DNA and RNA aptamers and their discovery using In-vivo Evolution & SELECT Protocols

From Lectures of:
Daniel Kalderon & Larry Chasin, Columbia University
and
Peter Quin, USC

Topics:

- **Aptamers**
- **SELECT**
- **DNA enzymes (DNAzymes)**
- **RNA enzymes (Ribozymes)**

Nucleic acid aptamers

Aptamers: **molecules that bind other molecules with good affinity and specificity.**

- **Usually these are proteins . . . . But they can also be RNA or DNA.**
- **That is, single stranded RNA or DNA molecules can and will fold up into secondary and tertiary structures depending on their sequence.**
- **DNA can be synthesized as very large numbers of different random sequences, and some may be aptamers for a specific target.**

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Some examples of aptamer targets

Small molecule Targets:

- Zn_2
- ATP
- adenosine
- cyclic AMP
- GDP
- FMN (RNA aptamer in E.coli)
- cocaine
- dopamine
- amino acids (arginine)
- porphyrin
- biotin
- organic dyes (cibacron blue, malachite green)
- neutral disaccharides (cellobiose & cellulose)
- oligopeptides
- aminoglycoside antibiotics (tobramycin)

Protein Targets

- thrombin
- HIVtat
- HIV rev
- Factor IX
- VEGF
- PDGF
- ricin
- large glycoproteins
- anthrax spores

Binding Affinity by Aptamers to their Targets

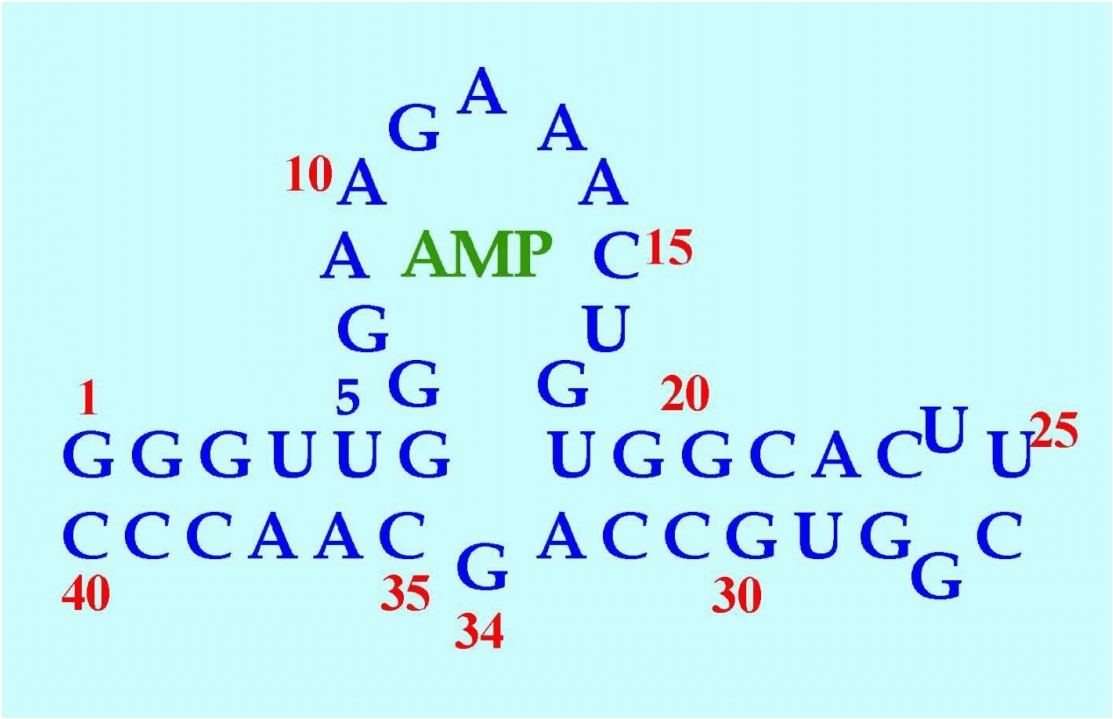
Table 1. Nucleic acid aptamers for which three-dimensional structures have been determined. ND, not determined.

Ligand	Nucleic acid*	Affinity K_d [μM]	3D structure†
Theophylline	RNA (*4*)	~0.3	NMR, 1EHT (*5*)
FMN	RNA (*6*)	~0.5	NMR, 1FMN (*7*)
AMP	DNA (*9*)	~6	NMR, 1AW4 (*12*)
	RNA (*8*)	~10	NMR, 1AM0, 1RAW (*10*, *11*)
Arginine	2 DNA (*15*)	~125	NMR, 1OLD, 2ARG (*18*, *20*)
	RNA (*16*)	~60	NMR, 1KOC (*19*)
Citrulline	RNA (*16*)	~65	NMR, 1KOD (*19*)
Tobramycin	2 RNA (*25*)	~ 0.009	NMR, 1TOB (*32*)
		~ 0.012	NMR, 2TOB (*33*)
Neomycin B	RNA (*26*)	~ 0.115	NMR, 1NEM (*34*)
HIV-1 Rev peptide	2 RNA (*40*)	~ 0.004	NMR, 1ULL, 484D (*41*, *42*)
HTLV-1 Rex peptide	RNA (*43*)	~ 0.025	NMR, 1C4J (*44*)
MS2 coat protein	3 RNA (*45*)	ND	X-ray, 5-7MSF (*45*, *46*)
Thrombin	DNA (*47*)	~ 0.025	NMR, 148D (*38*); x-ray, 1HAO (*39*)

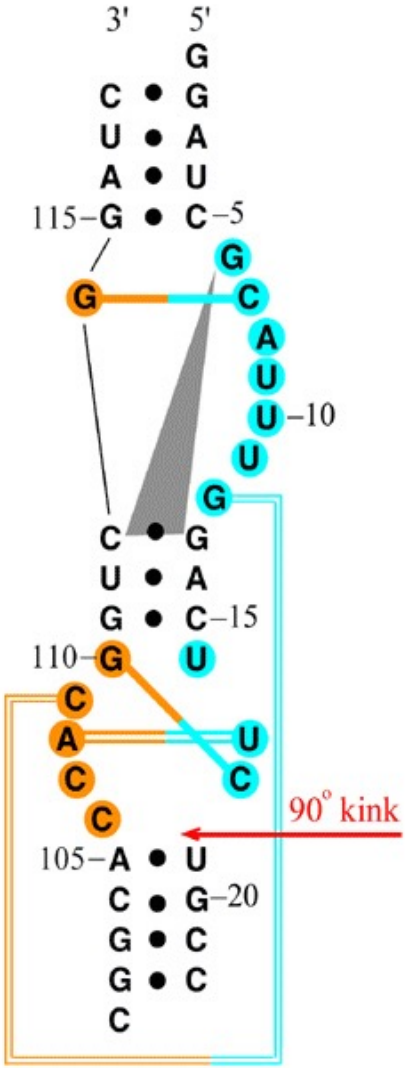
*The number of different sequences that have been studied is indicated. †The structure determination method (e.g., NMR, nuclear magnetic resonance) and the Protein Data Bank entry for the atomic coordinates are given.

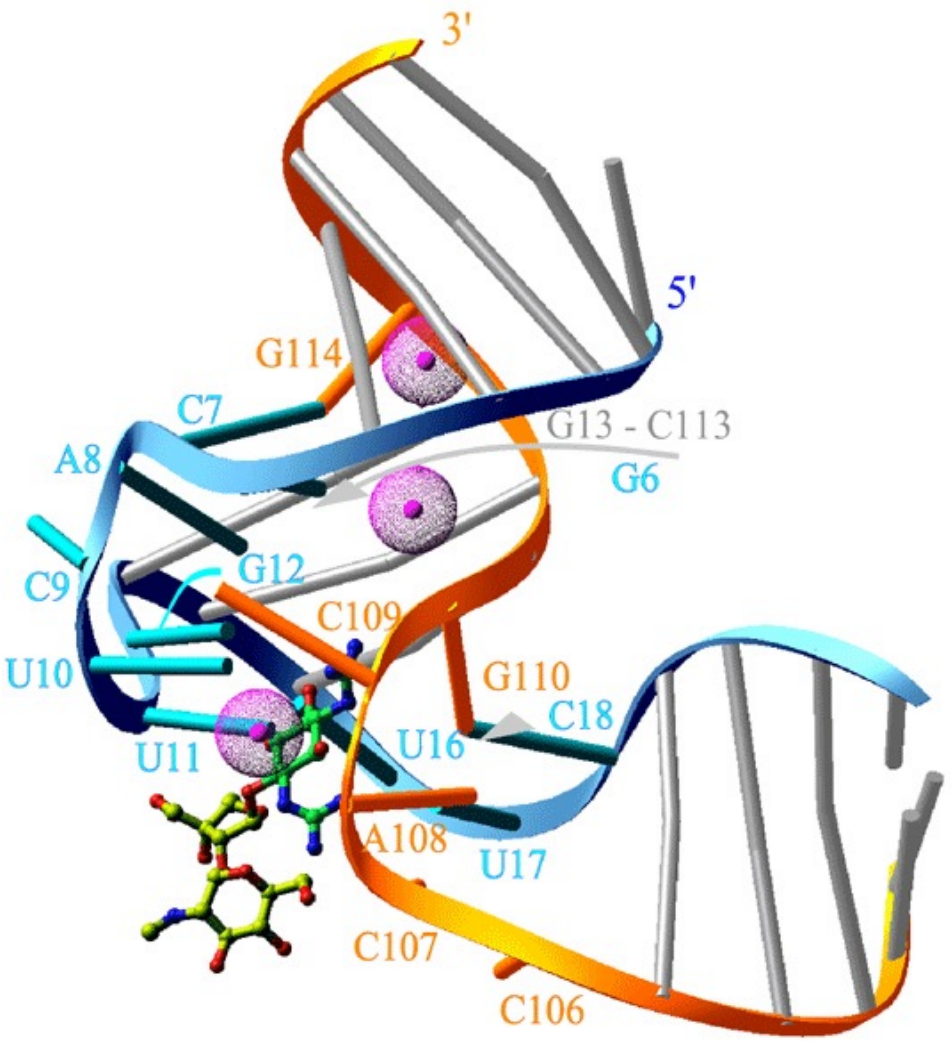
Hermann, T. and Patel, D.J.\2000. Adaptive recognition by nucleic acid aptamers. Science ***287:*** *820-825.*

Example: AMP-binding aptamer



Example: Streptomycin-binding aptamer





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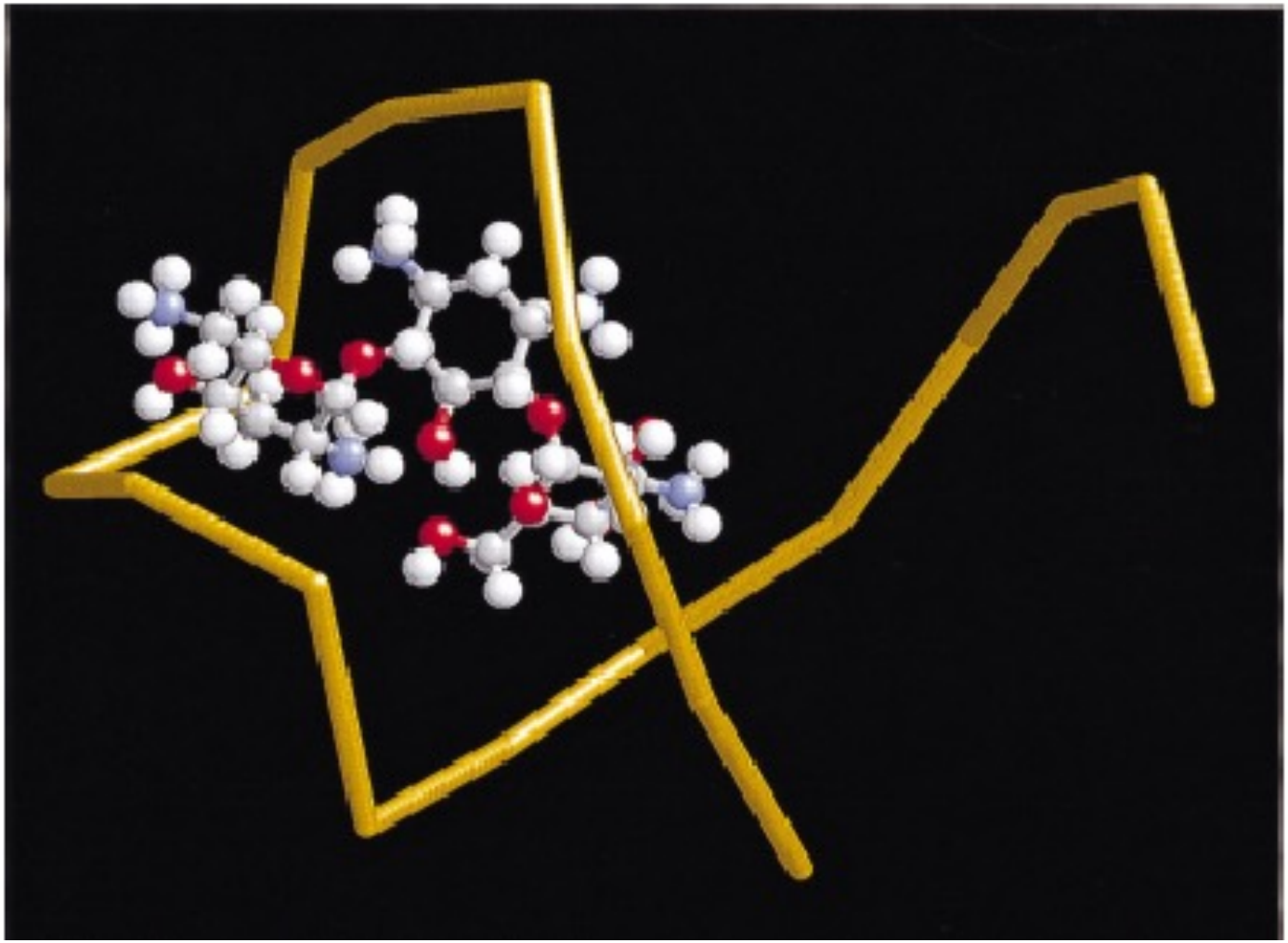
Example: Tobramycin (antibiotic) bound to its aptamer (backbone)



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Example:
An aptamer-gated nanorobot for targeted transport of molecular payloads

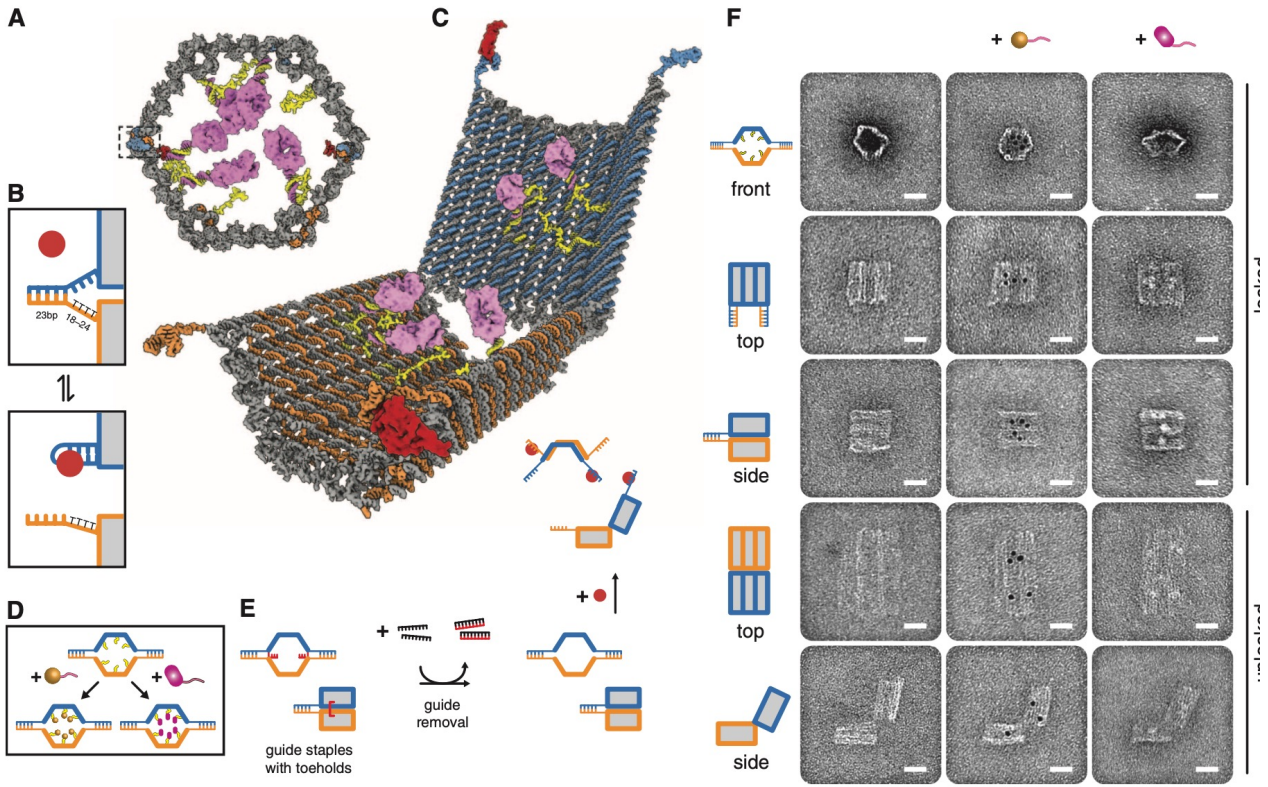


Fig. 1. Design and TEM analysis of aptamer-gated DNA nanorobot. (**A**) Schematic front orthographic view of closed nanorobot loaded with a protein payload. Two DNA-aptamer locks fasten the front of the device on the left (boxed) and right. (**B**) Aptamer lock mechanism, consisting of a DNA aptamer (blue) and a partially complementary strand (orange). The lock can be stabilized in a dissociated state by its antigen key (red). Unless otherwise noted, the lock duplex length is 24 bp, with an 18- to 24-base thymine spacer in the nonaptamer strand. (**C**) Perspective view of nanorobot opened by protein displacement of aptamer locks. The two domains (blue and orange) are constrained in the rear by scaffold hinges. (**D**) Payloads such as gold nanoparticles (gold) and antibody Fab´ fragments (magenta) can be loaded inside the nanorobot. (**E**) Front and side views show guide staples (red) bearing 8-base toeholds aid assembly of nanorobot to 97.5% yield in closed state as assessed by manual counting. After folding, guide staples are removed by addition of fully complementary oligos (black). Nanorobots can be subsequently activated by interaction with antigen keys (red). (**F**) TEM images of robots in closed and open conformations. Left column, unloaded; center column, robots loaded with 5-nm gold nanoparticles; right column, robots loaded with Fab´ fragments. Scale bars, 20 nm.

