

Classical and Statistical Thermodynamics

Key references: 1. Chapter 2 of van Holde (2nd Ed.)

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Thermodynamics

- The branch of science that describes the behavior of matter and transformations between different forms of energy on the macroscopic level.
- Classical thermodynamics
 - Describe the system in terms of bulk properties
 - A set of **Laws of Thermodynamics**
 - Abstract, but generally applicable
 - Does not make reference to the microscopic details
- Statistical thermodynamics (statistical mechanics)
 - Emphasize “bottom-up” approach
 - Provide links between microscopic details (typically not measurable) to macroscopic properties (measurable)
 - Increasingly important, but do not replace classical thermodynamics

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Fundamental Concepts

- **System** (all materials involved in the process under study) and surroundings (the rest of the universe)
 - Open vs close (transfer of **matter**)
 - Diathermal vs adiabatic (transfer of **heat**)
 - Expandable or constant-volume (transfer of **work**)
 - Isolated (neither matter nor energy exchange)
- **State** of the system
 - A thermodynamic state definable only for systems at **equilibrium**
 - A condition in which equilibrium exists between the system and its surroundings with respect to one or more system variables
 - System variables: extensive and intensive
 - pressure (P) and volume (V)
 - temperature (T): an abstract quantity, measured indirectly (°C, F, K, ...)
 - Ideal gas: $PV = nRT$, thus $T = PV/nR$ (absolute temperature)
 - concentration (C): an intensive variable (relative quantity)

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First Law of Thermodynamics

- Conservation of energy: heat (q), work(w), and (internal) energy (E)

$$\Delta E = q - w \quad dE = \delta q - \delta w$$
- Internal energy (E)
 - Macroscopically: only definable on relative scale (see Eq above)
 - Microscopically: multiple contributions including: kinetic, vibrational, rotational, (chemical) bonding, non-bonded interaction, nuclei (typically not relevant in chemistry/biochemistry) components
 - A **function of state**
 - Depends on the state only, regardless of the path of arrival
 - If only P dV work, $dE = \delta q - PdV$

$$= \delta q \quad \text{if constant } V$$

$$\Delta E = q_v$$

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Enthalpy

- Internal energy is related to q_v , what about q_p ?

$$\begin{aligned}
 dE &= \delta q - PdV \\
 d(E + PV) &= dE + PdV + VdP \\
 &= \delta q - PdV + PdV + VdP \\
 &= \delta q + VdP \\
 dH &= \delta q + VdP \\
 &= \delta q \quad \text{if constant } P \\
 \Delta H &= q_p
 \end{aligned}$$

- Enthalpy ($H=E+PV$): a state function that captures energy change at constant P
- For liquid phase processes (most biochemical processes), dV is small and thus $\Delta E \sim \Delta H$

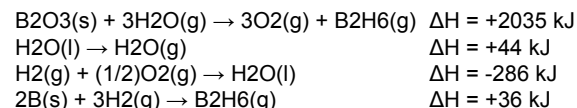
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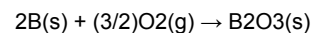
Hess's Law

- Enthalpy is a state function. Thus, the enthalpy change of a reaction is the same regardless of what pathway is taken to achieve the products, i.e., only the start and end states matter to the reaction, not the individual steps between.

Given:



Find the ΔH of:



Answer: $\Delta H = -1273 \text{ kJ}$

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Boltzmann Distribution



- A statistical view of the system at molecular level
 - Molecules (of the same kind) within the system are **indistinguishable**
 - At a given moment, each molecule might reside in one of the possible discrete **microscopic states** i with corresponding energy level e_i
 - For very large system (remember Avogadro Number: 6.02×10^{23}) at equilibrium, only **most probably state** is relevant.
- Boltzmann distribution**: specifies the most probable (i.e., equilibrium) probability of observing a certain energy state at a specified temperature

$$P_i \propto e^{-e_i / k_B T}$$

- k_B : Boltzmann constant ($=1.3806503 \times 10^{-23} \text{ J K}^{-1}$); $k_B = R/N_A$
- The most fundamental relationship in statistical mechanics (or statistical thermodynamics)

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Derivations of Boltzmann Distribution (1)

