

## **NANOBIOTECNOLOGIE – 4 CFU (32 ore)**

**-Nanosistemi e multivalenza: cooperatività**

**-Dimensioni e densità**

**-Funzionalizzazione**

**-Targeting**

**-Delivering**

**-Sensing**

**Esame scritto su 5 esercizi**

## Energie dei legami covalenti

C-O bond	81 kcal/mol	1.43 Å
C-C bond	86 kcal/mol	1.54 Å
C-H bond	103 kcal/mol	1.11 Å
C=C bond	143 kcal/mol	1.33 Å
C=O bond	165 kcal/mol	1.21 Å

Se confrontate con molte interazioni non covalenti sono caratterizzate da:

- Energie molto elevate
- Distanze piccole
- Forte dipendenza dall'orientazione

## Interazioni non covalenti

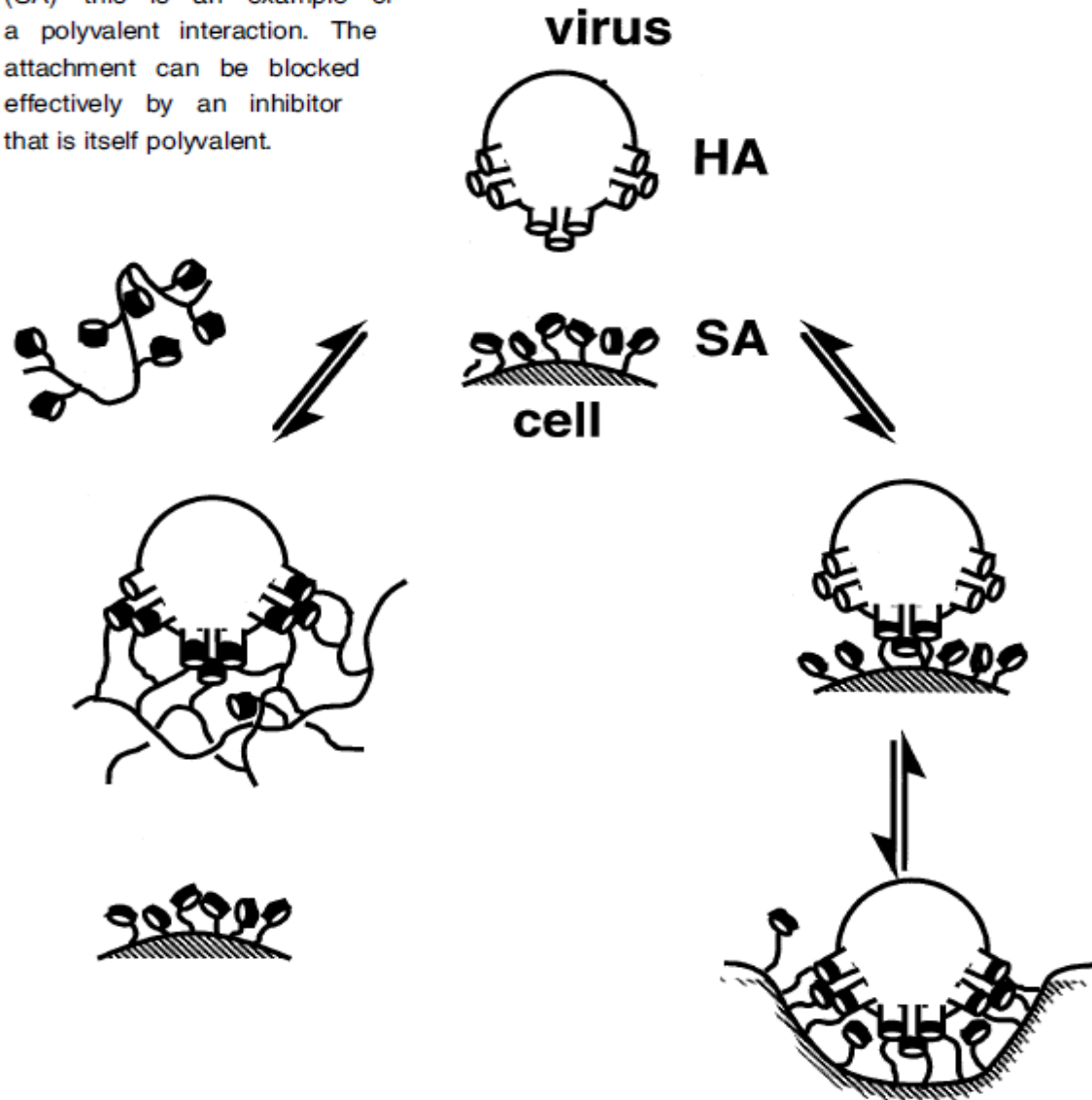
*(guidano la formazione delle interazioni supramolecolari)*

- idrofobiche <10 kcal/mol
- elettrostatiche ~5 kcal/mol
- legame ad idrogeno 2-10 kcal/mol
- $\pi$ - $\pi$  aromatiche 0-10 kcal/mol
- van der Waals 0.1-1 kcal/mol

**La forza intermolecolare che agisce tra due molecole è la somma di tutte le forze che esercitano l'una con l'altra.**

**Conclusione: molte interazioni devono operare in maniera concertata per portare ad energie simili a quelle di un legame covalente**

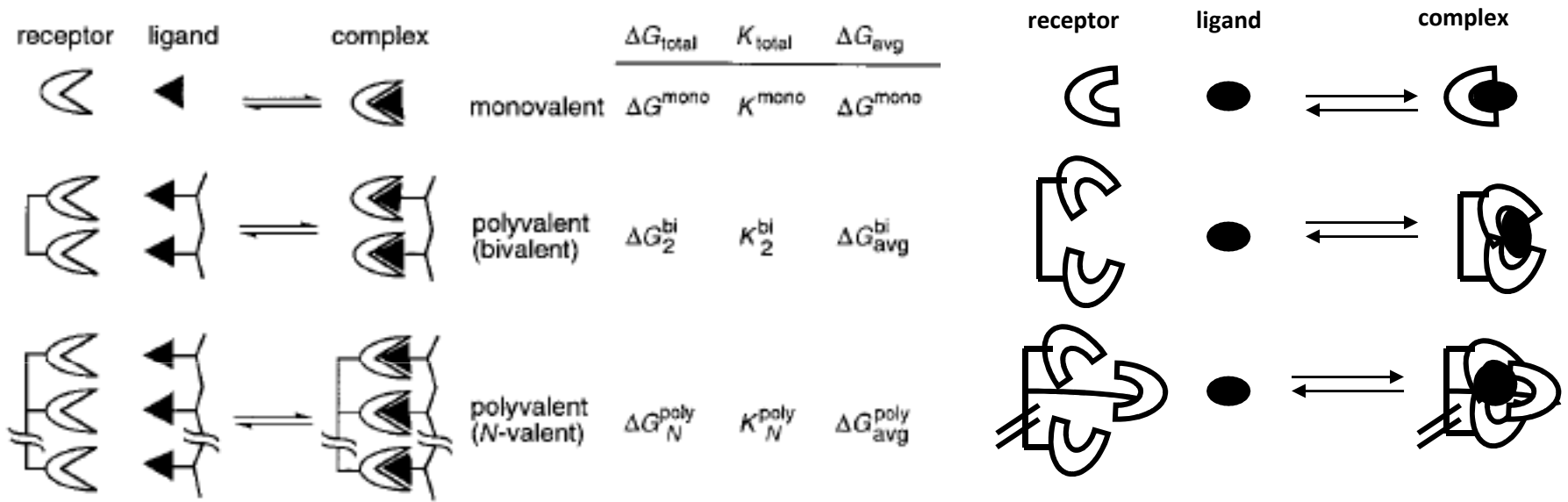
The attachment of an influenza virus to a target cell occurs through multiple simultaneous interactions between hemagglutinin (HA) and sialic acid (SA)—this is an example of a polyvalent interaction. The attachment can be blocked effectively by an inhibitor that is itself polyvalent.



**Dal punto di vista topologico un virus e la superficie di una cellula sono simili ad un nanosistema sintetico**

# Multiple interactions in binding: definitions

reference: *Angew. Chem. Int. Ed.* 1998, 37, 2754–2794



multiple binding sites:  
receptor interacts with  
a multivalent ligand

multiple binding sites:  
receptor interacts with  
a monovalent ligand

$$\Delta G_{avg}^{poly} = \Delta G_N^{poly} / N$$

$$\Delta G = -RT \ln(K)$$

$$K_N^{poly} = (K_{avg}^{poly})^N$$

$$\Delta G_{avg}^{poly} = \alpha \Delta G^{mono}$$

$$N \Delta G_{avg}^{poly} = \Delta G_N^{poly} = \alpha N \Delta G^{mono}$$

$$K_N^{poly} = (K_{avg}^{poly})^N = (K^{mono})^{\alpha N}$$

$$\alpha = \frac{\lg(K_N^{poly})}{\lg(K^{mono})^N}$$

$\alpha ?$

**$\alpha$ =degree of cooperativity**

**$\alpha > 1$ : positive cooperativity (synergistic)**

**$\alpha = 1$ : noncooperative (additive)**

**$\alpha < 1$ : negative cooperativity (interfering)**

**in all cases overall  
binding constants  
increase!**

→ when talking about cooperativity (in binding) we often consider this one as the typical situation.

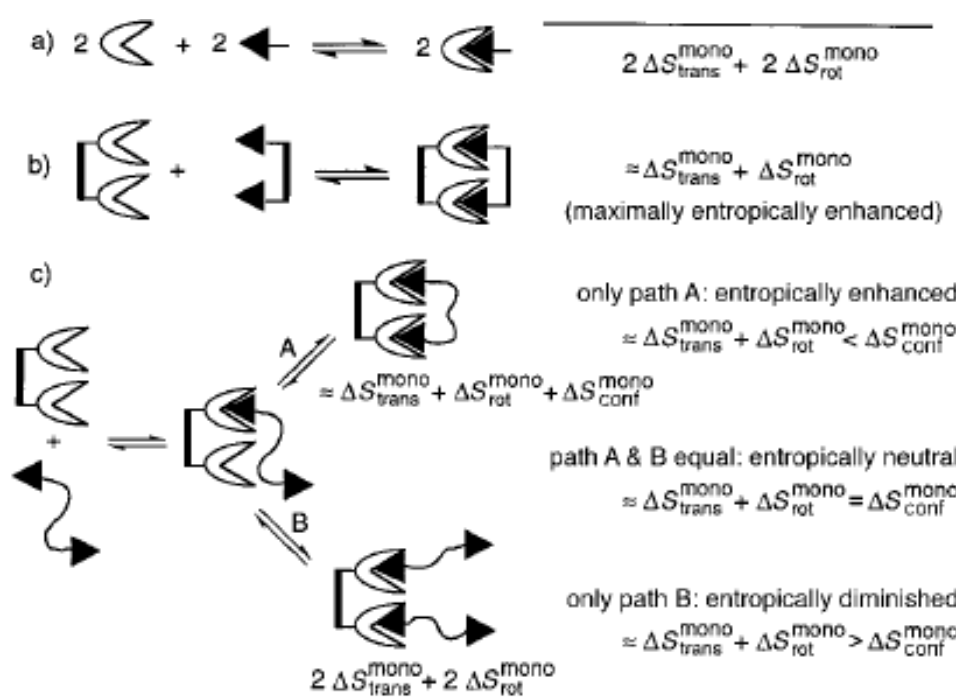
**On the contrary this is a rare situation!**

most of the available examples are characterized by  $\alpha < 1$

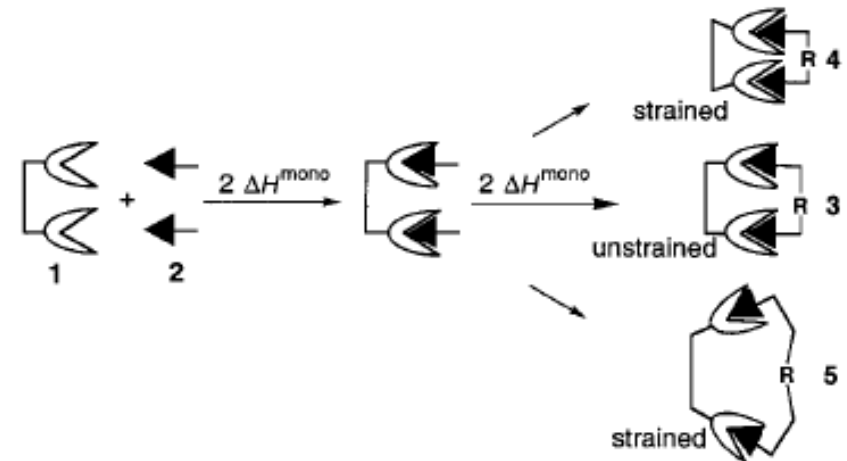
**Don't be fooled by the overall strength of binding which is always larger:**

$$\Delta G_N^{poly} = \Delta G^{mono} - RT \ln(\beta)$$

$$\beta = K_N^{poly} / K^{mono}$$



**Entropy**



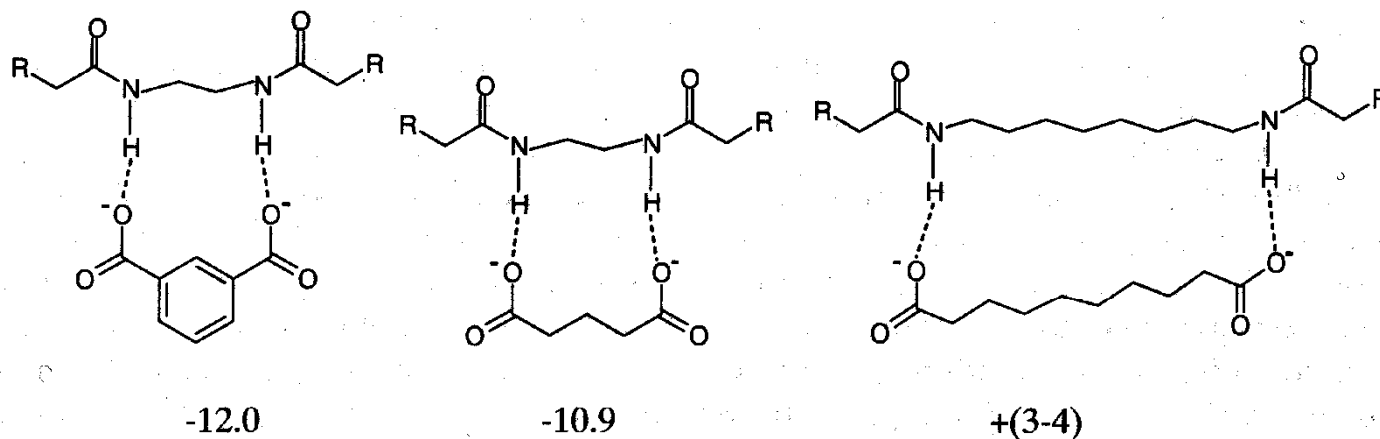
**Enthalpy**

Positive cooperativity is due to entropic and enthalpic contributions to binding.

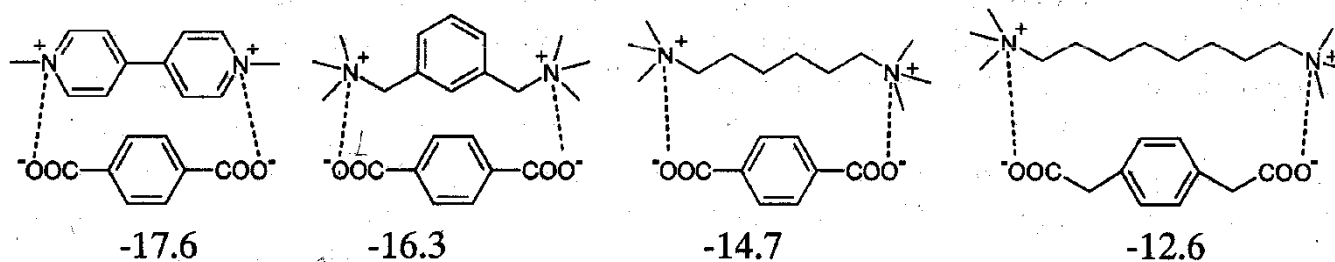
Entropy: loss of motion of the molecule, including internal rotation and vibrations (contribution already paid for in connecting together the recognition elements)

Enthalpy: secondary functional groups interactions, conformational changes, polarization of the interacting groups

## Il peso dell'entropia



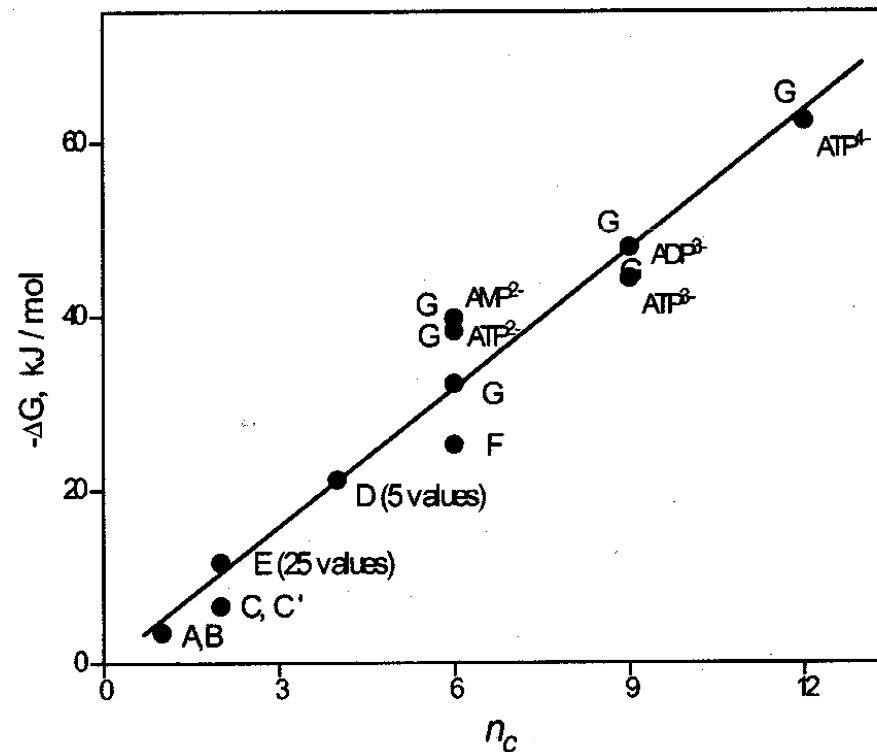
Ion pairs (in water at zero ionic strength)



Energie di associazione ( $\Delta G$  in  $\text{kJ mol}^{-1}$ ) tra molecole in seguito alla formazione di legami ad idrogeno o coppie ioniche in funzione del numero di legami singoli che separano i siti di interazione.

