Supplementary Material (ESI) for Analyst

Novel insights into the bottom-up mass spectrometry proteomics approach for the characterization of Pt-binding proteins: the insulin-cisplatin case study

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SUPPLEMENTARY DATA

The present document provides further information on the paper mentioned above.

Contents:

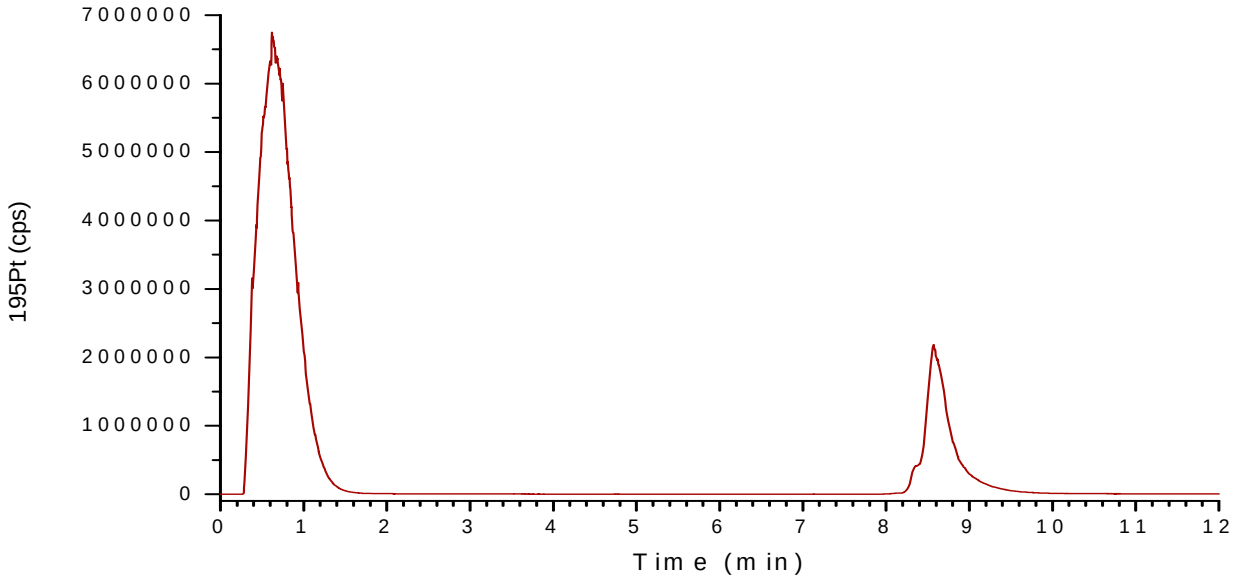

Figure S-1. HPLC(RP)-ICP-MS chromatogram showing the separation of unreacted cisplatin (tr= 0.3-1.3 min) from insulin-cisplatin adducts (tr: 8.2-9.2 min). ^{195}Pt was monitored.

