quality Control

**ITC Studies of two lots of anti-quinidine antibodies**

<table>
<thead>
<tr>
<th>Antibody Lot</th>
<th>Activity</th>
<th>$K_a \times 10^8$ M$^{-1}$</th>
<th>$\Delta H$ (Kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good</td>
<td>90%</td>
<td>3.3</td>
<td>-28</td>
</tr>
<tr>
<td>Bad</td>
<td>12%</td>
<td>3.3</td>
<td>-28</td>
</tr>
</tbody>
</table>

MicroCal Application Note (1997)
# ITC Applications

## Antibody Process Development and Control

ITC Studies of immobilized anti-quinidine antibodies

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Activity</th>
<th>$K_a \times 10^8 \text{ M}^{-1}$</th>
<th>$\Delta H \text{ (Kcal/mol)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good</td>
<td>90%</td>
<td>3.3</td>
<td>-28</td>
</tr>
<tr>
<td>Immobilized</td>
<td>36%</td>
<td>0.16</td>
<td>-24</td>
</tr>
</tbody>
</table>

MicroCal Application Note (1997)
ITC Applications
Enzyme Kinetics

Rate = $\frac{d[P]}{dt} = \frac{1}{V \cdot \Delta H_{\text{app}}} \frac{dQ}{dt}$
ITC Applications
Enzyme Kinetics

Typical concentrations
- Enzyme 25-100 pM
- Substrate 10-100 µM
  (2-20 µl per injection; 15-30 injections)

**ITC Products**

**VP-ITC**
- Cell volume 1.4 ml
- ~4-5 experiments/8 hours

**iTC**
- Cell volume 0.2 ml
- 10-15 experiments /8 hours
- 7x less sample than VP-ITC

**Auto-iTC**
- ~50 experiments/day
- 384 samples, unattended operation
- Filed upgradeable from iTC_{200}
- Available Mid 2008
DSC Theory

\[ \text{N} \rightleftharpoons \text{D} \]

\[ K_{\text{eq}} = \frac{\text{D}}{\text{N}} \]
DSC Theory

\[ G = -RT \ln K_{eq} \]

\[ G = H - T \Delta S \]
Microcalorimetry
DSC Applications

Domain structure
Formulation and drug stability
Yield improvement during chromatography
Improve yield during cell culture
Membrane studies
DSC Data

- Oligomers
- Shelf-Life/ Stability
- Binding
- Stabilizing Forces/
Energetics

Cp (kcal/mole/°C)

Temperature (°C)
Typical DSC Data

Data: SAMPLE5DSC_cp
Model: M2State
Chi^2 = 3361644.85449

Tm 1  60.00 ±0.1541
H1  1.14E5 ±2.44E3

Tm 2  69.83 ±0.0890
H2  1.47E5 ±2.21E3

kcal/mole

Temperature (°C)
## Liquid Formulation Stability Evaluation of Excipients

<table>
<thead>
<tr>
<th>Category</th>
<th>Exceipient</th>
<th>$T_m$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>48.1</td>
</tr>
<tr>
<td>Sugars</td>
<td>Manitol</td>
<td>46.7</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>49.6</td>
</tr>
<tr>
<td>Polymers / Polyols</td>
<td>PEG (300)</td>
<td>49.4</td>
</tr>
<tr>
<td></td>
<td>Ethanol (low)</td>
<td>48.7</td>
</tr>
<tr>
<td></td>
<td>Ethanol (high)</td>
<td>43.8</td>
</tr>
<tr>
<td>Salts</td>
<td>NaCl</td>
<td>53.1</td>
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<tr>
<td></td>
<td>CaCl$_2$</td>
<td>41.1</td>
</tr>
<tr>
<td>Surfactants</td>
<td>Pluronic F68</td>
<td>46.6</td>
</tr>
<tr>
<td></td>
<td>Tween 80</td>
<td>45.8</td>
</tr>
<tr>
<td>Glucose/NaCl</td>
<td></td>
<td>52.2</td>
</tr>
</tbody>
</table>

Antibody Stability
Effect of Glycosylation

Native $\rightarrow$ Partially Deglycosylated $\rightarrow$ Deglycosylated

**DSC PRODUCTS**

**VP-DSC**
Cell volume 0.5 ml  
~4 samples/8 hours

**Capillary DSC**
Cell volume 0.16 ml  
~50 samples per day  
Unattended operation  
Up to 576 samples on board
Conclusions

Improved sensitivity and data analysis software have enabled the lab scientist to use the techniques of microcalorimetry for many studies.

Heat is a universal detector of biological processes.

Microcalorimetry is one of the more versatile technologies available for characterization and analysis of biological molecules.