What is an Aptamer?

apto: "to fit"

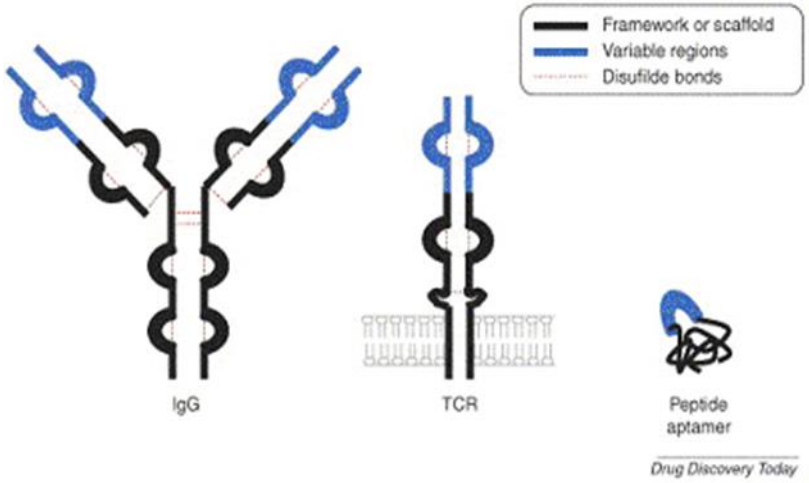
mer: "smallest unit of repeating structure"

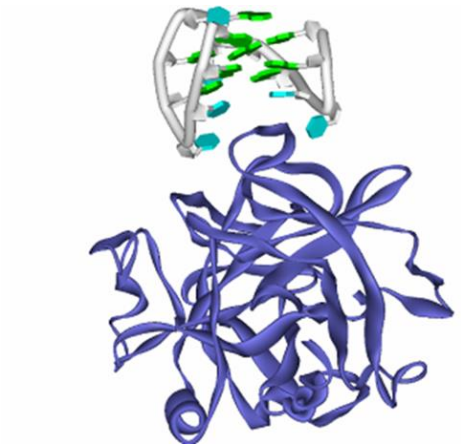
Aptamers are single stranded folded oligonucleotides that bind to molecular (protein) targets with high affinity and specificity

There are three types of aptamers: DNA, RNA, and peptide aptamers. All have very similar properties but are distinctly unique.

It is theoretically possible for aptamers to be used against any molecular target - aptamers have been selected against small molecules, toxins, peptides, proteins, viruses, bacteria, and even whole cells.

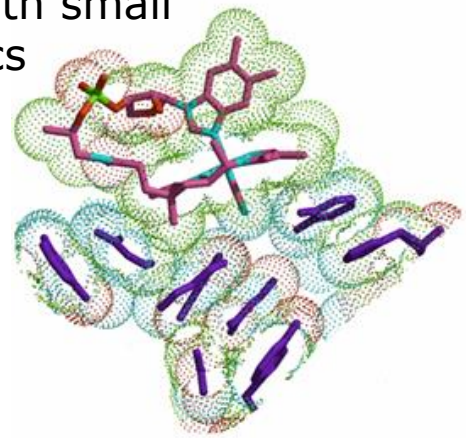
RNA aptamer	DNA aptamer	Peptide aptamer
Form complex secondary and tertiary structure	Form complex secondary and tertiary structure	Structure constrained by scaffold
Form diverse 3D structures	Less diverse 3D structure than RNA aptamer	3D structure constrained by scaffold
Bind target with the entire sequence	Bind target with the entire sequence	Bind target with variable region only
Biosensor, diagnostic, therapeutic applications	Biosensor, diagnostic, therapeutic applications	Biosensor, diagnostic, therapeutic applications

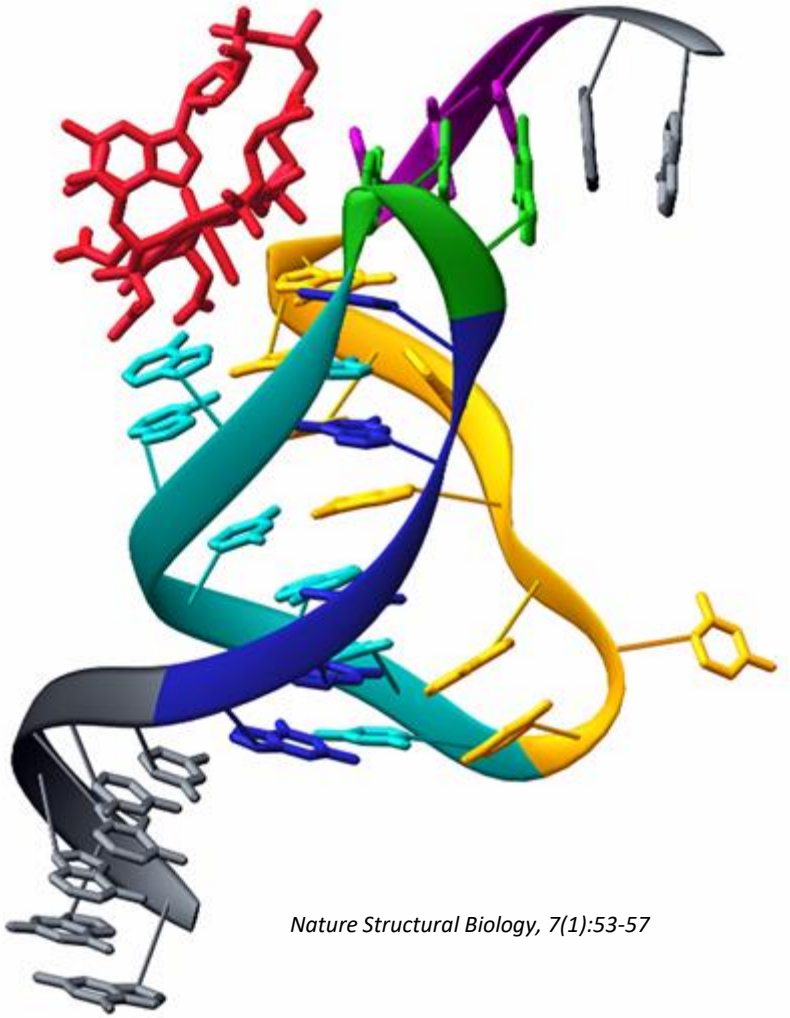




Aptamer Structure

- Unique tertiary structures allow aptamers to fold into stable scaffolds for carrying out molecular recognition
- van der Waals, hydrogen bonding, and electrostatic interactions drive high affinity target binding
- Designed to block protein-protein interactions
- Share properties of both small molecules and biologics





