DNA Superstructures

Self-Assembly of Cyclic Metal–DNA Nanostructures using Ruthenium Tris(bipyridine)-Branched Oligonucleotides**

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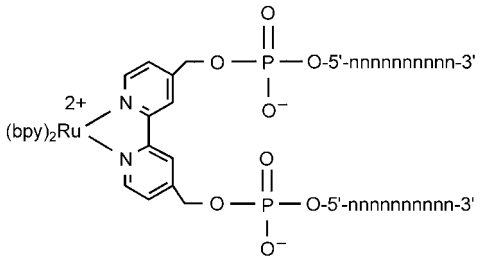
One of the promises of nanoscience is the creation of ordered structures that contain addressable molecular components, which are designed to accomplish complex operations.[1] However, whereas many functional molecular components have already been constructed, methods to assemble them in a deliberately designed manner on the nanometer scale have yet to be devised.[1,2] In this respect, DNA has emerged as a promising template to accomplish this task because of its uniquely selective self-assembly, ready programmability, and facile synthesis.[3–5] In principle, short (10–30-bases long) strands of DNA can rapidly self-assemble into relatively rigid, programmable, higher-order DNA structures.[5] Pioneering work by Seeman demonstrated the use of modified Holliday junctions as oligonucleotide-based vertices in the assembly of complex two- and three-dimensional nanostructures.[3a–c] DNA nanostructures have also been constructed[3] through the use of oligonucleotide–gold colloidal particles,[3d–f] biotin–avidin interactions,[3g] guanine quartets,[3h,i] and organic vertices.[3j–l]

We are interested in the creation of cyclic transition-metal–DNA nanostructures that contain short DNA duplexes as arms and transition-metal centers as vertices. In these well-defined supramolecular structures, DNA serves as a nanoscale rigid molecule to spatially position addressable transition metals, which have intrinsic properties such as luminescence and redox activity,[6] into an ordered array. Whereas transition metals have been used to generate 3D DNA networks,[4a–c] linear DNA arrays,[4d] and metalated DNA,[4g–h] to our knowledge, discrete cyclic metal–DNA structures have not been previously accessed.[4] Herein, we report the synthesis and properties of a branched ruthenium(II)–DNA complex, in which two parallel DNA strands are linked to a relatively rigid Ru^{II} tris(bipyridine) center.[9] Self-assembly of this molecule leads to the formation of a discrete metal–DNA cyclic nanostructure, which contains two DNA duplexes and



1: n=DNA nucleotide

two redox- and photoactive $[Ru(bpy)_3]^{2+}$ centers (bpy = bipyridine). Furthermore, we show that branched oligonucleotides based on the non-metalated ligand undergo less-selective association, which illustrates the role of the transition metal in this self-assembly process.

In a preliminary report,[10] we described the synthesis of an oligonucleotide-branched transition-metal complex through a convergent, solid-phase approach. In its initial design, a ruthenium(II) center was linked to two $d[T_{10}]$ DNA strands through flexible six-carbon spacers and two monodentate imidazole ligands. To better control the self-assembly of these complexes, we needed to expand this solid-phase strategy to access relatively rigid, branched DNA complexes with mixed DNA sequences.[11] The vertex used here was based on the complex, *cis*-$[Ru(bpy)_2$(4,4′-bis(hydroxymethyl)-2,2′-bipyridine)$][PF_6]_2$ (**2**, see Scheme 1),[8] which exhibits both luminescence and redox activity. Examination of a number of X-ray crystal structures reported for related complexes indicates that the ruthenium center should orient the 4,4′-carbon centers at an angle of $\approx 70°$ (C···Ru···C), whereas in the free ligand the carbon centers are directed in a transoidal fashion.[9] Complex **2** was first converted into the bis(phosphoramidite) derivative **3** by reaction with chloro(cyanoethyl)-*N*,*N*-diisopropylphosphoramidite.[7] In parallel, a number of DNA sequences were synthesized on a high-density controlled-pore glass (CPG, 56–64 $\mu mol\,g^{-1}$) support (Scheme 1). After the removal of the protecting dimethoxytrityl groups from the DNA sequences, the ruthenium complex **3** was added to the oligonucleotide-functionalized CPG support. After oxidation, the DNA-linked Ru complexes were then cleaved from the support.[7]