(vedi: *Angew. Chem. Int. Ed Eng.* 1998, 37, 826; *Chem. Eur. J.* 1999, 5, 1284)

Sperimentalmente si osserva che il costo entropico è molto maggiore quando si ha a che fare con interazioni covalenti che con interazioni non covalenti tra coppie ioniche (suggerimento: le interazioni tra coppie ioniche non comportano una totale “rigidificazione” del complesso). Nel caso di interazioni tra coppie ioniche si trova una relazione lineare tra  $-\Delta G$  e numero di interazioni



$$-\Delta G_{\text{mono}} = 5 \text{ kJ mol}^{-1}$$

è un esempio di sistema **non cooperativo** ( $\alpha=1$ )

Energie libere di complessazione ( $-\Delta G$  in  $\text{kJ mol}^{-1}$ ) in funzione del numero di interazioni tra coppie ioniche ( $n$ ) misurate in acqua.

noncooperative

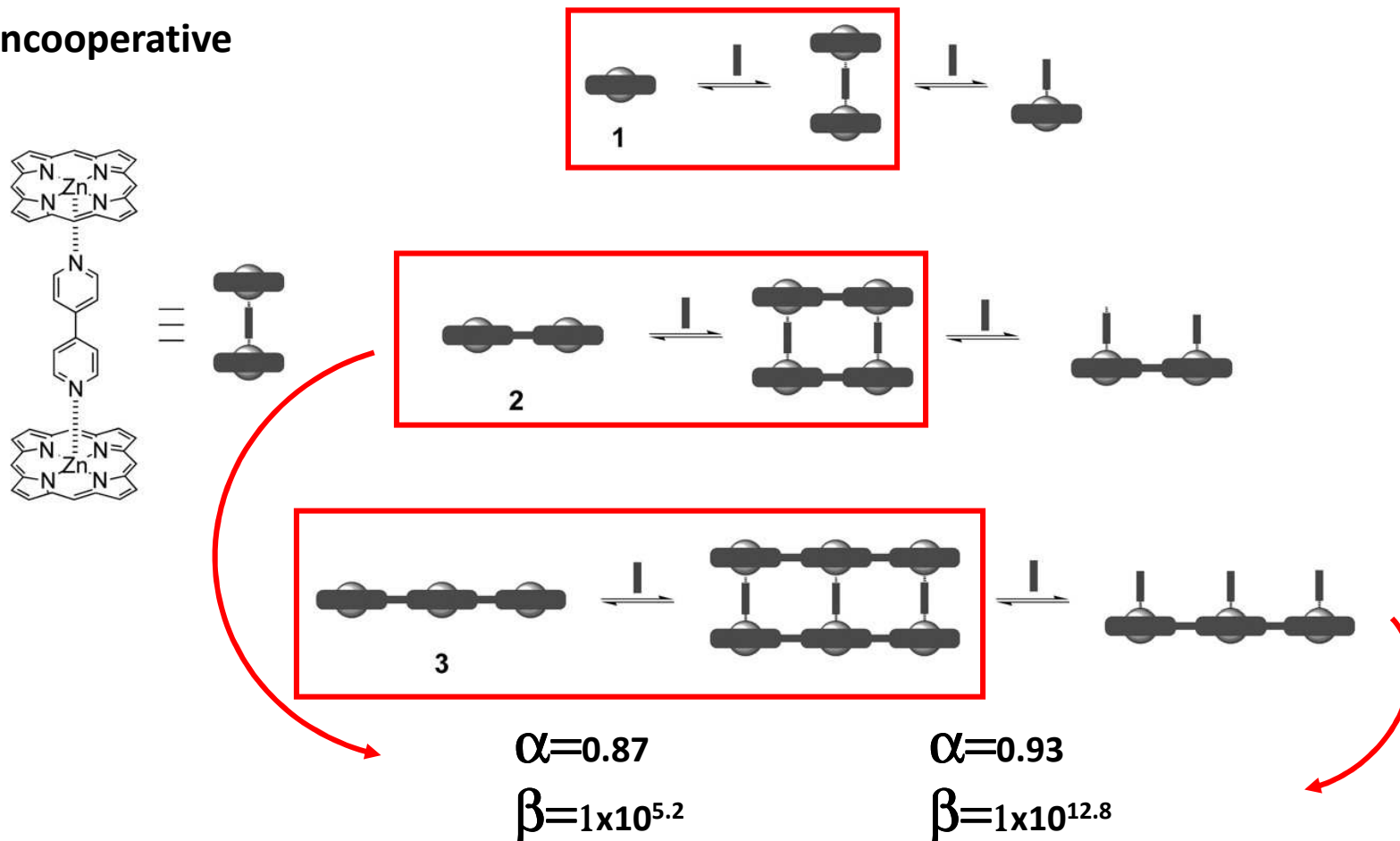
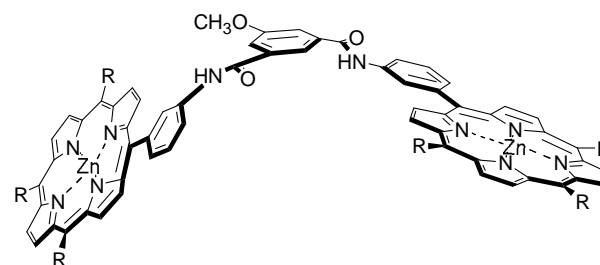
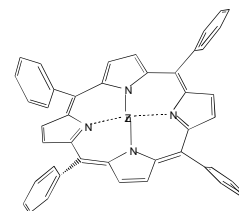
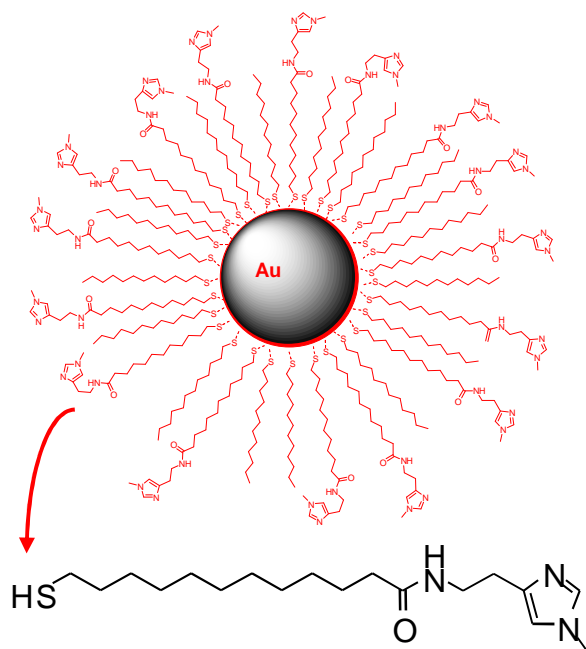


Table 1. Stability constants of the porphyrin ladder and enthalpy per interaction

Porphyrin	$K_{Mn}^{\dagger} M^{-1}$	$\log K_{open}^{\dagger}$	$\log K_{ladder}^{\dagger}$	$\Delta G_{ladder}^{\dagger} kJ \cdot mol^{-1}$	$\Delta H/interaction^{\dagger} kJ \cdot mol^{-1}$
1a	1,650	$3.6 \pm 0.1$	$6.3 \pm 0.1$	$-35.2 \pm 0.6$	$-33.4 \pm 3.3$
1b	3,750	$4.1 \pm 0.1$	$7.1 \pm 0.3$	$-39.8 \pm 1.7$	$-36.2 \pm 1.2$
2	2,120	$7.5 \pm 0.1$	$12.3 \pm 0.1$	$-68.8 \pm 0.8$	$-34.0 \pm 3.1$
3	2,620	$12.1 \pm 0.4$	$19.9 \pm 0.6$	$-111.2 \pm 3.4$	$-35.9 \pm 3.1$

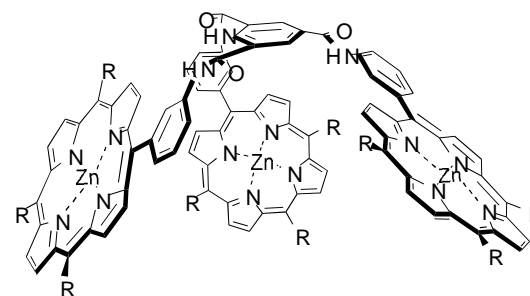
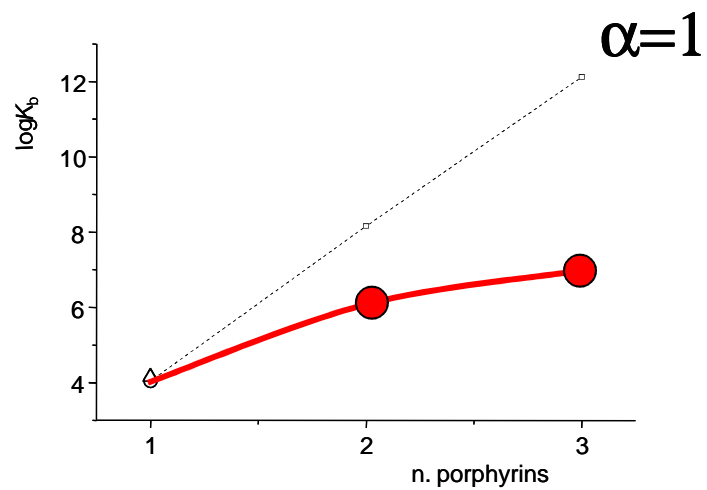
Errors are quoted at 95% of confidence.

## negative cooperativity



$$\alpha=0.75$$

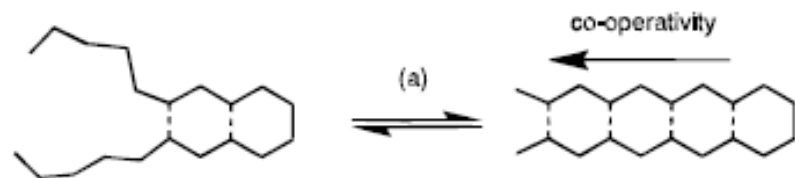
$$\beta=90$$



$$\alpha=0.58$$

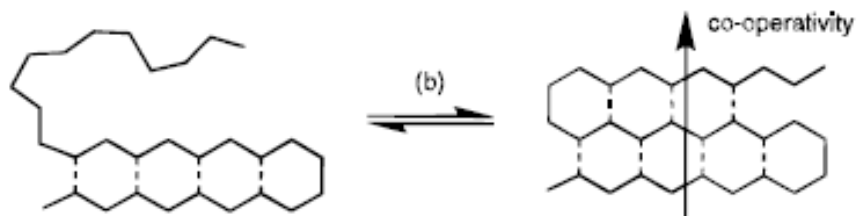
$$\beta=1000$$

2 $\beta$



K=0.42

3 $\beta$

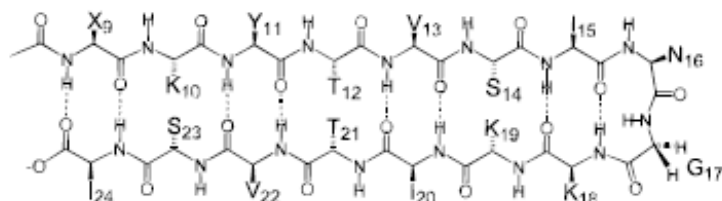


K=1

$\alpha=0.38$

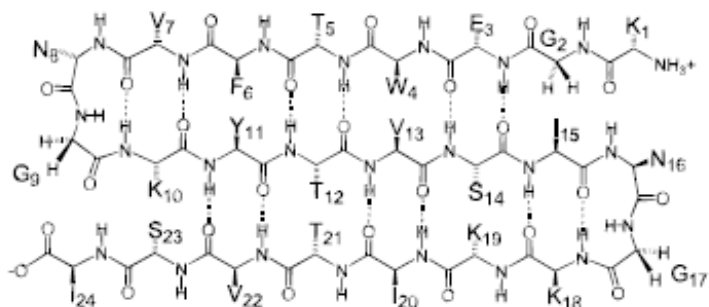
$\beta=2.4$

(a) X = G or K

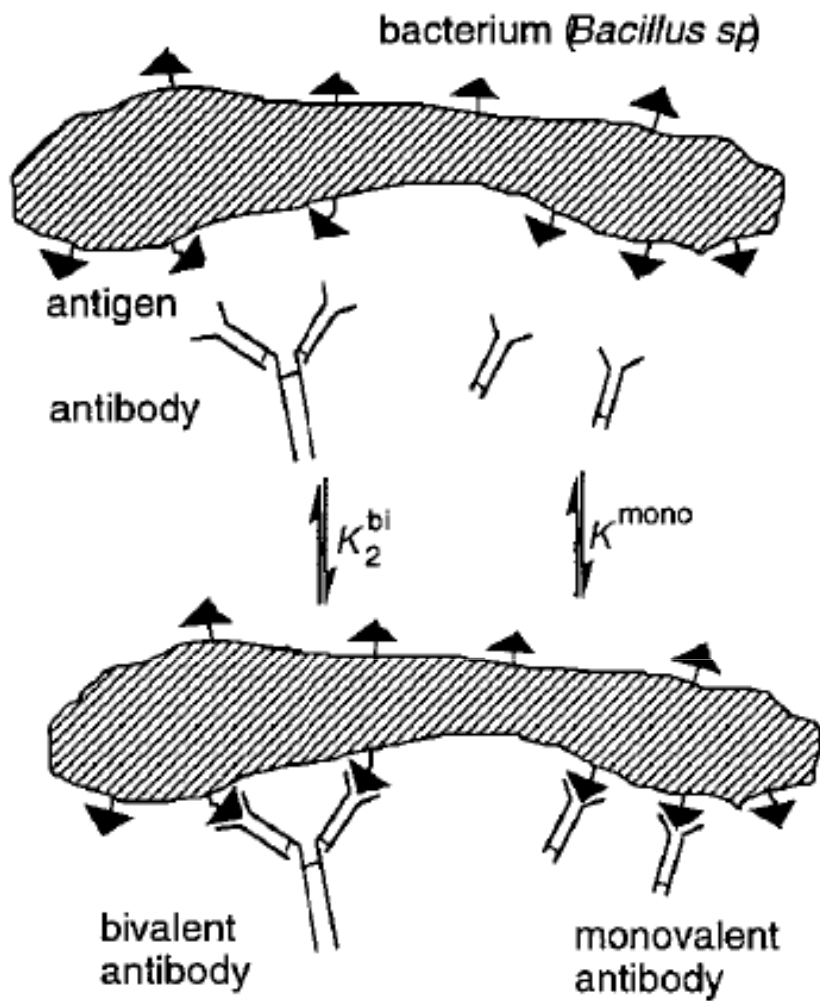


**negative cooperativity**

(b)



JACS 2000, 122, 8350-8356



Dato sperimentale:

$$K_2^{bi} = 30 K^{mono}$$

$$K_{avg}^{bi} = (30 K^{mono})^{1/2} < K^{mono}$$

$$\alpha < 1$$

$$\beta = 30$$

Figure 8. The binding of a bivalent antibody to ligands packed on the surface of a bacterium is negatively cooperative.<sup>[270]</sup>

Oligosaccharide	$K$ for complex of lectin/Gal-oligosaccharide ( $M^{-1}$ )
Gal( $\beta$ 1)OMe	$K^{\text{mono}} = 7 \times 10^4$
Gal( $\beta$ 1,4)GlcNAc( $\beta$ 1,2) Gal( $\beta$ 1,4)GlcNAc( $\beta$ 1,4) <span style="margin-left: 20px;">} Man</span>	$K_2^{\text{bi}} = 3 \times 10^7$
Gal( $\beta$ 1,4)GlcNAc( $\beta$ 1,2)Man( $\alpha$ 1,6) Gal( $\beta$ 1,4)GlcNAc( $\beta$ 1,2)Man( $\alpha$ 1,3) <span style="margin-left: 20px;">} Man</span> Gal( $\beta$ 1,4)GlcNAc( $\beta$ 1,4)	$K_3^{\text{tri}} = 2 \times 10^8$

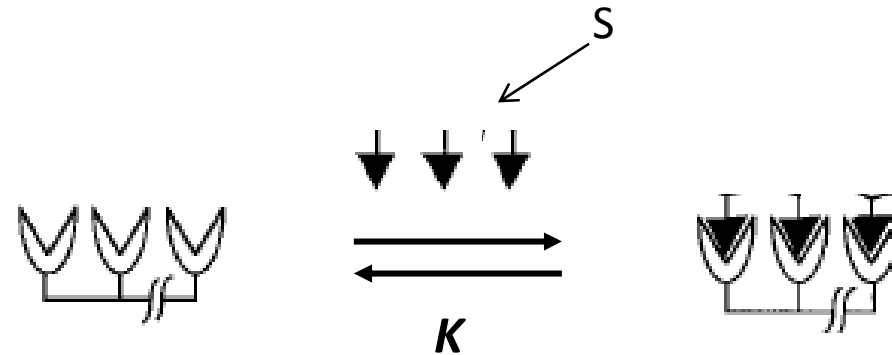
Figure 9. Binding of mono-, bi-, and trivalent Gal-terminated oligosaccharides to C-type mammalian hepatic lectins.<sup>[201]</sup>

$$K_2^{\text{bi}} < (K^{\text{mono}})^2 \quad \beta=420$$

$$K_3^{\text{tri}} < (K^{\text{mono}})^3 \quad \beta=2800$$

$$\alpha < 1$$

**Not considered:**



Typically “cooperativity” in these multiple interactions is due to conformational changes of the different sites of the polyvalent receptor (allosterism).

They are described by the Hill equation:

$$\log \frac{Y}{1-Y} = h \log[S] - \log K$$

Y = degree of saturation; h =Hill constant ( $h \leq$  number of sites;  $h > 1$  positive cooperativity;  $h = 1$  no cooperativity;  $h < 1$  negative cooperativity)

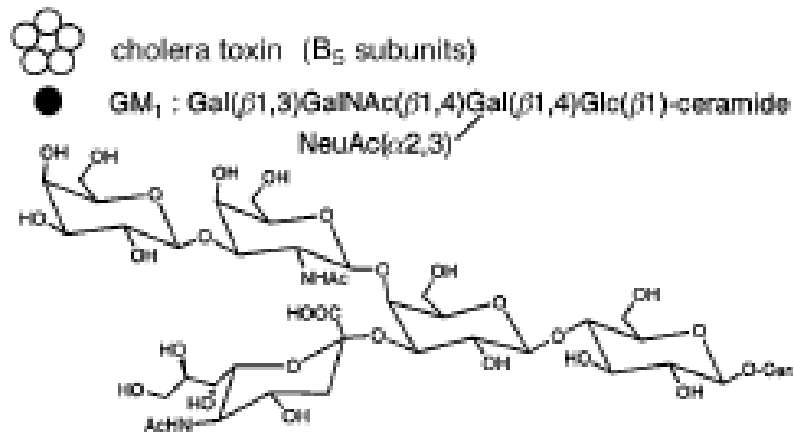










Figure 7. The binding of five molecules of GM<sub>1</sub> oligosaccharide to pentavalent cholera toxin occurs with enthalpically enhanced binding; that is, the binding of one GM<sub>1</sub> oligosaccharide increases the favorable enthalpy of binding of the next GM<sub>1</sub> oligosaccharide.<sup>1290</sup> Since the individual molecules of GM<sub>1</sub> oligosaccharide translate and rotate independently, the binding in this system is entropically neutral. *I* = number of ligands, *j* = number of contacts between GM<sub>1</sub> units, *q* = interaction energy (enthalpy) of two GM<sub>1</sub> units in contact.

