Supporting Information

for

Dynamics of Bleomycin Interaction with a Strongly Bound Hairpin DNA Substrate, and Implications for Cleavage of the Bound DNA

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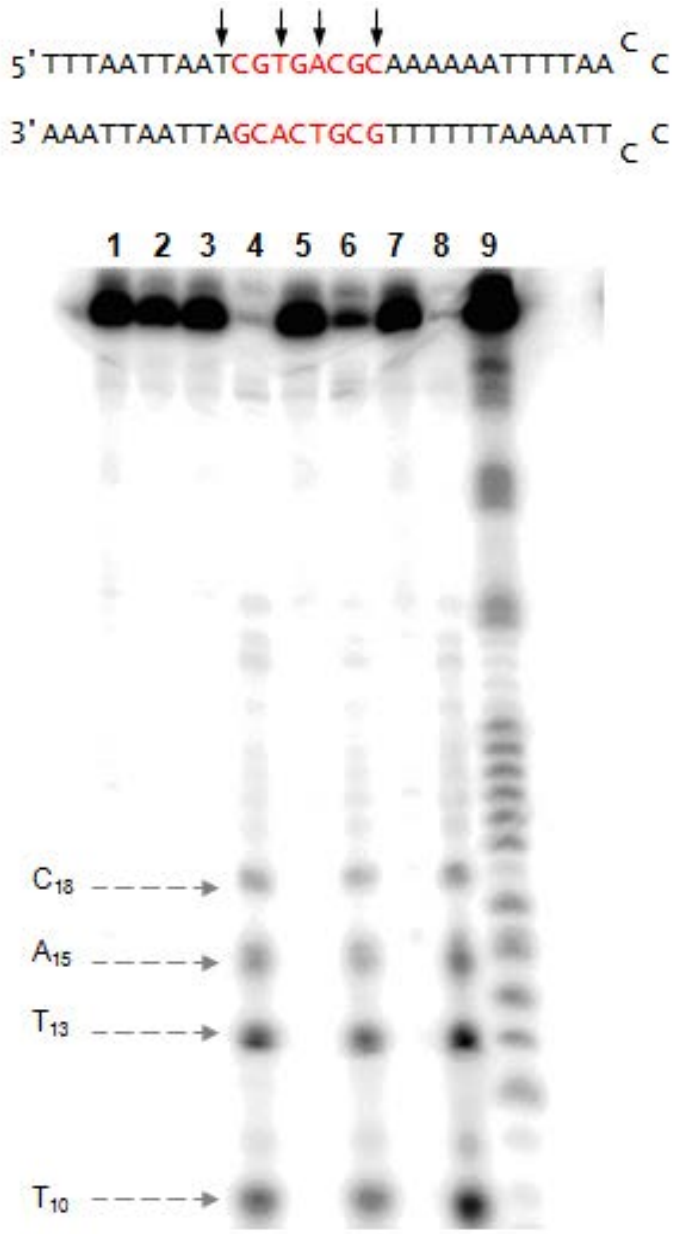

Figure S1. Sequence-selective cleavage of [5′-^{32}P]-end labeled 64-nt hairpin DNA **2** by BLM B_2. Lane 1, radiolabeled **2** alone; lane 2, 20 μM Fe^{2+}; lane 3, 5 μM BLM B_2; lane 4, 5 μM Fe(II)•BLM B_2; lane 5, 10 μM BLM B_2; lane 6, 10 μM Fe(II)•BLM B_2; lane 7, 20 μM BLM B_2; lane 8, 20 μM Fe(II)•BLM B_2; lane 9, G+A lane.

