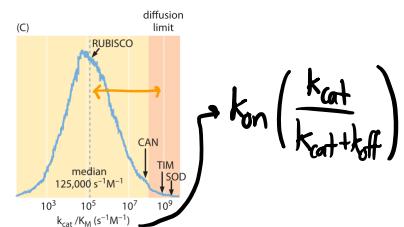


Most common Q: why so many enzymes below diffusion limit?



Last time: How do bacteria follow concentration gradients?



① Works @ low concentrations!

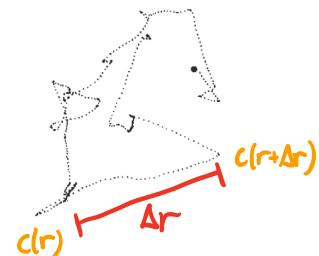
$$[\text{e.g. } C \approx 3 \text{nM} \approx 2 \text{ (\mu m)}^3]$$

⇒ discrete molecules matter!

②

Burg-Purcell Limit on precision of

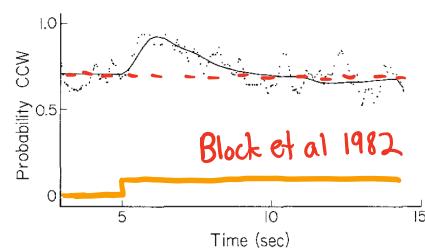
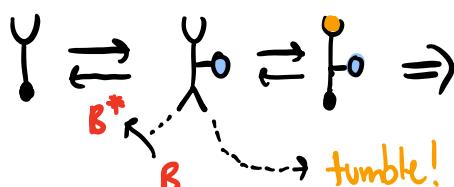
$$\text{Chemoreception: } \frac{\partial \log c}{\partial r} \gtrsim \frac{1}{\Delta r} \frac{1}{\sqrt{D_{act}}}$$



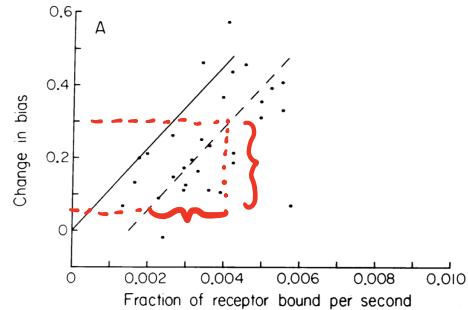
⇒ E. coli must swim to achieve necessary precision!

③

Simple pathways for measuring temporal gradients

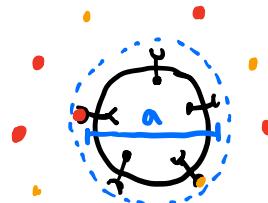


\Rightarrow even single bound receptor
 $(\sim 1/600)$ can produce
 large change in bias



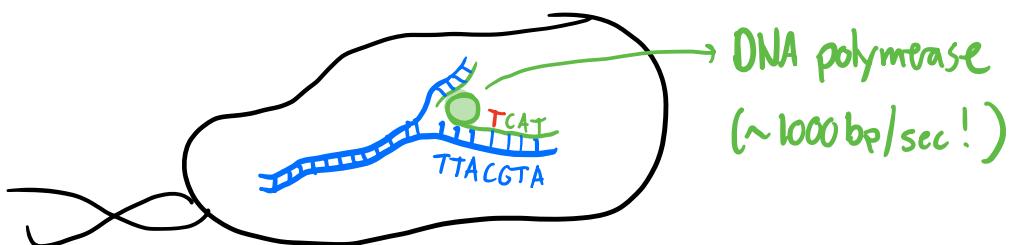
Today: does this high sensitivity make
 cells susceptible to errors?

E.g. bind wrong
 substrate?



\Rightarrow Lots of related error problems in cell biology...

① Copying genome (DNA) during cell division



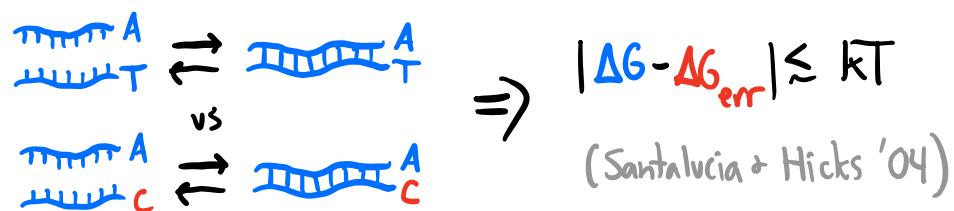
key insight: chemistry (i.e. free energy) favors
 correct pairs (A-T, C-G)

\Rightarrow errors in n=1 molecule cause permanent change in offspring!

