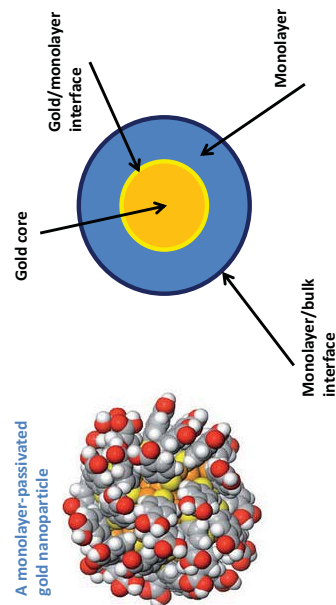
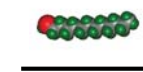


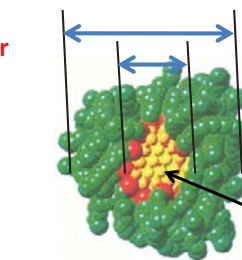


Striptease of a gold nanoparticle...

The gold core and the surrounding monolayer



functional unit

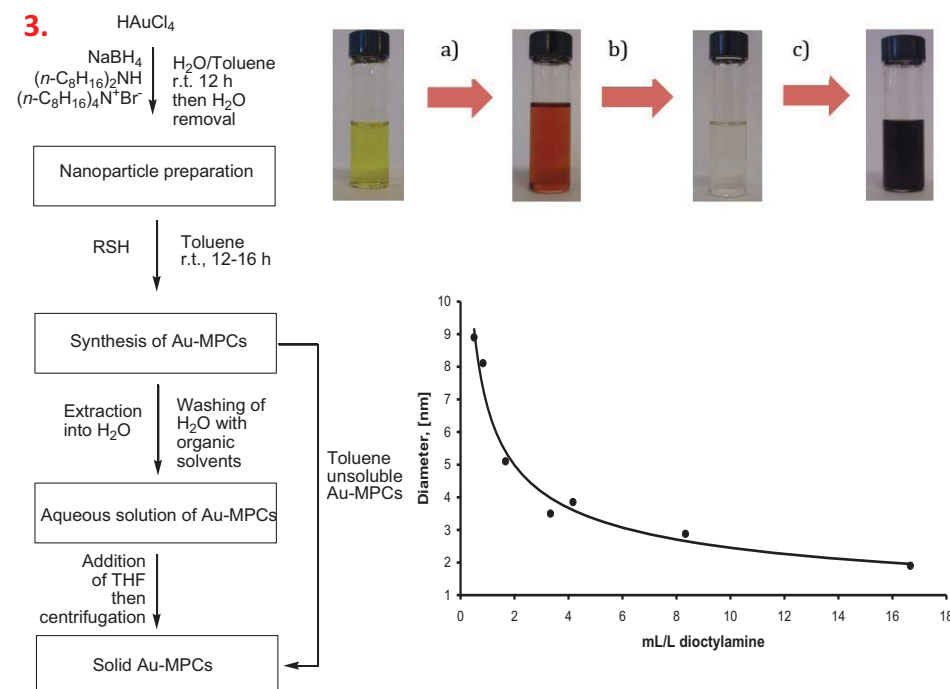


Gold core

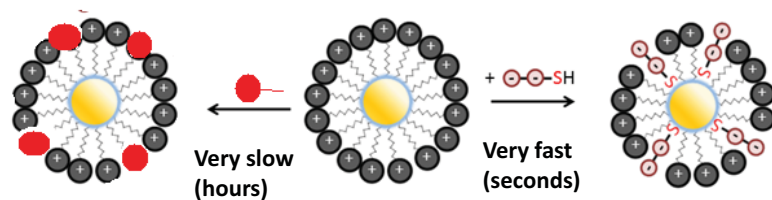
A multivalent aggregate:
its properties can be exploited to prepare
enzyme-like systems, functional proteins,
antigens, nanodrugs...

General strategies for the preparation of AuNp	Pros	Cons
1. Citrate reduction of Au(III) salts followed by passivation (Turkevich)	Very simple	Little control on size and dispersion
2. Two-phase NaBH_4 reduction with PTC catalyst and in situ passivation (Murray)	Relatively good control of size and distribution; excellent for lipophilic monolayer	Not suitable for hydrophilic monolayers; contamination by PTC catalyst
3. Secondary amine pre-passivation under phase transfer (our procedure)	Excellent control of size and distribution and of monolayer composition; suitable for hydrophilic monolayers	Contamination by secondary amines

In all cases ligand exchange is possible (post modification)



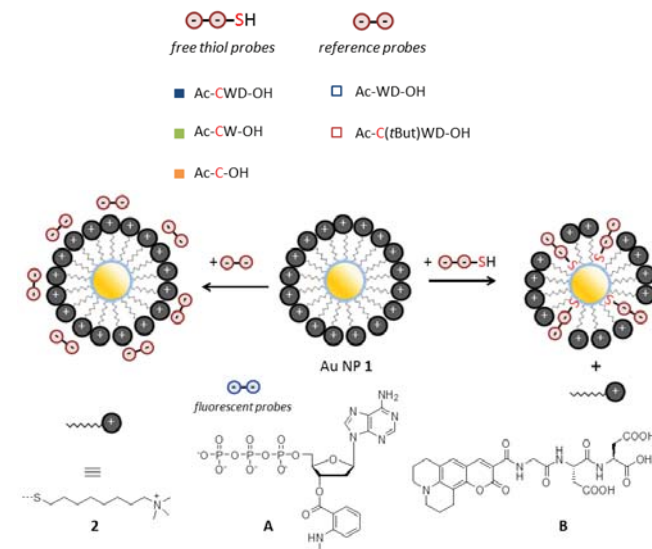
Ligand exchange rate depends on concentration on the surface of the monolayer



In any case it never reaches 100% exchange

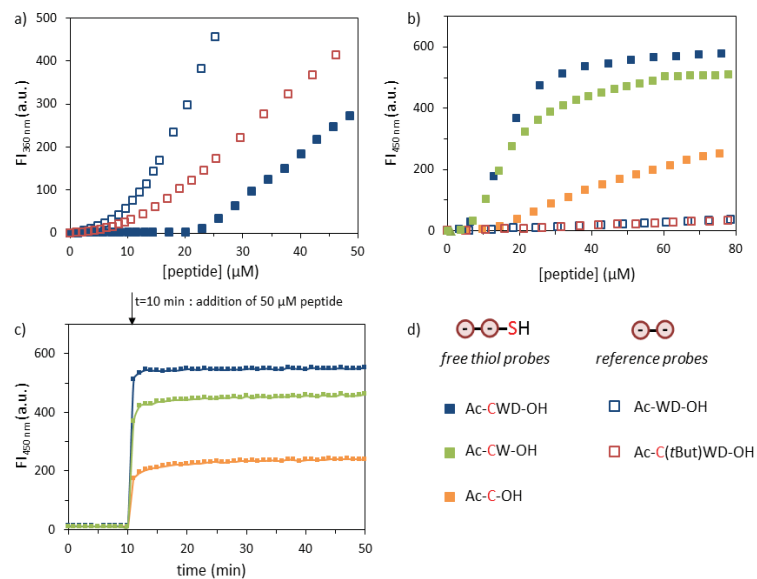
5

Ligand exchange requires thiol for the anchoring to the gold surface



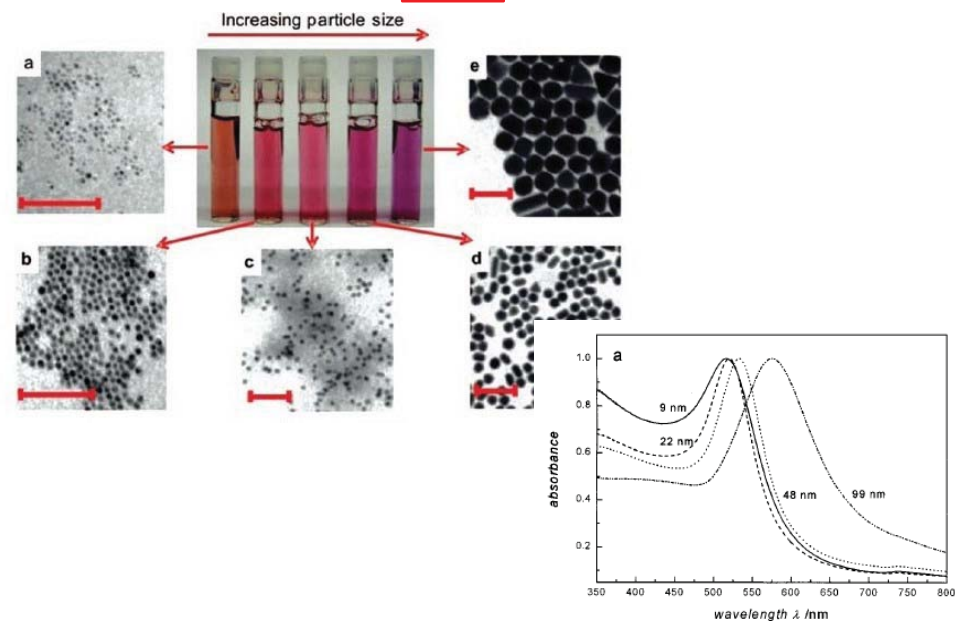
6

Probing the adhesion and the exchange



7

The electronic properties of the gold core

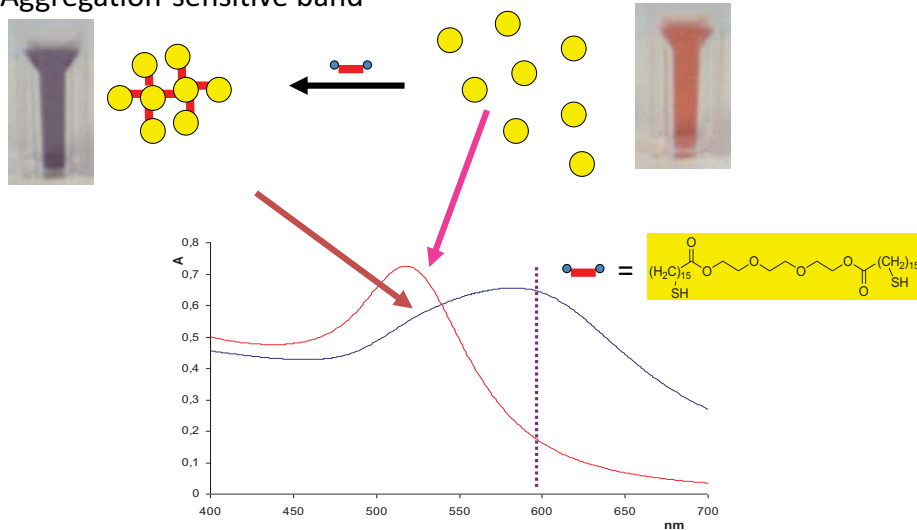


The plasmon resonance band absorbs > 500 nm and is size-dependent

8

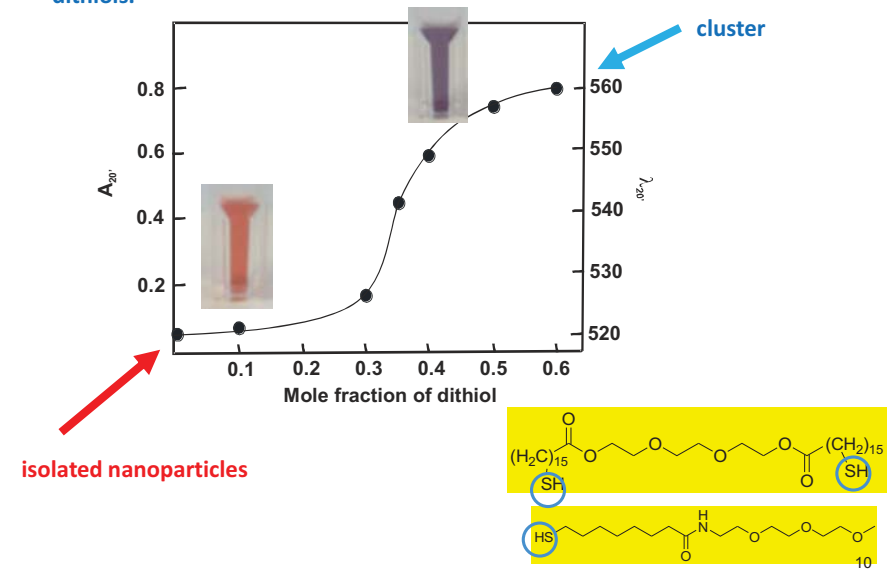
Gold colloids with diameter >3.5 nm: plasmon resonance

Aggregation-sensitive band



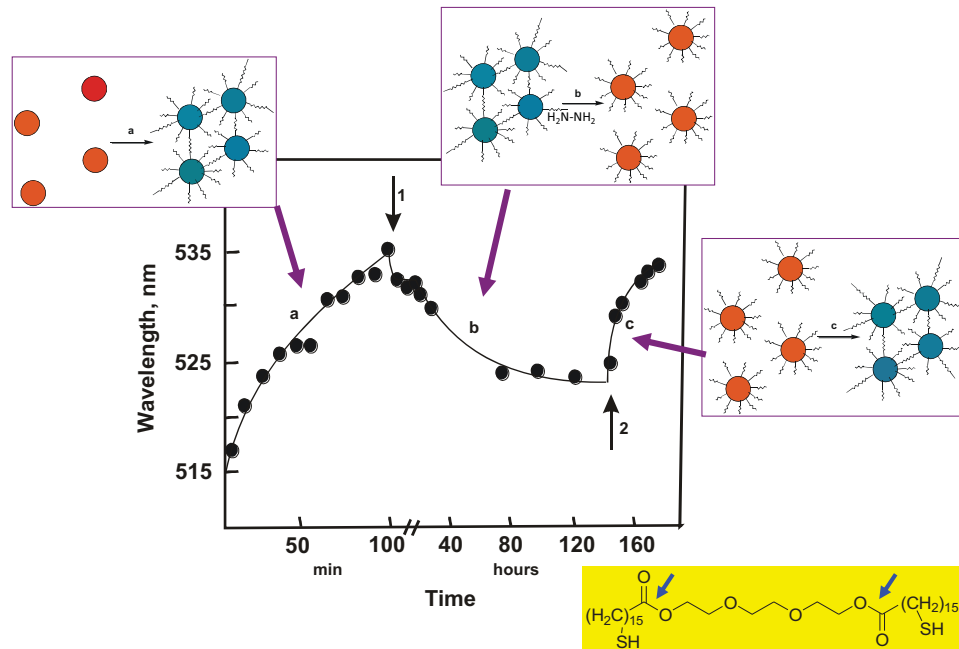
9

The aggregation process is cooperative as shown by the addition of a mixture of mono- and dithiols.



