Sensing through Signal Amplification

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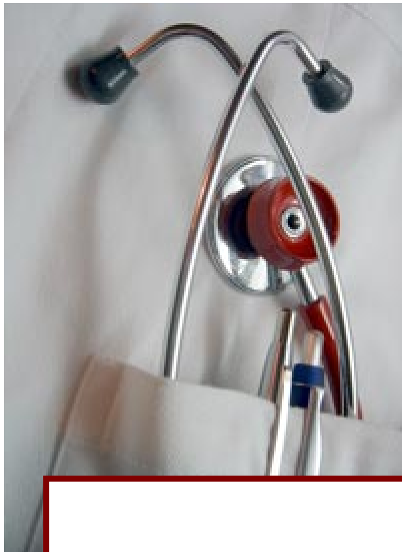
The importance of detecting ultralow (bio)chemical concentrations

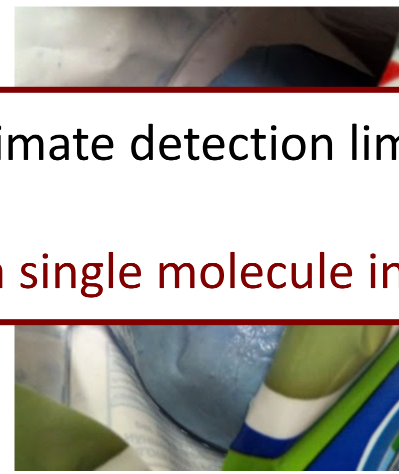


Disease diagnosis

DNA diagnostics

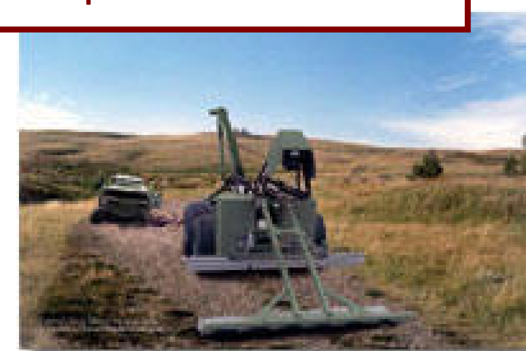
The ultimate detection limit

‘Naked eye detection of a single molecule in a complex mixture’



Food contamination

Environmental pollution

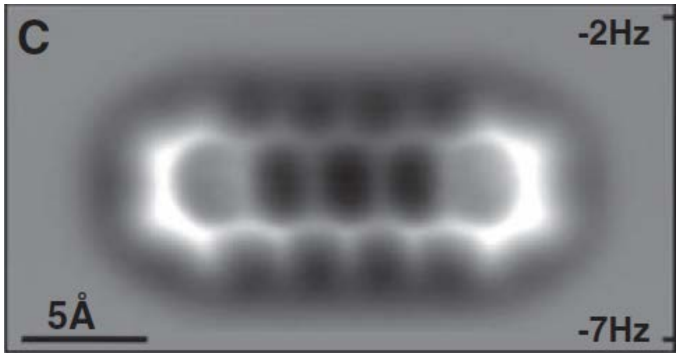


Explosives detection

..and more

Single molecule detection

TEM, SEM, AFM
SPR, SERS, X-ray

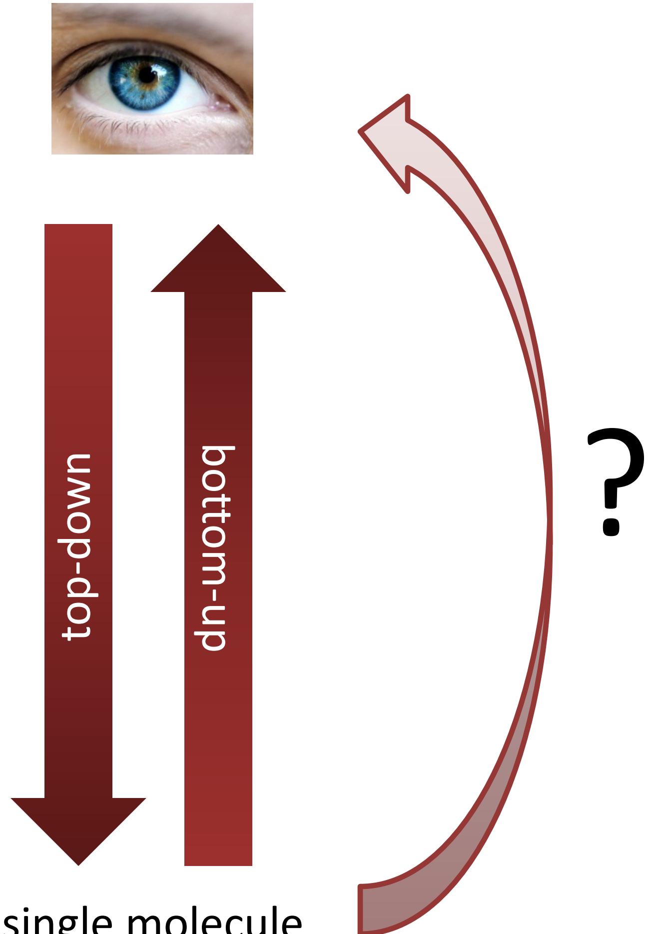


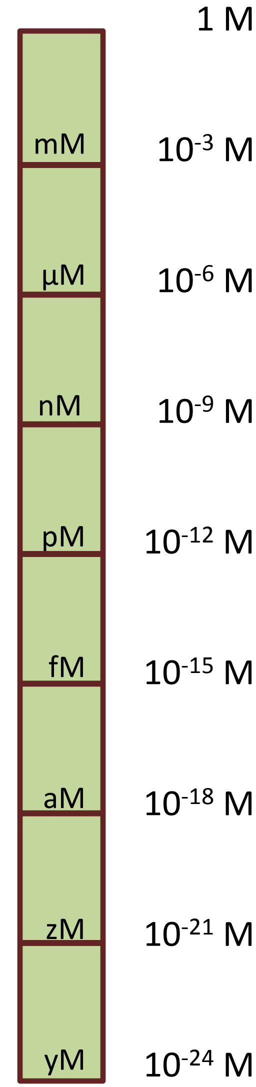
pentacene

Gross *et al. Science*, **2009**, *325*, 1110-1114

DNA smiley

Rothemund, *Nature*, **2006**, *440*, 297-302





1 molecule / 1 liter = 1.66×10^{-24} M = 1.66 yM

Earth

≈

5.97×10^{24} kg

=

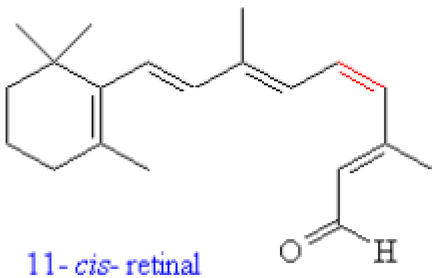
1 kg

Where is Wally ?

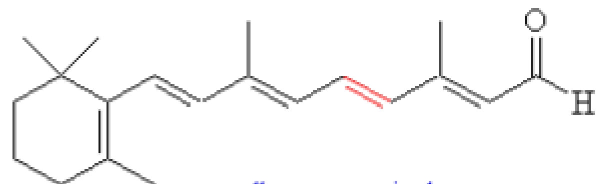

HEY, WALDO-WATCHERS!
SAW SOME TRULY TERRIFIC SIGHTS TODAY—SOMEONE BURNING TROUSERS WITH AN IRON; A LONG THIN MAN WITH A LONG THIN TIE; A GLOVE ATTACKING A MAN. PHEW! INCREDIBLE!
Waldo
WHERE'S WALDO? DEPARTMENT STORE
TO:
WALDO-WATCHERS
OVER THE MOON,
THE WILD WEST,
NOW

Signal amplification in Nature - catalysis

vertebrate visual response

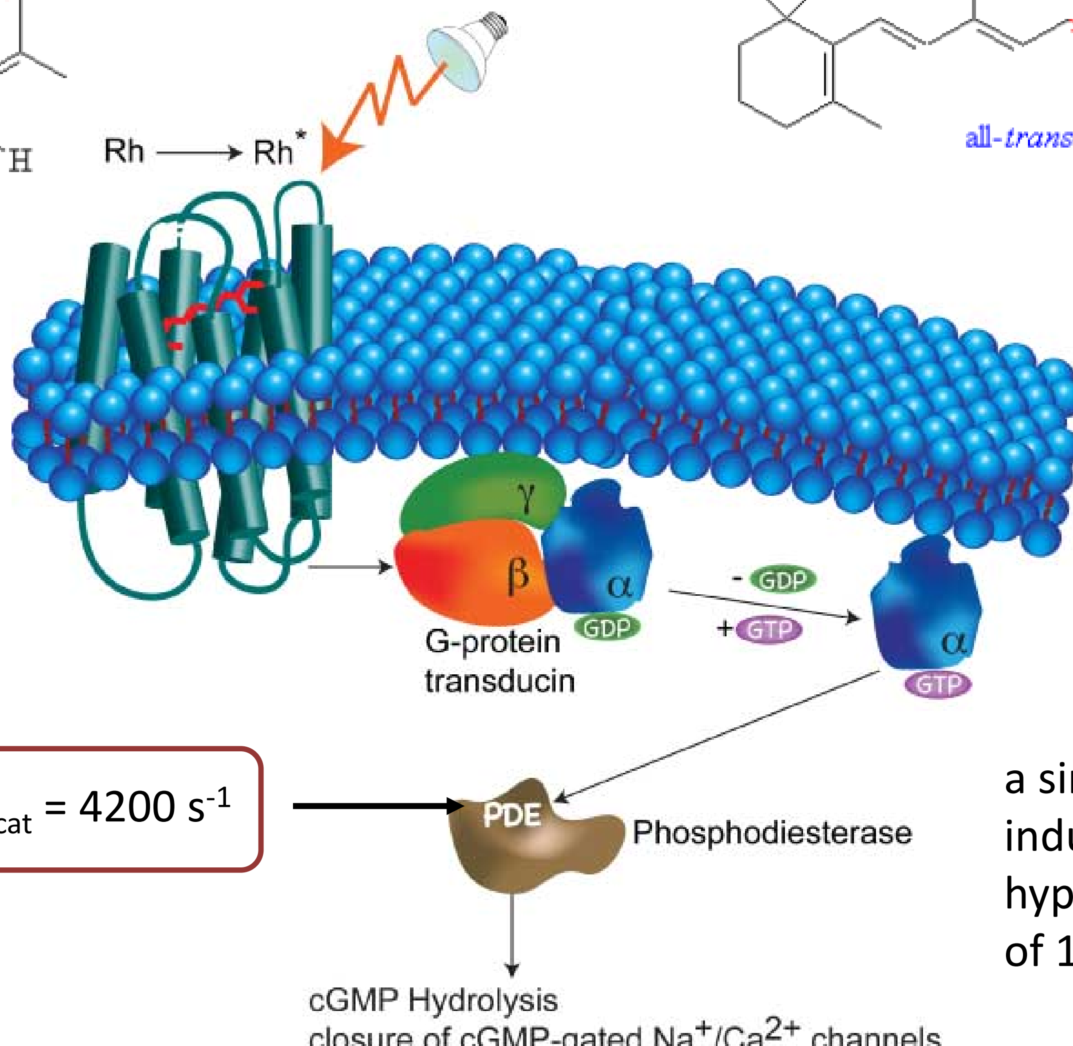


11-*cis*-retinal



all-*trans*-retinal

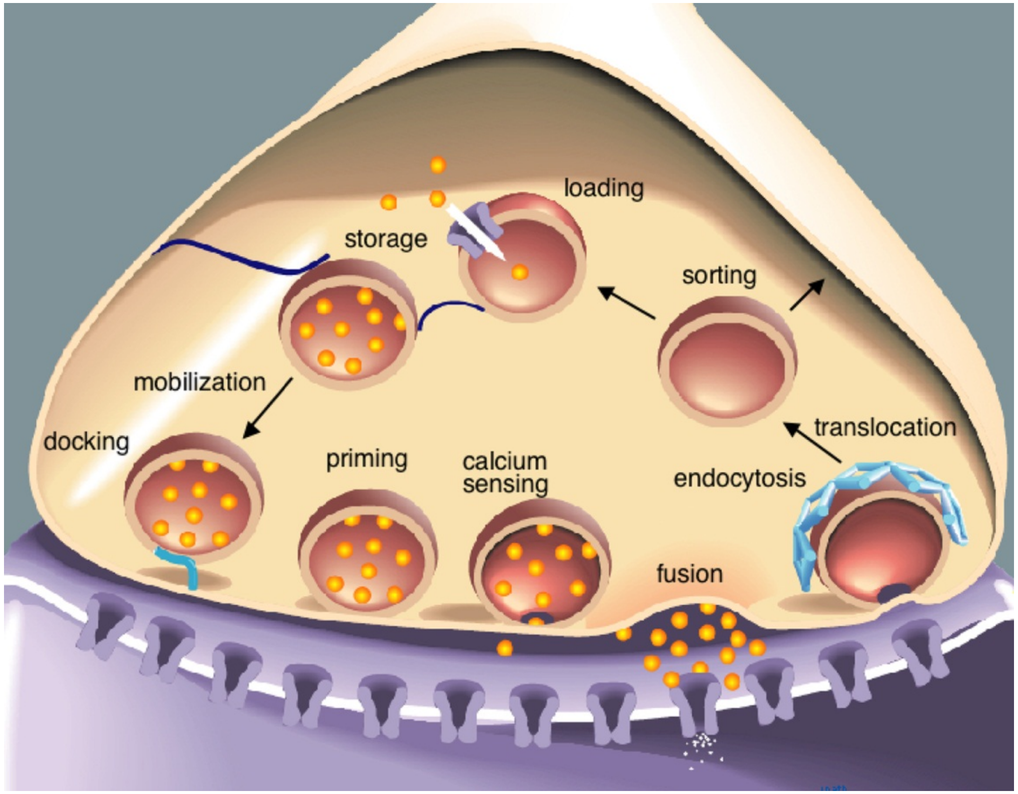
- Light hits a rod cell and isomerizes retinal.
- ↓ Rhodopsin converts to metarhodopsin II.
- ↓ Metarhodopsin II activates transducin.
- ↓ Transducin activates phosphodiesterase.
- ↓ Phosphodiesterase hydrolyzes cyclic GMP.
- ↓ Cyclic GMP is scarce, so Na^+ channels close.
- ↓ The membrane is hyperpolarized.
- ↓ An electrical impulse is sent to the brain.



$k_{cat} = 4200\ s^{-1}$

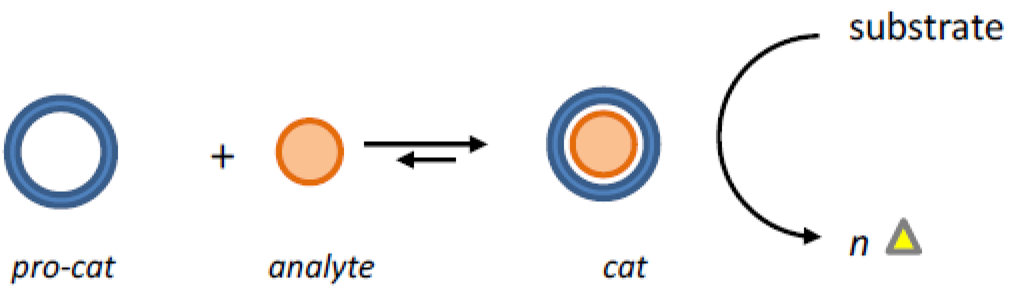
a single photon can induce a membrane hyperpolarization of 1 mV

Signal amplification in Nature - multivalency

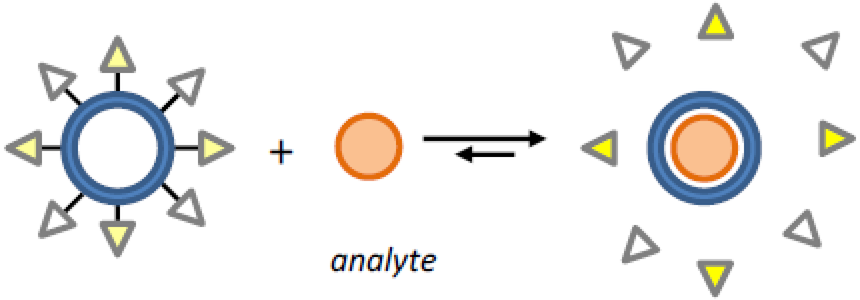


Supramolecular sensing systems

catalysis

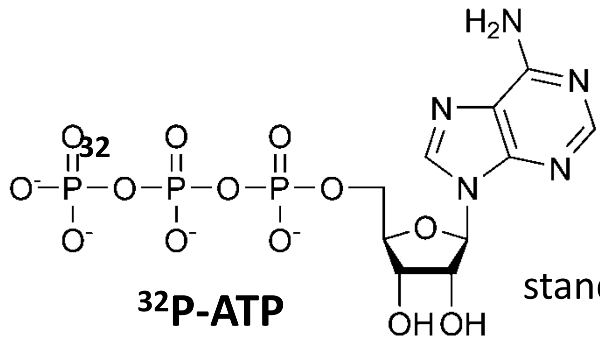


multivalency

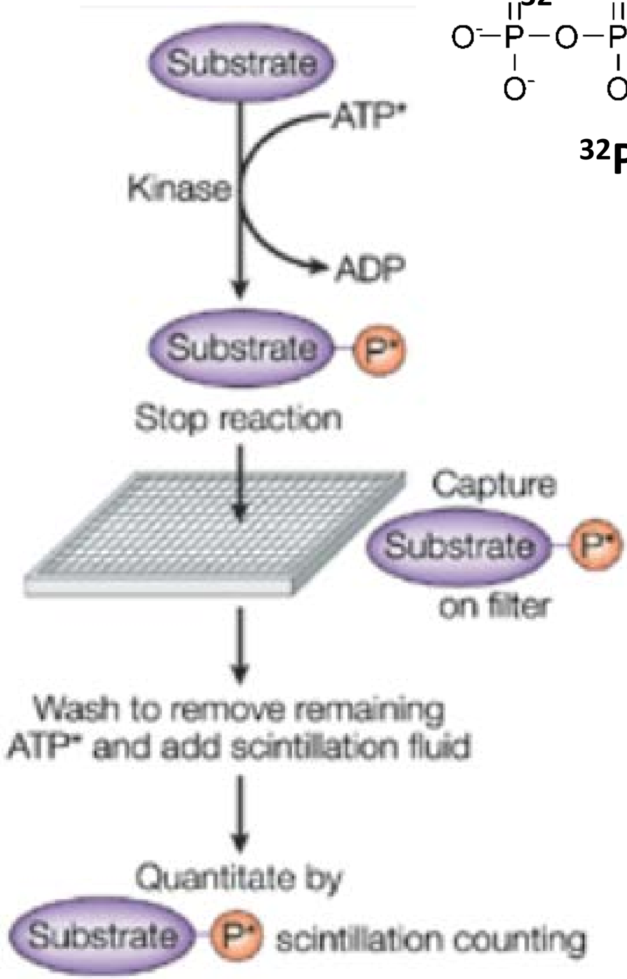


Prins *et al*. *Chem. Soc. Rev.* **2011**, *40*, 4480-4505

Signal amplification in assays : Radioactivity assays

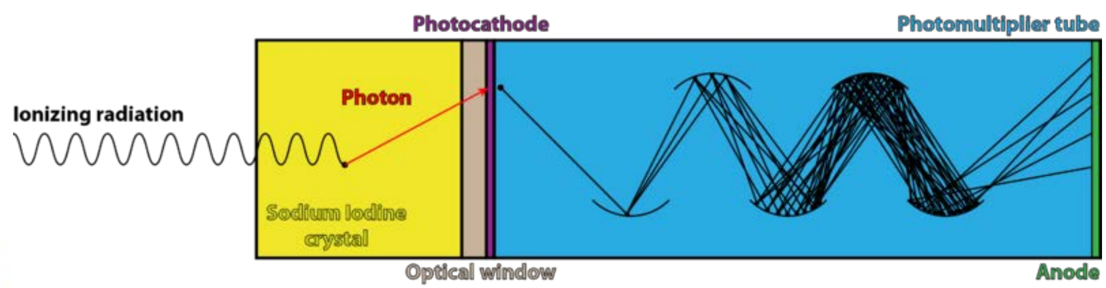


^{32}P-ATP



quantification

standard autoradiography, phosphorimaging, or liquid scintillation counting techniques



advantage

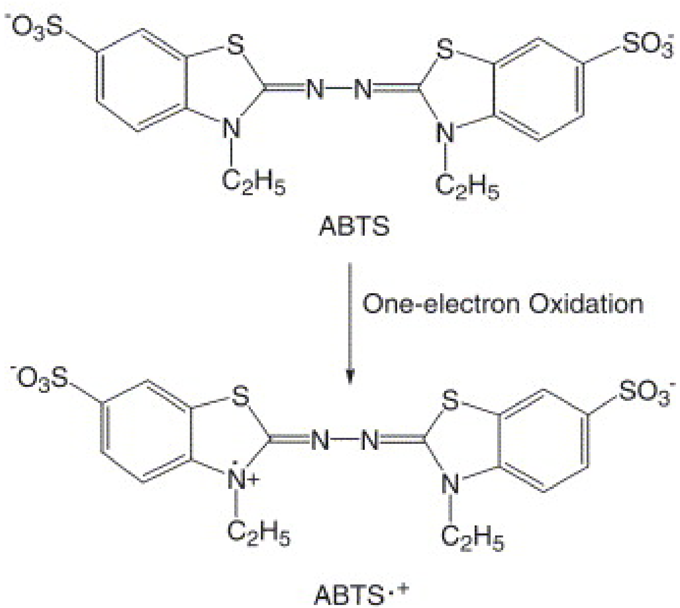
- high sensitivity
- high specificity (monitoring selective reactions)

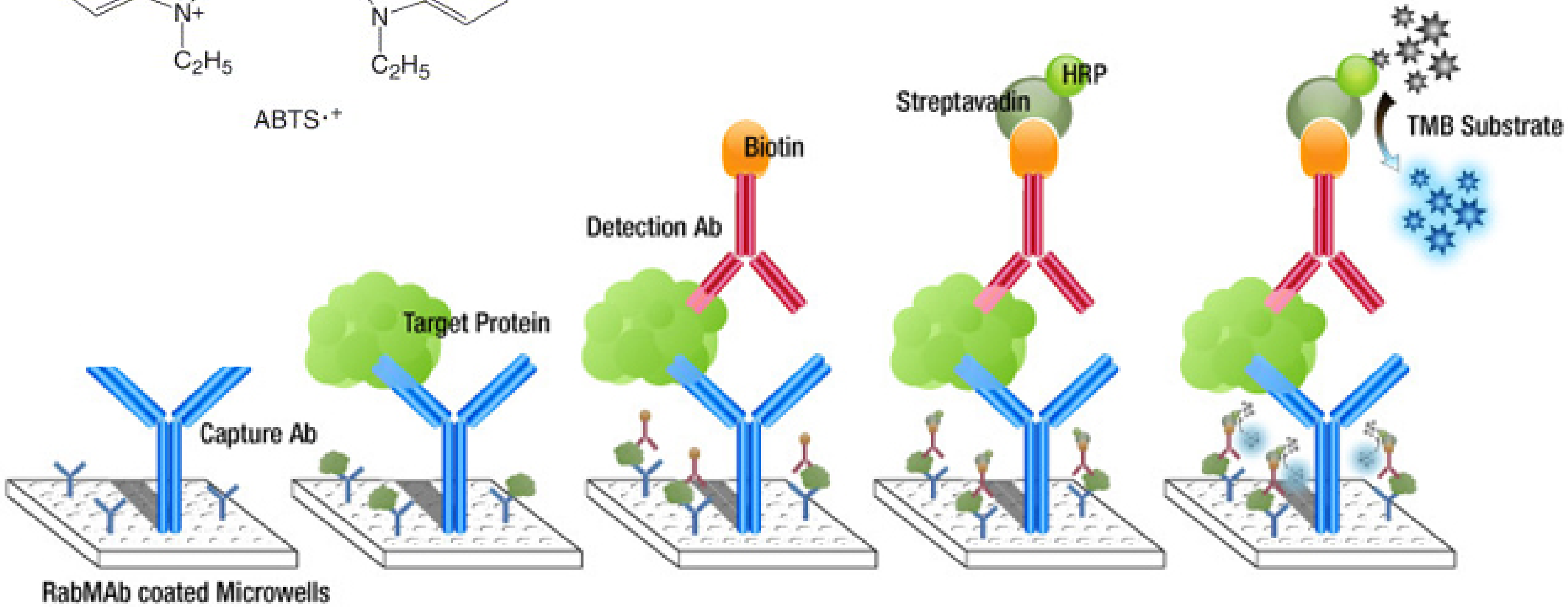
disadvantage

- radioactivity
- no high throughput

Yalow and Berson, *J. Clin.Invest.* **1960**, *39*, 1157-1175

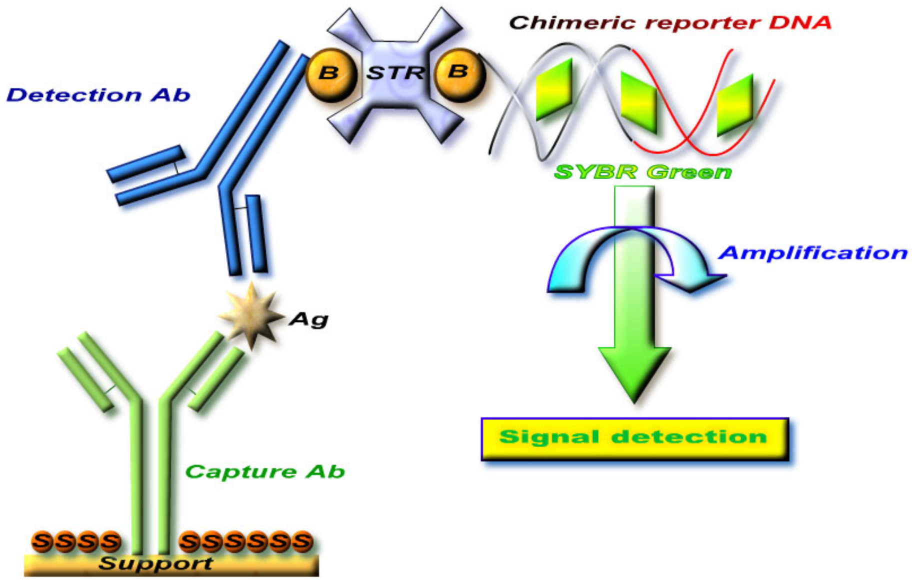
Signal amplification in assays : Enzyme-Linked ImmunoSorbent Assay (ELISA)





Engvall, Perlmann, *Immunochem..* **1971**, *8*, 871-874

Signal amplification in assays : ImmunoPCR

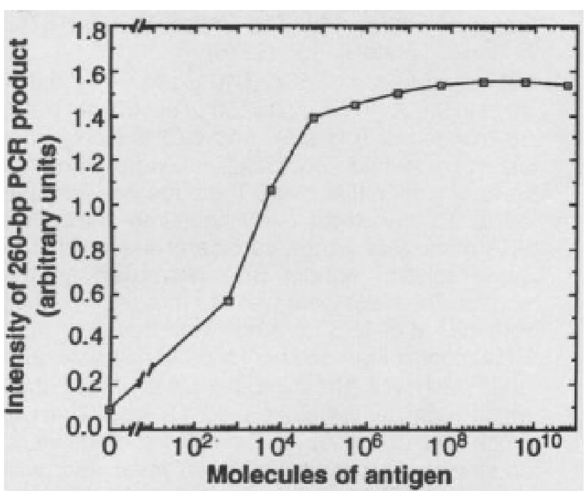


Analyte: bovine serum albumin (BSA)

