Concept review: Binding equilibria

Binding equilibria and association/dissociation constants

The binding of a protein to a ligand at equilibrium can be written as:

$$P + L \leftrightarrow PL$$

And so the equilibrium constant for association is:

$$K_a = K_{eq} = \frac{[PL]}{[P][L]} \qquad \text{units } M^{-1}$$

And the equilibrium constant for dissociation is:

$$K_d = \frac{[P][L]}{[PL]} \qquad \text{units M}$$

•A ligand is defined as a group, ion, or molecule coordinated to a central atom or molecule in a complex. We will arbitrarily say that the the smaller of the two molecules in the complex is the ligand and the larger is the receptor (typically a protein).
•Affinity is defined as the attractive force between two groups, ions, or molecules that causes them to enter into and remain in a non-covalent complex.

Binding equilibria and free energy

The free energy change upon binding at any given concentration is:

$$\Delta G = \Delta G^\circ + RT \ln K_a = \Delta G^\circ + RT \ln\left(\frac{[PL]}{[P][L]}\right)$$

At equilibrium, $\Delta G = 0$ and hence:

$$\Delta G^\circ = -RT \ln\left(\frac{[PL]_{eq}}{[P]_{eq}[L]_{eq}}\right) = -RT \ln\left(K_a\right) = +RT \ln\left(K_d\right)$$

- $\Delta G < 0$ indicates that the reaction is spontaneous in the forward direction and energy is released.
- $\Delta G = 0$ indicates that the reaction is at equilibrium and no further change can or will occur.
- $\Delta G > 0$ indicates that the reaction is spontaneous in the reverse direction.

- Recall that free energy relationships are in no way an indication of kinetic stability or instability. Spontaneous reactions do not necessarily have to occur quickly.

Binding equilibria and free energy

Biologically relevant interactions generally have ΔG values in the range of -5 to -10 kcal/mol.

Common language	K_d (M)	K_a (M^{-1})	ΔG° (kJ/mol)	ΔG° (kcal/mol)
No affinity (high millimolar)	> 10^{-1}	< 10^{1}	> -5.9	> -1.4
Very weak affinity (low millimolar)	10^{-3} to 10^{-1}	10^{1} to 10^{3}	-18 to –5.9	-4.3 to -1.4
Low affinity (high micromolar)	10^{-5} to 10^{-3}	10^{3} to 10^{5}	-30 to –18	-7.1 to –4.3
Moderate affinity (low micromolar)	10^{-6} to 10^{-5}	10^{5} to 10^{6}	-36 to –30	-8.5 to –7.1
High affinity (nanomolar)	10^{-9} to 10^{-6}	10^{6} to 10^{9}	-53 to -36	-13 to –8.5
Very high affinity (pico/femtomolar)	10^{-14} to 10^{-9}	10^{9} to 10^{14}	-83 to -53	-20 to -13
Effectively irreversible (low femtomolar)	< 10^{-14}	> 10^{14}	< -83	< -20

Biologically relevant interactions use multiple non-covalent interactions to obtain the required affinities.

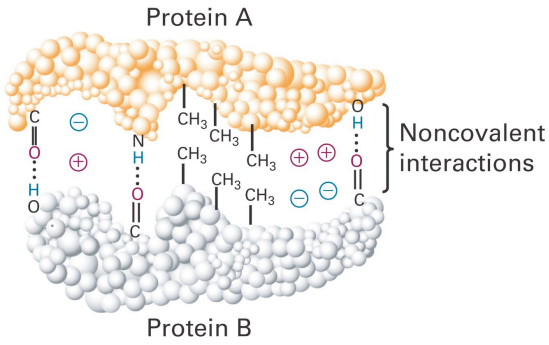
Strength (kcal/mole)*

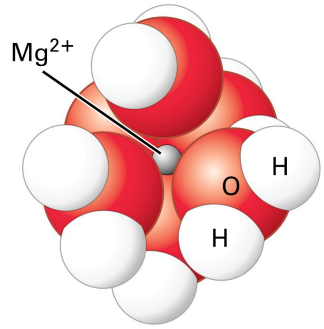
Bond Type	Length (nm)	In Vacuum	In Water
Covalent	0.15	90	90
Ionic	0.25	80	3
Hydrogen	0.30	4	1
van der Waals attraction (per atom)	0.35	0.1	0.1

