

A label-free G-quadruplex-based mercury detection assay employing the exonuclease III-mediated cleavage of T–Hg²⁺–T mismatched DNA

Wanhe Wang¹, Tian-Shu Kang², Philip Wai Hong Chan^{3,4}, Jin-Jian Lu², Xiu-Ping Chen², Chung-Hang Leung² and Dik-Lung Ma^{1,5}

¹ Department of Chemistry, Hong Kong Baptist University, Kowloon Tong, Hong Kong, China

² State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Macao, China

³ School of Chemistry, Monash University, Clayton, Victoria 3800, Australia

⁴ Department of Chemistry, University of Warwick, Coventry CV4 7AL, UK

⁵ Partner State Key Laboratory of Environmental and Biological Analysis, Hong Kong Baptist University, Hong Kong, China

E-mail: edmondma@hkbu.edu.hk and Duncanleung@umac.mo

Received 9 September 2015, revised 3 November 2015


Accepted for publication 4 November 2015

Published 17 November 2015



Abstract

We report herein the use of an exonuclease III and G-quadruplex probe to construct a G-quadruplex-based luminescence detection platform for Hg²⁺. Unlike common DNA-based Hg²⁺ detection methods, when using the dsDNA probe to monitor the hairpin formation, the intercalation of the dsDNA probe may be influenced by the distortion of dsDNA. This ‘mix-and-detect’ methodology utilized the G-quadruplex probe as the signal transducer and is simple, rapid, convenient to use and can detect down to 20 nM of Hg²⁺.

 Online supplementary data available from stacks.iop.org/STAM/16/065004/mmedia

Keywords: iridium(III), exonuclease III, G-quadruplex, mercury(II) ion

1. Introduction

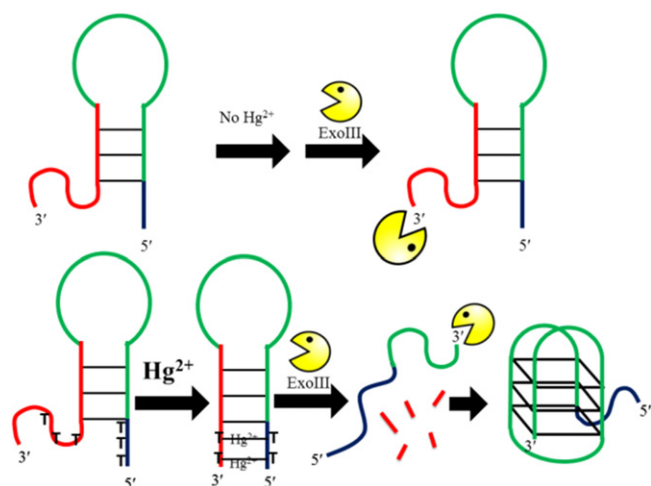
Mercury ions (Hg²⁺) are a hazardous pollutant produced by refineries, factories, power station or runoff from landfills. Long-term absorption of mercury may result in kidney damage, memory impairment and other severe health problems [1, 2]. Living organisms will absorb and then accumulate mercury in fatty tissue via eating and drinking. Therefore, exposure of the human body to mercury should be minimized due to potential metal accumulation. According to the United States Environmental Protection Agency, the

maximum safe concentration of mercury in drinking water is 2 ppb, the quantitative detection of which would require a very sensitive instrument [3–5]. Unfortunately, existing analytical methods for the sensitive detection of Hg²⁺ are not satisfactory for in-field use due to expensive instrumentation and labor-intensive sample preparation protocols. These instrumental methods include atomic absorption spectroscopy (AAS), inductively-coupled plasma mass spectrometry (ICP-MS) [6] and ion-selective electrodes [7, 8].

In 2004, Ono and co-workers discovered that Hg²⁺ is able to coordinate with thymine nucleobases [9–12]. This discovery has sparked a wave of novel Hg²⁺ detection methods, such as oligonucleotide-based luminescence [13–28], colorimetric [29–40], electrochemical [41, 42] and surface-enhanced Raman scattering methods [43–46]. Later on, they reported the crystal structure of a DNA duplex



Content from this work may be used under the terms of the Creative Commons Attribution 3.0 licence. Any further distribution of this work must maintain attribution to the author(s) and the title of the work, journal citation and DOI.



Scheme 1. Schematic representation of the ExoIII-assisted label-free G-quadruplex-based assay for Hg^{2+} .

containing the T– Hg^{II} –T mismatch, which showed that the T– Hg^{II} –T bond largely distorts DNA structure and also stabilizes B-form DNA [12]. Moreover, C– Ag^{I} –A [47] and C– Ag^{I} –T mismatches have also been reported [11].

