Functional DNA Nanotechnology in Sensing and Imaging

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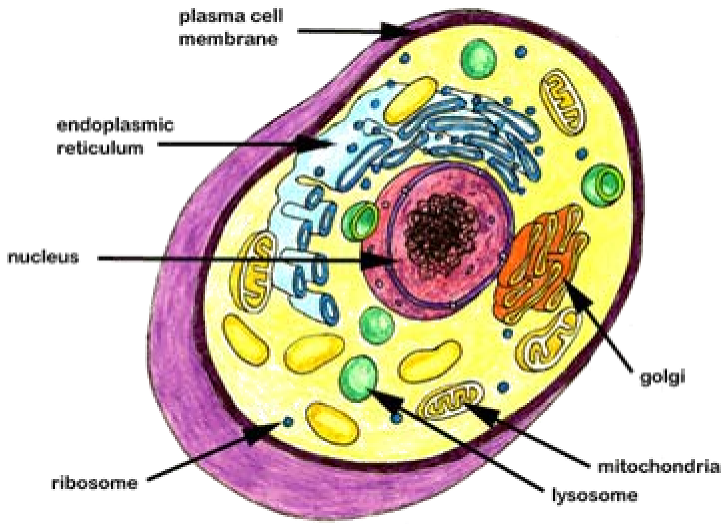
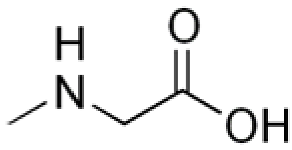
Science & Technology Gap in Nanobiotechnology





Sarcosine: Prostate cancer marker in urine (*Nature*, 2009, 457, 910)

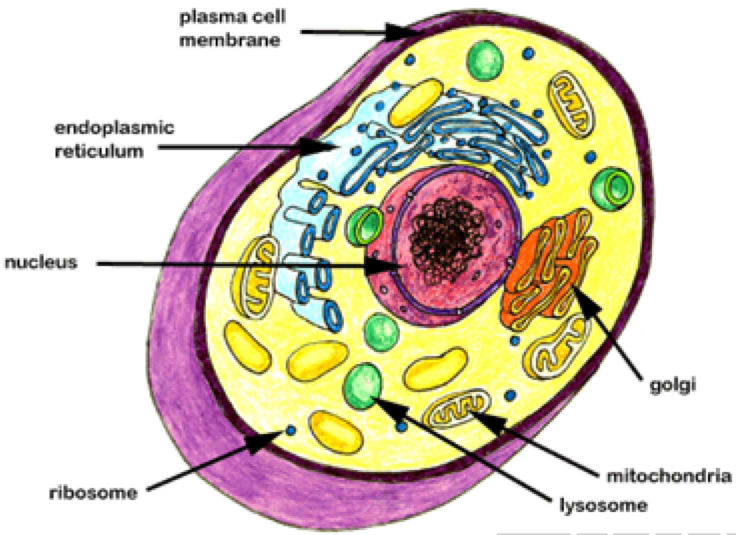
Well developed (relatively speaking): DNA, RNA and proteins

Less well developed:

- Carbohydrates
- Metabolites: metal ions: calcium, iron….
  organic molecules: ATP, NADH, hormone, receptor….
  (biomarkers for biological events such biomass conversion)
- Invasive species: metal ions: lead, mercury…
  organic molecules: dioxins, pesticides, PCBs, melamine..
  (biomarkers for diseases such as cancer)

A number of non-DNA/RNA/Protein biomarkers remain to be discovered and detected
Correlation of metabolites/biomarkers with DNA/RNA/Protein levels and with biological function is an unmet challenge in nanobiotechnology.

Metabolites/markers in biology and environment



- Essential Metal ions
- Bulk and essential non-metal ions
- Non-essential biological elements
- Toxic metal ions
- Radionuclides

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 Sb	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 Pb	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 Fm	101 Md	102 No		

Metabolites/markers are large in numbers, subtle in structural differences, small in quantities. Therefore they represent new unique challenges and opportunities in nanobiotechnology.

Instrumental Analyses for Metabolites/biomarkers

Inductively Coupled Plasma

Mass spectrometry

Advantages

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

Disadvantages

- ↓ Require sophisticated equipment, sample-pretreatment, and skilled operators
- ↓ difficult for in-situ, on-site, remote or real-time detection/imaging

Four key steps in designing sensors

? a general method to obtain molecules for any specific target (e.g., Pb^{2+}, Amphetamine, cocaine, Ricin, cancer)

? 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.

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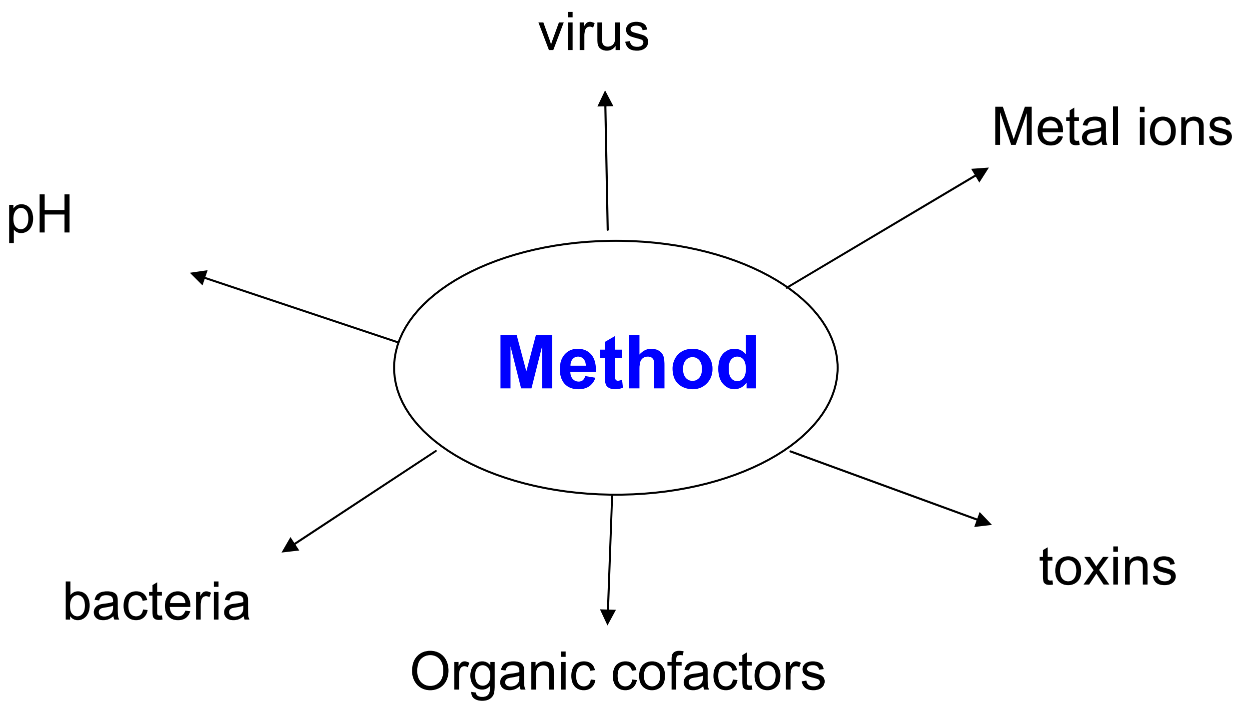
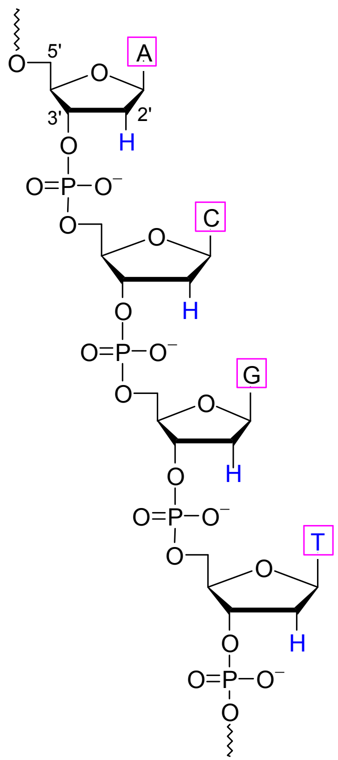


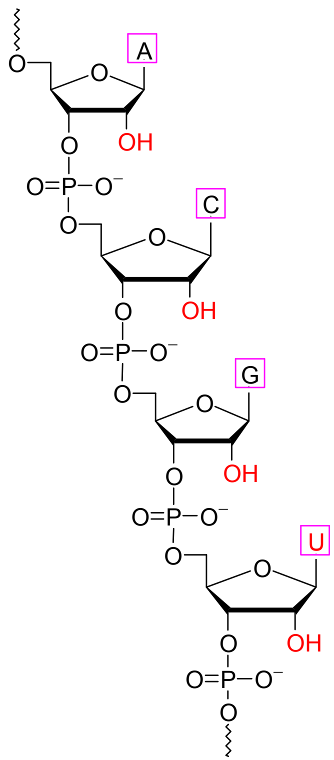
The success on designing sensors for one target (e.g., pH) cannot be translated into success for designing sensors for other target analytes. Until recently, antibody is the only method that is general enough for a broad range of targets, but antibodies are not very good at sensing small molecular metabolites and biomarkers.

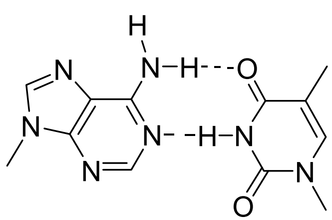
Functional DNA: a new paradigm in biology?

DNA

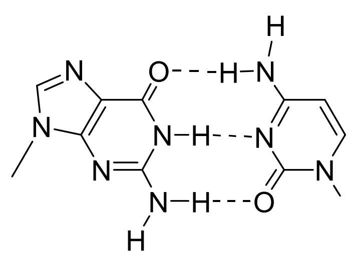


RNA



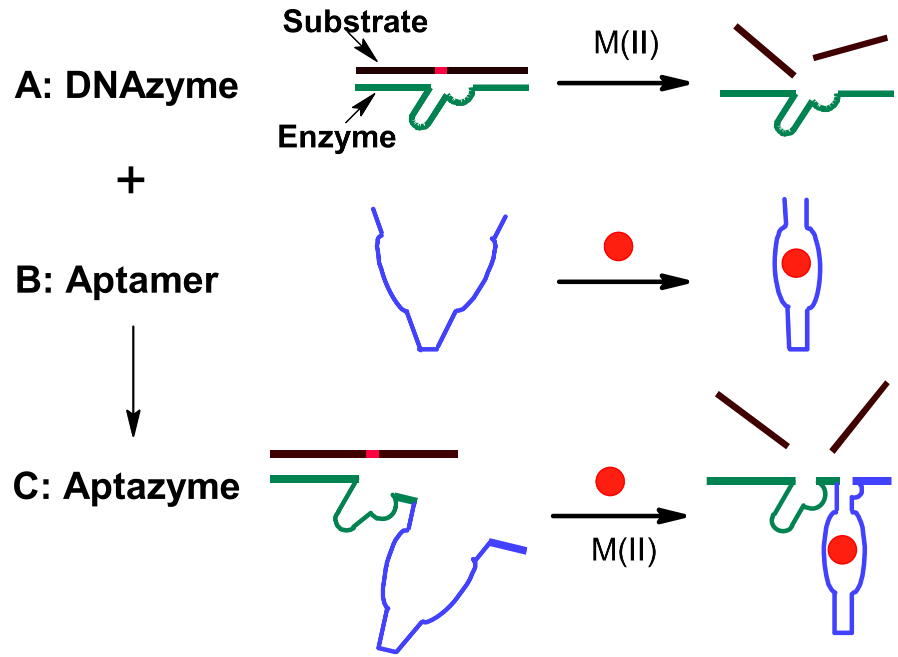


A — T



G — C

DNA/RNA = Protein enzymes
DNA/RNA = Antibodies

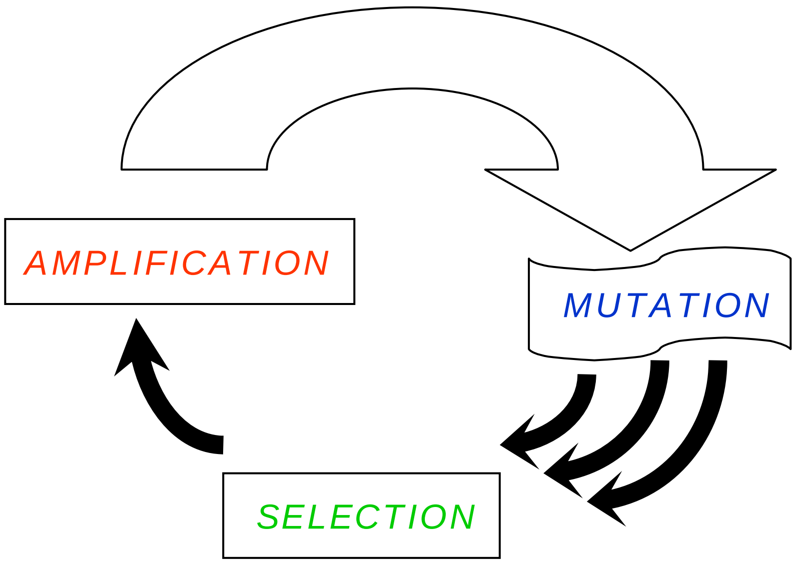


Kruger, K. *et al. Cell* 31, 147 (1982).
Guerrier-Takada, C. *et al. Cell* 35, 849 (1983).
Breaker, R.; Joyce, G. *Chem. Biol. 1*, 223 (1994).

