Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Platinated oligonucleotides: new probes forming ultrastable conjugates with graphene oxide

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1. Additional methods

Denaturing gel electrophoresis. To prepare 500 mL of a 15% denaturing polyacrylamide gel containing 8 M urea, the following solutions or powders were mixed: 240 g urea, 187.5 mL acrylamide/bisacrylamide (29:1, 40 %), 50 mL 10× Tris/boric acid/EDTA (TBE) buffer and 200 mL Milli-Q water. Warm the above mixture to 37 °C to dissolve the urea. The final volume was 500 mL by adding more Milli-Q water. To 22 mL of 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 8 M urea to increase the sample density. The gel was run at 500 V for 30 min.

Gel electrophoresis of AF-A_{15} with cisplatin. 1 μL of 10 μM AF-A_{15} was mixed with 20 μL different concentrations of cisplatin (0.2, 2, 5, 10, 25, 100 μM) for 16 h at room temperature. Then the samples were loaded into a denaturing 15% 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} or FAM-C_{15} with chloroauric acid trihydrate. 1 μL of 10 μM AF-A_{15} or FAM-C_{15} was mixed with 20 μL different concentrations of chloroauric acid trihydrate (0.2, 2, 5, 10, 25, 100 μM) for 1 h at room temperature. Then the samples were loaded into a denaturing 15% polyacrylamide gel.

Gel electrophoresis of AF-T_{15} with mercury perchlorate. 1 μL of 10 μM AF-T_{15} was mixed with 20 μL different concentrations of mercury perchlorate (0.2, 2, 5, 10, 25, 100 μM) for 1 h at room temperature. Then the samples were loaded into a denaturing 15% polyacrylamide gel.

Gel electrophoresis of FAM-C_{15} with silver nitrate. 1 μL of 10 μM FAM-C_{15} was mixed with 20 μL different concentrations of silver nitrate (0.2, 2, 5, 10, 25, 100 μM) for 1 h at room temperature. Then the samples were loaded into a denaturing 15% polyacrylamide gel.

2. Fluorescence quenching of FAM-A_{15} with $HAuCl_4$. To understand fluorescence quenching, 2 μL 100 μM FAM-A_{15} or free fluorescein were incubated with 100 μL 10 μM $HAuCl_4$ dissolved in 10 mM phosphate buffer. Then 1 μL 1 M KCN were added to the mixtures.

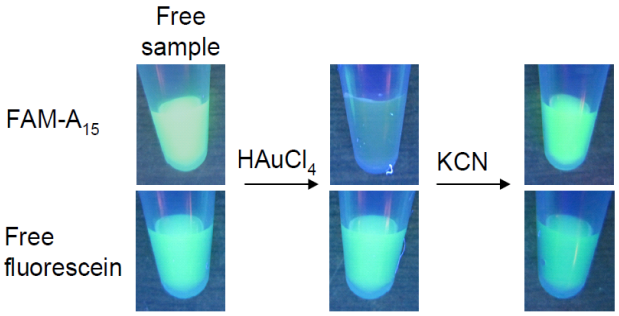

Figure S1. Fluorescence of FAM-A_{15} and free fluorescein reacting with $HAuCl_4$ and then add KCN to dissolve gold.

3. FAM-C_{15} with $HAuCl_4$. Since cytosine can also bind to gold tightly, we measured FAM-C_{15} reacting with $HAuCl_4$. Little shift was observed until 100 μM $HAuCl_4$ was added. This indicates that the complex is not very stable compared to the cisplatin adduct.

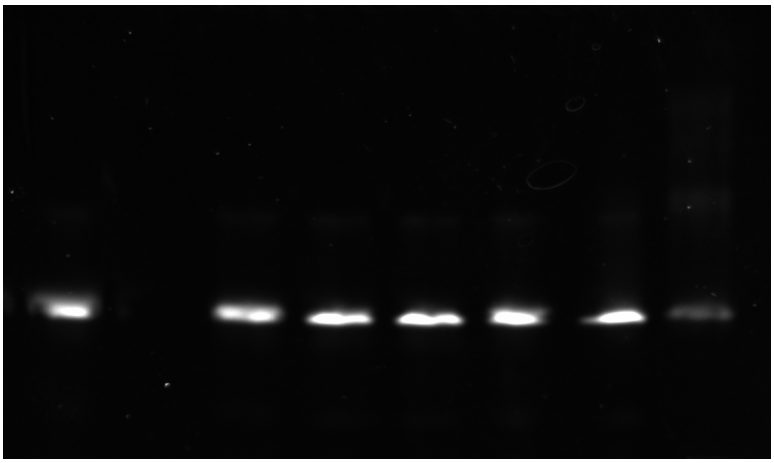

Figure S2. Denaturing gel images of FAM-C_{15} reacting with various concentrations of $HAuCl_4$. The first lane is the free DNA and the subsequent lanes containing increasing $HAuCl_4$ concentrations (0.2, 2, 5, 10, 25 and 100 μM).

