Supporting Information

for

$PtCl_2$(phen) Disrupts the Metal Ions Binding to Amyloid-β Peptide

Guolin Ma, Erqiong Wang, Hao Wei, Kaiju Wei, Pingping Zhu and Yangzhong Liu

Contents:

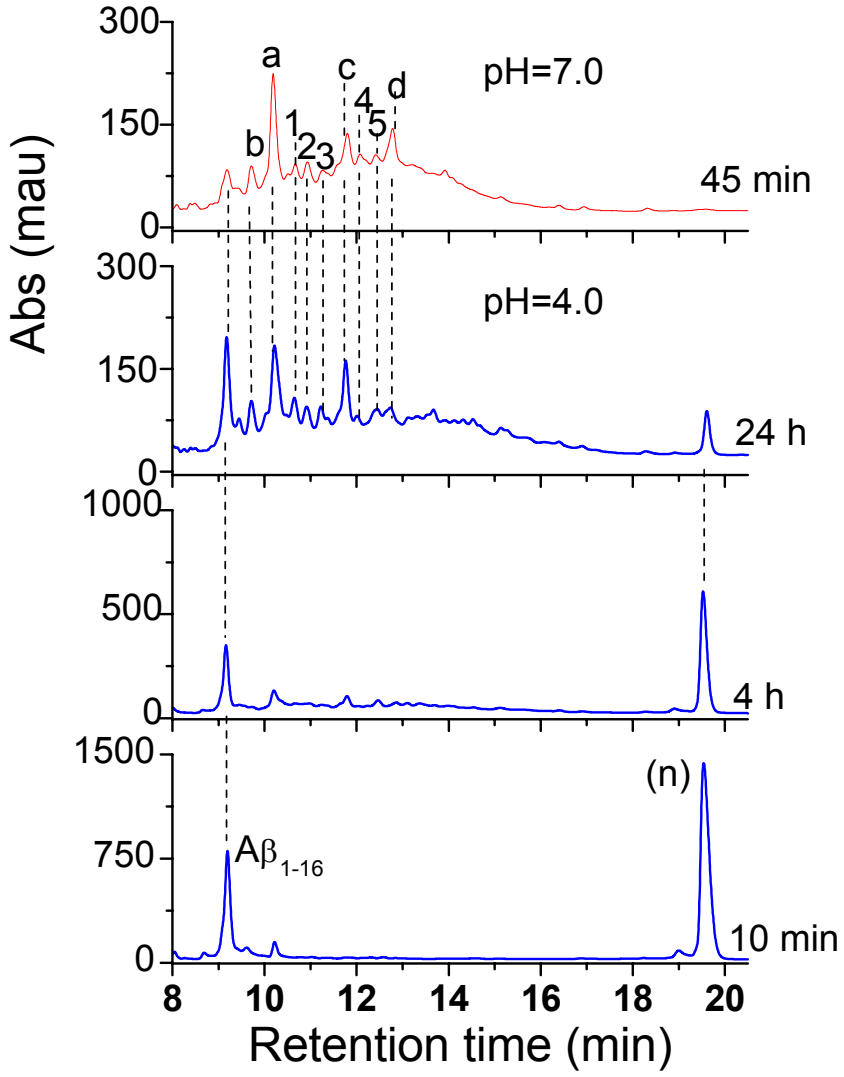

Figure S1. Comparison with the products of $A\beta_{1-16}$ with $[PtCl(phen)(DMSO)](NO_3)_2$ (**n**) at pH 7.0 (Red curve) and 4.0 (Blue curve) in DMSO/H_2O (v/v=2/1) solution at 25°C. The reaction rate declined apparently at pH 4.0; however, the products produced at pH 7.0 and 4.0 share the same retention time on HPLC profiles. This indicate that pH has no influence on products produced in the reaction of $A\beta_{1-16}$ with $PtCl_2(phen)$.

