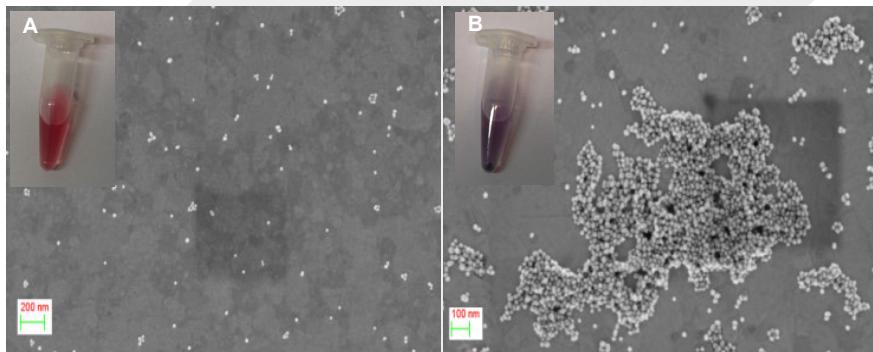
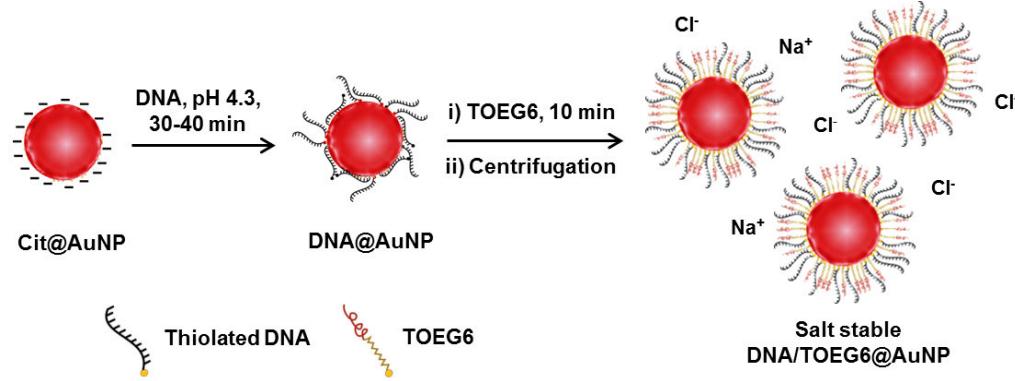
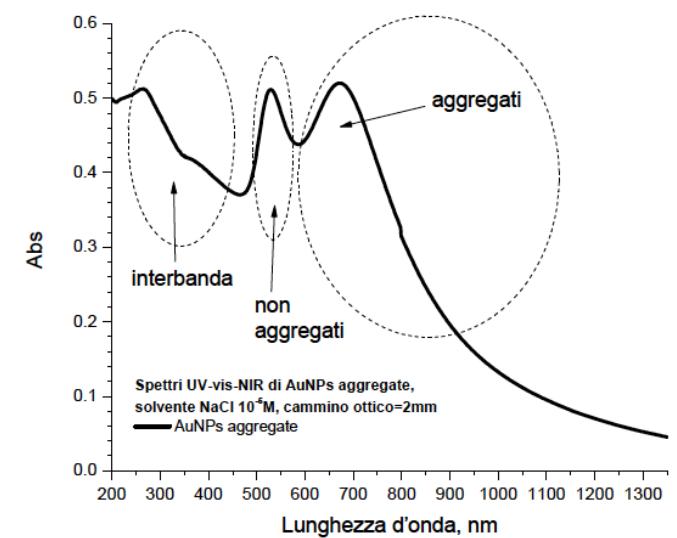
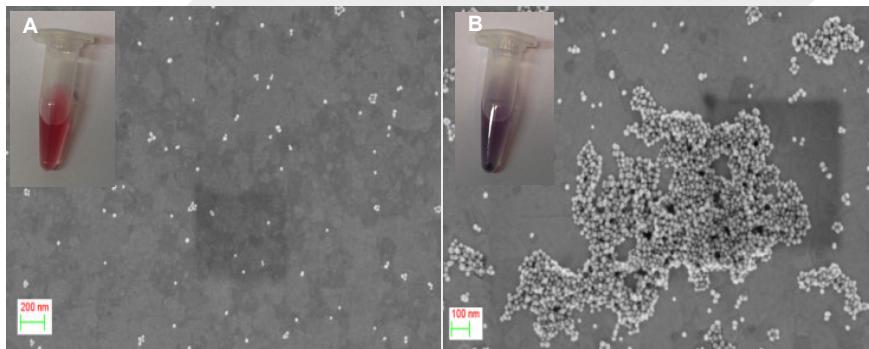
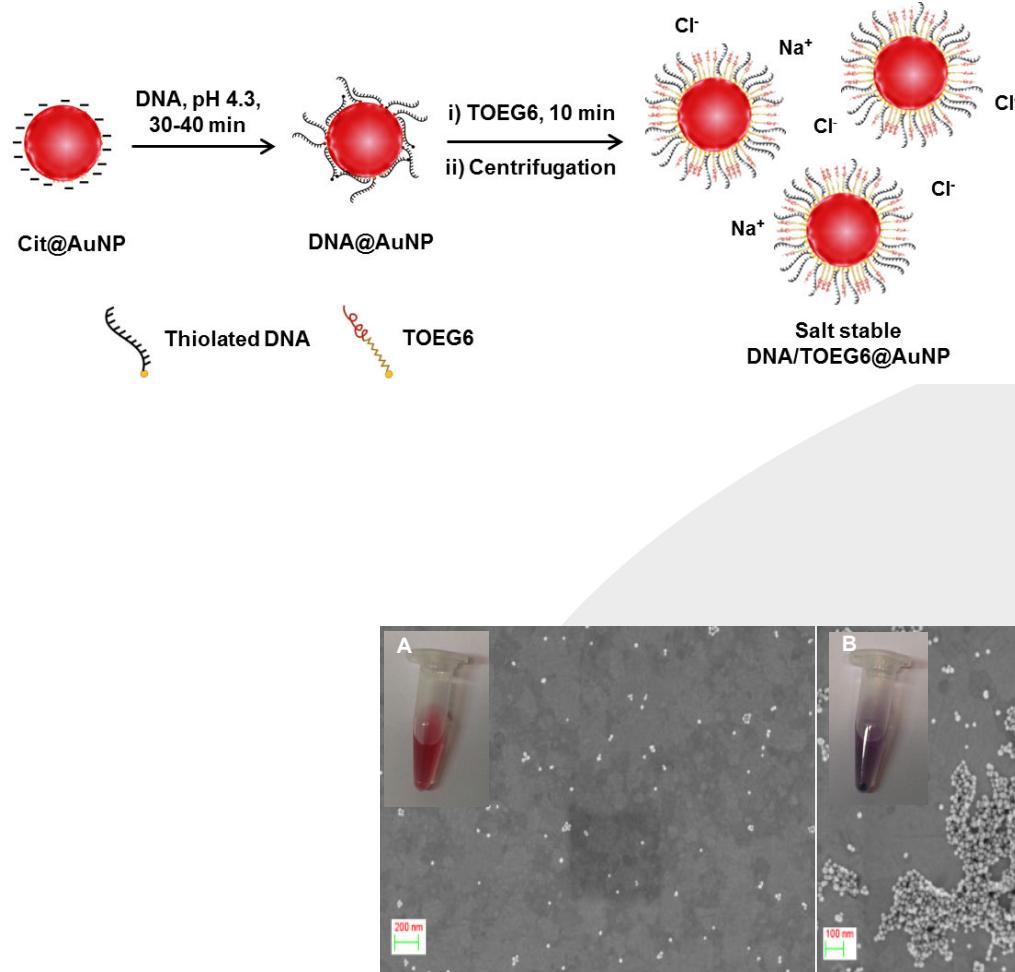


Parte 2: **Nanoparticelle metalliche funzionalizzate e il loro utilizzo in biotecnologia**

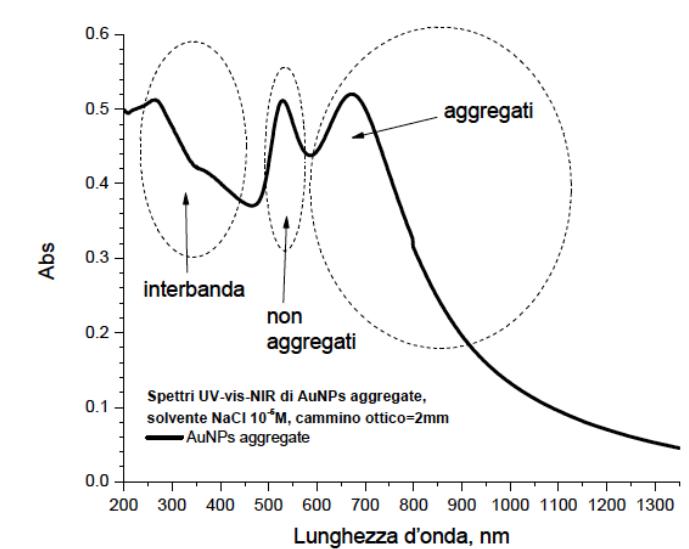


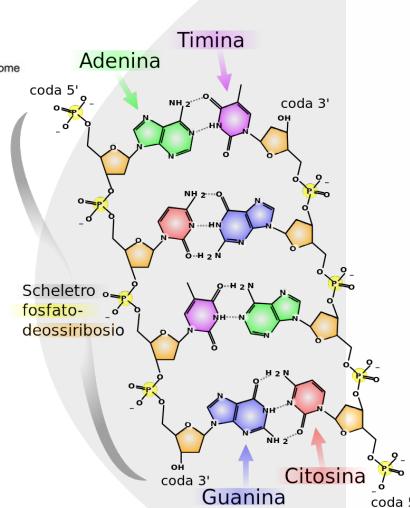
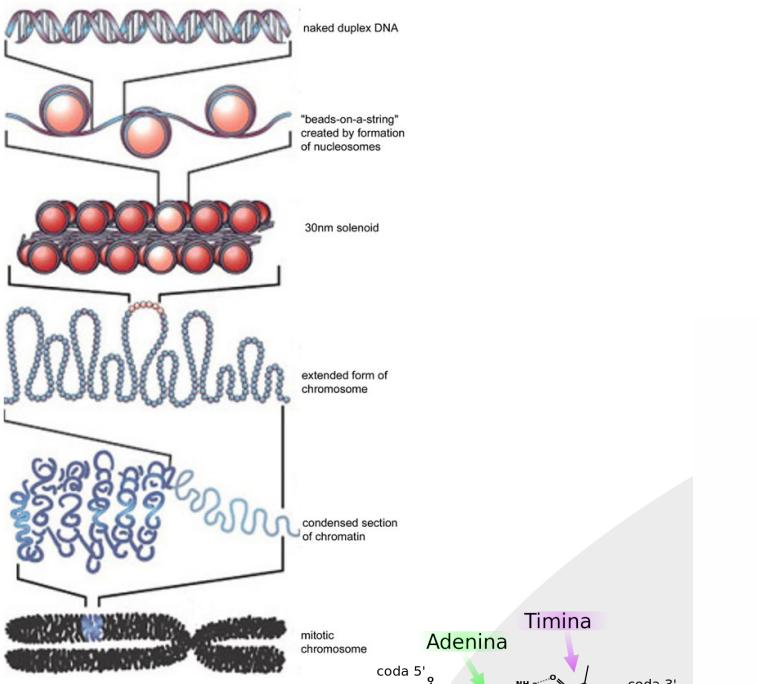
In generale, aggregati di NP si comportano come una NP più grossa.





