Luminescent platinum(II) complexes with functionalized N-heterocyclic carbene or diphosphine selectively probe mismatched and abasic DNA

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The selective targeting of mismatched DNA overexpressed in cancer cells is an appealing strategy in designing cancer diagnosis and therapy protocols. Few luminescent probes that specifically detect intracellular mismatched DNA have been reported. Here we used Pt(II) complexes with luminescence sensitive to subtle changes in the local environment and report several Pt(II) complexes that selectively bind to and identify DNA mismatches. We evaluated the complexes’ DNA-binding characteristics by ultraviolet/visible absorption titration, isothermal titration calorimetry, nuclear magnetic resonance and quantum mechanics/molecular mechanics calculations. These Pt(II) complexes show up to 15-fold higher emission intensities upon binding to mismatched DNA over matched DNA and can be utilized for both detecting DNA abasic sites and identifying cancer cells and human tissue samples with different levels of mismatch repair. Our work highlights the potential of luminescent Pt(II) complexes to differentiate between normal cells and cancer cells which generally possess more aberrant DNA structures.
The binding interactions between metal complexes and nucleic acids have been the subject of numerous studies in recent decades. These interactions are likely to lead to therapeutic (for example, anti-cancer) effects and/or be used for diagnostic (for example, luminescent probes) purposes. To date, the selective targeting of nucleic acids in cancer cells by chemotherapeutic metal complexes in a manner that minimizes off-target bindings and hence diminishes side effects remains a great challenge. Endeavours in this research area have led to luminescent metal complexes that show emission of up to 9.4 Å favor strong mismatched base pairs, whereas the other auxiliary bipyridine base pairs at mismatched sites, concomitantly with the ejection of the bulky ancillary ligand can be designed to hamper the intercalation of the platinum complex with well-matched DNA while exhibiting high binding affinity with mismatched DNA. This difference is possible because the mismatched sites possess larger binding pockets. We have previously described a luminescent [Pt(C^N^N)(NHC)]^+(HC^N^N=N=6-phenyl-2,2'-bipyridine, NHNC=N-heterocyclic carbene, 1b in the current report) complex with an NHC ligand almost perpendicular to the C-deprotonated C^N^N=N plane. This complex exhibits a weak binding affinity to matched DNA. Thus, varying the N-alkyl/ary1 substitution(s) of NHC, [Pt(C^N^N)(NHC)]^+ may generate a candidate scaffold to target mismatched DNA via cooperative π-stacking and groove binding interactions.

Here we describe several luminescent, mismatched DNA probes based on the [Pt(C^N^N)(NHC)]^+, [Pt(N^C^N)(NHC)]^+, and [Pt(C^N^N)(μ-dcpm)]^2 complexes. The mononuclear Pt(II) complex selectively detects DNA containing CC mismatches, and the dinuclear Pt(II) complex exhibits high selectivity towards several types of DNA mismatches. These luminescent Pt(II) complexes also selectively bind thermodynamically unstable abasic DNA, are able to differentiate cancer cells that have different levels of MMR capacity and can differentiate colon tumour tissue from normal tissue.

**Results**

**Structure and emission responses to CC mismatched DNA.** We synthesized and characterized a series of [Pt(C^N^N)(NHC)]^+ complexes with different alkyl chains, aromatic groups and hydrophilic alcohol moieties on NHC and/or on C-deprotonated C^N^N=N ligands (Fig. 1; details in Supplementary Information). In addition, we examined the emission responses of complexes that bind mismatched and matched DNA (Supplementary Fig. 1). The most unstable CC mismatched DNA was chosen in the primary screen because metalloinsertors display the highest binding to this type of mismatched DNA. We found that as the steric hindrance of the NHC ligand increased, the binding of the

![Figure 1](https://example.com/figure1.png)