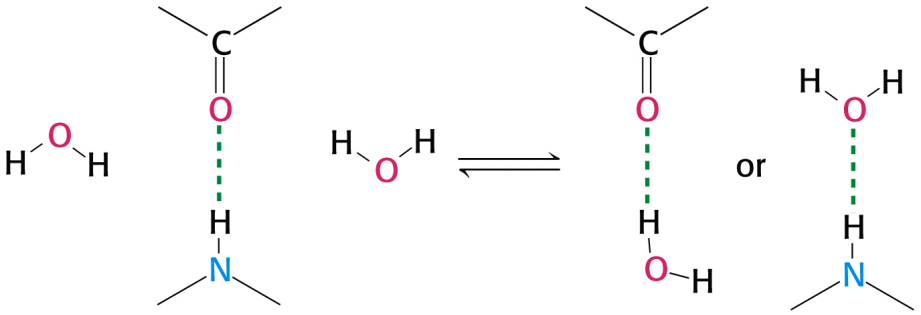
Molecular Biology of the Cell (NCBI bookshelf)

- The strength of a bond can be measured by the energy required to break it, here given in kilocalories per mole (kcal/mole).
- One kilocalorie is the quantity of energy needed to raise the temperature of 1000 g of water by 1°C. An alternative unit in wide use is the kilojoule, kJ, equal to 0.24 kcal.
- Individual bonds vary a great deal in strength, depending on the atoms involved and their precise environment, so that the above values are only a rough guide. Note that the aqueous environment in a cell will greatly weaken both the ionic and the hydrogen bonds between non-water molecules.
- The bond length is the center-to-center distance between the two interacting atoms; the length given here for a hydrogen bond is that between its two nonhydrogen atoms.
- Corollary: It requires at least ~5-10 H-bonds, or ~2-3 ionic bonds, or ~50-100 atoms of shape complementarity (van der Waals) to obtain biologically relevant affinities. Obviously, real interactions use all 3 types of interactions.

The properties of water are the reason why H-bonds and electrostatic interactions are much weaker than in a vacuum







Stryer and Lodish (NCBI bookshelf)

•Weak interactions are the key means by which molecules interact with one another—enzymes with their substrates, hormones with their receptors, antibodies with their antigens. The strength and specificity of weak interactions are highly dependent on the medium in which they take place, and most biological interactions take place in water.
•Water is an excellent solvent for polar molecules. Water greatly weakens electrostatic forces and hydrogen bonding between polar molecules by competing for their attractions.
•The dielectric constant of water is 80, so water diminishes the strength of electrostatic attractions by a factor of 80 compared with the strength of those same interactions in a vacuum. The dielectric constant of water is unusually high because of its polarity and capacity to form oriented solvent shells around ions. These oriented solvent shells produce electric fields of their own, which oppose the fields produced by the ions. Consequently, the presence of water markedly weakens electrostatic interactions between ions.
•Consider the effect of water on hydrogen bonding between a carbonyl group and the NH group of an amide. A hydrogen atom of water can replace the amide hydrogen atom as a hydrogen-bond donor, whereas the oxygen atom of water can replace the carbonyl oxygen atom as a hydrogen-bond acceptor. Hence, a strong hydrogen bond between a CO group and an NH group forms only if water is excluded.

