

Pt(II) compounds interplay with Cu(II) and Zn(II) coordination to the Amyloid- β peptide has metal specific consequences on deleterious processes associated to Alzheimer's Disease.

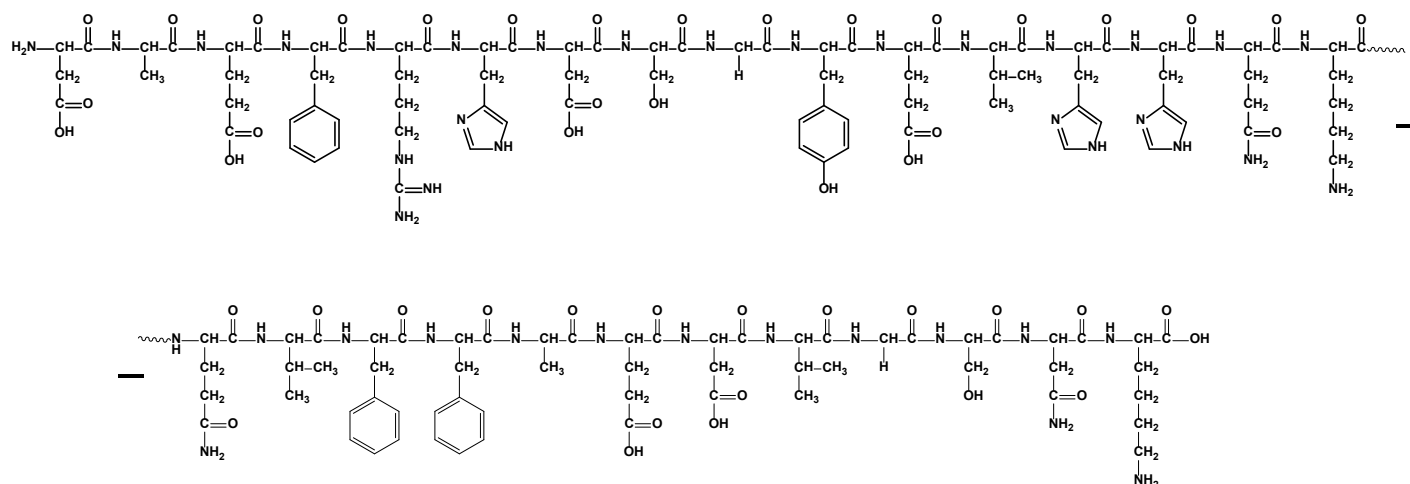
Fabrice Collin,^a Isabelle Sasaki,^b Hélène Eury,^b Peter Faller^b and Christelle Hureau^b

^a PHARMA-DEV, UMR 152 IRD-UPS, Université Paul Sabatier, 118 route de Narbonne, 31400 Toulouse, France.

^b LCC, CNRS UPR 8241, 205 route de Narbonne, 31077 Toulouse, France.
and Université Paul Sabatier, 118 route de Narbonne, 31400 Toulouse, France.

SUPPLEMENTARY INFORMATION

Experimental Section	p1
ROS data	p6
EPR data	p7
Aggregation data	p8
TEM pictures	p9
RMN data	p11
HPLC/MS data	p13



Scheme S1. A β 28 sequence.

1-Experimental Section

Chemicals and peptide preparation.

Peptides: A β 28 peptide (sequence DAEFRHDSGYEVHHQKLVFFAEDVGSNK) was bought from GeneCust (Dudelange, Luxembourg) with purity grade > 98%.

Stock solutions of peptide were prepared by dissolving the powder in milliQ water (resulting pH ~ 2) to reach a ~ 2 mM concentration. Peptide concentration was then determined by UV-visible absorption of Tyr10 considered as free tyrosine ($\epsilon_{276}-\epsilon_{296}$) = 1410 M⁻¹cm⁻¹ at pH 2. The stock solution was adjusted to pH 12. Then, the solutions were diluted down to the appropriate concentration in peptide. All pH values are given with a $\pm$ 0.2 pH unit error.

Copper solutions: Cu(II) used was from CuSO₄.5(H₂O) and purchased from Sigma. Stock solution of Cu(II) (~1M) was prepared in H₂O.

Zinc solutions: Zn(II) used was from Zn(SO₄).H₂O and purchased from Strem. Stock solution of Zn(II) (~0.1M) was prepared in H₂O.

Hepes and phosphate buffer were bought from Sigma-Aldrich.

Tris-d¹¹ buffer stock solution (500mM) was prepared in D₂O from [deuterated tris(hydroxymethyl)aminomethane] powder bought from Eurisotop and adjusted to required pH (see NMR experiments section).

ThioflavinT (ThT) was bought from Acros organics.

Platinum complexes

Commercial reagent grade chemicals, 1,10-phenanthroline (phen), 2,2'-bipyridine (bpy), 4,7-diphenyl-1,10-phenanthroline(Φ -phen) (Aldrich) and K₂PtCl₄ (Strem) were used. Pt(DMSO)₂Cl₂,¹ Pt(bpy)Cl₂,² Pt(Φ -Mepy)(DMSO)Cl,³ Pt(phen)Cl₂ and Pt(Φ -phen)Cl₂⁴ were prepared as previously described.

Liquid chromatography / mass spectrometry analysis (HPLC/MS)

The A β 28 peptide (50 μ M) was incubated with Pt complex (1.0 equivalent) in ammonium phosphate buffer (0.8 mM, pH 7.3) and DMSO 1%. HPLC/MS analysis was performed on an ion-trap mass spectrometer (LCQ DECA XP Max, ThermoFisher), equipped with an electrospray ionization source, coupled to a SpectraSystem HPLC system. Sample (10 μ L) was injected onto the column (Phenomenex, Synergi Fusion RP-C18, 250 $\times$ 1 mm, 4 μ m), at room temperature. Gradient elution was carried out with formic acid 0.1% (mobile phase A) and acetonitrile/water (80/20 v/v) formic acid 0.1% (mobile phase B) at a flow-rate of 50

$\mu\text{L}\cdot\text{min}^{-1}$. The mobile phase gradient was programmed with the following time course: 12% mobile phase B at 0 min, held 3 minutes, linear increase to 100% B at 15 min, held 4 min, linear decrease to 12% B at 20 min and held 5 min. The mass spectrometer was used as a detector, working in the full scan positive mode between 50 and 2 000 Da followed by data dependent scans of the two first most intense ions, with dynamic exclusion enabled. Isolation width was set at 4 Da and collision energy at 28% (units as given by the manufacturer), using wideband activation.

