A Cocktail of Cu^{2+}- and Zn^{2+}- Peptoid-Based Chelators can Stop ROS Formation towards Alzheimer's Disease Therapy

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Experimental Procedures

Materials:

Rink amide resin was purchased from Novabiochem. Trifluoroacetic acid (TFA) and zinc(II) sulfate monohydrate were purchased from Alfa Aesar. (S)-(-)-1-phenylethylamine (*N*spe), 8-hydroxy-2-quinolinecarbonitrile, 4'-chloro-2,2':6',2''-terpyridine, 2-picolylamine (*N*2pic), benzylamine (*N*pm), N,N-Diisopropylethylenediamine (*N*^{i}Pr$_2$ae), 2,2',2'',2'''-(Ethane-1,2-diyldinitrilo)tetraacetic acid (EDTA) were purchased from Acros. Bromoacetic acid and chloroacetic acid were purchased from MERCK. Copper sulfate pentahydrate, N,N'-diisopropylcarbodiimide (DIC), piperidine, HEPES buffer (sodium salt of 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid), Phosphate buffer, Acetic buffer, Sodium ascorbate, Trypsin-EDTA solution, n-Octanol, acetonitrile (ACN), Methanol (MeOH) and water and HPLC grade solvents were purchased from Sigma-Aldrich. Dimethylformamide (DMF) and dichloromethane (DCM) solvents were purchased from Bio-Lab Ltd. Peptide Aβ16 (DAEFRHDSGYEVHHQK) was bought from Genecust. All reagents and solvents were used without additional purification. 4'-chloro-2,2':6',2''-terpyridine amine (Terpy) and 8-hydroxy-2-quinolinemethylamine (HQ) were synthesized according to previously published procedures.[1]

Synthesis and purification of the peptoid oligomers:

Peptoid oligomers were synthesized manually at room temperature on Rink amide resin using a variation of a previously reported peptoid sub-monomer protocol.[2] Typically, 100 mg of resin was swollen in dichloromethane (DCM) for 40 minutes before initiating oligomer synthesis. De-protection of resin was performed by addition of 20% piperidine solution (1.5 ml in Dimethyl formamide (DMF)) and the reaction was allowed to shake at room temperature for 20 min. Piperidine was washed from the resin using DMF (10 mL g^{-1} resin) (3 x 1 min). Bromoacetylation was completed by adding 20 eq. bromoacetic acid (1.2 M in DMF, 8.5 mL g^{-1} resin) and 24 eq. of diisopropylcarbodiimide (DIC) (2 mL g^{-1} resin); this reaction was allowed to shake at room temperature for 20 min. Following the reaction, the bromoacetylation reagents were washed from the resin using DMF (10 mL g^{-1} resin) (3 x 1 min) and 20 eq. of submonomer amine (1.0 M in DMF, 10 mL g^{-1} resin) were added. The amine displacement reaction was allowed to shake at room temperature for 20 min and was followed by multiple washing steps (DMF, 10 mL g^{-1} resin). Bromoacylations and amine displacement steps were repeated until the desired peptoids were obtained. Incorporation of several amines required alteration of the general protocol by previously described procedure: Terpy[1], HQ[3], *N*^{i}Pr$_2$ae[4] and *N*2pic[5].
To cleave the peptoid oligomers from solid support for analysis, approximately 5 mg of resin was treated with 95% Trifluoroacetic acid (TFA) in water (40 mL g^{-1} resin) for 10 minutes. The cleavage cocktail was evaporated under nitrogen gas and the peptoid oligomers were re-suspended in 0.5 mL HPLC solvent (1:1 HPLC grade acetonitrile: HPLC grade water). To cleave the peptoid oligomers from solid support for preparative HPLC the beads were treated with 5ml of 95% TFA in water for 45 minutes. The cleavage cocktail was evaporated under low pressure, re-suspended in 5 mL HPLC solvent and lyophilized overnight.
Peptoid oligomers were analyzed by reversed-phase HPLC (analytical C18 column, 5 µm, 100 Å, 2.0x50 mm) on a Jasco UV-2075 instrument at 214 nm. A linear gradient of 5–95% acetonitrile (ACN) in water (0.1% TFA) over 10 min was used at a flow rate of 0.7 mL/min. Preparative HPLC was performed using a phenomenex C18 column (15µm, 100 Å 21.20x100mm) on a Jasco UV-2075 instrument at 230 nm. Peaks were eluted with a linear gradient of 5–95% ACN in water (0.1% TFA) over 50 min at a flow rate of 5 mL/min. Mass spectrometry was performed on a Waters LCT Premier mass, Bruker Compass HyStar and Advion expression mass under electrospray ionization (ESI), direct probe ACN: H_2O (70:30), flow rate 0.3 ml/min.

General method for water solubility test:

1 mg peptoid was taken in the Eppendorf and water was added gradually (e.g. 5 ul per addition) until a clear solution was obtained. The solubility test was repeated three times and average values are presented.

EPR studies:

EPR spectra were taken on a Bruker EMX-10/12 X-band digital spectrometer from 2300 G to 4200 G, 3G amplitude modulation, approximately 9.4 GHz, and at 203 K. Spectra were recorded using a microwave power of 5-20 mW. Sample were prepared in HEPES buffer (50 mM, pH =7.4) +10% of glycerol (v/v) as a cryoprotectant.

(2,2,6,6-Tetramethyl-1-piperidinyl)oxidanyl (TEMPO, g = 2.0058) was used as a reference for simulations. Spectra simulation and processing were performed with Bruker WIN-EPR and SimFonia Software.

UV/Visible spectroscopy:

In the titration experiments, 3 mL of solvent (un-buffered water or buffer) was first measured as a blank. Then, in a typical experiment, 10–20 µL of a peptoid solution (5mM in water) was added (to get 17–33 µM concentration) and then sequentially titrated with 2-4 µl aliquots of a metal ion solution (5 mM) of Cu(II) or Zn(II), in multiple steps, until the binding was completed, and the spectrum was measured again.
In the selectivity experiment, solutions containing mixtures of metal ions (1 equivalent of Cu(II) ions and 1–10 equivalents of Zn(II) ions) in 3 mL of HEPES buffer (50 mM, pH=7.4) were first measured as a blank. Then, peptoid was added (10 µL, 5 mM) and the spectrum was measured again.
All measurements were performed using Agilent Cary 60 UV-Vis spectrophotometer, a double beam, Czerny–Turner monochromator. Data processing was done with KaleidaGraph software.

Dissociation constant calculations
The dissociation constant for Cu^{2+} with peptoid **AD2** was measured by using UV-Vis spectroscopy following a competition method. The stock solutions of peptoid **AD2**, EDTA, and Cu^{2+} were prepared at 5 mM concentration in water. For EDTA, the pH value was maintained at pH = 7.0. In a competition experiment,[6, 7] peptoid **TB** and EDTA were taken in a 1 : 1 ratio at 12 µM and gradually titrated with Cu^{2+} up to 1.2 equivalents. The UV-Vis spectra were monitored in the 200‑800 nm range. Following the previously reported method, the slope between ([**AD2**]total/[Cu**AD2**]-1) and ([EDTA]total/[CuEDTA]-1) was calculated. The slope equals K_D(Cu**AD2**) x K_A(CuEDTA) x α(EDTA), where K_D(Cu**AD2**) is the dissociation constant of Cu**AD2**, K_A (CuEDTA) is the association constant of Cu-EDTA complexation and α(EDTA) is the pH correction factor for EDTA. UV measurements were performed using an Agilent Cary 60 UV-Vis spectrophotometer, a double beam, Czerny‑Turner monochromator.

Synthesis of metal complexes for MS analysis:

Samples for MS analysis were prepared shortly before measurements. In a typical experiment, a solution of peptoid oligomers or amyloid $A\beta_{1\text{-}16}$ (100–200 µL of 0.05 mM) in un-buffered water or HEPES buffer was treated with metal solution (5 mM in water) and the mixture was stirred for 30 minutes prior to MS analysis. Mass spectrometry analysis of the metal complexes was performed on a Waters LCT Premier mass spectrometer or Bruker Compass HyStar or Advion expression mass, under electrospray ionization (ESI), direct probe ACN : H_2O (70 : 30), flow rate 0.3 mL min^{-1}.

Circular dichroism spectroscopy:

Approximately, 500 µL solutions (5 mM in water) of lyophilized peptoids powders and metal solutions were prepared immediately before CD measurements. CD scans were performed at 25 °C(if not stated otherwise) at a concentration of 100 µM in a solution of phosphate buffer (50 mM, pH=7.4) or HEPES buffer (10 mM, pH=7.4). The spectra were obtained by averaging 4 scans per sample in a fused quartz cell (path length = 0.1 cm). Scans were performed over the 370 to 190 nm region using 50 nm min^{-1} scan rate. CD measurements were performed using a circular dichroism spectrometer Applied Photophysics Chirascan. Data processing was done with KaleidaGraph software.

ROS formation:

Reagents, except the peptoids, were commercially available and were used as received. All the solutions were prepared in milliQ water (resistance: 18.2 MΩ) immediately before experiments. A stock solution of HEPES buffer was prepared at 500 mm, pH 7.4, and then diluted to 50 mM. Phosphate buffer was prepared by mixing K_2HPO_4 and KH_2PO_4 to reach a stock solution of 500 mM, pH 7.4. Sodium ascorbate was prepared at 5 mm each day because of the quick degradation of the ascorbate. Stock solution of peptide Aβ16 with concentration about 10 mM was prepared and titrated by using the Tyr chromophore, with ε_{293}=2400 $cm^{-1}M^{-1}$ at basic pH. The stock solution was stored at 4°C. The solutions were diluted down to the appropriate concentration in peptide. All pH values are given with a ±0.2 pH unit error.

UV/Visible spectrophotometry for kinetics of ROS formation:

UV/Vis ROS kinetic experiments were recorded with a spectrophotometer Agilent Cary 60 UV-Vis spectrophotometer, a double beam, Czerny–Turner monochromator with external stirrer of cuvette holder, in 1 cm path length quartz cuvettes, with 800 rpm stirring. The samples were prepared from stock solutions of peptoids, $A\beta_{1-16}$ peptide, EDTA, Zn^{2+} and Cu^{2+}, diluted to 10(or 20), 10, 10 (or 20), 10 and 9 μM, respectively, in 50 mM HEPES solution, pH 7.4. Ascorbate was diluted to 100 μM.

Stability at the physiological pH and body temperature:

Stability of peptoids at the physiologically relevant pH and temperature was studied by UV-Vis spectroscopy. All measurements were performed using a circular dichroism spectrometer Applied Photophysics Chirascan with built in temperature controller. The stability experiments were performed at pH 7.4 (PBS, 50 mM) and 4.0 (Acetic buffer, 50 mM) at a concentration of 100 μM of peptoid in solution. All solutions of peptoids and buffers were freshly prepared prior to the experiment. In the typical experiment, the aliquot of peptoid was added to the buffer, mixed and its UV\Vis spectrum was recorded at the RT (25°C). Next, the sample holder was heated till 37°C, and the UV\Vis spectrum of the mixture was taken again. The temperature was kept constant at 37°C and the UV\Vis spectrum were recorded at 1, 24 and 72 hours after heating. The spectra were obtained by averaging 4 scans per sample in a fused quartz cell (path length = 0.1 cm). Scans were performed over the 370 to 190 nm region using 50 nm min^{-1} scan rate. Data processing was done with KaleidaGraph software.

Stability to proteolytic degradation:

Stability to proteolytic degradation was performed by previously described procedure. [8] Oligomers were dissolved in PBS buffer (50 mM, pH=7.4) at a concentration of 1 mg mL^{-1} and exposed to 0.1 μM trypsin. A control containing 1 mg mL^{-1} peptoid in PBS buffer alone was also performed. Samples were then incubated at 37 °C and pH was monitored at physiologically relevant levels (pH 7.4). At defined intervals (every 24 hours), aliquots were removed, and flash frozen in liquid nitrogen to quench the reaction, and then lyophilized for further analysis. Specifically, lyophilized samples were dissolved at 0.1 mg mL^{-1} in 50% ACN/50% water. An HPLC analysis of 10 minute gradient 5-95 % ACN/H_2O with 0.1% (v/v) TFA at a flowrate of 0.7 mL min^{-1} with the eluents measured at 214 nm was employed. Experiments were lasting for three days, and with each timepoints taken, stimuli were also replenished. Reactions were done in triplicate and degradation was monitored by decreasing main peak areas of chromatogram peaks in comparison to control. Specifically, spectra were extracted at 214 nm and the max absorbance and corresponding retention time of the control peaks were identified using built-in JASCO ChromNav Peak Analysis/Finder tool with a baseline at $y = 0$. The absorbance of each sample peak was normalized against its respective control at fixed retention time and plotted against incubation time.

logP_{ow} estimation by Shake-Flask Method:

Partition experiments were carried according to previously described procedure.[9] Samples were prepared by putting 500 μL of octanol in contact with 500 μL of PBS (50 mM, pH=7.4), which contained 10 μL of peptoid (5 mM). Each peptoid was measured in triplicate. The samples were allowed to equilibrate under gentle agitation for ~150 hours (to be sufficient for the system to reach equilibrium). After this point samples were taken from the PBS half and the octanol half and diluted to total volume of 1 mL to produce sufficient volume for spectroscopy. The concentration of peptoid remaining in the PBS and the octanol was measured individually by UV/Vis spectroscopy using calibration plots of the absorbance of the peptoid at λ_{max} as function of it's concentration in n-octanol/ACN or in PBS buffer (50 mM, pH=7.4). From these concentrations the ratio K of concentration of peptoid in PBS to concentration in octanol was calculated. UV/Vis spectra were measured on Agilent Cary 60 UV-Vis spectrophotometer, a double beam, Czerny–Turner monochromator.