The G-quadruplex is a DNA secondary structure which is formed from a guanine-rich DNA sequence with four or more G-tracts. It is stabilized by monovalent cations such as K^+ , Na^+ or NH_4^+ to form square-planar arrangements of guanine residues [48, 49]. Because of its rich structural polymorphism, the G-quadruplex has been widely used to construct analytical detection platforms [43, 44, 50–64] or various types of logic gates [65–67].

Exonuclease III (ExoIII) is a nuclease that catalyzes the stepwise removal of mononucleotides from the 3'-terminus of duplex DNA. Exo III has been used as a component of oligonucleotide-based detection platforms for different targets or as a tool for signal amplification [68, 69]. Interestingly, ExoIII has been reported to cleave DNA duplexes containing T– Hg^{2+} –T mismatches and release Hg^{2+} into solution [42].

In our previous study, we constructed AND, OR and INHIBIT logic gates utilizing Hg^{II} and Ag^{I} ions as signal inputs. The 'Klenow fragment' polymerase was utilized as one of the signal transducing elements that catalyzes the extension of designed DNA in the 5' to 3' direction to form a duplex product and displace the split G-quadruplex sequence in the presence of Ag^+ and Hg^{2+} [70]. Moreover, previous investigations demonstrated that the photophysical properties of iridium(III) complexes could be fine-tuned by changing the C \wedge N or N \wedge N donor ligand. We report herein a novel detection platform for Hg^{2+} using a novel iridium(III) complex. Unlike common detection methods using dsDNA probes to monitor the hairpin formation, which could be influenced by the distortion of dsDNA by mismatches, our approach utilized a G-quadruplex probe and is based on the newly discovered phenomenon that ExoIII cleaves DNA duplexes containing T– Hg^{2+} –T mismatches. The detection mechanism of this platform is outlined in scheme 1. The DNA probe contains a G-quadruplex-forming sequence (green line), and its complementary sequence (red line), as well as 3' and 5' polyT

(poly thymine) overhangs. The overall DNA structure assumes a hairpin formation with unhybridized 3' and 5'-termini. Since ExoIII is unable to recognize single-stranded DNA (ssDNA) as substrate, it will not cleave the unhybridized 3' overhang and the hairpin DNA will not be digested in the absence of Hg^{2+} . Upon exposure to Hg^{2+} however, the 3' and 5' polyT overhangs form T– Hg^{2+} –T bridges, creating a duplex DNA structure that is vulnerable to ExoIII digestion from the 3'-terminus (red line). ExoIII cleavage releases the G-quadruplex-forming sequence. The G-quadruplex structure binds with the luminescent G-quadruplex-selective iridium(III) complex **1**, with a switch-on emission response. Furthermore, the Hg^{2+} may be recycled as they are re-released into solution following the ExoIII cleavage step.

2. Experimental details

2.1. Materials

Reagents, unless specified, were purchased from Sigma Aldrich (St. Louis, MO, USA) and used as received. Iridium chloride hydrate ($\text{IrCl}_3 \cdot x\text{H}_2\text{O}$) was purchased from Precious Metals Online (Australia). All oligonucleotides were synthesized by Techdragon Inc. (Hong Kong, China).

2.2. Synthesis

Complex **1** was prepared according to (modified) literature methods and was characterized by proton nuclear magnetic resonance ($^1\text{H-NMR}$), $^{13}\text{C-NMR}$ and high resolution mass spectrometry (HRMS). Complex **1**. ^1H NMR (400 MHz, acetone- d_6) δ 8.80 (*d*, $J = 8.8$ Hz, 2H), 8.53 (*d*, $J = 8.8$ Hz, 2H), 8.14–8.09 (*m*, 4H), 7.89 (*d*, $J = 8.8$ Hz, 2H), 7.83–7.81 (*m*, 2H), 7.67–7.63 (*m*, 2H), 7.42–7.38 (*m*, 2H), 7.31–7.27 (*m*, 2H), 7.21–7.17 (*m*, 2H), 7.15–7.06 (*m*, 6H), 6.58 (*d*, $J = 7.6$ Hz, 2H); ^{13}C NMR (100 MHz, DMSO- d_6) δ 181.0, 161.3, 148.9, 148.8, 147.8, 142.1, 140.0, 132.5, 132.1, 131.7, 131.3, 129.9, 129.7, 129.6, 128.5, 127.5, 127.3, 126.9, 125.0, 123.9, 122.1, 118.1; HRMS: calculated for $\text{C}_{44}\text{H}_{28}\text{IrN}_4\text{S}_2$ [M-PF_6] $^+$ 869.1385; found 869.1332; analyzed ($\text{C}_{44}\text{H}_{28}\text{IrN}_4\text{S}_2\text{PF}_6$). C, H, N: calculated 52.12, 2.78, 5.53; found 52.34, 2.77, 5.58.

