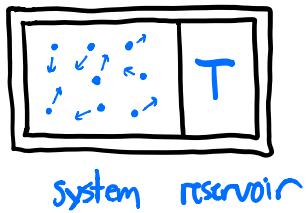


Last time: A crash course in Equilibrium Statmech

① Exchange energy:



Boltzmann Distribution

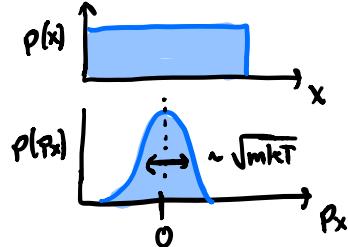
$$p(\vec{s}) = \frac{e^{-\frac{E(\vec{s})}{kT}}}{Z} \quad \begin{array}{l} \text{energy of microstate } \vec{s} \\ \text{temperature of reservoir} \\ \text{normalization const} \end{array}$$

↑
probability of microstate \vec{s}

Ideal Gas :

$$Z(N, V, T) \equiv \frac{1}{N!} \int \prod_{i=1}^N \frac{d\vec{x}_i d\vec{p}_i}{h_0^3} e^{-\frac{|\vec{p}_i|^2}{2m kT}}$$

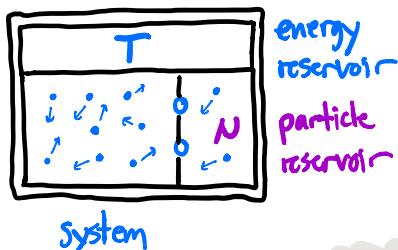
\downarrow



$$\ln Z(N, V, T) \approx N \log \left[\frac{V}{N} e \cdot c_0(m, kT) \right]$$

derived in supplemental note below...

② Exchange particles + energy



$$p(\vec{s}) \propto e^{-\frac{E(\vec{s}) + N(\vec{s})\mu}{kT}} \quad \begin{array}{l} \text{chemical potential} \\ \text{of reservoir} \end{array}$$

$$\frac{\text{Ideal gas}}{\text{gas}}: \quad \frac{\mu}{kT} \equiv -\frac{\partial \ln Z}{\partial N} = \log \left[\frac{c e}{c_0(m, kT)} \right] \quad \begin{array}{l} \text{concentration} \\ c = N/V \end{array}$$

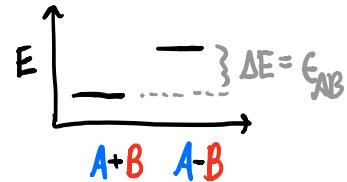
Today: How does this apply to biology?

⇒ will illustrate w/ a few case studies...

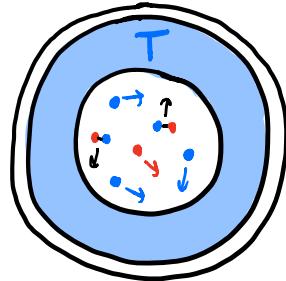
Case Study #1: How do cells build costly molecules?

⇒ suppose cell has precursors $A+B$

+ wants to build $A-B$ complex:



Question: can we get thermal noise to build this for us?



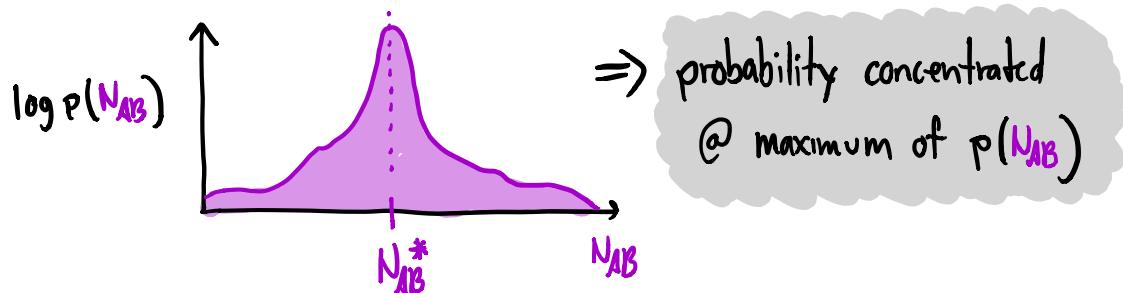
⇒ How many $A-B$ molecules (N_{AB}) @ thermal equilibrium, starting from $N_A = N_B = N_0$ @ $t=0$?

