

Chapter 14: Sedimentation Velocity

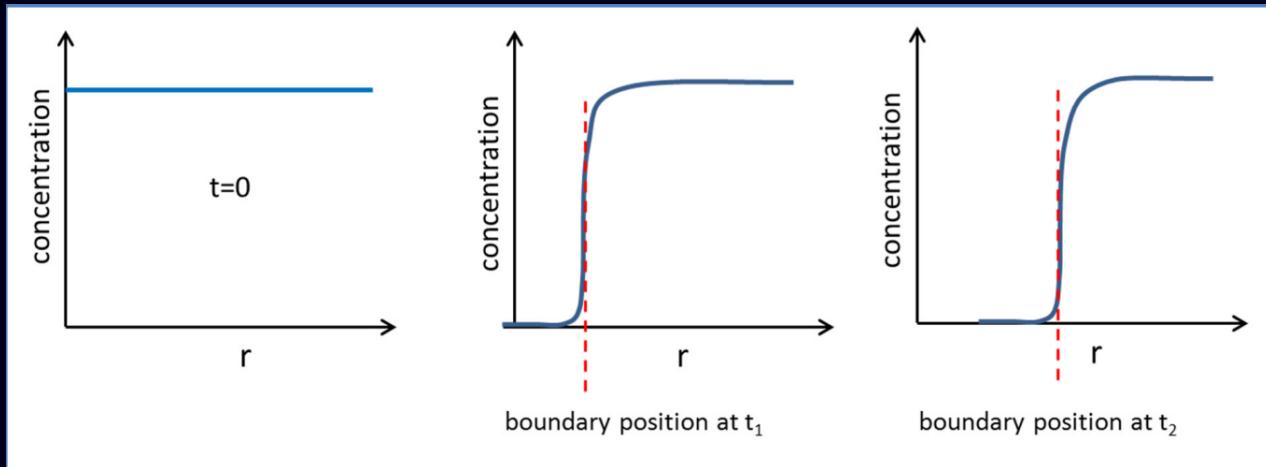
The external force (on a per molecule basis) due to centrifugation is $m\phi\omega^2r$, where ϕ is the **density increment**, ω is the **angular velocity**, m is the **mass**, and r is the **radius**. The **opposing frictional force** is $-fv$. When setting the sum of these forces to zero (terminal velocity), the equation yields:

$$V = \frac{m\phi\omega^2r}{f} \quad (1)$$

or, on a per mole basis:

$$V = \frac{M\phi\omega^2r}{N_A f} \quad (2)$$

How do you visualize velocity v in a velocity sedimentation experiment? If the sample begins with the macromolecule uniformly distributed (concentration is equal everywhere in tube), then when centrifugation begins, the **top region** (low r) of the sample will lose its macromolecules.



The sedimentation velocity v is the speed of the boundary: $\frac{\Delta r}{\Delta t}$

Meaning and Measurement of Sedimentation Coefficient, s

How does the sedimentation velocity v relate to molecular properties? From the equation $V = \frac{M\phi\omega^2 r}{N_A f}$, v is affected by both molecular properties (M) and experimental parameters (ω). We want to separate the two kinds of variables on different sides of the equation:

$$\frac{v}{\omega^2 r} = \frac{m\phi}{f} \quad (3)$$

Now, introduce **sedimentation coefficient** s to be equal to those quantities. s is obtained experimentally using $s = \frac{v}{\omega^2 r}$. s is obtained using molecular properties through:

$$s = \frac{M\phi}{N_A f} \text{ or } s = \frac{m\phi}{f} \quad (4)$$

If angular velocity $\omega \uparrow$, then the sedimentation velocity $v \uparrow$ from eq. (1). But the sedimentation coefficient s is unaffected. We know this is true because from **equation 4**, we can relate s to molecular properties without reference to experimental parameters.

Since v is defined as $\frac{dr}{dt}$ we can determine that:

$$s = \frac{\frac{dr}{dt}}{\omega^2 r} = \frac{\frac{d\ln(r)}{dt}}{\omega^2} \quad (5)$$

Measuring the position r of the boundary at a series of time points during the experiment and plotting them as **ln(boundary position)** vs. t should give a straight line with slope $s\omega^2$, which can be used to find s .

A special unit is used to express the value of the sedimentation coefficient (natural units are seconds). The **Svedberg**, S is defined as 10^{-13} sec.

Relating s to molecular properties

The sedimentation coefficient relates to molecular properties in two ways:

- dependence on mass
- frictional coefficient f (which depends on size \rightarrow mass)

Starting with $s = \frac{m\phi}{f}$, where $f = 6\pi\eta R \rightarrow s = \frac{m\phi}{6\pi\eta R}$

But R relates to volume and mass according to $V = \frac{4}{3}\pi R^3 \rightarrow R = (\frac{3V}{4\pi})^{\frac{1}{3}}$

Relate volume V to mass m by the density $\rightarrow m = V\rho$ and substitute V for $\frac{m}{\rho}$

$$s = \frac{m\phi}{6\pi\eta(\frac{3m}{4\pi\rho})^{\frac{1}{3}}}$$

$$s = m^{\frac{2}{3}}\left(\frac{\phi}{6\pi\eta}\right)\left(\frac{4\pi\rho}{3}\right)^{\frac{1}{3}} \text{ or } s = \left(\frac{M}{N_A}\right)^{\frac{2}{3}}\left(\frac{\phi}{6\pi\eta}\right)\left(\frac{4\pi\rho}{3}\right)^{\frac{1}{3}}$$

Do larger molecules sediment faster or slower than small molecules of equal density and similar shape? Centrifugal force on an object is proportional to mass, but opposing force is only $\frac{1}{3}$ power of the mass.

So larger molecules move faster, according to $\frac{2}{3}$ power of their mass.

This assumes that the protein or molecule is nearly spherical since we used Stoke's Equation. If the molecule of interest is **non-spherical**, then it will have the same centrifugal force but ↑ frictional force $\rightarrow \downarrow$ sedimentation coefficient s. This will result in a lower estimation for molecular weight.

Combining s and D (diffusion coefficient) to get molecular weight without a spherical assumption

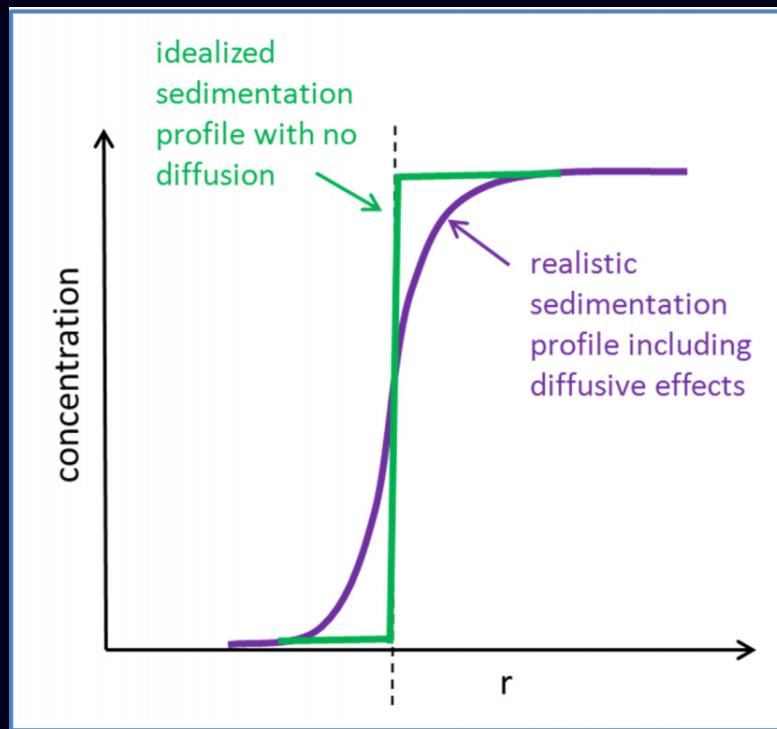
We can eliminate the assumption of spherical shape if we have measured values for s and D together. Having both values cancels frictional coefficient out. We know that $f = \frac{K_B T}{D}$ and from equation (4), $f = \frac{M\phi}{N_A s}$. If we equate the two together:

$$f = \frac{K_B T}{D} = \frac{M\phi}{N_A s}$$

$$M = \left(\frac{RT}{\phi}\right)\left(\frac{s}{D}\right)$$

Molecular weight relates only to the ratio of s to D, shape is no longer considered (frictional coefficient).

The diffusion coefficient D can be obtained from the sedimentation experiment itself. Under ideal conditions without diffusion, the concentration profile at the boundary would be perfectly steep. At a position just before the boundary layer, there will be no macromolecules. Right at the boundary layer, there will be a concentration of macromolecules that is the same all throughout the tube after that layer.



Chapter 15: Chemical Reaction Kinetics

Reaction Velocity, v

If we consider a reaction to be reactants → products, then reaction velocity v is the frequency (#/time) wth which the event is occurring per unit volume. The units of v are $\frac{\#}{\text{volume} \times \text{time}}$ or M/sec. There is only one velocity associated with the reaction, even though multiple reactants and products may be involved.

The reaction velocity is indicated equally by the rate of change of **any** of the species involved:



$$\begin{aligned}\frac{-d[A]}{dt} &= \alpha v \\ \frac{-d[B]}{dt} &= \beta v \\ \frac{d[C]}{dt} &= \gamma v \\ \frac{d[D]}{dt} &= \delta v\end{aligned}$$

Rearranging to isolate velocity v gives

$$v = -\left(\frac{1}{\alpha}\right)\left(\frac{d[A]}{dt}\right) = \left(\frac{1}{\beta}\right)\left(\frac{d[B]}{dt}\right) = \left(\frac{1}{\gamma}\right)\left(\frac{d[C]}{dt}\right) = \left(\frac{1}{\delta}\right)\left(\frac{d[D]}{dt}\right) = \dots$$

Evidently, if we measure the rate of change of concentration of some species, then we measured reacton velocity v .

Rate laws: how v depends on concentrations

Velocity of a reaction depends on how concentrated the reactants are. Besides concnetration, different reactions (different chemical species) will have different v according to likelihood of underlying chemical events → k

Combined dependence of v on rate constant k and concentrations → **rate law**.

Consider the equation: $A \xrightarrow{k} B$

The rate law is $v = k[A]$, this reaction is first order in A.

Now consider $2A \xrightarrow{k} B \Rightarrow v = k[A]^2$, and the reaction is second order in A.

