Supporting Information for

Understanding the Effect of Carbonate Ion on Cisplatin Binding to DNA

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Platination of plasmid DNA. Plasmid pBR322 was purchased from New England Biolabs and amplified in a 100 mL LB culture of *E. coli* XL1-Blue cells with ampicillin as a selecting agent. The plasmid was purified on a Maxi-prep column (Qiagen), ethanol precipitated, analyzed by agarose gel electrophoresis, and used for platination reactions. Platination reactions were performed using 19.2 μM DNA (with respect to bp), 23.8 mM of buffering agent ($NaHCO_3$, NaH_2PO_4, or HEPES), pH 7.4, and 5 mM NaCl. Cisplatin concentrations varied from 1.5 μM to 60 μM and r_f values, the ratio of added platinum per nucleotide, varied from 0.078 to 3.13. Reactions were incubated for 24 h at 37°C in the dark.

Measurement of r_b values. An aliquot of each sample was dialyzed for 24 h against 10 mM Tris-HCl, pH 7.4, 1 mM EDTA to remove unreacted platinum. DNA concentrations were quantified by measuring the absorbance of the dialyzed solutions at 260 nm on a Cary 50 Bio UV-visible spectrophotometer equipped with a microprobe (C Technologies Inc.). Platinum concentrations were measured by atomic absorption spectroscopy using an AAnalyst 300 equipped with an HGA-800 graphite furnace and operated through the AAWinLab interface, version 3.0 (Perkin Elmer, Wellesley, MA). A hollow cathode platinum lamp with 265.9 nm emission was used with a slit width of 0.70 nm. Pyrolysis was performed at 1200°C for 20 s and atomization at 2650°C for 5 s. AA samples were measured in duplicate using a calibration range of 20-80 μg/L and an r value of $\geq$ 0.998 for all calibration curves. For samples requiring dilution,

dialysis buffer was used. The dialysis buffer was analyzed by both AA and UV-Vis and no detectable amounts of platinum or DNA were observed.

Agarose gel analysis of platination. Samples were analyzed on 1% agarose gels (see Figure S1). Gels were stained with 1 μg/mL ethidium bromide and then destained in deionized water. Imaging was done on a Fluor-S (BioRad) and processed by using QuantityOne software (BioRad).