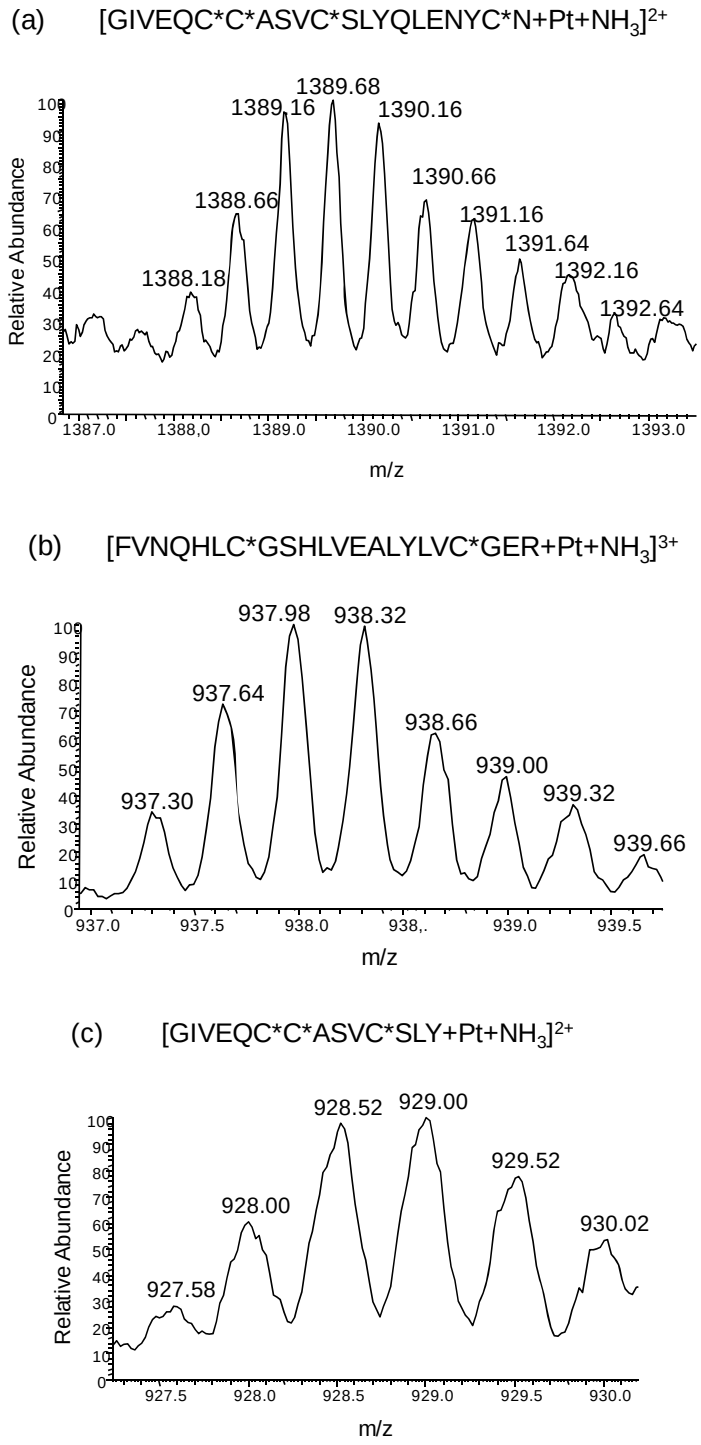

Figure S-2. Some platinum-bound peptide ions observed in the nESI-LIT MS of in a complete tryptic digestion of isolated insulin-cisplatin adducts (including previous treatment with Urea, DTT and IAA). These ions correspond to: (a) $[GIVEQC^*C^*ASVC^*SLYQLENYC^*N+Pt+NH_3]^{2+}$ at m/z 1388.18, (b) $[FVNQHLC^*GSHLVEALYLVC^*GER+Pt+NH_3]^{3+}$ at m/z 937.30, (c) $[GIVEQC^*C^*ASVC^*SLY+Pt+NH_3]^{2+}$ at m/z 927.58.