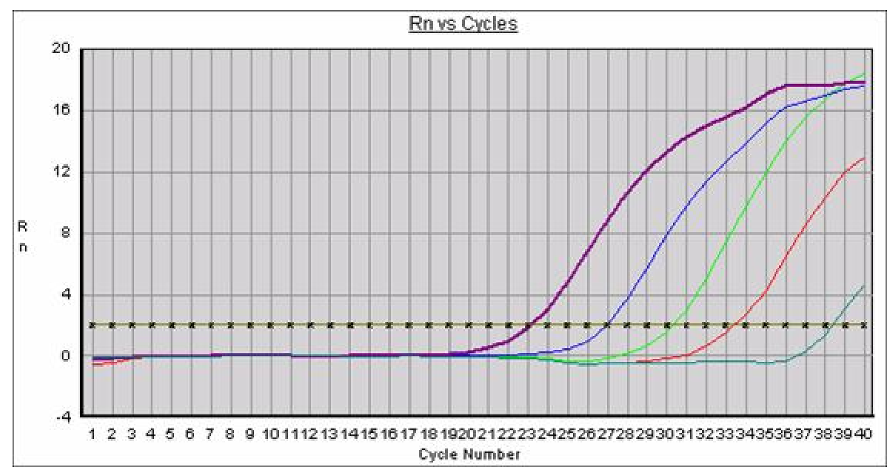
9.6×10^{-21} mol (in 45 µl)

detection:
gel+staining (*e.g.* ethidium bromide)

in situ by introducing fluorescent markers

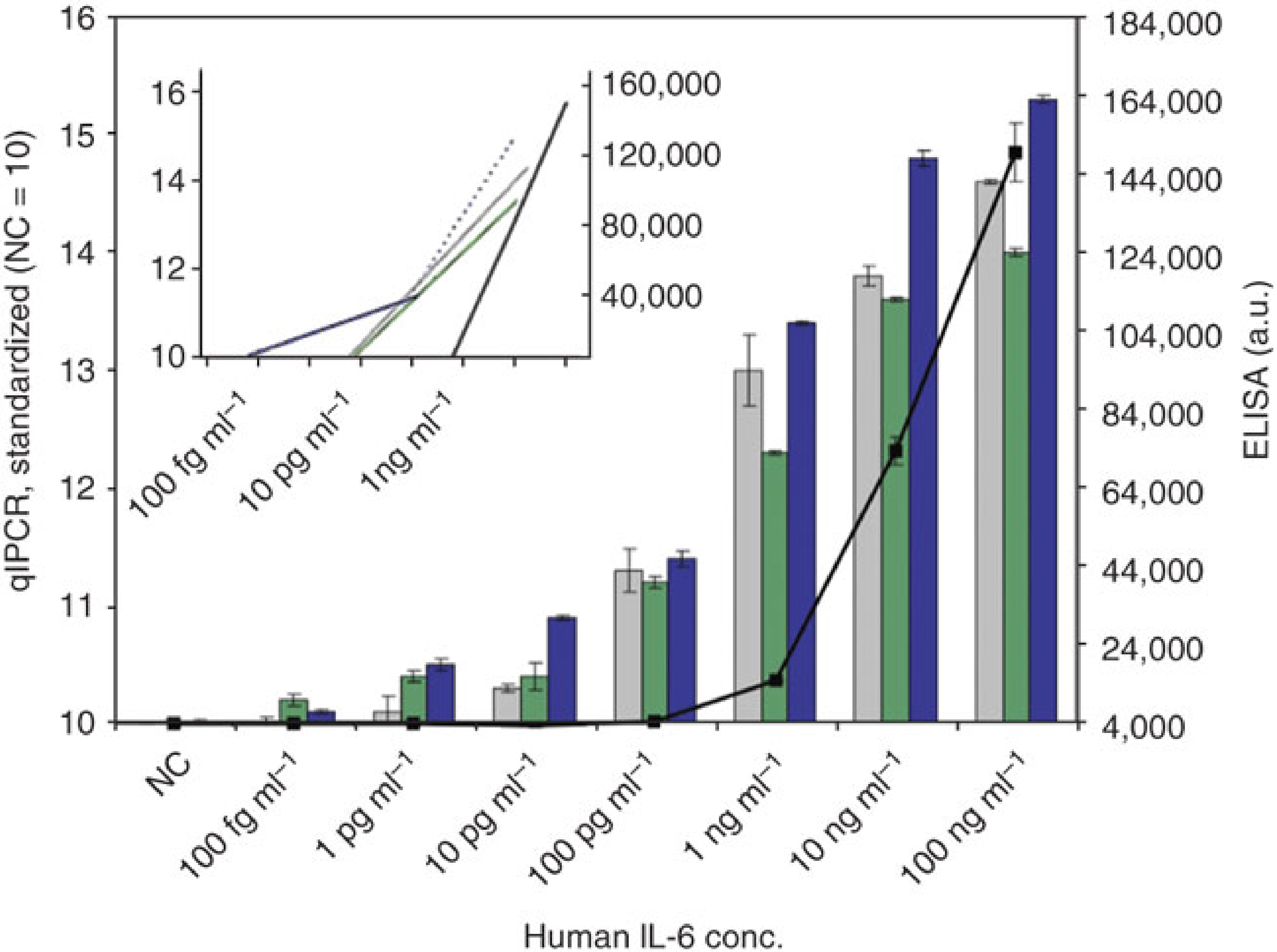


580 antigen molecules in sample
(x10^5 increased sensitivity compared to ELISA)



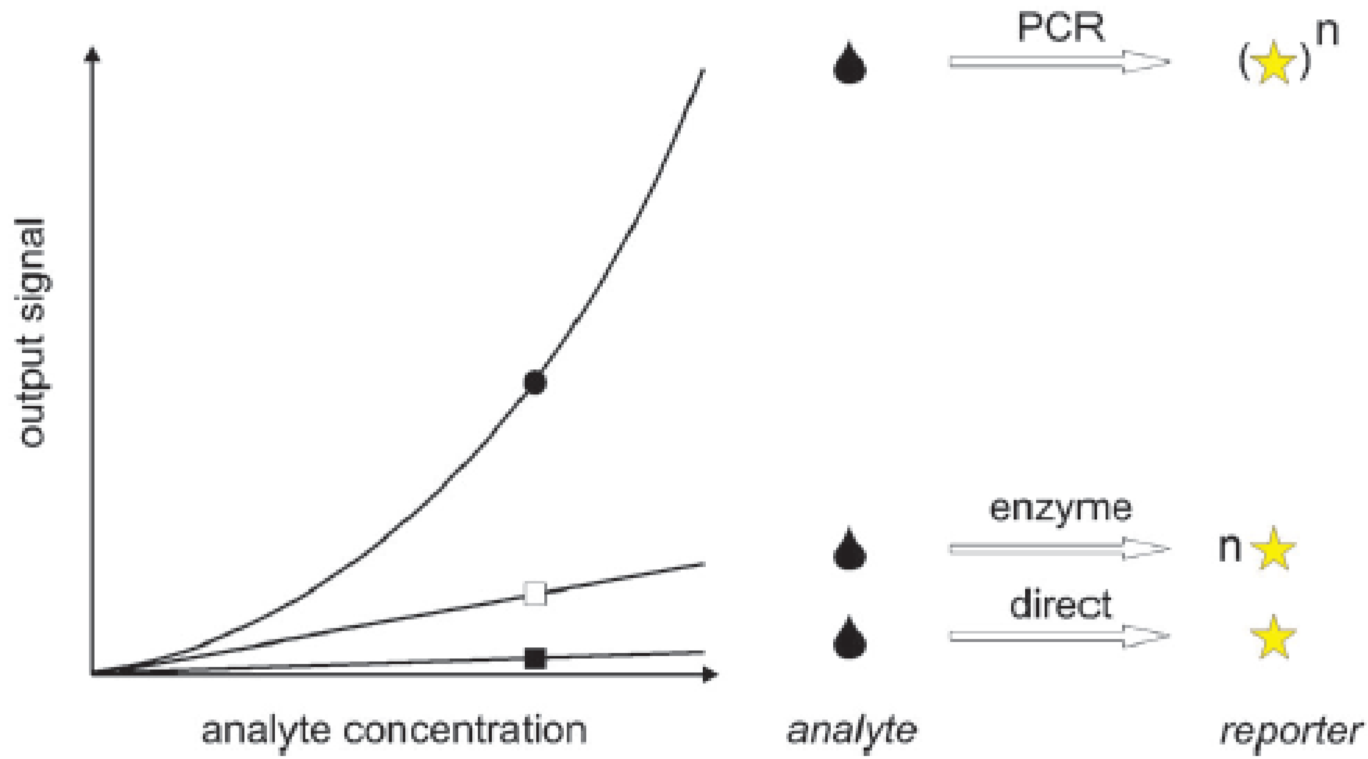
Cantor *et al.*, *Science*. **1992**, *258*, 120-122

ELISA *vs* immunoPCR



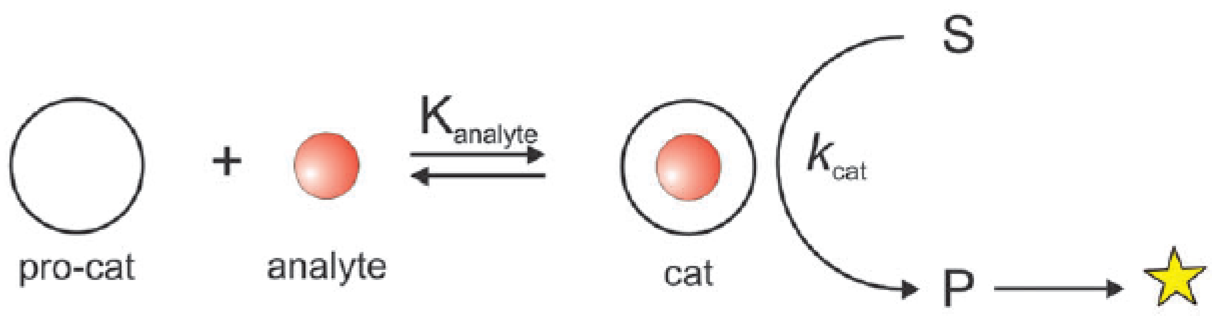
Niemeyer *et al*. *Nature Protocols*. **2007**, *2*, 1918-1930

Summary



output signal
analyte concentration
PCR
$(\star)^n$
enzyme
n
direct
analyte
reporter

Quantification of amplification by catalyst activation



operating conditions

- high $K_{analyte}$
- excess pro-cat ([cat]=$[A]_0$)

catalysis

- low background rate ($k_{uncat} << k_{cat}$)
- detectable P
- no product inhibition

$$\frac{d[P]}{dt} = k_{cat}[cat] = k_{cat}[A]_0$$

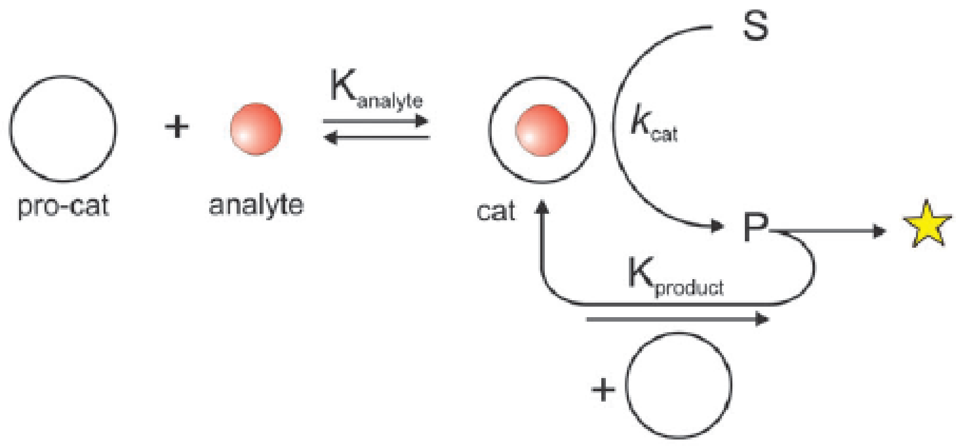
⇩

$$[P]_t = k_{cat}[A]_0 t$$

- linear relationship
- signal strength is time-dependent (high TOF)

Anslyn *et al*. *Angew. Chem. Int. Ed.* **2006**, *45*, 1190-1196

Quantification of amplification by autocatalysis



$$\frac{d[P]}{dt} = k_{cat}[cat] = k_{cat}[A]_0 + k_{cat}[P(t)]$$

operating conditions

- high $K_{analyte}$ and $K_{product}$
- excess pro-cat ($[cat]=[A]_0$)

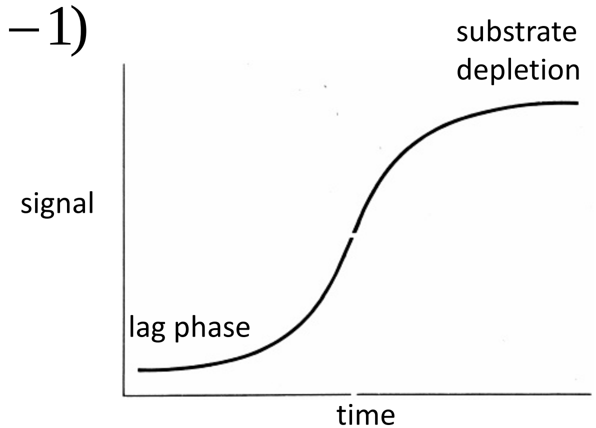
$$P(t) = P_0 e^{k_{cat} t} + A_0 (e^{k_{cat} t} - 1)$$

catalysis

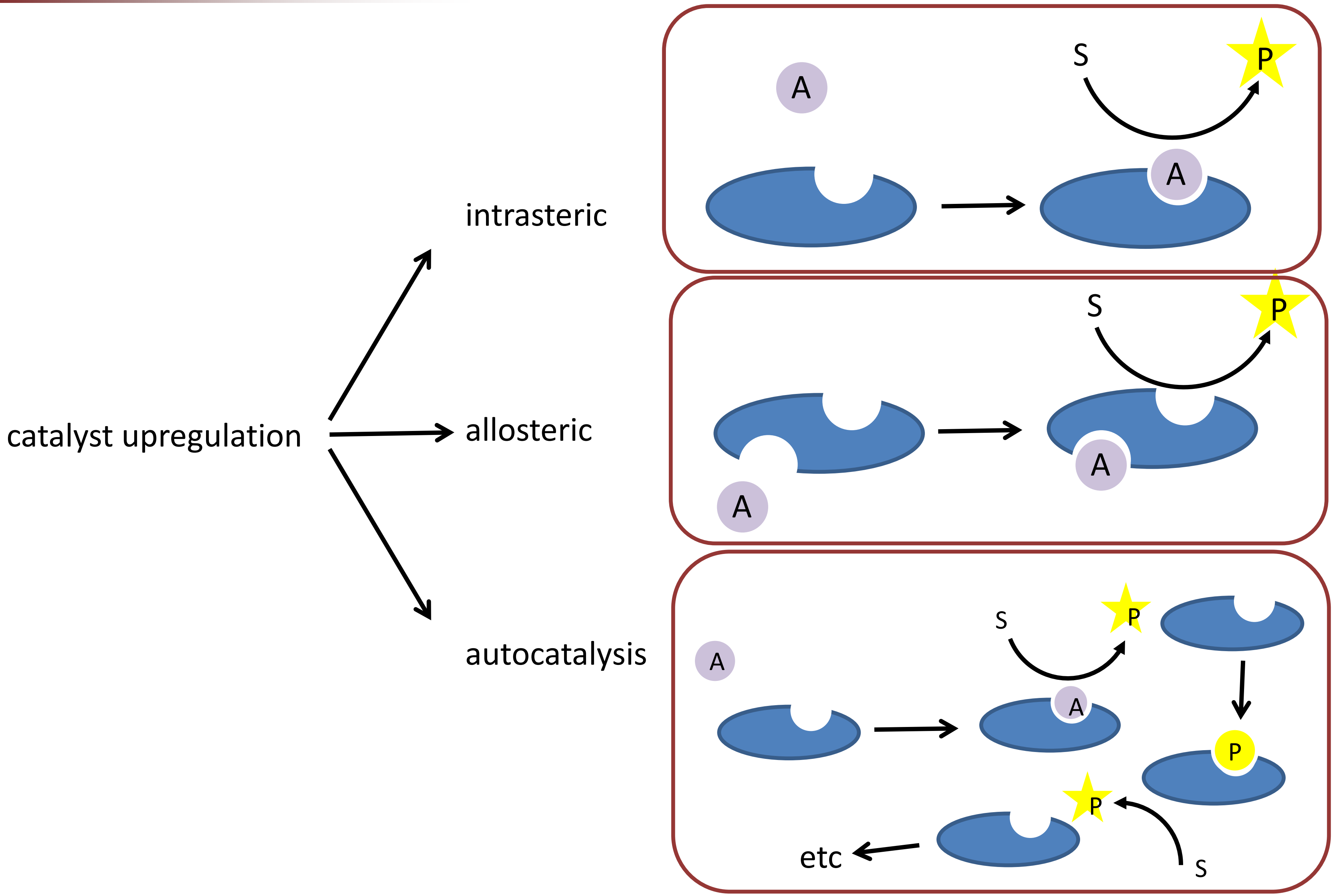
in case $P_0=0$ $\quad P(t) = A_0 (e^{k_{cat} t} - 1)$

- low background rate !! ($k_{uncat} << k_{cat}$)
- detectable P

- exponential increase (sigmoidal curve)

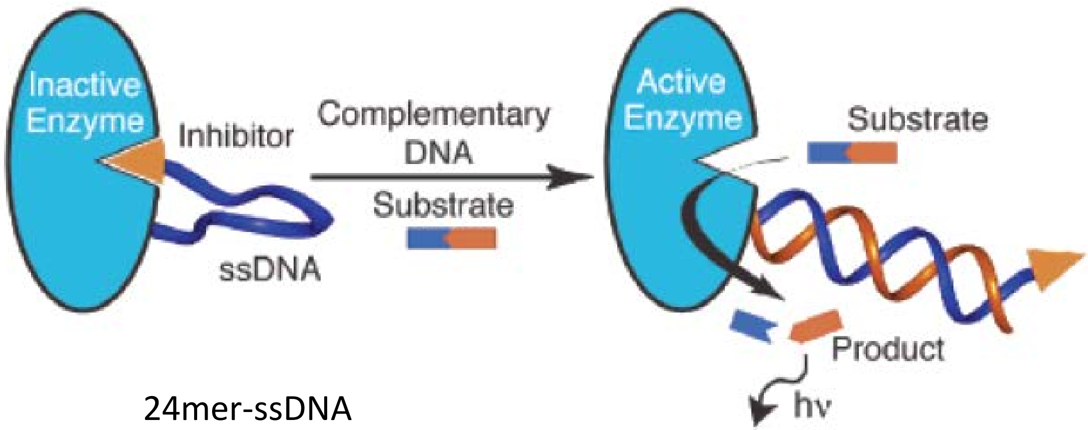


Overview examples



DNA detection using an engineered enzyme

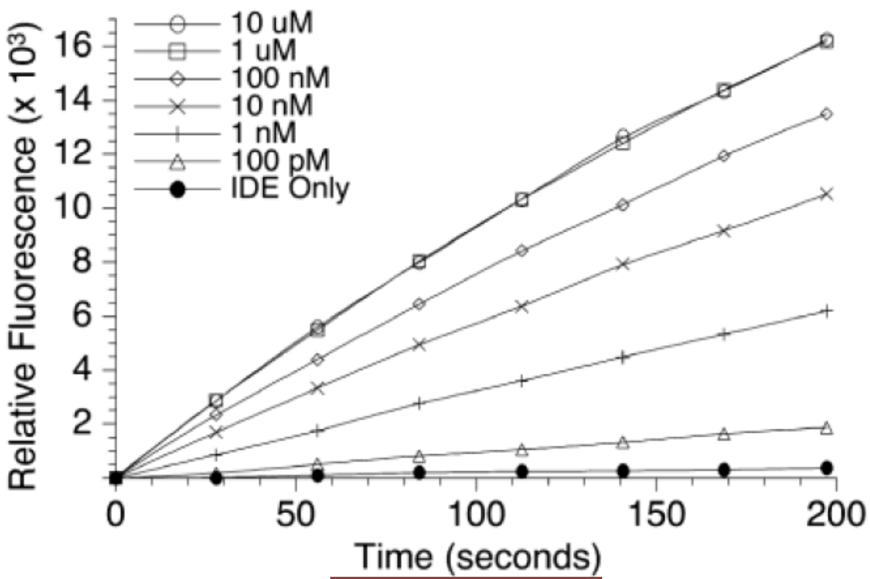
mM
µM
nM
pM
fM
aM
zM
yM

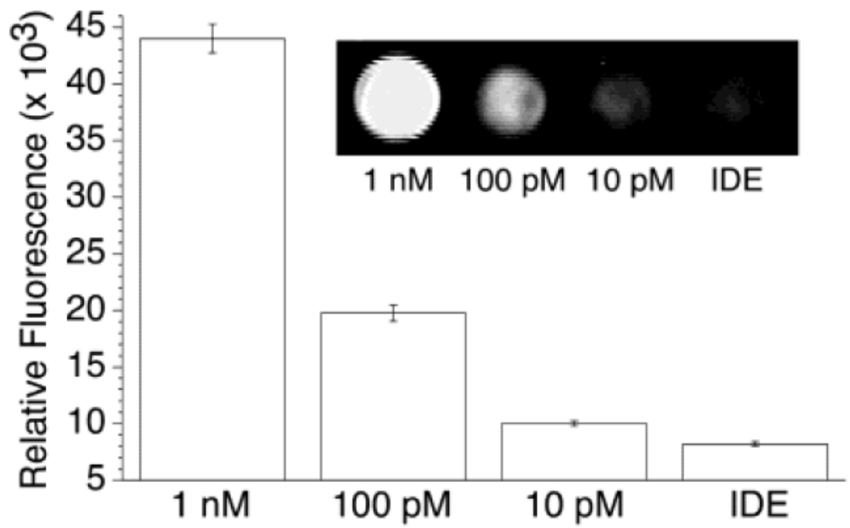


24mer-ssDNA

IDE construct
(*i*nhibitor-*D*NA-*e*nzyme)

Cereus neutral protease (CNP)
•compatible with DNA-tether
•high activity (k_{cat} = 165 s^{-1})



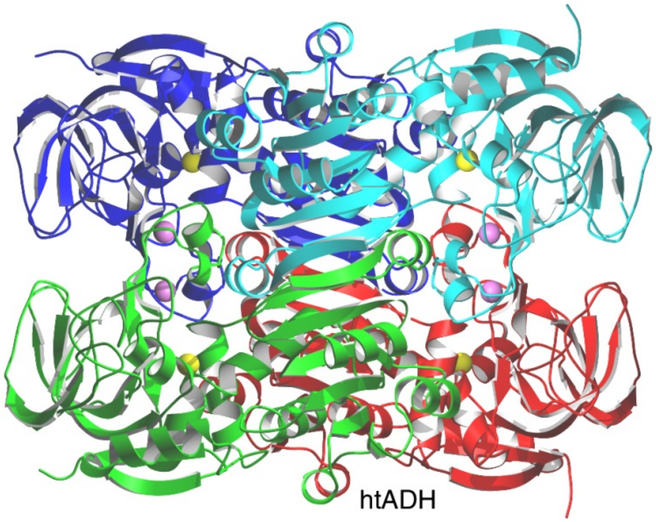


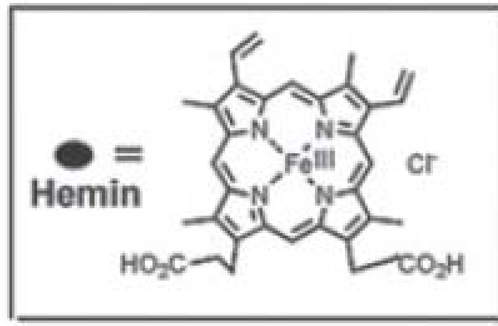
readout after 80 minutes

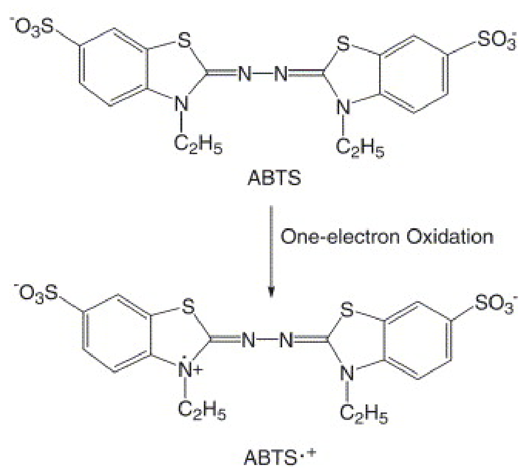
Issues: enzyme modification, synthesis

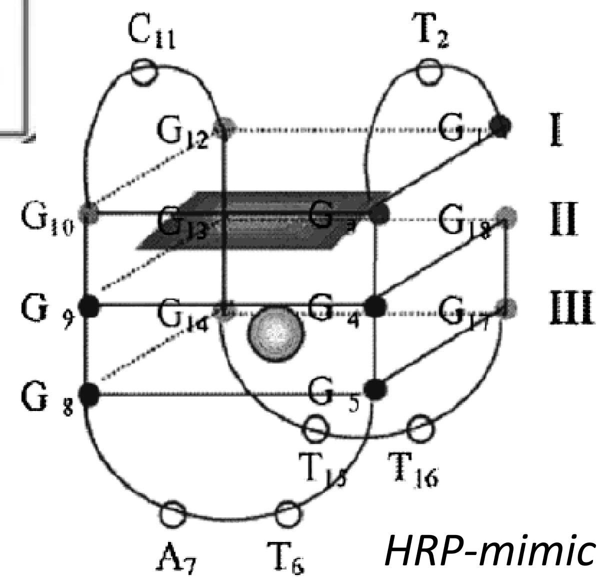
Ghadiri *et al.*, *J. Am. Chem. Soc.* **2003**, *125*, 344-345

Enzymes versus DNAzymes









HRP-mimic

enzymes

blue

($\varepsilon = 3.6 \times 10^4\ M^{-1}\ cm^{-1}$)

DNAzymes

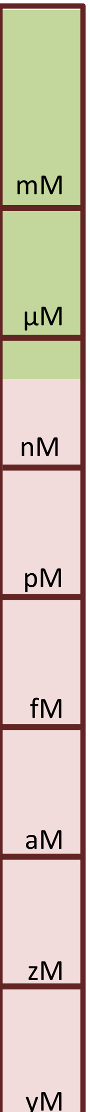
(DNA with catalytic activity)

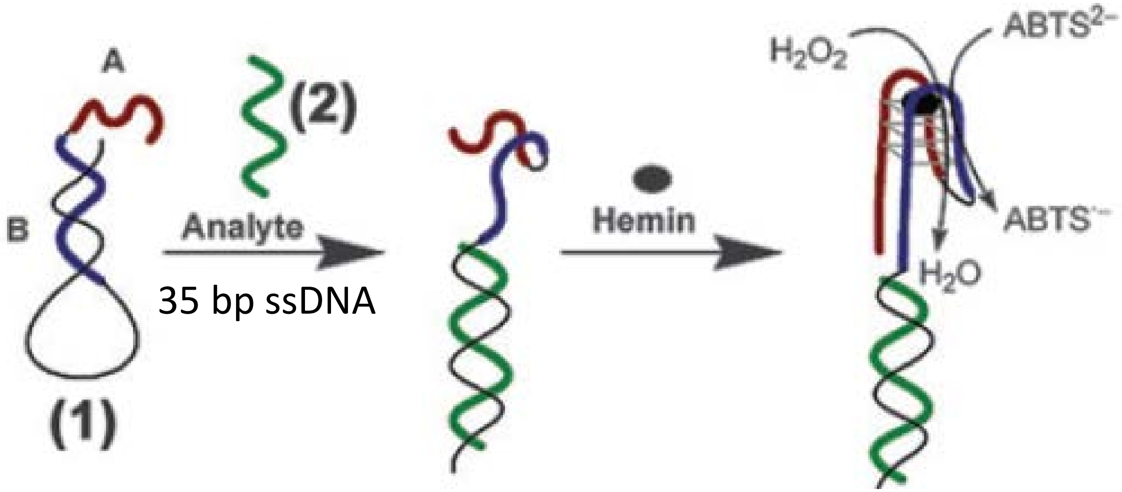
- high activity
- selective conditions
- isolation/preparation in small quantities

- nonnatural oligonucleotide-based catalysts
- higher chemical stability
- easy conjugation to other molecules (biotin)
- preparation in large quantities using PCR

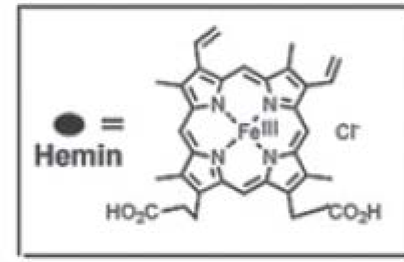
review: Willner *et al.*, *Chem. Soc. Rev.* **2008**, *37*, 1153-1165; Mauk, Sen *et al.* **2001**, *123*, 1337 - 1348

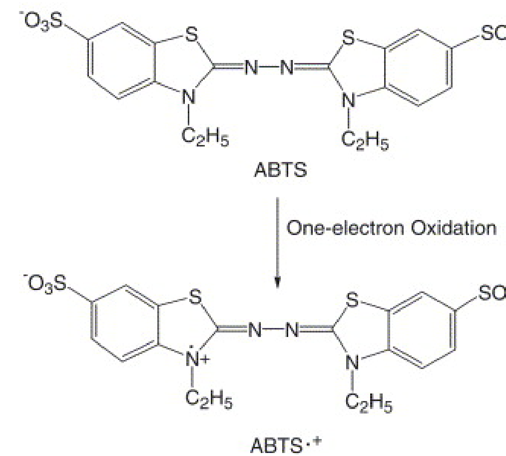
DNA detection using a DNAzyme

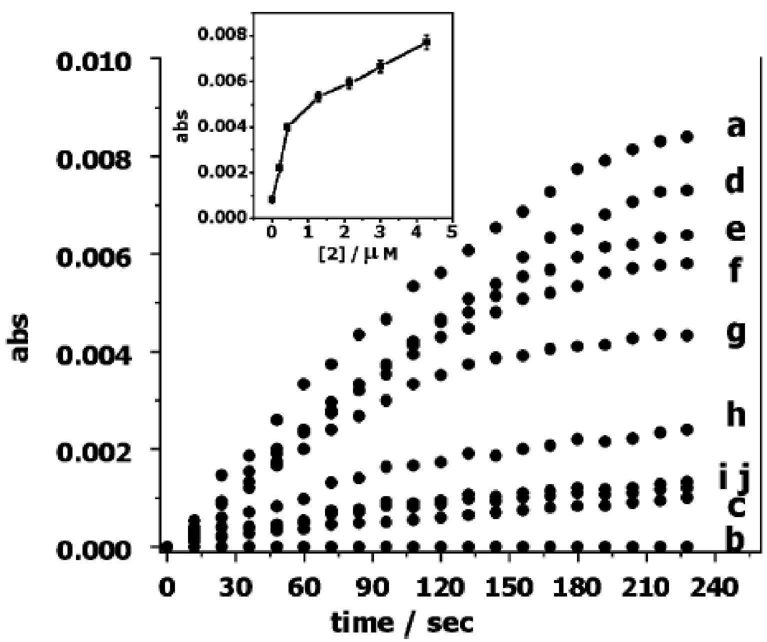




35 bp ssDNA







4.3 µM

↓

0.2 µM

mismatch DNA (single bp mismatch)