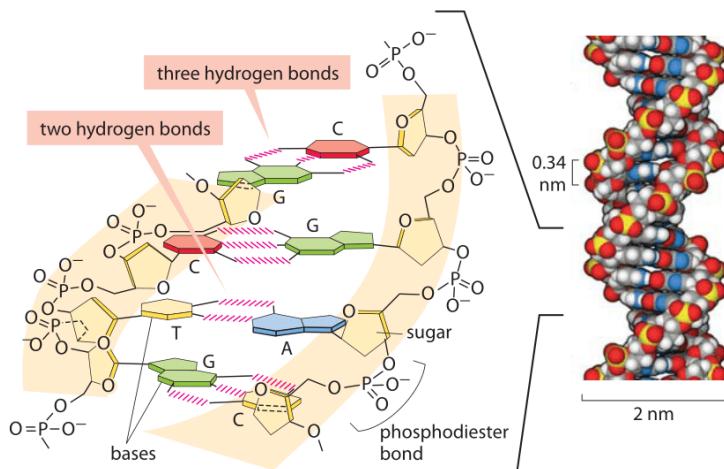
\Rightarrow How strong are these free energy differences?

\Rightarrow still a bit controversial... (Oertell et al 2016)

a) In vitro hybridization experiments:



b) Counting hydrogen bonds



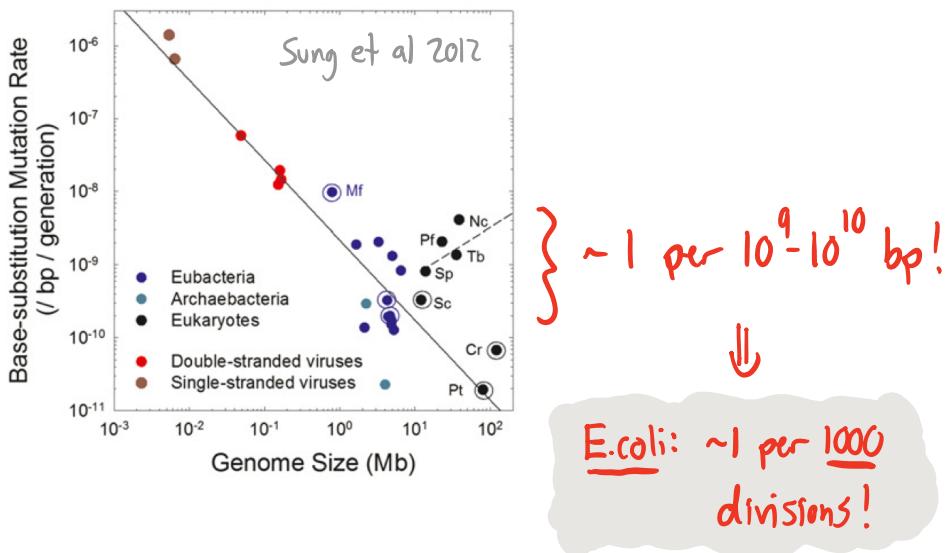
\Rightarrow mispairing \approx 1-2 hydrogen bonds ($5-10kT$)

\Rightarrow Naive Boltzmann factor @ equilibrium:

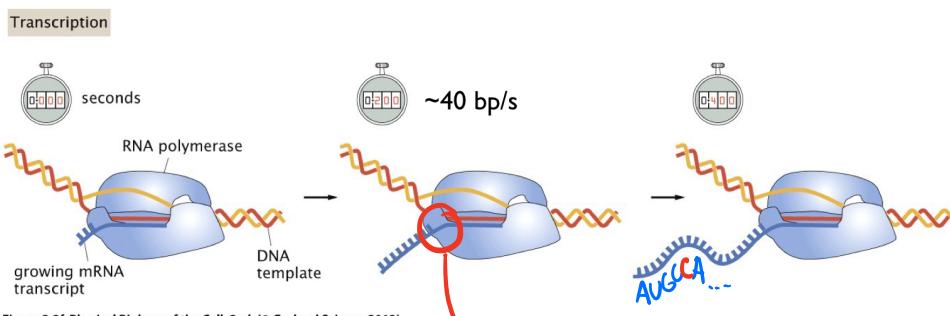
$$\frac{p(\text{error})}{p(\text{correct})} = \frac{\cancel{\int} e^{-\Delta G_{\text{err}}/kT}}{\cancel{\int} e^{-\Delta G/kT}} = e^{\frac{\Delta G - \Delta G_{\text{err}}}{kT}} \approx e^{-1} - e^{-10}$$

