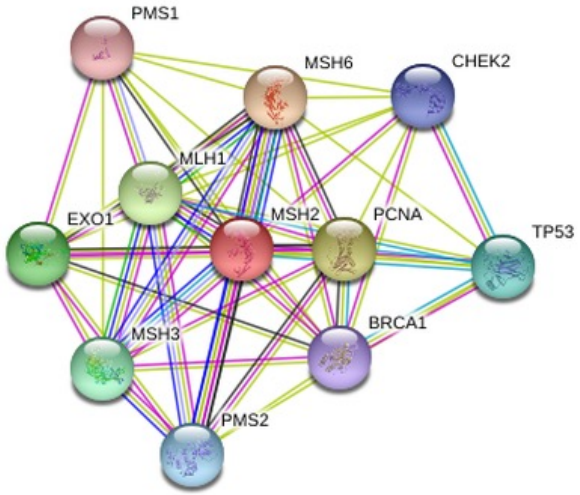
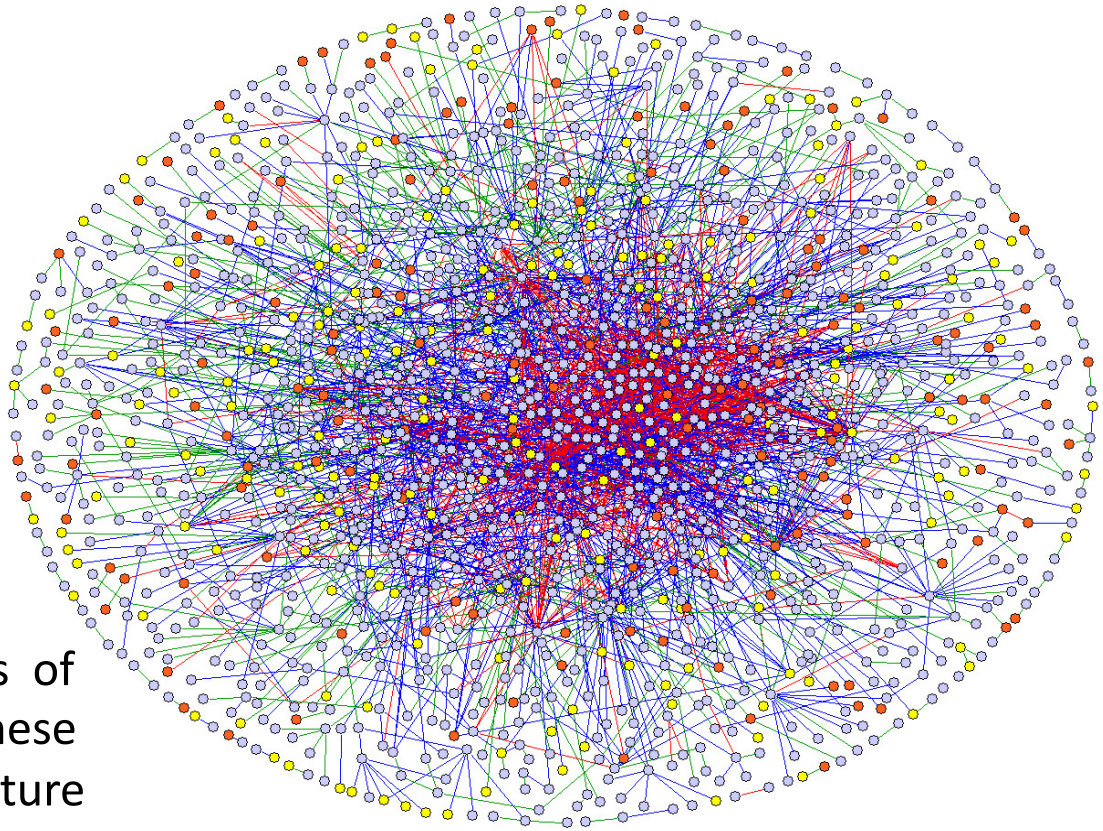
Protein-ligand interactions

BIOMOLECULAR INTERACTIONS

L'espressione e la regolazione delle componenti di una cellula e l'organizzazione dei pathways che sono alla base della sua funzionalità vengono controllati da una complessa rete di interazioni tra biomolecole



…quanto complessa??



Protein-protein human network

- Sistemi *in vitro*:
  - in soluzione
  - in superficie

Cell function and regulation depend on transient interactions among thousands of different macromolecules in the cell. These diagrams are useful, but a complete picture requires a deeper, more quantitative level of understanding.

Alberts, Bruce; Johnson, Alexander; Lewis, Julian; Morgan, David; Raff, Martin; Roberts, Keith; Walter, Peter.

Molecular Biology of the Cell

Garland Science

Chemical reactions in cells

Two opposing streams of chemical reactions occur in cells:

(1) the **catabolic pathways** break down foodstuffs into smaller molecules, thereby generating both a useful form of energy for the cell and some of the small molecules that the cell needs as building blocks

(2) the **anabolic, or biosynthetic, pathways** use the small molecules and the energy harnessed by catabolism to drive the synthesis of the many other molecules that form the cell.

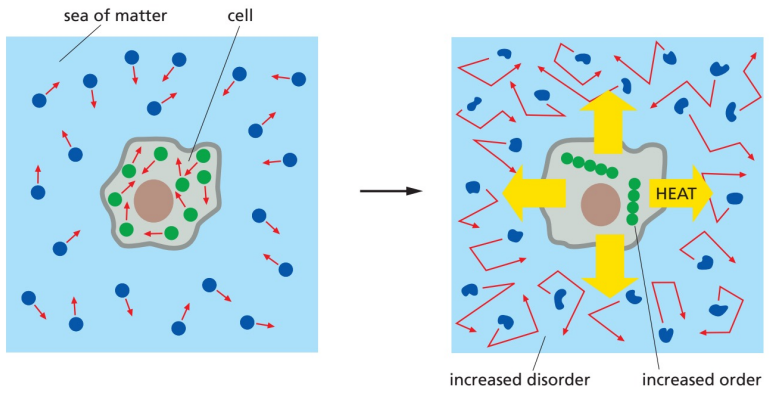
Together these two sets of reactions constitute the metabolism of the cell.

The general principles by which cells obtain energy from their environment and use it to create order are central to cell biology.

Living cells defy the second law of thermodynamics?

No: a cell is not an isolated system, it takes in energy from food, or as photons from the sun and uses it to generate order within itself, converting part of the energy it uses into heat.

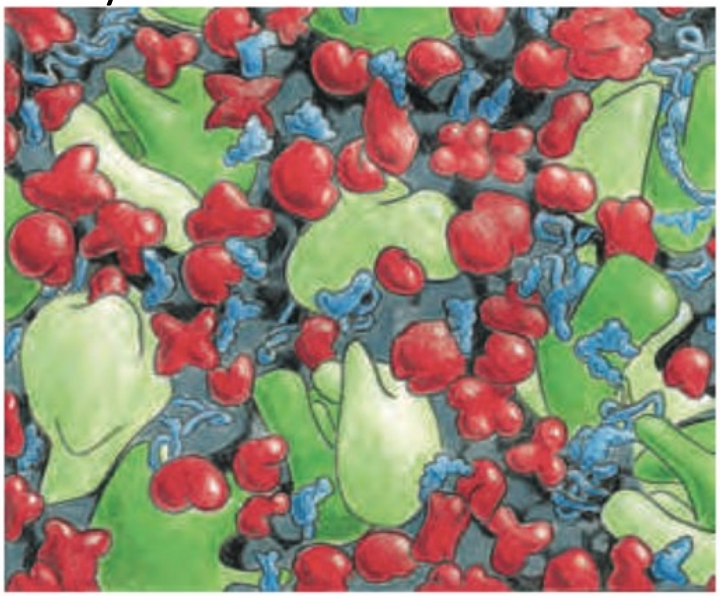
The heat is discharged into the cell's environment and disorders the surroundings.

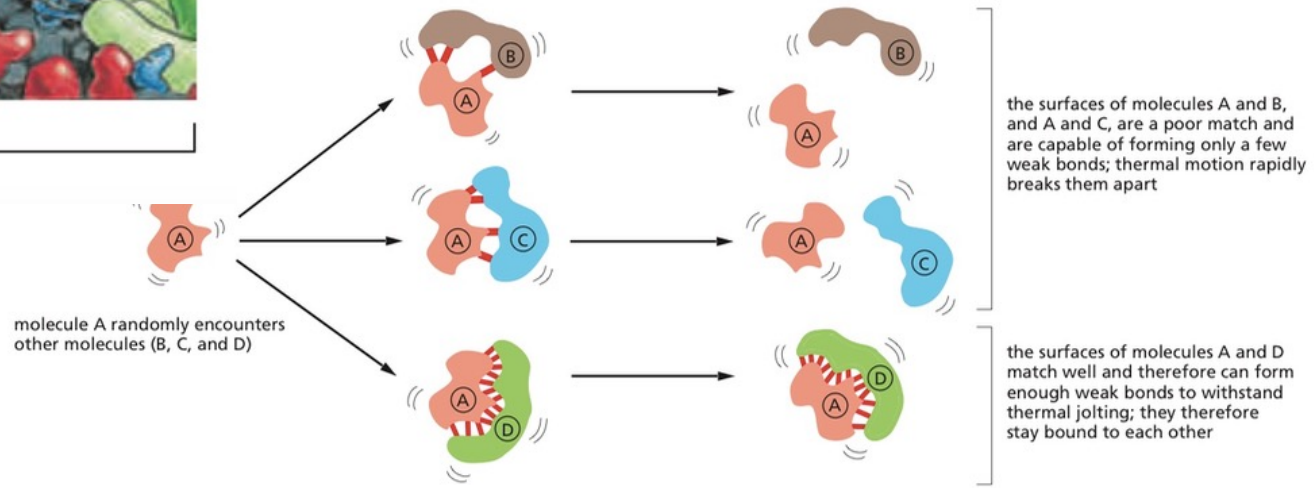


The total entropy—that of the cell plus its surroundings—increases, as demanded by the second law of thermodynamics.

Life inside a cell

Molecules in the cell are in a very crowded environment, in continual random thermal movements: rapid “faint” associations and dissociation between molecules are made. Such reactions allow for specific chemical reactions, including the catalytic action of enzymes





Oxidation and reduction reactions

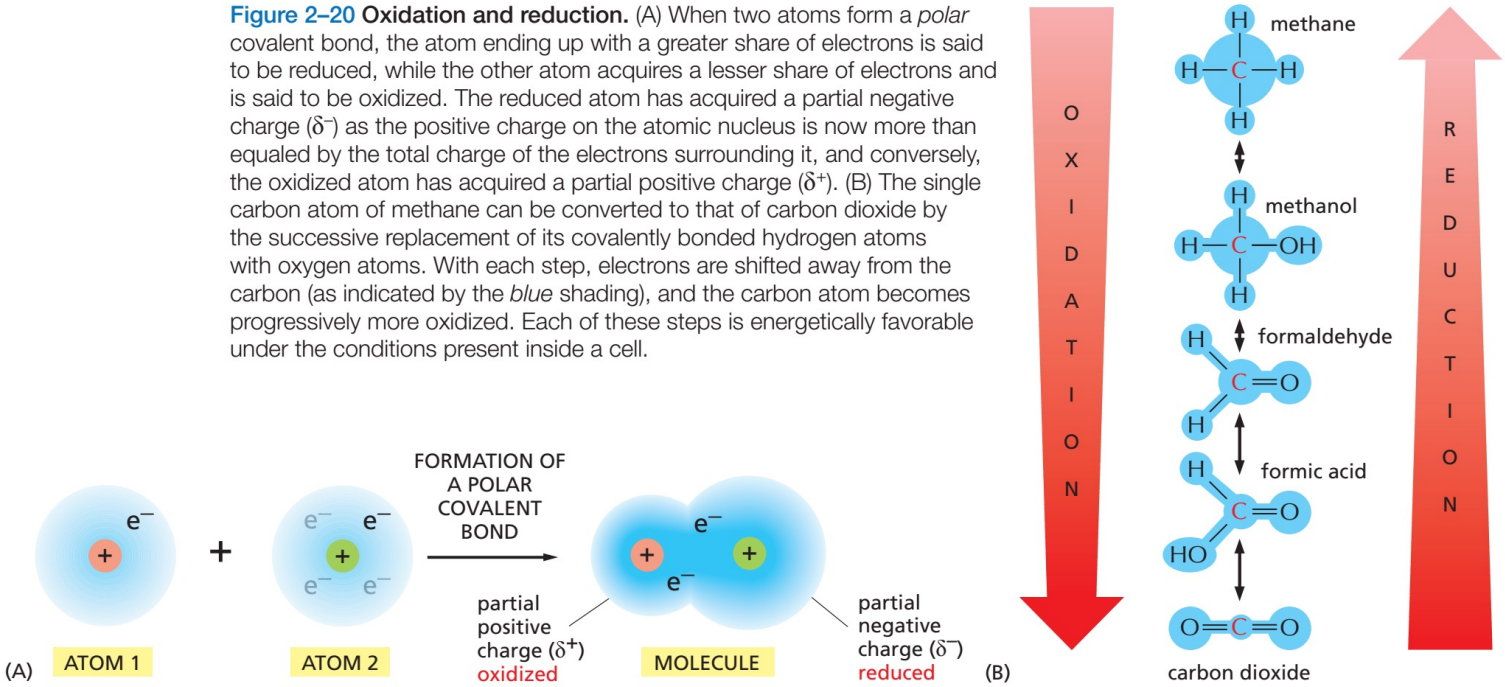


Figure 2–20 Oxidation and reduction. (A) When two atoms form a *polar* covalent bond, the atom ending up with a greater share of electrons is said to be reduced, while the other atom acquires a lesser share of electrons and is said to be oxidized. The reduced atom has acquired a partial negative charge (δ^-) as the positive charge on the atomic nucleus is now more than equaled by the total charge of the electrons surrounding it, and conversely, the oxidized atom has acquired a partial positive charge (δ^+). (B) The single carbon atom of methane can be converted to that of carbon dioxide by the successive replacement of its covalently bonded hydrogen atoms with oxygen atoms. With each step, electrons are shifted away from the carbon (as indicated by the *blue* shading), and the carbon atom becomes progressively more oxidized. Each of these steps is energetically favorable under the conditions present inside a cell.