If the equation is now $A + B \xrightarrow{k} C$, the rate law is now $v = k[A][B]$.

Relationship of rate constants to equilibrium constants

What about if we had reversible processes? Velocities of forward and reverse reactions depend on the concentrations of reactants and products, respectively. When the concentrations are reached where forward and reverse reactions are equal, no **net** conversion is occurring. This is **chemical equilibrium**. Consider the reaction:



The **forward** reaction velocity is $v = k_1[A]^2$

The **reverse** reaction velocity is $v = k_{-1}[B]$

At **equilibrium**, $k_1[A]^2 = k_{-1}[B]$ and $\frac{k_1}{k_{-1}} = \frac{[B]}{[A]^2}$

The ratio of the rate constants is equal to the equilibrium constant K.

Integrating Rate Laws

For simple reactions, we can integrate the differential equations that come from rate law to describe how concentrations of reactants and products change over time

1st Order Decay



To get a differential equation in terms of [A], combine two points:

- First need $v = \frac{-d[A]}{dt}$
- Also need $v = k[A]$

If we equate them together, we get the following:

$$\frac{-d[A]}{dt} = k[A]$$

$$\int \frac{d[A]}{[A]} = -k \int dt$$

$$\ln[A]|_{[A]_0}^{[A]} = -kt|_0^t$$

This gives us the familiar first order decay equations

$$\ln\left(\frac{[A]}{[A]_0}\right) = -kt \quad (8)$$

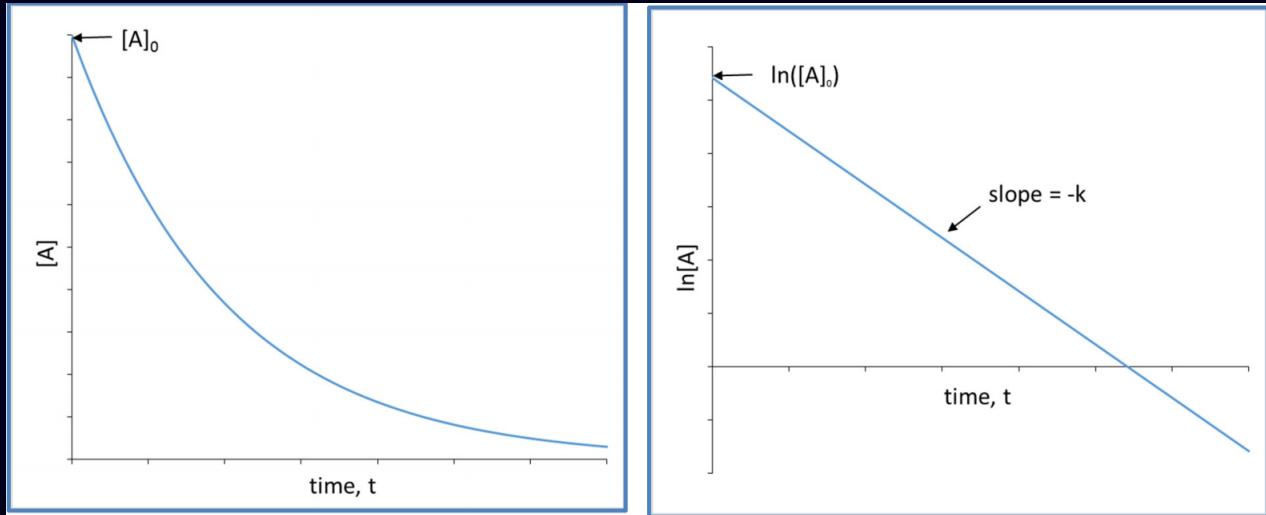


Figure 1: Behavior of $[A]$ over time is exponential, while $\ln[A]$ is linear, with slope $\rightarrow k$

Describing decay times for 1st order decay

Time scale of first order decay is described in terms of half-life, $t_{1/2}$, which is the time required for a reaction to reach 50% completion. Parameter τ is used to describe decay times. It gives the time required for a reaction to reach $\frac{1}{e}$ completion compared to initial condition: $\frac{[A]}{[A]_0} = \frac{1}{e}$

We can connect $t_{1/2}$ and τ with the following comparison:

$$\ln\left(\frac{1}{2}\right) = -kt_{\frac{1}{2}} \text{ to } \ln\left(\frac{1}{e}\right) = -1 = -k\tau \quad (9)$$

This will give:

$$t_{\frac{1}{2}} = \ln(2)\tau \quad (10)$$

For the simple first order decay reaction of [A]

$$t_{\frac{1}{2}} = \frac{\ln(2)}{k} \text{ and } \tau = \frac{1}{k}$$

Integrated rate law for a 2nd order irreversible reaction



Repeat the same process as above to get:

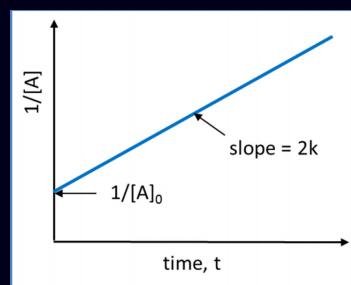
$$\frac{-d[A]}{dt} = 2k[A]^2$$

$$\int \frac{d[A]}{[A]^2} = -2k \int dt$$

$$-\frac{1}{[A]} \Big|_{[A]_0}^{[A]} = -2kt \Big|_0^t$$

$$\frac{1}{[A]} - \frac{1}{[A]_0} = 2kt$$

A plot of $\frac{1}{[A]}$ vs. time gives a straight line, where slope = k



Establishing a rate law from measured reaction velocities

A different way of experimentally examining a rate law is by evaluating the dependence of reaction velocity on concentrations.

If a reaction is **first order** in [A], then v will depend linearly on [A]. If [A] doubles, then v will also double.

If a reaction is **second order** in [A], then doubling [A] will quadruple the reaction velocity.

In general:

$$v = [A]^\alpha \quad (11)$$

Then for rate measurements made at two different concentrations:

$$\ln\left(\frac{v_2}{v_1}\right) = \alpha \ln\left(\frac{[A]_2}{[A]_1}\right) \quad (12)$$

Behavior of more complex reaction schemes

Now, we want to consider events that are more complex than one step reactions. Consider the following example:



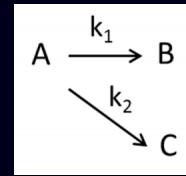
This gives the following equations:

$$\frac{d[A]}{dt} = -k_1[A]$$

$$\frac{d[B]}{dt} = k_1[A] - k_2[B]$$

$$\frac{d[C]}{dt} = k_2[B]$$

For another example:



The change in concentration as a function of time is given as:

$$\frac{d[A]}{dt} = -k_1[A] - k_2[A] = -(k_1 + k_2)[A]$$

$$\frac{d[B]}{dt} = k_1[A]$$

$$\frac{d[C]}{dt} = k_2[A]$$

Steady state assumptions for obtaining simple rate laws for complex reactions

For complex reactions, dependence of the rate of the overall reaction (rate law) can depend on concentrations of species that do not contribute to the reaction itself.

When sequential reactions are involved, simplified rate laws can be obtained by assuming steady state conditions.

Steady state is when the intermediate species (that do not contribute to overall reaction stoichiometry) concentrations have reached constant value, at least momentarily.

$$\frac{d[\text{Intermediate}]}{dt} = 0$$

Consider the following equation:



In this equation, the intermediate is B, and the overall reaction stoichiometry is $A \longrightarrow C$. Based on equation 13, we can then state that:

$$\begin{aligned} \frac{d[B]}{dt} &= k_1[A] - k_{-1}[B] - k_2[B] \\ &= k_1[A] - [B](k_{-1} + k_2) = 0 \end{aligned}$$

Now rearrange to isolate [B]:

$$[B] = \frac{k_1[A]}{k_2 + k_{-1}} \quad (14)$$

Now go back to the original reaction scheme. The overall reaction velocity is $v = \frac{d[C]}{dt}$, but we know that $\frac{d[C]}{dt} = k_2[B]$. Substituting [B] from equation 14 shows that:

$$v = \frac{k_1 k_2 [A]}{k_2 + k_{-1}} \quad (15)$$

From the equation 15 above, this 2-step reaction behaves as first order in [A] at steady state.

Enzyme Kinetics under a steady-state assumption

The following model is used to treat kinetics of a simple unimolecular enzyme reaction:



E is the free enzyme, S is the free/unbound substrate, ES is the enzyme-substrate complex, and P is the product. $\frac{k_1}{k_{-1}}$ describes how tightly the enzyme binds the substrate. K_{cat} describes the catalytic rate constant for formation of P.

The velocity of the overall reaction is described by $v = \frac{d[P]}{dt}$. According to the rate law, $v = k_{cat}[ES]$. [ES] is an intermediate, and so we need to replace [ES] by adopting steady state assumption (where $\frac{d[ES]}{dt} = 0$).

$$\frac{d[ES]}{dt} = k_1[E][S] - (k_{-1} + k_{cat})[ES] = 0$$

$$[ES] = \frac{k_1[E][S]}{k_{-1} + k_{cat}}$$

Now plug in [ES] from above into $v = k_{cat}[ES]$:

$$v = \frac{k_{cat} k_1 [E][S]}{k_{-1} + k_{cat}} \quad (17)$$

This equation is a start, but it only describes the reaction velocity in terms of the free enzyme concentration. In an experiment, we usually only have control over the total enzyme concentration. We want to rewrite the equation in terms of total enzyme concentration and in terms of the ratio of the reaction velocity to the maximum possible value ($[ES] = [E]_{total}$). The maximum velocity is $k_{cat} \times$ max value for $[ES]$, which is:

$$v_{max} = k_{cat}[E]_{total} = k_{cat}([ES] + [E]) \quad (18)$$

The ratio of reaction velocities relative to its maximum is given:

$$\frac{v}{V_{max}} = \frac{k_{cat}[ES]}{k_{cat}([E] + [ES])}$$

$$\frac{v}{V_{max}} = \frac{[ES]}{([ES] + [E])}$$

$$\frac{v}{V_{max}} = \frac{1}{1 + \frac{[E]}{[ES]}}$$

Taking the previous equation $[ES] = \frac{k_1[E][S]}{k_{-1} + k_{cat}}$ and rearranging so that it becomes $\frac{[E]}{[ES]} = \frac{k_{-1} + k_{cat}}{k_1[S]}$, substitute this equation above to give:

$$\frac{v}{V_{max}} = \frac{1}{1 + \frac{k_{-1} + k_{cat}}{k_1[S]}}$$

$$\frac{v}{V_{max}} = \frac{[S]}{[S] + \frac{k_{-1} + k_{cat}}{k_1[S]}}$$

$$\frac{v}{V_{max}} = \frac{[S]}{[S] + K_M} \longrightarrow K_M = \frac{k_{-1} + k_{cat}}{k_1}$$

Or, we can also convert from fractional velocity to v using equation 18 for V_{max} :

$$v = \frac{k_{cat}[E]_{total}[S]}{[S] + K_M} \quad (19)$$

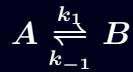
Relaxation kinetics: how systems approach equilibrium

The T-Jump Method

The temperature jump method was developed to study fast reactions and their "relaxation" back towards equilibrium. If energy is rapidly delivered to a solution containing reactant and product at equilibrium, the temperature of the system can be heated nearly instantaneously. Recalling the **van't Hoff equation**, if ΔH for the reaction is non-zero, then the equilibrium constant will be different at a new temperature. Then you can observe how fast the reaction returns to its new equilibrium.

