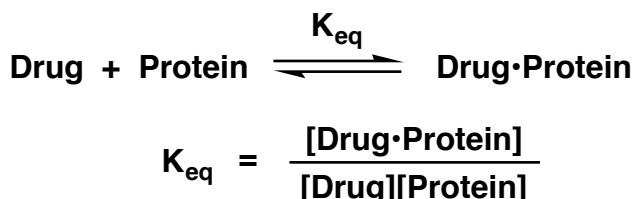


Drug-Target Binding

Equilibrium Binding and the Role of “Weak” Binding Forces in Drug Action

Drug-Target Binding: The Equilibrium Constant

The association of a drug with its target protein is the event that leads to medicinal activity. Therefore, the amount of drug•target(protein) complex formed by a drug ultimately determines the medicinal effects displayed by that compound. This is described by the equilibrium binding expression shown below.



Equilibrium Association Constant

Terminology: the value K_{eq} is the same as K_{assoc} (association constant) and K_B (binding constant). That is, $K_{eq} = K_a = K_B$

When $K_{eq} = 1$ we have a 50:50 mixture of $[\text{Drug}\cdot\text{Protein}]$ and $[\text{Drug}] + [\text{Protein}]$.

When $K_{eq} > 1$ we have greater amounts of $[\text{Drug}\cdot\text{Protein}]$.

When $K_{eq} < 1$ we have greater amount of “free” $[\text{Drug}]$ and $[\text{Protein}]$.

So, when considering K_{eq} for drug-target binding, *larger* numbers mean better drug binding. Typically, useful drugs will have a K_{eq} of $\geq 1 \times 10^6 \text{ M}^{-1}$ for association with their biological target.

Importantly, LeChatlier’s Principle tells us that adding more **Drug** to the system shown above will drive the equilibrium to the right – yielding greater amounts of the **Drug•Protein** complex. This is why increasing the dose of drug can yield a greater medicinal effect (but be careful, because greater doses also may yield greater side effects resulting from “off target” interactions).

Equilibrium Dissociation Constant

The dissociation constant $K_D = 1/K_B$. The K_D is a useful way to present the affinity of a drug for its biological target. This is because the number K_D quickly tells us the concentration of drug that is required to yield a significant amount of interaction with the target protein.

Specifically, when drug concentration equals K_D , the 50% of the target protein will exist in the drug-protein complex $[\text{Drug}\cdot\text{Protein}]$ and 50% of the protein will remain in the free form $[\text{Protein}]$. (This holds true under conditions where drug is present in excess relative to protein). Typically, useful drugs must display a $K_D \leq 1 \times 10^{-6} \text{ M}$ for the interaction with their biological target.

Based upon the discussion above, it is clear that, for a drug with a $K_D = 1 \times 10^{-6} \text{ M}$ against its target, it will be necessary to employ a dose that achieves micromolar concentrations of the drug in the human body. As we will see later in the course, such concentrations may be achieved with drug doses on the order of 200 mg. Many modern drugs display values for $K_D \leq 1 \times 10^{-9} \text{ M}$, meaning that mere nanomolar, or even picomolar, *in vivo* drug concentrations are required to elicit a medicinal effect. When considering the K_D for drugs, *smaller* numbers mean better binding.

On and Off Rates Are Typically Fast

Equilibrium constants are composed of two separate rate constants. $K_{eq} = k_{on}/k_{off}$ and $K_D = k_{off}/k_{on}$. In these expressions, k_{off} and k_{on} are *rate* constants, not *equilibrium* constants. Typically, both of these rate constants are large (e.g. in the range $\geq 1 \times 10^7 \text{ s}^{-1}$), meaning that association and dissociation are very fast on a laboratory timescale. Equilibrium for the binding of small molecules to biological macromolecules are usually established within microseconds of mixing the two components. On a practical level, this means that you don't have to *wait* for drug-target binding to occur. Usually, when a drug is mixed with a its protein target inhibition occurs immediately, with the extent of drug-protein association defined by K_{eq} .

