

# DNA-based biosensors for metal detection

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GWF meeting (Hamilton)

# Problem to solve: heavy metal contamination

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## Three Michigan officials criminally charged in Flint lead poisoning crisis

Charges against the three include tampering with evidence for allegedly changing lead water-test results.

BY MIKE HOUSEHOLDER AND ED WHITE THE ASSOCIATED PRESS

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A lead main service line is dug up and measured as contractors replace residential water line in Flint, Mich. Rachel Woolf/The Flint Journal-MLive.com via AP



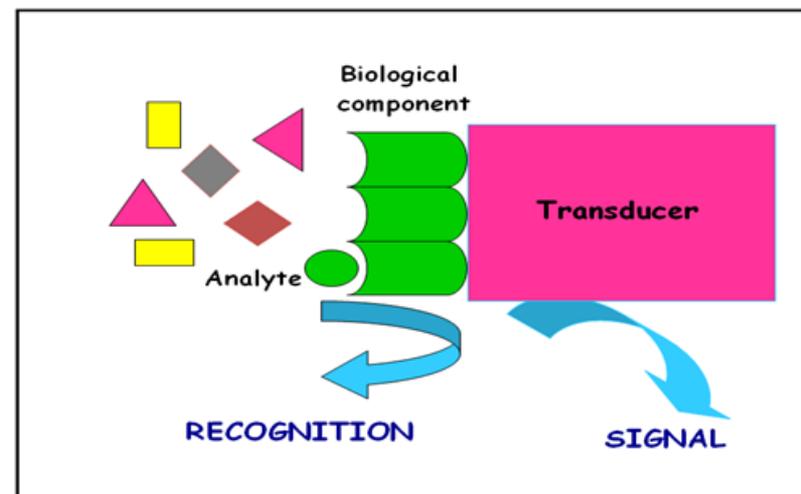
**LEAD IN THE WATER**

# Importance and challenges of metal detection

- **Environmental monitoring**
- **Food analysis**
- **Biomedical diagnosis**
  
- **Instrumentation analysis**
  1. **Highly accurate, reliable**
  2. **Multiplexed detection**
  3. **Industrial standard**
  4. **Expensive**
  5. **Long turnaround time**
  6. **Detects total metal**
  
- **(Bio)sensors**
  1. **Cost effective**
  2. **On-site, real-time detection**
  3. **Detects bioavailable metal**



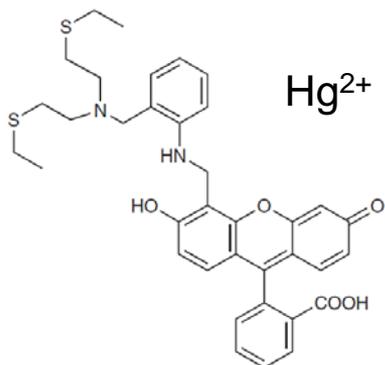
<http://userpage.chemie.fu-berlin.de>



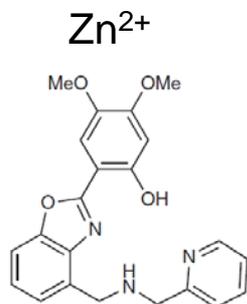
<http://www.nanoscience.imdea.org>

# Various metal ligands

## Small molecule fluorescent chelators



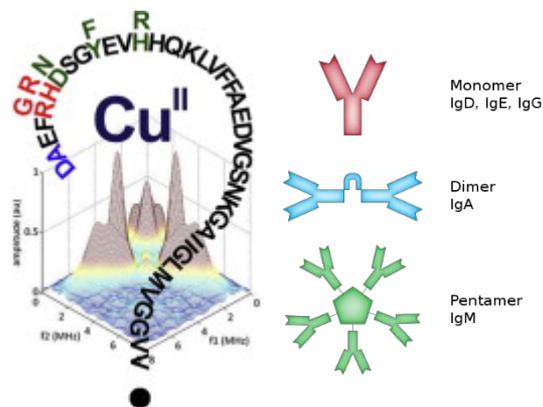
$\text{Hg}^{2+}$



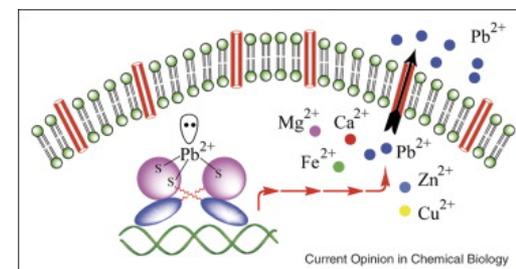
$\text{Zn}^{2+}$

*Nat. Chem. Biol.* **2008**, 4, 168-175

## Peptides, proteins, & antibodies

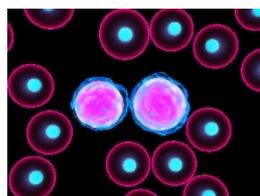
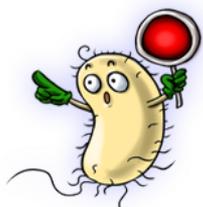


*Coord Chem Rev*, 256, 2012, 2175

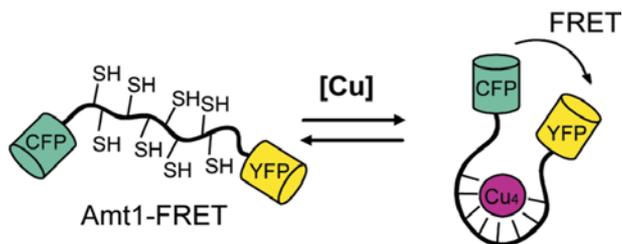


*Curr Opin Chem Biol*, 12, 2008, 214

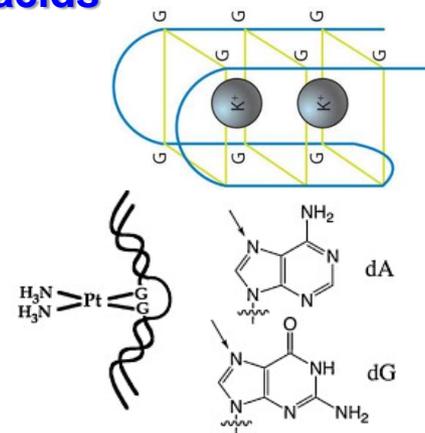
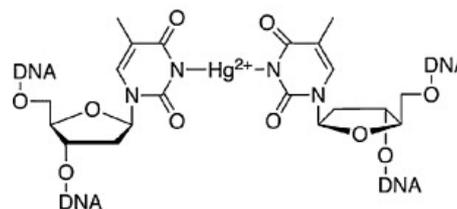
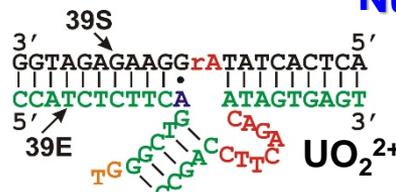
## Genetically engineered cells



