Electronic Supplementary Material (ESI) for Metallomics.

Supplementary Information (ESI) for

Methionine Oxidation of Amyloid Peptides by Peroxovanadium Complexes: Inhibition of Fibril Formation through a Distinct Mechanism

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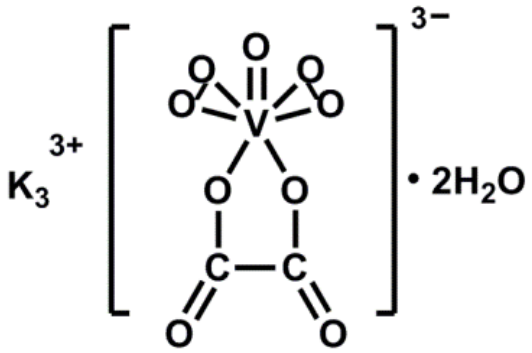

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Scheme S1. The molecular structure of complex **3**, $K_3[VO(O_2)_2(OX)]\cdot 2H_2O$.

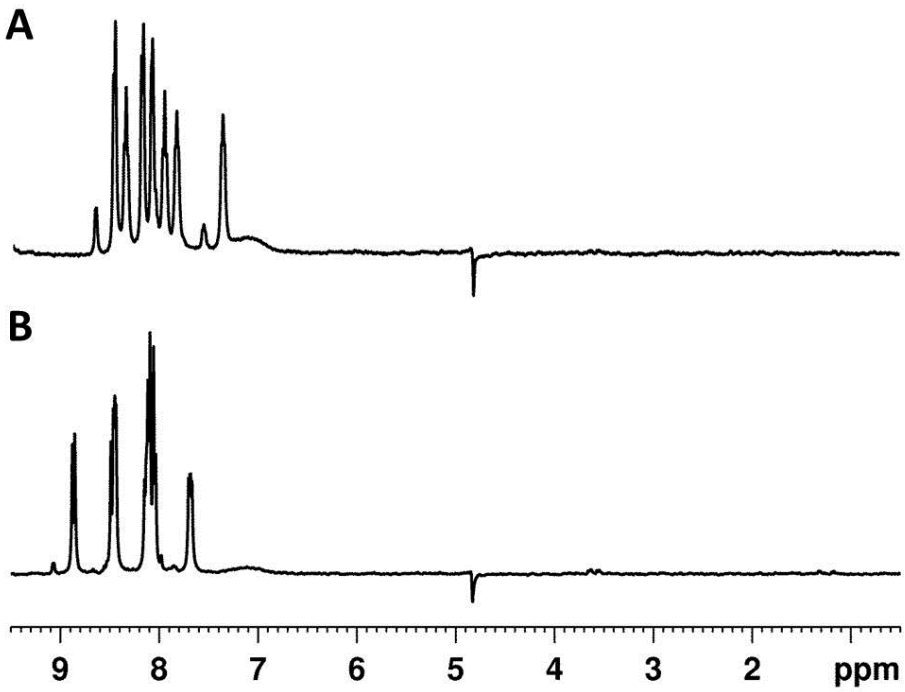

Figure S1. ^{1}H NMR spectra of the peroxovanadium complexes in 9:1 H_2O/D_2O at pH 5.7, 298 K. (A) complex **1** and (B) complex **2**.

