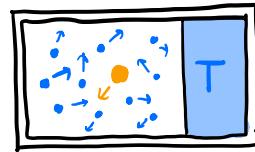


Announcements:

- ① advanced copy of notes on canvas (Week 5)
- ② new practice problem for today's material
- ③ Supplemental reading for today's material:
 - * Ch 15 of Physical Biology of the Cell
 - * Review article on Canvas "A perspective on enzyme catalysis"
 - * Raw data for myosin kinetic params (Ouellet et al 1952, canvas)

Last time:

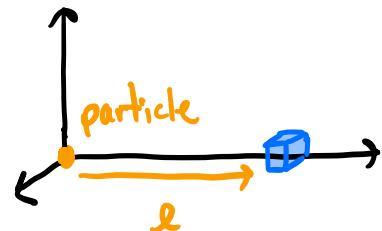
Applications of Diffusion



①

$$\text{Diffusion timescale } \tau_d = l^2/D$$

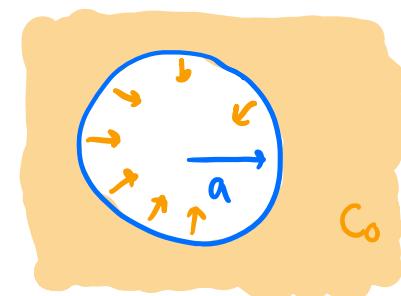
required to travel distance l



②

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

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



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

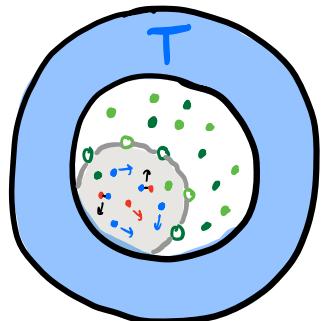
(normal brain weighs ~ 1.4 kg)

⇒ brain synthesizes & uses ~10x its weight in ATP every day!

②

Lingering question from Lecture 3:

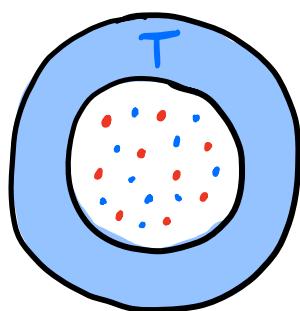
How do cells build costly molecules...



... while preventing others from reaching equilibrium?



Basic Setup:



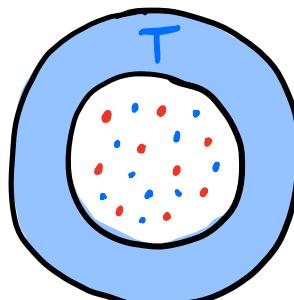
Different kinds of molecules (A, B, \dots) diffusing around in cell & undergoing chemical reactions...

- 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_i 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, $\tau_D \sim 10^{-2} s$)

\Rightarrow Dynamics can often be modeled w/ "rate equations"

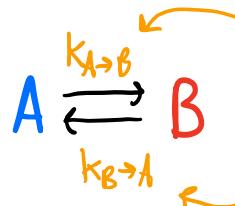
$$\frac{dc_A(t)}{dt} = F_A(c_A(t), c_B(t), \dots)$$

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

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

Example 1:

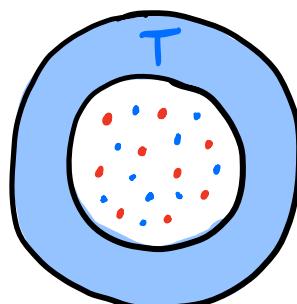
transformation:



"Rate constants"
"kinetic parameters."

