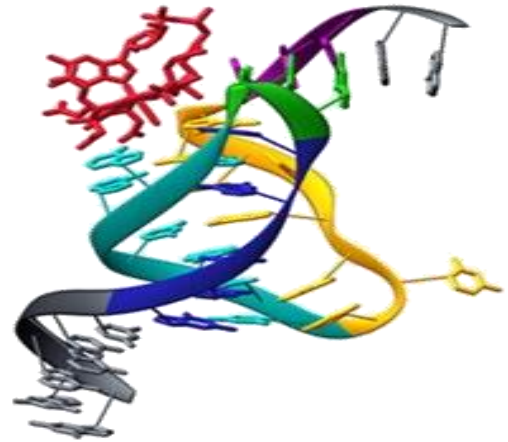
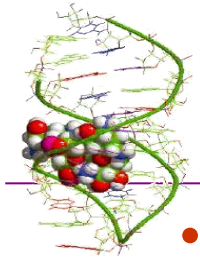
Department of
Bacteriology &Virology
medicine

isfahan university
of medical science

Use of Aptamer to detect pathogenic bacteria

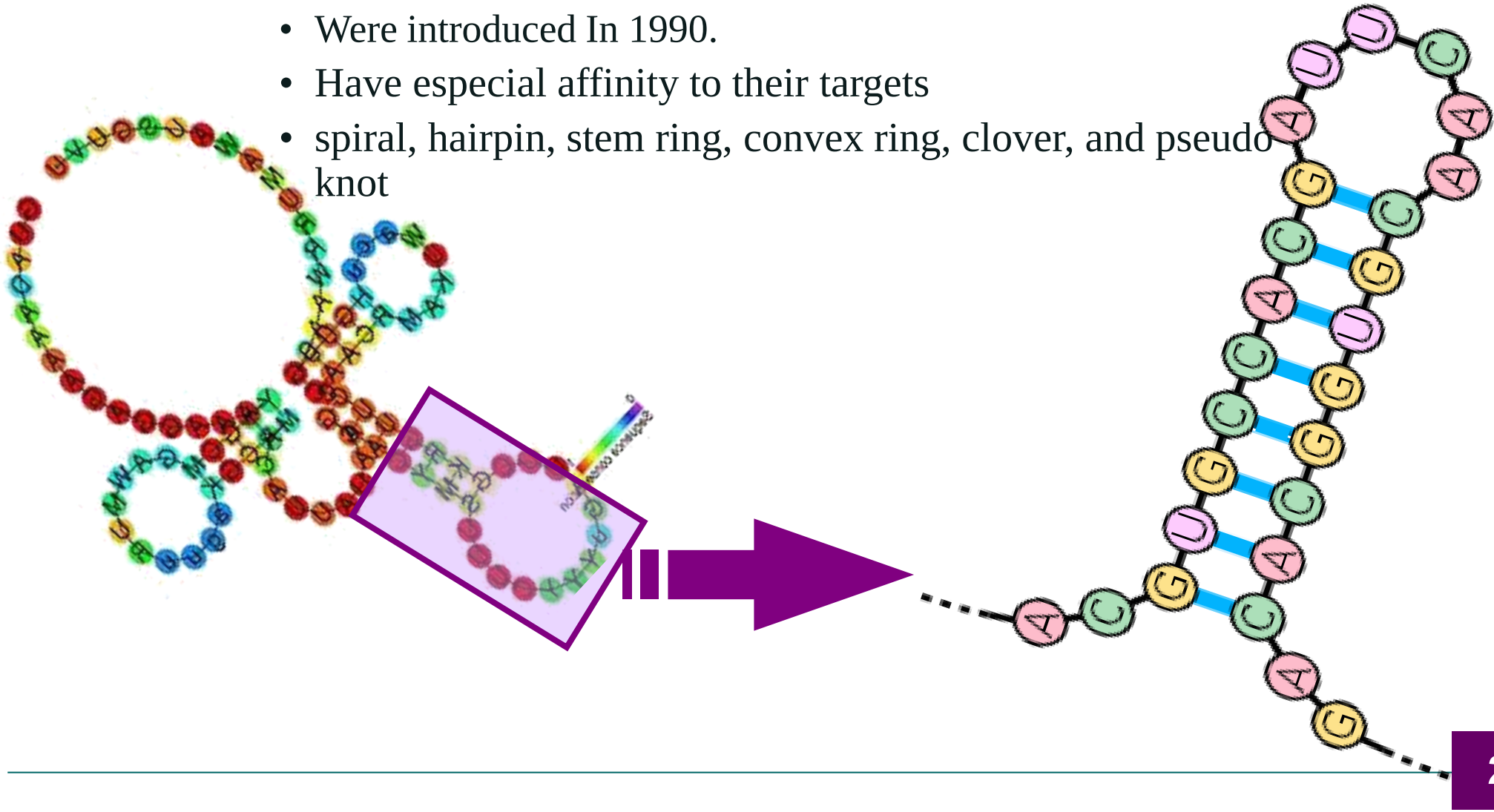


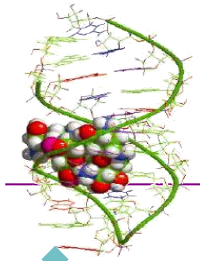
By : Arezoo Fallah



What is an aptamer?

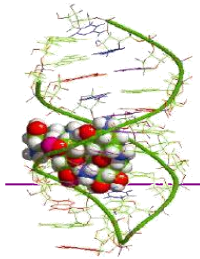
- Aptamers are ssDNA or RNA oligonucleotides
  - Were introduced In 1990.
  - Have especial affinity to their targets
  - spiral, hairpin, stem ring, convex ring, clover, and pseudo knot



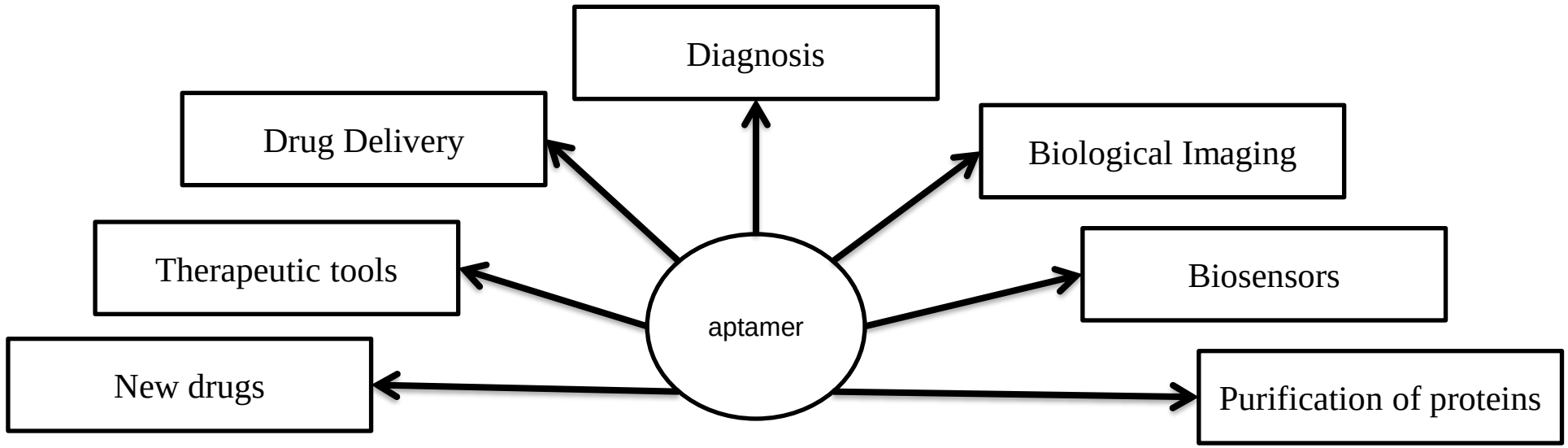


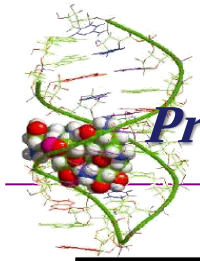
History

- Aptamers are synthetic, highly structured, single stranded DNA/RNA ligands
- Term “Aptamer”(Ellington&Szostak 1990) : *aptus* (to fit) + *mer* (part)
- The first SELEX experiment on single- stranded oligonucleotides was published by Tuerk and Gold in 1990
- 'Systematic evolution of ligands by exponential enrichment' (SELEX)
- A protocol in which vast libraries of single-stranded oligonucleotides are screened for desired activities



