SELEX and Artificial Ribozymes

Some definitions

SELEX: Systematic Evolution of Ligands by EXponential Enrichment (alternatively: in vitro selection, in vitro evolution)

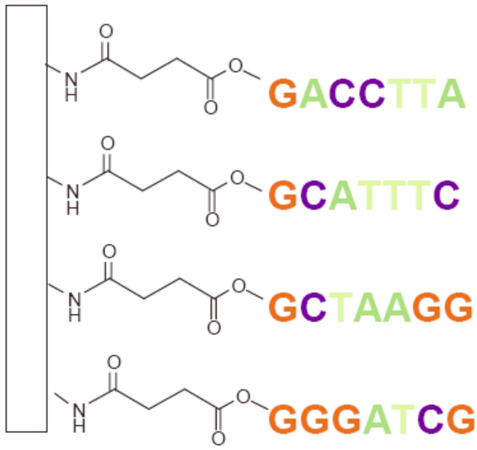
Aptamer: nucleic acid ligand (from Aptus: to fit)

Jack Szostak (Boston, MA)
Jerry Joyce (La Jolla, CA)
Larry Gold (Boulder, CO)

Combinatorial RNA libraries

- Straight-forward synthesis: use of nucleotide mixtures in automated solid phase synthesis,
- Enormous complexities: typically 10^{14} to 10^{16} different molecules,
- Nucleic acids can be enzymatically copied.

Library synthesis - basics



Combinatorial Chemistry!

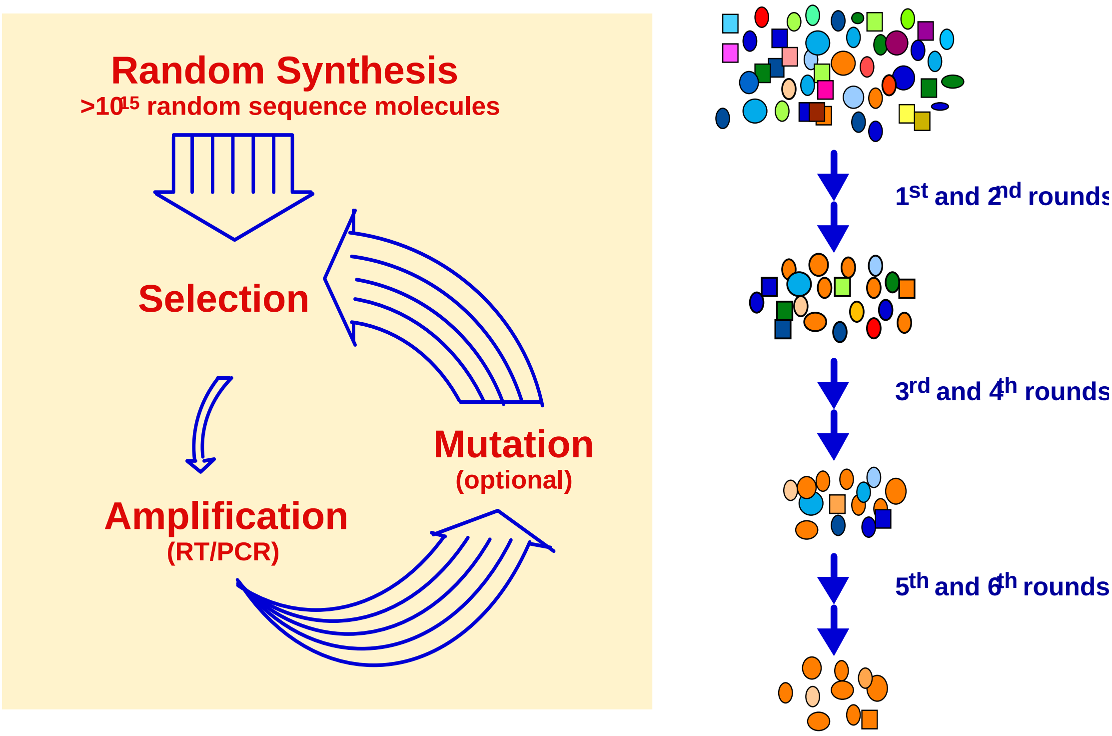
To meditate!

Size of the RNA fragment (nt)	Number of possible sequences	Mass of RNA required to explore all the possible sequences
20	1.1×10^{12}	0.012 µg
30	1.5×10^{18}	0.018 g
40	1.2×10^{24}	25 kg
100	1.6×10^{60}	8.4×10^{37} kg

1 mole = 6.022×10^{23} molecules

Mass of the solar system: 2.10^{30} kg

In vitro selection and evolution techniques



Ellington & Szostak (1990), Tuerk & Gold (1990), Beaudry & Joyce (1992)

SELEX

(I) Criteria of selection

(1) Purification by affinity chromatography (ligand, protein etc.)
(2) Purification by gel shift (protein, RNA ligand, peptide etc.)
(3) RNA self-modification leading to a change of size or attachment of biotin (followed by gel purification or streptavidin affinity column etc.)

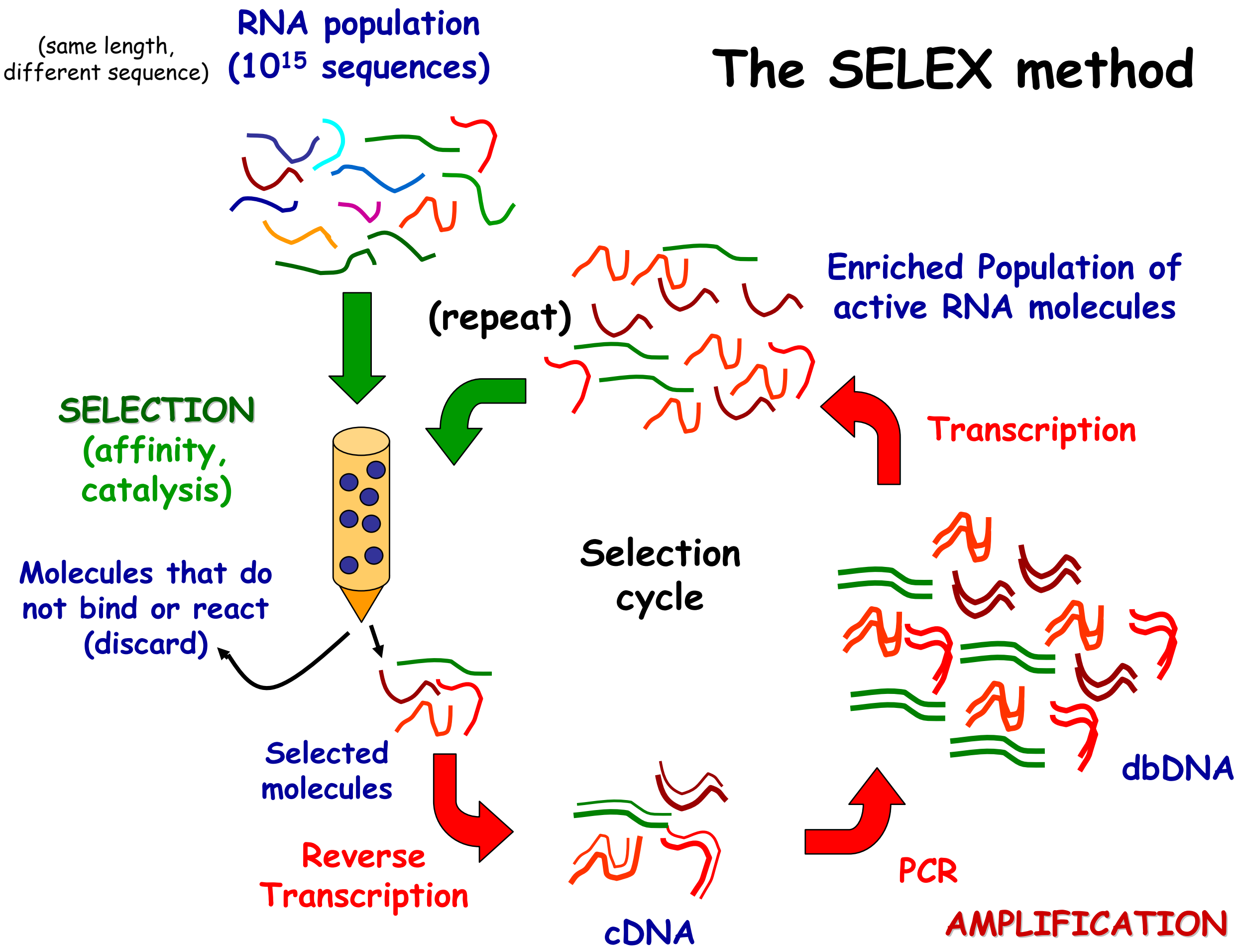
(II) Amplification

Once the selection is performed, the molecules that have been selected are amplified (first step: reverse transcription; second step: PCR)