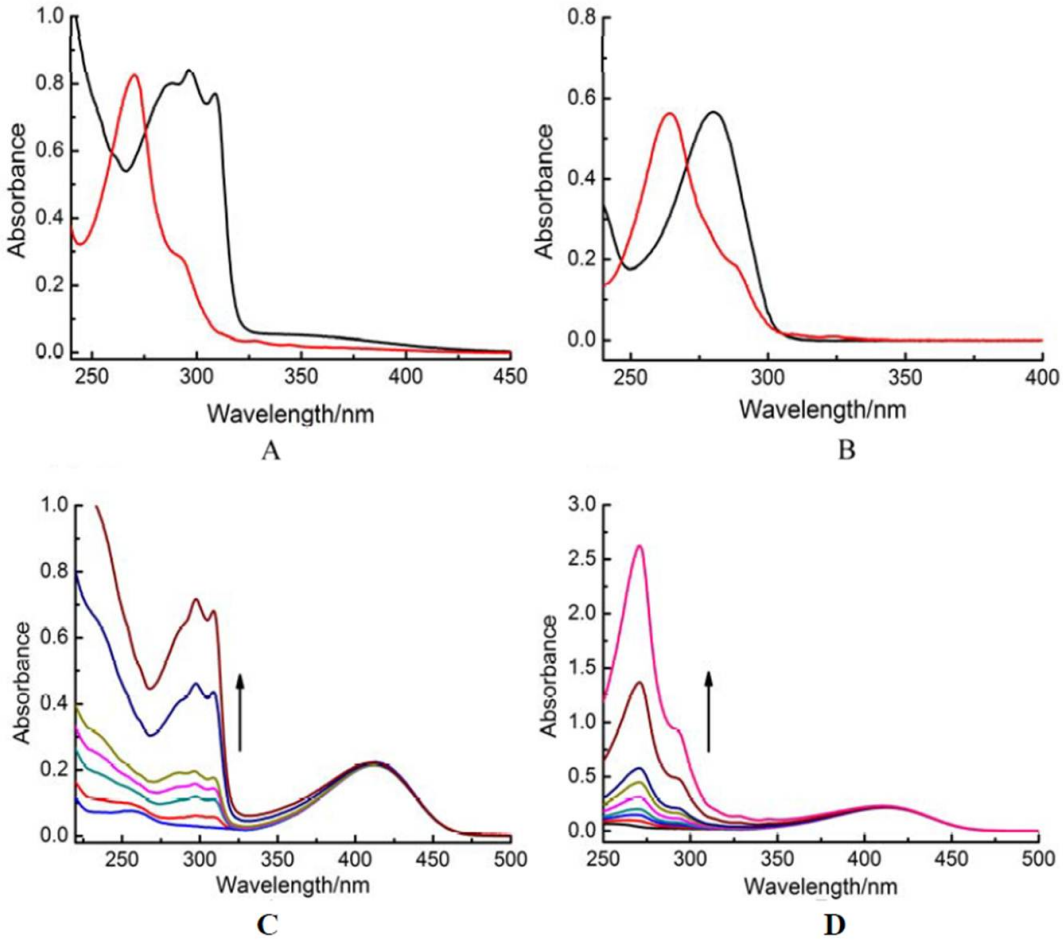

Figure S2. The UV spectrum of complex **1** (black) and **2** (red) respectively (A). The UV spectrum of bipyridine (black) and phenanthroline (red) respectively (B). And titration experiments, the UV spectra of ThT (10μM) with complex **1** (C) and **2** (D). The complex concentration was 0, 2, 4, 6, 10, 15, 20, 50 and 100 μM from bottom to top.