3. Results and discussion

Complex **1** is a cyclometalated iridium(III) complex: $[\text{Ir}(\text{pbt})_2(\text{biq})]\text{PF}_6$ (**1**, where *pbt* = 2-phenylbenzo[*d*]thiazole, *biq* = 2,2'-biquinoline, figure 1). The synthesis, characterization and photophysical properties of **1** are given in the supplementary information (table S1, figure S1). No significant change in the UV–visible absorption spectrum of **1** was observed in aqueous buffered solution over 48 h, indicating that the complex is stable in aqueous solution and may be suitable for long term use. By itself, complex **1** was weakly emissive in aqueous buffered solution (20 mM Tris, pH 7.0).

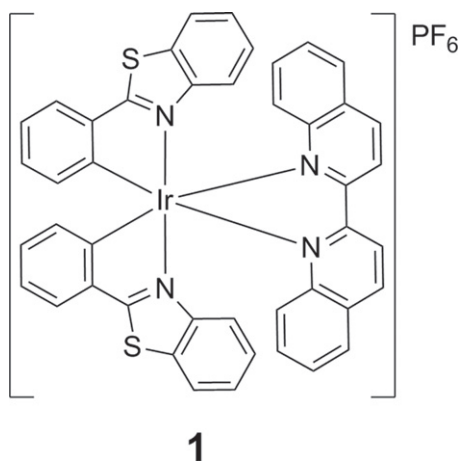


Figure 1. Chemical structure of cyclometalated iridium(III) complex **1**.

Encouragingly, we found that a significant increase in luminescence response of **1** can be triggered by the presence of G-quadruplex DNA, as shown by emission titration experiments. We then explored the effect of the addition of various types of DNA (figure 2). ssDNA or double-stranded DNA (ds17, ds26) had only a minor effect on the luminescence of **1**. However, in the presence of the Pu27 G-quadruplex, the luminescence of **1** was significantly increased (about 3-fold). We anticipate that the G-quadruplex shields the metal center of **1** from solvent quenching by non-radiative decay of the excited state, thus recovering ³MLCT phosphorescence. To validate this hypothesis, the G-quadruplex fluorescent intercalator displacement (G4-FID) assay was utilized to investigate the binding affinity of **1** for G-quadruplex and dsDNA. The result showed that only 2 μM of **1** could displace 50% of thiazole orange (TO) from G-quadruplex-TO assemble. On the other hand, only about 30% of TO could be displaced from dsDNA-TO assemble in the presence of 5 μM of complex **1** (figure 3). It outlines the G-quadruplex selective property of complex **1**.

As complex **1** displays the property of a G-quadruplex-selective probe, we sought to apply it in our Hg²⁺ sensing platform. Encouragingly, we observed that the presence of Hg²⁺ enhanced the luminescence intensity of the complex **1**-hairpin DNA system (figure 4). To validate that the luminescence enhancement is due to the switching of the DNA structure, we also sought to exclude the possibility of direct

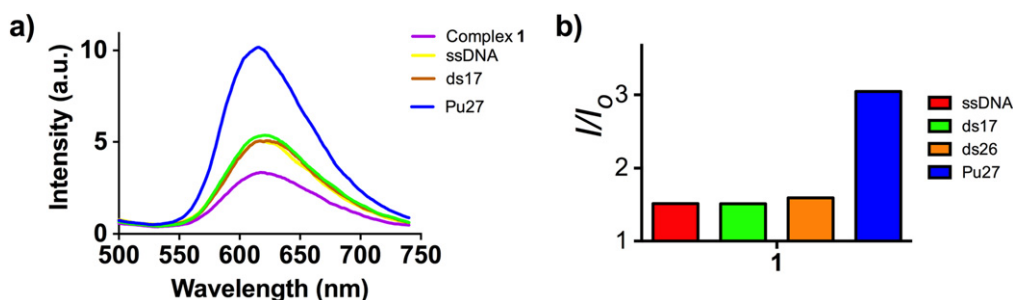


Figure 2. (a) Emission spectrum and (b) luminescence enhancement of complex **1** (1 μM) in the presence of 5 μM of ssDNA, ds17, ds26 or Pu27 G-quadruplex.

