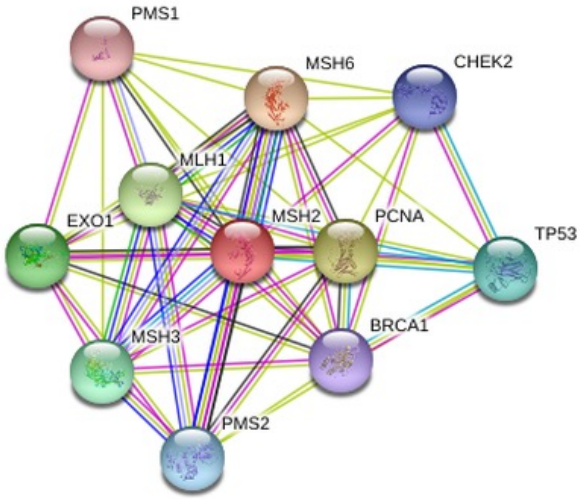
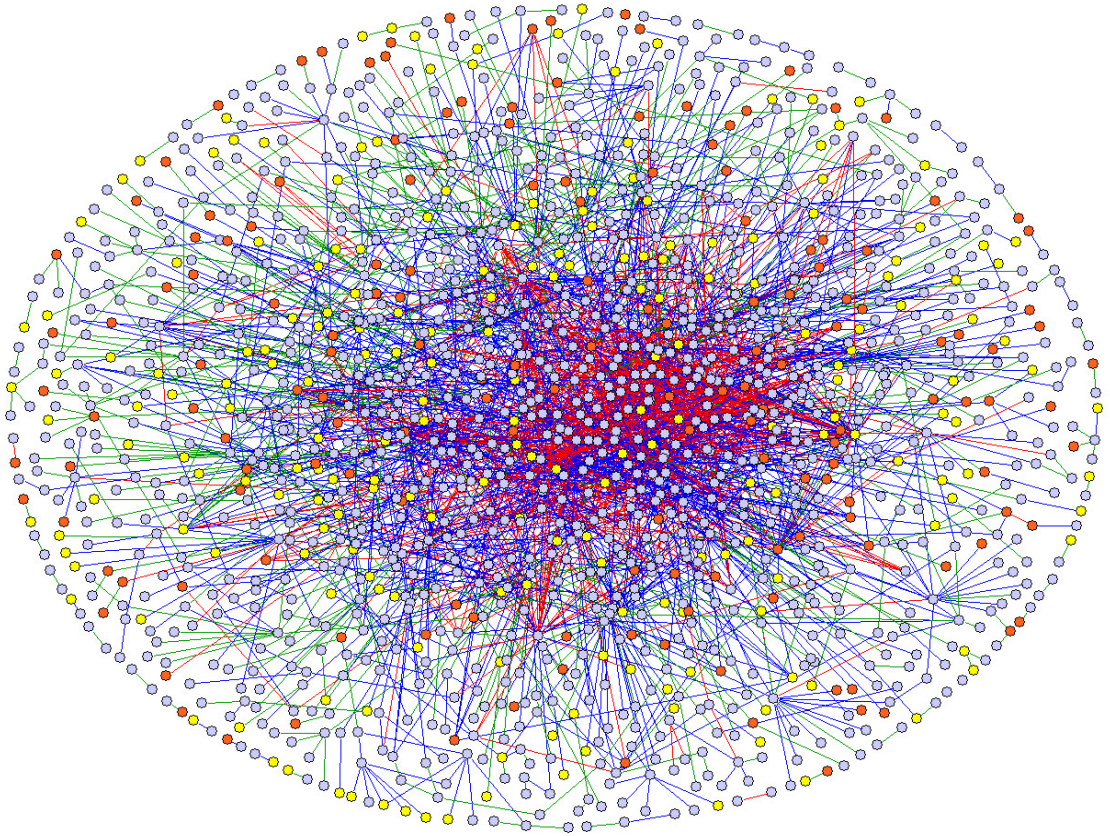
Protein-ligand interactions

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??

