Supplementary Information to

DNA or Protein? Capillary Zone Electrophoresis–Mass Spectrometry Rapidly Elucidates Metallodrug Binding Selectivity

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Materials and Methods

Materials

Chemicals (at least analytical grade), if not otherwise stated, were obtained from commercial suppliers and used as received. Ubiquitin (from bovine erythrocytes), [tri(acetylacetonato)cobalt(III)] (99.99% trace metals basis) and 3-nitrobenzyl alcohol (for mass spectrometry, ≥99.5%) were purchased from Sigma-Aldrich, ammonium bicarbonate (BioUltra, ≥99.5%), formic acid (p.a. for mass spectrometry) and ammonium hydroxide solution (≥25% in H_2O, eluent additive for LC-MS) from Fluka and acetic acid (99.8%, for analysis) from Acros. The lyophilized oligonucleotide 5′-dATTGGCAC-3′ (HPLC purified) was obtained from Integrated DNA Technologies (Coralville, USA) or Microsynth (Balgach, Switzerland), and methanol and 2-propanol (both HPLC grade) from Fisher. NaOH solution (1 M) was purchased from Agilent Technologies and inorganic standards (P, Pt, Ru, Re; 1000 ± 3 µg/mL) from CPI. Ultrapure water was obtained from a Millipore Advantage A10 system (18.2 MΩ, ≤5 ppb TOC, 185 UV, Darmstadt, Germany). Cisplatin, RM175 and [(cym)RuCl(maltolato)], where cym = η^6-p-cymene, were synthesized according to literature procedures.[1-3]

Instrumentation

Measurements were performed on a G7100 capillary electrophoresis system (Agilent Technologies, Waldbronn, Germany) equipped with a diode-array detector (190–400 nm). High resolution mass spectra were acquired on an ESI-qTOF mass spectrometer (maXis 4G UHR-TOF, Bruker, Bremen, Germany) and ICP-MS data were acquired on an Agilent 7800 or 8800 (Waldbronn, Germany).

Analyte Preparation

Stock solutions of ubiquitin (ub), DNA-oligomer (DNA, 200 µM each), and the metal complexes cisplatin, RM175 and [(cym)RuCl(maltolato)] (1 mM each) were prepared in water. Reaction solutions were prepared by adding the metallodrugs to the biomolecule mixture to yield final concentrations of 50 µM for each ub/DNA and 200 µM for the respective metallodrug. They were

incubated at 37 °C at constant shaking (400 rpm) in the dark. Aliquots were taken after 1 and 24 h of incubation.

CZE-MS

Capillaries (fused silica, undeactivated, 75 µm, Agilent Technologies, USA) were cut to a total length of 70 cm with a Shortix™ capillary column cutter (SGT, Singapore) to obtain a clean edge. About 5 mm of the protective polyimide outer capillary coating was removed at both ends of the capillaries by flaming. The UV/Vis detection window was self-made 23 cm from the inlet end. The capillary was wiped clean with isopropanol before installation. New capillaries were flushed (950 mbar) successively with HCl (1 M, 5 min), water (1 min), NaOH (1 M, 10 min), water (1 min) and BGE (20 min) before use. The capillary cassette temperature was 25 °C. We used NH_4HCO_3 (25 mM, pH 7.9) as background electrolyte (BGE). The separation voltage was 25 kV and capillaries were flushed with NaOH (1 M), water and BGE for 1 min each after each run. Typical instrument parameters can be found in Table S1.

CZE–ESI-MS. A coaxial sheath-flow CE–ESI-MS Sprayer Kit (Agilent Technologies) was used for CE hyphenation to the ESI mass spectrometer. The sheath liquid for ESI-MS experiments consisted of isopropanol, methanol, water, formic acid and 3-nitrobenzyl alcohol (25/25/50/0.2/0.5, v/v/v/v). The latter was added to obtain high charge states of the protein.[4] A syringe pump (KDS 1000, KD Scientific Inc., Holliston, USA) provided the sheath flow at 3 µL/min. Typical instrument parameters can be found in Table S1. Before injection, the capillary was rinsed with BGE for 1 min (950 mbar). Samples were hydrodynamically injected for 5 s at 50 mbar. The electropherograms in the figures were obtained by selecting the appropriate mass-to-charge signal including an offset of ±0.05 *m/z*.

