MicroCal®

Microcalorimetry for Life Science Research

Ultrasensitive Calorimetry for the Life Sciences™

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} M^{-1}

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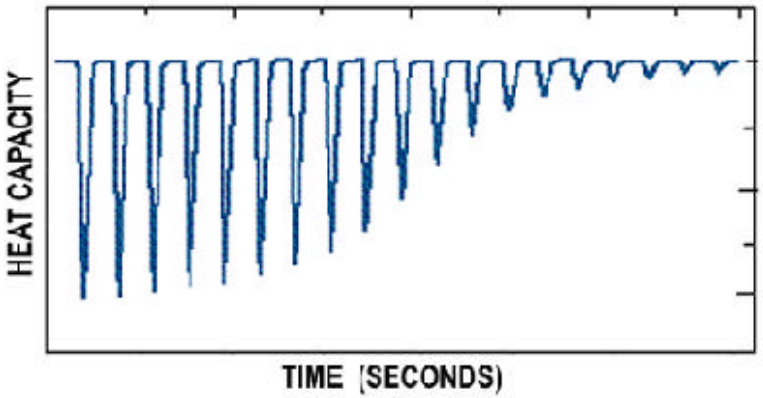
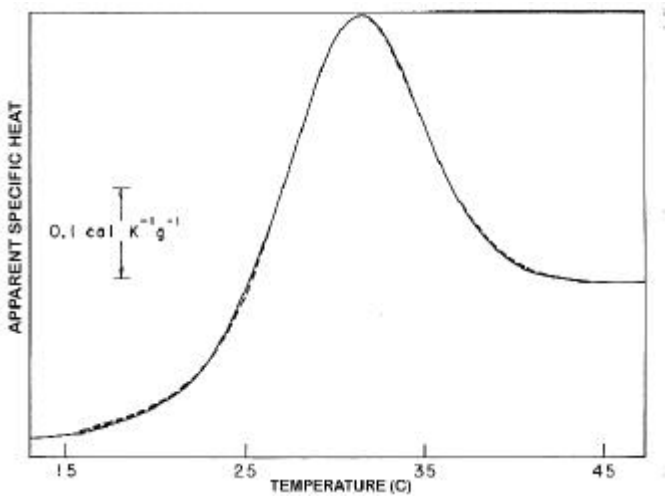
Differential Scanning and Isothermal Titration Calorimetry

ITC



DSC



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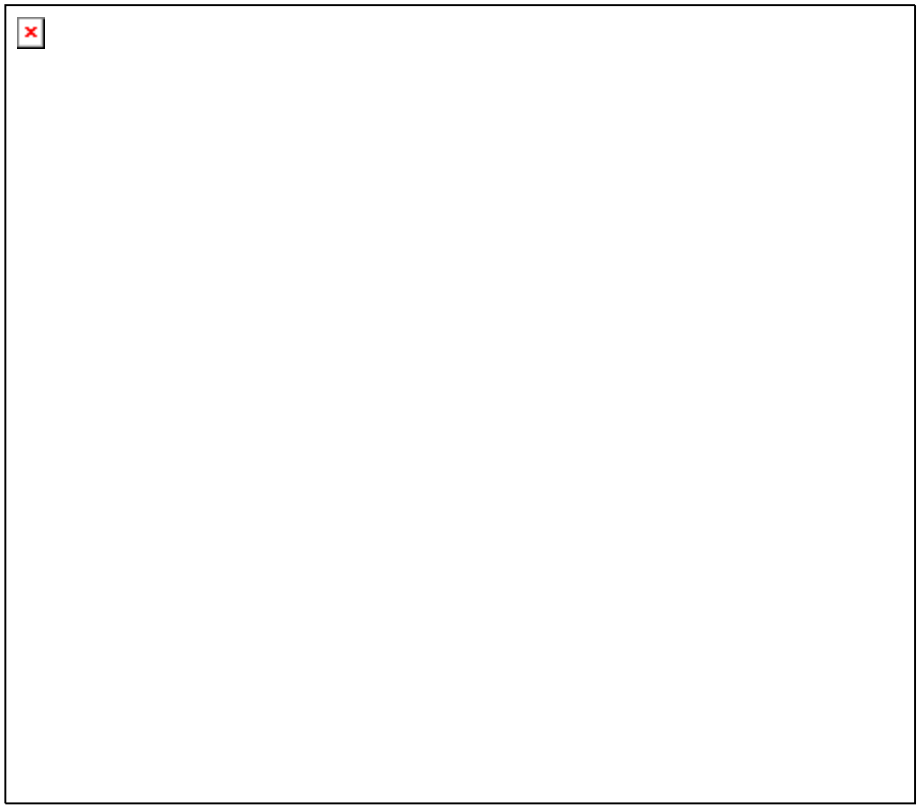
Measuring Temperature Changes in Calorimetry



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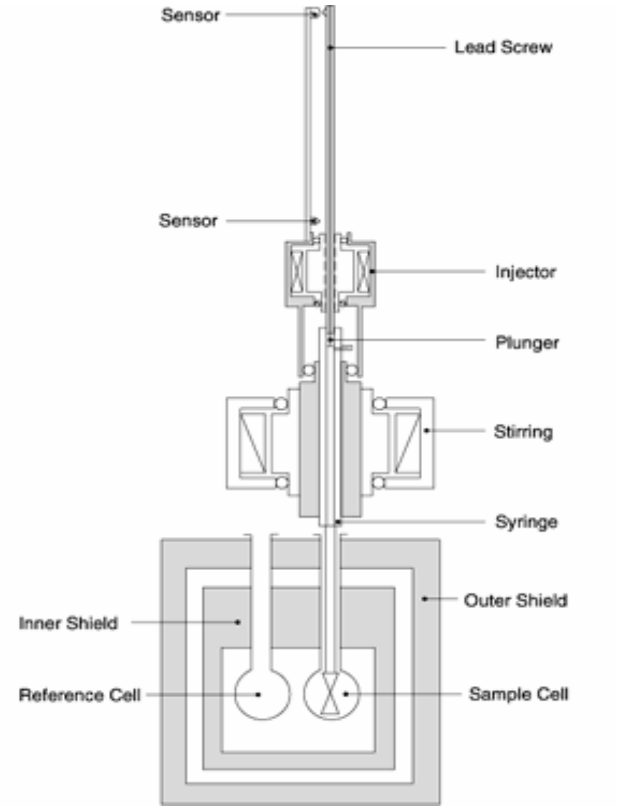
ITC