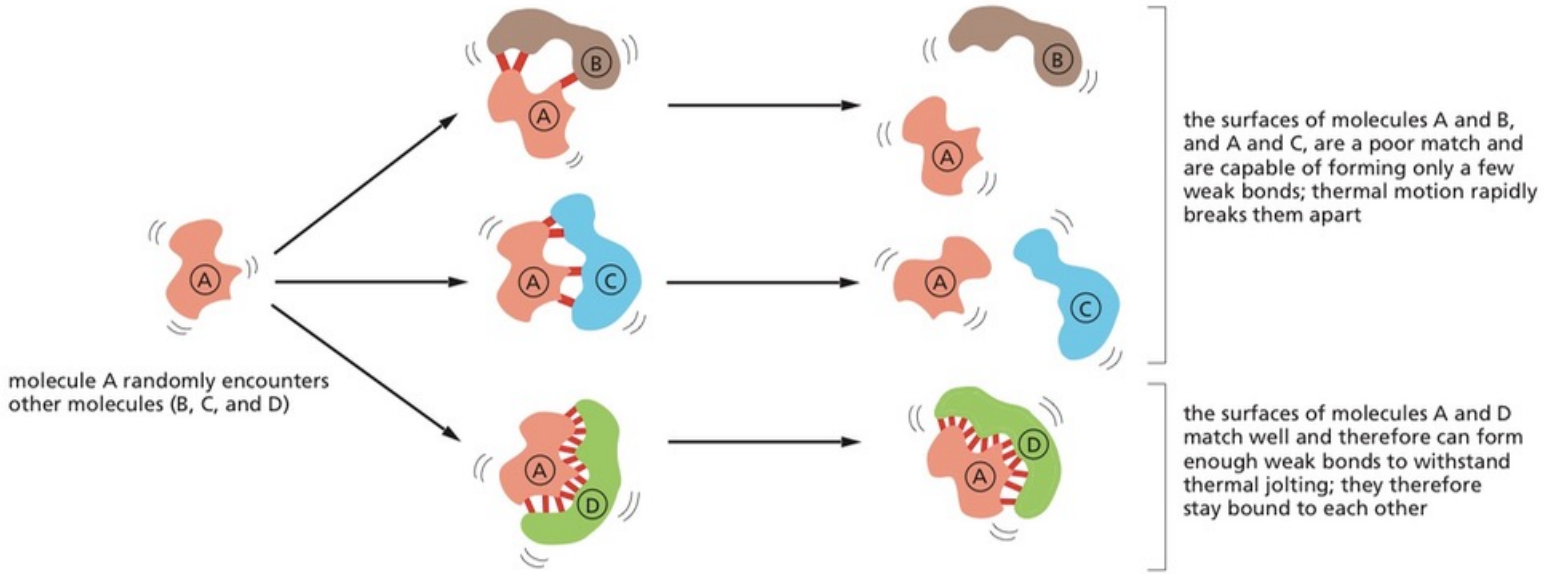
When a molecule in a cell picks up an electron (e –), it often picks up a proton (H+) at the same time (protons being freely available in water). The net effect in this case is to add a hydrogen atom to the molecule.

$A + e^- + H^+ \rightarrow AH$

Hydrogenation reactions are reductions, and the reverse, **dehydrogenation reactions are oxidations**. Cells use enzymes to catalyze the oxidation of organic molecules in small steps, through a sequence of reactions that allows useful energy to be harvested.

Molecular recognition

Molecular recognition refers to the process in which **biological macromolecules** interact with each other or with various **small molecules** through **noncovalent interactions** to form a specific **complex**. Characteristics:

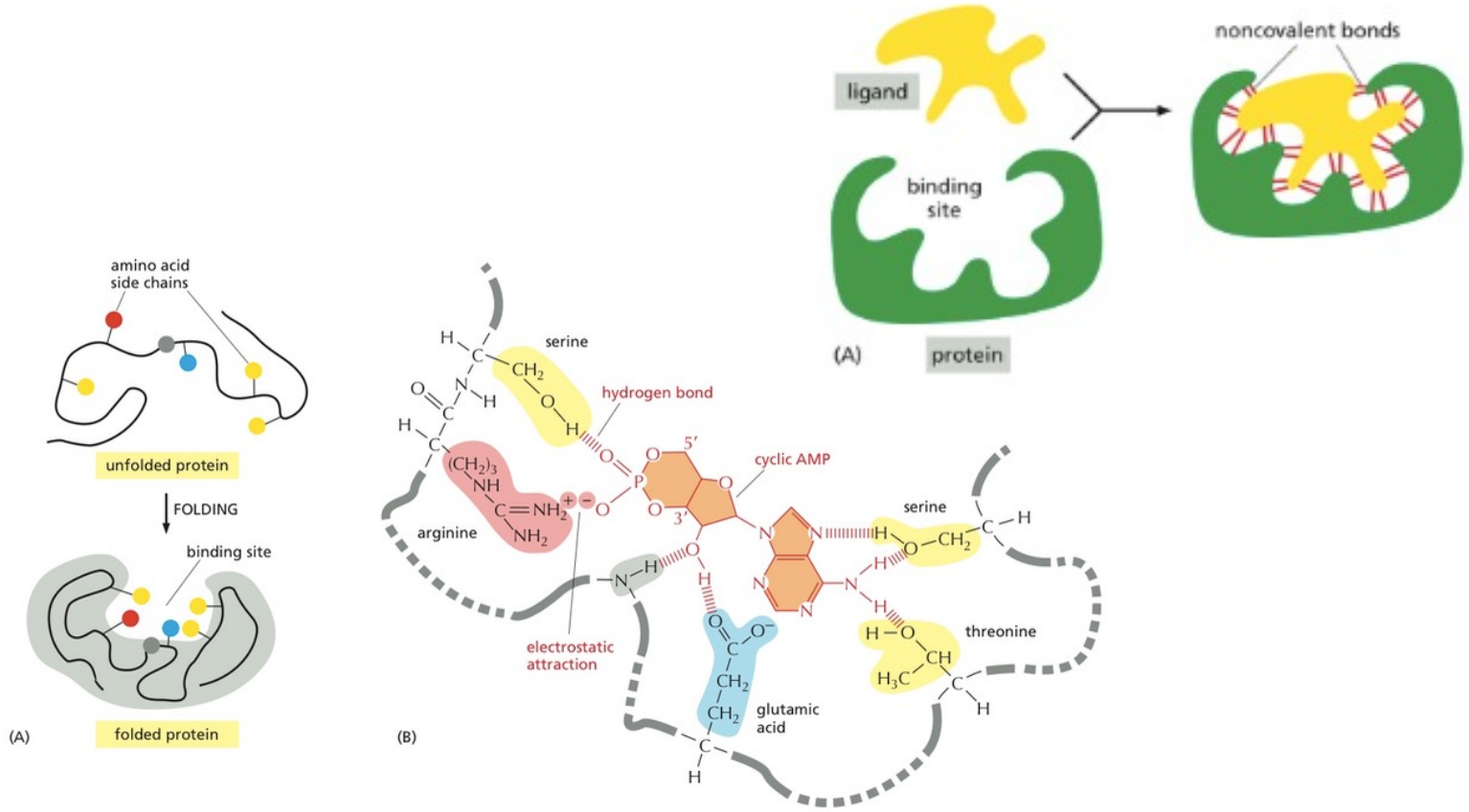


(i) **Specificity**. which distinguishes the highly specific binding partner from less specific partners;

(ii) **Affinity**. high concentration of weakly interacting partners cannot replace the effect of a low concentration of the specific partner interacting with high affinity

Molecular recognition

A **binding site** is a cavity made by aminoacids from different portions of the chain. Separated regions provide binding sites for different ligands, allowing for protein activity being regulated.

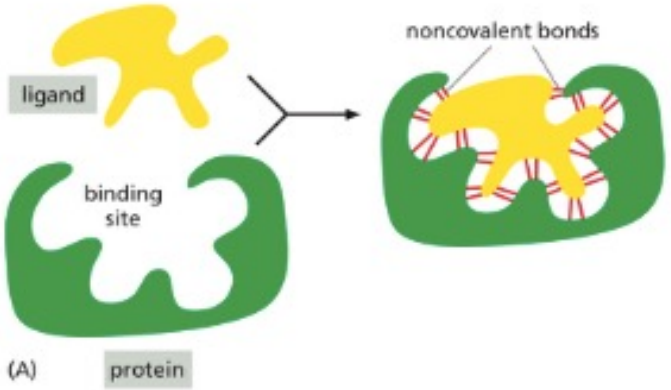


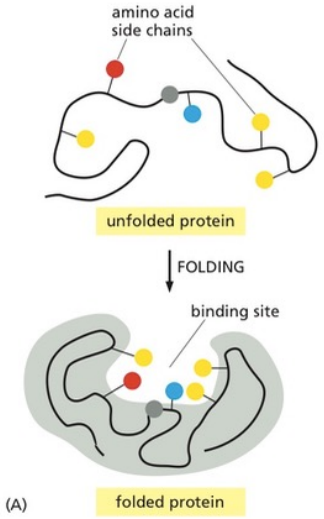
Selectivity depends on the set of non-covalent bonds (additive!!!) and the favorable hybdrophobic interactions that can form simultaneoulsly

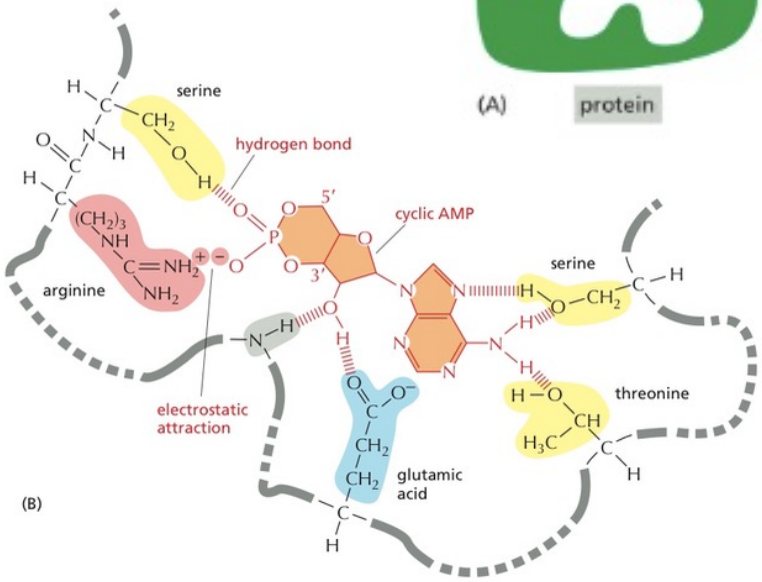
Molecular recognition

Ligand binding site are generally kept dry by the tendency of water molecules to for H-bond networks: is energetically unfavorable for a single water mol. to break the network and bind the protein.

Clustering of neighboring polar residues with the same charge induced by folding also favors positive ions to bind.

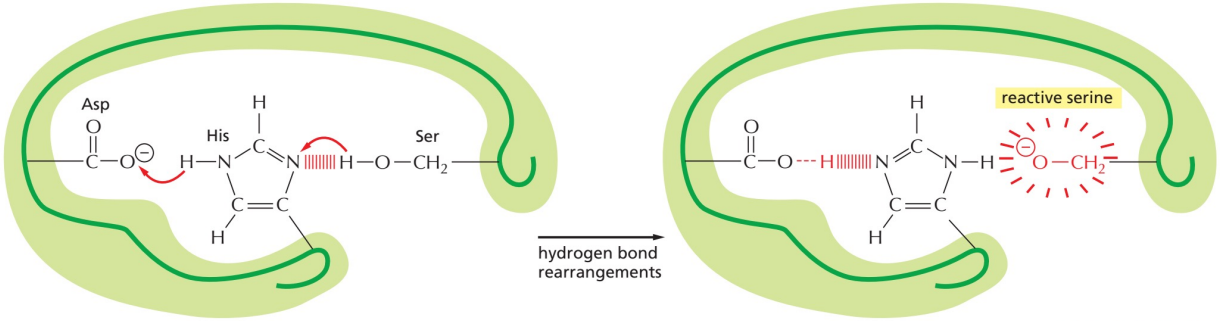






Selectivity depends on the set of non-covalent bonds (additive!!!) and the favorable hybdrophobic interactions that can form simultaneoulsly

Molecular recognition



Also, folding may promote H-bonding which makes even unreactive groups (i.e. serine) reactive.

Figure 3–39 An unusually reactive amino acid at the active site of an enzyme. This example is the "catalytic triad" Asp-His-Ser found in chymotrypsin, elastase, and other serine proteases (see Figure 3–12). The aspartic acid side chain (Asp) induces the histidine (His) to remove the proton from a particular serine (Ser). This activates the serine and enables it to form a covalent bond with an enzyme substrate, hydrolyzing a peptide bond. The many convolutions of the polypeptide chain are omitted here.