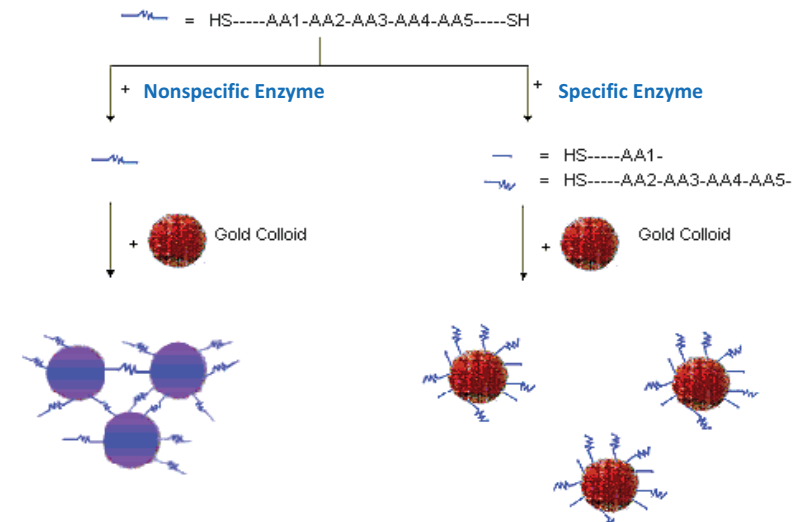
12

Aggregation de-aggregation experiment



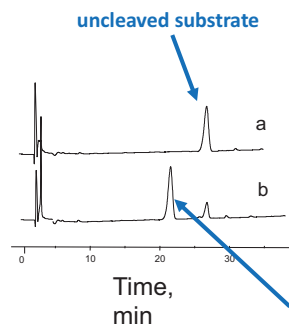
11

Indirect test for the presence of a protease

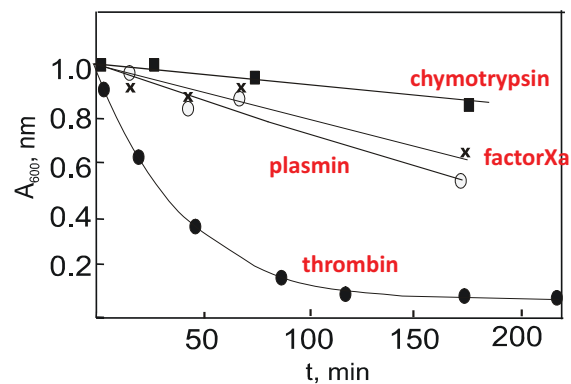


1. Test for thrombin

Substrate: Cys (SAC, NAc)-Gly-(D)Phe-Pro-Arg-Gly-Cys (SAC)

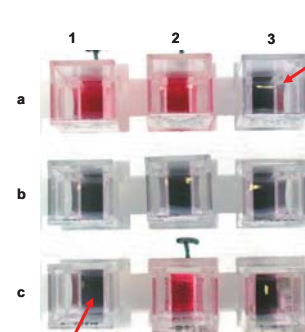


Ac-Cys(S-Ac)-Gly-(D)Phe-Pro-Arg-OH



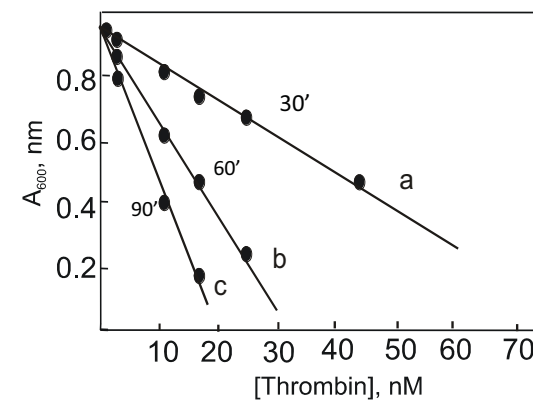
13

Guess where thrombin is?



none

[peptide]=62 μ M
[protease]=44.5 nM
pH8, 25°C, 60 min



Quantitative determination

14

2. Test for lethal factor (anthrax protease component)

15AA-3Ac

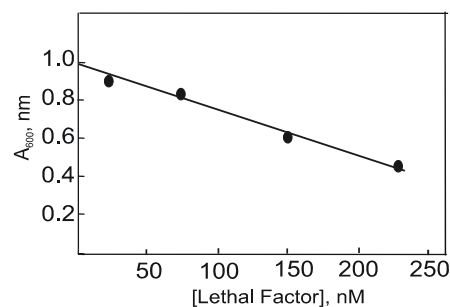
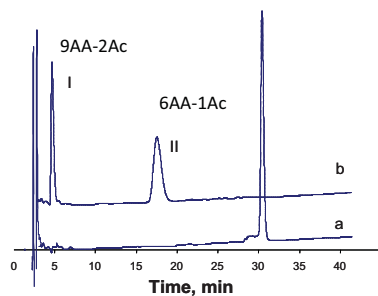
(N-Ac)C(S-Ac)-L-R-R-R-R-V-Y-P-Y-P-(Nor)L-E-L-C(S-Ac)-OH

LF

9AA-2Ac

6AA-1Ac

(N-Ac)C(S-Ac)-L-R-R-R-R-V-Y-P-OH + H-Y-P-(Nor)L-E-L-C(S-Ac)-OH

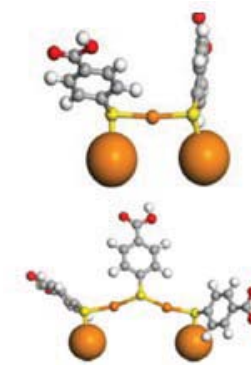
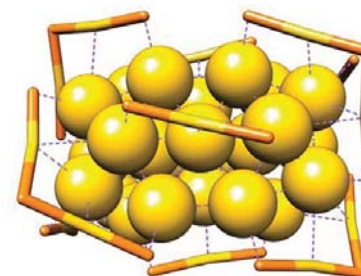


Quantitative determination

15

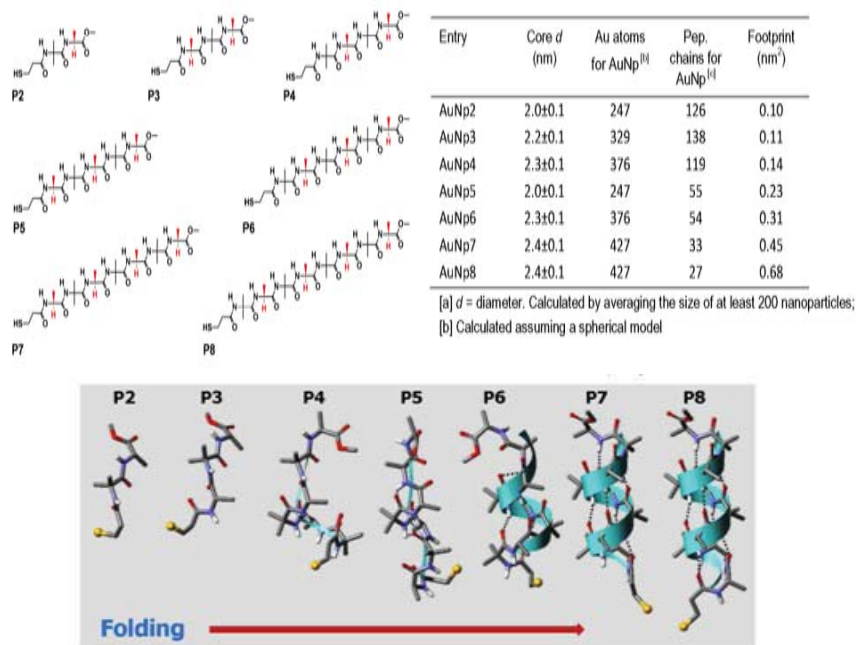
The chiral properties of the gold core:

- from the arrangement of atoms;
- from the surface Au atoms and -SR



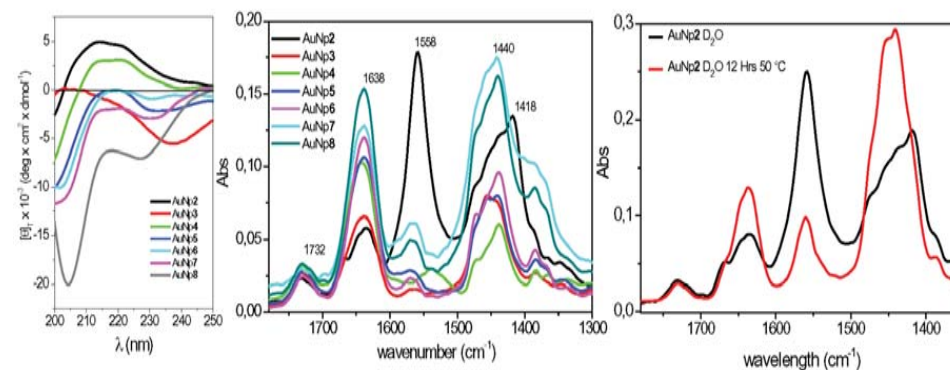
16

The gold surface of peptide-passivated AuNp



17

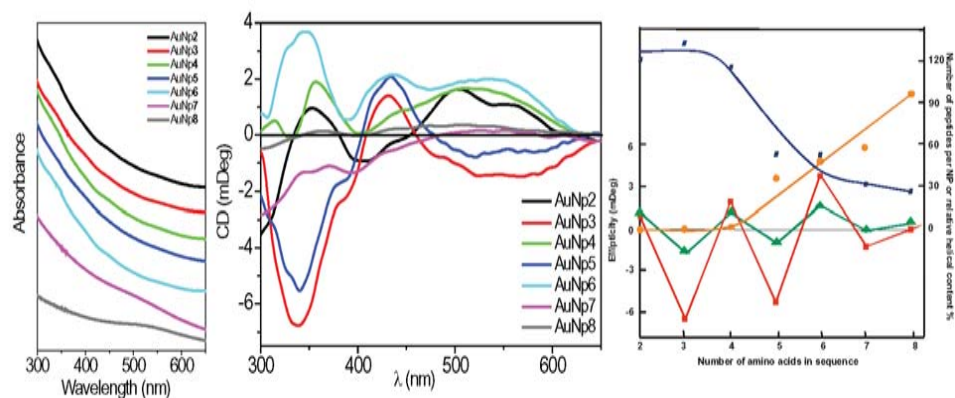
ECD and FT-IR properties once bound to the gold surface



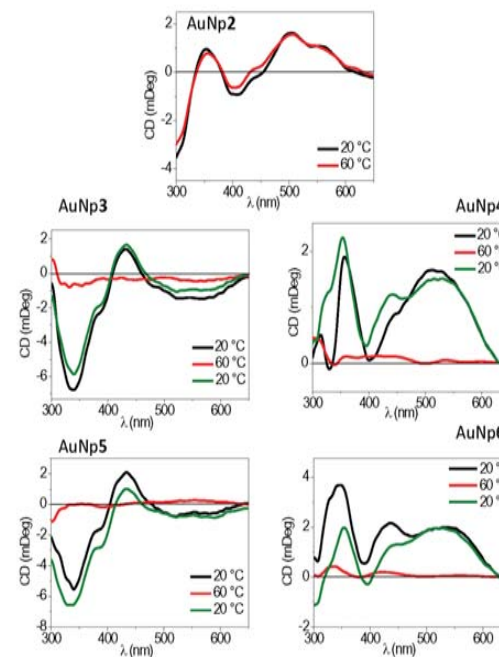
With A. Moretto and F. Mancin, ACS Nano, 2013