(III) Mutagenesis

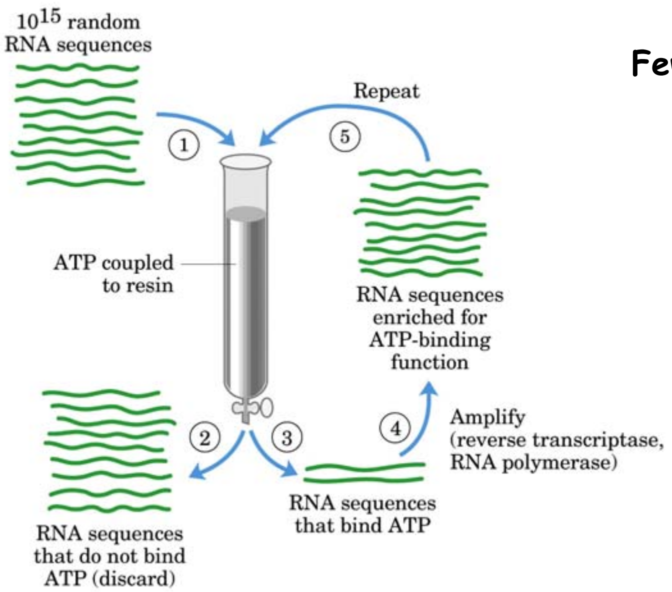
To improve sub-optimal population of molecules (evolution) by introducing random mutations

The SELEX method



In vitro selection and evolution methods (SELEX)

Jack Szostak (Boston, MA)
Jerry Joyce (La Jolla, CA)
Larry Gold (Boulder, CO)



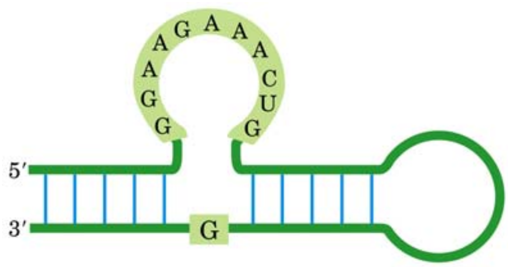
(a)

Few definitions

SELEX: **S**ystematic **E**volution of **L**igands by **Ex**ponential Enrichment

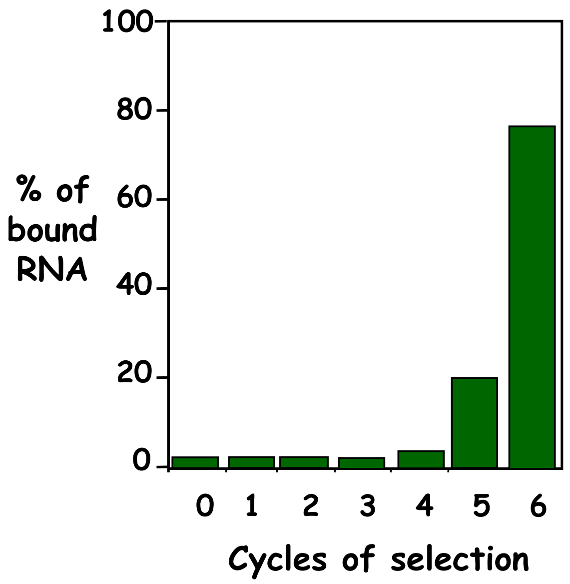
Aptamer: nucleic acid ligand (from Aptus: adjust)

Example: Selection of an ATP aptamer
(Sassanfar & Szostak (1993) Nature 364, 550)



ATP aptamer

Example of enrichment in active molecules of a population of RNA (eg bound to a dye column) by SELEX



little exercice

In theory, if the background noise is 1%, then the enrichment factor in active molecules (with an affinity of 100%) is 100 fold at each cycle.

If the starting population has 10^{13} different molecules and if a signal of 20% appears at the 5^{th} cycle, then there are more than 200 active molecules (with an affinity of 100%) in the population.

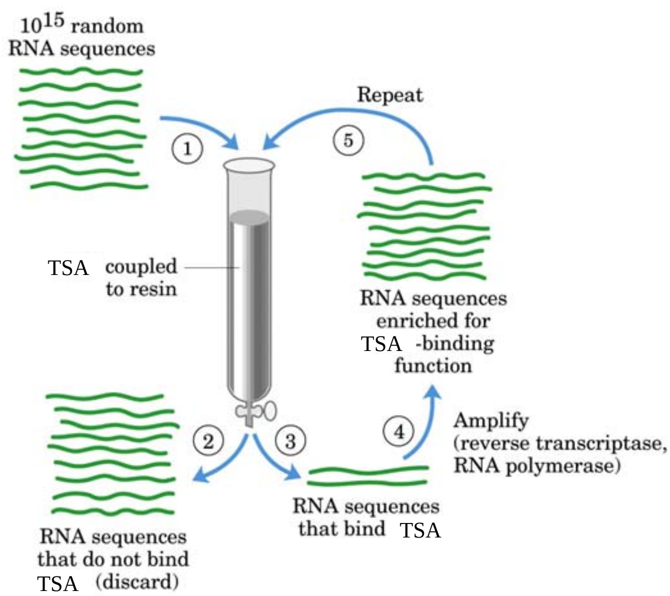
$$0,2 \times 10^{13}/100^5 = 200$$

Principles of library design

Artificial ribozymes - methodology

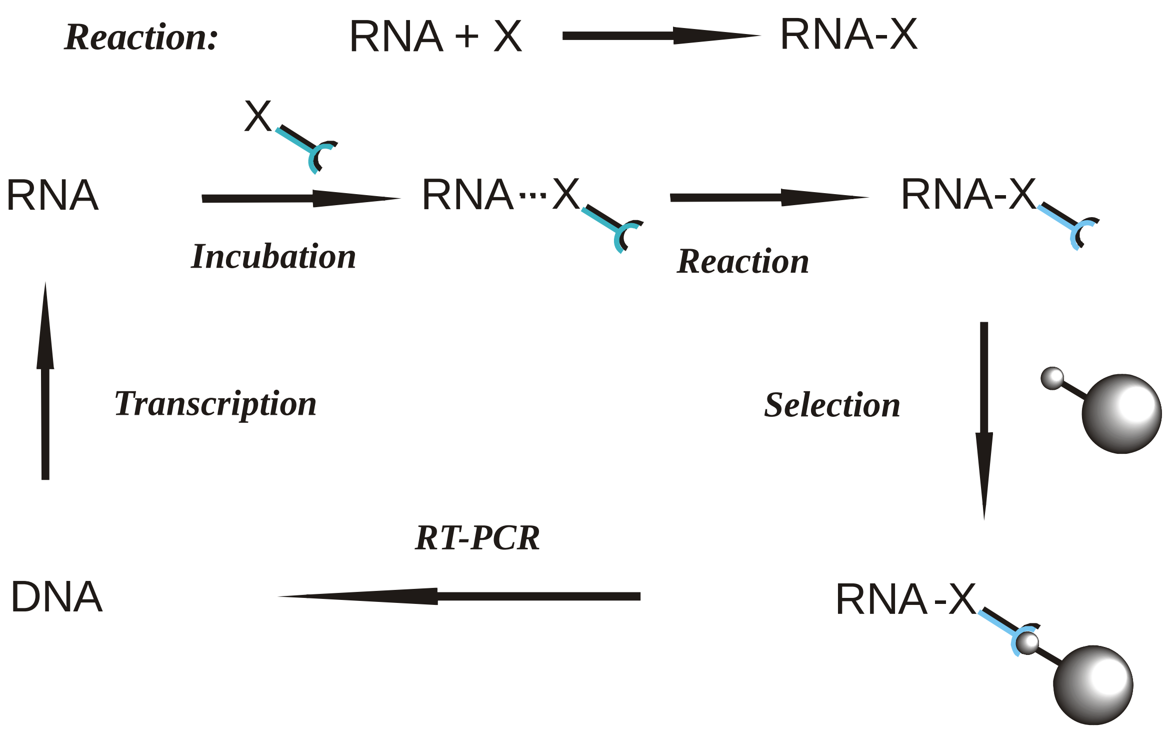
- SELEX against transition state analogs
- Direct selection
- Direct selection with tethered reactants

