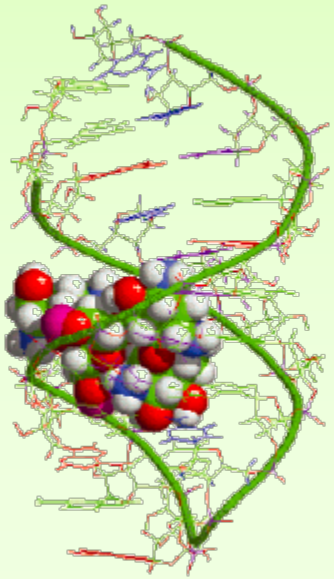
Gli APTAMERI e le LORO APPLICAZIONI



APTAMER TECHNOLOGY

What are aptamers?

" Aptamers (from the Latin *aptus*, to fit) are single-stranded nucleic acids which are capable of binding proteins or other small molecules"

- The technology of aptamer selection was discovered in 1990 by the groups of Tuerk & Gold and Ellington & Szostak.

- The aptamer selection techniques enable us to discover RNAs or DNAs (i.e. aptamers) that have affinity to virtually any desired targets (small molecules, proteins, carbohydrates, etc) even though they are not natural targets of DNAs or RNAs.

APTAMER TECHNOLOGY

Principle of aptamer technology

• RNA or DNA library is generated. In the library, the nucleic acid molecules are designed to contain randomized sequences in the center.

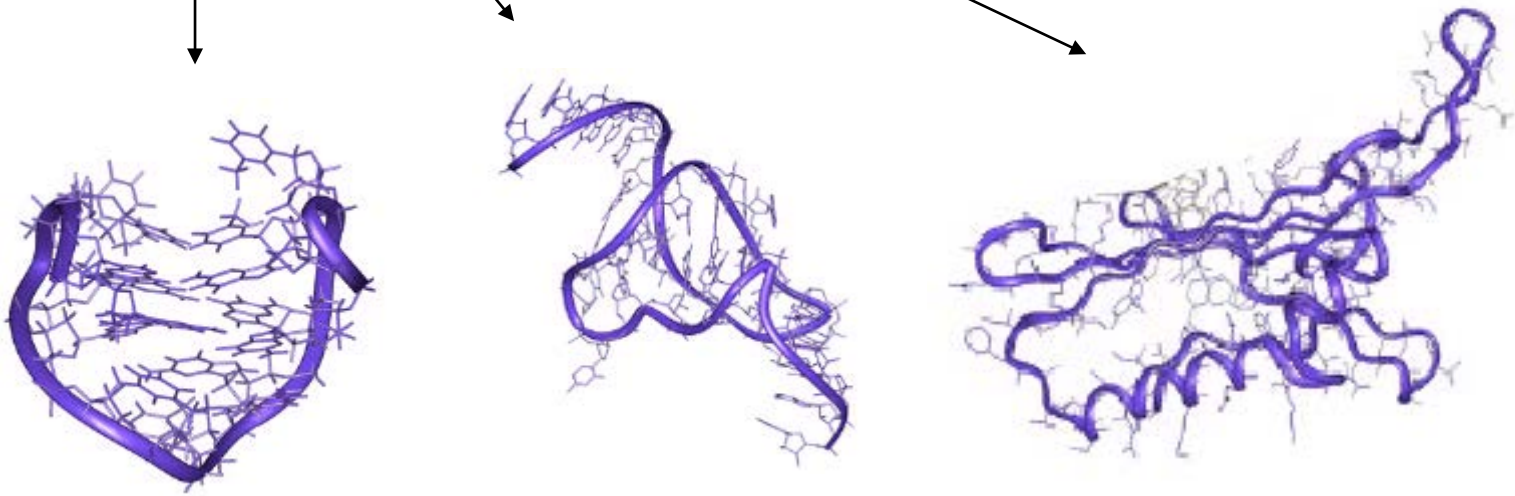
- If the randomized sequence is 30 nucleotides long, there will be 4^{30} or $\sim 10^{18}$ different nucleic acid molecules in the library.

About 100 mg to have 1 molecule for each sequence

APTAMER TECHNOLOGY

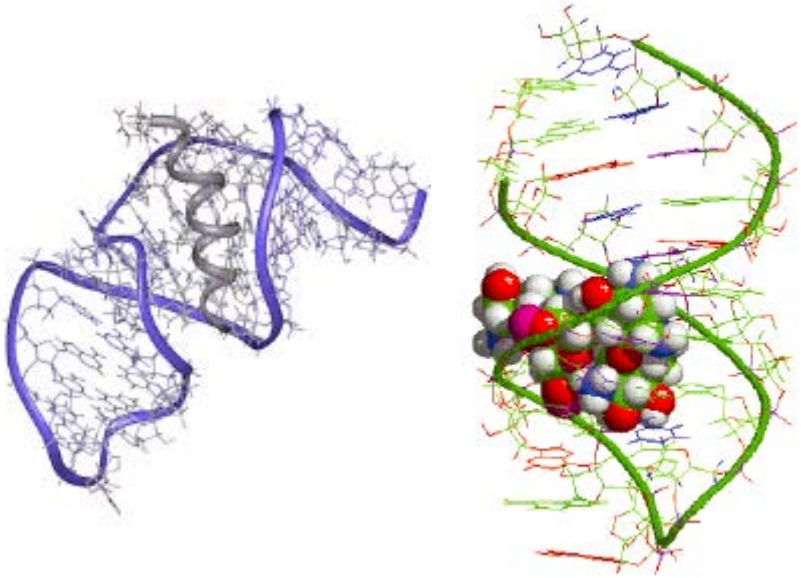
Single-stranded nucleic acids can fold into different structures using intramolecular interactions between bases, sugars and phosphate groups.

- hydrophobic (ring stacking)
- hydrogen bonding



APTAMER TECHNOLOGY

Out of 1,000,000,000,000,000,000 different molecules in the random library, there is a chance that some nucleic acid molecules (or aptamers) can fold into proper conformation able to bind tightly and specifically with the desired target.



Interactions between target and aptamer

- charge-charge interaction

- hydrogen bonding

- hydrophobic interaction

APTAMER TECHNOLOGY

How to select the aptamers with desired properties out of a complex library?

The aptamers are usually present at a very low abundance in the library, possibly only one molecule out of 1,000,000,000,000,000,000 different molecules in the library.

***in vitro* evolution**

or

SELEX (Systematic Evolution of Ligands by EXponential enrichment)

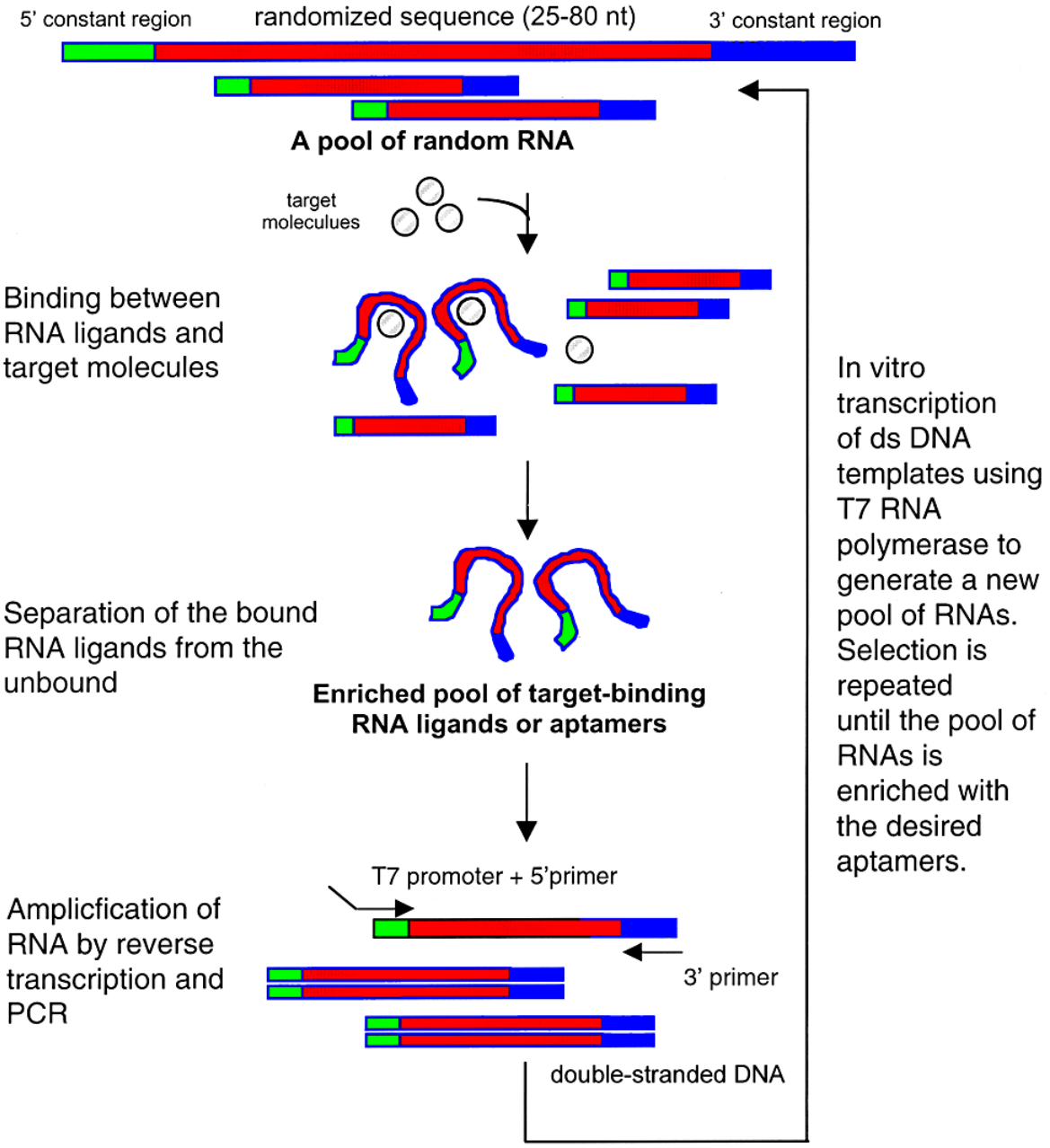
APTAMER TECHNOLOGY

***In vitro* evolution or**
SELEX (Systematic Evolution of Ligands by EXponential enrichment)

RNA library

~10^{16} - 10^{17} different sequences

APTAMER TECHNOLOGY



APTAMER TECHNOLOGY

Aptamers recognizing micro- and molecular targets

Small molecules:

antibiotics, amino acids, nucleotides

Proteins:

streptavidin, prion protein, DNA polymerase, reverse transcriptase, integrase, thrombin, VEGF, oncostatin, etc

Carbohydrates:

cellulose, dextran, sially Lewis X (blood group antigen)

Lipids:

cholic acid, farnesyl

Nucleic acids:

16s rRNA, tRNA, TAR element of HIV1

APTAMER TECHNOLOGY

Recognition of targets by aptamers can be very specific and strong

- Aptamers to L-arginine can differentiate between L- and D-arginine.

