

Highly Sensitive and Selective Fluorescent and Colorimetric Sensors for Radionuclides, Other Metal Ions, and Organic Molecules

Yi Lu

*Department of Chemistry, Biochemistry and
Center for Biophysics and Computational Biology
Beckman Institute for Advanced Science and Technology
University of Illinois at Urbana-Champaign
Urbana, IL 61801*

Measurement and Monitoring: A Major Challenge Facing ERSP

Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18				
Period																						
1	1 H																2 He					
2		3 Li	4 Be														5 B	6 C	7 N	8 O	9 F	10 Ne
3		11 Na	12 Mg														13 Al	14 Si	15 P	16 S	17 Cl	18 Ar
4	19 K	20 Ca		21 Sc	22 Ti	23 V	24 Cr	25 Mn	26 Fe	27 Co	28 Ni	29 Cu	30 Zn	31 Ga	32 Ge	33 As	34 Se	35 Br	36 Kr			
5	37 Rb	38 Sr		39 Y	40 Zr	41 Nb	42 Mo	43 Tc	44 Ru	45 Rh	46 Pd	47 Ag	48 Cd	49 In	50 Sn	51 Sh	52 Te	53 I	54 Xe			
6	55 Cs	56 Ba	*	71 Lu	72 Hf	73 Ta	74 W	75 Re	76 Os	77 Ir	78 Pt	79 Au	80 Hg	81 Tl	82 Ph	83 Bi	84 Po	85 At	86 Rn			
7	87 Fr	88 Ra	**	103 Lr	104 Rf	105 Db	106 Sg	107 Bh	108 Hs	109 Mt	110 Uun	111 Uuu	112 Uub	113 Uut	114 Uuq	115 Uup	116 Uuh	117 Uus	118 Uuo			
*Lanthanides		*		57 La	58 Ce	59 Pr	60 Nd	61 Pm	62 Sm	63 Eu	64 Gd	65 Tb	66 Dy	67 Ho	68 Er	69 Tm	70 Yb					
**Actinides		**		89 Ac	90 Th	91 Pa	92 U	93 Np	94 Pu	95 Am	96 Cm	97 Bk	98 Cf	99 Es	100 Em	101 Md	102 No					

Before ER: How many and how much (identification and quantification)?

Where and when (on-site, real-time with high spatial and temporal resolution)?

What species (e.g., different oxidation states with different bioavailability)?

During ER: How effective are the remediation methods?

After ER: Long-term monitoring.

Fundamental science: what structural features responsible for molecular recognition of different radionuclides and different oxidation states of the same radionuclide?

Current techniques

Atomic absorption spectrometry

Inductively coupled plasma mass spectrometry

Anodic stripping voltammetry

X-ray fluorescence spectrometry or microscope

Phosphorimetry

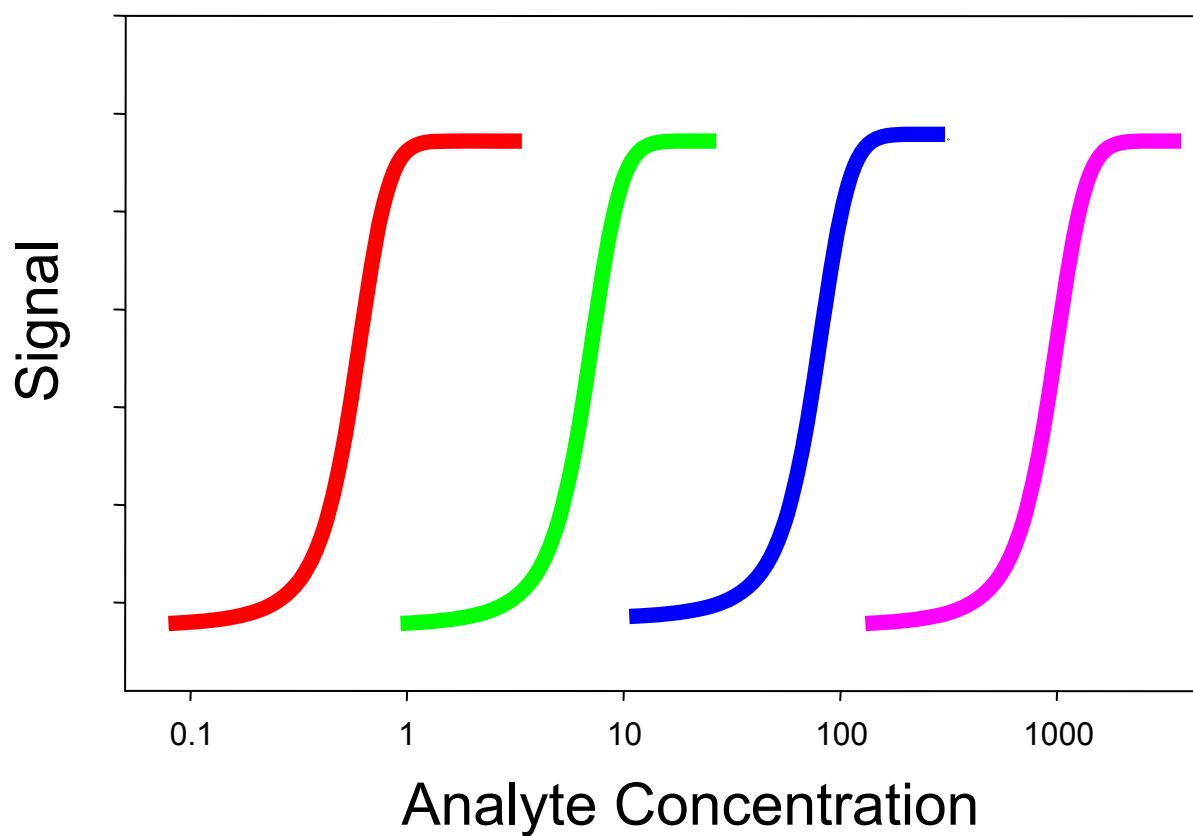
Advantages

- ↑ Industrial standard
- ↑ Highly sensitive (down to ~ppb or less)
- ↑ Detect a number of metal ions simultaneously

Disadvantages

- ↓ Require sophisticated equipment, sample-pretreatment, and skilled operators
- ↓ only detect total amount of metal ions (not bioavailable metals)
- ↓ difficult for in-situ, on-site, remote or real-time detection

Desired Features of Sensors

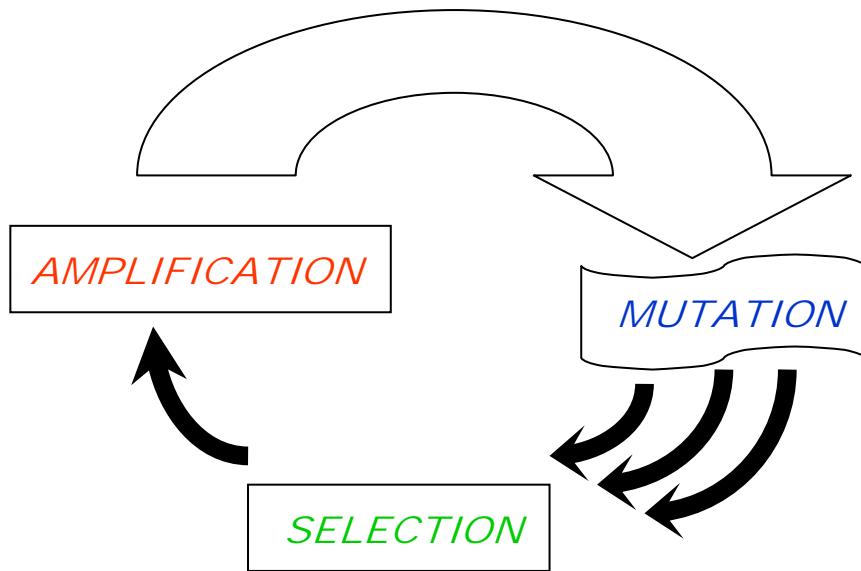


- low detection limit
- high sensitivity
- high selectivity
- wide dynamic range
- simple to use
- cost-effective

Four key steps in designing a sensor

- ? a **general** method to obtain molecules for a specific analyte (e.g., Pb^{2+} , Hg^{2+} , Amphetamine, Ricin)
- ? a **general** method to improve selectivity;
- ? a **general** method to transform molecular recognition into physical detectable signals without compromising the binding affinity and selectivity;
- ? a **general** method to fine-tune the dynamic range.

1. A general method to obtain molecules for any specific analyte



Advantages:

- High throughput (10^{14} - 10^{15} different sequences)
- Selective Amplification
- Improvement in each round
- Minimal cost (~ \$5/round)
- Short time (~ 2 days/round)

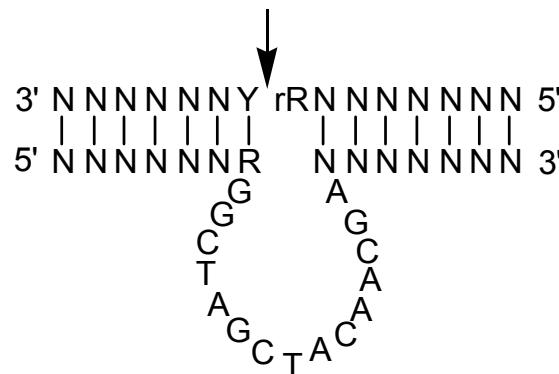
A. D. Ellington, J. W. Szostak, *Nature* 1990, 346, 818.
C. Tuerk, L. Gold *Science* 1990, 249, 505.
A. Beaudry, G. F. Joyce, *Science* 1992, 257, 635.
J. Liu, Y. Lu, *J. Fluoresc.* 2004, 14, 343.

Analytes recognized by selected DNA/RNA

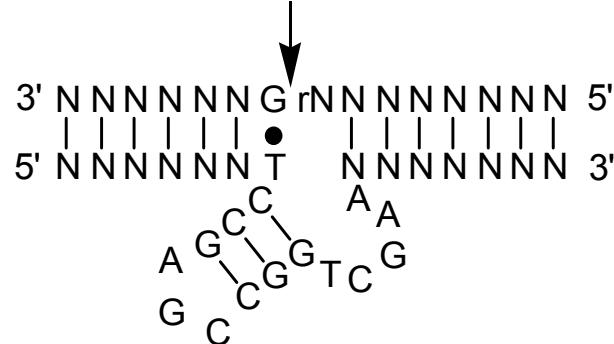
- Metal ions** (Mg(II), Ca(II), Pb(II), Co(II), Cr(VI), U(VI))
- Organic dyes** (Cibacron blue, reactive green 19)
- Amino acids** (L-Valine, D-Tryptophan)
- Nucleosides/nucleotides** (Guanosine, ATP)
- Nucleotide analogs** (8-oxo-dG, 7-Me-guanosine)
- Biological cofactors** (NAD, porphyrins, Vitamin B₁₂)
- Aminoglycosides** (Tobramycin, Neomycin)
- Antibiotics** (Streptomycin, Viomycin)
- Peptides** (Rev peptide)
- Enzymes** (Human Thrombin, HIV Rev Transcriptase)
- Growth cofactors** (Keratinocyte and Basic fibroblast)
- Antibodies** (human IgE)
- Gene regulatory factors** (elongation factor Tu)
- Cell adhesion molecules** (human CD4, selectin)
- Viral particles** (Rous sarcoma virus, Anthrax spores)

Starting with a pool of DNA with 10^{14} - 10^{15} different sequences, new catalytic DNA that are selective toward any analyte of choice can be obtained through a series of selection, amplification and mutation in the laboratory.