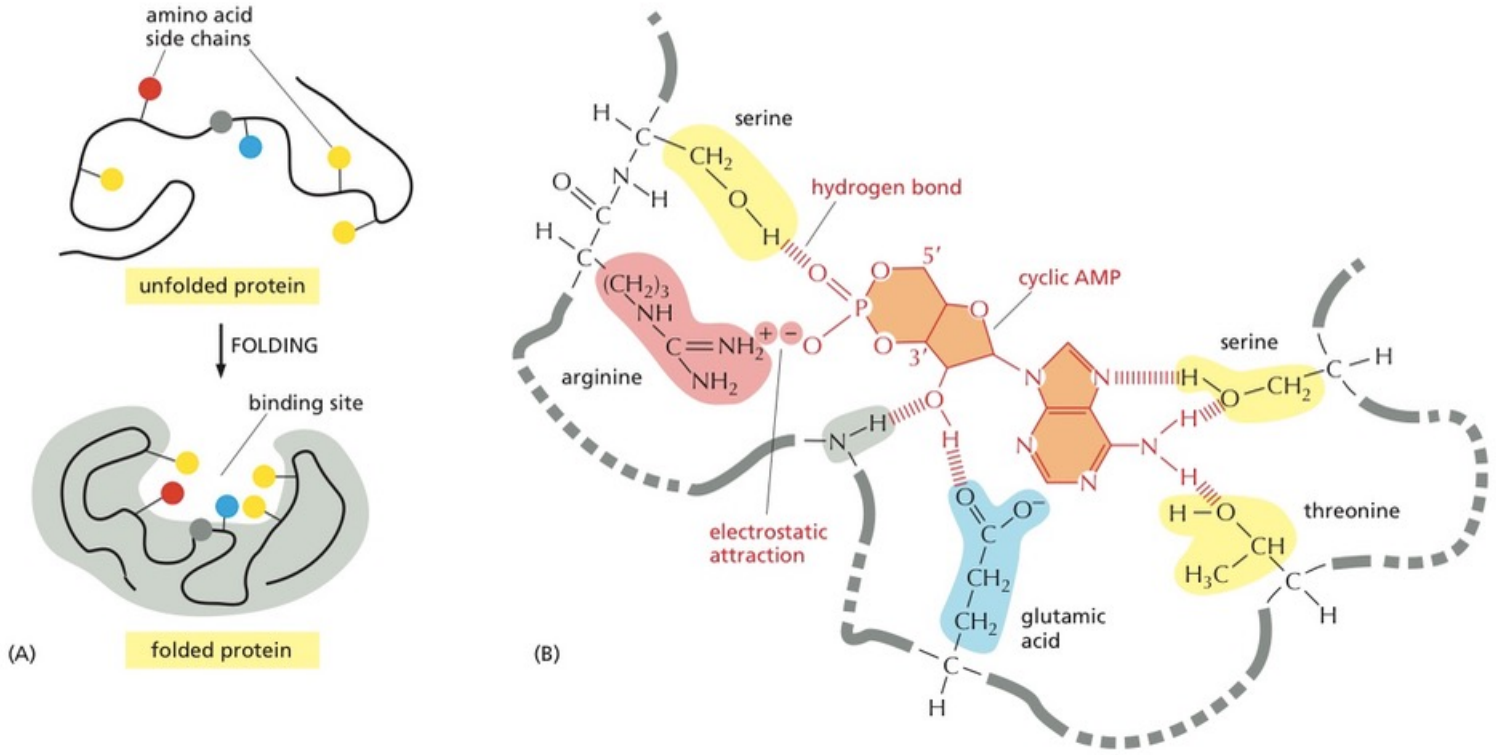


Protein-protein human network

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

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

Highest affinity macromolecules: Antibodies

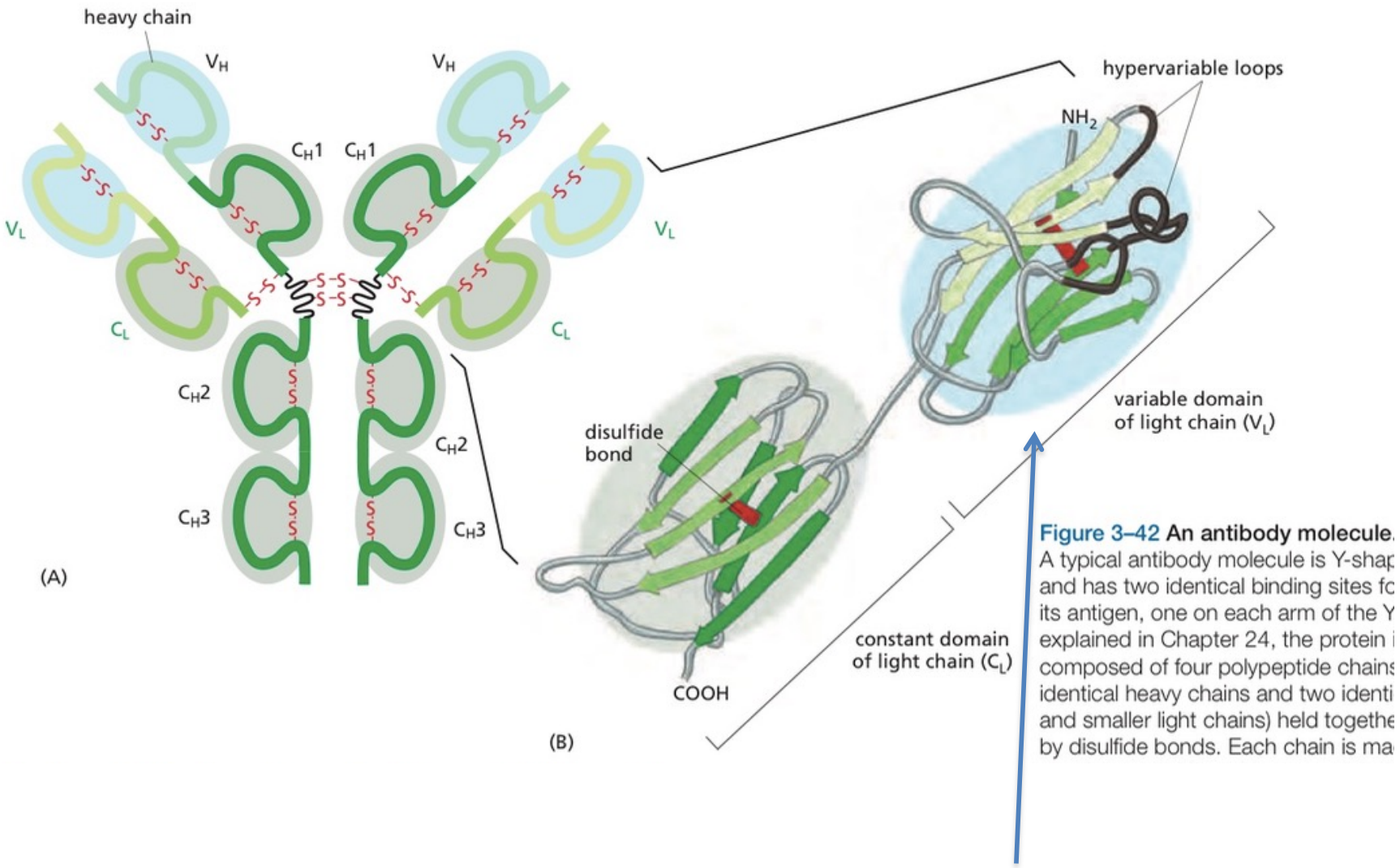
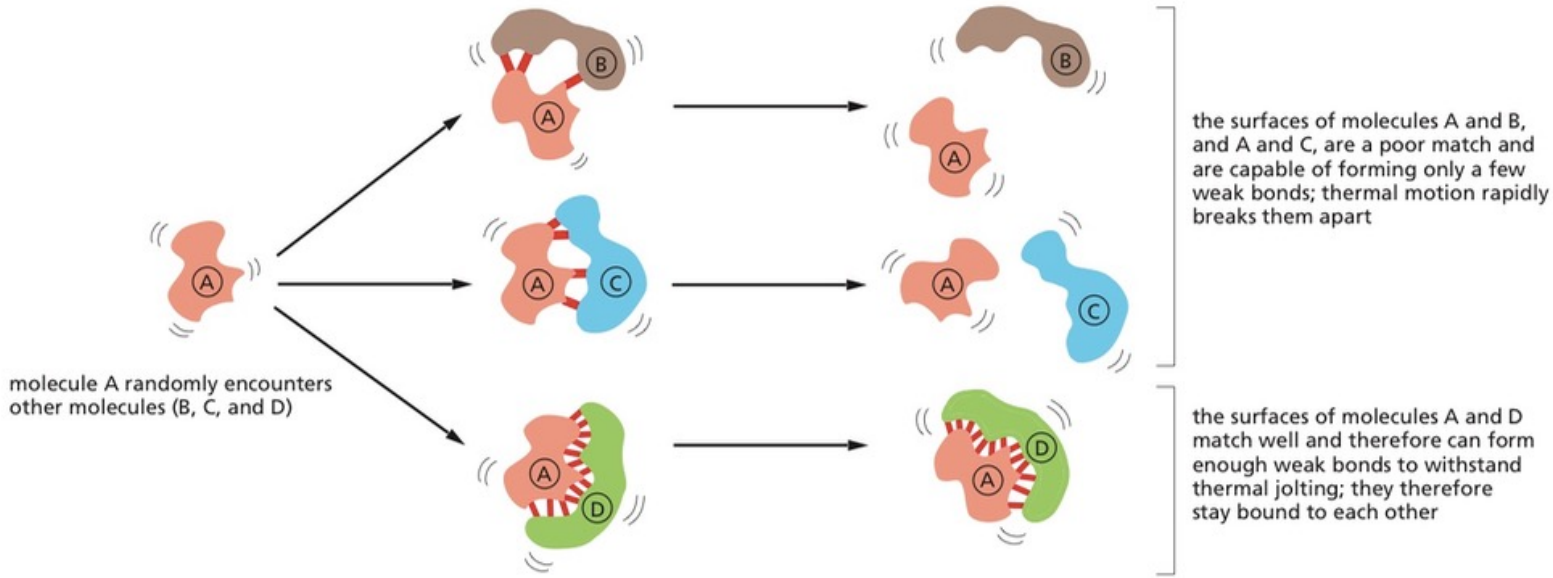


Figure 3–42 An antibody molecule.
A typical antibody molecule is Y-shap
and has two identical binding sites fo
its antigen, one on each arm of the Y
explained in Chapter 24, the protein i
composed of four polypeptide chains
identical heavy chains and two identi
and smaller light chains) held togethe
by disulfide bonds. Each chain is ma

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

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:

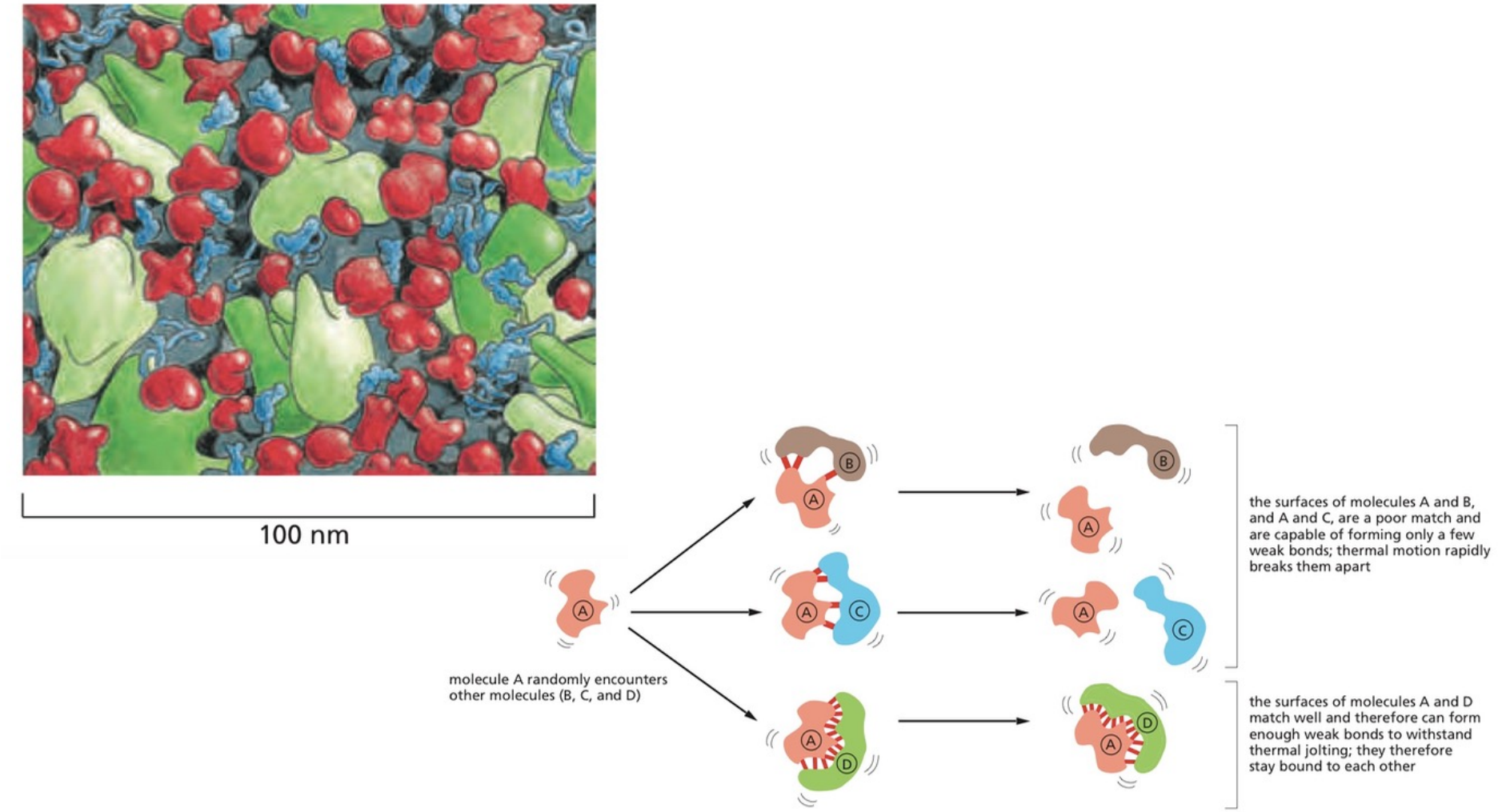


(i) **Specificity**. which distinguishes the highly specific binding partner from less specific partners;

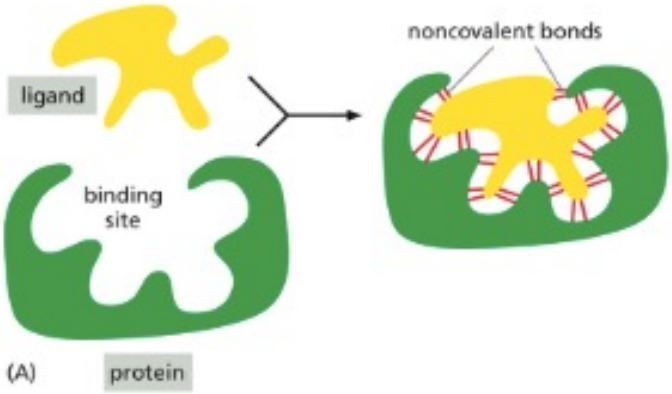
(ii) **Affinity**. high concentration of weakly interacting partners cannot replace the effect of a low concentration of the specific partner interacting with high affinity

Molecular recognition

Molecules in the cell are in a very crowded environment, in continual random thermal movements: rapid “faint” associations and dissociation between molecules are made.



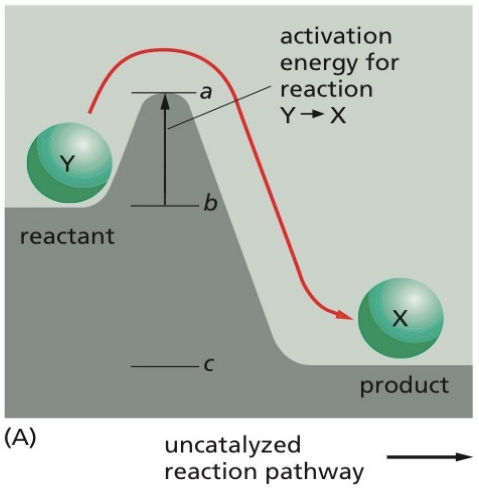
An examples: enzymes

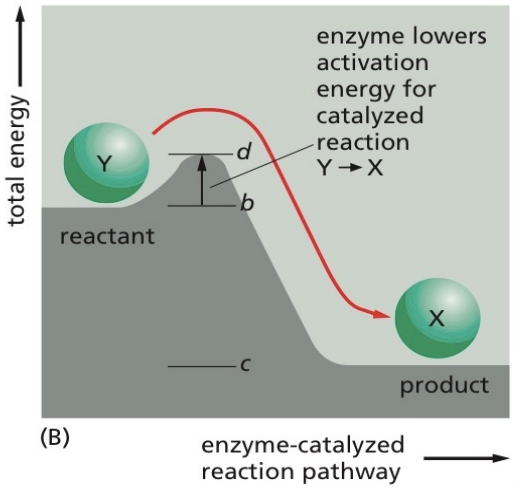


An **actin molecule** needs only to associate with other actin molecules to form a filament.

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.
Catalysis of organized sets of chemical reactions by enzymes creates and maintains the cell, making life.





Other examples: enzymes

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.