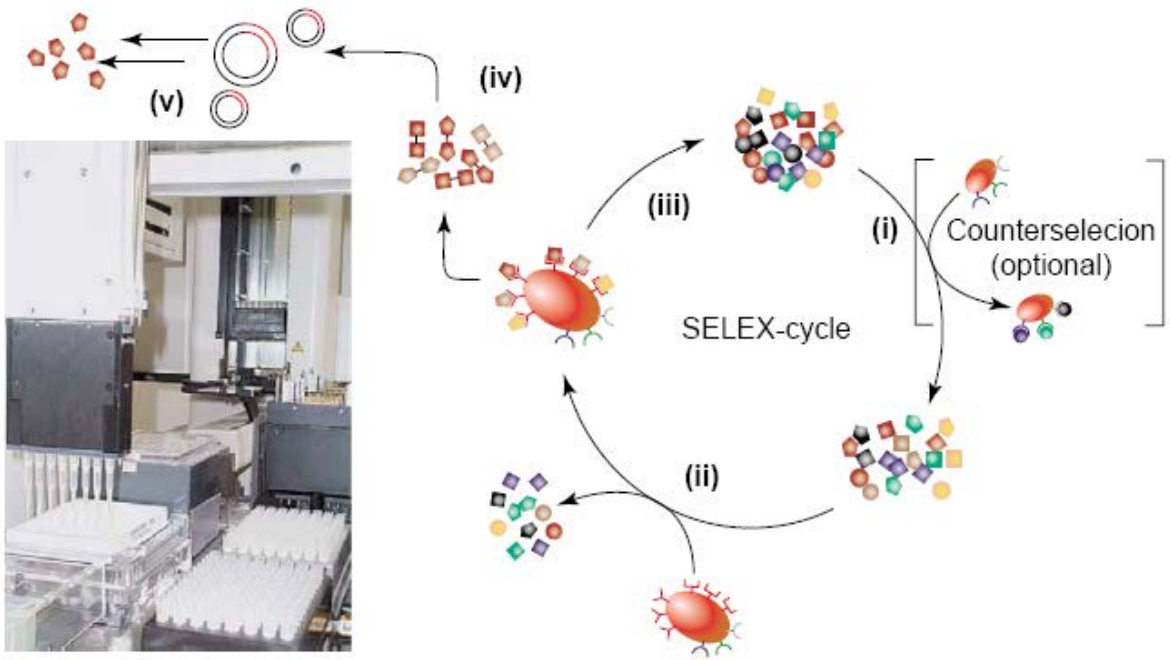
- Aptamers to dextran (repeating units of glucose linked via α-1,6 glucosidic bonds) do not bind to celluose (repeating units of glucose linked via β-1,6 glucosidic bonds)

- Dissociation constants can be very low (in nM or even pM ranges).

APTAMER TECHNOLOGY

Aptamers V.S. Antibodies

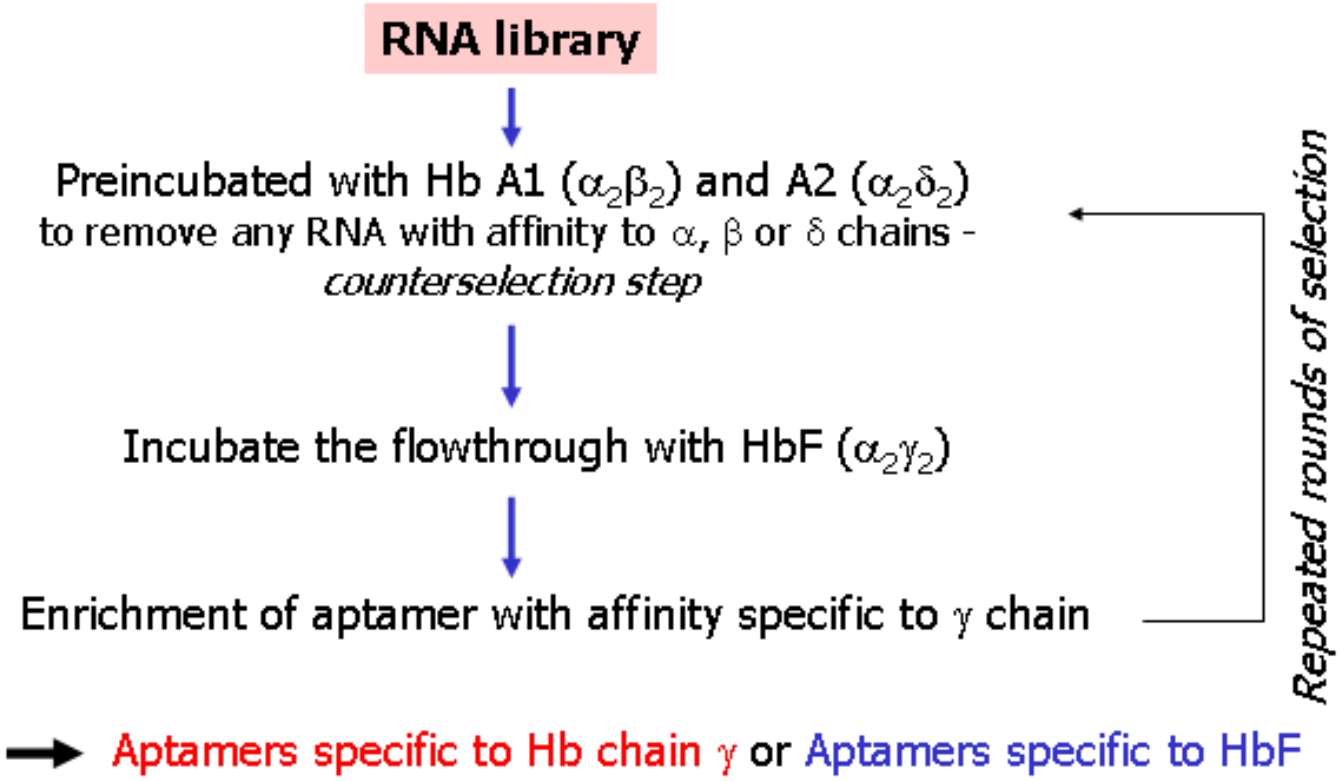
- Affinity and specificity of aptamers are comparable to those of antibodies.
- Aptamers are useful for some targets that may be less immunogenic or toxic.
- Aptamer selection is faster, cheaper and obviates the need of using animals.



APTAMER TECHNOLOGY

Aptamers V.S. Antibodies

- Aptamers with desired properties can easily be generated using appropriate selection strategies.



APTAMER TECHNOLOGY

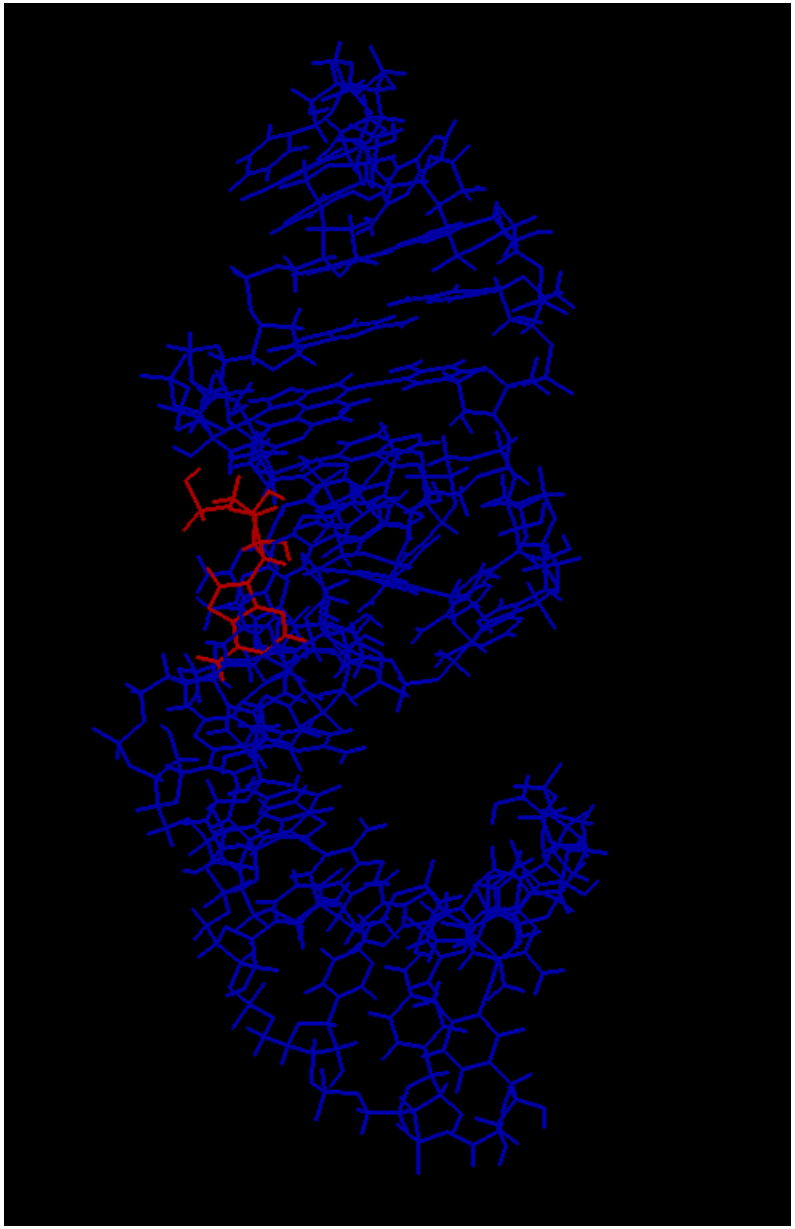
Aptamers V.S. Antibodies

- Aptamer has promising roles in therapy (good tissue penetration, shorter half-life, and less immunogenic).