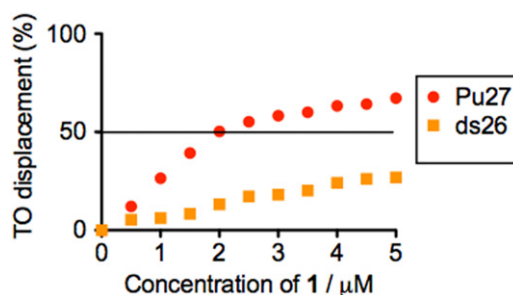


Figure 3. Percentage of TO displaced from DNA duplex ds26 or Pu27 G-quadruplex upon addition of complex **1**.

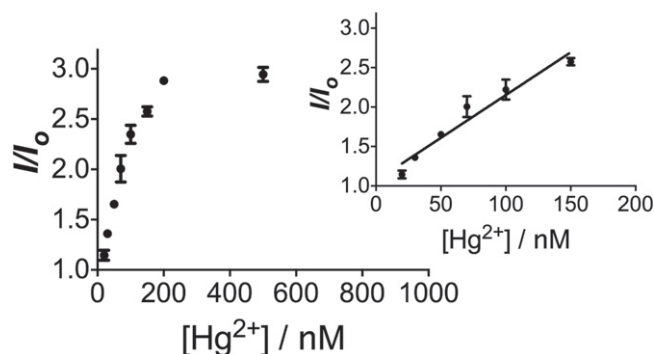


Figure 4. Emission enhancement of the system ([**1**] = 1 μM, [DNA] = 1.5 μM, [K⁺] = 100 mM) with increasing concentration of Hg²⁺.

interaction between complex **1** and Hg²⁺ as a contributor to the response. We found that no luminescence increase was observed for the system lacking hairpin DNA (figure S2). We propose that the observed luminescence enhancement of the system is due to the formation of the T-Hg²⁺-T mismatched duplex in the 3' and 5' overhangs, which allows ExoIII digestion of the hairpin DNA, resulting in the release of the G-quadruplex-forming sequence and the formation of a G-quadruplex structure that is recognized by the G-quadruplex-selective complex **1** with a switch-on emission response.

The linear detection range of the system was found to be from 20 to 200 nM of Hg²⁺ (figure 4). Maximal luminescence was reached at 200 nM. The detection limit of the present assay was determined to be 20 nM of Hg²⁺ by the 3σ method. The selectivity of this detection platform for Hg²⁺ over other metal ions was also evaluated. The results showed that the

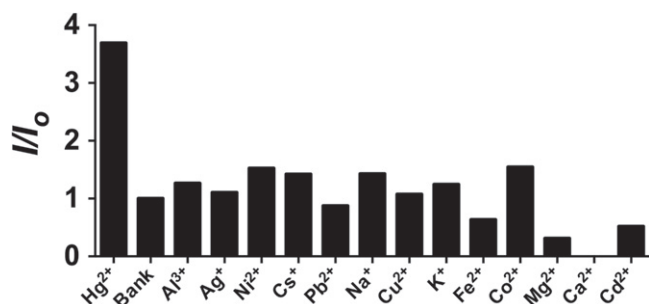


Figure 5. Relative luminescence intensity of the system ($[I] = 1 \mu\text{M}$, $[\text{DNA}] = 2 \mu\text{M}$) in the presence of 10 nM Hg^{2+} or 5-fold excess of other metal ions.

luminescence response of the system for Hg^{2+} was significantly stronger than that for 5-fold excess concentrations of the other metal ions (figure 5).

In order to investigate the effectiveness of this Hg^{2+} assay in a practical application, we applied it to the detection of Hg^{2+} in spiked natural water samples. No Hg^{2+} was detected in the natural water samples by ICP-MS. Therefore, the natural water samples (Nam Sang Wai River in Hong Kong) were diluted 50-fold with Tris buffer and spiked with various concentrations (20, 50, 100, 200 and 500 nM) of Hg^{2+} . The samples showed a gradual increase in luminescence intensity with Hg^{2+} (figure 6). This result demonstrates that our detection platform could potentially be further developed as a sensitive probe for natural water sample analysis of Hg^{2+} .

4. Conclusions

In conclusion, a novel luminescent iridium(III) complex which shows the G-quadruplex probe property, was investigated and utilized for the construction of an exonuclease-assisted, label-free G-quadruplex-based luminescence detection platform for Hg^{2+} . Compared with the modified DNA, the cost label-free approach using unmodified DNA is relatively low. Unlike common detection methods, when using the dsDNA probe to monitor the hairpin formation, the intercalation of the dsDNA probe may be influenced by the distortion of dsDNA. This ‘mix-and-detect’ methodology utilized the G-quadruplex probe as the signal transducer and it is simple, rapid, convenient to use and can detect down to 20 nM of Hg^{2+} , which is comparable to recently reported label-free DNA-based Hg^{2+} ion detection methods. For comparison, we have also summarized those reported methods in table S2. Furthermore, the potential application in a real water sample was also demonstrated, which shows the robustness of the system.