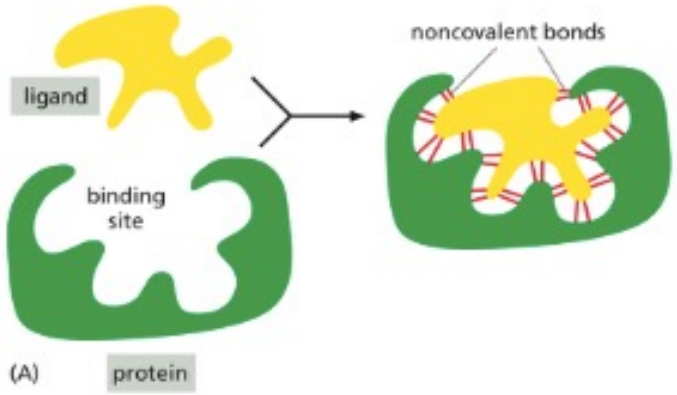
Equilibrium constant

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



Equilibrium constant

Protein–Ligand Binding Kinetics

At the equilibrium, the two reactions balance

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

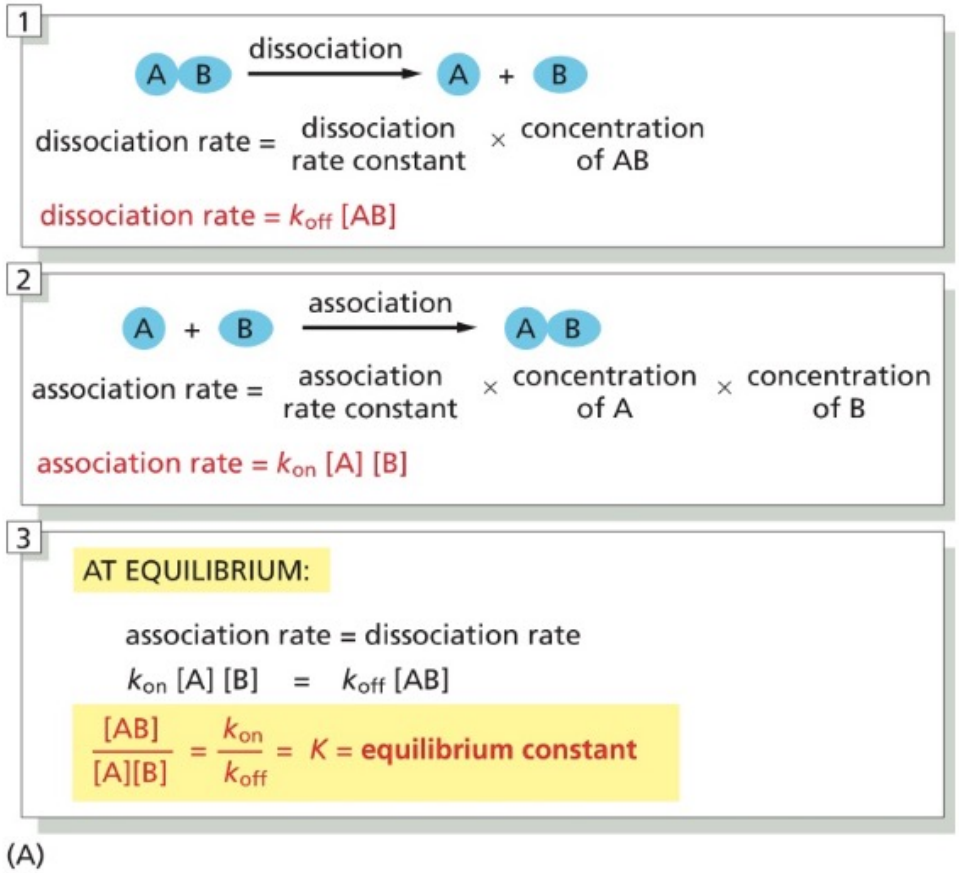
[..] is the **equilibrium concentration**

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



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_{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

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:

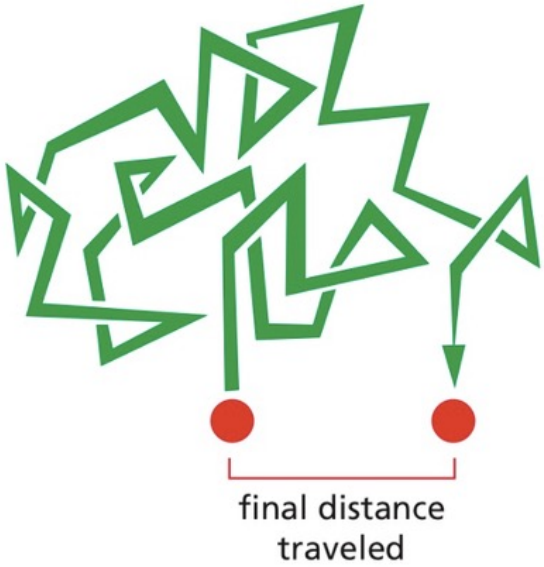
(1)the movement of a molecule from one place to another (translational motion)
(2)the rapid back-and-forth movement of covalently linked atoms with respect to one another (vibrations)
(3) rotations.

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.

Alberts, Bruce; Johnson, Alexander; Lewis, Julian; Morgan, David; Raff, Martin; Roberts, Keith; Walter, Peter. Molecular Biology of the Cell (Page 59). Garland Science. Kindle Edition.

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!



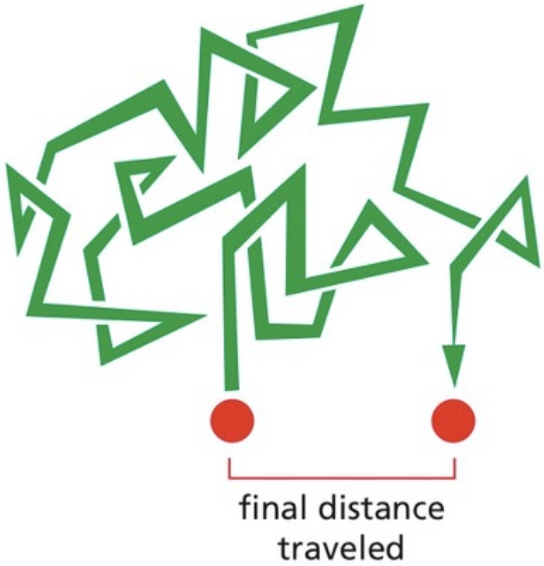
Diffusion with great heat exchange generates a molecule ***random walk***

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.

Alberts, Bruce; Johnson, Alexander; Lewis, Julian; Morgan, David; Raff, Martin; Roberts, Keith; Walter, Peter. Molecular Biology of the Cell (Page 59). Garland Science. Kindle Edition.

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

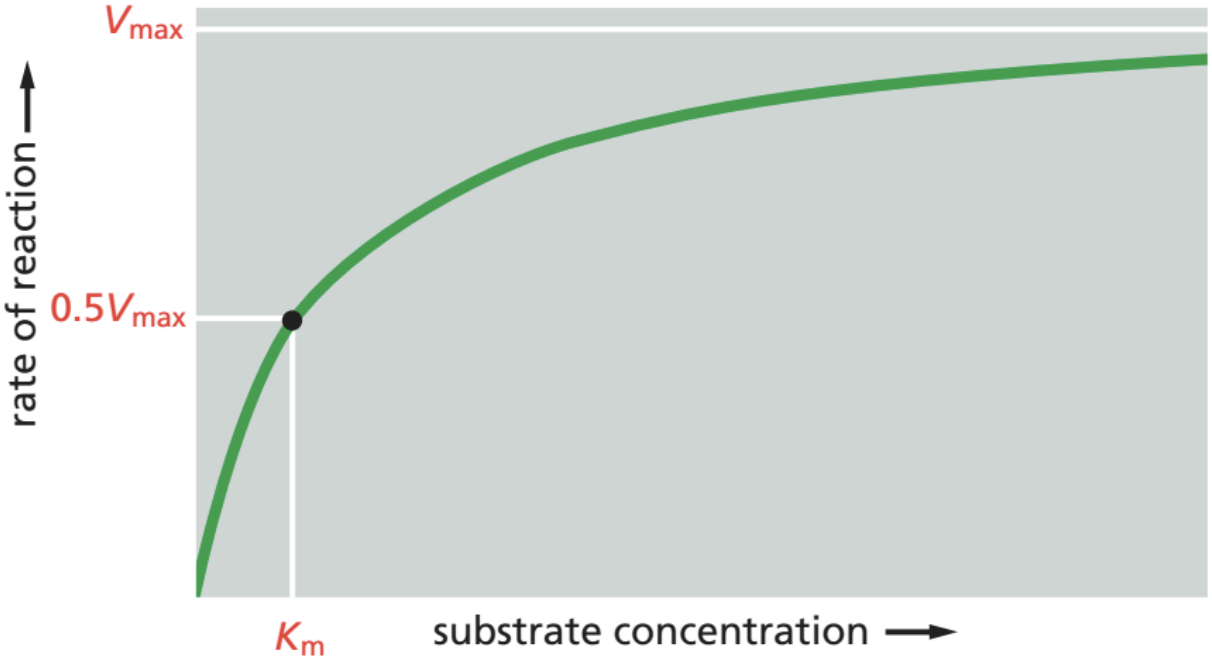
Water: 55.5 M

1 substrate mol per 10^5 water mol!

