Single and Two-Photon DNA-Based Fluorescence Sensors for Pb^{2+} and Hg^{2+}

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Abstract

Against the backdrop of contamination disasters akin to Flint, Michigan's, researchers are striving to develop new approaches with ever-increasing urgency for sensitive, selective, and continuous detection of heavy metal ions in water, especially Pb^{2+} and Hg^{2+}. Through this study, the researchers propose the development of single and two-photon fluorescence-based biosensors that rely upon specific oligonucleotides, coupled with a fluorophore, that form stable G-quadruplex structures at room temperature. Upon exposure to Pb^{2+} or Hg^{2+}, the DNA's existing metal ion is replaced by the heavy metal ion, quenching the fluorescence of the dye; the degree of decrease in fluorescence intensity is the figure of merit for assessing the sensitivity and the selectivity of the sensor. As such, the oligonucleotide T30695 demonstrated high selectivity for Pb^{2+} and Hg^{2+} ions with detection limits of 4.5 and 4 ppb respectively, both of which fall below the World Health Organization's maximum for hazardous concentrations. In addition, two-photon spectroscopy was shown to be more optimal for sensitivity, and due to its near-infrared excitation and low scattering, is still effective in muddy and opaque waters. The continuous monitoring of heavy metal ions requires—as the sensor is fluorescence based—a fiber optic cable, which in field application would be ideal for excitation and recording of fluorescence. Additionally, it was demonstrated that other oligonucleotide sequences, such as that of T-rich T30695, are unable to form G-quadruplex structures or display selectivity and sensitivity for the investigated metal ions. In summary, the research shows significant promise for development of an effective, single and two-photon, fluorescence-based DNA biosensor which will allow for continuous monitoring of toxic heavy metal ions in water sources for the purpose of avoiding potential disasters.

Introduction

Heavy metals are abundant, naturally occurring, metallic elements with densities at least five times greater than that of water. Their many applications in various fields of modern science have led to their ample distribution, as well as—relatively recently—their widespread environmental contamination. Copious numbers of anthropogenic and natural sources can introduce heavy metals into the biosphere, including industrial emissions from power plants and incinerators, forest fires, and even the urban lifestyle.

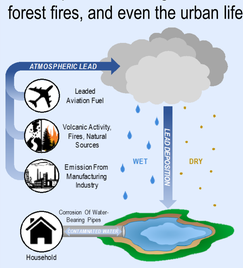

Figure 1: Depiction of Pb^{2+} pollution, contamination, and effect on humans.

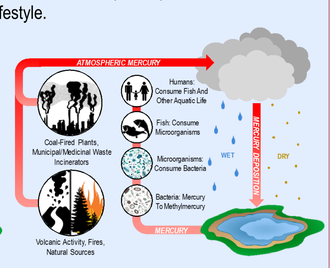

Figure 2: Depiction of Hg^{2+} pollution, contamination, and effect on humans.

Heavy metals such as lead and mercury have no known beneficial effects on organic life, and can cause serious, even fatal illness over time. Their disruption of crucial metabolic processes in the heart, brain, etc. stems from their potential for bioaccumulation. Furthermore, these metals' toxicities result from their ability to bind with cell components such as DNA and protein sites by displacing other vital minerals from their natural binding locations, causing cell malfunction, DNA damage, etc.

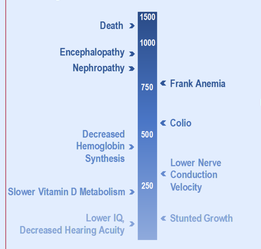

Figure 3: Effects of various lead concentrations in ppb within the bloodstream of children.

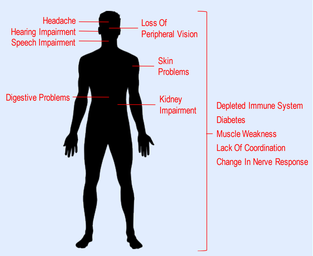

Figure 4: Effects of mercurial poisoning in different regions of the body. There is no "safe" limit of mercury exposure.

