

NEXT GENERATION SENSORS FOR CONTAMINANTS IN WATER: CATALYTIC DNA AS A MOLECULAR BEACON

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ABSTRACT

The mobile warfighter needs sensor technology that provides an immediate and unambiguous response to toxins in water. Heavy metals are a ubiquitous and troublesome class of pollutants, and lead (Pb) occupies a prominent position as a contaminant requiring constant attention. Especially due to its nature of its toxicity to humans and the environment, persistency, and bioaccumulation, anthropogenic sources of Pb from military operations require active monitoring and sensing to ensure soldier health protection and environmental compliance. We have created a selective and sensitive miniature sensor for Pb^{2+} by combining two significant advances: (a) catalytic DNA that is reactive only to Pb^{2+} and (b) nanoscale fluidic molecular gates that can manipulate fluid flows and perform molecular separations on tiny volumes of material.

INTRODUCTION

Capillary electrophoresis columns, now microfabricated in polydimethylsiloxane and polycarbonate, are capable of precisely controlled fluidic movement by application of an electric field across distal ends of the columns. A three dimensional arrangement of these channels can induce a user selectable fraction of a sample to pass through a novel molecular gate consisting of a thin polymeric membrane perforated with a number of long narrow channels, typically on the order of 100 nm in diameter. Specific recognition elements can be incorporated into these channels that cause a measurable response in the presence of a particular species. In this case, the interior of the channels can be chemically modified with a unique sequence of catalytic DNA that cleaves an associated strand of substrate DNA in the presence of lead. Tagging the substrate DNA with a fluorophore allows for detecting the substrate DNA fragments, thus providing a sensitive optical signal for the presence of lead. This research is concerned with all chemical aspects of design and function for a miniature sensor capable of remote, selective, and sensitive detection of bioavailable lead.

In 1994, DNA was shown through a technique called *in vitro* selection (*vide infra*) to carry out catalytic functions when single stranded (Breaker and Joyce, 1994). The DNAs (called catalytic DNA or DNA enzymes or deoxyribozymes) have proven capable of catalyzing many reactions including RNA/DNA-cleavage, ligation, phosphorylation, cleavage of phosphoramidate bonds, and porphyrin metallation (Breaker, 1997). Catalytic DNAs have shown great promise as anti-viral pharmaceutical agents against diseases such as AIDS and leukemia. Recently Lu and coworkers at Illinois demonstrated that catalytic DNAs can expand out of the realm of biological chemistry and into environmental monitoring by selectively reacting with Pb^{2+} in the presence of interfering cations (Li and Lu, 2000).

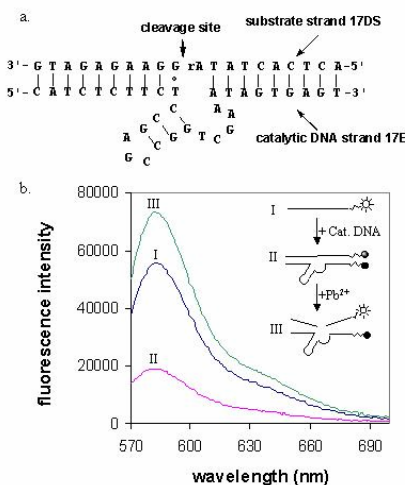


Figure 1. (a) Structure of the Pb-sensing catalytic DNA. (b) Fluorosensing mechanism and results.

