Supporting Information

FIRST CRYSTAL STRUCTURE FOR A GOLD CARBENE-PROTEIN ADDUCT

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Crystallization and X-ray diffraction data collection

We have tried to solve the structures of Au(NHC)Cl adducts with several model proteins, including hen egg white lysozyme (HEWL) and horse heart cytochrome c (Cyt c), which have been frequently used as model systems in the field of protein metalation [1-3], but attempts to obtain crystals of adducts formed between Au(NHC)Cl and these proteins by both cocrystallization and soaking procedures up to now failed. This is not surprising considering that HEWL and Cyt c seem to not interact with gold carbene compounds, as judged by ESI MS spectra [4]. On the contrary, crystals of the adduct of Au(NHC)Cl with thaumatin suitable for X-ray diffraction analysis were obtained by soaking procedure. In particular, thaumatin crystals were grown at 293 K using the hanging drop vapour diffusion method and 20 % polyethylene glycol, 0.2 M sodium tartrate at pH 7.2 as a precipitating agent (protein concentration 30 mg mL^{-1}). These crystals were transferred in a solution consisting of 15 % polyethylene glycol, 0.15 M sodium tartrate at pH 7.2, 5 mM Au(NHC)Cl and 25% DMSO. Crystals were kept in this solution for four days and then fished with nylon loops and flash-frozen at 100 K in a nitrogen gas produced by an Oxford Cryosystem (and maintained at 100 K during the data collection) using glycerol as a cryoprotectant. X-ray diffraction data were collected 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, first at 1.70 Å resolution and successively, using a second crystal, at 1.93 Å resolution. Data were integrated and scaled using HKL2000[5], following the indications of Karplus and Diederichs [6-7]. This

strategy has been used to improve the quality of the electron density maps around metal centre in the adducts formed in the reaction between HEWL and cisplatin [8-10]. Details of data collection statistics are reported in Table S1.

Structure resolution and refinement

The structure of the adduct formed in the reaction between thaumatin and Au(NHC)Cl was solved by molecular replacement using the PDB file 3QY5 [11], without water molecules and other ligands, as starting model. Inspection of the electron density maps calculated using this model clearly reveals the presence of many sites attributable to gold compound fragments. Inspection of anomalous electron density maps confirm our assignment. Peaks of anomalous map were interpreted as indicated in Table S3.

Conventional structural refinements were carried out with REFMAC5.7 [12]; model building and map inspections were performed using COOT [13]. Refinement statistics are reported in Table S1. Structure validation was carried out using Whatcheck [14]. Coordinates and structure factors were deposited in the Protein Data Bank under the accession code 5JVX.

References

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Table S1. Data collection and refinement statistics

	Au(NHC)Cl/Thaumatin	Au(NHC)Cl/Thaumatin (crystal 2)
PDB code	5JVX	not deposited
Data collection temperature (K)	100	100
Cryoprotectant used	glycerol	glycerol
Data reduction		
Space group	$P4_12_12$	$P4_12_12$
Unit cell parameters		
a=b (Å)	57.91	58.12
c (Å)	150.14	150.44
Molecules per asymmetric unit	1	1
Observed reflections	565795	135216
Unique reflections	27058	20287
Resolution (Å)	54.03-1.70 (1.73-1.70)	54.21-1.93 (1.96-1.93)
Completeness (%)	93.0 (80.0)	99.9 (100.0)
Rmerge	0.123 (1.745)	0.207 (1.438)
Rmerge in top intensity bin	0.052	0.080
Rpim	0.026 (0.479)	0.085 (0.689)
I/σ(I)	37.0 (1.7)	11.6 (1.1)
Multiplicity	20.9 (13.5)	6.7 (5.1)
$C_{1/2}$ last shell	0.594	0.359
CC* last shell	0.863	0.727
Refinement		
Resolution (Å)	54.03-1.70	54.21-1.93
number of reflections in working set	25708	19215
number of reflections in test set	1323	
Isotropic R-factor/Rfree/Rall (%)	17.0/18.7/17.1	16.7/23.0/17.0
Non-H atoms used in the refinement	2020	2010
Mean B-value (Å^2)	23.9	25.5
Au atom occupancy	0.65, 0.55, 0.35/0.35, 0.15, 0.35, 0.20	0.65, 0.55, 0.35/0.35, 0.20, 0.35, 0.30
Au atom B-factor (Å^2)	30.9, 28.3/ 42.6, 27.8, 52.3, 48.2, 31.6	28.2, 24.9, 41.6/24.6, 54.9, 49.7, 48.1
Rmsd bonds (Å)	0.009	0.018
Rmsd angles (°)	1.38	1.90
Ramachandran values (%) from Coot		
Preferred region	97.6	97.2
Allowed	2.3	2.2
Disallowed	0	0

Parentheses indicate information for highest resolution shell.