Results and Discussion

Table S1. Peptoid oligomer sequences and their molecular weights.

*N*spe = (S)-(+)-1-phenylethylamine, *N*he = ethanolamine, *N*^{i}Pr$_2$ae = N,N-Diisopropylethylenediamine, *N*2pic = 2-picolylamine, *N*pm = benzylamine, Terpy= 4'-yloxy-(2,2′:6′,2″-Terpyridine) ethylamine, HQ = 8-hydroxy-2-quinolinemethylamine,

Peptoid	Sequence	Molecular weight (M+H$^+$), [gr/mol]	
		Calculated	Found
AD1	*N*^{i}Pr$_2$ae-*N*spe-HQ-*N*spe-*N*spe-Terpy-*N*spe	1392.7292	1392.06
AD2	*N*^{i}Pr$_2$ae-*N*^{i}Pr$_2$ae-*N*spe-HQ-*N*spe-*N*spe-Terpy-*N*spe	1576.8868	1576.69
PT1	*N*2pic-Terpy-*N*pm	645.2932	645.3

ESI-MS spectra for peptoid oligomers

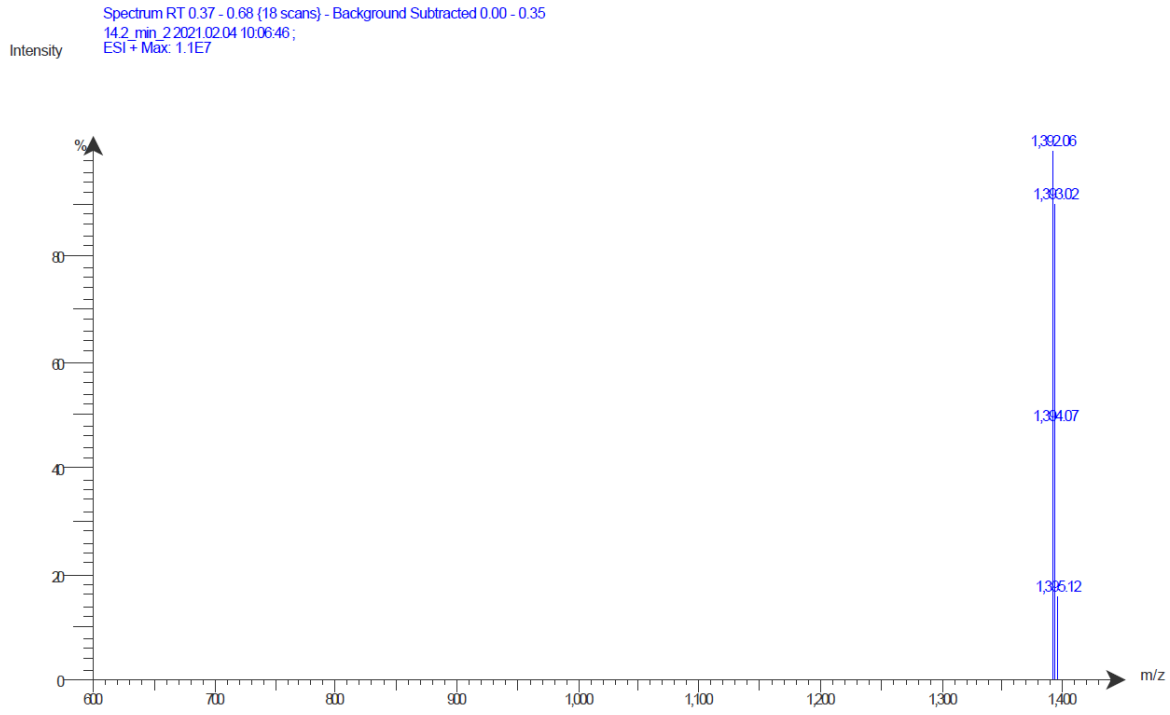

Fig. S1 ESI-MS spectrum of peptoid oligomer **AD1** in acetonitrile

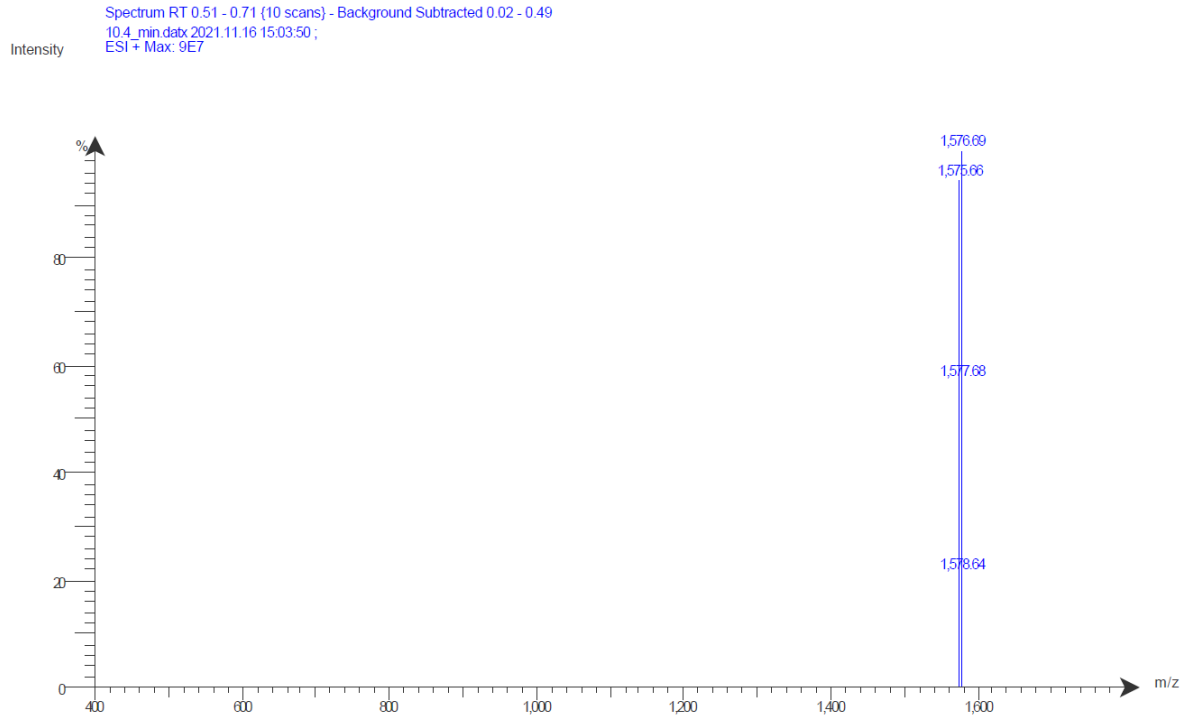

Fig. S2 ESI-MS spectrum of peptoid oligomer **AD2** in acetonitrile

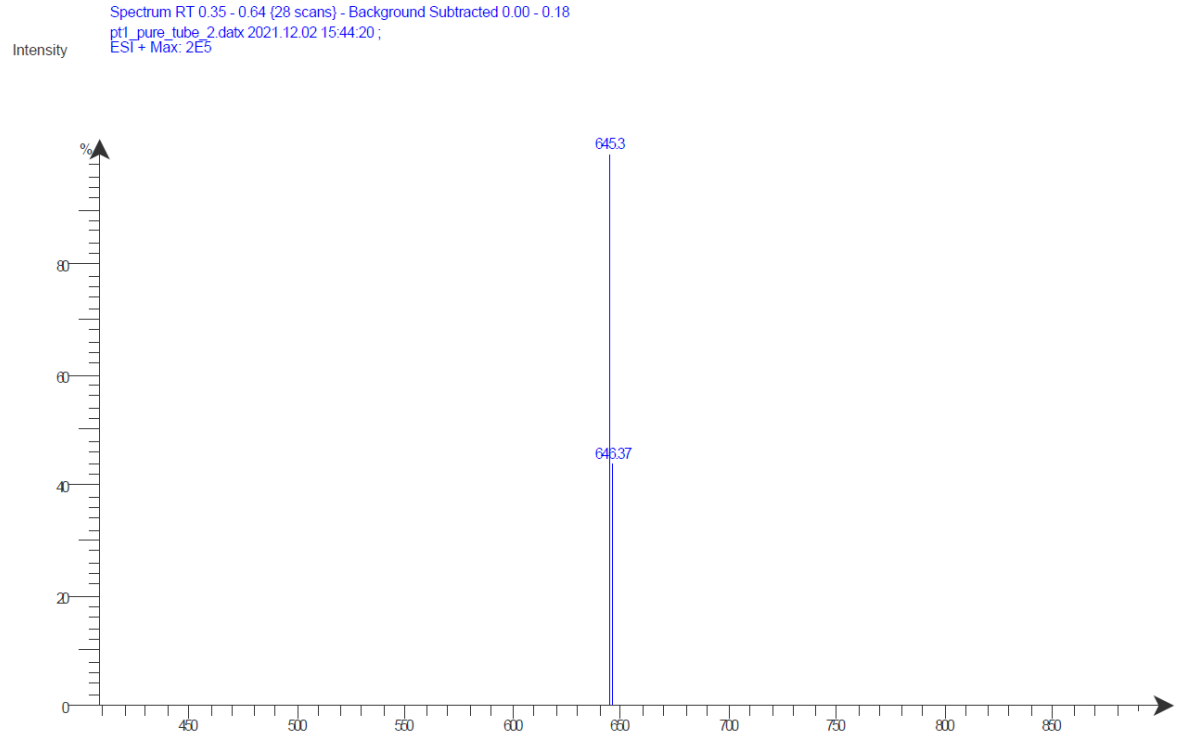

Fig. S3 ESI-MS spectrum of peptoid oligomer **PT1** in acetonitrile

HPLC analytical chromotograms for peptoid oligomers

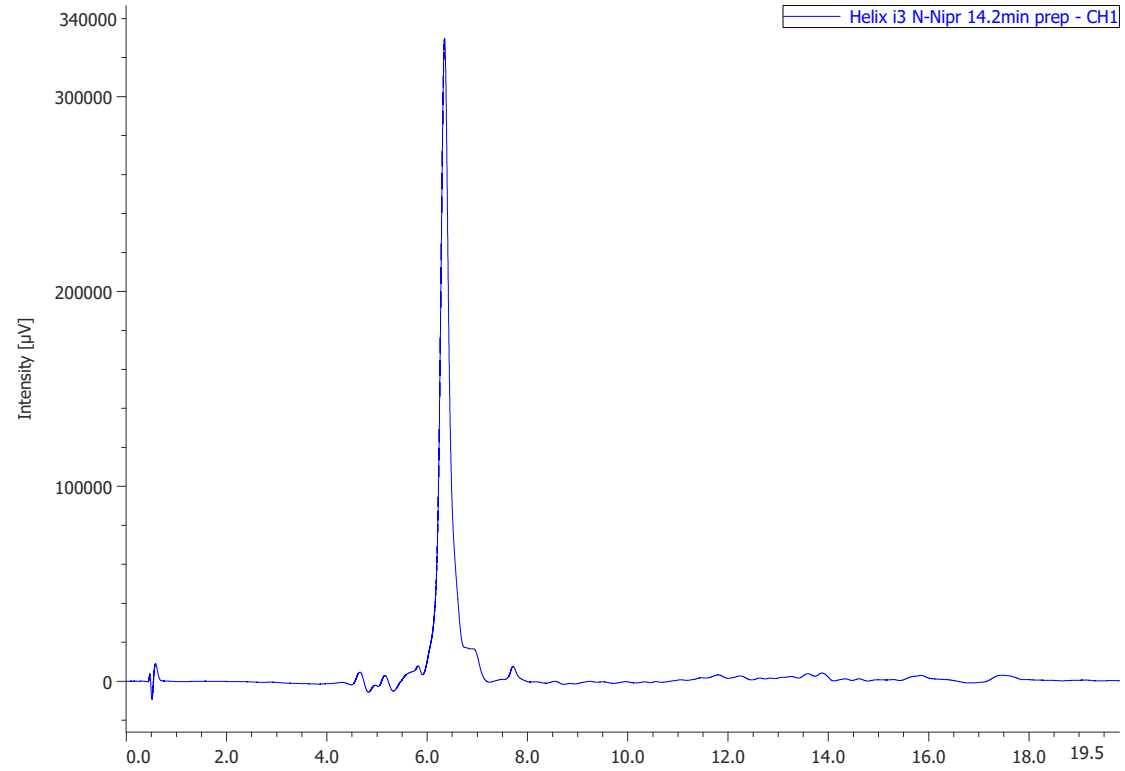

Fig. S4 HPLC chromotogram of peptoid oligomer **AD1** in acetonitrile

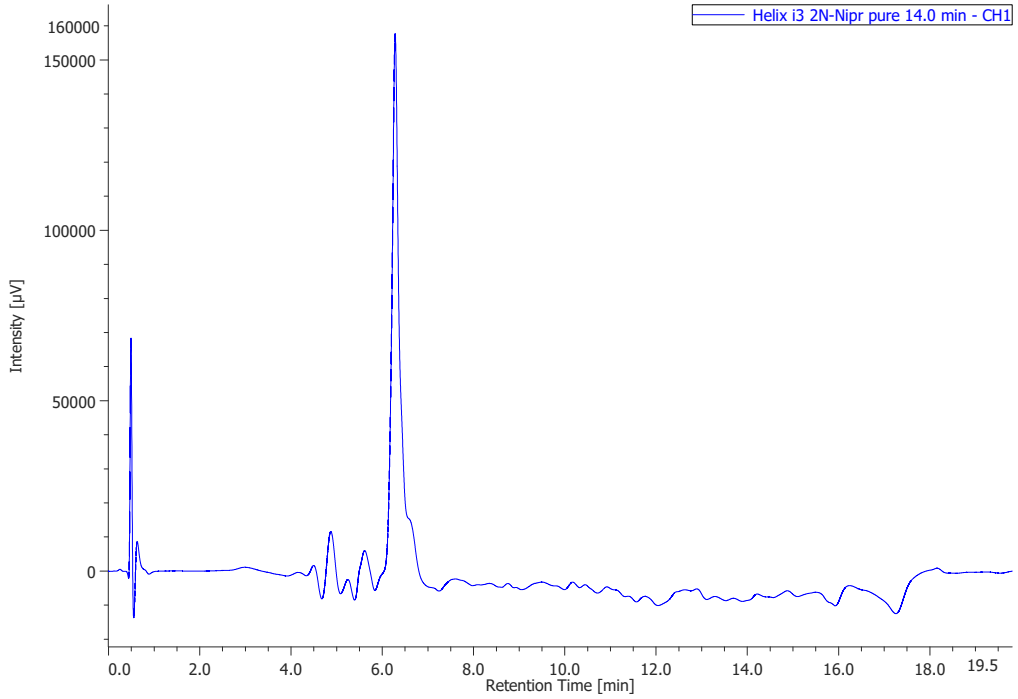

Fig. S5 HPLC chromotogram of peptoid oligomer **AD2** in acetonitrile

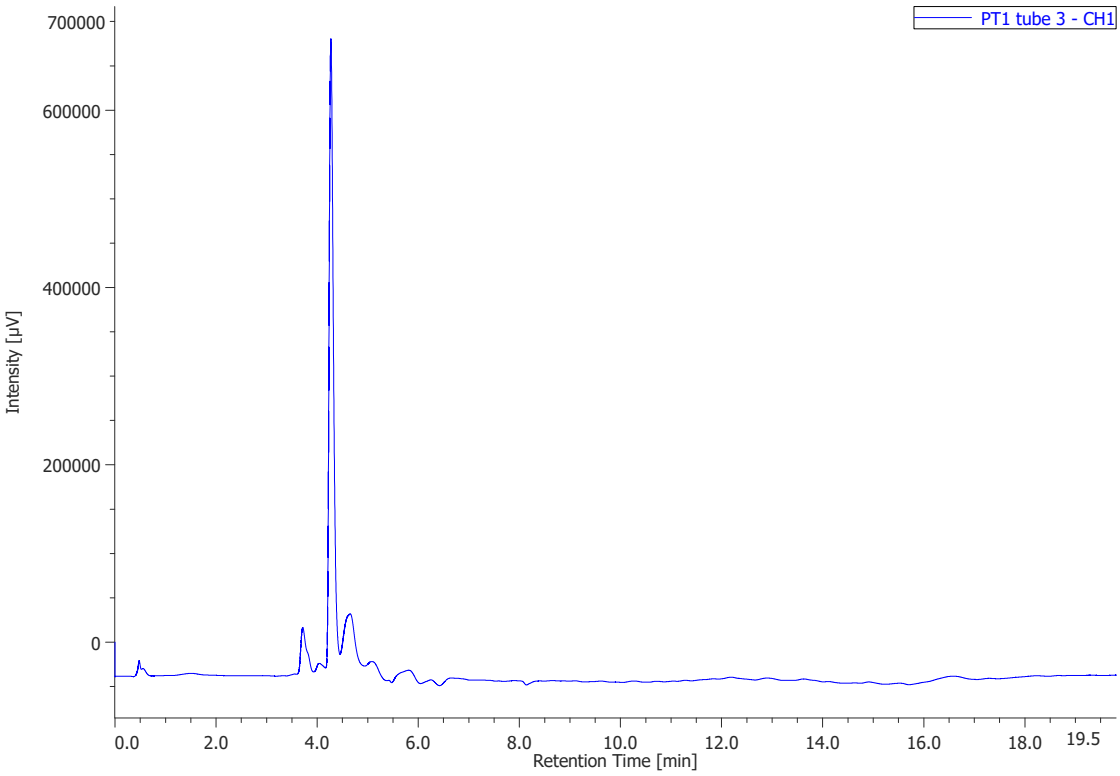

Fig. S6 HPLC chromotogram of peptoid oligomer **PT1** in acetonitrile

UV-Vis titration and selectivivty data for AD2

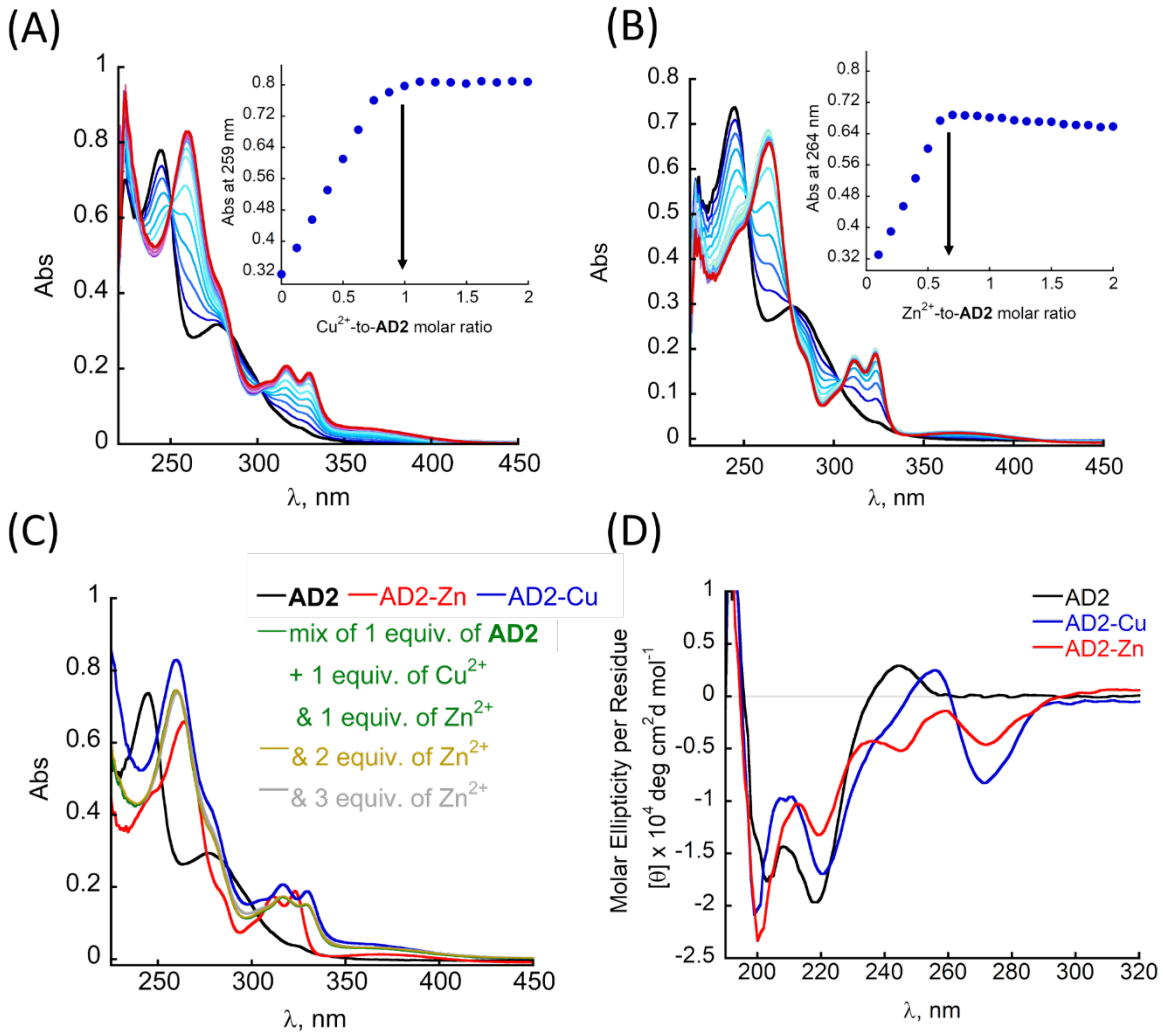

