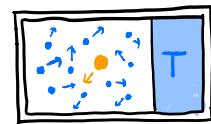


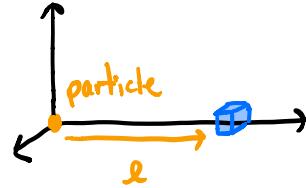
Last time: Applications of Diffusion



①

Diffusion timescale $\tau_d \equiv l^2 / D$

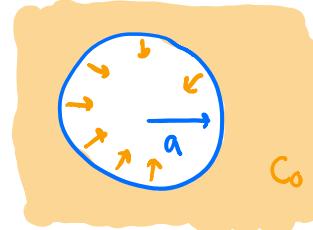
required to travel distance l



②

"Speed limit" $R_{\max} \equiv 4\pi D a c_0$

to capture particles via 3D diffusion



Today: Dynamics in physical space (diffusion)

↳ Dynamics in chemical space ("kinetics")

① Cells are "chemical factories"!

E.g. human brain uses ~ 400 kcal of energy every day

in units
of ATP



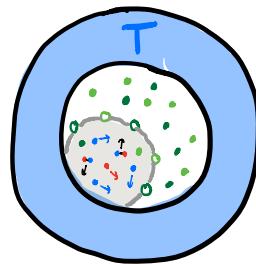
$$\frac{400 \text{ kcal}}{\text{day}} \cdot \frac{1 \text{ mole ATP}}{12 \text{ kcal}} \cdot \frac{500 \text{ g ATP}}{1 \text{ mole ATP}} \approx 17 \text{ kg ATP/day} !$$

(normal brain
weights ~ 1.4 kg)

\Rightarrow brain synthesizes & uses $\sim 10x$ its weight in ATP every day!

② Lingering question from Lecture 3:

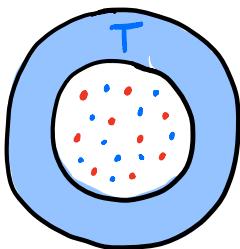
How do cells build costly molecules...



... while preventing others from reaching equilibrium?



Basic Setup:



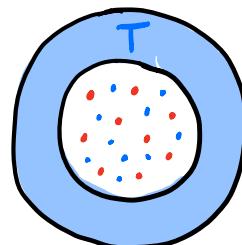
Different kinds of molecules (A, B, ...) diffusing around in cell + undergoing chemical reactions...

- e.g. ① "transformation" / "isomerization" $A \rightleftharpoons B$
② "synthesis" / "polymerization" $A + B \rightleftharpoons AB$
③ etc., etc.: $A + B + ATP \rightleftharpoons AB + ADP + P$

Key variables: $C_{A_i}(\vec{x}, t)$ = local concentration of A;
at position \vec{x} , time t

\Rightarrow after $\tau_D \sim \frac{l^2}{D}$, molecules have diffused across cell

$$\Rightarrow C_{A_i}(\vec{x}, t) \approx C_{A_i}(t)$$



(Recall: small molecules in E. coli, $\tau_D \sim 10^{-2} s$)

⇒ Dynamics can often be modeled w/ "rate equations"

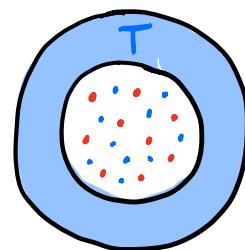
$$\frac{dc_A(t)}{dt} = F_A(c_A(t), c_B(t), \dots)$$

↔ or in molar units: $[A] \equiv \frac{c_A(t)}{1 \text{ mole/L}}$

$$\frac{d[A]}{dt} = G_A([A], [B], \dots) \Leftarrow \text{more conventional}$$

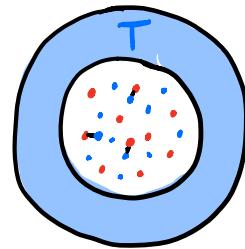


$$\Rightarrow \frac{d[B]}{dt} = +k_{A \rightarrow B}[A] - k_{B \rightarrow A}[B]$$



$$\Rightarrow \frac{d[A]}{dt} = -k_{A \rightarrow B}[A] + k_{B \rightarrow A}[B]$$