Ma come si fanno aggregare le NP in modo controllato?
Per esempio con il DNA

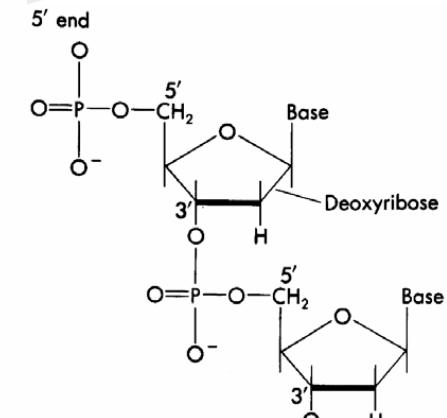
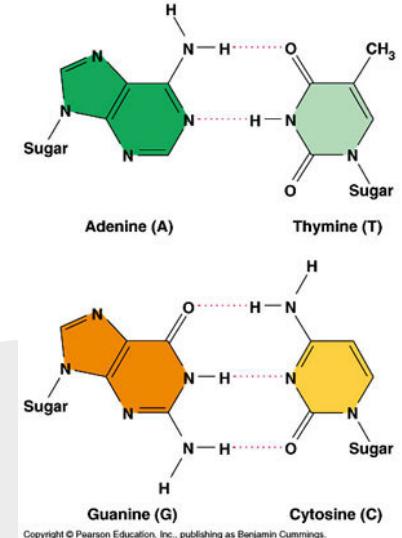
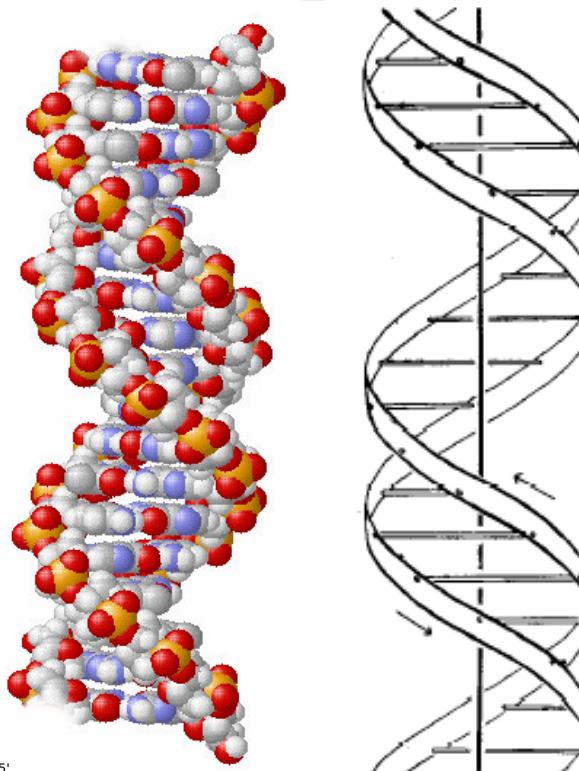




DNA

Base pairing

5' TAGACTACGCATAACGCCT 3'
3' ATCTGATGCGTATGCGGA 5'



DNA Biotechnology

- **restriction enzymes:**

cut DNA at specific sequences

- **ligases:**

link two DNA pieces covalently

- **helicase:**

unwinds DNA

- **topoisomerases:**

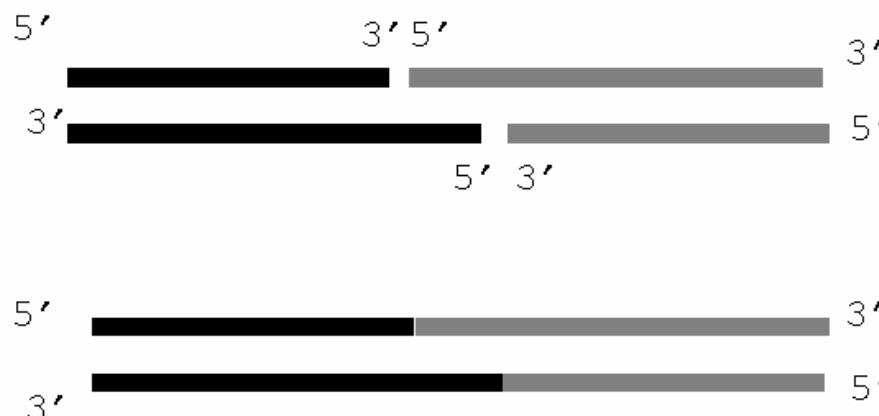
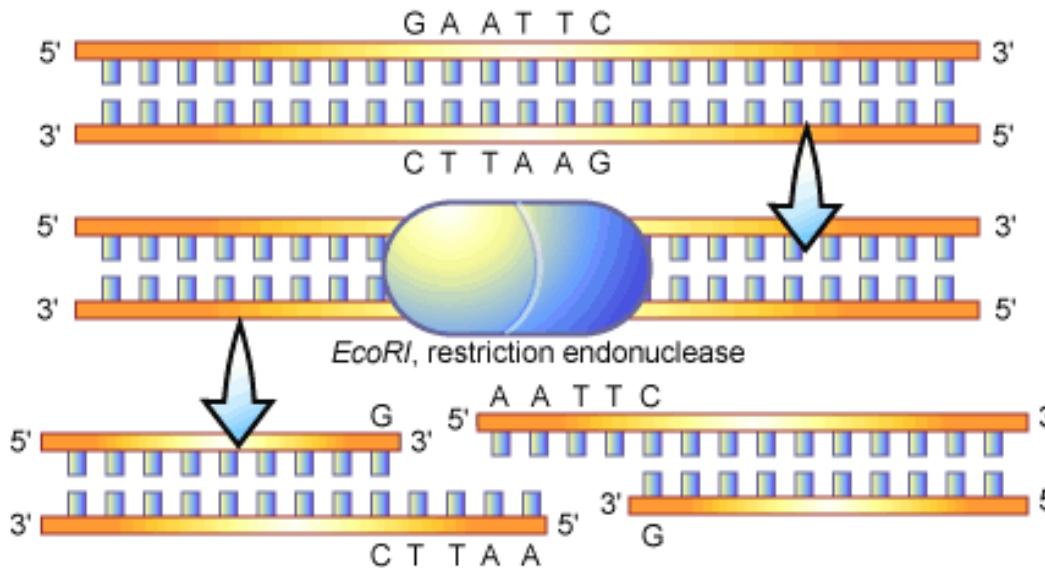
Change topology (linking, winding number)

- **DNA/RNA polymerases:**

make copies

- **DNA binding proteins:**

help in recombination, function as transcriptional modulators, etc.



Courtesy of Friedrich C. Simmel, LMU, Munich

Ligation of sticky ends

DNA Biotechnology

It is the combination of in vitro hybridization and synthetic branched DNA that leads to the ability to use DNA as a construction material

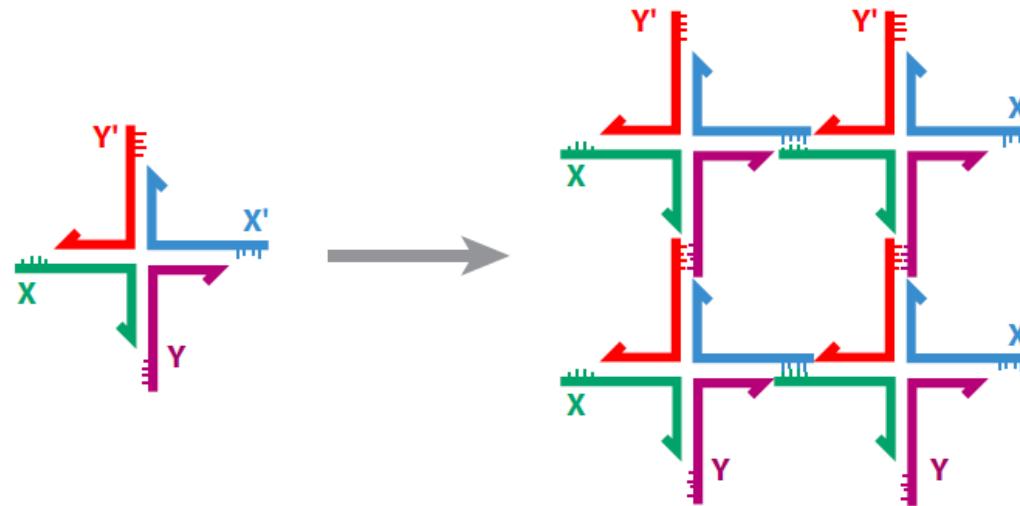
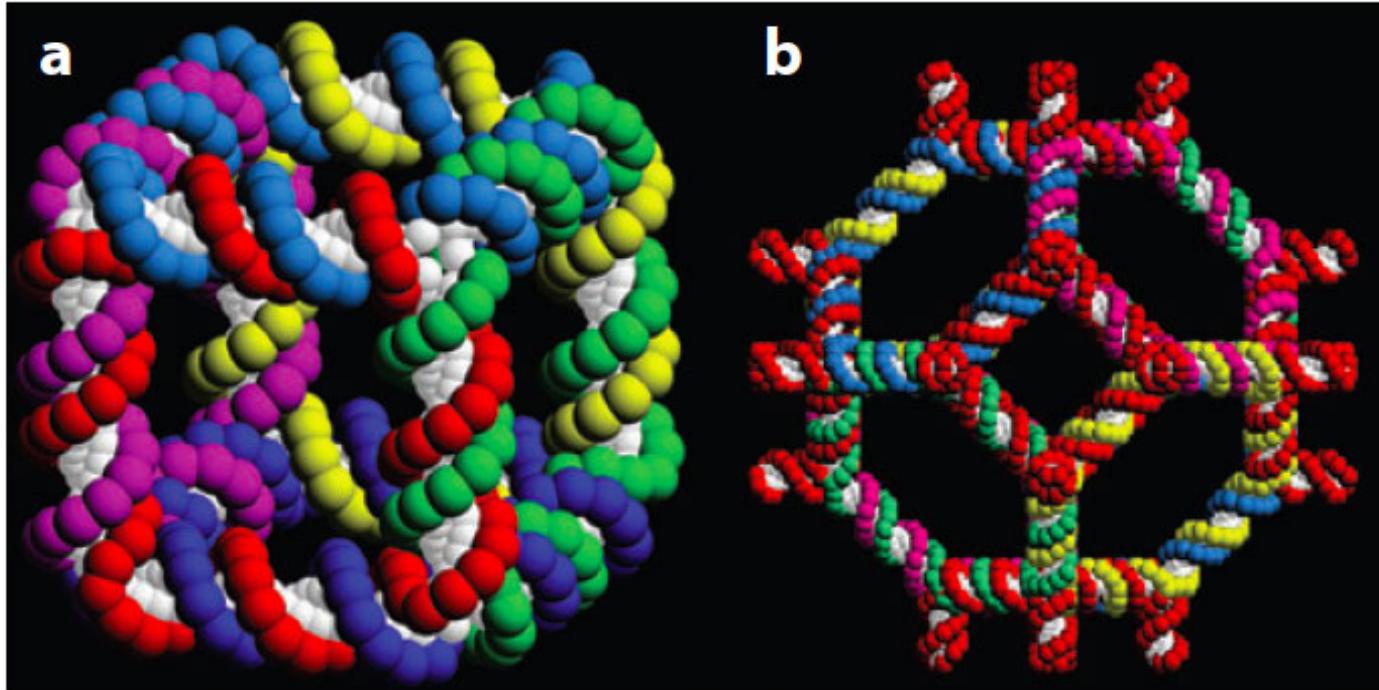


Figure 2

Self-assembly of branched DNA molecules to form larger arrangements. The image on the left shows a four-arm branched junction made from four differently colored strands. Its double helical domains are tailed in 5' sticky ends labeled (clockwise from the left) X, Y', X', and Y; the sticky ends are indicated by small extensions from the main strand (our convention is to represent 3' ends by arrowheads or, as here, by half arrowheads). The primed sticky ends complement the unprimed ones. The image on the right shows how four of these junctions can self-assemble through this complementarity to yield a quadrilateral. The sticky ends have come together in a complementary fashion. Note, this assembly does not use up all the available sticky ends, so that those that are left over could be used to generate a lattice in two dimensions (2D) and, indeed, in 3D.



