DNA-Catalyzed Sequence-Specific Hydrolysis of DNA

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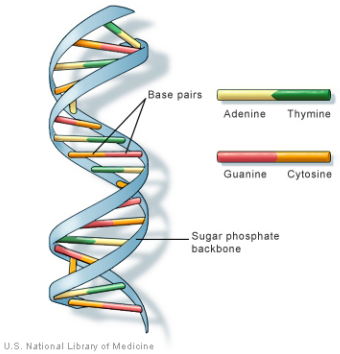
November 18, 2009

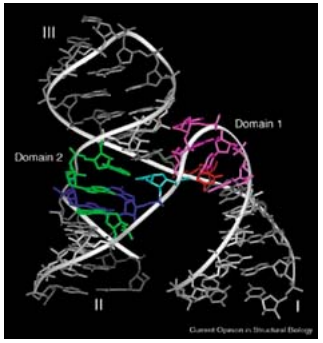
Deoxyribozymes

DNA double helix

Effective for storing genetic information



Take away one of the strands and DNA can fold into complex molecular structures



Hammerhead ribozyme

Single-stranded DNA molecules that can fold into complex molecular structures suitable for catalysis

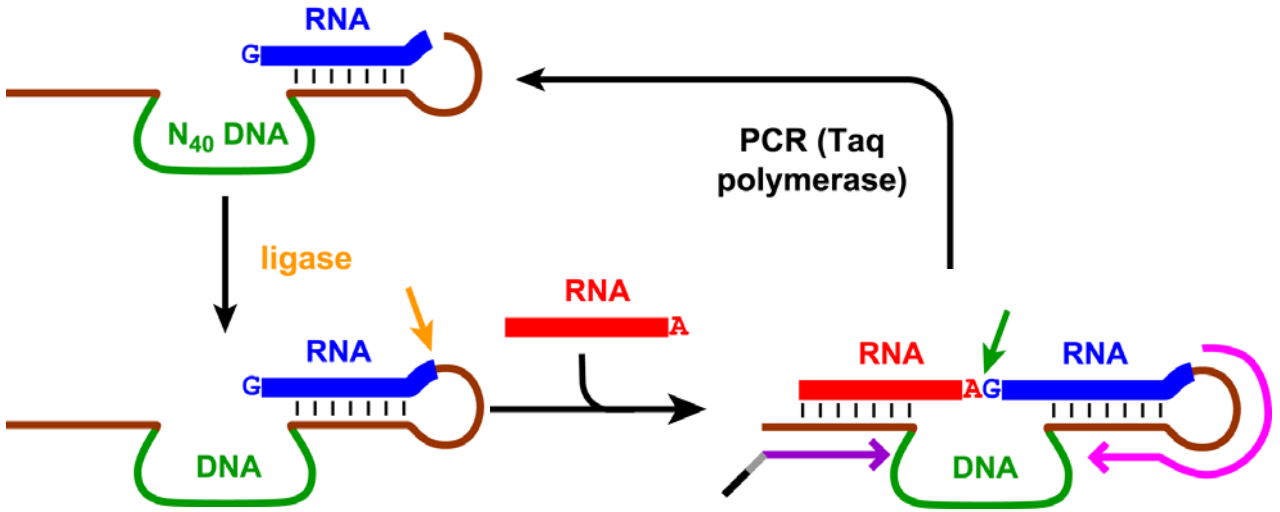
Advantages of deoxyribozymes

- **Highly stable**
- **Function under non-physiological conditions**
- **PCR enables simple selection process**
- **Can be identified to catalyze reactions between any pair of substrates**

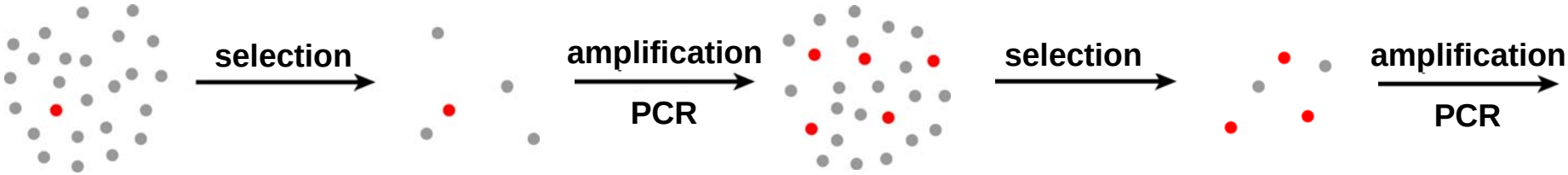
However, natural deoxyribozymes are unknown

Deoxyribozymes identified by in vitro selection

General scheme for selection of RNA- ligating deoxyribozyme



Enriching catalytically active molecules with every round of selection

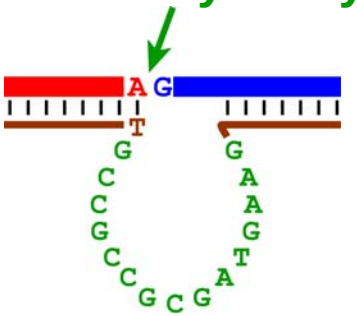


The world of DNA catalysis

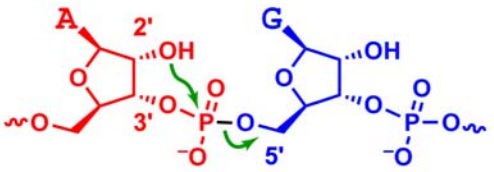
- **First deoxyribozyme identified in 1994**