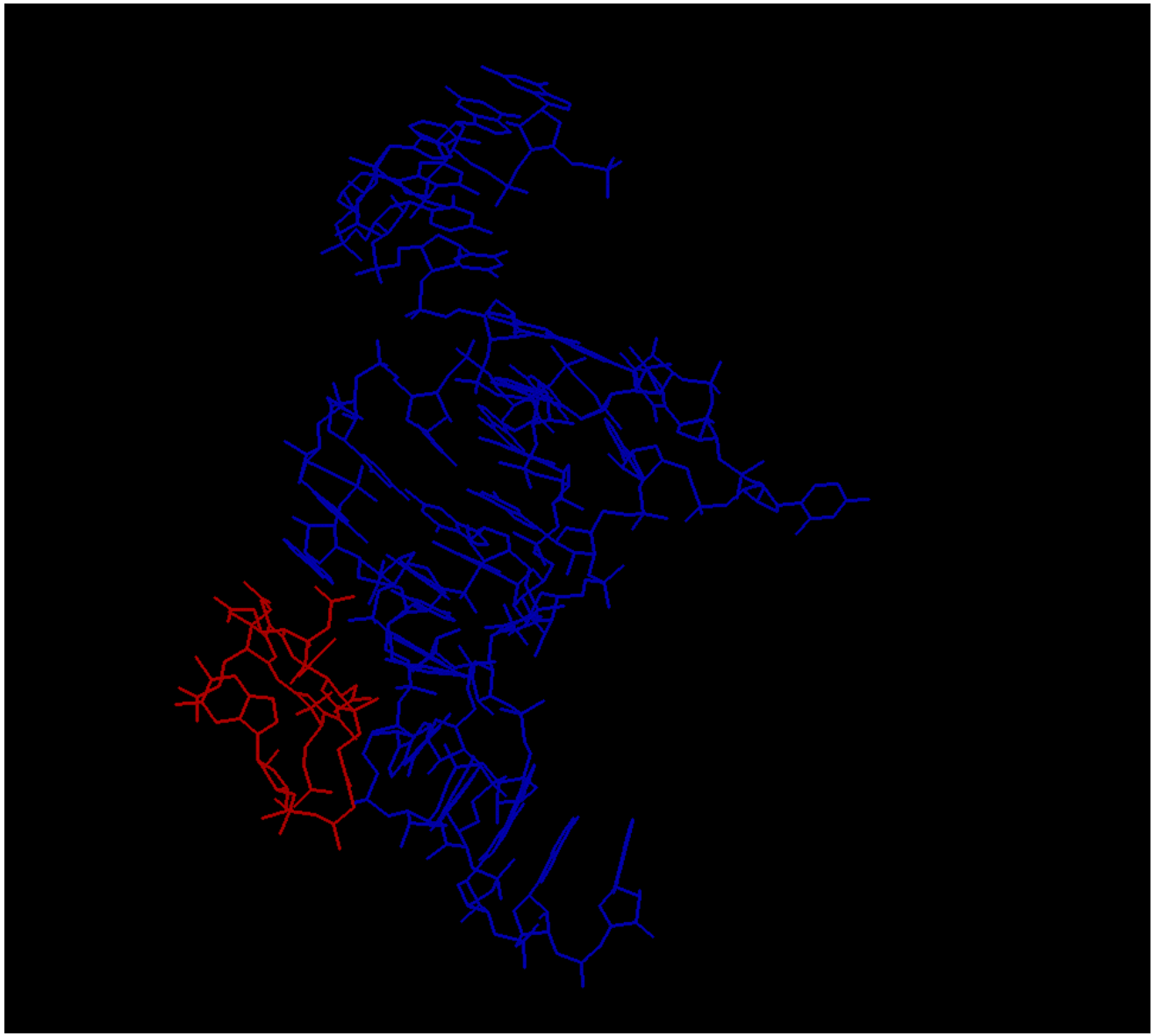
- Modifications of aptamers are much easier, compared to antibodies.

Aptamers as an emerging class of molecules that rival antibodies in therapeutic, diagnostic, and research applications.

ATP Aptamer with AMP



Aptamer for Vitamin B12



APTAMERS IN THERAPEUTICS

Uses of aptamers in therapeutics :

Target inhibition (to bind and block activities of target molecules)

Target molecules

- extracellular targets

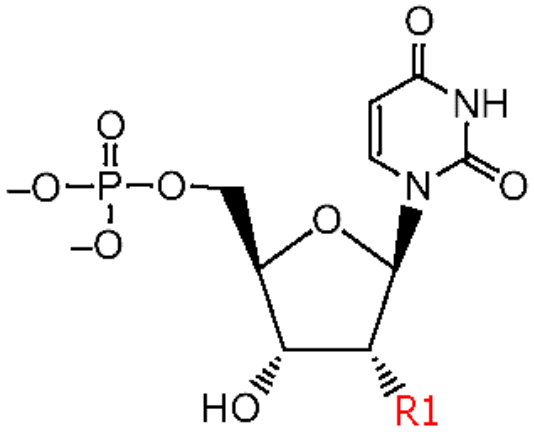
- intracellular targets

Nuclease-resistant aptamers for targeting extracellular targets

• Unmodified RNA and DNA are subject to rapid nuclease degradation.

Half-life of RNA in plasma ~ seconds

• Replacement of natural pyrimidines with their 2'-modified nucleotide increase RNA stability.



R1 = fluoro, amino, O-methyl, or O-allyl

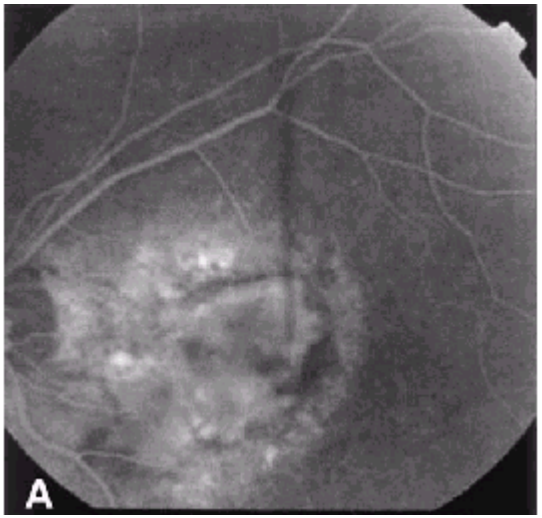
APTAMERS IN THERAPEUTICS

Example of extracellular target inhibition by aptamers

Anti-VEGF aptamers (VEGF= vascular endothelial growth factor)

Ruckman J, Green LS, Beeson J, et al. *J Biol Chem.* 1998;273:20556–20567.

VEGF plays important roles in some eye disorders (e.g. choroidal neovascularization or diabetic retinopathy)



- increase blood vessel formation -> bleeding
- increase vascular permeability -> exudation

↓

Loss of vision

Inhibition of VEGF is the mechanism of action of anti-angiogenic/anticancer drugs **Bevacizumab** (commercial name Avastin, monoclonal antibody) and **Ranibizumab** (commercial name Lucentis, monoclonal antibody Fab)

APTAMERS IN THERAPEUTICS

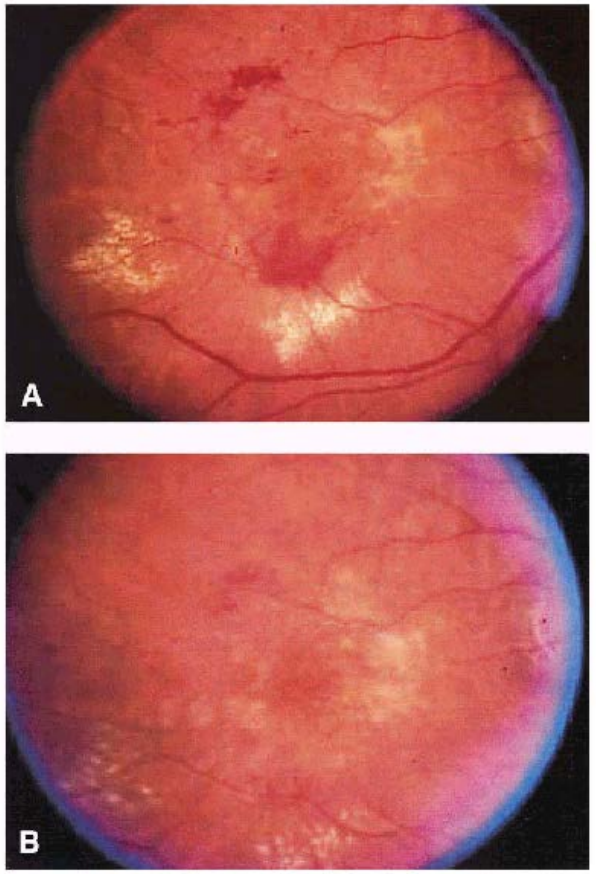
Anti-vascular Endothelial Growth Factor Therapy for Subfoveal Choroidal Neovascularization Secondary to Age-related Macular Degeneration

Phase II Study Results

The Eyetech Study Group

Ophthalmology

Volume 110, Number 5, May 2003



Anti-VEGF aptamers are safe and ~ 80% of the treated eyes show stabilization or improvement of vision.

APTAMER AS A TOOL IN RESEARCH

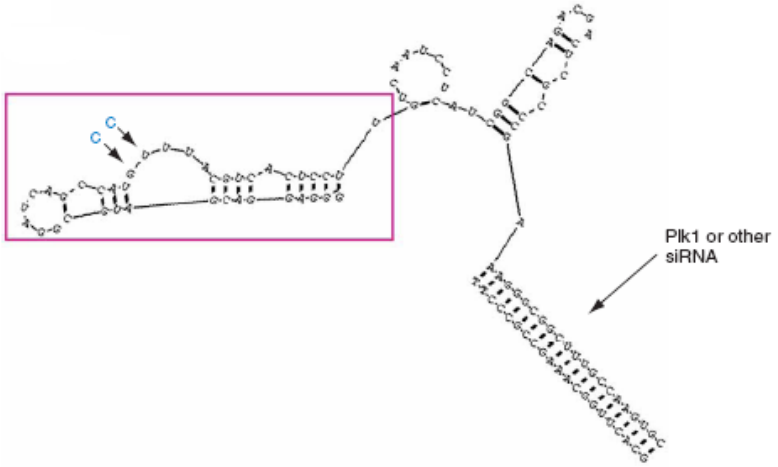
Other applications of aptamer in systems biology research