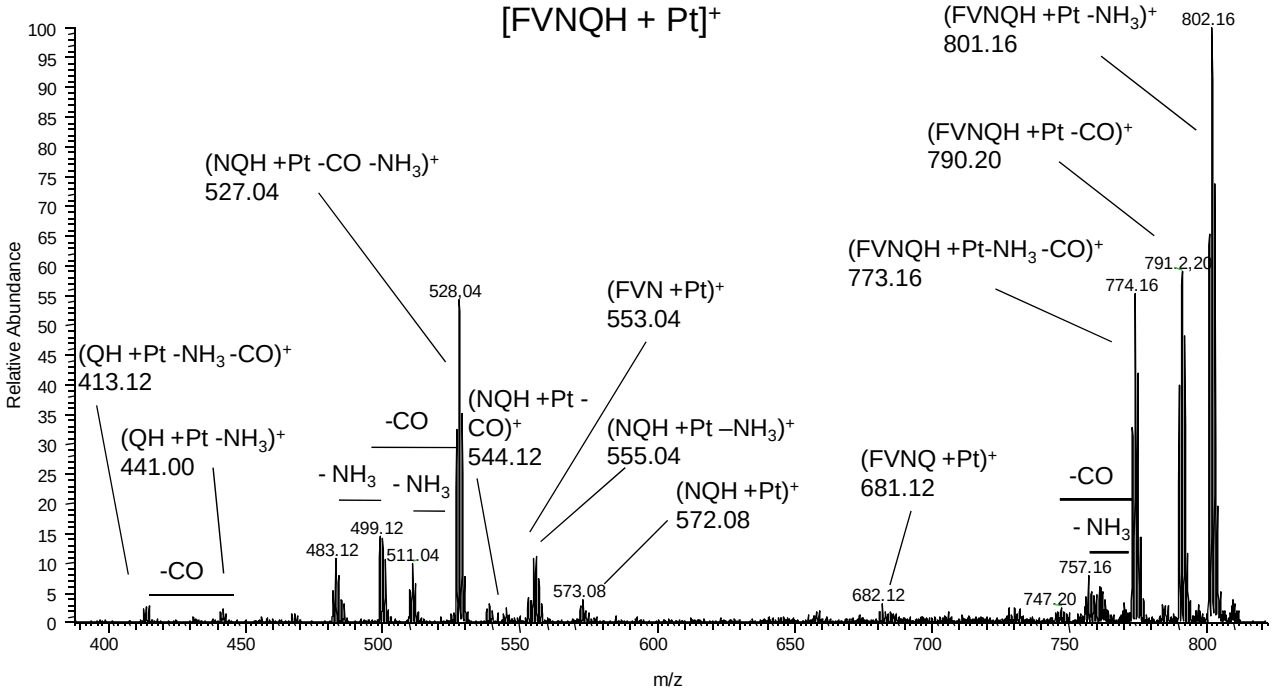

Figure S-3. CID-MS^3 of the platinated fragment ion at m/z 818.00 (1+) observed in the MS^2 of the platinum-containing peptide at m/z 1048.5 (2+). Transition: 1048.50(2+) → 818.16 (1+). The sequence of the precursor ion and the main fragment ions are shown.