The products of this convergent synthesis were isolated by using denaturing polyacrylamide gel electrophoresis (PAGE), and their structures were analyzed by using PAGE, anion-exchange HPLC, and MALDI-TOF mass spectrometry. The fastest moving band in the gel was identified as unmodified DNA, and the next band was identified as the mono(DNA)-functionalized Ru complex **4** (Scheme 1), which resulted from the coupling of the ruthenium vertex **3** to only one oligonucleotide strand on the solid support. The slowest moving species was confirmed as the desired DNA-branched ruthenium conjugate **1** by MALDI-TOF MS. In parallel to the

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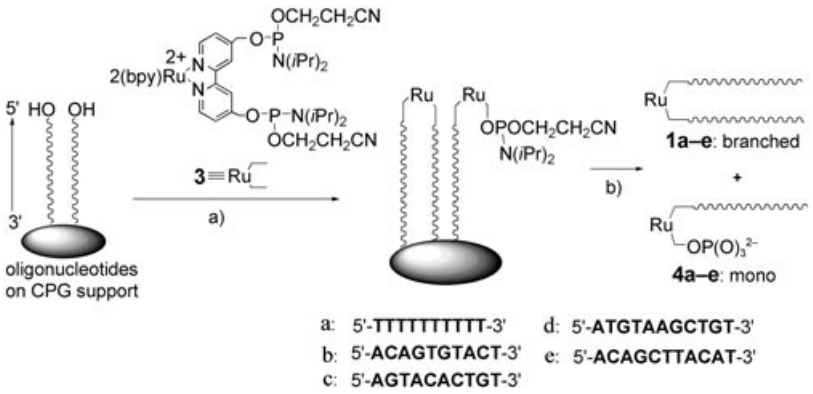


Scheme 1. Convergent synthesis of the mono- and branched oligonucleotide–Ru(II) complexes; a) tetrazole, room temperature, 2 h; b) I_2, pyridine, H_2O, then conc. NH_4OH, 12 h, 55 °C.

generation of the ruthenium–DNA conjugates, DNA-branched bipyridine conjugates **5d–e** were also prepared by a similar solid-phase convergent strategy and were isolated and purified by denaturing PAGE (Figure 1).[7] The UV/Vis absorption spectra of the Ru–DNA conjugates **1** exhibited transitions at $\lambda =$ 260 nm which are characteristic of the heterocyclic DNA bases. The bipyridine π–π^* and metal-to-ligand charge-transfer (MLCT) transitions were observed at $\lambda \approx 280$ and 454 nm, respectively.[7] Upon irradiation at $\lambda = 454$ nm, the fluorescence spectra of the ruthenium–oligonucleotide conjugates **1** showed an emission peak at $\lambda = 615$ nm which was identical to that of the unconjugated Ru complex **2** (Figure 2c).[7] Thus, the fluorescence properties of the ruthenium–bipyridine species are retained in the DNA-branched Ru conjugates.

Because of the branched architecture of the Ru–DNA complexes **1**, it was important to assess the propensity of the DNA arms to form stable duplexes upon hybridization with their complementary partners. Thermal denaturation experiments of complex **1b** in the presence of two equivalents of the complementary oligonucleotide **c** showed the conventional sigmoidal melting-temper-

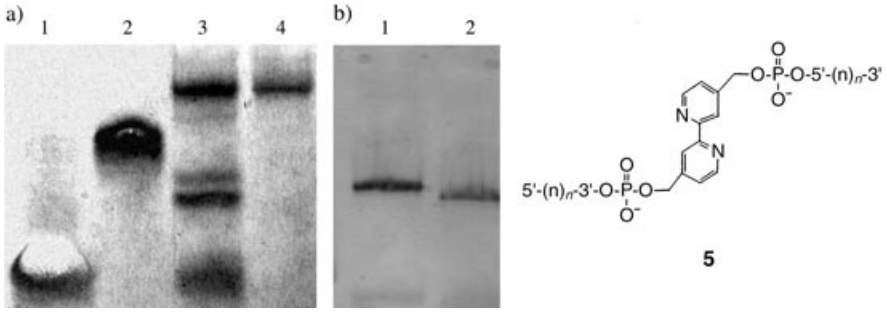


Figure 1. a) Denaturing with 24% PAGE: lane 1) 11-mer control DNA, d; lane 2) 21-mer control DNA; lane 3) crude mixture of **1d**; lane 4) $[Ru(bpy)_3]^{2+}$–bis(DNA) **1d.** b) Denaturing with 24% PAGE: lane 1) bpy–bis(DNA) **5d**; lane 2) bpy–bis(DNA) **5e**. n = DNA nucleotide.