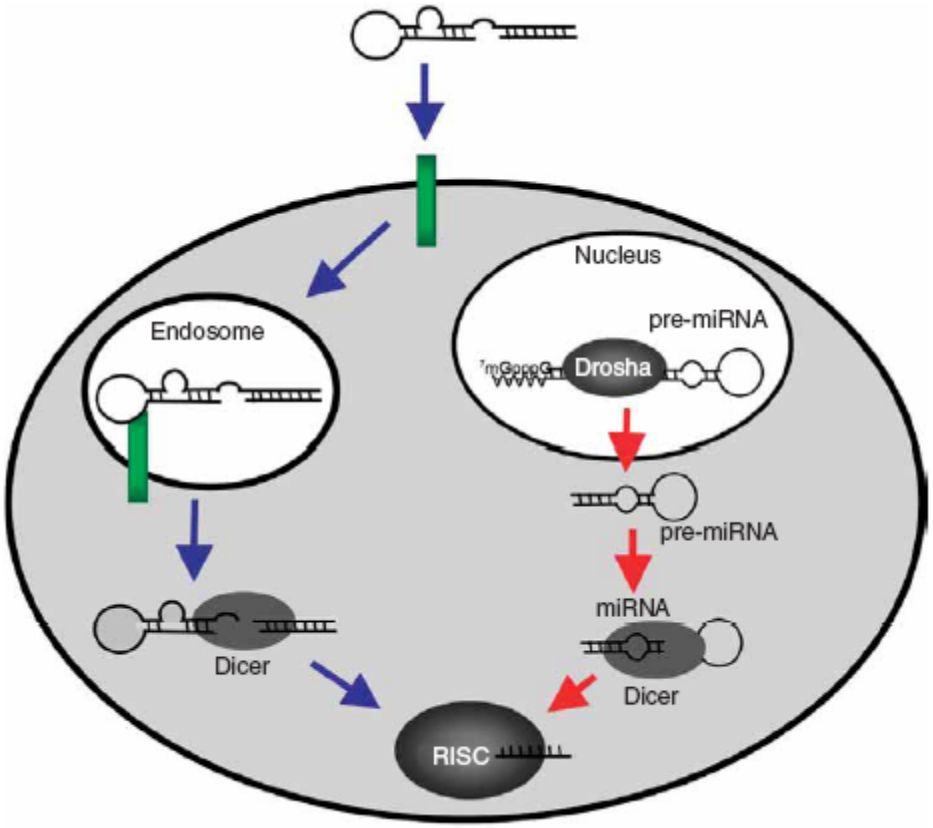
Cell type–specific delivery of siRNAs with aptamer-siRNA chimeras

James O McNamara II[1,3], Eran R Andrechek[2,3], Yong Wang[1], Kristi D Viles[1], Rachel E Rempel[2], Eli Gilboa[1], Bruce A Sullenger[1] & Paloma H Giangrande[1]

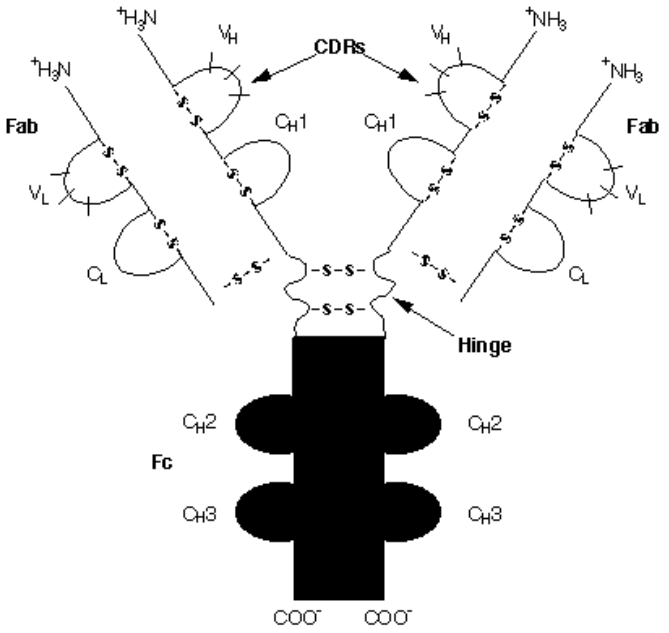
VOLUME 24 NUMBER 8 AUGUST 2006 NATURE BIOTECHNOLOGY

anti-prostate specific membrane antigen aptamer





Examples of intracellular target inhibition by aptamers



Antibodies contain intramolecular disulfide bonds, which may not form properly in the reducing environment of cytoplasm.

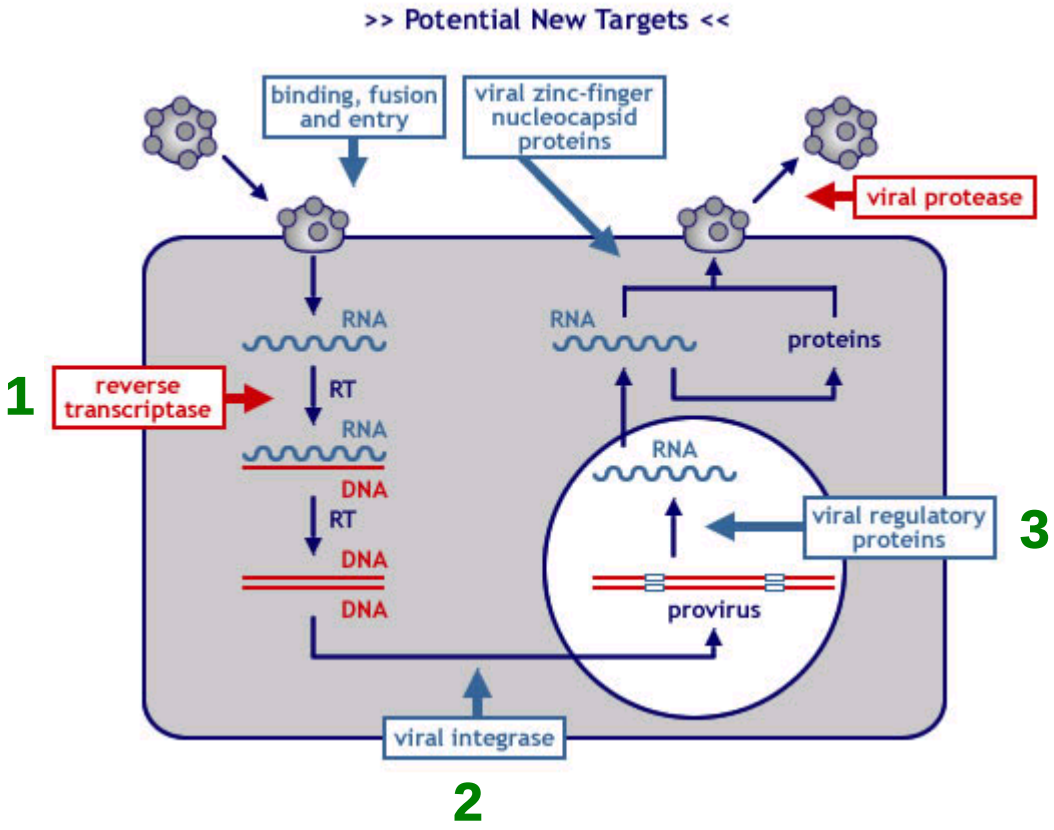
↓

reduced binding activity towards the targets

RNA aptamers can be used inside the cell instead of antibodies to block the functions of intracellular targets.

→ *Applications in gene therapy*

Aptamers against protein components of HIV



1. Anti reverse transcriptase
2. Anti-integrase
3. Anti-TAT, Anti Rev

Example: anti-reverse transcriptase aptamers

Potent Inhibition of Human Immunodeficiency Virus Type 1 Replication by Template Analog Reverse Transcriptase Inhibitors Derived by SELEX (Systematic Evolution of Ligands by Exponential Enrichment)

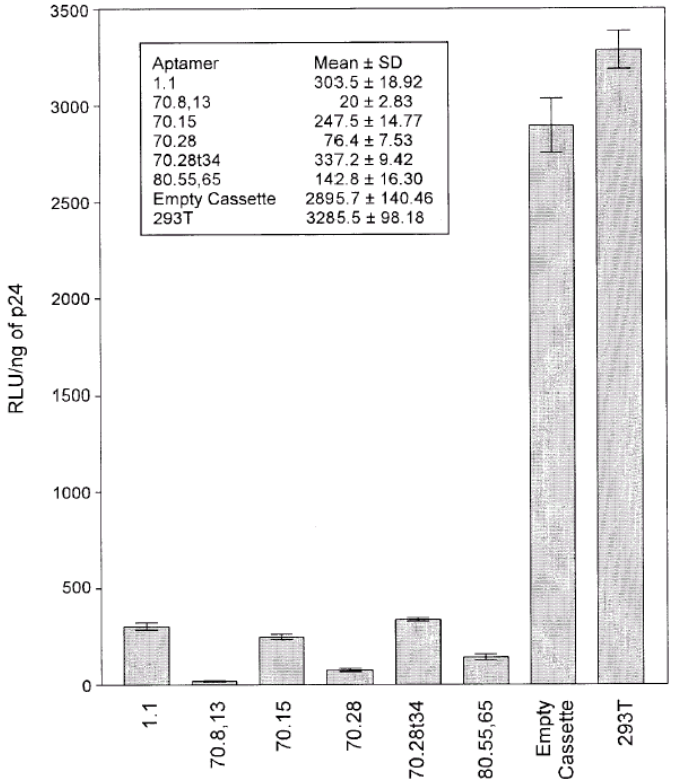
Pheroze Joshi and Vinayaka R. Prasad*

Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York 10461

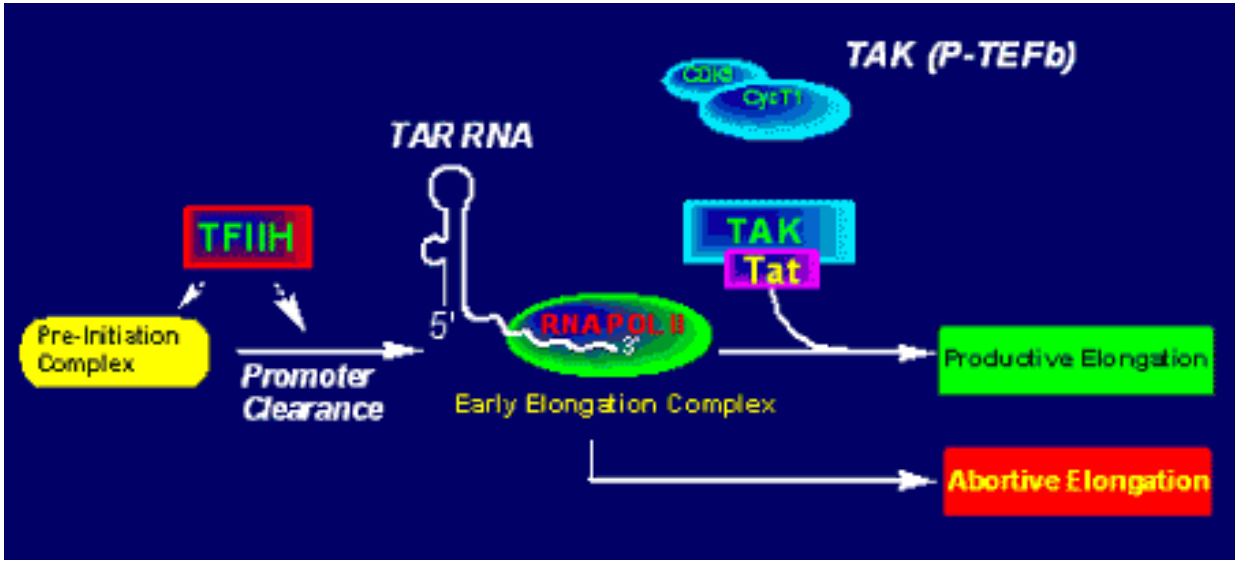
JOURNAL OF VIROLOGY, July 2002, p. 6545–6557