Temperature Range: 2°-80°C

Cell Volume: 1.3 or 0.2 ml

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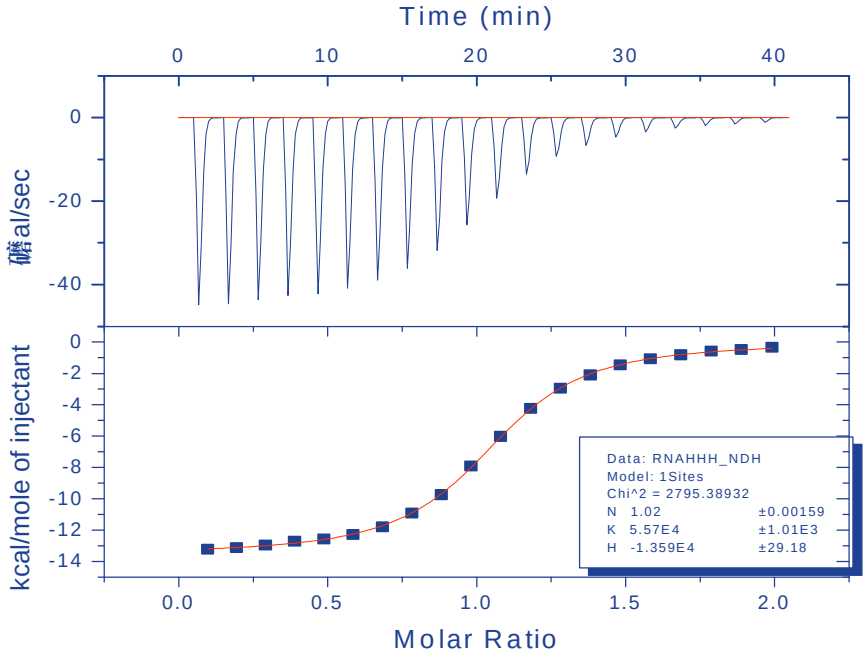
Representative ITC Data



ITC
Theoretical Basis

$$\Delta G = -RT \ln K_B$$

$$\Delta G = \Delta H - T\Delta S$$

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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 (Δ H, Δ 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

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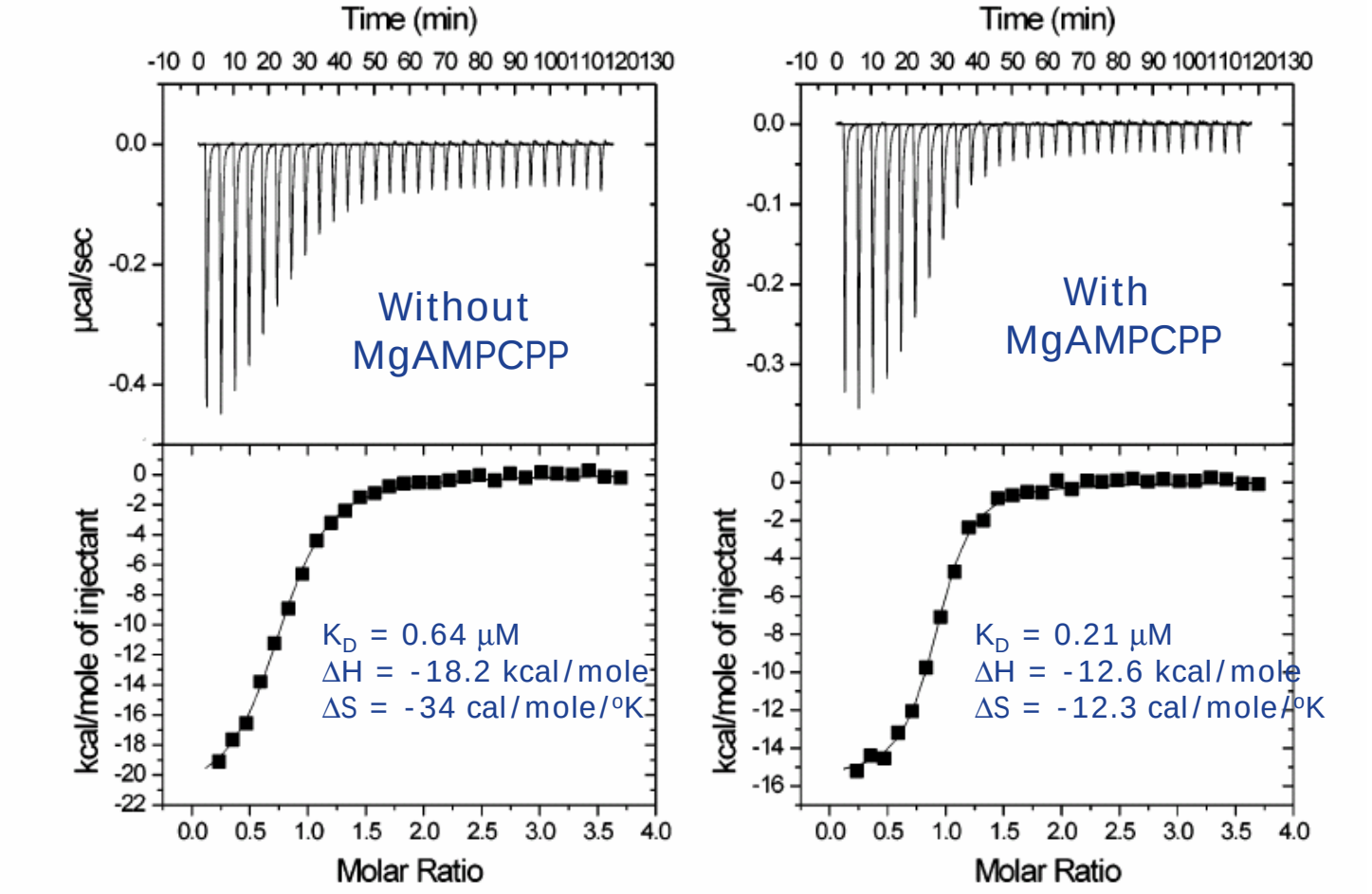
Protein-ligand interactions

