Materials and analytical techniques: Both HEWL (L7651) and RNase A (R5500) were purchased from Sigma-Aldrich and used without further purifications; *t*-$PtCl_2$(dma)(ma) was synthesized as previously reported.[1]

ESI-MS. A solution of *t*-$PtCl_2$(dma)(ma) (10^{-4} M) with HEWL or RNase (3:1 metal/protein ratio) was incubated at 37°C for 72 h and ESI MS spectra were recorded after 24 and 72 h. After a 20-fold dilution with water, ESI MS spectra were recorded by direct introduction at 5 µl/min flow rate in an Orbitrap high-resolution mass spectrometer (Thermo, San Jose, CA, USA), equipped with a conventional ESI source. The working conditions were the following: spray voltage 3.1 kV, capillary voltage 45 V, capillary temperature 220°C, tube lens voltage 230 V. The sheath and the auxiliary gases were set, respectively, at 17 (arbitrary units) and 1 (arbitrary units). For acquisition, Xcalibur 2.0. software (Thermo) was used and monoisotopic and average deconvoluted masses were obtained by using the integrated Xtract tool. For spectrum acquisition, a nominal resolution (at m/z 400) of 100,000 was used.

Crystallization, data collection, structure solution and refinement

Crystals of the adduct between HEWL and *t*-$PtCl_2$(dma)(ma) were obtained by cocrystallization experiments, using a 15 mg mL^{-1} protein solution and a protein-metallodrug molar ratio of 1:10. Crystals suitable for X-Ray diffraction analysis grew from solutions containing 0.6 M $NaNO_3$, 20% ethylene glycole and 0.1 M sodium acetate, at pH 4.2 in few days. Crystals of the adduct between RNase A and *t*-$PtCl_2$(dma)(ma) were obtained by soaking experiments: we first grew ligand-free RNase A crystals, by using a reservoir solution containing 20% PEG4000 and 10 mM sodium citrate pH 5.0 and a protein solution at 20 mg mL^{-1} concentration, then we soaked these crystals with solutions containing 22% PEG4000 and an excess of the Pt compound at 1:10 protein to metal molar ratio. Crystals were incubated for five days prior to data collection. In the case of both HEWL and RNase A, the hanging drop vapour diffusion method was used, by mixing 1µL of protein with 1µL of reservoir solution.

Crystals were fished with nylon loops and the surrounding solvent was completely removed prior to flash cooling at 100K in supercooled N_2 gas (Oxford Cryosystems). Diffraction data of the adducts between *t*-$PtCl_2$(dma)(ma) and the two model proteins were collected in-house at the Institute of Biostructures and Bioimages – CNR, Naples, Italy, on a Saturn 944 CCD detector. The X-ray radiation used was Cu Kα radiation from a Rigaku MicroMax-007 HF generator. Diffraction data were processed and scaled by using HKL2000.[2] Crystals of HEWL-[*t*-$PtCl_2$(dma)(ma)] adduct belong to the tetragonal space group $P43_12$ and diffract up to 2.5 Å resolution, whereas those of RNase A-[*t*-$PtCl_2$(dma)(ma)]

adduct belong to the monoclinic space group C2 and diffract up to 2.0 Å resolution. Details of data collection and processing statistics are in Table 1.

The structure of both the adducts with the Pt compound were solved by Fourier difference method, using the PDB files 193L[3] and 1JVT[4] as starting models for HEWL and RNase A adducts, respectively. Refinement was performed with Refmac5,[5] model building and map analysis were performed with Wincoot.[6] The analysis of Fourier difference maps, calculated with (Fo–Fc) and (2Fo–Fc) coefficients at various stages of refinement, allowed the positioning of two Pt compounds for HEWL molecule and four Pt compounds for each RNase A molecule in the asymmetric unit. Occupancy of metallodrugs was manually adjusted monitoring Rfactor/Rfree, B values and positive and negative peaks in the (Fo-Fc) and (2Fo-Fc) electron density maps.

The final model of HEWL-[*t*-$PtCl_2$(dma)(ma)] has R-factor and Rfree values of 0.179 and 0.259, respectively; RNase A-[*t*-$PtCl_2$(dma)(ma)] refines up to R-factor and Rfree values of 0.214 and 0.275. At the end of refinement, the geometry of protein structures was monitored using Wincoot[6] and CCP4[7] routines. Statistics and parameters of the refinement are given in Table 2. In the case of HEWL-[t-PtCl2(dma)(ma)], the quality of the electron density maps does not allow to obtain information on the Pt ligands. On the other hand, the inspection of the electron density maps of the RNase A-[t-PtCl2(dma)(ma)] adduct unambiguously demonstrates that Pt complex retains the nitrogen ligands upon the binding. In this last protein-metallodrug complex, it should be noted that a peak of electron density is found at a distance of 2.6 Å from the Pt center bound to His105. We have modelled this peak as the fourth, loosely coordinated, ligand of Pt, but we cannot exclude that this peak is due to the presence of a water molecule at partial occupancy.

All the figures were prepared with PyMOL (http://pymol.org). The coordinates of the structures have been deposited in the Protein Data Bank (entry 4QGZ for HEWL adduct and 4QH3 for RNase A adduct).

Table 1. X-ray diffraction data-collection and processing statistics of the adducts formed when *t*-$PtCl_2$(dma)(ma) reacts with HEWL and RNase A.

