Review Presentation about Mercury Biosensor

Reporter: zouyan
Advisor: Prof Zhao
Dr. Wei

Backgroud

- **Mercury**: a liquid metal with a high thermal conductivity, has many unique properties that make it useful in more than 3000 industrial applications, ranging from fungicides and bactericides in the agriculture industry(alkyl mercury compounds) to heat transfer agents and catalysts in the chemical industry.

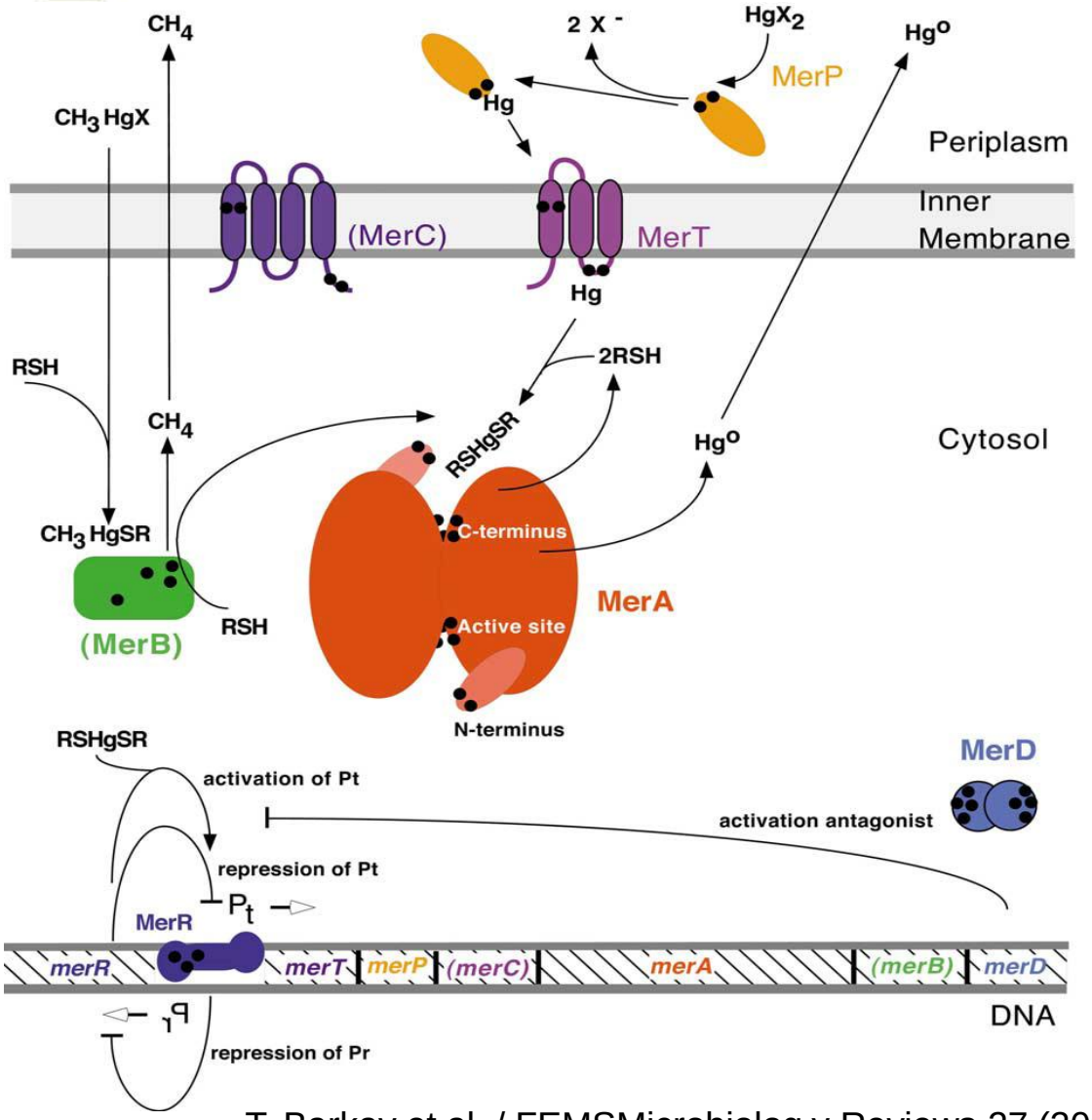
Backgroud

- However, mercury, as a highly toxic compound, caused heavy environmental contamination and deleterious effects on human health.

Detection method of mercury

- **Physical**: AAS, AES, GC-MS
- **Chemical**: inductively coupled plasma spectrometry
  electrochemical method
  disadvantage: expensive instruments
  complicated operation
  secondary pollution
- **Biosensor**: whole-cell sensor
  protein-based sensor
  oligonucleotide-Based

