Enzymes Biological Catalysts

Michelis - Menten Scheme

$$E + S \underset{k_b^1}{\overset{k_f^1}{\rightleftharpoons}} ES \underset{k_b^2}{\overset{k_t^2}{\rightleftharpoons}} E + P$$

$$\mathcal{E} + S \underset{\Longleftarrow}{\Longrightarrow} \mathcal{E}S \underset{\Longleftarrow}{\Longrightarrow} \mathcal{E} + P$$

$$\frac{d[S]}{dt} = -k_f^1 [\mathcal{E}][S] + k_b^1 [\mathcal{E}S]$$

$$\frac{d[P]}{dt} = k_f^2 [\mathcal{E}S] - k_b^2 [\mathcal{E}][P]$$

$$\frac{d[\mathcal{E}S]}{dt} = k_f^1 [\mathcal{E}][S] + k_b^2 [\mathcal{E}][P]$$
$$- k_b^1 [\mathcal{E}S] - k_f^2 [\mathcal{E}S]$$

$$\frac{d[S]}{dt} = -k_f^1[E][S] + k_b^1[ES]$$

$$\frac{d[P]}{dt} = k_f^2[ES] - k_b^2[E][P]$$

$$\frac{d[ES]}{dt} = k_f^1[E][S] + k_b^2[E][P]$$
$$- k_b^1[ES] - k_f^2[ES]$$

Total Enzyme is conserved

$$[E]_0 = [ES] + [E]$$

$$[E] = [E]_0 - [ES]$$

$$\frac{d[ES]}{dt} = k_f^1[E][S] + k_b^2[E][P]$$
$$- k_b^1[ES] - k_f^2[ES]$$

$$[E] = [E]_0 - [ES] \qquad \nearrow$$

$$\frac{d[ES]}{dt} = k_f^1\left([E]_0 - [ES]\right)[S]$$
$$+ k_b^2\left([E]_0 - [ES]\right)[P]$$

$$- (k_b^1 + k_f^2)[ES]$$
$$= -[ES]\left[k_f^1[S] + k_b^2[P] + k_b^1 + k_f^2\right] \quad \overset{\text{steady}}{\underset{\text{approx}}{\text{state}}}$$
$$+ k_f^1[E]_0[S] + k_b^2[E]_0[P]$$

$$[ES]_{SS} = \frac{(k_f^1[S] + k_b^2[P])[E]_0}{k_f^1[S] + k_b^1 + k_b^2[P] + k_f^2}$$

$$E + S \rightleftharpoons ES \qquad [E] = [E]_0 - [ES]$$

$$v = -\frac{d[S]}{dt} = k_f^1[E][S] - k_b^1[ES]$$

$$= k_f^1([E]_0 - [ES])[S] - k_b^1[ES]$$

$$v = \frac{\left(k_f^1 k_f^2 [S] - k_b^1 k_b^2 [P]\right)[\mathcal{E}]_0}{k_f^1 [S] + k_b^1 + k_b^2 [P] + k_f^2}$$

approximations

① Steady state, $[\mathcal{E}] << [S]$

② measure initial rate

$$[S] \approx [S]_0, \quad [P] \approx 0$$

$$v_{inital} \approx \frac{k_f^1 k_f^2 [S]_0 [\mathcal{E}]_0}{k_f^1 [S]_0 + k_b^1 + k_f^2}$$

$$v_{inital} \approx \frac{k_f^1 k_f^2 [S]_0 [E]_0}{k_f^1 [S]_0 + k_b^1 + k_f^2}$$

