Sensing the heat:

Calorimetry

- Background & history
- Instrumentation and measuring principles
- Examples and case stories

Peter Westh
Roskilde University, Denmark
PWESTH@RUC.dk

Thermodynamics

Is a wonderful structure with no contents

Aharon Katchalsky

For the (experimentally convenient) (P,T,n_i) variable system

Equilibrium state	1st derivatives	2nd derivatives (response functions)	3rd derivatives
G	S {T} (H {S})	H_i {T,n_i}	H_{i-j} {T,n_i,n_j}
	V {P}	V_i {P,n_i}	V_{i-j} {P,n_i,n_j}
	μ_i {n_i}	C_p {T,T}	dC_p/dT {T,T,T}
		α {P,T}	Etc etc
		κ {P,P}	
		μ_{i-j} {n_i, n_j}	

Koga (2007) Solution Thermodynamics: a differential approach. Elsevier.

For membranous (colloidal) systems perhaps a fourth variable: Area ($dG/dA=\gamma$)

Thermodynamic relationships

The (T,P,n_i) ensemble where Gibbs free energy is "the king"

Enthalpy relates to the temperature dependence of the G-function

$$\left(\frac{\partial\left[G/T\right]}{\partial T}\right)_p = -\frac{1}{T^2}H$$

Enthalpy and internal energy can be derived from temperature measurements

$$q_p = c_p \Delta T \qquad \Delta H = q_p$$

$$q_v = c_v \Delta T \qquad \Delta U = q_v$$

$$\left(\frac{\partial\left[G/T\right]}{\partial T}\right)_p = -\frac{1}{T^2}H$$

Needless Thermodynamics ?

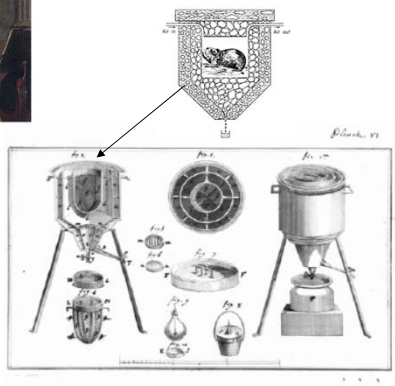
- These thermodynamic relationships however are rarely implicitly applied in modern biocalorimetry
- Rather, heat reports binding, folding, aggregation, adsorption, activation, growth, ...
- The challenge is often that the "active" component is very dilute – hence, a lot of water must be heated and ΔT is small

Calorimetry
The pro´s and con`s of application

PRO
- **Universally applicable**
- **No probe/no special sample preparation**
- **Quantitative**
- **Non-specific**

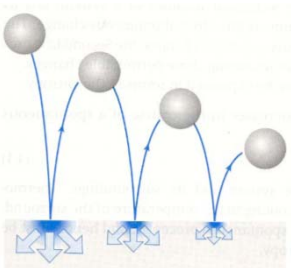
CON
- **No structure information**
- **Moderate sensitivity**
- **Low through-put**
- **Non specific**

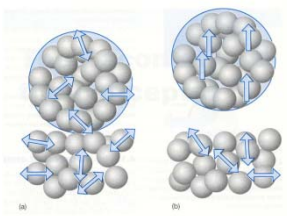
Basics and history



WORK and HEAT

$$\Delta U = w + q$$





- One of the oldest analytical principles still in use – Lavoisier had rather precise calorimeters by 1780.
- Readily measured thermodynamic function.
- Heat cannot be measured – temperature can.
- Heat is NOT at state function – enthalpy and internal energy are.

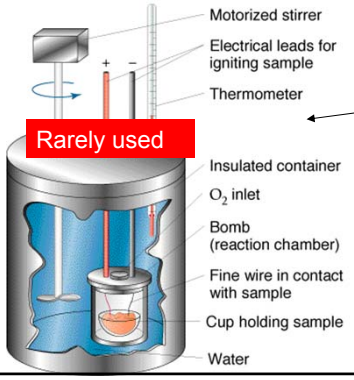
Measuring principles

Detect temperature - calculate heat

For p or V constant

heat = enthalpy or internal energy

Bomb-calorimeter

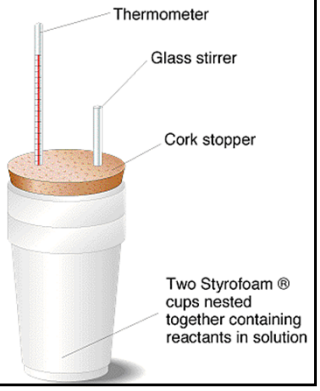


Rarely used

$\Delta H = q_p$

$\Delta U = q_v$

Coffe cup-calorimeter



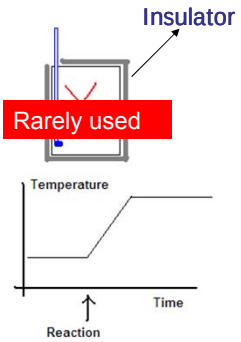
Measuring μJ or nJ heats !

The measuring cell in a typical commercial calorimeter has a heat capacity of ~10J/K. Hence 1 μJ will increase its temperature by 10^{-6}J $(10J/K)^{-1}$ = 10^{-7} K. How does one measure that ?