Fig. S7 UV-Vis titration of **AD2** (33 μM) with (A) Cu^{2+} (B) Zn^{2+} in HEPES buffer (50 mM, pH = 7.4). Insets: metal-to-peptoid ratio plots, constructed from the corresponding UV-Vis titration (C) UV-Vis spectra of **AD2** (33 μM), it's Cu^{2+} and Zn^{2+} complexes, and the complexes formed upon the addition of a mixture of 1 equiv. of Cu^{2+} and n equiv. of Zn^{2+} (n=1 green line, n=2 yellow line, n=3 grey line) (D) CD spectra of **AD2** (100 μM) and it's corresponding metal complexes.
Note: For Fig. 7A, inset, the metal-to-peptoid ratio plot consists of two slopes with different degree of steepness: sharp steep slope between 0 to 0.7 equiv. of Cu^{2+} added, followed by not steep slope from 0.7 to 1 equiv. of Cu^{2+}. This phenomenon is attributed to the kinetics of the Cu-peptoid complex formation during titration in the buffer conditions. The Job Plot experiment was performed to determine the exact stoichiometry for this complex.

Binding constant determination by competition experiment with EDTA

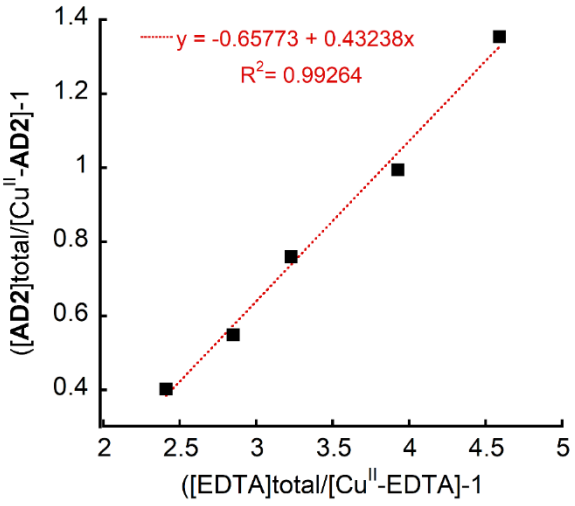

Fig. S8. Binding affinity determination by competition method with EDTA.[6,7] The experiment has been executed at pH = 7.0, using EDTA as a competitor agent. $CuSO_4$ is used as a metal ions source in the experiment. The formation constant for EDTA should be corrected for EDTA's acid-base properties in pH 7, which could be done by calculating the fraction, α(EDTA).[10]

Dissociation constant calculation for Cu**AD2**:

Slope = $K_D(Cu^{2+}$-**AD2**$) \cdot K_A(Cu^{2+}$ -EDTA)$\cdot$ α(EDTA), for Cu^{2+}-**AD2** K_D is 1.43 x 10^{-16} M

[K_D: Dissociation constant of Cu^{2+}-**AD2** complex, K_A: Association constant of Cu^{2+}-EDTA (6.309 x 10^{18} M^{-1}), and α(EDTA) is the pH correction factor].