<http://2010.igem.org/Team:Peking/Project/Biosensor>

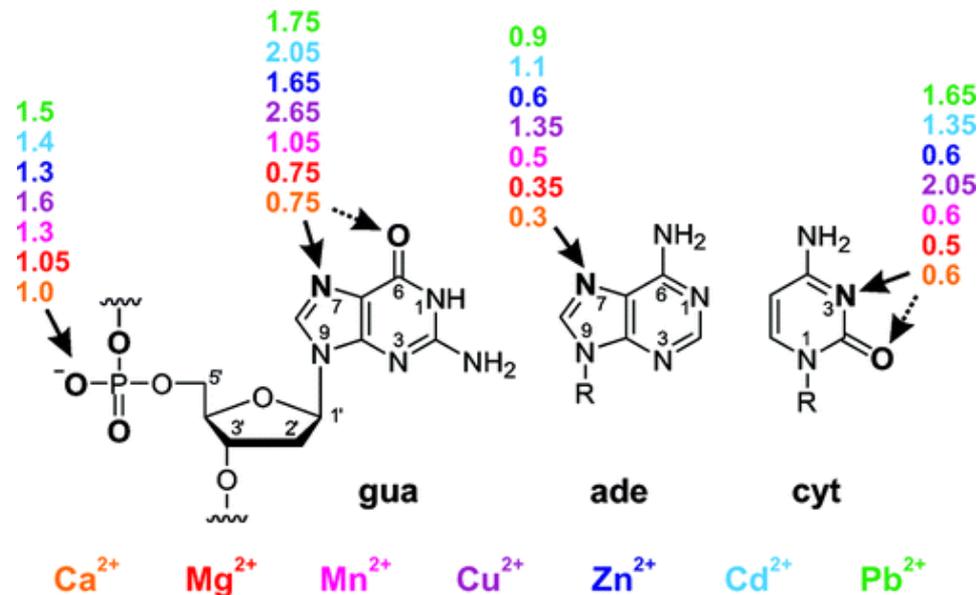
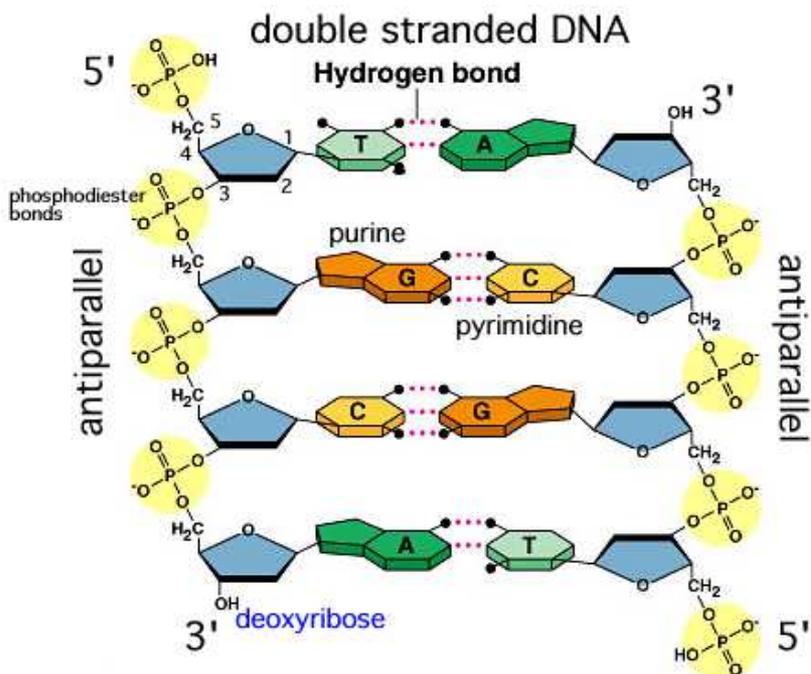


## Nucleic acids



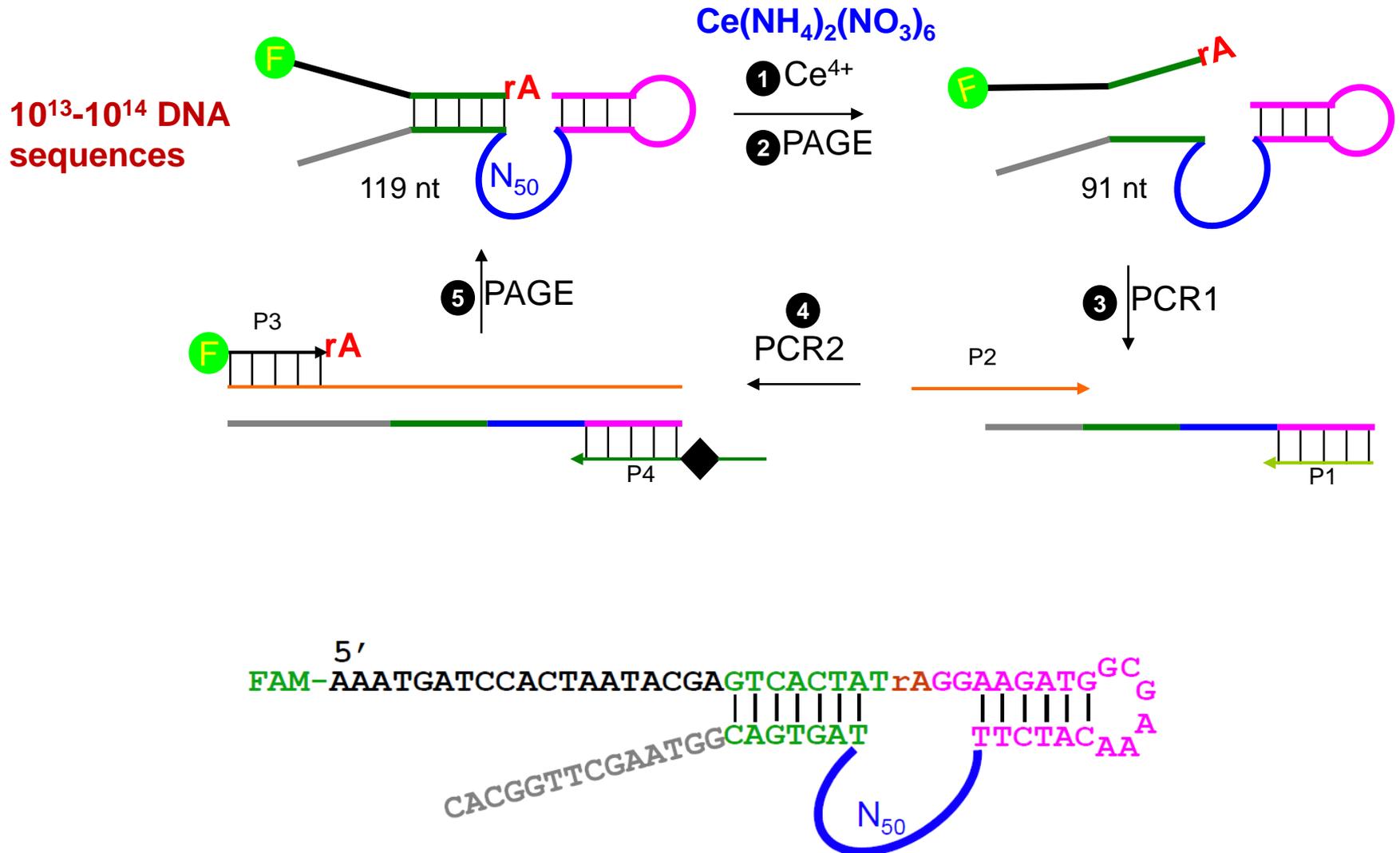
Highly stable, cost effective, easy to modify,  
combinatorial selection + rational design

