

Lecture 14: Kinetics of Non-Covalent Biomolecular Interactions

This lecture covers : Significance, typical values and diffusion limit, approach to equilibrium, and multivalency

Noncovalent Interactions

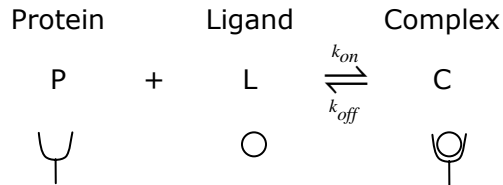


Figure 1. Protein-ligand binding.

Association rate = $k_{on} C_p C_L$

Dissociation rate = $k_{off} C_c$

@ equilibrium, $k_{on} C_p C_L = k_{off} C_c$

$$\frac{C_p C_L}{C_c} = \frac{k_{off}}{k_{on}} = K_d$$

\swarrow
 $\frac{1}{s}$

\searrow
 $\frac{L}{mol\ s}$

In general, for protein-protein interactions, $k_{on} \approx 10^5\ mol^{-1}s^{-1}$

half-time for complex dissociation $\tau_{1/2} = \frac{\ln 2}{k_{off}}$

$\frac{K_d}{\mu M}$	$\frac{\tau_{1/2}}{ms}$	<u>types</u>
mM	milliseconds	non-specific stickiness
μM (micromolar)	milliseconds-seconds	cell surface, multi valent
nM	minutes-hours	antibodies, enzymes
pM	hours-weeks	growth factors
fM (femtomolar)	weeks-months	hycholase inhibitors

\downarrow stronger interactions

Fractional saturation $Y = \frac{C_c}{C_{p,o}} = \frac{C_c}{C_c + C_p}$

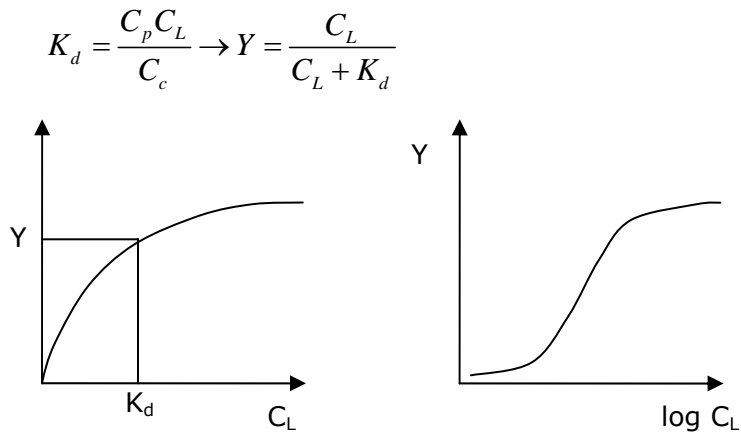


Figure 2. Left: Graph of fractional saturation versus ligand concentration. Right: Graph of fractional saturation versus the logarithm of ligand concentration.

If $C_{p,o} \approx C_{L,o}$, then at equilibrium, $C_L \neq C_{L,o}$

$$Y = \frac{C_{L,o} - yC_{p,o}}{C_{L,o} - yC_{p,o} + K_d}$$

$$Y = \frac{K_d + C_{L,o} + C_{p,o} - \sqrt{(K_d + C_{L,o} + C_{p,o})^2 - 4C_{p,o} C_{L,o}}}{2C_{p,o}}$$

If instead $C_{L,o} \gg C_{p,o}$, $C_L \approx C_{L,o}$

$$Y = \frac{C_{L,o}}{C_{L,o} + K_d}$$

How quickly is equilibrium reached?

$$\frac{dC_c}{dt} = k_{on} C_L C_p - k_{off} C_c$$

If $C_{L,o} \gg C_{p,o}$ "pseudo-1st order"

$$k_{on} C_L = k_{on} C_{L,o}$$

$$C_{p,o} = C_p + C_c$$

↙ (complexed)

$$C_p = C_{p,o} - C_c$$

$$\begin{aligned} \frac{dC_c}{dt} &= k_{on} C_{L,o} C_p - k_{off} C_c = k_{on} C_{L,o} (C_{p,o} - C_c) - k_{off} C_c \\ &= k_{on} C_{p,o} C_{L,o} - (k_{on} C_{L,o} + k_{off}) C_c \end{aligned}$$

$$\Rightarrow C_c(t) = C_{p,o} \frac{C_{L,o}}{C_{L,o} + K_d} (1 - e^{-k_{obs}t})$$

$$k_{obs} = k_{on} C_{L,o} + k_{off}$$

$$\frac{\ln 2}{k_{obs}} = \text{half-time for reaching equilibrium}$$

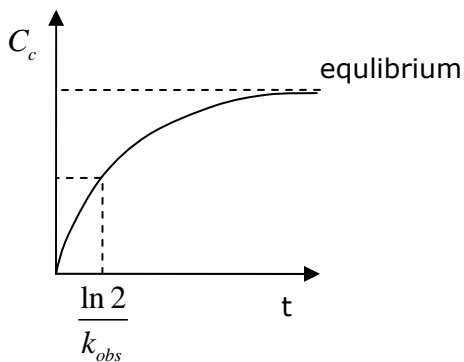


Figure 3. Concentration of complex versus time. Equilibrium is approached at long times.