$$\text{bottom} = k_f^1 \left([S]_0 + \frac{k_b^1 + k_f^2}{k_f^1} \right)$$

units of
concentration
$\downarrow$

$$v_{initial} = \frac{k_f^2 [S]_0 [E]_0}{[S]_0 + \frac{k_b^1 + k_f^2}{k_f^1}}$$

$$K_M = \frac{k_f^2 + k_b^1}{k_f^1}$$

$\uparrow$
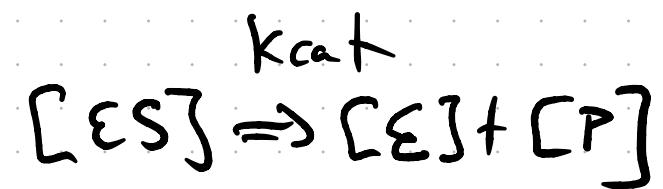
Michaelis constant

At early times

$$V_{initial} = \frac{k_2 [S]_0 [E]_0}{[S]_0 + K_m}$$

$$k_2 = k_{cat}$$

$$[ES] \xrightarrow{k_{cat}} [E] + [P]$$

2 limits

$[S]_0 << K_m$

$V_{initial}$ is proportional to $[S]_0$

$[S]_0 >> K_m$
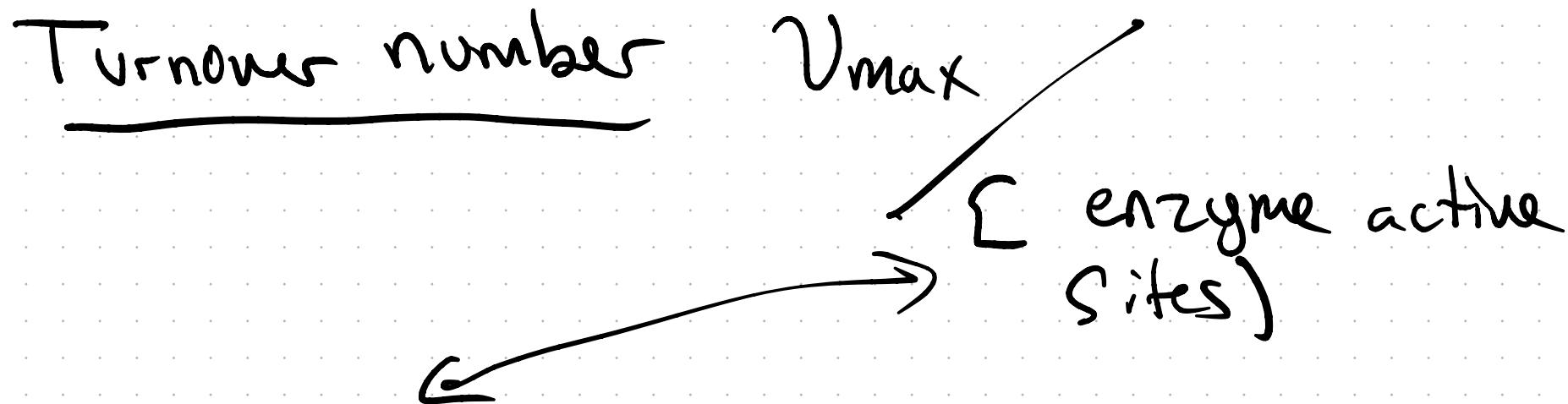
$V_{initial}$ is 0th order in $[S]_0$

$V_{max} = V_{initial} = k_{cat} [E]_0 \Leftarrow$ maximum rate

$$V_{initial} = \frac{V_{max}[S]_0}{[S]_0 + K_m}$$

if $[S]_0 = K_m$

$$V_{initial} = V_{max}/2 \quad \leftarrow \text{ can do this to find } K_m$$

Turnover number

V_{max} / [enzyme active sites)

$$[\text{active sites}] = [E]_0 \cdot (\# \text{ of sites/enzyme})$$

If # sites/enzyme = 1
then turnover number $= k_{cat}$

Catalytic Efficiency – how effectively
   an enzyme converts substrate to
   product

$$\epsilon = \frac{k_{cat}}{K_m} \quad - \text{units of } \frac{1}{M} \cdot \frac{1}{s}$$

range of $\epsilon \sim 1 - 10^{10} \frac{1}{Ms}$ $\quad \underset{\text{diffusion limited}}{\underline{10^8 - 10^{10}}}$

$$G = \frac{k_{cat}}{K_m} = \frac{k_{cat}}{\dfrac{k_{cat} + k_b'}{k_f'}} = \frac{k_f'}{1 + k_b'/k_{cat}}$$

