Cooperativity and Allostery

Special vignette done for Hernan Garcia class.

Two topics:
1) Cooperative Binding
2) special category of molecules exhibiting cooperativity - allosteric molecules
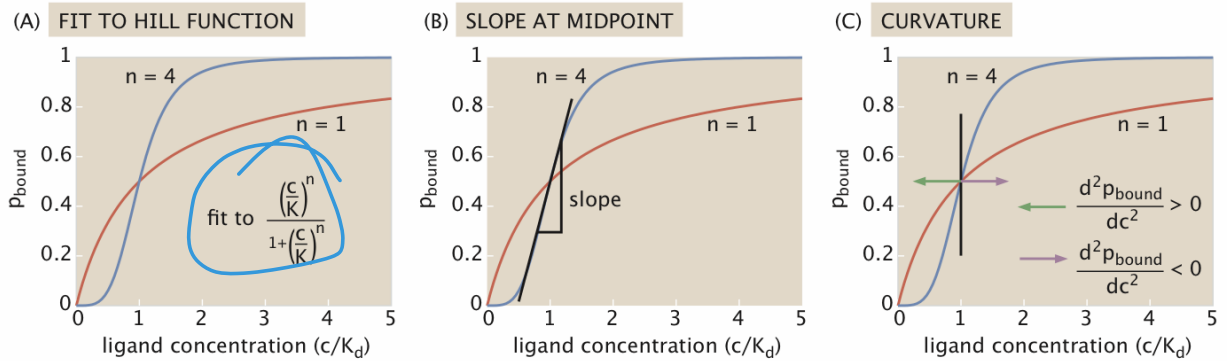
Generic ideas on cooperativity

(A) FIT TO HILL FUNCTION

fit to $\dfrac{\left(\frac{c}{K}\right)^n}{1+\left(\frac{c}{K}\right)^n}$

n = 4

n = 1

p_{bound}

ligand concentration (c/K_d)

(B) SLOPE AT MIDPOINT

n = 4

n = 1

slope

p_{bound}

ligand concentration (c/K_d)

(C) CURVATURE

n = 4

n = 1

$\dfrac{d^2 p_{bound}}{dc^2} > 0$

$\dfrac{d^2 p_{bound}}{dc^2} < 0$

p_{bound}

ligand concentration (c/K_d)

Figure 2.14

Three variations on the theme of cooperativity. (A) Fit to a Hill function. (B) Evaluated from the slope at the midpoint between the minimum and maximum values of the activity curve. (C) Determined from the curvature of the binding (or activity) curve.
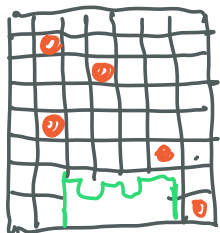
$$P_{bound}([L]) = \frac{\left(\frac{[L]}{K_d}\right)}{1 + \frac{[L]}{K_d}}$$
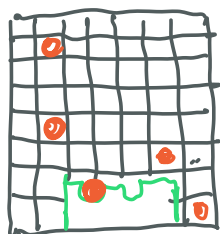
STATES	ENERGY	MULTIPLICITY	STATISTICAL WEIGHT

$$L\epsilon_{sol}$$
$$-\underline{L\epsilon_{sol}}$$
$$0$$

$$\frac{\Omega^{L}}{L!} \times \frac{L!}{\Omega^{L}} = 1$$
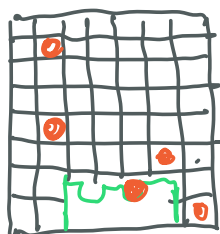
$$\mathbb{1}$$

$$(L-1)\epsilon_{sol} + \epsilon_b$$
$$-\underline{L\epsilon_{sol}}$$
$$\epsilon_b - \epsilon_{sol}$$

$$\frac{\Omega^{L-1}}{(L-1)!} \times \frac{L!}{\Omega^{L}} = \frac{L}{\Omega}$$

$$\frac{L}{\Omega} e^{-\frac{(\epsilon_b - \epsilon_{sol})}{k_B T}}$$
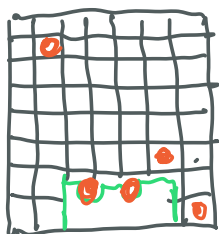$$\beta = \frac{1}{k_B T}$$

