DNA biosensors

Principles of DNA biosensors

❖**Nucleic acid hybridization**

➢ **Perfect match**

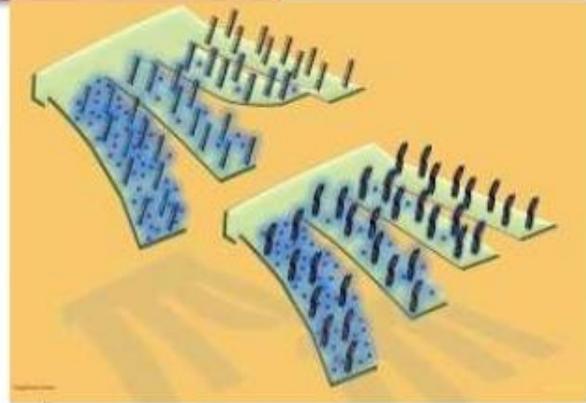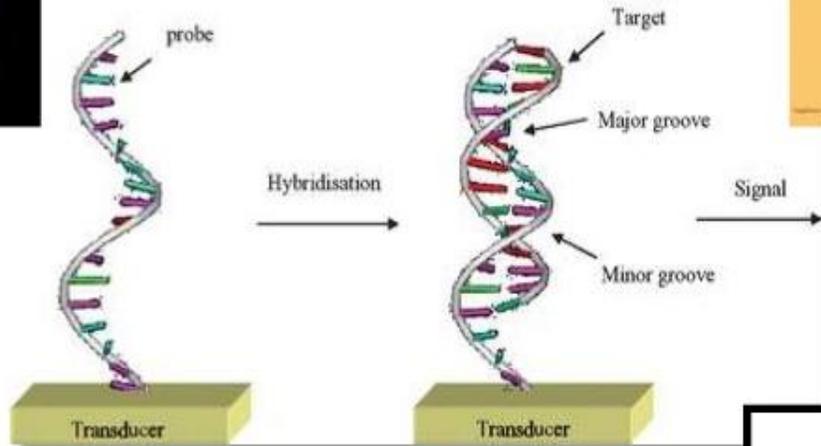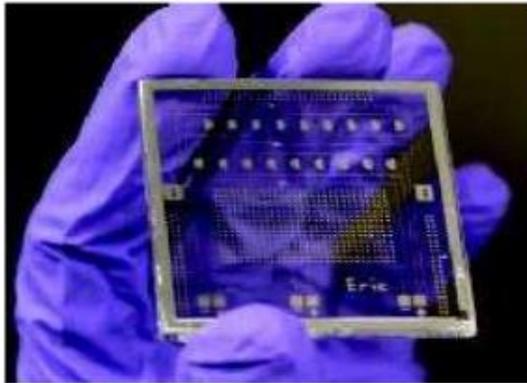   stable dsDNA, strong hybridization
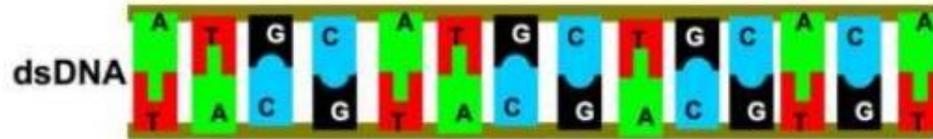
➢ **One or more base mismatches**

   weak hybridization
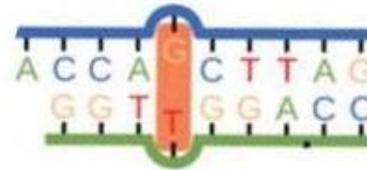
❖ Forms of DNA Biosensors

- Electrodes

- Chips

- Crystals

❖ Types of DNA Based Biosensors

- Optical

- Electrochemical

- Piezoelectric

Immobilization of DNA Probe onto Transducer Surface

- ➤ **simple adsorption**
  onto carbon surfaces

Immobilization of DNA Probe onto Transducer Surface

➤ Thiolated DNA for self assembly onto gold (or platinum) transducers

SAM conjugation

Thiol modified DNA

Au or Pt Surface
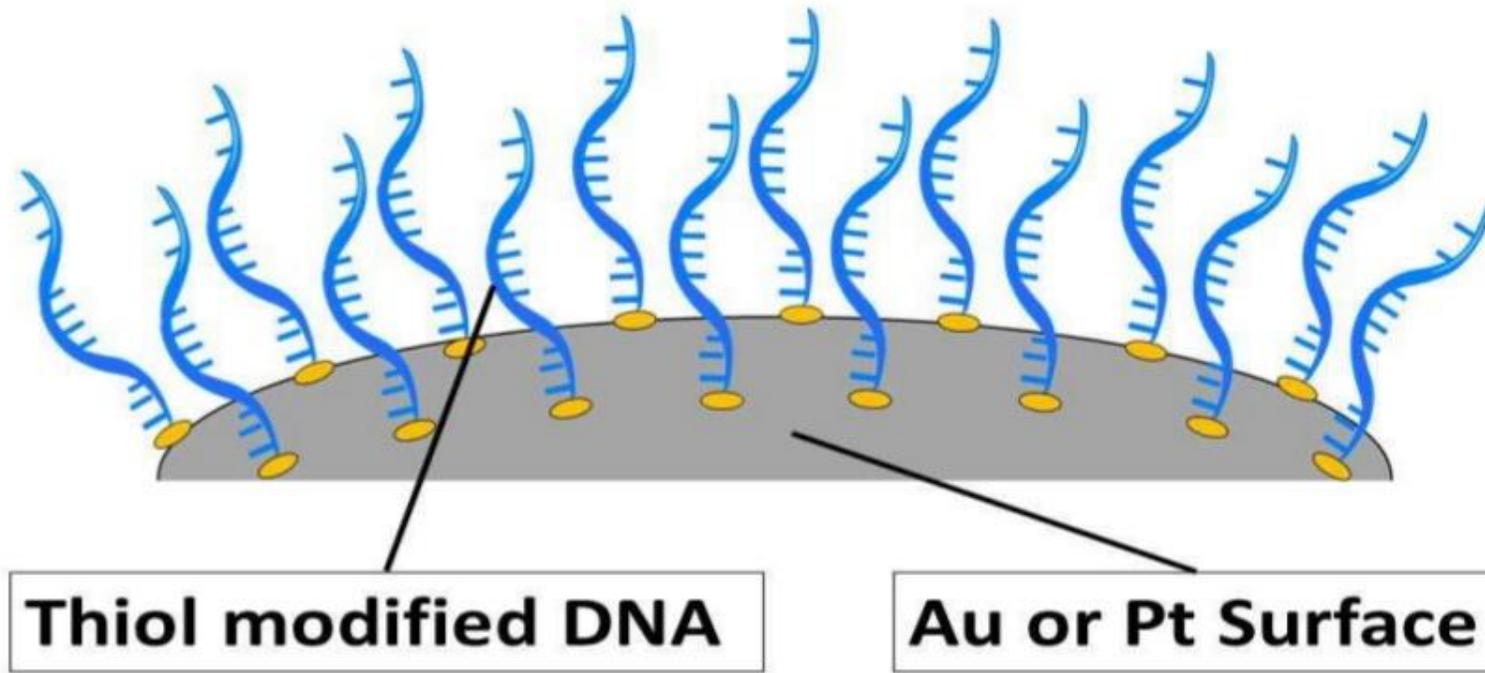
Immobilization of DNA Probe onto Transducer Surface

➤ Covalent linkage to the gold surface via functional **alkanethiol-based** monolayers
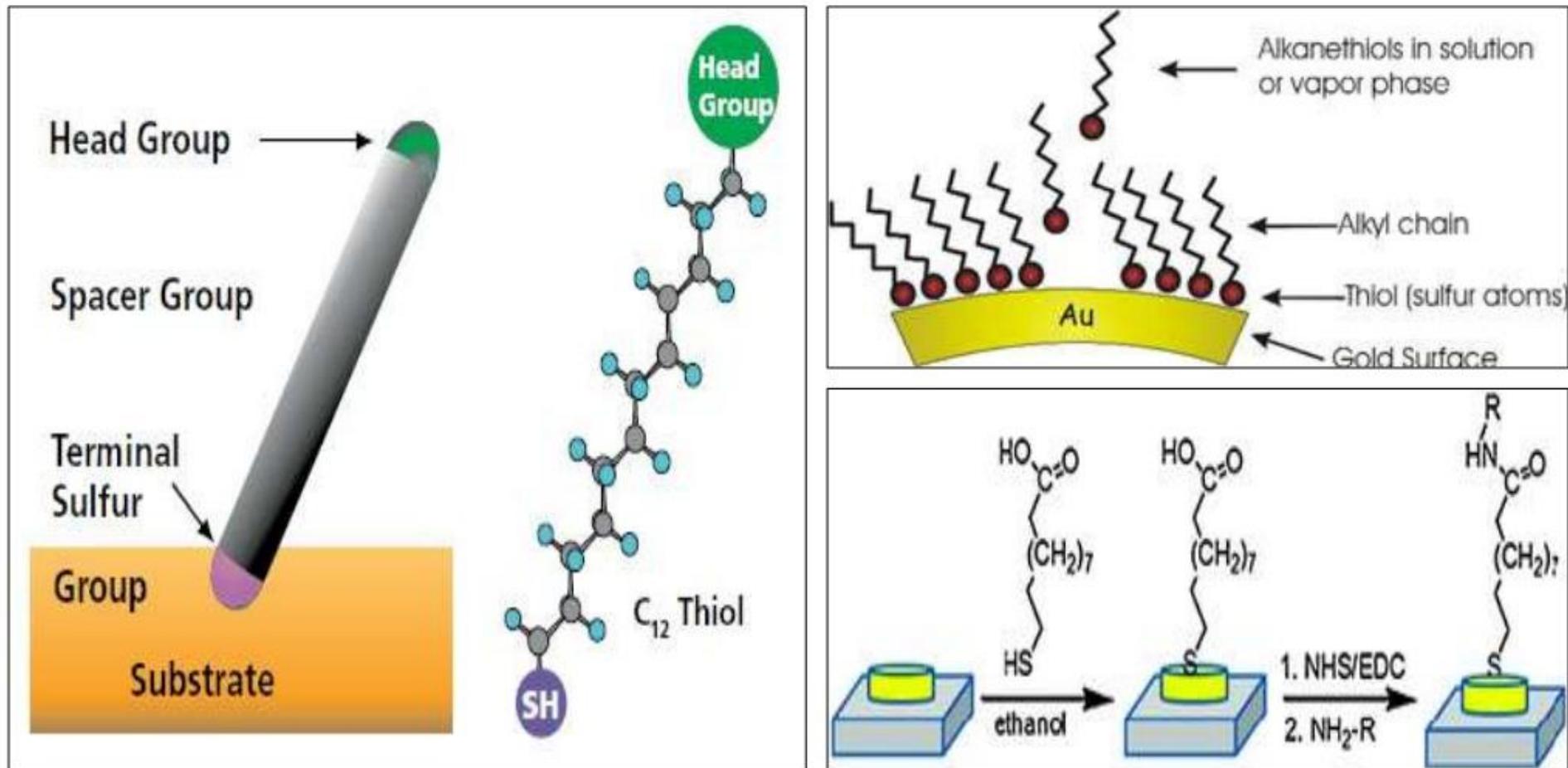
Immobilization of DNA Probe onto Transducer Surface

➢ Use of biotinylated DNA for complex formation with a surface-confined avidin or streptavidin
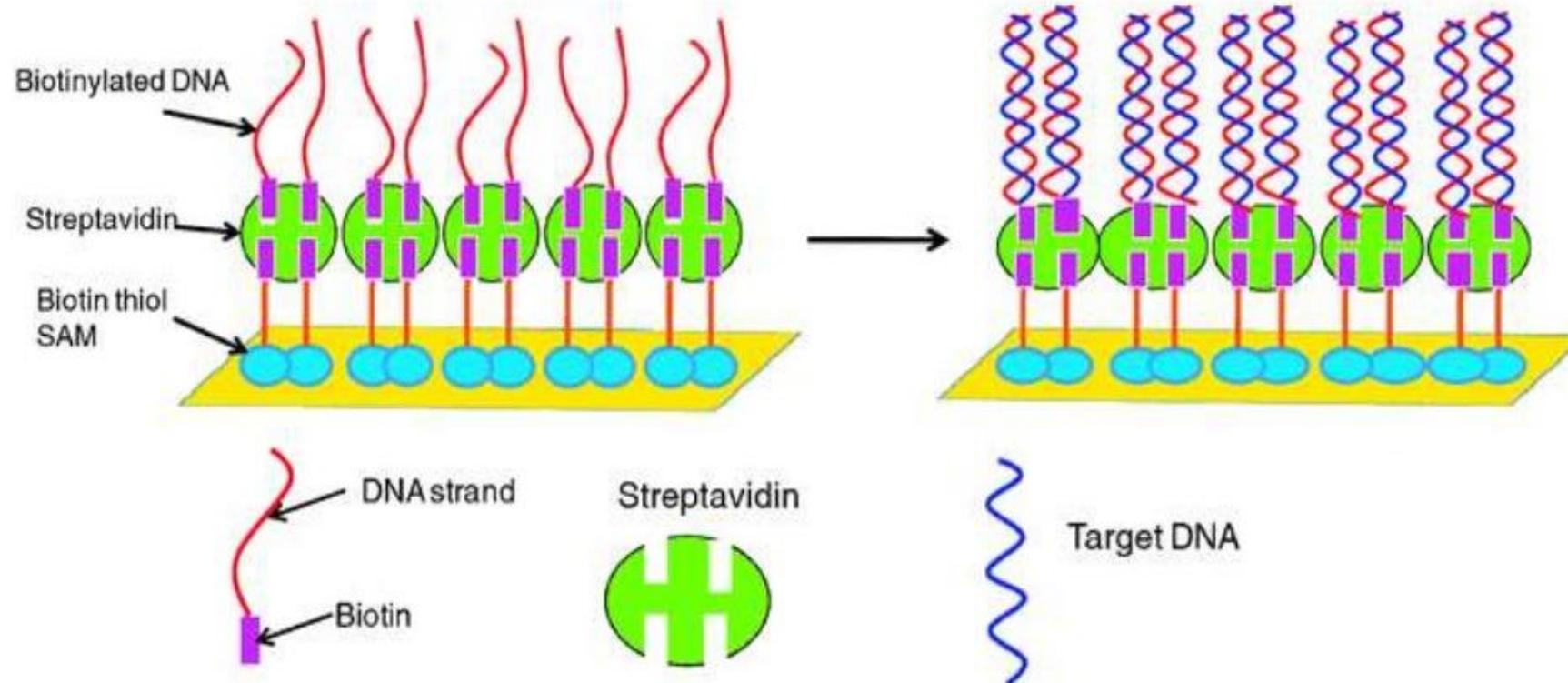
Tecniche innovative ed ultrasensibili PCR-free per la diagnosi precoce di acidi nucleici in biopsia liquida

Noemi Bellassai

Webinar, **28 Aprile 2021**

Liquid biopsy

Non-invasive test based on the **detection of biomarkers** related to specific disease circulating in body fluids (blood, plasma, serum, urine, saliva, synovial fluid etc.).

Circulating tumor DNA

Tumor-associated antigen

Circulating tumor cells

Point mutation

Exosome

Protein

CH_3 CH_3

DNA Methylation

CH_3

MicroRNA
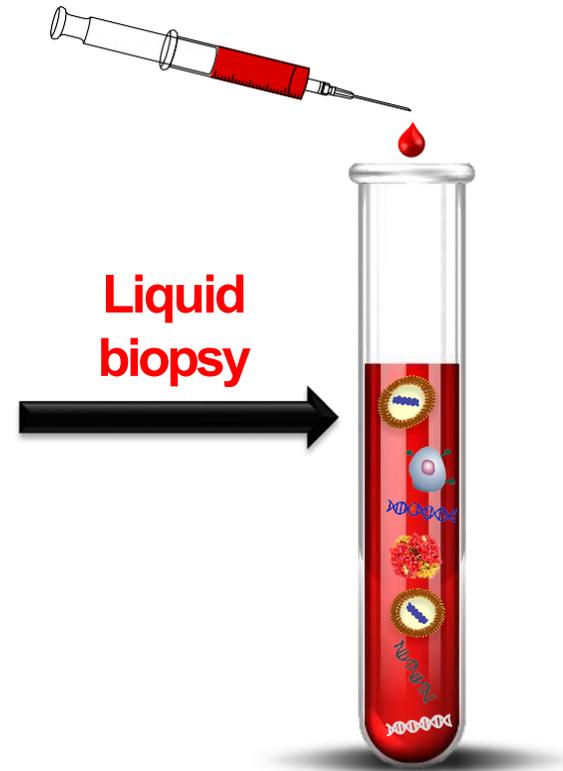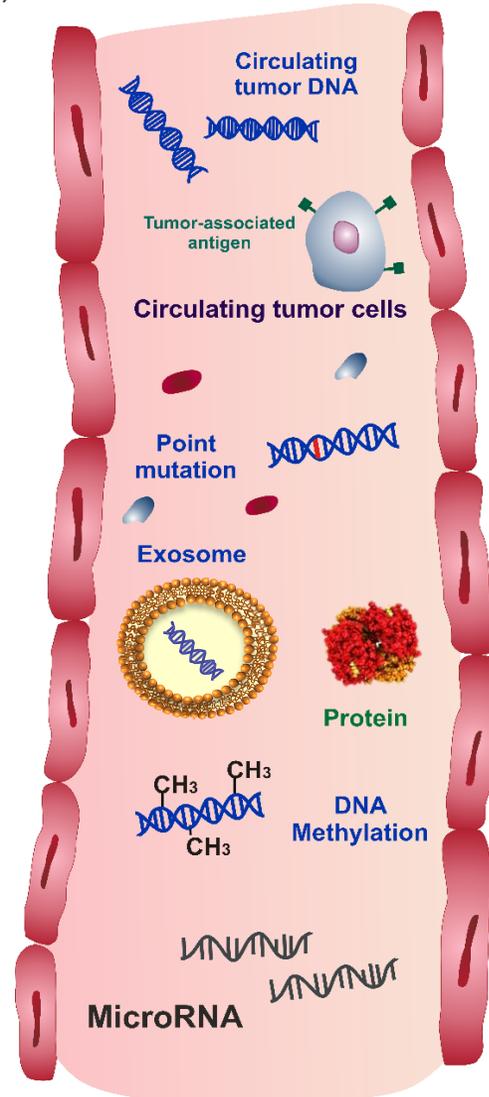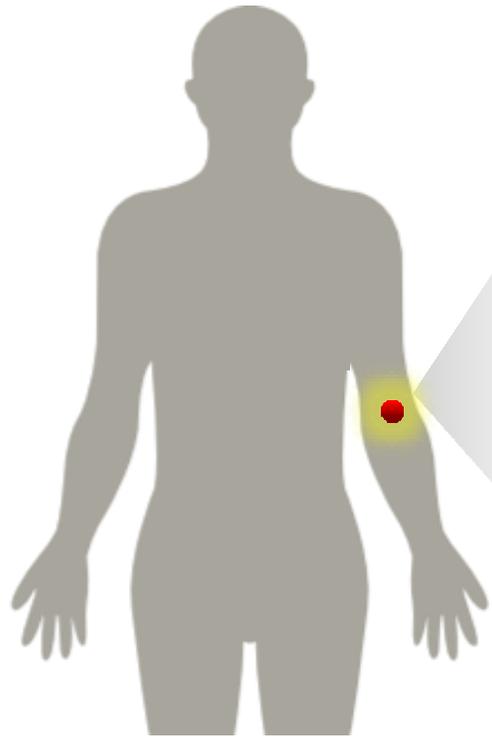
Liquid biopsy

ctDNA

miRNA

Protein

CTCs

Bellassai et al., Front Chem **2019**, 7, 570

Liquid biopsy

CTCs ctDNA

Noninvasive blood sample

Real-time liquid biopsy

Screening and early detection of cancer
EGFR mt in ctDNA (NSCLC)
CTC counts (NSCLC)

Real-time monitoring of therapy
CTC counts (BC)
KRAS mt on ctDNA (CRC)
AR mt on ctDNA (PC)

Stratification and therapeutic intervention
HER2 or ER expression on CTCs (BC)
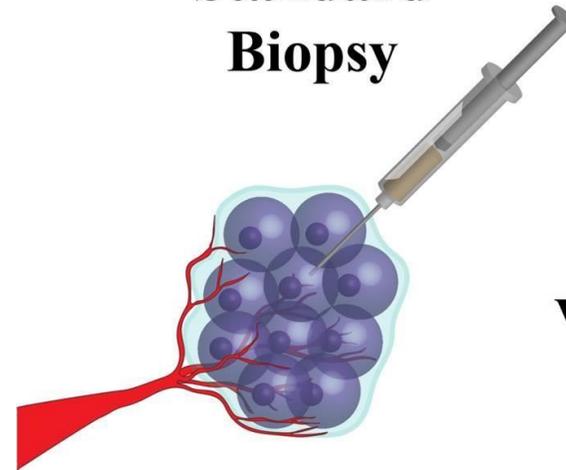CTC counts (BC) - METABREAST trial

Clinical applications

Therapeutic targets and resistance mechanisms
KRAS mt (CRC)
EGFR mt (NSCLC),
Lack of ER expression (BC)
AR mt or ARv7 expression (PC)

Risk for metastatic relapse (prognosis)
CTC counts in solid tumors
(e.g., breast, prostate, colorectal, lung, bladder cancers)
KRAS mt on ctDNA (CRC)

PERSONALIZED TREATMENT

Alix-Panabières et al., Cancer Discov. **2016**; 6(5), 479

Standard Biopsy

VS.

Liquid Biopsy

Time-Intensive Procedure
Localized Sampling of Tissue
Not Easily Obtained
Some Pain/Risk
Invasive
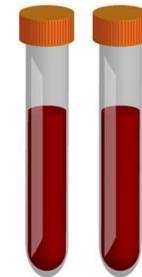
Quick
Comprehensive Tissue Profile
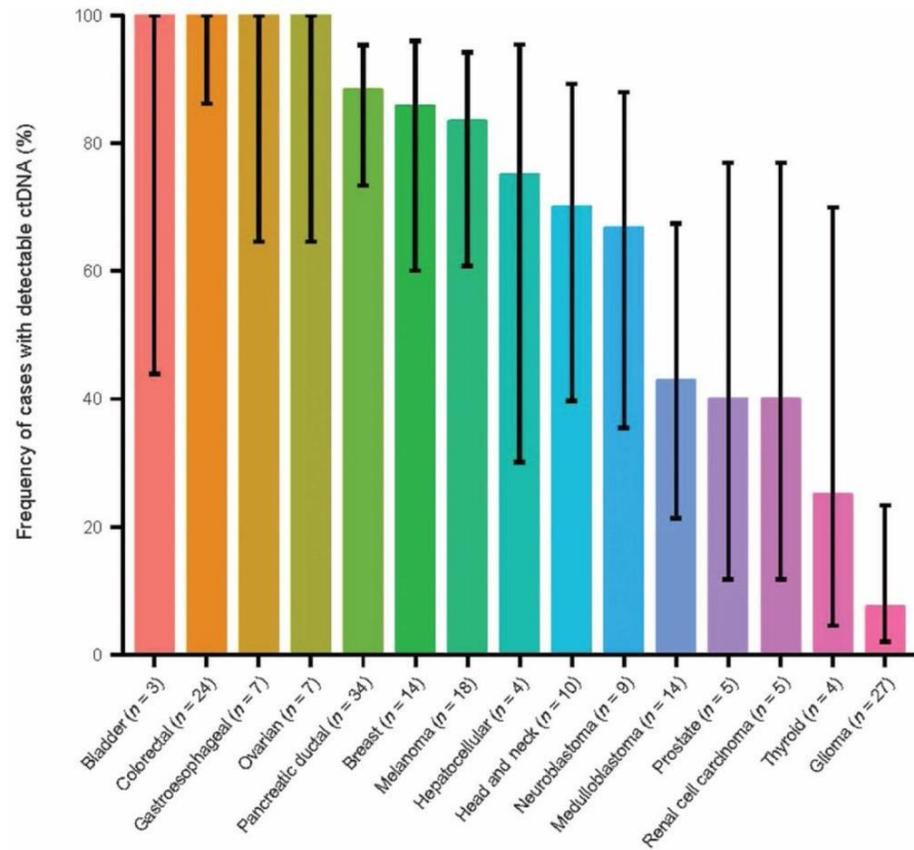Easily Obtained
Minimal Pain/Risk
Minimally Invasive

Lovly et al. **2016**. Circulating Tumor DNA. My Cancer Genome (Updated February 8).

