Electronic Supplementary Information

Recognition of Guanines at a Double Helix-Coil Junction in DNA by a Trinuclear Copper Complex

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Materials. Oligonucleotides containing 7-deazaguanosine (dzG) were kindly synthesized by Dr. J. G. Muller and Prof. C. J. Burrows at the University of Utah. Other oligonucleotides were purchased from Invitrogen and purified by 20% denaturing polyacrylamide gel electrophoresis (acrylamide-bisacrylamide 19:1, 7 M urea). DNA concentrations were determined from their absorbance at 260 nm and their estimated extinction coefficients [www.idtdna.com/SciTools/SciTools.aspx]. 3-Mercaptopropionic acid (MPA) and diethyl dithiocarbamic acid (DDTC) were purchased from Sigma-Aldrich. **L** and complex **1** were prepared as described previously [K. J. Humphreys, K. D. Karlin, S. E. Rokita, J. Am. Chem. Soc,. 2002, 124, 8055-8066]. Unless indicated, all other chemicals were obtained from commercial sources.

Synthesis of ligand *L'*. Preparation of **L'** followed a protocol similar to that developed for a related ligand [Hofmann et al., *Dalton Trans.,* **2003**, 2979-2985]. To 2-(chloromethyl)pyridinium hydrochloride (1.97 grams, 12 mmol) in 0.5 mL of water was added 3 mL of 20% aq. NaOH while stirring under N_2. To the resulting red solution was added 307 mg (3 mmol) of 1, 5-pentanediamine, 3 mL of 20% aq. NaOH and 20 mg of hexadecyltrimethylammonium chloride. This mixture was stirred vigorously at room temperature for 24 hours whereupon the solution turned red-gray. Extraction with CH_2Cl_2 (3 X 20 mL) was followed by washing with 20 mL of H_2O and the organic layer was dried over Na_2SO_4. The solvent was evaporated and a red-brown oil (ligand) was obtained. The desired product was purified by column chromatography (Al_2O_3) ,and the first fraction was collected as a red oil in 45% yield (630 mg). R_f = 0.43 (CH_2Cl_2:EtOAc = 1:1) ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.50 (4H, d), 7.52 - 7.64 (8H, m), 7.13 (4H, dd), 3.83 (8H, s), 2.55 (4H, m), 1.50 (4H, m), 1.21 (2H, m).

Synthesis of the copper complex of *L'* ($[Cu_2(\mathbf{L'})(H_2O)_4](ClO_4)_4 \cdot H_2O$). The ligand (***L'***) (250 mg, 0.50 mmol) was dissolved in 5 mL of dry MeOH, and added to a methanolic solution of $Cu(ClO_4)_2 \cdot 6H_2O$ (166 mg, 0.45 mmol). The mixture was stirred at room temperature for 2 hours. The solution was filtered through a medium frit, and the filtrate was collected and layered with dry Et_2O overnight. This solution was filtered through a fine frit and a blue-purple precipitate was collected in 88% yield (935 mg). Anal. calcd. for ($C_{29}H_{44}N_6Cu_2Cl_4O_{21}$): C, 31.96; H, 3.88; N, 7.71. Found: C, 31.79; H, 3.70; N, 7.42. Based on structures of similar systems, it is likely that each copper(II) ion is bound by the three N-donors of the dipicolylamine moiety plus two water molecules. Uv/Vis (MeOH): 655 nm (106 M^{-1} cm^{-1}), 685 nm (63.5 M^{-1} cm^{-1}).

Preparation, oxidation and characterization of oligonucleotides. Oligonucleotides were radiolabeled at their 5'-termini by [γ-^{32}P]ATP (Amersham Bioscience) and T4 polynucleotide kinase (New England Biolabs) following standard procedures. All DNA including the ^{32}P-labeled strand was annealed in phosphate buffer (pH 7.5) by heating to 90° C followed by slow cooling to room temperature. Oligonucleotides (0.1 μM, 90 nCi) were incubated in the alternative presence and absence of MPA (10 mM) and Cu^{II} complexes (0 - 1.5 μM) for 15 min at ambient temperature and then quenched by addition of DDTC (10 mM). DNA was precipitated with 3 M sodium acetate (pH 5.5) and ethanol, and dried under reduced pressure. Samples were resuspended in loading buffer (8 M urea, 40% sucrose, 0.025% bromophenol blue, 0.025% xylene cyanol FF) and applied to a 20% denaturing polyacrylamide gel. Gels were visualized by phosphorimagery and quantified using ImageQuant software (Molecular Dynamics, Sunnyvale, CA).and Scion Image (Scion Corporation, Frederick, MD).

