Supporting Information

Citrate inhibition of cisplatin reaction with DNA studied by fluorescently labeled oligonucleotides: implication for selectivity towards guanine

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1. Materials and Methods.

Chemicals. All the DNA samples were purchased from Integrated DNA Technologies Inc. (IDT, Coralville, IA). The non-labeled and FAM-labeled DNAs were purified by standard desalting. DNAs labeled with other fluorophores were purified by HPLC. Cis-diammineplatinum(II) dichloride (cisplatin), carboplatin, oxaliplatin, and sodium hexachloroplatinate(IV) hexahydrate were obtained from Sigma-Aldrich (St Louis, MO). Tetramethylethylenediamine (TEMED), ammonium persulfate (APS), and acrylamide/bis-acrylamide (29:1) solution 40% (w/v) were obtained from Bio Basic Inc (Markham, ON, Canada). 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), trisodium citrate, and sodium phosphate were purchased from Mandel Scientific Inc (Guelph, ON, Canada). Milli-Q water was used to prepare all the buffers and solutions. All other reagents and solvents were of analytical grade and used as received.

Non-denaturing gel electrophoresis. To prepare a non-denaturing 12.5% polyacrylamide gel, the following solutions were mixed: 6.6 mL acrylamide/bisacrylamide (29:1, 40 %), 2.2 mL 10× Tris/boric acid/EDTA (TBE) buffer, and 13.2 mL milli-Q water. The total volume was 22 mL. To this solution, 50 μL of 10% (w/v) APS and 20 μL TEMED were added to initiate polymerization. This solution was then quickly transferred between two glass plates and a comb was inserted to make the gel. The gel was formed after 1 h. To load a gel, 10 μL of each sample was mixed with 10 μL 30% (w/v) glycerol to increase the sample density. The gel was run at 500 V for 30 min.

Gel electrophoresis for fluorophore-labeled DNA/cisplatin. In a typical reaction, 1 μL of 10 μM FAM-labeled A_{15}, T_{15}, C_{15} or G_{15} was respectively mixed with 20 μL cisplatin (10, 25, 50, 100, or 250 μM) for 16 h at room temperature. Then the samples were loaded into a non-denaturing 12.5%

polyacrylamide gel. To study T_{15} DNA labeled with different fluorophores, 1 μL of 10 μM FAM-T_{15} or AF-T_{15}, was respectively mixed with 20 μL of 250 μM cisplatin for 2 h at room temperature. Then the samples were loaded into a non-denaturing 12.5% polyacrylamide gel. The gels were then documented using a Bio-Rad Chemidoc MP imaging system using epi-light excitation.

Gel electrophoresis of AF-A_{15} and AF-T_{15} with cisplatin. 1 μL of 10 μM AF-A_{15} or AF-T_{15} was mixed with 20 μL different concentrations of cisplatin (10, 25, 50, 100, 250 μM) for 16 h at room temperature. Then the samples were loaded into a non-denaturing 12.5% polyacrylamide gel.

Gel electrophoresis of AF-A_{15} with cisplatin in citrate. 20 μL of 250 μM cisplatin first incubated with different concentration of citrate buffer (1, 0.01, 0.05, 0.1, 0.5, 1,5,10 mM) at pH 7 for 24 h at room temperature. Then 1 μL 10 μM AF-A_{15} added for another 16 h. The samples were loaded into a non-denaturing 12.5% polyacrylamide gel. To study the effect of pH, 20 μL of 250 μM cisplatin first incubated with citrate buffer (10 mM, pH=3, 4, 5, 6, 7, 9, 11) for 24 h at room temperature. Then 1 μL of 10 μM AF-A_{15} added for another 16 h. The samples were loaded into a non-denaturing 12.5% polyacrylamide gel.

Gel electrophoresis of mixture of AF-A_{15} and FAM-G_{15} with cisplatin. 20 μL of 250 μM cisplatin was mixed with 1 μL 10 μM AF-A_{15} and 1 μL 10 μM FAM-G_{15}. The mixture was incubated for different time (0.5, 1, 2, 4, 16 h) at room temperature. 1 μL 10 μM AF-A_{15} and 1 μL 10 μM FAM-G_{15} mixture in 20 μL Milli-Q water performed as a control. The samples were loaded into a non-denaturing 12.5% polyacrylamide gel.