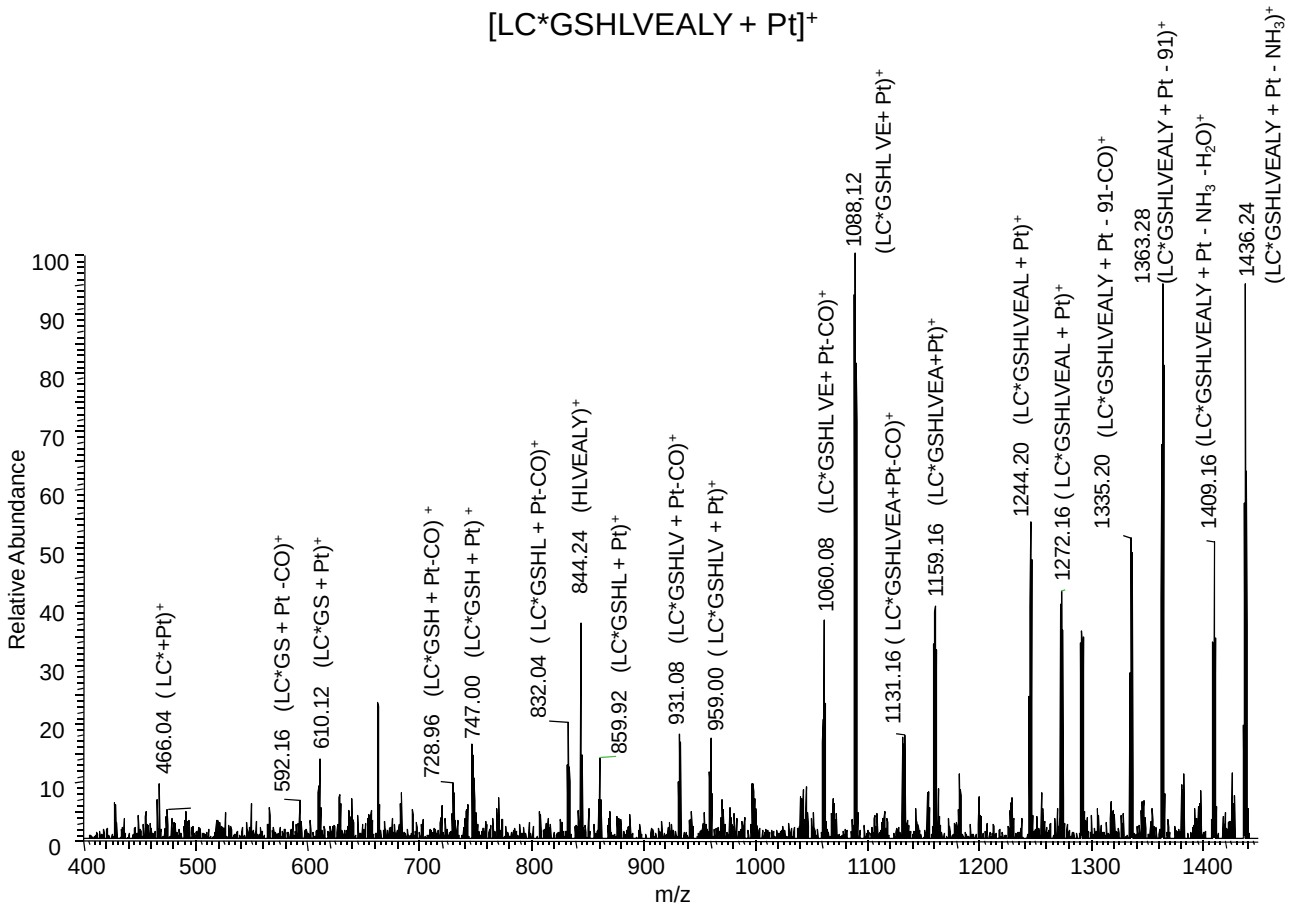

Figure S-4. CID-MS^3 of the platinated fragment ion at m/z 1453.52 (1+) observed in the MS^2 of the platinum-containing peptide at m/z 1048.50 (2+). Transition: 1048.50(2+) → 1453.52 (1+). The sequence of the precursor ion and the main fragment ions are shown.

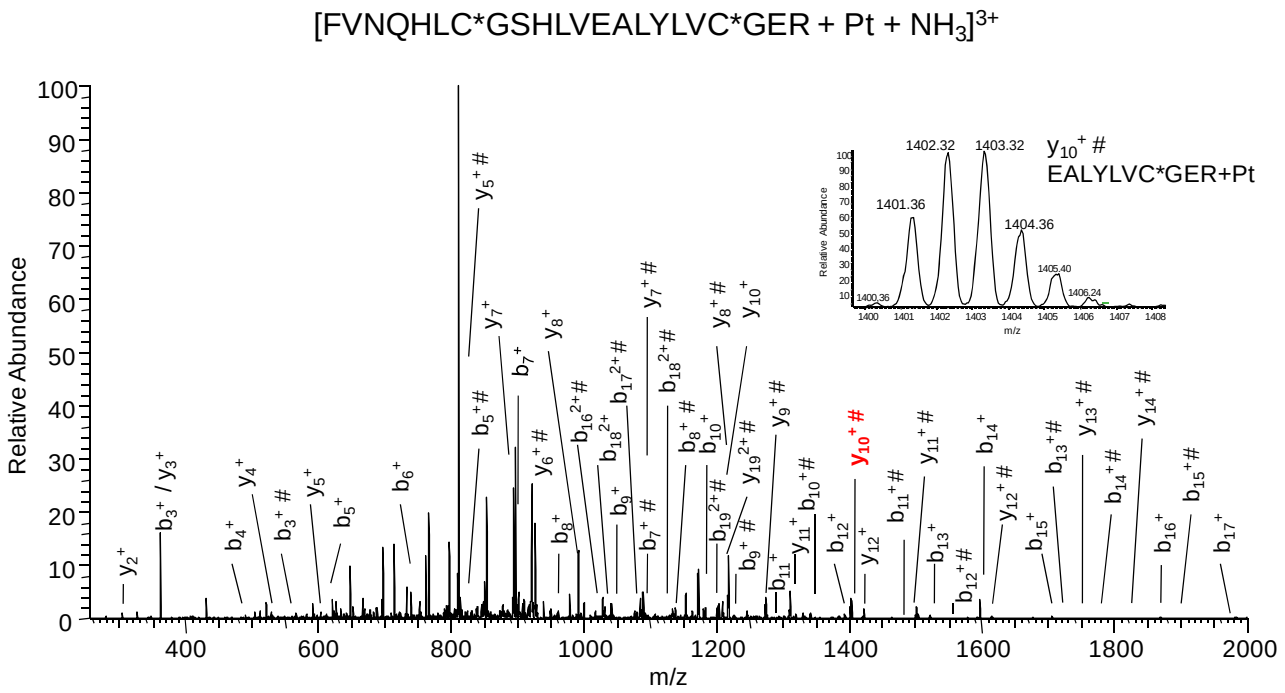

Figure S-5. CID-MS^2 spectrum for the 3+ ion at m/z 937.30, corresponding to [FVNQHLC*GSHLVEALYC*GER+Pt+NH$_3$]$^{3+}$, observed in the nESI-LIT MS analysis of a complete overnight tryptic digestion of isolated insulin-cisplatin adducts. # denotes a platinum-containing fragment ion. Inset: platinum-containing fragment ion observed at m/z 1401.36 (1+). The sequence of the precursor ion is shown and the main fragment ions have been assigned.