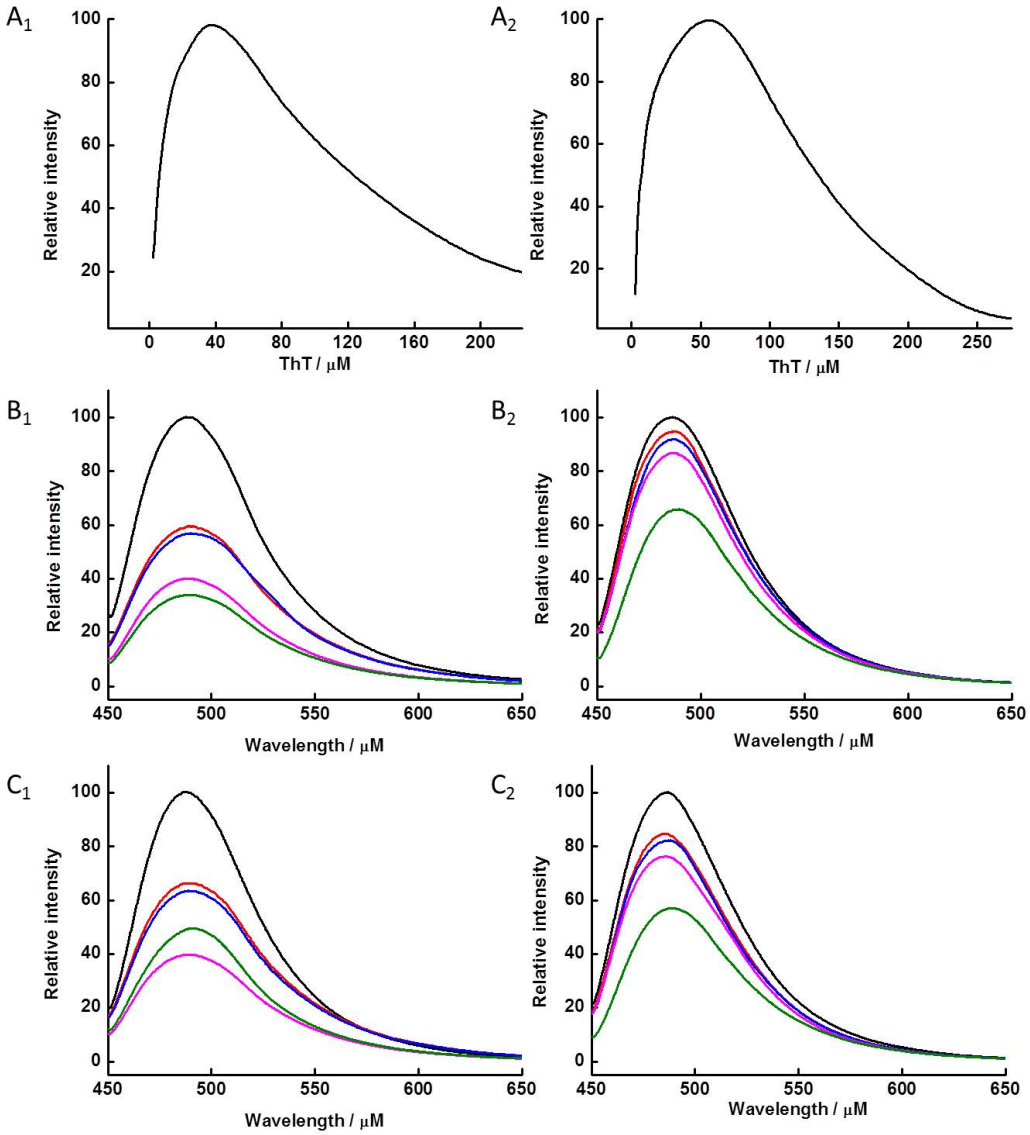

Figure S3. The competitive effects of ThT and V complex on interaction with aggregated peptides. First, the working curve of ThT concentration to PrP106-126 (A_1) and Aβ1-42 (A_2). Then, the inhibitory effects of complex **1** (B_1 and B_2) and **2** (C_1 and C_2) on aggregated peptide with ThT. The left panel represents the interaction between V complex and PrP106-126 (B_1 and C_1), while the right panel represents the interaction between V complex and Aβ1-42 (B_2 and C_2). From A_1 and A_2, the suitable ThT concentration used for PrP106-126 and Aβ1-42 are 40 and 60μM, respectively. The peptide concentration is 10μM for PrP106-126 and Aβ1-42 both. The concentration of vanadium complex used are 3μM (red), 6μM (blue), 10μM (magenta), and 20μM (olive) respectively.

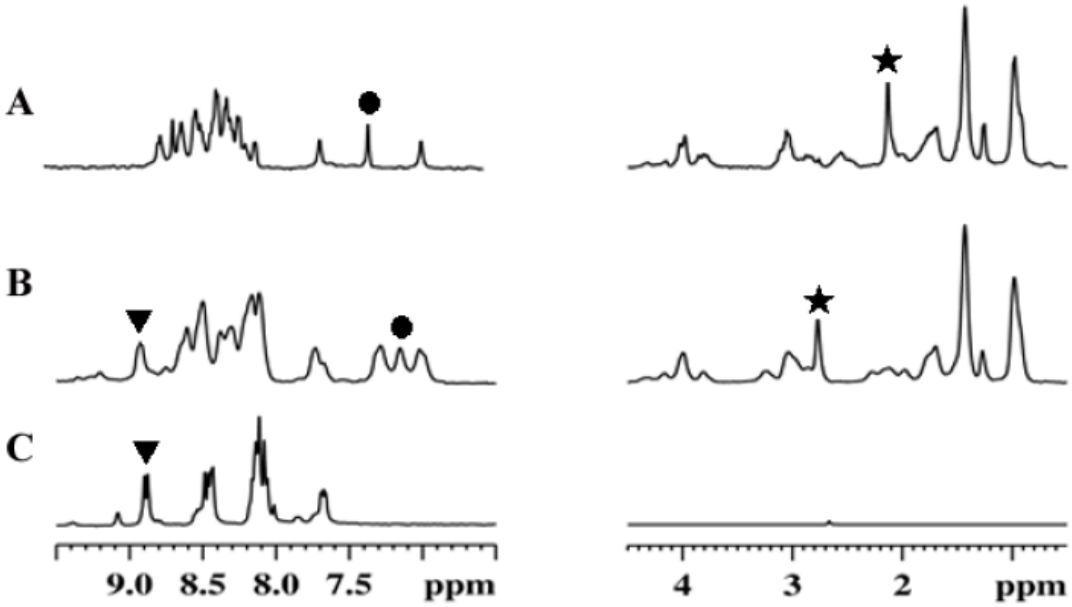

Fig. S4 ^{1}H NMR spectra of PrP106-126 in 9:1 H_2O/D_2O at pH 5.7, 298 K. (A) PrP106–126 alone; (B) 0.25mM PrP106-126 in the presence of **2** (5.0 equiv); (C) complex **2** itself. The signal at 2.08 ppm (star) representing the $C_{\delta}H_{s}$ group of methionine is significantly shifted when incubated with the complex. The signal at 7.38 ppm (dot) represents the CδHs of His111, which is clearly perturbed by addition of **2**. The resonance peak at 8.9 ppm from the compound (inverted triangle) was also perturbed, which implied the interaction of vanadium complex with the peptide.