Douglas SM, Bachelet I, Church GM. A logic-gated nanorobot for targeted transport of molecular payloads. Science 2012; 335:831-4; PMID:22344439; http://dx. doi.org/10.1126/science.1214081

Example: Programming DNA-Based Biomolecular Reaction Networks on Cancer Cell Membranes

Uses Aptamers to bind DNA hairpin devices to Cell Membrane Proteins

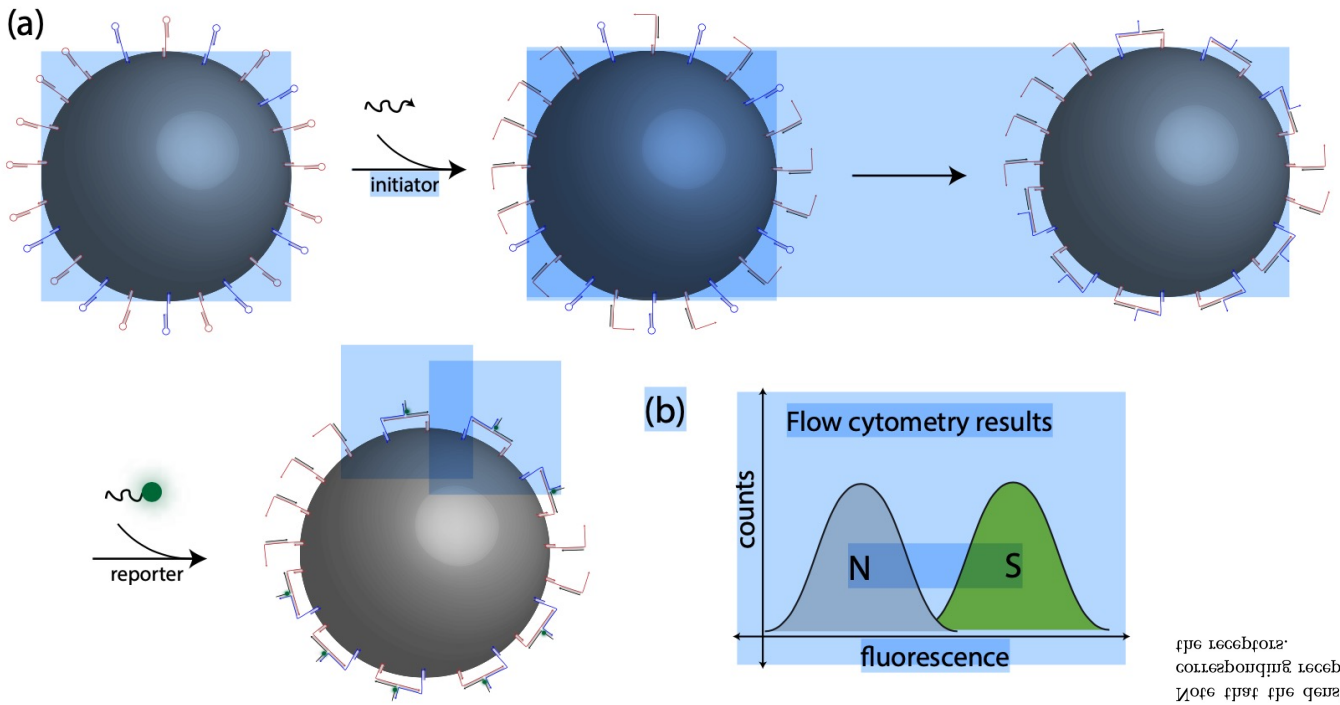


Figure 1: A high-level description of our architecture. (a) An example reaction network by our architecture. This is a 5-level linear cascade reaction network, and there are two types of origami in the network that are indicated by two different colors, where each node is a DNA origami sheet connected with a DNA aptamer. Here a DNA aptamer is a DNA strand that can be rationally designed[35] and recognize a particular cell membrane receptor which can range from small molecules to proteins.[32−35] Each node targets a designated cancer cell (the Vero shape) membrane receptor that mix the nodes with the cancer cells in a reaction buffer. It node targeted receptors exist on the membrane, node nodes will be localized on the membrane by aptamer-receptor binding. When introducing the reaction network, we raise a DNA aptamer-aptamer-receptor binding. When operating the reaction network, we first wash off the free nodes in the buffer to exclude potential non-localized reactions. By introducing the initiator strands, the 5-level linear cascade reaction is started. First, the initiator opens up the leg region by DNA strand displacement. Then, the output strand from the leg region opens up the sole region. The output strand from the sole region can hybridize with a reporter DNA strand (conjugated with a fluorophore), such that the cancer cells are labeled by the fluorophore and can be recognized by flow cytometry. (b) Group "2": cancer cells labeled by fluorophore via aptamer-receptor reaction network on the membrane. Group "N": cancer cells of the same type without fluorophore. Note that the density of each node on the cell membrane is determined by the density of the corresponding receptor, and the nodes can move on the cell membrane because of the diffusion of the receptors.

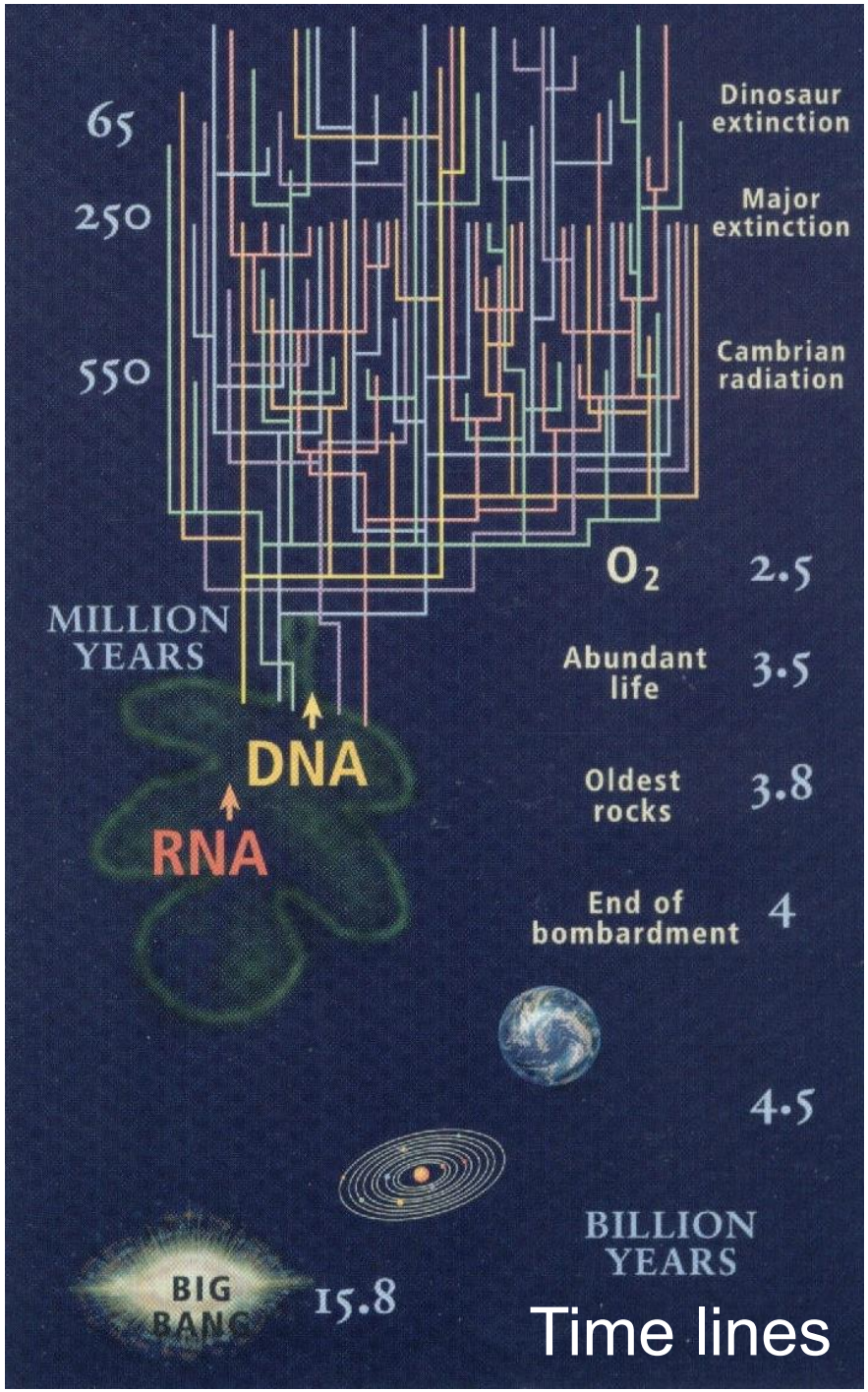
Tianqi Song, Shalin Shah, Hieu Bui, Sudhanshu Garg, Abeer Eshra, Ming Yang, and John Reif,
Programming DNA-Based Biomolecular Reaction Networks on Cancer Cell Membranes,
Journal of the American Chemical Society (JACS), Vol. 141, No. 42, pp. 16539-16543. (Oct 2019).
\ https://doi.org/10.1021/jacs.9b05598

But how can we discover aptamers?

=> Use Evolution !!

The RNA World Hypothesis

- RNA has the essential properties needed for life: it can serve both as a repository of information (in its sequence of nucleotides) and as a catalyst.
- RNA may have supported cellular or pre-cellular life – the "RNA World"
- The RNA world evolved into the protein/DNA world of today.



The RNA World, 2nd Ed.

Evolution of Nucleic acid aptamers

- Aptamers can be selected from among these molecules based on their ability to bind an immobilized ligand. The tiny fraction found by chance to be able to bind to your favorite ligand can by amplified by PCR (along with background molecules).

- Re-iteration of the procedure will enrich for the aptamer until they dominate the population. At this point they can be cloned and sequenced.

- RNA molecules can be selected by synthesizing them from a randomized DNA population using the T7 promoter appended to each DNA molecule.

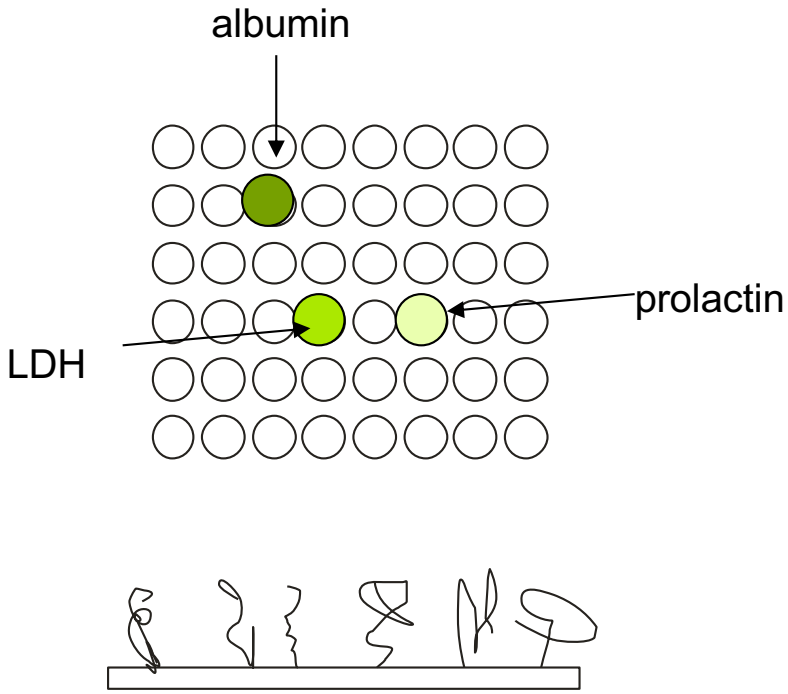
- This enrichment procedure is just the SELEX method described earlier for finding the RNA substrate for RNA binding proteins. In this case it's the same procedure, looked from the opposite point of view: not what RNA will the protein bind best, but what RNA binds the protein best.

SELEX Procedure for Evolving Aptamers

C. Tuerk and L. Gold. "Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase," Science, 249:505-10, 1990

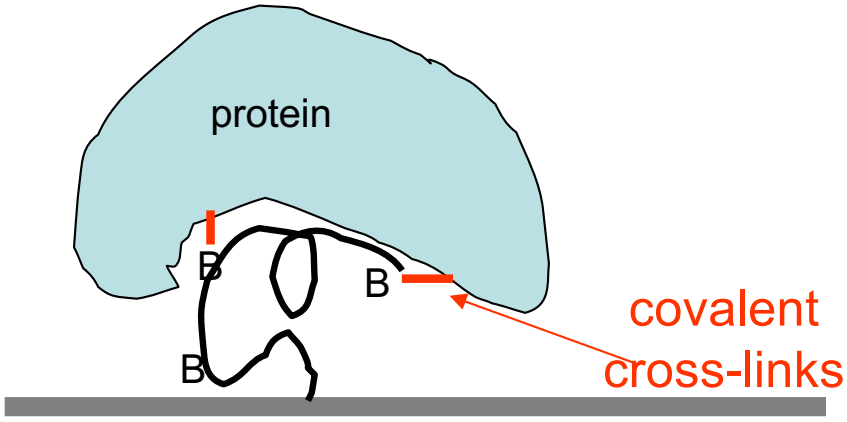
More recently:

Somalogic, Inc.: Photoaptamers



Wash stringently to
Produce a low background.

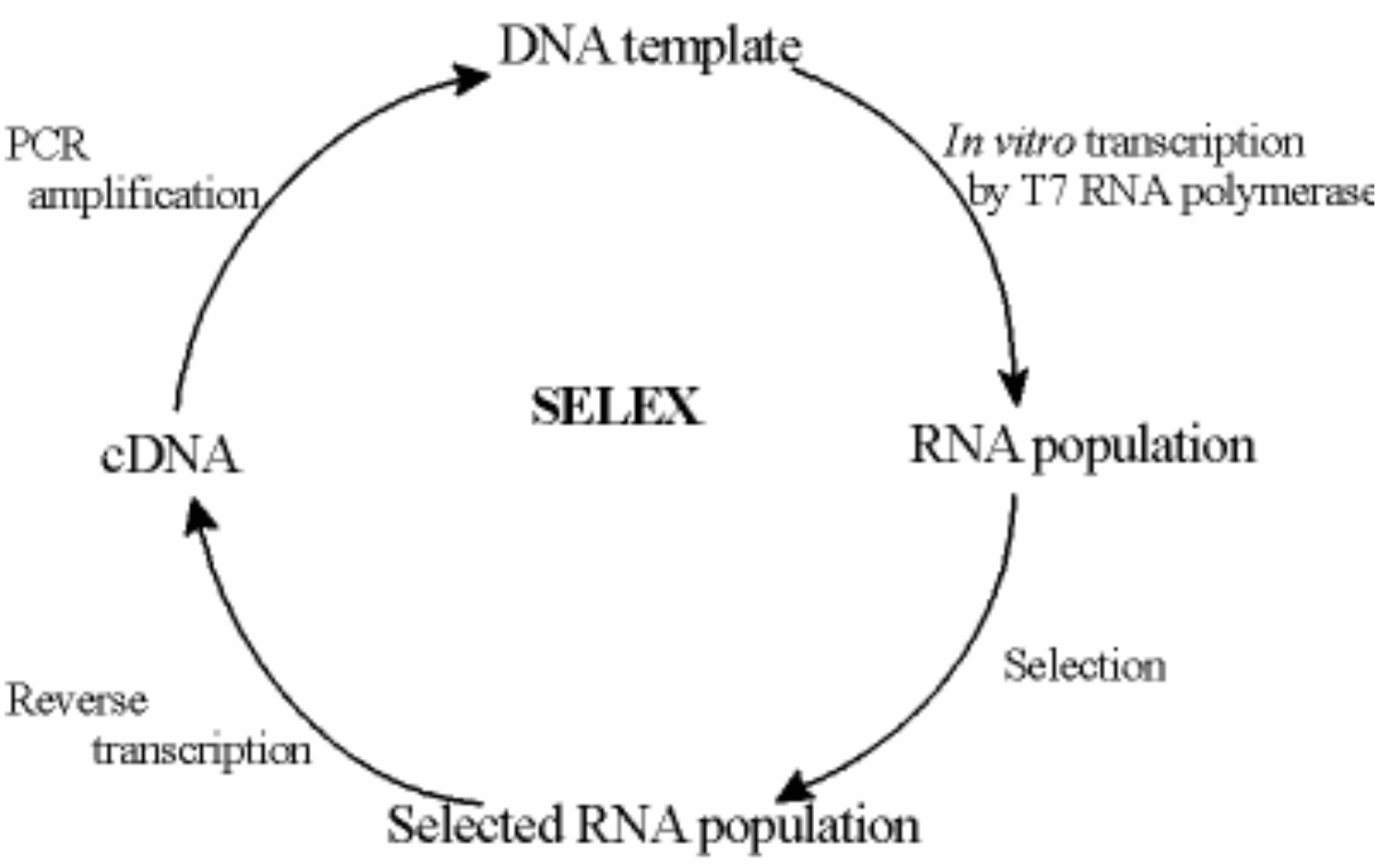
Stain with a protein-specific
sensitive fluosecent stain
(e.g, for primary amine groups)



In Vitro Selection & Evolution

- SELEX: Systematic Evolution of Ligands by EXponential enrichment. Also known as “In Vitro Selection” or “In Vitro Evolution”.
- Allows simultaneous screening of $> 10^{15}$ individual nucleic acid molecules for different functionalities.
- Developed in 1990 in the laboratories of Gerald F. Joyce (La Jolla), Jack W. Szostak (Boston), and Larry Gold (Boulder).
- An important and wildly used tool in molecular biology.

for a review, see Silverman, 2003, RNA, 9: 377-83



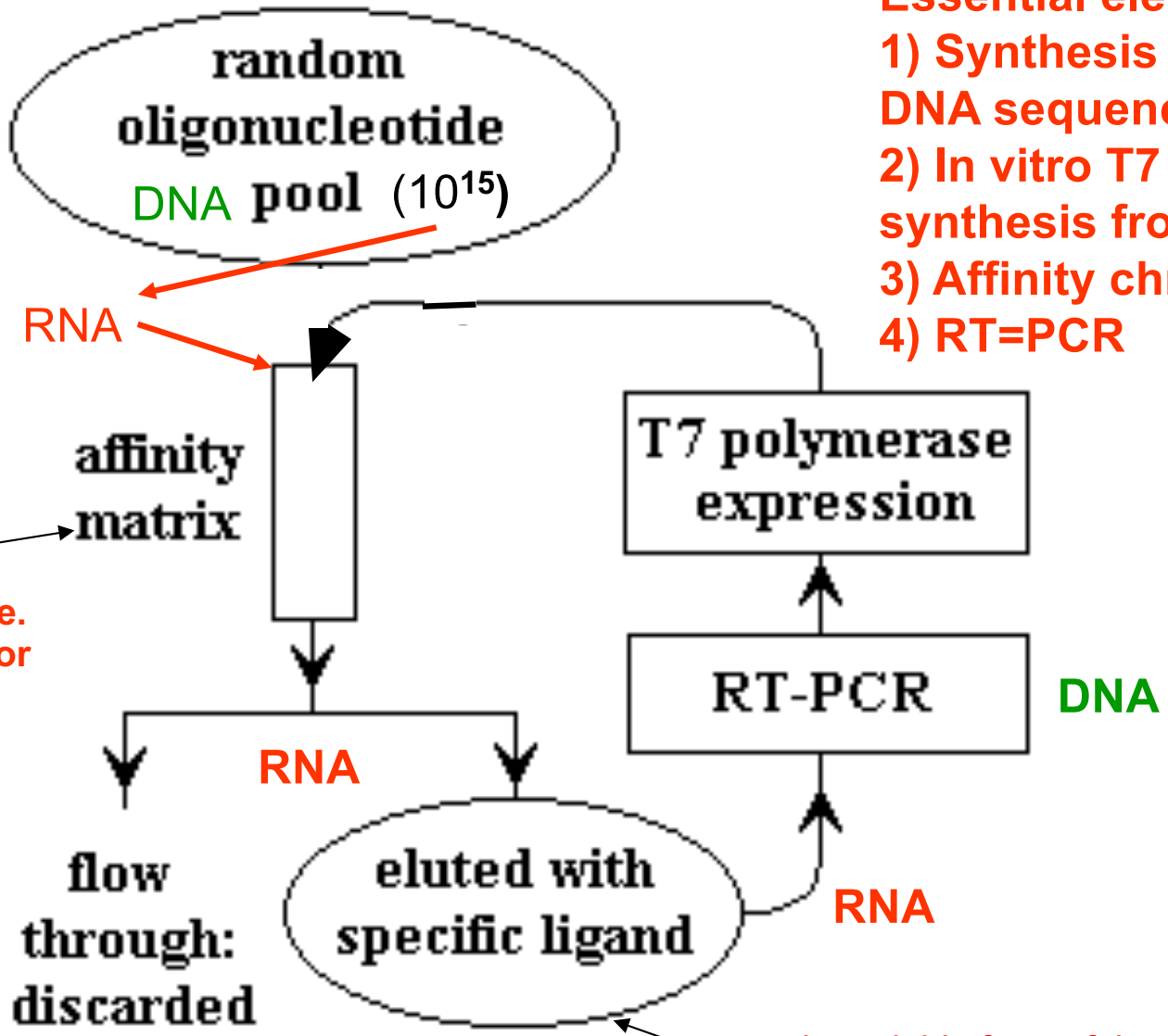
The general strategy:

1) Introduction of genetic variation or mutations.
2) Selection of variant molecules best suited for a target function.
3) Amplification of the selected molecules.