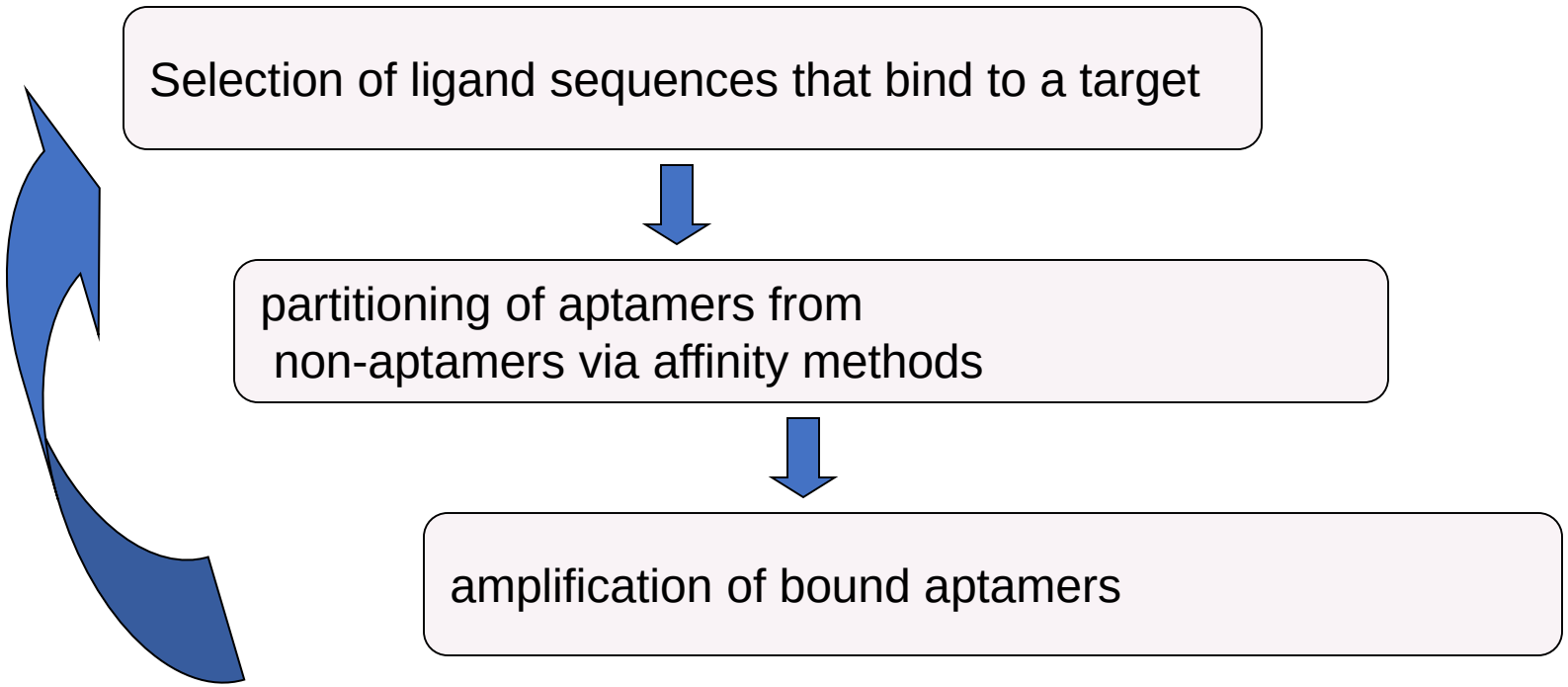
Nature Structural Biology, 7(1):53-57

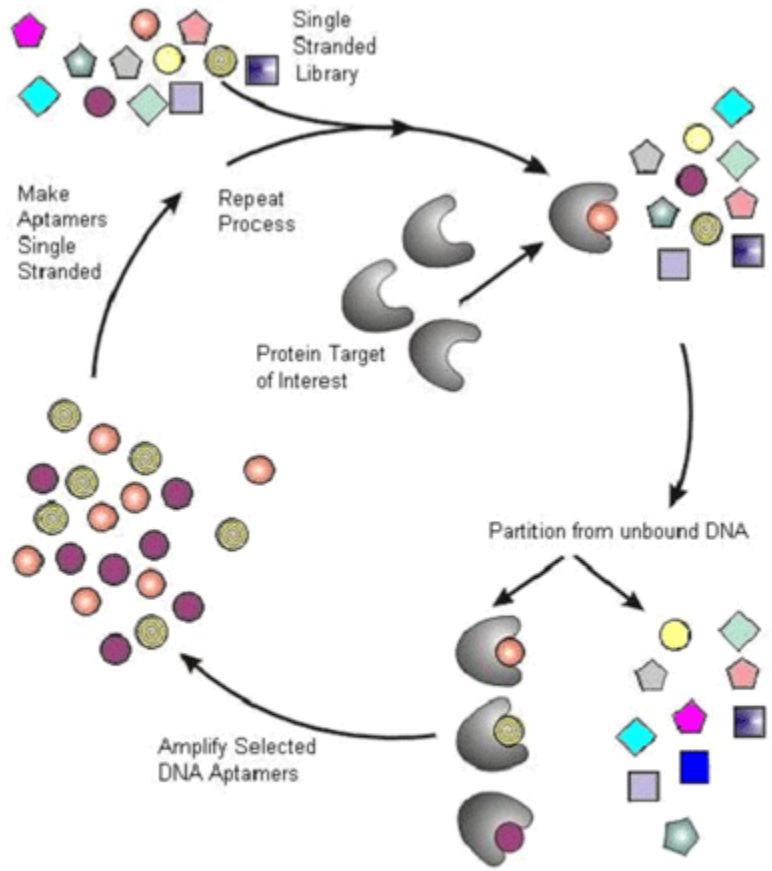
- SELEX (Systematic Evolution of Ligands by Exponential Enrichment)

  Tuerk and Gold (1990) *Science* 249, p505-510

SELEX (systematic evolution of ligands by exponential enrichment) is a process that involves the progressive purification from a combinatorial library of nucleic acid ligands with a high affinity for a particular target by repeated rounds of partitioning and amplification.

Three Processes





SELEX: Systematic Evolution of Ligands by Exponential Amplification
Tuerk, C. & Gold, L. (1990) Science 249, 505-510

BioProcessing JOURNAL
TRENDS & DEVELOPMENTS IN BIOPROCESS TECHNOLOGY

Synthetic Antibodies: *The Emerging Field of Aptamers*

By SUZY KEDZIERSKI, MAKAN KHOSHNEJAD, and G. THOMAS CALTAGIRONE*

Introduction

The current global market for aptamers is approximately $99 million annually and is anticipated to increase at an astonishing compound annual growth rate (CAGR) of 106.3% for the next five years resulting in an estimated value of $3.7 billion by the year 2017.[1]

Sometimes referred to as a "synthetic antibody," an aptamer is a nucleic acid or peptide molecule that binds to a target or antigen with high affinity and specificity. Aptamers have a wide range of applications including diagnostics, therapeutics, forensics, and biodefense. To date, hundreds of aptamer sequences have been identified and can now be chemically synthesized in the lab on demand, faster and less expensively, without the traditional issues associated with producing recombinant antibodies. This article will review aptamer technology, its advantages and limitations, as well as highlight a few of its many applications in the life sciences.

About Aptamers

Aptamers are single-stranded DNA or RNA oligonucleotides (short strands of nucleic acids) or peptides that have been engineered through a selection process to exhibit exceptional binding affinity and specificity to their target or antigen. Figure 1 shows the schematic of aptamer production and some of the shapes aptamers possess. Typical aptamer targets include heavy metals, small organics, peptides, proteins, tissues, and organs.

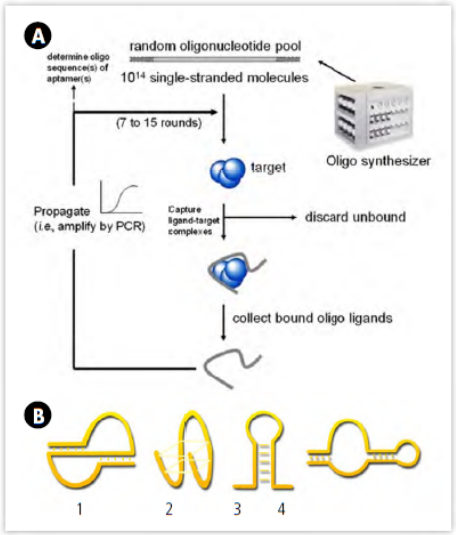
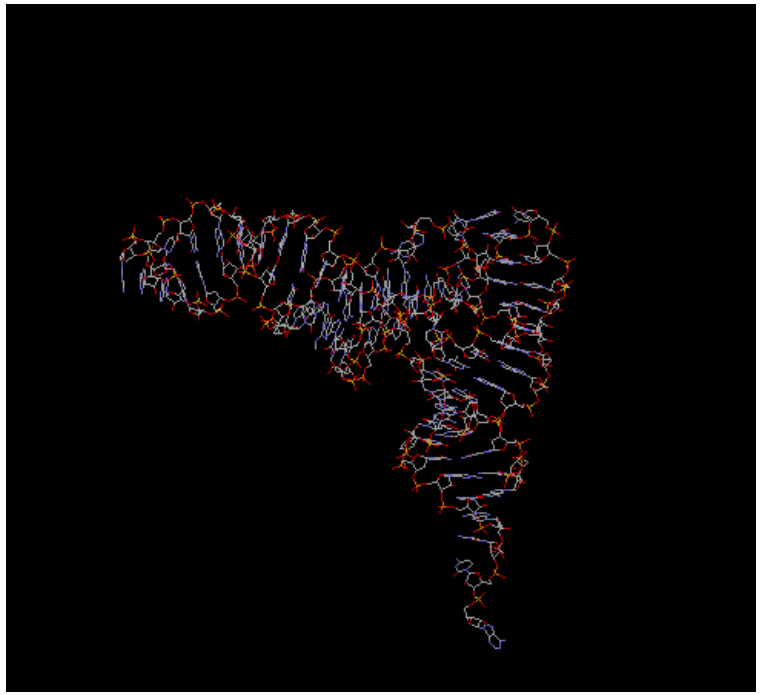


FIGURE 1. Illustration of aptamer production and various aptamer shapes. **(A)** A random library of 10^{13-14} oligonucleotides synthetized and used for selection against a target molecule (*e.g.*, protein). The bound oligonucleotides are collected and amplified using PCR. This selection step is repeated many times, followed by identification of the candidates with a DNA sequencer. **(B)** The conformational shapes of aptamers contribute to their specificity. The following are structural conformations of various aptamers: (1) pseudoknot (ligand for HIV-1 reverse transcriptase); (2) G-quartet (ligand for thrombin); (3) hairpin (ligand for bacteriophage for T4 polymerase); and (4) stem loop/bulge (ligand for adenosine-5′-triphosphate [ATP]).[2]



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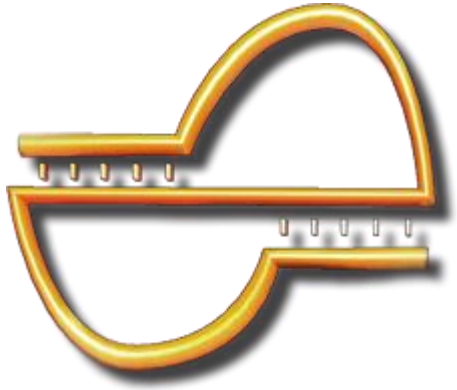
G. Thomas Caltagirone, PhD, President and CEO, Aptagen, LLC
Email: marketing@aptagen.com; Phone: (717) 278-2436; Website: www.aptagen.com

Examples of Aptamer Shapes

A.



B.

C.

D.

