Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Determination of the binding sites for oxaliplatin on insulin using mass spectrometry-based approaches

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Experimental:

SEC-ICP-MS

LC-MS/MS (MS^E and data search using PLGS and Excel)

E**xperimental:**

SEC-ICP-MS

An Agilent 1100 HPLC (Agilent Technologies, Waldbronn, Germany) fitted with a BioSeP-SEC-S 2000 guard column 30 x 4.6 mm, (Phenomenex, Taastrup, Denmark) and a BioSep 2000-S size exclusion column, 5 µm particles, 300 x 4.6mm (Phenomenex, Taastrup, Denmark). The mobile phase was 50 mM NH_4HCO_3, pH 7.8 with a flow of 350 µL min^{-1} and 10 µL sample diluted with mobile phase was injected using the autosampler from the HPLC system. The Pt signal was recorded using an ICP-MS, Elan 6000 (Perkin Elmer, Concord, ON, Canada).The spray chamber was a PC^3 cyclonic spray chamber (Elemental Scientific Inc, Omaha, NE, USA) fitted with a Mira Mist parallel nebulizer (Burgener Research, Mississauga, ON, Canada). The nebulizer gas was adjusted externally to a flow of around 1 L min^{-1}. The ICP-MS settings were: Lens voltage: 7V, RF power: 900W, dwell time: 100 ms and the Pt signals were recorded at m/z 195 and 196. Data were processed using WSearch32 V1.6.2005 (a freely available mass spectral data reduction program).

LC-MS/MS (MS^E and data search using PLGS and Excel)

Low and elevated energy MS spectra were both acquired from m/z 50 to 1990 for 0.48 s each with a 0.02-s interscan delay. In low energy MS mode, data were collected at a constant collision energy of 4 eV. In elevated energy MS mode, the collision energy was ramped from 15 to 40 eV during each 0.48 s data collection cycle with one complete cycle of low and elevated energy data acquired every 1.0 s. The radio frequency applied to the quadrupole mass analyzer was adjusted such that ions from m/z 300 to 1990 were efficiently transmitted, ensuring that any ions less than m/z 300 observed in the LC-MS data only arose from dissociations in the collision cell. DIA LC-MS^E raw data files were processed using ProteinLynx GlobalServer (PLGS) version 2.4 (Waters Corporation) and subsequent database searching was performed using the Ion Accounting algorithm[1], embedded in PLGS, searching the supplied human SwissProt database and as well a specific database with the porcine insulin A chain and B chain included. The search tolerances were set to automatic (typically 10 ppm for precursor and 25 ppm for product ions), with oxidation of methionine, and deamidation of asparagine and glutamine set as variable modifications. Furthermore, to search for peptides with oxaliplatin, masses corresponding to attachment of Pt(dach) and Pt(dach)+OH (307.07 Da and 324.07 Da) were included as variable modifications and also masses 306.07 Da and 323.07 Da to allow for loss of a proton upon binding to insulin. For certain samples acrylamide and carbamidomethyl modification for cysteine residues were set as fixed modifications. Other settings included, minimum number of peptide matches (3), minimum number of product ion matches per peptide (3), minimum number of product ion matches per protein (5), maximum number of missed V8 cleavage sites (2), and maximum false positive rate (5%). PLGS was configured to also output results as csv-files for further analysis in Excel (Microsoft Corporation, Redmond, WA).

1. Li, G. Z.; Vissers, J. P. C.; Silva, J. C.; Golick, D.; Gorenstein, M. V.; Geromanos, S. J. (2009). Proteomics 9: 1696-1719.

Fig. S1: MALDI-TOF-MS spectrum of intact insulin-oxaliplatin analyzed by MALDI-ToF-MS (Voyager-DE)

