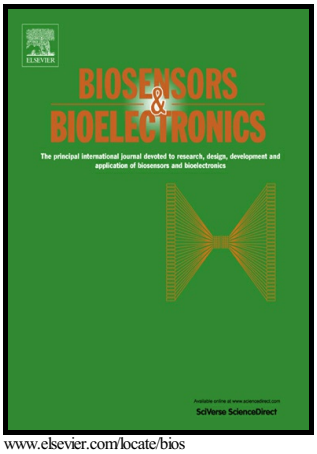


www.elsevier.com/locate/bios

T-T mismatch-driven biosensor using triple functional DNA-protein conjugates for facile detection of Hg^{2+}

Ruoyu Wang, Xiaohong Zhou, Hanchang Shi, Yi Luo

PII: S0956-5663(15)30635-7
DOI: http://dx.doi.org/10.1016/j.bios.2015.11.082
Reference: BIOS8212

To appear in: *Biosensors and Bioelectronic*

Received date: 17 September 2015
Revised date: 25 November 2015
Accepted date: 27 November 2015

Cite this article as: Ruoyu Wang, Xiaohong Zhou, Hanchang Shi and Yi Luo, T-T mismatch-driven biosensor using triple functional DNA-protein conjugates fo facile detection of Hg^{2+}, *Biosensors and Bioelectronic* http://dx.doi.org/10.1016/j.bios.2015.11.082

T-T mismatch-driven biosensor using triple functional DNA-protein conjugates for facile detection of Hg^{2+}

Ruoyu Wang [a], Xiaohong Zhou [a*], Hanchang Shi [a] and Yi Luo[a]

[a] State Key Joint Laboratory of ESPC, Center for Sensor Technology of Environment and Health, School of environment, Tsinghua University, Beijing 100084, China;

Corresponding author: xhzhou@mail.tsinghua.edu.cn.

Abstract: We report herein a T-T mismatch-driven biosensor using triple functional DNA-protein conjugates for facile detection of mercury ions (Hg^{2+}) based on evanescent wave fluorescence excitation. Fluorescein-labeled DNA strands and streptavidin molecules were conjugated using heterobifunctional crosslinkers，and the obtained conjugates were named as "Hg^{2+} dependent conjugates, HDCs". Initially hybridized with quencher-labeled DNA (Q-DNA) strands, HDCs showed low evanescent wave-induced fluorescence emission signals; However, in the presence of Hg^{2+}, the DNA moieties of HDCs tended to form hairpin structures stabilized by T-T mismatches, releasing Q-DNA strands, which was accompanied by increases in the fluorescent signals. The novel detection strategy enables the fluorescent detection of mercury ions with high specificity and a low detection limit of 1.06 nM in a facile way.

Keywords: DNA-protein conjugates; Mercury; T-T mismatch; Evanescent wave; Optical fiber biosensor

1 INTRODUCTION

Mercury is a highly toxic metal that can cause adverse damage in brain, kidney, immune system and many other body parts from long exposure (Liu and Lu 2007). Sources of mercury emissions include both natural and anthropogenic activities. The annual global release of mercury into the environment was estimated to be 5,000-8,000 metric tons (UNEP 2013). Based on the fact that the majority of these human releases of mercury have associated with coal-burning based industrial sites, and the most common state of toxic mercury is Hg^{2+} ion in aqueous solution, it is especially useful to develop highly sensitive and selective sensors for Hg^{2+} ions. Recently, DNA-based Hg^{2+} biosensing techniques have become a hot topic in environmental and analytical science. Compared with antibody, the traditional immune recognition element, DNA is easier to produce, more stable and cost-effective.