The Relationship Between K_{eq} and ΔG

The size of an equilibrium association constant is determined by the energetics of the interaction between the two partners. Is there an energetic “payoff” when the two partners interact – or are they energetically “happier” just swimming around by themselves in solution? If there is an energetic payoff they bind to each other and the size of the binding constant is determined by the size of that energetic payoff. The association of drugs with their macromolecular targets in the cell depends on the formation of energetically favorable weak bonding interactions between the two partners. The equation that relates the free energy of binding (ΔG) to K_{eq} is shown below:

$$\Delta G = -RT \ln K_{eq}$$

The free energy of binding (ΔG), in turn, can be broken down into component parts, as shown in the equation below:

$$\Delta G = \Delta H - T\Delta S$$

In this equation:

ΔH is the *enthalpy of binding*. In a simple view, enthalpy of binding reflects energetic gains of bond-making, the costs of bond-breaking, bond-angle-strain, and steric effects (steric effects are molecular “crowding and bumping” in the drug•protein complex).

ΔS is the *entropy of binding*. Entropy is the measure of disorder in the system. More disorder (greater entropy) is favored. In the simple view of an association process like the one shown at the top of this page, drug-target binding is entropically disfavored because two free molecules always possess greater disorder (greater total amount of rotational and translational freedom, for example) than a single molecule (or a molecular complex like drug•protein). Remember, though, that changes in the order/disorder of *solvent* molecules like water (not shown in the equation at the top of the page) often represent the dominant contribution to the free entropy of binding. Thus, if target-bound water molecules are “released” to the bulk upon drug binding, the free entropy of binding can be favorable! Note that the entropic contribution to ΔG is temperature dependent. Entropic contributions are greater at high temperature (this makes sense: molecules are more disordered at high temperature... spinning faster, flying faster, vibrating harder).

What Is Drug-Target “Binding” on a Molecular Level?

First of all, when we discuss “binding” of drugs to their biological targets, we usually mean *reversible, noncovalent* binding not *irreversible, covalent* bonding. Remember, *covalent* bonding involves the formation of a strong bond between two atoms. For example, a C-C bond is worth about 85 kcal/mol. Bonds of this strength do not spontaneously break with the thermal energies available at room temperature (24 °C) or physiological temperature (37 °C).

In contrast, noncovalent binding of drugs to their macromolecular targets in cells involves the formation of ensembles (sets) of *weak, noncovalent* bonds between complementary functional groups on the surfaces of the drug and the biological target. These bonds are so weak that their formation is reversible; that is, they are constantly forming and breaking at physiological temperatures. What types of weak bonds are we talking about here? Answer: Ionic/electrostatic interactions, Hydrogen bonds, van der Waals forces, and hydrophobic effects. These interactions each are typically worth between 0.5-10 kcal/mol. These weak bonds are highly distance-dependent and direction-dependent. So, the participating functional groups must be close to each other and aligned exactly right to gain an energetic “payoff”. Drugs typically bind in concave “pockets” or “clefts” on the surface of their macromolecular targets. (Often, drugs bind to the same concave pocket where the endogenous ligand for the target protein normally “sits down”.) In order to achieve strong binding, the drug must “fit snugly” into this binding pocket and must position a chemically appropriate array of functional groups in close proximity to the protein functional groups that line the pocket. In order for a drug to bind tightly with its biological target, the two must possess – “*shape and functional group complementarity*”.

Much of the modern drug discovery and drug design effort involves the systematic search for small organic molecules (MW ~ 200) that possess the appropriate *shape and functional group complementarity* required for high affinity binding to an “active site” pocket of a selected target protein.

Calculating How Changes in ΔG affect K_{eq} : Small Changes in ΔG Have Significant Effects on K_{eq}

To calculate the change in K_{eq} due to a change in the free energy of binding:

$$(\Delta G_1 - \Delta G_2) = (-RT \ln K_{eq1}) - (-RT \ln K_{eq2})$$

$$\text{Rearranges to: } \Delta \Delta G = -RT \ln K_1/K_2$$

Where: $\Delta \Delta G$ = the change in free energy of binding
 R = 0.001987 kcal/mol K
 T is in degrees Kelvin
 at 25 °C, the term RT = 0.59 kcal/mol