While accurate, current techniques for monitoring detection of heavy metals, such as atomic absorption spectrometry, are quite expensive and oft require complicated, multistage sample preparation. In addition, heavy metal concentrations also vary significantly as functions of time, sometimes within periods as short as an hour. Thus remains the necessity for a novel, sensitive, and selective approach for the continuous, on-site detection of heavy metals to reduce potential health disasters such as that which struck Flint, Michigan.

Research Objective

Major Objective:

- Develop a technique for continuous detection of heavy metal ions in reliant on single and/or two-photon fluorescence-based DNA sensors
- Employ a specific oligonucleotide that displays sensitivity and selectivity for specific heavy metal ions (i.e. Pb^{2+} or Hg^{2+}) through significant changes in fluorescence

Research Approach:

- Analyze fluorescence data from single and two-photon spectroscopy and the corresponding cross-sections in order to determine the most effective means of detecting the presence of heavy metal ions.

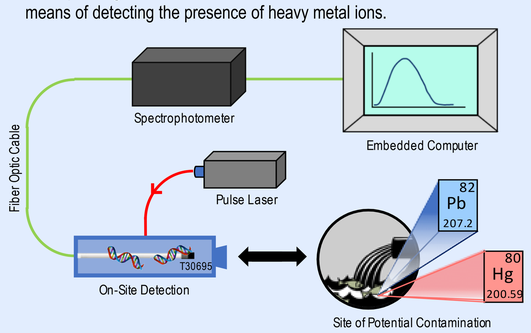

Figure 5: Schematic representation of an ideal, general concept for on-site detection of heavy metal ions using fiber optic cable with laser excitation.

Research Approach

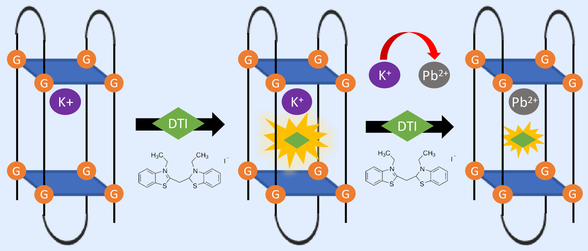

Figure 6: Schematic representation of the use of G-quadruplex based DNA sensors to "detect" presence of heavy metal ions through quenching the fluorescence of the fluorophore.

Investigated Systems

Oligonucleotides:

T30695: 5'-GGGTGGGTGGGTGGGT-3'

T-rich T30695: 5'-TTTTTTGGGTGGGTGGGTGGGTTTTTTT-3'

Spectroscopic Techniques:

1) Use of UV/Vis absorption spectrophotometer
2) Study of single-photon fluorescence
3) Usage of two-photon spectroscopy to determine 2PA fluorescence cross-sections
4) Circular Dichroism (CD)

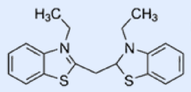

Figure 7: Molecular structure of the fluorophore, diethylthiacyanine iodide (DTI dye).

Results

Interaction of G-quadruplex DNA (T30695/DTI) with Pb^{2+}

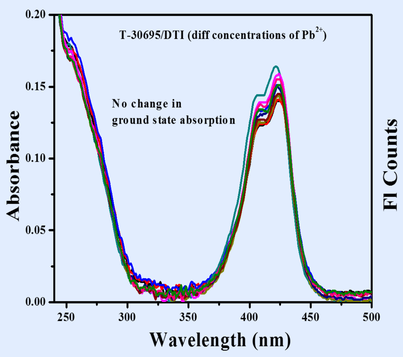

Figure 8: Optical absorbance spectra of T-30695/DTI at varying Pb^{2+} concentrations. Lack of significant change in absorption of both the dye (at 420 nm) and DNA (at 260 nm) suggests little to no structural changes.