$$(L-1)\epsilon_{sol} + \epsilon_b$$
$$\epsilon_b - \epsilon_{sol}$$

$$\frac{\Omega^{L-1}}{(L-1)!} \times \frac{L!}{\Omega^{L}} = \frac{L}{\Omega} \Rightarrow \frac{L}{\Omega} e^{-\beta \Delta\epsilon}$$

$$(L-2)\epsilon_{sol} + 2\epsilon_b + \epsilon_{int}$$
$$\uparrow$$
$$2(\epsilon_b - \epsilon_{sol}) + \epsilon_{int}$$
cooperativity
energy

$$\frac{\Omega^{L-2}}{(L-2)!} \times \frac{L!}{\Omega^{L}} \approx \left(\frac{L}{\Omega}\right)^2$$

$$\left(\frac{L}{\Omega}\right)^2 e^{-2\beta\Delta\epsilon}$$
$$e^{-\beta\epsilon_{int}}$$

$$P_0 = \cfrac{1}{1 + 2\left(\frac{L}{\Omega}\right)e^{-\beta\Delta\epsilon} + \left(\frac{L}{\Omega}\right)^2 e^{-2\beta\Delta\epsilon} e^{-\beta\epsilon_{int}}} \Big\} Z$$

$$P_{1k} = \cfrac{\left(\frac{L}{\Omega}\right)e^{-\beta\Delta\epsilon}}{Z} \qquad P_2 = \cfrac{\left(\frac{L}{\Omega}\right)^2 e^{-2\beta\Delta\epsilon} e^{-\beta\epsilon_{int}}}{1 + 2\left(\frac{L}{\Omega}\right)e^{-\beta\Delta\epsilon} + \left(\frac{L}{\Omega}\right)^2 e^{-2\beta\Delta\epsilon} e^{-\beta\epsilon_{int}}}$$

STATES	ENERGY	WEIGHTS
	0	1
	$\varepsilon_b - \mu$	$e^{-\beta(\varepsilon_b - \mu)}$
	$\varepsilon_b - \mu$	$e^{-\beta(\varepsilon_b - \mu)}$
	$2\varepsilon_b - 2\mu + \varepsilon_{int}$	$e^{-\beta(2\varepsilon_b - 2\mu + \varepsilon_{int})}$

Figure 2.15

States and weights for a nonallosteric two-site receptor. There are a total of four different states, corresponding to the two choices of occupancy for each of the two sites. The doubly occupied state includes an interaction energy ε_{int} to account for cooperativity.

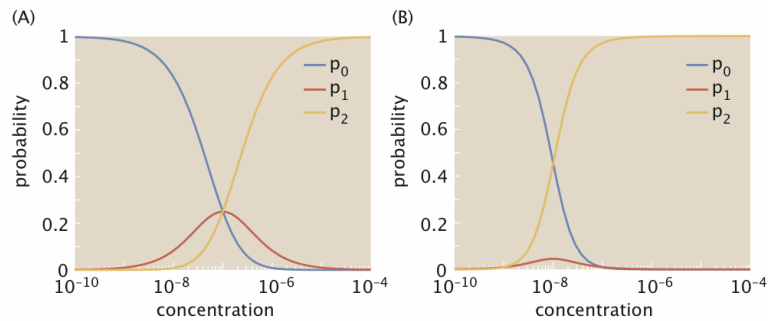*Cooperativity ⟹ suppression of singly occupied state*
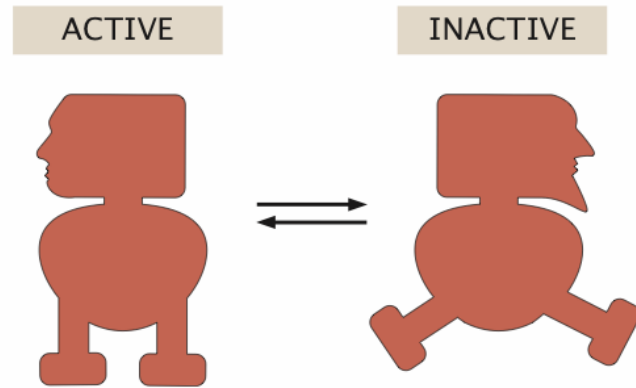

Figure 2.16

Probabilities of the different states of the nonallosteric two-state receptor. (A) Probabilities of the different states of occupancy for the case where there is no cooperativity ($\varepsilon_{int} = 0$, $\omega = 1$) between the binding sites. Note that since there are two states of single occupancy, we have $p_0 + 2p_1 + p_2 = 1$. (B) Probabilities of the different states of occupancy of the receptor for the case where the cooperativity is characterized by $\varepsilon_{int} = -4.6$ $k_B T$ and $\omega \approx 100$. In the presence of cooperativity, the probability of the singly bound intermediate is suppressed, suggesting the approximation of ignoring that state altogether, as in a Hill function.

