Metal-containing DNA hairpins as hybridization probes†

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A Ru^{II}–based emission, while almost entirely quenched in a Ru^{II}/Os^{II} heterodimetallated DNA hairpin, is dramatically restored upon hybridization to a complementary oligonucleotide, while hybridization to an oligonucleotide that contains a single mismatch results in significantly lower emission intensity.

Luminescent polypyridine transition metal complexes have been gaining interest as molecular probes for bioanalytical applications.[1] Several important features distinguish emissive coordination compounds from their organic counterparts: (a) they are typically chemically inert and photostable, (b) their photophysical characteristics can be tuned while maintaining very similar structural features, (c) their photophysical properties are commonly insensitive to environmental changes (*e.g.* pH, ionic strength), although functional groups can be appended to affect sensing capabilities, (d) they exhibit rather large Stokes' shifts, and (e) their excited states are relatively long-lived, a feature that facilitates their utilization in polarization assays with relatively high molecular weight assemblies. Most of these traits can be attributed to their unique excited-state manifold, where the emission results from a metal-to-ligand-charge-transfer (MLCT) state.[2]

Oligonucleotides containing emissive transition metal complexes have become an important tool for the study of energy- and electron-transfer processes in nucleic acids.[3,4] In an early contribution, we have reported the synthesis of Ru^{II}- and Os^{II}-containing nucleosides **1** and **2** and their corresponding phosphoramidites (Fig. 1).[5] The Ru^{II} nucleoside exhibits a long-lived excited state in phosphate buffer pH 7.0 (τ = 1.08 μs) associated with a relatively high emission quantum efficiency (ϕ = 0.051). In contrast, the Os^{II}-containing nucleoside is quite non-emissive in an aqueous environment (τ = 0.027 μs, ϕ = 1×10^{-4}) and serves as a quencher of the Ru^{II} excited state.[6,7] Since this donor/acceptor interaction is distance-dependent,[5] we have envisioned its application for the detection of DNA hybridization events. In this contribution we demonstrate the utilization of novel metal-containing oligonucleotides as hybridization probes following the molecular beacons principle (Fig. 2).[8]

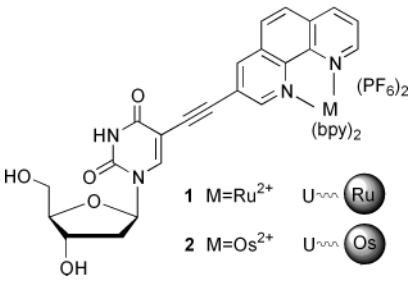

Fig. 1 Metal-containing nucleosides **1** and **2** serve as a donor and an acceptor, respectively.

† Electronic supplementary information (ESI) available: synthesis, enzymatic degradation, composition analysis and thermal denaturation studies of oligonucleotides. See http://www.rsc.org/suppdata/cc/b1/b100036p/

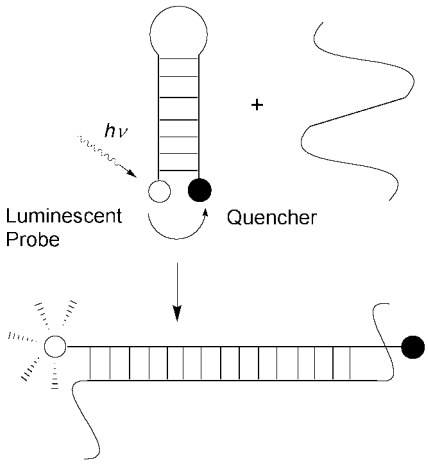

Fig. 2 General principle of molecular beacons technology: the quenched emission of a donor is restored upon hybridization to a target oligonucleotide.

The oligonucleotides used for this study are shown in Fig. 3.‡ The heterodimetallated hairpin **3**, which holds the donor and acceptor in close proximity, possesses a single mismatch in its 8-mer stem and a stable T_4 loop. Upon hybridization to its perfect complementary oligonucleotide **4a**, a 20-mer duplex **3·4a** with an additional six base pairs is formed, while placing the quencher away from the Ru^{II} donor (Fig. 3).§ Thermal denaturation studies confirm the enhanced stability of duplex **3·4a** when compared to the hairpin **3** (Table 1 and Fig. 4).