- Consider a three-state system (e_1, e_2, e_3). At a given T , let's write down the probability of finding the system in these states as p_1, p_2 and p_3 .
- Since energy is a relative quantity, $p_i/p_j = f(e_i - e_j)$
- $p_3/p_2 = f(e_3 - e_2) = f(e_3 - e_1)/f(e_2 - e_1)$
- ... this means that $f(x+y) = f(x)f(y)$
- What kind of functional form would give rise to such property?
- ... exponential functions!
- ... $p_i = \alpha \exp(-\beta e_i)$
- ... this is exactly what (not how) Boltzmann derived 150 years ago! It turns out that $\beta = 1/k_B T$

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Derivations of Boltzmann Distribution (2)

- Considering an **isolated** system of N with a total energy restricted to $E=Me$ where each particle might access energy levels are ne , with $n=0, 1, 2, \dots$

$$n_0 + n_1 + n_2 + \dots = N$$

$$e n_1 + 2e n_2 + 3e n_3 + \dots = Me$$

- Boltzmann postulated that if one can observe such an assembly over a long period of time, each microstate will occur with equal probability and that one will find that the number of occurrences for any particular set of distribution is proportional to the number of corresponding microstates, which is given by:

$$W = \frac{N!}{n_0! n_1! n_2! \dots}$$

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Derivations of Boltzmann Distribution (2)

- A case of $N=7$, $E=7e$, the total number of possible states: 1716

Table for the possible arrangements of 7 atoms and 7 allowed energy levels:

Case	n_0	n_1	n_2	n_3	n_4	n_5	n_6	n_7	W , number of microstates
1	6							1	7
2	5	1					1		42
3	5		1			1			42
4	5			1	1				42
5	4	2				1			105
6	4	1	1		1				210
7	4	1		2					105
8	4		2	1					105
9	3	3			1				140
10	3	2	1	1					420
11	3	1	3						140
12	2	4		1					105
13	2	3	2						210
14	1	5	1						42
15		7							1

$$W_1 = \frac{7!}{1!6!} = \frac{5040}{720} = 7$$

$$W_{10} = \frac{7!}{1! \cdot 1! \cdot 2! \cdot 3!} = \frac{5040}{12} = 420$$

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Derivations of Boltzmann Distribution (2)

- For very large N , only the most probable microstate matters!
- Maximize W or equivalently $\ln W$ with N and E restriction

$$\ln N! \cong N \ln N - N$$

Thus, $\ln W$ can be expressed as follows:

$$\ln W \cong N \ln N - \sum_i n_i \ln n_i - \left(N - \sum_i n_i \right)$$

The term inside the parenthesis is zero since $N = \sum_{i=0}^{\infty} n_i$.

Thus,

$$\ln W \cong N \ln N - \sum_i n_i \ln n_i$$

Taking the differential (recall that N is constant):

$$d(\ln W) = -\sum_i (1 + \ln n_i) dn_i = -\sum_i \ln n_i dn_i$$

where the fact that $\sum_i dn_i = dN = 0$, has already been taken into account.

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Derivations of Boltzmann Distribution (2)

$$d(\ln W) = -\sum_i (1 + \ln n_i) dn_i = -\sum_i \ln n_i dn_i$$

where the fact that $\sum_i dn_i = dN = 0$, has already been taken into account.

The next step involves the use of Lagrange undetermined multipliers. It is known that:

$$dE = 0 = \sum_i \epsilon_i dn_i \quad \text{and} \quad dN = 0 = \sum_i dn_i$$

Thus, we can add the above equations freely to $d(\ln W)$. For any constant α and β the following should be true:

$$d(\ln W) = -\sum_i \ln n_i dn_i - \alpha \sum_i dn_i - \beta \sum_i \epsilon_i dn_i$$

At maximum W or maximum $\ln W$, $d(\ln W) = 0$.

$$0 = -\sum_i (\alpha + \beta \epsilon_i + \ln n_i) dn_i$$

This equation is true only if each term in the summation is zero:

$$0 = (\alpha + \beta \epsilon_i + \ln n_i)$$

$$\ln n_i = -\alpha - \beta \epsilon_i$$

$$n_i = e^{-\alpha} e^{-\beta \epsilon_i}$$

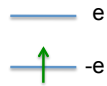
Does it make physical sense?