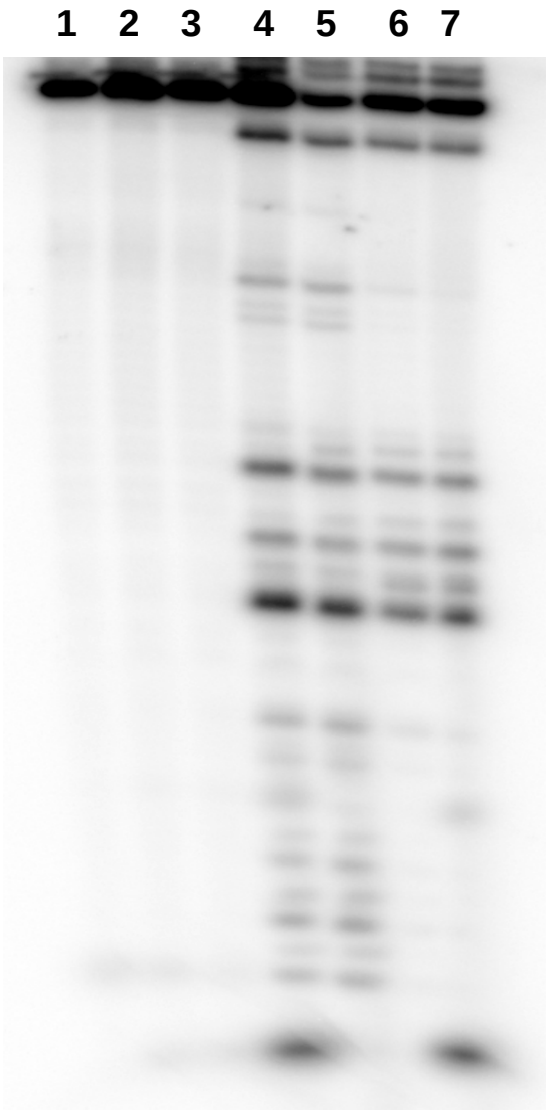

Figure S2. Sequence-selective cleavage of [5′-^{32}P]-end labeled 64-nt hairpin DNA **2** by BLM B_2 in the presence of varying concentrations of NaCl. Lane 1, radiolabeled DNA **2** alone; lane 2, 5 μM Fe^{2+}; lane 3, 5 μM BLM B_2; lane 4, 5 μM Fe(II)•BLM B_2; lane 5, 5 μM BLM B_2 + 10 mM NaCl; lane 6, 5 μM Fe(II)•BLM B_2 + 50 mM NaCl; lane 7, 5 μM BLM B_2 + 150 mM NaCl. All cleavage reactions were carried out in the presence of 10 mM Tris-HCl, pH 8.0.

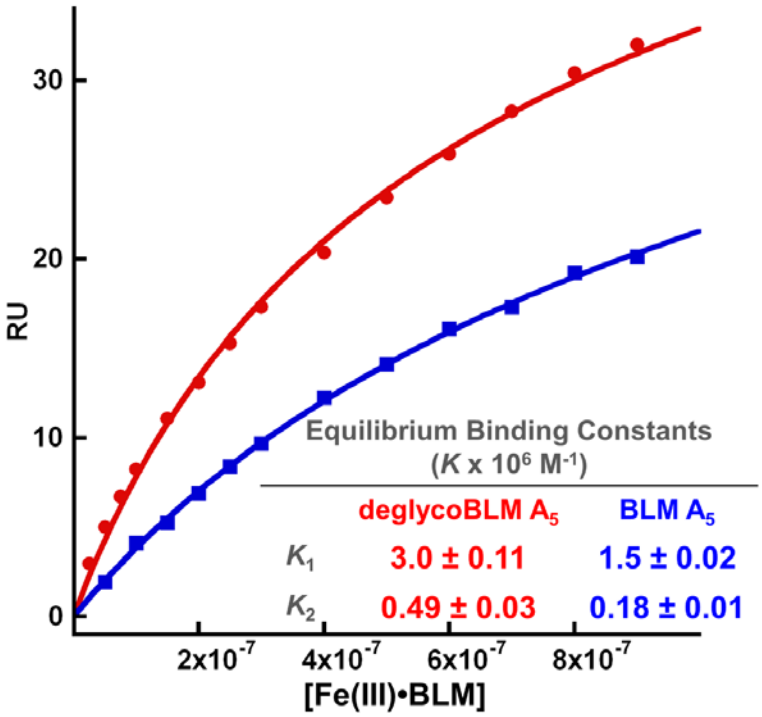

Figure S3. SPR steady-state equilibrium binding plots of BLM A_5 (blue) and deglycoBLM A_5 (red) with DNA **2** at 10 mM NaCl and 25 °C. The steady-state response values were fitted as a function of free ligand concentration to a two-site interaction model. The equilibrium binding affinities are listed in the inset.