Application of SELEX:

- Engineering an enzyme with a novel function.
- Investigating the RNA world hypothesis.
- Designing molecules for clinical applications.

SELEX: Systematic Evolution of Ligands by Exponential Enrichment for RNA (or DNA)



Essential elements:

1) Synthesis of randomized DNA sequences
2) In vitro T7 mediated RNA synthesis from DNA
3) Affinity chromatography
4) RT=PCR

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Building Random DNA Library for SELEX:

A library of random 40-mers (DNA sequences with 40 bases) synthesized, between 2 arbitrary 20-mers (PCR sites)

- Total number of possible 40-mers: $4^{40} = 10^{24}$
- Practical limit = 10^{15} = ~ 2 nmoles = ~ 50 ug DNA

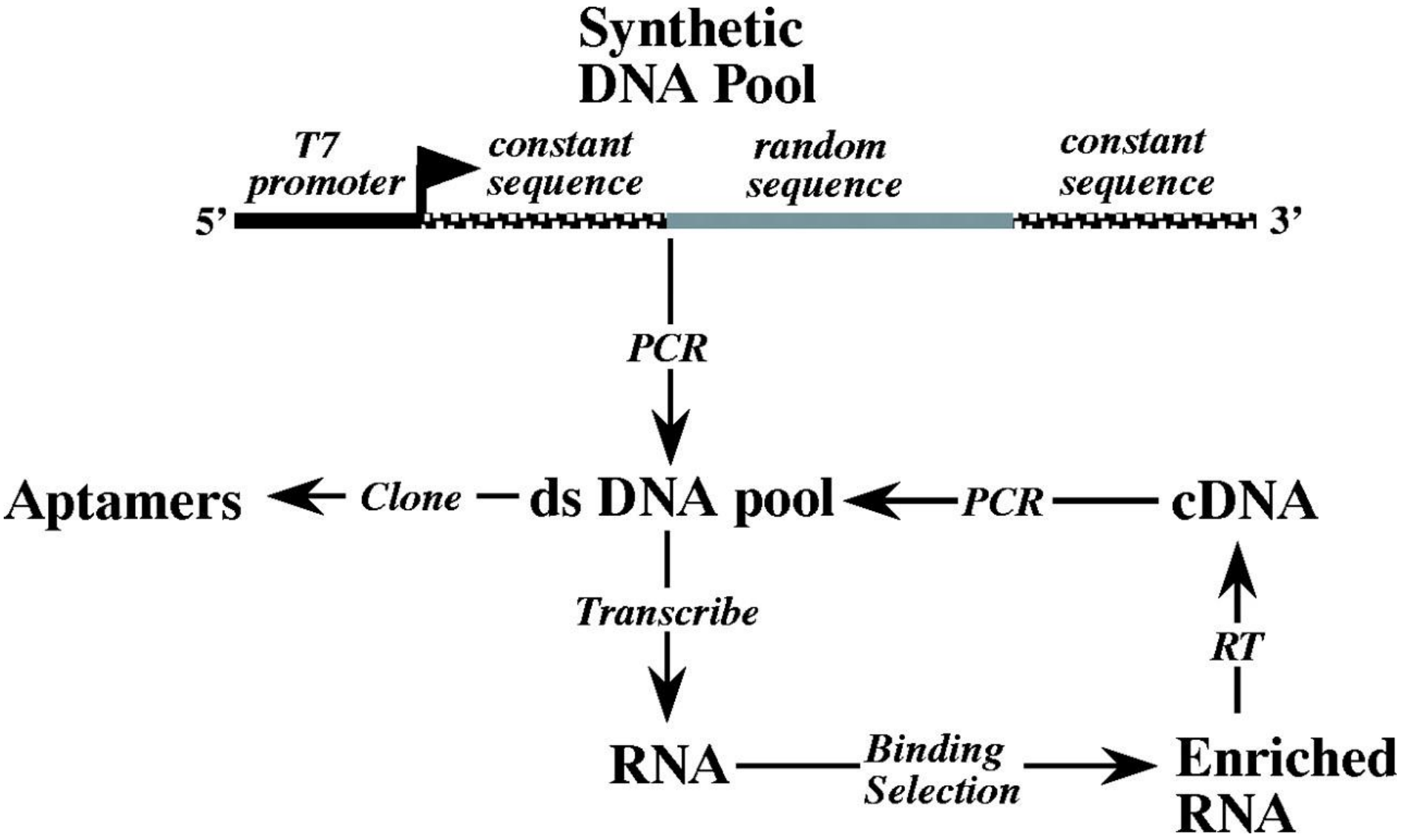
- 10^{15} is a large number.
  Very large (e.g., 500,000 times as many as all the unique 40-mers in the human genome.)

- These 10^{15} sequences are known as "sequence space"

- Each DNA molecule of these 10^{15} (or RNA molecule copied from them) can fold into a particular 3-D structure, but we know little as yet about these structures.

- But can select the molecules that bind to our target by: AFFINITY CHROMATOGRAPHY

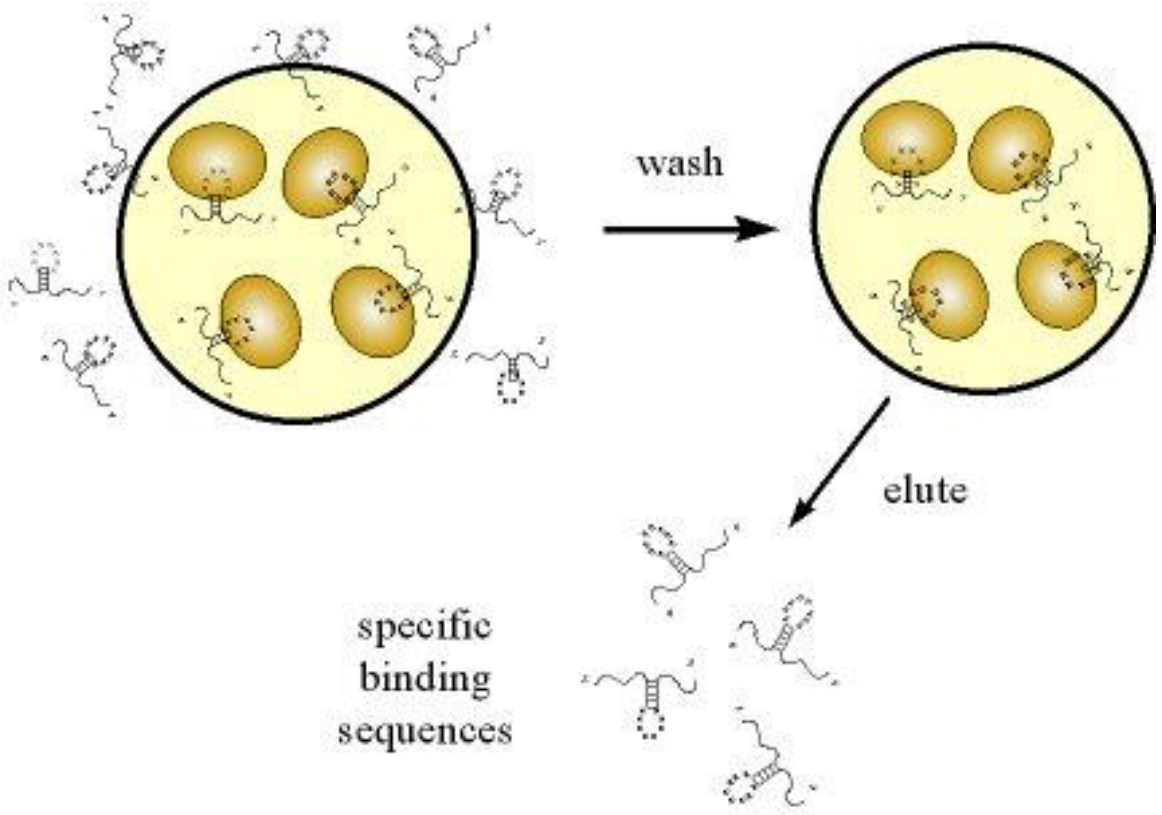
General scheme for **SELEX:**



SELEX Example:

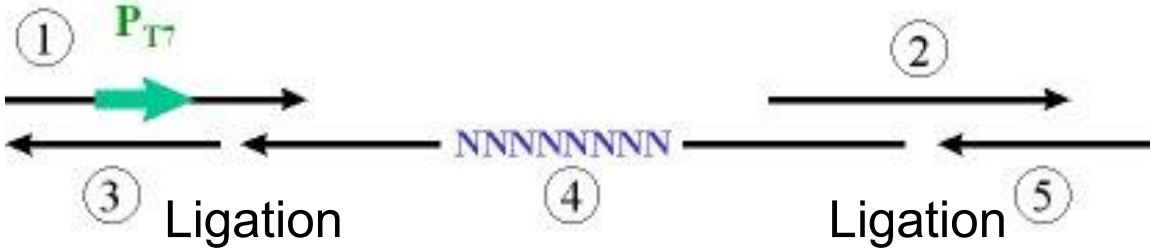
Example: Aptamer selected for binding to a protein, T4 DNA polymerase (Tuerk C, Gold L. 1990. *Science* 249:505-10)

- Selection via binding to T4 DNA polymerase immobilized on nitrocellulose filters
- The filters are washed to remove non-specifically bound RNA molecules
- Tight (specific) binding RNA molecules are eluted from the T4 DNA pol protein and are collected.



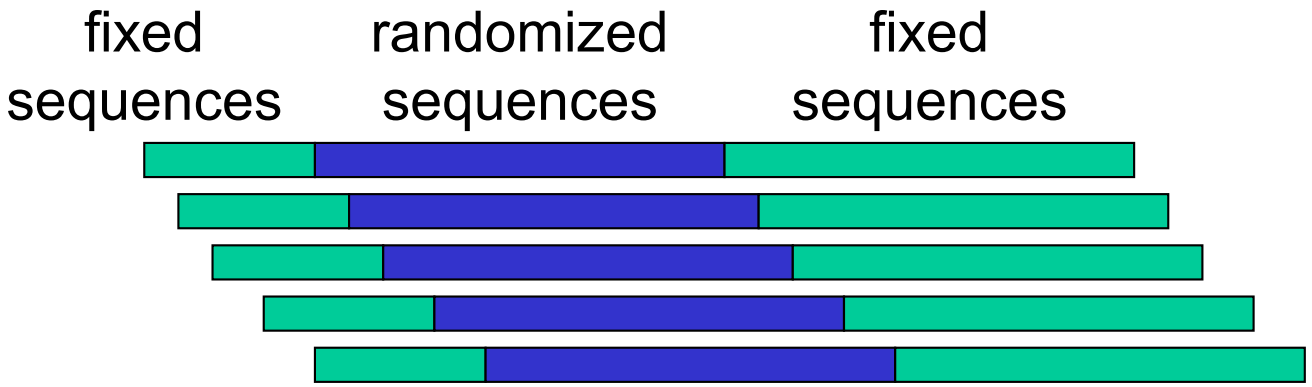
Construction of the randomized RNA pool for **SELEX:**

DNA oligo synthesis



Ligation to generate the DNA template

In vitro transcription
RNA pool

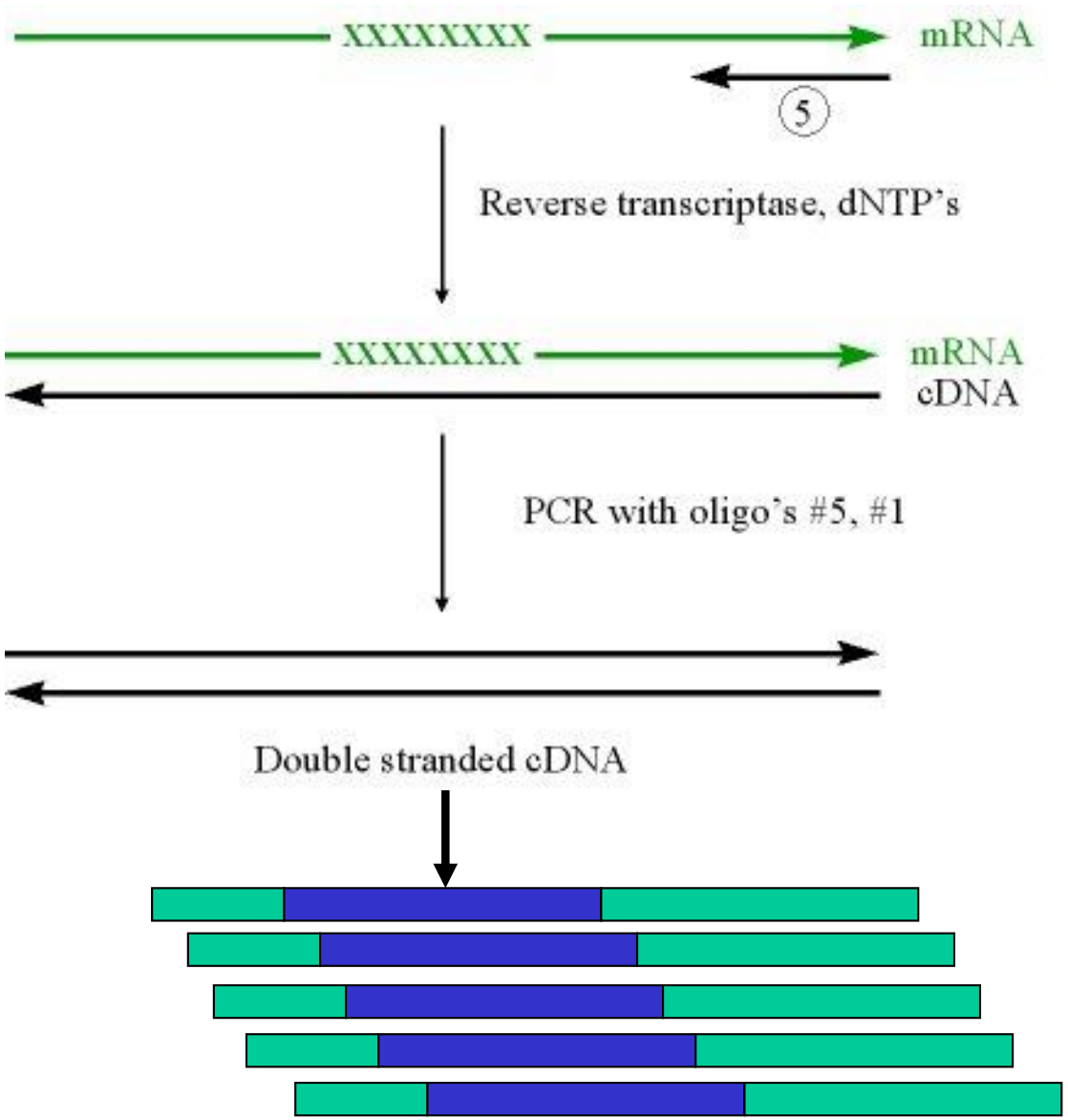


pool size = 4^N

N = 8, pool = 65,536; N = 40, pool ~ 10^{24}; N = 100, pool ~10^{60}

Amplification: for **SELEX:**

- The eluted RNA molecules are converted to single strand cDNA using oligonucleotide #5 as a primer, and adding Reverse transcriptase and dNTP's.
- Duplex DNA is produced from the single strand cDNA by PCR using oligonucleotides #1 and #5.
- The duplex DNA is amplified via PCR using primer #1 and #5.
- In vitro transcription generated a new pool of RNA with increased activity.