# DNA for metal binding

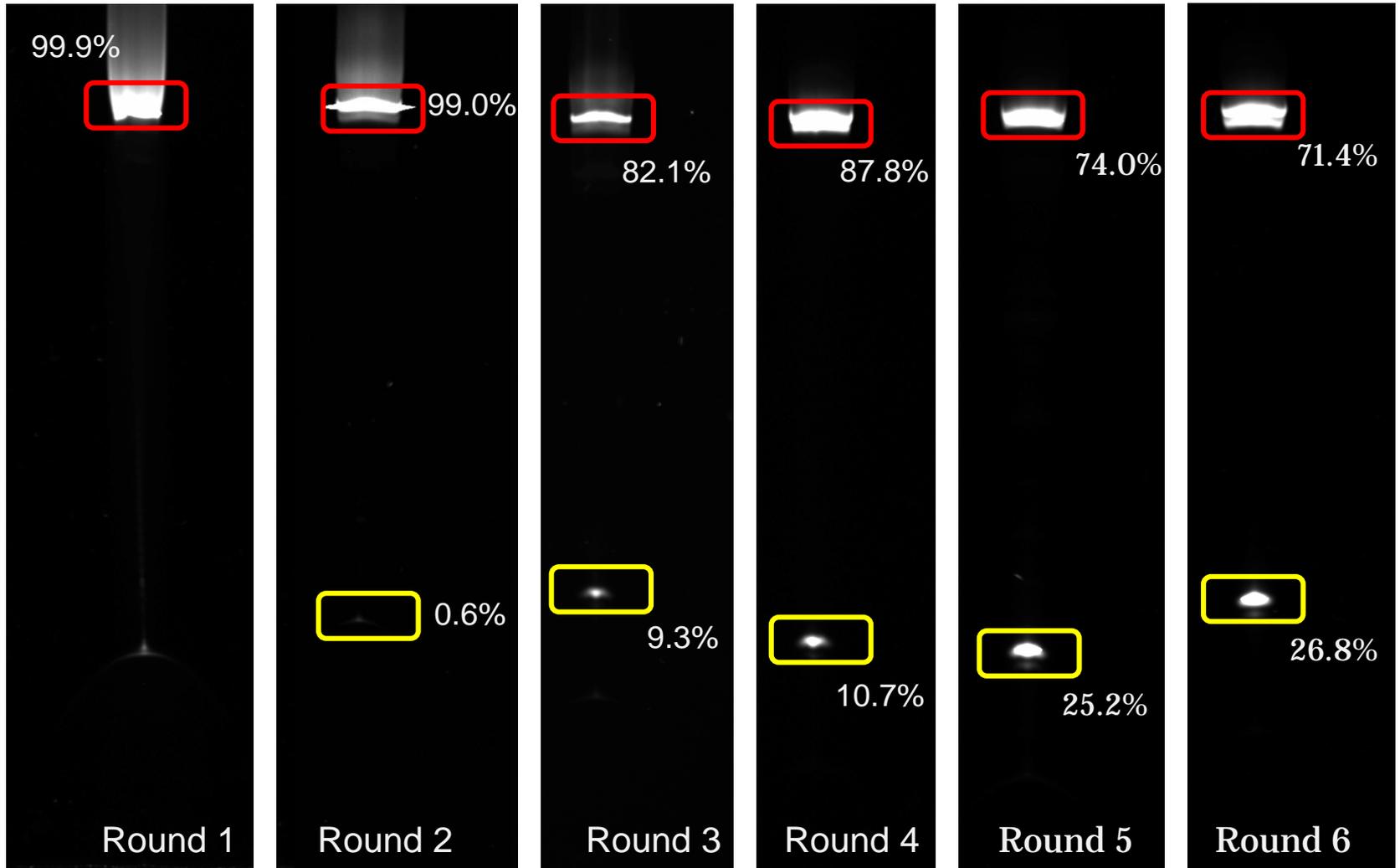


- Phosphate: electrostatic interaction, binds hard Lewis acids
- Bases: metal coordination

