

Supporting Information

Cisplatin binding to angiogenin protein: new molecular pathways and targets for the drug anticancer activity

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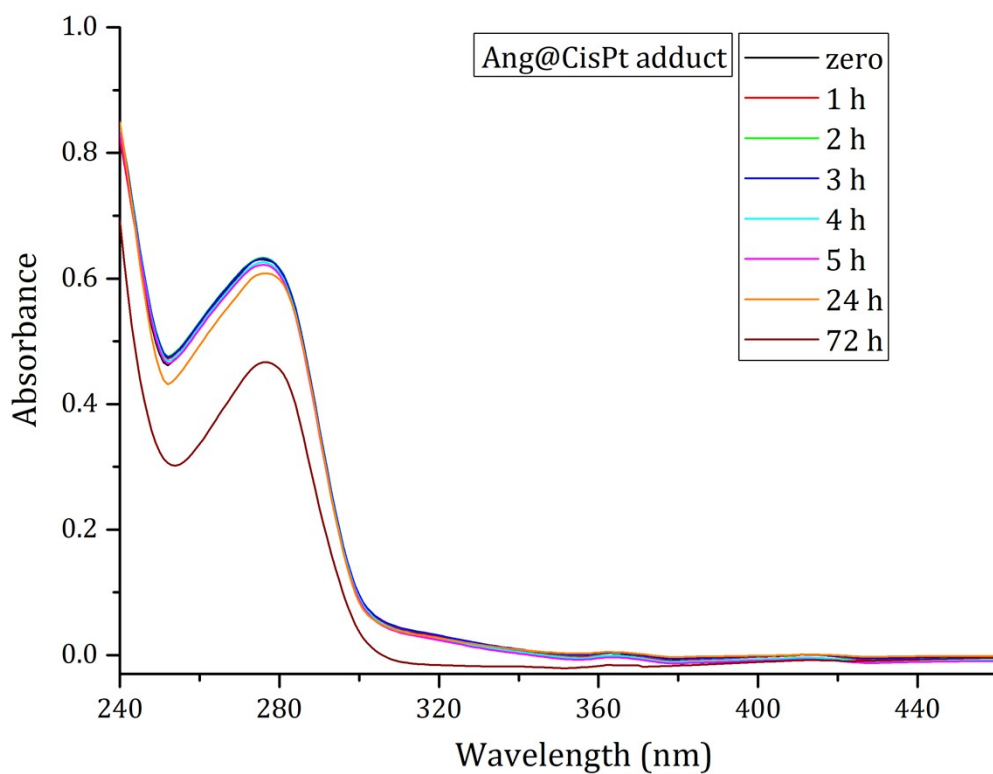
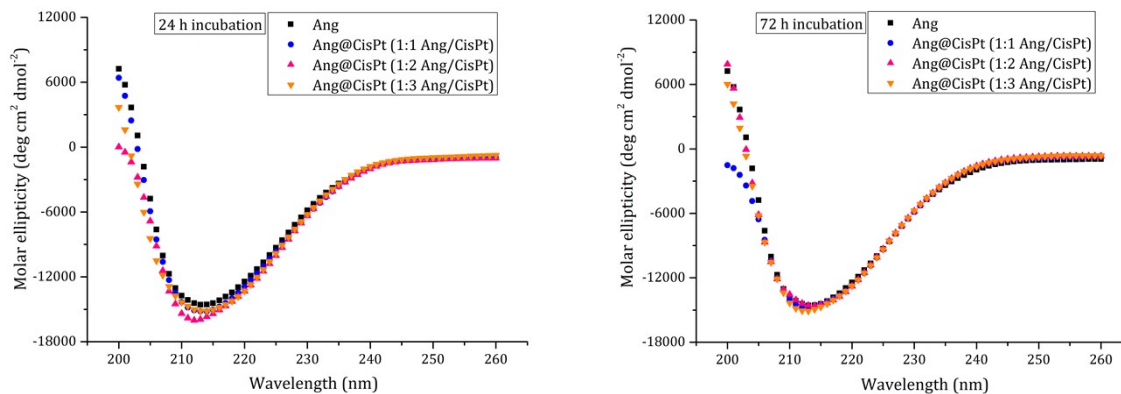


Figure S1. UV-vis absorption spectra of the Ang@CisPt adduct obtained upon incubation of the protein with the metal compound in a 1:3 protein to metal molar ratio. Spectra were collected at different incubation times in 20 mM sodium citrate buffer pH 5.1. Experimental settings: protein concentration of 0.5 mg mL^{-1} ($35 \text{ }\mu\text{M}$), range 240–500 nm, bandwidth 2.0 nm, scanning speed 200 nm min^{-1} , data pitch 1.0 nm, optical path-length quartz cell 0.1 cm.