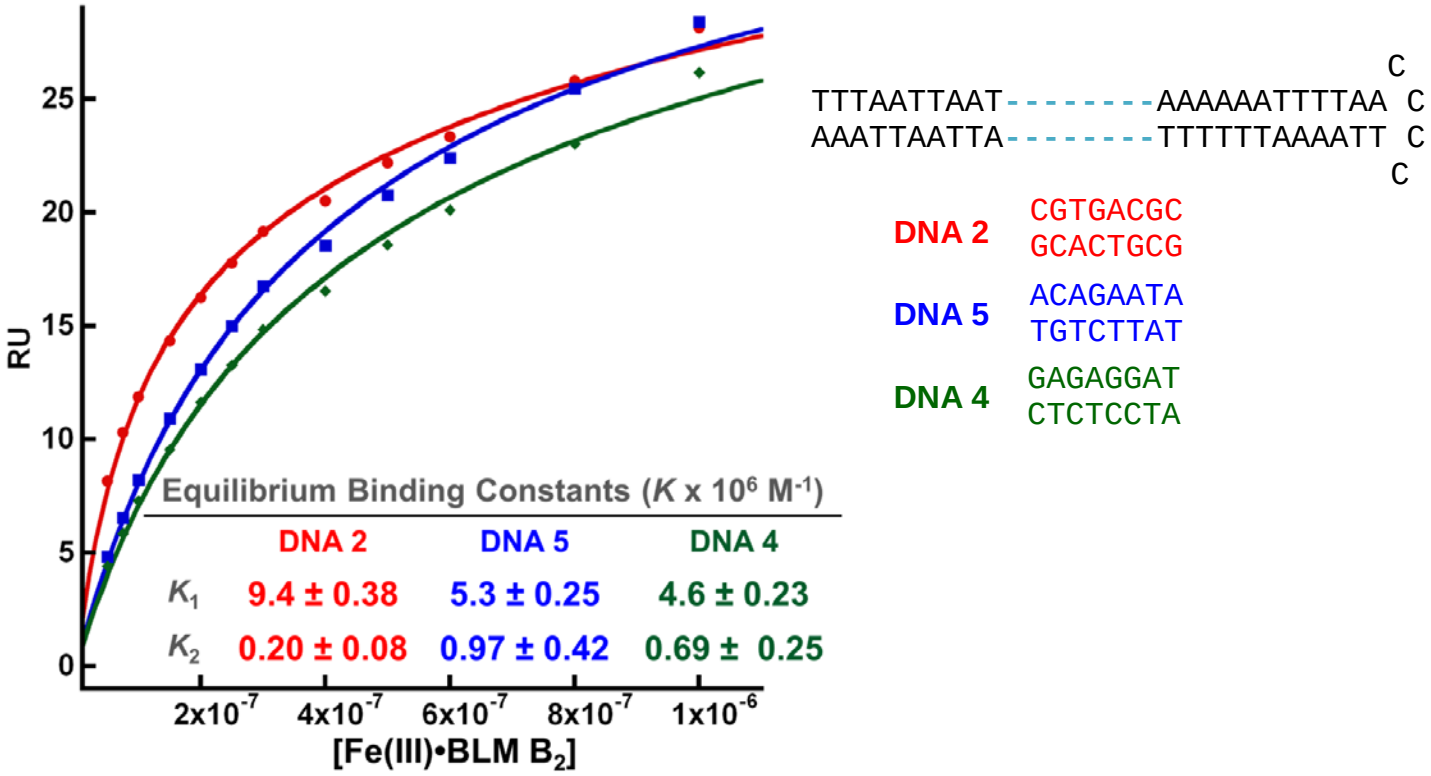

Figure S4. SPR steady-state equilibrium binding plots of Fe(III)•BLM B_2 with different DNA sequences at 10 mM NaCl and 15 °C. The steady-state response values were fitted as a function of free ligand concentration to a two-site interaction model. The equilibrium binding affinities are listed in the inset.