# In vitro selection of a cerium-dependent DNAzyme



# Selection progress



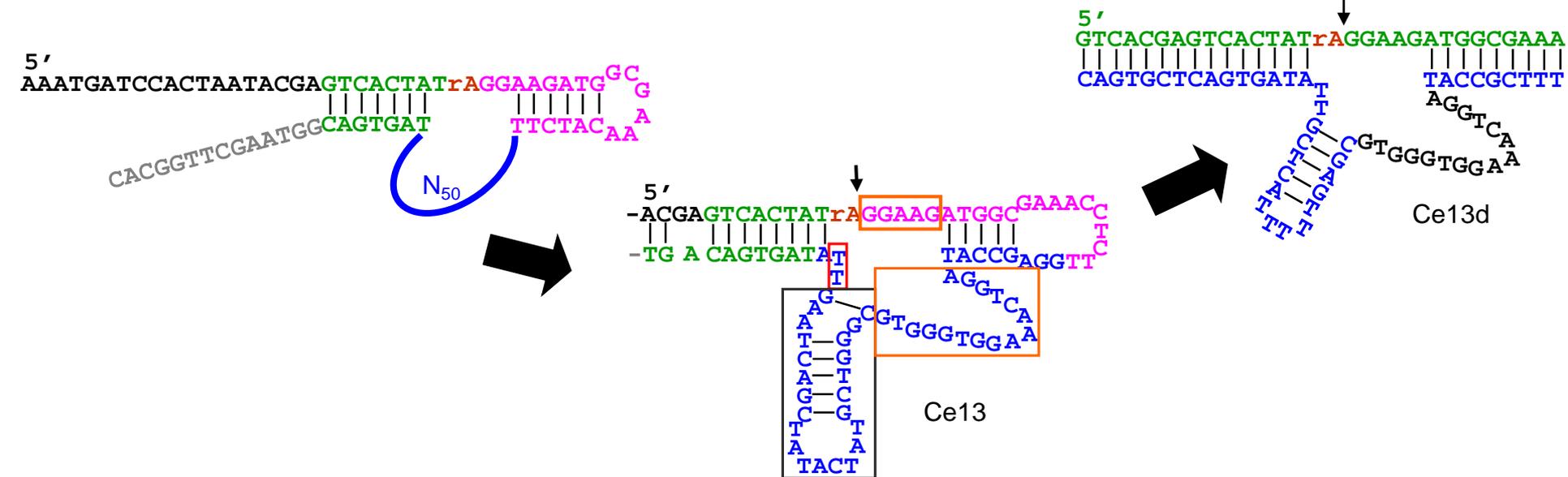
# Sequence alignment

Cleavage site

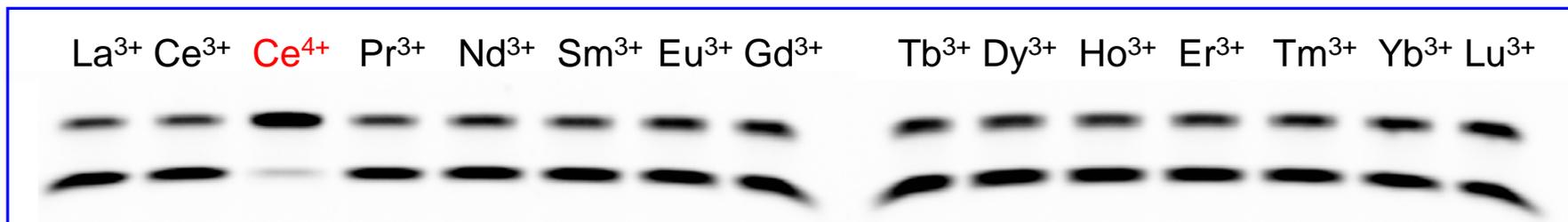
Start of the N<sub>50</sub> region

```

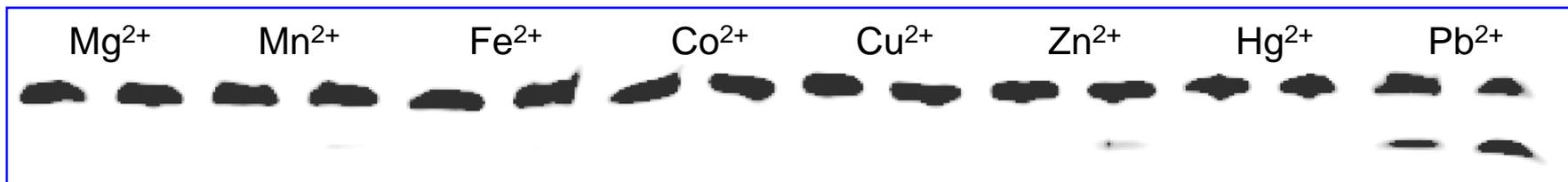
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CTGCAGAATTCTAATACGAGTCACTATAGGAAGAT --- GCGAAACATT- GGAG --- CCATAGGTCAAAGGTAGGTGCG- GTC --- GTATC-ATATCG- ACTAA ---
CTGCAGAATTCTAATACGAGTCACTATAGGAAGAT --- GCGAAACAT --- GGAG --- CCATAGGTCAAAGGTAGGTGCGAGTC --- GTATC-ATATCG- ACTAA ---
CTGCAGAATTCTAATACGAGTCACTATAGGAAGAT --- GCGAAACATCTT GGAG --- CCATAGGTCAAAGGTAGGTGCGGGTC --- GTATC-ATATCG- ACTAA ---
CTGCAGAATTCTAA- ACGAGTCACTATAGGAAGAT --- GCGAAACATCCT GGAG --- CCATAGGTCAAAGGTAGGTGCGGGTC --- GTATC-ATATCG- ACTA ---
CTGCAGAATTCTAATACGAGTCACTATAGGAAGAT --- GCGAAACATCTT GGAG --- CCATAGGTCAAAGGTAGGTGCGGGTC --- GTATC-ATATCG- ACTA ---
CTGCAGAATTCTAATACGAGTCACTATAGGAAGAT --- GCGAAACATCTT GGAG --- CCATAGGTCAAAGGTAGGTGCGGGTC --- GTATC-ATATCG- ACYA ---
CTGCAGAATTCTAATACGAGTCACTATAGGAAGAT --- GCGAAACATCTT GGAG --- CCATAGGTCAAAGGTAGGTGCGGGTC --- GTATC-ATATCG- ACCA ---
CTGCAGAATTCTAATACGAGTCACTATAGGAAGAT --- GCGAAACATCTT GGAG --- CCATAGGTCAAAGGTWGGTGCKGGYS --- KWWYM-WWWYCR-MYWA ---
CTGCAGAATTCTAATACGAGTCACTATAGGAAGAT --- GCGAAACATCTT- TA- --- CAAGGAACAATAATGGGGTTCGGGT --- ATA-TTGTCGTACCG ---
CTGCAGAATTCTAATACGAGTCACTATAGGAAGAT --- GCGAAACATCTT- TA- --- CGAACGGTTAAGAAAAGTGACTTATC --- CAGTGGTTATCTGACTA ---
CTGCAGAATTCTAATACGAGTCACTATAGGAAGAT --- GCGAAACATCTT- TACG- --- A- CGTCA- --- TCCCAAACAGG- CCATTAAA- AAAAGGATATAAG ---
CTGCAGAATTCTAATACGAGTCACTATAGGAAGAT --- GCGAAACATCTT- TACG- --- A- CGTCA- --- TCCTAAACAGG- CCATTAAA- AAAAGGATATAAG ---
CTGCAGAATTCTAATACGAGTCACTATAGGAAGAT --- GCGAAACATCTT- AACG- --- AGTGTAAGATCTCCCTGAAAGG- C- AGAATG- CAAAGTACAC ---
CTGCAGAATTCTAATACGAGTCACTATAGGAAGAT --- GCGAAACATCTT- AACG- --- AGTGTAAGATCTCCCTGAAAGG- C- AGAATG- CAAAGTACAC ---
CTGCAGAATTCTAATACGAGTCACTATAGGAAGAT --- GCGAAACATCTT- TACG- --- AGAGTAG- --- TCATTTAAA- --- TTAA- CAAAGTACACTGACGCAA ---
CTGCAGAATTCTAATACGAGTCACTATAGGAAGAT --- GCGAAACATCTT- T- --- TTC- --- GCGTAAATGACCGTATTCATG- C- GAATA- GGACATACG ---
CTGCAGAATTCTAATACGAGTCACTATAGGAAGAT --- GCGAAACATCTT- TACGACTCCGGGGCATGAACCACGATGGCCAT --- ATA- TAACGAATG ---
CTGCAGAATTCTAATACGAGTCACTATAGGAAGAT --- GCGAAACATCTCACAAG --- GGGGTGTATTAT- TCACGCGGATAACGTTAATA- CATGGTAC ---
  
```



# A general lanthanide-dependent DNAzyme

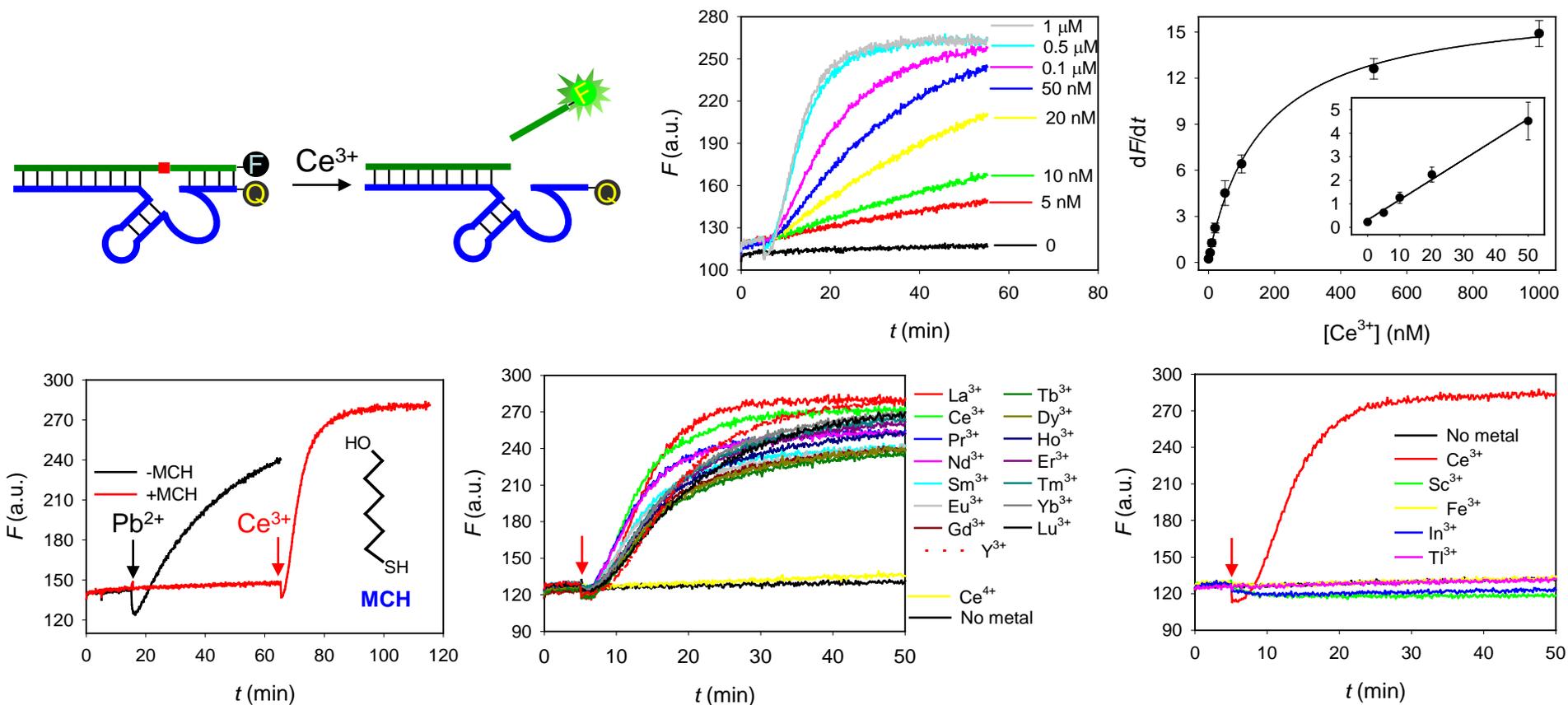


Left lane: 10uM  
Right lane: 100uM



- Y<sup>3+</sup> shows similar activity like other lanthanides
- Pb<sup>2+</sup> is the only active divalent metal ion