- Given the two state system shown to the right

$$n_+ \sim \exp(-e/kT) \quad n_- \sim \exp(e/kT)$$

$$P_+ = \frac{\exp(-e_i/kT)}{\exp(-e/kT) + \exp(e_i/kT)}$$

$$P_- = \frac{\exp(e_i/kT)}{\exp(-e/kT) + \exp(e_i/kT)}$$



- When temperature is near zero ($T \rightarrow 0$), $p_+ \rightarrow 0$, $p_- \rightarrow 1$
- When temperature approaches infinity, $p_+ = p_- = 0.5$

Partition Function

- Given the Boltzmann distribution, the probability of observing any energy state is given as:

$$P_j = \frac{n_j}{N} = \frac{\exp(-e_j/kT)}{\sum_i \exp(-e_i/kT)} = \frac{1}{Q} \exp(-e_j/kT)$$

- Q is defined as the (canonical) **partition function**
- If considering degeneracy of energy states

$$Q = \sum_i g_i \exp(-e_i/kT)$$

- Contains **all** information about the system (g_i and e_i)! All classical thermodynamics quantities can be derived from Q
- How to compute Q?
 - Theory and simulation

Statistical Thermodynamics

- Thermodynamic **ensembles**: a collection of identical units (i.e., atoms or molecules) of a system
 - A theoretical concept that relate microscopic properties to corresponding thermodynamics properties
 - Equivalence of ensemble average and time average
- Canonical ensemble: N, V and T are constant
- (Internal) energy $E = \langle E \rangle = N \langle e \rangle$

$$P_j = \frac{1}{Q} \exp(-e_j/kT) = \frac{1}{Q} \exp(-\beta e_j)$$

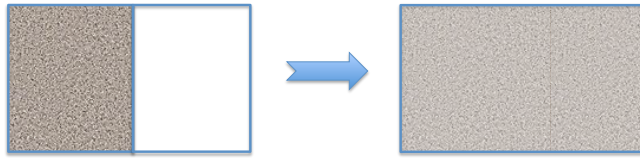
$$\begin{aligned} \langle e \rangle &= \sum_j e_j P_j = \frac{1}{Q} \sum_j e_j \exp(-\beta e_j) \\ &= - \frac{d \ln Q}{d\beta} = k_B T^2 \frac{d \ln Q}{dT} \end{aligned}$$

Spontaneous Processes

- Real-world processes have a natural direction of change.
 - Heat transfers from high T to low T
 - (Ideal) gas spontaneously fills up all the accessible volume
- Energy and enthalpy do not provide information such directionality of change.
 - Above processes occur naturally in isolated systems where energy does not change (First Law of Thermodynamics)
- What quantities would help us to predict the direction of change in complex systems?
 - It is all about probability!**
- Concept of **entropy** and **Second Law** of Thermodynamics
 - Original concept comes of studies of heat engine (conversion between heat and work)
 - Statistical derivation: provides entropy a tangible meaning

Entropy

- A measure of internal heat content, or, the unavailability of a system's energy to do work ($S=q_{\text{rev}}/T$).
- Based on the same logic that is used for deriving Boltzmann distribution, equilibrium will lie where the probability is at maximum.



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Entropy

- Statistical definition (1): $S = k_B \ln W$ (1)
 - Extensive: $S = S_1 + S_2$
 - A function of state (as W is)
- Statistical definition (2): $S = -k_B \sum_i p_i \ln p_i$ (2)
- Two definitions are equivalent

$$W = \frac{N!}{n_0!n_1!n_2!\dots}$$

$$\ln W = (N \ln N - N) - \sum_i (n_i \ln n_i - n_i)$$

$$= N \ln N - \sum_i n_i \ln n_i$$

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Relation of Classical and Statistical Entropy

$$dS = k_B d \ln W = -k_B d \sum_i n_i \ln n_i$$

$$= -k_B \sum_i \ln n_i dn_i \quad (\text{remember } \sum_i dn_i = 0)$$

$$n_i = n_1 \exp(-e_i/k_B T) \quad (\text{in reference to } e_1 = 0)$$

$$\ln n_i = \ln n_1 - \frac{e_i}{k_B T}$$

$$dS = k_B \sum_i \frac{e_i}{k_B T} dn_i = \frac{1}{T} \sum_i e_i dn_i = \frac{dq_{\text{rev}}}{T}$$

- The statistical definition of entropy thus provides a clearly defined microscopic meaning of the classical entropy.