$\Rightarrow \gtrsim 1$ error every 10^4 bp!

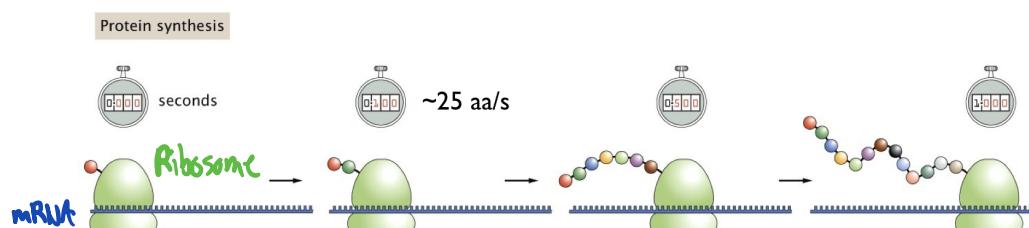
\Rightarrow Actual mutation rates =



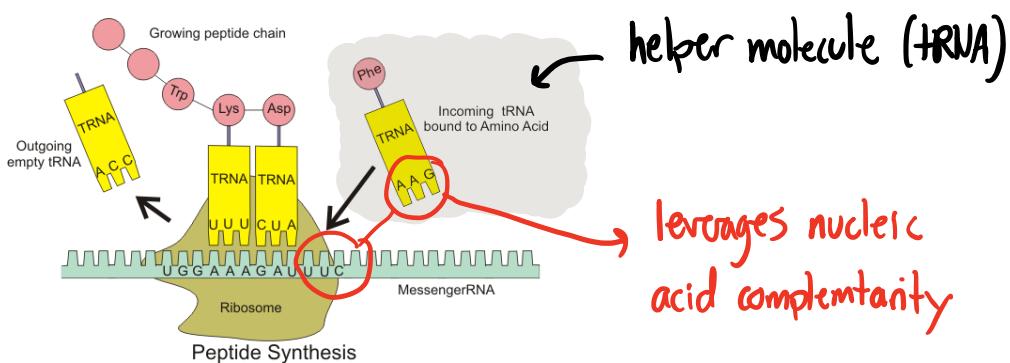
② Errors in transcription & translation:



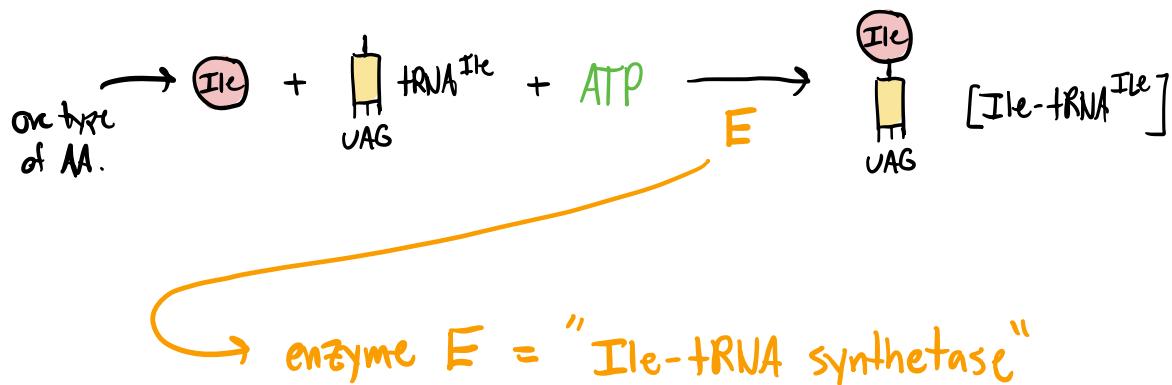
→ leverages similar chemical complementarity as DNA



⇒ How does this work?