MS Data Analysis

The generated tandem MS data was searched using the SEQUEST algorithm against the human A β peptide sequence. Dynamic modifications were specified according to the expected mass shift due to the coordination of Pt complex to the A β 28 peptide (see Table S1).

Fluorescence spectroscopy

Fluorescence spectra were measured with a FLUOstar Optima (BMG Labtech) or a SAFAS multiplate reader.

Aggregation of the A β 28 peptide was monitored by thioflavine-T fluorescence (excitation at 440 nm, emission at 490 nm). The peptide and the Pt complexes were first mixed together and then the aggregation process was triggered by the addition of Zn. Significant effect of varying the incubation time of the peptide and Pt complexes (up to one hour) wasn't observed.

Taking into account that false-positive effect can be observed when using ThT to monitor aggregation,⁵ specific controls were performed. The possibility that the ThT Fluorescence decrease observed in presence of complexes **1** to **3** was due to inner-filter effect (either due to the absorption of the Pt complexes themselves or to presence of high turbidity induced by the Pt complexes) was ruled out on the basis of UV-Vis measurement at 440 nm (no absorption at this wavelength). In addition, as explained in the full-text, the Pt complexes interact with residues 11-13, while ThT interact within the hydrophobic core of the peptide. Hence, the possibility that the Pt complexes that induce the decrease of the ThT fluorescence take the place of ThT was ruled out.

Regarding complexes **4** and **5** for which no effect on the fluorescence was observed, the possibility of interference with ThT was not considered since it was shown by other

techniques (ESI-MS, NMR (see below)...) that those two complexes do not interact with the peptide even in absence of ThT.

Transmission electron microscopy

Peptide samples (20 μ L), aggregated for 45h, were applied on EM grids (copper grids recovered with a carbon film), washed with 20 μ L of Milli-Q water, and negatively stained with an aqueous solution (20 μ L) of uranyl acetate (1% w/w). Samples were air-dried and examined with a JEOL 1011 transmission electron microscope operating at an accelerating voltage of 100 kV.

EPR Experiments

Electron Paramagnetic Resonance (EPR) data were recorded using an Eleksys E 500 Bruker spectrometer, operating at a microwave frequency of approximately 9.5 GHz. All spectra were recorded using a microwave power of 50 mW across a sweep width of 150 mT (centred at 310 mT) with modulation amplitude of 1 mT. Experiments were carried out at 110 K using a liquid nitrogen cryostat.

EPR samples were prepared from stock solution of peptide diluted down to 1.0 mM in hepes buffer 20mM, pH 7.4. 1.0 equiv. of $^{63}\text{Cu(II)}$ from in-lab prepared 0.1 M $^{63}\text{Cu(NO}_3)_2$ stock solution was added to the A β peptide pre-incubated with the Pt complexes. Samples were frozen in quartz tube after addition of 10% glycerol as a cryoprotectant and stored in liquid nitrogen until used.

RMN Experiments

1D ^1H were recorded on a Bruker Avance 500 spectrometer equipped with a 5 mm triple resonance inverse Z-gradient probe (TBI 1H, 31P, BB). Suppression of the water signal was achieved with WATERGATE or presaturation sequences. Diffusion measurements were made using the stimulated echo pulse sequence with bipolar gradient pulses.

UV-Vis Experiments

UV-Vis experiments were recorded on a Hewlet Packard Agilent 8453 UV-Vis spectrometer at 20°C.

Ascorbate consumption experiments.

Ascorbate (Asc) consumption was monitored by UV-Vis. Intensity of the Asc absorption band at $\lambda = 265 \text{ nm}$ ($\epsilon = 14\,500 \text{ M}^{-1}.\text{cm}^{-1}$) was monitored as a function of time, in 50 mM hepes buffer, pH 7.4 containing 100 μM of Asc, 7 μM of the A β 16 peptide incubated for one hour with 7 μM of Pt complexes. Reaction was started by addition of 5 μM of Cu(II).

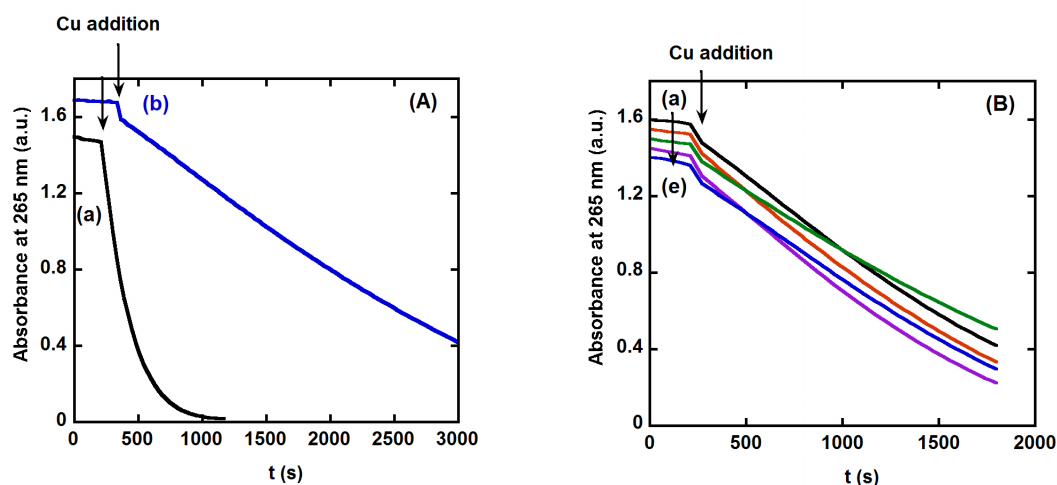


Figure S1. Ascorbate (Asc) consumption of Cu(II) (Panel A, (a)) and of Cu(Aβ28) (Panel A, (b)) and of Cu(Aβ16) in presence of complexes **1** to **5** (panel B, spectra (a) to (e), respectively). [Cu(II)] = 5 μM, [Aβ16] = 7 μM and [Pt complexes] = 7 μM. [Asc] = 100 μM, [hepes] = 50mM, pH 7.4. Note that spectra (a) to (e) in panel B were arbitrarily moved up or down for a better clarity of the figure.