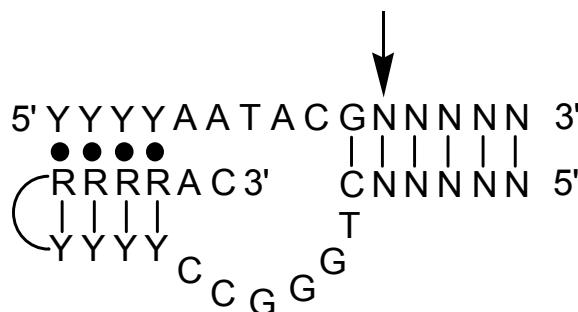
Examples of in vitro selected Catalytic DNA



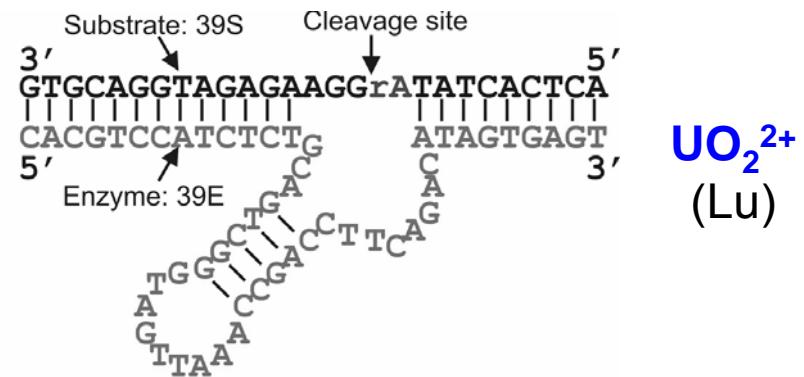
Mg^{2+}
(Joyce)



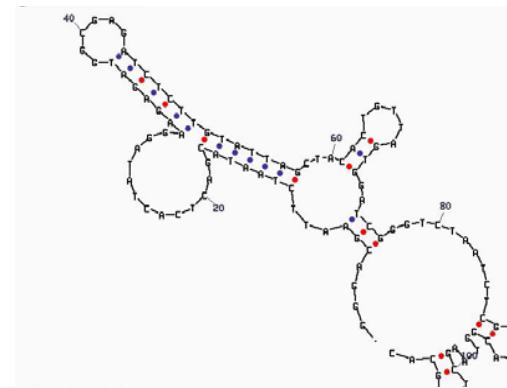
Pb^{2+}
(Lu, Joyce)



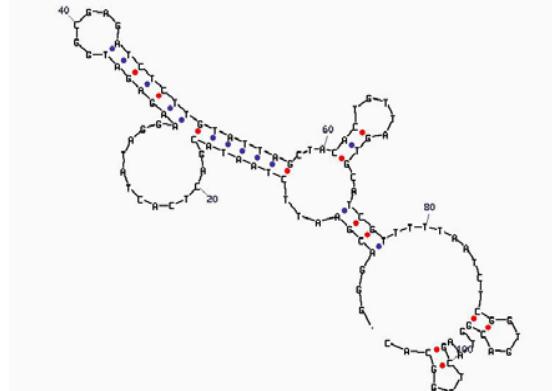
Cu^{2+}
(Breaker)



UO_2^{2+}
(Lu)



Zn^{2+}
(Lu)

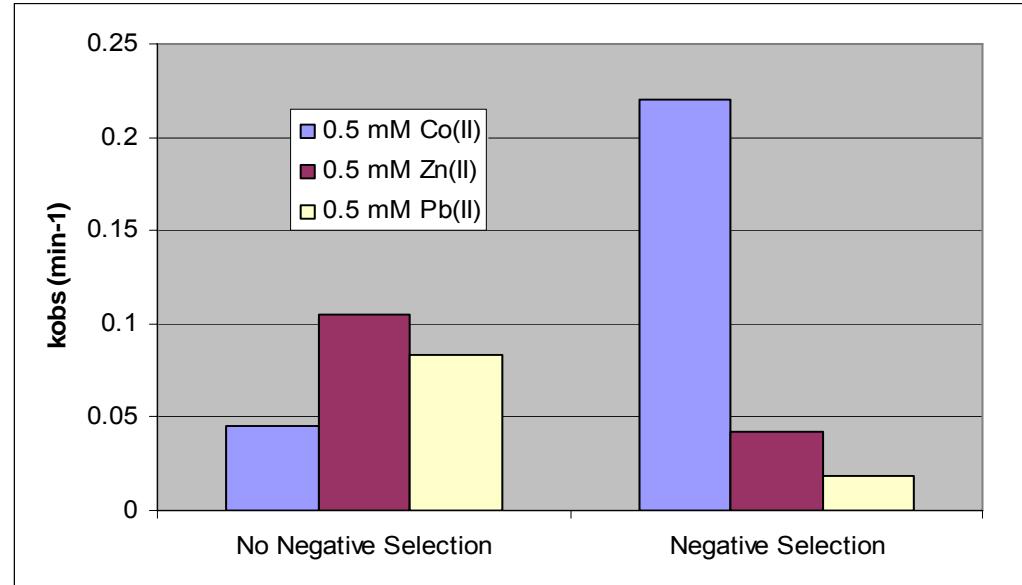
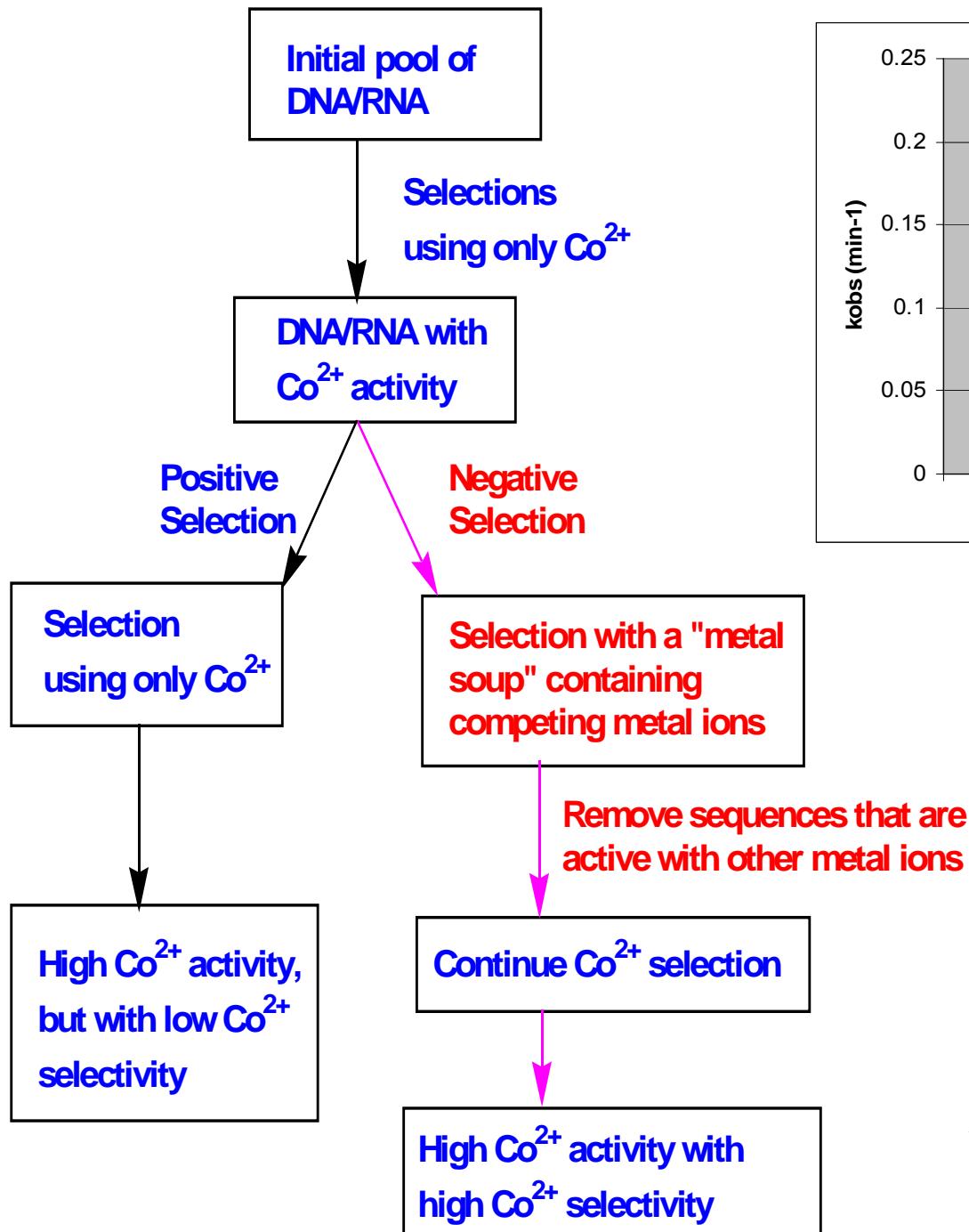


Co^{2+}
(Lu)

Specific sequence/code
= specificity

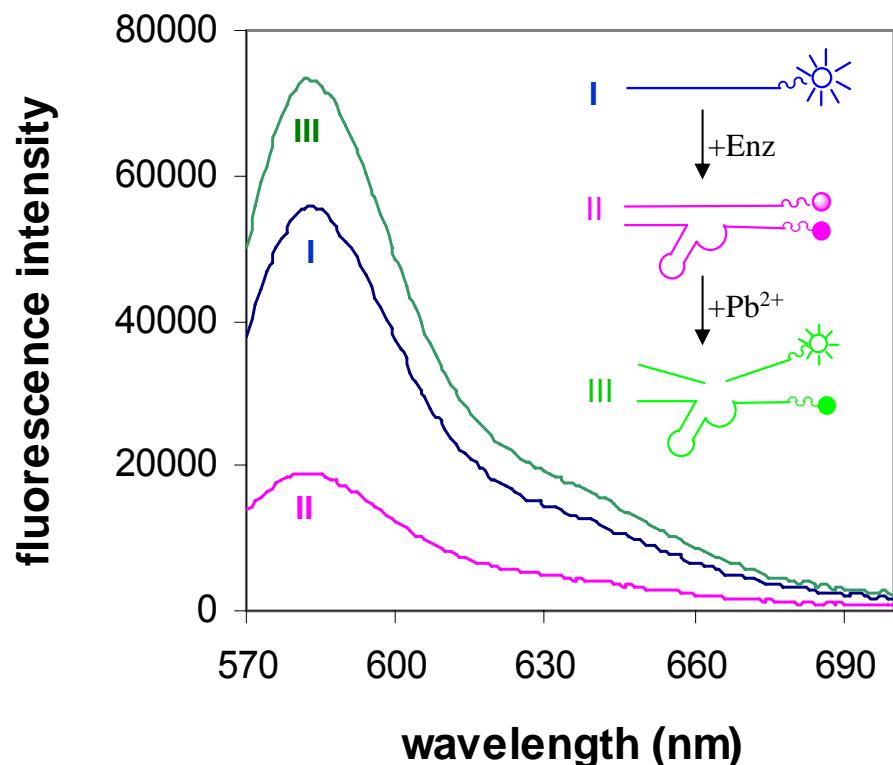
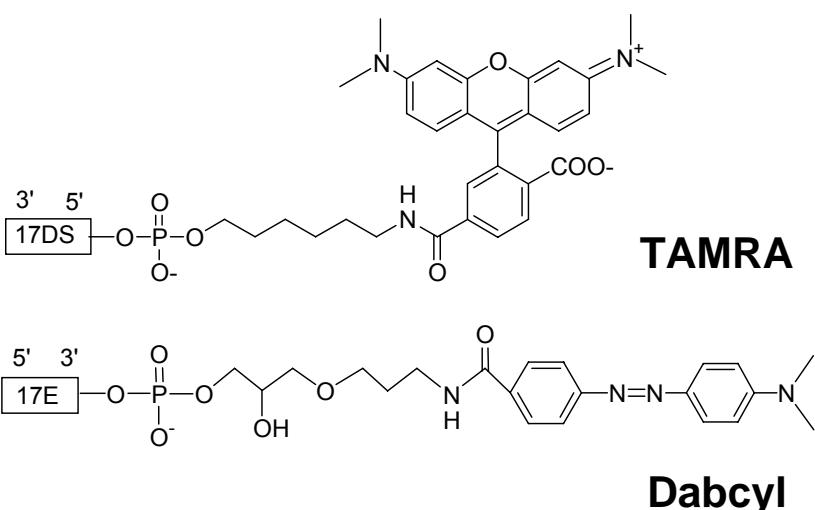
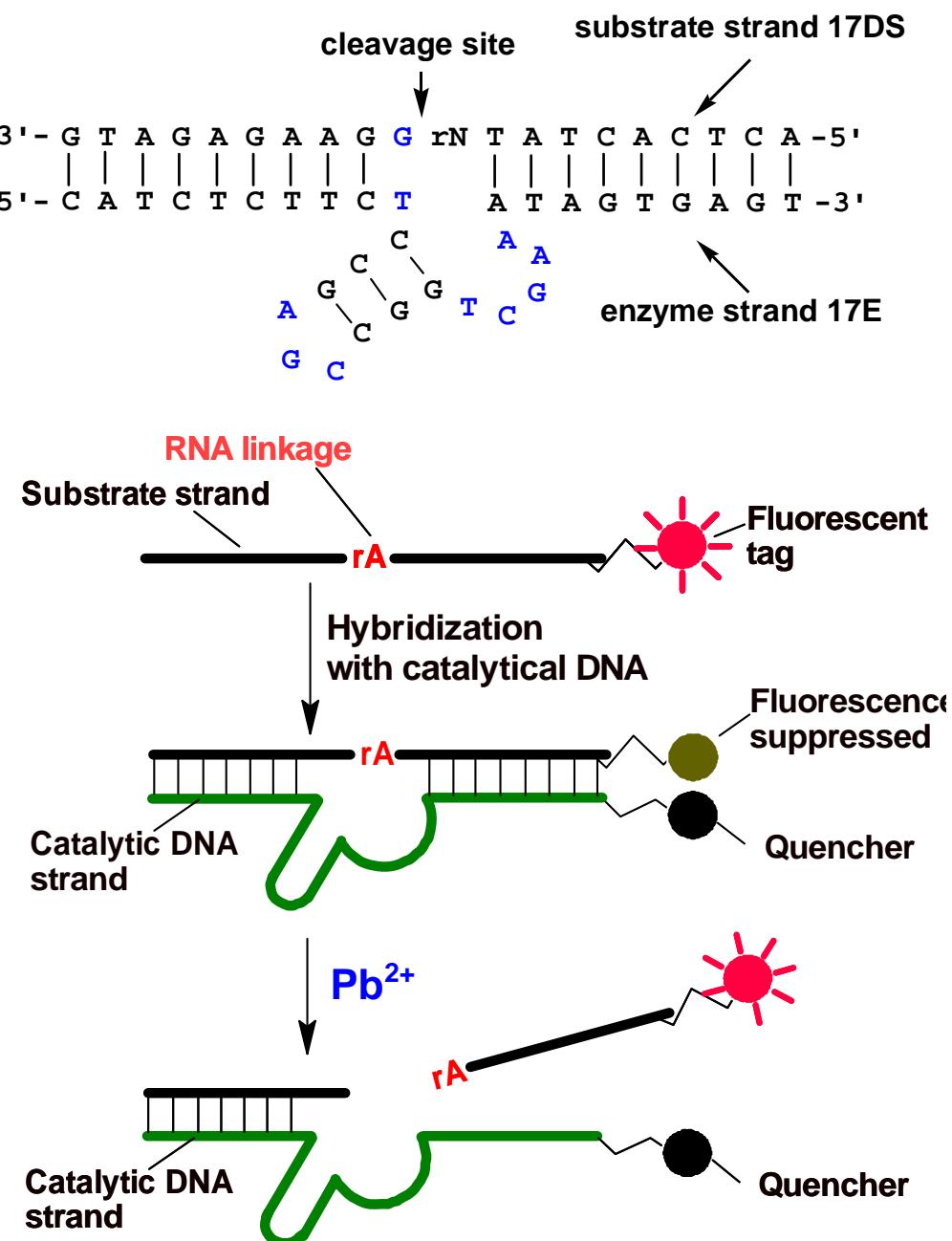
Lu, Y. *Chem. Euro. J.* 8, 4588-4596 (2002).