In 2004, Ono and Togashi first reported that mercury ions can bind in between two T-bases, promoting T-T mismatch (Ono and Togashi 2004) (Sebera et al. 2013; Torigoe et al. 2010, Uchiyama et al. 2012). They applied this discovery to design a fluorescent Hg^{2+} sensor. In their design, a dual-labeled T-rich DNA strand would fold into a hairpin structure in the presence of Hg^{2+} ions, leading to a decrease in fluorescence intensity. Being smartly designed, their sensor reached a detection limit of 40 nM, but was still some distance away from the toxic level regulated by EPA, i.e. 10 nM Hg^{2+} ions in drinking water. To further improve the sensitivity of this

mismatch-driven mercury sensing strategy, intensive studies applying auxiliary materials were developed, using photo-induced electron transfer (Zhang et al. 2015), graphene oxide (Qiu et al. 2015), molecular beacons (Hui Boon et al. 2014; Xu et al. 2011), Sybr Green I (Wang and Liu 2008), and long lifetime fluorescence quantum dots with gold nanoparticles (Huang et al. 2013). Though more sensitive and selective, most of these reported sensors are limited to homogeneous detections, which, to a certain extent, are still controversial to be called as true biosensors due to lack of solid phases (Wang and Wolfbeis 2013). With less conceptual dispute, surface-based biosensors have been highlighted in practical uses because they are able to work as independent portable devices for pollutants detection when compared with traditional large-scale instrument like fluorescence spectrometer and HPLC, etc. (Ligler 2009). Besides, integrated surface-based biosensors usually contain regenerable sensing regions, which are beneficial to achieve cheap, automatic, on-line and facile detection. Within such biosensors, functional DNAs have also become powerful tools in detecting small molecules and metal ions in water (Lan and Lu 2012; Liu et al. 2015; Roh et al. 2011; Toh et al. 2015; Torabi and Lu 2014).

When designing a surface-based biosensor, it is important to keep a subtle balance between its increasing regenerable ability and the decreasing activities of interfacial materials. To meet this challenge, we reported the use of DNA-protein conjugates as triple functional signal probes in both aptamer- and DNAzyme-based detections using an optical-fiber based evanescent wave biosensor (Wang et al. 2015a; Wang et al.

2015b). As the name suggests, the synthesized DNA-protein conjugates undertake three roles, acting simultaneously as signal conversion, recognition and report elements, and exhibit advantages of both protein and DNA moiety. Specifically, the conjugates consisted of a streptavidin (SA) moiety and functional DNA strands, and were hybridized to DNA modified magnetic beads (MBs). Upon the addition of targets, conjugates were released from MBs due to structure-switching processes or the existence of other competitive DNAs, and were further captured by a desthiobiotin-modified optical-fiber. By replacing direct DNA-target separation with the gentler SA-desthiobiotin separation, the sensing fiber could be reused for more than 250 times. Although with reasonable designs, MBs can be powerful tools in a large number of biosensing applications (Palecek and Fojta 2007; Tekin and Gijs 2013), in our previous work, the whole work depended on chemical modifications of MBs with amino-DNAs, which may lead to sensing deviations due to batch-to-batch variations, and may suffer from nonspecific adsorption at different levels if not well handled (Fuentes et al. 2006; Lermo et al. 2007; Lund et al. 1988). Also, the earlier reported detection relied on two structure-switching processes, which were complicated and time-consuming when compared with those one-step detection strategies. More importantly, the introduction of MBs into the biosensing strategy would hinder the on-line use of the evanescent wave biosensor system, thus largely limiting its application to long-term and unattended fields.