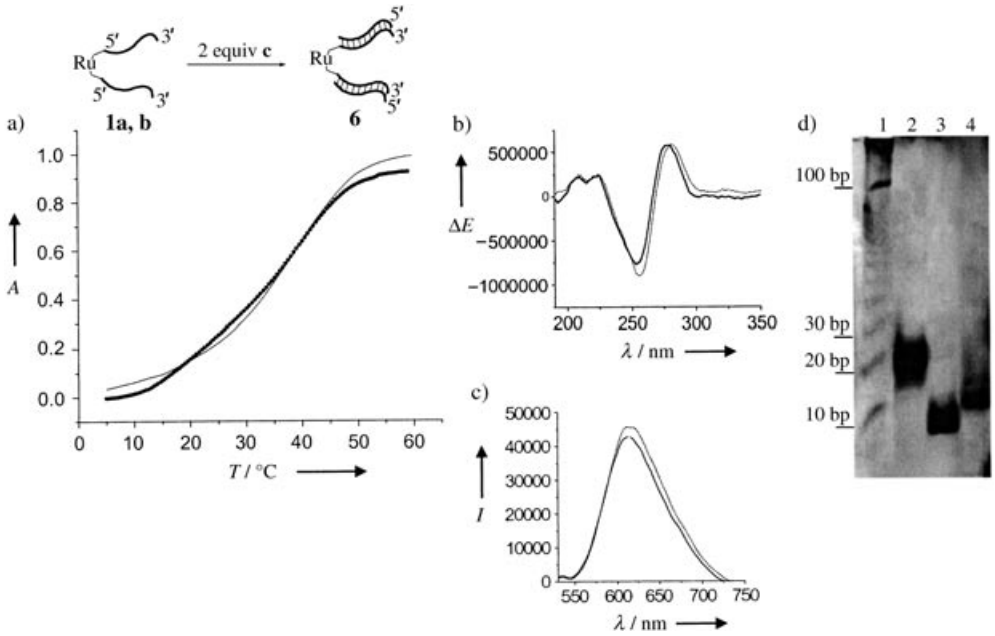
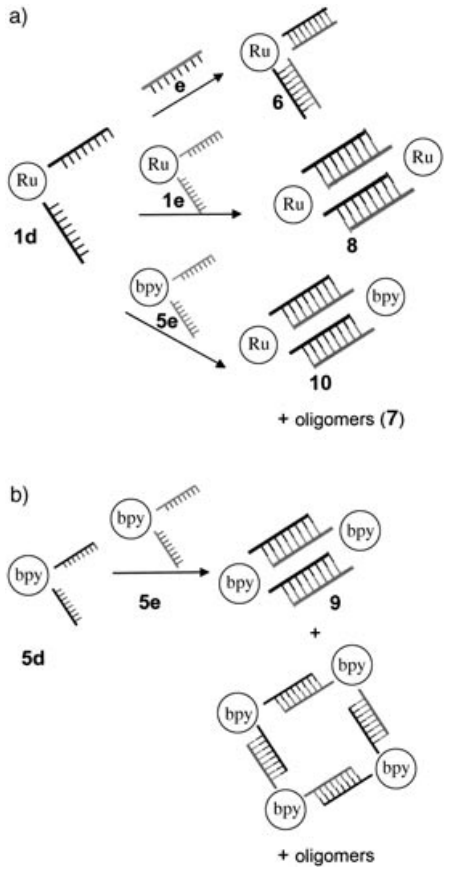