Different Applications of Aptamers

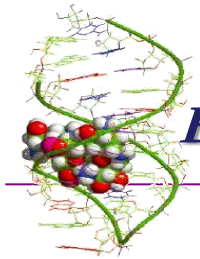




Progress of aptamers for diseases' therapy in on-going or completed clinical trials

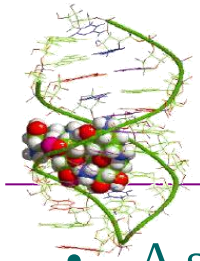
Table 4. Progress of aptamers for diseases' therapy in on-going or completed clinical trials.

Name	Form	Target	Condition	Phase
Pegaptanib sodium (Macugen)	27-nt RNA	VEGF (Vascular Endothelial Growth Factor)	Age-related macular degeneration	Approved
E10030	29-nt DNA	PDGF (Platelet-Derived Growth Factor)	Age-related macular degeneration	Phase III
REG1 (RB006 and RB007)	37-nt RNA	Coagulation factor IXa	Coronary artery disease	Phase III
ARC1905	38-nt RNA	C5 (Complement component 5)	Age-related macular degeneration	Phase III
AS1411	26-nt DNA	Nucleolin	Acute myeloid leukemia	Phase II
ARC1779	39-nt DNA	A1 domain of von Willebrand factor	Von Willebrand disease/thrombotic thrombocytopenic/purpura	Phase II
NOX-E36	40-nt RNA	CCL2 (Chemokine C-C motif Ligand 2)	Chronic inflammatory diseases/type 2 diabetes mellitus/systemic lupus erythematous	Phase II
NOX-A12	45-nt RNA	CXCL12 (Chemokine C-X-C motif Ligand 12)	Multiple myeloma and non-Hodgkin lymphoma/autologous or hematopoietic stem cell transplantation	Phase II
NU172	26-nt DNA	Thrombin	Heart disease	Phase II
NOX-H94	44-nt RNA	Hepcidin peptide hormone	Anemia/end-stage renal disease/inflammation	Phase II
ARC19499	32-nt RNA	TFPI (Tissue Factor Pathway Inhibitor)	Hemophilia	Phase I



Examples of recently developed aptamers for the diagnosis of human diseases.

Name	Target	K_d (nM)	Sensitivity	Specificity
		Cancers		
XL-33	Metastatic colon cancer cells (SW620)	0.7	81.7% (n = 71 metastatic colon cancer tissues)	66.7% (n = 18 non-metastatic colon cancer tissues)
yl19	Cholangiocarcinoma cells (QBC-939)	42.4	-	100% (n = 6 cancer cell lines)
LXL-1	Metastatic breast cancer cells (MDA-MB-231)	44.0	76% (n = 34)	100% (n = 8 cancer cell lines)



A schematically represented biosensor

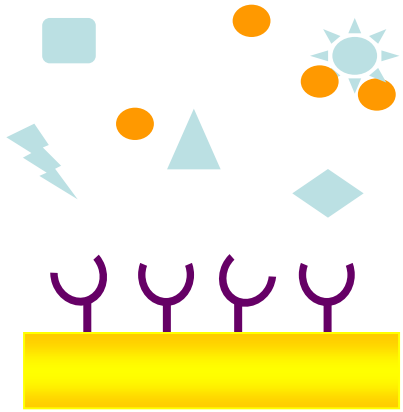
- A sensor is a device that transforms environmental information, ranging from the concentration of a specific sample component to total composition analysis, into an analytically useful signal.