These drawbacks motivate us to find an alternative method with comparable sensing performances but without the introduction of MBs. In the original systems, MBs were used to separate released and unreleased conjugates, i.e., only released conjugates could trigger evanescent wave induced emission (EWIE) signals, the interrupting background signals were naturally suppressed due to magnetic separation. To remove MBs, a solution must be provided to keep background relatively low. Considering that the nature of our EWIE signals belongs to fluorescence, we assume that the use of quenchers will be a suitable choice. Herein, we intend to use fluorescein-labeled T-rich DNA strands and quencher-labeled complementary DNA strands to explore a more concise way for Hg^{2+} ions detection with SA-DNA conjugates (we name them "Hg^{2+}-dependent conjugates", HDCs, in the following context). In addition to having specific binding sites for Hg^{2+}, we choose HDCs as sensing elements for the following reasons: First, conjugating DNA strands to protein molecules like SA is a well-developed technique. Unlike other linking strategies (Au-SH, click chemistry, etc.), no specific nanomaterial or metal catalyst is needed for such conjugations (Niemeyer 2010). Second, one SA molecule may attract more than one DNA strands, leading to amplified signals. Finally, the SA-dethiobiotin recognition kinetics are fast and reversible (Hirsch et al. 2002; Tominaga et al. 2008). To sum up, the major concern of this work is to develop a simplified T-T mismatch-driven surface-based Hg^{2+} sensing system using DNA-protein conjugates as signal probes. The generated evanescent wave fluorescence excitation signals were collected on an optical fiber

104 based platform. Detection performances including detection limits, selectivity,
105 reusability and applications in real water samples were fully evaluated.

106 ## 2 EXPERIMENTAL SECTION

107 ### 2.1 Materials

108 See supplementary information for chemicals, DNA sequences, buffers and the
109 schematic diagram of the optical fiber based EWIE biosensing platform used in this
110 work (Page S2-S4). The preparations of the desthiobiotin-modified optical-fiber and
111 the HDCs are also listed in the supplementary information (Page S4-S6).

112 ### 2.2 Sensing mechanism

113 Fig. 1a depicts the design of this T-T mismatch-driven mercury sensor using HDCs. A
114 HDC consists of a streptavidin moiety and several fluorescein-labeled DNA strands
115 (strands A) that are designed to hybridize with excess strands B (quencher-labeled,
116 Q-DNA), ensuring low background signals in the absence of Hg^{2+}. Strand A contains
117 five self-complementary base pairs (bps) separated by seven T–T mismatches (in blue).
118 In the presence of Hg^{2+}, strand A is bound to form a hairpin structure stabilized by the
119 T–T mismatch, releasing Q-DNA strands, which is accompanied by an increase in the
120 fluorescent intensity. This will be reflected by a signal increase when detected in the
121 EWIE platform (Fig.1b). Normally the more Hg^{2+} ions exist, the higher signal will
122 appear, exhibiting a typical "turn-on" sensing mode.

Since the reported T-rich DNA strands exhibit high mercury-binding affinities and structure-switching activities in the presence of competitive strands (Wang et al. 2008), we hypothesized that when conjugated with SA, they may maintain sensing properties because of structural flexibility. To test this hypothesis, the EWIE platform was utilized to reveal the hybridization-induced quenching and the mismatch-stabilized structure switching (Fig. 2). Before tests, HDCs were synthesized based on earlier reported strategies (Wang et al. 2015b; Xiang and Lu 2012), and a desthiobiotin-modified tapered optical-fiber was set inside the flow cell of the EWIE sensing platform. 2 μL of HDC standard solution and 2 μL of 100 μM Q-DNA were added to 450 μL of MOPS buffer (10 mM MOPS, 0.75 M $NaNO_3$, pH 7.5), then the obtained mixture were rotated for hybridization at room temperature. 1 h later, small volume of concentrated Hg^{2+} solution was added to the mixture, making its final mercury concentration at 500 nM, then the obtained mixture was rotated for another 1 h at room temperature. The EWIE signal traces recorded automatically before and after the Q-DNA/Hg^{2+} addition are shown in Fig 2a. As expected, the fluorescent intensity significantly decreased after the addition of Q-DNA. Quantitatively, about 9.5X increase of the integrated peak area was observed after Hg^{2+} addition (Fig. 2b). In contrast, no such increase was observed without adding mercury ions. Such results suggested that the used DNA strands exhibited mismatch activity had ability to bind Hg^{2+}. This allowed us to further utilize HDC to construct a facile mercury biosensor.