Combinatorial biology: a general method to obtain DNA/RNA for a specific target

***In vitro* Selection**

Systematic Evolution of Ligands by Exponential Enrichment (SELEX)



Advantages:

- High throughput (10^{14}-10^{15} different sequences)
- Selective Amplification
- Improvement in each round
- Minimal cost
- Short time

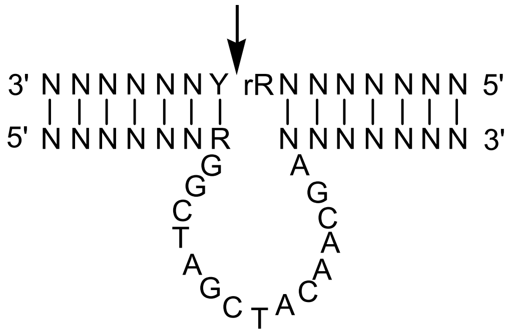
Ellington, A. D.; Szostak, J. W. *Nature 346*, 818(1990).
Tuerk, C.; Gold, L. *Science 249*, 505 (1990).
Beaudry, A. A.; Joyce, G. F. *Science 257*, 635 (1992).

Molecules Recognized/bound by Selected DNA/RNA

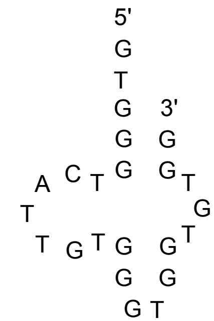
Analyte/target type	Examples
Metal ions	Mg(II), Ca(II), Mn(II), Pb(II), Hg(II), U(VI)
Organics	Cibacron blue, reactive green 19
Amino acids	L-Valine, D-Tryptophan
Nucleosides/nucleotides	Guanosine, ATP
Nucleotide analogs	8-oxo-dG, 7-Me-guanosine
Biological cofactors	NAD, FMN, porphyrins, Vitamin B_{12}
Aminoglycosides	Tobramycin, Neomycin
Antibiotics	Streptomycin, Viomycin
Peptides	Rev peptide
Enzymes	Human Thrombin, HIV Rev Transcriptase
Growth cofactors	Karatinocyte GF, Basic fibroblast GF
Antibodies	human IgE
Gene regulatory factors	elongation factor Tu
Cell adhesion molecules	human CD4, selectin
Intact viral particles	Rous sarcoma virus, Anthrax spores

Juewen Liu and Yi Lu, *J. Fluoresc*. 14, 343-354 (2004).

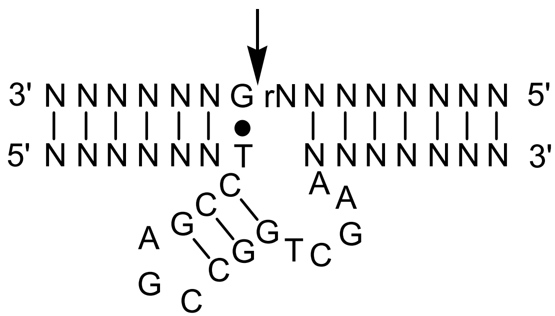
Examples of in vitro selected DNAzymes



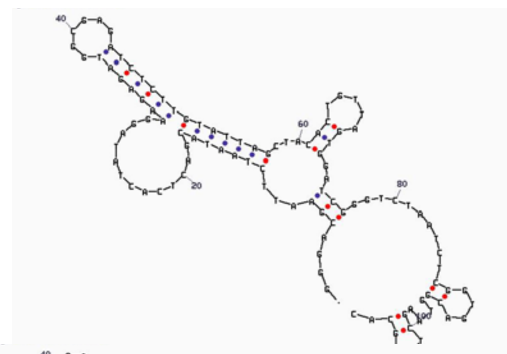
Mg^{2+}
(Joyce)



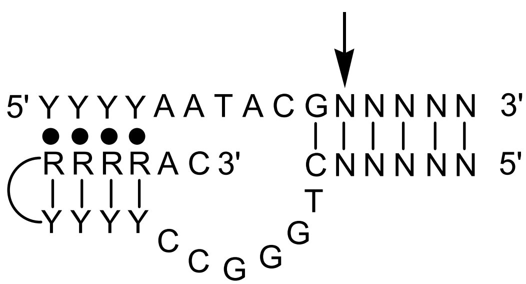
Heme
(Sen, Li)



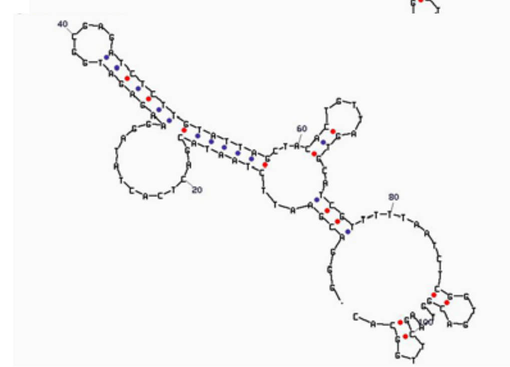
Pb^{2+}
(Lu, Joyce)



Zn^{2+}
(Lu)



Cu^{2+}
(Breaker)



Co^{2+}
(Lu)

Sen, D. & Geyer, C.R. *Curr. Opin. Chem. Biol.* 2, 680-7 (1998).
Li, Y.; Breaker, R. R. *Curr. Opin. Struct. Biol. 9*, 315-323 (1999).
Lu, Y. *Chem. Euro. J.* 8, 4588-4596 (2002).

Why using functional DNA in sensing?

- environmentally benign
- cost effective
- stable under rather harsh conditions
  - DNA is ~1,000-fold more stable to hydrolysis than proteins and ~ 100,000-fold more stable than RNA
  - Globular shape:
    - More resistant to nucleases
    - Less likely to bind other molecules
- can be denatured and renatured many times (for manufacturing and storage)
- Can be readily modified for signal transduction
- can be genetically engineered to be delivered to a specific location

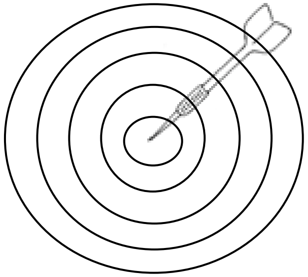
Juewen Liu, Zehui Cao, and Yi Lu, *Chem. Rev.* (in press).

Four key steps in designing sensors

√ a general method to obtain molecules for a specific analyte;

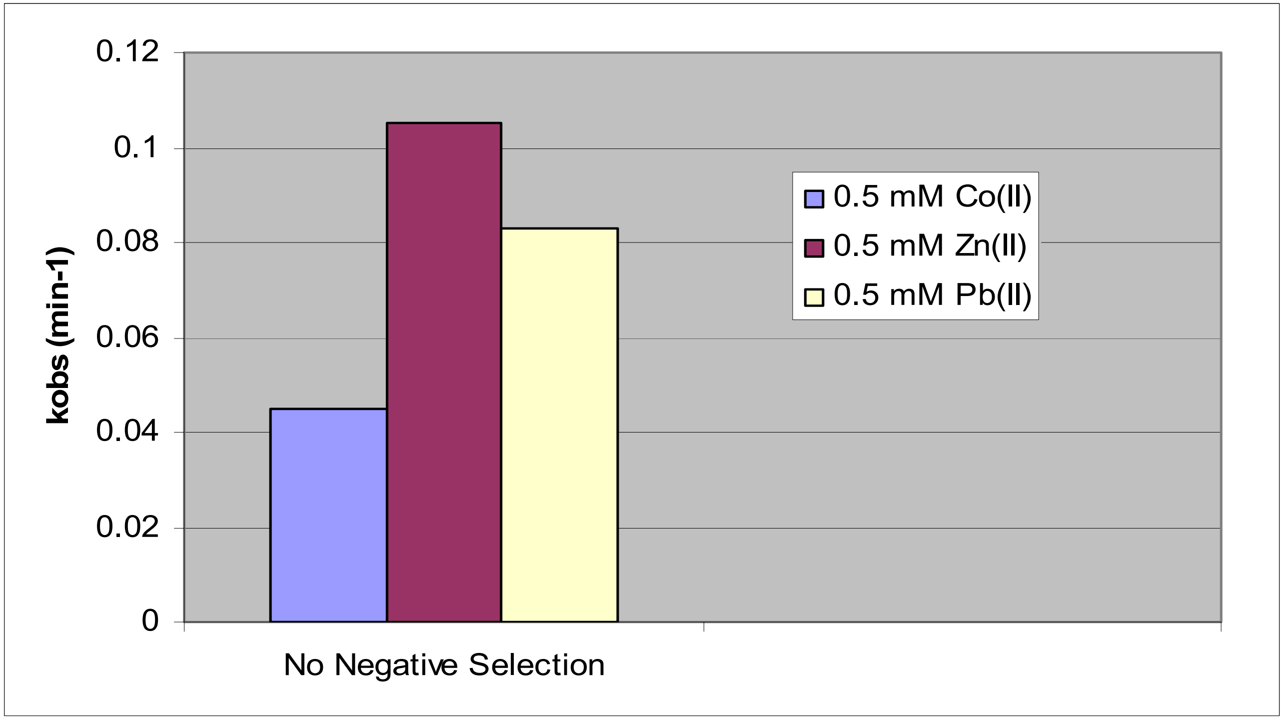
? a general method to improve selectivity;

What most want to do



What most end up doing

Problems with Selectivity in *in vitro* Selection

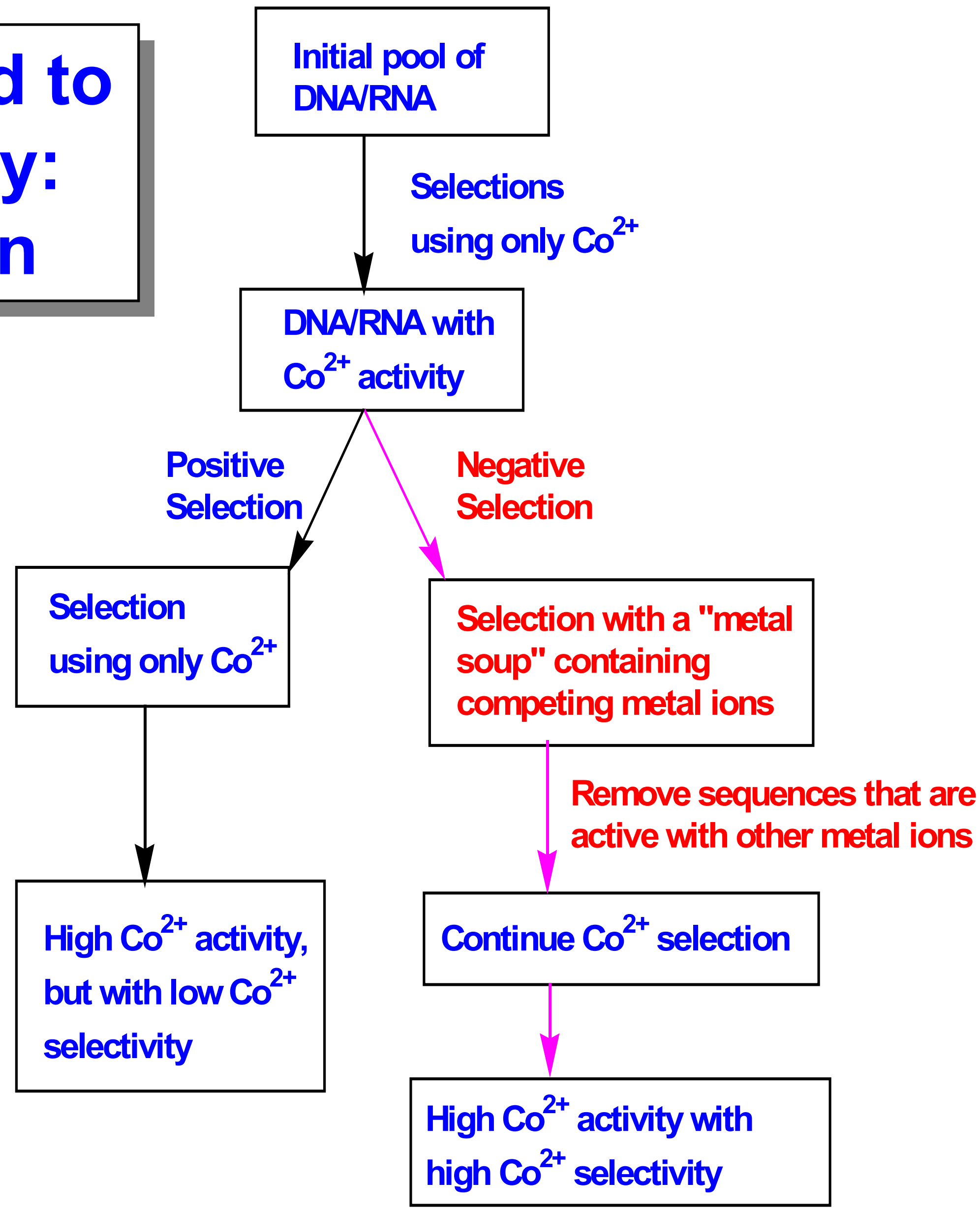


- **Sequences selected with Co^{2+} show high activity with Zn(II) and Pb(II)**
- **Must reduce or remove unwanted activity to improve metal selectivity**