Table S2 Summary of binding affinities for Cu^{2+} and Zn^{2+}

Compound	Binding affinity (K_A) at pH = 7.0, [M^{-1}]		Reference
	Cu^{2+}	Zn^{2+}	
PT1[a]	4.55 x 10^{11}	5.41 x 10^{12}	[5]
AD2	7.00 x 10^{15}	N.D.	*This work*
$A\beta_{1-16}$	10^9	10^5-10^6	[11,12]

Note: N.D. - not determined

[a] Binding affinities were calculated from the values of K_D(Cu**PT1**) and K_D(Zn**PT1**), which were obtained by competition experiment with EDTA at pH = 7.0 and reported in reference [5]

HR ESI-MS data for Cu^{2+} complexes with AD2

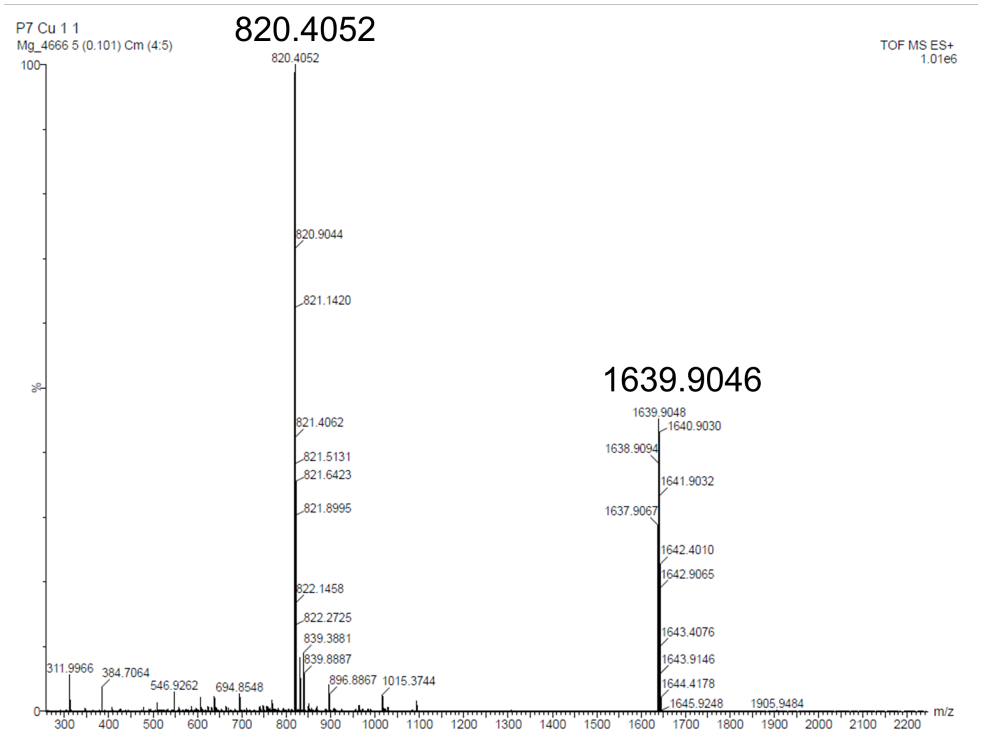

Fig. S9 ESI-MS traces of mixture of 1 equiv. of **AD2** with 1 equiv. of Cu^{2+} in un-buffered water (pH=7.0), suggesting formation of 1:1 Cu(II)**AD2**

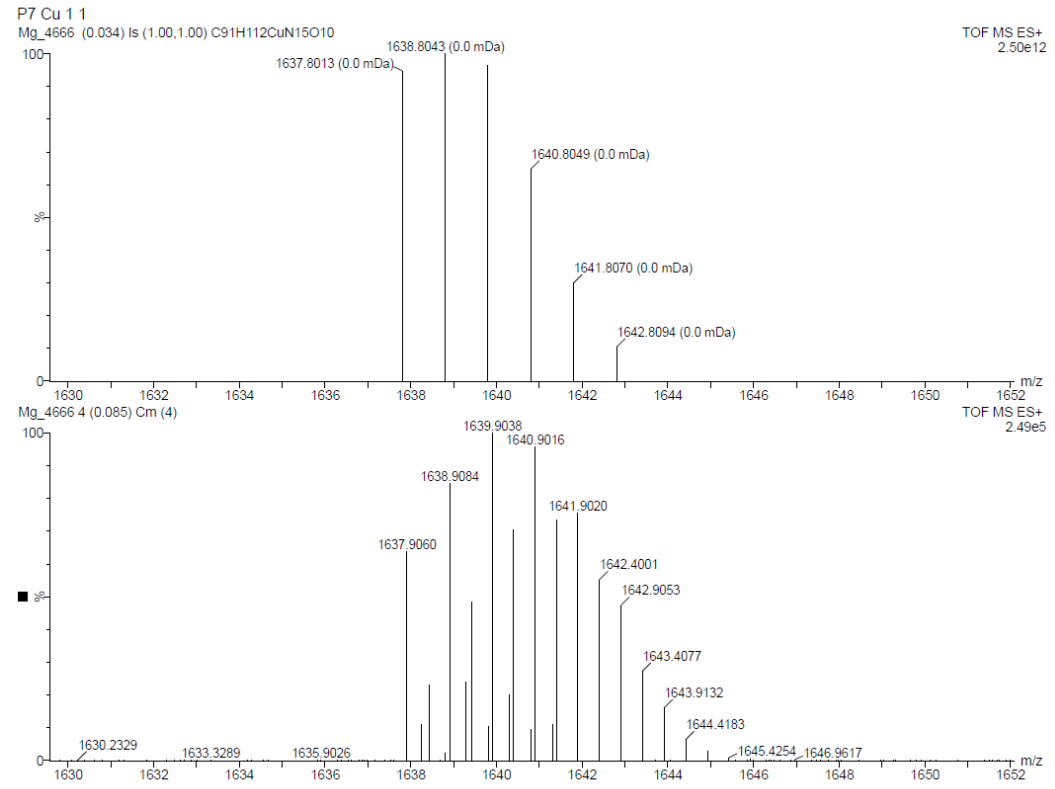

Fig. S10 ESI-MS m/z traces of Cu(II)**AD2** (bottom) and calculated ESI-MS spectrum (top).

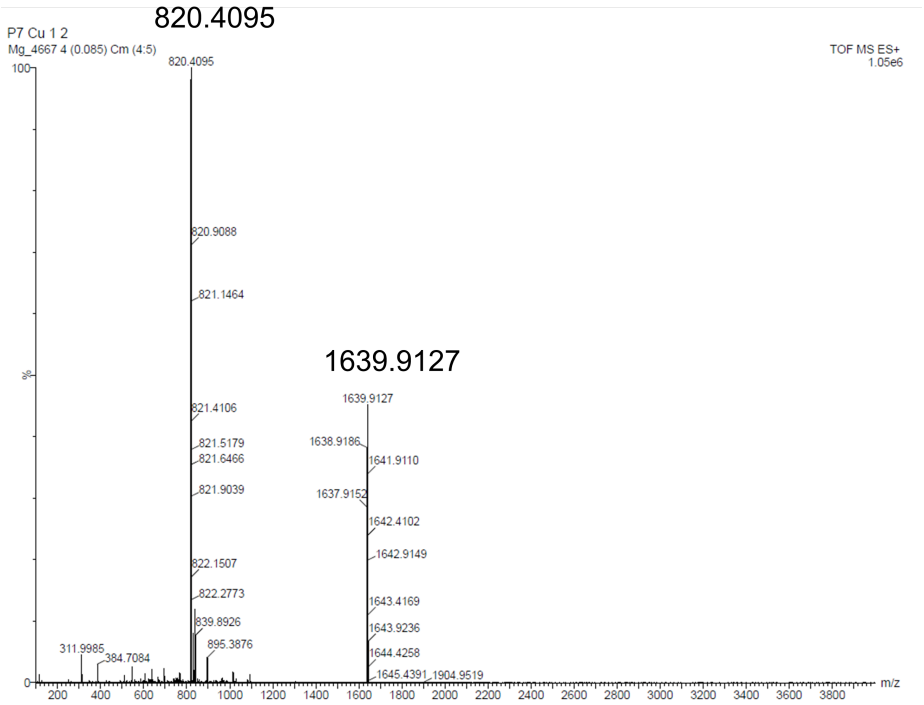

Fig. S11 ESI-MS traces of mixture of 2 equiv. of **AD2** with 1 equiv. of Cu^{2+} in un-buffered water (pH=7.0), suggesting formation of 1:1 Cu(II)**AD2**

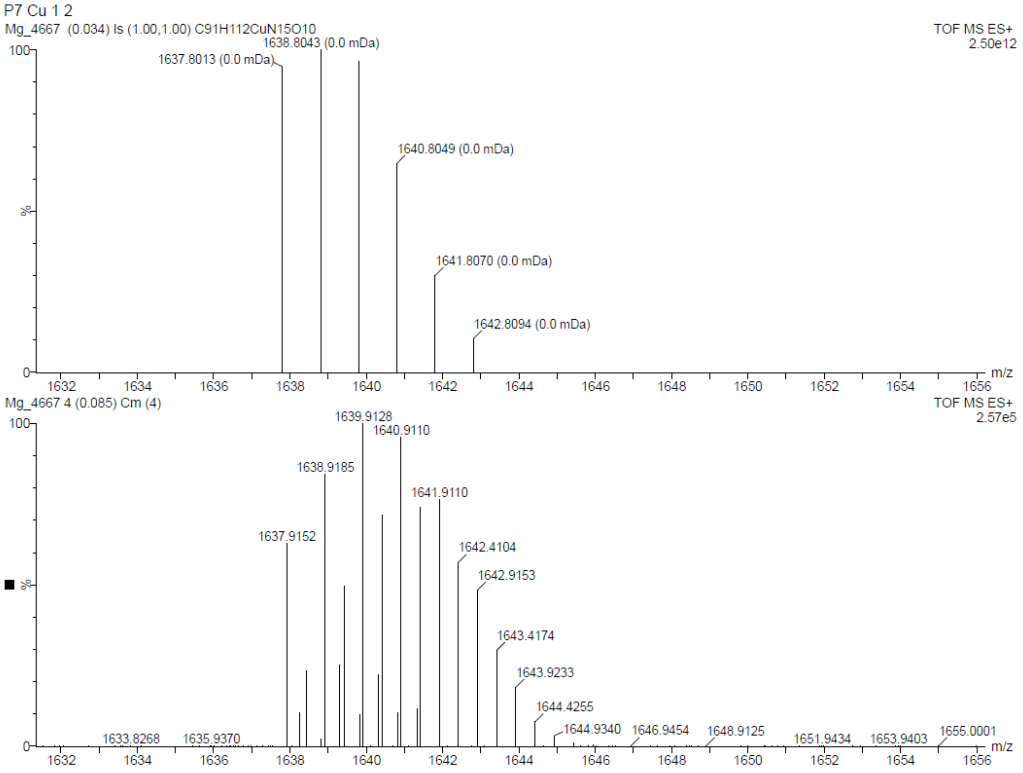

Fig. S12 ESI-MS m/z traces of Cu(II)**AD2** (bottom) and calculated ESI-MS spectrum (top).

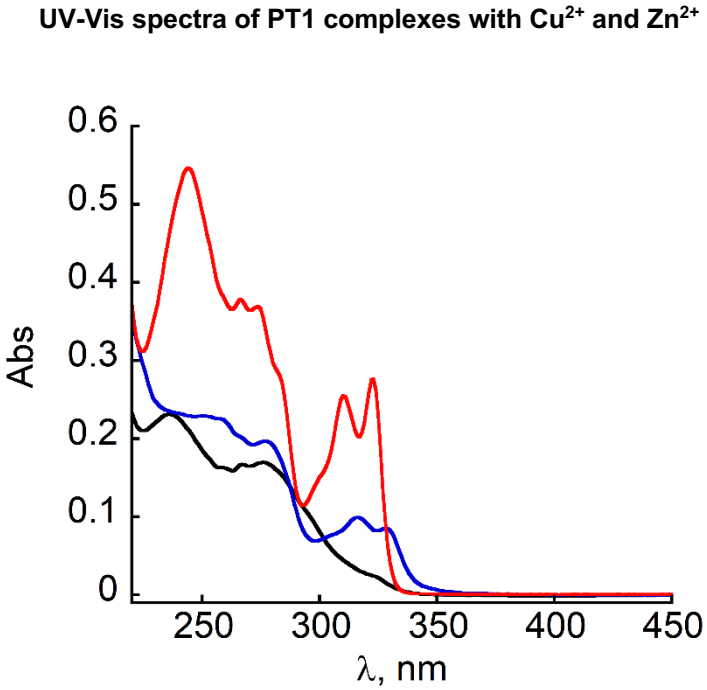

Fig. S13 UV-Vis spectra of free **PT1** (17 μM, black), and its 1:1 complexes with Cu^{2+} (blue) and Zn^{2+} (33 μM, red) in HEPES buffer (50 mM, pH = 7.4).

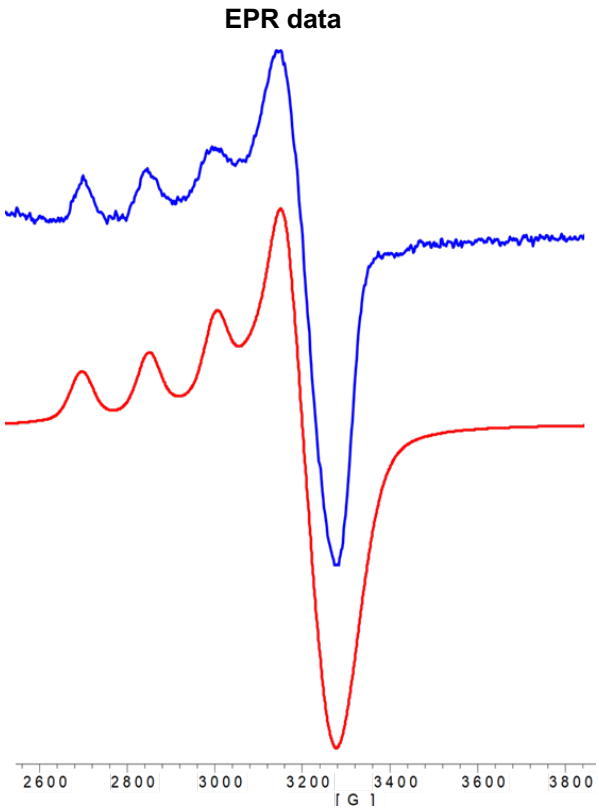

Fig. S14 X-band EPR spectra of peptoid copper Cu(II)**AD2** complex in frozen solution state in HEPES (50 mM, pH = 7.4) buffer (blue line) and the corresponding simulated spectra (red line) measured at 203 K. Reference- (2,2,6,6-Tetramethyl-1-piperidinyl)oxidanyl (TEMPO, g = 2.0058).