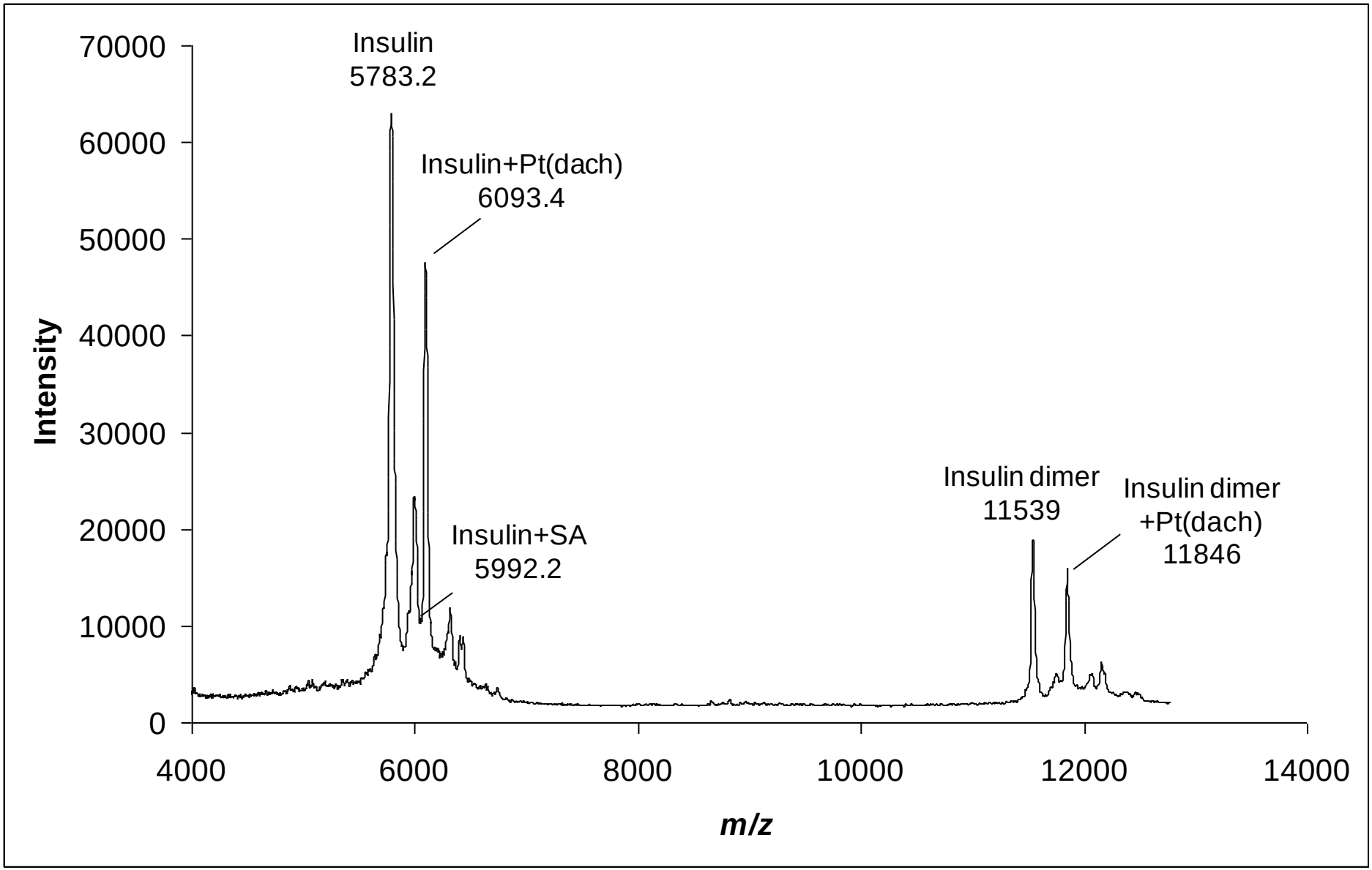

Fig. S2: SEC-ICP-MS chromatogram of reduced insulin-oxaliplatin complex as well as free oxaliplatin