Sosa et al., Nat. Rev. Cancer.**2014**; 14:611-622

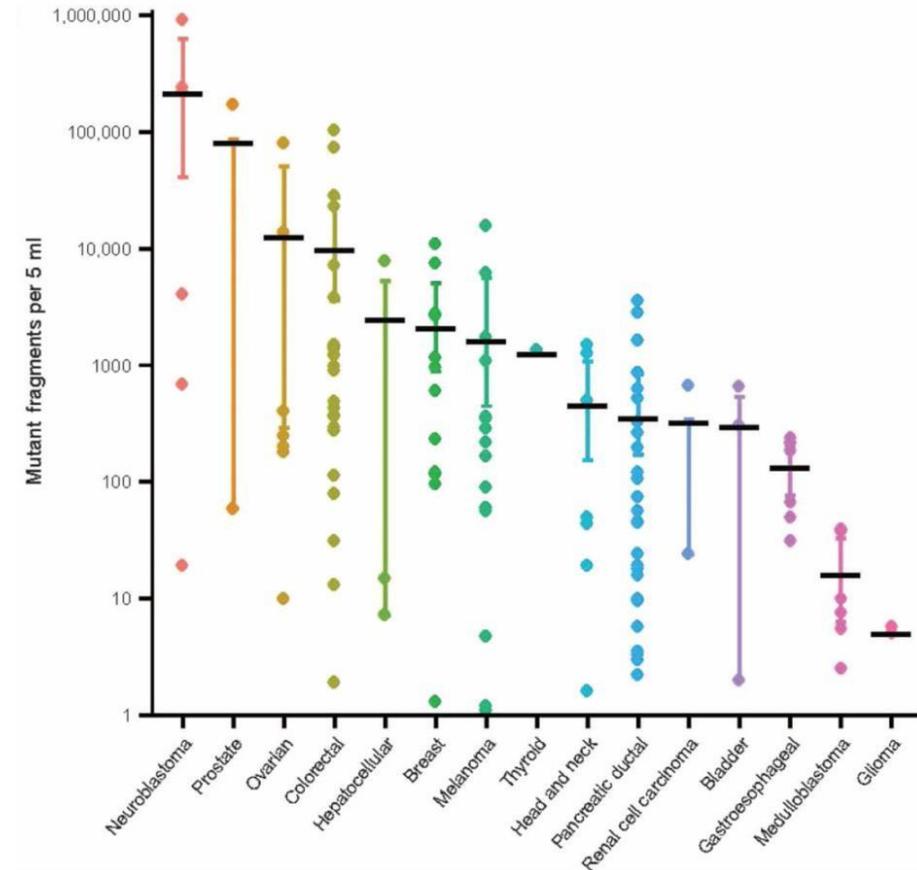# Liquid biopsy for early diagnosis disease

The opportunity
Circulating tumour DNA (ctDNA) is easy accessible and can be detected in most metastatic cancers
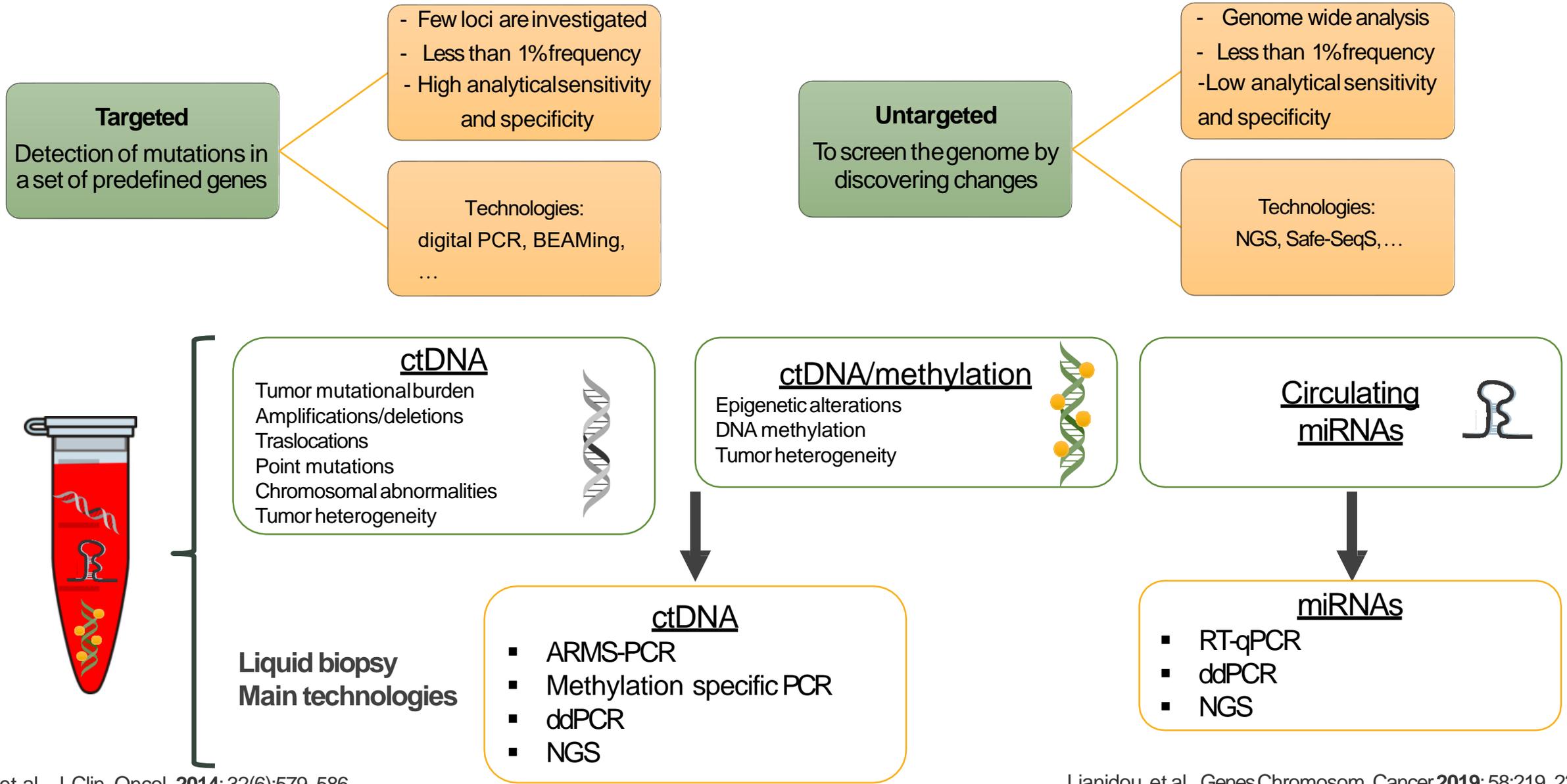
The challenge
ctDNA is often only present at low levels

Bettegowda et al., *Sci Transl Med.* **2014**, 19, 6(224)

Detection of nucleic acid biomarkers

Targeted
Detection of mutations in a set of predefined genes

- Few loci are investigated
- Less than 1% frequency
- High analytical sensitivity and specificity

Technologies:
digital PCR, BEAMing, …

Untargeted
To screen the genome by discovering changes

- Genome wide analysis
- Less than 1% frequency
- Low analytical sensitivity and specificity

Technologies:
NGS, Safe-SeqS, …

ctDNA
Tumor mutational burden
Amplifications/deletions
Traslocations
Point mutations
Chromosomal abnormalities
Tumor heterogeneity

ctDNA/methylation
Epigenetic alterations
DNA methylation
Tumor heterogeneity

Circulating miRNAs

Liquid biopsy
Main technologies

ctDNA
- ARMS-PCR
- Methylation specific PCR
- ddPCR
- NGS

miRNAs
- RT-qPCR
- ddPCR
- NGS

Diaz et al., J. Clin. Oncol. **2014**; 32(6):579–586

Lianidou et al., Genes Chromosom. Cancer **2019**; 58:219–232

Target Amplification Methods

- **Polymerase chain reaction (PCR)**

  - **PCR using specific probes**

  - **RT PCR**

  - **Nested PCR-increases sensitivity, uses two sets of amplification primers, one internal to the other**

  - **Multiplex PCR-two or more sets of primers specific for different targets**

  - **Arbitrarily Primed PCR/Random Primer PCR**

- **Isothermal methods**

Polymerase chain reaction (PCR) Inventor
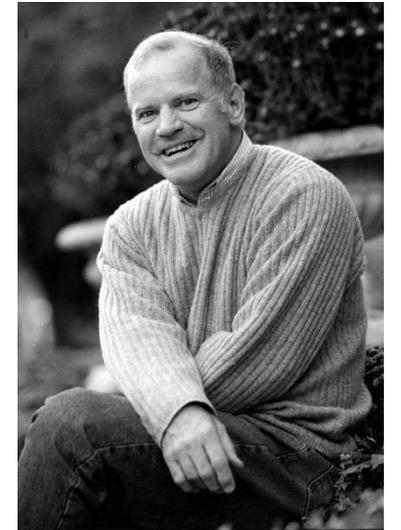
Kary Banks Mullis (1944-2019)

Nobel Prize in Chemistry 1993

Beyond PCR ... Isothermal amplification

**Nucleic acids amplification operated at a <u>constant temperature</u>**

❑ **Implementation in point-of-care devices is simplified**

❑ **Can be performed under simple conditions (e.g., water bath)**

❑ **Many isothermal amplification methods are available providing exponential or linear amplification**

❑ **Enzymatic and enzyme-free isothermal amplification methods are available**

Giuffrida M.C. et al., *Biosens Bioelectron*, **2017**, 90, 174-186.

Method	Temp (℃)	Reaction time (min)	Amplification	Target	Primers	Main applications
LAMP	60-65	30-60	10^9	dsDNA hundred base-pair long	4-6	Bacteria, Viruses
RPA	25-42	5-20	$10^9 - 10^{11}$	dsDNA ssDNA RNA	2	Pathogens, Viruses
NASBA	~41	90-120	10^9	RNA	2	Bacteria, Pathogens
RCA	30-65	60-120	10^3 linear 10^9 expon.	ssDNA	1	Plasmid, Viruses
NEEA	54-58	15-30	10^9	dsDNA RNA	2	Viruses, RNA DNA
HDA	37-60	60-120	10^6	dsDNA	2	Biomarkers, Viruses

Loop mediated isothermal amplification (LAMP)

➢ Amplification takes place at a single temperature (65°C)
   (No need of thermal cycler)

➢ Uses polymerase with high strand displacement activity
   (Bacillus stearothermophilus Bst DNA Polymerase instead of *Taq*Poly)

➢ Amplification efficiency is high (up to 10^9)

➢ Can be also used for RNA templates by addition of reverse transcriptase
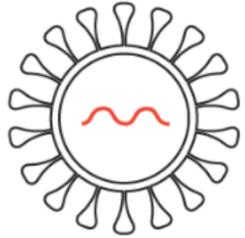
Loop mediated isothermal amplification (LAMP)
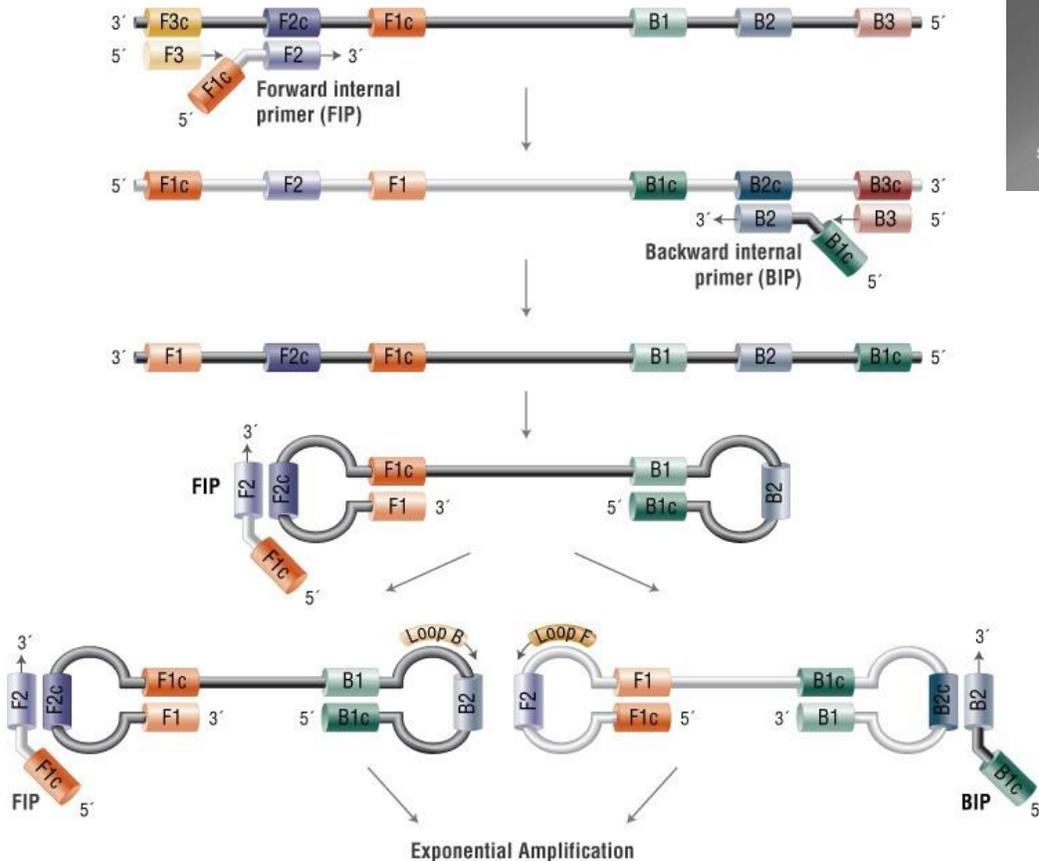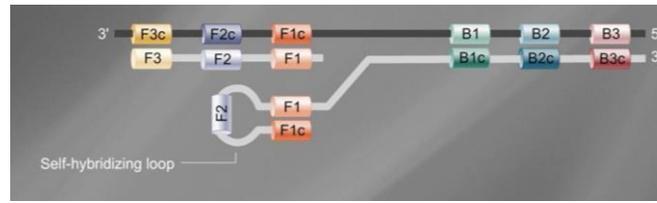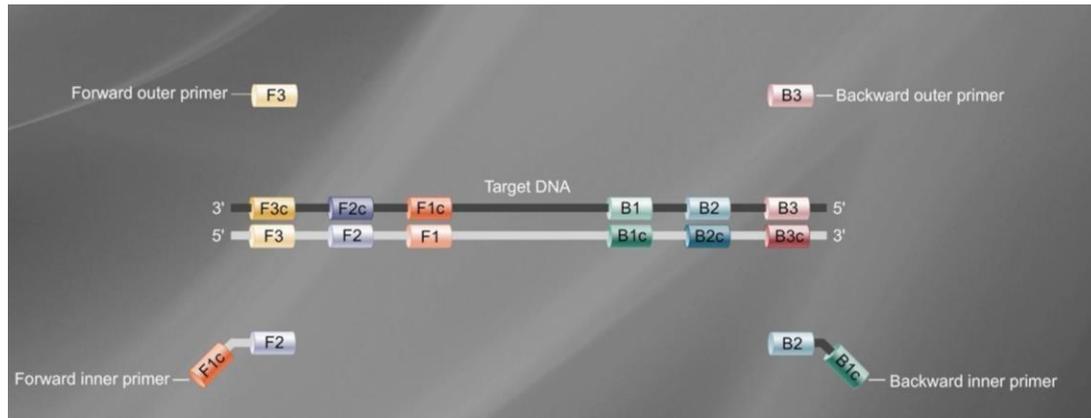
Loop-mediated isothermal amplification (LAMP) uses 4-6 primers recognizing 6-8 distinct regions of target DNA.

A strand-displacing DNA polymerase initiates synthesis and 2 of the primers form loop structures to facilitate subsequent rounds of amplification.

https://www.youtube.com/watch?v=L5zi2P4lggw

LAMP-Based SARS-CoV-2 Testing Methods

SARS-CoV-2 Rapid Colorimetric LAMP Assay Kit
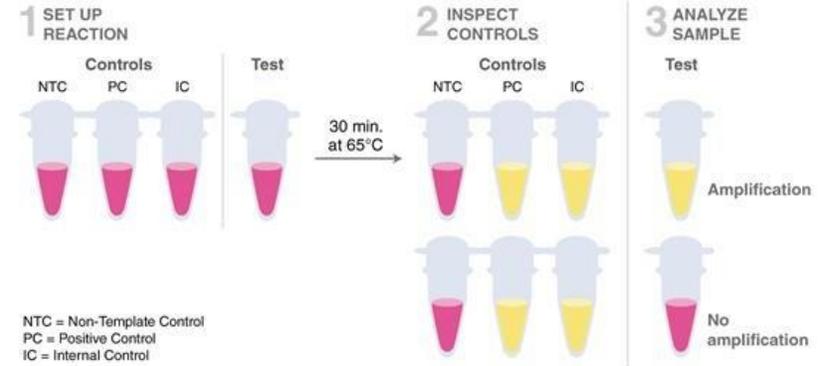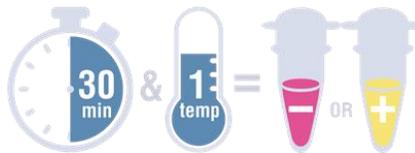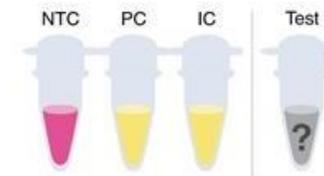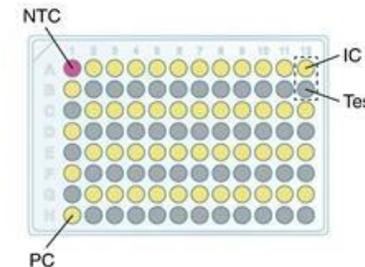
Molecular beacon-assisted isothermal circular strand displacement polymerization (ICSDP)

Isothermal amplification
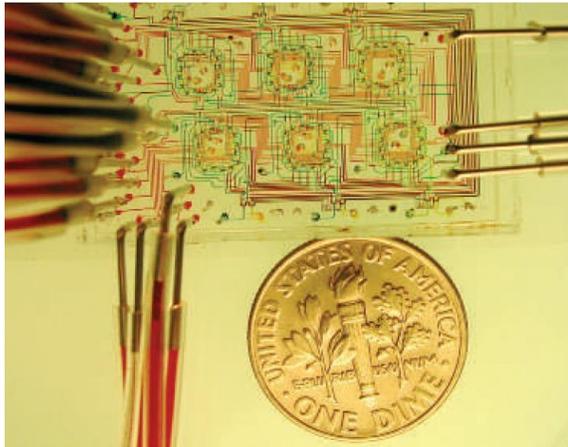Isothermal circular strand displacement polymerization. Displaced target available for a new cycle.
Linear amplification

Primer and **target** interaction with open molecular beacon

Amplification starts with polymerase

One more copy of target

DNA polymerase

target

primer

Molecular beacon

F Q

Guo Q. et al., *Nucleic Acids Res.* **2009**, 37, e20

Giuffrida M.C. et al., *Anal. Bioanal. Chem.* **2015**, 407, 6, 1533-1543
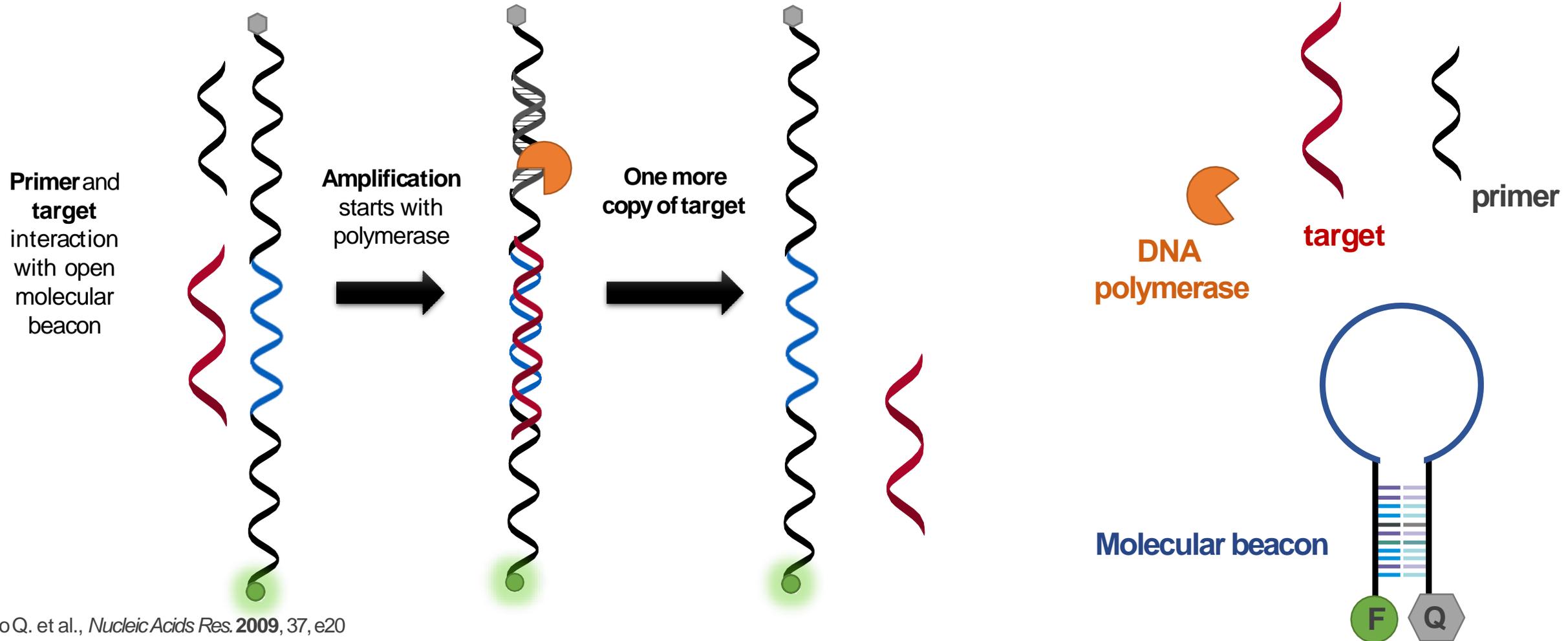
MICROFLUIDICS

It is the science and technology of systems that process or manipulate small (10^{-9} to 10^{-18} litres) amounts of fluids, using channels with dimensions of tens to hundreds of micrometres.
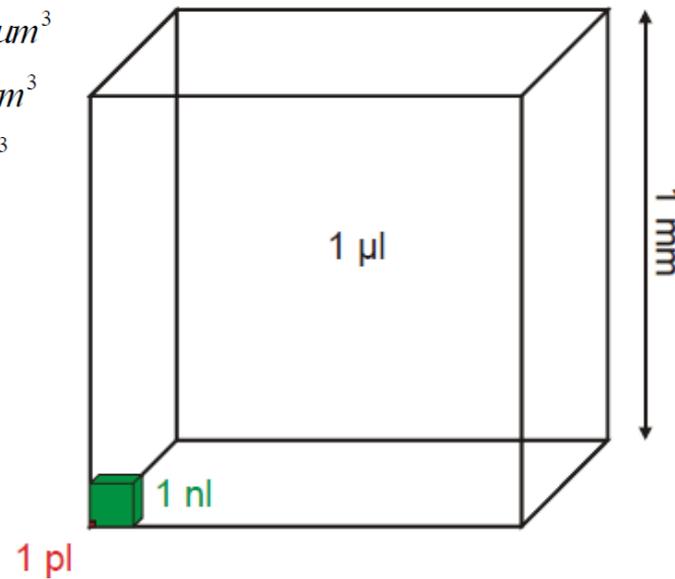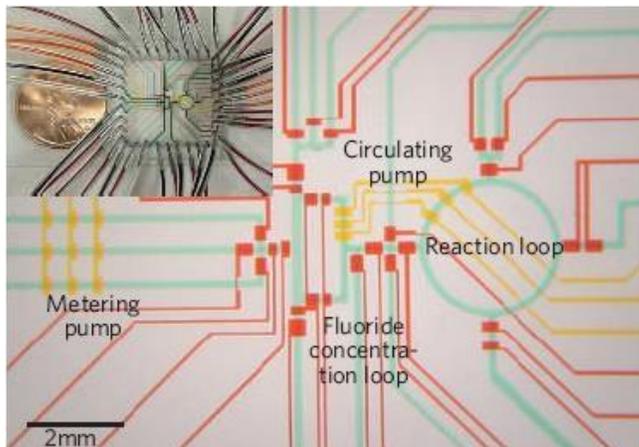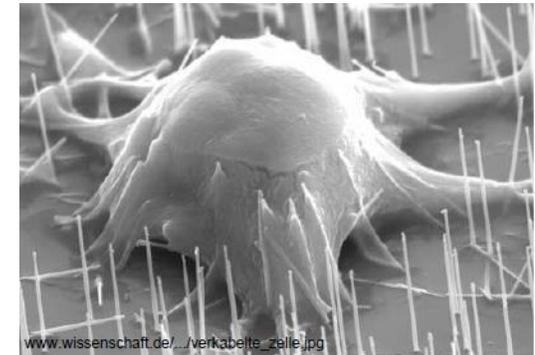
$$1l = 1dm^3$$
$$1\mu l = 1mm^3$$
$$1nl = 100\mu m^3$$
$$1pl = 10\mu m^3$$
$$1fl = 1\mu m^3$$

1 µl

1 mm

1 nl

1 pl

Typical size of a cell 1-30 µm

www.wissenschaft.de/.../verkabelte_zelle.jpg

Drug inhaler, Droplet diameter ~ 5 µm

Circulating pump

Reaction loop

Metering pump

Fluoride concentration loop

2mm

Whitesides, G. *Nature* **2006**, 442, 368–373

MICROFLUIDICS

Newtonian fluid → laminar flow
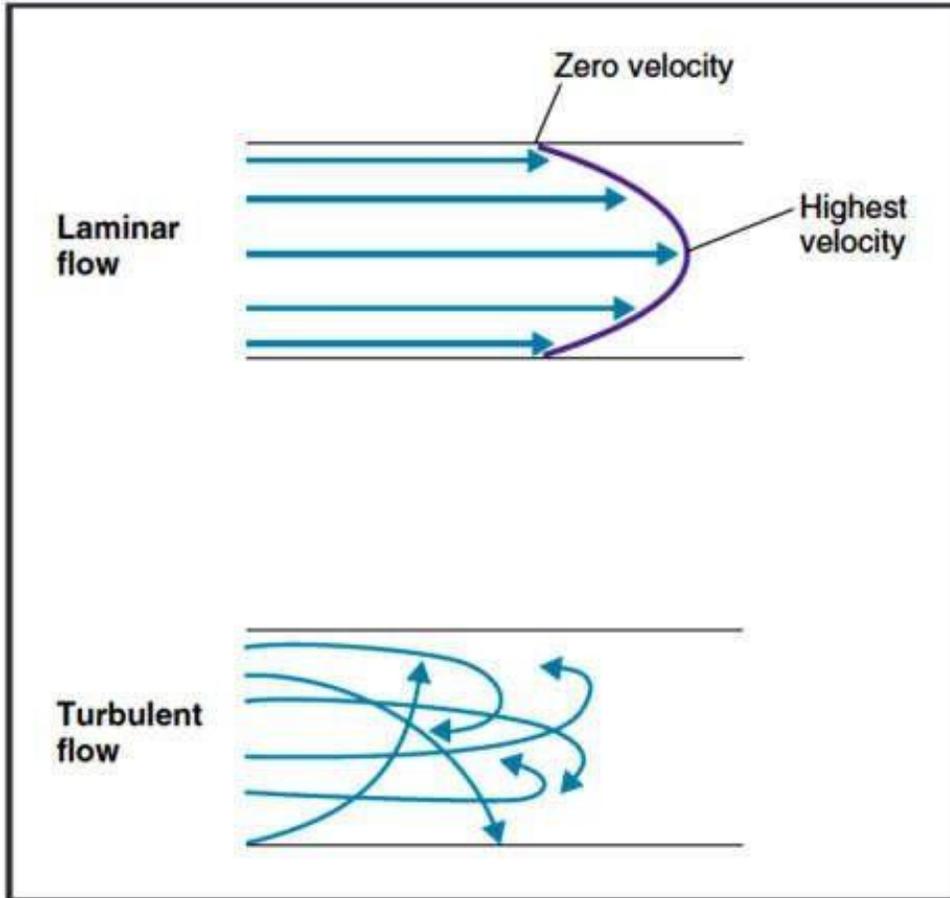
Model for the description of the motion of fluids

- **Non-dimensional Navier-Stokes equation**

$$\frac{\rho UL}{\mu}\left(\frac{\partial \mathbf{u}'}{\partial t'}+\mathbf{u}'\nabla\mathbf{u}'\right)=-\nabla p'+\eta\nabla^2\mathbf{u}'+\mathbf{f}'$$
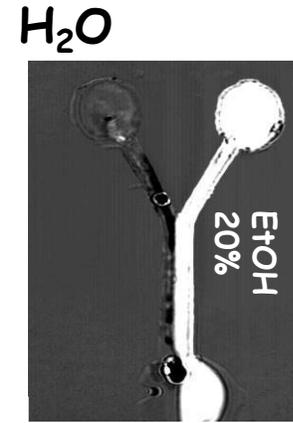
Reynolds number

