Electronic Supplementary Material (ESI) for Metallomics.

Electronic Supporting Information

for

Cisplatin Reacts with Histone H1 and the Adduct Forms Ternary Complex with DNA

Contents

A

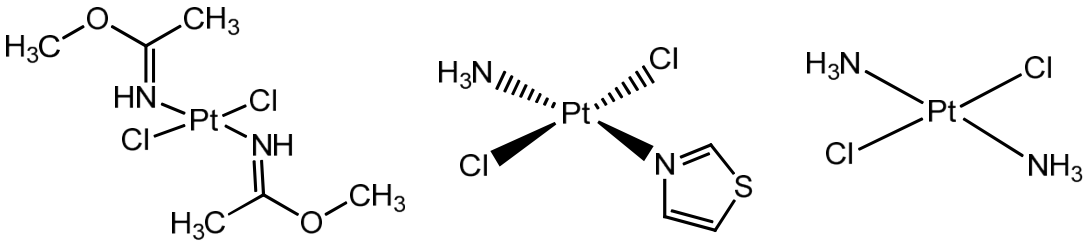
histone H1.4

MSETAPAAPA APAPAEKTPV KKKARKSAGA AKRKASGPPV SELITKAVAA SKERSGVSLA ALKKALAAAG YDVEKNNSRI KLGLKSLVSK GTLVQTKGTG ASGSFKLNKK AASGEAKPKA KKAGAAKAKK PAGAAKKPKK ATGAATPKKS AKKTPKKAKK PAAAAGAKKA KSPKKAKAAK PKKAPKSPAK AKAVKPKAAK PKTAKPKAAK PKKAAAKKK

histone H1.0

MTENSTSAPA AKPKRAKASK KSTDHPKYSD MIVAAIQAEK NRAGSSRQSI QKYIKSHYKV GENADSQIKL SIKRLVTTGV LKQTKGVGAS GSFRLAKSDE PKKSVAFKKT KKEIKKVATP KKASKPKKAA SKAPTKKPKA YPVKKAKKKL AATPKKAKKP KTVKAKPVKA SKPKKAKPV PKAKSSAKRA GKKK

B



trans-EE *trans*-PtTz transplatin

Scheme S1 Protein sequences and platinum complexes used in this work. (A) Sequences of human histones H1.4 and H1.0. The sequences used in this work are highlighted in yellow. (B) Structures of *trans-EE*, *trans*-PtTz, and transplatin.

Table S1 Sequence information in construction of the $H1_{N90}$ plasmid

cDNA	ATGTCCGAGACTGCGCCTGCCGCGCCCGCTGCTCCGGCCCCTGCCGAGAAGACTCCCGT GAAGAAGAAGGCCCGCAAGTCTGCAGGTGCGGCCAAGCGCAAAGCGTCTGGGCCCCC GGTGTCCGAGCTCATTACTAAAGCTGTTGCCGCCTCCAAGGAGCGCAGCGGCGTATCTT TGGCCGCTCTCAAGAAAGCGCTGGCAGCCGCTGGCTATGACGTGGAGAAAAACAACAG CCGCATCAAGCTGGGTCTCAAGAGCCTGGTGAGCAAG
PCR primers	Forward primer: CGCGGATCCGAGAACCTGTACTTCCAATCCATGTCC GAGACTGCGCCT Reverse primer: CCGCTCGAGGGATTGGAAGTACAGGTTCTCCTTGCT CACCAGGCTCTT