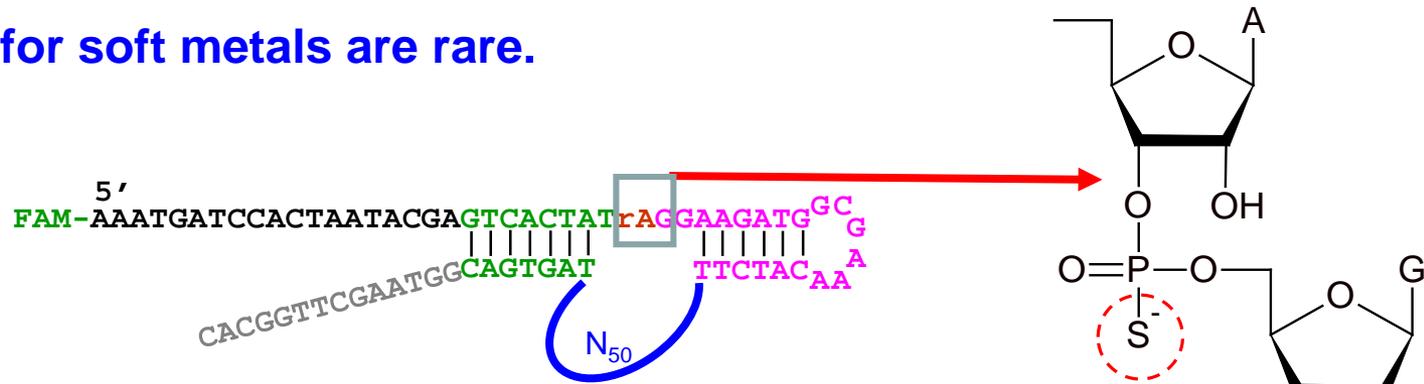
# Highly sensitive lanthanide detection





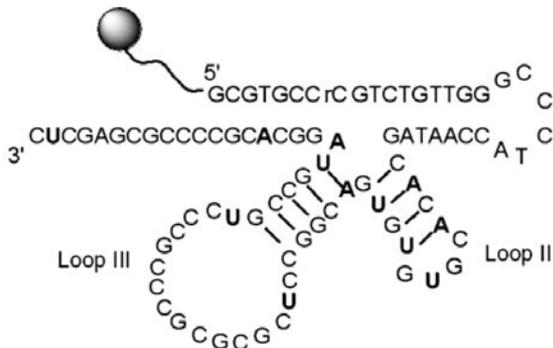
# Selection of DNAzymes using a PS-modified library

So far, DNAzymes for soft metals are rare.



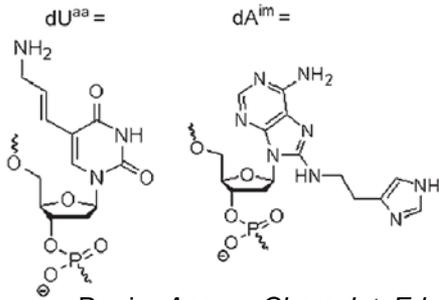
Solution 1: modified bases

Solution 2: modified phosphate backbone



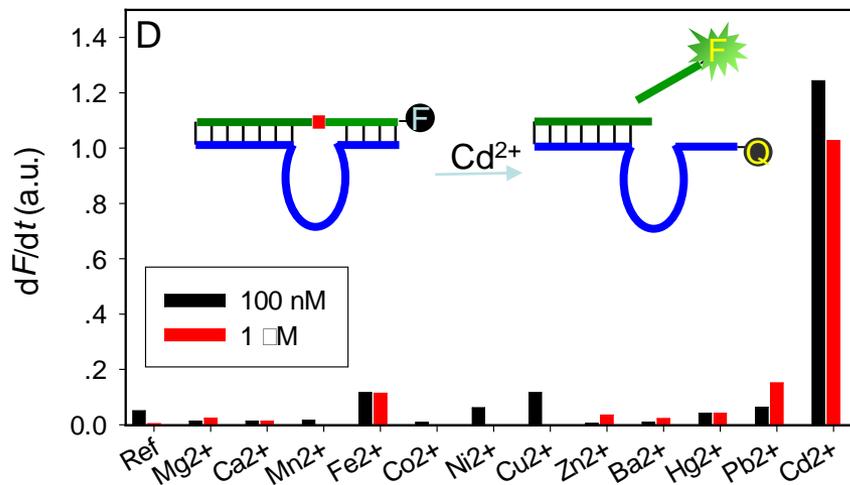
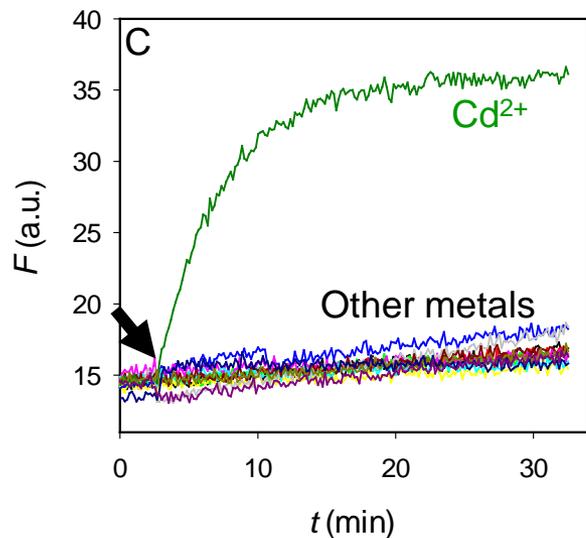
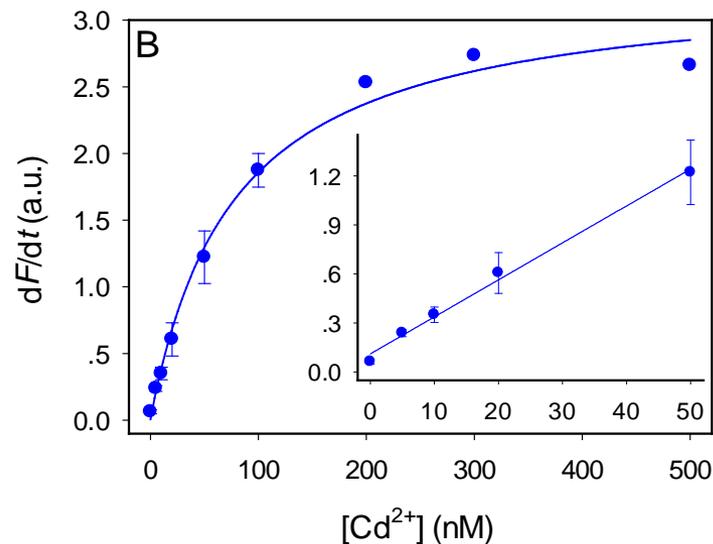
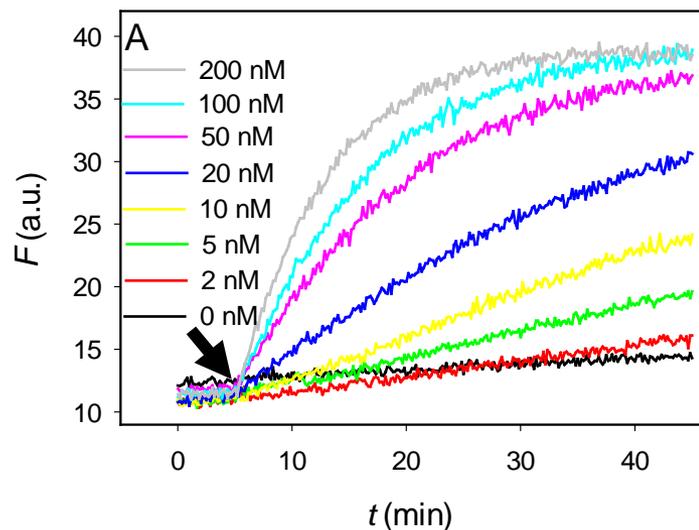
Can we get new DNAzymes that can discriminate different thiophilic metals?

Single modification made at the fixed region.