2. A general method to improve sensor selectivity

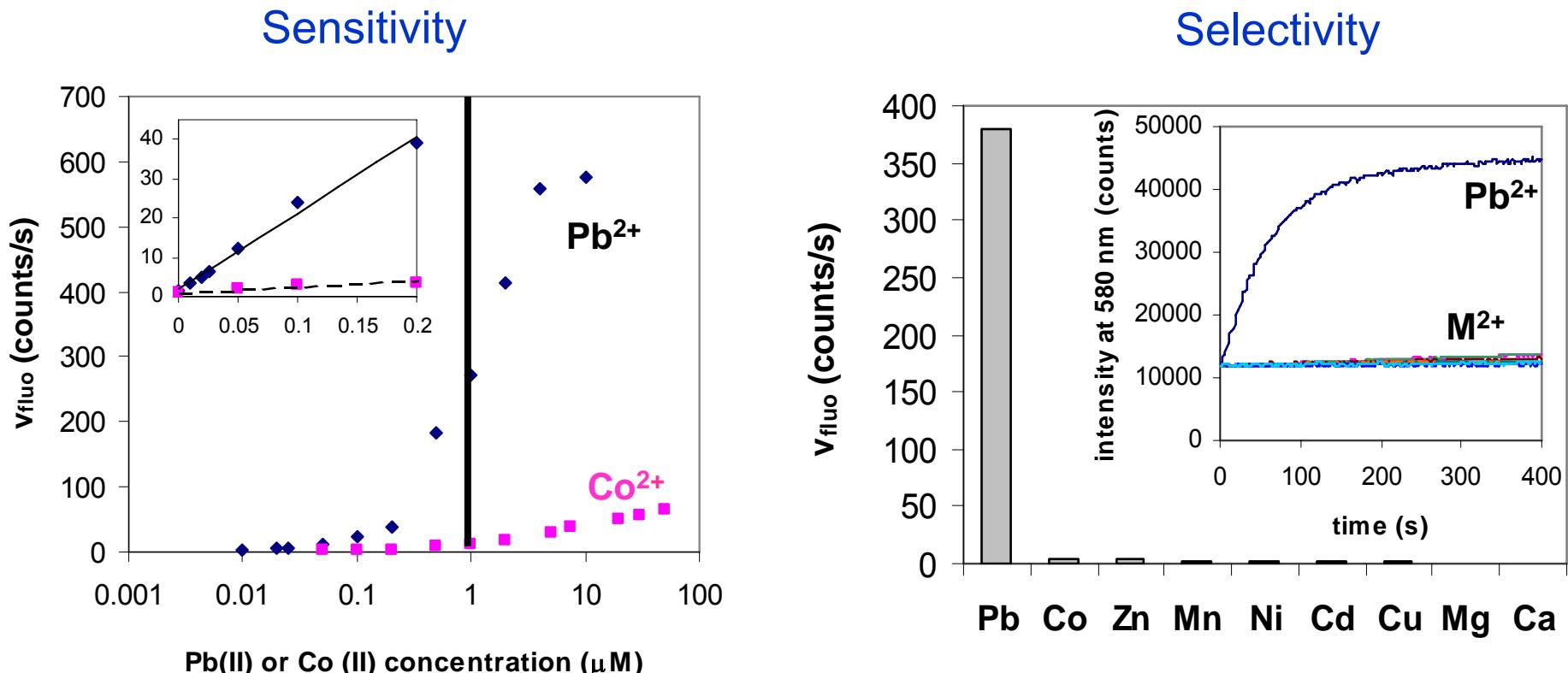


Applying a “negative” selection strategy in *in vitro* selection or SELEX, one can improve sensor’s analyte selectivity. This method can be generally applied to any selection method.

3. A general method to convert catalytic DNA into fluorescent sensors using catalytic molecular beacon



A Highly Sensitive and Selective DNAzyme Biosensor for Pb^{2+}



Dynamic range: 1 nM (0.2 ppb) to 4 μM (800 ppb)
(Lead toxic level defined by US CDC: 500 nM (100 ppb))
(Lead toxic level defined by US EPA: 75 nM (15 ppb))

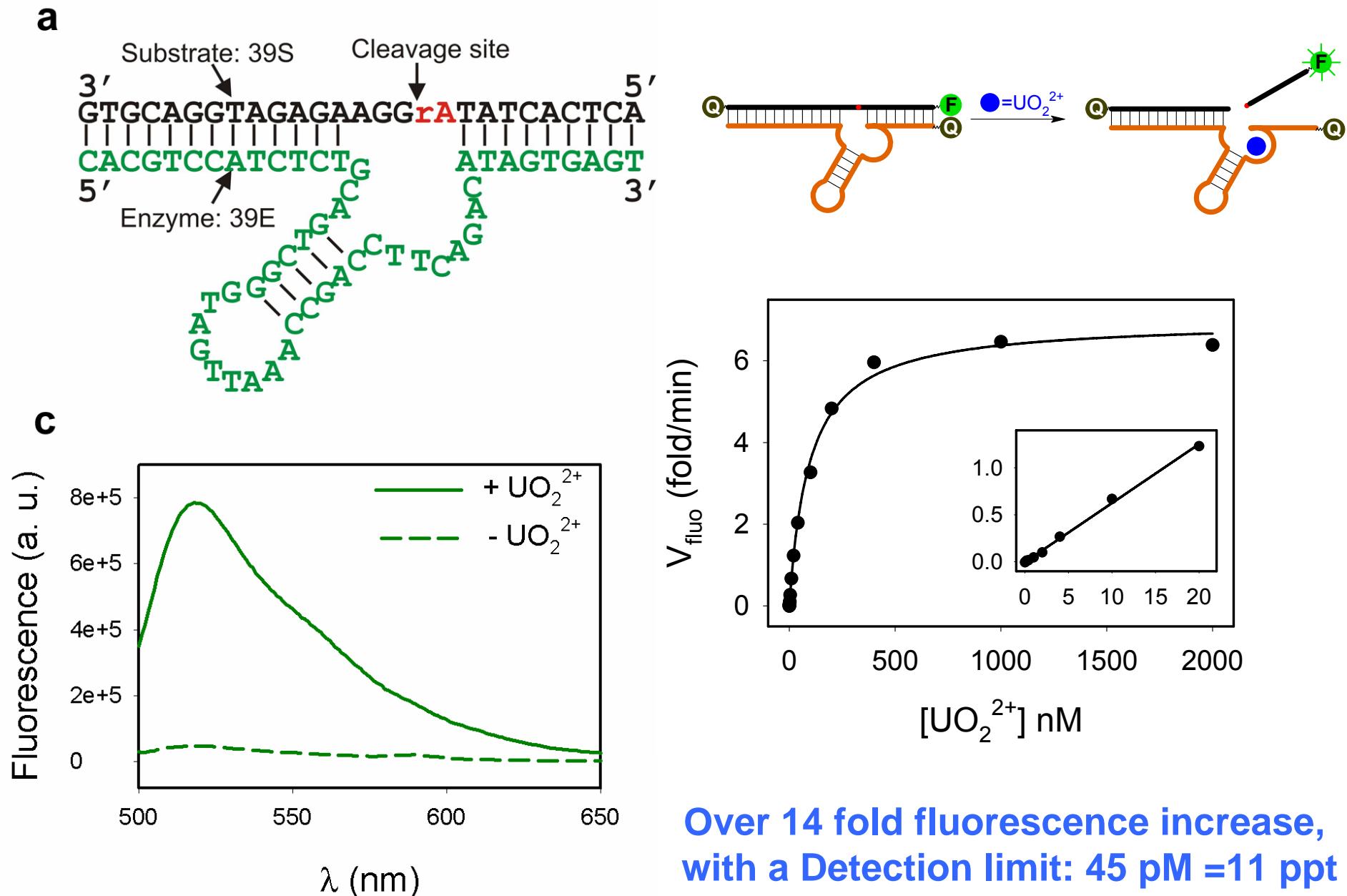
Li, J.; Lu, Y. *J. Am. Chem. Soc.* 122, 10466-10467 (2000).

Liu, J.; Lu, Y. *Anal. Chem.* 75, 6666 – 6672 (2003).