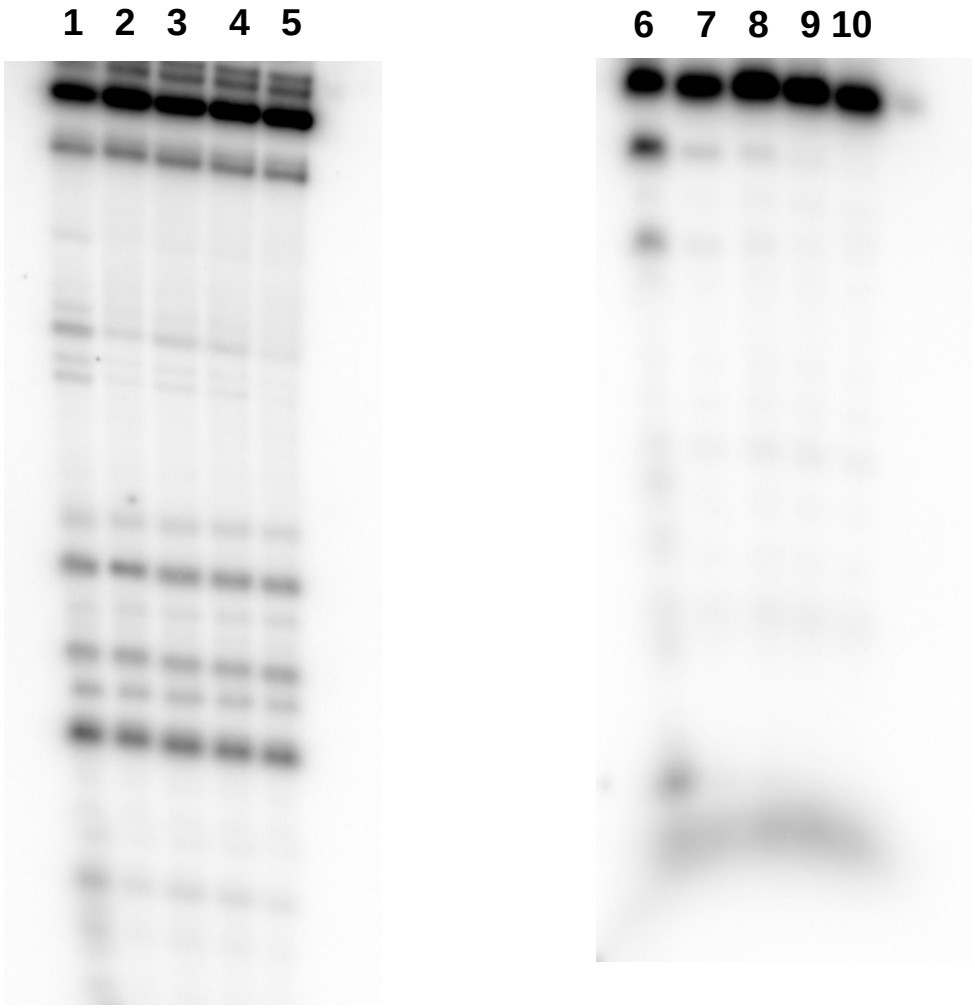

Figure S5. (Left panel) Sequence-selective cleavage of [5′-^{32}P]-end labeled 64-nt hairpin DNA **2** by 2.5 μM Fe(II)•BLM B_2 in the presence of varying concentrations of an efficiently cleaved 16-nt DNA[7a,14] (5'-CGCTTTAAAAAAAGCG-3'). Lane 1, 2 pmol radiolabeled DNA **2** ; lane 2, 2 pmol radiolabeled DNA **2** + 2 pmol unlabeled 16-nt DNA; lane 3, 2 pmol radiolabeled DNA **2** + 4 pmol unlabeled 16-nt DNA; lane 4, 2 pmol radiolabeled DNA **2** + 8 pmol unlabeled 16-nt DNA; lane 5, 2 pmol radiolabeled DNA **2** + 16 pmol unlabeled 16-nt DNA. (Right panel) Sequence-selective cleavage of [5′-^{32}P]-end labeled 16-nt hairpin DNA by 2.5 μM Fe(II)•BLM B_2 in the presence of varying concentrations of DNA **2**. Lane 6, 2 pmol radiolabeled 16-nt DNA; lane 7, 2 pmol radiolabeled 16-nt DNA + 2 pmol unlabeled DNA **2**; lane 8, 2 pmol radiolabeled 16-nt DNA + 4 pmol unlabeled DNA **2**; lane 9, 2 pmol radiolabeled 16-nt DNA + 8 pmol unlabeled DNA **2**; lane 10, 2 pmol radiolabeled 16-nt DNA + 16 pmol unlabeled DNA **2**. All cleavage reactions were carried out in the presence of 50 mM Tris-HCl, pH 8.0.