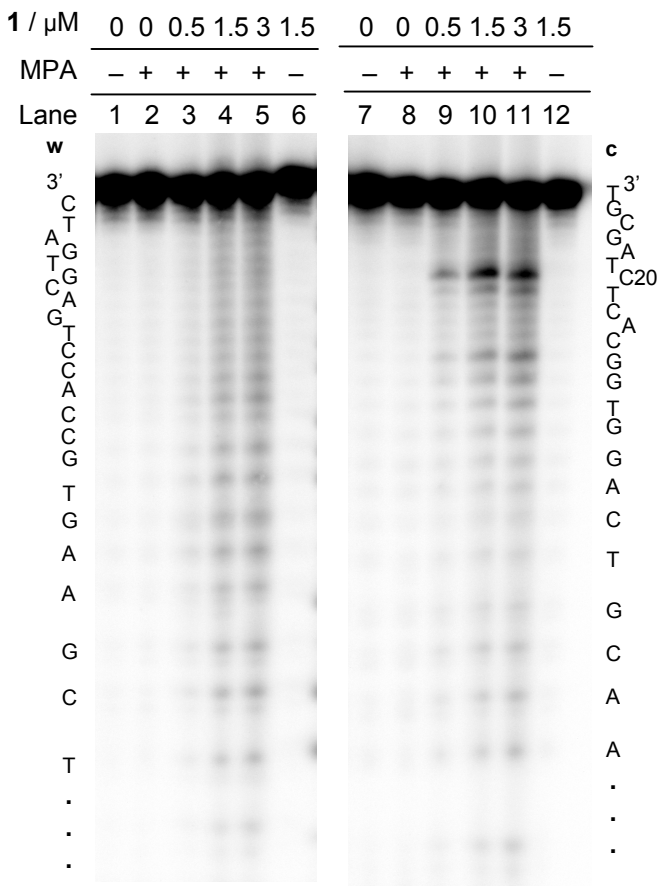

Figure S1. Phosphoimage of a denaturing 20% polyacrylamide gel showing scission products of DS **5**. 5'-End labeled Watson strand (**w**, lanes 1-6) and Crick strand (**c**, lanes 7–12) were alternatively annealed with the complementary strand (0.1 μM) in 10 mM sodium phosphate (10 mM, pH 7.5) and incubated with complex **1** in the presence (+) and absence (–) of 10 mM MPA for 15 min at ambient temperature. Reaction was then quenched by 10 mM DDTC.

