

## Reading for lecture 5

1. **Thermal Energy**
  2. **Diffusion**
  3. **Reaction Rates**
  4. **Catalysis**
- Howard, Chapters 4,5
  - Voet and Voet, Chapter (3), 13
  - Berg, H.C., "Random Walks in Biology" 152pp Princeton University Press, ISBN 0-691-00064-6

$$p_i = \frac{1}{Z} e^{-\frac{u_i}{kT}}$$

$$Z = \sum_i e^{-\frac{u_i}{kT}}$$

$$k = 1.38 \times 10^{-23} \text{ J/K (sometimes "k}_B\text{")}$$

$$kT = 4.1 \times 10^{-21} \text{ J} \sim 4 \text{ pN nm (at } 25^\circ \text{ C)}$$

Covalent bond:  $\sim 600 \text{ pN nm} = 150 \text{ kT}$

Hydrogen bond:  $\sim 30 \text{ pN nm} = 7.5 \text{ kT}$

Photon (green,  $\lambda=500 \text{ nm}$ ):  $397 \text{ pN nm} \sim 100 \text{ kT}$

## Thermal Energy

### **Boltzmann's Law**

[ Boltzmann's Equation ] (2)

Several ways to derive/justify this (Statistical Mechanics)

Based on the postulate that all micro-states are equally probable

Boltzmann distribution maximises  $W$  with the constraints  $S(N_i) = N$  and  $S(N_i E_i) = E$

Combining two systems into a larger system: probabilities multiply, energies add.

$Z$  ("partition function") is a normalizing factor

**$kT$  is a natural unit of energy**

Covalent bonds are unlikely to break (  $\exp(150) = 1064.5$  )

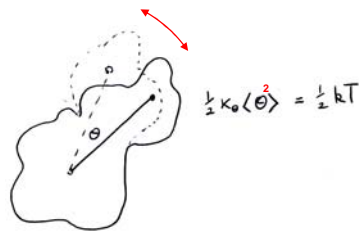
**Hydrogen bonds (and protein folds) are thermally labile (  $\exp(7.5) = 1800$  )**

Principle of Equipartition of Energy:

$\frac{1}{2} kT$  per degree of freedom

$$\left( \begin{array}{l} u(x) = \frac{1}{2} kx^2 \\ \langle u \rangle = \int_{-\infty}^{\infty} u(x) p(x) dx \\ = \frac{1}{2} k \int_{-\infty}^{\infty} x^2 \cdot \frac{1}{Z} e^{-\frac{kx^2}{2kT}} dx \end{array} \right)$$

.....



The diagram shows a protein structure with a red arrow indicating a degree of freedom. The equation  $\frac{1}{2} k_B \langle \theta^2 \rangle = \frac{1}{2} kT$  is written next to it.

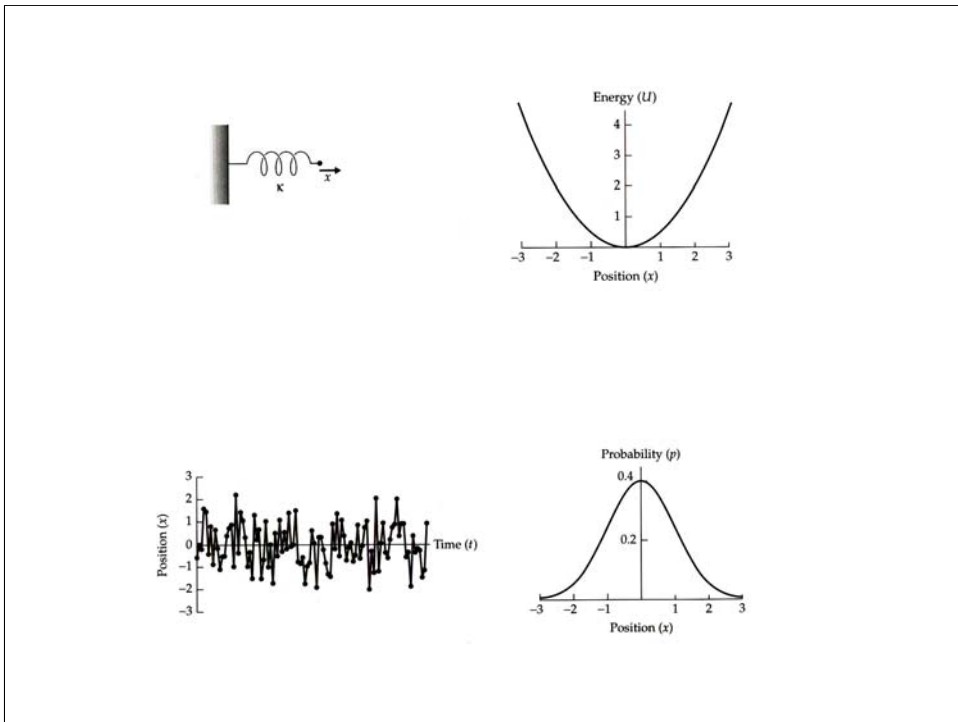
[Equipartition of Energy] (3)