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

Alberts, Bruce; Johnson, Alexander; Lewis, Julian; Morgan, David; Raff, Martin; Roberts, Keith; Walter, Peter. Molecular Biology of the Cell (Page 59). Garland Science. Kindle Edition.

Basic Concepts and Thermodynamic Relationships



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 ($\mathbf{K_m}$) is a measure of how tightly the substrate is bound, with a large value of K_m corresponding to weak binding.

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.

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

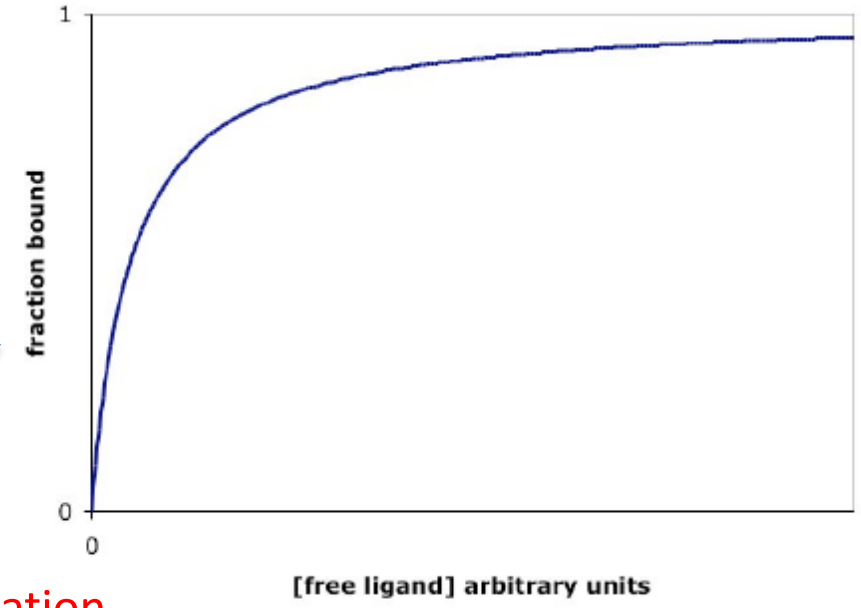
$$substituting [PX]$$

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

$$eliminate [P]$$

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

Langmuir equation



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?

Legge di Fick e di legge di conservazione

N particelle browniane, ossia grandi rispetto alle particelle del fluido in cui si muovono, ciascuna avente una posizione $\vec{x}_\alpha$ e una velocita $\vec{v}_\alpha = \frac{d\vec{x}_\alpha}{dt}$ con $1 \leq \alpha \leq \mathrm{N}$.

Definiamo la concentrazione c:

$$c(\vec{x}, t) = \sum_{\alpha=1}^{N} \delta(\vec{x} - \vec{x}_\alpha(t))$$

E la densita' di corrente media:

$$\vec{j}(\vec{x}, t) = \sum_{\alpha=1}^{N} \delta(\vec{x} - \vec{x}_\alpha(t))\vec{v}_\alpha(t)$$

Legge di Fick e di legge di conservazione

Se il sistema e' isolato, vale la legge di conservazione del numero di particelle, che da' una legge di continuita':

$$\frac{\partial c}{\partial t} = -\vec{\nabla} \cdot \vec{j}$$

In assenza di forze esterne:

$$< c(\vec{x}, t) >= costante$$

In presenza di forze esterne o fluttuazioni statistiche: ci aspettiamo che il sistema tenda a ritornare all'equilibrio.
Assumiamo, empiricamente, che la reazione del sistema avvenga secondo la cosidetta **legge di Fick**, per cui si crea una corrente di particelle nel verso opposto al gradiente iniziale

$$\vec{\nabla} c \neq 0 \qquad \vec{j} \propto -\vec{\nabla} c \rightarrow \vec{j} = -D\vec{\nabla} c \qquad [D] = \frac{m^2}{s}$$

dove **D e' detto coefficiente di diffusione** ed in generale dipende da posizione e tempo

Legge di Fick e di legge di conservazione

Si ottiene: $$\frac{\partial c}{\partial t} = -\vec{\nabla} \cdot \vec{j} = \vec{\nabla} \cdot (D\vec{\nabla} c) = D\vec{\nabla}^2 c$$

Vera se D e' indipendente dalla diffusione.
Nel caso 1-dimensionale:

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2}$$

La soluzione c (x,t) e' una gaussiana. Se inizialmente tutte le particelle sono condensate in un punto, cioe' $\mathrm{c(x,t=0)} = \mathrm{N}\delta(\mathrm{x})$ si ottiene l'equazione di diffusione:

$$c(x,t) = \frac{1}{(4\pi Dt)^{\frac{1}{2}}} e^{-\frac{x^2}{4Dt}}$$

Legge di Fick e di legge di conservazione

Da: $$< x^2 >= \frac{1}{N} \int x^2 c(x,t) dx = 2Ddt$$

Si ottiene: $$D = \lim_{x \to \infty} \frac{< (x(t) - x(0))^2 >}{2dt}$$

Formula empirica della diffusione, derivate da Einstein.

Equazione di Langevin

Nel quadro della descrizione teorica dei processi diffusivi, nel primo decennio del XX secolo, accanto ad Einstein si inserisce il fisico francese P.Langevin (1872-1946).
Nella descrizione del moto browniano, Langevin parte dalle equazioni del moto.[3]
Si consideri una particella browniana immersa in un fluido, di raggio a, $10^{-9}m < a < 5\times10^{-7}m$ (essa ha quindi dimensioni maggiori rispetto agli atomi del fluido in cui è immersa). Il suo moto è pertanto più lento di quello degli atomi ed è il risultato di collisioni rapide e casuali con essi, dovute a fluttuazioni di densità del fluido.
Considerando il moto unidimensionale, l'equazione di Newton della particella di massa m e velocià $v(t)$ è

$$m\frac{dv(t)}{dt} = F(t) \tag{2.11}$$

dove F(t) è la forza totale agente sulla particella al tempo t, causata dall'interazione di questa con il mezzo circostante. Tale forza è dominata da un termine di attrito viscoso $-\gamma v(t)$ dove $\gamma = 6\pi\eta a$, con a raggio della particella ed η viscosità del fluido. Ci si aspetta anche un contribuito casuale alla forza F(t), denotato con $\xi(t)$, dovuto alle fluttuazioni casuali della densità del fluido.
Dunque le equazioni del moto della particella browniana, dette *equazioni di Langevin* sono

$$\frac{dx(t)}{dt} = v(t) \qquad\qquad \frac{dv(t)}{dt} = -\frac{\gamma}{m}v(t) + \frac{\xi(t)}{m} \tag{2.12}$$

dove la variabile stocastica $\xi(t)$ dà l'effetto di rumore dovuto al fluido.