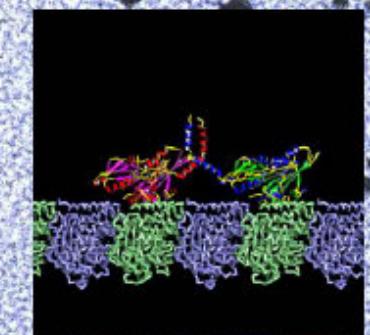
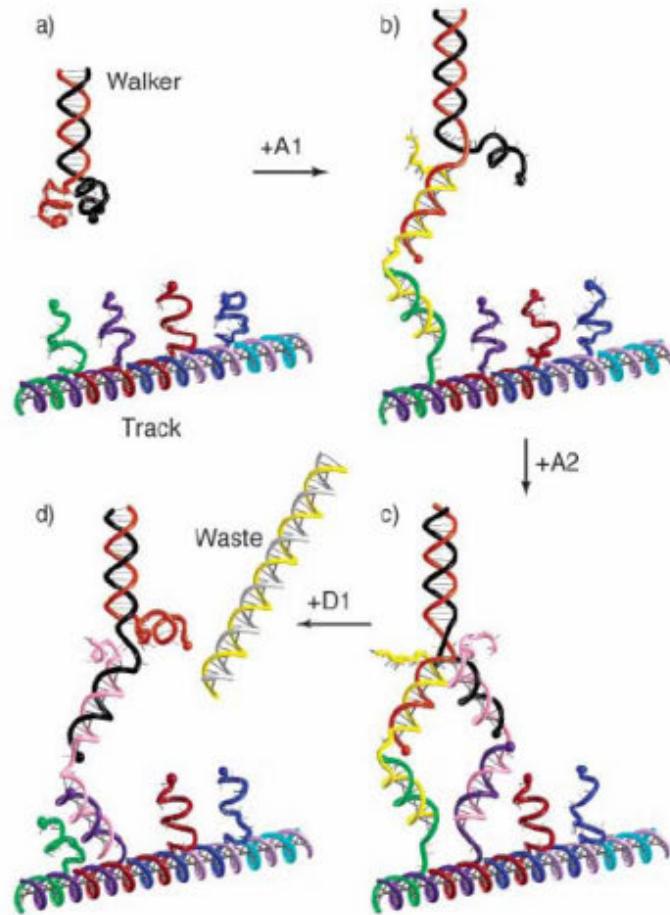
DNA molecule with the connectivity of a cube. The six backbone strands are represented by the colored balls and the bases are all drawn in white. Note that the molecule is a hexacatenane, with the six strands linked to each other. Each single strand corresponds to a face of the molecule

DNA can be used to:

- Build nanostructures
- guide materials synthesis
- construct nanomechanical devices
- perform computations

Locomotion

A simple DNA walker walks along a track



Kinesin on Microtubule,
Mandelkow lab

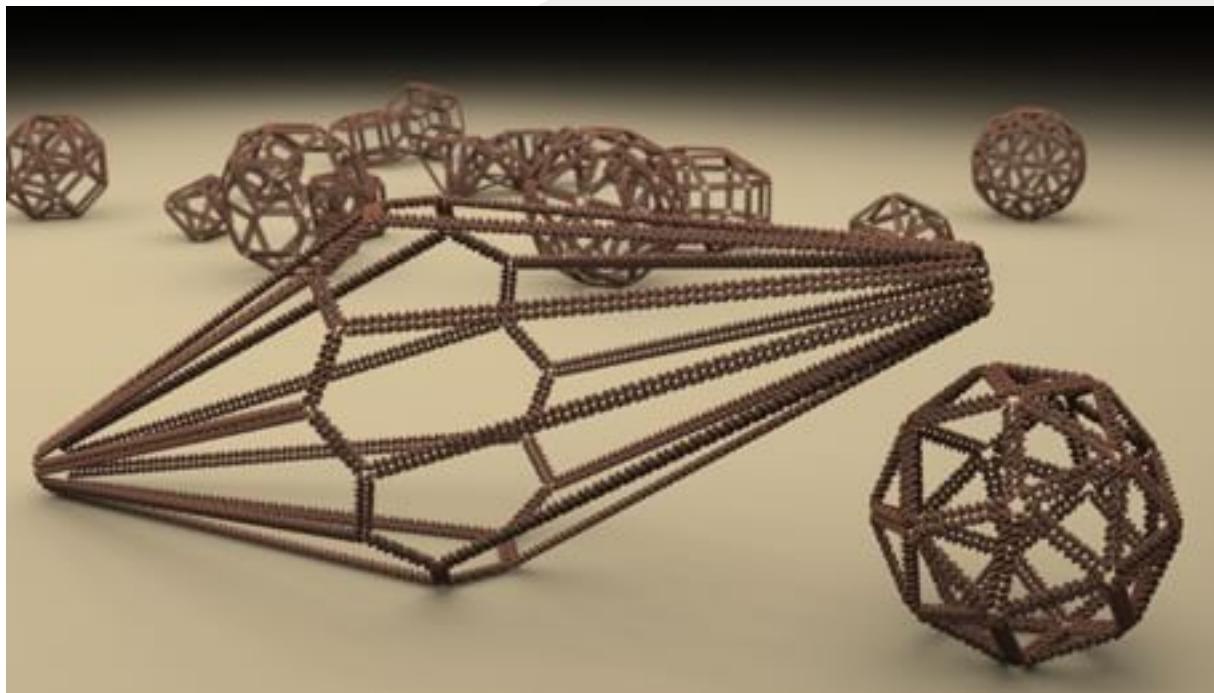
Shin & Pierce,
JACS 126, 10834 (2004)

Nanoroadmap, Rome 2004

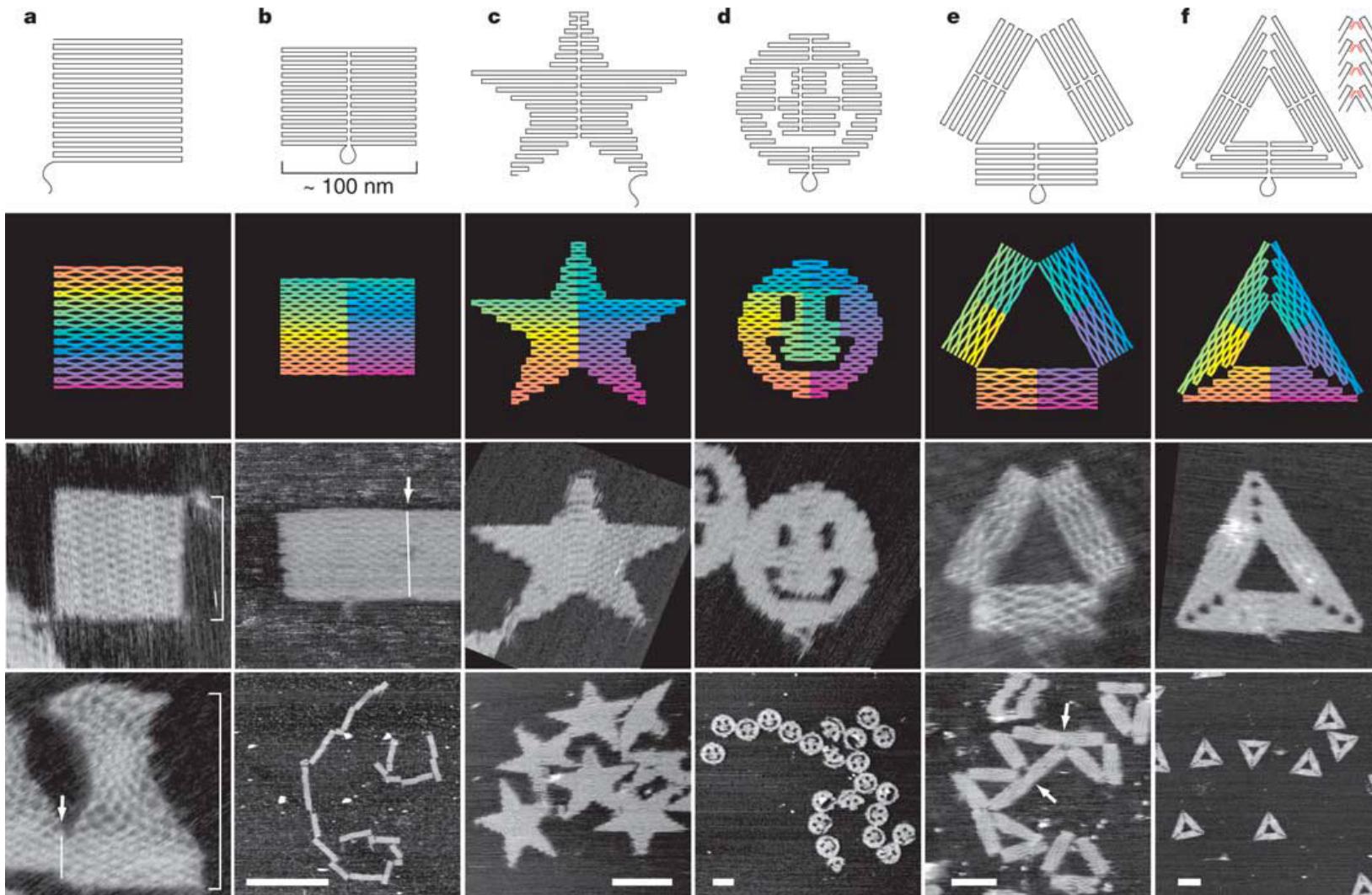
23

L'evoluzione ha dotato il DNA di una capacità straordinaria di **codificare le informazioni**:

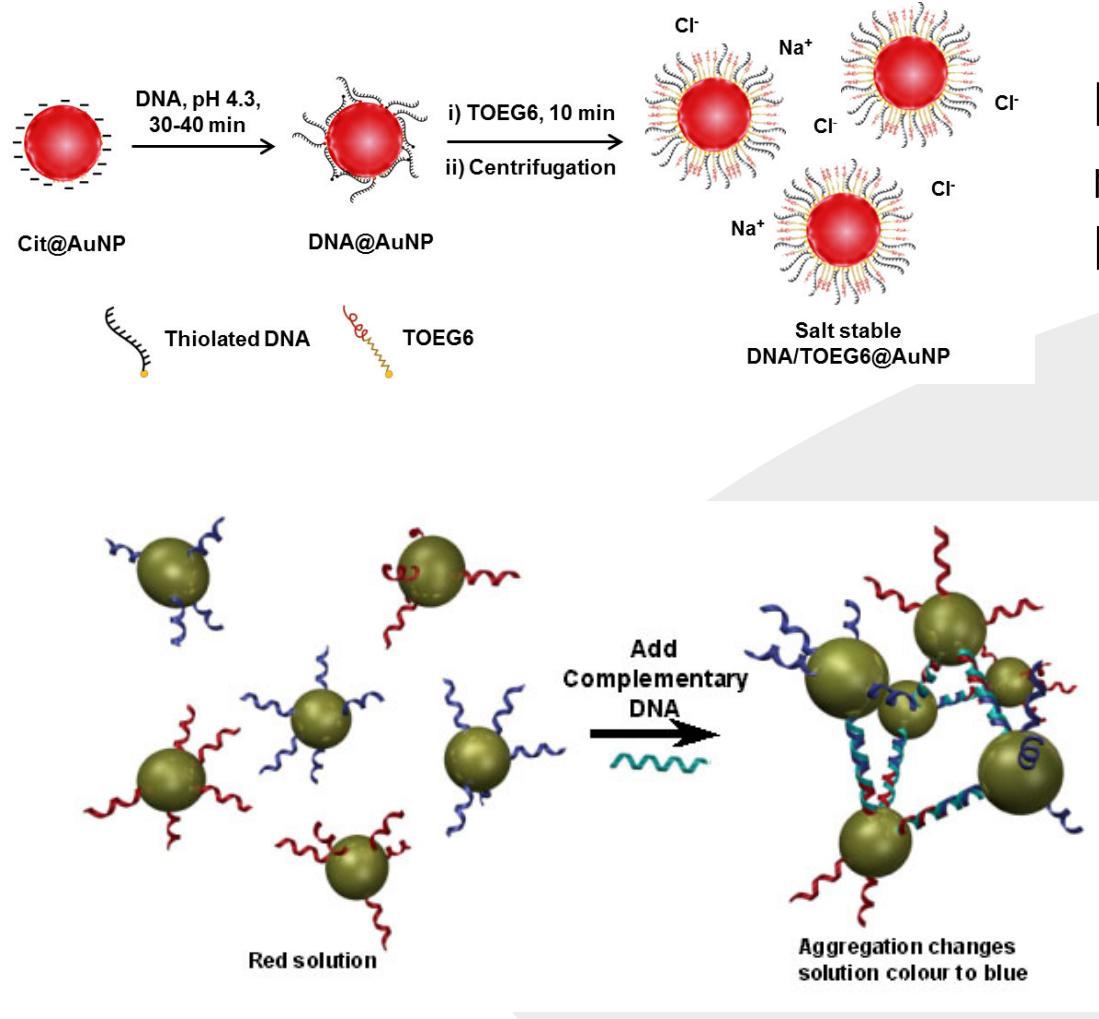
un solo grammo di DNA può memorizzare circa **700 terabyte**, cioè 700.000 gigabyte d'informazione, corrispondente a circa 14.000 Blu-ray disc. E questo può durare milioni di anni, come dimostrano le ricerche di recupero e di sequenziamento del DNA di antiche specie animali e vegetali. Ecco perché, una delle applicazioni più interessanti, anche se di là da venire, degli origami a DNA è la memorizzazione di dati. (Le Scienze, 2016)