Allosteric Molecules

(A)

(B)

ACTIVE		INACTIVE

ion channels	enzymes	receptors

INACTIVE

ACTIVE
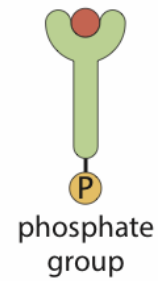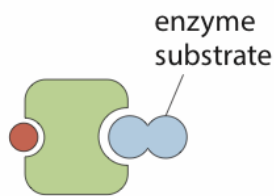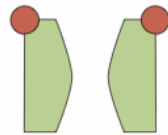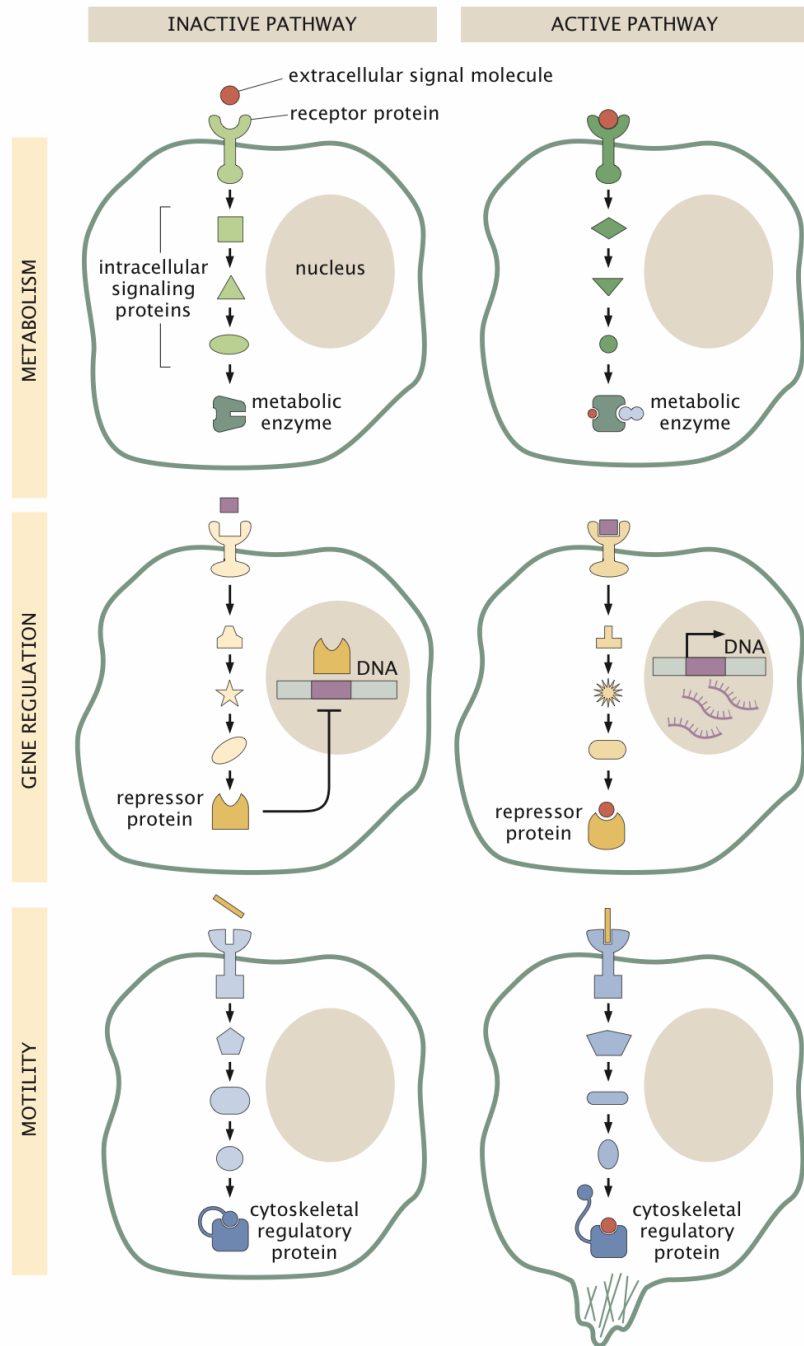

enzyme
substrate

phosphate
group

Figure 1.3

Signaling pathways and allostery. Each panel shows a schematic representation of inactive and active signaling pathways. In each case, an extracellular ligand binds to a receptor resulting in a cascade involving intracellular signaling proteins. These proteins in turn influence a variety of other proteins that can carry out specific biological processes, including activating metabolic enzymes (top panel), turning on the transcription of key genes (middle panel), or turning on cytoskeletal polymerization in particular regions within the cell (bottom panel).