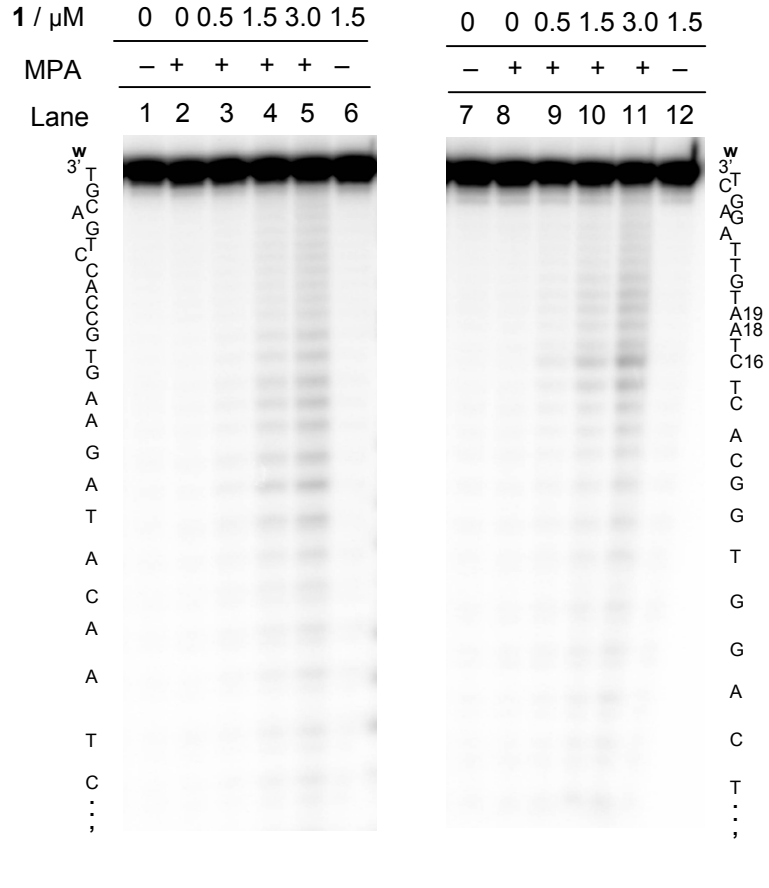

Figure S2. Phosphoimage of 20% denaturing polyacrylamide gels showing scission products of DS **6**. 5'-^{32}P-labeled Watson strand (**w**, lanes 1–6) and Crick strand (**c**, lanes 7–12) were alternatively annealed with the complementary strand (0.1 μM) in 10 mM sodium phosphate (10 mM, pH 7.5) and incubated with complex **1** in the presence (+) and absence (–) of 10 mM MPA for 15 min at ambient temperature. Reaction was then quenched by 10 mM DDTC.

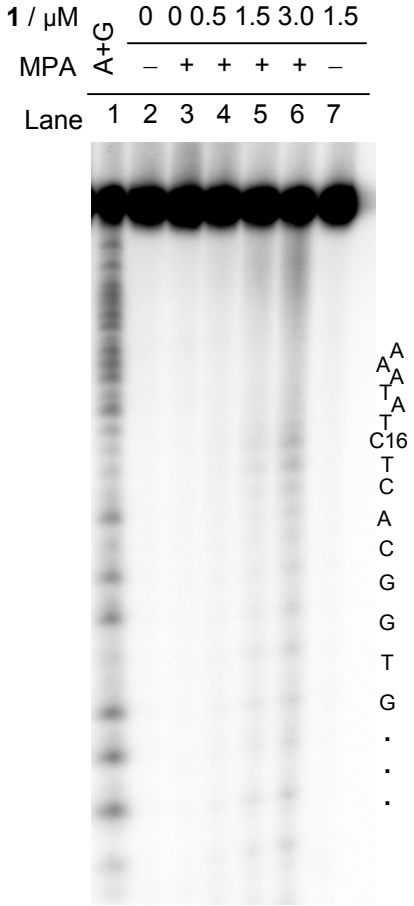

Figure S3. Phosphoimage of a denaturing 20% polyacrylamide gel showing scission products of 5'-^{32}P labeled SS **1**. The DNA (0.1 μM) in 10 mM sodium phosphate (10 mM, pH 7.5) was incubated with complex **1** in the presence of 10 mM MPA for 15 min at ambient temperature, and quenched with 10 mM DDTC.

