Reactions of Metallodrugs with Proteins: Selective Binding of Phosphane-Based Platinum(II) Dichlorides to Horse Heart Cytochrome c Probed by ESI MS coupled to Enzymatic Cleavage.

Supplementary Information

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Experimental Details

General

All syntheses were carried out using standard vacuum-line and Schlenk techniques under argon atmosphere. All chemicals and solvents used were obtained from commercial suppliers and were used as received: horse heart cytochrome c (Sigma C7752); hen egg white lysozyme (Sigma L7651); endoproteinase Asp-N (Sigma, P3303); Trypsin (Promega, V5111); NH_4HCO_3 (Fluka, 09830). The diphosphane Pt(II) dichloride complexes were prepared from dichloro-(1,5-cyclooctadienyl)-platinum(II) similar to literature procedures[9]. Stock solutions of **1** and **2** for the protein interaction studies were prepared in DMSO.

Recording of the ESI MS spectra

The incubated samples were diluted with water (20-fold dilution). ESI-MS spectra were recorded via direct introduction of the samples (flow rate 5 µL/min) in an LTQ-Orbitrap high-resolution mass spectrometer (Thermo, San Jose, CA, USA), equipped with a conventional ESI source. The specrometer's working conditions were: 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; monoisotopic and average deconvoluted masses were obtained by using the integrated Xtract tool. A nominal resolution (at m/z 400) of 100.000 was used for spectrum acquisition.