INACTIVE PATHWAY

ACTIVE PATHWAY

METABOLISM

GENE REGULATION

MOTILITY

extracellular signal molecule

receptor protein

intracellular signaling proteins

nucleus

metabolic enzyme

metabolic enzyme

DNA

repressor protein

DNA

repressor protein

cytoskeletal regulatory protein

cytoskeletal regulatory protein

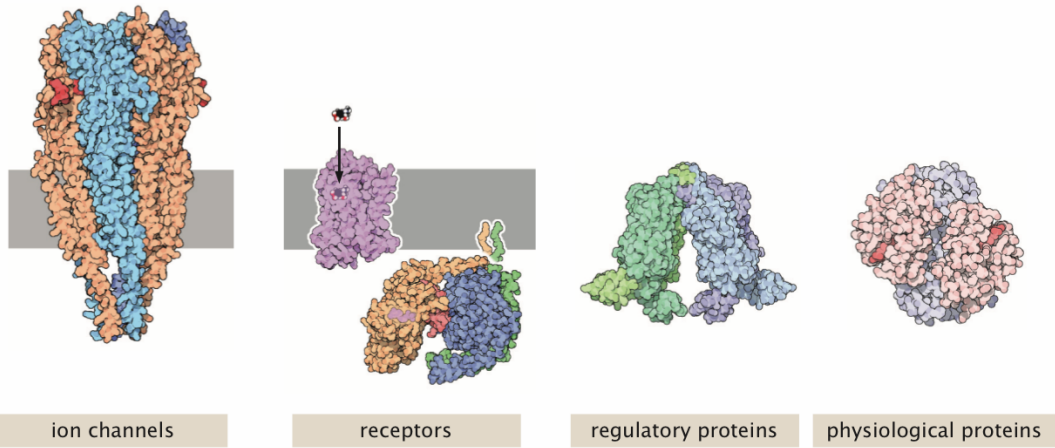| ion channels | receptors | regulatory proteins | physiological proteins |

Figure 1.18

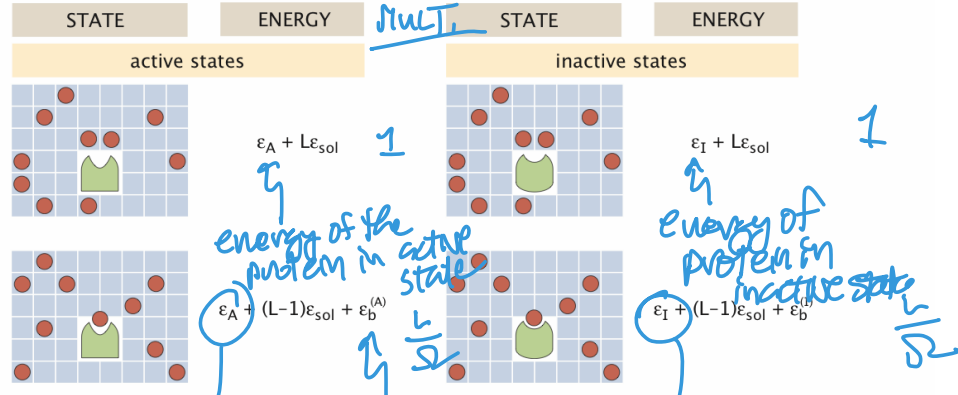Structures of MWC molecules. Many different macromolecules can exist in distinct conformational states including ion channels, such as the nicotinic acetylcholine receptor shown here, G protein–coupled receptors such as the adrenergic receptor shown here, transcription factors such as the Lac repressor shown here, and proteins relevant to human physiology such as hemoglobin. Illustrations courtesy of David Goodsell.