Table S2. Details of Au(NHC)Cl binding sites

Site	Au ligands	Binding site	Occupancy	B-factor (Å^2)	Au-N distance (Å)	Au-C distance (Å)	N-Au-C angle (°)	Location and Interactions of NHC ligand with surrounding protein residues (Å)
Au1	NHC	N-terminus	0.65	30.9 34.7-37.7	1.6	1.9	177.2	On the surface of the protein. Stacking interactions with π system of side chain of Asn40 (distances between 3.0 and 4.5 Å)
Au2	NHC	NZ Lys49	0.55	28.3 26.5-28.5	1.6	1.9	177.8	The ring is packed between side chains of Phe80 and of Lys106 (3.8-4.5 Å). Bu is in contact with side chains of Leu87 (3.3 Å), Trp51 (2.9-3.1 Å), Thr85 (3.0 Å) and in short contact with main chain atoms of Gly81 (2.7-3.6 Å)
Au3A	NHCA	NZ Lys106A	0.35	42.6	2.4	2.0	178.9	The ring is sitted on the side chain of Pro83 (3.5 Å). Bu is almost parallel to Arg82 side chain (4.3 Å)
Au3B	NHCB	NZ Lys106B	0.35	27.8	2.1	2.0	178.7	Sitted on the disulphide bridge Cys149-Cys158 (3.5 Å). Bu is is short contact with side chain of Ile105 (3.4-3.6 Å) and main chain atoms of Tyr157 (3.0-3.5 Å)
Au4	X	NZ Lys97B	0.15	52.3	2.1			n.a.
Au5	X	NH1 Arg8	0.35	48.4	2.2			n.a.
Au6	wat	NH2 Arg175	0.20	31.6	2.2			n.a

X=undefined. Distances are in Å and B-factors in Å^2.

Table S3. Peak Size and attribution of the anomalous map peaks in the structure of Thaumatin/Au(NHC)Cl

	σ	Assignment
Peak	25.3	Au1
Peak	23.5	Au2
Peak	16.3	Au3B
Peak	8.9	Au6A
Peak	8.8	Au3A
Peak	7.9	Au5
Peak	5.6	Cl^-
Peak	5.4	SG Cys56
Peak	5.2	SG Cys177
Peak	5.1	SD Met112
Peak	5.0	SG Cys158
Peak	4.8	Au4
Peak	4.7	SG Cys126
Peak	4.7	SG Cys193
Peak	4.6	SG Cys145
Peak	4.6	SG Cys204
Peak	4.4	SG Cys121
Peak	4.4	SG Cys66
Peak	4.4	SG Cys134

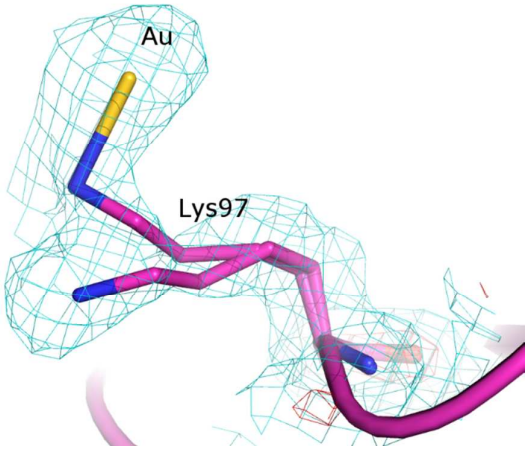


Figure S1. Details of the gold binding site close to Lys97 side chain. Lys97 adopts two different conformations. 2Fo-Fc electron density maps are contoured at 0.5σ (cyan) level

