# New Biorecognition Elements

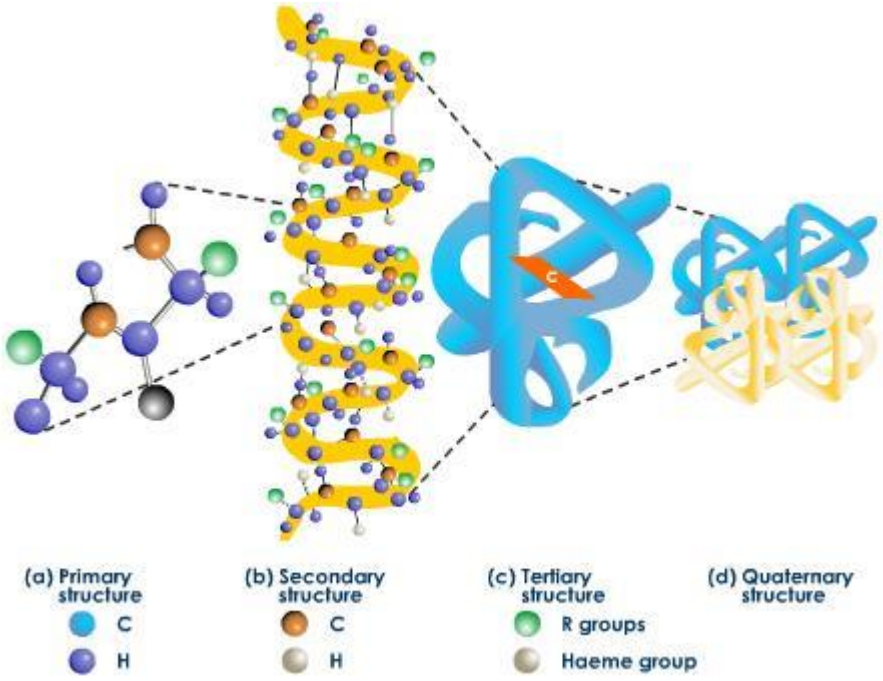
- Aptamers
- Molecularly imprinted polymers (MIPs)
- Proteins:
  - Antigens
  - Lectins (carbonhydrate-binding proteins)
- Lipids
- Carbohydrates
- Peptide nucleic acids (PNAs)
- New approaches for known materials

# Aptamers

- Single stranded folded oligonucleotides (RNA/DNA) and peptide that bind to molecular targets with high affinity and specificity.
- They range in size from 20 to 80 bases(6-26 kDa).
- Based on their three-dimensional structures, aptamers can well-fittingly bind to a wide variety of targets from single molecules to complex target mixtures or whole organisms with high affinity and specificity.
- Small RNA/DNA molecules can form secondary and tertiary structures capable of specifically binding their targets

The primary structure is the specific sequence of amino acids in a protein.
The secondary structure is twisted into a helical shape because of interaction between amino acids in the polypeptide chain.
The tertiary structure is when the protein twists and bends into a complex three dimensional shape.
The quarternary structure of the protein is when some proteins composed of more than one polypeptide chain join together.

- This is the primary, secondary, tertiary, and quarternary structures of hemoglobin.



## *COMPARISON OF RNA, DNA AND PEPTIDE APTAMERS:*

| RNA APTAMER | DNA APTAMER | PEPTIDE APTAMER |
|---|---|---|
| FROM COMPLEX SECONDARY AND TERTIARY STRUCTURE | FROM COMPLEX SECONDARY AND TERTIARY STRUCTURE | STRUCTURE CONSTRAINS BY SCAFFOLED |
| FROM DIVERSE 3D STRUCTURE | LESS DIVERSE 3D STRUCTURE THAN RNA APTAMER | 3D STRUCTURE CONSTRAINTS BY SCAFFOLD |
| BIND TARGET WITH THE ENTIRE SEQUENCE | BIND TARGET WITH THE ENTIRE SEQUENCE | BIND TARGET VARIABLE REGION ONLY |
| BIOSENSEOR, DIAGONOSTIC, THERAPEUTICS APPLICATIONS | BIOSENSEOR, DIAGONOSTIC, THERAPEUTICS APPLICATIONS | BIOSENSEOR, DIAGONOSTIC, THERAPEUTICS APPLICATIONS |

# Aptamers

Essentially a chemical equivalent of antibodies BUT have additional advantages:

| Aptamer | Antibodies |
|---|---|
| Aptamer are oligonucleotide and protein | Antibodies are protein in nature. |
| Investigator determines target site of protein | Immune system determines target site of protein. |
| Wide variety of chemical modifications to molecule for diverse functions | Limited modifications of molecule |
| No evidence of immunogenicity. | Significant immunogenicity |
| They are more stable at high temperature and they can be regenerated easily after denaturation. | Temperature sensitive |
| Entire selection is a chemical process carried out in vitro and can therefore target any protein . | Selection requires a biological system, therefore difficult to raise antibodies to toxins (not tolerated by animal) or non-immunogenic target. |
| Aptamers are single stranded DNA or RNA oligonucleotide or peptides. | Antibodies are monoclonal or polyclonal. |

Aptamers bind to variety of lignads such as nucleic acid, proteins, small organic compounds, and even entire organisms.

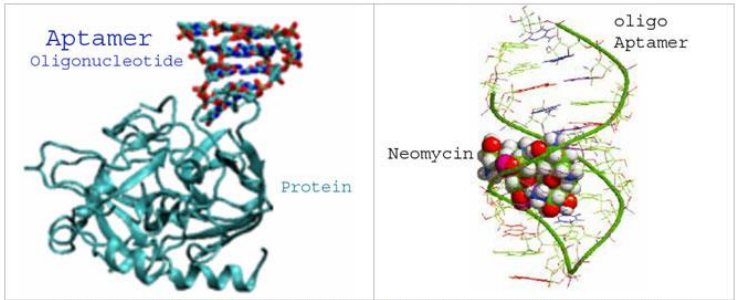
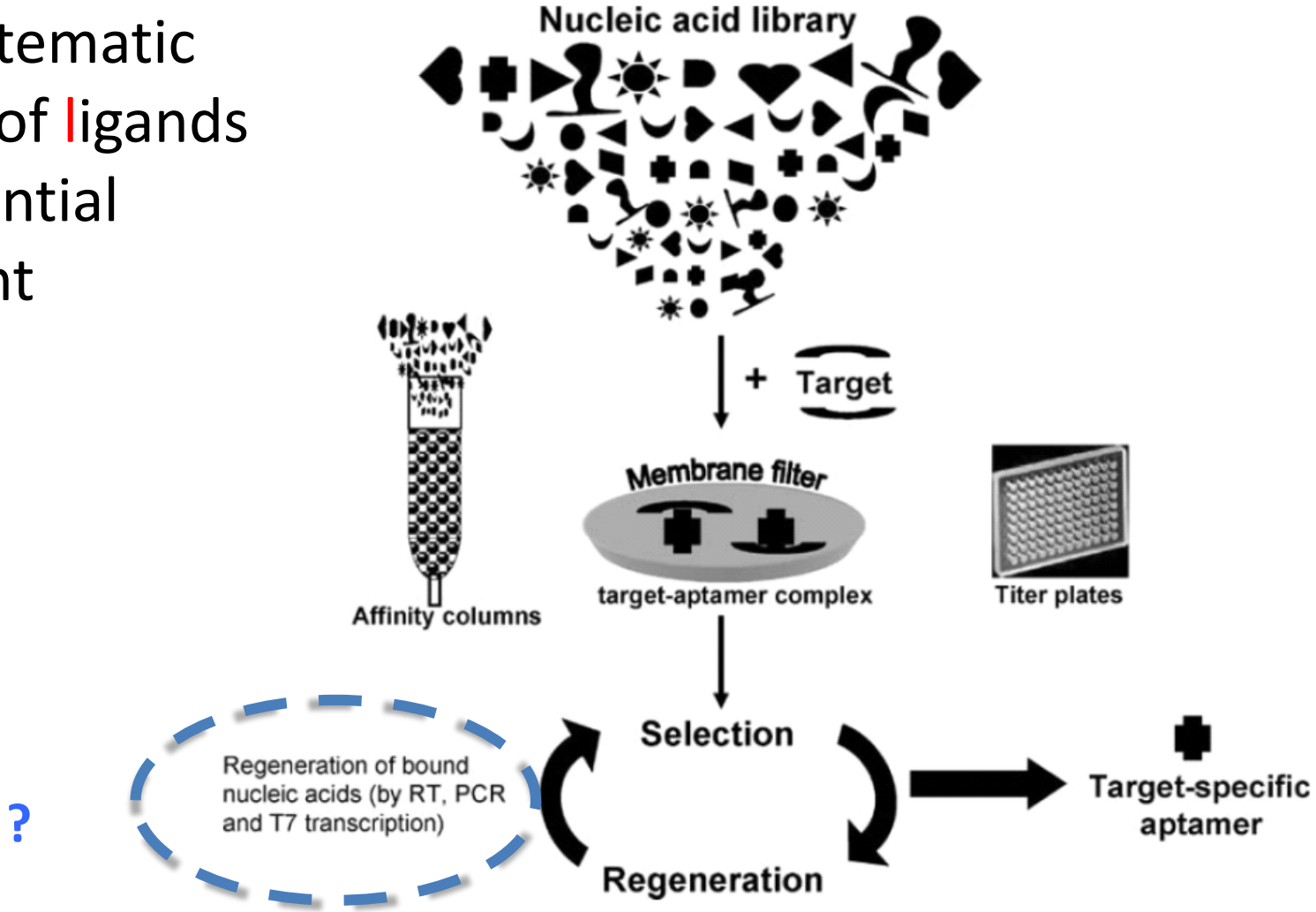