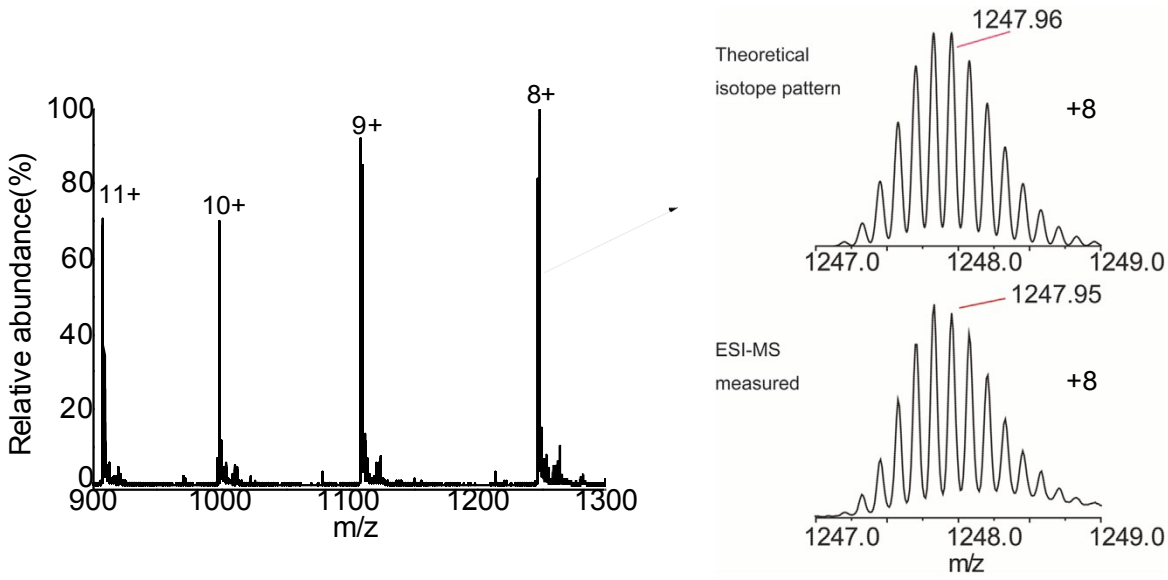


Figure S1. ESI-MS spectra of $H1_{N90}$. The theoretical isotopic pattern is given for comparison.

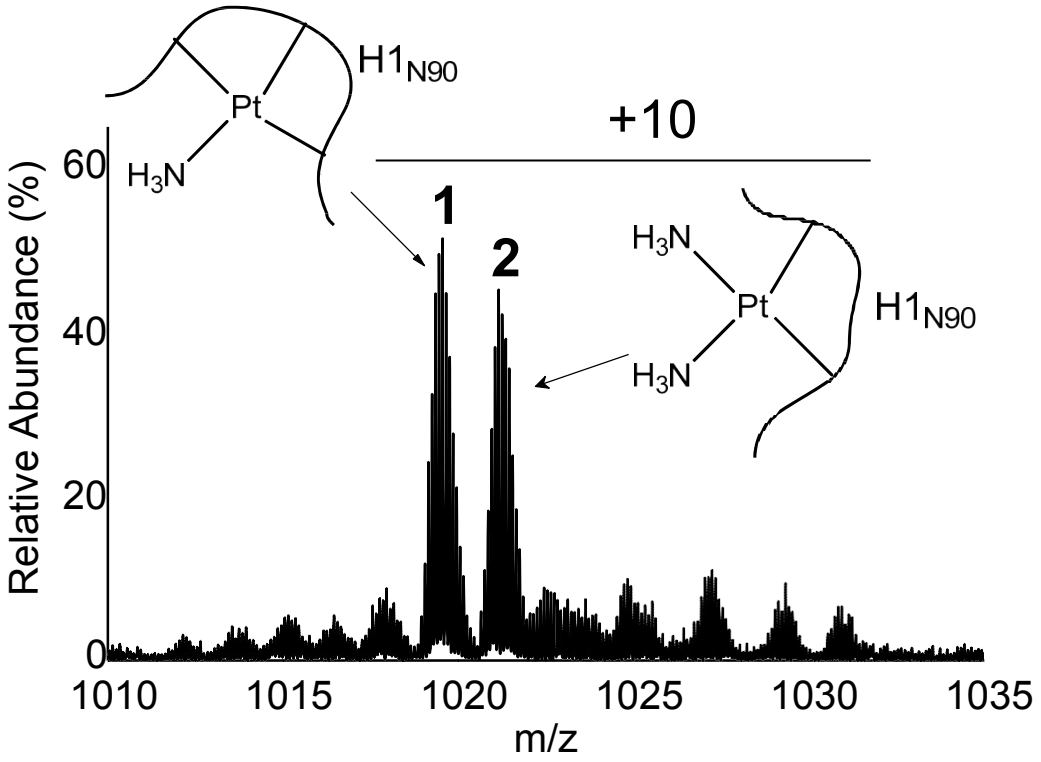


Figure S2. ESI-MS spectra of $H1_{N90}$ reacted with equimolar cisplatin. The selected region shows products with +10 charges, $Pt(NH_3)(H1_{N90})$ and $Pt(NH_3)_2(H1_{N90})$ were the two major products.