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Relation to Partition Function

$$S = -k_B \sum_i p_i \ln p_i$$

$$= -k_B \sum_i \frac{e^{-e_i/k_B T}}{Q} \ln \frac{e^{-e_i/k_B T}}{Q}$$

$$= k_B (\ln Q + \langle E \rangle / k_B T) \quad \langle E \rangle = -\frac{d \ln Q}{d\beta} = k_B T^2 \frac{d \ln Q}{dT}$$

$$= \frac{\partial}{\partial T} (k_B T \ln Q)$$

$$= -\frac{\partial A}{\partial T} \quad A = -k_B T \ln Q$$

A: Helmholtz free energy

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What does entropy capture?

- Correct interpretations: as given by the definitions
 - A measure of internal heat content (the unavailability of a system's energy to do work)
 - A statistical measure of the probability for a given macro state
 - A measure of uncertainty (from information theory)
- Confusing/incorrect interpretations
 - A measure of “disorder” or “randomness”
 - A measure of “complexity”

$$S = k_B \ln W$$

$$S = -k_B \sum_i p_i \ln p_i$$

Calculation of Entropy

- Example: isothermal expansion of ideal gas from V_1 to V_2

1. Classical approach $dE = dq - dw = 0$

$$dq = dw = PdV = RT \frac{dV}{V}$$

$$\Delta S = \frac{q_{\text{rev}}}{T} = R \int_{V_1}^{V_2} \frac{dV}{V} = R \ln \left(\frac{V_2}{V_1} \right)$$

2. Statistical approach: $V_1 = n_1 V_0$ $V_2 = n_2 V_0$

$$W_1 \propto n_1^N \quad W_2 \propto n_2^N$$

$$\begin{aligned} \Delta S &= k_B \ln W_2 - k_B \ln W_1 \\ &= k_B \ln \left(\frac{n_2^N}{n_1^N} \right) = R \ln \left(\frac{n_2}{n_1} \right) = R \ln \left(\frac{V_2}{V_1} \right) \end{aligned}$$

Second Law of Thermodynamics

- In an **isolated system**, entropy reaches maximum at equilibrium (the most probable distribution). In another word, $\Delta S > 0$ provides a criterion for spontaneous change (**only if** in an isolated system, i.e., without energy exchange in the form of heat or work).

“Derivation”: at equilibrium, an infinitesimal change is reversible.

$$dE = dq - dw = TdS - PdV$$

In addition, for isolated system, $dE=0$, $dw=0$. Thus,

$$dS = 0$$

therefore, entropy is either at maximum or minimum.

Free Energies

- At constant P and T (NPT): $dP = dT = 0$

$$V dP - S dT = 0 \quad dE = T dS - P dV$$

$$(V dP - S dT) + dE - (T dS - P dV) = 0$$

$$dE + V dP + P dV - (S dT + T dS) = 0$$

$$d(E + PV - TS) = 0$$

- If we define a new quantity $G = E + PV - TS = H - TS$,
 $dG = 0$ for a reversible process at NPT
- G is referred to as “**Gibbs free energy**”, which is at minimum at equilibrium under NPT condition (most common).
- Similarly, **Helmholtz free energy**, $A = E - TS$, is at minimum at equilibrium under NVT condition.
- The free energies provide the direction of spontaneous changes under corresponding conditions.

Free Energy and Probability

- Remember, from the statistical mechanical point of view, direction of spontaneous processes is given by the direction of maximum probability.
 - for isolated systems, the probability of a macro-state is given by entropy. i.e., $W = \exp(S/k_B)$