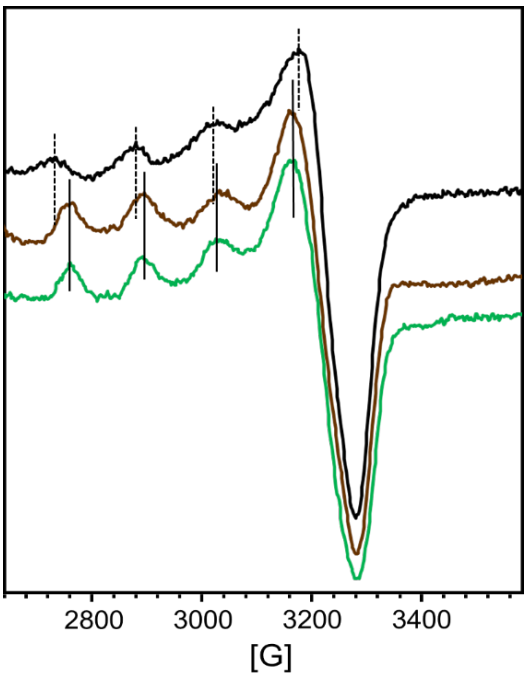

Fig. S15 EPR spectra of Cu^{2+} + **AD2** (green), $A\beta_{1-16}$ + Cu^{2+} + Zn^{2+} (black), $A\beta_{1-16}$ + Cu^{2+} + Zn^{2+} + **AD2** + EPR tube frozen after one hour (brown) Conditions: = [**AD2**] = [$A\beta_{1-16}$] = [Zn^{2+}] = 280 μM, [Cu^{2+}] = 250 μM, [HEPES]= 50 mM (pH=7.4). Recording conditions: T = 203 K, v = 9.5 GHz, modulation amplitude = 3 G, microwave power: 20 mW.

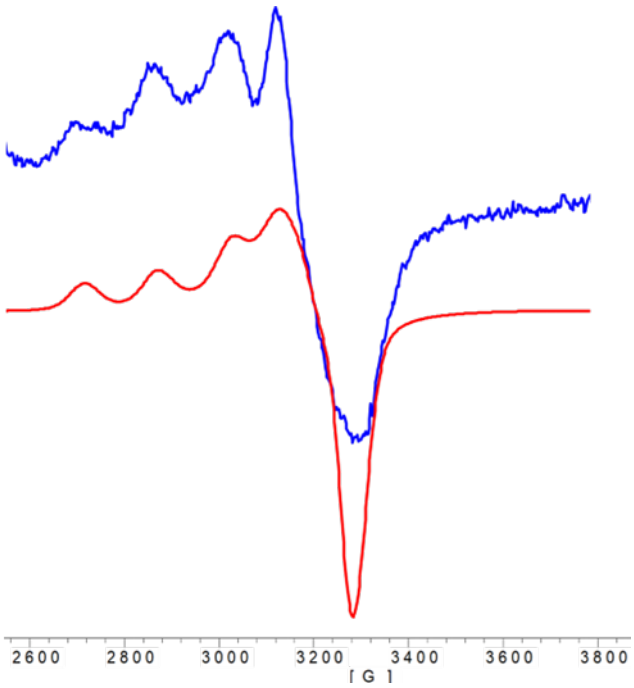

Fig. S16 X-band EPR spectra of peptoid copper Cu(II)**PT1** complex in frozen solution state in HEPES (50 mM, pH = 7.4) buffer (blue line) and the corresponding simulated spectra (red line) measured at 203 K. Reference- (2,2,6,6-Tetramethyl-1-piperidinyl)oxidanyl (TEMPO, g = 2.0058). Recording conditions: T = 203 K, v = 9.5 GHz, modulation amplitude = 3 G, microwave power: 20 mW.

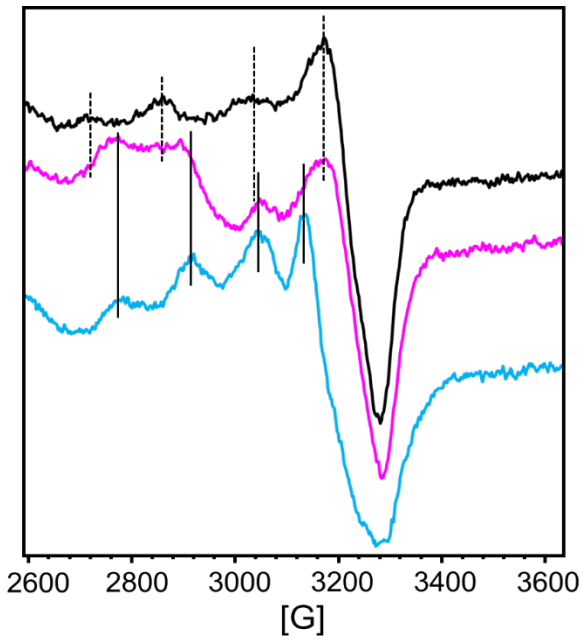

Fig. S17 EPR spectra of Cu^{2+} + **PT1** (cyan), $A\beta_{1-16}$ + Cu^{2+} + Zn^{2+} (black), $A\beta_{1-16}$ + Cu^{2+} + Zn^{2+} + **PT1** EPR tube frozen after one hour (pink) Conditions: = [**PT1**] = [$A\beta_{1-16}$] = [Zn^{2+}] = 280 μM, [Cu^{2+}] = 250 μM, [HEPES]= 50 mM (pH=7.4). Recording conditions: T = 203 K, v = 9.5 GHz, modulation amplitude = 3 G, microwave power: 20 mW.

MS data for Cu^{2+} and Zn^{2+} extraction from Cu,Zn-Aβ complex by PT1

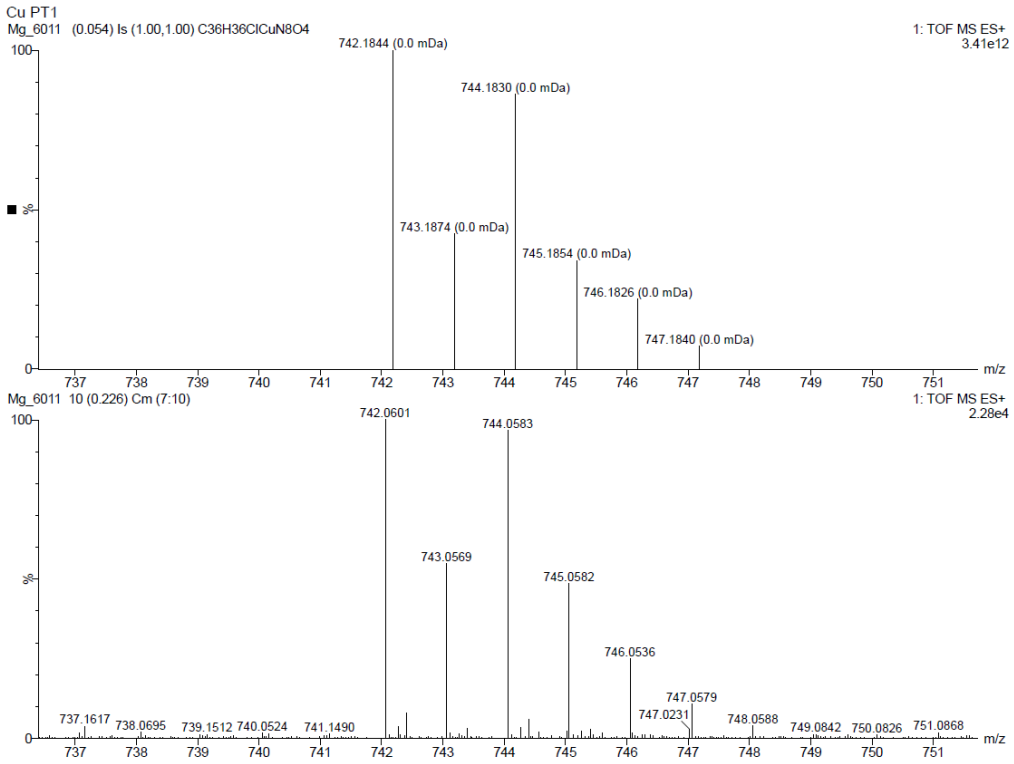

Fig. S18 ESI-MS m/z traces of 1 equiv. of **PT1** with 1 equiv. of Cu^{2+} in HEPES buffer (50 mM, pH=7.4), suggesting the formation of 1:1 Cu(II)**PT1**-Cl (bottom) and calculated ESI-MS spectrum (top). Coordination of Cu**PT1** to Cl is plausible as HCl was used for adjusting pH during HEPES buffer preparation.

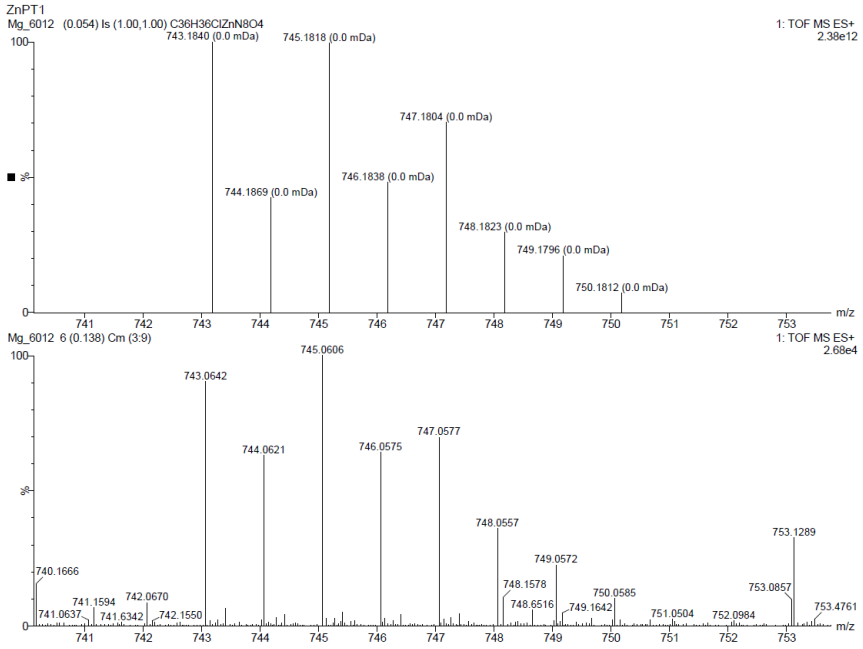

Fig. S19 ESI-MS m/z traces of 1 equiv. of **PT1** with 1 equiv. of Zn^{2+} in HEPES buffer (50 mM, pH=7.4), suggesting the formation of 1:1 Zn(II)**PT1**-Cl (bottom) and calculated ESI-MS spectrum (top). Coordination of Zn**PT1** to Cl is plausible as HCl was used for adjusting pH during HEPES buffer preparation.

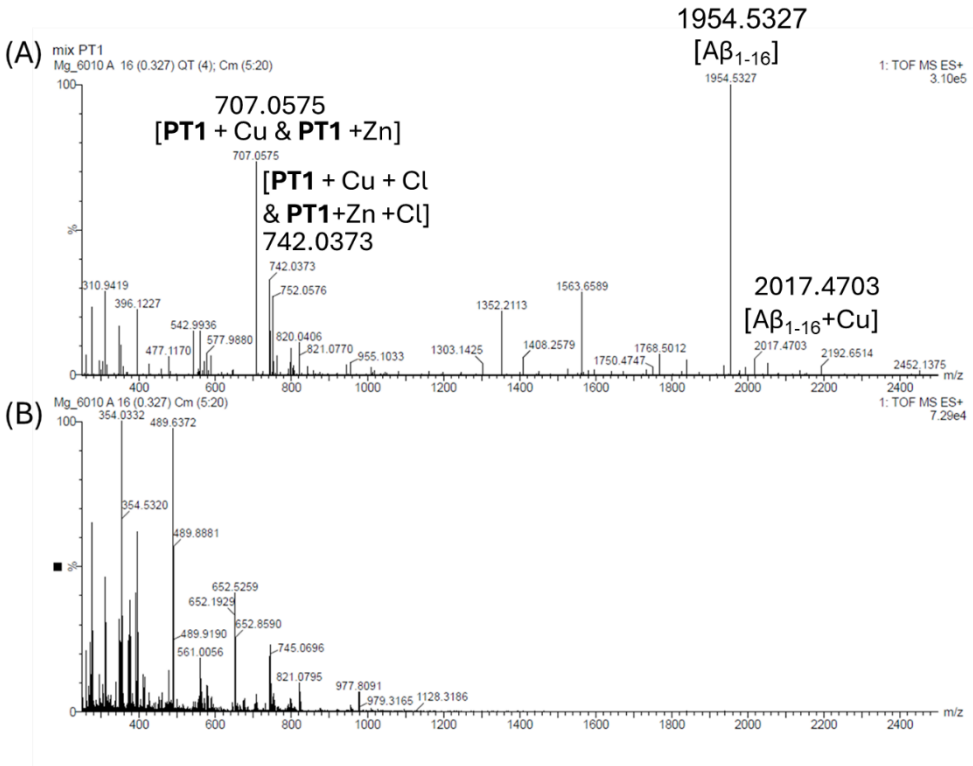

Fig. S20 ESI-MS m/z traces of the mixture of 1 equiv. of Cu,Zn$Aβ_{1-16}$ with 1 equiv. of **PT1** in HEPES buffer (50 mM, pH=7.4) (A) projection of the full masses, generated by the built-in Waters LCT Premier mass software analysis of the fragmentation of the measured spectrum (B) raw data