A. Pseudoknot (ligand for HIV-1 reverse transcriptase)
B. G-quartet (ligand for thrombin)
C. Hairpin (ligand for bacteriophage for T4 polymerase)
D. Stem loop/bulge (ligand for ATP)

taken from McGown, et.al. (1995)

Antibody VS aptamer in therapeutic use

Advantages of antibodies
• Pharmacokinetic and other systemic properties of antibodies are often sufficient to support product development
• Comparatively long circulating half-lives
• Not susceptible to nuclease degradation
• Antibody technologies are widely distributed

Limitations of antibodies
• Antibodies are produced biologically in a process
• Viral or bacterial contamination
• Large size limits

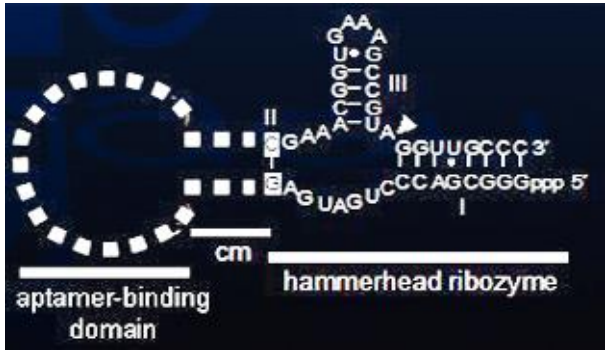
Advantages of aptamers
• Aptamers are produced chemically in a readily scalable process
• Chemical production process is not prone to viral or bacterial contamination
• Non-immunogenic
• Smaller size allows more efficient entry into biological compartments

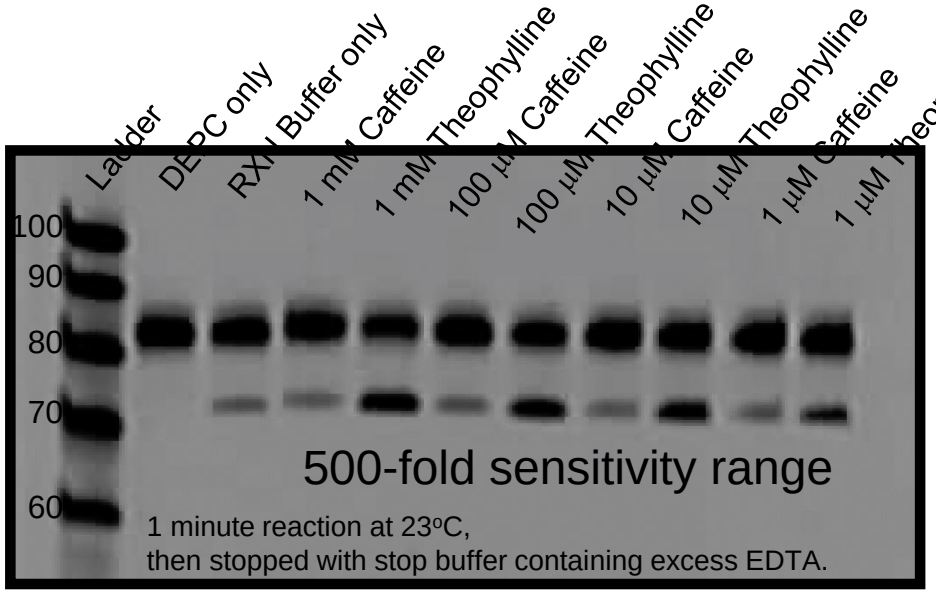
• **Limitations of aptamers**
• Pharmacokinetic and other systemic properties are variable and often hard to predict
• Shorter half-life
• Unmodified aptamers are highly susceptible to serum degradation

Apta-switch™

(aptamer that produces a self-cleavage output signal)

Target	Example
Metal Ions	Co^{2+}, Ni^{2+}, Cd^{2+} Zn^{2+}, Mn^{2+}
Small Organics	Caffeine
Peptides	Rev Peptide
Proteins	Phosphorylated ERK2, Unphosphorylted ERK2

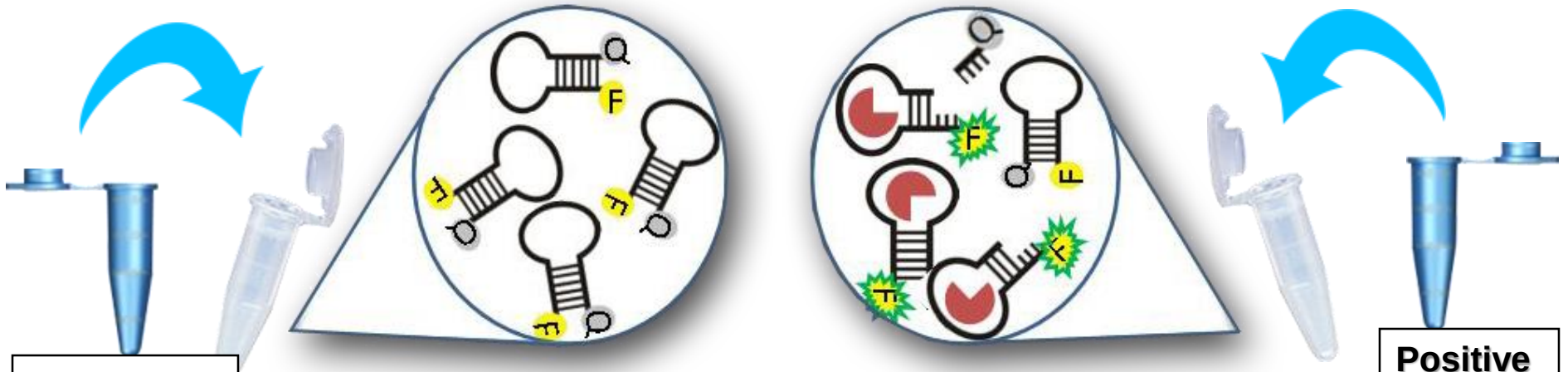




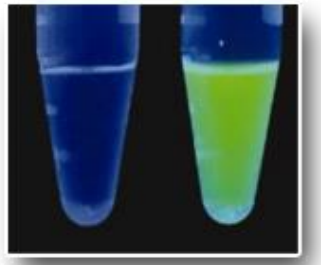
Specificity Against Theophylline vs. Caffeine

Apta-beacon™ Diagnostic Assay

(simple 1-step reaction, free in-solution)