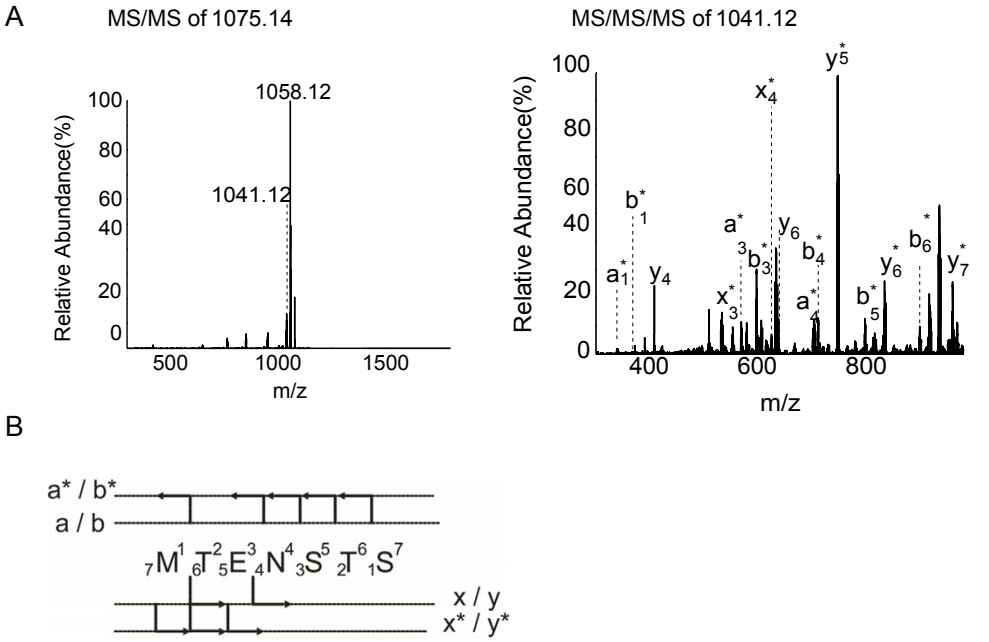


Figure S3. Tandem MS spectrometry of the platinated product M1 from the reaction of $H1_{N7}$ with cisplatin. The asterisk indicates the platinum containing fragments. (A) ESI-MS/MS and MS/MS/MS spectra from the CID experiment on the single charged peptide at m/z 1075.14 and 1041.12. (B) The fragmentation scheme of the MS/MS/MS spectrum. The black arrows denote the fragment ions observed in the ESI-MS/MS/MS spectra.

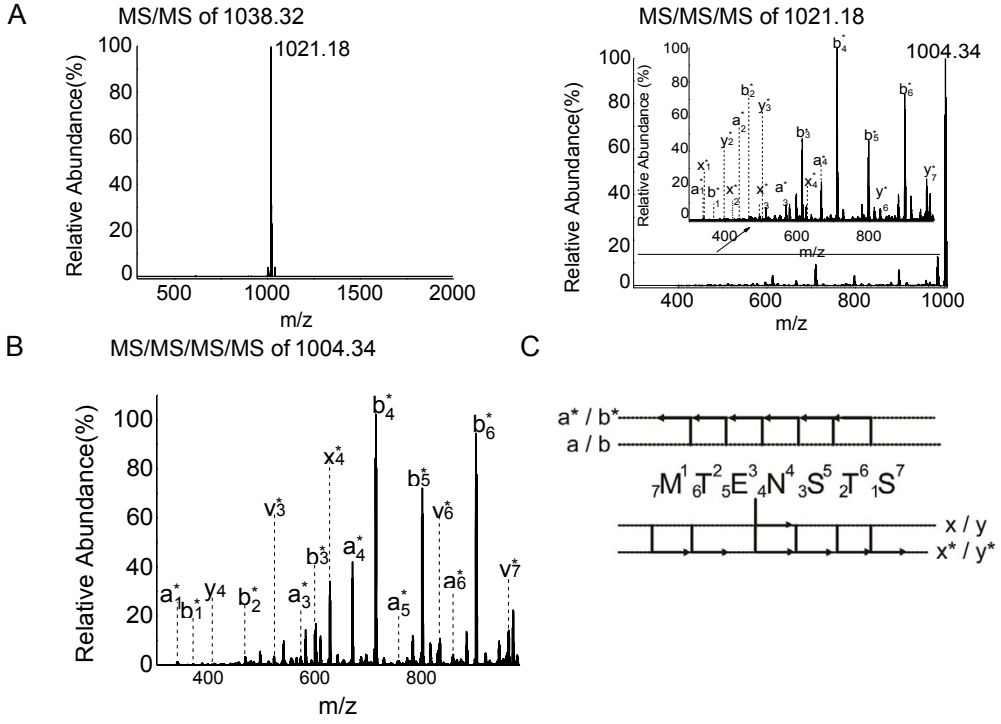


Figure S4. ESI-MS/MS, MS/MS/MS and MS/MS/MS/MS spectra of the product M3, showing the fragments from the ion at m/z 1038.32 (A), 1021.18 (A) and 1004.34 (B). Fragmentation schemes based on the spectra (A) and (B) are shown in (C).