Recently, they reported a new application for catalytic DNAs as biosensors for metal ions, specifically Pb^{2+} . This application is based on the observation that catalytic DNA, obtained through *in vitro* selection, can be used to

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bind metal ions with high affinity and specificity (Breaker and Joyce, 1994; Li et al. 2000; Santoro et al. 2000). Therefore, the activity of a selected catalytic DNA can be used to measure the identity and quantity of the specific metal ion. The biosensor consists of a catalytic DNA capable of base-pairing to a DNA substrate containing a single ribonucleotide residue (labeled rA in Fig. 1a). When a fluorophore, *e.g.* carboxytetramethylrhodamine (TAMRA), is attached to the 5'-end of the DNA substrate, the fluorescence signal at 580 nm is quenched by its proximity to a fluorescence quencher, *e.g.* 4-(((4-dimethylamino)-phenyl)azo)benzoic acid (Dabcyl), at the nearby 3'-end of the catalytic DNA. In the presence of Pb^{2+} , the fluorescence emission of TAMRA increases dramatically ($\sim 400\%$), due to the cleavage of the substrate DNA and subsequent separation of the fluorophore from the quencher (Fig. 1b inset). This is followed by the release of substrate DNA fragments and Pb^{2+} . This system represents a new class of metal ion sensors and is the first example of using the powerful tools of combinatorial molecular biology to identify a cation-specific catalytic DNA for sensing of metal ions. It combines the high selectivity of catalytic DNA (> 80 fold for Pb^{2+} over other divalent metal ions, see Fig. 2) with the ultralow background and resulting high sensitivity of fluorescence detection, and it can be applied

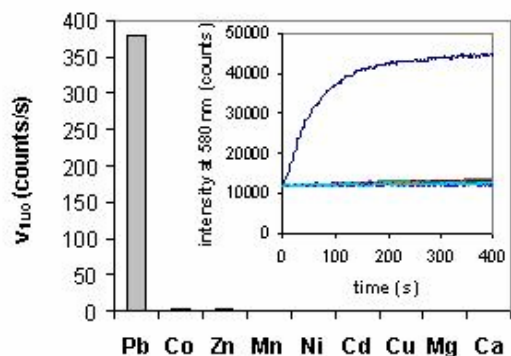


Figure 2. Sensor selectivity.

to quantitative detection of Pb^{2+} over a concentration range of three orders of magnitude. The sensitivity and selectivity of the system can be altered by using different fluorescence/quencher pairs for different sensors. The system is easily regenerated by washing away the cleaved products and adding new substrate DNA to the catalytic DNA strand -- a fact that will be exploited to regenerate active material in the proposed sensor. Finally, catalytic DNA specific for other metal ions and with various detection ranges can be isolated by varying the catalytic DNA selection conditions in the combinatorial search, making this approach extensible to other metal ions and small organic molecules. Thus, the approach outlined here is targeted not only at the development of a specific

sensor for Pb^{2+} but also toward *rapid development of a whole class of highly specific sensors*.

Bohn *et al.* have developed a new approach to fluidic control, in which a 'molecular gate' is constructed from a thin (typically $5\ \mu\text{m}$) polymeric membrane perforated with a number ($\sim 10^8\ \text{cm}^{-2}$) of long narrow (typical aspect ratios of 25-250) channels, *viz.* Fig. 3. These structures exhibit unique and tunable electrokinetic flow properties, because the product of the channel diameter, a , and the inverse Debye length, κ , is ~ 1 . When $\kappa a < 1$, the electric double layer extends throughout the pore (Saksena and Zydney, 1995; Basu and Sharma, 1997; Kim and Stevens, 1997), and the mobile counterions filling the channel

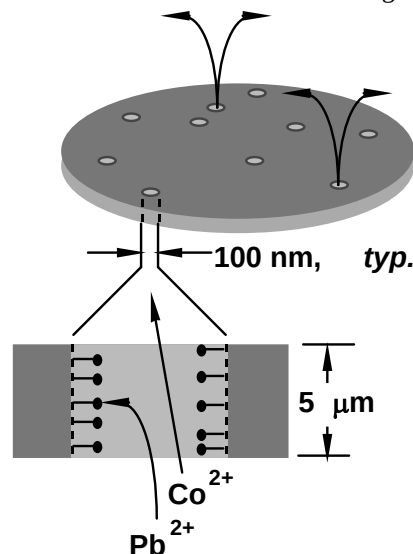


Figure 3. Schematic diagram of the molecular gate used as an intelligent nanofluidic processor