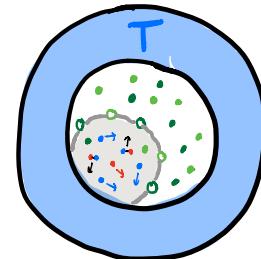
$$\Rightarrow \frac{d}{dt}([A] + [B]) = 0 \Rightarrow [A] + [B] = \text{const.} \checkmark$$



$$\Rightarrow \frac{d[AB]}{dt} = k_{\text{on}} [A][B] - k_{\text{off}} [AB]$$

↓ rate of bumping into each other in space

$$\Rightarrow [A] + [AB] = \text{const} \quad \& \quad [B] + [AB] = \text{const}$$



$$\frac{d[AB]}{dt} = +k_{\text{on}} [A][B][\text{ATP}] - k_{\text{off}} [AB][\text{ADP}][P] = 0$$

\Rightarrow What can we learn from these rate eqns?

$$\Rightarrow @ \text{ equilibrium: } \frac{d[AB]}{dt} = 0$$

$$\Rightarrow \frac{[AB][ADP][P]}{[A][B][ATP]} = \frac{k_{on}}{k_{off}}$$

\Rightarrow Showed in Lecture 3: $\frac{[AB][ADP][P]}{[A][B][ATP]} = e^{\frac{\Delta G_0^{ATP} - \Delta G_0^{AB}}{kT}}$

Change in free energy
in "standard conditions"

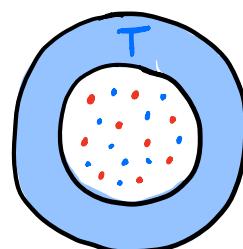
\Rightarrow connection between rate constants and free energy: $k_{on}/k_{off} = e^{\Delta G_0/kT}$

\Rightarrow Rate eqns are consistent w/ equilibrium statmech...
... but also provide info about dynamics!

\Rightarrow Easiest to see w/ $A \rightleftharpoons B$ example:

$$\Rightarrow \frac{d[B]}{dt} = k_{A \rightarrow B}[A] - k_{B \rightarrow A}[B]$$

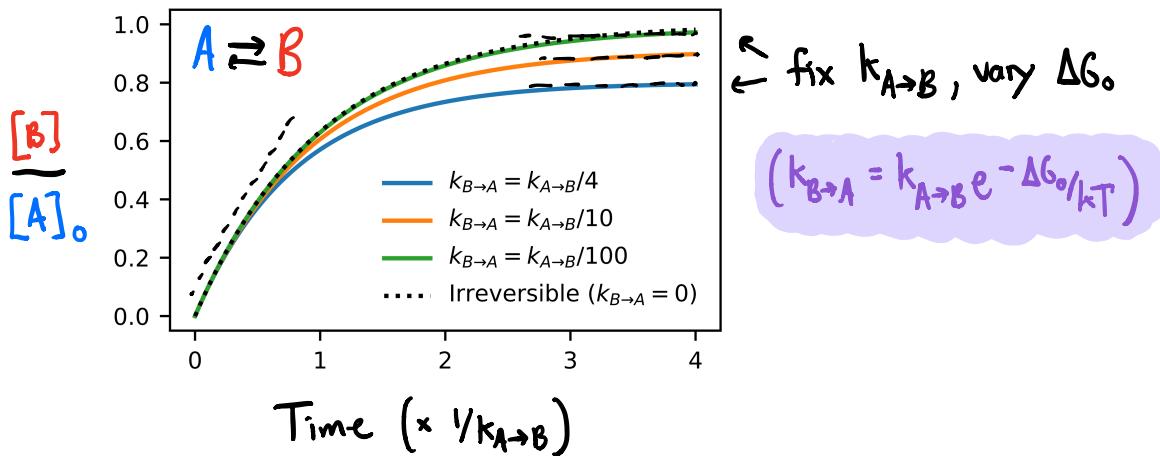
$$+ [A] + [B] = \text{const}$$



\Rightarrow Solution:

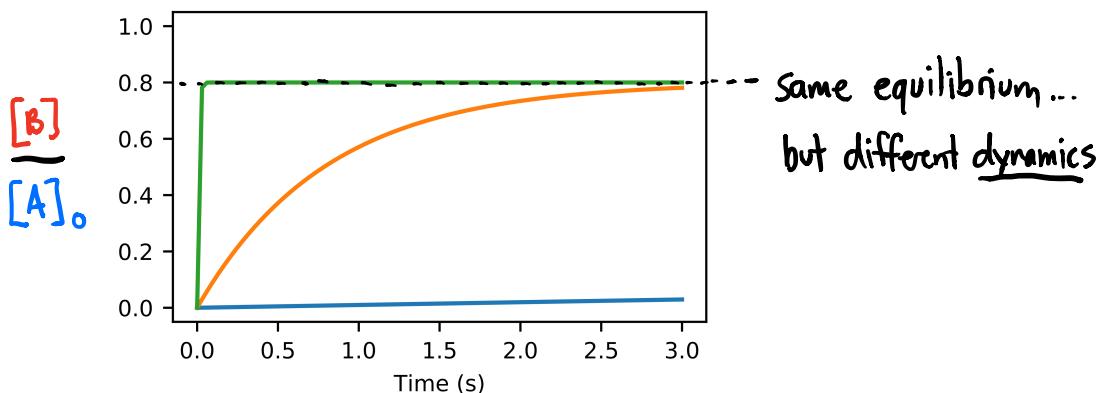
$$[B] = [A]_0 \cdot \frac{k_{A \rightarrow B}}{k_{A \rightarrow B} + k_{B \rightarrow A}} \left[1 - e^{-(k_{A \rightarrow B} + k_{B \rightarrow A})t} \right]$$

(starting w/ $[B] = 0$, $[A] = [A]_0$ @ $t = 0$)



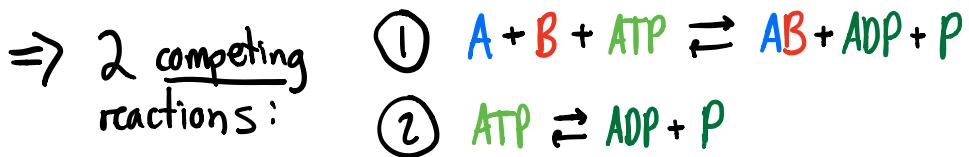
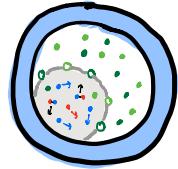
\Rightarrow "Irreversibility" ($A \rightarrow B$) $\approx \Delta G_0 \gg kT$

\Rightarrow Can also fix ΔG_0 , but vary $k_{A \rightarrow B}$:



\Rightarrow if we care about behavior of specific rxn (e.g. $A \rightleftharpoons B$)
rates have huge effect on what other rxns "look like"

Example: building costly molecules w/ ATP



$$\Rightarrow \frac{d[AB]}{dt} = +k_{\text{on}}^{AB}[A][B][\text{ATP}] - k_{\text{off}}^{AB}[AB][\text{ADP}][P]$$

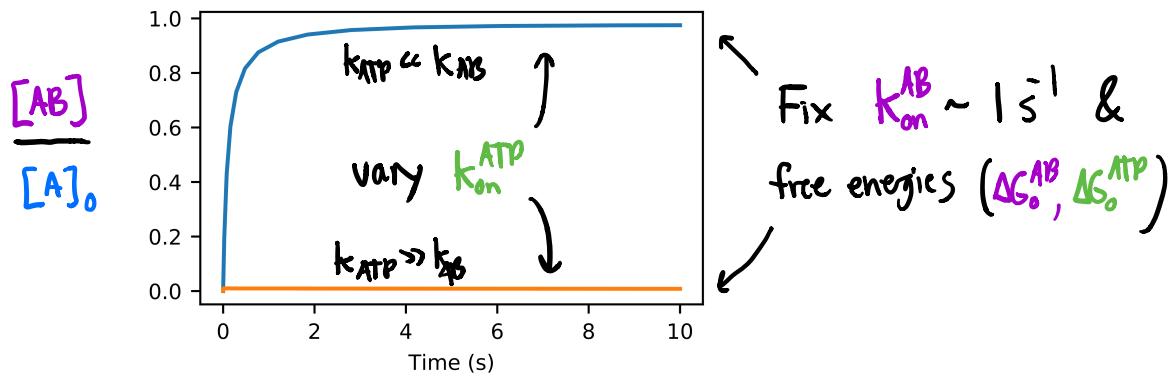
$$\frac{d[\text{ATP}]}{dt} = -\frac{d[AB]}{dt} + k_{\text{on}}^{\text{ATP}}[\text{ADP}][P] - k_{\text{off}}^{\text{ATP}}[\text{ATP}]$$