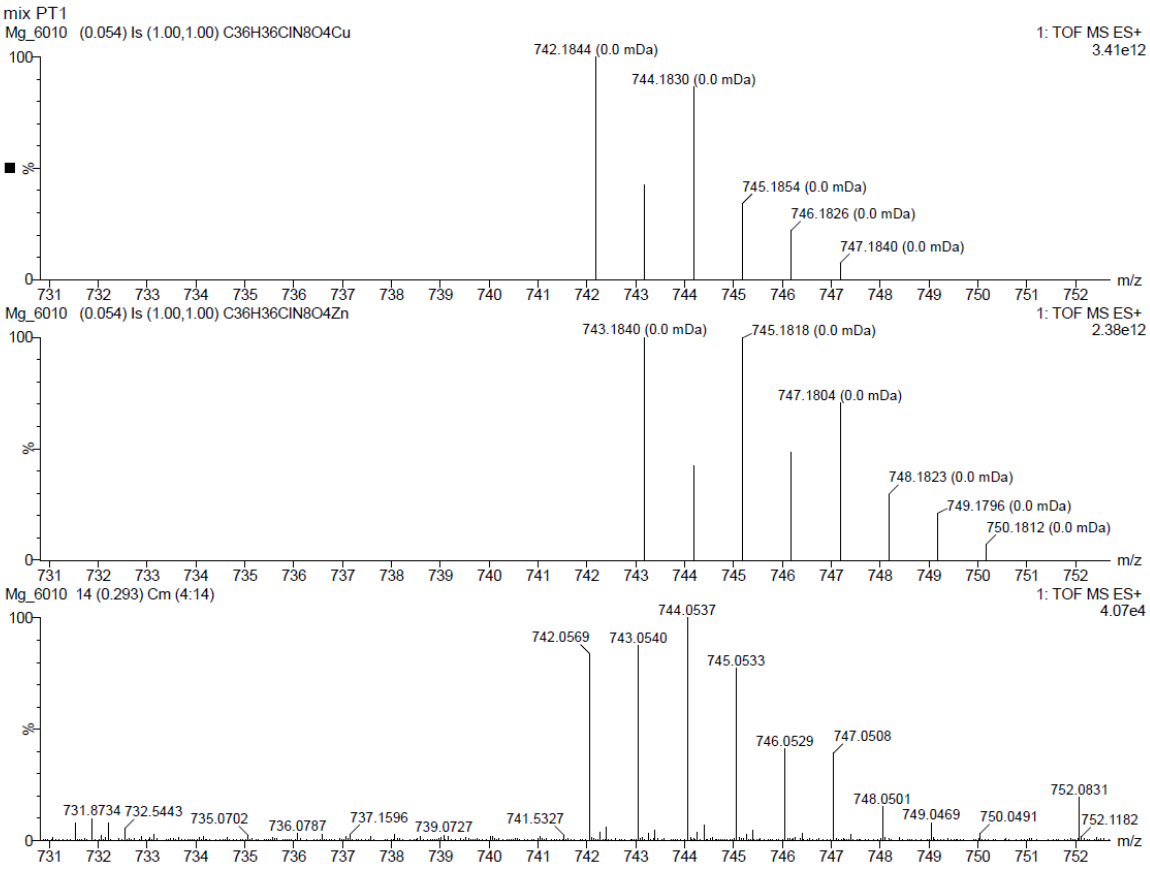

Fig. S21 ESI-MS m/z traces of the mixture of 1 equiv. of Cu,Zn$A\beta_{1\text{-}16}$ with 1 equiv. of **PT1** in HEPES buffer (50 mM, pH=7.4) (bottom) and calculated ESI-MS spectrum of Cu**PT1** (top) and Zn**PT1** (middle).

Table S3 Summary of the fragments assignments from Fig. S20B

m/z	Assignment	Contains Metal	Reference
354.0332	m/2z of [**PT1**+Cu] & [**PT1**+Zn]	Yes	This work
489.6372	m/3z, Unknown fragment	No	This work
561.0056	m/z, Unknown fragment	No	This work
652.5259	m/3z, [$A\beta_{1\text{-}16}$+3H]	No	[13]
821.0795	m/z, [**PT-1**+Zn+OAc+3H_2O]	Yes	[5]
977.8091	m/2z, [$A\beta_{1\text{-}16}$+2H]	No	[13]

MS data for Cu^{2+} and Zn^{2+} extraction from Cu,Zn-Aβ complex by cocktail mixture of AD2 and PT1

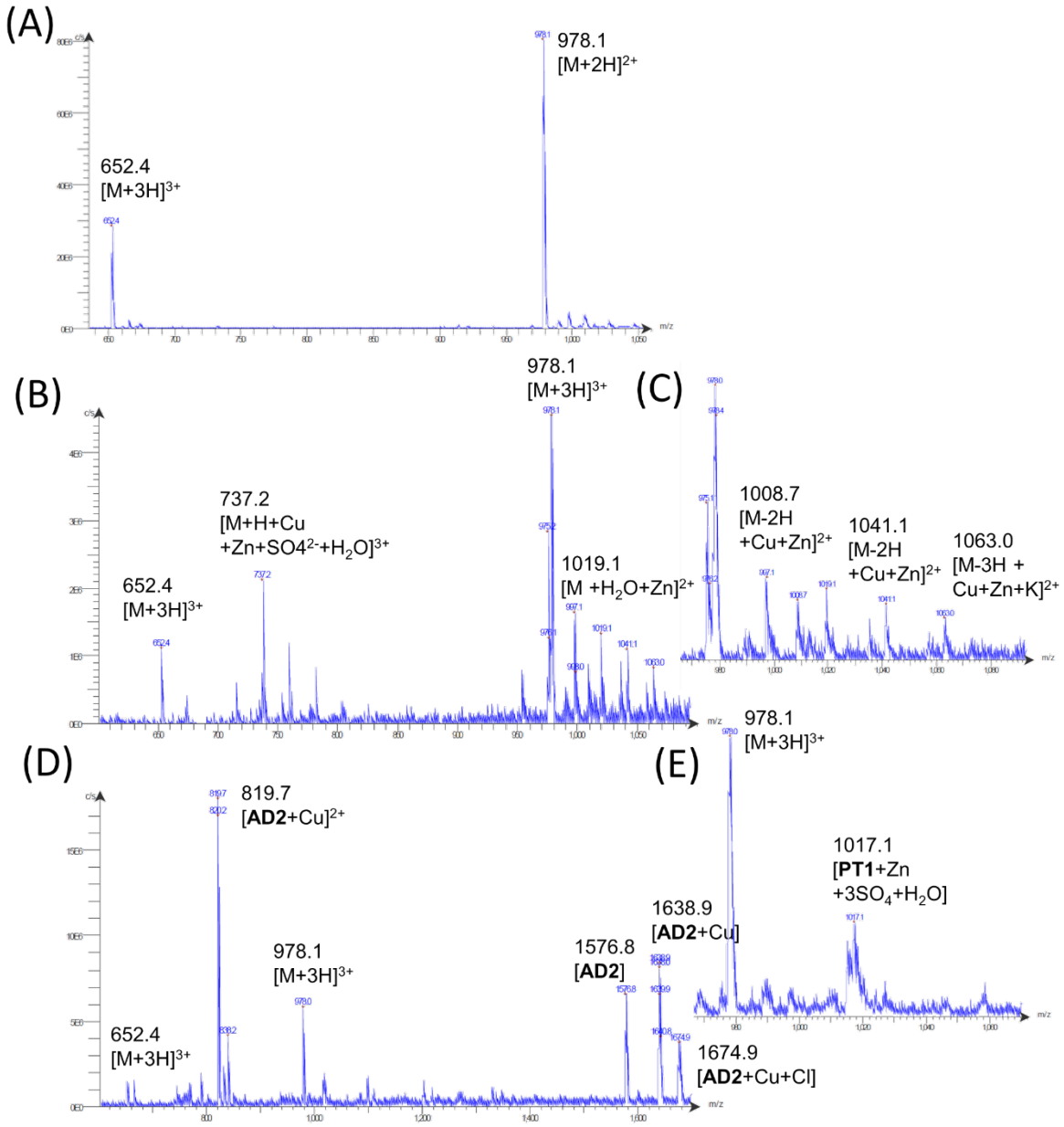

Fig. S22 ESI-MS m/z traces of (A) metal-free $Aβ_{1-16}$ (B) mixture of 1 equiv. of $Aβ_{1-16}$, 1 equiv. of Zn^{2+} and 0.9 equiv. of Cu^{2+} (C) zoom in of panel B in range 970-1070 m/z (D) mixture of $Aβ_{1-16}$, 1 equiv. of Zn^{2+} and 0.9 equiv. of Cu^{2+}, that was treated with 1 equiv. of **AD2** and 1 equiv. of **PT1** for 1 hour (E) zoom in of panel B in range 970-1070 m/z

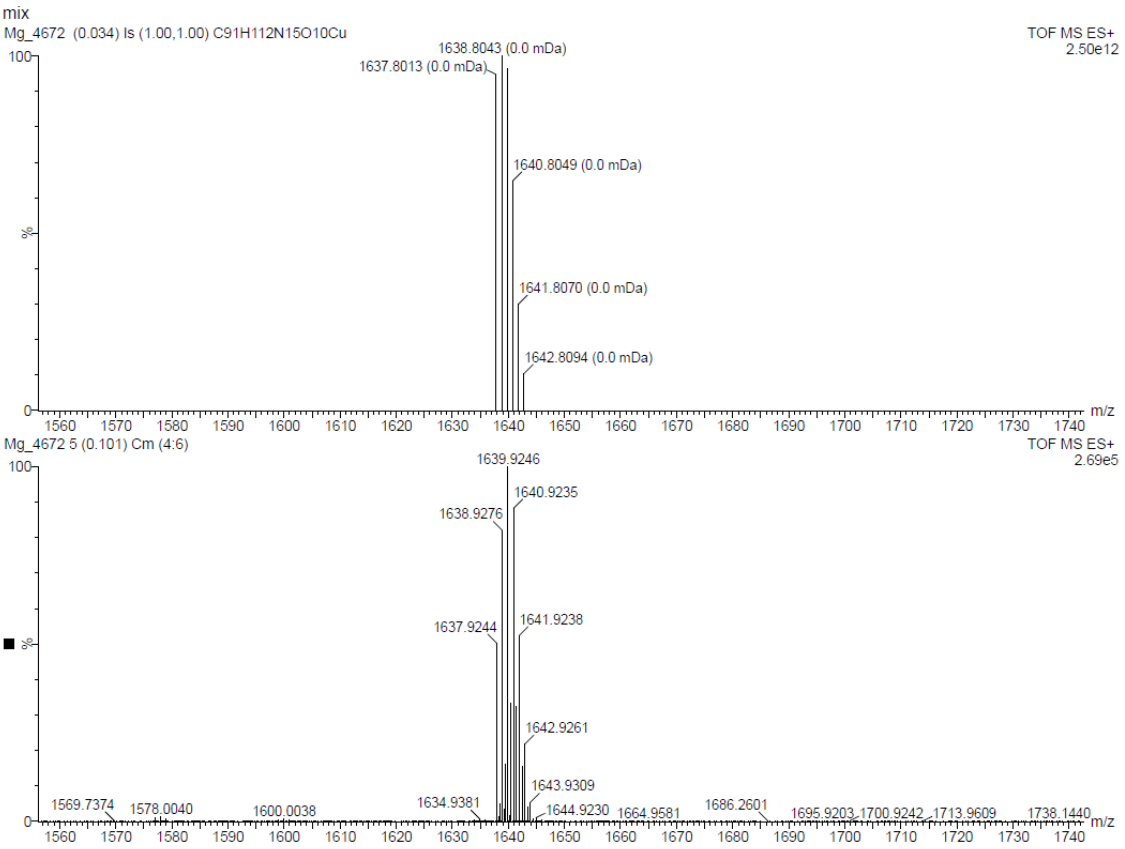

Fig. S23 ESI-MS m/z traces of Cu(II)**AD2** from Fig. S32D (bottom) and calculated ESI-MS spectrum (top)

