Supplementary Information

Platination of the copper transporter ATP7A involved in anticancer drug resistance

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1. Geometry optimization of the Pt-moieties in II and A-C models. DFT-based geometry optimizations were carried out on the reduced model compounds *cis*-[Pt(SH$_2$)$_2$(NH$_3$)$_2$]$^{2+}$ (with NH$_3$ *trans* to SH$_2$), *trans*-[Pt(SH$_2$)$_2$(NH$_3$)$_2$]$^{2+}$ (with NH$_3$ *trans* to NH$_3$), and [Pt(SH$_2$)(NH$_3$)$_3$]$^{2+}$, using the Gaussian09 code.[1] We used the hybrid B3LYP[2] recipe for the exchange−correlation functional and the 6-31G(d,p) basis set with polarization functions[3-6] for the S, N and H atoms and the LanL2DZ basis set[7, 8] for the Pt atom. The structural parameters resulting from this geometry optimization are reported in Table S1.

2. Classical MD simulations. 250ns- and 400ns-long classical MD simulations were carried out for **II** and **A-C** Pt-models and **I** apo model, respectively, using the NAMD code.[9] For the peptide, water molecules, and counterions we used, respectively, the AMBER parm99 force field[10] with the "Stony Brook" modification[11] for the backbone torsions, the TIP3P[12] water model, and the Smith&Dang force field.[13] For the Pt-moieties we built an Amber-type force field. The equilibrium bond lengths, the equilibrium bond angles, and the equilibrium improper dihedral angles of the four bonds involving the Pt atom in each adduct were fixed to the values obtained from the optimized structures of the corresponding reduced model compounds (Table S1). Equilibrium bond angles $\widehat{\text{CB-S-Pt}}$ and $\widehat{\text{CA-N-Pt}}$ have been fixed according to the experimental values observed by X-ray spectroscopy for the complex Atox1-cisplatin[14] in the monomeric form (Table S2). The force constants of the bonded interaction terms of the Amber-like force field have been guessed, as far as possible, according to the typical values obtained in our previous study on the Ctr1-cisplatin complex[15] (Table S3 and S4). The partial charges for the atoms in the Pt-moieties in the models were calculated by performing a standard RESP parameterization[16] based on gas phase DFT calculations (same level of theory as for the optimization step) of the reduced model-compounds (see Table S5). In particular, the charges of the SH$_2$ and NH$_3$ groups, which are replaced by bonds with the S and N atoms of the cysteine residues in the models, have been transferred to the Pt atom (see Table S6). Cys19 and Cys22 in adduct **II**, and Cys22 in adduct **A**, have been modeled using the deprotonated cysteine residue CYM (charge -1) of the Amber library; while to model Cys19 in **A** and **B** and Cys22 in **C**, we have modified the residue CYM by removing the HN atom bound to the nitrogen and distributing its charge, plus an additional -1 charge, over the rest of the atoms, in order to get a final charge

of -2. This modified residue is indicated as CYN in Fig. 1. This leads to a null total charge for the Pt-moiety of **II B**, and **C** and to a charge -1 for model **A**.

MD simulations were carried out in the NPT ensemble with a target temperature of 300 K and a target pressure of 1 bar by using Langevin dynamics with a coupling coefficient of 1 ps and the Langevin piston Nosé-Hoover algorithm[17] with an oscillation period of 100 fs and a damping time scale of 50 fs. The SETTLE algorithm[18] was employed to constrain the water dynamics. Periodic boundary conditions were applied. Long-range electrostatic interactions were evaluated using the Particle Mesh Ewald (PME) method.[19] The cutoff for the real space part of the PME and for the van der Waals interactions was set to 12 Å. The integration time step was set to 1 fs with a sampling time step of 10 ps.