⇒ must have: $N_A = N_0 - N_{AB}$, $N_B = N_0 - N_{AB}$

Probability of N_{AB} macrostate is:

$$p(N_{AB}) \propto \left(\frac{\int d\vec{x} d\vec{p} \dots}{(N_0 - N_{AB})!} \right)^{N_0 - N_{AB}} \left(\frac{\int d\vec{x} d\vec{p} \dots}{(N_0 - N_{AB})!} \right)^{N_0 - N_{AB}} \left(\frac{\int d\vec{x} d\vec{p} \dots}{N_{AB}!} \right)^{N_{AB}} e^{-\frac{\epsilon_{AB} N_{AB}}{kT}}$$

$$\propto \exp \left[\ln Z_A(N_0 - N_{AB}) + \ln Z_B(N_0 - N_{AB}) + \ln Z_{AB}(N_{AB}) - \frac{\epsilon_{AB} N_{AB}}{kT} \right]$$



$$0 = \left. \frac{\partial \log p(N_{AB})}{\partial N_{AB}} \right|_{N_{AB}^*} = \underbrace{\frac{\partial \ln Z_A}{\partial N_A}(-1) + \frac{\partial \ln Z_B}{\partial N_B}(-1) + \frac{\partial \ln Z_{AB}}{\partial N_{AB}}}_{= \frac{\mu_A}{kT}} - \frac{\epsilon_{AB}}{kT}$$

$$= \frac{\mu_A}{kT} = \log \left(\frac{c_A}{c_A^0} \right) \Big|_{N_A = N_0 - N_{AB}^*}$$

$$c_A \equiv \frac{N_A}{V}$$

$$\Rightarrow \log \left(\frac{c_{AB}}{c_{AB}^{\circ}} \frac{c_A^{\circ}}{c_A} \frac{c_B^{\circ}}{c_B} \right) = - \frac{E_{AB}}{kT} \quad @ \text{ equilibrium}$$

\Rightarrow often conventional to define quantity ΔG :

$$\frac{\Delta G}{kT} = \frac{\Delta E + \mu_{AB} - \mu_A - \mu_B}{kT} = \frac{\Delta E}{kT} + \log \left(\frac{c_{AB}}{c_{AB}^{\circ}} \frac{c_A^{\circ}}{c_A} \frac{c_B^{\circ}}{c_B} \right)$$

change in "free energy" of $A+B \rightarrow AB$ reaction

\Rightarrow previous result implies that $\Delta G = 0 @ \text{ equilibrium}$

Payoff: can measure ΔG @ other concentrations,
 (harder to measure ΔE , c_{AB}°, \dots , separately)

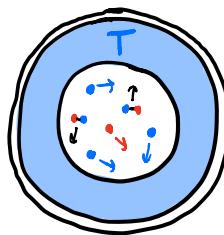
\Rightarrow e.g. when $c_{AB} = c_A = c_B = \frac{1 \text{ mole}}{1 \text{ liter}} = 1 \text{ "Molar"} = 1 \text{ M}$

$\Rightarrow \Delta G \equiv \Delta G_0$ change in free energy
 in "standard conditions"

\Rightarrow if define molar concentrations $[A] \equiv \frac{c_A}{M}$, etc.