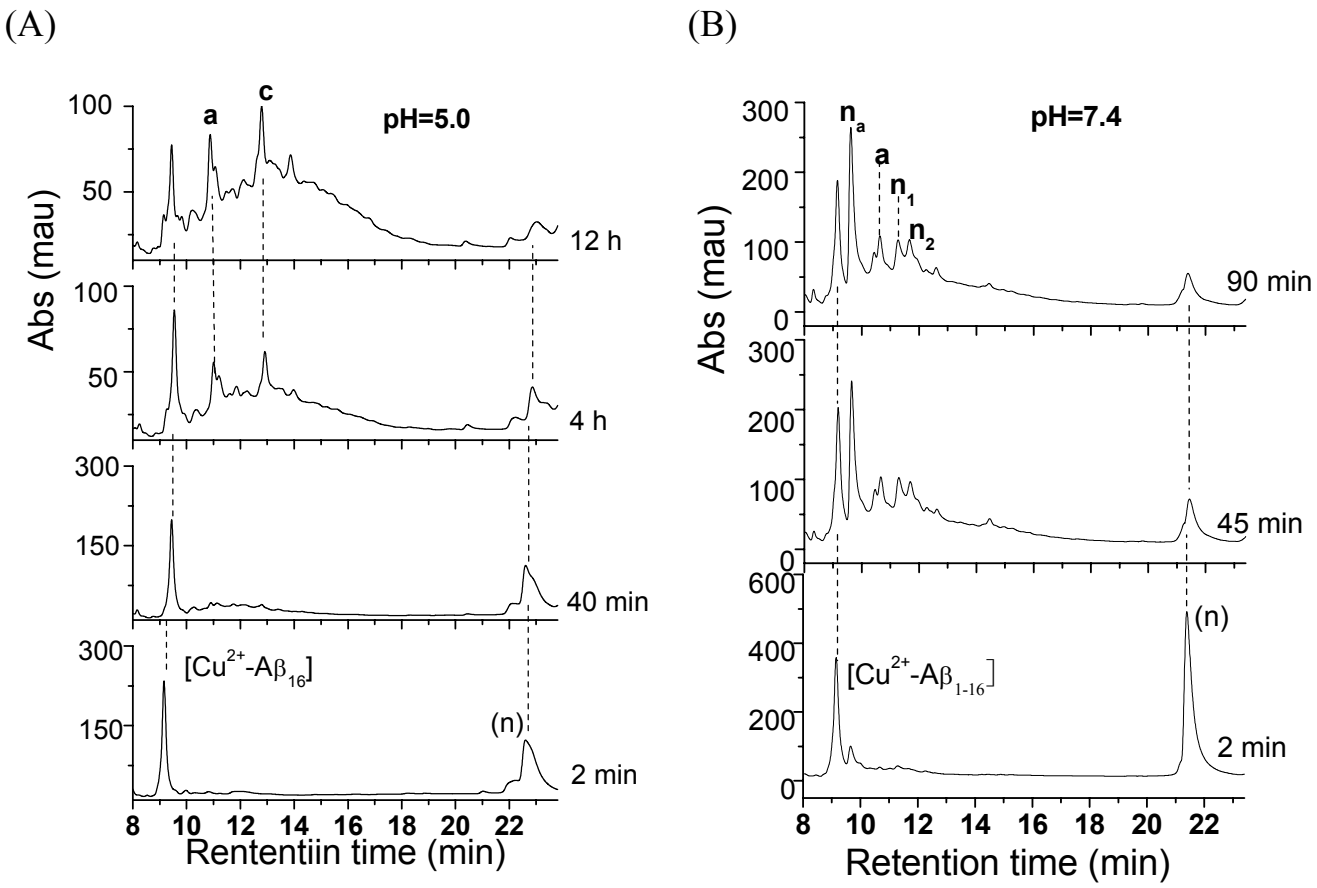

Figure S2. HPLC profiles for the reaction of $A\beta_{1\text{-}16}$ with $PtCl_2(phen)$ at 298K in DMSO/H_2O (v/v=1/2) solution in the presence of 2.5-fold molar excess of Cu^{2+} ions. (A) pH 5.0; (B) pH 7.4. The molar ratio of Cu^{II}:$A\beta_{1-16}$:Pt^{II} was 2.5:1:1 in the reaction system. HEPES has no influence on the reaction.

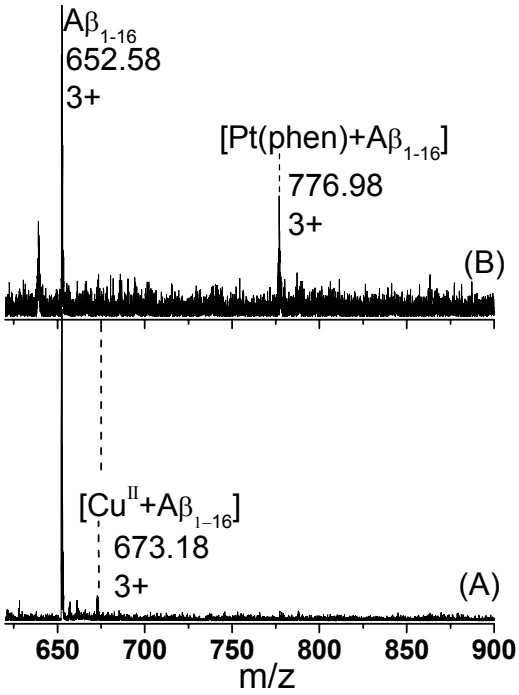

Figure S3. ESI-MS analysis of [Cu^{II}-$A\beta_{1-16}$] (A) and the products from the reaction of $PtCl_2$(phen) (B). The reaction was performed in DMSO/H_2O (v/v=1/2) at 298K pH 7.4 for 4 hours. 2.5-fold molar equvilant Cu^{2+} ions were present in the reactions.

