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DNA Mismatch Detection by Resonance Energy Transfer between Ruthenium(II) and Osmium(II) Tris(2,2′-bipyridyl) Chromophores

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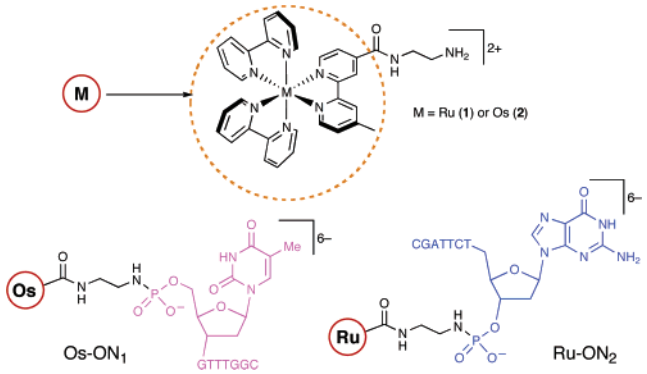
Octahedral tris-chelate complexes $[M^{II}(bpy)_3]^{2+}$ (M = Ru or Os, bpy = 2,2′-bipyridyl), covalently attached to the 3′- and 5′-phosphates of two oligonucleotides, are juxtaposed when hybridized contiguously to a fully complementary DNA target. Visible metal-to-ligand charge-transfer (MLCT) excitation of the $[Ru^{II}(bpy)_3]^{2+}$ unit leads to resonance energy transfer to the MLCT state of the $[Os^{II}(bpy)_3]^{2+}$ moiety, with the energy transfer efficiency depending on the degree of hybridization. The extent of attenuation of the intense red luminescence from the Ru^{II} chromophore hence allows highly sensitive structural probing of the assembly and constitutes a novel approach to DNA sensing which is capable of detecting mutations.

The development of reliable, highly sensitive methods for in situ detection of oligonucleotides (oligos) and other biomacromolecules is of major current interest.[1] DNA sensors are used in medical diagnostics, genetic mutation identification, and gene delivery monitoring. A common approach which avoids the need for long oligo probes is to use split-probes whereby signal generation (e.g., luminescence)[2] follows the simultaneous hybridization to a target of two shorter complementary fragments. Such a strategy has been used, for example, in the chemical labeling of DNA following resonance energy transfer.[3] Split-probe DNA sensors exist based on excimers,[4] exciplexes,[5] and dual fluorescence.[6] Related systems with surface-attached capture probes have been used in optical scanometric,[7] electrochemical,[8] fluorescence,[9] surface plasmon resonance,[10] and diffraction-grating-based[11] DNA detection.

Energy transfer can occur between metal complexes, with energy donors based on $[Ru^{II}(bpy)_3]^{2+}$ (bpy = 2,2′-bipyridyl) and acceptors based on $[Os^{II}(bpy)_3]^{2+}$ or similar species being especially well-studied. Many examples of such systems with covalently linked chromophores are known[12] and energy transfer processes of this type can also occur in supramolecular arrays.[13] Long-range energy transfer between Ru^{II} and Os^{II} polypyridyls in DNA assemblies has been studied, with either intercalating Ru^{II} and Os^{II} 7,8-dimethyl-dipyridophenazine derivatives[14] or 1,10-phenanthroline derivatives covalently attached to complementary oligos.[15]



We report a new split-probe DNA detection method using short-range energy transfer between Ru^{II} and Os^{II} chro-

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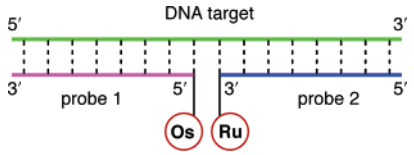

Figure 1. Split-probe system (Os−ON_1)·16-mer·(Ru−ON_2). **Os** and **Ru** represent the $[Os^{II}(bpy)_3]^{2+}$ and $[Ru^{II}(bpy)_3]^{2+}$ units, respectively. DNA target = 5′pdGCCAAACACAGAATCG3′; oligo probe 1 (ON_1) = 5′pdT-GTTTGGC; oligo probe 2 (ON_2) = dCGATTCTG3′p.

mophores (Figure 1). This system offers an attractive combination of visible excitation outside the range of biological absorption with virtually no auto-fluorescence background. In the current study we used a 16-mer target and two 8-mer probes. A split-probe system involving sensitized visible emission from a Tb^{III} complex has been described,[16] and other related lanthanide-based systems exist.[17] However, we are unaware of any examples of such sensors based on energy transfer from $[Ru^{II}(bpy)_3]^{2+}$ or related chromophores, or that use two metals. The precursor complexes **1** and **2** were prepared as described elsewhere,[18] and coupled to the oligos to give Os−ON_1 and Ru−ON_2 by following previously reported procedures.[19]

Under 465 nm metal-to-ligand charge-transfer (MLCT) excitation, a solution of Ru−ON_2 emits at λ_{max} = 646 nm, which is not significantly affected by addition of Os−ON_1, showing that the two partners do not interact in the absence of a DNA target. However, subsequent addition of the 16-mer causes a ca. 28% decrease in the luminescence intensity (Figure 2A), indicating self-assembly of (Os−ON_1)·16-mer·(Ru−ON_2) and concomitant energy transfer. A control experiment with the unmodified ON_1 shows an emission intensity somewhat higher than that from free Ru−ON_2 and much higher than that from (Os−ON_1)·16-mer·(Ru−ON_2). The emission intensity decrease caused by the presence of the Os^{II} unit is ca. 46% when compared with the control.[20] DNA melting temperature profiles with detection at 260 nm for (Os−ON_1)·16-mer·(Ru−ON_2) (Figure S1 in the Supporting Information), (ON_1)·16-mer·(Ru−ON_2), and (ON_1)·16-mer·(ON_2) show sigmoidal transitions, evidencing tandem duplex formation, with respective T_m values of 33, 30, and 28 °C.