18

Chiroptical properties



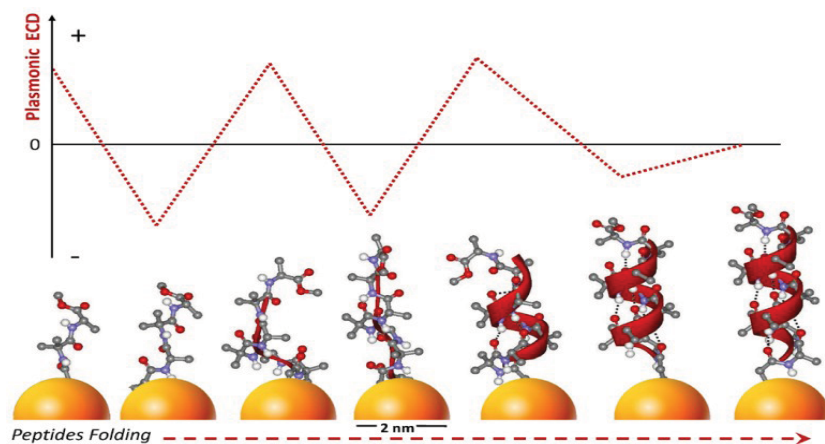
19



Chiroptical properties in the plasmon resonance absorption wavelength are temperature dependent in a reversible way

20

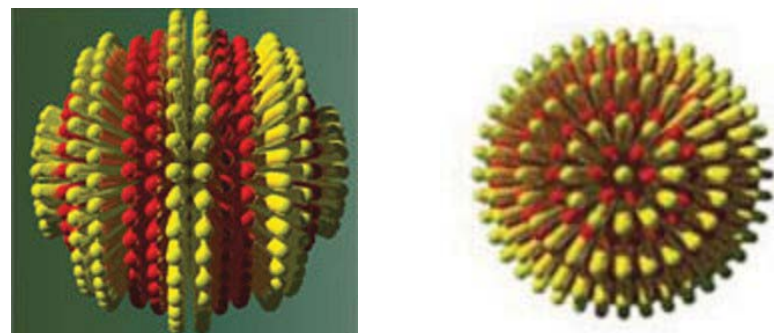
Gold surface vs bulk solvent interaction



With A. Moretto and F. Mancin, in press

21

The monolayer: control of its topology

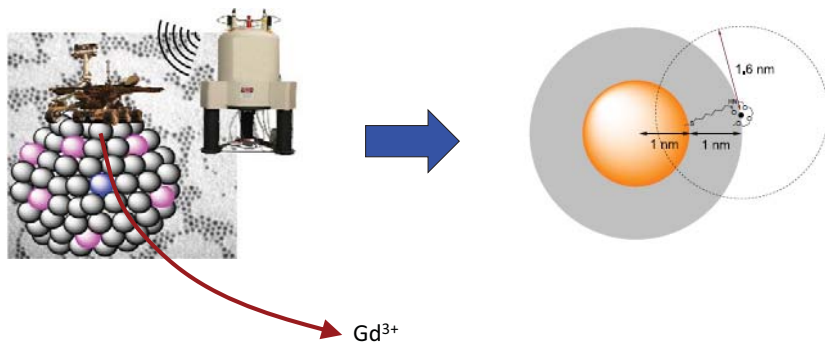


22

Tools to investigate the monolayer structure

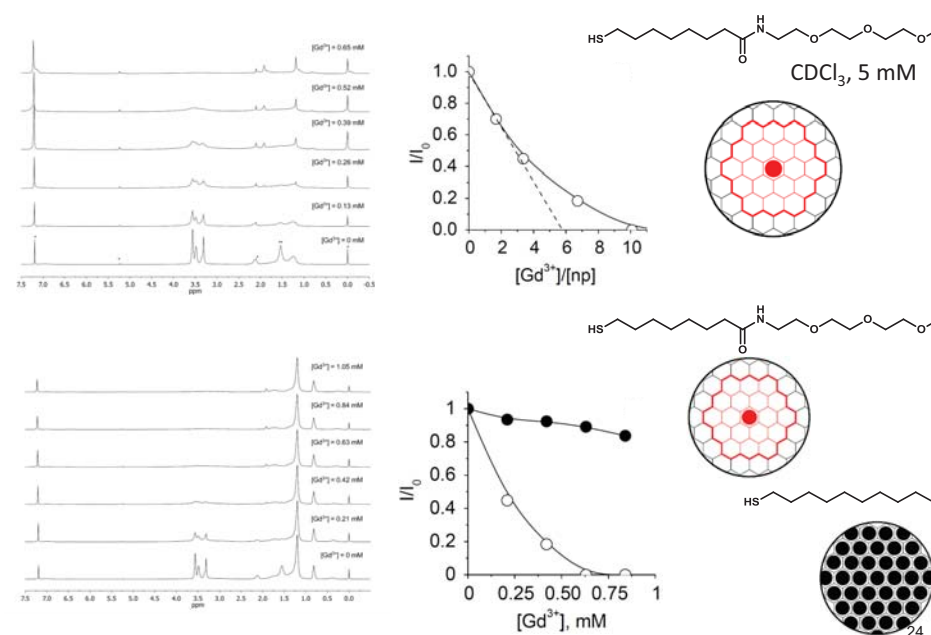
- STM (F. Stellacci, MIT)
- EPR (M. Lucarini, Bologna)
- Catalytic activity (L.J. Prins, Padova)
- Surface binding (L.J. Prins, Padova)

NMR ?



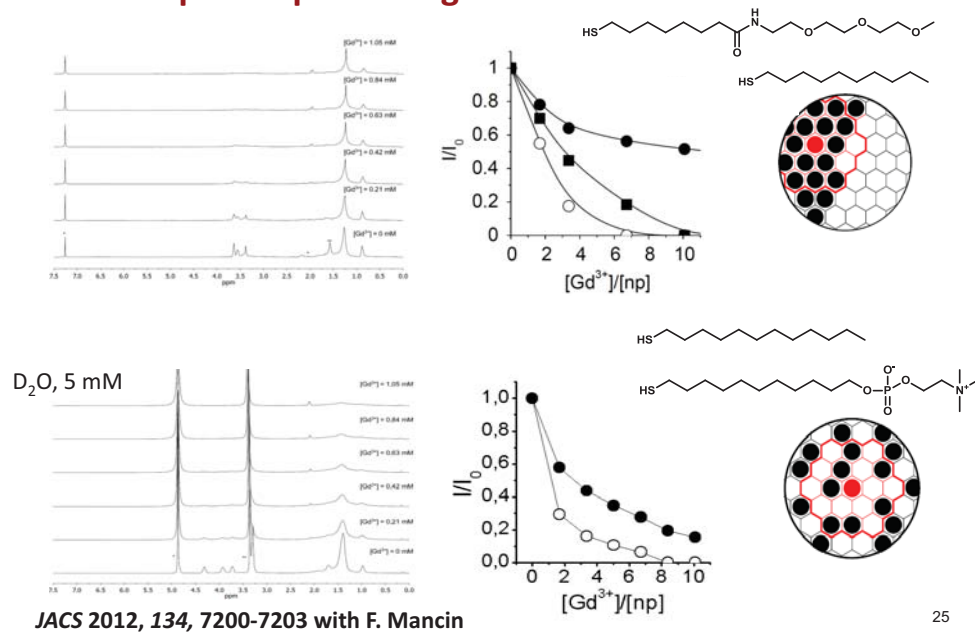
23

Gd³⁺ PRE (paramagnetic relaxation enhancement) probe



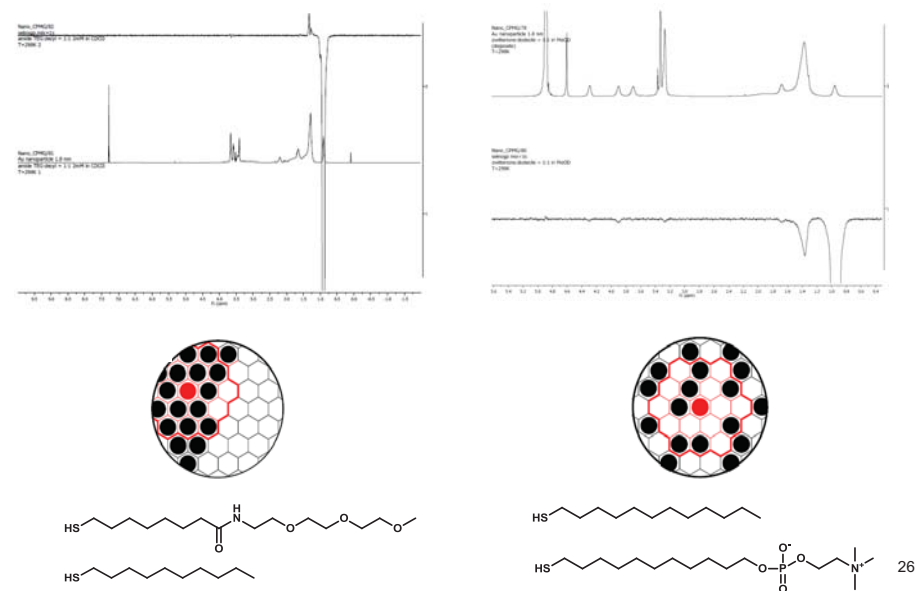
24

Gd³⁺ PRE probe: patterning



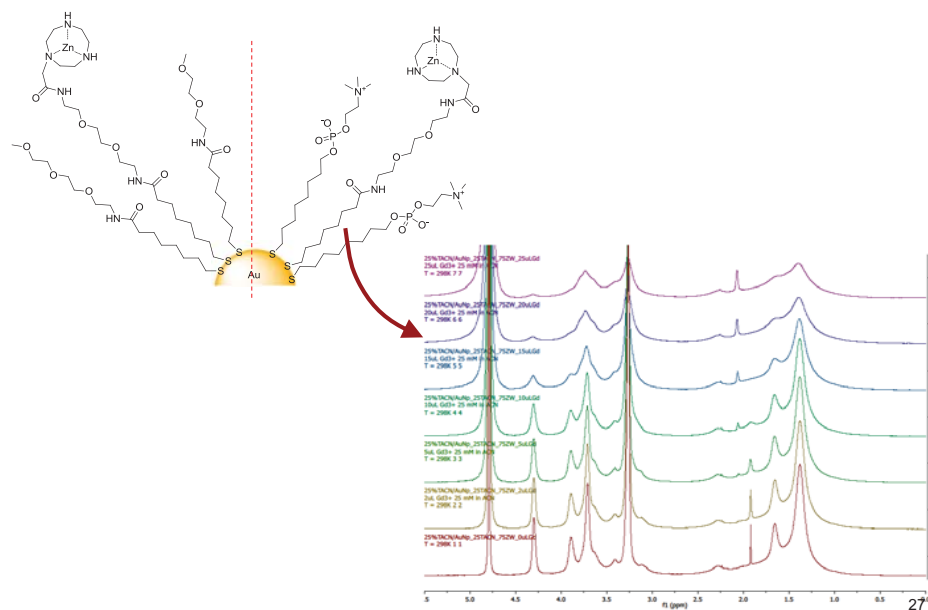
25

Controls: NOE



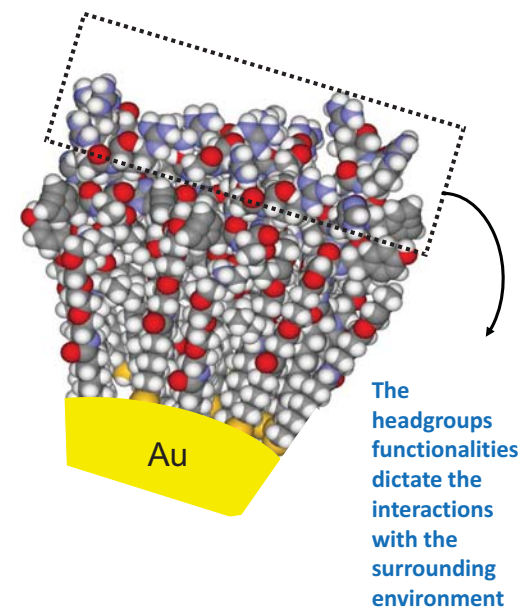
26

Gd³⁺ PRE probe: patterning



27

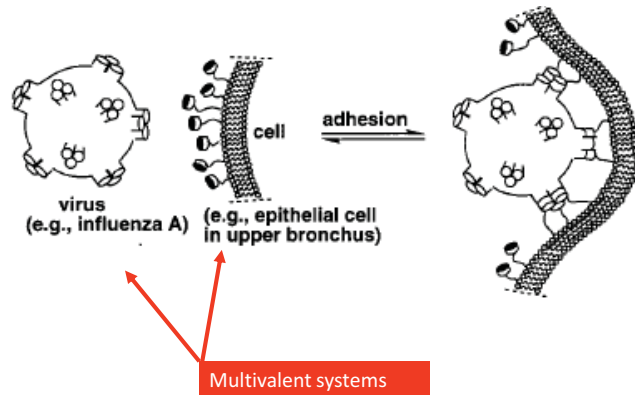
a more realistic representation of the mixed monolayer covering the gold core



28

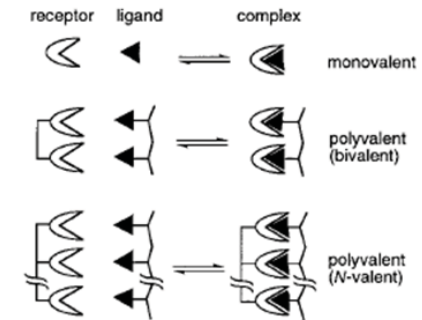
The monolayer interface: Multivalency/recognition

A poor binding is transformed into a strong one by taking advantage of multiple weak interactions



29

Cooperativity in recognition



$$\alpha = \frac{\lg(K_N^{multi})}{\lg(K_{inter}^{mono}) + \lg(K_{intra}^{mono})^{N-1}} \quad (1)$$

30

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 $\alpha > 1$ as the typical situation.

On the contrary this is a rare situation!