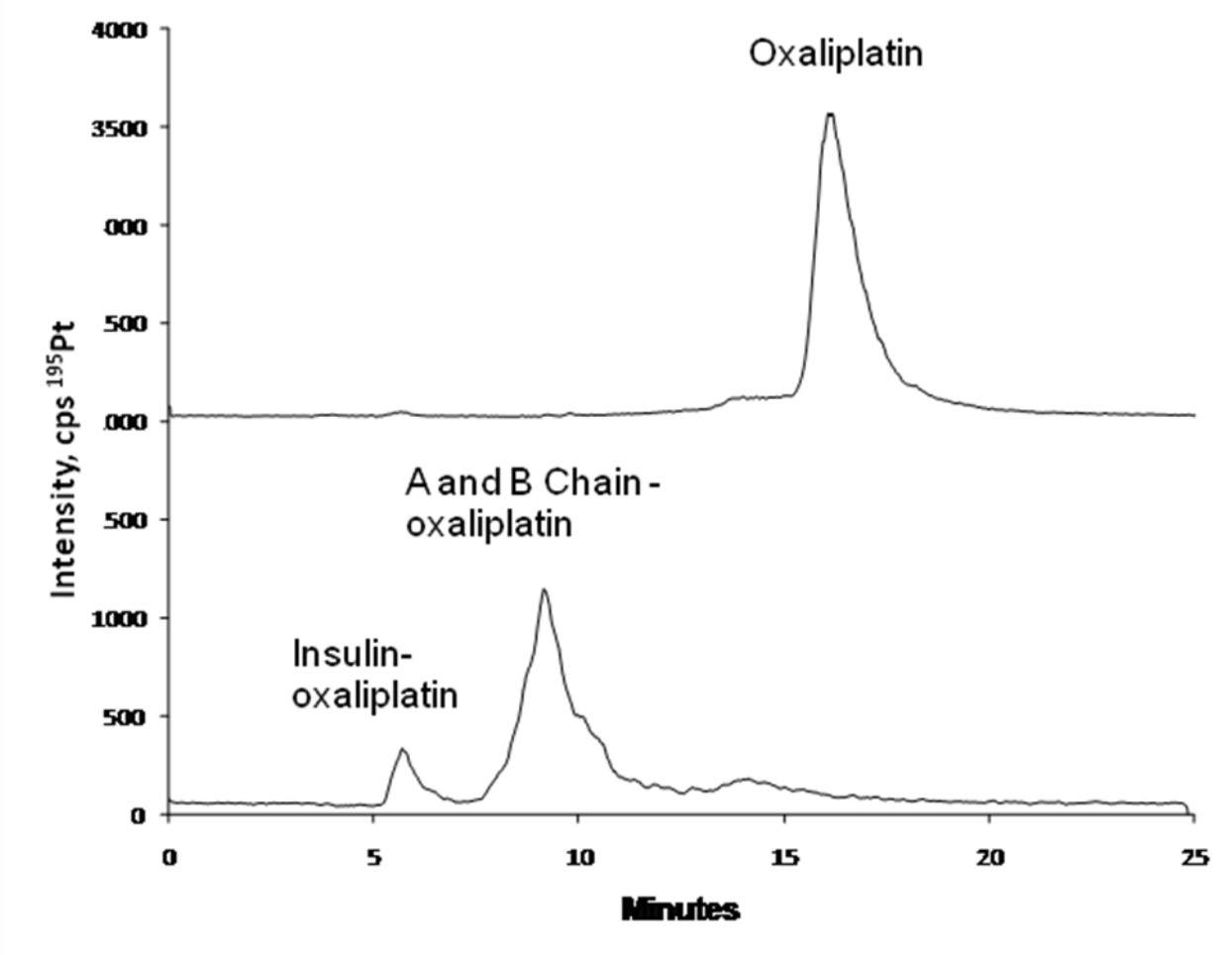

Fig. S3: The deconvoluted nESI-Q-ToF- mass spectrum of reduced insulin-oxaliplatin. A denotes the A chain and B denotes the B chain of insulin