Maximum, k_f' or k_{cat} big

or k_b' small

Introduction to Statistical thermodynamics
(Barrick chapter 9)

$$\frac{K_{on}}{K_{off}} = K_{eq} = e^{-\Delta G^\circ / RT}$$
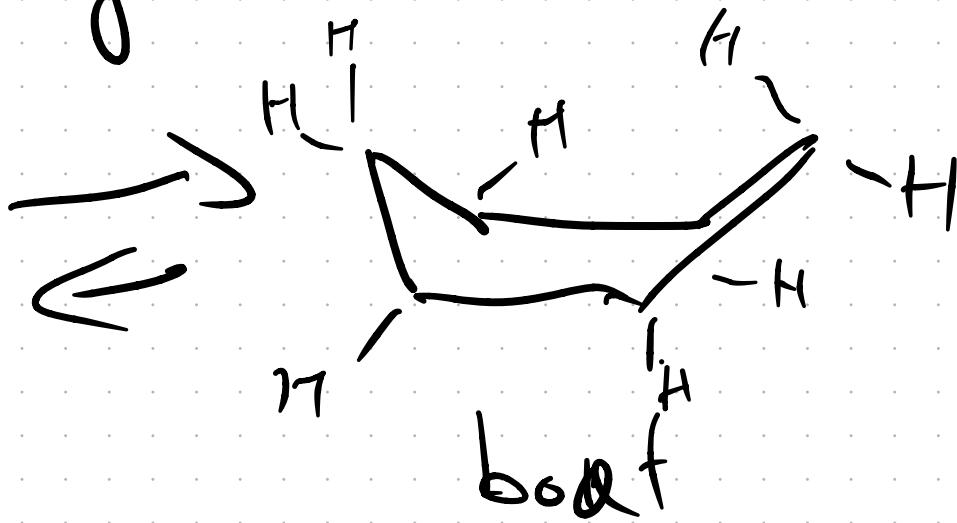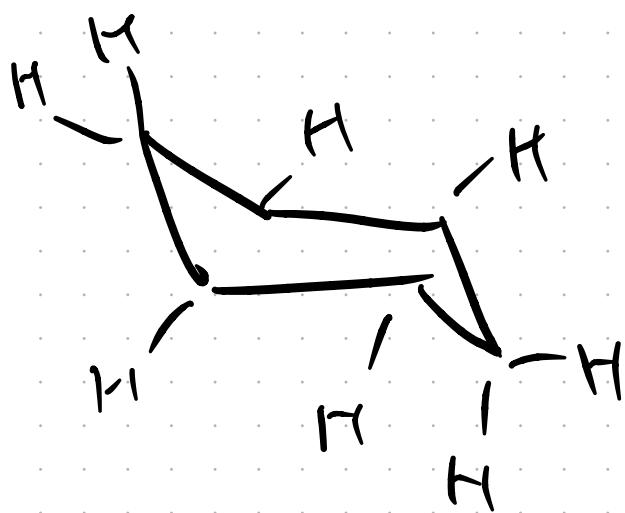
Didn't see how this arrises
    for a collection of molecules