In vitro transcription
RNA pool

Results and Analyses for **SELEX:**

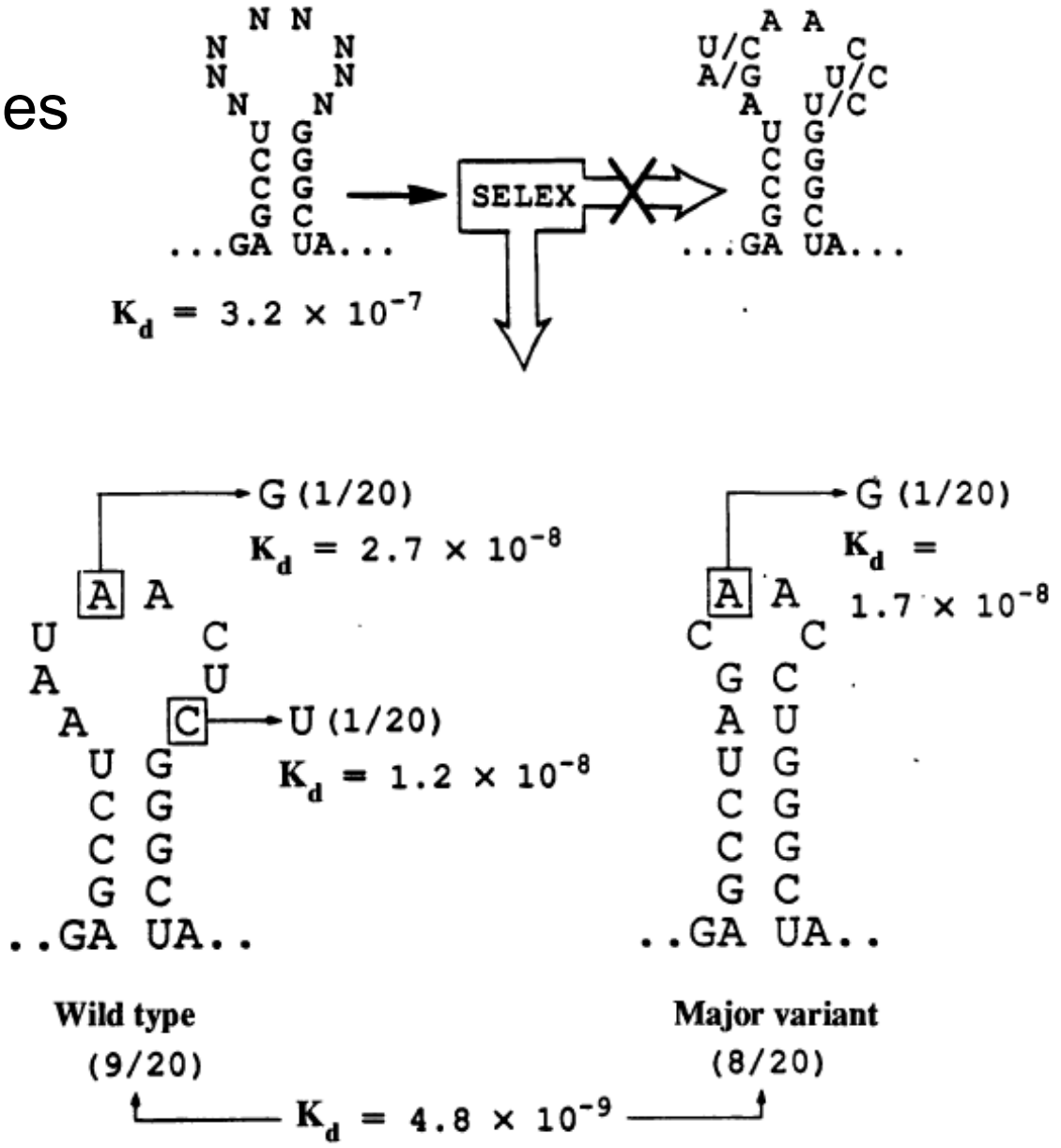


Fig. 8. Summary of results. The use of SELEX did not yield the apparent consensus one would expect from the batch sequences shown in Fig. 5, but yielded the wild-type and major variant species with three single mutants. The frequencies of each species in the 20 isolates tested are shown with the approximate K_d's derived from the filter binding assays shown in Fig. 7.

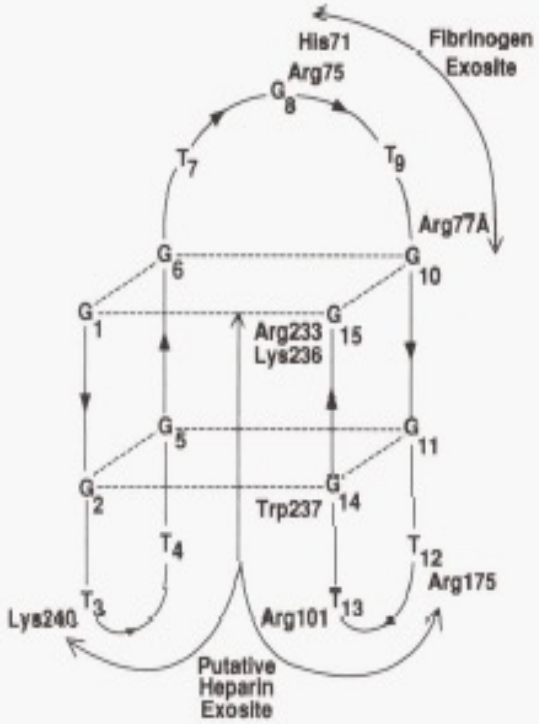


FIG. 1. Schematic of the folded 15-mer DNA and its interaction with α-thrombin.

G-quartets dominate the structure of antithrombin DNA aptamers

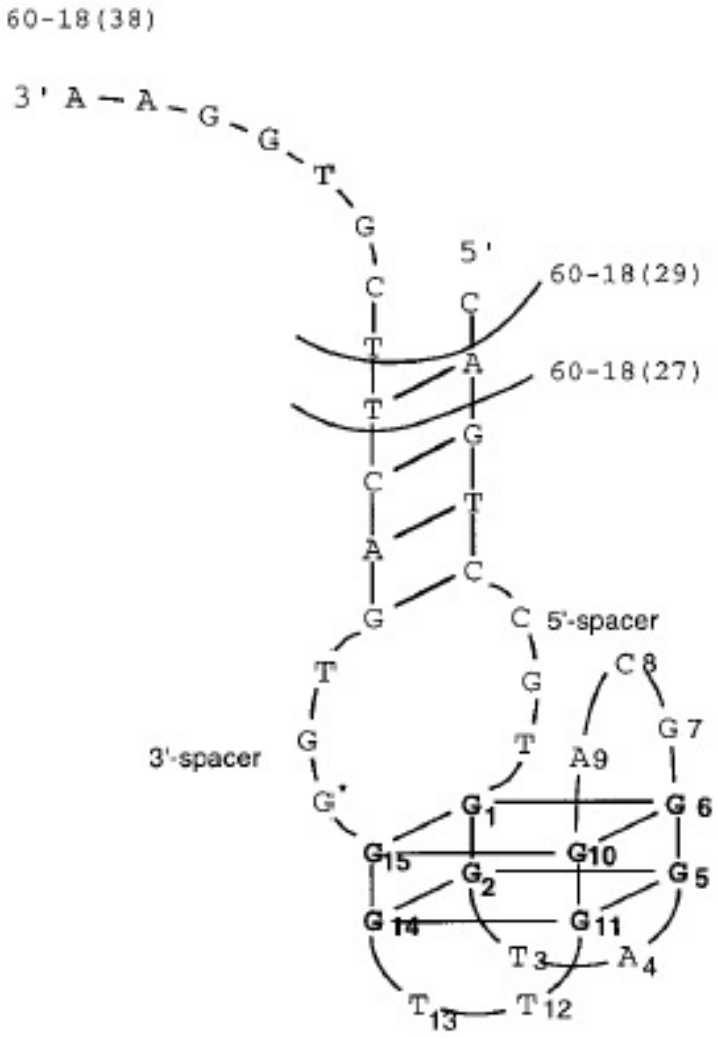


Figure 4. Proposed secondary structure of the thrombin DNA ligand 60-18[29] is shown. The 15 nucleotides of the G-quadruplex core sequence are numbered. Conserved G nucleotides required for G quadruplex formation are indicated in bold. The three nucleotides of the 5′- and 3′-spacer regions are indicated. An * indicates the first G residue of the 3′-spacer involved in high-affinity binding.

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In vitro evolution of peptide/protein using **SELEX:**

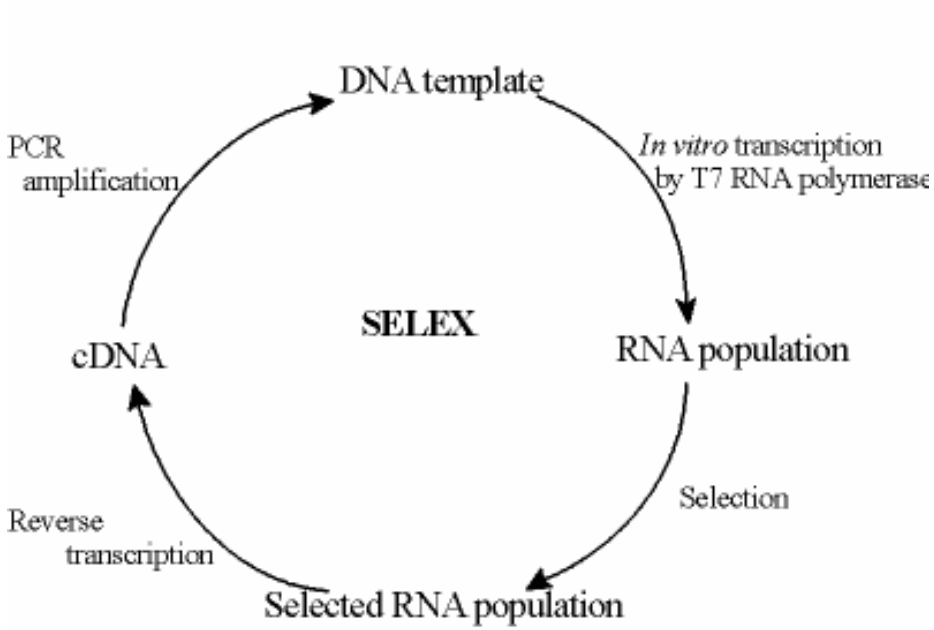
transcription **translation**

DNA ⇌ **RNA** → **Protein**

reverse transcription

❖ A peptide sequence can NOT "reverse-translate" to DNA/RNA, a different scheme of amplification is needed to link function (being selected) and sequence.

❖ Current Methods: Phage display; mRNA display; …



SELECT for Ribozymes (RNA enzymes)

Ribozyme: **a ribonucleic acid (RNA) enzyme that catalyzes a chemical reaction**

Ribozymes = RNA enzymes

1982 Tom Cech: Tetrahymena rRNA intron is self-spliced out
(Guanosine [GR] + Mg++)

Altman and Pace: Ribonuclease P is an RNP:
RNA component alone can process the 5' ends of tRNAs

Mitochondrial group I introns (GR –catalyzed) also can self-splice

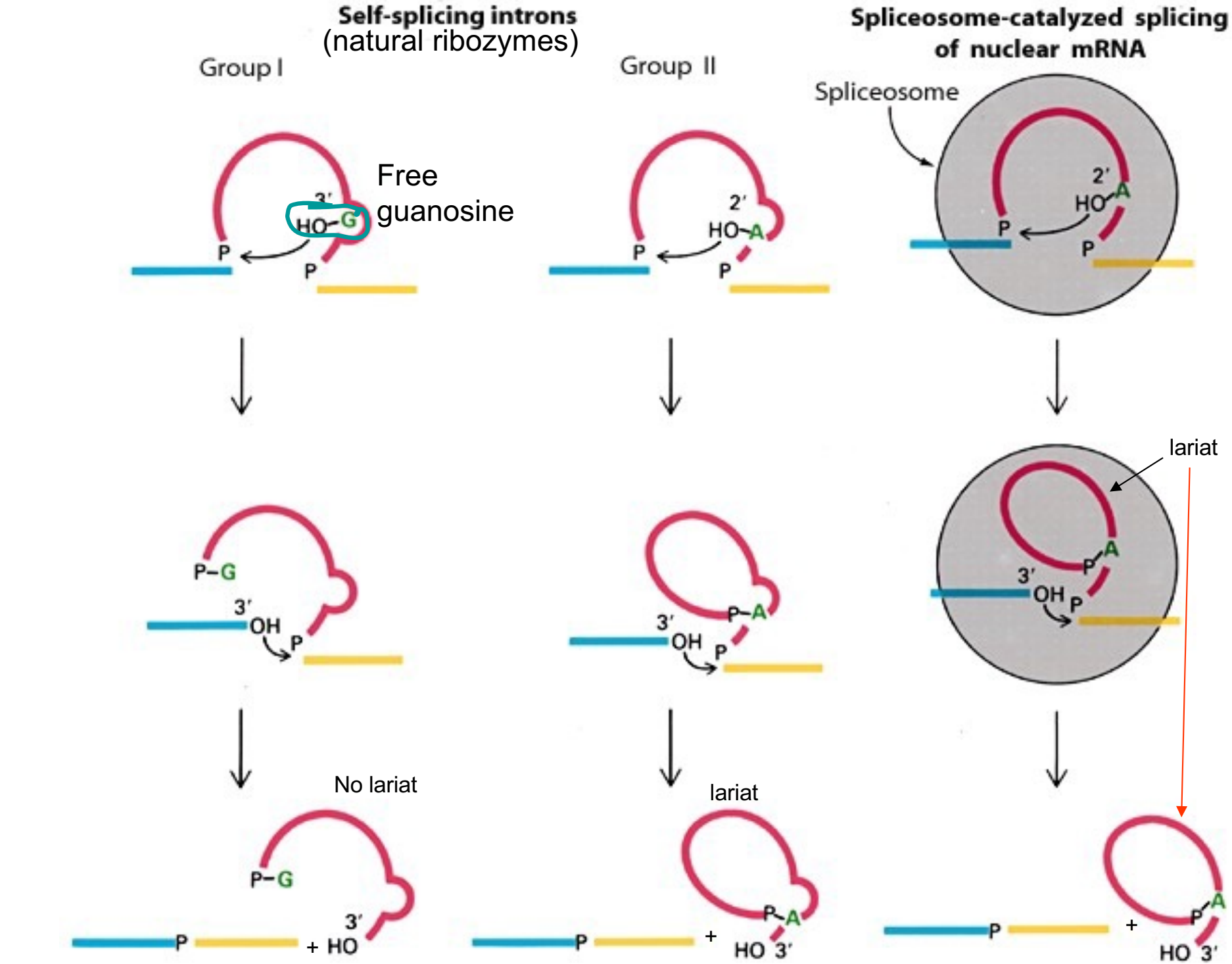
Then group II introns in mitochondria (lariat-formers)

Mutations (100's) revealed required attributes:

- Internal guide sequence
- GR-binding site
- secondary structure

Conserved base analysis (100's) → confirms structure

X-ray diffraction: a few 3-D structures


Self-splicing introns
(natural ribozymes)
Group I
Group II
Spliceosome-catalyzed splicing
of nuclear mRNA
Spliceosome
Free
guanosine
lariat
No lariat
lariat
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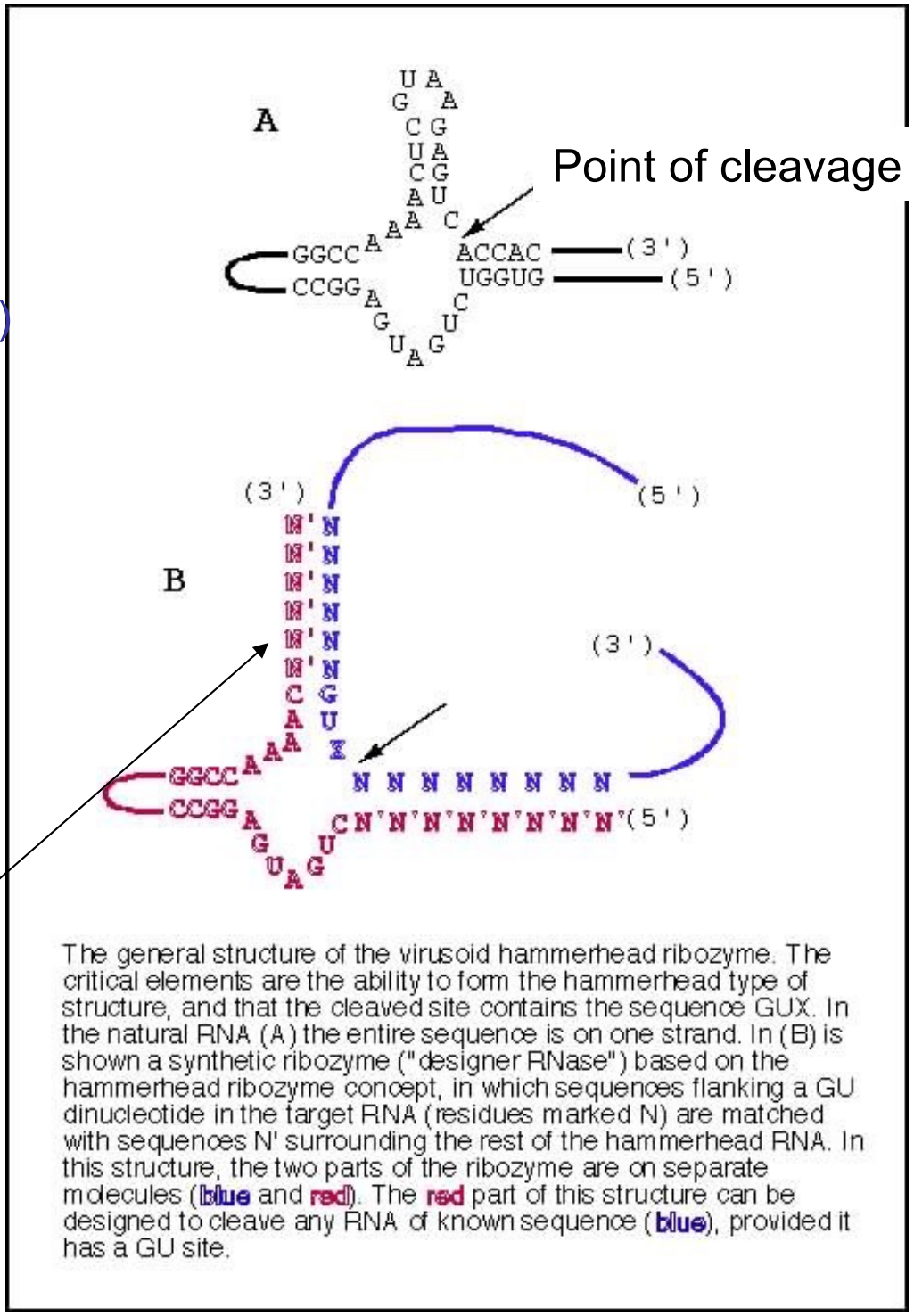
Hammerhead Ribozymes:

Hammerhead ribozyme (RNase) can cleave in cis ("hammer head" is upside down)

Synthetic variation: cleaves in *trans*

You are in charge of what it will cleave (you fill in the N's)



The general structure of the virusoid hammerhead ribozyme. The critical elements are the ability to form the hammerhead type of structure, and that the cleaved site contains the sequence GUX. In the natural RNA (A) the entire sequence is on one strand. In (B) is shown a synthetic ribozyme ("designer RNase") based on the hammerhead ribozyme concept, in which sequences flanking a GU dinucleotide in the target RNA (residues marked N) are matched with sequences N' surrounding the rest of the hammerhead RNA. In this structure, the two parts of the ribozyme are on separate molecules (**blue** and **red**). The **red** part of this structure can be designed to cleave any RNA of known sequence (**blue**), provided it has a GU site.