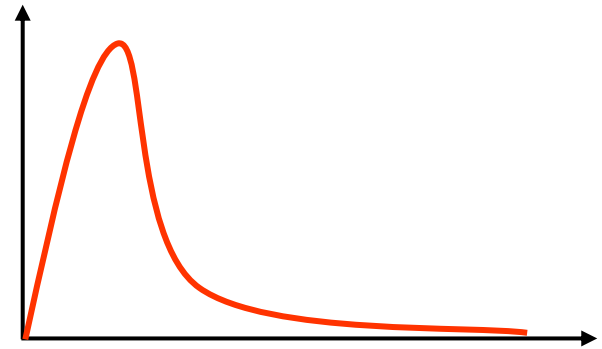
Recognition Part

- Antibody
- Enzyme
- Aptamer

Transducer

- Electrochemical
- Thermal
- Optical
- Mass Changes

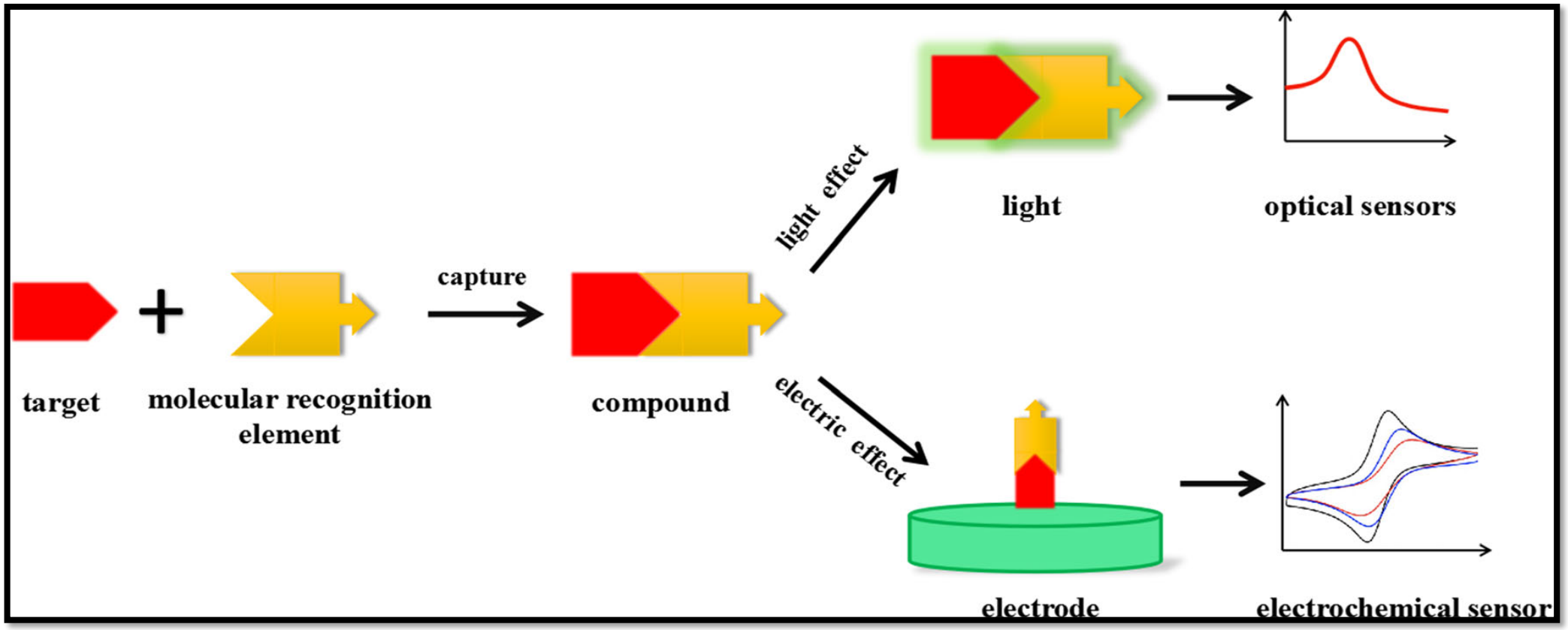




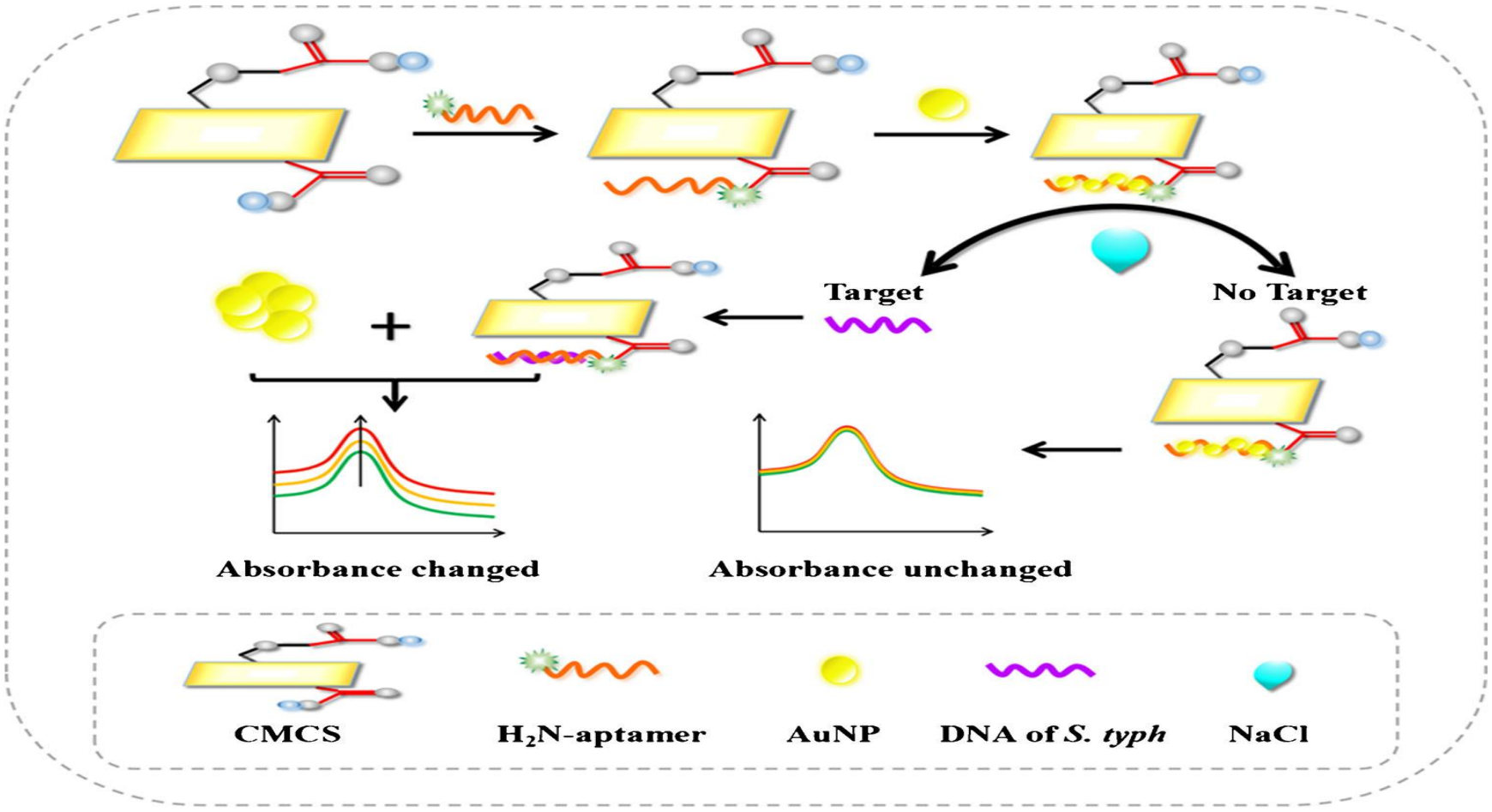
Receptor Transducer Recorder

Combination of aptamer and biosensor

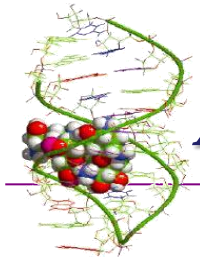
- principle of optical biosensor and electrochemical biosensor



optical aptamer biosensor

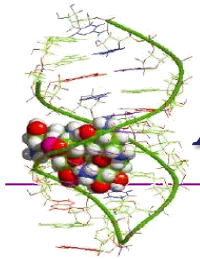


The schematic diagram of optical aptamer biosensor for detecting S. typimurium.
Reprinted with permission from Yi et al. (2019)



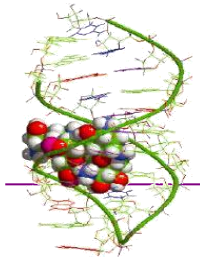
Aptamers versus antibodies

آنتی بادی	آپتامر	خصوصیات
بازه بین pM تا µM	بازه بین pM تا µM	**میل ترکیبی اتصال**
• فرایند تولید نیازمند یک سیستم بیولوژیک است • وجود آلودگی در فرایند تولید ممکن است بر روی کیفیت محصول نهایی تاثیر بگذارد • تولید آنتی بادی علیه یک ماده سمی که برای حیوان قابل تحمل نیست دشوار است • برای تولید آنتی بادی علیه اهداف غیر ایمونوژن می بایست اهداف را کانژوگه نمود یا به نحوی تغییر داد.	• شیمیایی • قابل سنتز در مقیاس بالا • نیازمند دورهای تکراری در آزمایشگاه جهت جداسازی یک آپتامراختصاصی علیه یک هدف شناخته شده • قابل جداسازی علیه تقریبا هر نوع هدفی • مقرون به صرفه	**فرایند تولید**
• ایمونوژنیسیتی بالا	• غیر ایمونوژنیک	**ایمنی زایی**