Statistical thermodynamics connects
   <u>average</u> properties of sets of molecules
    with <u>Macroscopic</u> thermodynamic properties
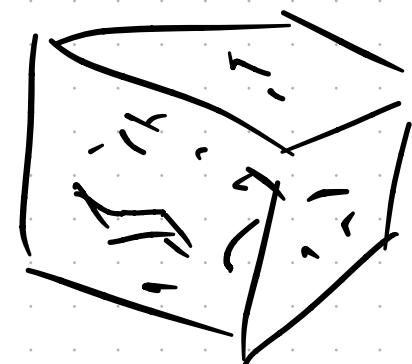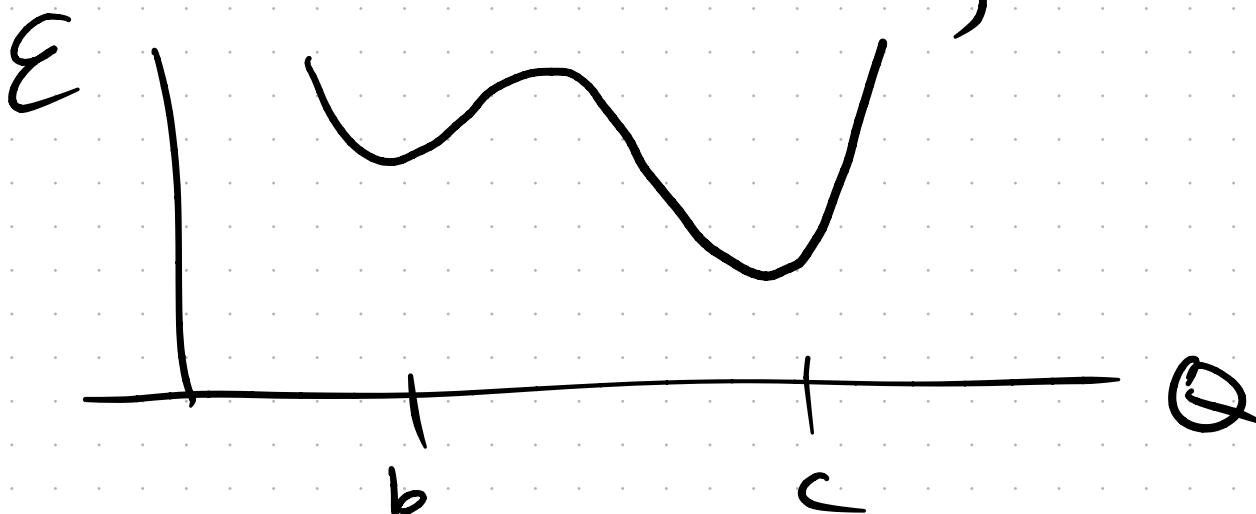
Molecular Example $A \rightleftharpoons B$
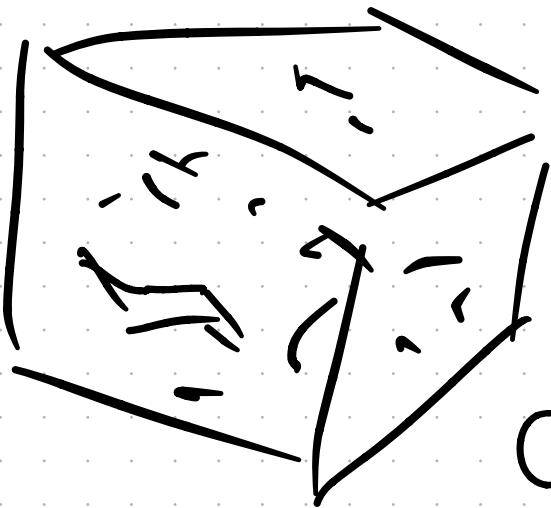
Molecule that goes between 2 states

