Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Crystal X-ray Structure for the Complex formed in the Reaction between Oxaliplatin and Lysozyme

*Luigi Messori, Tiziano Marzo and Antonello Merlino**

Crystallization, Data collection and refinement

Oxaliplatin and hen egg white lysozyme were obtained from Sigma. Crystals of HEWL in the presence of Oxaliplatin were grown by hanging-drop vapor diffusion technique at 298 K mixing a protein solution containing 15 mg mL^{-1} of HEWL incubated for 24 h with an excess of Oxaliplatin (protein to metal drug ration 1:10) with equal volumes of reservoir solution. Best crystals grow within 2-4 days from the following conditions: 0.6 M $NaNO_3$, 0.1 M sodium acetate pH 4.4 and 20 % ethylene glycol. X-ray diffraction data were collected from a single crystal at the CNR Institute of Biostructure and Bioimages, Naples, Italy, using a Saturn944 CCD detector equipped with CuKα X-ray radiation from a Rigaku Micromax 007 HF generator. Crystals were slowly dehydrated at air [1] and flash-frozen at 100 K using nitrogen gas produced by an Oxford Cryosystem (and maintained at 100 K during the data collection) without using cryoprotectants [2], following the procedure used in other works [3]. Data set was processed and scaled using the HKL2000 package [4]. Data collection statistics are reported in Table S1.
The structure was solved by molecular replacement method, using the PDB file 4J1A [5], without water molecules and ligands, as starting model. The refinement was carried out with Refmac5.7 [6], model building and map inspections were performed using Coot [7]. 5% of the data was used for calculation of the R-free value. After several rounds of refinement using the maximum likelihood option in Refmac, manual adjustments of side-chain atoms and addition of water molecules to the coordinates, the structure converged to Rfactor of 0.180 and to Rfree of 0.235. Refinement statistics are reported in Table S1. Structure validation has been carried out using Procheck [8]. Coordinates and structure factors were deposited in the Protein Data Bank (PDB code 4PPO).
Distances between Pt and the other ligands are reported in Table S2.

ESI-MS experiments

A solution of Oxaliplatin ($3*10^{-4}$ mol L^{-1}) with HEWL (3:1 complex/protein molar ratio) was incubated in ammomium acetate buffer solution 20 mM pH=6.8 at 37°C for 72 h and ESI MS spectra were recorded after 6, 24, 48, 72 h (Fig. S1). After a 20-fold dilution with water, ESI MS spectra were recorded by direct introduction at 5 µL min^{-1} 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.