Equazione di Langevin

dove la variabile stocastica $\xi(t)$ dà l'effetto di rumore dovuto al fluido. Se trascurassimo tale componente stocastica, l'equazione (2.12) diverrebbe

$$\frac{dv(t)}{dt} = -\frac{\gamma}{m}v(t) \tag{2.13}$$

con soluzione

$$v(t) = e^{-\frac{t}{\tau}}v(0) \qquad\qquad \tau = \frac{m}{\gamma} \approx 10^{-3}s \tag{2.14}$$

dove τ è il tempo di rilassamento tipico della velocità della particella browniana.

Secondo l'uguaglianza (2.14), la velocità della particella dovrebbe tendere a zero per tempi lunghi. Ciò non può essere vero in quanto in equiliibrio, per il teorema di equipartizione vale

$$< v^2(t) >_{eq} = \frac{kT}{m} \tag{2.15}$$

mentre da (2.14) risulta

$$< v^2(t) >_{eq} = e^{-\frac{2t}{\tau}} < v^2(0) >_{eq} \to 0 \tag{2.16}$$

Quindi $\xi(t)$ in (2.12) è necessaria per avere il corretto equilibrio.

Il modo browniano è dovuto alle collisioni rapide e casuali tra particelle colloidali e molecole del fluido circostante, causate da fluttuazioni di densità di quest'ultimo. Si suppone che la forza durante tale collisione vari in modo estremamente rapido nel tempo di qualsiasi osservazione; ci si aspetta dunque, per la componente casuale della forza F

$$< \xi(t) >_{\xi} = 0 \tag{2.17}$$

Equazione di Langevin

Si dimostra matematicamente, applicando il teorema del limite centrale a una distribuzione di variabili casuali indipendenti, che la componente casuale della forza ha una distribuzione gaussiana con media zero.[3] A differenza dell'equazione di diffusione (2.7), che è un'equazione deterministica, l'equazione di Langevin è stocastica,in quanto include una forza casuale. Ogni soluzione di tale equazione del moto stocastica è una differente traiettoria casuale.

I processi di diffusione browniana sono caratterizzati, dunque, da due proprietà[4]:

- la crescita lineare nel tempo dello spostamento quadratico medio (MSD da mean-squared displacement)

$$< \mathbf{r}^2(t) >= 2dDt \tag{3.1}$$

  dove d è la dimensione spaziale e D il coefficiente di diffusione.

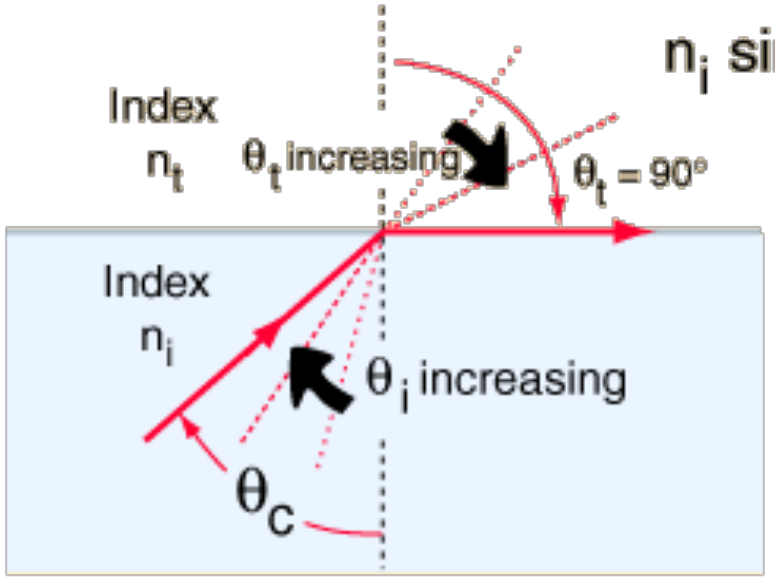
- la funzione densità di probabilità di trovare la particella che diffonde, alla posizione $\mathbf{r}$ al tempo t è una gaussiana

$$P(\mathbf{r}, t) = \frac{1}{(4\pi Dt)^{d/2}} e^{-\frac{\mathbf{r}^2}{4Dt}} \tag{3.2}$$

Come si determina sperimentalmente
la cinetica di binding?

SURFACE PLASMON RESONANCE (SPR)

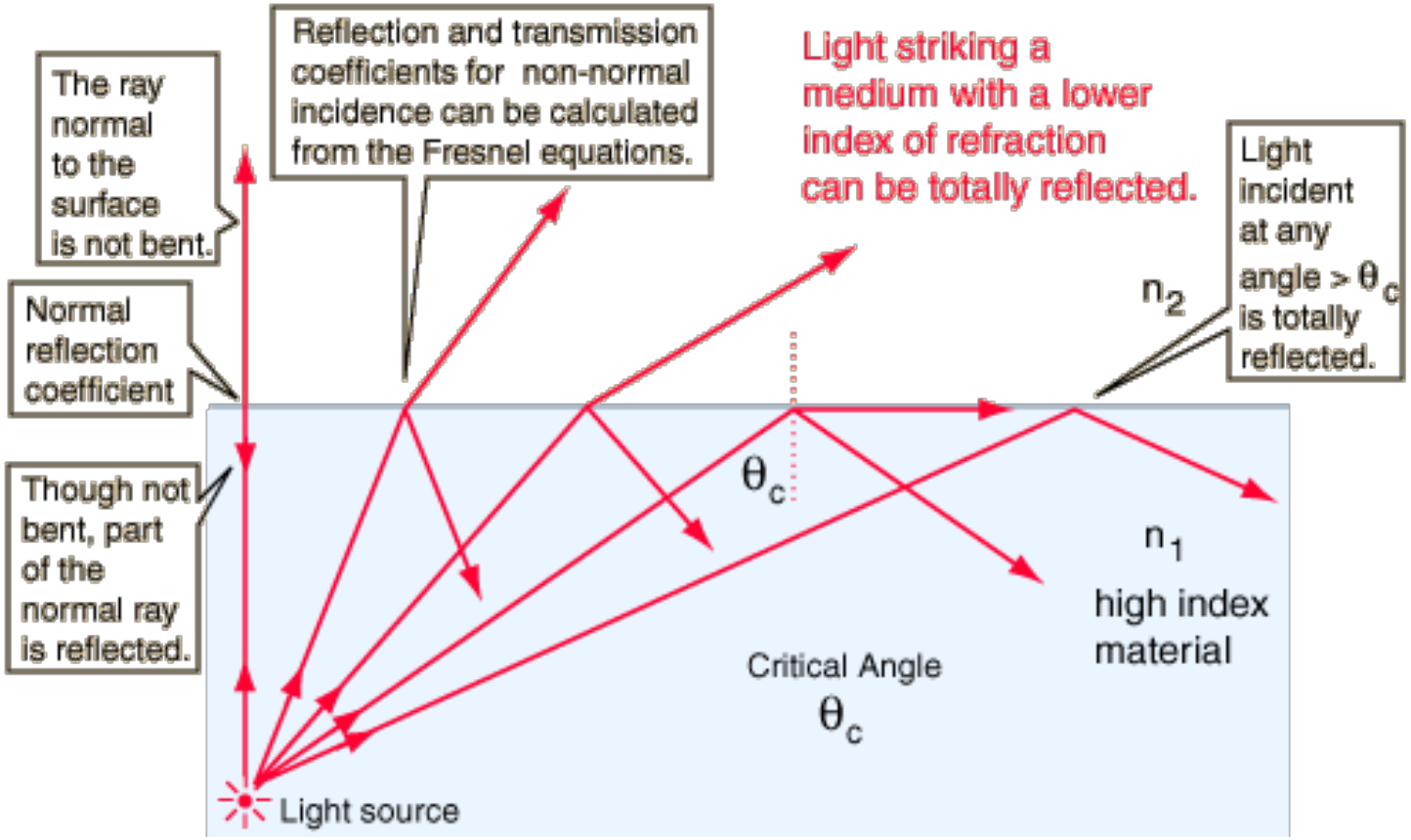
Surface Plasmon Resonance (SPR)



