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Electronic Supplementary Information.

Copper(II) coordination properties of the Aβ(1-16)$_2$ peptidomimetic: an experimental evidence of intermolecular macrochelate complex species in the Aβ dimer.

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ESI-MS investigations of Copper(II) distribution among the various mono- and polynuclear species.

We used ESI Mass spectrometry as the method for the assessment and elucidation of copper binding and stoichiometry of the metal complexes of the Aβ(1-16)$_2$ peptide. High resolution ESI-MS spectra (FT-MS) were carried out at different metal to peptide ratios and different pH values. A part of the FT-MS spectrum obtained at 1:1 metal to ligand ratio and at pH 7.0 is reported in **Fig. S1.a** as an example. Stoichiometries were assigned by comparison of the experimental (**Fig. S1.b and S1.c)** and simulated isotopic distribution pattern (**Fig. S1.d and S1.e)**. Absolute intensity values of m/z signals corresponding to the complex species identified at pH 7.0, were deduced by peak lists of each spectrum (**Table S1**) and reported in **Table S2**. The relative oligomer species intensity were then computed by dividing the sum of the intensity of a given complex species by the sum of the intensities of all Aβ(1-16)$_2$ containing ions (i.e. relative mononuclear species at 1:1 metal to peptide ratios is: [$7357012_{(P+Cu)}$ + $1978162,1_{(P+Cu+Na)}$ + $3613334,3_{(P+Cu+K)}$ + $1022971_{(P+Cu+2K)}$] / [$2660867_{(P)}$ + $585871,3_{(P+Na)}$ + $1066661_{(P+K)}$ + $7357012_{(P+Cu)}$ + $1978162,1_{(P+Cu+Na)}$ + $3613334,3_{(P+Cu+K)}$ + $1022971_{(P+Cu+2K)}$ + $7751906.0_{(P+2Cu)}$ + $2033766,9_{(P+2Cu+Na)}$ + $3663851,8_{(P+2Cu+K)}$ + $575625,52_{(P+2Cu+Na+K)}$+ $863438,28_{(P+3Cu)}$] = 0,46; P indicates the Aβ(1-16)$_2$ peptide). The relative percentage of all the oligonuclear species formed at different metal to peptide ratios and at pH 7,0 are reported in **Table S3**. Absolute intensity values of m/z signals corresponding to the complex species identified at pH 8,5 and the relative percentage of all the oligonuclear species formed at different metal to peptide ratios were reported in **Table S4** and **Table S5** respectively. The distribution of copper(II) among the various mono- and polynuclear species was reported in **Figure S2** (pH 7.0) and **Figure S3** (pH 8.5).

Evidences of Cu(I) coordination by ESI-MS measurements

As already explained in this work, dinuclear species are characterized by copper(II) binding to the N-terminal binding sites of peptide fragments while macrochelate coordination by interchain imidazole moieties may occur only in trinuclear complexes. ESI-MS spectra reported in this work provided some evidences of Cu(I) coordination when macrochelate complex species form in Aβ(1-16)$_2$. This is best represented by isotopic distribution of peaks corresponding to dinuclear (m/z 608.035) and trinuclear (m/z 623.514) species of Cu(II)–Aβ(11-16)$_2$ system reported in **Fig. S5.a**. In trinuclear species, the measured isotopic profile (**Fig. S5.b**) overlaids with the theoretical isotopic pattern (**Fig. S5.c**) only when the complexes formula corresponding to [Aβ(11-16)$_2$+2Cu(II)+Cu(I)+H]$^{4+}$ (blue isotopic pattern) and [Aβ(11-16)$_2$+3Cu(II)]$^{4+}$ (red isotopic pattern) are considered. This result strongly suggests that a fraction of the metal ions, in trinuclear species, are coordinated as Cu(I). On the other hand, the isotopic distribution of the dinuclear species (**Fig. S5.d**) was quite similar to the theoretical isotopic distribution of [Aβ(11-16)$_2$ + 2Cu(II)]$^{4+}$ (**Fig. S5.e**) suggesting that all copper ions are coordinated as Cu(II). It was not clear how Cu(I) complexes can form in trinuclear species. Reduction of Cu(II) to Cu(I) may occur in the mass spectrometer because generation of ions in ESI source are related to an electrochemical process. Nevertheless, only when the reduced metal ions form stable complexes, it reasonable to suppose that they can be detected by mass spectrometer. Therefore, the data reported here argue for a Cu(I) coordination only when interchain imidazole macrochelates are involved in the metal coordination. The Aβ(11-16)$_2$ fragment possesses two high affinity copper(II) binding sites at its N-terminal side. The presence of His residue at the third position is the main feature explaining this high affinity. It can also figure out that the third copper ion may be engaged with the two His residues at position 14. This situation may reproduce what reported by Shearer et Szalai who hypothesized a linear coordination environment for Cu(I) generated by the two histidine 13 and 14.[50]