Acknowledgments

This work is supported by Hong Kong Baptist University (FRG2/14-15/004), the Health and Medical Research Fund

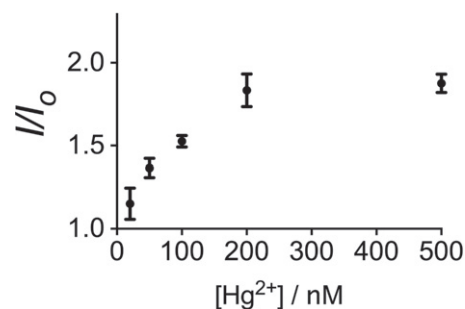


Figure 6. Luminescence response of the system to increasing concentrations (20, 50, 100, 200 and 500 nM) of Hg^{2+} in a 50-fold diluted river water sample.

(HMRF/13121482 and HMRF/14130522), the Research Grants Council (HKBU/201811, HKBU/204612 and HKBU/201913), the French National Research Agency/Research Grants Council Joint Research Scheme (A-HKBU201/12), State Key Laboratory of Environmental and Biological Analysis Research Grant (SKLP-14-15-P001), National Natural Science Foundation of China (21575121), Guangdong Province Natural Science Foundation (2015A030313816), Hong Kong Baptist University Century Club Sponsorship Scheme 2015, Interdisciplinary Research Matching Scheme (RC-IRMS/14-15/06), the State Key Laboratory of Synthetic Chemistry, the Science and Technology Development Fund, Macao SAR (098/2014/A2 and 007/2014/AMJ), the University of Macau (MYRG091 (Y3-L2)-ICMS12-LCH, MYRG2015-00137-ICMS-QRCM and MRG023/LCH/2013/ICMS).

References

- [1] Sekowski J W, Malkas L H, Wei Y and Hickey R J 1997 Mercuric ion inhibits the activity and fidelity of the human cell DNA synthesome *Toxicol. Appl. Pharm.* **145** 268–76
- [2] Tchounwou P B, Ayensu W K, Ninashvili N and Sutton D 2003 Review: environmental exposure to mercury and its toxicopathologic implications for public health *Environ. Toxicol.* **18** 149–75
- [3] Nolan E M and Lippard S J 2008 Tools and tactics for the optical detection of mercuric ion *Chem. Rev.* **108** 3443–80
- [4] Ma D-L, Chan D S-H, Man B Y-W and Leung C-H 2011 Oligonucleotide-based luminescent detection of metal ions *Chem. Asian. J.* **6** 986–1003
- [5] Ma D-L, He H-Z, Leung K-H, Zhong H-J, Chan D S-H and Leung C-H 2013 Label-free luminescent oligonucleotide-based probes *Chem. Soc. Rev.* **42** 3427–40
- [6] Hatch W R and Ott W L 1968 Determination of submicrogram quantities of mercury by atomic absorption spectrophotometry *Anal. Chem.* **40** 2085–7
- [7] Salaün P and van den Berg C M G 2006 Voltammetric detection of mercury and copper in seawater using a gold microwire electrode *Anal. Chem.* **78** 5052–60
- [8] Passariello B, Barbaro M, Quaresima S, Casciello A and Marabini A 1996 Determination of mercury by inductively coupled plasma—mass spectrometry *Microchem. J.* **54** 348–54
- [9] Ono A and Togashi H 2004 Highly selective oligonucleotide-based sensor for mercury(II) in aqueous solutions *Angew. Chem. Int. Edn* **43** 4300–2