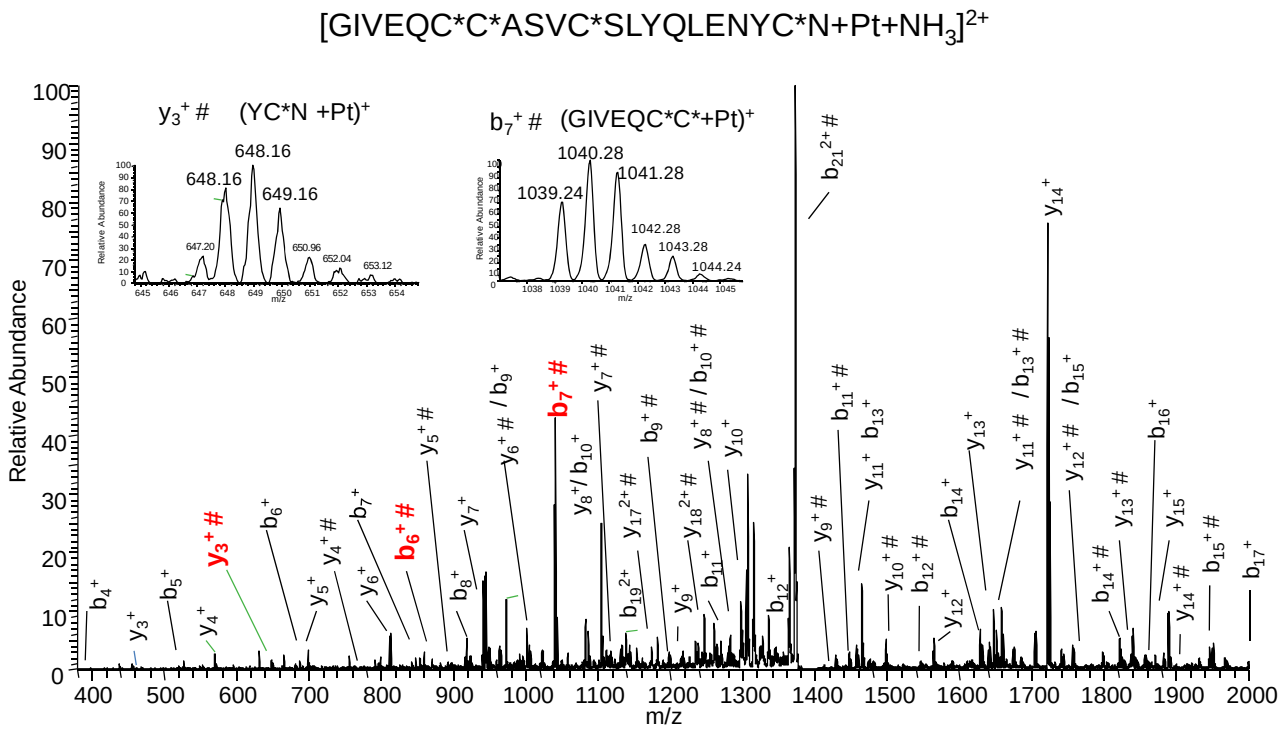

Figure S-6. CID-MS^2 spectrum for the 2+ ion at m/z 1388.18, corresponding to [GIVEQC*C*ASVC*SLYQLENYC*N+Pt+NH$_3$]$^{2+}$, observed in the nESI-LIT MS analysis of a complete overnight tryptic digestion of isolated insulin-cisplatin adducts. # denotes a platinum-containing fragment ion. Inset: relevant platinum-containing fragment ions observed at m/z 648.16 (1+) and 1039.24 (1+). The sequence of the precursor ion is shown and the main fragment ions have been assigned.