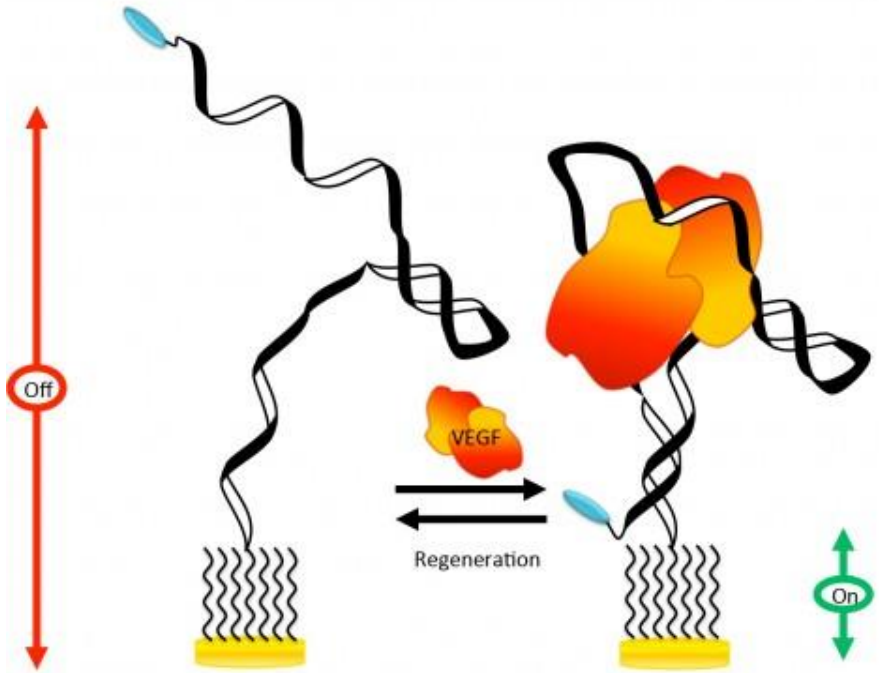


No Incubation or wash steps = total time << 1 minute

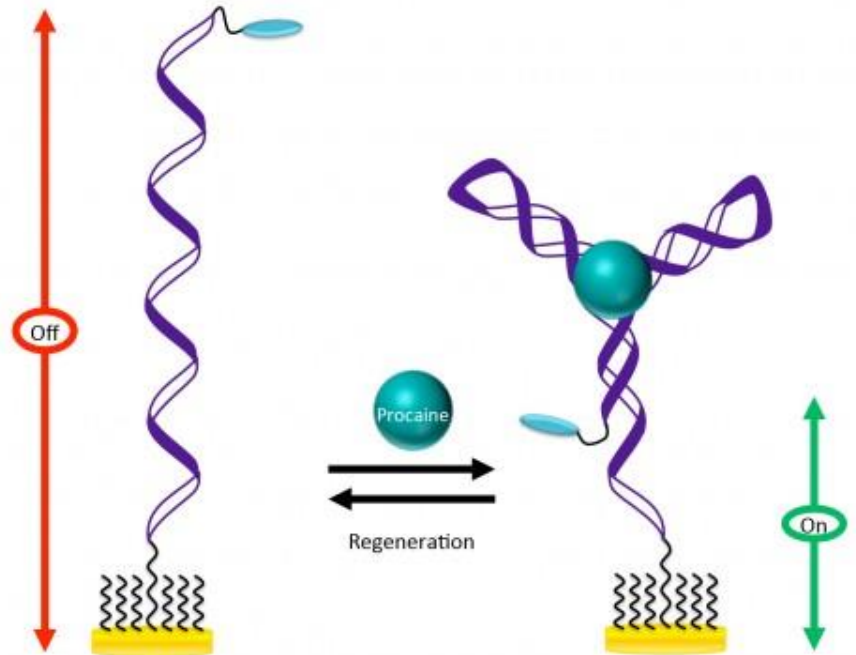


Quantitate based on a titration of controls

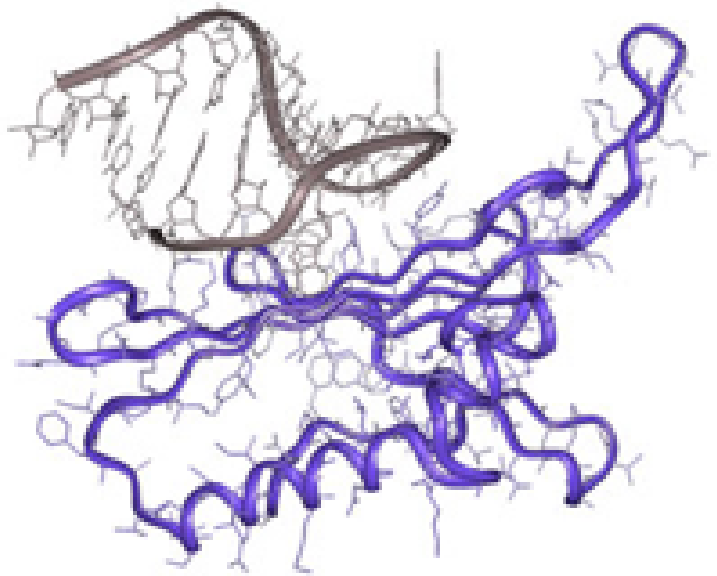
Vascular Endothelial Growth Factor (VEGF) Aptamer Probe Protein Sensor



Cocaine Binding Aptamer (CBA) Probe Small Molecule Sensor



Vitamin b12-binding RNA aptamer



RNA Aptamer Complexed with MS2 Coat Protein

The Ellington Lab Aptamer Database

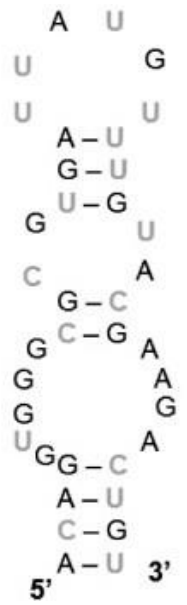
Aptamers are DNA or RNA molecules that have been selected from random pools based on their ability to bind other molecules. *Aptamers* have been selected which bind nucleic acid, proteins, small organic compounds, and even entire organisms. These novel molecules have many potential uses in medicine and technology.

The *Aptamer Database* is a comprehensive, annotated repository for information about aptamers and *in vitro* selection. This resource is provided to collect, organize and distribute all the known information regarding aptamer selection.

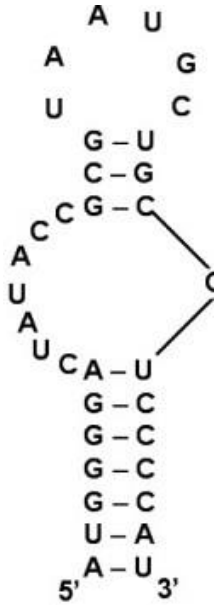
Aptamer database: http://aptamer.icmb.utexas.edu/index.php