- [10] Tanaka Y, Oda S, Yamaguchi H, Kondo Y, Kojima C and Ono A 2006 15N–15N J-coupling across HgII: direct observation of HgII-mediated T–T base pairs in a DNA duplex *J. Am. Chem. Soc.* **129** 244–5
- [11] Kondo J, Yamada T, Hirose C, Okamoto I, Tanaka Y and Ono A 2014 Crystal structure of metallo DNA duplex containing consecutive Watson–Crick-like T–HgII–T base pairs *Angew. Chem. Int. Edn* **53** 2385–8
- [12] Funai T, Nakamura J, Miyazaki Y, Kiriu R, Nakagawa O, Wada S-i, Ono A and Urata H 2014 Regulated incorporation of two different metal ions into programmed sites in a duplex by DNA polymerase catalyzed primer extension *Angew. Chem. Int. Edn* **53** 6624–7
- [13] Li M, Wang Q, Shi X, Hornak L A and Wu N 2011 Detection of mercury(II) by quantum dot/DNA/gold nanoparticle ensemble based nanosensor via nanometal surface energy transfer *Anal. Chem.* **83** 7061–5
- [14] Wang J and Liu B 2008 Highly sensitive and selective detection of Hg²⁺ in aqueous solution with mercury-specific DNA and Sybr green I *Chem. Commun.* 4759–61
- [15] Wang Z, Heon Lee J and Lu Y 2008 Highly sensitive ‘turn-on’ fluorescent sensor for Hg²⁺ in aqueous solution based on structure-switching DNA *Chem. Commun.* 6005–7
- [16] Zhang L, Li T, Li B, Li J and Wang E 2010 Carbon nanotube–DNA hybrid fluorescent sensor for sensitive and selective detection of mercury(II) ion *Chem. Commun.* **46** 1476–8
- [17] Chan D S-H, Lee H-M, Che C-M, Leung C-H and Ma D-L 2009 A selective oligonucleotide-based luminescent switch-on probe for the detection of nanomolar mercury(II) ion in aqueous solution *Chem. Commun.* 7479–81
- [18] Liu J and Lu Y 2007 Rational design of ‘turn-on’ allosteric DNzyme catalytic beacons for aqueous mercury ions with ultrahigh sensitivity and selectivity *Angew. Chem. Int. Edn* **46** 7587–90
- [19] Chiang C-K, Huang C-C, Liu C-W and Chang H-T 2008 Oligonucleotide-based fluorescence probe for sensitive and selective detection of mercury(II) in aqueous solution *Anal. Chem.* **80** 3716–21
- [20] Liu M, Wang Z, Zong S, Chen H, Zhu D, Wu L, Hu G and Cui Y 2014 SERS Detection and removal of mercury(II)/silver(I) using oligonucleotide-functionalized core/shell magnetic silica sphere@Au nanoparticles *ACS Appl. Mater. Interfaces* **6** 7371–9
- [21] Dave N, Chan M Y, Huang P-J J, Smith B D and Liu J 2010 Regenerable DNA-functionalized hydrogels for ultrasensitive, instrument-free mercury(II) detection and removal in water *J. Am. Chem. Soc.* **132** 12668–73
- [22] Deng L, Ouyang X, Jin J, Ma C, Jiang Y, Zheng J, Li J, Li Y, Tan W and Yang R 2013 Exploiting the higher specificity of silver amalgamation: selective detection of mercury(II) by forming Ag/Hg amalgam *Anal. Chem.* **85** 8594–600
- [23] Zhu G, Li Y and Zhang C-Y 2014 Simultaneous detection of mercury(II) and silver(I) ions with picomolar sensitivity *Chem. Commun.* **50** 572–4
- [24] Xu H, Zhu X, Ye H, Yu L, Liu X and Chen G 2011 A simple ‘molecular beacon’-based fluorescent sensing strategy for sensitive and selective detection of mercury(II) *Chem. Commun.* **47** 12158–60
- [25] Wu Y, Zhan S, Xu L, Shi W, Xi T, Zhan X and Zhou P 2011 A simple and label-free sensor for mercury(II) detection in aqueous solution by malachite green based on a resonance scattering spectral assay *Chem. Commun.* **47** 6027–9
- [26] Li H, Zhai J and Sun X 2011 Nano-C60 as a novel, effective fluorescent sensing platform for mercury(II) ion detection at critical sensitivity and selectivity *Nanoscale* **3** 2155–7
- [27] Zhu Z, Xu L, Zhou X, Qin J and Yang C 2011 Designing label-free DNA sequences to achieve controllable turn-off/on fluorescence response for Hg²⁺ detection *Chem. Commun.* **47** 8010–2