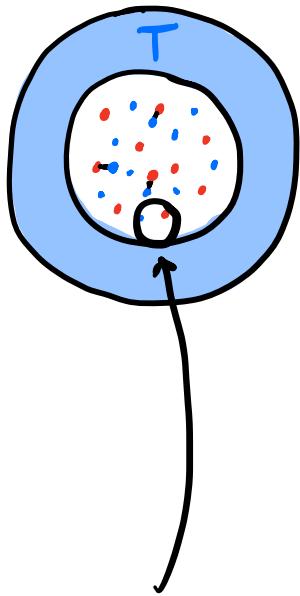
$$\Rightarrow \frac{d[B]}{dt} = +k_{A \rightarrow B}[A] - k_{B \rightarrow A}[B]$$

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



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

Example 2: synthesis: $A + B \xrightleftharpoons[k_{\text{off}}]{k_{\text{on}}} AB$

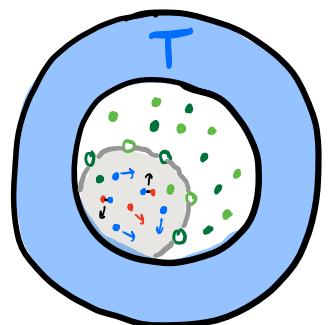


$$\Rightarrow \frac{d[AB]}{dt} = k_{\text{on}} [A][B] - k_{\text{off}} [AB]$$

rate of bumping into each other in space

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

Example 3: $A + B + ATP \xrightleftharpoons[k_{\text{off}}]{k_{\text{on}}} AB + ADP + P$



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

\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:

$$\frac{[AB][ADP][P]}{[A][B][ATP]} = e^{\frac{\Delta G_{o}^{ATP} - \Delta G_{o}^{AB}}{kT}}$$

Change in free energy
in "standard conditions"

\Rightarrow connection between rate constants

and free energy: $k_{on}/k_{off} = e^{-\Delta G_o/kT}$

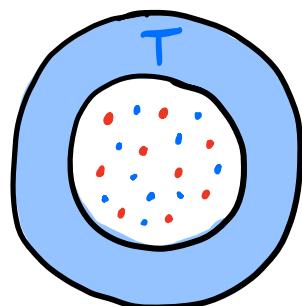
\Rightarrow Rate eqns are consistent w/ equilibrium statmech...

... but also provide info about dynamics!