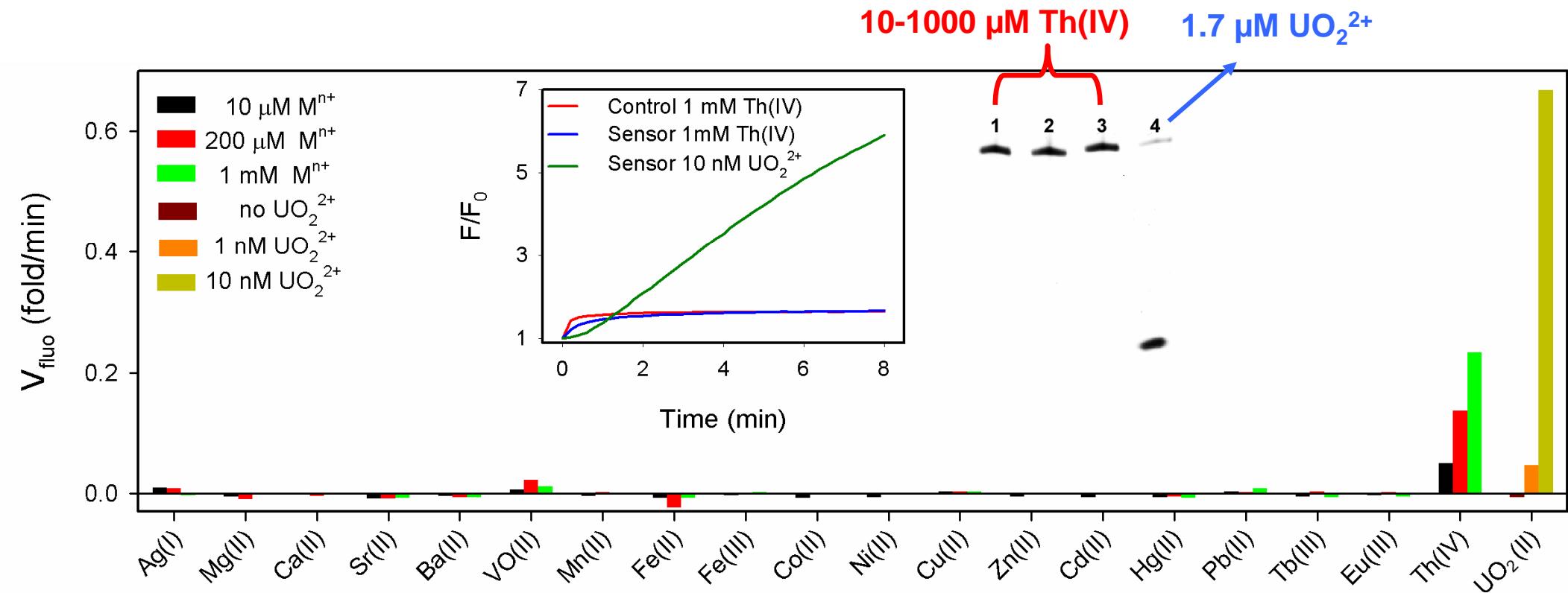
Lu, Y. et al., *Biosensors & Bioelectronics* 18, 529-540 (2003).

Swearingen, C. B. et al., *Anal. Chem.* 77, 442-448 (2005).

Selection and design of a UO_2^{2+} sensor



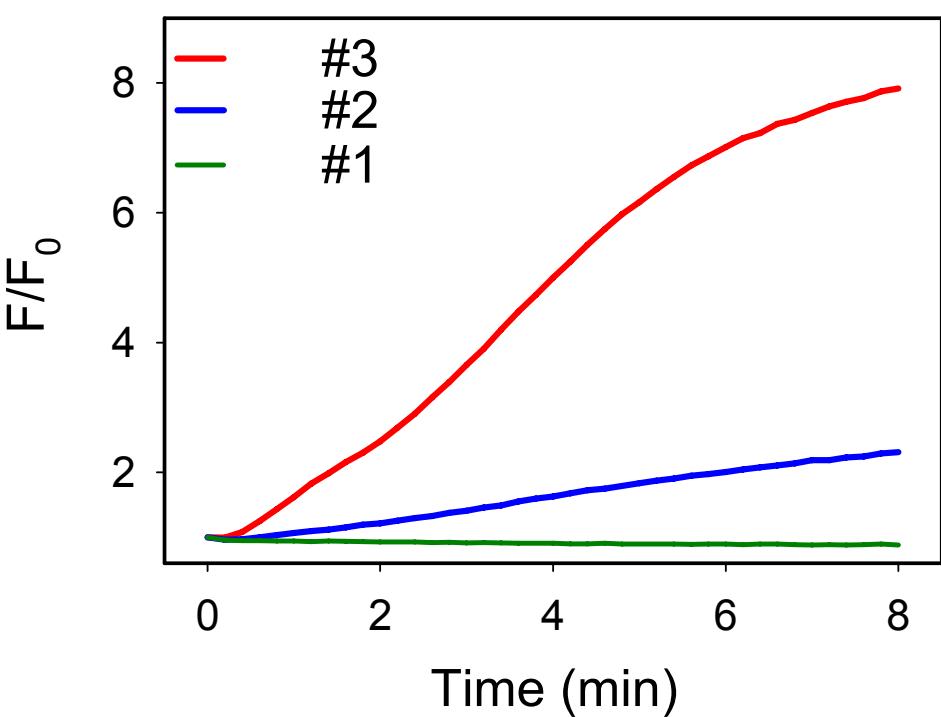
Over 1 Million Fold Selectivity



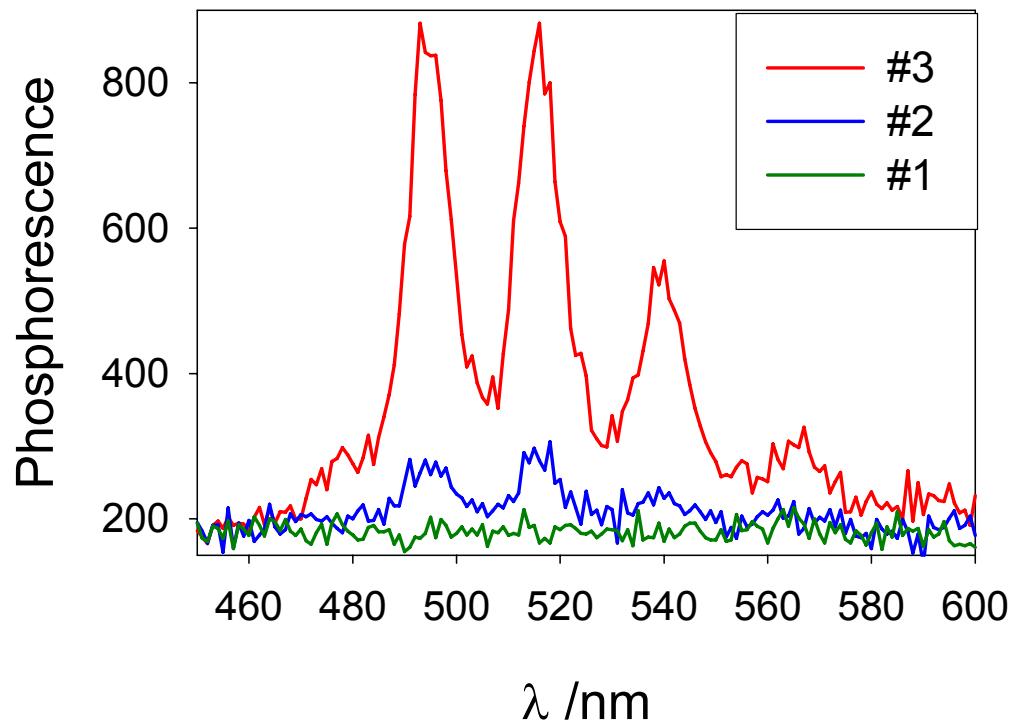
Only Th(IV) showed slight fluorescence increase
Due to interaction with the fluorophore, no cleavage activity

Uranyl Detection in NABIR FRC Soil Samples

300×diluted
Catalytic DNA sensor response

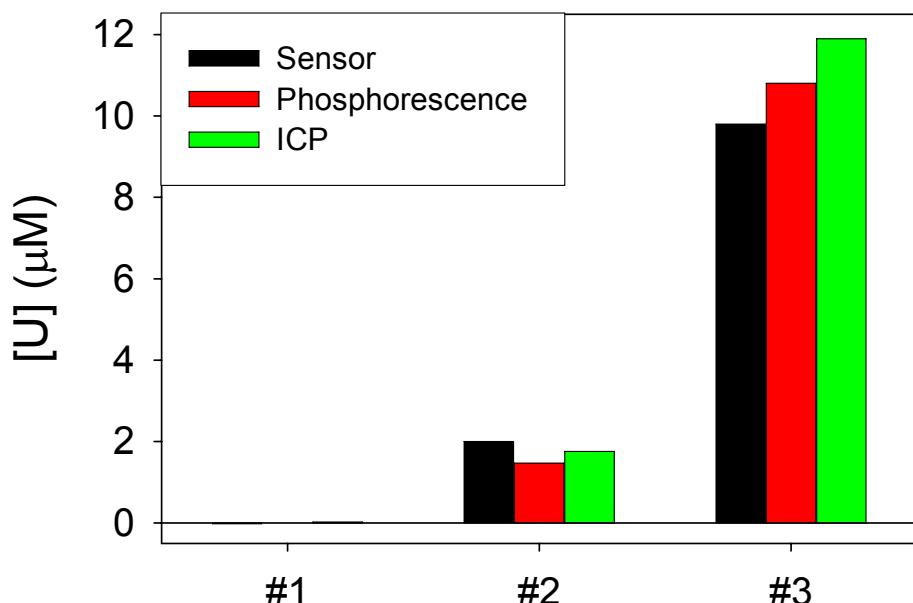


50×diluted
Phosphorescence in 10% H_3PO_4



We followed a sample extraction procedure established by P. Zhou and B. Gu,
Environ. Sci. Technol. 39, 4435-4440 (2005).