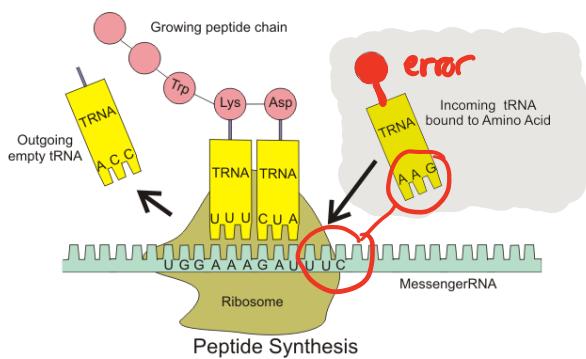
\Rightarrow tRNAs must also be "charged" w/ correct AA!



\Rightarrow tRNA synthetase can also mess up :

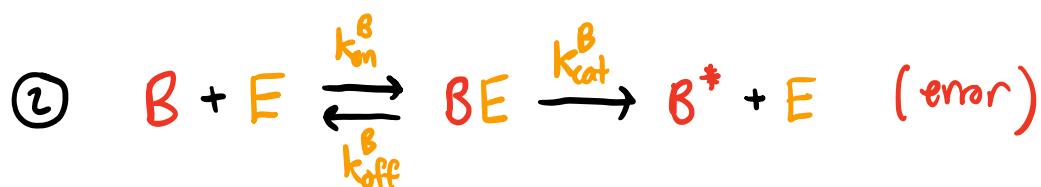
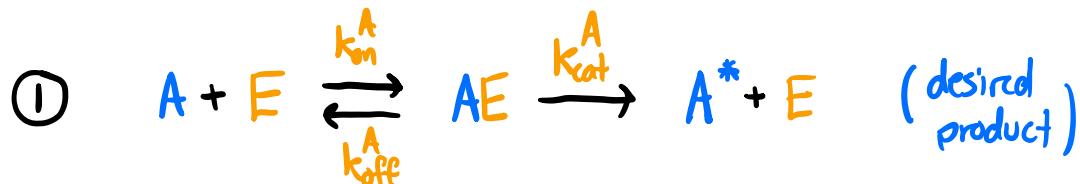


\Rightarrow leads to errors @ translation:



Question: what controls these basal error rates
(+ how might cells improve on them?)

Simplesst scenario : Michaelis-Menten Reaction
w/ 2 competing substrates



Rate
Eqns:

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

$$\frac{d[B E]}{dt} = k_{\text{on}}^B [B][E] - k_{\text{off}}^B [BE] - k_{\text{cat}}^B [BE] = 0$$

\Rightarrow @ steady state :

$$[AE] = \frac{k_{\text{on}}^A [A][E]}{k_{\text{off}}^A + k_{\text{cat}}^A}$$

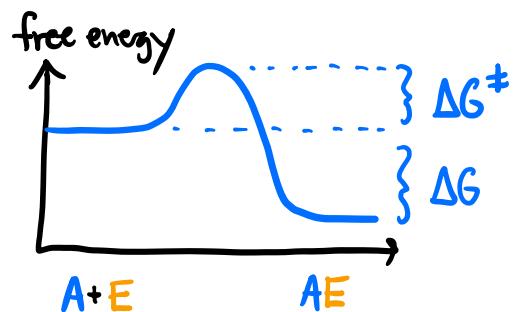
$$[BE] = \frac{k_{\text{on}}^B [B][E]}{k_{\text{off}}^B + k_{\text{cat}}^B}$$

$$\Rightarrow \rho = \frac{\text{rate of error product}}{\text{rate of correct product}} = \frac{\frac{d[B^+]}{dt}}{\frac{d[A^+]}{dt}} = \frac{k_{\text{cat}}^B [BE]}{k_{\text{cat}}^A [AE]}$$

$$= \frac{[B]}{[A]} \times \left(\frac{\frac{k_{\text{on}} k_{\text{cat}}^B}{k_{\text{off}}^B + k_{\text{cat}}^B}}{\frac{k_{\text{on}} k_{\text{cat}}^A}{k_{\text{off}}^A + k_{\text{cat}}^A}} \right)$$

\Rightarrow Question: what sets k_i^A vs k_i^B ?