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

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

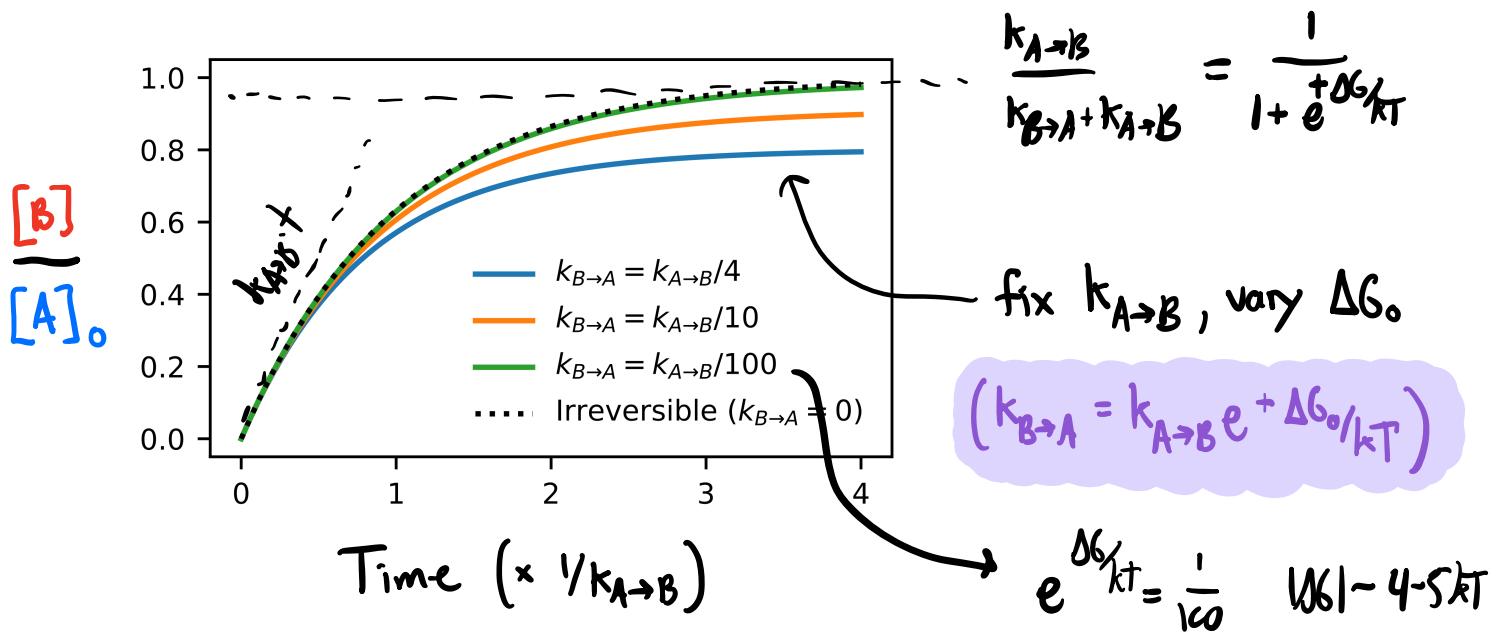
$$+ [A] + [B] = \text{const}$$



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

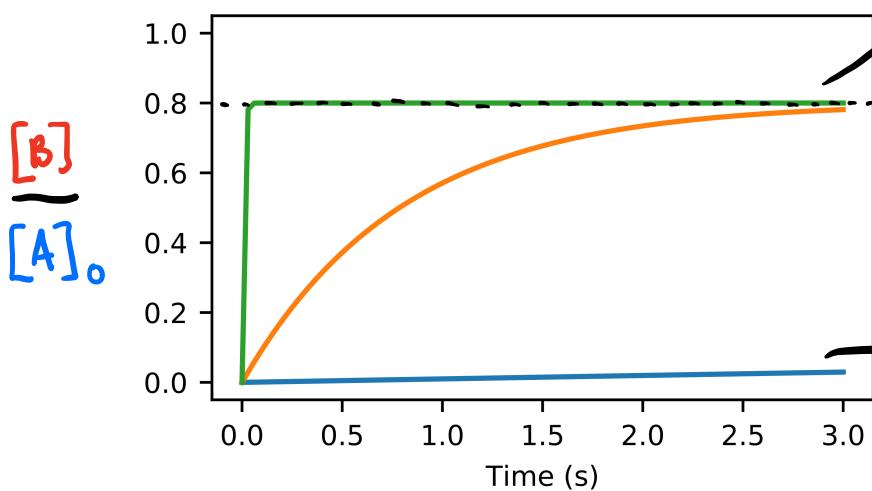
(supplemental)
note

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



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

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



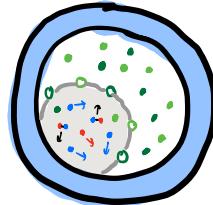
$k_{A \rightarrow B} \gg 1 \text{ s}^{-1}$
instant equilibrium.

Same equilibrium ($1/1 + e^{\Delta G_0 / kT}$)
but different dynamics!

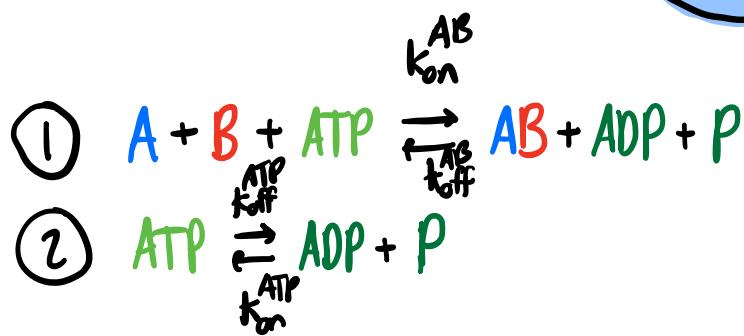
$k_{A \rightarrow B} \ll 1 \text{ s}^{-1}$
 \rightarrow frozen @ initial value.