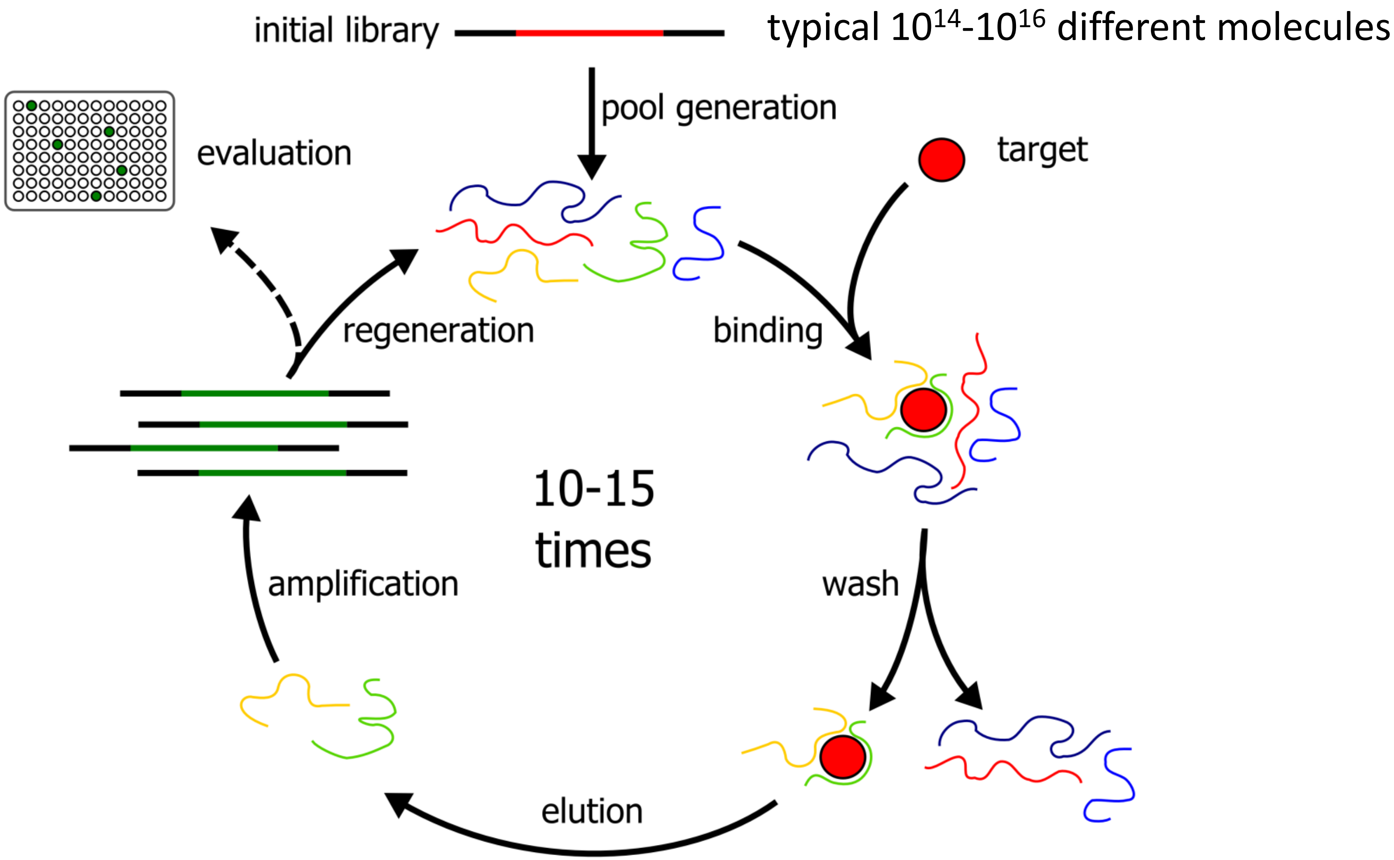
control (no analyte)

control (no beacon)

Willner *et al.*, *J. Am. Chem. Soc.* **2004**, *126*, 7430-7431

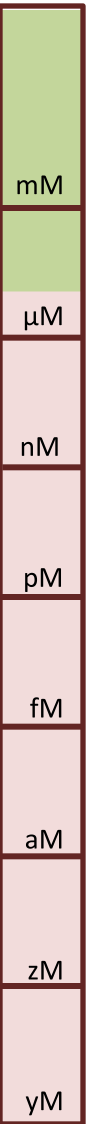
Detection of small molecules using aptazymes

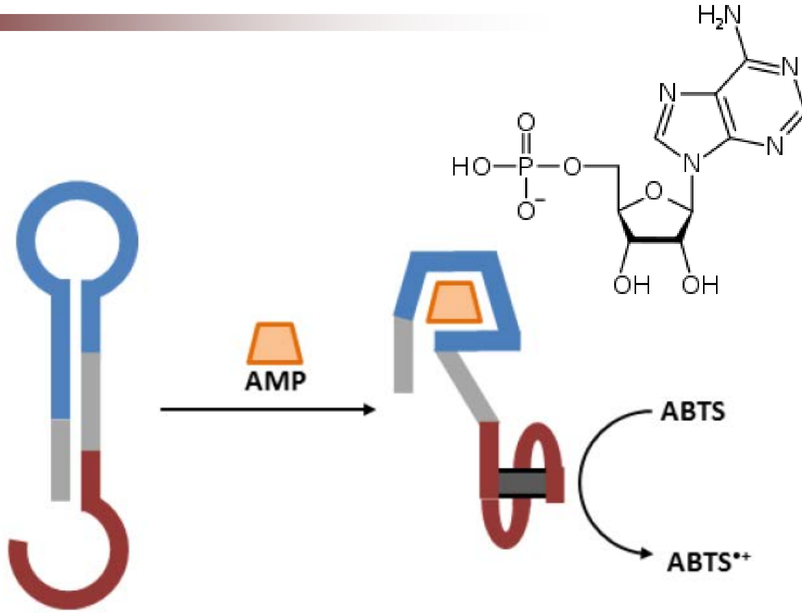
Aptamers are nucleic acid molecules (DNA/RNA) with specific binding functionality.
In the presence of targets, aptamers adopt a complementary 3D conformation which can preferentially bind the targets
Aptazymes are rational combination of DNAzymes/ribozymes and aptamers.

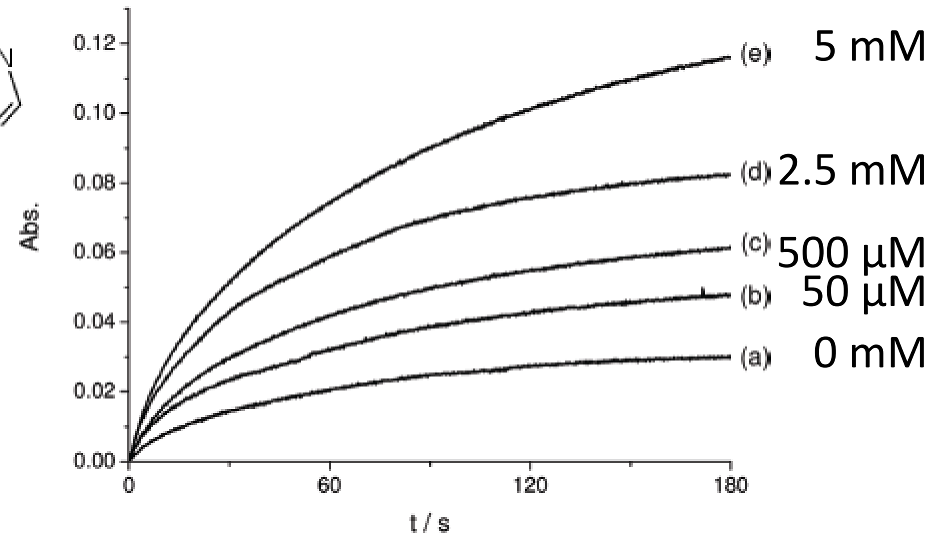


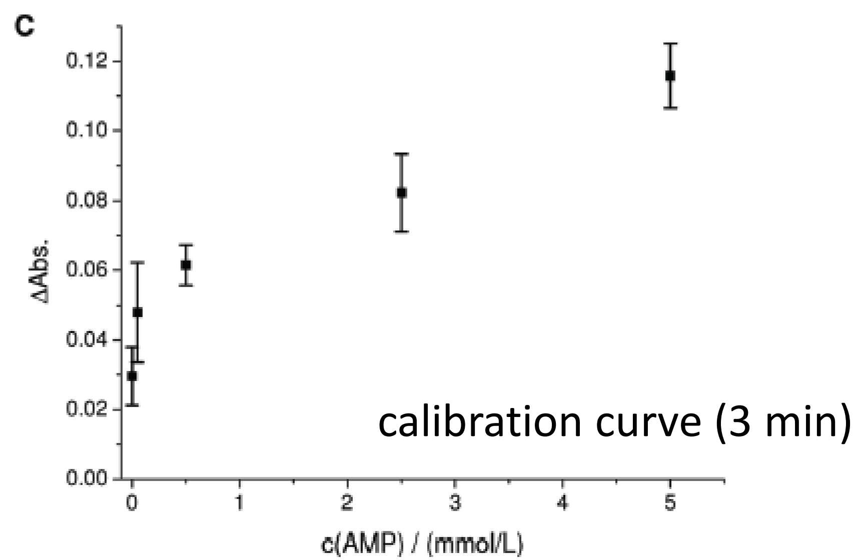
SELEX
Systematic Evolution of Ligands by EXponential Enrichment

Detection of AMP using an aptazyme









Reason for low sensitivity

AMP-aptamer complex formation competes with duplex formation.

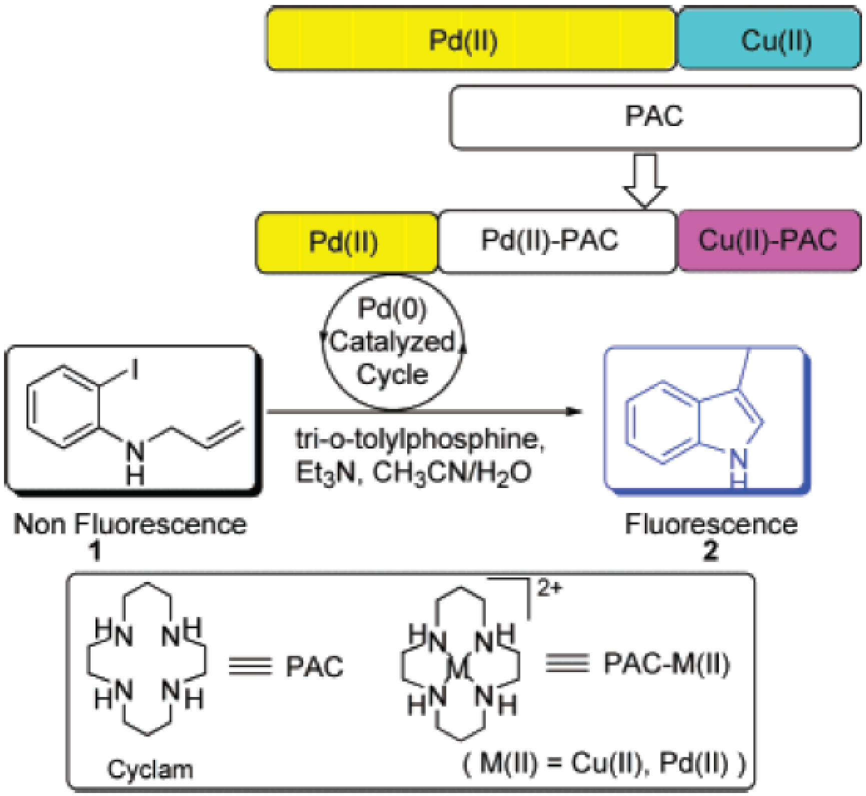
Reported: stability of the stem duplex ($\Delta G°$ = -9.62 kcal·mol^{-1}) was adjusted to that of the aptamer-AMP complex (K_{eq} = 1.67 × 10^{5} M^{-1}; , $\Delta G°$ = 7.1 kcal·mol^{-1}).

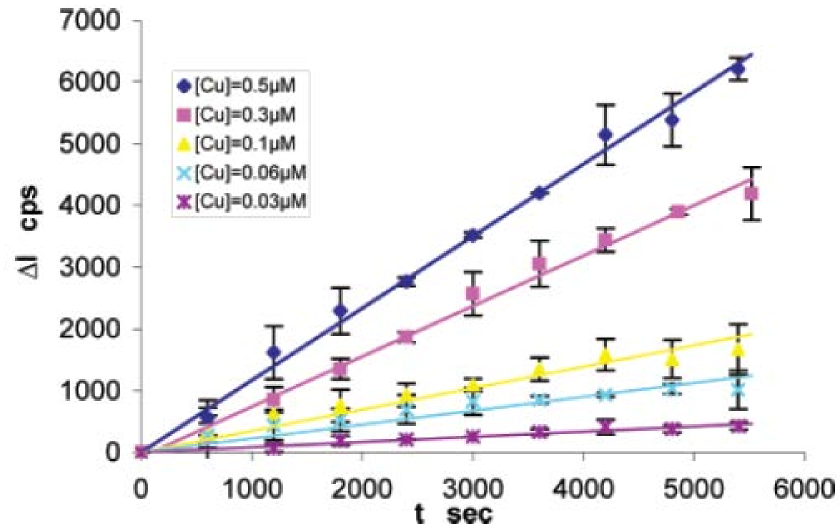
Willner *et al.*, *Anal. Chem.* **2009**, *81*, 9114-9119

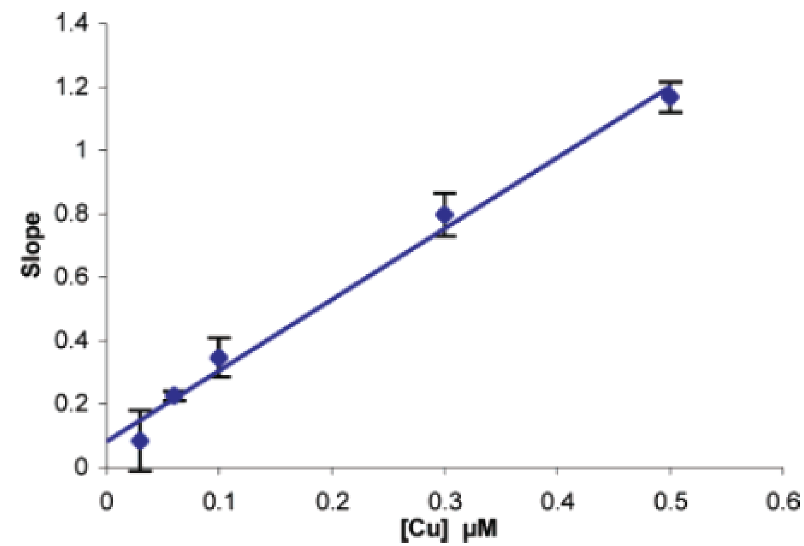
Metal detection using a synthetic system

mM
µM
nM
pM
fM
aM
zM
yM

$K_{Cu(II)\bullet PAC} >> K_{Pd(II)\bullet PAC}$







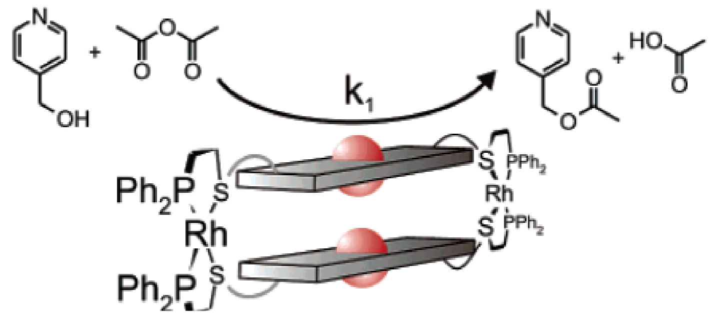
30 nM analyte requires 90 minutes

The higher the binding affinity (series Cu(II), Ni(II), Co(II), and Cd(II)), the greater the initial rate observed (selectivity).

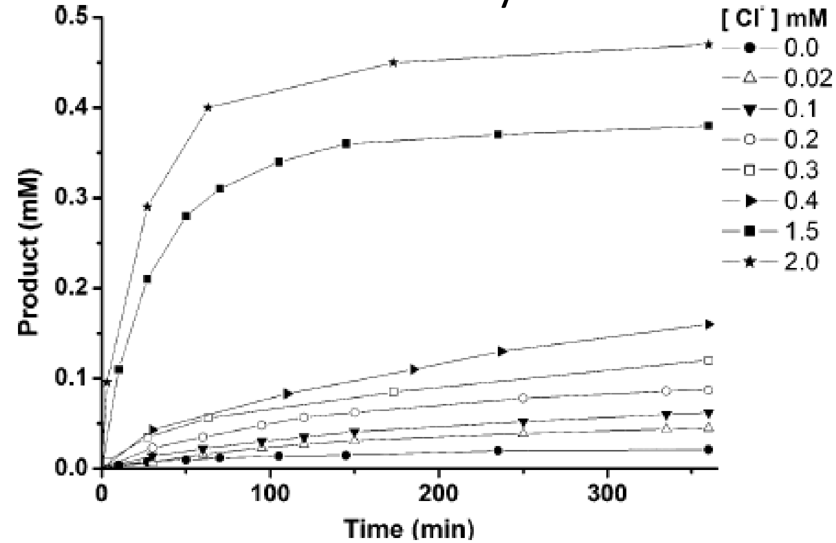
Anslyn *et al.*, *J. Am. Chem. Soc.* **2004**, *126*, 14682-14683

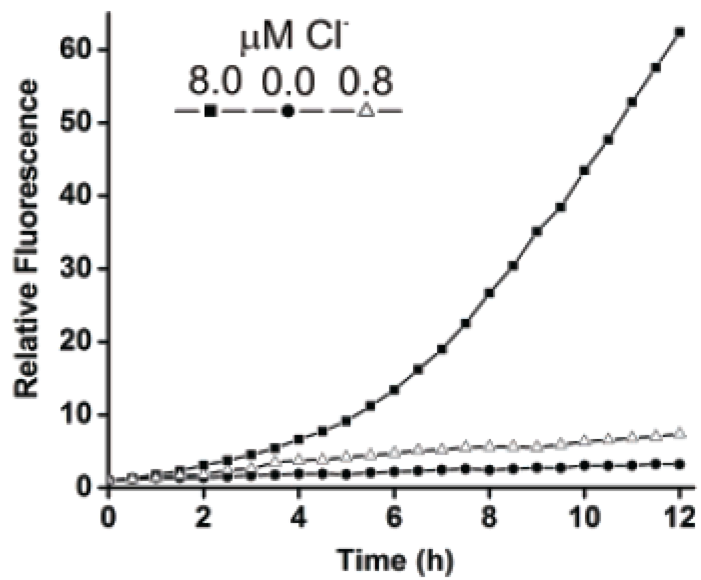
Small molecule detection using allosteric control

mM
µM
nM
pM
fM
aM
zM
yM



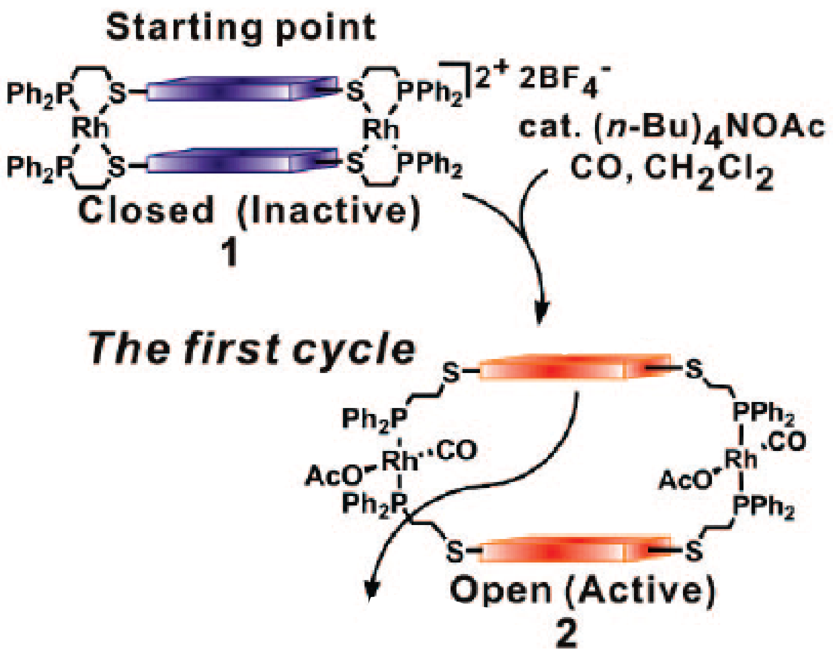
measured by GC





6 hours

Mirkin *et al.*, *J. Am. Chem. Soc.* **2005**, *127*, 1644-1645



Mirkin *et al.*, *J. Am. Chem. Soc.* **2008**, *130*, 11590-11591

PCR-like amplification

mM

µM

nM

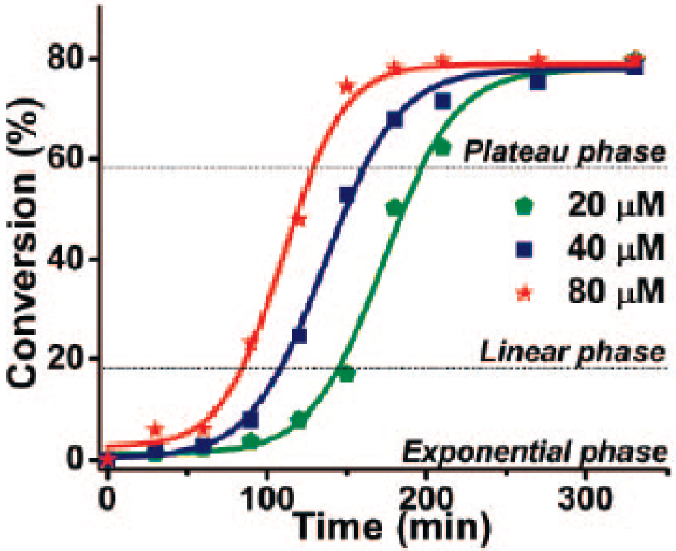
pM

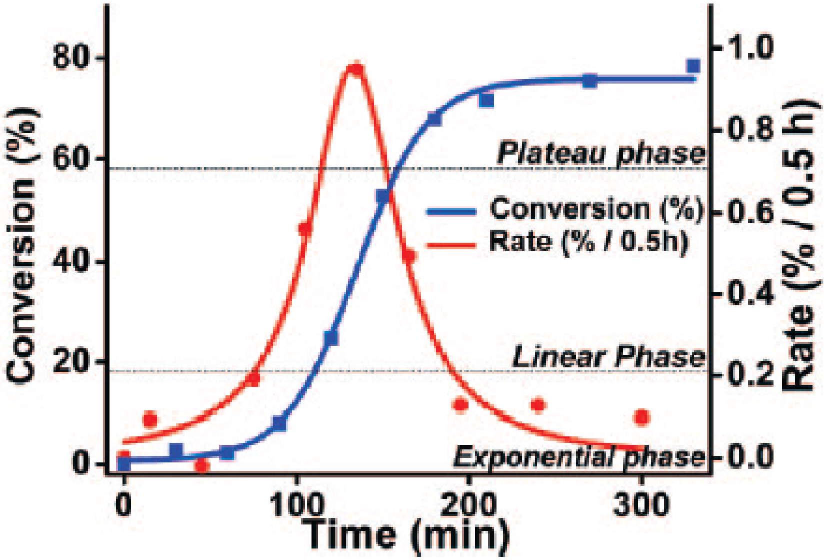
fM

aM

zM

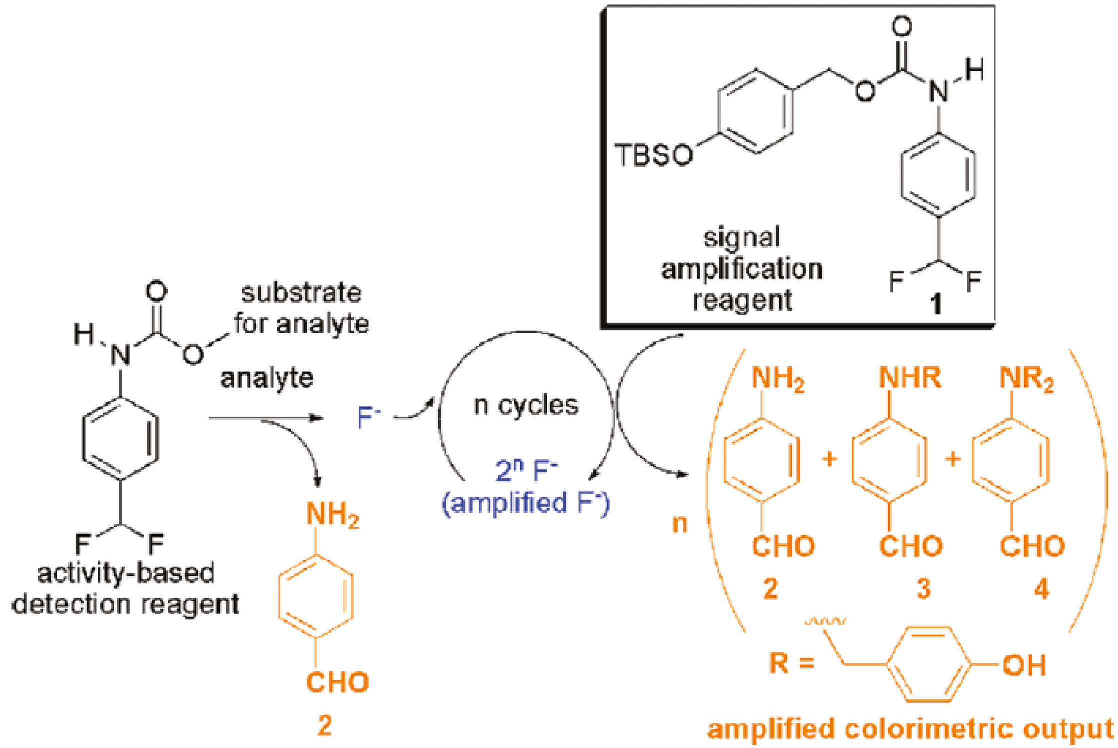
yM

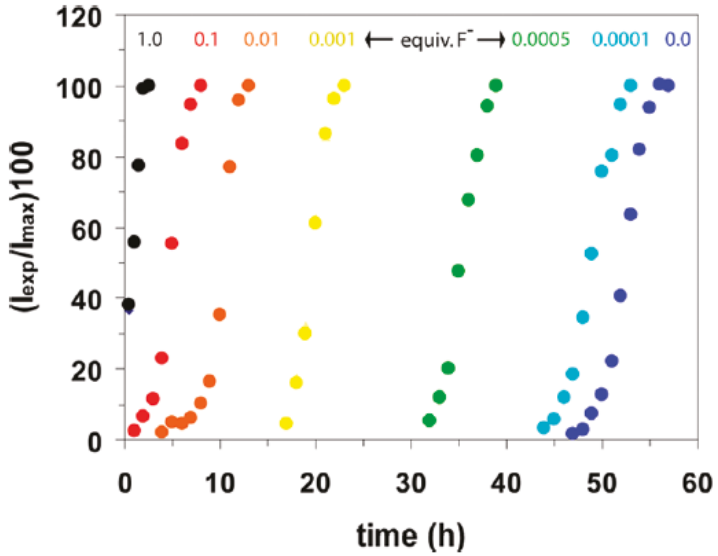




Mirkin *et al.*, *J. Am. Chem. Soc.* **2008**, *130*, 11590-11591

A Two-Component Small Molecule System for the detection of Pd(II)





response to F⁻

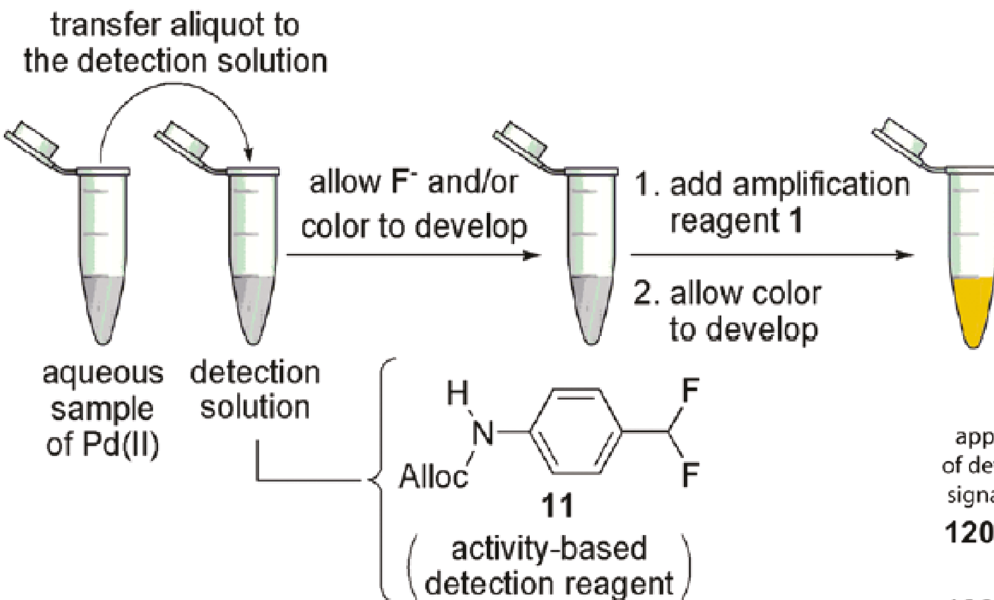
$$\alpha = \frac{(I_{amp}-I_{background})}{I_{initial}}$$