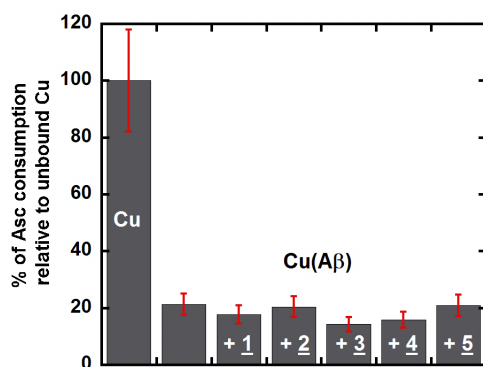


Figure S2. Effect of the Pt(II) complexes **1** to **5** on the consumption of Ascorbate (Asc) by the Cu(Aβ16) complex (same conditions as in Figure S1). The values represent the initial slope of the decay in Asc absorption at 265 nm.

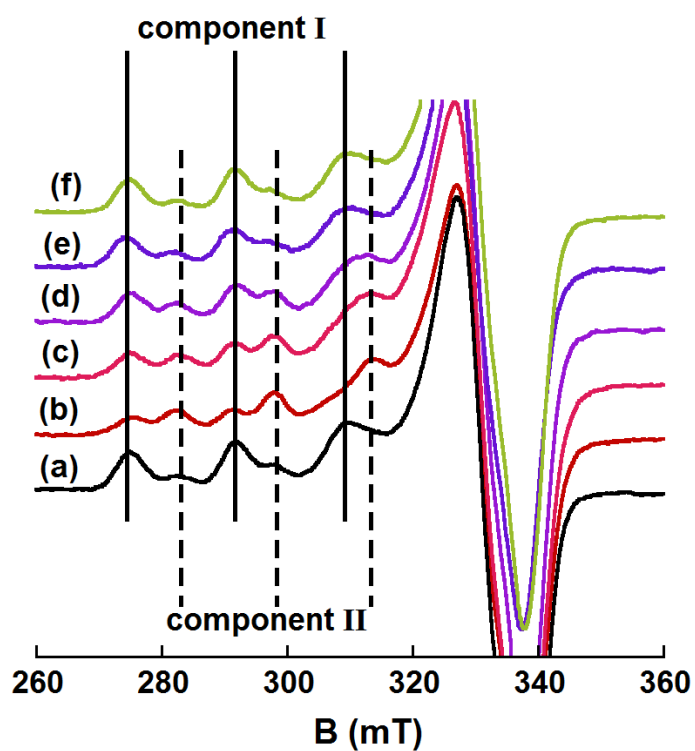


Figure S3. EPR spectra of 200 μ M A β 28 incubated with 1.0 equivalent of Pt complexes for 1 hour at room temperature, without Pt complexes (a) and in presence of (b) **1**, (c) **2**, (d) **3**, (e) **4**, (f) **5**, followed by addition of 1.0 equivalent of Cu(II). [hepes] = 20 mM, pH 7.4, with 10% glycerol. ν = 9.5 GHz, microwave power = 50 mW, modulation amplitude = 1 mT, T = 110K.

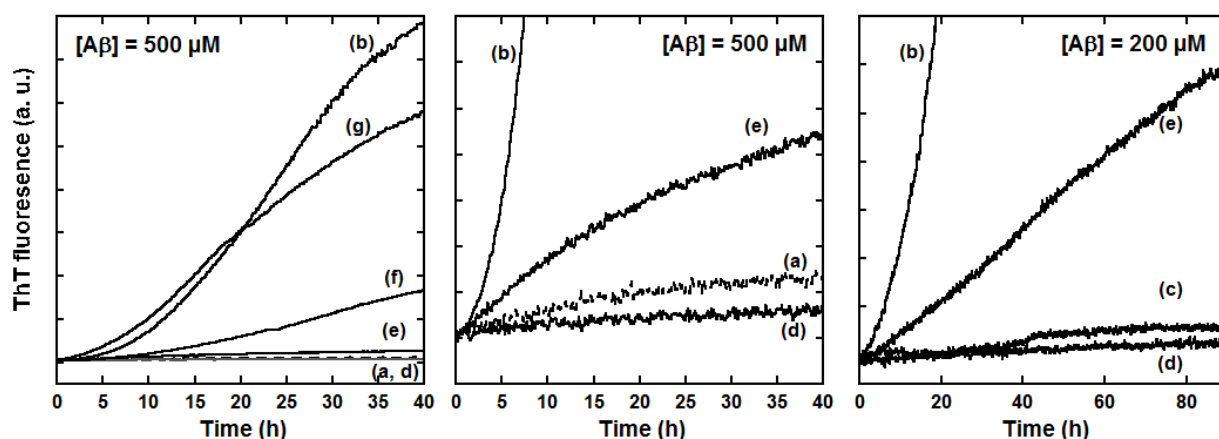


Figure S4. ThT fluorescence as a function of time of (a) apo-Aβ28, Zn_{0.5}(Aβ28) (b) and Zn_{0.5}(Aβ28) in presence of 1, (c), 2 (d), 3 (e), 4 (f) and 5 (g). Left and centre panels: [Aβ28] = 500 μM; [Zn] = 250 μM, [Pt-complexes] = 550 μM, 5% DMSO (v/v). Right panel: [Aβ28] = 200 μM; [Zn] = 100 μM [Pt-complexes] = 220 μM, 2% DMSO (v/v). Phosphate buffer (10 mM, pH 7.4,) [ThT] = 10 μM. Complex 1 is not sufficiently soluble at 500 μM in presence of Aβ28 to be studied by fluorescence. Hence the measurements were also performed at 200 μM. Note that no effect of the incubation time between the Pt complexes and the Aβ28 prior to the triggering of the aggregation by the addition of Zn was observed.