Figure 2. a) Thermal denaturation curves for complex **1**: relative absorbances at $\lambda = 260$ nm of the control duplex **b**–**c** (——) and the oligonucleotide-branched ruthenium complex **1b** with two equivalents of the single-stranded oligonucleotide **c** (•••••). b) CD spectra of the oligonucleotide-branched ruthenium complex **1b** hybridized to 2 equivalents of the complementary oligonucleotide **c** (——) and the control duplex, **b**–**c** (-----) in TMS (Tris, 50mM; $MgCl_2$, 10mM; NaCl, 100 mM; pH 8) buffer solutions. c) Steady-state emission spectra of the conjugate **1b** (-----) and **1b** hybridized with 2 equivalents of complement **c** (——) in TMS buffer solutions at 5 °C. d) Native PAGE image (TMS buffer): lane 1) 10–330 base-pair ladder; lane 2) Ru–bis(duplex) **6**; lane 3) control duplex **d**–**e**; lane 4) oligonucleotide-branched ruthenium complex **1d**. bp = base-pair.

ature curves for a DNA duplex (Figure 2a).[7] Interestingly, a slight increase (1–4 °C) in the melting temperature was observed for the oligonucleotides in the Ru–DNA conjugates **1** relative to the melting temperature of the Watson–Crick control duplex.[5] Thermal denaturation experiments were also carried out for the mono(DNA)-functionalized Ru complexes **4**, which do not possess a branched architecture. These complexes exhibited a similar enhancement in duplex stability (melting temperatures were increased by 4 °C) which indicates that the positively charged Ru center is likely responsible for this effect in both the mono- and the branched DNA–Ru^{II} complexes.[7] Further evidence for the formation of a B-DNA duplex was obtained by circular dichroism (CD) spectroscopy (Figure 2b).[12]

Figure 2d shows an image from the native PAGE analysis of the hybridization product of complex **1d** with two equivalents of a complementary DNA, **e** (Scheme 2). This



Scheme 2. a) Discrete $[Ru(bpy)_3]^{2+}$–DNA nanostructures through the self-assembly of **1,** and b) discrete bpy–DNA nanostructures from **5**.

gives rise to a band with electrophoretic mobility similar to that of a duplex that contains 40 nucleotides in the molecular-weight marker ladder. This band can thus be assigned to the Ru–bis(duplex) conjugate **6** (that contains 44 bases, Scheme 2).[13] Molecular modeling studies of complex **6** show no apparent steric barrier to the formation of two duplex arms at the 4,4′-positions of the bipyridine ligand.[7] Ru–bis(duplex) **6** was examined by fluorescence spectroscopy; upon excitation at 454 nm, an emission at $\lambda = 616$ nm was observed which showed a $\approx 6\%$ decrease in its intensity relative to that of the unhybridized DNA–Ru complex **1** (Figure 2c).[14] Thus, upon hybridization with their complementary partners, the branched Ru^{II} complexes form stable bis(duplex) systems and retain their fluorescence properties.

In light of the ability of complexes **1** to form stable duplexes, we explored the self-assembly of two complementary Ru^{II}-branched oligonucleotides. In principle, this association can result in either discrete cyclic structures or linear oligomeric/polymeric species.[15] We anticipated that the relative rigidity of the ruthenium vertex in complexes **1** would result in a more efficient formation of cyclic products. Self-assembly was first examined by heating equimolar solutions of the DNA-branched Ru complex **1d** and its complement **1e** to 90 °C, followed by slow cooling of the mixture to 4 °C (1.8 °C min^{-1}, 12 h). PAGE analysis revealed the formation of products of extremely slow mobility which correspond to at least 330 base-pairs for the molecular-weight marker (Scheme 2, Figure 3a). This points to the formation of