State	No. of ligands ( <i>I</i> )	Configuration	$I\Delta G - j\Delta q$
1	0		0
2	1		$\Delta G$
3	2		$2\Delta G$
4	2		$2\Delta G - \Delta q$
5	3		$3\Delta G - \Delta q$
6	3		$3\Delta G - 2\Delta q$
7	4		$4\Delta G - 3\Delta q$
8	5		$5\Delta G - 5\Delta q$

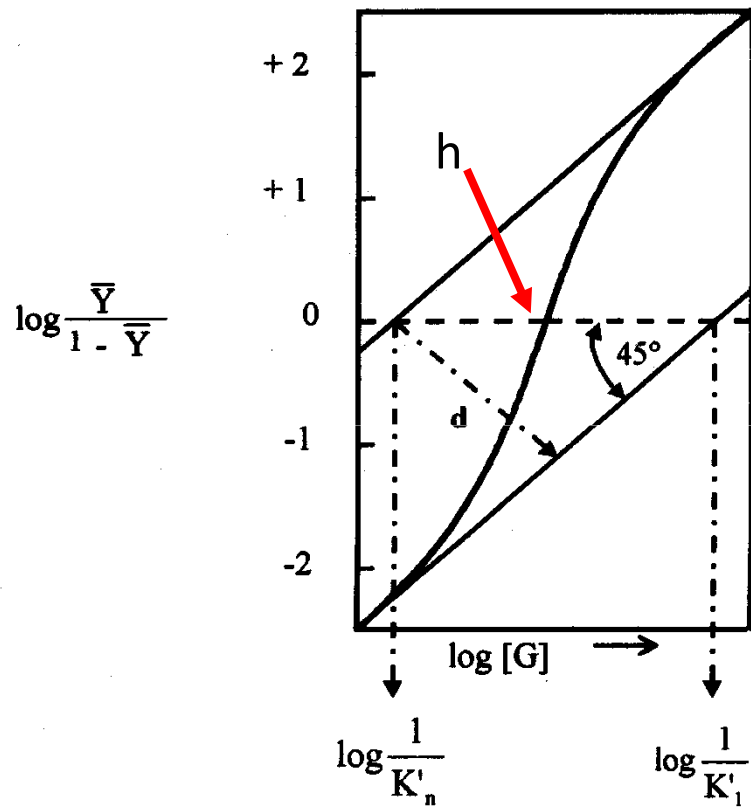
(GM1 = porzione saccaridica del ganglioside GM1)

**Un possibile esempio di cooperatività positiva ( $\alpha > 1$ )**

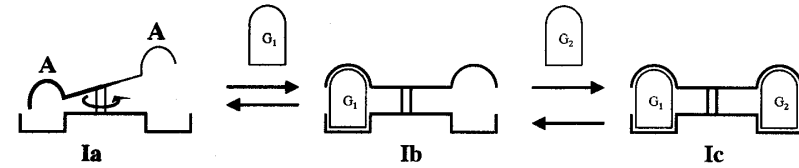
## Grafico di Hill

$$\log \frac{Y}{1-Y} = h \log[S] - \log K$$

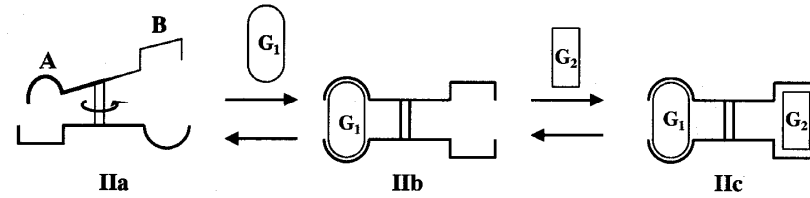
G=S



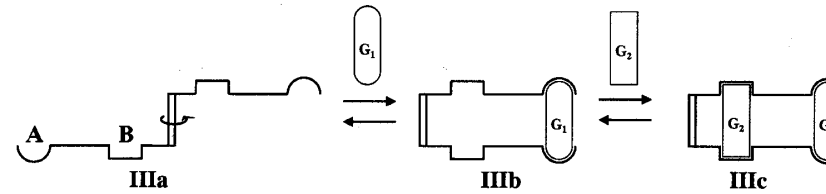
**Esempi di interazioni  
di tipo allosterico**



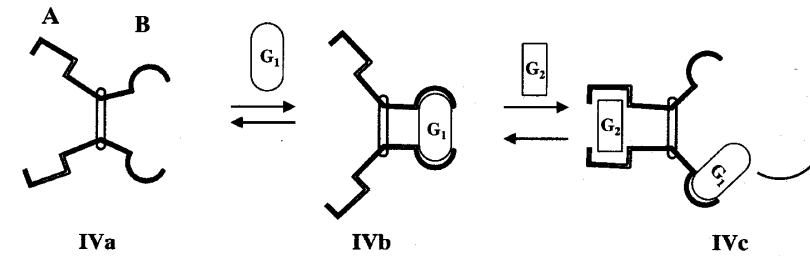
**Homotropic, positive cooperativity**



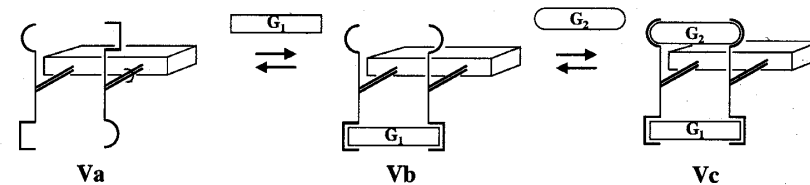
**Heterotropic, positive cooperativity**



**Heterotropic, positive cooperativity**



**Heterotropic, negative cooperativity**



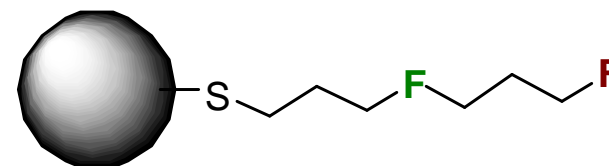
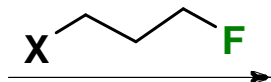
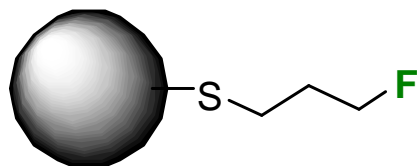
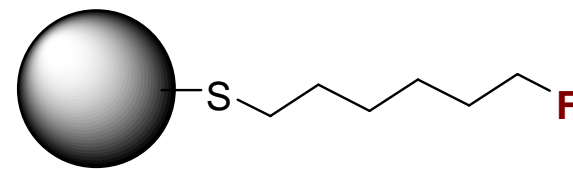
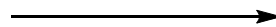
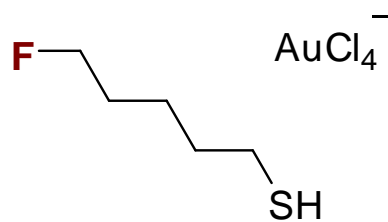
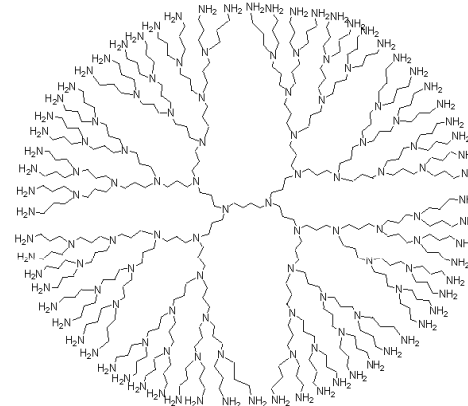
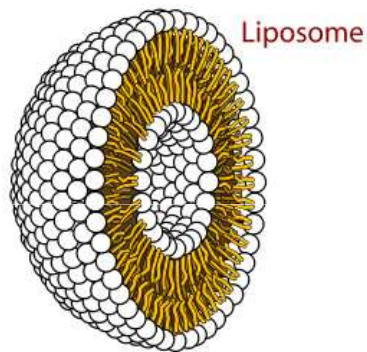
**Homotropic, positive cooperativity**

# Coniugazione di una biomolecola ad un nanosistema

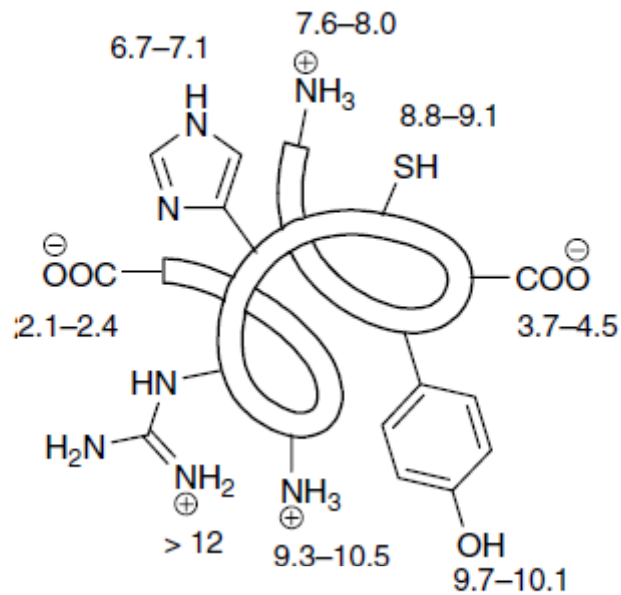
## Nanosistema

Polimero organico, dendrimero,  
lipide: gruppi funzionali già presenti  
nei singoli monomeri

Cluster inorganico: richiede  
funzionalizzazione o in fase di sintesi  
o successivamente

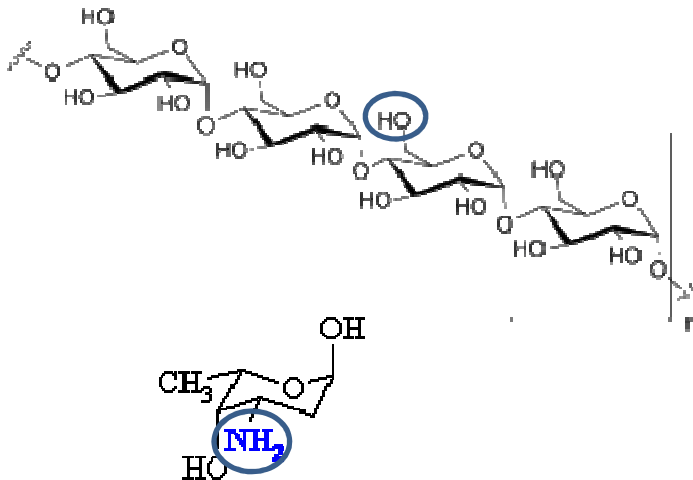


## Coniugazione di una biomolecola ad un nanosistema

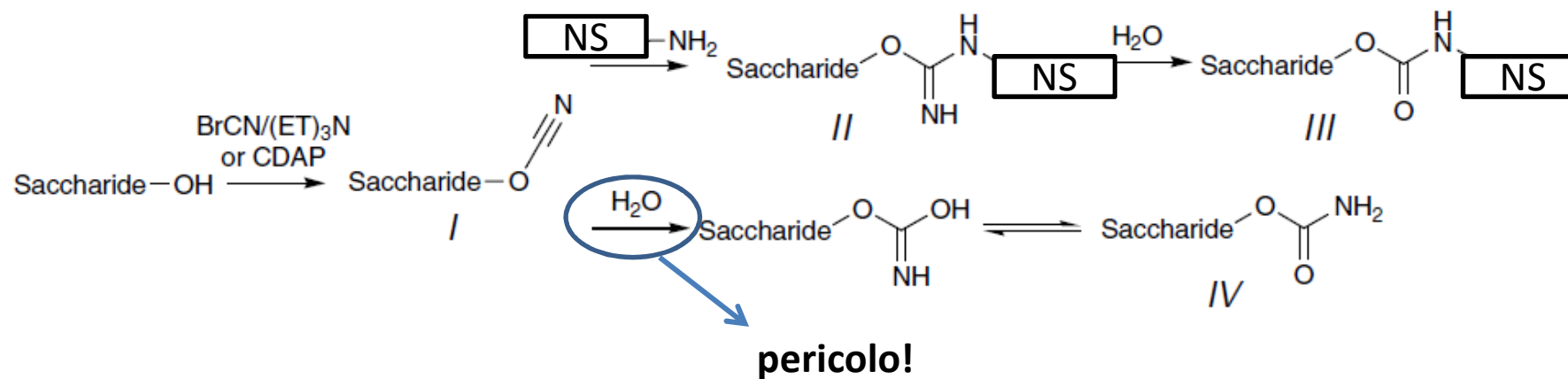


Gruppi funzionali presenti in una proteina o in un oligosaccaride: ci consentono l'ancoraggio al nanosistema ma la chimica necessaria può non essere banale.

I gruppi funzionali che servono per la coniugazione dipendono dalle condizioni operative

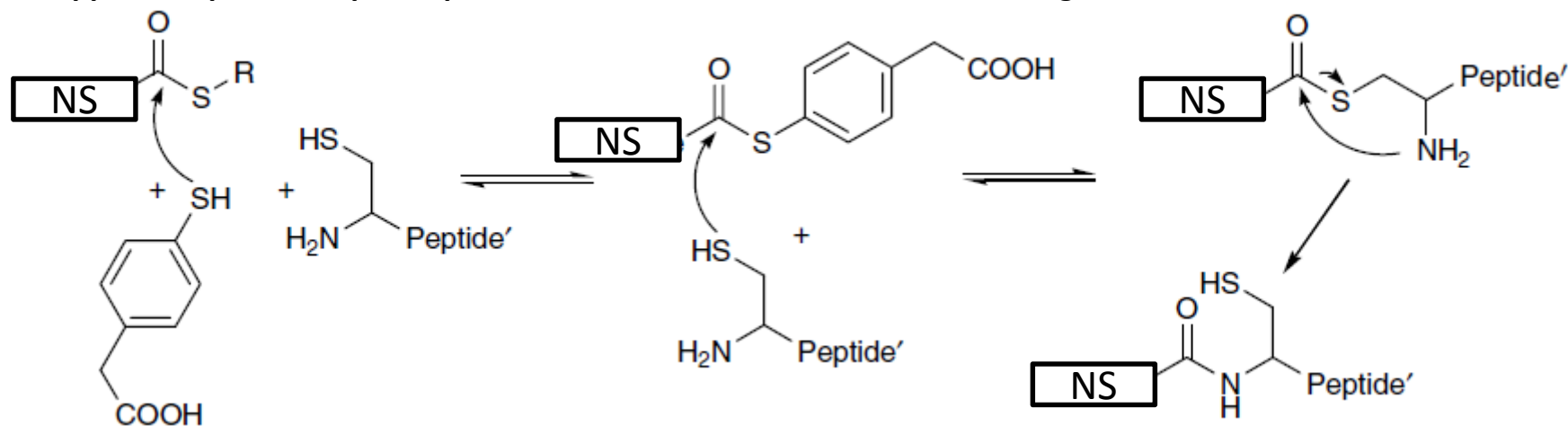


## Coniugazione di un oligosaccharide

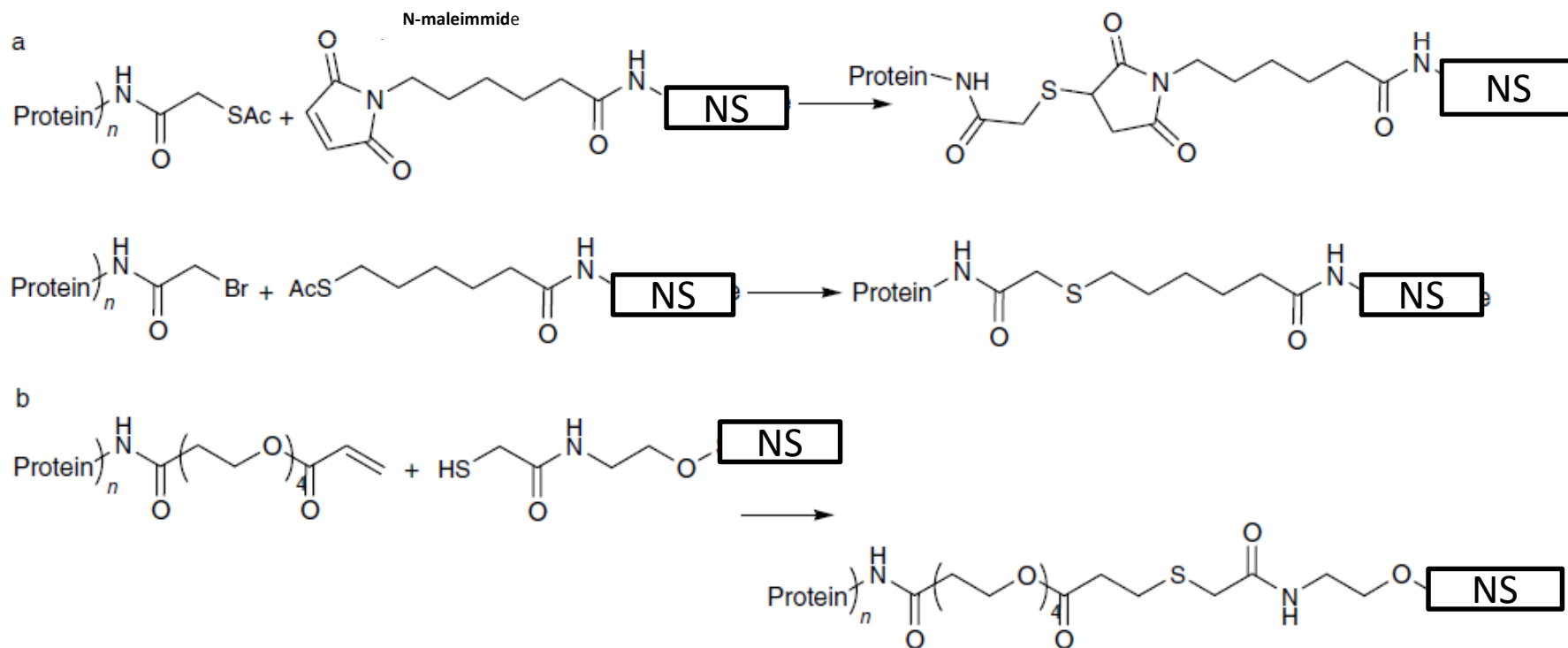


In generale l'ambiente nel quale si conduce la coniugazione non deve presentare gruppi funzionali competitivi con la reazione di coniugazione stessa!