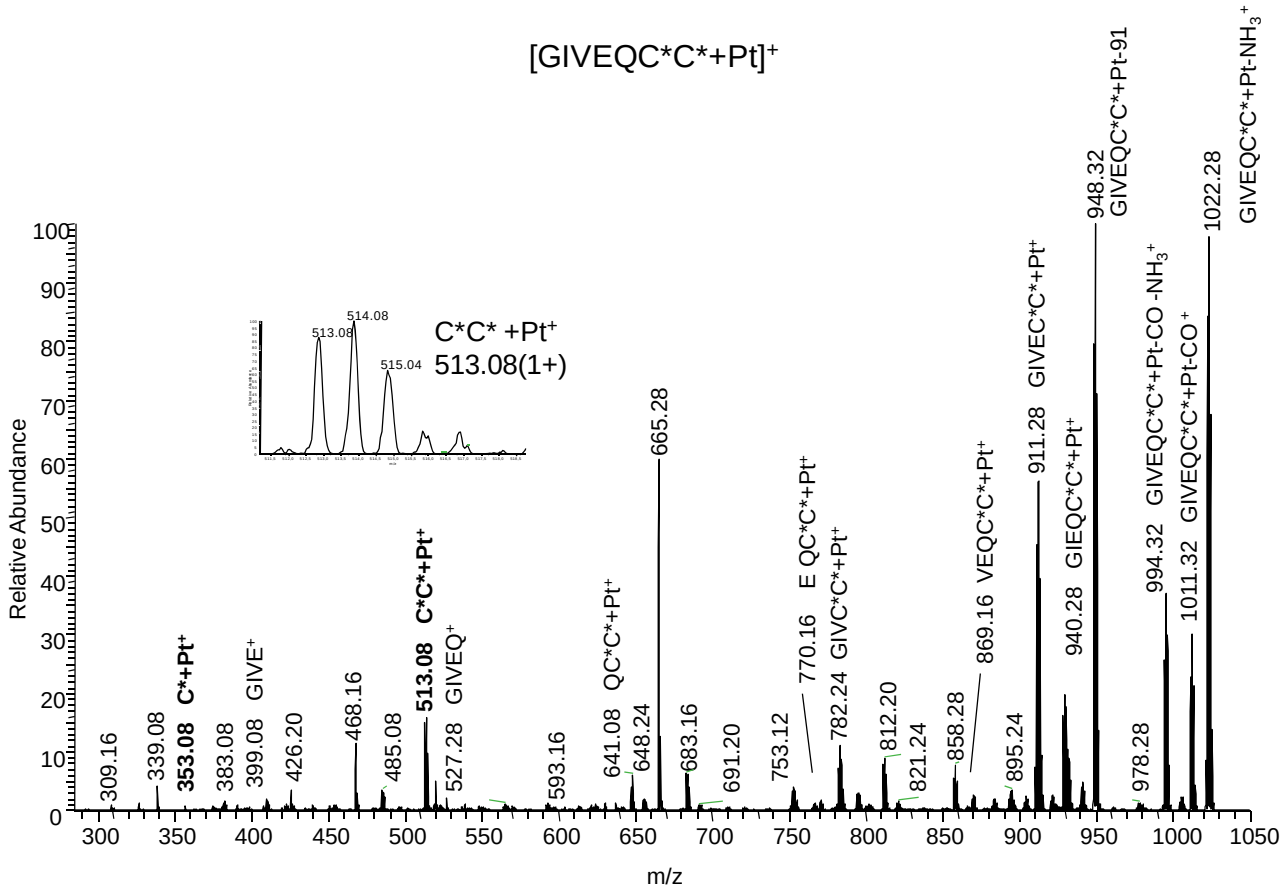

Figure S-7. CID-MS^3 of the platinated fragment ion at m/z 1039.24 (1+) observed in the MS^2 of the platinum-containing peptide at m/z 1388.18 (2+). Transition: 1388.18(2+) → 1039.24 (1+). The sequence of the precursor ion and the main fragment ions are shown. Inset: Pt- fragment ion at m/z 513,08 (1+).