Equilibrium:

$$\frac{[AB]}{[A][B]} = e^{-\Delta G_0^{AB}/kT}, \quad \frac{[\text{ATP}]}{[\text{ADP}][P]} = e^{-\Delta G_0^{\text{ATP}}/kT}$$

\Rightarrow i.e. ATP doesn't seem to do anything...
("equilibrium = death")

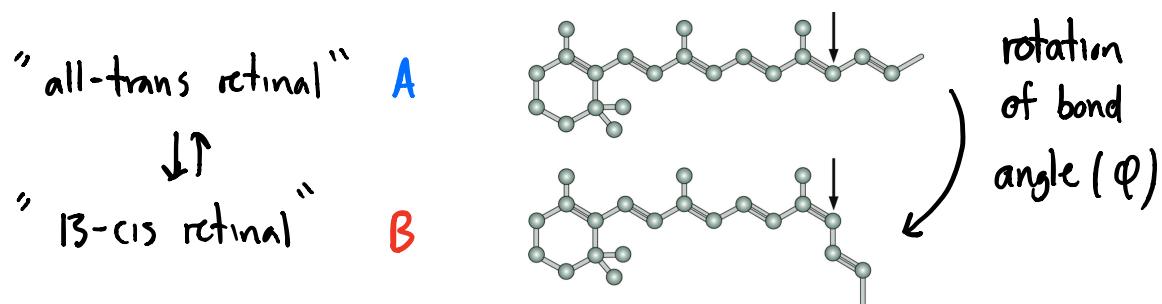
\Rightarrow But timescales (k_{on}^{AB} vs k_{on}^{ATP}) have huge effect!



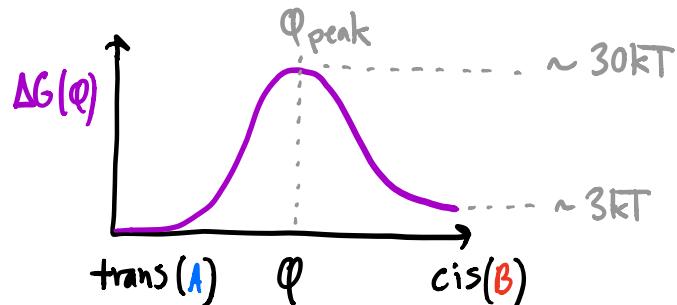
\Rightarrow de facto "equilibrium" (i.e. over some finite timescale)
can strongly depend on quantitative rates (k_{on}, k_{off})

\Rightarrow Next: What sets these rates?

Example: isomerization of retinal (photosynthesis, vision, ...)



can map out free energy "landscape"



"reaction coordinate"

⇒ Question: what controls rate of $A \rightarrow B$?

⇒ Heuristic argument: $k_{A \rightarrow B} \approx C \cdot \Pr(\varphi_{\text{peak}})$

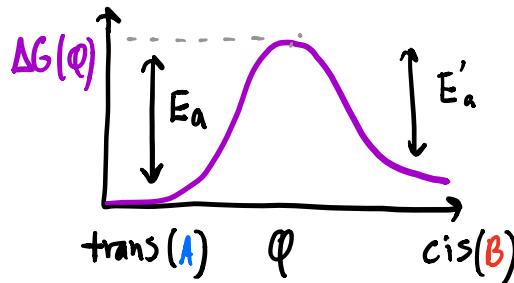
(since $\varphi < \varphi_{\text{peak}}$ mostly $\rightarrow A$, $\varphi > \varphi_{\text{peak}} \rightarrow B$)

+ Boltzmann dist'n: $\Pr(\varphi) \propto e^{-\Delta G(\varphi)/kT}$

⇒ "Arrhenius equation"

$$k_{A \rightarrow B} = A \cdot e^{-E_a/kT}$$

"activation energy"
"basal rate" of motion

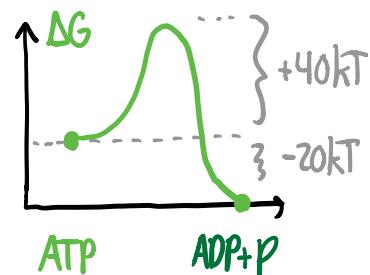


\Rightarrow activation energies $\gg kT$ can really slow things down!