TABLE 1. Dissociation constants (K_d) of various aptamers for interaction with HIV-1 RT and their ability to inhibit RT activity in vitro (IC_{50})

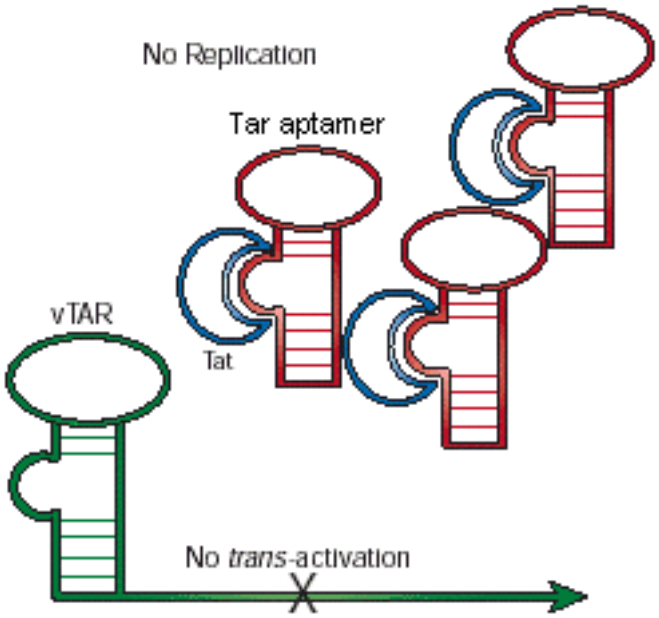
RNA aptamer	K_d (nM)	IC_{50} (nM)	Length (nt)[a]
70.8,13	27	89	78
70.15	63	159	73
80.55,65	129	143	79
70.12,16	184	210	71
70.28	201	254	77
80.10	180	309	89
70.28t34	219	327	67
80.18	197	334	74
1.1	270	607	65
70.24,67	2,001	>1,000	101



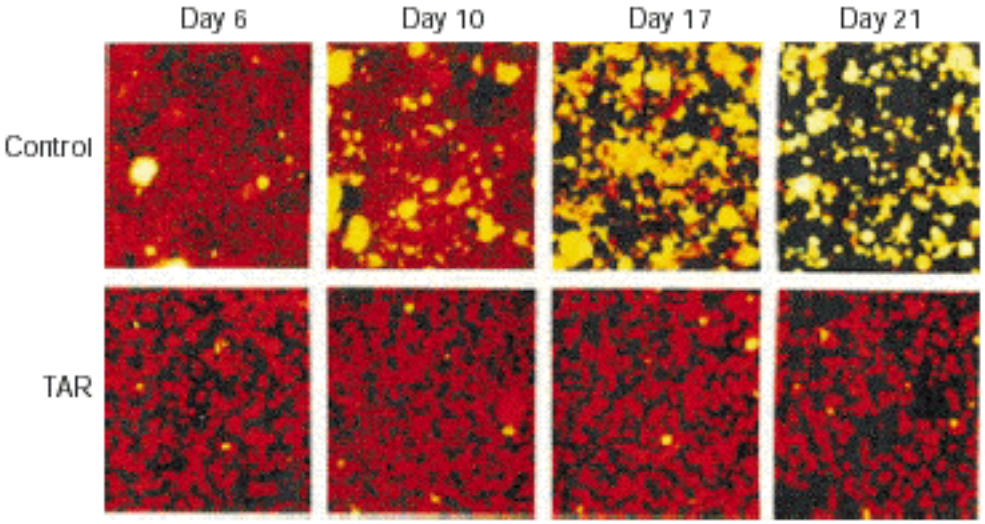
Example: anti-Tat aptamers



Tat-TAR binding leads to productive transcription of HIV1 genome, activating viral gene expression.



Anti-Tat aptamers act as TAR decoys. They compete for Tat, which subsequently inhibits Tat-TAR interactions and stops viral gene expression and replication.



Expression of anti-Tat aptamers can render cells resistant to HIV. TAR decoy- and control vector-containing CD4+ T cells were challenged with HIV-1 and viral spread through the cultures was monitored by immunofluorescent staining of cells (yellow cells) at various days following infection

Aptamers for targets of all types

Table 1: DNA and RNA aptamers for various targets indicating known dissociation constants (K_ds). [1]

Molecular Target		Scaffold	K_d	Reference
Class	Molecule			
Ions	K^+	DNA	76 nM	[76]
	Hg^{2+}	DNA	-	[77]
	Cu^{2+}	DNA	-	[78]
	Zn^{2+}	DNA	-	[79]
	UO_2^{2+}	DNA	-	[80]
	Pb^{2+}	DNA	-	[81, 82]
Organic Dyes	Malachite green	RNA	0.8 μM	[83]
	Reactive green 19	DNA	33 μM	[84]
	Sulforhodamine B	DNA	0.66 μM	[85]
Nucleotides	ATP / Adenosine	RNA	1 μM	[86]
& nucleobases	ATP / Adenosine	DNA	6 μM	[87]
	Guanosine	RNA	32 μM	[88]
	Theophylline	RNA	0.11 μM	[89]
	cAMP	RNA	10 μM	[90]
	7-methyl GTP	RNA	0.5 μM	[91]
	Guanine/Xanthine	RNA	1.8 μM	[92]
Amino acids	Arginine	RNA	0.33 μM	[93]
	Arginine	DNA	2.5 mM	[94]
	Citrulline	RNA	62 μM	[95]
	Valine	RNA	12 mM	[96]
	Tryptophan	RNA	18 μM	[97]
	L-tyrosinamide	DNA	45 μM	[98]
Antibiotics	Tetracycline	RNA	0.8 nM	[99]
	Tobramycin	RNA	0.8 nM	[100]
	Viomycin	RNA	12 μM	[101]
	Chloramphenicol	RNA	2.1 μM	[102]
	Neomycin	RNA	0.1 μM	[103]
	Lividomycin	RNA	< 0.2 μM	[104]
	Streptomycin	RNA	~ 1 μM	[105]
	Kanamycin	RNA	< 0.2 μM	[104, 106]
Bioactive small	Dopamine	RNA	2.8 μM	[107]
molecules	Peptide (substance P)	RNA	0.19 μM	[108]
	cholic acid,	DNA	20 μM	[109]
	(R)-thalidomide	DNA	1 μM	[110]
	17β-estradiol	DNA	n.d.	[111]
	Cocaine	DNA	5 μM	[112]
	Aspartame	DNA	n.d.	[113]
Transition-state	Diels Alder reaction	RNA	3.5 mM	[114]
analogs	Bridged biphenyl isomerization	RNA	542 μM	[115]
Oligosaccharides	Cellobiose	DNA	0.1-10 μM	[116]
	Sialyllactose	DNA	4.9 μM	[117]
Toxins	Ricin	DNA	58 nM	[118]
	Abrin toxin	DNA	28 nM	
	BotulinuM neurotoxin	DNA	3 nM	[119]
	Ochratoxin A	DNA	0.36 μM	[120]
Cofactors	Cyanocobalamin	RNA	0.09 μM	[121]
	Flavin	RNA	0.5 μM	[122]
	NAD	RNA	2.5 μM	[123]
	RMP-biotin	RNA	2 μM	[124]
	N-methylmesoporphyrin IX	RNA	14 μM	[125]
	N-methylmesoporphyrin IV	DNA	0.5 μM	[126]
	Factor XII	DNA	~ 1 μM	[127]
	Coenzyme A	RNA	1 μM	[128]
	S-adenosyl methionine	RNA	n.d.	[129]
Peptides	Vasopressin	DNA	0.85 μM	[130]
	RGD	DNA	n.d.	[131]
	Neuropeptide Y	DNA	0.3 – 10 μM	[132]
	Pepocin	RNA	~20 nM	[133]
Proteins	Nucleolin	DNA		[134]
	Thrombin	DNA	25-200 nM	[135]
	factor VIIa	RNA	11 nM	[136]
	Factor IXa	RNA	0.58 nM	[137]
	von Willebrand factor	RNA	1.4 nM	[138]
	PrPc	RNA	n.d.	[139]
	PrPc	DNA	10 nM – 23 μM	[140]
	Aβ	RNA	29-48 nM	[141]
	β2microglobulin	RNA	10 nM	[142]
	BACE-1	RNA	280 nM	[143]
	PSMA	RNA	2.1 nM	[144]
	neutrophil elastase	DNA	17 nM	[145]
	Taq DNA polymerase	DNA	7-36 pM	[146]
	Protein kinase C-δ	DNA	122 nM	[147]
	P-selectin	RNA	19-39 μM	[148]
	L-selectin	DNA	1.8 nM	[149]
	Sialyl Lewis X	RNA	85 μM	[150]
	Human PLA2	RNA	1.7 nM	[151]
	Interferon-γ	RNA	2.7	[152]
Hormones,	Human VEG-F 165	2'FPy RNA	49-130 pM	[153]
Growth factors	Angiopoietin-2	RNA	2.2 nM	[154]
& cell-surface	GnRH	Spiegelmer	20 nM	[155]
receptors	Ghrelin	Spiegelmer	45-90 nM	[156]
	PDGF B-chain	DNA	10 nM	[157]
	TGFβRIII	RNA	1.5-2.5 nM	[158]
	4-1BB	RNA	40 nM	[159]
	OX40	RNA	8 nM	[160]
	Human bFGF	DNA	16-560 pM	[161]
	HER3/ErbB-3	RNA	45-400 nM	[162]
	Transferrin receptor	DNA	nM range	[163]
	Transferrin receptor	RNA	nM range	[163]
	MEN2A receptor	2'F-Py RNA	30-100 nM	[164]
	CTLA-4 (T-cell)	RNA	10 nM	[165]
Transcription	E2F isoforms	2' F-Py RNA	0.2-5 nM	[166, 167]
Factors	NFκB	DNA	n.d.	[168]
Antibodies	Human IgE	DNA	9 nM	[169]
	Human IgE	RNA	30 nM	[169]
	Anti-insulin receptor AbMA20	RNA	30 nM	[170]
	mAb to acetyl choline receptor	RNA	6-60 nM	[171]
	Rat mAb 198	RNA	25 nM	[172]
Anti-viral targets	Influenza virus surface glycoproteins	DNA	n.d.	[173]
	HIV MN envelope glycoprotein	DNA	1 nM	[174]
	HIV-1 TAR element	DNA	20-50 nM	[175]
	HIV-1 reverse transcriptase	DNA	180 pM	[176]
	HIV-1 Tat	RNA	n.d.	[177]
	HIV-2 Tat	RNA	n.d.	[178]
	Hepatitis C NS3	RNA	120 nM	[179]
	HIV-1 RNase H	DNA	n.d.	[180]
Whole cells	anthrax spores	DNA	-	[181]
	YPEN-1 endothelial cells	DNA	-	[182]
	PC12 cells	DNA	-	[183]
	U-251 glioblastoma (tenascin-C)	2'F-py RNA	5 nM	[184]
	Trypanosoma cruzi	RNA	172 nM	[185]
	SCLC cells	DNA	-	[186]
	Jurkat T leukemia cell,	DNA	-	[187]
	B-cell tumor cells	DNA	-	[188]
	CCRF-CEM leukemia cells	DNA	-	[189]