$$n_i \sin\theta_c = n_t \sin 90°$$

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

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

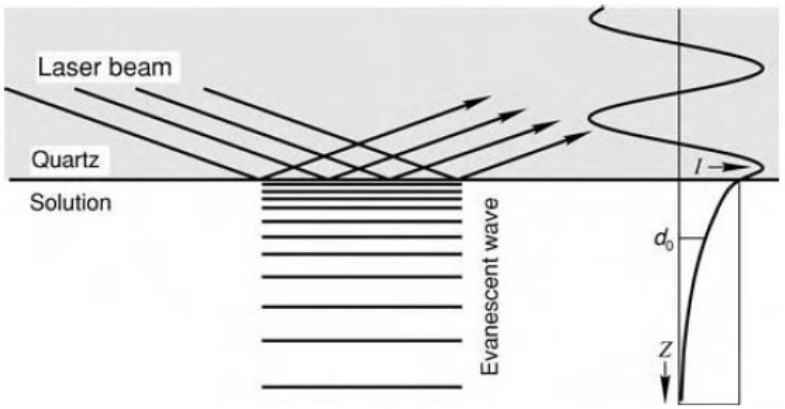
Although the incident light beam is totally internally reflected under these conditions, an electromagnetic field penetrates a small distance into medium 2. The intensity of this field decays exponentially with the distance z from the interface:

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

with a characteristic penetration depth

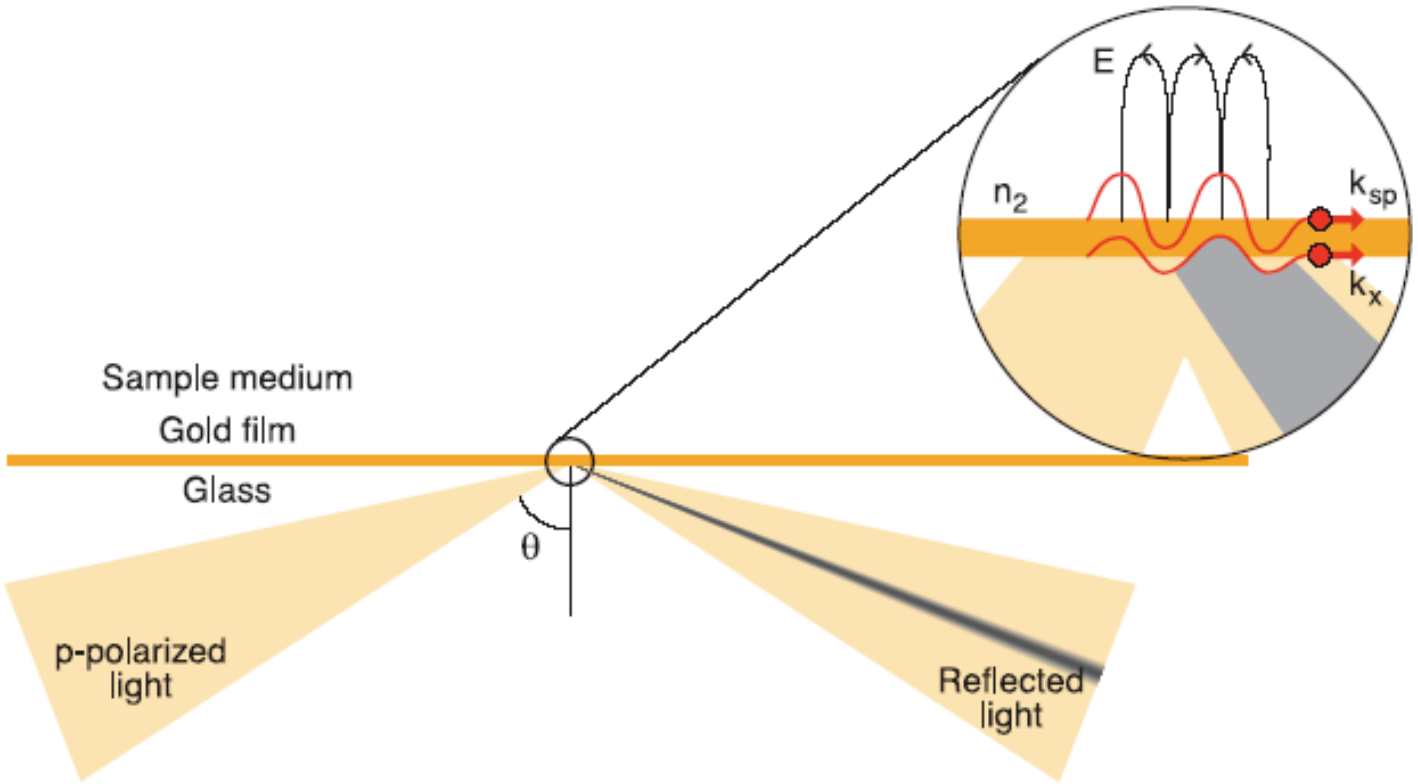
$$d_p = \frac{\lambda_0/n_1}{4\pi\sqrt{\sin^2\theta - (n_2/n_1)}} \tag{D}$$

This field is often called the 'evanescent' wave.



