Equilibrium in Solution

The chemical potential for molecules in solution is given by a formula that is very similar to that for ideal gases:

$$\mu_A(T, p, c_A) = \mu_A^o(T, p) + RT \ln c_A = \mu_A^o(T, p) + RT \ln [A]$$

The precise definition of the standard chemical potential $\mu_A^o(T, p)$ is now more complicated; it is defined at a given pH, salt concentration, etc…, all solution properties that need to be defined in advance. We will not go through those and take it as a given that the standard state is appropriately defined.

Given a standard chemical potential $\mu_A^o(T, p)$, then the analysis that we did for the ideal gas follows straight through and we find for a solution process

$$\nu_A\, A(g, T, p) + \nu_B\, B(g, T, p) = \nu_C\, C(g, T, p) + \nu_D\, D(g, T, p)$$

that following the ideal gas analysis in our previous lecture

$$\Delta G(\varepsilon) = \varepsilon\left[\nu_C \mu_C^o(T) + \nu_D \mu_D^o(T)\right] - \left[\nu_A \mu_A^o(T) + \nu_B \mu_B^o(T)\right] + RT \ln\left(\frac{[C]^{\nu_C}[D]^{\nu_D}}{[A]^{\nu_A}[B]^{\nu_B}}\right)$$

and the equilibrium constant K comes out through

$$\Delta G_{rxn}^{\circ} = -RT \ln K, \qquad K = e^{-\Delta G^{\circ}/RT}$$

Where $K = Q_{eq} = \frac{[C]^{\nu_C}[D]^{\nu_D}}{[A]^{\nu_A}[B]^{\nu_B}}$ at equilibrium as before, and where the concentrations Q are equilibrium concentrations.

Temperature dependence of K (or K_p)

$$\ln K(T) = -\frac{\Delta G^\circ}{RT} \quad \Rightarrow \quad \frac{d\ln K}{dT} = \frac{d}{dT}\left(-\frac{\Delta G^\circ}{RT}\right) = \frac{\Delta G^\circ}{RT^2} - \frac{1}{RT}\frac{d\Delta G^\circ}{dT}$$

But at <u>fixed pressure and/or solutions properties</u> (p = 1 bar, pH constant, etc..)

$$\frac{d\Delta G^\circ}{dT} = \left(\frac{\partial \Delta G^\circ}{\partial T}\right)_{1\ \text{bar, pH constant, etc...}}$$

and from fundamental equation

$$dG = -SdT + Vdp \quad \Rightarrow \quad \left(\frac{\partial G}{\partial T}\right)_p = -S \quad \Rightarrow \quad \left(\frac{\partial \Delta G^\circ}{\partial T}\right)_p = -\Delta S^\circ(T)$$

$$\therefore \quad \frac{d\ln K}{dT} = \frac{\Delta H^\circ(T) - T\,\Delta S^\circ(T)}{RT^2} + \frac{1}{RT}\Delta S^\circ(T)$$

$$\boxed{\frac{d\ln K(T)}{dT} = \frac{\Delta H^\circ(T)}{RT^2}}$$

<u>Integrating</u>: $\ln K(T_2) = \ln K(T_1) + \int_{T_1}^{T_2} \frac{\Delta H^\circ(T)}{RT^2} dT$

At constant p: $\Delta H^\circ(T) = \Delta H^\circ(T_1) + \Delta C_p(T - T_1)$

$$\ln K(T_2) = \ln K(T_1) + \int_{T_1}^{T_2} \frac{\Delta H^\circ(T_1) + \Delta C_p(T - T_1)}{RT^2} dT$$

Over small T ranges, $\Delta C_p(T - T_1)$ can be <u>assumed small</u> and <u>ΔH° independent of T.</u>

$$\Rightarrow \quad \boxed{\ln K(T_2) \approx \ln K(T_1) + \frac{\Delta H^\circ}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right) = \ln K(T_1) + \frac{\Delta H^\circ}{R}\left(\frac{T_2 - T_1}{T_1 T_2}\right)}$$

If $\Delta H^\circ(T) < 0$ (Exothermic) $T_2 > T_1$ means $K_p(T_2) < K_p(T_1)$

The equilibrium shifts toward reactants

If $\Delta H^\circ(T) > 0$ (Endothermic) $T_2 > T_1$ means $K_p(T_2) > K_p(T_1)$

The equilibrium shifts toward products

This is Le Chatelier's principle for Temperature

- Example: The Haber process

$$\tfrac{1}{2}\, N_2(g, T, p) + 3/2\, H_2(g, T, p) = NH_3(g, T, p)$$

$$\Delta H^\circ_{rxn}(298\ K) = -46.21\ kJ/mol$$
$$\Delta G^\circ_{rxn}(298\ K) = -16.74\ kJ/mol$$

$$K_p = \frac{p_{NH_3}}{p_{H_2}^{3/2} p_{N_2}^{1/2}} = p^{-1} \frac{X_{NH_3}}{X_{H_2}^{3/2} X_{N_2}^{1/2}} = e^{\frac{16{,}740\ J/mol}{(8.314\ J/K\text{-}mol)(298\ K)}} = 860$$

For p = 1 bar this is pretty good, lots of product. However, the reaction at room T is slow (this is kinetics, not thermodynamics). Raising T to 800 K can speed it up. But since $\Delta H^\circ(T) < 0$ (exothermic), Le Chatelier tells us that the equilibrium will shift toward the reactants.