Selection against transition state analogs



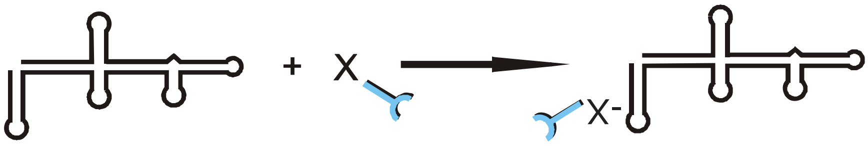
(a)

Direct selection of RNA catalysts

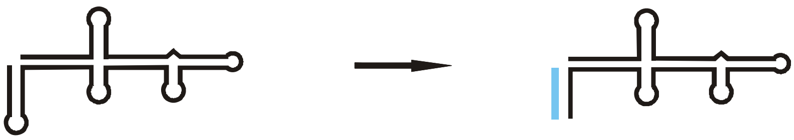


Direct selection of RNA catalysts

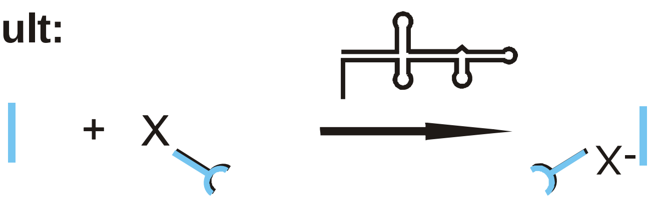
Result of the selection:



Engineering



Result:



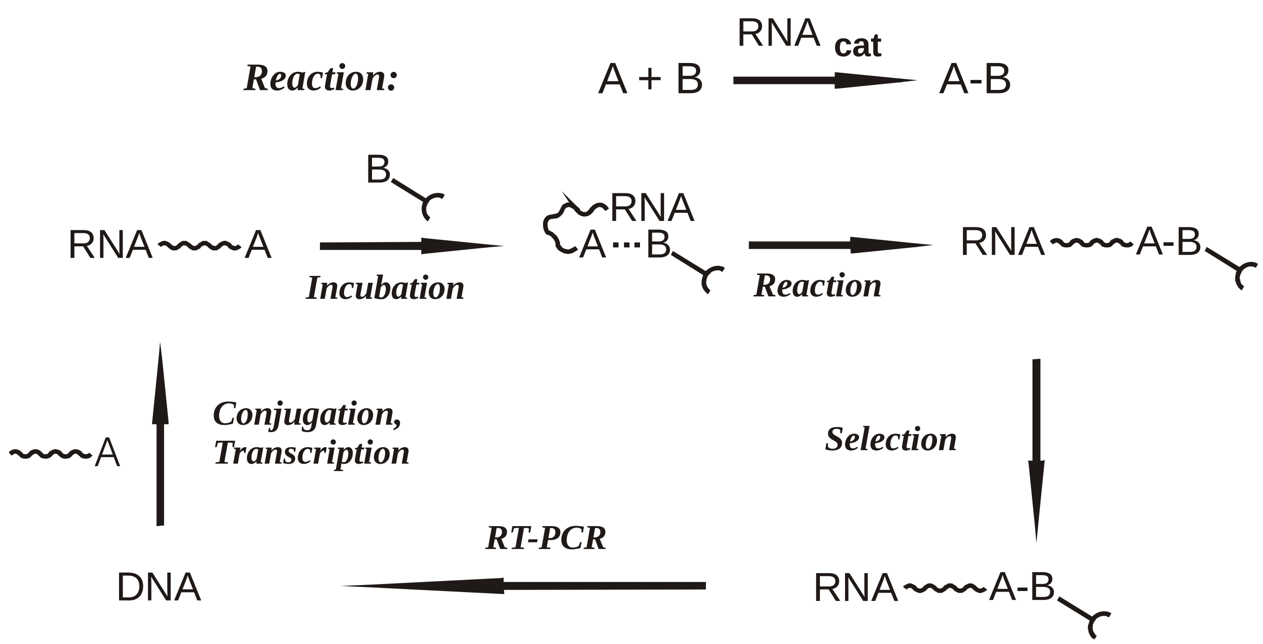
Problem:

Suitable only for modifying reactions of RNA.

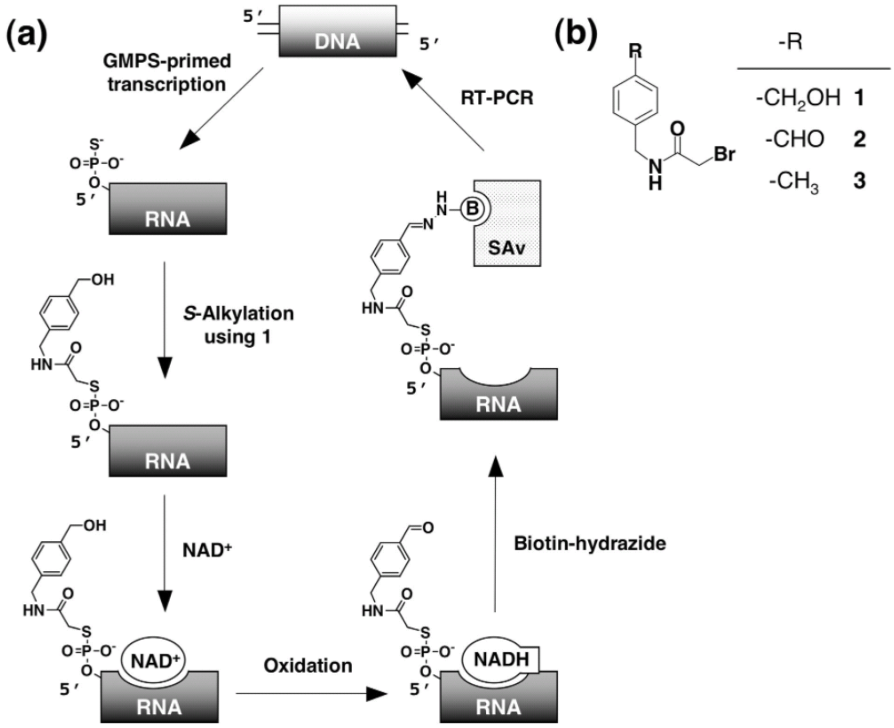
Challenge:

A + B → AB

Direct selection with tethered reactants



Example: a redox ribozyme



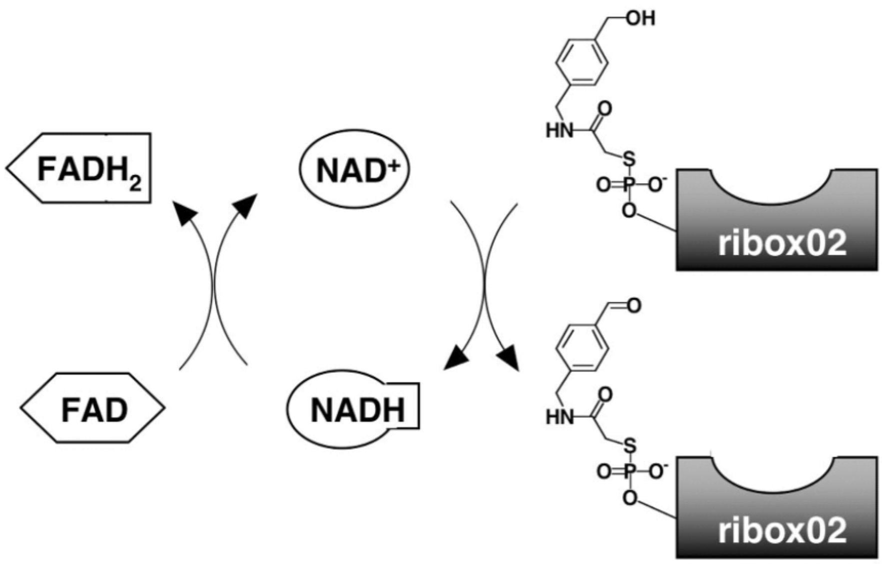
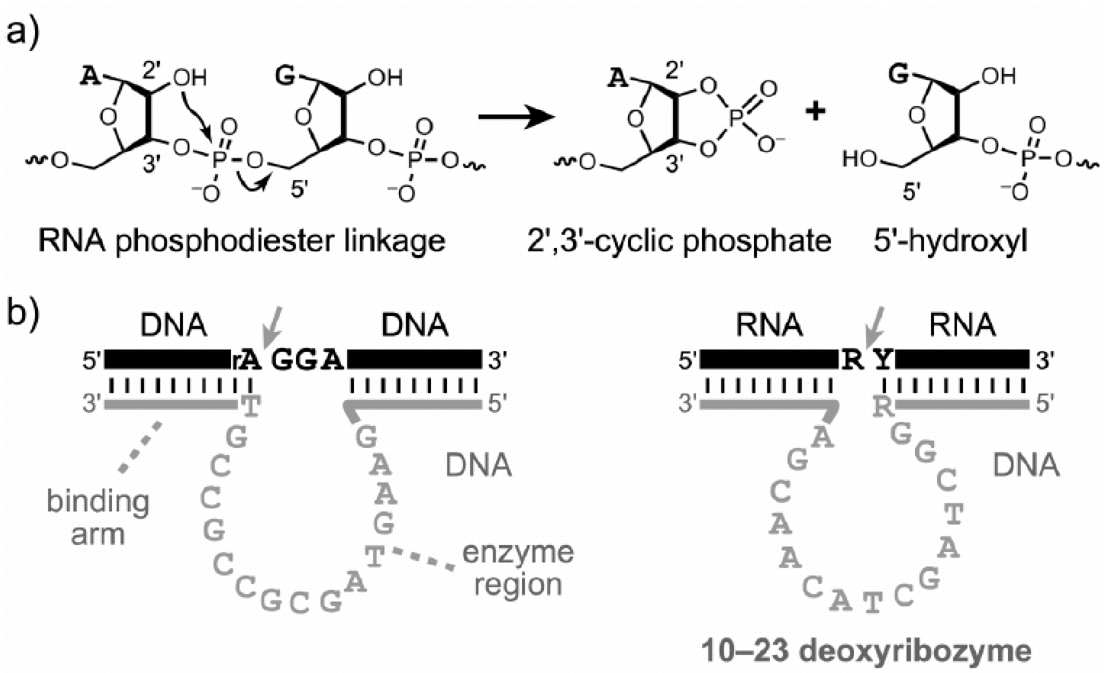
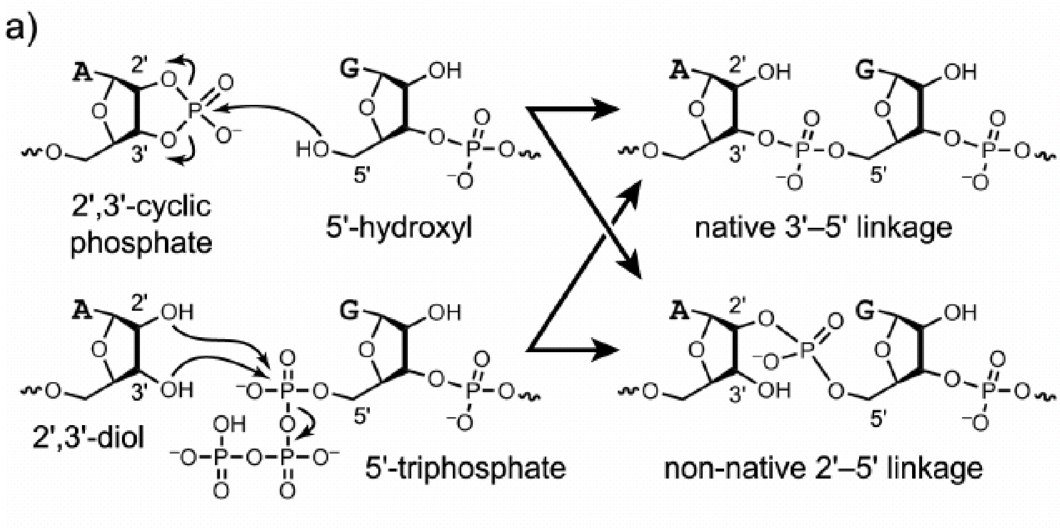


Fig. 9 A multicomponent redox system involving **1**-ribox02, NAD^+/NADH, and FAD/$FADH_2$ [31].

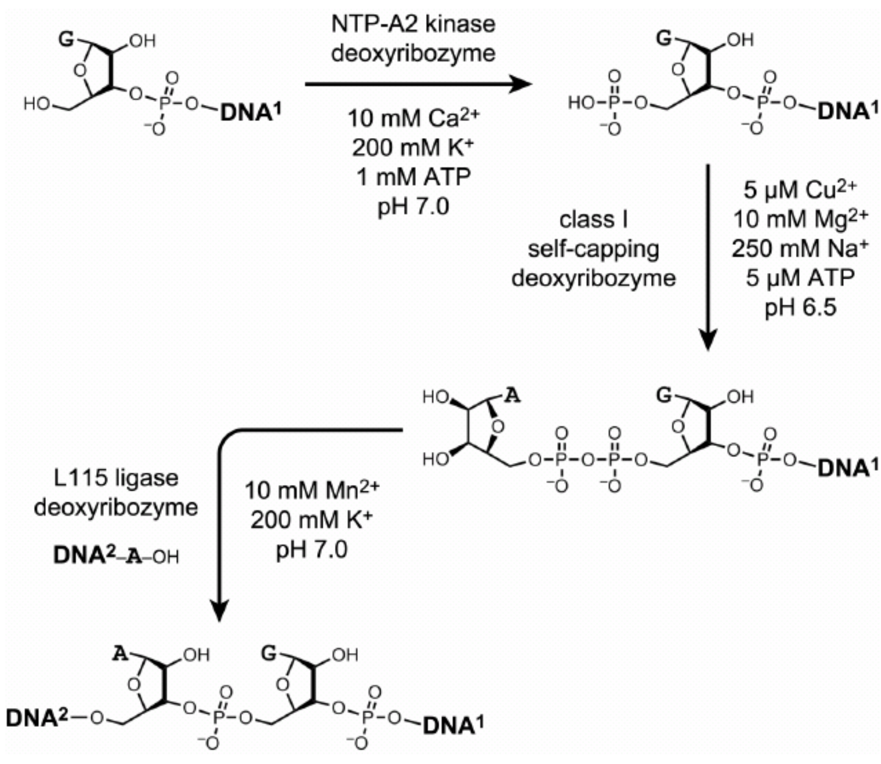
Deoxyribozymes

- Discovery
- Scope
- Limitations





S.K. Silverman



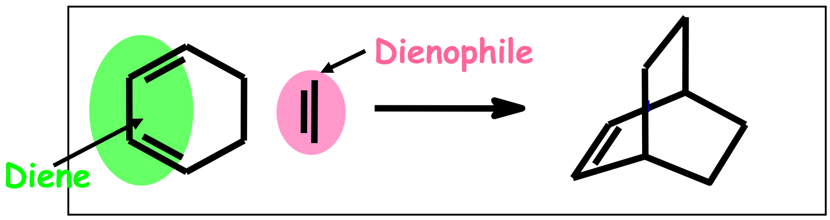
S.K. Silverman

RNA-catalyzed Diels-Alder reactions

Rationales

- Exporation of the catalytic bandwidth of RNA
- Understand structural and mechanistic principles
- Compare with protein enzymes, deduce general governing principles

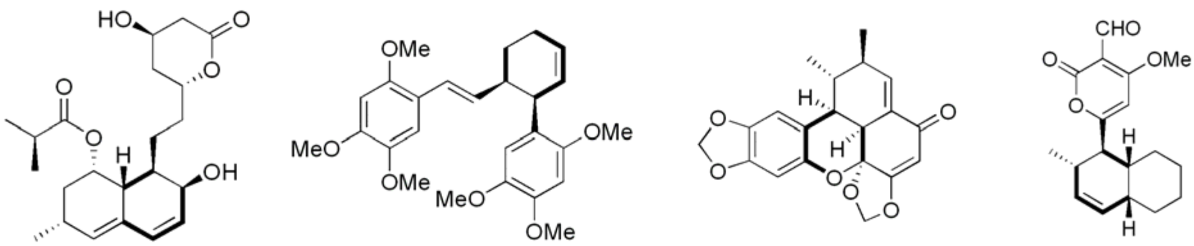
The Diels-Alder reaction



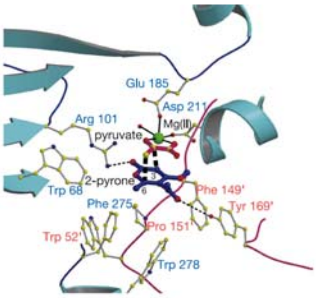
Otto Diels (1876-1952) Kurt Alder (1902-1958)