Figure 1. NMR structures of specific aptamers of a large protein and a small molecule

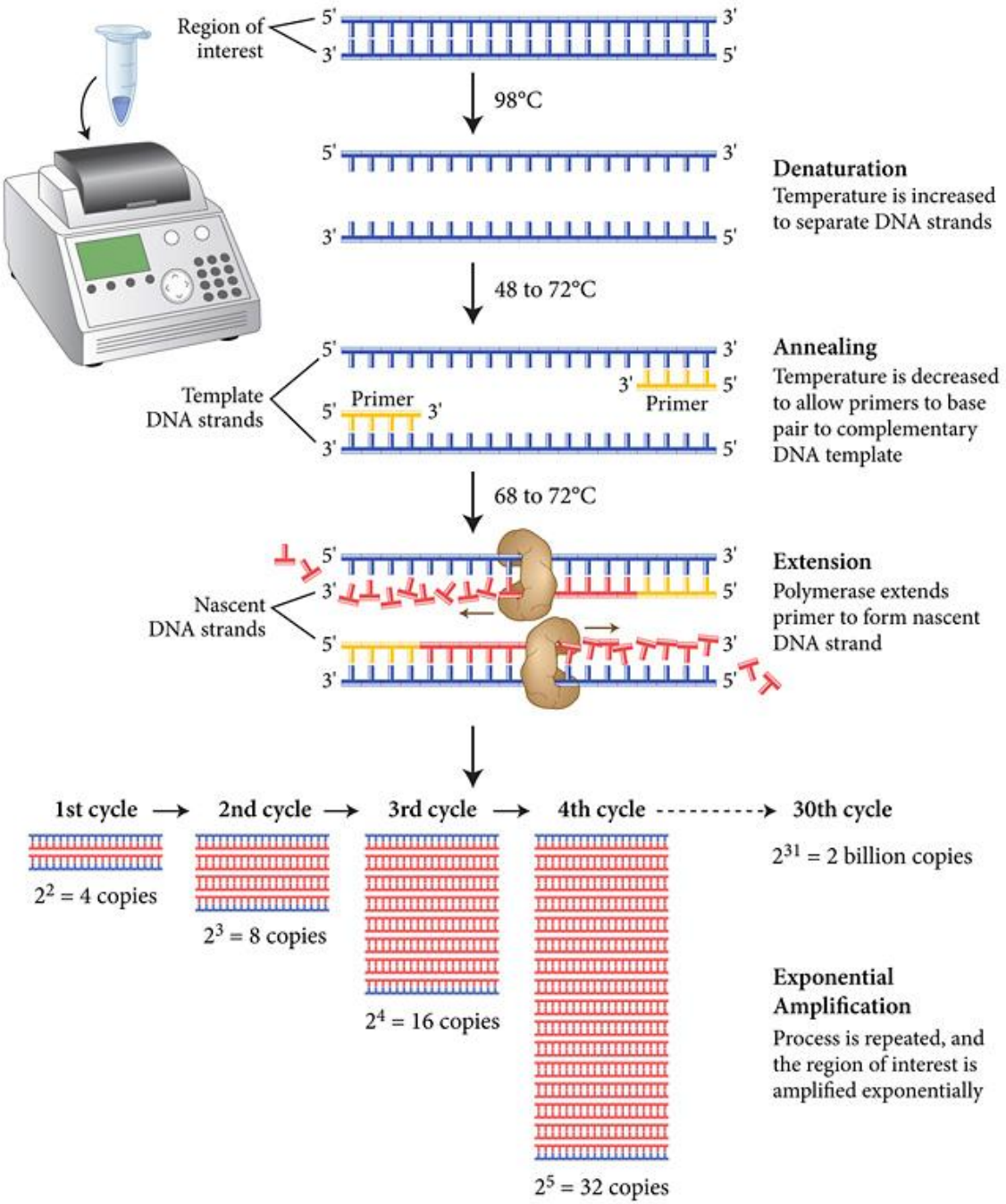
# Aptamers

SELEX: systematic evolution of ligands by exponential enrichment



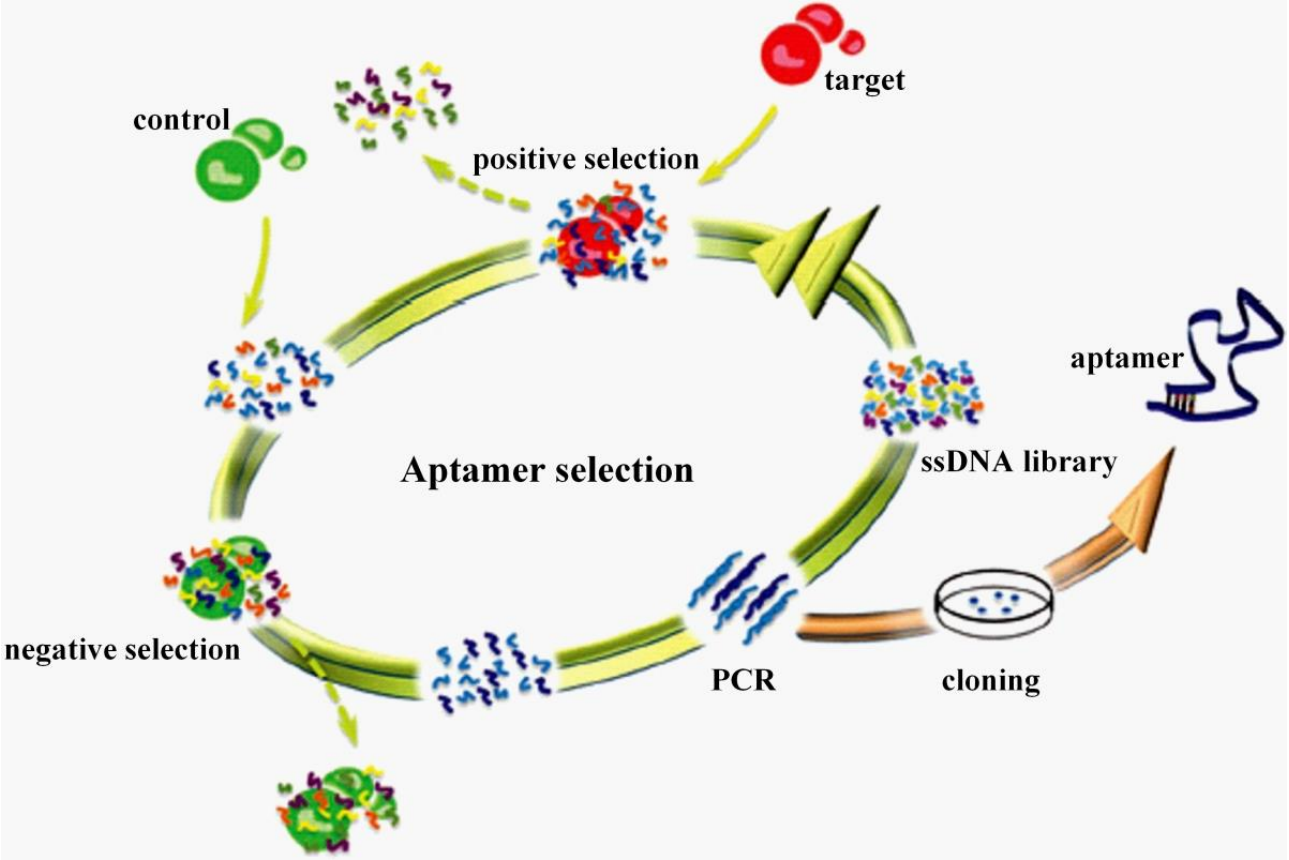

*Gopinath, 2007*

# Principle of Polymerase chain reaction ( PCR)



# Aptamers

Aptamer selection for cancer cells
(*http://sitemaker.umich.edu/ntr/aptamers*)