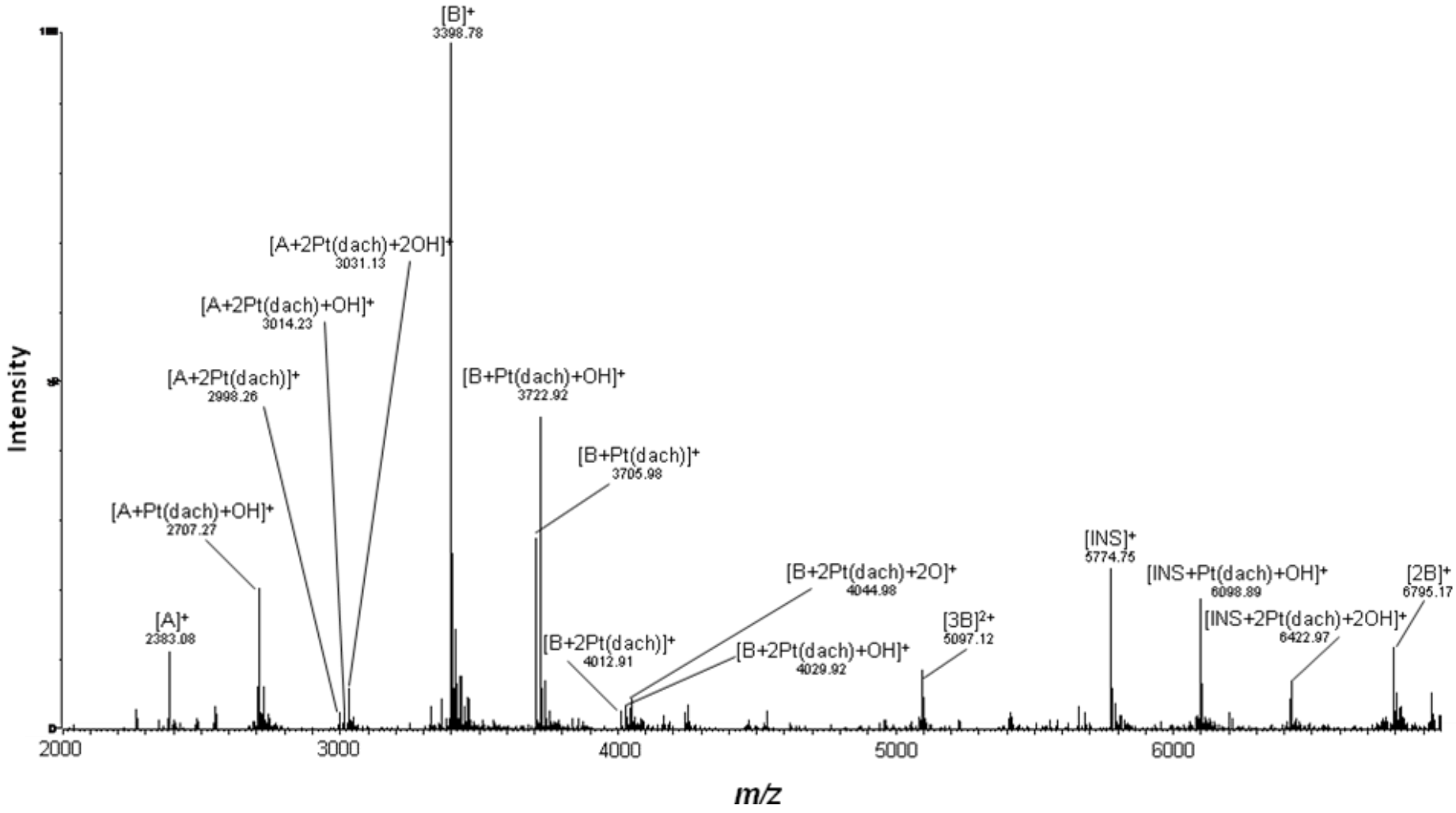

Fig. S4: MALDI-ToF MS/MS spectrum of a: B1+Pt(dach) at *m/z* 1789.71, b: A1+Pt(dach) at *m/z* 724.26