determine transport. At the other limit, $\kappa a > 1$, the electrical double layer is mostly collapsed, and normal ion migration effects dominate. Thus, flow can be controlled by (a) direction of the applied bias, (b) sign and surface density of the immobile charge on the wall, *i.e.*, pH and chemical derivatization, and (c) the magnitude of κa . Because κ is controlled by solution ionic strength, control of flow in these nanometer channels is exceptionally versatile (Kemery et al., 1998; Steehler et al., 1998). These nanoscale porous materials with well-defined cross-sectional geometry are an excellent choice for fluidic handling at low levels due to several related factors. (a) The separations capacity factor, k' , which scales like the surface-to-volume ratio, is huge. Comparing a $20\ \mu\text{m}$ i.d. wall-coated open tubular column with a $10\ \text{nm}$ thick coating to a $200\ \text{nm}$ i.d. nanopore with the same coating, the increase in capacity factor is a factor $> 10^2$. (b) Nanopores have fundamentally different properties than their larger μm -scale analogs, because

characteristic length scales that describe important physico-chemical phenomena are approximately equal to the dimensions of the nanochannels. (c) Nanopores are ideally suited to making intelligent interconnects between microfluidic elements, because the interconnect itself can be made to be integral to the intelligent movement of biomolecules, and it is simple to integrate and to interface with existing microfluidic technologies. Combining catalytic DNA with molecular gates to achieve a Nanofluidic Intelligent Processor (NIP) will exploit all three of these important characteristics.

METHODS

All DNA is purchased from Integrated DNA Technologies (IDT) (Coralville, IA) or from Trilink BioTechnologies, Inc. (San Diego, CA). Prepolymer and curing agent (Sylgard 184, Dow Corning Corp. Midland, MI) and polycarbonate nuclear track-etched (PCTE) membranes with a hydrophilic wetting layer of poly(vinylpyrrolidone) (Osmonics, Minnetonka, MN) were used in the PDMS (poly(dimethylsiloxane)) chip. These PCTE membranes are 10 microns thick with 200 nm diameter pores at a pore density of 3×10^8 pores / cm² and are used as the molecular gate membranes. Microfluidic devices (shown in Figures 4 and 5) were fabricated from poly(dimethylsiloxane) (PDMS) using standard photolithographic and replica molding methods (McDonald et al., 2000). For the transport device (Figure 4), two identical channels were orthogonally oriented on a PDMS microchip and separated at the intersection by a nanocapillary array interconnect, a PCTE membrane containing 200 nm diameter cylindrical pores. PCTE membranes act as an electronically gateable valve preventing fluid flow between the microchannels unless the proper voltage scheme is applied to the terminal ends of channels, forcing electrokinetic fluid flow through the membrane (Kuo et al., 2003a, 2003b; Cannon et al., 2003; Kuo et al., 2004, Tulock et al., 2004). For the separation device (Figure 5), an additional channel is added perpendicular to the horizontal channel (Kuo et al., 2003b; Cannon et al., 2003). Analyte-containing sample is introduced through injection channel and metal ions are spatially separated during travel along the separation channel based on differences in electrophoretic mobility of the metal ion. This allows the target metal ions to be selectively discriminated and collected at the collection channel.

RESULTS

Chip design.

We designed two types of chips for these experiments. Figure 4 shows the simpler design that contains two channels, a source channel and a detection channel. For this transport device, the general chip design

consists of the thin PCTE nanocapillary array layer sandwiched between two crossed PDMS microfluidic channels. Crossed microfluidic channels were fabricated from poly(dimethylsiloxane) (PDMS) using standard rapid prototyping protocols for PDMS (Duffy et al., 1998). Channels used in this experiment were 100 μ m wide, 60 μ m deep and 1.4 cm long for the transport devices. A reservoir PDMS layer was sealed on the top of the sandwiched device and fluidic connection to the various channel layers was accomplished by punching small holes through the PDMS channel layers. The small rectangle is the PCTE membrane containing 200-nm diameter cylindrical pores. This device was used for lead characterization. Applied voltages across the channel arms force solution to flow from one reservoir to another along the channels, in this case, positively charged cationic species would move from high positive voltages

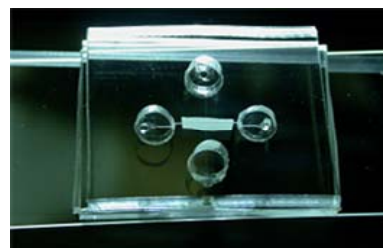


Figure 4: Optical image of the two channel PDMS gated injection and transport device.

through the membrane to ground.