Positive selection involves the isolation of a target cell population by using an aptamer that specifically binds that population.
Negative selection, however, involves the depletion of all cell types except your cell type of interest

# Aptamer biosensors

**Table 1.** Aptasensor for pathogen-associated molecules detection.

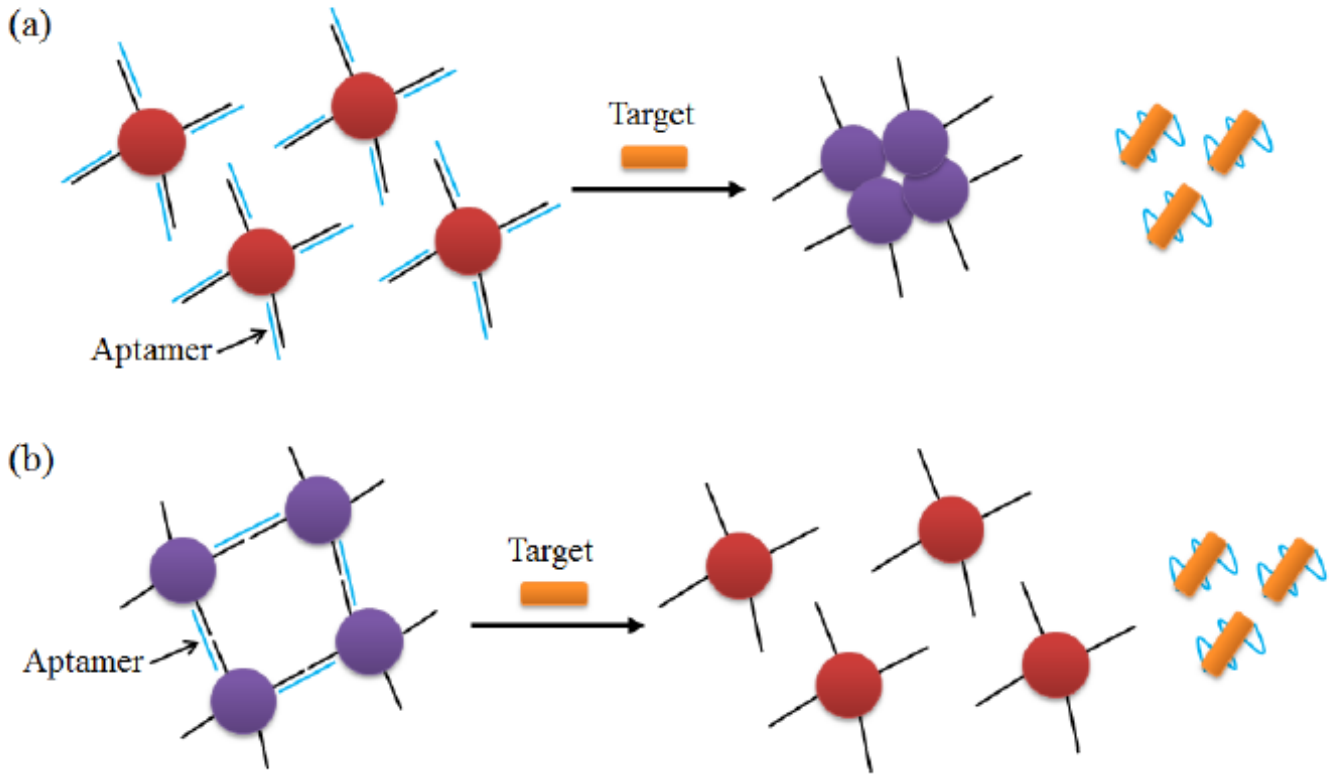
| Target | Aptamer (DNA/RNA) | Method of detection | Reference |
|---|---|---|---|
| Anthrax toxin | DNA | Single-walled carbon nanotubes (label free detection) | Cella *et al.*, 2010 |
| Botulinum neurotoxin | DNA | Electrochemical sensor (label:HRP) | Fang Wei & Chih-Ming Ho, 2009 |
| Cocaine | DNA | MBET aptamer sensor (label:FITC) | Zhou *et al.*, 2012 |
| *E.coli* O157:H7 | RNA | fluorescence microscopy (label:dT-FAM) | Maeng *et al.*, 2012 |
| Lead | DNA | Aptamer-functionalized colloidal photonic crystal hydrogel (CPCH) films | Fen Ye *et al.*, 2012 |
| Mercury | DNA | Graphene oxide-based fluorescent sensor (label-free detection) | Ming Li *et al.*, 2012 |
| *Staphylococcus aureus* | RNA | Fluorescence microscopy (label:dT-FAM) | Maeng *et al.*, 2012 |
| *Salmonella typhimurium* | RNA | Aptamer-immobilized ELISA (label: Biotin) | Seung Ryul Han & Seong-Wook Lee, 2013 |

*Song, 2008 for Aptamer based biosensors*
*Song, 2012 for Aptamers and Their Biological Applications*

# Aptamer biosensors

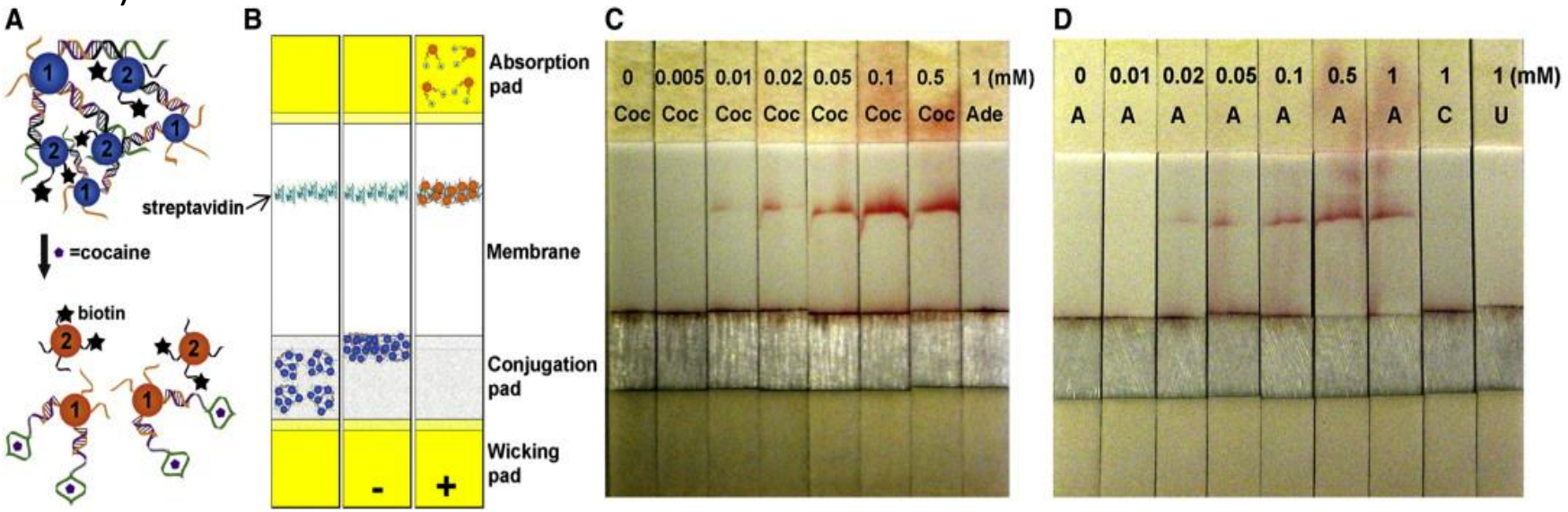
e.g. ATP, cocaine, $Pb^{2+}$ and $K^{+}$ biosensors

**Figure 7.** Schematic illustrations of optical aptasensors using AuNPs. (**a**) Aptamer release and AuNP aggregation by target binding; (**b**) Aptamer release and AuNP disaggregation by target binding.




Colorimetric-Based Optical Aptasensor

# Ex. **Simple dip stick tests based on labeled AuNP-aptamer lateral flow device**(Cocaine detection)




(A) Schematic illustration of cocaine aptamer induced AuNP disassembly and incorporation of such system onto the lateral flow device. Aggregates of AuNPs functionalized with both cocaine aptamer and biotin are disassembled in the presence of cocaine due to the binding between aptamer and cocaine.