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

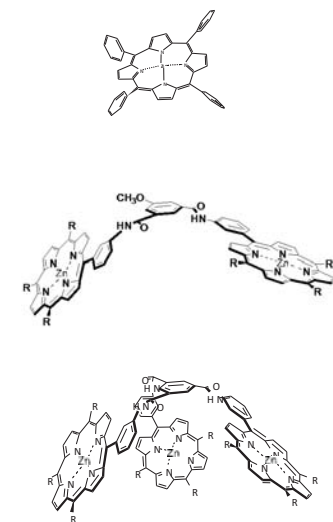
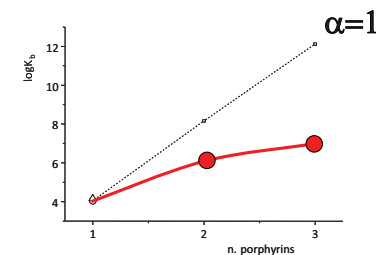
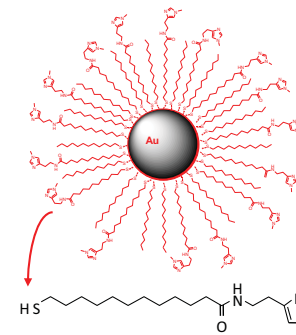
Don't be fooled by the overall strength of binding which is easily larger:

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

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

31

Cooperativity in multivalent systems

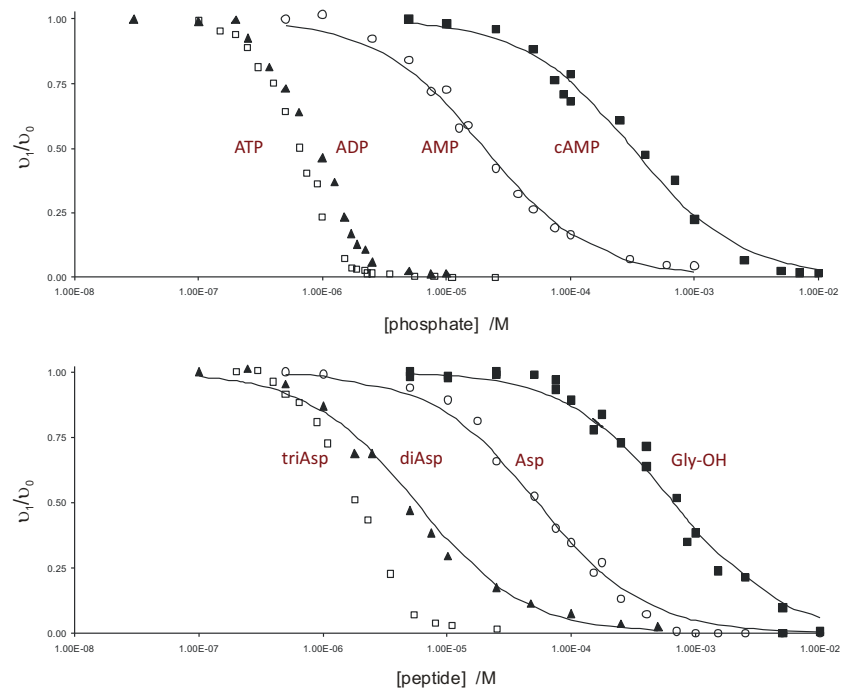


$\alpha=0.75$
 $\beta=90$

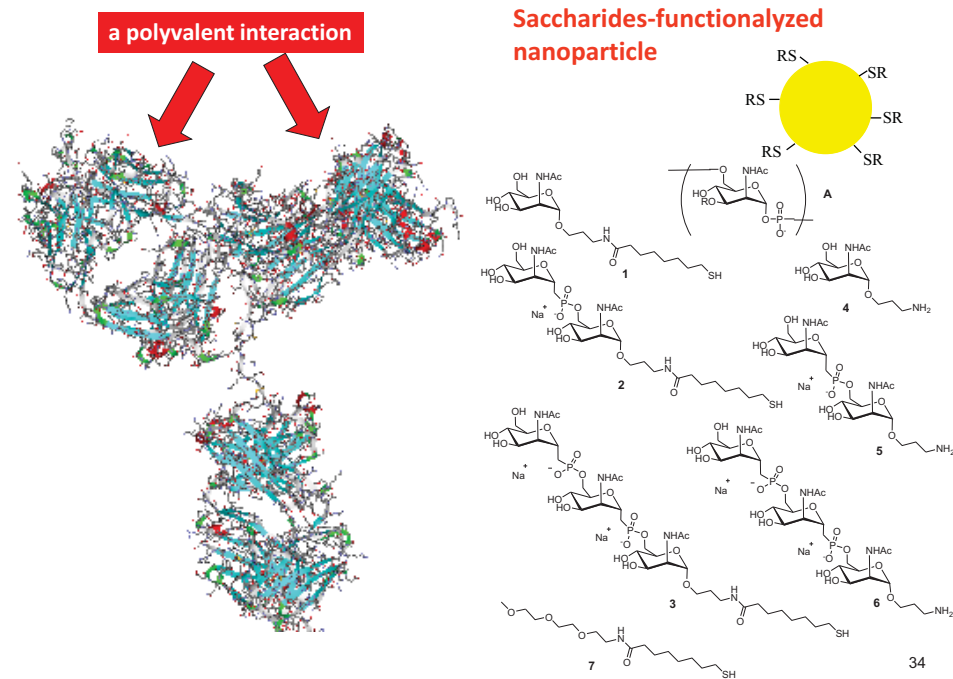
$\alpha=0.58$
 $\beta=1000$

32

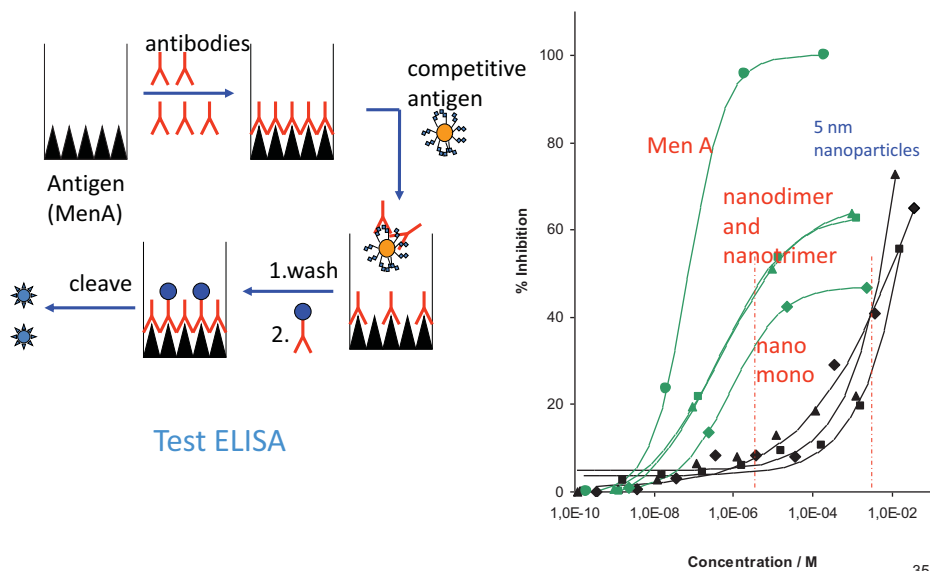
Binding of a polyanion to a cationic Au-NP increases with the increase of charge



33

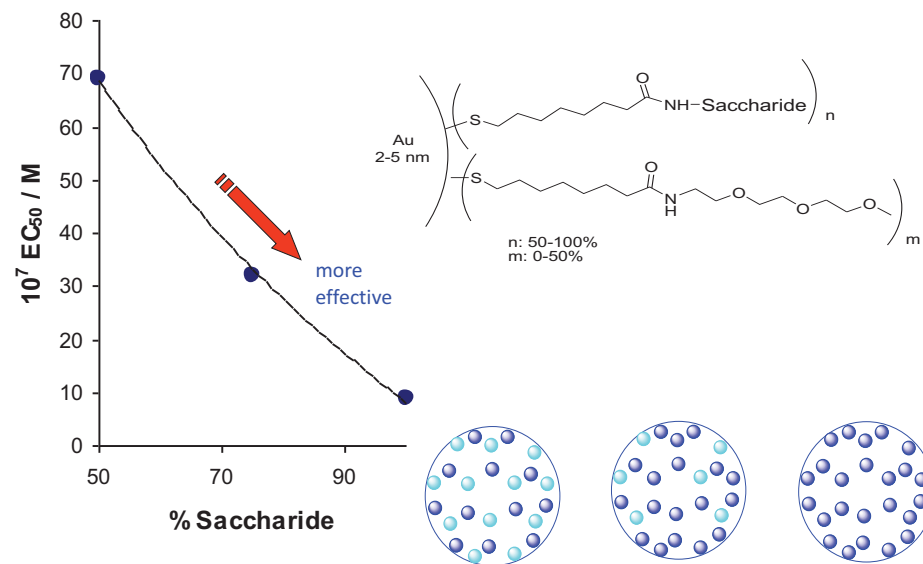


Amplification of the interaction between the antibody and the saccharide-functionalized nanoparticle



35

Efficiency depends on saccharide concentration on the monolayer

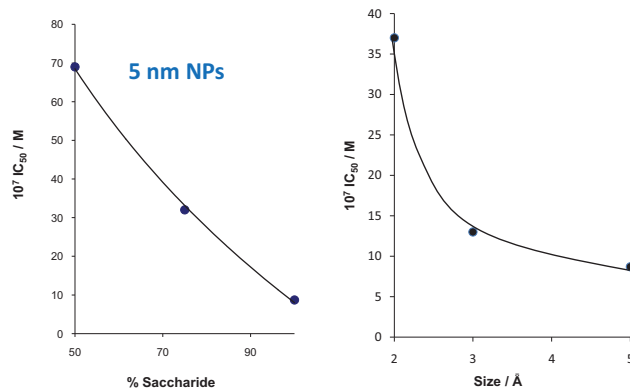


The most concentrated is the most effective

Adv. Mater. 2008, 20, 4348-4352; with Luigi Lay and Grazia Lombardi

36

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

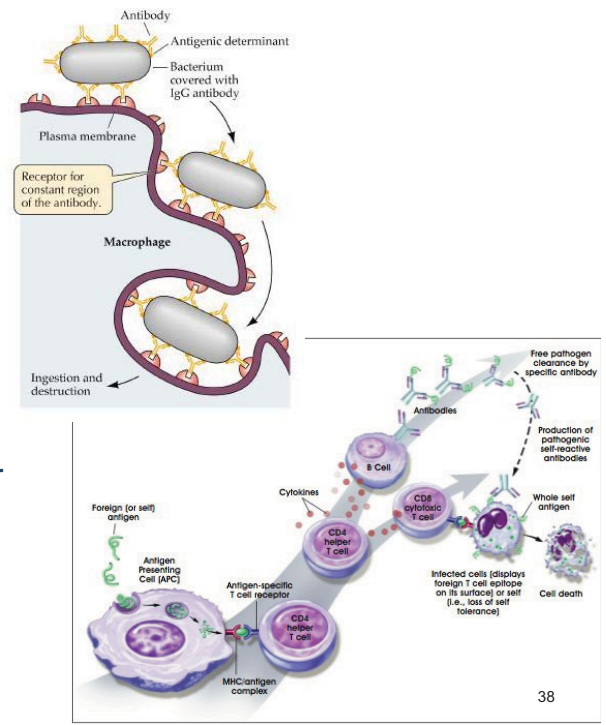


Di-saccharide-functionalized NPs

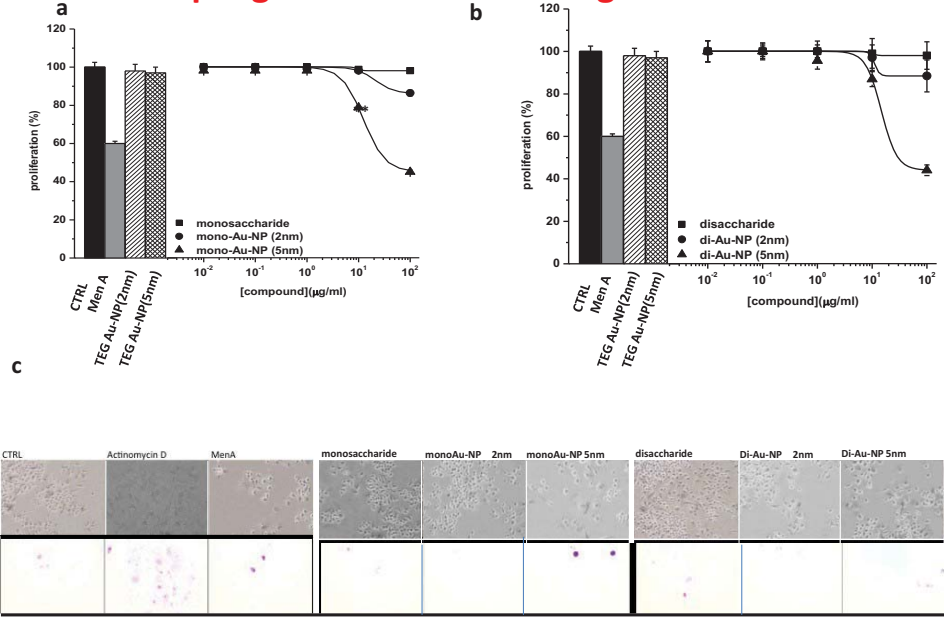
But are these Glyconanoparticles immunogenic?

