

# Nanoscale

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

*Accepted Manuscripts* are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the [Information for Authors](#).

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard [Terms & Conditions](#) and the [Ethical guidelines](#) still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

# An oligonucleotide-based label-free luminescent switch-on probe for RNA detection utilizing a G-quadruplex-selective iridium(III) complex†

Dik-Lung Ma,<sup>\*,a</sup> Sheng Lin,<sup>a</sup> Ka-Ho Leung,<sup>a</sup> Hong-Zhang He,<sup>a</sup> Hai-Jing Zhong,<sup>b</sup> Li-Juan Liu,<sup>b</sup> Lihua Lu,<sup>a</sup> Daniel Shiu-Hin Chan,<sup>a</sup> Anne Bourdoncle,<sup>c,e</sup> Jean-Louis Mergny<sup>c,d</sup> and Chung-Hang Leung<sup>b</sup>

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

DOI: 10.1039/b000000x

We report herein the synthesis and application of a novel G-quadruplex-selective luminescent iridium(III) complex for the construction of an oligonucleotide-based, label-free, rapid and convenient luminescent RNA detection platform.

Ribonucleic acid (RNA) plays fundamental roles in transcription and translation processes in living cells. mRNA is responsible for the delivery of genetic information from the nucleus to the ribosomes of the cell, where it is translated into proteins. A special type of noncoding RNA, microRNA, is involved in the transcriptional and post-transcriptional regulation of gene expression, *via* hybridisation with complementary mRNA molecules. The abnormal expression or mutation of various types of RNA has been associated with a range of human diseases, such as cancer, mitochondrial disease, muscle disease and neurodegenerative disease.<sup>1-6</sup> Commonly-used detection methods for RNA include Northern blotting,<sup>7</sup> microarray technology<sup>8</sup> and real-time polymerase chain reaction (RT-PCR).<sup>9, 10</sup> However, these methods tend to be unwieldy, labor-intensive, time-consuming, costly or may necessitate stringent safety measures to control radiographic exposure. Therefore, the development of rapid, simple and low-cost detection platforms for RNA detection is highly desirable. In recent years, DNA-based techniques utilizing luminescent,<sup>11-18</sup> colorimetric<sup>19</sup> or electrochemical<sup>20</sup> signals for the construction of RNA detection platforms have been reported.<sup>21</sup>

The G-quadruplex is a DNA secondary structure consisting of square-planar arrangements of guanine nucleobases stabilized by Hoogsteen hydrogen bonding and monovalent cations.<sup>22-25</sup> Since the rich structural polymorphism of the G-quadruplex motif,<sup>26-28</sup> this structure widely act as a versatile signal transducing element in the construction of DNA-based detection platforms for metal ions,<sup>29</sup> DNA,<sup>30-33</sup> small molecules,<sup>34-36</sup> protein<sup>37, 38</sup> and enzyme activity.<sup>39-42</sup> Meanwhile, luminescent heavy metal complexes have attracted tremendous interest in sensory applications<sup>43-47</sup> due to their following advantages: (i) their long-lived phosphorescence allows them to be distinguished in highly fluorescent environments with the use of time-resolved spectroscopy, (ii) their large Stokes shifts can prevent self-quenching, and (iii) their modular synthesis allows their photophysical and molecular recognition properties to be easily tuned without labour-intensive synthetic protocols.<sup>48-51</sup>

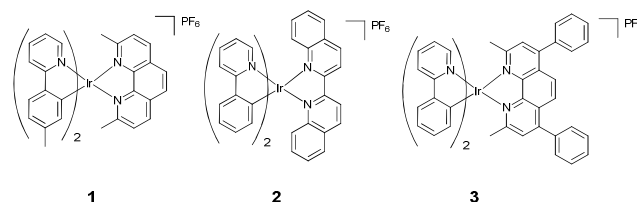
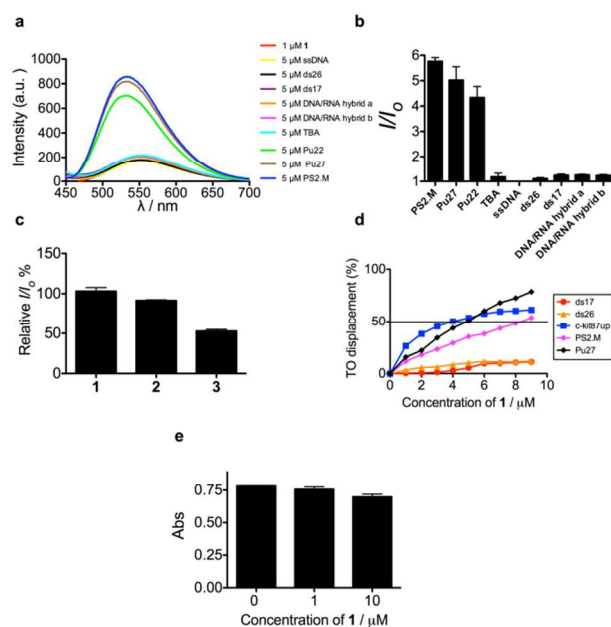


Fig. 1 Chemical structures of cyclometallated iridium(III) complexes 1, 2 and 3.