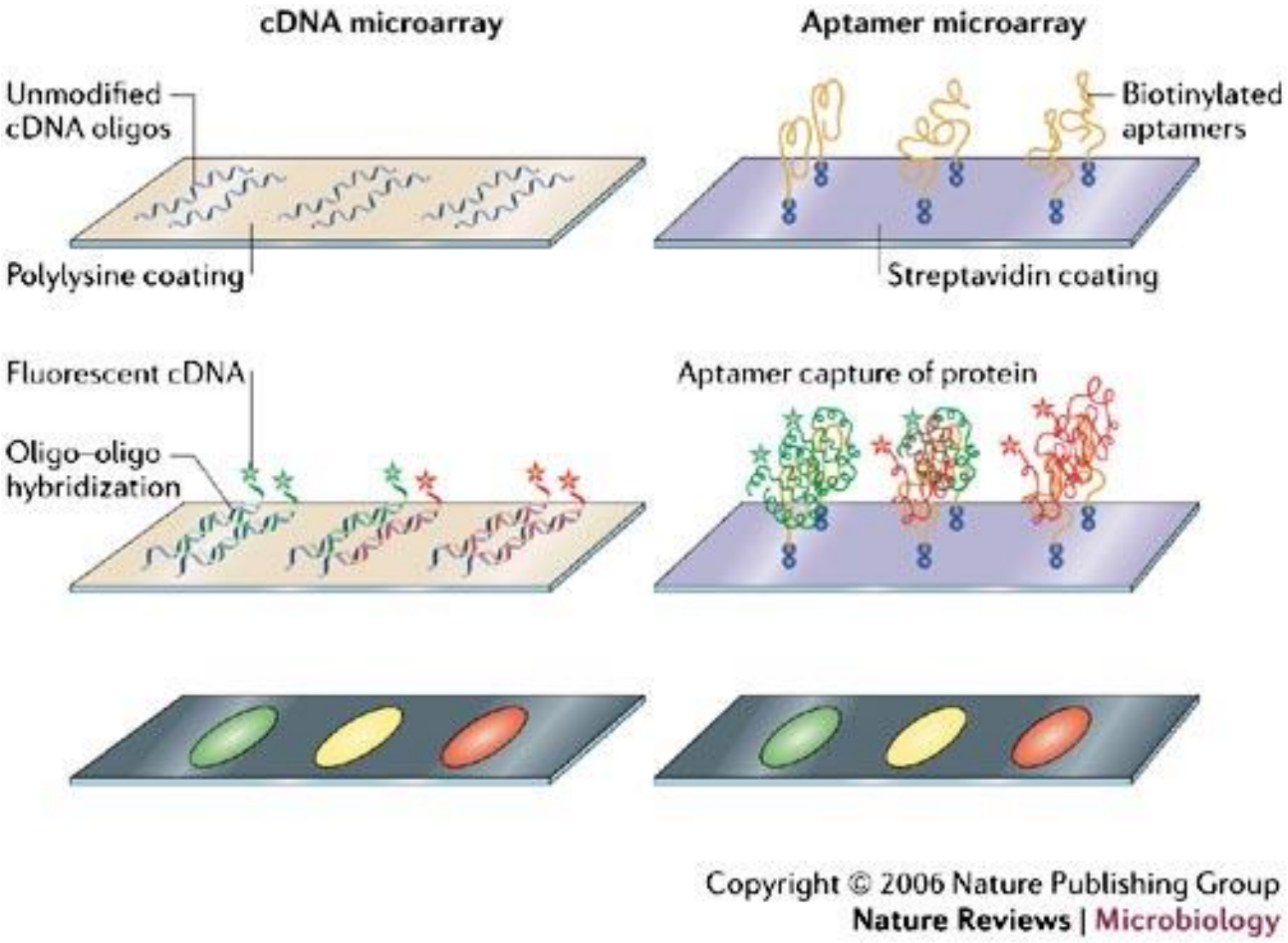
(B) The structure of lateral-flow device; the device is composed of a wicking pad, a glass fiber conjugation pad, a membrane, and an absorption pad. Biotin labeled AuNP aggregates containing cocaine aptamers are dropped onto the conjugation pad and the wicking pad is dipped into a sample solution with cocaine. The solution flows along the conjugation pad, rehydrates and induces disassembly of AuNP aggregates.

(C) The dipstick test for cocaine. In the presence of cocaine, disassembled AuNPs with biotin are captured onto streptavidin immobilized on the membrane, producing a red line.

(D) The lateral flow device can be generally applied with other aptamers, such as adenosine aptamers, for adenosine sensing. Reproduced with permission of ref. 124. Copyright Wiley-VCH Verlag GmbH & Co. KGaA.

J. Liu, D. Mazumdar, Y. Lu A Simple and Sensitive "Dipstick" Test in Serum Based on Lateral Flow Separation of Aptamer-Linked Nanostructures Angew. Chem. Int. Ed., 45 (2006), p. 7955

# Aptamer biosensors





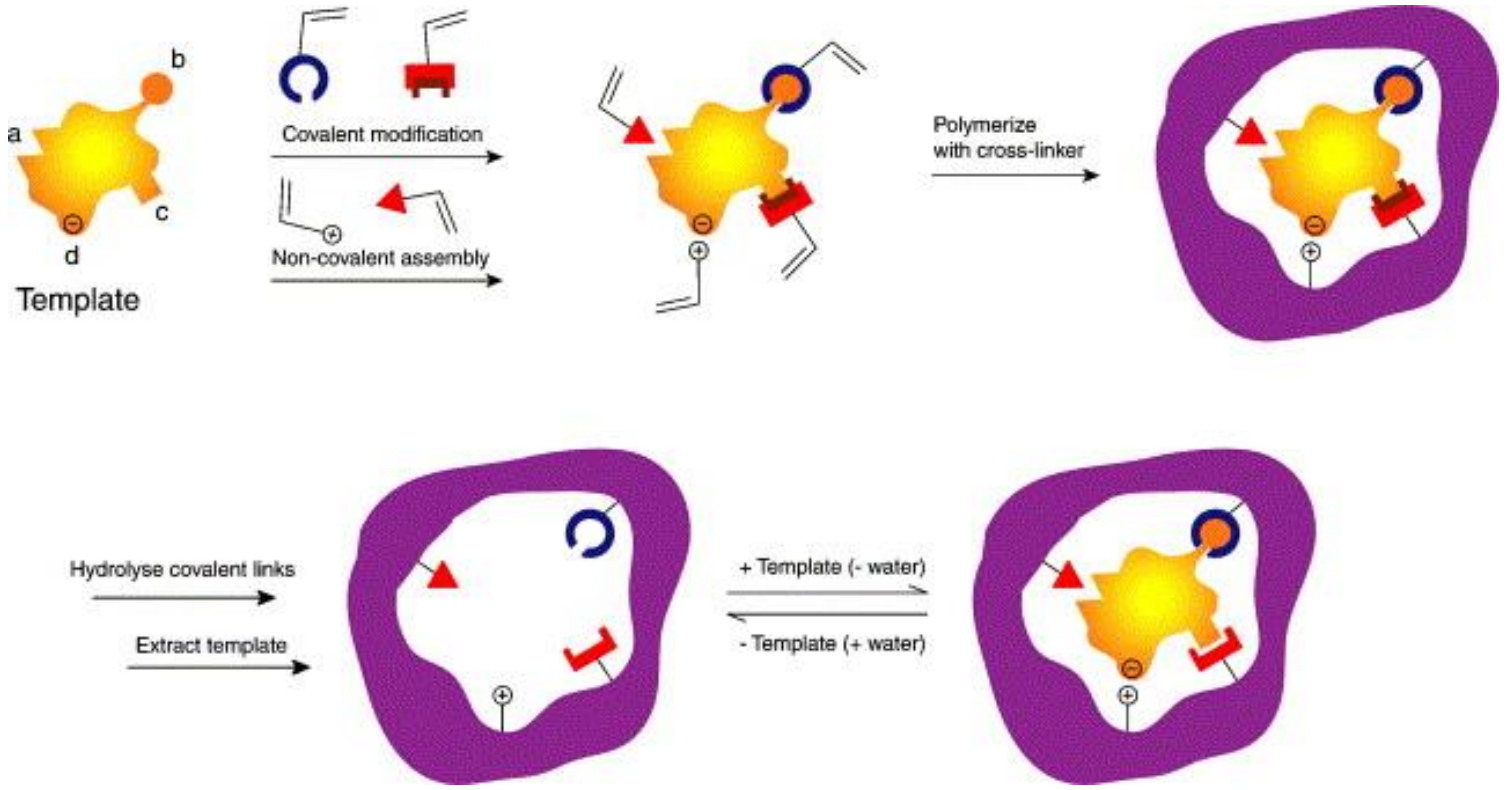
Nature Reviews | Microbiology

*Bunka, 2006*

# Molecularly imprinted polymers (MIP)

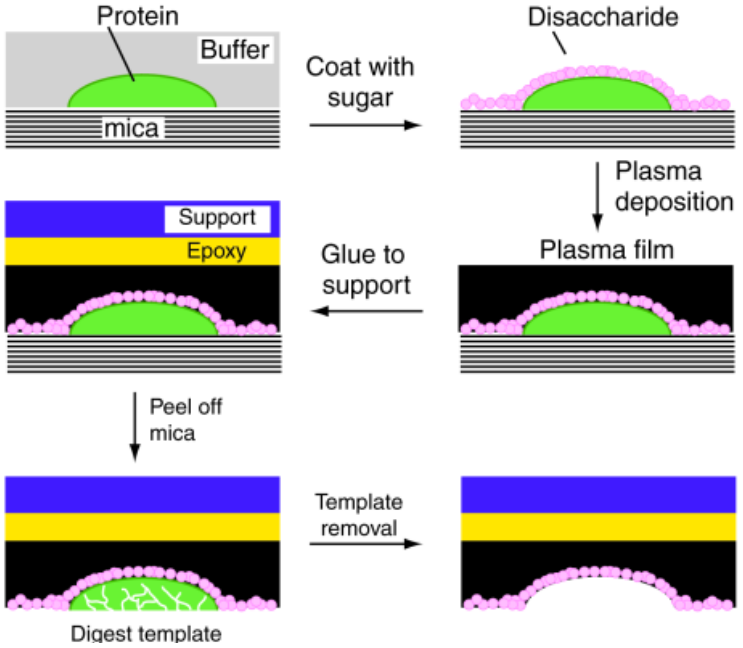
**Bossi A, 2007 & http://nice.asu.edu/nano/molecular-imprinted-polymers-mip**