A second design is shown in Figure 5. A three channel design allows more flexibility. The injection channel can be constantly replenished with sample from an environmental source, such as river water. For analysis, voltages are altered to inject a portion of the injection channel volume onto the long, horizontal separation channel. This channel is designed for

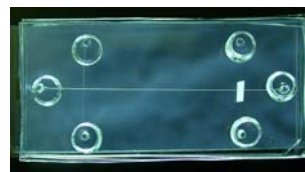


Figure 5: A three channel PDMS device.

electrophoretic separation of analytes. When the analyte of interest is at the intersection of the detection channel with the microchambers, voltage switching sends the analyte band into the microchamber of choice where the catalytic DNA is contained. Solutions can be moved from one area of the chip to another by proper application of voltages across the reservoir ends.

Electrophoretic control of solution movement.

Using a two channel sensor (Figure 4), we demonstrated the movement of Pb^{2+} from a reservoir, through the source (vertical) channel, through the molecular gate membrane, and into the detection (horizontal) channel. The injection channel was filled with background electrolyte solution (BGE) and the reservoirs were filled with Pb^{2+} in BGE. The detection channel was filled with the DNAzyme system shown in Figure 1a (hybridized system with enzyme strand (17E) and a substrate strand (17DS)) in BGE. Microfluidic

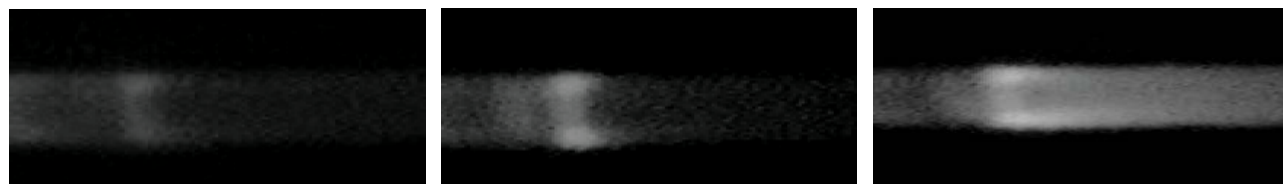


Figure 6. Fluorescence image series for gated injection of Pb^{2+} band across the array of 200 nm diameter capillaries.

transport was achieved by applying bias across different reservoirs. To inject Pb^{2+} solution into the detection channel, a positive high voltage was applied to the two reservoirs at the ends of the source channel while grounding the waste reservoir of the detection channel. With 200 nm pore diameter nanocapillary array interconnections, this forward bias condition causes flow from the injection channel through the nanocapillary array to the collection channel. The sequential images in Figure 6 captures the injection band at the intersection of the two cross-channels. The fluorescence steadily increases with time as the Pb^{2+} band moves through the horizontal channel. The catalytic DNA thereby transduces the lead concentration into a fluorescence intensity.

Figure 7 shows results from using the three channel chip (Figure 5). With appropriate electrical biases, we were able to prove controlled movement of solutions along multiple channels. By switching the voltage bias configurations, a versatile fluidic manipulation was demonstrated. The injection and separation channel (Fig.

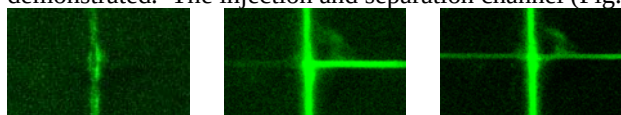


Figure 7: Fluorescent images of lead injections into the collection channel from background to the on (inject) state to the off state.