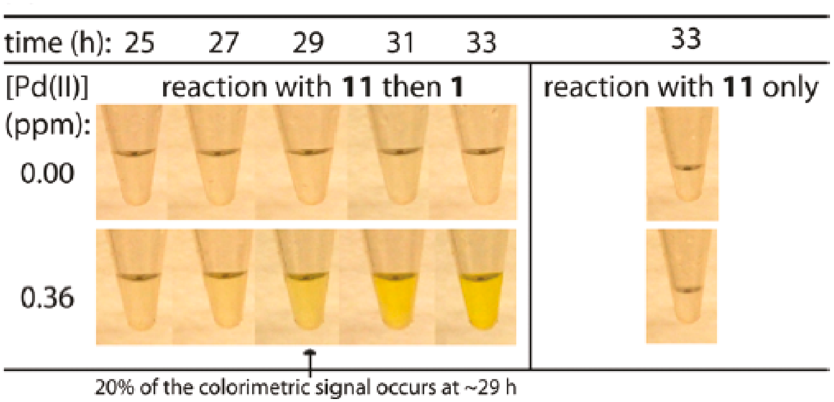
α reaches 2168(35 for 0.0005 equiv F⁻)
corresponding to 11 amplification cycles

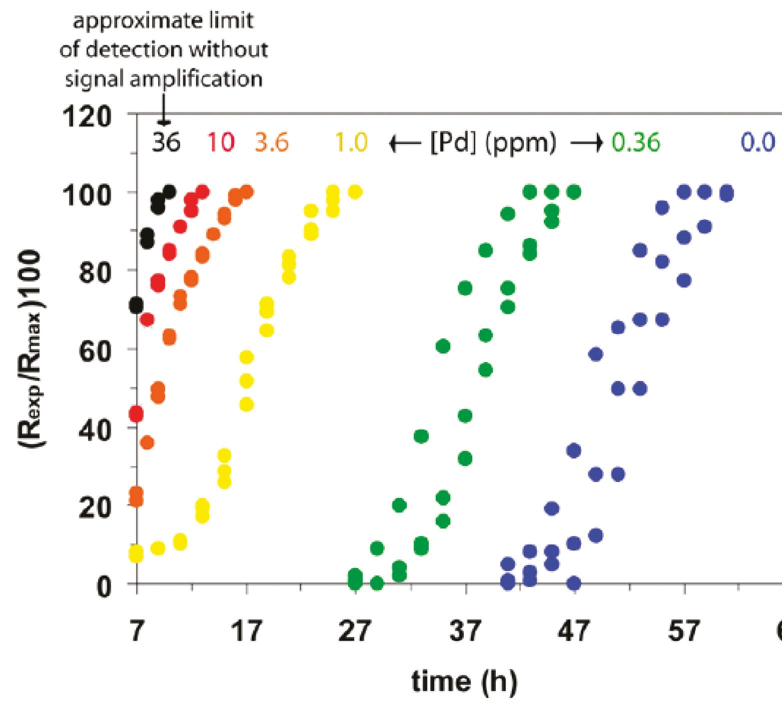
Phillips *et al.*, *J. Am. Chem. Soc.* **2011**, *133*, 5170-5173

Assay development

mM
µM
nM
pM
fM
aM
zM
yM

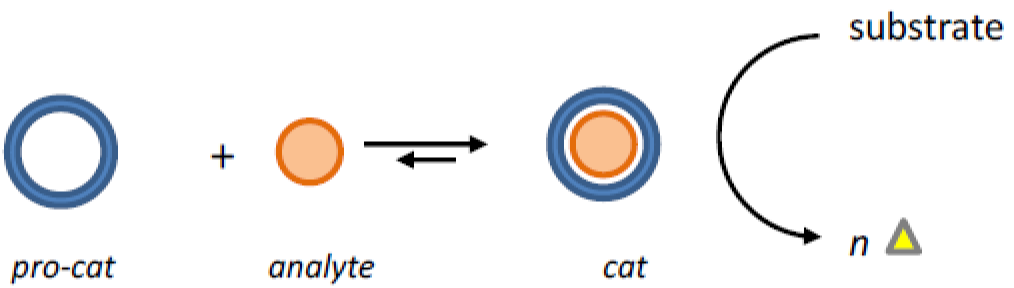






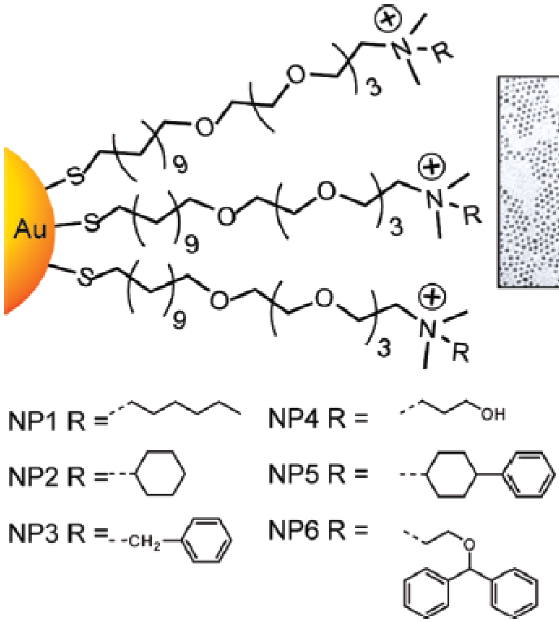
Phillips *et al.*, *J. Am. Chem. Soc.* **2011**, *133*, 5170-5173

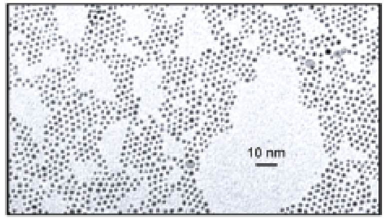
catalysis

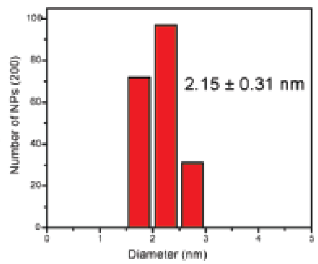


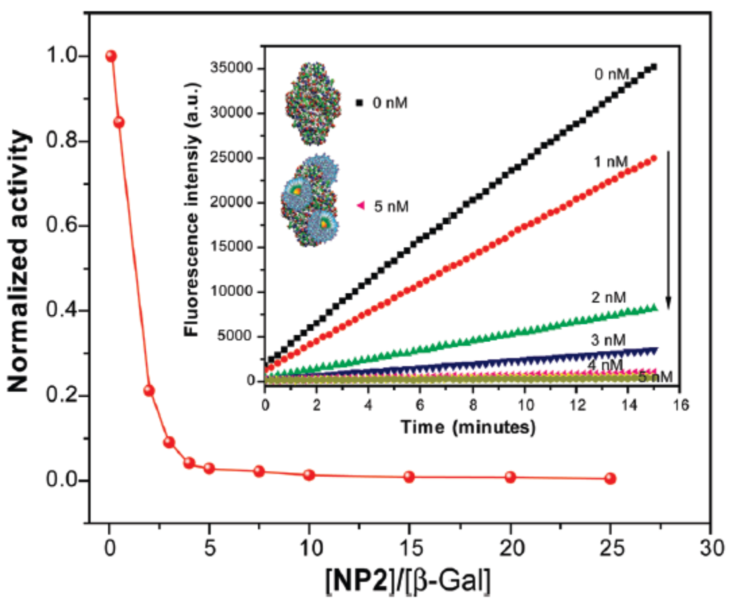
from the sensing of small molecules
to
the sensing of proteins

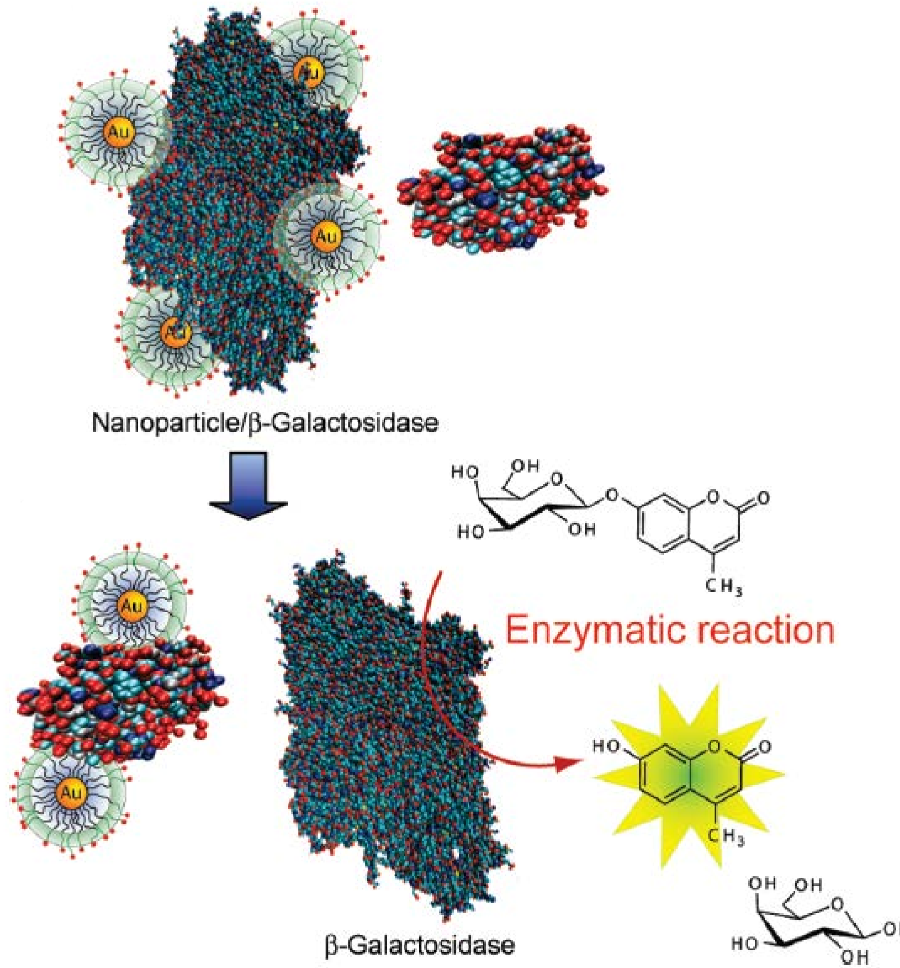
Nanoparticle based detection of proteins









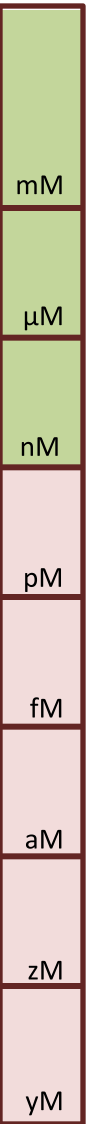


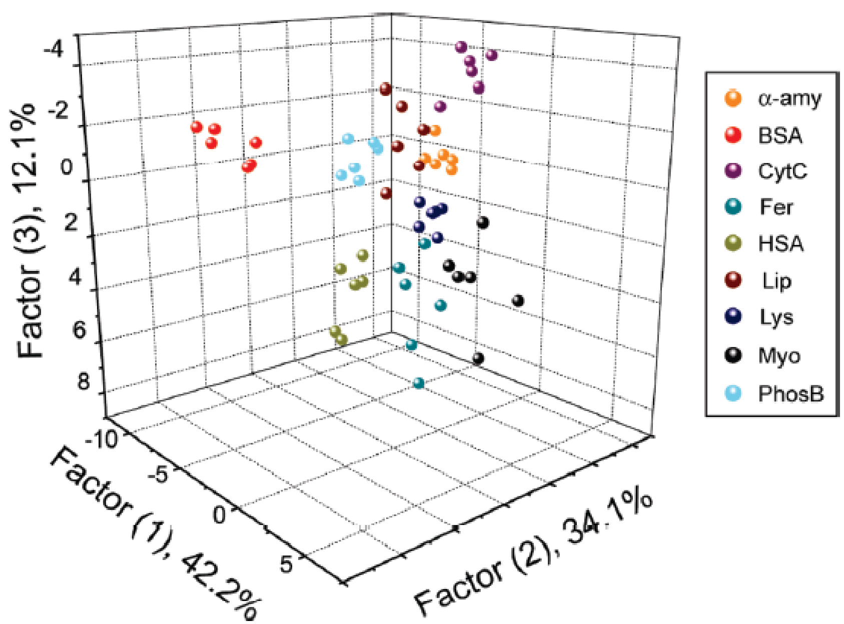
Rotello *et al.*, *J. Am. Chem. Soc.* **2010**, *132*, 5285-5289

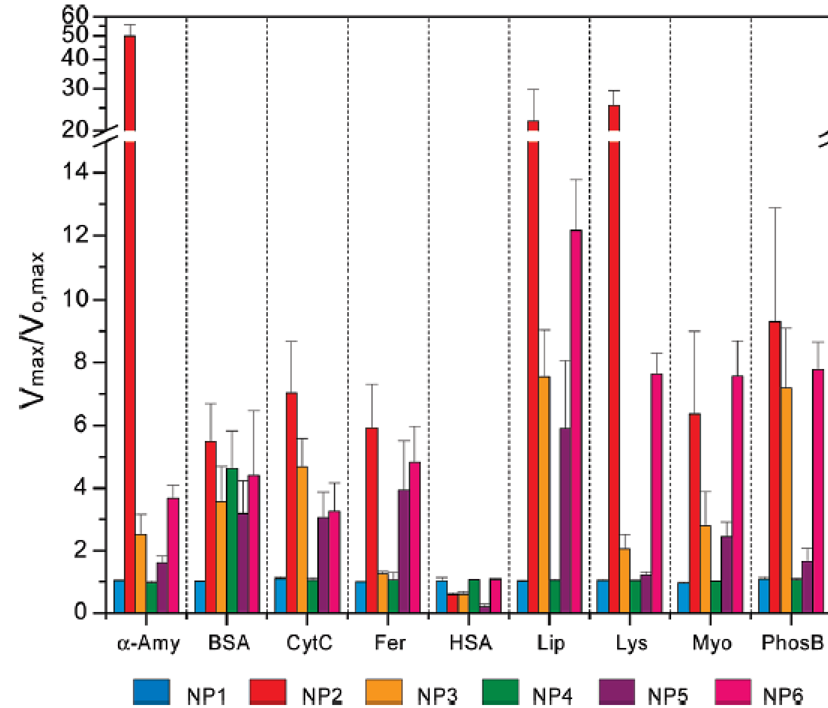
Table 1. Physical Properties of the Proteins Used As Sensing Targets in Phosphate Buffer Solution at pH 7.4[18a]

protein[a]	M_w (kDa)	pI
α-*amylase* (α-*Am*)	50.0	5.0
bovine serum albumin (BSA)	66.3	4.8
cytochrome *c* (CytC)	12.3	10.7
ferritin (Fer)	750.0	4.5
human serum albumin (HSA)	69.4	5.2
lipase (Lip)	58.0	5.6
lysozyme (Lys)	14.4	11.0
myoglobin (Myo)	17.0	7.2
alkaline phosphatase (PhosB)	140.0	5.7

[a] Proteins in *italics* are commonly found in human urine.



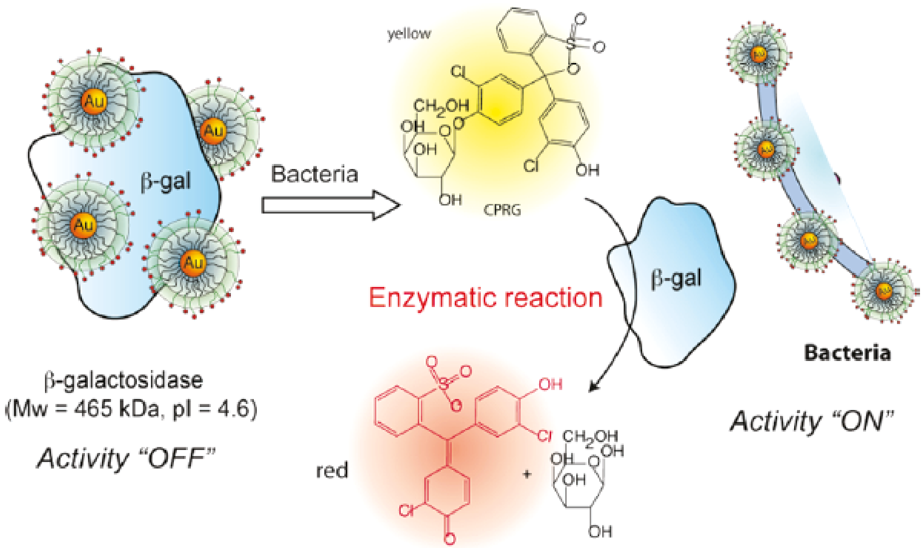


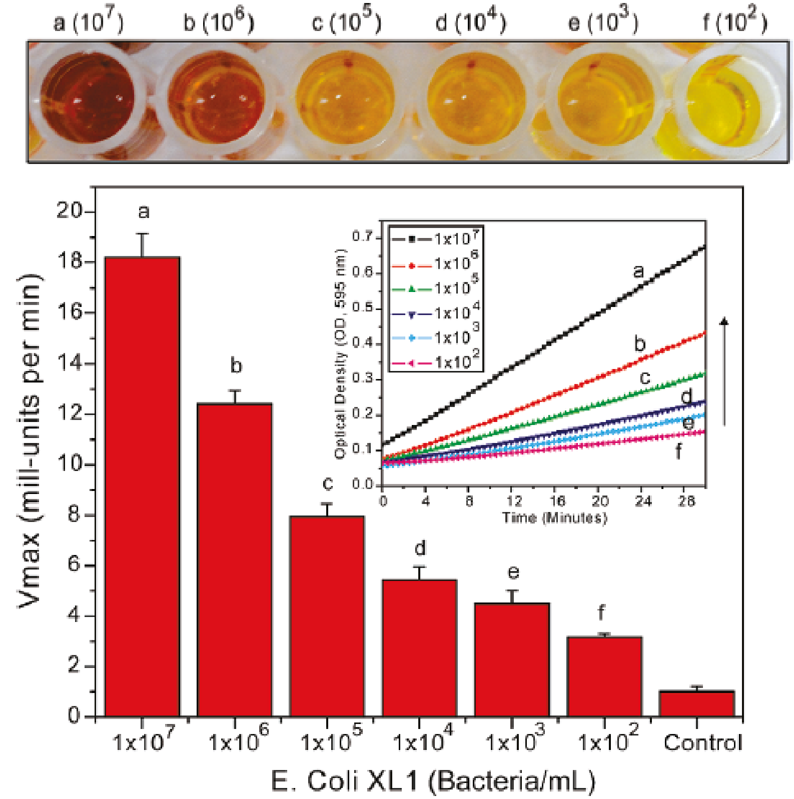


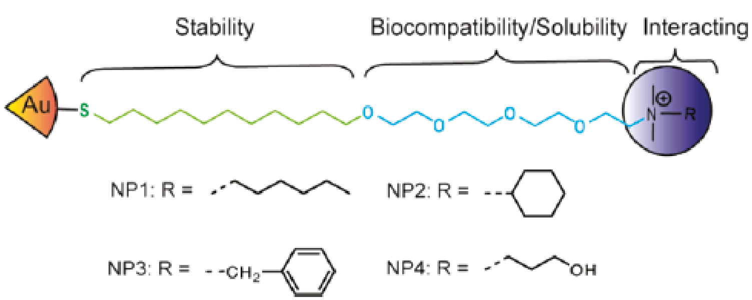
proteins at 1 nM concentration

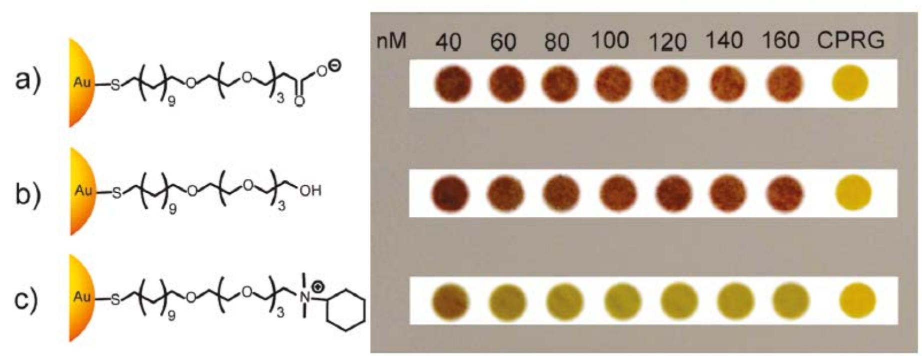
Rotello *et al.*, *J. Am. Chem. Soc.* **2010**, *132*, 5285-5289

Sensing of bacteria



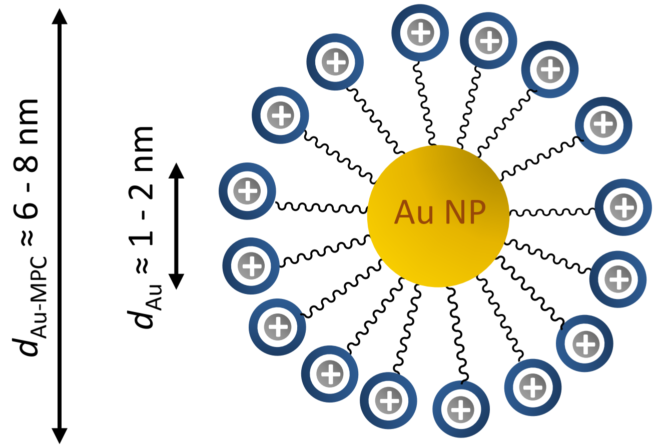




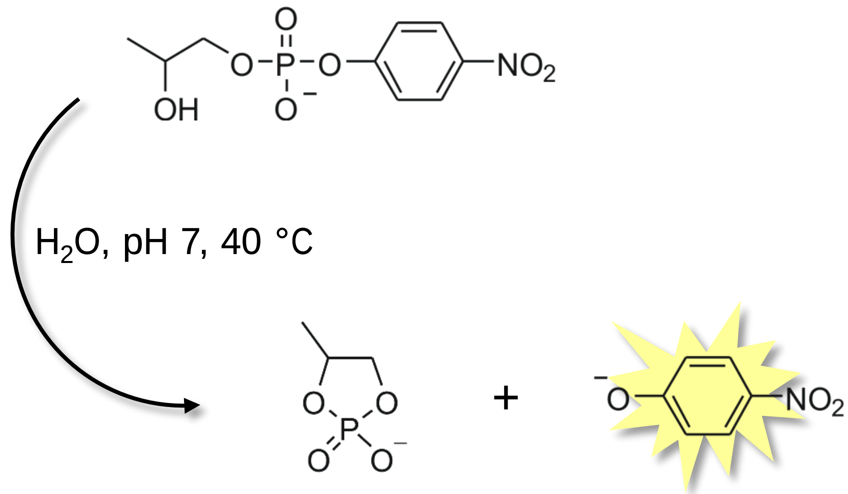


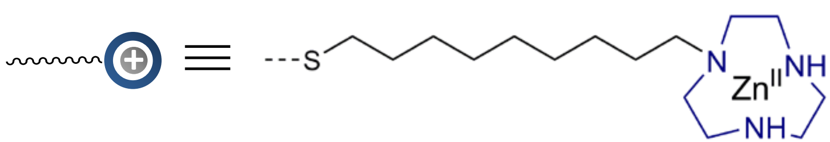
Rotello *et al.*, *J. Am. Chem. Soc.* **2011**, *133*, 9650-9653

Catalytic nanoparticles for sensing

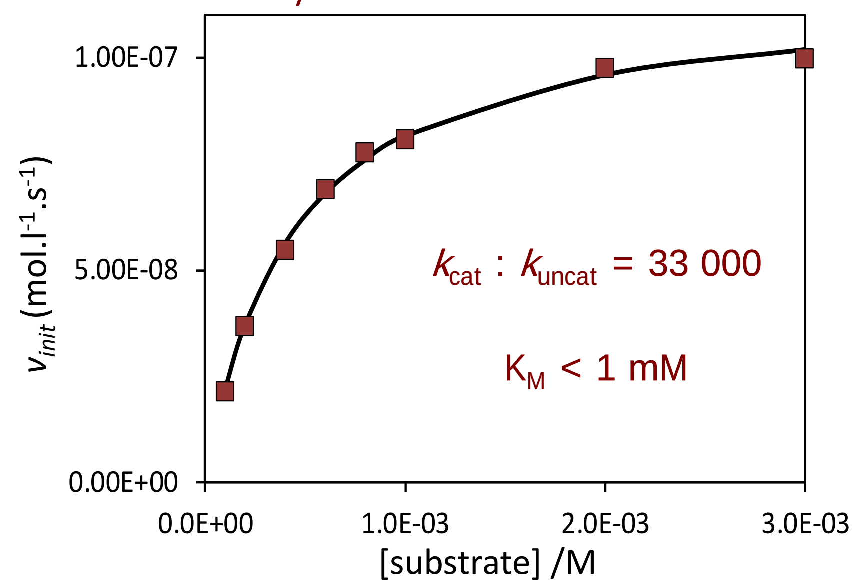


~ 70 head groups



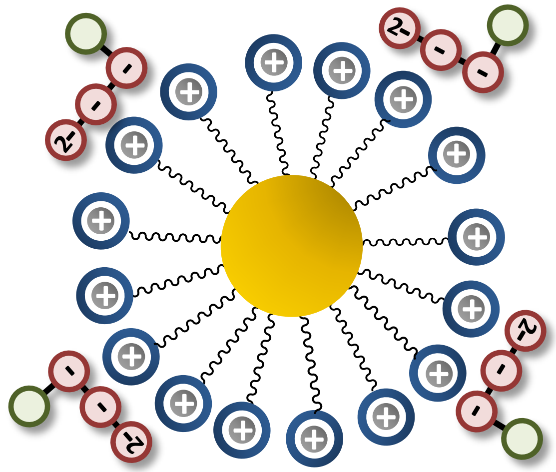


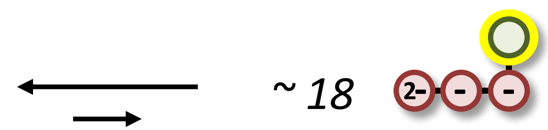
enzyme-like saturation behaviour



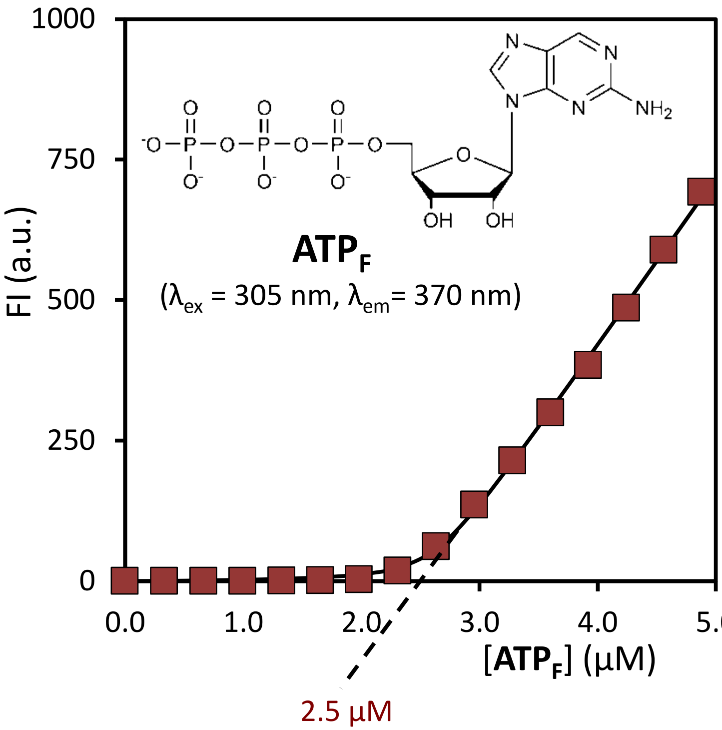
Scrimin *et al.* *Angew. Chem. Int. Ed.*, **2004**, *43*, *Chem. Eur. J.*, **2011**, *17*, 4879-4889

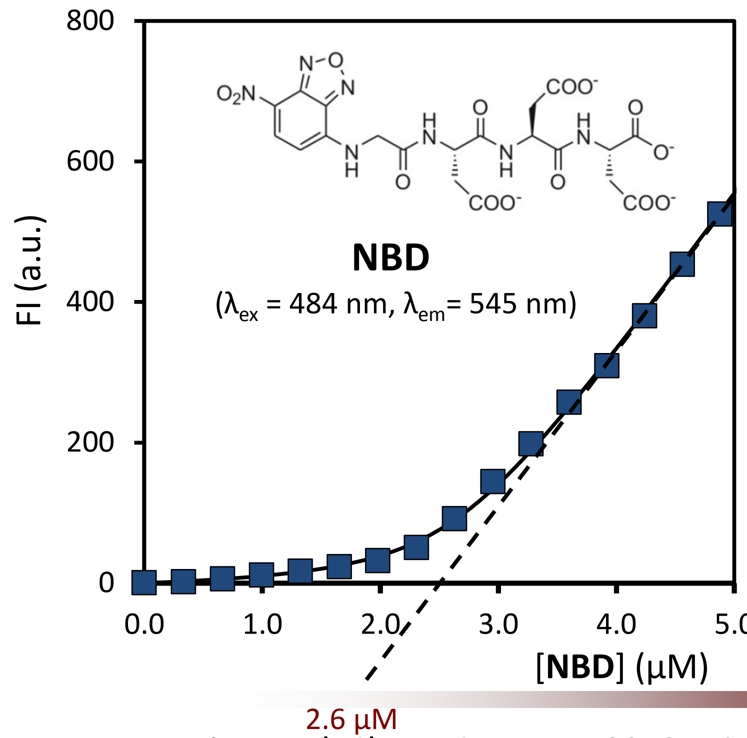
Monolayer protected Au nanoparticles: cationic surface





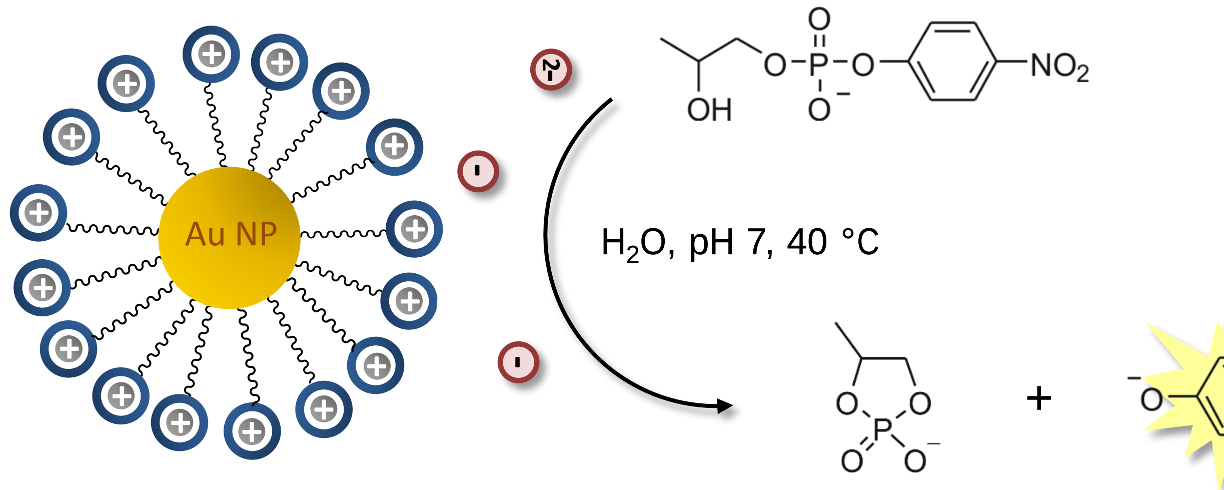
[TACN •Zn(II)] = 10 µM
pH = 7.0, T = 25 °C.

