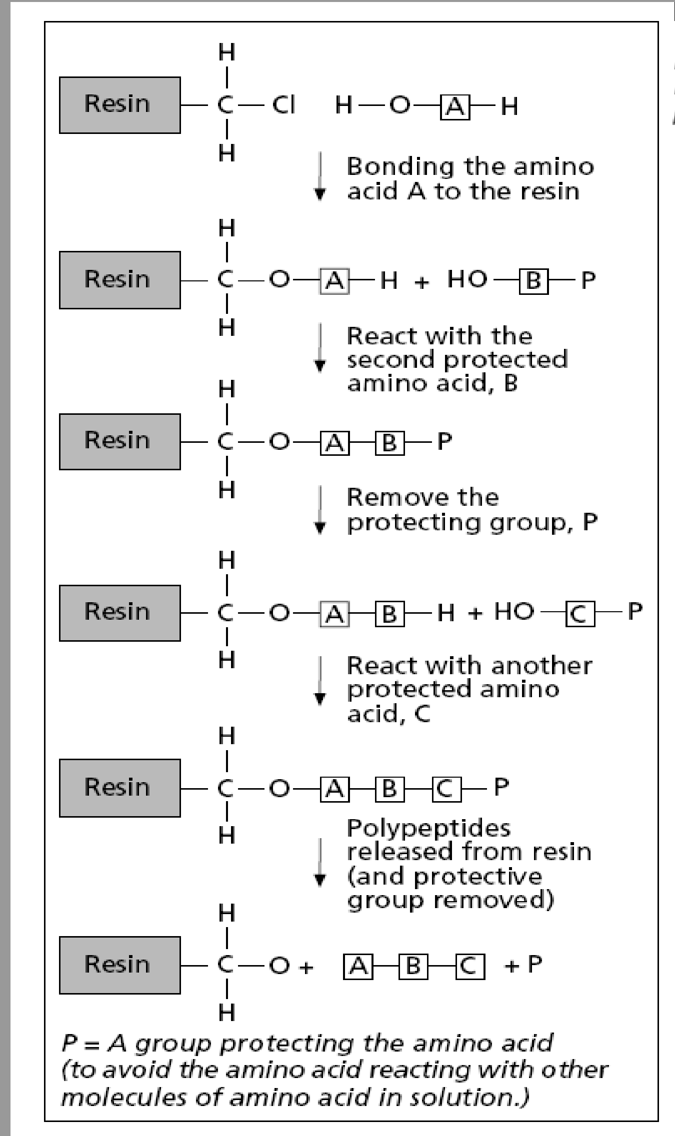
Recettori Biomimetici

Chimica combinatoriale

Studi di dinamica molecolare

- MIP (polimeri a stampo molecolare)
- Peptidi
- Aptameri

Sintesi di aminoacidi via split and mix su resina

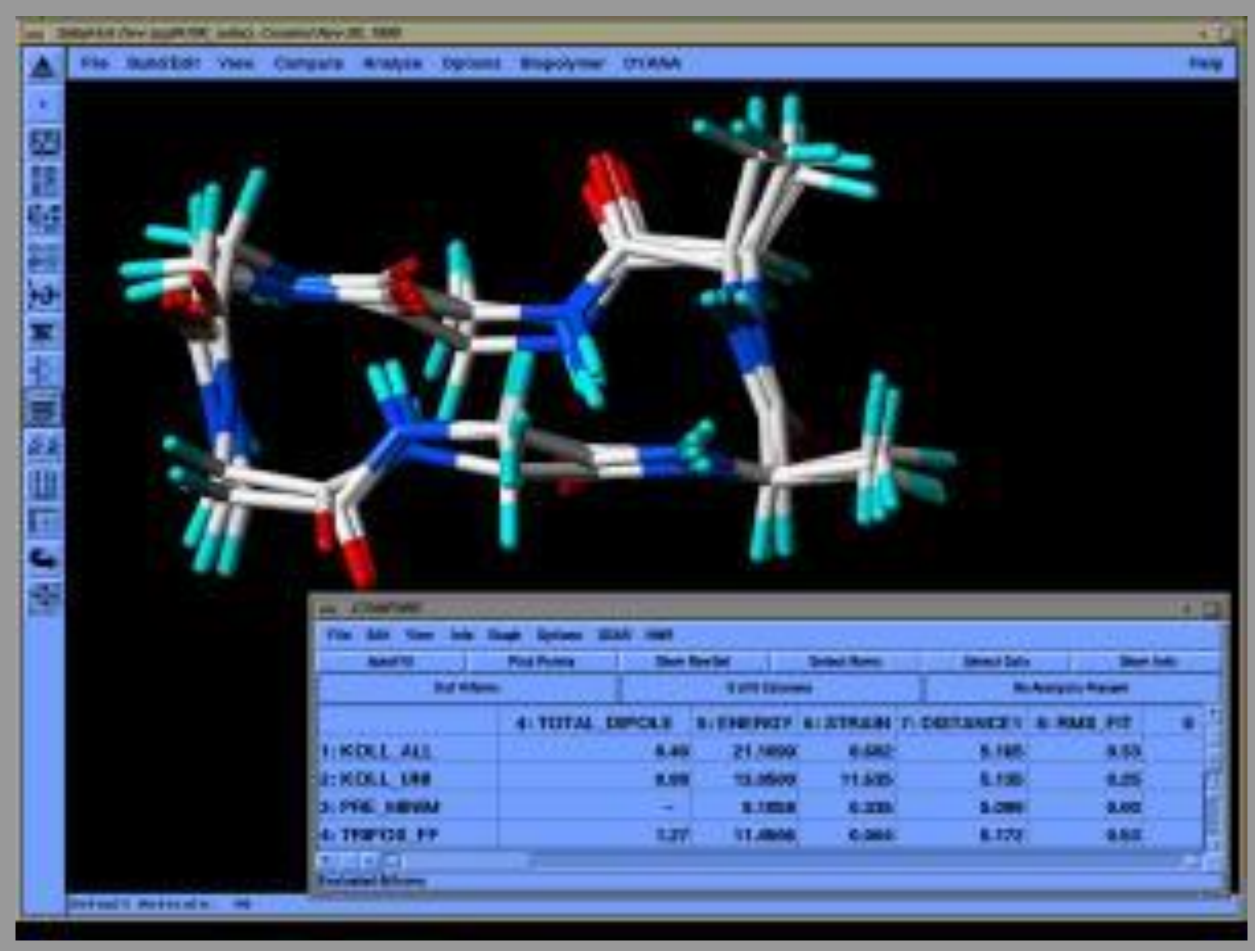


Split synthesis

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

Dinamica molecolare

Creazione di ligandi sulla base di informazioni presenti in database, p. es. strutture cristallografiche, sequenze primarie etc.



Biomimetic Approach

- Starting from the biological structure we thought to reproduce with natural amino acids the proper shape of binding dock

- Our 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

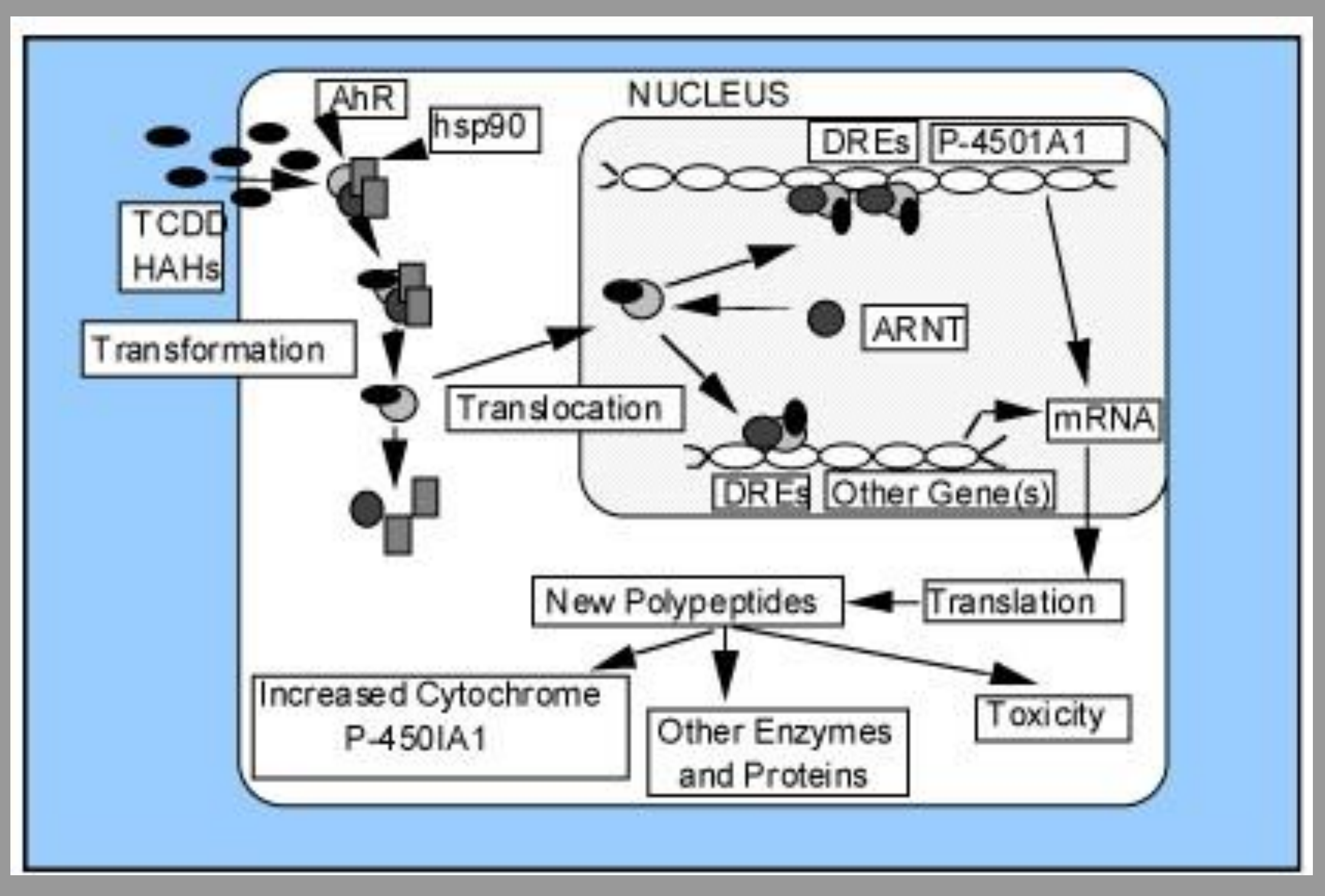
DIOSSINE E COMPOSTI DIOSSINO-SIMILI

Le diossine (TCDDs), i furani (TCDFs) e i policlorobifenili (PCBs), composti diossino-simili, fanno parte di un gruppo di composti chimici noti come Persistent Organic Pollutants (POPs). Grazie all'elevata lipofilicità hanno la capacità di bioaccumalarsi con conseguenze per la salute umana.
La catena alimentare rappresenta la principale fonte di esposizione per l'uomo a tali contaminanti.

DIOSSINE **FURANI**

Attualmente, l'unico metodo accettato per determinare la concentrazione di diossine nei campioni alimentari è la gas-cromatografia ad alta risoluzione accoppiata alla spettrometria di massa (GC-HRMS)

Interaction Dioxin-Ah receptor

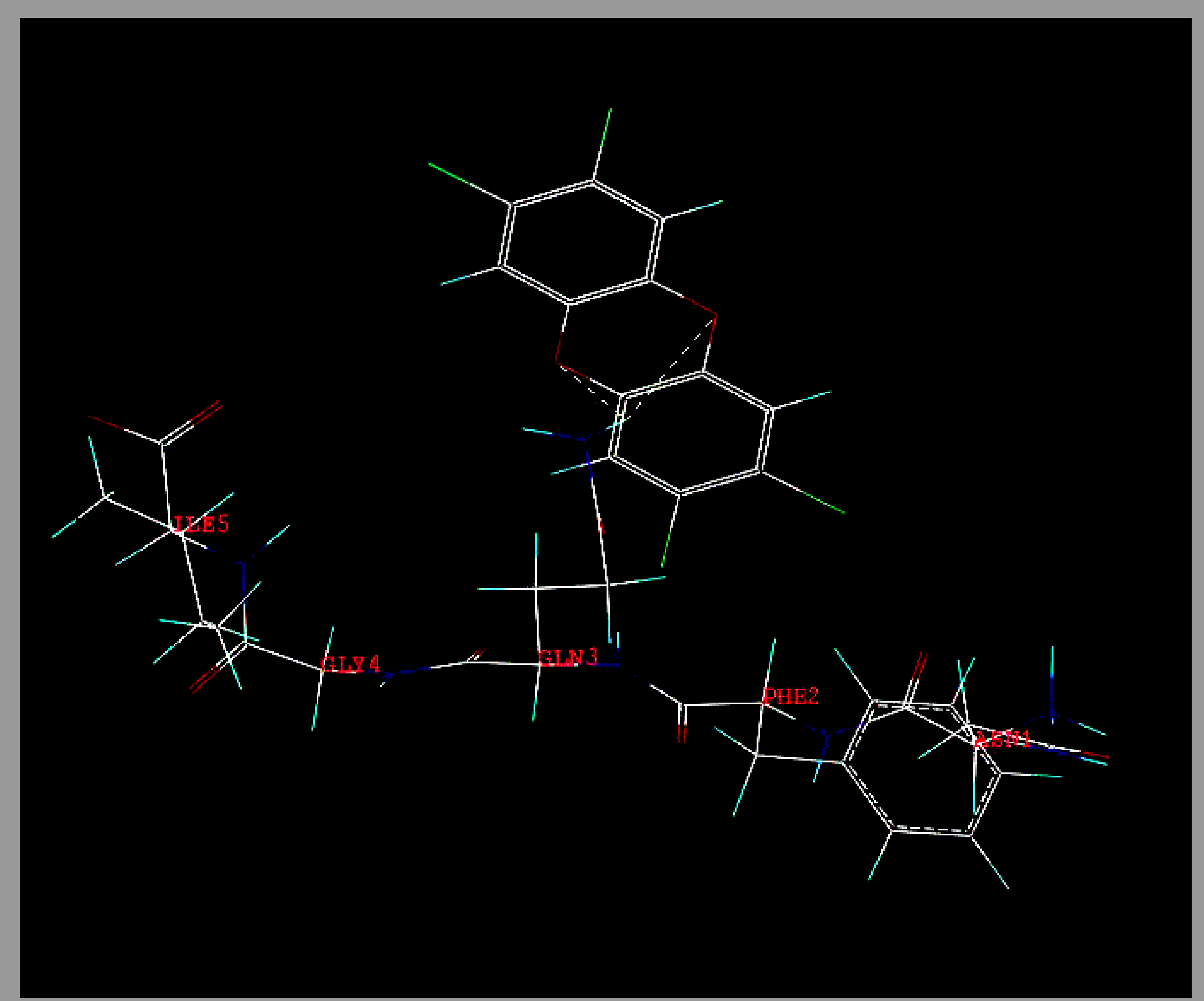


Leaprfrog® algorithm results using pentapeptides library versus TCDDs.