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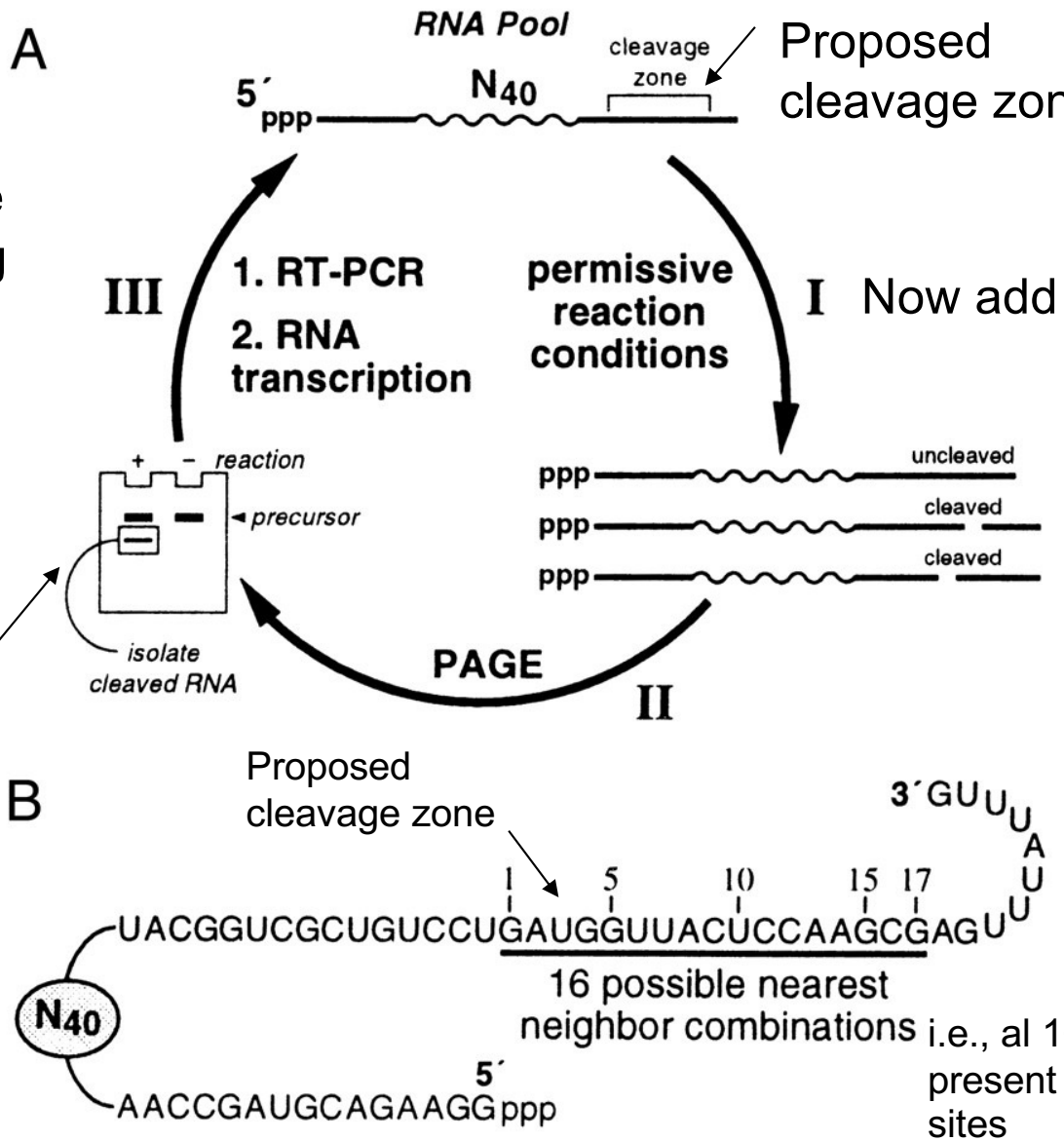
You can use SELEX to isolate new artificial ribozymes

Tang, J. and Breaker, R.R. 2000.
Structural diversity of self-cleaving ribozymes.
Proc Natl Acad Sci U S A **97:** 5784-5789.

10^{15} DNA molecules with T7 promoter

Keep molecules under non-permissive conditions so they stay intact (without Mg++)



RT -> cDNA: Cleavage zone is rebuilt by being part of the primer.

Isolate the successfully cleaved by size on gels

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Columbia University

Example: Creating new ribozymes: “Isolation of New Ribozymes from a Large Pool of Random Sequences.”

Bartel & Szostak, SCIENCE, 261 (1993) 1411

- ❖ Iterative in vitro selection; isoate a new class of catalytic RNAs from a large pool of random-sequence RNA molecules.
- ❖ Ribozymes ligate two RNA molecules that are aligned on a template by catalyzing the attack of a 3'-hydroxyl on an adjacent 5'-triphosphate-a reaction;
- ❖ Rxn similar to that employed by the familiar protein enzymes that synthesize RNA.
- ❖ In vitro evolution gave ribozymes with reaction rates 7 million times faster than the uncat-alyzed reaction rate.

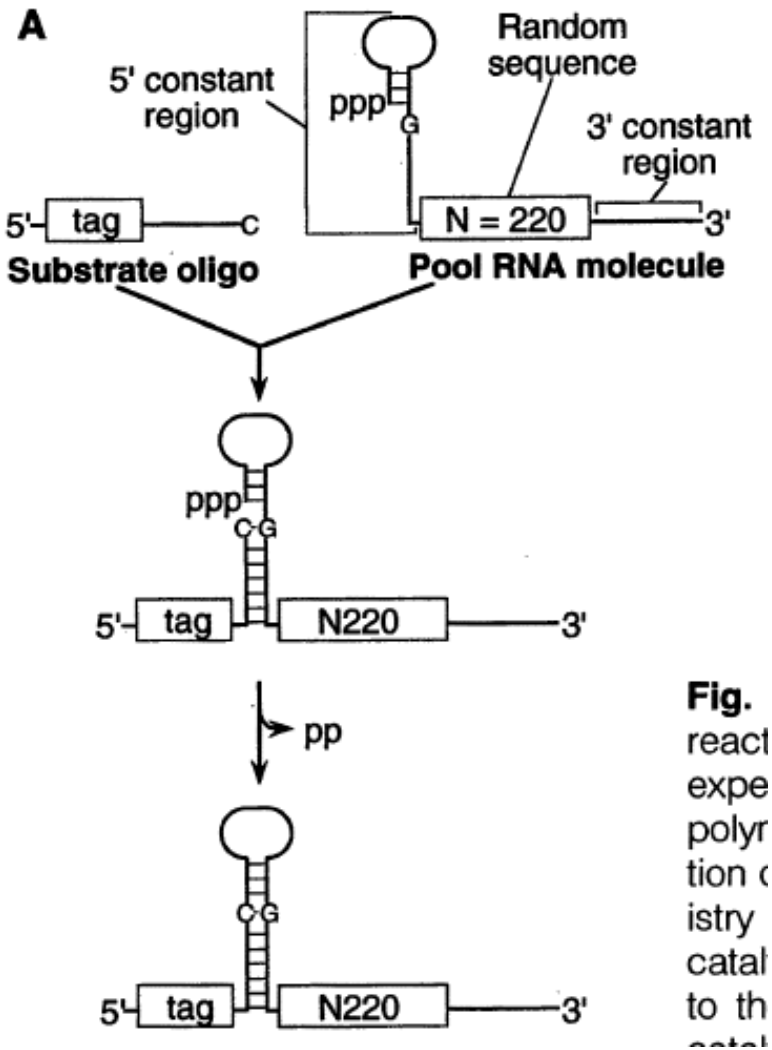
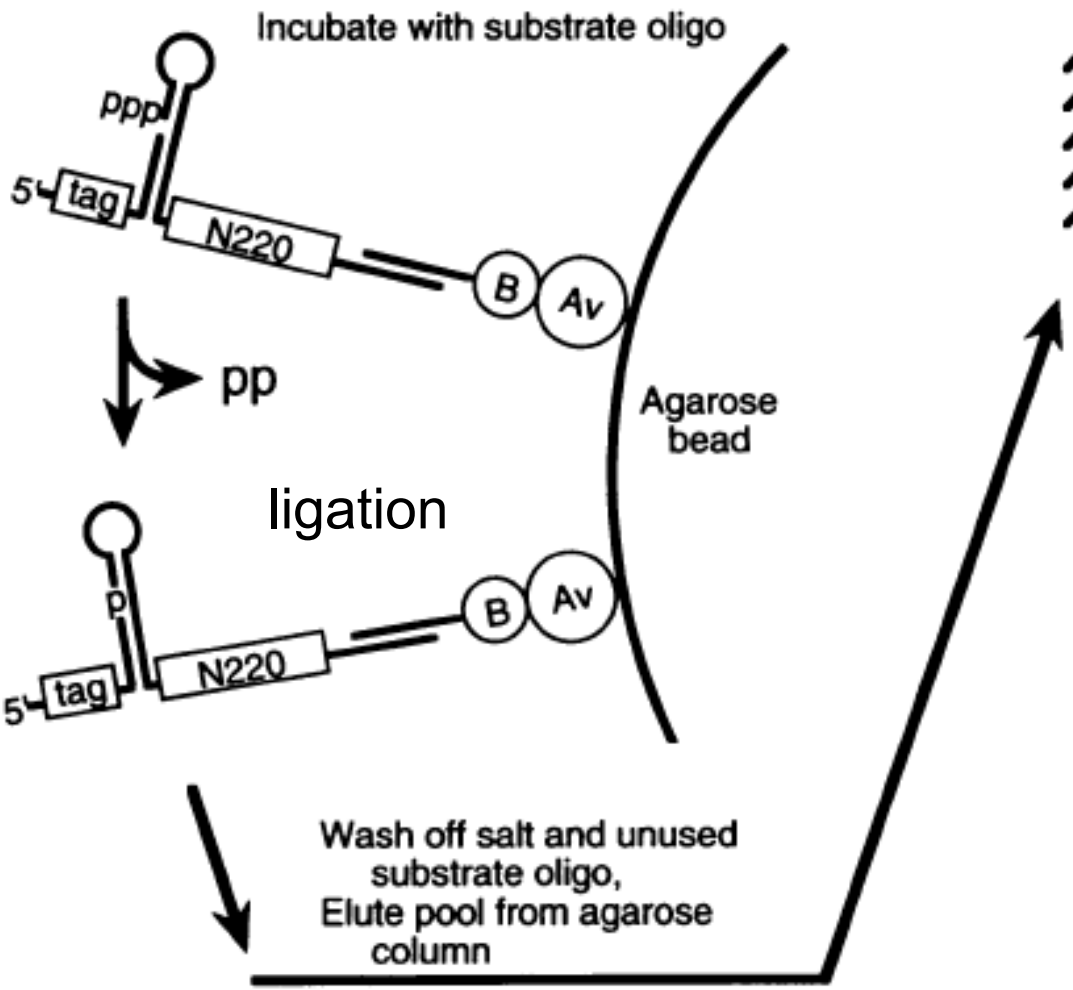
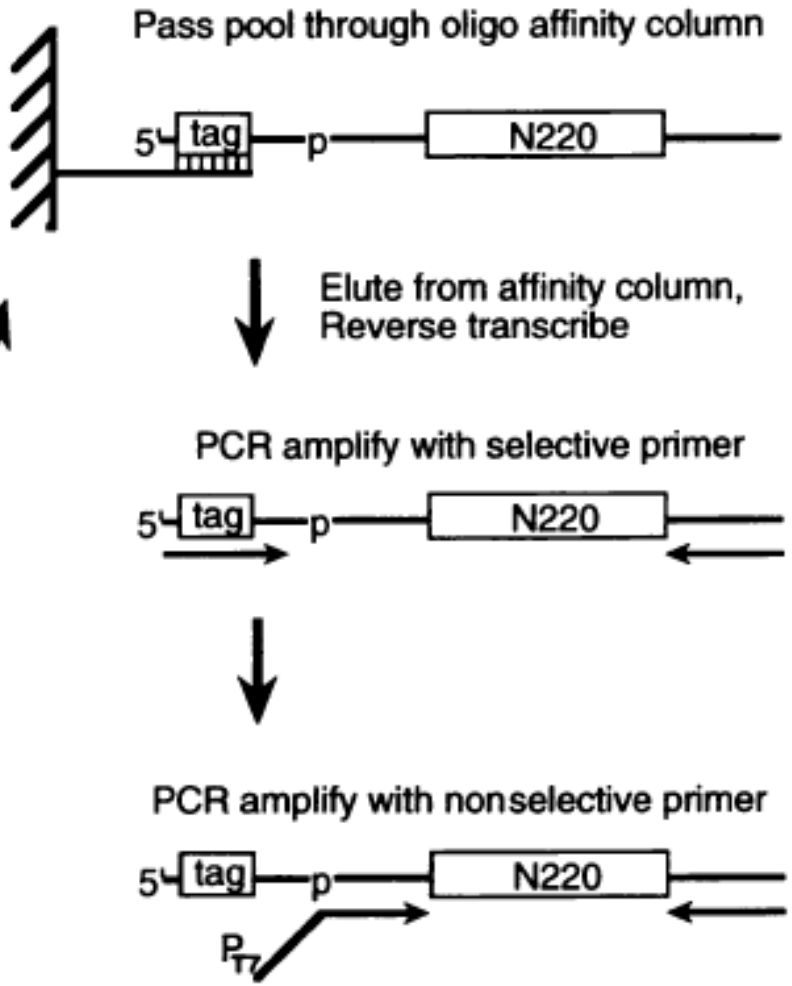


Fig. react expe polyr tion istry catal to th

Selection Scheme



selection



Ribozyme Catalyzed

Un-Catalyzed

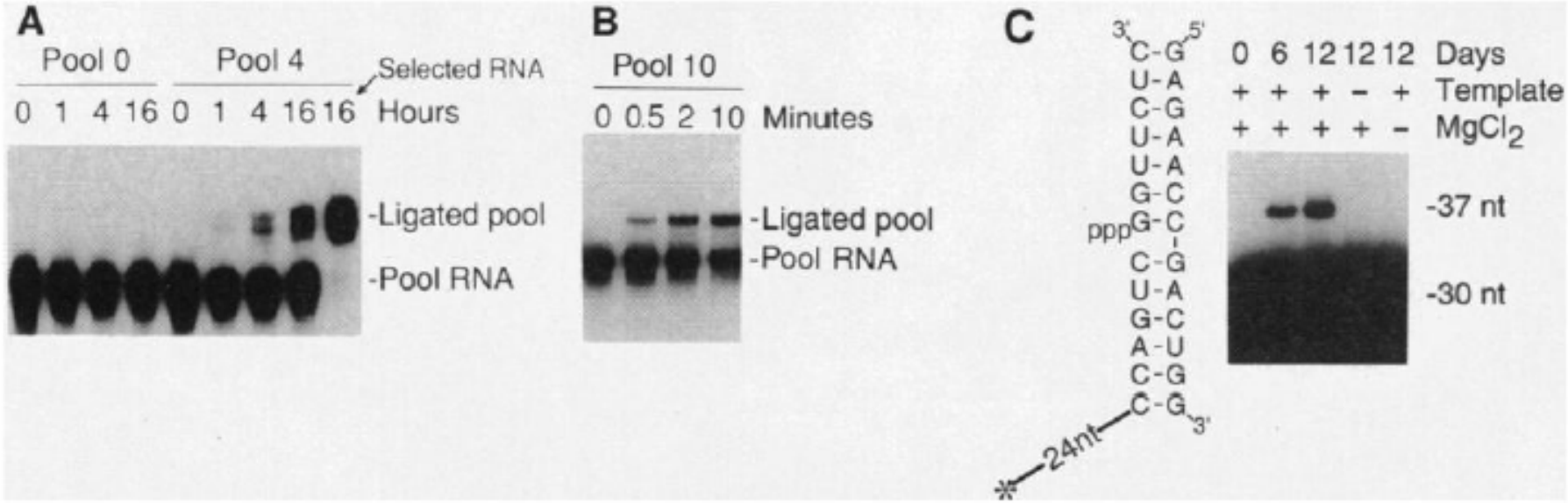


Fig. 4. (**A**) Time course of the catalyzed reaction. Phosphorus-32–labeled RNA of pools 0 and 4 was immobilized on agarose beads and incubated with substrate oligonucleotide. At the indicated time samples of the reaction were removed and stopped by addition of EDTA. RNA was eluted from the beads and separated by electrophoresis on a 4 percent acrylamide–7 M urea gel. A portion of the 16-hour pool 4 reaction was enriched for ligated molecules on an oligonucleotide affinity column ("selected RNA"). Immobilization, ligation, elution, and affinity selection conditions were as described for the first rounds of selection (Fig. 3). PhosphorImager (Molecular Dynamics) or Betagen (IntelliGenetics) scans were used for ^{32}P quantitation throughout. (**B**) Time course of pool 10 RNA ligation. Uniformly labeled pool RNA (0.4 μM) was annealed (*33*), and then incubated at 25°C with substrate oligonucleotide (2.5 μM) in the ligation buffer of the initial rounds (Fig. 3). Samples of the reaction were stopped with EDTA, and the RNA was separated on a 6 percent acrylamide–7 M urea gel. (**C**) Time course of the uncatalyzed reaction and template and magnesium dependence of this reaction. The 30-nt molecule that had been used as a substrate

Example of Ribozyme Evolution:

RNA Polymerase

Evolution of RNA Polymerase:

From ligase to RNA polymerase: “RNA-Catalyzed RNA Polymerization: Accurate and General RNA-Templated Primer Extension”