Mercury Resistance System



narrow-spectrum mercury resistance（lack B）: inorganic mercury

broad-spectrum mercury resistance : inorganic mercury & organic mercury

merR

↓

merTP(C)A(B)D

T. Barkay et al. / FEMSMicrobiolog y Reviews 27 (2003) 355-384

Recombinant Whole-cell Bacterial Hg(II) Sencor

Anal. Chem. **1995,** *67,* 667−669

A Luminescence-Based Mercury Biosensor

Marko Virta,*,† Jorma Lampinen,† and Matti Karp‡

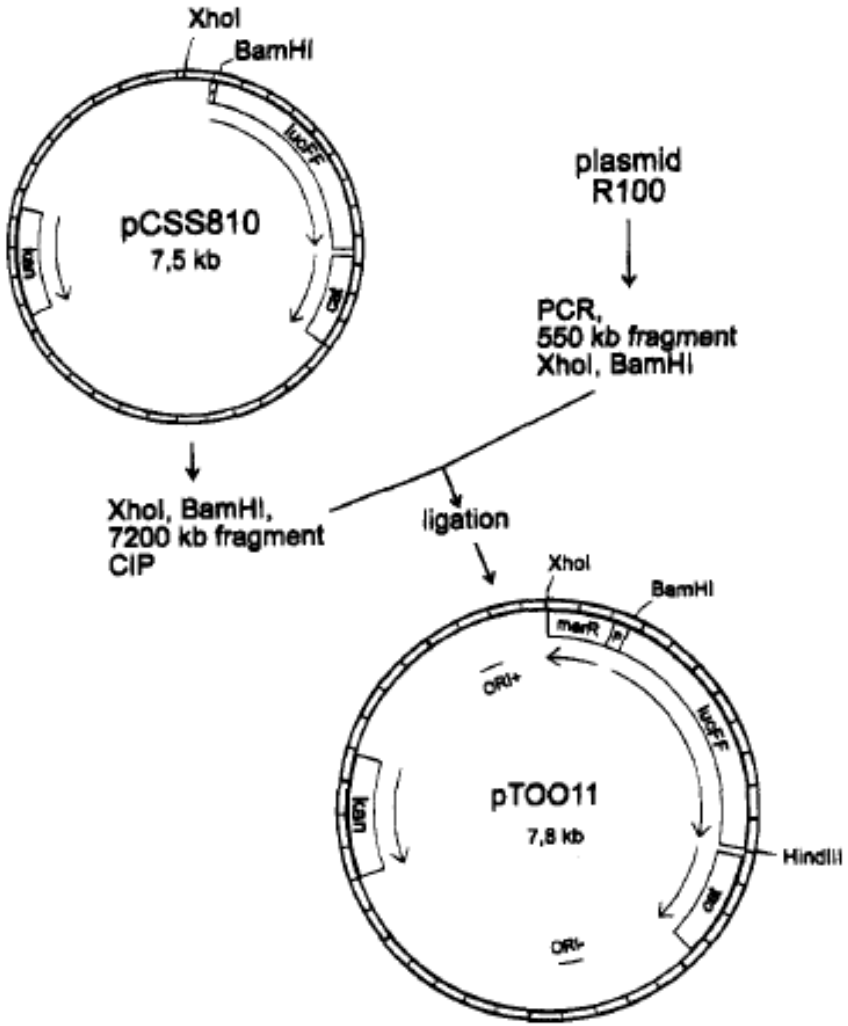
Department of Biochemistry and Pharmacy, Abo Akademi University, P.O. Box 66, FIN-20521 Turku, Finland, and Department of Biotechnology, University of Turku, Tykistokatu 6, FIN-20520 Turku, Finland

Biosensor: mer operon — firefly luciferase

The fkefly luciferase catalyses the reaction:

$$ATP + O_2 + \text{D-luciferin} \longrightarrow AMP + PP_i + CO_2 + \text{oxyluciferin} + \text{light(560nm)}$$

Mechanism



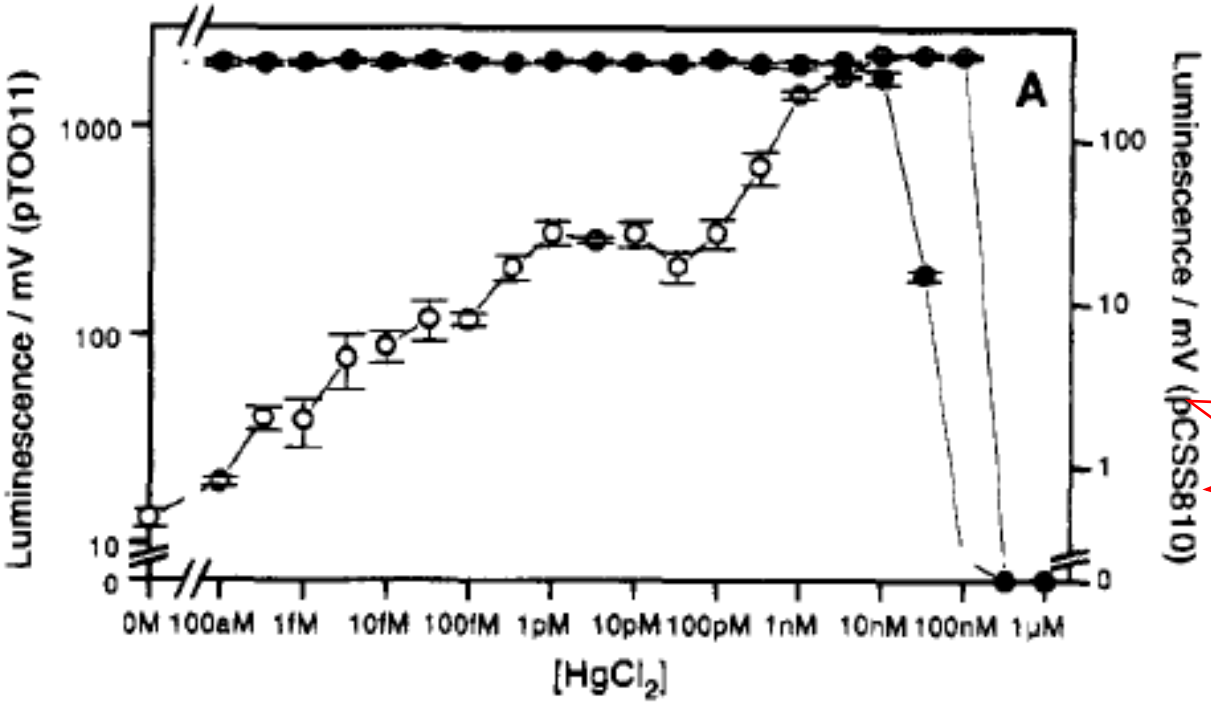
PmerR :