PEPTIDES	2,3,7,8-TCDD binding score [kJ/mol]	number of hydrogen bonds	Type of Nitro-group bonded with the bridge hydrogen
*A) [N] Asn-Phe-**Gln**-Gly-Ile [C]*	*-127.8*	*2*	*$CONH_2$*
*B) [N] Asn-Phe-**Gln-Gly**-Gln [C]*	*-119.7*	*2*	*CONHR*
[N] Asn-**Phe-Gln**-Gly-Asn [C]	-114.3	1	**CON**HR
[N] **Gln**-Phe-**Gln**-Gly-Arg [C]	-102.6	2	$CONH_2$/ $CONH_2$
[N] Phe-**Phe-Gln**-Gly-Arg [C]	-97.6	1	CONHR
[N] Asp-Phe-**Gln**-Gly-**Arg** [C]	-97.3	2	$CONH_2$ / NH_2
[N] Arg-Phe-Gln-Gly-**Arg** [C]	-90.4	2	NH_2/NH_2
[N] **Asn**-Phe-Gln-Gly-Asp [C]	-83.7	1	NH_3
[N] Asn-Phe-**Gln-Gly**-Arg [C]	-83.5	1	CONHR
*C) [N] Asn-Phe-**Gln-Gly**-Phe [C]*	*-80.8*	*1*	*CONHR*
[N] **Ile-Phe**-Gln-Gly-Arg [C]	-66.1	1	CONHR
PEPTIDES	**1,4,6,9-TCDD binding score [kJ/mol]**	**number of hydrogen bonds**	**Type of Nitro-group bonded with the bridge hydrogen**
*B) [N] Asn-Phe-**Gln-Gly**-Gln [C]*	*-122.80*	*1*	*$CONH_2$*
[N] Asn-Phe-**Gln**-Gly-Asn [C]	-116.02	1	NH_2
[N] Ggnl-Phe**Gln**-Gly-Arg [C]	-113.85	2	NH_3
[N] Phe-Phe-**Gln**-Gly-Arg [C]	-111.17	2	$CONH_2$
[N] **Asp**-Phe-Gln-Gly-Arg [C]	-110.46	1	$CONH_2$
*A) [N] Asn-Phe-**Gln**-Gly-Ile [C]*	*-95.48*	*2*	*$CONH_2$*
[N] Arg-Phe-**Gln**-Gly-Arg [C]	-93.72	3	$CONH_2$
[N] **Asn**-Phe-Gln-Gly-Asp [C]	-92.97	2	$CONH_2$
*C) [N] **Asn**-Phe-Gln-Gly-Phe [C]*	*-92.88*	*1*	*NH_3*
[N] Asn-Phe-**Gln**-Gly-Arg [C]	-83.68	1	NH_3
[N] Ile-Phe-Gln-Gly-**Arg** [C]	-66.23	1	CONHR

The aminoacids involved in hydrogen bonds with dioxin are reported in bold.
The peptides selected for the experiments are highlighted. The Kobayashi's backbone is underlined (in red).

Interaction between Asn-Phe-Gln-Gly-Ile and 2,3,7,8-TCDD through a double hydrogen bond between dioxin's oxygen and the hydrogen bonded with the N amide group from Gln3.



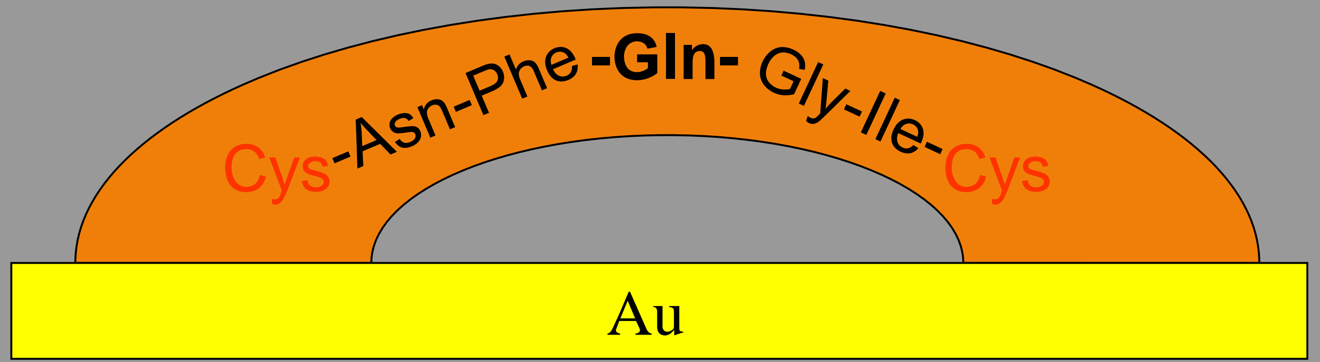
Immobilization

A

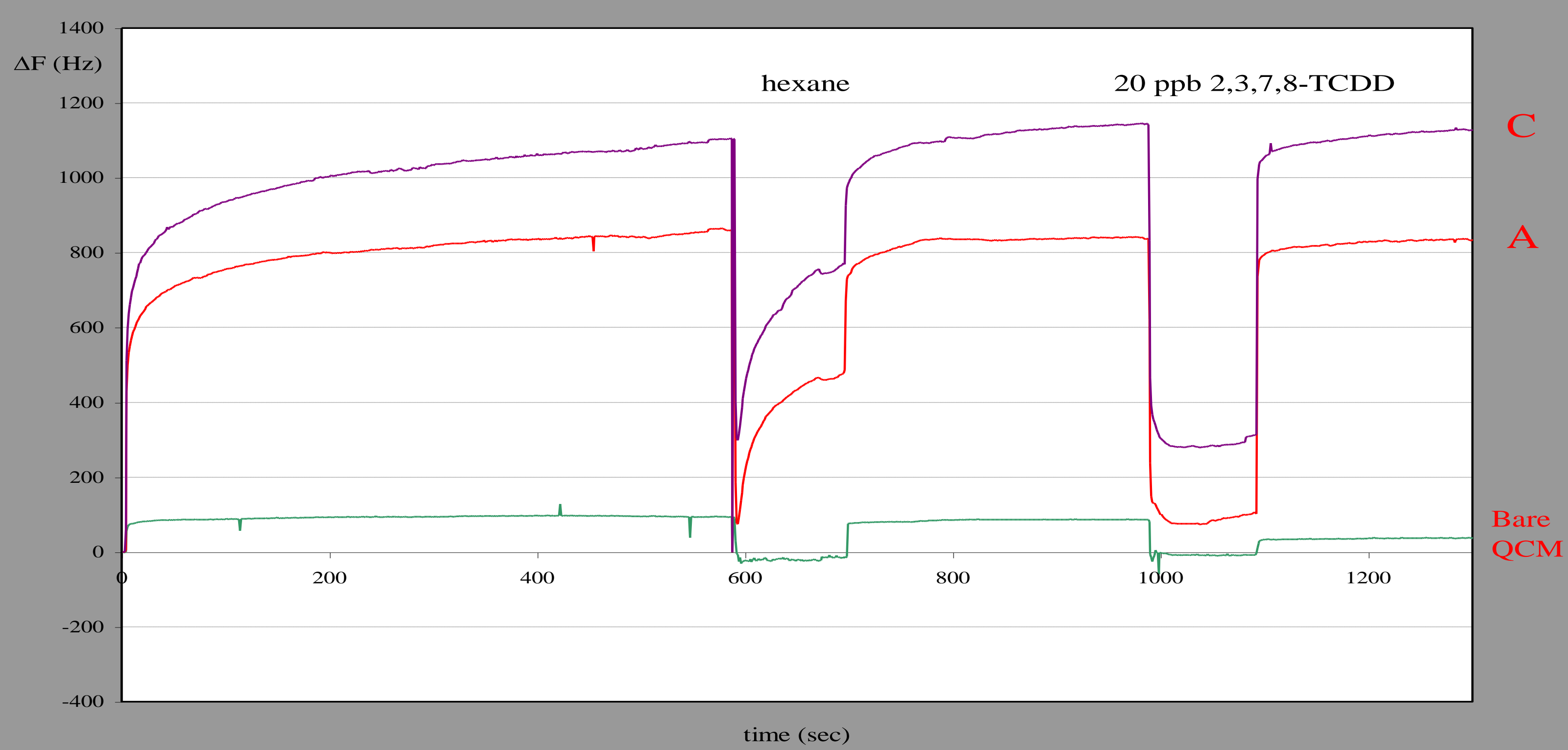
[N] Cys **-**Asn-Phe-**Gln**-Gly-Ile-Cys**[C]**

B

[N] Cys**-**Asn-Phe-**Gln-Gly**-Phe-Cys **[C]**

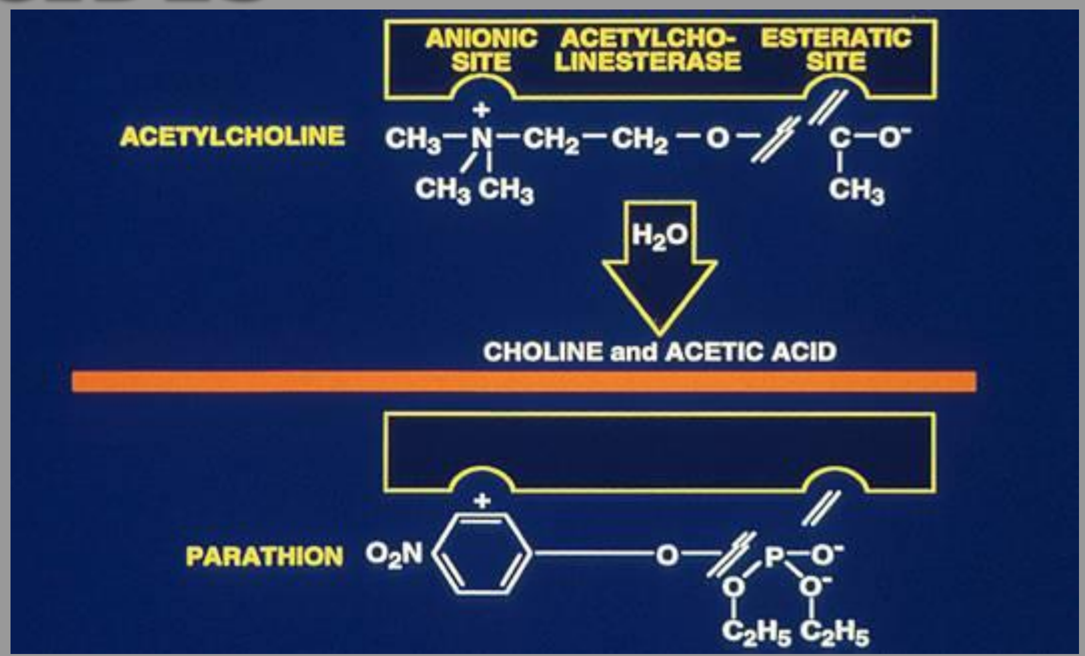


Experimental results



The sensor response of the QCM modified with oligopeptides A and C compared to the shift frequency obtained using the bare QCM. The hexane was the solvent of the dioxin stock solution, (2,3,7,8,-TCDD 20 ppb).

BIOMIMETIC RECEPTORS FOR PESTICIDES



Carbamate

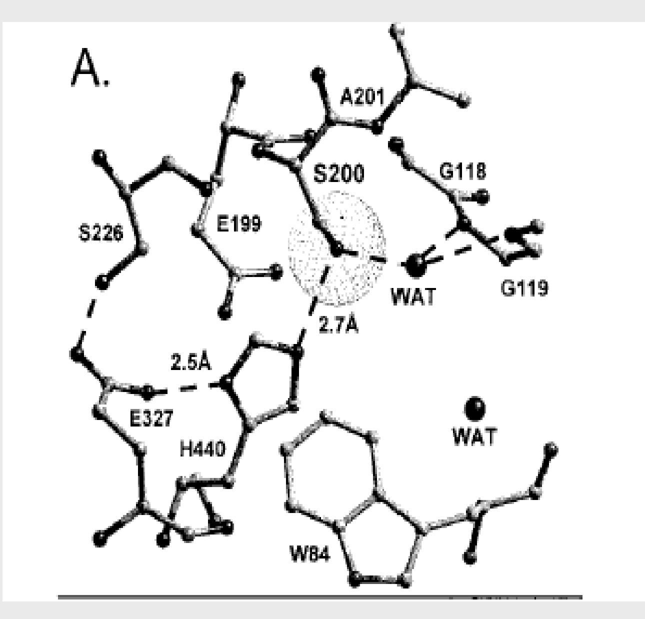
organophosphate

✓Mechanism of AChE inhibition

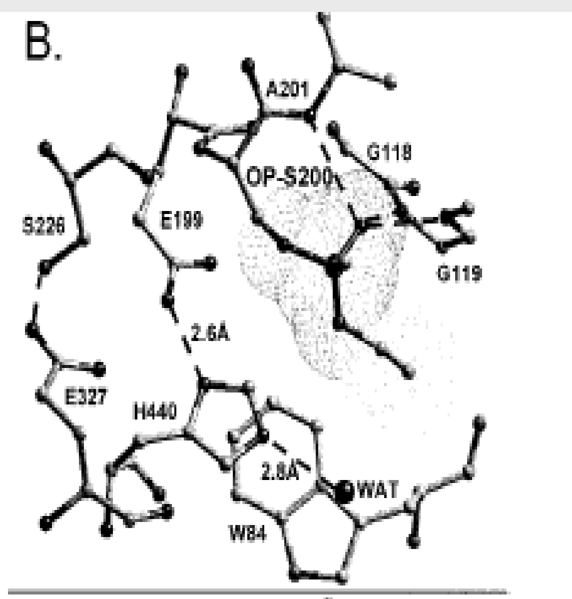
AChE, the target enzyme of pesticides, **is an efficient serine hydrolase** that catalyzes the breakdown of acetylcholine (ACh)