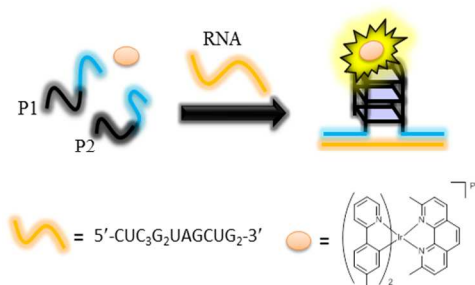
We report herein the synthesis and application of a novel G-quadruplex-selective luminescent iridium(III) complex **1** [Ir(ppy)<sub>2</sub>(dmph)]PF<sub>6</sub> (where ppy = 2-(p-tolyl)pyridine; dmph = 2,9-dimethyl-1,10-phenanthroline) for the sensitive detection of RNA. The mechanism of this assay is depicted in Scheme 1. Two oligonucleotides, P1 and P2, each contain a split G-quadruplex-forming sequence (black line) and a complementary sequence (blue line) that hybridizes with the target RNA (orange line). Upon addition of the target RNA, the two split G-quadruplex sequences will be brought into close proximity, and an intermolecular G-quadruplex can be formed. The formation of the G-quadruplex structure is recognized by the G-quadruplex-selective iridium(III) complex with an enhanced luminescence response, allowing the system to function as a switch-on luminescent probe for RNA.

The spectroscopic and photophysical characterization of the novel iridium(III) complex **1** are presented in the Electronic Supporting Information (Table S1, Fig. S1 and S2). We first investigated the luminescence behavior of the iridium(III) complex **1** towards different types of DNA. Complex **1** was weakly emissive in aqueous buffered solution. Encouragingly, the luminescence of complex **1** was significantly enhanced in the presence of various G-quadruplex DNA sequences (PS2.M, Pu27 and Pu22). A *ca.* 5.5-fold enhancement in the luminescence of complex **1** was observed in the presence of 5 μM of PS2.M, while *ca.* 4-fold enhancements were observed for Pu27 or Pu22 G-quadruplex DNA. In contrast, no significant changes in the luminescence of complex **1** was observed upon the addition of 5 μM of ssDNA, dsDNA or DNA/RNA hybrid duplex (Fig. 2a and 2b).

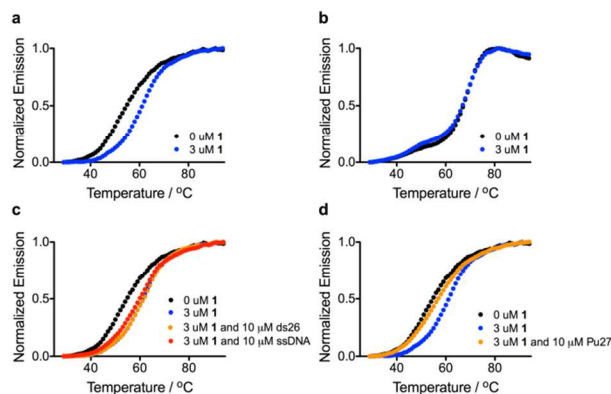


**Fig. 2** (a) Emission spectrum of complex **1** (1  $\mu\text{M}$ ) in the presence of 5  $\mu\text{M}$  of ssDNA, ctDNA or various G-quadruplexes. (b) Luminescence enhancement of complex **1** (1  $\mu\text{M}$ ) in the presence of 5  $\mu\text{M}$  of ssDNA, dsDNA or various G-quadruplexes. (c) Luminescence enhancement of complexes towards intermolecular G-quadruplex. (d) G4-FID titration curves of DNA duplexes (ds17 and ds26) or G-quadruplexes (PS2.M, Pu27 and *c-kit87up*). (e) Inhibition activity of complex **1** (1 or 10  $\mu\text{M}$ ) on the peroxidase activity of G-quadruplex/hemin complexes (1  $\mu\text{M}$  of G-quadruplex, 1  $\mu\text{M}$  of hemin, 2 mM of ABTS, and 2 mM of  $\text{H}_2\text{O}_2$ ).