4. Additional mass spectra. To study the binding kinetics of cisplatin toward the oligonucleotides, freshly prepared cisplatin (in Milli-Q water) was incubated with the single-stranded oligonucleotide A_{15} in an effective complex/oligonucleotide ratio of 100:20 μM for up to 50 h at 37 °C in a dry bath incubator (Isotemp 2050FS, Fisher Scientific) in the dark. Aliquots of 25 μL were taken after 6 and 50 h and stored at -20 °C until analysis by mass spectrometry. The thawed samples were diluted

immediately prior to analysis 1:10 with a 1.1 mM solution of ammonium acetate in methanol/water/2-propanol giving a ratio of 65/30/5 and a final DNA concentration of 2 μM. Mass spectra were obtained using a Micromass Q-TOF Ultima Global mass spectrometer using electrospray ionization (ESI).

The ESI mass spectrum of the free A_{15} DNA incubated at 37 °C for 50 h is shown in Figure S3A. The major peaks can be assigned to the variously charged species of the DNA, while little degradation products were observed. For example the m/z = 926.18 peak is the DNA containing five negative charges and the high resolution isotope pattern of this peak is shown in Figure S3B, confirming this peak carries five charges.

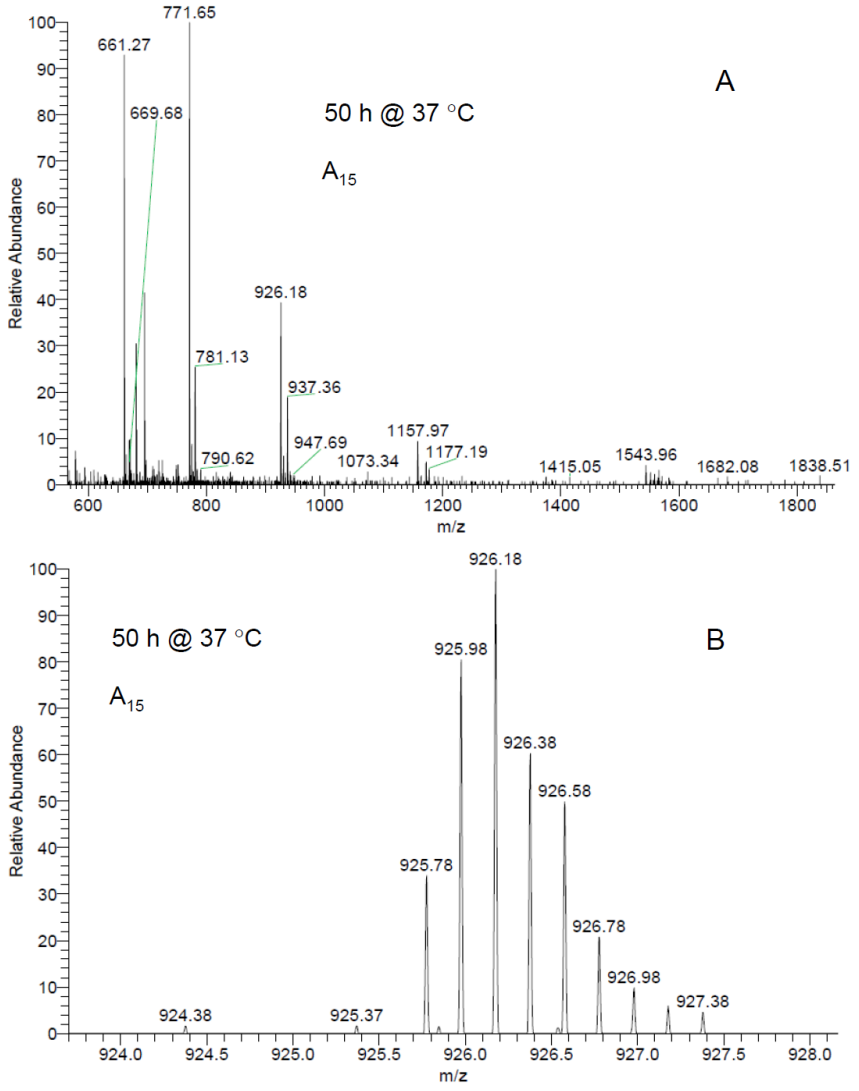

Figure S3. (A) ESI mass spectrum of non-platinated single-stranded oligonucleotide A_{15} incubated at 37 °C for 50 h. (B) Characteristic isotope distribution of A_{15} oligonucleotide signals with five negative charges.

The high resolution DNA/cisplatin mixture spectra for species carrying five negative charges are shown in Figure S4.

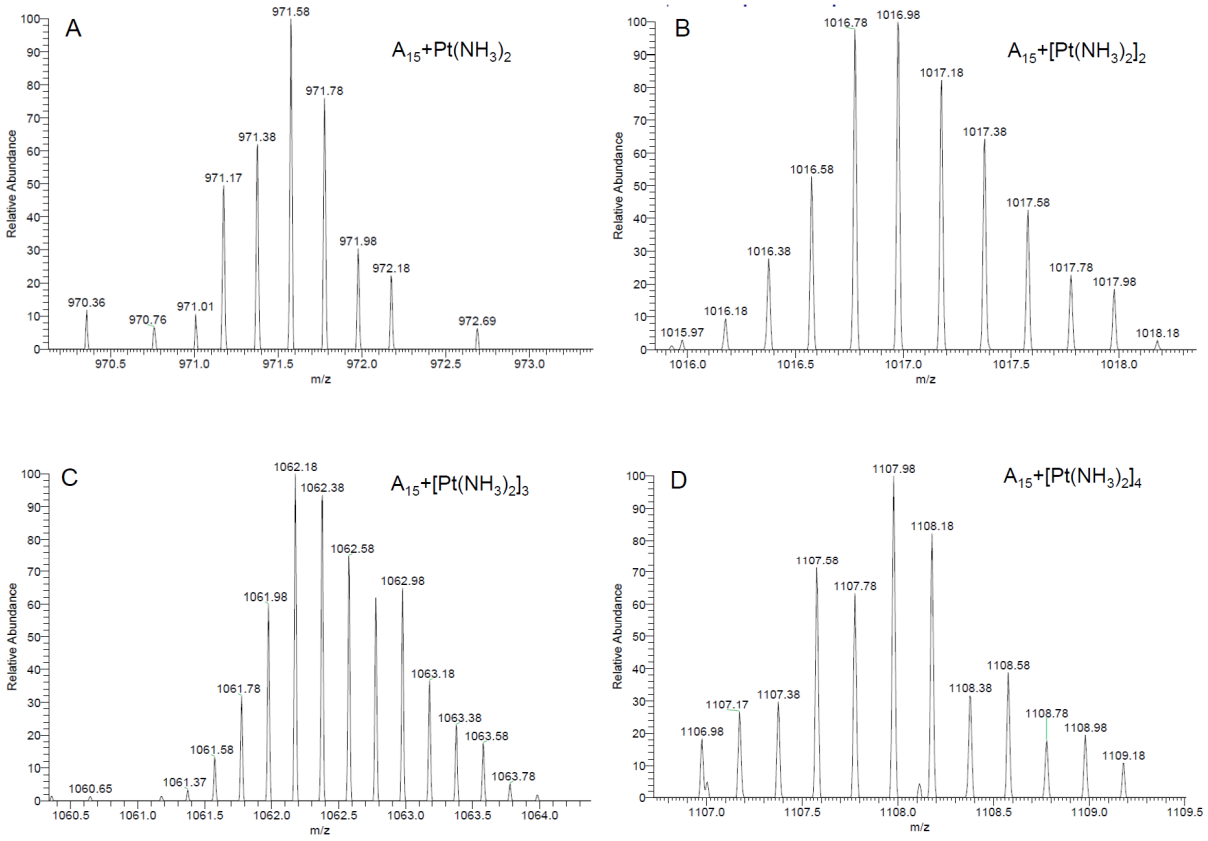

Figure S4. The peak patterns of platinated single-stranded oligonucleotide with five negative charges. (A) A_{15}+ $Pt(NH_3)_2$, (B) A_{15}+ $[Pt(NH_3)_2]_2$, (C) A_{15}+ $[Pt(NH_3)_2]_3$ and (D) A_{15}+ $[Pt(NH_3)_2]_4$. Effective spraying conditions (1 mM ammonium acetate and 2 μM oligonucleotide in methanol/water/2-propanol = 65:30:5) lead to negatively charged species.

