APPENDIX: SYNTHESIS OF A LONG-LIFETIME BINARY MOLECULAR BEACON[§]

A1.1: INTRODUCTION

Ruthenium complexes possess long luminescent lifetimes (~ 1 μs). When the Ru complex functions as the donor in a resonance energy transfer (RET) pair, its long lifetime is inherited by the RET acceptor. Here, we describe the synthesis of a long-lifetime molecular beacon consisting of two probes: (1) $Ru(DIP)_2(bpy')^{2+}$ (where bpy′= 4-(3-carboxypropyl)-4′-methyl-2,2′-bipyridine) tethered to the 3′ end of a DNA oligonucleotide and (2) the organic fluorophore Cy5 tethered to the 5′ end of a DNA oligonucleotide. The two strands are complementary to adjacent regions of the target. In the presence of target DNA or RNA, the two fluorophores are brought together and energy transfer between Ru and Cy5 occurs (**Figure A1.1**). Described elsewhere, detection by these probes was studied by time-resolved emission measurements, and the luminescence in the presence of target is temporally well distinguished from the intense, but shorter-lived autofluorescence of cellular media.[1]

A1.2: EXPERIMENTAL PROTOCOLS

A1.2.1: PROBE SEQUENCE

The probe sequences are complementary to a region of *Aplysia californica* sensorin mRNA. A region low in secondary structure was selected as the target for the

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[§] Adapted from the supporting information to Martí, A. A.; Puckett, C. A.; Dyer, J.; Stevens, N.; Jockusch, S.; Ju, J.; Barton, J. K.; Turro, N. J. Inorganic-organic hybrid luminescent binary probe for DNA detection based on spin-forbidden resonance energy transfer. *J. Am. Chem. Soc.* **2007,** *129,* 8680–8681.