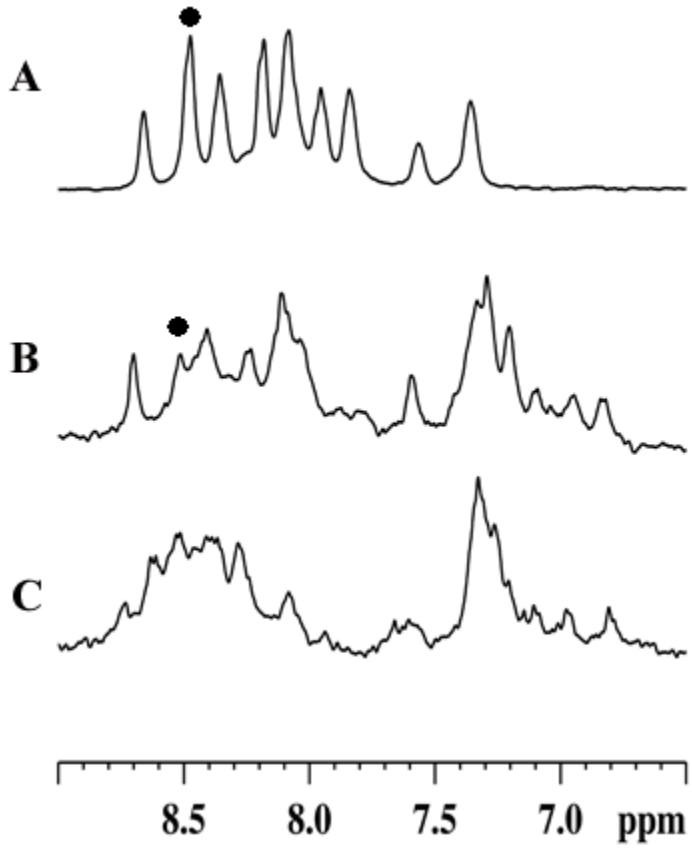

Figure S5. Portion of ^{1}H NMR spectra for complex **1** in the absence (A) and presence of A$\beta_{1\text{-}42}$ (B). The resonance peak from the complex (dot) is affected by addition of the peptide. (C) Portion of ^{1}H NMR spectrum of the peptide alone. The peptide concentration was 0.25 mM and 5.0 equivalent amounts of complex **1** were added in the solution.

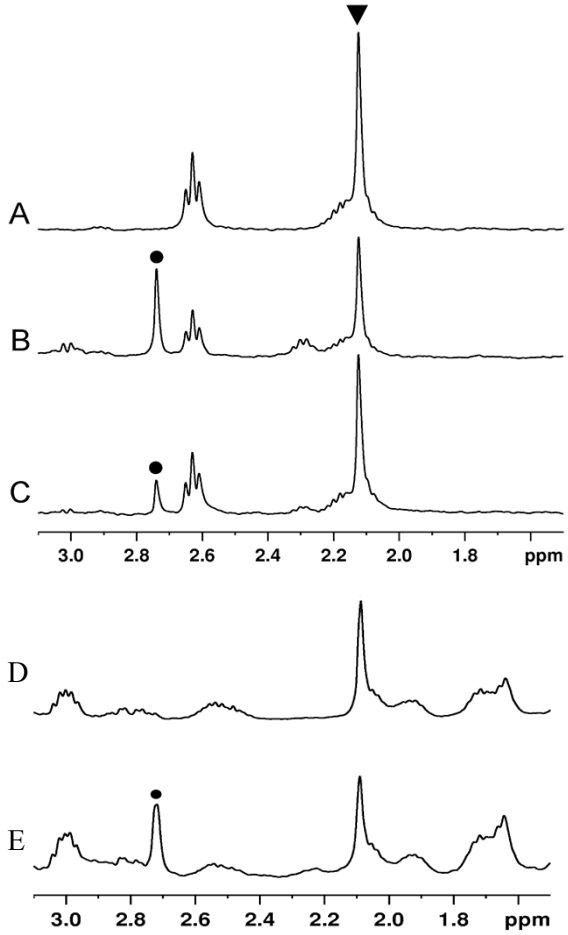

Figure S6. The up-field portion of ^{1}H NMR spectra for methionine in the absence (A) and presence of complex **1** (B) and **2** (C). The signal at 2.12 ppm (inverted triangle) represents the methyl protons of methionine. The signal at 2.73 ppm (dot) represents the methyl protons of oxidized methionine when incubated with the vanadium complex. Moreover, the up-field portion of ^{1}H NMR spectra for PrP106-126 in the absence (D) and presence of H_2O_2 (E). The peak at 2.72 ppm (dot) is assigned to the methyl protons of oxidized methionine.