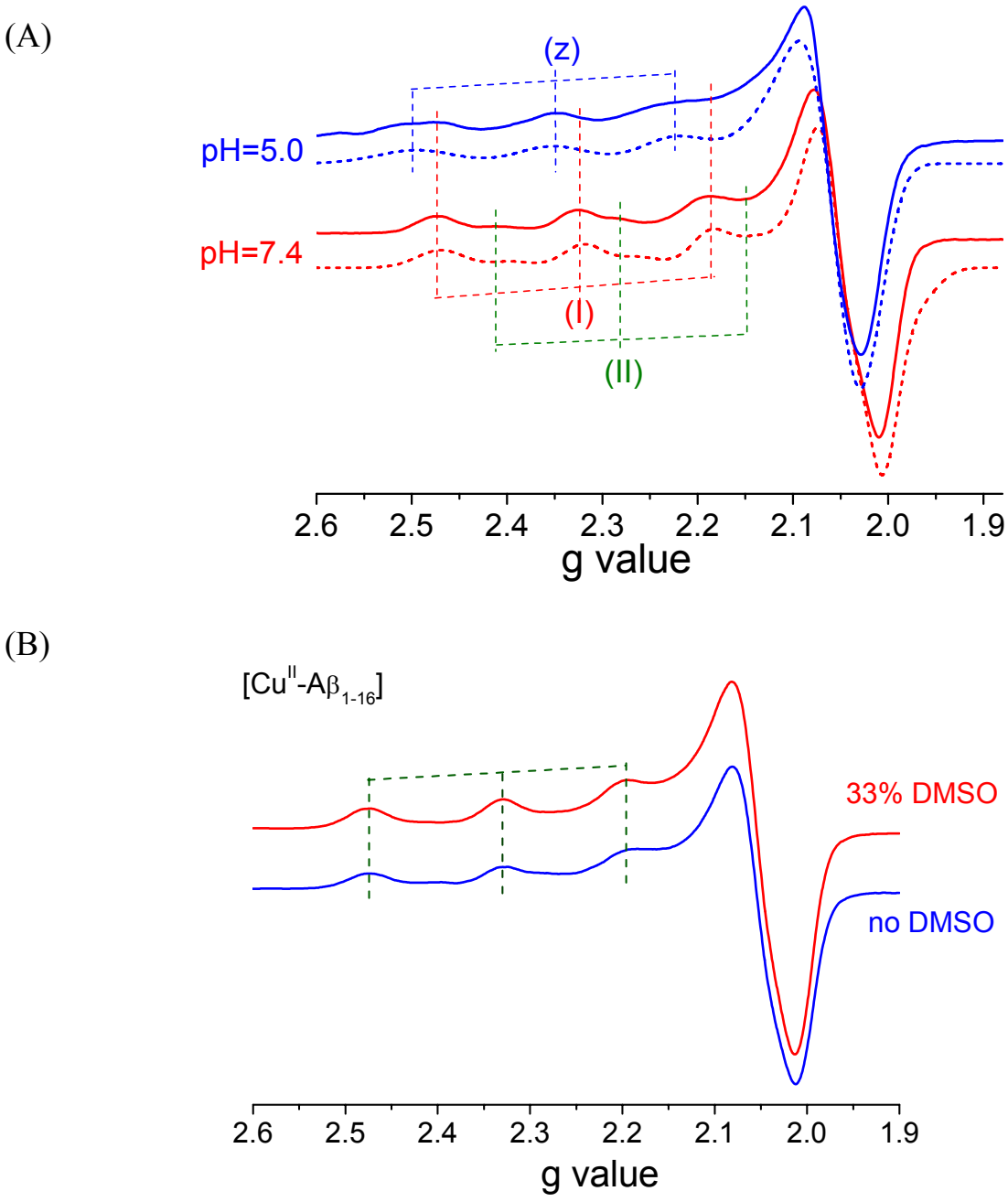

Figure S4. EPR spectra the [Cu^{II}-$A\beta_{1-16}$] complex. **(A)** the measured spectra (solid lines) and the simulation curves (dash lines) of the [Cu^{II}-$A\beta_{1-16}$] complex at pH 5.0 (blue) and 7.4 (red). **(B)** Verifying the effect of DMSO on the EPR spectra. 240 μM Cu^{2+} and 300 μM $A\beta_{1-16}$ in 5 mM HEPES buffer, pH=7.4.

Near physiological pH, two different binding modes of [Cu^{II}-$A\beta_{1-16}$] complexes are present usually noted mode (**I**) and (**II**). The mode (**II**) is stable at high pH ($7.8 \leq pH \leq 9.3$).[1] Moreover, the mode (**z**) is observed in the spectrum at pH 5.0. The signal of mode z was observed dominantly at lower pH (4.0).[1] The EPR data of these binding modes are listed on Table S1.

Table S1. EPR data of [Cu^{II}-Aβ$_{1-16}$] corresponding to different coordination modes.

[Cu^{II}-Aβ$_{1-16}$]	$g_{//}$ [a]	$A_{//}$ [a]	Proposed Binding Sites	Reference
Mode (I)	**2.275**	**184**	**N/A**	**This work**
	2.262	184	[NH_2^{D1}, CO^{D1-A2}, N_{Im}^{H6}, $N_{Im}^{H13/H14}$]	1-4
	2.272	171	[NH_2^{D1}, CO^{D1-A2}, N_{Im}^{H6}, $N_{Im}^{H13/H14}$]	5-7
Mode (II)[b]	**2.228**	**170**	NH_2^{D1}, CO, N^-, N_{Im}^{H13}	**This work**
	2.226	161	[NH_2^{D1}, CO^{A2-E3}, $N^{-(D1-A2)}$, N_{Im}]	1-4
	2.227	157	[CO^{A2}, N_{Im}^{H6}, N_{Im}^{H13}, N_{Im}^{H14}]	5-7

[a] Spin hamiltonian parameters of [Cu^{II}-Aβ$_{1-16}$] complexes were determined from simulations of the CW-EPR Spectra in Figure S4 using program Hyperfine Spectrum.[8]

[b] The binding mode (II) was still controversial.