Figures

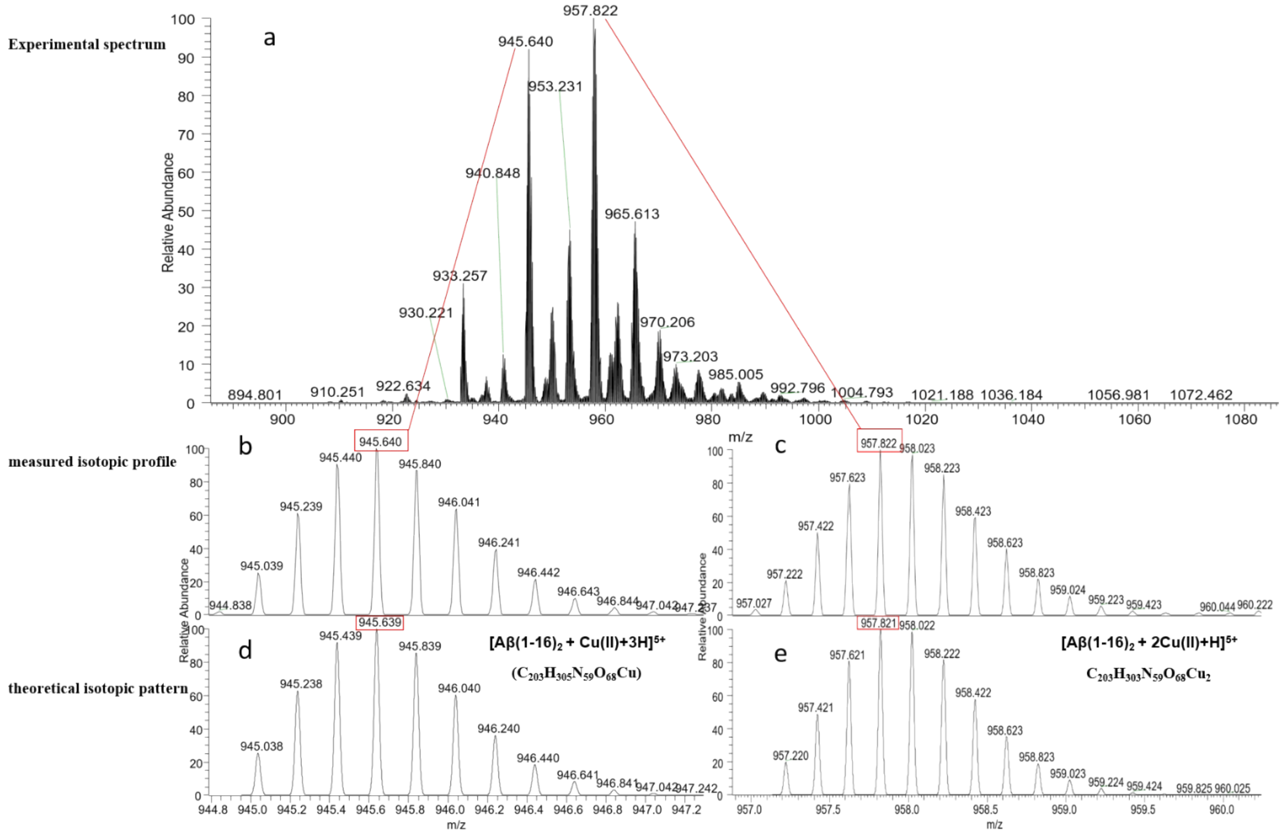

Fig. S1. **a)** ESI Mass spectrum of Cu(II)–Aβ(1-16)$_2$ system; **b)** isotopic distribution of peak corresponding to $[A\beta(1\text{-}16)_2 +Cu(II)+3H]^{5+}$ (m/z 945.640); isotopic distribution of peak corresponding to $[A\beta(1\text{-}16)_2 +2Cu(II)+H]^{5+}$ (m/z 957.822); **d)** theoretical