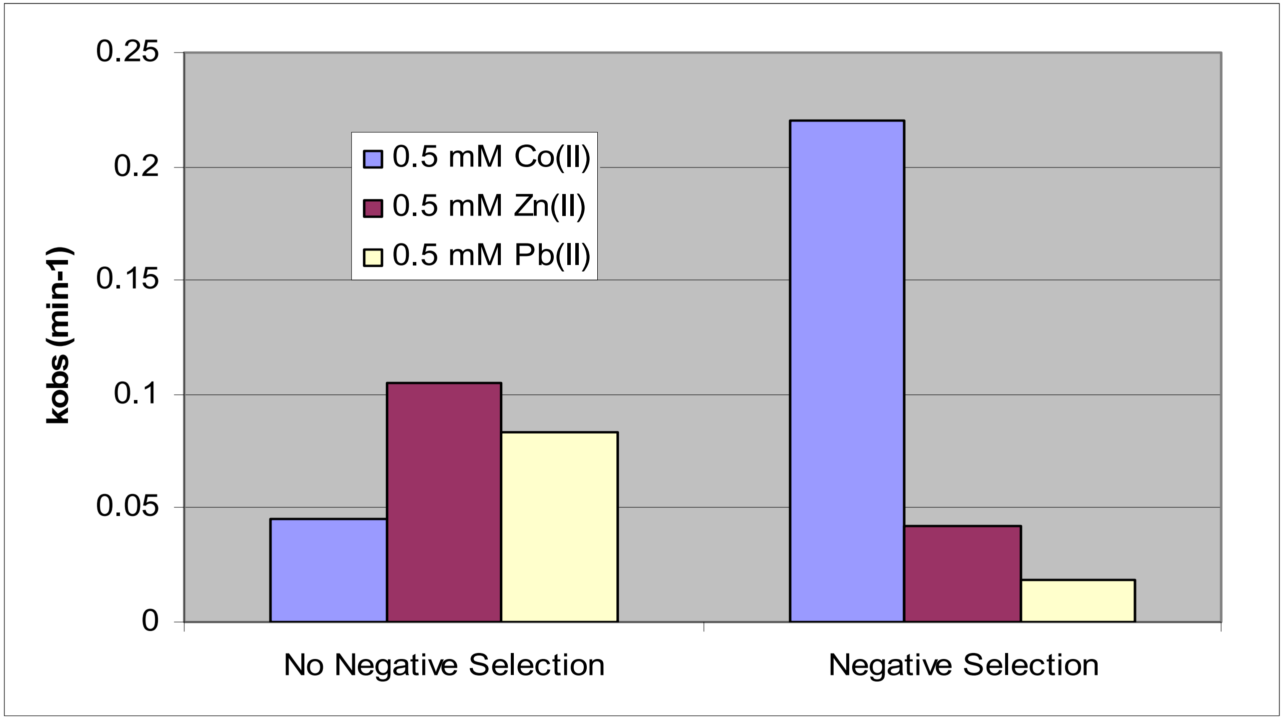
2. A general method to improve selectivity: negative selection

- Incorporate negative selection to reduce “unwanted” activity
- Can be performed in parallel with positive selection for comparison

P. J. Bruesehoff, J. Li, A. J. Augustine III, and Y. Lu, *Combinatorial Chemistry and High Throughput Screening*, *5*, 327-335 (2002).



Improved Metal Ion Selectivity after Negative Selection



P. J. Bruesehoff, J. Li, A. J. Augustine III, and Y. Lu, *Combinatorial Chemistry and High Throughput Screening*, *5*, 327-335 (2002).

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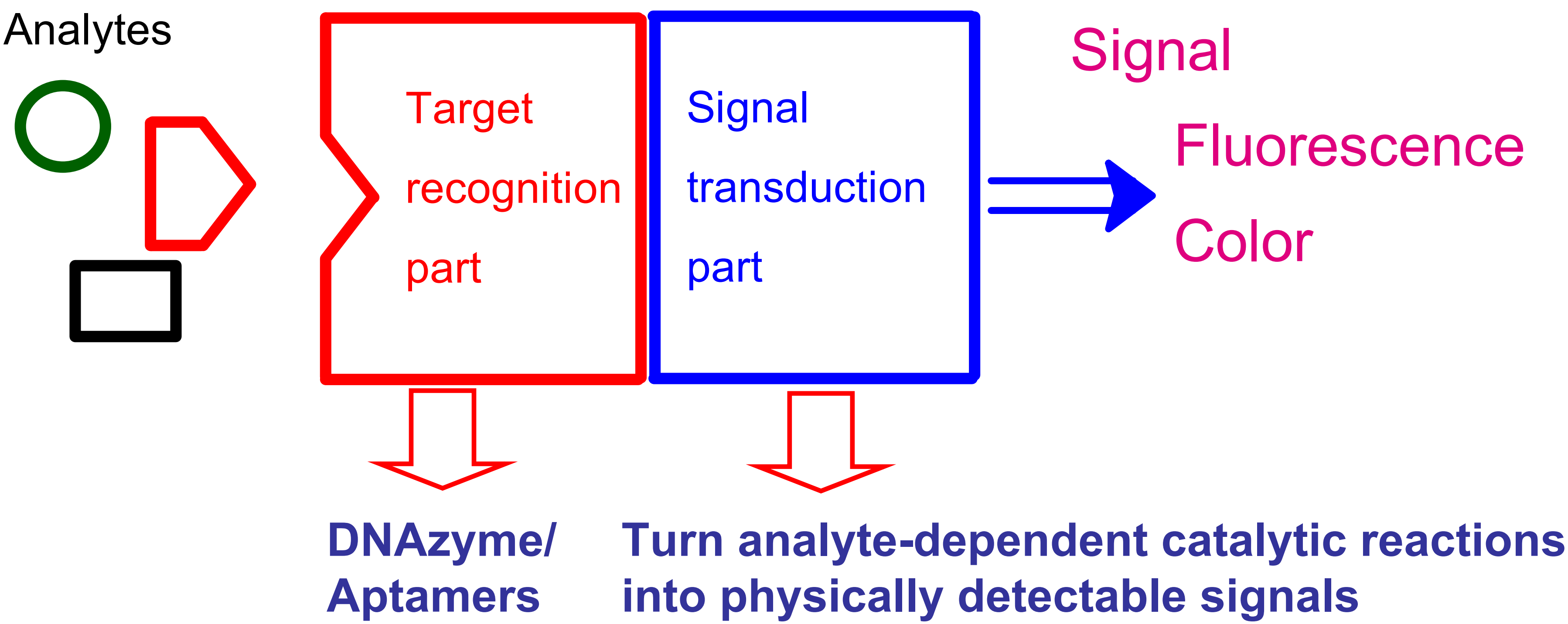
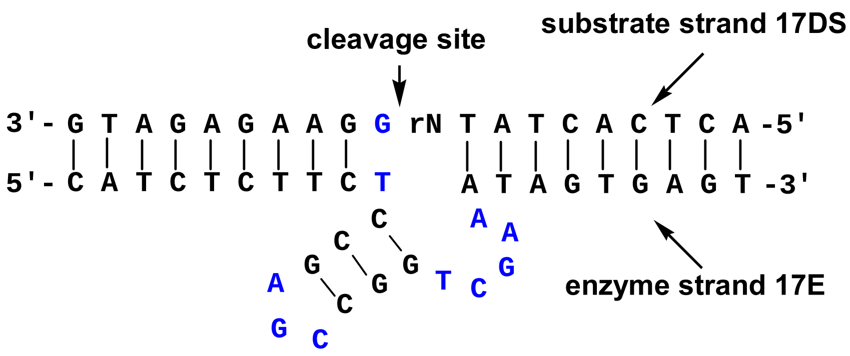
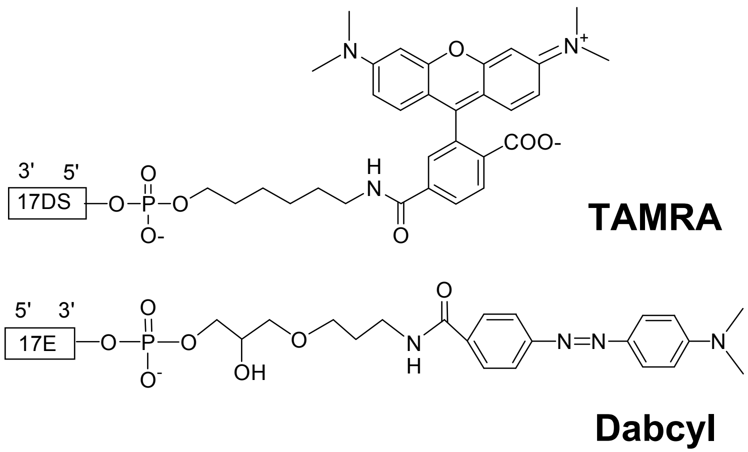
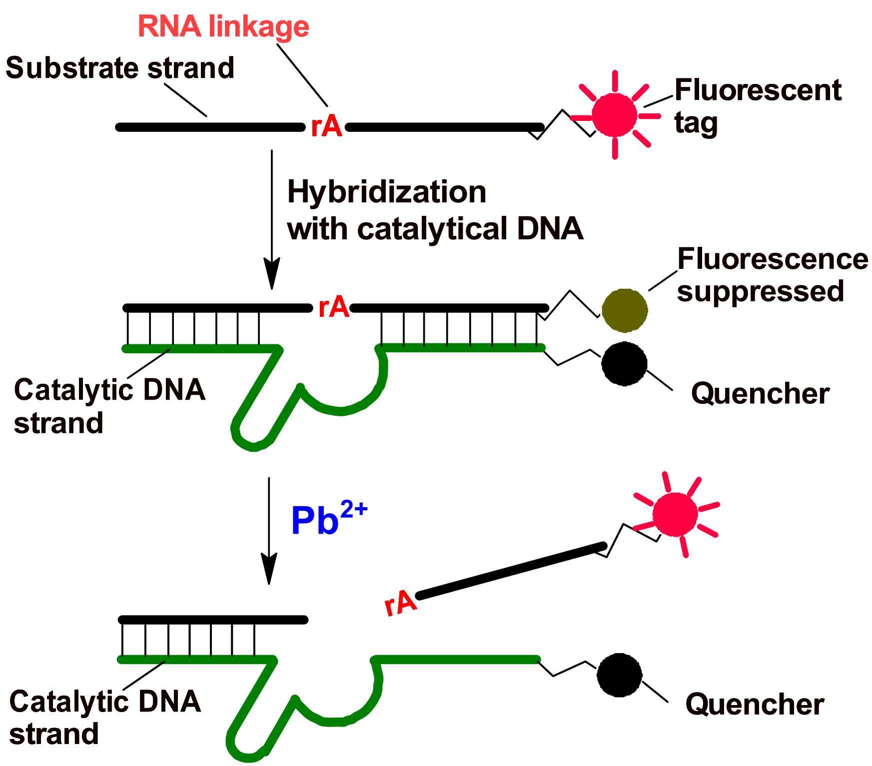
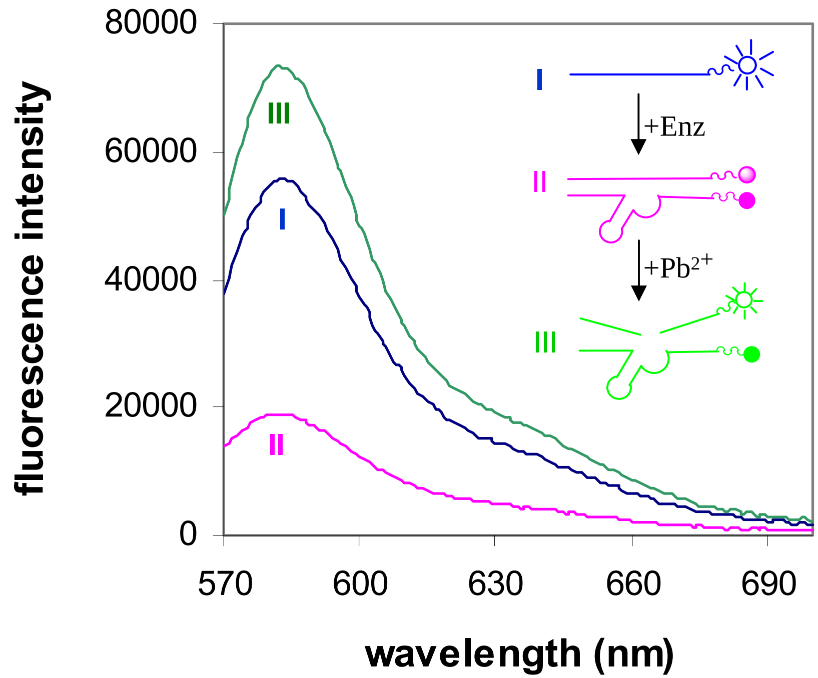


A general method to convert DNAzymes into fluorescent sensors using catalytic beacon