Aptamers versus antibodies

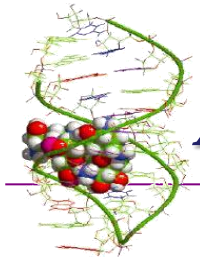
خصوصیات	آپتامر	آنتی بادی
پایداری	• مقاوم به حرارت • قابلیت بازگشت به کانفورماسیون اصلی پس از کاهش درجه حرارت • پایداری در مقابل سیکل‌های دناتوراسیون/رناتوراسیون • حساس به نوکلئاز	• حساس به حرارت • دناتوراسیون غیر قابل برگشت در مواجهه با شرایط سخت • مقاوم در برابر تجزیه نوکلئاز
زمان ماندگاری	• نگهداری راحت و آسان در حالت پودر لیوفیلیزه یا نگهداری به عنوان محلول در منهای ۲۰ درجه برای مدت نامحدود	• زمان ماندگاری محدود
کانفورماسیون	• بدون ساختار در فضای محلول و فولد شدن به یک ساختار سه بعدی پس از اتصال	• دارای ساختار معین حتی بدون حضور لیگاند
اعمال تغییرات شیمیایی در یک ناحیه خاص	• فرایند ساده و آسان اعمال تغییرات • نشاندار کردن/کانژوگاسیون مواد شیمیایی هم در حین سنتز و هم پس از سنتز قابل انجام است	• محدودیت در اعمال تغییرات شیمیایی
تثبیت	• تثبیت ساده هم از ناحیه '۵ و هم از ناحیه '۳	• معمولا جهت گیری های مولکول آنتی بادی به صورت تصادفی دیده می شود
اندازه	• اندازه کوچک به آپتامر اجازه می دهد به راحتی در ساختارهای بیولوژیک وارد شوند. • حساس به فیلتراسیون کلیه/ نیازمند تغییرات (مودیفیکاسیون) بیشتر نظیر پگیلاسیون (PEG) می باشد.	• اندازه بزرگ اجازه فیلتراسیون کلیه را نمی دهد اما همین اندازه بزرگ از سویی دیگر در دسترس قرار گیری آنتی بادی را برای بسیار از ساختارهای بیولوژیکی مهار می‌کند



Appropriate specificity

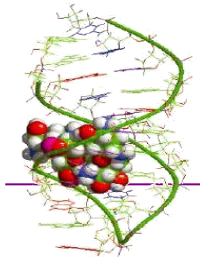
Select for the desired target form.

Small size of aptamers:
Used to bind to multiple epitopes simultaneously without physical constraint.

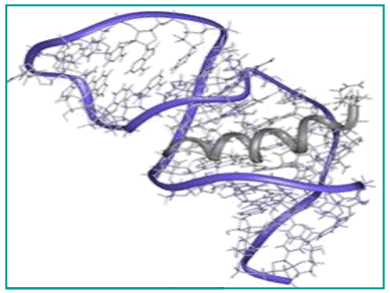


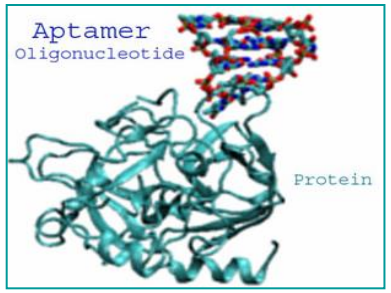
Aptamer advantages for diagnostic producers.

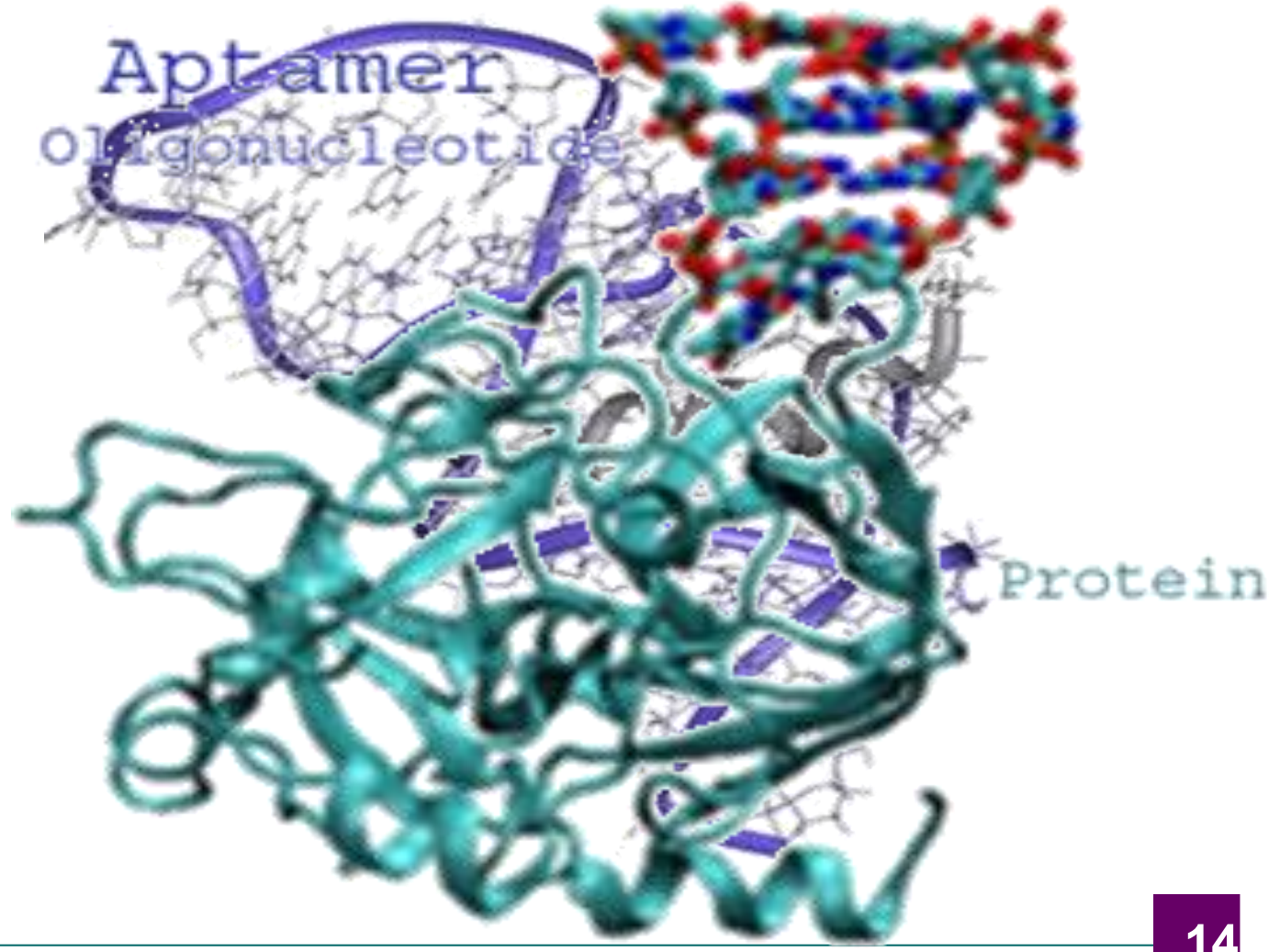
	Aptamers	Antibodies
Production time	1 week	6 months
Quality assurance	simple	extensive
shipping	ambient	-4C
Target Range	Wide: Ions, Cell, Toxins	Narrow: Immunogenics
Chemical Modification	Easy	Limited

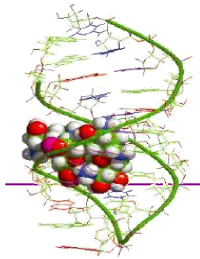


Aptamer-target interaction





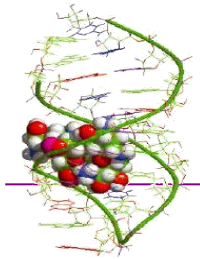




Aptamer-target interaction

❖The binding forces mediating the aptamer–target interactions, such as

- **hydrogen bonding**
- **electrostatic interaction**
- **hydrophobic effect**
- **vanderWaals forces**