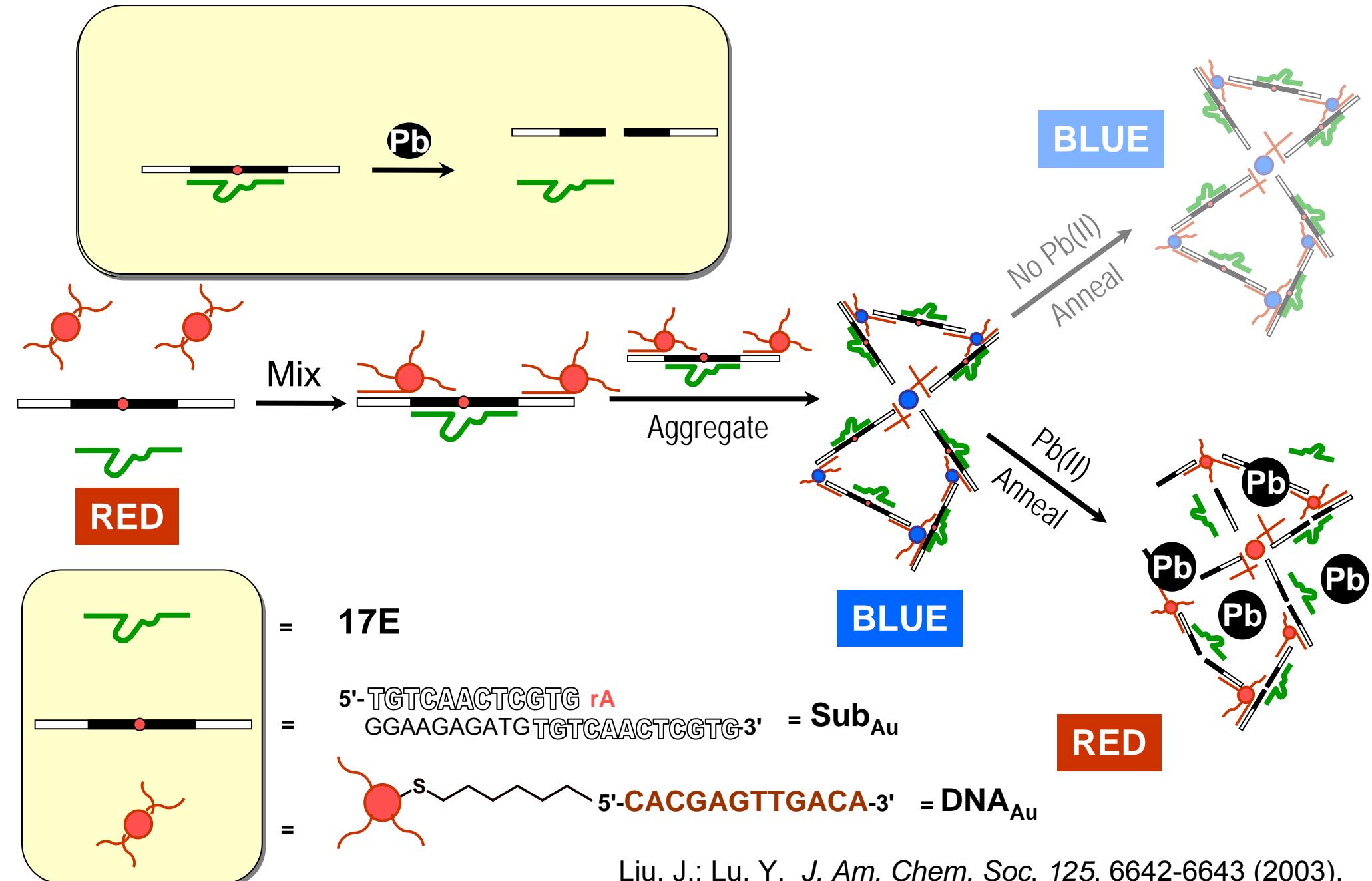
Further comparison



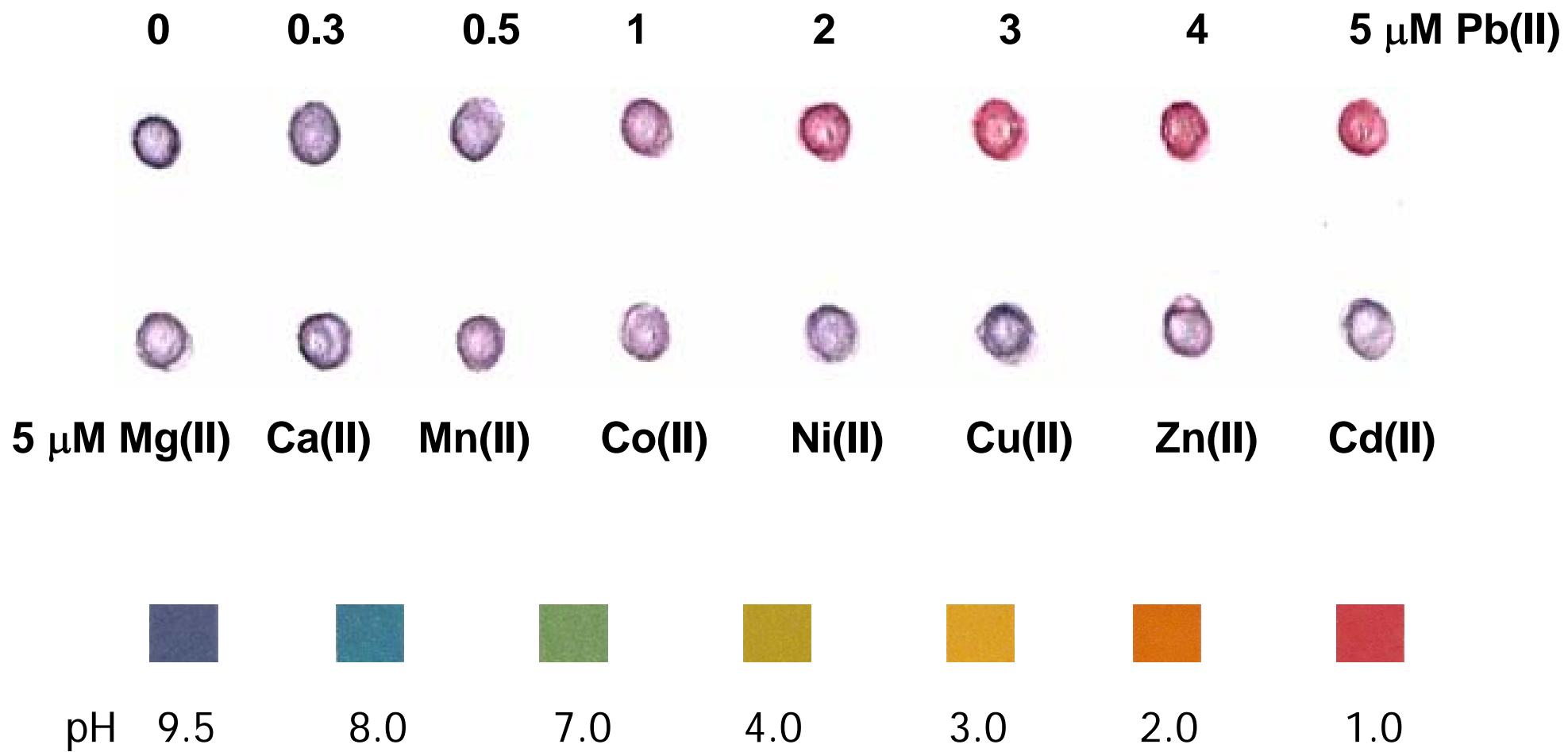
Performance is comparable to
ICP and phosphorescence

Method	Detection Limit (pM)	Detection Limit (ppt)
X-ray Fluorescence	11,760,000	2,800,000
Atomic Absorption Spectrometry	336,000	80,000
EPA MCL	126,000	30,000
ICP-Atomic Emission Spectrometry	8,400	2,000
Antibody fluorescence	1,000	238
ICP-Mass Spectrometry	420	100
Stripping Voltammetry	100	24
Catalytic DNA Sensor	45	11
Phosphorimetry	42	10
Kinetic Phosphorimetry	4.2	1

Design of a Simple Colorimetric Biosensor



pH Indicator like Operation



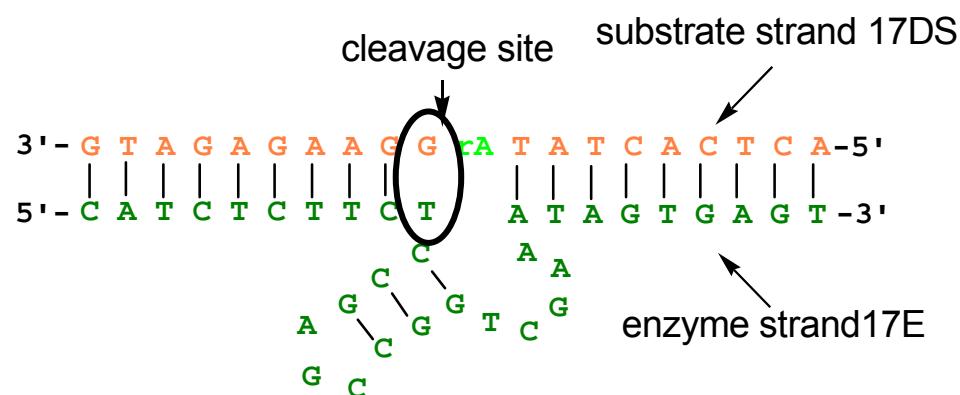
Liu, J.; Lu, Y. *J. Am. Chem. Soc.* 125, 6642-6643 (2003).
Liu J.; Lu, Y. *Chem. Mater.*, 16, 3231 (2004);
Liu J.; Lu, Y. *J. Am. Chem. Soc.* 126, 12298 (2004).

Four key steps in designing metal sensors

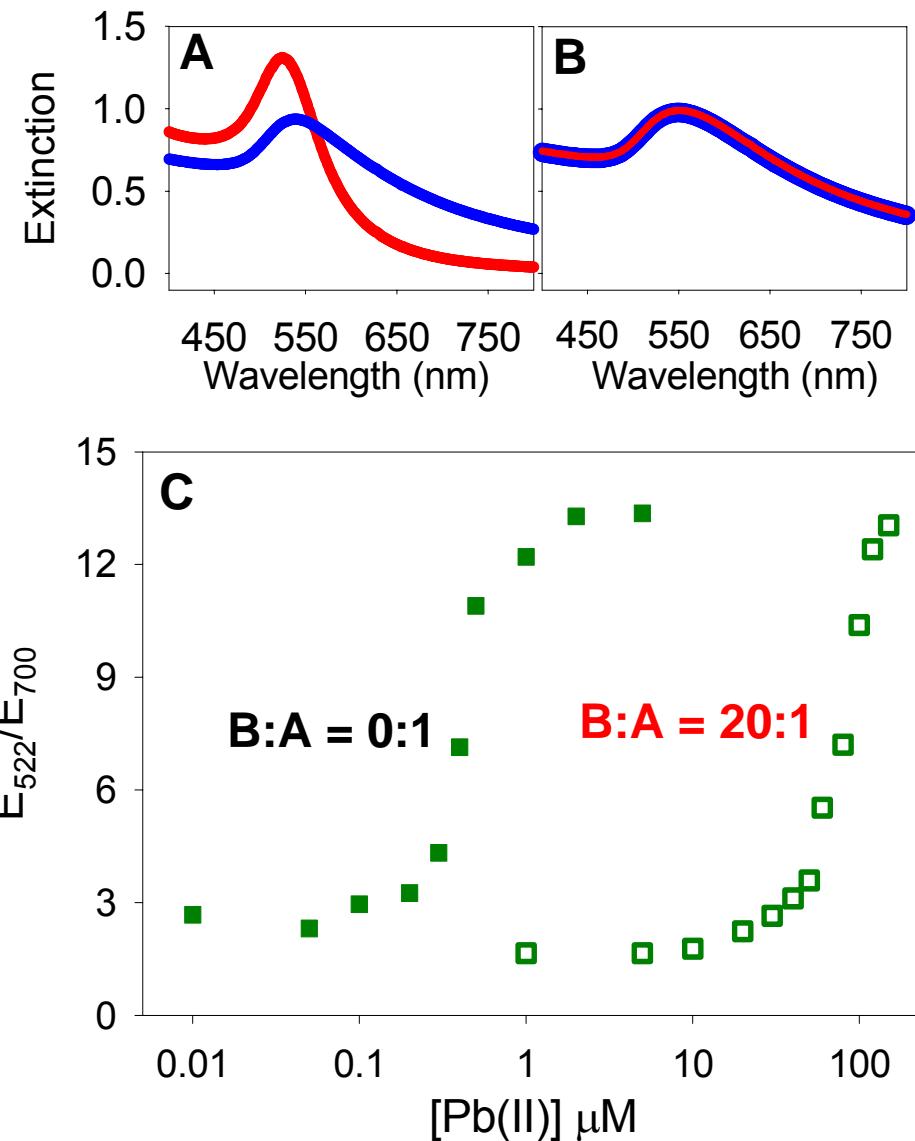
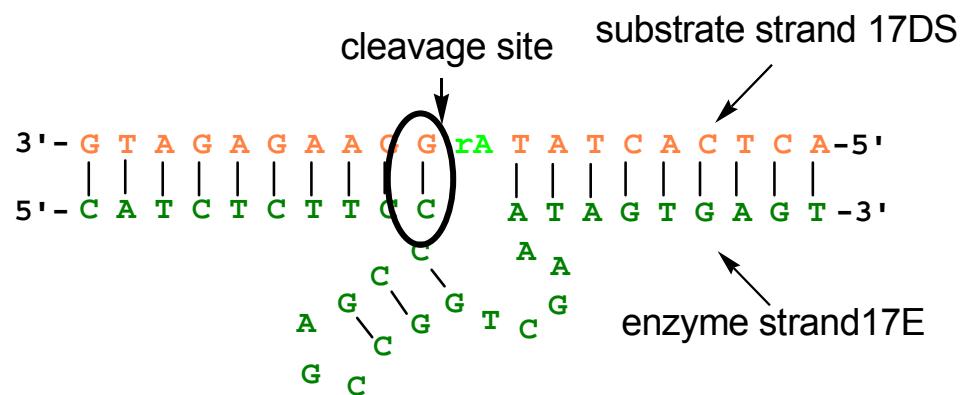
- ✓ a general method to obtain molecules for a specific analyte;
- ✓ a general method to improve selectivity;
- ✓ a general method to transform molecular recognition into physical detectable signals without compromising the binding affinity and selectivity;
- ? a general method to fine-tune the dynamic range.