**Figure 1 | Chemical structures and proposed binding modes.** Platinum(II) complexes 1a–1o, 2a, 3, 4 and 5a–5c. The counteranions for all complexes are CF_3SO_3^-. The inset shows the molecular design of Pt(II) complexes tested for binding mismatched DNA.
Pt(II) complexes to matched DNA decreased, whereas the binding with CC mismatched DNA remained high for certain bulky NHC ligands (Fig. 2; the DNA sequences in all the experiments are depicted in the Supplementary Table 2). For example, for the [Pt(C3N4N)(NHC)]+ type complexes of 1e (−CH3), 1f (−C6H5), 1g (−C5H11), 1h (−C6H13), 1i (−C4H9), and 1j (−CH2Ph), which contain the same N3-benzyl group but different N1-substituents on the NHC ligands (Table 1), an increase in the length of the N1-alkyl was accompanied by a marked decrease in emission enhancement towards matched DNA (IΩM), from 38.9-fold to <3-fold. Although increasing the chain length from the N1-methyl of 1e to the N1-ethyl of 1f led to a decrease in emission enhancement in the case of mismatched DNA (IΩMM), the emission intensity of 1g, which bears N3-propyl, was much stronger than that of 1f, suggesting that N3-alkyl favours the groove binding of the Pt(II) complex to DNA. This may enhance the tightness of the insertion binding mode, as proposed. Further lengthening the alkyl chain to n-butyl, n-pentyl and n-hexyl gradually decreased the binding with mismatched DNA. Comparing the IΩMM/IΩM ratios, 1c, containing N3-(n-butyl) and N3-benzyl substitutions on the NHC (Fig. 1) showed the most significantly different emission responses between CC mismatched DNA and matched DNA. We characterized the structure of this complex by 1H–1H correlation spectroscopy (COSY) and nuclear Overhauser effect spectroscopy (NOESY) nuclear magnetic resonance (NMR; Supplementary Fig. 2). As shown in Fig. 3a,c, the emission intensity of 1c (λmax = 535 nm) in a Tris-buffered solution increased by 26.4-fold in the presence of 1 equivalent of a hairpin DNA oligomer with a CC mismatch29 (Fig. 3c). However, the emission of 1c was enhanced only 2.6-fold upon the addition of the same amount of the hairpin DNA, having the cognate sequence with the mismatched bases replaced by matched GC pairs. In another mismatched DNA model using a 17-mer double-stranded DNA (dsDNA) formed by two complementary oligomers44, the emission intensity of 1c displayed 27.5-fold and 2.8-fold increases in emission intensity upon the addition of CC mismatched 17-mer dsDNA and matched 17-mer dsDNA, respectively (Supplementary Fig. 3). In the literature, a rhodium–Oregon Green conjugate has been reported to exhibit a ∼3.2-fold higher emission intensity in the presence of CC mismatched DNA compared with matched DNA47. In a control experiment, the classical DNA intercalator, ethidium bromide (EB), showed increases in emission intensity with hairpin mismatched DNA and matched DNA of 12.7-fold and 14.1-fold, respectively, revealing a 0.9-fold difference (Supplementary Fig. 4). Under similar conditions, 1a, containing a less bulky N-methyl substitution on the NHC, displayed similar 8.5-fold and 7.1-fold emission enhancements for hairpin CC mismatched DNA and matched DNA, respectively (1.2-fold difference; Supplementary Fig. 5). This result can be explained by the fact that the less bulky N-methyl substituted NHC does not impose enough steric hindrance to diminish the binding of the Pt complex with matched DNA, resulting in similarly high enhancement in emission intensity for both matched and mismatched DNA. Conversely, the more bulky N3-(n-butyl) and N3-benzyl groups diminished the binding of 1c with matched DNA, while a much stronger binding affinity with mismatched DNA was still maintained.

Characterizations of bindings to CC mismatched DNA. The following experiments were performed to shed light on the DNA-binding interactions of the novel complexes. In ultraviolet/visible absorption titration experiments, upon the addition of dsDNA containing a CC mismatch to 1c in a Tris-buffered solution, a hypochromicity of ~34% at λ = 335 nm, together with isosbestic spectral changes, was observed (Supplementary Fig. 6a); however, only a minor hypochromicity of ~12% was detected at the same wavelength for 1c when matched dsDNA with a similar sequence was used instead (Supplementary Fig. 6b). Isothermal titration calorimetry (ITC) experiments (Fig. 3b) revealed that the titration of CC mismatched DNA with 1c resulted in a significant exothermic reaction, revealing binding between the two components, with a dissociation constant (Kd) of 51.5 ± 8.0 μM. In contrast, the reaction between 1c and matched DNA was a very weak exothermic reaction, with a Kd estimated to be >600 μM. In a similar experiment, the Kd values of the binding of 1a to mismatched and matched DNA were 66.2 ± 15.7 and 56.4 ± 13.6 μM, respectively (Supplementary Fig. 7a,b). The Kd values of the binding of EB to mismatched DNA and matched DNA were 1.32 ± 0.24 and 1.60 ± 0.28 μM, respectively (Supplementary Fig. 8a,b). 1H NMR and two-dimensional (2D) NOESY, total correlation spectroscopy (TOCSY) and COSY NMR experiments were performed using a self-complementary DNA oligonucleotide, 5′-C5G3G2A4T3C4G5-3′, containing CC mismatches in D2O (phosphate buffer, pH 6.1; Fig. 4a–d; Supplementary Figs 9 and 10)48. In the range of 6.8–8.0 p.p.m., the aromatic 1H signals of C5 and A4 show substantial shifts, whereas those of the other bases remain nearly unshifted in the presence of 1c (1H NMR in Fig. 4a). In addition, in the 2D H1′ × aromatic NOESY spectrum of 1c with DNA at a 1:1 ratio, a sequential NOESY walk (Fig. 4d), which showed a marked shift at A4 and C5 compared with DNA only (Fig. 4b), was found in combination with cytokine H6/H5 TOCSY (Supplementary Fig. 9) and H1′ × H2′/H2″ TOCSY (Fig. 4c) NMR. At [1c][DNA] ratios of 0.25:1 and 0.5:1 (Supplementary Fig. 10), a gradual disappearance of C5 and A4 aromatic protons of complex-free DNA and the subsequent appearance of new C5 and A4 aromatic protons of 1c-bound DNA in the NOESY spectra were found. Thus, both one-dimensional and 2D NMR results indicate the possible binding of 1c at the mismatched site. Collectively, the more significant the hypochromicity in ultraviolet/visible spectral changes, the higher the amount of heat generation, and the marked shifts of NMR signals at the mismatched site together revealed that 1c preferentially binds to DNA containing CC mismatches. In the emission quenching experiments using [Cu(phen)]2+ (phen = 1,10-phenanthroline), a minor-groove-specific quencher, the emission intensity of 1c in the presence of CC mismatched DNA gradually decreased upon increasing the amount of [Cu(phen)]2+ (Supplementary...
with different adjacent base pairs.