3 RESULTS AND DISCUSSION

3.1 Detection

After a series of optimization procedures (supplementary information, Page S7), 2 μL of HDC standard solution and 2 μL of 100 μM Q-DNA were added to 450 μL MOPS buffer and the obtained mixture were kept at room temperature for 1 h for hybridization. After that, a small volume of concentrated Hg^{2+} solution was added. After vortexing, the mixture was rotated at room temperature for 0.5 h. Then the sensor solution prepared above was transferred to the fiber-based evanescent wave platform to continue the EWIE measurement. Fig. S1b shows a typical EWIE signal recorded by the inset computer software. After the sensor solutions were treated with Hg^{2+} of various concentrations, net peak areas of the EWIE signals were calculated. As shown in Fig.3, higher concentrations of Hg^{2+} resulted in larger peak areas. To quantify targets, net peak area values were related to targets' concentrations, resulting in a logistic-fitted calibration curve. Based on the $3\alpha_{blank}$/slope rule, the limit of detection (LOD) toward Hg^{2+} is 1.06 nM, which is lower than US EPA defined mercury toxicity level in drinking water (10 nM). A linear relationship (y=2160.94x-3866.07, R=0.996) between the net peak areas and mercury concentrations is observed in the range of 75-1000 nM (Fig. 3a, inset). Theoretically, the detection can also be conducted using a regular fluorescence spectrometer instead of the EWIE platform, of which the detection limit is 20 nM, nearly 20-fold higher than the LOD obtained using the EWIE platform (Fig. S6). The comparison between

two obtained LODs proves the superiority of the current established EWIE biosensor, i.e. the exponential decay of evanescent field strength confines excited signals to a narrow distance from the fiber's surface (usually one half of the excitation wavelength), which shields possible background interferences from other components in bulk phase and increases the sensitivity (Golden et al. 1992; Taitt et al. 2005; Zhou et al. 2014).

Besides, the selectivity and sensitivity of Hg^{2+} detection between the biosensor with and without MBs have been compared, please see section 8 of the supplementary information (Page S9-S11). The LOD obtained using MBs based method is 2.1 nM, about 2-fold higher than that of this MBs-free EWIE based strategy (1.06 nM). The latter also exhibited a slightly broader linear range. Taking the preparation work into consideration, the modification of MBs was time-consuming, requiring a five-hour room-temperature coupling and an overnight incubation at low temperature.

3.2 Selectivity

To determine the selectivity of the sensor, 500 nM of mercury ions and its competitive metal ions were individually added to the test sample. After the identical standard detection progress (as described in section 3.1), the EWIE signals were monitored and analyzed. As shown in Fig. 3b, among the tested ions, only Hg^{2+} resulted in significant signal increase, suggesting excellent selectivity of the proposed mercury biosensor. This selectivity is comparable with other fluorescence-based method using Ag/SiO_2

184 nanoparticles (Pang et al. 2015) and small organic molecules (Huo et al. 2010; Zhang
185 et al. 2014).

186 3.3 Determination of Hg^{2+} in real water samples

187 To verify the applicability of this method, we further tested this T-T mismatch-driven
188 mercury biosensor with bottled water from the local supermarket (C'estbon purified
189 drinking water, 350 mL), tap water from our laboratory and pond water collected in
190 Tsinghua University campus. First, mercury-spiked water samples were preliminary
191 purified with 0.22 μm syringe-driven filters, then they were mixed with 10X MOPS
192 buffer (v/v =9/1). For each test, 2 μL of HDC standard solution and 2 μL of 100 μM
193 Q-DNA were added to 450 μL prepared water sample, the obtained mixtures were
194 rotated for 0.5 h at room temperature. Then the mixtures were injected successively
195 into the flow cell of the optical fiber-based evanescent wave biosensor for EWIE
196 detection. As shown in Table 1, Hg^{2+} concentrations calculated from the working
197 curve are close to the original spiked values in three water samples with satisfactory
198 recovery rates ranged from 85% to 95%, indicating that the established biosensor is
199 able to detect mercury in real water samples with a simple pre-treatment.