For this to occur they must induce:

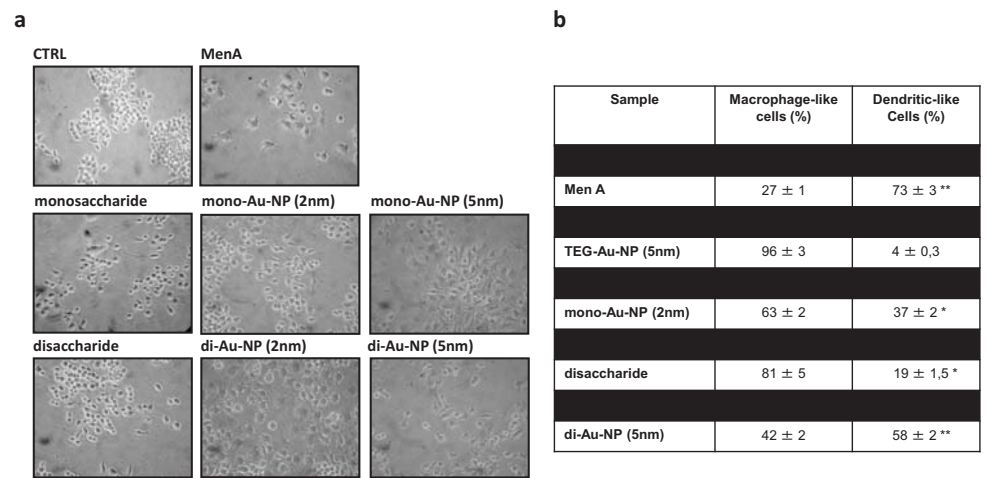
- a) the activation of macrophages;
- b) macrophages differentiation into dendritic-like cells;
- c) the activation of effector functions in the same cells
- d) the stimulation of T cell response and interleukine-2 release.

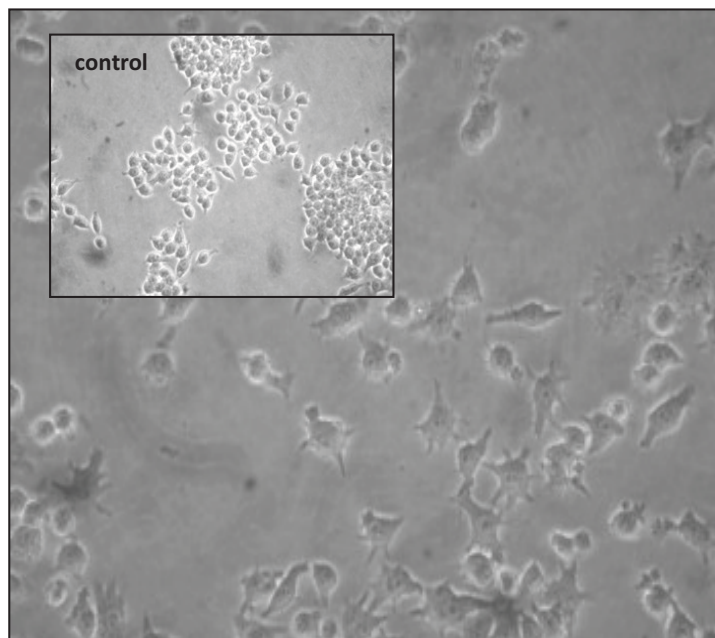


Macrophage activation and cell growth inhibition



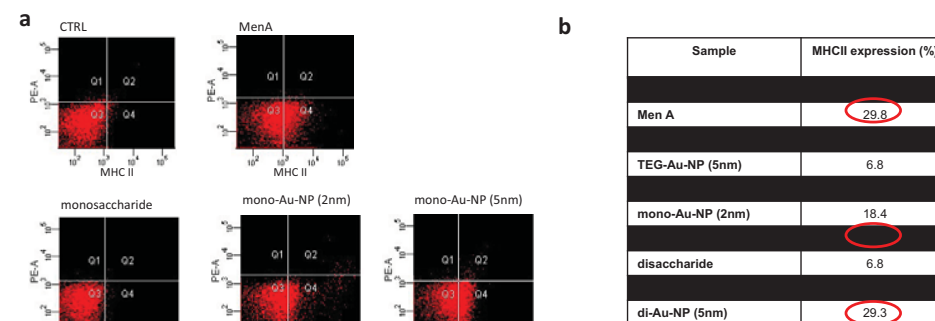
Macrophage differentiation into dendritic cells





41

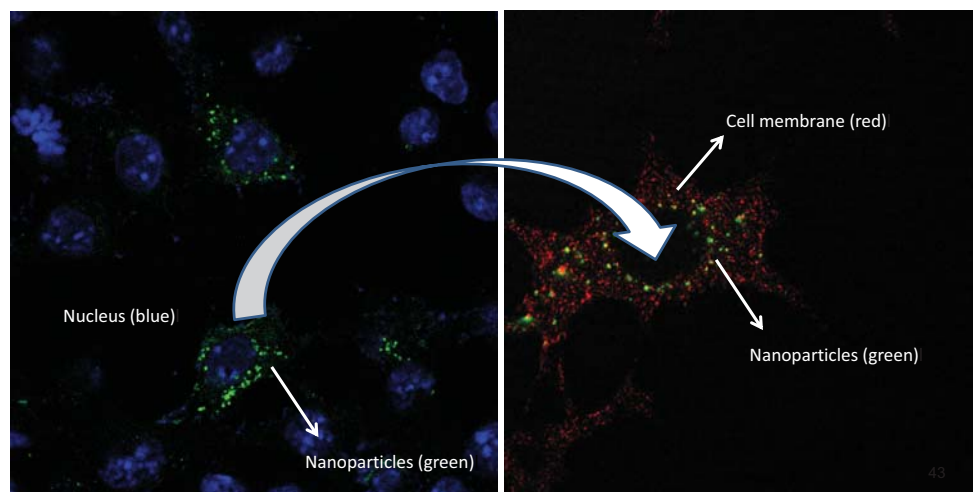
Activation of effector functions in the dendritic-like cells



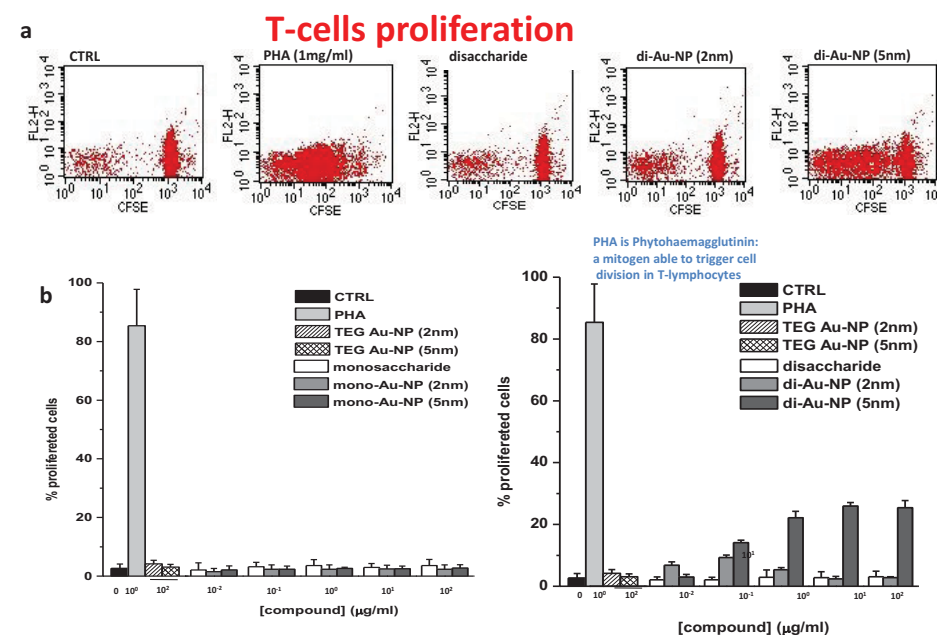
Determination of the membrane expression of class II major histocompatibility complex molecules (MHCII), which represents a typical marker of mature antigen-presenting cells

42

The nanoparticles enter into the cell crossing the macrophages membrane. The nanoparticles are in the cytoplasm and surround the nucleus



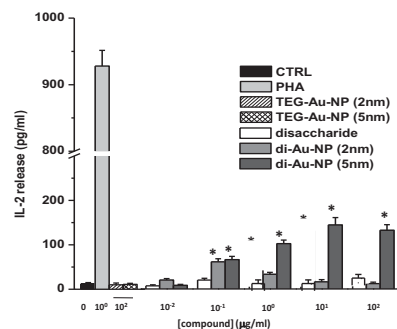
43



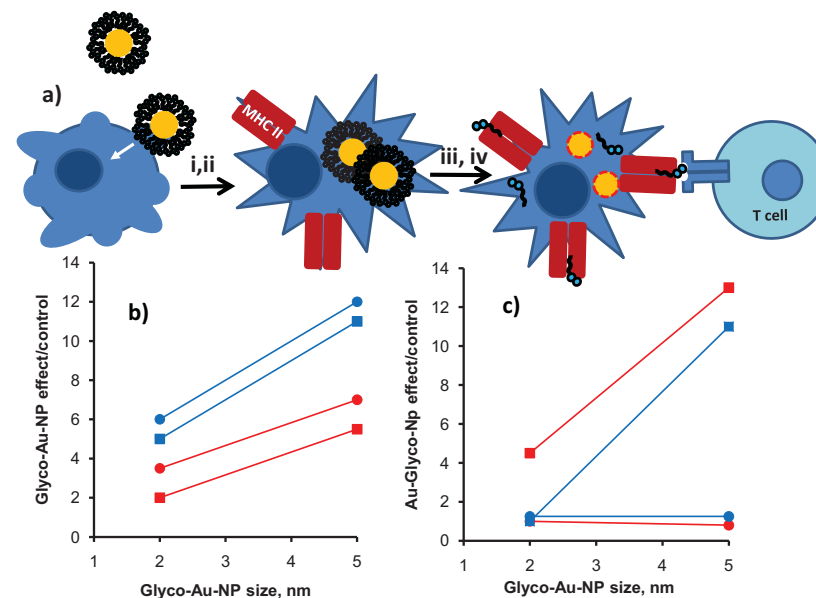
The generated T cell responses may be essential for developing a functional T cell memory following vaccination.

44

....and Interleukin 2 (IL-2) release



Antigen binding to the T cell receptor stimulates the secretion of IL-2, and the expression of IL-2 receptors IL-2R. IL-2 is necessary for the development of T cell immunologic memory



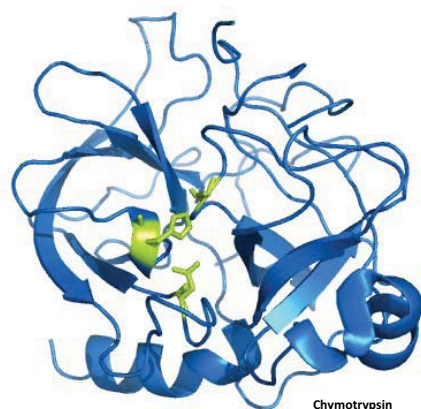
45

Nanoscale, 2013, 5, 392-402 with G. Lombardi and L. Lay

46

The monolayer interface: Multivalency/catalysis

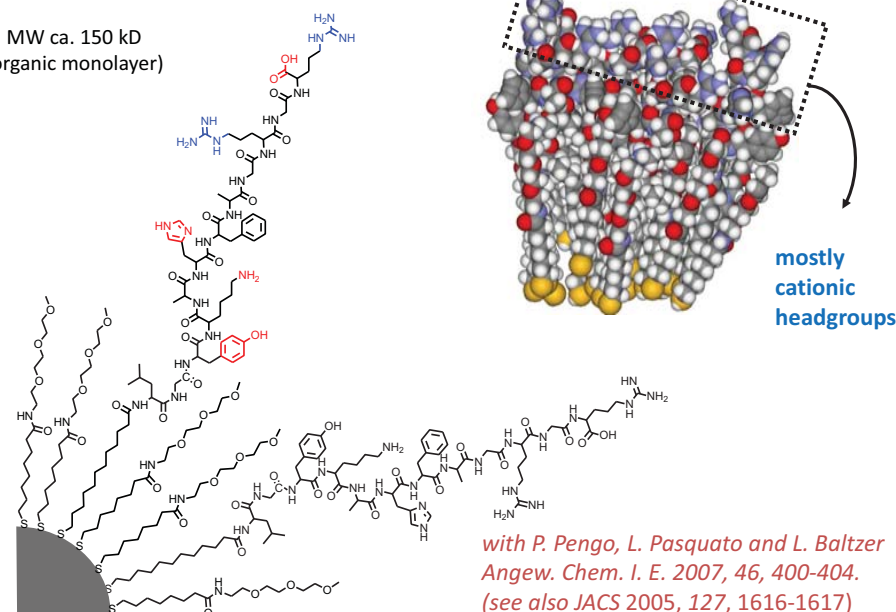
In the catalytic site of a protease several functional groups operate in a concerted fashion