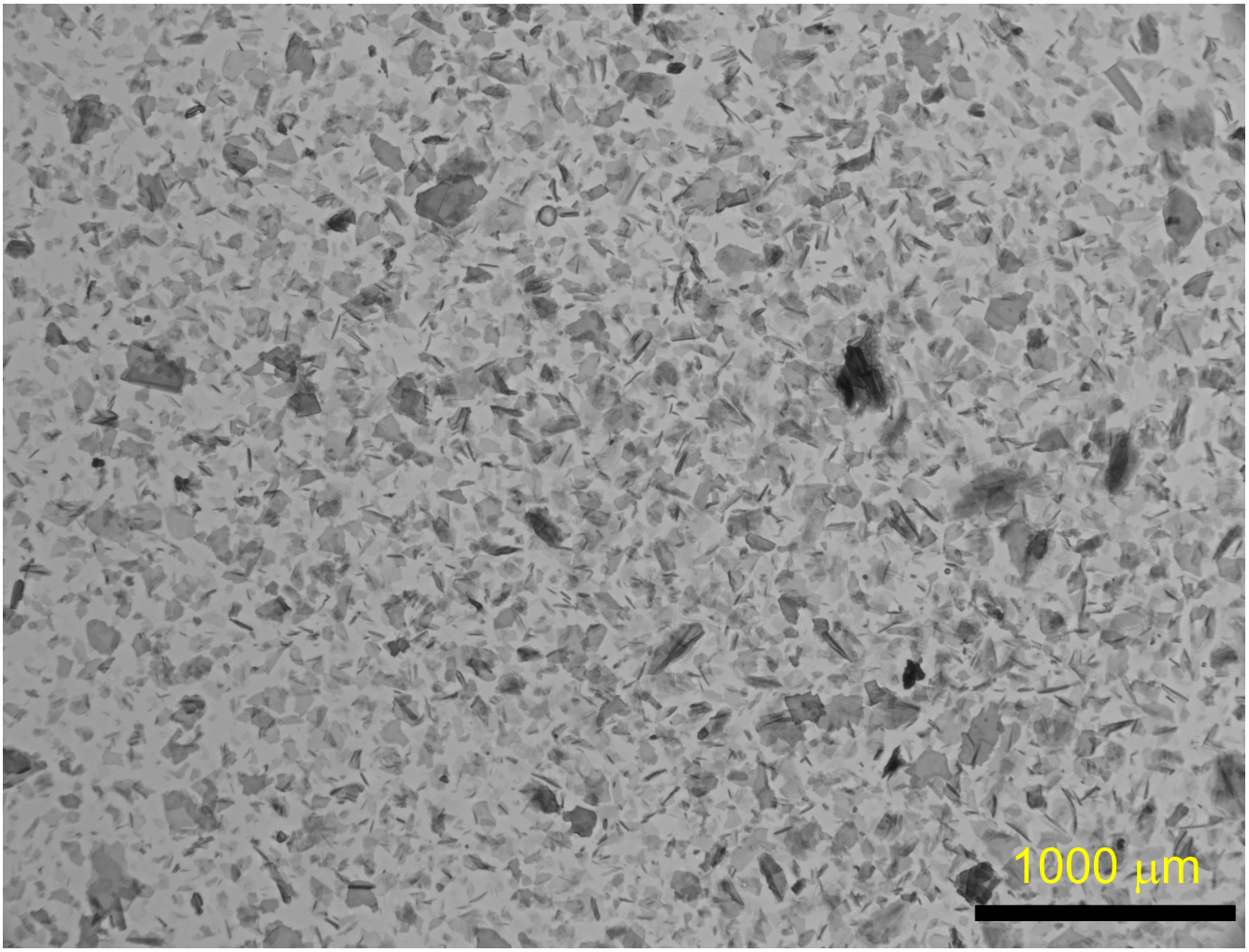

Figure S5. A transmission optical micrograph of the GO sheets.