Tris buffer solution upon the addition of different types of DNA. M, match; MM, mismatch. (Fig. 3c) After binding to different concentrations of CC mismatched DNA (0.75 mM) and matched DNA (M), the interaction between CC mismatched DNA and matched DNA was observed. We also examined the emission responses of 1c towards all the CC mismatched DNA sequences.

Table 1 | A comparison of structure–emission response relationships of [Pt(C^N^N)(NHC)]CF₃SO₃ complexes containing N-benzyl groups towards matched and mismatched DNA.

| Complex | 1e | 1f | 1g | 1c | 1h | 1i | 1j |
|---------|----|----|----|----|----|----|----|
| R¹      | -CH₂Ph |
| R²      | -CH₃ | -C₂H₅ | -nC₃H₇ | -nC₄H₉ | -nC₅H₁₁ | -nC₆H₁₃ | -CH₂Ph |
| Iₘ⁺     | 38.9 | 8.7 | 9.3 | 2.6 | 2.3 | 2.8 | 3.2 |
| IₘMM⁺   | 66.2 | 22.2 | 42.0 | 26.4 | 8.2 | 7.0 | 3.5 |

*Iₘ and IₘMM denote the relative emission intensities of the Pt(II) complexes towards matched and mismatched DNA, respectively.

Figure 3 | Binding of 1c with mismatched DNA. (a) The emission spectra of complex 1c (5 µM) in a Tris buffer solution (50 mM NaCl and 2 mM Tris, pH 7.5) after binding to different concentrations of CC mismatched DNA (MM) and matched DNA (M). (b) Plot of integrated ITC data for the exothermic interaction between CC mismatched DNA (0.75 mM) and 1c (0.1 mM). (c) Changes in emission intensity (± s.e.m.) at 535 nm of complex 1c (5 µM) in the Tris buffer solution upon the addition of different types of DNA. M, match; MM, mismatch. (d) Relative emission enhancement of 1c towards CC MM DNA with different adjacent base pairs.

Targeting thermodynamically more stable DNA mismatches. For recognition of the more stable DNA mismatches, an increase in the binding affinity of the Pt(II) complex with DNA is required. The dinuclear analogues of 1c, which contain an N^C^N instead of a C^N^N ligand (Fig. 1), exhibited 4.5-fold higher emission responses towards 1 equivalent of CC mismatched DNA than matched DNA (Supplementary Fig. 12), consistently with the selective probe for CC mismatched DNA.

Fig. 11), indicating the binding of the complex through the minor groove29. Indeed, a favourable binding mode between 1c and CC mismatched DNA has also been identified in molecular docking experiments (Supplementary Fig. 12), consistently with the insertion of the Pt(C^N^N) plane between base pairs and the favourable minor-groove binding interaction of the N₁⁻(n-butyl) and N₃-benzyl moieties. Complex 1d, which contains an N¹^C¹^N instead of a C^N^N ligand (Fig. 1), exhibited 4.5-fold higher emission enhancement (15.0-fold higher than matched DNA). These experiments suggest that 1c is a selective probe for CC mismatched DNA.

The emission responses of 1c towards all other types of DNA mismatches, including CT, AC, TT, AG, AA and GG mismatches, all of which are more thermodynamically stable than CC mismatches30, were tested (Fig. 3c). However, no significant difference in emission responses between these mismatched DNA and matched DNA was observed. We also examined the emission responses of 1c to CC mismatched DNA having 16 different adjacent base pairs (Fig. 3d). Complex 1c displayed higher emission responses to all the CC mismatched DNA sequences than the cognate matched DNA, with adjacent 5′-ACA-3′ revealing the highest emission enhancement (15.0-fold higher than matched DNA). These experiments suggest that 1c is a selective probe for CC mismatched DNA.