F: 5'-TTAAGGATCCCCTCATAGTTAATTTCTCCTC TTTTGGATTGGATAG-3'

R: 5'-ATATCTCGAGCTAAGGCATAGCTGACCT-3'

electroporation → E.coli MC1061 cell

Results



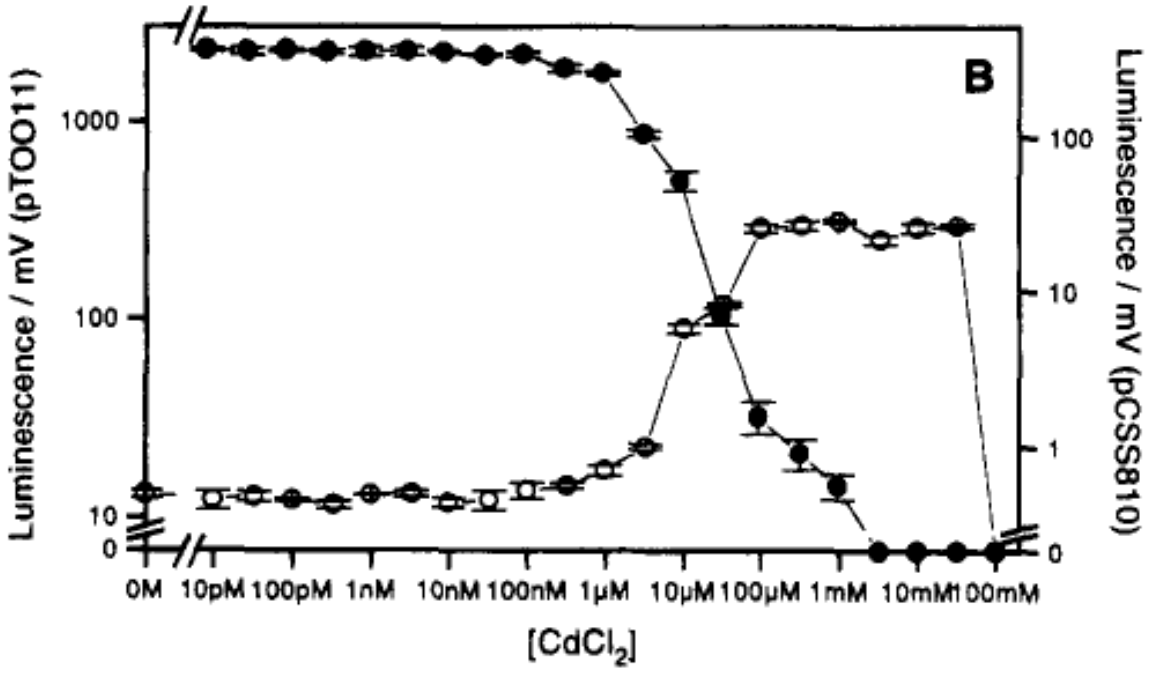
○ MC1061/pT0011

● MC1061/pCSS810

the limit detection of mercury : 0.1fm

- The luminescence increased with increasing $HgCl_2$ concentration in a linear manner to concentration of 0.1 μM.

- the only ion that was observed to interfere with the measurement was cadmium



○ MC1061/pT0011

● MC1061/pCSS810

- Cd induced remarkable luminescence too, however, the concentration needed for induction was almost 10^7-fold that of Hg.