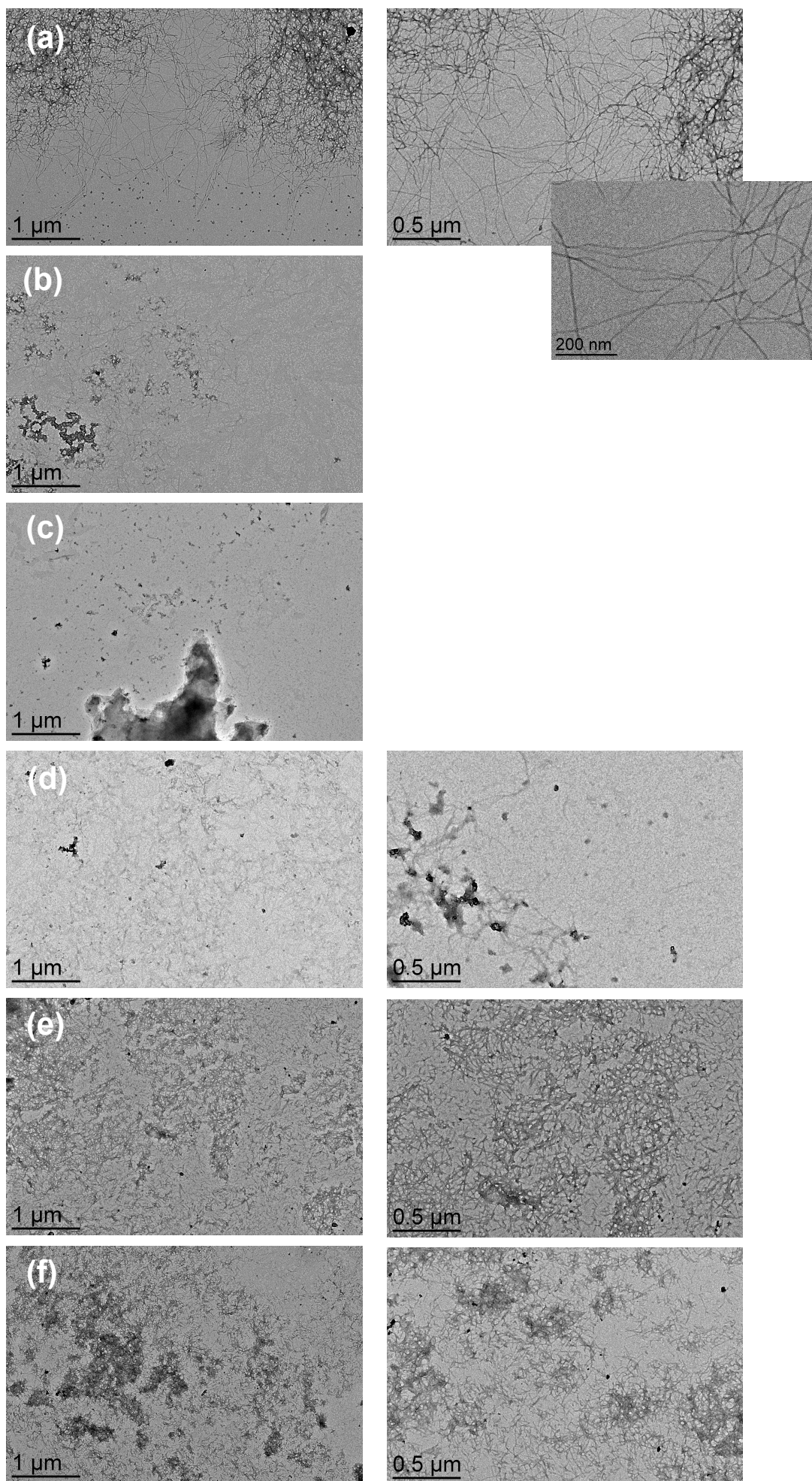


Figure S5. Representative Transmission Electron Microscopy (TEM) pictures of fibrils generated from A β 28 peptide, at room temperature. Phosphate buffered (10 mM, pH 7.4) aqueous solution of 500 μ M A β 28, 250 μ M Zn(II), 5% DMSO (v/v) and 10 μ M Thioflavin T, without (a) or with 550 μ M of Pt complexes (b) 1, (c) 2, (d) 3, (e) 4, and (f) 5 at various magnification. The A β 28 peptide was incubated with the Pt complexes for one hour prior to the experiment, and the aggregation triggered by the addition of Zn. Evolution of the samples were monitored by ThT experiments and taken for TEM analysis after 45 h.