We also compared the luminescence response of complex **1** towards the G-quadruplex formed by the association of P1 and P2 in the presence of the target RNA, with two other G-quadruplex-selective iridium(III) complexes (**2** and **3**). We found that complex **1** showed the highest luminescence response to the intermolecular G-quadruplex out of all the complexes tested, suggesting that complex **1** is the ideal choice for this sensing platform (Fig. 2c). To further validate the suitability of complex **1** as a G-quadruplex-selective probe, G-quadruplex fluorescent intercalator displacement (G4-FID) and fluorescence resonance energy transfer (FRET) melting assays were employed to evaluate the binding affinity and selectivity of complex **1** for G-quadruplex DNA.<sup>52</sup> Although the emission region of thiazole orange (TO) (510–750 nm) and complex **1** (500–700 nm) overlap, complex **1** is not expected to interfere in the G4-FID assay due to its very low absorbance at the excitation wavelength of TO (501 nm). The results of the G4-FID assay indicated that complex **1** could displace TO from G-quadruplex structures with  $\text{G}^4\text{DC}_{50}$  values (half-maximal concentration of compound required to displace 50% TO from DNA) of < 9  $\mu\text{M}$ , while the displacement of TO from dsDNA was less than 50% even at the highest concentration of **1** tested (Fig. 2d). The selective binding of complex **1** towards G-quadruplex DNA was further analysed by FRET-melting assays. The melting temperature ( $\Delta T_m$ ) of the F21T G-quadruplex was increased by about 6  $^\circ\text{C}$  upon the addition of complex **1** (Fig. 3a). By comparison, no significant change in the melting temperature of F10T dsDNA was observed at the same concentration of complex **1** (Fig. 3b). Additionally, the stabilization effect of complex **1** towards the F21T G-



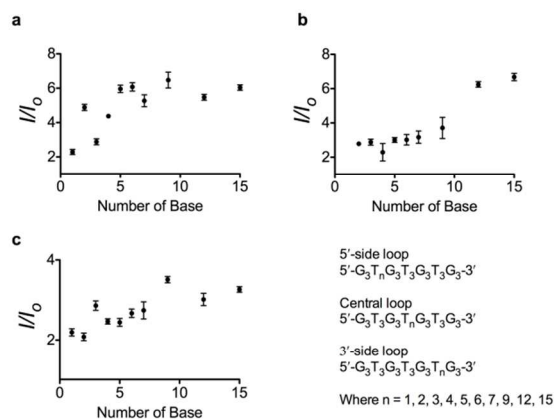
**Scheme 1.** Schematic representation of the split G-quadruplex-based luminescent turn-on detection platform for RNA using the G-quadruplex-selective iridium(III) complex **1**.



**Fig. 3** (a) Melting profile of F21T G-quadruplex DNA (0.2  $\mu\text{M}$ ) in the absence and presence of **1** (3  $\mu\text{M}$ ). (b) Melting profile of F10T dsDNA (0.2  $\mu\text{M}$ ) in the absence and presence of **1** (3  $\mu\text{M}$ ). (c) Melting profile of F21T (0.2  $\mu\text{M}$ ) in the absence and presence of **1** (3  $\mu\text{M}$ ) and ds26 (10  $\mu\text{M}$ ) or ssDNA (10  $\mu\text{M}$ ). (d) Melting profile of F21T (0.2  $\mu\text{M}$ ) in the absence and presence of **1** (3  $\mu\text{M}$ ) and Pu27 (10  $\mu\text{M}$ ).

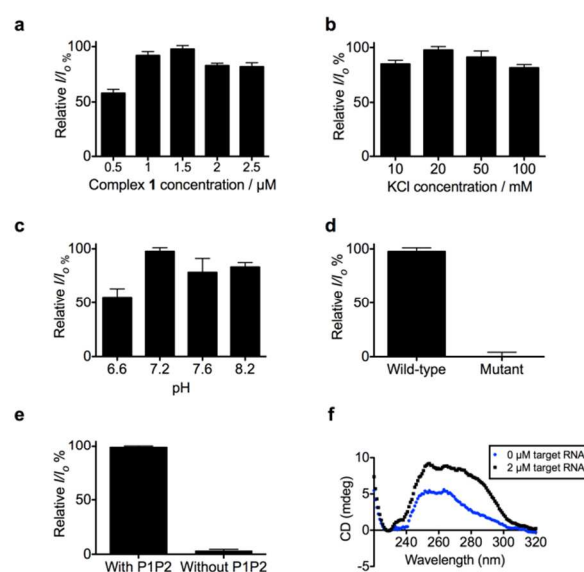
quadruplex was not significantly perturbed by the addition of 50-fold higher concentration of unlabeled competitor dsDNA (ds26) or ssDNA (Fig. 3c). This indicates that complex **1** binds selective to G-quadruplex DNA over dsDNA or ssDNA. However, the addition of a 50-fold excess of an unlabeled G-quadruplex competitor Pu27 abrogated the stabilizing effect of complex **1** on the F21T G-quadruplex, as it is able to sequester complex **1** and prevent its interaction with the labeled G-quadruplex (Fig. 3d). Taken together, these results demonstrate the ability of complex **1** to discriminate between G-quadruplex DNA and dsDNA or ssDNA.