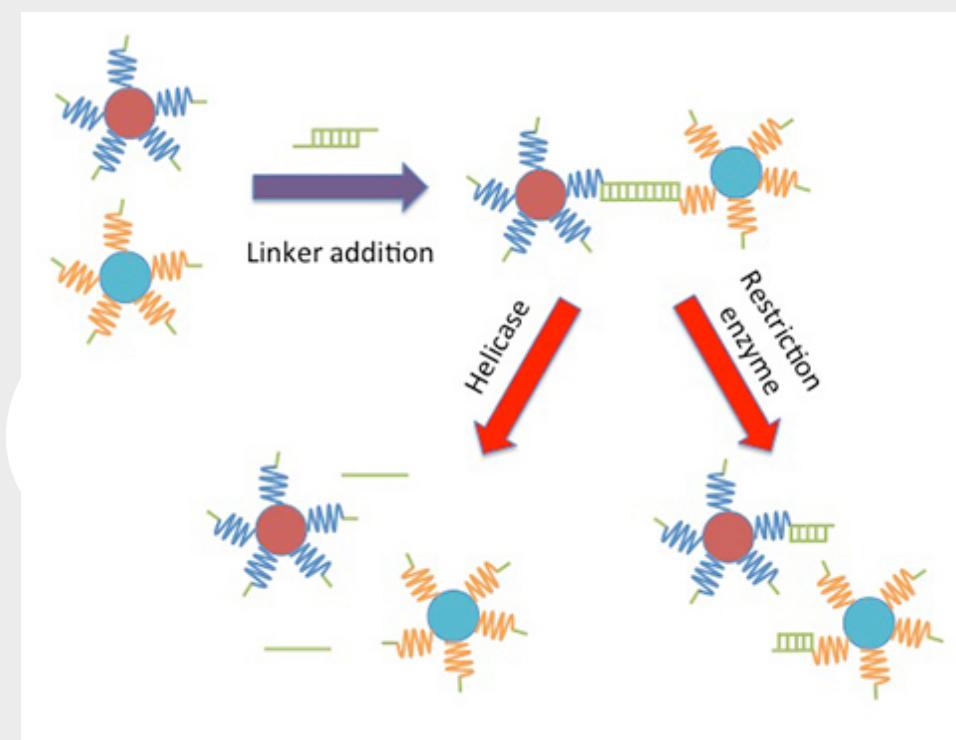
DNA origami

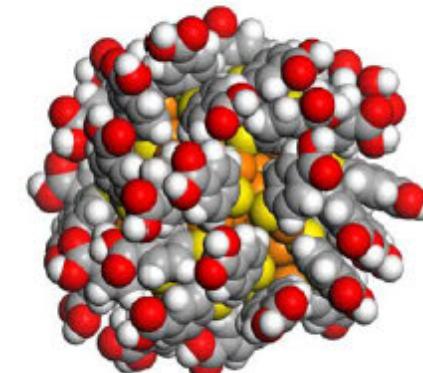
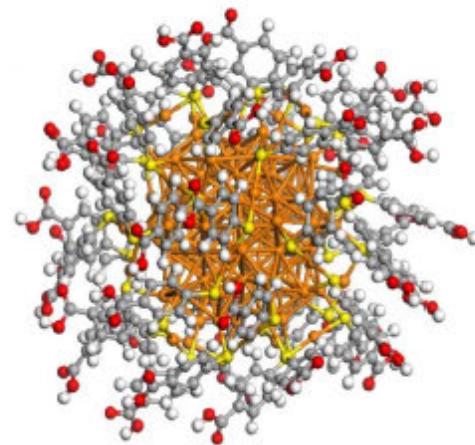
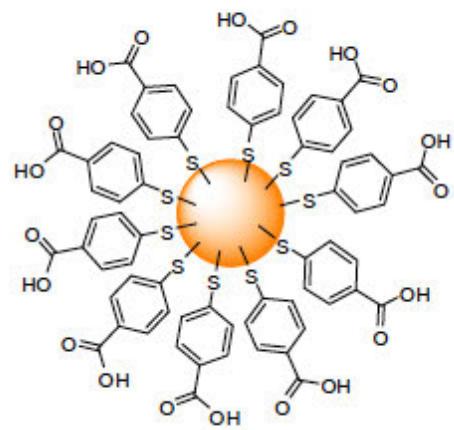
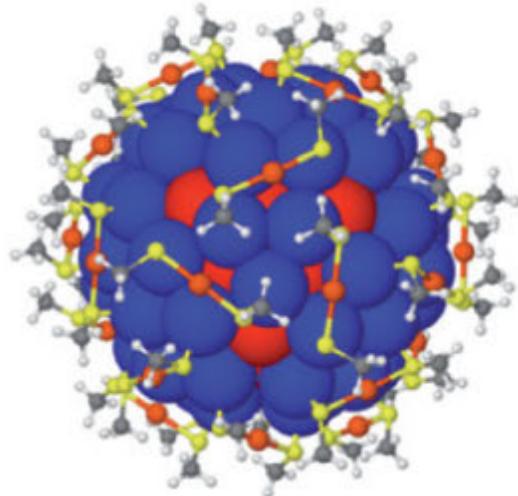


https://www.youtube.com/watch?v=Trg2__Lgnco



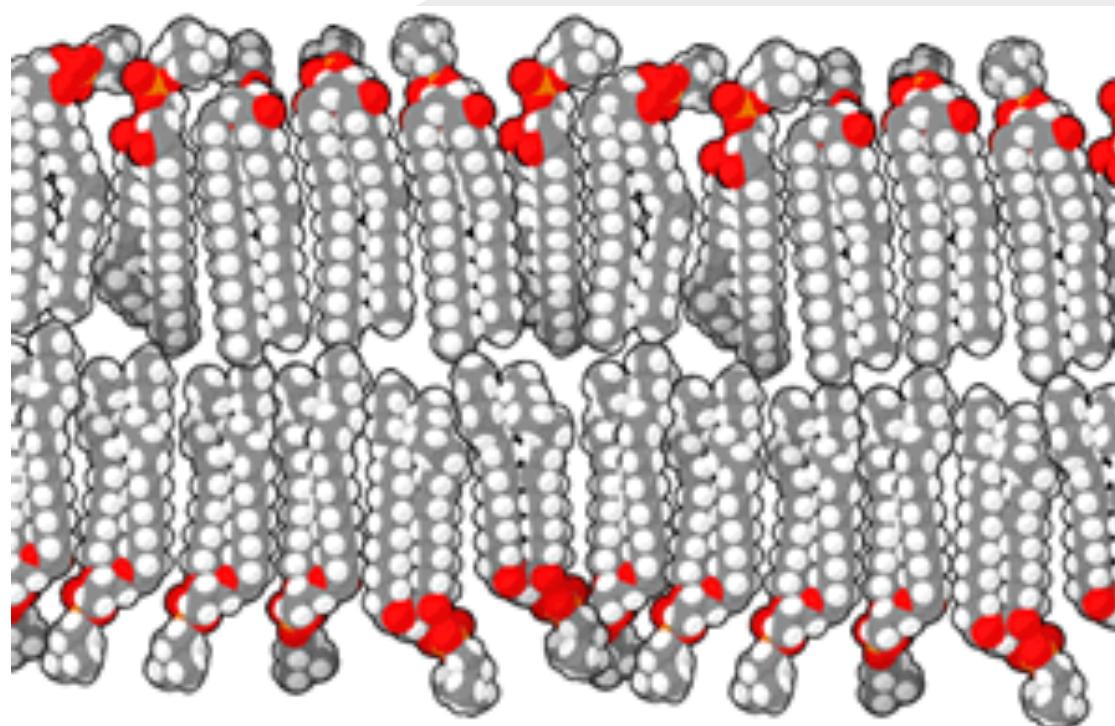
Ma come si fanno aggregare le NP in modo controllato?
Per esempio con il DNA





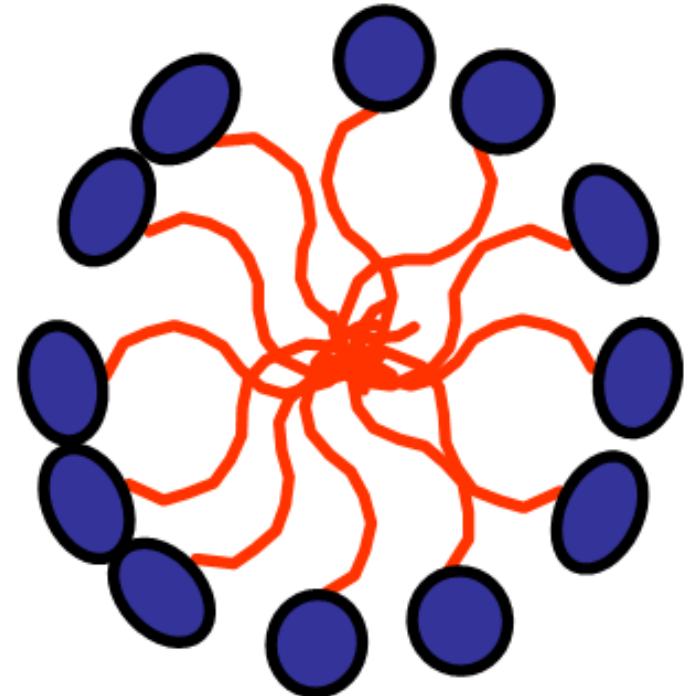
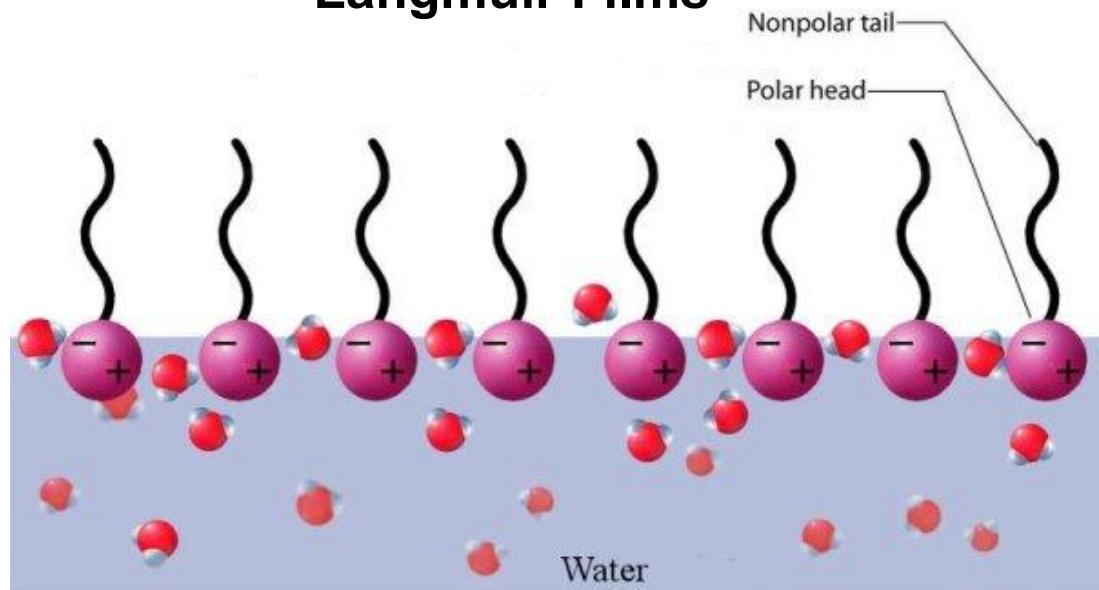
Self-assembly

Membranes are made of strongly anisotropic molecules
Strongly anisotropic molecules like to self-organizing



Self-organized monolayers (on liquid surfaces)