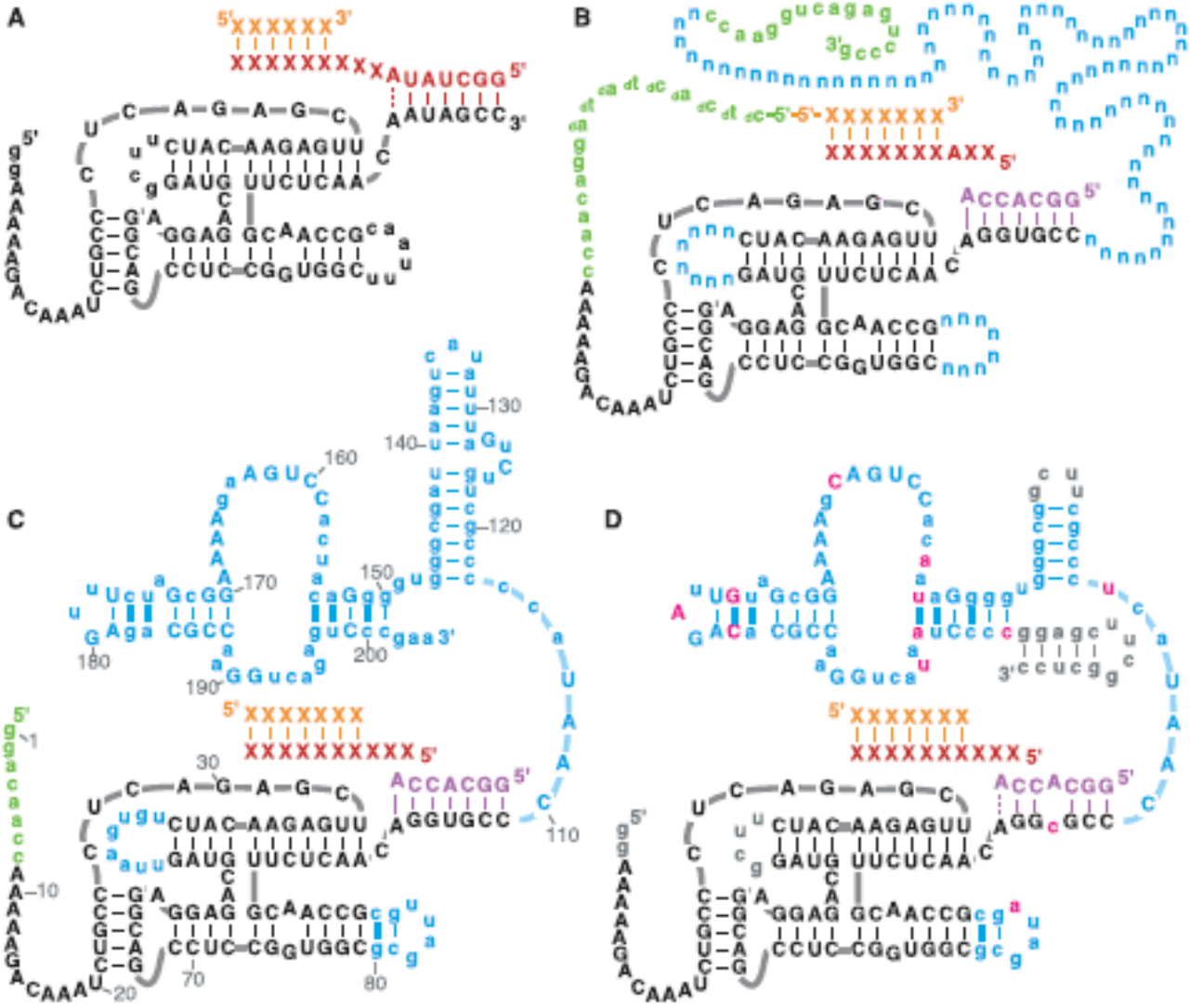
Science ***292, 1319 (2001)***

(A) A limited polymerase based on the ligase core (black line). *Template-ribozyme pairing required*

(B) Randomized pool

(C) Round 10, polymerase; no pairing required;

(D) Further improved polymerase



Evolution of RNA Polymerase:

Crystal Structure of the Catalytic Core of an RNA-polymerase Ribozyme (SCIENCE, 326, (2009), 1271)

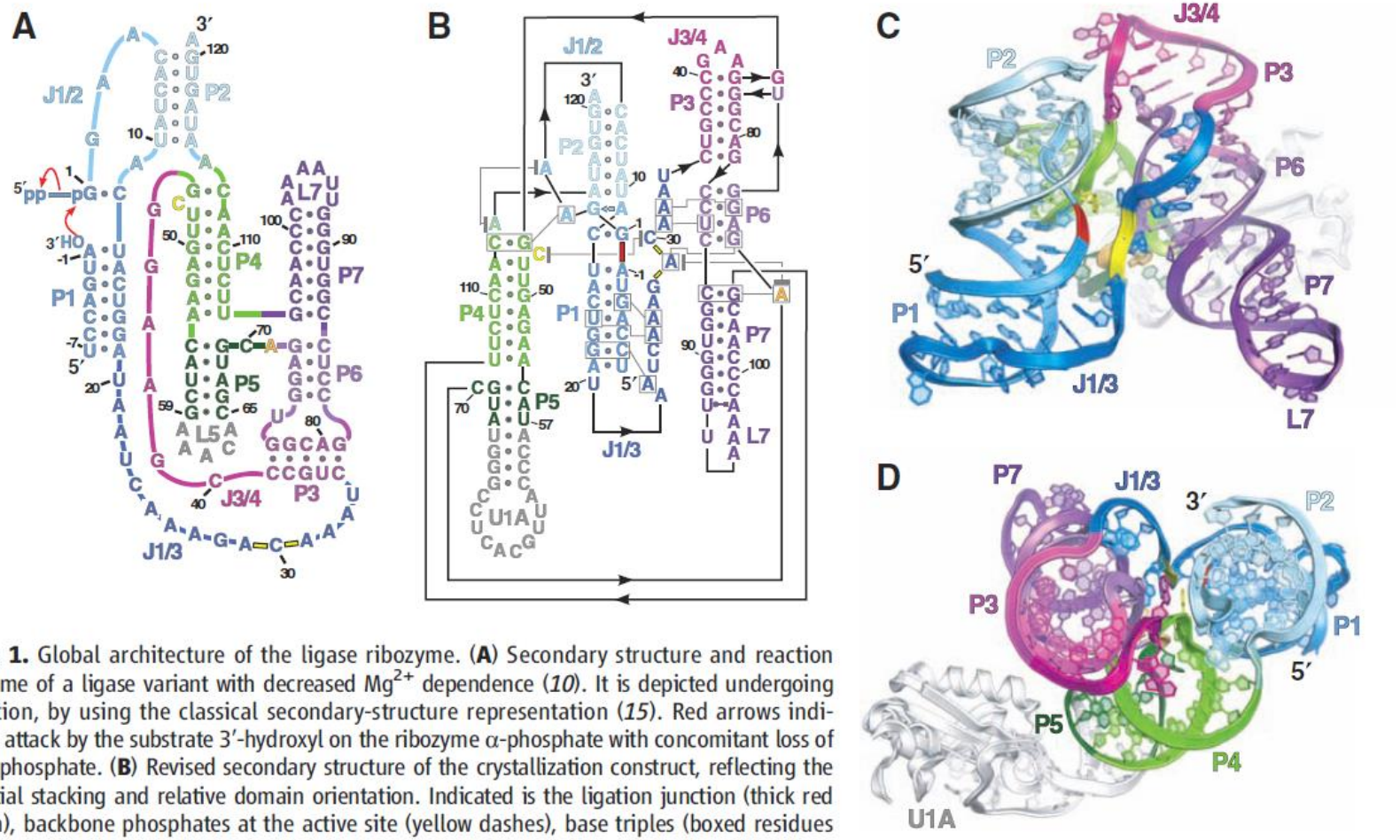


Fig. 1. Global architecture of the ligase ribozyme. (**A**) Secondary structure and reaction scheme of a ligase variant with decreased Mg^{2+} dependence (*10*). It is depicted undergoing ligation, by using the classical secondary-structure representation (*15*). Red arrows indicate attack by the substrate 3′-hydroxyl on the ribozyme α-phosphate with concomitant loss of pyrophosphate. (**B**) Revised secondary structure of the crystallization construct, reflecting the coaxial stacking and relative domain orientation. Indicated is the ligation junction (thick red dash), backbone phosphates at the active site (yellow dashes), base triples (boxed residues connected with gray lines), and stacking interactions (residues vertically aligned or connected with gray lines terminating in gray bars). Nucleotides numbered as in (A); those in gray were added to facilitate crystallization. Base-pair geometries indicated using nomenclature of (*27*). (**C**) Ribbon representation of ligase structure, as if peering into the active site (yellow) and ligation junction (red). (**D**) Top-down view, relative to (C).

Evolution of RNA Polymerase:

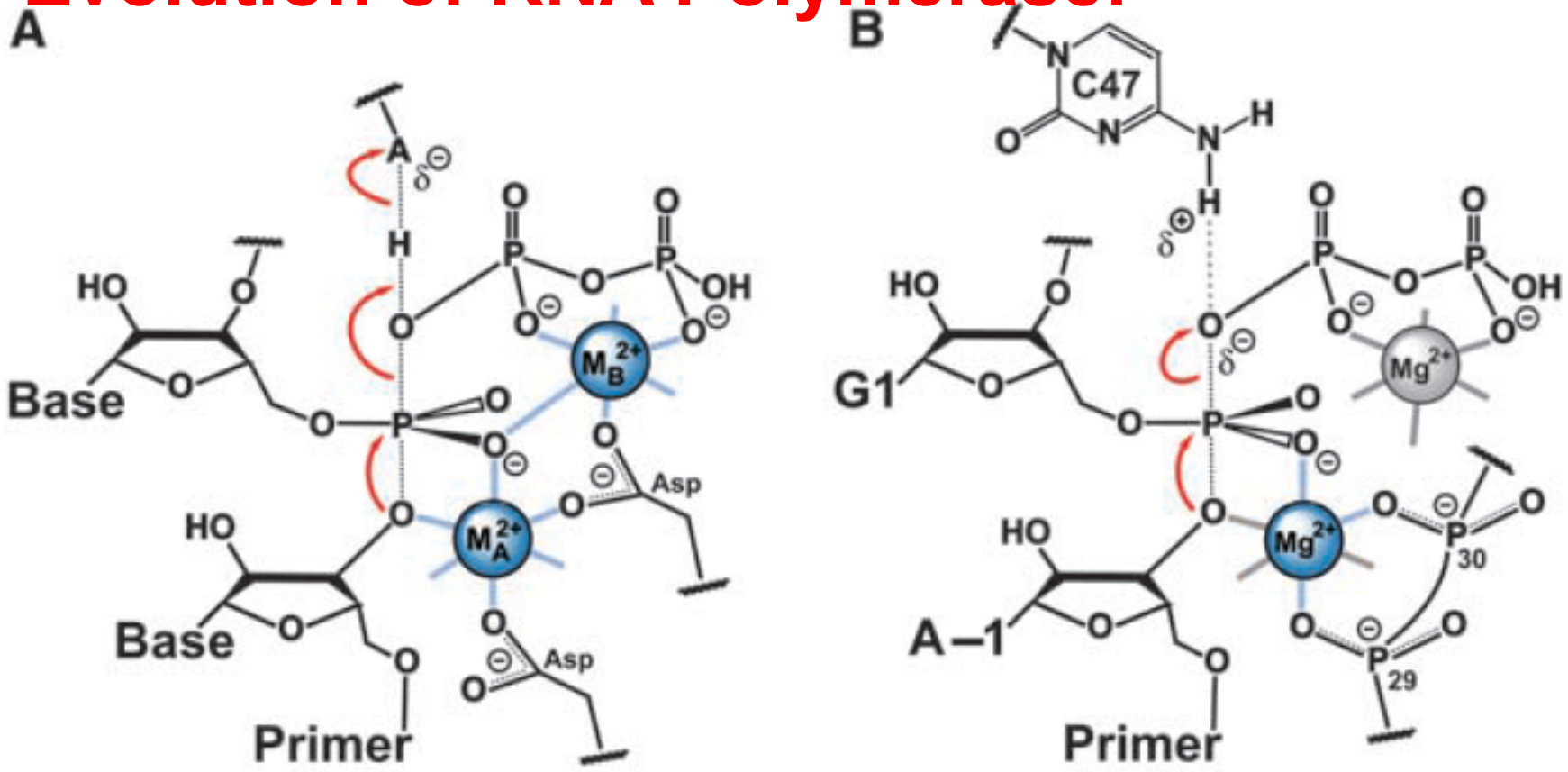


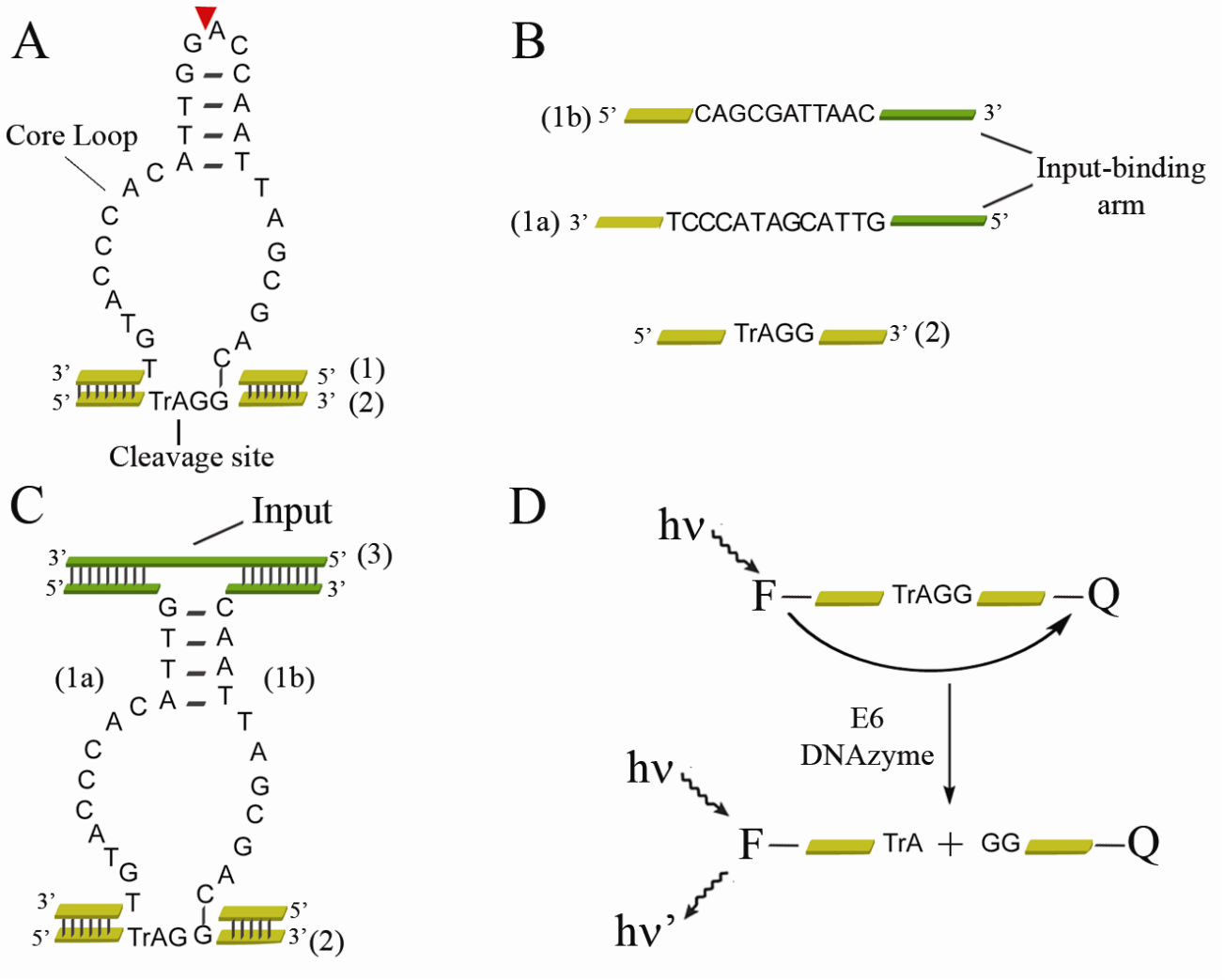
Fig. 4. Transition-state stabilization by polymerases built from either protein or RNA. (**A**) Catalysis by proteinaceous polymerases (*23*, *24*). Indicated are bonds formed or broken during the transition state (red arrows), coordination of catalytic metal ions, M_A and M_B (blue solid lines), and an active-site acid (A•••H). (**B**) Model for catalysis by the ligase ribozyme. Notation as in (A), with the addition of a hydrogen bond between C47 N4 and the leaving group (dashed gray line). Some magnesium ligands are not specified; for those that are, relative orientations are unknown. A proposed contact to the reactive phosphate pro-R_P oxygen (*28*) and two speculative contacts implied by NAIM are in blue. Metal ion and coordinations not supported (or refuted) by structural or biochemical evidence are in gray.

SELECT for DNAzymes (DNA enzymes)

DNAzymes: are DNA oligonucleotides that
can catalyze specific chemical reactions, such as restriction cuts.

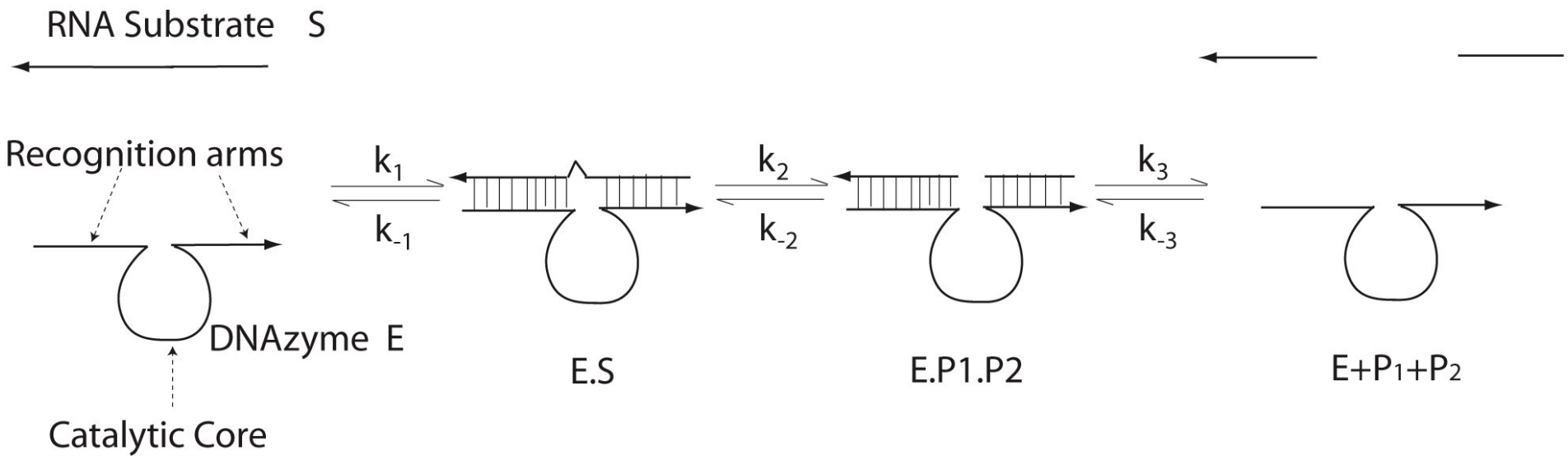
- **DNAzymes can be discovered by In vitro selection or In vitro evolution**
- **DNAzymes are also named: Deoxyribozymes, DNA enzymes or catalytic DNA.**

DNAzyme



(from Wilner)

DNAzyme kinetics



- 2nd step is rate determining
- Requires metal ion as cofactor
- $k_2 >> k_{-2}$, $k_1 >> k_{-1}$, $k_3 >> k_2$

[Santoro]

SELECT for DNAzymes (DNA enzymes)

- Start with 10^{15} DNA molecules again
- Select for enzyme activity:
    - E.g., cleaves itself off a solid support in the presence of Mg++
- Many different activities have been selected.
  Most have to do with nucleic acid transformations;
  RNase, ligase, kinase, etc.
  But not all (C-C bond formation possible).
- Generally much slower than protein enzymes.
- Most work has been on RNases
  (usually associated with the word “ribozymes”)