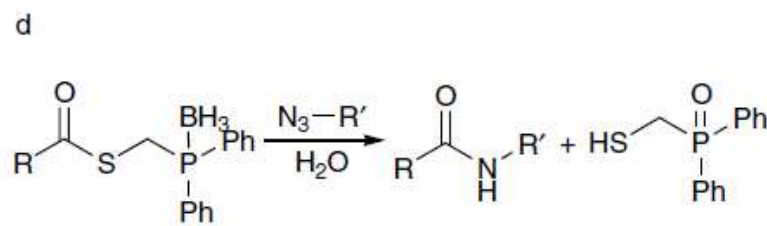
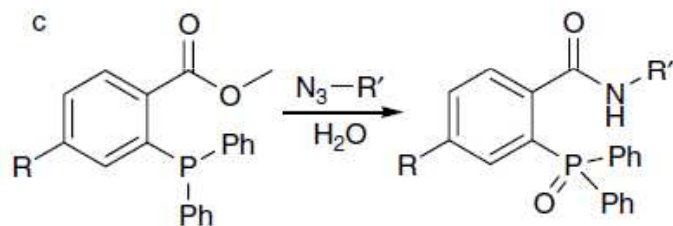
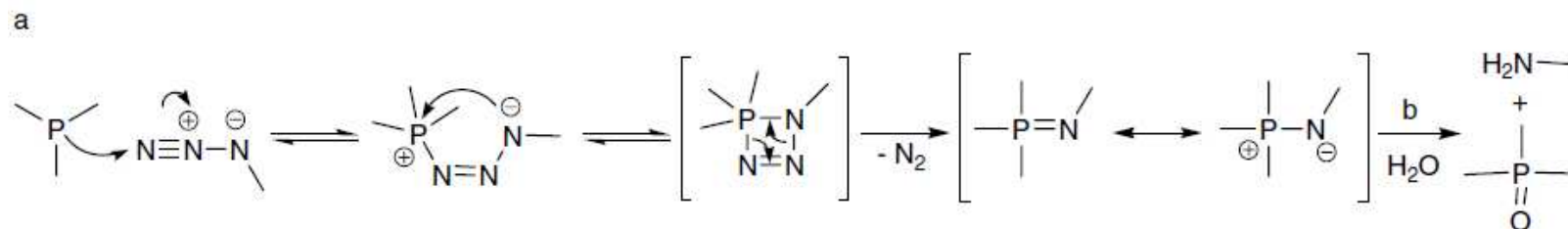
La formazione di un legame peptidico può essere condotta sfruttando le classiche strategie di accoppiamento; L'approccio qui sotto riporta quella che viene chiamata "native chemical ligation"



In queste funzionalizzazioni la proteina può essere sostituita da un aminooligosaccaride

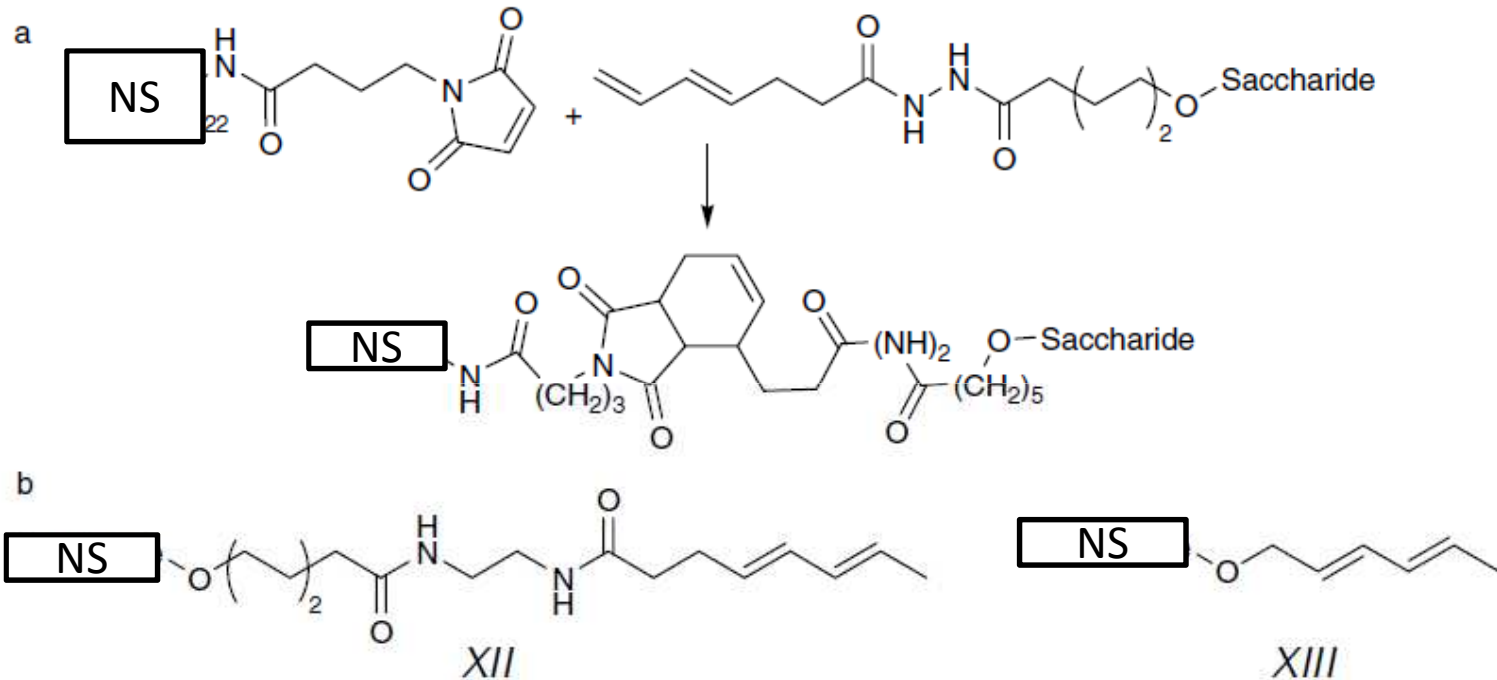


La reazione a-b è la reazione di Staudinger che permette di ottenere un gruppo amminico a partire da una azide; quest'ultima si ottiene facilmente per sostituzione nucleofila di un alogenuro. La reazione c e la d sono invece le reazioni di formazione di un legame peptidico che può essere condotta in situ. Queste reazioni si chiamano "Staudinger ligations".

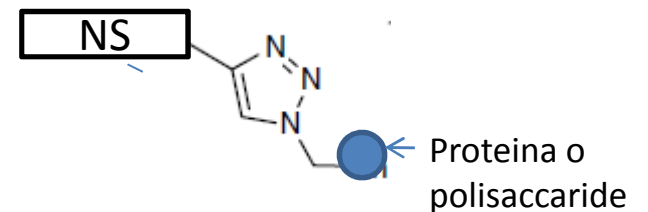


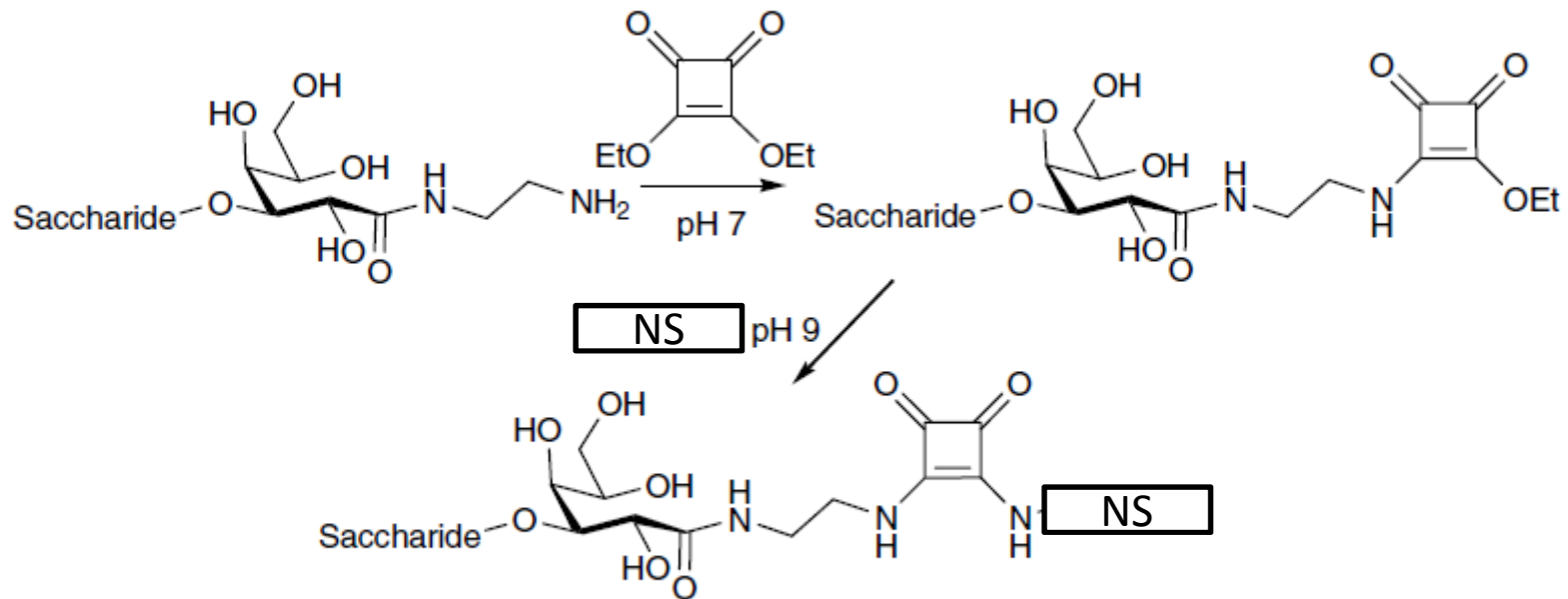
(a) Formation of ylide from azide and phosphine. In the presence of water (b) the reaction continues to form amine and phosphine(V) oxide in the so called Staudinger reaction, or Staudinger reduction. If there is a suitable electrophil, the reaction continues toward the Staudinger ligation. Example of a "nontraceless" Staudinger ligation (c) and a "traceless" Staudinger ligation (d) employing VIII-like and X, respectively.

Una possibile alternativa è usare una reazione di cicloadizione come in questo caso tra un diene ed un alchene povero di elettroni; le reazioni di cicloadizione sono reazioni poco sensibili al solvente.

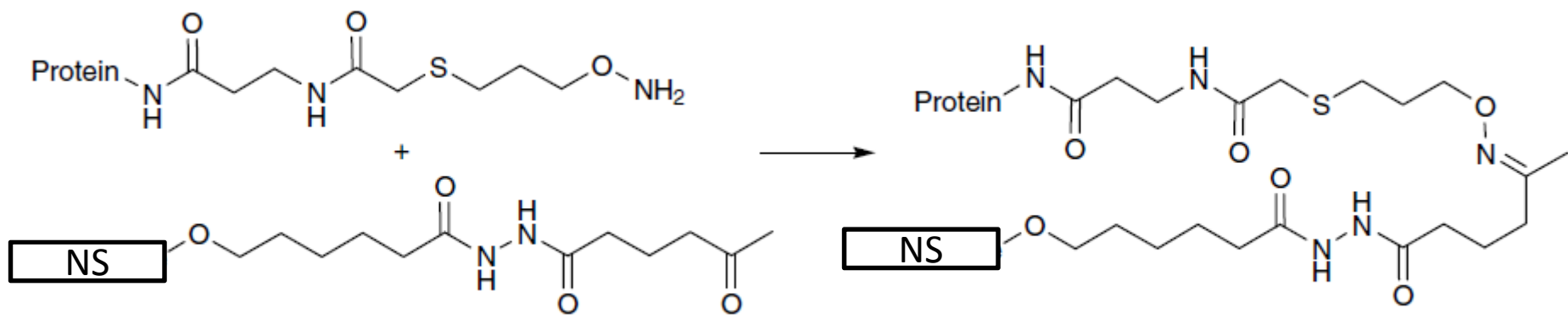


Questo prodotto si forma invece da una reazione di cicloadizione nota come 1,3-dipolare. I due partners sono un alchino e una azide: viene chiamata “click reaction”





Use of DES for *Streptococcus* oligosaccharide antigen and protein coupling via diethyl squarate.



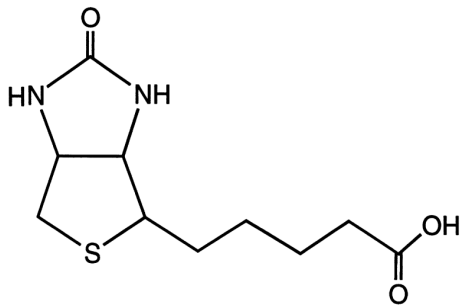
Ketohexanoyl derivative of the hexasaccharide reaction with aminosylated BSA.

**Table 2.** Selected coupling methods, molecule per protein substitution comparison; substitution is expressed as a mole ratio, and as the percentage of coupled linker functional groups

Coupling method	Protein	Hapten and linker f. group	Molar ratio (Reacted groups/%)	Coupling conditions	Time	Yield /%	Merits/(+) Handicaps/(-)	Reference
Reductive amination	TT-NH <sub>2</sub>	Maltotriose	6.0-20	r.t.; pH 8.0	7-14 d	n.a.	(+) Simple chemistry (-) Not universal, long reaction time	Pawlowski et al. (1999)
	BSA-NH <sub>2</sub>	Maltotriose	9.9	r.t.; pH 8.0	14 d	n.a.		Pawlowski et al. (1999)
	TT-NH <sub>2</sub>	Lipooligosaccharide, V	18.9	37 °C; pH 9	4 d	36?		Mieszala et al. (2003)
	TT-NH <sub>2</sub>	Lipooligosaccharide, VF	15.5	37 °C; pH 9	4 d	36?		Mieszala et al. (2003)
Thioether bond formation, nucleophilic substitution	TT-(L)-Br	Dextran, 93 kDa	11.2 (43)	r.t.; pH 8.5	2 d	n.a.	(+) Efficiency	Pawlowski et al. (1999)
	TT-(L)-Br	Maltotriose	214 (82)	r.t.; pH 8.5	2 d	n.a.		Pawlowski et al. (1999)
	TT-(L)-Br	Polysaccharide, 6 kDa	16.0 (73)	r.t.; pH 8.5	n.a.	7 <sup>a</sup>		Pawlowski et al. (2000)
	TT-(L)-SH	Polyglutamic acid-Br	11	r.t.; pH 7.4	1.5 h	n.a.		Kubler-Kielb et al. (2006)
	TT-(L)-Br	Polyglutamic acid-SH	16	r.t.; pH 7.4	1.5 h	n.a.		Kubler-Kielb et al. (2006)
	TT-(L)-Br	Polysaccharide-SAc	10 (30)	r.t.; pH 8.3	30 h	57		Grandjean et al. (2009)
	BSA-(L)-Br	Polysaccharide-SAc	2 (7)	r.t.; pH 8.3	30 h	53		Grandjean et al. (2009)
	BSA-(L)-Br	Tetrasaccharide-SH	8 (53)	r.t.; pH 7.8	n.a.	n.a.		Leung et al. (2009)
Thioether bond formation, addition reaction	BSA-(L)-=	Oligosaccharide-SH, Fig. 7b	8	pH 8.9	8 h	~ 100	(+) Simple purification, high yield, reaction time	Deiadek et al. (2008)
	TT-(L)-SAc	Oligosaccharide-maleimide, Fig. 7a	14	r.t.; pH 6	2 h	48 <sup>b</sup>		Grandjean et al. (2009)
	BSA-(L)-SAc	Oligosaccharide-maleimide	4	r.t.; pH 6	2 h	72		Grandjean et al. (2009)
Thioether bond formation, radical thiol-ene coupling	BSA-(L)-SAc	glycopeptide=	9 (38)	20 °C; ACVA; h <sup>v</sup>	8 h	n.a.	(+) reaction time, selectivity (-) αs-initiator, or high temperature	Wittrock et al. (2007)
	BSA-(L)-=	glycopeptide-SH	8 (32)	h <sup>v</sup>		n.a.		
Acide-alkyne coupling	CPMV-N <sub>2</sub>	Dye-yne	60 (100)	4 °C; pH 8	16 h	94-96	(+) Selectivity, % of derivatization, yield (-) additives	Wang et al. (2003)
	CPMV-yne	Dye-N <sub>2</sub>	48 (80)	4 °C; pH 8	16 h	80		Wang et al. (2003)

Esiste anche la possibilità di coniugare il nanosistema alla proteina o al polisaccaride attraverso un “legame” non covalente. Questa interazione però deve essere molto forte, cioè essere caratterizzata da un valore di  $-\Delta G$  molto elevato ( $>20$  kcal/mole; cioè una costante di associazione di almeno  $1 \times 10^{14} \text{ M}^{-1}$ ).

Questa situazione si verifica per l'interazione biotina/(strept)avidina

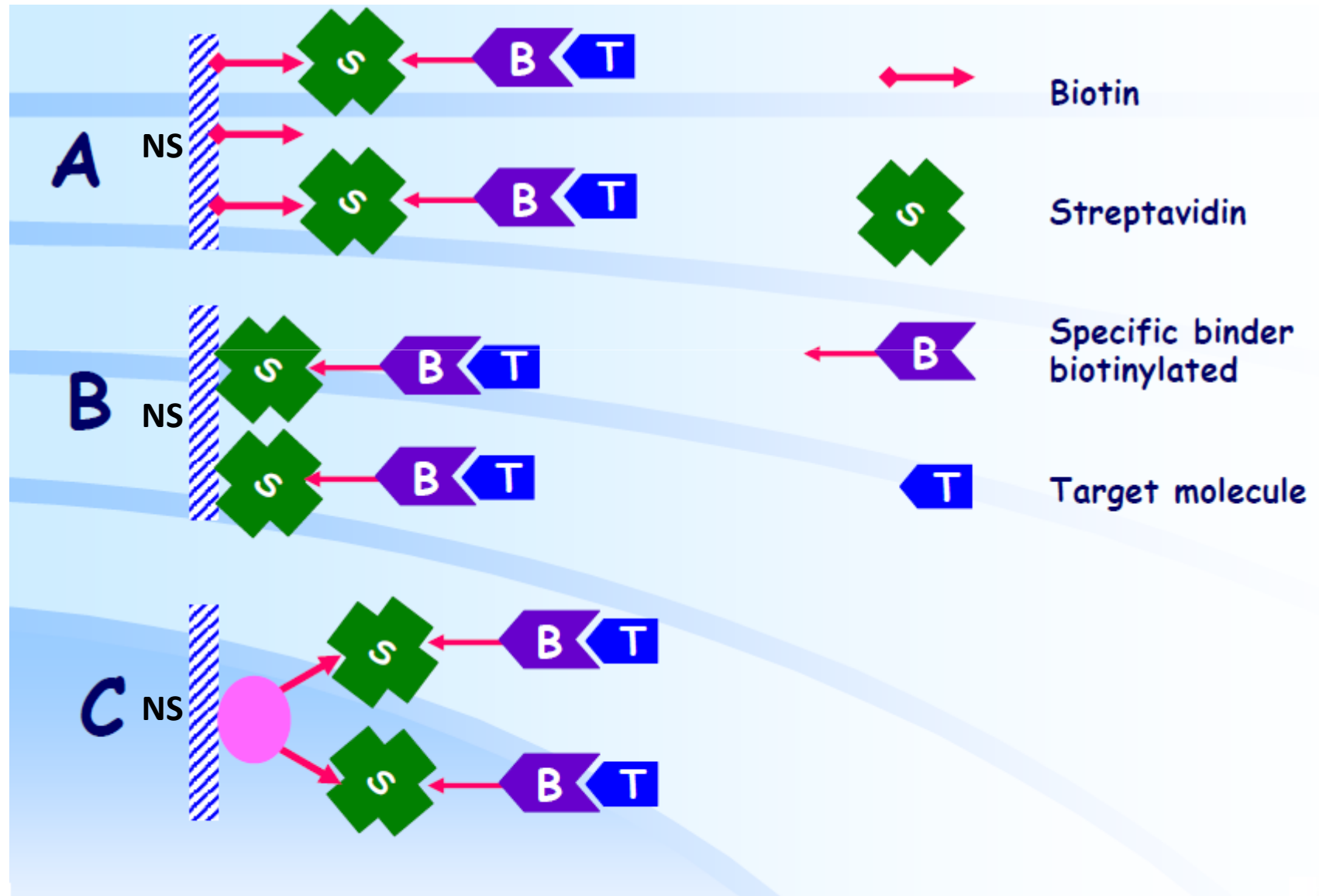


Biotina

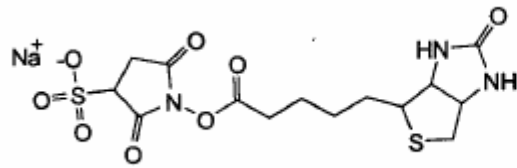
### Confronto tra avidina e streptavidina

	Avidin	Streptavidin
Molecular weight	66 kDa	60 kDa
Subunits	4	4
Binding sites for biotin/mol	4	4
Isoelectric point	~10.5	5–8
Carbohydrate residue	Rich	Absent
Tyrosine	6/subunit	1/subunit
Affinity for biotin	$1 \times 10^{15} \text{ M}^{-1}$	$1 \times 10^{15} \text{ M}^{-1}$

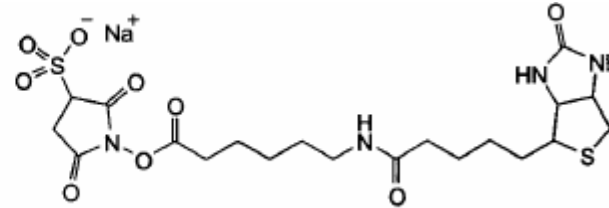
# Strategie per l'interazione nanosistema/target mediate dalla complessazione biotina/(strept)avidina



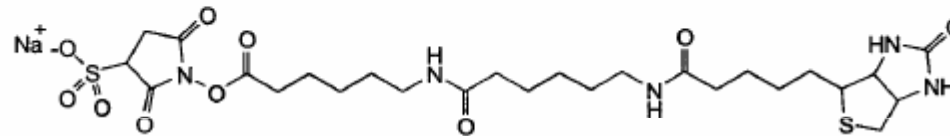
## Strategie di coupling via estere attivo (nucleofilo tipico: ammina primaria o secondaria)