- For a system coupled with NPT conditions,

$$P_i \propto \exp(-G_i/RT) = \exp(-H_i/RT) \exp(S_i/R)$$

- For a system coupled with NVT conditions,

$$P_i \propto \exp(-A_i/RT) = \exp(-E_i/RT) \exp(S_i/R)$$

Statistical Meaning of Free Energies

- Directly defined by the probability of a macro-state
- Further related to the properties of microscopic states through the partition function
- For example:

$$E = -\frac{d \ln Q}{d\beta} = k_B T^2 \frac{d \ln Q}{dT}$$

$$S = \frac{\partial}{\partial T} (k_B T \ln Q) = k_B \ln Q + k_B T \frac{\partial \ln Q}{\partial T}$$

$$A = E - TS$$

$$= k_B T^2 \frac{d \ln Q}{dT} - T(k_B \ln Q + k_B T \frac{\partial \ln Q}{\partial T})$$

$$= -k_B T \ln Q$$

Other Thermodynamic Properties

- Classical thermodynamics relationships can be used to derived the statistical definitions of all properties
- For example:

$$P = -\left(\frac{\partial A}{\partial V}\right)_{N,T} = k_B T \left(\frac{\partial \ln Q}{\partial V}\right)_{N,T}$$

$$H = E + PV$$

$$G = A + PV$$

$$C_v = \left(\frac{\partial E}{\partial T}\right)_{N,V}$$

$$C_p = \left(\frac{\partial H}{\partial T}\right)_{N,P}$$

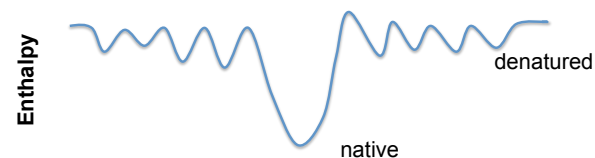
$$E = -\frac{d \ln Q}{d\beta}$$

$$S = \frac{\partial}{\partial T} (k_B T \ln Q)$$

$$A = -k_B T \ln Q$$

Enthalpy and Entropy Compensation

- $\Delta G = \Delta H - T\Delta S$
- Consider an example of protein folding:

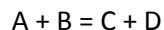


$$\Delta H_f < 0 \quad \Delta T_f < 0$$

- Low temperature, $\Delta G_f = \Delta H_f - T\Delta S_f < 0$ (folding)
(the favored state is not the more "random" denatured state!)
- At folding temperature T_m , $\Delta G_f = 0$ ($p_f = p_u = 0.5$)
- High temperature, $\Delta G_f = \Delta H_f - T\Delta S_f > 0$ (unfolding)

Equilibrium Constant

- Remember that given a reaction:



the quantity: $K = [C][D]/[A][B]$ is a constant that is commonly referred to as an “equilibrium constant”. But why a constant?

- Proof:**

- First, needs to define a “standard state” (commonly 298K, 1atm, 1M). Then for a solution with a concentration [A],

$$G = G^0 + RT \ln ([A]/[A]^0) = G^0 + RT \ln [A]$$

- Therefore,

$$\Delta G = \Delta G^0 + RT \ln ([C][D]/[A][B])$$

- At equilibrium, $\Delta G = 0$. Thus,

$$\Delta G^0 + RT \ln ([C][D]/[A][B]) = 0$$

$$\Delta G^0 = - RT \ln K$$

$$K = [C][D]/[A][B] = \exp (- \Delta G^0/RT)$$

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Thermochemistry

- Experimental determination of the thermodynamic parameters such as ΔG , ΔH and ΔS .
- ΔG can be directly calculated from K_{eq}
 - Often done by fitting to a titration curve
 - Require choosing a measurable that responds to binding or (bio-) chemical reaction
- Determination of ΔH and ΔS
 - Van’t Hoff Relationship
 - Calorimetry
 - Theory and simulation: not reliable at quantitative level

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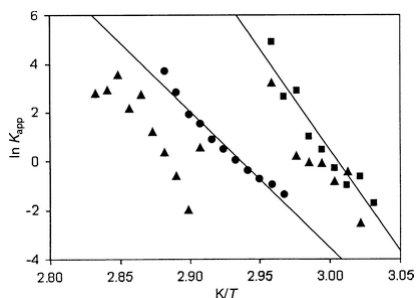
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Van’t Hoff Relationship

- The classical approach, require multiple measurements of K_{eq} at different temperatures and certain assumptions

$$\ln K_{eq} = - \frac{\Delta G^0}{RT} = - \frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R}$$