Acetylcholine + $H_2O \rightarrow$ choline + acetic acid

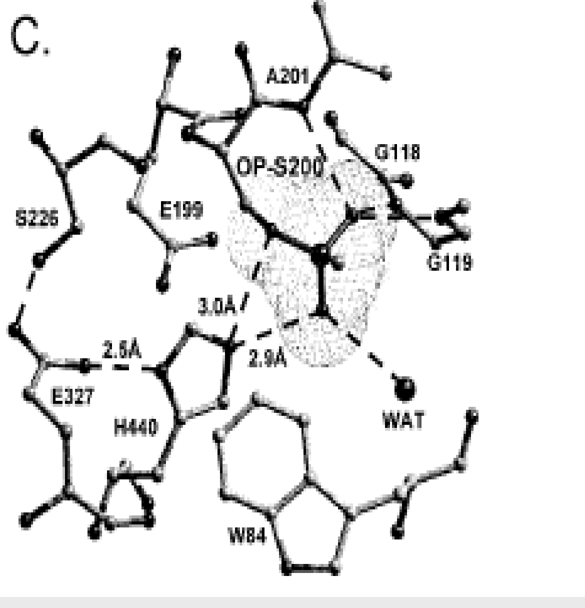
How pesticides work



Native structure: the active site, including the catalytic triad (S200-H440-E327) and the oxyanion hole (-NH of G118, G119, and A201)



Pro-aged structure: Phosphonylation triggers a conformational change for H440 that disrupts the H-bond to E327



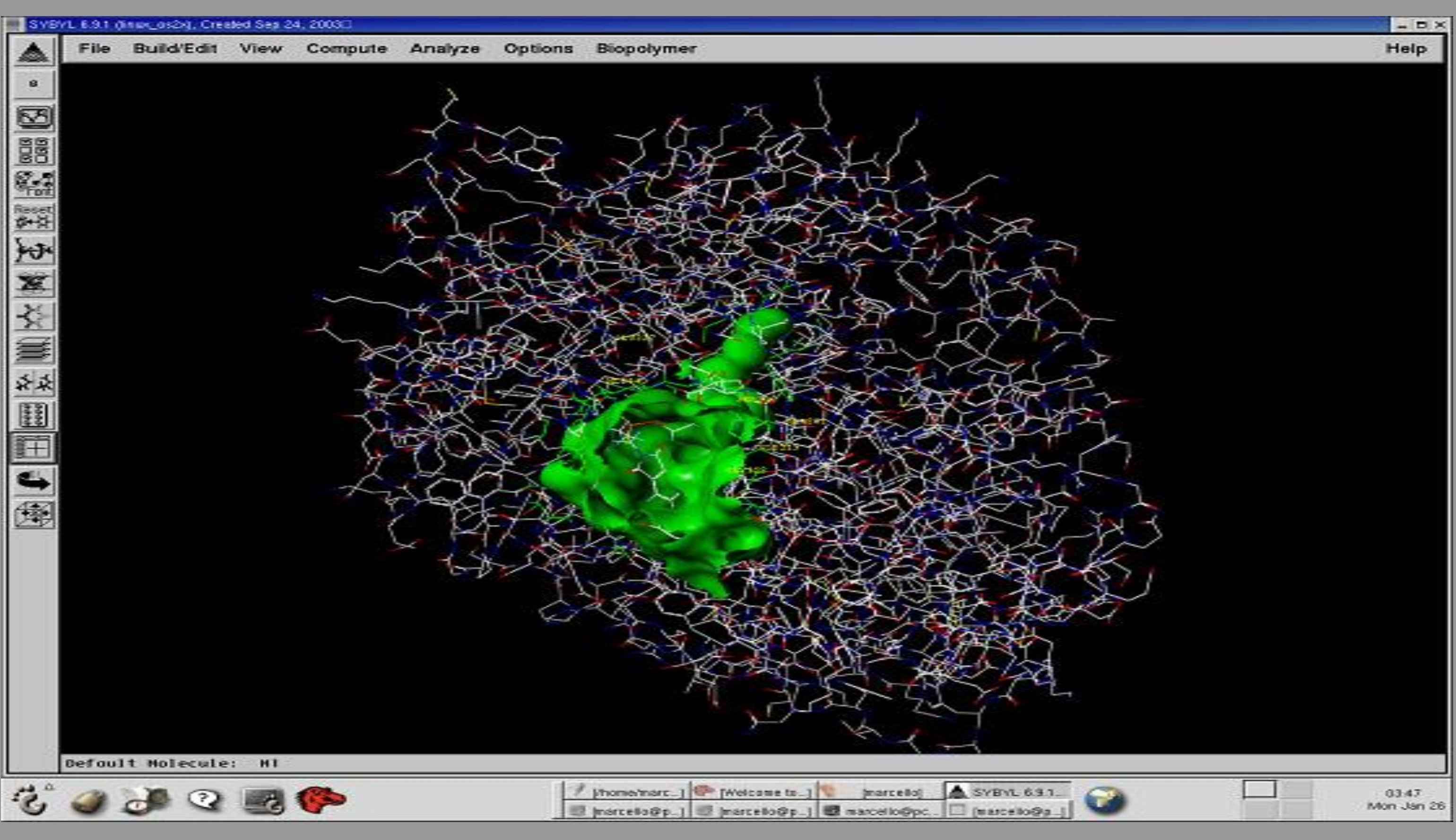
Aged structure: For reaction of AChE with VX and most phosphonates, aging predominates, and dealkylation results in movement of H440 to the negatively charged pocket formed by E327 Ox, S200 Ox, and one anionic oxygen of the dealkylated OP

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

❖Computational screening

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

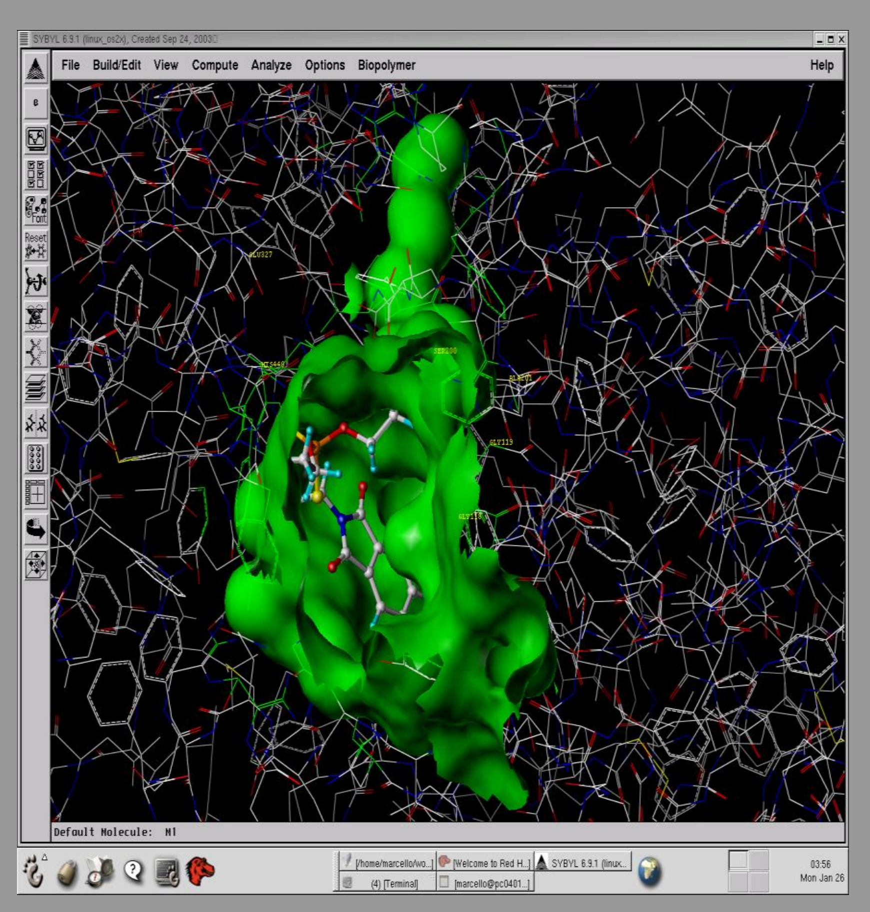
Methylphosphonylated Acetylcholinesterase (Aged) Obtained By Reaction With O-Ethyl-S-[2-[Bis(1-Methylethyl) Amino]Ethyl] Methylphosphonothioate (Vx) conventional X-ray crystallography resolution [Å]: 2.40

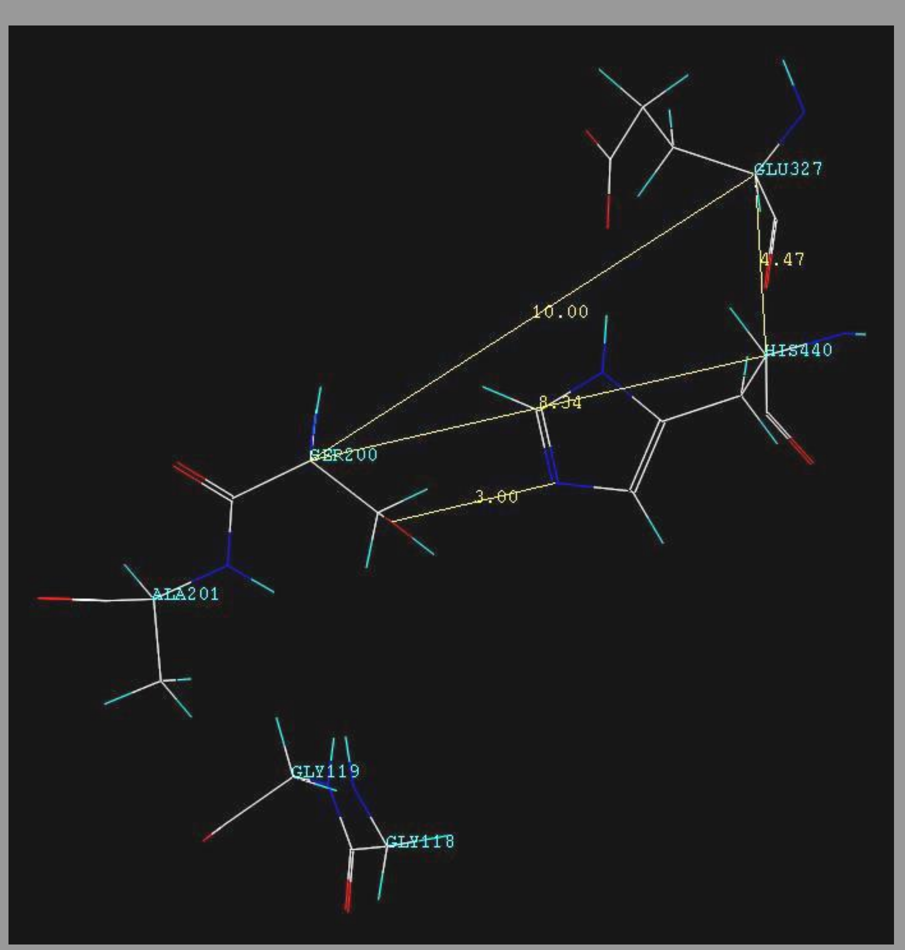


In green the molecular electrostatic potential distribution on the surface of the enzyme binding pocket

✓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**

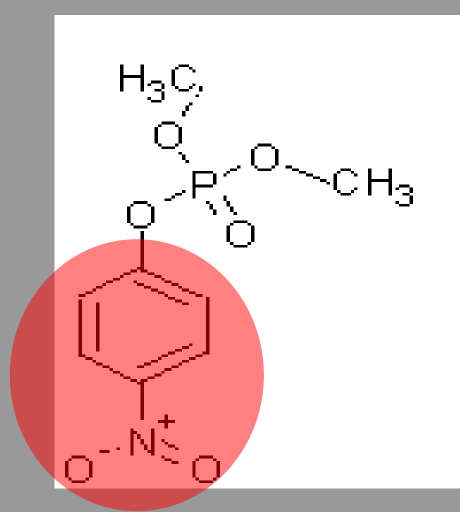
✓Simulated binding results vs paraoxon of the tetrapeptides selected for experimental screening

	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**

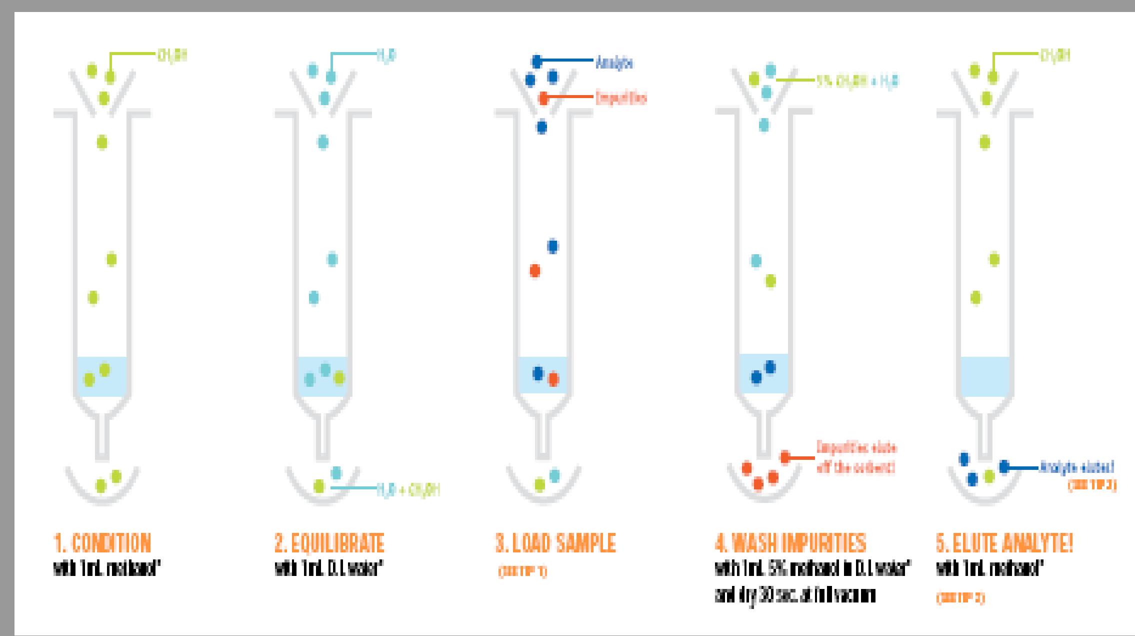
→ **B** **His-Gly-Ser-Ala**

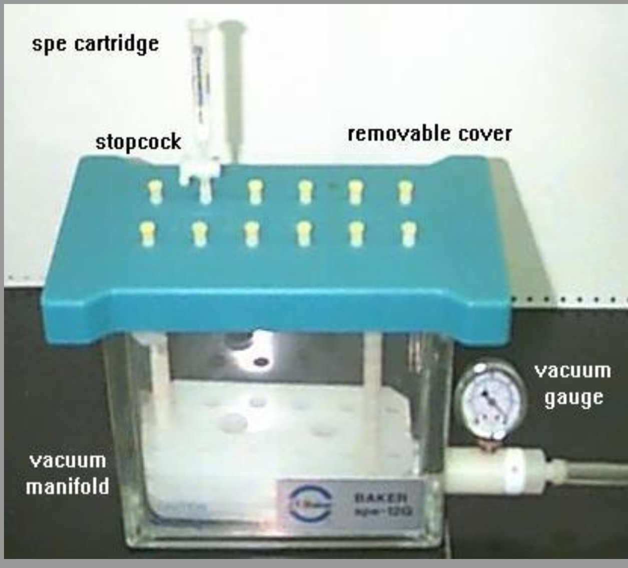
→ **C** **Glu-Pro-Ser-Ala**

→ **D** **His-Glu-Pro-Ser**

NC **Glu-His-Ser-Gly**

✓Pre-analytical applications: selective affinity columns (Extraction or purification)