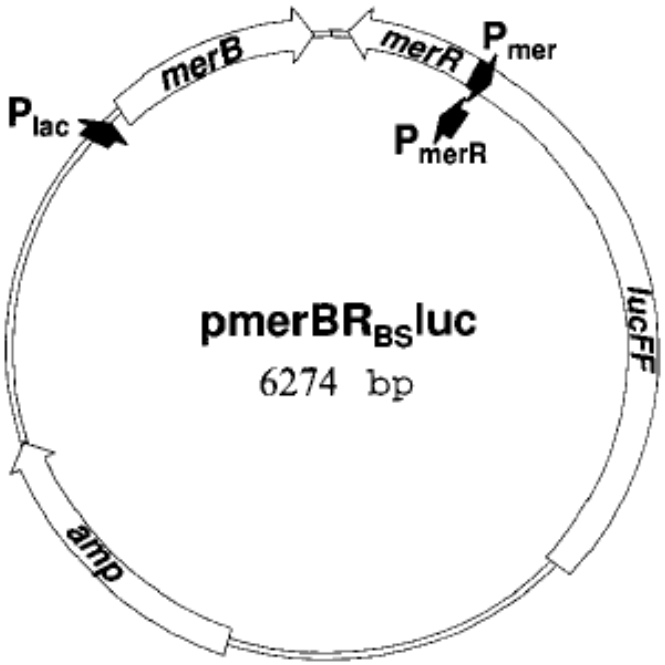
Anal. Chem. **2001,** *73,* 5168−5171

Detection of Organomercurials with Sensor Bacteria

Angela Ivask,[†,‡] Kaisa Hakkila,[‡] and Marko Virta[*,‡]

National Institute of Chemical Physics and Biophysics, Tallinn, Estonia, and Department of Biotechnology, University of Turku, Turku, Finland

Mechanism



recepter — reporter (lucFF)

PmerB:

5'-ATATCACGTGATGAAGCTCGCCCCATATAT-3'

5'-AATTCTCGAGGCTGCGAATCCGATGCCGGT-3'

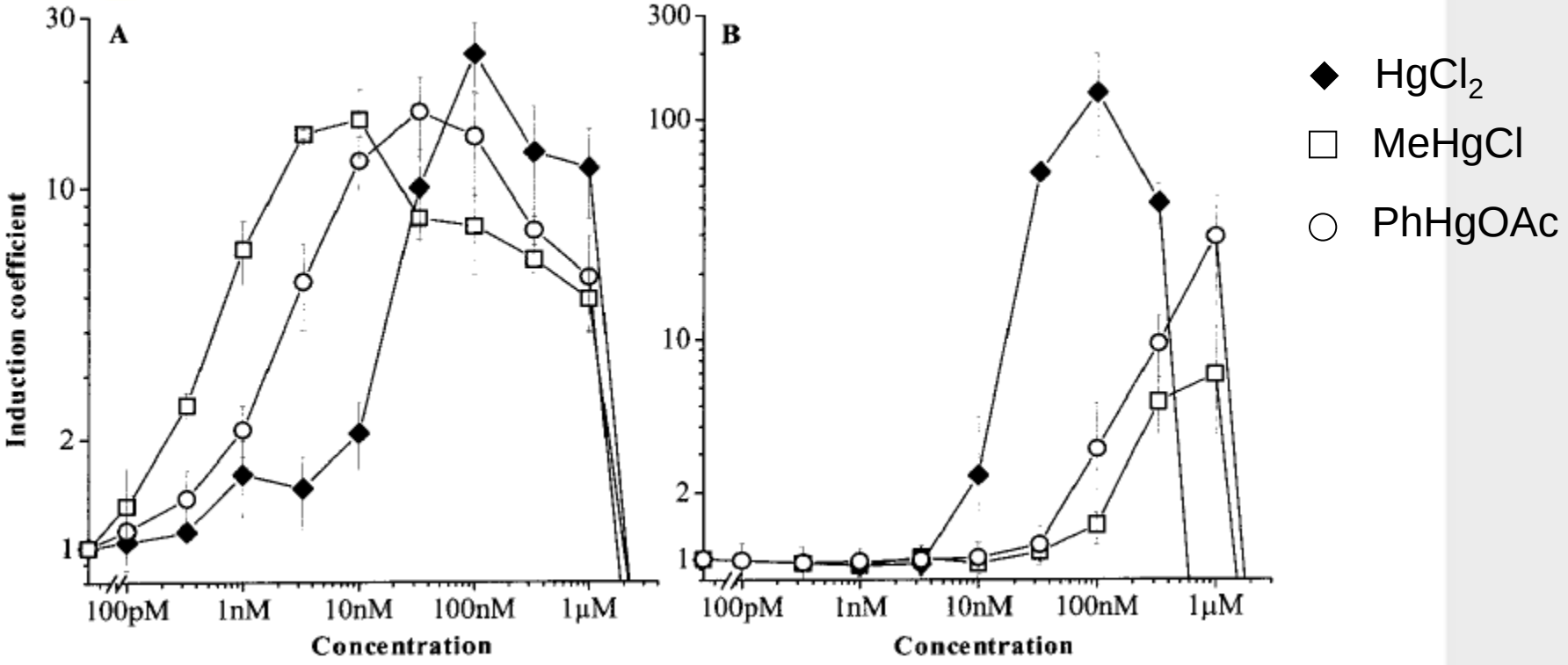
PmerR(mer):

5'-ATATCTCGAGTCCTCAGCATAGTACCGGGA-3'

5'-TTAAGGATCCCCTCATACGCTTGTCCTTTCAAA-3'