Interestingly, complex **1** showed only weak luminescence enhancement in the presence of the thrombin-binding aptamer (TBA) G-quadruplex (Fig. 2a and 2b). TBA has previously been shown to readily accommodate planar aromatic ligands, but not ribbon-like molecules.<sup>52</sup> This result suggests that complex **1** may bind outside the G-tetrad for the other G-quadruplexes.<sup>53</sup> In order to explore whether complex **1** mainly bound to the G-quadruplex structure by end-stacking, we investigated the ability of complex **1** to inhibit the peroxidase activity of the G4/hemin ensemble. Hemin has been described as an end-stacking binding ligand that forms a peroxidase-like DNAzyme upon binding to G-quadruplex DNA. Therefore, ligands that also bind to the termini of the G-quadruplex motif will compete with hemin, and thereby decrease the generation of the colored product generated by the DNAzyme-catalysed oxidation of ABTS<sup>2-</sup>.<sup>54, 55</sup> Interestingly, our



**Fig. 4** Luminescence enhancement of complex 1 as a function of loop size (a) 5'-side loop, (b) central loop and (c) 3'-side loop (in nucleotides).

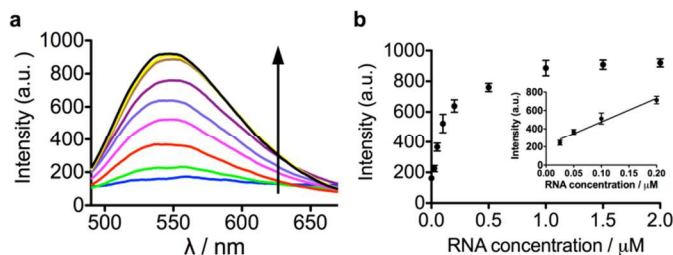
results showed that the peroxidase activity of the G4/hemin complex was only slightly decreased by the addition of up to 10  $\mu\text{M}$  of complex 1 (Fig. 2e). This suggests that the end-stacking is not the predominant interaction between complex 1 and G-quadruplex DNA. The ability of complex 1 to displace TO but not hemin from G-quadruplex DNA could be due to the fact that the porphyrin-like hemin is a strong G-quadruplex binding ligand.<sup>56</sup> Thus, complex 1, which exhibited a  $G^4DC_{50}$  value of about 4–8  $\mu\text{M}$  against TO in the G4-FID assay, may not have been able to displace hemin under the conditions used in the experiment. Alternatively, TO may bind to the G-quadruplex at multiple sites, as has been reported for the human telomeric G-quadruplex ( $5'-G_3(T_2AG_3)_3-3'$ ) by Teulade-Fichou, Mergny and co-workers.<sup>52</sup> Therefore, complex 1 may have been able to displace TO from different binding sites on the G-quadruplex, while not being an avid-enough ligand to dislodge the strong end-stacking ligand hemin from the terminal G-tetrad. To examine the role of the G-quadruplex loops in the binding interaction of complex 1, we investigated the luminescence response of complex 1 towards various G-quadruplex DNA structures with different loop sizes. The G-quadruplex topologies of the sequences utilized in this experiment were validated by Mergny *et al.*<sup>57</sup> We explored G-quadruplex sequences containing a 5'-side loop ( $5'-G_3T_nG_3T_3G_3T_3G_3-3'$ ), a central loop ( $5'-G_3T_3G_3T_nG_3T_3G_3-3'$ ) or a 3'-side loop ( $5'-G_3T_3G_3T_3G_3T_nG_3-3'$ ), with loop sizes ranging from 1 to 15 nucleotides ( $n = 1, 2, 3, 4, 5, 6, 7, 9, 12, 15$ ). The results showed that the luminescence intensity of complex 1 generally increased with greater loop size, regardless of the location of the loop (Fig. 4). The luminescence enhancement of complex 1 increased from 2.0 to 6.5-fold for the 5'-side loop and central loop, and from 2.0 to 3.5-fold for the 3'-side loop, as the loop size was increased from 1 to 15 nt. Interestingly, the maximal luminescence enhancement for both the central and side loops occurred at a loop size of about 9 to 12 nt. This result suggests that the G-quadruplex loop may play an important role in the G-quadruplex-complex 1 interaction, and is consistent with previous work by Qu and co-workers who showed that the nature of the loop region could affect the binding interaction between ligands and G-quadruplex DNA.<sup>58</sup> Encouraged by the superior selectivity of complex 1 for G-quadruplex DNA, we sought to utilize complex 1 for the