- Assuming that ΔH and ΔS are temperature independent (often a strong assumption in biochemical/biophysical processes)
- “safer” to calculate $\Delta S = (\Delta H - \Delta G)/R$

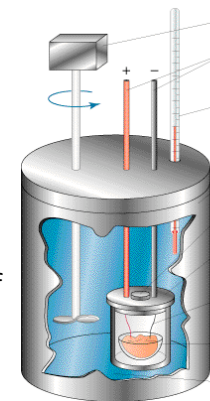


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Calorimetry

- “the quantitative measurement of heat”
- Depending on the setup, either q_v or q_p
 - Probe ΔE (bomb) or ΔH (constant pressure)
 - Biochemical systems often require the ability to study very small volumes (microcalorimetry)
- A **calorimeter** measures the heat into or out of the sample
- A **differential calorimeter** measures the heat of the sample relative to a reference
- A **differential scanning calorimeter (DSC)** does all the above and heats the sample with a linear temperature ramp
- A **Isothermal Titration Calorimetry (ITC)** measures the heat absorbed or released by a (bio)chemical reaction

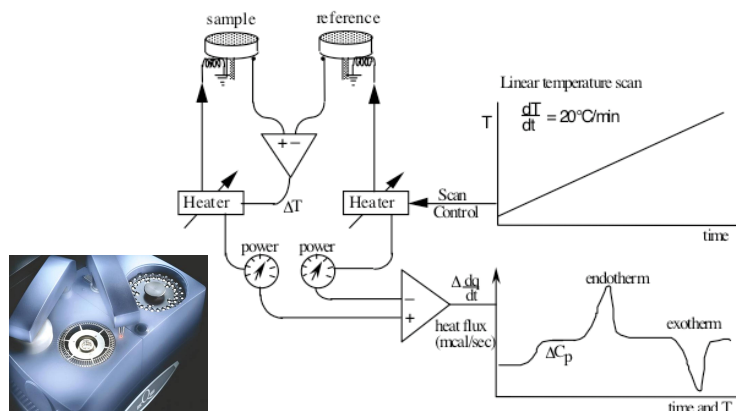


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Differential Scanning Calorimetry (DSC)

- The difference in heat flow to the sample and a reference at the same temperature, is recorded as a function of temperature.



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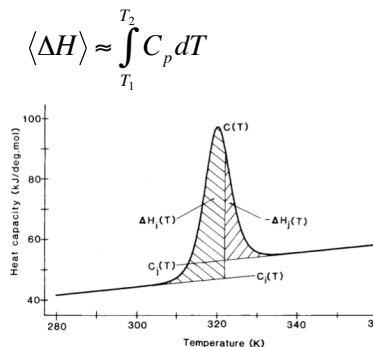
Analysis of DSC

- Mostly for monitoring heat effects associated with phase transitions and chemical reactions as a function of temperature (such as protein folding)

- Basic properties are:

$$C_p = dq_p / dT = dH / dT$$

- Substantial complications arise during the transition range where a mixture of potentially many (micro-) states might contribute to the observed C_p and deconvolution becomes highly non-trivial



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Two-State Analysis of DSC

- Two-state reversible transition assumed

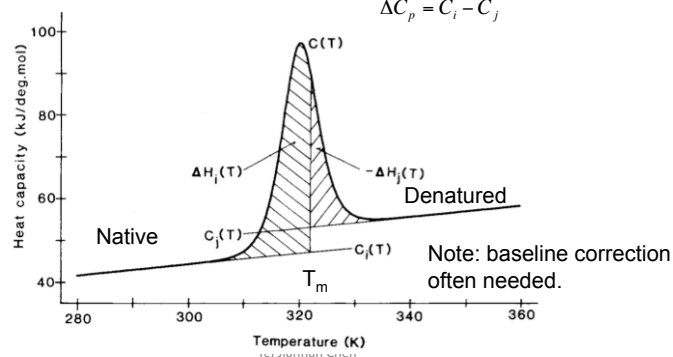
$$\Delta H^0(T_m) \approx \langle \Delta H^0 \rangle \propto \int_{T_i}^{T_j} (C_p - C_i) dT$$

$$\Delta H^0(T) = \Delta H^0(T_m) + \int_{T_m}^T \Delta C_p dT$$

$$\Delta S^0(T_m) = \Delta H^0(T_m) / T_m$$

$$\Delta S^0(T) = \Delta S^0(T_m) + \int_{T_m}^T \Delta C_p / T dT$$

$$\Delta C_p = C_i - C_j$$



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Relation to the van't Hoff Enthalpy