Indeed: $K_p(800\ K) = 0.007$

What to do? $\Rightarrow$ Note above $K_X = \boxed{p} K_p$

Again use Le Chatelier, but with pressure! If we increase p, Eq. shifts toward products.

$\Rightarrow$ Run reaction at high T and high p

For p = 1 bar, T = 800 K, $K_p = 0.007$

$$K_X = \frac{X_{NH_3}}{X_{H_2}^{3/2} X_{N_2}^{1/2}} = (1)K_p = 0.007$$

But at p = 100 bar, $K_X = (100)K_p = 0.7$ much better!

- Heterogeneous Equilibria

If a product or reactant is a solid or liquid, it will not appear in the ratio of partial p's for K_p or in the concentrations if the equilibrium is in solution. However, it must be used in ΔG.

Why? Take ν_A A(s) + ν_B B(g) = ν_C C(l) + ν_D D(g)

The solid and liquid are not mixed – they are pure states.

$$\Delta G = \left[\nu_C \mu_C (s, \text{pure}, p) + \nu_D \mu_D (g, \text{mix}, p)\right] - \left[\nu_A \mu_A (l, \text{pure}, p) + \nu_B \mu_B (g, \text{mix}, p)\right]$$

And for (l) or (s) $\mu_C (\text{pure}, p) \approx \mu^o (\text{pure})$ (no p-dependence)

$$\Rightarrow \quad \Delta G = \nu_C \mu_C^o + \nu_D \mu_D^o - \nu_A \mu_A^o - \nu_B \mu_B^o + RT \ln \frac{p_D^{\nu_D}}{p_B^{\nu_B}} = \Delta G^o + RT \ln Q$$

$$\therefore \quad K_p = \left[\frac{p_D^{\nu_D}}{p_B^{\nu_B}}\right]_{Eq.}$$ No A or C involved.

But we still have $\Delta G^\circ_{rxn} = \nu_C \mu_C^\circ + \nu_D \mu_D^\circ - \nu_A \mu_A^\circ - \nu_B \mu_B^\circ$

and $\ln K_p = -\frac{\Delta G^\circ_{rxn}}{RT}$

e.g. the decomposition of limestone

$CaCO_3\,(s) = CaO\,(s) + CO_2\,(g)$ T = 25°C

Calculate equilibrium vapor pressure at room T and elevated T.
Data at 25°C:

Substance	$CaCO_3$ (s)	CaO (s)	CO_2 (g)
μ° (kJ/mol)	-1128.8	-604.0	-394.36
$\Delta\bar{H}_f^\circ$ (kJ/mol)	-1206.9	-635.09	-393.51

At equilibrium,

$$\Delta G = \mu(CaO, s) + \mu(CO_2, g) - \mu(CaCO_3, s)$$
$$= \mu^\circ(CaO, s) + \mu^\circ(CO_2, g) + RT \ln p_{CO_2} - \mu^\circ(CaCO_3, s)$$
$$= \Delta G^\circ + RT \ln K_p \qquad \text{where } K_p = p_{CO_2} \text{ (at eq.)}$$

The equilibrium constant includes only the gas, but ΔG° includes the solids too.

ΔG° (kJ/mol) = -604.0 – 394.4 – (-1128.8) = 130.4 kJ/mol
ΔH° (kJ/mol) = -635.1 – 393.5 – (-1206.9) = 178.3 kJ/mol

Equilibrium pressure:

$$\ln K_p = -\frac{\Delta G^\circ}{RT} = -\frac{130,400\ \text{J/mol}}{(8.314\ \text{J/K-mol})(298.15\ \text{K})} = -52.50$$

$$K_p = 1.43 \times 10^{-23}\ \text{bar}$$

Nothing there at room T ! Try 1100 K:

$$\ln p_{CO_2}(1100\ \text{K}) \approx \ln p_{CO_2}(298\ \text{K}) + \frac{\Delta H^\circ}{R}\left(\frac{1}{1100\ \text{K}} - \frac{1}{298\ \text{K}}\right)$$

$$= -52.50 - \frac{178,300\ \text{J/mol}}{8.314\ \text{J/K-mol}}\left(\frac{1}{1100\ \text{K}} - \frac{1}{298\ \text{K}}\right) = 0.17$$

$$p_{CO_2}(1100\ \text{K}) \approx 0.84\ \text{bar}$$

There's probably some change in $\Delta \bar{H}_f^\circ$ over such a wide T range, but clearly the equilibrium shifts dramatically.

Equilibrium: Application to Drug Design

Based on "Rational cytokine design for increased lifetime and enhanced potency using pH-activated histidine switching," Sarkar, Lowenhaupt, Horan, Boone, Tidor, and Lauffenburger, Nature Biotechnology **20**, 908 (2002).

