L. LIGAND BINDING

Equilibrium binding of Receptors to Ligands

The interaction of myoglobin with oxygen is one example of a common and important phenomenon in biochemistry – the specific recognition of one molecule by another. It is key that the insulin receptor only recognize its one select "ligand", insulin, out of all the molecules in the cell. Metabolic enzymes must recognize a single (or very few) molecules upon which to exert catalysis. Antibodies must selectively bind to only those molecules that come from outside the body – it they attack the body itself, havoc ensues. To each molecule a task, and to that task must the molecule be true.

The Dissociation Constant

Most of these interactions are equilibrium phenomena. A receptor (typically, but not always, a protein) reversibly binds a ligand, which is commonly, but not always, a smaller molecule. In fact some receptor-ligand interactions are between two quite sizable proteins. The equilibrium is typically described as a dissociation reaction:

$$R \bullet L \leftrightarrows R + L$$

Where R•L is the receptor-ligand complex (the two objects non-covalently associated with each other), and R and L are the free receptor and ligand. Accordingly, the following equilibrium constant holds.

$$K_d = \frac{[R][L]}{[R \bullet L]}$$

Where K_d is the so-called "dissociation constant", reflecting the degree of dissociation of the receptor and ligand from each other.

The numerical values of dissociation constants are typically given in units of concentration (though Dan Gerrity will tell you, quite rightly, that equilibrium constants are unitless). The reason for this convenience will appear shortly, but for now it's worth noting that in biology, the value of K_d can vary dramatically from one receptor ligand pairing to another. Typical values range from 10^{-15} M to 10^{-6} M, which reflect free energies of dissociation (ΔG_{dissoc}) from +21 kcal/mol (positive and unfavorable) to +8 kcal/mol (still unfavorable, but not as much so). However, binding can be as tight as to give zeptomolar K_d values (10^{-21} M) and weak enough to be in the millimolar range (10^{-3} M).

Determining the Dissociation Constant

The most common means of determining the K_d for a given R•L complex is to vary the concentration of ligand in a solution containing a fixed, low concentration of the receptor.[1] At each concentration of ligand, the fraction of bound receptor is measured. That fraction (Y) is represented as:

$$Y = \frac{[R \bullet L]}{[R]_{total}}$$

Where $[R]_{total}$ is the combined quantity of free *and* bound receptor in the assay. The following derivation allows Y to be related to the concentration of ligand in solution.

$$Y = \frac{[R \bullet L]}{[R]_{total}} = \frac{[R \bullet L]}{[R] + [R \bullet L]} = \frac{[R][L]/K_d}{[R] + [R][L]/K_d}$$

$$Y = \frac{[L]}{K_d + [L]}$$

I leave the last bit of algebra there for your amusement. The equation that results is a relationship that causes Y to span values from 0 to 1 (fully unbound to completely bound), but a fraction of 1 is only approached at very, very high concentrations of ligand (Figure O.1). Of particular interest is that:

When $[L] = K_d$

$$Y = \frac{[L]}{K_d + [L]} = \frac{K_d}{K_d + K_d} = 0.5$$

This means that K_d can be interpreted as *the ligand concentration that leads to 50% occupancy of the receptor's binding site*. The lower the value of K_d, the less ligand required to achieve 50% occupancy, indicating a higher affinity between receptor and ligand (which of course is implied by the nature of the equilibrium constant, but it never hurts to repeat it).

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[1] The low concentration of receptor is key to a simplifying assumption that the concentration of free ligand is equal to the total concentration of added ligand. That is, the amount of ligand actually bound to the receptor should be negligible.

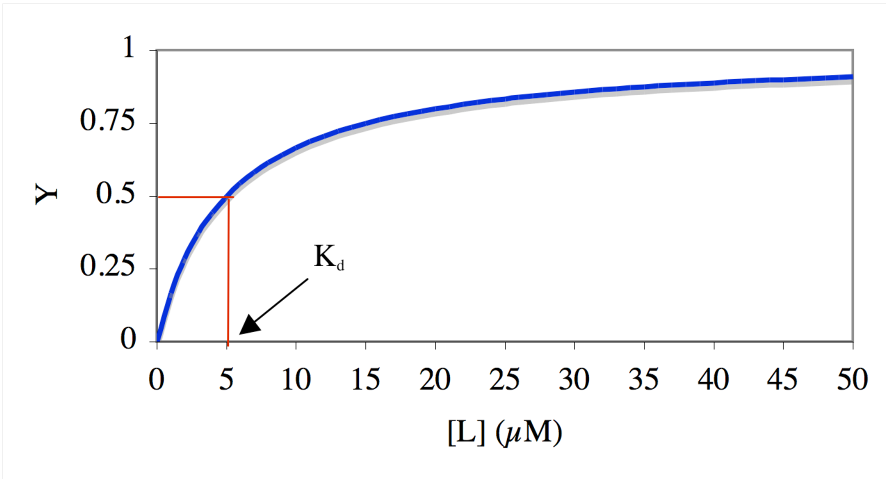

Figure L.1. Plot of fraction bound receptor (Y) vs. the concentration of ligand. Note that the K_d can be identified from this plot as the concentration of ligand that yields 50% bound receptor. Note also that even at ligand concentrations 10-fold higher than the K_d, only 90% of the receptor is bound.