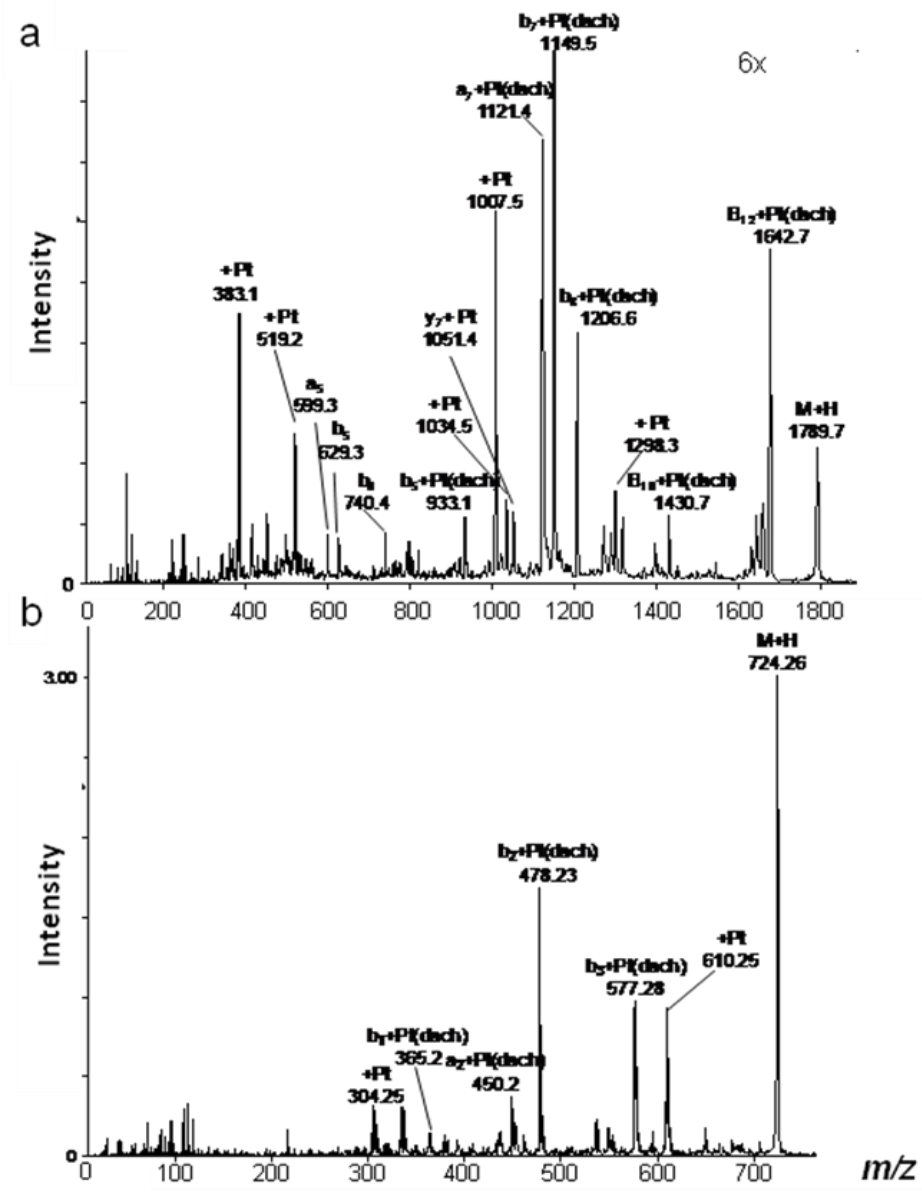

Fig. S5: MALDI-ToF-ToF-MS/MS spectrum of B1+Pt(dach)+acrylamide at m/z 1860.7

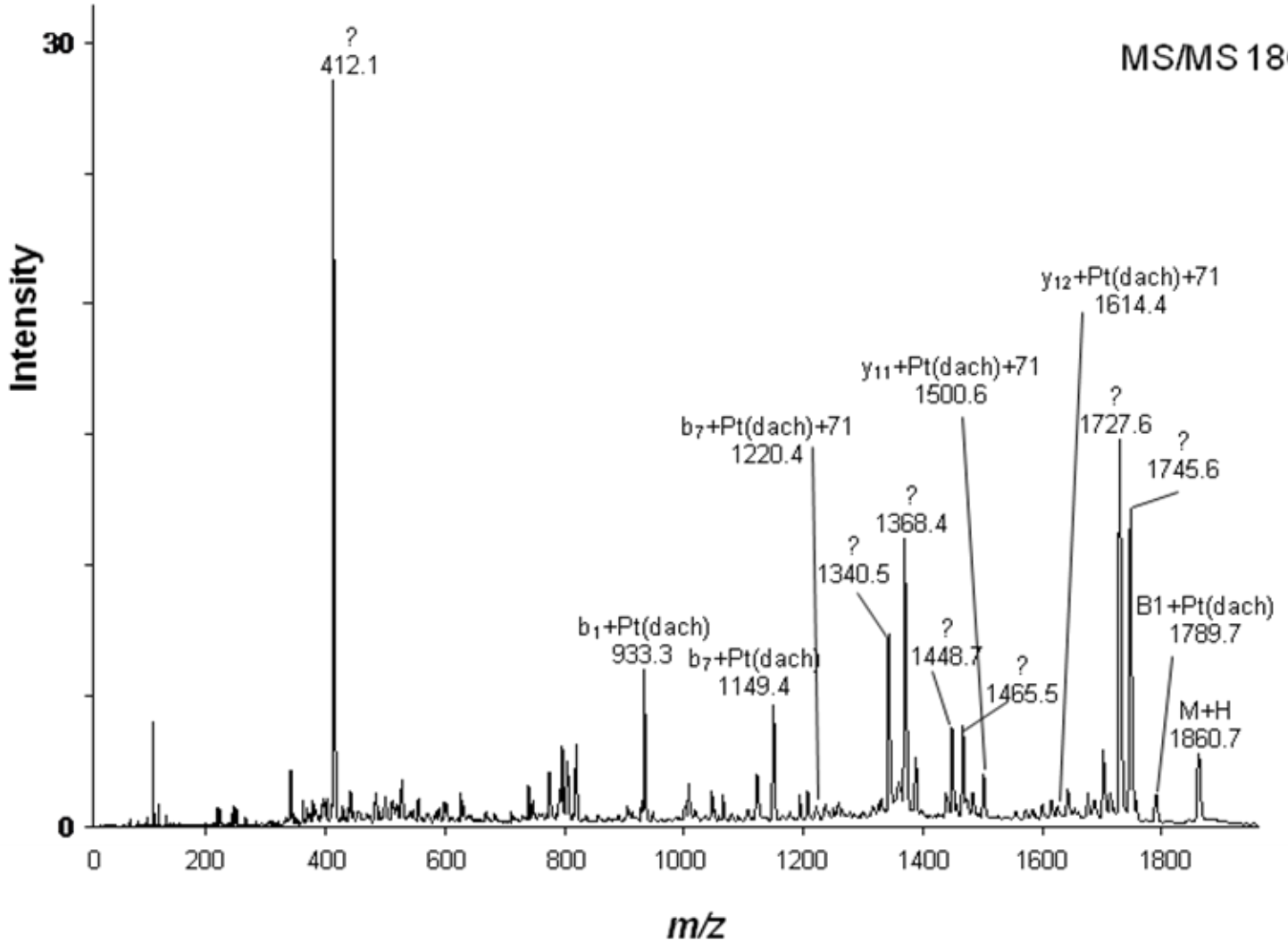

Fig. S6: MALDI-ToF-ToF- MS/MS spectrum of B1+Pt(dach) at m/z 1789.71

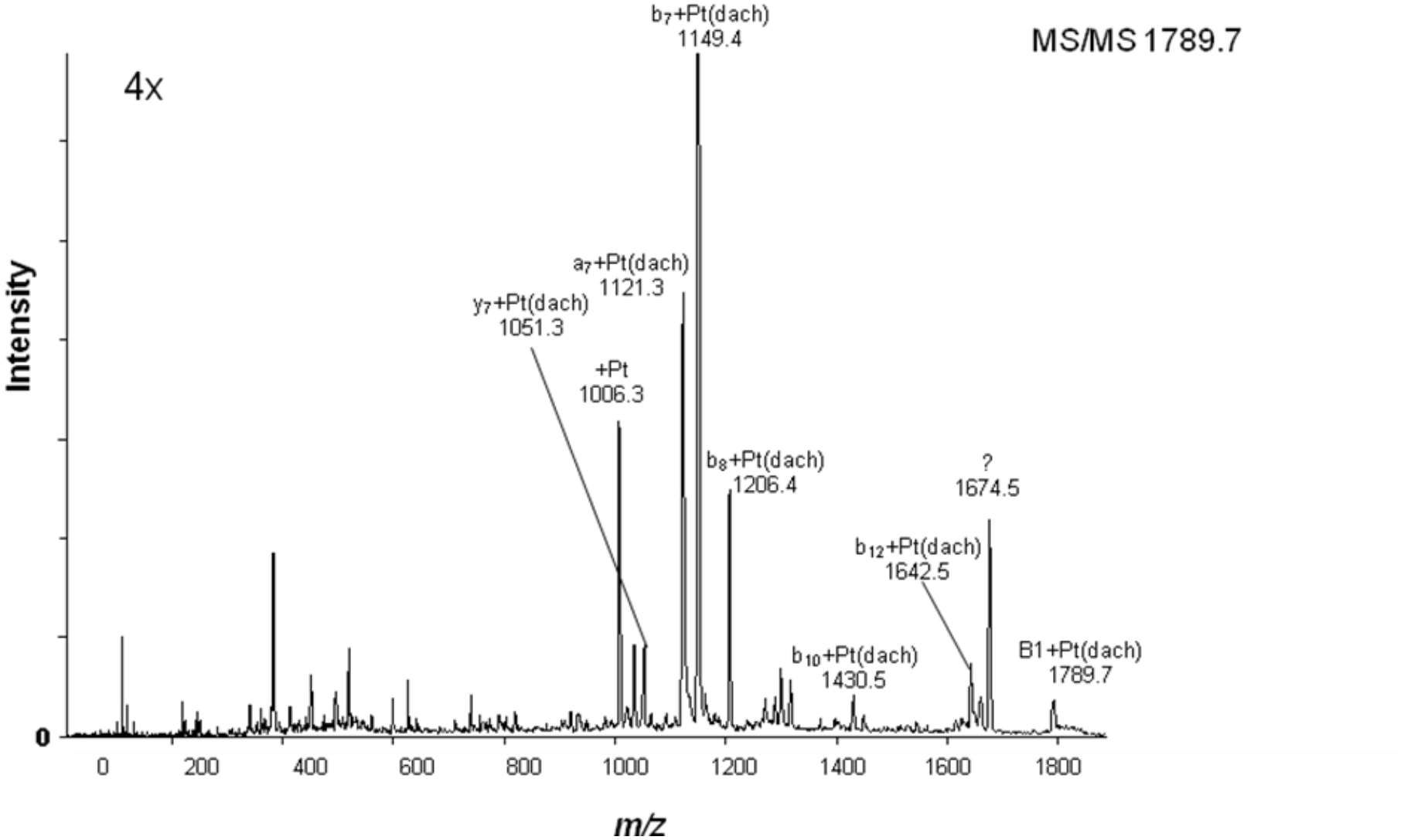

Table S1: Overview of peptides formed after digestion only, and after both digestion and reduction of insulin-oxaliplatin identified with MALDI-ToF-ToF-MS/MS. The bottom part shows the peptides with Pt(dach) attached after incubation of digested only, and both digested and reduced peptides with oxaliplatin for 2 hours at 37 °C