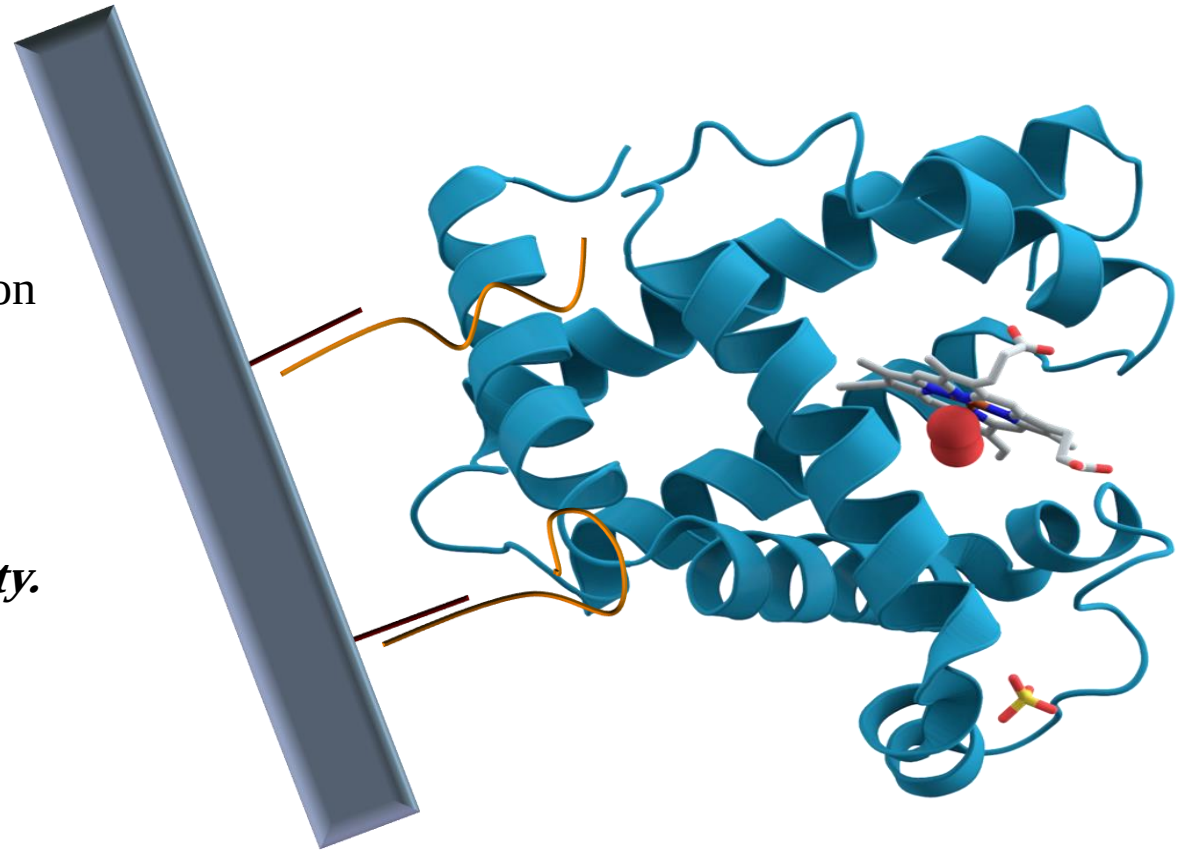


Combined capture aptamers

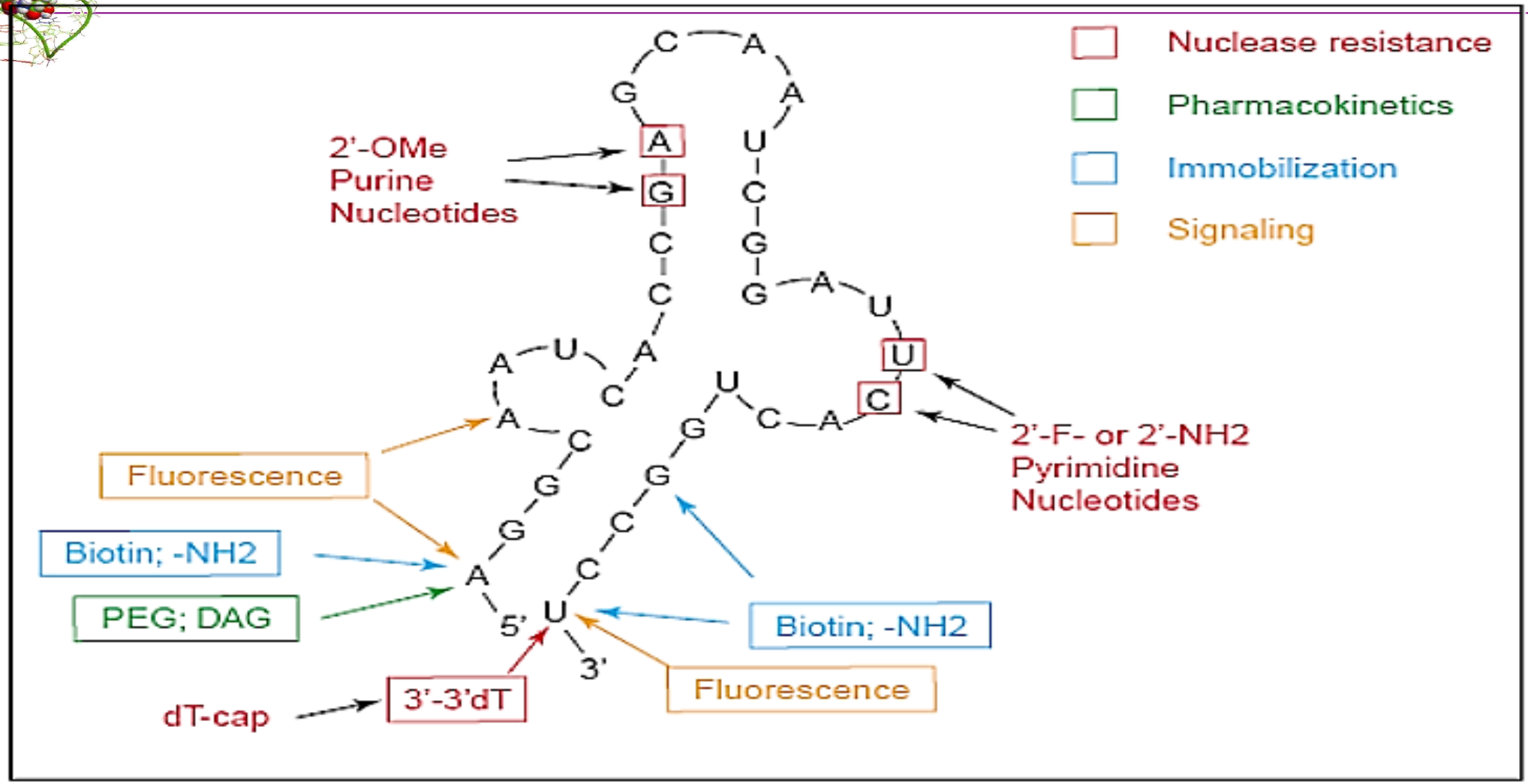
The aptamers for different epitopes on target protein.

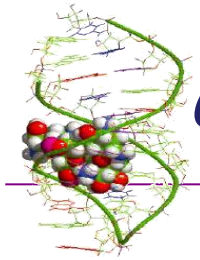
Mixed together on capture surface.

Combined binding increases affinity.