Figure 2.10

States and energies of the simplest MWC molecule. The left column shows the active states of the MWC molecule, and the right column shows the inactive states. Here we account for the energies of the ligand in solution and acknowledge the difference in binding energy of a ligand depending upon whether it is in the active ($\varepsilon_b^{(A)}$) or the inactive ($\varepsilon_b^{(I)}$) state.

STATE	ENERGY		STATE	ENERGY
active states			inactive states	
	$\varepsilon_A + L\varepsilon_{sol}$			$\varepsilon_I + L\varepsilon_{sol}$
	$\varepsilon_A + (L-1)\varepsilon_{sol} + \varepsilon_b^{(A)}$			$\varepsilon_I + (L-1)\varepsilon_{sol} + \varepsilon_b^{(I)}$

(handwritten annotations:) MULTI. 1, 1 ; energy of the protein in active state ; energy of protein in inactive state ; $\frac{L}{\Omega}$; $\frac{L}{\Omega}$; E_{active} ; binding energy of ligand in active state ; $E_{inactive}$

$$\Delta\varepsilon_A = \varepsilon_b^{(A)} - \varepsilon_{sol}$$
$$\Delta\varepsilon_I = \varepsilon_b^{(I)} - \varepsilon_{sol}$$

$$P_{active}([L]) = \frac{\left(1 + \frac{L}{\Omega}e^{-\beta\Delta\varepsilon_A}\right)e^{-\beta E_{active}}}{\left(1 + \frac{L}{\Omega}e^{-\beta\Delta\varepsilon_A}\right)e^{-\beta E_{active}} + \left(1 + \frac{L}{\Omega}e^{-\beta\Delta\varepsilon_I}\right)e^{-\beta E_{inactive}}}$$

STATES	ENERGY	WEIGHTS	STATES	ENERGY	WEIGHTS
active states			inactive states		
	ε_A	$e^{-\beta\varepsilon_A}$		ε_I	$e^{-\beta\varepsilon_I}$
	$\varepsilon_A + \varepsilon_b^{(A)} - \mu$	$e^{-\beta\varepsilon_A}e^{-\beta(\varepsilon_b^{(A)}-\mu)}$		$\varepsilon_I + \varepsilon_b^{(I)} - \mu$	$e^{-\beta\varepsilon_I}e^{-\beta(\varepsilon_b^{(I)}-\mu)}$

Figure 2.11

States and weights of the simplest MWC molecule using the chemical potential concept. The left column shows the active states of the MWC molecule, and the right column shows the inactive states of the MWC molecule. Those states with a bound ligand incur a cost μ to take the ligand out of the reservoir that is partially compensated for by the binding energy ε_b.

(A)   energies ($k_B T$ units)   concentration (M)

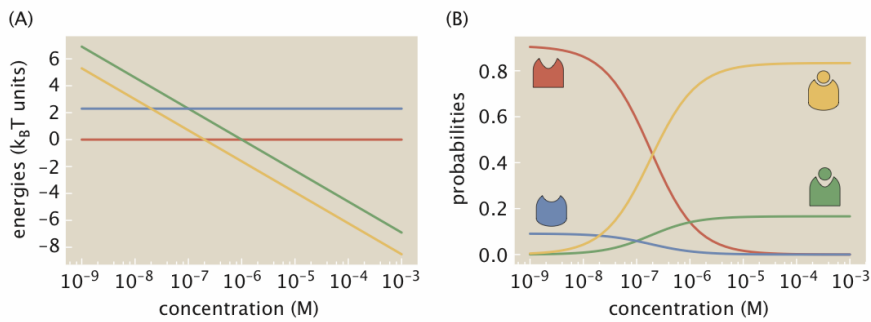(B)   probabilities   concentration (M)

Figure 2.12

Energies and probabilities of different states as a function of concentration of ligand. (A) Free energies of the four states of the simplest MWC molecule as a function of ligand concentration. (B) Probabilities of the four states as a function of ligand concentration. The crossing points in these graphs correspond to those concentrations at which the energies of two states cross. Parameters used in the plots are $\varepsilon_I = 2.3\, k_B T$, $\varepsilon_A = 0$, $K_I = 2 \times 10^{-8}$ M, and $K_A = 10^{-6}$ M.