Catalysis of Diels-Alder reactions in Nature

•Secondary metabolites thought to be biosynthesized via Diels-Alder reaction:



Mevinolin (*Aspergillus terreus*) | **Alflabene** (*Alpinia flabellata*) | **Carpanone** (*Cinnamomum sp.*) | **Solanapyrones A** (*Alternaria solani*)



Ose et al. *Nature* 2003

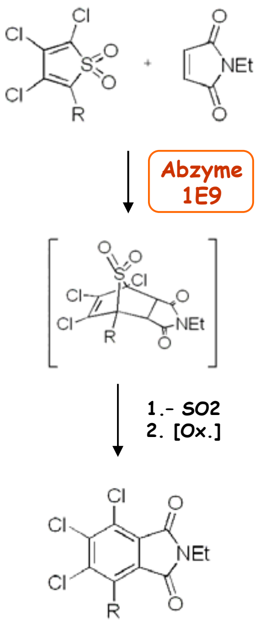
First solved structure of an enzyme which is assumed to catalyze a Diels Alder Reaktion by Ose *et al.*:

•Macrophomate Synthase (MPS)

To date, there is no solid proof for the existence of Diels-Alderases in Nature.

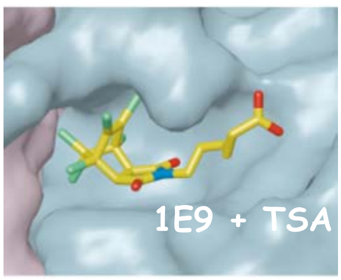
Artificial biopolymeric Diels-Alder catalysts

catalytic antibodies
(Abzymes)

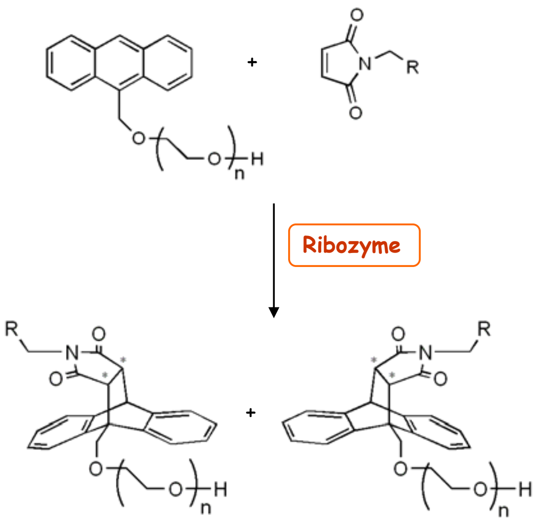


Involved Interactions:

- hydrophobic interactions
- 1 hydrogen bond



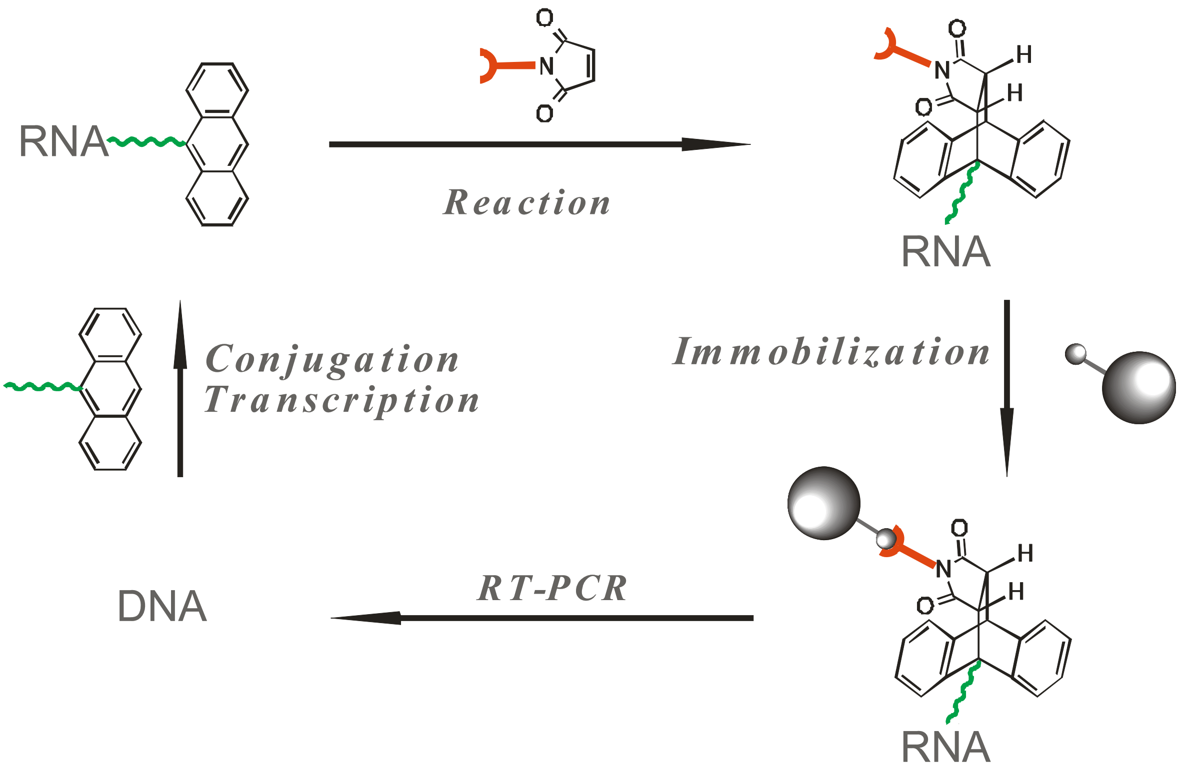
catalytic RNA
(Ribozymes)



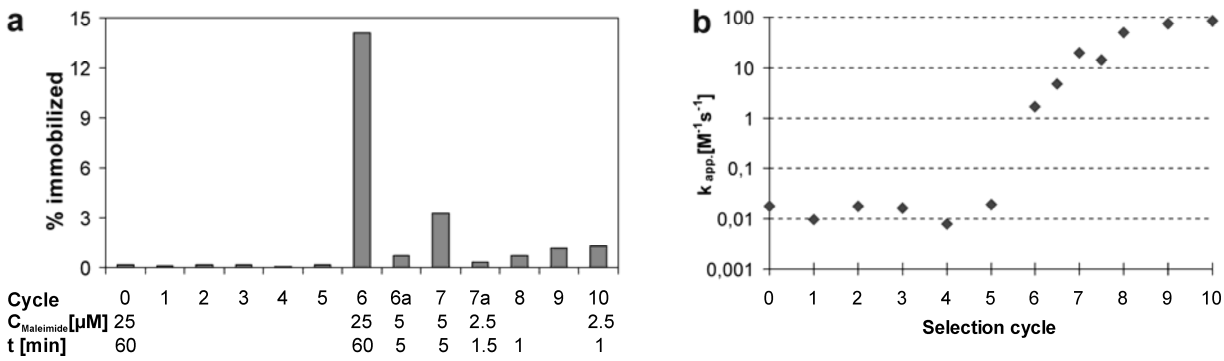
structure and mechanism ?