Highest affinity macromolecules: Antibodies

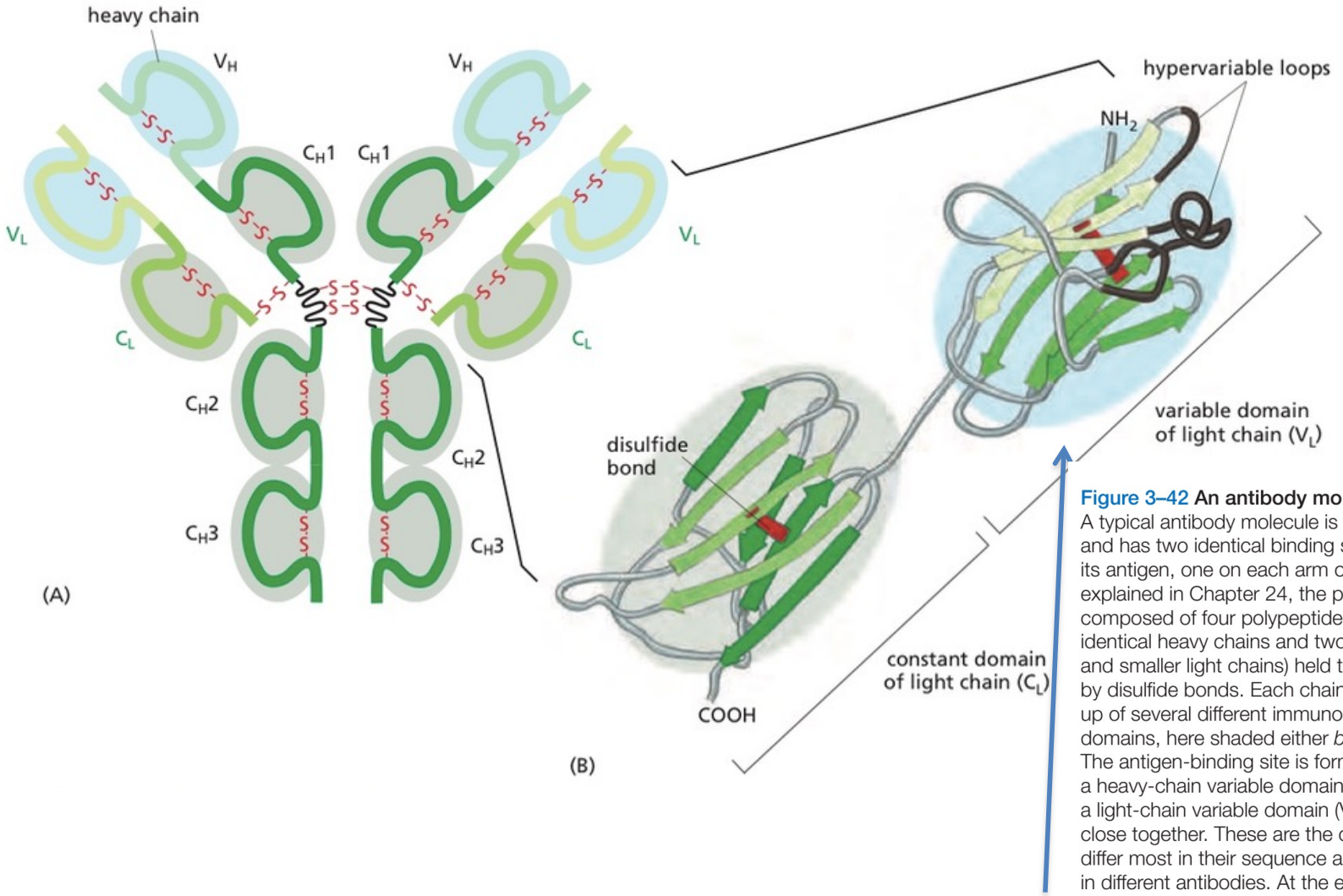


Figure 3–42 An antibody molecule. A typical antibody molecule is Y-shaped and has two identical binding sites for its antigen, one on each arm of the Y. As explained in Chapter 24, the protein is composed of four polypeptide chains (two identical heavy chains and two identical and smaller light chains) held together by disulfide bonds. Each chain is made up of several different immunoglobulin domains, here shaded either *blue* or *gray*. The antigen-binding site is formed where a heavy-chain variable domain (V_H) and a light-chain variable domain (V_L) come close together. These are the domains that differ most in their sequence and structure in different antibodies. At the end of each of the two arms of the antibody molecule, these two domains form loops that bind to the antigen (see Movie 24.5).

Two equivalent binding sites for the antigen, one per each arm

An examples: enzymes

The chemical reactions that a cell carries out would normally occur only at much higher temperatures than those existing inside cells.
Each reaction requires a specific boost in chemical reactivity. The control is exerted through specialized biological catalysts.
These are almost always proteins called enzymes, although RNA catalysts also exist, called ribozymes.

For **enzymes,** ligand binding is only a necessary first step in their function.
Enzymes cause the chemical transformations that make and break covalent bonds in cells. They bind to one or more ligands, called **substrates**, and convert them into one or more chemically modified products, doing this over and over again with amazing rapidity.

Enzymes speed up reactions, often by a **factor of a million** or more, without themselves being changed—that is, they act as catalysts that permit cells to make or break covalent bonds in a controlled way.

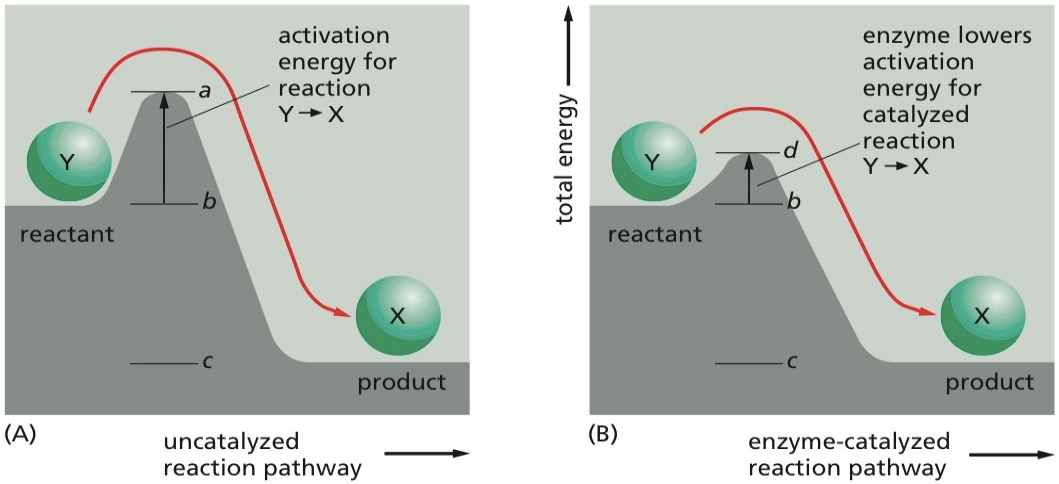
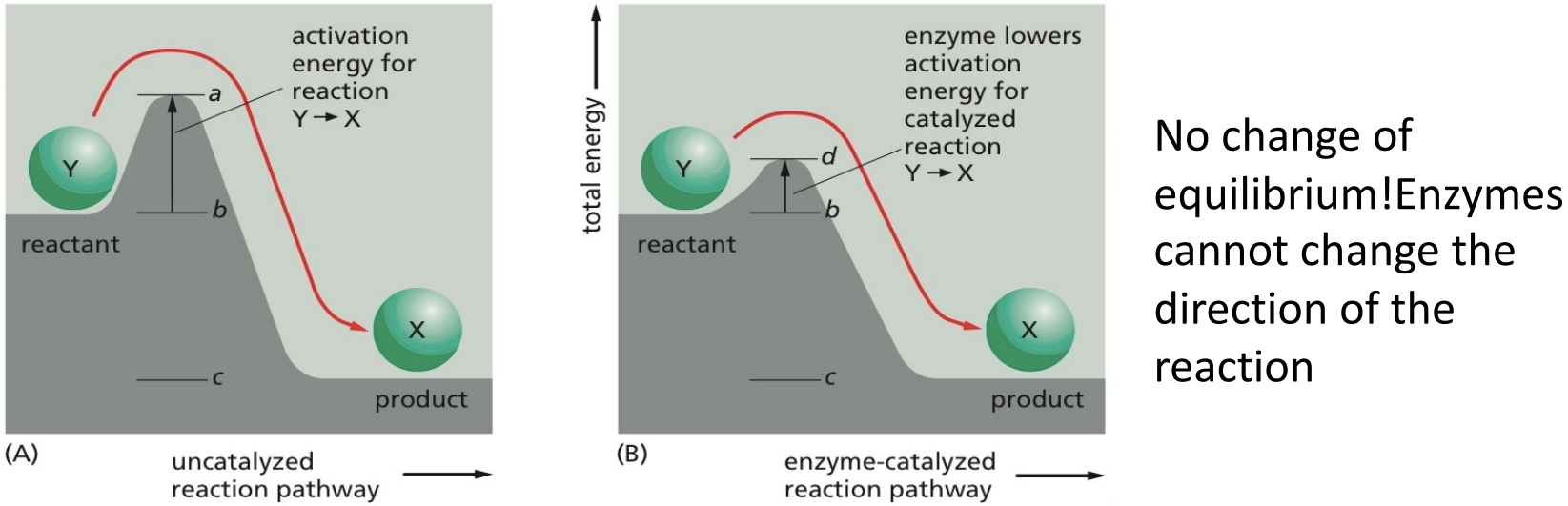


TABLE 3–1 Some Common Types of Enzymes

Enzyme	Reaction catalyzed
Hydrolases	General term for enzymes that catalyze a hydrolytic cleavage reaction; *nucleases* and *proteases* are more specific names for subclasses of these enzymes
Nucleases	Break down nucleic acids by hydrolyzing bonds between nucleotides. *Endo–* and *exonucleases* cleave nucleic acids *within* and *from the ends of* the polynucleotide chains, respectively
Proteases	Break down proteins by hydrolyzing bonds between amino acids
Synthases	Synthesize molecules in anabolic reactions by condensing two smaller molecules together
Ligases	Join together (ligate) two molecules in an energy-dependent process. DNA ligase, for example, joins two DNA molecules together end-to-end through phosphodiester bonds
Isomerases	Catalyze the rearrangement of bonds within a single molecule
Polymerases	Catalyze polymerization reactions such as the synthesis of DNA and RNA
Kinases	Catalyze the addition of phosphate groups to molecules. Protein kinases are an important group of kinases that attach phosphate groups to proteins
Phosphatases	Catalyze the hydrolytic removal of a phosphate group from a molecule
Oxido-Reductases	General name for enzymes that catalyze reactions in which one molecule is oxidized while the other is reduced. Enzymes of this type are often more specifically named *oxidases*, *reductases*, or *dehydrogenases*
ATPases	Hydrolyze ATP. Many proteins with a wide range of roles have an energy-harnessing ATPase activity as part of their function; for example, motor proteins such as *myosin* and membrane transport proteins such as the *sodium–potassium pump*
GTPases	Hydrolyze GTP. A large family of GTP-binding proteins are GTPases with central roles in the regulation of cell processes

Enzyme names typically end in "-ase," with the exception of some enzymes, such as pepsin, trypsin, thrombin, and lysozyme, that were discovered and named before the convention became generally accepted at the end of the nineteenth century. The common name of an enzyme usually indicates the substrate or product and the nature of the reaction catalyzed. For example, citrate synthase catalyzes the synthesis of citrate by a reaction between acetyl CoA and oxaloacetate.

Enzymes



No change of equilibrium!Enzymes cannot change the direction of the reaction

Any molecule requires activation energy—a kick over an energy barrier—to undergo a chemical reaction that leaves it in a more stable state.

In a cell, the kick is delivered by an unusually energetic random collision with surrounding molecules—more violent as the temperature is raised.

-Enzymes aid the kick over energy barriers, controlling chemical reactions in cells.

-Each enzyme binds tightly to one or more molecules, the substrates, and holds them in a way that greatly reduces the activation energy of a particular chemical reaction that the bound substrates can undergo.

-Enzymes increase the rate of chemical reactions (up to 10^{14} times!) because they allow a much larger proportion of the random collisions with surrounding molecules to kick the substrates over the energy barrier.

Basic Concepts and Thermodynamic Relationships

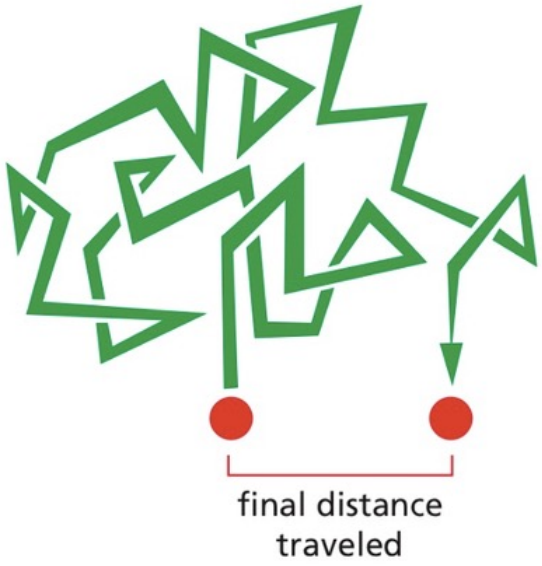
Enzyme can process 1000 mol. per second. Meaning they bind a new substrate in a fraction of a milliseconds.
But enzymes and their substrates are present in relatively small numbers in a cell.
How do they find each other so fast?
Rapid binding is possible because the **motions caused by heat energy are enormously fast at the molecular level,** generating:

(1) the movement of a molecule from one place to another (translational motion)
(2) the rapid back-and-forth movement of covalently linked atoms with respect to one another (vibrations)
(3) rotations.

All of these motions help to bring the surfaces of interacting molecules together.
The rates of molecular motions can be measured by a variety of spectroscopic techniques.

Basic Concepts and Thermodynamic Relationships

A large globular protein is constantly tumbling, rotating about its axis about a million times per second.
Molecules are also in constant translational motion, which causes them to explore the space inside the cell very efficiently by wandering through it—a process called ***diffusion.*** A small organic molecule, for example, takes only about one-fifth of a second on average to diffuse a distance of 10 µm, or the whole cell!

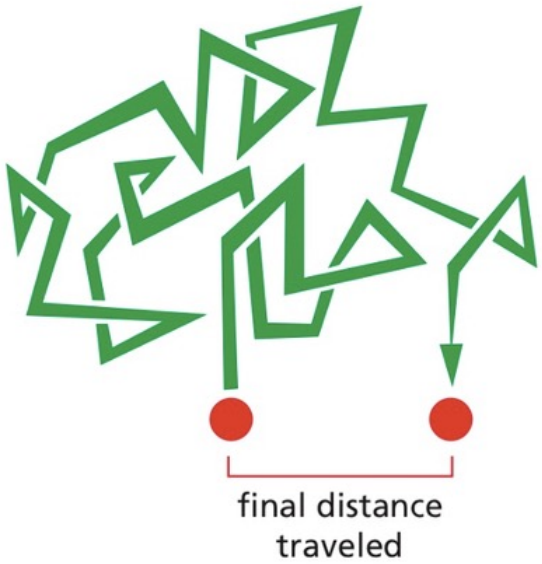


Diffusion with great heat exchange generates a molecule ***random walk***

The **average net distance** that each molecule travels from its starting point is **proportional to the square root of the time** involved.
if it takes a molecule 1 second on average to travel 1 µm, it takes 4 seconds to travel 2 µm, 100 seconds to travel 10 µm, and so on.

Basic Concepts and Thermodynamic Relationships

Since enzymes move more slowly than substrates in cells, we can think of them as sitting still. **The rate of encounter of each enzyme molecule with its substrate will depend on the concentration of the substrate**



Abundant substrates : 0.5 mM

Water: 55.5 M

1 substrate mol per 10^5 water mol!

However, will face 100.000 random collisions per second by the substrate

The inside of a cell is very crowded . Nevertheless, experiments in which fluorescent dyes are injected into cells show that small organic molecules diffuse through the watery gel of the cytosol nearly as rapidly as they do through water.

Brownian motion

Il moto casuale di una piccola particella (con diametro dell'ordine del micron) immersa in un fluido, dovuto ad urti tra la particella e le molecole del fluido stesso, e chiamato moto browniano.