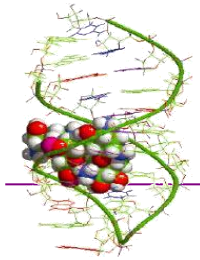
Chemical modifications of aptamers





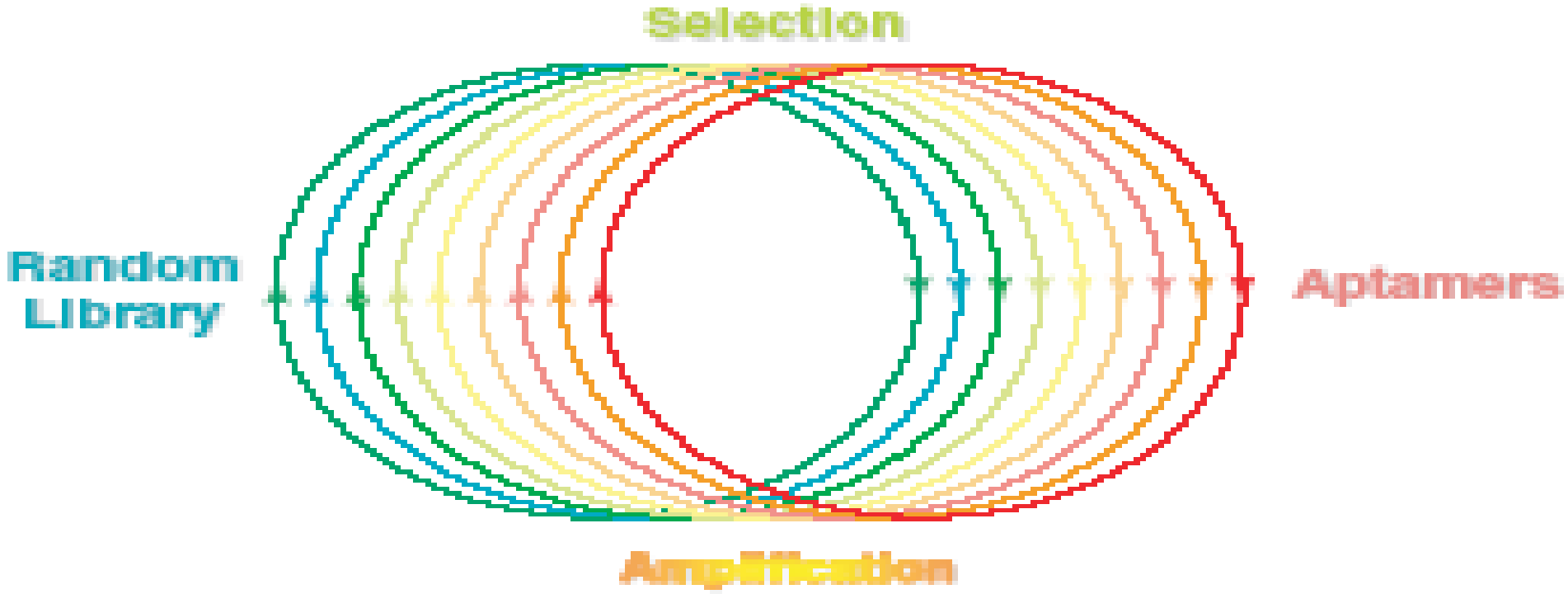
Quantitative Diagnostic Methods for Aptamers

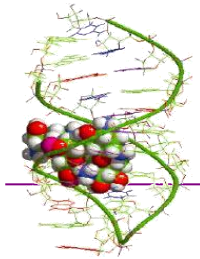
- **ELISA**
- **Flow cytometry**
- **Capillary tube electrophoresis**
- **High-performance liquid chromatography (HPLC)**
- **MALDI_TOF**



SELEX

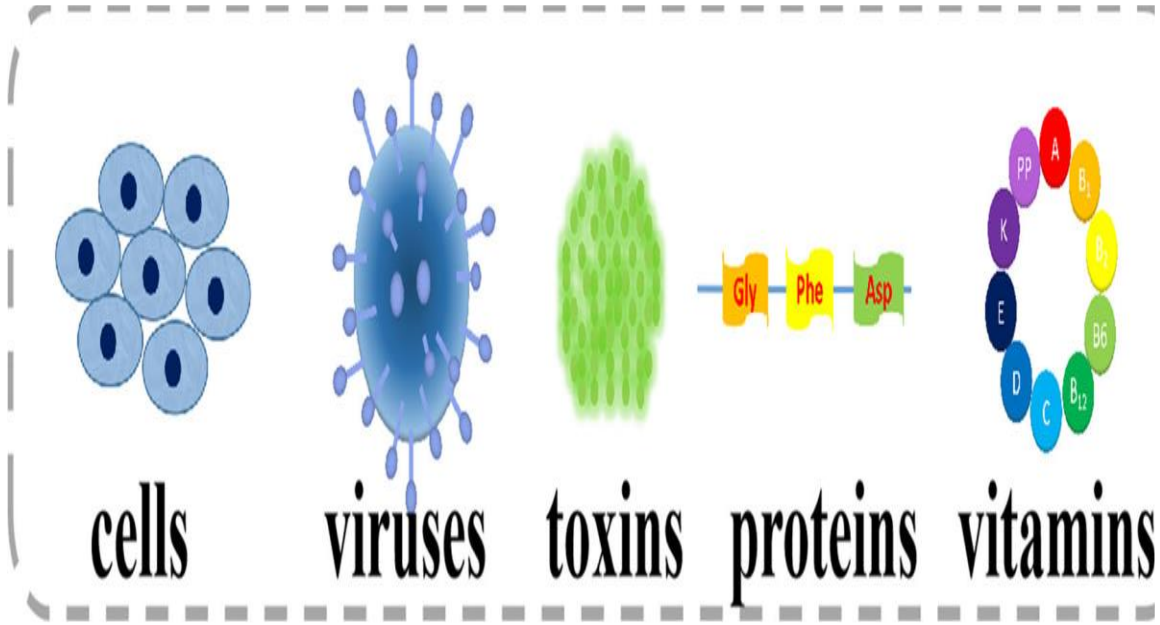
Systematic Evolution of Ligands by Exponential enrichment