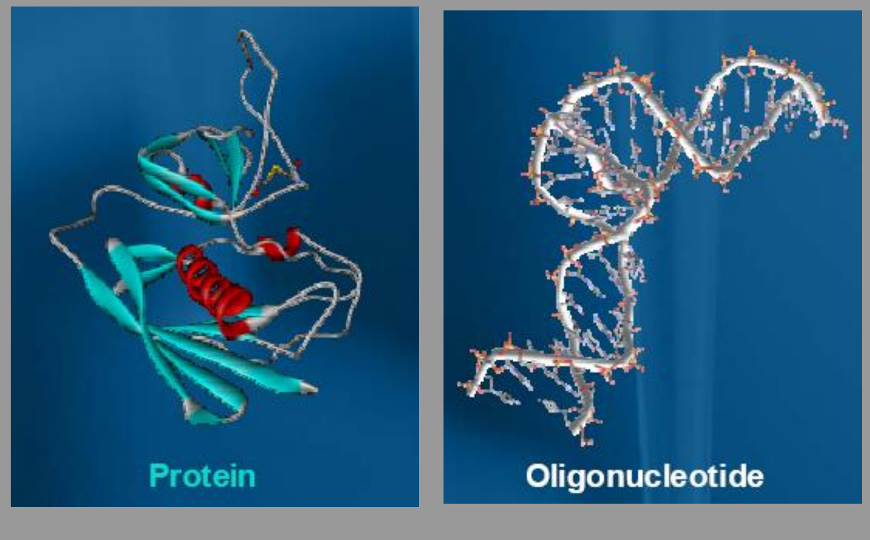


is a technique enabling purification of a biomolecule with respect to biological function or individual chemical structure. The substance to be purified is specifically and reversibly adsorbed to a ligand (binding substance), immobilized by a covalent bond to a chromatographic bed material (matrix). 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.

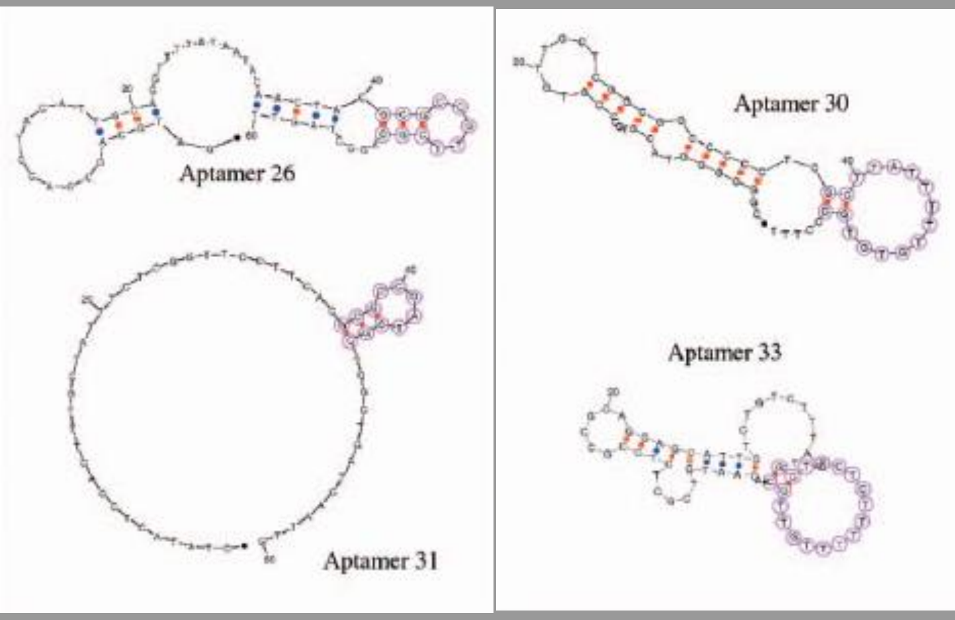
Aptamers are oligonucleotides (DNA or RNA molecules) that can bind with high affinity and specificity to a wide range of target molecules (proteins, peptides, drugs, vitamins and other organic or inorganic compounds).

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)

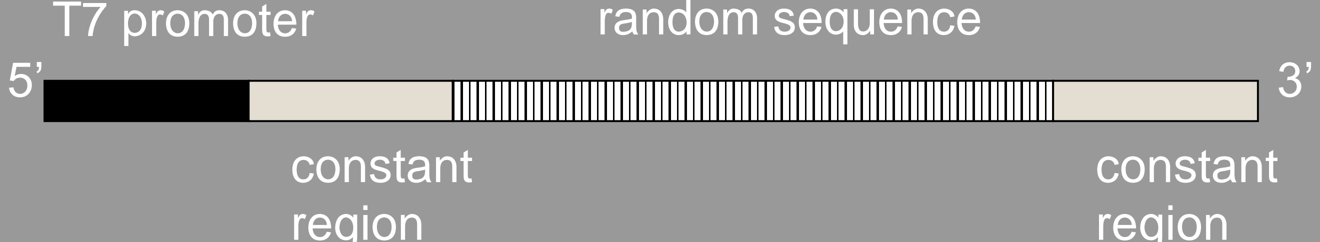
Their name is derived from the Latin word "**aptus**" which means "to fit".



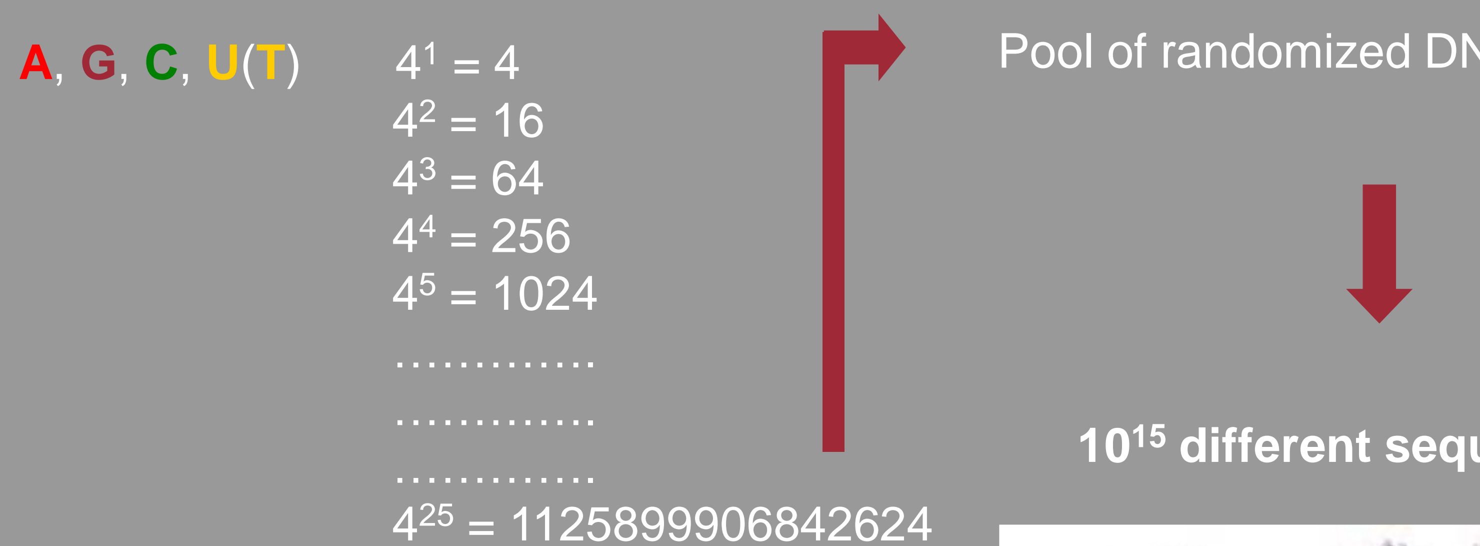
Similar to proteins short oligonucleotides can adopt complex three-dimensional structures



Starting point: Combinatorial oligonucleotide library

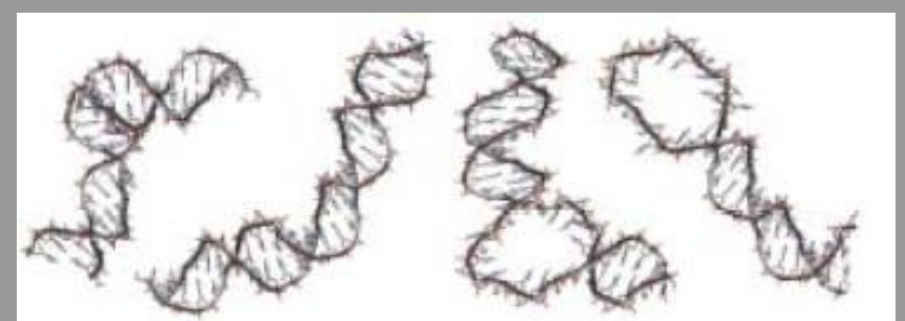


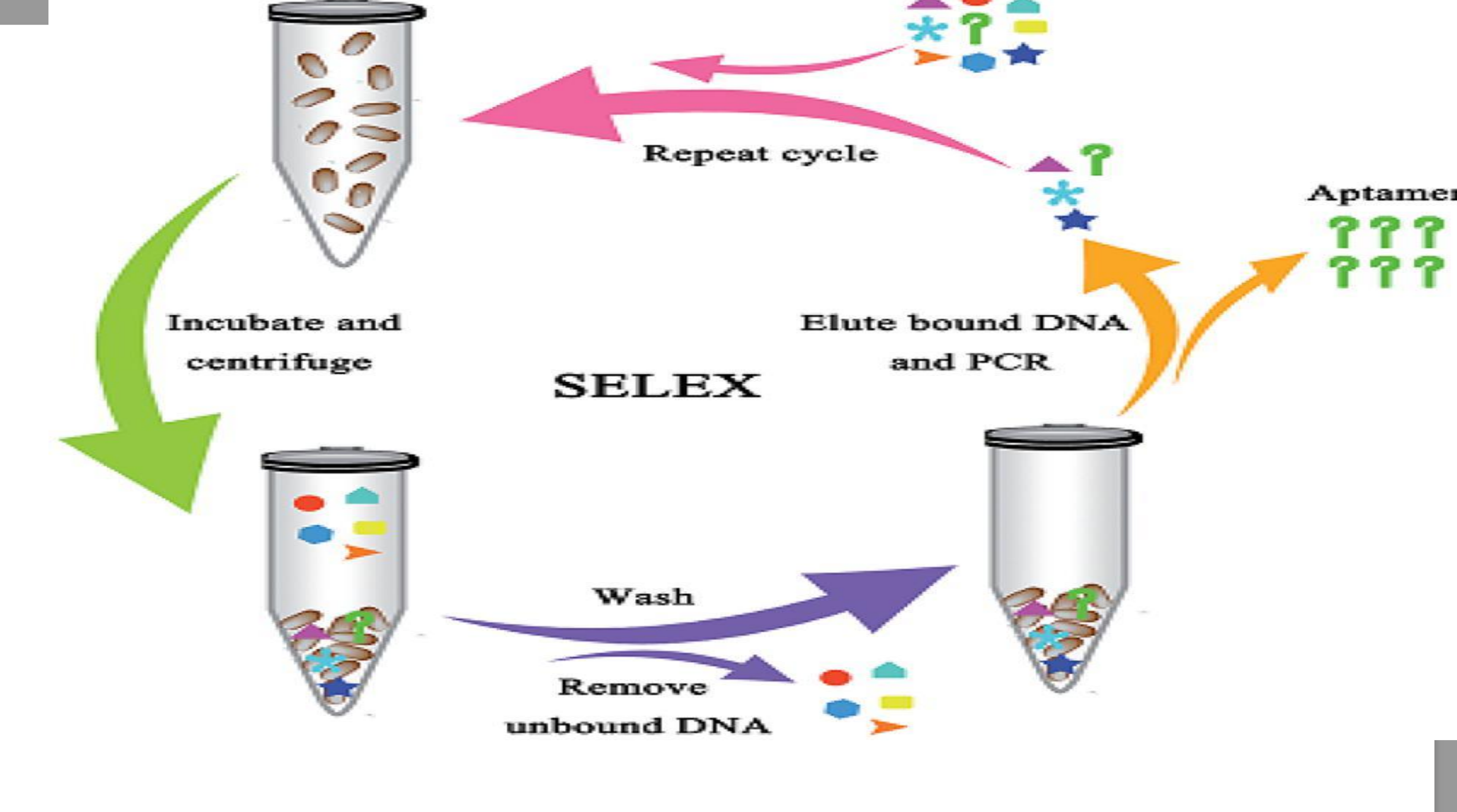
A library containing a 25-nucleotide random region is represented by 4^{25} (~10^{15}) individual sequences available for partitioning.
Normally, the starting round contains **10^{14}-10^{15} individual sequences**.