Tobromycin binding to aminoglycoside nucleotidyltransferase(2")



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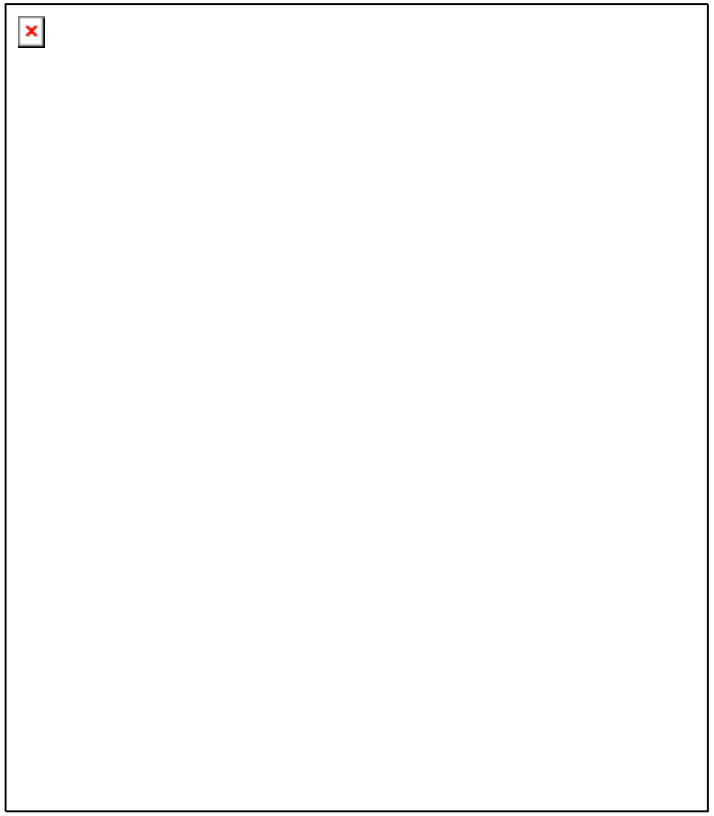
Wright and Serpersu, Biochemistry 44, 11581-11591 (2005)

Protein-Protein interactions



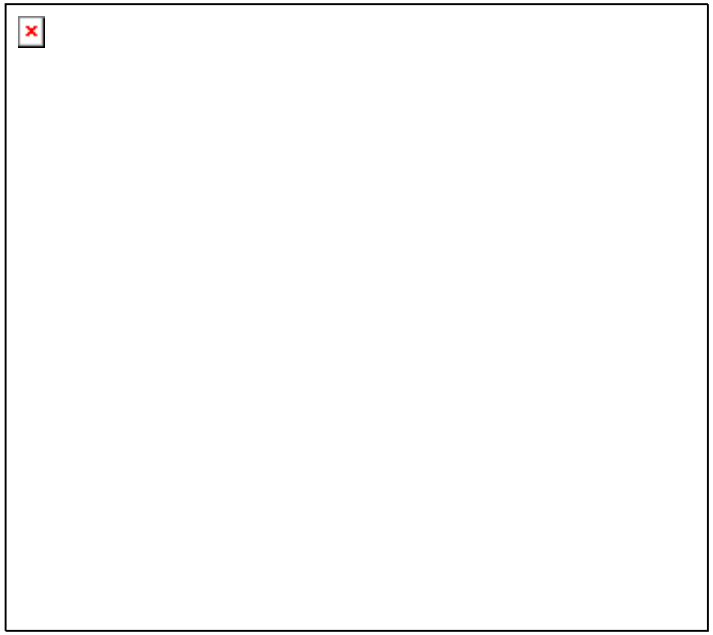
C-terminal domain of nuclear RNA auxiliary factor (U2AF^{65}-UHM) binding to spliceosomal component mutant SF3b155-W7 (shown) or wild-type SF3b155

	SF3b155-W7	Wild-type SF3b155
K_D (μM)	2.50	2.83
ΔG (kcal/mol)	-7.8	-7.7
ΔH (kcal/mol)	-14.9	-9.4
ΔS (cal/mole/^{o}K)	-23.4	-5.6

Thickman et al, J. Mol. Biol. 356, 664-683 (2006)