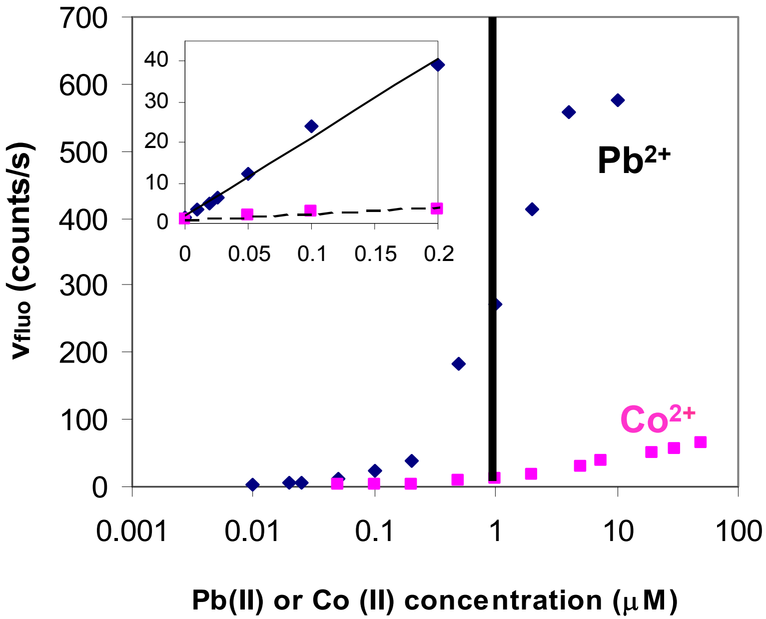




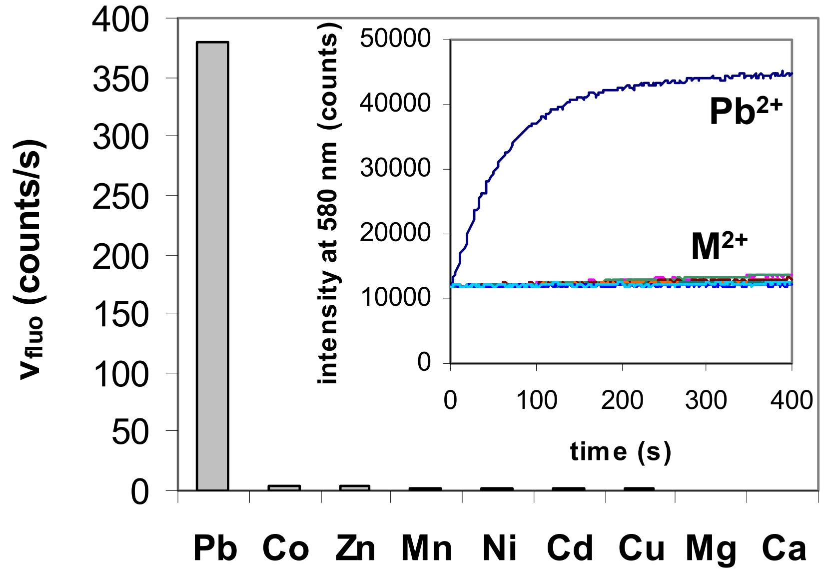
Li, J.: Lu, Y. *J. Am. Chem. Soc., 122,* 10466-10467. (2000).

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

Sensitivity



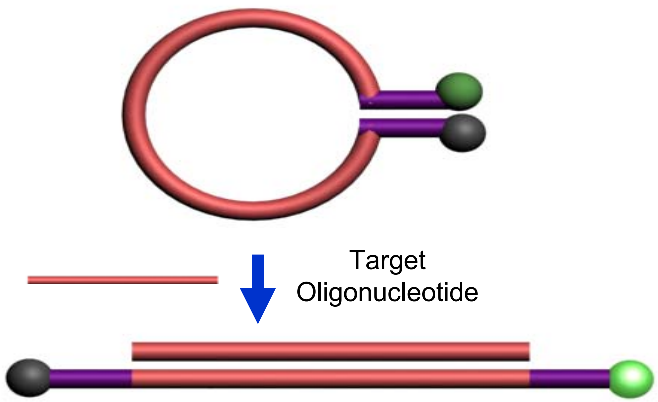
Selectivity

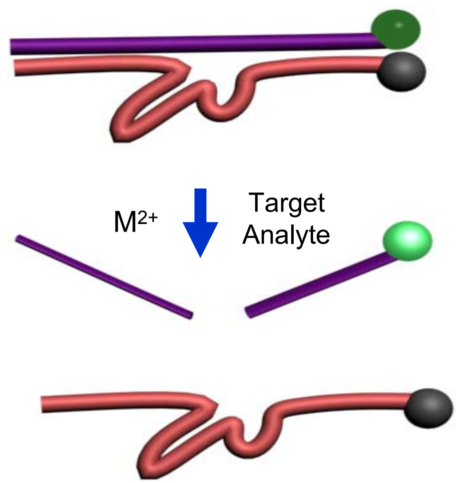


Dynamic range: 1 nM (0.2 ppb) to 4 μM (800 ppb)
(Lead toxic level defined by US CDC: 500 nM (100 ppb))
(Lead toxic level defined by US EPA: 75 nM (15 ppb)

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 & Bioeletronics* **18**, 529-540 (2003).
Swearingen, C. B. et al., *Anal. Chem*. 77, 442-448 (2005).

Molecular Beacon vs. Catalytic Beacon





Molecular beacon

Catalytic beacon

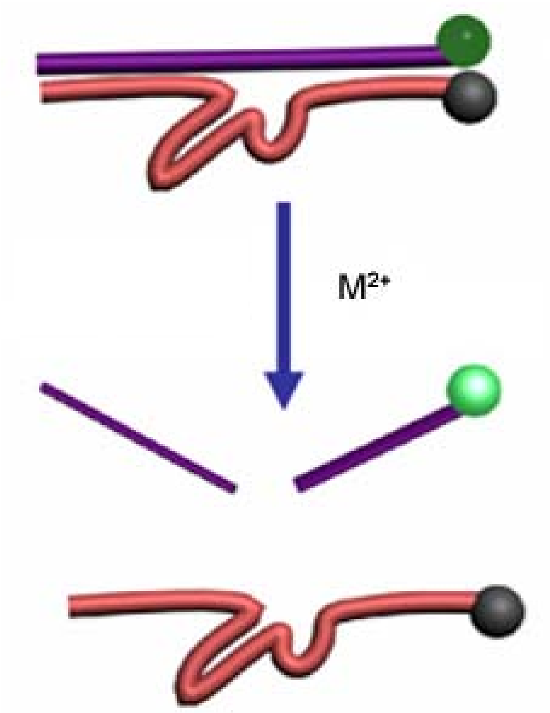
Can detect DNA/RNA only
No signal amplifications

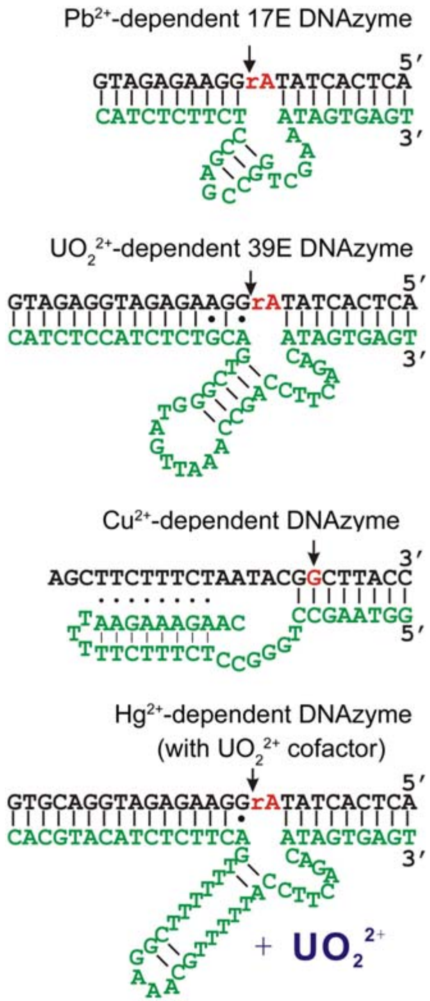
Can detect a broad range of targets
Signal amplifications

S. Tyagi and F. R. Kramer, *Nature Biotech.* 14, 303-308 (1996).
J. Li and Y. Lu, J. Am. Chem. Soc. 122, 10466-10467 (2000).

A general method to convert DNAzymes into fluorescent sensors using catalytic beacon for a broad range of metal ions

Fluorescent sensors
Based on catalytic beacon





Pb^{2+}: Detection limit: 1 nM
EPA MCL: 75 nM

UO_2^{2+}: Detection limit: 45 pM
EPA MCL: 126 nM

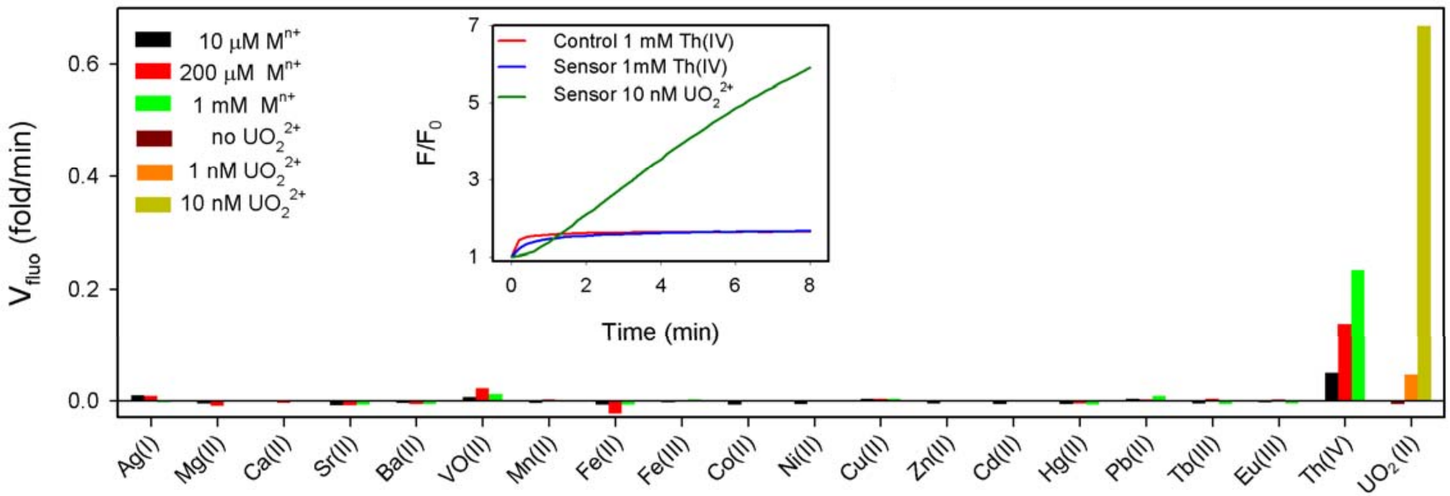
ICP-MS: 420 pM

Cu^{2+}: Detection limit: 35 nM
EPA MCL: 20 μM

Hg^{2+}: Detection limit: 2.4 nM
EPA MCL: 10 nM

Li, J.: Lu, Y. J. Am. Chem. Soc., 122, 10466-10467. (2000).
J. Liu, et al. *Proc. Natl. Acad. Sci* 104, 2056 (2007).
J. Liu and Y. Lu J*. Am. Chem. Soc*. 129, 9838 (2007).
J. Liu and Y. Lu, *Angew. Chem., Int. Ed.,* 46,7587 (2007).

High specificity or selectivity

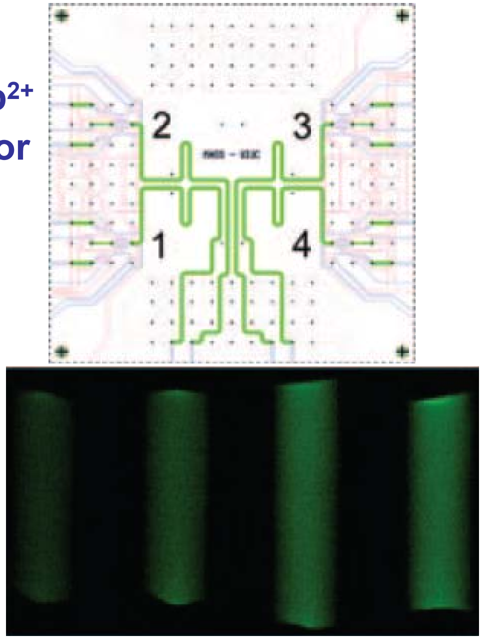


Over 1 million fold selectivity over Th(IV),
and hundreds of millions fold selectivity over other metal ions

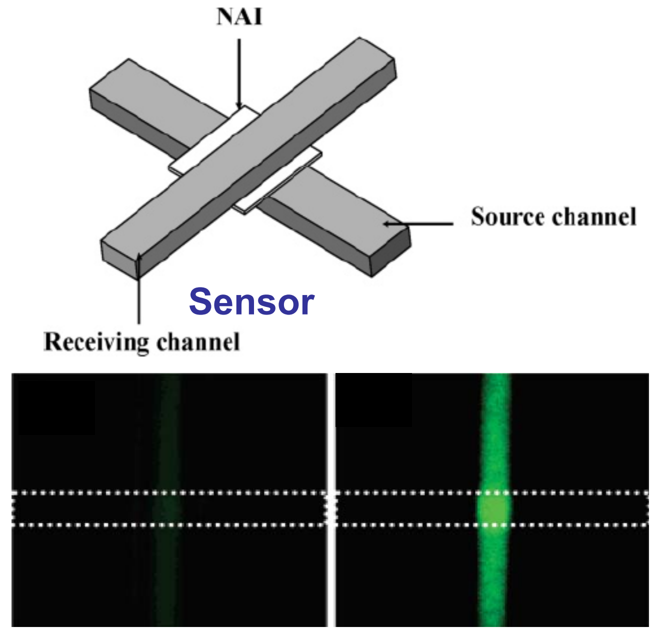
Juewen Liu, Andrea K. Brown, Xiangli Meng, Donald M. Cropek, Jonathan D. Istok, David B. Watson, and Yi Lu, *Proc. Natl. Acd. Sci. USA* 104, 2056 (2007).