[1] n.d.: not determined; -: not applicable

Review di Krishnan e Simmel 2010 (preliminary version of paper)

Aptamers to targets with potential applications

Target	References	Comments
Toxins		
Ricin, pepocin, gypsphilin	144	Have different sequence from natural rRNA targets
Enzymes		
Thrombin	7	First identification
	138, 145	Use in vivo
	146	Better than heparin against clot-bound thrombin
	81	An RNA aptamer against thrombin
Activated plasma protein C	74	
HIV-1 reverse transcriptase	69, 96	
Other retroviral reverse transcriptases	107, 147	
HIV-1 integrase	95, 148	
Protein kinase C	108	
Human neutrophil elastase	124, 149, 150	Identification of inhibitory aptamer when conjugated to weak peptide inhibitor of enzyme. Effective in an animal model of lung injury
Hepatitis C virus NS3 protease/helicase	103, 151, 152	
Yersinia protein tyrosine phosphatase	153	
Phospholipase A2	100	Inhibited contractions of guinea pig pleural strip in vitro
2′,5′-Oligoadenylate synthetase	121	
Angiogenin (ribonuclease activity)	154	DNA aptamer prevents angiogenesis and cell proliferation
Adhesion and recognition molecules		
L-selectin	155	DNA aptamers inhibit cellular adhesion and rolling in vitro and trafficking in vivo
P-selectin	156	Affinity (20 pM) is 10^6-fold better than the natural ligand sialyl Lewis X. Unlike the latter, discriminate against E- and L-selectins by 10^4–10^5
CD4	38, 115	Disrupts immune recognition in vitro
Rhinovirus capsid protein	157	Intended to block infectivity of virus
Growth factors and hormones		
Basic fibroblast growth factor	83	
VEGF	14, 84, 140	Inhibit induction of permeability in vivo. 2′-F modification and lipid derivatization
		Improve stability and pharmacological properties

Target	References	Comments
NGF[a]	82	
PDGF	99, 139	Effective in vivo as a PEG conjugate
Keratinocyte growth factor	13	
γ-Interferon	158, 159	
Substance P	76	
Vasopressin	18	
Regulatory proteins and oncogenes		
E2F transcription factor	86	Prevents binding to DNA and entry into S phase
K-ras	101	Raised against farnesylated peptide
Raf-1	102	
P210bcr-abl	160	DNA aptamer introduced into chronic myelogenous leukemia cells by electroporation reduced cell proliferation
MDM2 oncoprotein	161	
HIV-1 Rev	67, 88, 143, 162–164	These are Rev-responsive, even though unrelated to natural Rev-response element. Are inhibitory to HIV-1 growth in cell cultures
HTLV-I Tax	39	
Miscellaneous		
IgG against human insulin receptor	104	Possible approach to blocking a common form of antibody-mediated, autoimmune diabetes
IgE	12	Bind Fc portion of antibody, preventing interaction with cell surface receptor. Possible role in allergy therapy
Hamster PrP[a]	165	

[a] NGF, nerve growth factor; PrP, prion protein.

Reports some examples of aptamers able to inhibit in vitro and/or in vivo the target molecule

Aptamer	Target molecule	Aptamer activity		Diagnostic or therapeutic application
		in vitro	in vivo	
PKC-6 PKC-10	protein kinase CβII	inhibition of the enzyme autophosphorylation	nd	nd
9A	Raf-1 RBD	inhibition of the Ras-induced Raf-1 activation	nd	nd
NX1838	$VEGF_{165}$	inhibition of VEGF binding to VEGF receptor	inhibition of the VEGF-induced vascular permeability	administration in humans in phase-I clinical trials
PDGF-B aptamer	PDGF-B	inhibition of PDGF binding to PDGF receptor	reduction of tumor interstitial fluid pressure	administration in tumor-bearing rats
NX21909	neutrophil elastase	inhibition of the enzyme	inhibition of lung injury and neutrophil influx	imaging of inflammation
Toggle-25	thrombin	inhibition of plasma clot formation and platelet activation	nd	nd
XAP	anti-NES antibody	inhibition of binding to the NES receptor	inhibition of the Rev-dependent export	nd
AL6-A	angiogenin	inhibition of the ribonucleolytic activity of angiogenin	nd	nd

Where determined, the diagnostic or therapeutic effect of the aptamer is reported. nd, not determined.

APTAMER TECHNOLOGY

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- Pharmacologic Properties
- Production Properties
- Chimeric Aptamers
- Aptamers vs. Antibodies
- Examples

THERAPEUTICS

Aptamers are oligonucleotides that bind to molecular targets in a manner conceptually similar to antibodies. Through the SELEX process, aptamers have been identified against numerous target types including growth factors, enzymes, immunoglobulins, receptors, viral proteins and others. The Archemix preclinical portfolio includes aptamers directed to a wide range of validated therapeutic targets (see Examples). Aptamers are similar to therapeutic antibodies, and as such, have a number of desirable characteristics for use as therapeutics, including biological efficacy, high specificity and affinity, and excellent pharmacokinetic properties. In addition, they offer specific competitive advantages such as:

- aptamers are produced by an entirely *in vitro* process, which allows for the generation of initial therapeutic

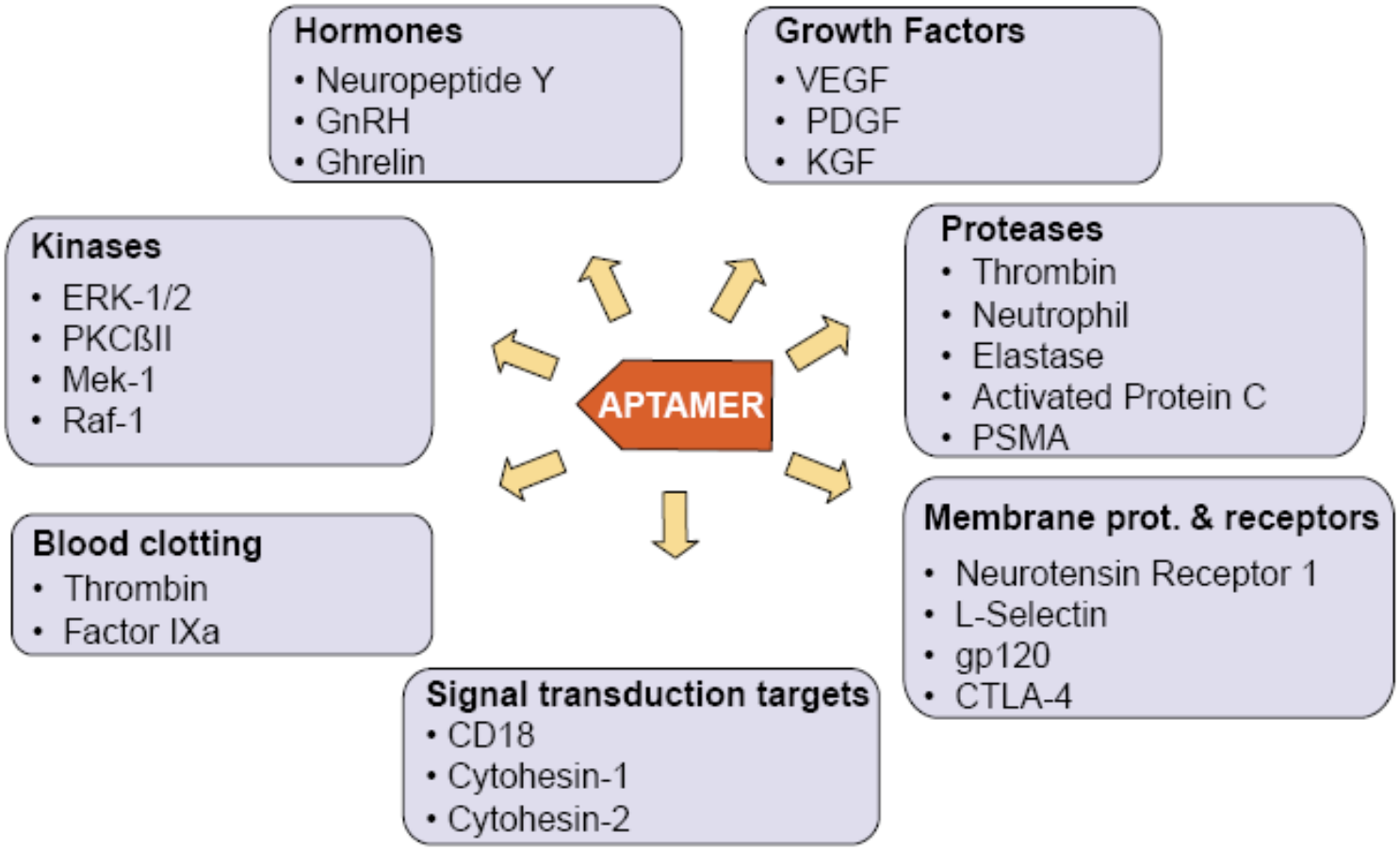
APTAMERS

Aptamers are a new class of therapeutic molecules with substantial advantages over existing protein therapeutics.

Click to see larger image.