47

A peptide-functionalized Au-NP has a protein-like structure

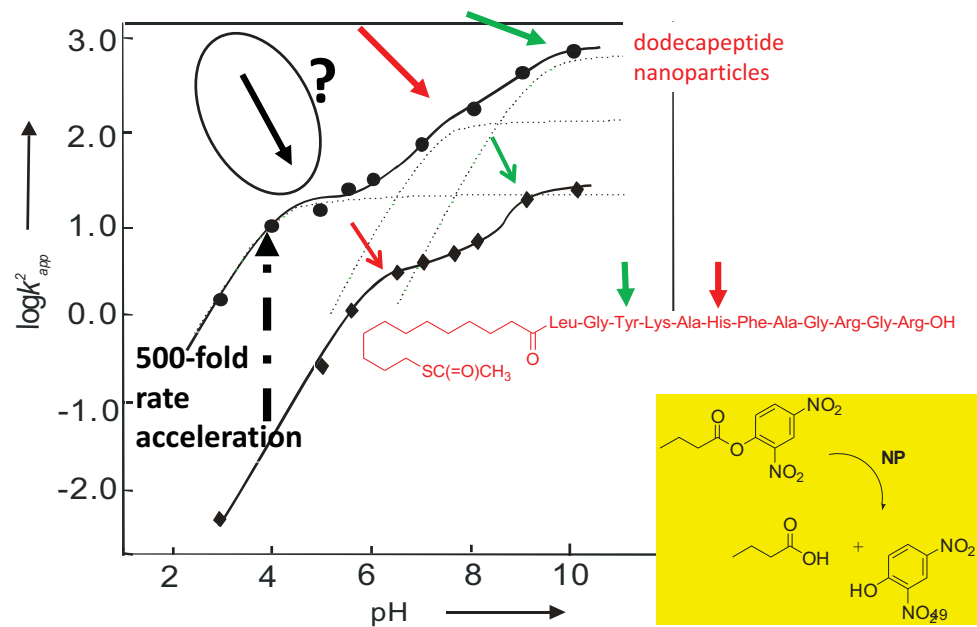
MW ca. 150 kD
(organic monolayer)



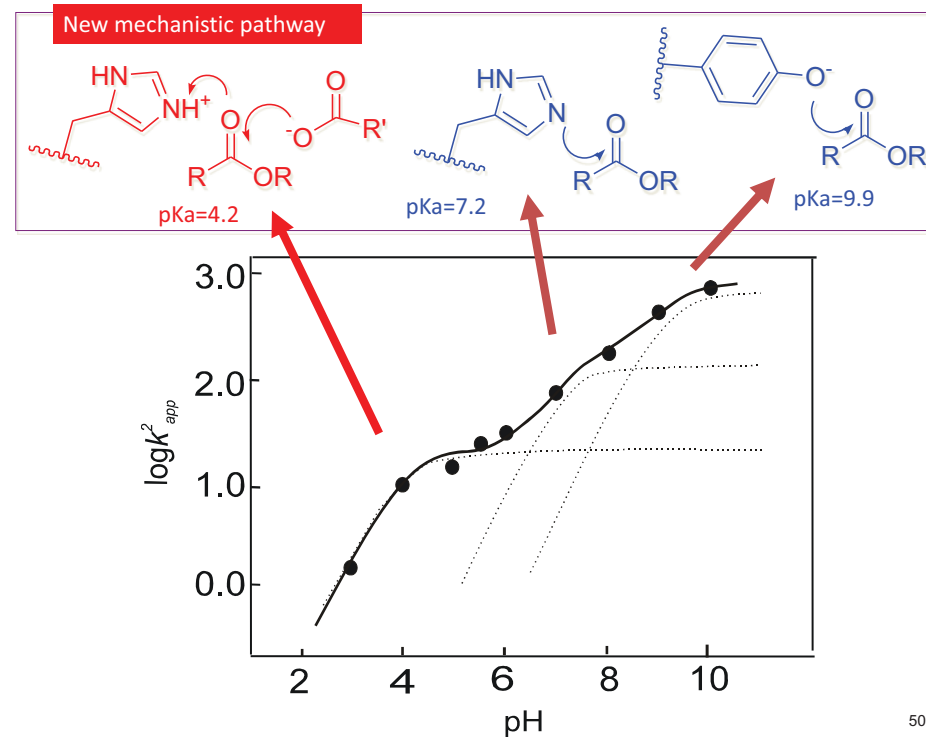
with P. Pengo, L. Pasquato and L. Baltzer
Angew. Chem. I. E. 2007, 46, 400-404.
(see also *JACS* 2005, 127, 1616-1617)

48

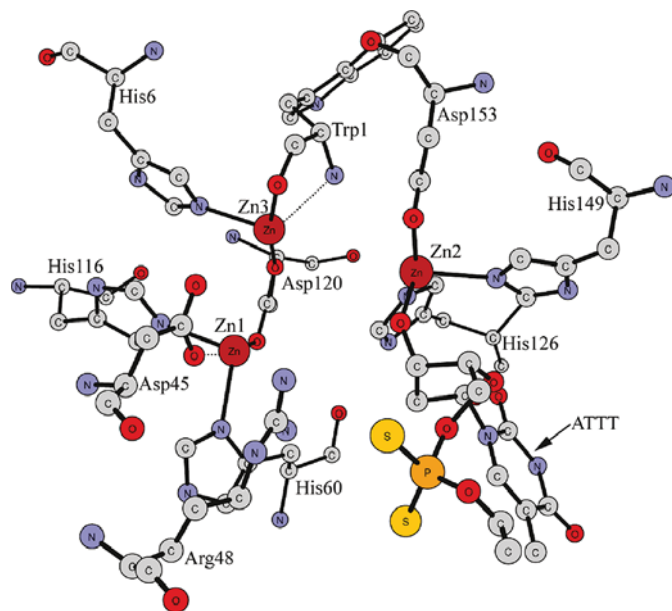
at least three nucleophilic species involved



New mechanistic pathway

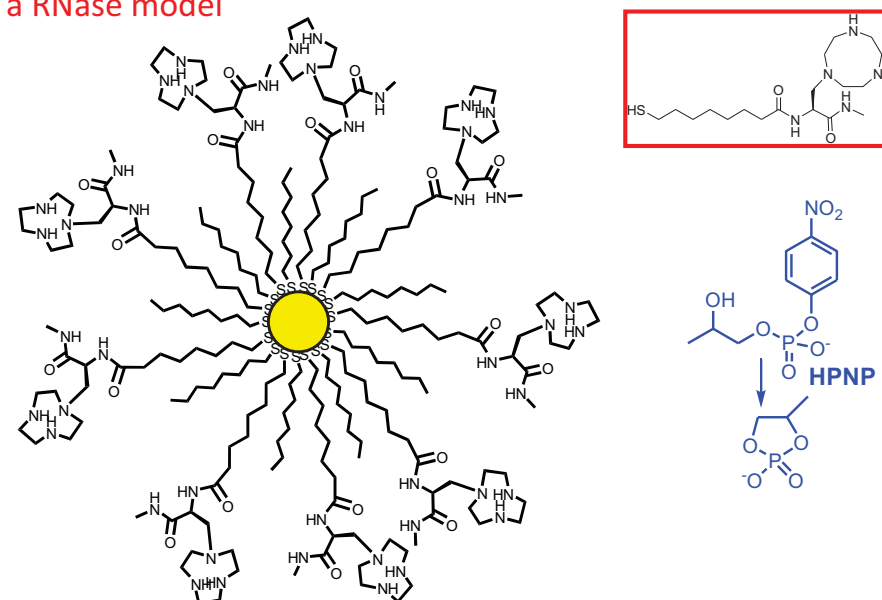


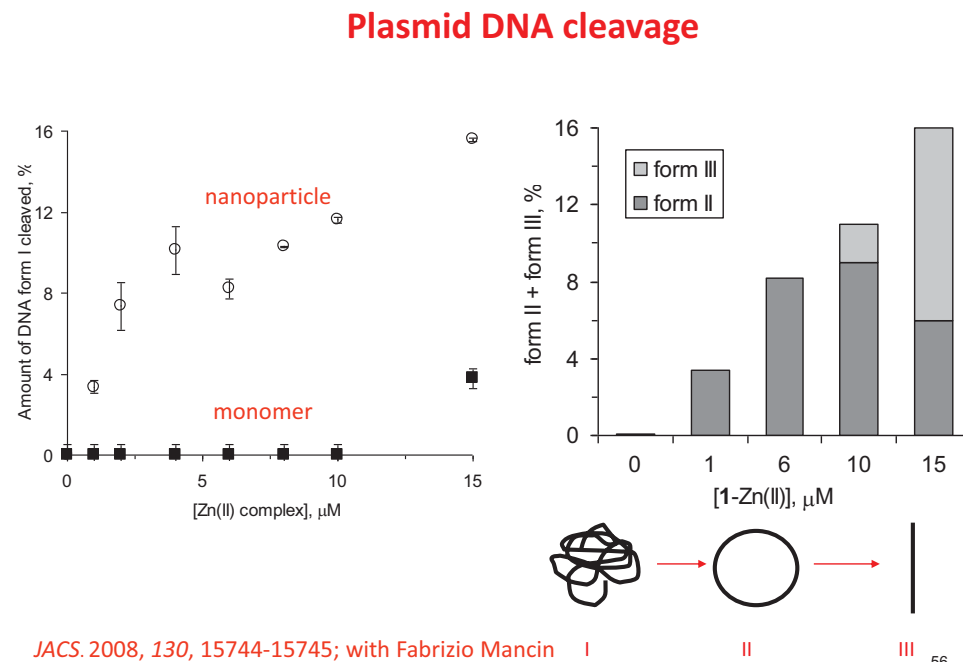
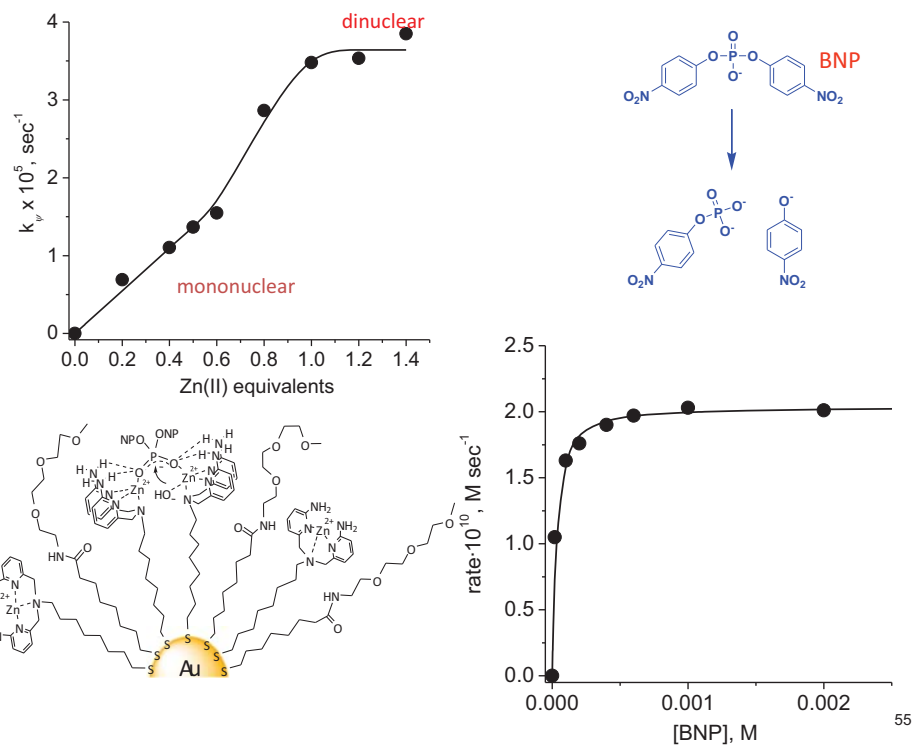
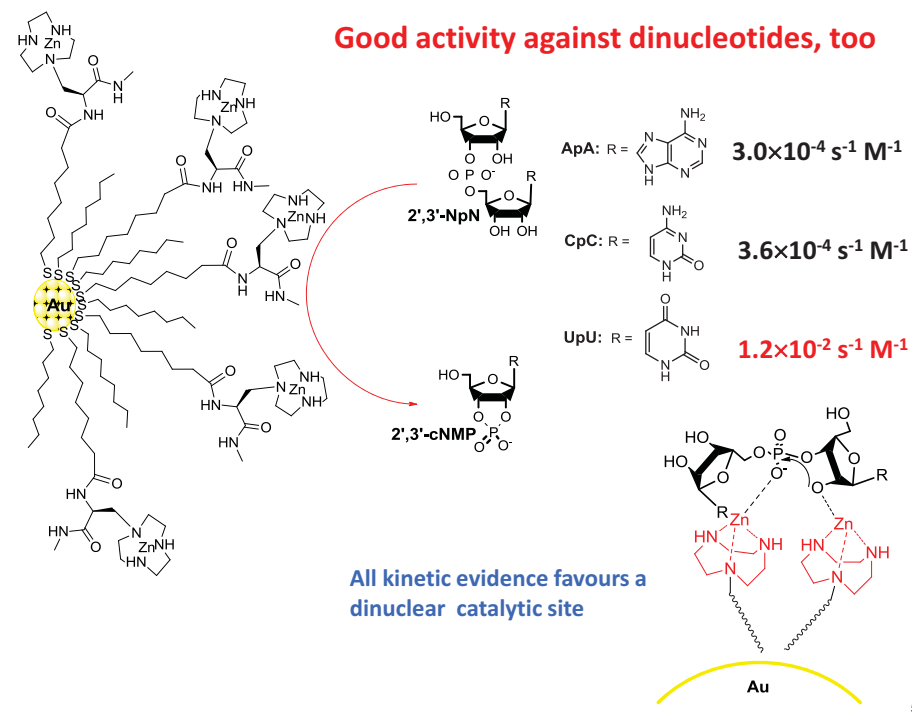
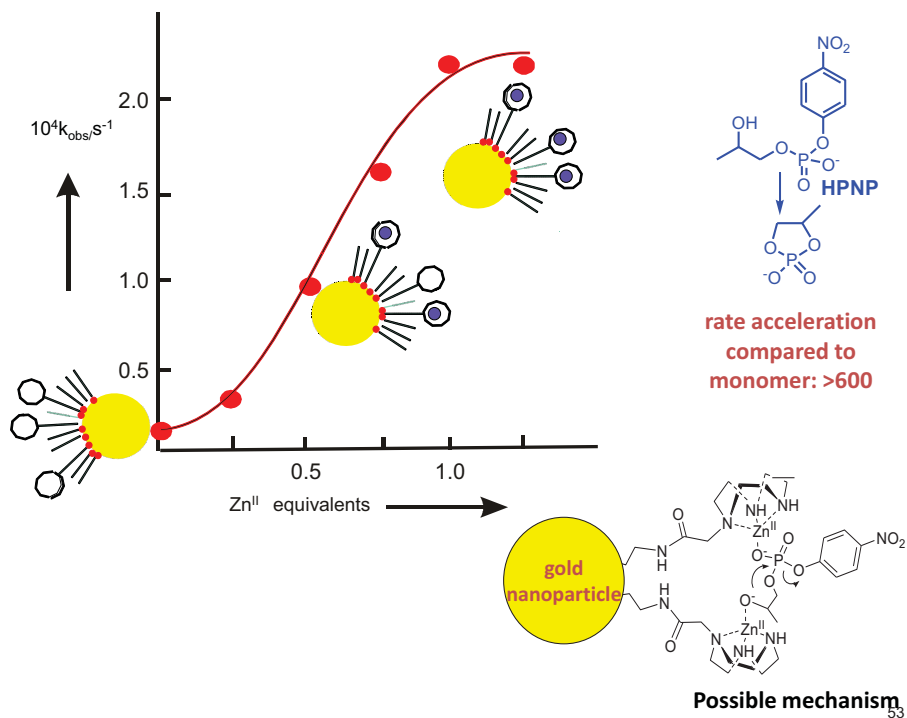
A cluster of three Zn(II) ions is present in the active site of nuclease P1



