

## 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



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  $\Delta G^\circ$  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  $^{125}\text{I}$ ) and added to cells under conditions that prevent the ligand from being internalized ( $4^\circ\text{C}$ ). 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,

$$K_D = \frac{([R]_T - [C]_{eq})[L]_0}{[C]_{eq}}$$

or

$$\frac{[C]_{eq}}{[L]_0} = \frac{[R]_T}{K_D} - \frac{[C]_{eq}}{K_D}$$

The value of  $K_D$  (and thus  $\Delta G^0$ ) can be obtained by measuring the concentration of complexes formed at various initial ligand concentrations  $[L]_0$  (through the radioactive labeling) by plotting  $\frac{[C]_{eq}}{[L]_0}$  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  $[L]_o \ll K_D$  then  $\frac{[C]_{eq}}{[R]_T} \cong \frac{[L]_o}{K_D}$ ,

when  $[L]_o \gg K_D$  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  $K_D(\text{pH}5.5) > K_D(\text{pH}7.4)$ .

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

	$K_D(\text{pH } 7.4), \text{ pM}$	$K_D(\text{pH } 5.5) / K_D(\text{pH } 7.4)$
WT	$270 \pm 90$	$1.7 \pm 0.5$

Since  $\Delta G^\circ = -RT \ln K_D$ , we can get the difference of  $\Delta G^\circ$ '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:  $0.53 \pm 0.3$  (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:

	$K_D(\text{pH } 7.4), \text{ pM}$	$K_D(\text{pH } 5.5) / K_D(\text{pH } 7.4)$
WT	$270 \pm 90$	$1.7 \pm 0.5$
D110H	$370 \pm 450$	$4.4 \pm 0.8$
D113H	$320 \pm 130$	$6.8 \pm 2.4$

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)