Structure of the plasmid pmerBRBSluc ⟶ Escherichiacoli MC1061

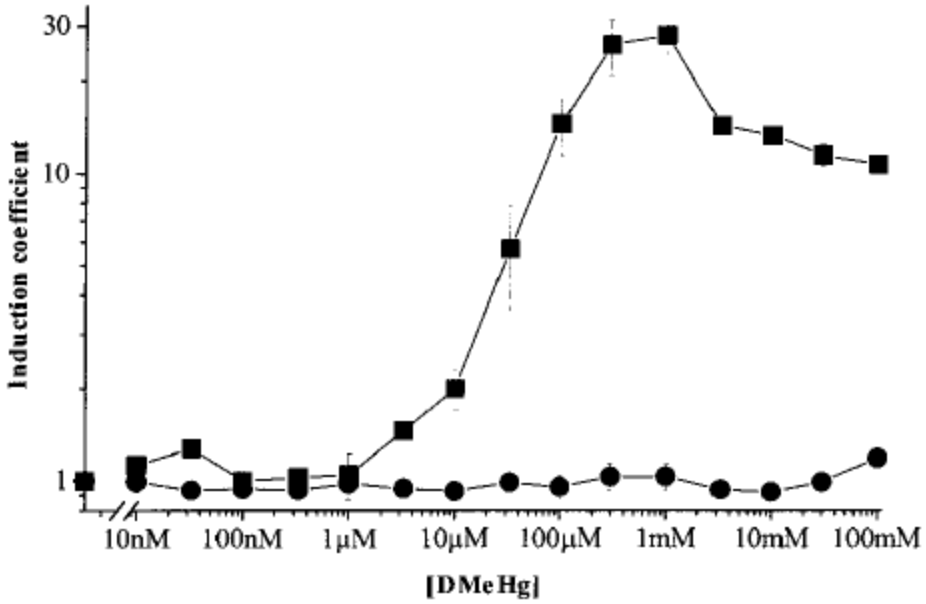
Results



pmerBR$_{BS}$luc pT0011

A：The lowest concentration that caused noticeable responce was $HgCl_2$ (10 nM), MeHgCl (0.2 nM) and PhHgOAc (1 nM) .

B：The concentrations were 10nM ($HgCl_2$), 0.1nM (MeHgCl and PhHgOAc).



■ MC1061(pmerBRBSluc)

● MC1061(pTOO11)

- Response to DMeHg, but the increase of luminescence was seen only with MC1061(pmerBRBSluc).

Protein-Based Hg(II) Detectors

J|A|C|S
COMMUNICATIONS

Published on Web 03/03/2007

Design of an Emission Ratiometric Biosensor from MerR Family Proteins: A Sensitive and Selective Sensor for Hg^{2+}

Seraphine V. Wegner, Ayse Okesli, Peng Chen, and Chuan He*

Department of Chemistry, The University of Chicago, 5735 South Ellis Avenue, Chicago, Illinois 60637

Received November 21, 2006; Revised Manuscript Received February 15, 2007; E-mail: chuanhe@uchicago.edu

(19) **United States**
(12) **Patent Application Publication**
Lu et al.

(10) **Pub. No.: US 2011/0123982 A1**
(43) **Pub. Date: May 26, 2011**

(54) **NUCLEIC ACID BASED FLUORESCENT SENSOR FOR COPPER DETECTION**

(76) Inventors: **Yi Lu**, Champaign, IL (US); **Juewen Liu**, Kitchener (CA)

(21) Appl. No.: **12/598,070**

(22) PCT Filed: **Jul. 16, 2008**

(86) PCT No.: **PCT/US2008/070177**

§ 371 (c)(1), (2), (4) Date: **Dec. 22, 2010**

Related U.S. Application Data

(60) Provisional application No. 60/950,062, filed on Jul. 16, 2007.

Publication Classification

(51) **Int. Cl.**
C12Q 1/68 (2006.01)
C07H 21/00 (2006.01)

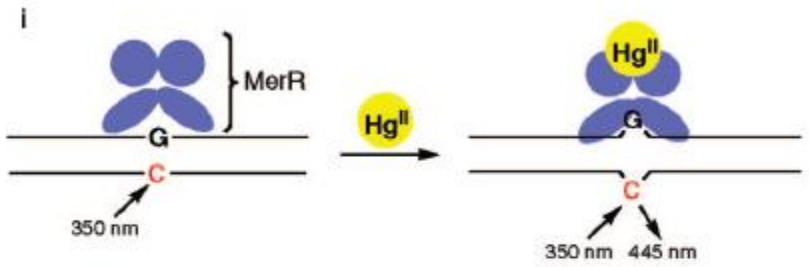
(52) **U.S. Cl.** **435/6**; 536/22.1

(57) **ABSTRACT**

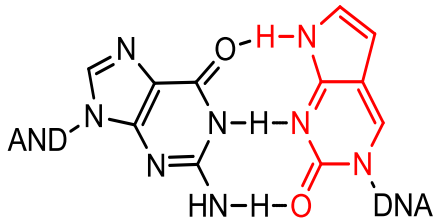
A nucleic acid enzyme responsive to copper, comprising an oligonucleotide comprising a nucleotide sequence of SEQ ID NO:1, wherein the nucleic acid enzyme is not self-cleaving.