Figure 2.13
States and weights for the simplest MWC molecule. Comparison of the states and weights in statistical mechanics language and thermodynamic language.
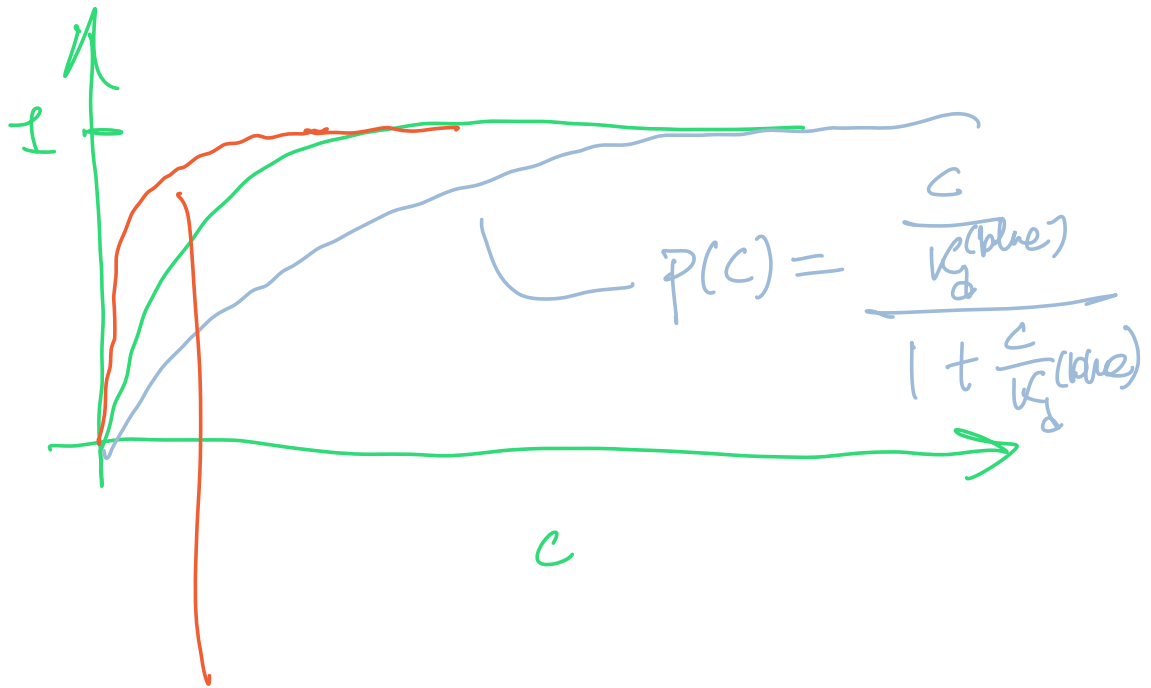
	STATE	ENERGY	BOLTZMANN WEIGHT	THERMODYNAMIC WEIGHT
ACTIVE STATES		ε_A	$e^{-\beta\varepsilon_A}$	$e^{-\beta\varepsilon_A}$
		$\varepsilon_A + \varepsilon_b^{(A)} - \mu$	$e^{-\beta\varepsilon_A}\, e^{-\beta\varepsilon_b^{(A)}}\,\dfrac{c}{c_0}$	$e^{-\beta\varepsilon_A}\,\dfrac{c}{K_A}$
INACTIVE STATES		ε_I	$e^{-\beta\varepsilon_I}$	$e^{-\beta\varepsilon_I}$
		$\varepsilon_I + \varepsilon_b^{(I)} - \mu$	$e^{-\beta\varepsilon_I}\, e^{-\beta\varepsilon_b^{(I)}}\,\dfrac{c}{c_0}$	$e^{-\beta\varepsilon_I}\,\dfrac{c}{K_I}$

$$P_{active}([L]) = \frac{\left(1 + \dfrac{[L]}{K_d^{(A)}}\right)e^{-\beta\varepsilon_{Active}}}{\left(1 + \dfrac{[L]}{K_d^{(A)}}\right)e^{-\beta\varepsilon_{Active}} + \left(1 + \dfrac{[L]}{K_d^{(I)}}\right)e^{-\beta\varepsilon_{Inactive}}}$$

$P_{active} = 1$

leakiness

$\leftarrow$ saturation

$[L]^*$

$[L]$

Data Collapse

$$P(C) = \frac{\frac{C}{K_d}(blue)}{1 + \frac{C}{K_d}(blue)}$$

c

$$P_{bound} = \frac{\frac{C}{K_d}(red)}{1 + \frac{C}{K_d}(red)}$$

$$x \equiv \frac{C}{K_d}$$

$$p(x) = \frac{x}{1+x}$$

c/K_d