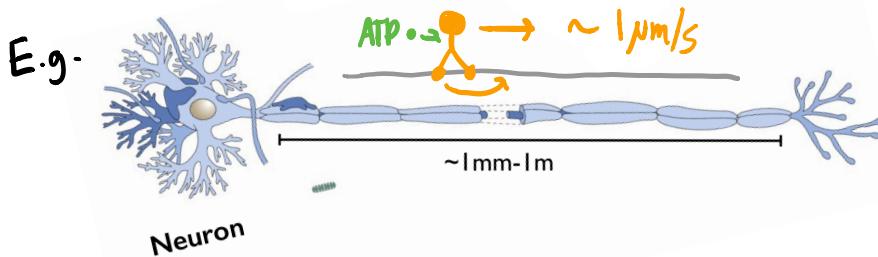
e.g. $\text{trans} \rightarrow \text{cis}$ isomerization, $E_a \sim 30kT \Rightarrow k_{A \rightarrow B} \sim 10^3 \text{ s}^{-1}$

e.g. $\text{ATP} \rightarrow \text{ADP} + \text{P} \Rightarrow E_a \sim 40kT$

$$\Rightarrow k_{\text{off}}^{\text{ATP}} \sim A e^{-E_a/kT} \sim 10^{-4} \text{ s}^{-1}$$



\Rightarrow But life happens faster than this!



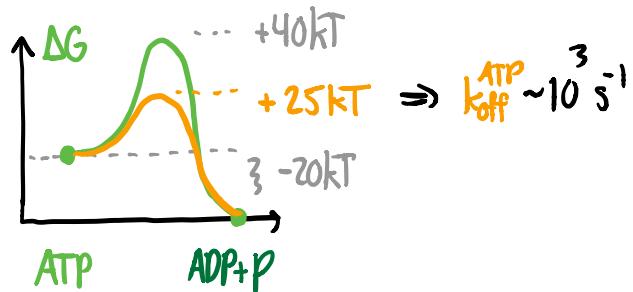
Question: How do cells modify reaction rates?

\Rightarrow change temp?

$$\frac{k_{\text{off}}(T')}{k_{\text{off}}(T)} = e^{-\frac{E_a}{kT'} + \frac{E_a}{kT}}$$

$$\Rightarrow 25^\circ \rightarrow 75^\circ \text{C} \Rightarrow 300 \rightarrow 350 \text{ K} \Rightarrow k_{\text{ATP}}^{\text{off}} \sim 0.03 \text{ s}^{-1}$$

Answer: cells use enzymes to lower activation energies



$$\Rightarrow \frac{k_{off}^{ATP}}{k_{off}^{ADP}} \approx e^{-\frac{E_a}{kT} + \frac{E_a}{kT}} \approx e^{-25 + 40} \sim 10^7$$

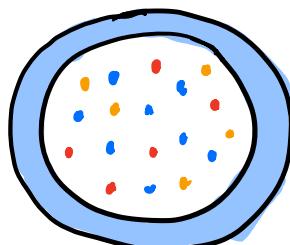
\Rightarrow How do enzymes do it? \Rightarrow lots of ways!

e.g. holding molecules close, bending them, shielding H₂O

\Rightarrow exploit extended size of protein

\Rightarrow How do enzymes fit into rate eqn framework?

\Rightarrow "Michaelis-Menten kinetics"



Basic idea: uncatalyzed reaction \Rightarrow catalyzed reaction



\hookrightarrow usually ≈ 0

Rate eqns: $\frac{d[AE]}{dt} = k_{\text{on}}[A][E] - k_{\text{off}}[AE] - k_{\text{cat}}[AE]$

$$\frac{d[B]}{dt} = +k_{\text{cat}}[AE] \quad + \quad [E] + [AE] = \text{const}$$

Key approximation: separation of timescales

$\Rightarrow A + E \rightleftharpoons AE$ often faster than $AE \rightarrow B + E$