Chair ← lower energy

boat

what if we could
watch this molecule
and see its cfg. vs time

Can do this with a simulation

Assume all molecules obey newton's equations

$$F_i = m_i a_i \quad \text{for every atom}$$
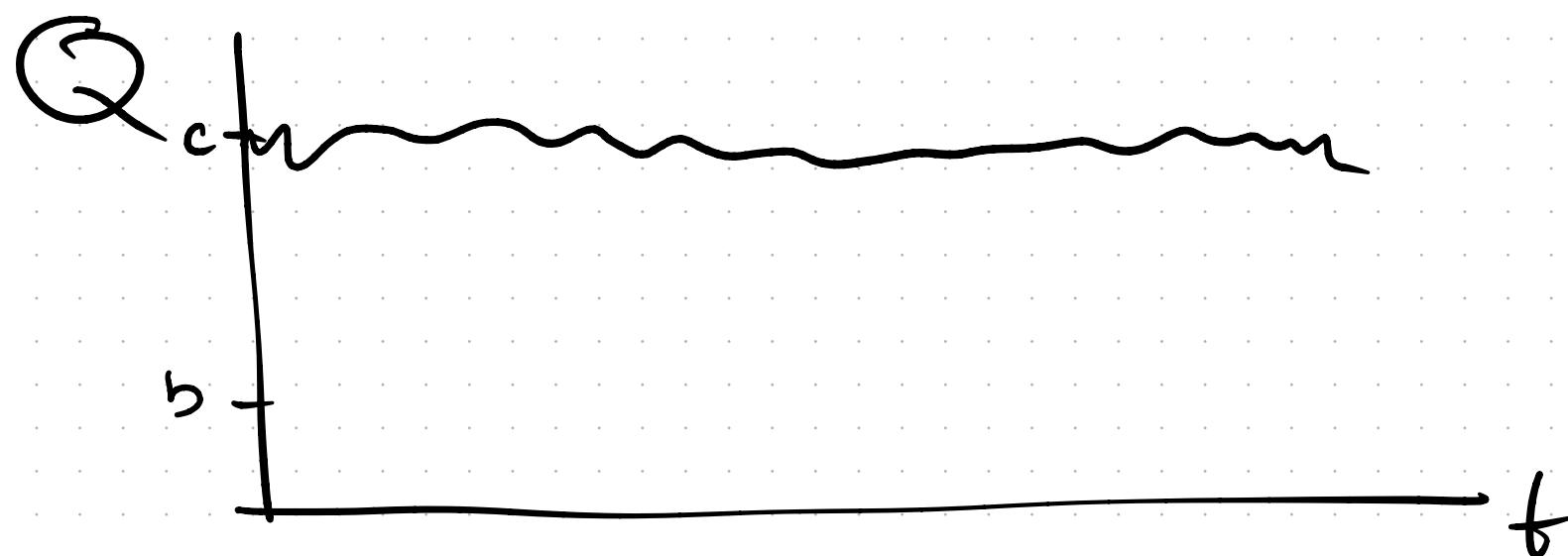
$$F_i = -\frac{\partial U}{\partial \vec{x}_i} \quad U(\vec{x}) \quad \text{force field}$$