Mechanism

a) Turn-On



II:



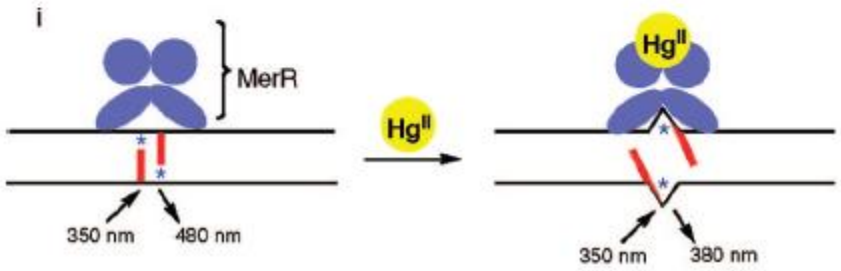
G-----C

III:

5'-CCTTGACTCCGTACATGAGTACGGAAGTCCC-3'

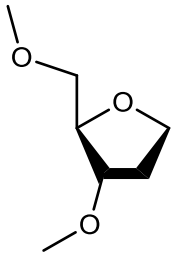
3'-GGAACTGAGGCATGTACTCATGCCTTCAGGG-5'

b) Ratiometric

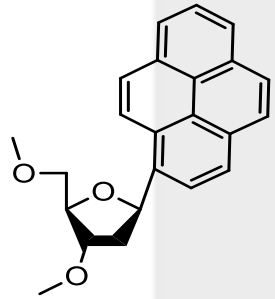


II:

*: **Ab:**



I:

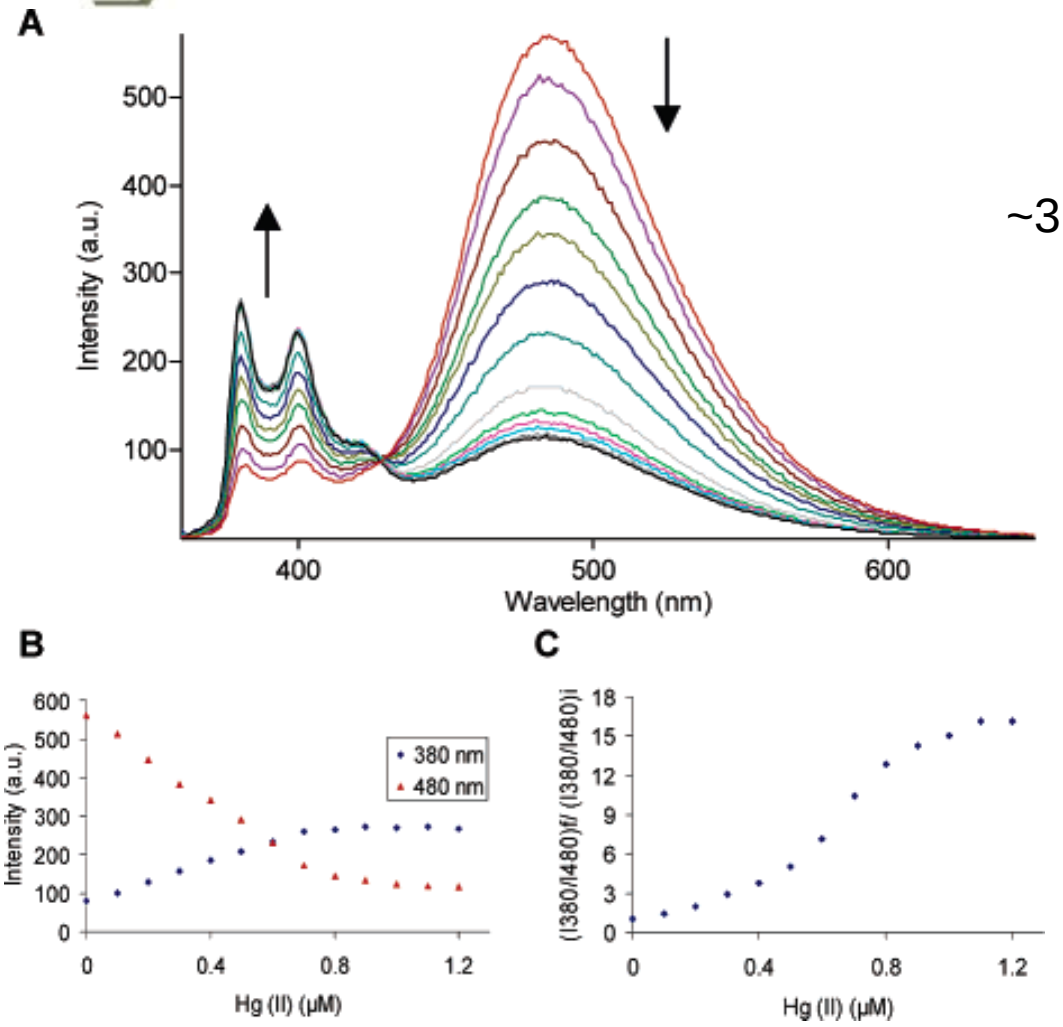


III:

5'-CCTTGACTCCGTACA*AGTACGGAAGTCCC-3'

3'-GGAACTGAGGCATGT*TCATGCCTTCAGGG-5'