4. A general method to tunable Dynamic Range

A. Active DNA

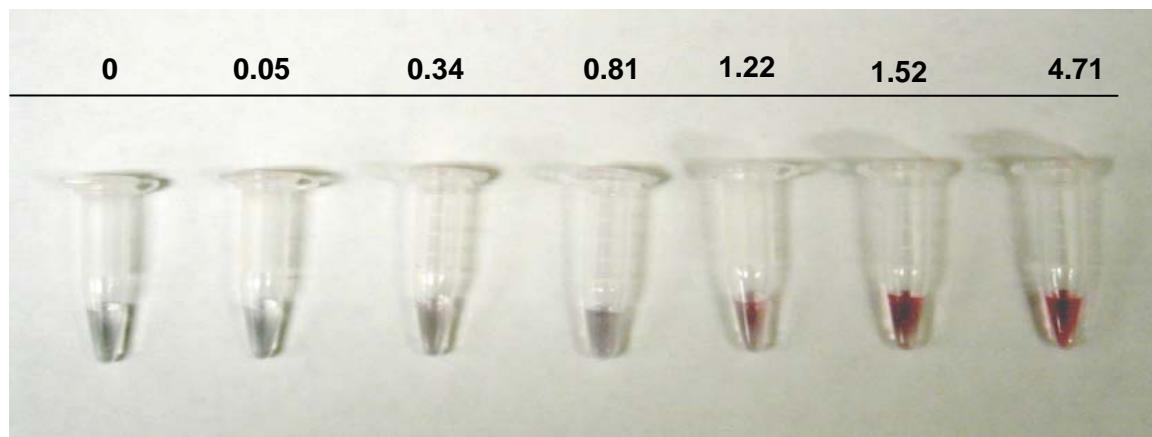
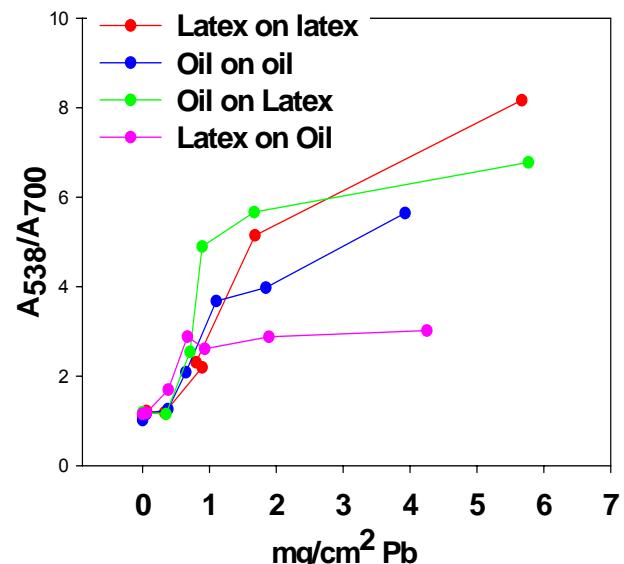
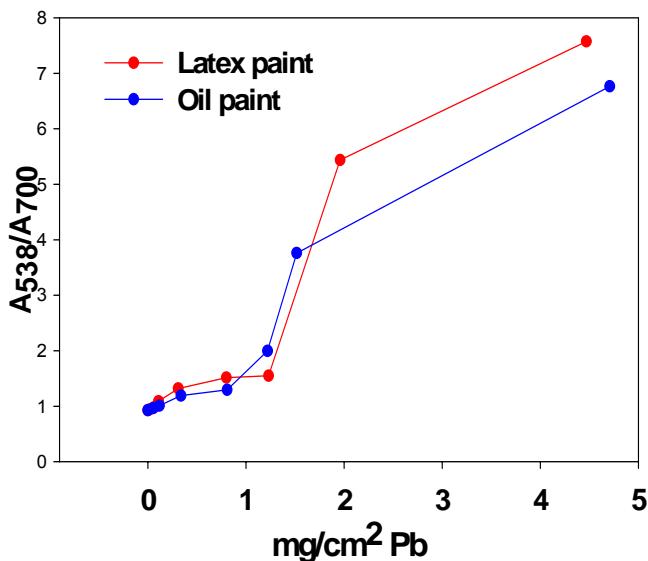
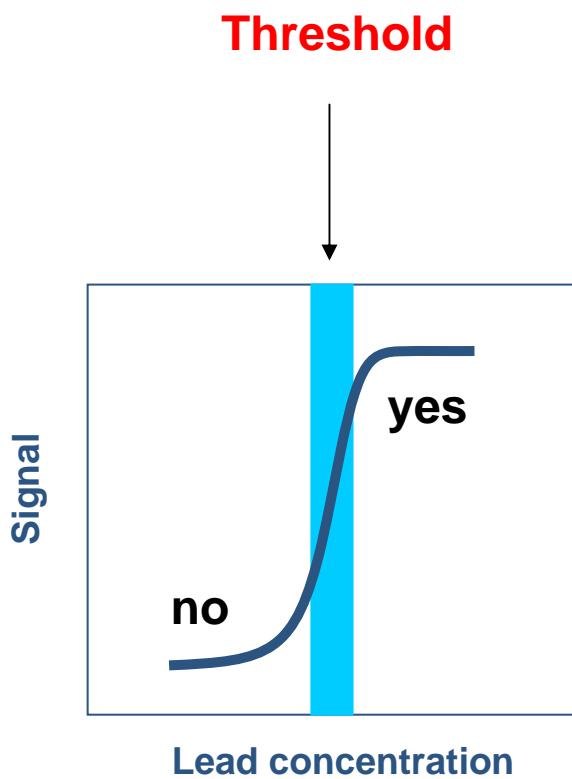


B. Inactive DNA



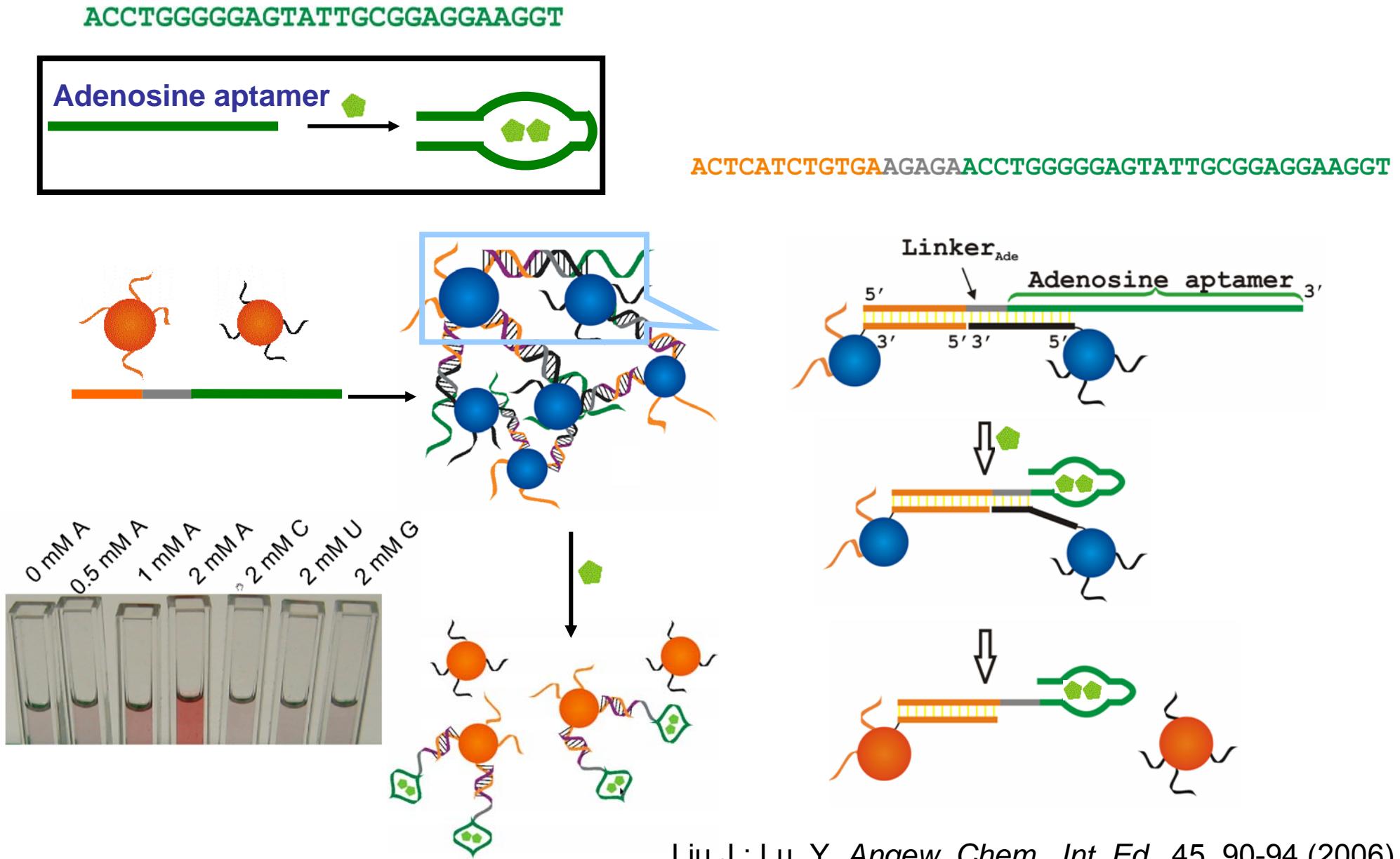
Brown, A. K.; Li, J.; Pavot, C. M.-B.; Lu, Y. *Biochemistry* 42, 7152-7161 (2003).
Liu, J.; Lu, Y. *J. Am. Chem. Soc.* 125, 6642-6643 (2003).