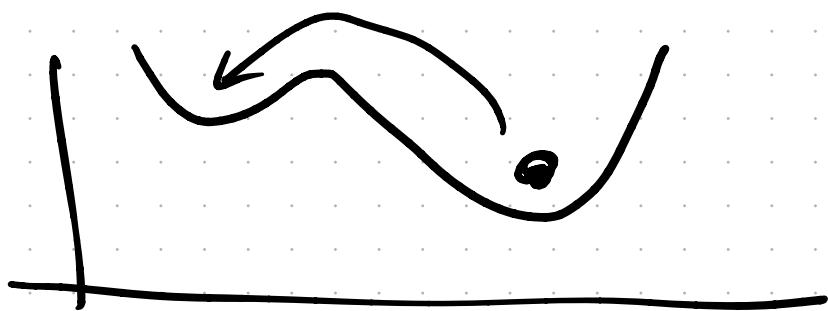
Problem is that real systems have $N \sim 10^{23}$
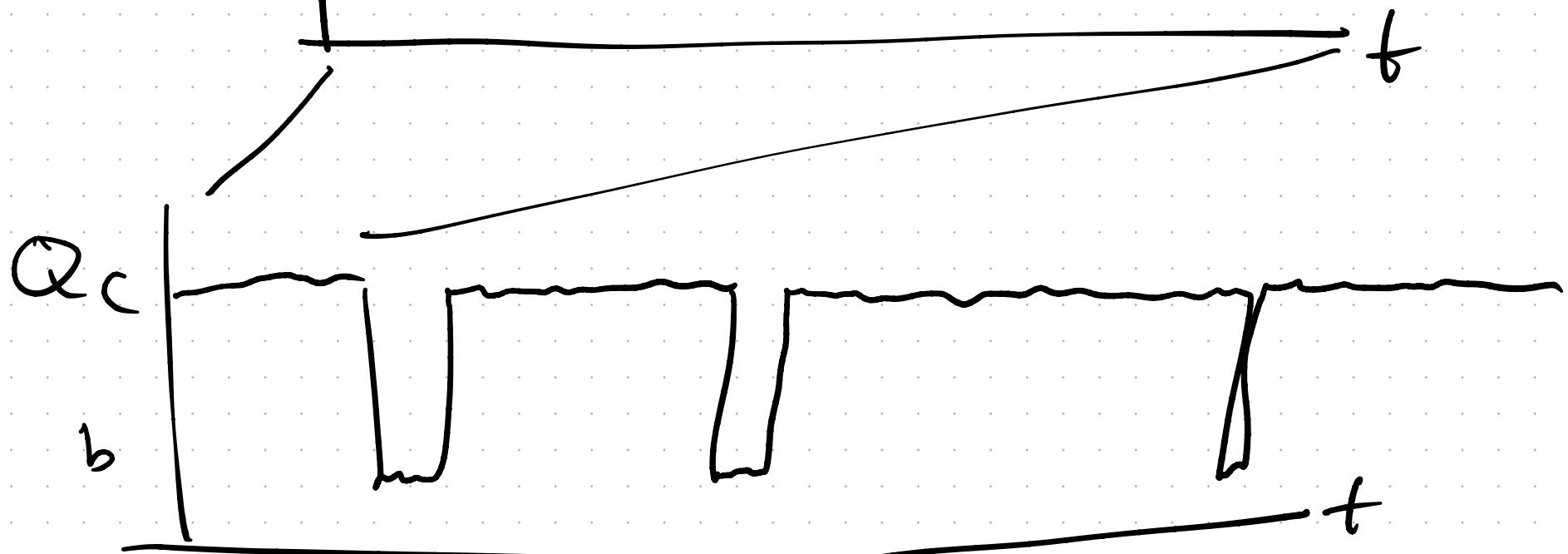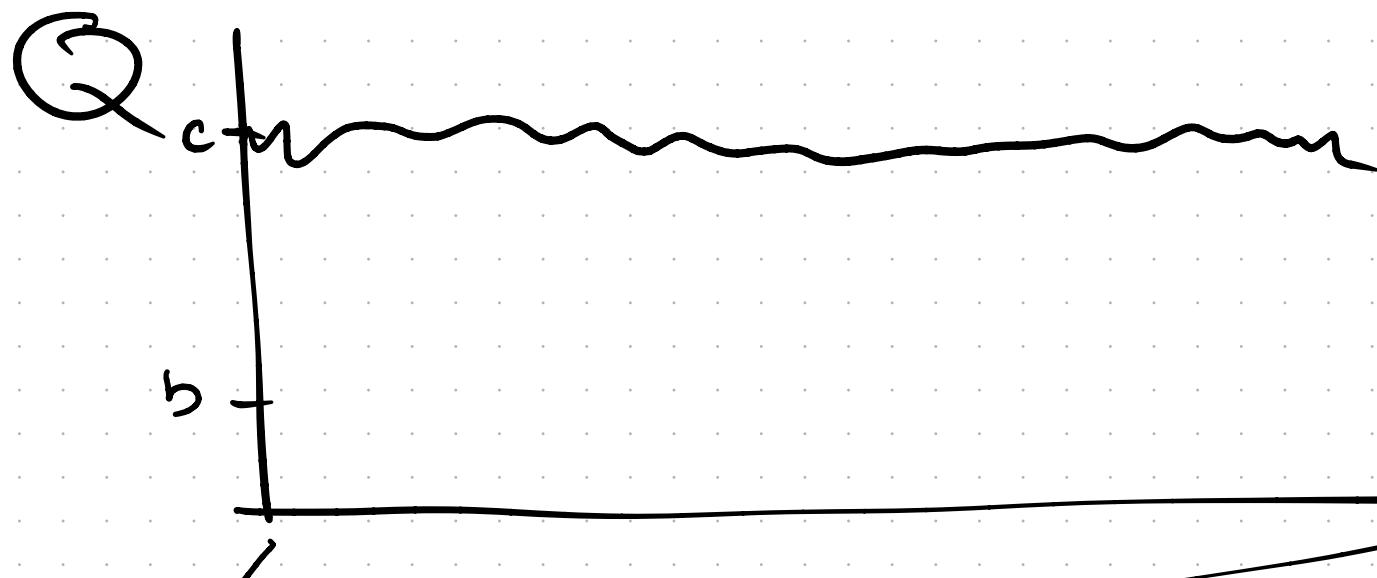
Because real systems are large we will treat them statistically
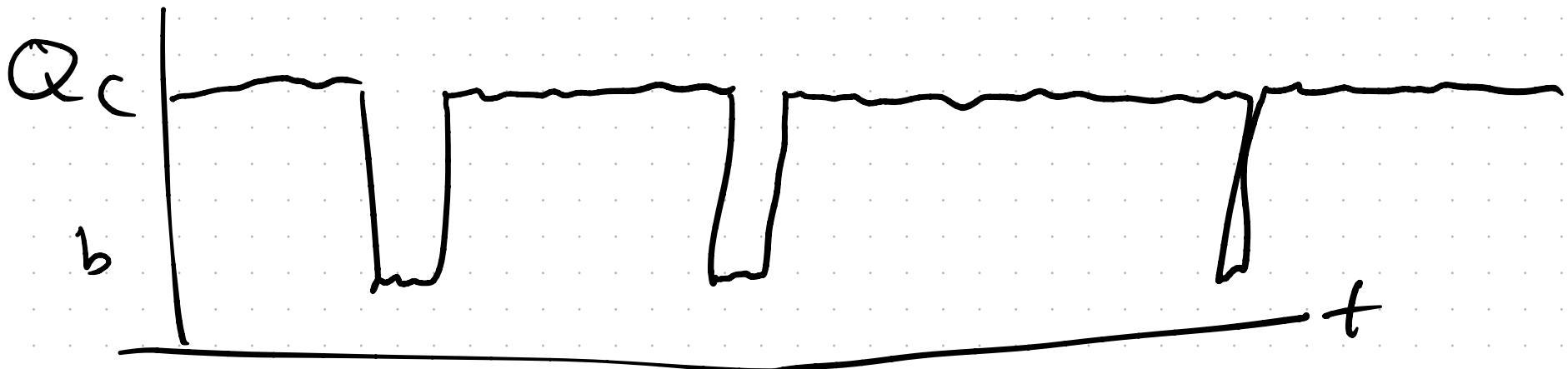
What would happen if we watch a 2 state molecule in H_2O