Results



~5-fold decrease in pyrene eximer

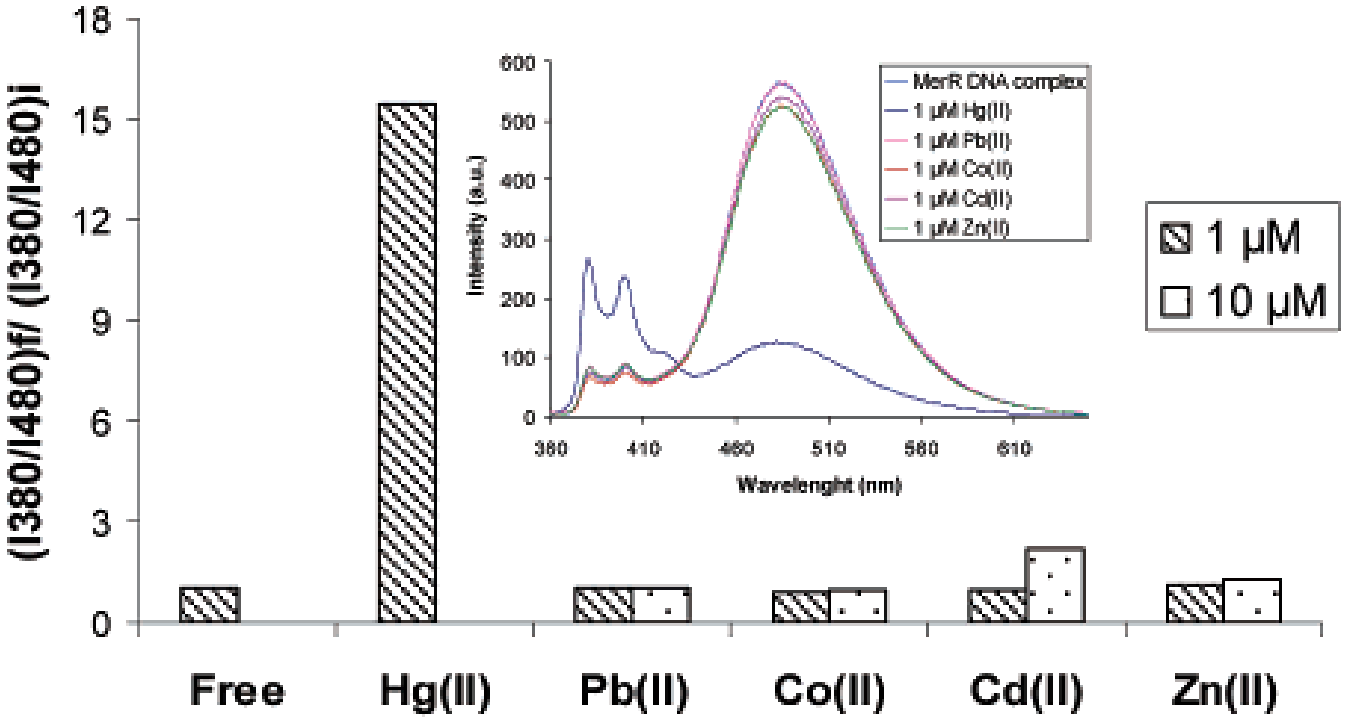
~3.3-fold increase in pyrene monomer

↓

~15-fold ratiometric response

Fluorescence response of MerR-DNA complex to the addition of Hg^{2+} at 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9,1.0, 1.1, and 1.2 μM.

Results



Ratiometric response for different metal ions at 1 and 10µM.

Oligonucleotide-Based Hg(II) Sensor

Molecular Sensors

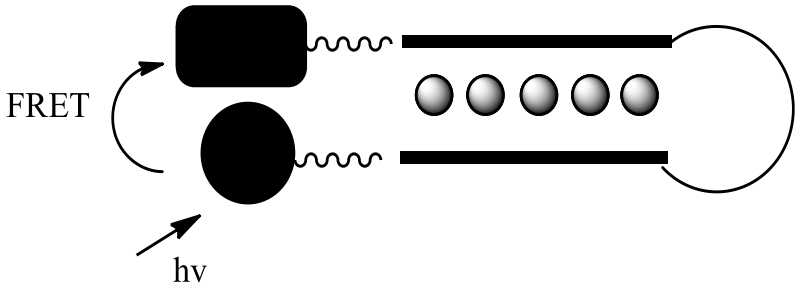
Highly Selective Oligonucleotide-Based Sensor for Mercury(II) in Aqueous Solutions**

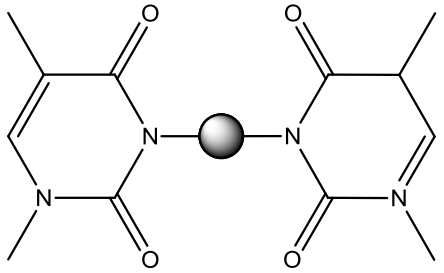
Mechanism

Hg-binding sequence — Hg-binding sequence

D-ODN-F: 5'-dabcyl-d(**TTCTTTCTTCCCCTTGTTTGTT**)-(6-fluorescein)-3'