CZE–ESI-MS². In-source collison-induced dissociation (ISCID) energy was increased (90 – 170 eV) for top-down fragmentation experiments. All other parameters were similar to CZE–ESI-MS runs.

CZE–ICP-MS. A coaxial sheath-flow CE–ESI-MS Sprayer Kit (Agilent Technologies) was used for CE hyphenation to the ICP mass spectrometer in combination with a custom-built interface. The sheath liquid for ICP-MS experiments was 20 mM acetic acid containing 20 ppb Re as external standard and was provided by the nebulizer pump with an uptake of 0.03 rpm or 0.1 rpm. A solution

of [(triacetylacetonato)cobalt(III)] with a cobalt concentration of 50 mg/L was used as an internal standard that was diluted to a final concentration of 5 mg/L cobalt in the reaction mixtures. Typical instrument parameters can be found in Table S1 Before injection, the capillary was rinsed with BGE for 1 min (950 mbar). Samples were hydrodynamically injected for 10 s at 50 mbar. On the Agilent 7800 phosphorous was tracked as ^{31}P (for cisplatin and RM175) and as ^{47}PO on the 8800 ICP-MS (for [(cym)RuCl(maltolato)]). To lower the spectroscopic interferences for phosphorous an oxygen cell gas flow of 30% was applied.

Data and statistical analysis

ESI mass spectra data were analysed by ESI Compass 1.3 DataAnalysis 4.0 (Bruker Daltonics). Mass spectra of DNA and ubiquitin were deconvoluted by the maximum entropy deconvolution algorithm with automatic data-point spacing and an instrument resolving power of 30000. Data analysis was carried out with Microsoft Excel. The CZE–ICP-MS experiments were baseline subtracted by subtracting the average baseline signal between 0.5–2.0 min. Peak area was calculated by using trapezoid rule, and summation between peak valleys was obtained with the perpendicular drop method. Each experiment was carried out independently three times and these data sets were used for calculating standard deviations (SDs).

Table S1 Typical instrument parameters

CE system	Agilent G7100
Capillary	fs capillary, i.d. 75 µm, length 70 cm
Background electrolyte	25 mM NH_4HCO_3, pH 7.9
Separation Voltage	25 kV
Sample Injection	Hydrodyn., ICP: 50 mbar, 10s
	ESI: 50 mbar, 5s

ICP-MS system	Agilent 7800
RF power	1550 W
Sample depth	8 mm
Plasma gas	15 L/min
Carrier gas flow	1.05 L/min
Auxilary Gas	0.9 L/min
Monitored isotopes	^{31}P, ^{59}Co, ^{185}Re, ^{195}Pt or ^{102}Ru
Integration time [s]	0.2 (^{31}P), 0.1 (^{59}Co), 0.01 (^{185}Re), 0.1 (^{195}Pt or ^{102}Ru)
Measurement statistics	100 sweeps

ICP-MS system	Agilent 8800
RF power	1550 W
Sample depth	8 mm
Plasma gas	15 L/min
Carrier gas flow	1.30 L/min
Monitored isotopes	^{47}PO, ^{48}SO, ^{59}Co, ^{185}Re, ^{195}Pt or ^{102}Ru
Integration time [s]	0.2 (^{47}PO), 0.1 (^{59}Co), 0.1 (^{185}Re), 0.1 (^{102}Ru)
Measurement statistics	100 sweeps
Cell gas flow	30% O_2

ESI-MS system	Bruker maXis qTOF
capillary voltage	4.5 kV
gas flow	0.4 bar
dry gas	4 L/min
dry heater	180 °C
quadrupole energy	4 eV
collision energy	8.0 eV
ion cooler transfer time	70 µs
Spectra Rate	1 Hz
ISCID Energy	0 eV or 90 – 170 eV