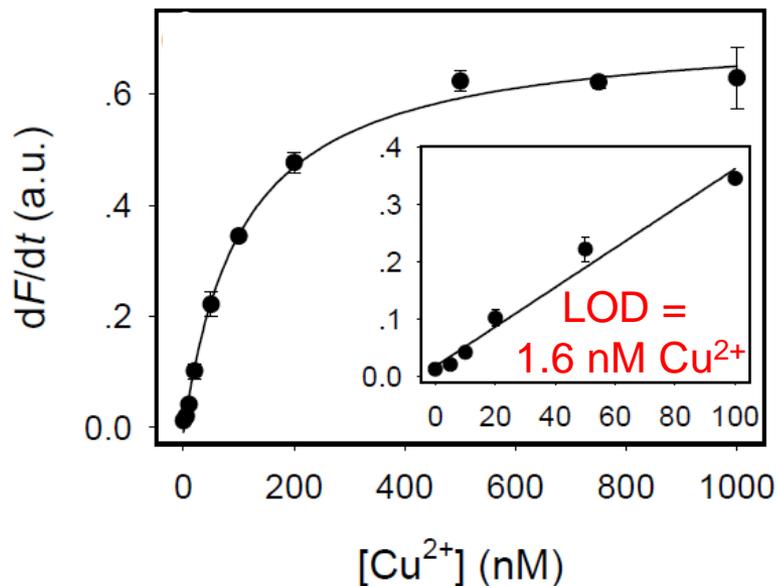
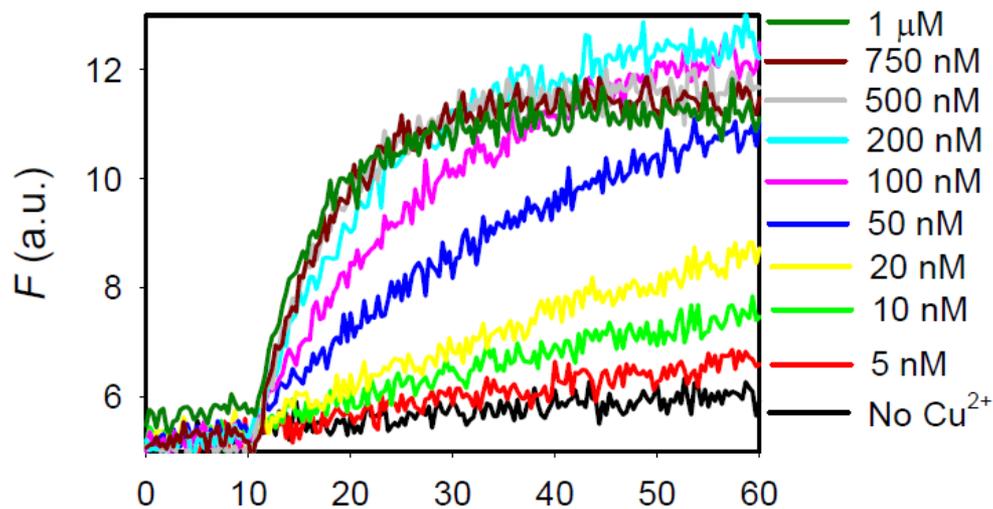
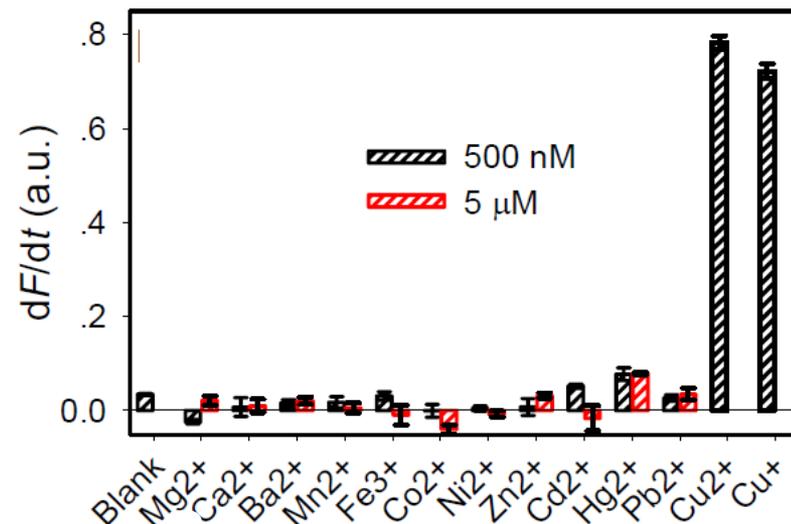
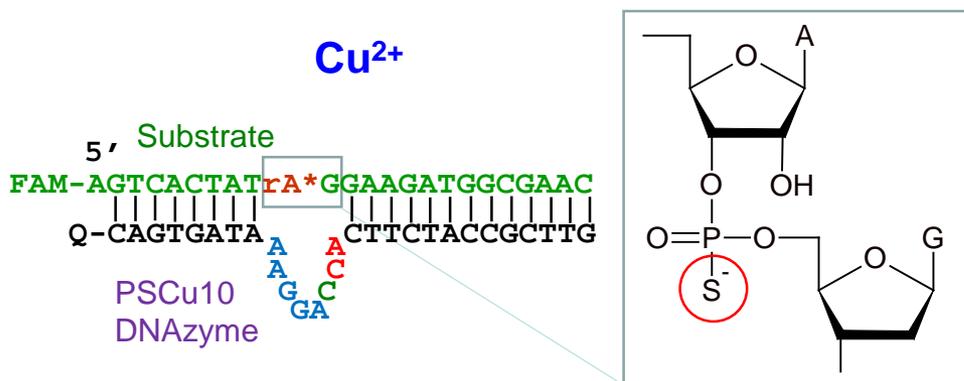