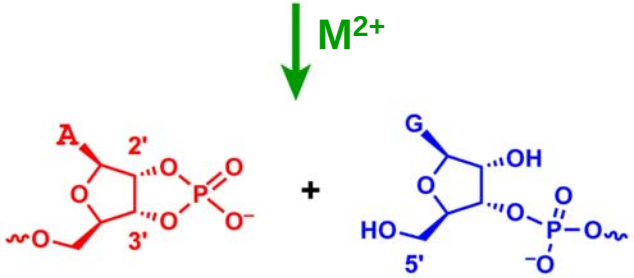
Note: First publication describing PCR: 1988

Deoxyribozymes that cleave RNA



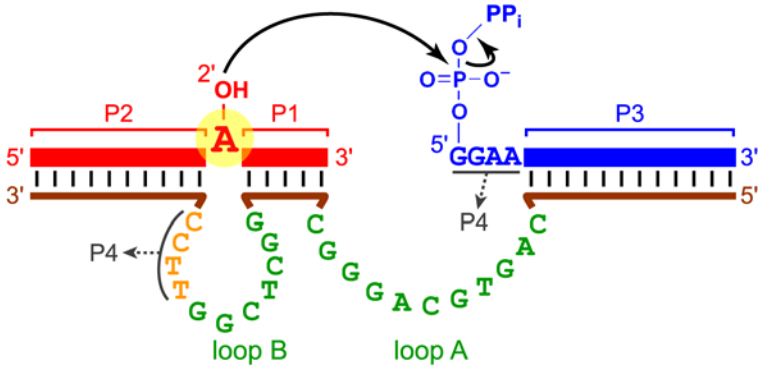
DNA enzyme





R. Breaker & G. Joyce (1994)

Deoxyribozymes that form branched RNA



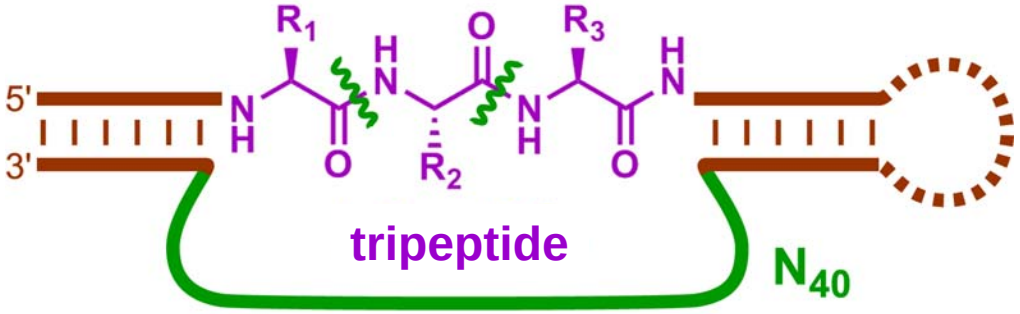
7S11 deoxyribozyme

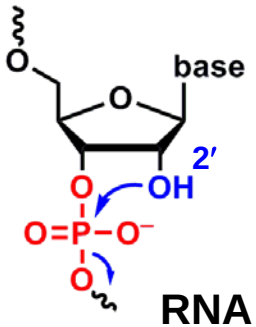
R.L. Coppins & S.K. Silverman (2004)

Deoxyribozymes catalyzing:

- DNA/RNA ligation
- DNA phosphorylation
- DNA adenylation
- Diels-Alder reaction
- Nucleopeptide linkage formation