\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 2 competing reactions:



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

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

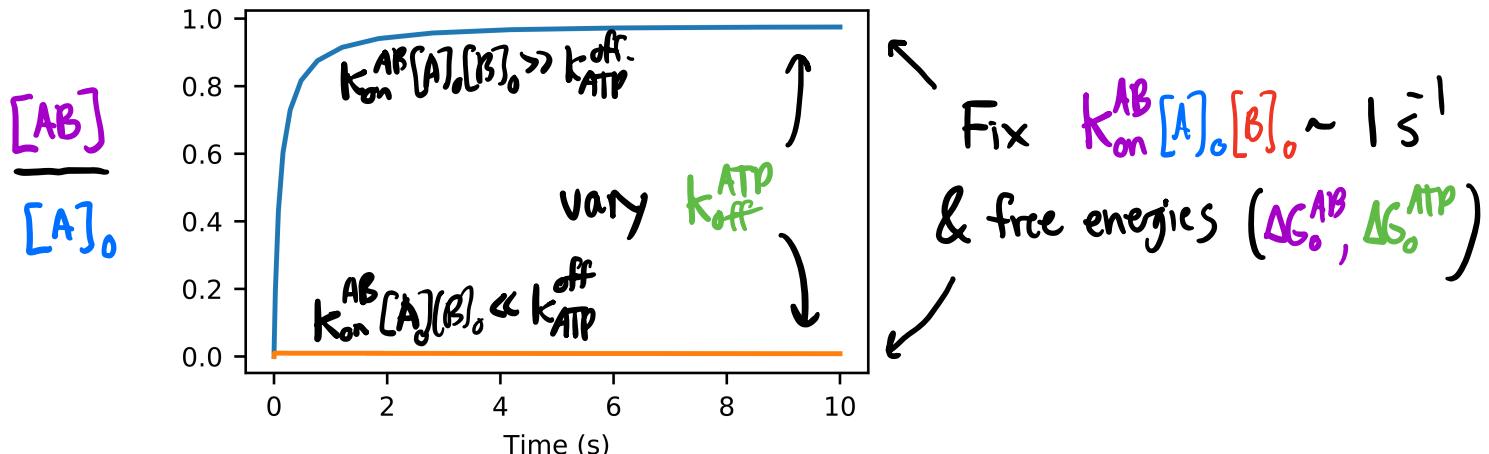
Equilibrium:

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

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

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

see code in Canvas.



\Rightarrow de facto "equilibrium" (i.e. over some finite timescale)

can strongly depend on quantitative rates ($k_{\text{on}}, k_{\text{off}}$)

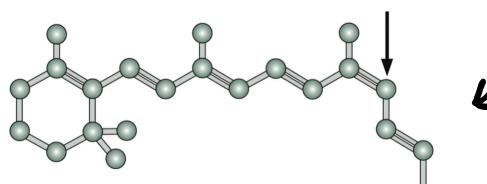
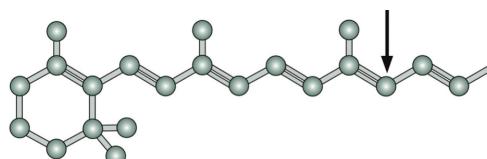
\Rightarrow Next: What sets these rates?