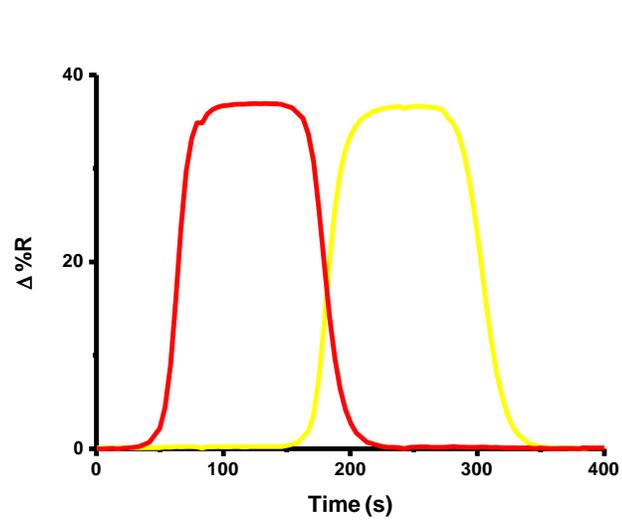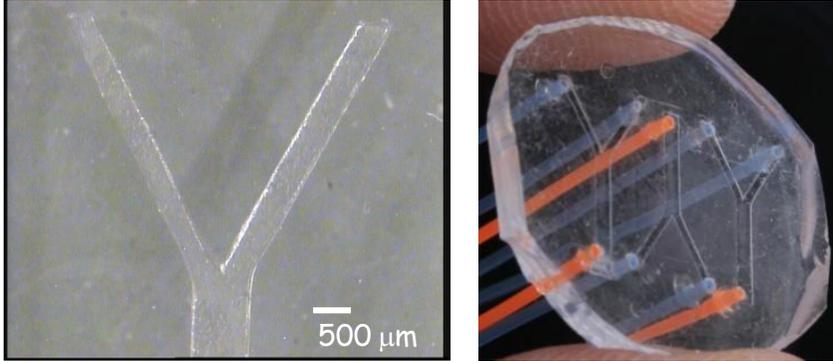$$\text{Re}=\frac{\rho UL}{\mu}\approx\frac{\text{Inertial forces}}{\text{Viscous forces}}$$

Re>2000
Turbulent
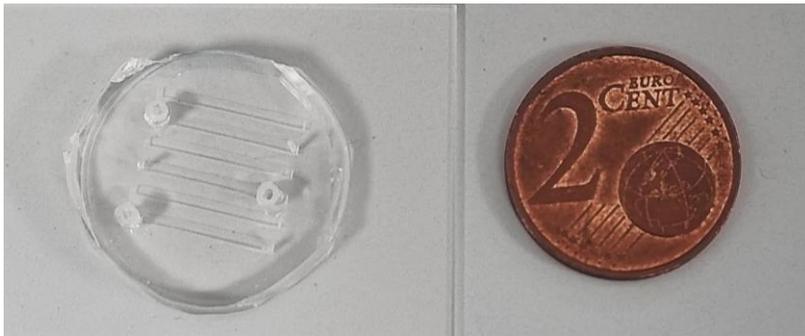
Re<2000
Laminar

MICROFLUIDICS: devices fabrication

Fabrication of microfluidic device by PDMS replica molding
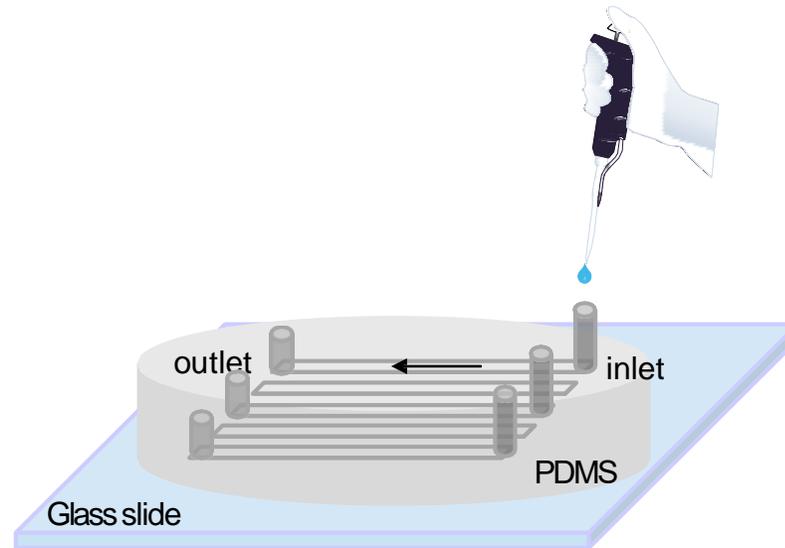

500 μm

Δ %R
Time (s)

H_2O

EtOH 20%

Re≈100

Laminar flow

Immiscible liquids

microfluidic channels (14×0.4×0.8mm)

< 1 μL of sample volume

Parallel microchannels
for multiple detection

outlet   inlet

PDMS

Glass slide

D'Agata R. et al., *Biosens. Bioelectron.* **2010**, 25(9):2095-2100.

MICROFLUIDICS: devices fabrication

Fabrication of microfluidic device by PDMS
(polydimethylsiloxane) replica molding

Master with different design
in plastic or silicon wafer

Micropatterned
layer

Add prepolymer and cure

Peel away PDMS

PDMS device with
microfluidic pattern

Embossed layer
of polymer

Weibel et al. *Nature Reviews: Microbiology* **2007**, 5, 209.
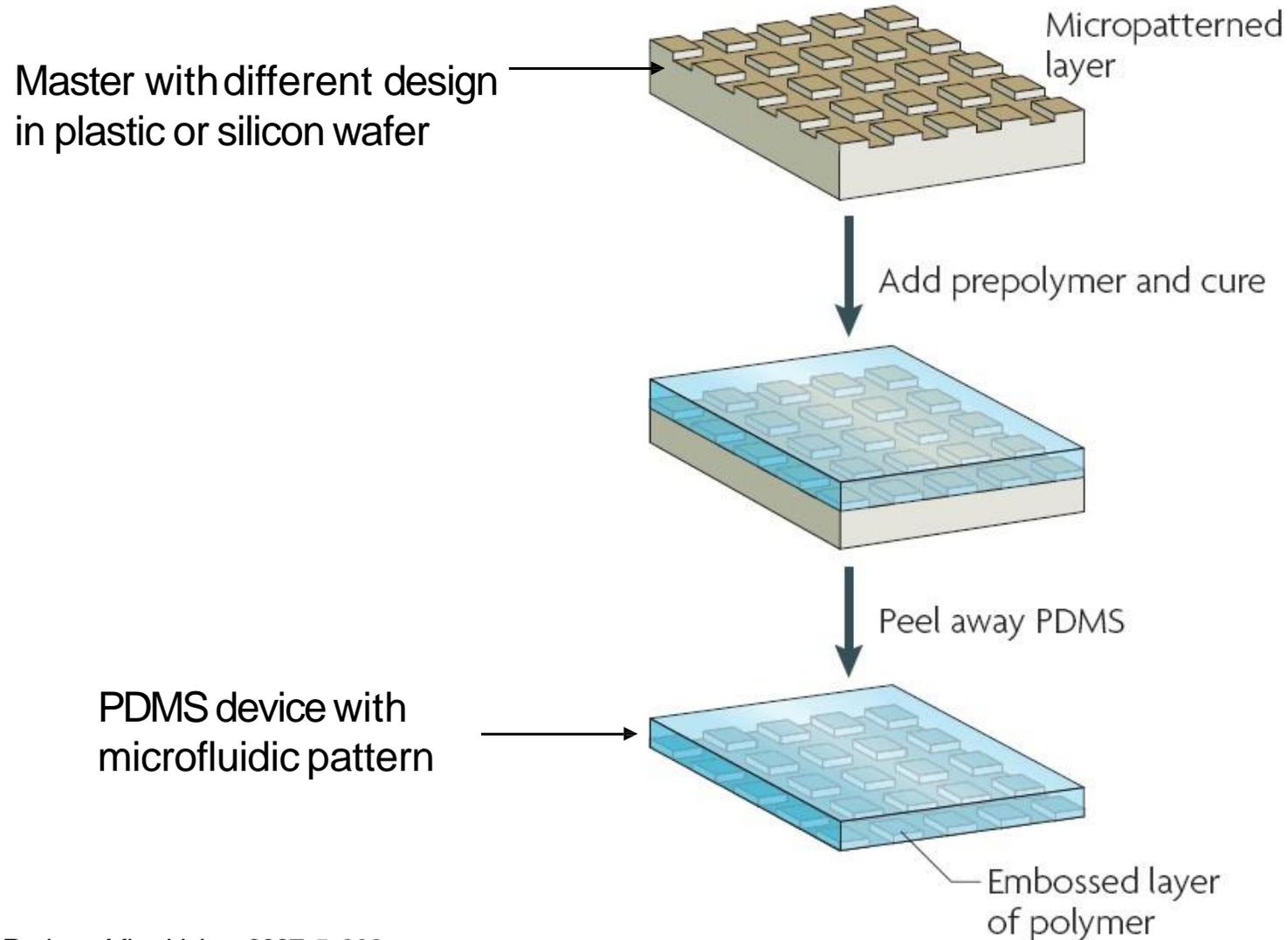
MICROFLUIDICS: Why?

- **Small sample volume**
- **Miniaturization**

- **Reduction of analysis time**

- **Parallel devices and faster processes**

- **High-throughput**

- **Integration and portable devices (lab-on-a-chip, micro Total Analysis Systems μTAS)**

Fluid

Microchannel

Prof. Lee, ETH Zurich
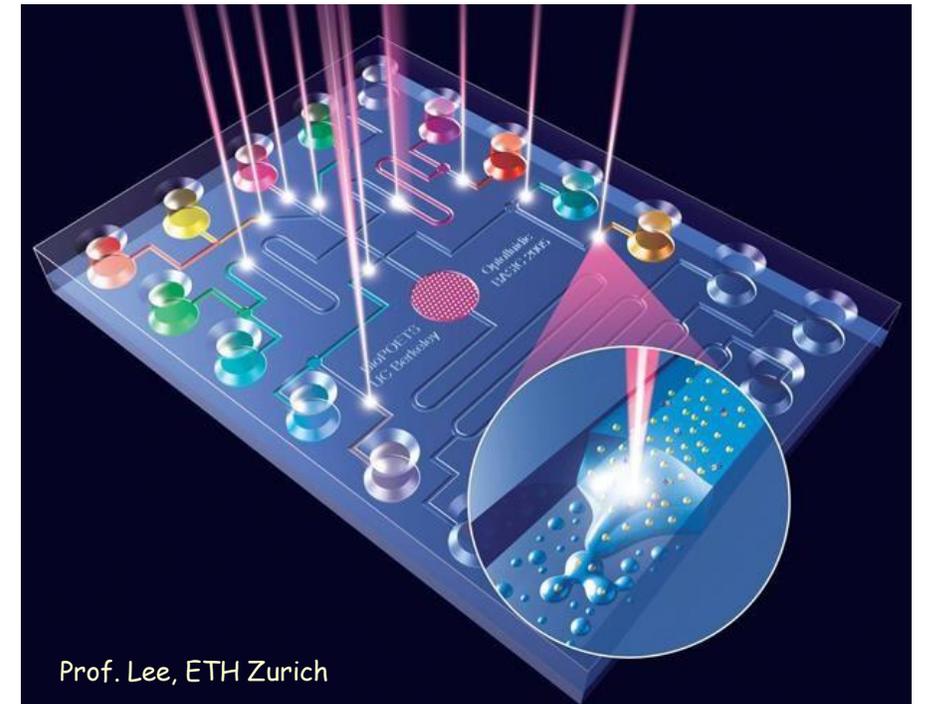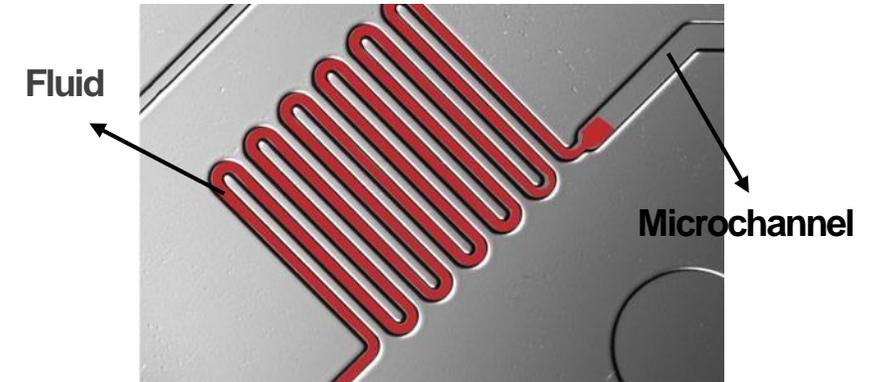
microRNA (miRNA)

- **Single-stranded, non-coding RNA molecules**

- **Key-role in protein expression**

- **mRNA silencing**

- **Remarkable stability when released into biofluids**

Challenges for miRNA detection
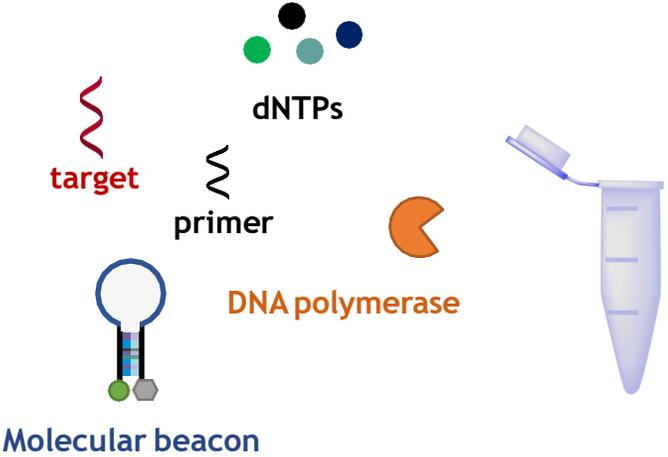
- Analytes are present at low concentrations

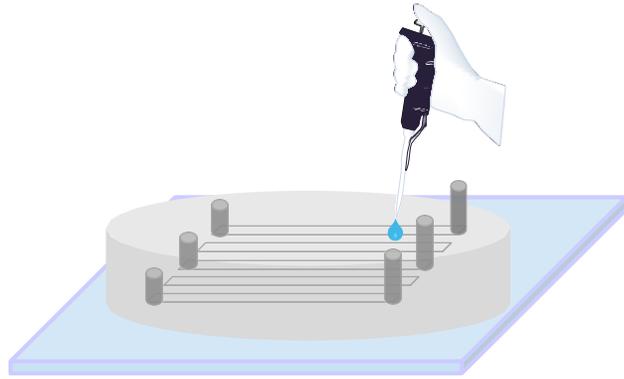 **Biomarker levels: fg mL^{-1} - ngmL^{-1}**

- Short length sequence

  Length: 19-23 nt

- High sequence homology

Mendell J.T. et al., *Cell* **2012**, 148, 1172-1187

Tu M. et al., *Sci. Rep.* **2016**, 6, 25032

D'Agata R. et al., *Anal. Bioanal. Chem.* **2019**, 411: 4425

Microfluidic lab-on-a-chip platform for liquidbiopsy: microRNA
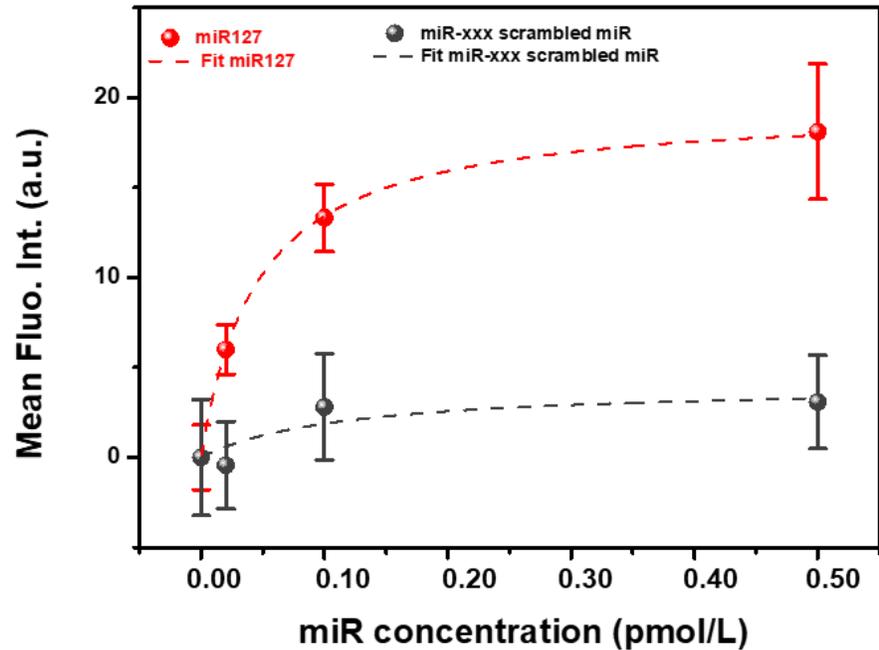

target

dNTPs

primer

DNA polymerase

Molecular beacon

ICSDP amplification

30°C 2 hr
constant temperature

Fluorescence detection

microRNA-127 biomarker for chronic joint disease

miR127
Fit miR127

miR-xxx scrambled miR
Fit miR-xxx scrambled miR

20

10

0

Mean Fluo. Int. (a.u.)

0.00   0.10   0.20   0.30   0.40   0.50

miR concentration (pmol/L)

- Low sample volume
- No time consuming
- High selectivity in buffer
- Discrimination in synovial fluid
- Low detection

Optical biosensors: Surface Plasmon Resonance

Electromagnetic radiation in resonance with surface plasmon oscillation.

Surface plasmon polaritons : quasi-particles resulting from the coupling of surface plasmons and photons

ε_2

50 nm ~

ε_1

θ

ε_0

Kretschmann configuration
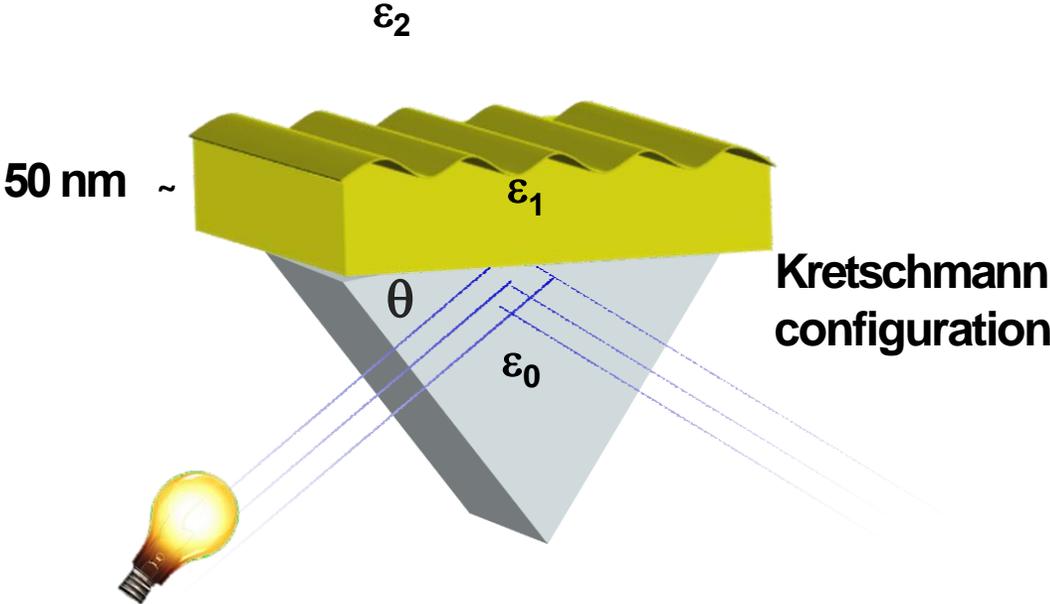
Plasmon wave vector = radiation wave vector

$$K_{SPP} = \frac{\omega}{c}\sqrt{\frac{\varepsilon'_1 \varepsilon_2}{\varepsilon'_1 + \varepsilon_2}} = \frac{\omega}{c}\sqrt{\varepsilon_0}\, sen\,\vartheta = K_{Light}$$

Radiation wave vector

$$k_{Light} = \frac{\omega}{c}\sqrt{\varepsilon_0}\, sen\,\theta$$

Snell's Law

$$\sin\theta = \frac{\varepsilon_1}{\varepsilon_0}$$

Optical biosensors: Surface Plasmon Resonance
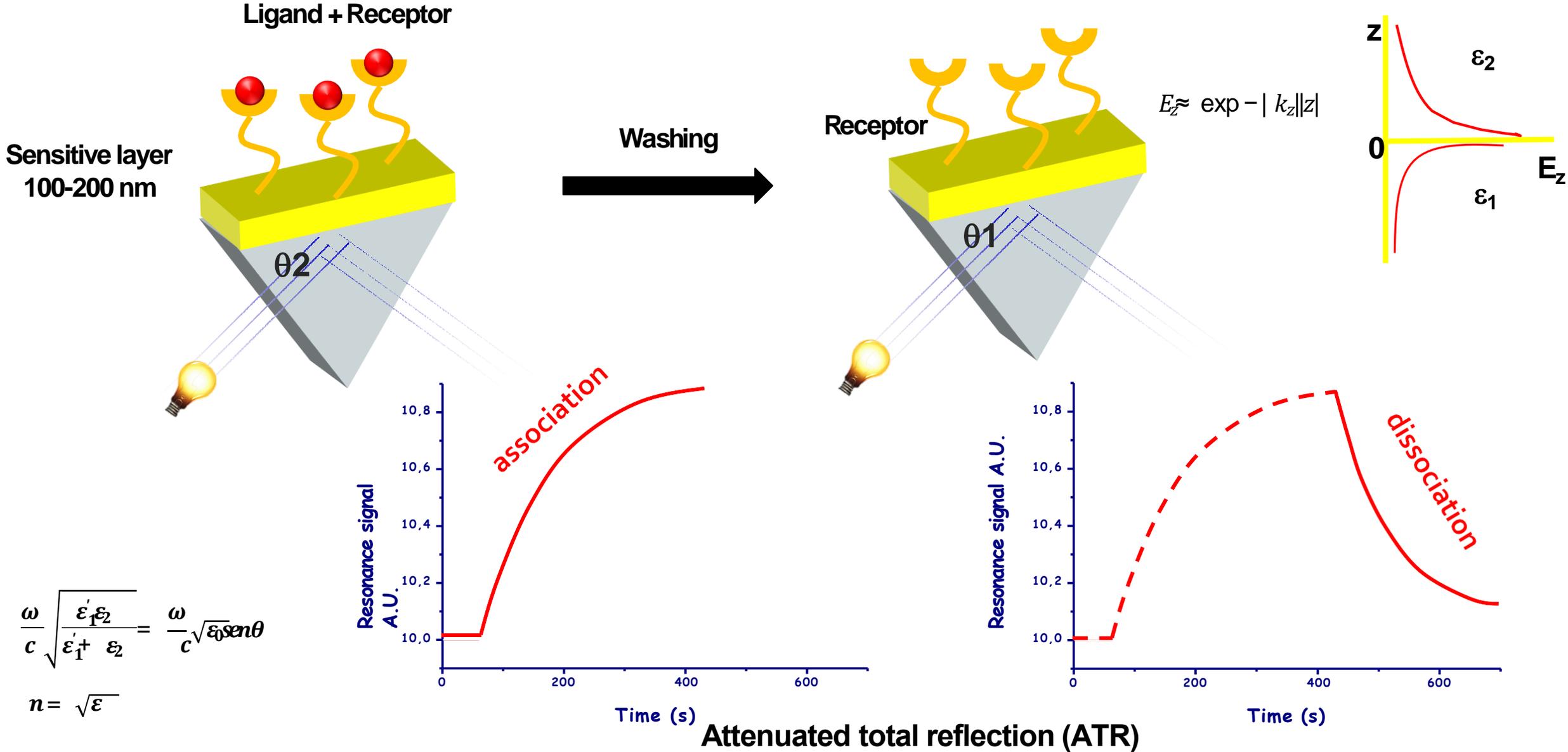

Receptor

Ligand (biomolecule)