Targeting thermodynamically more stable DNA mismatches. For recognition of the more stable DNA mismatches, an increase in the binding affinity of the Pt(II) complex with DNA is required. The dinuclear analogues of 1c, which contain an additional positive charge and a larger molecular dimension, are expected to have higher DNA-binding affinities as a result of stronger ionic bonding interactions and possibly a better geometry to fit into the pocket at the mismatched site. Therefore, we examined the emission responses of a series of doubly charged dinuclear platinum complexes with two Pt^{II}(C^N^N) moieties linked by bridging bis(NHC) or diphasine ligands (Fig. 1) in the presence of hairpin mismatched and matched DNA. Among the dinuclear Pt(II) complexes, [Pt^{II}_2(C^N^N)(μ-dcpm)]^{2+} 2 (Fig. 1; the X-ray crystallographic structure is depicted in
mismatched DNA (Fig. 2). Figure 5a and Supplementary Fig. 15 illustrate that the emission intensity of 2 at λ = 634 nm was elevated 50-fold upon the addition of only 0.5 equivalents of hairpin DNA containing a CC mismatch but enhanced only 8.2-fold in the presence of the same amount of matched DNA, that is, a 6.1-fold difference in emission responses.

The binding affinity of 2 with DNA was examined by ITC. The interaction of 2 was much more exothermic with CC mismatched DNA than with matched DNA (Supplementary Fig. 16). Notably, the K_d of the binding of 2 to CC mismatched DNA was found to be 7.0 ± 2.1 μM, which is 7.4-fold higher than that of 1c. The K_d of the binding of 2 with matched DNA was negligible. In addition, the melting temperature (T_m) of DNA was determined by the change in absorbance (OD_{260nm}) of DNA by increasing 6.8 °C for mismatched DNA in the presence of 2 at an equimolar concentration (Supplementary Fig. 17a). However, only a 0.5 °C increase in melting temperature was found for matched DNA in the presence of 2 (Supplementary Fig. 17b).

Complex 2 also exhibited higher emission responses to CC mismatched DNA containing 16 different adjacent sequences (Fig. 5b), with a nearby 5'-TCT-3' sequence generating the highest fold increase in emission (13.5-fold higher than matched DNA). In addition to its response to CC mismatches, 2 also showed higher emission responses to several other types of mismatched DNA over matched DNA, and the emission increased 4.9-fold for CT, 3.8-fold for AC and 3.4-fold for TT compared with matched DNA but no higher for other mismatches (AG, 1.8-fold; GT, 1.4-fold; AA, 1.4-fold; GG, 0.8-fold; Fig. 5a). Such differences in the emission responses between mismatched and matched DNA partially correlated with the thermodynamic stability of the DNA base pairs, CC ≤ AC ≤ CT < AA ≤ TT < GA ≤ GT < GG [49], suggesting an insertion binding mode [50]. The emission response to AC was slightly lower than that of CT, and the response to AA was lower than that of TT; these observations are not fully consistent with the trend of thermodynamic stability of mismatched DNA. The lower emission response towards A-containing AC and AA mismatches may have been caused by the weak coordination of the N7 of purine in adenine at the mismatch site to the Pt complex, leading to decrease of emission intensity (note that the pyrimidine N3 in C and T is not accessible to the Pt(II) complexes due to steric hindrance). We performed hybrid quantum mechanics/molecular mechanics (QM/MM) calculations to study the mode of binding between 2 and mismatched DNA. As shown in Fig. 5c, 2 bound to mismatched DNA through an insertion binding mode at the minor groove (this result is also consistent with the results of a [Cu(phen)_2]^2+ quenching experiment, Supplementary Fig. 18); the C^N^N planes interacted with nearby GC and AT base pairs by π-stacking interactions (inter-planar distance between the C^N^N plane and the base pairs of ~3.2 Å). The QM/MM simulation also indicated that the dinuclear structure better fit the mismatched sites, which have larger pockets (the inter-planar separation between nearby base pairs at mismatched sites can be >9 Å), but not the matched site, which contains less space between base pairs.

We also tested the emission responses of 2 to dsDNA of poly(dC)-poly(dG), poly(dC-dG)_2, poly(dA)-poly(dT) and poly(dA-dT)_2; single-stranded DNA of oligo(dT)_20 and oligo(dC)_20 dsRNA and bovine serum albumin, all of which were observed to have at least 4.5-fold lower emission intensity than that of CC mismatched DNA (Fig. 5d). Notably, the emission intensity of 2 with RNA containing a CC mismatch was 3.3-fold higher than that with matched RNA (Fig. 5d).

**Photophysical properties upon binding to mismatched DNA.** To better understand the increase in emission upon binding with...
mismatched DNA, we measured the emission spectra of the Pt(II) complex (1c or 2) in buffer solutions containing CC mismatched DNA under air or argon. Under Ar, no significant changes in emission intensity were found compared with that under air conditions (Supplementary Fig. 19). The two Pt(II) complexes were weakly emissive in buffer solutions, but their emission intensities were significantly higher in acetonitrile, even under air (11-fold for 1c, 9-fold for 2, Supplementary Fig. 20a,b; the emission intensity was found to increase with decreasing donor strength of various solvents, as shown in Supplementary Fig. 20c). Thus, the increased emission could not be accounted for by the shielding of oxygen quenching upon mismatched DNA binding to the Pt(II) complexes. In addition, variations of pH from 5 to 11 did not evoke a significant change in emission intensity (Supplementary Fig. 21). We propose that the shielding of quenching by a buffer solution and/or a more ordered structure (Supplementary Fig. 20c). Hence, time-resolved emission spectroscopy could account for the emission enhancement51,52.