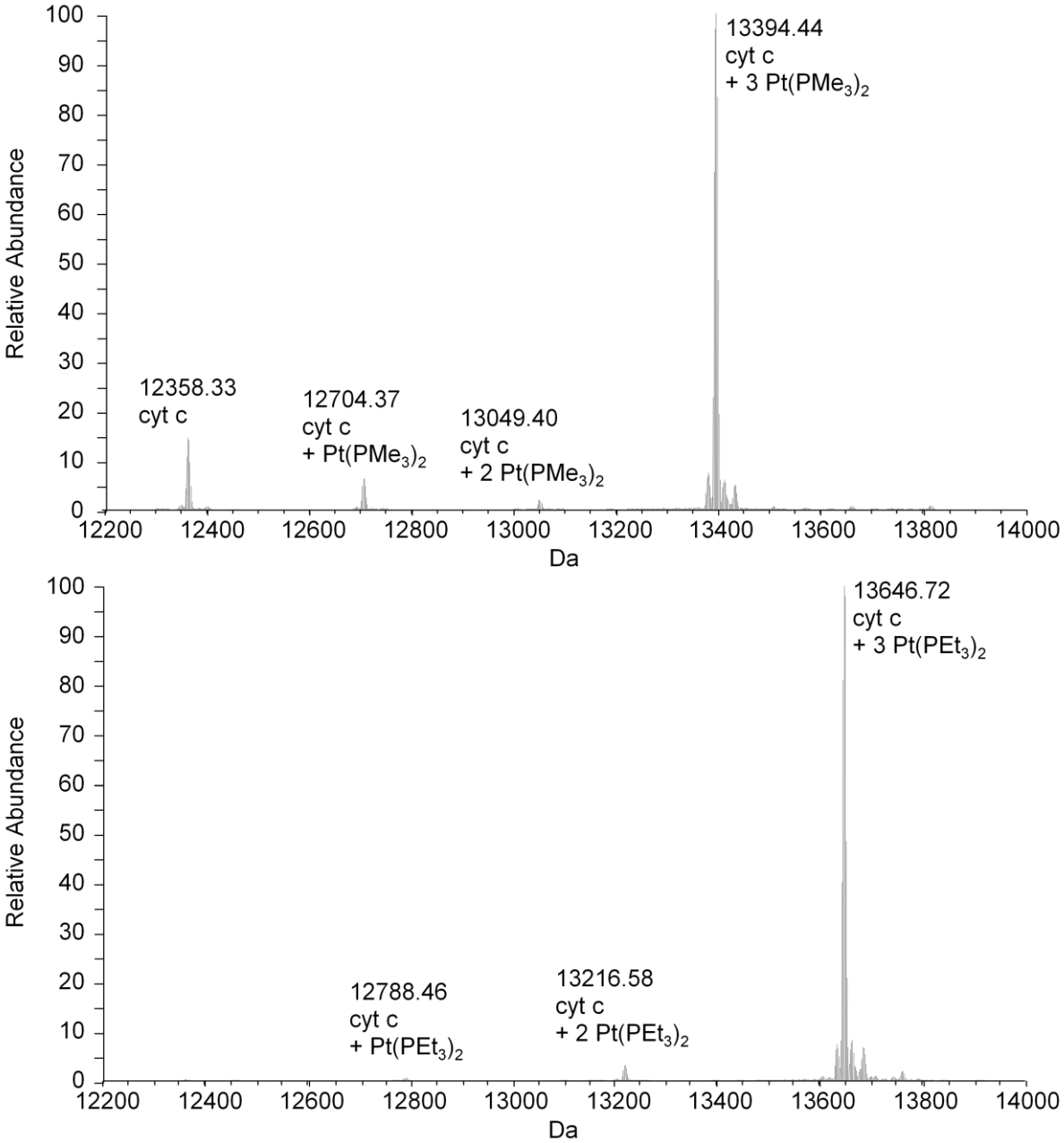

Fig. S1 Deconvoluted ESI-MS spectra of $(Me_3P)_2PtCl_2$ (top) and $(Et_3P)_2PtCl_2$ (bottom) after 96h incubation with cyt c (3:1 ratio). Both compounds show clear preference to form 3:1 adducts.