Structures of the anti-coagulant aptamers

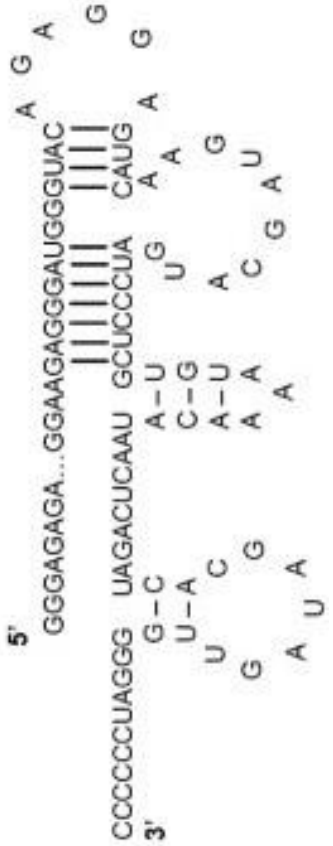
Anti-Thrombin



Anti-Factor IXa

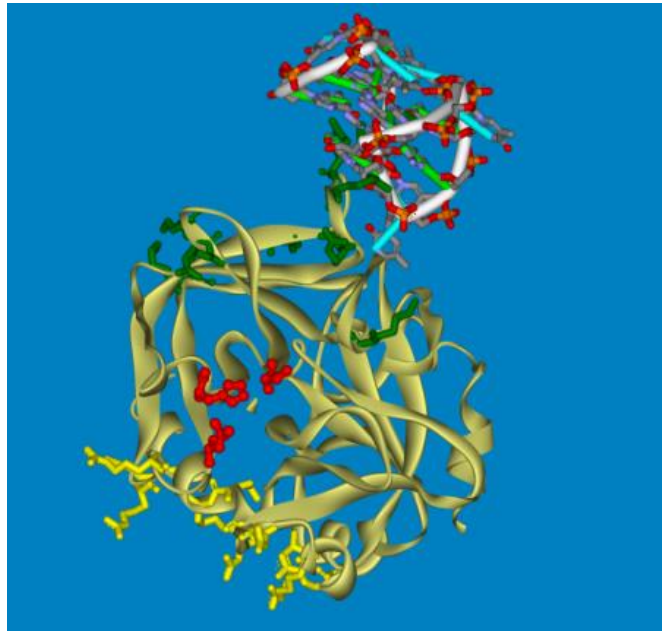


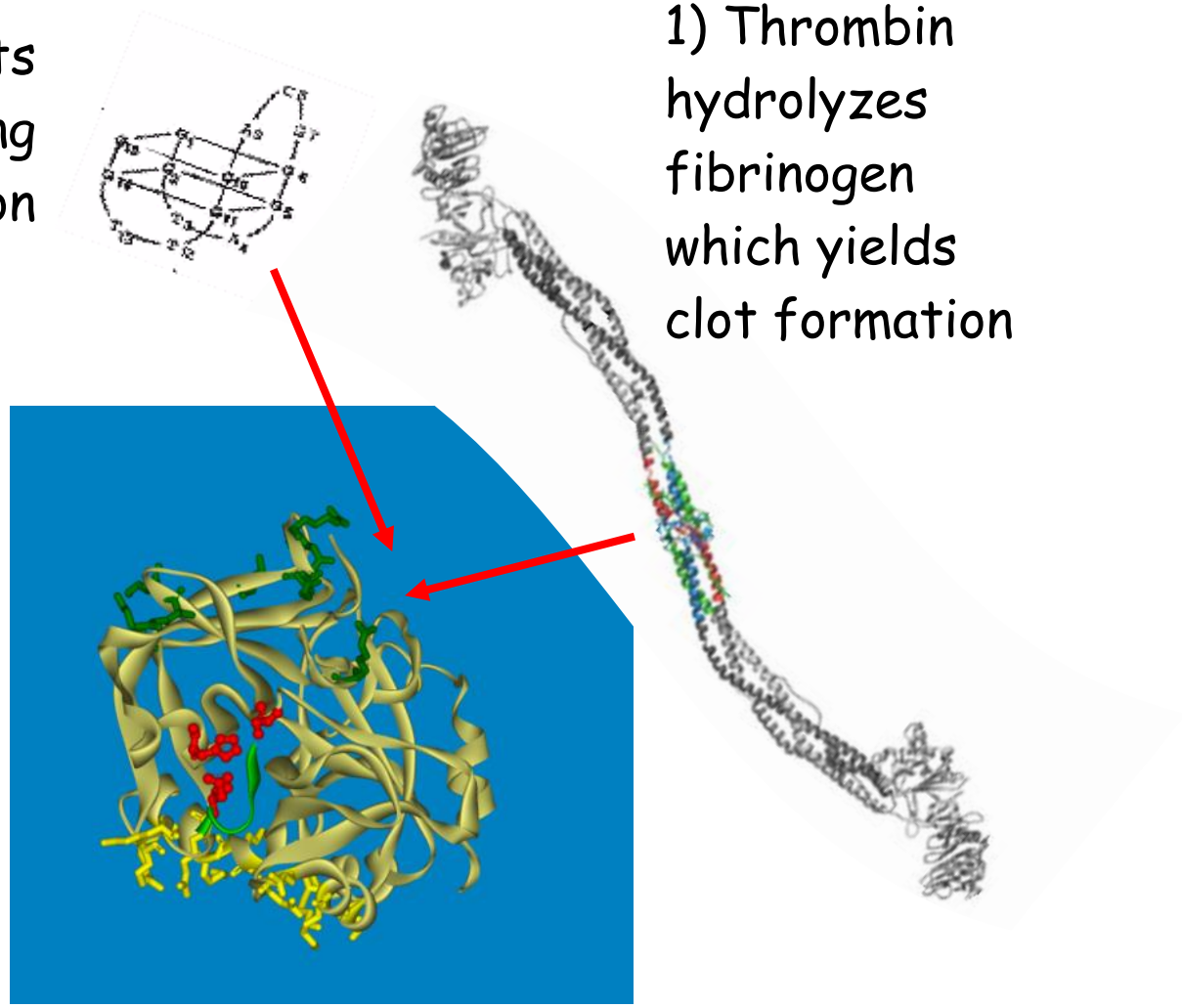
Anti-Factor VIIa



DNA aptamer and Thrombin

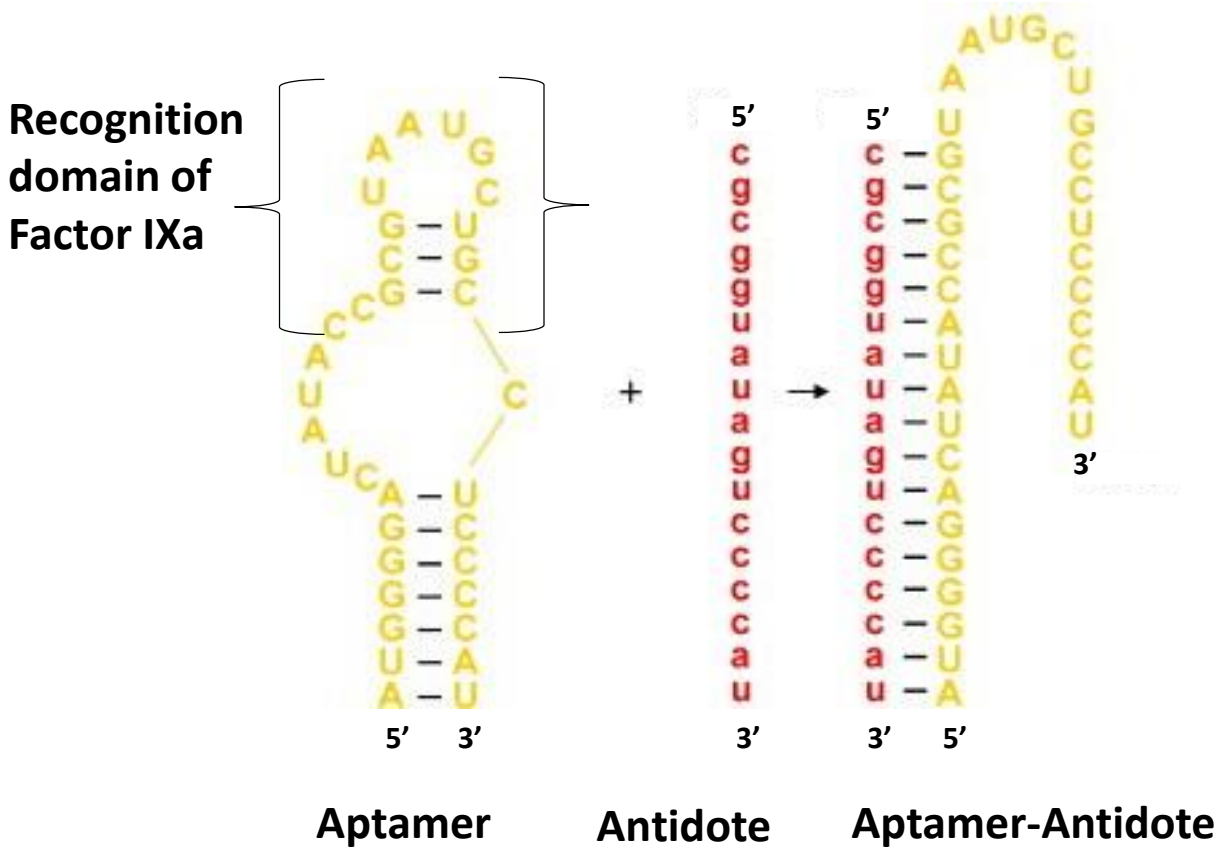
2) Aptamer inhibits fibrinogen binding and clot formation





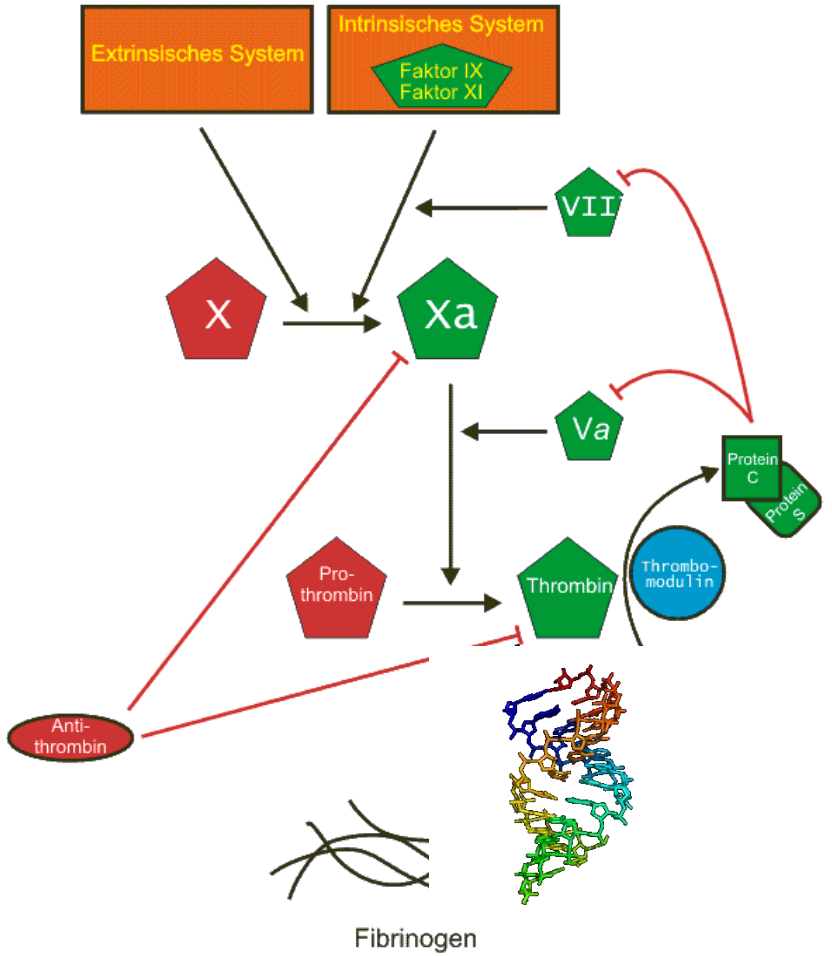
1) Thrombin hydrolyzes fibrinogen which yields clot formation

Mechanism of action between the aptamer anti-factor IXa and its antidote

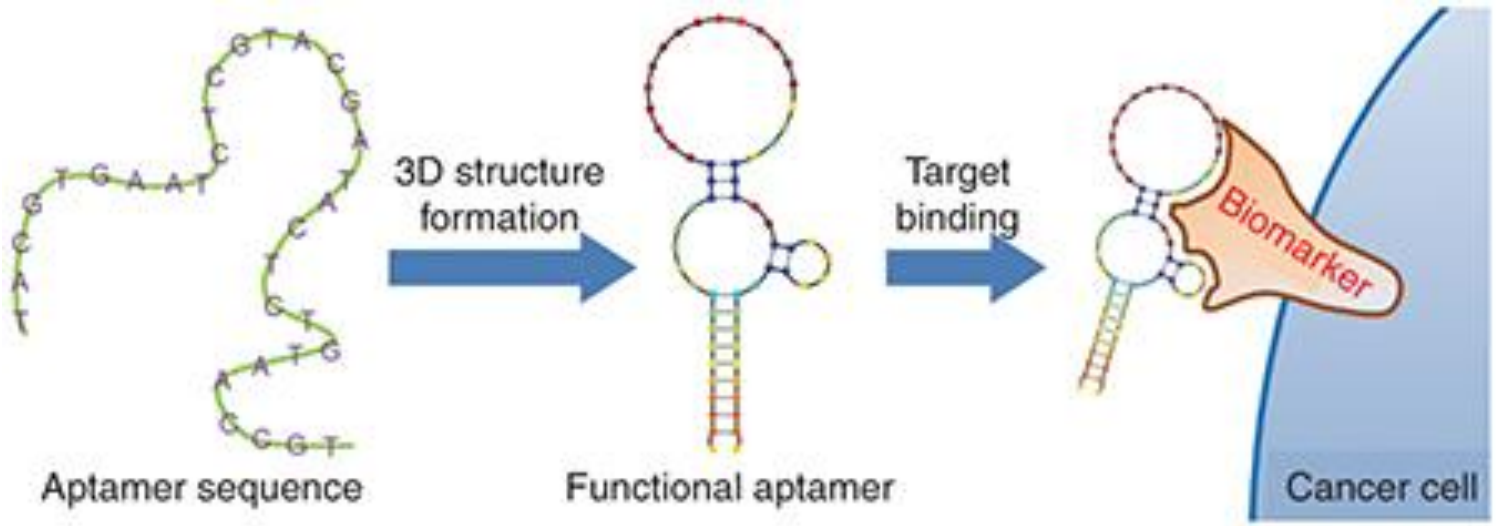


Unique opportunity – fast antidot

Aptamer blocks thrombin, and antidot blocks aptamer, restoring coagulation



Fibrin mesh for clotting


3D structure formation
Target binding
Biomarker
Aptamer sequence
Functional aptamer
Cancer cell