Table S2 Experimental (m_{exp}) and theoretical (m_{theor}) masses of the detected species during CZE–ESI-MS experiments. The mass error is given in parts per million (Δppm).

compound	species	m_{exp}	m_{theor}	Δppm
Cisplatin	$[DNA + 3H]^{3+}$	803.8227	803.8237	1.2
	$[DNA + Pt(NH_3)_2 + H]^{3+}$	879.8235	879.8254	2.2
	$[DNA + Pt(NH_3)_2Cl + 2H]^{3+}$	891.8143	891.8170	3.0
	$[DNA + 2Pt(NH_3)_2 - H]^{3+}$	955.4902	955.4923	2.2
	$[DNA + Pt(NH_3)_2 + Pt(NH_3)_2Cl]^{3+}$	967.8155	967.8190	3.6
	$[DNA + 2\{Pt(NH_3)_2Cl\} + H]^{3+}$	979.8060	979.8096	3.7
	$[DNA + 3\{Pt(NH_3)_2\} - 3H]^{3+}$	1031.1568	1031.1597	2.8
	$[DNA + 2\{Pt(NH_3)_2\} + Pt(NH_3)_2Cl - 2H]^{3+}$	1043.4814	1043.4852	3.6
	$[ub + 8H]^{8+}$	1071.4592	1071.4608	1.5
	$[ub + Pt(NH_3)_2 + 6H]^{8+}$	1099.9579	1099.9613	3.1
RM175	$[(bip)Ru(en) - H]^{+}$	315.0434	315.0433	0.3
	$[(bip)Ru(en)Cl]^{+}$	351.0198	351.0197	0.3
	$[DNA + 3H]^{3+}$	803.8187	803.8237	6.2
	$[DNA + (bip)Ru(en) - H]^{3+}$	908.4995	908.5032	4.1
	$[DNA + 2\{(bip)Ru(en)\} - H]^{3+}$	1013.1803	1013.1823	2.0
	$[ub + 8H]^{8+}$	1071.4603	1071.4608	0.5
	$[ub + (bip)Ru(en) + 6H]^{8+}$	1110.7154	1110.7155	0.1
	$[DNA + 3\{(bip)Ru(en)\} - 3H]^{3+}$	1117.5243	1117.5278	3.1
	$[2DNA + 4\{(bip)Ru(en)\} - 3H]^{5+}$	1215.6161	1215.6176	1.2
	$[DNA + 3H]^{3+}$	803.8187	803.8237	6.2
[(cym)RuCl(maltolato)]	$[(cym)Ru(maltolato)]^{+}$	361.0382	361.0377	1.4
	$[ub + (cym)Ru + 6H]^{8+}$	1100.7147	1100.7108	3.5
	$[ub + 8H]^{8+}$	1071.5908	1071.5861	4.4
	$[DNA + 2\{(cym)Ru\} - H]^{3+}$	959.8269	959.8239	3.1
	$[DNA + 3H]^{3+}$	803.8274	803.8237	4.6

Table S3 Experimental (m_{exp}) and theoretical (m_{theor}) masses of the selected cisplatin–biomolecule adducts during CZE–ESI-MS^2 experiments. Mass errors are given in parts per million (Δppm). Capital N is a nucleoside monophosphate, n is a nucleobase, HO-N is a nucleoside, and N-f is a nucleoside 3´,5´-diphosphate methylene furane.