Familiar from molecular velocity distributions in a gas for Kinetic Theory

Factor of half only if energy is quadratic function of degree of freedom (eg  $\sim kx^2$  or  $mvx^2$ ). But always  $\langle U \rangle \sim kT$ .

Proteins are constantly in motion

For proteins or other particles in water, this energy is due to constant collisions with water molecules...



[Brownian Motion] (4)

Tethered Brownian motion (optical tweezers in lecture 10)

Free Brownian Motion  $\gg$  Diffusion...

(No. per unit area in bin  $x$ )  $C(x) \Delta x$

(Nett no. moving from L->R)

$$J(x) \Delta t = \frac{1}{2} [ C(x) - C(x+\Delta x) ] \Delta x$$

$-\Delta C$

So:  $J(x) = -\frac{1}{2\Delta t} \Delta C \frac{\Delta x^2}{\Delta x} = -\frac{\Delta x^2}{2\Delta t} \left( \frac{\Delta C}{\Delta x} \right)$

Limit ( $\Delta x \rightarrow 0$ ):

$J(x) = -D \frac{\partial C}{\partial x}$ 

**Fick's Law**

$D = \frac{\Delta x^2}{2\Delta t}$ 

**Diffusion Coefficient**

Conservation of particle number:

$\frac{\partial C}{\partial t} \left( = -\frac{\partial J}{\partial x} \right) = D \frac{\partial^2 C}{\partial x^2}$ 

**Diffusion Equation**

## Diffusion

[Diffusion as Random Walk ] (5)

Assumption is that in some characteristic time  $Dt$ , each particle will move either way at random into the next "bin" of width  $Dx$ .

Particles flow from where there are many to where there are few.

$Dx$  (~mean free path?) and  $Dt$  (~ m.f.p. / mean velocity?) are not well defined, but  $D$  can be measured by comparing observed concentration and flux to solutions of the Diffusion Equation.

(NB: the constant  $D$  is proportional to  $Dx^2 / Dt$ . Different from "normal" translational motion where  $\mathbf{v} = Dx / Dt$  is constant.)

How far and how fast do molecules diffuse?

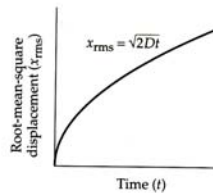
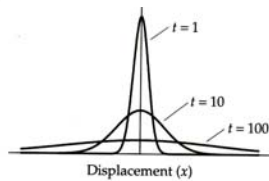
$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} \quad \text{Diffusion Equation}$$

One solution:  $C(x,t) = \frac{C_0}{\sqrt{4\pi Dt}} \exp\left\{-\frac{x^2}{4Dt}\right\} : t > 0$  (Test it by substitution)

At time  $t$ :  $C \propto \exp\left\{-\frac{x^2}{2\sigma^2}\right\}$ ,  $\sigma^2 = 2Dt = \langle x^2 \rangle$

**Gaussian Distribution**

Concentration



$$C(x,0) = C_0 \delta(x)$$

(b.c. – molecule[s] released at  $x=0$  at  $t=0$ )

How far and how fast do molecules diffuse?

[ Free diffusion ] (6)

$(\langle x^2 \rangle)^{1/2} = x_{rms} = (2Dt)^{1/2}$  is a measure of how far the particles get, on average, in time  $t$ .

For short times and distances, diffusion is very fast

K<sup>+</sup> ion in water goes 1 micron in 0.25 ms, 0.1mm in 2.5s

For long times and distances, diffusion is very slow.

