

## Background:

Heavy metals are an ubiquitous and troublesome class of pollutants, and lead (Pb) occupies a prominent position as a contaminant requiring constant attention due to its numerous toxicological effects over a wide range of exposure. Lead is a RCRA metal, and its presence often defines a waste as hazardous. It is also an EPA Urban Air Toxic, meaning its emissions are regulated under the Clean Air Act Amendment of 1990. Anthropogenic sources of Pb from military operations require active monitoring and sensing to ensure environmental compliance and protection. Despite the recognized adverse effects of lead on aquatic and terrestrial biota, its presence is not actively monitored, in large part due to the lack of a field product that meets all requirements for remote, real-time, in-situ measurement of lead in groundwater.

## Objective:

This project aims to create a highly selective and sensitive miniaturized sensor for bioavailable lead ( $Pb^{2+}$ ) by combining two recent advances: (1) catalytic deoxyribonucleic acid (DNA) that is reactive only to  $Pb^{2+}$  and which can be tagged to produce fluorescence and (2) nanoscale fluidic molecular gates that can manipulate fluid flow and perform molecular separations on tiny volumes of material. This work will develop both the chemistry needed to combine  $Pb^{2+}$ -specific catalytic DNA with the molecular gates and the protocol for separating, sensing, and quantifying  $Pb^{2+}$  in a complex matrix.

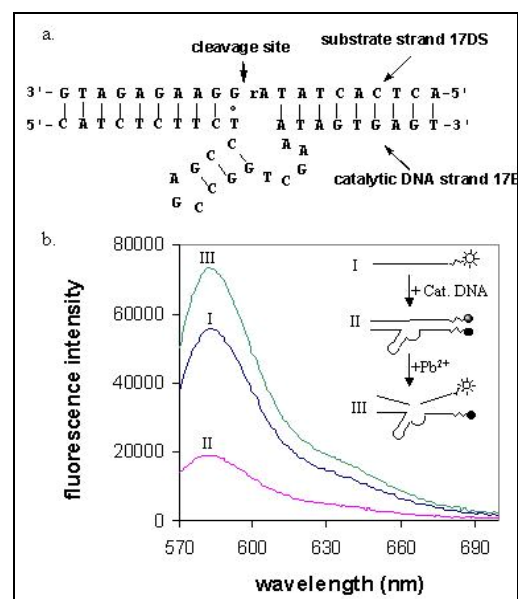
## Summary of Process/Technology:

Building on the capability of microfabricated, capillary electrophoresis columns in polydimethylsiloxane to precisely control fluidic movement, a three-dimensional arrangement of these channels will be used to induce a sample to pass through a molecular gate consisting of a thin polymeric membrane. Specific recognition elements that cause a measurable response in the presence of a particular species will be incorporated into these channels. In this case, the interior of the channels will be chemically modified to bind a unique sequence of DNA, obtained through in vitro selection, that is highly selective toward  $Pb^{2+}$ . It will cleave an associated strand of substrate DNA in the presence of  $Pb^{2+}$ . Tagging the substrate DNA with a fluorophore allows for detection of the substrate DNA fragments, thus providing a sensitive optical signal for the presence of  $Pb^{2+}$ . This research will combine these scientific advances into a miniaturized sensor that is capable of remote, selective, and sensitive detection of the presence of  $Pb^{2+}$ .

## Benefit:

Several key operating characteristics make this sensor modality stand apart. The strategy outlined can apply to any analyte for which a combinatorial binding sequence can be

identified. The catalytic DNA can collect analyte for as long as is needed to generate a usable signal, thereby increasing the sensitivity of the sensors. The substrate DNA can be released and regenerated, allowing repeated unattended use in the field. Unlike other chemically-based sensors, the waste stream produced from operation of this device is exceedingly small, and only non-toxic DNA fragments are added. The sensor can be extremely rugged. In particular, it is insensitive to episodic loss of liquid analyte stream so it can survive periods without liquid input, such as periods of fluctuating groundwater levels.



(a) Structure of the  $Pb^{2+}$  sensing catalytic DNA. (b) Fluoresensing mechanism and preliminary results from unbound DNA.

## Accomplishments:

This SEED project began in FY 2002. Accomplishments will be noted upon completion of the project.

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