Discovery of:
DNA and RNA aptamers
DNA enzymes (DNAzymes)
RNA enzymes (Ribozymes)
using
In-vivo Evolution & SELECT Protocols

From Lectures of:
Daniel Kalderon & Larry Chasin, Columbia University
and
Peter Quin, USC
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)
Some examples of aptamer targets

**Small molecules**

- $\text{Zn}_2$
- ATP
- adenosine
- cyclic AMP
- GDP
- FMN (and an RNA aptamer is found naturally in E.coli)
- cocaine
- dopamine
- amino acids (arginine)
- porphyrin
- biotin
- organic dyes (cibacron blue, malachite green)
- neutral disaccharides (cellobiose, and cellulose)
- oligopeptides
- aminoglycoside antibiotics (tobramycin)

**Proteins**

- thrombin
- HIVtat
- HIV rev
- Factor IX
- VEGF
- PDGF
- ricin
- large glycoproteins such as CD4
- anthrax spores (?)

Daniel Kalderon & Larry Chasin, Columbia University
Some examples of aptamer targets

Zn$_2$
ATP
adenosine
cyclic AMP
GDP
FMN (and naturally in E.coli)
cocaine
dopamine
amino acids (arginine)
porphyrin
biotin
organic dyes (cibacron blue, malachite green)
neutral disaccharides (cellobiose)
oligopeptides
aminoglycoside antibiotics (tobramycin)
proteins (thrombin, tat, rev, Factor IX, VEGF, PDGF, ricin)
large glycoproteins such as CD4
anthrax spores
Table 1. Nucleic acid aptamers for which three-dimensional structures have been determined. ND, not determined.

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

*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.

Example: AMP-binding aptamer
Example: Streptomycin-binding aptamer
Example: Tobramycin (antibiotic) bound to its aptamer (backbone)
Example: A 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 Aptimers to bind DNA hairpin devices to Cell Membrane Proteins

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,
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.

More recently: Somalogic, Inc.: Photoaptamers

Wash stringently to produce a low background.

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

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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

Have a random 40-mer synthesized, between 2 arbitrary 20-mers (PCR sites)

\[
4^{40} = 10^{24}
\]

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. We know little as yet about these structures.

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

Previously discussed SELEX in terms of finding the substrate sequence(s) for an RNA binding protein. Here: select an RNA sequence that can bind any target of interest (protein, small molecule).
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

Random oligonucleotide pool ($10^{15}$)

Affinity matrix

T7 polymerase expression

RT-PCR

DNA

RNA

Ligand is immobilized here. Small molecule or large molecule.

e.g., the soluble form of the immobilized affinity column material

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General scheme

Synthetic DNA Pool

T7 promoter

5' constant sequence random sequence constant sequence 3'

PCR

Aptamers ← Clone — ds DNA pool ← PCR → cDNA

Transcribe

RNA — Binding Selection → Enriched RNA

RT
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

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 $\sim 10^{24}$; $N = 100$, pool $\sim 10^{60}$
Amplification:

- 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.
Results and Analyses

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.
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.
In vitro evolution of peptide/protein

- 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; …
mRNA display for in vitro selection of peptide and protein

**mRNA display.** An mRNA template (black line) covalently attached to puromycin is used to program an *in vitro* translation reaction. After protein synthesis, the puromycin enters the ribosome *in cis* to form a covalent mRNA-protein fusion.

Richard Roberts
RNA aptamers are unstable in vivo (bloodstream)
DNA aptamers are more stable but still can be destroyed by DNases.

Modification to protect:
2’ F-YTP \( (Y = \text{pyrimidine}) \)
2’ NH_2-YTP

But not substrates for PCR enzymes.
OK for T7 RNA polymerase and reverse transcriptase.
So: *Isolation of an RNase-resistant aptamer* \(10^{15}\)

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**Diagram:**

- DNA synthesizer
- T7 promoter
- Random
- PCR site
- T7 polymerase, 2’ F-CTP + 2’ F-UTP
- 2’ F-YTP
- Affinity chromatography
- Selection
- Enriched stable aptamer
- Final product after N iterations

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Natural enantiomers: peptides = L-amino acids
nucleic acids = D-ribose

Spiegelmers
for more stable RNA aptamers (spiegel = mirror)

Synthesize a D-amino acid version of your peptide target

L-RNA is resistant to nucleases

Ordinary D-ribose nucleic acid

Noxxon (Germany)
First products: Anti-CGRP Anti-Grehlin
Extended Example of an RNA Aptamer:

**Therapeutic use of an aptamer that binds to and inhibits clotting factor IX**


Factor IX acts together with Factor VIIIa to cleave Factor X, thus activating it in a step in the blood coagulation cascade leading to a clot. 
-Thus inhibition of Factor IX results in inhibition of clot formation. Desirable during an angioplasty, for example. 
-The usual anti-coagulant used in angioplasty is heparin, which has some toxicitiy and is difficult to control. 

Inverted T at 3’ end (3’-3’) slows exonucleolytic degradation ( R-3’ O-P-O-3’-R-T )
Anti-Factor IX RNA aptamer isolated by SELEX

Kd for Factor IX = 0.6 nM

$F_{IXa} + F_{VIIIa}$ cleaves $F_X$

Aptamer inhibits this activity

Clotting time increase

Conjugate to polyethyleneglycol to increase bloodstream lifetime

PEG = polyethyleneglycol polymer, appended to decrease clearance rate.

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An **antidote** to stop the anti-clotting action if a patient begins to bleed. Would be an improvement over heparin. Just use the complementary strand (partial) as an antidote. The 2 strands find each other in the bloodstream!