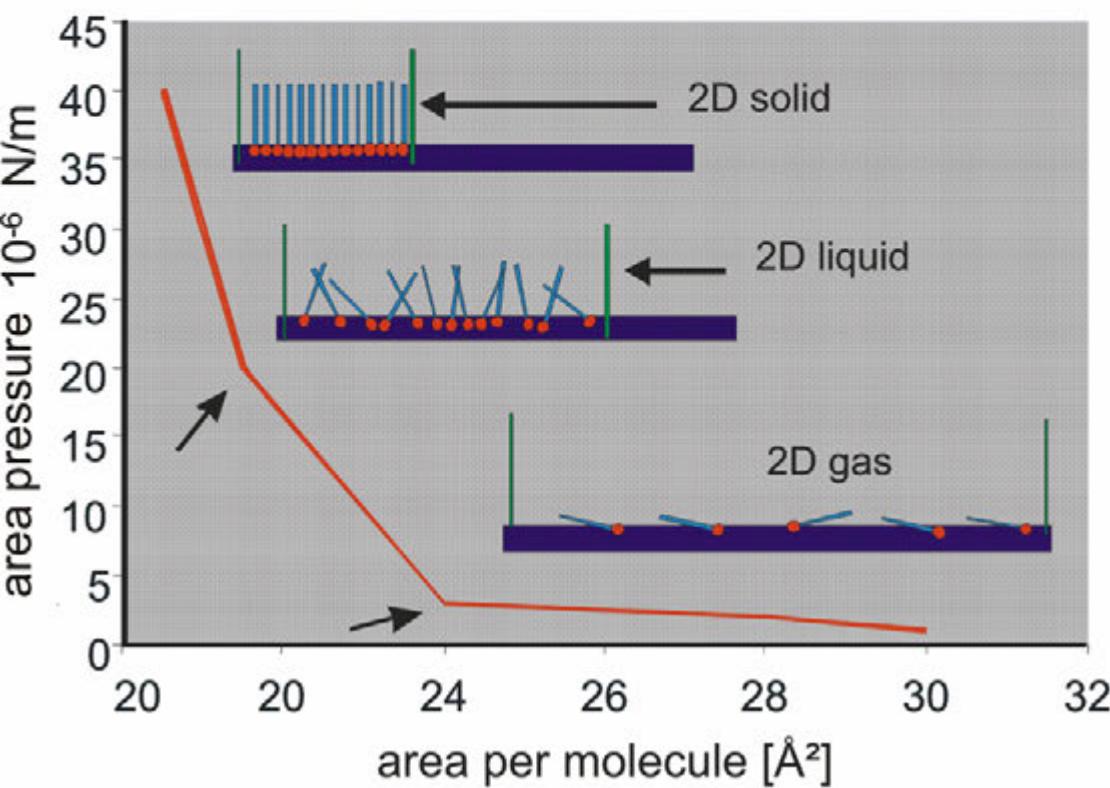
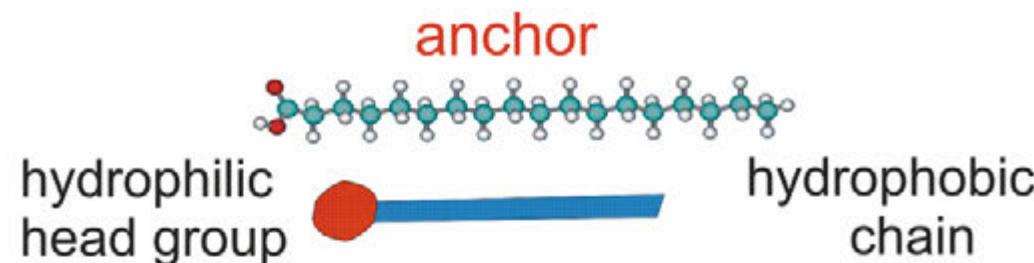
Langmuir Films



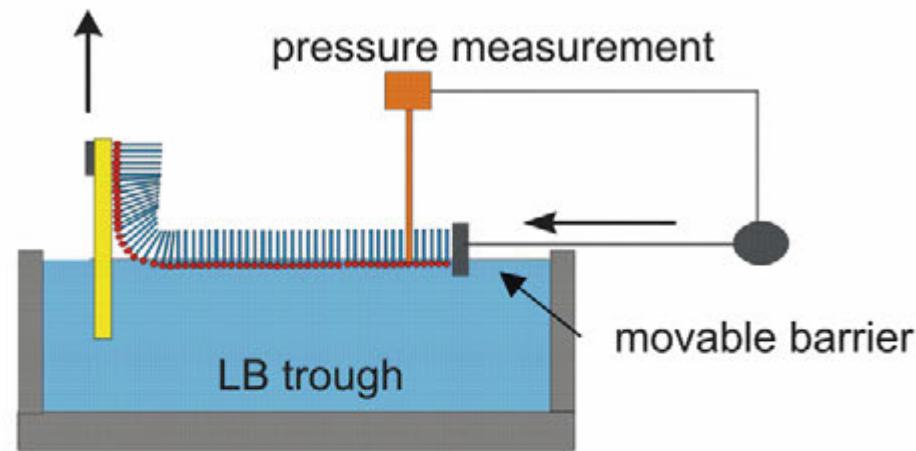
The term “molecular self-assembly” refers to spontaneous formation of an ordered molecular overlayer on the surface, often proceeding through several consecutive stages where 1D and 2D ordered structures can also exist.

Thermodynamically, molecular self-assembly proceeds toward the state of lower entropy , and must therefore be compensated by a sufficient decrease of enthalpy due to intermolecular and molecule-surface interactions.

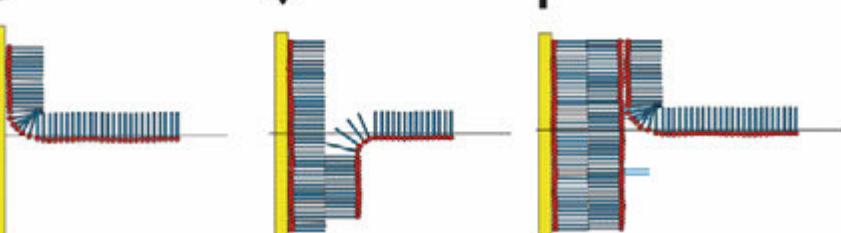
Self-organized monolayers (on solid surfaces)



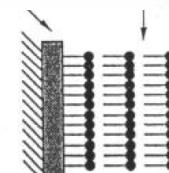
transfer of LB films on substrates



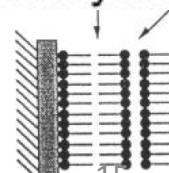
production of multilayers



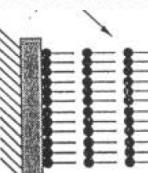
>1000 layers possible



X-type on a hydrophobic surface

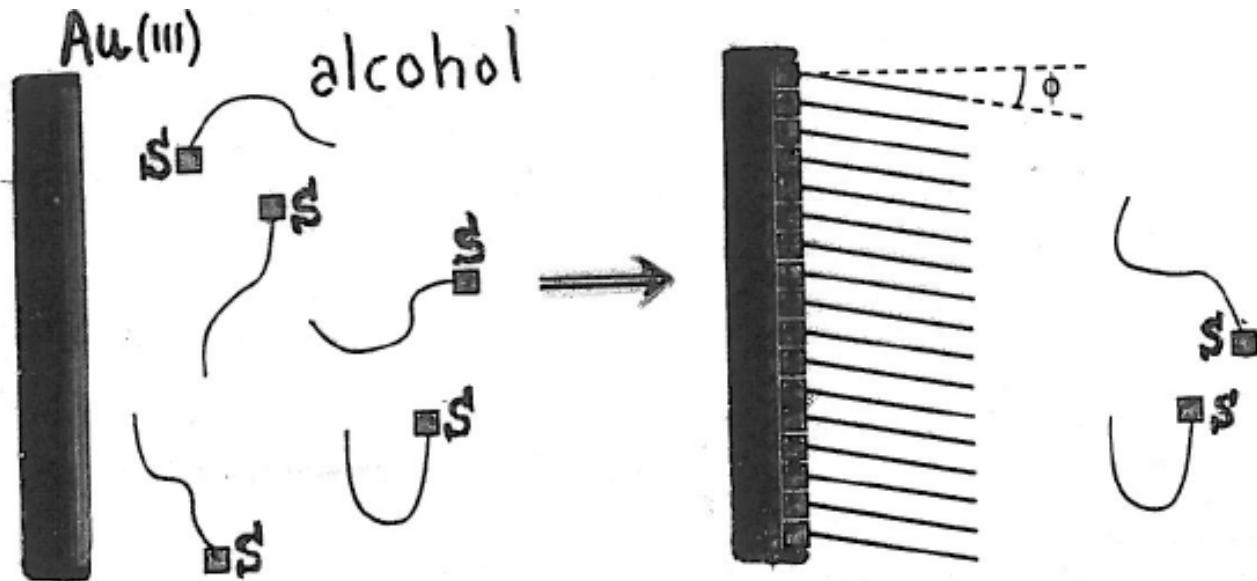


Y-type on a hydrophilic surface



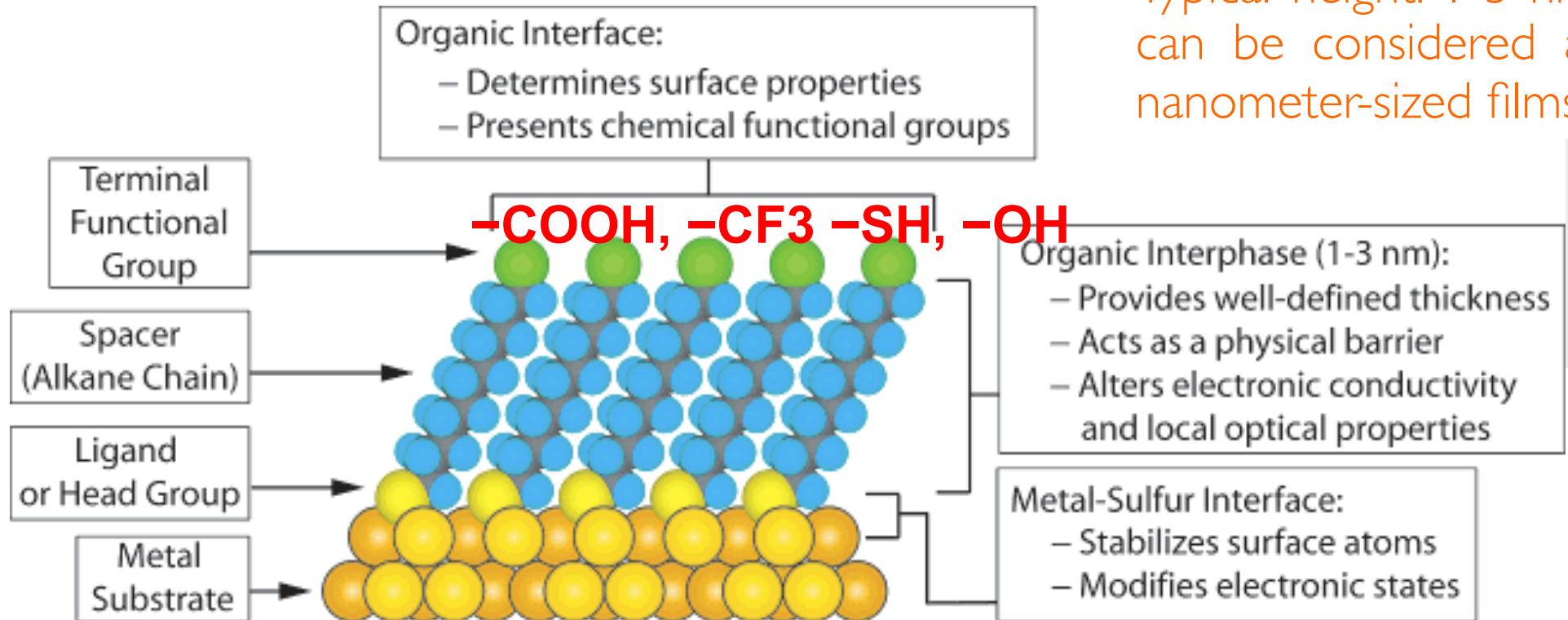
Z-type on a hydrophilic surface

Self-organized monolayers (on solid surfaces)



The simplest example of self-assembling is given by self-assembled monolayers (SAMs): SAMs are distinguished from surfactant layers by the fact that one end of the molecules is designed to have a favorable and specific interaction with the surface of interest. This results in the formation of a stable monolayer

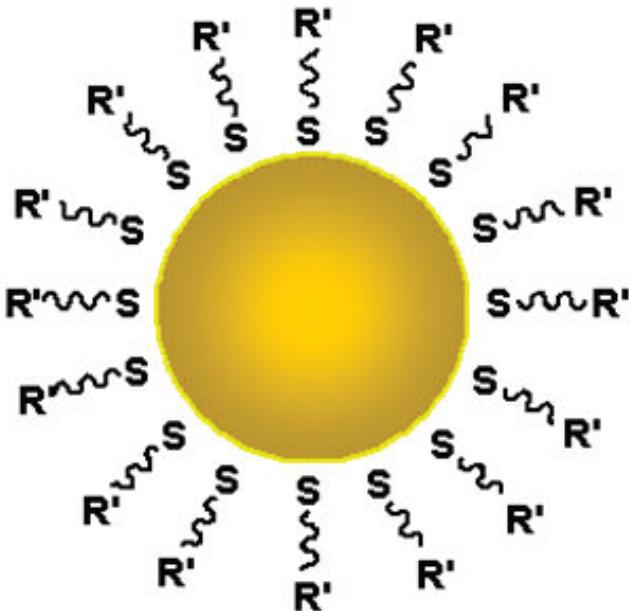
Alkanethiol SAMs



The high affinity of thiols for noble and coinage metal surfaces makes it possible to generate surfaces with useful and tunable chemical function.