Cooperativity in catalysis

a RNase model





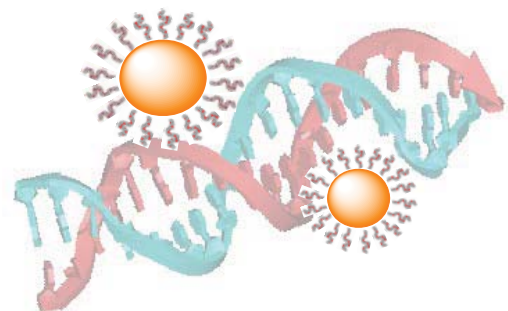
JACS. 2008, 130, 15744-15745; with Fabrizio Mancin

CHEMISTRY

Snip it with zinc

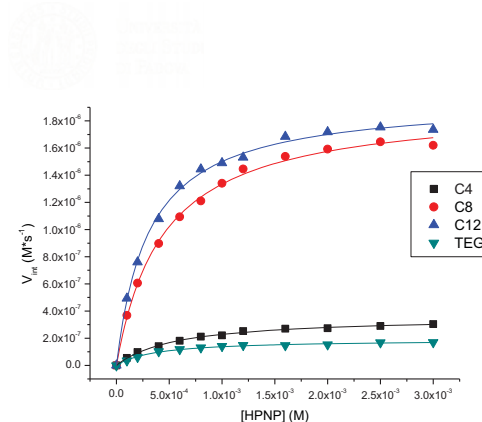
J. Am. Chem. Soc. doi:10.1021/ja801794t (2008)
The covalent bonds that string DNA's nucleotide subunits together are tough, so breaking them requires high temperatures or a catalyst. Now Fabrizio Mancin and Paolo Scrimin at the University of Padova in Italy and their colleagues describe how to make a DNA-cleaving catalyst that works 100 times faster than one of the most efficient synthetic alternatives.

Their method relies on self-assembling sulphur-containing ligands. These are spread across a surface of gold nanoparticles and attach many zinc-based catalysts to each nanoparticle. Because this generates many catalytic sites close together, the substance can cut both strands of DNA at the same point, which enzymes find easy but artificial catalysts have not previously managed.



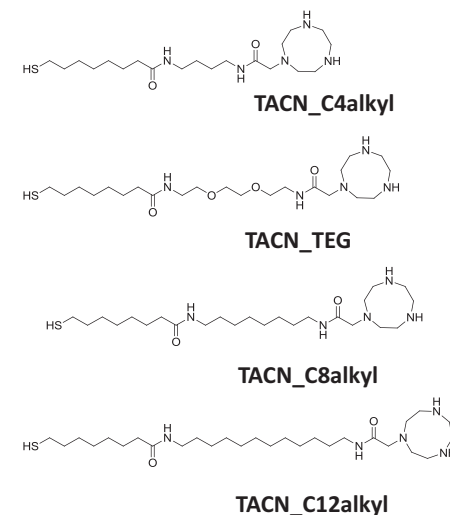
Likely the gold nanoparticles are able to interact with both strands: cooperativity and multivalency are both exploited!

57



Experimental conditions: [TACN] = 2.00×10^{-5} M;
[Zn²⁺] = 2.00×10^{-5} M, [HEPES] = 0.01 M; pH = 7.5; T = 40°C

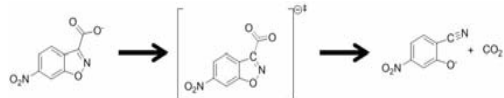
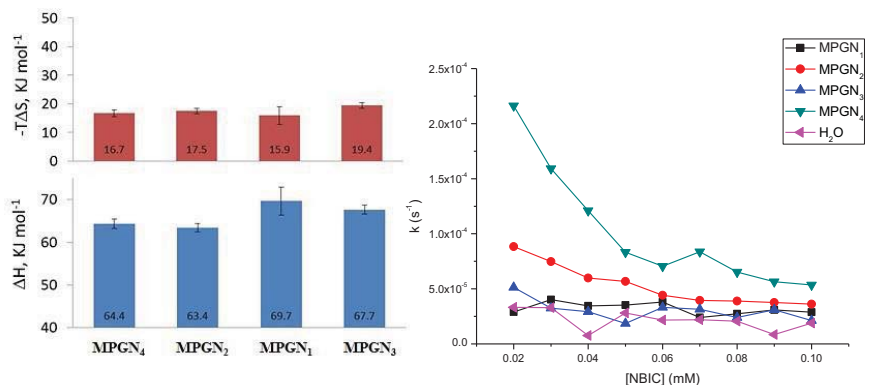
NPs	K_M [mM]	k_{cat} [$\times 10^{-3}$ s ⁻¹]	k_{cat}/K_M [M ⁻¹ s ⁻¹]
C4	0.576 ± 0.032	36.0 ± 0.7	62 ± 4
C8	0.440 ± 0.002	192.2 ± 2.7	437 ± 7
C12	0.307 ± 0.014	196.2 ± 2.2	638 ± 30
TEG	0.381 ± 0.031	19.0 ± 0.4	50 ± 4



with Marta DIEZ CASTELLNOU
J. Am. Chem. Soc. 2014, 136, 1158–1161

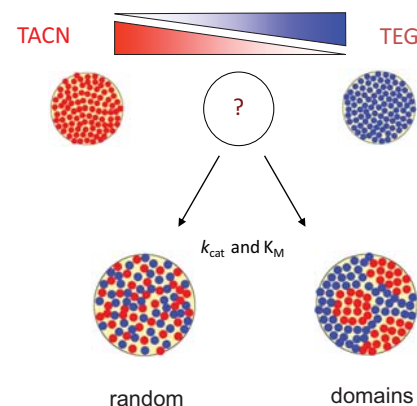
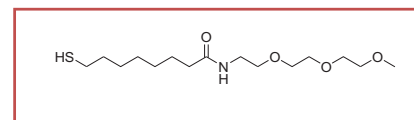
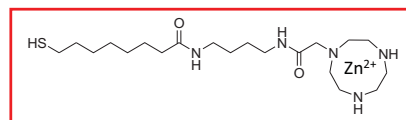
58

Enthalpic and polarity of the monolayer control



59

The source of catalysis

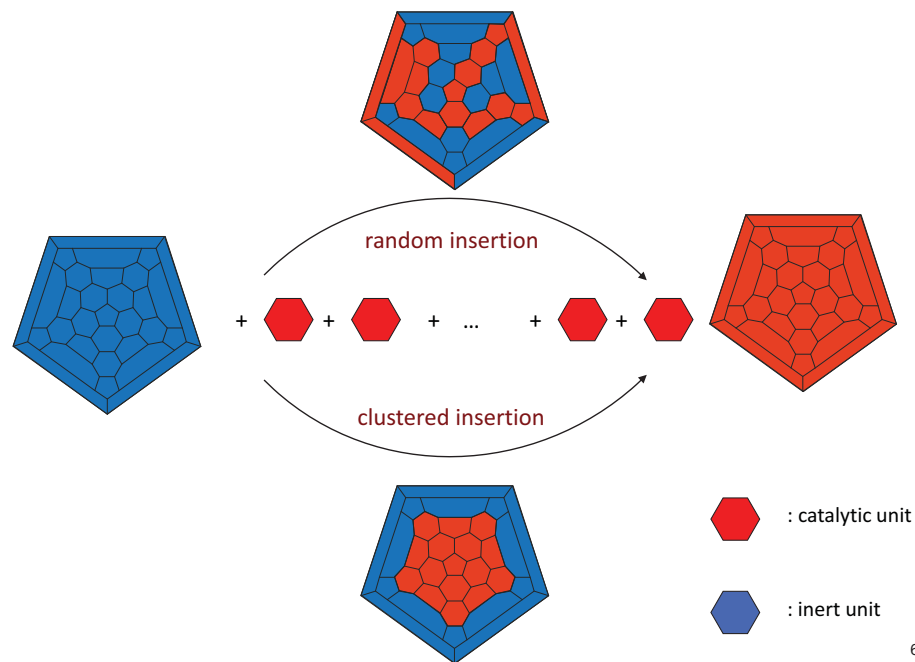


Batch	TACN (%)
I	100
II	74
III	56
IV	35
V	17
VI	10
VII	5

JACS 2008, 130, 5699–5709 and *Chem. Eur. J.* 2011, 17, 4879 – 4889 with L. Prins