Seeking DNA catalysts that cleave peptide bonds

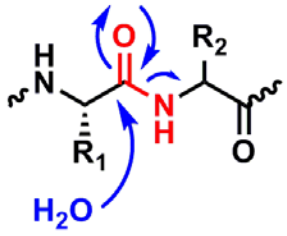




RNA

$t_{1/2}$ ~10 years

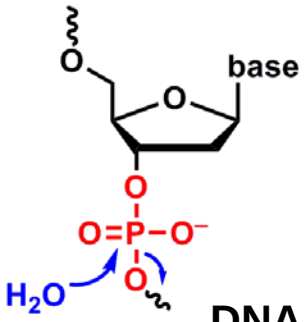
less stable than



Protein

$t_{1/2}$ ~200 years

less stable than



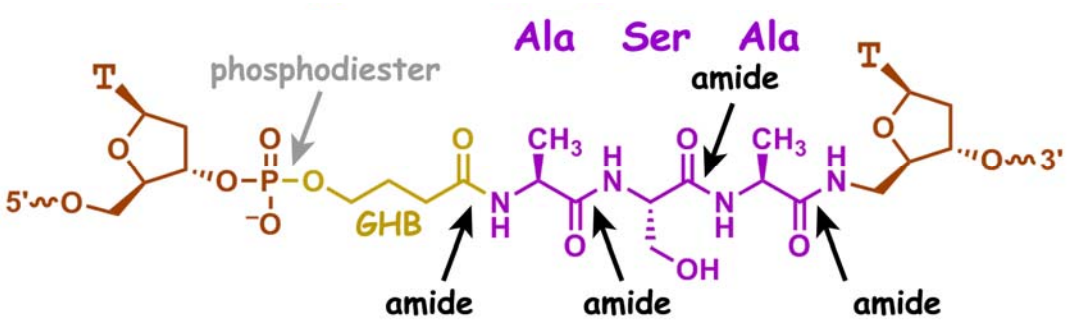
DNA

$t_{1/2}$ ~30 million years

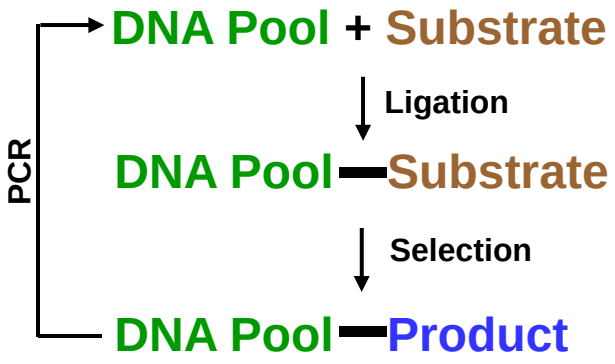
Why search for deoxyribozymes that cleave peptide bonds?

- **Fundamental interest: Amide bond is at least 20-fold more stable than RNA phosphodiester bond**
- **Practical application: Need for artificial proteases**

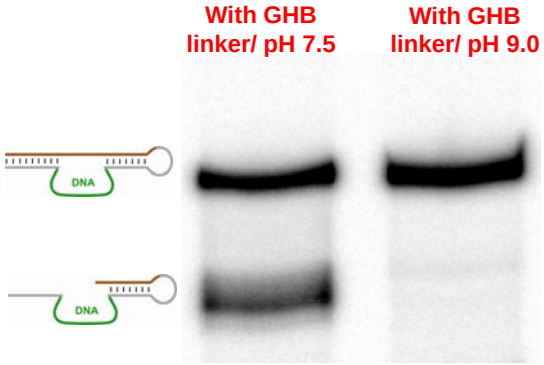
Substrate for selection



Selection performed

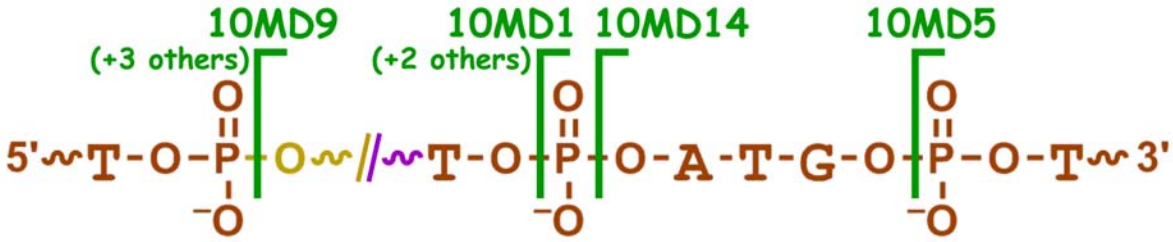


Round 8 Selection gel:



- 36 clones
- 7 distinct sequences

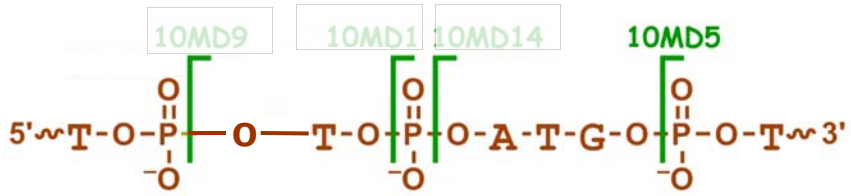
DNA hydrolysis!

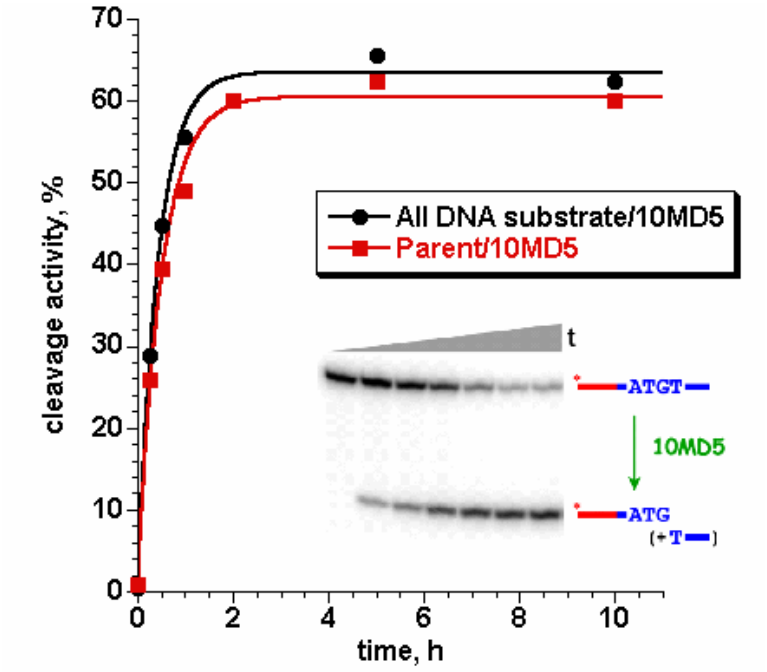


- DNA phosphodiester bond is at least 10^5-fold more stable than amide bond
- Rate enhancements of the order of 10^{12}