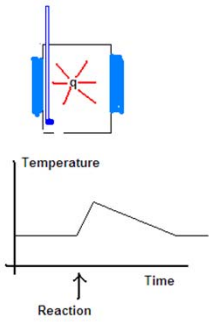
- All biocalorimeters are “coffe cup” instruments (i.e. measure H, not U)
- Two principles:

Adiabatic Calorimetry

•High sensitivity
•Cp must be measured
•elaborate



Rarely used



Heat flow Calorimetry

•Cp irrelevant
•Faster to use
•Possibility of feed-back

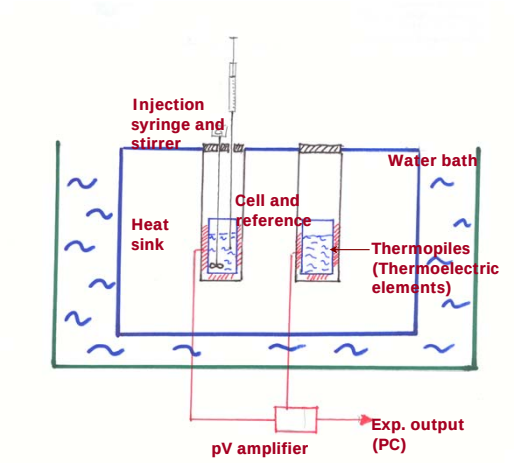
Two types of heat flow calorimeters have revolutionized biochemical applications

- Differential Scanning Calorimetry (DSC)
- Isothermal Titration Calorimetry (ITC)

DSC	ITC
Measures heat required to linearly increase T	Measures heat of mixing (titrand into titrate)
Constant composition – temperature perturbed	Constant T – composition perturbed
Thermal breakdown, denaturation, phase transitions	Ligand binding, receptor studies, adsorption, kinetics

ITC

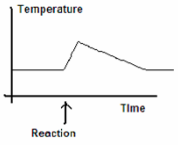
Passive heat flow calorimetry



Principles of a heat conduction or ”passive” calorimeter.

Basic out-put

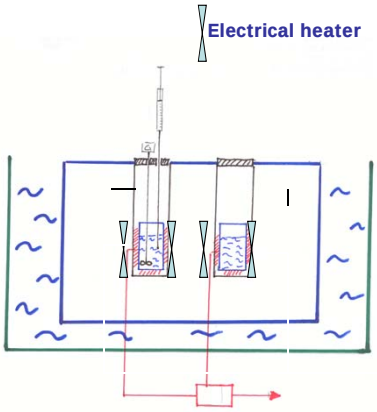
$\Delta T = T_{cell} - T_{ref}$



τ ~ 10-100 sec

+++
Readily converted into enthalpy
Versatile
- - - -
Low time resolution
Broad peaks

Next Generation
Power compensated ITC (~1990)

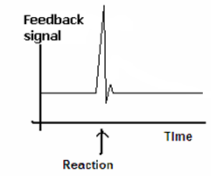


The feed-back system sustains a constant and very small ΔT between cell and reference.
Net ref⇒cell heat flow

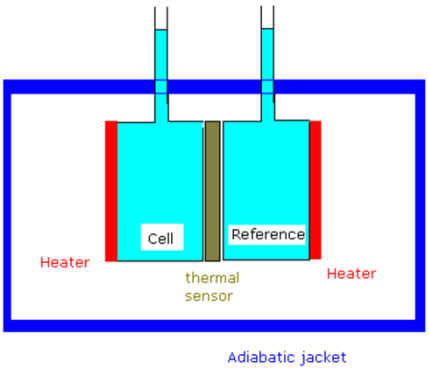
Exothermic process is compensated out by (fast) adjustment of the feed-back heaters.

+++
Fast responce, high sensitivity
-- - -
Narrow applicability,

Feed-Back Control



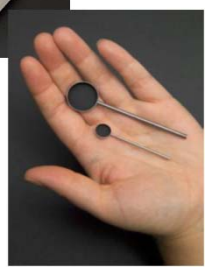
Semi-adiabatic, feed-back calorimetry (no water bath)



1. The temperature of the ADIABATIC JACKET is adjusted so that it is equal to the temperature of the reference.

2. The CELL is heated so that it is slightly warmer than the REFERENCE. This heat signal is the PRIMARY OBSERVABLE of the experiment.

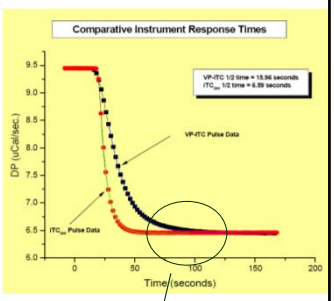
ITC_{200}
a modern instrument

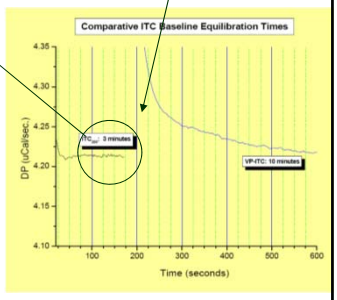


Noise level ~0.002μCal/sec or about 0.01μW.

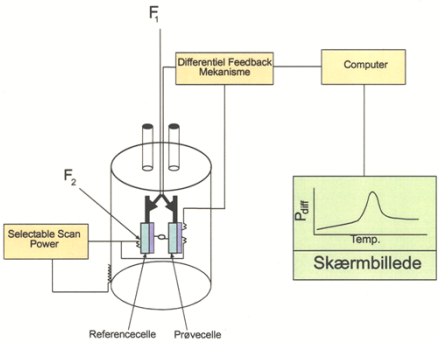
The heat capacity is about 3 J/K – detection level ~0.1μJ

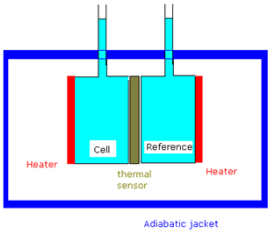
Hence the the thermal noise is about $1x10^{-7}/3 \sim 3x10^{-8}$K !