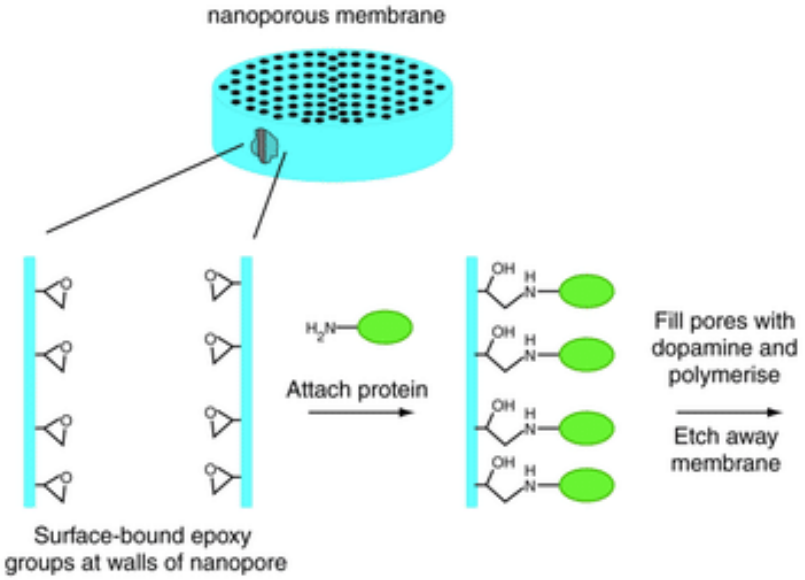
- Creation of **synthetic receptors** with binding constants comparable to natural receptors, but capable of withstanding much harsher conditions
- Also known as biomimetic receptors, synthetic antibodies, plastic receptors/antibodies, etc

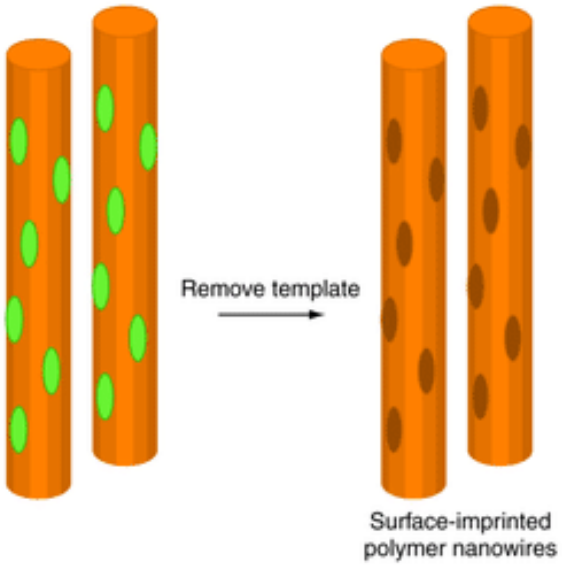


# Molecularly imprinted polymers (MIP)

Some fabrication techniques: see more in *Whitcombe, 2011*



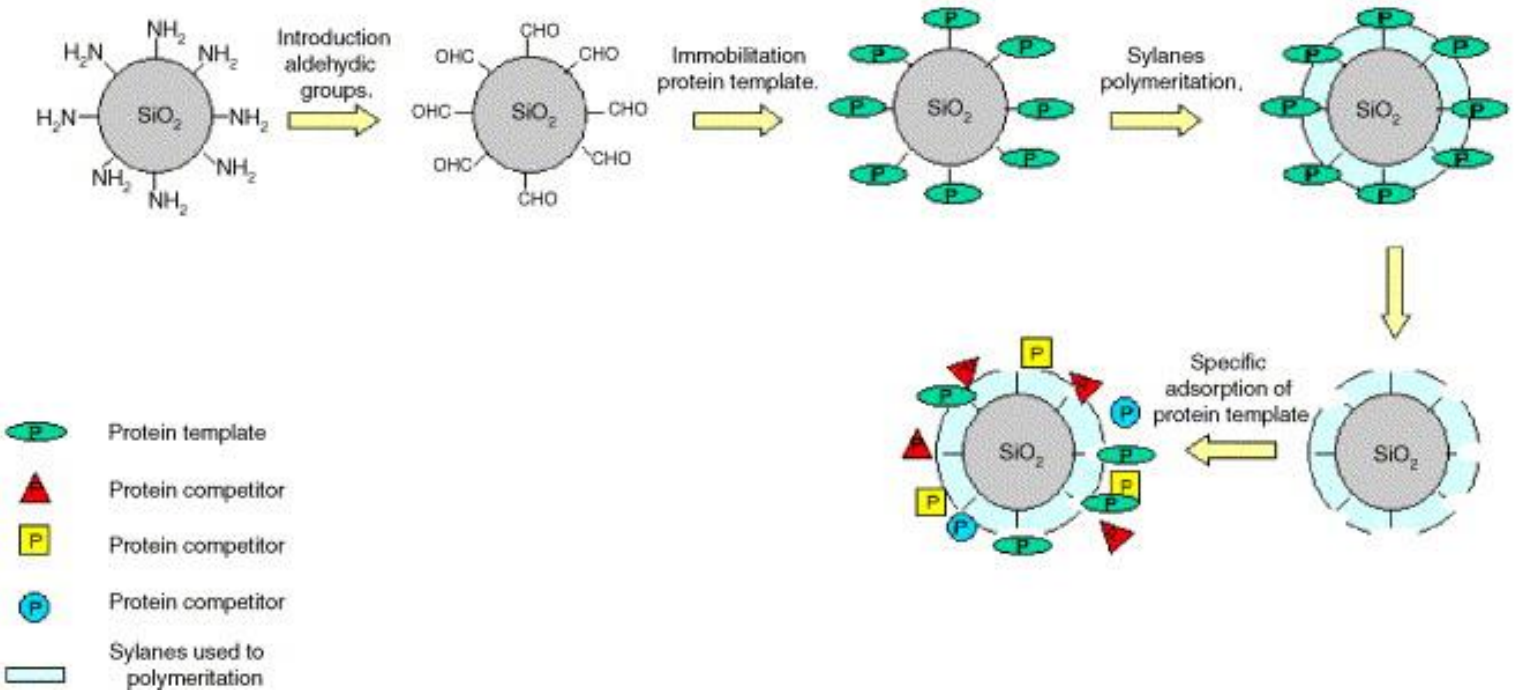



# Molecularly imprinted polymers (MIP)

**Bossi A, 2007 & http://nice.asu.edu/nano/molecular-imprinted-polymers-mip**

Silica-based materials imprinted for protein recognition

- Haemoglobin template is performed
- Competitive binding experiments: MIPs were able to bind the template specifically, while other proteins such as transferin and chymotrypsinogen A, where practically non-absorbed



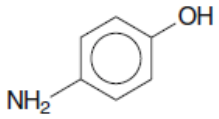
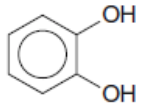
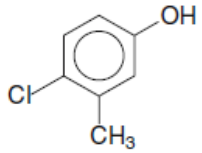
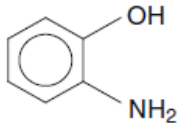
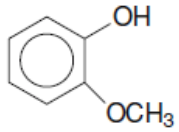
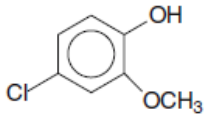
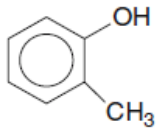
# MIP

Specificity?