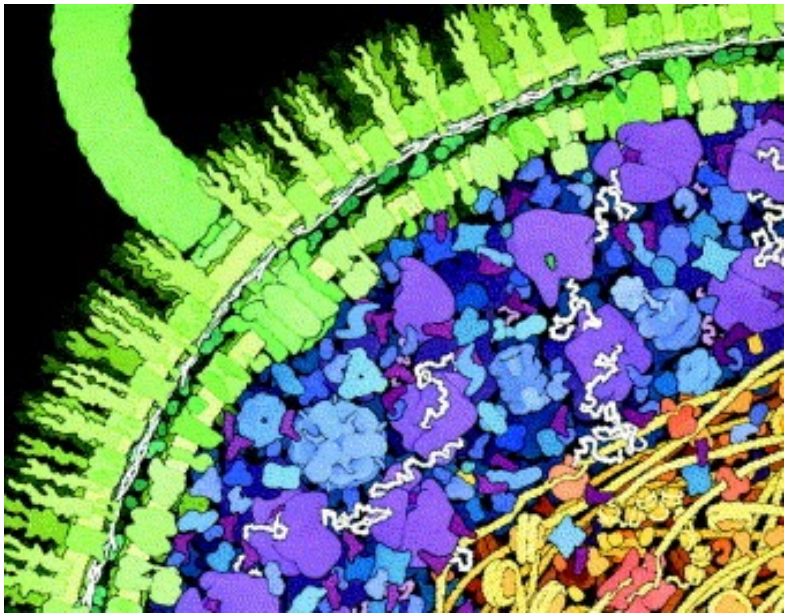
Il **moto browniano** di una particella colloidale `e una sequenza di passi casuali della posizione e dell'orientazione della particella stessa. Tale sequenza `e chiamata **diffusione** ed `e descritta da un'equazione,che permette di comprendere come la posizione della particella evolve nel tempo.

Come si deriva l'equazione di diffusione per particelle all'equilibrio, ovvero in assenza di forze esterne?

Cell crowding and diffusion constant

Brownian motion

Owing to thermal energy, macromolecules are in permanent chaotic motion



A particle at absolute temperature T has on the average a kinetic energy associated with movement along each axis of $kT/2$, where k is Boltzmann's constant. A particle of mass m and velocity v_x on the x- axis has a kinetic energy $mv_x^2/2$.

On the average $\langle\, mv_x^2/2 \,\rangle = kT/2$, where $\langle\ \rangle$ denotes an average over time or over an ensemble of similar particles. From this relationship we can compute the mean-square velocity as

$$\langle v_x^2 \rangle = kT/m \qquad \text{(A)}$$

and the root-mean-square velocity as

$$\langle v_x^2 \rangle^{1/2} = (kT/m)^{1/2} \qquad \text{(B)}$$

Cell crowding and diffusion constant

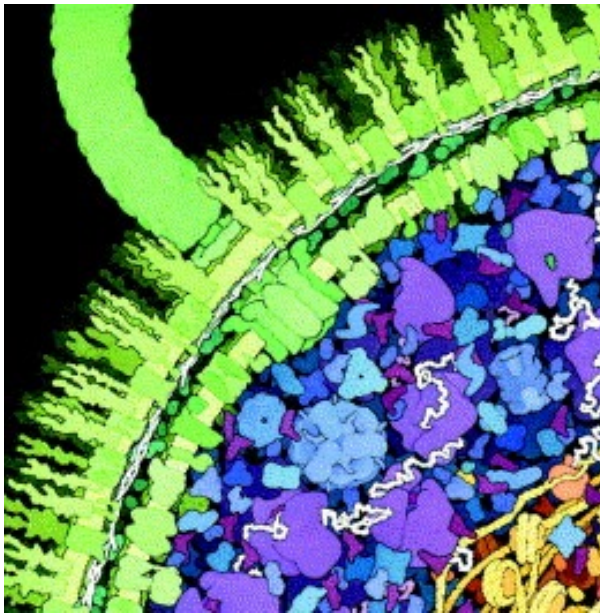
Sucrose in a vacuum

Sucrose has a molecular mass of 342 Da. This is the mass in grams of one mole or 6.02×10^{23} molecules; the mass of one molecule is $m = 5.7 \times 10^{-23}$ g. The value of kT at room temperature, 293 K, is 4.04×10^{-14} g cm^2 s^{-2}. Therefore, $\langle u_x^2 \rangle^{1/2} = 8.3 \times 10^3$ cm s^{-1}. If collisions were absent the sucrose molecule would cross a typical swimming pool in about 1 s. According to Eq. (B) in Comment D3.2 the velocity of small particle is inversely proporational to the root-square of its molecular weight.

Biological macromolecules

For proteins with a typical molecular mass of of 20 kDa the velocity is 1.09×10^3 cm s^{-1}. For the DNA with a molecular mass of 900 000 kDa the velocity is 33 cm s^{-1}.

Cell crowding and diffusion constant



Brownian motion

Albert Einstein provided a theoretical explanation of Brownian motion in 1905, which helped confirm the atomic theory of matter. His work led to the development of the diffusion equation, linking the motion to the diffusion coefficient D, which measures how fast particles spread out over time:

$$\langle x^2 \rangle = 2Dt$$

where:

- $\langle x^2 \rangle$ is the mean squared displacement of the particle,
- D is the diffusion coefficient,
- t is time.

Einstein's work was later expanded upon by Jean Perrin, who experimentally verified the atomic nature of matter through his observations of Brownian motion.

D measures the rate of diffusion
expressed as unit of area per unit of time!

Cell crowding and diffusion constant

Brownian motion

Consider that motions in the x-, y- and z-directions are independent. If $\langle x^2 \rangle = 2D_1 t$, then $\langle y^2 \rangle = 2D_2 t$, $\langle z^2 \rangle = 2D_3 t$. In two dimensions, the square of the distance from the origin to the point (x, y) is $r^2 = x^2 + y^2$; therefore

$$\langle r^2 \rangle = 4Dt \tag{D3.3}$$

where D is the average of the diffusion coefficients D_1 and D_2 in a two-dimensional random walk. In three dimensions, $r^2 = x^2 + y^2 + z^2$, and

$$\langle r^2 \rangle = 6Dt \tag{D3.4}$$

where D is average of the D_1, D_2 and D_3 diffusion coefficients in a three-dimensional random walk. Again, knowledge of the $\langle r^2 \rangle$ is sufficient to determine D and vice versa.

D measures the rate of diffusion
expressed as unit of area per unit of time!

Cell crowding and diffusion constant

Comment D3.3 Biologist's box: Diffusion and time

From Eq. (D3.2) we can calculate the instantaneous velocity of a small particle. Consider a few examples.

Urea in water

The diffusion coefficient of urea in water is 118 $cm^2\ s^{-1}$ (Table D3.3). A particle with such a diffusion coefficient diffuses a distance $x = 10^{-4}$ cm in a time $t = x^2/2D = 5 \times 10^{-4}$ s, or about 0.5 ms. It diffuses a distance $x = 1$ cm in a time $t = x^2/2D = 5 \times 10^5$ s, or about 14 h. It is clear that diffusive transport takes a long time when distances are large.

Proteins in water

A particle with a diffusion coefficient D of the order $10^{-6}\ cm^2\ s^{-2}$ (a small globular protein) diffuses a distance $x = 10^{-4}$ cm in a time $t = x^2/2D = 5 \times 10^{-3}$ s, or about 5 ms. It diffuses a distance $x = 1$ cm in a time $t = x^2/2D = 5 \times 10^5$ s, or about 138 h.

Tobacco mosaic virus in water

The tobacco mosaic virus has a small diffusion coefficient ($0.44 \times 10^{-7}\ cm^2\ s^{-2}$) and diffuses very slowly: it travels a distance $x = 0.5$ cm in a time $t = x^2/2D = 2.5 \times 10^6$ s, or about 700 h. This explains why the moving boundary method in which the minimal required diffusing distance is about a few millimetres is never used for measurements of diffusion coefficients of such large molecules.

Diffusion constant

Fick’s first equation

The diffusion constant D is defined by **Fick's Law of Diffusion**, which describes the movement of particles from regions of higher concentration to regions of lower concentration. For **one-dimensional diffusion**, Fick’s first law can be written as:

$$J = -D\frac{dC}{dx}$$

where:

- J is the diffusion flux (the amount of substance moving through a unit area per unit time),
- D is the diffusion coefficient (or constant),
- $\frac{dC}{dx}$ is the concentration gradient (change in concentration C over distance x).

This means the diffusion flux is proportional to the concentration gradient, and the proportionality constant is the diffusion constant D.

Fick’s first equation correlates the flux to a concentration gradient

Diffusion constant

Einstein Relation for Diffusion:

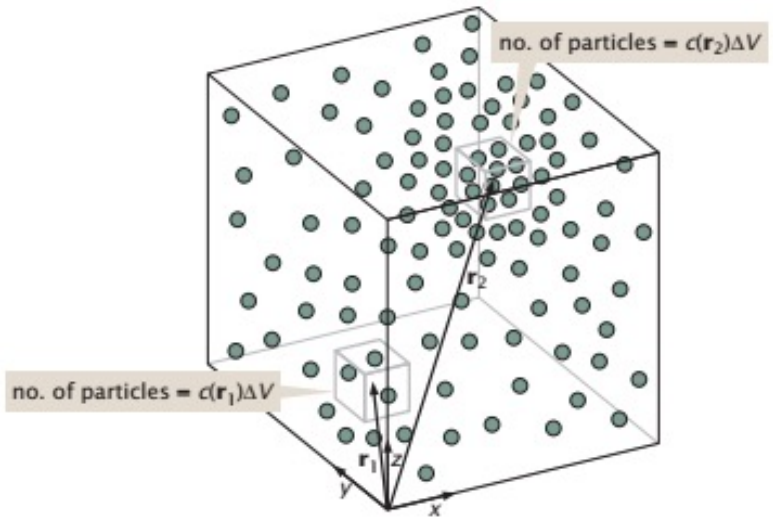
For a small particle undergoing Brownian motion in a fluid, the diffusion constant is related to the temperature, viscosity of the fluid, and the size of the particle. This relationship is given by the **Stokes-Einstein equation**:

$$D = \frac{k_B T}{6\pi\eta r}$$

where:

- D is the diffusion constant,
- k_B is the Boltzmann constant ($1.38 \times 10^{-23}\ \mathrm{J/K}$),
- T is the absolute temperature,
- η is the dynamic viscosity of the fluid,
- r is the radius of the particle.

Diffusive dynamics (Fick's second equation)

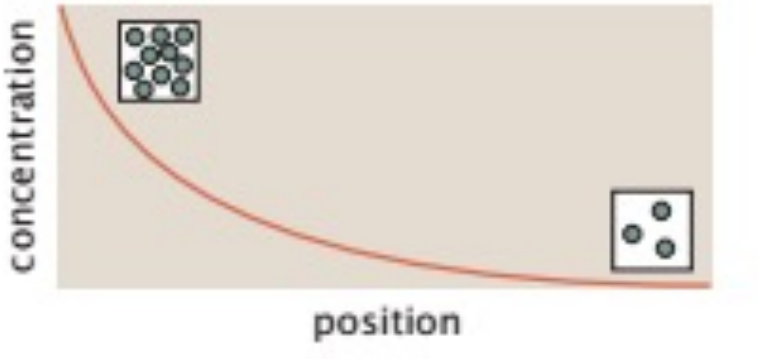


Partcles' number conserved

we divide space up into a bunch of small boxes, large enough to include many molecules, but small enough so that the density is nearly uniform over the scale of the box.
We use the notation c(**r**,t) to signify the concentration in a box centered at position **r** in three-dimensional space (with units of number of particles per unit volume) and c(x,t) to signify the concentration field in one-dimensional problems (with units of number of particles per unit length).

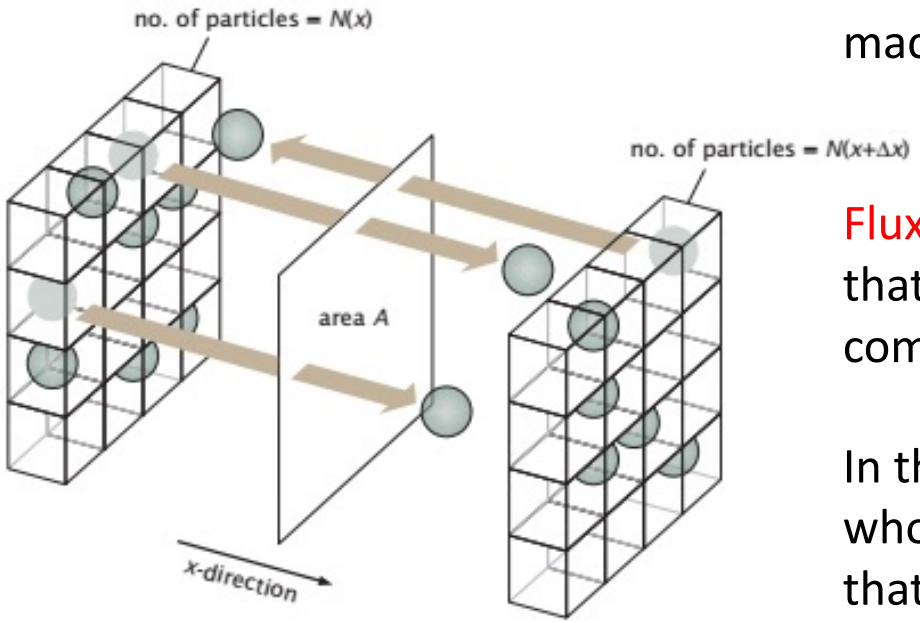
"Concentration gradient" is a spatial variation in the concentration field.

simple concentration profile where on the left-hand side of the domain of interest, the concentration of the molecule of interest is high, while on the right-hand side of the domain of interest, the concentration is low



Phillips, Rob; Kondev, Jane; Theriot, Julie; Garcia, Hernan. Physical Biology of the Cell (Page 516). CRC Press.

Diffusive dynamics



The other key quantity of interest for our macroscopic description of diffusion is the flux.

Flux can be seen as the net number of molecules that cross area A per unit time. That is the component of the flux vector in that direction.

In three dimensions, the flux is actually a vector whose components give the flux across planes that are perpendicular to the x-, y-, and z directions.

The goal of our thinking is to determine what amounts to an “equation of motion” that tells how the concentration field changes in both space and time.