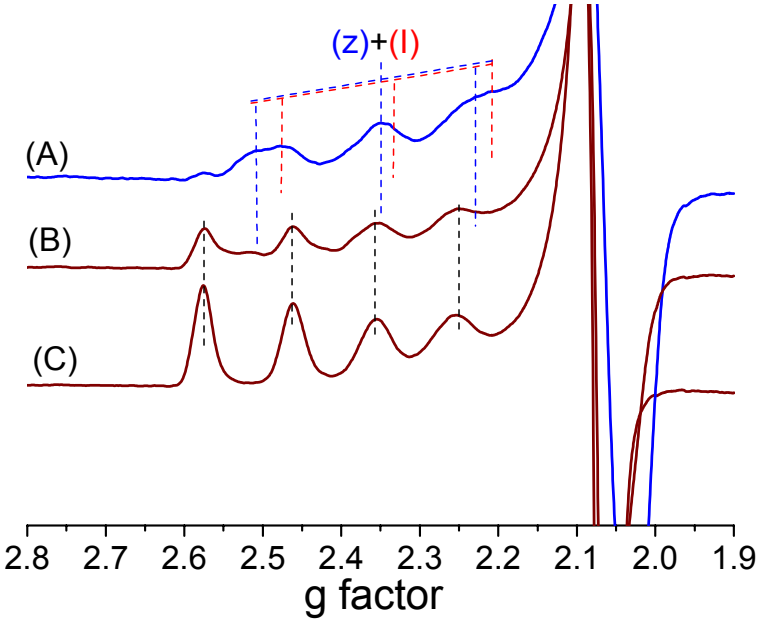

Figure S5. Selected $g_{//}$ region of CW-EPR spectra. (A) 150 μM [Cu^{II}-$A\beta_{1-16}$] complex was prepared at the molar ratio Cu^{2+}:$A\beta_{1-16}$=0.8:1 in ultrapure water, pH=5.0. Mode (z) and (I) are present in solution; (B) Reaction of 150 μM [Cu^{II}-$A\beta_{1-16}$] with equal molar $PtCl_2$(phen) mixed at pH=5.0. The EPR spectrum was recorded after 12 hours reaction; (C) 150 μM $Cu(NO_3)_2$ in ultrapure water at pH=5.0.

The signals of [Cu^{II}-$A\beta_{1-16}$] decreased after the reaction with $PtCl_2$(phen) at pH 5.0. The product signals were consistent with $Cu(NO_3)_2$ in the aqueous, suggesting that the binding of $PtCl_2$(phen) released Cu^{2+} from $A\beta_{1-16}$ peptide at low pH.