$\Rightarrow [AE]$ in local equilibrium: $\frac{d[AB]}{dt} \approx 0$

$$\Rightarrow \frac{[AE]}{[A][E]} \approx \frac{k_{\text{on}}}{k_{\text{off}} + k_{\text{cat}}} = \frac{1}{K_m} \quad \text{"Michaelis constant"}$$

+ finite # enzymes:

$$[E] + [AE] = [E]_0$$

$$\Rightarrow \frac{[AE]}{[A]([E]_0 - [AE])} = \frac{k_{on}}{k_{off} + k_{cat}} \equiv \frac{1}{K_m}$$

after
some
algebra

$$[AE] = \frac{[E]_0 [A]}{K_m + [A]} \Rightarrow$$

$K_m \approx$ concentration
where ~50% of
enzymes are bound

$$\Rightarrow \text{combine w/ } \frac{d[B]}{dt} = k_{cat} [AE] \dots$$

\Rightarrow

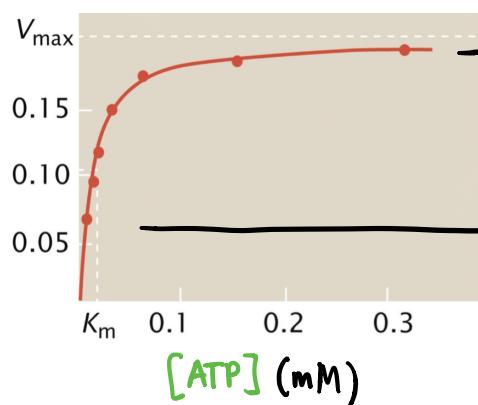
"Michaelis-Menten equation"

$$\frac{d[B]}{dt} = \underbrace{k_{cat} [E]_0}_{V_{max}} \times \frac{[A]}{K_m + [A]}$$



E.g. myosin

 (in vitro)



$$\frac{d[B]}{dt} = k_{cat} [E]_0 \quad (\text{enzyme limited})$$

$$\frac{d[B]}{dt} \approx \frac{k_{cat}}{K_m} [E]_0 \cdot [A] \quad (\text{substrate limited})$$

\Rightarrow given $[E]_0$, can estimate $k_{cat} \sim 5 \text{ s}^{-1}$

\Rightarrow could we build a super fast motor protein?

\Rightarrow as $k_{cat} \rightarrow \infty \Rightarrow \text{rate} \approx \frac{k_{cat}}{K_m} [A] \rightarrow k_{on} [A]$

\Rightarrow But eventually limited by diffusion!

$$R_{max} \approx 4\pi D a c_A$$



$$\Rightarrow \frac{k_{cat}}{K_m} \lesssim 4\pi D a \cdot 1 \frac{\text{mole}}{\text{L}}$$

$$\sim \frac{10 \cdot (10 \mu\text{m})^2 \cdot (1 \text{ nm}) \cdot 10^{24}}{1000 (\text{cm})^3} \sim 10^9 \frac{\text{reactions}}{\text{scc.}}$$

Next time: how do real enzymes compare to this fundamental limit?

Supplemental Note: Analytical solution of $A \rightleftharpoons B$

we start from the rate equation:

$$\frac{d[B]}{dt} = k_{A \rightarrow B}[A] - k_{B \rightarrow A}[B] + [A]_0 + [B]_0$$

$$\Rightarrow \frac{d[B]}{dt} = k_{A \rightarrow B}[A]_0 - (k_{A \rightarrow B} + k_{B \rightarrow A})[B]$$

$$\Rightarrow \frac{d}{dt} \left[[B] e^{(k_{A \rightarrow B} + k_{B \rightarrow A})t} \right] = k_{A \rightarrow B} [A]_0 \cdot e^{(k_{A \rightarrow B} + k_{B \rightarrow A})t}$$

$$\Rightarrow [B] e^{(k_{A \rightarrow B} + k_{B \rightarrow A})t} - [B]_0 = \int_0^t k_{A \rightarrow B} [A]_0 \cdot e^{(k_{A \rightarrow B} + k_{B \rightarrow A})t} dt$$

$$\Rightarrow [B] = [A]_0 \cdot \frac{k_{A \rightarrow B}}{k_{A \rightarrow B} + k_{B \rightarrow A}} \left[1 - e^{-(k_{A \rightarrow B} + k_{B \rightarrow A})t} \right] \checkmark$$