Diffusive dynamics

in one dimension, flux is linearly related to concentration gradient:

$$j = -D\frac{\partial c}{\partial x}, \qquad (13.1)$$

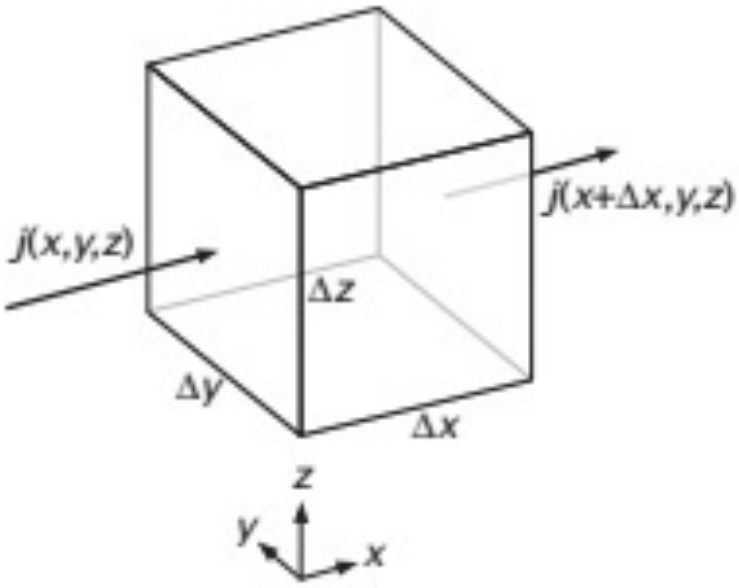
J = current density, number of particles crossing unit area/ unit time
D = diffusion coefficient

$$[j] = \frac{1}{\text{length}^2 \times \text{time}},$$

$$\left[\frac{\partial c}{\partial x}\right] = \frac{\text{number of particles/length}^3}{\text{length}} = \frac{\text{number of particles}}{\text{length}^4}.$$

[D] = length^2/time, indipendent on dimensionality of space!

Diffusive dynamics



The basic strategy is to assess how many particles enter or leave at the face at position x and similarly across the face at position x + Δx.

We define:
Nbox(x, y, z,t)
as the number of particles in the box at time t and note that this can be computed as
Nbox(x, y, z,t) = c(x, y, z,t)ΔxΔyΔz.

Since mass is conserved (that is, we are not yet thinking about the case where there are reactions that can alter the number of particles of a given species), the change in Nbox(x, y, z,t) can only arise from the fluxes across the faces of the box.

Diffusive dynamics

First, note that the change in the number of particles, N_{box}, per unit time is the change in concentration per unit time times the volume of the box, and can be written as

$$\frac{\partial N_{\text{box}}}{\partial t} = \frac{\partial c}{\partial t}\Delta x\,\Delta y\,\Delta z. \tag{13.4}$$

By mass conservation, this result has to be equal to the number of particles going into the box per unit time, $j(x, y, z)\Delta y\,\Delta z$, *minus* the number of particles going out of the box per unit time, $j(x + \Delta x, y, z)$ $\Delta y\,\Delta z$, and is reckoned as

$$\frac{\partial c}{\partial t}\Delta x\,\Delta y\,\Delta z = j(x, y, z)\Delta y\,\Delta z - j(x + \Delta x, y, z)\Delta y\,\Delta z. \tag{13.5}$$

We can then Taylor-expand $j(x + \Delta x, y, z)$ to first order in Δx (see the discussion of Taylor expansions on p. 215) to give

$$\frac{\partial c}{\partial t}\Delta x\,\Delta y\,\Delta z \approx j(x, y, z)\Delta y\,\Delta z - \left[j(x, y, z) + \frac{\partial j}{\partial x}\Delta x\right]\Delta y\,\Delta z. \tag{13.6}$$

If we now collect terms, the local statement of conservation of mass can be written as

$$\frac{\partial c}{\partial t} = -\frac{\partial j}{\partial x}. \tag{13.7}$$

Diffusive dynamics

Note that the significance of this equation is that it is a statement about the relation between the flux and concentration in every little neighborhood of the volume of interest.
By combining the statement of mass conservation (Equation 13.7) and the relation between flux and concentration gradient (Equation 13.1), we can generate a very useful relation, namely:

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2}, \qquad (13.8)$$

which is the classic law of diffusion in one dimension.
Note that to derive this particular form of the diffusion equation, we had to assume that D is independent of concentration.
This single equation embodies two key ideas, namely, (i) mass conservation and (ii) a material law relating flux and concentration.
Note that the first of these ideas is independent of material particulars, while Fick's law need not be satisfied in all circumstances since flux might depend on concentration in a more complicated, nonlinear fashion.

Diffusive dynamics: biological consequences

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0.

In particular, if at time t = 0 we start with N molecules in an infinitesimally small region around x = 0, the concentration profile will evolve in the following way:

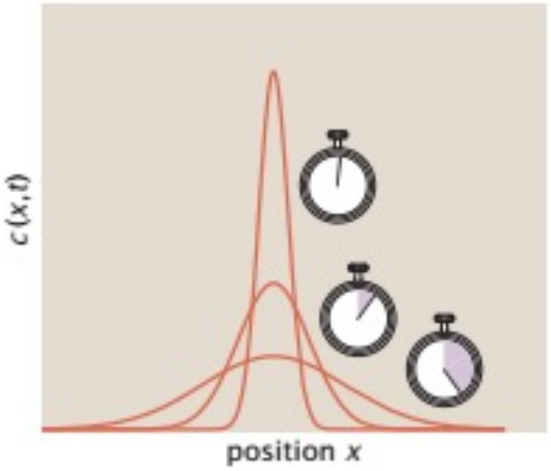


Figure 13.15: Time evolution of the concentration field. The plot shows the solution for the diffusion equation at different times for an *initial* concentration profile that is a spike at $x = 0$.

$$c(x,t) = \frac{N}{\sqrt{4\pi Dt}} e^{-x^2/4Dt}. \qquad (13.32)$$

denoted as Green's Function of the Diffusion equation

By dividing by N, this equation can then be interpreted as giving the probability density for finding a particle between x and x + dx.

This equation for the concentration tells us that the profile has the form of a Gaussian. The width of the Gaussian is √ 2Dt and hence it increases as the square root of the time.

One of the most beautiful features of a solution like this is that once it is known, by exploiting the linearity of the diffusion equation itself, we are then free to write the solution for an arbitrary initial distribution of diffusing molecules.

Diffusive dynamics: biological consequences

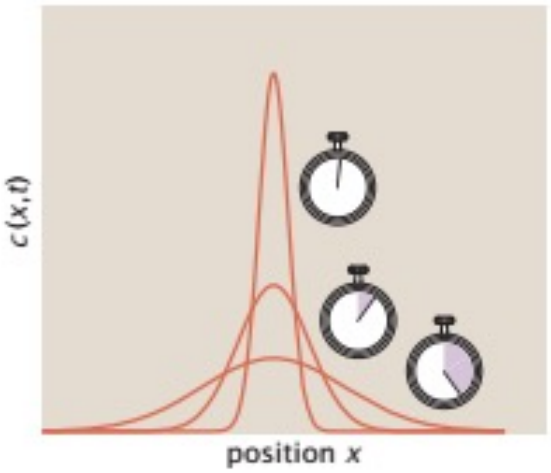


Figure 13.15: Time evolution of the concentration field. The plot shows the solution for the diffusion equation at different times for an *initial* concentration profile that is a spike at $x = 0$.

Note in Figure 13.15 that the mean position of the concentration distribution does not change with time.
This corresponds to the absence of a drift term.
One of the most interesting quantities to feature is the width of the distribution, $\langle x^2 \rangle$, which broadens over time. Since the distribution is Gaussian, we can essentially read off the dynamics of the width, but we take this opportunity to compute it explicitly since it is instructive both physically and mathematically:

$$\langle x^2 \rangle = \frac{\int_{-\infty}^{+\infty} x^2 \frac{N}{\sqrt{4\pi Dt}} e^{-x^2/4Dt}\, dx}{N} = \frac{1}{\sqrt{4\pi Dt}} \int_{-\infty}^{+\infty} x^2 e^{-x^2/4Dt}\, dx, \quad (13.33)$$

where we have made use of the probability distribution for finding a particle at position x at time t, which is related to the concentration distribution, Equation 13.32, by c(x,t)/N. This integral brings to:

$$\langle x^2 \rangle = \frac{1}{\sqrt{4\pi Dt}} \int_{-\infty}^{+\infty} x^2 e^{-x^2/4Dt}\, dx = \frac{1}{\sqrt{4\pi Dt}} \frac{\sqrt{\pi}}{2} (4Dt)^{3/2} = 2Dt. \quad (13.34)$$

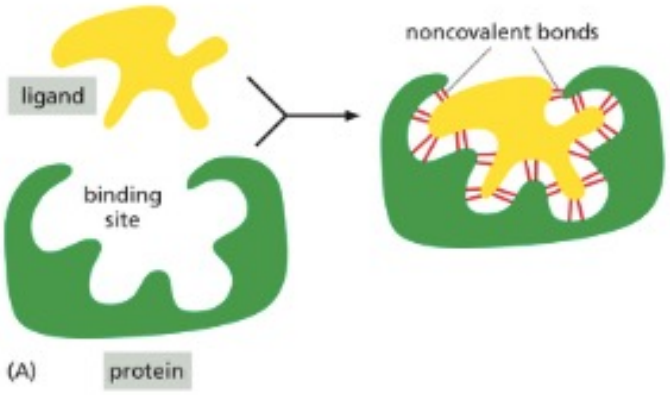
this result reveals how diffusion times scale with the square of the distance over which diffusion must act.

Equilibrium constant as a measure of binding strenght

Protein–Ligand Binding Kinetics

describes the process underlying the association between the protein and ligand, particularly focusing on the rate at which these two partners bind to each other

$$P + L \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} PL$$



k_{on} and k_{off} are the kinetic rate constants

k_{on} depends on the random thermal movement of molecules. When poorly matching mol collide they bind and dissociate with the same rapidity. The more noncolvalent bonds form, the longer the binding persists.

Different biological actions, require different persisting time for the mol. bonding!

In most experiments the fraction of molecules bound to the protein – let's call this fraction q – is the parameter that is easiest to measure. The situation here is similar to that for the protein melting curves. The exact physical nature of the signal does not really matter; all that matters is that the signal is linearly proportional to the fraction of ligands bound.

$$\theta = \frac{[PX]}{[P_{total}]} = \frac{[PX]}{[P]+[PX]}$$

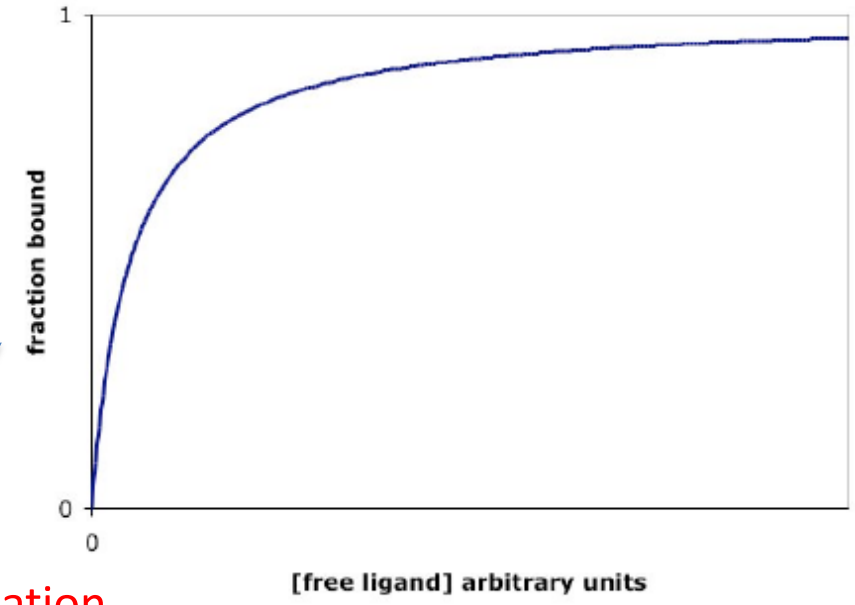
$$substituting [PX]$$

$$\theta = \frac{K[P][X]}{[P]+K[P][X]}$$

$$eliminate [P]$$

$$\theta = \frac{K[X]}{1+K[X]}$$

Langmuir equation



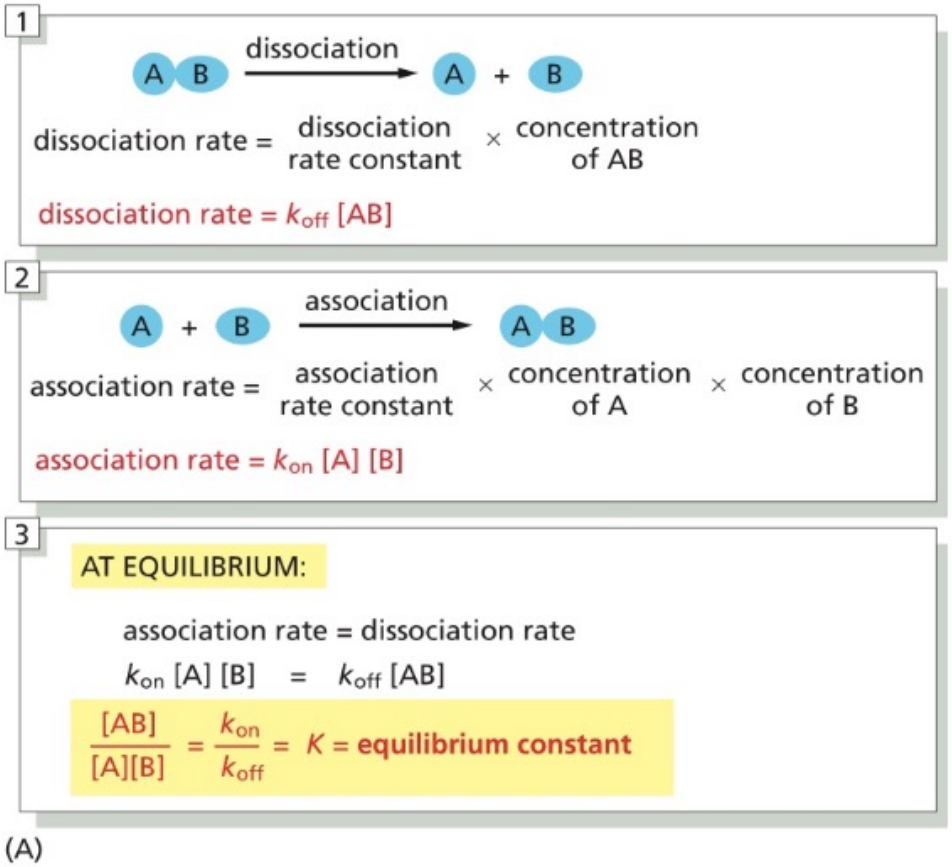
Equilibrium constant as a measure of binding strenght

Example: a population of identical **antibody** molecules suddenly encounters a population of **ligands** diffusing in the fluid surrounding them.

At frequent intervals, one of the ligand molecules will bump into the binding site of an antibody and form an antibody–ligand complex. The population of antibody–ligand complexes will therefore increase, but not without limit: over time, a second process, in which individual complexes break apart because of **thermally induced motion**, will become increasingly important.