We used time-resolved emission spectroscopy to examine the binding between the Pt complexes and DNA. As shown in Supplementary Fig. 22, the emission of 2 in the presence of CC mismatches had a lifetime of 2.3 μs, whereas in the presence of matched DNA, the lifetime is composed of a major portion of short decay (<100 ns) and a small amount of 1.2-μs decay. The difference in emission intensity, $I_{\text{mismatch}}/I_{\text{match}}$, was observed to increase with increasing decay time, from 2.3-fold at t = 0 to 11.8-fold at t = 10 μs (Supplementary Fig. 23). Therefore, time-resolved emission spectroscopy may be a useful strategy to achieve higher sensitivity than steady-state emission spectroscopy for the detection of mismatched DNA using luminescent Pt(II) complexes.

**Targeting abasic DNA.** The abasic (AP) site, in which a purine or a pyrimidine base is missing in a nucleotide, represents another type of thermodynamically unstable DNA defect50. In the presence of a 27-mer dsDNA oligomer containing an abasic site50 (AP_C with a complementary base of C), the emission intensity of an equimolar amount of 1c at $\lambda_{\text{max}} = 535$ nm exhibited a 32.2-fold increase (Supplementary Fig. 24). This elevation in emission was even higher than that observed for the binding of 1c with CC mismatched DNA with similar sequences (26.4-fold). For comparison, only a 4.7-fold increase in emission was detected when 1c was incubated with normal 27-mer dsDNA with a cognate sequence. In the presence of other abasic sites of AP_A, AP_T and AP_G, the emission intensities of 1c were increased by 14.8, 21.1 and 14.4 times, respectively. Complex 2 exhibited a 32.2-fold increase (Supplementary Fig. 24). This property is also found in Rh(III)-based metalloinsertors, in which the metal complex binds to thermodynamically unstable DNA structures through an insertion binding mode50.

**Detection of genomic DNA base pair defects.** The human colorectal carcinoma cell line HCT116 is known to be MMR deficient, owing to mutations in the MMR-associated hMLH1 gene, whereas HCT116 cells containing a transferred, normal chromosome 3 (denoted as HCT116N) restore MMR activity53. We found that 2 showed significantly stronger emission at $\lambda_{\text{max}} = 634$ nm in the presence of genomic DNA isolated from HCT116 cells compared with that from HCT116N cells ($P < 0.01$, n = 3; Fig. 6a; Supplementary Fig. 27). We then measured and
As shown in Fig. 6c–f, after labelling with Pt(II) complexes with cytoplasmic contents, then washed to minimize the fluorescence background due to HCT116N cancer cells were permeabilized by digitonin and to accumulate in cytoplasmic compartments, the HCT116 and microscopy. Because the Pt(II) complexes are lipophilic and tend mismatched DNA in permeabilized cell samples by fluorescence detection of mismatched DNA in cancer cells. (a) Changes in emission intensity (± s.e.m.) of 2 (5 μM) at 634 nm in an aqueous buffer solution (50 mM NaCl and 2 mM Tris, pH 7.5) after binding to genomic DNA extracted from different cancer cell lines. (b) Plot of fold of increased emission versus reported mutation rates in different cancer cell lines, lg (mutation rate); a linear fit is shown. Fluorescence microscopy studies of HCT116 (d) and HCT116N (f) cells pretreated with digitonin and stained with 10 μM 2; (c,e) bright-field images of HCT116 and HCT116N cells, respectively. (g) Plot of emission responses (fold of increase in emission ± s.e.m.) of 2 versus different concentrations of DNA extracted from colon tumour tissue or adjacent normal tissue. All the concentrations of genomic DNA are shown as base pair concentrations.

We next tested whether 2 could be used to selectively detect mismatched DNA in permeabilized cell samples by fluorescence microscopy. Because the Pt(II) complexes are lipophilic and tend to accumulate in cytoplasmic compartments, the HCT116 and HCT116N cancer cells were permeabilized by digitonin and then washed to minimize the fluorescence background due to interactions of the Pt(II) complexes with cytoplasmic contents. As shown in Fig. 6c–f, after labelling with 2, the emission signal of the HCT116 cells was significantly stronger than that of the HCT116N cells. In addition, complex 2 emitted significantly stronger signals in cancerous HCT116 cells compared with those in the immortalized normal human colon mucosal epithelial cell line NCM460 (ref. 55; Supplementary Fig. 28); complex 2 also consistently exhibited significantly lower emission responses in the presence of the DNA isolated from NCM460 cells compared with that from HCT116 cells (Supplementary Fig. 27). The differences in emission intensity were not caused by variations in cellular uptake of Pt(II) complexes as shown by inductively coupled plasma mass spectrometer measurements (Supplementary Fig. 29). For comparison, no significant differences in the emission properties were found between the two cell lines labelled with the general DNA intercalator EB (Supplementary Fig. 30).