Pb²⁺ Detection in Paint

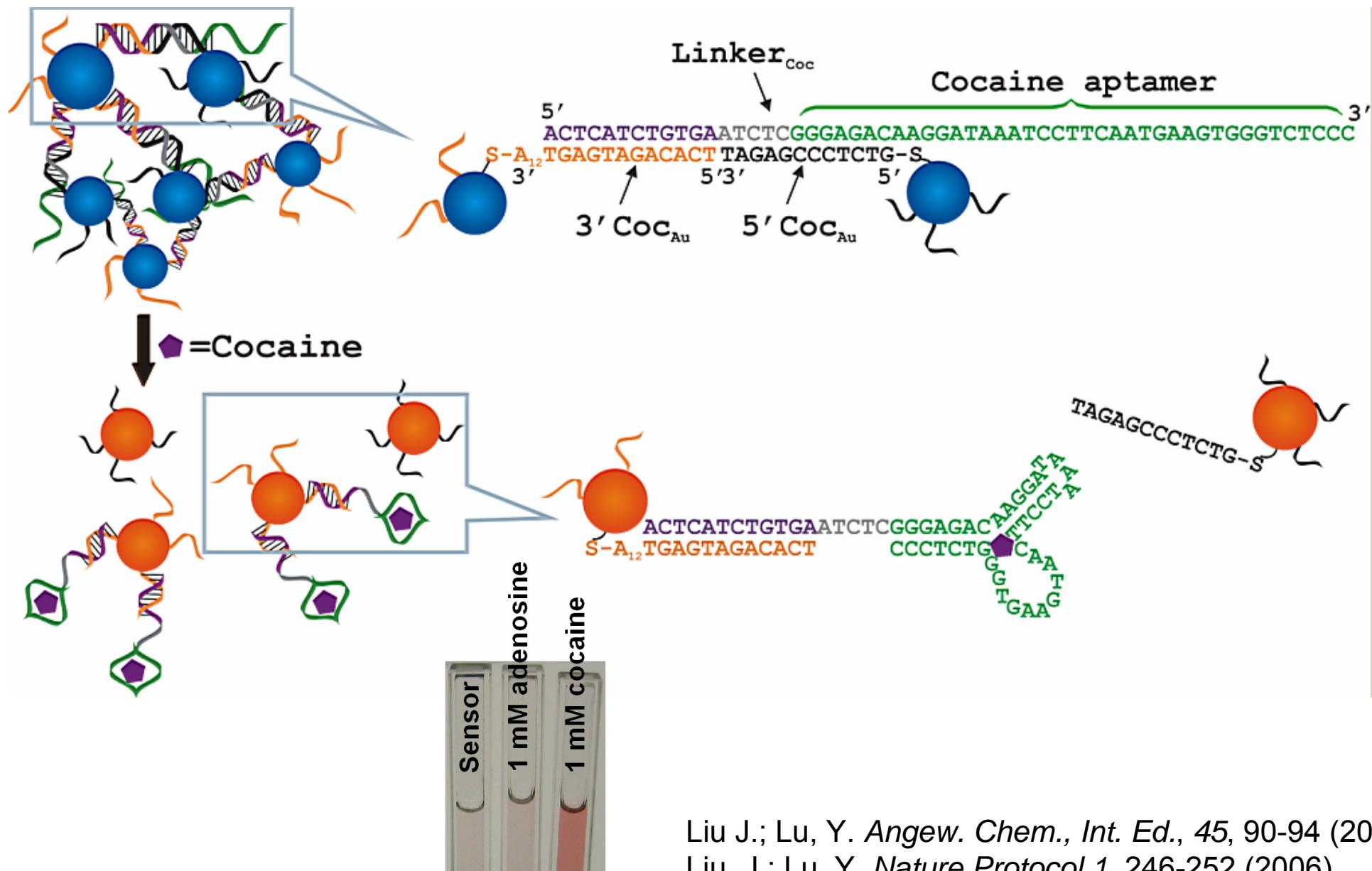


mg Pb / cm² paint

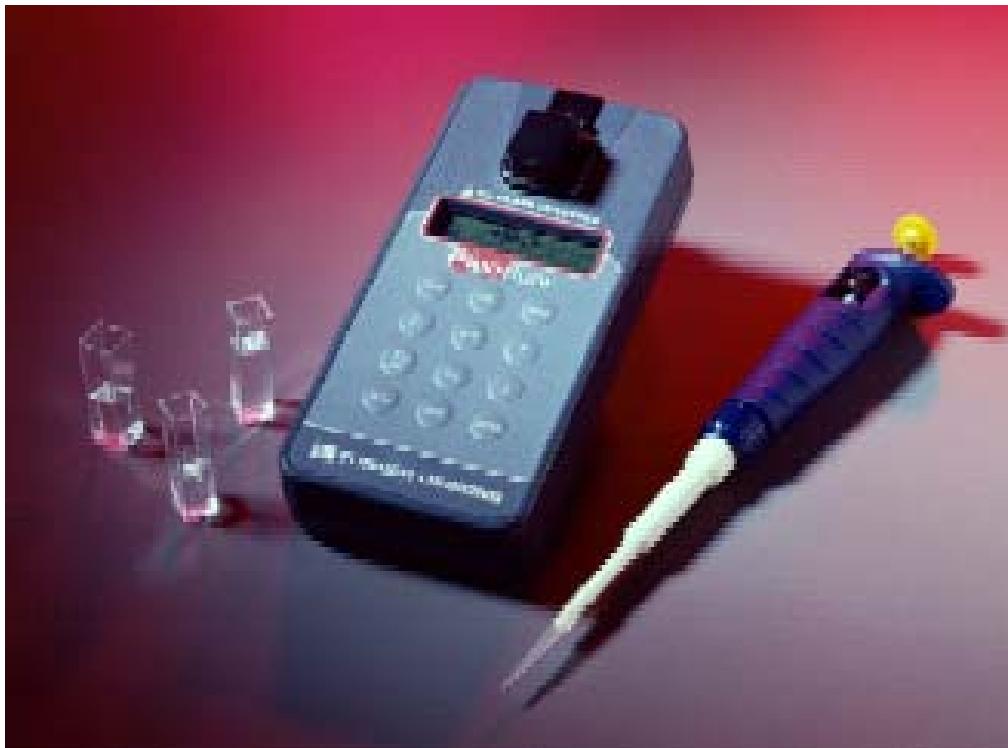
Beyond metal ion sensors



The method is general: a colorimetric cocaine sensor



Portable spectrometers for more accurate on-site, real-time detection and quantification



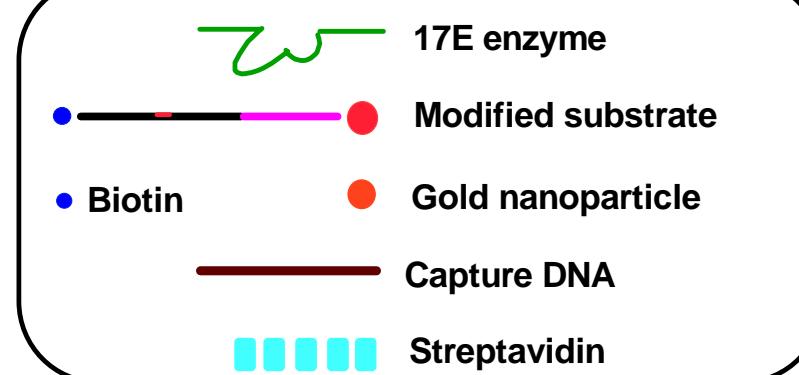
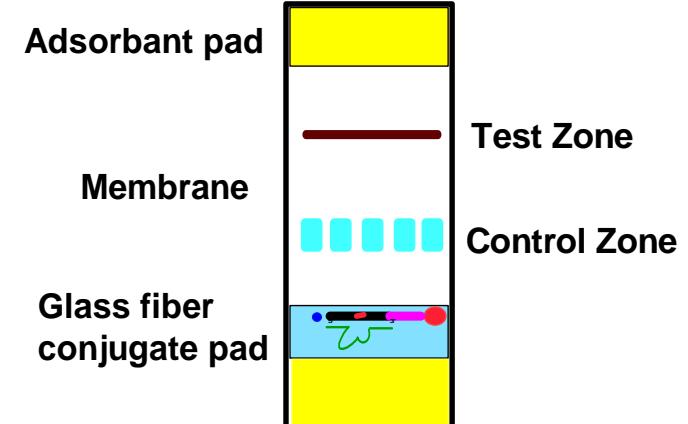
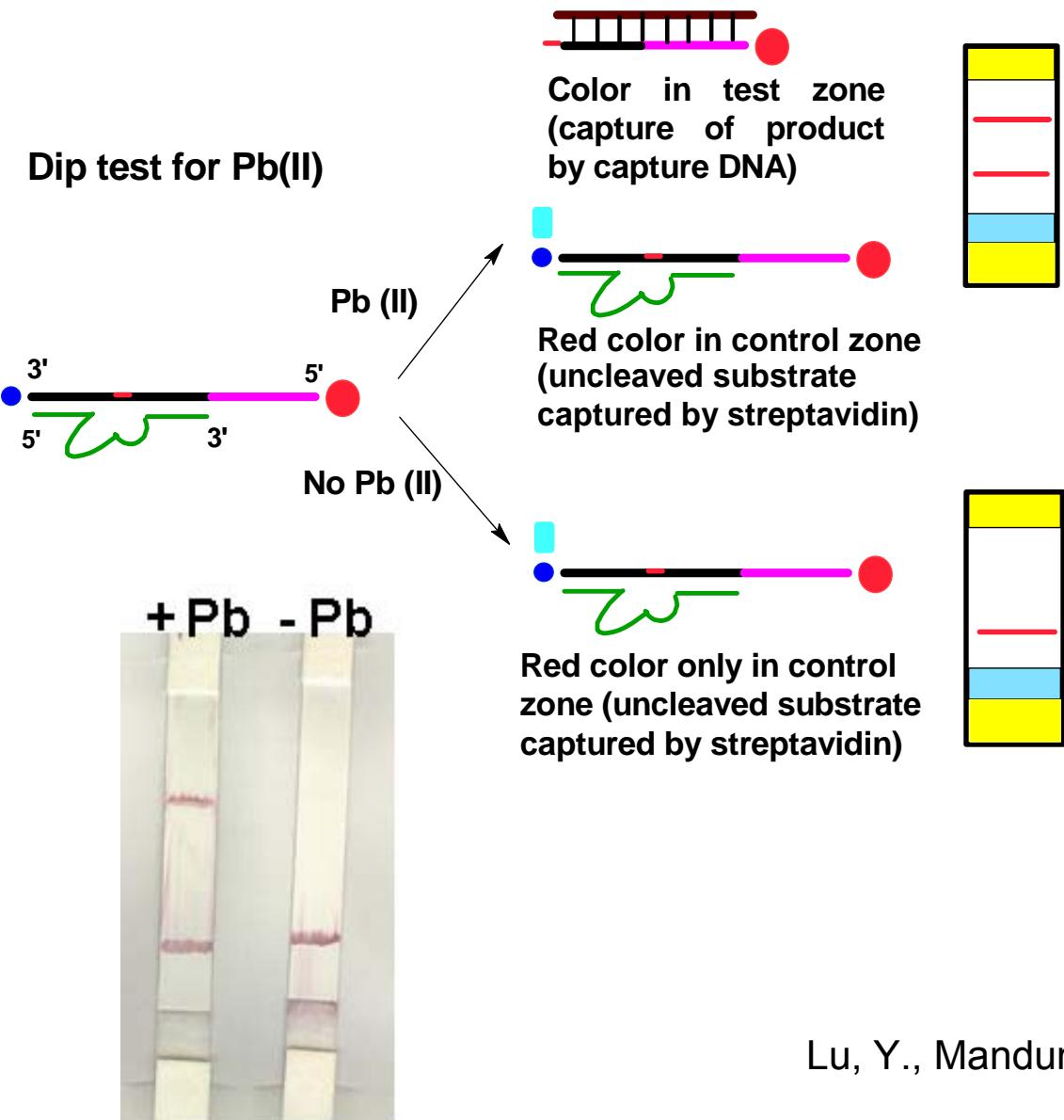
Fluorometer



UV-vis

Litmus paper for Pb²⁺

Dip test for Pb(II)