- Different assumptions involved in DSC analysis and van't Hoff relationship. Nonetheless, $\Delta H_{DSC}^0(T)$ and $\Delta H_{vH}^0(T)$ can be related.
- Assuming a reversible two-state system, the (base-line corrected) calorimetry enthalpy change,

$$\Delta H_i^0(T_x) = \int_{T_i}^{T_x} [C_p(T) - C_i(T)] dT = \int_{T_i}^{T_x} \Delta C_i(T) dT$$

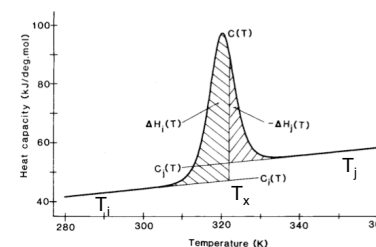
$$\approx f_i \langle \Delta H \rangle \approx f_i \int_{T_i}^{T_j} \Delta C_p dT$$

$$\Delta H_j^0(T_x) \approx -f_j \int_{T_i}^{T_x} \Delta C_p dT$$

$$K(T) = \frac{f_j}{f_i} \approx -\frac{\Delta H_j^0(T)}{\Delta H_i^0(T)}$$

$$\Delta H_{vH}^0(T) = -RT^2 \frac{d \ln K(T)}{dT} = -RT^2 \left(\frac{\Delta C_i(T)}{\Delta H_i^0(T)} - \frac{\Delta C_j(T)}{\Delta H_j^0(T)} \right)$$

Disagreement of $\Delta H_{DSC}^0(T)$ and $\Delta H_{vH}^0(T)$ indicates non two - state behavior



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Major Sources of Errors

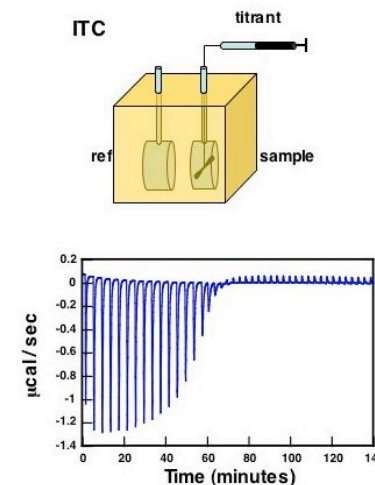
- Calibration
- Contamination
- Sample preparation – how sample is loaded into a pan
 - Need to minimize thermal gradient
- Residual solvents and moisture.
- Thermal lag
 - Heating/Cooling rates
 - Sample mass
- Processing errors

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Isothermal Titration Calorimetry

- Mostly used for understanding inter-molecular interactions
- Incremental addition over time of a ligand (or a binding partner) into a solution of a protein (or another partner), until no excess heat measurable (no more free protein)
- Measure the heat required to maintain the temperature



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Analysis of ITC

- Much simpler compared to DSC



$$q = \Delta H^0(T) n_{PL} = \Delta H^0(T) V [PL]$$

$$= \Delta H^0(T) V [P_T] \frac{K_a [L]}{1 + K_a [L]}$$

$$[PL] = [P_T] \frac{K_a [L]}{1 + K_a [L]}$$

$$q_i = \Delta H^0(T) V [P_T] \left(\frac{K_a [L]_i}{1 + K_a [L]_i} - \frac{K_a [L]_{i-1}}{1 + K_a [L]_{i-1}} \right)$$

$$[L]_i = 0.5([L_T] - [P_T] \pm \sqrt{([L_T] - [P_T] - 1/K_a)^2 - 4[L_T]})$$

Define a ratio $R = [L_T]/[P_T]$, fitting of q_i vs R will yield both K_a and $\Delta H^0(T)$. Free energy and entropy changes then follow.