The speed at which equilibrium is achieved depends on the forward and backward rate constants of the reaction.

Consider a simple system:



Let x be the distance of each species from equilibrium. \bar{A} will be $[A]_{equilibrium}$ at the new temperature, same for \bar{B} . The concentrations of A and B are related to the equilibrium conc. by:

$$\begin{aligned}[A] &= \bar{A} + x \\ [B] &= \bar{B} - x\end{aligned}$$

Now examine the approach to equilibrium by writing an equation for time dependence of x .

$$\begin{aligned}\frac{d[A]}{dt} &= \frac{d[\bar{A} + x]}{dt} = \frac{dx}{dt} \\ \frac{d[A]}{dt} &= -k_1[A] + k_{-1}[B] \\ \frac{d[A]}{dt} &= -k_1[\bar{A} + x] + k_{-1}[\bar{B} - x] \\ &= k_{-1}\bar{B} - k_1\bar{A} - x(k_1 + k_{-1})\end{aligned}$$

The term $k_{-1}\bar{B} - k_1\bar{A} = 0$ because $\frac{\bar{B}}{\bar{A}} = K = \frac{k_1}{k_{-1}}$

So, after setting the above to 0, the equation yields:

$$\frac{dx}{dt} = -(k_1 + k_{-1})x \quad (20)$$

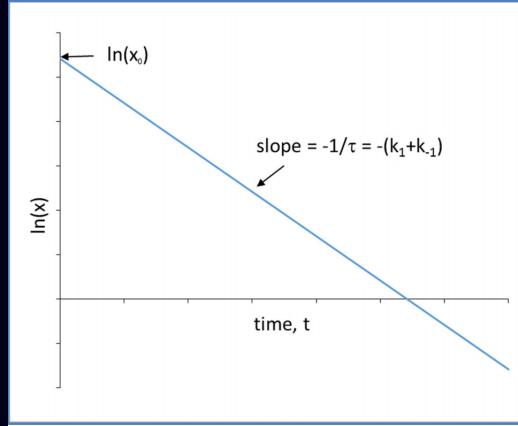
X follows first order kinetics. Skipping the familiar details for handling a first order differential equation:

$$x = x_o^{-\left(k_1 + k_{-1}\right)} \text{ and } \ln\left(\frac{x}{x_0}\right) = -(k_1 + k_{-1})t \quad (21)$$

Convert these to even more general forms:

$$x = x_0 e^{\frac{-t}{\tau}} \text{ and } \ln\left(\frac{x}{x_0}\right) = \frac{-t}{\tau} \quad (22)$$

where $\tau = \frac{1}{k_1 + k_{-1}}$



If you can find [A] or [B] as a function of time, then you can also find x as a function of time.

$$x = [A] - \bar{A} = \bar{B} - [B]$$

You can use this to measure τ , and thus also $k_1 + k_{-1}$

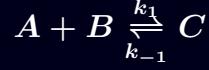
$$1/\tau = k_1 + k_{-1} \text{ and } k_1 = Kk_{-1}$$

$$1/\tau = Kk_{-1} + k_{-1} = (K + 1)k_{-1}$$

$$k_{-1} = \frac{1}{\tau(K + 1)} \text{ and } k_1 = \frac{K}{\tau(K + 1)}$$

Higher Order Reactions Approaching Equilibrium

Now consider this second order reversible reaction:



There is only one transformation, so the distance can still be described by x :

$$[A] = \bar{A} + x$$

$$[B] = \bar{B} + x$$

$$[C] = \bar{C} - x$$

We can use the same approach from the above to describe the change in concentrations:

$$\begin{aligned}\frac{d[A]}{dt} &= \frac{d(\bar{A} + x)}{dt} = \frac{dx}{dt} \\ \frac{d[A]}{dt} &= -k_1(\bar{A} + x)(\bar{B} + x) + k_{-1}(\bar{C} - x) \\ &= k_{-1}\bar{C} - k_1\bar{A}\bar{B} - x(k_1(\bar{A} + \bar{B}) + k_{-1}) - k_1x^2\end{aligned}$$

$k_{-1}\bar{C} - k_1\bar{A}\bar{B}$ cancels to 0, and if we are close enough to equilibrium then x is small, so we can neglect the x^2 term. So,

$$\frac{dx}{dt} = -(k_1(\bar{A} + \bar{B}) + k_{-1})x \quad (23)$$

So, the distance from equilibrium x shows first order behavior close to equilibrium, with $\tau = \frac{1}{k_1(\bar{A} + \bar{B}) + k_{-1}}$

Kinetics from Single Molecule Studies

For a unimolecular event, we can get a sense for the rate constant by looking at how long the molecule persists in its current state before undergoing a reaction.

Think of this as the "waiting time" before a reaction or conformational change occurs. While reactions are random, the average waiting time should be shorter for a process with a higher rate constant.

Consider $A \rightarrow B$ with rate constant k.

How long before any molecule A turns into B? Treat the reaction in bulk:

$$\frac{A}{A_0} = e^{\frac{-t}{\tau}} \text{ where } \tau = \frac{1}{k} \quad (24)$$

$\frac{A}{A_0}$ can be considered the probability that any molecule A will **not** convert at time t.

The probability that molecule A reacts precisely at time t is $P(A)_{react} = \frac{1}{\tau} e^{\frac{-t}{\tau}}$.

To get the average time at which a molecule of A reacts (aka waiting time), we need to get the average value of t by weighting all possible values of t by probability of reaction at time t.

To do this, multiply t by the probability of reaction at time t, then integrate from t = 0 to infinity.

$$\text{waiting time} = \int_{t=0}^{t=\infty} (t(\frac{1}{\tau})e^{\frac{-t}{\tau}}) dt \quad (25)$$

If we can measure how long it takes a single molecule to undergo a transition, then we have measured τ .

Chapter 16: Kinetic Theories and Enzyme Catalysis

Now we want to look at what determines the rate constant, k . What makes some reactions innately fast or slow? There are two main models that try to explain the mechanisms of chemical reactions and their rate constants.

The Arrhenius Equation

This is often used to discuss reaction rates in terms of **molecular collisions**. The rate constant k is determined by:

$$k = A e^{-\frac{E_a}{RT}} \quad (26)$$

A is the **frequency factor** and E_a is an **activation energy**. We already know that the frequency of collisions in a reaction is dependent on concentrations. The frequency factor in the equation for k accounts for other phenomena ie. the dependence of molecular velocities and consequently collision rates on temperature, and the dependence of reaction probability on the orientation of the colliding molecules.

E_a describes a lower bound for energy that reactants must have for reaction to occur. Why does E_a enter the equation for k as an exponential term? **Boltzmann Distribution!**

If $N(E) \propto e^{\frac{-E}{RT}}$, then we can find the fraction of molecules having energy at least as high as E_a by taking the ratio of area that falls under the curve and has $E \geq E_a$ divided by entire area under the curve.

$$\frac{\int_{E_a}^{\infty} e^{\frac{-E}{RT}}}{\int_0^{\infty} e^{\frac{-E}{RT}}} = \frac{-RT e^{\frac{-E}{RT}}|_{E_a}^{\infty}}{-RT e^{\frac{-E}{RT}}|_0^{\infty}}$$

$$\frac{e^{-\frac{E_a}{RT}}}{1} = e^{-\frac{E_a}{RT}}$$

A key element of Arrhenius equation: **the rate constant depends strongly on the height of an energy barrier.** It also depends on the temperature.

The dependence of k on T can be used to evaluate the activation energy in the Arrhenius model.

$$\frac{d(\ln(k))}{dt} \cong \frac{d(-\frac{E_a}{RT})}{dT} = \frac{E_a}{RT^2}$$

or

$$\begin{aligned} \frac{d(\ln(k))}{d(\frac{1}{T})} &\cong \frac{d(-\frac{E_a}{RT})}{d(\frac{1}{T})} = \frac{d(-\frac{E_a}{RT})}{-\frac{d(T)}{T^2}} \\ &= \frac{-E_a}{RT^2} = \frac{-E_a}{R} \end{aligned}$$

Eyring Transition State Theory

This method provides a slightly different way of looking at things that are more explicit about the occurrence of high energy species during a single reaction event. A single reaction step:



is broken down in terms of two steps.

- First step: unstable high energy species (the transition state)
- Second step: the transition breaks down to product.

The double-dagger symbol indicates the **transition state**.



The rate constant for breakdown of transition state is approximately the frequency of molecular vibrations, which is on the order of $\frac{k_B T}{h}$, where h is **Planck's Constant**

$$k_{breakdown} = \frac{k_B T}{h} \text{ and } v = \frac{d[C]}{dt} = k_{breakdown}[AB^\ddagger] \quad (28)$$

Then, the velocity of the reaction scheme above is:

$$v = \frac{d[C]}{dt} = \frac{k_B T}{h}[AB^\ddagger] \quad (29)$$

If we assume that the first step (formation of the transition state) is at equilibrium, then $\frac{k_+}{k_-} = K \ddagger = \frac{[AB \ddagger]}{[A][B]}$, so, $[AB \ddagger] = K \ddagger [A][B]$. If we substitute this value, then the reaction velocity would be $v = \frac{k_B T}{h} K \ddagger [A][B]$

Match this up to the simple rate law we would write for a single elementary reaction $\rightarrow v = k[A][B]$. The Eyring Model gives:

$$k = \frac{k_B T}{h} K \ddagger$$

The rate constant k is largely dependent on the equilibrium constant $K \ddagger$ for forming the transition state. We can also write $K \ddagger$ in terms of free energy for reaching the transition state:

$$K \ddagger = e^{\frac{-\Delta G}{RT}}$$

Substituting this gives us the equation:

$$k = \frac{k_B T}{h} e^{\frac{-\Delta G}{RT}} \quad (30)$$

While this is different than the **Arrhenius Equation**, it is similar in that it shows the exponential dependence on an energy barrier.