We further examined the ability of the Pt(II) complex to detect mismatched DNA of primary human tumours of colon cancer which is frequently characterized by MMR deficiency. DNA samples were extracted from colorectal adenocarcinoma (well to moderately differentiated) and the surrounding normal tissues of a patient (see details in the Methods section). The results of the emission responses of 2 (5 μM) to the DNA from the tumour and normal tissues are shown in Fig. 6g. In the presence of 0.3–1.9 μM of DNA extracted from normal colon tissues, weak emission responses of up to 1.3-fold of the initial emission intensity were detected; in contrast, the emission intensity increased in a concentration-dependent manner in the presence of 0–1.6 μM of DNA extracted from colon tumour tissues, showing up to a 5.5-fold increase in emission at 1.9 μM. Thus, there was a difference between the emission responses of 2 to the DNA of human colon tumour tissue and the normal adjacent tissue.

In conclusion, we have identified two classes of luminescent Pt(II) complexes that can be used for the detection of DNA mismatches and abasic DNA sites. These Pt(II) complexes are able to differentiate between cells with different levels of MMR activity. Importantly, the differential emission responses of the Pt(II) complexes to DNA from human colon cancer tissues and normal colon tissue suggest that the Pt(II) complexes are promising candidates for tumour diagnosis via simple emission spectroscopy measurements of DNA extracts. The selective targeting of DNA mismatches also lends the complexes therapeutic potential for cancer treatment, and they may be used as scaffolds in the design of new anti-cancer agents for the treatment of MMR-deficient cancers.
Metcalfe, C. & Thomas Jim, A. Kinetically inert transition metal complexes that reversibly bind to DNA. Coord. Chem. Rev. 211, 317–351 (2000).

Ma, D.-L., He, H.-Z., Leung, K.-H., Chen, D. S.-H. & Leung, C.-H. Bioactive Luminescent Transition-Metal Complexes for Biomedical Applications. Angew. Chem. Int. Ed. 52, 7666–7682 (2013).

Zeglis, B. M., Pierre, V. C. & Barton, J. K. Metallo-intercalators and metallo-inserters. Chem. Commun. 4565–4579 (2007).

Richards, A. D. & Rodger, A. Synthetic metallocompounds as agents for the control of DNA structure. Chem. Soc. Rev. 36, 471–483 (2007).

Lo, K. K. W. in Photofunctional Transition Metals Complexes. Vol. 123 (ed. Yan, W. V. W.) 204–245 (Springer-Verlag, 2007).

Gao, F., Chao, H. & Ji, L.-N. DNA binding, photo cleavage, and topoisomerase inhibition of functionalized ruthenium(II)-polypyridine complexes. Chem. Rev. 105, 1962–1979 (2005).

Cromans, D. S. Platinum complexes of terpyridine: interaction and reactivity with biomolecules. Coord. Chem. Rev. 253, 1495–1516 (2009).

Komor, A. C. & Barton, J. K. The path for metal complexes to a DNA target. Chem. Commun. 49, 3617–3630 (2013).

Gorlach, A., Ketter, N. & Teulade-Fichou, M.-P. Finding needles in a haystack: recognition of mismatched base pairs in DNA by small molecules. Chem. Soc. Rev. 43, 3630–3665 (2014).

Ma, D.-L., Che, C.-M. & Yan, S.-C. Platinum(II) complexes with dipyrrolophenazine ligands as human telomerase inhibitors and luminescent probes for G-quadruplex DNA. J. Am. Chem. Soc. 131, 1835–1846 (2009).

Murat, P., Singh, Y. & Defrancq, E. Methods for investigating G-quadruplex DNA structure and interactions. Chem. Soc. Rev. 40, 5293–5307 (2011).

Carlson, C. B., Stephens, O. M. & Real, P. A. Recognition of double stranded RNA by proteins and small molecules. Biopolymers 70, 86–102 (2003).

Zou, T. et al. Luminescent cyclometalated palladium(II) complex forms emissive intercalating adducts with double-stranded DNA and RNA: differential emissions and anticancer activities. Angew. Chem. Int. Ed. 53, 10119–10123 (2014).

Pierre, V. C., Kaiser, J. T. & Barton, J. K. Insights into finding a mismatch through the structure of a mispaired DNA bound by a rhodium intercalator. Proc. Natl Acad. Sci. USA 104, 429–434 (2007).

Song, H., Kaiser, J. T. & Barton, J. K. Crystal structure of [(Rh(bpy)2dpz)2]+ bound to mismatched DNA reveals side-by-side metalion-sense interactions. Chem. Sci. 3, 4515–4520 (2012).

Kinsella, T. J. Coordination of DNA mismatch repair and base excision repair processing of chemotherapy and radiation damage for targeting resistant cancers. Clin. Cancer Res. 15, 1853–1859 (2009).