ITC Applications

DNA triplex formation



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

$K_D = 1.1x10^{-8}$

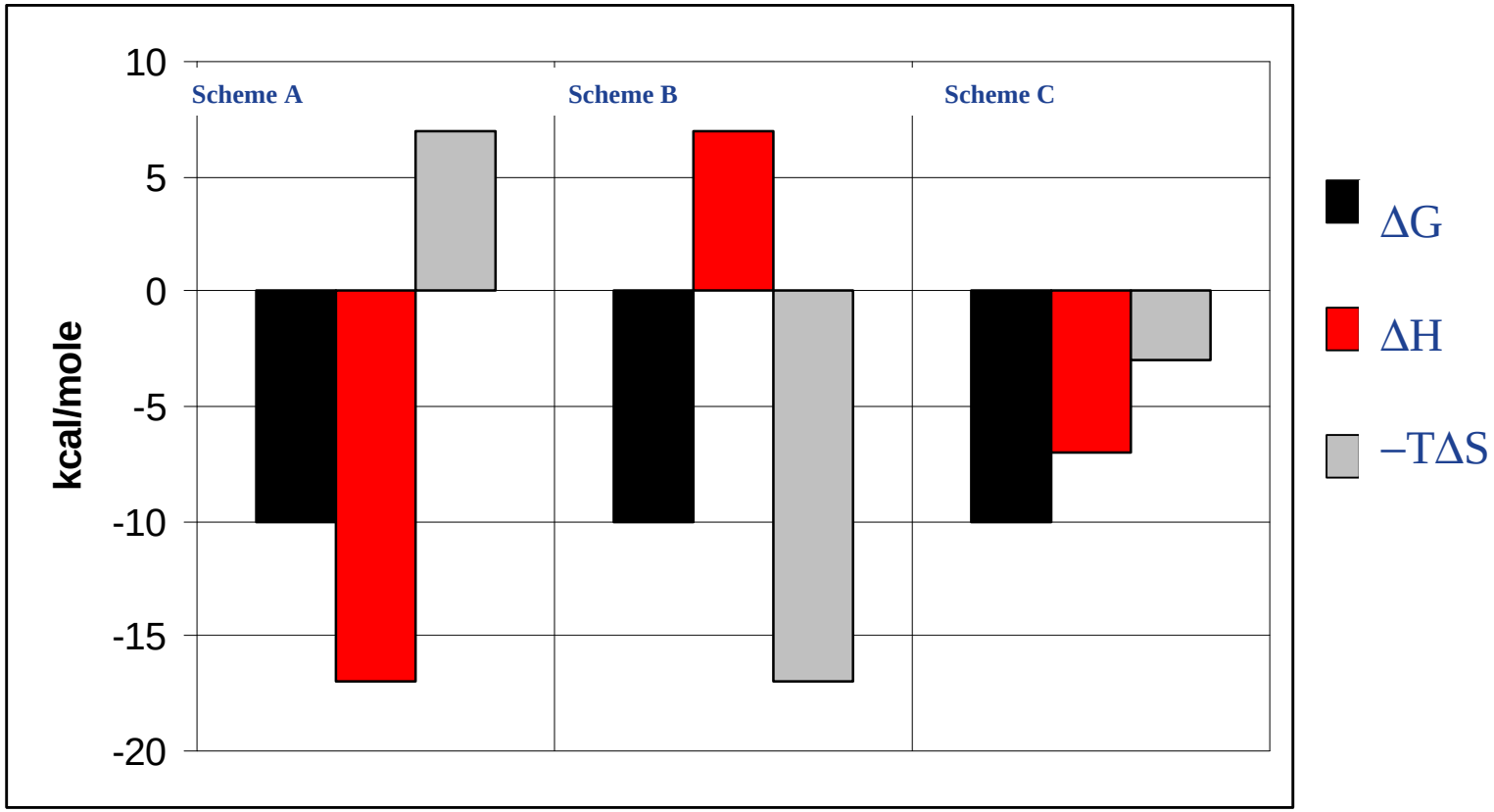
DG = -10.8 kcal/mole

DH = -83.8 kcal/mole

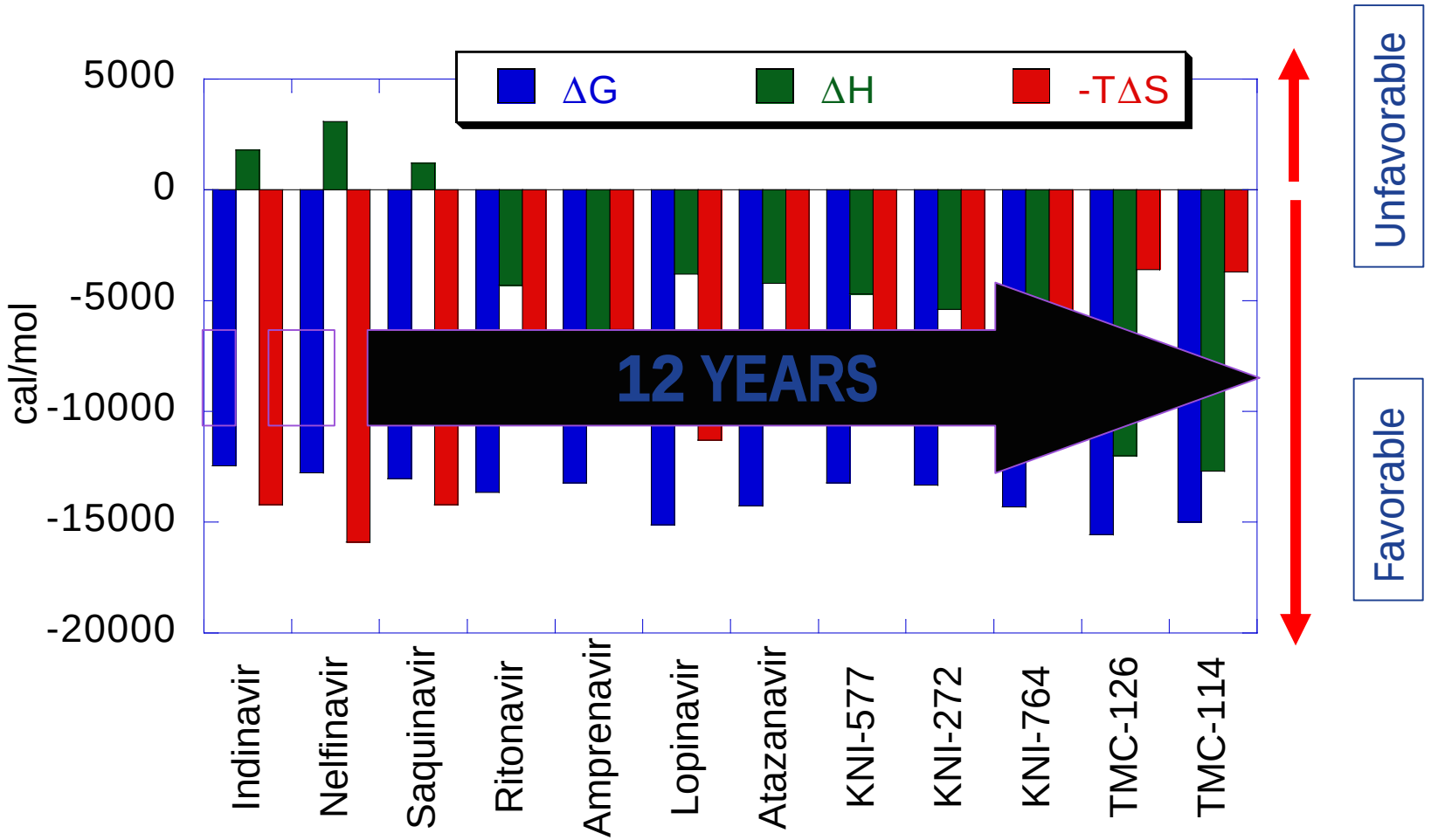
DS = -24.5 e.u

Kamiya et al., JACS 118,4532-4538

Thermodynamic Signatures for Drug Binding



The Evolution of HIV-1 Protease Inhibitors



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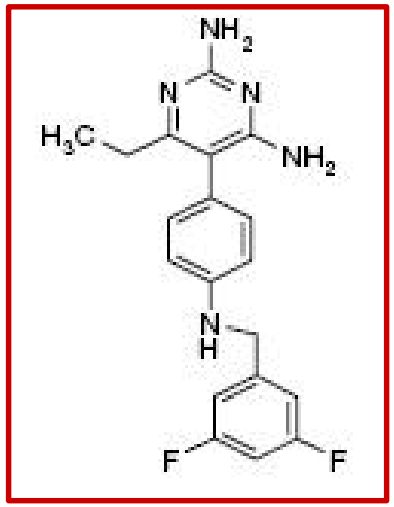
Velazquez-Campoy et al (2003) Current Drug Targets Infectious Disorders, 3 311
Ohtaka et al (2004) Int. J. Biochem. Cell Biol. 36 1787

Optimizing Diaminopyrimidine Renin Inhibitors

Aided by ITC Data

Sarver, et al, Anal. Biochem. (2007) 360, 30-40

The Starting Point – A “Weak” Binder