Gel electrophoresis of mixture of AF-A_{15} and FAM-G_{15} with cisplatin in citrate. 20 μL of 250 μM cisplatin first incubated with citrate buffer (final concentration 10 mM) at pH 7 for 24 h at room temperature. Then 1 μL 10 μM AF-A_{15} and 1 μL 10 μM FAM-G_{15} added and the mixture was incubated for different time (0.5, 1, 2, 4, 16 h) at room temperature. 1 μL 10 μM AF-A_{15} and 1 μL 10 μM FAM-G_{15} mixture in 20 μL citrate buffer (10 mM, pH 7) performed as a control. The samples were loaded into a non-denaturing 12.5% polyacrylamide gel.

Mass spectrometry. Sample containing 500 μM cisplatin and 500 μM citrate were dissolved in Mill-Q water without additional buffer. After vigorous mixing and overnight reaction in the dark, the sample was mixed with water-methanol (1:1) containing 0.1% formic acid to facilitate ionization. Mass spectra were obtained using a Micromass Q-TOF Ultima Global mass spectrometer using electrospray ionization (ESI).

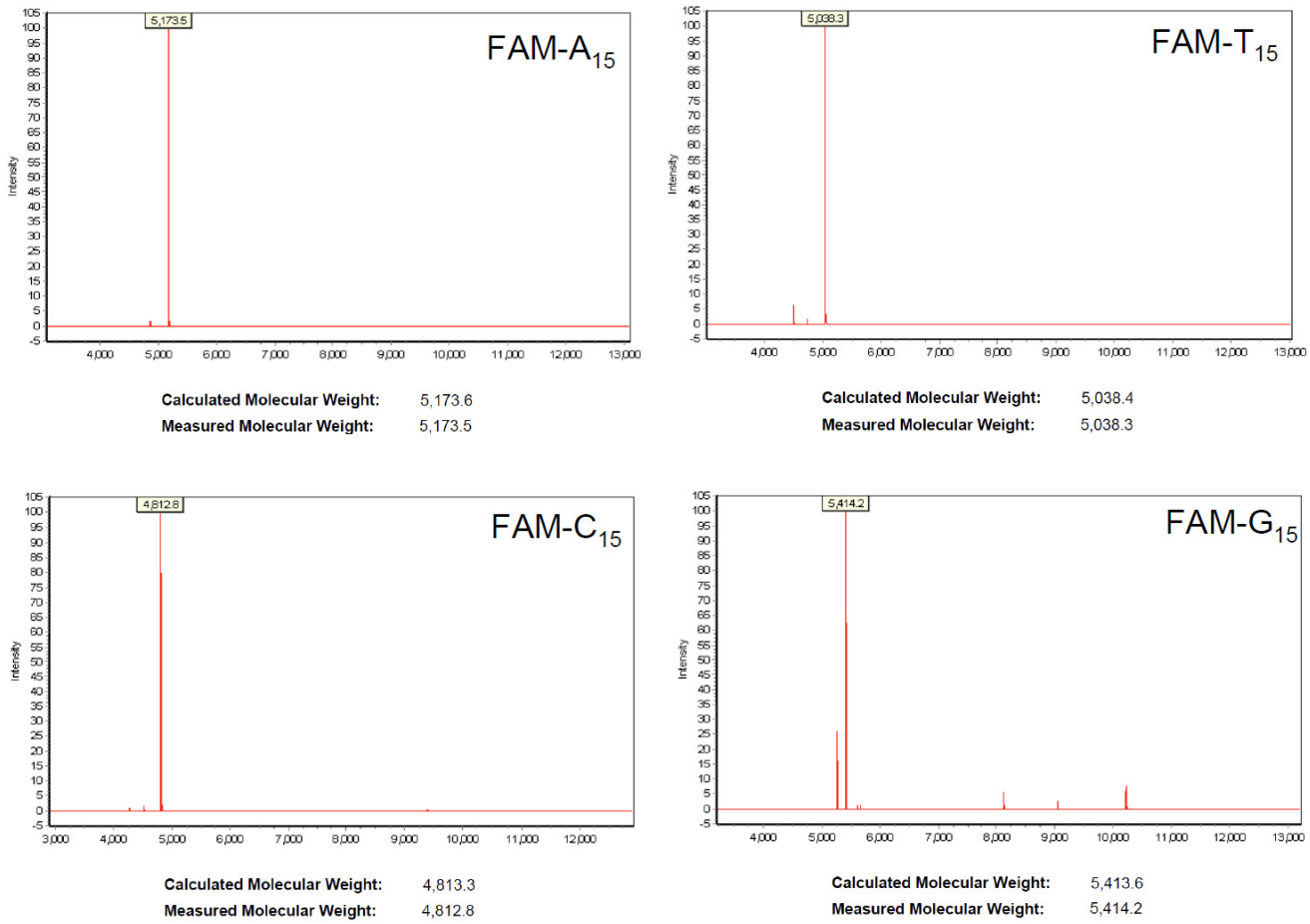

Figure S1. Mass spectrometry of FAM-labeled DNA samples used in this work. FAM-G_{15} has peaks corresponding to shorter sequences and intermolecular complexes, explaining the smeared gel. The spectra were supplied by IDT as proof for quality control.

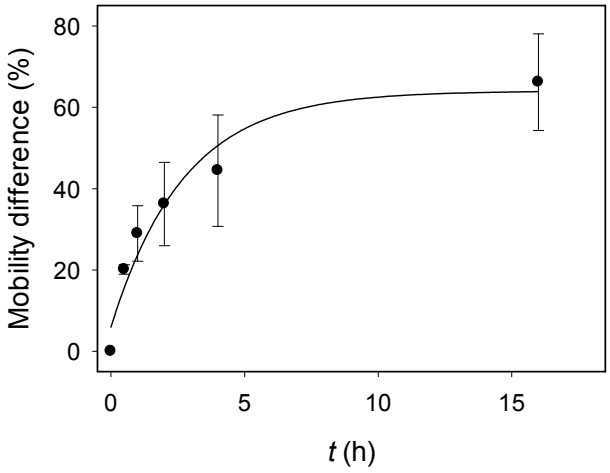

Figure S2. Kinetics of cisplatin reacting with AF-A_{15} quantified using gel electrophoresis. The distance between the free DNA and the center of each band with Pt was measured and compared to the free DNA mobility.

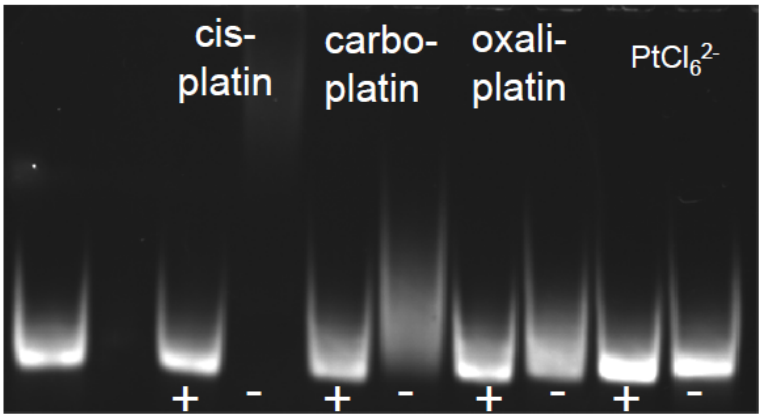

Figure S3. Reaction of platinum-based compounds with AF-A_{15} in the presence or absence of 10 mM citrate (denoted by the '+' and '-' signs) after 48 h. For all the gels, the first lane on the left is the free DNA without cisplatin. The same reaction condition as that for Figure 3B in the main paper was used but with 48 h reaction time. A moderate reaction is observed with carboplatin.