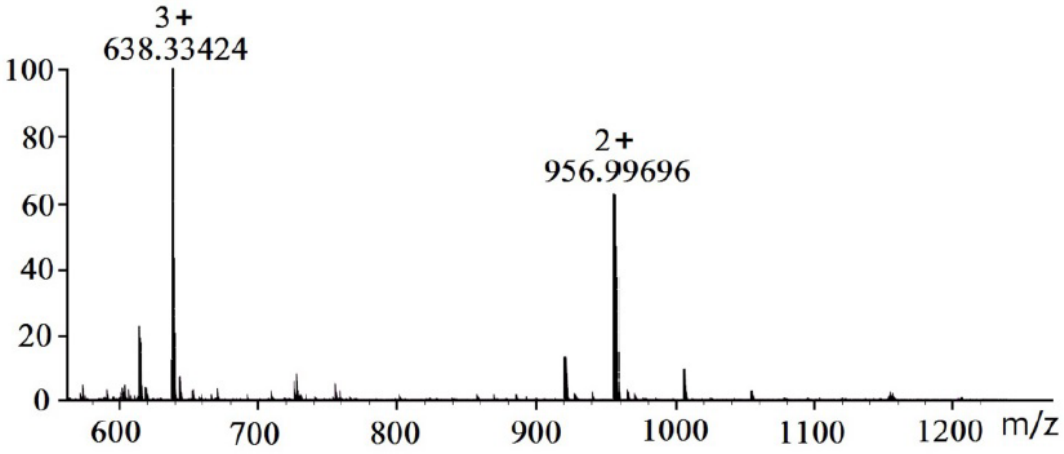

Figure S7. ESI-MS spectrum of PrP106-126 alone. The peaks at 956.99(2+) and 638.33(3+) correspond to the expected mass of the peptide.

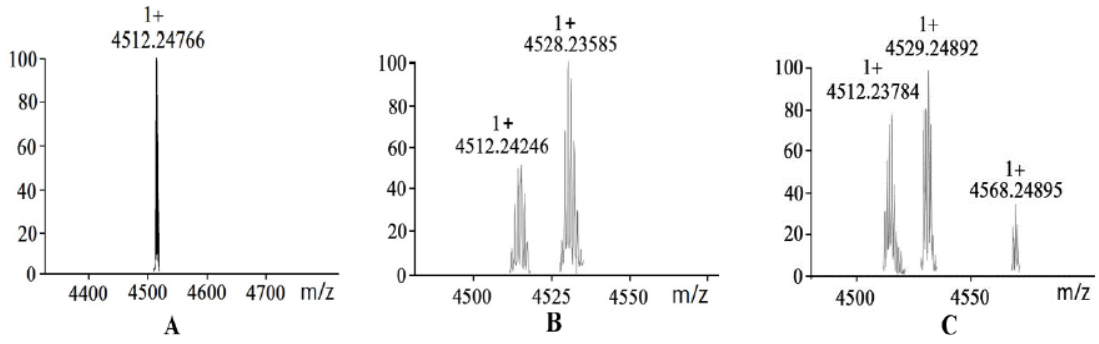

Figure S8. ESI-MS spectra of $A\beta_{1\text{-}42}$ (50μM) in the absence (A) and presence of complex **1** (B) and **2** (C). The complex concentration is 500μM in solution.