13) were filled with BGE and the collection channel was filled with 500 nM DNAzyme. After verifying complete filling, one of the reservoirs of the injection channel was replaced with 10 μ M Pb^{2+} . Figure 7A shows a sequence of fluorescence images of electrokinetically injected Pb^{2+} forced into the detection (horizontal) channel containing

the DNAzyme. The first picture is the background state where slight fluorescence is observed due to substrate DNA adhesion to the membrane. The middle picture shows the migration and injection of the lead solution into the detection channel whereupon the DNAzyme reacts to cleave the substrate DNA and release fluorophores. We note that the negatively charged cleaved DNA moves toward the highest positive bias. The off state (right picture) shows the eventual clear out of the fluorescent fragments after injection is stopped. The images show the electrophoretic control of Pb^{2+} and cleaved DNA through the microchannels via nanocapillary array. These results were obtained using a laser induced fluorescence

detection scheme. Future control of the migration and collection of the fluorescent substrate fragments will further enhance our detection limits.

Calibration Curve with Lead Solution.

Using the two channel system (Figure 4), additional experiments were performed to investigate the reproducibility of low lead concentrations and the quantitative behavior of the chip. Figure 8 shows the reproducibility of transfer experiments in which 100 nM Pb^{2+} is transferred from the source channel to the detection channel across a PCTE molecular gate membrane. The source channel was filled with 100 nM Pb^{2+} in BGE and the detection channel was filled with 500 nM DNAzyme. Fluorescence was detected by laser-induced fluorescence (LIF) excited with 488-nm radiation

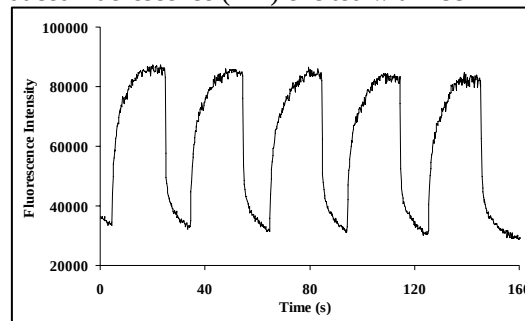


Figure 8: Migration of Pb^{2+} across PCTE nano-capillary arrays with 200 nm-diameters connecting to PDMS channels.

from an argon ion laser. Excellent reproducibility was obtained using this low lead concentration. The detection limit was evaluated by repetitive injection of 50 nM Pb^{2+}

standard solution. From the baseline noise during the off state and the fluorescence intensity of 50 nM Pb^{2+} at during the on state, the detection limit (signal-to-noise ratio of 3:1) was determined to be 11 nM (2.2 ppb) which is lower than the 72 nM (15 ppb) action level in drinking water recommended by the U.S. Environmental Protection Agency. These results demonstrate that the combination of electrokinetically actuated measurement cycles on a microfluidic device and a Pb^{2+} -selective DNzyme produce a device sensitive enough to monitor lead in drinking water or ground water. A calibration curve (Figure 9) was constructed by measuring

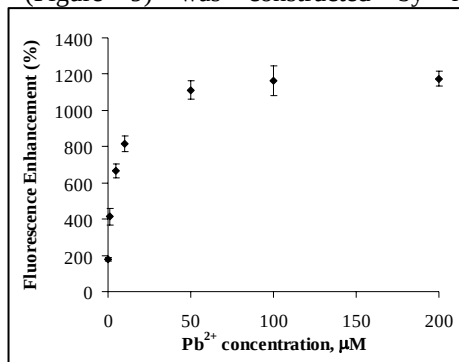


Figure 9: Calibration curve of the DNzyme-based Pb^{2+} detection system.

fluorescence intensities using 7 different concentrations Pb^{2+} solutions varying in concentration from 100 nM to 200 μM . The plot of fluorescence intensity versus $[\text{Pb}^{2+}]$ agreed well with the response rate versus $[\text{Pb}^{2+}]$ curve using a conventional spectrofluorometer (Liu and Lu, 2003) suggesting reliable performance of on-chip DNzyme assays for Pb^{2+} .