We can also look at the temperature difference of $\ln(k)$ for the Eyring equation like we did for the Arrhenius:

$$\begin{aligned} \frac{d \ln(k)}{dT} &\cong \frac{d(\frac{-\Delta G \ddagger}{RT})}{dT} \\ &= \frac{d(\frac{-\Delta H \ddagger + \Delta S}{RT})}{dT} \\ &= \frac{\Delta H \ddagger}{RT^2} \end{aligned}$$

The activation energy in the Arrhenius Equation relates closely to the transition state enthalpy in the Eyring model.

Catalysis by lowering the transition state energy

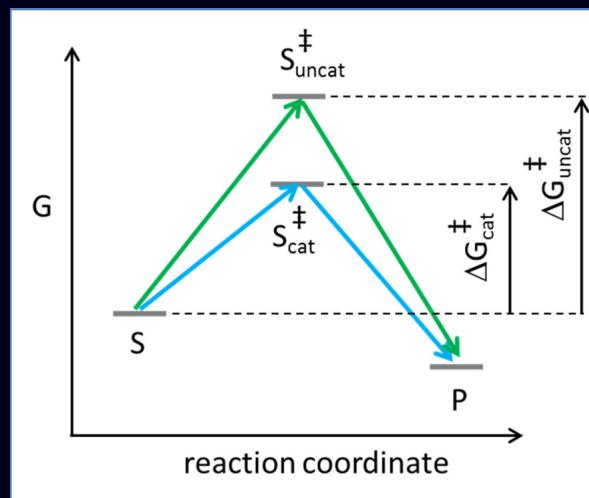
The Eyring transition state model provides a way to look at catalysis in terms of transition state energies.

Let the rate constant for the uncatalyzed reaction = k_{uncat} , and the rate constant for catalyzed reaction = k_{cat} . Using the **Eyring equation** for the rate constant, we can write out the ratio of two rate constants:

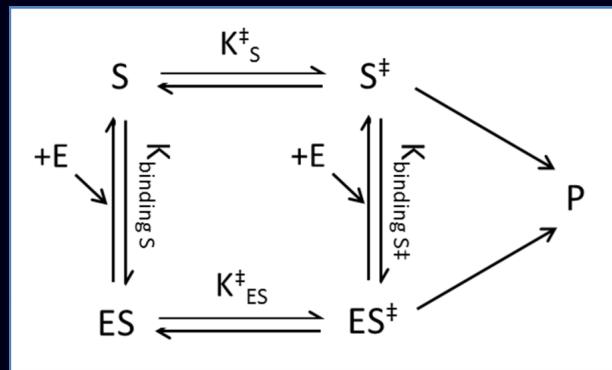
$$\begin{aligned} \frac{k_{cat}}{k_{uncat}} &= \frac{\frac{k_B T}{h} K_{cat}^{\ddagger}}{\frac{k_B T}{h} K_{uncat}^{\ddagger}} \\ &= \frac{K_{cat}^{\ddagger}}{K_{uncat}^{\ddagger}} \\ &= \frac{e^{-\frac{\Delta G_{cat}^{\ddagger}}{RT}}}{e^{-\frac{\Delta G_{uncat}^{\ddagger}}{RT}}} \\ &= e^{\frac{-(\Delta G_{cat}^{\ddagger} - \Delta G_{uncat}^{\ddagger})}{RT}} \end{aligned}$$

If a catalyst lowers the transition state energy for a reaction by an energy that amounts to $10RT$, then the reaction will be sped up by a factor of $e^{10} \rightarrow 22,000$.

How is it that a catalyst lowers the transition state energy of a reaction?



The surface configuration of the enzyme is complimentary to the unstable molecule with only transient existence (intermediate state), or the "activated complex" for the reaction that is catalyzed by that reaction. The enzyme would show attraction for the substrate, which would become attached to it in its active surface region. The substrate would be strained, and typically deform into the configuration of the activated complex. The activated complex then reassumes the original configuration or turns into the product.



We can relate the binding events in the presence of an enzyme to the formation of the transition state. The reactions on the **top** describe the reaction in the **absence** of the enzyme. The reactions on the bottom describe reaction with enzyme. The ratio between the rate constants with and without enzymes is:

$$\frac{k_{cat}}{k_{uncat}} = \frac{K_{ES}^{\ddagger}}{K_S^{\ddagger}}$$

By providing a binding surface that is complimentary to the transition state form for substrate, the equilibrium constant for reaching the transition state is increased by mass action → reaction is sped up.

The ratio for how much a reaction is sped up is:

$$\frac{k_{cat}}{k_{uncat}} = \frac{K_{ES}^{\ddagger}}{K_S^{\ddagger}} = \frac{K_{bindingS^{\ddagger}}}{K_{bindingS}}$$

By this equation: the enzyme speeds up its reaction by binding exceptionally tightly to the transition state form of the substrate. This is what lowers the free energy of the transition state.

Practical Consequences of Enzymes Binding Tightly to the Transition State

Transition state analogues as enzyme inhibitors

If an enzyme speeds up a reaction by a factor of 1000, then by the math above, the enzyme binds the transition state form of the substrate ($S\ddagger$) more tightly than it binds the substrate.

A drug molecule that looks like the $S\ddagger$ will bind tightly to the enzyme and thus act as an inhibitor. The main challenge is that the transition state is unstable. The main goal is to find a compound that looks similar to the transition state, but is still stable.

Creating new enzymes from a natural antibody repertoire

The goal was to create novel enzymes that would catalyze useful chemical types of reactions that no natural enzymes had evolved to carry out.

If you can find an antibody sequence with a high affinity for the transition state of a desirable reaction, then you have found an enzyme for that reaction.

Computational Enzyme Design

Instead of designing a novel protein from scratch, take a natural protein that has a surface cleft suitable for binding a compound of about the right size. Then modify the amino acid sequence mainly within the binding site cleft. No need to create transition state analogs, and instead only requires an accurate model for what the transition state is likely to look like. Computer programs can then design reasonable models of transition states.

The most challenging part is designing amino acid changes into a protein so that the TS is tightly bound. One issue concerns the calculation of free energies for large systems like proteins and their complexes.

Changing amino acid sequences can also cause loss of stability and aggregation, or make alternate (non-native) configurations more stable than the intended structure.

Kinetic Parameters of Natural Enzymes

Part of the complexity concerns the saturation behavior of enzyme kinetics.

$$v = \frac{[E_{total}]k_{cat}[S]}{[S] + K_M}$$

An enzyme that has a high k_{cat} may not be great if it does not bind substrate well (i.e. if K_M is high). The best conditions is to have **high k_{cat}** and **low K_M** .

The ratio of $\frac{k_{cat}}{K_M}$ is considered the general measure of efficiency of an enzyme. k_{cat} is the maximum velocity at any substrate concentration. $\frac{k_{cat}}{K_M}$ is the slope of the velocity curve in its linear region well below saturation. We are evaluating the standard Michaelis-Menten velocity equation (above) at $[S] \ll K_M$.

Typically, K_M values exhibited by natural enzymes tend to be roughly in the same range as natural cellular concentration of the substrate on which they operate.

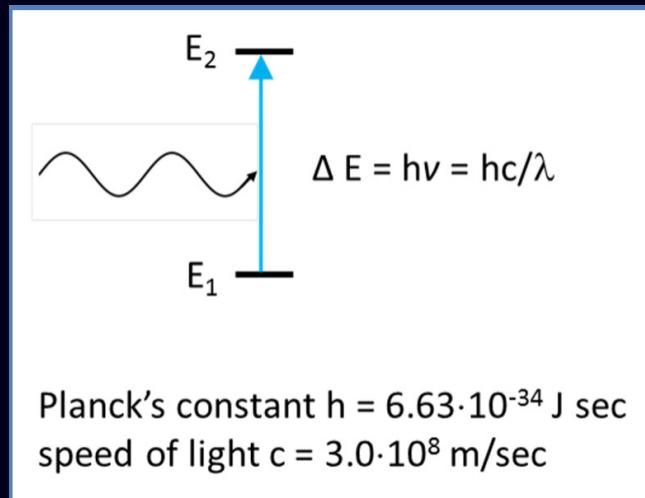
Chapter 17: Introduction to Biochemical Spectroscopy

Energy Transitions

From quantum mechanics, we know that molecules can exist only in discrete energy states. Transitions between one energy state and another can be driven by absorption/emission of electromagnetic radiation (photons) if the energy of the photon matches the energy difference of the transition.

The relationship between frequency or wavelength of radiation and energy is:

$$E = hv = \frac{hc}{\lambda}$$



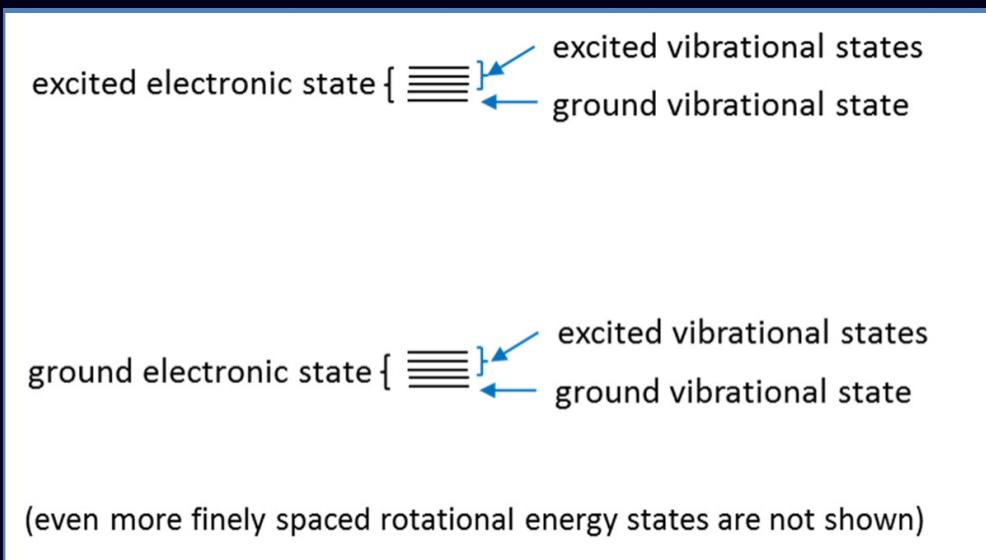
Simple molecules (like single atoms) show very sharp absorption and emission bands.