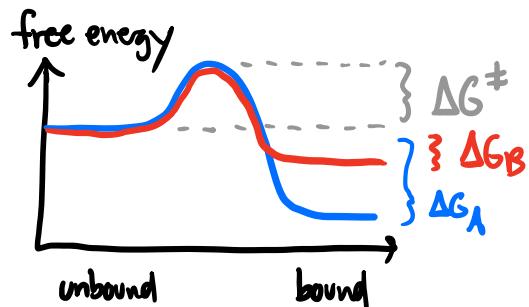
Lecture 6:
Arrhenius Law



$$\Rightarrow k_{\text{on}}^A \propto e^{-\frac{\Delta G^‡}{kT}}$$

$$k_{\text{off}}^A \propto e^{-\frac{\Delta G^‡}{kT} + \frac{\Delta G_A}{kT}}$$

for similar substrates,
might expect that:



$$\Rightarrow k_{on}^B \approx k_{on}^A \equiv k_{on}$$

$$k_{off}^A \approx k_{on} e^{+\frac{\Delta G_A}{kT}}$$

$$k_{off}^B \approx k_{on} e^{+\frac{\Delta G_B}{kT}}$$

$$\Rightarrow \text{Similarly for catalytic step: } k_{cat}^A \approx k_{cat}^B \approx k_{cat}$$

\Rightarrow e.g. bonds formed @ different part of molecule than determines enzyme binding.

\Rightarrow e.g. limited by other processes (ATP hydrolysis)

\Rightarrow Putting everything together:

$$\frac{\text{relative error rate, } \rho}{\text{rate, } r} = \frac{[BE]}{[AE]} = \frac{[B]}{[A]} \times \frac{k_{on} e^{+\frac{\Delta G_A}{kT}} + k_{cat}}{k_{on} e^{+\frac{\Delta G_B}{kT}} + k_{cat}} \geq \frac{[B]}{[A]} e^{-\frac{\Delta G_B - \Delta G_A}{kT}}$$

(lower bound achieved when $k_{cat} \ll k_{off}^A, k_{off}^B$)

\Rightarrow big problem if " ΔG " $\approx 10kT$ & need ultra low error rates

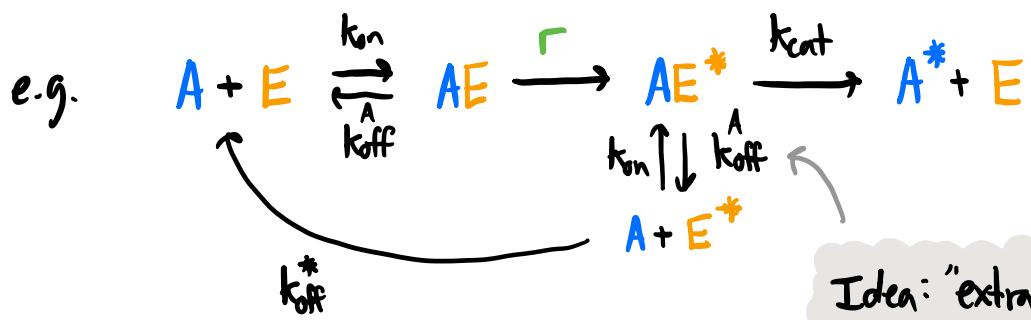
Question: can cells evade this thermodynamic limit
w/ simple molecular toolkit?

One potential mechanism: "kinetic proofreading"

⇒ originally proposed by Hopfield ('74) & Ninio ('75)

⇒ later extended to many different settings

Basic idea: add a "spurious" irreversible step
after binding but before catalysis:



\Rightarrow now relative error rate, $\rho \equiv [BE^+] / [AE^+]$

New rate equations:

$$\frac{d(AE)}{dt} = k_{on}^A[A][E] - k_{off}^A[AE] - r(AE) = 0$$

$$\frac{d(AE^+)}{dt} = r(AE) + k_{on}^A[A][E^+] - k_{off}^A[AE^+] - k_{cat}[AE^+] = 0$$

$$\frac{d(E^+)}{dt} = -k_{on}^A[A][E^+] + k_{off}^A[AE^+] - k_{off}^*[E^+] = 0 \quad + \text{versions w/ } B \text{ substrate ...}$$