DSC





Detector and feed-back system is similar to ITC.

The primary observabel is the difference in POWER (i.e. measured in watts=J/sec) supplied when the cell and reference are heated at the same constant rate.

The cell/reference assembly is housed in an ADIABATIC JACKET

DSC

fundamental relationships

The differential POWER or HEAT FLOW (e.g. in μW) reflects the HEAT CAPACITY of the sample

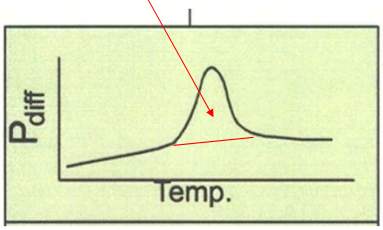
$$J_{heat} = C_P m \frac{\partial T}{\partial t}$$

$$\left(\frac{\partial H}{\partial T}\right)_P = C_P$$

For example heating 1 g H2O, 1 K/s requires:
J = 4.2 J/g K 1 g 1 K/s = 4.2 J/s= 4.2 W

Differential powers can be measured into the μW to nW-range

It follows that the area under the peak is the enthalpy change, ΔH, of the transition



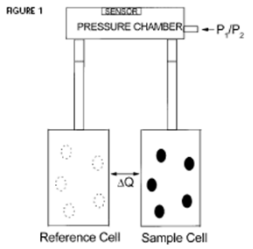
ITC and DSC are by far the most common calorimetric instruments in biophysical and biochemistry labs to

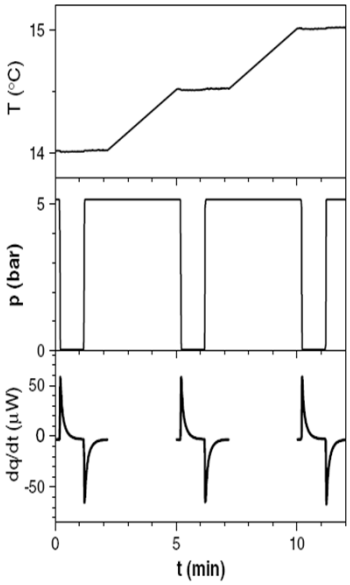
We will return to applications of these later!

Fisrt some

More specialized instruments

Pressure perturbation DSC





Measures

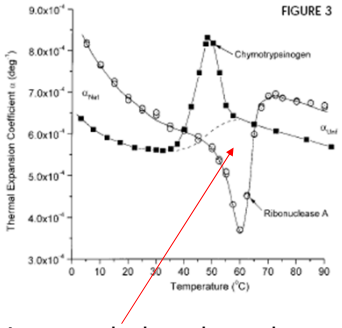
HEAT OF COMPRESSION

Which is tantamount to

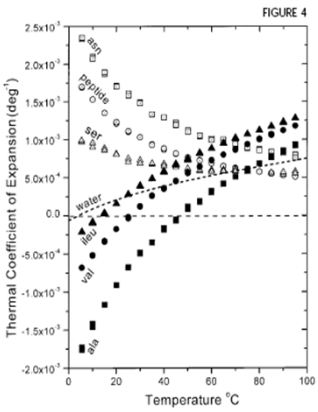
THERMAL EXPANSIVITY

$$\left.\frac{\partial Q_{\text{rev}}}{\partial p}\right|_T = -T\left.\frac{\partial V}{\partial T}\right|_p$$

Pressure perturbation DSC - examples



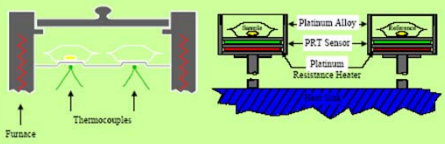
Area equals the volume change, ΔV, for the denaturation



Slope reveals some properties of the water hydrating the amino acid side chain

Temperature Modulated DSC

Comparison of Heat Flux and Power Compensation DSC



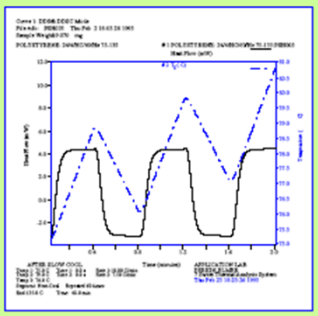
Heat Flux DSC *Power Compensation DSC*

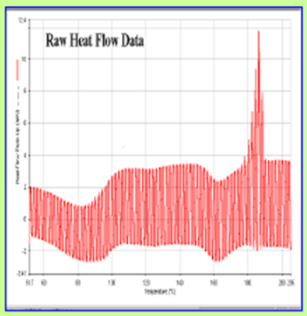
Temperature Modulated Differential Scanning Calorimeter (TMDSC)

Temperature Modulated Differential Scanning Calorimetry (TMDSC) is a term used to describe a DSC techniques whereby a sample is subjected to a superposition of a linear and a periodic temperature program (perturbation). From analysis of the heat flow response, the specific heat capacity can be determined. The response to the linear part of the perturbation yields the total heat capacity while the response to the linear part yields the complex specific heat capacity.