200 3.4 Regeneration

201 Compared with disposable sensing probes, reusable biosensors need fewer preparation
202 steps and shorter operating time. Large quantities of samples could be continuously
203 detected, which paves the way toward automatic on-line monitoring of pollutants.

In the light of easily reversible binding between desthiobiotin and streptavidin, the sensing surface of the desthiobiotin-modified fiber could be regenerated using acid washing buffer. We took one standard EWIE test about every 50 times to test the regeneration ability of this sensing surface, and four representative signal traces were shown in Fig. 3(c). During the whole test procedure, the fiber was reused for at least 200 times, and the signal recoveries ranged from 94.3 % to 99.0 %. This result suggests that this proposed biosensor is applicable for Hg^{2+} detection with enough accuracy and reusability.

4 CONCLUSIONS

In conclusion, we have developed a facile method for sensitive detection of Hg^{2+} using triple functional DNA-SA conjugates as sensing elements. Under optimized conditions, the addition of Hg^{2+} promotes fluorescein-labeled DNA strands folding into hairpin structures, thus turning on the fluorescent signal by separating the fluorescein and its quencher. This gives rise to the amount of fluorescent molecules, reflected by an increase in EWIE signals. Through this approach, Hg^{2+} can be detected at the LOD of 1.06 nM with high selectivity and good applicability in real water samples. No other accessory materials were introduced to the system, this T-T mismatch-driven Hg^{2+} biosensor is facile and effective with high regeneration cycles.

ACKNOWLEDGEMENT

We thank associate professor Yu Xiang from the Department of Chemistry in

Tsinghua University and PHD candidate Lan-hua Liu from the School of Environment for insightful discussions. This research is supported by the Major Scientific Equipment Development Project of China (2012YQ030111).

REFERENCES

Fuentes, M., Mateo, C., Rodriguez, A., Casqueiro, M., Tercero, J.C., Riese, H.H., Fernández-Lafuente, R., Guisán, J.M., 2006. Detecting minimal traces of DNA using DNA covalently attached to superparamagnetic nanoparticles and direct PCR-ELISA. Biosensors and Bioelectronics 21(8), 1574-1580.

Golden, J.P., Thompson, R.B., Ligler, F.S., Shriver-Lake, L.C., Anderson, G.P., 1992. Fluorometer and tapered fiber optic probes for sensing in the evanscent wave. Optical Engineering 31(7), 1458-1462.

Hirsch, J.D., Eslamizar, L., Filanoski, B.J., Malekzadeh, N., Haugland, R.P., Beechem, J.M., Haugland, R.P., 2002. Easily reversible desthiobiotin binding to streptavidin, avidin, and other biotin-binding proteins: uses for protein labeling, detection, and isolation. Anal. Biochem. 308(2), 343-357.

Huang, D., Niu, C., Ruan, M., Wang, X., Zeng, G., Deng, C., 2013. Highly sensitive strategy for Hg^{2+} detection in environmental water samples using long lifetime fluorescence quantum dots and gold nanoparticles. Environmental science & technology 47(9), 4392-4398.

Hui Boon, T., Huanan, W., Xinbing, Z., Li, S.F.Y., 2014. Detection of Hg^{2+} using molecular beacon-based fluorescent sensor with high sensitivity and tunable dynamic range. Sensors and Actuators B: Chemical 195, 623-629.

Huo, F.J., Sun, Y.Q., Su, J., Yang, Y.T., Yin, C.X., Chao, J.B., 2010. Chromene "lock", thiol "key", and mercury(ii) ion "hand": A single molecular machine recognition system. Organic Letters 12(21), 4756-4759.