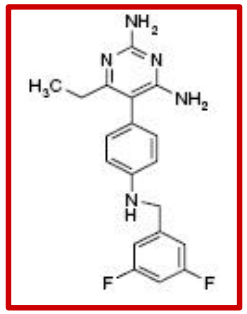
Compound 1

IC_{50}	=	6.6 μM
K_d	=	3.6 μM
? H	=	-9.50 kcal/M
T? S	=	-2.00 kcal/M

The IC_{50} and K_d are not very exciting, but the ? 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 ? H is consistent with the strong network of H-bonds.

The low IC_{50} and K_d are consistent with unoccupied S2 and S3 pockets.

So what do we do now?

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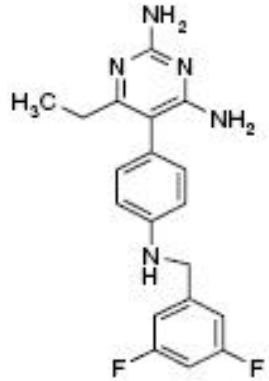
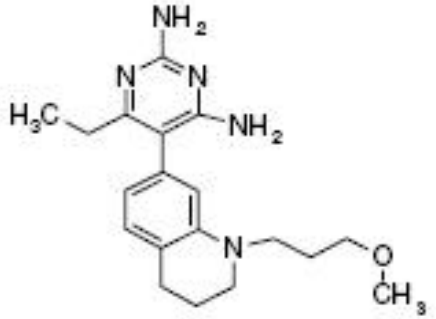
Dramatic Improvement in Enzyme Activity, Binding Affinity and Enthalpy





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

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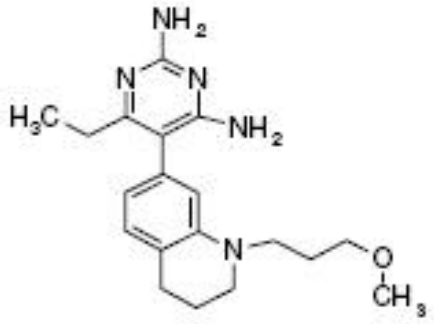
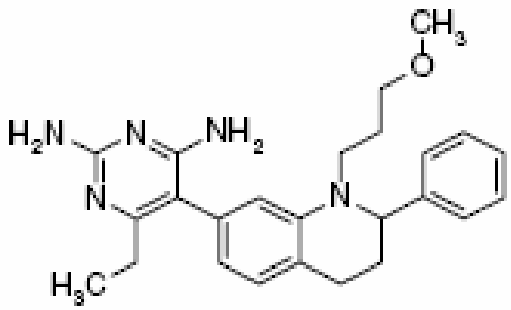
What Was the Result?