\Rightarrow solve @ steady state (algebra exercise; supplemental notes)

\Rightarrow when k_{off}^* is fast ($k_{off}^* \gg k_{on}^A[A], k_{on}^B[B]$)

$$[AE] \approx \frac{k_{on}^A[A][E]}{k_{off}^A + r}$$

$$[AE^+] \approx \left(\frac{r}{k_{off}^A + k_{cat}} \right) [AE]$$

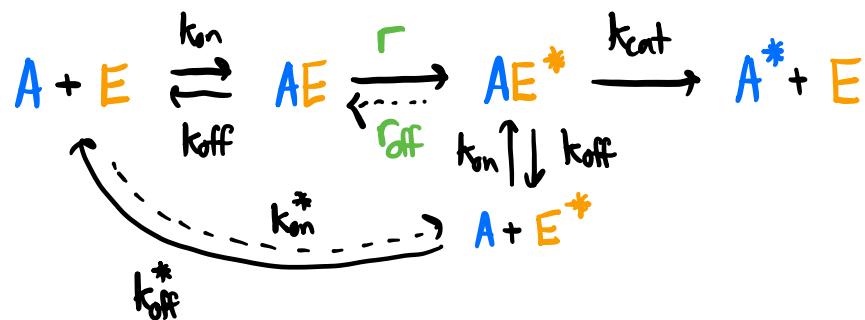
extra factor off k_{off}^* !

$$\Rightarrow \text{relative error rate } \rho = \frac{[BE^+]}{[AE^+]} \geq \frac{[B]}{[A]} e^{-\frac{2(\Delta G_A - \Delta G_B)}{kT}}$$

(achieved when $r \ll k_{off}^*$, $k_{cat} \gg k_{off}^*$)

\Rightarrow i.e. squares error rate ($10^{-2} \rightarrow 10^{-4}$, $10^{-4} \rightarrow 10^{-8}!$)

\Rightarrow Irreversibility was crucial! E.g. reversible version:



\Rightarrow Same calc'n (exercise) yields $\frac{[BE^*]}{[AE^*]} = \frac{[\beta]}{[A]} e^{\frac{\Delta G_A - \Delta G_B}{kT}}$

\Rightarrow how to ensure irreversibility ($r_{off} \ll r$)?

\Rightarrow burn free energy! ($r_{off} = r e^{+\frac{\Delta G_r}{kT}}$)

e.g. ATP hydrolysis: $AE + ATP \rightarrow AE^* + AMP, P, \text{etc...}$

\Rightarrow ΔG_{ATP} not used to build product,
but to limit reversibility!

\Rightarrow i.e. "wasted" energy is feature not bug!

Kinetic Proofreading



Benefits: enhances specificity above thermodynamic limit

Costs: ① slower processivity:

$$\Rightarrow \text{Slowdown} = \frac{k_{\text{cat}} [AE^*]}{k_{\text{cat}} [AE]} = \frac{r}{k_{\text{off}}} \approx 1$$

if proofreading
is effective
($r \ll k_{\text{off}}$)

② "wastes" energy!



$$\frac{\text{energy using rxns / product}}{k_{\text{cat}} [AE^*]} = \frac{r [AE]}{k_{\text{off}}} = \frac{k_A}{k_{\text{cat}}} \gtrsim 1$$

if proofreading
is effective
($r \ll k_{\text{off}}$)

\Rightarrow Hopfield: can be used as test for proofreading!

Regular M.M.