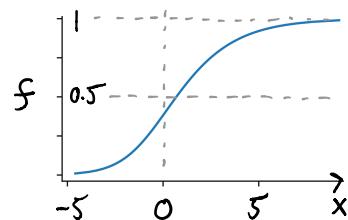
can rewrite our result as :

$$\Rightarrow \log\left(\frac{[AB]}{[A][B]}\right) = -\frac{\Delta G^\circ}{kT}$$



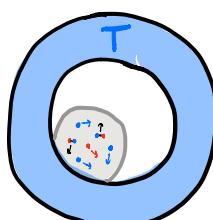
\Rightarrow can also write as function of $f \equiv \frac{N_{AB}}{N_0}$ (fraction molecules converted)

$$\log\left[\frac{f}{(1-f)^2}\right] = \underbrace{-\frac{\epsilon_{AB}}{kT} + \log\left[\frac{\frac{N_0}{V} \cdot c_{AB}^\circ}{c_A^\circ \cdot c_B^\circ}\right]}_{x < 0}$$



\Rightarrow depends on absolute concentration!

(not just relative #s of $A+B$ vs $A-B$)



Prefer to run in smaller volume?

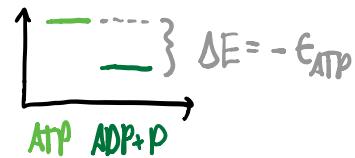
\Rightarrow but when $\Delta E > 0$, hard to beat 50-50...

How do cells get around this limitation?

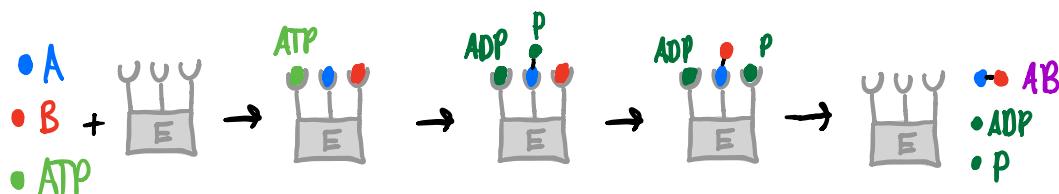
⇒ one method: couple to energy releasing reaction

often w/ ATP = "energy currency of cell"

ATP hydrolysis: $\text{ATP} \rightleftharpoons \text{ADP} + \text{P}$

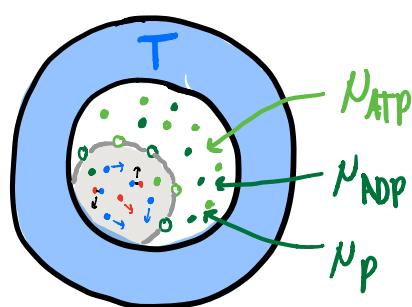


⇒ coupling often implemented using enzyme (E):



⇒ Let's imagine that cell has
big reservoir of $\text{ATP} (+\text{ADP}, \text{P})$

$$\Rightarrow \frac{\mu_{\text{ATP}}}{kT} = \log\left(\frac{c_{\text{ATP}}}{c_{\text{ADP}}}\right), \text{ etc}$$



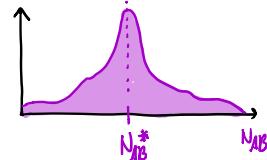
\Rightarrow Probability of N_{AB} macrostate is now:

$$p(N_{AB}) \propto \exp \left[\ln Z_A(N_0 - N_{AB}) + \ln Z_B(N_0 - N_{AB}) + \ln Z_{AB}(N_{AB}) - \frac{\epsilon_{AB} N_{AB}}{kT} \right]$$