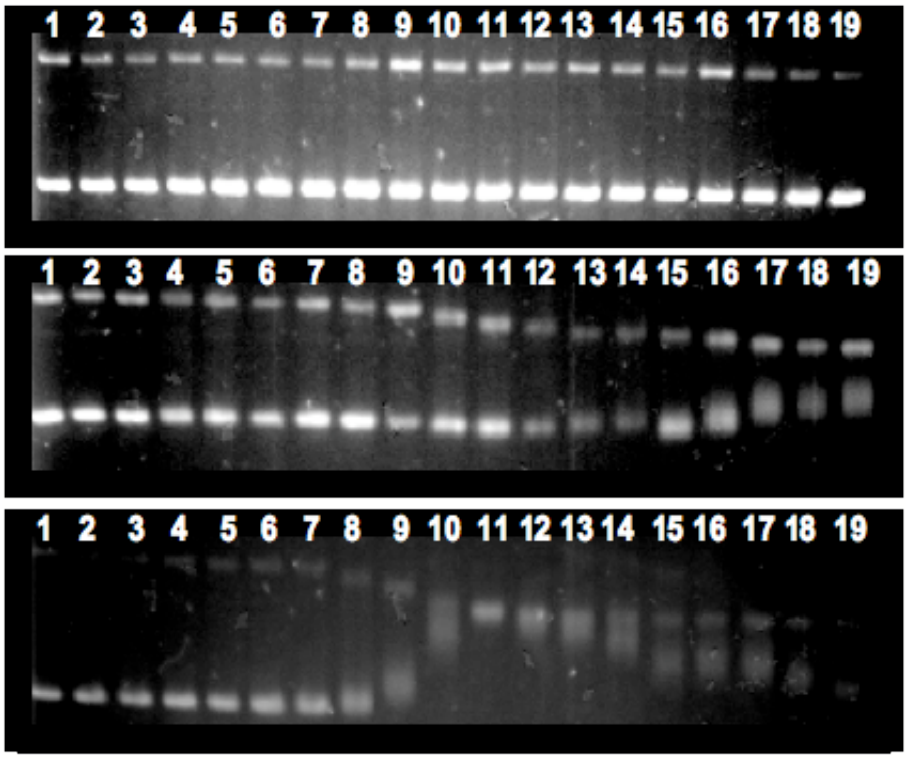

Figure S1. 1% agarose gels showing DNA platinated at r_f values from 0.078 to 3.13 (r_f increases from left to right). Lane numbers and their corresponding r_b values are as follows: Top (platination in carbonate buffer): 1, 0.0069; 2, 0.0046*; 3, 0.0058; 4, 0.0056; 5, 0.0060; 6, 0.0069; 7, 0.0072; 8, 0.013; 9, 0.029; 10, 0.043; 11, 0.058; 12, 0.056*; 13, 0.047; 14, 0.061; 15, 0.078; 16, 0.069*; 17, 0.071; 18, 0.080; 19, 0.075*. Middle (platination in phosphate buffer): 1, 0.0069*; 2, 0.0090; 3, 0.0088; 4, 0.0099; 5, 0.012; 6, 0.010; 7, 0.013; 8, 0.022; 9, 0.055*; 10, 0.048; 11, 0.078*; 12, 0.075; 13, 0.078; 14, 0.097; 15, 0.10; 16, 0.11; 17, 0.10; 18, 0.12; 19, 0.13. Bottom (platination in HEPES buffer): 1, 0.012; 2, 0.013; 3, 0.015; 4, 0.021; 5, 0.023; 6, 0.019; 7, 0.034*; 8, 0.043; 9, 0.091*; 10, 0.10; 11, 0.14; 12, 0.13*; 13, 0.14; 14, 0.15*; 15, 0.17; 16, 0.17; 17, 0.19; 18, 0.16; 19, 0.15. Asterisks indicate where a measured data point was not available, in which cases the value was extrapolated from a logarithmic fit of the r_b versus r_f curve (data not shown).

Reaction of cisplatin with a 14mer DNA. The oligonucleotide was allowed to react with cisplatin (1.2 equiv) in 24 mM carbonate, phosphate, or HEPES buffer, at either pH 6.8 or 7.4, and in the presence of 5 mM NaCl in O-ring sealed Eppendorf tubes. The cisplatin concentrations investigated were 10, 20, 40, 100, and 200 μM. Cisplatin solutions were freshly prepared at a concentration of 3 mM in 41 mM NaCl and used immediately. All reactions were incubated at 37°C for 24 h in the dark and then analyzed by IE-HPLC using an Agilent 1200 series instrument. Separation was accomplished on a 6.2 x 80 mm Agilent Zorbax Oligo column with 80:20 water:acetonitrile containing 25 mM sodium phosphate, pH 7. Peaks were eluted with a NaCl gradient of 0.05 to 0.25 M over 70 minutes. Product peaks at 27.0 and 28.5 min and the starting material peak at 45.4 min were collected with an automated fraction collector for charac-