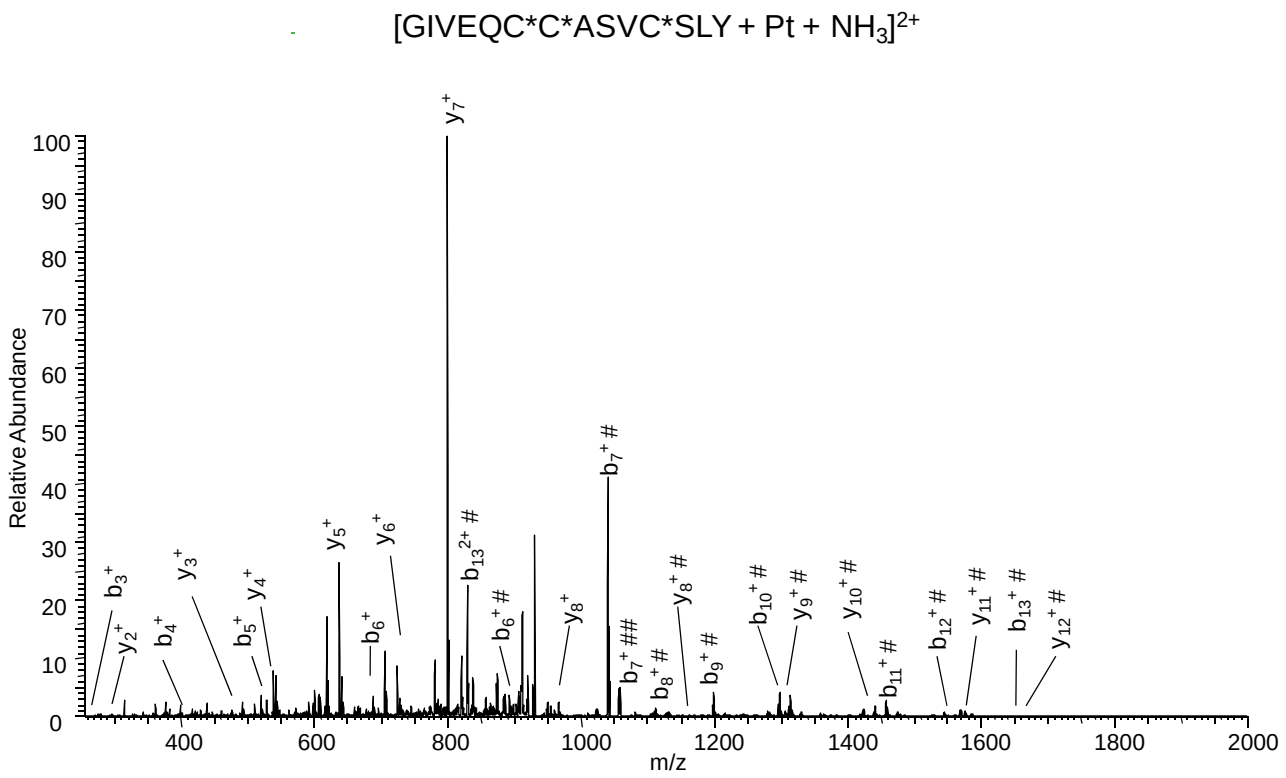

Figure S-8. CID-MS^2 spectrum for the 2+ ion at m/z 927.58, corresponding to $[GIVEQC*C*ASVC*SLY+Pt+NH_3]^{2+}$, observed in the nESI-LIT MS analysis of a complete overnight tryptic digestion of isolated insulin-cisplatin adducts. # denotes a platinum-containing fragment ion. The sequence of the precursor ion is shown and the main fragment ions have been assigned. # denotes platinum-containing ions.