The analysis for equilibrium that we have used for reactions involving breaking and making covalent bonds applies equally well to reactions such as those involved in ligand-receptor binding, where the ligand and receptor are proteins

$$R + L = C$$

where R is the receptor, L is the ligand, and C is the receptor-ligand complex. The interactions between these proteins typically involve multiple non-covalent interactions, including hydrogen bonds, hydrophobic interactions, and electrostatic interactions. The equilibrium constant and Gibbs free energy change for the reaction are related in the usual way

$$\Delta G^\circ = -RT \ln K_a$$

where the equilibrium constant K_a is called the association constant

$$K_a = \frac{[C]}{[R][L]}$$

The standard state needed to characterize ΔG° is defined at a set of specific reference conditions (pH, salt concentration, etc….). By convention, the reverse process (the dissociation) is used to characterize the strength of ligand binding through the equilibrium constant K_D, also called the dissociation constant

$$K_D = \frac{[R][L]}{[C]}$$

The lower the K_D, the better the ligand (the tighter the binding).

In an experiment the ligand is typically labeled radioactively (e.g. with ^{125}I) and added to cells under conditions that prevent the ligand from being internalized (4°C).The ligand is usually in great excess compared to the number of receptors, so that at equilibrium $[L]=[L]_0$ is a good approximation (the ligand concentration is effectively unchanged during the process).

If $[R]_T$ is the total concentration of receptors, then $[R]_T=[R]+[C]$, so that at equilibrium,

$$K_D = \frac{\left([R]_T - [C]_{eq}\right)[L]_o}{[C]_{eq}}$$

or

$$\frac{[C]_{eq}}{[L]_o} = \frac{[R]_T}{K_D} - \frac{[C]_{eq}}{K_D}$$

The value of K_D (and thus ΔG^o) can be obtained by measuring the concentration of complexes formed at various initial ligand concentrations $[L]_0$ (through the radioactive labeling) by plotting $\frac{[C]_{eq}}{[L]_o}$ as a function of $[C]_{eq}$ (a Scatchard plot). The slope gives $\frac{1}{K_D}$.

The fraction of receptors occupied is

$$\frac{[C]_{eq}}{[R]_T} = \frac{1}{\left(1 + \frac{K_D}{[L]_o}\right)}$$

Note, when $[L]_o << K_D$ then $\frac{[C]_{eq}}{[R]_T} \cong \frac{[L]_o}{K_D}$,

when $[L]_o >> K_D$ then $\frac{[C]_{eq}}{[R]_T} \cong 1 - \frac{K_D}{[L]_o}$

Although free energies for receptor-ligand binding reactions are generally determined experimentally (through K_D), it is possible to computationally estimate the changes in free energy that accompany point mutations in one of the amino acids in the ligand. This approach

can be used to design a new and "better" drug that binds with an affinity that improves its properties. An example of such a designed mutated ligand is an improved version of Granulocyte-Colony Stimulating Factor (GCSF). GCSF is a protein drug that is used to treat chemotherapy patients and stimulates the growth of white blood cells.

It is desirable to have GCSF bind tightly to its receptor at the cell surface (at pH 7.4) as this signals the cell to produce the desired proteins. But when the complex C is internalized in the cell in endosomal compartments (pH 5.5), it is desirable for GCSF to fall off its receptor to be recycled back to the solution to be used again instead of being degraded within the endosome. Thus a design principle for an improved mutant GCSF is weaker binding at pH 5.5 (inside cell) than at pH 7.4 (cell surface), or in other words $K_D(pH5.5) > K_D(pH7.4)$.

For the wild type (WT) GCSF the data gives:

	K_D(pH 7.4), pM	K_D(pH 5.5)/ K_D(pH 7.4)
WT	270±90	1.7±0.5

Since $\Delta G^\circ = -RT \ln K_D$, we can get the difference of ΔG°'s for the dissociation reaction at the different pH's

$$\left[\Delta G^\circ(\text{pH } 7.4) - \Delta G^\circ(\text{pH } 5.5)\right]/RT$$

WT: 0.53±0.3 (measured from K_D values)

Calculations were performed on several mutants and two showed appreciable differences in free energies

D110H:	8.3 (calculated)
D113H:	17 (calculated)

These mutant GCSF molecules were synthesized and evaluated for binding to the GCSF receptor with the following results:

	K_D(pH 7.4), pM	K_D(pH 5.5)/ K_D(pH 7.4)
WT	270±90	1.7±0.5
D110H	370±450	4.4±0.8
D113H	320±130	6.8±2.4

The mutants do bind more weakly than does the wild type at low pH, and thus have the potential to be better drugs (in fact, in animal trials the mutants have much longer half-lives than the wild type). Differences in free energies for the mutants can be obtained from the experimental K's:

$$\left[\Delta G^\circ(\text{pH } 7.4) - \Delta G^\circ(\text{pH } 5.5)\right]/RT$$

WT:	0.53±0.3 (measured from K_D values)
D110H:	1.5±0.2 (measured from K_D values)
D113H:	1.9±0.4 (measured from K_D values)