P-aminophenol detection

*Santos, 2007*

**Table 5** Relative responses to phenol compounds that are structurally analogous to 4-aminophenol

| Compound | Structures | Current peak/μA | Response (%) |
|---|---|---|---|
| 4-aminophenol |  | 0.459 | 100 |
| Catechol |  | 0.127 | 27.6 |
| 4-chloro-3-methylphenol |  | 0.049 | 10.6 |
| 2-aminophenol |  | 0.028 | 6.1 |
| guaiacol |  | 0.031 | 6.8 |
| chloroguaiachol |  | 0.031 | 6.8 |
| 2-cresol |  | 0.029 | 6.4 |
| mixture of phenol compounds | - | 0.331 | 72.1 |

# MIP

Nicotine biosensor (*Krupadam, 2013*)

- Natural receptor (AChE) excellent selective binding at pH 7.0 but drastic reduction (40-60 %) in others
- MIPs work in wide pH range (6.5-8.9)

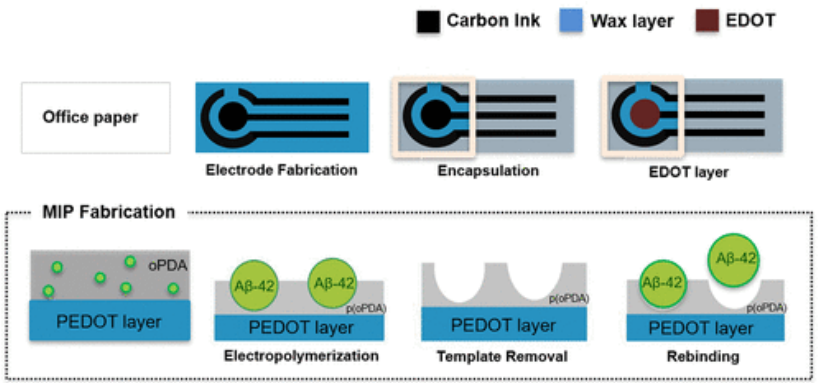
*Table 3. Selective binding of nicotine and its analogues to the nicotine imprinted polymers and AChE.*

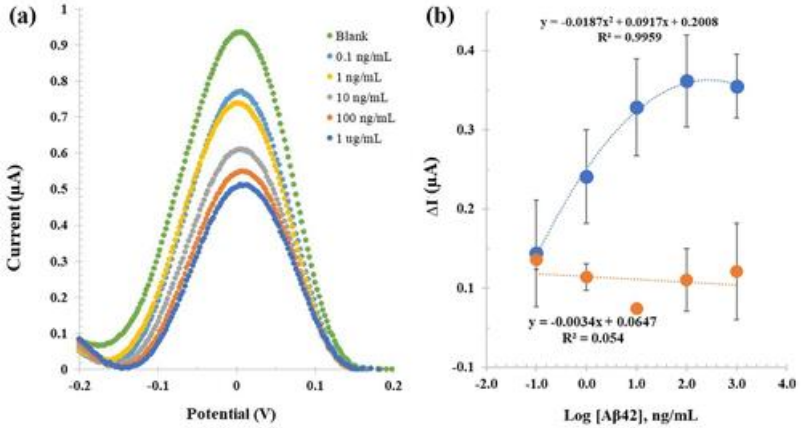
| S.No | Nicotine and its analogues | MIP-1 | MIP-2 | MIP-3 | AChE |
|---|---|---|---|---|---|
| 1 | Nicotine | 100 | 100 | 100 | 100 |
| 2 | Myosine | 28 | 33 | 15 | 22 |
| 3 | Cotinine | 24 | 35 | 12 | 15 |
| 4 | Epinephrine | 17 | 21 | 6 | 11 |
| 5 | Physostigmine | 16 | 18 | 5 | 12 |

# Paper-Based Platform with an In Situ Molecularly Imprinted Polymer for β-Amyloid

May 15, 2020
https://doi.org/10.1021/acsomega.0c00062

- Alzheimer's disease (AD) is one of the most common forms of dementia affecting millions of people worldwide.
- A biosensor using paper as a platform for the electrochemical recognition of peptide amyloid β-42 (Aβ-42), a biomarker for AD present in blood, associated with visible differences in the brain tissue was proposed.
- The sensor layer relies on a molecularly imprinted polymer as a biorecognition element, created on the carbon ink electrode's surface by electropolymerizing a mixture of the target analyte (Aβ-42) at neutral pH 7.2.




SWV measurements of the (a) MIP/CI-HME-based biosensor and the corresponding calibration curve (b) and MIP (blue dots) and NIP sensing layer (orange dots) calibration curve. Different concentrations of Aβ-42 (ng/mL) in PBS buffer.

# MIP vs Natural Biomolecules

| Natural Biomolecules | MIPs |
|---|---|
| Low stability | High chemical and physical stability |
| High price | Inexpensive |
| Poor performance in non-aq. media | Work in organic solvents |
| Different operational requirement (pH, T) | Minimal operational requirements |
| For limited number of analytes | Virtually for any compound |
| Poor compatibility with micromachining technology and miniaturisation | Polymers are fully compatible with micromachining technology |
| Soluble | Most of them are insoluble |

# Antigen Arrays

*Robinson WH (2006)*

Production of high-affinity antibodies is a hallmark of many autoimmune and infectious diseases

- **Autoimmune disease**

  e.g. Detection of blood autoantibodies in the diagnosis of rheumatoid arthritis (RA)

- **Cancer** (certain anti-tumor antibody responses can predate the development of clinically detectable disease)

  e.g. 22 peptides were identified as targets of the antibody response in prostate cancer. Blood antibody reactivity against these 22 peptides provided 82% sensitivity and 88% specificity for the diagnosis of prostate cancer

- **Infectious disease**

  e.g. Infections with Epstein Barr virus (EBV), hepatitis B virus (HBV) and human immunodeficiency virus (HIV), can be diagnosed by detection of host antibody responses

- **Allergic disease**

# Antigen Arrays

*Robinson WH (2006)*

What will be the outcome of profiling of autoantibodies in an antigen array in autoimmune disease??

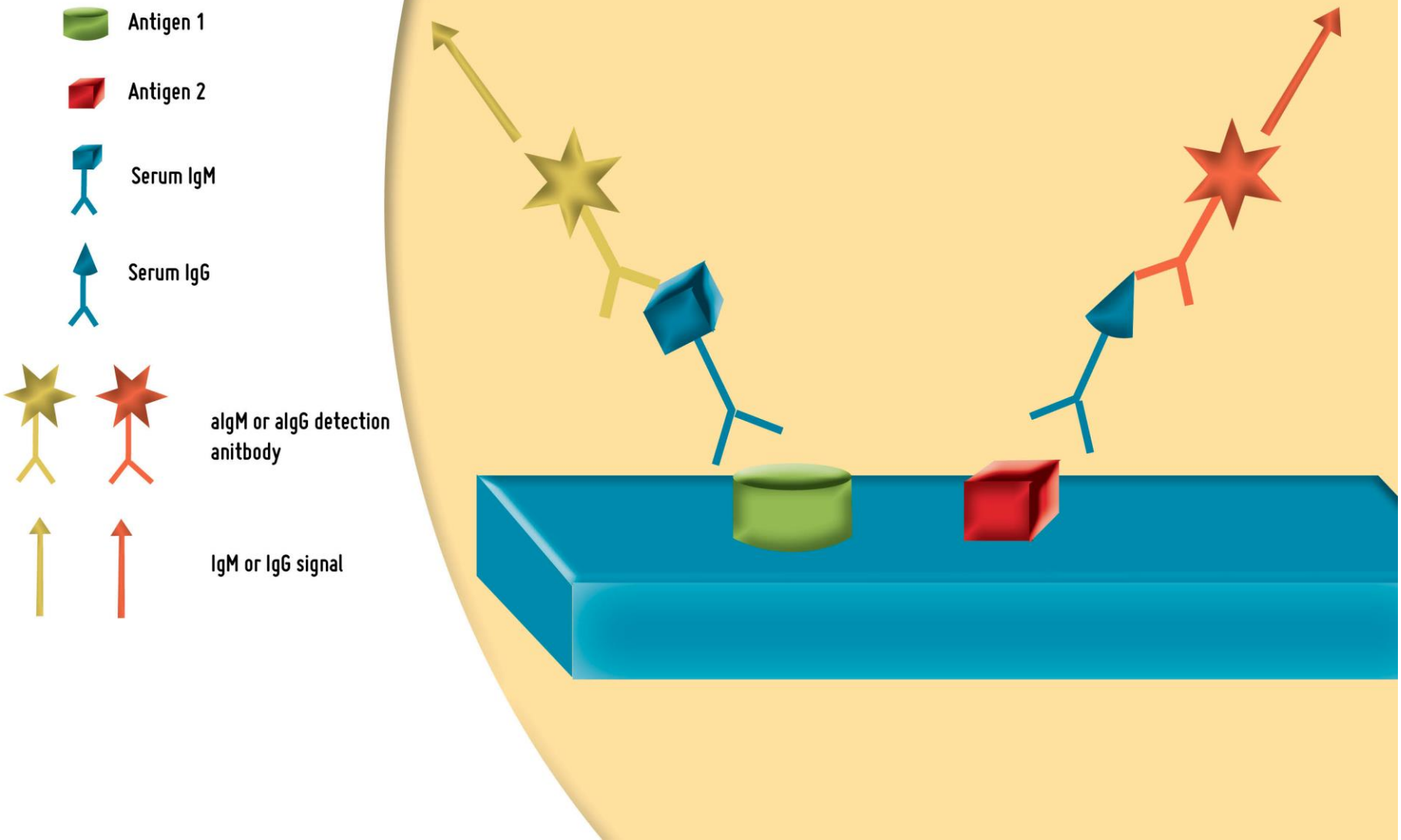
(i) identification of molecular subtypes of disease based on differences in the specificity of the autoimmune response;