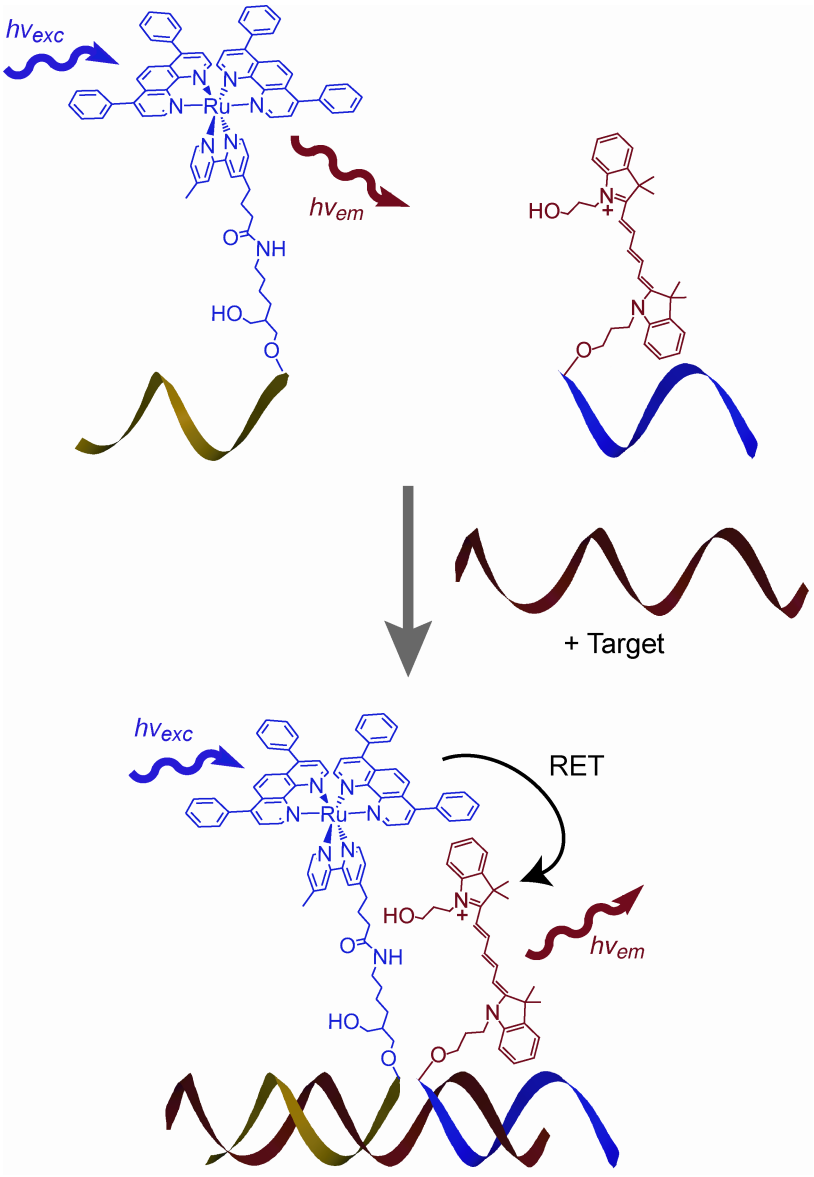

Figure A1.1: Detection of DNA by a binary molecular beacon. When the probes are free in solution, only emission from the ruthenium complex is observed. In the presence of target, $Ru(DIP)_2(bpy')^{2+}$ and Cy5 are brought into close proximity, a condition favorable for resonance energy transfer (RET), and mainly Cy5 emission is observed.

binary probe based on the modeled secondary structure. The modeling details have been reported elsewhere.[2]

Ru-probe: 5′-AAG TTG ATC AAG TTG GT-($Ru(DIP)_2(bpy')^{2+}$)-3′

Cy5-Probe-1: 5′-Cy5-TAT GTT TCA CTG GAT GA-3′

Cy5-Probe-2: 5′-Cy5-ATG TTT CAC TGG ATG A-3′

Cy5-Probe-3: 5′-Cy5-TTC ACT GGA TGA-3′

Target: 5′-TCA TCC AGT GAA ACA TAC AGC ACC AAC TTG ATC AAC TT-3′

A1.2.2: PROBE SYNTHESIS

A1.2.2.1: SYNTHESIS OF $[Ru(DIP)_2(bpy')]Cl_2$

$Ru(DIP)_2Cl_2$ was synthesized in analogous fashion to the published synthesis of $Ru(bpy)_2Cl_2$.[3] $Ru(DIP)_2(bpy')^{2+}$ was prepared by refluxing 41 mg of $Ru(DIP)_2Cl_2$ (49 μmol) and 16.4 mg (64 μmol) of 4-(3-carboxypropyl)-4′-methyl-2,2′-bipyridine (prepared according to the published procedure)[4] in 10 mL of 1:1 ethanol:water for 3 h. The mixture was cooled to ambient temperature and the ethanol removed *in vacuo*. The solution was diluted with water (20 mL) and filtered. The complex was precipitated as the PF_6^- salt by addition of NH_4PF_6, then returned to the Cl^- salt using a Sephadex DEAE anion exchange column. ESI-MS (cation): 511.3 *m/z* (M^{2+}) obsd, 511.1 *m/z* (M^{2+}) calcd. $\varepsilon_{440} = 35{,}200\ M^{-1}\ cm^{-1}$ in water, as determined by inductively coupled plasma mass spectrometry (ICP-MS) measurements.

A1.2.2.2: SYNTHESIS OF THE RU-PROBE

$Ru(DIP)_2(bpy')^{2+}$ was tethered to the 3′-end of DNA by first coupling the complex to amine-modified beads, followed by DNA synthesis and cleavage of the Ru-DNA conjugate from the beads (**Figure A1.2**).[5] The Fmoc group was removed from 3′-amino-modifier C7 CPG 500 beads (Glen Research) by incubation with 20% piperidine in DMF for 15 min. The beads were rinsed with DMF and CH_3CN, dried *in vacuo*, then placed under $Ar_{(g)}$. To the beads (2 μmol), $[Ru(DIP)_2(bpy')]Cl_2$ (4.5 mg, 4 μmol), HBTU (1.5 mg, 4 μmol), HOBT (0.6 mg, 4 μmol), and DIEA (2 μL, 12 μmol) in anhydrous DMF (1.5 mL) were added. The reaction mixture was shaken for 30 min at ambient temperature. The beads were rinsed with DMF, CH_3CN, and CH_2Cl_2, then divided into two aliquots and transferred into two DNA synthesis columns. DNA was synthesized using an ABI 3400 DNA synthesizer. The DNA was cleaved from the beads and deprotected with conc. NH_4OH (2 h at ambient temperature, 6 h at 60 °C). The Ru-DNA conjugate was purified by HPLC using a gradient of 5:95 to 65:35 (acetonitrile:50 mM ammonium acetate) over 30 min. The DMT was removed with 80% acetic acid for 15 min, followed by addition of ethanol, and removal of solvent *in vacuo*. The Ru-DNA conjugate was purified once more by HPLC. MALDI-TOF: 6473 *m/z* (M^+) obsd, 6477 *m/z* (M^+) calcd.