Ligand + Receptor

θ1

θ2

RU

80

40

θ1

Degrees or Energy

RU

80

40

θ1

θ2

Degrees or Energy

$$\frac{\omega}{c}\sqrt{\frac{\varepsilon_1'\varepsilon_2}{\varepsilon_1' + \varepsilon_2}} = \frac{\omega}{c}\sqrt{\varepsilon_0}\,sen\theta$$
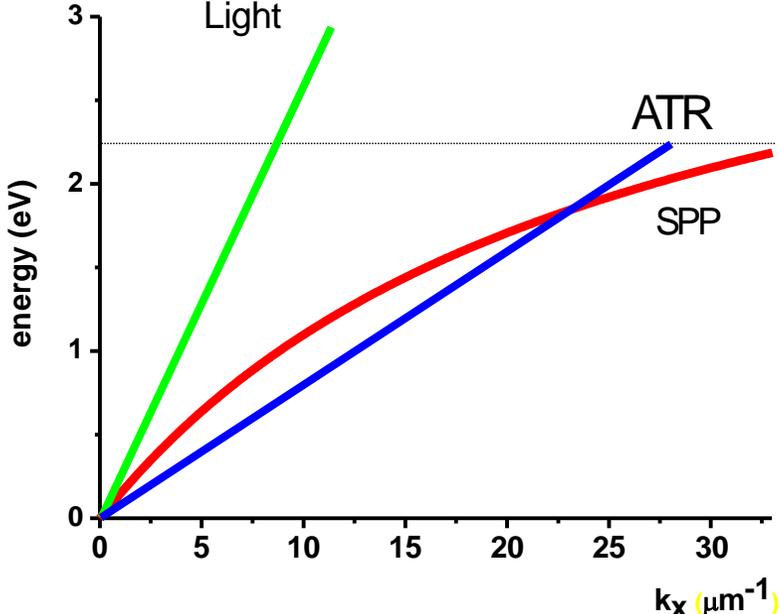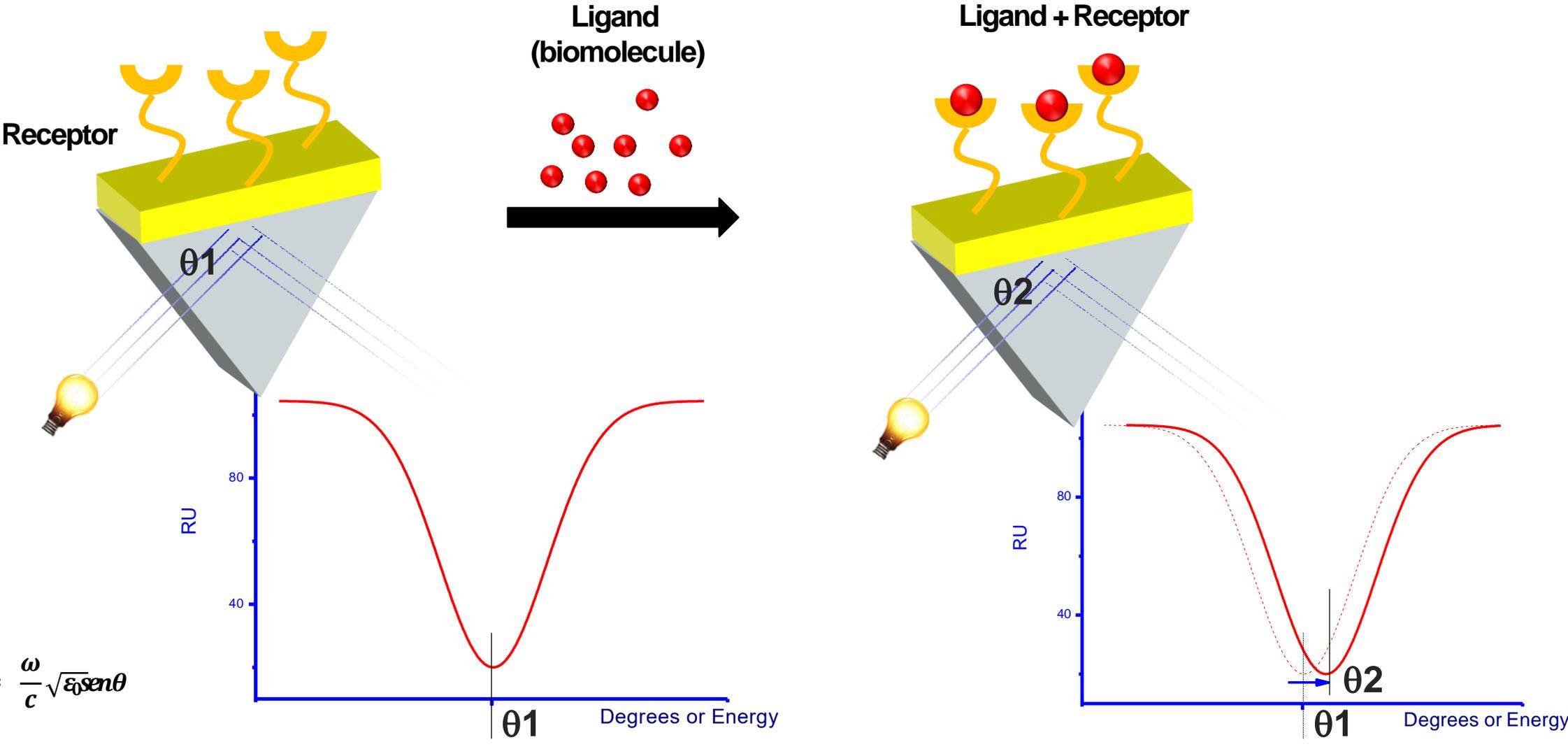
$$n = \sqrt{\varepsilon}$$

Attenuated total reflection (ATR)

Optical biosensors: Surface Plasmon Resonance

Ligand + Receptor

Sensitive layer 100-200 nm

$\theta 2$

Washing

Receptor

$\theta 1$

$E_z \approx \exp - |k_z||z|$

z

ε_2

0

E_z

ε_1

$$\frac{\omega}{c}\sqrt{\frac{\varepsilon_1'\varepsilon_2}{\varepsilon_1' + \varepsilon_2}} = \frac{\omega}{c}\sqrt{\varepsilon_0} \, sen\theta$$

$$n = \sqrt{\varepsilon}$$

association

dissociation

Resonance signal A.U.

Time (s)

Attenuated total reflection (ATR)

Surface Plasmon Resonance Imaging (SPRI)

- **Real-time analysis**
- **Label-free**
- **High sensitivity**
- **Multi-analyte monitoring**

Sensitivity can be improved up to nM-fM

PCR-free method!

Kretschmann configuration

CCD

$$\%R = 100\times \frac{0.85\, I_p}{I_s}$$
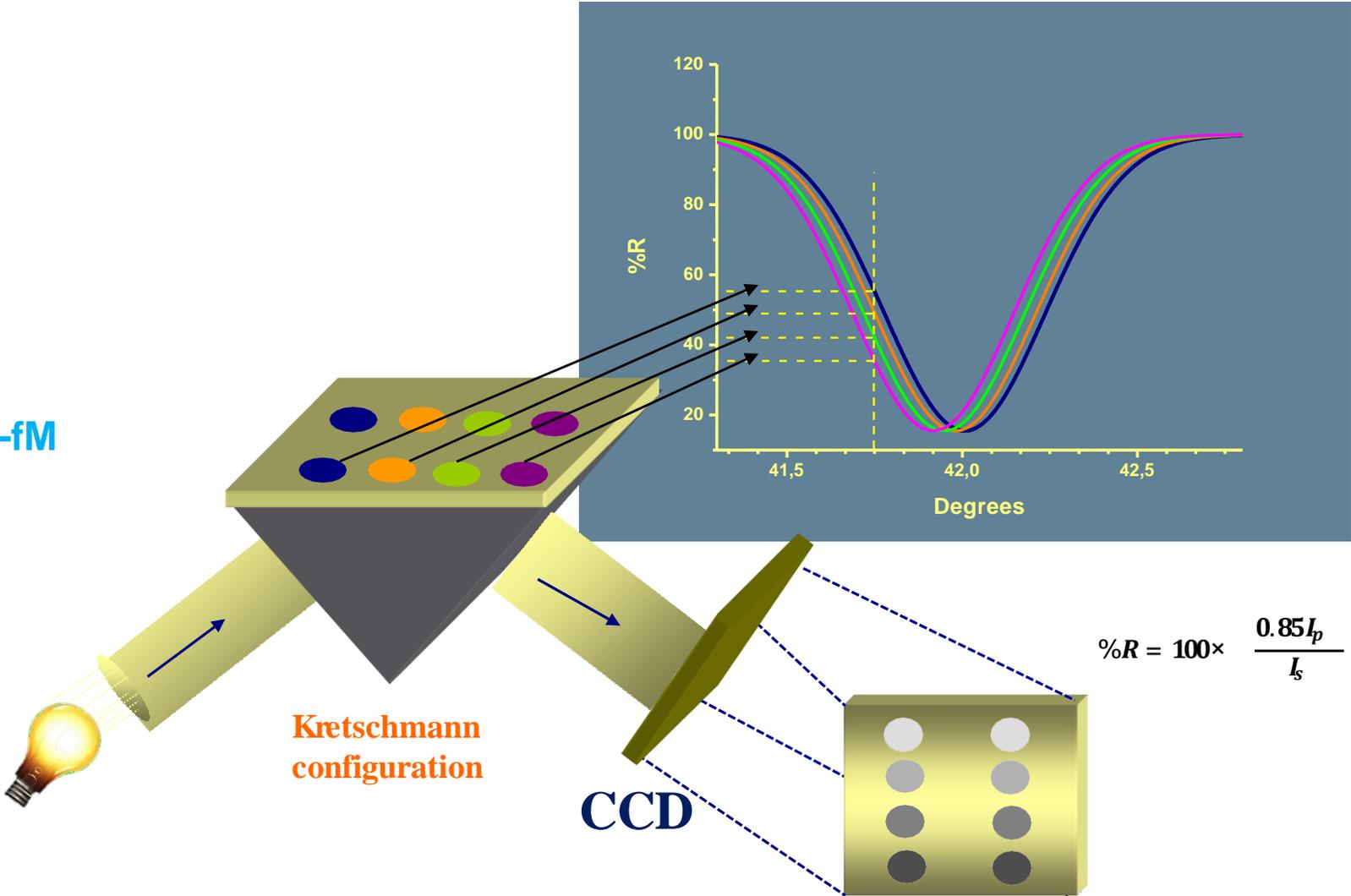
Rothenhäusler et al. Surface–plasmon microscopy. **1988**, Nature 332, 615–617 D'Agata at al. Anal Bioanal Chem. **2013**; 405(2-3):573-84.

Surface Plasmon Resonance Imaging (SPRI)

The lateral resolution of a SPR image is limited by the surface plasmon decay distance L_x that is the distance on the surface by which the intensity of the field associated to plasmons decreases by a 1/e factor.

$$L_x = \frac{1}{2k \, k''_x}$$

k''_x is the immaginary part of the x component of the wave-vector

For gold:
L_x=0.1 μm at λ=488 nm Å, L_x=10 μm at λ=647 nm
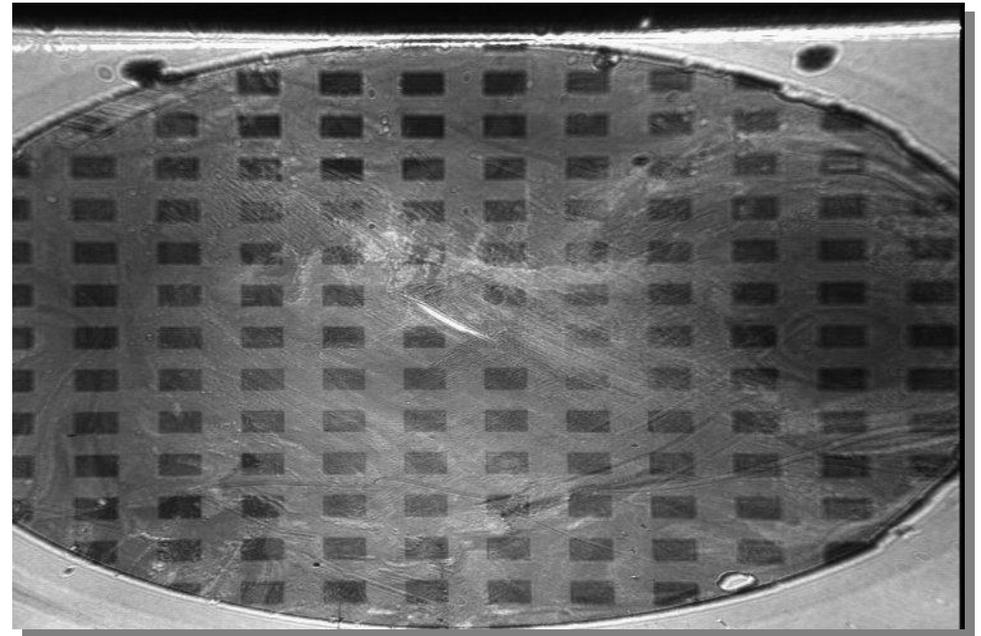
Surface Plasmon Resonance Imaging (SPRI)

Example of SPRI image

D'Agata at al. Organic & Biomolecular Chemistry, **2006**, 4; 610.
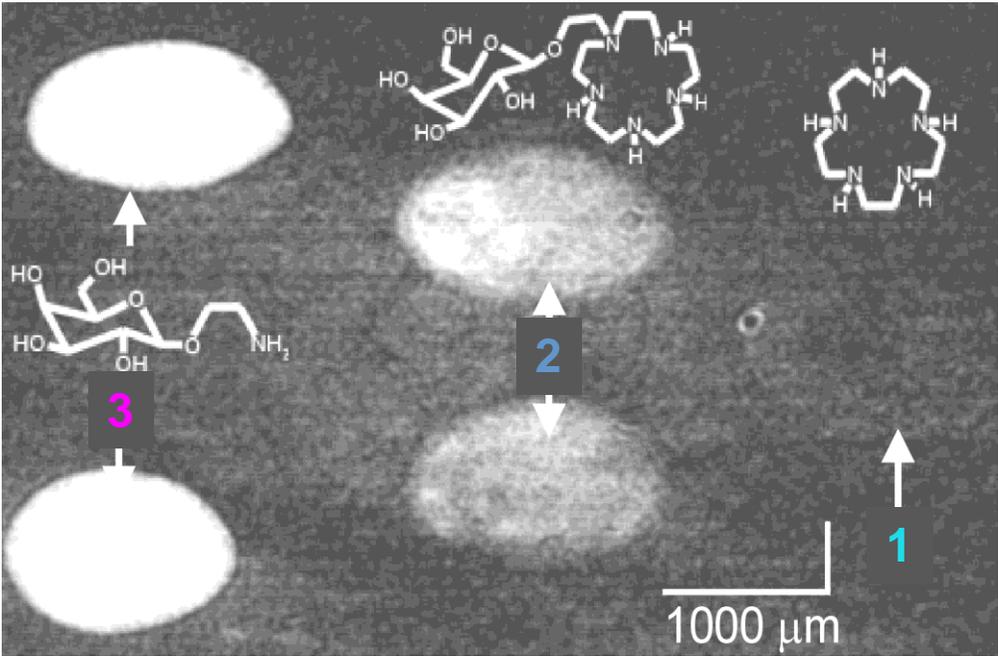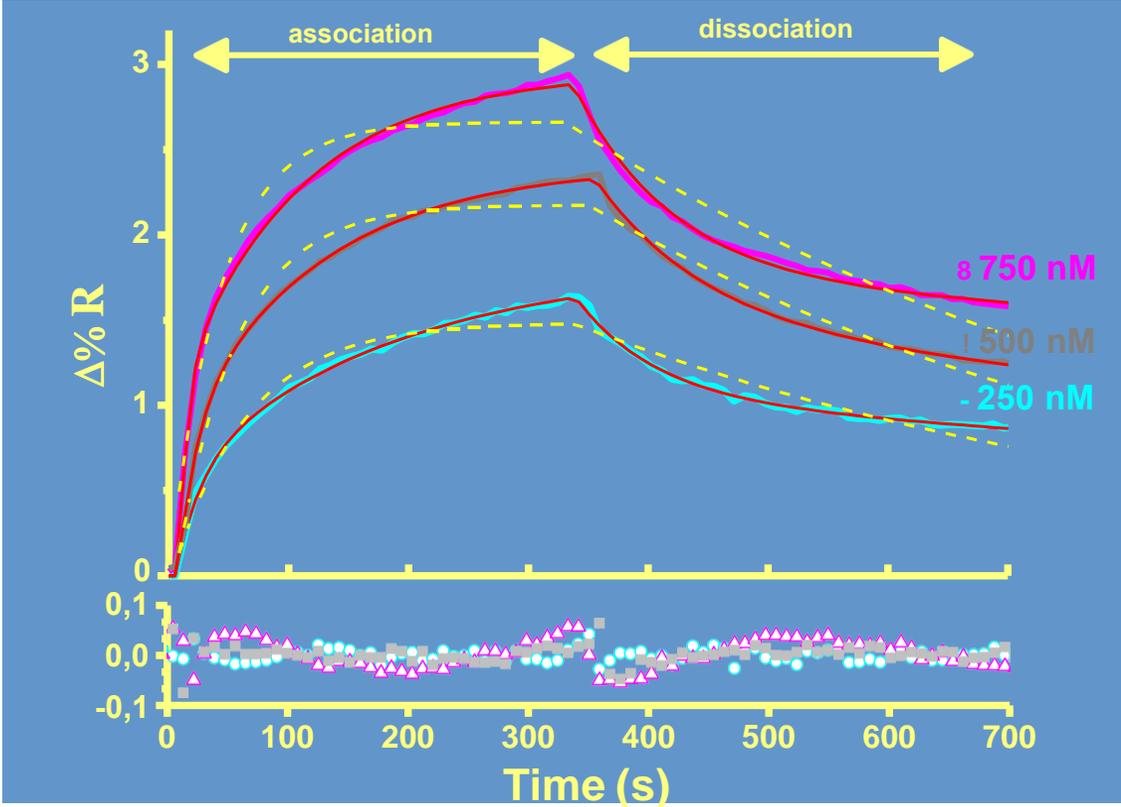
Microfluidic lab-on-a-chip plasmonic platform: detection of DNA

ssDNA-36mer
1μM

%R

Time (sec.)

Microfluidic channels

DNA-DNA
hybridization (100%)

DNA-DNA
partial hybridization

DNA-DNA
no hybridization
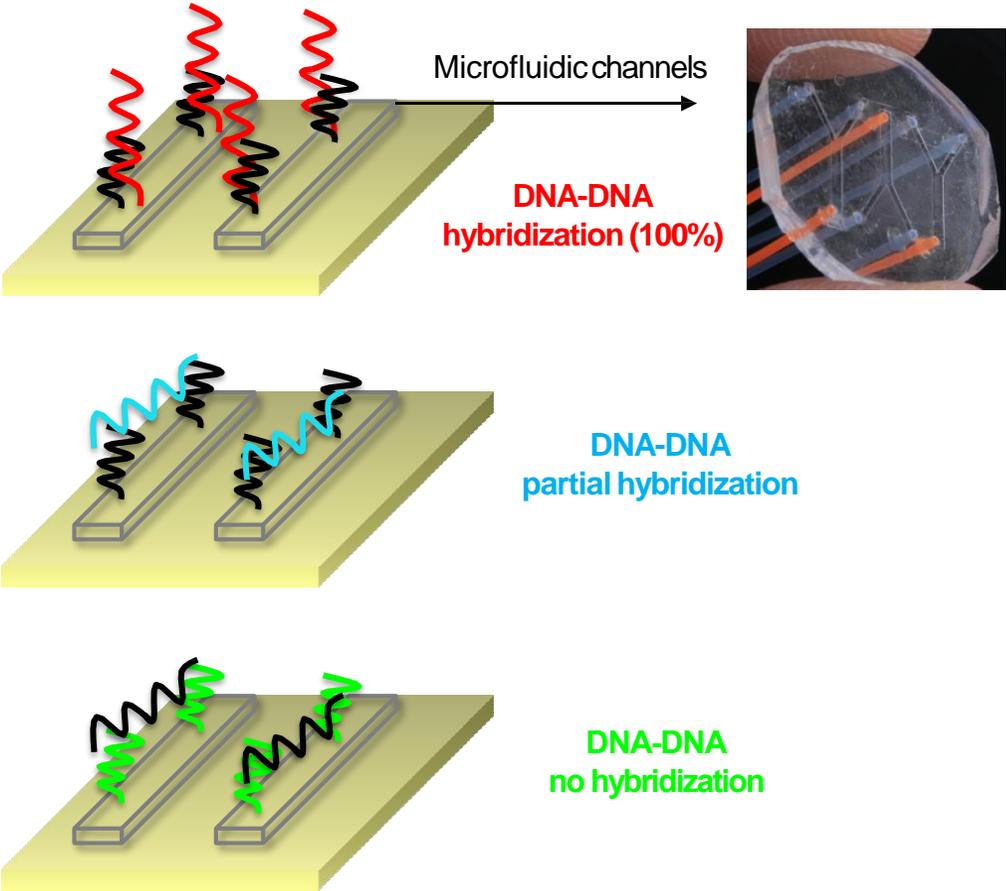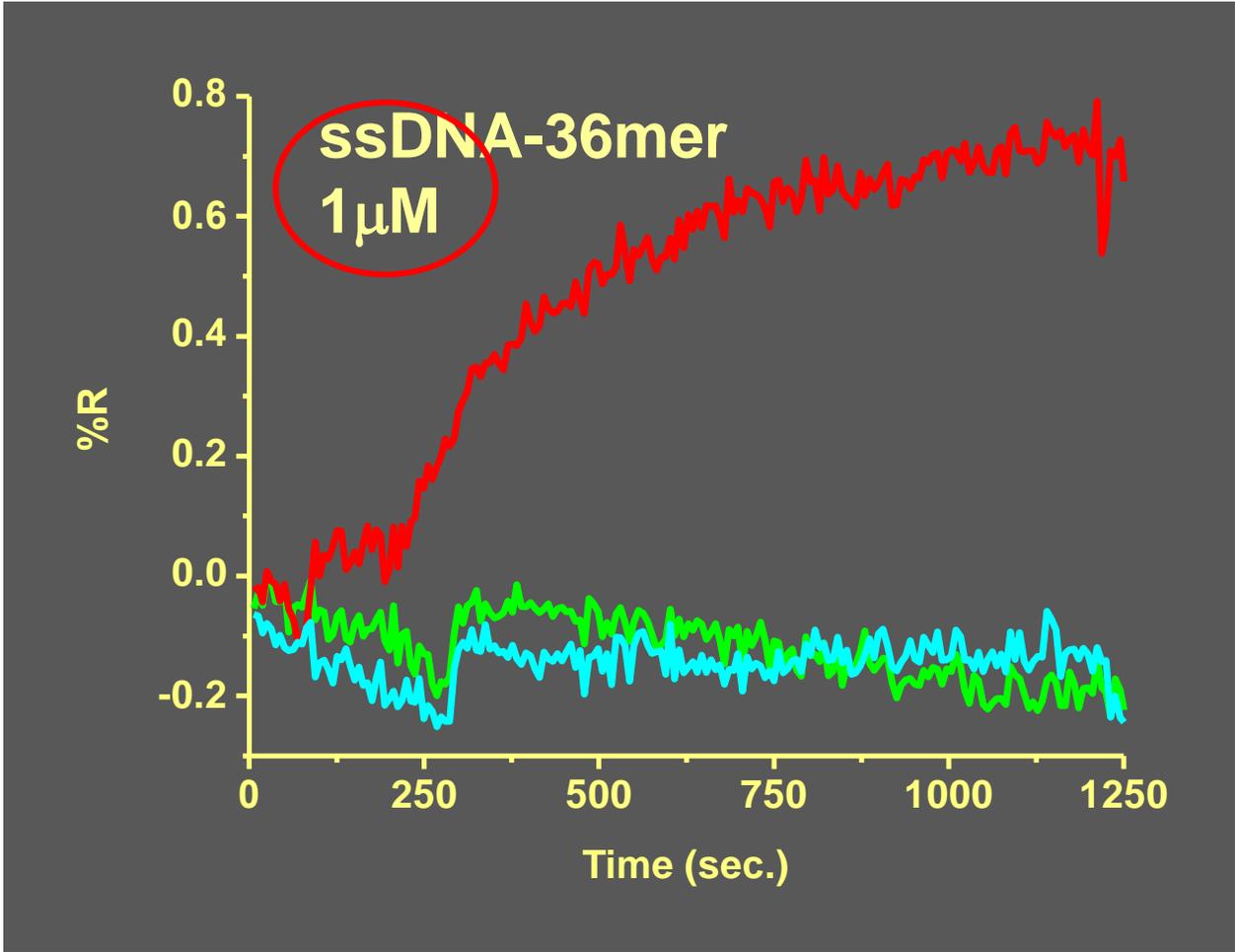
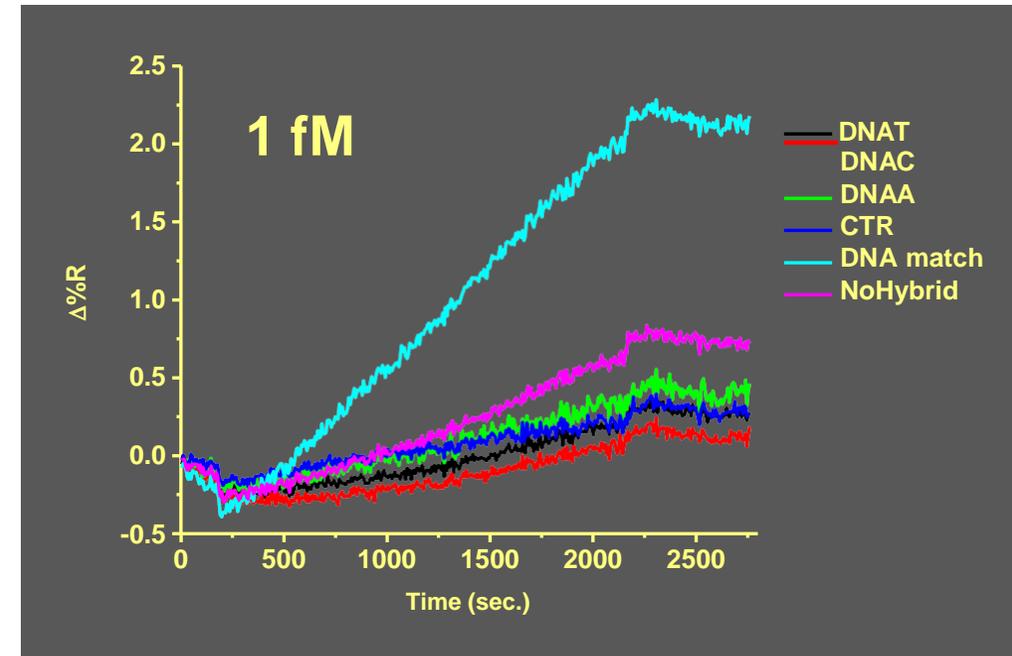———— 5'-LL-AAACCCTTAATCCCA-3' PROBE

———— 3'-TTTGGGAATTAGGGTTTTTTTTTTTTCGTCGAATAGCA-5'
ssDNA-36mer-match

Nanoparticle amplification-SPRI

Surface Plasmon resonance
Gold nanoparticles (NPs) + gold surface
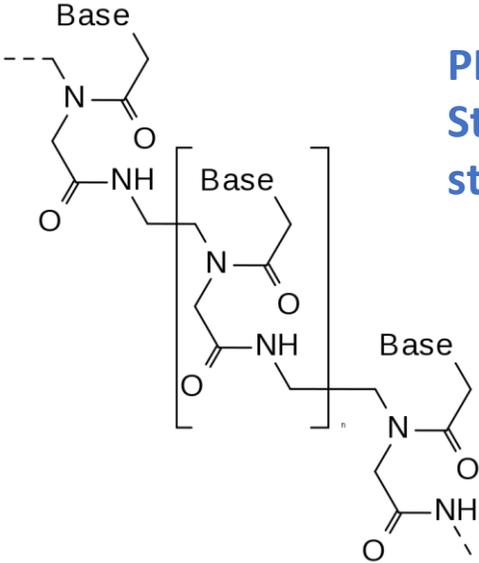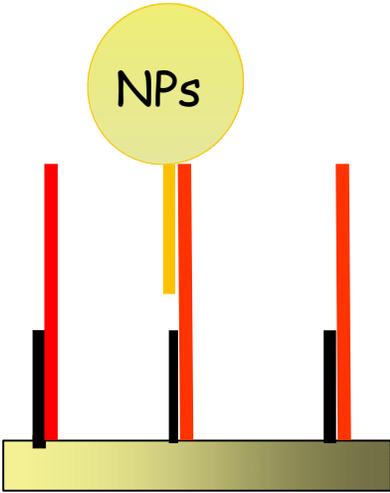
PEPTIDE NUCLEIC ACID –PNA
Stronger affinity for complementary strands of DNA