They only undergo transitions at very narrow wavelengths —> **Rydberg Series**.

However, complex molecules have complex spectra. Presence of multiple atoms in a molecule introduces dependence of energy on nuclear positions.

Nuclear motions give rise to vibrational energy states. The energy differences in vibrational states is much smaller than those between electronic states. Within vibrational states are even smaller rotational states. This hierarchical level of complexity allows for a huge number of transitions with closely spaced energies.

As a result, absorption and emission spectra for larger molecules are complex and more continuous in nature rather than discrete.



Consider an energy associated with wavelength of 400 nm in the violet region of the visible spectrum.

$$E = h\nu = \frac{hc}{\lambda} = 4.1 \times 10^{-21} J$$

This is equivalent to about $120 K_B T$. According to the Boltzmann equation ($e^{-\frac{E}{K_B T}}$), the probability of a molecule being in this excited state rather than ground state is basically zero.

Repeat this for a typical vibrational transition. Consider a typical stretch where $\lambda = 1.9 \mu M$. The energy is about $1.4 \times 10^{-19} J$ or about $25 K_B T$. The probability is much higher here.

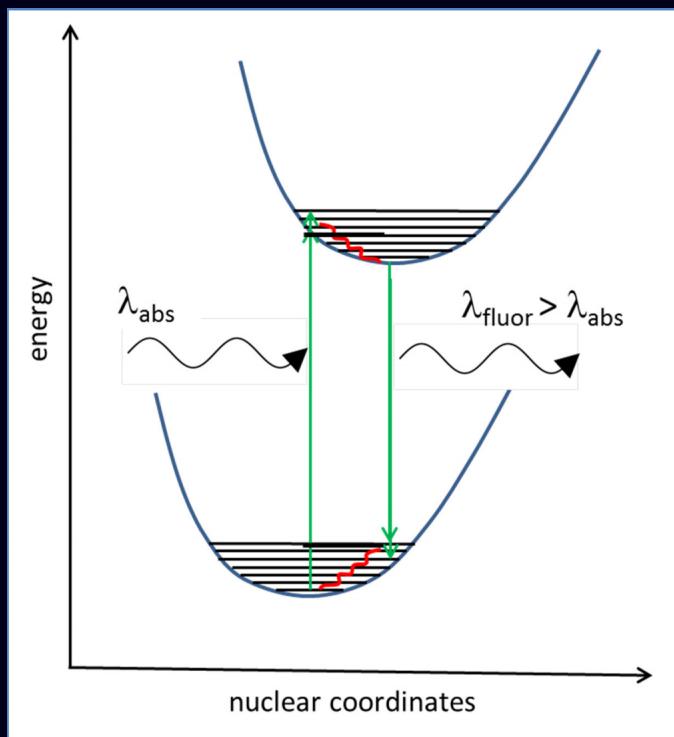
Conclusion: At ordinary temperatures and unless otherwise excited, molecules almost exclusively populate the lowest vibrational state of the lowest electronic state. **A high probability transition requires the initial energy state to be well-populated.**

Fluorescence

Our analysis from above tells us that an absorption transition usually happens from the ground vibrational state of the ground electronic state.

The next question is: to what higher electronic energy state is a molecule likely to be excited by absorption.

General Idea: Lowest energy nuclear positions for a molecule are slightly different for different electronic states.



Consider the image below. The two black curves indicate the classical energy of a molecule as a function of its nuclear positions in two different energy states. The minimum energies occur at slightly different nuclear positions. These are illustrated by the green lines coming from the lowest horizontal line in each curve.

The width of the lines illustrate the range of nuclear positions that are allowed in each vibrational state. The timescale for photon absorption is much shorter than the timescale for nuclear motions. This means that electronic transitions happen "vertically" in the diagram shown.

The Franck – Condon Principle : When a molecule is undergoing an electronic transition (like ionization), the nuclear configuration of the molecule experiences no significant change.

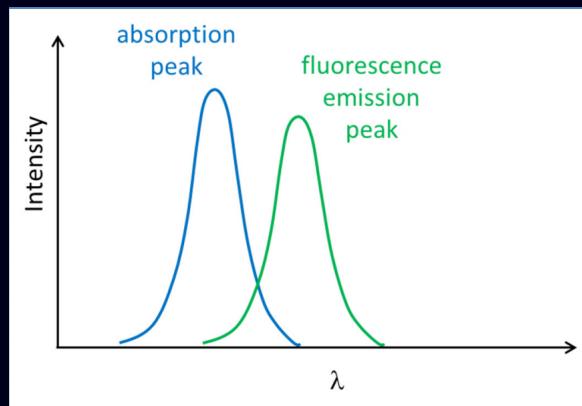
Nuclei are much bigger than electrons, and electronic transitions happen faster than the nuclei can respond. When the nuclei finally realigns itself with the new electronic configuration, it then undergoes a **vibration**.

If an electronic transition must occur without appreciable movement of nuclei, then it must occur to a vibrational state for which initial nuclear positions are allowable.

After excitation, according to the Boltzmann equation, a molecule must return to the ground state. This can occur with the emission of a photon. This is known as **fluorescence**. The timescale for fluorescence is typically in the $10^{-5} - 10^{-8}$ sec range. This is long enough for thermal vibrations and collisions to allow the molecule to descent to lower vibrational states

within excited electronic state, before returning to the ground electronic state.

The **key consequence** is that the fluorescence emission spectrum for a molecule is shifted to lower energy and longer wavelength compared to absorption spectrum. This is known as **Stoke's Shift**.



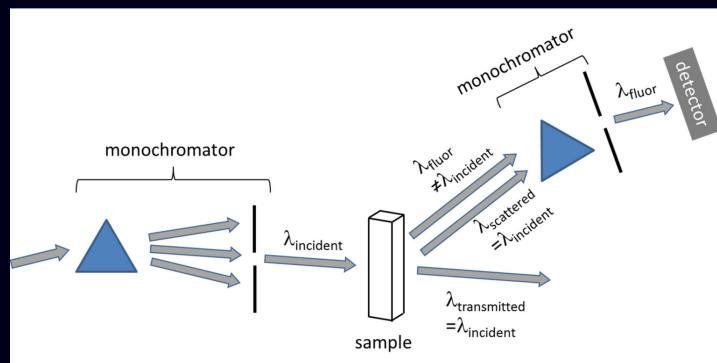
Uses and Advantageous Properties of Fluorescence

Fluorescence offers a high degree of sensitivity for detecting and measuring concentrations of specific molecules. The high sensitivity of fluorescence comes from two factors.

First is the shift in wavelength from incident wavelength to emission wavelength. In an absorption experiment involving dilute molecule, you must analyze a small difference between two large numbers: the number of photons transmitted by a blank compared to number transmitted by the sample.

But, in a fluorescence experiment, the change in wavelength makes it possible to analyze the number of emitted photons without interference from transmitted photons, which have the same wavelength as the incident light.

This requires a second monochromator placed between the sample and the detector, and a first monochromator is required between the light source and the sample. Extra level of sensitivity comes from the ability to monitor fluorescence in a direction different from the path of the transmitted beam. Scattered photons travel in all directions including the same path as fluorescent photons. But, since their wavelength is the same as the incident wavelength, they are distinguishable.



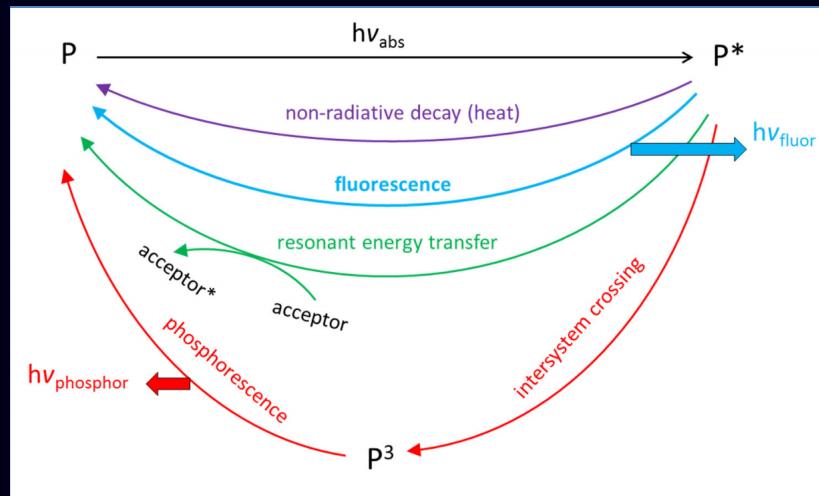
Proteins typically have natural fluorescence owing to the presence of tryptophan amino acids. A particularly useful feature of fluorescence is its sensitivity to chemical environment. The greater sensitivity to environment for fluorescence compared to absorbance relates in part to the longer time scale of fluorescence. In general, increased flexibility and environmental polarity lead to lower fluorescent intensity. The peak emission wavelength can also be affected.

For example, fluorescence of tryptophan increases by a factor of 4 in a low polarity solvent (like DMSO, $\epsilon = 35$) compared to water ($\epsilon = 80$). Tryptophan resides almost always become less flexible and more rigidly held in the folded state of a protein, thus leading to higher fluorescence.

Kinetics of Fluorescence and competing routes for return to ground state

After a molecule has been driven to an excited state by absorbing a photon, there are several routes it can take to return to ground state. The relative rates of these processes relates directly to which pathways dominate for a given molecule.

If the rate constant for fluorescent emission is higher than the rate constants for other processes, then most of the excited molecules will return to the ground state by way of fluorescent emission.

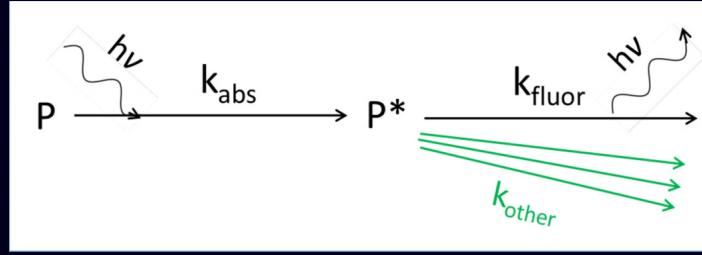


A number of phenomena affect fluorescence, including chemical environment, so fluorescence can be used to monitor various events that alter the environment of a fluorophore in solution.