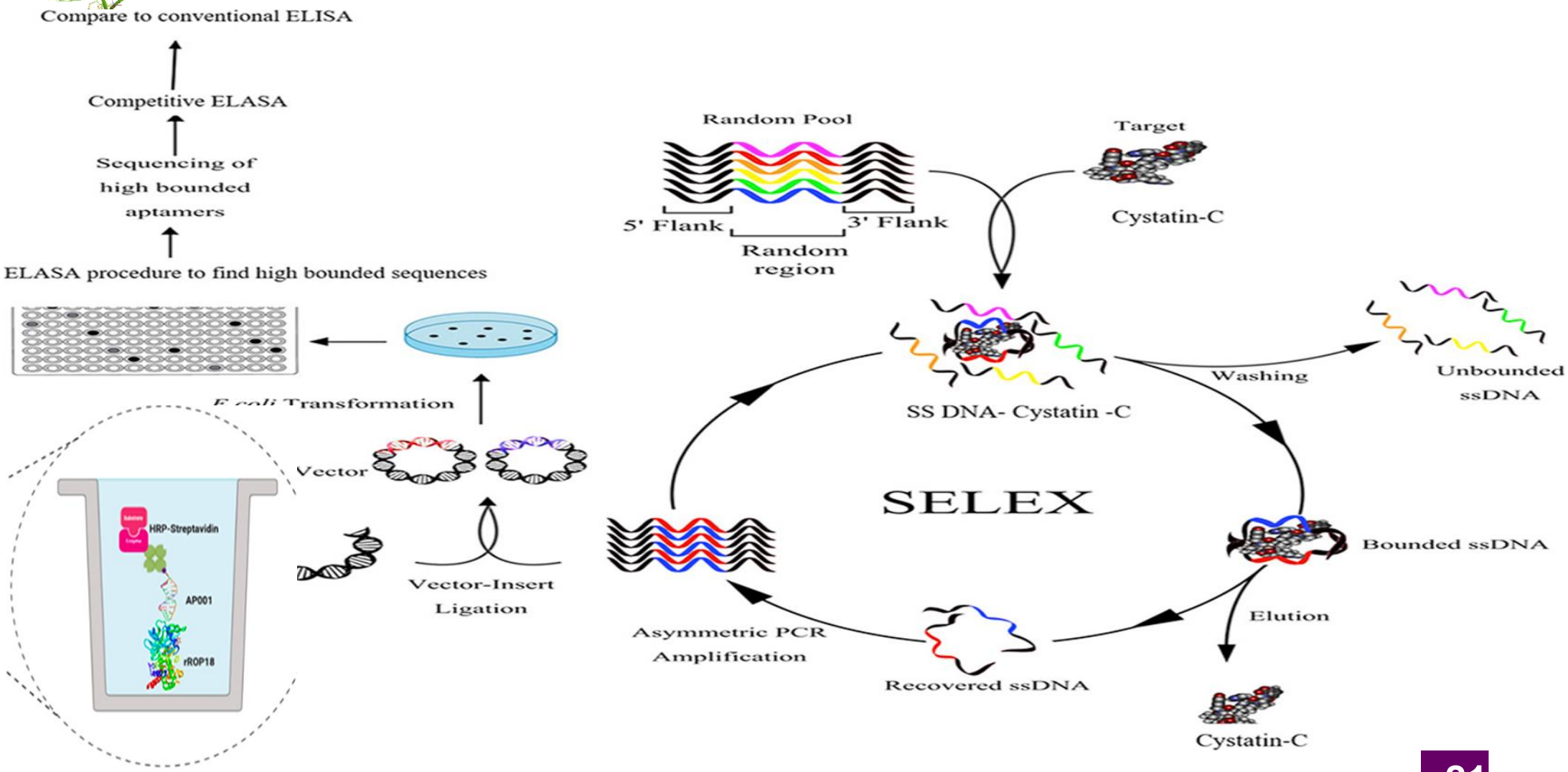


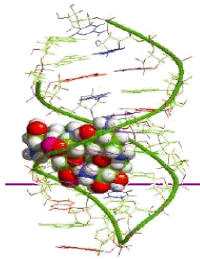
SELEX components (cont.)

- Target
  - Small molecules
  - Bimolecules
  - Whole cells
  - Whole organisms



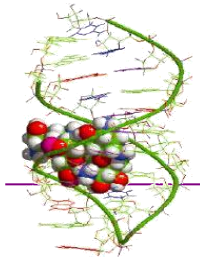
SELEX process





Separation techniques

- **Filtration (nitrocellulose, Dialysis)**
- **Chromatography(size exclusion,Affinity)**
- **Electrophoresis(PAGE, Agarose)**
- **Magnetic beads**
- **Precipitation(immunoprecipitation, centrifuge)**
- **SPR**
- **AFM:** High cost

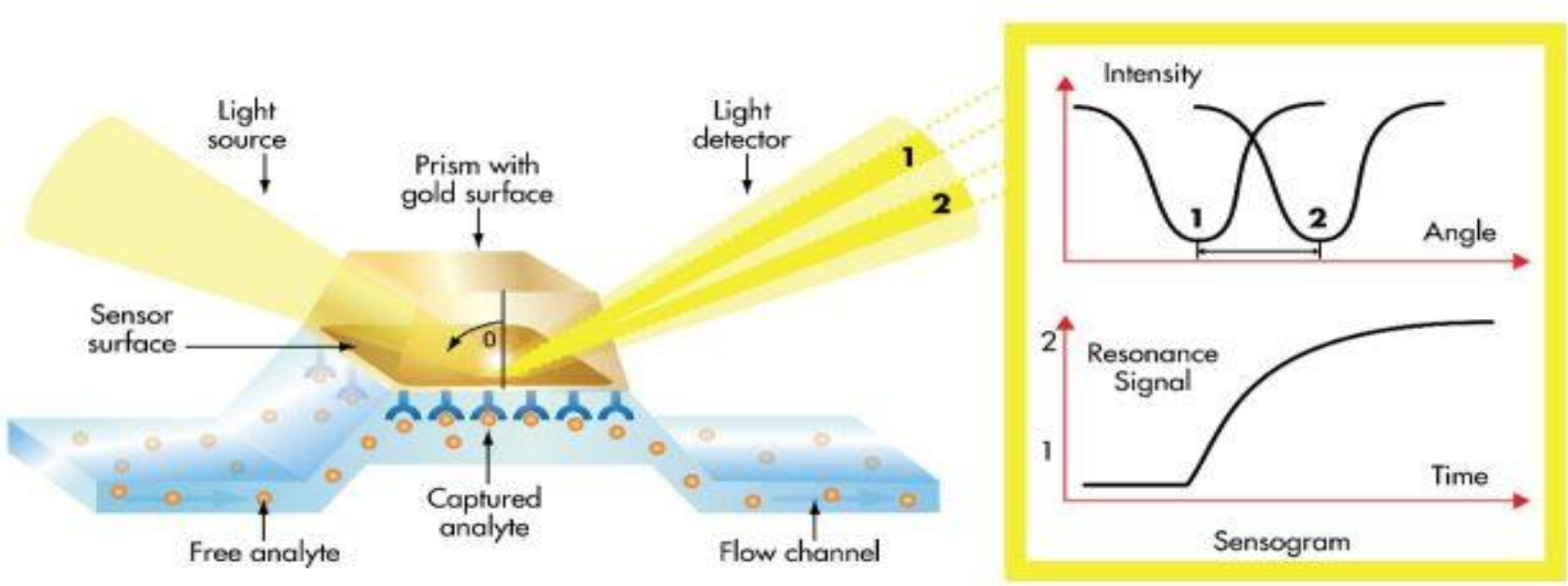


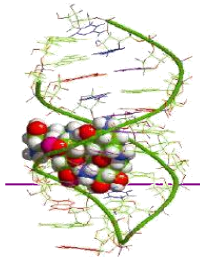
SELEX

- Negative SELEX
- Counter SELEX

Measurement of KD values and LOD

SPR analysis

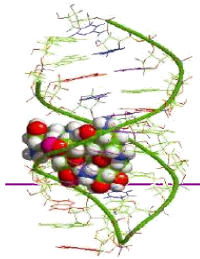




Aptamer-based technology for the identification of food pathogens

Table 1 Examples of aptamers application to different targets in different areas

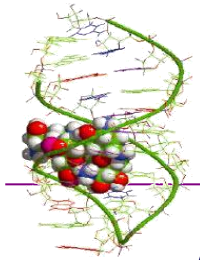
Target	Method	Sample
Streptococcus pyogenes	Cell-SELEX	Cooked chicken
Salmonella typhimurium	Cell-SELEX	Pasteurized milk
Salmonella typhimurium and Vibrio parahemolyticus	Cell-SELEX	Frozen shrimp, chicken breasts
Salmonella	–	Pork
Escherichia coli	Cell-SELEX	Milk and tap water and pond
Staphylococcus aureus	–	Fresh fish
Staphylococcus aureus	Cell-SELEX	Pork meat
Staphylococcus aureus	Cell-SELEX	Milk
Listeria monocytogenes	SELEX	Liced beef, chicken, turkey
Campylobacter jejuni	SELEX	Live cell
Lactobacillus acidophilus	Cell-SELEX	Oxidized PSi Fabry-Pérot thin films
Francisella tularensis	SELEX	*Bacterial antigen*