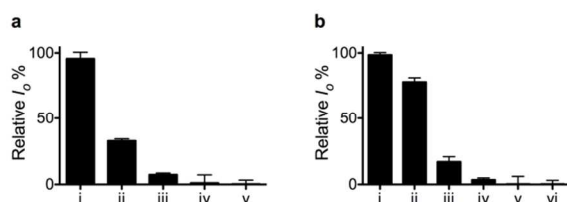
**Fig. 5.** Relative luminescence intensity of the system. (a) ( $[P1]$  and  $[P2] = 2 \mu\text{M}$ ) with different concentrations of 1 (0.5, 1, 1.5, 2 and 2.5  $\mu\text{M}$ ). (b) ( $[1] = 1.5 \mu\text{M}$ ,  $[P1]$  and  $[P2] = 2 \mu\text{M}$ ) with different concentrations of KCl (10, 20, 50 and 100 mM). (c) ( $[1] = 1.5 \mu\text{M}$ ,  $[P1]$  and  $[P2] = 2 \mu\text{M}$ ) with different buffer pH values (6.6, 7.2, 7.6 and 8.2). (d) Relative luminescence intensity of 1.5  $\mu\text{M}$  of complex 1 upon addition of 2  $\mu\text{M}$  of the target RNA in the presence and absence of 2  $\mu\text{M}$  each of P1 and P2. (e) Luminescence intensity of the system ( $[1] = 1.5 \mu\text{M}$ ,  $[KCl] = 20 \text{ mM}$ ) in the presence of P1/P2 (wild type) and P1mut/P2mut (mutant). Error bars represent the standard deviations of the results from three independent experiments. (f) CD spectrum of 2  $\mu\text{M}$  each of P1 and P2 in the absence and presence of 2  $\mu\text{M}$  of target RNA.

development of the G-quadruplex-based RNA detection assay. A preliminary experiment showed that the luminescence of complex 1 was significantly enhanced when P1-P2 was annealed with 45 target RNA sequence. We presume that the luminescence enhancement of complex 1 was due to the formation of the DNA/RNA hybrid that brings the intermolecular G-quadruplex sequences into close proximity, leading to the subsequent formation of the split G-quadruplex structure that interacts 50 strongly with complex 1.

After optimization of the pH, concentration of complex 1 and KCl (Fig. 5a–c), we performed an emission titration experiment with increasing concentrations of target RNA. Encouragingly, the luminescence intensity of complex 1 was enhanced as the 55 concentration of target RNA was increased (Fig. 6). The system exhibited a linear range of detection for RNA from 25 to 200 nM, with a maximal luminescence reached at 1  $\mu\text{M}$  of the target RNA. Although the assay showed a modest 5-fold maximal luminescence enhancement at saturating RNA concentrations, it 60 was highly sensitive for RNA, with a detection limit of 25 nM using the  $3\sigma$  method. To validate the mechanism of our sensing platform, we utilized mutant split G-quadruplex sequences ( $P1_{\text{mut}}$  and  $P2_{\text{mut}}$ ) that lack consecutive guanine bases, and are thus unable to fold into a G-quadruplex upon hybridization to the 65 target RNA. No significant change in the luminescence of complex 1 was observed when  $P1_{\text{mut}}$  and  $P2_{\text{mut}}$  were incubated with the target RNA (Fig. 5d). We also conducted an emission



**Fig. 6** (a) Emission spectrum of the system ( $[1] = 1.5 \mu\text{M}$ ,  $[P1]$  and  $[P2] = 2 \mu\text{M}$ ,  $[\text{KCl}] = 20 \text{ mM}$ ) in the presence of increasing concentrations of target RNA. (b) Luminescence response of the system at  $\lambda = 547 \text{ nm}$  vs. RNA concentration. Inset: linear plot of the change in luminescence intensity at  $\lambda = 547 \text{ nm}$  vs. RNA concentration. Error bars represent the standard deviations of the results from three independent experiments.



**Fig. 7** (a) Luminescence intensity of the system ( $[1] = 1.5 \mu\text{M}$ ,  $[P1]$  and  $[P2] = 2 \mu\text{M}$ ,  $[\text{KCl}] = 20 \text{ mM}$ ) for detection of  $1 \mu\text{M}$  of target i) RNA, ii) single-base mismatched RNA strand, iii) two-base mismatched RNA strand, iv) three-base mismatched RNA strand and v) random coil RNA strand. (b) Luminescence intensity of the system ( $[1] = 1.5 \mu\text{M}$ ,  $[P1]$  and  $[P2] = 2 \mu\text{M}$ ,  $[\text{KCl}] = 20 \text{ mM}$ ) for detection of  $1 \mu\text{M}$  of i) target RNA, ii) corresponding DNA, iii) single-base mismatched DNA strand, iv) two-base mismatched DNA strand, v) three-base mismatched DNA strand and vi) random coil DNA strand.

experiment with RNA and complex **1** in the absence of P1 and P2. No enhancement was observed upon addition of  $2 \mu\text{M}$  of target RNA (Fig. 5e), indicating that the luminescence response of complex **1** was dependent on the presence of P1 and P2. Circular dichroism (CD) spectroscopy was further utilized to verify the conformational change of P1/P2. The characteristic G-quadruplex signals that appeared in the presence of the target RNA were consistent with previous reports (Fig. 5f).<sup>59</sup> These results indicate that the luminescence enhancement of the system is dependent upon the formation of the G-quadruplex motif induced by the target RNA.