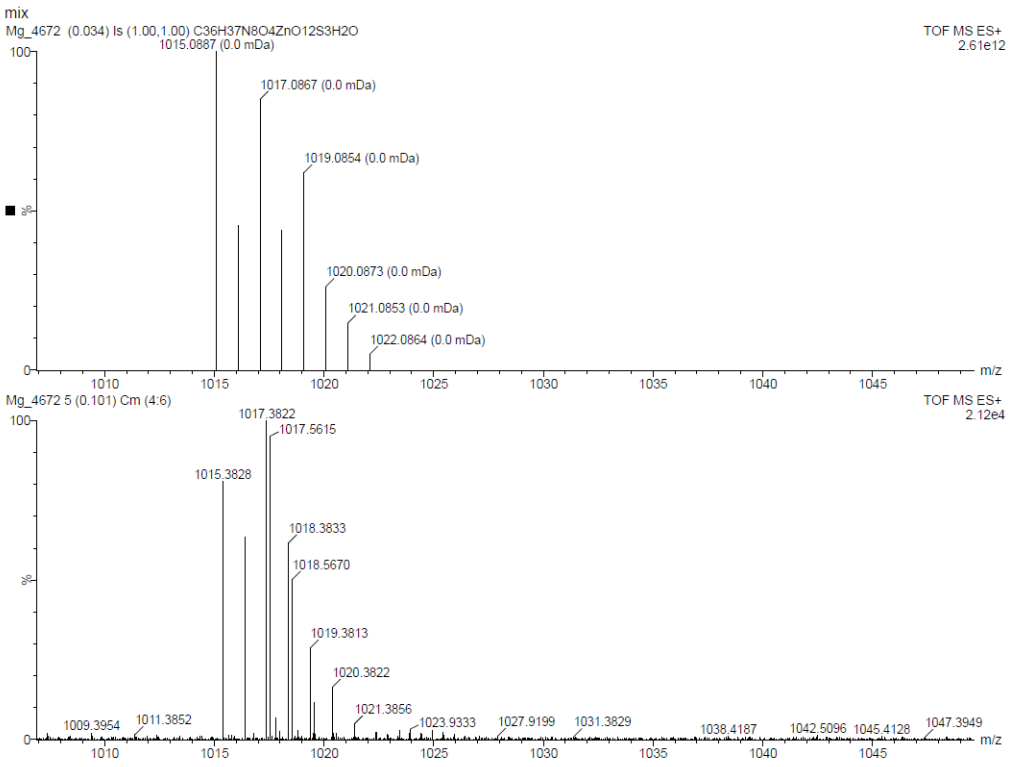

Fig. S24 ESI-MS m/z traces of Zn(II)**PT1** from Fig. S32D (bottom) and calculated ESI-MS spectrum (top)

CD data for Cu^{2+} and Zn^{2+} extraction from Cu,Zn-Aβ complex by cocktail mixture of AD2 and PT1

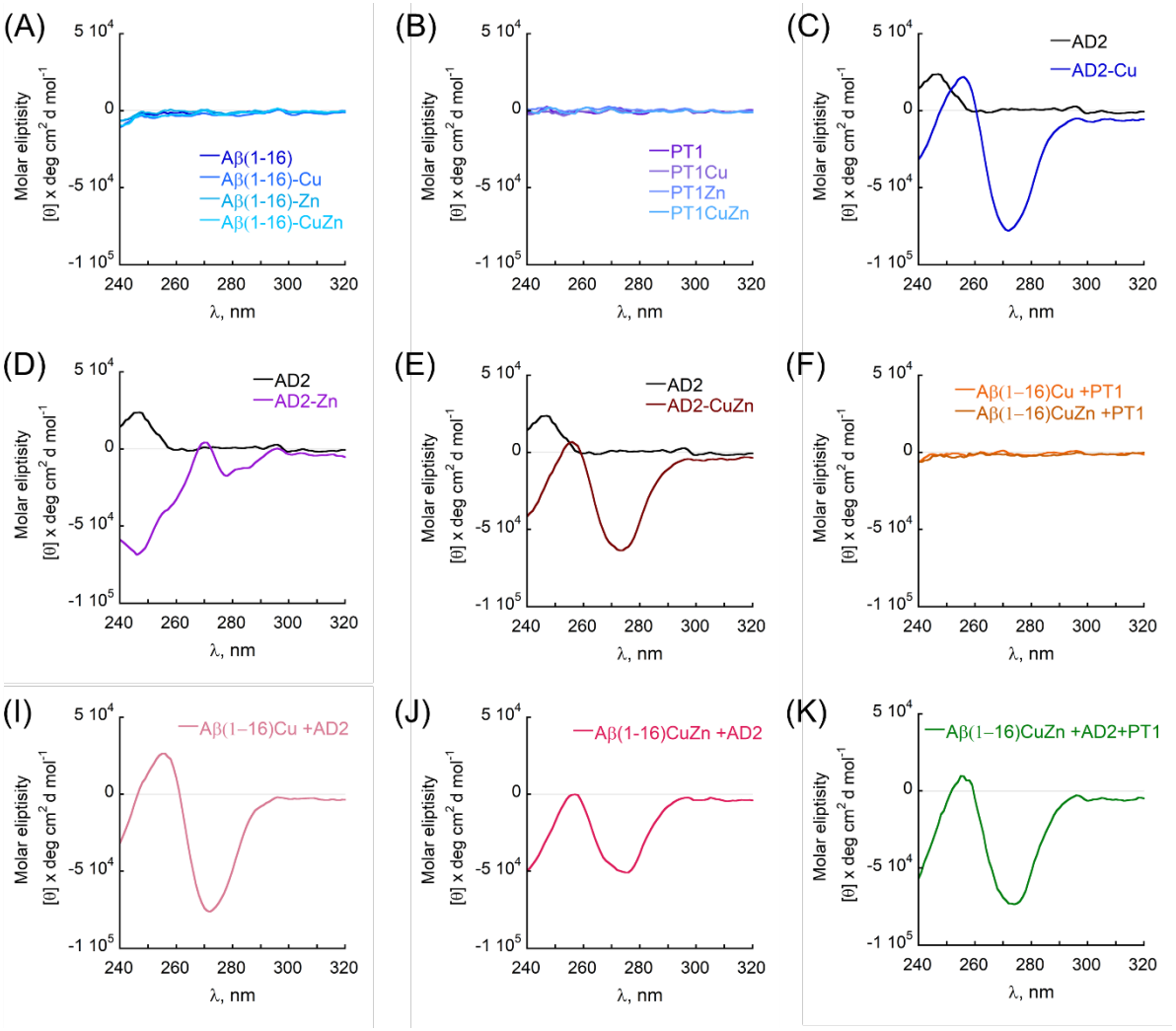

Fig. S25 CD spectra of (A) $Aβ_{1-16}$ and its metal complexes (B) **PT1** and it's metal complexes (C) **AD2** and its Cu^{2+} complex (D) **AD2** and its Zn^{2+} complex (E) mixture of 0.9 equiv. of Cu^{2+} and 1 equiv. of Zn^{2+} with 1 equiv. of **AD2** (F) mixture of 1 equiv. of $CuAβ_{1-16}$ or $CuZnAβ_{1}$-16,1 hour after addition of 1 equiv. of **PT1**(I) mixture of 1 equiv. of $CuAβ_{1-16}$ 1 hour after addition of 1 equiv. of **AD2** (J) mixture of 1 equiv. of $Aβ_{1-16}$, 1 equiv. of Zn^{2+} and 0.9 equiv. of Cu^{2+}, 1 hour after addition of 1 equiv. of **AD2** (K) mixture of 1 equiv. of $Aβ_{1-16}$, 1 equiv. of Zn^{2+} and 0.9 equiv. of Cu^{2+}, 1 hour after addition of 1 equiv. of **AD2** and 1 equiv. of **PT1** Conditions (A) [HEPES] =10 mM, pH = 7.4, $[Aβ_{1-16}] = [Zn^{2+}] = 100$ μM, $[Cu^{2+}] = 90$ μM; (B,C,D): [HEPES] =10 mM, pH = 7.4 [**AD2**]=[**PT1**]=$[Cu^{2+}]$=$[Zn^{2+}] = 100$ μM (E-K): [HEPES] =10 mM, pH = 7.4 $[Aβ_{1-16}]$=[**AD2**]=[**PT1**] =$[Zn^{2+}] = 100$ μM, $[Cu^{2+}] = 90$ μM.

The principles and detailes of the CD studies of Cu^{2+} and Zn^{2+} extraction from Aβ complex by peptoids were described in our previous work[14]. Similarly, in this study, only the Cu**AD2** complex has a single strong minimum band near 270 nm, while other components either do not have a signal at all in this range, or have a signal of opposite sign (Zn**AD2**). Therefore, obtaining single minimum near 270 nm in competition experiments will clearly indicate the exclusive formation of Cu**AD2**.

Stability of the peptoids at physiological pH and temperature

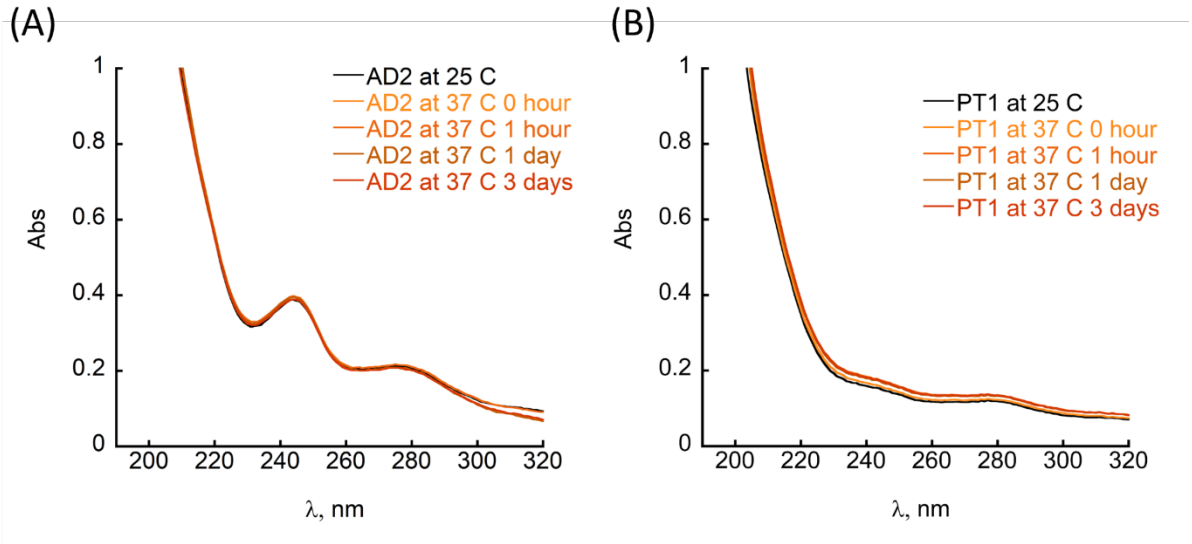

Fig. S26 Stability of the UV-Vis spectra of the peptoid (A) **AD2** and (B) **PT1** in acetate buffer (50 mM, pH 4.0) at 25°C and 37°C with respect to time. Duration of experiment – 72 hours.

n-octanol/water partition coefficient Pow determination by shake-flask method

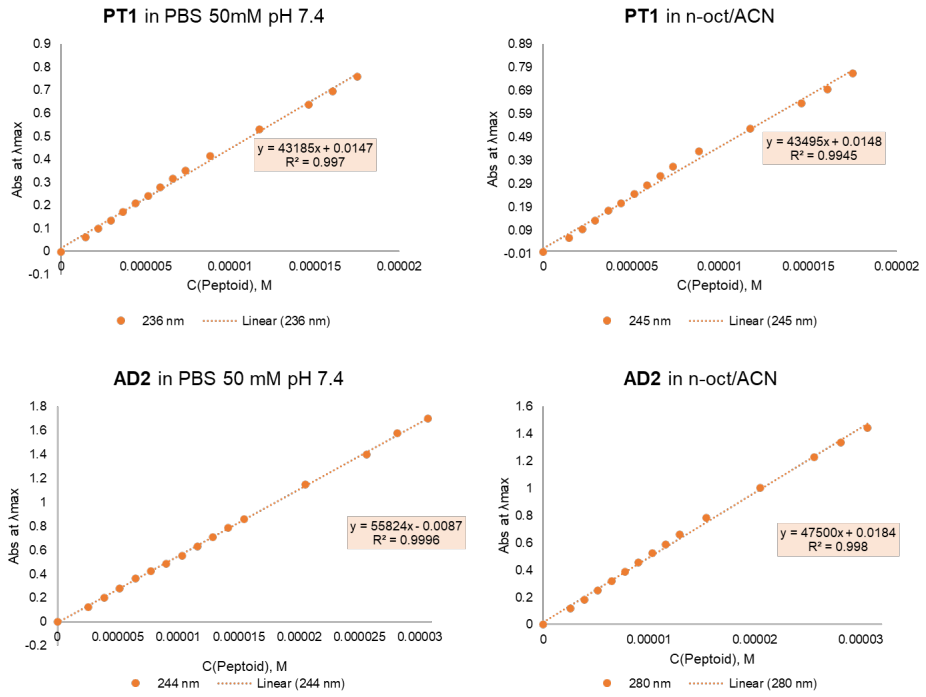

Fig. S27 Calibration curves built from UV-Vis spectra of peptoids **AD2** and **PT1** in PBS (50 mM, pH = 7.4) and n-octanol/ACN (1:1).

#	compound	$C_{average}$(PBS)	$C_{average}$(n-octanol)	$logP_{ow}$	BBB penetration prediction
1	**PT1**	$2.52 \cdot 10^{-6}$ M	$8.34 \cdot 10^{-6}$ M	0.519±0.013	+
2	**AD2**	$1.68 \cdot 10^{-6}$ M	$1.88 \cdot 10^{-5}$ M	1.054±0.049	+

Fig. S28 Partition coeficient $logP_{ow}$ values obtained from the Shake-Flask method. Concentrations of the peptoid remaining in the PBS and the octanol fractions were measured individually by UV/Vis spectroscopy using calibration plots (see Fig. S21) of the absorbance of the peptoid at λ_{max} as function of it's concentration in n-octanol/ACN or in PBS buffer (50 mM, pH=7.4). From these concentrations the ratio K of concentration of peptoid in PBS to concentration in octanol was calculated. $logP_{ow}$ values represented here are derived from 3 independent measurements under identical conditions.