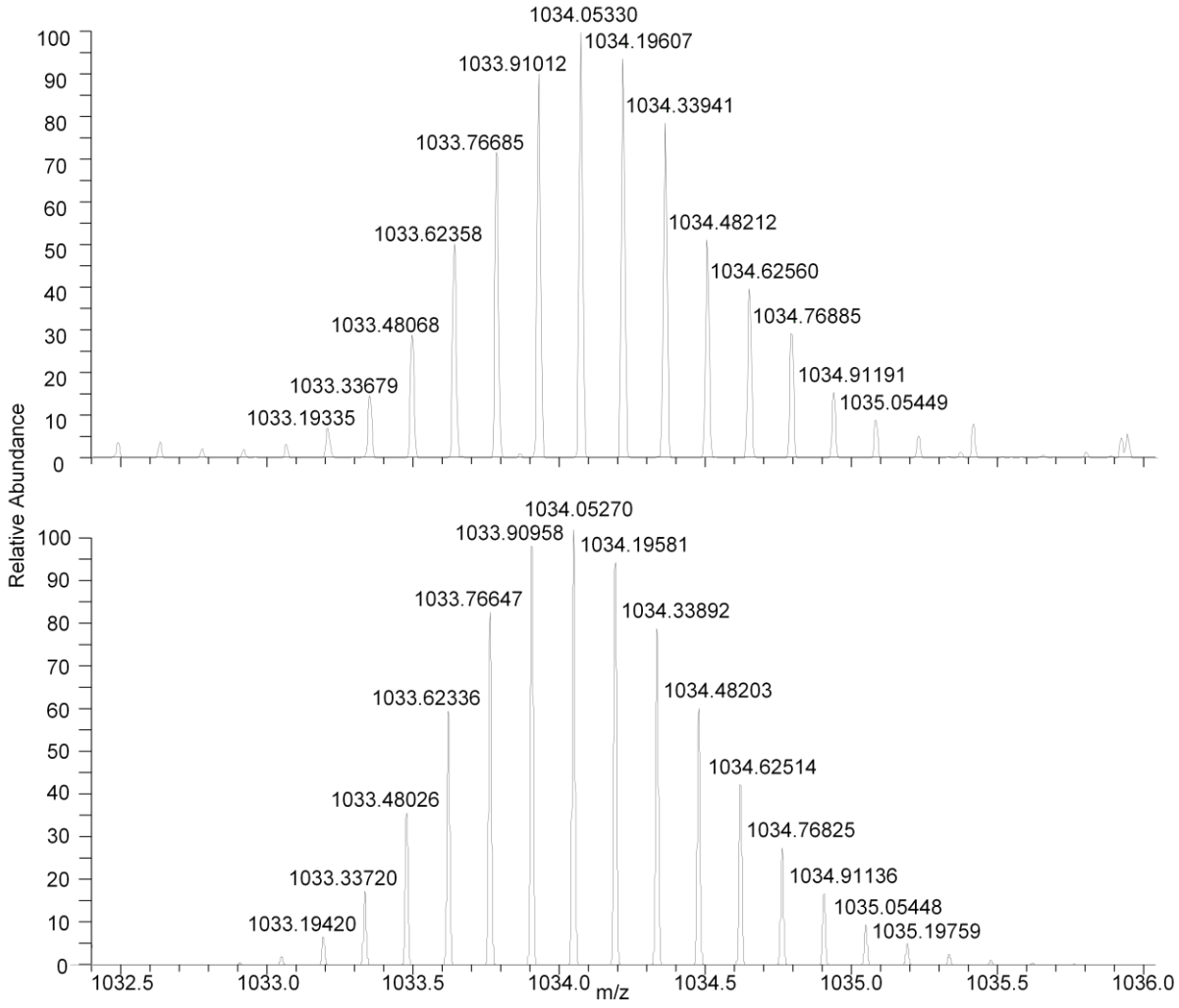

Fig. S2 Experimental (top) and theoretical (bottom) isotopic pattern for the fragment [cyt c(G1-T49)$^{+}$ + $C_{36}H_{90}P_6Pt_3^{6+}$]$^{7+}$ at charge state +7. Binding of 3 units $Pt(PEt_3)_2$ to the segment of cyt c with amino acids 1-49 is proven.

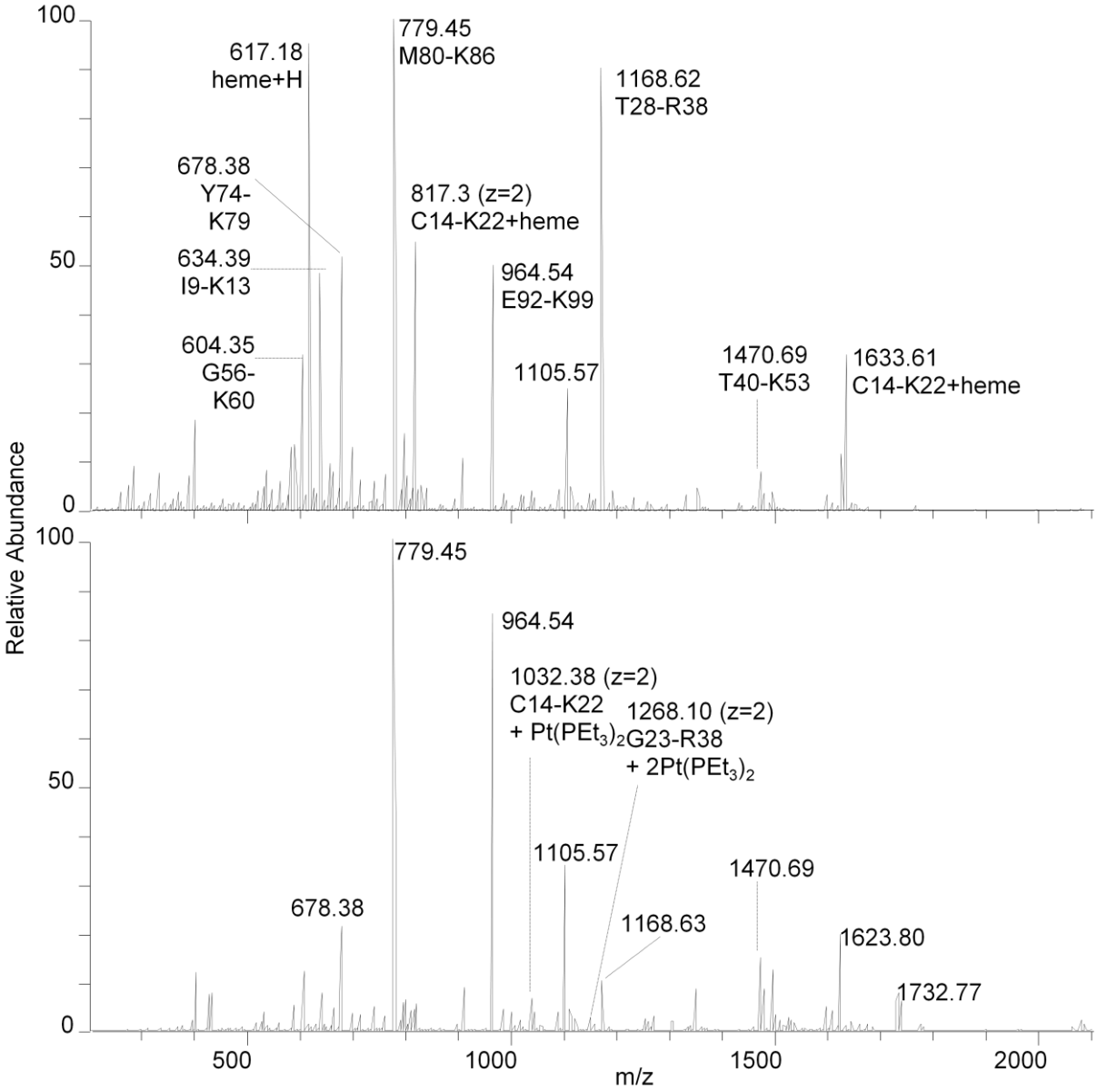

Fig. S3 Trypsin digestion pattern of free (top) and platinated (bottom) cyt c. The main signals are assigned according to literature.[1,2]