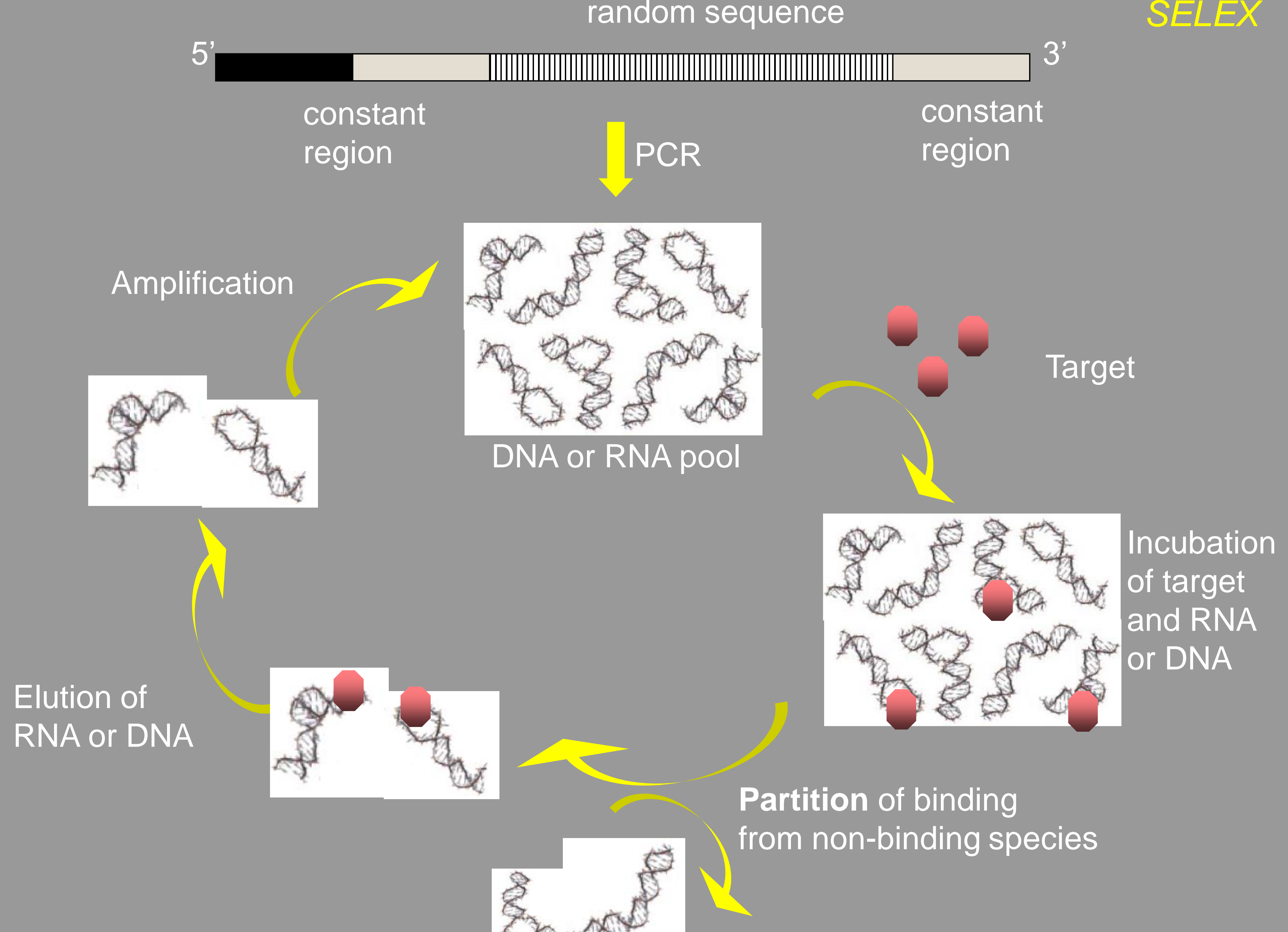
Pool of randomized DNA or RNA

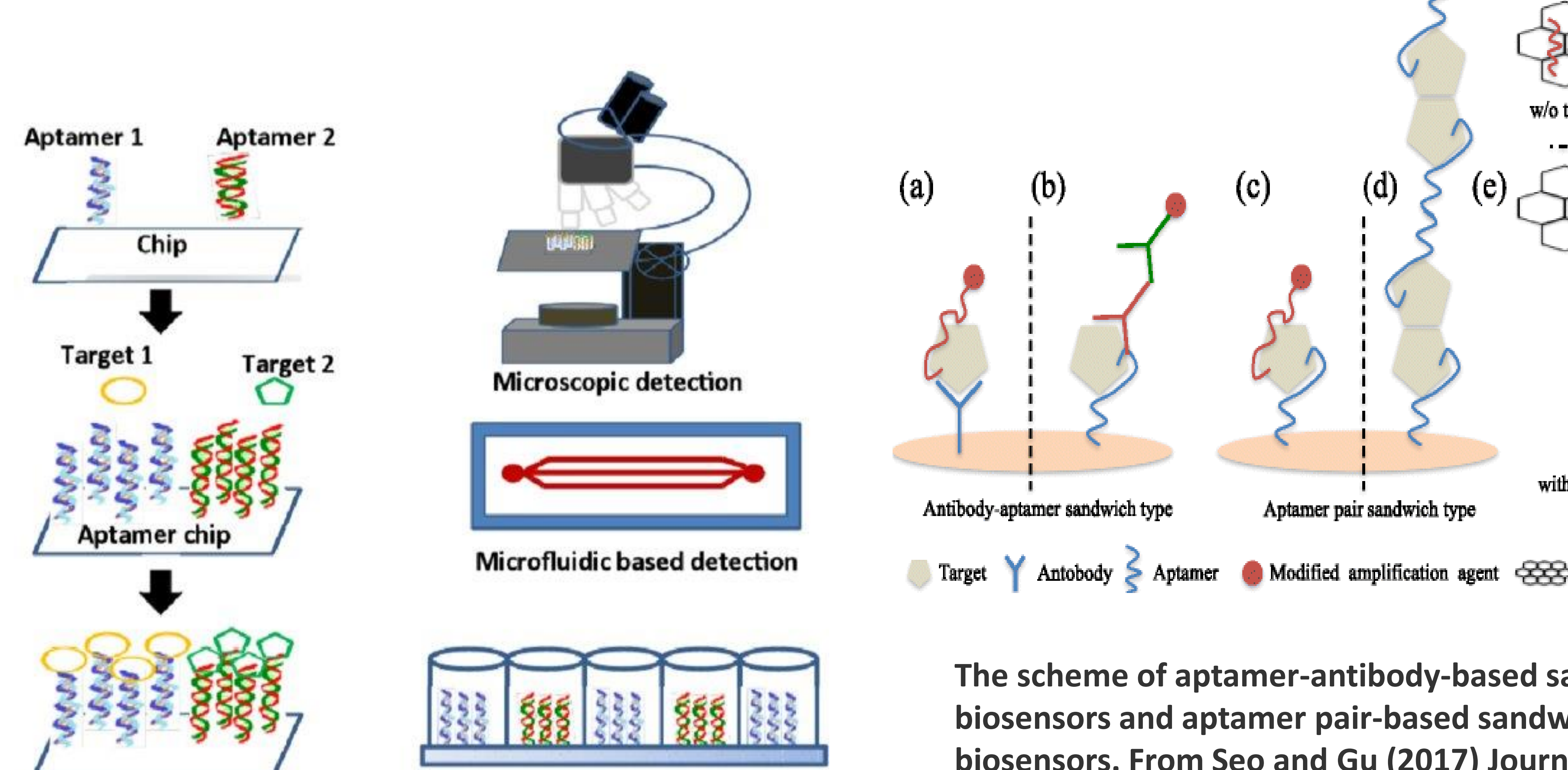
10^{15} different sequences!!!!





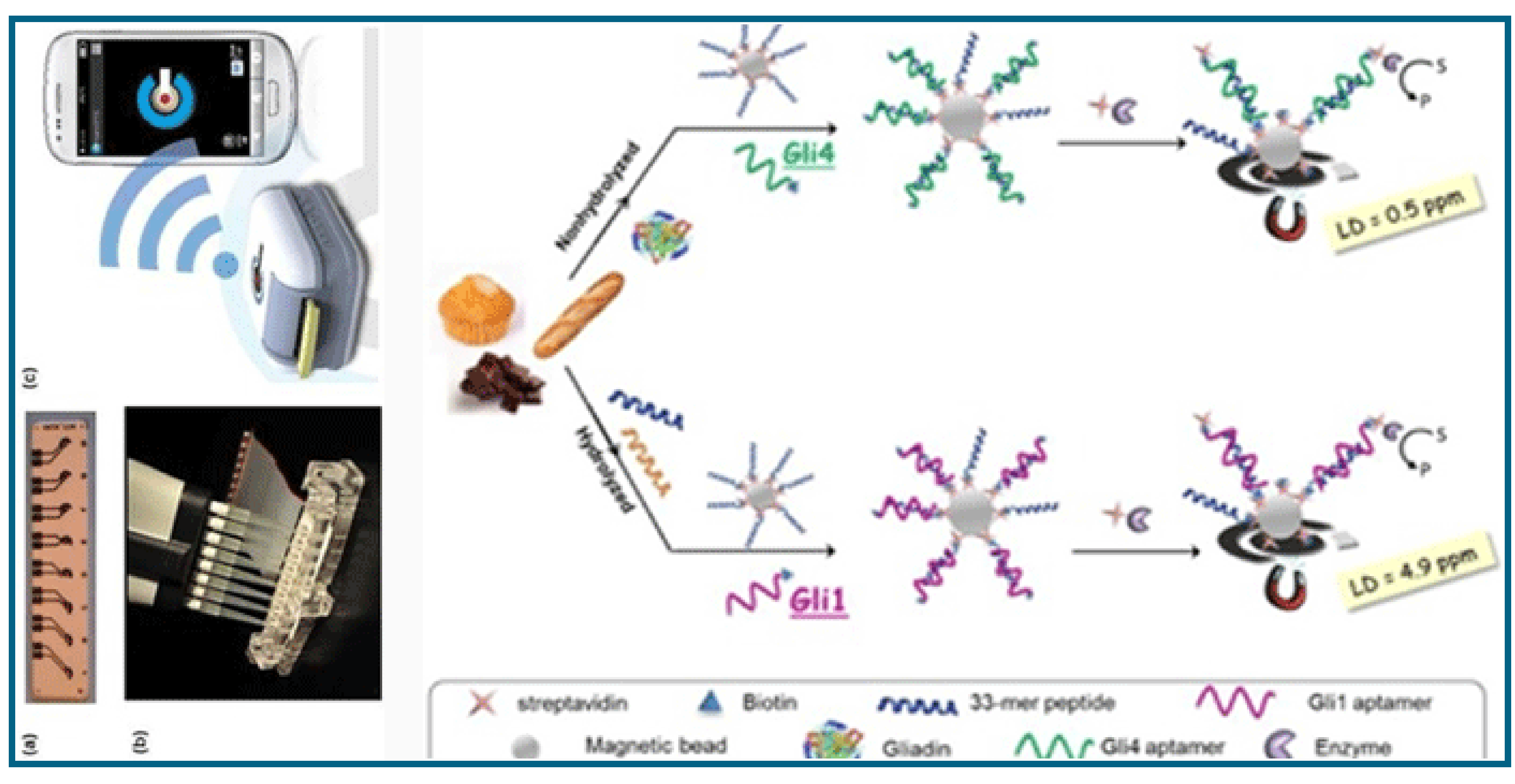
Repeat cycle
Aptamer
Incubate and centrifuge
Elute bound DNA and PCR
SELEX
Wash
Remove unbound DNA

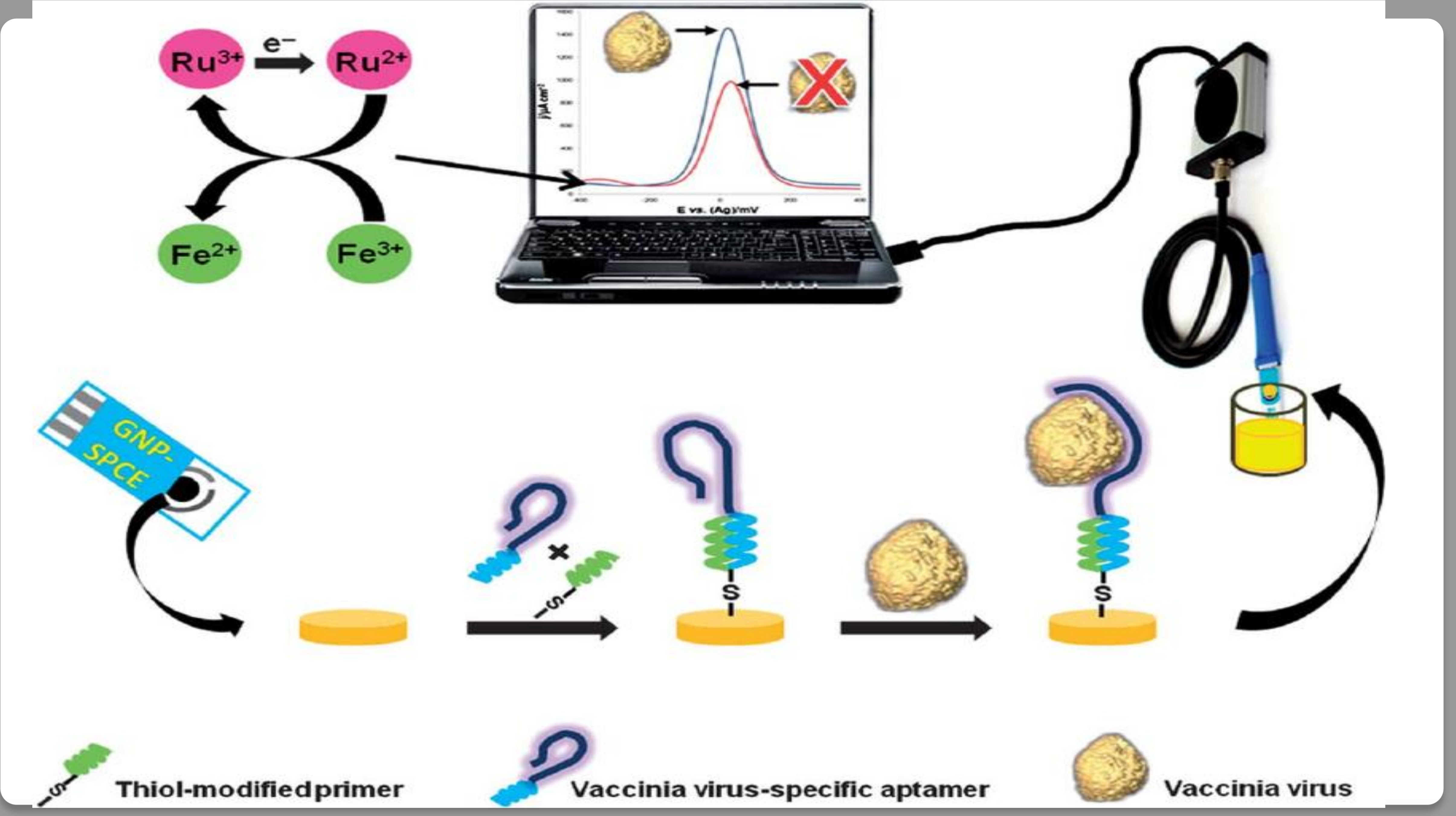

SELEX
random sequence
5'
3'
constant region
constant region
PCR
Amplification
DNA or RNA pool
Target
Incubation of target and RNA or DNA
Elution of RNA or DNA
Partition of binding from non-binding species