APTAMER AS A TOOL IN RESEARCH

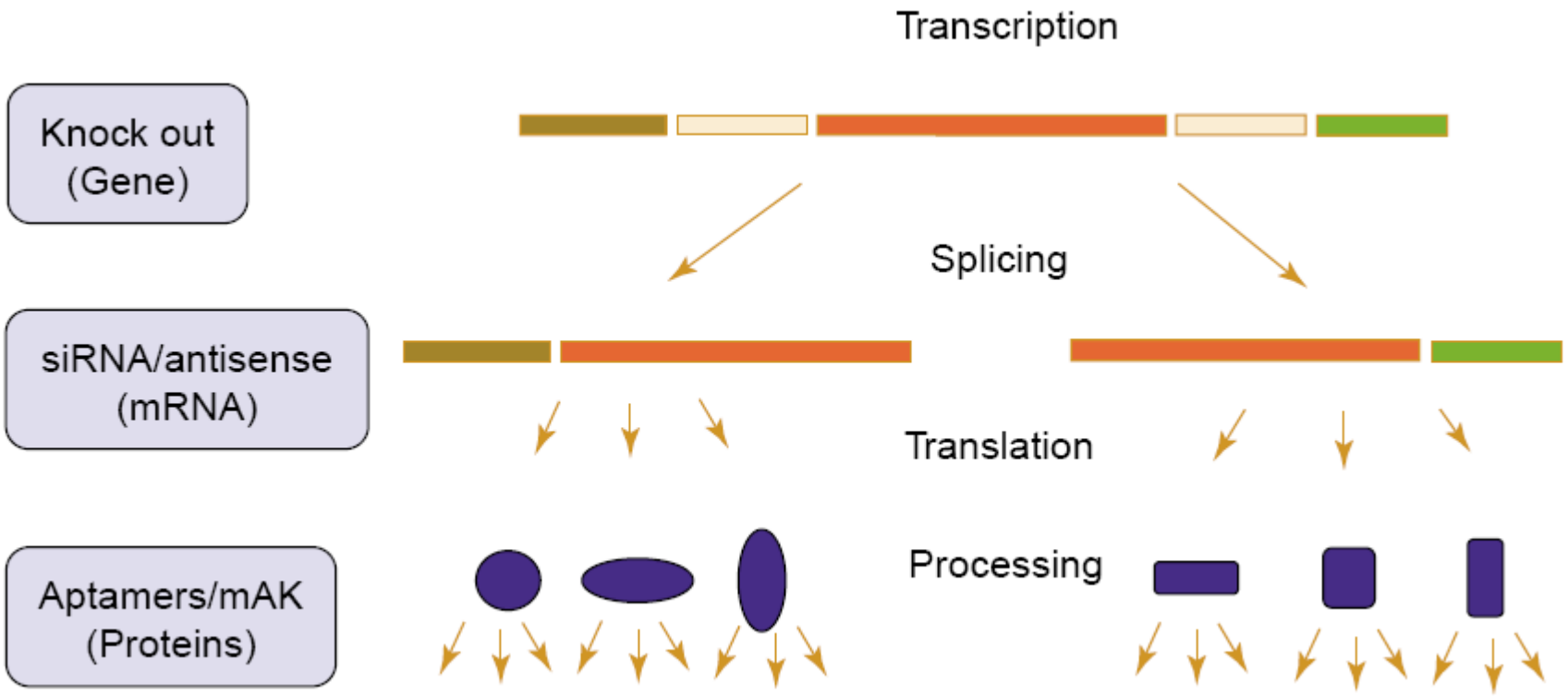
Aptamers as a tool to study the target functions



Aptamers can be used to antagonize the target proteins as a means of elucidating their biological roles.

APTAMER AS A TOOL IN RESEARCH

Aptamers as a tool to study the target functions



The results obtained from the studies using aptamers can be complementary to those using siRNA or gene knockout.

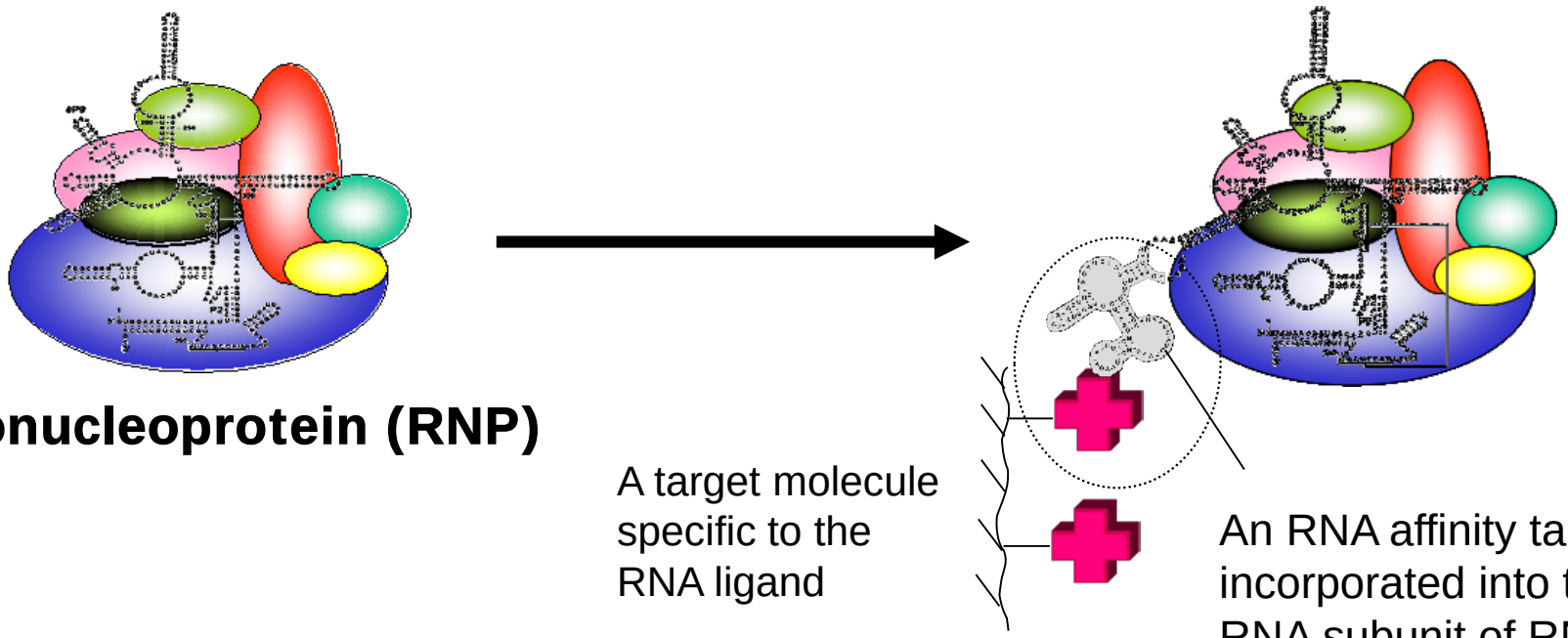
APTAMER AS A TOOL IN RESEARCH

RNA aptamers as affinity tags to identify the protein-RNA interactions

- Many of noncoding RNAs have been identified in humans but the protein subunits or functions of most noncoding RNAs are not yet known.

The need for RNA affinity tags

to enable specific isolation of the noncoding RNAs and their subunits to study the functions and subunit components.



Ribonucleoprotein (RNP)

A target molecule specific to the RNA ligand

An RNA affinity tag is incorporated into the RNA subunit of RNP.

APTAMER AS A TOOL IN RESEARCH

RNA aptamers as affinity tags to identify the protein-RNA interactions

• RNA aptamers against various matrices have been developed to be used as RNA affinity tags.

Streptavidin aptamers: Affinity tags for the study of RNAs and ribonucleoproteins

CHATCHAWAN SRISAWAT and DAVID R. ENGELKE
Department of Biological Chemistry, The University of Michigan, Ann Arbor, Michigan 48109-0606, USA

RNA (2001), *7*:632–641. Cambridge University Press. Printed in the USA.

Sephadex-binding RNA ligands: rapid affinity purification of RNA from complex RNA mixtures

Chatchawan Srisawat, Irwin J. Goldstein and David R. Engelke*

Nucleic Acids Research, 2001, Vol. 29, No. 2 e4

StreptoTag: A novel method for the isolation of RNA-binding proteins

MONIKA BACHLER,[1] RENÉE SCHROEDER,[1] and UWE VON AHSEN[2]

RNA (1999), *5*:1509–1516. Cambridge University Press. Printed in the USA.

• The RNA affinity tags enable a specific isolation of ribonucleoproteins in a native state with preserved activity.

APTAMER AS A TOOL IN RESEARCH

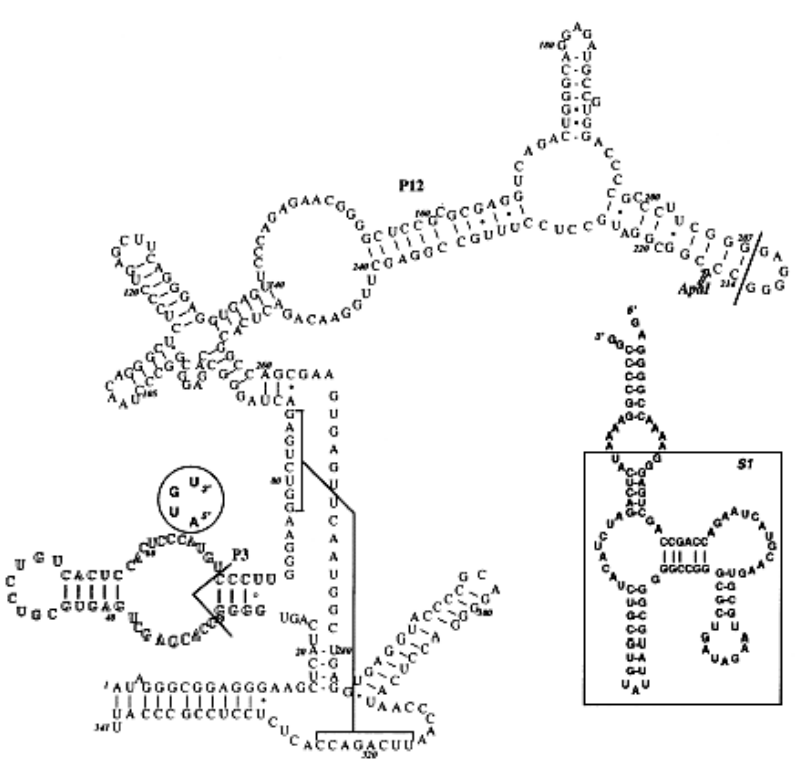
RNA aptamers as affinity tags to identify the protein-RNA interactions

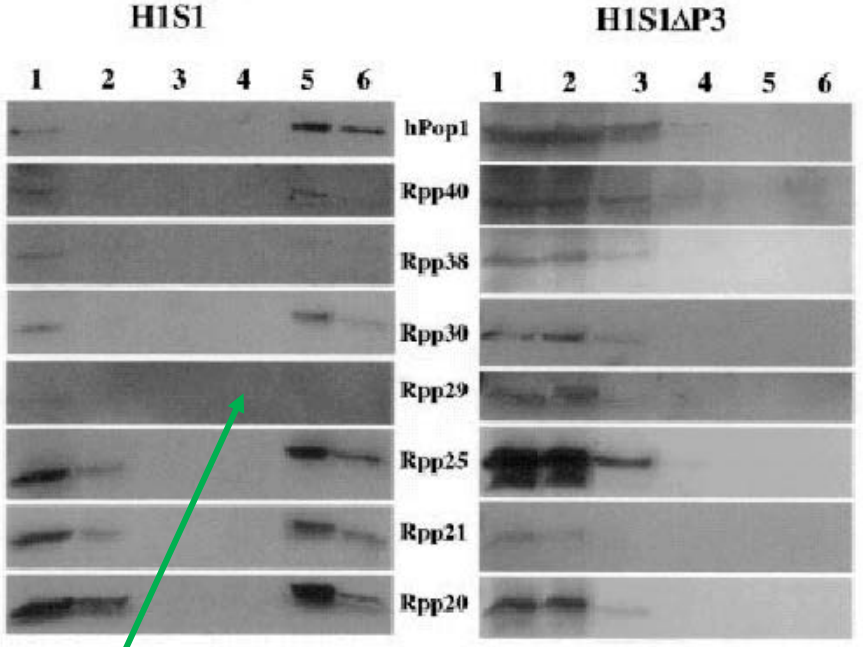
Partial reconstitution of human RNase P in HeLa cells between its RNA subunit with an affinity tag and the intact protein components

Yong Li and Sidney Altman*

Department of Molecular, Cellular and Developmental Biology, Yale University, 266 Whitney Avenue, New Haven, CT 06511, USA

Nucleic Acids Research, 2002, Vol. 30 No. 17





Frazioni di RNaseP

western blot

APTAMER AS A TOOL IN RESEARCH

RNA aptamers as affinity tags to identify the protein-RNA interactions

- Commercially-available RNP purification kit using RNA aptamer tags.