Digested and digested and reduced peptides identified with MALDI-ToF-ToF-MS

Peptide	$M+H^+$	Ions identified	Binding site
B1	1482.66	$b_1 - b_{12}$, y_6- y_{10}	
B1 + Pt(dach)	1789.71	(b_5, a_7, b_7 b_8, b_{10}, b_{12}, y_7) + Pt(dach)	Histidine5 or cysteine7
B1 + 2 Pt(dach)	2096.75	b_7 +Pt(dach)	
B2	867.36	$b_2 - b_7$, $y_3 - y_6$	
B3	1086.53	$b_1 - b_8$, y_1- y_5	
B3 + Pt(dach)	1393.65	None with Pt(dach)	
A1	417.50		
A1 + Pt(dach)	724.26	(b_2, b_3, a_2, a_3, and possible b_1) + Pt(dach)	N-terminus
A2	513.10		
B1-A2	2968.20	a_5,b_5, a_6 b_6, b_7 –SH_2	
B1-A2 + Pt(dach)	3275.37	(Bb_5, Bb_7, B1) + Pt(dach)	Histidine5
B1A1*A2	3366.42	Bb_5, Bb_6	
B1-A1*A2+Pt(dach)	3673.50	None with Pt(dach)	
B1A2*	2321.87	Bb_1 –Bb_7	
B1A2* + Pt(dach)	2628.93	(Bb_5, Bb_7, B1)+ Pt(dach)	Histidine5
B2-A3	1377.57	$B2b_2 - B2b_5$, $B2A3y_3$, $B2A3y_4$, $A2By_4$- $A2By_6$	
B2-A3 +Pt(dach)	1684.61	None with Pt(dach)	

Peptides with Pt(dach) after incubation of digested insulin and digested and reduced insulin with oxaliplatin for 2 hours

Peptide	$M+H^+$	Ions with Pt(dach) attached	Binding site
B1 +Pt(dach)	1789.76	a_7, b_7, b_8, b_{10}, b_{12}	Cysteine7?
A2-B3 +Pt(dach)	1684.62	None	
B1-A2+Pt(dach)	3275.36	Bb_5, Bb_7, B1	Histidine5?
B3+Pt(dach)	1393.63	None	

* Denotes a mis-cleaved peptide