(ii) identification of the pre-disease autoantigen targets, thereby providing insights into the mechanisms underlying the initiation of autoimmunity;

(iii) application of antibody profiles to develop and guide antigen-specific therapy

- Diagnostic and prognostic utility

# Antigen Arrays



# Lipid microarrays

***Kanter, 2006***

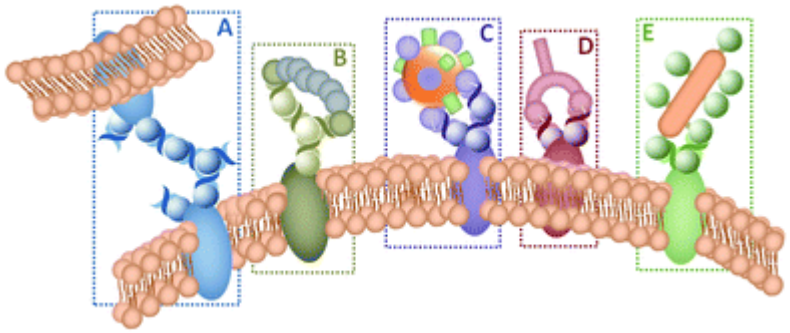
Identify anti-lipid antibodies in multiple sclerosis (MS)

- lipid microarrays containing 50 distinct brain and myelin lipids
- myelin arrays are used to profile anti-lipid antibody responses in cerebral spinal fluid (CSF) derived from a MS and 10 other neurologic disease control patient samples

➢ autoimmune responses to sulfatide and other lipids are present in individuals with multiple sclerosis

# Carbohydrate Arrays

***Pashkuleva and Reis, 2010***




Carbohydrates in recognition events with another cell (A), toxins (B), viruses (C), antibodies (D) and bacteria (E)

to provide both satisfying selectivity and quantitative performance:

(i) carbohydrates must be present in a regular and homogeneous manner so that all immobilized ligands have equal activity toward the analytes present in the solution;

(ii) the density of the immobilised carbohydrates must be very well controlled: many carbohydrate-analyte interactions are polyvalent in nature and their affinity is extremely sensitive to the density and orientation of individual carbohydrates

*Wang, 2002*; polysaccharides and other microbe-derived molecules serve as the main antigenic structures which host cells recognize and mount a response

# Lectin biosensor

***Rahaie and Kazami, 2010***

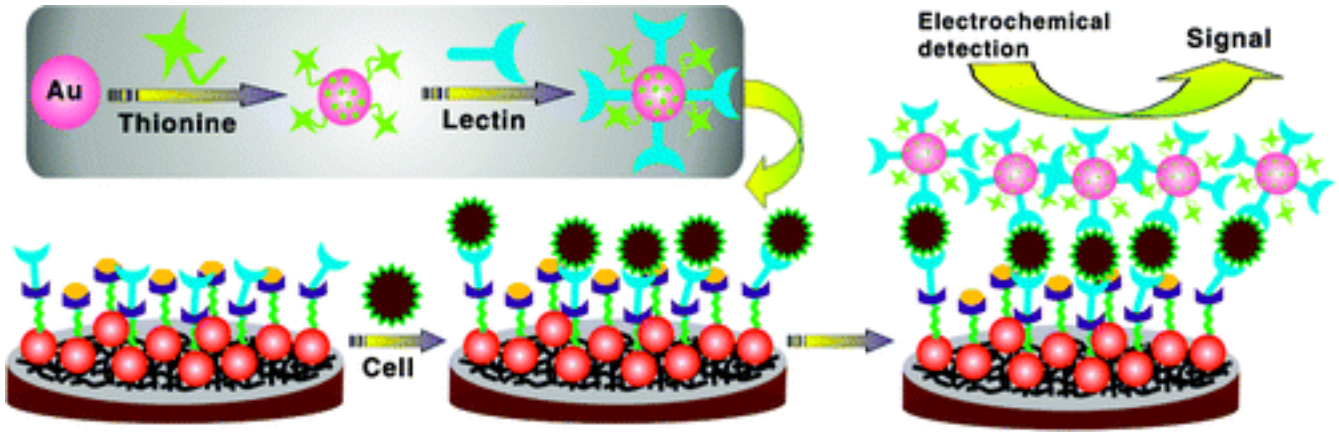
- **Lectins:** A protein family that generally exhibit strong binding to specific carbohydrate moieties (glycans)
- In addition, particular structural profiles of glycans and their recognition by lectins are attributed to disease progression → diagnostic tool
- Applications in cancer cell and pathogen detection

# Lectin biosensor

**Zhang, 2010**

- Lectin-based biosensor to detect cancer-associated glycosylation
- Mannose and sialic acid expression on normal and cancer cells derived from human lung, liver, and prostate.
- Sandwich format: combining the lectin-based biosensor with the {lectin−Au−Th} bioconjugates for signal amplification.

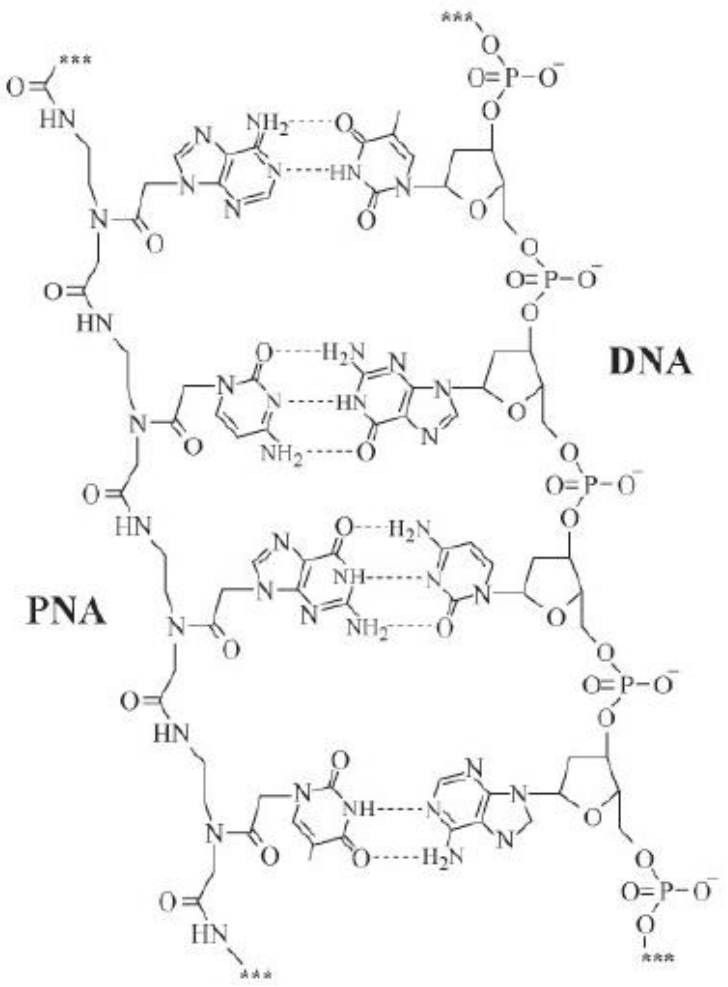
➢ The proposed strategy demonstrated that mannose exhibited high expression levels in both normal and cancer cells, while sialic acid was more abundant in cancer cells as compared to normal ones.