+ $\frac{\epsilon_{ATP} N_{AB}}{kT} + \frac{\mu_{ATP} N_{AB}}{kT} - \frac{\mu_{ADP} N_{AB}}{kT} - \frac{\mu_p N_{AB}}{kT}$

new terms
from ATP

\Rightarrow maximum of $\log p(N_{AB})$ now occurs when

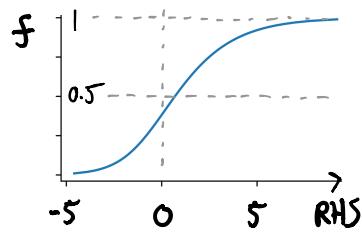


$$\begin{aligned} \frac{\partial \log p(N_{AB})}{\partial N_{AB}} &= \frac{\mu_A}{kT} + \frac{\mu_B}{kT} - \frac{\mu_{AB}}{kT} - \frac{\epsilon_{AB}}{kT} + \frac{\epsilon_{ATP}}{kT} \\ &\quad + \frac{\mu_{ATP}}{kT} - \frac{\mu_{ADP}}{kT} - \frac{\mu_p}{kT} = 0 \end{aligned}$$

\Rightarrow can rearrange to write $f \equiv \frac{N_{AB}}{N_0}$ as :

$$\log \left[\frac{f}{(1-f)^2} \right] = - \frac{\Delta G_o^{AB}}{kT} + \log \left[\frac{N_0}{V} \frac{1}{IM} \right]$$

$$+ \frac{\Delta G_o^{ATP}}{kT} + \log \left(\frac{[ATP]}{[ADP][P]} \right)$$



$\Delta G_o^{ATP} \approx 12kT \Rightarrow$ extra free energy can allow A-B to form!

\Rightarrow but depends on concentrations of ATP, ADP, P!

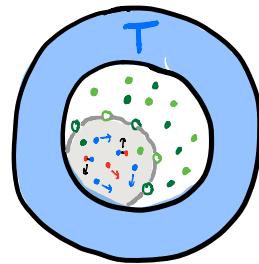
@ cellular conditions: $[ATP] \approx 5 \times 10^{-3}$, $[ADP] \approx 5 \times 10^{-3}$, $[P] \approx 10^{-2}$

$$\Rightarrow \Delta G^{ATP} = \Delta G_o^{ATP} + kT \log \left(\frac{[ATP]}{[ADP][P]} \right) \approx 20kT$$

But : if keep same # of ATP molecules & add more ADP/P

\Rightarrow can drive $\Delta G^{ATP} < \Delta G^{AB} \Rightarrow N_{AB} \ll N_0$

\Rightarrow i.e. can't prevent thermal noise
from running $A+B \rightarrow A-B$ in reverse!



Crucial question: what sets $[ATP]$, $[ADP]$, $[P]$ in cell?

\Rightarrow Another application of Equilibrium statmech?

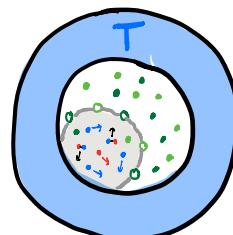
\Rightarrow if whole cell was in equilibrium:

$$\log\left(\frac{[ATP]}{[ADP][P]}\right) = -\frac{\Delta G_{\circ}^{ATP}}{kT} \Rightarrow \Delta G^{ATP} = 0$$

$$\Rightarrow \log\left(\frac{[AB]}{[A][B]}\right) = -\frac{\Delta G_{\circ}^{AB}}{kT}$$

back to
where we
started!

True equilibrium = death!



Some components
must be held
out-of-equilibrium

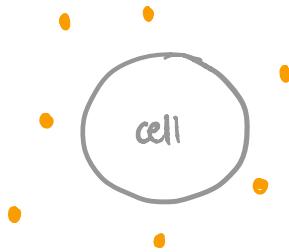
Question:

How to set things up to reach equilibrium
for some components ($A + B + ATP \rightleftharpoons A \cdot B + ADP + P$)
but not for all ($ATP \rightleftharpoons ADP + P$)?

\Rightarrow will see how later in course (dynamics)

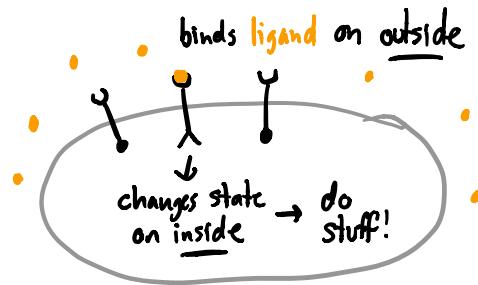
Case Study #2:

How do cells measure the state of the environment?