M. Chandra, A. Sachdeva, and S. K. Silverman, *Nat. Chem. Biol.* **2009**, *5*, 718-720

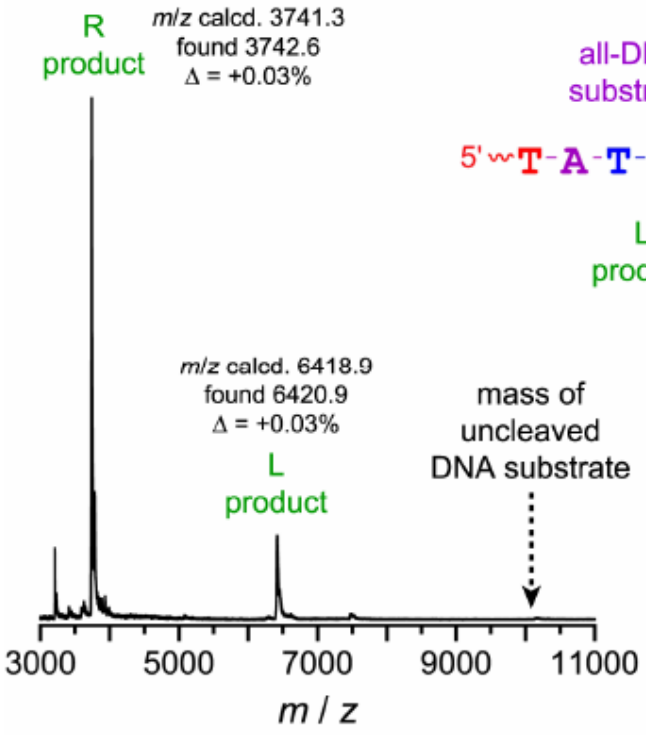
Peptide portion of substrate dispensable for activity of 10MD5





k_{obs} 2.7 h^{-1} ($t_{1/2}$ 15 min)

Identity of the products formed from 10MD5 cleavage reaction confirmed by mass spectrometry



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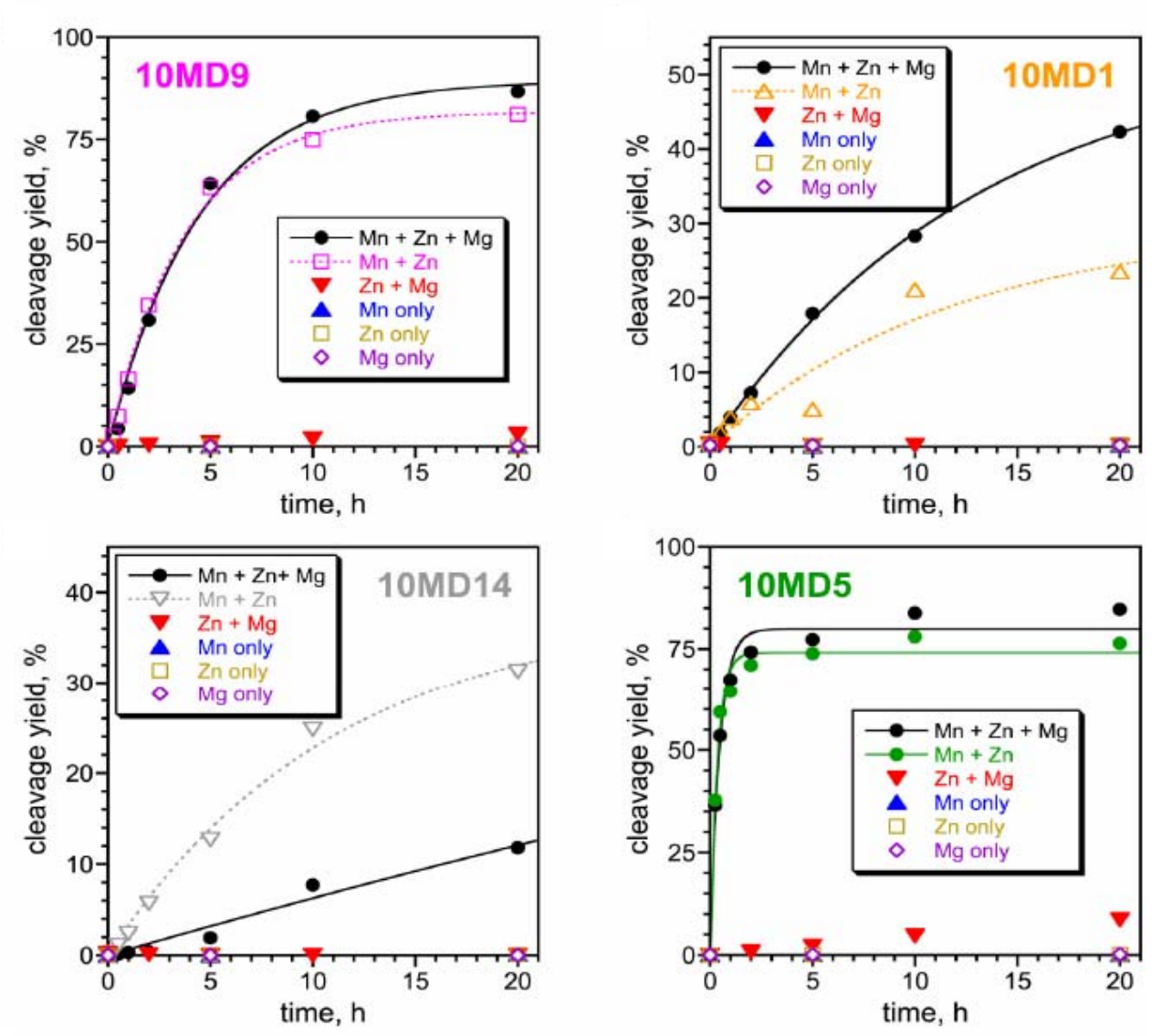
Characterization of 10MD deoxyribozymes