Once molecules adsorbed on the surface, the formation of ordered and closely packed arrangement starts. It depends upon the intermolecular interactions, such as van der Waals, dipole or $\pi - \pi$ interactions.

The terminal group confers specific properties to the surface (hydrophobic/hydrophilic)

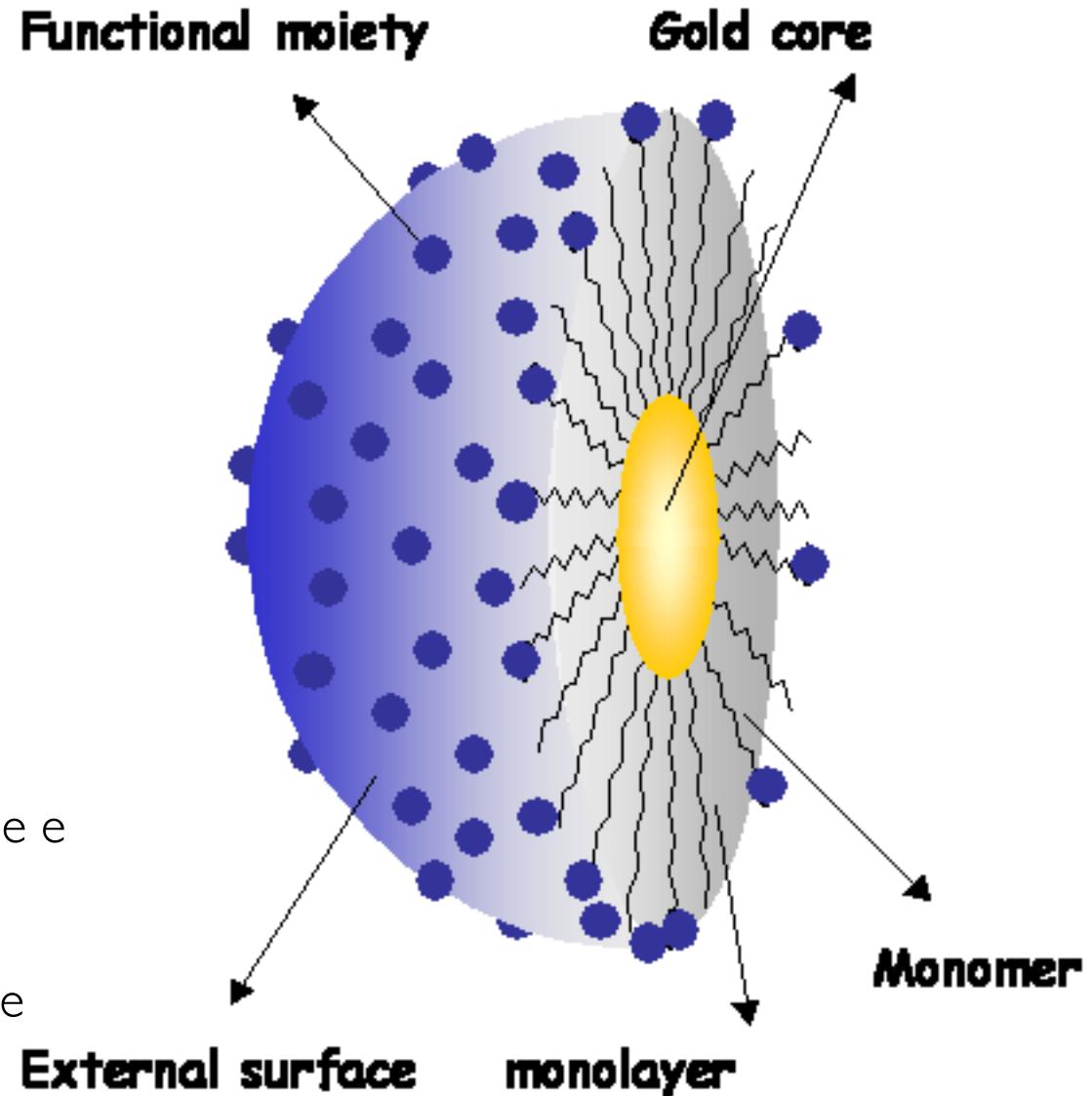


L'anima metallica:

la dimensione determina le proprietà ottiche e elettroniche del materiale

Il SAM:

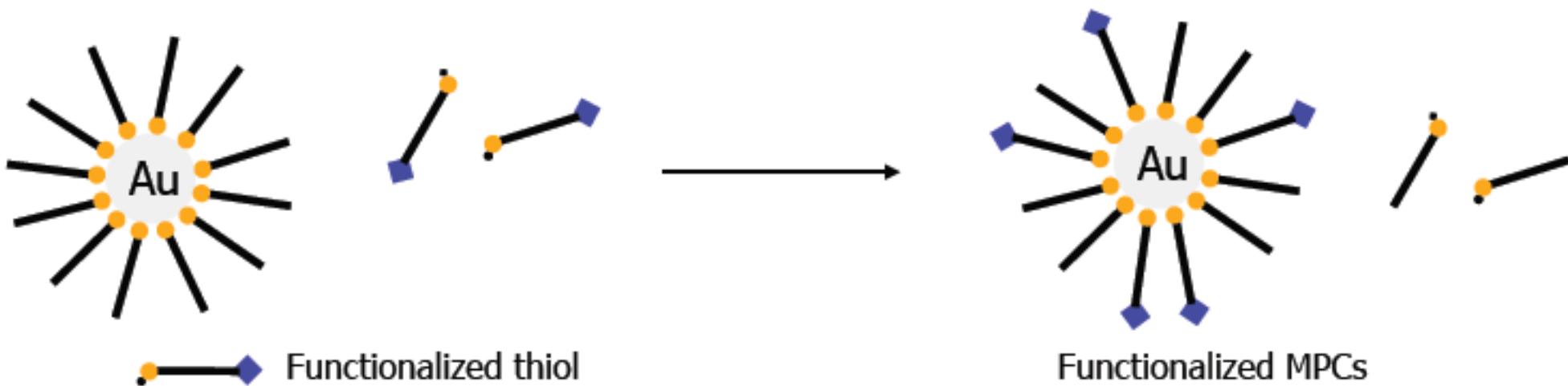
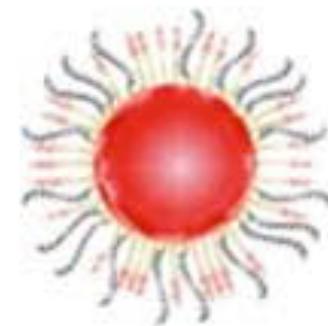
è responsabile della stabilità, della solubilità e delle interazioni delle NP con l'ambiente (funzionalità)



- synthesis using a mixture of thiols

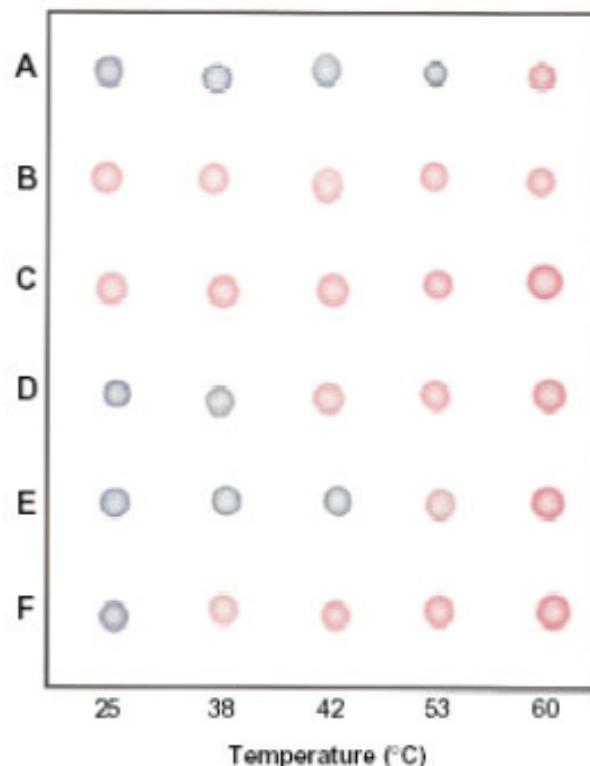
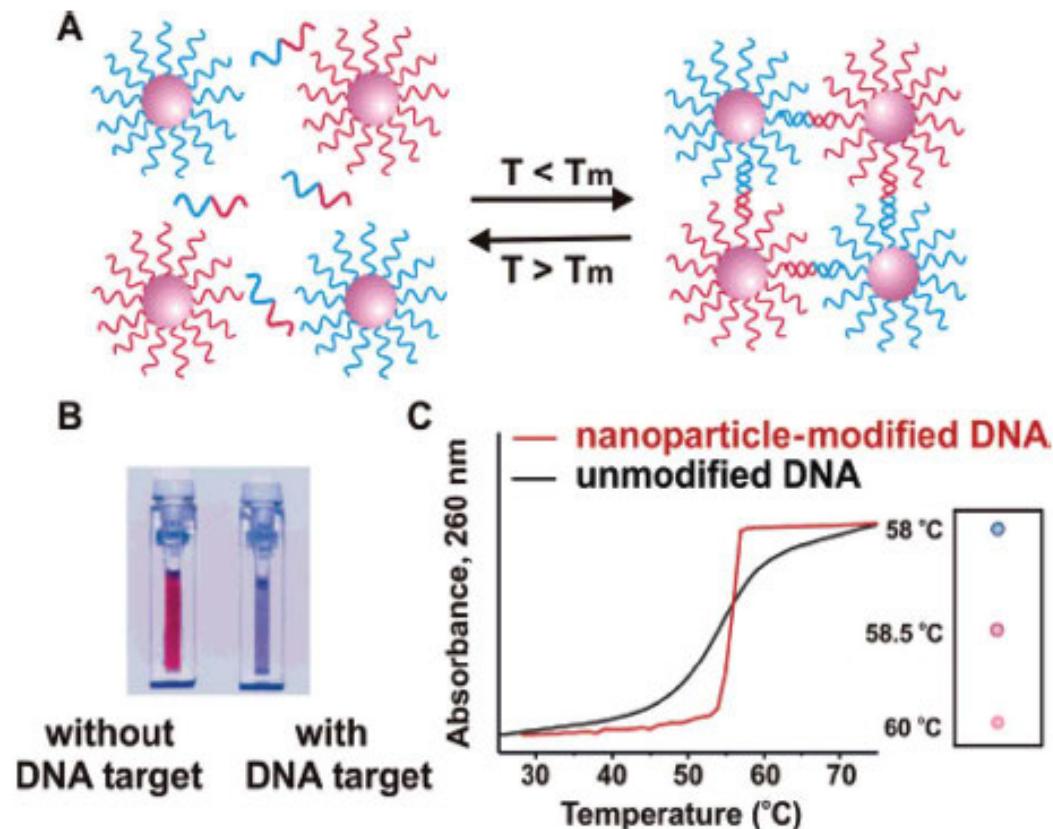
thiols should survive under the reaction conditions