We can lump together the various non-fluorescent pathways for return to ground state under a single rate constant, k_{other} . Various events can then be analyzed in terms of the effects they have on the relative rates of k_{fluor} and k_{other} .

With respect to kinetic treatments, fluorescence experiments can be of two essentially different types:

1. Under continuous illumination where steady state behavior is assumed
2. Following a brief pulse of incident light, after which time-dependent measurements are made.



Constant Illumination

Under constant illumination, the concentration of the excited fluorophore (P^*) is not changing. Setting $\frac{d[P^*]}{dt} = 0$:

$$\frac{d[P^*]}{dt} = 0$$

$$= k_{abs}[P] - (k_{fluor} + k_{other}[P^*])$$

Rearranging to obtain an expression for $[P^*]$:

$$[P^*] = \frac{k_{abs}[P]}{k_{fluor} + k_{other}}$$

Then, the fluorescent intensity I_{fluor} is given by:

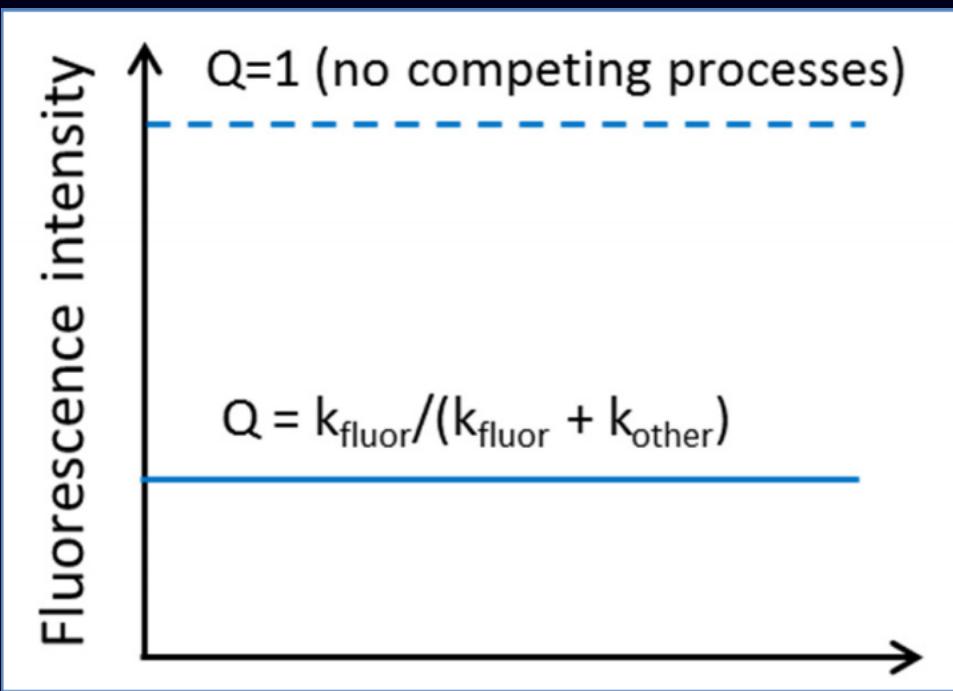
$$I_{fluor} = k_{fluor}[P^*]$$

$$= \frac{k_{abs}[P] * k_{fluor}}{k_{fluor} + k_{other}}$$

Since the rate of photon absorption is $k_{abs}[P]$, the ratio of number of photons emitted to the number absorbed - a fractional quantity known as the **quantum yield Q** - is given by:

$$Q = \frac{k_{fluor}}{k_{fluor} + k_{other}}$$

The quantum yield and intensity of fluorescence observed under constant illumination is decreased by events in solution that increase the rates of non-fluorescent "other" pathways for return to ground state.



An example is shown above. The binding of a fluorescent molecule to a protein would decrease the non-fluorescent pathways by reducing mobility of the fluorophore and thereby increase the quantum yield along with the steady state fluorescence intensity.

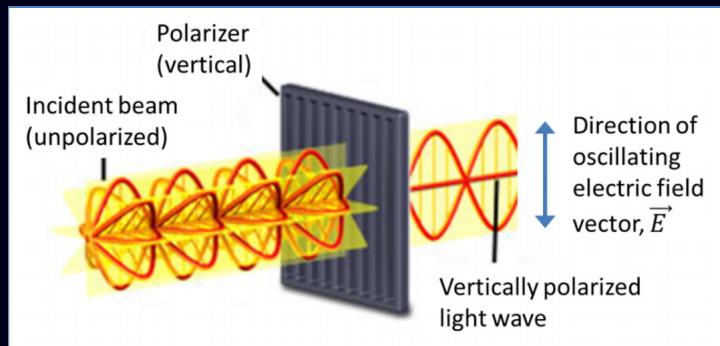
Time-resolved Fluorescence

An excitation pulse can be applied and the fluorescence intensity (which must decay back to zero) can be monitored over time. The same kinetic scheme as above can be used if we remove the continuous absorption event. This becomes a simple case of exponential decay with a total rate constant of $k_{\text{fluor}} + k_{\text{other}}$ and a decay time of:

$$\tau = \frac{1}{k_{\text{fluor}} + k_{\text{other}}}$$

Chapter 18: Special Topics in Biochemical Spectroscopy

Polarization and Selection Rules



Orientation plays an important role in spectroscopy. The effects become apparent in spectroscopy experiments conducted using polarized light. Light emitted from an ordinary source (light bulb) carries photons whose electric field vectors point in all possible directions perpendicular to the direction of travel.

We know that for a photon of light to be absorbed and cause a transition from an initial state to a final state that the energy of the photon must be correct (discrete quantum levels). But the **direction of its electric field vector** is also important.

In absorption spectroscopy, the extinction coefficient relates to the strength/probability of a transition by being proportional to the square of a transition dipole moment, $\vec{\mu}$. In the general form of the transition dipole moment:

$$\vec{\mu} = \int \psi_i \vec{x} \psi_f d\vec{x}$$

where \vec{x} describes a general position vector in space, and ψ_i and ψ_f are the quantum mechanical wavefunctions for the initial and final energy states.

We can divide these into individual directions in x, y, z components. The probability of absorbing a photon polarized along the x direction is related to the x component of the transition dipole moment, evaluate as:

$$\mu_x = \int \psi_i x \psi_f dx$$

$$\mu_y = \int \psi_i y \psi_f dy$$

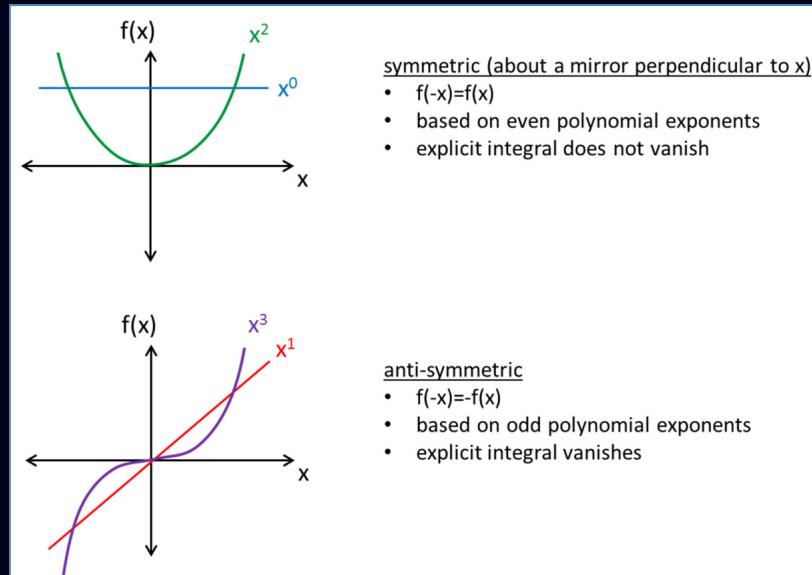
$$\mu_z = \int \psi_i z \psi_f dz$$

Analyzing whether electronic transitions can(not) occur when the light is polarized in certain directions can be simplified by using a treatment that considers the symmetry vs. anti-symmetry of the initial and final wavefunctions.

A reminder for symmetric and anti-symmetric functions:

- Symmetric: $f(x) = f(-x)$
- Anti-symmetric: $f(-x) = -f(x)$

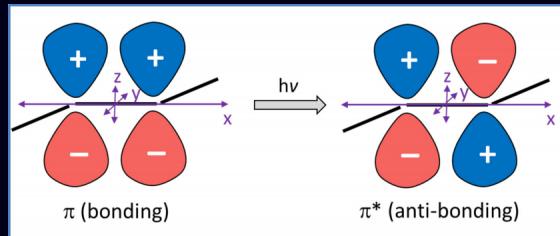
Functions with even exponents ($x^0, x^2, x^4\dots$) are symmetric, while functions in the odd power ($x^1, x^3, x^5\dots$) are anti-symmetric.



Odd (anti-symmetric) functions integrate to zero while even (symmetric) functions do not.

In 3D, where integration would be over all dimensions, the result integrates to zero if the function is odd with respect to **any** of the three spatial variables.

Consider an electronic transition between a π bonding molecular orbital and π^* anti-bonding molecular orbital. Transitions are common in conjugated double bond systems. Both molecular orbitals are effectively combinations of two side-by-side p orbitals.



Signs of the two p orbitals are aligned in the π , but oppositely oriented in the π^* molecular orbital. This creates an extra nodal plane in the latter case.

Since even/odd(ness) is an exponential property, products of functions behave according to the addition of even and odd numbers. Even + Even = Even. Even + Odd = Odd. Odd + Odd = Even.

We need to make a separate analysis to consider transition dipole component for light polarized in each possible direction.

For example, in the case of light polarized along the x direction, the term 'x' in the middle of the integral can be understood as being $x^1y^0z^0$, which is thus odd with respect to x, and even with respect to y and z. We can construct a table to analyze μ_x :

w/r/t axis	$\vec{\Psi}_i$	$x = x^1y^0z^0$	$\vec{\Psi}_f$	total
x	even	odd	odd	even
y	even	even	even	even
z	odd	even	odd	even

By looking at this table, we can see that the total function inside the integral is even across all three variables. Thus we can say that a $\pi \rightarrow \pi^*$ transition can occur by absorption of a photon polarized along x. This transition is **allowed**. Note that the allowable transition for polarization along x does not mean that the photon's direction of travel is along x. In fact, the direction of travel would have to be \perp to x.