E.g. want to detect concentration of target chemical ("ligand")

⇒ one common sol'n:
"receptor" proteins
embedded in membrane

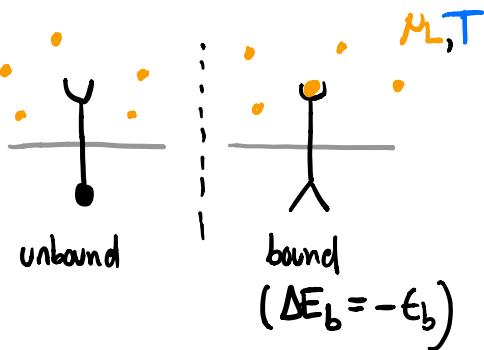


E.g. *atoS* gene (E.coli): binds acetoacetate

⇒ turns on acetoacetate metabolism ⇒ growth!

⇒ can we model this process w/ stat mech?

Focus on single receptor:



Boltzmann distribution:

$$\rho(\ddagger) = \frac{1}{Z} e^{-\frac{(-\epsilon_b)}{kT} + \frac{\mu_L \cdot 1}{kT}} \quad ; \quad \rho(\ddagger) = \frac{1}{Z} e^{-\epsilon_b + 0 \cdot \mu_L}$$

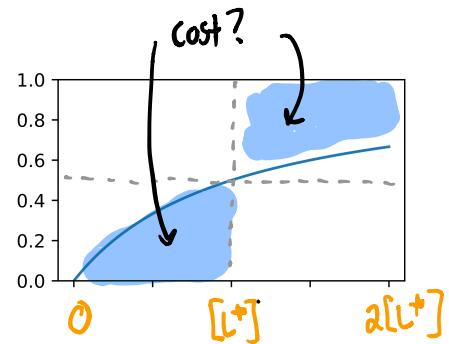
$$\Rightarrow \rho(\ddagger) = \frac{e^{\frac{\epsilon_b}{kT} + \log\left(\frac{c_L}{c_{L^*}}\right)}}{1 + e^{\frac{\epsilon_b}{kT} + \log\left(\frac{c_L}{c_{L^*}}\right)}} = \frac{[L]}{K_m + [L]}$$

where $K_m \equiv \exp\left[-\frac{\epsilon_b}{kT} + \log\left(\frac{c_{L^*}}{[L]}\right)\right]$ ← single free parameter

\Rightarrow If want to detect when $[L]$ reaches $[L^*]$,

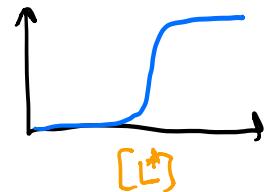
\Rightarrow can pick K_m s.t. $\rho(\ddagger) \approx \frac{1}{2}$

$$\Rightarrow P(\text{on}) = \frac{[L]}{[L^+] + [L]}$$



Problem: fixing set point $[L^*]$ also fixes responsiveness!

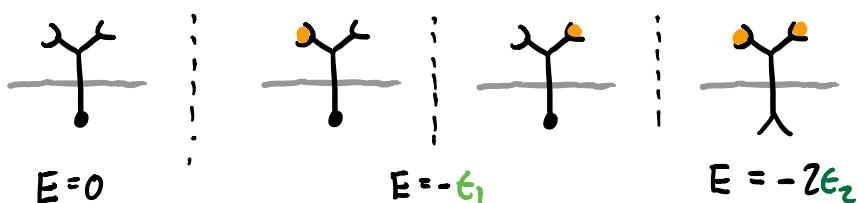
\Rightarrow would love to have something like a switch:



\Rightarrow how can cells implement this behavior using our simple statmech toolbox?