Miniaturization of the Fluorescent Sensor for remote or in vivo monitoring

Pb^{2+} Sensor



0 0.5 2 10 μM Pb^{2+}



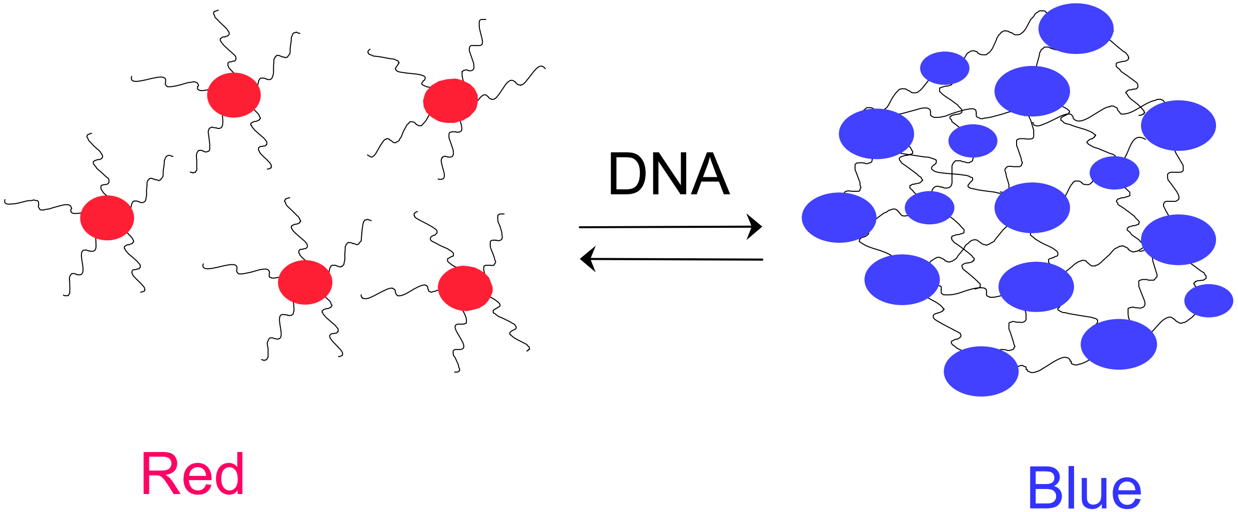
$-Pb^{2+}$ $+Pb^{2+}$

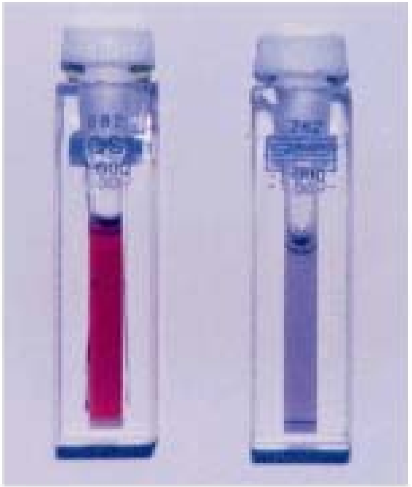
- The DNAzyme is compatible with microfluidic channels
- Very small sample consumption (< 1 nL)
- Automated detection possible
- Continuous monitoring possible

Shaikh K.; Ryu K.; Doluch E.; Nam J.; Liu J.; Thaxton C.; Chiesl T.; Barron A.; Lu Y.; Mirkin C.; Liu C. *PNAS*, **2005**, 102, 9745.

Chang I.; Tulock J.; Liu J.; Kim W.; Cannon D.; Lu Y.; Bohn P.; Sweedler J.; Cropek D., *Environ. Sci. Technol*, **2005**, 39, 3756.

Colorimetric Sensing based on DNA-functionalized nanoparticles

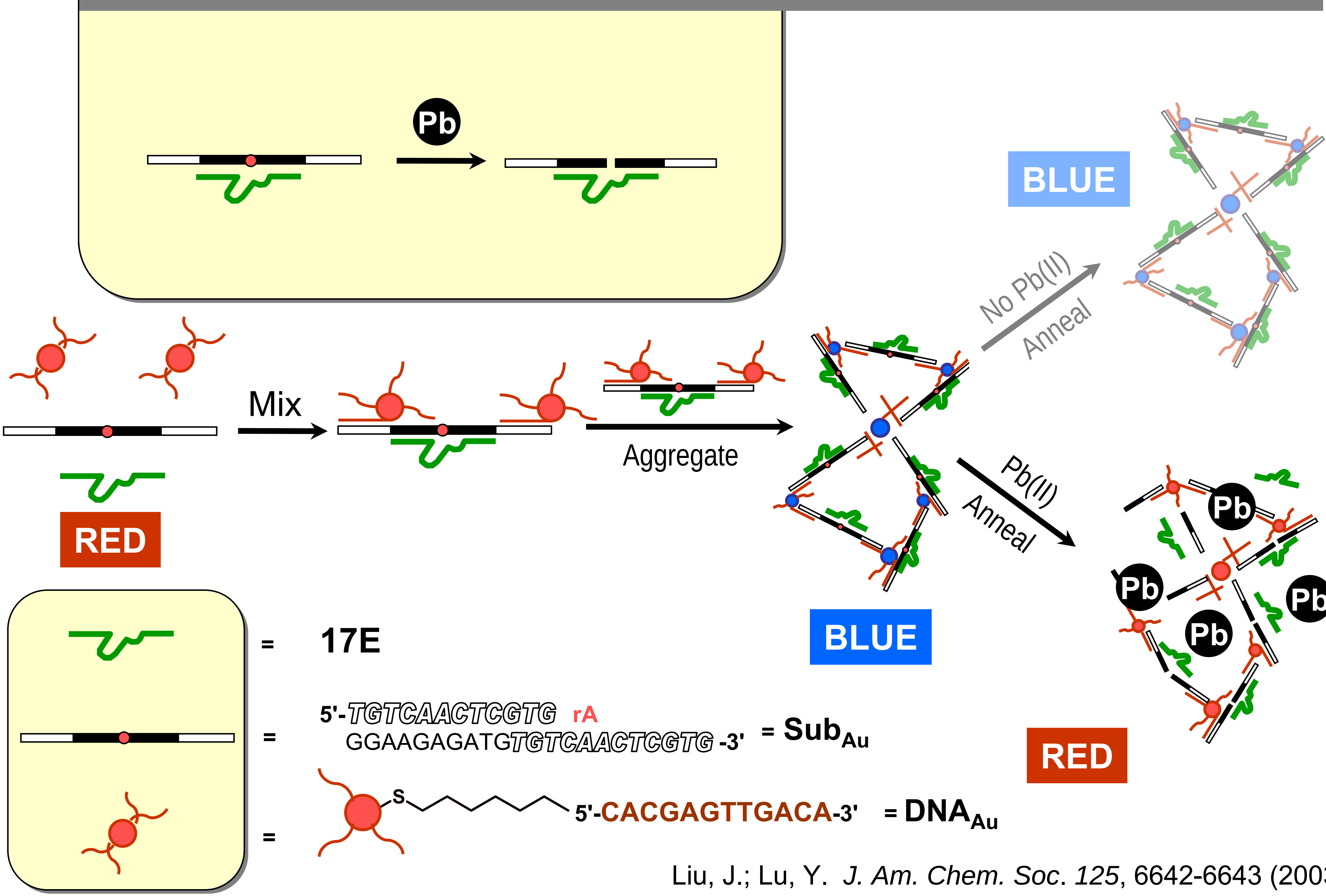




C. A. Mirkin, et al. *Nature* 382, 607-609 (1996).
R. Elghanian,et al. *Science 277*, 1078-1080 (1997).

Next Steps:
How to expand the technology beyond DNA detection?

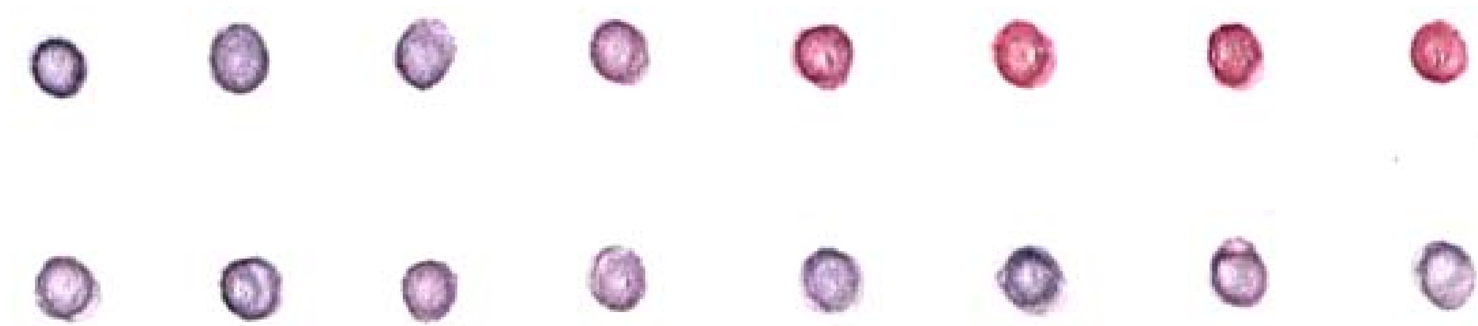
Design of a Simple Colorimetric Biosensor



Liu, J.; Lu, Y. *J. Am. Chem. Soc. 125*, 6642-6643 (2003).

pH Indicator like Operation

0 0.3 0.5 1 2 3 4 5 μM Pb(II)



5 μM Mg(II) Ca(II) Mn(II) Co(II) Ni(II) Cu(II) Zn(II) Cd(II)

pH 9.5 8.0 7.0 4.0 3.0 2.0 1.0

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).

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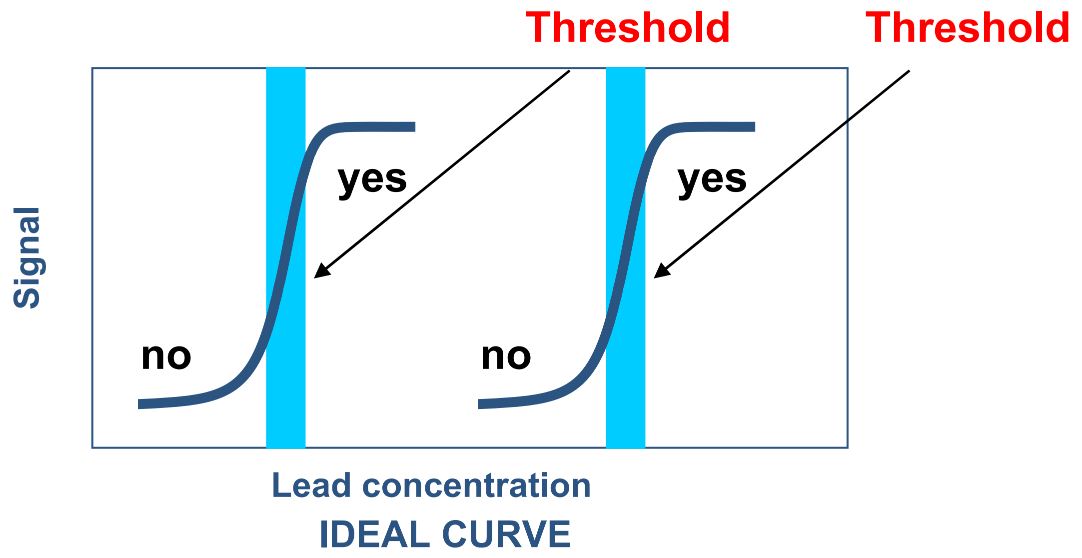
√ a general method to improve selectivity;

√ 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.