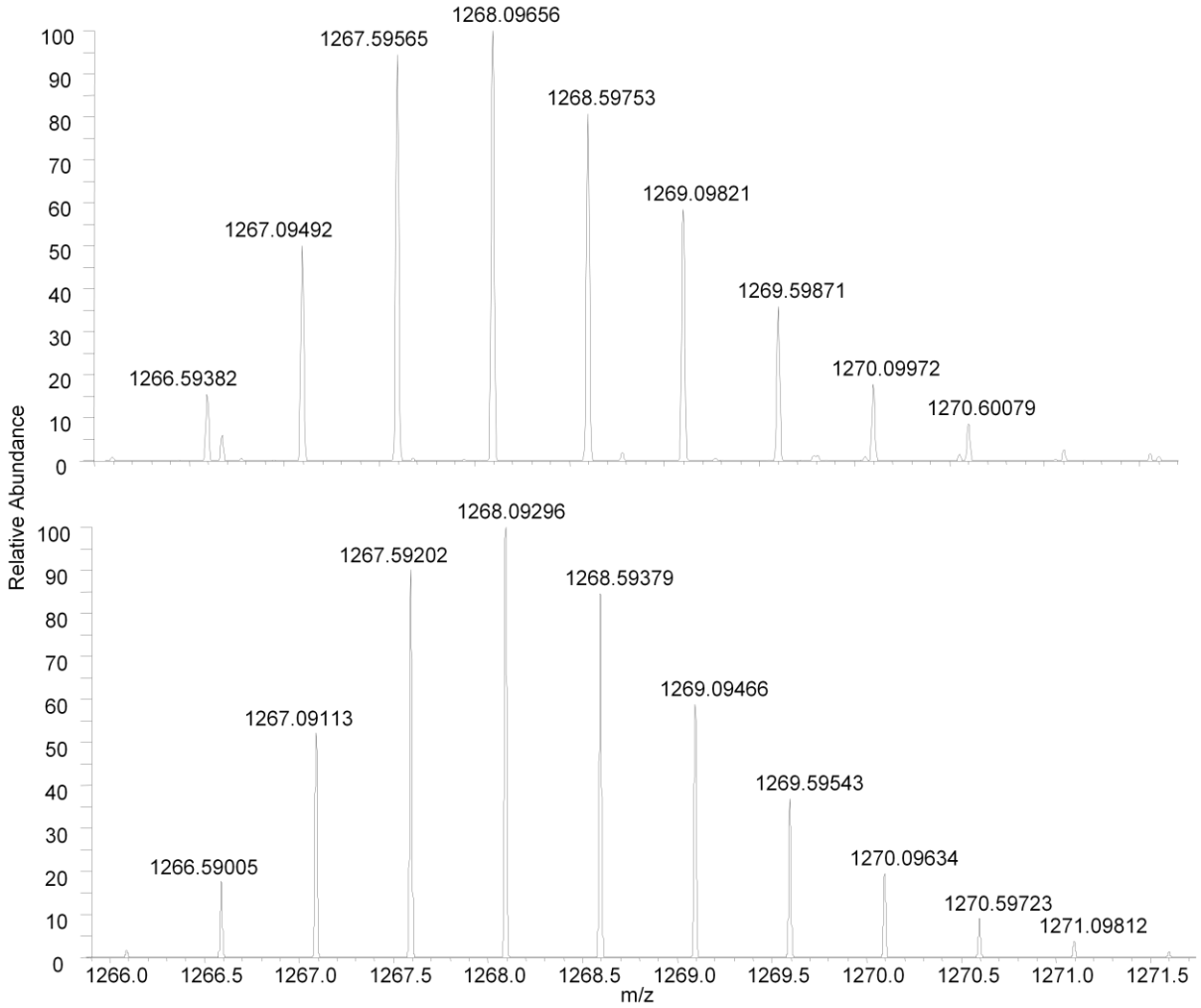

Fig. S4 Experimental (top) and theoretical (bottom) isotopic pattern for the fragment [cyt c(G23-R38) + 2Pt$(PEt_3)_2^{2+}$ -2H^+]$^{2+}$ at charge state +2. Amino acids H26 and H33 are determined possible binding sites for Pt.