Lan, T., Lu, Y., 2012. Metal ion-dependent DNAzymes and their applications as biosensors. In: Sigel, A., Sigel, H., Sigel, R.K.O. (Eds.), Interplay between Metal Ions and Nucleic Acids, pp. 217-248. Springer, Po Box 17, 3300 Aa Dordrecht, Netherlands.

Lermo, A., Campoy, S., Barbé, J., Hernández, S., Alegret, S., Pividori, M.I., 2007. In situ DNA amplification with magnetic primers for the electrochemical detection of food pathogens. Biosensors and Bioelectronics 22(9–10), 2010-2017.

Ligler, F.S., 2009. Perspective on optical biosensors and integrated sensor systems. Analytical

chemistry 81(2), 519-526.

Liu, J., Lu, Y., 2007. Rational design of "turn-on" allosteric DNAzyme catalytic beacons for aqueous mercury ions with ultrahigh sensitivity and selectivity. Angewandte chemie international edition 46(40), 7587-7590.

Liu, L.H., Zhou, X.H., Shi, H.C., 2015. Portable optical aptasensor for rapid detection of mycotoxin with a reversible ligand-grafted biosensing surface. Biosensors and Bioelectronics 72, 300-305.

Lund, V., Schmid, R., Rickwood, D., Robbiati, F., Homes, E., 1988. Assessment of methods for covalent binding of nucleic acids to magnetic beads, DynabeadsTM, and the characteristics of the bound nucleic acids in hybridization reactions. Nucleic Acids Res. 16(22), 10861-10880.

Niemeyer, C.M., 2010. Semisynthetic DNA–protein conjugates for biosensing and nanofabrication. Angewandte Chemie International Edition 49(7), 1200-1216.

Ono, A., Togashi, H., 2004. Highly selective oligonucleotide-based sensor for mercury(II) in aqueous solutions. Angewandte Chemie International Edition 43(33), 4300-4302.

Palecek, E., Fojta, M., 2007. Magnetic beads as versatile tools for electrochemical DNA and protein biosensing. Talanta 74(3), 276-290.

Pang, Y., Rong, Z., Xiao, R., Wang, S., 2015. "Turn on" and label-free core-shell Ag@SiO_2 nanoparticles-based metal-enhanced fluorescent (MEF) aptasensor for Hg^{2+}. Scientific Reports 5, 9451.

Qiu, H., Wu, N., Zheng, Y., Chen, M., Weng, S., Chen, Y., Lin, X., 2015. A robust and versatile signal-on fluorescence sensing strategy based on SYBR Green I dye and graphene oxide. International journal of nanomedicine 10, 147-156.

Roh, Y.H., Ruiz, R.C., Peng, S., Lee, J.B., Luo, D., 2011. Engineering DNA-based functional materials. Chemical Society reviews 40(12), 5730-5744.

Sebera, J., Burda, J., Straka, M., Ono, A., Kojima, C., Tanaka, Y., Sychrovsky, V., 2013. Formation of a thymine-Hg^{II}-thymine metal-mediated DNA base pair: Proposal and theoretical calculation of the reaction pathway. Chem.-Eur. J. 19(30), 9884-9894.

Taitt, C.R., Anderson, G.P., Ligler, F.S., 2005. Evanescent wave fluorescence biosensors. Biosensors & bioelectronics 20(12), 2470-2487.

Tekin, H.C., Gijs, M.A.M., 2013. Ultrasensitive protein detection: a case for microfluidic magnetic bead-based assays. Lab Chip 13(24), 4711-4739.

Toh, S.Y., Citartan, M., Gopinath, S.C.B., Tang, T.-H., 2015. Aptamers as a replacement for antibodies in enzyme-linked immunosorbent assay. Biosensors and Bioelectronics 64(0), 392-403.

Tominaga, R., Sivakumar, M., Tanaka, M., Kinoshita, T., 2008. Visible detection of biotin by thin-film interference: thickness control through exchange reaction of biotin/dethiobiotin–avidin bonding. Journal of Materials Chemistry 18(9), 976.