Binding equilibrium

$$K_d = \frac{[P][L]}{[PL]} \quad \text{and} \quad [P] = [P]_o - [PL]$$

Solving for [PL]:

$$K_d = \frac{([P]_o - [PL])[L]}{[PL]} = \frac{[P]_o[L]}{[PL]} - [L]$$

$$\boxed{[PL] = \frac{[P]_o[L]}{K_d + [L]}}$$

As with protein (P): $[L] = [L]_o - [PL]$

But if $[L]_o >> [PL]$ (as is almost always the case) then:

$$[L] = [L]_o$$

The concentrations of bound protein is then given by:

$$\boxed{[PL] = \frac{[P]_o[L]_o}{K_d + [L]_o}}$$

- Draw a to scale graph of [PL] vs [L]o
- Consider [L]o = 0
- Consider [L]o = Kd
- Consider [L]o >> Kd

Determining K_d: Direct plot

It is common to rearrange this equation to give the fraction bound (ν):

$$\nu = \frac{[PL]}{[P]_o} = \frac{[L]_o}{K_d + [L]_o} \quad \text{(Langmuir isotherm)}$$

If the protein has n independent binding sites with the same affinity for the ligand then the average number of L bound per protein P becomes:

$$\boxed{\nu = \frac{n[L]_o}{K_d + [L]_o}}$$

When this sort of data is generated experimentally, computer software with curve fitting functions would be used to fit the data to the equation.

- As with [PL] vs. [L], a plot of v vs. [L] is hyperbolic

Determining K_d: Recipricol plot

Before everybody had a computer on their desk, graphical approaches were used to extract n and K_d from binding data.

$$\frac{1}{\nu} = \frac{K_d}{n[L]} + \frac{1}{n}$$

"Benesi-Hildebrand Plot"

- This is an equation of the form $y = mx + b$
  - m = slope = K_d/n
  - b = Y-intercept (x=0) = 1/n
  - -b/m = X-intercept (y=0) = $-1/K_d$

•Other types of plot include:
• v vs. log[L] (Bjerrum plot)
• v/[L] vs. v (Scatchard plot)

Association and dissociation kinetics

An intermolecular association can be described with two rate constants:

$$P + L \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} PL$$

$$v_{forward} = k_{on}[P][L]$$

units of $v_{forward}$ and $v_{reverse}$ are Ms^{-1}
units of k_{on} are $M^{-1}s^{-1}$

$$v_{reverse} = k_{off}[PL]$$

units of k_{off} are s^{-1}

At equilibrium $v_{forward} = v_{reverse}$
and so:

$$k_{off}[PL] = k_{on}[P][L]$$

$$\boxed{\frac{[PL]}{[P][L]} = K_{eq} = K_a = \frac{1}{K_d} = \frac{k_{on}}{k_{off}}}$$

The ratio of the on rate (k_{on}) to the off rate (k_{off}) is equal to the equilibrium constant (K_{eq}) for the association reaction.

•For tight binding interactions, Keq >> 1, Kd << 1, and kon is very large (in the order of $10^{8\text{-}9}$ $M^{-1}s^{-1}$) and koff must be very small (10^{-2} - 10^{-4} s^{-1}).To get a more intuitive understanding of Kd's, it is often easier to think about the rate constants which contribute to binding and dissociation. It can be shown mathematically that the rate at which two simple object associate depends on their radius and effective molecular weight. The maximal rate at which they will associate is the maximal rate at which diffusion will lead them together. Let us assume that the rate at which P and L associate is diffusion limited. The theoretical kon is about 10^8 M-1s-1. Therefore, if we know the Kd we can estimate the first order rate constant for dissociation, koff.
•We can also determine koff experimentally. Imagine the following example. Adjust the concentrations of P and L such that Po << Lo and Lo>> Kd. Under these conditions of ligand excess, P is entirely in the bound from, PL. Now at t = 0, dilute the solution so that Lo << Kd. The only process that will occur here is dissociation, since negligible association can occur given the new condition. If you can measure the biological activity of PL, then you could measure the rate of disappearance of PL with time, and get koff. Alternatively, if you could measure the biological activity of P, the rate at which activity returns will give you koff. For a first order rate constant, the half-life of the reaction can be calculated by the expression: $k = 0.693/t_{1/2}$.