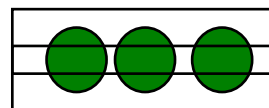
Immobilization of DNA

There are two inherent advantages associated with surface immobilization of the catalytic DNA sensor moiety. First, since hybridization of the enzyme strand with the substrate strand would occur in a specific reaction zone, background fluorescence would not be observed if the detection zone is isolated from the reaction zone. Noise in the background fluorescence is a fundamental factor determining a limit of detection especially for a low level of Pb^{2+} detection. To this end, reducing the background level due to higher hybridization efficiency can be accomplished with immobilization. Second, the surface-immobilized DNzyme may be regenerated and used multiple times (Swearingen et al., 2005). Hybridization of surface-bonded DNA has been shown to be reversible, enabling reuse of the chip for successive measurements (Levicky et al., 1998).

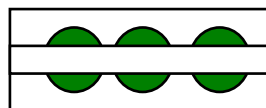
A strategy to develop a microfluidic chip with an internal reference for lead detection is illustrated in Figure

10: $\text{Pb}(\text{II})$ -specific DNzyme with fluorescent dye on one end and an amine moiety on the other end is immobilize onto a superaldehyde[®] glass surface. To form the enclosed microfluidic chip, a PDMS substrate that contains the microchannel structure and sample ports is plasma treated, aligned, and then placed in direct contact with the glass substrate. The hybridization channel passed through partially each of the predeposited probe sites. The immobilized DNzyme is then hybridized with the complementary quencher-containing substrate strand in an incubation chamber. The fluorescence in the channel is quenched upon hybridization, while the fluorescence that sealed under PDMS acts as an internal reference. In the presence of lead, a fluorescent signal can be detected upon the reaction of lead ion with the substrate strand and release of the quencher. The sensing chemistry can be regenerated several times by hybridization of DNzyme with its complementary substrate strand in the channel.

1. Immobilization of fluorescein labeled DNzyme onto glass. PDMS channel is assembled on top of the spots.



2. Hybridization of quencher labeled substrate DNA in channel.



3. Reaction with Pb^{2+} will cleave the substrate and release the fluorophore.

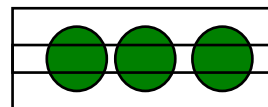
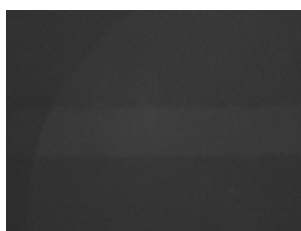


Figure 10. Internal standard scheme

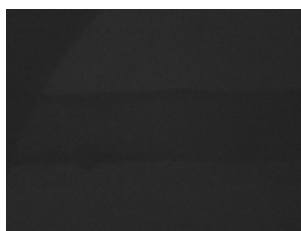
Amino and fluorophore modified DNzyme was hand spotted onto SuperAldehyde[®] glass slides (Telechem International, Inc., Sunnyvale, CA). Since the slides are already covered with surface aldehyde groups, the amino terminated DNzyme should readily bind to this surface. The slide was allowed to dry for 12 hrs at room temperature ($\sim 25^\circ\text{C}$) at $<30\%$ relative humidity. The spotted slides were then rinsed with 2 x 0.1% SDS, 2 x dH_2O for 2 min each wash at room temperature to remove unbound DNA, followed by 5 min gentle mixing in 500 ml NaBH_4 reducing solution to block the unreacted surface. After the blocking treatment, the slides were

rinsed with 2 x dH₂O, 1 x 0.1% SDS, then transferred to boiling dH₂O at 100 °C for 3 min, followed by plunging the slides into ice cold 100% ethanol for 30 sec to fix the denatured DNA.