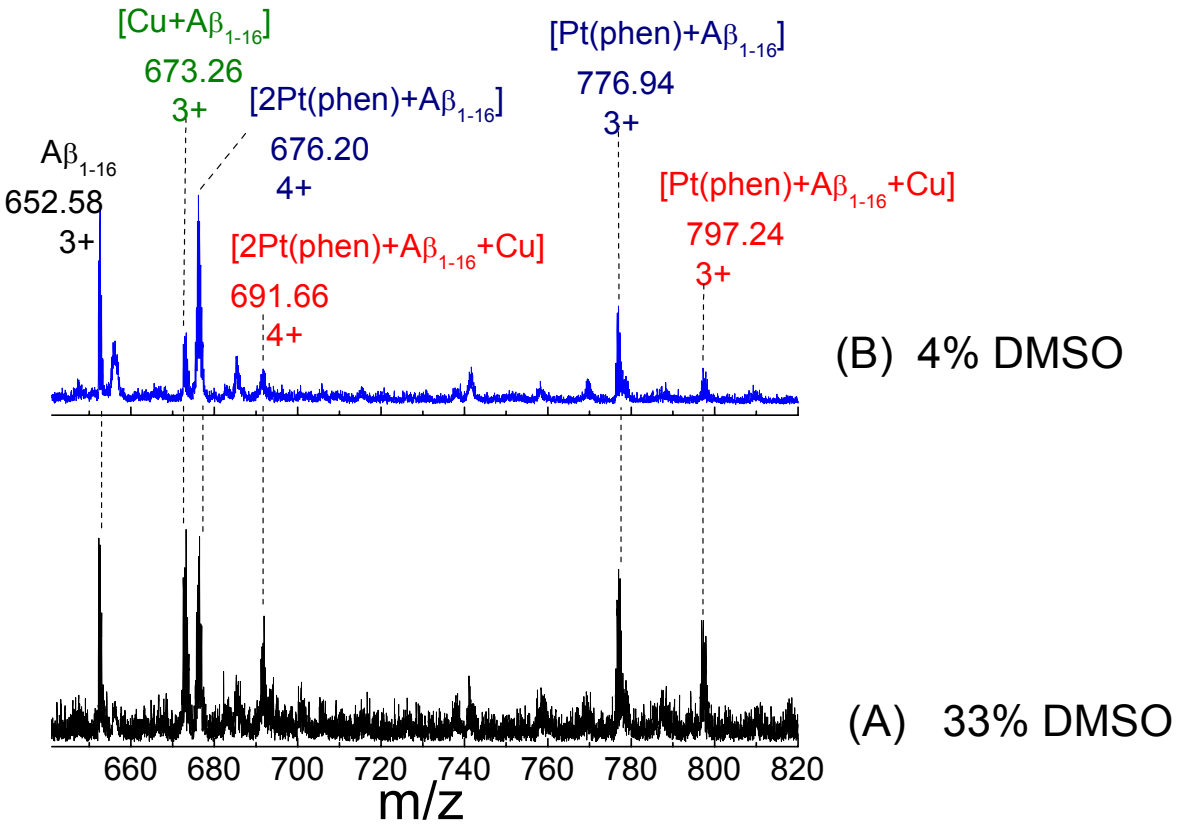

Figure S6. Comparison of the DMSO concentrations on the platination adducts. Reactions were performed in 0.15 mM $A\beta_{1-16}$ with equimolar $PtCl_2$(phen) in the presence of 2.5-fold of Cu^{2+}. (A) 33% DMSO (v/v); (B) 4% DMSO(v/v). The reactions were carried out at 298K, pH 5.0 for 6 hours before ESI-MS measurements. The same products were formed in these two reactions, although the relative abundance of products varied slightly in the two reactions. This result shows that 33% DMSO does not influence the reaction of $PtCl_2$(phen) with the [Cu^{II}-$A\beta_{1-16}$] complex.

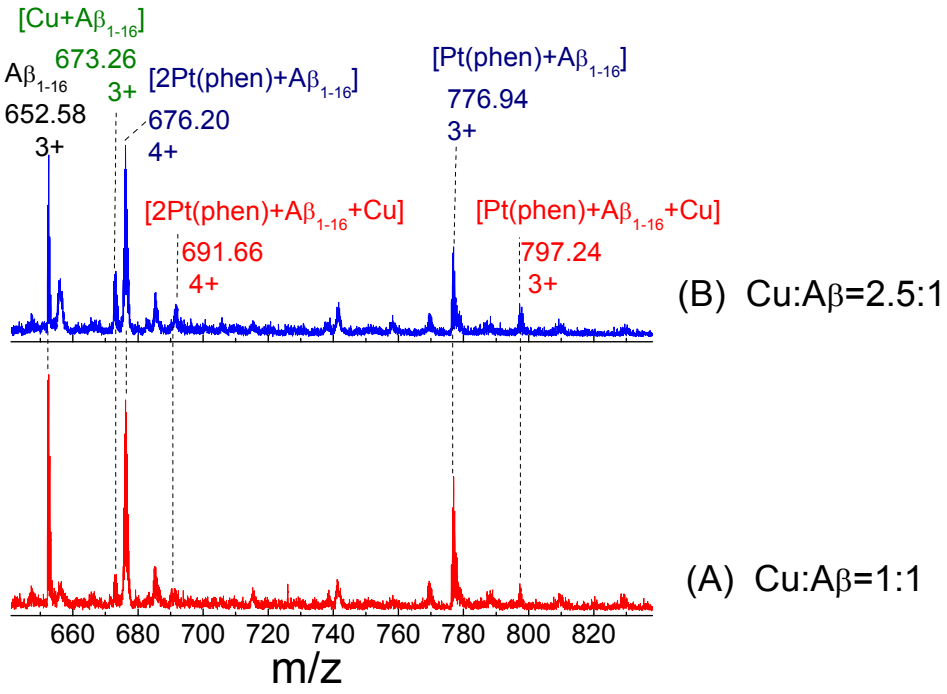

Figure S7. Comparison of the Cu(II) ratios on the platination adducts. The ratios of copper are labeled in the figure. Reactions were performed in 0.15 mM $A\beta_{1-16}$ with equimolar $PtCl_2$(phen) at 298K, pH 5.0 for 6 hours before ESI-MS measurements. 4% DMSO(v/v) are present in the reaction system. This result shows that the copper containing adducts are only slightly lower in the reaction with 1:1 copper ratio.