What metal ion cofactors are required for optimal activity?

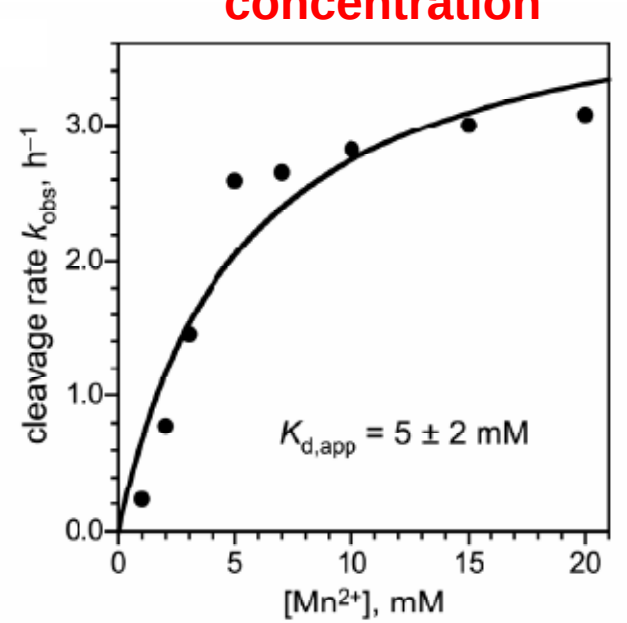


- **Most of these enzymes were heterobimetallic**

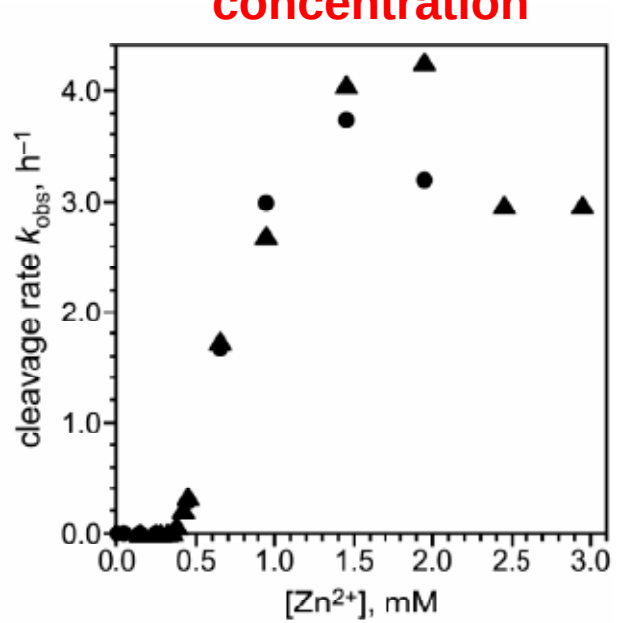
M. Chandra, A. Sachdeva, and S. K. Silverman, *Nat. Chem. Biol.* **2009**, *5*, 718-720