- [28] Zhu X, Chen L, Lin Z, Qiu B and Chen G 2010 A highly sensitive and selective ‘signal-on’ electrochemiluminescent biosensor for mercury *Chem. Commun.* **46** 3149–51
- [29] Li T, Dong S and Wang E 2009 Label-free colorimetric detection of aqueous mercury ion (Hg²⁺) using Hg²⁺-modulated G-quadruplex-based DNazymes *Anal. Chem.* **81** 2144–9
- [30] Xu X, Wang J, Jiao K and Yang X 2009 Colorimetric detection of mercury ion (Hg²⁺) based on DNA oligonucleotides and unmodified gold nanoparticles sensing system with a tunable detection range *Biosens. Bioelectron.* **24** 3153–8
- [31] Liu C-W, Hsieh Y-T, Huang C-C, Lin Z-H and Chang H-T 2008 Detection of mercury(II) based on Hg²⁺-DNA complexes inducing the aggregation of gold nanoparticles *Chem. Commun.* 2242–4
- [32] Li T, Li B, Wang E and Dong S 2009 G-quadruplex-based DNzyme for sensitive mercury detection with the naked eye *Chem. Commun.* 3551–3
- [33] Xue X, Wang F and Liu X 2008 One-step, room temperature, colorimetric detection of mercury (Hg²⁺) using DNA/nanoparticle conjugates *J. Am. Chem. Soc.* **130** 3244–5
- [34] Darbha G K, Singh A K, Rai U S, Yu E, Yu H and Chandra Ray P 2008 Selective detection of mercury (II) ion using nonlinear optical properties of gold nanoparticles *J. Am. Chem. Soc.* **130** 8038–43
- [35] Liu C-W, Huang C-C and Chang H-T 2008 Control over surface DNA density on Gold nanoparticles allows selective and sensitive detection of mercury(II) *Langmuir* **24** 8346–50
- [36] Chen G-H, Chen W-Y, Yen Y-C, Wang C-W, Chang H-T and Chen C-F 2014 Detection of mercury(II) ions using colorimetric gold nanoparticles on paper-based analytical devices *Anal. Chem.* **86** 6843–9
- [37] Zuo X, Wu H, Toh J and Li S F Y 2010 Mechanism of mercury detection based on interaction of single-strand DNA and hybridized DNA with gold nanoparticles *Talanta* **82** 1642–6
- [38] Wei Q, Nagi R, Sadeghi K, Feng S, Yan E, Ki S J, Caire R, Tseng D and Ozcan A 2014 Detection and spatial mapping of mercury contamination in water samples using a smartphone *ACS Nano* **8** 1121–9
- [39] You J, Hu H, Zhou J, Zhang L, Zhang Y and Kondo T 2013 Novel cellulose polyampholyte–gold nanoparticle-based colorimetric competition assay for the detection of cysteine and mercury(II) *Langmuir* **29** 5085–92
- [40] Kanayama N, Takarada T and Maeda M 2011 Rapid naked-eye detection of mercury ions based on non-crosslinking aggregation of double-stranded DNA-carrying gold nanoparticles *Chem. Commun.* **47** 2077–9
- [41] Wu D, Zhang Q, Chu X, Wang H, Shen G and Yu R 2010 Ultrasensitive electrochemical sensor for mercury(II) based on target-induced structure-switching DNA *Biosens. Bioelectron.* **25** 1025–31
- [42] Xuan F, Luo X and Hsing I M 2013 Conformation-dependent exonuclease III activity mediated by metal ions reshuffling on thymine-rich DNA duplexes for an ultrasensitive electrochemical method for Hg²⁺ detection *Anal. Chem.* **85** 4586–93
- [43] Du Y, Li B and Wang E 2012 ‘Fitting’ makes ‘sensing’ simple: label-free detection strategies based on nucleic acid aptamers *Acc. Chem. Res.* **46** 203–13
- [44] Wang F, Lu C-H and Willner I 2014 From cascaded catalytic nucleic acids to enzyme–DNA nanostructures: controlling reactivity, sensing, logic operations, and assembly of complex structures *Chem. Rev.* **114** 2881–941
- [45] Lu C-H, Willner B and Willner I 2013 DNA nanotechnology: from sensing and DNA machines to drug-delivery systems *ACS Nano* **7** 8320–32
- [46] Willner I, Shlyahovsky B, Zayats M and Willner B 2008 DNazymes for sensing, nanobiotechnology and logic gate applications *Chem. Soc. Rev.* **37** 1153–65