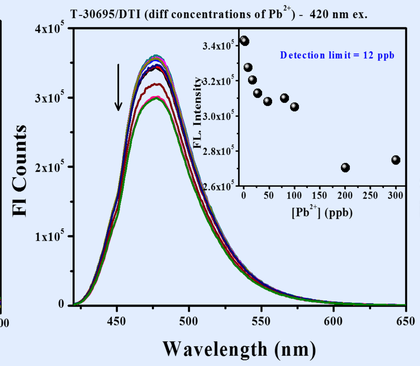

Figure 9: Fluorescence spectra of T30695/DTI upon exposure to different Pb^{2+} concentrations. One-photon excitation begins at 420 nm, and sensitive change in fluorescence takes place at 475 nm as shown in the inset.

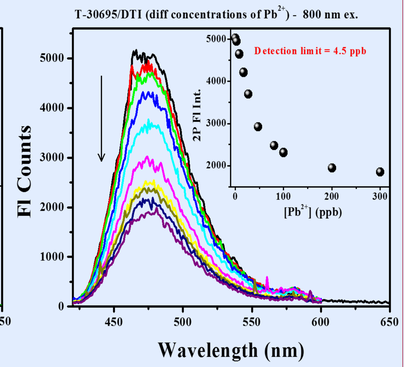

Figure 10: Fluorescence spectra of T30695/DTI upon exposure to different Pb^{2+} concentrations. Two-photon excitation begins at 800 nm, and sensitive change in fluorescence takes place at 475 nm as shown in the inset.

Interaction of G-quadruplex DNA (T30695/DTI) with Hg^{2+}

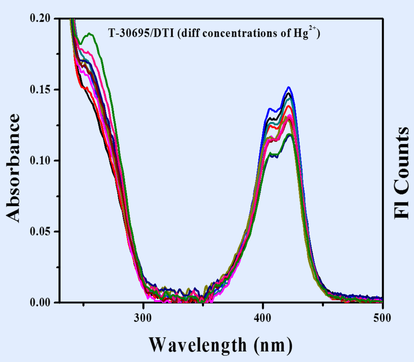

Figure 11: Optical absorbance spectra of T-30695/DTI at varying Pb^{2+} concentrations. Lack of significant change in absorption of both the dye (at 420 nm) and DNA (at 260 nm) suggests little to no structural changes.

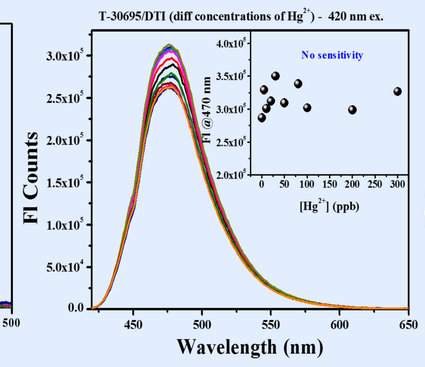

Figure 12: Fluorescence spectra of T30695/DTI upon exposure to different Hg^{2+} concentrations. One-photon excitation is at 420 nm, and non-sensitive change in fluorescence takes place at 475 nm as shown in the inset.

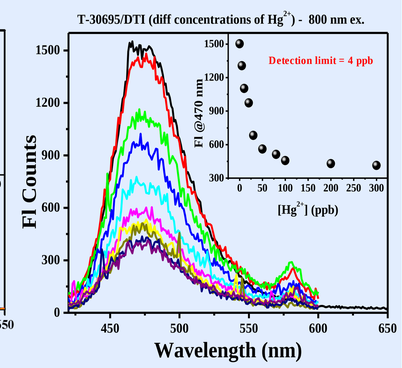

Figure 13: Fluorescence spectra of T30695/DTI after exposure to different Hg^{2+} concentrations. Two-photon excitation begins at 800 nm, and sensitive change in fluorescence takes place at 475 nm as shown in the inset.