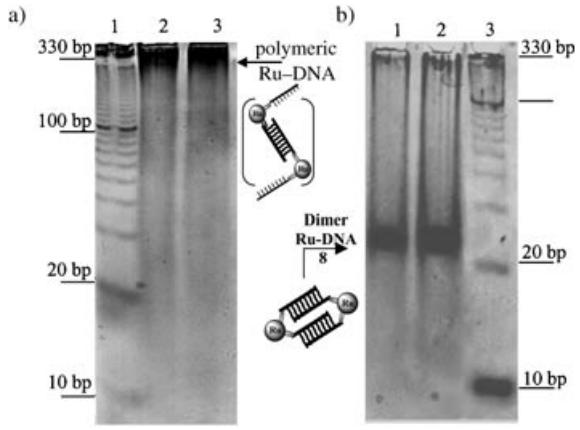


Figure 3. Native PAGE analyses: a) Hybridization from 90 to 4 °C overnight: lane 1) 10–330 base-pair ladder; lane 2) solution of **1d–1e** (20 μM) in TMS; lane 3) solution of **1d–1e** (40 μM) in TMS. b) Hybridization at 4 °C overnight: lane 1) solution of **1d–1e** (40 μM) in TMS; lane 2) solution of **1d–1e** (20 μM) in TMS; lane 3) 10–330 base-pair ladder.

linear polymeric DNA–Ru species **7** (Figure 3a). Interestingly, when the self-assembly of **1d** with **1e** was carried out under milder conditions (4 °C, 12 h), one major discrete product formed along with small amounts of polymeric species. The mobility of this Ru–DNA structure corresponds to a 44-base molecular-weight marker and is consistent with the formation of a dimer, **8**, which contains two DNA duplexes (44 nucleotides) and two $[Ru(bpy)_3]^{2+}$ vertices (Scheme 2, Figure 3b). To determine whether this dimer was an open, linear product or a cyclic molecule, enzymatic digestion with Mung Bean Nuclease was conducted.[16] This enzyme is selective to the degradation of single-stranded DNA. It is thus expected to degrade open, oligomeric species that contain single DNA strands, but to leave closed, cyclic species that contain double DNA strands intact. Under conditions that cause the degradation of single-stranded

DNA, the dimer **8** remained unmodified by the enzyme.[7] Thus, the self-assembly of the DNA-branched Ru complexes **1** generates a discrete metal–DNA cyclic nanostructure.[17]

To probe the role that the transition-metal ion plays in the self-assembly process, a hybridization experiment was carried out with the unmetalated bipyridine–bis(DNA) conjugates **5**. The DNA-branched bipyridine molecules **5** are expected to show a higher flexibility than the DNA-branched Ru complexes **1** and a twist of the two DNA strands into a transoidal arrangement.[9] Samples **5d** and **5e** were combined under similar hybridization conditions (4 °C, 12 h, Scheme 2, Figure 4). In addition to a band that was assigned to a dimeric

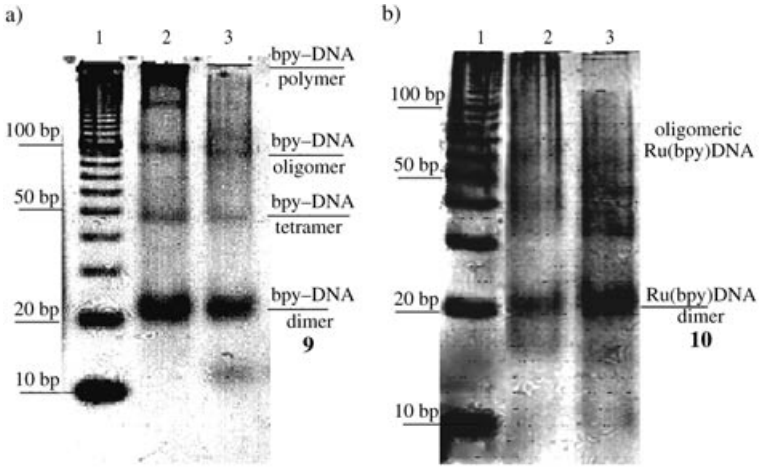


Figure 4. Native PAGE analyses: a) Hybridization with unmetalated-bipyridine–DNA conjugates **5** at 4 °C overnight: lane 1) 10–330 base-pair ladder; lane 2) solution of **5d–5e** (20 μM) in TMS; lane 3) solution of **5d–5e** (20 μM) in TMS with added enzyme. b) Hybridization at 4 °C overnight of oligonucleotides from the metalated complex **1** with those of the unmetalated conjugates **5**: lane 1) 10–330 base-pair ladder; lane 2) solution of **5d–1e** (20 μM) in TMS; lane 3) solution of **5d–1e** (20 μM) in TMS solution with added enzyme.