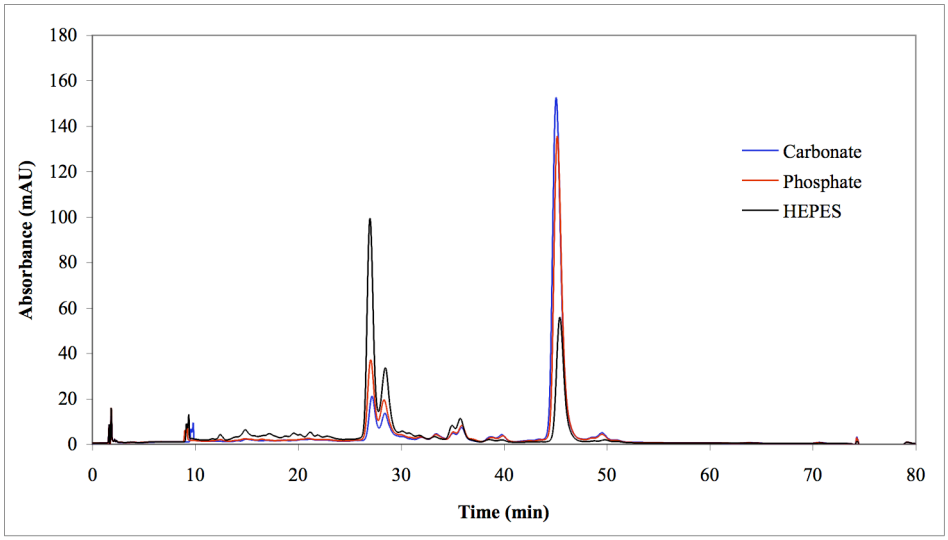

Figure S2. HPLC chromatograms of the reaction of 40 μM cisplatin with the 14mer, 5'-TTC-ACCGGAATTCC-3', in 24 mM carbonate, phosphate, or HEPES buffer, pH 7.4, and 5 mM NaCl. The major product elutes at 27.0 min, and a minor peak appears at 28.5 min. The unplatinated oligonucleotide elutes at 45.4 min.

terization (see Figure S2). Combined fractions were dialyzed against water in the dark at 4°C and then lyophilized to dryness. Figure S3 depicts the yields of platinated oligonucleotide as a function of cisplatin concentration for each buffer system. All yields are reported relative to the reaction in HEPES buffer, which is unaffected by variations in cisplatin concentration or pH. The same products were attained under all reaction conditions.

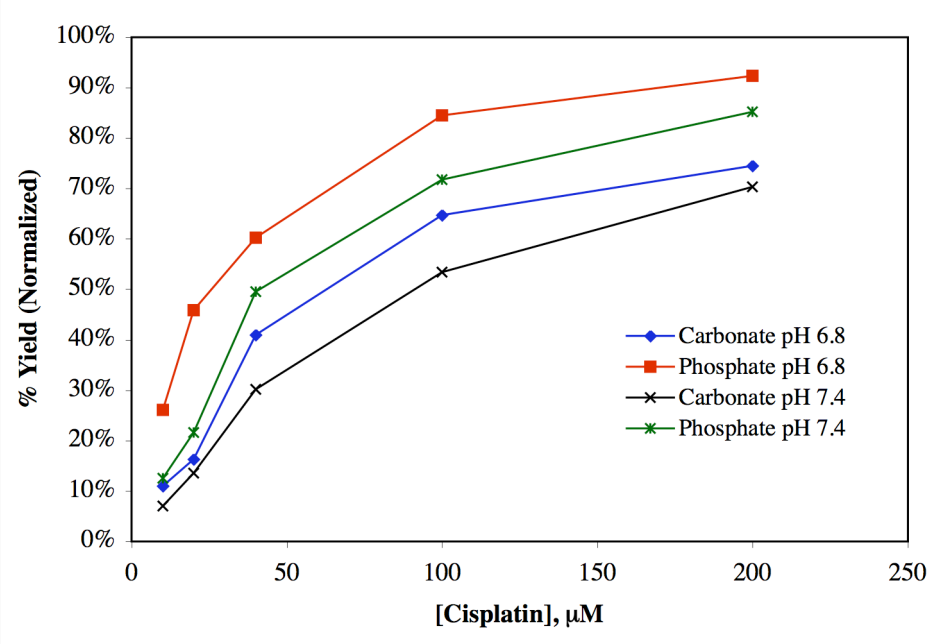

Figure S3. Yield of platinated 14mer ssDNA in 24 mM buffer, normalized to the yield in HEPES buffer, as a function of cisplatin concentration. The presence of both carbonate and phosphate ions strongly inhibits the reaction at low concentrations of cisplatin.