Variation of catalytic activity of 10MD5 with Mn^{2+} and Zn^{2+} concentration

Variation with of k_{obs} Mn^{2+} concentration



Variation with of k_{obs} Zn^{2+} concentration



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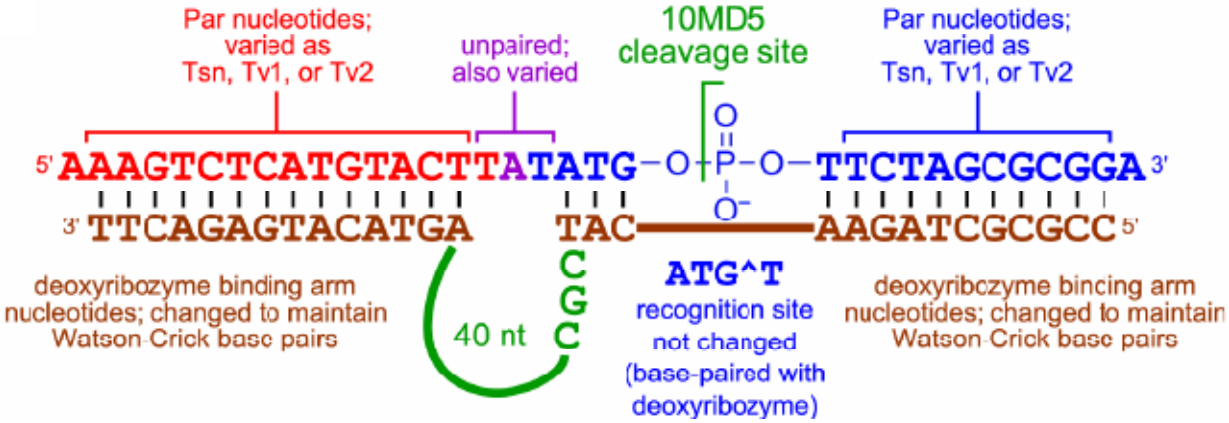
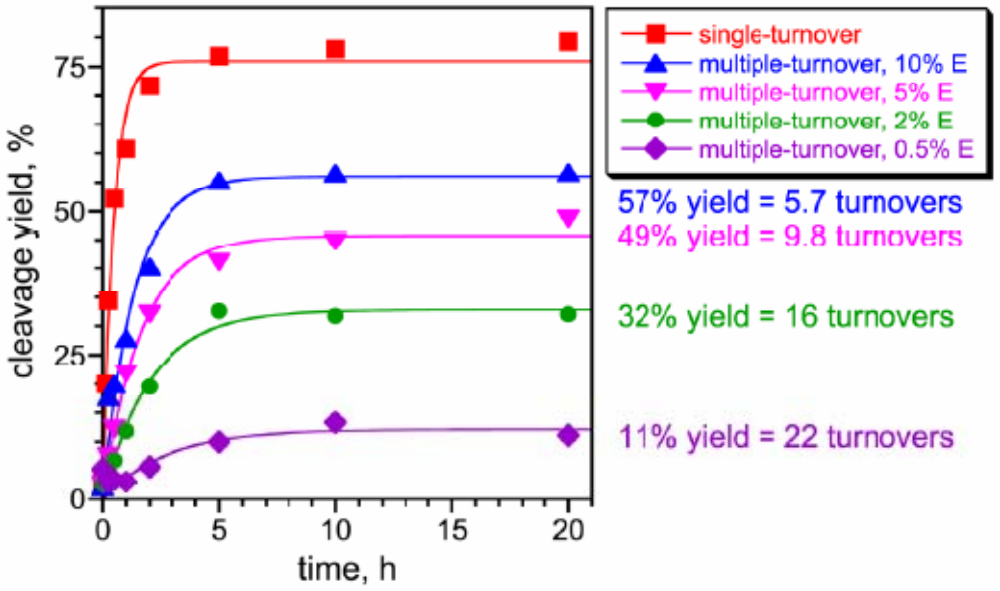
Identification of recognition site

Watson-Crick co-variation experiments



Capable of multiple turnover

- **DNA catalysts usually suffer from product inhibition**
- **Binding arms of 10MD5 were optimized for least product inhibition**