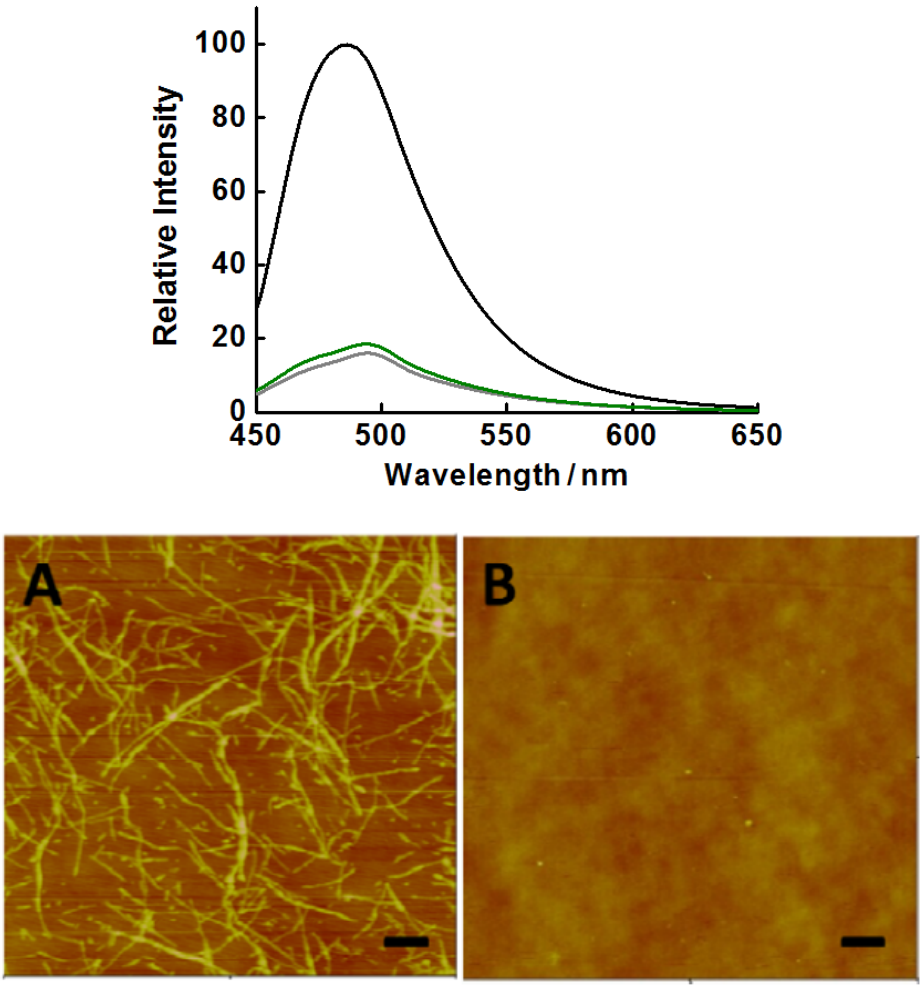

Figure S9. Upper: Evaluation of the ability of complex **3** to inhibit PrP106-126 aggregation as measured by ThT fluorescence. PrP106-126 was incubated with ThT in the absence (black) and presence of **3** (green). The peptide concentration was 10 μM and the vanadium complex was 30 μM. The control (ThT only) is shown in gray. Lower: AFM morphology of PrP106-126 in the absence (A) and presence of complex **3** (B). The scale bar is 500 nm.

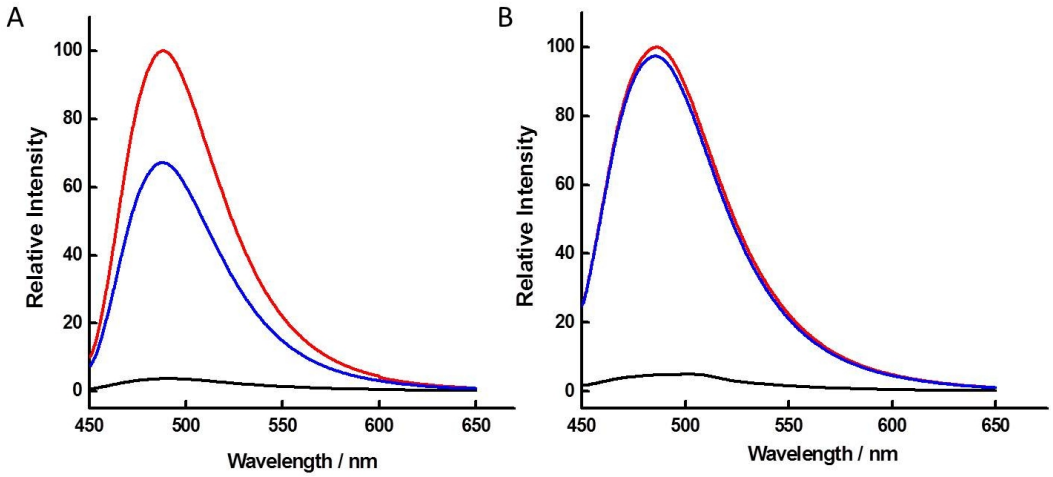

Figure S10. Evaluation of H_2O_2 to inhibit amyloid peptides aggregation as measured by ThT fluorescence at phosphate buffer pH 7.5. (A) PrP106-126 (100 μM) was incubated with ThT (100 μM) in the absence (red) and presence of H_2O_2 (100 μM) (blue). (B) $A\beta_{1-42}$ (10 μM) was incubated with ThT (10 μM) in the absence (red) and presence of H_2O_2 (10 μM) (blue). The control (ThT only) is shown in black.