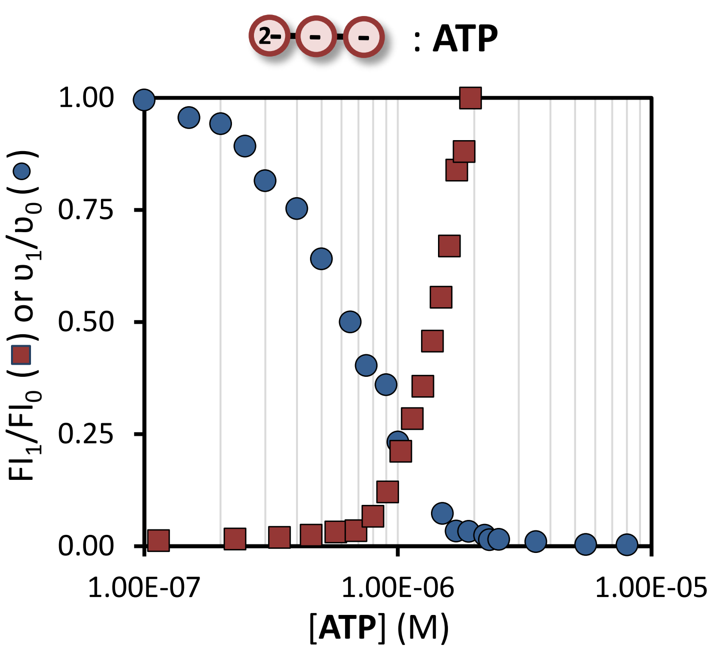


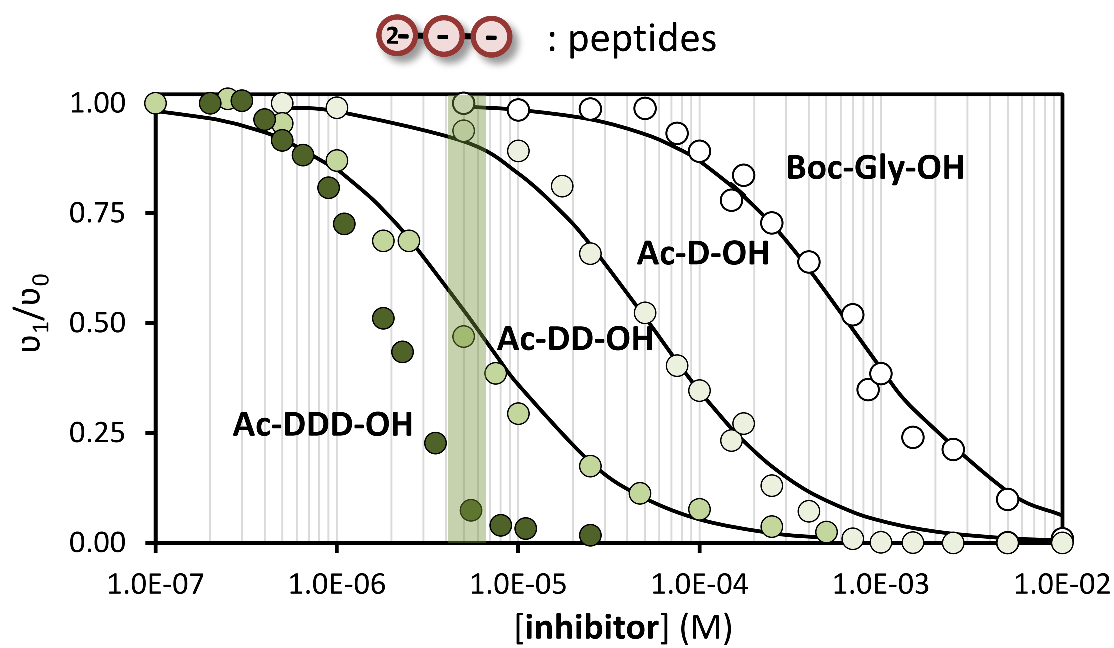


Prins *et al. Chem. Commun.* **2012**, *48*, 1916-1918

Catalytic signal generation

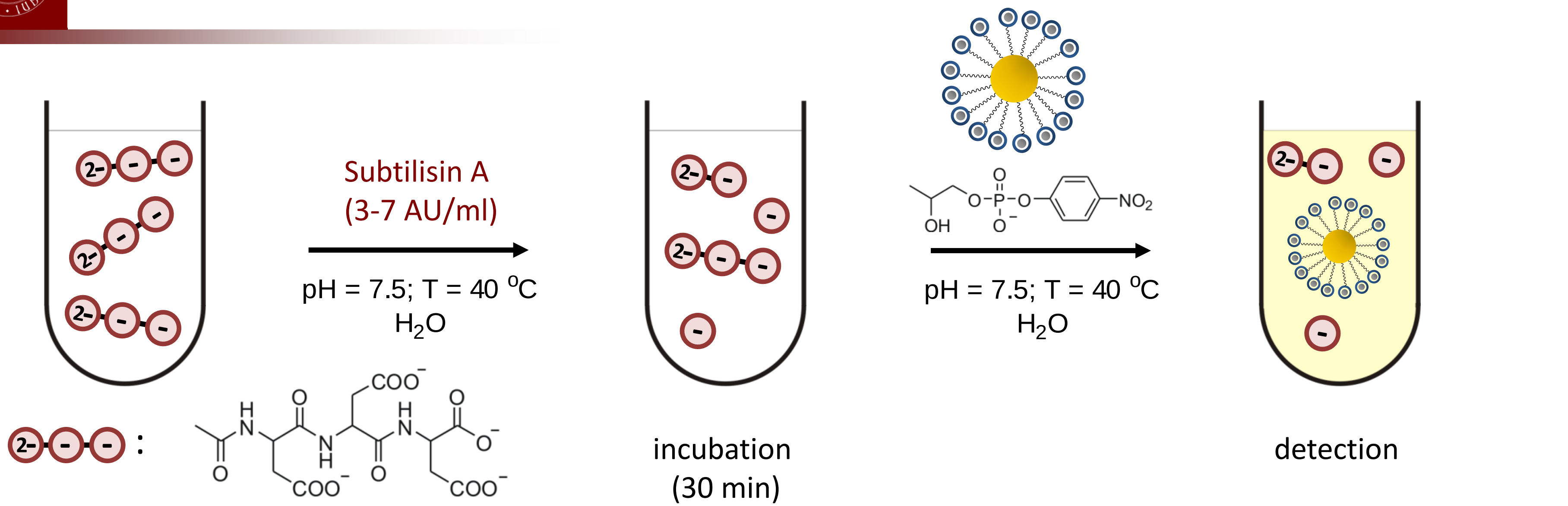


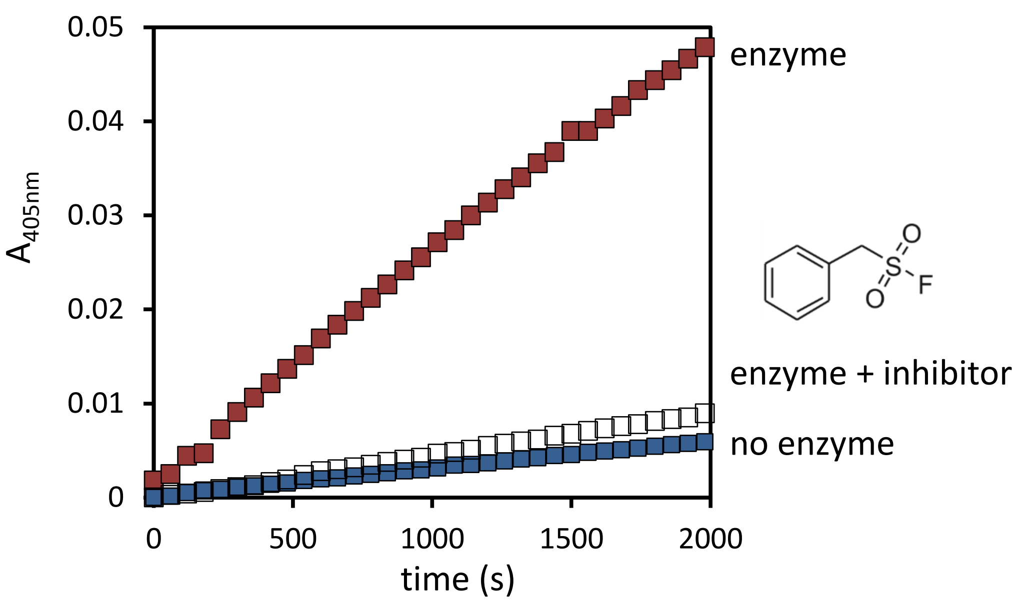


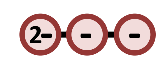
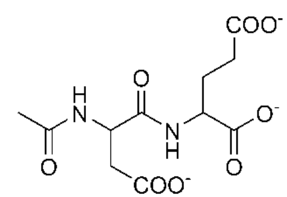
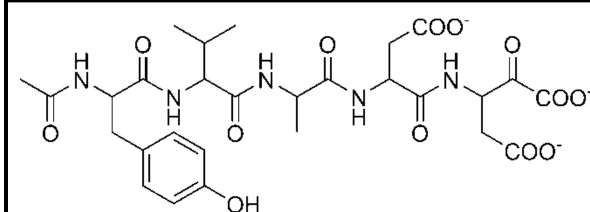


[TACN •Zn(II)] = 5 µM, [substrate] = 1 mM, [HEPES] = 10 mM, pH = 7.0, T = 25 °C.

Enzyme assay

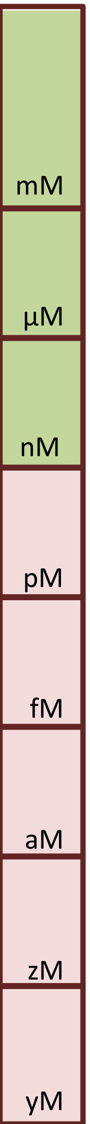


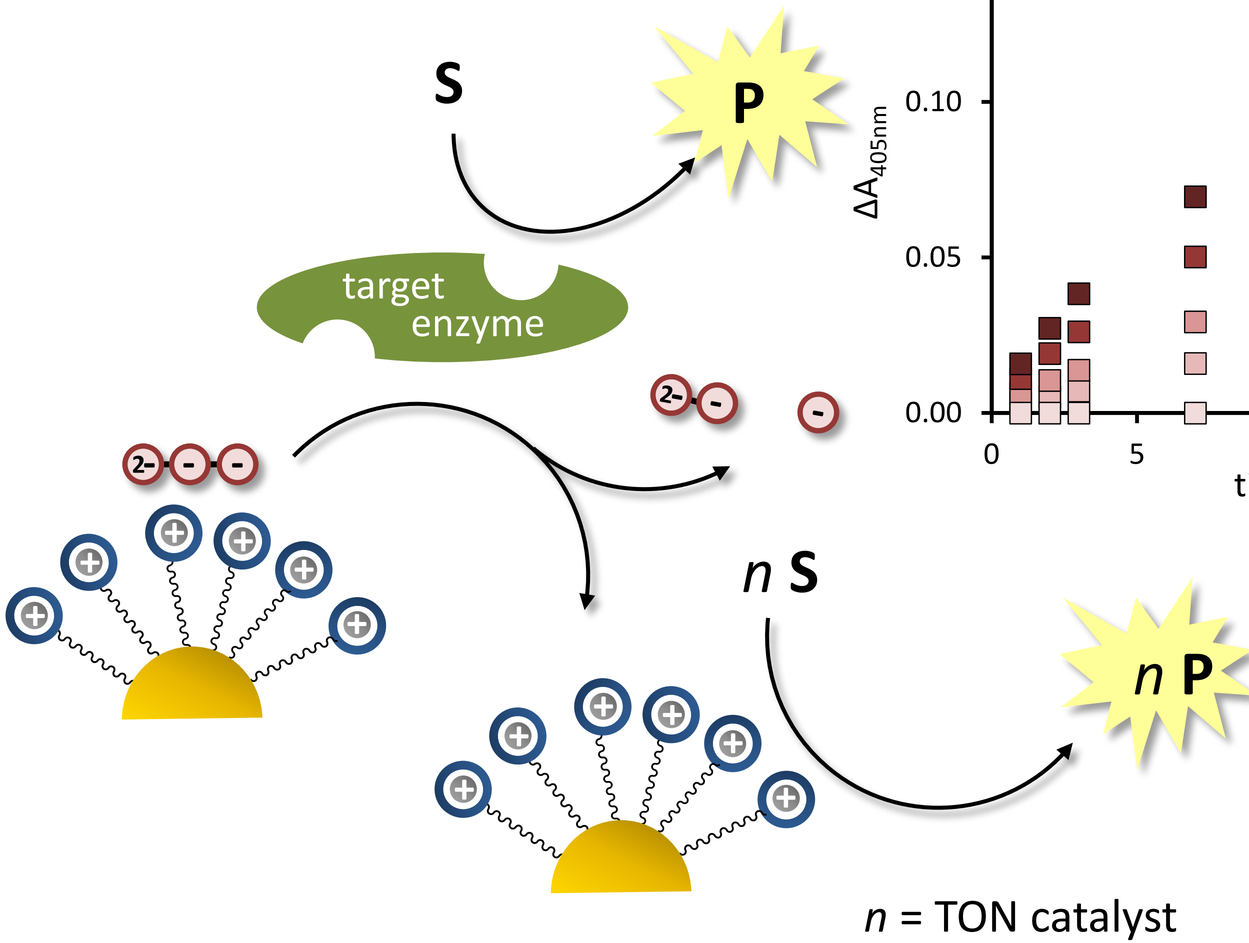


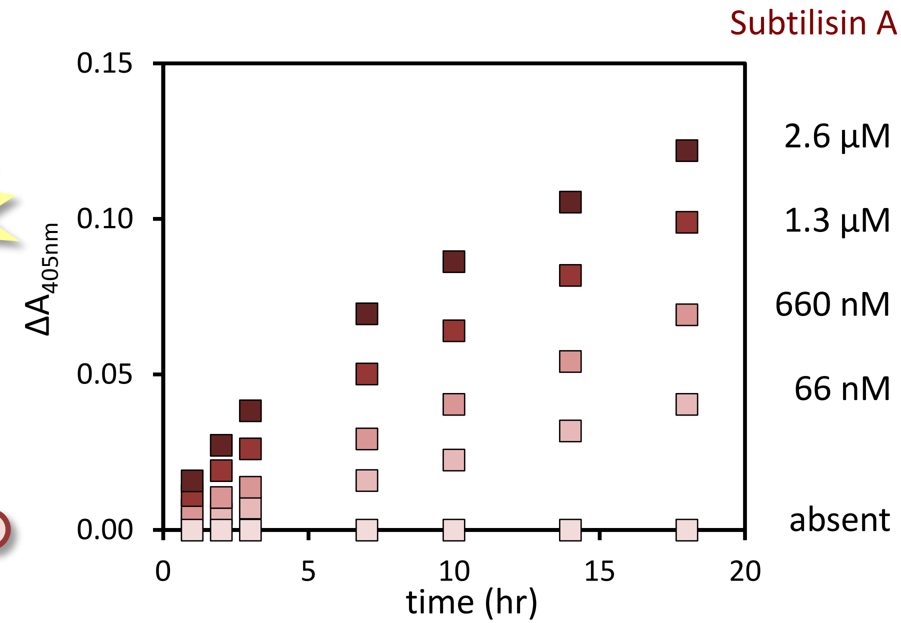
	enzyme
	GCP II
	Caspase-1

Prins *et al. Angew. Chem. Int. Ed.*, **2011**, *50*, 2307 –2312; PCT 2012085080

Sensing through signal amplification



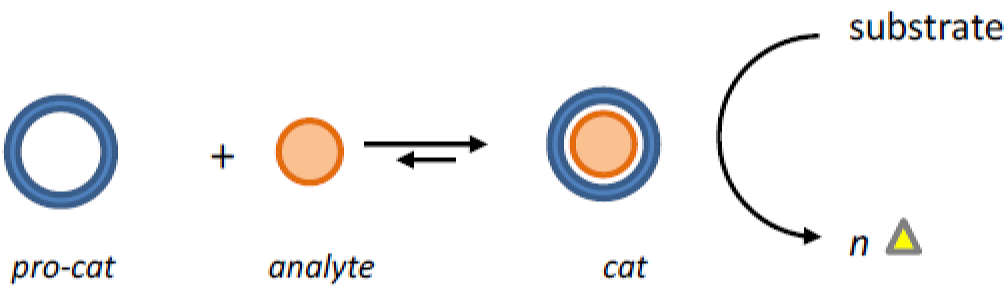




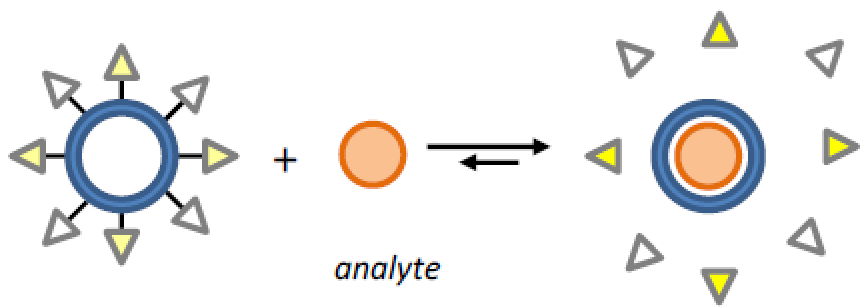
Importance: the analyte (enzyme) triggers a cascade of catalytic events

Supramolecular sensing systems

catalysis

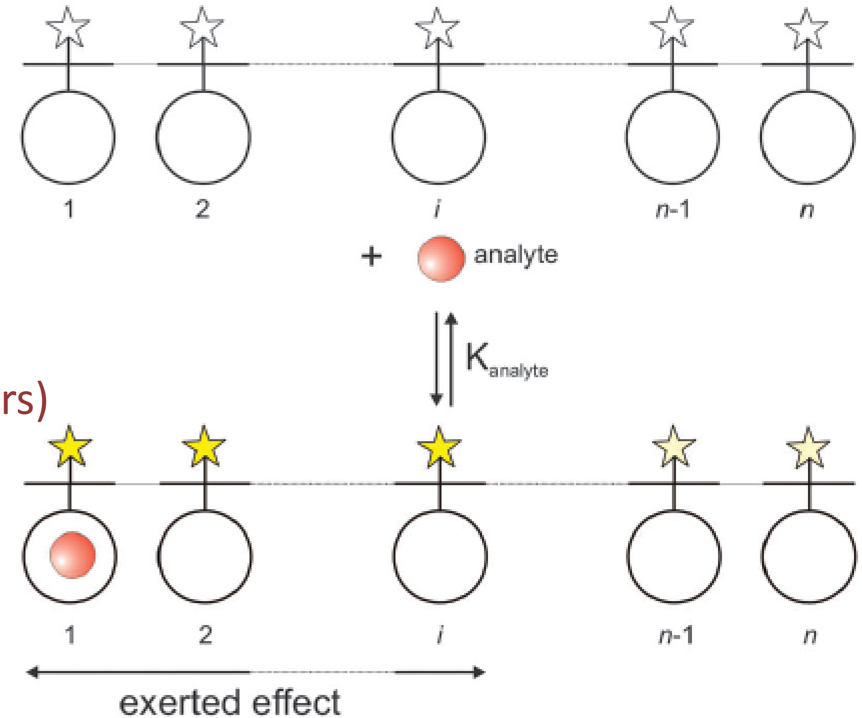


multivalency



Multivalency

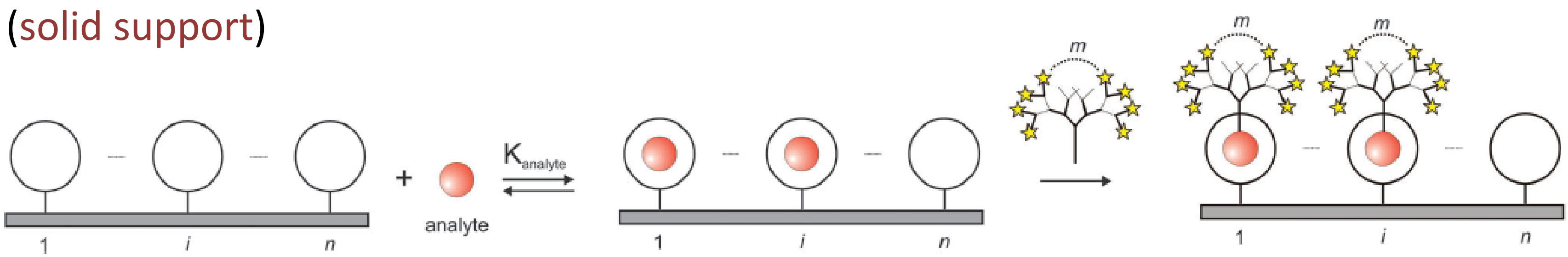
A



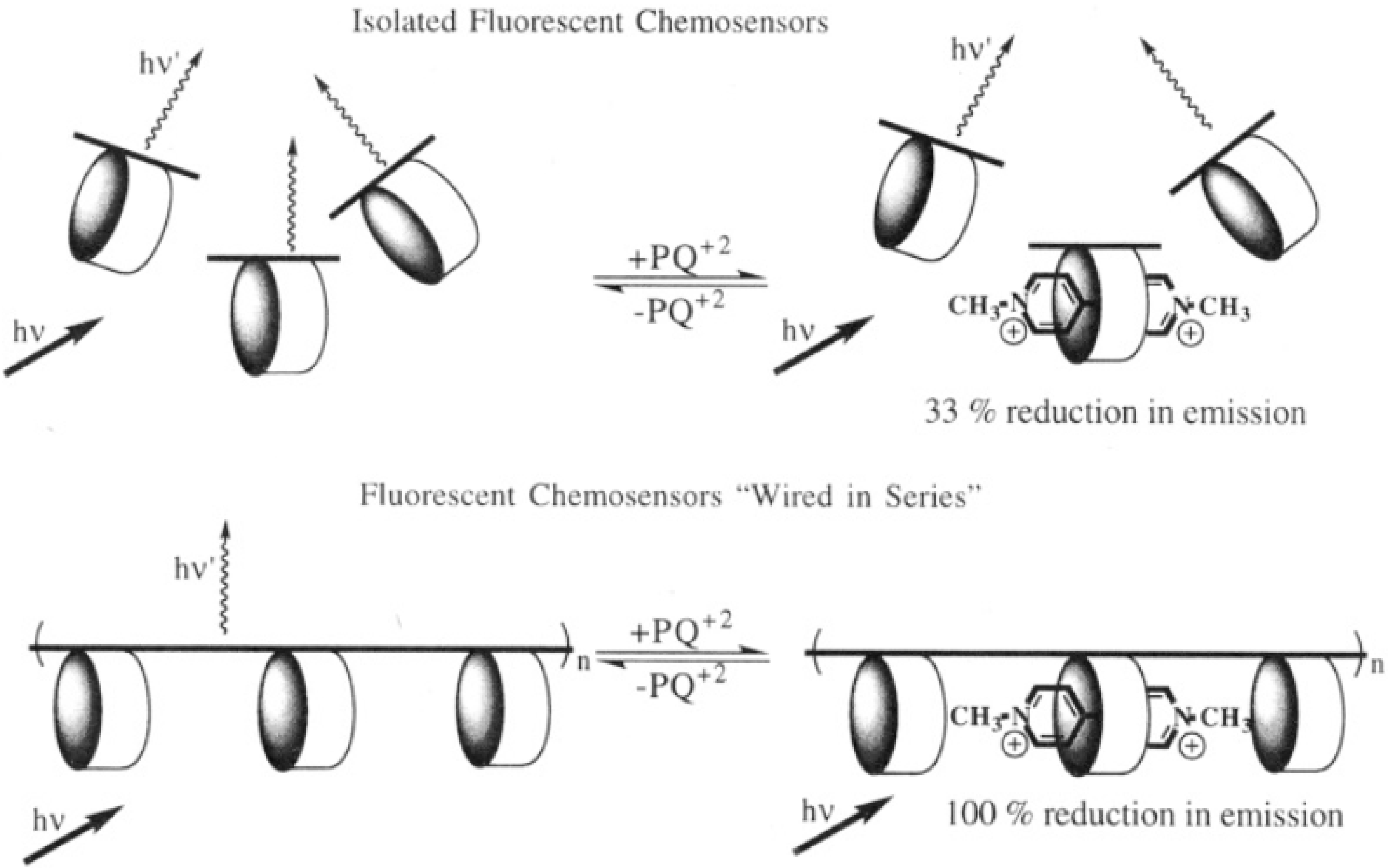
collective property (polymers)

- electron conductivity
- helicity
- stability

B (solid support)



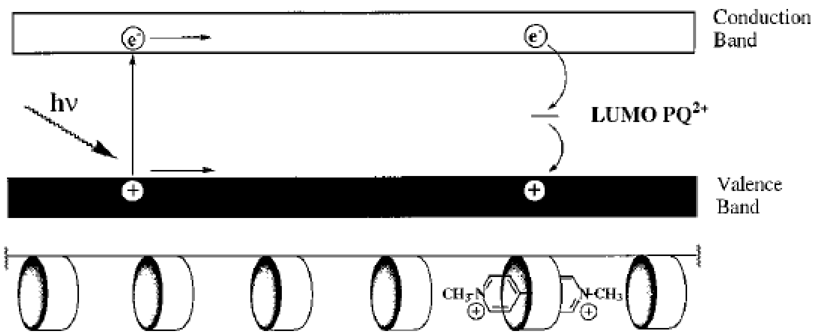
The ‘Molecular Wire’ approach



Swager *et* al. *Acc.Chem.Res.*, **1998**, 201-207

The ‘Molecular Wire’ approach

conjugated fluorescence polymer

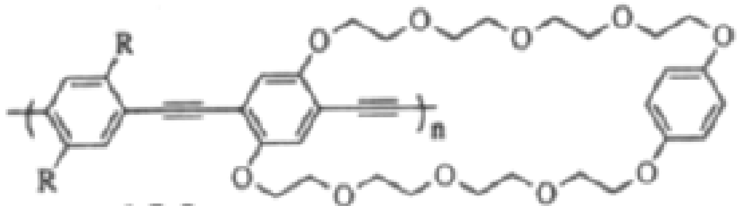


Key issues:

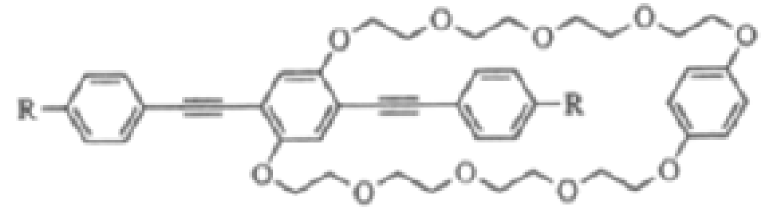
- multitude of binding sites (guest binding at low concentrations)
- very high apparent binding constant
- polymerization degree
- mobility of the excited state
- fluorescence lifetime

Small molecule sensing

mM
µM
nM
pM
fM
aM
zM
yM

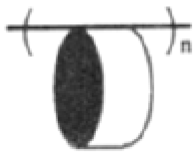


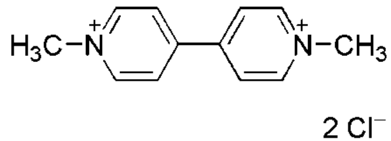
6, 7, 8
R=CON(C_8H_{17})$_2$

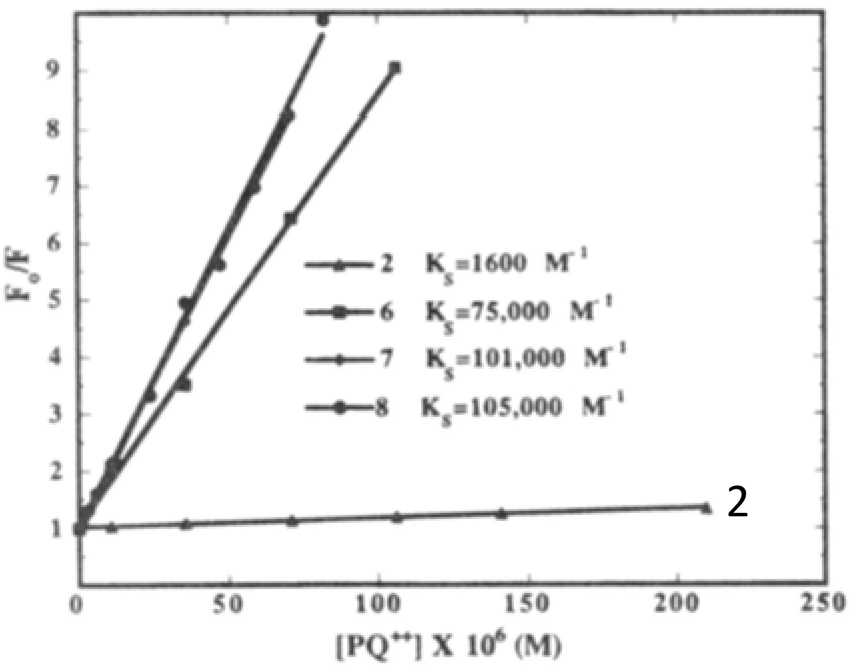


2
R=O$C_{12}H_{25}$

control





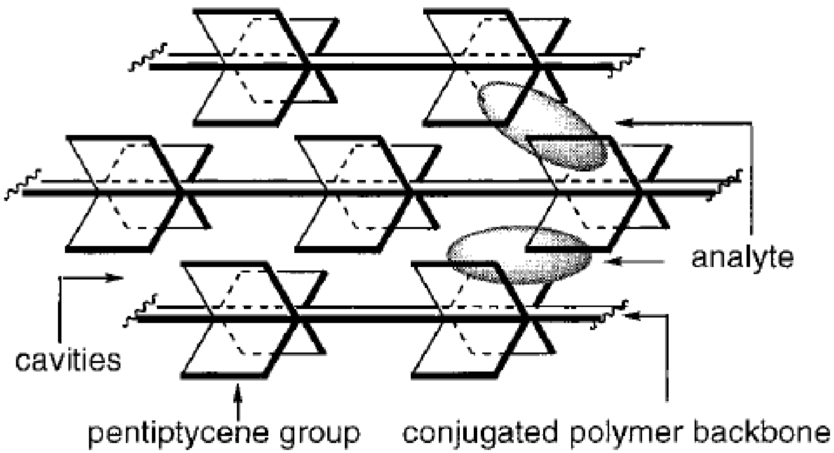


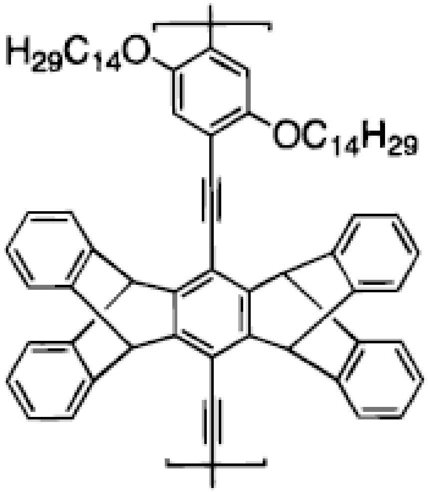
Mean molecular weight (PDI)
6: 31 100 (1.6)
7: 65 400 (1.6)
8: 122 500 (1.8)

Stern-Volmer plots (F0/F=1-K[PQ^{2+}])

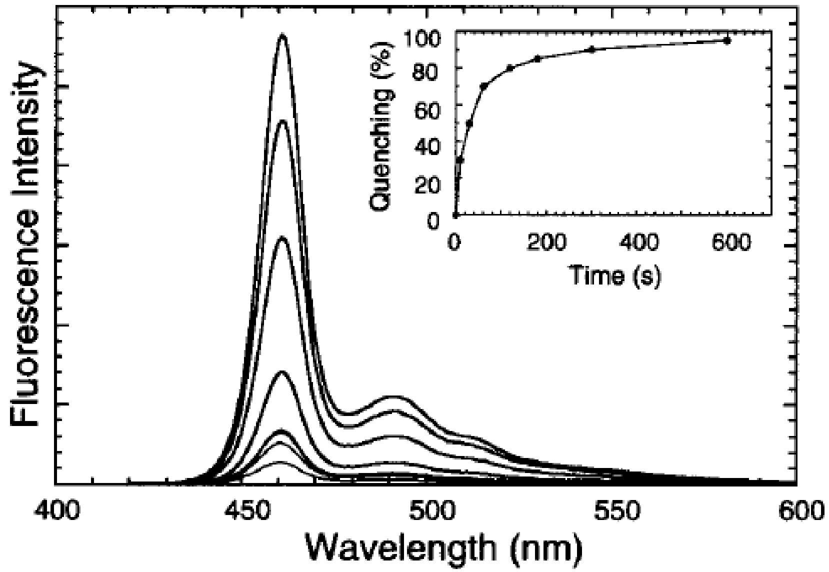
Swager *et* al. *J. Am. Chem. Soc.*, **1995**, 7017-7018

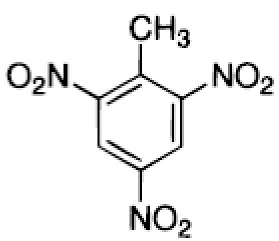
Polymer sensor materials





1. No π-stacking of polymer backbones, thus high fluorescence quantum yields and spectroscopic stability in thin films.
2. Second, reduced interpolymer interactions enhance the solubility
3. The cavities generated allow diffusion of small organic molecules into the films.

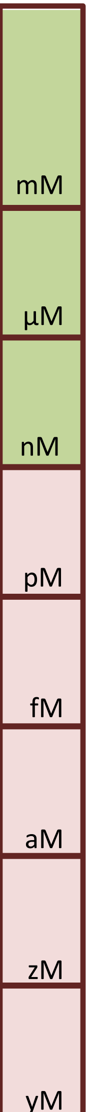


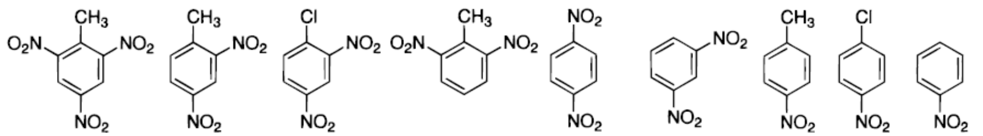


TNT

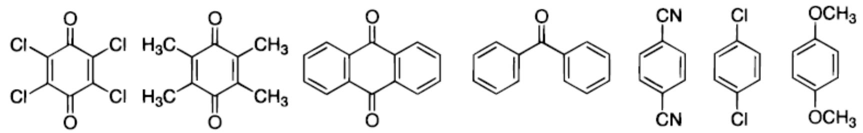
Swager *et* al. *J. Am. Chem. Soc.*, **1998**, *120*, 11864-11873

Fluorescent Porous Polymer Films as TNT Chemosensors

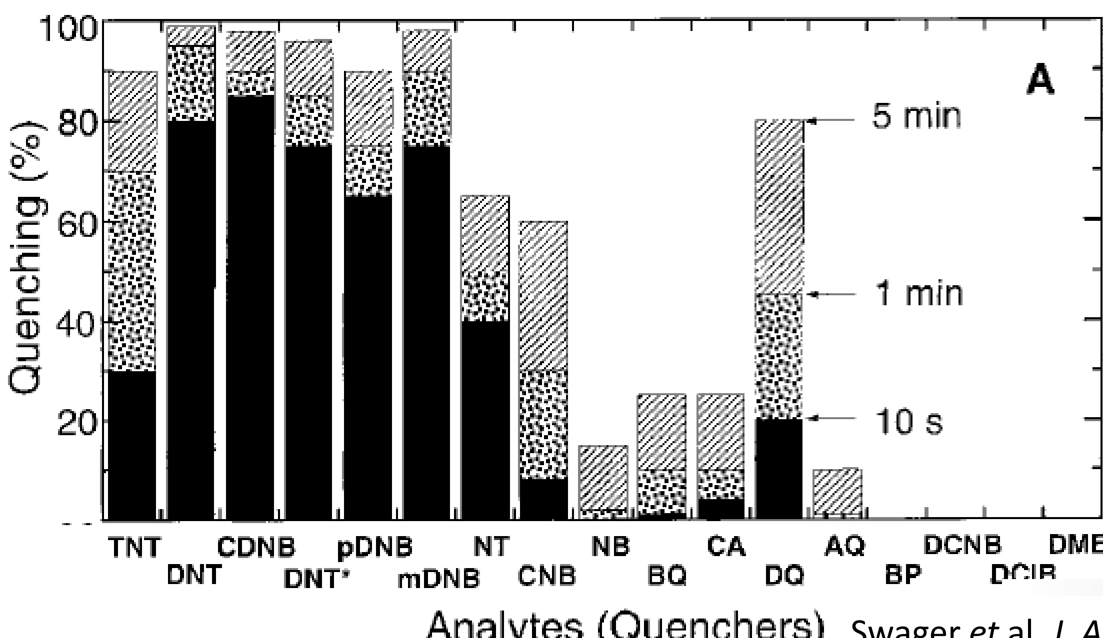




	TNT	DNT	CDNB	DNT*	pDNB	mDNB	NT	CNB	NB
rel. VP	1	18	10	70	3	110	2×10^4	3×10^3	3×10^4
E_{red} (V)	-0.7	-1.0	-0.8	-1.0	-0.7	-0.9	-1.2	-1.1	-1.15



	BQ	CA	DQ	AQ	BP	DCNB	DCIB	DMB
rel. VP	1×10^5	950	360	0.015	200	700	1×10^6	1×10^4
E_{red} (V)	-0.5	0.0	-0.8	-0.9	-1.6	-1.7	-1.8	< -2

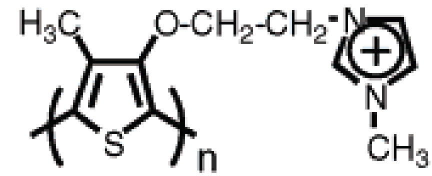


vapour pressure (VP) TNT = 10 ppb at 25 °C

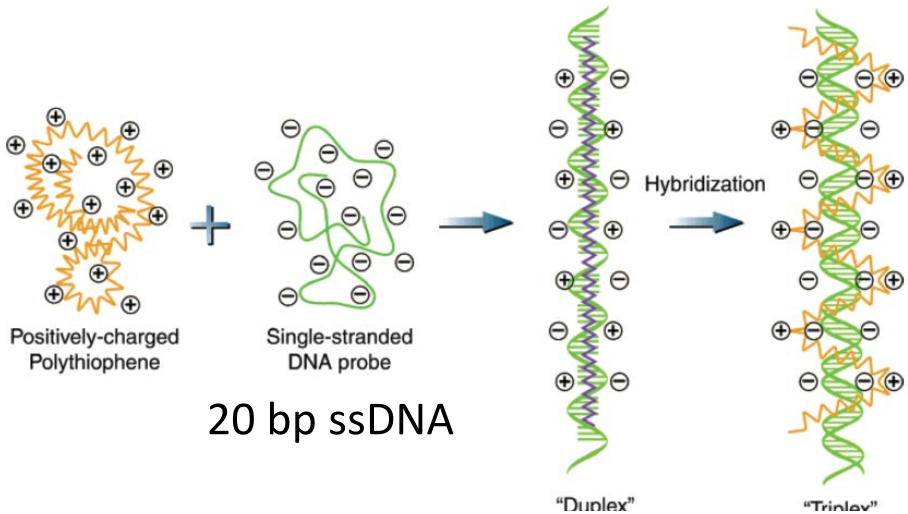
Swager *et al.* *J. Am. Chem. Soc.*, **1998**, *120*, 11864-11873

Fluorescent Polymers for DNA detection

mM
µM
nM
pM
fM
aM
zM
yM



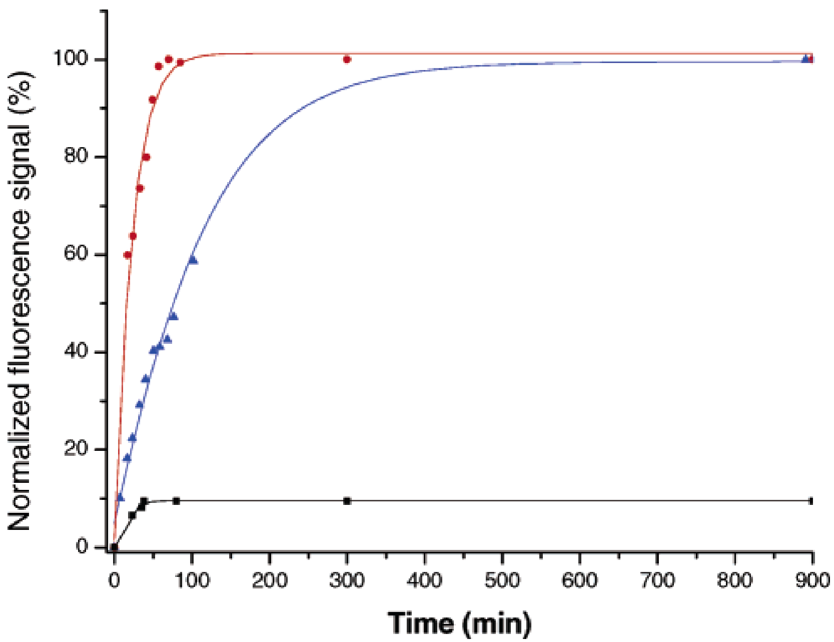
6 - 10 kDa (PDI:1.2-2.9)



20 bp ssDNA

duplex form/aggregate
decreased fluorescence

triplex form
increased fluorescence

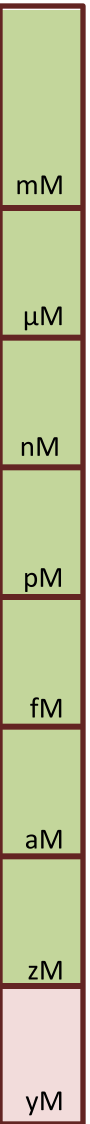


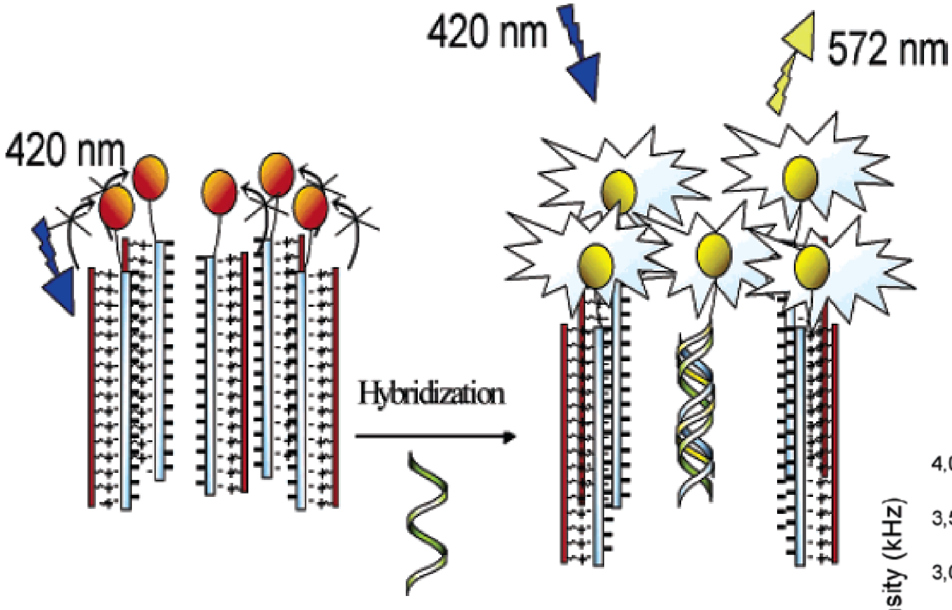
red: target
blue: one mismatch
black: double mismatch

The limit of detection for the perfectly matched 20-mer target is 310 molecules, or 0.54 zmol, in a volume of 150 μL.

Boudreau, Leclerc *et* al. *J. Am. Chem. Soc.*, **2004**, *126*, 4240-4242

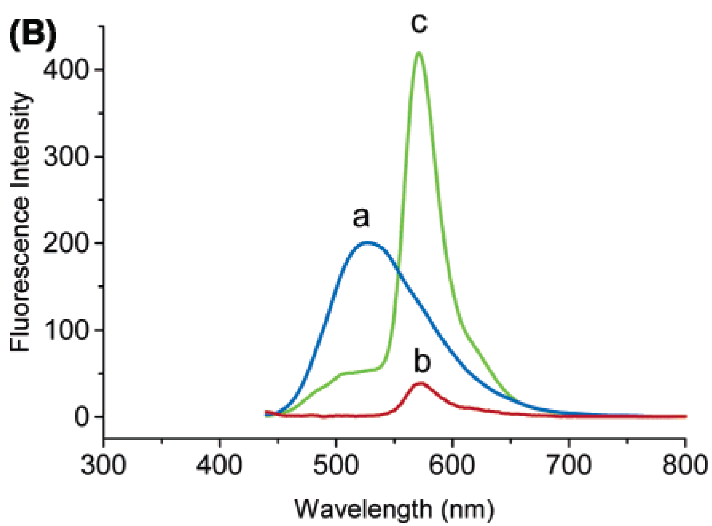
Ultrasensitive detection using fluorescence chain reaction (FCR)



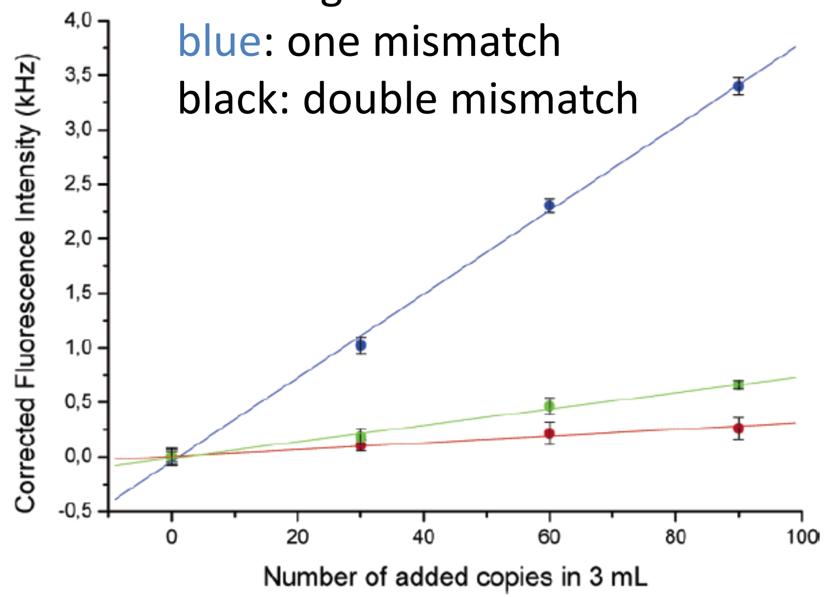


capture probe is labeled with a fluorophore (Alexa Fluor 546)

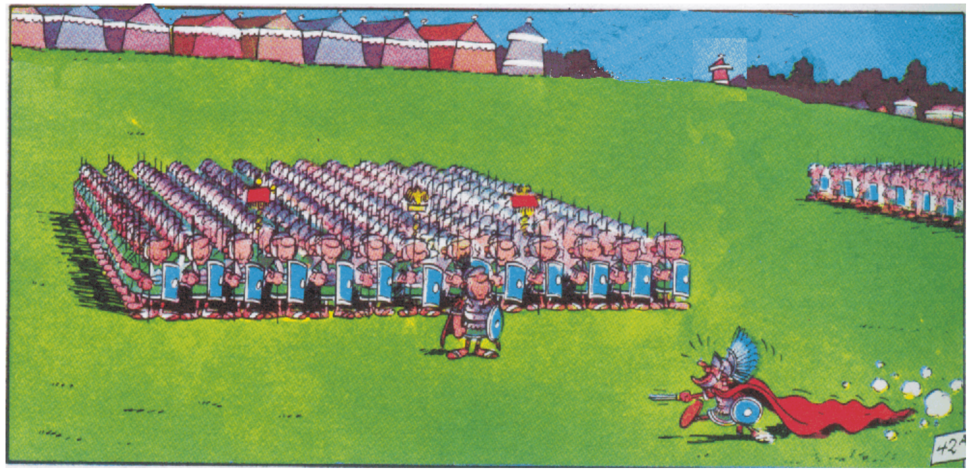
large number of capture probes are available ($x10^{10}$ copies)



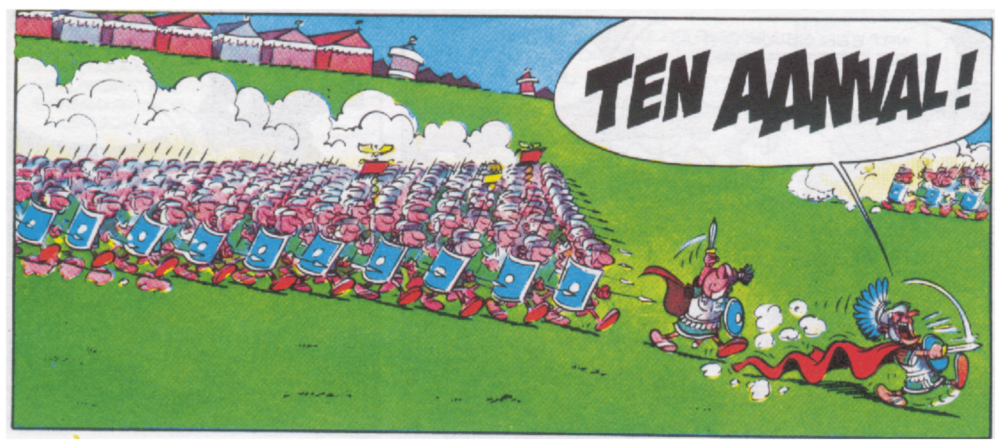
red: target
blue: one mismatch
black: double mismatch



Boudreau, Leclerc *et* al. *J. Am. Chem. Soc.*, **2005**, *127*, 12673-12676



carré

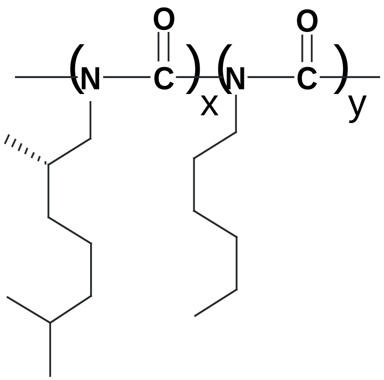


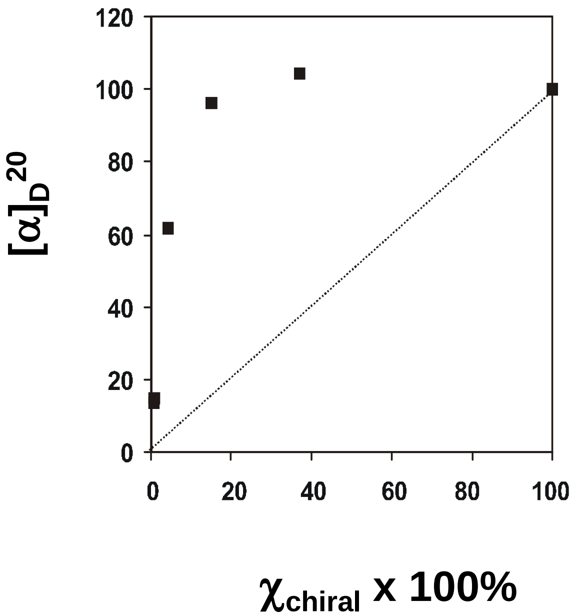
triangle

circle !