A linear gradient in T with a sine wave or zigzag superimposed

TMDSC: Close-up of Raw Data

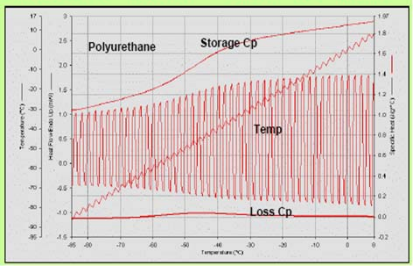




In-phase and out-of-phase heat capacity single out different response/relaxation processes

TMDSC: Determination of Real and Imaginary Components of Complex Heat Capacity

Storage $Cp = Cp' = |Cp| \cos \varphi$ *Loss* $Cp = Cp'' = |Cp| \sin \varphi$



TMDSC Curve Types

- Total Cp
- Storage Cp
  - Specific heat capacity which is instantaneous (in phase) on the time scale of the dynamic experiment
  - Energy storage contributions from molecular vibration, rotation, and translation involving existing structures
  - Does not include relaxation enthalpy, heat of moisture loss, or enthalpy of curing as long as the corresponding heat flow rates are independent on temperature changes during modulation
- Loss Cp

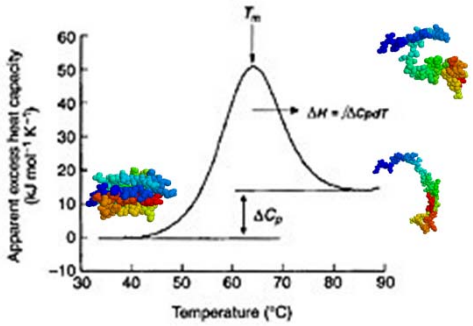
Heat: The universal detector

Some classical and modern uses of calorimetry in bio- and colloidal sciences

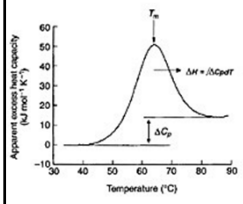
DSC
equilibrium unfolding of globular proteins

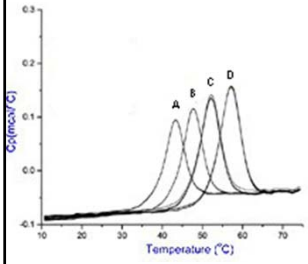
By 1975 the (semi) adiabatic DSC instruments developed at the Protein Research Instute, Moscow, reached a quality adequate to directly monitor the thermal unfolding of small globular proteins. This application remains the most common use of calorimetry in biochemistry.

General output:



DSC – an easy access to much information





Assumption:  N ⇆ D K= [D]/[N]

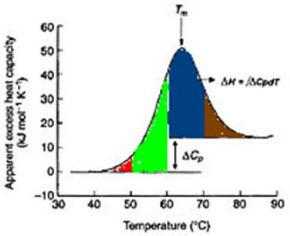
T_m: Temperature where K=1 ([D]=[N])

ΔH: Enthalpy of transition (total area using "step" shaped baseline)

ΔS°: At T_m, ΔG°=0 hence ΔS°=ΔH/T

ΔCp : D-N difference in heat capacity. dΔH/dT=ΔCp

Check your assumption: The Vant´t Hoff analysis



Divide the peak area into T-partitioned slices

Determine the equilibrium constant at each temperature
E.g. At 50°C:
fraction denatured = red area/total area
Native fraction (total area-red area)/total area

Hence: K(50°C)= red area/(total area – red area)

Van´t Hoff equation

$$\frac{d\ln K}{d(1/T)} = -\frac{\Delta H^o}{R} \Rightarrow$$

Plot calculated lnK values against 1/T

The slope is -ΔH°/R

$$\ln\left(\frac{K_2}{K_1}\right) = -\frac{\Delta H^\circ_m}{R}\left[\frac{1}{T_2} - \frac{1}{T_1}\right]$$

Investigate an extensive temperature interval

The definition: C_p=dH/dT leads to Kirchkoff´s law

$$\Delta H(T_2) = \Delta H(T_1) + \int_{T_1}^{T_2} \Delta C_P dT$$

If ΔC_p is independent of T

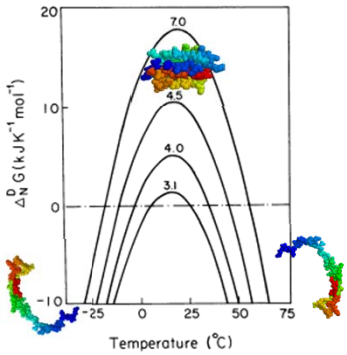
$$\Delta H(T_2) = \Delta H(T_1) + \Delta C_p (T_2 - T_1)$$

And equivalently for the entropy change ΔS

$$\Delta S(T_2) = \Delta S(T_1) + \Delta C_P \int_{T_1}^{T_2} \frac{1}{T} dT$$

$$\Delta S(T_2) = \Delta S(T_1) + \Delta C_P \ln\left[\frac{T_2}{T_1}\right]$$

The use of Kirchkoff´s equation on DSC (and other) data lead to counterintuitive results!



Proteins denature upon both heating and cooling

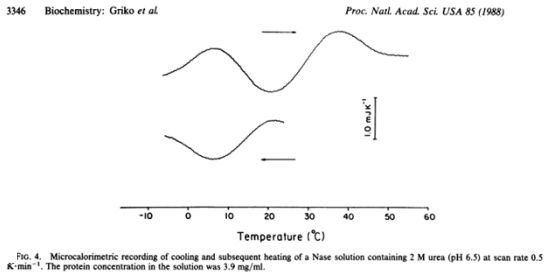
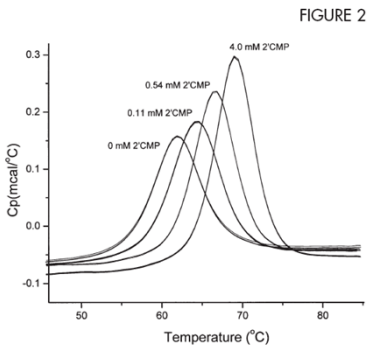
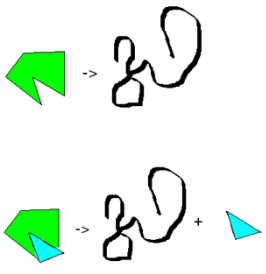


FIG. 4. Microcalorimetric recording of cooling and subsequent heating of a Nase solution containing 2 M urea (pH 6.5) at scan rate 0.5 K·min^{-1}. The protein concentration in the solution was 3.9 mg/ml.