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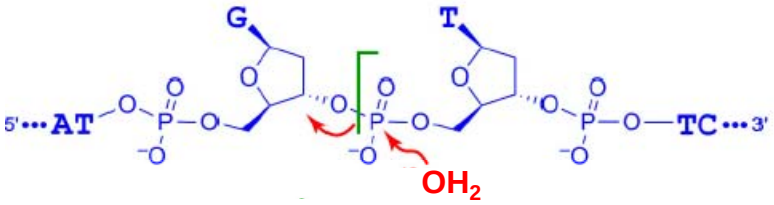
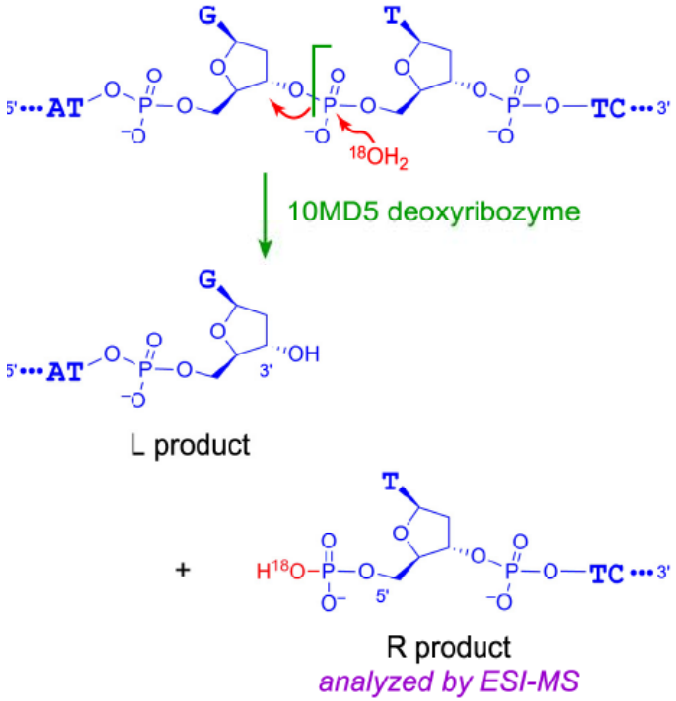
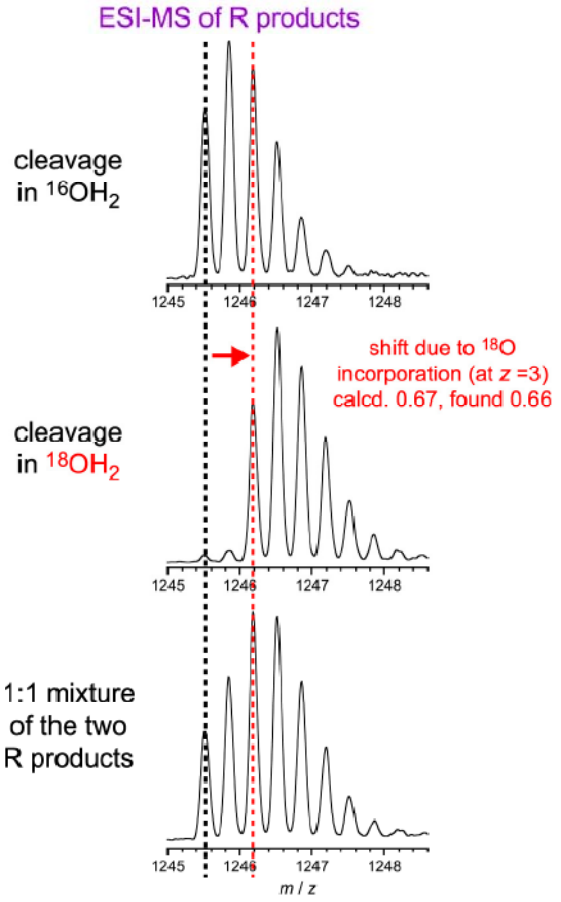
Hydrolysis Vs Oxidative Cleavage

- H_2O as a nucleophile



Monitoring the fate of oxygen atom in water





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Conclusions

- Numerous deoxyribozymes were identified that catalyze sequence-specific hydrolysis of single-stranded DNA
- Rate enhancements of the order of 10^{12} relative to uncatalyzed reaction
- These deoxyribozymes require two metal ion cofactors, Mn^{2+} and Zn^{2+} for their catalytic activity

Future Efforts

- Double stranded DNA cleavage

Could DNA catalyst be artificial restriction enzyme?

(The work horse of molecular biology)

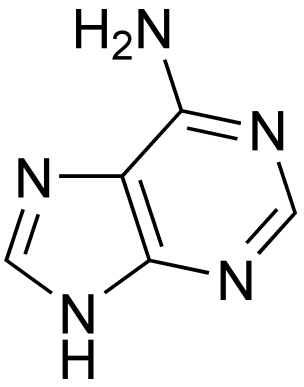
Acknowledgements

- **Prof. Scott K. Silverman**
- **Silverman Lab**
- **Organizers of CBD S&T Conference**

Funding

National Institutes of Health
National Science Foundation

Defense Threat Reduction Agency
David and Lucile Packard Foundation



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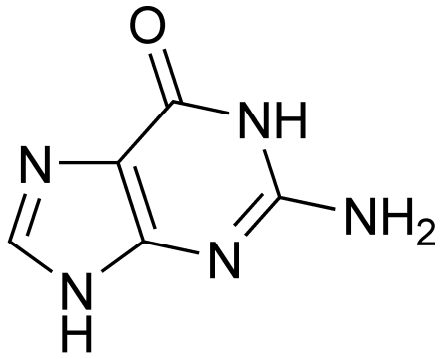

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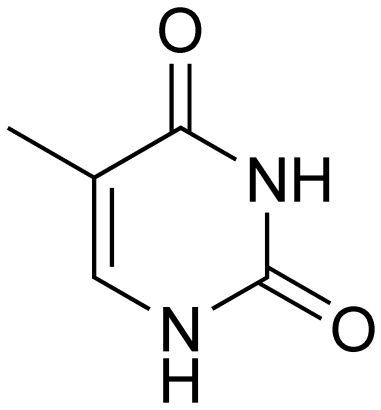