Base

Base

Base

NPs

NPs

5'-GCAGCTTATCGT-3'-Biotin

ssDNA-12merC_6Biotin
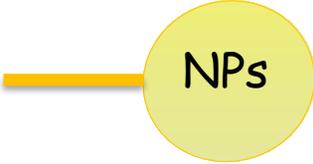
5'-LL-AAACCCTTAATCCCA-3'   PNA-15mer

3'-TTTGGGAATTAGGGTTTTTTTTTTTCGTCGAATAGCA-5'
ssDNA-36mer-match

D'Agata et al., *ChemBioChem* **2008**, 9, 2067
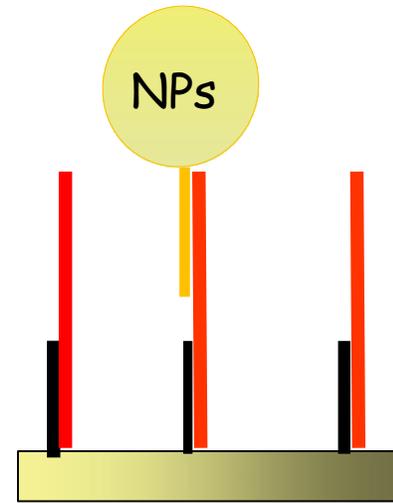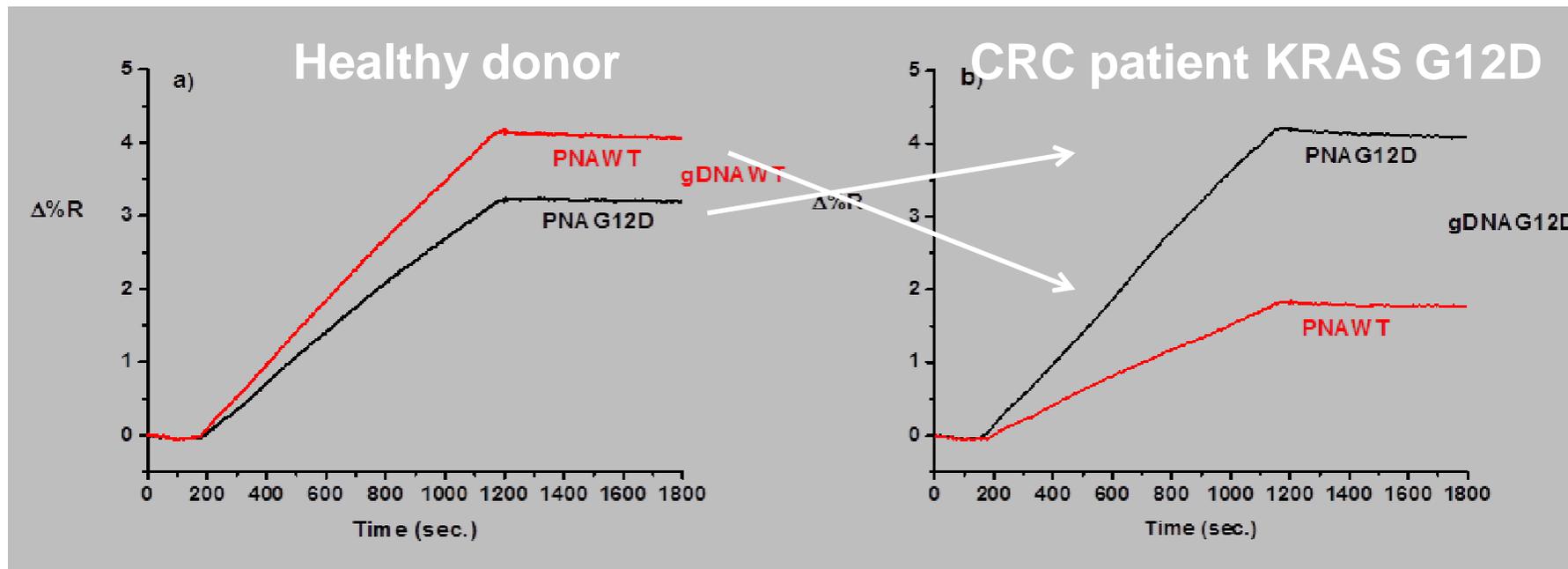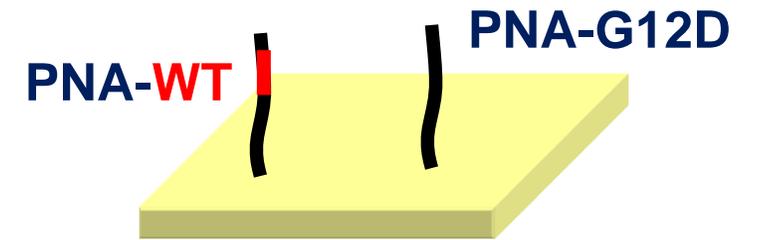
Nanoparticle amplification-SPRI: SNPs detection
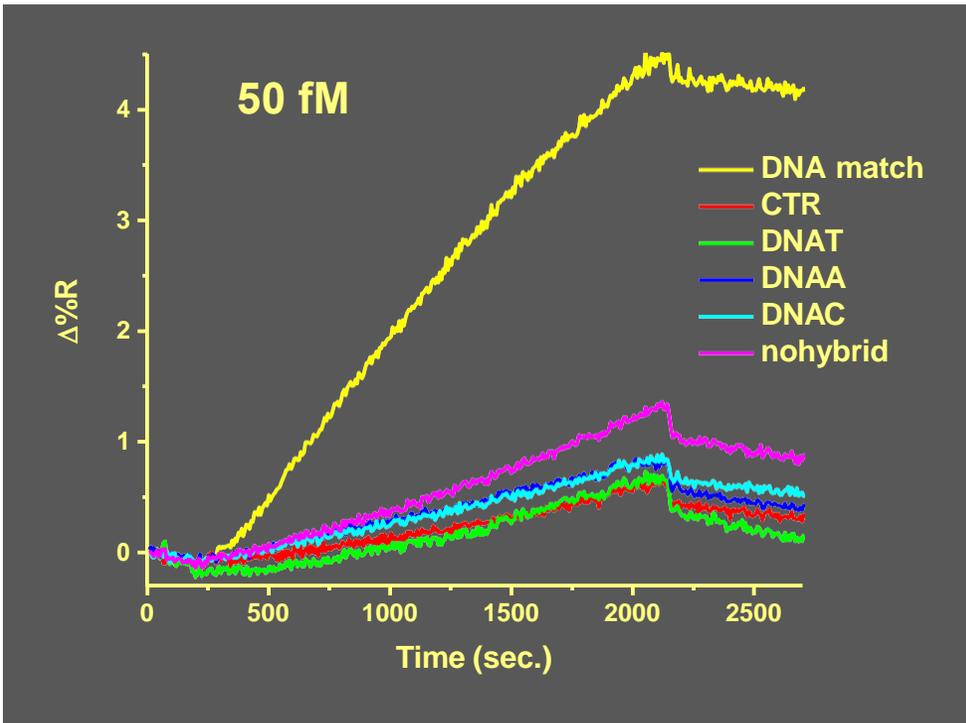
**PCR-free detection of KRAS mutations
(Plasma from colorectal cancer patients)**

PNA-G12D

PNA-WT

YouTube https://youtu.be/88n3IRsWTm8

D'Agata et al., Biosens. Bioelectron., **2020**, 170, 112648
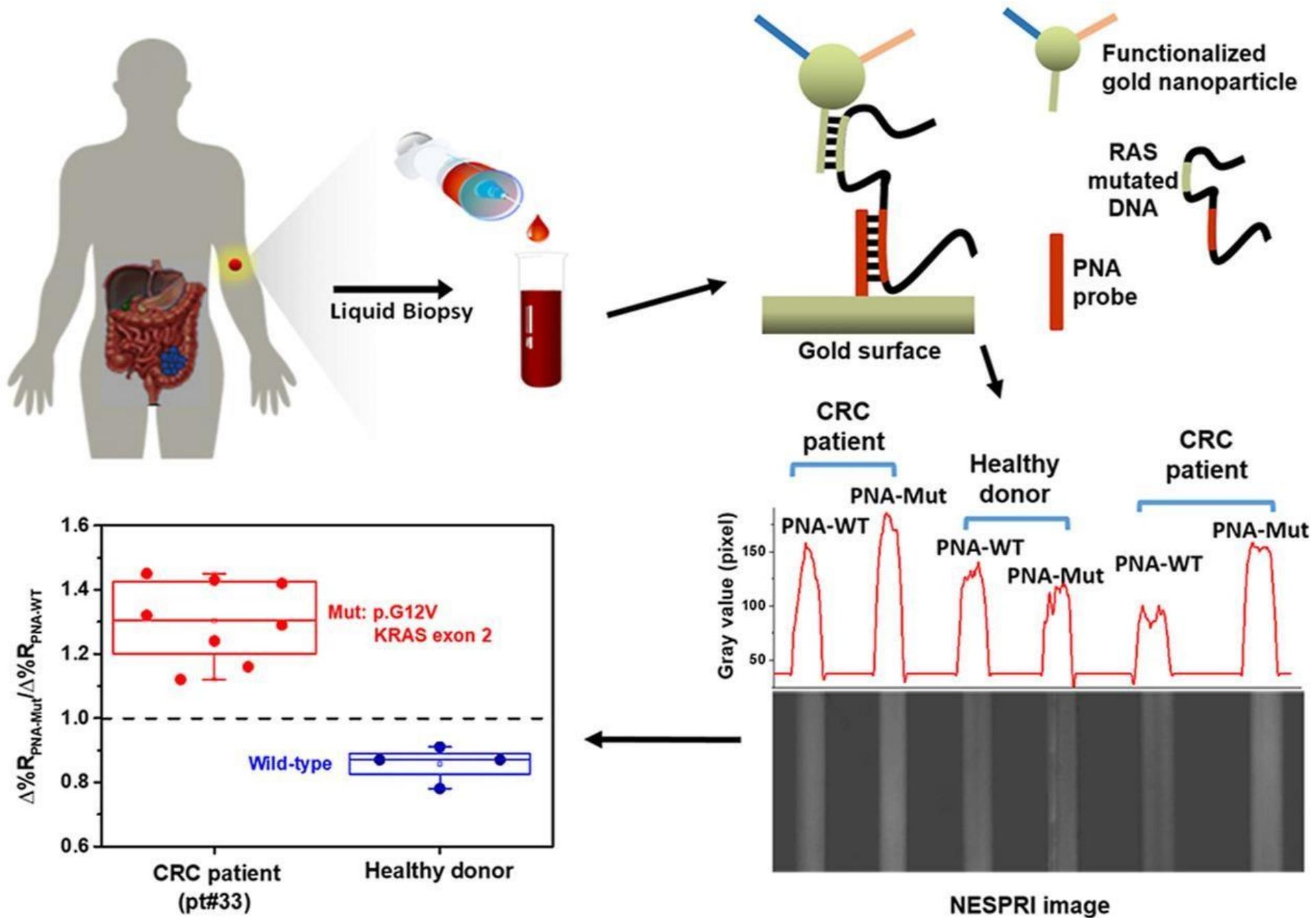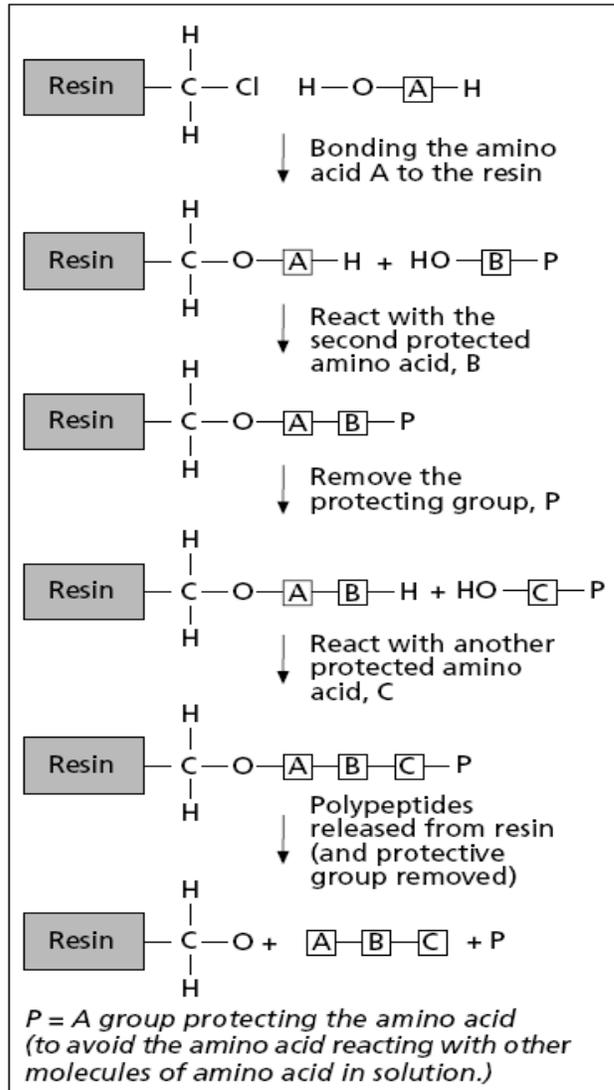
Biomimetic receptors

Used for biosensors or for sample preparation/purification

Obtained via combinatorial chemistry and/or molecular modelling

- Peptides

- Aptamers

- MIP (Molecularly Imprinted Polymers)

Combinatorial chemistry approach: Synthesys of aminocids via split and mix

Left diagram:

H—O—[A]—H

Resin—C—Cl

Bonding the amino acid A to the resin

Resin—C—O—[A]—H + HO—[B]—P

React with the second protected amino acid, B

Resin—C—O—[A]—[B]—P

Remove the protecting group, P

Resin—C—O—[A]—[B]—H + HO—[C]—P

React with another protected amino acid, C

Resin—C—O—[A]—[B]—[C]—P

Polypeptides released from resin (and protective group removed)

Resin—C—O + [A]—[B]—[C] + P

P = A group protecting the amino acid (to avoid the amino acid reacting with other molecules of amino acid in solution.)

Right table:

Split synthesis

| Stage	Reaction vessel 1 (A)	Reaction vessel 1 (B)	Reaction vessel 1 (C)	
1	Resin + A	Resin + B	Resin + C	3 compounds
		MIX		
2	Resin-A+A Resin-B+A Resin-C+A	Resin-A+B Resin-B+B Resin-C+B	?	9 compounds
		MIX		
3	Resin-A-A+A Resin-B-A+A Resin-C-A+A Resin-A-B+A Resin-B-B+A Resin-C-B+A Resin-A-C+A Resin-B-C+A Resin-C-C+A	Resin-A-A+B Resin-B-A+B Resin-C-A+B Resin-A-B+B Resin-B-B+B Resin-C-B+B Resin-A-C+B Resin-B-C+B Resin-C-C+B	Resin-A-A+C Resin-B-A+C Resin-C-A+C Resin-A-B+C Resin-B-B+C Resin-C-B+C Resin-A-C+C Resin-B-C+C Resin-C-C+C	27 compounds
		MIX		

Biomimetic Approach

➢   Starting from the biological structure it is possible to reproduce with natural amino acids the proper shape of binding dock

➢   The biomimetic approach relies on the design and development of artificial oligopeptides as a mimic of the biological binding site by using molecular modeling
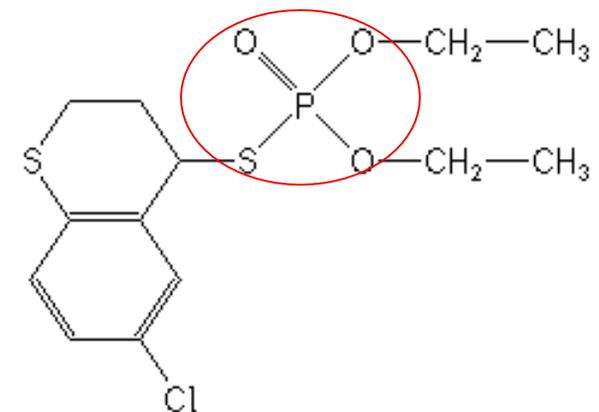
✓**Why oligopeptides?**

➢Nature exploited aminoacids structures to obtain the most of receptors

➢Oligopeptides have the advantage of informatics help from the point of the crystallographic informations from native proteins

➢Great number of combinations using 20 aminoacids which can do any binding traps

BIOMIMETIC RECEPTORS FOR PESTICIDES
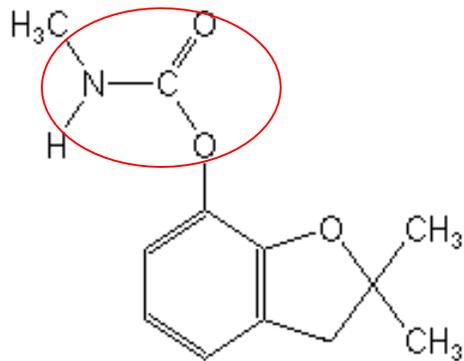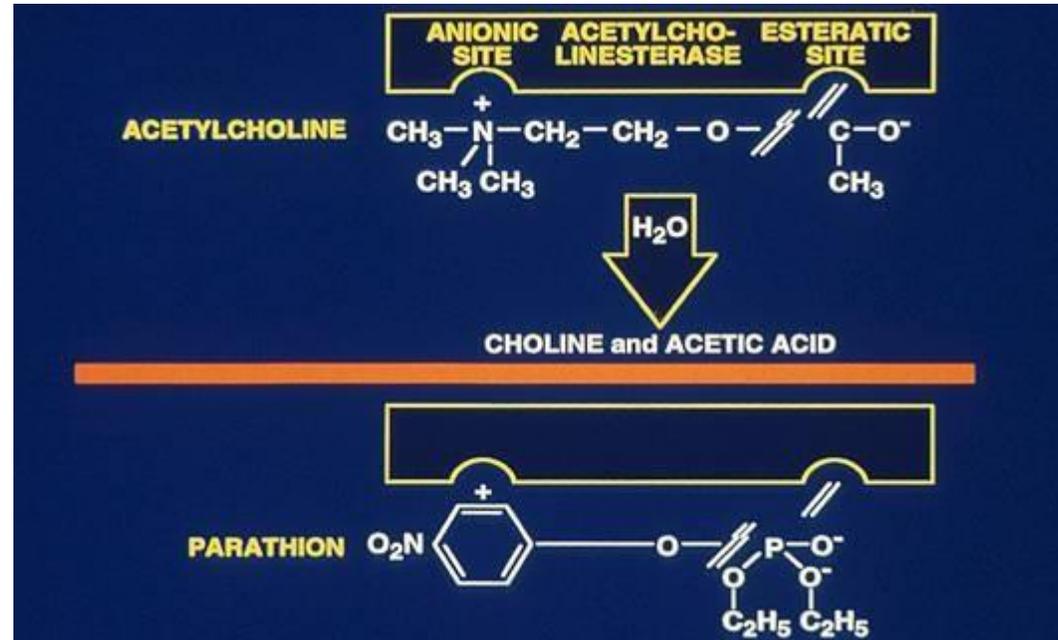
Carbamate

organophosphate

✓ Mechanism of AChE inhibition

AChE, the target enzyme of pesticides, **is an efficient serine hydrolase** that catalyzes the breakdown of acetylcholine (ACh)
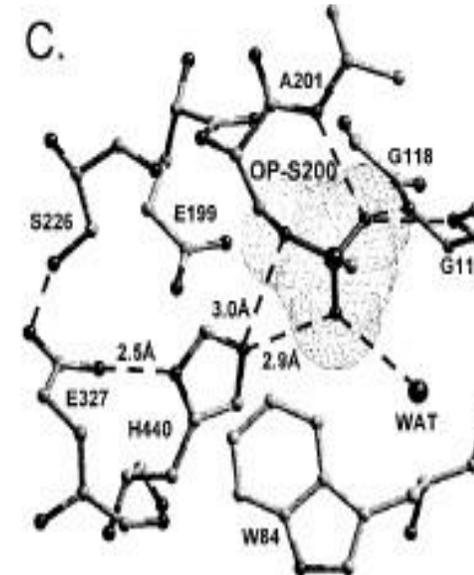
Acetylcholine + H₂O → choline + acetic acid

How pesticides work
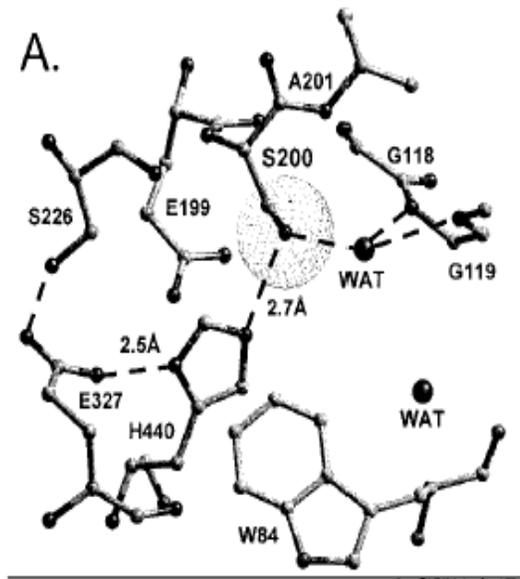
A. Native structure: the active site, including the catalytic triad (S200-H440-E327) and the oxyanion hole (-NH of G118, G119, and A201)
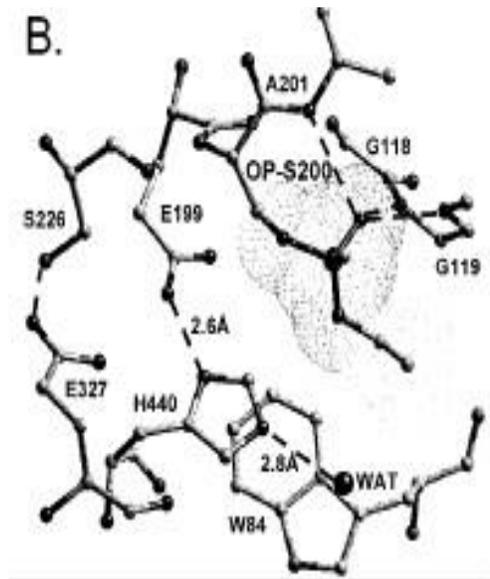
B. Pro-aged structure: Phosphonylation triggers a conformational change for H440 that disrupts the H-bond to E327

C. Aged structure: For reaction of AChE with VX and most phosphonates, aging predominates, and dealkylation results in movement of H440 to the negatively charged pocket formed by E327 Ox, S200 Ox, and one anionic oxygen of the dealkylated OP

From *Millard et al J.Am.Chem.Soc. 121, (1999)*

❖ Computational screening

## ✓ AChE-OP crystallographic structure (PDB ID: 1VXO)

Methylphosphonylated Acetylcholinesterase (Aged) Obtained By Reaction With O-Ethyl-S-[2-[Bis(1-Methylethyl)
Amino]Ethyl] Methylphosphonothioate (Vx) conventional X-ray crystallography resolution [Å]: 2.40
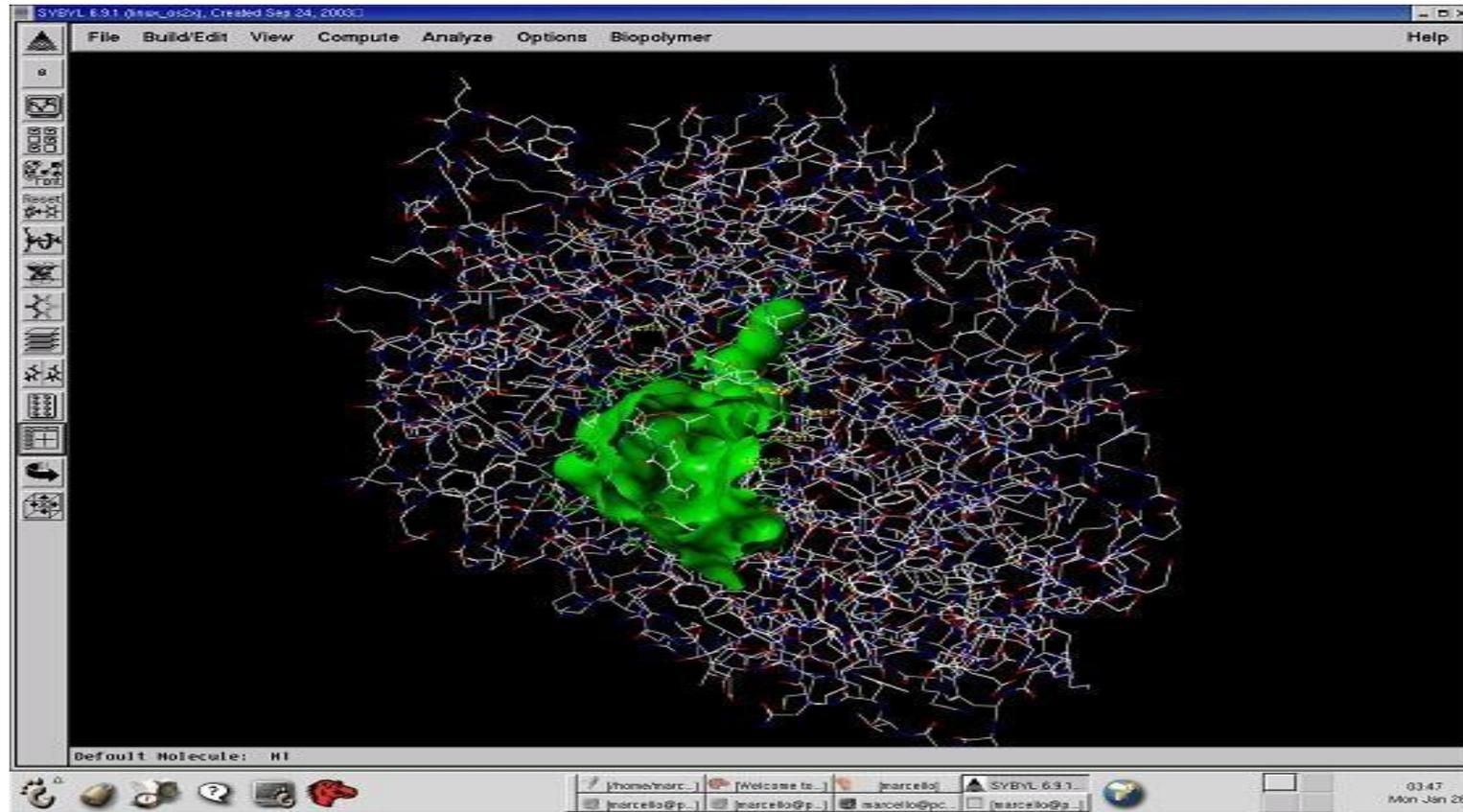
In green the molecular electrostatic potential distribution on
the surface of the enzyme binding pocket