SPR

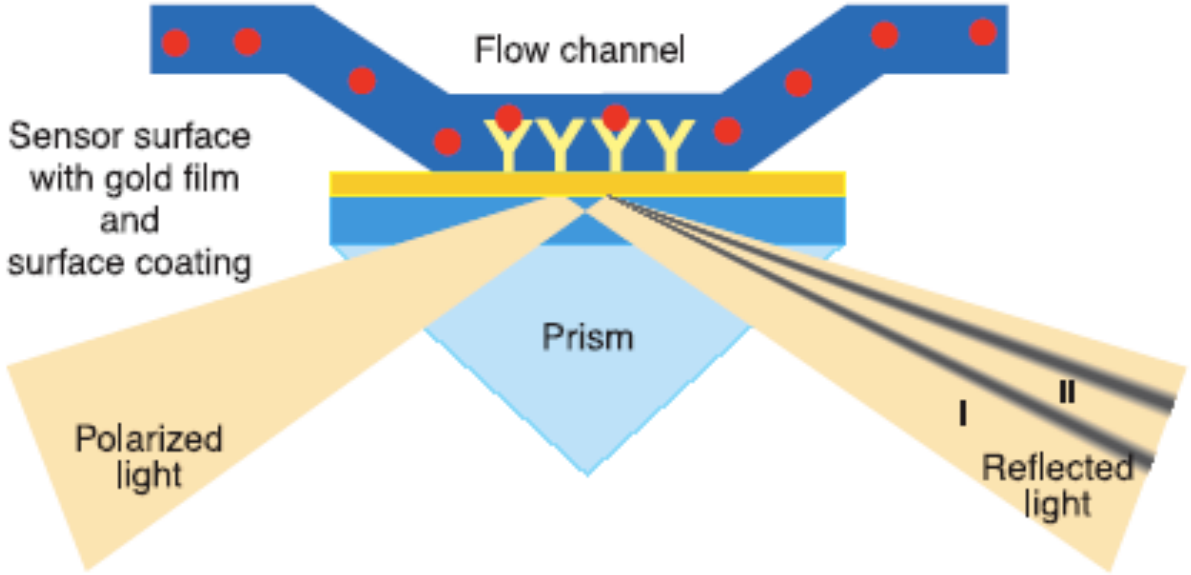
Lo strato d'oro è di circa 50 nm

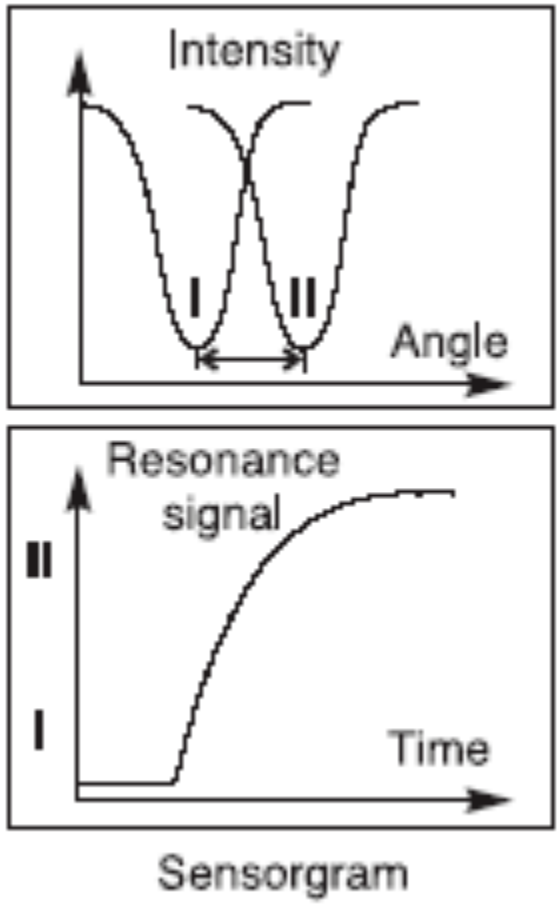


If the interface between the two media is coated with a thin layer of a suitable conducting material, such as a metal, the p-polarized component of the evanescent field wave penetrates the metal layer. In fact, at a specific angle of incidence, plane polarised light excites the delocalised surface electrons (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.

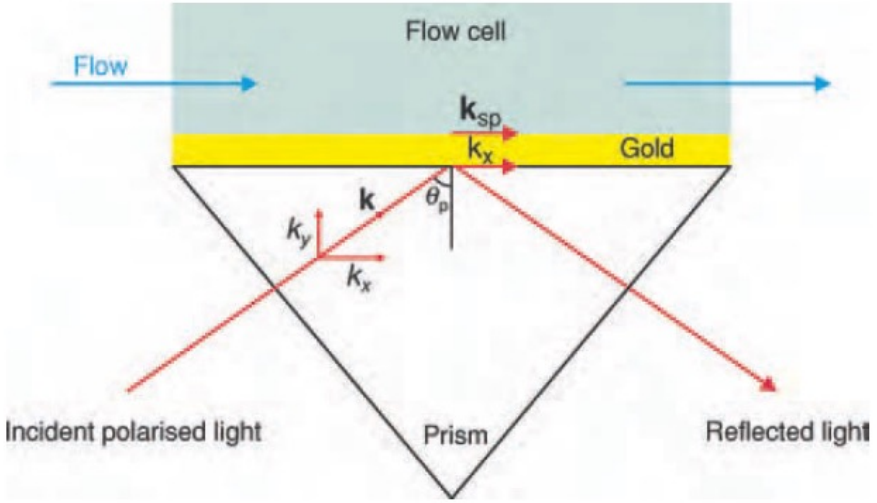
Surface plasmon resonance

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:

$$k_x = k_{sp}$$

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

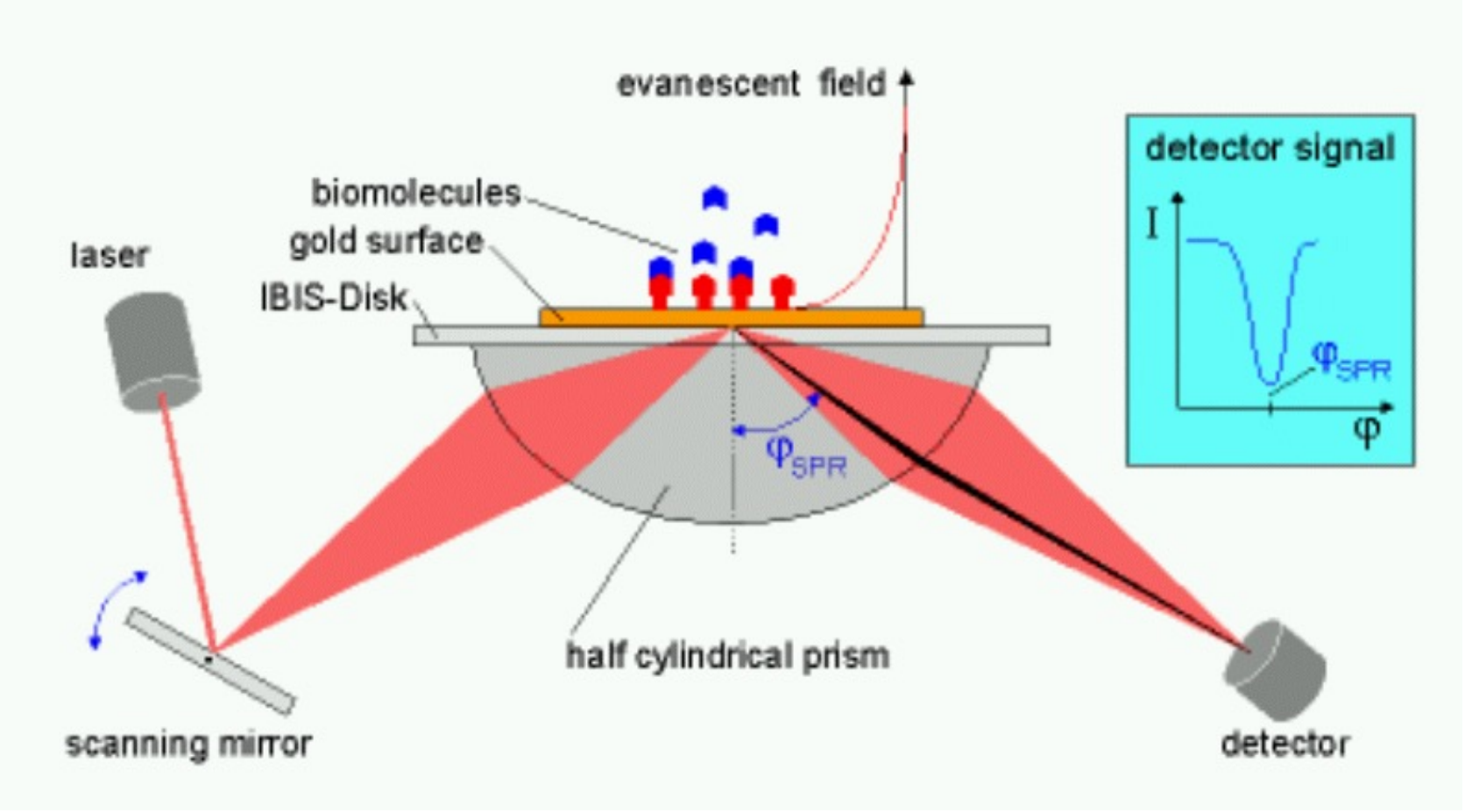
$$n_{glass} sin\theta_p = \sqrt{\frac{\varepsilon_{metal} \times \varepsilon_{cell}}{\varepsilon_{metal} + \varepsilon_{cell}}}$$



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.