3. NMR chemical shifts. For ^{1}H nuclei the chemical shielding constants σ were converted to NMR chemical shifts δ using the shielding constant of tetramethylsilane (TMS):[20]

$$\delta^{\mathrm{H}}(\mathrm{X}) = \sigma^{\mathrm{H}}(\mathrm{TMS}) - \sigma^{\mathrm{H}}(\mathrm{X}). \tag{1}$$

$\sigma^{\mathrm{H}}(\mathrm{TMS})$ is the ^{1}H chemical shielding calculated for an isolated TMS molecule using the same DFT level of theory.

To reduce systematic errors in calculating the chemical shifts of ^{13}C and ^{15}N nuclei we introduced secondary references, namely C_2H_6 for ^{13}C,[20] and cisplatin, $Pt(NH_3)_2Cl_2$ in water, for ^{15}N. Indeed, it has been shown that the core electrons contribution to the chemical shifts is almost constant,[21] and using as a reference a compound with similar coordination, the error due to the frozen core approximation essentially cancels out.[22]

Knowing the experimental value of the chemical shift ($\delta_{\mathrm{exp}}^{\mathrm{C}}$) of C_2H_6 relative to TMS, the ^{13}C chemical shielding of the standard reference, $\sigma^{\mathrm{C}}(\mathrm{TMS})$, can be expressed in terms of this experimental value and of the computed chemical shielding of the secondary reference, yielding for the ^{13}C chemical shifts

$$\delta^{\mathrm{C}}(\mathrm{X}) = \sigma^{\mathrm{C}}(\mathrm{TMS}) - \sigma^{\mathrm{C}}(\mathrm{X}) = \delta_{\mathrm{exp}}^{C}(C_2H_6) + \sigma^{C}(C_2H_6) - \sigma^{C}(\mathrm{X}). \tag{2}$$

The value for $\delta_{\mathrm{exp}}^{\mathrm{C}}(C_2H_6)$ relative to TMS was taken from ref. [23].

Applying the same procedure, the ^{15}N chemical shifts were computed using as a secondary reference cisplatin, $Pt(NH_3)_2Cl_2$, for which we know the experimental value of chemical shift $\delta_{exp}^N\left(Pt\left(NH_3\right)_2Cl_2\right)$ relative to ammonia,[24]

$$\delta^N(X) = \sigma^N\left(NH_3\right) - \sigma^N(X) = \delta_{exp}^N\left(Pt\left(NH_3\right)_2Cl_2\right) + \sigma^N\left(Pt\left(NH_3\right)_2Cl_2\right) - \sigma^N(X) \quad (3)$$

4. QM/MM simulations and ^{15}N NMR chemical shielding calculation of the reference compound cisplatin, Pt(NH₃)₂Cl₂. The molecule $Pt(NH_3)_2Cl_2$ was built using the Antechamber package.[25] The Gaussian 09 program[1] was used to optimize the structure of $Pt(NH_3)_2Cl_2$ and to calculate the RESP charges.[16] The system was inserted in a box of ~1600 water molecules. 2 ns-long classical MD simulations were performed with the same setup described in the Methods section (see main text). During the MD simulations, the structure of $Pt(NH_3)_2Cl_2$ was kept fixed. Then, 10 ps-long QM/MM simulations were carried out based on the last MD snapshots. The QM part is the $Pt(NH_3)_2Cl_2$ molecule and the MM part is the solvent. The QM/MM simulation procedure was the same as that of the platinated proteins (see the main text). 100 equidistant snapshots were extracted from the QM/MM simulations for the ^{15}N chemical shielding calculation.