Cold denaturation is a general phenomenon of paramount fundamental (and little practical) importance

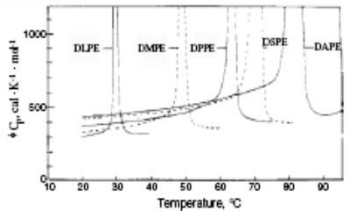
Interactions of proteins and other molecules effects the thermogram

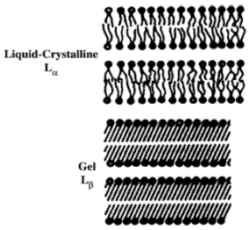




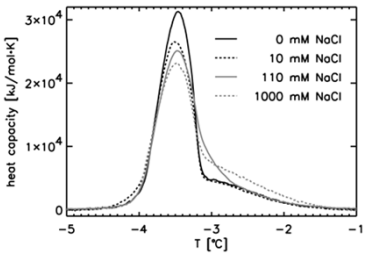
The binding of a ligand to the native state brings abour stabilization – The dicplacement of the peak along with the change in transition enthalpy quantifies the binding strength

DSC and Lipidmembranes: phase diagrams

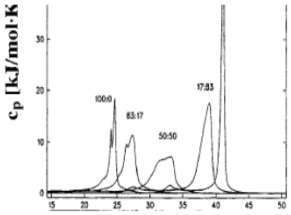




Blume (1983) *Biochem.* **22**; 5436.



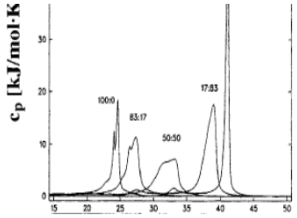
Böckman et al (2003) *Biophys J.* **85**, 1647



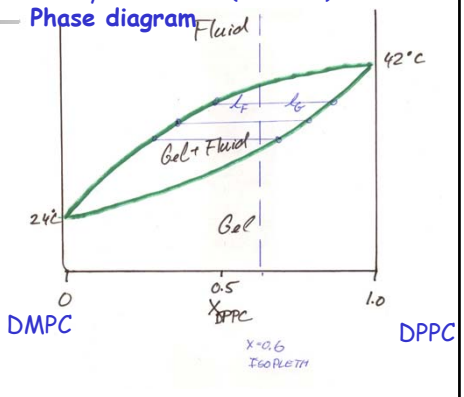
Schrader et al (2002) *J.Phys.Chem.* ***106***, *6581*

DSC and the lever rule

Schrader et al (2002) *J.Phys.Chem.* ***106***, *6581*



Binary membrane (two PCs)
Phase diagram



$$\text{Lever rule}: \frac{n_F}{n_G} = \frac{l_G}{l_F}$$

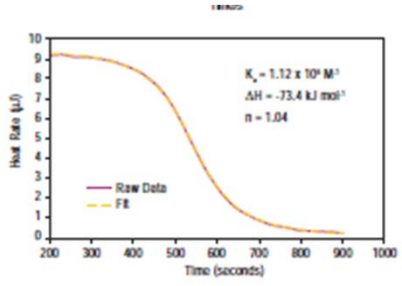
The ratio n_F/n_G quantifies the conversion of gel to fluid phase and is hence reflected in the calorimetric heat flow

ITC

- **Principle: Detect the heat during gradual increase of ligand concentration and concomitant occupation of sites**

http://protein-solutions.dk/itc.html

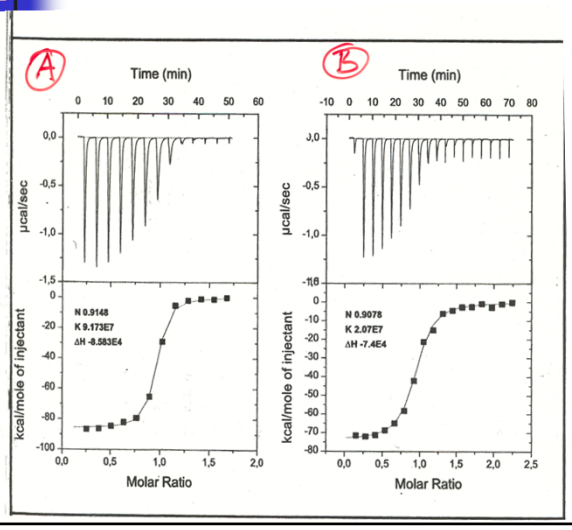
Continous injection measurements



Classical ITC – Specific host guest interaction

Principle:

Detect the heat during gradual increase of ligand concentration and concomitant occupation of sites

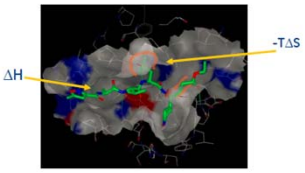


Hybridization of complementary 15-mer DNA strands.