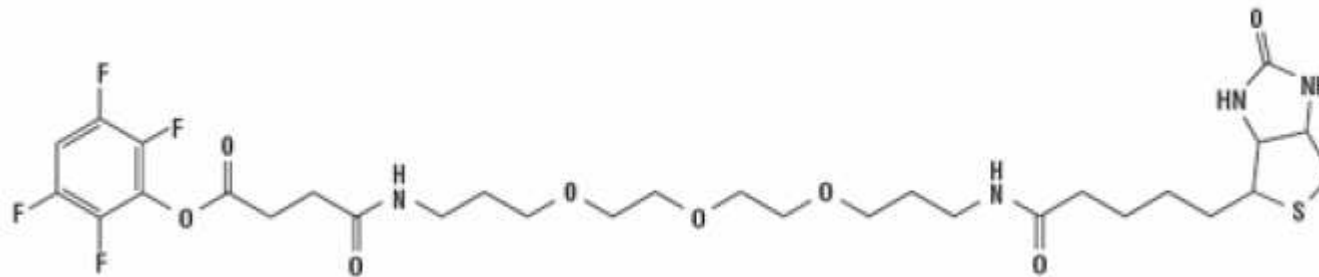
Sulfo-NHS-Biotin



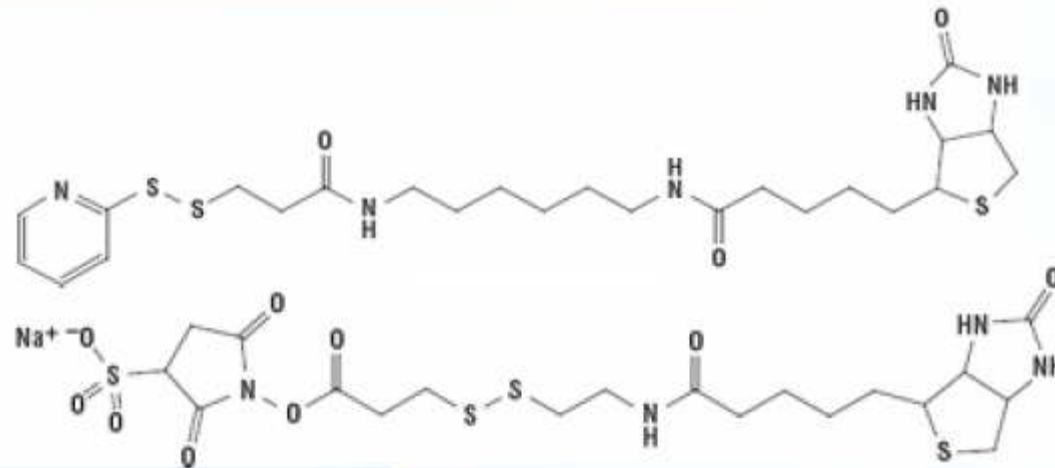
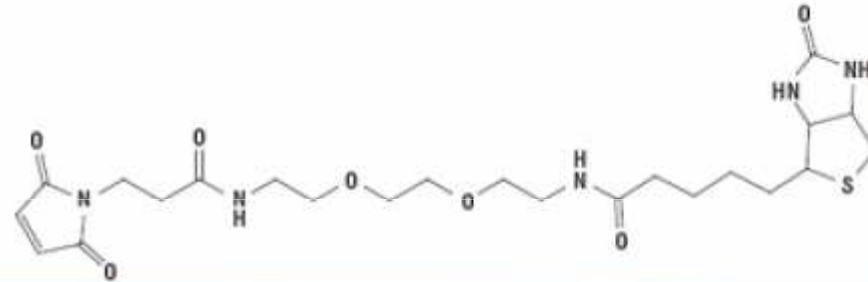
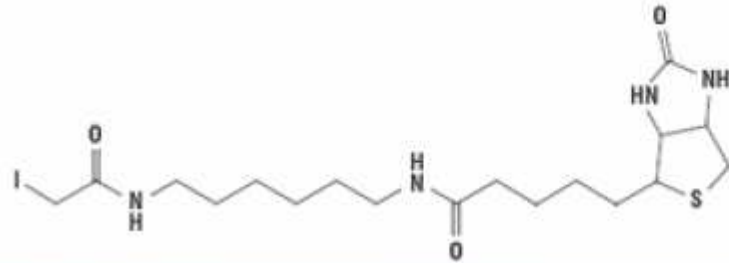
Sulfo-NHS-LC-Biotin



Sulfo-NHS-LC-LC-Biotin



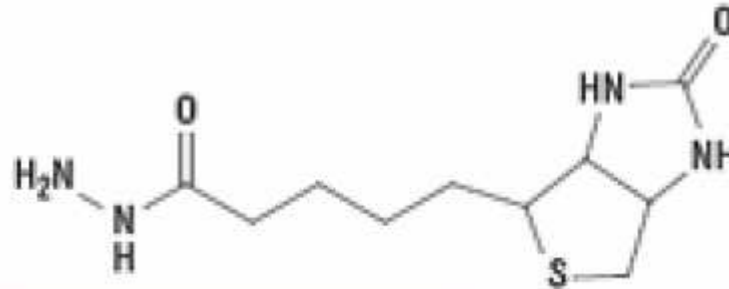
## Strategie di coupling con tioli



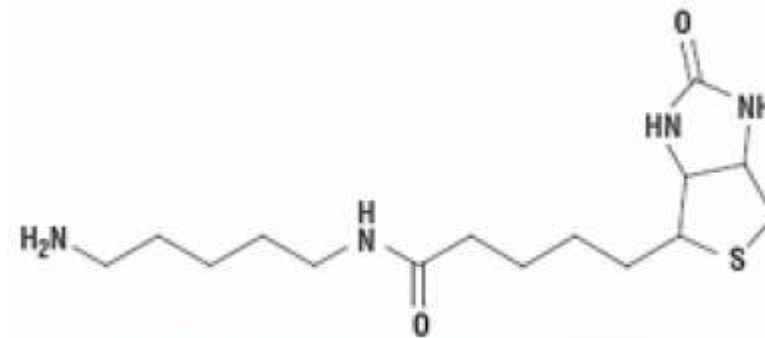
**Strategie di coupling con:**

**a) carboidrati; b) derivati di acidi carbossilici**

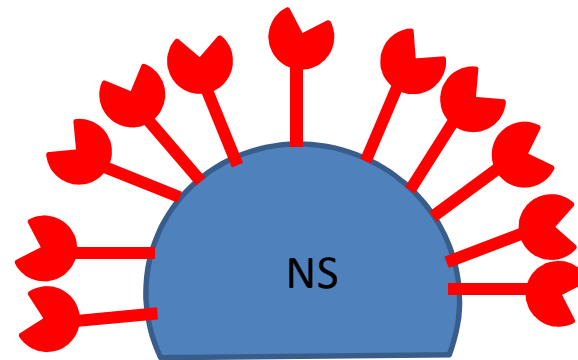
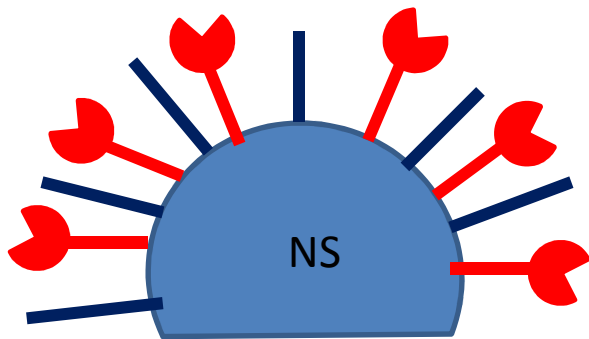
**a) Idrazoni**



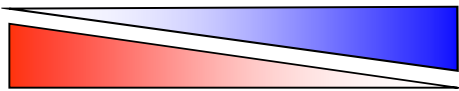
**b) Ammidi**



# Ruolo delle dimensioni del nanosistema e della densità dei gruppi funzionali nella complessazione con il target

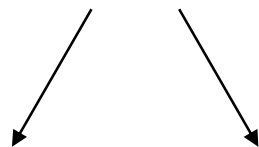
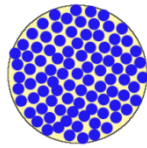
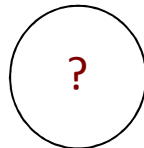
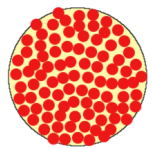


Gruppo funzionale

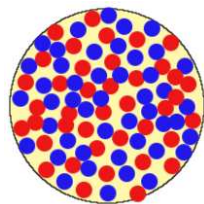


Gruppo inerte

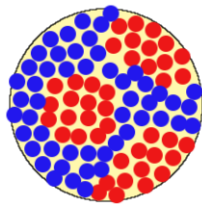
# RUOLO DELLA % DI FUNZIONALIZZAZIONE DEL NS



$K_d$



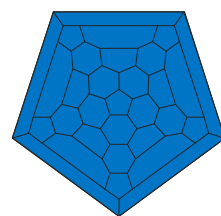
random



domini

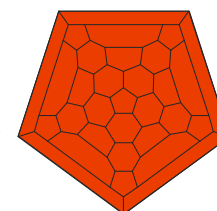
Gruppi funzionali (attivi) possono mescolarsi con unità inerti in maniera casuale o formando domini

Queste due situazioni possono essere rappresentate da una superficie nella quale esagoni o pentagoni sono affiancati gli uni agli altri

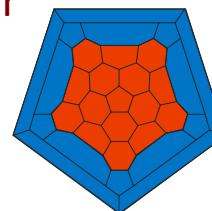


+ + + ... + +

Inserimento casuale



Inserimento a cluster



: unità funzionale

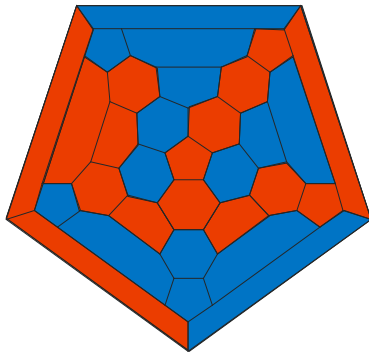
: unità inerte

# Analisi (semplice target bivalente)

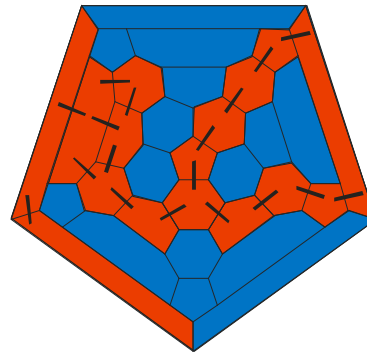
Affinità per singolo sito assunta costante ( $\alpha=1$ )

Siti potenziali  
' $K_d$ '

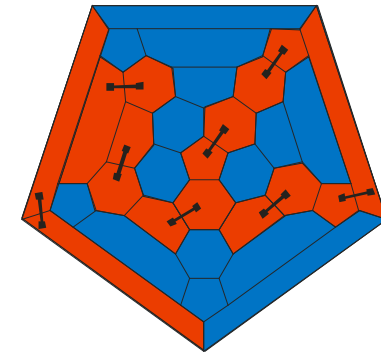
Siti saturabili  
'*molecole di target max*'



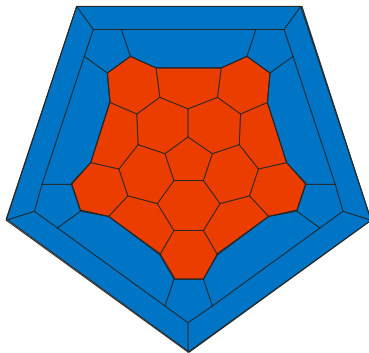
random



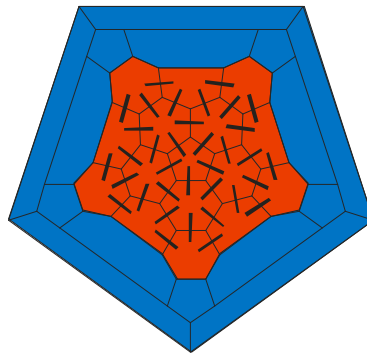
18



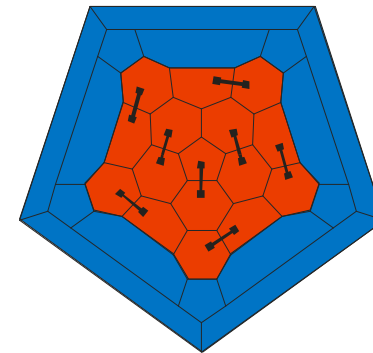
8



clustering

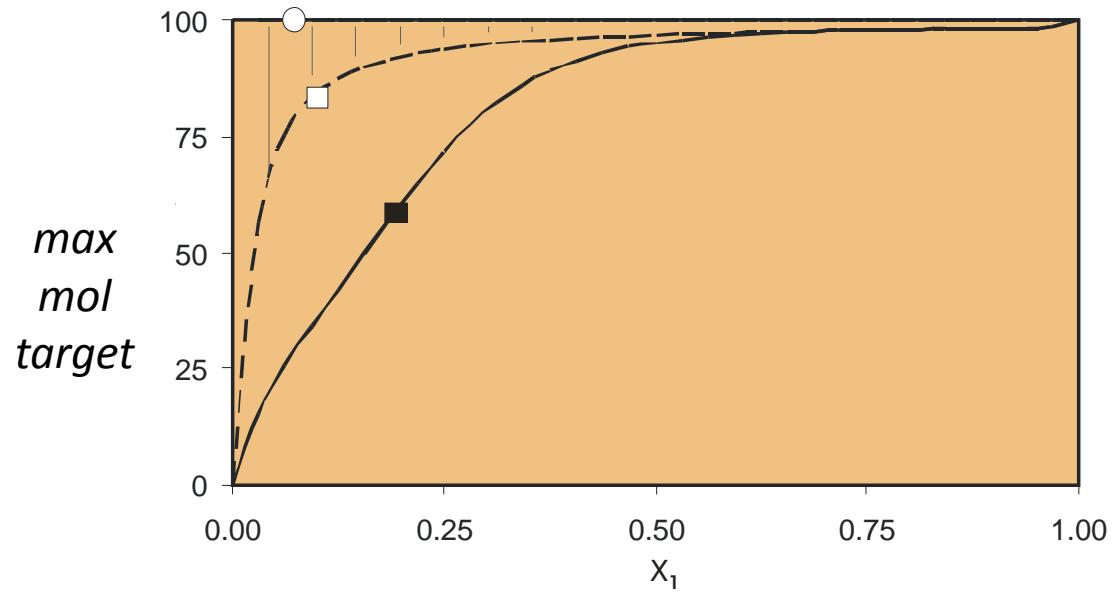


35

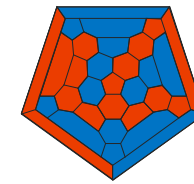


8

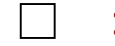
# Che cosa mi aspetto



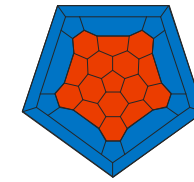
:



**random**



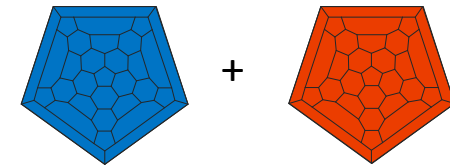
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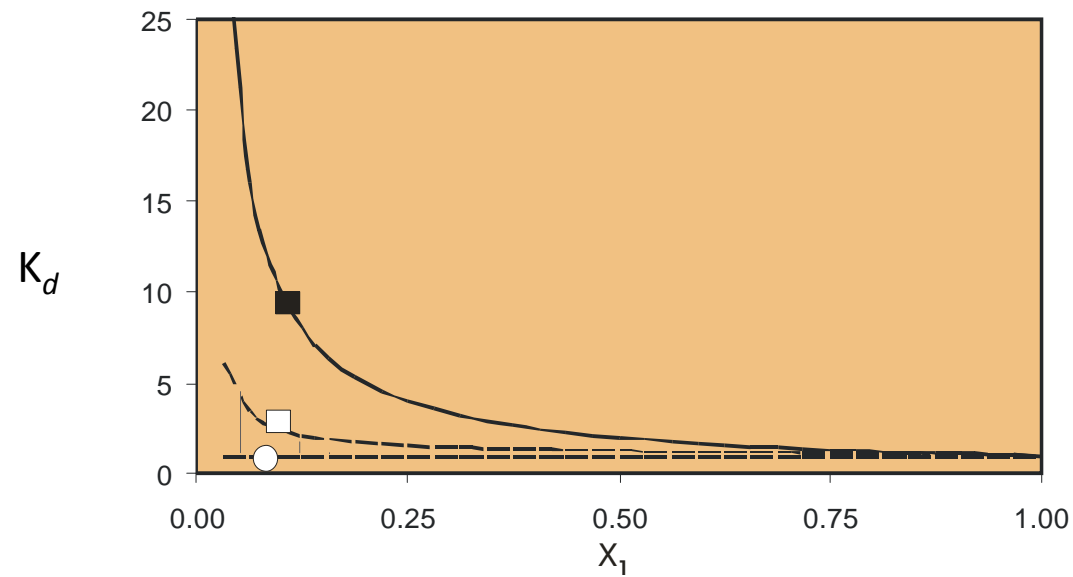
**clustering**



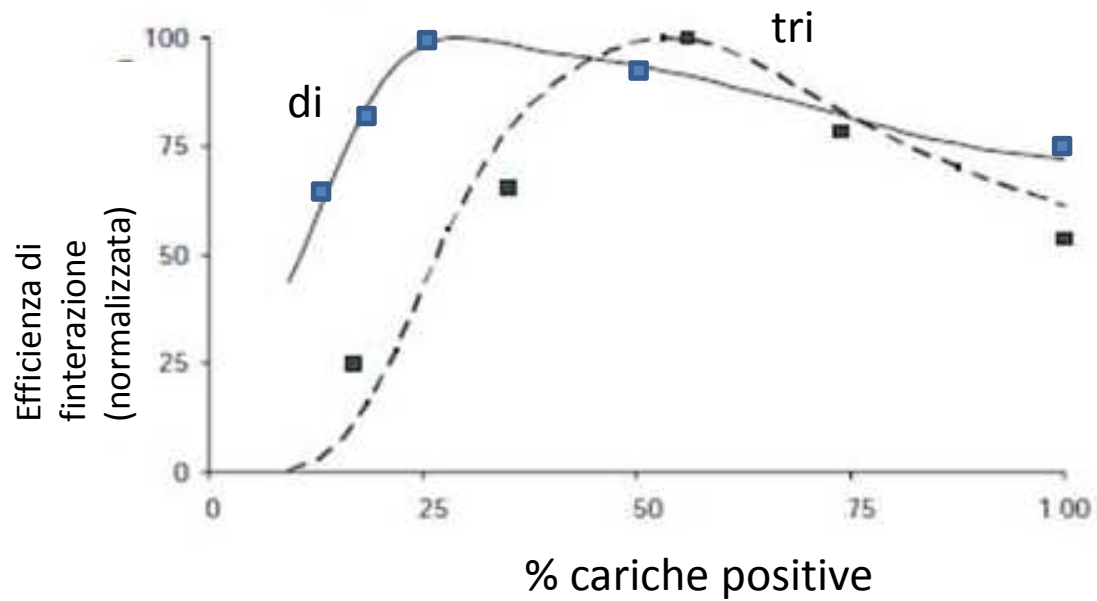
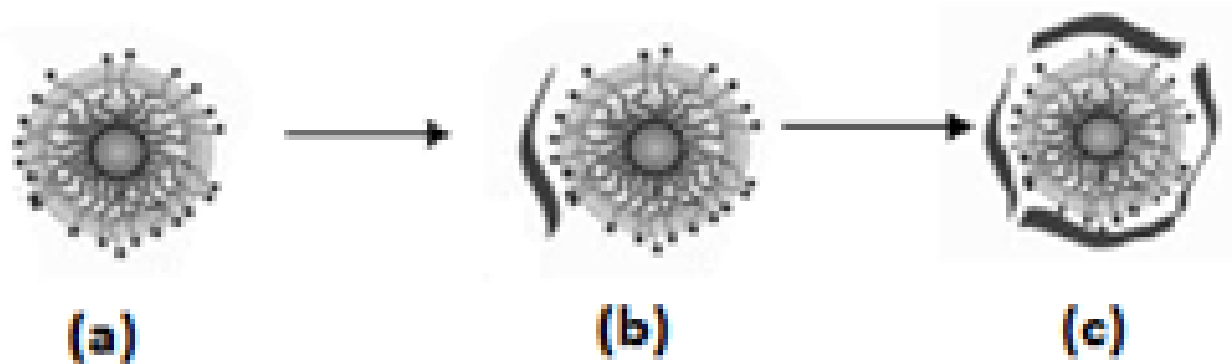
:

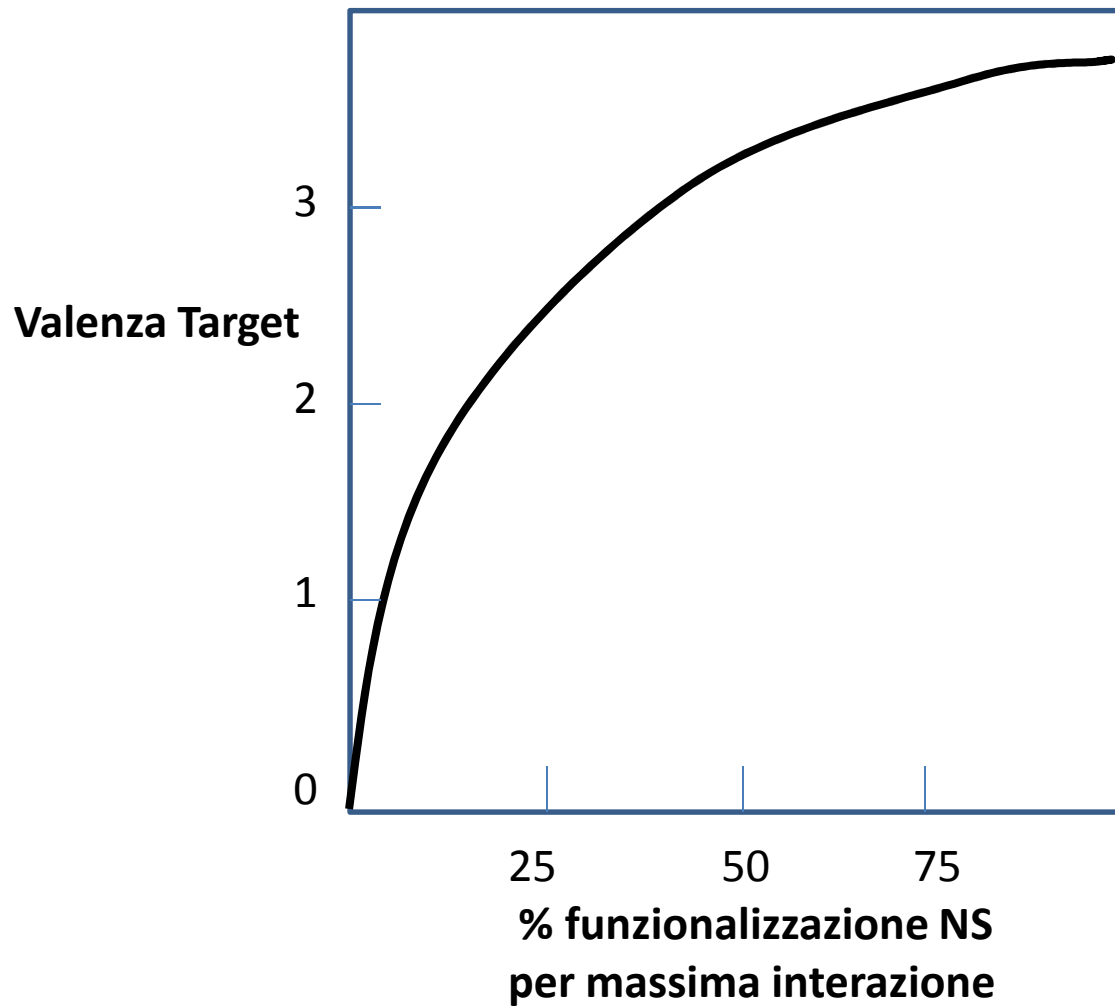


**self-sorting**



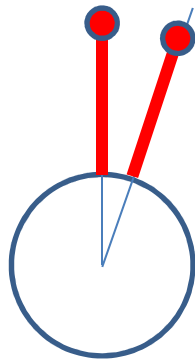
## Binding di un oligonucleotide ad una nanoparticella cationica



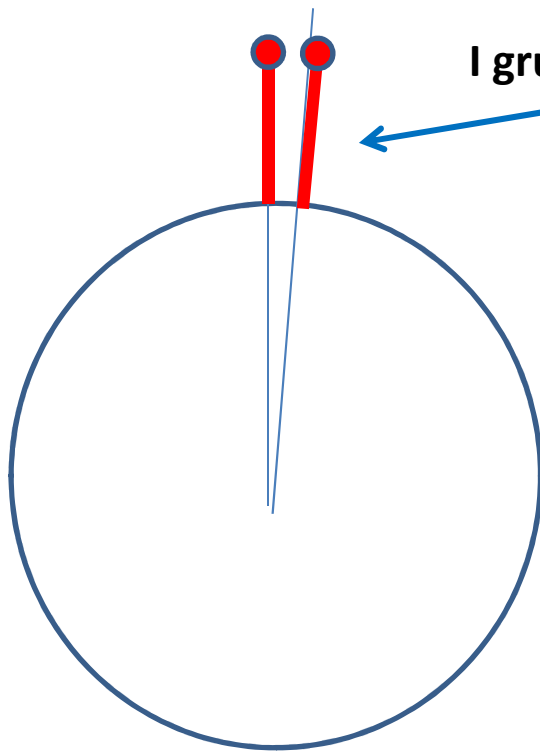


**La conclusione è che  
per target ad alta  
valenza conviene usare  
un NS ad alta % di  
funzionalizzazione**

## RUOLO DELLA DIMENSIONE DEL NS



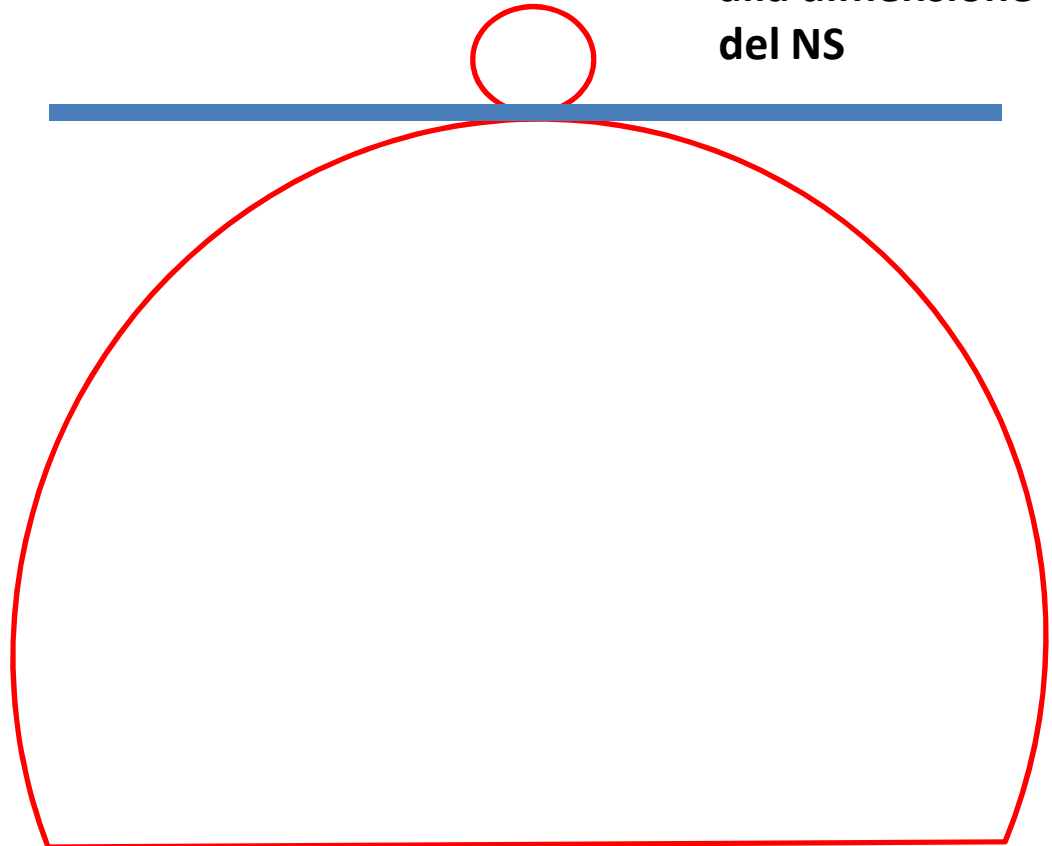
I gruppi funzionali sono più lontani



I gruppi funzionali sono più vicini

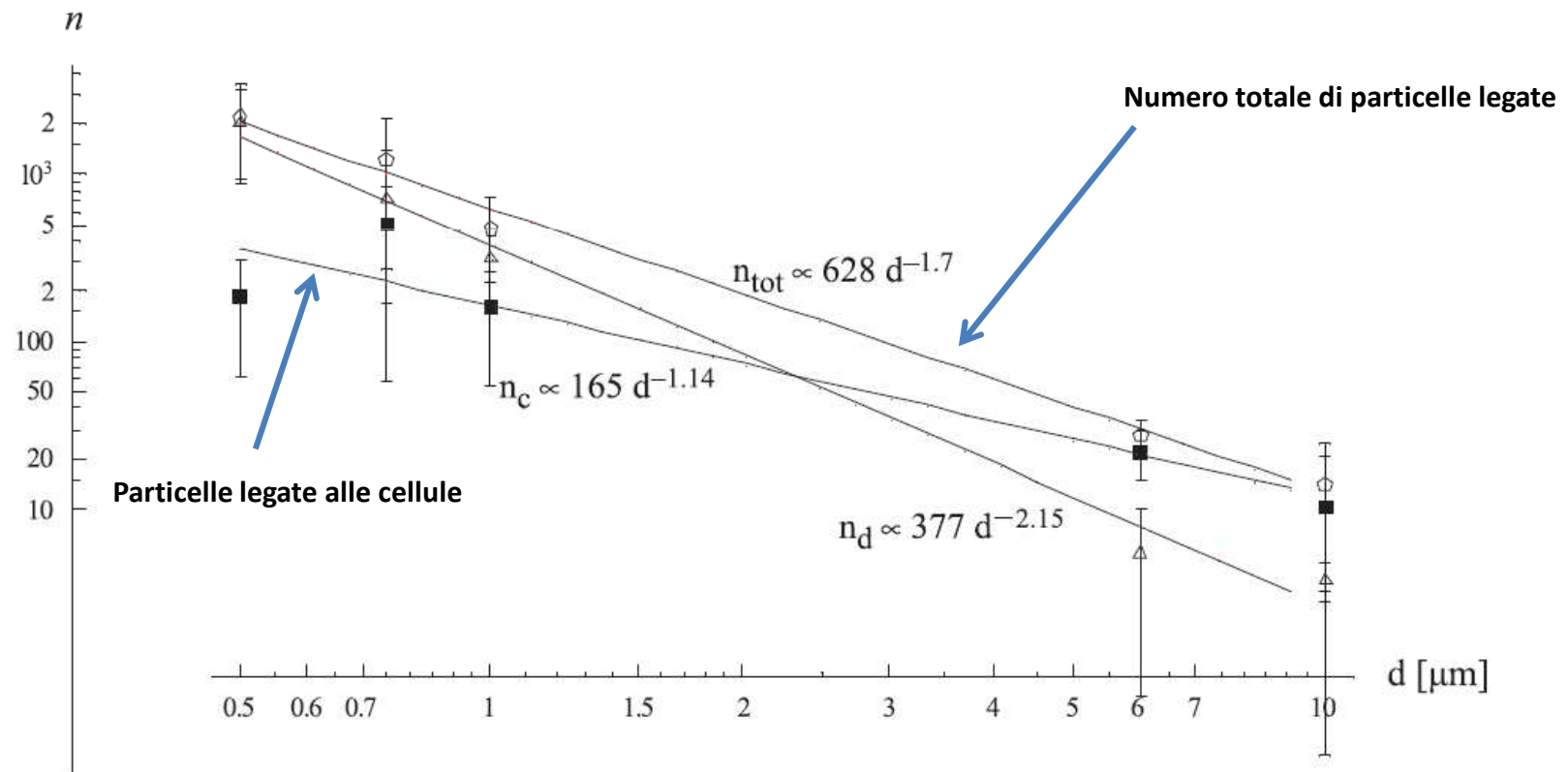
Il “peso” relativo della frazione funzionale è però maggiore nel NS più piccolo

La superficie di contatto con il target è direttamente proporzionale alla dimensione del NS



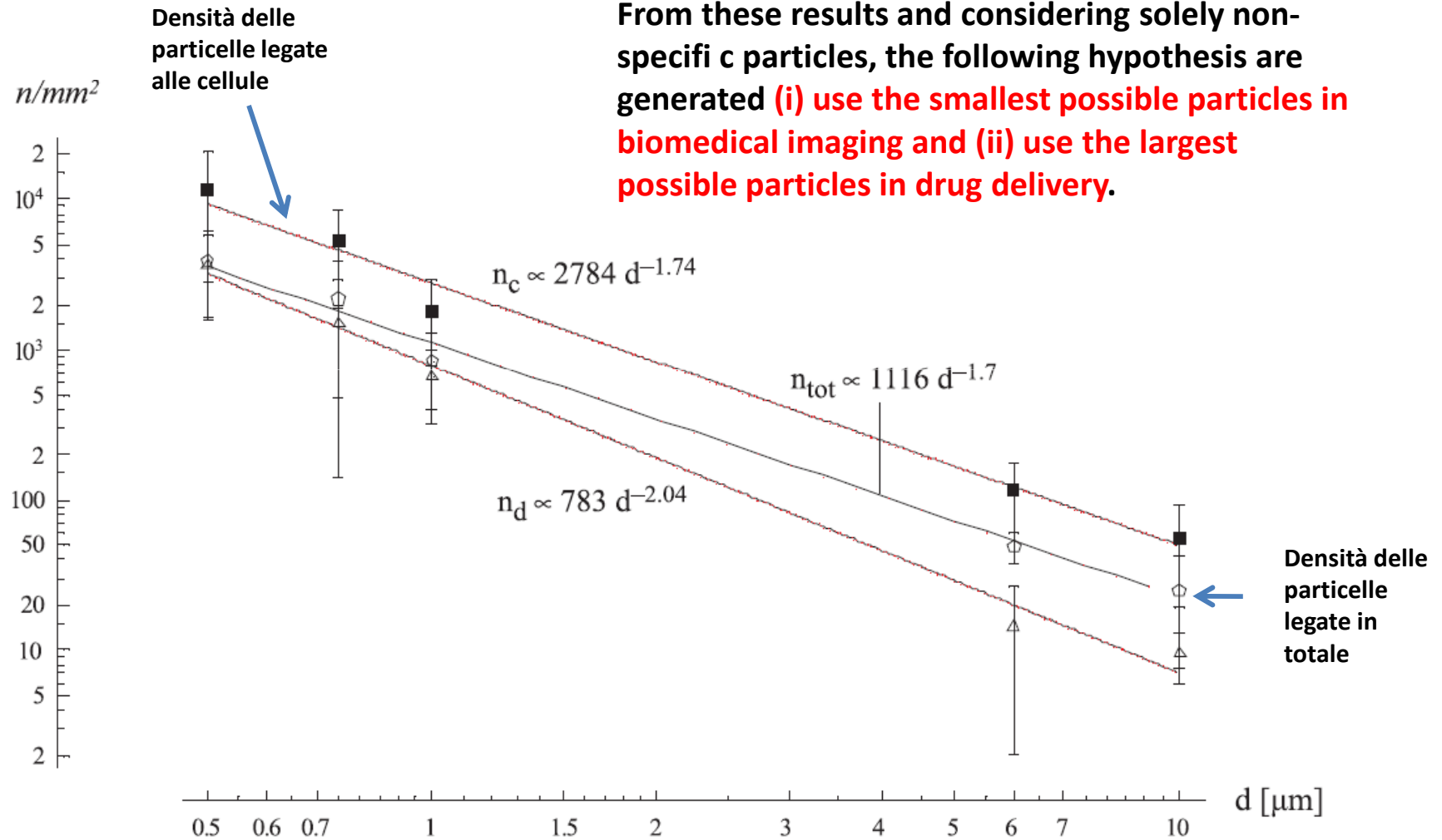
In this work, the focus is on spherical particles **not coated with ligand molecules** and with a size ranging from 0.5  $\mu\text{m}$  up to 0.10  $\mu\text{m}$ . The adhesive performances of these particles is assessed in terms of the number of particles adhering non-specifically to a confluent layer of endothelial cells under fixed hydrodynamic conditions in a parallel plate flow chamber.

Particelle commerciali di polistirene (Fluoresbrite)



**Figure 4** The variation of the total number of particles ( $n_{\text{tot}}$ , white pentagons), of the number of particles adherent to the cells ( $n_c$ , black boxes), of the number of particles adherent to the borosilicate dish ( $n_d$ , white triangles) with the particle diameter  $a$  at the end of the experiment (800 sec).

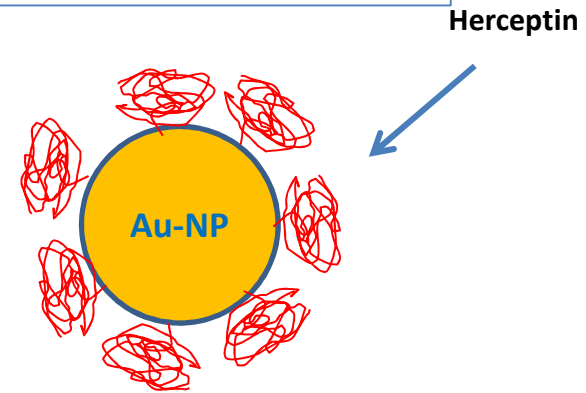
**Conclusions: (i) the absolute number of particles adherent to the cell layer per unit surface decreases with the size of the particle as  $d^{-1.7}$ ; (ii) the volume of the particles adherent per unit surface increases with the size of the particles as  $d^{+1.3}$ . From these results and considering solely non-specific particles, the following hypothesis are generated (i) use the smallest possible particles in biomedical imaging and (ii) use the largest possible particles in drug delivery.**



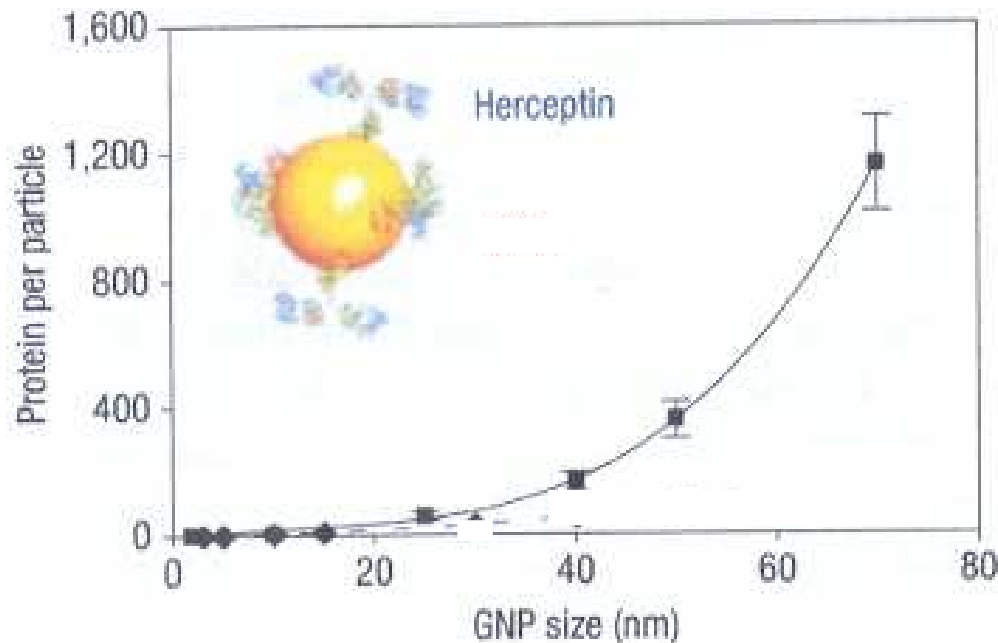
**Figure 5** The variation of the surface density of the total number of particles ( $\bar{n}_{tot}$  white pentagons), of the number of particles adherent to the cells ( $\bar{n}_c$  black boxes), of the number of particles adherent to the borosilicate dish ( $\bar{n}_d$  white triangles) with the particle diameter  $a$  at the end of the experiment (800 sec).