CELL-SELEX Technology

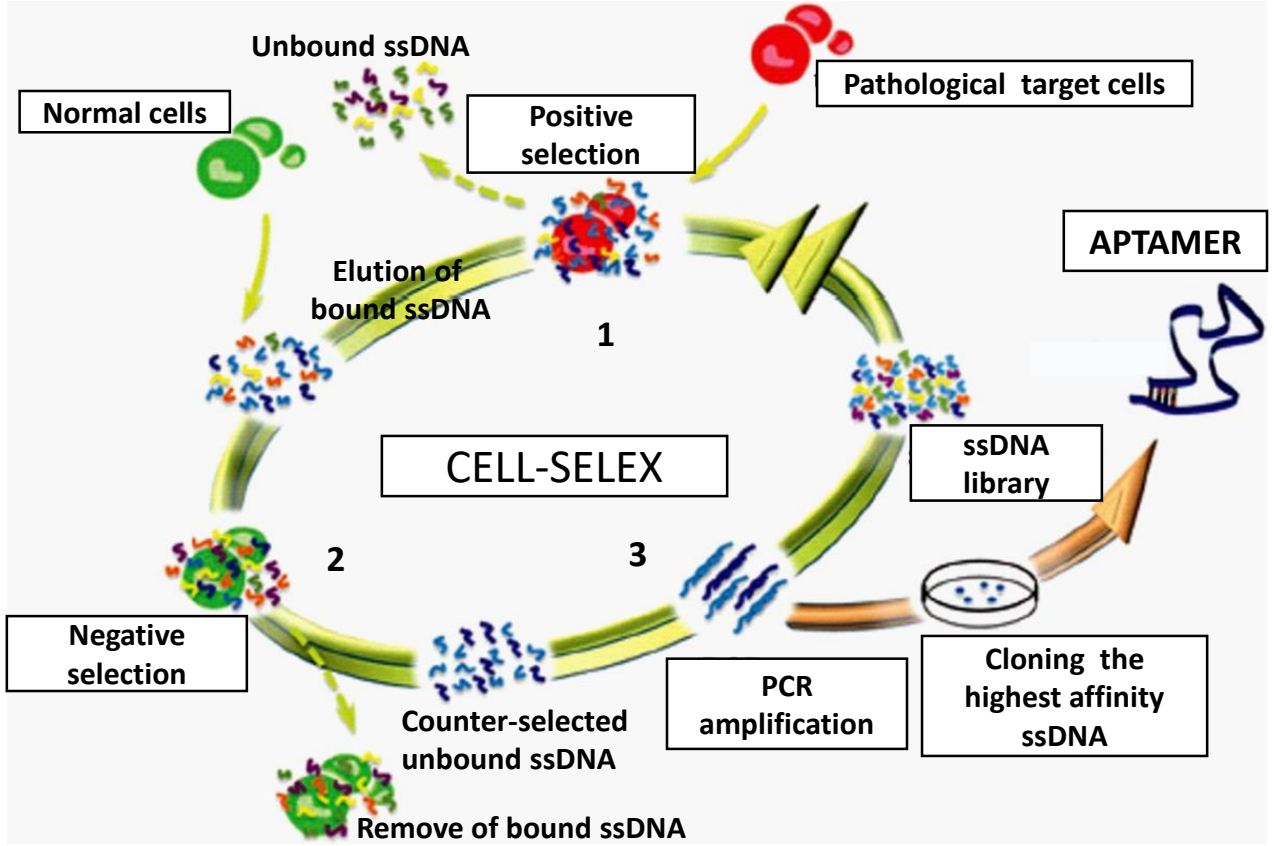


Figure 1

A list of therapeutic aptamers undergoing clinical trials

Aptamer	Molecular target	Sponsor	Medical indications	Current status
ARC1779	Activated von Willebrand Factor (vWF)	Archemix Corporation	Purpura; Thrombotic Thrombocytopenic; Von Willebrand Disease Type-2b	Phase 2 completed
ARC1905	Complement factor C5	Ophthotech Corporation	Age-Related Macular Degeneration	Phase 1 completed
ARC19499	Tissue Factor Pathway Inhibitor (TFPI)	Baxter Healthcare Corporation	Hemophilia	Phase 1 terminated
AS1411	Nucleolin	Antisoma Research	Leukemia, Myeloid	Phase 2 completed
			Metastatic Renal Cell Carcinoma	Phase 2 status is unknown
E10030	Platelet-derived growth factor (PDGF)	Ophthotech Corporation	Age-Related Macular Degeneration	Phase 3 recruiting participants
NOX-E36	Monocyte Chemoattractant Protein-1 (MCP-1)	NOXXON Pharma AG	Type 2 Diabetes Mellitus; Albuminuria	Phase 2 completed
NOX-A12	Stromal Cell-Derived Factor-1	NOXXON Pharma AG	Multiple Myeloma; Chronic Lymphocytic Leukemia	Phase 2 recruiting participants
NOX-H94	Hepcidin	NOXXON Pharma AG	Anemia of Chronic Disease	Phase 2 completed
NU172	Thrombin (Factor IIa)	ARCA Biopharma	Heart Disease	Phase 2 status is unknown
REG1	Coagulation factor IX	Regado Biosciences	Coronary Artery Disease	Phase 3 recruiting participants

Anti-VEGF

- there are mainly three injections available with us for treatment.
- These are :

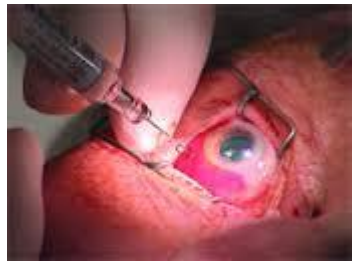
1-Lucentis (Ranibizumab)

2- Avastin (bevacizumab)

3-Macugen(pegaptanib)

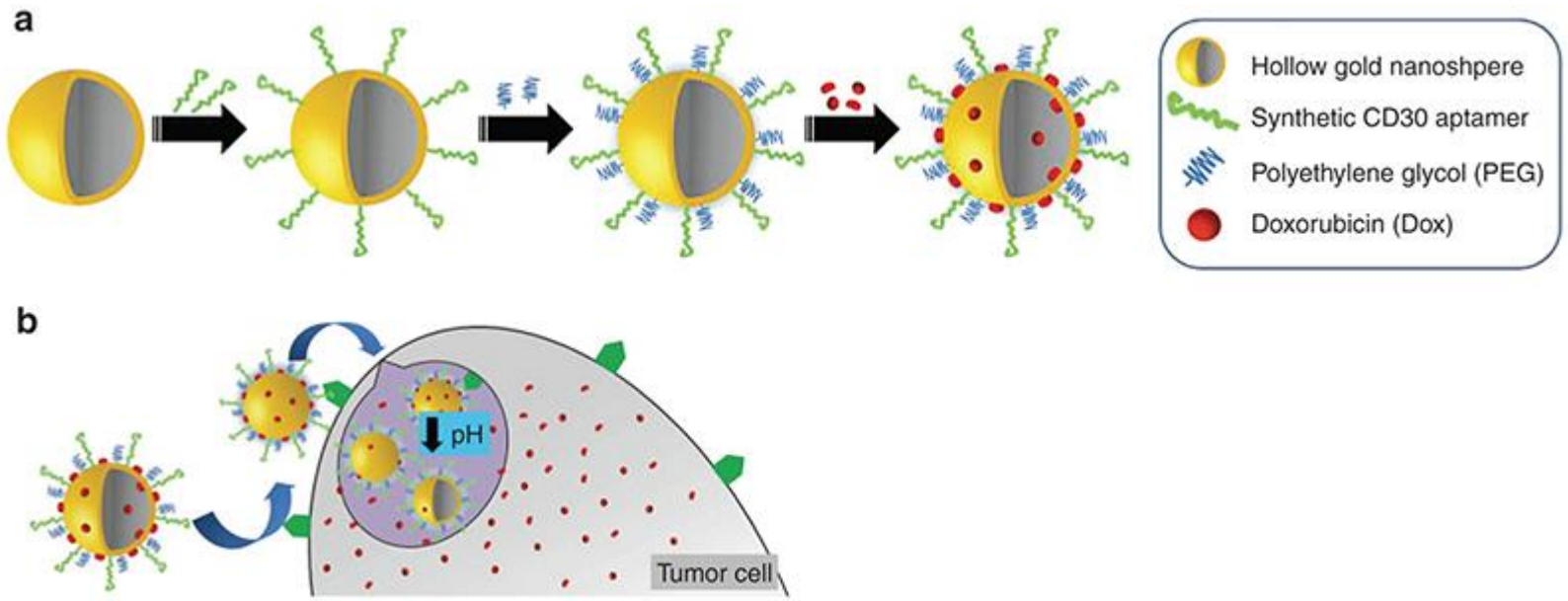
Pegaptanib

- **Pegaptanib** sodium injection (brand name **Macugen**) is an anti-angiogenic medicine for the treatment of neovascular (wet) age-related macular degeneration (AMD).
- It was discovered by Gilead Sciences and licensed in **2000** to EyeTech Pharmaceuticals.
- Approval was granted by the U.S. Food and Drug Administration (FDA) in December **2004**.