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

"all-trans retinal" A



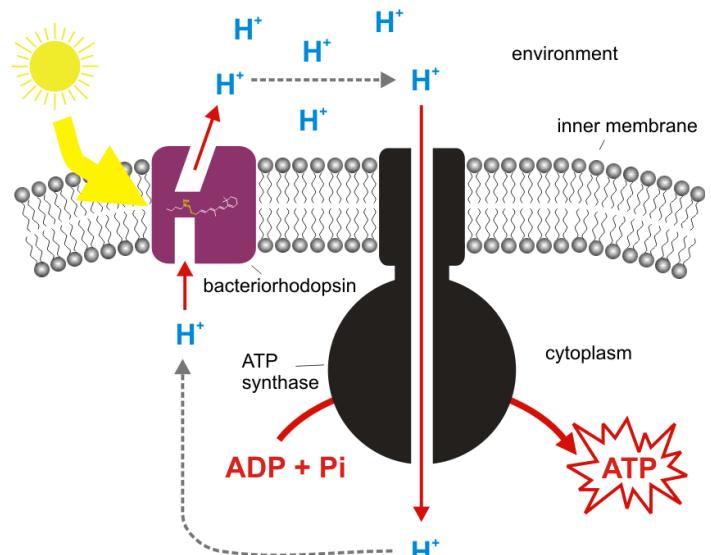
"13-cis retinal" B



rotation
of bond
angle (ϕ)



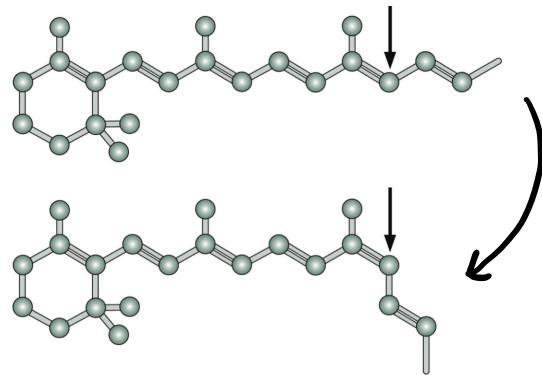
San Francisco Bay Salt Ponds



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

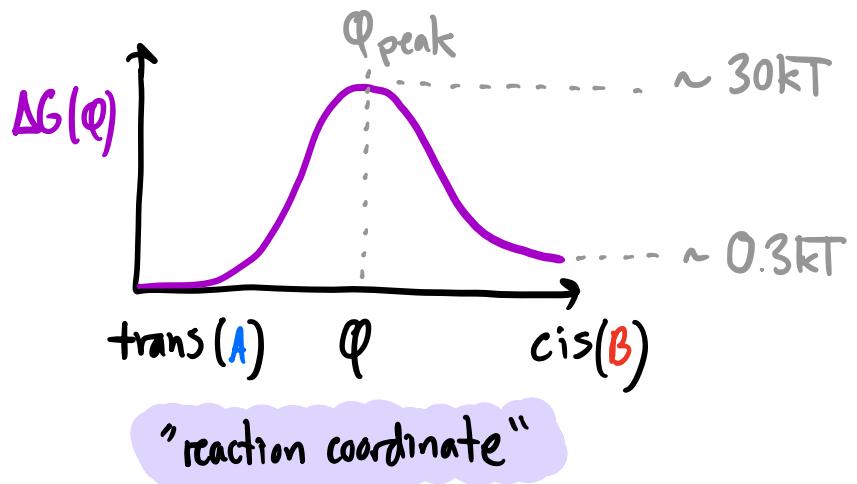
"all-trans retinal" A

$\downarrow \uparrow$
"β-cis retinal" B



rotation
of bond
angle (ϕ)

can map out free energy "landscape"



⇒ 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"

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

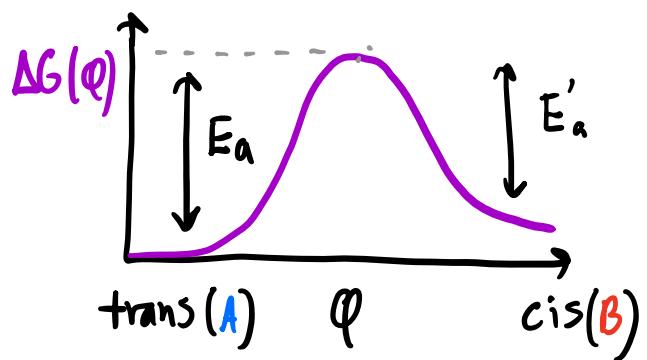
"activation energy"