Dual TRAP™ RNP Purification Kit

Catalog Number- **ASA - 100**

CytoStore's Tandem RNP Affinity Purification (Dual TRAP™) kit comprises a patent-pending, dual RNA tagging system that facilitates the convenient purification and enrichment of RNA molecules complexed with specifically associated proteins, RNA's and other small molecules. The novel use of two RNA aptamers in tandem as tags provides for simple, reversible and high-affinity binding to the separation matrices while avoiding common problems associated with conventional RNP purification methodologies, such as non-specificity and difficulty in purifying low-abundance proteins.

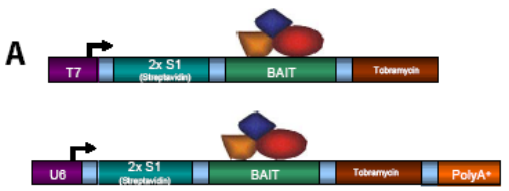
The Dual TRAP™ methodology has been successfully validated in Drosophila, yeast, and mammalian systems.

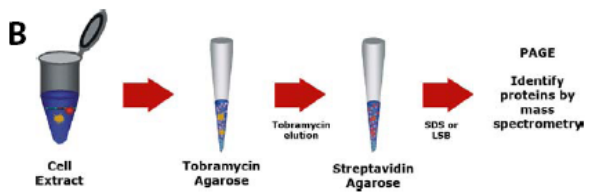
How It Works

Using the pTRAP vector provided, user-selected RNA "bait" sequences are tagged with two distinct RNA aptamers, S1 and tobramycin. The tagged sequences can be expressed either *in vitro* or *in vivo* (the latter requires subcloning the bait cassette into a suitable expression vector). After incubating transcription reactions with a cell lysate, the newly formed RNP's are passed sequentially through two separate affinity columns, alternately containing bound tobramycin and streptavidin. After final washing, the bound RNP's are eluted from the second affinity matrix, and are ready for further characterization on SDS gels and/or via mass spectrometry.

Key Benefits

- Avoids isolation of more abundant, but non-specific, proteins – a common occurrence with single-affinity binding methods
- Two-step purification achieves approximately 10^6-fold increased purity, while still allowing for high-yield recovery of approximately 70% of target RNP's
- Dual RNA aptamer tags allow for high-affinity, specific and reversible binding to separation matrices yielding a highly enriched RNP fraction for further study
- As few as one molecule per prokaryotic cell, or 100 molecules per eukaryotic cell [estimated], can be isolated and identified. In contrast, the best single tagging systems can only purify more abundant proteins.



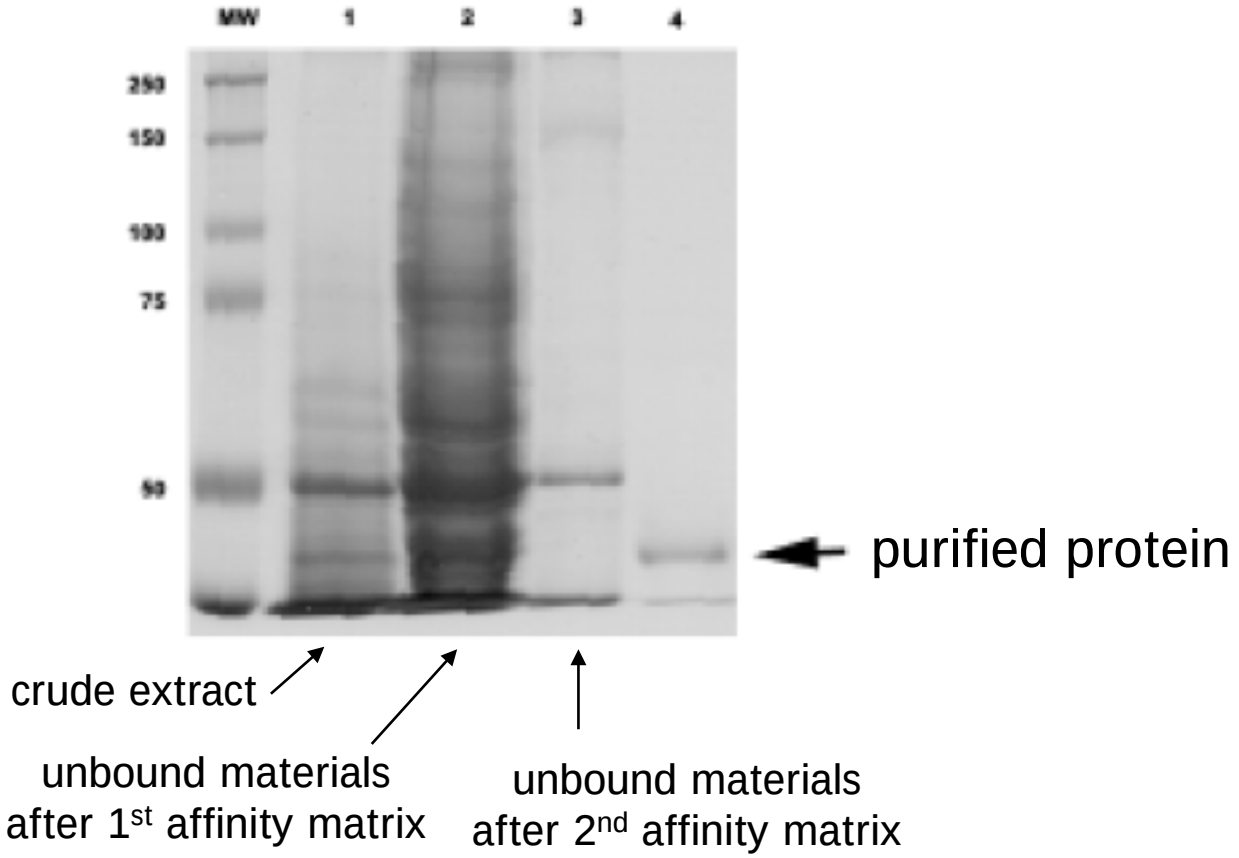


Dual TRAP™ tagging. Figure A shows S1 and tobramycin tags flanking a RNA "bait" sequence. The top version is for *in vitro* transcription; the bottom version shows a hypothetical *in vivo* expression construct. Figure B shows the Dual TRAP™ purification procedure.

APTAMER AS A TOOL IN RESEARCH

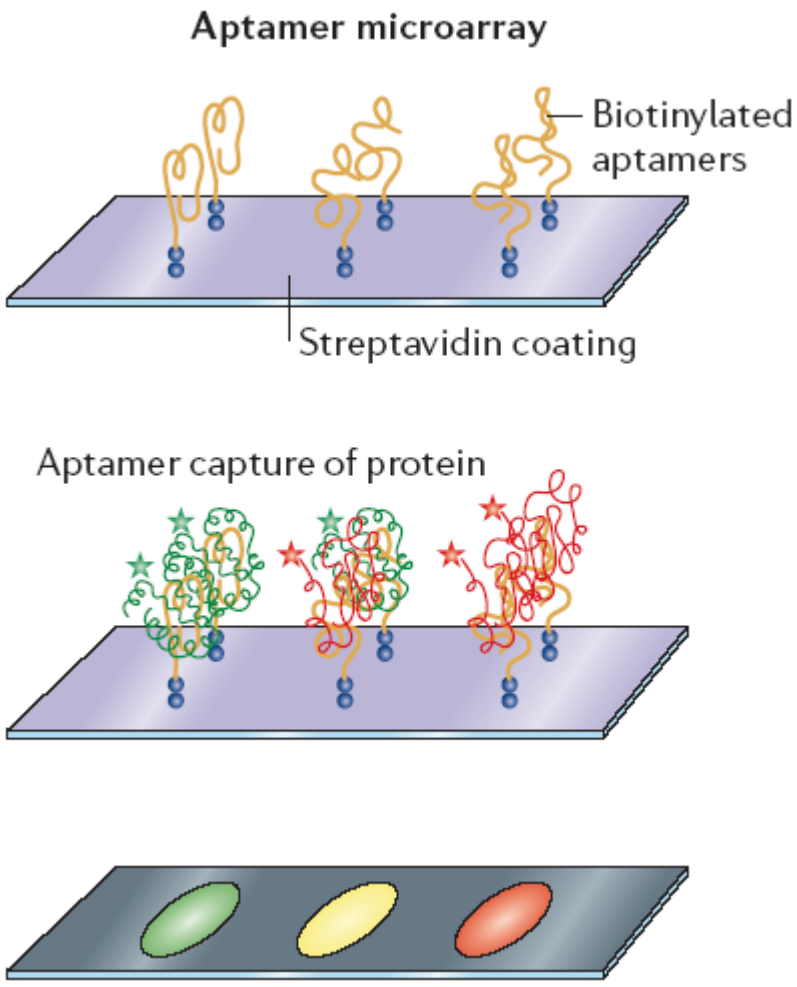
RNA aptamers as affinity tags to identify the protein-RNA interactions

• Drosophila nanos mRNA 3'UTR sequence tagged with the RNA aptamers can be specifically isolated with its interacting protein after a tandem affinity purification.



APTAMER AS A TOOL IN RESEARCH

Aptamer microarray for protein profiling



Advantages of aptamer-based over antibody-based microarray:

- high cross-reactivity between antibodies and other proteins.

- higher cost and labor-intensive processes in generating antibodies.

- aptamer microarrays can be easily regenerated for repeated uses.

- Simple protein stains can be used for detection with aptamer microarrays.

Uses of aptamers in diagnostics :

Using aptamers in various applications conventionally used with antibodies (e.g. immunoassays, diagnostic imaging).

- cheap and easy to produce
- easily modified (biotinylation, crosslinking)

Aptamer-Based Folding Fluorescent Sensor for Cocaine

Milan N. Stojanovic,* Paloma de Prada, and Donald W. Landry*

J. Am. Chem. Soc. **2001,** *123,* 4928−4931