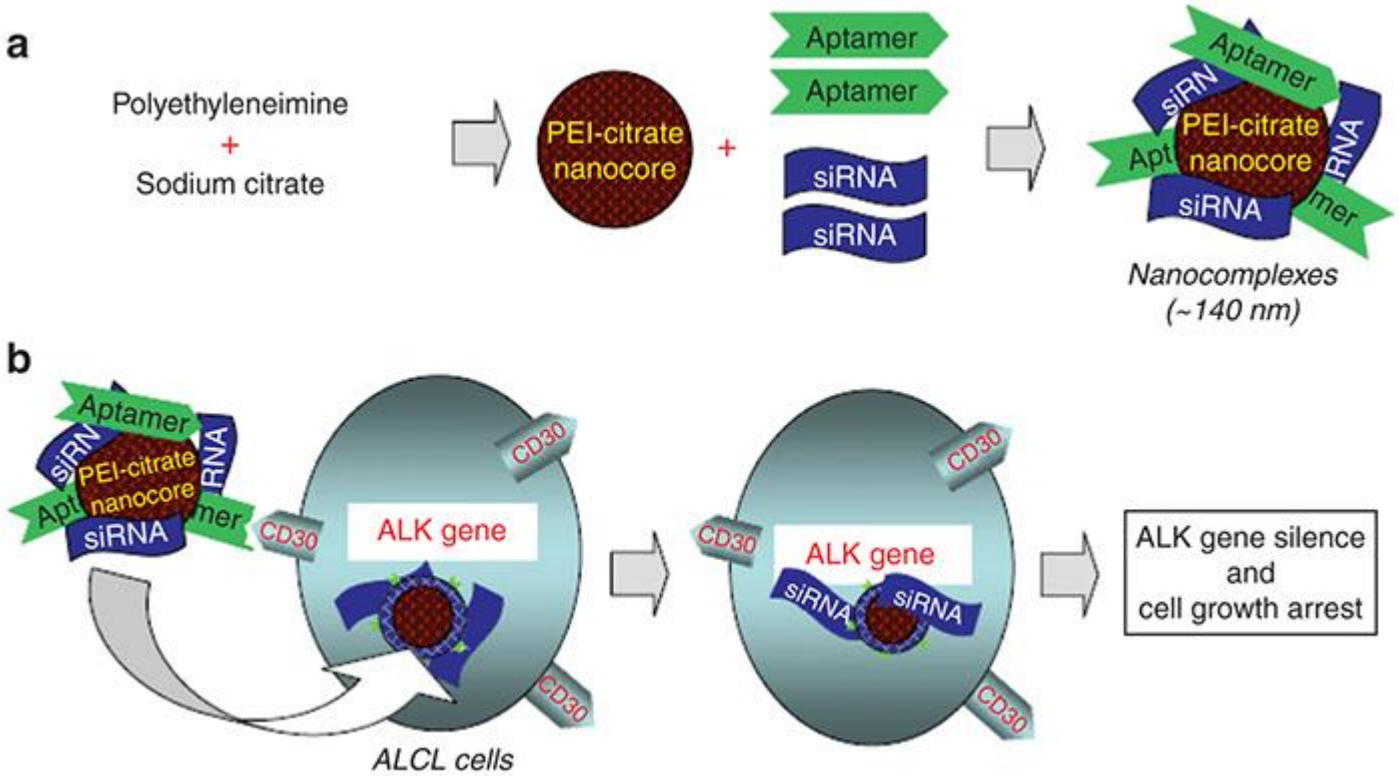


Aptamers specifically targeting cell surface biomarkers used in cancer therapy

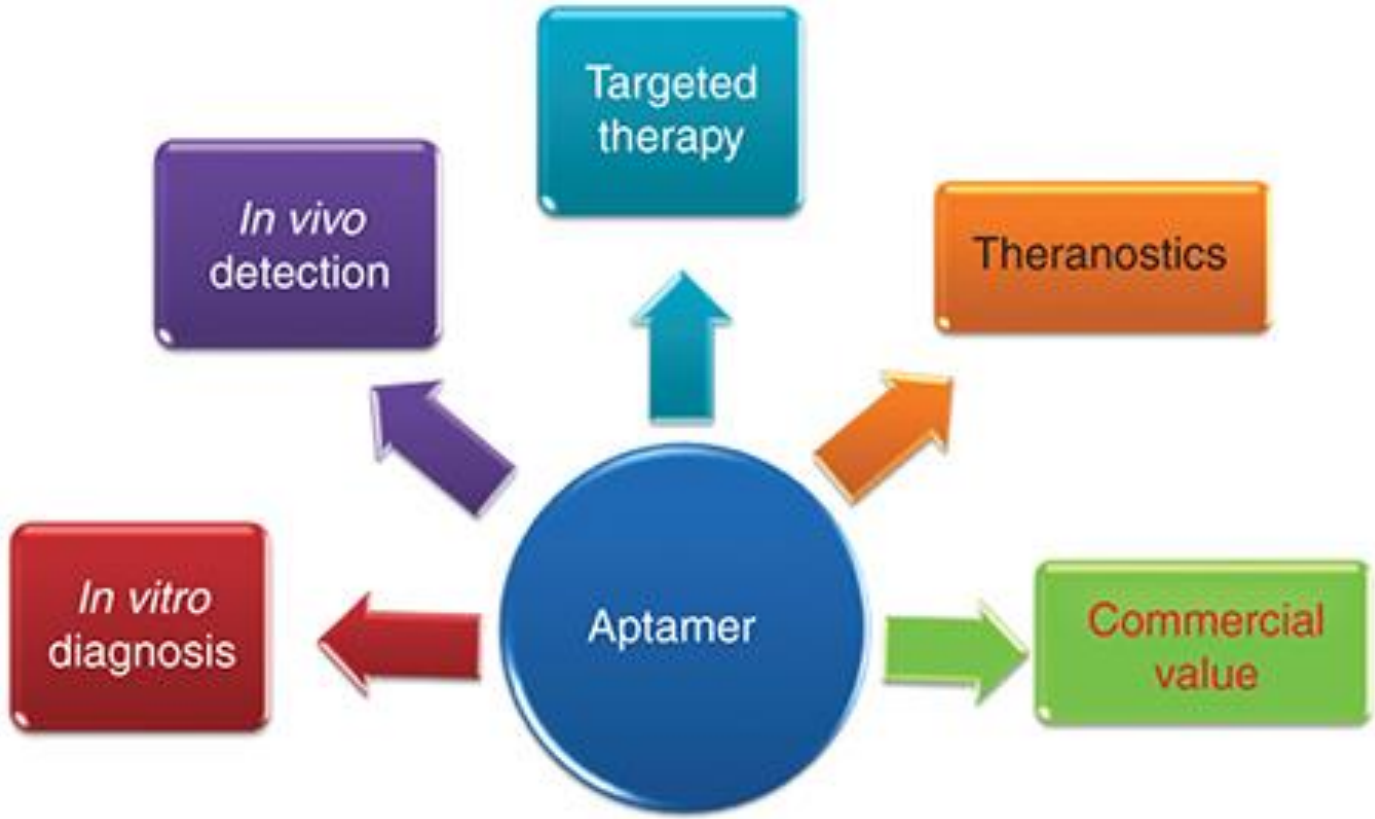
Cell surface biomarker	SELEX method	Aptamer	Applications
Alkaline phosphatase placental-like 2 (ALPPL-2)	Cell-SELEX	RNA	Pancreatic carcinoma diagnosis or therapy[46]
AXL	Cell-SELEX	RNA	Inhibitory aptamer for AXL-dependent cancer[59,123]
B-cell activating factor receptor (BAFF-R)	Protein-SELEX	RNA	Targeting aptamer for BAFF-R-dependent cancer therapy[104]
Carcinoembryonic antigen (CEA)	Protein-SELEX	RNA	Inhibition of CEA-mediated cancer metastasis[124]
CD16α (FcγRIIIα)	Hybrid-SELEX	DNA	Targeting CD16α for immunotherapy[113]
CD28	Protein-SELEX	RNA	Agonistic aptamer that enhances cellular immune response against lymphoma[125]
CD30	Protein-SELEX or Hybrid-SELEX	RNA and DNA	Targeting or immunotherapy of T-cell lymphoma[23,126]
CD44	Protein-SELEX	RNA and DNA	Targeting aptamer for cancer stem cells[31,127,128]
CD71 (Transferrin receptor)	Internalized-SELEX	RNA	Targeting of CD71-dependent cancer[37]
CD124 (IL-4Rα)	Protein-SELEX	DNA	Blocking CD124 and inducing Myeloid-derived suppressor cells (MDSCs) apoptosis[129]
CD133	Cell-SELEX	RNA	Aptamer that targets cancer stem cells[34]
c-MET	Protein-SELEX	DNA	Targeting aptamer for c-MET-driven cancer[113]
EGFR (ErbB1/HER1)	Cell-SELEX or Protein-SELEX	RNA	Antagonist for EGF-dependent cancer proliferation[52,130]
ErbB2/HER2	Protein-SELEX or Cell-SELEX or Internalized-SELEX	RNA and DNA	Targeting of HER2-driven cancer for therapy or diagnosis[43,56,64,115,131]
ErbB3/HER3	Protein-SELEX	RNA	Inhibition of heregulin-induced growth of MCF7 cells[132]
E-Selectin	Protein-SELEX	DNA	Targeting of cancers with upregulated E-Selectin expression for diagnosis or therapy[133–135]
EpCAM	Protein-SELEX	DNA and RNA	Targeting of EpCAM-expressing cancer cells for diagnosis or therapy[136,137]
Fractalkine (CX3CL1)	Protein-SELEX	DNA	Antagonist for Fractalkine-related inflammatory diseases or cancer[138]
HPV-16 E7	Protein-SELEX	RNA	HPV-infected cervical cancer therapy or diagnosis[139]
Immunoglobin Heavy Mu Chain (IGHM)	Cell-SELEX	DNA	Targeting aptamer for Burkitt lymphoma diagnosis and therapy[47,48]
Integrins- αvβ3	Protein-SELEX	RNA	Inhibition of integrin-dependent cancer cell proliferation[140]
Matrix metalloprotease 9 (MMP-9)	Protein-SELEX	RNA	Targeting aptamer for MMP-9 to promote cancer diagnosis or therapy[141]
MUC1	Protein-SELEX	DNA	MUC1-targeted aptamer that enhances cancer diagnosis or therapy[65,76,142]
Necleolin	Non-SELEX	DNA	Targeting or biotherapy for nucleolin-expressing cancers[15]
Prostate specific membrane antigen (PSMA)	Protein-SELEX	RNA and DNA	Targeting aptamer used in prostate cancer therapy or diagnosis[69,143]
PTK7	Cell-SELEX	DNA	Targeting aptamer for acute lymphoblastic leukemia therapy or diagnosis[11,49]
RET	Cell-SELEX	RNA	Neutralizing aptamer that inhibits RET-dependent intracellular signaling pathway[35]
Tenascin-C	Hybrid-SELEX	RNA	Aptamer that targets Tenascin-C-driven cancer for therapy or diagnosis[36]