- Ligand exchange



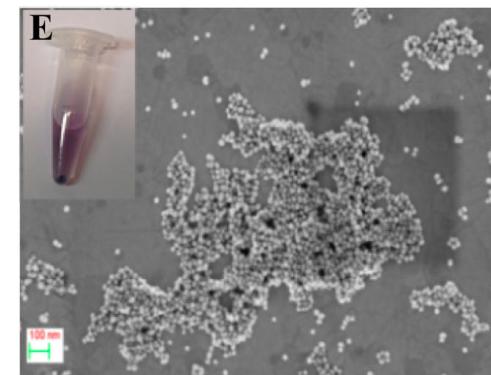
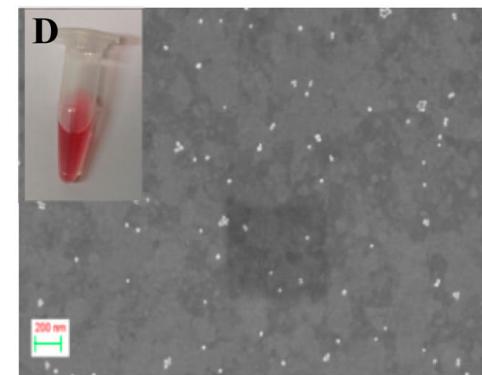
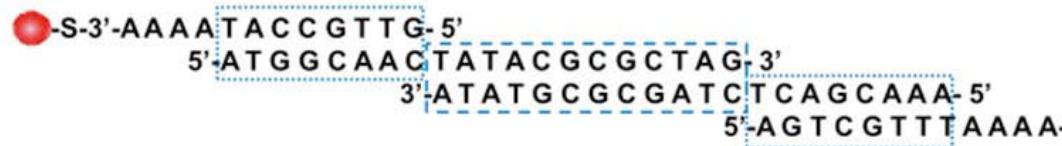
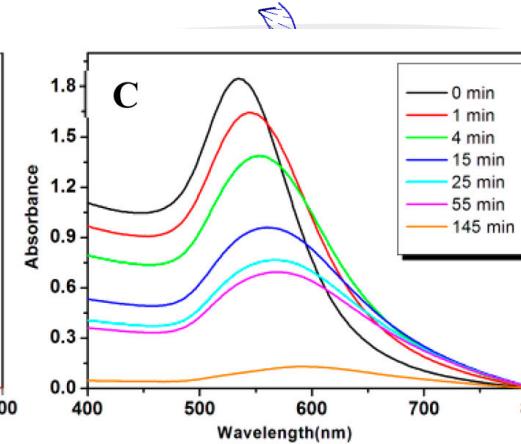
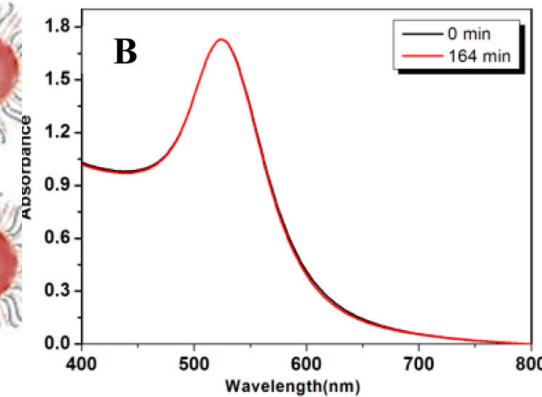
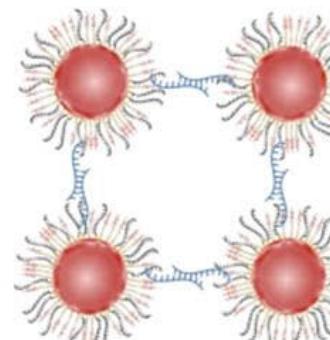
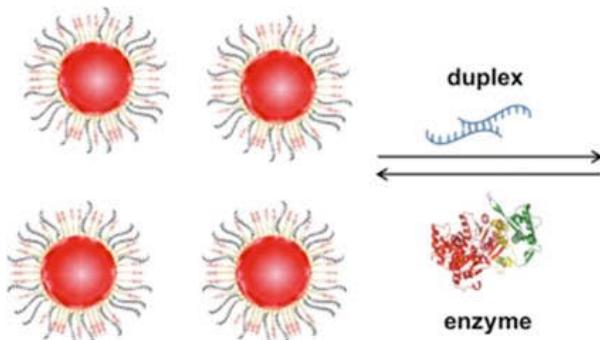
Hostetler, M. J.; Green, S. J.; Murray, R. W. *J. Am. Chem. Soc.*, 1996, 118, 4212 - 4213.

Gold nanoparticles: detection

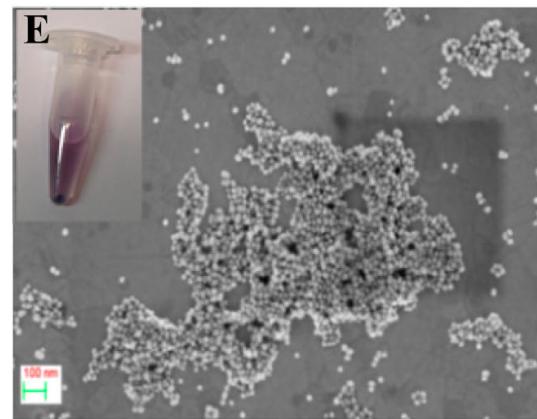
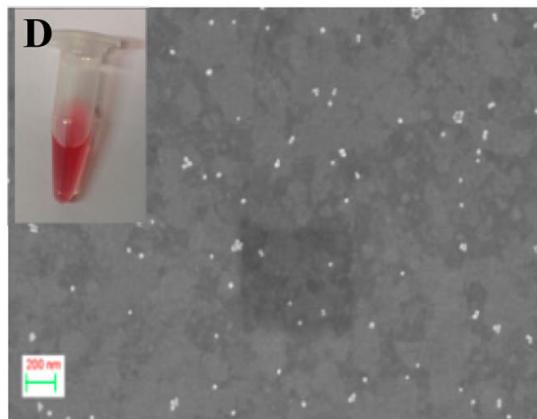
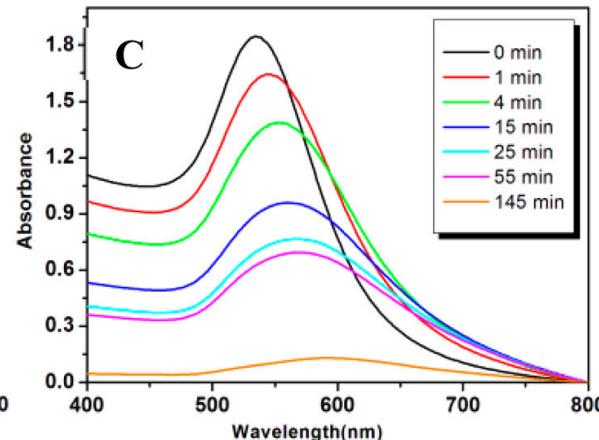
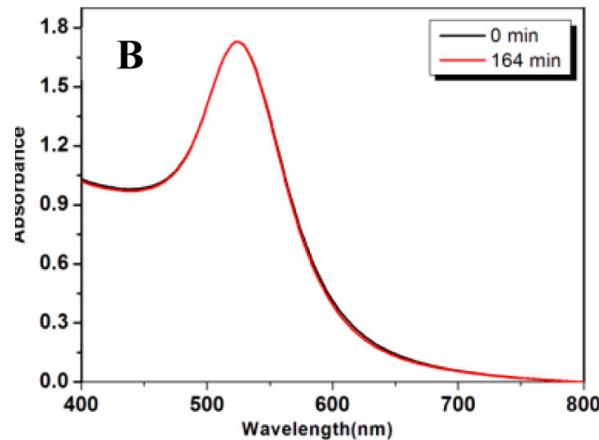


Selective polynucleotide detection for the target probes :
(A) complementary target; (B) no target; (C) complementary to one probe; (D) a 6-bp deletion; (E) a 1-bp mismatch; and (F) a 2-bp mismatch. Nanoparticle aggregates were prepared in a 600- μ l thin-walled Eppendorf tube by addition of 1 μ l of a 6.6 μ M oligonucleotide target to a mixture containing 50 μ l of each probe (0.06 μ M final target concentration). The mixture was frozen (5 min) in a bath of dry ice and isopropyl alcohol and allowed to warm to room temperature. Samples were then transferred to a temperature controlled water bath, and 3- μ l aliquots were removed at the indicated temperatures and spotted on a C₁₈ reverse phase plate.

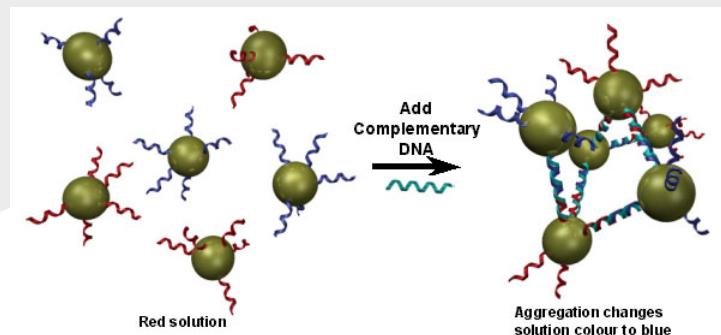
Reazioni enzimatiche: elicasi



Reazioni enzimatiche: elicasi

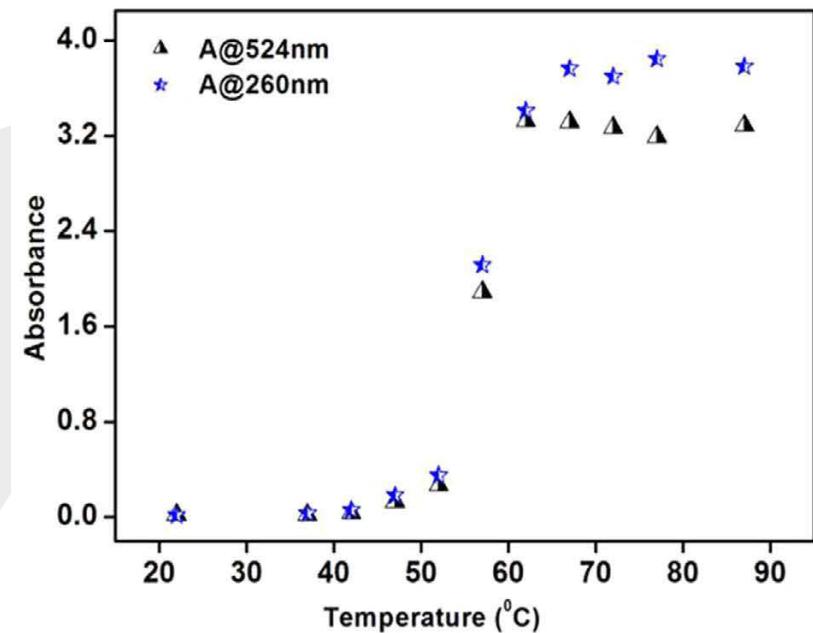
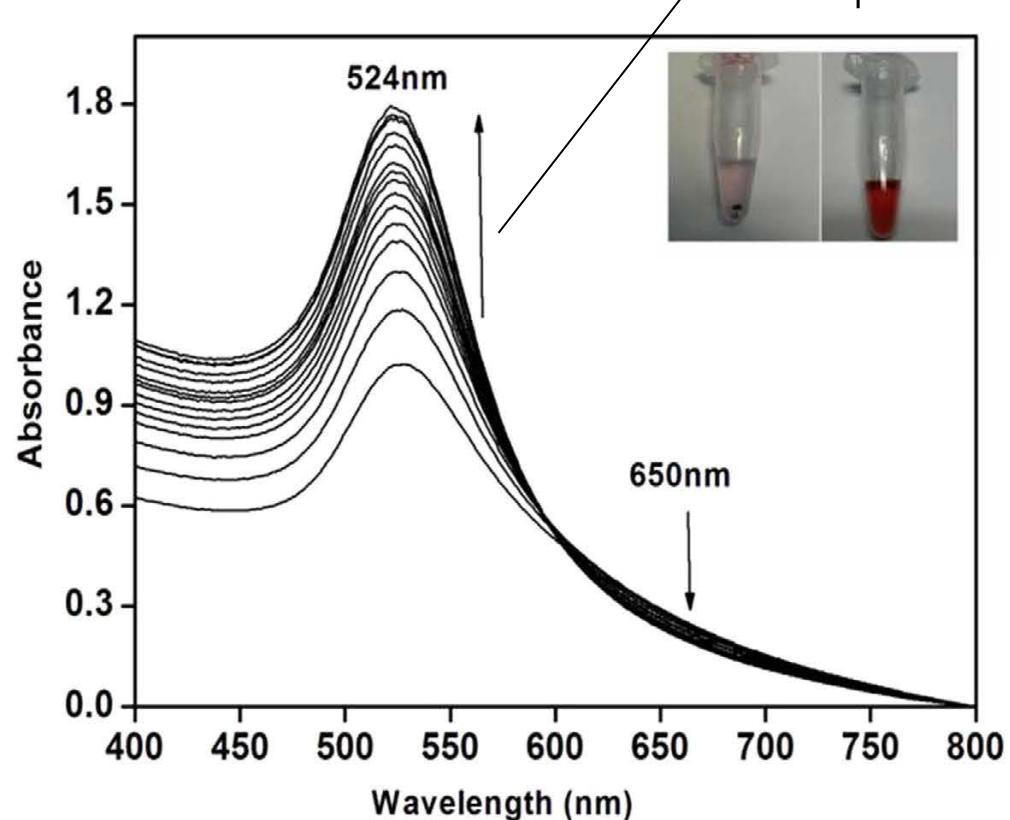


Aggregazione di NP



Esperimento di melting (senza enzima)