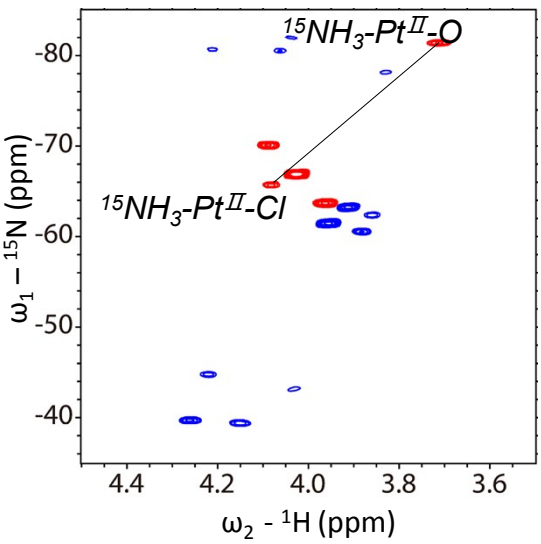


Figure S5. NMR spectra of ^{15}N-cisplatin in the reaction with H1$_{N7}$. Overlay of ^{1}H,^{15}N-HSQC NMR spectra of ^{15}N labeled cisplatin before (red) and after (blue) the incubation with 1.2 molar equivalents of H1$_{N7}$ in 10 mM phosphate buffer at 37 °C for 23.5 h.

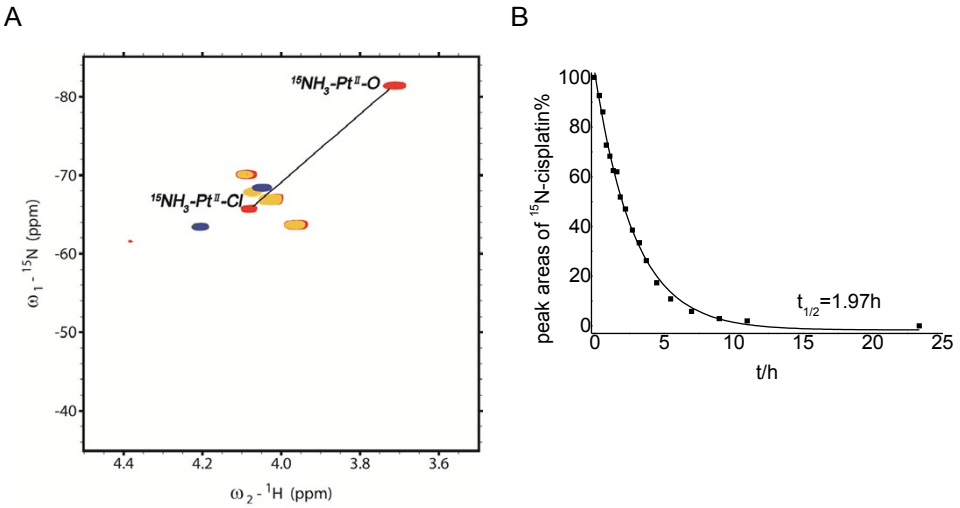


Figure S6. NMR spectra monitoring the reaction of ^{15}N-cisplatin with dsDNA2. (A) Overlay of ^{1}H,^{15}N-HSQC NMR spectra of ^{15}N labeled cisplatin (1 mM) incubated with equimolar dsDNA2 at 37 °C for 0 h (red), 2 h (gold) and 23.5 h (blue) in 10 mM PB (pH 7.0). (B) Plot of the ratio of unreacted cisplatin to initial cisplatin versus time for the reaction of cisplatin with dsDNA2.