Formulation of the Apt-HAuNS-Dox nanoscale drug-delivery system and the mechanism of pH-dependent drugs release. (**a**) A schematic illustration of Apt-HAuNS-Dox synthesis. Aptamers and PEG were conjugated to the surface of HAuNS sequentially via covalent S-Au bonds, followed by loading with doxorubicin through a charge force. (**b**) Our hypothesis is that the Apt-HAuNS-Dox NPs selectively **target lymphoma cells** via the aptamer-mediated biomarker interaction, resulting in internalization and intracellular delivery into lysosomes. Due to their low pH sensitivity, lysosomal microenvironment triggers a rapid Dox release and initiates tumor cell apoptosis. PEG, polyethylene glycol; NPs, nanoparticles.



Development of a tumor cell type-selective and cancer gene-specific nanocomplex for ALCL cells. (**a**) A nano-sized carrier core structure was initially formed via aggregation of polyethyleneimine (PEI) and cross linking with sodium citrate (PEI-citrate nanocore). The synthetic RNA-based CD30 aptamers and *ALK* siRNA were then incorporated onto the PEI-citrate nanocore to form the nanocomplex. (**b**) When the functional RNA nanocomplex is added to cultures, the aptamer component will selectively target CD30-positive ALCL cells. Aptamer-mediated cell binding will facilitate intracellular delivery of the nanocomplex. The siRNA component will subsequently silence the cellular *ALK* gene, resulting in the growth arrest of ALCL cells. **ALCL, anaplastic large cell lymphoma**.


Targeted therapy
In vivo detection
Theranostics
In vitro diagnosis
Aptamer
Commercial value

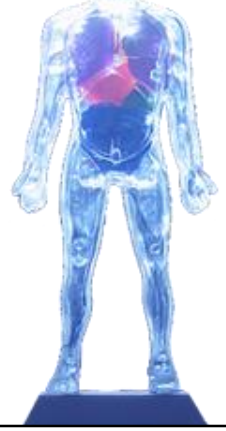
Reasons for Failures of Aptamer Drug Candidates

Typical Aptamer Strategy: Develop aptamers in vitro against a known protein target of interest to block disease pathway.

however…

In vitro selected aptamers do not necessarily operate/function in vivo as therapeutic candidates.

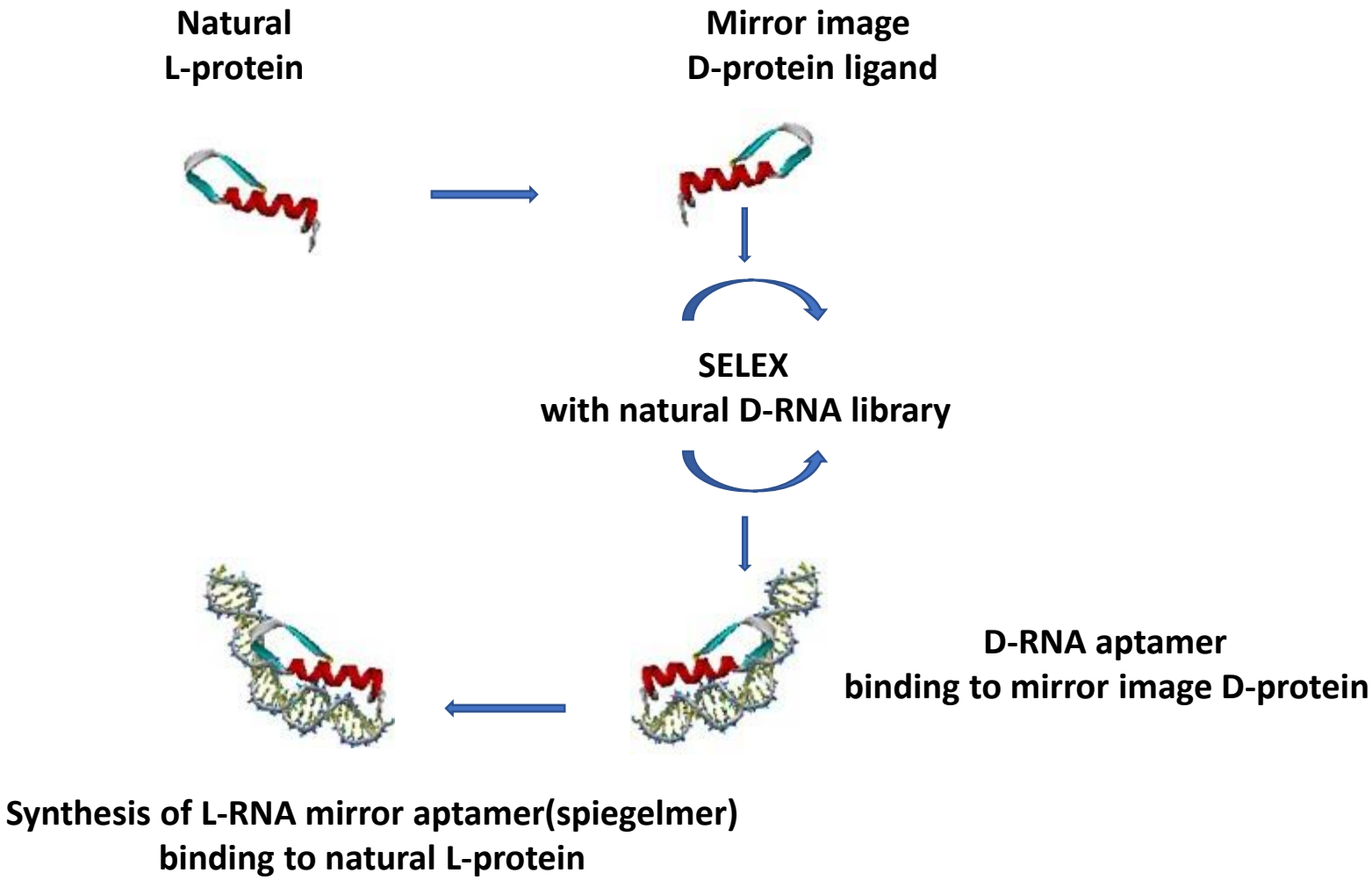
Aptamers are sensitive to the environmental conditions in which they are selected.



The Conventional Paradigm in preclinical development is deficient.

DELIVERY is always an issue!

http://images.google.com/images?q=drug+discovery&btnG=Search&hl=en&lr=&ie=UTF-8


Natural
L-protein
Mirror image
D-protein ligand
SELEX
with natural D-RNA library
D-RNA aptamer
binding to mirror image D-protein
Synthesis of L-RNA mirror aptamer(spiegelmer)
binding to natural L-protein

History of oligonucleotide therapeutics.