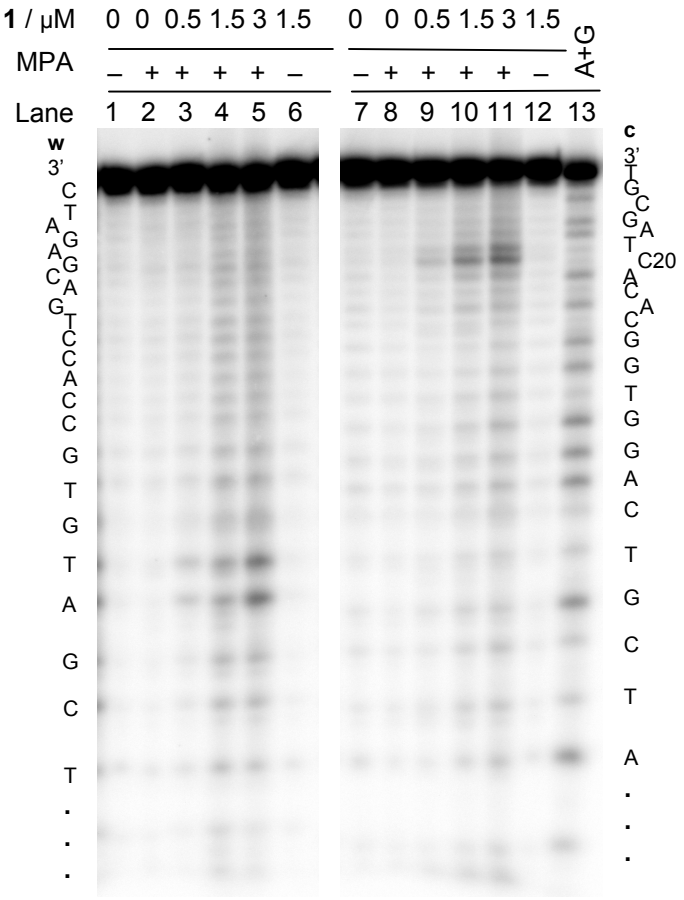

Figure S4. Phosphoimage of a denaturing 20% polyacrylamide gel showing scission products of DS **7**. 5'-^{32}P-labeled Watson strand (**w**, lanes 1–6) and Crick strand (**c**, lanes 7–12) was annealed with the complementary strand (0.1 μM) in 10 mM sodium phosphate (10 mM, pH 7.5) and incubated with complex **1** in the presence (+) and absence (–) of 10 mM MPA for 15 min at ambient temperature. Reaction was then quenched by 10 mM DDTC.

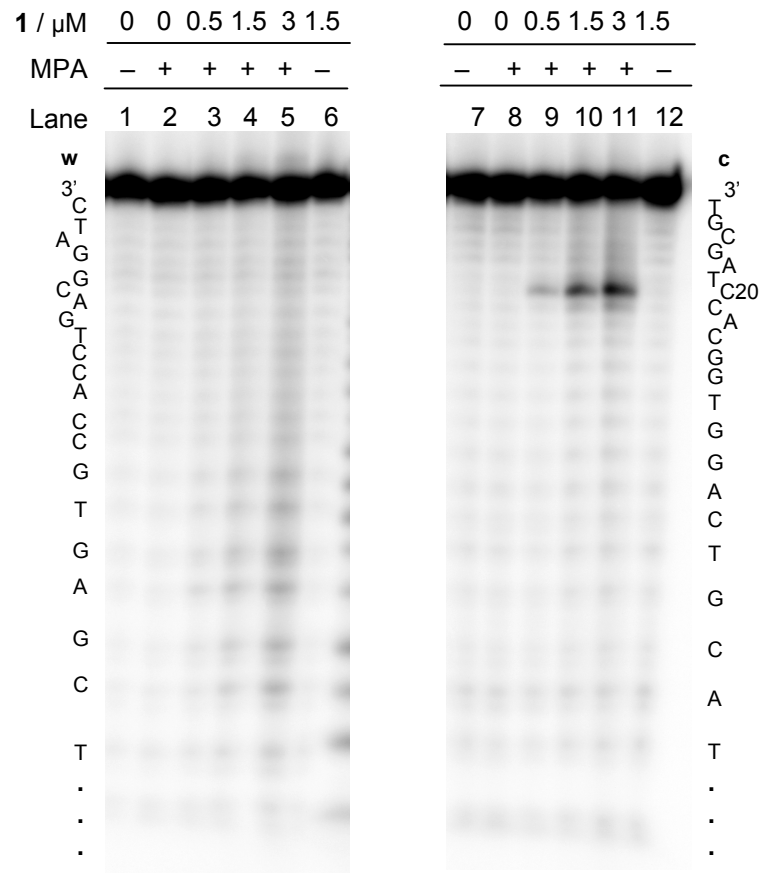

Figure S5. Phosphoimage of a denaturing 20% polyacrylamide gel showing scission products of DS **8**. 5'-^{32}P-labeled Watson strand (**w**, lanes 1–6) and Crick strand (**c**, lanes 7–12) were alternatively annealed with the complementary strand (0.1 μM) in 10 mM sodium phosphate (10 mM, pH 7.5) and incubated with complex **1** in the presence (+) and absence (–) of 10 mM MPA for 15 min at ambient temperature. Reaction was then quenched by 10 mM DDTC.