Seelig & Jäschke, *Chem. Biol.* **1999** (6) 167-176

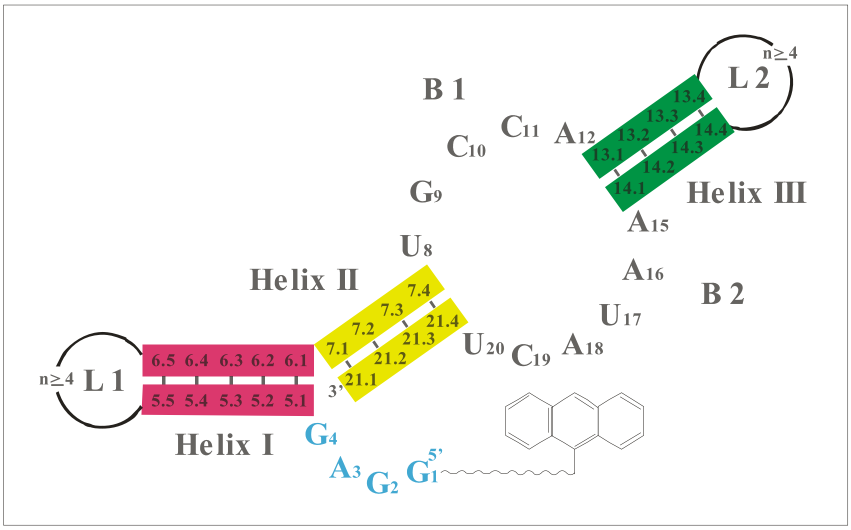
In vitro selection of Diels-Alderase ribozymes



Progress of the selection

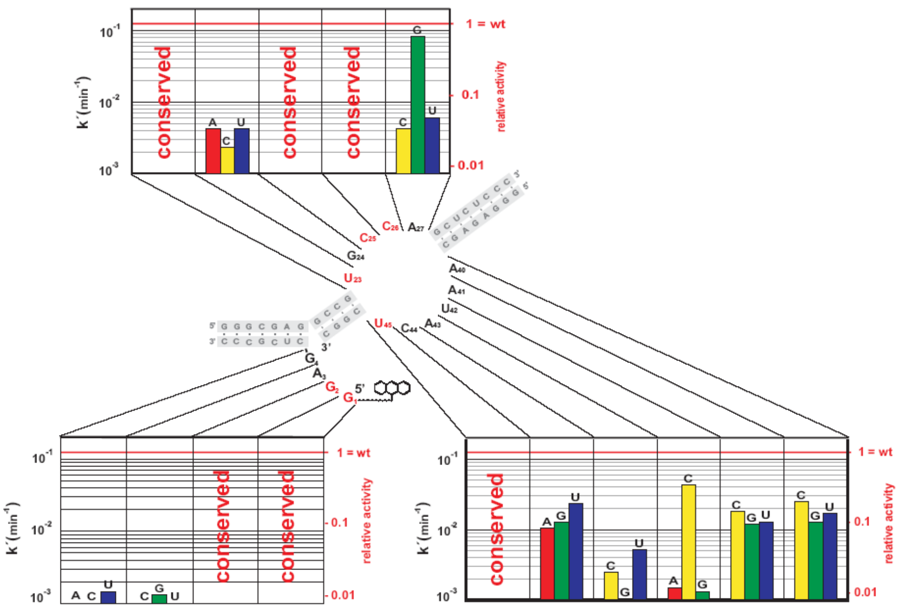


Catalytic minimal motiv



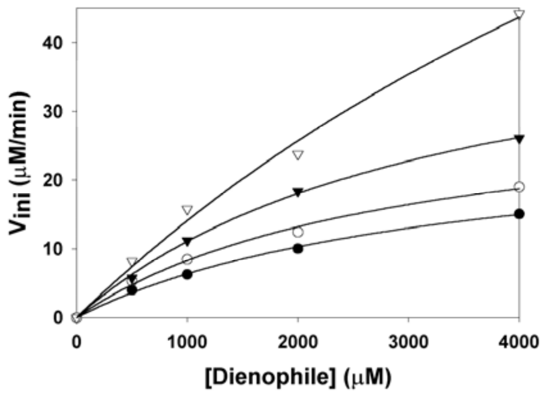
Seelig & Jäschke, *Chem. Biol.* **1999** (6) 167-176

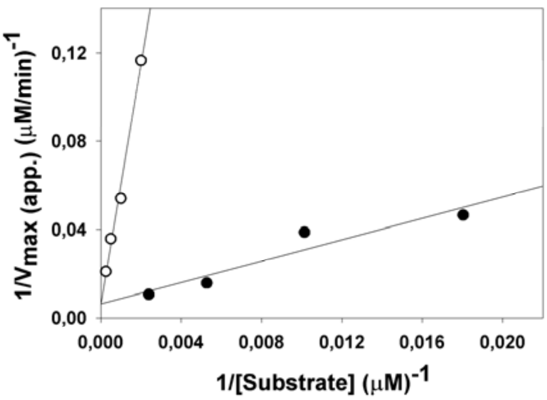
Probing the secondary and tertiary structure



Keiper, Bebenroth, Seelig, Westhof & Jäschke, *Chem. Biol.* **2004** (11) 1217-1227 .

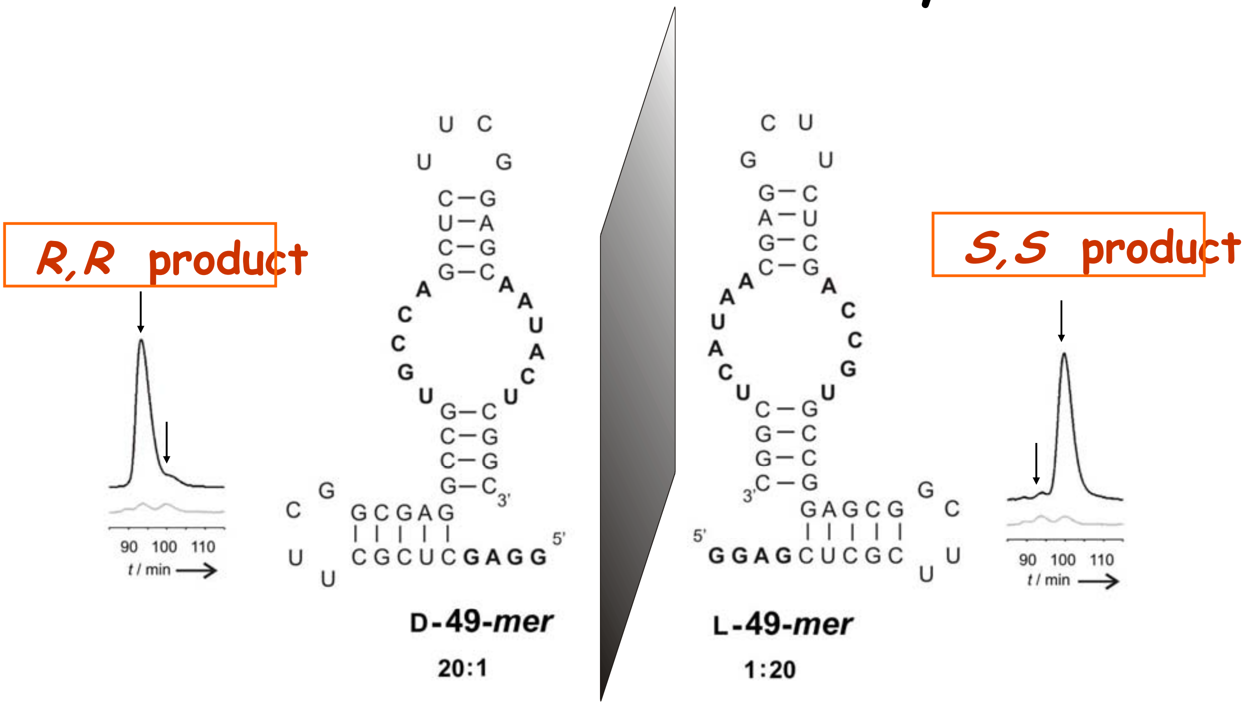
The hallmarks of enzymatic catalysis – saturation behaviour & multiple turnovers





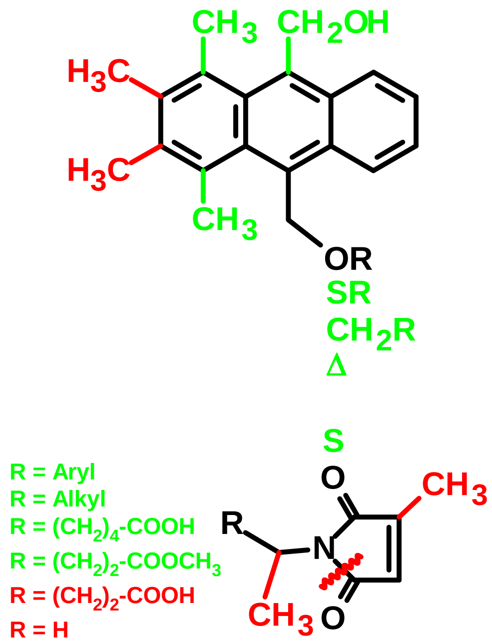
Seelig, B., Keiper, S. Stuhlmann, F. & Jäschke, A. *Angew. Chem.* **2000** (112) 4764-4768.