K<sup>+</sup> ion goes 1 m in 8 years

Lord Kelvin's demonstration in Glasgow is still going after more than a Century

Drift velocity due to external force:  $v = \frac{F}{\gamma}$  (Low Reynold's Number)  $\gamma = 6\pi\eta r$  Stokes' Law

↓  
Viscous Drag coefficient

Diffusion with external force:  $J = -D \frac{\partial C}{\partial x} + \frac{F}{\gamma} C$  ;  $F = -\frac{\partial u}{\partial x}$

At Equilibrium:  $J = 0$  &  $C(x) = C_0 e^{-\frac{u(x)}{kT}}$

$\frac{\partial C}{\partial x} = \frac{1}{D\gamma} \left( -\frac{\partial u}{\partial x} \right) C$

$\frac{\partial C}{\partial x} = -\frac{1}{kT} \cdot \frac{\partial u}{\partial x} \cdot C$

Equating expressions:  $D\gamma = kT$  or  $D = \frac{kT}{\gamma}$  **Einstein Relation**

Video of big and small beads?

Diffusion coefficient can be related to viscous drag coefficient using the Einstein Relation

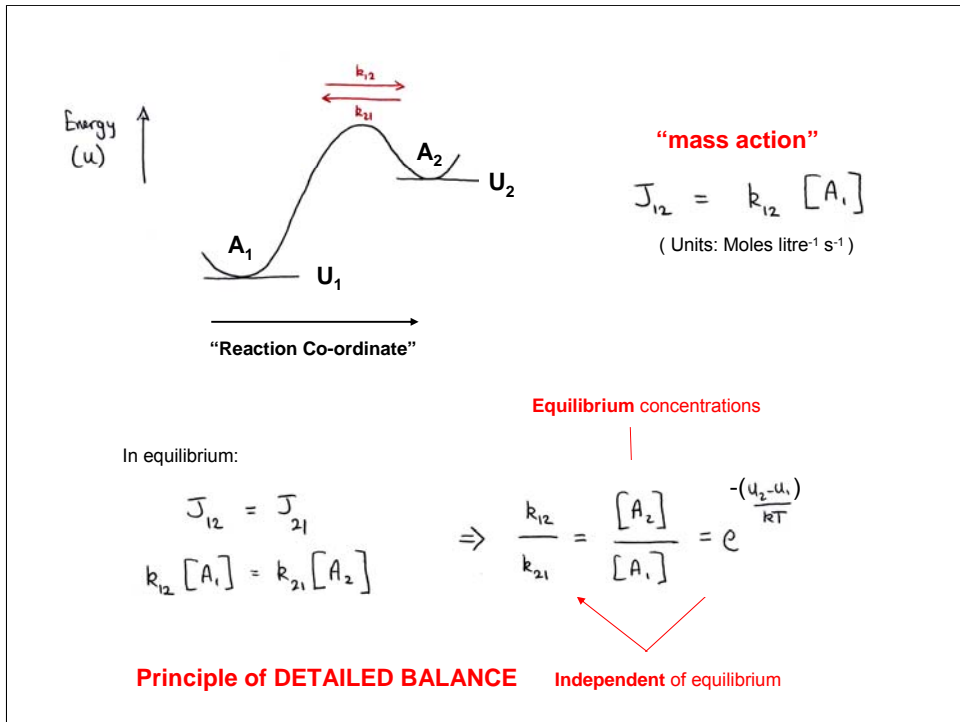
[ Einstein Relation ] (7)

D is constant, so the expression applies even out of equilibrium (when  $J \neq 0$  and concentration does not obey Boltzmann distribution).

Can calculate D from size or vice versa. Shape doesn't matter much for small particles.

Small particles diffuse faster – less averaging of molecular collisions.

(nb. Effective size for ions includes “solvation shell” of water molecules)



## Reaction Rates

So far we've talked about particles (which could be molecules) and how they are constantly in motion due to thermal agitation.

Thermal energy can also make them change into something else – “uni-molecular” reaction.

Or make them collide and react with each other – “bi-molecular” reaction.

What can we say about these reactions?

[ Reaction diagram ] (8)

“Reaction co-ordinate”

Treating it as **instantaneous** transition between states works if transitions are fast compared to typical lifetimes in each state.

Law of **Mass action**: Number of molecules that make a transition is proportional to the number that are available. (Sort of obvious).