✓Design of the oligopeptides library as possible receptors

The geometry of the binding pocket was investigated to create oligopeptides library
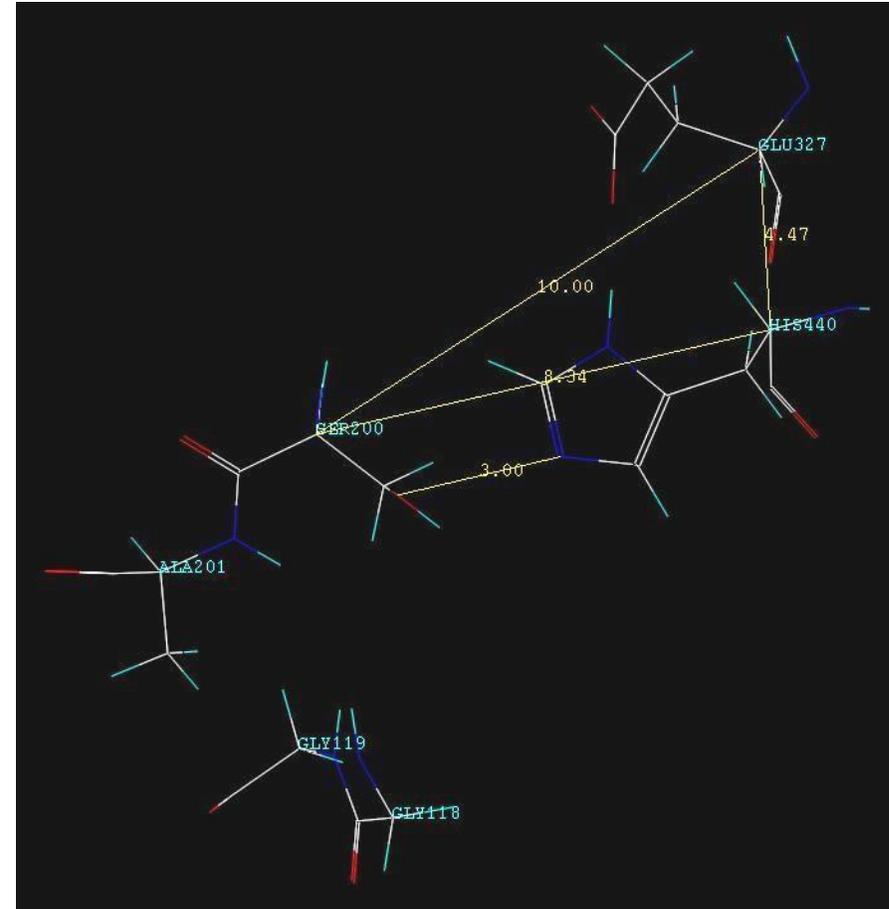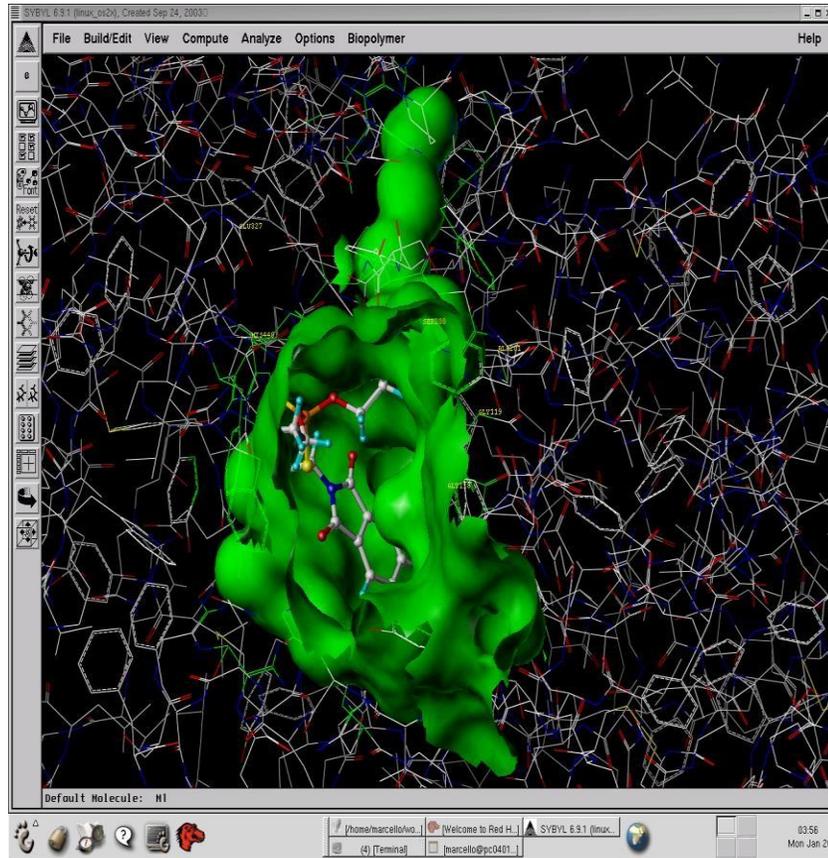
Three dimensional coordinates of the asymmetric carbon (Cα) of each aminoacid involved in the binding pocket were calculated in order to reproduce the geometry observed

✓ Tetrapeptides library

> ➤ easy to synthesise

> ➤ more possibility to preserve in solution the secondary structure predicted

•A series of tetrapeptides, containing the possible combinations of the catalytic triad (SER 200, HIS 440, GLU 327) and the catalytic oxyanion hole (GLY 118 GLY 119 ALA 201) was drawn

•The proper geometry of binding pocket was achieved using alternatively a GLY or a PRO residue

Library
(24 tetrapeptides)

Ser-Gly-His-Glu	Glu-Gly-Ser-Ala
Ser-Gly-Glu-His	His-Gly-Ser-Ala
His-Glu-Gly-Ser	Gly-Pro-Ser-Ala
Glu-His-Gly-Ser	Ser-Ala-Pro-Glu
Ser-Pro-His-Glu	Ser-Ala-Pro-His
Ser-Pro-Glu-His	Ser-Ala-Pro-Gly
His-Glu-Pro-Ser	Glu-Pro-Ser-Ala
Glu-His-Pro-Ser	His-Pro-Ser-Ala
Gly-Gly-Ser-Ala	Gly-Ser-Gly-Ala
Ser-Ala-Gly-Glu	Ala-Gly-Ser-Gly
Ser-Ala-Gly-His	Ser-Gly-Pro-Ala
Ser-Ala-Gly-Gly	Ala-Pro-Gly-Ser

✓ **Simulated binding results vs paraoxon of the tetrapeptides selected for experimental screening**

	A Ser-Ala-Gly-Glu	B His-Gly-Ser-Ala	C Glu-Pro-Ser-Ala	D His-Glu-Pro-Ser
Binding Score (KJ/mol)	38	73	21	93

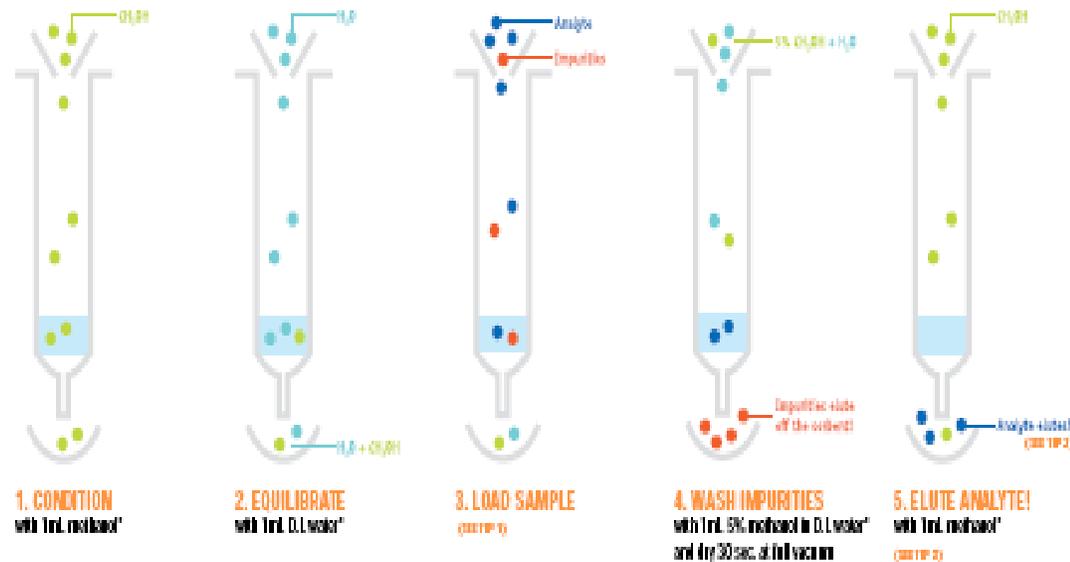Negative control (NC): Glu-His-Ser-Gly
Primary sequence of AChE catalytic triad

PARAOXON
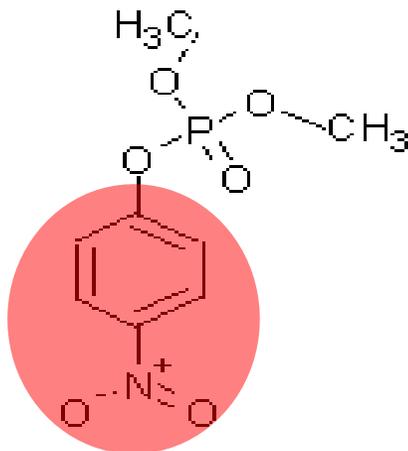
A Ser-Ala-Gly-Glu

B His-Gly-Ser-Ala

C Glu-Pro-Ser-Ala

D His-Glu-Pro-Ser

NC Glu-His-Ser-Gly

✓Pre-analytical applications: selective affinity columns   (Extraction or purification)
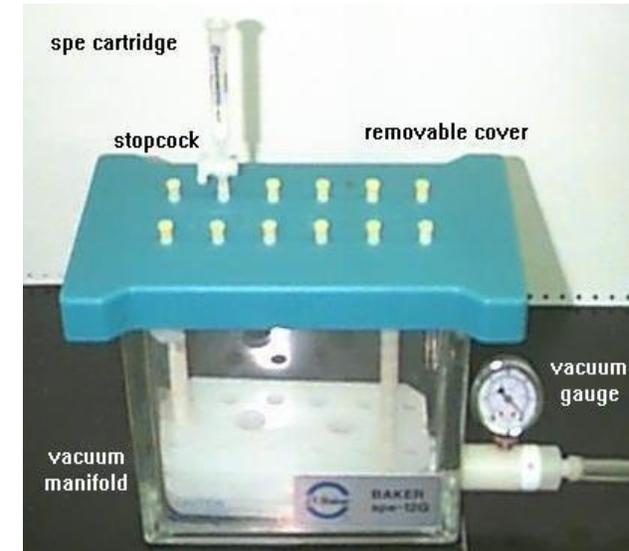
is a technique enabling purification of a biomolecule with respect to biological function or individual chemical structure. The substance to be purified is specifically and reversibly adsorbed to a ligand (binding substance), immobilized by a covalent bond to a chromatographic bed material (matrix). Samples are applied under favourable conditions for their specific binding to the ligand. Substances of interest are consequently bound to the ligand while unbound substances are washed away. Recovery of molecules of interest can be achieved by changing experimental conditions to favour desorption.

Article

Computationally Designed Peptides for Zika Virus Detection: An Incremental Construction Approach

Marcello Mascini [1,2,*] , Emre Dikici [3,4], Marta Robles Mañueco [3], Julio A. Perez-Erviti [5],
Sapna K. Deo [3,4], Dario Compagnone [2] , Joseph Wang [6], José M. Pingarrón [1]
and Sylvia Daunert [3,4,7,*]

- Zika infection is known to cause neurological problems to pregnant women and potentially cause microcephaly and other congenital malformations and diseases to the unborn child. Zika affects, both male and females and it has been reported that the virus can be transmitted sexually through semen and vaginal fluids.

- The Zika virus is a mosquito-borne flavivirus, and due to the lack of specific antibodies/binders that can be used in immunoassays for diagnosis of the disease, these immunoassays present cross-reactivity with other flaviviruses and arboviruses. It is well established that ZIKV has many common genetic sequences and protein structures with other flaviviruses, like DENV, West Nile virus or Chikungunya. This limits the use of immunoassays for the detection of human pathogens within the flavivirus genus.

- The flavivirus envelope protein is responsible for virus entry and represents a major target for neutralizing antibodies. The Zika virus structure is similar to other known flaviviruses structures except for the ~10 amino acids that surround the Asn-154 glycosylation site found in each of the 180 envelope glycoproteins that make up the icosahedral shell
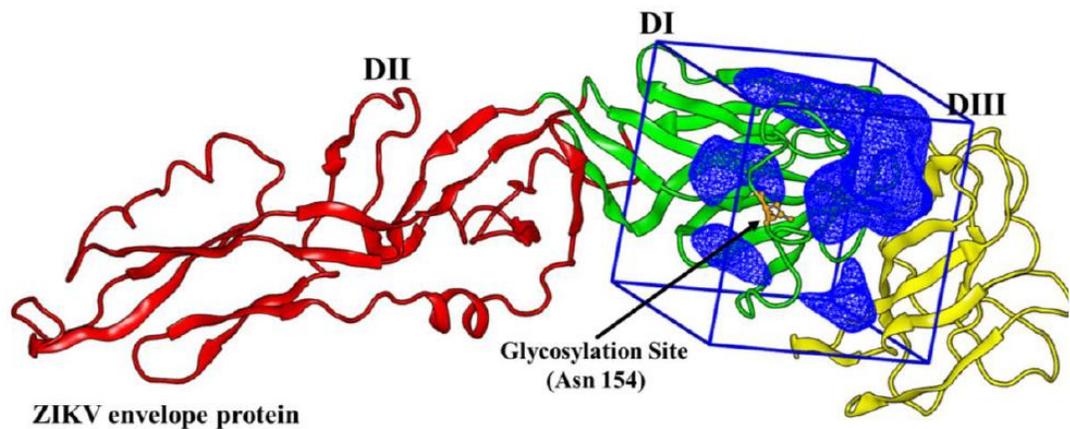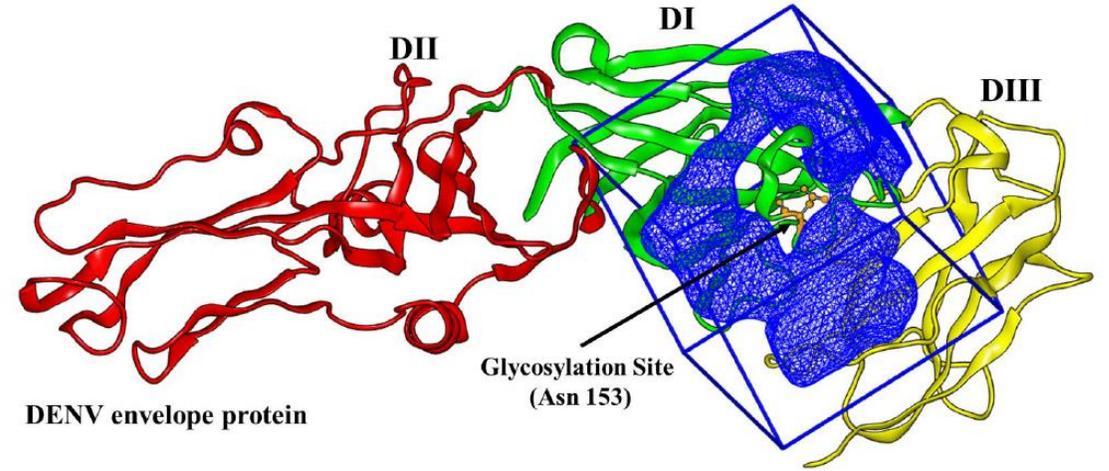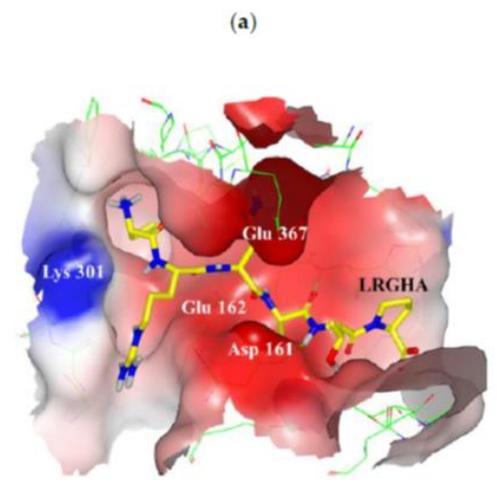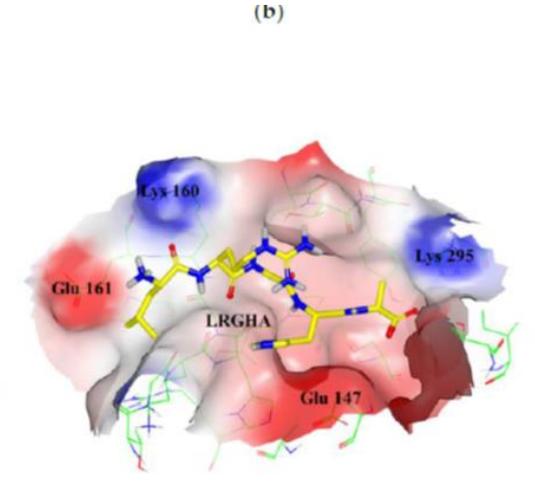
Glycosylation site
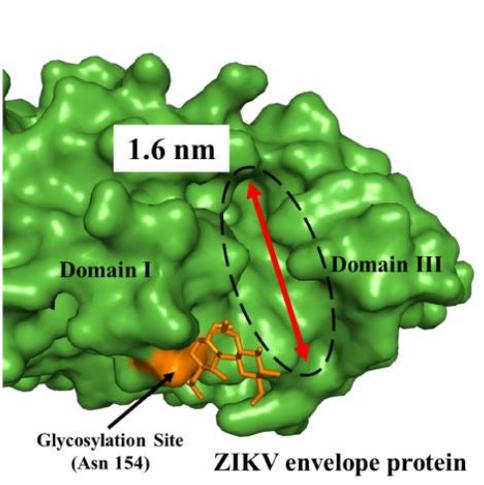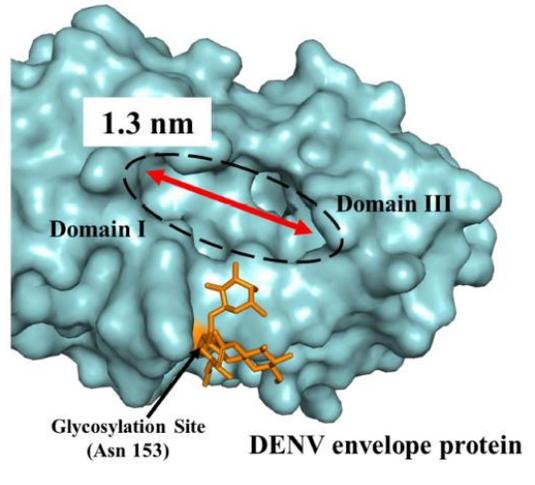
Molecular docking

Figure 4. Cont.

Figure 5. Cont.

8 different peptides selected, sinthesyzed, biotynilated and tested with direct ELISA test using Avidin-HRP

i.e. inactivated virus onto ELISA microwells , reaction with peptides, incubation with Avidin-HRP
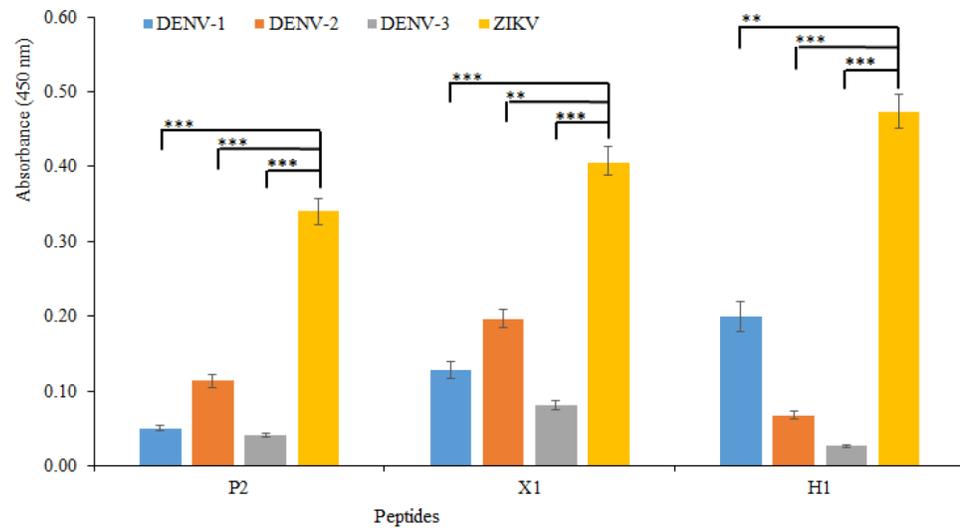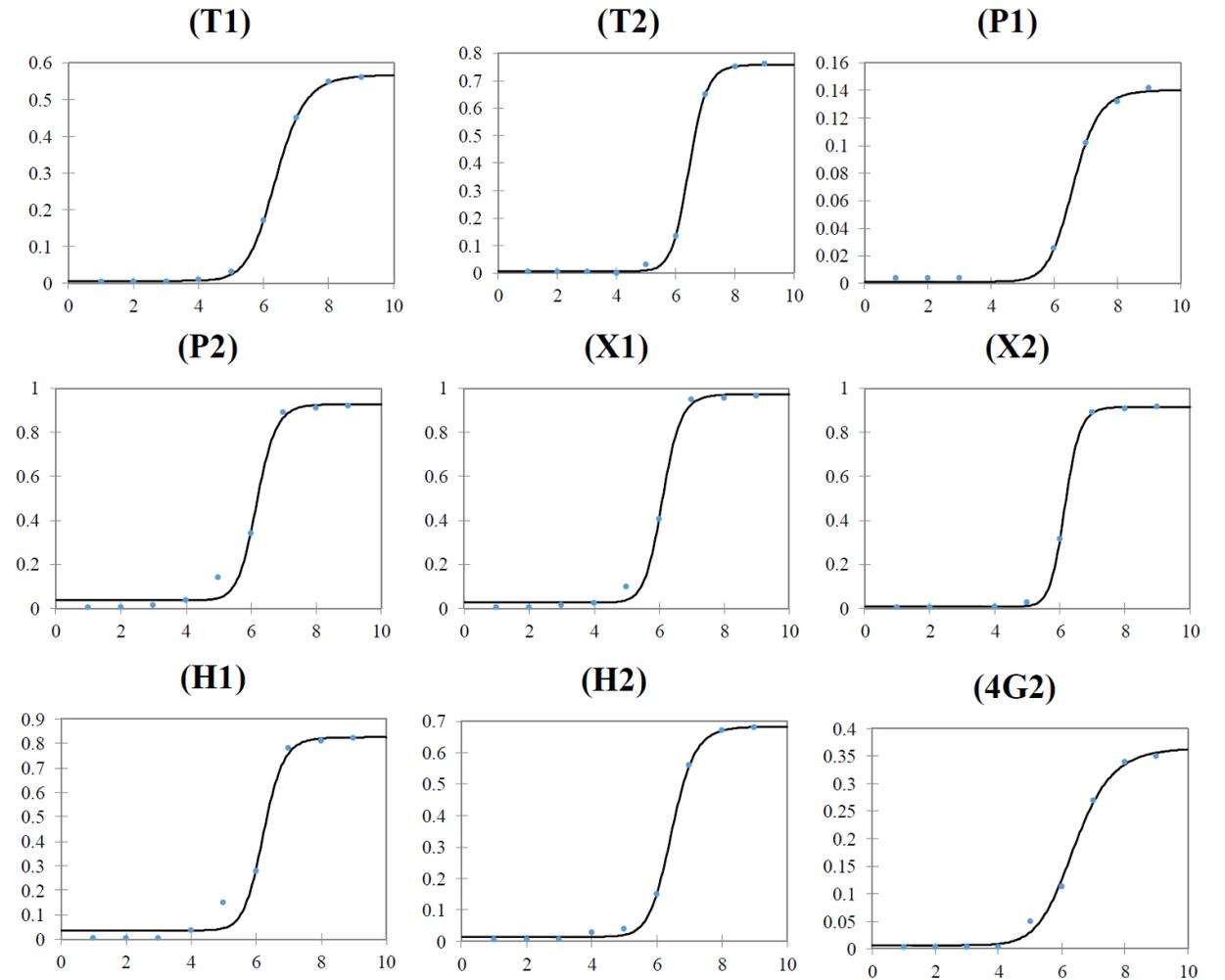
Figure 6. Sigmoidal ZIKV particles concentration response trend of the ELISA assay obtained using the eight peptides and antibody 4G2. Y-axis = absorbance (450nm); X-axis = log [ZIKV], copies/mL.

Figure 7. Cross-reactivity study. In the ELISA direct assay, the spectrophotometric absorbance signals were obtained by using the best three peptides (P2, X1, and H1) binding the ZIKV and three serotypes of DENV (DENV-1, -2, and -3) at the concentration of 10^6 copies/mL. Statistical significance between ZIKV and DENV serotypes (1–3) was calculated using two-way analysis of variance. Different p values were indicated by **($p < 10^{-3}$) or ***($p < 10^{-4}$).

Aptamers are oligonucleotides (DNA or RNA molecules) that can bind with high affinity and specificity to a wide range of target molecules (proteins, peptides, drugs, vitamins and other organic or inorganic compounds).

They were "discovered" in 1990 by the development of an in vitro selection and amplification technique, known as SELEX (Systematic Evolution of Ligands by Exponential enrichment).
(Ellington et al., **Nature** 346, 818; Tuerk and Gold, **Science** 249, 505)

Their name is derived from the Latin word "**aptus**" which means "to fit".