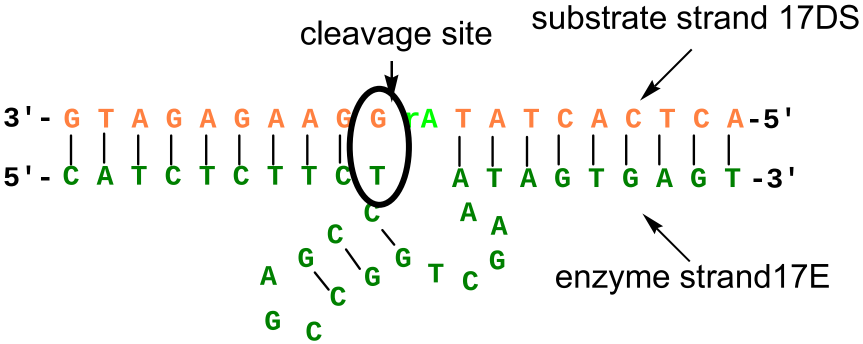
The need for tunable dynamic range

- 22 million old houses in the US alone have used lead paint
- US federal "thresholds" are 1.0 mg/cm^2 and 0.5%
- Current lead detection kits are based on $Na_2S \bullet 9H_2O$ or sodium rhodizonate.
- A study of available kits showed low rates of both false positive and false negative results when compared to laboratory analytical results using the federal thresholds (www.hud.gov)
- Current kits cannot tune the dynamic range

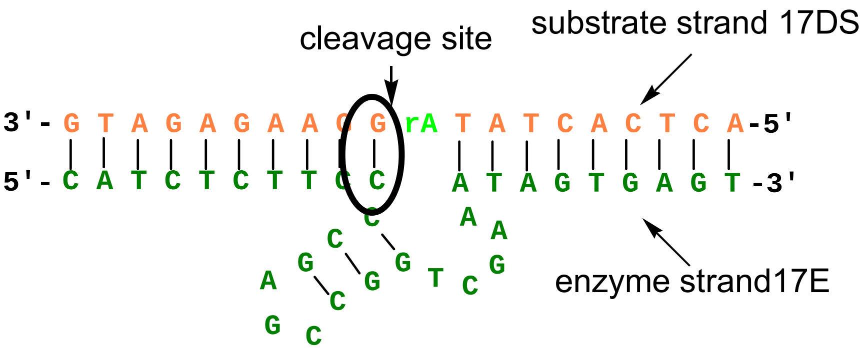


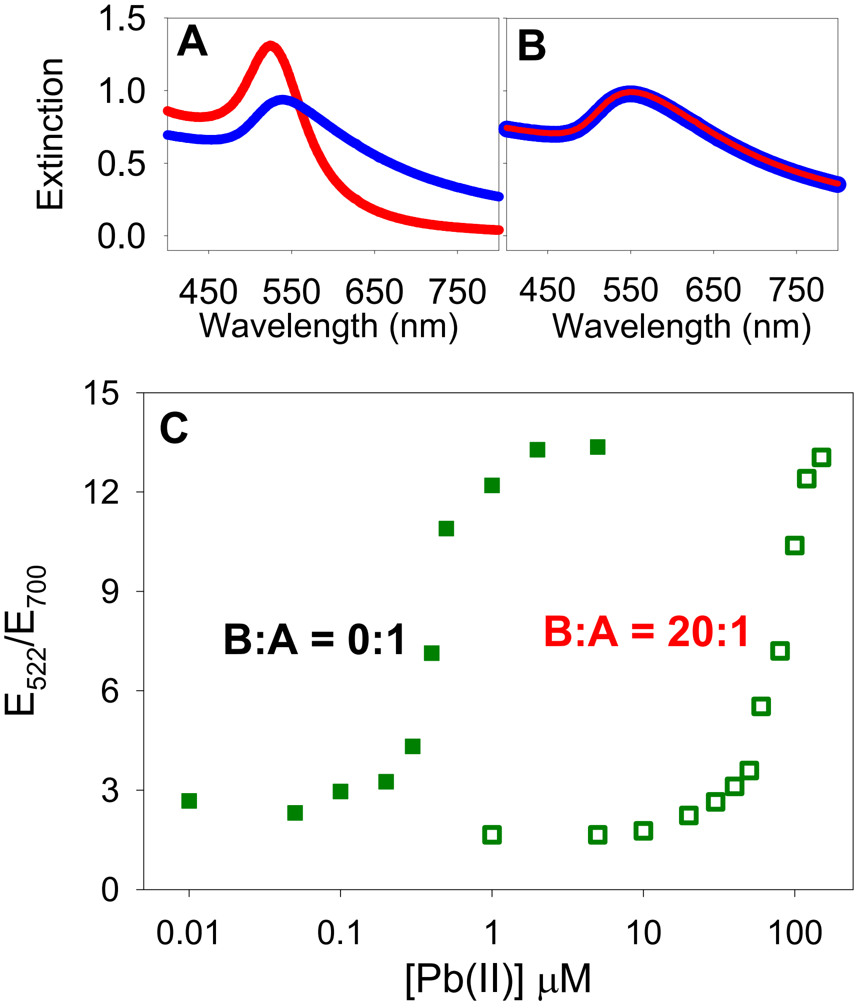
A Colorimetric Biosensor with Tunable Dynamic Range

A. Active DNA



B. Inactive DNA



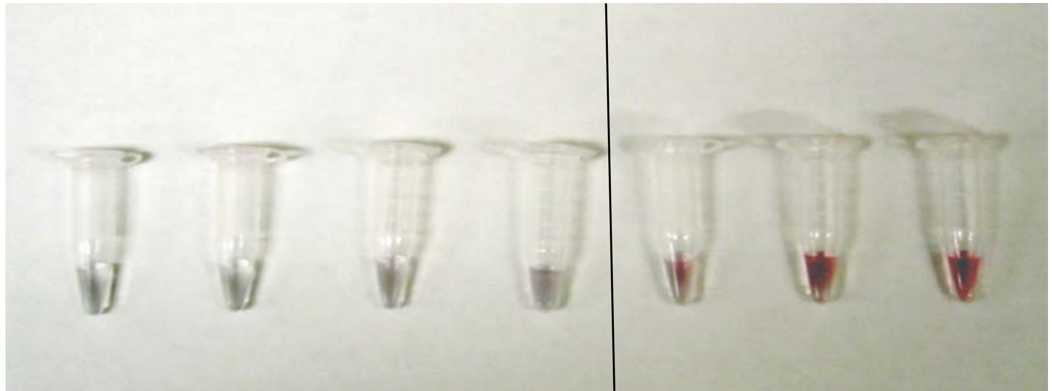


Brown, A. K.; Li, J.; Pavot, C. M.-B.; Lu, Y. *Biochemistry 42*, 7152-7161 (2003).
Liu, J.; Lu, Y. *J. Am. Chem. Soc. 125*, 6642-6643 (2003).

Pb^{2+} Detection in Paint

1.0 mg/cm² (threshold)

qualitative

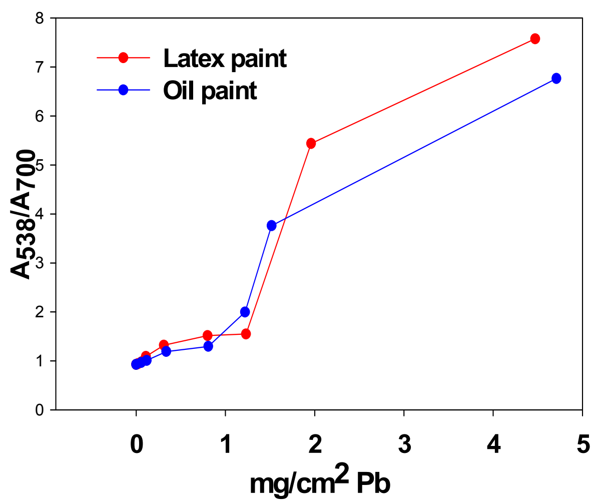


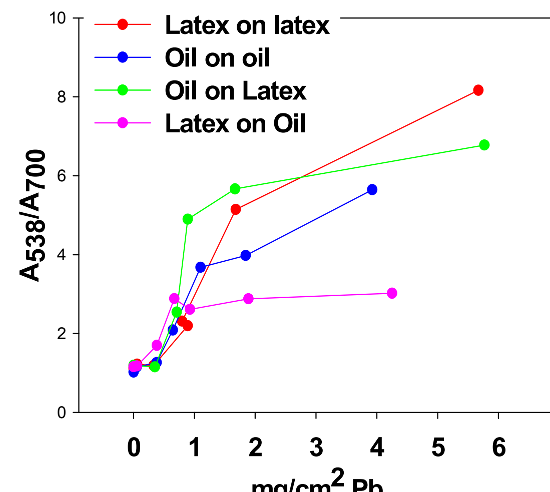
0 | 0.05 | 0.34 | 0.81 | 1.22 | 1.52 | 4.71

mg Pb / cm² paint

Portable colorimeter

quantitative

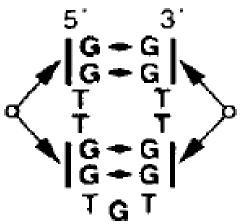




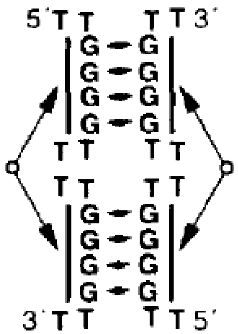
The tunable sensor allowed our Pb sensor to change color right at the federal threshold of 1.0 mg/cm², and to work with different types of paints.

Beyond metal and catalytic DNA-based detection

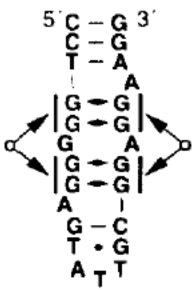
Thrombin



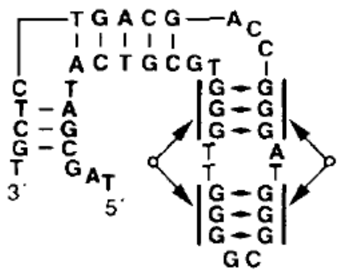
HIV gp120



ATP



HNE



Metal ions (Mg(II), Ca(II), Pb(II), Zn(II))

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, FMN, porphyrins, Vitamin B_{12})

Aminoglycosides (Tobramycin, Neomycin)

Antibiotics (Streptomycin, Viomycin)

Peptides (Rev peptide)

Enzymes (Human Thrombin, HIV Rev Transcriptase)

Growth cofactors (Karatinocyte GF, Basic fibroblast GF)

Antibodies (human IgE)

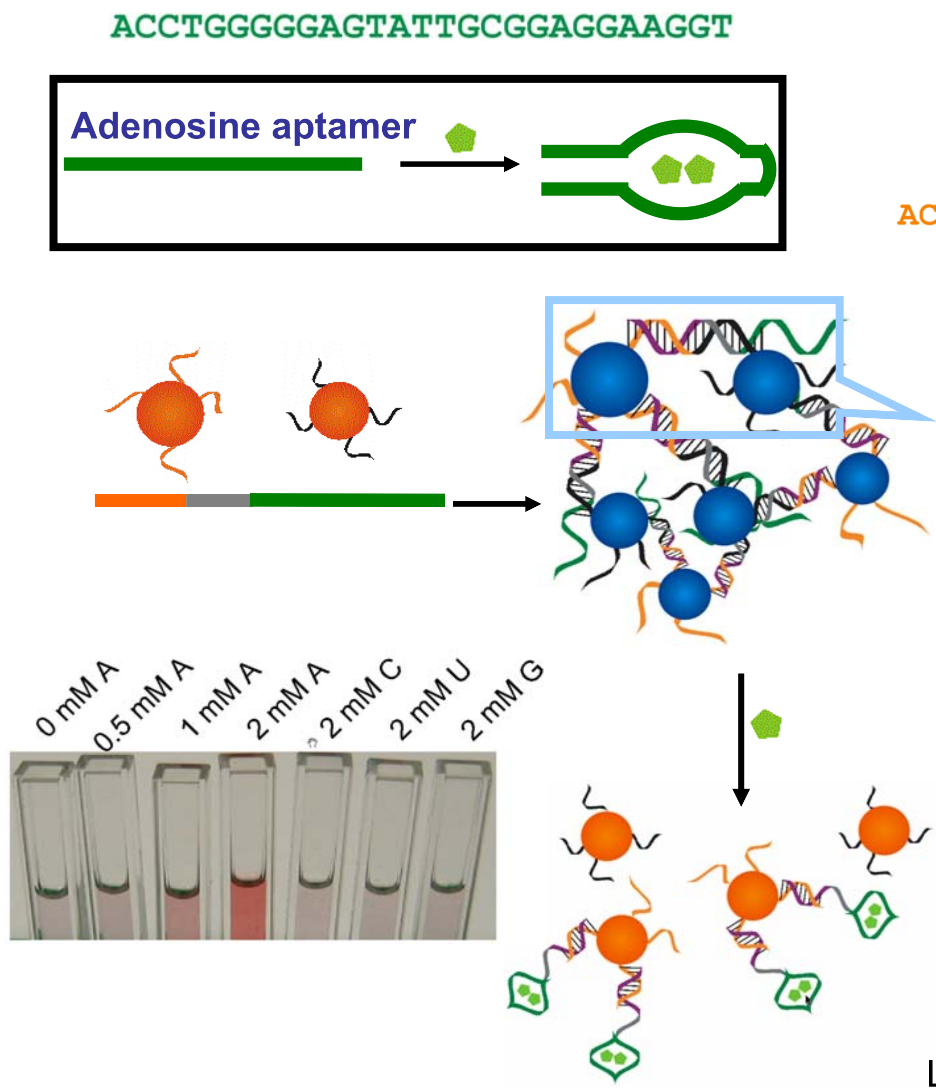
Gene regulatory factors (elongation factor Tu)

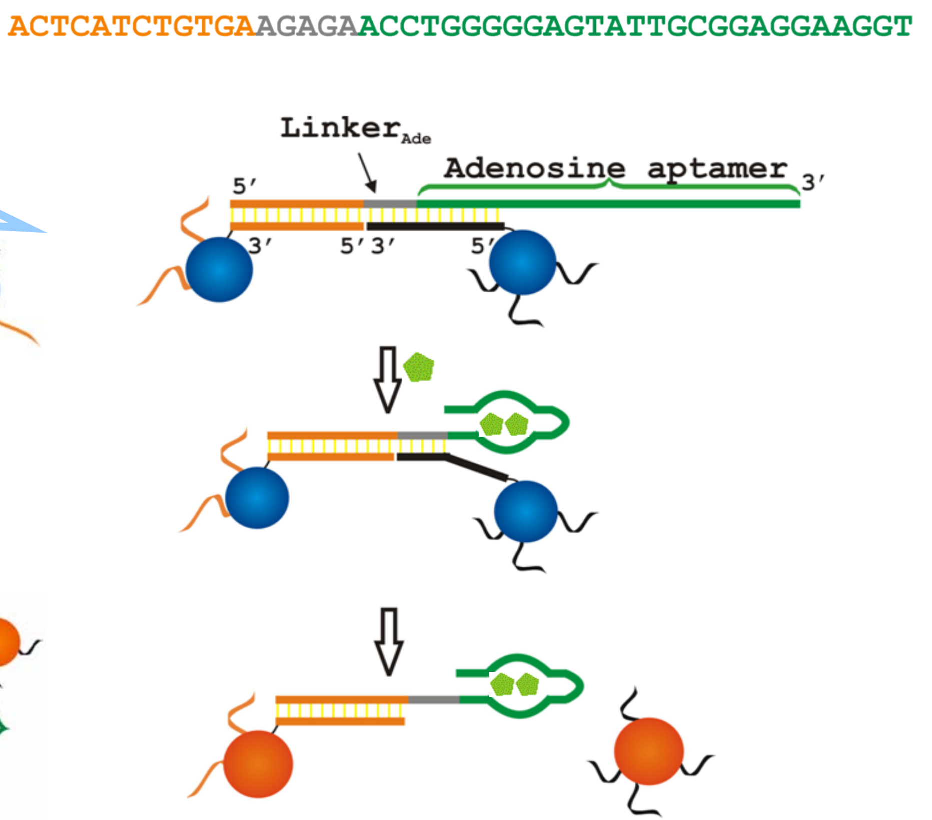
Cell adhesion molecules (human CD4, selectin)

Intact viral particles (Rous sarcoma virus, Anthrax spores)

Juewen Liu and Yi Lu, *J. Fluoresc*. 14, 343-354 (2004).