- [47] Funai T, Miyazaki Y, Aotani M, Yamaguchi E, Nakagawa O, Wada S-i, Torigoe H, Ono A and Urata H 2012 Ag(I) ion mediated formation of a C–A mispair by DNA polymerases *Angew. Chem. Int. Edn* **51** 6464–6
- [48] Davis J T 2004 G-quartets 40 years later: from 5'-GMP to molecular biology and supramolecular chemistry *Angew. Chem. Int. Edn* **43** 668–98
- [49] Zhou J, Bourdoncle A, Rosu F, Gabelica V and Mergny J-L 2012 Tri-G-quadruplex: controlled assembly of a G-quadruplex structure from three G-rich strands *Angew. Chem. Int. Edn* **51** 11002–5
- [50] Hu D, Pu F, Huang Z, Ren J and Qu X 2010 A Quadruplex-based, label-free, and real-time fluorescence assay for RNase H activity and inhibition *Chem. Eur. J.* **16** 2605–10
- [51] Zhao C, Wu L, Ren J and Qu X 2011 A label-free fluorescent turn-on enzymatic amplification assay for DNA detection using ligand-responsive G-quadruplex formation *Chem. Commun.* **47** 5461–3
- [52] Qu K, Zhao C, Ren J and Qu X 2012 Human telomeric G-quadruplex formation and highly selective fluorescence detection of toxic strontium ions *Mol. Biosyst.* **8** 779–82
- [53] He H-Z, Chan D S-H, Leung C-H and Ma D-L 2013 G-quadruplexes for luminescent sensing and logic gates *Nucleic Acids Res.* **41** 4345–59
- [54] Chen Z, Lin Y, Zhao C, Ren J and Qu X 2012 Silver metallization engineered conformational switch of G-quadruplex for fluorescence turn-on detection of biothiols *Chem. Commun.* **48** 11428–30
- [55] Peng Y, Wang X, Xiao Y, Feng L, Zhao C, Ren J and Qu X 2009 i-Motif quadruplex DNA-based biosensor for distinguishing single- and multiwalled carbon nanotubes *J. Am. Chem. Soc.* **131** 13813–8
- [56] Hu D, Huang Z, Pu F, Ren J and Qu X 2011 A label-free, quadruplex-based functional molecular beacon (LFG4-MB) for fluorescence turn-on detection of DNA and nuclease *Chem. Eur. J.* **17** 1635–41
- [57] Xu H, Gao S, Yang Q, Pan D, Wang L and Fan C 2010 Amplified fluorescent recognition of G-quadruplex folding with a cationic conjugated polymer and DNA intercalator *ACS Appl. Mater. Interfaces* **2** 3211–6
- [58] Zhu C, Wen Y, Li D, Wang L, Song S, Fan C and Willner I 2009 Inhibition of the *in vitro* replication of DNA by an aptamer–protein complex in an autonomous DNA machine *Chem. Eur. J.* **15** 11898–903
- [59] Li D, Song S and Fan C 2010 Target-responsive structural switching for nucleic acid-based sensors *Acc. Chem. Res.* **43** 631–41
- [60] Zhou J, Amrane S, Korkut D N, Bourdoncle A, He H-Z, Ma D-L and Mergny J-L 2013 Combination of i-Motif and G-quadruplex structures within the same strand: formation and application *Angew. Chem. Int. Edn* **125** 7896–900
- [61] Ma D-L, Chan D S-H and Leung C-H 2014 Group 9 organometallic compounds for therapeutic and bioanalytical applications *Acc. Chem. Res.* **47** 3614–31
- [62] Leung C-H, Chan D S-H, He H-Z, Cheng Z, Yang H and Ma D-L 2012 Luminescent detection of DNA-binding proteins *Nucleic Acids Res.* **40** 941–55
- [63] Lin S, Gao W, Tian Z, Yang C, Lu L, Mergny J-L, Leung C-H and Ma D-L 2015 Luminescence switch-on detection of protein tyrosine kinase-7 using a G-quadruplex-selective probe *Chem. Sci.* **6** 4284–90
- [64] Leung K-H, He H-Z, He B, Zhong H-J, Lin S, Wang Y-T, Ma D-L and Leung C-H 2015 Label-free luminescence switch-on detection of hepatitis C virus NS3 helicase activity using a G-quadruplex-selective probe *Chem. Sci.* **6** 2166–71
- [65] Ji L, Guo Y, Hong S, Wang Z, Wang K, Chen X, Zhang J, Hu J and Pei R 2015 Label-free detection of Pb²⁺ based on aggregation-induced emission enhancement of Au-nanoclusters *RSC Adv.* **5** 36582–6
- [66] Guo Y, Zhou L, Xu L, Zhou X, Hu J and Pei R 2014 Multiple types of logic gates based on a single G-quadruplex DNA strand *Sci. Rep.* **4** 7315
- [67] Xu L, Hong S, Sun N, Wang K, Zhou L, Ji L and Pei R 2015 Berberine as a novel light-up i-Motif fluorescence ligand and its application to design molecular logic systems *Chem. Commun.* at press (doi:10.1039/C5CC08242K)
- [68] Su X, Zhu X, Zhang C, Xiao X and Zhao M 2012 *In situ*, real-time monitoring of the 3' to 5' exonucleases secreted by living cells *Anal. Chem.* **84** 5059–65
- [69] Song C and Zhao M 2009 Real-time monitoring of the activity and kinetics of T4 polynucleotide kinase by a singly labeled DNA-hairpin smart probe coupled with λ exonuclease cleavage *Anal. Chem.* **81** 1383–8
- [70] Ma D-L, Lin S, Lu L, Wang M, Hu C, Liu L-J, Ren K and Leung C-H 2015 G-quadruplex-based logic gates for Hg^{II} and Ag^I ions employing a luminescent iridium(III) complex and extension of metal-mediated base pairs by polymerase *J. Mater. Chem. B* **3** 4780–5