Daniel Kalderon & Larry Chasin, Columbia University

Allosteric ribozymes

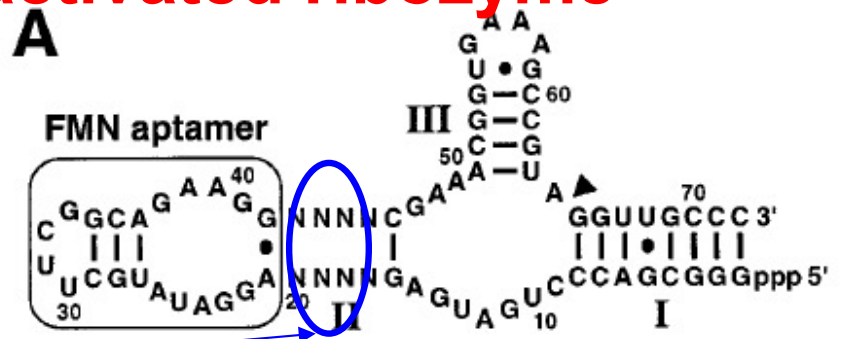
- **Allosteric: the alteration of the activity of a protein through the binding of an effector molecule at a specific site.**
- **Ribozyme: a ribonucleic acid (RNA) enzyme that catalyzes a chemical reaction**
- **Combine an aptamer and a ribozyme → Allosteric ribozyme**
- **Catalytic activity can be controlled by ligand binding !**
- **Positive or negative.**
- **Modular**
- **Molecular switches, biosensors**

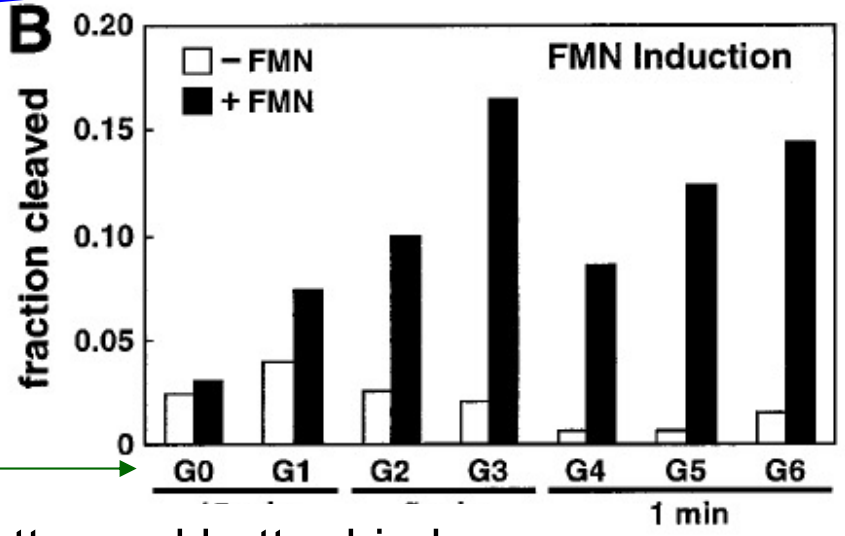
Daniel Kalderon & Larry Chasin, Columbia University

Selection of an allosterically activated ribozyme

Isolation of aptamer-ribozyme combinations that respond to ligand binding.

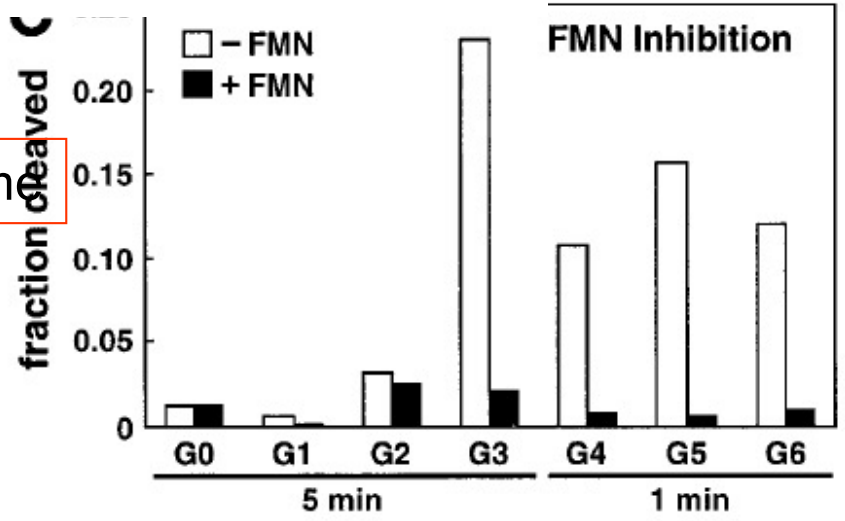
Randomize the "communication module"





Iterations

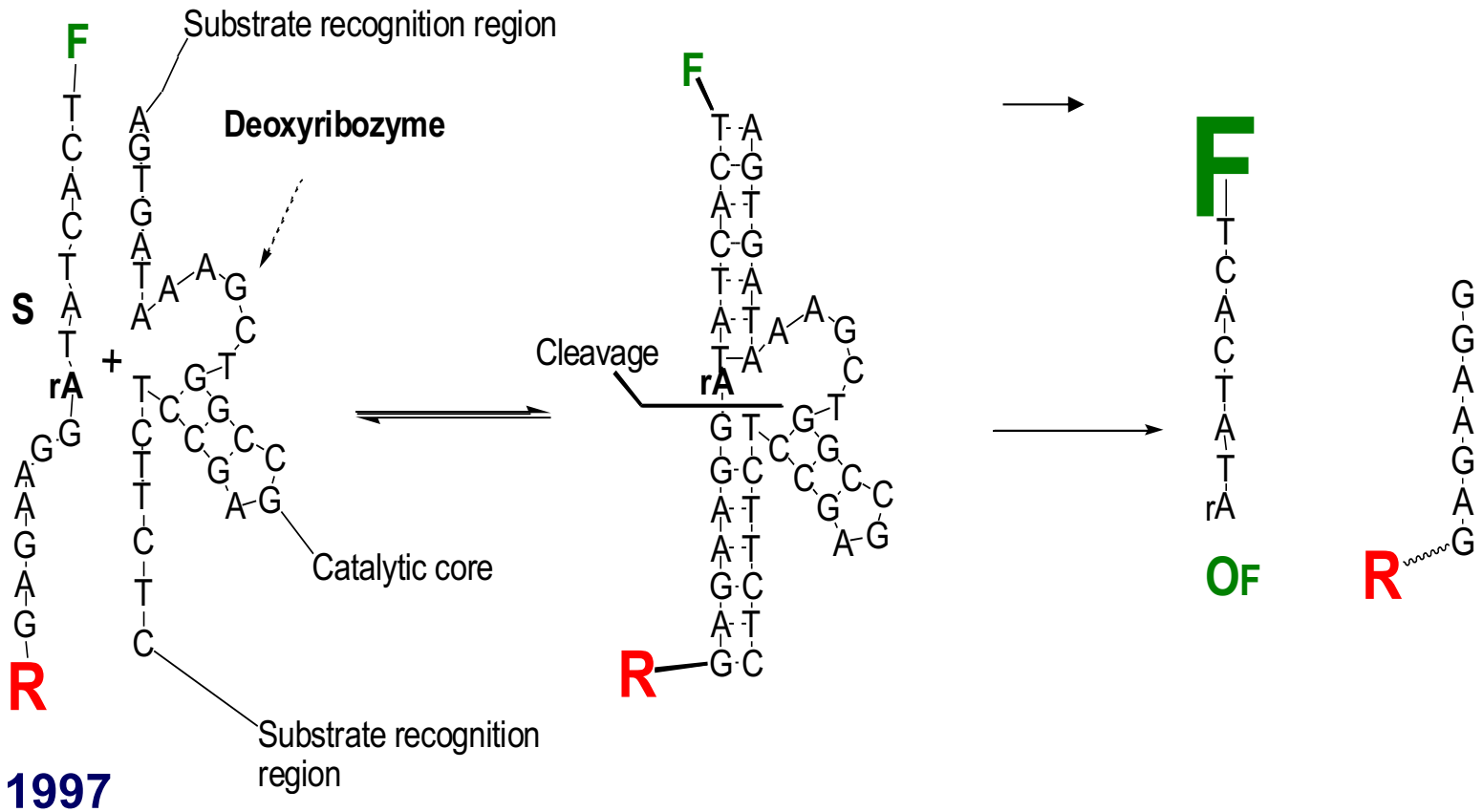
Select with decreasing activation times for better and better binders.



Selection of an allosterically ***inhibited*** ribozyme

Soukup, G.A. and Breaker, R.R. 1999. Engineering precision RNA molecular switches. *Proc Natl Acad Sci U S A* **96:** 3584-3589.

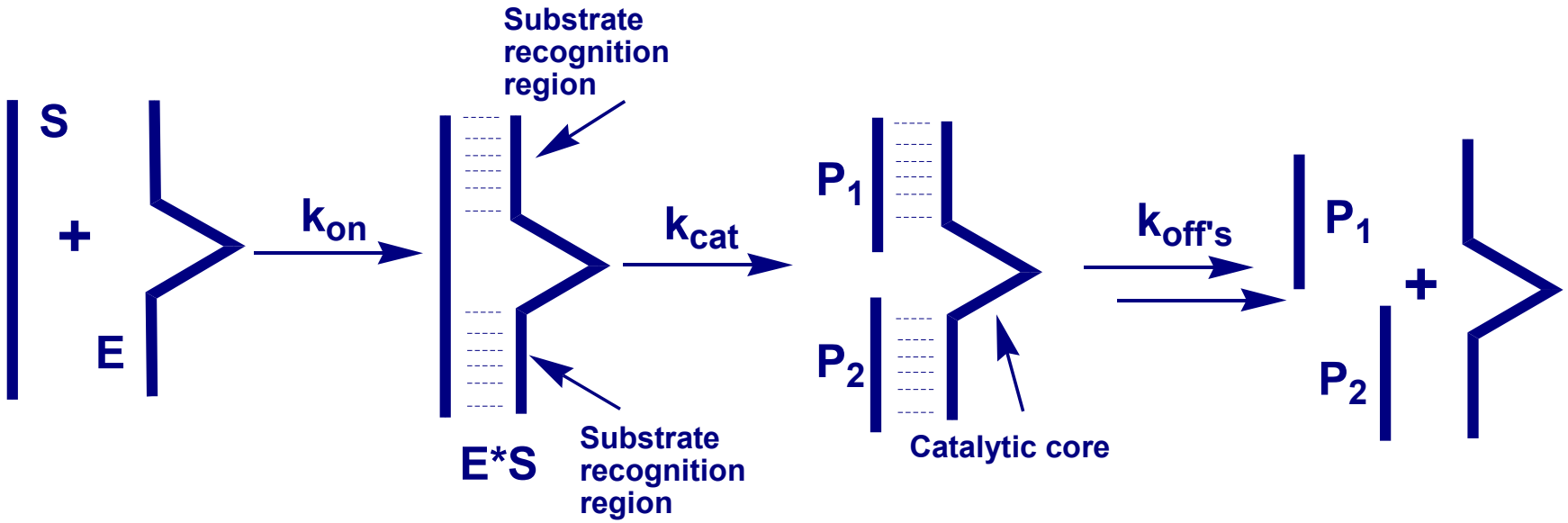
Ribozymes used to cut double stranded DNA at Recognition Sites:

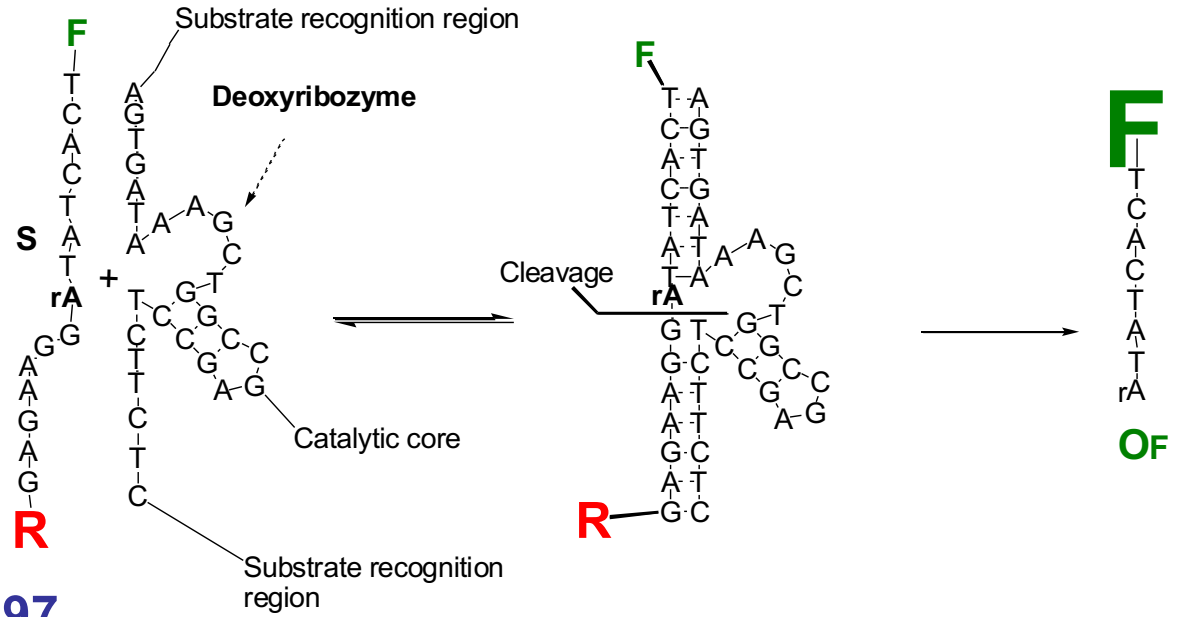


Joyce 1995, 1997

Milan N. Stojanovic
Columbia University

Ribozymes used to cut double stranded DNA at Recognition Sites:



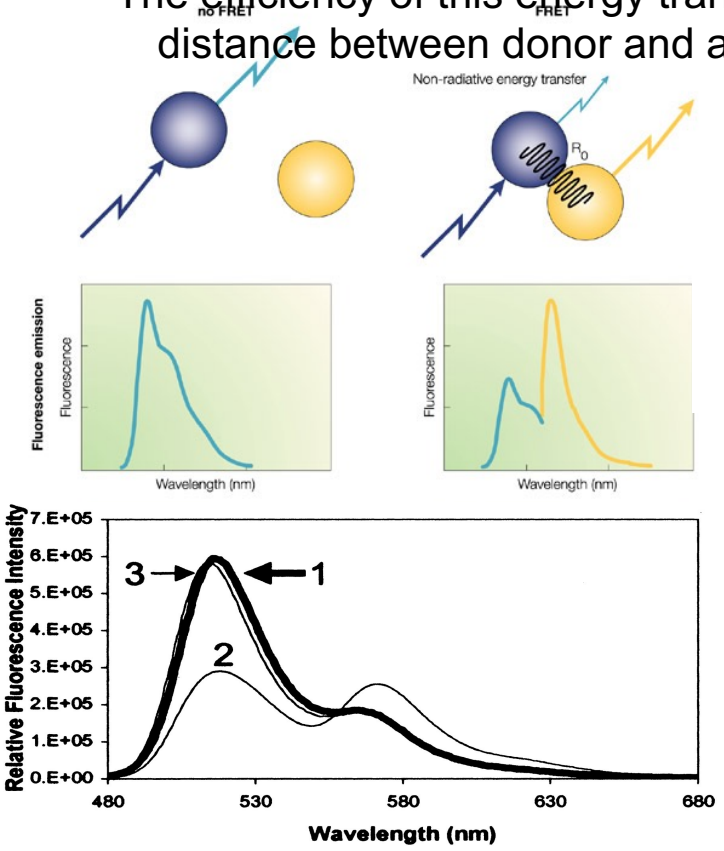


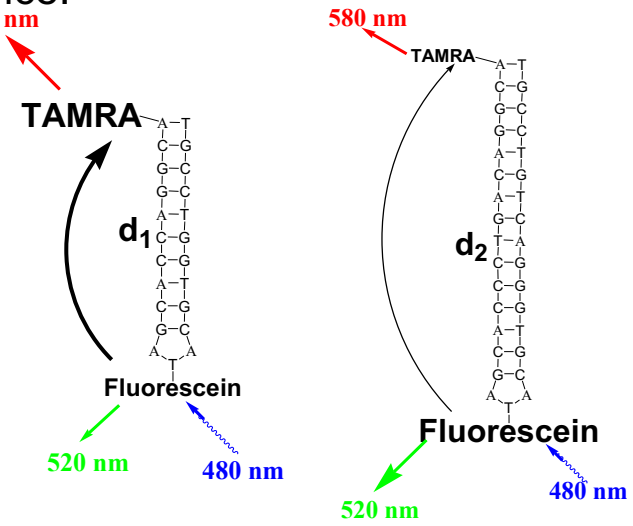
Joyce 1995, 1997

Milan N. Stojanovic
Columbia University

Förster or fluorescence resonance energy transfer (FRET)

- FRET is mechanism describing energy transfer between two light-sensitive molecules (chromophores).
- A donor chromophore, initially in its electronic excited state, may transfer energy to an acceptor chromophore through nonradiative dipole–dipole coupling.
- The efficiency of this energy transfer is inversely proportional to the sixth power of the distance between donor and acceptor, making FRET extremely sensitive to small changes in distance.