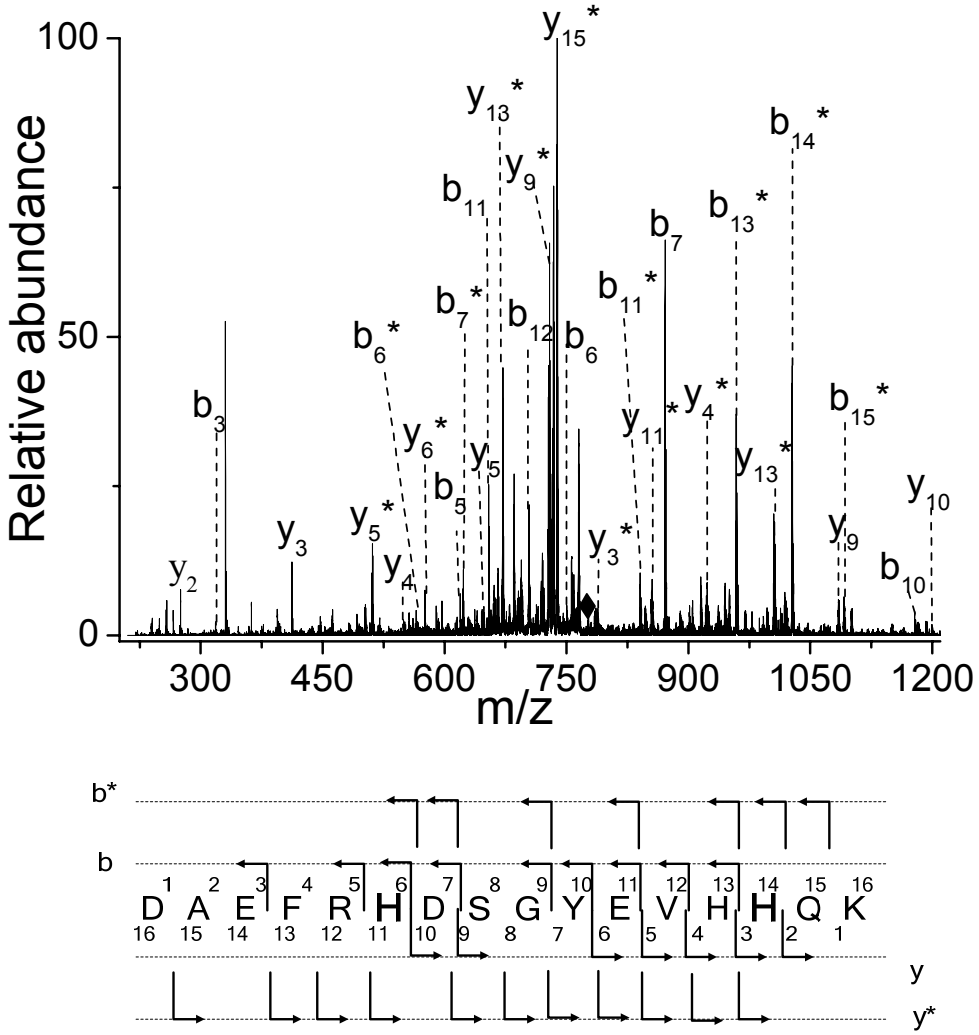

Figure S8. ESI-MS/MS spectrum of the product [Pt(phen)+A$\beta_{1\text{-}16}$] (**a**) at m/z 777.20 (top) and the fragmentation scheme from the MS/MS spectrum (bottom). The precursor ion was denoted ♦ on the spectra. His6 and His14 were potential binding sites.

In the MS/MS spectrum of **a**, the distribution of fragments is nearly identical to that of $\mathbf{n_a}$. The smallest platinated fragment b_6* and the largest free peptide y_{10} indicated that His6 should be a binding site; while the y_3* and b_{13} suggest that His14 is another binding site. This result was consisting with our previous report.[9] The adducts $\mathbf{n_a}$ and **a** share the same binding sites His6 and His14. Our EPR experiments also confirmed that $PtCl_2$(phen) changed Cu^{2+} coordination sphere and could not released Cu^{2+} at pH 7.4. As a result, the molecular formula of $\mathbf{n_a}$ was confirmed as [Pt(phen)+A$\beta_{1\text{-}16}$+Cu^{II}].