Biosensor

Surface plasmon resonance (label-free)

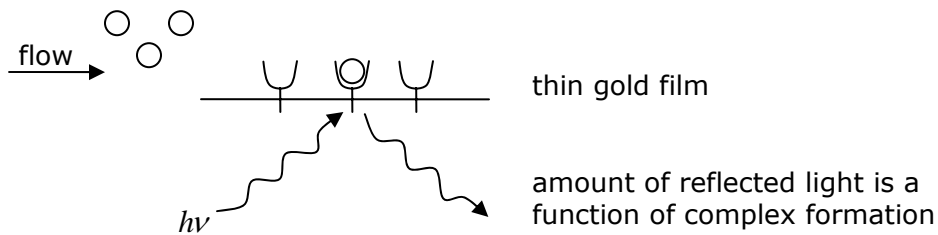


Figure 4. Schematic of how surface plasmon resonance works.

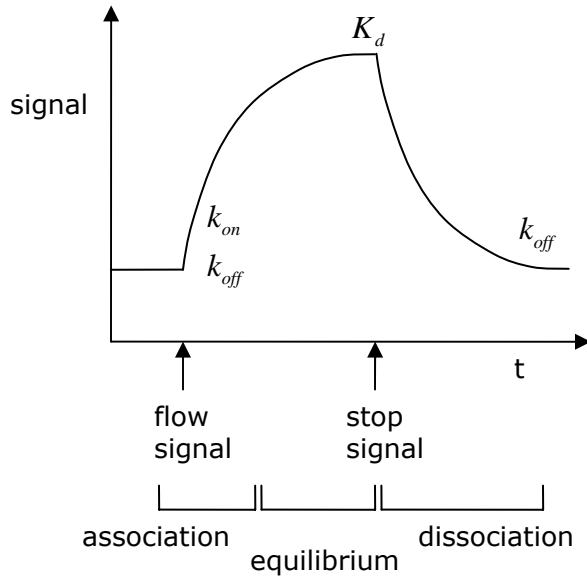


Figure 5. Signal of detector versus time.

redundant estimates: k_{off} in both association & dissociation, $K_d = \frac{k_{off}}{k_{on}}$ in equilibrium phase

best approach: fit one set of parameters to three phases of experiment. (global least squares)

Multivalency (Avidity)

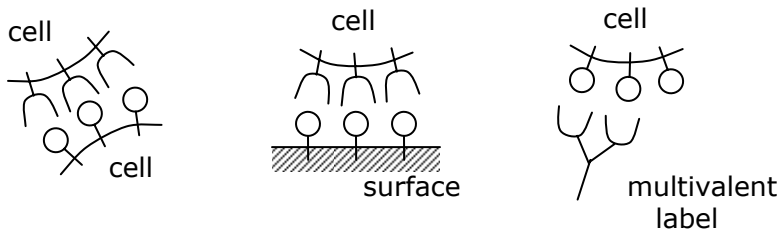


Figure 6. Three examples of multiple protein-ligand binding.

How does multivalency effect apparent interaction strength?

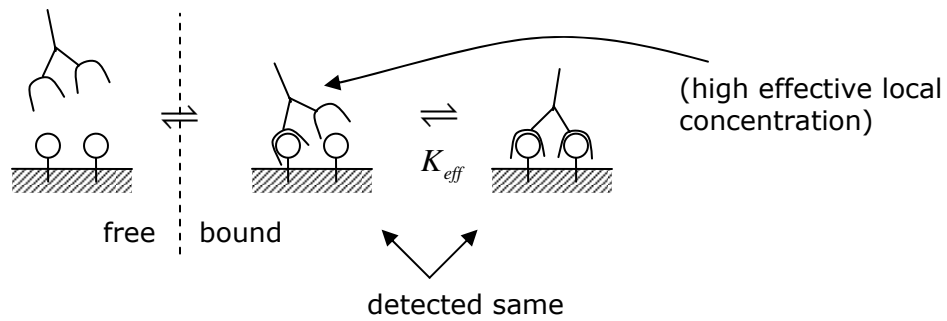


Figure 7. Multivalent binding equilibrium.