Torabi, S.F., Lu, Y., 2014. Functional DNA nanomaterials for sensing and imaging in living cells. Current Opinion in Biotechnology 28(0), 88-95.

Torigoe, H., Ono, A., Kozasa, T., 2010. Hg^{II} Ion specifically binds with T:T mismatched base pair in duplex DNA. Chemistry – A European Journal 16(44), 13218-13225.

Uchiyama, T., Miura, T., Takeuchi, H., Dairaku, T., Komuro, T., Kawamura, T., Kondo, Y., Benda, L., Sychrovsky, V., Bour, P., Okamoto, I., Ono, A., Tanaka, Y., 2012. Raman spectroscopic detection of the T-Hg^{II}-T base pair and the ionic characteristics of mercury. Nucleic Acids Res 40(12), 5766-5774.

UNEP, 2013. Global mercury assessment 2013: Sources, emissions, releases, and environmental transport.

Wang, J., Liu, B., 2008. Highly sensitive and selective detection of Hg^{2+} in aqueous solution with mercury-specific DNA and sybr green I. Chemical communications(39), 4759-4761.

Wang, R.Y., Xiang, Y., Zhou, X.H., Liu, L.H., Shi, H.C., 2015a. A reusable aptamer-based evanescent wave all-fiber biosensor for highly sensitive detection of Ochratoxin A. Biosensors & bioelectronics 66, 11-18.

Wang, R.Y., Zhou, X.H., Shi, H.C., 2015b. Triple functional DNA–protein conjugates: Signal probes for Pb^{2+} using evanescent wave-induced emission. Biosensors and Bioelectronics 74, 78-84.

Wang, X.D., Wolfbeis, O.S., 2013. Fiber-optic chemical sensors and biosensors (2008-2012). Analytical chemistry 85(2), 487-508.

Wang, Z., Heon Lee, J., Lu, Y., 2008. Highly sensitive "turn-on" fluorescent sensor for Hg^{2+} in aqueous solution based on structure-switching DNA. Chemical communications(45), 6005-6007.

Xiang, Y., Lu, Y., 2012. Using commercially available personal glucose meters for portable quantification of DNA. Analytical chemistry 84(4), 1975-1980.

Xu, H.F., Zhu, X., Ye, H.Z., Yu, L.S., Liu, X.X., Chen, G.N., 2011. A simple "molecular beacon"-based fluorescent sensing strategy for sensitive and selective detection of mercury (II). Chemical communications 47(44), 12158-12160.

Zhang, J.R., Huang, W.T., Zeng, A.L., Luo, H.Q., Li, N.B., 2015. Ethynyl and π-stacked thymine–Hg^{2+}–thymine base pairs enhanced fluorescence quenching via photoinduced electron transfer and simple and sensitive mercury ion sensing. Biosensors and Bioelectronics 64(0), 597-604.

Zhang, Y.B., Yang, Y.T., Hao, J.S., Yin, C.X., Huo, F.J., Chao, J.B., Liu, D.S., 2014. Spectroscopic study of the recognition of 2-quinolinone derivative on mercury ion. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 132, 27-31.

Zhou, X.H., Liu, L.H., Xu, W.Q., Song, B.D., Sheng, J.W., He, M., Shi, H.C., 2014. A reusable evanescent wave immunosensor for highly sensitive detection of bisphenol A in water samples. Scientific Reports 4, 4572.