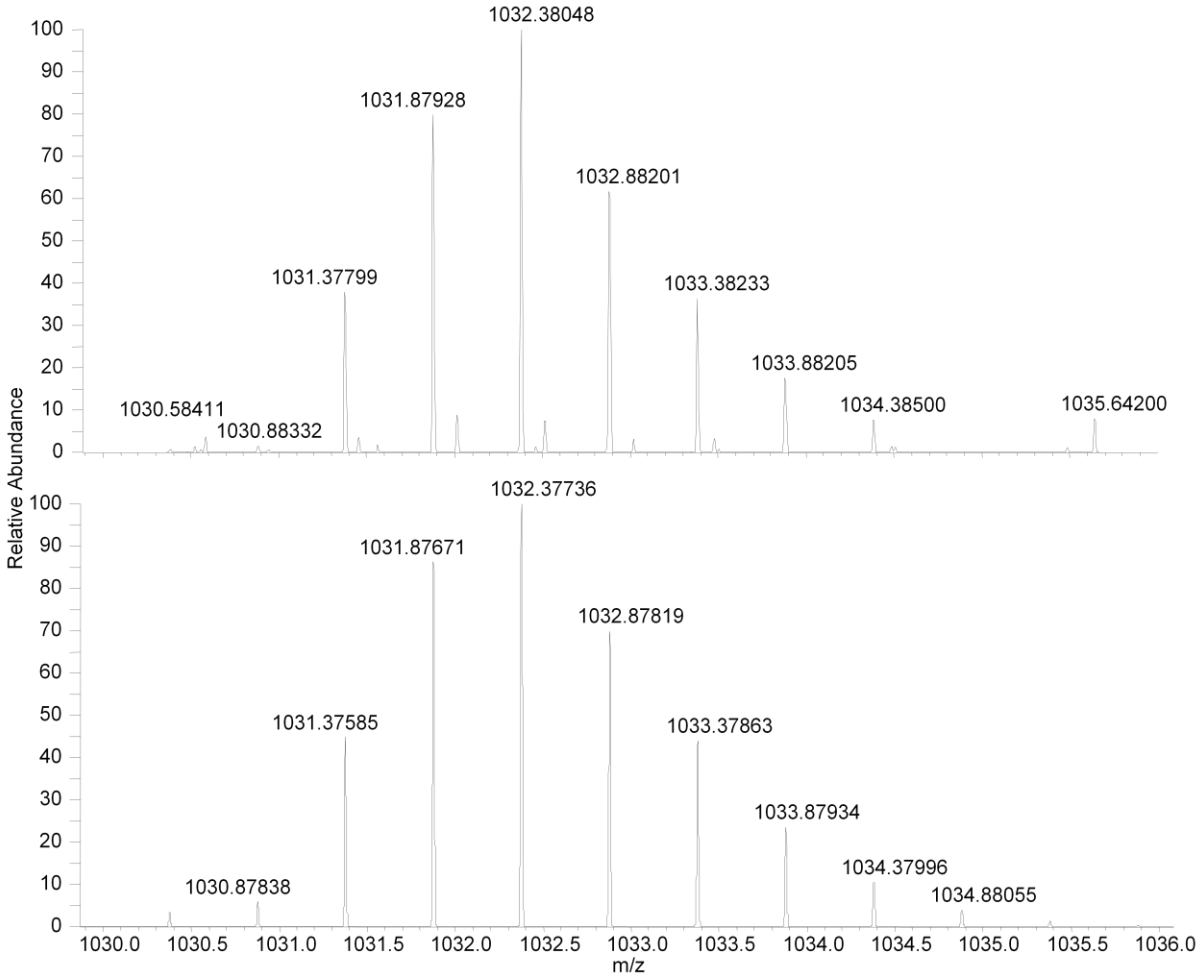

Fig. S5 Experimental (top) and theoretical (bottom) isotopic pattern for the fragment [cyt c(C14-K22)$^{+}$ + Pt(PEt$_3$)$_2^{2+}$ -1H^{+}]$^{2+}$ at charge state +2. Amino acid H18 is assigned as binding site, since the two cysteines, C14 and C17, are still covalently bound to the heme group.