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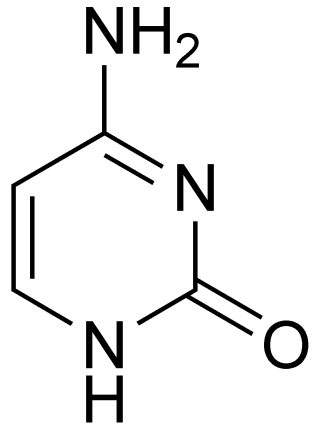
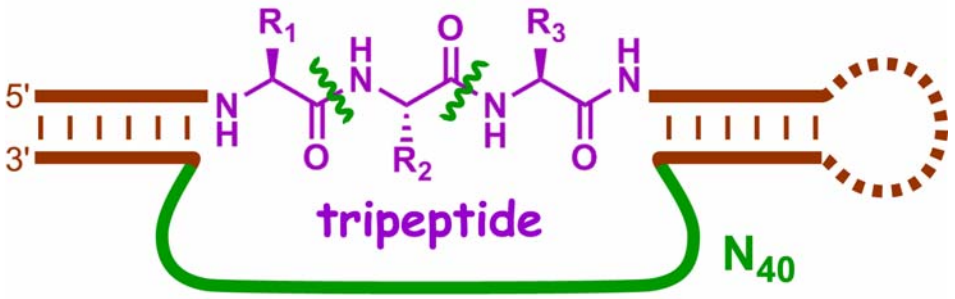
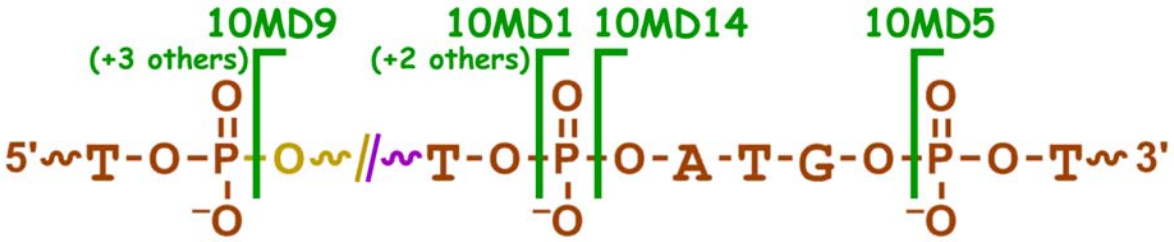


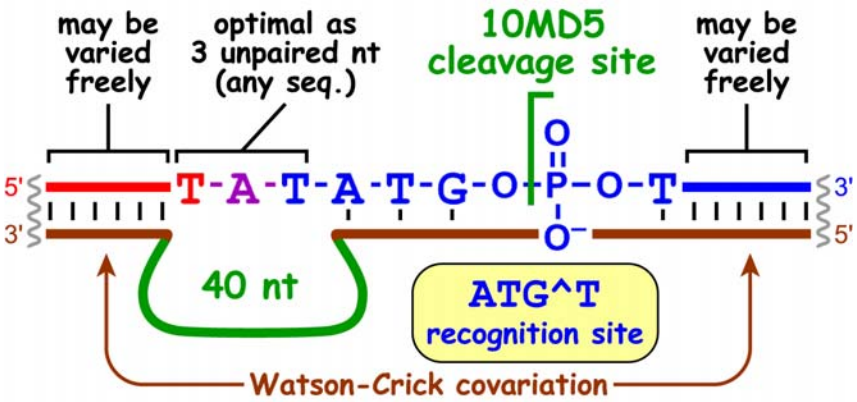
C

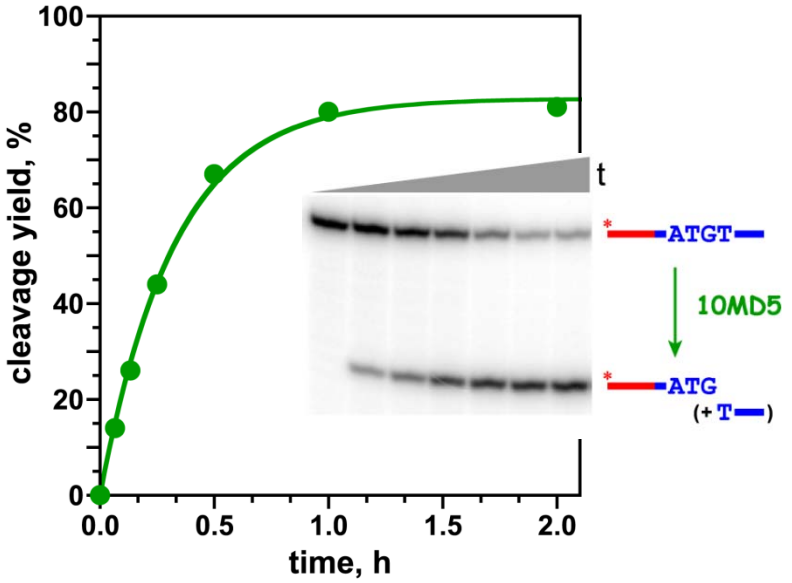
Seeking deoxyribozymes with proteolytic activity





DNA hydrolysis!





Chandra, M.; Sachdeva, A.; Silverman, S.K. *Nat. Chem. Biol.* **2009**, *5*, AOP (Aug. 16).

deoxyribozyme	k_{obs} , h^{-1} (substrate **D**)	k_{obs} , h^{-1} (substrate **P**)
10MD9	$<3 \times 10^{-4}$	0.26
10MD1	$<3 \times 10^{-4}$	0.14
10MD14	3.6×10^{-3}	0.14
10MD5	2.3	2.0