Compound 2



Compound 3

IC_{50}	=	691 nM
K_d	=	535 nM
? H	=	-14.50 kcal/M
T? S	=	- 5.87 kcal/M

IC_{50}	=	58 nM
K_d	=	79 nM
? H	=	-10.00 kcal/M
T? S	=	- 0.23 kcal/M

Another 10-fold improvement!

ITC Applications

Antibody Quality Control

ITC Studies of two lots of anti-quinidine antibodies

Antibody Lot	Activity	K_a (x 10^8 M^{-1})	DH (Kcal/mol)
Good	90%	3.3	-28
Bad	12%	3.3	-28

MicroCal Application Note (1997)

ITC Applications

Antibody Process Development and Control

ITC Studies of immobilized anti-quinidine antibodies

Antibody	Activity	K_a (x 10^8 M^{-1})	DH (Kcal/mol)
Good	90%	3.3	-28
Immobilized	36%	0.16	-24

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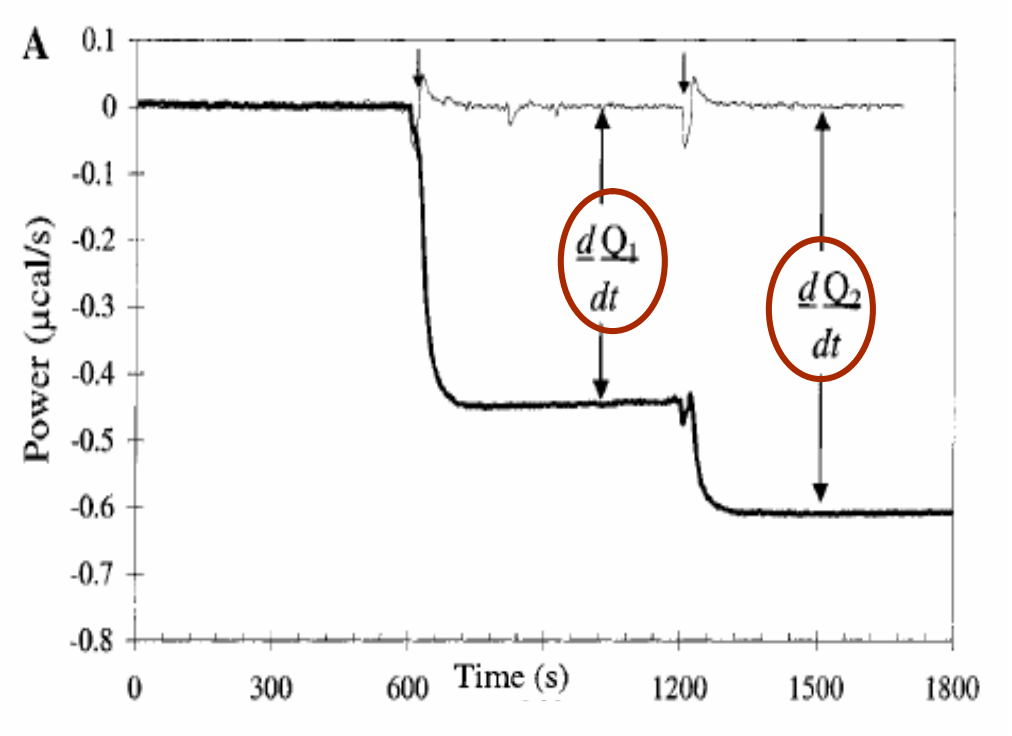
MicroCal Application Note (1997)

ITC Applications
Enzyme Kinetics



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

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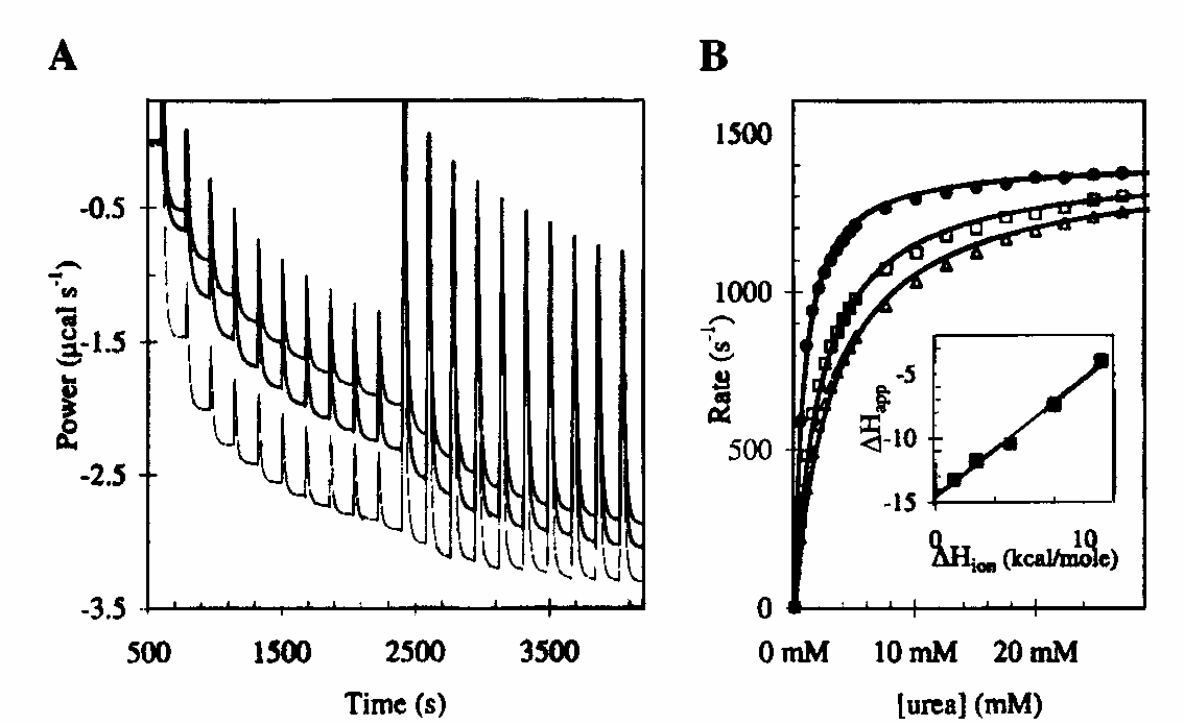
ITC Applications
Enzyme Kinetics