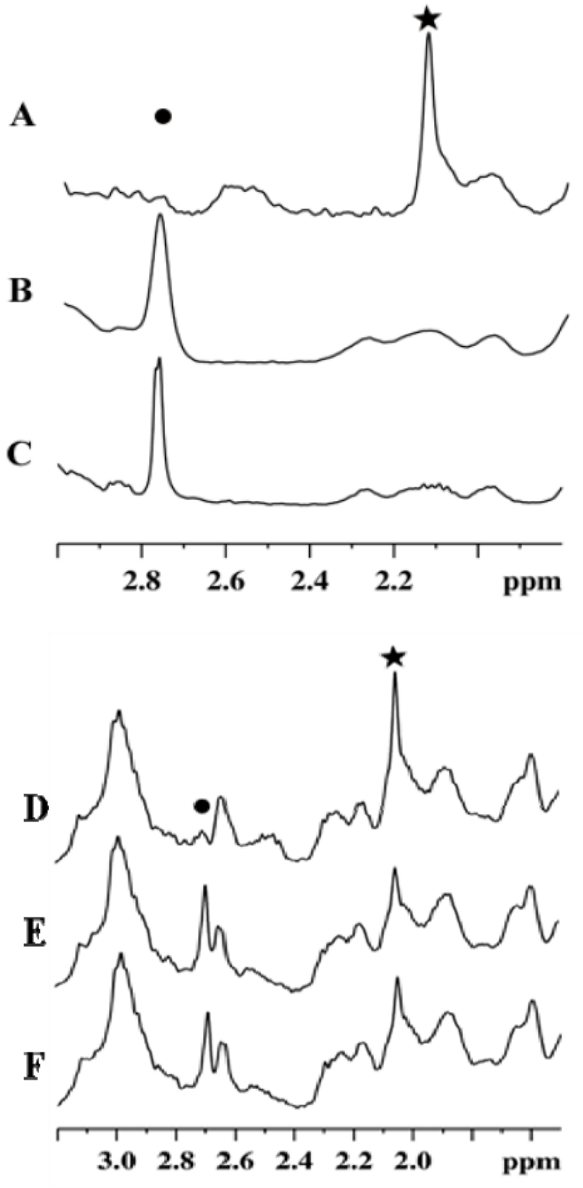

Figure S11. ^{1}H NMR spectra of 0.25 mM PrP106-126 in 9:1 H_2O/D_2O at pH 5.7, 298 K. (A) PrP106–126; (B) PrP106-126 in the presence of complex **1** (5.0 equiv); (C) PrP106-126 in the presence of complex **1** (5.0 equiv) and DTT (5.0 equiv). The signal at 2.08 ppm (star) representing the $C_\delta Hs$ group of methionine is significantly shifted when incubated with the complex **1**. After further incubation with DTT for 12h, the signal at 2.72 ppm (dot) slightly changed. Moreover, $_1$HNMR spectra of 0.25 mM $A\beta_{1\text{-}42}$ in 9:1 H_2O/D_2O at pH 5.7, 298 K. (D) $A\beta_{1\text{-}42}$; (E) $A\beta_{1\text{-}42}$ in the presence of complex **1** (5.0 equiv); (F) $A\beta_{1\text{-}42}$ in the presence of complex **1** (5.0 equiv) and DTT (5.0 equiv). The signal at 2.08 ppm (star) representing the $C_\delta Hs$ group of methionine is significantly shifted when incubated with the complex **1**. After further incubation with DTT for 12h, The signal at 2.72 ppm (dot) slightly changed. In addition, the results of complex **2** are similar to the mentioned above.