The selectivity of the method for the target RNA versus other RNA sequences was also examined. The intensity of perfectly complementary target RNA is about 3 times higher than that of a single-base mismatched sequence (Fig. 7a). Furthermore, only negligible effects on the luminescence of complex **1** were observed in the presence of RNA sequences bearing 2 to 3 base substitutions, or with random coil RNA. Although a relatively high luminescence enhancement was observed with the corresponding DNA sequence, the system showed strong selectivity for the target RNA over DNA sequences containing two or more base substitutions or random coil DNA (Fig. 7b).

miRNA networks control the expression of a significant fraction of the human transcriptome, and the upregulation or downregulation of particular miRNA sequences has been implicated in the pathogenesis of various diseases.<sup>60</sup> Furthermore,

mutations in miRNA or in the miRNA-binding sites of mRNAs can also disrupt normal mRNA function.<sup>61</sup> For example, miR-15a and miR-16-1 deletion mutants were unable to block the expression of the oncogene BCL2 in cases of B-cell chronic lymphocytic leukemia.<sup>62</sup> Additionally, the rs2910164 mutation in miR-146a has been associated with a number of cancers through increased binding to BRCA1 and other genes.<sup>63, 64</sup> Therefore, given its ability to discriminate between mRNA mutants differing by only a single base, this assay (with the aid of total cellular RNA extraction) could potentially be used as a diagnostic platform to detect target RNA sequences associated with a variety of human diseases.

In conclusion, we have synthesized a novel G-quadruplex-selective iridium(III) complex and have explored its utility for the construction of the first metal and G-quadruplex-based luminescent assay for RNA detection. Our “mix-and-detect” sensing platform is rapid, simple, convenient to use, cost-effective and does not require the covalently labeling of oligonucleotides. It can detect down to  $25 \text{ nM}$  of target RNA without any signal amplification or fluorescent labeling of oligonucleotides and can effectively discriminate between RNA sequences differing by a single base.

This work is supported by Hong Kong Baptist University (FRG2/11-12/009), Centre for Cancer and Inflammation Research, School of Chinese Medicine (CCIR-SCM, HKBU), the Health and Medical Research Fund (HMRF/13121482), 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), the Science and Technology Development Fund, Macao SAR (001/2012/A and 103/2012/A3) and the University of Macau (MYRG091(Y2-L2)-ICMS12-LCH and MYRG121(Y2-L2)-ICMS12-LCH).

## Notes and references

<sup>a</sup> Department of Chemistry, Hong Kong Baptist University, Kowloon Tong, Hong Kong, China. E-mail: edmondma@hkbu.edu.hk

<sup>b</sup> State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Macao, China

<sup>c</sup> ARNA Laboratory, University of Bordeaux, F-33000 Bordeaux, France

<sup>d</sup> INSERM U869, IECEB, F-33600 Pessac, France

<sup>e</sup> Univ. Poitiers, 40 avenue du recteur Pineau, 86000 Poitiers (France)

† Electronic Supplementary Information (ESI) available: experimental details and supplementary spectral data. See DOI: 10.1039/b000000x/

- C. M. Croce, *Nat. Rev. Genet.*, 2009, **10**, 704.
- J. Jiang, E. J. Lee, Y. Gusev and T. D. Schmittgen, *Nucleic Acids Res.*, 2005, **33**, 5394.
- A. W. Wark, H. J. Lee and R. M. Corn, *Angew. Chem. Int. Ed.*, 2008, **47**, 644.
- L. Levinger, M. Mörl and C. Florentz, *Nucleic Acids Res.*, 2004, **32**, 5430.
- P. Conforti, C. Ramos, B. L. Apostol, D. A. Simmons, H. P. Nguyen, O. Riess, L. M. Thompson, C. Zuccato and E. Cattaneo, *Mol. Cell. Neurosci.*, 2008, **39**, 1.
- T. M. Wheeler and C. A. Thornton, *Curr. Opin. Neurol.*, 2007, **20**, 572.
- G. S. Pall, C. Codony-Servat, J. Byrne, L. Ritchie and A. Hamilton, *Nucleic Acids Res.*, 2007, **35**, e60.
- P. T. Nelson, D. A. Baldwin, L. M. Scearce, J. C. Oberholtzer, J. W. Tobias and Z. Mourelatos, *Nat. Meth.*, 2004, **1**, 155.