Rate constants in units of s<sup>-1</sup> so that  $k dt$  is the **fraction** of molecules in a given state that make a transition in a given time.

**Notation**:  $[A]$  is the concentration of A in units “**Molar**” = **moles per litre**

1 mole is Avogadro's number of molecules

Avogadro's number is HUGE and is chosen so that one mole weighs the same number of grams as the sum of atomic numbers for the molecule (“formula weight”)

As before, ratio of rate constants is **always**  $\exp(DU / kT)$ , even out of equilibrium.



$$G = U - TS \quad \text{"Free energy"}$$

("Gibbs" Free energy only if  $U = H = U_0 + PV$ )

$$p_i \propto e^{-\frac{G}{kT}} = e^{-\frac{U}{kT}} \cdot e^{\frac{S}{k}}$$

Free energy includes statistical weights

$$\text{but: } S_i = k \ln W_i \Rightarrow p_i \propto W_i e^{-\frac{U}{kT}}$$

Free energy indicates the spontaneous direction of a reaction

$$\Delta G_{\text{system}} = \Delta U_{\text{system}} - T \Delta S_{\text{system}}$$

$$\text{but: } \Delta U_{\text{system}} = \Delta Q = -T \Delta S_{\text{surroundings}}$$

$$\begin{aligned} \text{so: } \Delta G_{\text{system}} &= -T (\Delta S_{\text{system}} + \Delta S_{\text{surroundings}}) \\ &= -T \Delta S_{\text{universe}} \\ &< 0 \end{aligned}$$

## Free Energy

Each state is really an ensemble of **microstates**

eg. Quantum mechanical energy levels

eg. Brownian motion – various positions of a protein domain within one “state”

If a “state” has a molecule free to diffuse, it will have many more microstates than if that molecule is stuck down.

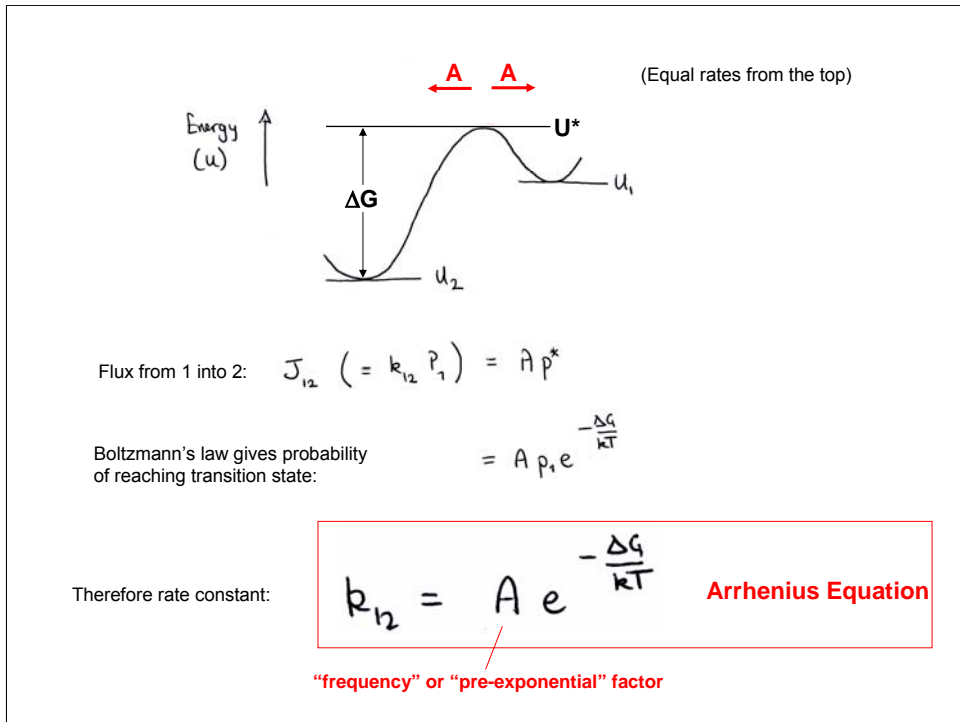
For proteins the state boundaries are “drawn on” to the energy landscape

The more microstates, the more probably the macro-state – **Entropy Increases**

[ Free Energy ] (9)

“free” in the sense of being available to do work without violating the 2nd law of thermodynamics.