compound	species	m_{exp}	m_{theor}	Δppm
ub + $Pt(NH_3)_2$	$[^1MQIF_a{}^4 + Pt - 2H]^+$	685.2149	685.2132	2.5
	$[^1MQIF_b{}^4 + Pt - 2H]^+$	713.2125	713.2081	6.2
DNA + $Pt(NH_3)_2$	$[2g + Pt(NH_3) - H]^+$	513.0793	513.0817	4.7
	$[G + Pt - H]^+$	523.0081	523.0090	1.7
	$[G + Pt(NH_3) - H]^+$	540.0356	540.0355	0.2
	$[G\text{-}f + Pt(NH_3)_2 - H]^+$	735.0667	735.0652	2.0
	$[GG + Pt(NH_3)]^+$	869.0892	869.0881	1.3
	$[TG\text{-}f + Pt(NH_3) - H]^+$	1022.086	1022.0848	1.2
	$[TTG\text{-}f + Pt(NH_3) - H]^+$	1326.1279	1326.1309	2.3
	$[TTG\text{-}f + Pt(NH_3)_2 - H]^+$	1343.1517	1343.1574	4.2
	$[TTGG\text{-}f + Pt(NH_3) - H]^+$	1655.1753	1655.1834	4.9
	$[TTGG\text{-}f + Pt(NH_3)_2 - H]^+$	1672.2055	1672.2099	2.6
	$[HO\text{-}ATTGG\text{-}f + Pt(NH_3) - H]^+$	1888.2554	1888.2750	10.4
DNA + $Pt(NH_3)_2Cl$	$[g + PtCl]^+$	381.9876	381.9823	13.9
	$[g + Pt(NH_3)Cl]^+$	399.0112	399.0089	5.8
	$[G\text{-}f + Pt(NH_3) - H]^+$	718.0389	718.0387	0.3
	$[G\text{-}f + Pt(NH_3)]^+$	755.0161	755.0152	1.2
	$[G\text{-}f + Pt(NH_3)_2Cl]^+$	772.0418	772.0418	0.0
	$[TTG\text{-}f + Pt(NH_3)_2Cl]^+$	1380.1333	1380.1340	0.5
	$[HO\text{-}TTG\text{-}f + Pt(NH_3)_2Cl]^+$	1613.2284	1613.2254	1.9
	$[TTGG\text{-}f + Pt(NH_3)Cl]^+$	1692.1596	1692.1601	0.3
	$[TTGG\text{-}f + Pt(NH_3)_2Cl]^+$	1709.1821	1709.1866	2.6
DNA + 2{$Pt(NH_3)_2$}	$[a + Pt(NH_3) - H]^+$	346.0381	346.038	0.3
	$[g + Pt(NH_3)_2 - H]^+$	379.0596	379.0589	1.8
	$[A + Pt - H]^+$	507.0153	507.014	2.6
	$[2g + Pt(NH_3) - H]^+$	513.0838	513.0817	4.1
	$[G + Pt - H]^+$	523.0095	523.0090	1.0
	$[G + Pt(NH_3) - H]^+$	540.0403	540.0355	8.9
	$[GG + Pt(NH_3)]^+$	869.0846	869.0881	4.0
	$[DNA - C + 2Pt(NH_3)_2 - 2H]^{2+}$	1377.2163	1377.2131	2.3
	$[HO\text{-}ATTGGCA + 2Pt(NH_3)_2 - 2H]^+$	1328.1954	1328.1947	0.5
	$[TTGG\text{-}f + Pt(NH_3) - H]^+$	1655.1949	1655.1834	6.9

Table S3 Cont.

compound	species	m_{exp}	m_{theor}	Δppm
DNA + 3{$Pt(NH_3)_2$}	$[a + Pt(NH_3) - H]^+$	346.0376	346.038	1.2
	$[g + Pt(NH_3)_2 - H]^+$	379.0560	379.0589	7.7
	$[2g + Pt - H]^+$	496.0576	496.0557	3.8
	$[A + Pt - H]^+$	507.0149	507.014	1.8
	$[2g + Pt(NH_3) - H]^+$	513.0847	513.0817	5.8
	$[G + Pt - H]^+$	523.0130	523.0090	7.6
	$[G + Pt(NH_3) - H]^+$	540.0429	540.0355	13.7
	$[HO\text{-}ATTGGCA + 2Pt(NH_3)_2 + Pt(NH_3) - 4H]^{2+}$	1433.1802	1433.1825	1.6

Table S4 Experimental (m_{exp}) and theoretical (m_{theor}) masses of selected RM175–biomolecule adducts during CZE–ESI-MS^2 experiments. Mass errors are given in parts per million (Δppm) Capital N is a nucleoside monophosphate, n is a nucleobase, HO-N is a nucleoside, and N-f is a nucleoside 3´,5´-disphosphate methylene furane.