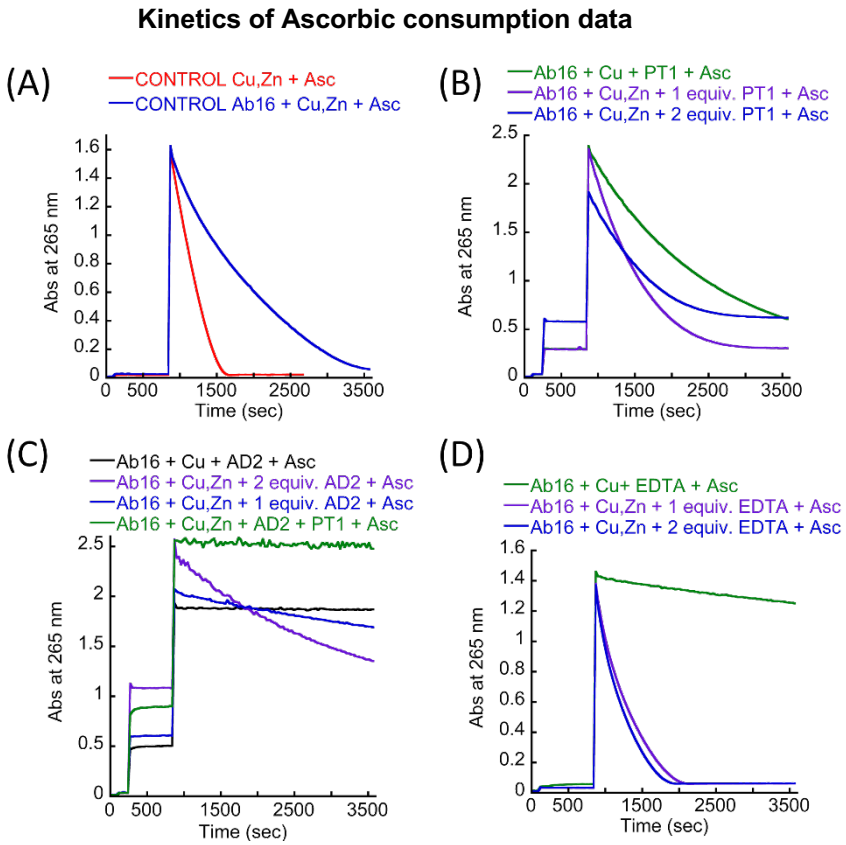

Fig. S29 Kinetics of ascorbate consumption, followed by UV/Visible spectroscopy at 265 nm

(A): Controls Cu^{2+} + Zn^{2+} + Asc (red curve), $A\beta_{1\text{-}16}$ + Cu^{2+} + Zn^{2+} + Asc (blue curve);

(B): $A\beta_{1\text{-}16}$ + Cu^{2+} + **PT1** + Asc (green curve), $A\beta_{1\text{-}16}$ + Cu^{2+} + Zn^{2+} + 1 equiv. **PT1** + Asc (purple curve), $A\beta_{1\text{-}16}$ + Cu^{2+} + Zn^{2+} + 2 equiv. **PT1** + Asc (blue curve);

(C): $A\beta_{1\text{-}16}$ + Cu^{2+} + **AD2** + Asc (black curve), $A\beta_{1\text{-}16}$ + Cu^{2+} + Zn^{2+} + 1 equiv. **AD2** + Asc (blue curve), $A\beta_{1\text{-}16}$ + Cu^{2+} + Zn^{2+} + 2 equiv. **AD2** + Asc (purple curve), $A\beta_{1\text{-}16}$ + Cu^{2+} + Zn^{2+} + **AD2** + **PT1** + Asc (green curve);

(D) $A\beta_{1\text{-}16}$ + Cu^{2+} + EDTA + Asc (green curve), $A\beta_{1\text{-}16}$ + Cu^{2+} + Zn^{2+} + 1 equiv. EDTA + Asc (purple curve), $A\beta_{1\text{-}16}$ + Cu^{2+} + Zn^{2+} + 2 equiv. EDTA + Asc (blue curve),

Conditions: [**AD2, PT1,** EDTA (1 equiv.)]=[$A\beta_{1\text{-}16}$] =[Zn^{2+}] = 10 μM, [**AD2, PT1,** EDTA (2 equiv.)] = 20 μM, [Cu^{2+}] = 9 μM, [Asc] = 100 μM, [HEPES] = 50 mM, pH=7.4, with background subtraction of signal at 800 nm.

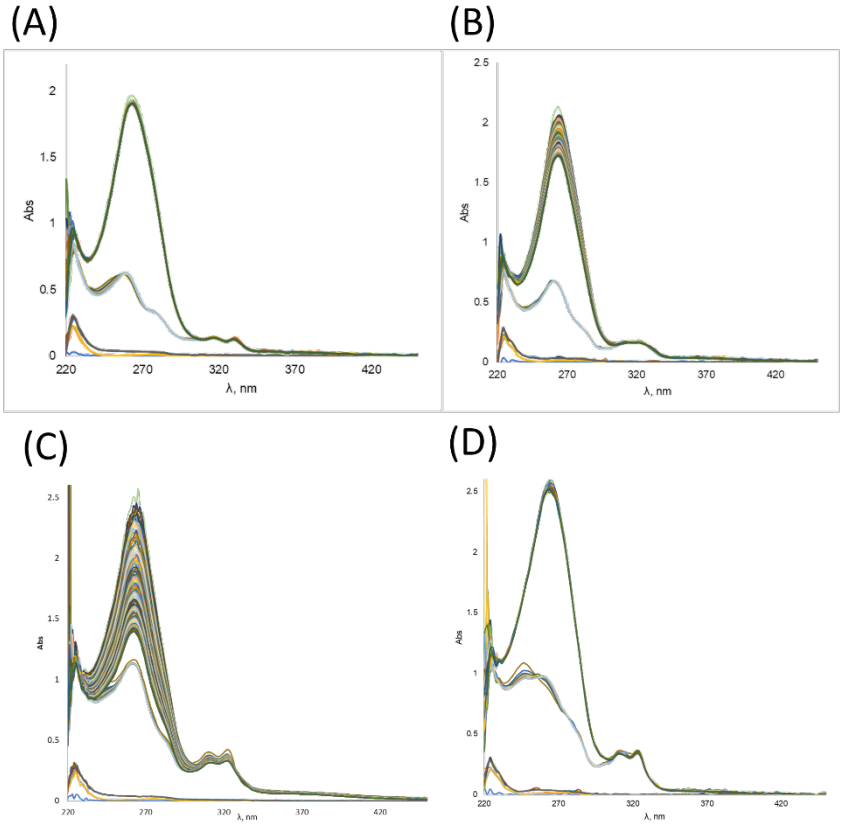

Figure S30. UV-Vis full spectra of kinetics of ascorbic consumption for (A) $A\beta_{1-16}$ + Cu^{II} + **AD2** + Asc (B) $A\beta_{1-16}$ + Cu^{II} + Zn^{II} + 1 equiv. **AD2** + Asc (C) $A\beta_{1-16}$ + Cu^{II} + Zn^{II} + 2 equiv. **AD2** + Asc (D) $A\beta_{1-16}$ + Cu^{II} + Zn^{II} + **AD2** + **PT1** + Asc. Additions were performed at: 31 sec – $A\beta_{1-16}$, 121 s - - Cu^{2+}, Zn^{2+} , 271 sec – **AD2, PT1,** 871 sec - Asc.

Conditions: 1 equiv. of [**AD2**] = [**PT1**] = [$A\beta_{1-16}$] =[Zn^{II}] = 10 μM, 2 equiv. of [**AD2**] = 20 μM, [Cu^{II}] = 9 μM, [Asc] = 100 μM, [HEPES] = 50 mM, pH=7.4, with subtraction of the background signal at 800 nm.

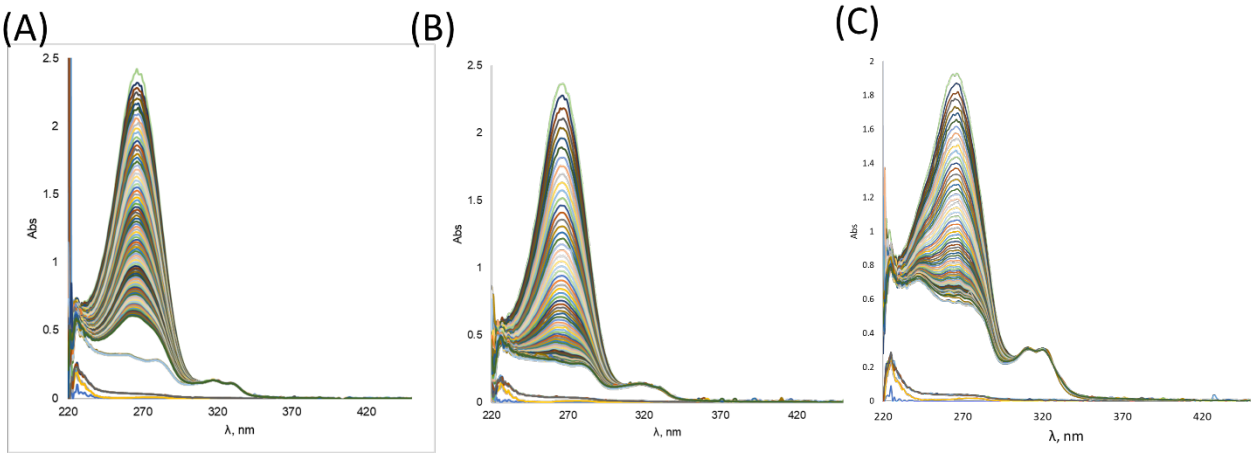

Figure S31. UV-Vis full spectra of kinetics of ascorbic consumption for (A) $A\beta_{1-16}$ + Cu^{II} + **PT1** + Asc (B) $A\beta_{1-16}$ + Cu^{II} + Zn^{II} + 1 equiv. **PT1** + Asc. (C) (B) $A\beta_{1-16}$ + Cu^{II} + Zn^{II} + 2 equiv. **PT1** + Asc. Additions were performed at: 31 sec – $A\beta_{1-16}$, 121 s - - Cu^{2+}, Zn^{2+} , 271 sec – **PT1,** 871 sec - Asc

Conditions: 1 equiv. of [**PT1**] = [$A\beta_{1-16}$] =[Zn^{II}] = 10 μM, , 2 equiv. of [**PT1**] = 20 μM [Cu^{II}] = 9 μM, [Asc] = 100 μM, [HEPES] = 50 mM, pH=7.4, with subtraction of the background signal at 800 nm.

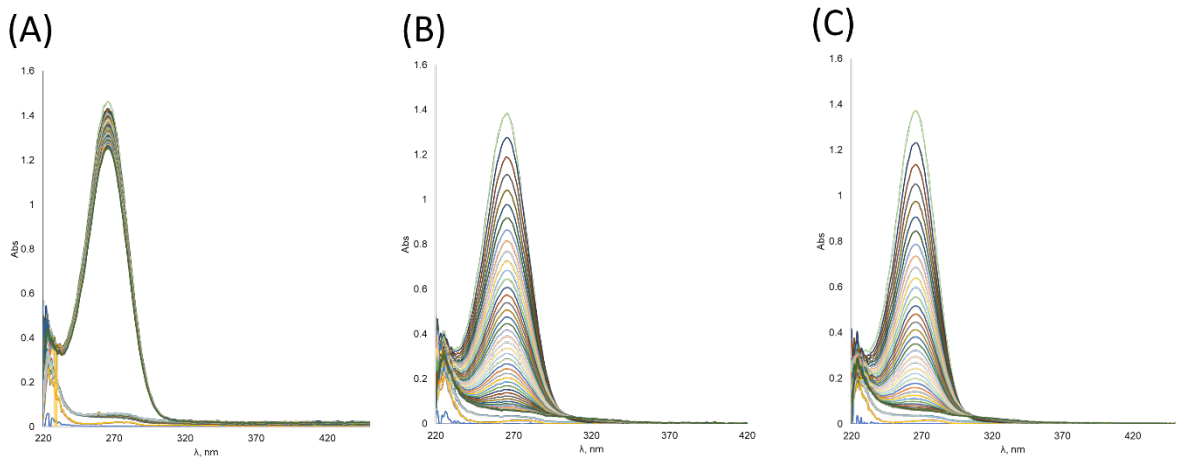

Figure S32. UV-Vis full spectra of kinetics of ascorbic consumption for (A) $A\beta_{1\text{-}16}$ + Cu^{II} + EDTA + Asc (B) $A\beta_{1\text{-}16}$ + Cu^{II} + Zn^{II} + 1 equiv. EDTA + Asc. (C) (B) $A\beta_{1\text{-}16}$ + Cu^{II} + Zn^{II} + 2 equiv. EDTA + Asc. Additions were performed at: 31 sec – $A\beta_{1\text{-}16}$, 121 s - - Cu^{2+}, Zn^{2+} , 271 sec – EDTA**,** 871 sec - Asc

Conditions: 1 equiv. of [EDTA] = [$A\beta_{1\text{-}16}$] =[Zn^{II}] = 10 μM, , 2 equiv. of [EDTA] = 20 μM [Cu^{II}] = 9 μM, [Asc] = 100 μM, [HEPES] = 50 mM, pH=7.4, with subtraction of the background signal at 800 nm.

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