Eventually, any population of antibody molecules and ligands will reach a steady state, or equilibrium, in which the number of binding (association) events per second is precisely equal to the number of “unbinding” (dissociation) events.

Equilibrium constant as a measure of binding strenght



(A)

Conveniently we define the EQUILIBRIUM CONSTANT K (also known as association constant or binding constant) as a measure of the strength of the binding.

Half of the binding sites will be occupied by ligand when the ligand's concentration (in moles/liter) reaches a value that is equal to **1/K**

K has units of liter/moles

k_{on} describes how many productive collisions occur per unit time per protein at a given concentration

k_{off} can differ by orders of magnitude (even for different DNA sequences) because it depends on the strength of the noncovalent bonds formed between A and B

Equilibrium constant as a measure of binding strenght

Protein–Ligand Binding Kinetics

At the equilibrium, the two reactions balance

$$k_{on}[P][L] = k_{off}[PL]$$

[..] is the **equilibrium concentration**

We define **the binding constant K_b** (M^{-1}) and the **dissociation constant K_d** (M) as:

$$K_b = \frac{k_{on}}{k_{off}} = \frac{[PL]}{[P][L]} = \frac{1}{K_d}$$

Therefore, the fast binding rate accompanied by a slow dissociation rate will give a high/low binding/dissociation constant and, hence, a high binding affinity.

Equilibrium constant as a measure of binding strenght

From the concentrations of the ligand, antibody, and antibody–ligand complex at equilibrium, we can calculate a convenient measure of the strength of binding—**the equilibrium constant (K) .**

The equilibrium constant for a reaction in which two molecules (A and B) bind to each other to form a complex (AB) has units of liters/mole, and half of the binding sites will be occupied by ligand when that ligand's concentration (in moles/liter) reaches a value that is equal to 1/K.

This equilibrium constant is larger the greater the binding strength, and it is a direct measure of the free-energy difference between the bound and free states

Equilibrium constant

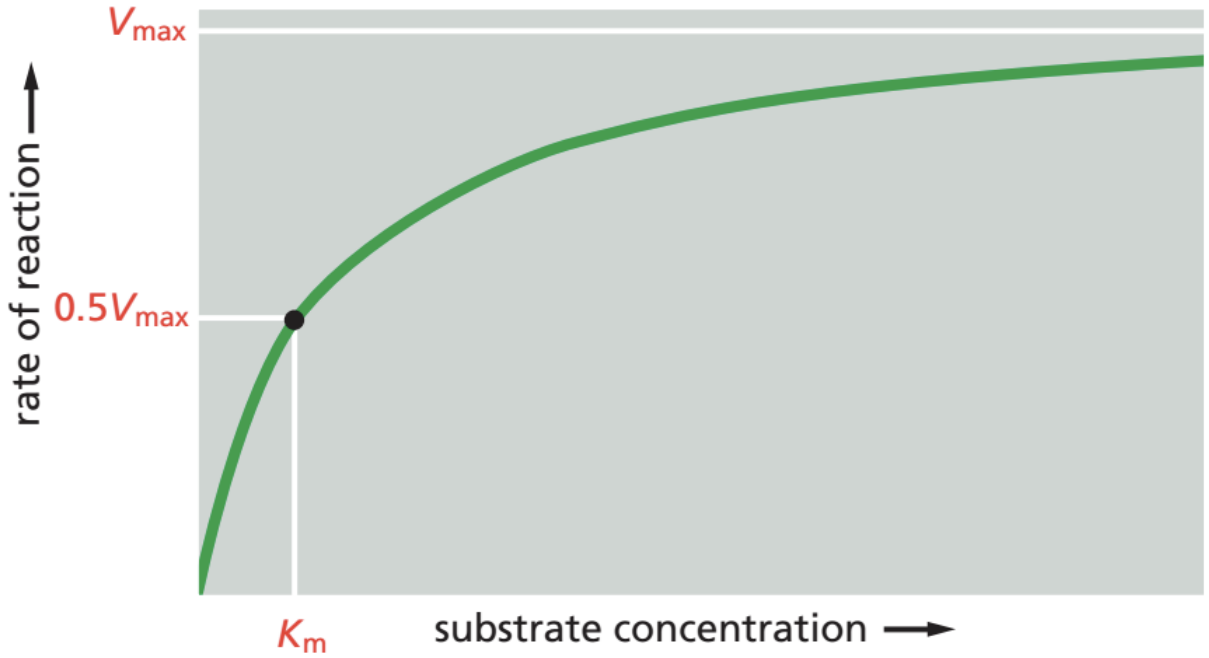
Enzymes are the most selective and powerful catalysts known. An understanding of their detailed mechanisms provides a critical tool for the discovery of new drugs, for the large-scale industrial synthesis of useful chemicals, and for appreciating the chemistry of cells and organisms.

A detailed study of the rates of the chemical reactions that are catalyzed by a purified enzyme—more specifically how these rates change with changes in conditions such as the concentrations of substrates, products, inhibitors, and regulatory ligands—allows biochemists to figure out exactly how each enzyme works. For example, this is the way that the ATP-producing reactions of glycolysis, were deciphered—allowing us to appreciate the rationale for this critical enzymatic pathway.

Enzyme kinetics, which has been indispensable for deriving much of the detailed knowledge that we now have about cell chemistry.

Enzyme kinetics

For enzume, ligand binding is only a necessary first step in their function. E + S → ES → EP → E + P.



Turnover number = V max/ Enzyme concentration is often about 1000 substrate molecules processed per second

The rate of an enzyme reaction (V) increases as the substrate concentration increases until a maximum value (V max) is reached. At this point all substrate-binding sites on the enzyme molecules are fully occupied, and the rate of reaction is limited by the rate of the catalytic process on the enzyme surface.

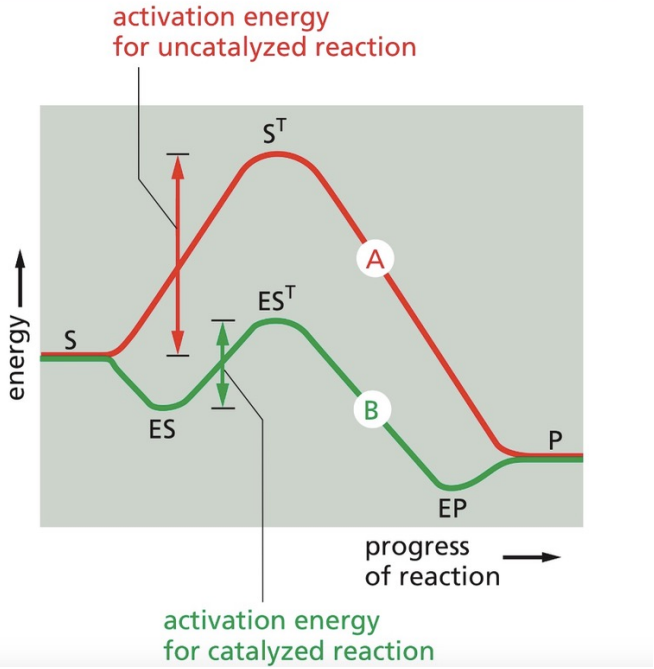
For most enzymes, the concentration of substrate at which the reaction rate is half-maximal (**K_m**) is a measure of how tightly the substrate is bound, with a large value of K_m corresponding to weak binding.

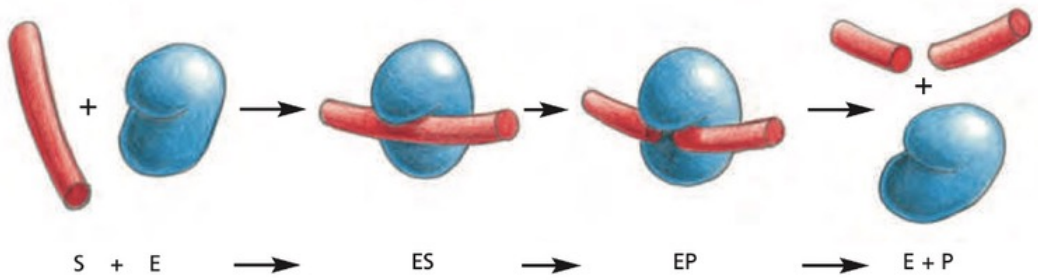
Equilibrium constant

Many enzymes have only one substrate, which they bind and then process to produce products. In this case, the reaction is written as

Here we have assumed that the reverse reaction, in which E + P recombine to form EP and then ES, occurs so rarely that we can ignore it. In this case, EP need not be represented, and we can express the rate of the reaction—known as its velocity, V, as where [ES] is the concentration of the enzyme–substrate complex, and kcat is the turnover number, a rate constant that has a value equal to the number of substrate molecules processed per enzyme molecule each second. But how does the value of [ES] relate to the concentrations that we know directly, which are the total concentration of the enzyme, [E o], and the concentration of the substrate, [S]? When enzyme and substrate are first mixed, the concentration [ES] will rise rapidly from zero to a so-called steady-state level,

Steady state enzyme kinetics

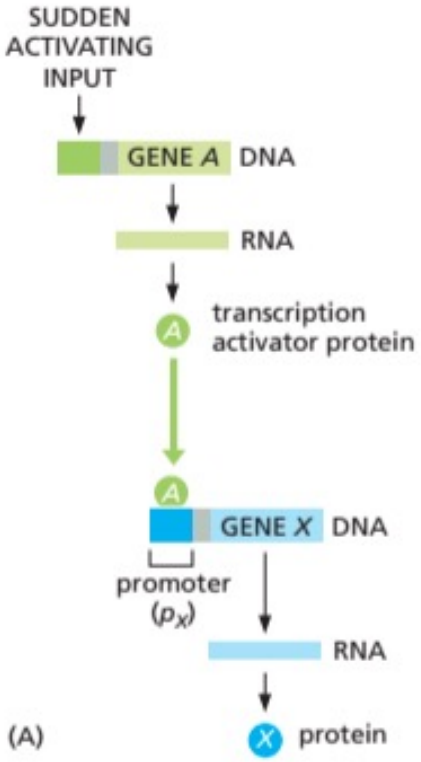




WHY ANALYZE THE KINETICS OF ENZYMES?

Enzymes are the most selective and powerful catalysts known. An understanding of their detailed mechanisms provides a critical tool for the discovery of new drugs, for the large-scale industrial synthesis of useful chemicals, and for appreciating the chemistry of cells and organisms. A detailed study of the rates of the chemical reactions that are catalyzed by a purified enzyme—more specifically how these rates change with changes in conditions such as the concentrations of substrates, products, inhibitors, and regulatory ligands—allows biochemists to figure out exactly how each enzyme works. For example, this is the way that the ATP-producing reactions of glycolysis, shown previously in Figure 2–48, were deciphered—allowing us to appreciate the rationale for this critical enzymatic pathway.

In this Panel, we introduce the important field of enzyme kinetics, which has been indispensable for deriving much of the detailed knowledge that we now have about cell chemistry.



(A)

$$A + p_X \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} A{:}p_X$$

rate of complex formation $= k_{on}[A][p_X]$

rate of complex dissociation $= k_{off}[A{:}p_X]$

(B)

at steady state:

$$k_{on}[A][p_X] = k_{off}[A{:}p_X]$$

$$[A{:}p_X] = \frac{k_{on}}{k_{off}}[A][p_X] = K[A][p_X] \qquad \textbf{Equation 8–1}$$

(C)

$$[p_X^T] = [p_X] + [A{:}p_X]$$

substituting $[p_X]$ from the above equation into Equation 8–1 yields:

$$[A{:}p_X] = K[A]([p_X^T] - [A{:}p_X])$$

$$[A{:}p_X](1 + K[A]) = K[A][p_X^T]$$

$$[A{:}p_X] = \frac{K[A]}{1 + K[A]}[p_X^T] \qquad \textbf{Equation 8–2}$$

(D)

$$\textit{bound fraction} = \frac{[A{:}p_X]}{[p_X^T]} = \frac{K[A]}{1 + K[A]} \qquad \textbf{Equation 8–3}$$

(E)

Transient behavior

Calculation of all $[A{:}p_X]$ values as a function of time, using Equation 8–4, allows us to determine the rate at which $[A{:}p_X]$ reaches its steady-state value. Because this value is attained asymptotically, it is often most useful to compare the times needed to get to 50, 90, or 99 percent of this new steady state. The simplest way to determine these values is to solve Equation 8–4 with a method called numerical integration, which involves plugging in values for all of the parameters (k_{on}, k_{off}, etc.) and then using a computer to determine the values of $[A{:}p_X]$ over time, starting from given initial concentrations of $[A]$ and $[p_X]$. For $k_{on} = 0.5 \times 10^7$ sec^{-1} M^{-1}, $k_{off} = 0.5 \times 10^{-1}$ sec^{-1} ($K = 10^8$ M^{-1} as above), and $[p_X^T] = 10^{-10}$ M, it takes $[A{:}p_X]$ about 5, 20, and 40 seconds to reach 50, 90, and 99 percent of the new steady-state value following a sudden tenfold change in $[A]$ (Figure 8–73B). Thus, a sudden jump in $[A]$ does not have instantaneous effects, as we might have assumed from looking at the cartoon in Figure 8–72A.

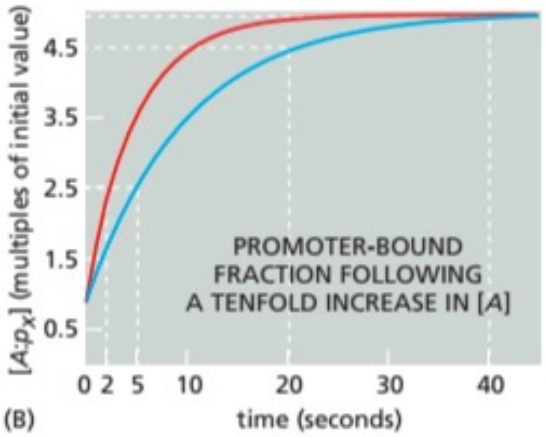
Differential equations therefore allow us to understand the transient dynamics of biochemical reactions. This tool is critical for achieving a deep understanding of cell behavior, in part because it allows us to determine the dependence of the dynamics inside cells on parameters that are specific to the particular molecules involved. For example, if we double the values of both k_{on} and k_{off}, then Equation 8–1 (Figure 8–72C) indicates that the steady-state value of $[A{:}p_X]$ does not change. However, the time it takes to reach 50% of this steady state after a ten-fold

$$\frac{d[A{:}p_X]}{dt} = \text{rate of complex formation} - \text{rate of complex dissociation}$$

$$\frac{d[A{:}p_X]}{dt} = k_{on}[A][p_X] - k_{off}[A{:}p_X] \qquad \text{Equation 8–4}$$

(A)

Figure 8–73 Using differential equations to study the dynamics and steady-state behavior of a biological system. (A) Equation 8–4 is an ordinary differential equation for calculating the rate of change in the formation of bound promoter complex in response to a change in other components. (B) Formation of $[A{:}p_X]$ after a tenfold increase in $[A]$, as determined by solving Equation 8–4. In *blue* is the solution corresponding to $k_{on} = 0.5 \times 10^7$ sec^{-1} M^{-1} and $k_{off} = 0.5 \times 10^{-1}$ sec^{-1}. In this case, it takes $[A{:}p_X]$ about 5, 20, and 40 seconds to reach 50, 90, and 99 percent of the new steady-state value. For the *red curve*, the k_{on} and k_{off} values are doubled, and the system reaches the same steady state more rapidly.