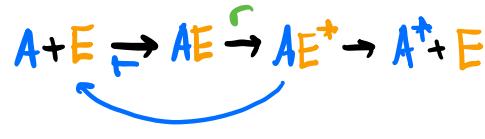


vs



\Rightarrow same used energy
for correct vs error

Kinetic Proofreading

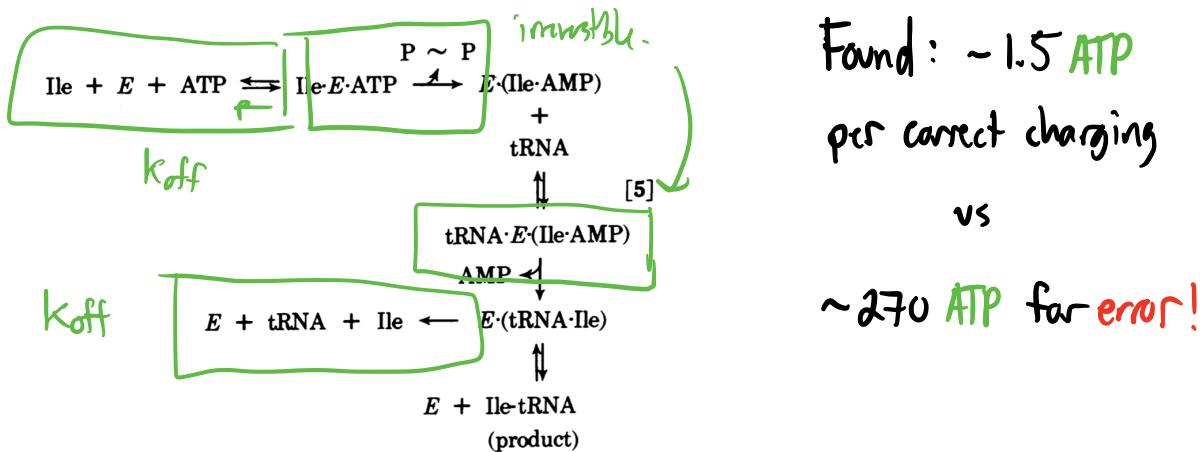


vs



\Rightarrow more burned energy
per error product!

\Rightarrow applied to tRNA charging:



\Rightarrow consistent w/ proofreading hypothesis...

\Rightarrow potential explanation for complicated rxn.

Supplemental Notes: Error rate for kinetic proofreading

@ equilibrium, rate equations become:

$$(i) \quad \frac{d[AE]}{dt} = k_{on}^A[A][E] - k_{off}^A[AE] - r[AE] = 0$$

$$(ii) \quad \frac{d[AE^*]}{dt} = r[AE] + k_{on}^A[A][E^*] - k_{off}^A[AE^*] - k_{on}^B[B][E^*] = 0$$

$$(iii) \quad \frac{d[E^*]}{dt} = -k_{on}^A[A][E^*] + k_{off}^A[AE^*] - k_{off}^B[BE^*] \\ -k_{on}^B[B][E^*] + k_{off}^B[BE^*] - k_{off}^A[E^*] = 0$$

(iv, v) same as Eqs (i) & (ii) w/ $A \leftrightarrow B$

\Rightarrow From Eq (iii), we have:

$$[E^*] = \frac{k_{off}^A[AE^*] + k_{off}^B[BE^*]}{k_{on}^A[A] + k_{on}^B[B] + k_{off}^A + k_{off}^B}$$

\Rightarrow from Eqs i + iv, we have:

$$[AE] = \frac{k_{on}^A}{k_{off}^A + r} [A][E], \quad \text{same w/ } A \leftrightarrow B$$

\Rightarrow substituting into Eqs (ii) + (v), we have

$$\begin{aligned} \frac{k_{on}^A}{k_{off}^A + r} [A][E] &= \left[\left(\frac{k_{off}^* + k_{on}^B[B]}{k_{off}^* + k_{on}^A[A] + k_{on}^B[B]} \right) k_{off}^A + k_{cat} \right] [AE^*] \\ &\quad + \left(\frac{k_{on}^A[A]}{k_{off}^* + k_{on}^A[A] + k_{on}^B[B]} \right) k_{off}^B [BE^*] \end{aligned}$$

(and same for $A \leftrightarrow B$)

\Rightarrow can solve for $[AE^*]$ + $[BE^*]$ (but messy...)

\Rightarrow if k_{off}^* is "fast" (i.e., $k_{off}^* \gg k_{on}^A[A], k_{on}^B[B]$)

then eqs reduce to:

$$[AE^*] = \frac{k_{on}^A}{K_{off}^A + r} \times \frac{r}{k_{off}^B + k_{cat}} \times [A][E]$$

(and same w/ $A \leftrightarrow B$)

$$\Rightarrow \rho = \frac{[BE^*]}{[AE^*]} = \frac{k_{on}^B[B]}{k_{on}^A[A]} \left(\frac{\frac{A}{k_{off}^A + r}}{\frac{B}{k_{off}^B + r}} \right) \left(\frac{\frac{A}{k_{off}^B + k_{cat}}}{\frac{B}{k_{off}^A + k_{cat}}} \right)$$