The scheme of aptamer-antibody-based sandwich-type biosensors and aptamer pair-based sandwich-type biosensors. From Seo and Gu (2017) Journal of Biological Engineering 11:11

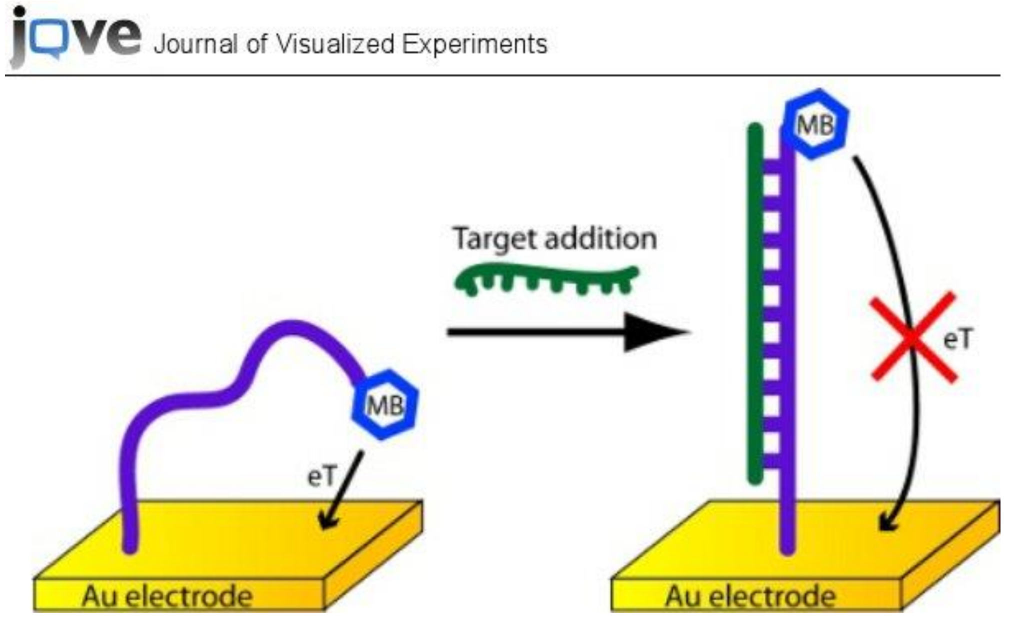
Misura di allergeni utilizzando microbeads magnetiche

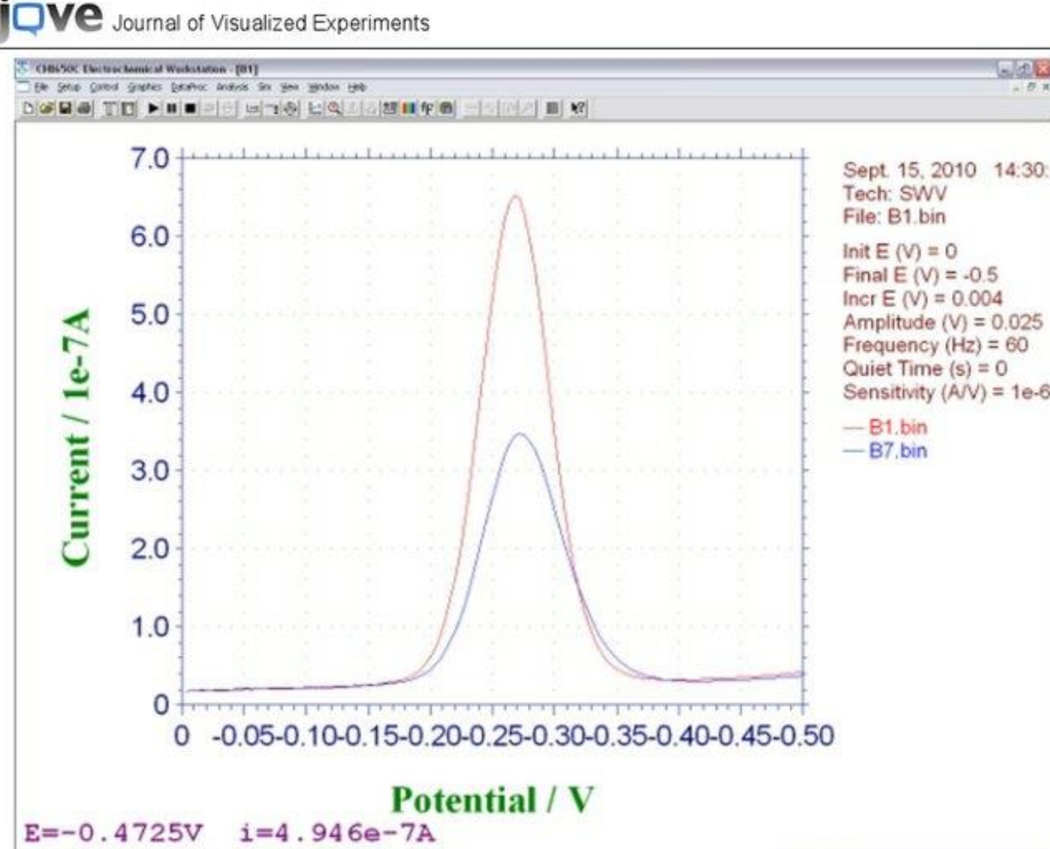


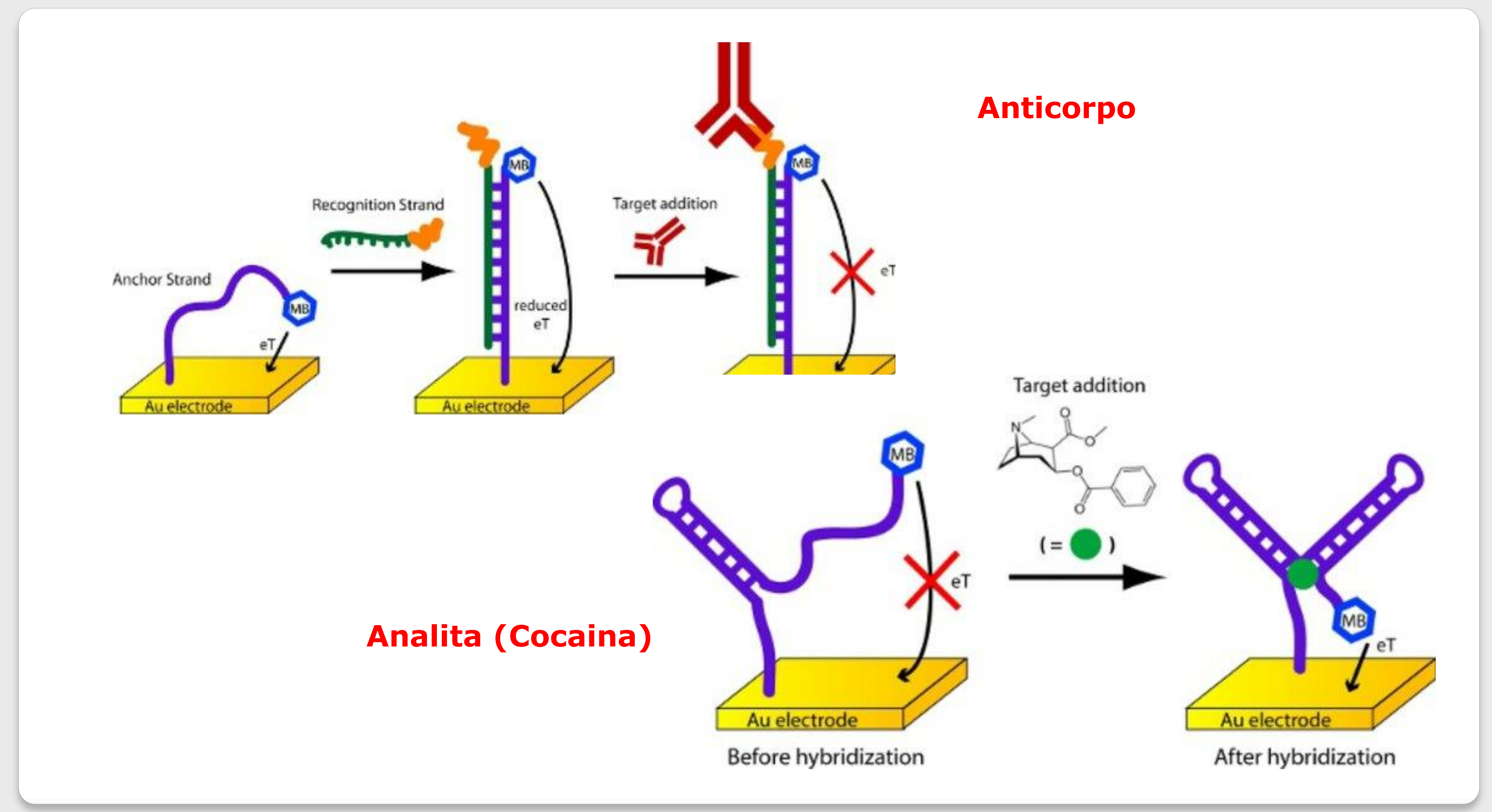


Ru3+
e−
Ru2+
Fe2+
Fe3+
E vs. (Ag)/mV
GNP-SPCE
S
S
S
Thiol-modifiedprimer
Vaccinia virus-specific aptamer
Vaccinia virus