Tables

Table S1. Structural parameters of the reduced model-compounds mimicking the Pt-environment of **II** and **A-C** optimized by the Gaussian09 code at the DFT level in gas phase. Bond lengths are expressed in Angstrom and bond angles in degree.

cis-[Pt(SH$_2$)$_2$(NH$_3$)$_2$]$^{+2}$ (NH$_3$ $trans$ to SH$_2$)	$trans$-[Pt(SH$_2$)$_2$(NH$_3$)$_2$]$^{+2}$ (NH$_3$ $trans$ to NH$_3$)	[Pt(SH$_2$)(NH$_3$)$_3$]$^{+2,}$
Pt-S 2.4	Pt-S 2.4	Pt-S 2.4
Pt-N 2.1	Pt-N 2.1	Pt-N 2.1 (N $trans$ to S)
		Pt-N 2.11
S-Pt-S 91	S-Pt-N 90	S-Pt-N 91
N-Pt-N 89		N-Pt-N 89
Improper dihedral		
S-Pt-S-N 180	N-Pt-S-N 180	S-Pt-N-N 180
S-Pt-N-N 180	S-Pt-N-S 180	N-Pt-N-N 180
N-Pt-N-S 180	N-Pt-S-N 180	N-Pt-N-S 180
N-Pt-S-S 180	S-Pt-N-S 180	N-Pt-S-N 180

Table S2. Equilibrium bending angles (degree) $\overset{\frown}{\text{CB-S-Pt}}$ and $\overset{\frown}{\text{CA-N-Pt}}$ used in the pseudo-Amber potential for **II** and **A-C**. The values were chosen according to those obtained by X-ray spectroscopy for Atox1 in complex with cisplatin.[14]

	II	**A**	**B**	**C**
CA19-N19-Pt		106	106	
CA22-N22-Pt				106
CB19-SG19-Pt	112	102	102	
CB22-SG22-Pt	112	112		102

Table S3. Harmonic force constants of bond stretching (kcal mol^{-1}Å^{-2}) and angle bending (kcal mol^{-1}rad^{-2}) used to build the pseudo-Amber potential of the platinated moieties of **II** and **A-C**. The NE atom refers to the nitrogen atom of the amino group. The values were chosen according to the typical values obtained for the Pt-moiety of platinated-Ctr1 through the force matching procedure.[15]

	II	**A**	**B**	**C**
Pt-SG	70	70	70	70
Pt-NE	100	100	100	100
SG-Pt-SG	40			
SG-Pt-NE	40	40	40	40
SG-Pt-N		40	40	40
NE-Pt-NE	40		40	40
NE-Pt-N			40	40
CA-N-Pt		20	20	20
CB-SG-Pt	20	20	20	20

Table S4. Force constants (kcal mol^{-1}) of improper dihedral angles used to build the pseudo-Amber potential of the platinated moieties of **II** and **A-C**. The NE atom corresponds to the nitrogen of the amino group. The values were chosen according to the typical values obtained for the Pt-moiety of platinated-Ctr1 through the force matching procedure.[15]

	Phase (degree)	No. of barriers	**II**	**A**	**B**	**C**
SG-Pt-SG-NE	180	2	10			
N-Pt-SG-NE	180	2		10	10	10
SG-Pt-N-SG	180	2		10		
SG-Pt-N-NE	180	2			10	10

Table S5. RESP charges (in fraction of electronic charges) of the reduced model compounds used to build the pseudo-Amber potential of the Pt-moieties in **II** and **A-C** calculated via the Gaussian09 code.

Atom names	$[Pt(SH_2)_2(NH_3)_2]^{+2}$ NH$_3$ *cis* to NH$_3$ relevant to **II**	$[Pt(SH_2)_2(NH_3)_2]^{+2}$ NH$_3$ *trans* to NH$_3$ relevant to **A**	$[Pt(SH_2)(NH_3)_3]^{+2}$ relevant to **B** and **C**
Pt	-0.049165	0.061409	-0.039637
N	-0.321582	-0.5286	-0.350504
S	0.100847	0.076768	0.095513
HS	0.204966	0.221684	0.205611
HN	0.278461	0.325920	0.287157

Table S6. RESP charges (in fraction of electronic charges) of the Pt-moieties in **II** and **A-C** obtained from the RESP charges of the reduced model compounds reported in Table S5. The charges of the SH$_2$ and NH$_3$ groups, that in the models are replaced by bonds with the S and N atoms of the deprotonated cysteine residues, have been distributed on the Pt atom.