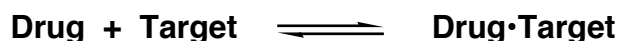
Example: For this example, let us assume that, for drug **A**, $K_{eq} = 1$. Let’s see how the equilibrium binding constant changes at 25 °C if we make an analog (**B**) that retains all the binding abilities of **A** and *adds one new hydrophobic interaction that is worth 2.3 kcal/mol*. (Remember, according to the conventions of physical chemistry, a favorable binding energy is expressed as a *negative* number.)

$$\text{In this case: } \Delta \Delta G = \Delta G_1 - [\Delta G_1 + (-2.3 \text{ kcal/mol})] = 2.3 \text{ kcal/mol} = (-0.59 \text{ kcal/mol}) \ln 1/K_2$$

$$\text{Giving: } -3.898 = \ln 1/K_2 \text{ which, in turn, becomes, } 0.020 = 1/K_2$$

$K_2 = 50$ Conclusion: A small change in binding energy results in a large (50-fold) change in the equilibrium binding constant!

The binding constant can tell us how much drug is bound to target via the relationships shown below.



$$K_{\text{eq}} = \frac{[\text{Drug}\cdot\text{Target}]}{[\text{Drug}][\text{Target}]}$$

Let's consider how changes in binding constant affect the amount of drug that ends up bound to its biological target ($D_{\text{free}}/D_{\text{total}}$).

In the equations below: Total Drug = D_{total} ; Free Drug = D_{free} ; Bound Drug = $D\cdot T$; Target = T

The following is true: $T_{\text{total}} = T_{\text{free}} + D\cdot T$ thus... $D\cdot T = T_{\text{total}} - T_{\text{free}}$

So the equation for K_{eq} above can be rewritten:

$$K_{\text{eq}} = (T_{\text{total}} - T_{\text{free}})/(D_{\text{free}})(T_{\text{free}})$$

Multiply both sides by the denominator ($D_{\text{free}})(T_{\text{free}})$ to get:

$$K_{\text{eq}}(D_{\text{free}})(T_{\text{free}}) = (T_{\text{total}} - T_{\text{free}})$$

Adding T_{free} to each side gives:

$$K_{\text{eq}}(D_{\text{free}})(T_{\text{free}}) + T_{\text{free}} = T_{\text{total}}$$

Dividing each side by T_{free} gives:

$$K_{\text{eq}}(D_{\text{free}}) + 1 = T_{\text{total}}/T_{\text{free}}$$

Invert each side to get the USEFUL EXPRESSION for the ratio of free target to total target in the system:

$$1/(K_{\text{eq}}D_{\text{free}} + 1) = T_{\text{free}}/T_{\text{total}} \quad (\text{Eqn 1})$$

Now we need to estimate values for T (cellular concentration of the biological target) and D_{total} (the total concentration of drug achieved in the cell). A reasonable "generic" estimate for the concentration of a biological target (like a receptor protein) might be about 1×10^{-8} M (10 nM). An estimate for a "reasonably attainable" drug concentration is 1×10^{-6} M (1 μ M). Note: Because drug concentration is in large excess over target concentration, we can estimate that $D_{\text{free}} \sim D_{\text{total}}$ in our equations. So, let's plug-and-chug these numbers through Eqn 1.

Let's use these equations and estimated concentrations to see how much target is bound by drug for a couple of different binding constants. We calculated on the previous page that addition of one interaction worth 2.3 kcal/mol increased the K_{eq} by a factor of 50. This is true no matter what the "starting" K_{eq} . So, in this example, let's say that we have a drug with a (realistic) binding constant of 1×10^6 M and compare this to an analog that "gains" one additional hydrogen bond worth 2.3 kcal/mol which increases the binding constant by 50 times (to 50×10^6 M). We can calculate how changing the binding constant affects the amount of drug bound to its target!

For $K_{\text{eq}} = 1 \times 10^6$ M, $T_{\text{free}}/T_{\text{total}} = 0.5$ (50% of the target is free – not bound by drug)

For $K_{\text{eq}} = 50 \times 10^6$ M, $T_{\text{free}}/T_{\text{total}} = 0.02$ (only 2% of the target is free! Target is mostly bound by drug!!)

Addition of a mere 2.3 kcal/mol of binding energy took us from 50% of target bound to 98% of target bound! That could make the difference between a poor drug and a good drug!!!