Column A represents data for perfectly complementary strands

Column B shows data with one mismatch

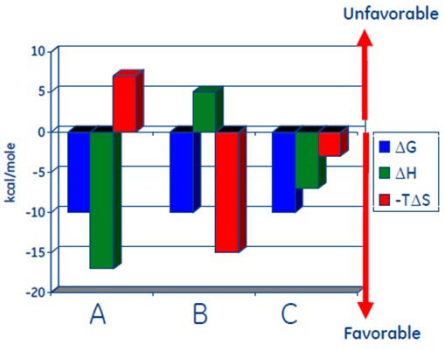
Mechanistic interpretation



A. Good hydrogen bonding with unfavorable conformational change

B. Binding dominated by hydrophobic interaction

C. Favorable hydrogen bonds and hydrophobic interaction



ITC results are used to get insights into mechanism of binding

Fundamental data analysis

1. Reaction: $P + L \leftrightarrow PL$, Law of mass action: $K=[PL]/([P][L])$

2. Mass conservation: $[P]_{tot} = [P] + [PL]$ & $[L]_{tot} = [L] + [PL]$

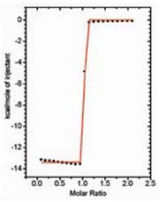
3. Heat of reaction: $P + L \leftrightarrow PL$, ΔH
   hence $q = \Delta n_{PL}\ \Delta H\ V_{cell}$

In the simplest case ($P + L \leftrightarrow PL$), q(i) becomes a quadratic equation in $[P]_{tot}$

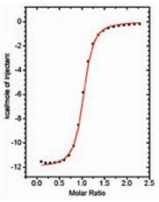
Limitations

Window of binding strength typically 10^3-10^9 M^{-1}

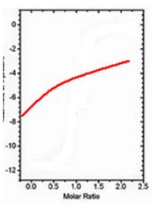
Examples of variable binding constants. $\Delta H = 13 kJ/mol$, $n = 1$



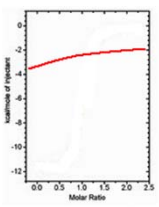
Too strong



Perfect



Hard



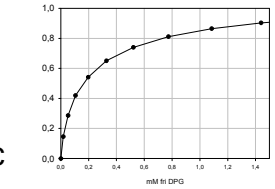
Too weak

Advanteges

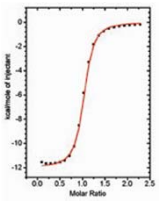
- High resolution (in [L])
- Quite fast
- Several thermodynamic parameters in one trial
- No labiling

Degree of saturation

$\Theta = K[L]/(1+K[L])$



ITC output

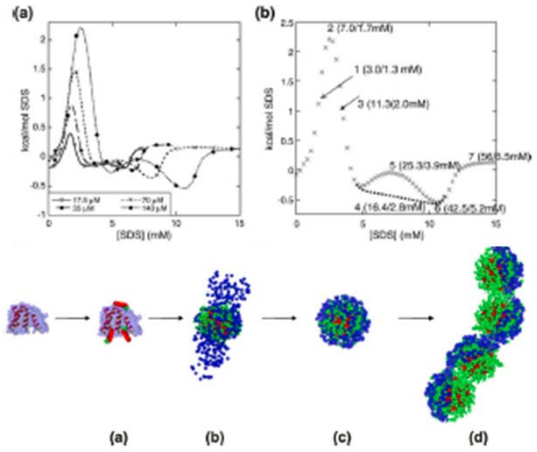


Detecting association

- **"Integral method"**: the primary observable scales with the population of a given species (e.g. bound ligand). Examples include spectroscopy, hydrodynamics, selective electrodes.
- **"Differential method"**: The primary observable scales with the change of a species upon a small (concentration) perturbation. ITC

ITC and SAXS

inherently complementary

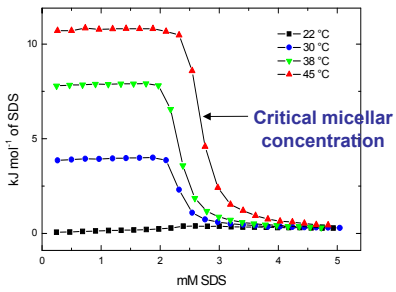


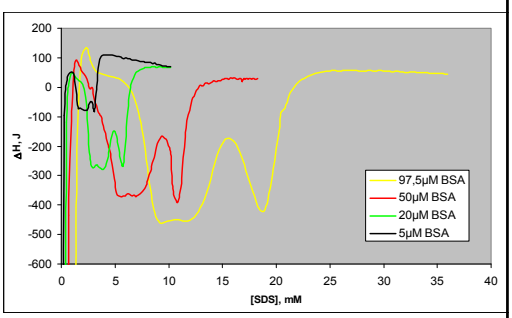
Andersen et al 2009

The SDS-BSA system

Titration of 98mM SDS into buffer
- Precise determination of CMC
- No heat of micellization at 22°C
- Temp. dependence of CMC

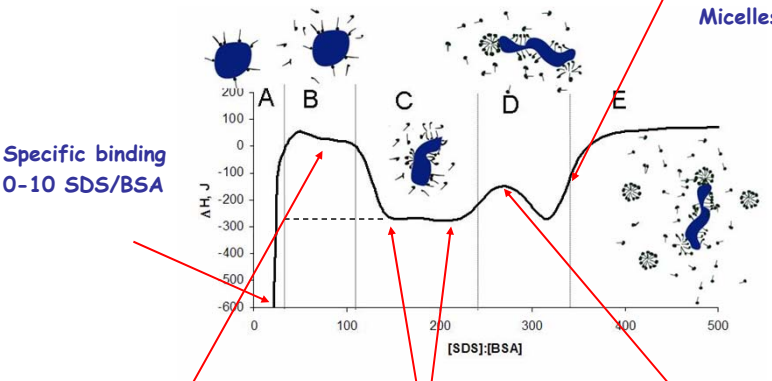
98mM SDS into BSA solutions at 22°C
- Numerous characteristic transitions
- No effect of micellization