Martin, S. A., Lord, C. J. & Ashworth, A. Therapeutic targeting of the DNA mismatch repair pathway. Clin. Cancer Res. 16, 5107–5113 (2010).

Bridge, G., Rashid, S. & Martin, S. A. DNA mismatch repair and oxidative DNA damage: implications for cancer biology and treatment. Cancers 6, 1597–1614 (2014).

Lothe, R. A. Microsatellite instability in human solid tumors. Mol. Med. Today 3, 61–68 (1997).

Hoeijmakers, J. H. J. Genome maintenance mechanisms for preventing cancer. Nature 411, 366–374 (2001).

Loeb, L. A., Loeb, K. R. & Anderson, J. P. Multiple mutations and cancer. Proc. Natl Acad. Sci. USA 100, 776–781 (2003).

Iyer, R. R., Pluciennik, A., Burdett, V. & Modrich, P. L. DNA mismatch repair: functions and mechanisms. Chem. Rev. 106, 302–323 (2006).

Jackson, B. A., Alekseyev, V. Y. & Barton, J. K. A versatile mismatch recognition agent: specific cleavage of a plasmid DNA at a single base mispair. Biochemistry 48, 4655–4666 (1999).

Zeglis, B. M., Pierre, V. C., Kaiser, J. T. & Barton, J. K. A bulky rhodium complex bound to an adenosine-adenosine DNA mismatch: general architecture of the metalion-sense binding mode. Biochemistry 48, 4247–4253 (2009).

Lim, M. H., Song, H., Ohlson, E. D., Dervan, E. E. & Barton, J. K. Sensitivity of Ru(bpy)2dpz2+ luminescence to DNA defects. Inorg. Chem. 48, 5392–5397 (2009).

McConnell, A. J. et al. Luminescent properties of ruthenium(II) complexes with sterically expansive ligands bound to DNA defects. Inorg. Chem. 51, 12511–12520 (2012).

Chen, H., Yang, P., Yuan, C. & Pu, X. Study on the binding of base-mismatched oligonucleotide d(GCGAGCG) by cobalt(III) complexes. Eur. J. Inorg. Chem. 2005, 3141–3148 (2005).

Fu, C., Harms, K. & Zhang, L. Meggers, E. DNA mismatch recognition by a hexacoordinate silicon sandwich–ruthenium hybrid complex. Organometallics 33, 3219–3222 (2014).

Yang, X. L. et al. Imidazole-imidazole pair as a minor groove recognition motif: Ru(bpy)2dpz2+ mismatched base pairs. Nucleic Acids Res. 27, 4183–4190 (1999).