Atom names	$[Pt(NH_3)_2]^{+2}$ in **II**	$[Pt(NH_3)]^{+2}$ in **A**	$[Pt(NH_3)_2]^{+2}$ in **B** and **C**
Pt	0.972393	1.489432	0.978065
N	-0.321582	-0.5286	-0.350504
HN	0.278461	0.325920	0.287157

Table S7. Structural parameters of the Pt-moieties in **II** and **A-C**, obtained from QM/MM simulations. The values correspond to an average over 150 snapshots. The number in parenthesis is the standard deviation. S1 and S2 refer to the sulfur atom of Cys19 and Cys22, respectively. Bond lengths are expressed in Angstrom and angles in degree.

	II	**A**	**B**	**C**
Pt-N		2.11 (0.05)	2.11 (0.05)	2.07 (0.05)
Pt-N1	2.12 (0.05)	2.13 (0.05)	2.11 (0.05)	2.11 (0.05)
Pt-N2	2.14 (0.06)		2.14 (0.06)	2.12 (0.06)
Pt-S1	2.37 (0.05)	2.37 (0.05)	2.35 (0.05)	
Pt-S2	2.38 (0.06)	2.43 (0.06)		2.41 (0.07)
S1-Pt-S2	91 (4)			
N-Pt-N2			96 (4)	94 (4)
N1-Pt-N2	88 (3)		86 (3)	89 (3)
N1-Pt-S1	95 (3)	89 (4)	91 (3)	
N1-Pt-S2		82 (3)		93 (4)
N-Pt-S1		83 (3)	86 (2)	
N-Pt-S2		106 (3)		84 (3)
N2-Pt-S2	86 (3)			
improper				
	N1-Pt-N2-S2 175 (3)	N1-Pt-S2-N 176 (3)	N1-Pt-N2-N 176 (4)	N1-Pt-N2-N 176 (3)
	N2-Pt-S2-S1 176 (3)	N-Pt-S1-N1 175 (3)	N2-Pt-N-S1 174 (4)	N2-Pt-N-S2 173 (5)
	S1-Pt-N1-N2 176 (3)	S1-Pt-N1-S2 172 (5)	N-Pt-S1-N1 176 (3)	N-Pt-S2-N1 176 (4)
	S2-Pt-S1-N1 175 (3)	S2-Pt-N-S1 172 (5)	S1-Pt-N1-N2 175 (3)	S2-Pt-N1-N2 173 (5)

Table S8.: NMR chemical shifts (in ppm) of ^{15}N (top) and of ^{1}H (bottom) of the ammine ligands in free and Mnk1-coordinated cisplatin (**II**, **A-C**), calculated by using the BLYP functional with the norm-conserving pseudopotentials of the Martins−Troullier type. The calculations are compared with the experimental data. The numbers reported in parentheses are the standard deviations. Note that cisplatin, PtCl2(^{15}NH3)2, is used as a secondary reference in the theoretical calculations. The experimental values of the chemical shifts of the N and H nuclei of the ammine groups are generically indicated as N* and H* respectively, since the stereospecific assignment is lacking.