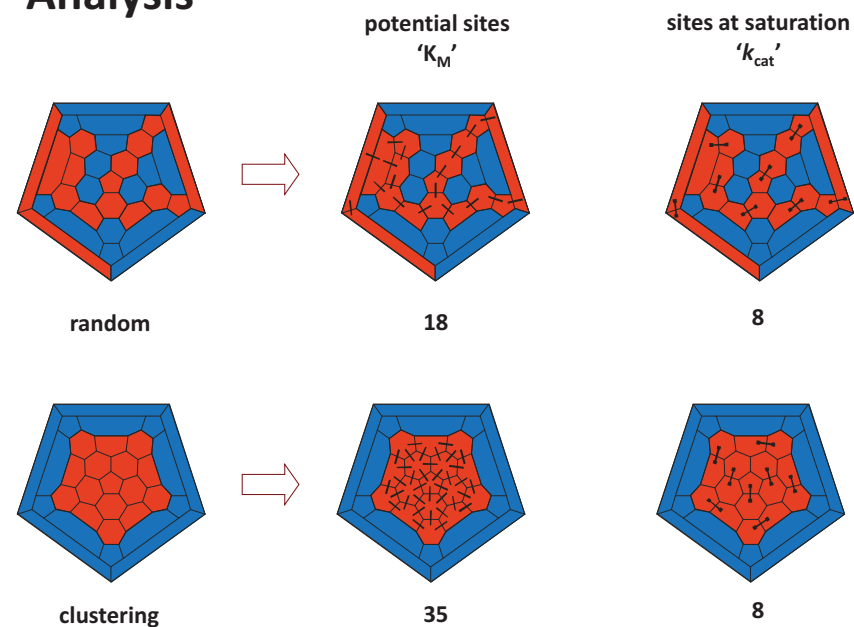
60

A simple model assuming k_{cat} and K_M , per catalytic site constant



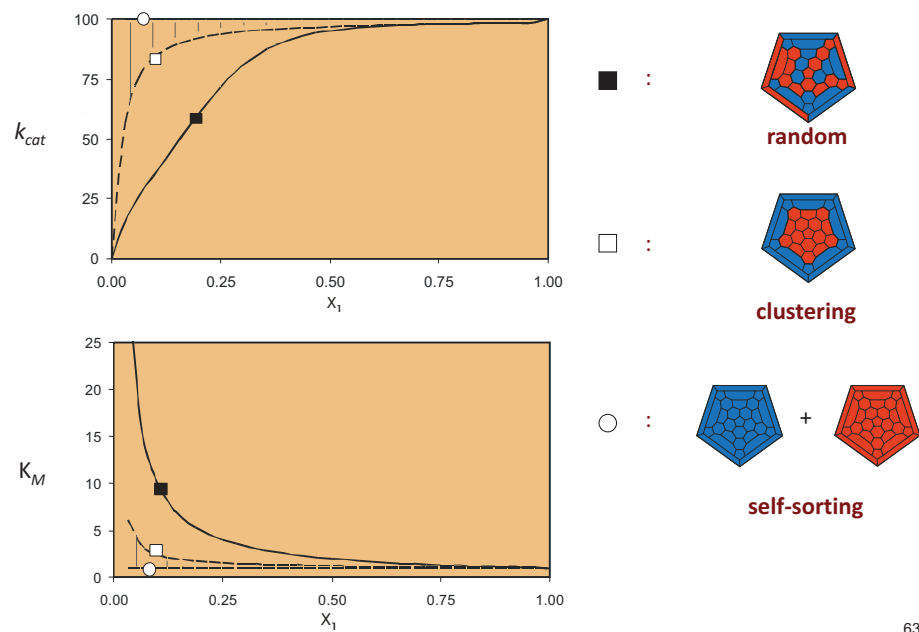
61

Analysis



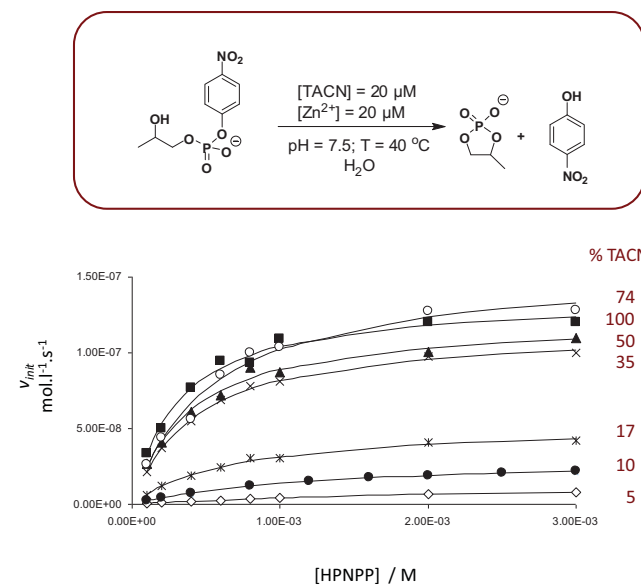
62

Anticipated behavior



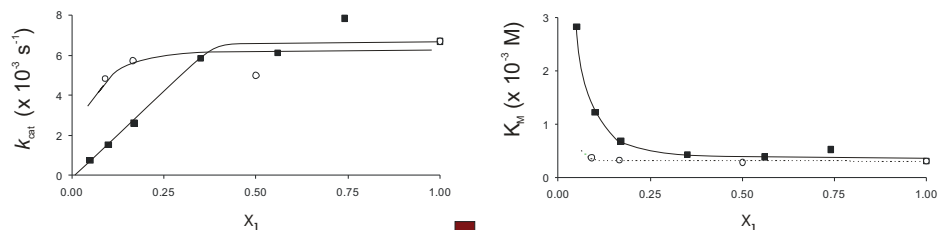
63

Catalytic activity



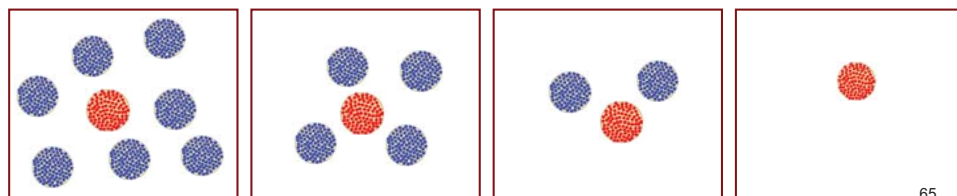
64

Organization of the monolayer

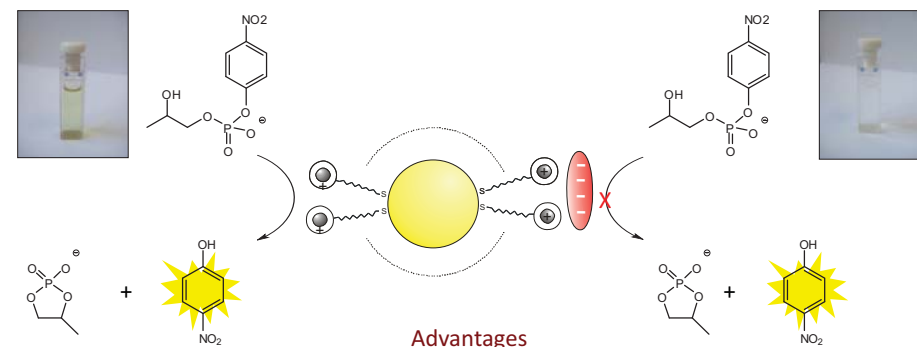


RANDOM DISTRIBUTION

Control experiment:



The monolayer interface: Multivalency/recognition/catalysis

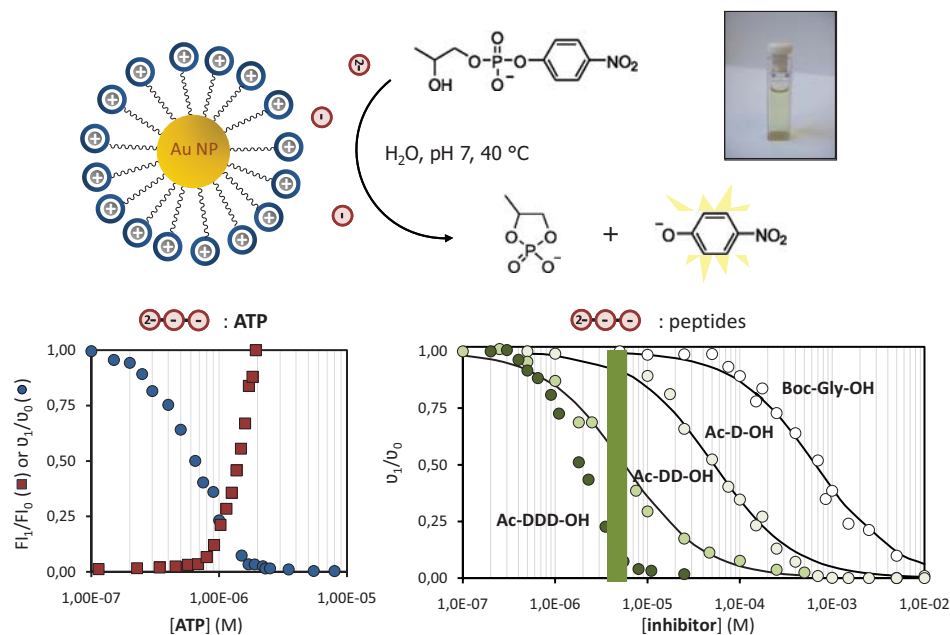


Advantages

- Visible detection
- Fast
- No background reaction
- Relative high affinity of HPNPP (sub mM)
- Low catalyst concentration (nM)

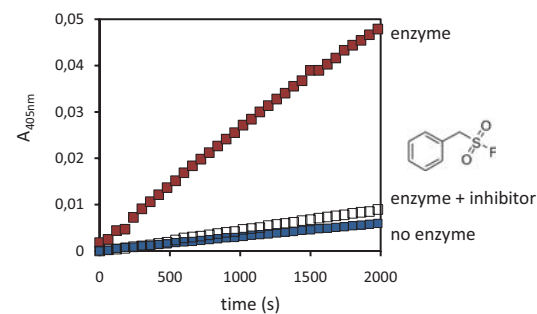
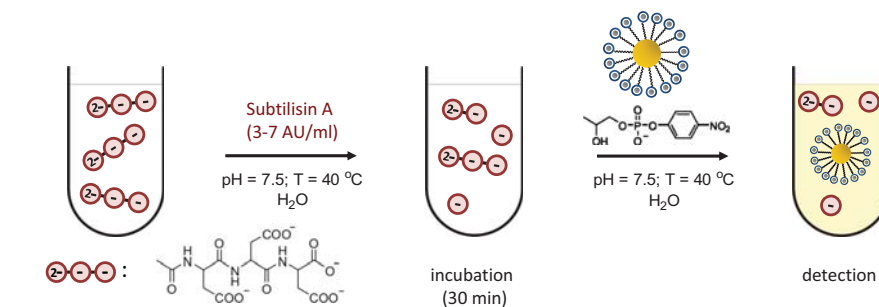
66

Catalytic signal amplification



[TACN • Zn(II)] = 5 μ M, [substrate] = 1 mM, [HEPES] = 10 mM, pH = 7.0, T = 25 $^{\circ}$ C.

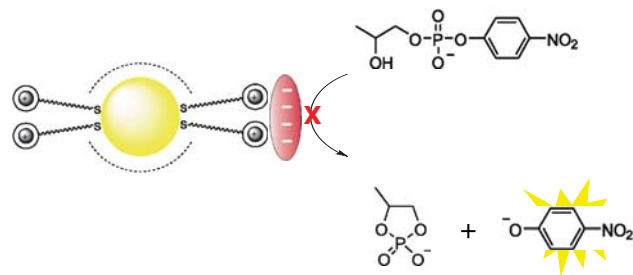
67



enzyme	enzyme
GCP II	
Caspase-1	

ACIE 2011, 50, 2307–2312 with L. Prins

68



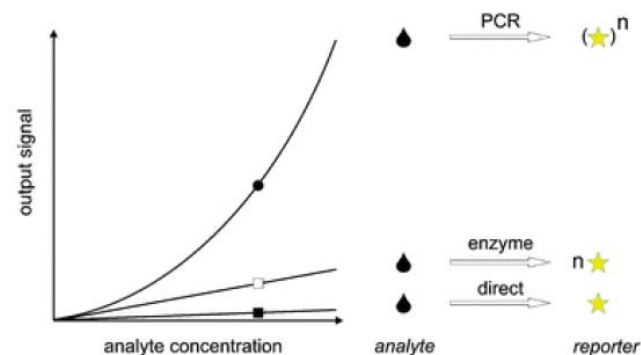
Criterion

difference in binding affinity between substrates and products of enzyme activity

Enzyme	Role	Substrate (K_{dis} [M])	Product(s) (K_{dis} [M])	Optimal assay concentration [M] ^[9]
asparaginase	metabolism	asparagine (1.2×10^{-3})	aspartate (8.0×10^{-5})	2.5×10^{-4} (58)
Glutamase	metabolism	glutamine (2.4×10^{-3})	glutamate (8.0×10^{-5})	1.0×10^{-4} (52)
Glutamate carboxypeptidase II	neurotransmission	N-acetyl-L-aspartyl-L-glutamate (9.0×10^{-7})	N-acetyl-L-aspartate (6.0×10^{-5}) and glutamate (8.0×10^{-5})	5.0×10^{-6} (65)
caspase 1	cell apoptosis	AcNH-YVADD (5.0×10^{-5}) ^[9]	AcNH-YVAD (1.0×10^{-4}) ^[9] and aspartate (8.0×10^{-5})	5.0×10^{-6} (42)

69

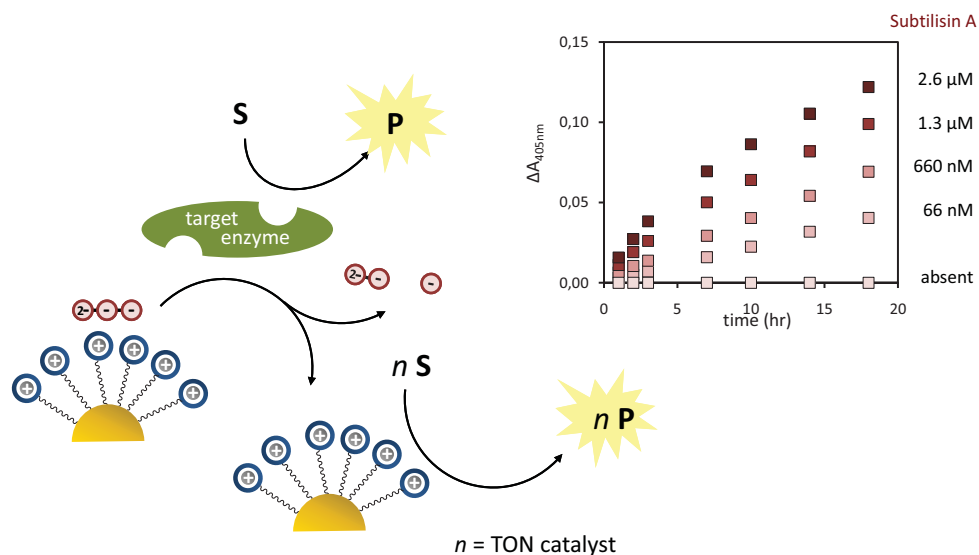
Signal amplification: the higher, the better...



The strength of the output signal as a function of the analyte concentration depends on reading out system's conditions: direct read out (■), enzymatic amplification (□), PCR amplification (●)⁵.

70

Sensing through signal amplification



71

Acknowledgments

PhDs:

Giovanni_Zaupa
Marta Dal Molin
Matteo Graziani
Davide Zaramella
Andrea Lovato
Luca Baù
Marta Diez Castellnou

PostDocs:

Flavio Manea
Renato_Bonomi
Anna Sansone
Patrizia Galzerano
Giulio Gasparini
Satadru Jha

Collaborations:

Lars Baltzer
Grazia Lombardi
Luigi Lay
Lucia Pasquato
Claudia Sissi
Roger Stromberg
Patrizia Pontisso



Alessandro
Moretto
Fabrizio
Mancin

Leonard J
Prins



72