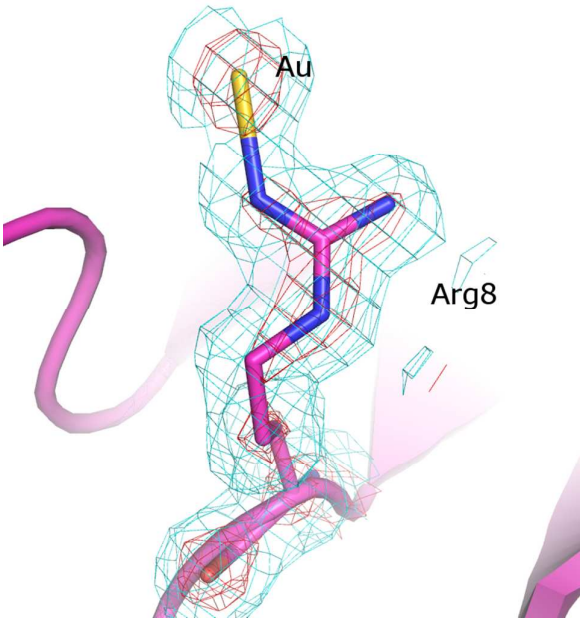


Figure S2. Details of the gold binding site close to Arg8 side chain. 2Fo-Fc electron density maps are contoured at 0.5σ (cyan) and 3.0 σ level (red).

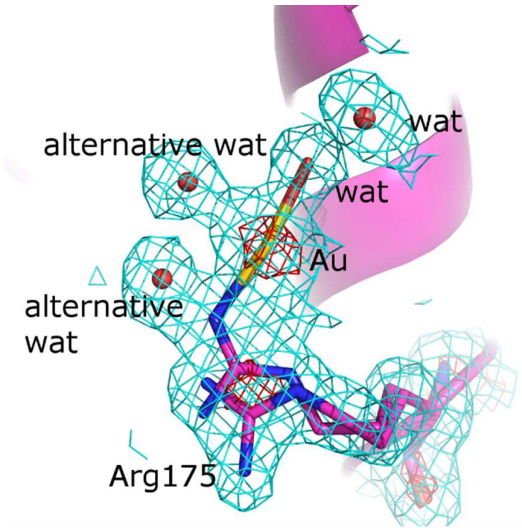
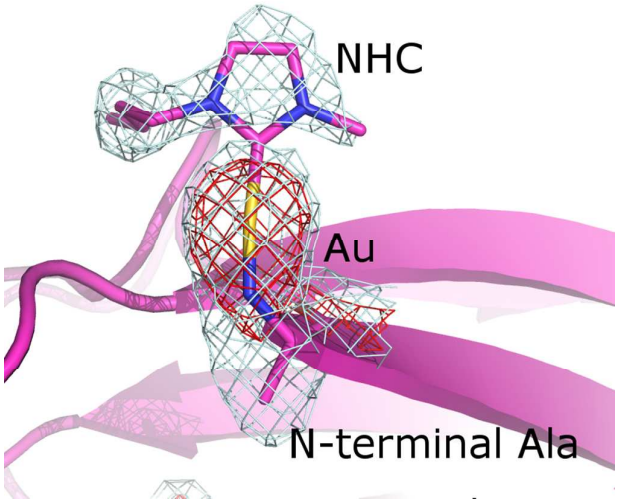


Figure S3. Details of the gold binding site close to Arg175 side chain. Arg175 adopts two different conformations. 2Fo-Fc electron density maps are contoured at 1.0σ (cyan) and 4.0 σ level (red).

A



B

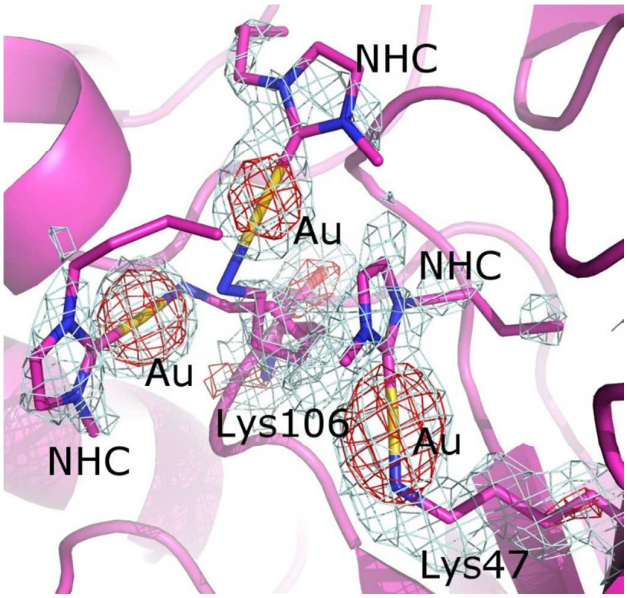


Figure S4. Details of the binding site of Au(NHC)Cl to thaumatin in the structure refined at 1.93 Å resolution. Au centres coordinated to N-terminal amine (A) and lysine residues (B) are shown. The binding of the gold compound is always associated with the loss of the Cl^- ligand. 2Fo-Fc electron density maps are contoured at 3.0σ (red) and 0.8σ (cyan) level.