$d_1 < d_2$, FRET is stronger

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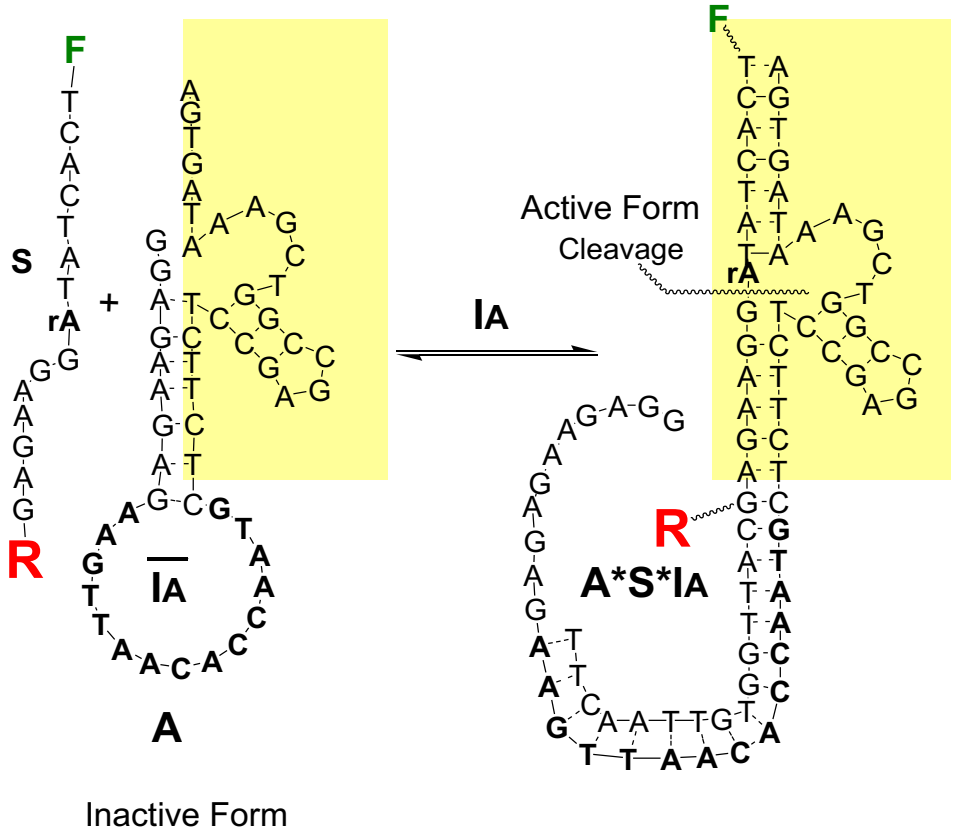
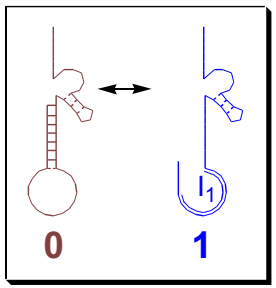
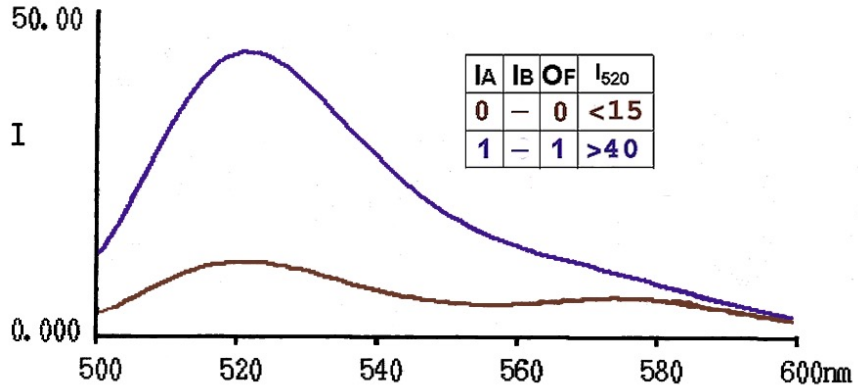
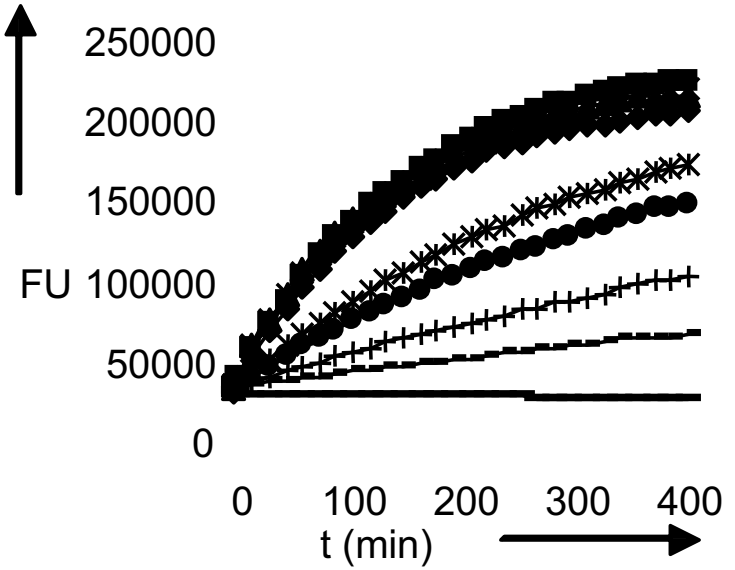
Catalytic Molecular Beacons as Sensor Gates Detector









Joyce 1995, Breaker 1999, Tyagi, Kramer 1996

Stojanovic et al., *ChemBioChem* **2001**
Stojanovic et al., *J. Am. Chem. Soc.* **2002**

Using an allosteric ribozyme to create a chemical sensor

Frauendorf, C. and Jaschke, A. 2001. Detection of small organic analytes by fluorescing molecular switches. Bioorg Med Chem ***9:*** *2521-2524.*

Start with a theophylline-dependent ribozyme:

Analogy:
A molecular "beacon" that respond to nucleic acid hybridization

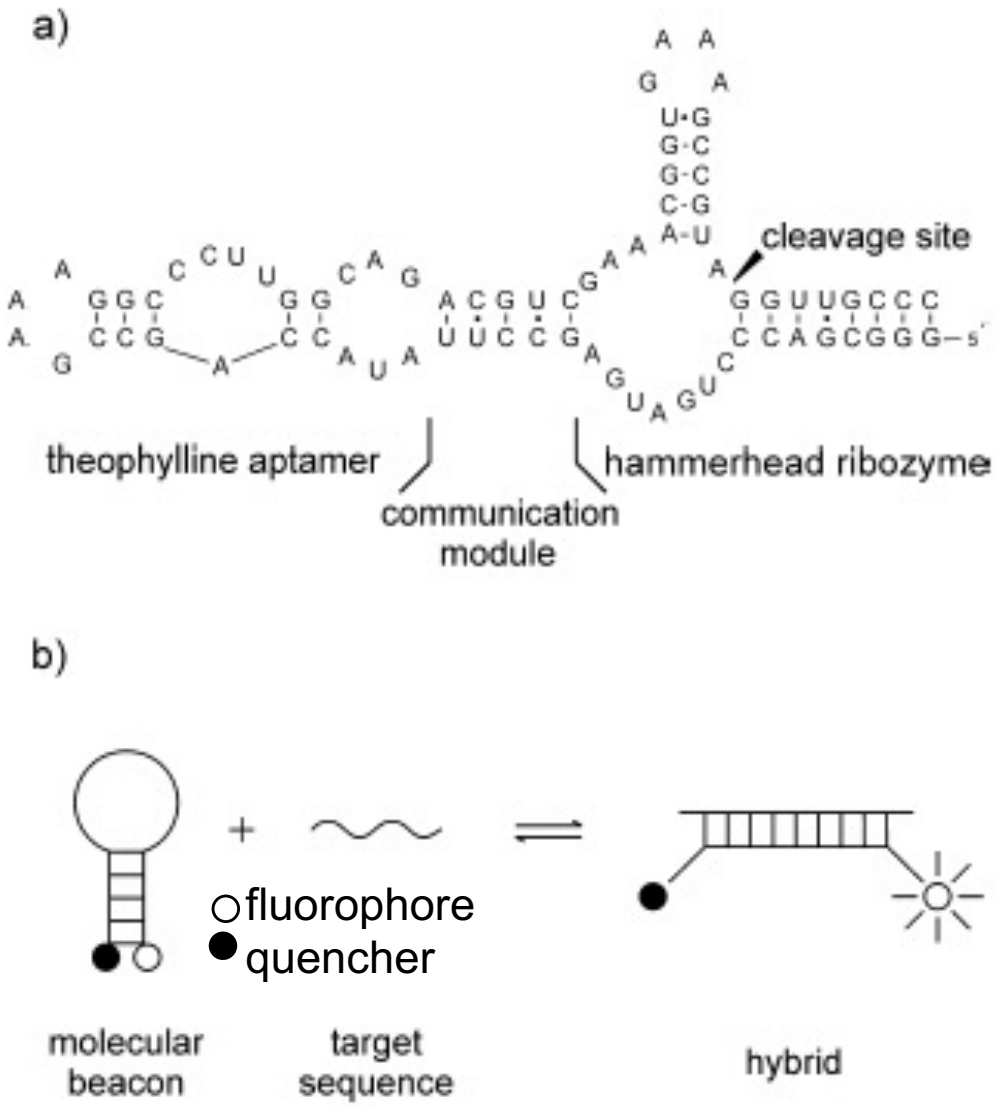
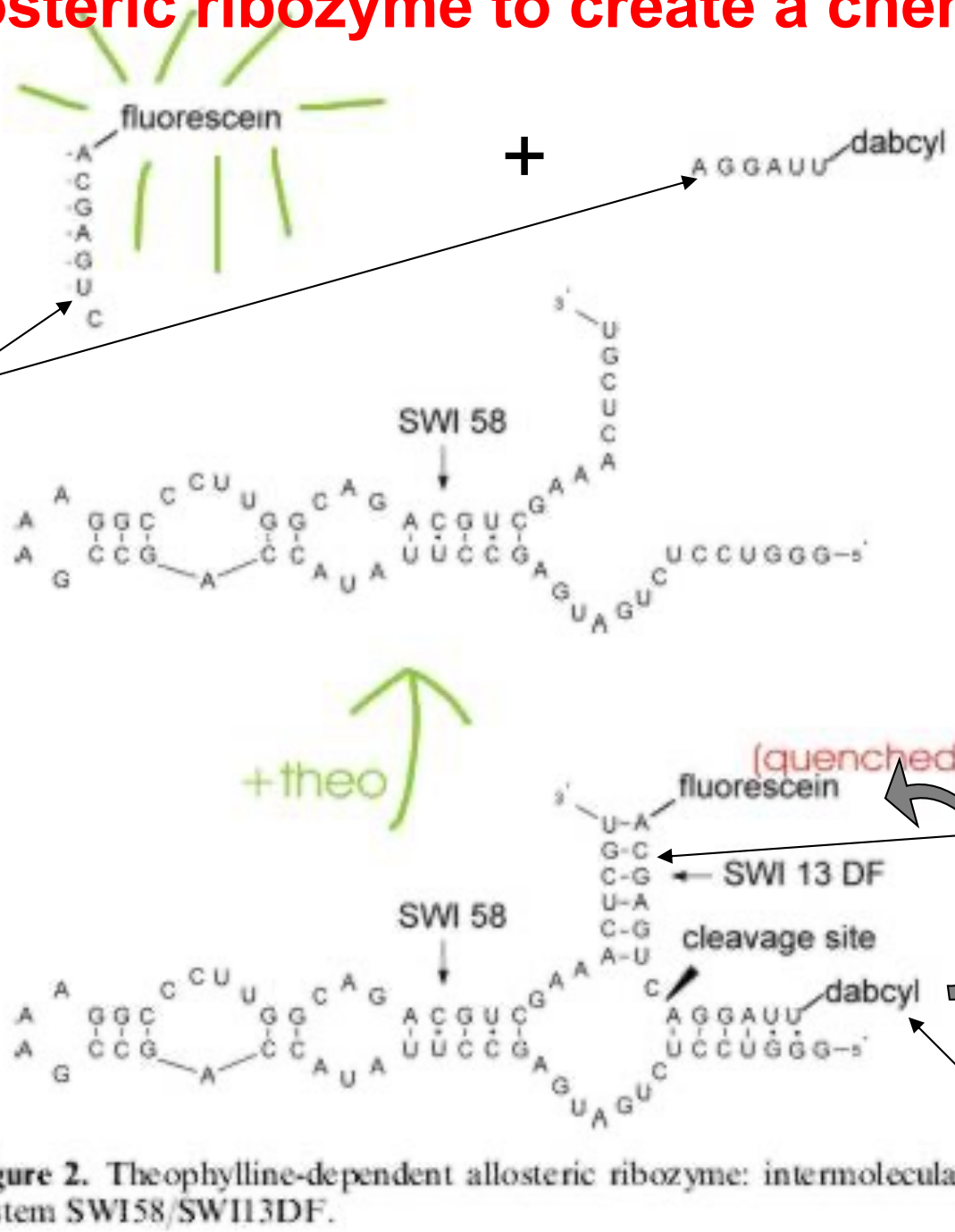


Figure 1. (a) Theophylline-dependent allosteric ribozyme[6] and (b) operating principle of molecular beacons.

Using an allosteric ribozyme to create a chemical sensor



Too short to maintain a stable duplex structure with SWI 58

Separate substrate molecule (in trans), fluorescently tagged

Nearby quenching group kept close by hybridization

Figure 2. Theophylline-dependent allosteric ribozyme: intermolecular system SWI58/SWI13DF.

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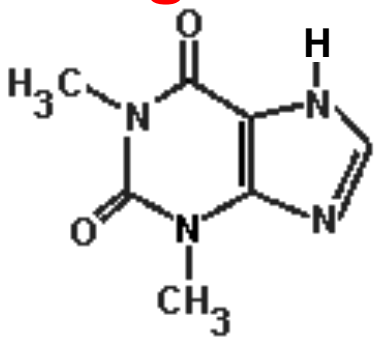
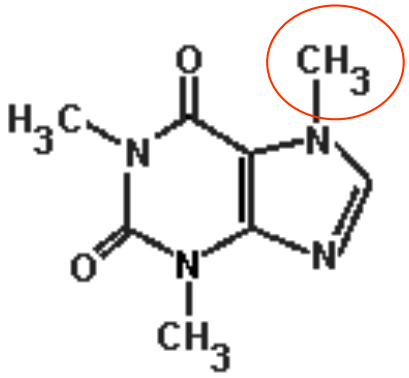
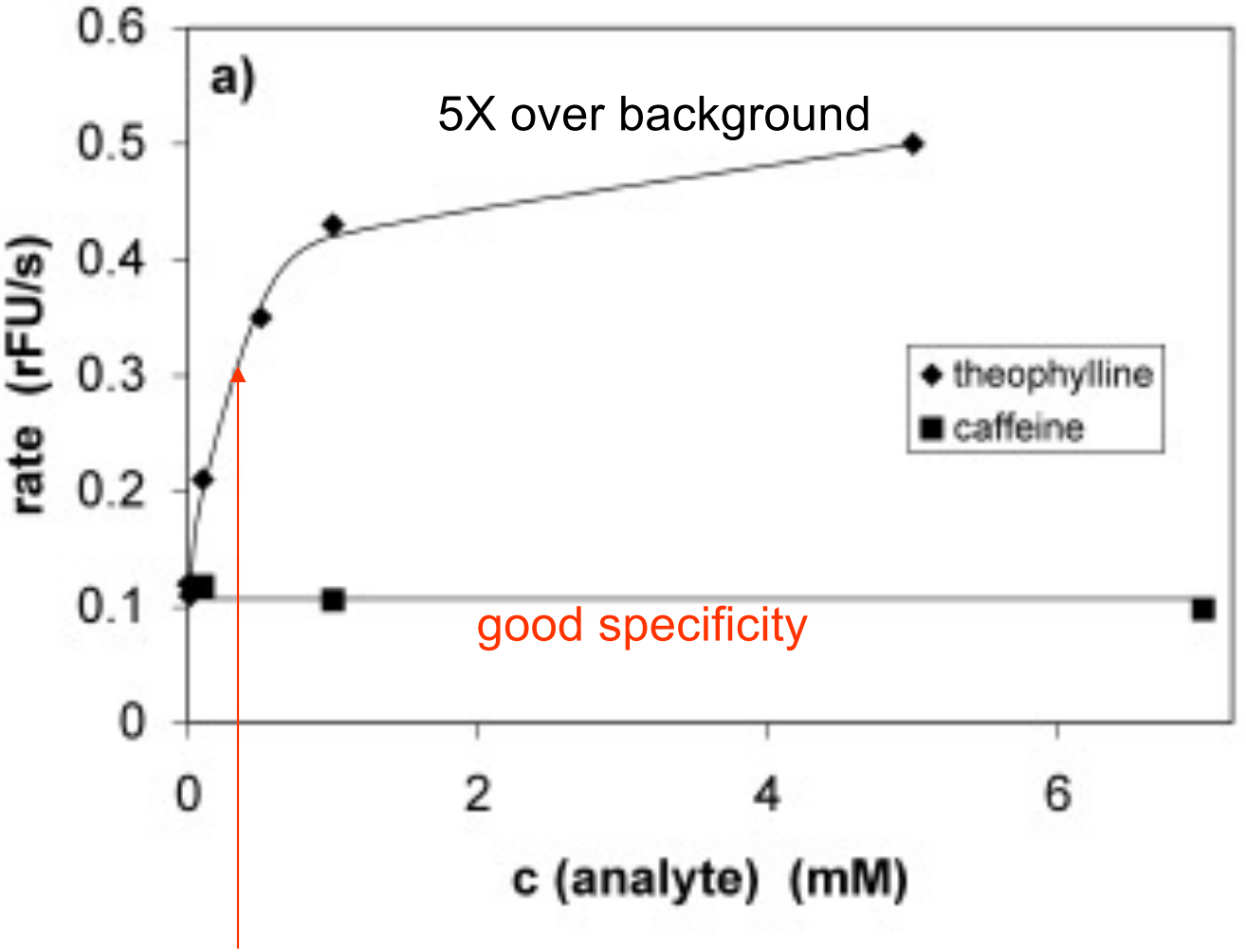
Using an allosteric ribozyme to create a chemical sensor



theophylline



caffeine



Not so sensitive (0.3 mM)

Daniel Kalderon & Larry Chasin, Columbia University

Some DNAzyme activities

Emilsson, G. M. and R. R. Breaker (2002). Deoxyribozymes: new activities and new applications.
Cell Mol Life Sci **59**(4): 596-607.

Table 1. Reactions and rate constants of deoxyribozymes.

Chemical reaction: Enzyme name	Cofactor/covalent modifications	Maximum k (min^{-1})	Rate enhancement	Ref.
RNA transesterification:			over spontaneous reaction	
G5 dominant clone	Pb^{2+}	1	10^5	29
E6	Mg^{2+}	0.04	10^5	38
Mg5/8-17/17E[a]	Zn^{2+}	1.35	10^8 calc	73–76
10–23	Mg^{2+}	5	10^5 calc.	31, 32
Na8	–	0.0067	10^8	27
HD2	L-histidine	0.2	10^6	28
16.–11	imidazole-modified DNA, Zn^{2+}	4.3	10^8 calc.	42
9_{25}–11	imidazole-, amine-modified DNA	0.044	10^6 calc.	44
DNA cleavage:				
Class II	Cu^{2+}	0.2	10^7	49–51
Rd10 dominant clone	phosphoramidate substrate Mg^{2+}, oligonucleotide	n/a	10^3	54
N-glycosylase activity:				
10–28	Ca^{2+}	0.2	10^6	53
Phosphorylation:				
NTP-A2.1	Ca^{2+}	0.012	10^9	59
Adenylation:				
Class I	Cu^{2+}, Mg^{2+}	0.0048	10^{10}	62
DNA ligation:				
E47	imidazole substrate, Zn^{2+}	0.07	10^5	65
L78[b]	AMP-capped substrate, Mn^{2+}	0.0001	10^5	64
C14	phosphorothioester linkage formation	0.023	10^2	67
Porphyrin metalation:				
PS5.M	–	1.3	10^3	55, 77

Compare protein enzymes,
Typically 6000 on this scale
(100/sec)