Detection of Mercury(II) and Lead(II) with Graphene Oxide-Based Biosensors

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Presentation Outline

• Background and motivation
• Fluorescent sensors based on energy and charge transfer
• Graphene oxide
• Detection of Hg(II) with graphene oxide as the fluorescent quencher
• Detection of Pb(II) with graphene oxide as fluorophore
Sources of Toxic Heavy Metals

- Toxic heavy metals: Pb\(^{2+}\), Hg\(^{2+}\), As\(^{3+}\), Cd\(^{2+}\),
Hg (II) and Pb(II)

- Very toxic heavy metals;
- not biodegradable;
- accumulated in vital organs through the food chain;
- cause human illness and dysfunction

- EPA: <15 ppb Pb$^{2+}$ in drinking water
- EPA: <2 ppb Hg$^{2+}$ in drinking water
Approaches For Heavy Metal Detection

Lab-based methods for heavy metal measurement

- Cold-vapor atomic absorption spectrometry (CV-AAS);
- Cold-vapor atomic fluorescence spectrometry (CV-AFS);
- Inductively coupled plasma-mass spectroscopy (ICP-MS);
- X-ray absorption spectroscopy

http://serc.carleton.edu/research_education/geochemsheets/techniques/MCICPMS.html
Approaches for Heavy Metal Detection

Disadvantages of Lab-based analytical method

- labor-intensive, time-consuming
- laboratory-based large-scale instruments
- require large sample volume
- need Professional operation
- cannot be used as portable instruments for on-site detection or point-of-care use
FRET: Förster Resonance Energy Transfer

- Two dye molecules in a proximity
- Non-radiative energy transfer from an excited energy donor to an energy acceptor
- Dipole–dipole interactions
- Energy transfer efficiency:
  - spectral overlap between donor emission and acceptor absorption
  - $1/d^6$
  - ~10 nm

This slide is adapted from the KAIST 's presentation
The relationship between the energy transfer efficiency and the distance (R) between the energy donor and the energy acceptor is given by the equation:

$$E = \frac{R_0^6}{R_0^6 + r^6}$$

where $R_0$ is the Förster distance at which the energy transfer is 50%.

This figure is adapted from the KAIST’s presentation.
Fluorophores for FRET Sensors

Typical organic dyes as donors and acceptors in FRET systems
Our Approach on Fluorescent Sensors

**Energy donor**

- Avoid photo-bleaching
- Broad excitation spectrum
- High quantum yielding
- Potential of multiplex detection

**Energy acceptor**

- Graphene oxide

**Replacement of organic donor with inorganic quantum dots**

- Avoid photo-bleaching
- Broad excitation spectrum
- High quantum yielding
- Potential of multiplex detection

**Replacement of organic acceptor with graphene oxide**

- Avoid photo-bleaching
- Longer distance of energy transfer
- Higher efficiency?
- Simple immobilization of ssDNA and enzyme
Mechanism of Energy Transfer from Quantum dot to Graphene Oxide

Fluorescence quenching mechanism

- The distance was controlled by changing the dsDNA length

\[ E = \frac{1}{1 + \left(\frac{d}{d_0}\right)^4} \]

- \( d_0 = 9.7 \) nm

fluorescence quenching due to nano-surface energy transfer (NSET) rather than FRET.
Mechanism of Energy Transfer from Quantum dot to Graphene Oxide

Nano-metal surface energy transfer (NSET):
Persson & Lang, Phys. Rev. B 1982, 26, 5409

Dipole-dipole interaction induces the electronic interband transition in the energy acceptor

Energy transfer efficiency ($E$):

$$E = 1 - \frac{F_{DA}}{F_D} = 1 - \frac{\tau_{DA}}{\tau_D}$$

$$E = \frac{1}{1 + (d/d_0)^4}$$
Graphene Oxide: Emerging Biosensing Material

- large two-dimensional substrate
- excellent water solubility
- composed of earth-abundant elements;
- do not contain any toxic materials
- strong non-covalent interaction between biomolecules and GO (π-π interaction, electrostatic interaction, hydrogen bonding)
- Weak interaction between dsDNA and graphene oxide

Characterization of Graphene Oxide (GO)

AFM image

FT-IR

Raman spectrum

height profile
“Turn-on” Fluorescent Sensing of Pb$^{2+}$

(a) Fluorescent aptamer-quantum dot

(b) Weak fluorescent aptamer-quantum dot on graphene oxide

Aptamer: 5’-NH$_2$-(CH$_2$)$_6$-GGGTGGGGTGHTGGGTGGGGT-3’

(c) Fluorescent aptamer-quantum dot with Pb$^{2+}$

Graphene oxide (GO)
“Turn-on” Fluorescent Sensing of Pb$^{2+}$

Add graphene oxide to the quantum dot solution to form QD-aptamer-GO

Fluorescence quenching kinetics

Time-dependent relative fluorescence intensity of the GO/aptamer-QD ensemble in the presence of 100 nM Pb$^{2+}$. 