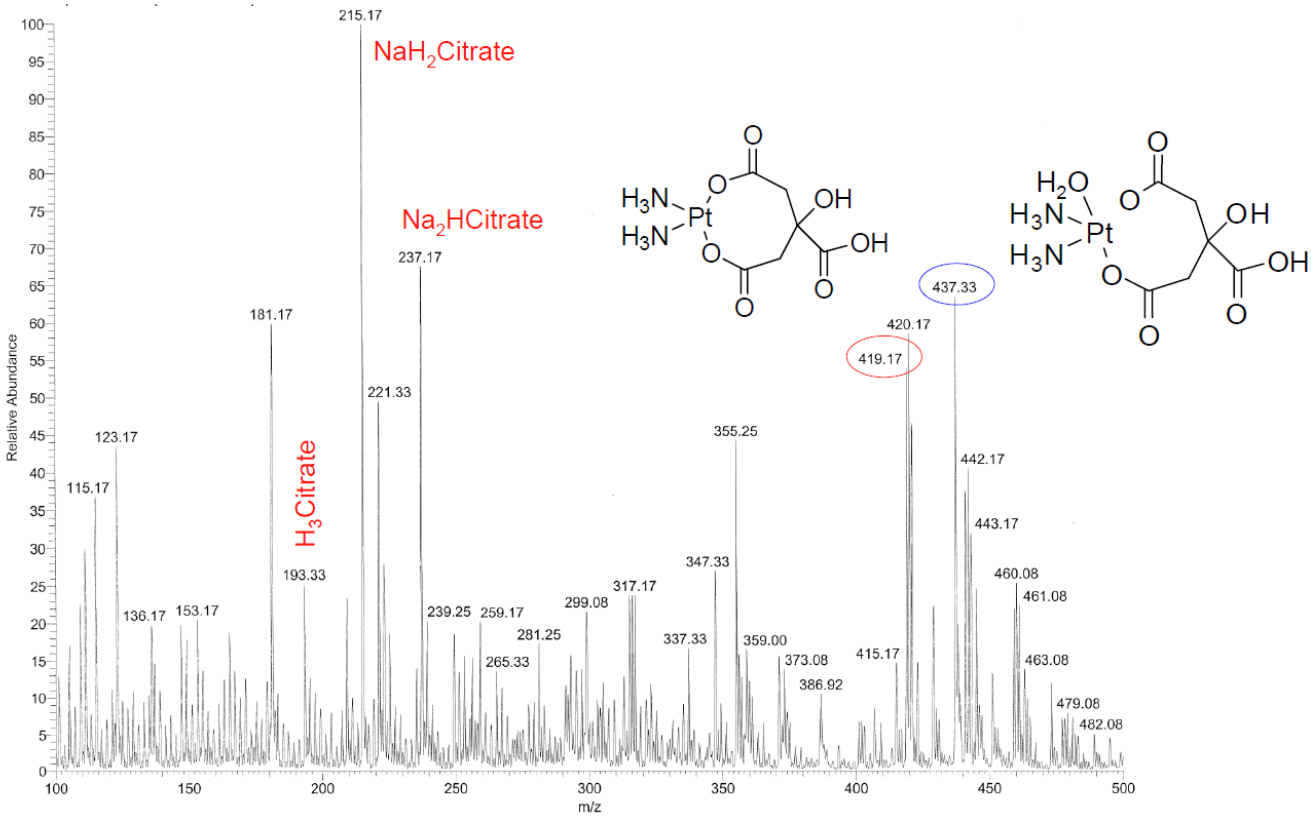

Figure S4. ESI (+) spectrum of cisplatin reacting with citrate. The structures of the main citrate associated species are also shown.

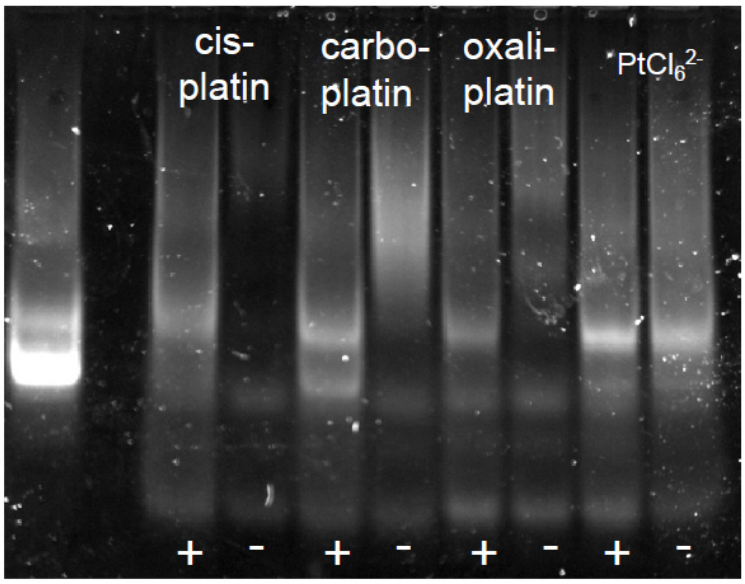

Figure S5. Reaction of platinum-based compounds with FAM-G_{15} in the presence or absence of 10 mM citrate (denoted by the '+' and '-' signs) after 24 h. For all the gels, the first lane on the left is the free DNA without cisplatin. All the samples with platinum compounds showed reaction and citrate still showed moderate inhibition effect on cisplatin and carboplatin.