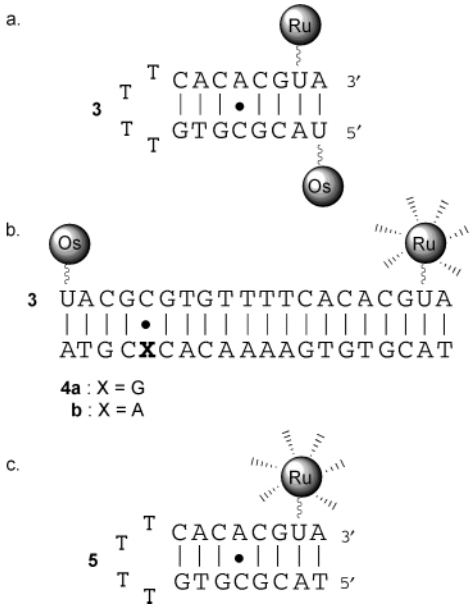

Fig. 3 Oligonucleotides used in this study: (a) Ru^{II}/Os^{II} heterodimetallated hairpin **3**, (b) duplexes **3•4a** and **3·4b** formed upon hybridization of **3** to the perfect (**4a**) and single-mismatch (**4b**) complement, respectively, and (c) a control Ru^{II}-containing hairpin **5**.

Table 1 Thermal denaturation and photophysical data

Sample	T_m/°C[ac]	I_{em} (arbitrary units)[bc]
3	69	5
3·4a	70	89
3·4b	70	38
5	69	100
5·4a	65	99
5·4b	69	98

[a] Melting temperature, ±1 °C. [b] Percent luminescence intensity (with respect to **5**), ±3%, 25 °C. [c] In 10 mM phosphate buffer pH 7.0 with 100 mM NaCl.

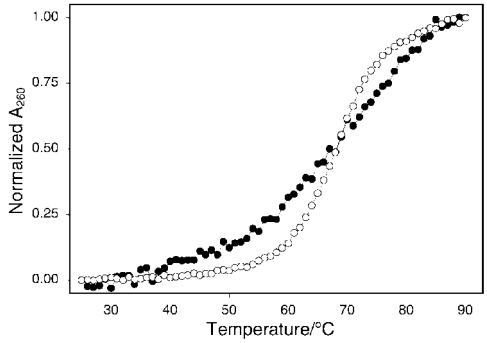

Fig. 4 Thermal denaturation curves of **3** (●) and **3·4a** (○).

Hybridization of hairpin **3** to oligonucleotide **4b** yields a less stable duplex **3·4b** that contains a single AC mismatch (Table 1 and ESI).

Steady-state emission spectra of iso-absorptive oligonucleotide solutions are shown in Fig. 5.§ The Ru-based emission intensity of the dimetallated hairpin **3** increases 17-fold when it hybridizes to its complementary strand to give duplex **3·4a**. The presence of a non-complementary strand **4b**, whose sequence differs from the perfect complement by merely one base, does not fully restore the Ru-based emission of the probe. The emission intensity of duplex **3·4b** is only 43% of the luminescence intensity of the perfect duplex **3·4a**. Hence, the hairpin hybridization probe **3** is able to distinguish between the target strand and a strand that contains a single mutation. We attribute this difference to the less favorable association between the unmatched oligonucleotides that is likely to lead to an equilibrium mixture of the quenched hairpin **3** and emissive

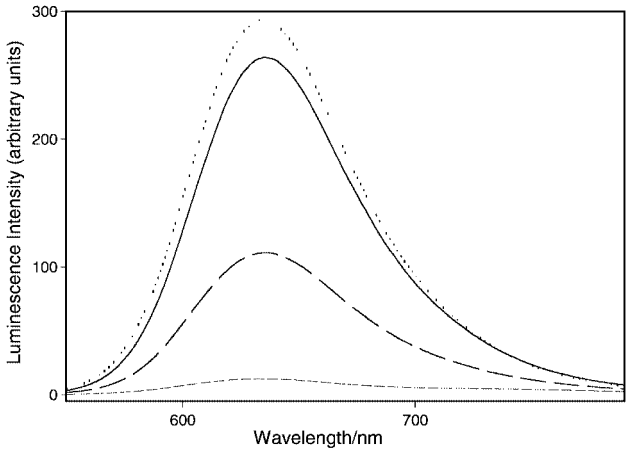

Fig. 5 Steady-state emission spectra of **3** (- - -), **3·4a** (—), **3·4b** (– – –) and **5** (···).

duplex **3·4b**.¶ Importantly, the luminescence of oligonucleotide **5**, the Ru^{II}-only control, does not significantly change in the presence of the complementary strand **4a** or the non-complementary strand **4b** (Table 1).‖

To the best of our knowledge, this is the first example of metallated hairpin oligonucleotides that serve as 'metallo-beacons'. The unique photophysical features of coordination compounds and their chemical compatibility with functional groups found on biomolecules hold great promise for future studies. In particular, advanced designs that include modulated emission and tailored quenchers that can undergo electron-transfer processes can further enhance the sensitivity and dynamic range of metal-based hybridization probes.