Aptamer-based technology for the identification of food pathogens

Table 1 Method for detecting microorganisms by optical aptamer biosensor

Detection method	Detection objects (sample type)	Strategy	LOD	Ref
Colorimetry	*S. typimurium* (milk)	Gold nanoparticles modify aptamer	56 cfu/mL	Ma et al. (2017)
Colorimetry	*V. parahemolyticus* (water)	Horseradish peroxidase catalytic substrate	10 cfu/mL	Wu et al. (2015)
Colorimetry	*E. coli* (food extract)	Gold nanoparticles bind to target DNA fragments	< 1 logcfu/g	Quintela et al. (2015)
Colorimetry	*S. typimurium* (serum)	The binding affinity and specificity of aptamer are determined by fluorescence binding assays	10^2 cfu/mL	Lavu et al. (2016)
Colorimetry	H3N2 virus (pure virus)	Enzyme catalyze hydrogen peroxide to reduce gold ions	11.16 μg/mL	Chen et al. (2016)
Colorimetry	Patulin (food extract)	An enzyme-chromogenic substrate system	48 pg/mL	Wu et al. (2016)
Fluorescence	*S. enteritidis* (food and water)	Fluorescent dye bind with a G-quadruplexes	60 cfu/mL	Zhang et al. (2016)
Fluorescence	*H. pylori* (milk)	Aptamer-CuInS2 quantum dots sensor	0.46 pmol/L	Liu et al. (2017)
Fluorescence	*S. sonnei* (food extract)	A sandwich complex pair of SS-3 and SS-4	10^3 cells/mL	Song et al. (2017)
Fluorescence	*S. aureus* (whole blood)	Aptamer-functionalized silica magnetic nanoparticles	682 cfu/mL	Borsa et al. (2016)
Fluorescence	H1N1 virus (pure virus)	A sandwich-based aptamer assay is performed on an integrated microfluidic system automatically	0.032 HAU	Tseng et al. (2016)
Fluorescence	aflatoxin M1 (milk)	The quenching-dequenching mechanism	5.0 ng/kg	Sharma et al. (2016)
Absorbance	*L. monocytogens* (milk)	Vancomycin and aptamer recognize *L. monocytogenes* at different sites	5.4×10^3 cfu/mL	Zhang et al. (2016)
Chemilumine-scence	*E. coli* (milk)	*E. coli* O157:H7 bind with aptamer-conjugated 6-FAM	4.5×10^3 cfu/mL	Khang et al. (2016)



Haemophilus influenzae

Media and reagents

Random DNA library and primers

PCR amplification

ssDNA preparation

Bacterial cell SELEX

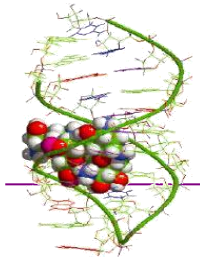
Flow cytometric analysis of aptamer-binding affinity

Detection of Hib

Aptamers binding assay

Prediction of secondary structures

Candidate sequence detection in clinical samples



Shigella sonnei

Bacterial strains and reagents

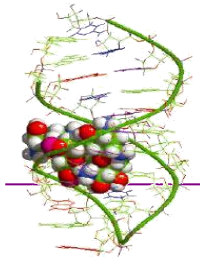
Oligonucleotide library and primers

Whole bacterial cell SELEX procedure

Cloning and sequencing

Enzyme-Linked Aptamer Sedimentation Assay (ELASA)

Statistical analyses



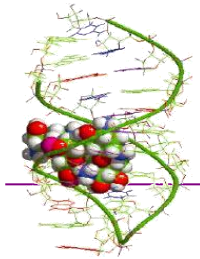
Bacillus anthracis ⇒ spore

Campylobacter jejuni ⇒ *Cross Reaction*

Bacillus thuringiensis ⇒ *QDS* quantum dots

Bacillus thuringiensis ⇒ *CNTS* carbon nanotubes

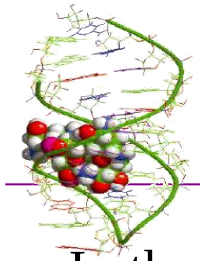
Salmonella ⇒ *S-ps8_4 -Pilli IVB*



Staphylococcus aureus

Staphylococcus aureus enterotoxin a

Staphylococcus aureus enterotoxin b

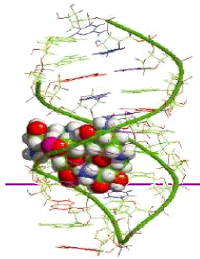


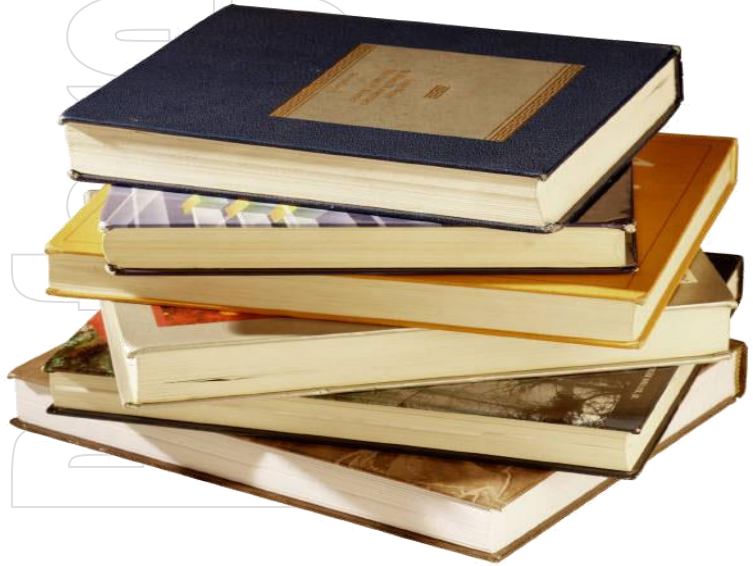
Conclusions

- In the near future, it will be getting easier and quicker to obtain aptamers with high affinity and specificity for clinical use, with the development of new instruments and software, combining high-throughput sequencing with high-throughput binding analysis.

- Aptamers are also ideal targeting ligands for targeted therapy, due to their possession of the high affinity and specificity ability.

- Various aptamer-based drug delivery systems such as aptamer-chemotherapy agents, aptamer-siRNA/shRNA/miRNA, aptamer-antibody, aptamer-enzyme and aptamer-nanoparticles have been established to specifically deliver the drug to the expected sites, therefore reducing the possibility of side effects caused by the off-target effects.





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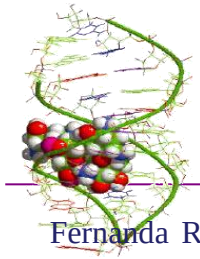

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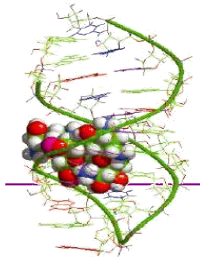

Thanks for your attention