A2.2.2.3: SYNTHESIS OF THE CY5-PROBES

DNA was synthesized using ‘ultramild’ reagents with Cy5 was added at the 5′-end, using a Cy5 phosphoramidite (Glen Research). The MMT group was removed by

the DNA synthesizer. The DNA was cleaved and deprotected with 0.05 M potassium carbonate in methanol for 4 h at ambient temperature. To the supernatant, 1.5 equivalents by volume 2.0 M TEAA were added. The solution was concentrated *in vacuo* and desalted using a Nap10 column (GE Healthcare), eluting with water. The Cy5-DNA conjugate was purified by HPLC using a gradient of 5:95 to 65:35 (acetonitrile:50 mM ammonium acetate) over 30 min. MALDI-TOF: Cy5-Probe-1, 5748 *m/z* (M^+) obsd, 5749 *m/z* (M^+) calcd; Cy5-Probe-2, 5442 *m/z* (M^+) obsd, 5445 *m/z* (M^+) calcd; Cy5-Probe-3, 4193 *m/z* (M^+) obsd, 4194 *m/z* (M^+) calcd.

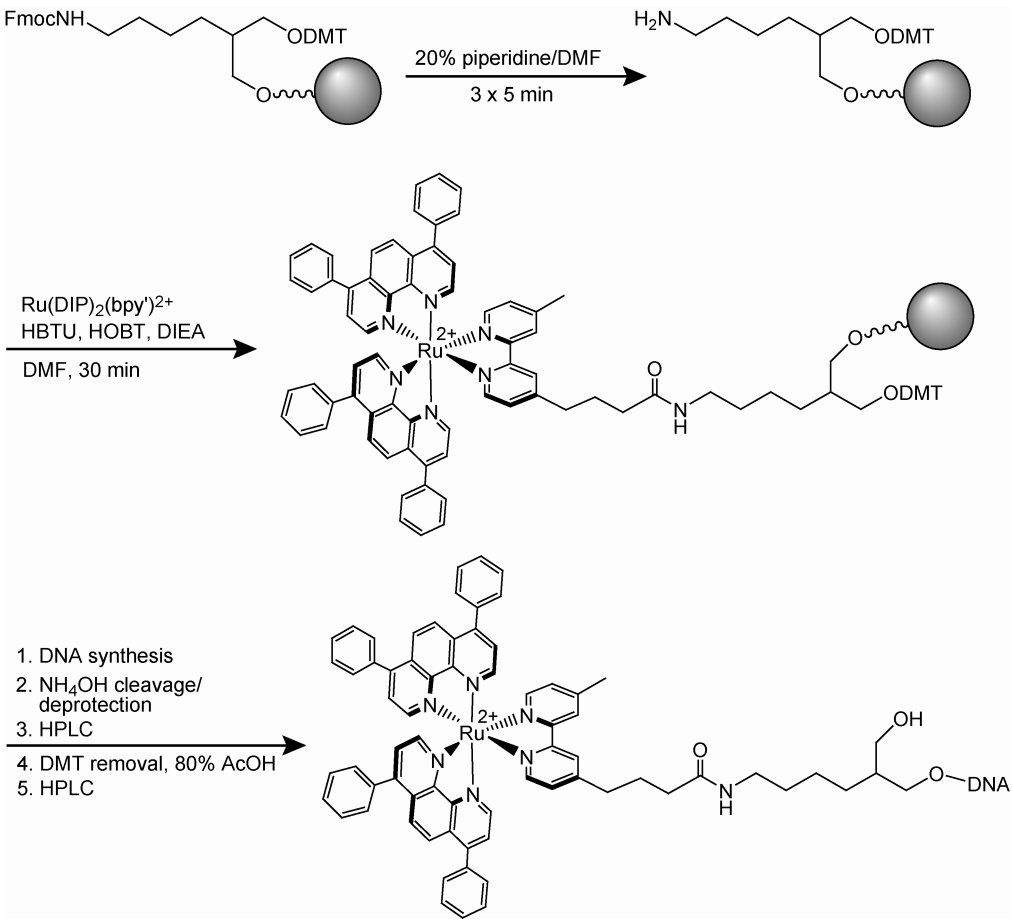

Figure A1.2: Synthesis of the Ru-probe.

A1.3: REFERENCES

1. Martí, A. A.; Puckett, C. A.; Dyer, J.; Stevens, N.; Jockusch, S.; Ju, J.; Barton, J. K.; Turro, N. J. *J. Am. Chem. Soc.* **2007,** *129,* 8680–8681.
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