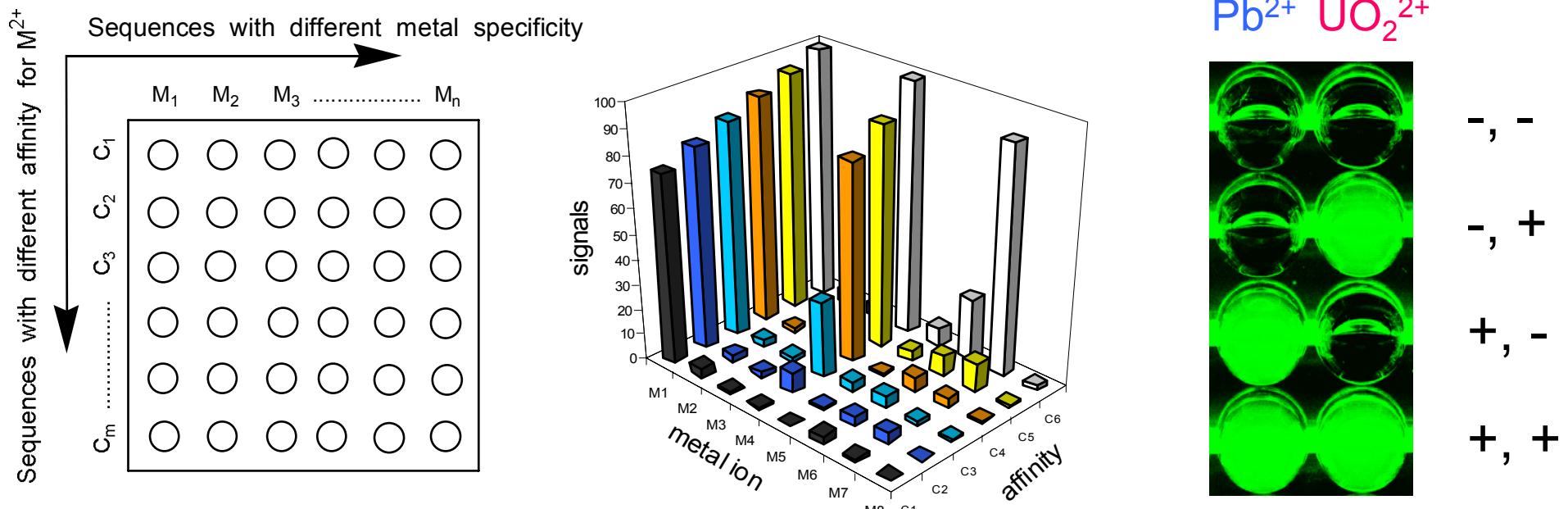


Summary

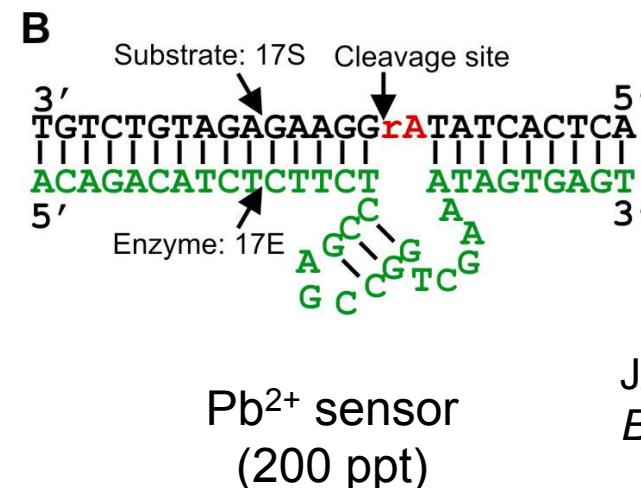
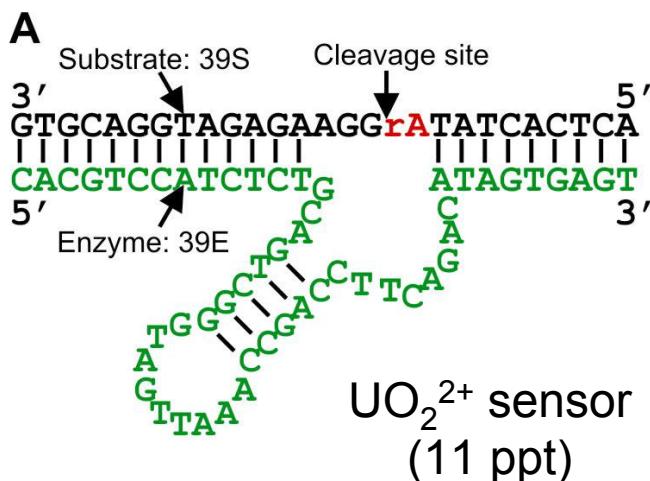
- To obtain sensitive and selective biosensors, we have demonstrated the following general strategies:
 - to obtain sensing molecules;
 - to improve selectivity;
 - to convert molecular recognition event into physically detectable signals (e.g., fluorescence and colorimetric);
 - to tune the dynamic range.
- The new catalytic DNA fluorescent and colorimetric sensors
 - are stable and cost-effective;
 - are highly sensitive and selective;
 - can detect bioavailable metal ions;
 - can be applied to detection and quantification of any metal ions in any different oxidation states;
 - can be apply for detection of organic contaminants;
 - allow real-time, on-site (or remote) sensing.

Future directions

Applications: DNAzyme microarrays



Fundamental Science: Structural features for selectivity



JACS, 124, 15208 (2002).
Biochemistry 42, 7152 (2003).

Acknowledgments

DNA/RNA Lab at UIUC

Graduate Students:

Andrea K. Brown

Peter J. Bruesehoff

Hee-Kyung Kim

Juewen Liu

Kevin E. Nelson

Daryl P. Wernette

Lynette Cunningham

Jing Li

Caroline Pavot

Postdoctoral fellows:

Daisuke Miyoshi

Wenchao Zheng

Xiaotang Wang

Lab for Fluorescence Dynamics

Dr. Robert Clegg

Sophia Breusege

Dr. Frank Stuhmoier

FRC, Oak Ridge National Laboratory

Dr. David Watson

Dr. Kenneth Lowe

Ms. Marcella (Sally) Mueller

Dr. Jack Istok

Ms. Mandy Michalsen

Funding

Department of Energy

Office of Science

ERSP program

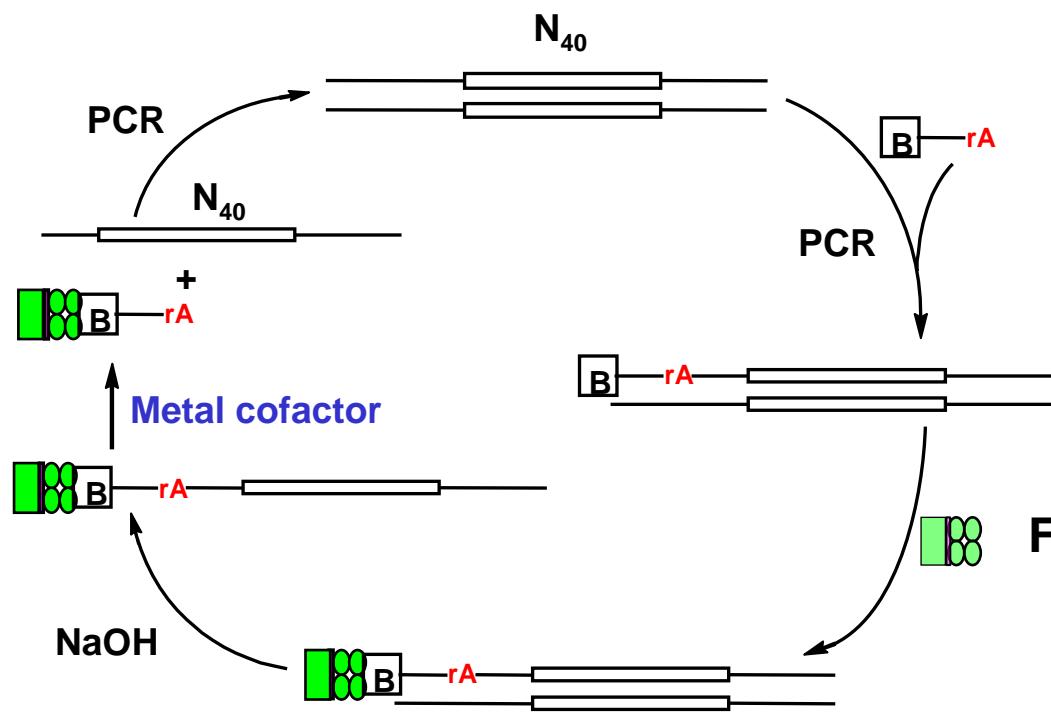
(DEFG02-01-ER63179)

Advantages of Catalytic DNA-based biosensors

- environmentally benign
- cost effective
- stable under rather harsh conditions
- can be denatured and renatured many times
- allow combinatorial search for desired metal-binding properties
- adaptable to optic fiber and microarray chip technology
- unlimited by the choice of fluorophore/optical tags
- easy to attach fluorophore/optical tags to any desired position
- activity-based detection immune to source fluctuation and electronic drift
- highly sensitive (down to ppt) and selective (more than 1 million fold)
- allows on-site, real-time detection and quantification with high spatial (< cm) and time (< min.) resolution.
- detect not only different metal ions, but also different oxidation states of the same metal ions, allowing sensing of bioavailable toxic metal ions.

Lu, Y. *Chem. Euro. J.* 8, 4588-4596 (2002).

A typical in vitro selection protocol



In vitro selection with $[M^{n+}]$ as metal ion cofactor

↓
Cloning and sequencing

↓
Introducing mutations

↓
Further selection by decreasing reaction time and $[M^{n+}]$

↓
Cloning and sequencing

↓
Characterization

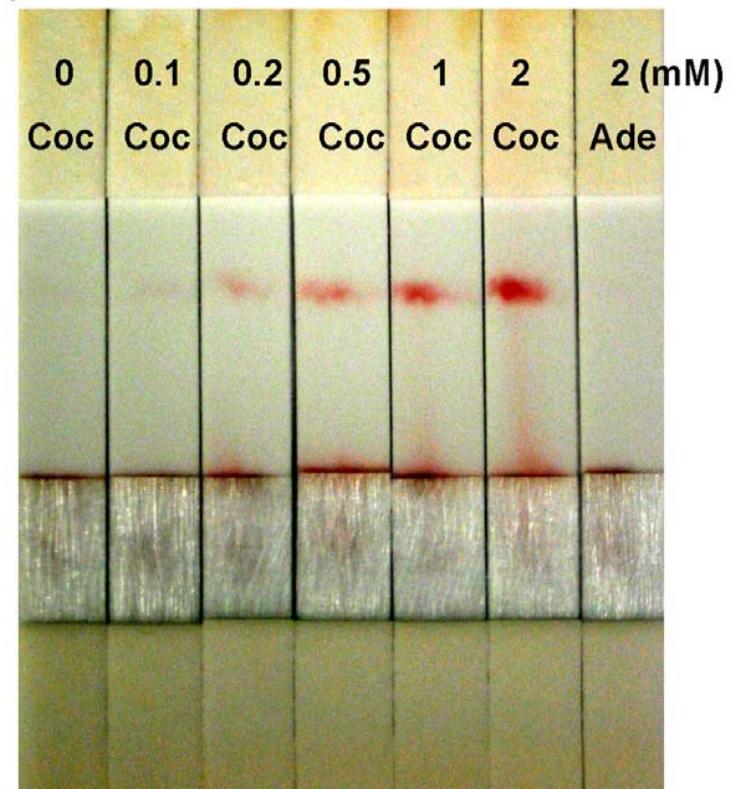
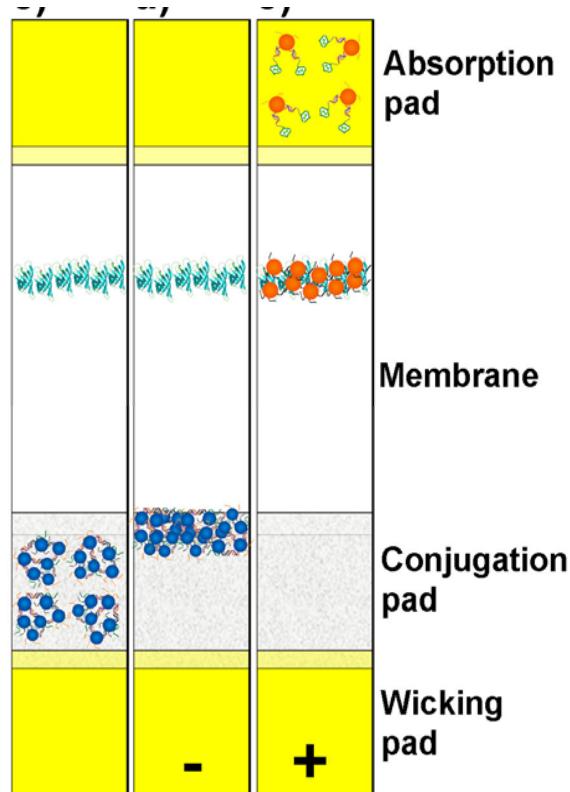
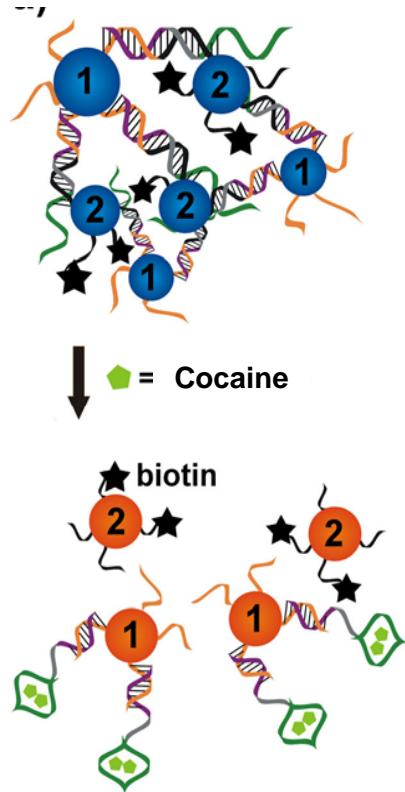
R. R. Breaker, G. F. Joyce *Chem. & Biol.* 1, 223 (1995).

J. Li, W. Zheng, A. H. Kwon, and Y. Lu

Nucleic Acids Res. 28, 481 (2000).

Adaptable to any metal ion and any oxidation state of the same metal ion with tunable activity, and affinity

An even more simple “dip-stick” test



In undiluted human blood serum