Single and Two-photon Fluorescence Sensitivity of T-30695/DTI to Pb^{2+} and Hg^{2+}

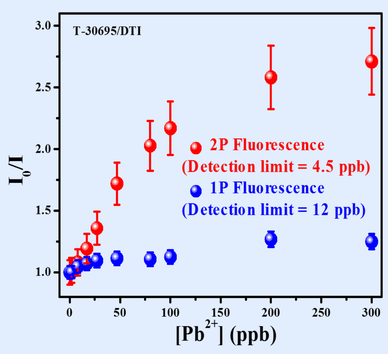

Figure 14: A comparison of the change in the single and two-photon fluorescence sensing of Pb^{2+} at different concentrations. Better sensitivity was observed with two-photon excitation.

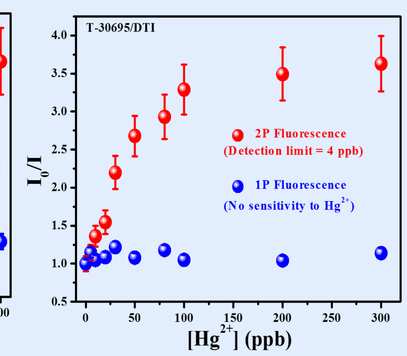

Figure 15: A comparison of the change in the single and two-photon fluorescence sensing of Hg^{2+} at different concentrations. Better sensitivity was observed with two-photon excitation.

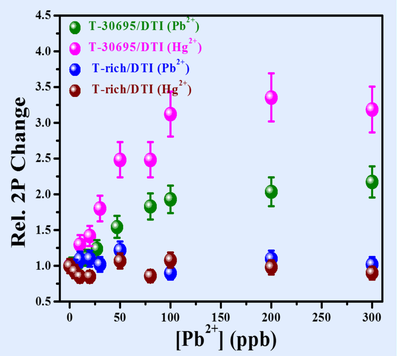

Figure 16: A comparison of 2P/1P cross-section ratio in determining sensitivity of T30695 and T-rich T30695 for Pb^{2+} and Hg^{2+} detection. T-rich T30695 is essentially ineffective.

Selectivity of T30695/DTI for Toxic Metal Ions

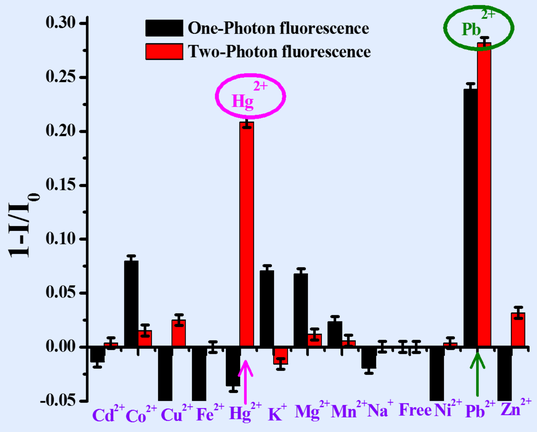

Figure 17: A demonstration of T30695's selectivity of Pb^{2+} and Hg^{2+} over other common metals for both 1P and 2P excitations.

Canonical Structures of DNA with Metal Ions

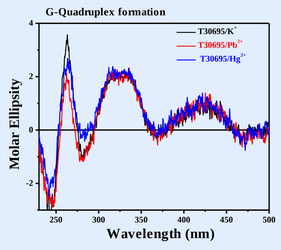

Figure 18: The circular dichroism measurements of the T30695 oligonucleotide with K^+, Hg^{2+}, and Pb^{2+} shows the formation of a G-quadruplex structure.