![Graph showing fluorescence quenching kinetics]
"Turn-on" Fluorescent Sensing of Pb\(^{2+}\)

Add graphene oxide to the quantum dot solution to form QD-aptamer-GO

Fluorescence vs. GO concentration

Relative fluorescence intensity at 567 nm as a function of the GO concentration. \(F_0\) and \(F\) are the fluorescence intensity at 567 nm before and after addition of GO ((300 nM aptamer-QD, 0.3 mM NaCl, 0.1 mM ethylenediamine, pH=7.0))
“Turn-on” Fluorescent Sensing of Pb$^{2+}$

Fluorescence emission vs. Pb$^{2+}$ concentration

Fluorescence spectra of the GO/aptamer-QD ensemble assay upon addition of Pb$^{2+}$ from 0-1000 nM (300 nM aptamer-QDs, 200 µg/mL GO, 10 mM PBS, 0.3 M NaCl, 0.1 mM ethylenediamine, and pH=7.0);
"Turn-on" Fluorescent Sensing of Pb$^{2+}$

Limit of detection (LOD) = 90 pM

Sensor response vs. Pb$^{2+}$ concentration

Relative fluorescence intensity of the GO/aptamer-QD ensemble assay as a function of Pb$^{2+}$ concentration. Inset: the linear range of the sensing assay.
“Turn-on” Fluorescent Sensing of Pb$^{2+}$

Selectivity toward Pb$^{2+}$

Selectivity of the GO/aptamer-QD ensemble assay toward Pb$^{2+}$ over other metal ions of 500 nM ([Pb$^{2+}$]=50 nM, [other metal ions]=500 nM). $F_0$ and $F$ are the fluorescence intensity at 567 nm in the presence and absence of Pb$^{2+}$ or other metal ions, respectively.
Detection of Pb\(^{2+}\) in river water

The fluorescent response of the GO/aptamer-QD ensemble upon the addition of Pb\(^{2+}\) in the solution containing river water (300 nM aptamer-QD, 200 μg/mL GO, 0.3 M NaCl, 0.1 mM ethylenediamine and pH=7.0). The insert is the linear response range of the assay.

LOD = 260 pM

River water: PBS solution=1:1 v/v

"Turn-on" Fluorescent Sensing of Pb\(^{2+}\)
Graphene oxide acts as not only an energy acceptor (quencher) but also an interesting energy donor (fluorophore)
Graphene Oxide: Emerging Biosensing Material

$\text{SP}^2$ carbon-carbon matrix
$\text{SP}^3$ carbon atoms linked to different oxygen-containing groups:
- hydroxyl (-OH), carboxyl (-COOH), carbonyl (C=O), epoxy (C-O-C)
Optical properties of graphene oxide is dependent on functional groups.

Absorption spectra of graphene oxide (GO), KOH-treated GO and HNO₃-treated GO.

XPS spectra of KOH-GO and HNO₃-GO.
Graphene Oxide: Emerging Biosensing Material

graphene oxide (GO), KOH-treated GO and HNO₃-treated GO
Graphene Oxide: An Amazing Fluorophore

Fingerprinting fluorescence of graphene oxide is associated with functional groups

- $\sigma^* \rightarrow n$ electronic transition of C-OH
- $\pi^* \rightarrow \pi$ transition of the $sp^2$ carbon atoms.
- $\pi^* \rightarrow n$ transition of the C=O associated groups
“Turn-off” Fluorescent Sensing of Hg\(^{2+}\)

Quenching fluorescence of GO via electron transfer

Graphene oxide (GO)

ssDNA aptamer:

\[
5'-\text{NH}_2-(\text{CH}_2)_6\text{TTTCTTTCTCGGTGGTTTGT}-3' 
\]

dsDNA with Hg\(^{2+}\):

\[
5'-\text{NH}_2-(\text{CH}_2)_6\text{TCTTTCTCGGTGGTTTGTGG}-3' 
\]

Hg\(^{2+}\) induced hairpin structure
“Turn-off” Fluorescent Sensing of Hg$^{2+}$

**Fluorescence quenching kinetics**

(Left) Time dependent-fluorescence emission spectra of graphene oxide (100 mg/L) in PBS solution (0.3 M NaCl, 0.1 mM ethylenediamine, pH=7) with 20 nM Hg$^{2+}$; (Right) time-dependent fluorescence intensity at 600 nm. The loading of DNA on GO was around 10 nmol/g GO.
“Turn-off” Fluorescent Sensing of Hg$^{2+}$

Limit of detection (LOD) = 0.92 nM

(Left) Fluorescence emission spectra of aptamer-graphene oxide (100 mg/L) in PBS solution (0.3 M NaCl, 0.1 mM ethylenediamine, pH=7); (Right) fluorescence intensity at 600 nm of aptamer-GO conjugates and GO in the presence of various Hg$^{2+}$ concentrations (0, 0.1, 0.2, 0.5, 1, 2, 10, 20, 50, 100, 500 and 1000 nM). Inset shows the linear region.
“Turn-off” Fluorescent Sensing of Hg$^{2+}$

Sensor’s selectivity toward Hg$^{2+}$

Fluorescence quenching efficiency of aptamer-GO conjugates in various environmental metal ions ([Hg$^{2+}$]=50 nM, other metal ions have a concentration of 200 nM).
Lab-on-Chip for Heavy Metal Detection

- integrate sensors with microfluidic units into single chip to form a lab-on-chip system

Collaborated with Dr. Yuxin Liu
Summary

- “Turn-on” fluorescence detection of Pb$^{2+}$ was developed with GO as super fluorescence quencher and CdSe/ZnS quantum dots attached by Pb$^{2+}$ specific ssDNA;

- The developed fluorescent sensor for Pb$^{2+}$ detection has a LOD of 90 pM and excellent selectivity toward Pb$^{2+}$

- A fluorescent sensor for Hg$^{2+}$ detection was developed by the electron transfer from GO to the bounded Hg$^{2+}$

- The Hg$^{2+}$ sensor shows a LOD as low as 0.92 nM and excellent selectivity.
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