compound	Species	m_{exp}	m_{theor}	Δppm
DNA + (bip)Ru(en)	$[g + (bip)Ru - H]^+$	406.0263	406.0241	5.4
DNA + 2{(bip)Ru(en)}	$[(bip)Ru(en) - H]^+$	315.0488	315.0433	17.5
	$[(bip)Ru + H_2PO_4]^+$	352.9588	352.9515	20.7
	$[c + (bip)Ru - H]^+$	366.0238	366.0179	16.1
	$[t + (bip)Ru - H]^+$	381.0236	381.0176	15.7
	$[a + (bip)Ru - H]^+$	390.0345	390.0292	13.6
	$[g + (bip)Ru - H]^+$	406.0279	406.0241	9.4
	$[AC + (bip)Ru - H]^+$	875.0877	875.0896	2.2
	$[AC + (bip)Ru(en) - H]^+$	935.1547	935.1584	4.0
	$[CAC + (bip)Ru - H]^+$	1164.1365	1164.1363	0.2
	$[TTGGCAC + (bip)Ru]^{2+}$	1215.6729	1215.6709	1.6
	$[CAC + (bip)Ru(en) - H]^+$	1224.2172	1224.2051	9.9
	$[TTGGCAC + (bip)Ru(en)]^{2+}$	1245.7034	1245.7053	1.5
	$[HO\text{-}ATT\text{-}f + (bip)Ru - H]^+$	1274.1609	1274.1620	0.9
	$[DNA - c + (bip)Ru(en)]^{2+}$	1306.7274	1306.7294	1.5
	$[HO\text{-}ATT\text{-}f + (bip)Ru(en) - H]^+$	1333.2236	1333.2230	0.5
	$[DNA + (bip)Ru(en)]^{2+}$	1362.2491	1362.2511	1.5
	$[DNA - c + 2(bip)Ru - en - 2H]^{2+}$	1433.7099	1433.7137	2.7
	$[DNA - c + 2(bip)Ru(en) - 2H]^{2+}$	1463.7479	1463.7481	0.1
	$[GCAC + (bip)Ru - H]^+$	1493.1880	1493.1891	0.7
	$[DNA + 2(bip)Ru(en) - 2H]^{2+}$	1519.2702	1519.2697	0.3

Table S4 Cont.

compound	Species	m_{exp}	m_{ex}	Δppm
DNA + 2{(bip)Ru(en)}	$[DNA + 2(bip)Ru(en) + Na - H]^{2+}$	1530.7589	1530.7646	3.7
	$[GCAC + (bip)Ru(en) - H]^{+}$	1553.2536	1553.2579	2.8
	$[HO\text{-}ATTG\text{-}f + (bip)Ru - H]^{+}$	1603.2136	1603.2148	0.7
	$[HO\text{-}ATTG\text{-}f + (bip)Ru(en) - H]^{+}$	1663.2836	1663.2836	0.0
	$[HO\text{-}ATTGG\text{-}f + (bip)Ru - H]^{+}$	1932.2673	1932.2677	0.2
	$[HO\text{-}ATTGG\text{-}f + (bip)Ru(en) - H]^{+}$	1992.3356	1992.3365	0.5
DNA + 3{(bip)Ru(en)}	$[(bip)Ru(en) - H]^{+}$	315.0499	315.0433	20.9
	$[(bip)Ru + H_2PO_4]^{+}$	352.9573	352.9515	16.4
	$[c + (bip)Ru - H]^{+}$	366.0243	366.0179	17.5
	$[t + (bip)Ru - H]^{+}$	381.0225	381.0176	12.9
	$[a + (bip)Ru - H]^{+}$	390.0336	390.0292	11.3
	$[g + (bip)Ru - H]^{+}$	406.0278	406.0241	9.1
	$[AC\text{-}OH + (bip)Ru - H]^{+}$	875.0854	875.0896	4.8
	$[DNA - c + 3(bip)Ru(en) - 3H]^{3+}$	975.4967	975.4959	0.8
	$[DNA + (bip)Ru(en)]^{2+}$	1362.2481	1362.2511	2.2
	$[DNA - c + 2(bip)Ru(en) - 2H]^{2+}$	1463.7465	1463.7481	1.1
	$[GCAC + (bip)Ru - H]^{+}$	1493.1854	1493.1891	2.5
	$[DNA + 2(bip)Ru(en) - 2H]^{2+}$	1519.2693	1519.2697	0.3
	$[GCAC + (bip)Ru(en) - H]^{+}$	1553.2569	1553.2579	0.6
	$[HO\text{-}ATTG\text{-}f + (bip)Ru - H]^{+}$	1603.2162	1603.2148	0.9
	$[HO\text{-}ATTG\text{-}f + (bip)Ru(en) - H]^{+}$	1663.2844	1663.2836	0.5
	$[HO\text{-}ATTGG\text{-}f + (bip)Ru - H]^{+}$	1932.2660	1932.2677	0.9