In contrast with the perfectly matched system, using the 3′-mismatched target 5′GCCAAACACAGAAT***A***G3′ (Figure 2B) affords a much smaller (ca. 17%) decrease in the 646 nm emission intensity on assembly with Os−ON_1 and Ru−ON_2, indicating some hybridization of Ru−ON_2 with the mismatched target region at 10 °C. The melting profile of this system has transitions at 14 and 36.5 °C (Figure S2). The lower temperature transition is attributable to dissociation of the mismatched Ru−ON_2 probe, whereas the higher temperature transition most likely corresponds with dissociation of the perfectly matched Os−ON_1. Hence, discrimination between perfect match and mismatch can be achieved by changing the temperature so as to almost completely avoid hybridization of the mismatched probe (see below). Perhaps surprisingly, using a 5′-mismatched target (5′pdGCCA***G***A-CACAGAATCG3′) leads to an only very slightly smaller decrease in emission intensity when compared with the perfectly matched system. However, further experiments with 16-mers having double mismatches at their 5′- and 3′-ends (5′G***A***CA***G***ACACAGAATCG3′ and 5′GCCAAACACAG-***G***AT***G***G3′, respectively) show no significant decreases in the 646 nm emission, indicating the absence of any split-probe assemblies (Figure 2C). Indeed, the 5′-doubly mismatched system actually shows a slight *increase* in the Ru-based MLCT emission (Figure S3). Our approach can therefore discriminate double mismatches, even at 10 °C.

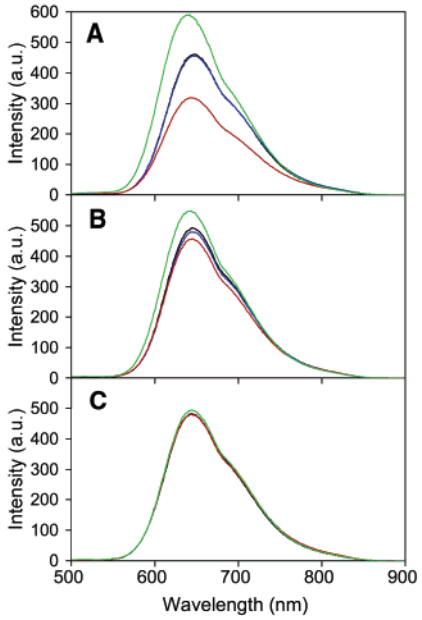

Figure 2. Emission spectra recorded at 10 °C with 465 nm excitation: (A) with the perfectly matched target; (B) with the 3′-mismatched target; and (C) with the 3′-doubly mismatched target. Black = Ru−ON_2; blue = Ru−ON_2 + Os−ON_1; red = (Os−ON_1)·16-mer·(Ru−ON_2); green = control (ON_1)·16-mer·(Ru−ON_2). Experimental conditions: 10 mM Tris, pH 7.0, 0.1 M NaCl.

In light of the melting temperature profiles of the perfect and mismatched systems, we have carried out further experiments at 23 °C, which allows ca. 80% hybridization of the perfectly matched probes and substantially diminishes probe binding in the mismatched region(s). For the perfectly matched (Os−ON_1)·16-mer·(Ru−ON_2), the decrease in emission intensity is considerably smaller at 23 °C (Figure S4; change = ca. 27%) when compared with that observed at 10 °C, presumably due to partial duplex dissociation

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(20) Note that all subsequently quoted emission decreases refer to the relative differences between the full (Os−ON_1)·16-mer·(Ru−ON_2) assembly and the Os-free control.

induced by elevated temperature. However, at 23 °C, the effects of introducing mismatches into the targets also become relatively more pronounced. The decrease in emission intensity observed with the 3′-mismatched target drops to ca. 6% (Figure S5), while that with the 5′ mismatched target is ca. 14% (Figure S6), showing more effective discrimination when compared with the perfect system. As expected at this temperature, emission spectra of the doubly mismatched systems also show no evidence for energy transfer following self-assembly. The results obtained for the split-probe systems with mismatched targets compared with the perfectly matched system are summarized in Figure 3.

This proof-of-principle study demonstrates that DNA split-probe systems based on luminescent MLCT complex chromophores can detect mismatches. We are currently refining this sensitive and selective DNA detection procedure and extending its scope to include other chromophoric complexes and biological targets.

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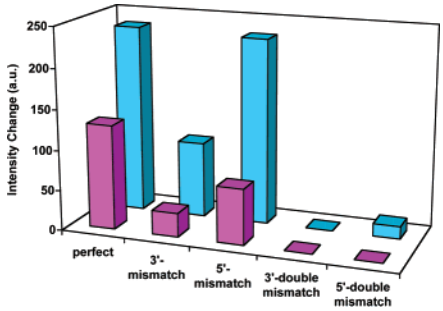

Figure 3. Summary of changes in emission intensities measured at 646 nm between the full (Os−ON_1)·16-mer·(Ru−ON_2) split-probe systems and their Os-free controls. Abbreviations refer to the 16-mer targets. Blue = experiments performed at 10 °C; purple = experiments performed at 23 °C. Note that only the magnitudes of the intensity changes are shown; these correspond with decreases in all cases (with respect to their controls), with the exception of the 5′-doubly mismatched system at 10 °C, for which an increase is observed.

Supporting Information Available: Further experimental details and Figures S1−S6 (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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