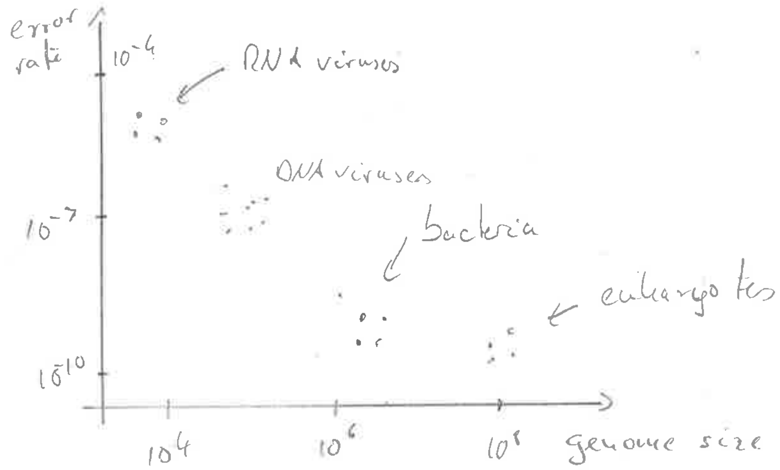
Kinetic proofreading

2017-12-12 ①

- many biochemical reactions are extremly accurate!
- DNA replication as low as 1 in 10^{10}
- how is such accuracy achieved?
  what are the costs?



- rough scaling: $\mu \sim \frac{1}{L}$
- necessary to enable error free replication with high prob.
- $e^{-\mu L}$
- other wise, Muller's Ratchet kicks in....

- how is high accuracy achieved?

Energy of discrimination

consider a set of competing reactions

$$C + c \underset{k_c}{\overset{k_c'}{\rightleftharpoons}} C_c \xrightarrow{W} \text{correct}$$

diffusion limited on rates:

$$k = k_c' = k_D'$$

$$D + c \underset{k_D}{\overset{k_D'}{\rightleftharpoons}} D_c \xrightarrow{W} \text{wrong}$$

polymerisation independent of identity (W)

Transition state population

$$[C_c](W + k_c) = [C]k_c$$

Product formation: $W[cC] = W \frac{[C]k}{W+k_c}$

ratio of correct to wrong product:

$$\frac{W[cC]}{W[cD]} = \frac{[C](k_D+W)}{[D](k_c+W)} \longrightarrow \frac{[C]}{[D]}\frac{k_D}{k_c} = \frac{[C]}{[D]} e^{-\Delta G/kT}$$

energy of discrimination

- ΔG is the difference in binding free energy of C/D state
- wrong nucleotide pairings only cost 2-5 kT $\quad e^{-5} \simeq 0.01 \gg 10^{-9}$
- how do cells achieve high accuracy?

Intermediate states

$$X + c \rightleftharpoons X_c \underset{W'}{\overset{W}{\rightleftharpoons}} X_c^* \xrightarrow{V} \text{product}$$

$$X_c^* \rightleftharpoons X + c$$

- just adding an intermediate doesn't work
  → if $X_c \rightleftharpoons X_c^*$ is an equilibrium reaction, nothing is gained
  → discrimination is still $e^{-\Delta G/kT}$
- need irreversible step?

$$X + C \rightleftharpoons X_c \xrightarrow[\text{irreversible}]{W,\ ATP \to ADP+P} X_c^* \xrightarrow{V} \text{product}$$

$$X_c^* \xrightarrow{\text{now small}} X + C$$

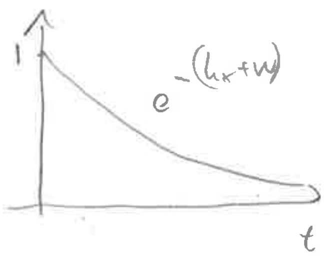
- can be achieved by coupling to ATP hydrolysis
- often triggers conformational transitions — museum metaphor
- now X_c is already biased towards C
- $X_c \to X_c^*$ can achieve another factor $\exp(-\Delta G/kT)$

$\Rightarrow$ enrichment of correct product by $e^{-2\Delta G/kT}$

$\Rightarrow$ more intermediate steps increase accuracy even further

Effectively a delay

- consider freshly formed X_c complex
- probability of still being present



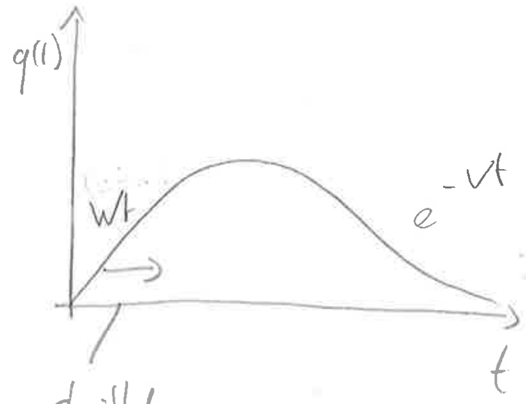
$$\frac{dp}{dt} = -(k_x + W)\,p(t) \quad \Rightarrow \quad p(t) = e^{-(k_x+W)t}$$

- second intermediate is populated by $p(t)$

$$\frac{dq(t)}{dt} = W p(t) - V q(t) = W e^{-(k_x+W)t} - V q(t)$$

$$\Rightarrow \quad q(t) = W e^{-Vt} \int_0^t e^{-(V-W-k_x)t'} \, dt'$$

$$= \frac{W e^{-Vt}}{W + k_x - V} \left[1 - e^{-(W+k_x-V)t}\right]$$

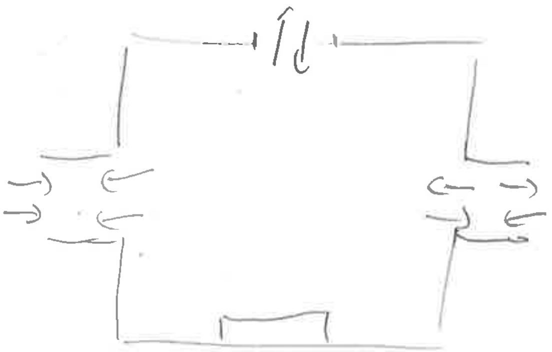


shifted away from 0

$\Rightarrow$ delay gives more time to discriminate $\quad e^{-(k_c-k_0)t}$

Museum metaphor

- consider the challenge: "Identify Holbein fans in the Kunstmuseum".



Holbein fans stay 10 min
others 1 min

but there only 10% Holbein fans