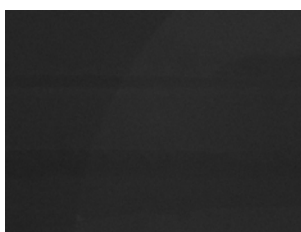
Standard rapid prototyping and soft lithography processes were used to fabricate the upper PDMS layer with embedded channels and reservoirs. The sealing between PDMS layer and glass was achieved by oxygen plasma treatment of the PDMS surface for 20 s at 55 W with O₂ pressure of ca. 500 mTorr. The channel is 100 µm wide x 100 µm deep x 20 mm long.



a. Before hybridization



b. After hybridization



c. After the reaction with Pb²⁺

Figure 11. Fluorescence images of the microfluidic channel with immobilized catalytic DNA.

Figure 11 shows the fluorescence images of the assembled channel. The top image shows the upper quadrant of a spot bisected by a horizontal channel. The fluorescence increase in the channel could be caused by the reflection index difference between the PDMS and the air. The immobilized DNAzyme is then hybridized with the complementary quencher-containing substrate strand in an incubation chamber. The fluorescence in the channel is quenched upon hybridization (Figure 11b) and

the channel appears dark, while the fluorescence sealed under PDMS remains unchanged. In the presence of lead, a fluorescent signal can be detected upon the reaction of lead ion with the substrate strand and release of the quencher (Figure 11c). The sensing chemistry can be regenerated by reintroducing the complementary substrate strand into the channel. This device is an important step toward detecting multiple heavy metal ions with a single injection by introducing different DNAzymes at different locations in the detection channel on a microfluidic-nanofluidic molecular gated device.

CONCLUSIONS

This work will produce a prototype sensor having all desired characteristics of a remote field sensor. Several key operating characteristics make this sensor modality stand apart. Repetitive analyte delivery cycles can be realized, meaning that the catalytic DNA can react with analyte for as long as is needed to generate a usable signal, increasing sensitivity. Second, the substrate DNA can be released and regenerated, allowing repeated unattended use in the field. Third, unlike other chemically based sensors, the waste stream produced from operation of this device is exceedingly small, (mL/year) and only non-toxic DNA fragments are added. Finally, 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 might be encountered with groundwater sources that periodically dry up. Finally, the strategy outlined here is completely general and a sensor can be constructed for *any* analyte for which a combinatorial binding sequence can be identified.

Successful performance of this sensing platform increases the relevance of this research beyond Pb²⁺ to include creation of field sensors for other chemicals of interest such as Al, Hg, Cd, and depleted uranium. This platform offers the possibility of incorporating multiple sensing locations in one device; thus, by incorporating different metal-ion selective DNAzymes into a single microfluidic device, multiple species can be determined simultaneously. This work begins our efforts to create rugged devices for rapid monitoring of drinking water for the soldier in the field.

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REFERENCES

Basu, S. and Sharma, M. M., 1997: An Improved Space-Charge Model for Flow Through Charged Microporous Membranes, *J. Membr. Sci.*, **124**, 77-91.