$$K_{c \to b} = A e^{-G^{\ddagger}/RT}$$

Q_c

b

t
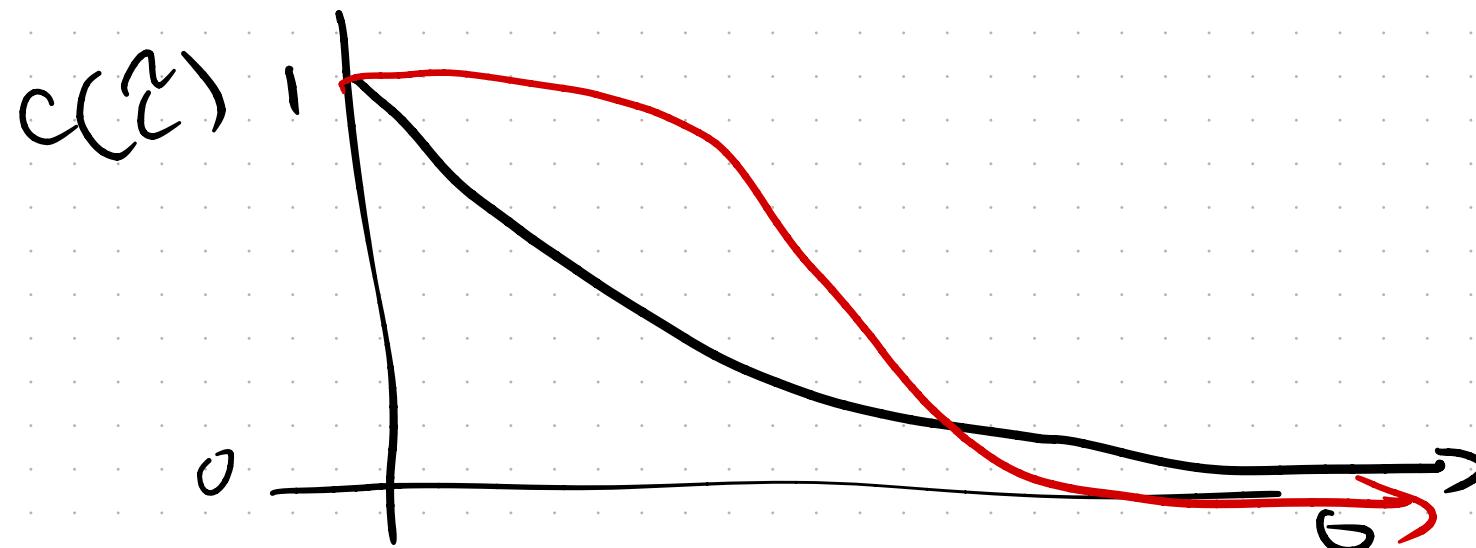
Auto correlation function
tells us over what period of time
is a molecule's configuration correlated
with itself