"basal rate"
of motion

→ Hard! QM

$$\text{Simple cases: } A = \frac{kT}{h} = 6 \text{ ps}^{-1}$$

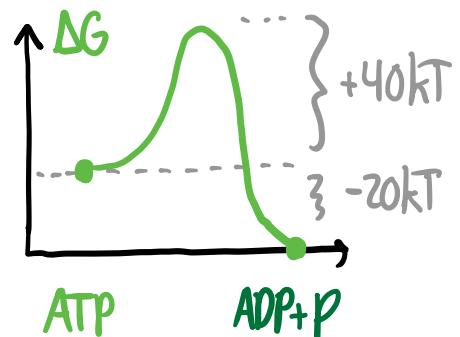


\Rightarrow activation energies $\gg kT$ can really slow things down!

e.g. trans \rightarrow cis retinal, $E_a \sim 30kT \Rightarrow k_{A \rightarrow B} \sim A e^{-E_a/kT} \sim 1 s^{-1}$
 \downarrow
 $6 \text{ ps}^{-1} e^{-30}$

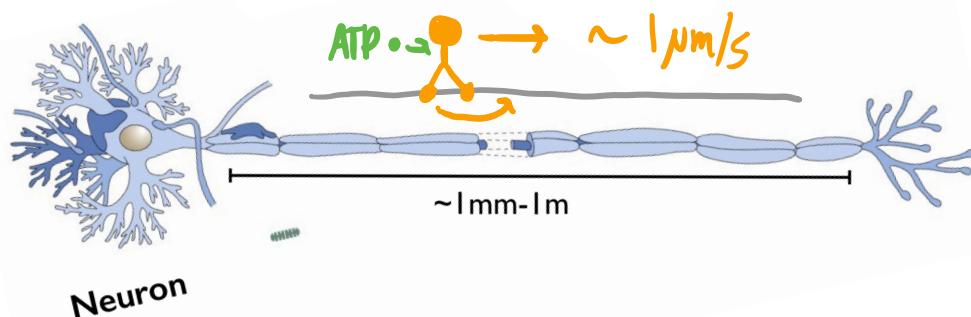
e.g. $\text{ATP} \rightarrow \text{ADP} + \text{P} \Rightarrow E_a \sim 40kT$

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



\Rightarrow But life happens faster than this!

E.g.



Question: How do cells modify reaction rates?

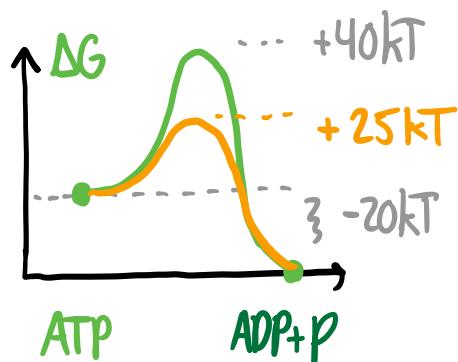
\Rightarrow change temp?

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

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

Answer: cells use enzymes to lower activation energies

E.g. myosin



$$10^{-4} \text{ s} \rightarrow 10^3 \text{ s}$$

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

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

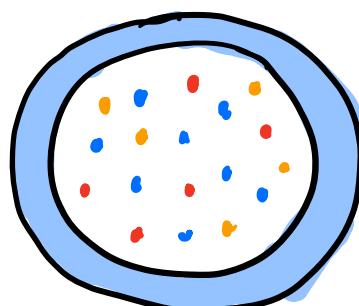
e.g. holding molecules close, bending them, shielding H₂O

\Rightarrow exploit extended size of protein

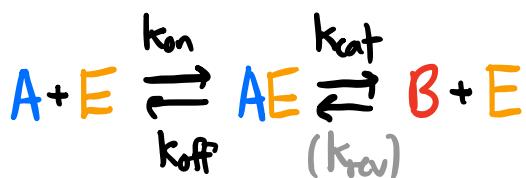
see review
in Canvas

\Rightarrow How do enzymes fit into rate eqn framework?