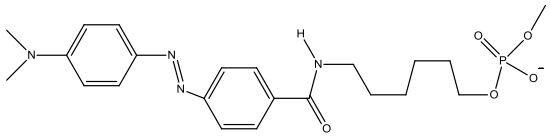
linker



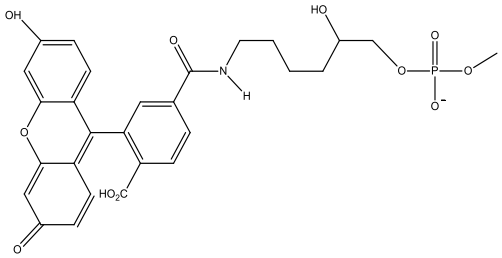


T-Hg-T

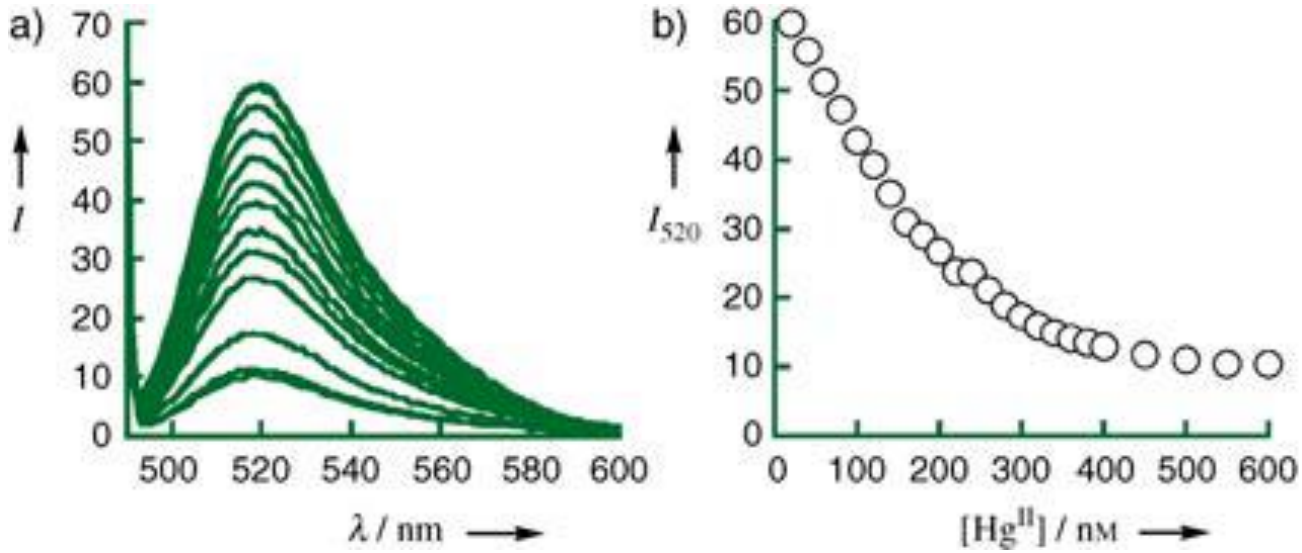
dabcyl =



Fluorescein derivative =

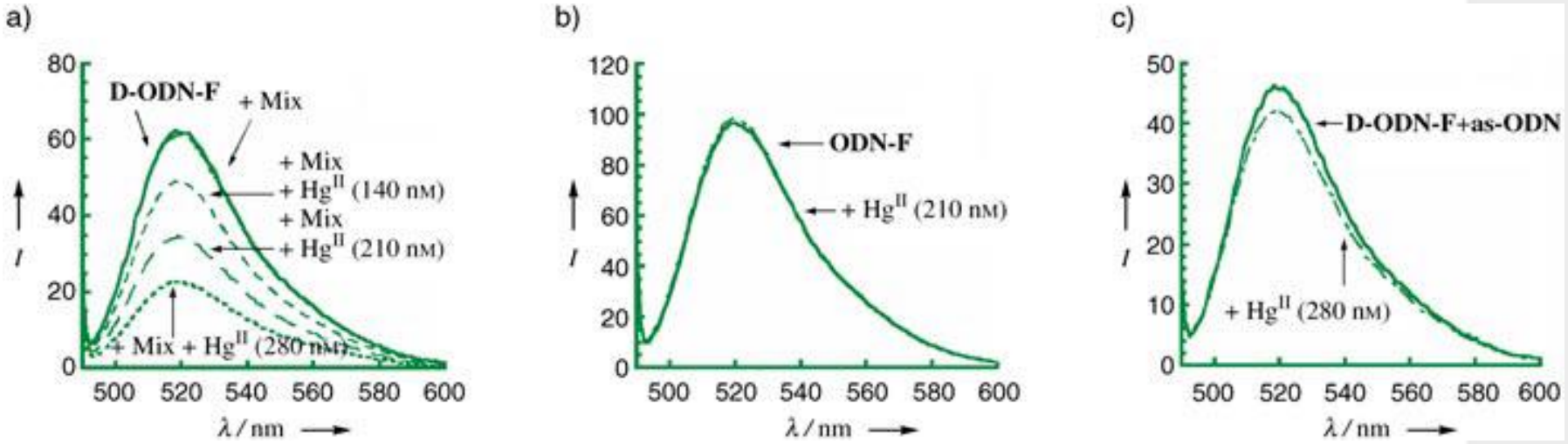


Results



- A: Fluorescence response of D-ODN-F (10 nm) upon addition of HgII ion. The intensity of the fluorescence decreased as the concentration of Hg^{II} increased.
- B: Fluorescence emission intensity (520 nm) versus Hg^{II} concentration.

Results



- a: a mixture of heavy metal ions and appropriate concentration of $Hg(ClO_4)_2$.
- b: contrast of ODN-F
- A contrast of as-ODN(no D and F)

Thank You !