L.L. Hansen et al unpublished

The calorimetric fingerprint: structural interpretation



Saturation (230 SDS/BSA, 1.2 g/g protein)
Micelles in the bulk

Specific binding
0-10 SDS/BSA

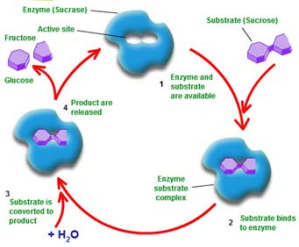
N to D transition
25-50 SDS/BSA

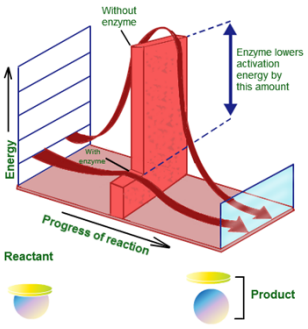
Binding to D
50-100 SDS/BSA

Elongation transition
110 SDS/BSA

Arleth et al

Enzyme kinetics





- Extraordinary catalysts, enhancing reaction velocities with 10^5 to 10^{17}, without being consumed
- Very specific discriminating between substrates
- Have developed to lower activation energy selectively for reactions needed for cell survival

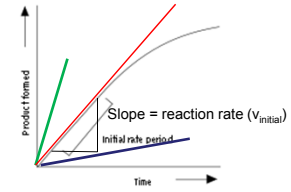
RUC Roskilde Universitet
Roskilde University www.ruc.dk

Basic kinetics

$$S \rightarrow P$$

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_2} E + P$$

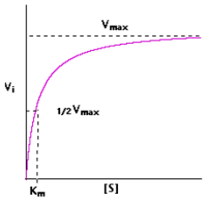
Initial rate, and substrate concentration



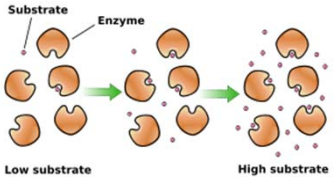
Connected values of $v_{initial}$ and [S]

If $\frac{d[ES]}{dt} \approx 0$ **(steady state)**

$$V = \frac{V_{max}[S]}{K_m + [S]}$$



V_i = initial velocity (moles/time)
[S] = substrate concentration (molar)
V_{max} = maximum velocity
K_m = substrate concentration when V_i is one-half V_{max} (Michaelis-Menton constant)



RUC Roskilde Universitet Roskilde University www.ruc.dk

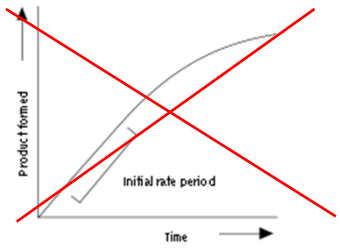
Enzyme assays

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_2} E + P$$

Conventional approach:
- Measure [P] – or [S]
- Use two time-points and $v_0 \sim \Delta[P]/\Delta t$ to find the slope

Common methods:
- Spectroscopy
- Chromatography
- Labeling

.....



Isothermal Calorimetry:
- Measures the rate directly
- Label free
- Continous (real-time data)

Fundamental equation:

$$HF = \frac{d[P]}{dt} \Delta H V_{cell}$$

So once the (apparent) enthalpy change is established the

REACTION RATE IS DIRECTLY GIVEN BY THE PRIMARY OBSERVABLE: HEAT FLOW

Differentiation inevitably generates noise !

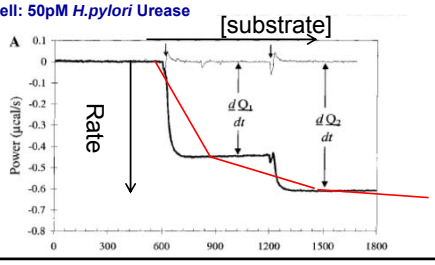
RUC Roskilde Universitet Roskilde University www.ruc.dk

Isothermal Calorimetry and enzyme activity

- Detecting the heat of the catalyzed reaction: heat flow J/s = ΔH x rate
- Rate –not concentration – is the primary observable – sensitivity ~pmol sec^{-1}
- Real time – complex time courses readily characterized
- No probe, fluorophore etc required
- Works in "complex systems" (opaque, multi-phase (colloid), insol. Substrate, side reactions)

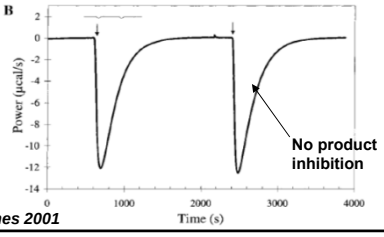
Substarte (syringe) → enzyme (cell)
Constant substrate concentration

Syringe: 40mM Urea, 3µl injections (88µM in cell)
Cell: 50pM *H.pylori* Urease



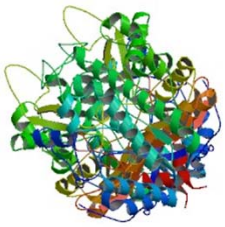
Substarte (syringe) → enzyme (cell)
Substrate depletion

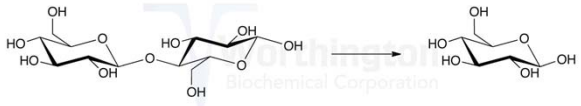
Syringe: 25mM Urea, 18µl injections (513µM in cell)
Cell: 4nM *H.pylori* Urease