Protein
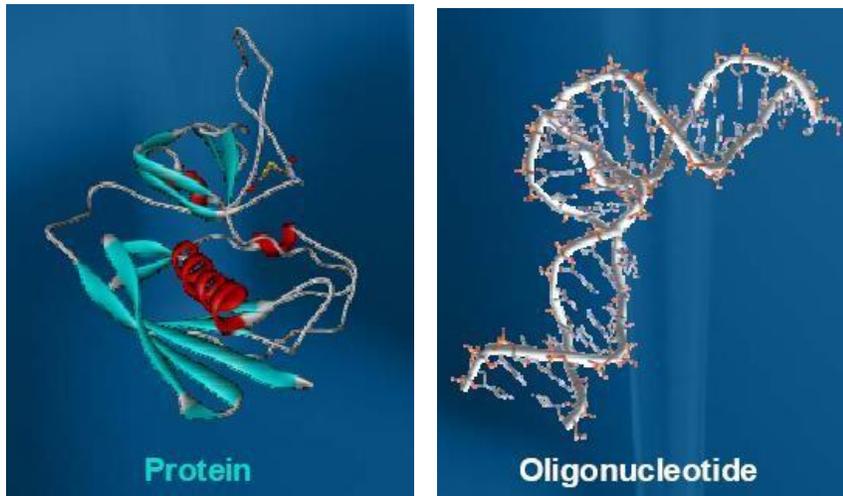
Oligonucleotide

Similar to proteins short oligonucleotides can adopt complex three-dimensional structures

Aptamer 26

Aptamer 30

Aptamer 33

Aptamer 31

T7 promoter   random sequence

5' ▮▮▮▮▮▮▮▮▮░░░░░░▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮░░░░░ 3'

constant   constant
region   region
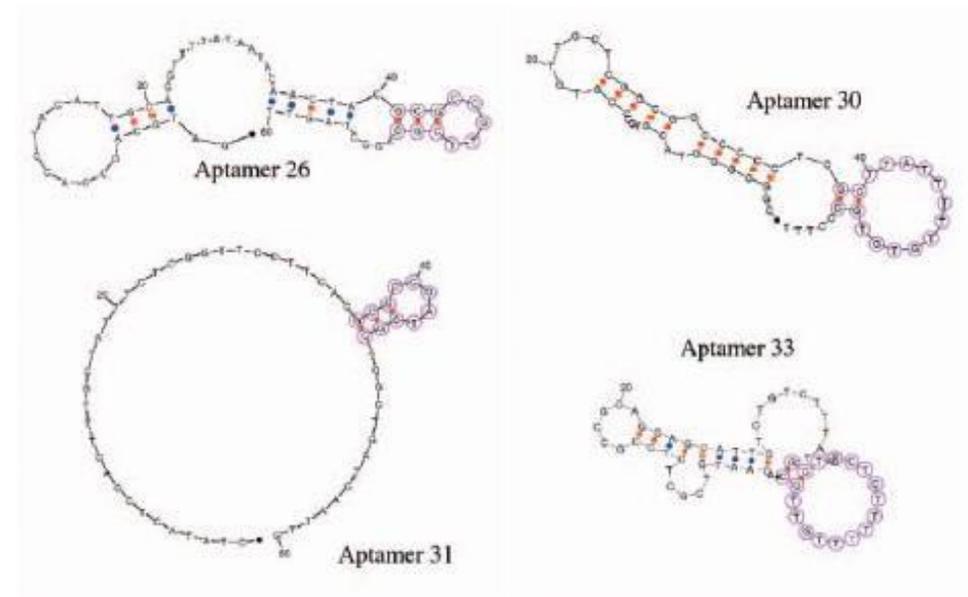
A library containing a 25-nucleotide random region is represented by 4^{25} ($\sim 10^{15}$) individual sequences available for partitioning.

Normally, the starting round contains **10^{14}-10^{15} individual sequences**.

A, **G**, **C**, **U**(**T**)

$4^1 = 4$
$4^2 = 16$
$4^3 = 64$
$4^4 = 256$
$4^5 = 1024$
....
....
....
$4^{25} = 1125899906842624$

Pool of randomized DNA or RNA

10^{15} different sequences!!!!

ELSEVIER
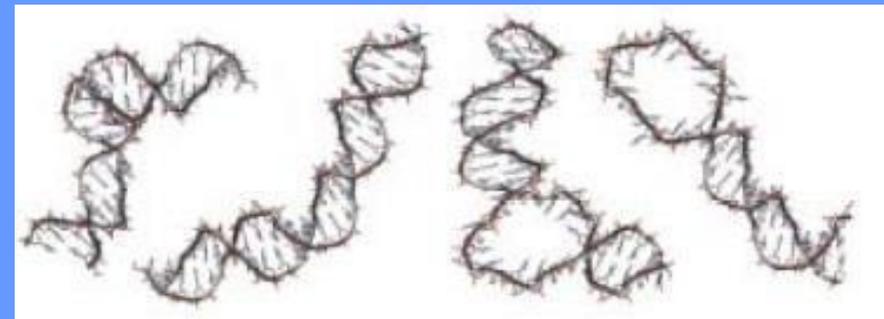
Review

Aptamers used for biosensors and targeted therapy

Yi Ning, Jue Hu, Fangguo Lu *

Department of Microbiology, The Medicine School of Hunan University of Chinese Medicine, Changsha, Hunan, 410208, PR China

A

Targeted-immobilized affinity beads

DNA/ RNA library

Washing (nonbinding)

Elution (binding)

Cloning and sequencing

TGTGGCGTGTCTCACCTGGAATCGTAGCT
AGGAATGCCAATGTTGCAAGATACGTAGC
GGAATCTCGGTATCGTTAATCGCTATCGTT

Regeneration

Unbound sequences

Bound sequences

Amplification

B

SMB Streptavidin coated Magnetic Beads

F Fluorescein

Target

Streptavidin Magnetic Beads

Combinatorial Library

N₆₀   96nt

5'-   -3'

ssDNA oligonucleotides

enriched ssDNA pool

2. - n. SELEX round

1. SELEX round

Purification of ssDNA

PAGE

Binding

dsDNA

FluMag-SELEX Process

Washing

last SELEX round

PCR Amplification

Primers

Elution Heat

Removal of unbound oligonucleotides

Cloning and Sequencing

Fig. 5. Various signals generated by aptasensor based on structure-switching designs. (A) A schematic representation of the fluorescent aptasensor for thrombin assay. Thrombin-induced structure change of the aptamer from quenching-state into G-quartet structure could lead to fluorescence enhancement. Fig. 5A adapted from ref. [100]; (B) A schematic representation of the electrochemical aptasensor for thrombin assay. Before adding the thrombin, MB covalently labeled onto aptamer could transfer electron with the electrode surface due to the flexible conformation of the aptamer. Upon adding the thrombin A, G-quaduplex structure was formed and the MB moiety was far away from the electrode surface, resulting in the electrochemical signal-off. Fig. 5B adapted from ref. [105]; (C) A schematic representation of the colorimetric aptasensor for adenosine assay. Gold nanoparticles are functionalized with aptamer. Addition of the adenosine results in nanoparticles linking together and aggregating, thus causing the change in color. Fig. 5C adapted from ref. [107]. Copyright (2007) American Chemical Society.

Plastic Antibody

Polymer   Template Analyte

Antigens

Antigen
Antigen-binding site

Natural Antibody
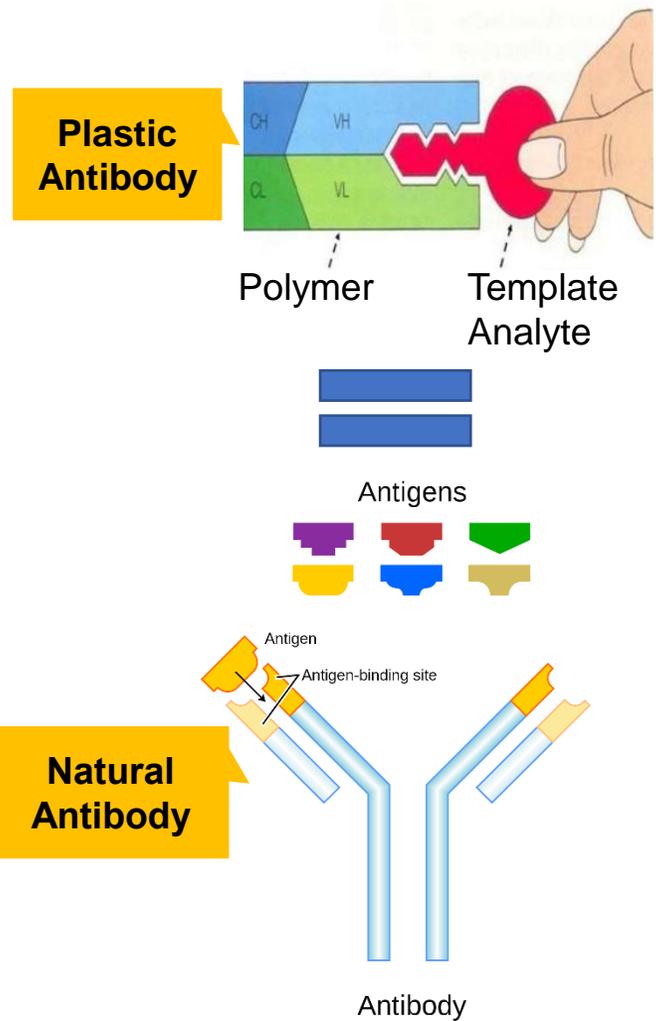
Antibody

Molecularly imprinted polymers (MIPs) are synthetic receptors for a targeted molecule. As such, they are analogues of the natural antibody–antigen systems

Crosslinking agent

Self-assembly

Polymerization

Template

Functional monomers

Removal of template

Rebinding template
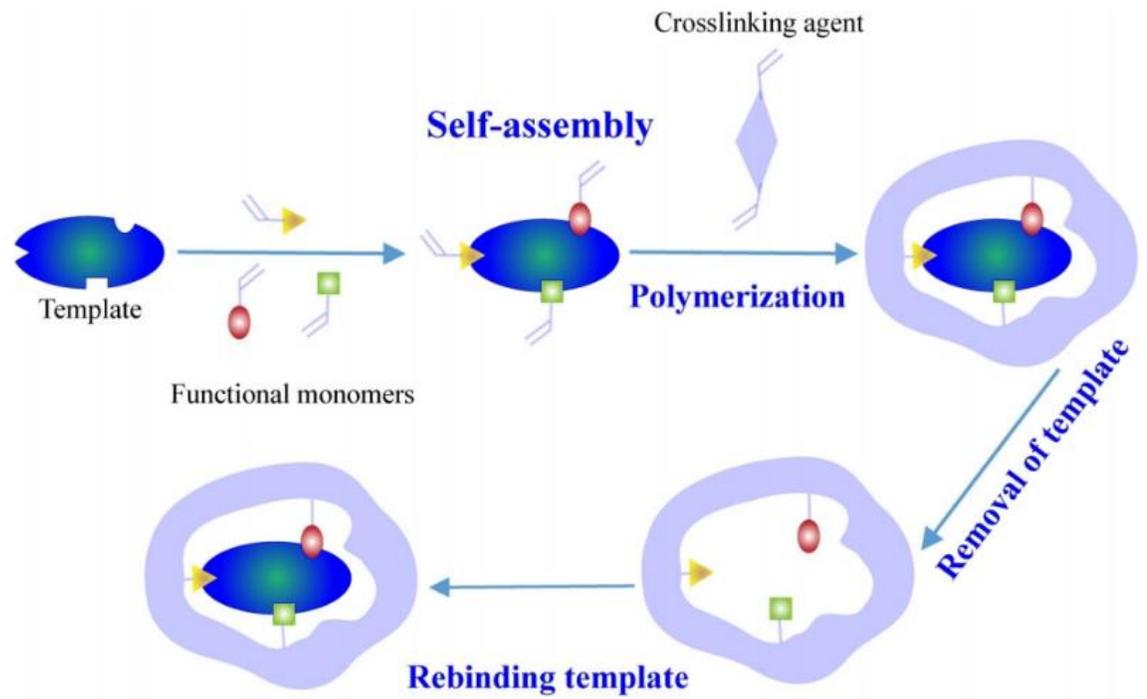
Scheme 1. Schematic representation of the synthesis of molecu-larly Imprinted Polymers (MIPs).

MIP-State of the art

MIPs Applications

MIPs are excellent materials with high selectivity and are widely used for:

Sample preparation in bio analytical methods
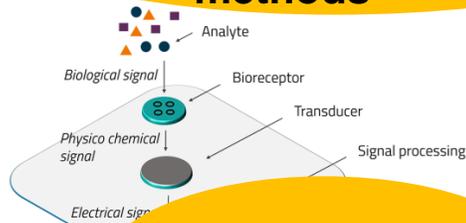
Sensors applications
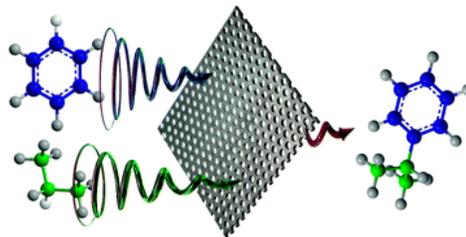
Catalysis

Chromatography
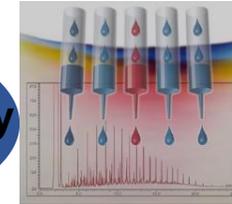
Drug delivery

Solid phase extraction
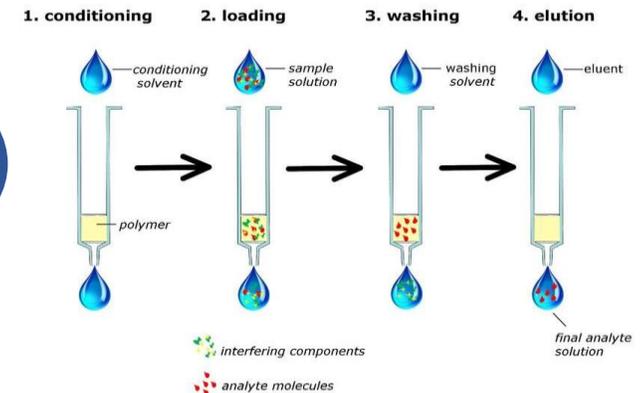
Advantages of MIPs

❖ High **selectivity** and **affinity** for the **target molecule** used in the imprinting procedure.

Compared to biological systems such as proteins and nucleic acids MIP has:

Higher physical robustness

Strength

Long storage life of the polymers

Resistance to elevated temperature and pressure

Low cost

Inertness towards acids, bases, metal ions and organic solvents

Int J Mol Sci. 2011; 12(9): 5908–5945
Chem. Rev. 2000, *100,* 2495-2504
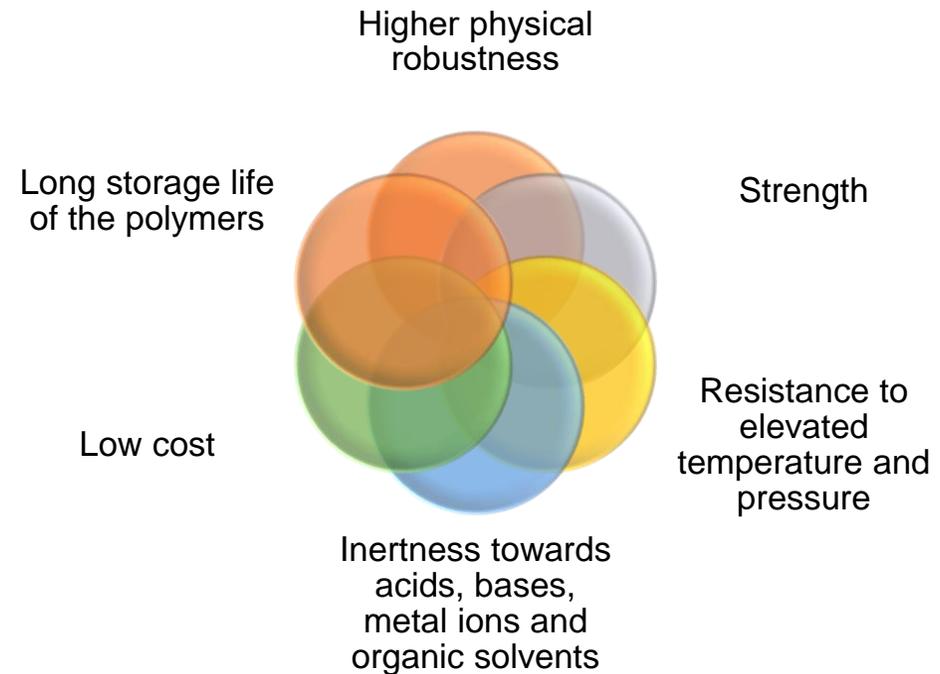
MIPs Synthesis

Components of MIP Mixture

Monomers

Polymerization

Polymer

Initiator

Porogen (Solvent)

Template (Target analyte)

Crosslinking monomer

Functional monomer

Cross-link

Polymer Chain A

Polymer Chain B

Polymer Chain C
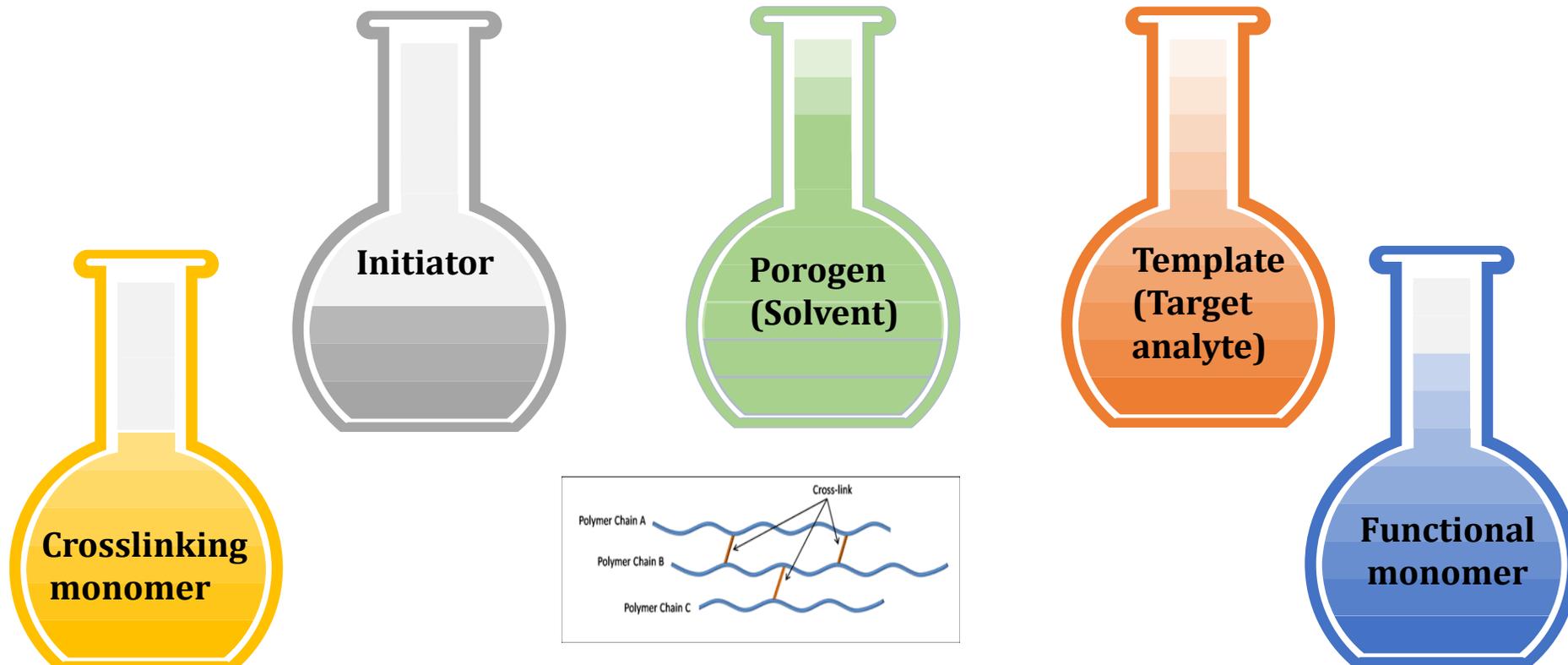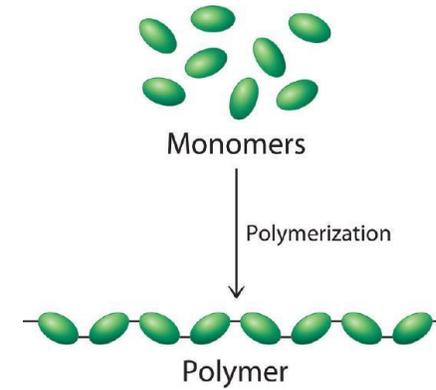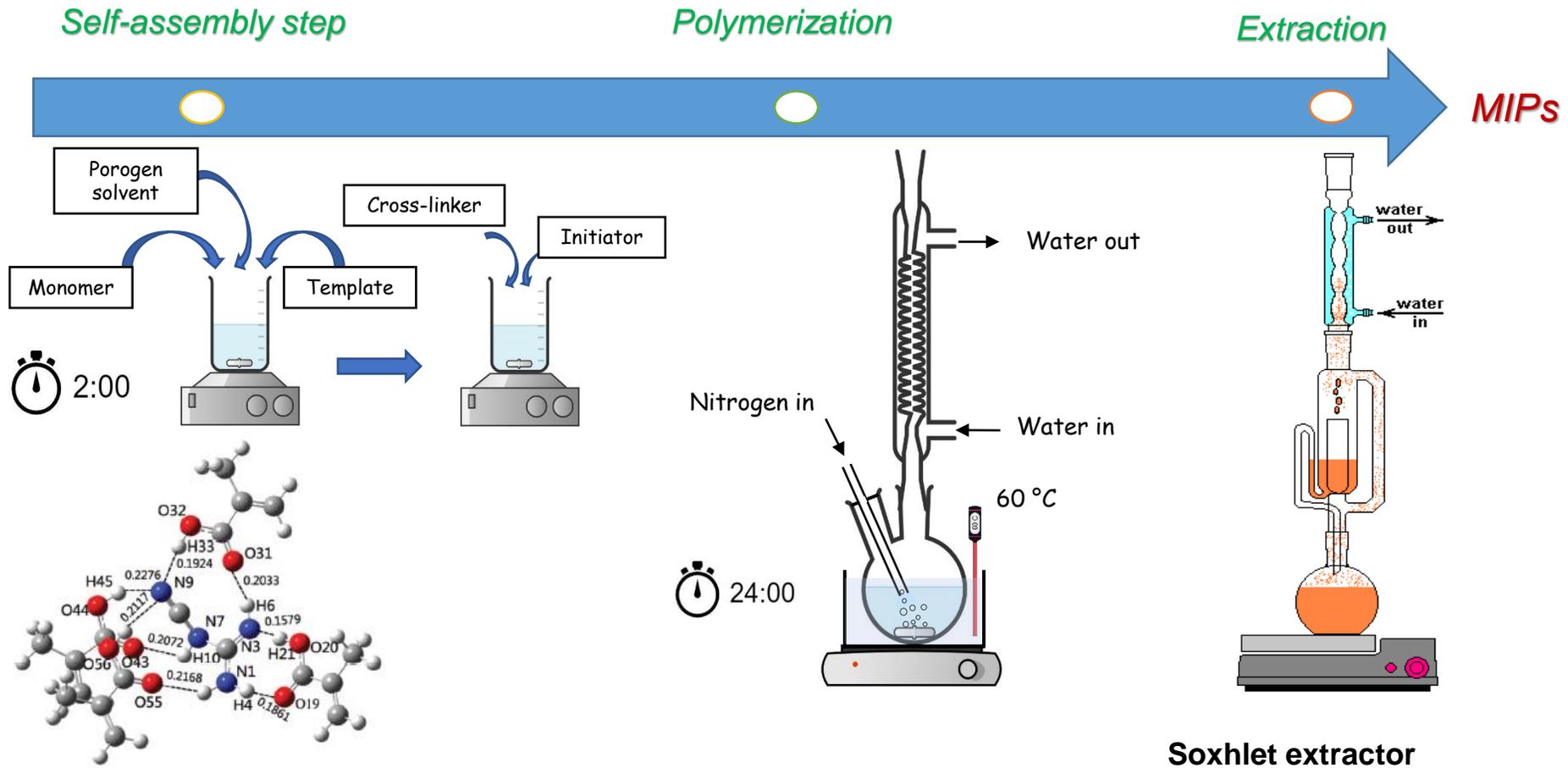
MIPs Synthesis

MIP synthesis

Sulfamethoxazole MIPs Synthesis
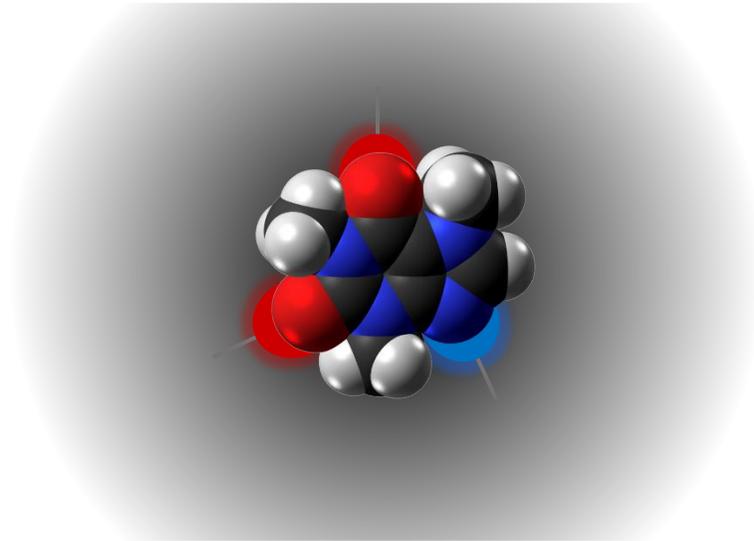
Selective rebinding

Template (Sulfamethoxasole)
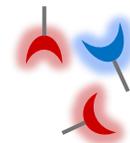
Monomer (Methacrylamide MMA)

MIPs Synthesis
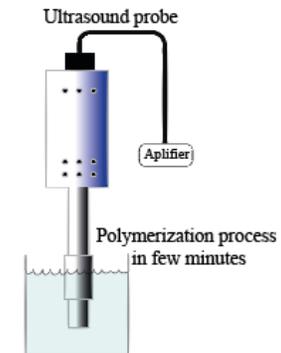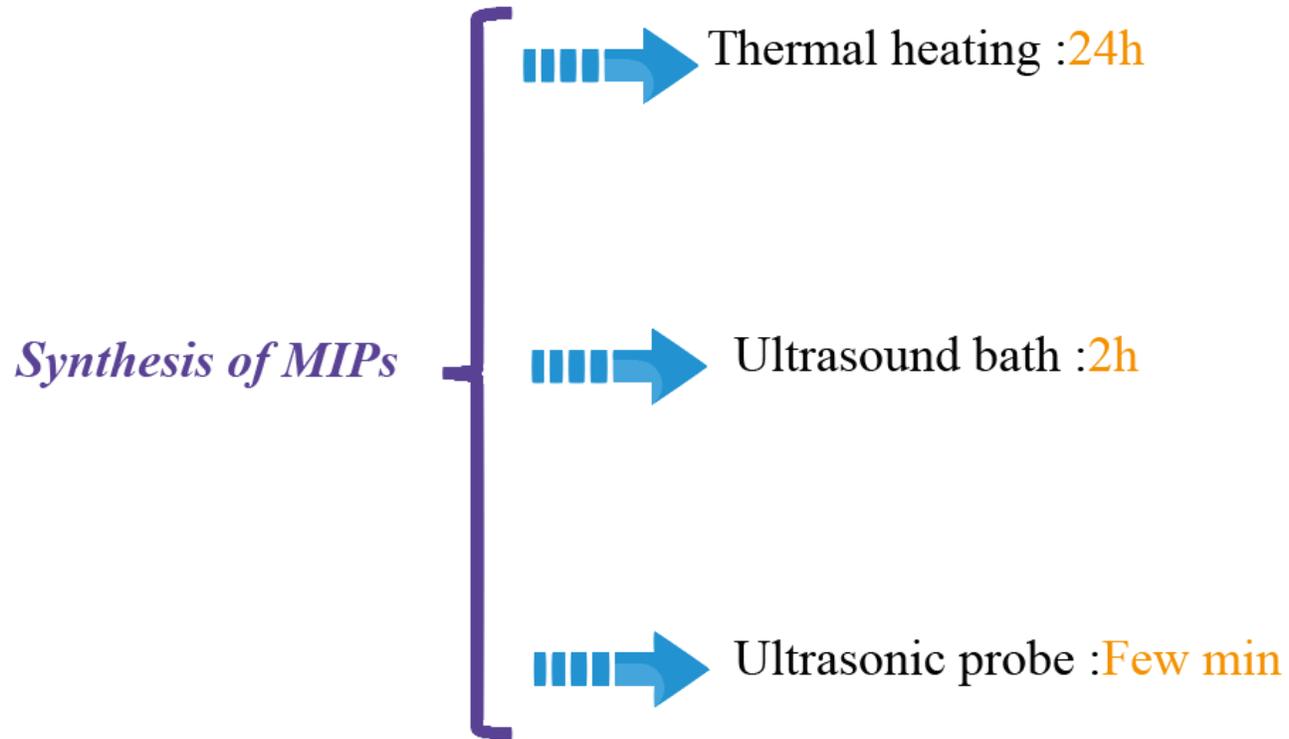
Figure : Synthesis of magnetic molecularly imprinted polymer

03 MIP-Synthesis

Theoretical optimizations prior to MIP s synthesis

Selection of the functional monomer

Prepolymer	E_Monomer (Hartree)	E Complex	ΔE (kcal/mol)
Sulfamethoxasole:SMX	-1169.32	-	
SMX-Acrylamide	-245.92	-1415.29	-31.37
SMX- 4-vinyl pyridine	-323.88	-1493.25	-31.37
SMX-Methacrylic acid	-304.788	-1474.13	-13.80
SMX-Methacrylamide	-285.03	-1454.41	-37.65

Selection of the solvent

Complexes monomer-template-solvent	E_complex (Hartree)
SMX- Methacrylamide-ETOH	-1608.60
SMX- Methacrylamide-DMSO	-2004.76
SMX- Methacrylamide-DMF	-1701.53
SMX- Methacrylamide-ACETONE	-1646.392
SMX- Methacrylamide-ACETONITRILE	-1586.45
SMX- Methacrylamide-TOLUENE	-1724.49
SMX- Methacrylamide-WATER	-1530.38
SMX- Methacrylamide-METHANOL	-1569.47

Methacrylamide -SMX have highest interaction energy in DMSO solvent due to the formation of a more stable complex.