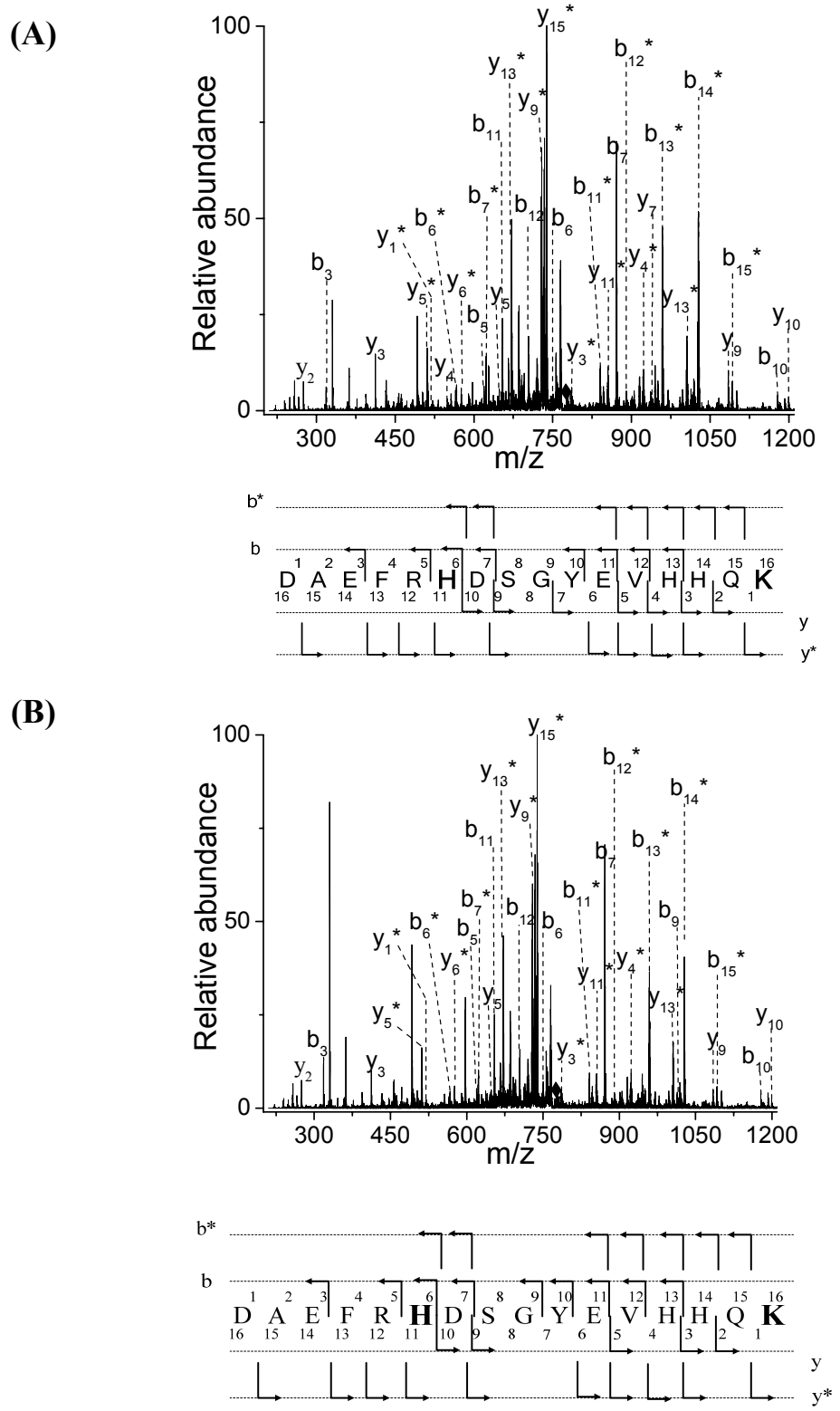

Figure S9. ESI-MS/MS spectra of the products $\mathbf{n_1}$ (A) and $\mathbf{n_2}$ (B) at m/z 777.20. The fragmentation schemes are given under the MS/MS spectra. The precursor ion was denoted ♦ on the spectra. $\mathbf{n_1}$ and $\mathbf{n_2}$ share the same binding sits His6 and Lys16. The different retention time of $\mathbf{n_1}$ and $\mathbf{n_2}$ on HPLC profiles may suggest the influence of the copper coordination.

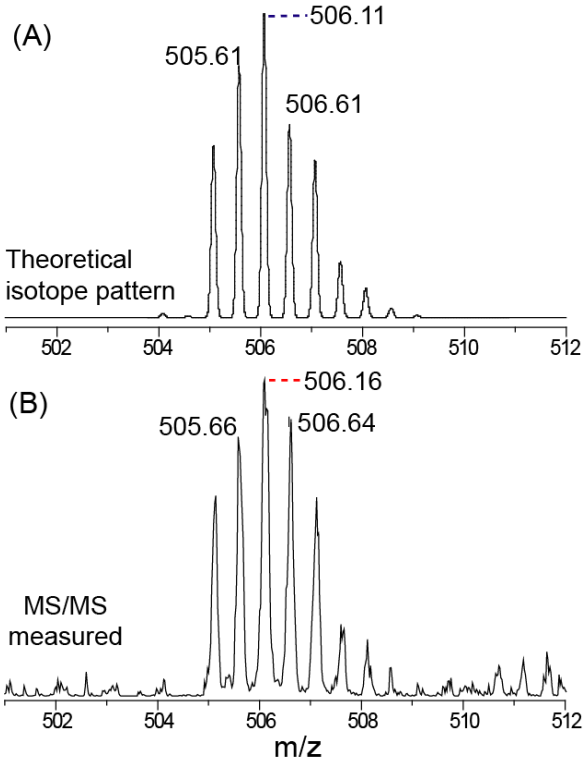

Figure S10. Isotopic distributions of the fragment ion x_4** [HHQK+Pt(phen)+Cu]$^{2+}$ produced in Figure 6. (A) Theoretical calculated isotope pattern, m/z = 506.11, z = 2+. The isotopic distribution was simulated by software IsoPro 3.0 with the fragment formula: $[C_{36}H_{41}N_{12}O_7PtCu]^{2+}$. (B) Measured ESI-MS spectrum, m/z =506.16, z = 2+.

In the MS/MS process, peptide bond breakage between Cα-C, C-N and N-Cα yields six different product ions. Nomenclature for polypeptide fragments: a_n, b_n and c_n are N-terminal fragments; x_n, y_n and z_n are C-terminal fragments.[10]