We can also evaluate transition dipoles for light polarized along y or z:

w/r/ t axis	$\vec{\Psi}_i$	$y = x^0y^1z^0$	$\vec{\Psi}_f$	total		w/r/ t axis	$\vec{\Psi}_i$	$z = x^0y^0z^1$	$\vec{\Psi}_f$	total	
x	even	even	odd	odd		x	even	even	odd	odd	
y	even	odd	even	odd		y	even	even	even	even	
z	odd	even	odd	even		z	odd	odd	odd	odd	

Because the total function is odd for at least one variable, the integral will eventually vanish to 0. This means that μ_y and μ_z will vanish. These transitions are **forbidden**. The $\pi \rightarrow \pi^*$ transition cannot happen via absorption of a photon polarized along y or z.

Example of absorption of polarized light by an oriented pigment

Although light may only be absorbed when the electric field is oriented in a particular direction relative to the chromophore, the effects of this are often not evident in experiments done in solution, since the absorbing chromophore is present in all possible orientations.

The dependence of absorption on direction of polarization can sometimes be seen in a crystalline sample where the chromophore exists in the same orientation throughout the crystal specimen.

Fluorescence Experiments with Polarized Light

Interesting things occur when a sample absorbs polarized light and then reemits photons by fluorescence. We will assume that if a molecule absorbs a photon polarized in a specific direction, then by fluorescence, it will emit a photon polarized in the same direction **if the molecule has not changed its orientation** between absorption and emission.

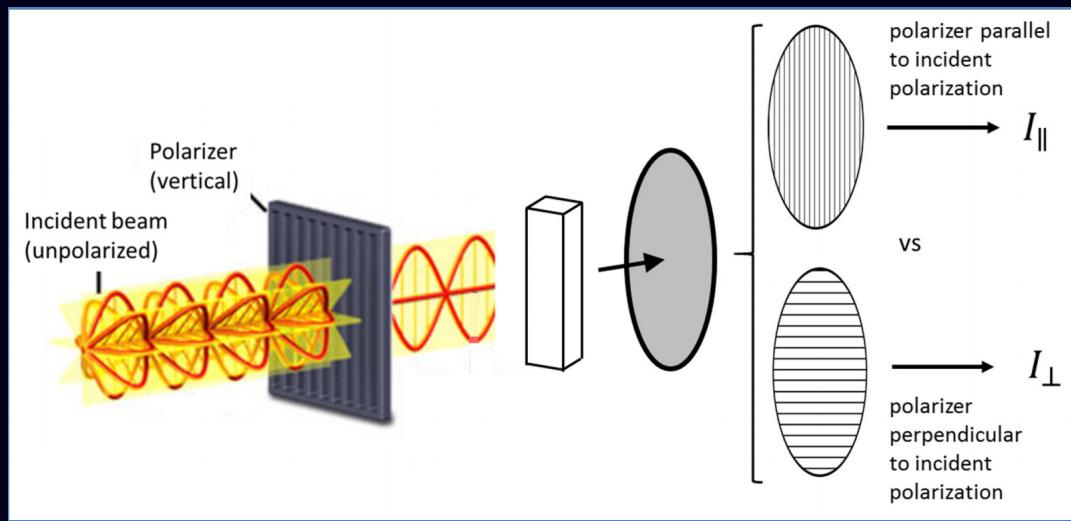
How do you expect a molecule to rotate in the time between photon absorption and re-emission via fluorescence? This depends on the relative rates of fluorescence and molecule rotation in solution.

If random molecular rotations occur slowly relative to fluorescence, then fluorescent photons will be polarized in the same direction as the incident (polarized) light. If the rotations

instead happen quickly relative to fluorescence, then emitted photons will have electric field vectors oriented in all directions equally.

Thus, it is possible to learn about the relative rates of fluorescence vs molecular rotation by studying the degree to which emitted photons are polarized in the same way as the incident light. If the timescale for fluorescence is known then the time scale for molecular rotations can be determined.

There are some essential features of a fluorescence polarization or fluorescence anisotropy experiment. Two polarizing filters are required: one before the sample, and one after the sample. Monochromators are also required to select appropriate wavelengths for the incident and emitted photons being detected. This allows to measure the relative intensity of emitted light that is polarized in different directions; this is a measure of how much molecules have rotated after absorption and before emission.



The intensity of light emitted parallel to the incident light is denoted I_{\parallel} , and the intensity emitted perpendicular to the incident light is denoted I_{\perp} . The unitless measure of how much stronger the parallel emission is compared to the perpendicular is described by **fluorescence anisotropy**, r . r is defined by:

$$r = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + 2I_{\perp}} \longrightarrow (0 \leq r \leq 1)$$

If r is close to 0 (no anisotropy) then the intensity is the same for \parallel and \perp emission. This means that the rate of molecular rotation is much faster than the rate of fluorescence. If r is close to 1 (perfect anisotropy), then the emission in the \perp orientation is negligible. The rate of fluorescence is much faster than the rate of molecular rotation.

How does fluorescence anisotropy relate to relative rates of fluorescence and molecular rotation. Unimolecular rates be described (reciprocally related) decay times. The decay time for changes in molecular orientation is referred to as rotational correlation time, denoted as τ_{rot} .

Fluorescence decay time is denoted as τ_{fluor} . Perrin's equation states that:

$$\frac{r}{r_0} = \frac{\tau_{rot}}{\tau_{rot} + \tau_{fluor}}$$

This equation shows that if the decay time for rotation is much longer than decay time for fluorescence ($\tau_{rot} \gg \tau_{fluor}$), then anisotropy (r) would be 1. r would instead be 0 if the decay time for rotation was much shorter than for fluorescence. r_0 is needed to deal with an imperfect alignment of emitted and absorbed photons that occurs even without any molecular rotation.

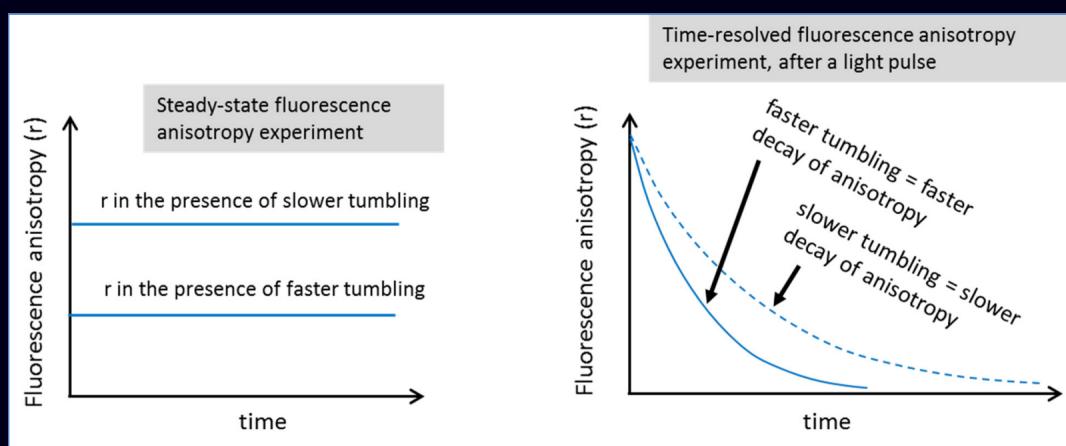
The rotational correlation is directly related to molecular size by:

$$\tau_{rot} = \frac{\eta V}{RT}$$

where η is viscosity, and V is molecular volume.

In practice, fluorescence anisotropy experiments are often used not to estimate molecular volumes, but in a more qualitative way, comparing the degree of anisotropy before and after some potential binding event. Two kinds of experiments are possible as illustrated. Under constant illumination (steady-state), events that lead to slower rotational tumbling give an increase in fluorescent anisotropy (r), since less tumbling occurs prior to fluorescent emission. The event **must** cause a change in the rotational correlation time of the fluorescent molecule.

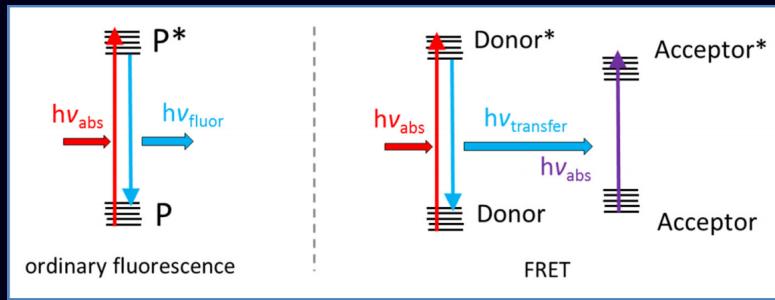
In a time-resolved experiment following an incident pulse, anisotropy will decay more slowly if the rotational tumbling is slower.



Fluorescent Resonant Energy Transfer (FRET)

Sometimes, an excited chromophore can return to ground state not by emission, but by transferring exciton energy to a nearby chromophore. The efficiency of this process depends on two main factors.

1. The degree of overlap between the emission spectrum of the first chromophore (donor) and the absorption spectrum of the second (acceptor).

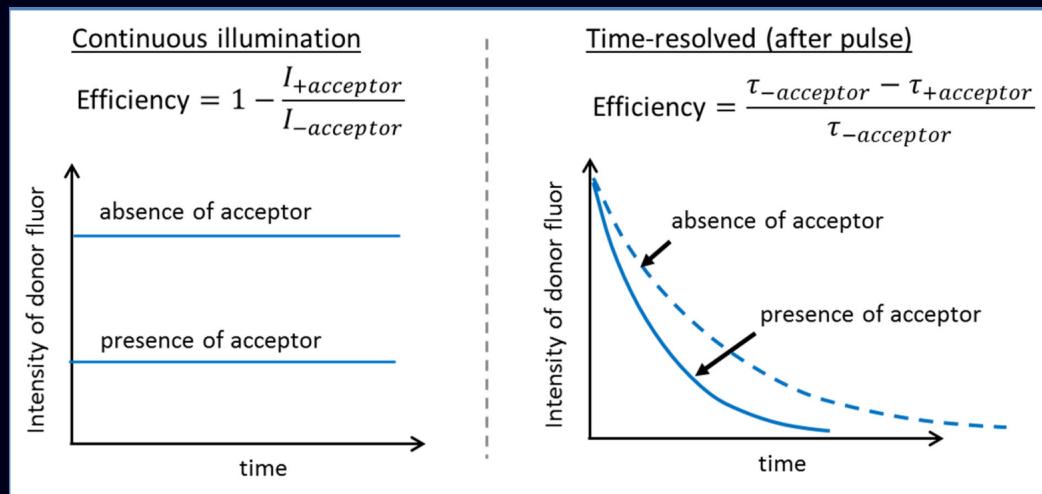


According to the Förster Equation, transfer efficiency depends strongly on separation (R) between donor and acceptor:

$$Efficiency = \frac{1}{1 + (\frac{R}{R_0})^6}$$

R_0 is particular for the donor and acceptor pair and depends chiefly on the quality of the spectral overlap between the donor emission and acceptor absorbance. When $R = R_0$, the efficiency is $\frac{1}{2}$. A good donor will have a relatively high value of R_0 . FRET experiments are useful mainly in the $10 - 100 \text{ \AA}$ range.

