Electronic Supplementary Information

Medicinal Gold Compounds form Tight Adducts with the Copper Chaperone Atox-1: an ESI MS investigation.

Experimental section

Materials. The various gold(III) complexes were synthesized as previously described (see the respective refs). Auranofin was purchased at Vinci-Biochem. Copper transport protein Atox1 was purchased from Giotto Biotech.

ESI Mass Spectrometry. For the analysis Atox-1 was dissolved (10^{-4} M) in 25 mM tetramethylammonium acetate buffer (TMeAmAc), pH 7.4 containing DTT (1:5 protein:DTT molar ratio). Then the three gold (III) complexes and Auranofin were added (1:1 metal/protein ratio) to the solution and incubated at room temperature for 24 or 72h. After a 10-fold dilution with 1% HCOOH, ESI-MS spectrum was recorded by direct introduction at 5 µl/min flow rate in an LTQ-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 and capillary temperature 220 °C. 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.

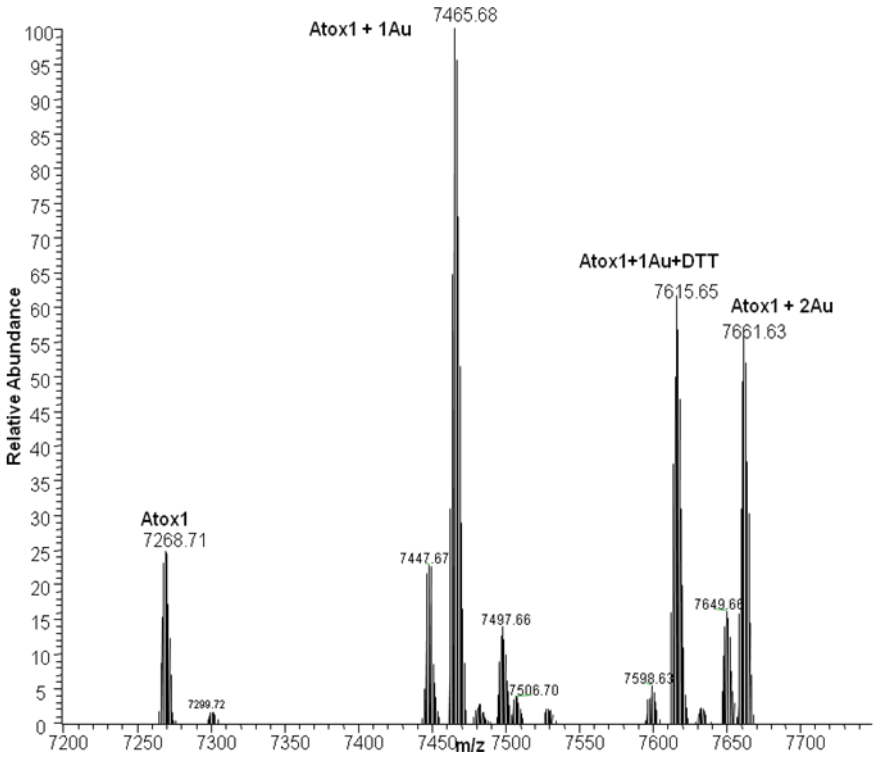

Figure S1. LTQ-Orbitrap ESI-MS spectrum of Atox-1 sequentially treated with $CuCl_2$ and Aubipyc (1:2:1 molar ratio) at room temperature. Incubation time 24 h (see: I. Anastassopoulou, L. Banci, I. Bertini, F. Cantini, E. Katsari, A. Rosato, *Biochemistry* 2004, **43**, 13046-53 for details of sample preparation).

Table S1

Experimental and theoretical values for the main ESI MS peaks

Peaks	Exp. value	Theor. monoisotopic value	Theor. average value
a	7269,7	7265.7	7270.4
b	7465,7	7462.7	7467.4
c	7661,6	7659.6	7664.3
d	7583,7	7580.8	7585.5