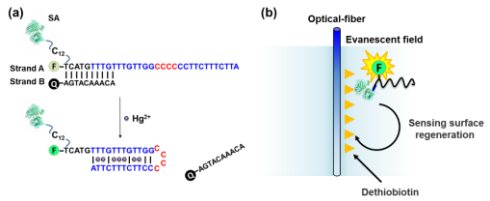


Fig. 1 (a) Scheme of the T-T mismatch-driven biosensor using triple functional DNA-protein conjugates for Hg^{2+}detection. (b) Schematic diagram of the sensing surface. Briefly, the surface of the optical fiber (in blue) is functionalized with receptor molecules (yellow triangles), to which the fluorescein-labeled DNA-SA conjugates could be captured via affinity reactions, thus exposing the fluorophores in the evanescent field (in light blue). See support information for details about the optical fiber based evanescent wave induced emission (EWIE) biosensing platform.

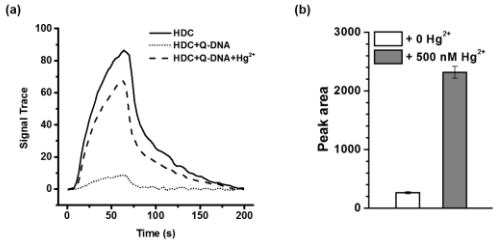


Fig. 2 (a) Original EWIE signal traces. (b) EWIE signal peak areas of the sensor in the absence of and after the addition of 500 nM Hg^{2+} for 60 min.

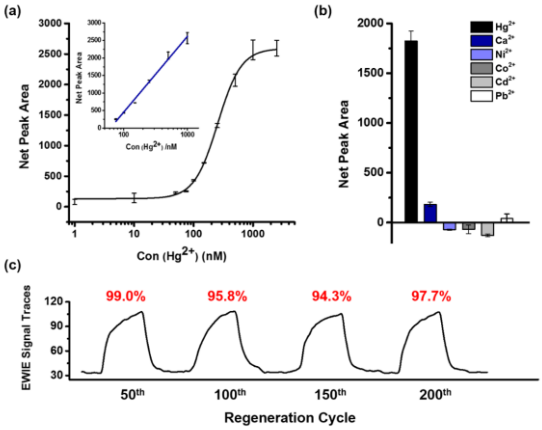
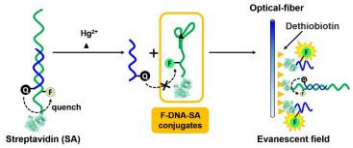


Fig. 3 (a) Variations of net peak areas with different Hg^{2+} concentration and logarithmic fitted calibration plots (Net peak area equals to integrated peak area minus relative background area). Inset: the linear range (75 nM-1000 nM). (b) Selective detection of Hg^{2+} against other five competitive metal ions. Each data is the average of at least three independent experimental results. (c) Regeneration signal traces of the sensing surface. Recoveries was labeled in red.



For TOC only

Table 1 Determination of Hg^{2+} in three different water samples (n=3)

Sample	Hg^{2+} (nM)		Recovery (%) [c]
	Spiked	Detected Mean[a] ± SD[b]	
Bottled water	0	＜1	-
	100	94.53 ± 2.16	95
	150	142.62 ± 4.85	95
Tap water	0	＜1	-
	100	89.92 ± 2.80	90
	150	127.03 ± 2.11	85
Pond water	0	＜1	-
	150	140.14 ± 1.44	93
	200	176.66 ± 1.18	88

[a] Mean value of three individual determinations

[b] Standard deviation

[c] Note that all the calculated recoveries were below 100%, the probable reason might be the existence of chloride ions (Cl^-) in tap water and pond water from chlorination. Since K_{sp} (Hg_2Cl_2) = 1.10×10^{-18}, the calculated results might be lower than spiked values.

Highlights

1. An evanescent wave-induced emission sensing system was applied to Hg^{2+} detection using synthesized DNA-protein conjugates as triple functional probes.
2. One facile structure-switching process driven by T-T mismatch separated quencher-labeled DNA from the fluorescent conjugates.
3. Advantages featured by T–Hg^{2+}–T mismatch enable the system attractive.
4. A low detection limit of 1.06 nM for Hg^{2+} and applications in detecting real water samples with high recoveries were reported.
5. Fiber probe surface can be reused for over 200 times.