species **9**, which contains two bipyridine moieties and two DNA duplexes, several bipyridine assemblies that ranged from tetramers to significant amounts of higher-order oligomers/polymers were observed (Figure 4, Scheme 2). Enzymatic digestion of the hybridized products did not affect the discrete bands that correspond to the dimer and tetramer, which indicates that they therefore correspond to cyclic products. These results are in contrast to the behavior of the DNA-branched Ru complexes **1,** which form the dimer **8** as the sole cyclic product, and illustrate the active role of the transition-metal ion in the self-assembly of these higher-order DNA structures.

Finally, to create hybrid DNA structures that contain both $[Ru(bpy)_3]^{2+}$ units and unfunctionalized bipyridine vertices, the self-assembly of the DNA-branched Ru complex **1d** with the DNA-branched bipyridine **5e** was examined (4 °C, 12 h, Scheme 2). This led to the formation of a cyclic dimer **10**, which contains two DNA duplexes and one ruthenium–bipyridine and one bipyridine vertex, along with higher molecular-weight oligomeric species (Figure 4, Scheme 2). The cyclic nature of the dimer **10** was also confirmed by enzymatic digestion with Mung Bean Nuclease. As they contain a bipyridine ligand as one of their vertices, structures **9** and **10** can potentially be further functionalized with other transition-metal ions (such as Cu^+, Ag^+, and Zn^{2+}), and thus they have the potential to form hybrid multimetallic DNA nanostructures and networks.[4]

In conclusion, we have demonstrated the solid-phase, convergent synthesis of DNA-branched Ru^{II} complexes **1** and their self-assembly into the first cyclic metal–DNA nanostructures.[18] These supramolecular structures contain two DNA duplex arms and two relatively rigid photo- and electroactive $[Ru(bpy)_3]^{2+}$ vertices. We have also shown that branched DNA complexes with unmetalated bipyridine vertices undergo less-selective self-assembly, which illustrates the role of the transition metal in this process. The transition-metal units in these nanostructures are readily addressable by means of light or electrical energy. Thus, this study represents a new method to use the selective association of DNA to organize functional molecular components on the nanometer scale. Efforts towards understanding the factors that govern the self-assembly process, the isolation of these cyclic structures and the study of their properties, as well as scanning probe microscopy experiments are currently underway.

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[12] The product from the hybridization of complex **1b** with 2 equivalents of complementary DNA exhibits a characteristic CD spectrum of a B-DNA: a negative band at $\lambda = 253$ nm, a crossover point at $\lambda = 265$ nm, and a positive band at $\lambda = 277$ nm (Figure 2b, solid line). This is very similar to the CD spectrum of the control duplex, **b**–**c**, which exhibits a negative band at $\lambda = 255$ nm, a crossover point at $\lambda = 267$ nm, and a positive band at $\lambda = 280$ nm (Figure 2b, dashed line); N. Berova, K. Nakanishi, R. W. Woody, *Circular Dichroism: Principles and Applications*, *2nd Ed.*, Wiley-VCH, Weinheim, **2000**.

[13] The products of the hybridization of the mono(DNA)-functionalized Ru complexes **4** with their single-stranded DNA complements are observed to have similar electrophoretic mobility to that of a control duplex. Thus, the positive charge of the Ru center does not appear to affect the migration of the Ru–DNA samples.

[14] Please see the Supporting Information for changes in the fluorescence spectra of the Ru–bis(homopolymeric) sequence, **1a**, upon hybridization to complementary DNA. The results show a 10% decrease in fluorescence intensity upon hybridization.

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[17] See Supporting Information for results of molecular modeling studies of **8**. The local energy minimum for **8** was obtained using the AMBER force field method.

[18] **Note added in Proof:** After acceptance of this manuscript, a communication about the synthesis of DNA triangles with vertices of iron(II) bis(terpyridine) complexes has appeared: J. S. Choi, C. W. Kang, J. W. Yang, Y.-G. Kim, H. Han, *J. Am. Chem. Soc.* **2004**, *126*, 8606 – 8607.