Typical concentrations

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

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Todd and Gomez, Anal. Biochem. 296, 179-187 (2001)

ITC Products

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

iTC_{200}
Cell volume 0.2 ml
10-15 experiments /8 hours
7x less sample than VP-ITC

Auto-**iTC_{200}**

~50 experiments/day
384 samples, unattended operation
Filed upgradeable from iTC_{200}
Available Mid 2008

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DSC Theory

$$K_{eq} = \frac{D}{N}$$

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DSC Theory

$$? \; G = -RT \ln K_{eq}$$

$$? \; G = ? \; H - T? \; S$$

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Microcalorimetry
DSC Applications

Domain structure

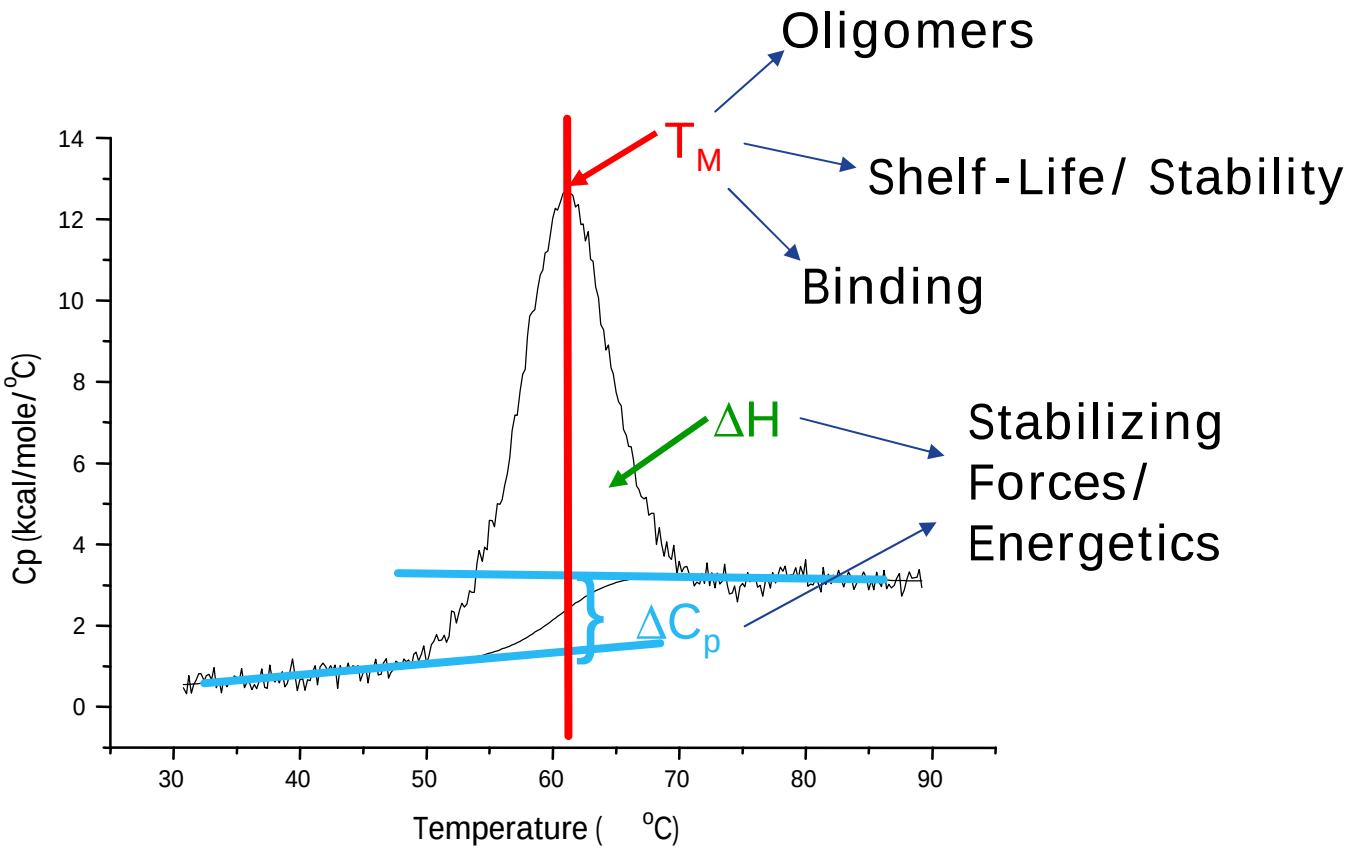
Formulation and drug stability

Yield improvement during chromatography

Improve yield during cell culture

Membrane studies

DSC Data