9. J. Li, B. Yao, H. Huang, Z. Wang, C. Sun, Y. Fan, Q. Chang, S. Li, X. Wang and J. Xi, *Anal. Chem.*, 2009, **81**, 5446.
10. C. K. Raymond, B. S. Roberts, P. Garrett-Engele, L. P. Lim and J. M. Johnson, *RNA*, 2005, **11**, 1737.
- 5 11. E. M. Harcourt and E. T. Kool, *Nucleic Acids Res.*, 2012, **40**, e65.
12. H. Dong, J. Zhang, H. Ju, H. Lu, S. Wang, S. Jin, K. Hao, H. Du and X. Zhang, *Anal. Chem.*, 2012, **84**, 4587.
13. Y.-Q. Liu, M. Zhang, B.-C. Yin and B.-C. Ye, *Anal. Chem.*, 2012, **84**, 5165.
- 10 14. Y. Tu, W. Li, P. Wu, H. Zhang and C. Cai, *Anal. Chem.*, 2013, **85**, 2536.
15. P. Shah, A. Rørvig-Lund, S. B. Chaabane, P. W. Thulstrup, H. G. Kjaergaard, E. Fron, J. Hofkens, S. W. Yang and T. Vosch, *ACS Nano*, 2012, **6**, 8803.
- 15 16. L. Cui, Z. Chen, Z. Zhu, X. Lin, X. Chen and C. J. Yang, *Anal. Chem.*, 2013, **85**, 2269.
17. Y. Tu, P. Wu, H. Zhang and C. Cai, *Chem. Commun.*, 2012, **48**, 10718.
18. B.-C. Yin, Y.-Q. Liu and B.-C. Ye, *J. Am. Chem. Soc.*, 2012, **134**, 5064.
- 20 19. Y. Wen, Y. Xu, X. Mao, Y. Wei, H. Song, N. Chen, Q. Huang, C. Fan and D. Li, *Anal. Chem.*, 2012, **84**, 7664.
20. H. Dong, S. Jin, H. Ju, K. Hao, L.-P. Xu, H. Lu and X. Zhang, *Anal. Chem.*, 2012, **84**, 8670.
- 25 21. H. Dong, J. Lei, L. Ding, Y. Wen, H. Ju and X. Zhang, *Chem. Rev.*, 2013, **113**, 6207.
22. Z. Chen, K.-w. Zheng, Y.-h. Hao and Z. Tan, *J. Am. Chem. Soc.*, 2009, **131**, 10430.
23. S. Neidle, *Curr. Opin. Struct. Biol.*, 2009, **19**, 239.
- 30 24. Y. Zhao, Z.-y. Kan, Z.-x. Zeng, Y.-h. Hao, H. Chen and Z. Tan, *J. Am. Chem. Soc.*, 2004, **126**, 13255.
25. Y. Xue, Z.-y. Kan, Q. Wang, Y. Yao, J. Liu, Y.-h. Hao and Z. Tan, *J. Am. Chem. Soc.*, 2007, **129**, 11185.
26. Y. Krishnan-Ghosh, D. Liu and S. Balasubramanian, *J. Am. Chem. Soc.*, 2004, **126**, 11009.
- 35 27. Y. Krishnan and F. C. Simmel, *Angew. Chem. Int. Ed.*, 2011, **50**, 3124.
28. S. Pitchaiya and Y. Krishnan, *Chem. Soc. Rev.*, 2006, **35**, 1111.
29. K. Qu, C. Zhao, J. Ren and X. Qu, *Mol. Biosyst.*, 2012, **8**, 779.
- 40 30. D.-L. Ma, H.-Z. He, K.-H. Leung, H.-J. Zhong, D. S.-H. Chan and C.-H. Leung, *Chem. Soc. Rev.*, 2013, **42**, 3427.
31. C. Zhao, L. Wu, J. Ren and X. Qu, *Chem. Commun.*, 2011, **47**, 5461.
32. H.-Z. He, D. S.-H. Chan, C.-H. Leung and D.-L. Ma, *Nucleic Acids Res.*, 2013, **41**, 4345.
- 45 33. S. Deng, L. Cheng, J. Lei, Y. Cheng, Y. Huang and H. Ju, *Nanoscale*, 2013, **5**, 5435.
34. Y. Peng, X. Wang, Y. Xiao, L. Feng, C. Zhao, J. Ren and X. Qu, *J. Am. Chem. Soc.*, 2009, **131**, 13813.
35. Z. Chen, Y. Lin, C. Zhao, J. Ren and X. Qu, *Chem. Commun.*, 2012, **48**, 11428.
- 50 36. C. Wang, J. Wu, C. Zong, H. Ju and F. Yan, *Analyst*, 2011, **136**, 4295.
37. D. Lin, J. Wu, F. Yan, S. Deng and H. Ju, *Anal. Chem.*, 2011, **83**, 5214.
- 55 38. M. Yin, Z. Li, Z. Liu, J. Ren, X. Yang and X. Qu, *Chem. Commun.*, 2012, **48**, 6556.
39. X. Su, X. Zhu, C. Zhang, X. Xiao and M. Zhao, *Anal. Chem.*, 2012, **84**, 5059.
40. X. Su, C. Zhang, X. Zhu, S. Fang, R. Weng, X. Xiao and M. Zhao, *Anal. Chem.*, 2013, **85**, 9939.