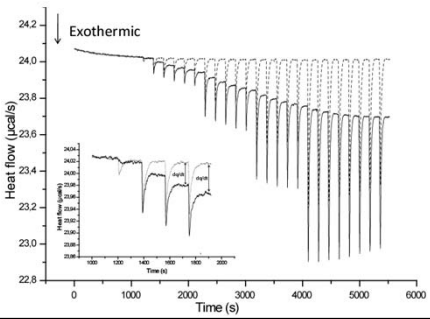


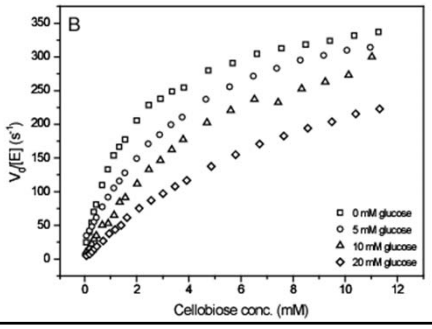
Todd & Gomes 2001

Beta glucosidase
product inhibition





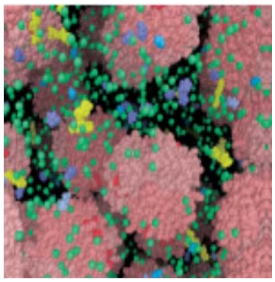




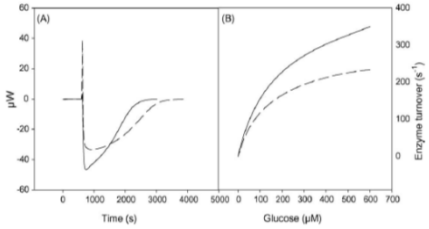
ITC and enzyme activity: Crowding

Hexokinase in buffer and 250 mg/ml serum albumin

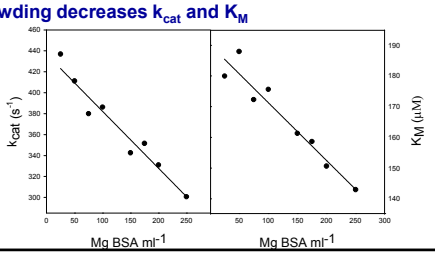
Diffusion, binding, enzyme flexibility etc is affected by crowded surroundings



Inside a red cell

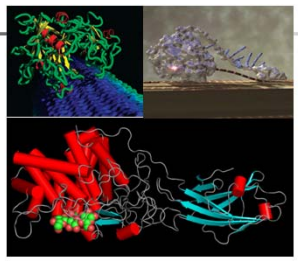


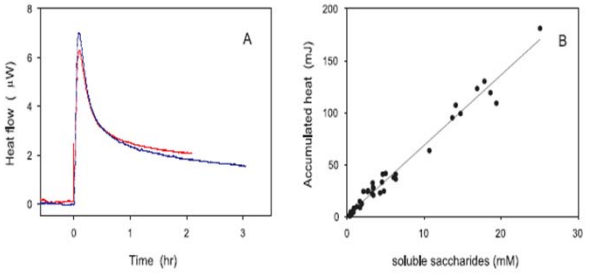
Crowding decreases k_{cat} and K_M

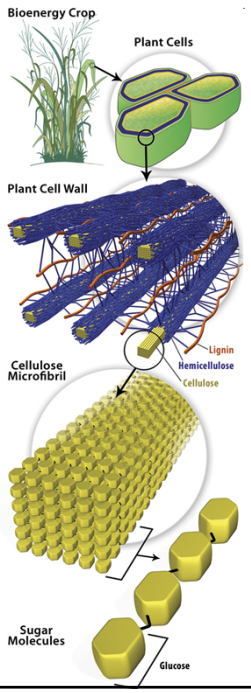


Olsen et al 2006

Enzyme activity against insoluble substrates: hydrolysis of cellulose

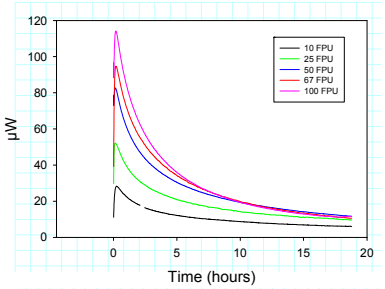


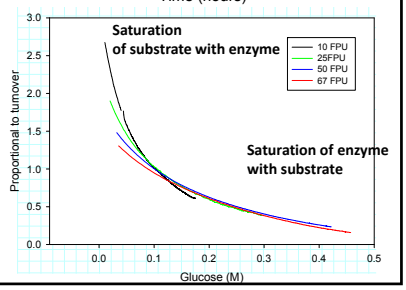




Calorimetry and high solid samples

30 % (w/w) biomass





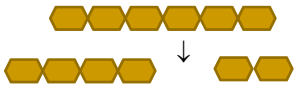
Turnover vs. product concentration

Low ΔH – then what?

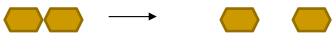
Signal amplification

Hydrolysis of glycosidic bonds is associated with moderate heat

Hydrolysis of cellulose



BG ~3 kJ/mol



GOx ~80 kJ/mol 0_2 +   -COOH + H_20_2

Catalase ~100 kJ/mol H_20_2 → H_20 + 0_2

If Gox/catalase/BG is added to the solution, the calorimetric signal from e.g. CBH is amplified by a factor of ~130

Other examples of ITC applications

Meatbolism – measuring the heat of live
- physiology
- medicine
- Fermentation technology
- etc etc

Drug formulation
- heat production = instability
- Rapid assesment of deday constant

Hydration and hydration driven transitions
- determine critical moisture levels

Calorimetry
The pros and cons of application

PRO
- **Universally applicable**
- **No probe / no special sample preparation**
- **Quantitative**
- **Non-specific**

CON
- **No structure information**
- **Moderate sensitivity**
- **Low through-put**
- **Non specific**