Goscinny & Uderzo *Asterix en de Britten* 1966

The ‘Sergeants-and-Soldiers’ principle in polymers

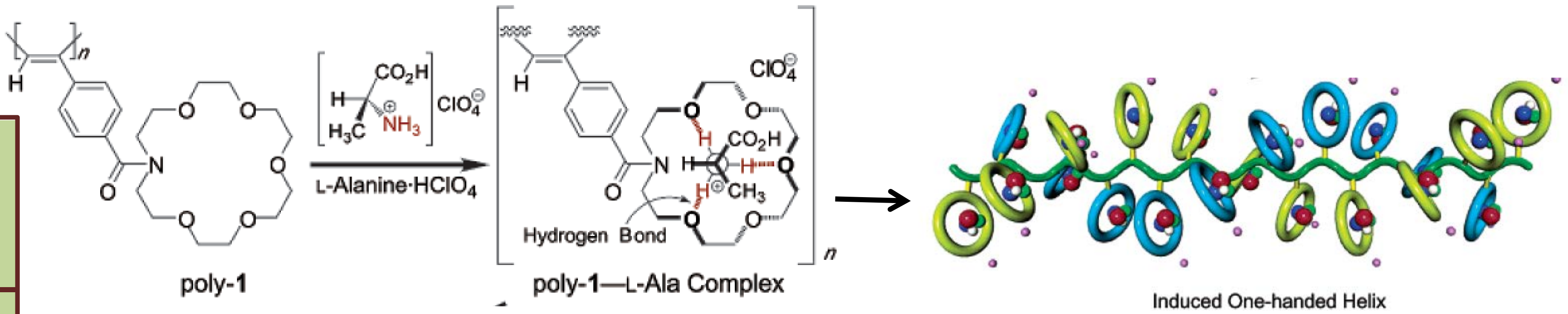




Green *et al. J. Am. Chem. Soc.*, **1989**, *111*, 6452-6453

The use of ‘Sergeants-and-Soldiers’ for sensing

mM
µM
nM
pM
fM
aM
zM
yM

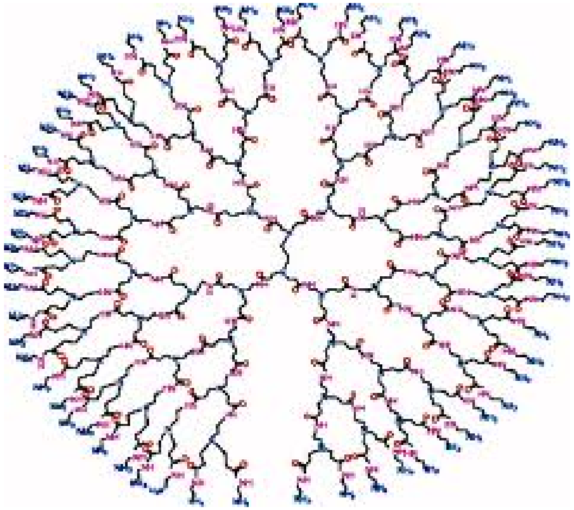


Yashima *et al. J. Am. Chem. Soc.*, **2003**, *125*, 1278-1283

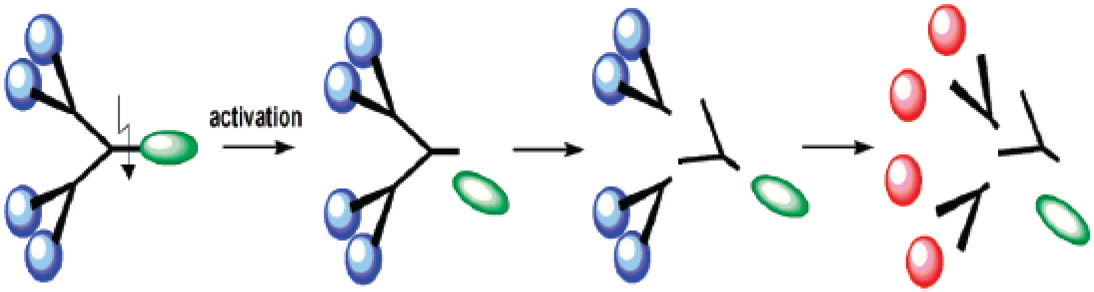
Detection of small enantiomeric excesses

majority rules

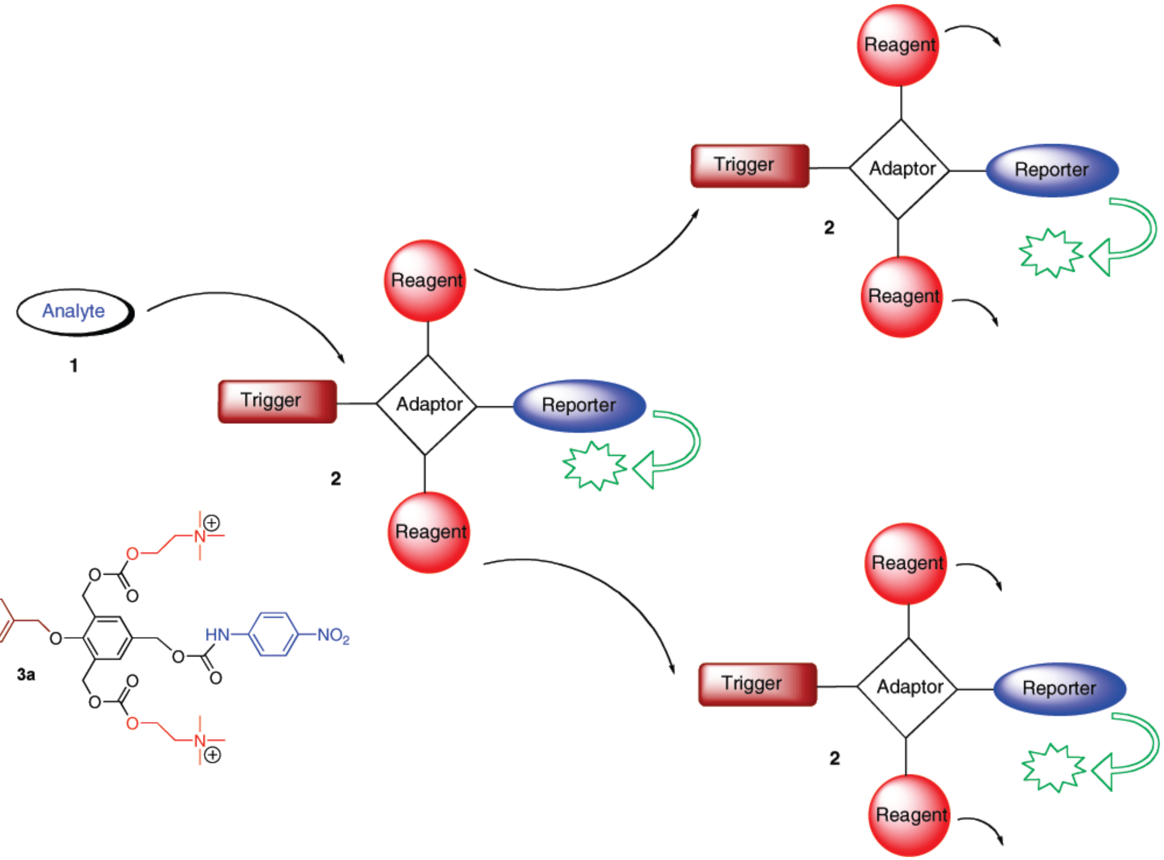
Yashima *et al. J. Am. Chem. Soc.*, **2003**, *125*, 1278-1283

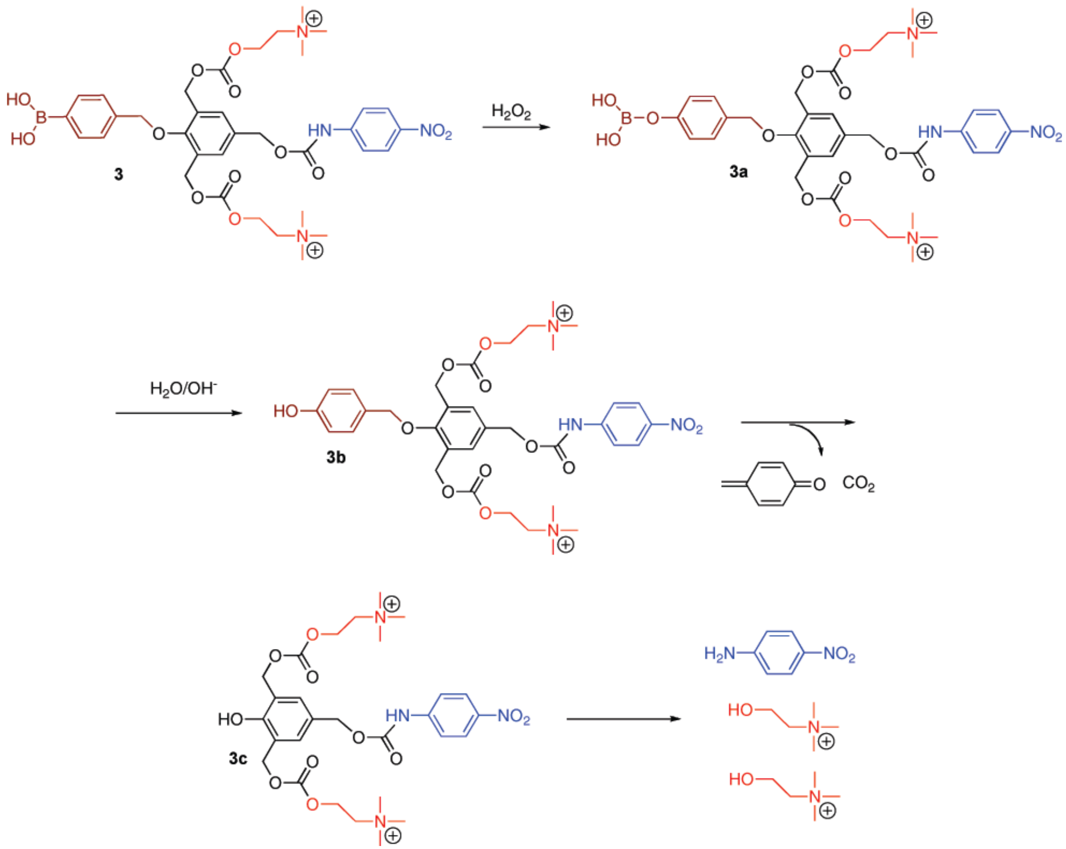


Dendrimers are repetitively branched molecules. The name comes from the Greek word "δένδρον" (pronounced dendron), which translates to "tree".



Dendritic chain reaction





Shabat *et al.* *J. Am. Chem. Soc.*, **2009**, *131*, 9934-9936

Sensing of H_2O_2

mM

µM

nM

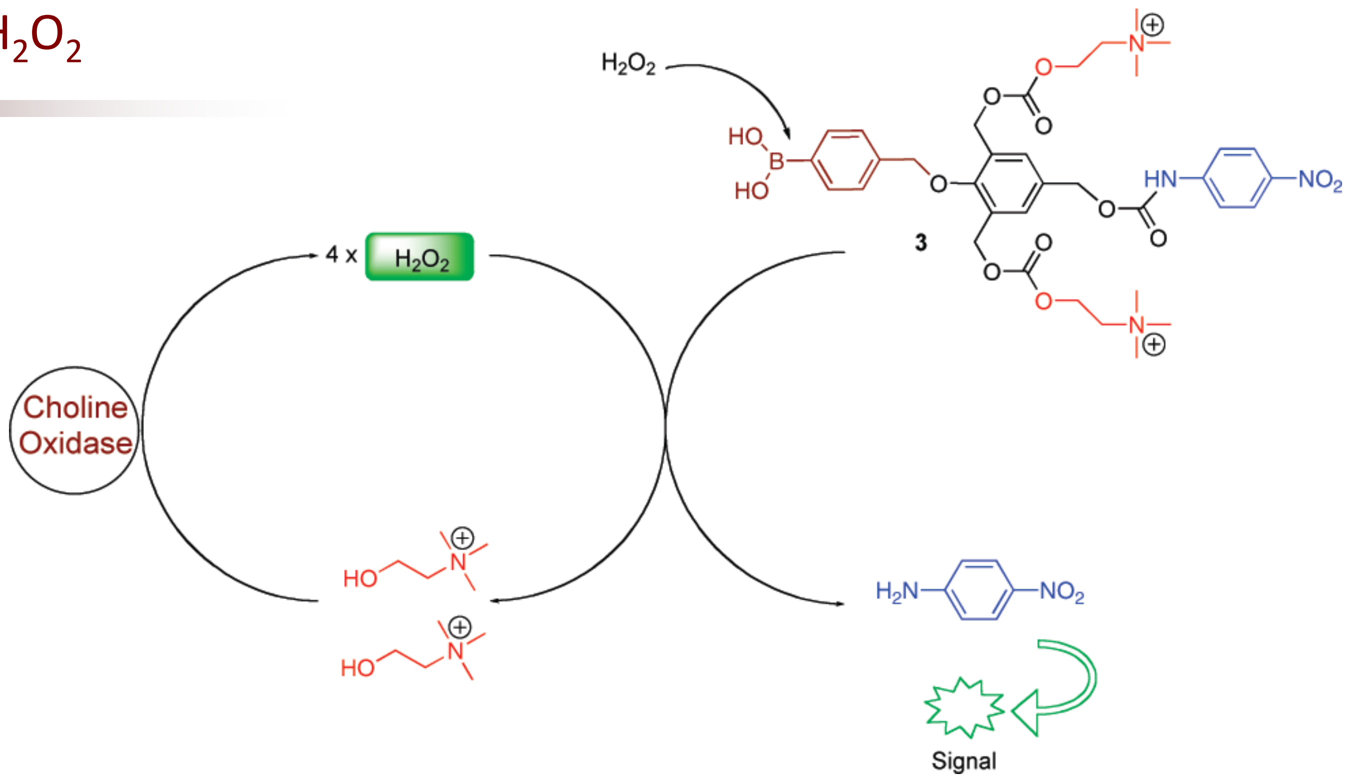
pM

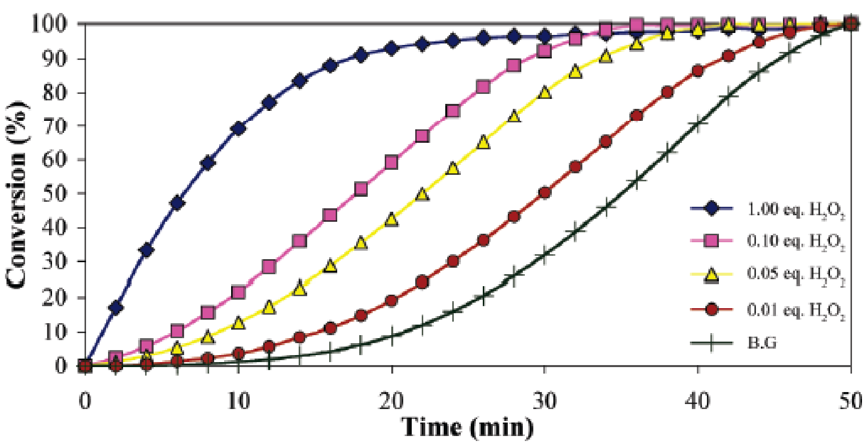
fM

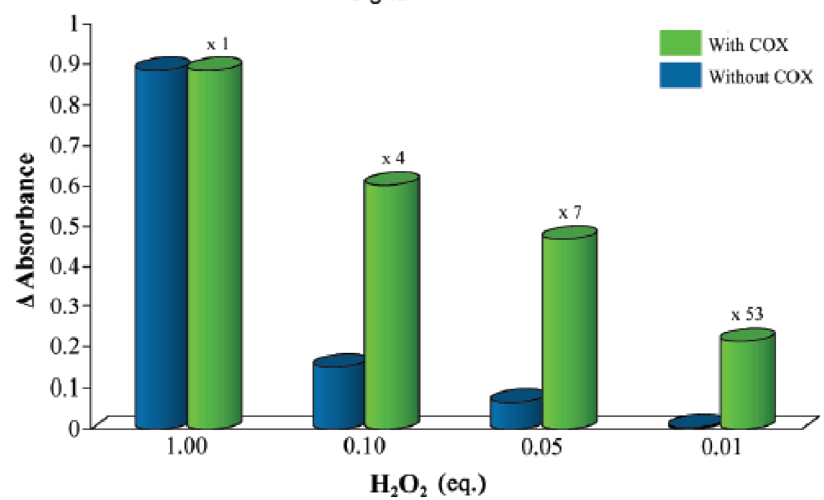
aM

zM

yM

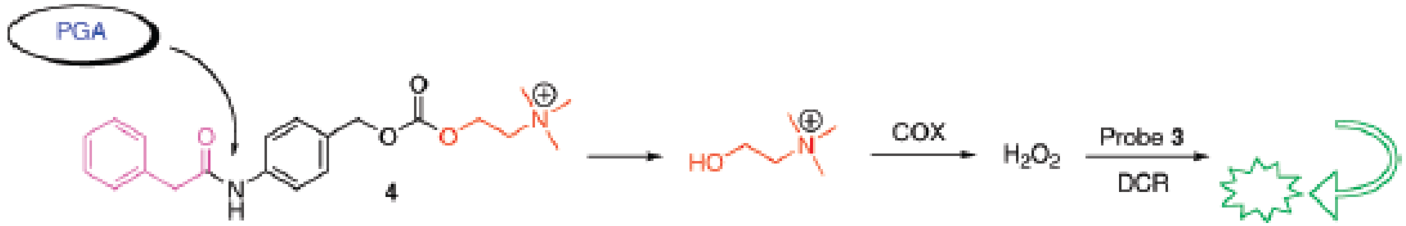


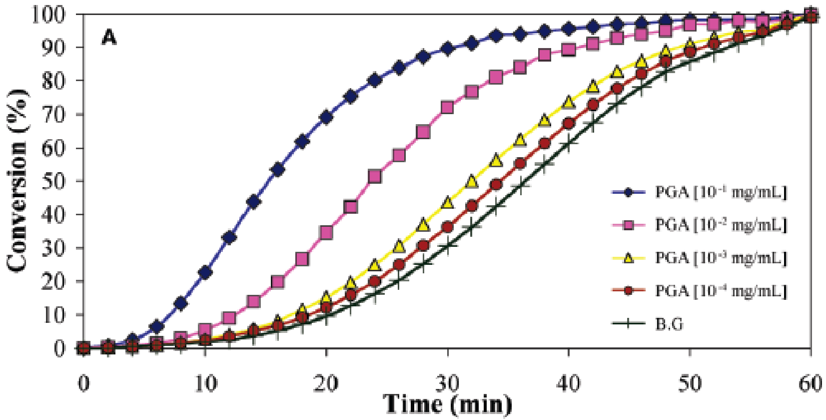




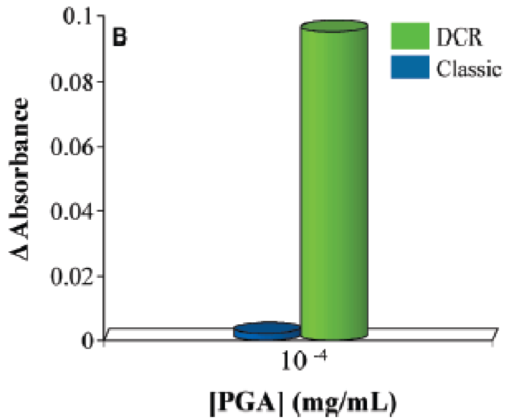
Shabat *et al. J. Am. Chem. Soc.*, **2009**, *131*, 9934-9936

Adaptation for enzyme detection





PGA
penicillin-G-amidase

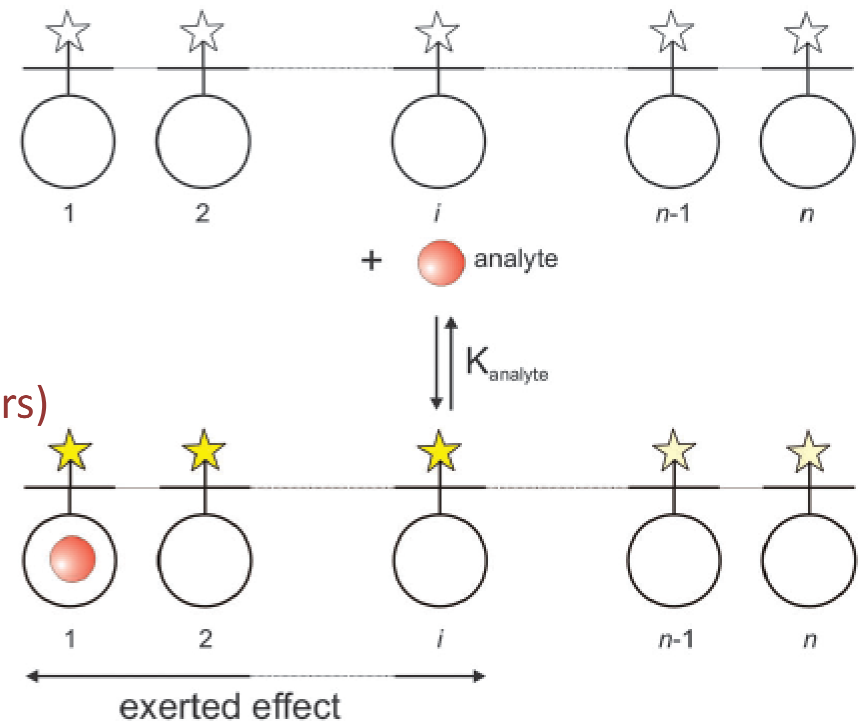


Critical issue
intrinsic stability of the probe

Shabat *et al. J. Am. Chem. Soc.*, **2009**, *131*, 9934-9936

Multivalency

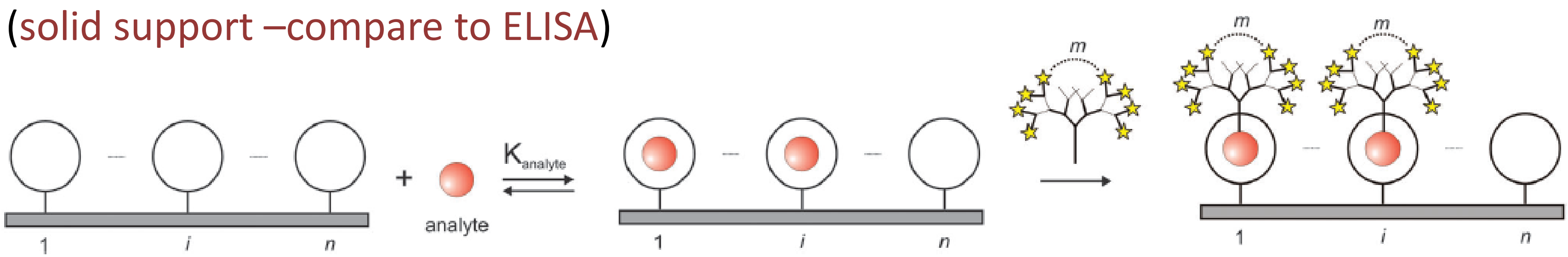
A



collective property (polymers)

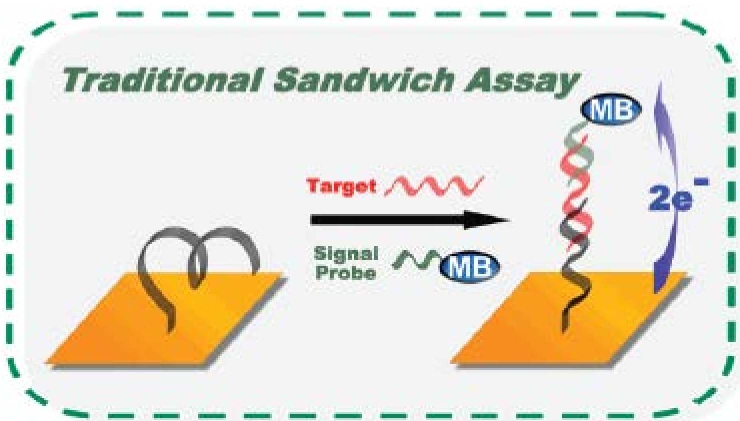
- electron conductivity
- helicity
- stability

B (solid support –compare to ELISA)

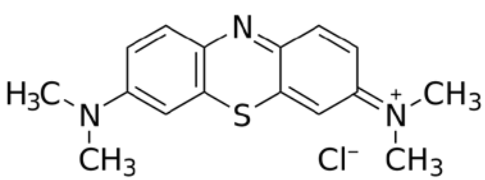


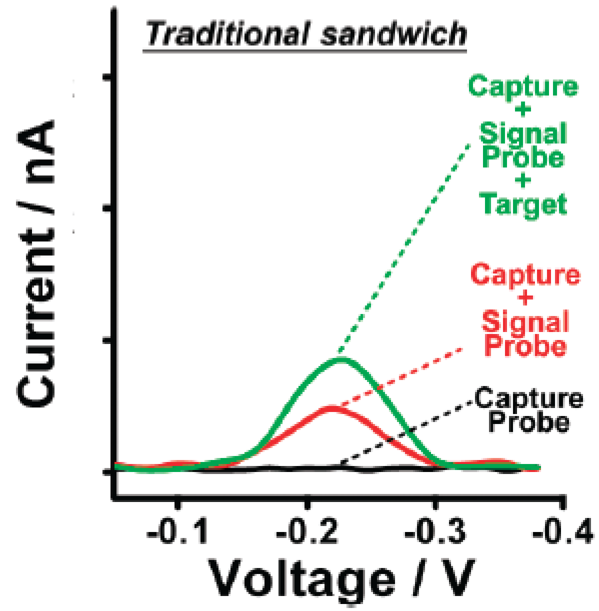
Sandwich assay for oligonucleotide detection

mM
µM
nM
pM
fM
aM
zM
yM



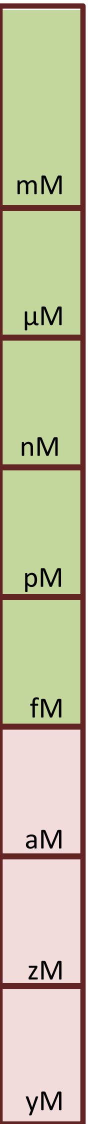
MB: methylene blue

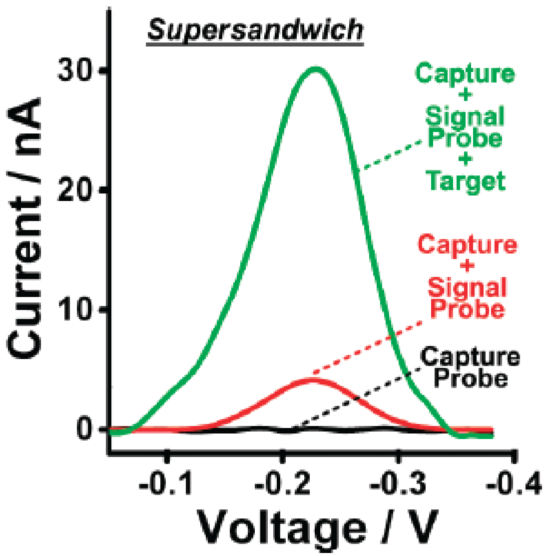


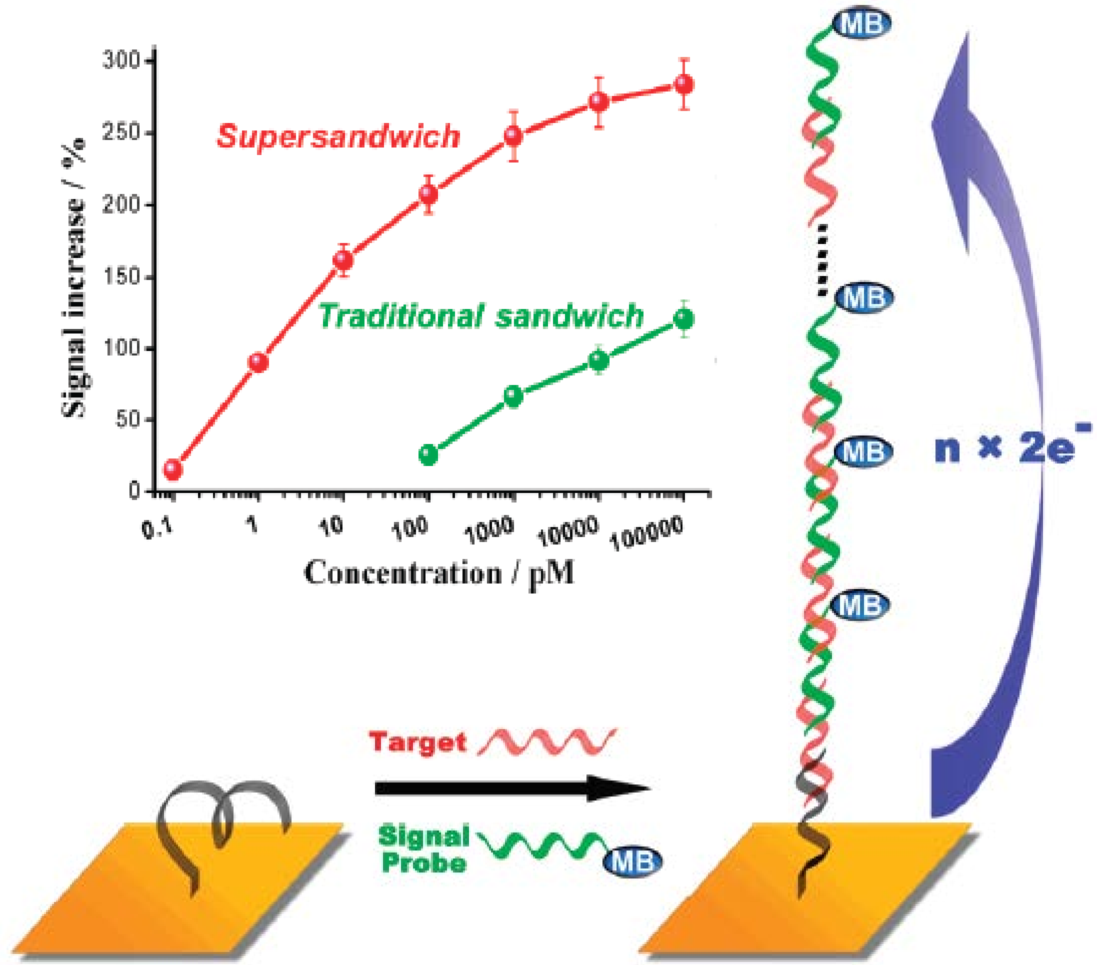


Zhuo, Plaxco, Heeger *et al. J. Am. Chem. Soc.*, **2010**, *132*, 14346-14348

A Supersandwich assay for oligonucleotide detection

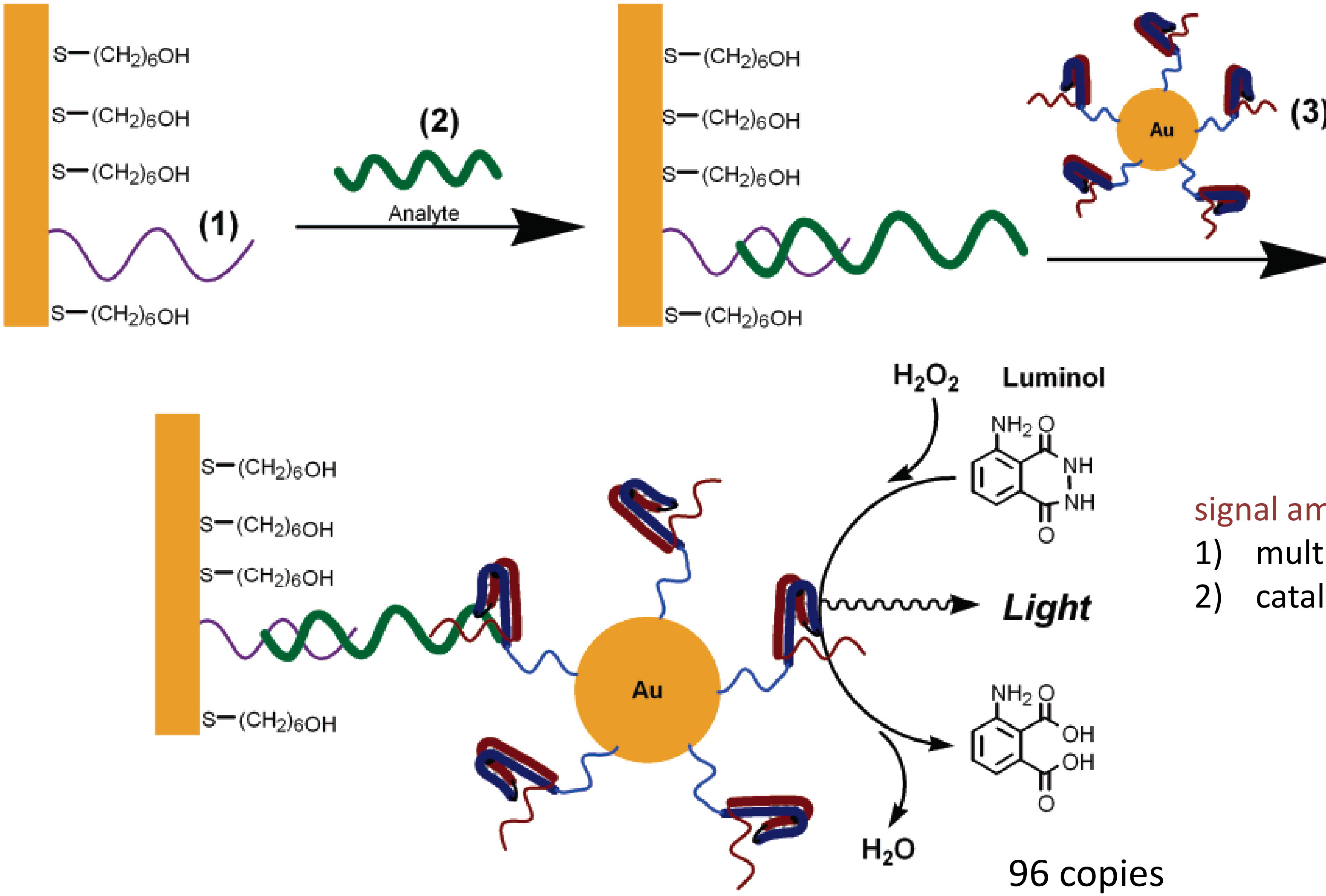






Zhuo, Plaxco, Heeger *et al. J. Am. Chem. Soc.*, **2010**, *132*, 14346-14348

DNAzyme-functionalized Au NPs for the amplified detection of DNA



signal amplification

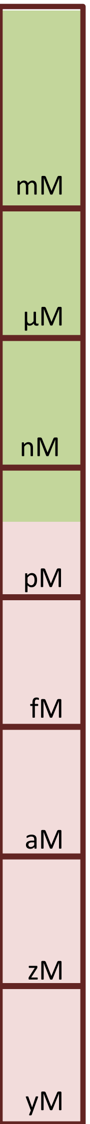
1) multivalency
2) catalysis

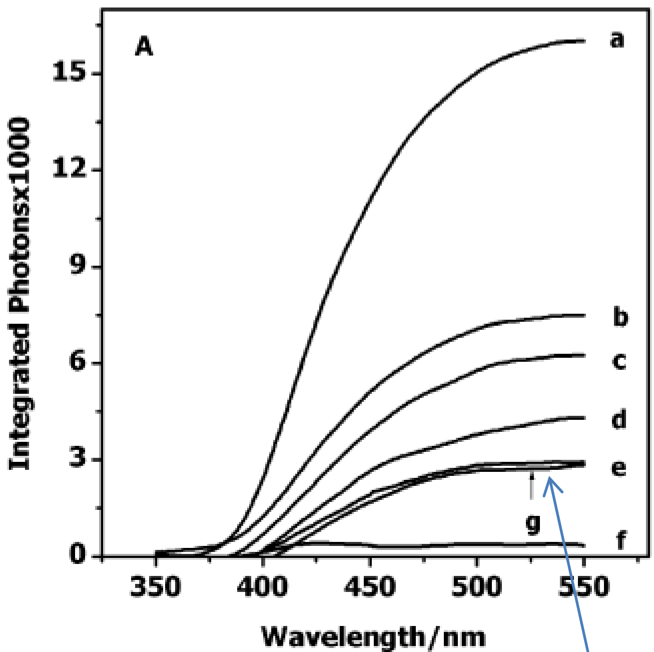
96 copies

(1) = 5'-HS$(CH_2)_6$CGATTCGGTACTGG-3'
(2) = 5'-TTGAGCATGCGCATTATCTGAGCCAGTACCGAATCG-3'
(3) = 5'-ATGCGCATGCTCAAT$_{10}$GGGTAGGGCGGGTTGGGT$_{17}$$(CH_2)_6$SH-3'
(3a) = 5'-ATGCGCATGCTCAATTTGGGTAGGGCGGGTTGGG-3'