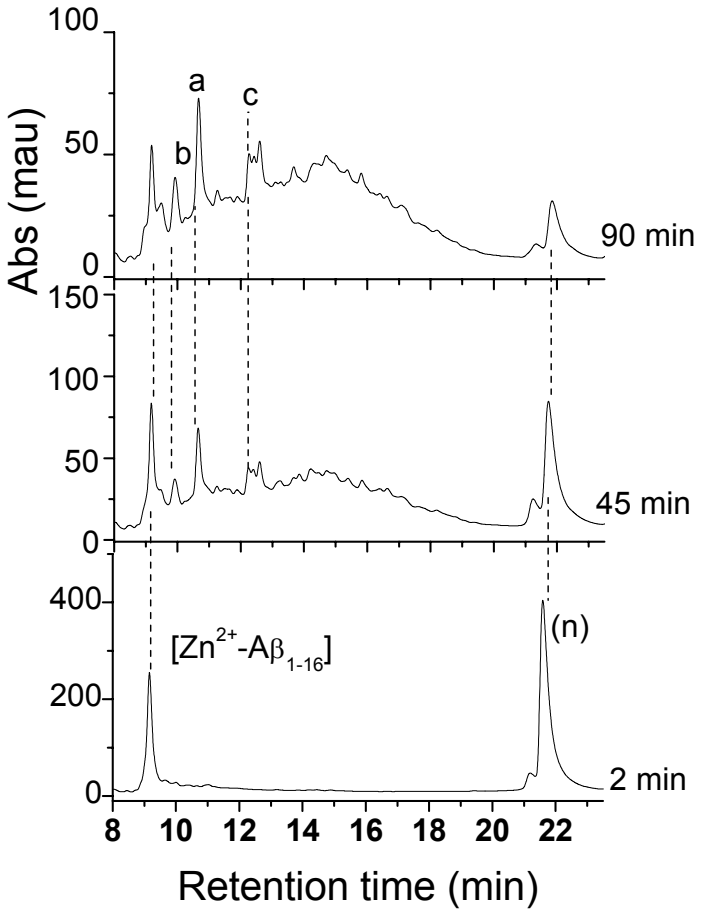

Figure S11. HPLC profiles for the reaction of [Zn^{II}-$A\beta_{1-16}$] with equimolar [PtCl(phen)(DMSO)](NO_3) (**n**) in DMSO/H_2O (v/v=1/2, pH 6.0) at 298K. The molar ratio of Zn^{II}:$A\beta_{1-16}$:Pt(II) was 2.5:1:1 in the reaction system.

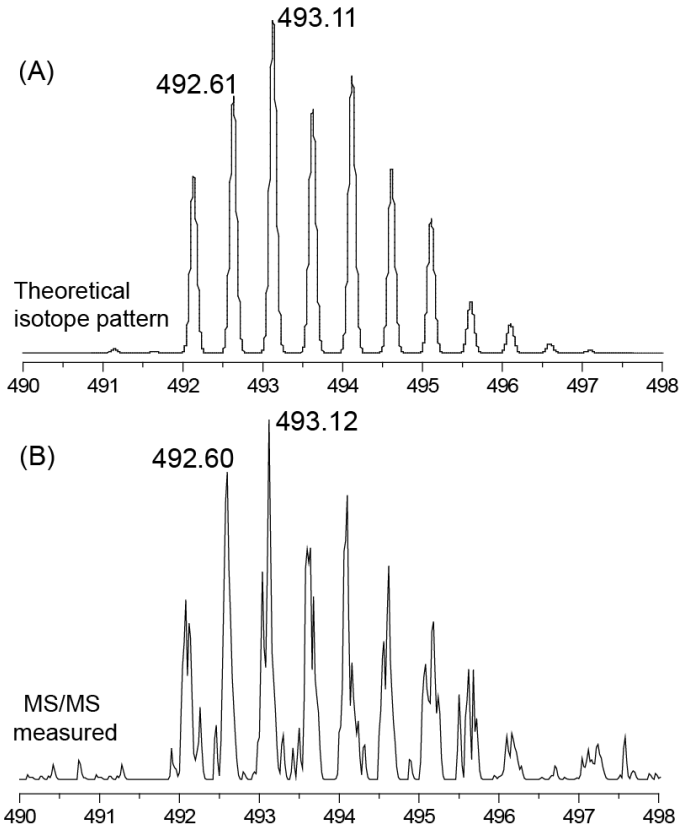

Figure S12. Isotopic distributions for fragment ion y_4** [HHQK+Pt(phen)+Zn]$^{2+}$ produced in Figure 9. (A) Theoretical calculated isotope pattern, m/z = 493.11, z = 2+. The isotopic distribution was simulated by software IsoPro 3.0 with the formula $[C_{35}H_{42}N_{12}O_6PtZn]^{2+}$. (B) Measured ESI-MS spectrum, m/z = 493.12, z = 2+.

Reference

1. B. Alies, H. Eury, C. Bijani, L. Rechignat, P. Faller and C. Hureau, *Inorg. Chem.*, 2011, **50**, 11192-11201.
2. H. Eury, C. Bijani, P. Faller and C. Hureau, *Angew. Chem. Int. Ed.*, 2011, **50**, 901-905.
3. C. Hureau, Y. Coppel, P. Dorlet, P. L. Solari, S. Sayen, E. Guillon, L. Sabater and P. Faller, *Angew. Chem. Int. Ed.*, 2009, **48**, 9522-9525.
4. P. Dorlet, S. Gambarelli, P. Faller and C. Hureau, *Angew. Chem. Int. Ed.*, 2009, **48**, 9273-9276.
5. S. C. Drew, C. L. Masters and K. J. Barnham, *Plos One*, 2010, **5**, e15875.
6. S. C. Drew, C. J. Noble, C. L. Masters, G. R. Hanson and K. J. Barnham, *J. Am. Chem. Soc.*, 2009, **131**, 1195-1207.
7. S. C. Drew and K. J. Barnham, *Accounts Chem Res*, 2011, **44**, 1146-1155.
8. W. R. Hagen, *CRC Press, Boca Raton, Fl, USA*, 2009.
9. G. Ma, F. Huang, X. Pu, L. Jia, T. Jiang, L. Li and Y. Liu, *Chem. Eur. J.*, 2011, **17**, 11657-11666.
10. H. Steen and M. Mann, *Nat. Rev. Mol. Cell. Bio.*, 2004, **5**, 699-711.