(B)

Transient behavior

$$\text{transcription rate} = \beta \frac{K[A]}{1 + K[A]}$$

$$\text{protein production rate} = \beta \cdot m \frac{K[A]}{1 + K[A]}$$

$$\text{protein degradation rate} = \frac{[X]}{\tau_X}$$

(A)

$$\frac{d[X]}{dt} = \text{protein production rate} - \text{protein degradation rate}$$

$$\frac{d[X]}{dt} = \beta \cdot m \frac{K[A]}{1 + K[A]} - \frac{[X]}{\tau_X} \qquad \text{Equation 8–5}$$

(B)

at steady state:

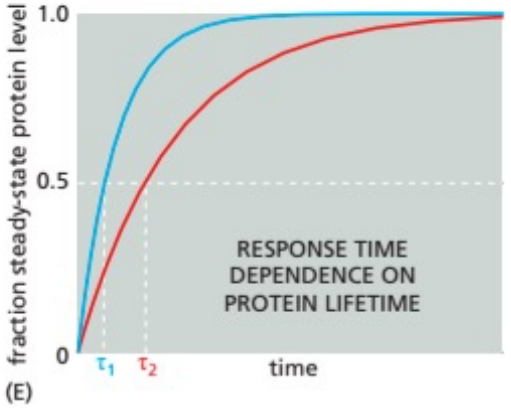
$$[X_{st}] = \beta \cdot m \frac{K[A]}{1 + K[A]} \cdot \tau_X \qquad \text{Equation 8–6}$$

(C)

$$[X](t) = [X_{st}](1 - e^{-\frac{t}{\tau_X}})$$

(D)

change in $[A]$ in our example changes from about 5 seconds to 2 seconds (see Figure 8–73B). These insights are not accessible from either cartoons or equilibrium equations. This is an unusually simple example; mathematical descriptions such as differential equations become more indispensible for understanding biological interactions as the number of interactions increases.



(E)

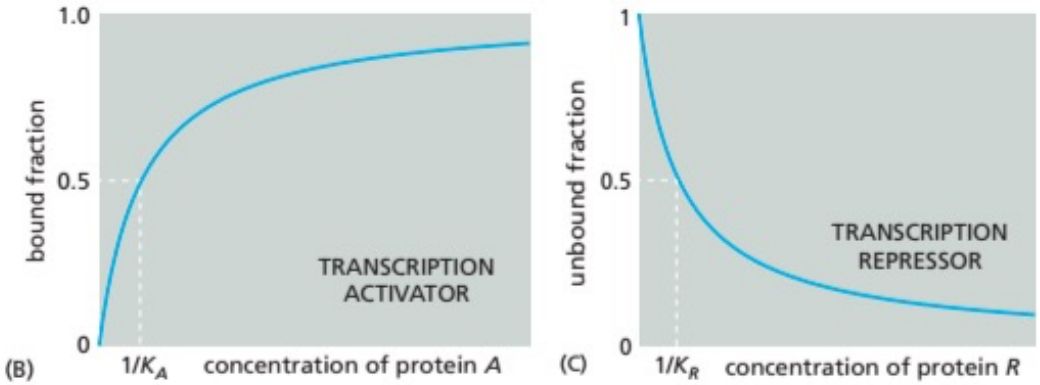
Figure 8–74 Effect of protein lifetime on the timing of the response.
(A) Equations for calculation of the rates of gene *X* transcription, protein *X* production, and protein *X* degradation, as explained in the text. (B) Equation 8–5 is an ordinary differential equation for calculating the rate of change in protein *X* in response to changes in other components. (C) When the rate of change in protein *X* is zero (steady state), its concentration can be calculated with Equation 8–6, revealing a direct relationship with protein lifetime (τ). (D) The solution of Equation 8–5 specifies the concentration of protein *X* over time as it approaches its steady-state concentration. (E) Response time depends on protein lifetime. As described in the text, the time that it takes a protein to reach a new steady state is greater when the protein is more stable. Here, the *blue line* corresponds to a protein with a lifetime that is 2.5-fold shorter than the lifetime of the protein in *red*.

Transient behavior

$$\text{bound fraction} = \frac{K[R]}{1 + K[R]}$$

$$\text{unbound fraction} = 1 - \text{bound fraction} = \frac{1}{1 + K[R]}$$

(A)



$$\text{protein production rate} = \beta \cdot m \frac{1}{1 + K[R]}$$

$$\frac{d[X]}{dt} = \beta \cdot m \frac{1}{1 + K[R]} - \frac{[X]}{\tau_X} \qquad \text{Equation 8–7}$$

$$[X_{st}] = \beta \cdot m \frac{1}{1 + K[R]} \cdot \tau_X$$

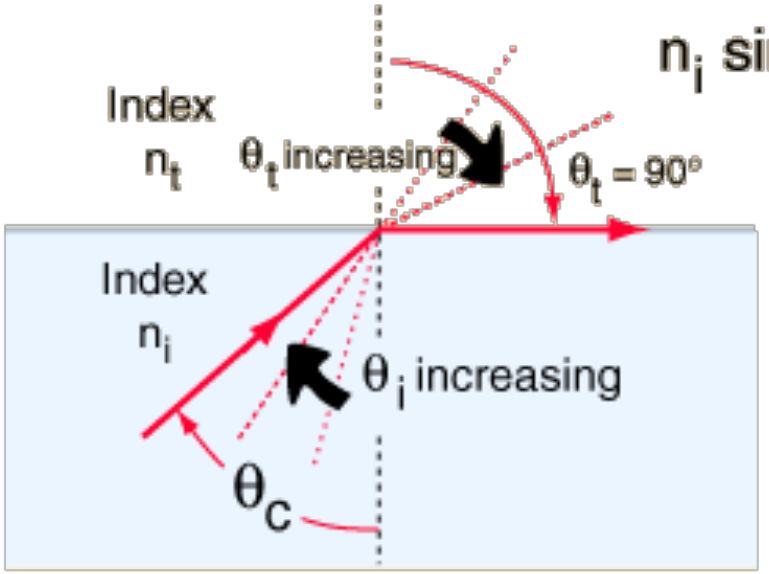
(D)

Figure 8–75 How promoter occupancy depends on the binding affinity of a transcription regulator protein. (A) The fraction of a binding site that is occupied by a transcription repressor *R* is determined by an equation that is similar to the one we used for a transcription activator (see Figure 8–72E), except that in the case of a repressor we are interested primarily in the unbound fraction. (B) For a transcription activator *A*, half of the promoters are occupied when $[A] = 1/K_A$. Gene activity is proportional to this bound fraction. (C) For a transcription repressor *R*, gene activity is proportional to the unbound fraction of promoters. As indicated, this fraction is reduced to half of its maximal value when $[R]=1/K_R$. (D) As in the case of the transcription activator *A* (see Figure 8–74), we can derive equations to assess the timing of protein *X* production as a function of repressor concentrations.

Come si determina sperimentalmente
la cinetica di binding?

SURFACE PLASMON RESONANCE (SPR)

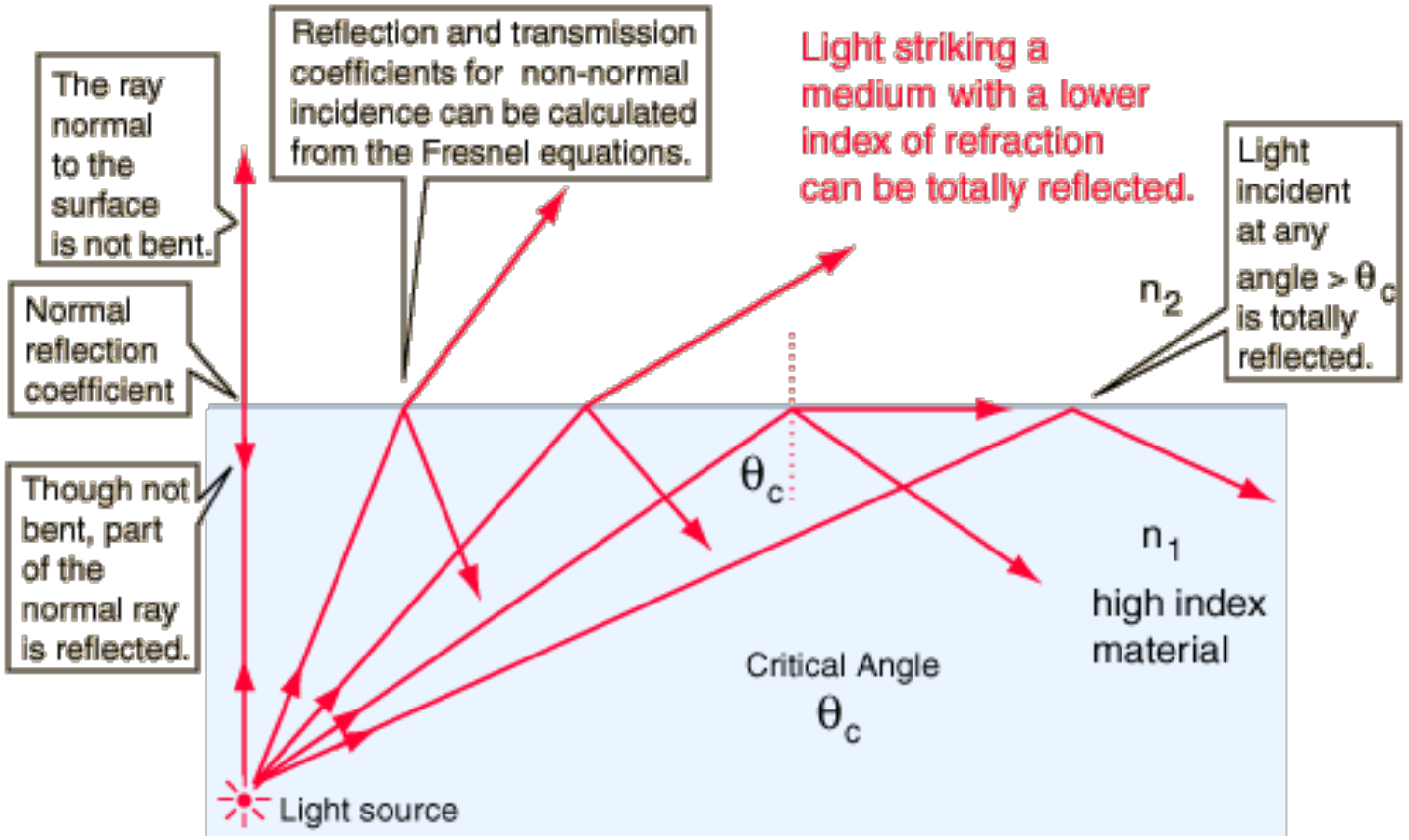
Surface Plasmon Resonance (SPR)



$$n_i \sin\theta_c = n_t \sin 90°$$

Threshold condition for total internal reflection.

TOTAL INTERNAL REFLECTION



Evanescent wave

When light propagating through a medium of high-refractive index encounters an interface with a medium of lower refractive index, it is either reflected or refracted according to Snell's law:

$$n_1 \sin\theta_1 = n_2 \sin\theta_2 \tag{A}$$

where n_1 and n_2 are the refractive indices of the high- and low-refractive index media, and θ_1 and θ_2 are the angles of incidence relative to the normal to the interface. When $n_2 > n_1$ and θ_1 is greater than the critical angle, θ_c, total internal reflection occurs in medium 1. The critical angle is

$$\theta_c = \sin^{-1}(n_2/n_1) \tag{B}$$

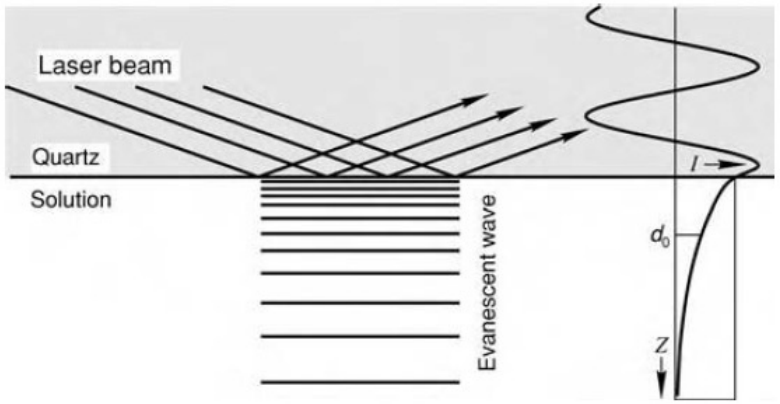
Although the incident light beam is totally internally reflected under these conditions, an electromagnetic field penetrates a small distance into medium 2. The intensity of this field decays exponentially with the distance z from the interface:

$$I(z) = I_0 \exp(-z/d_p) \tag{C}$$

with a characteristic penetration depth

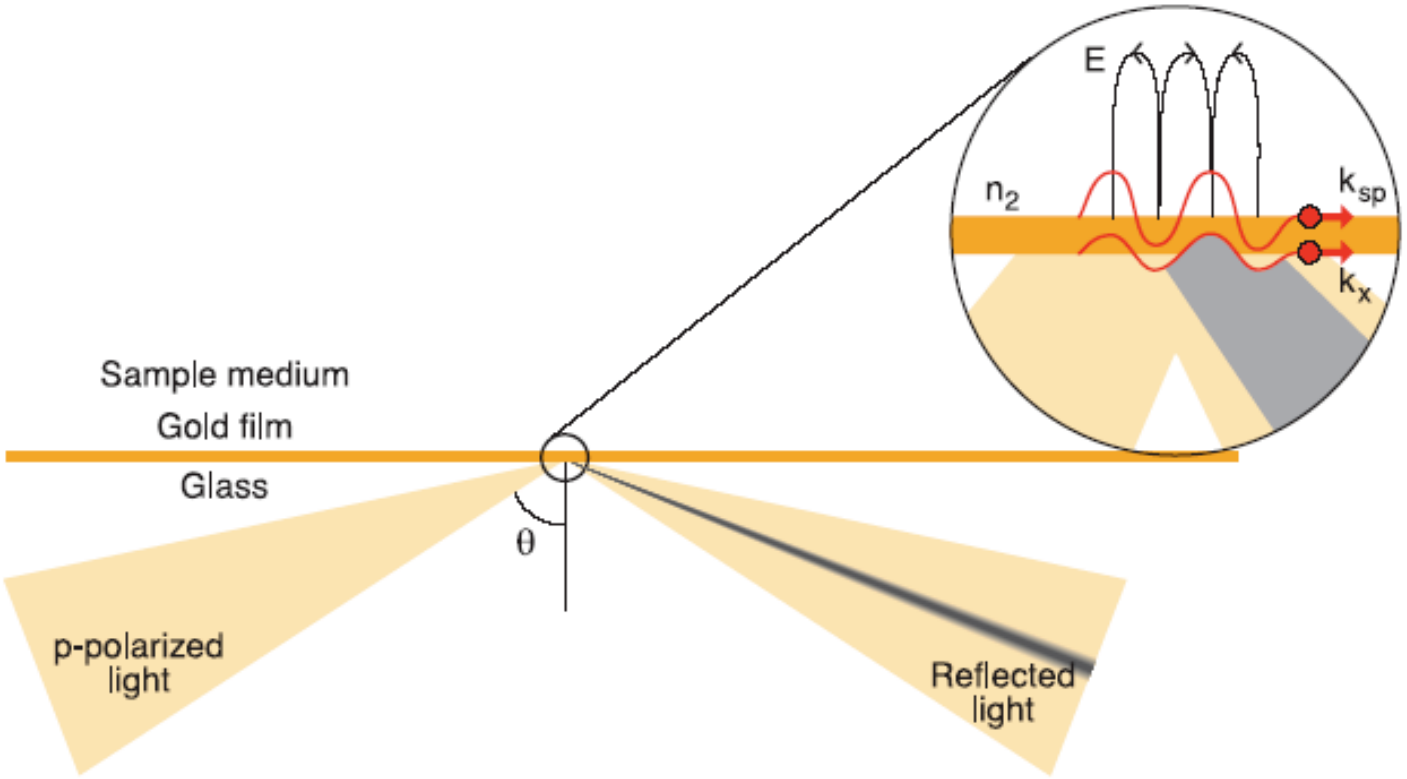
$$d_p = \frac{\lambda_0/n_1}{4\pi\sqrt{\sin^2\theta - (n_2/n_1)}} \tag{D}$$

This field is often called the 'evanescent' wave.



SPR

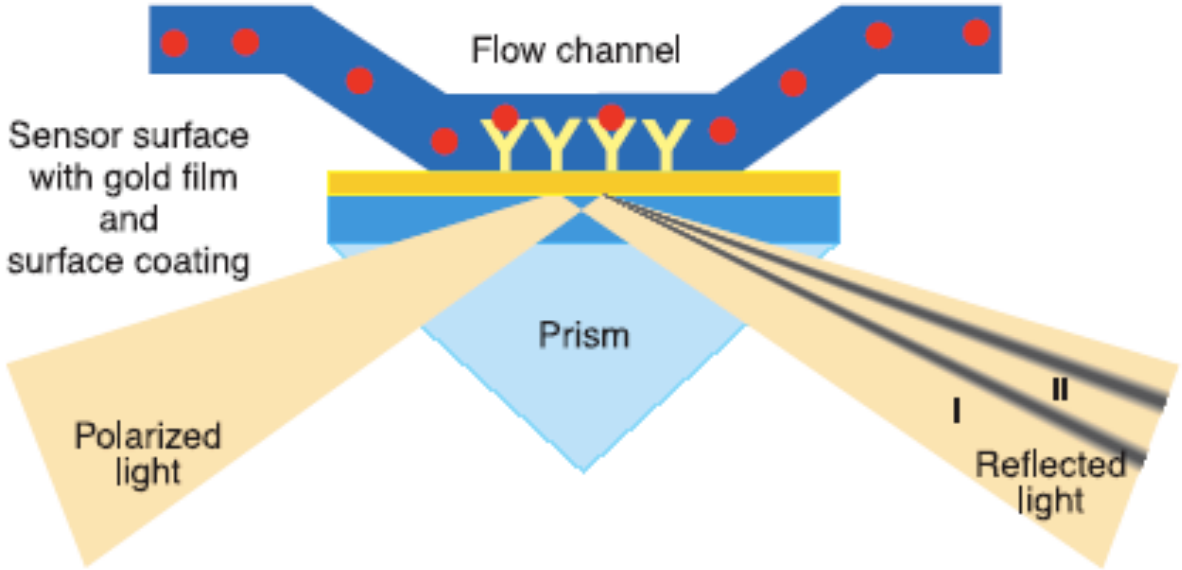
Lo strato d'oro è di circa 50 nm

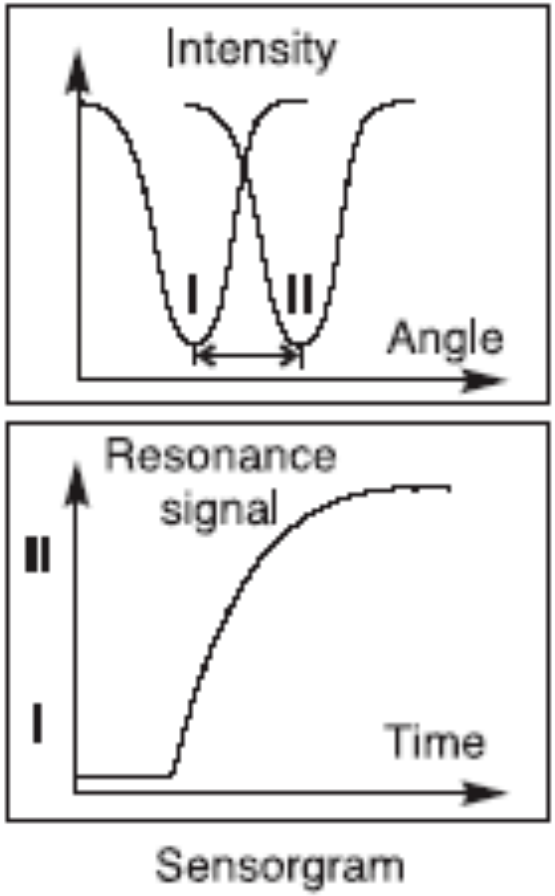


If the interface between the two media is coated with a thin layer of a suitable conducting material, such as a metal, the p-polarized component of the evanescent field wave penetrates the metal layer. In fact, at a specific angle of incidence, plane polarised light excites the delocalised surface electrons (or **plasmons**) of the metal, which results in a larger evanescent wave. As a consequences, at this angle of resonance the intensity of the reflected light decreases drastically due to the energy transferred to the plasmons.

SPR

Lo strato d’oro è di circa 50 nm





Sensorgram

In the same way, the velocity (and therefore the momemtum) of the plasmons is changed when the composition of the medium changes. Because of the change in momentum, the angle of incident light at which the resonance occurs changes. This can be measured very precise . This type of SPR is known as resonant angle or angular SPR and is commonly used. On the other hand, at a fixed angle of incident light, the wavelength can be varied until resonance occurs. This is known as resonant wavelength SPR or spectral SPR and is not used widely.

Several biosensors have been designed around the phenomenon of SPR. Due in large part to its relative simplicity and high sensitivity, this method has become very popular.

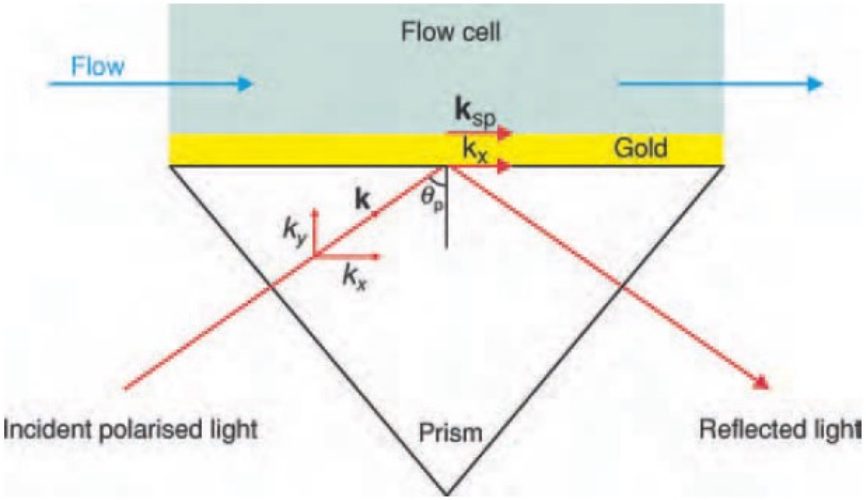
Surface plasmon resonance

The conditions for surface plasmon excitation at the interface between the metal and the biochemical solution are achieved by matching the projection of the wave vector of the incident light in the direction of the interface (k_x) and wave vector (k_{sp}) of the surface plasmon oscillation and are given by:

$$k_x = k_{sp}$$

$$k_x = \frac{\omega}{c}\sqrt{\varepsilon_{prism}} \times \sin\theta, \qquad k_{sp} = \frac{\omega}{c}\sqrt{\frac{\varepsilon_{metal} \times \varepsilon_{cell}}{\varepsilon_{metal} + \varepsilon_{cell}}}$$

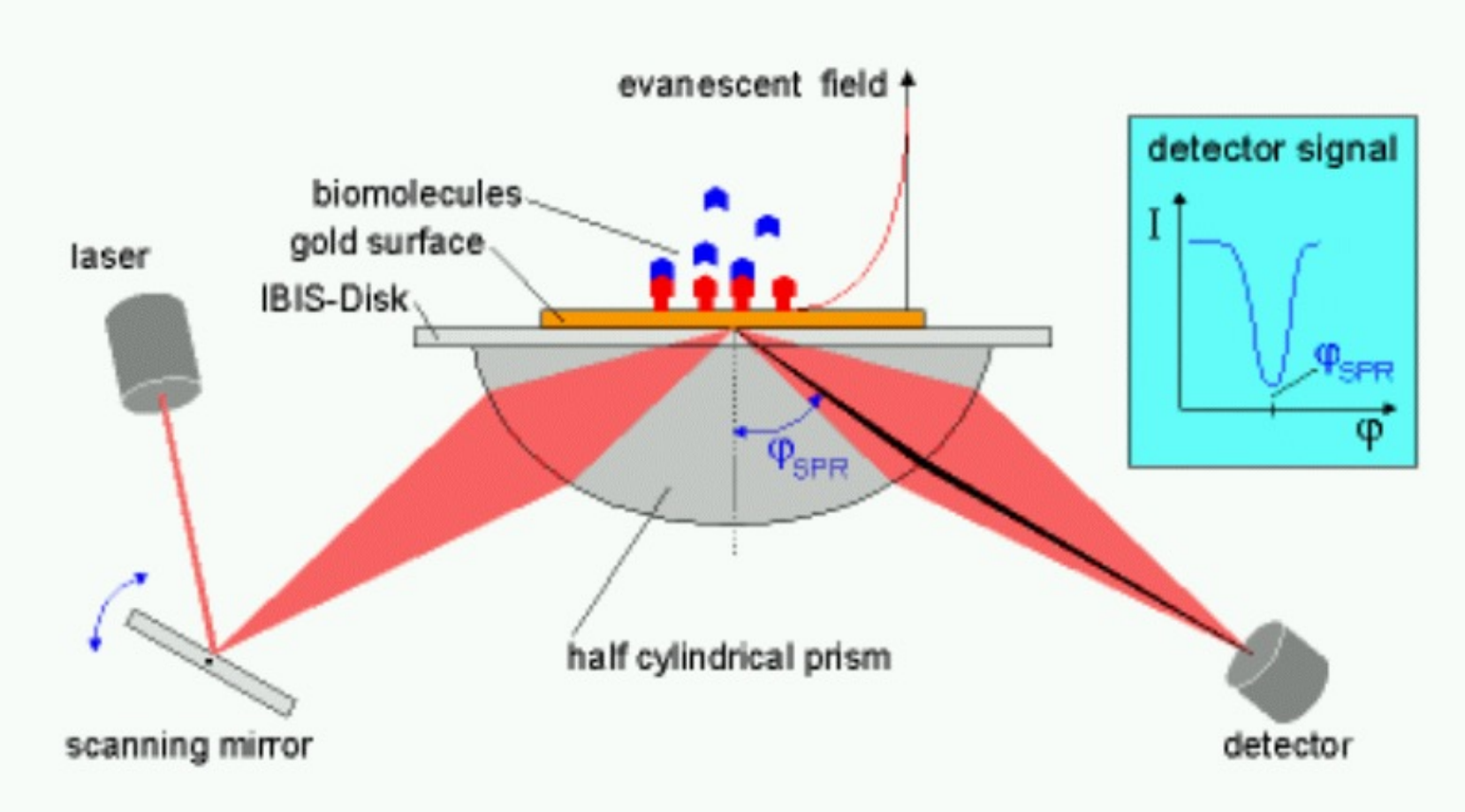
$$n_{glass} sin\theta_p = \sqrt{\frac{\varepsilon_{metal} \times \varepsilon_{cell}}{\varepsilon_{metal} + \varepsilon_{cell}}}$$



where ω is the angular frequency of the incident wave, c is the speed of light, and ε is the wavelength-dependent complex dielectric permittivity. **The incidence angle θp at which SPR conditions are satisfied** therefore **depends on the refractive index of the material** on the non-illuminated side of the metal (the flow cell in this case). The prism enables a range of incidence angles to be observed simultaneously in a wedge of light beams. When the resonance condition is satisfied, there is a strong absorption dip within the angular dependence of the wedge of reflected light. At optical wavelengths, the SPR condition is fulfilled by several metals, of which gold and silver are the most commonly used.

La velocità dei plasmoni, ovvero il momento, cambia quando cambia la composizione del mezzo. Per esempio, se delle molecole vanno a localizzarsi sul film di oro.

Il cambiamento di momento dei plasmoni, porta ad uno spostamento angolare della risonanza: l'angolo di incidenza della luce per vedere la risonanza cambia



Surface plasmon resonance

The resonance is influenced by the refractive index in the evanescent wave path.
The refractive index beyond the penetration distance of about 600 nm (the wavelength of the light) therefore does not affect the experimental outcome. The signal, measured in resonance units (RU), directly correlates with the amount of protein interacting near the surface (typically 1000 RU = 1 ng bound protein mm^{-2}). Results are plotted as a sensorgram, which represents changes in resonance signal as a function of time.