Figure S1 Experimental and simulated mass spectra of selected fragments of the DNA + $Pt(NH_3)_2Cl$ mono-adduct depicting the additional isotopic pattern of chloride.

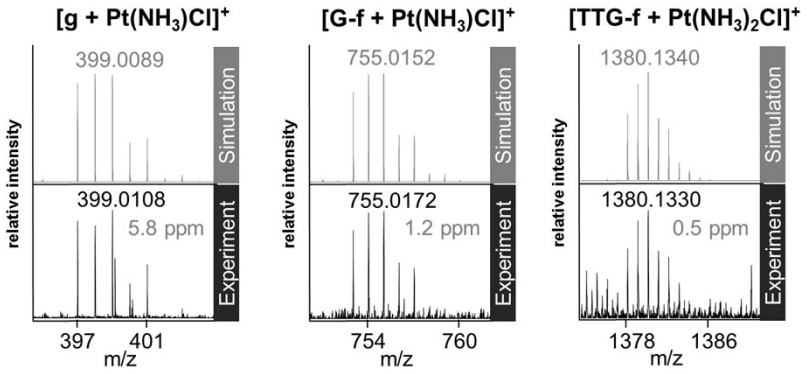


Figure S2 Top-down mass spectrum of the ubiquitin-cisplatin mono-adduct ub + $Pt(NH_3)_2$.

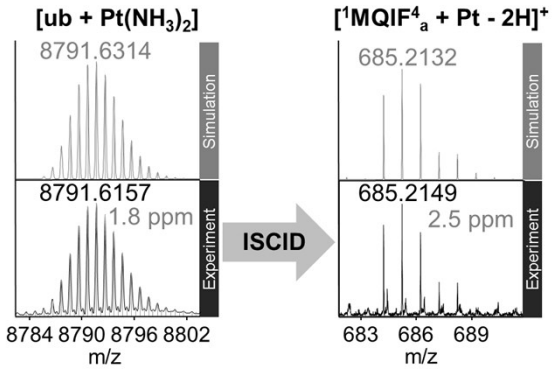
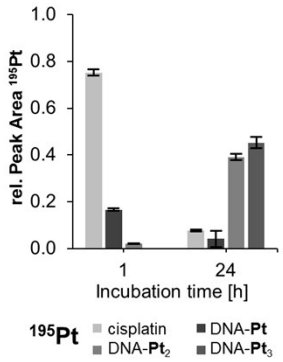
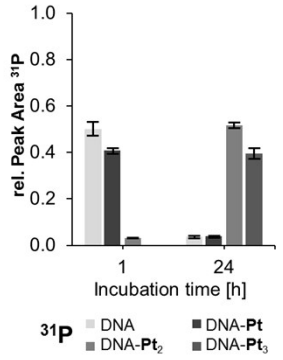


Figure S3 Plot of the relative peak areas of the ^{195}Pt and ^{31}P traces (n = 3) corresponding to selected species from the interaction between cisplatin and the oligonucleotide.