## Ruolo delle dimensioni nell'interazione con il target biologico: NS funzionalizzati

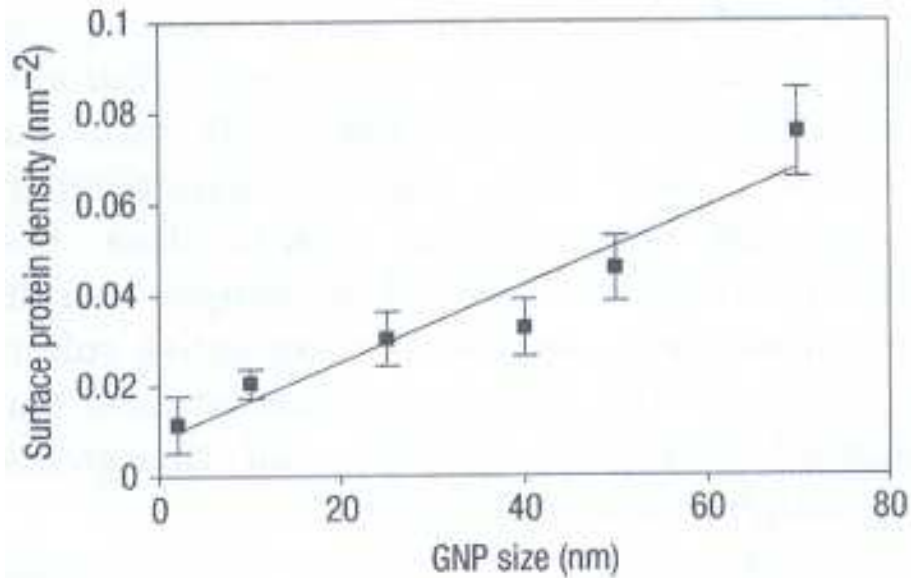
**Herceptin (Trastuzumab)** is a monoclonal antibody that interferes with the HerB2 receptor, a tyrosine kinase. The HerB receptors are proteins that are embedded in the cell membrane and communicate molecular signals from outside the cell to inside the cell, and turn genes on and off. The HerB proteins regulate cell growth, survival, adhesion, migration, and differentiation—functions that are amplified or weakened in cancer cells. In some cancers, notably some breast cancers, HerB2 is over-expressed, and, among other effects, causes breast cells to reproduce uncontrollably



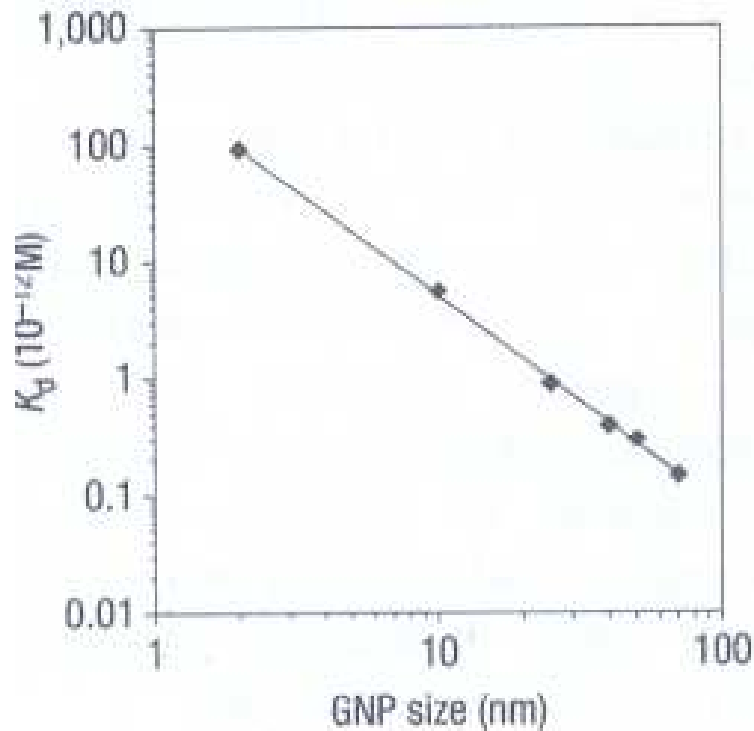
**Target: HerB2 receptor over expressed by SK-BR-3 human breast cancer cells**



**Andamento della quantità di proteine legata alle NP in funzione delle loro dimensioni.**



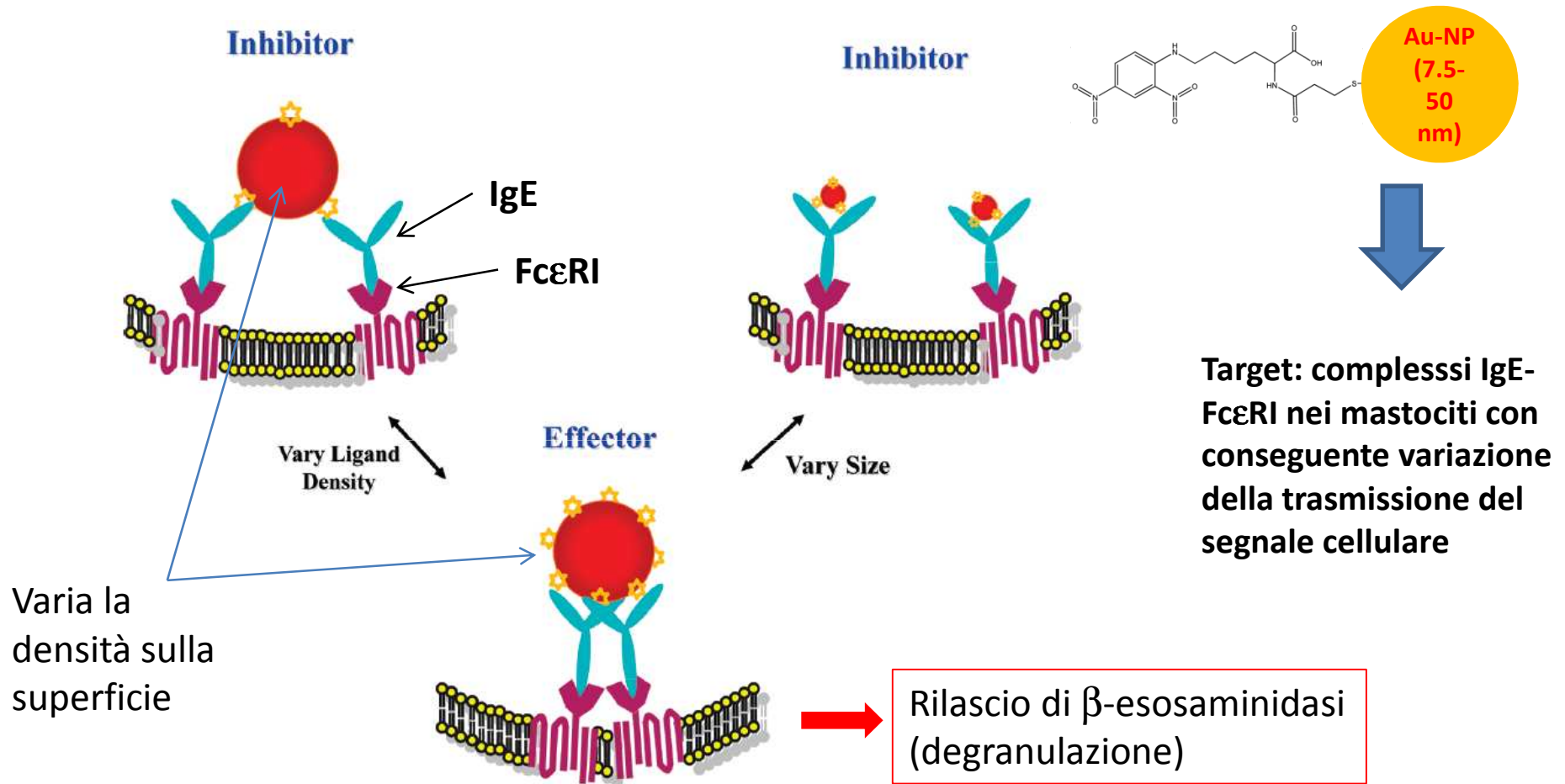
**Densità della proteina sulla superficie della NP in funzione della dimensione (è possibile che vi siano modi diversi di complessazione!)**

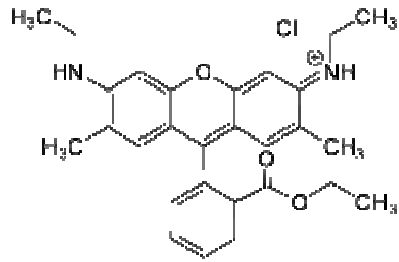


**L'affinità per il target dipende dalla dimensione: NP più grandi hanno una affinità maggiore.  
L'affinità si correla con la densità della proteina sulla NP.**

**ATTENZIONE: l'affinità è misurata in funzione della concentrazione di NP e NON di proteina!**

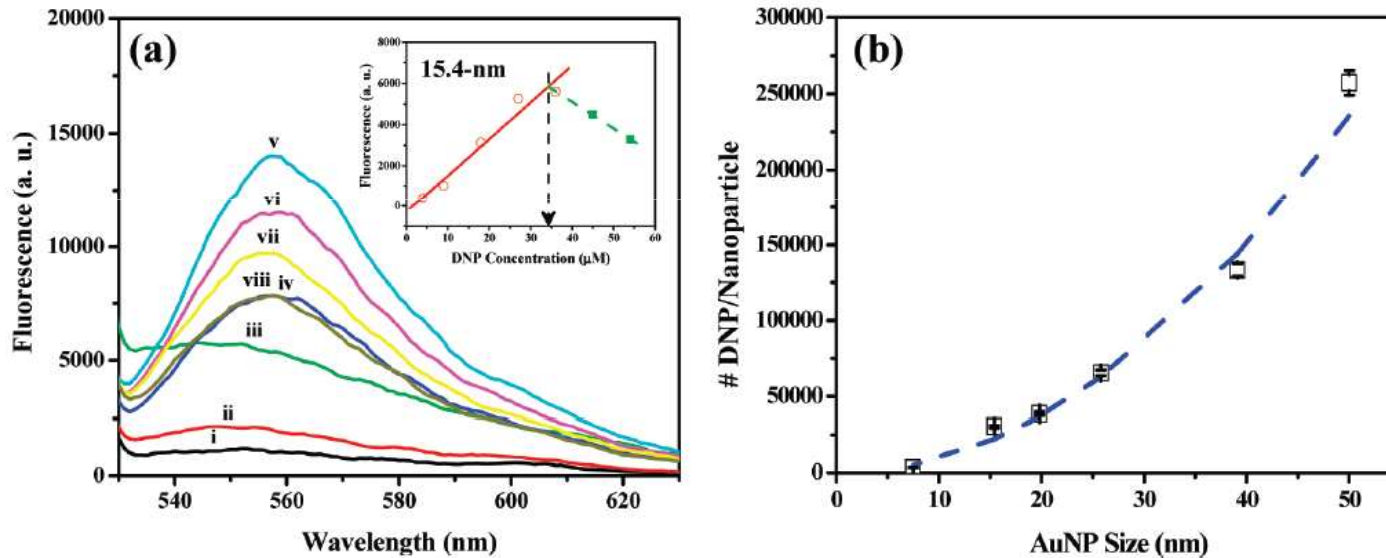
<sup>a</sup> Multivalent gold nanoparticles (AuNPs) were generated through covalent attachments of thiolated dinitrophenyl (DNP) onto AuNPs of various sizes. The attachment of multiple ligands onto the nanoparticle surface allows the formation of DNP molecules with different architectural features based on particle sizes and ligand densities, which, in turn, allows the selective control of specific interactions between DNP and IgE–FcεRI complexes in RBL-2H3 mast cells and the attendant alteration of cellular signal transduction.



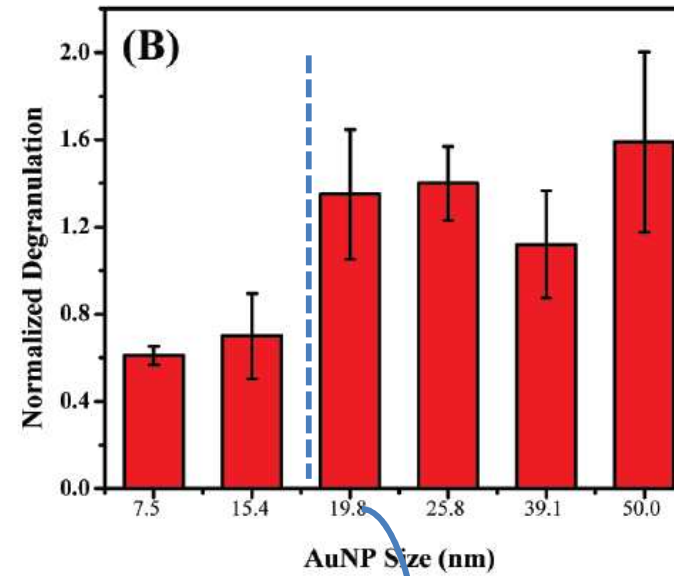
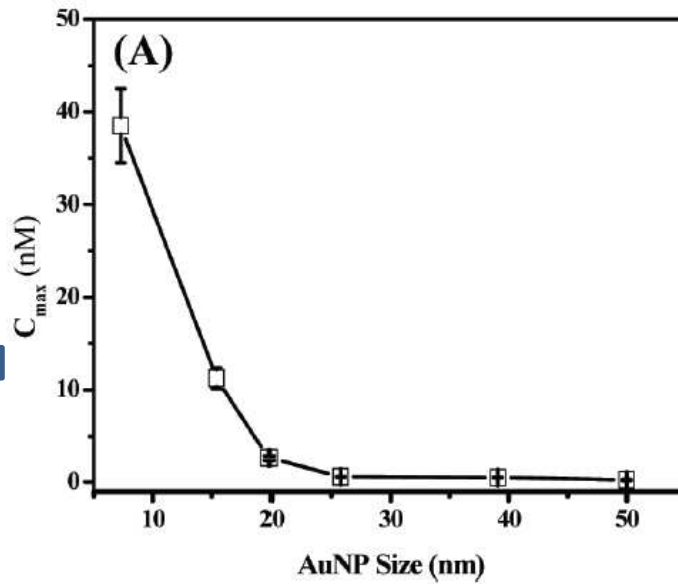


Rodamina 6G

**Determinazione del grado di copertura delle Au-NP con il tiolo funzionalizzato con il dinitro derivato mediante spostamento della Rodamina 6G dalla superficie.**



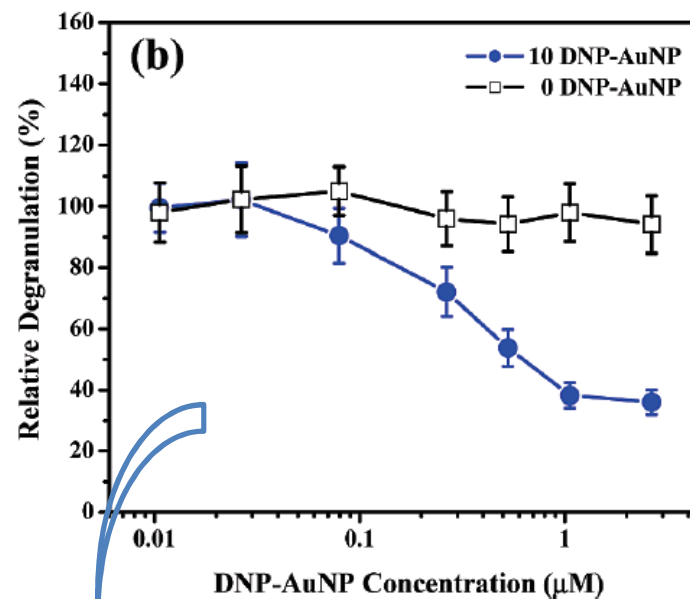
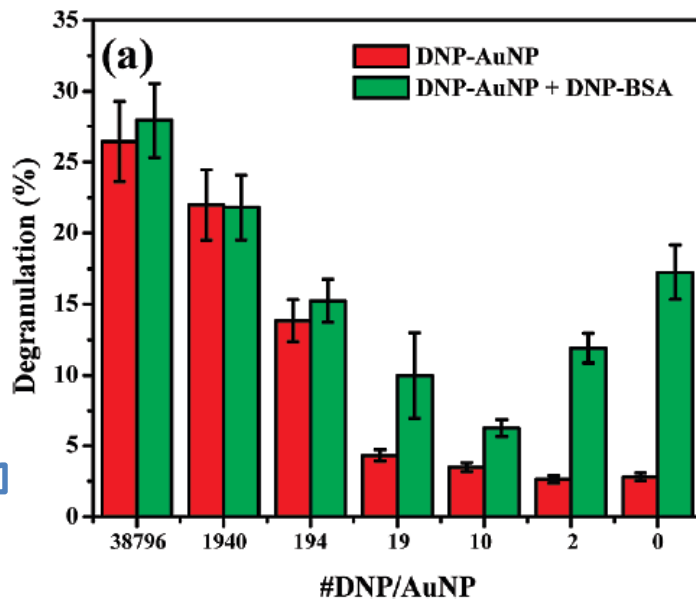
**Figure 1.** (a) Fluorescence spectra of R6G–AuNP (15.4 nm) solutions in the absence (i) and presence of thiolated DNP with (ii) 4, (iii) 9, (iv) 18, (v) 27, (vi) 36, (vii) 45, and (viii) 54  $\mu\text{M}$ . Inset: plot of fluorescence signal at 555 nm versus the concentration of DNP. (b) DNP loading analysis. DNP loading as a function of nanoparticle size (squares, experimental measurement; dashed line, calculation assuming constant DNP coverage density).



**Figure 3.** Degranulation of IgE-sensitized RBL-2H3 mast cells stimulated by DNP–AuNPs. Effect of (a)  $C_{max}$  and (b) degranulation response for different sizes of DNP–AuNPs.