The hallmarks of enzymatic catalysis - Stereoselectivity



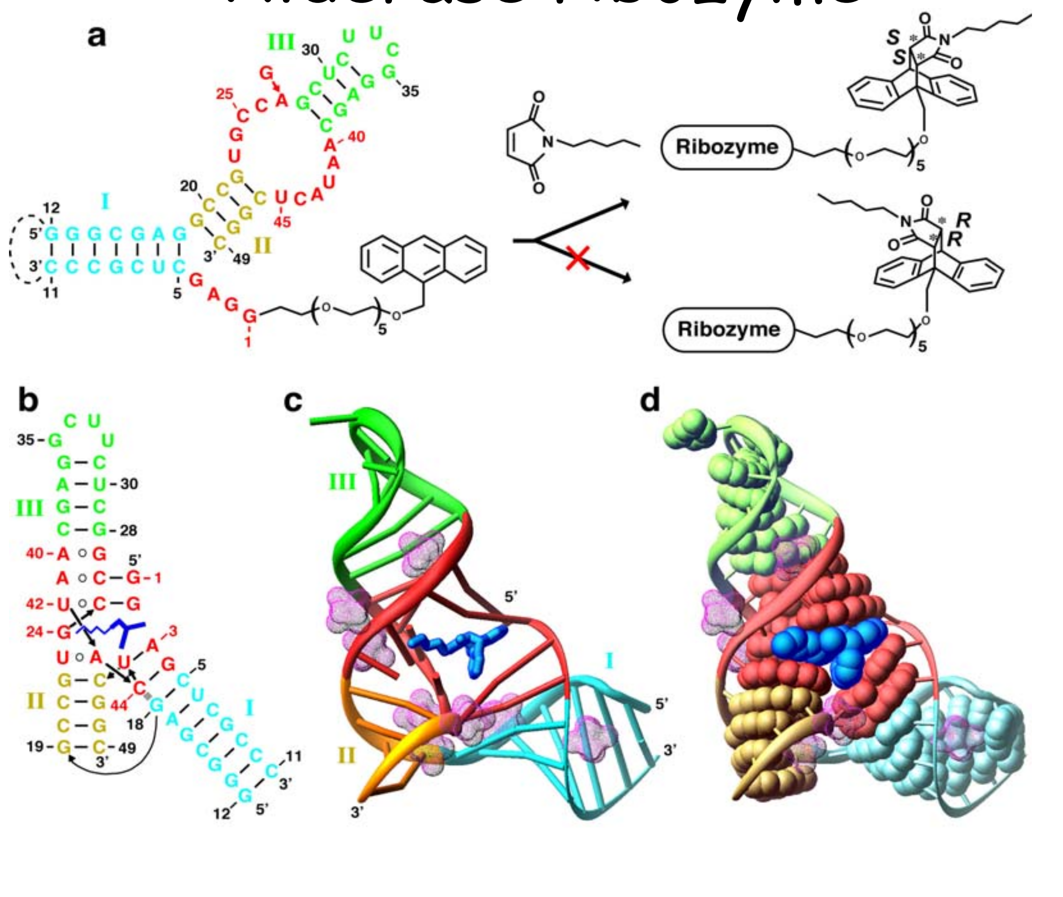
Seelig, B., Keiper, S. Stuhlmann, F. & Jäschke, A. *Angew. Chem.* **2000** (112) 4764-4768.

RNA – substrate interactions

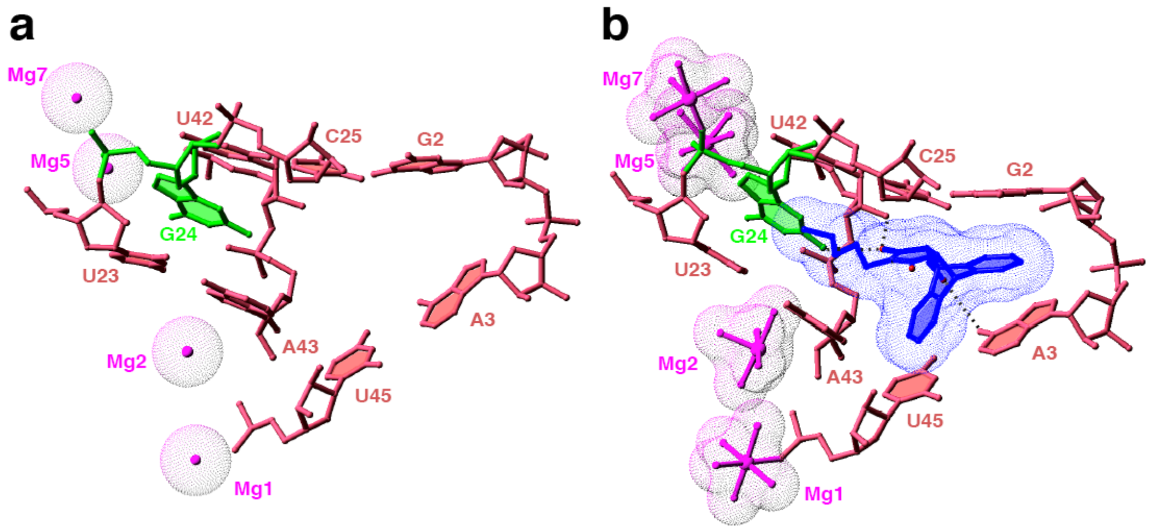


Stuhlmann & Jäschke *J. Am. Chem. Soc.* **2002** (124) 3238-3244.

The crystal structure of the Diels-Alderase ribozyme

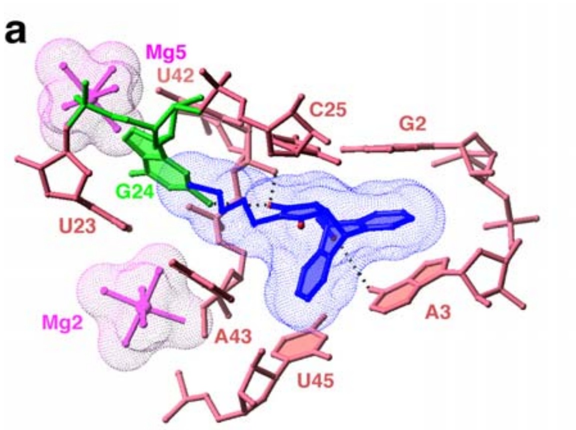


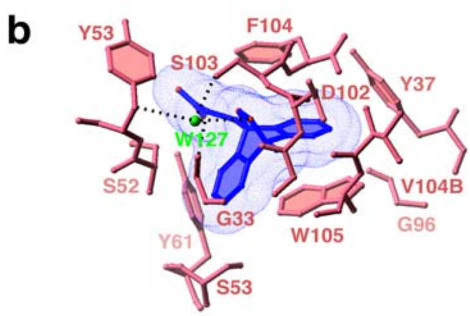
RNA – product interactions



Serganov, A., Keiper, S., Malinina, L., Tereschko, V., Skripkin, E., Höbartner, C., Polonskaia, A., Phan, A. T. , Wombacher, R., Micura, R., Dauter, Z., Jäschke, A., Patel, D. J.: *Nature Struct. Mol. Biol.* **2005** (12) 218-224.