One Mechanism: **cooperativity**

\Rightarrow suppose receptor can bind multiple particles @ once:



Boltzmann factors become:

$$\rho(\text{L}) = \frac{e^{0+0}}{Z} \quad ; \quad \rho(\text{L}^*) = \frac{e^{\frac{E_1}{kT} + \frac{\mu_L \cdot 1}{kT}}}{Z} \quad ; \quad \rho(\text{L}^{\bullet}) = \frac{e^{\frac{2E_2}{kT} + \frac{\mu_L \cdot 2}{kT}}}{Z}$$

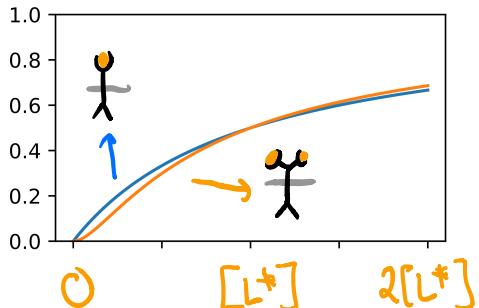
" $\rho(\text{L}^{\bullet})$

$$\Rightarrow \rho(\text{L}^{\bullet}) = \left(\frac{[L]}{k_m} \right)^2 / \left[1 + \left(\frac{[L]}{k_m} \right) e^{\frac{E_1 - E_2}{kT}} + \left(\frac{[L]}{k_m} \right)^2 \right]$$

where $k_m = \exp \left[-\frac{E_2}{kT} + \log \left(\frac{Q^0}{k_m} \right) \right]$

\Rightarrow Behavior depends on E_1 vs E_2 :

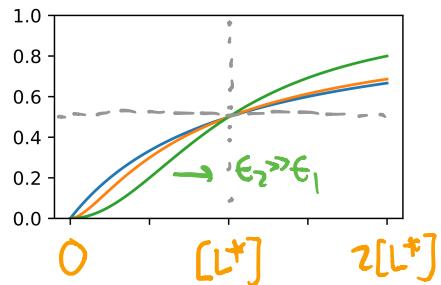
① If $\underbrace{E_1 = E_2}_{\text{"independent binding"}}$ $\Rightarrow \rho(\text{L}^{\bullet}) = \left(\frac{[L]}{k_m + [L]} \right)^2 = \left(\rho(\text{L}^*) \right)^2$



$$= \left[\frac{[L]}{(\sqrt{2}-1)[L^*] + [L]} \right]^2$$

② if $\epsilon_2 \gg \epsilon_1 \Rightarrow \rho(\frac{x}{\lambda}) = \frac{([\text{L}]/[\text{L}^*])^2}{1 + ([\text{L}]/[\text{L}^*])^2}$

\Rightarrow much more sensitive:

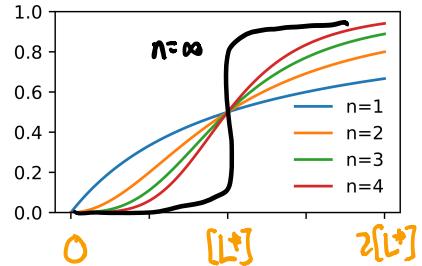


\Rightarrow can extend to more ligands

$$\rho\left(\frac{\text{L}_1 \text{L}_2 \dots \text{L}_n}{\lambda}\right) \approx \frac{([\text{L}]/[\text{L}^*])^n}{1 + ([\text{L}]/[\text{L}^*])^n}$$

when $\epsilon_n \gg \binom{n}{k} \epsilon_k$

"Hill function"

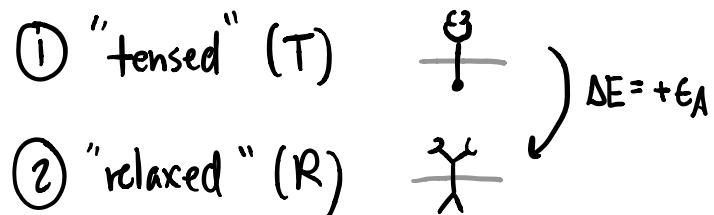


(e.g. hemoglobin binding oxygen, $n=4$)

Problem: how can a receptor distinguish
n-1 vs n bound ligands when $n \gg 1$?