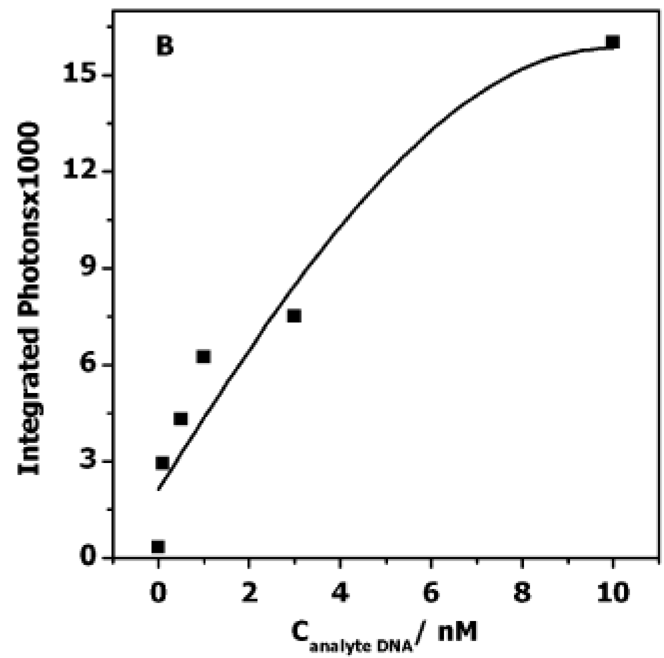
Willner *et al*. *Nano Lett*. **2004**, *4*, 1683-1687

Response curves





calibration curve

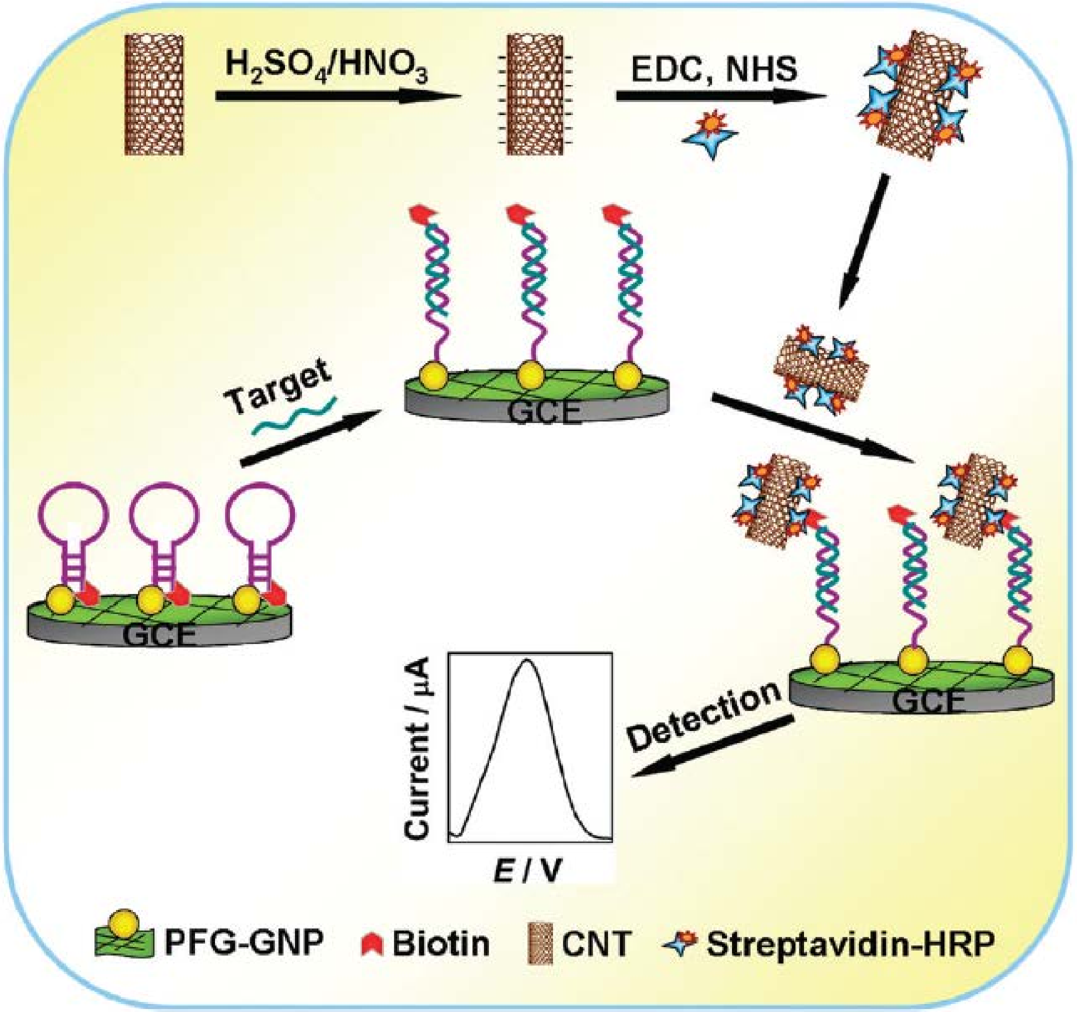


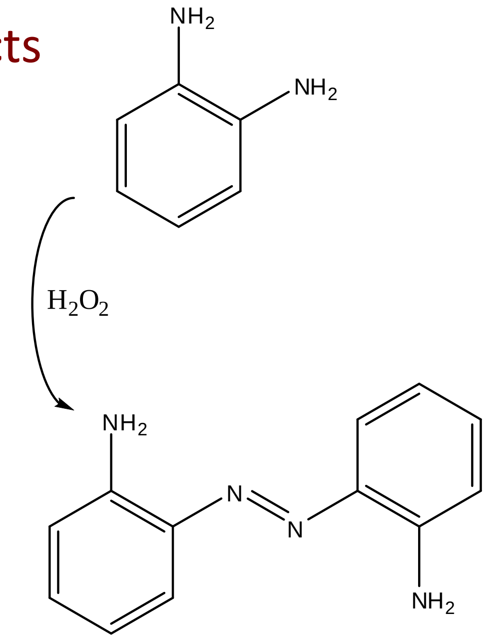
reference without NP

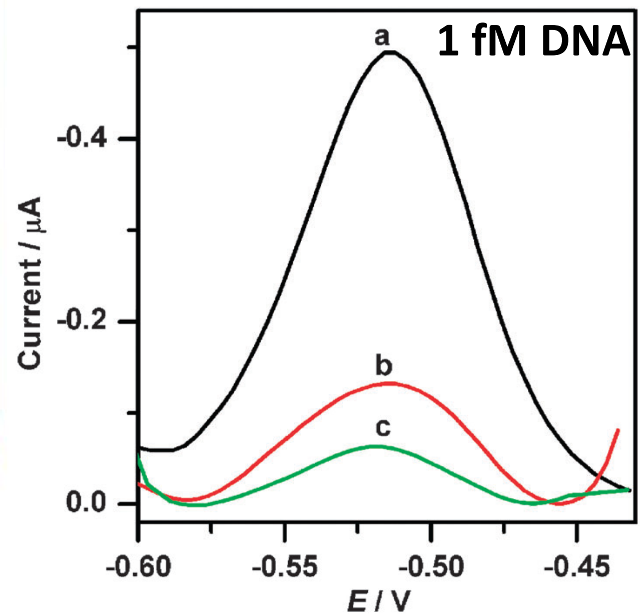
NPs cause 10-fold increase in sensitivity

Willner *et al*. *Nano Lett*. **2004**, *4*, 1683-1687

Signal amplification using multivalent CNT-HRP constructs





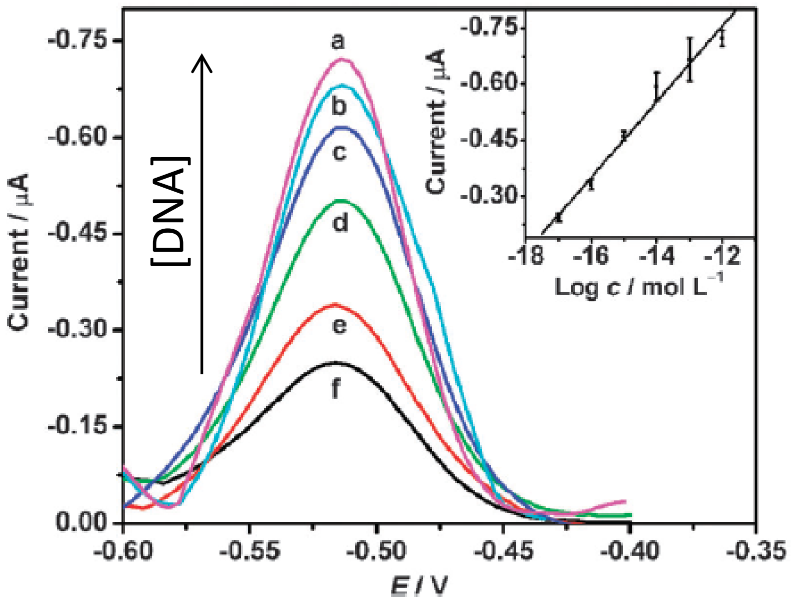


a) streptavidin–HRP–CNTs probe; b) streptavidin-HRP; c) streptavidin–HRP–CNTs probe (no DNA)

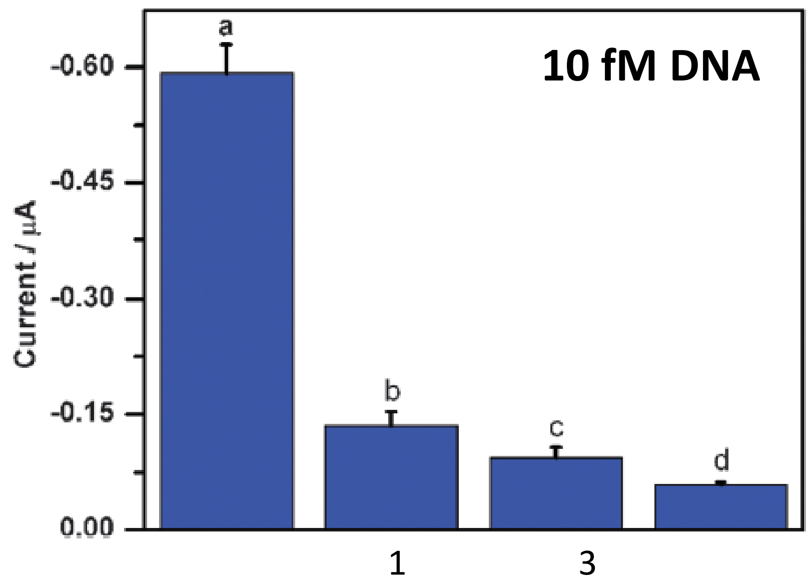
Lei, Ju *et al*. *Chem. Commun.* **2011**, *47*, 5220-5222

Amperometric detection of DNA

mM
µM
nM
pM
fM
aM
zM
yM

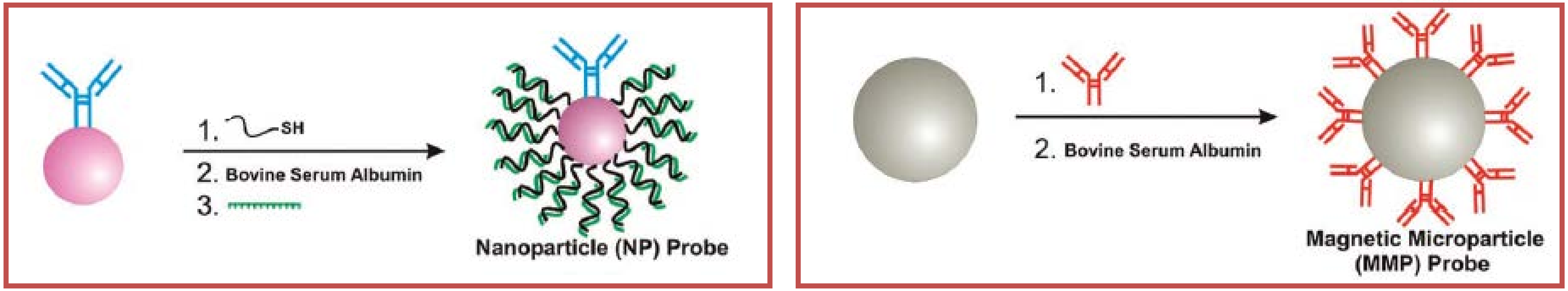


a= 1 pM
b= 100 fM
c= 10 fM
d=1 fM
e=100aM
f = 10 aM



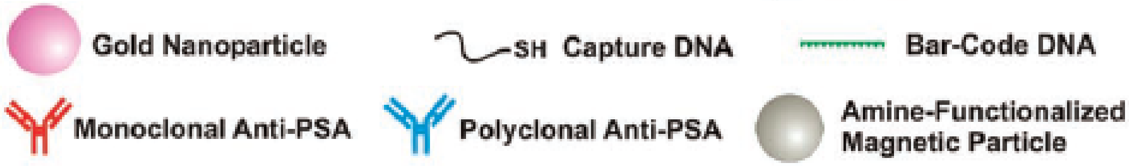
Lei, Ju *et al*. *Chem. Commun.* **2011**, *47*, 5220-5222

Bio-bar Codes (assay components)



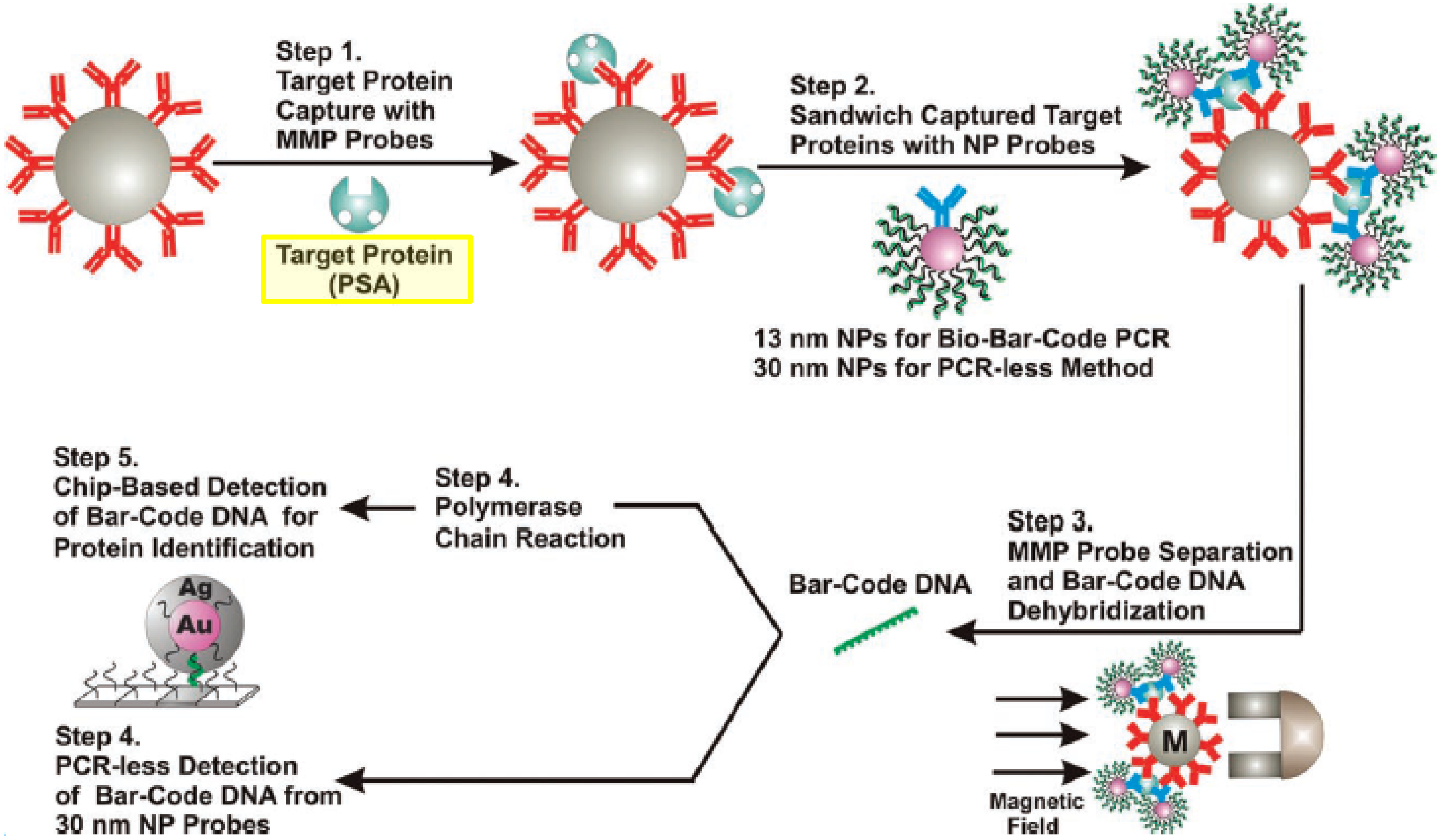
NP: 13 - 30 nm diameter

MMP: 1 µm diameter



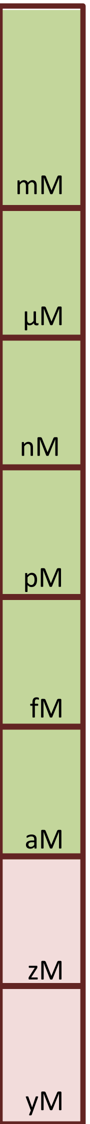
Mirkin *et al*. *Science*. **2003**, *301*, 1884-1886

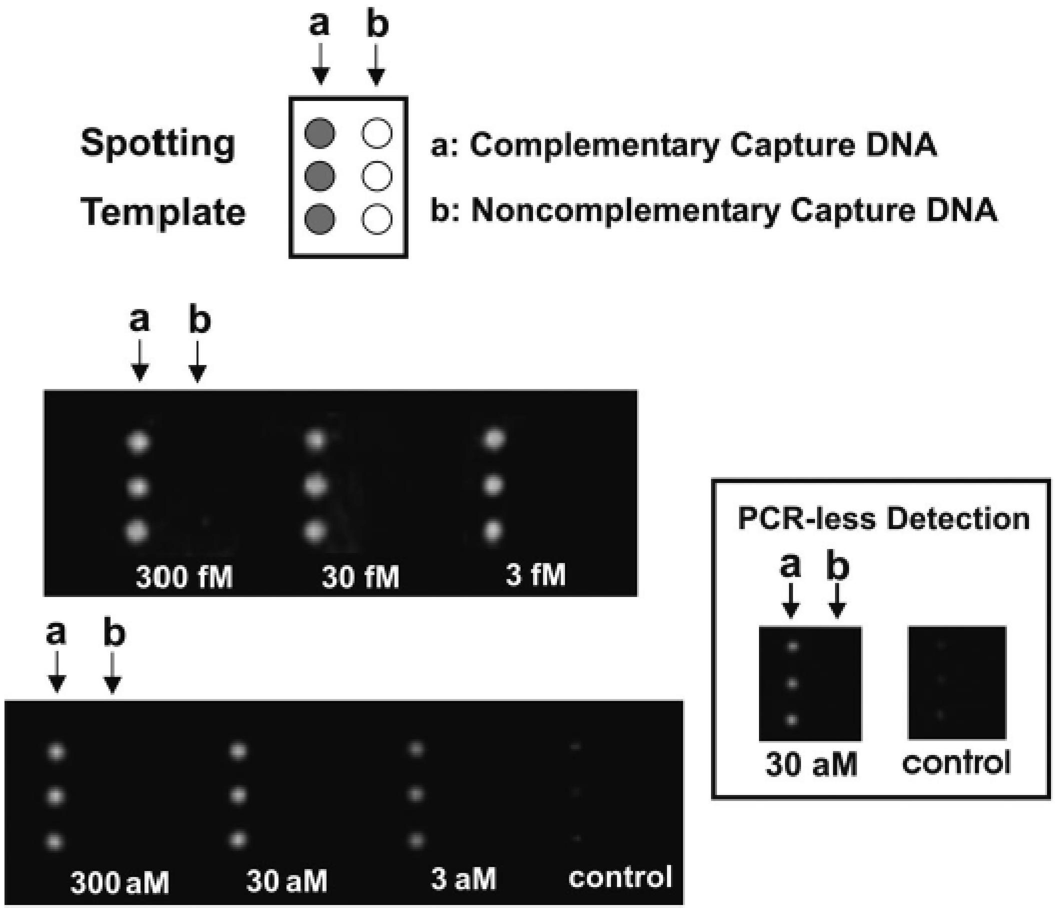
Detection of proteins using nanoparticle-based bio-bar codes



Mirkin *et al*. *Science*. **2003**, *301*, 1884-1886

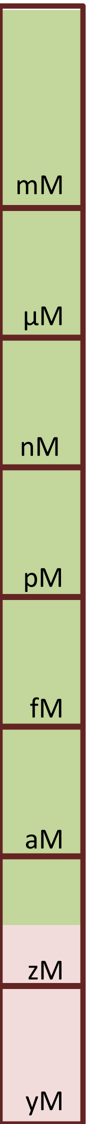
Signal production

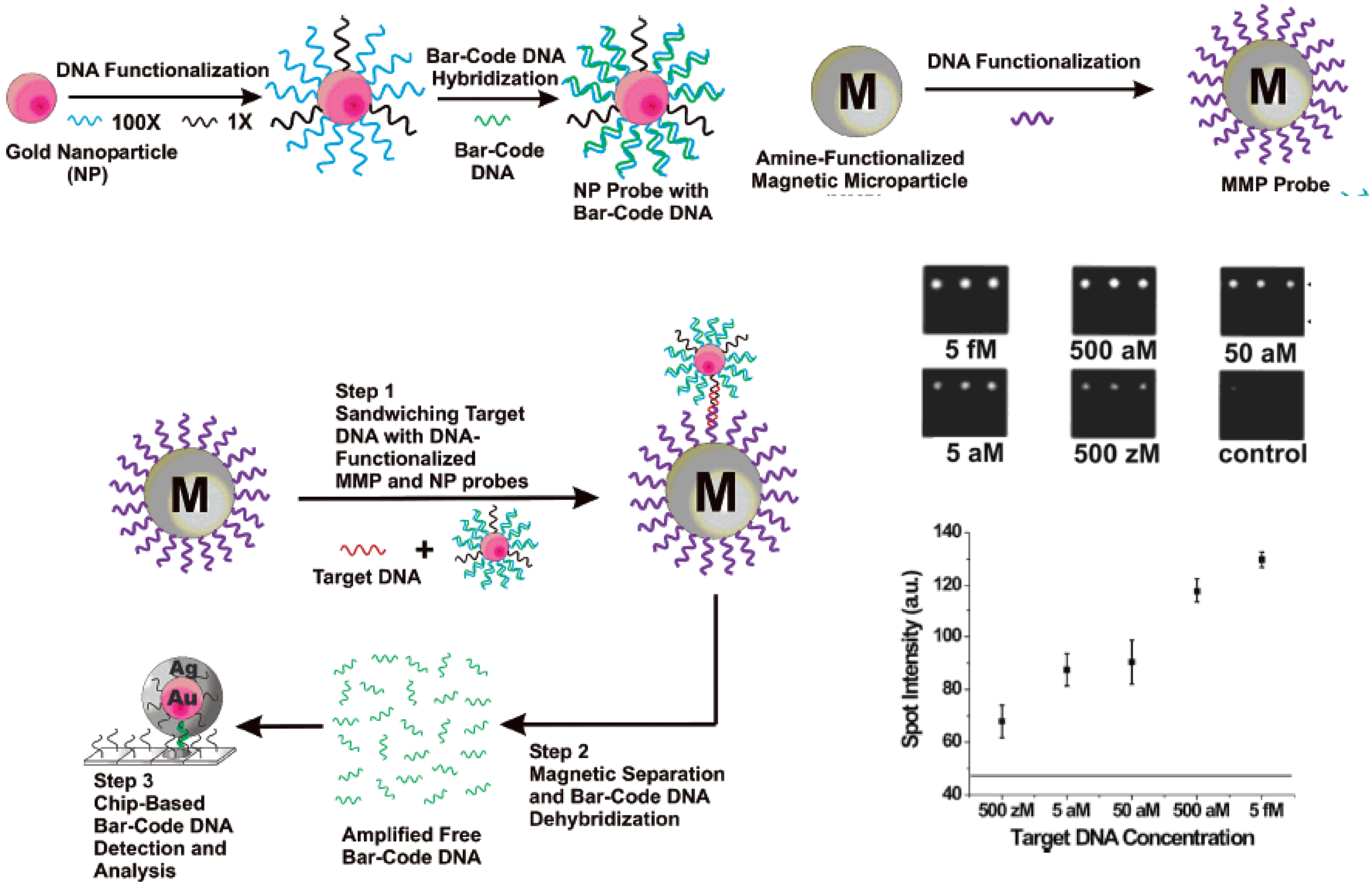




Mirkin *et al*. *Science*. **2003**, *301*, 1884-1886

Detection of DNA using nanoparticle-based bio-bar codes





Mirkin *et al*. *J.Am.Chem.Soc.* **2004**, *126*, 5932-5933

Concluding

Signal amplification

- catalysis
  (difficult to reach extremely low concentrations – autocatalysis)
- multivalency
  (essential)

Future

- cascade events
- beyond DNA, simple devices and readout

Acknowledgements

Funding:

erc European Research Council
239898

cost
CM0703: Systems Chemistry
CM0905: Organocatalysis

MARIE CURIE ACTIONS
READ-289723