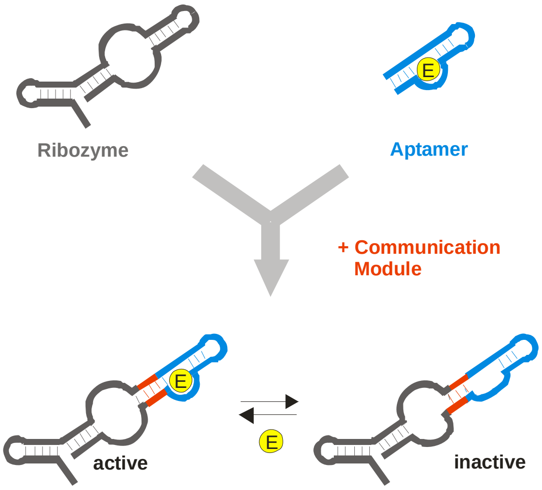
Comparison with Protein Diels-Alderases



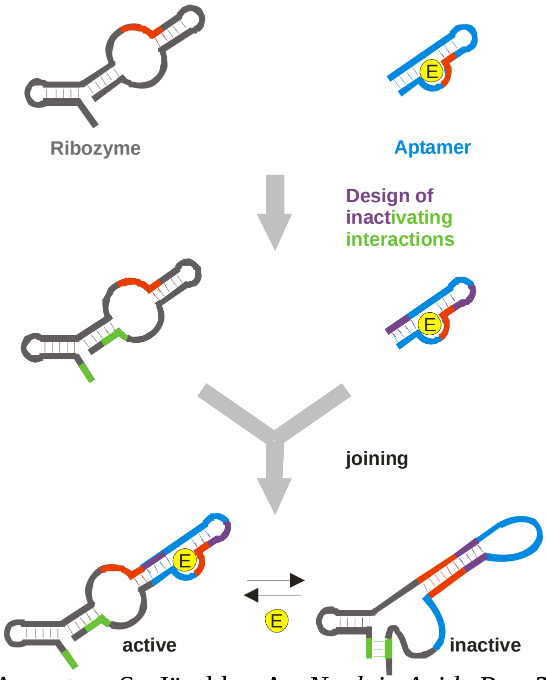


Hugot *et al. PNAS* 2002 (**99**) 9674-9678.

Can we use this structural information for reaction control?

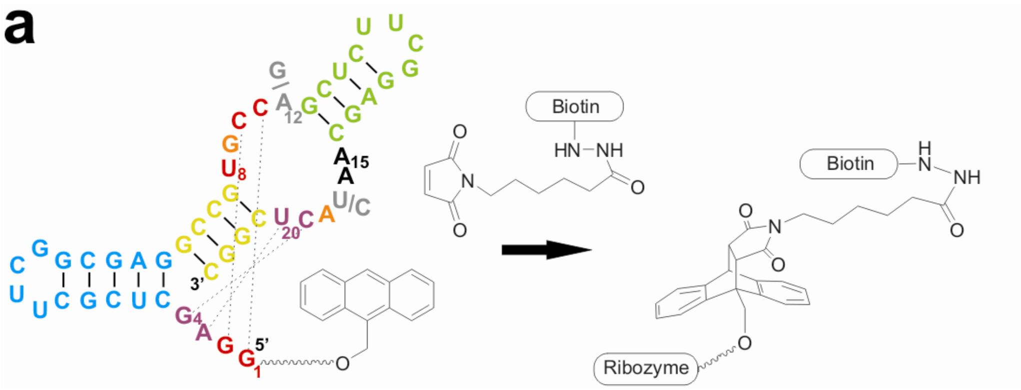


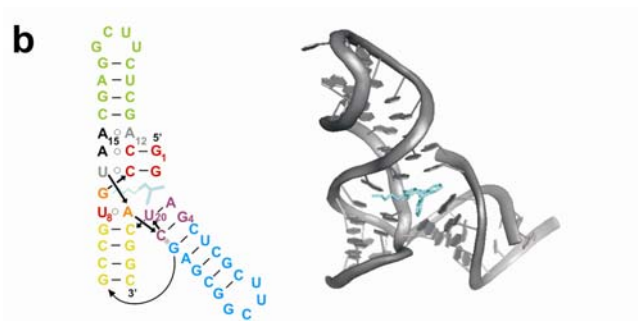
Helm, M., Petermeier, M., Ge, B., Fiammengo, R., Jäschke, A.: *J. Am. Chem. Soc.* **2005** (127) 10492-93.

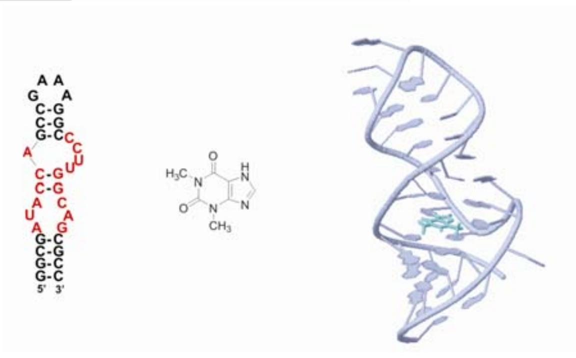


Amontov, S., Jäschke, A.: *Nucleic Acids Res.* **2006** 2006 (34) 5032-38; Petermeier, M., Jäschke, A. *Org. Biomol. Chem.* **2009** (7) 288-292.

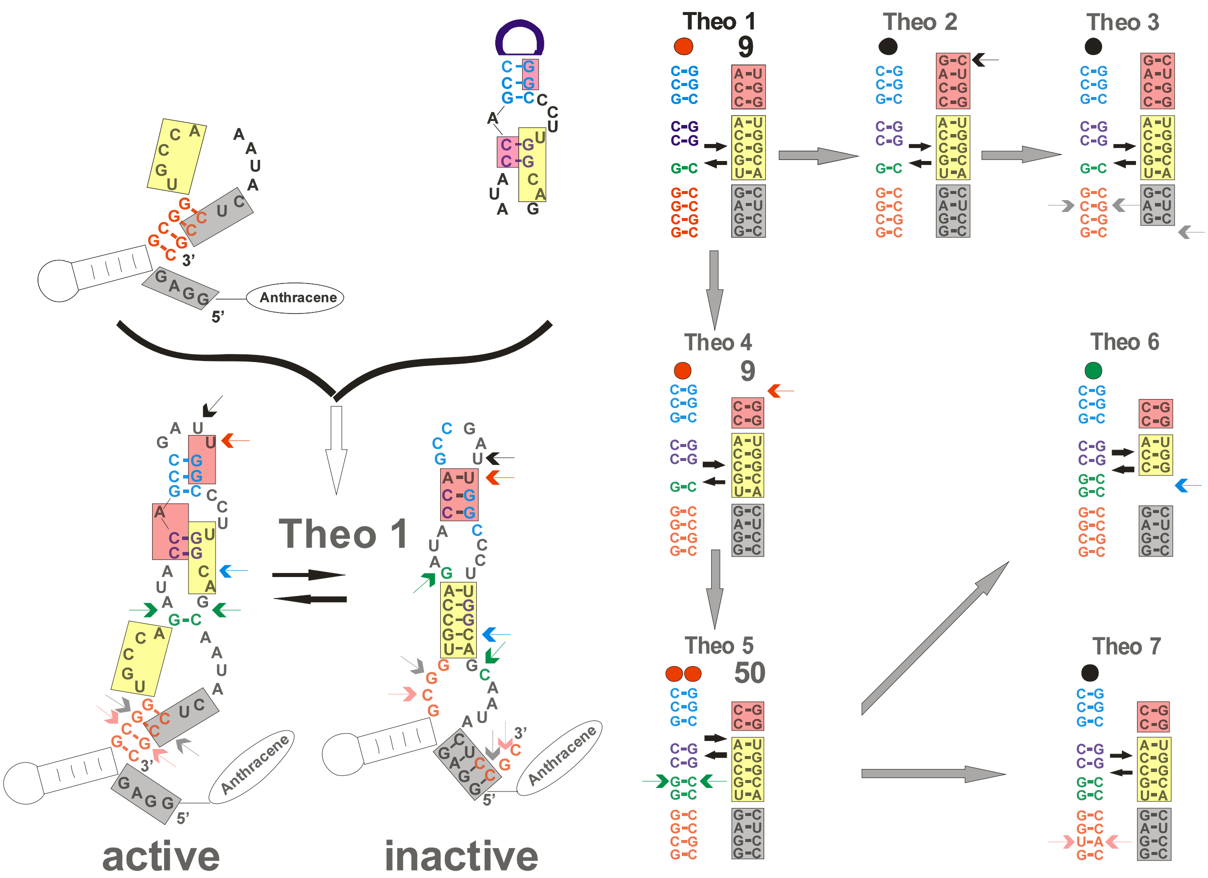
Allosteric Diels-Alderase ribozymes







Theophylline aptamer



Theophylline-dependent Diels-Alderase ribozyme