Experimental

Materials. Terminal deoxynucleotidyl transferase was purchased from Roche Applied Science. T4 polynucleotide kinase was obtained from New England Biolabs. All synthetic oligonucleotides, purified by ion exchange, were purchased from Integrated DNA Technologies. Radiolabeled nucleotides were purchased from Perkin Elmer Life Sciences. BLM solutions were prepared by dissolution in water immediately prior to use. $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ was purchased from Sigma Aldrich Chemicals and used to prepare fresh Fe^{2+} solutions immediately prior to use. Chelex 100 was purchased from Sigma Aldrich and used to remove adventitious Fe^{2+} from solutions prior to experiments.

Methods. Polyacrylamide gel electrophoresis was carried out in 90 mM Tris-borate buffer, pH 8.3, containing 5 mM EDTA. Cleavage sites were confirmed by comparison with the reaction products obtained by the Maxam-Gilbert G + A lanes produced using standard sequencing protocols. Analysis of the polyacrylamide gels was carried out by phosphorimager analysis using a Molecular Dynamics Storm 820 Phosphorimager.

[3′-^{32}P] End Labeling and Purification of 64-nt Hairpin DNAs. [3′-^{32}P]-end labeling was carried out by combining 10 pmol of the 64-nt hairpin DNA, 0.06 mCi [α-^{32}P]cordycepin (specific activity 5000 Ci (185 TBq)/mmol) and 400 units of recombinant terminal transferase in 40 μL (total volume) of 25 mM Tris-HCl, pH 6.6, containing 200 mM potassium cacodylate, 2.5 mM $CoCl_2$ and 0.25 mg/mL bovine serum albumin. The reaction mixture was incubated at 37 °C for 1 h. The [3′-^{32}P]-end labeled 64-nt hairpin DNA was purified by 16% polyacrylamide gel electrophoresis at 1800 V for 2.5 h.

[5′-^{32}P] End Labeling and Purification of Hairpin DNA. Ten pmol of 64-nt hairpin DNA was [5′-^{32}P]-end labeled by incubation with 20 units of T4 polynucleotide kinase and 0.06 mCi [γ-^{32}P]ATP (specific activity 6000 Ci (222 TBq)/mmol) in 50 μL (total volume) of 70 mM Tris-HCl buffer, pH 7.6, containing 10 mM $MgCl_2$ and 5 mM dithiothreitol. The reaction mixture was incubated at 37 °C for 1 h followed by heat inactivation of the enzyme at 65 °C for 20 min. The [5′-^{32}P]-end labeled 64-nt hairpin DNA was purified by 16% polyacrylamide gel electrophoresis at 1800 V for 2.5 h.

Sequence-Selective Cleavage of the Radiolabeled Hairpin DNA by BLM A_5. A sample of 5′- or 3′-^{32}P end labeled hairpin DNA (50,000 cpm) was treated with the appropriate concentrations of Fe^{2+} and BLM solutions in 5 μL (total volume) of 10 mM Na cacodylate buffer, pH 7.0. Reactions were incubated at 25 °C for 30 min, followed by removal of the supernatant under diminished pressure. Ten μL of denaturing gel loading buffer containing 98% formamide, 2 mM EDTA, 0.25% (w/v) bromophenol blue and 0.25% (w/v) xylene cyanol was added to the DNA pellet. The resulting solution was heated at 90 °C for 10 min, followed by chilling on ice. Five μL of each sample was loaded onto a denaturing gel (16% polyacrylamide, 7 M urea) and run at 1800 V for 2.5 h. Gels were visualized using a phosphorimager.

(14) Akiyama, Y.; Ma, Q.; Edgar, E.; Laikhter, A.; Hecht, S. M. *Org. Lett.* **2008**, *10*, 2127.