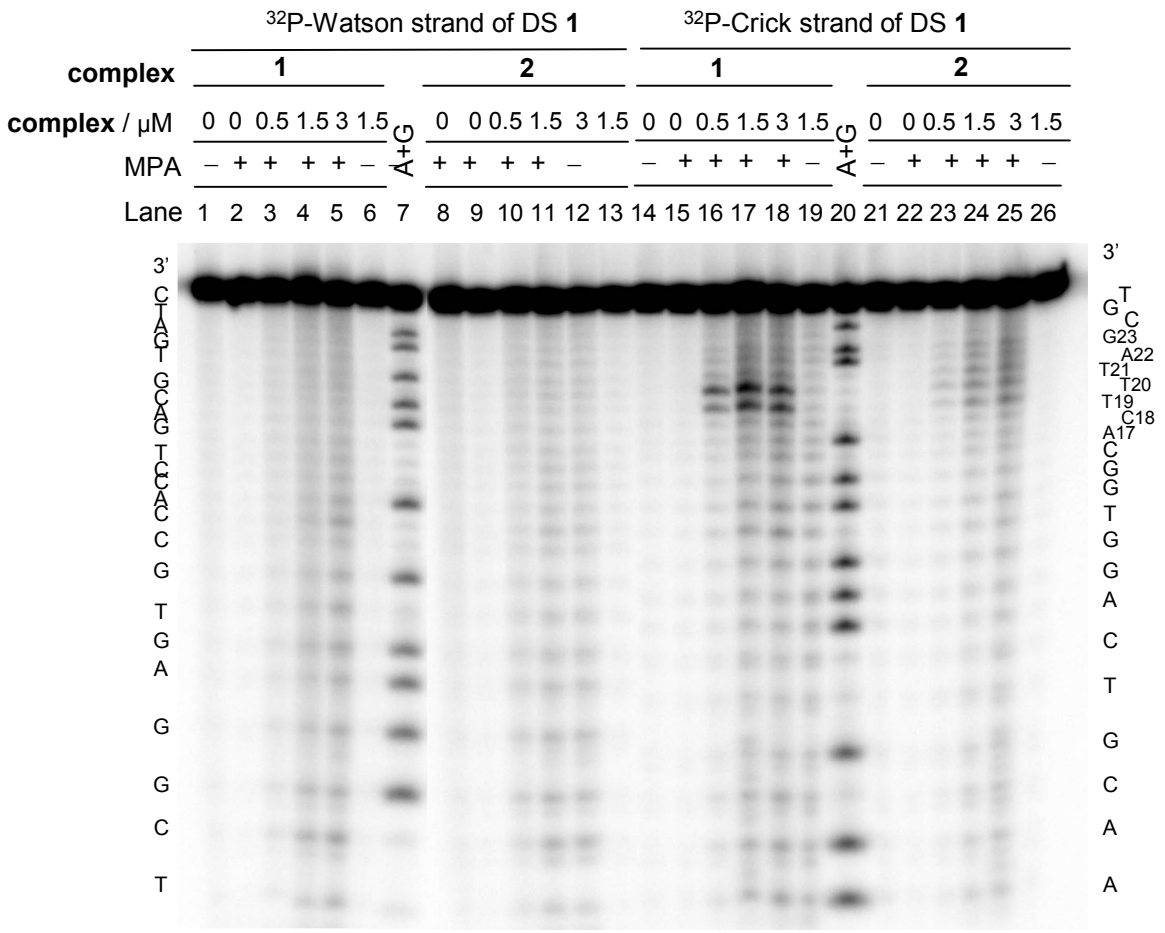

Figure S6. Phosphoimage of a 20% polyacrylamide gel showing scission products of DS **1**. 5'-^{32}P-labeled Watson strand (**w**, lanes 1–13) and Crick strand (**c**, lanes 14–26) were alternatively annealed with the complementary strand (0.1 μM) in 10 mM sodium phosphate (10 mM, pH 7.5). This samples were then incubated with complex **1** (lanes 1–6 and 14–19) or complex **2** (lanes 8–13 and 20–26) for 15 min at ambient temperature in the presence (+) or absence (–) of 10 mM MPA. Reaction was quenched by addition of 10 mM DDTC.