**Concentrazione di Au-NP alla quale si osserva la massima degranulazione**

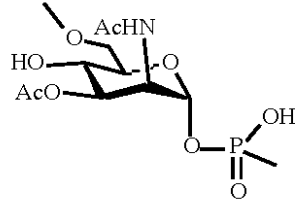
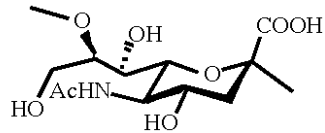
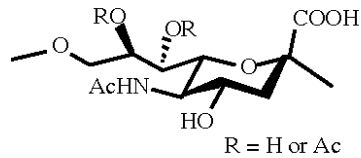
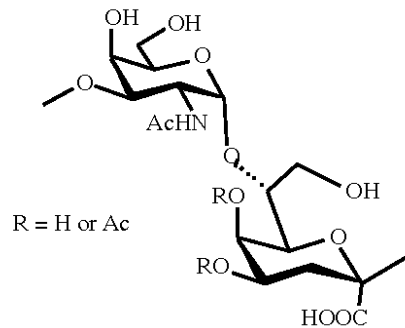
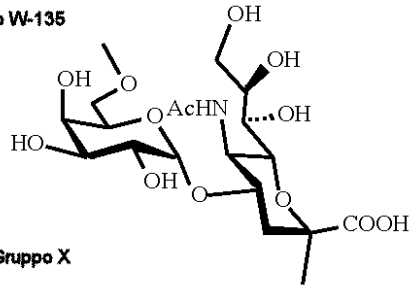
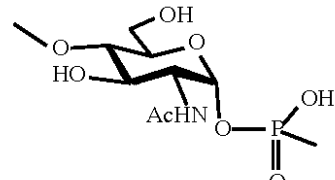
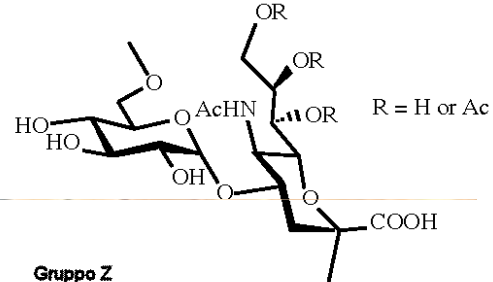
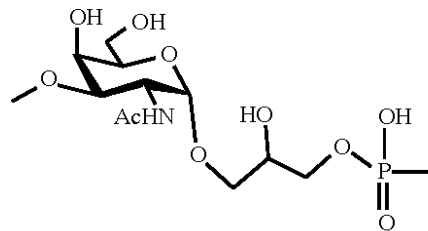
**Valore limite di dimensione per ottenere il massimo effetto di degranulazione**



**Figure 4.** (a) Degranulation of IgE-sensitized RBL-2H3 mast cells stimulated by DNP-AuNPs (19.8 nm) of different ligand densities. For inhibition studies, cells were preincubated with DNP-AuNPs of 2.7 nM for 1 h at 37 °C and then stimulated by 0.1 μg/mL DNP-BSA for one additional hour. (b) Inhibition of degranulation stimulated by 0.1 μg/mL DNP-BSA, with DNP-AuNPs of (□) 10 and (●) 0 DNP on one nanoparticle.

**Risultati ottenuti  
variando la  
concentrazione di tioli  
funzionali su Au-NP di  
19.8 nm**

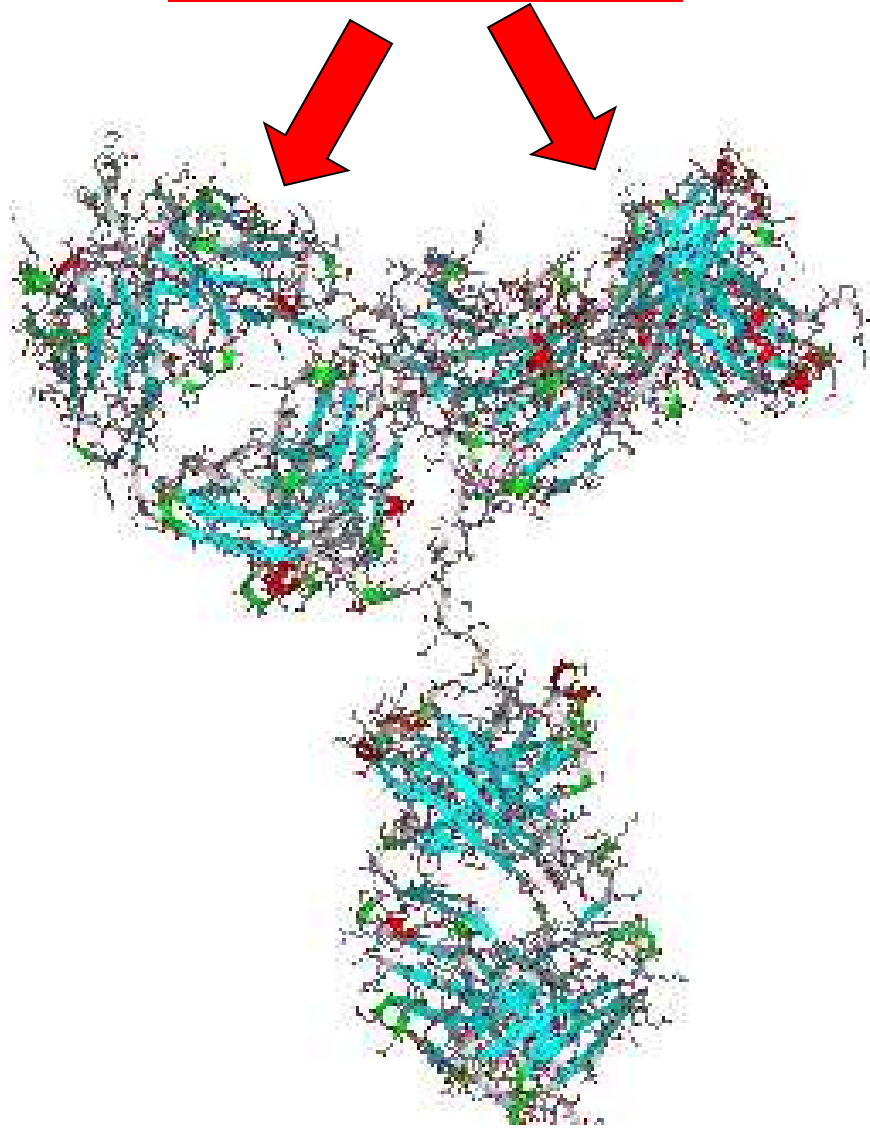
**Grafico dose/risposta  
nell'inibizione della  
degranulazione da parte di un  
sistema multivalente costituito  
da BSA-DNP**

**Gruppo A****Gruppo B****Gruppo C****Gruppo 28e****Gruppo W-135****Gruppo X****Gruppo Y****Gruppo Z**

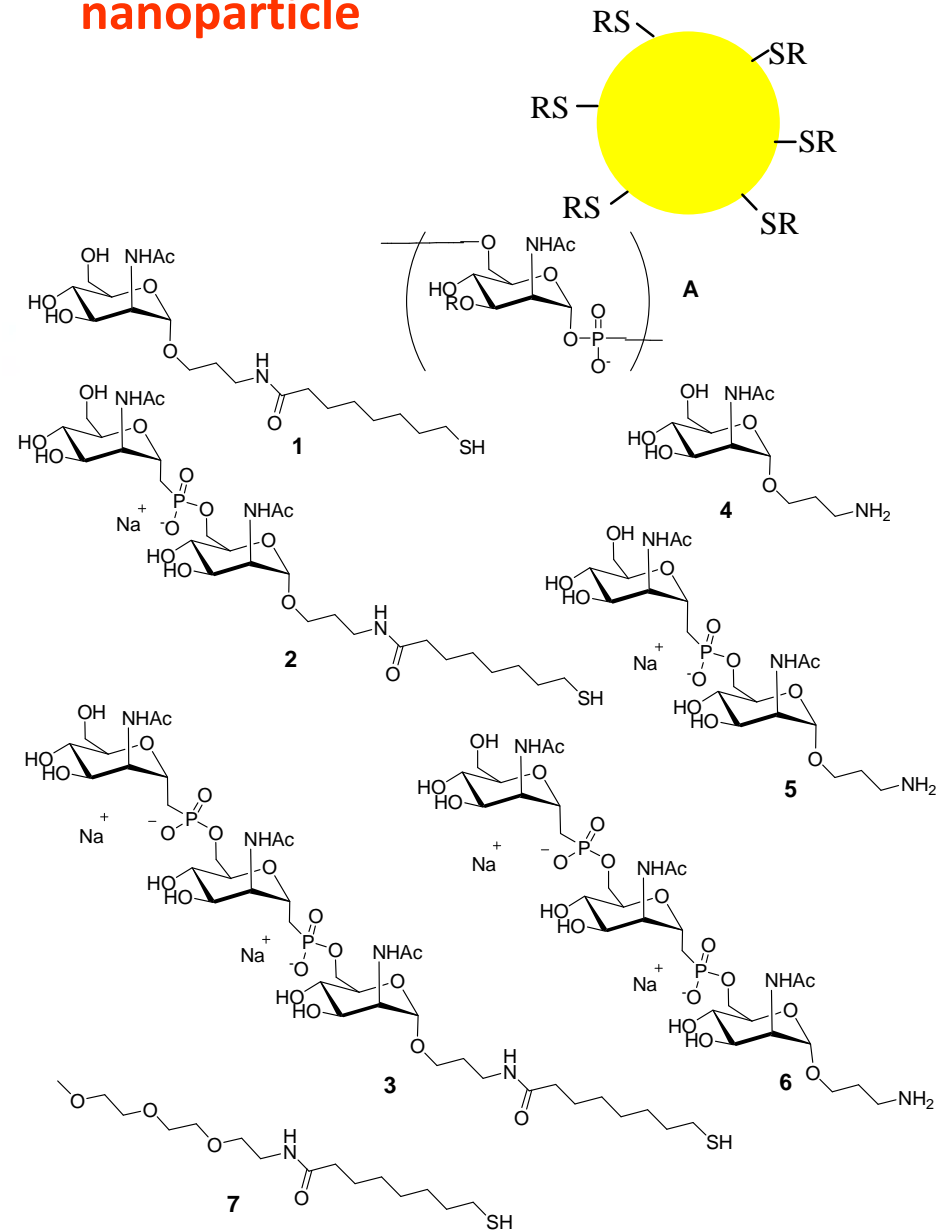
The family *Neisseriaceae* consists of Gram-negative aerobic bacteria from fourteen genera. The genus *Neisseria* contains two important human pathogens, *N. gonorrhoeae* and *N. meningitidis*. *N. gonorrhoeae* causes gonorrhea, and *N. meningitidis* is the cause of meningococcal meningitis. *N. meningitidis* infections have a low prevalence and high mortality.

*Neisseria meningitidis* has 13 clinically significant serogroups. These are classified according to the antigenic structure of their polysaccharide capsule.

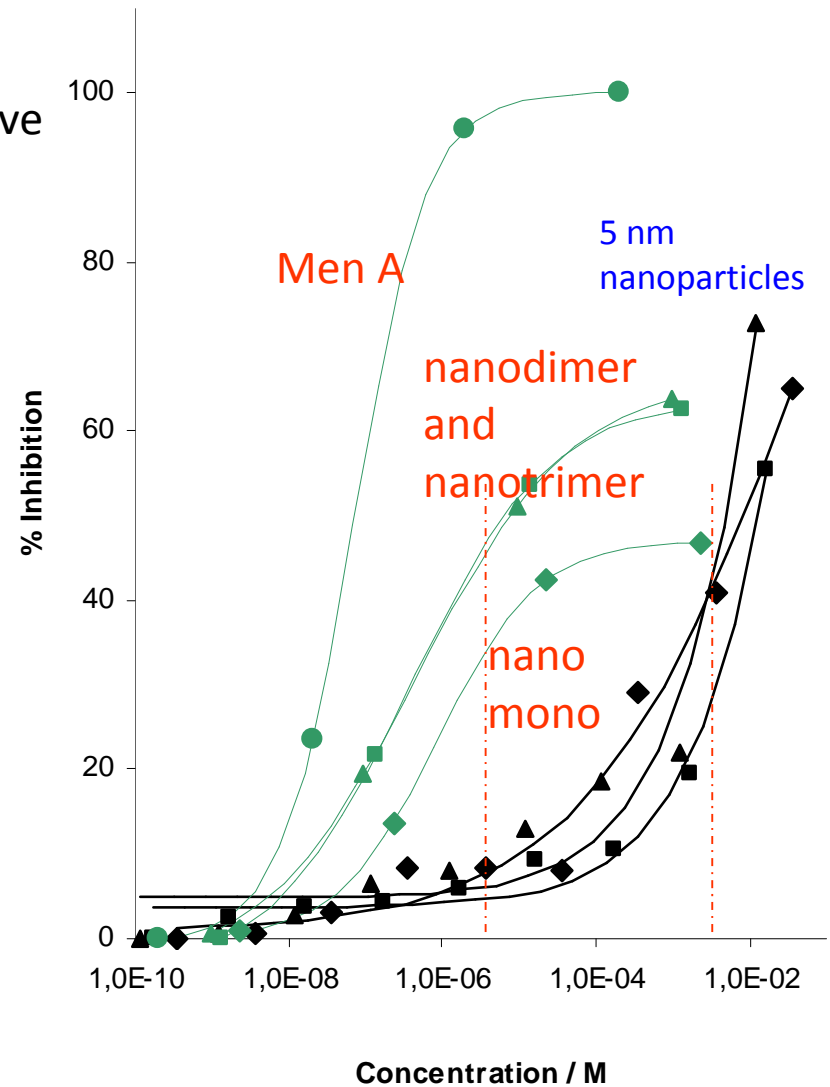
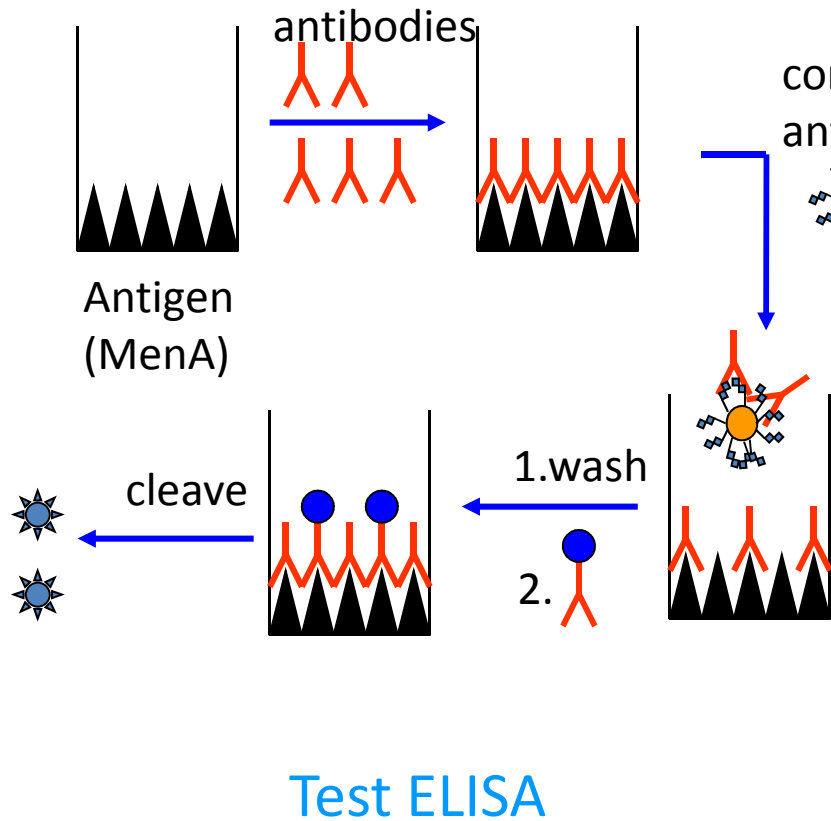
a polyvalent interaction



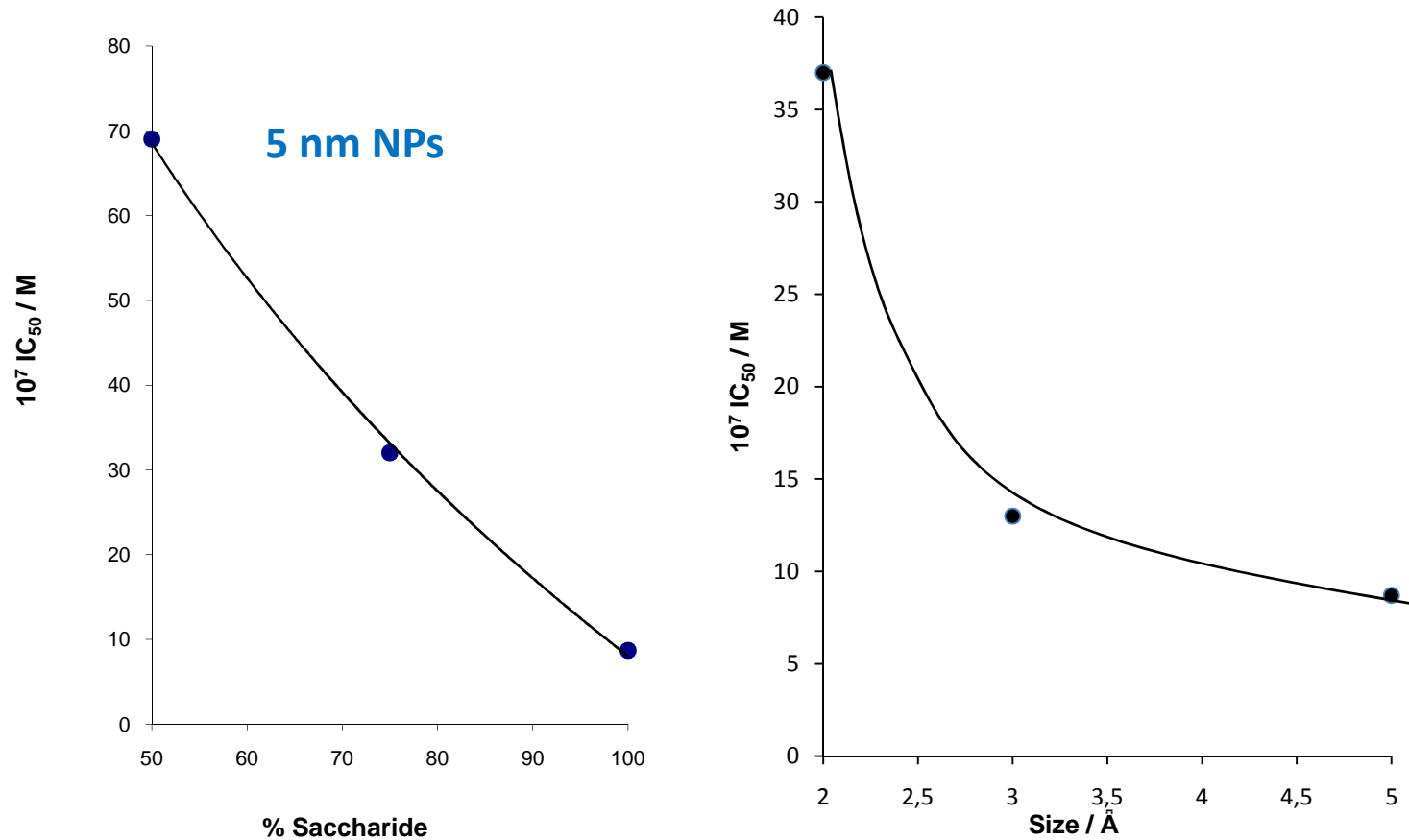
## Saccharides-functionalized nanoparticle



# Amplification of the interaction between the antibody and the saccharide-functionalized nanoparticle



The strength of the interaction depends on the density of the saccharides on the monolayer and on the size of the nanoparticle: the largest 100% functionalized nanoparticles show the strongest binding with the antibodies



**Di-saccharide-functionalized NPs**