Disgregazione degli assemblati di NP: aumento del picco a 524 nm

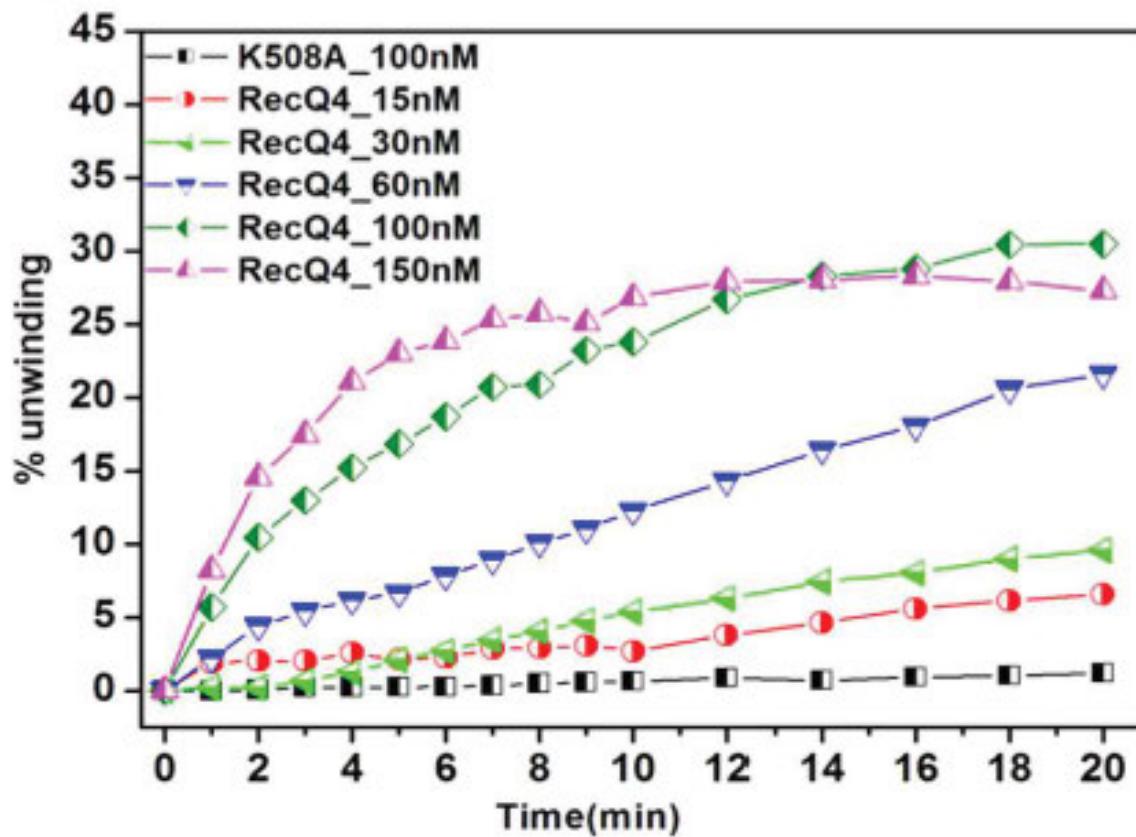


524 nm: AuNP 20 nm SPR peak
260 nm: free DNA SPR peak

Disgregazione degli assemblati di NP in presenza dell'enzima (e di ATP)

Aumento del picco a 524 nm rispetto a quello a 650 nm

a



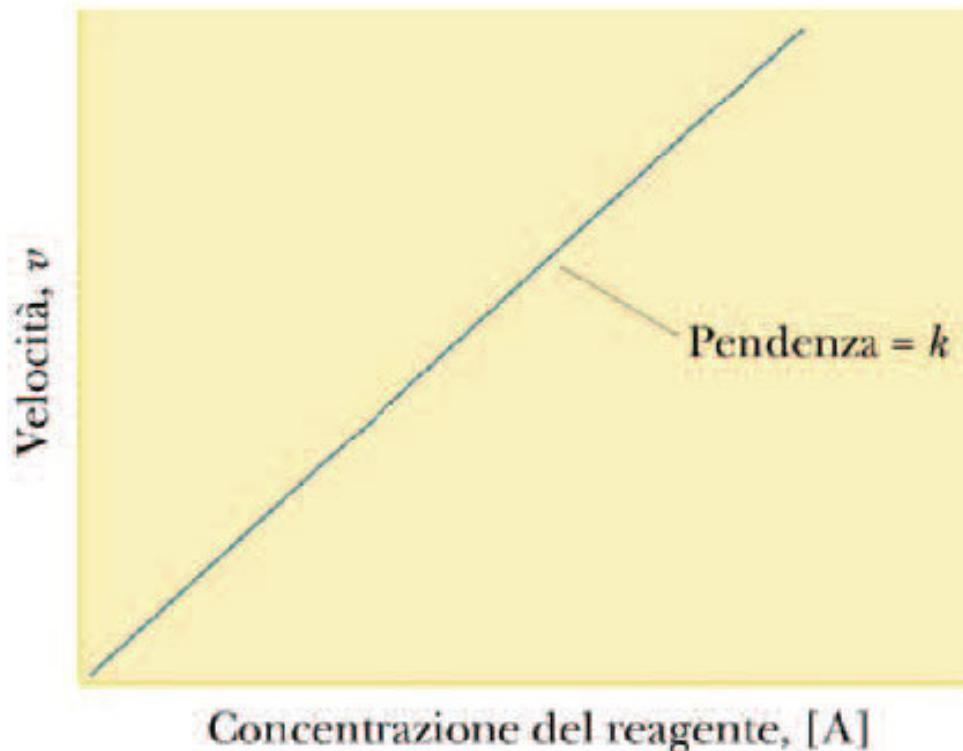
$$\% \text{ of unwinding} = \left(\frac{A@524\text{nm}:A@650\text{nm}}{A@524\text{nm}:A@650\text{nm for } 100\% \text{ unwound state}} \right) \times 100$$

Cinetica chimica

- reazione del **primo ordine**: $A \rightarrow P$

velocità della reazione $v = d[P]/dt = -d[A]/dt = k[A]$ $T = \text{cost}$

- $v (\text{Ms}^{-1}) \Rightarrow k = \text{costante di velocità (primo ordine)} (\text{s}^{-1})$



Cinetica chimica

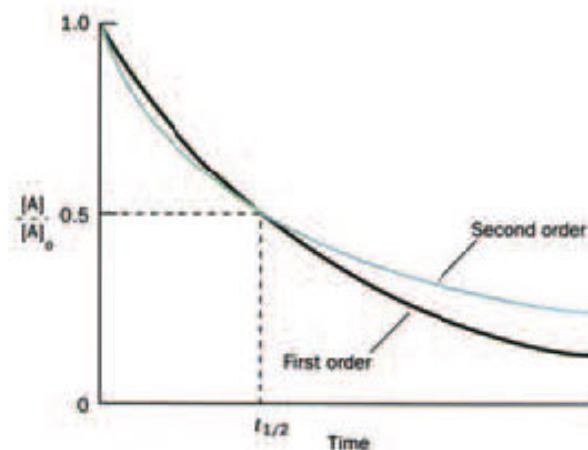
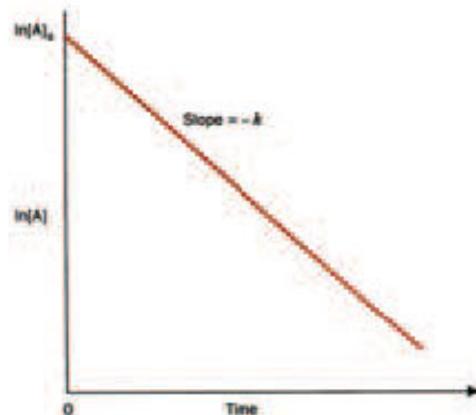
Equazione di velocità

- descrive il progredire di una reazione nel tempo

- reazione del **primo ordine**: $v = -d[A]/dt = k[A]$

$$d[A]/[A] = -k dt \Rightarrow d \ln[A] = -k dt \Rightarrow \int_{[A_0]}^{[A]} d \ln[A] = -k \int_0^t dt$$

$$\Rightarrow \ln[A] - \ln[A_0] = -kt \quad \text{eq. lineare} \quad \Rightarrow [A] = [A_0] \exp(-kt)$$



Cinetica enzimatica

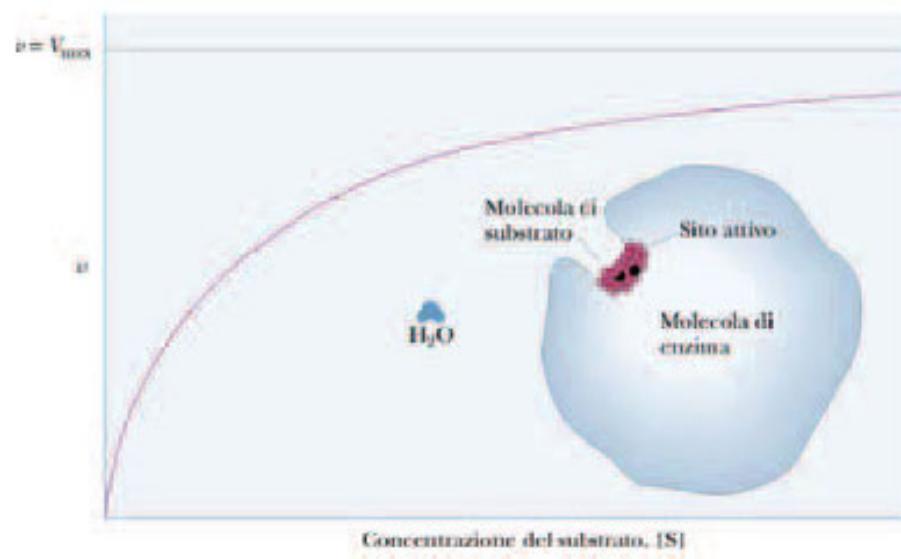
Reazione catalizzata da enzimi:

(reazioni ad 1 substrato)

curva di saturazione del substrato

- $[S]$ bassa $\Rightarrow v$ proporzionale ad $[S]$
- $[S]$ aumenta $\Rightarrow v$ non aumenta proporzionalmente ad $[S]$
- $[S]$ alta $\Rightarrow v$ indipendente da $[S]$ \Rightarrow **effetto di saturazione**

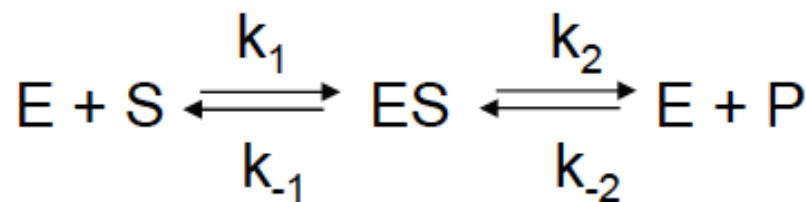
ogni molecola di enzima ha il sito di legame del substrato occupato da S



reazione del primo ordine non catalizzata

quindi quando $[S]$ è elevata la v diventa indipendente da $[S]$, cioè è di ordine zero rispetto al substrato

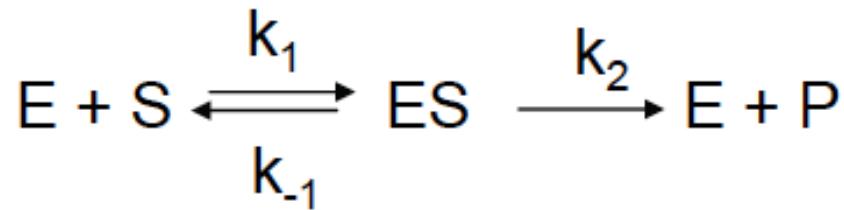
⇒ la reazione può essere pensata divisa in 2 reazioni elementari:



k_i = costanti di velocità per le reazioni

Assunzione 1)

- l'enzima E ed il substrato S si associano reversibilmente a formare il **complesso enzima-substrato ES (“complesso di Michaelis”)**
- il prodotto P si forma in un secondo momento, quando ES si rompe a dare E + P
- se $[S]$ è elevata, tutto l'enzima è nella forma ES la **seconda tappa limita la velocità di reazione**, che sarà insensibile ad aumenti di $[S]$



Assunzione 2): la seconda reazione sia irreversibile

⇒ misure di **velocità iniziale v_0**

- v_0 si ha fintanto che <10% del substrato è stato trasformato in prodotto (cioè prima che P si accumuli, visto che la velocità inversa è prop. a [P])

⇒ si minimizzano gli effetti delle reazioni reversibili (inibizione da parte del prodotto e progressiva inattivazione)



Elettra
Sincrotrone
Trieste





Elettra
Sincrotrone
Trieste