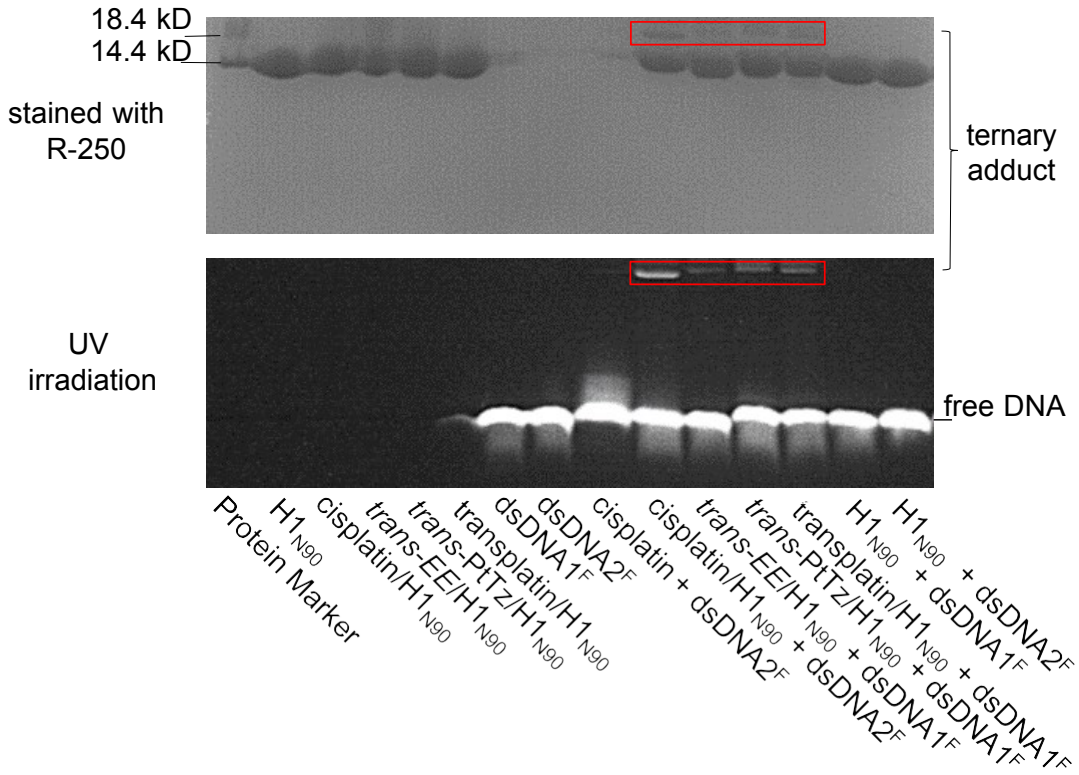


Figure S7. Tricine-SDS-PAGE analysis of the reaction of platinated $H1_{N90}$ with the FAM 5' labeled dsDNA. Platinated-H1 obtained with different compounds (cisplatin, *trans-EE, trans*-PtTz, and transplatin) were reacted with $dsDNA2^F$ or $dsDNA1^F$ (Scheme 1C) for 24 h in 50 mM HEPES (pH 6.8) at 37 °C; 0.2 mM platinated/$H1_{N90}$ and 0.1 mM $dsDNA2^F$ or 0.1 mM $dsDNA1^F$ were used. The red boxes show the bands of the protein-Pt-DNA ternary products.

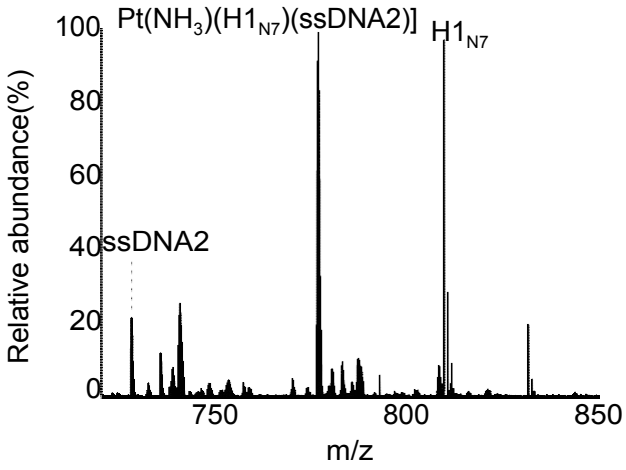


Figure S8. ESI-MS spectra of cisplatin/$H1_{N7}$ incubated with dsDNA2. The reaction was performed using 0.4 mM M2 and 0.04 mM dsDNA2 in 10 mM NH_4OAc (pH 6.8).