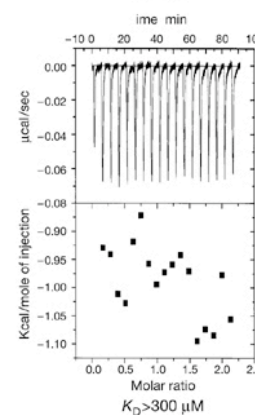
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B the yeast exosome



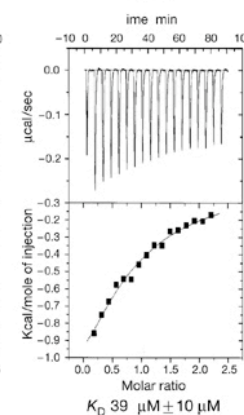
SsRrp4
A₇ RNA



C



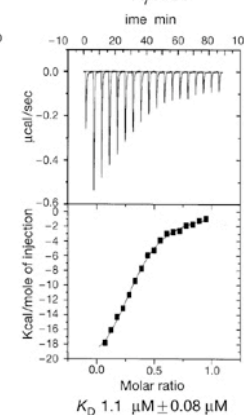
SsRrp41-Rrp42
A₇ RNA



D



SsRrp41-Rrp42-Rrp4
A₇ RNA



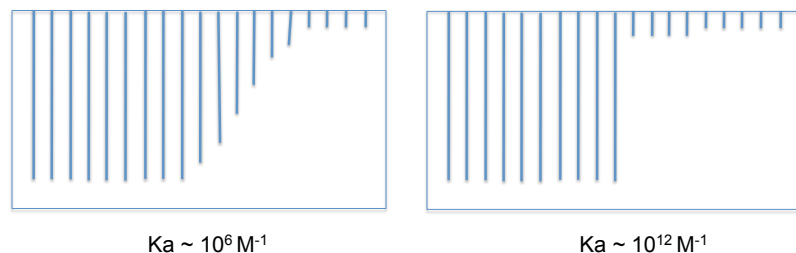
EMBO reports 8, 1, 63–69 (2007)

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Limitations of ITC

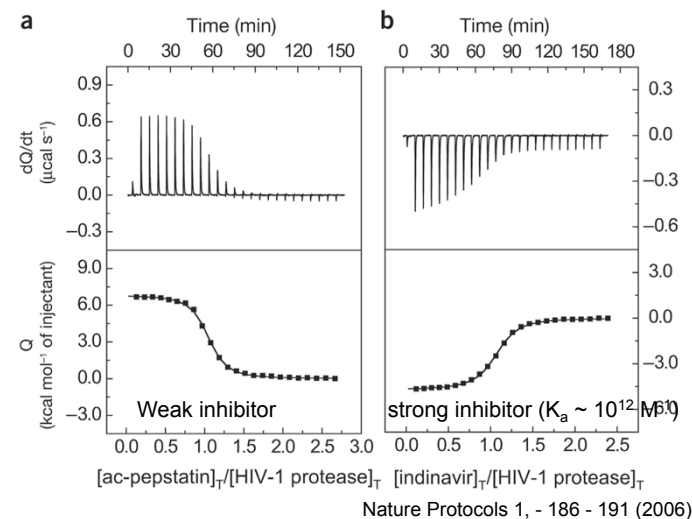
- Difficult for studying very strong binders
 - Each incremental of ligand becomes completely bound until protein is fully consumed; no transition to fit!
 - Upper limit $K_a \sim 10^9 \text{ M}^{-1}$
 - Might be overcome by so-called **displacement** titration



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Displacement ITC



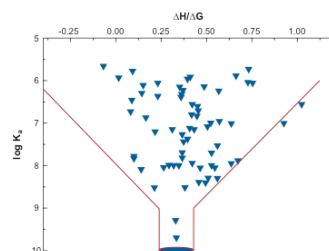
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Calorimetry for Drug Design

- Both DSC and ITC are powerful tools for obtaining thermodynamic data critical for drug design
- However, both are time-consuming and laborious and special designs are necessary for the need of high-throughput
 - e.g., ThermoFluor™ technology: less quantitative
- Whether binding is enthalpy or entropy driven can provide important insights for lead optimization (and such info can not be obtained from structural data)
- Replacement ITC for tight binders

Ref: "Calorimetry and Thermodynamics in Drug Design" by J. B. Chaires, Annu. Rev. Biophys. 2008. 37:135–51 .



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