\Rightarrow "Michaelis-Menten kinetics"



Basic idea: uncatalyzed reaction \Rightarrow catalyzed reaction



\hookrightarrow usually ≈ 0

(try)

Rate eqns: $\frac{d[AE]}{dt} = k_{\text{on}}[A][E] - k_{\text{off}}[AE] - k_{\text{cat}}[AE] \approx 0$

$$\frac{d[B]}{dt} = +k_{\text{cat}}[AE] \quad + \quad [E] + [AE] = \text{const} = [E]_0$$

Key approximation: separation of timescales

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

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

$$\Rightarrow \frac{[AE]}{[A][E]} \approx \frac{k_{\text{on}}}{k_{\text{off}} + k_{\text{cat}}} \equiv \frac{1}{K_M}$$

"Michaelis constant"

+ finite # enzymes:

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

$$\Rightarrow \frac{[AE]}{[A]([E]_0 - [AE])} = \frac{1}{K_m}$$

after
some
algebra
(exercise).

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

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

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

\Rightarrow "Michaelis-Menten equation"

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



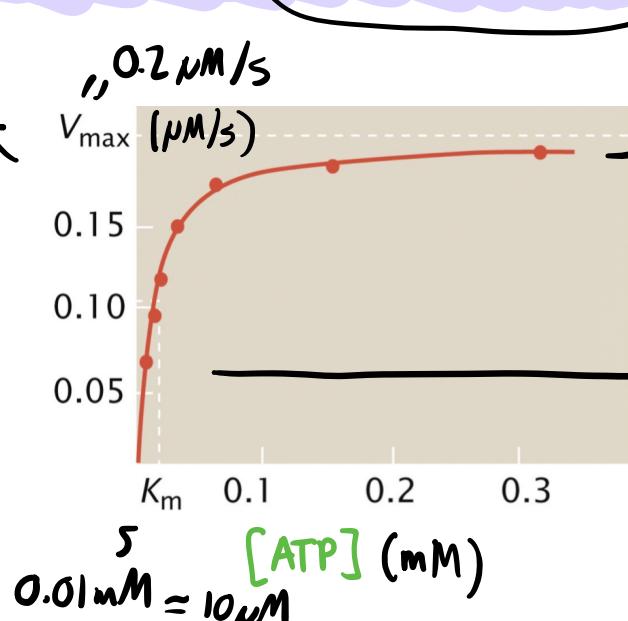
||



$\frac{d[B]}{dt}$
E.g. myosin



(in vitro)



$k_{cat}(E, A)$

$$\frac{d[B]}{dt} = k_{cat} [E]_0 \equiv V_{max}, \text{ enzyme limited, saturated regime.}$$

$$\frac{d[B]}{dt} \approx \frac{k_{cat}}{K_m} [E]_0 \cdot [A]$$

linear regime

substrate limited regime.

\Rightarrow given $[E]_0 \approx 0.1 \mu M$, can estimate k_{cat} :

$$k_{cat} = \frac{V_{max}}{[E]_0} \approx \frac{0.2 \mu M/s}{0.1 \mu M} = 2 s^{-1}$$

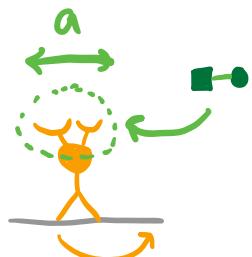
\Rightarrow Question: could we build a super fast motor protein?

\Rightarrow other ATPases as fast as $k_{cat} \sim 10^3 s^{-1}$

\Rightarrow Could evolution do even better?

Next time: eventually limited by diffusion!

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



Supplemental Reading: ① Ch 15 of Physical Biology of the Cell

② Review article on Canvas "A perspective on enzyme catalysis"

③ Raw data for myosin kinetic params (Ouellet et al 1952, canvas)

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

we start from the rate equation:

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

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

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

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

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