One solution: allostery ("MWC model")

Idea: suppose receptor has 2 conformational states:



\Rightarrow ligands bind independently in both states,
 but w/ different binding energies ($\Delta E = -\epsilon_R, -\epsilon_T$)

$$\Rightarrow \frac{p(+)}{p(+)} = \frac{p(\text{---}) + p(\text{---}) + p(\text{---}) + p(\text{---})}{p(\text{---}) + p(\text{---}) + p(\text{---}) + p(\text{---})}$$

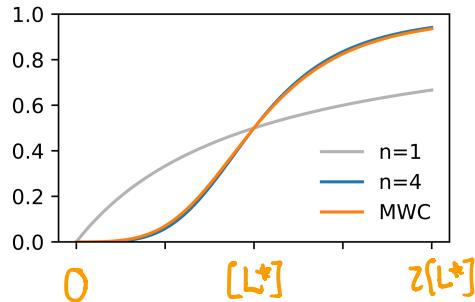
$$= e^{-\frac{\epsilon_A}{kT}} \frac{\left[1 + e^{\frac{-\epsilon_R + N_L}{kT}} \right]^N}{\left[1 + e^{\frac{-\epsilon_T + N_L}{kT}} \right]^N} \quad (\text{since everything is independent!})$$

$$\Rightarrow \frac{P(+)}{P(-)} = e^{-\frac{\epsilon_A}{kT}} \left[\frac{1 + [L]/K_m}{1 + ([L]/K_m) e^{\frac{\epsilon_T - \epsilon_R}{kT}}} \right]^n$$

$$\text{where } K_m = \exp \left[-\frac{\epsilon_R}{kT} + \log \left(\frac{e^{\epsilon_T}}{1 + e^{\epsilon_T}} \right) \right]$$

$$\Rightarrow \text{when } \frac{\epsilon_A}{n} \gg kT \quad \epsilon_R - \epsilon_T - \frac{\epsilon_A}{n} \gg kT$$

\Rightarrow looks like
Hill function:



\Rightarrow achieved w/ very simple implementation (just 2 states)

\Rightarrow for more info (+ more bio examples)

see Chapter 7 of Physical Biology of the Cell

Supplemental Note: Calculating $Z(N,V,T)$ for ideal gas

Recall: ① microstate $\vec{s} = (\vec{x}_1, \vec{p}_1, \dots, \vec{x}_N, \vec{p}_N)$

② energy $E(\vec{s}) = \sum_{i=1}^N \frac{|\vec{p}_i|^2}{2m}$

\Rightarrow From Boltzmann distribution $\left[p(\vec{s}) = \frac{e^{-E(\vec{s})/kT}}{Z} \right]$

$$\begin{aligned} Z(N,V,T) &= \frac{1}{N!} \iint \cdots \int \prod_{i=1}^N \frac{dx_i d\vec{p}_i}{h_0^3} e^{-\sum_{i=1}^N \frac{|\vec{p}_i|^2}{2mkT}} \\ &= \frac{V^N}{N! h_0^{3N}} \left[\left(\int dp e^{-\frac{p^2}{2mkT}} \right)^{3N} \right] \end{aligned}$$

3N P_x, P_y, P_z for each particle

can recognize as Gaussian distribution \sim mean 0, variance mkT

$$\Rightarrow \ln Z(N,V,T) = N \log V + \frac{3N}{2} \log \left(\frac{2\pi mkT}{h_0^2} \right) - \underbrace{\log N!}_{\log N! \approx N \log N - N}$$

$$\log N! \approx N \log N - N$$

$$\Rightarrow \ln Z(N, V, T) = N \log \left[\frac{V}{N} e^{\underbrace{\left(\frac{2\pi m k T}{h_0^2} \right)^{3/2}}_{C_0(m, kT)}} \right] \quad \checkmark$$