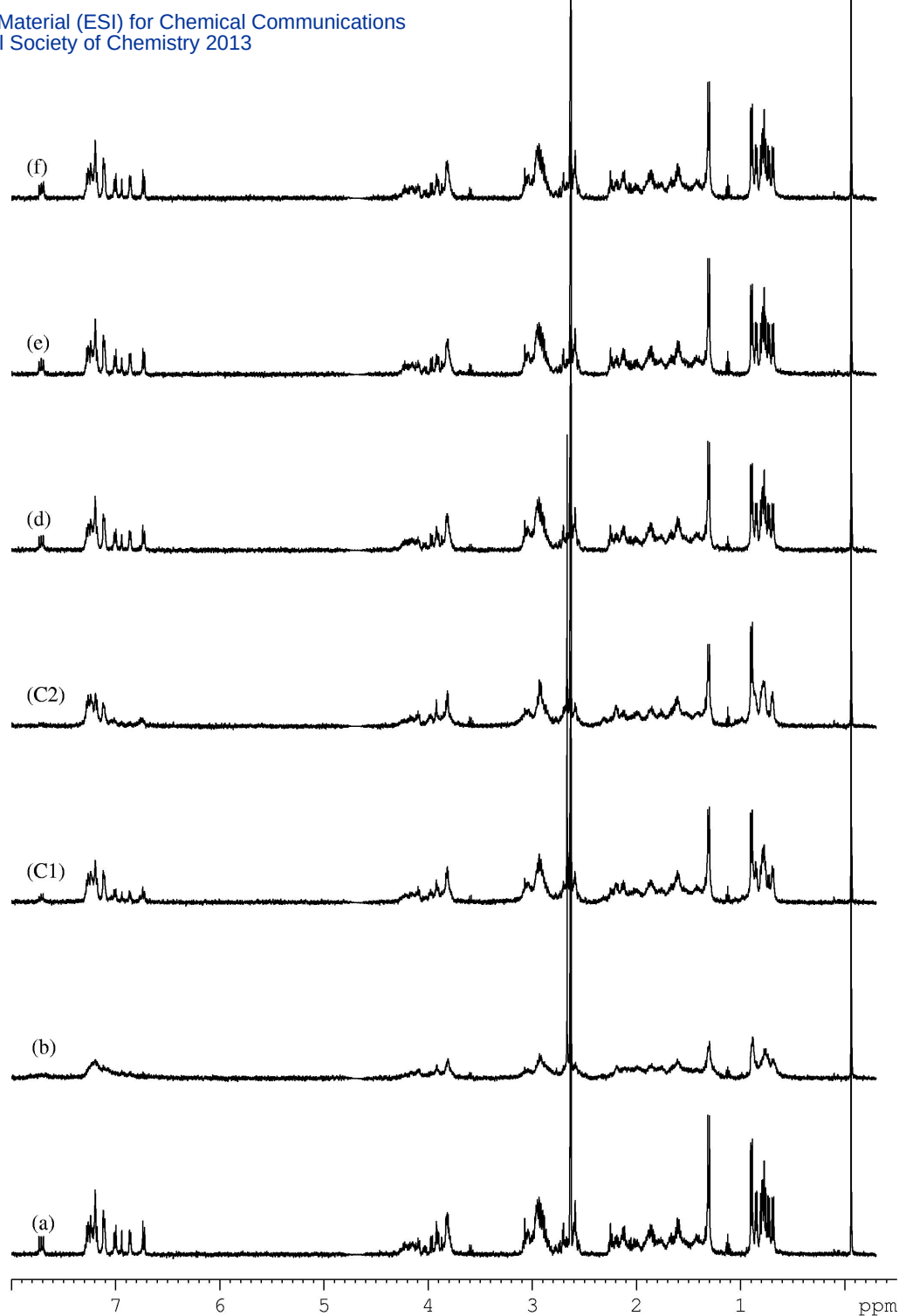


Figure S6. ^1H NMR spectra of (a) $\text{A}\beta_{28}$; (b) $\text{A}\beta_{28}$ after addition of one equiv. of complex **1**; (c1) complex **2** after 3 min; (c1) complex **2** after 60 min incubation; (d) complex **3**; (e) complex **4**; (f) complex **5**. For complexes 1 and 3 - 5 no difference were observed between 3 min or 60 minutes of incubation. Phosphate buffer (10 mM, pH 7.4, D_2O), $T = 298\text{ K}$, $[\text{A}\beta_{28}] = 100\text{ }\mu\text{M}$; $\nu = 500\text{ MHz}$.

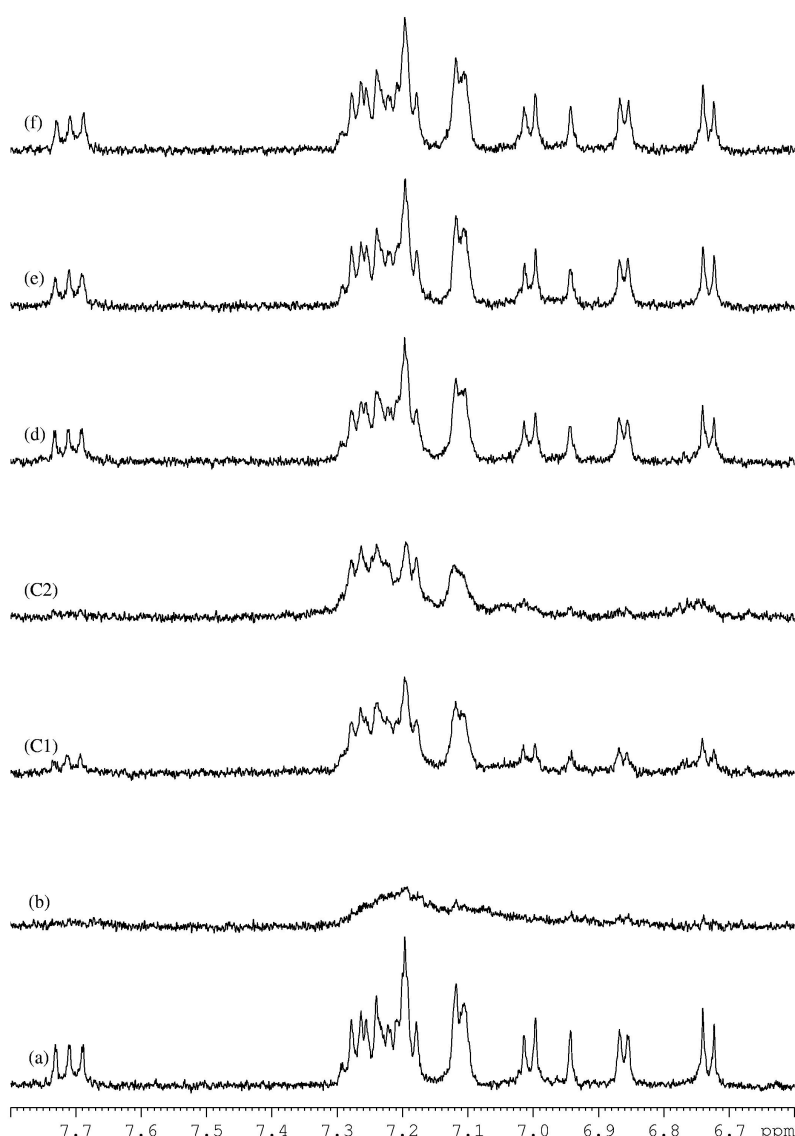


Figure S7. Aromatic region of ^1H NMR spectra of (a) A β 28; (b) A β 28 after addition of one equiv. of complex **1**; (c1) complex **2** after 3 min; (c2) complex **2** after 60 min incubation; (d) complex **3**; (e) complex **4**; (f) complex **5**. For complexes **1** and **3** - **5** no difference were observed between 3 min or 60 minutes of incubation. Phosphate buffer (20 mM, pH 7.4, D $_2$ O), T = 298 K, [A β 28] = 100 μM ; DMSO 2%; ν = 500 MHz.

Aromatic protons of the A β 28 His residues are the most affected by the addition of the Pt complexes (δ = 7.7 and 6.9 ppm). **1** and **2** are the most effective in inducing the vanishing of the NMR signals, **3** induces only a small decrease of the NMR signals and **4** and **5** have no effect. It is worth noting that the Tyr10 aromatic protons (δ = 7.0 and 6.7 ppm) are also broadened by the presence of the Pt complexes, in line with the implication of Glu11 in Pt binding.