- Breaker, R. R., 1997: DNA Enzymes, *Nat. Biotechnol.*, **15**, 427-431.
- Breaker, R. R. and Joyce, G. F., 1994: Inventing and Improving Ribozyme Function – Rational Design Versus Iterative Selection Methods, *Trends in. Biotechnol.*, **12**, 268-275.
- Cannon, D. M., Jr., Kuo, T.-C., Bohn, P. W., and Sweedler, J. V., 2003: Nanocapillary Array Interconnects for Gated Analyte Injections and Electrophoretic Separations in Multilayer Microfluidic Architectures, *Analyt. Chem.*, **75**, 2224-2230.
- Duffy, D.C., McDonald, J.C., Schueller, O.J.A., and Whitesides, G.M., 1998: Rapid Prototyping of Microfluidic Systems in Poly(dimethylsiloxane), *Anal. Chem.*, **70**, 4974-4984.
- Kemery, P. J., Steehler, J. K., and Bohn, P. W., 1998: Electric Field Mediated Transport in Nanometer Diameter Channels, *Langmuir*, **14**, 2884-2889.
- Kim, K. J. and Stevens, P. V., 1997: Hydraulic and Surface Characteristics of Membranes with Parallel Cylindrical Pores, *J. Membr. Sci.*, **123**, 303-314.
- Kuo, T.-C., Cannon, D. M., Jr, Shannon, M. A., Bohn, P. W., and Sweedler, J. V., 2003a: Hybrid Three-dimensional Nanofluidic/Microfluidic Devices Using Molecular Gates, *Sens. Actuators, A*, **102**, 223-233.
- Kuo, T.-C., Cannon, D. M., Jr., Chen, Y.N., Tulock, J. J., Shannon, M. A., Sweedler, J. V., and Bohn, P. W., 2003b: Gateable Nanofluidic Interconnects for Multilayered Microfluidic Separation Systems, *Analyt. Chem.*, **75**, 1861-1867.
- Kuo, T.-C., Kim, H.-K., Cannon, D. M., Jr, Shannon, M. A., Sweedler, J. V., and Bohn, P. W., 2004: Nanocapillary Arrays Effect Mixing and Reaction in Multilayer Fluidic Structures, *Angew. Chem.*, **43**, 1862-1865.
- Levicky, R., Herne, T. M., Tarlov, M. J., and Satija, S. K., 1998: Using Self-assembly to Control the Structure of DNA Monolayers on Gold: A Neutron Reflectivity Study, *J. Am. Chem. Soc.*, **120**, 9787-9792.
- Li, J., and Lu, Y., 2000: A Highly Sensitive and Selective Catalytic DNA Biosensor for Lead Ions, *J. Am. Chem. Soc.*, **122**, 10466-10467.
- Li, J., Zheng, W., Kwon, A. H., and Lu, Y., 2000: In Vitro Selection and Characterization of a Highly Efficient Zn(II)-dependent RNA-cleaving Deoxyribozyme, *Nucleic Acids Res.*, **28**, 481-488.
- Liu, J.W. and Lu, Y., 2003: A Colorimetric Lead Biosensor Using DNAzyme-Directed Assembly of Gold Nanoparticles, *J. Am. Chem. Soc.*, **125**, 6642-6643.
- McDonald, J. C., Duffy, D. C., Anderson, J.R., Chiu, D. T., Wu, H.K., Schueller, O. J. A., and Whitesides, G. M., 2000: Fabrication of Microfluidic Systems in Poly(dimethylsiloxane), *Electrophoresis*, **21**, 27-40.
- Saksena, S. and Zydney, A. L., 1995: Pore-size Distribution Effects on Electrokinetic Phenomena in Semipermeable Membranes, *J. Membr. Sci.*, **105**, 203-215.
- Santoro, S. W., Joyce, G. F., Sakthivel, K., Gramatikova, S., and Barbas, C. F., III, 2000: RNA Cleavage by a DNA Enzyme with Extended Chemical Functionality, *J. Am. Chem. Soc.*, **122**, 2433-2439.
- Steehler, J. K., Lu, W., Kemery, P. J., and Bohn, P. W., 1998: Electric Field Induced Permeability Modulation in Pure and Mixed Langmuir-Blodgett Multilayers of Hemicyanine Dyes and Octadecanoic Acid on Nanoporous Solid Supports, *J. Membr. Sci.*, **139**, 243-257.
- Swearingen, C.B., Wernette, D. P., Crokep, D.M., Lu, Y., Sweedler, J. V., and Bohn, P. W., 2005: Immobilization of a Catalytic DNA Molecular Beacon on Au for Pb(II) Detection, *Analyt. Chem.*, **77**, 442-448.
- Tulock, J. J., Shannon, M. A., Bohn, P. W., and Sweedler, J. V., 2004: Microfluidic Separation and Gateable Fraction Collection for Mass-limited Samples, *Analyt. Chem.*, **76**, 6419-6425.