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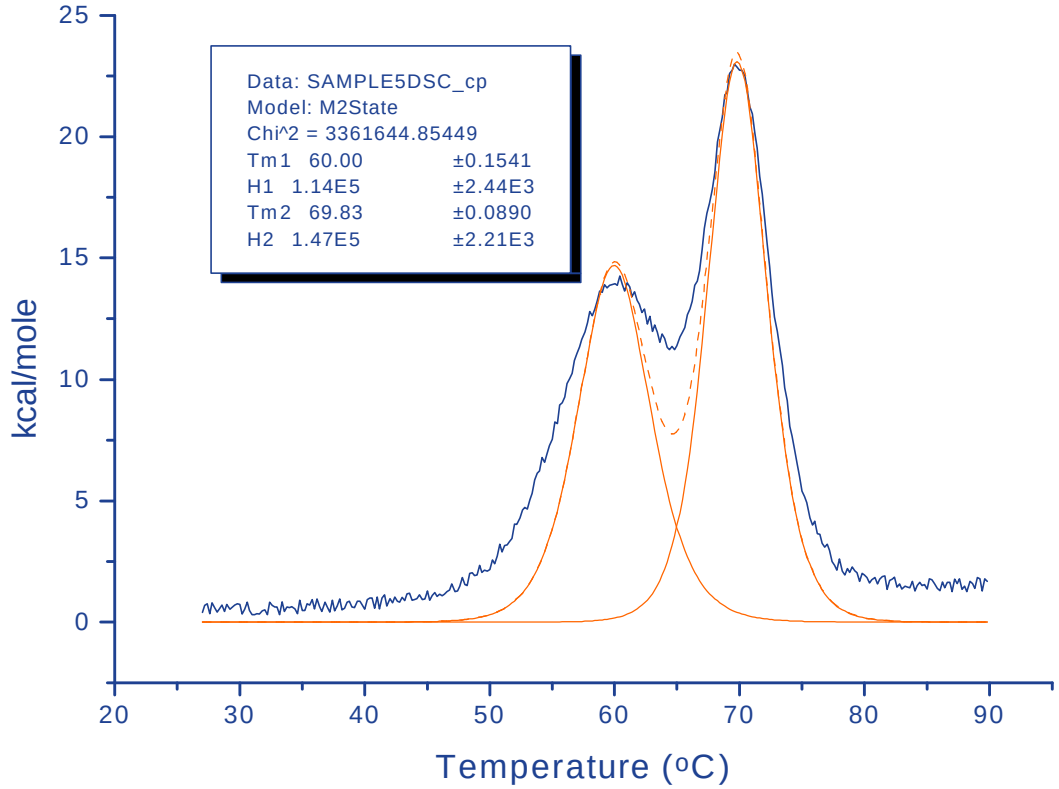
Typical DSC Data



Liquid Formulation Stability
Evaluation of Excipients

	Excepient	T_m (°C)
Control		**48.1**
Sugars	Manitol	46.7
	Glucose	**49.6**
Polymers / Polyols	**PEG (300)**	**49.4**
	Ethanol (low)	**48.7**
	Ethanol (high)	43.8
Salts	**NaCl**	**53.1**
	$CaCl_2$	41.1
Surfactants	Pluronic F68	46.6
	Tween 80	45.8
Glucose/NaCl		**52.2**

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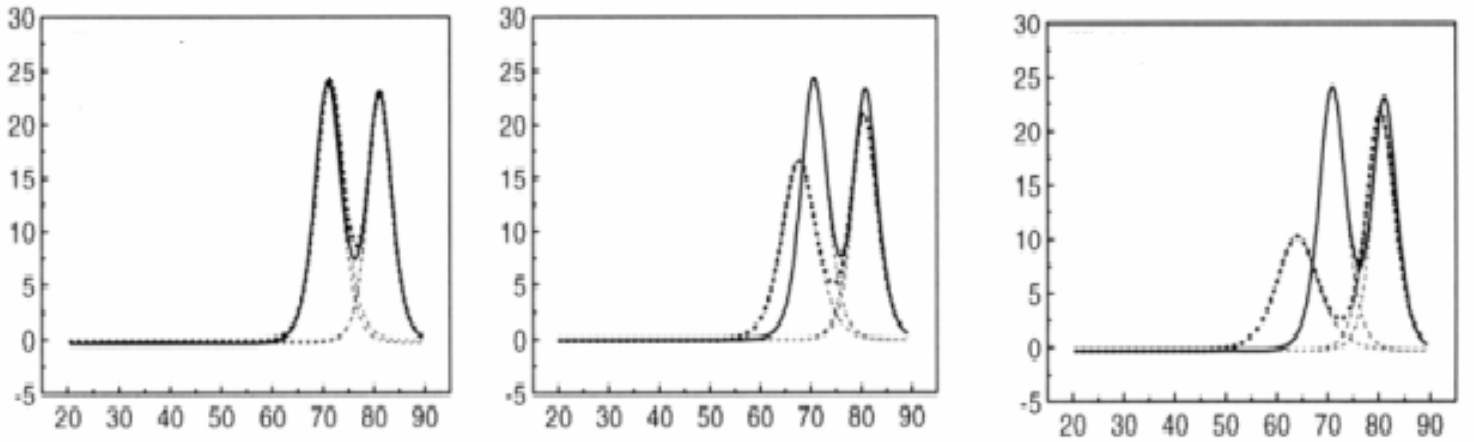
Excerpted from: Remmele, et. al., Pharmaceutical Res. 15, 200-208 (1998)

Antibody Stability
Effect of Glycosylation

Native → Partially Deglycosylated → Deglycosylated



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Mimura, et al, J. Biol. Chem. 276, 45539-45547 (2001)

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

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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.