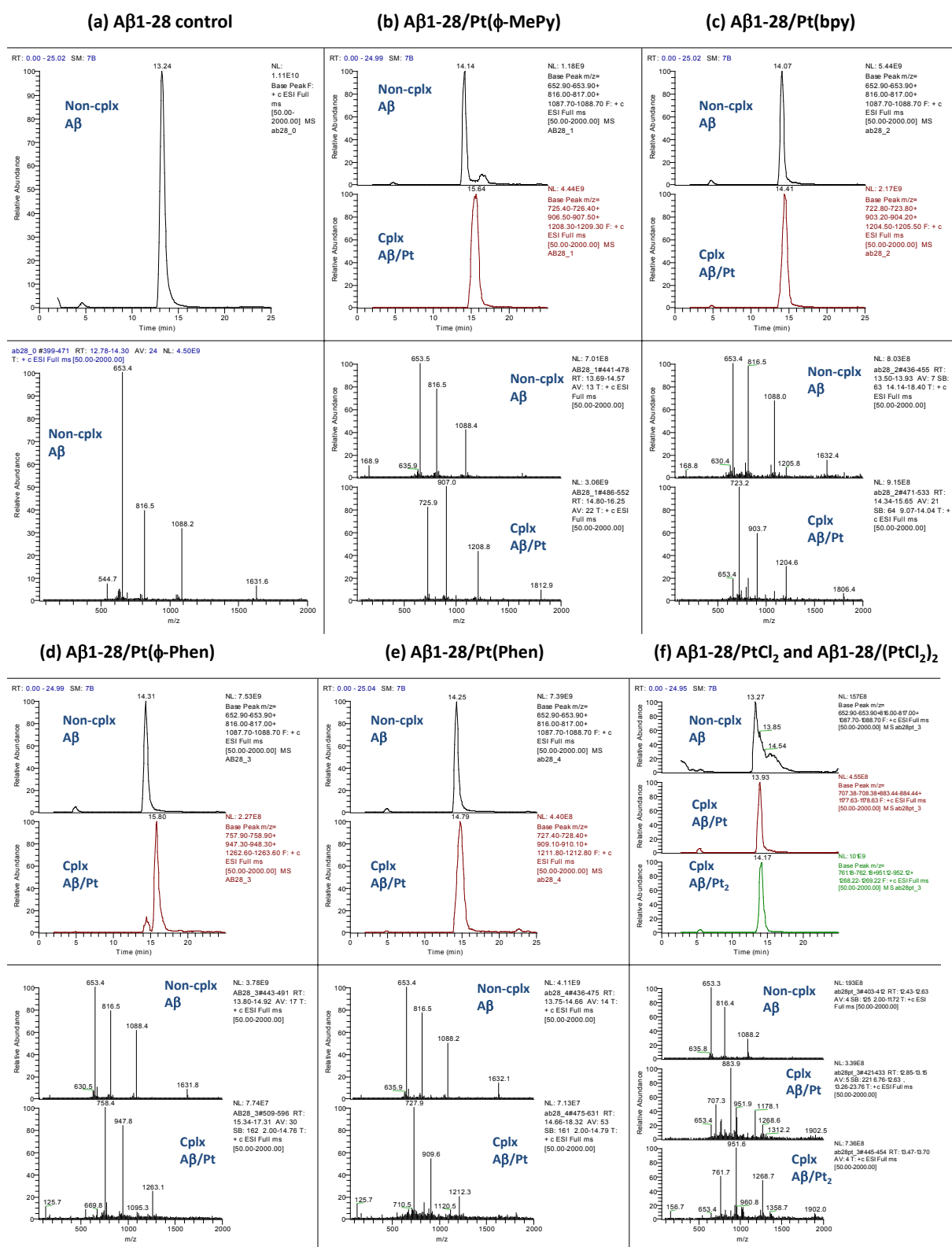


Figure S8. Trace chromatograms (top) and corresponding mass spectra (bottom) for aqueous solution of A β 28 (a) alone or complexed with (b) **1**, (c) **3**, (d) **5**, (e) **4**, and (f) **2**. [A β 28] = 50 μ M, [Pt complexes] = 50 μ M.

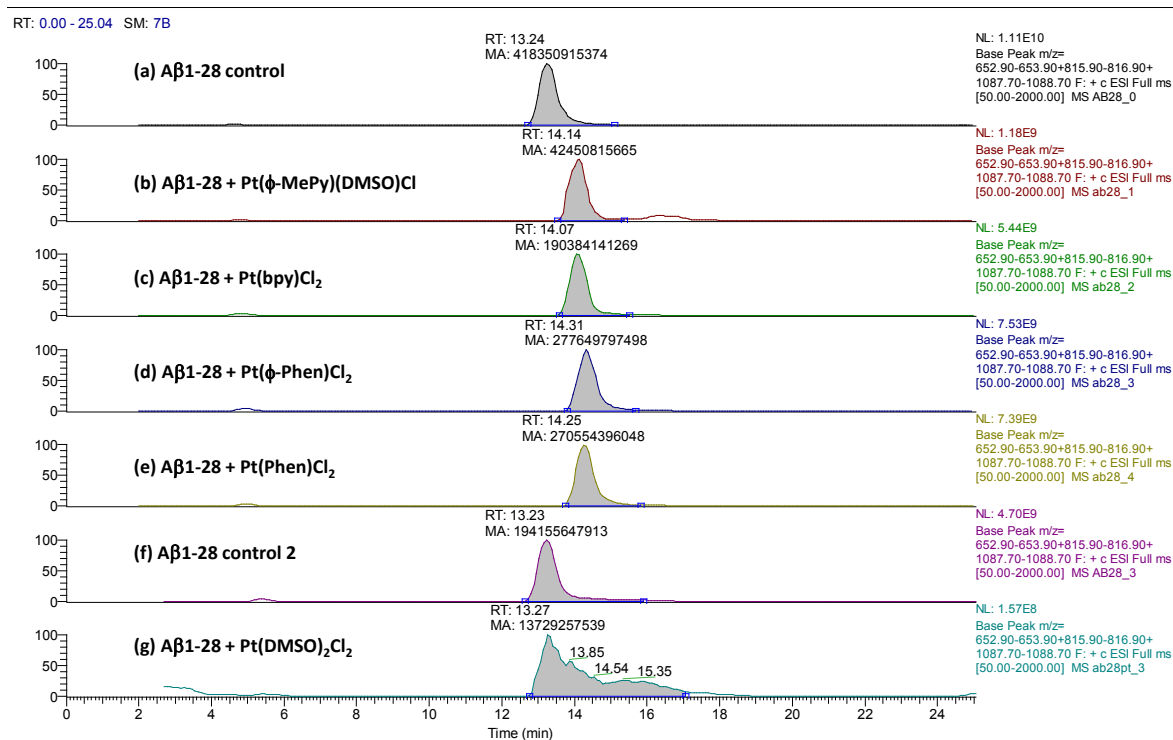


Figure S9. Trace chromatogram of the remaining non-complexed Aβ28 (m/z 653.4⁵⁺, 816.4⁴⁺ and 1088.2³⁺) for aqueous solutions of 50 μM Aβ1-28 (a, f) alone or complexed with (b) **1**, (c) **3**, (d) **5**, (e) **4** and (g) **2**. [Aβ28] = 50 μM, [Pt complexes] = 50 μM.