$C(\tau)$ small τ $C = 1$

long τ $C = 0$

$C(\tau)$

Compare $Q(t)$ to $Q(t+\tau)$

calculate $\langle Q \rangle = \dfrac{1}{N_t} \displaystyle\sum_{i=1}^{N_t} Q(t_i)$

$\delta Q(t) = Q(t) - \langle Q \rangle$
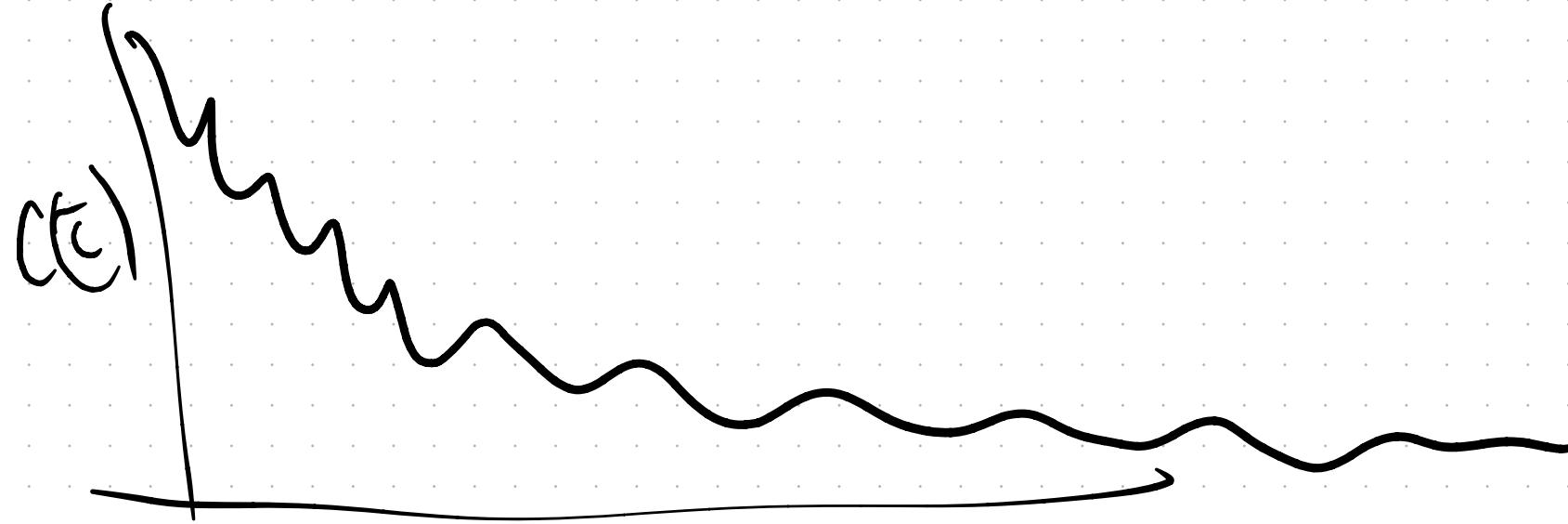
$\langle \delta Q(t)^2 \rangle = \langle (Q(t) - \langle Q \rangle)^2 \rangle = Var(Q)$

$$C(\tau) = \frac{\langle \delta Q(t_i + \tau) \, \delta Q(t_i) \rangle}{\langle \delta Q(t_i) \, \delta Q(t_i) \rangle}$$

@ $\tau = 0$ $C(0) = 1$

@ $\tau \to \infty$ turns $C(\tau \to \infty) = 0$

$$C(\tau) = \frac{\frac{1}{N} \sum_{i=1}^{N} \delta Q(t_i + \tau) \, \delta Q(t_i)}{\frac{1}{N} \sum_{i=1}^{N} (\delta Q(t_i))^2}$$

$C(t)$

define reaction time

$$\tau_{rxn} = \int_0^\infty C(\tau) d\tau \equiv \tau_{rxn}$$

$C(t) \approx e^{-a\tau}$ then $a = 1/\tau_{rxn}$