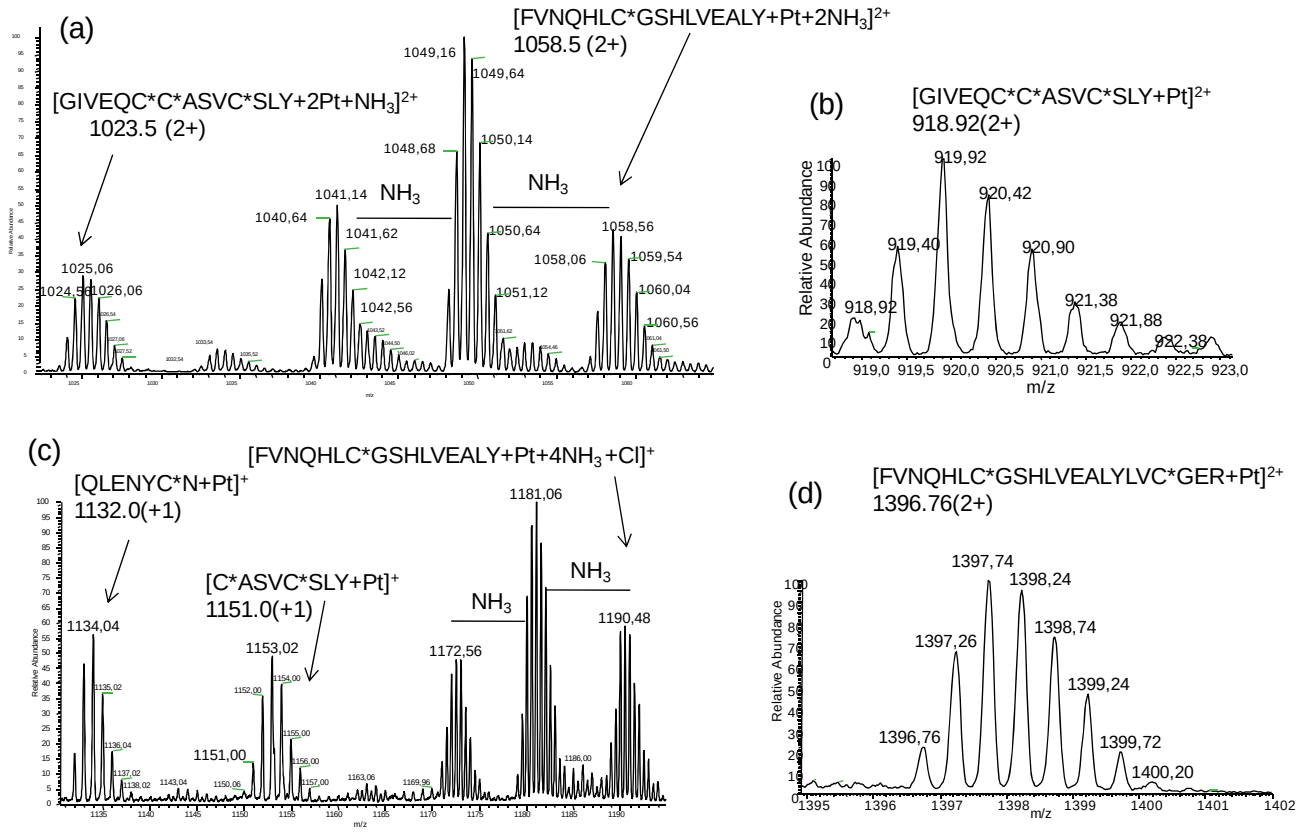

Figure S-9. (a,b,c,d) Zoom scans of platinum-containing ions observed in the nESI-LIT MS of a 1:5 incubation of tryptic insulin peptides with cisplatin (0.1%TFA, 37ºC, 96h).

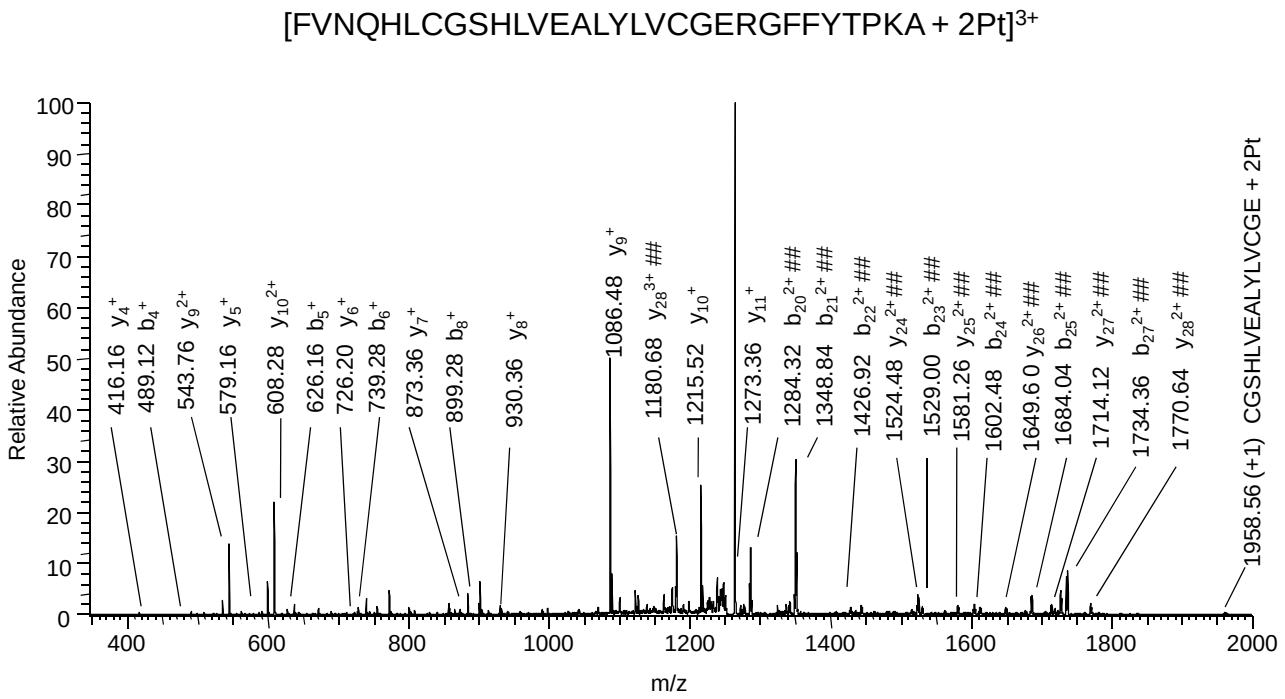

Figure S-10. CID-MS^2 spectrum for the diplatinated ion at m/z 1261.36 (3+), corresponding to [FVNQHLCGSHLVEALYLVCGERGFFYTPKA + 2Pt]$^{3+}$, observed in the nESI-LIT MS analysis of a insulin treated with 8M Urea and 10 mM DTT followed by incubation with an excess of cisplatin. The sequence of the precursor ion is shown and the main fragment ions have been assigned. ## represents diplatinated ions.