Use G instead of U for all rate calculations where each state is really an ensemble of states.



### [ Transition Rates ] (10)

Transition state has high energy and low probability => transitions can be treated as instantaneous.

Arrhenius equation immediately satisfies the requirement  $k_{12}/k_{21} = \exp(DG / kT)$ ,

Predicted temperature dependence is verified experimentally

Arrhenius Plot:  $\ln(\text{rate})$  vs  $1/T$

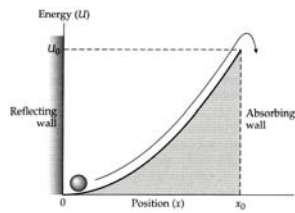
•What is the frequency factor ?

**Eyring Theory:** Quantum mechanical oscillations

$$A \approx \frac{kT}{h} = 6 \times 10^{12} \text{ s}^{-1}$$

Appropriate for single-bond transitions, electronic transitions, light absorption

**Kramers Theory:** Diffusion out of energy well



•Solve Diffusion Equation for this potential, particle starting at  $x=0$ .

$t_0$  = mean time to the top

$\varepsilon$  = probability to go over rather than come back

$$A = \frac{\varepsilon}{2t_0} \quad ; \quad t_0 = \frac{\gamma}{\kappa} \left( \frac{\pi kT}{4\Delta G} \right)$$

Appropriate for protein shape changes – except that potentials are not really quadratic.

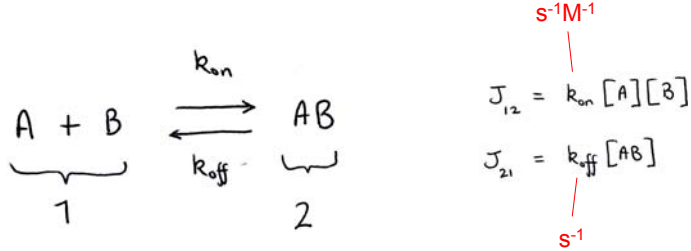
[ Frequency Factor ] (11)

Eyring and Kramers theories are both approximate

Protein rate constants are typically  $\sim 10^{-3} \text{ s}^{-1}$ , which gives  $\Delta G \sim 22 \text{ kT}$ . ( $\sim 4$  H-bonds)

In practice, rate constants are measured empirically rather than calculated

Bi-molecular Reactions: Simple Binding



At equilibrium:

$$J_{12} = J_{21} \Rightarrow \frac{[A][B]}{[AB]} = \frac{k_{\text{off}}}{k_{\text{on}}} = K_m$$

Dissociation Constant (units, M)

[Bi-molecular Reactions] (12)

( $k_{\text{on}}$  has units of  $\text{s}^{-1}\text{M}^{-1}$  : “Bi-molecular” rate constant)

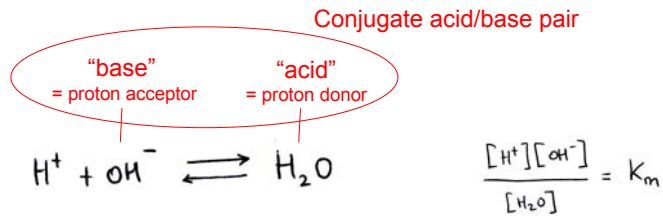
$K_m$  the probability of dissociation.

Diffusion-limited collision rates can be calculated (see Berg Ch 3)

Actual reaction rates are 100-1000x slower, as molecules still have to surmount the activation barrier after they find each other.

100-1000x gives  $\sim 5kT$ , but activation barriers may be higher than this since once molecules are close they will have many encounters before diffusing (far) apart.

Water acid-base equilibrium:



In fact, water dissociates very little (low  $K_m$ ), and:

$$[\text{H}^+][\text{OH}^-] = 10^{-14} \text{ M}^2$$

$$[\text{H}_2\text{O}] = 55.5 \text{ M}$$

Define: **pH = -log<sub>10</sub>[H<sup>+</sup>]**

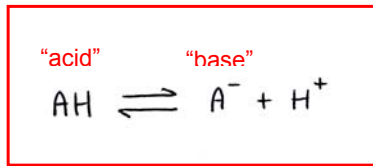
(In neutral (pure) water, pH = 7  
as there are equal concentrations  
of H<sup>+</sup> and OH<sup>-</sup>)