# Highly sensitive Cd<sup>2+</sup> detection



# Highly sensitive Cu<sup>2+</sup> detection

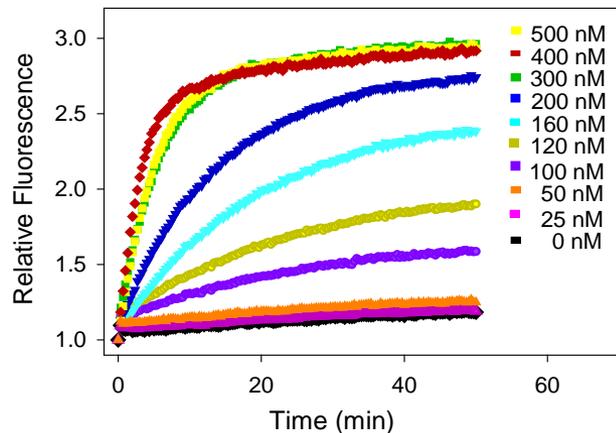


# Highly sensitive Ag<sup>+</sup> detection (using normal DNA)

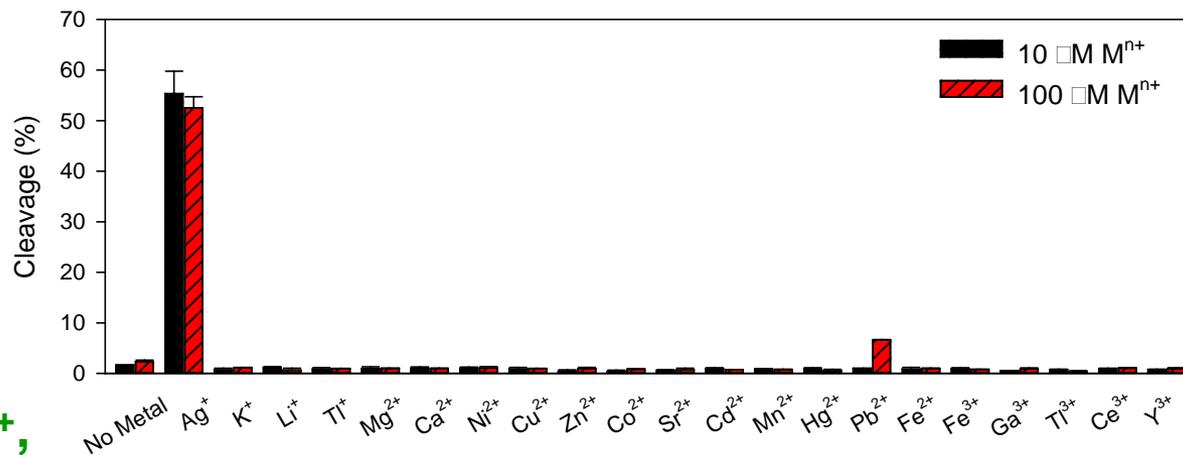


Only 52  
sequences  
out of >50,000

Efficient, 0.41 min<sup>-1</sup>

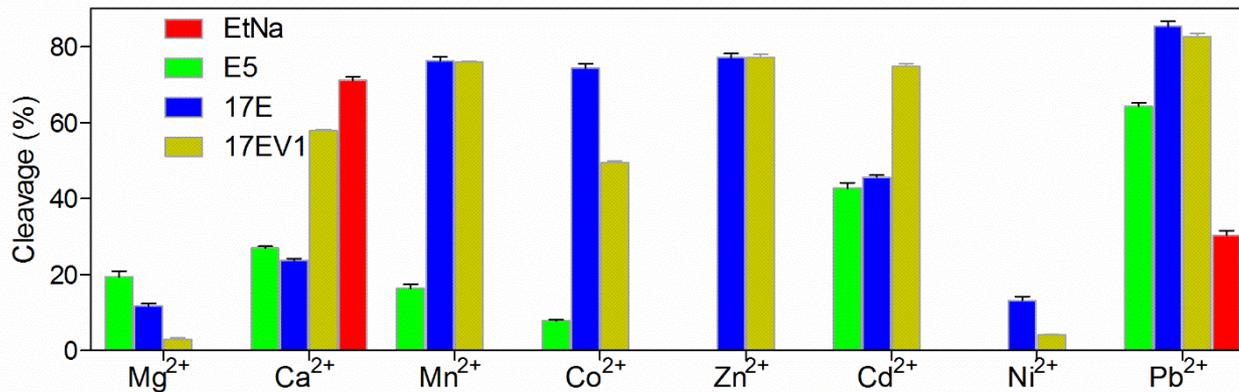
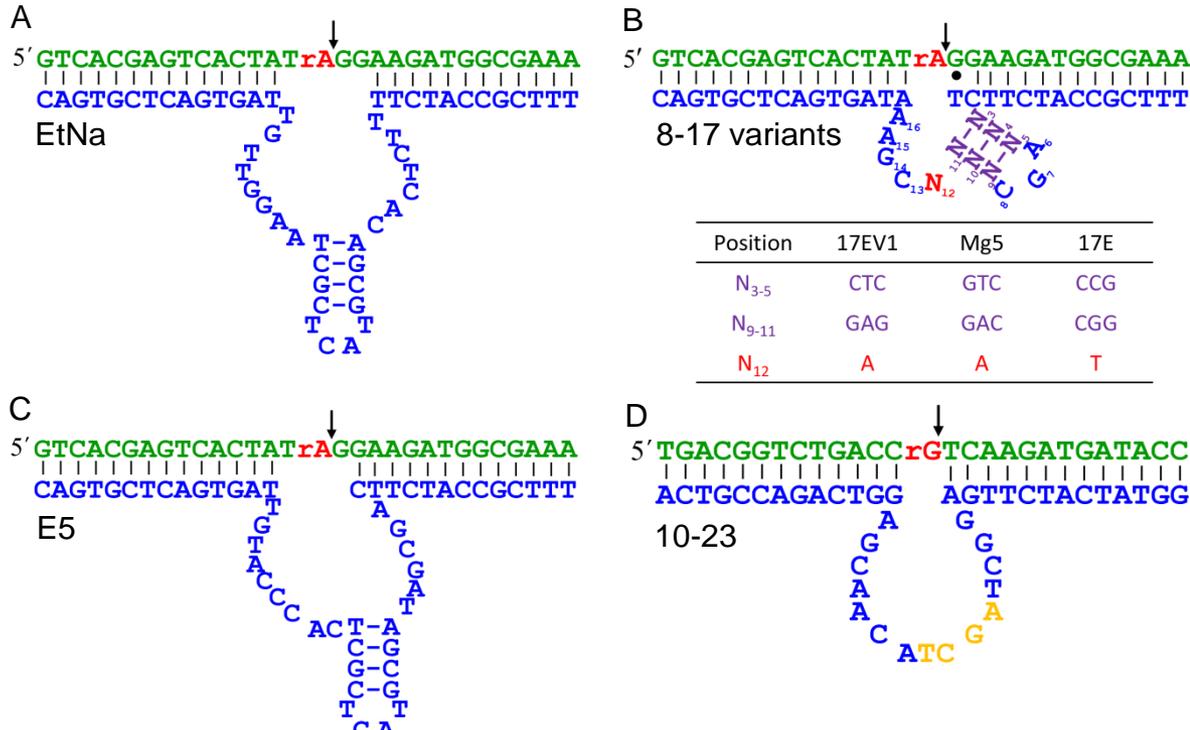


Detection limit =  
24 nM Ag<sup>+</sup>

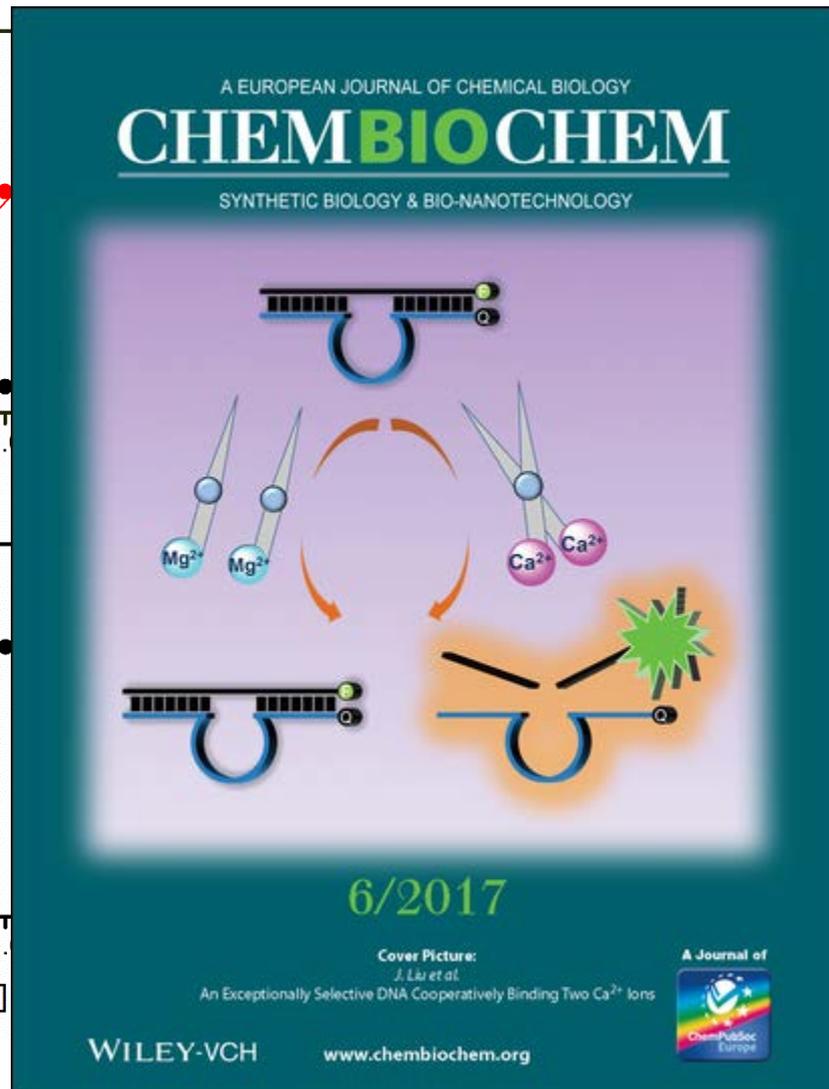
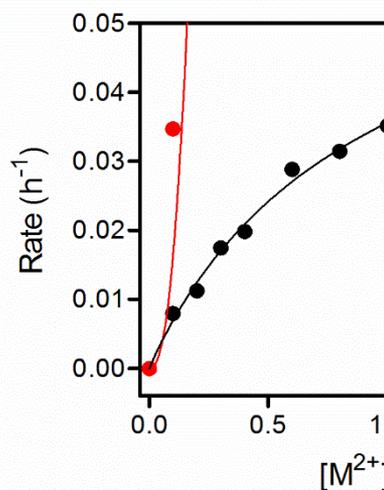
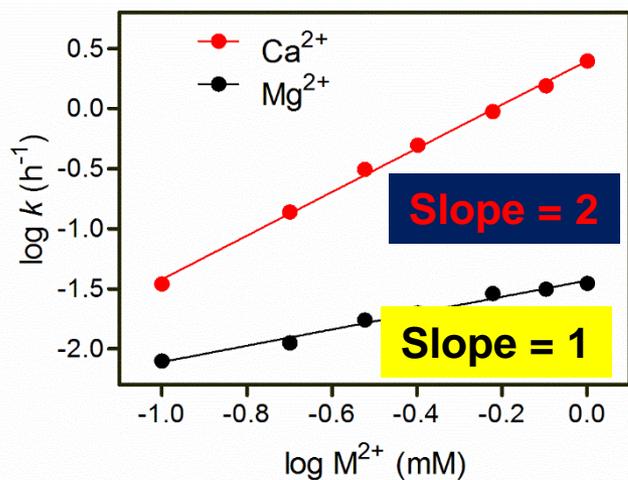
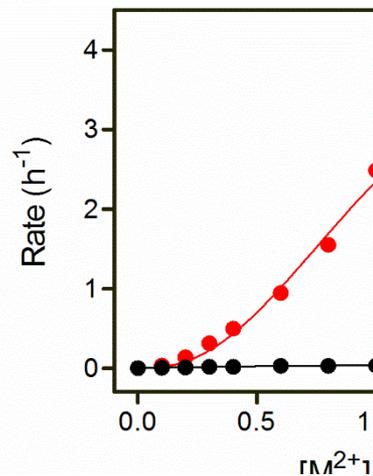
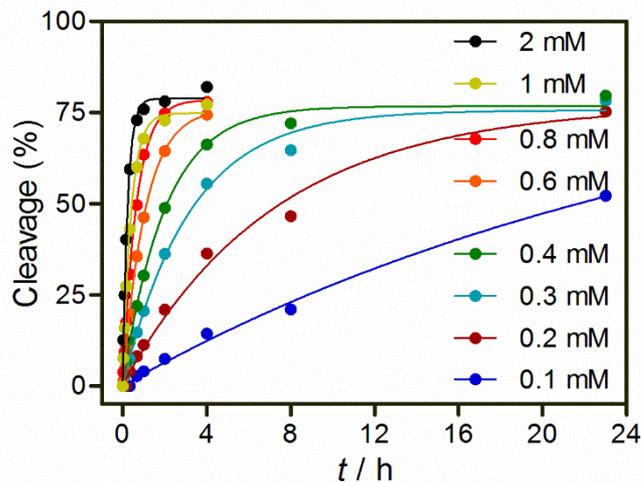