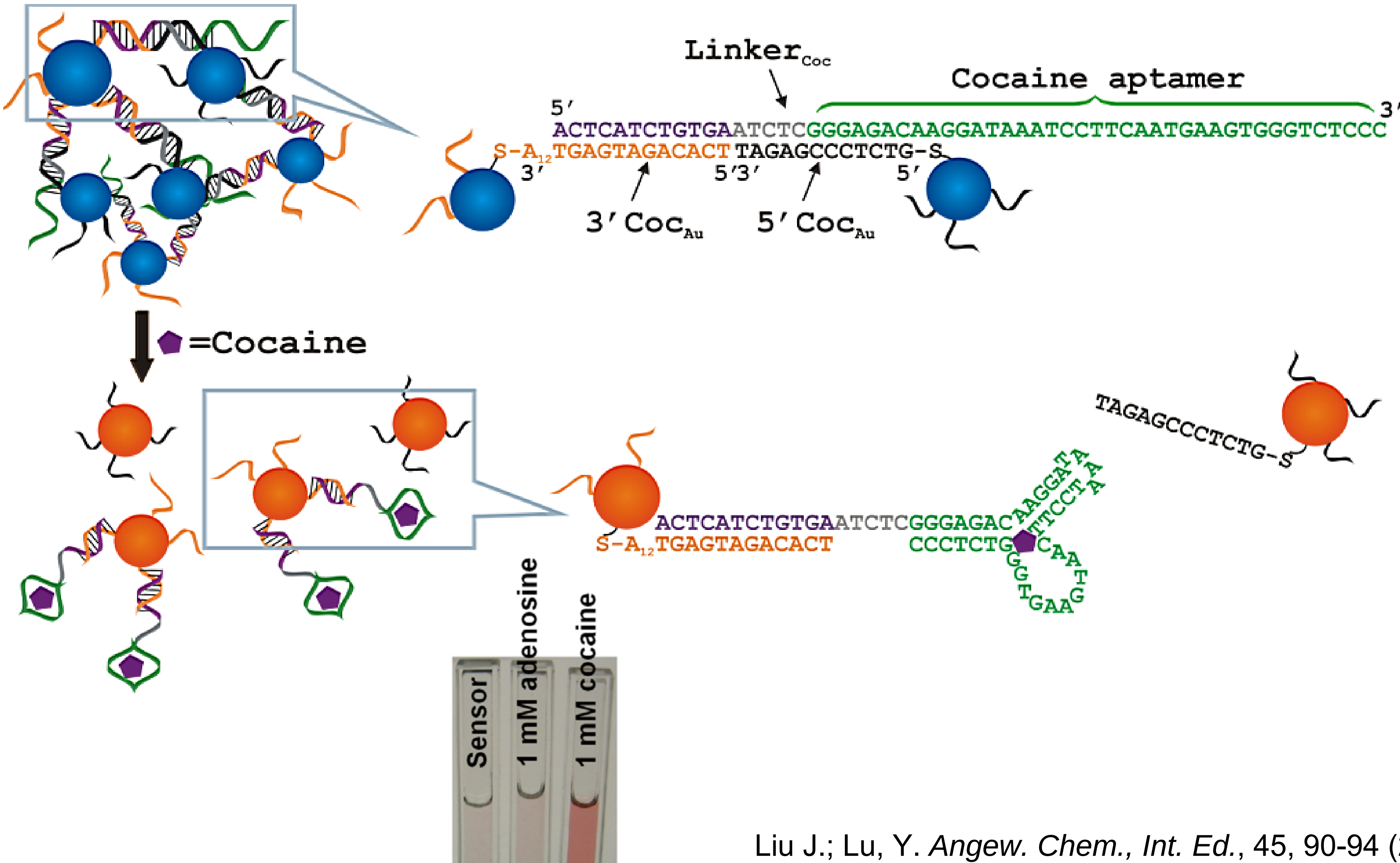
Aptamer sensors based on binding only





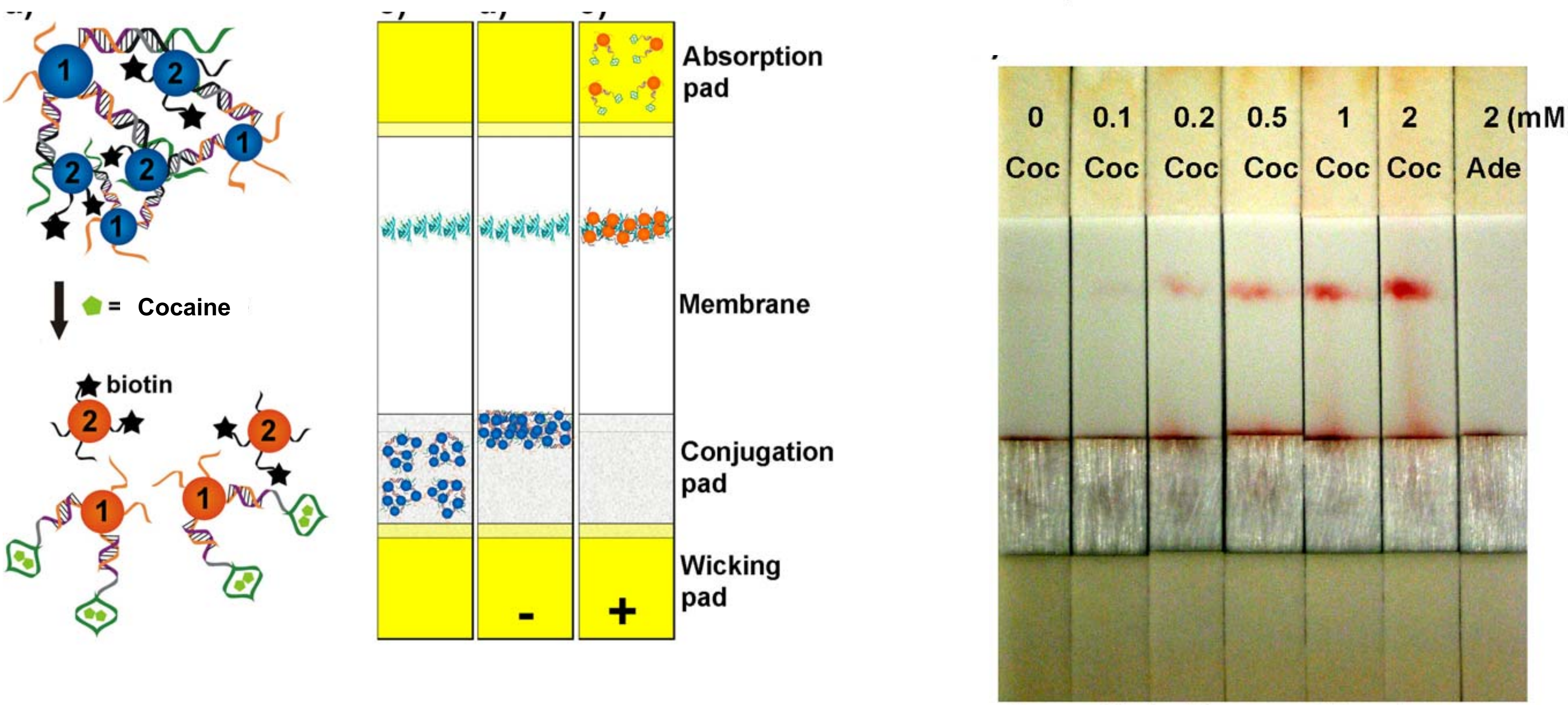
Liu J.; Lu, Y. *Angew. Chem., Int. Ed.*, 45, 90-94 (2006).
Liu, J.; Lu, Y. *Nature Protocol* 1, 246-252 (2006).

The method is general: a colorimetric cocaine sensor



Liu J.; Lu, Y. *Angew. Chem., Int. Ed.*, 45, 90-94 (2006).
Liu, J.; Lu, Y. *Nature Protocol* 1, 246-252 (2006).

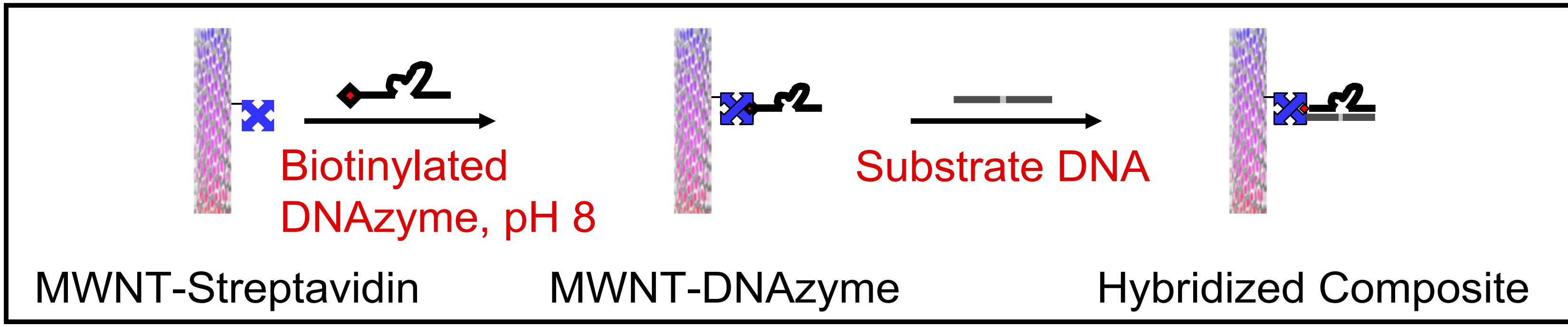
A dipstick test using functional DNA nanoparticles

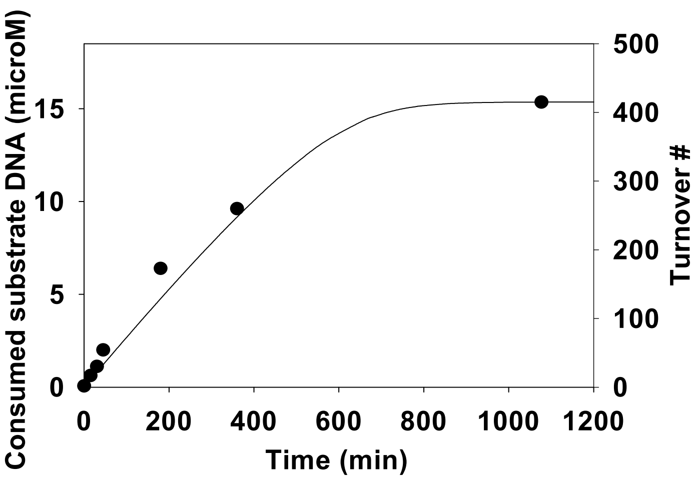


In undiluted human blood serum

Juewen Liu, Debapriya Mazumdar and Yi Lu, *Angew. Chem., Int. Ed. 45,* 7955 –7959 (2006).