#666-693 RT:15.30-15.68 NL: 1.36E8

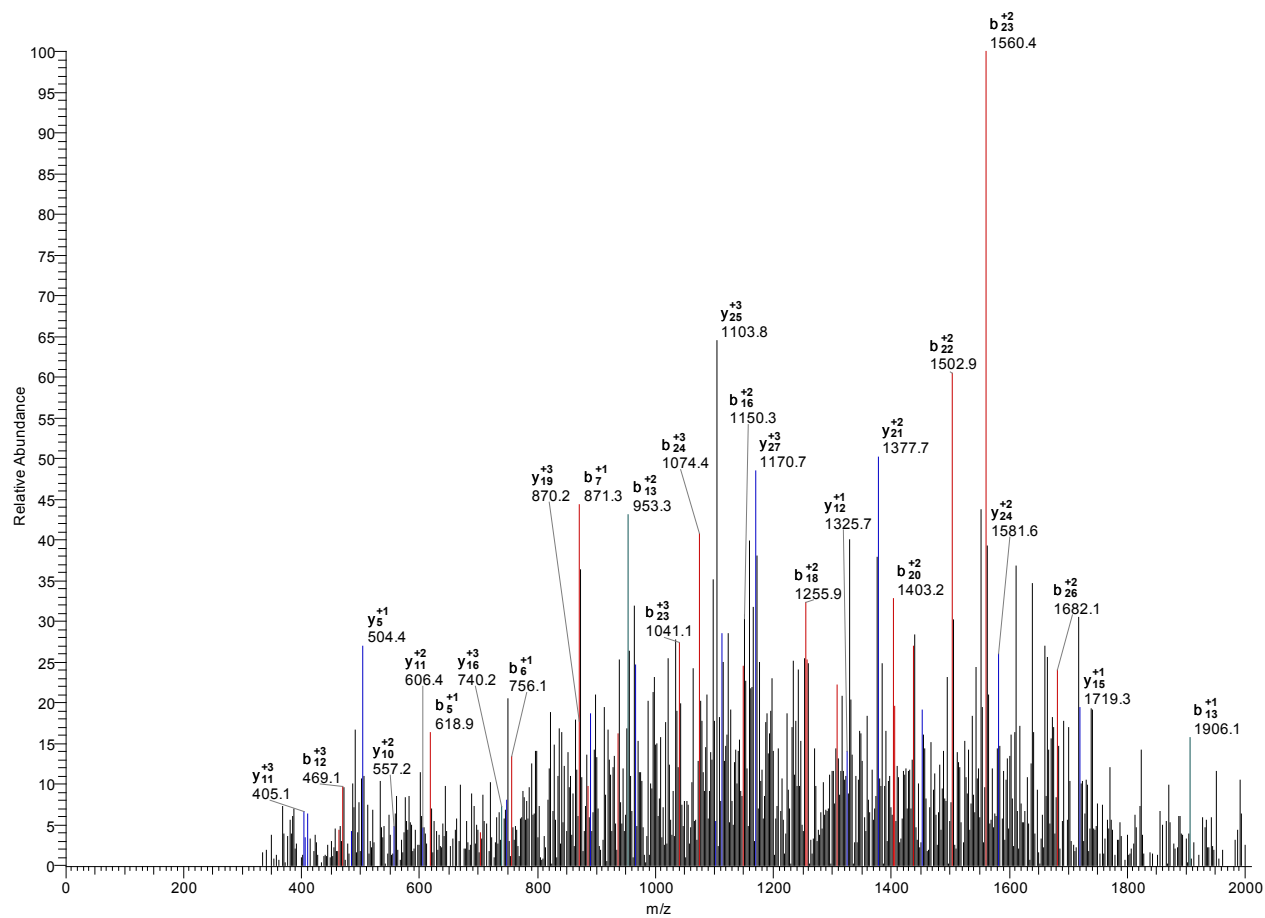


Figure S10. MS/MS spectrum of parent ion m/z 1208.08 (triply charged ion of A β 28/Pt(ϕ -MePy) complex), identification of H13 coordination.