	HEWL-*t*-$PtCl_2$(dma)(ma)	RNase A-*t*-$PtCl_2$(dma)(ma)
Space group	$P4_32_12$	C2
Cell parameters		
a(Å)	77.920	100.824
b(Å)	77.920	32.537
c(Å)	36.921	67.237
β(°)	90	
Resolution range (Å)	55.1 – 2.50 (2.54 – 2.50)	67.9 – 2.00 (2.03-2.00)
Observations	92052	39621
Unique reflections	4246	14538
Completeness (%)	100.0 (100.0)	96.5 (95.4)
I/σ(I)	16.0 (4.1)	22.1 (2.4)
Redundancy	21.7 (17.4)	2.7 (1.9)
Rmerge (%)	11.2 (83.5)	8.2 (48.4)
Mosaicity	1.7	2.0

Values in parenthesis refer to highest resolution shell

Table 2. Refinement statistics of the adducts formed when *t*-$PtCl_2$(dma)(ma) reacts with HEWL and RNase A.

	HEWL-*t*-$PtCl_2$(dma)(ma)	RNase A-*t*-$PtCl_2$(dma)(ma)
PDB code	4QGZ	4QH3
Resolution limits (Å)	55.1-2.51	67.9 – 2.00
No. of reflections used in refinement	4019	13780
No. of reflections in working set	3826	13046
No. of reflections in test set	193	734
R factor/Rfree	0.179/0.259	0.214/0.275
No. of protein atoms	1012	1847
No. of water molecules	62	89
No. of ligand atoms	8	48
R.m.s.d. from ideal values		
Bond lengths (Å)	0.019	0.014
Bond angles (°)	1.91	1.62
Average B factors (Å^2)		
Protein	52.17	45.73
Ligand	44.20	52.39
Solvent	49.48	38.68
Ramachandran values (%)		
Most favoured/ Additional allowed	96.0/4.0	92.1/6.2
Generously allowed/ Disallowed	0/0	1.7

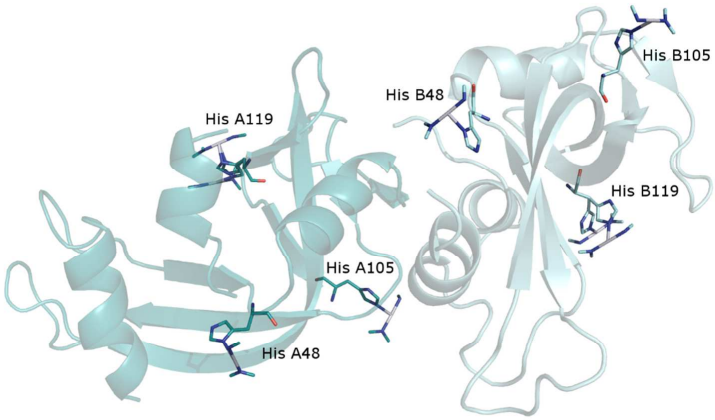

Figure S1. Cartoon representation of the asymmetric unit in the crystals of the adduct between RNase A and [*t*-$PtCl_2$(dma)(ma)]. The side chains of His48, His105 and His119, which are the residues involved in the Pt compound recognition, are shown along with Pt and its ligands. The two RNase A molecules in the asymmetric unit are coloured in green and light green.

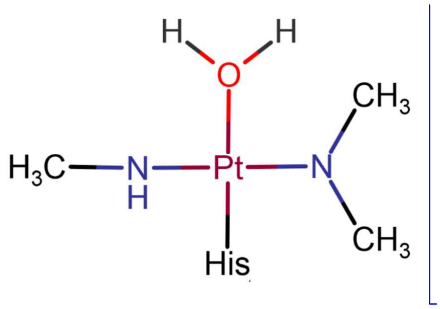

Figure S2. Schematic representation of the [*t*-$PtOH_2$(dma)(ma)] fragment bound to His105 upon the interaction between RNase A and [*t*-$PtCl_2$(dma)(ma)].

Comment [p1]: NH2(CH3) there is one hydrogen missing

Comment [p2]: NH(CH3)2… there is also one hydrogen missing

REFERENCES

[1] L. Cubo, A. G. Quiroga, j. Zhang, D. S. Thomas, A. Carnero, C. Navarro-Ranninger, S. J. Berners-Price *Dalton Trans.* 2009, 3457-3466.

[2] Otwinowski Z., Minor W. *Method Enzymol* 1997, **276**, 307-326.

[3] Vaney, M.C., Maignan, S., Riès-Kautt, M., Ducriux, A. *Acta Crystallogr D Biol Crystallogr*. 1996, **52**, 505-17.

[4] Vitagliano, L., Merlino, A., Zagari, A., Mazzarella, L. *Proteins* 2002, **46**, 97-104.

[5] Murshudov, G.N., Skubak, P., Lebedev, A.A., Pannu, N.S., Steiner, R.A., Nicholls, R.A., Winn, M.D., Long, F., Vagin, A.A. *Acta Crystallogr D Biol Crystallogr*. 2011, **67,** 355-367.

[6] Emsley, P., Lohkamp, B., Scott, W.G., Cowtan, K. *Acta Crystallogr D Biol Crystallogr* 2010, **66**, 486-501

[7] Collaborative Computational Project, Number 4. 1994. "The CCP4 Suite: Programs for Protein Crystallography". A *Acta Crystallogr D Biol Crystallogr* **50**, 760-763