**Monovalent, thiophilic Ag<sup>+</sup>,  
but it works very well!  
This is a surprise!**

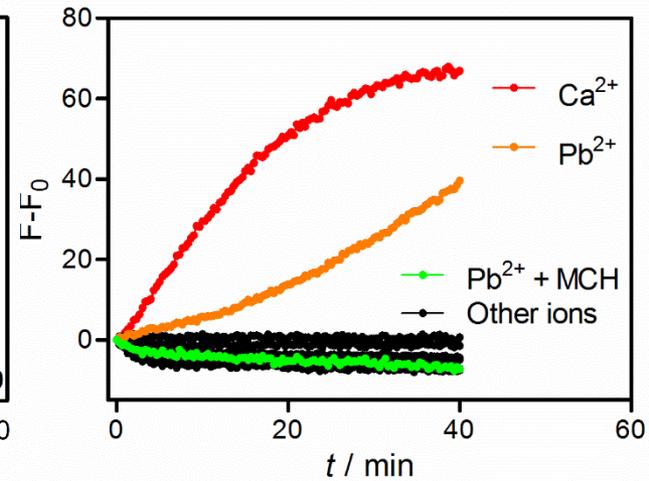
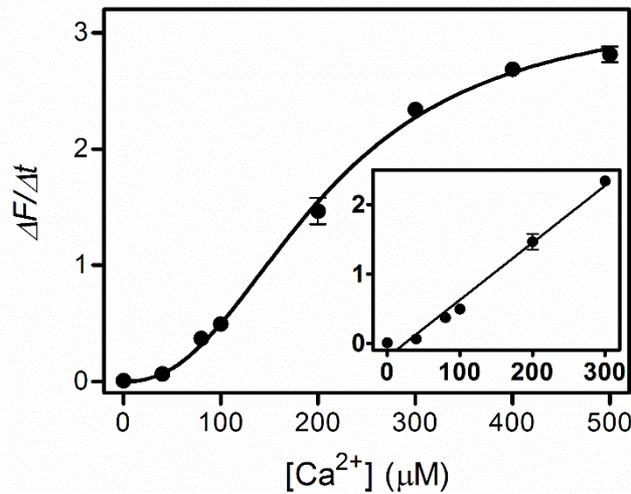
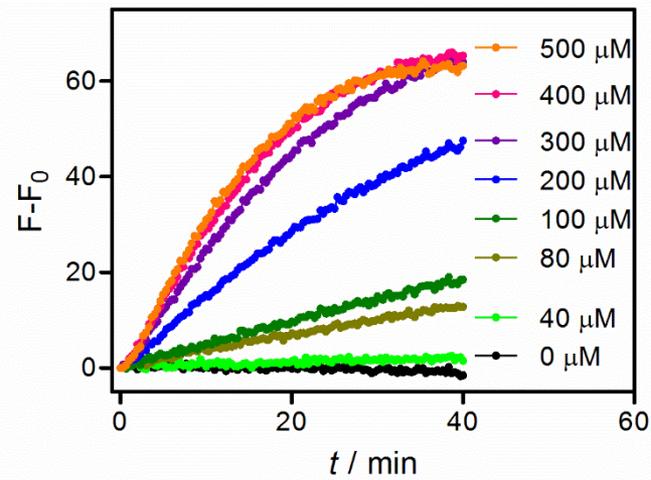
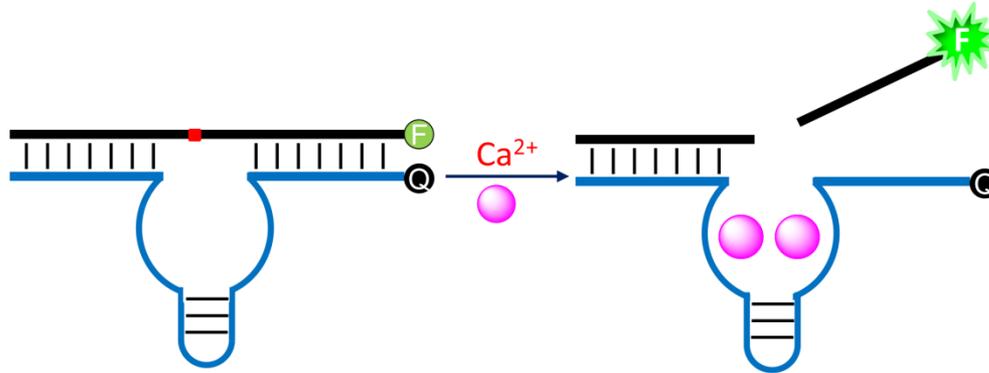
# EtNa has the best Ca<sup>2+</sup> specificity



# EtNa binds two $\text{Ca}^{2+}$ but only one $\text{Mg}^{2+}$



# Ca<sup>2+</sup> detection



Limit of Detection = 17  $\mu\text{M}$  Ca<sup>2+</sup>



# Acknowledgments



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Arsalan Beg Menhaj

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Jenny Lin

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Erin Kelly Firas El-Hamed  
Marissa Wu  
Murat Kiy  
Youssef Helwa  
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THANK  
YOU