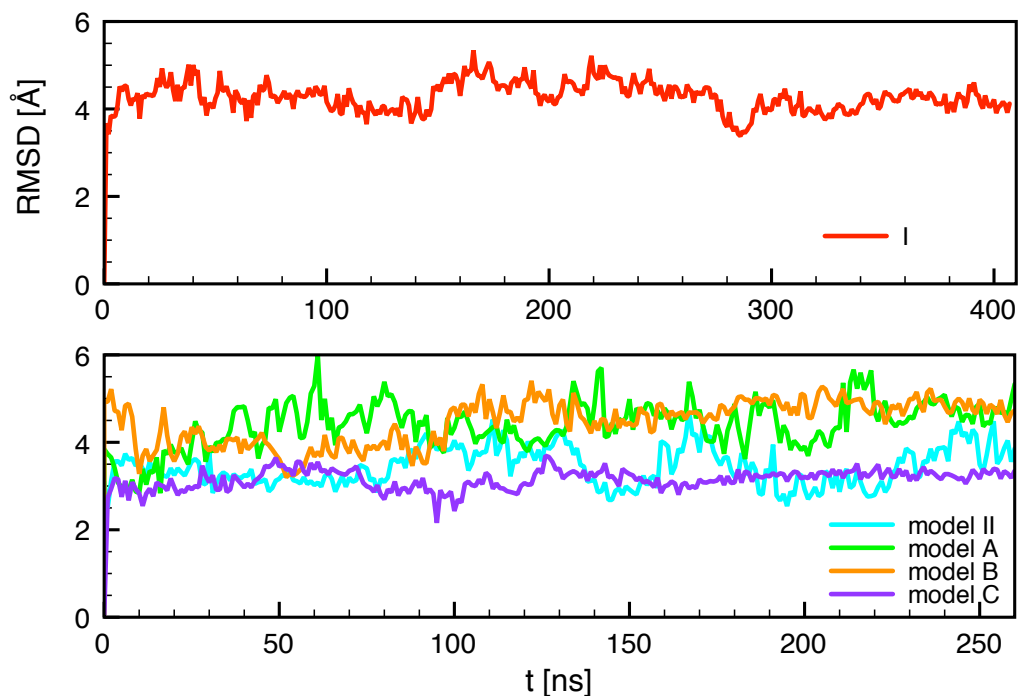| Atom name	Cisplatin	**II**	**A**	**B**	**C**	Atom name	Cisplatin experimental value	Mnk1-coordinated Cisplatin experimental value
N1	ref	-66.3(5.6)	-74.8(6.1)	-72.1(5.3)	-74.7(5.8)	N*	-67.1 / -67.1	-35.8/ -42.8
N2	ref	-61.4(5.6)		-66.6(6.4)	-66.5(6.4)			

Figures

Figure S1: Root-mean-square deviation from the initial conformations for **I** (top) and (**II**, **A-C**) (bottom) during preliminary MD simulations.
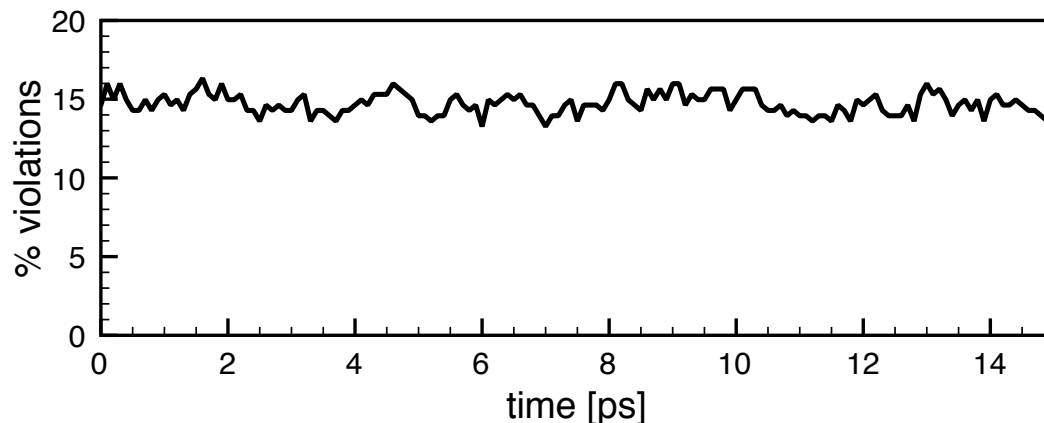
Figure S2: Violated NMR restraints during QM/MM simulations of **I**.

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