#566-593 RT:12.83-13.27 NL: 9.44E7

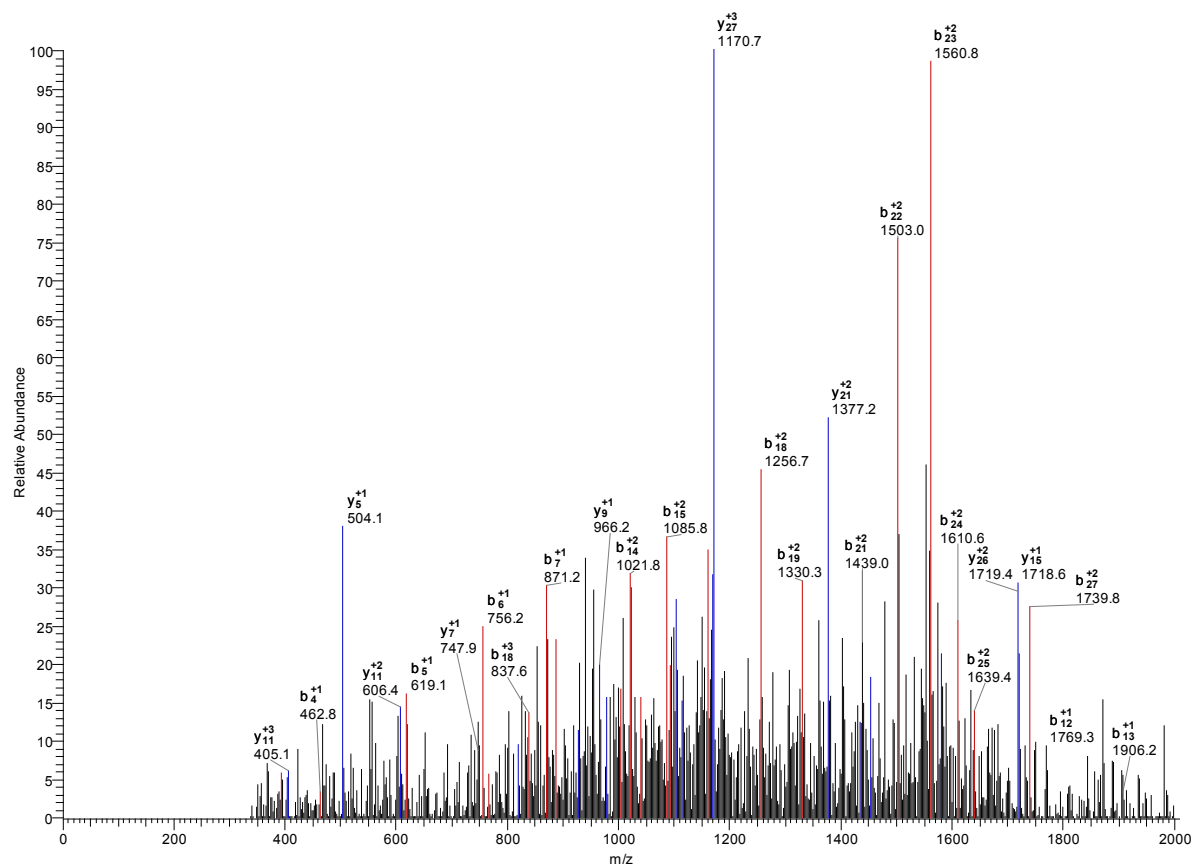
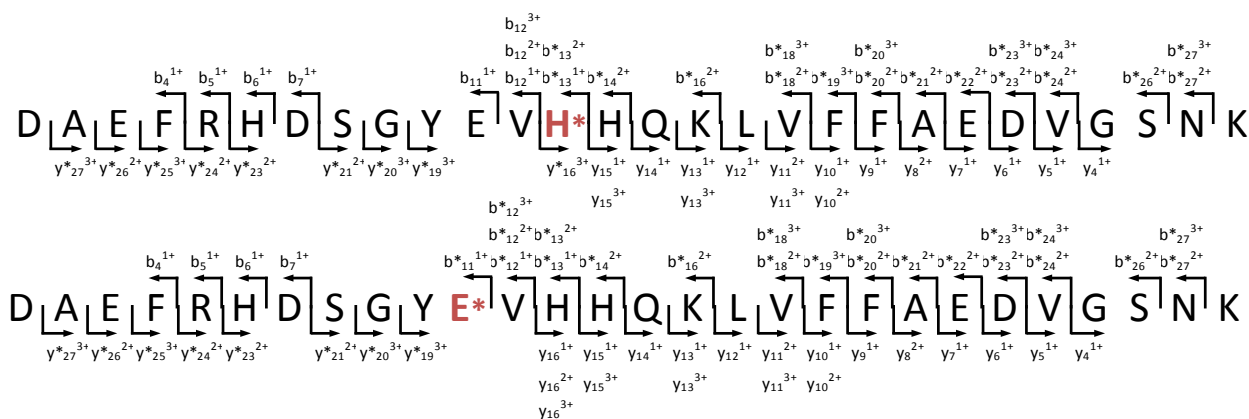


Figure S11. MS/MS spectrum of parent ion m/z 1208.08 (triply charged ion of Aβ₂₈/Pt(φ-MePy) complex), identification of E11 coordination.



Scheme S2. Fragmentation scheme of the A β 28/Pt(ϕ -MePy) complex deduced from top-down tandem mass spectra (see Supplementary Information, Figures S7 & S8) of the triply-charged parent ion at m/z 1208.8, including both mono-, di- and triply-charged ions of Pt-free ($b_n^{1,2,3+} / y_n^{1,2,3+}$) and platinated ($b_n^{*1,2,3+} / y_n^{*1,2,3+}$) fragments.

Table S1. Experimental mass of detected Pt(Aβ28) complexes, calculated from the multiply charged parent ions of Aβ28 and Pt(Aβ28) complexes (Figures S5-S6), along with the corresponding mass shift and complexation yield.

Observed complex	t _r (min)	m/z ^(charge) (Th) ^a	m _{exp} ^b (amu)	Δm _{exp} (amu) (expected Δm ^c)	Complex. yield ^d
Aβ1-28 control	13.2	1631.6 ²⁺ , 1088.2 ³⁺ , 816.5 ⁴⁺ , 653.4 ⁵⁺	3262.5 ± 2	-	-
Aβ1-28/Pt(φ-MePy)	15.6	1812.9 ²⁺ , 1208.8 ³⁺ 907.0 ⁴⁺ , 725.9 ⁵⁺	3624.9 ± 1	362.4 (362.5)	90 %
Aβ1-28/PtCl ₂	13.9	1178.1 ³⁺ 883.9 ⁴⁺ , 707.9 ⁵⁺	3538.9 ± 21	276.4 (265-269)	93 %
Aβ1-28/(PtCl ₂) ₂	14.2	1902.0 ²⁺ , 1268.7 ³⁺ 951.6 ⁴⁺ , 761.7 ⁵⁺	3804.3 ± 6	541.8 (530-538)	
Aβ1-28/Pt(bpy)	14.4	1806.4 ²⁺ , 1205.0 ³⁺ 903.7 ⁴⁺ , 723.3 ⁵⁺	3611.1 ± 1	348.6 (350.0)	54 %
Aβ1-28/Pt(Phen)	14.8	1818 ²⁺ , 1212.3 ³⁺ 909.5 ⁴⁺ , 727.9 ⁵⁺	3634.8 ± 2	372.3 (374.0)	35 %
Aβ1-28/Pt(φ-Phen)	15.8	1263.1 ³⁺ , 947.8 ⁴⁺ 758.4 ⁵⁺	3788.0 ± 7	525.5 (526.0)	34 %

^a experimental mass/charge ratio of the multiply charged parent ions of Aβ1-28 and Aβ1-28/Pt complexes (Figure S5, Supplementary information).

^b experimental mass calculated from multicharged ions ; uncertainties given at the 95% confidence level.

^c expected mass shift is calculated with monoisotopic mass of ¹⁹⁵Pt (the most abundant isotope of platinum) and of both isotopes of chlorine (³⁵Cl and ³⁷Cl).

^d complexation yield calculated by $[1 - A_{\beta\text{cplx}}/A_{\beta\text{control}}]$, where $A_{\beta\text{cplx}}$ is the peak area of remaining non-complexed Aβ1-28 and $A_{\beta\text{control}}$ is the peak area of control Aβ1-28 ; peak area are determined from trace chromatograms of Figure S6 (Supplementary information).

References

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