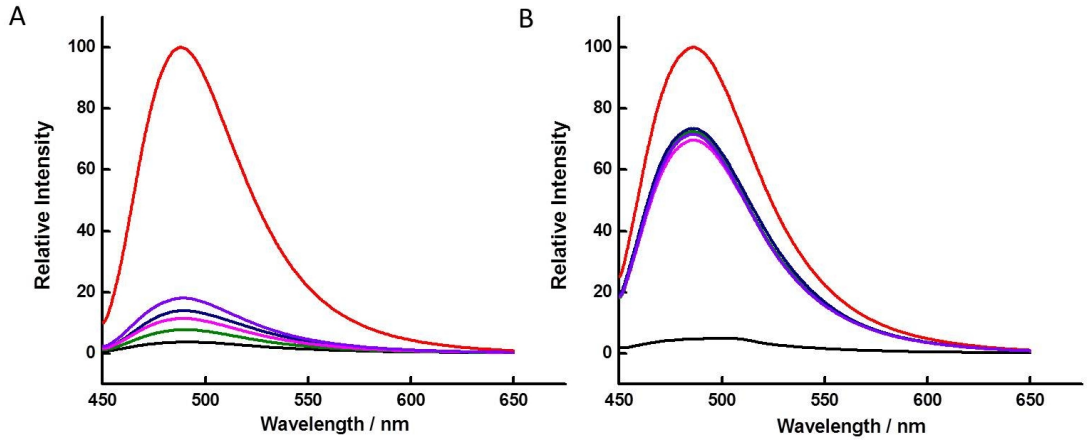

Figure S12. Effects of DTT reduction on amyloid protein aggregation as measured by ThT fluorescence at phosphate buffer pH 7.5. (A) PrP106-126 (100μM) was incubated with ThT(100μM) in the absence (red) and presence of **1**(100μM) (olive) and **2** (100μM) (magenta) and presence of **1**(100μM) with DTT(100μM) (navy) and **2** (100μM) with DTT(100μM) (violet). (B) $A\beta_{1\text{-}42}$(10μM) was incubated with ThT(10μM) in the absence (red) and presence of **1**(10μM) (olive) and **2**(10μM) (magenta) and presence of **1**(10μM) with DTT(10μM) (navy) and **2** (10μM) with DTT(10μM) (violet) . The control (ThT only) is shown in black.

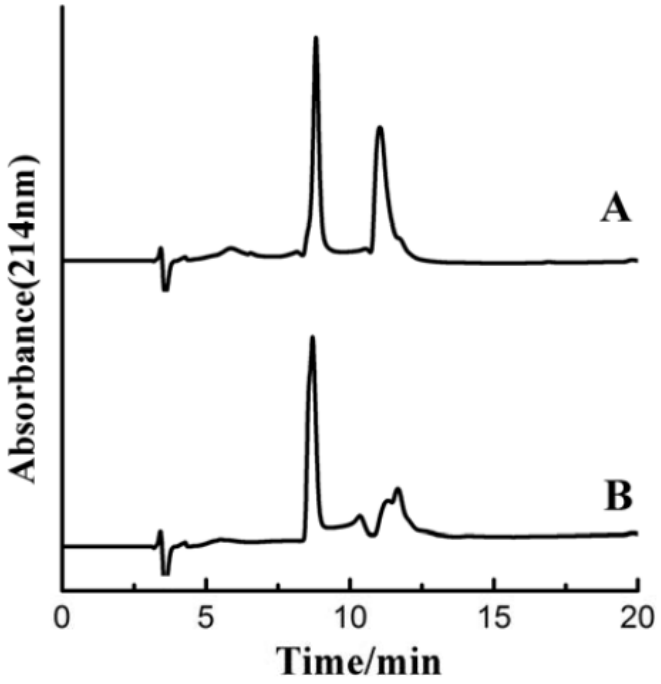

Figure S13. HPLC trace of complex **1**(A) and **2**(B).

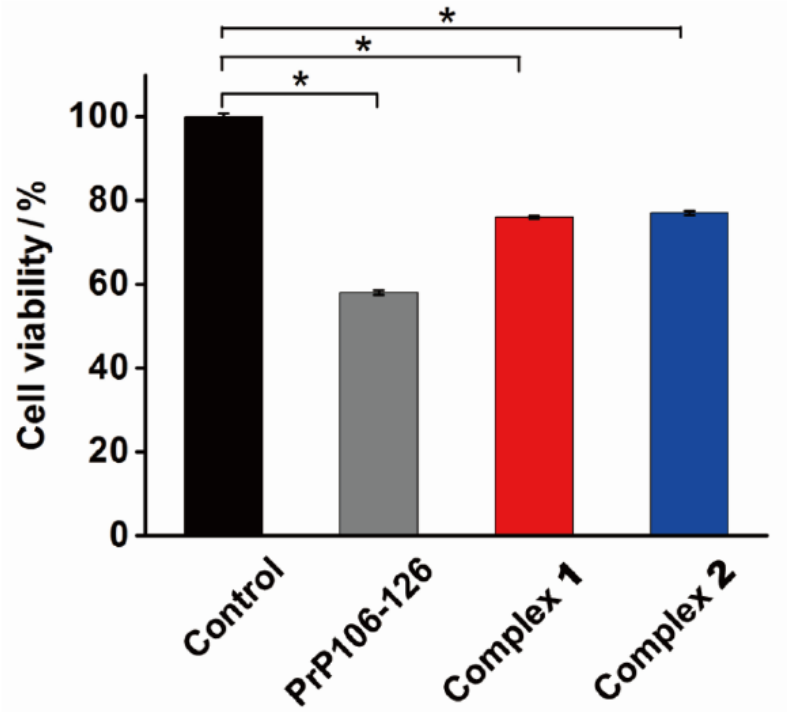

Figure S14. Cell viability was monitored using the MTT assay. SH-SY5Y cells were treated with different peroxovanadium complexes at 10 μM (red column for **1** and blue column for **2**) to detect the cytotoxicity of V complex independently. The cytotoxicity test of treating with PrP106-126 alone (grey column) was used for comparison. The data are shown as means ± S.E.M. with n=5. *p $<$ 0.05 (by one-way/Bonferroni ANOVA-post hoc test).