A

B

Figure S2. Circular dichroism spectra of the Ang@CisPt adduct obtained upon incubation of the protein with the metal compound at different protein to metal ratio (1:1, 1:2 and 1:3) compared to the spectrum of the metal-free protein (black squares) in 20 mM sodium citrate buffer pH 5.1 after 24 h (panel A) and 72 h (panel B) of incubation at 37 °C. Experimental settings: protein concentration of 0.2 mg mL⁻¹ (14 μM), range 200-250 nm, scanning speed 50 nm min⁻¹, bandwidth 2.0 nm, resolution 1.0 nm, sensitivity 50 mdeg, and response 2 s.

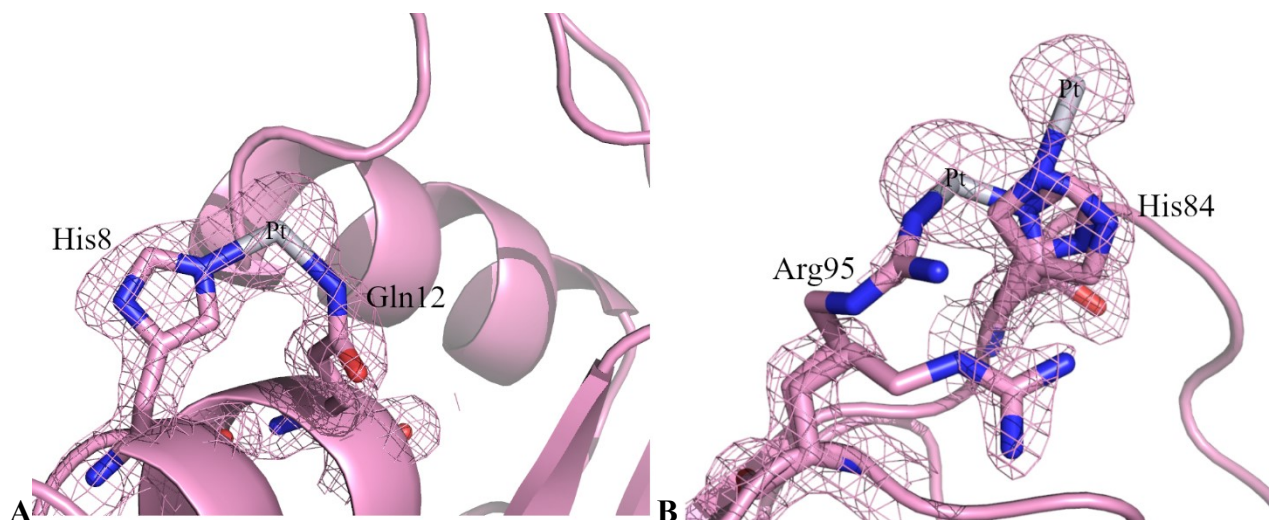


Figure S3. Pt binding sites in the structure of oxaliplatin/Ang²³. The metal center binds the side chains of His8 and Gln12 (A) and His84 and Arg95 (B). 2Fo-Fc electron density maps are contoured at 1.0 σ (light pink).