In human plasma

**Antidote 5-2 design = the open squares**

**16-fold excess**

**duplexed free aptamer**

**Scrambled antidote**

**Ratio of anti- to aptamer**

**+Oligomer 5-2**

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Antithrombin aptamer antidote tested in human serum

- Antidote acts fast (10 min)
- Antidote lasts a long time
- Need 10X antidote

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In serum of patients with heparin-induced thrombocytopenia

(heparin can no longer be used)

Reduced clotting
Reversed by antidote

Figure 5 Antidote-controlled anticoagulation of plasma from patients with HIT. a, b, The activity of aptamer Peg-9.3t and antidote 5-2 were tested in plasma from haemodialysis-dependent patients diagnosed with HIT (a) and in plasma from patients suffering from thromboembolic complications of HIT (b). Plasma samples were treated as indicated: aptamer, 125 nM Peg-9.3t; antidote, 1.25 uM AO 5-2; and mutant aptamer, 125 nM 9.3tM. Data is reported in seconds (s) and is the average ± range of duplicate measurements.

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Other Examples of RNA Aptamers:
Macugen: an RNA aptamer that binds VEGF and is marketed for adult macular degeneration (wet type)

From the label:

Pegaptanib sodium is represented by the following structural formula:

Where R is and contains a PEG chain of ~ 450 ethylene glycol units.


The molecular formula for pegaptanib sodium is C294H342F13N107Na28O188P28[C2H4O]n (where n is approximately 900) and the molecular weight is approximately 50 kilodaltons.

Macugen is formulated to have an osmolality of 280-360 mOsm/Kg, and a pH of 6–7.

VEGF = vascular endothelial growth factor

Aptamer vs, prostate cancer cell membrane antigen (PMSA), conjugated to rhodamine

Potential use as an anticancer diagnostic, and therapeutic.
Gold, L. et al.,
Aptamer-Based Multiplexed Proteomic Technology for Biomarker Discovery
PLoS ONE, 1 December 2010, Volume 5, e15004

SomaLogic, Inc.
SELECT for
Ribozymes (RNA enzymes)
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
  - Free guanosine
  - No lariat

- Group II
  - +
  - + (natural ribozymes)

Spliceosome-catalyzed splicing of nuclear mRNA

- Spliceosome
  - lariat
  - lariat

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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 GU. 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


**Diagram:**
- **A:**
  - RNA Pool
  - Proposed cleavage zone
- **I:**
  - Now add Mg++
  - 10^{15} DNA molecules with T7 promoter
  - Keep molecules under non-permissive conditions so they stay intact (without Mg++)
- **II:**
  - Proposed cleavage zone
  - Selecting for cleavage anywhere in the zone
- **III:**
  - 1. RT-PCR
  - 2. RNA transcription
  - Isolate cleaved RNA

**Steps:**
- RT -> cDNA: Cleavage zone is rebuilt by being part of the primer.
- Isolate the successfully cleaved by size on gels

**Note:**
- Daniel Kalderon & Larry Chasin, Columbia University
- UACGGUCGCUGUCUCGAUGGUUACUCCAAGCGAG
- 16 possible nearest neighbor combinations i.e., all 16 dinucleotides present as possible cleavage sites
Bartel & Szostak, SCIENCE, 261 (1993) 1411

- Iterative in vitro selection; isolate 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.
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
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
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).

Peter Quin, USC
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 $(A\cdots\cdots H)$. (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)
(but much slower than Ribozymes)
DNAzymes are DNA oligonucleotides that can catalyze specific chemical reactions, such as restriction cuts.

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

(from Wilner)
DNAzyme kinetics

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

[Santoro]
New synthetic ribozymes, and DNAzymes

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”)

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Combine an aptamer and a ribozyme → Allosteric ribozyme

Catalytic activity can be controlled by ligand binding!

Positive or negative.

Modular

Molecular switches, biosensors
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

Engineering precision RNA molecular switches. 
Using an allosteric ribozyme to create a chemical sensor

Reading

Start with a theophylline-dependent ribozyme:

**Analogy:**
A molecular “beacon” that respond to nucleic acid hybridization

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*Figure 1.* (a) Theophylline-dependent allosteric ribozyme and (b) operating principle of molecular beacons.
Nearby quenching group kept close by hybridization

Too short to maintain a stable duplex structure with SWI 58

Separate substrate molecule (in trans), fluorescently tagged

Daniel Kalderon & Larry Chasin, Columbia University
5X over background

Theophylline

Caffeine

5X over background

Not so sensitive
(0.3 mM)

good specificity

daniel kalderon & larry chasin, columbia university
**Some DNAzyme activities**


<table>
<thead>
<tr>
<th>Table 1. Reactions and rate constants of deoxyribozymes.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical reaction: Enzyme name</td>
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<tr>
<td>-------------------------------</td>
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<tr>
<td>RNA transesterification: G5 dominant clone</td>
</tr>
<tr>
<td>E6</td>
</tr>
<tr>
<td>Mg5/8-17/17E$^a$</td>
</tr>
<tr>
<td>10–23</td>
</tr>
<tr>
<td>Na8</td>
</tr>
<tr>
<td>HD2</td>
</tr>
<tr>
<td>16–11</td>
</tr>
<tr>
<td>9$_{25}$–11</td>
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<tr>
<td>DNA cleavage: Class II Rd10 dominant clone</td>
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<tr>
<td></td>
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<tr>
<td>N-glycosylase activity: 10–28</td>
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<tr>
<td>Phosphorylation: NTP-A2.1</td>
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<tr>
<td>Adenylation: Class I</td>
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<tr>
<td>DNA ligation: E47</td>
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<tr>
<td>L.78$^b$</td>
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<tr>
<td>C14</td>
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<td>Porphyrin metalation: PS5.M</td>
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</tbody>
</table>

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