	rel. Peak Area ^{195}Pt			
	1h	**RSD**	**24h**	**RSD**
cisplatin	0.753 ±0.013	1.7	0.078 ±0.004	4.7
DNA-**Pt**	0.166 ±0.006	3.7	0.041 ±0.035	84.8
DNA-Pt_2	0.021 ±0.002	7.7	0.391 ±0.012	3.2
DNA-Pt_3	-	-	0.452 ±0.034	7.5



	rel. Peak Area ^{31}P			
	1h	**RSD**	**24h**	**RSD**
DNA	0.495 ±0.045	9.1	0.032 ±0.010	32.3
DNA-**Pt**	0.402 ±0.015	3.6	0.033 ±0.009	25.9
DNA-Pt_2	0.033 ±0.008	25.3	0.527 ±0.026	5.0
DNA-Pt_3	-	-	0.402 ±0.019	4.6

Figure S4 Selected fragments from the top-down analysis of a reaction mixture containing RM175 and the oligonucleotide. The metallated adducts selected for top-down analysis correspond to DNA + 3{(bip)Ru(en)} (**A**) and DNA + {(bip)Ru(en)} (**B**).

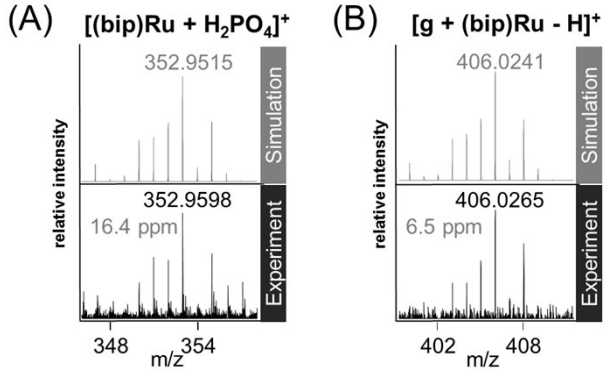


Figure S5 Extracted ion electropherograms (EIEs) of the [(cym)RuCl(maltolato)]-biomolecule adducts by CZE-ESI-MS (**A**) and CZE-ICP-MS (**B**) after incubation with a mixture of oligonucleotide and protein (1:1) for 1 and 24 h.

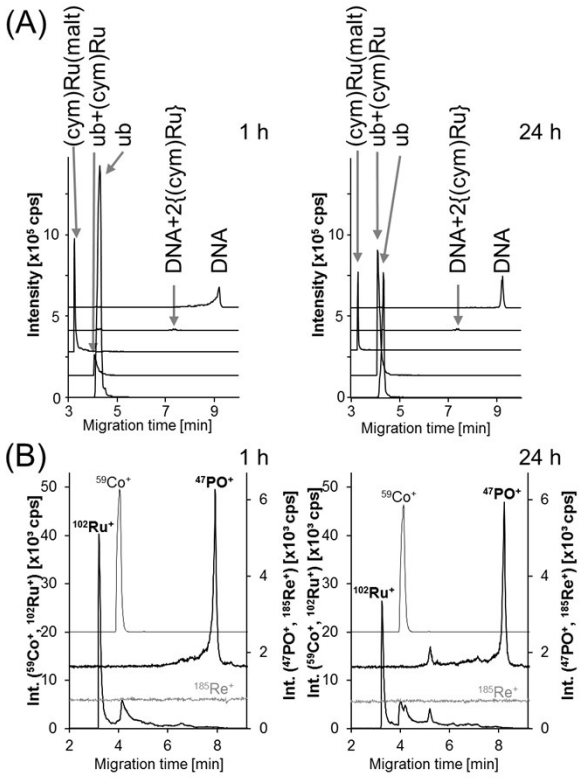
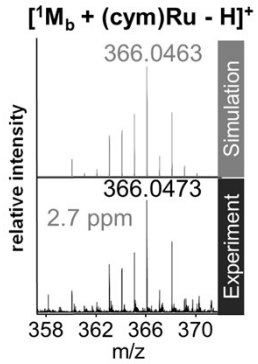


Figure S6 Selected fragment from the top-down analysis of the ub + (cym)Ru adduct from the reaction identifying Met1 as the primary binding site of [(cym)RuCl(maltolato)] on ub.



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