[ pH ] (13)

Very low concentrations of H<sup>+</sup> and OH<sup>-</sup>

However, pH is very important as it dictates whether “charged” molecules really are charged...

general acid-base equilibrium:



$$\frac{[\text{A}^-][\text{H}^+]}{[\text{AH}]} = K_m$$
$$\Rightarrow \frac{[\text{A}^-]}{[\text{AH}]} = \frac{K_m}{[\text{H}^+]}$$

Take log of both sides:

$$\log_{10} \left( \frac{[\text{A}^-]}{[\text{AH}]} \right) = -\log_{10} [\text{H}^+] + \log_{10} K_m$$

Define:  $\text{pK} = -\log_{10}[\text{K}_m]$

$$= \text{pH} - \text{pK}$$

[ acid/base equilibrium ] (14)

pK is the pH at which a site has equal probability of being charged or neutral.

High pH = low [H<sup>+</sup>] = sites tend to lack protons unless they have high pK

High pK sites are more likely to be protonated – eg NH<sub>3</sub><sup>+</sup> not NH<sub>2</sub>

Low pK sites are more likely to be de-protonated – eg COO<sup>-</sup> not COOH

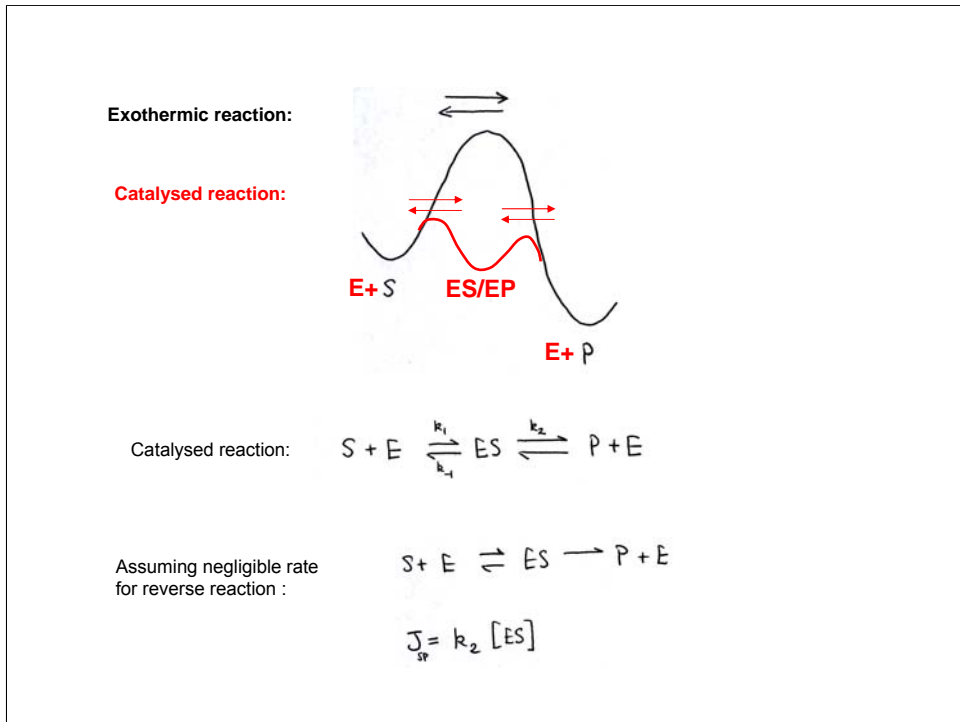
“Strong acids” like HCl have low pK – nearly all H become H<sup>+</sup>, so pH goes low.

“Strong alkalis” (Na)OH have high pK (~13 for the “base” OH<sup>-</sup>, compare slide 13) – nearly all OH<sup>-</sup> mop up a proton, so pH goes high.

“Weak acids” like acetic acid (pK close to 7) act as “**buffers**” at pH values close to their pK.

Small amounts of extra H<sup>+</sup> added (as “strong acid” eg HCl) are “mopped up” by small shifts in the fraction of dissociated weak acid.

Buffering is extremely important in biology: keeps pH steady (usually close to 7) and therefore charged groups on proteins etc in their correct state.



## Catalysis

[ Catalysis] (15)

Nearly all reactions in biology are catalysed

Usually extremely specific – only the right enzyme catalyses the right reaction.