Looking at our scheme from the previous chapter showing the routes for return to ground state, we can see that energy transfer by FRET is a phenomenon that competes with the fluorescent emission from the donor. Thus the presence of the acceptor chromophore reduces donor fluorescence and speeds decay of excited donor and its fluorescence.



The experiments can be done in two ways, either as a constant illumination (where fluorescent intensity) is decreased in the presence of acceptor chromophore, or as a time-resolved experiment, where the speed of decay is increased by the acceptor and characteristic decay time is decreased time.

FRET experiments are useful when detecting proximity or approximating the distance between two molecules or functional groups might be informative. Unless one of the two components of interest is naturally fluorescent, this involves labelling both components (one with donor fluorophore, one with acceptor).

FRET in Biology

FRET plays a key role in photosynthesis. The key step that converts light energy and chemical energy in photosynthesis takes place in a transmembrane protein complex known as the photosynthetic reaction center (RC). The RC binds a 'special pair' of chlorophyll molecules in a parallel and partially overlapping arrangement. This group makes the special pair suitable for participating in the primary photochemical event.

After the pair is excited, instead of returning to ground state, an electron leaves the special pair and jumps along a path of neighboring pigment cofactors (chlorophylls and carotenoids) bound to the protein. This leads the electron across the membrane and generates an electrochemical difference between the two sides. This forms the basis for chemical energy conversion in photosynthesis.

RC alone is not suited for absorbing photons that are hitting the photosynthetic membrane everywhere and with a broad range of wavelengths. Other transmembrane proteins known as light harvesting complexes (LH) bind a large number of pigment molecules and surround the RC.

LH proteins are designed to hold their pigment molecules in very specific positions with respect to each other. This allows the pigment molecules to absorb photons efficiently throughout the membrane, and then transfer that exciton energy (FRET) to pigment molecules closer to RC. Each transfer loses some energy, but ultimately the energy is delivered to the special pair in RC in order to drive the primary photochemical event.

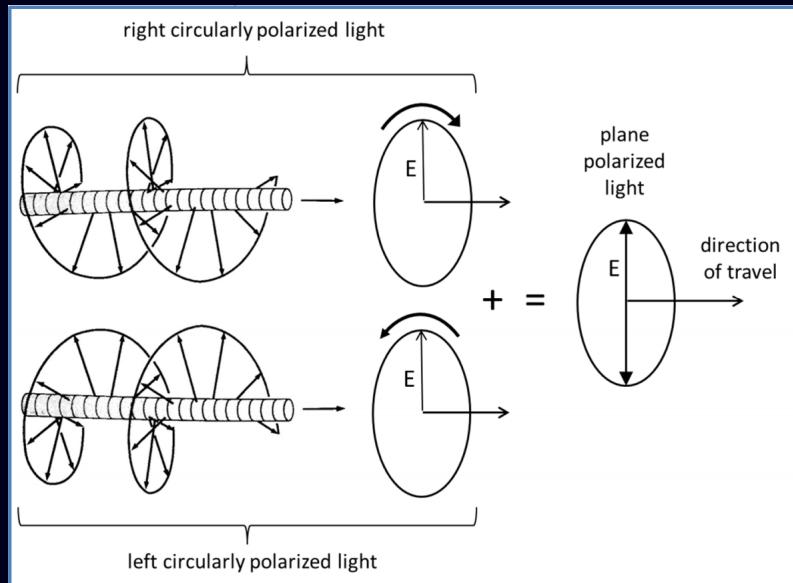
Spectroscopy of Chiral Molecules: Optical Rotation and Circular Dichroism

Chiral molecules exhibit special spectroscopic phenomena that show when they interact with polarized light.

Circularly Polarized Light

In plane polarized light, the electric field vector oscillates in a plane. A vector that oscillates up and down vertically can be generated by the sum of two vectors that rotate in a circle in opposite directions at equal frequency.

When they are both vertical (up or down), they add to give a vertical result, whereas when they are horizontal they oppose each other and cancel.



From the figure above, the 'right' component forms a right-handed helix, while the 'left' component forms the left-handed helix. The sense of the rotation is that a fixed observer looking towards the source will see the direction of the E field vector. **Clockwise in time** for right circularly polarized light. **Counter-clockwise for left circularly polarized light.**

By considering plane polarized light as a sum of two circular components (and as helical waves) we can see why chiral molecules might interact differently with left vs right polarized light. Helices are chirals, as is a biological macromolecule. Think of your feet and shoes (both of which are chiral). A particular shoe interacts differently with each foot.

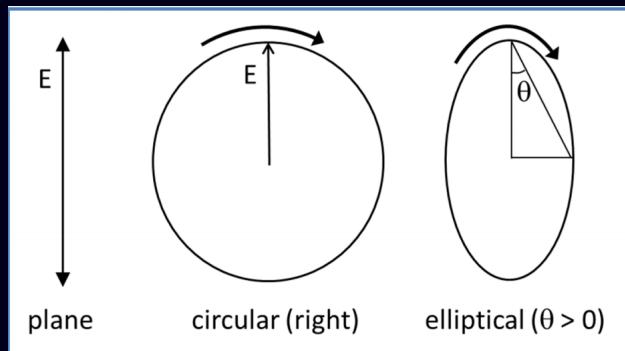
What are the distinct kinds of interactions that a chiral molecule (foot) can make with a chiral wave (shoe)? Two effects are the differences in absorption and index of refraction.

Circular Dichroism

If the left and right circularly polarized components (within a beam of plane polarized light) are absorbed to the same extent when passing through a sample, then the transmitted light should naturally remain plane polarized.

But if one component is absorbed more strongly than the other, this forms the basis for circular dichroism (CD). For example, if the left circularly polarized component is absorbed slightly more, then the remaining transmitted beam should have a slightly larger component of the right circularly polarized type. If we add up oppositely rotating vectors of unequal magnitude, we get an elliptical shape for the resulting path of the electric field vector.

The magnitude of circular dichroism is described by a parameter referred to as **ellipticity**, θ .



θ is the angle formed by a line between the tips of the transmitted electric field vectors in the \perp directions of maximum and minimum magnitude. The ellipticity can be measured by a CD spectrophotometer, this requires additional polarizers fromt eh sample and the detector.

Ellipticity effect originates from a difference in extinction coefficients (how strongly a substance absorbs light at a particular wavelength). The equation for θ in terms of absorbance for left vs right is:

$$\begin{aligned}\theta &= \frac{2.303(A_L - A_R)}{4} \\ &= \frac{2.303}{4} dC(\epsilon_L - \epsilon_R)\end{aligned}$$

where A = absorbance, ϵ = extinction coefficient, d is the path length of the light through sample, and C is the molar concentration. Recall from Beer's Law that $A = \epsilon Cd$.

According to the equation above, higher absorption of the left light (which means higher right component transmitted) yields a clockwise-rotating elliptical field vector \rightarrow positive θ . 2.303 comes from $\ln(10)$ which relates to the conventional use of \log_{10} .

Optical Rotation

The CD effect arises from differences in absorption. A different effect arises when left and right circularly polarized components travel through the sample at different speeds. What happens when light goes through a sample more slowly? The frequency of the wave is unchanged, but the wavelength changes. The speed of light is inversely dependent on the index of refraction, n . The index of refraction here may be different for the left component compared to the right:

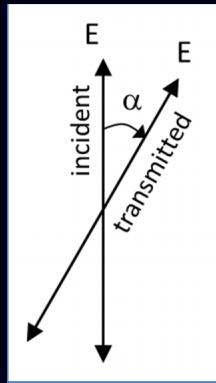
$$c_L = \frac{c_0}{n_L} c_R = \frac{c_0}{n_R}$$

where c_0 is the speed of light in a vacuum.

Then, $\lambda_L = \frac{c_L}{v} = \frac{c_0}{n_L v}$. How many oscillatory cycles does a light beam make when it passes through a sample of thickness d . The answer is $\frac{d}{\lambda}$.

The angular rotation in radians would be $\frac{2\pi d}{\lambda_L}$. After substituting λ with the above equation, the angular rotation is then $\frac{2\pi d n_L v}{c_0} = \frac{2\pi d n_L}{\lambda}$.

The final orientation of the polarization direction is determined by the sum of left and right vectors, whose angle is the average of the two component vectors. The resulting transmitted wave should be rotated according to half the difference between their separate angles of rotation.



This gives for the angle of rotation of the polarized beam:

$$\alpha = \frac{\pi d}{\lambda} (n_L - n_R)$$

According to this equation, the optical rotation angle α is positive if the index of refraction is higher for the left circularly polarized light. This means that its speed through the sample will be slower, thus the wave will oscillate further compared to the right circularly polarized light.

If you look at the above figure, you notice that if the left polarized light rotates further as a function of position along the direction of travel (α positive), it will rotate clockwise. This is opposite of the counterclockwise rotation of the E field vector seen by the fixed observer for left-handed. If $n_L - n_R > 0$, then the rotation of the electric field vector is clockwise.

If the optical rotation is expressed as a molar quantity by dividing by concentration and normalized for path length, an equation for molar optical rotation is obtained:

$$[\alpha] = \frac{100\alpha}{Cd} = \frac{100\pi}{\lambda C} (n_L - n_R)$$

As with the CD effect, for most molecules in solution, the difference in index of refraction ($n_L - n_R$) is very small. But the path length d is about 10^4 or more times longer than the wavelength λ , that the optical rotation is non-negligible. Optical rotation can be used to identify chiral molecules.

Optical rotation and circular dichroism are interrelated

Both optical rotation and circular dichroism are related to each other and they occur in the same molecular sample. Both arise from complex relationships between electric transition dipole moments and magnetic dipole moments in a molecule.