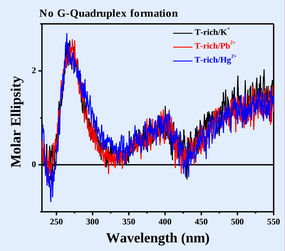

Figure 19: The circular dichroism measurements of T-rich T30695 oligonucleotide with K^+, Hg^{2+}, and Pb^{2+} shows that no G-quadruplex formation can be observed.

Conclusions

- Two novel DNA oligonucleotide sequences, T30695 and T-rich T30695, were selected for their ability to form G-quadruplex structures in order detect, with sensitivity and selectivity, the toxic heavy metal ions Pb2+ and Hg2+.
- DTI dye was found to be an efficient fluorophore for this mode of detection, with the ability to both interact with the DNA via intercalation and to demonstrate notable fluorescence changes upon binding to toxic heavy metal ions.
- T30695/DTI showed sensitivity to Pb2+ with both one-photon (12 ppb) and two-photon excitation (4.5 ppb); sensitivity under two-photon excitation was far better than that under one-photon excitation, which may be ascribed to the enhancing of two-photon cross-sections by interaction of the DNA backbone electric field.
- T30695/DTI lacked sensitivity to Hg2+ under one-photon excitation; far better sensitivity was observed with two-photon excitation (4 ppb). This—again—can be ascribed to the orientation of the G-quadruplex structures' electric fields along with the orientation of the fluorophore.
- T30695/DTI was found to be highly selective for only the heavy metal ions Pb2+ and Hg2+. Such selectivity may be attributed to the size of the mercury and lead metal ions, which more effectively stabilized the G-quadruplex structure.
- T-rich T30695/DTI showed neither sensitivity nor selectivity for Hg2+ or Pb2+ as it does not form a G-quadruplex, as evidenced by CD measurements.

References

1. Brochin, R., Leone, S., Phillips, D., Shepard, N., Zisa, D., & Angerio, A. (2008). The Cellular Effect of Lead Poisoning and Its Clinical Picture. Retrieved February 10, 2017, from https://blogs.commons.georgetown.edu/journal-of-health-sciences/issues-2/previous-volumes/vol-5-no-2-december-2008/the-cellular-effect-of-lead-poisoning-and-its-clinical-picture/
2. Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B. B., & Beeregowda, K. N. (2014). Toxicity, mechanism and health effects of some heavy metals. Retrieved March 14, 2017, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4427717/
3. Singh, R., Gautam, N., Mishra, A., & Gupta, R. (2011). Heavy metals and living systems: An overview. Retrieved March 14, 2017, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3113373/
4. Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2014). Heavy Metals Toxicity and the Environment. Retrieved January 15, 2017 from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4144270/
5. Xu, L., Shen, X., Hong, S., Wang, J., Zhang, Y., Wang, H., . . . Pei, R. (2015). Turn-on and label-free fluorescence detection of lead ions based on target-induced G-quadruplex formation. *Chem. Commun.*, 51(38), 8165-8168. doi:10.1039/c5cc01590
6. Zhan, S., Wu, Y., Liu, L., Xing, H., He, L., Zhan, X., . . . Zhou, P. (2013). A simple fluorescent assay for lead(II) detection based on lead(II)-stabilized G-quadruplex formation. *RSC Advances*, 3(38), 16962. doi:10.1039/c3ra42621a
7. Zhan, S., Wu, Y., Luo, Y., Liu, L., He, L., Xing, H., & Z P. (2014). Label-free fluorescent sensor for lead ion detection based on lead(II)-stabilized G-quadruplex formation. Analytical Biochemistry, 462, 19-25. doi:10.1016/j.ab.2014.01.010
8. Zhan, S., Xu, H., Zhang, D., Xia, B., Zhan, X., Wang, L., Lv, J., & Zhou, P. (2015). Fluorescent detection of Hg2+ and Pb2+ using GeneFinder™ and an integrated functional nucleic acid. *Biosensors and Bioelectronics*, 72, 95-99. doi:10.1016/j.bios.2015.04.021