Protein machines have complicated mechanisms – can be thought of as a series of catalysed reactions.

Michaelis-Menten:

$$[ES] = -k_{off} [ES] + k_{on} [S][E]$$

total enzyme:

$$[E]_T = [E] + [ES]$$

$$\therefore [ES] = -k_{off} [ES] + k_{on} [S] ([E]_T - [ES])$$

$$= -(k_{off} + k_{on} [S]) [ES] + k_{on} [S] [E]_T$$

steady state:

$$0 = -k_{off} [ES] + k_{on} [S] [E]_T$$

$$k_{off} [ES] = k_{on} [S] [E]_T$$

$$\frac{[ES]}{[E]_T} = \frac{k_{on} [S]}{k_{off} + k_{on} [S]}$$

$$\frac{[ES]}{[E]_T} = \frac{[S]}{K_m + [S]} \quad \& \quad J_{sp} = \frac{V_{max} [S]}{K_m + [S]}$$

## [ Catalysis rates – Michaelis Menten Kinetics ] (16)

(Derivation in problem set)

This is a typical **saturation** curve

Same relation for bound fraction in simple binding/unbinding reactions

As  $S \rightarrow \infty$ ,  $J \rightarrow C$ , proportional to  $E_t$  : saturating substrate concentration, all enzyme has substrate.

As  $S \rightarrow 0$ ,  $J \rightarrow (V_{max}/K_m)S$  : proportional to  $S$

When  $S = K_m$ , rate is half-maximal, and half of enzyme has substrate.

Much more complex steady-state kinetics can be done.

Many reactants and products

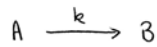
Sequences of reactions

These calculations give overall reaction rates, but DO NOT say anything about details of the mechanism

HOWEVER, they do allow possible mechanisms to be tested by comparing predicted rates to measured rates. – Lectures 9 and 10.



**Transient kinetics:**

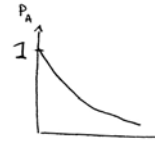


Probability of particle  
still being in state A  
at time t :

$$\frac{dP_A}{dt} = -kP_A$$

$$\Rightarrow P_A = A e^{-kt}$$

( b.c. :  $P(0) = 1$ , so  $A=1$  )



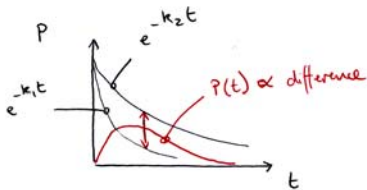
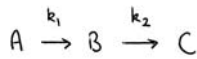
Probability of particle  
Making a transition between  
time t and t+dt :

$$P_t(t) = k P_A(t)$$

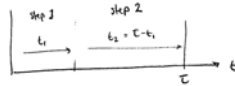
( mean lifetime =  $\int t \cdot P_t dt = 1/k$  )

[ Interval length distributions – single-step] (17)

Sequential steps:



Two successive steps



Let  $P_1(t) = k_1 e^{-k_1 t}$   $\left[ \begin{array}{l} \text{normalization: } \int_0^\infty P_1(t) dt = 1 \\ \text{+ to } P_2(t) \text{ via } P_1(t) \text{ solution} \end{array} \right]$   
 $P_2(t) = k_2 e^{-k_2 t}$

$$\begin{aligned} P(t) &= \int_0^t dt_1 P_1(t_1) P_2(t-t_1) \\ &= k_1 k_2 e^{-k_2 t} \int_0^t e^{-(k_1 - k_2)t_1} dt_1 \\ &= \frac{k_1 k_2}{-(k_1 - k_2)} e^{-k_2 t} \left[ e^{-(k_1 - k_2)t_1} \right]_0^t \\ &= \frac{-k_1 k_2}{k_1 - k_2} e^{-k_2 t} \left[ e^{-(k_1 - k_2)t} - 1 \right] \\ &= \frac{k_1 k_2}{k_1 - k_2} \left[ e^{-k_2 t} - e^{-k_1 t} \right] \end{aligned}$$

check  $\rightarrow$  symmetric if  $k_1$  and  $k_2$  are swapped.

[ Interval length distributions – double-step] (18)

Transient kinetics gives more information

Can either think of it as non-steady-state reaction rate

OR as the probability that a certain molecule reacts at a given time.

More on this in problem sets and in lecture 10.