Nakatani, K., Sando, S., Kumawasa, H., Kikuchi, J. & Saito, I. Recognition of guanine-guanine mismatches by the dimeric form of 2-amino-1,8-naphthyridine. J. Am. Chem. Soc. 123, 12650–12657 (2001).
35. David, A. et al. DNA mismatch-specific base flipping by a bisacidine macrocycle. *ChemBioChem* **4**, 1326–1331 (2003).
36. Chan, K. H., Chen, F. M. & Chou, S. H. Solution structure of the ActD–5′-CCGTTGTG-3′ complex: drug interaction with tandem G:T mismatches and hairpin loop backbone. *Nucleic Acids Res.* **31**, 2622–2629 (2003).
37. Nakatani, K. et al. Small-molecule ligand induces nucleotide flipping in (CAG)n trinucleotide repeats. *Nat. Chem. Biol.* **1**, 39–43 (2005).
38. Ono, A. et al. Specific interactions between silver(I) ions and cytosine-cytosine pairs in DNA duplexes. *Chem. Commun.* **4825–4827** (2008).
39. Jourdan, M., Granzhan, A., Guillot, R., Dumy, P. & Teulade-Fichou, M.-P. Double threading through DNA: NMR structural study of a bis-naphthalene macrocycle bound to a thymine-thymine mismatch. *Nucleic Acids Res.* **40**, 5115–5128 (2012).
40. Takada, T., Ashida, A., Nakamura, M. & Yamana, K. Cationic perylenediimide left-handed twist in CGG triplet repeats. *Nucleic Acids Res.* **40**, 4284–4294 (2013).
41. Lo, Y.-S., Tseng, W.-H., Chuang, C.-Y. & Hou, M.-H. The structural basis of actinomycin D-binding induces nucleotide flipping out, a sharp bend and a left-handed twist in CGG triplet repeats. *Nucleic Acids Res.* **41**, 12287–12295 (2013).
42. Jennette, K. W., Lippard, S. J., Vassiliades, G. A. & Bauer, W. R. Metallointercalation reagents. 2-Hydroxyethanethiolato(2,2′,2″-terpyridine)-platinum(II) monocation binds strongly to DNA by intercalation. *Proc. Natl Acad. Sci. USA* **71**, 3839–3843 (1974).
43. Che, C.-M., Yang, M., Wong, K.-H., Chan, H.-L. & Lam, W. Platinum(II) complexes of dipyridophenazine as metallointercalators for DNA and potent cytotoxic agents against carcinoma cell lines. *Chem. Eur. J.* **5**, 3350–3356 (1999).
44. Wang, P. et al. Specific blocking of CRB/DNA binding by cyclometalated platinum(II) complexes. *Angew. Chem. Int. Ed.* **50**, 2554–2558 (2011).
45. Frezza, M. et al. In vitro and in vivo antitumor activities and DNA binding mode of five coordinated cyclometalated organoplatinum(II) complexes containing biphosphine ligands. *J. Med. Chem.* **54**, 6166–6176 (2011).
46. Sun, R.-Y. et al. Luminescent cyclometalated platinum(II) complexes containing N-Heterocyclic carbene ligands with potent in vitro and in vivo anti-cancer properties accumulate in cytoplasmic structures of cancer cells. *Chem. Sci.* **2**, 728–736 (2011).
47. Zeglis, B. M. & Barton, J. K. A mismatch-selective bifunctional rhodium-oregon green conjugate: a fluorescent probe for mismatched DNA. *J. Am. Chem. Soc.* **128**, 5654–5655 (2006).
48. Cordier, C., Pierre, V. C. & Barton, J. K. Insertion of a bulky rhodium complex into a DNA cytosine-cytosine mismatch: an NMR solution study. *J. Am. Chem. Soc.* **129**, 12287–12295 (2007).
49. SantaLucia, J. & Hicks, D. The thermodynamics of DNA structural motifs. *Annu. Rev. Biophys. Biomol. Struct.* **33**, 415–440 (2004).
50. Zeglis, B. M., Boland, J. A. & Barton, J. K. Targeting abasic sites and single base bulges in DNA with metallointercalators. *J. Am. Chem. Soc.* **130**, 7530–7531 (2008).
51. Tong, G. S.-M. & Che, C.-M. Emissive or nonemissive? A theoretical analysis of the phosphorescence efficiencies of cyclometalated platinum(II) complexes. *Chem. Eur. J.* **15**, 7225–7237 (2009).
52. Lo, K.-K.-W., Choi, A. W.-T. & Law, H.-T. Applications of luminescence inorganic and organometallic transition metal complexes as biomolecular and cellular probes. *Dalton Trans.* **41**, 6021–6047 (2012).
53. Koi, M. et al. Human chromosome 3 corrects mismatch repair deficiency and microsatellite instability and reduces N-Methyl-N-nitro-N-nitosoguanidine tolerance in colon tumor cells with homoygous MLH1 mutation. *Cancer Res.* **54**, 4308–4312 (1994).
54. Glaab, W. E. & Tindall, K. R. Mutation rate at the hprt locus in human cancer cell lines with specific mismatch repair-gene defects. *Carcinogenesis* **18**, 1–8 (1997).
55. Moyer, M., Manzano, L., Merriman, R., Stauffer, J. & Tanzer, L. NCM460, a normal human colon mucosal epithelial cell line. *In Vitro Cell. Dev. Biol. Anim.* **32**, 315–317 (1996).
56. Pierre, V. C., Kaiser, J. T. & Barton, J. K. Insights into finding a mismatch through the structure of a mispaired DNA bound by a rhodium intercalator. *Proc. Natl Acad. Sci. USA* **104**, 429–434 (2007).
57. Neves, M. A. C., Totrov, M. & Abagyan, R. Docking and scoring with ICM: the benchmarking results and strategies for improvement. *J. Comput. Aided Mol. Des.* **26**, 675–686 (2012).
58. Valiev, M. et al. NWChem: A comprehensive and scalable open-source solution for large scale molecular simulations. *Comput. Phys. Commun.* **181**, 1477–1489 (2010).
59. Zhao, Y. & Truhlar, D. G. A new local density functional for main-group thermochemistry, transition metal bonding, thermochemical kinetics, and noncovalent interactions. *J. Chem. Phys.* **125**, 194101 (2006).
60. Harisharan, P. C. & Pople, J. A. The influence of polarization functions on molecular orbital hydrogenation energies. *Theor. Chim. Acta* **28**, 213–222 (1973).
61. Hay, P. J. & Wadt, W. R. Ab initio effective core potentials for molecular calculations. Potentials for K to Au including the outermost core orbitals. *J. Phys. Chem.* **82**, 299–310 (1985).
62. Cornell, W. D. et al. A second generation force field for the simulation of proteins, nucleic acids, and organic molecules. *J. Am. Chem. Soc.* **117**, 5179–5197 (1995).
63. Hambley, T. W. van der Waals radii of Pt(II) and Pd(II) in molecular mechanics models and an analysis of their relevance to the description of axial M −H (−C), M −H (−N), M −S, and M −M (M=Pd(II) or Pt(II)) interactions. *Inorg. Chem.* **37**, 3767–3774 (1998).

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**Author contributions**

T.Z. and C.-M.C. designed the research. S.K.F., T.Z. and C.-N.L and C.-M.C. analysed the data, and S.K.F., T.Z. and C.-M.C. performed the work. The authors declare no competing financial interests.

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