Mass spectrometry. ESI mass spectra were collected on an Agilent 1100 SL MSD trap in negative ion mode. The oligonucleotides were desalted on-line by HPLC. Samples were passed through an Agilent Extend C18 2.1 x 150 mm, 3.5 µm column using a gradient of 5 mM ammonium acetate pH 5.2 and acetonitrile at 0.2 mL/min. The mass spectrum of the major platinated product is shown in Figure S4.

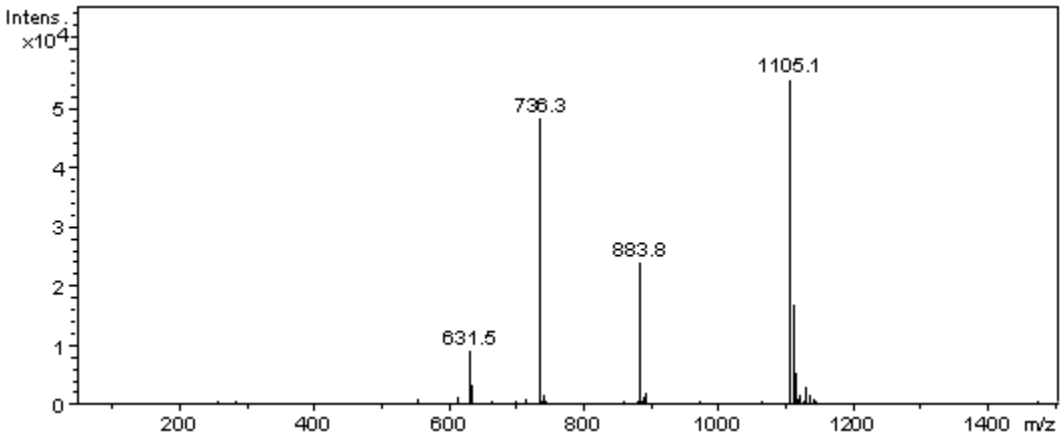

Figure S4. ESI (-) spectrum of major product peak. 631.5 m/z = $[M - 7H]^{7-}$, 736.3 m/z = $[M - 6H]^{6-}$, 883.8 m/z = $[M - 5H]^{5-}$, 1105.5 m/z = $[M - 4H]^{4-}$. Found mass = 4424.9 ± 1.7 Da. Calculated mass of 14mer with $\{Pt(NH_3)_2\}^{2+}$ adduct = 4425.8 Da.

Enzymatic digestion. In 100 µL of digestion buffer (1 mM $ZnCl_2$, 20 mM Na acetate pH 5.2), each of the two collected products and the unplatinated 14mer (5 nmol each) were mixed with 10 µL (10 U) of nuclease P1. The samples were incubated at 37°C overnight. To each sample was added 5 µL of 1.5 M Tris-HCl, pH 8.8, and 1 µL (10 mU) of calf intestinal phosphatase (CIP). After 4 h of incubation at 37°C, 6 µL of 0.1 N HCl was added, and the samples were vortexed and centrifuged at 13,500 RPM for 5 min. Each sample was analyzed by LC-MS on a Supelcosil LC-18-S, 2.1 x 250 mm, 5 µm column. Peaks areas were normalized to account for differences in extinction coefficients at 260 nm, and then peak ratios were calculated relative to the thymine peak. Thymine is the least reactive base towards cisplatin, so the peak area should remain constant upon platination. Peak identifications are based on the mass spectral data. Digest chromatograms of the major platinated product and unplatinated oligo are shown in Figures S5 and S6, respectively.