Extension to other types of nanomaterials: Nanotubes



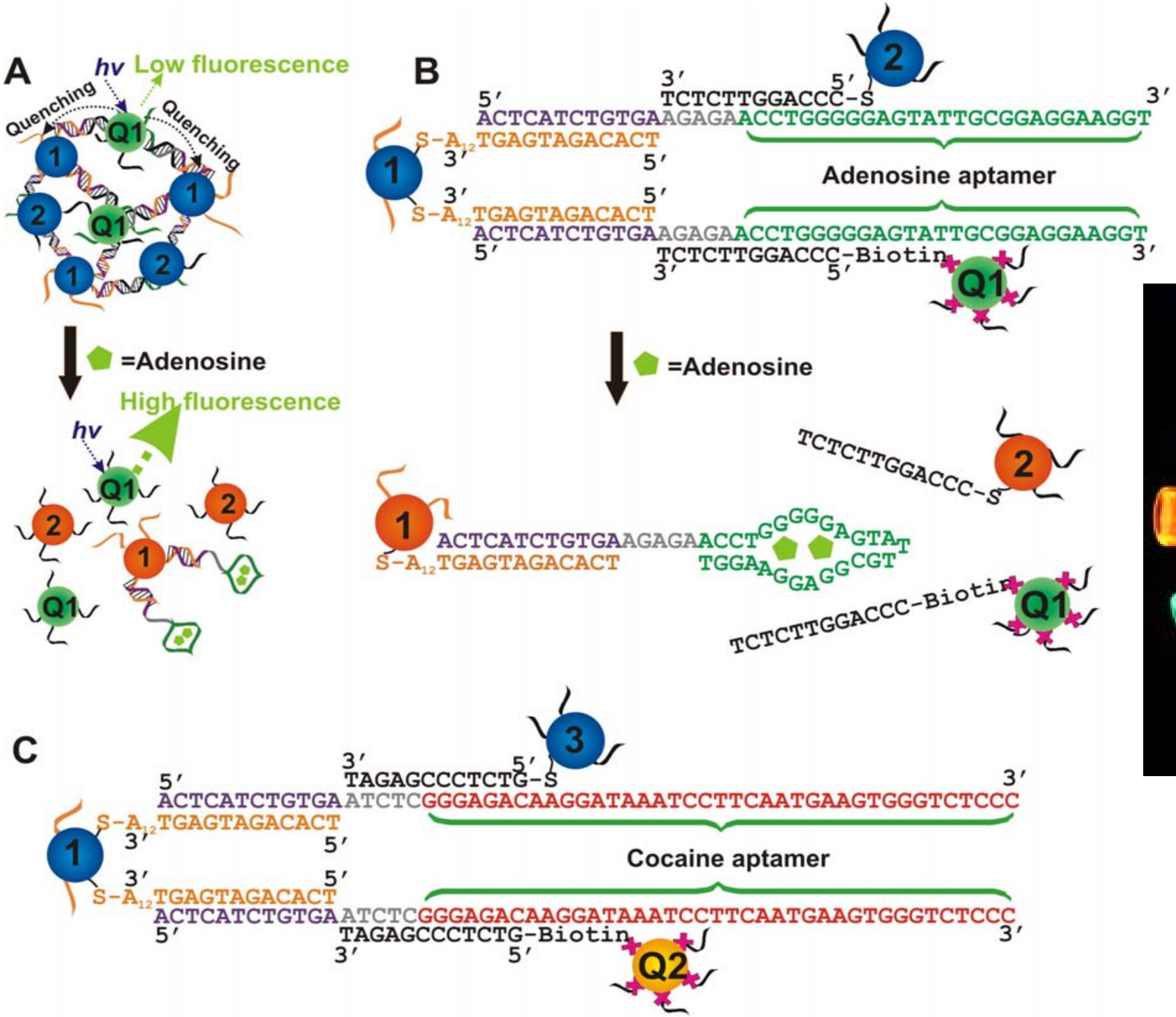


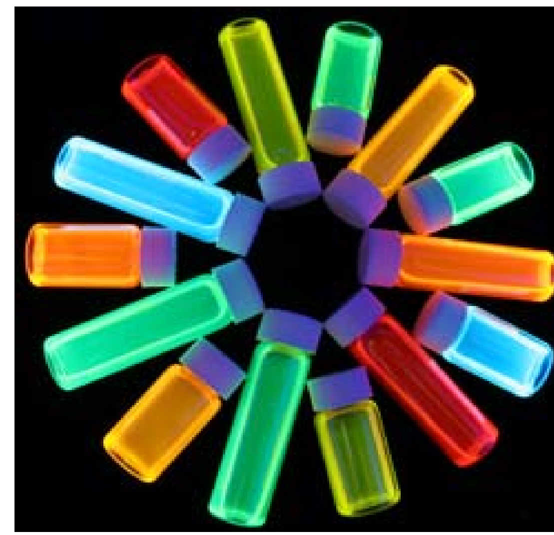
Multiple turnover behavior was fit to the integrated Michaelis-Menten equation.

	Immobilized onto MWNT-DNAzyme composite	*In solution phase*
k_{cat}	0.83 ±0.07 /min	2.85 ±0.29 /min
K_M	2.21 ±0.57 μM	2.60 ±0.80 μM
k_{cat} /K_M	0.38 /(min·μM)	1.10 /(min·μM)

Tae-Jin Yim, et al., *J. Am. Chem. Soc*. 127, 12200-12201 (2005).

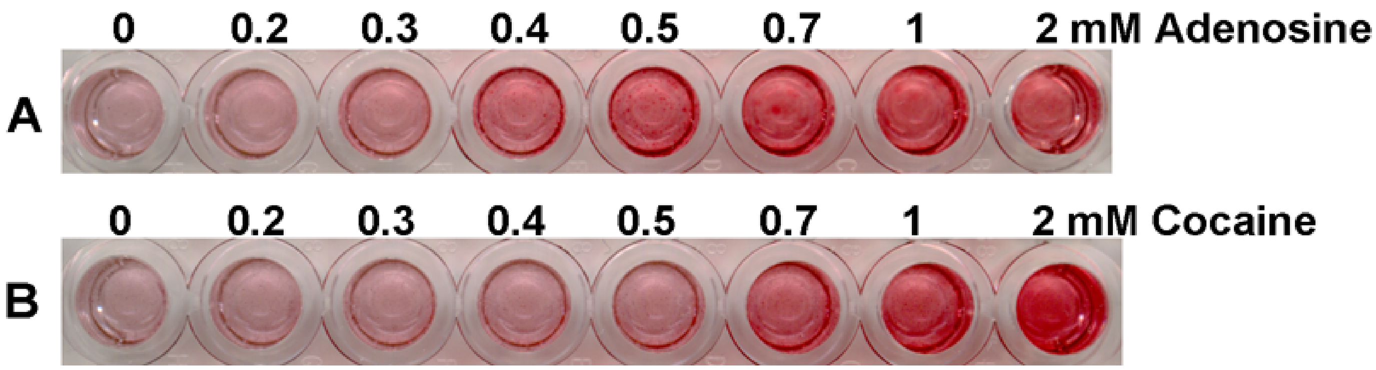
Extension to other types of nanomaterials: Quantum dots

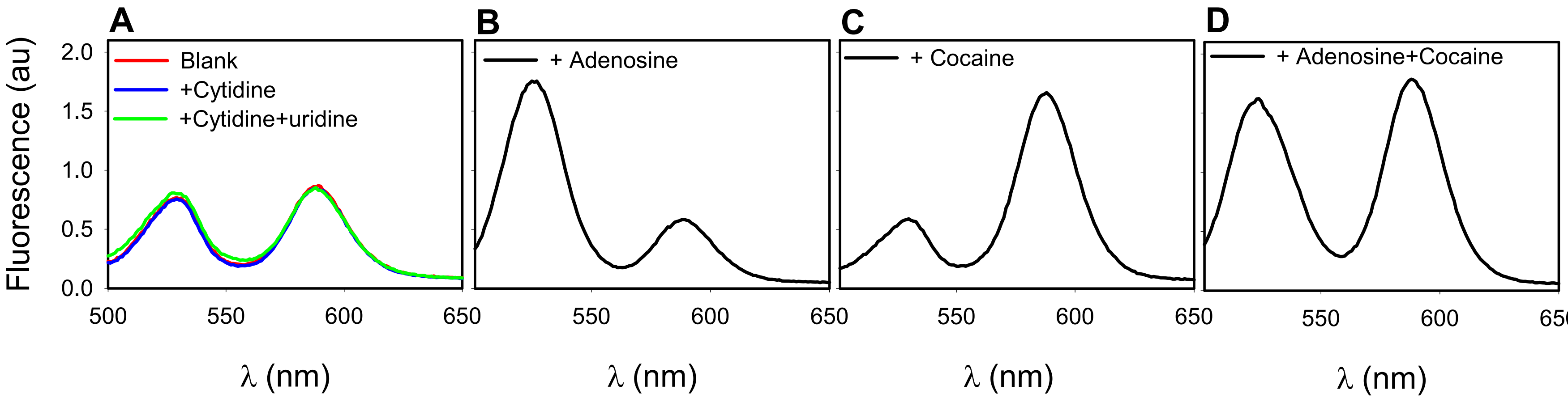




J. Liu, H.-H. Lee, and Y. Lu, *Anal. Chem*. 79, 4120-4125 (2007).

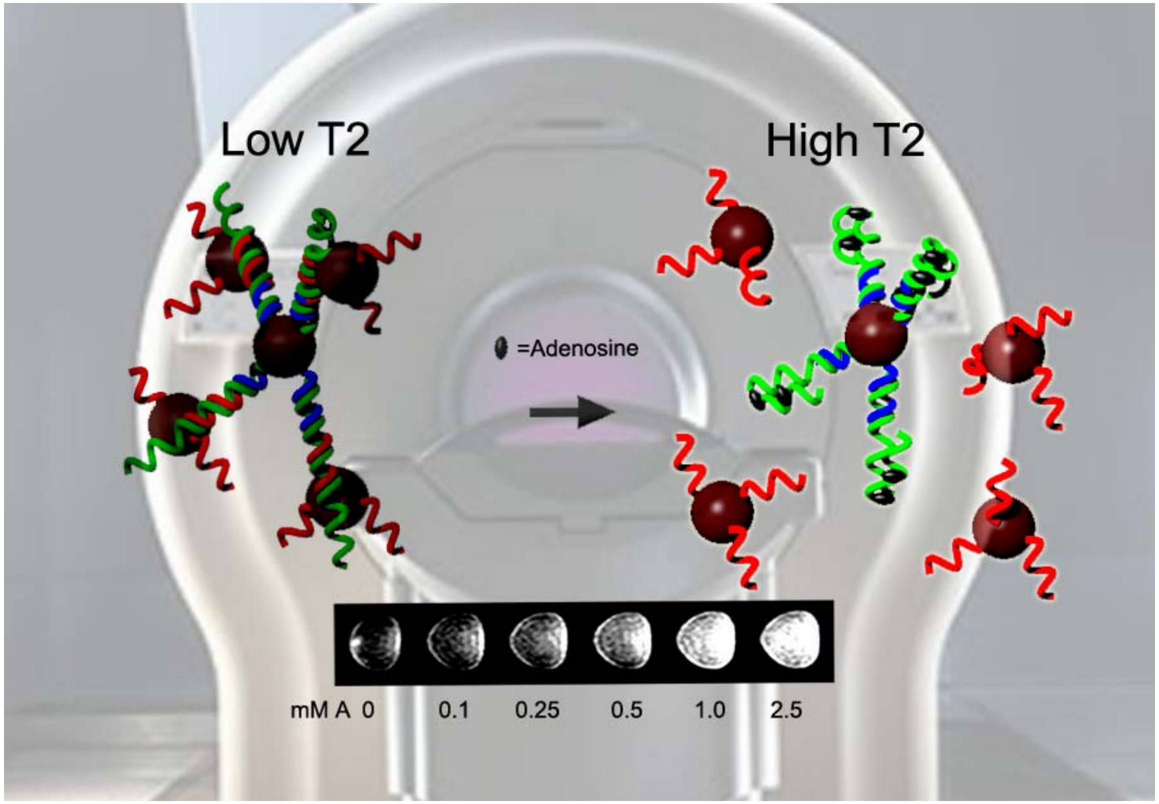
Simultaneous qualitative (colorimetric) and quantitative (fluorescent) sensing of multiple analytes in "one-pot"





J. Liu, H.-H. Lee, and Y. Lu, *Anal. Chem*. 79, 4120-4125 (2007).

Smart MRI agents for *in vivo* applications: Functional DNA-directed assembly of supermagnetic iron oxide nanoparticles:



M. Yigit, D. Mazumdar, H.-K. Kim, J. H. Lee, B. Odintsov, and Y. Lu *ChemBioChem* 8, 1675 -1678 (2007).
Mehmet Veysel Yigit, Debapriya Mazumdar, and Yi Lu, Bioconjugate Chem. 19, 412-417 (2008).

Summary

- To obtain sensors for a broad range of targets, general strategies have been developed to
  - to obtain sensing molecules;
  - to improve selectivity;
  - to convert molecular recognition event into physically detectable signals;
  - to tune the dynamic range.

- The combination of functional DNA and nanotechnology resulted in sensors that
  - are stable and cost-effective.
  - are highly sensitive and selective.
  - have tunable dynamic range
  - can be applied to detection and quantification of a broad range of targets (metal ions, organic molecules, proteins and cells….)
  - allow real-time, on-site (or remote) sensing.

Acknowledgments

DNA/RNA Lab at UIUC

Graduate Students:

Ying He	Andrea K. Brown
Hannah Ihms	Peter J. Bruesehoff
Tian Lan	Hee-Kyung Kim
Darius Brown	Jing Li
Nandini Nagraj	Jung Heon Lee
Eric Null	Juewen Liu
Zidong Wang	Kevin E. Nelson
Brian Wong	Daryl P. Wernette
Weichen Xu	Mehmet V. Yigit
Hang Xing	Debapriya Mazumdar
Yu Xiang	Seyed-Fakhreddin Torabi

Postdoctoral fellows:

Hui Wei	Zehui Cao
Juanzuo Zhou	Geng Lu
	Xiangli Meng
	Daisuke Miyoshi
	Wenchao Zheng

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Margaret Shyr, Dr. Paul Braun (MatSE, UIUC)
Abhijit Mishra, Gerard C. L. Wong (MatSE, UIUC)
Rong Tong and Jianjun Cheng (MatSE, UIUC)
Robert Barry, Dr. Pierre Wiltzius (Beckman, UIUC)
Tae-Jin Yim, Dr. Jonathan S. Dordick (CheE, RPI)
Carla B. Swearingen, Dr. Paul W. Bohn (Chem, UIUC)
Kashan A. Shaikh, Dr. Chang Liu (ECE, UIUC)
In-Hyoung Chang, Dr. Donald M. Cropek (CERL, DoD)

Funding:

NIH, DOE, NSF, DOD, HUD, EPA, & ISTC