- 60 41. D. Hu, Z. Huang, F. Pu, J. Ren and X. Qu, *Chem. Eur. J.*, 2011, **17**, 1635.
42. C.-H. Leung, H.-J. Zhong, H.-Z. He, L. Lu, D. S.-H. Chan and D.-L. Ma, *Chem. Sci.*, 2013, **4**, 3781.
- 65 43. Q. Zhao, F. Li and C. Huang, *Chem. Soc. Rev.*, 2010, **39**, 3007.
44. Q. Zhao, T. Cao, F. Li, X. Li, H. Jing, T. Yi and C. Huang, *Organometallics*, 2007, **26**, 2077.
45. J. Liu, Y. Liu, Q. Liu, C. Li, L. Sun and F. Li, *J. Am. Chem. Soc.*, 2011, **133**, 15276.
- 70 46. M. R. Gill and J. A. Thomas, *Chem. Soc. Rev.*, 2012, **41**, 3179.
47. V. Gonzalez, T. Wilson, I. Kurihara, A. Imai, J. A. Thomas and J. Otsuki, *Chem. Commun.*, 2008, 1868.
48. Y. Yang, Q. Zhao, W. Feng and F. Li, *Chem. Rev.*, 2012, **113**, 192.
49. Q. Zhao, C. Huang and F. Li, *Chem. Soc. Rev.*, 2011, **40**, 2508.
- 75 50. C. Li, M. Yu, Y. Sun, Y. Wu, C. Huang and F. Li, *J. Am. Chem. Soc.*, 2011, **133**, 11231.
51. C. Metcalfe and J. A. Thomas, *Chem. Soc. Rev.*, 2003, **32**, 215.
52. D. Monchaud, C. Allain, H. Bertrand, N. Smargiasso, F. Rosu, V. Gabelica, A. De Cian, J. L. Mergny and M. P. Teulade-Fichou, *Biochimie*, 2008, **90**, 1207.
- 80 53. F. Hamon, E. Largy, A. Guédin-Beaurepaire, M. Rouchon-Dagois, A. Sidibe, D. Monchaud, J.-L. Mergny, J.-F. Riou, C.-H. Nguyen and M.-P. Teulade-Fichou, *Angew. Chem. Int. Ed.*, 2011, **50**, 8745.
54. X. Cheng, X. Liu, T. Bing, Z. Cao and D. Shangquan, *Biochemistry*, 2009, **48**, 7817.
- 85 55. B. Jin, X. Zhang, W. Zheng, X. Liu, C. Qi, F. Wang and D. Shangquan, *Anal. Chem.*, 2013, **86**, 943.
56. J. Seenisamy, S. Bashyam, V. Gokhale, H. Vankayalapati, D. Sun, A. Siddiqui-Jain, N. Streiner, K. Shin-ya, E. White, W. D. Wilson and L. H. Hurley, *J. Am. Chem. Soc.*, 2005, **127**, 2944.
- 90 57. A. Guédin, J. Gros, P. Alberti and J.-L. Mergny, *Nucleic Acids Res.*, 2010, **38**, 7858.
58. H. Yu, C. Zhao, Y. Chen, M. Fu, J. Ren and X. Qu, *J. Med. Chem.*, 2009, **53**, 492.
- 95 59. J. Zhu, L. Zhang and E. Wang, *Chem. Commun.*, 2012, **48**, 11990.
60. S. Volinia, M. Galasso, S. Costinean, L. Tagliavini, G. Gamberoni, A. Drusco, J. Marchesini, N. Mascellani, M. E. Sana, R. Abu Jarour, C. Despouts, M. Teitell, R. Baffa, R. Aqeilan, M. V. Iorio, C. Taccioli, R. Garzon, G. Di Leva, M. Fabbri, M. Catozzi, M. Previati, S. Ambs, T. Palumbo, M. Garofalo, A. Veronese, A. Bottoni, P. Gasparini, C. C. Harris, R. Visone, Y. Pekarsky, A. de la Chapelle, M. Bloomston, M. Dillhoff, L. Z. Rassenti, T. J. Kipps, K. Huebner, F. Pichiorri, D. Lenze, S. Cairo, M.-A. Buendia, P. Pineau, A. Dejean, N. Zanesi, S. Rossi, G. A. Calin, C.-G. Liu, J. Palatini, M. Negrini, A. Vecchione, A. Rosenberg and C. M. Croce, *Genome Res.*, 2010, **20**, 589.
- 105 61. A. Bhattacharya, J. D. Ziebarth and Y. Cui, *Nucleic Acids Res.*, 2013, **41**, D977.
62. D. J. Allendorf and R. S. Davis, *JAMA*, 2011, **305**, 95.
- 110 63. K. Jazdzewski, E. L. Murray, K. Franssila, B. Jarzab, D. R. Schoenberg and A. de la Chapelle, *Proc. Natl. Acad. Sci. U.S.A.*, 2008, **105**, 7269.
64. J. Shen, C. B. Ambrosone, R. A. DiCioccio, K. Odunsi, S. B. Lele and H. Zhao, *Carcinogenesis*, 2008, **29**, 1963.
- 115