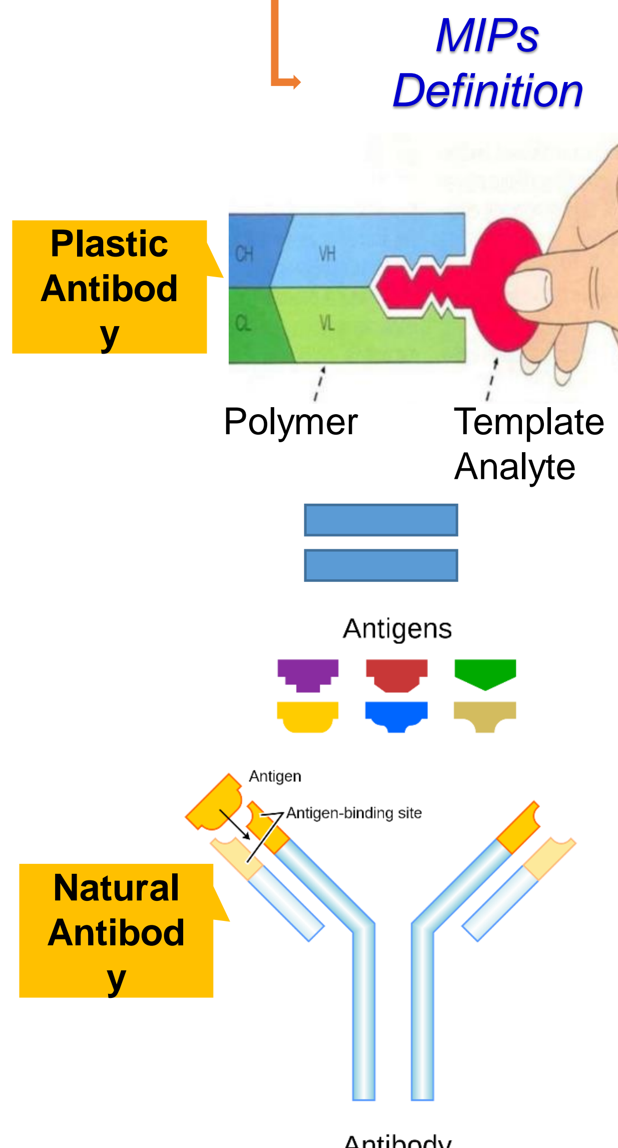
Label free electrochemical detection





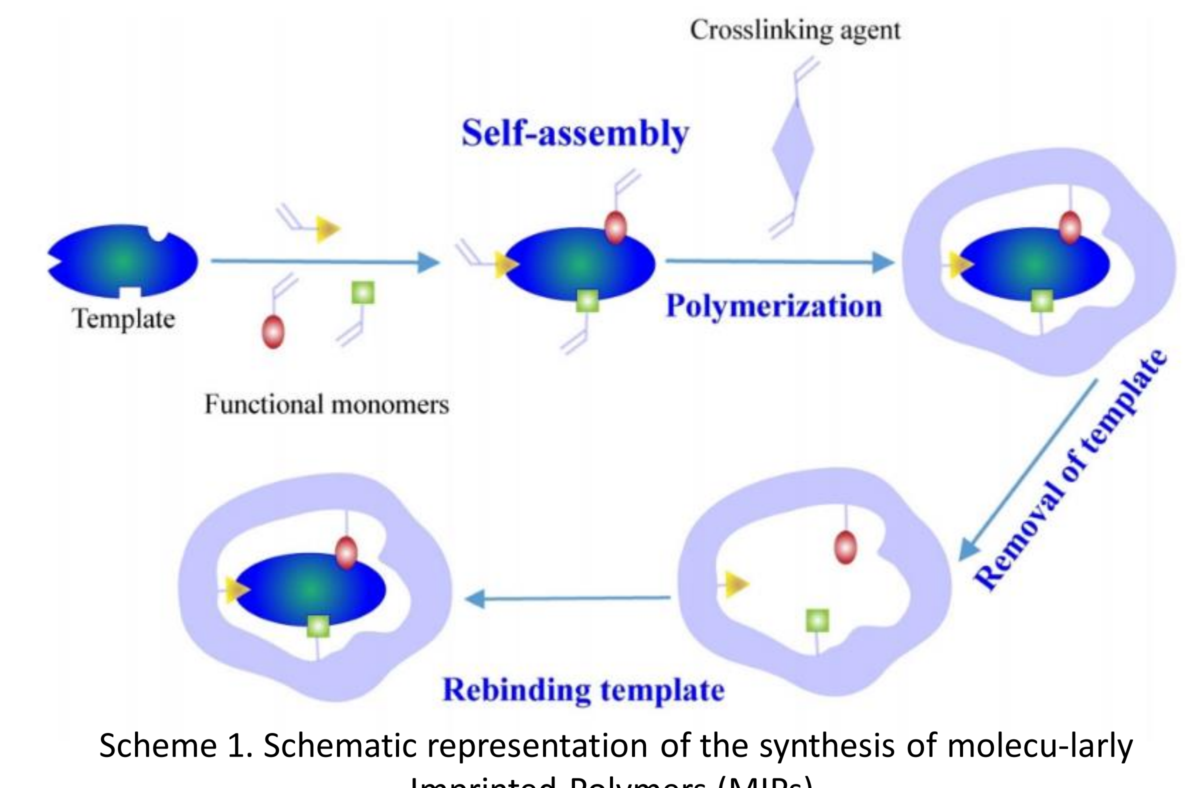

Anticorpo
Recognition Strand
Target addition
Anchor Strand
MB
eT
reduced
eT
Au electrode
Au electrode
Target addition
(=)
Analita (Cocaina)
Au electrode
Au electrode
Before hybridization
After hybridization

MIP-State of the art



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

DOI: 10.1021/acs.chemrev.8b00171 Chem. Rev. 2019, 119, 94–119



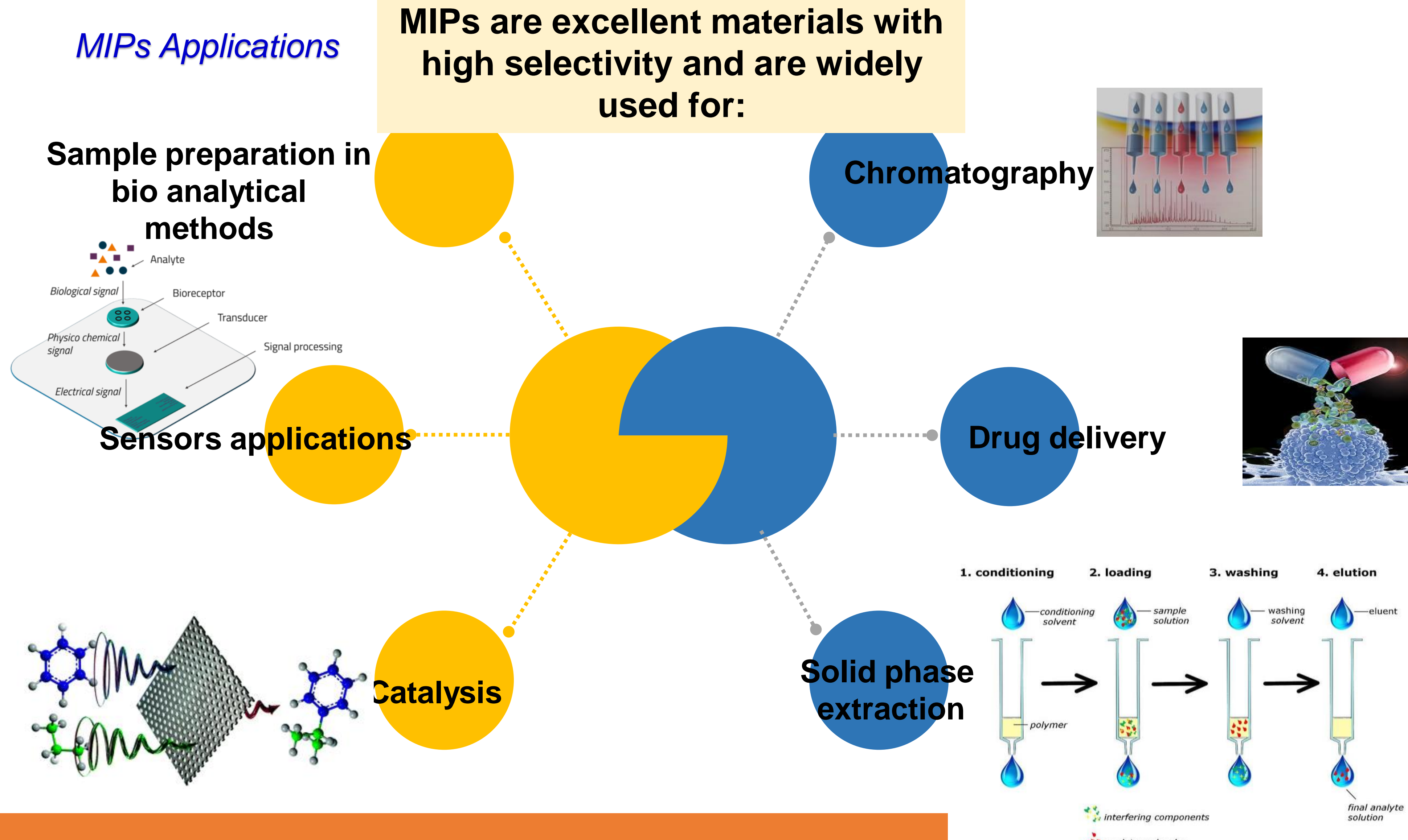
Scheme 1. Schematic representation of the synthesis of molecu-larly Imprinted Polymers (MIPs).

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

MIP-State of the art

MIPs Applications

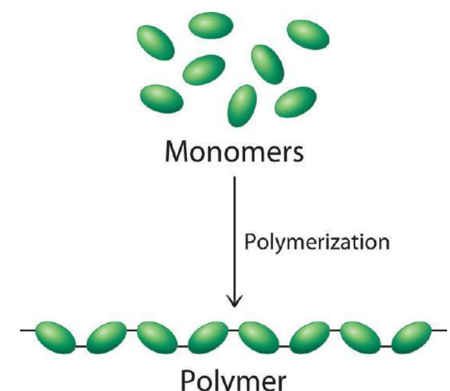
MIPs are excellent materials with high selectivity and are widely used for:



02 MIP-State of the art

MIPs Synthesis

Components of MIP Mixture



Initiator

Porogen (Solvent)

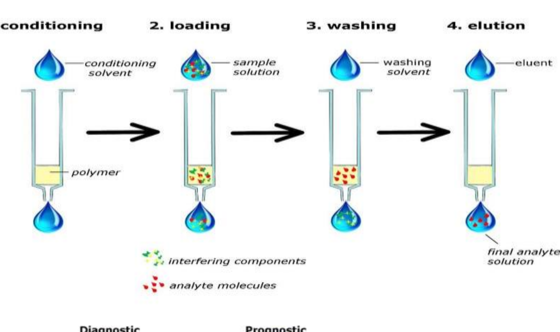
Template (Target analyte)

Crosslinking monomer

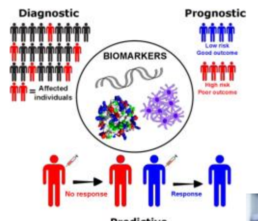
Functional monomer

Cross-link
Polymer Chain A
Polymer Chain B
Polymer Chain C

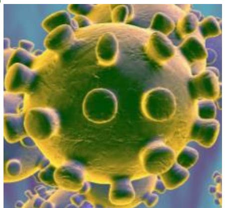
MIPs Applications



- Non-sensing Applications (purification of biological and chemical reagent
- Gas Sensing Applications (volatile organic carbons, VOCs)
- Liquid/Solution Sensing Applications:



  - Biomarkers (prostate-specific antigen (PSA), cancer antigen 125….)
  - Pharmaceutical and Drugs of Abuse Detection (cocaine, a range of antibiotics….)
  - Environmental Sensing and Pesticide
  - Detection Food Analysis (mycotoxins…)
  - Explosives Detection(pentaerythritol tetranitrate (PETN), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX))
  - Pathogen Detection (viruses, bacteriophage …)
  - Chiral Molecule Detection

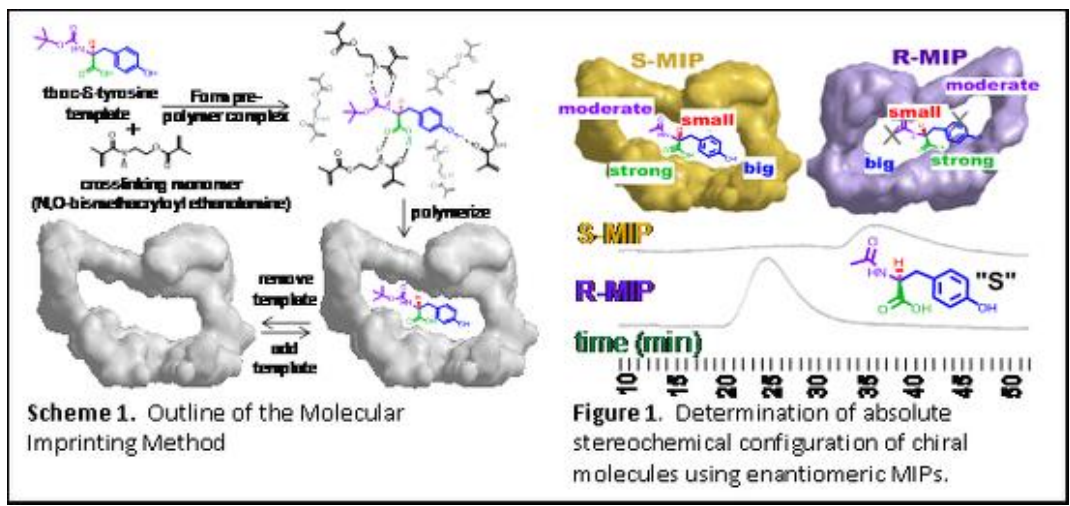


DOI: 10.1021/acs.chemrev.8b00171 Chem. Rev. 2019, 119, 94−119

MIP-State of the art

Advantages of MIPs

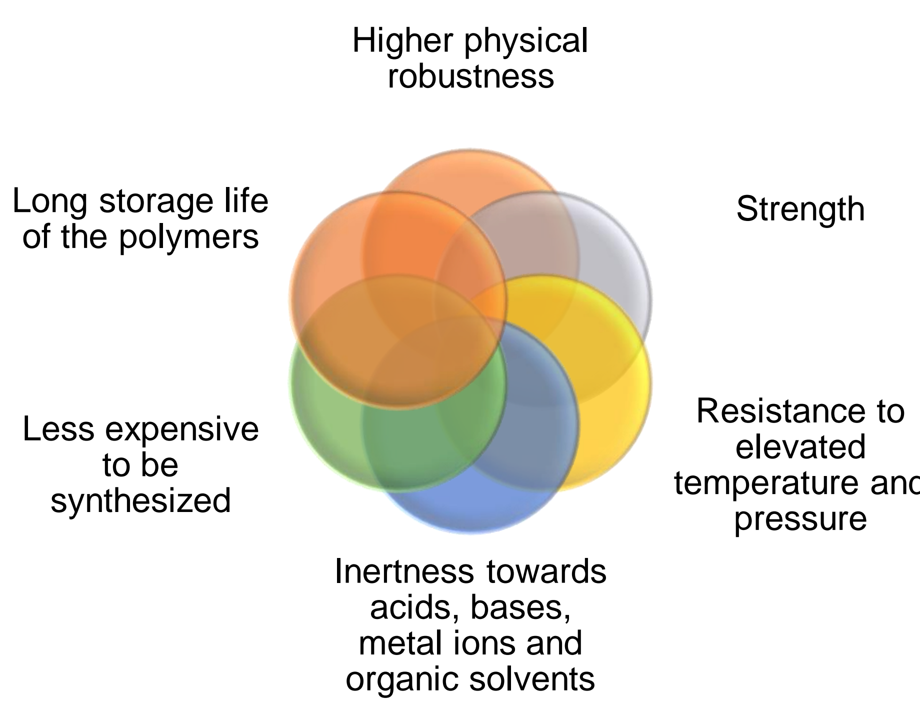
❖ High **selectivity** and **affinity** for the **target molecule** used in the imprinting procedure.



Scheme 1. Outline of the Molecular Imprinting Method

Figure 1. Determination of absolute stereochemical configuration of chiral molecules using enantiomeric MIPs.