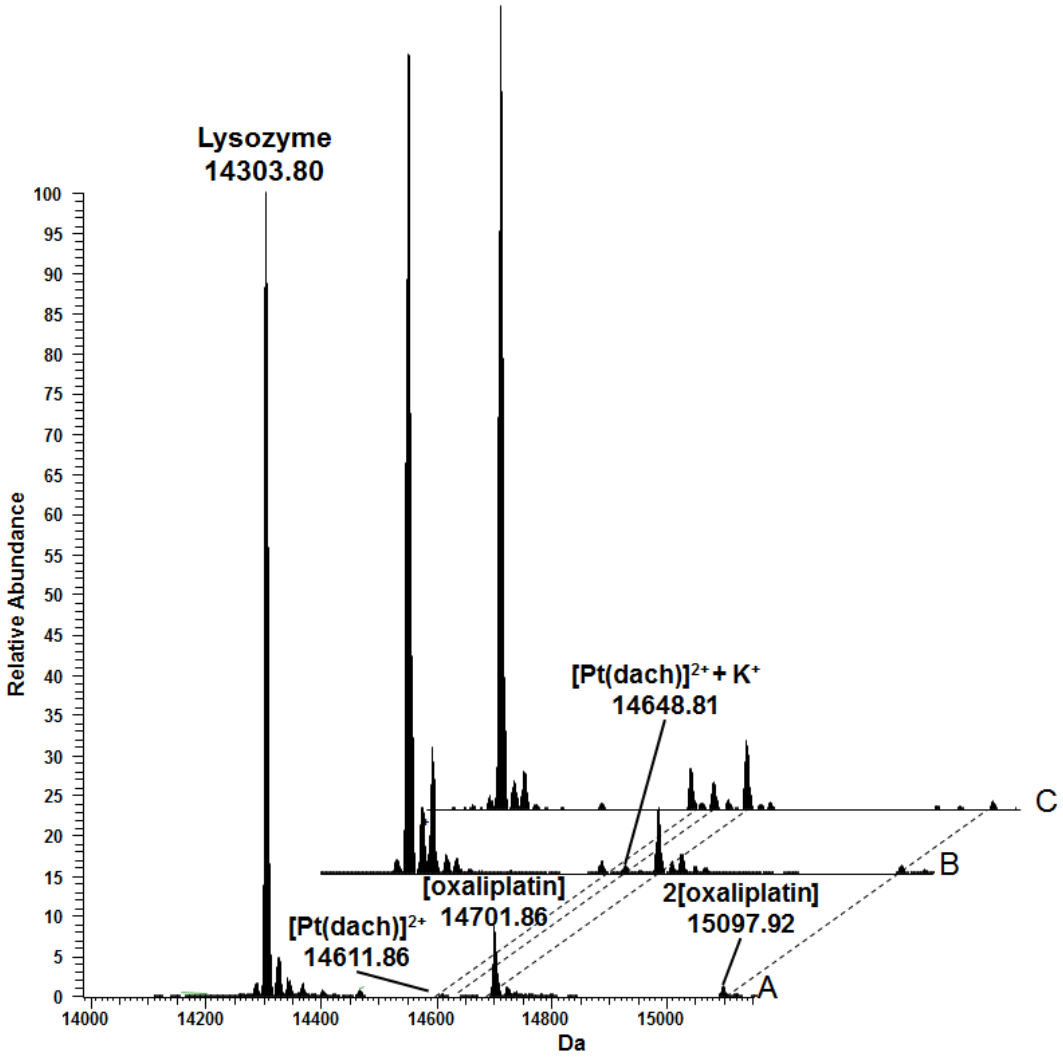

Fig S1. Deconvoluted ESI MS of HEWL treated with $3*10^{-4}$ mol L^{-1} Oxaliplatin (metal:protein ratio = 3:1 in 20 mmol L^{-1} ammonium acetate buffer, pH 6.8) recorded after 6h (A), 24h (B), 48h (C) of incubation at 37°C.