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Notes and references

‡ Oligonucleotides were synthesized using the standard solid-phase phosphoramidite chemistry as previously described.[5] Purification was accomplished by preparative polyacrylamide gel electrophoresis and reversed-phase HPLC. The composition of each oligonucleotide was confirmed by enzymatic digestion followed by quantitative HPLC analysis of the resulting nucleosides. See ESI for experimental details.

§ Solutions of the oligonucleotides in 10 mM phosphate buffer, pH 7.0 containing 100 mM NaCl were heated to 90 °C for 5 min and slowly cooled to rt prior to measurements. Emission spectra of degassed solutions were measured upon excitation at 456 nm. The spectra were converted to an energy scale (cm^{-1}) and typically integrated between 520 and 850 nm.

¶ Supporting evidence can be found in the thermal denaturation curves of the corresponding oligonucleotides. See ESI.

‖ It is of interest to mention that the Ru-based emission in the Ru^{II}/Os^{II} perfect duplex **3·4a**, where the metal complexes are separated by 17 base pairs, is quenched by 10% in comparison to the Ru^{II}-only perfect duplex **5·4a**. This is consistent with the anticipated distance-dependent interaction between these coordination compounds.[5]

1 J. R. Lackowicz, *Principles of Fluorescence Spectroscopy*, Kluwer Academic/Plenum Publishers, New York, 1999, pp. 573–594.

2 A. Juris, V. Balzani, F. Barigelletti, S. Campagna, P. Belser and A. Von Zelewsky, *Coord. Chem. Rev.*, 1988, **84**, 85; J.-P. Sauvage, J.-P. Collin, J.-C. Chambron, S. Guillerez, C. Coudret, V. Balzani, F. Barigelletti, L. De Cola and L. Flamigni, *Chem. Rev.*, 1994, **94**, 993; V. Balzani, A. Juris, M. Venturi, S. Campagna and S. Serroni, *Chem. Rev.*, 1996, **96**, 659; F. Barigelletti, L. Flamigni, J.-P. Collin and J.-P. Sauvage, *Chem. Commun.*, 1997, 333.

3 For selected examples, see: C. J. Murphy, M. R. Arkin, Y. Jenkins, N. D. Ghatlia, S. H. Bossmann, N. J. Turro and J. K. Barton, *Science*, 1993, **262**, 1025; T. J. Meade and J. F. Kayyem, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 352; P. J. Dandliker, R. E. Holmlin and J. K. Barton, *Science*, 1997, **275**, 1465; I. Ortmans, S. Content, N. Boutonnet, A. Kirsch-De Mesmaeker, W. Bannwarth, J.-F. Constant, E. Defrancq and J. L'homme, *Chem. Eur. J.*, 1999, **5**, 2712; T. T. Williams, D. T. Odom and J. K. Barton, *J. Am. Chem. Soc.*, 2000, **122**, 9048.

4 For overview articles, see: R. E. Holmlin, P. J. Dandliker and J. K. Barton, *Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 2714; K. E. Erkkila, D. T. Odom and J. K. Barton, *Chem. Rev.*, 1999, **99**, 2777; M. W. Grinstaff, *Angew. Chem., Int. Ed.*, 1999, **38**, 3629.

5 D. J. Hurley and Y. Tor, *J. Am. Chem. Soc.*, 1998, **120**, 2194.

6 D. J. Hurley and Y. Tor, submitted.

7 Polypyridine Ru^{II} and Os^{II} complexes are a well-established couple for the study of energy and electron transfer processes. The transfer of electronic excitation from Ru^{II} to Os^{II} centers is energetically allowed and has been demonstrated in multinuclear systems. See ref. 2 and F. Barigelletti and L. Flamigni, *Chem. Soc. Rev.*, 2000, **29**, 1.

8 S. Tyagi and F. R. Kramer, *Nature Biotech.*, 1996, **14**, 303; S. Tyagi, D. P. Bratu and F. R. Kramer, *Nature Biotech.*, 1998, **16**, 49; L. G. Kostrikis, S. Tyagi, M. M. Mhlanga, D. D. Ho and F. R. Kramer, *Science*, 1998, **279**, 1228; S. A. E. Marras, F. R. Kramer and S. Tyagi, *Genet. Anal-Biomol. Eng.*, 1999, **14**, 151; L. J. Brown, J. Cummins, A. Hamilton and T. Brown, *Chem. Commun.*, 2000, 621.