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MIPs synthesis optimizations

Optimization of time and amplitude of synthesis was done to select the best parameters for MIP-Ultrasound probe synthesis

	Parameters	Comment	Polymer quality
MMA -MIP 22-07-2020	10 MIN /20A	Polymer was formed	++
MMA -NIP 22-07-2020	10 MIN /20A	Polymer was formed	++
MMA -MIP 23-07-2020	7. 5MIN /30A	Polymer was formed	+++
MAA-NIP 23-07-2020	7. 5MIN /30A	Polymer was formed	+++
MMA-MIP 23-07-2020	5 MIN /20A	Polymer was formed	++++
MMA-NIP 23-07-2020	5 MIN /20A	Polymer was formed	++++

5 min as time of synthesis and 20 as pulse amplitude was selected

Graph of the un-retained template
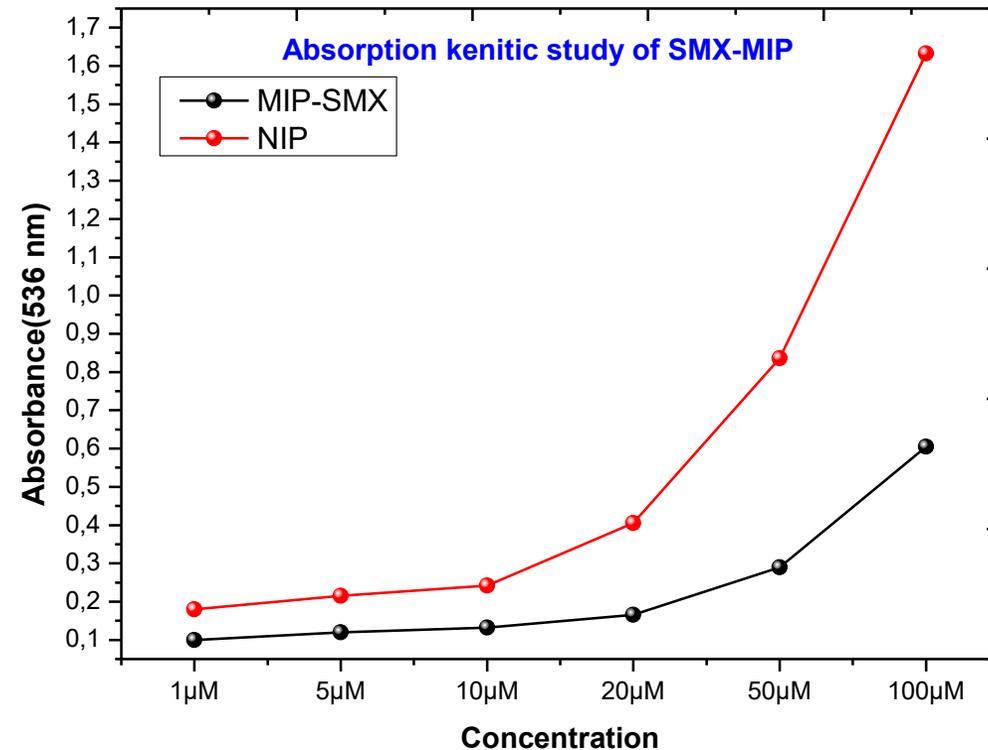
MIP has higher capacity to capture the template compared to non imprinted polymer

MIP based electrochemical sensors and nanomaterials
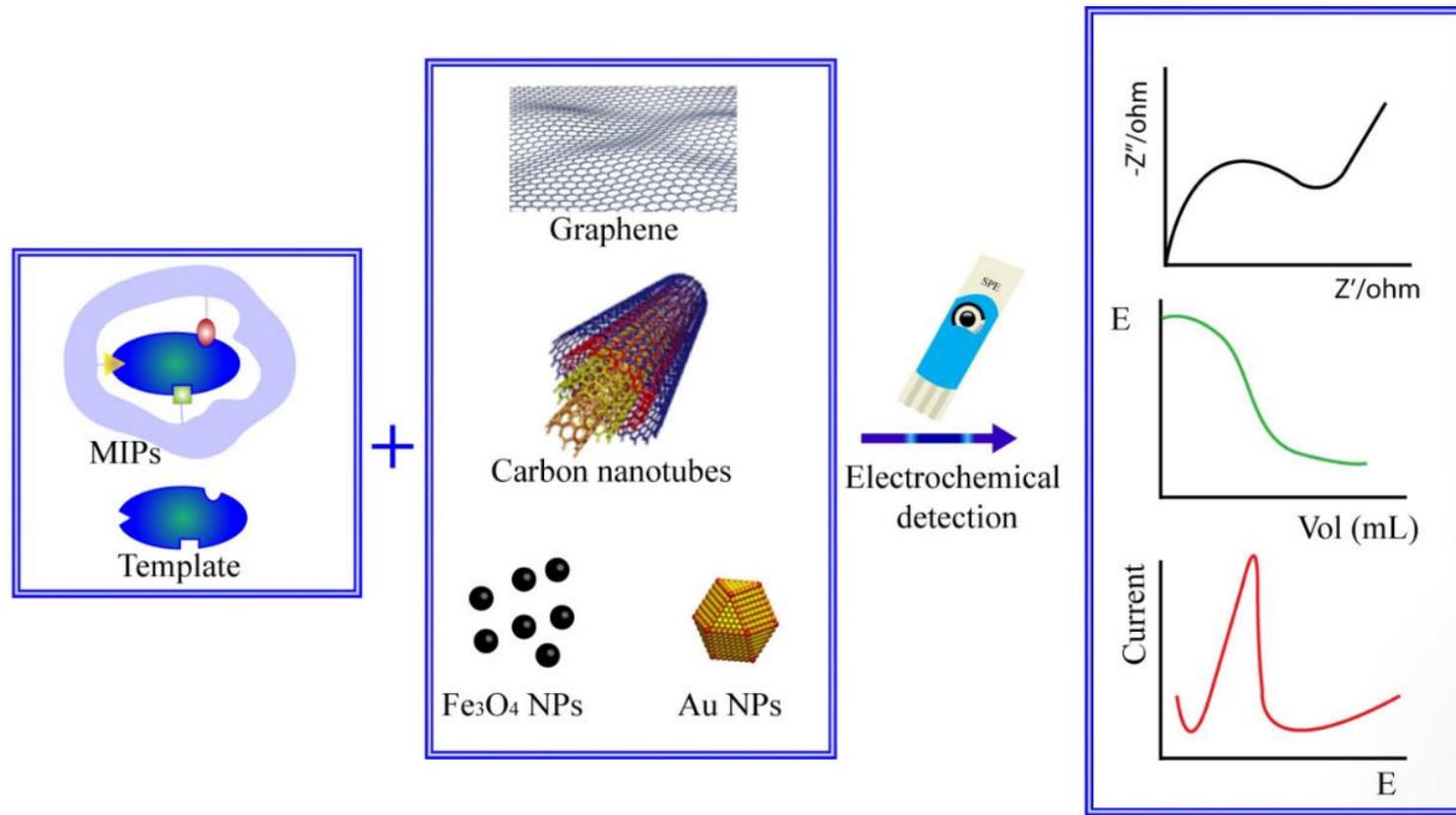
Scheme of MIP based electrochemical sensors and nanomaterials.

Abdellatif Ait Lahcen[a] and Aziz Amine[a],2018*

MIP based electrochemical sensors and nanomaterials
Electrosynthesis of MIPs

Modification with nanomaterials

Electrosynthesis of MIP

Desorption

Adsorption

- Recognition site
- Targeted analyte (template)
- Nanomaterials
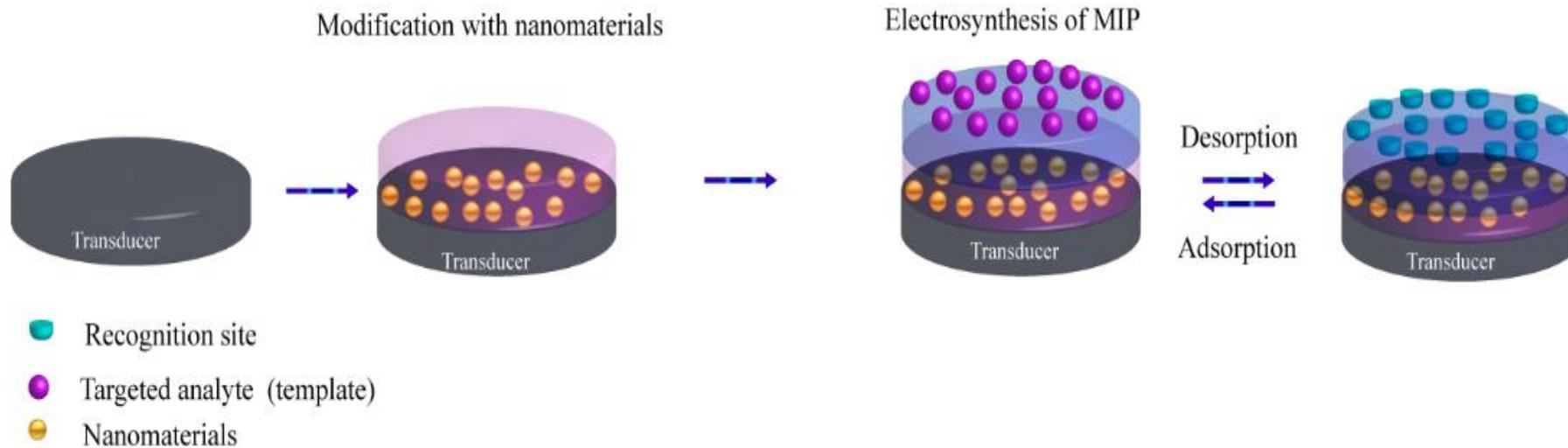
Initiator

Crosslinking monomer

Template (Target analyte)

Porogen (Solvent) Buffer

Functional monomer

MIP based electrochemical sensors and nanomaterials

Target — Template

Electrochemical behaviour — Non-electroactive — Electroactive

Detection — Voltametric measurement of redox marker ferricyanide — Impedance spectroscopy — Direct electrochemical detection

MIP-MEPS based sensing strategy for the selective assay of dimethoate. Application to wheat flour samples

MIP-MEPS based sensing strategy for the selective assay of dimethoate. Application to wheat flour samples

MIP-MEPS based sensing strategy for the selective assay of dimethoate. Application to wheat flour samples

ΔIpa (%)	Repeatability (RSD %)	Reproducibility (RSD %)
0.5 nM dimethoate (n=3)	0.68	2.72
1 nM dimethoate (n=3)	0.95	5.51

ΔIpa (%) for malathion, parathion and paraoxon after the rebinding step was negligible; **omethoate** gave a response of **23%**.
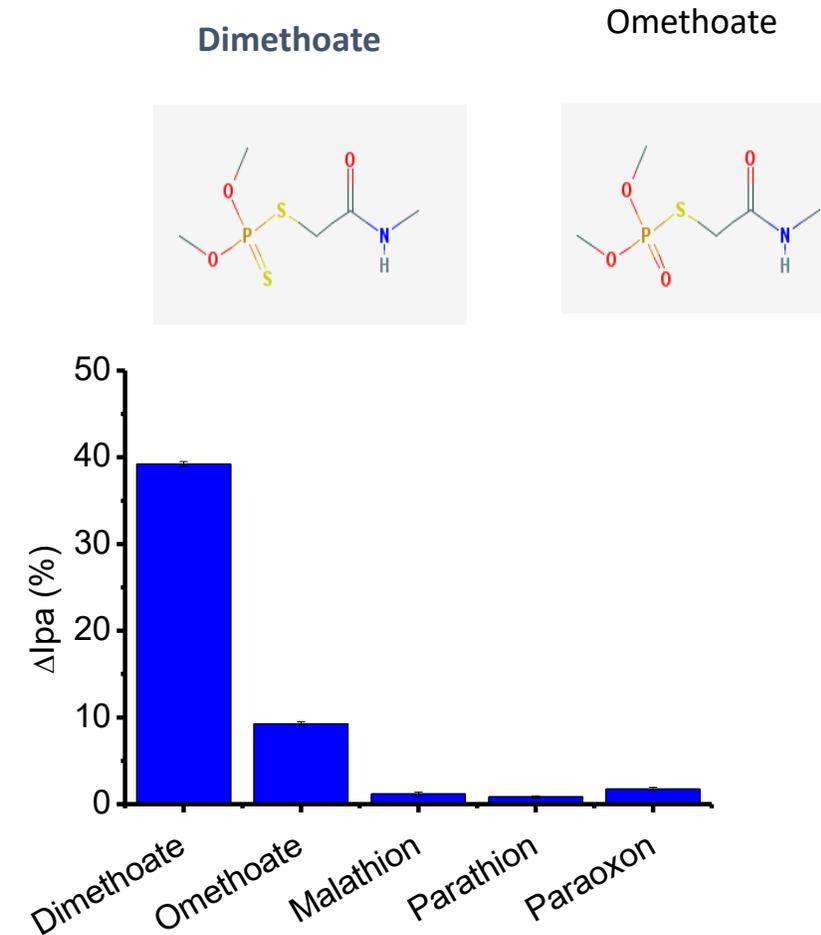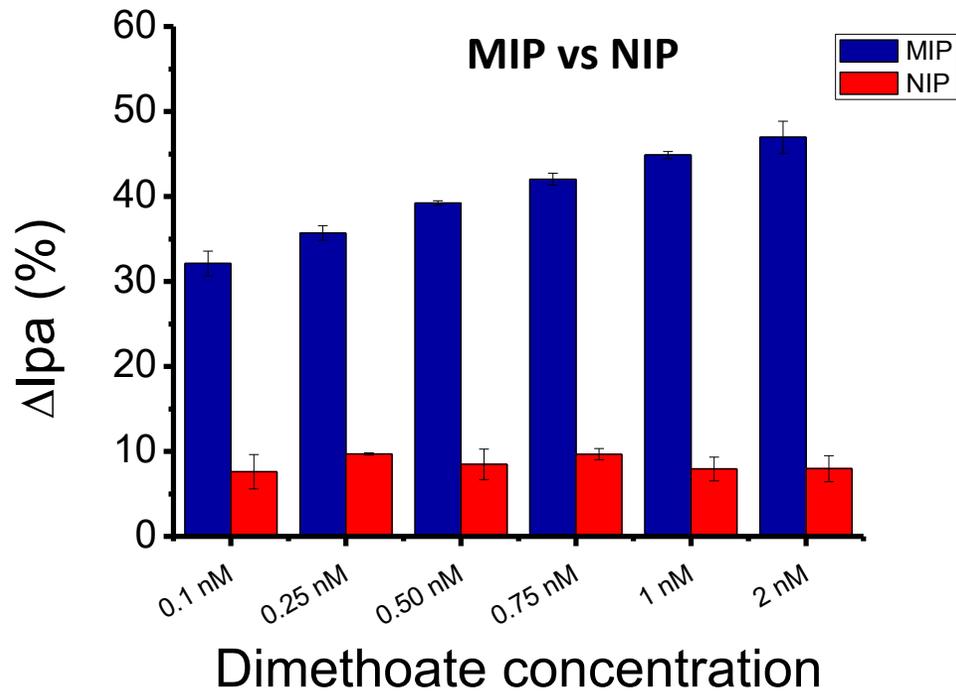
MIP-MEPS based sensing strategy for the selective assay of dimethoate. Application to wheat flour samples

Wheat flour samples: MIP vs. UHPLC-MS/MS

Wheat flour samples → MEPS (HDVB) → MIP-GCE detection
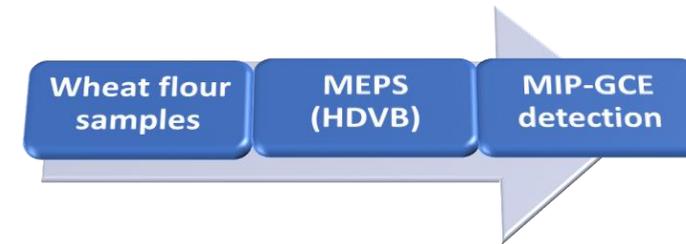
Samples	MIP-GCE RELATIVE ERROR (%) of dimethoate concentration (µg kg^{-1})	MIP-GCE SD of dimethoate concentration (µg kg^{-1})
Wheat flour spiked with dimethoate 0.5 MRL	+13.5	0.52
Wheat flour spiked with dimethoate 0.5 MRL + mix	+4.6	2.37
Wheat flour spiked with dimethoate MRL	-21.1	1.24
Wheat flour spiked with dimethoate MRL + mix	-21.2	1.36
Wheat flour spiked with dimethoate 1.5 MRL	+16.7	0.74
Wheat flour spiked with dimethoate 1.5 MRL + mix	-0.4	1.69
Wheat flour spiked with dimethoate MRL + omethoate (1:1)	+3.5	2.70
Wheat flour spiked with dimethoate MRL + omethoate (1:10)	-15.5	0.86

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Chlorpyriphos

Electrochromic Molecular Imprinting Sensor for Visual and Smartphone-Based Detections

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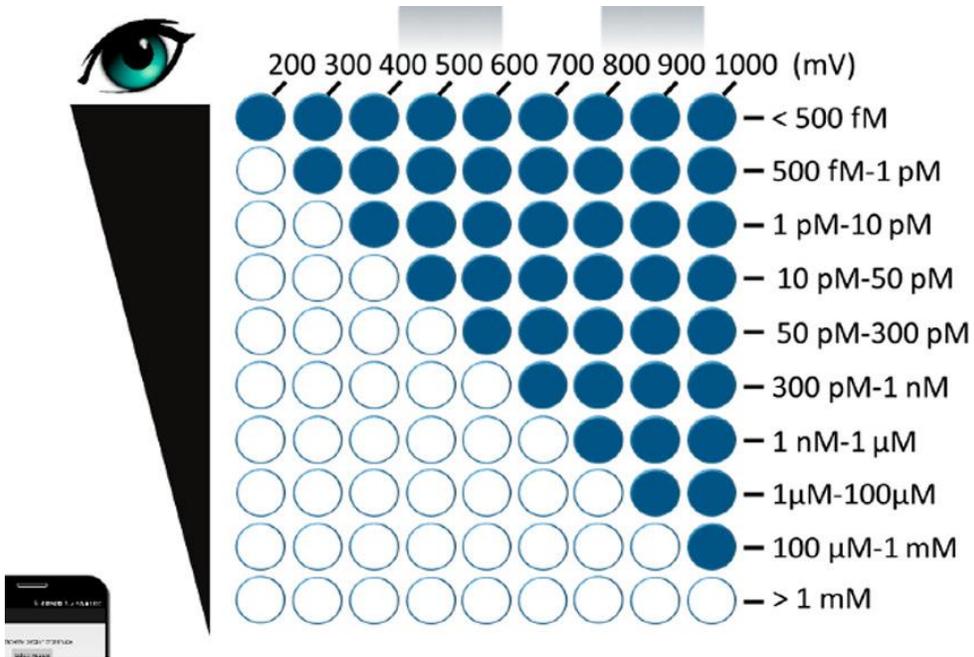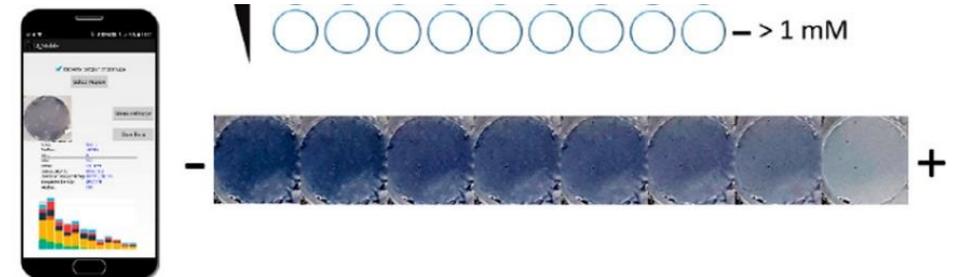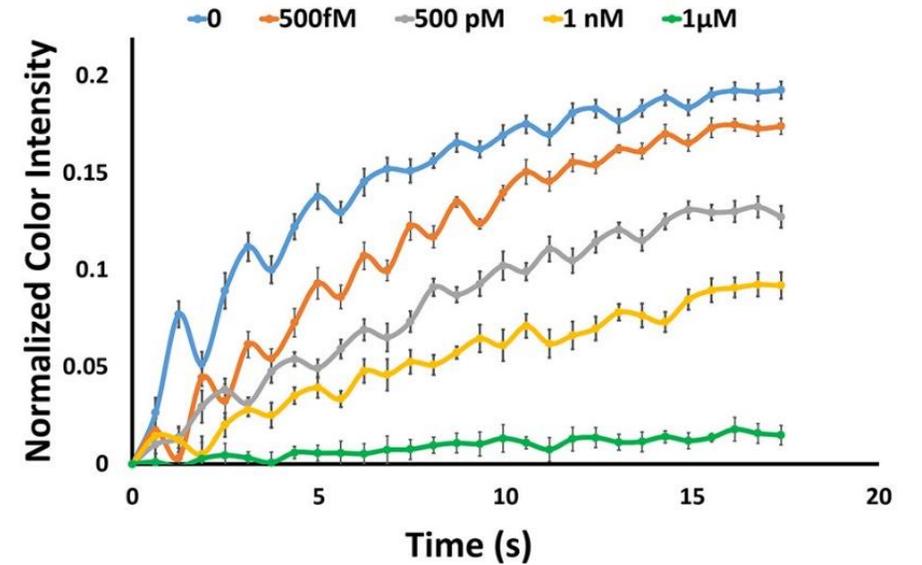
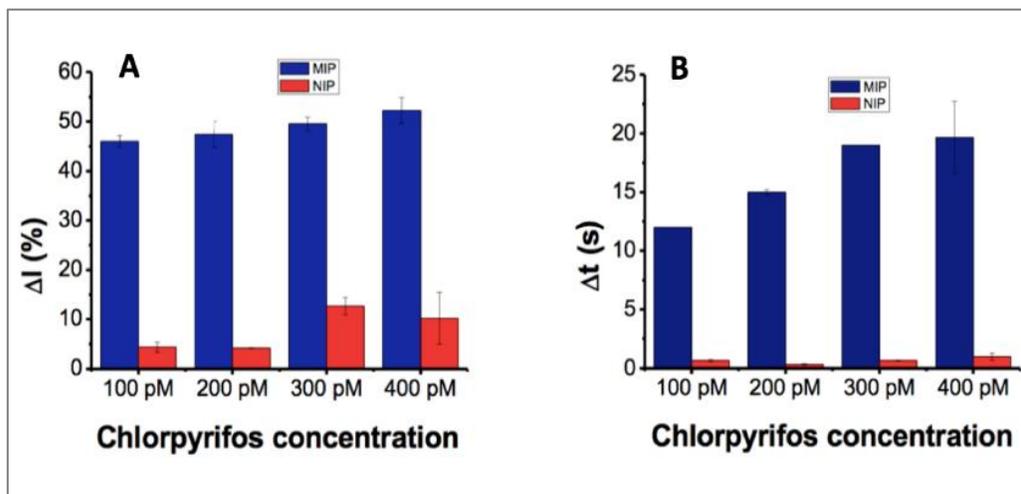# Electrochromic Molecular Imprinting Sensor for Visual and Smartphone-Based Detections

MIP vs NIP

SELECTIVITY (500 mV-1000 mV)

Recovery values of chlorpyrifos in spiked drinking water samples (n = 3) using the current response

Added (Spiked)	Found	Recovery (%)	RSD (%)
500 fM	517.19 fM	103.44 ± 16.14	15.60
500 pM	471.45 pM	94.29 ± 17.92	19.00
1 nM	0.99 nM	99.50 ± 19.90	20.00
1 μM	0.98 μM	97.55 ± 25.87	26.52
1 mM	1.07 mM	106.57 ± 15.30	14.36