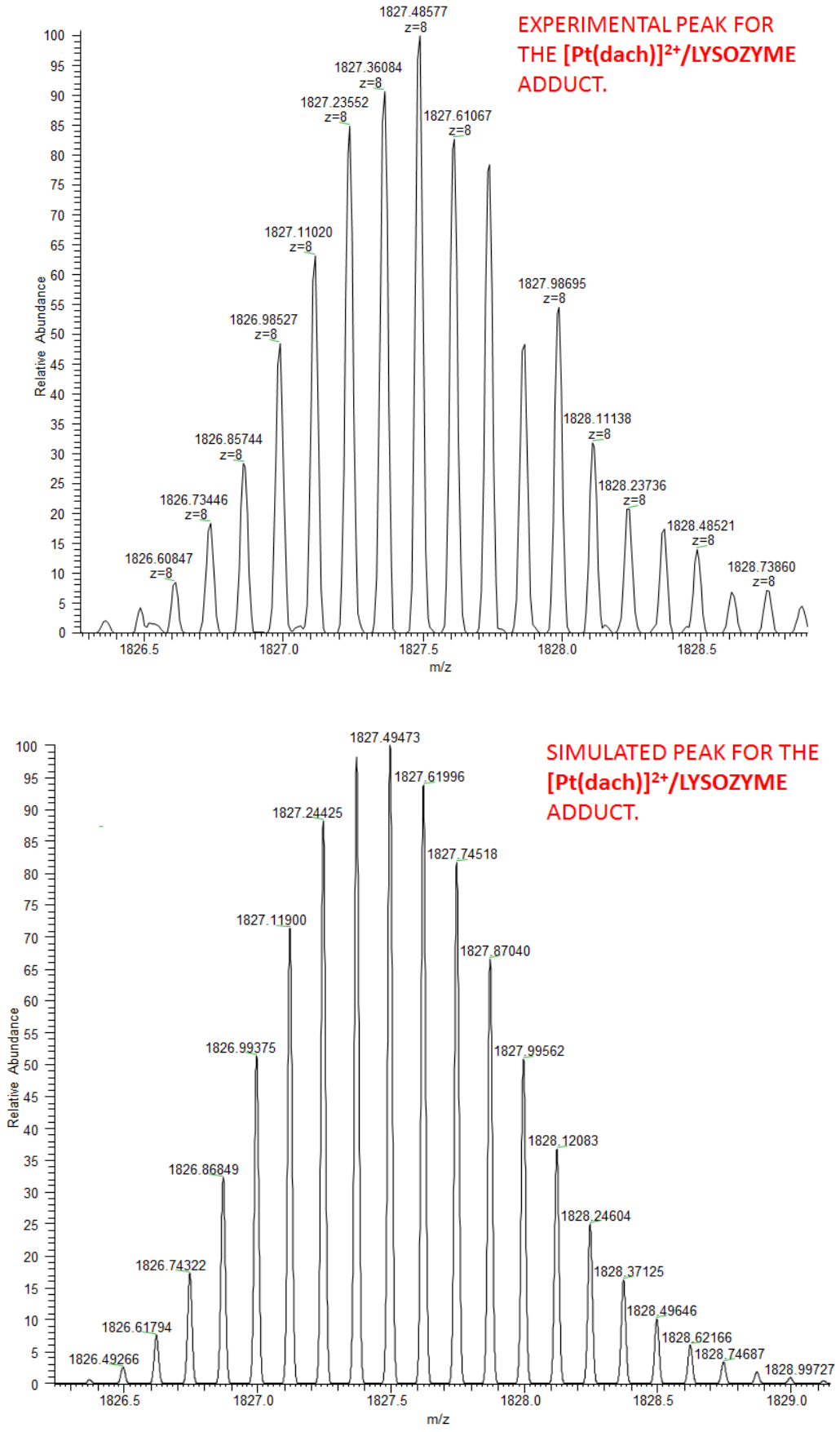

Fig S2. Direct comparison of the experimental ESI MS peak for the $[Pt(dach)]^{2+}$/LYSOZYME species with simulated isotopic pattern

References:

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Table S1. Data collection and refinement statistics for the Oxaliplatin-HEWL structure

PDB code	4PPO
Data-collection	
Space group	$P4_32_12$
Unit cell parameters	
a=b,c (Å)	77.05,37.29
Molecules per asymmetric unit	1
Observed reflections	44549
Unique reflections	12001
Resolution (Å)	50-1.73 (1.76-1.73)
Completeness (%)	97.8 (96.8)
Rmerge	0.088 (0.393)
I/σ(I)	11.8 (2.8)
Multiplicity	3.7 (2.3)
Refinement	
Resolution (Å)	30.8-1.73
number of reflections in working set	11406
number of reflections in test set	570
R factor/Rfree (%)	0.180 (0.235)
Number of non-H atoms used in the refinement	1163
Occupancy of Pt ion	0.70
B-factor of Pt ion	48.3
Ramachandran values (%)	
Most favoured/ Additional allowed	88.5/11.5
Generously allowed/ Disallowed	0/0
R.m.s.d. bonds (Å)	0.019
R.m.s.d. angles(Å)	1.85

Table S2. Selected bond lengths (Å) and angles (°) for the Oxaliplatin binding site in the adduct formed with HEWL, in the crystal structure of the Oxaliplatin (CSD code CUHKEV) [9] and in the structure of the adduct with 1,2-d(GpG) (PDB code 1IHH) [10].

	In the adduct	in the CSD structure [9]	in the structure of the adduct with 1,2-D(GpG) [10].
PDB code	4PPO	CUHKEV	1IHH
Pt-OD1 Asp119	2.05	-	-
Pt-O	1.98	2.01-2.03	-
Pt-N	1.98-2.10	2.04-2.06	2.03-2.04
Pt-N (DNA)	-		1.94-1.98
N-Pt-O angle (°)	94.2	98.4-95.4	
O-Pt-O angle (°)	94.5	82.5	
N-Pt-N angle (°)	86.0	83.9	84.0
N-Pt- N(DNA) angle (°)	-	-	79.6-97.7
N (DNA)-Pt-N(DNA) angle (°)	-	-	98.4