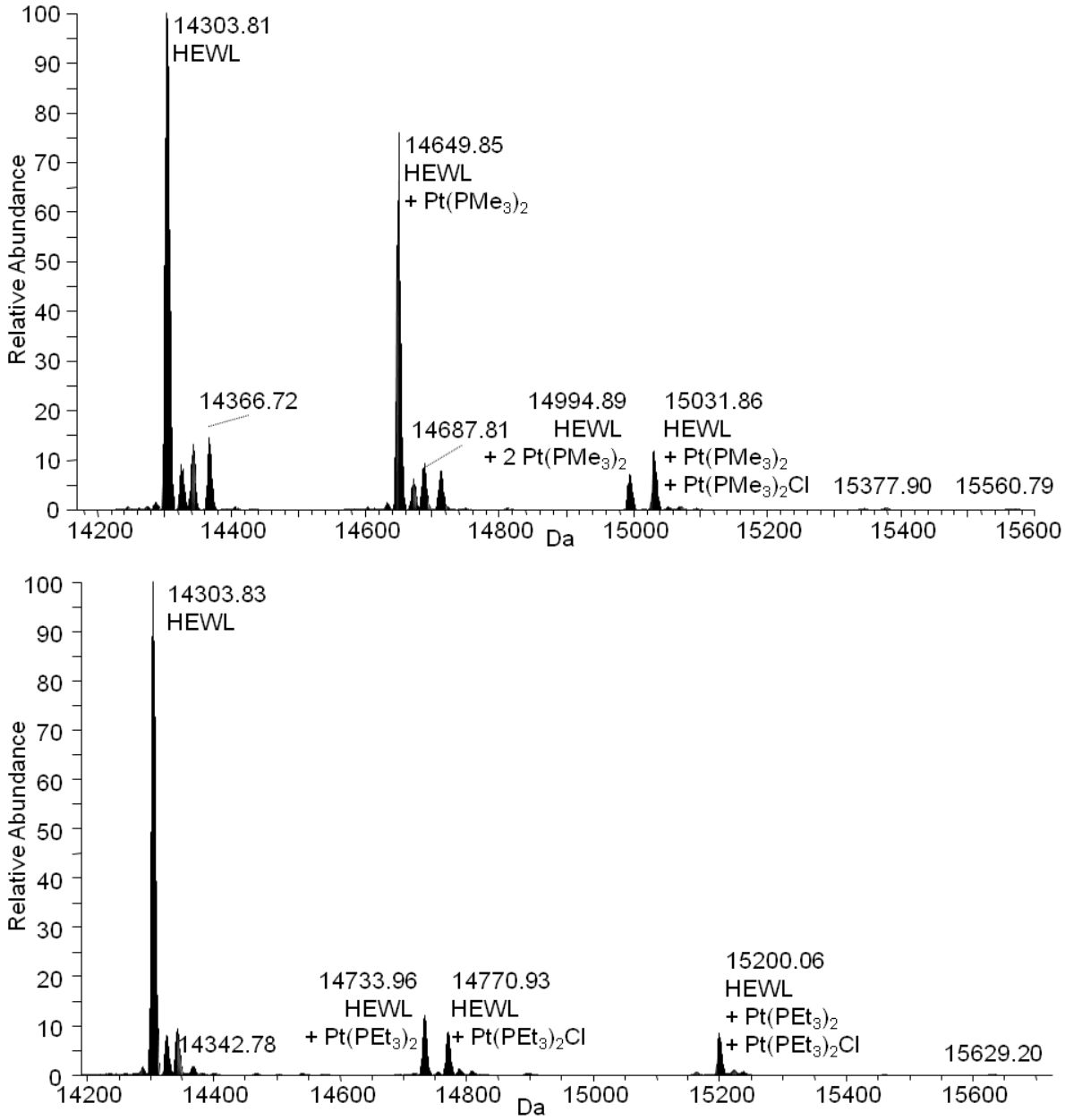

Figure S6. Deconvoluted ESI-MS spectra of $(Me_3P)_2PtCl_2$ (top) and $(Et_3P)_2PtCl_2$ (bottom) after 96h incubation with HEWL (3:1 ratio). Both compounds show interaction with HEWL to a much lesser and less defined extent than when incubated with cyt c.

Literature:

1. S. C. Henderson, S. J. Valentine, A. E. Counterman, and D. E. Clemmer, *Anal. Chem.*, 1999, 71, 291-301.
2. J. M. Busnel, S. Descroix, T. Le Saux, S. Terabe, M. C. Hennion, and G. Peltre, *Electrophoresis*, 2006, 27, 1481–1488.