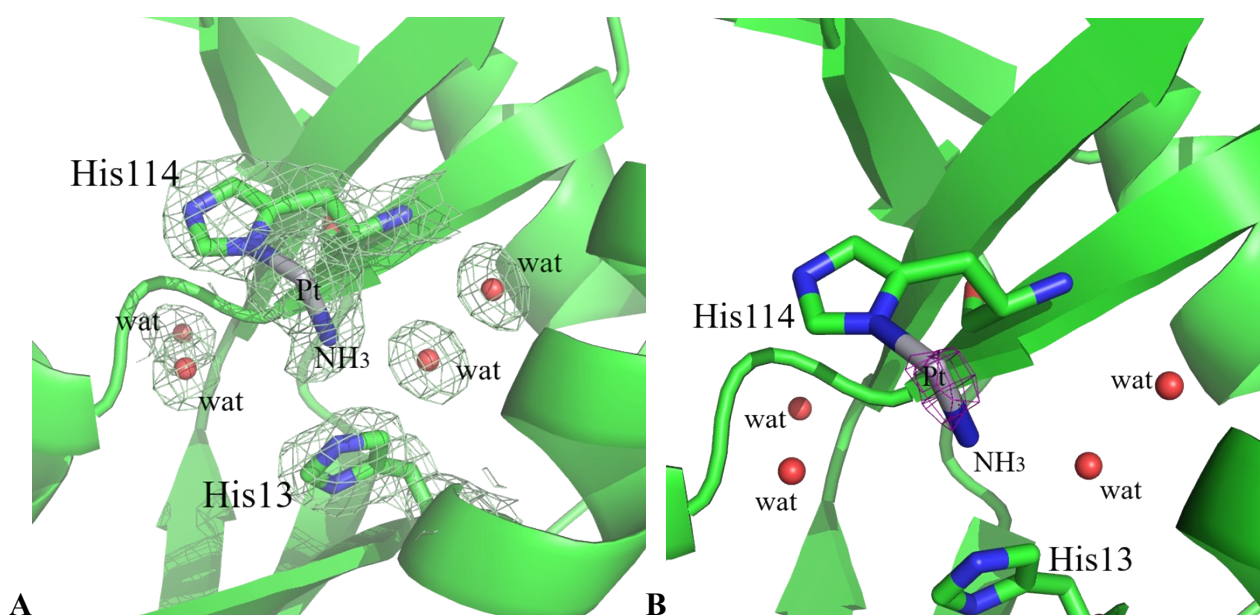


Figure S4. Details of the CisPt fragment binding site in the Ang@CisPt adduct formed in a second crystal. In panel A, 2Fo - Fc electron density maps are contoured at the 1.0 σ level (green); in panel B anomalous difference electron density map is reported at 2.0 σ level (violet).

Table S1. Data collection and refinement statistics of the structures of two Ang@CisPt adducts*Data collection*

PDB code	8003	8004
Soaking time	5 days	1 month
Space group	P 2 ₁ 2 ₁ 2	P 2 ₁ 2 ₁ 2
a (Å)	85.32	85.25
b (Å)	37.48	37.51
c (Å)	38.33	38.16
α β γ (°)	90.0	90.0
Resolution range (Å)	42.66 - 1.76	42.63-1.99
	1.79-1.76	1.99-2.02
Observations	131250 (3414)	85963 (2091)
Unique reflections	12229 (535)	8572 (414)
Completeness (%)	95.0 (83.2)	96.0 (98.1)
Redundancy	10.7 (6.4)	10.0 (5.1)
†Rmerge (%)	0.083 (0.664)	0.083 (0.581)
Average I/σ(I)	19.1 (2.3)	17.1 (2.4)
CC _{1/2}	1.00 (0.856)	0.996 (0.927)
Anom. completeness (%)	94.6 (81.7)	95.3 (97.6)
Anom. Multiplicity	5.8 (3.4)	5.5 (2.7)

Refinement

Resolution range (Å)	42.66-1.76	42.62-1.99
N. of reflections (working set)	11495	8145
N. of reflections (test set)	619	557
R-factor/R-free (%)	24.54/29.04	31.02/36.72
N. of residues	122	122
N. of atoms	1124	1051
Average B-factors (Å ²)		
All atoms	20.82	23.29
Pt atoms	47.87	63.66
R.m.s. deviations		
Bond lengths (Å)	0.011	0.006
Bond angles (°)	1.724	1.526
Ramachandran statistics (Validation Report)		
Favoured	119 (96%)	110 (91%)
Allowed	4 (3%)	10 (8%)
Outliers	1 (1%)	1 (1%)

†Rmerge = $\frac{\sum h \sum i |I(h,i) - \langle I(h) \rangle|}{\sum h \sum i I(h,i)}$, where I(h,i) is the intensity of the ith measurement of reflection h and $\langle I(h) \rangle$ is the mean value of the intensity of reflection h.

Criteria used in determination of resolution cut:

R_{pim} ≤ 0.6000

I/σ(I) ≥ 2.00

CC(1/2) ≥ 0.3000

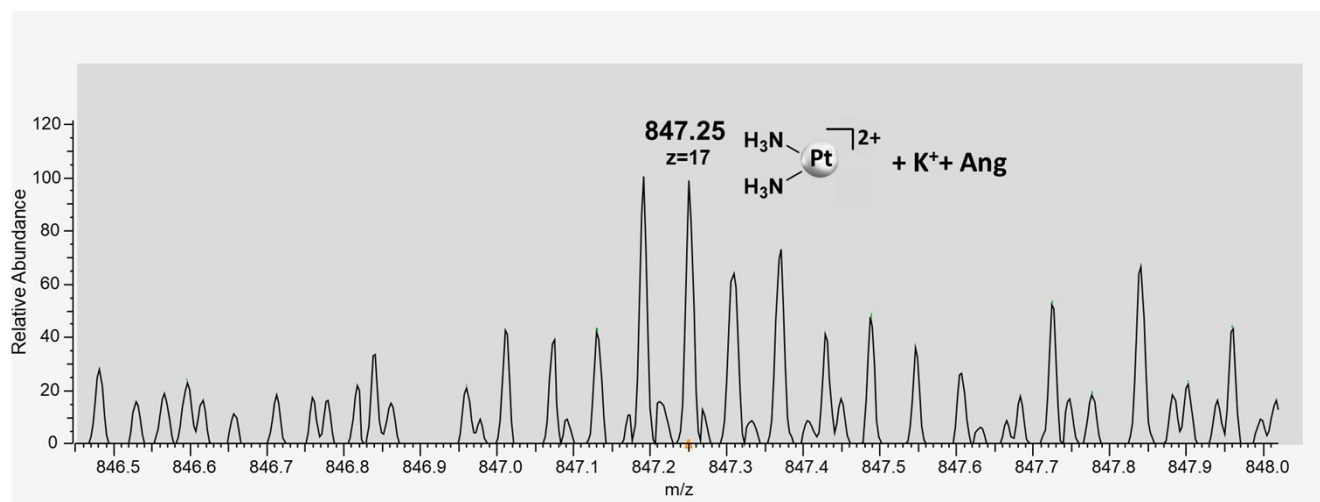
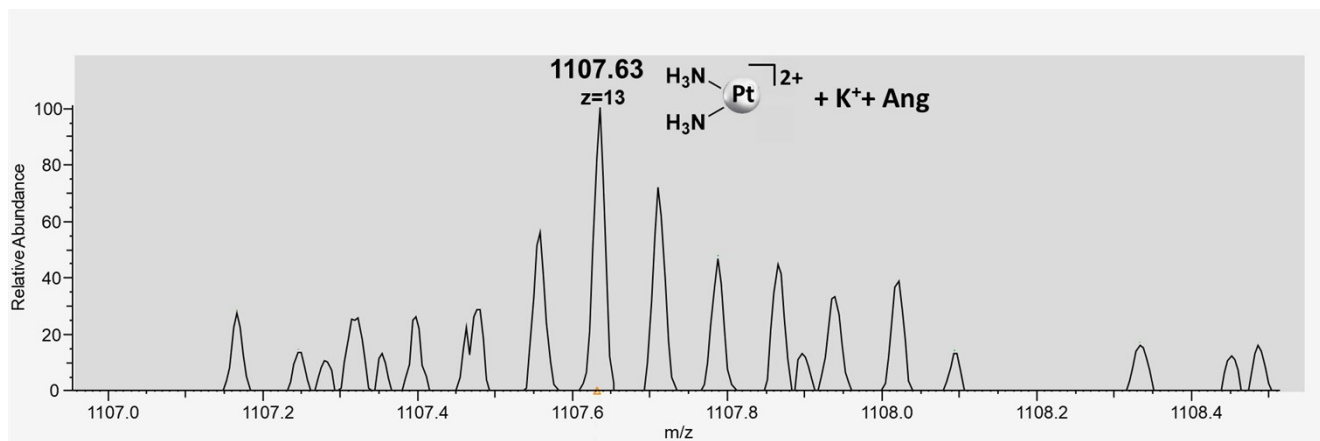
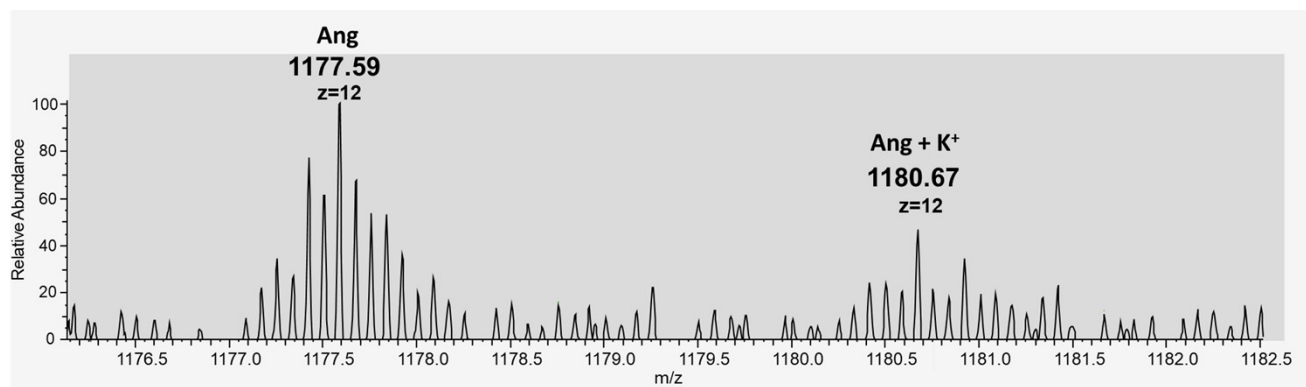


Figure S5. Mass spectra of Ang 10^{-6} M in water (top). The peak at m/z 1177.59 corresponds to the protein where the first amino acid residue, i.e. glutamine, is present as pyroglutamic acid. Middle and bottom: Ang (10^{-6} M) incubated with CisPt (protein to metal molar ratio 1:5) in water at 37 °C for 48 h.