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



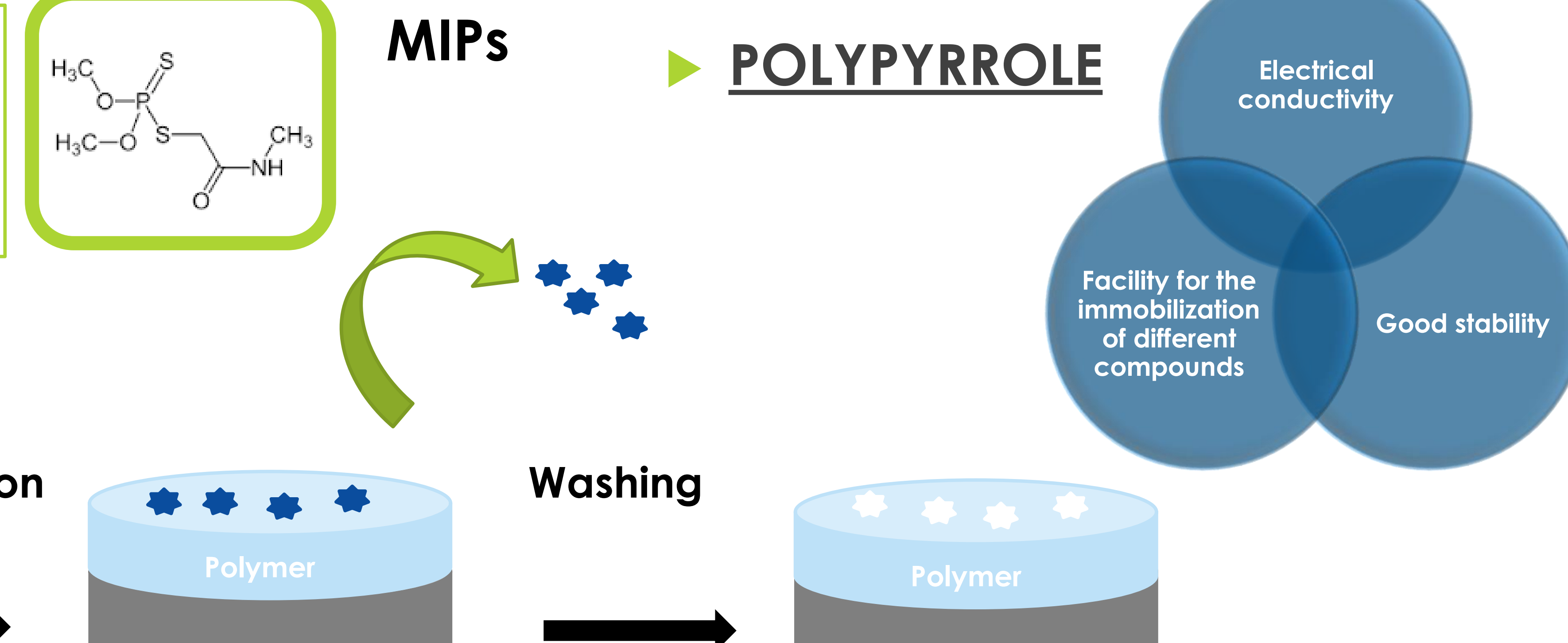
Int J Mol Sci. 2011; 12(9): 5908–5945

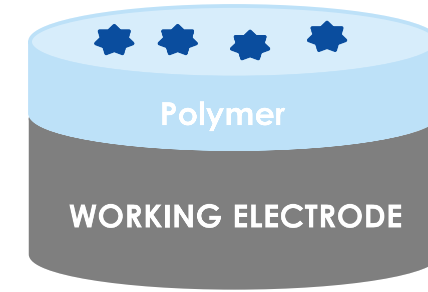
Chem. Rev. 2000, *100,* 2495-2504

AIM OF THE WORK:

- development of **molecularly imprinted polymer** (**MIP**) **based sensors** for the detection of **dimethoate**;
- development of a rapid, simple, sensitive, selective and portable **screening method**, for the detection of **dimethoate** residues in **wheat flour**, based on the **combination** of **MIP** electrochemical sensor with the microextraction by packed sorbent (**MEPS**).

DIMETHOATE: Organophosphate pesticide (OP).
Inhibitory effect on the function of the **enzyme acetylcholinesterase** (AChE) that hydrolyses the neurotransmitter acetylcholine and this effect leads to a **pathologic excess of acetylcholine in the body.**





Polymerization

WORKING ELECTRODE

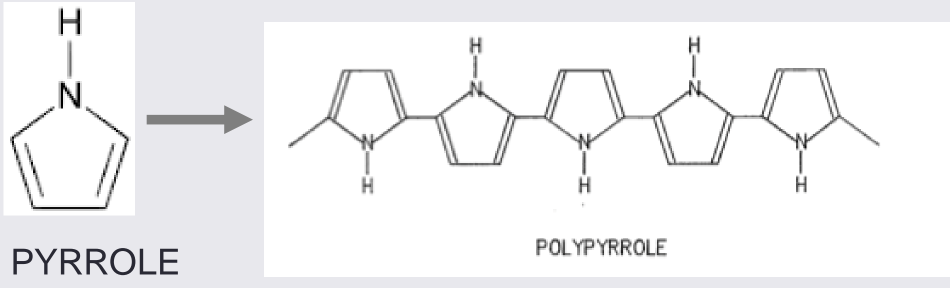
Template

Monomer

PYRROLE (Py)

The electropolymerization of polypyrrole (PPy) has been widely used for the preparation of molecularly imprinted electrochemical sensors (Da Silva et al., 2014; Jara-Ulloa et al., 2013; Zhou et al., 2012), due to:

- biocompatibility;
- facility of the immobilization of different compounds;
- good stability;
- ability to transduce energy arising from interaction of analyte and analyte-recognizing-site into electrical signals that are easily monitored;
- ability to protect electrodes from interfering materials;
- easy ways for electrochemical deposition on the surface of any type of electrodes.



CV in phosphate buffer pH 6.8
Potential range from -400 mV to 1500 mV
Scan rate: 50 mV/s
10 cycles

MIP-MEPS based sensing strategy for the selective assay of dimethoate

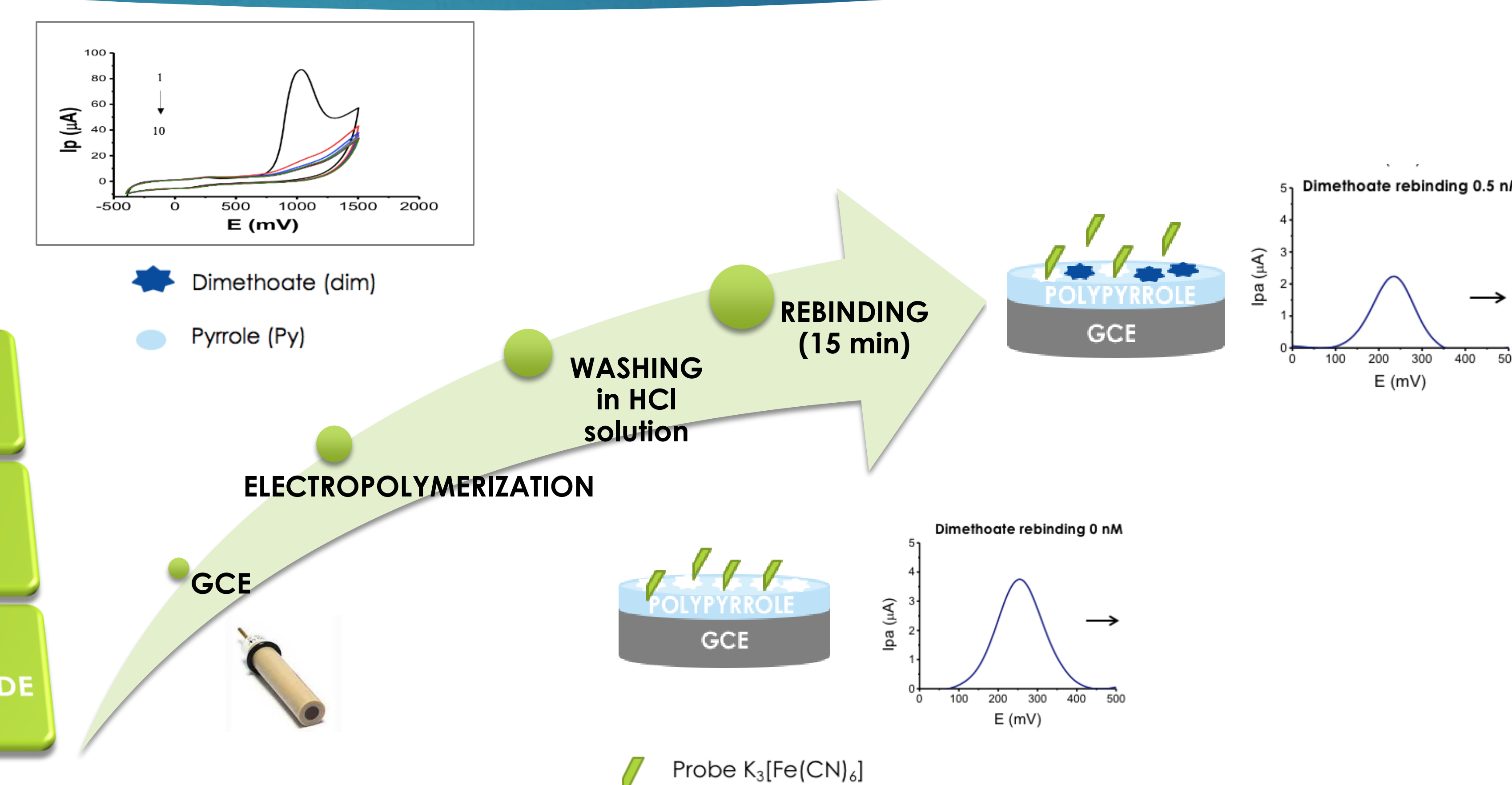
DIMETHOATE MONITORING IN WHEAT FLOUR

SAMPLE PREPARATION

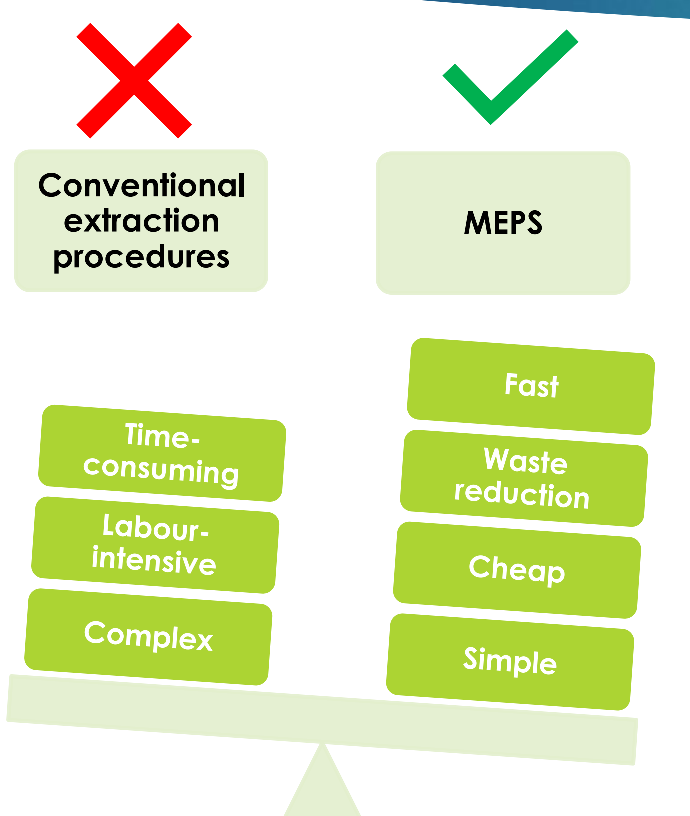
ANALYTE DETECTION

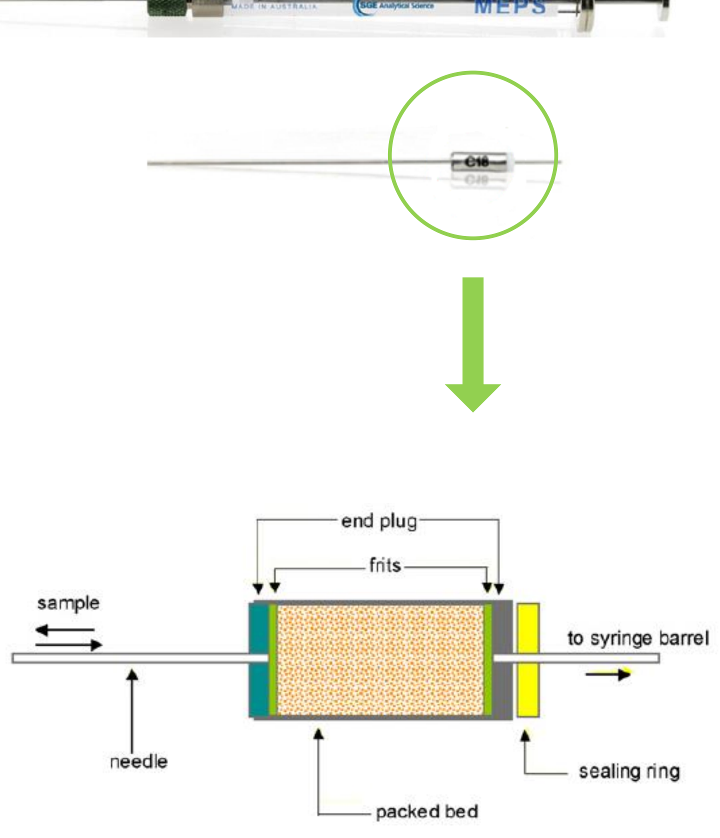
MICROEXTRACTION BY PACKED SORBENT (MEPS)

MIP-GLASSY CARBON ELECTRODE



MICROEXTRACTION BY PACKED SORBENT (MEPS)





SUMMARY OF RESULTS: analytical application of the sensor

Wheat flour samples → MEPS (HDVB) → MIP-GCE detection

MEPS
ACTIVATION: 100 µL of methanol for three times
CONDITIONING: by flushing two times with a 90:10 (v:v) water-acetonitrile solution
LOADING OF THE SAMPLE
WASHING: 100 µL of water for three times
ELUTION: 100 µL of acetonitrile for three times

MIP-GCE
The sensor was placed in 100 µL of eluate for 15 minutes at room temperature for the rebinding step.

Electrochemical measurements.

Relative error (RE %) of dimethoate concentrations detected in spiked wheat flour samples by the MIP-GCE (SWV) with respect to the dimethoate concentrations detected by the UHPLC-MS/MS; **standard deviation (SD) of dimethoate concentrations** detected in spiked wheat samples (n = 6) by the MIP-GCE (SWV).

Samples	MIP-GCE RE (%)	MIP-GCE SD
Wheat flour spiked with dimethoate **0.5 MRL**	+13.5	0.5
Wheat flour spiked with dimethoate **0.5 MRL + mix**	+4.6	2.4
Wheat flour spiked with dimethoate **MRL**	-21.1	1.2
Wheat flour spiked with dimethoate **MRL + mix**	**-21.2**	1.4
Wheat flour spiked with dimethoate **1.5 MRL**	**+16.7**	0.7
Wheat flour spiked with dimethoate **1.5 MRL + mix**	-0.4	1.7
Wheat flour spiked with dimethoate **MRL + omethoate (1:1)**	+3.5	2.7
Wheat flour spiked with dimethoate **MRL + omethoate (1:10)**	-15.5	0.9