# Peptide Nucleic Acids (PNAs)

- PNAs are DNA mimics in which a peptide-like repeat of (2-aminoethyl) glycine unit
- Bind to their complementary nucleic acid sequences with higher thermal stability and specificity than the corresponding deoxyribooligonucleotides
- However, they are more destabilized by single-base mismatches than are DNA/DNA hybrids

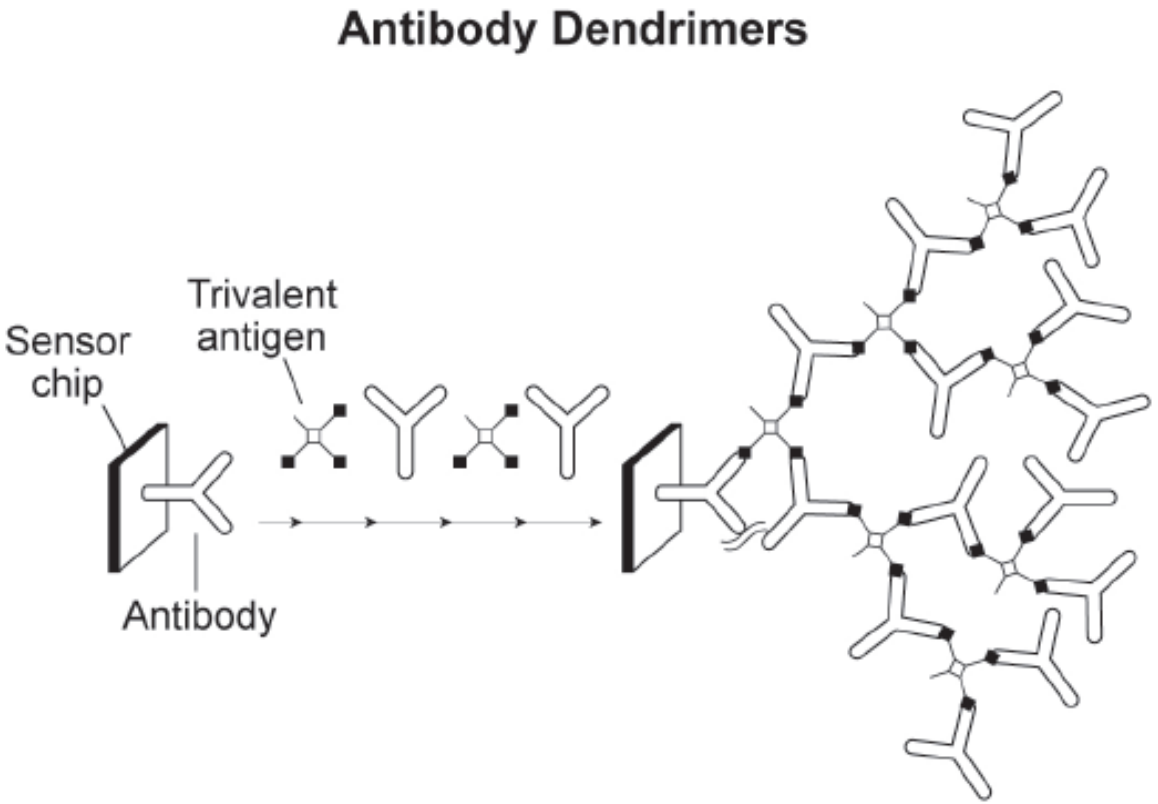
➢ PNA biosensors in stead of DNA sensors for higher selectivity



# New Approaches using Known Materials

Yamaguchi and Harada, 2003

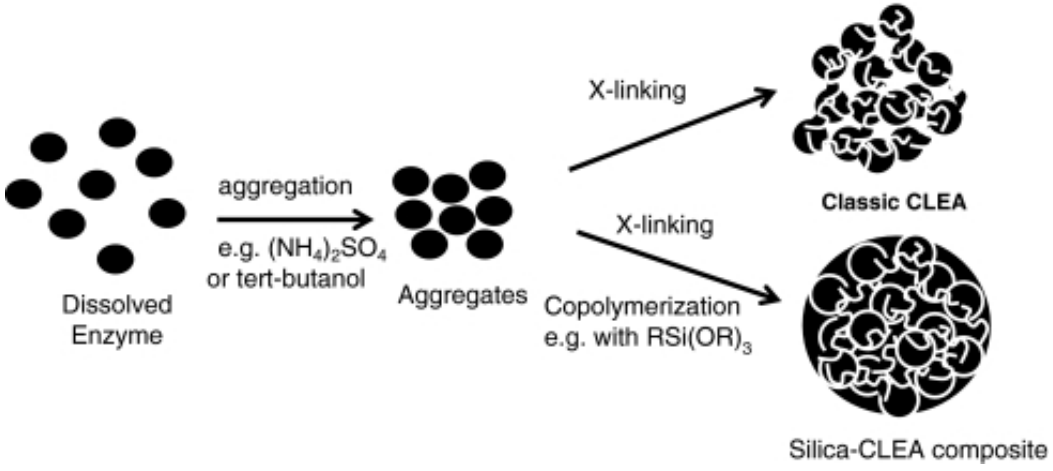
To increase the signal intensity



# New Approaches using Known Materials

***Cross-linked enzyme aggregates (CLEAs) and Cross-linked enzyme crystals (CLECs)***

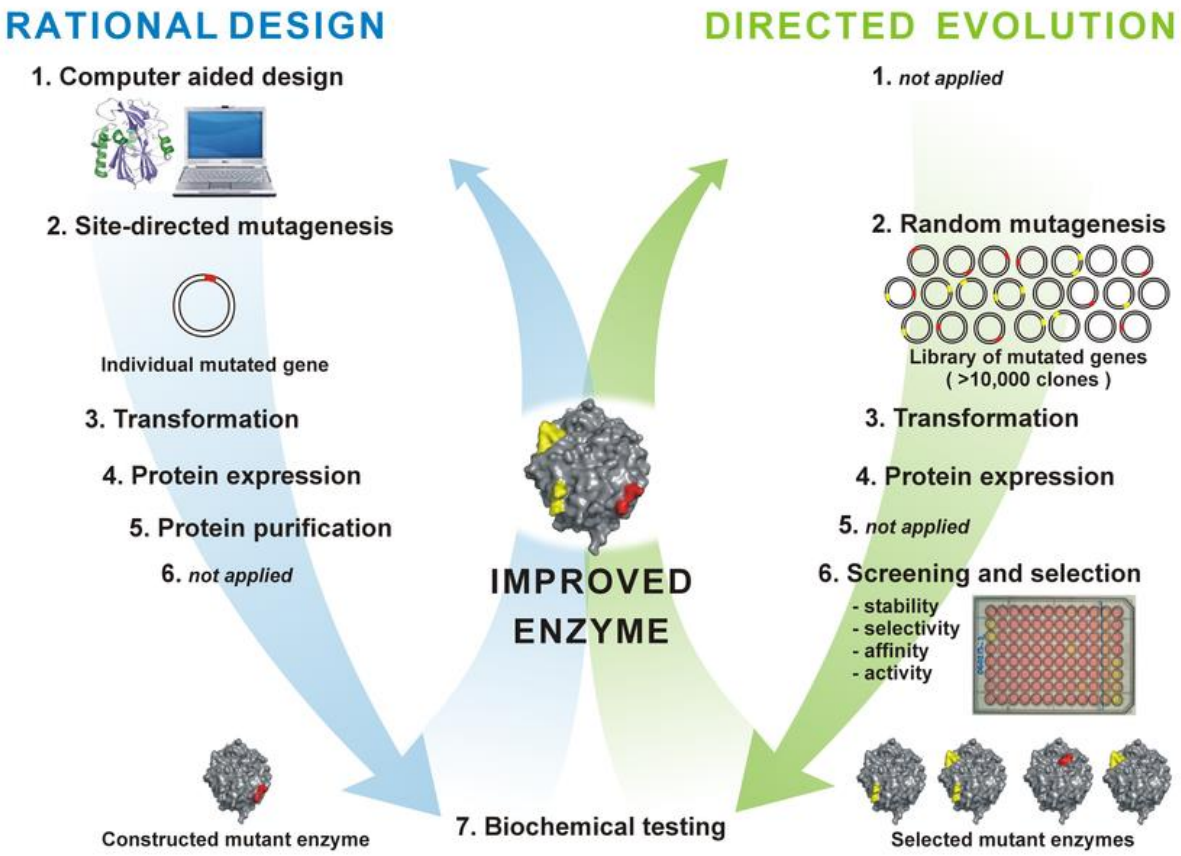
- easily prepared from crude enzyme extracts,
- generally exhibit improved storage and operational stability towards denaturation by heat, organic solvents, and autoproteolysis
- possibility to co-immobilize two or more enzymes to provide CLEAs that are capable of catalyzing multiple biotransformations, independently or in sequence as catalytic cascade processes.



# New Approaches using Known Materials

**Protein engineering**: proteins are engineered at the genetic level to design new protein molecules either from scratch or by making calculated variations on a known structure.

- **In rational protein design** - detailed knowledge of the structure and function of the protein is used to make desired changes.
- **In directed evolution** (non-rational design), random mutagenesis is applied to a protein, and a selection regime is used to pick out variants that have the desired qualities




For a better recognition molecule:

- Improve protein stability
- Modify cofactor requirement
- Increase enzyme activity
- Modify enzyme specificity