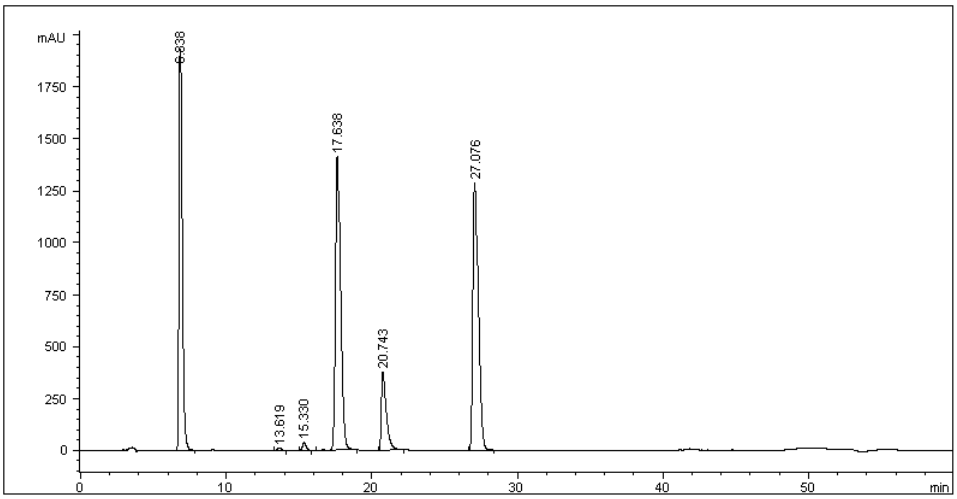

Figure S5. HPLC chromatogram of the major product peak after enzymatic digestion. Peaks were identified by their mass spectra as dC (6.6 min), dG (15.3 min), T (17.6 min), *cis*-Pt(NH_3)$_2$-d(pGpG) (20.7 min), and dA (27.1 min). Peak ratios (C/G/A/T): 4.8/0.1/2.8/4.0. The bifunctional nature of the *cis*-Pt(NH_3)$_2$-crosslink is confirmed by the nearly complete loss of free dG peak.

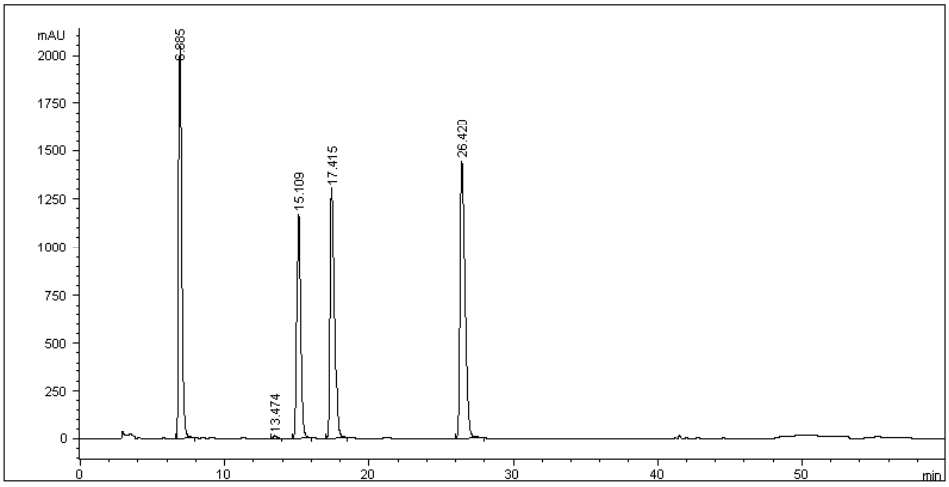

Figure S6. HPLC chromatogram of unplatinated 14mer after enzymatic digestion. Peaks were identified by their mass spectra as dC (6.9 min), dG (15.1 min), T (17.4 min), and dA (26.4 min). Peak ratios (C/G/A/T): expected: 5/2/3/4, found: 4.9/2.2/3.0/4.0.