isotopic distributions of $[A\beta(1\text{-}16)_2 +Cu(II)+3H]^{5+}$ **e)** theoretical isotopic distribution of $[A\beta(1\text{-}16)_2 + 2Cu(II)+H]^{5+}$.

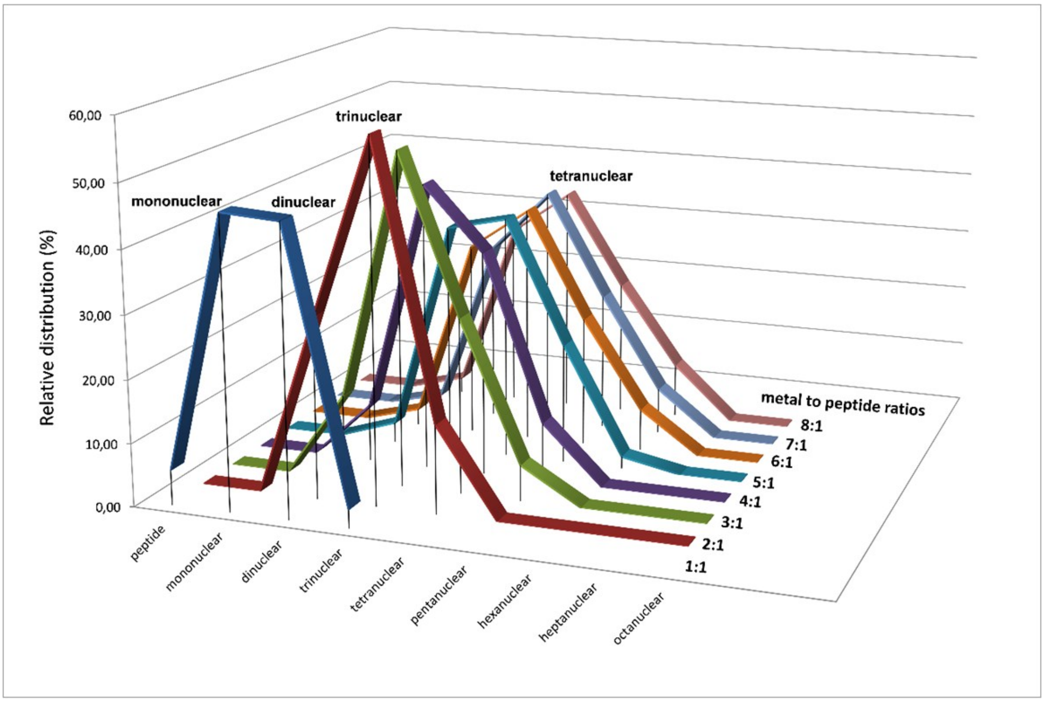

Fig. S2. Relative distribution of oligonuclear copper(II) complex species of the Cu(II)–Aβ(1-16)$_2$ system at different metal to ligand ratios and pH 7.0.

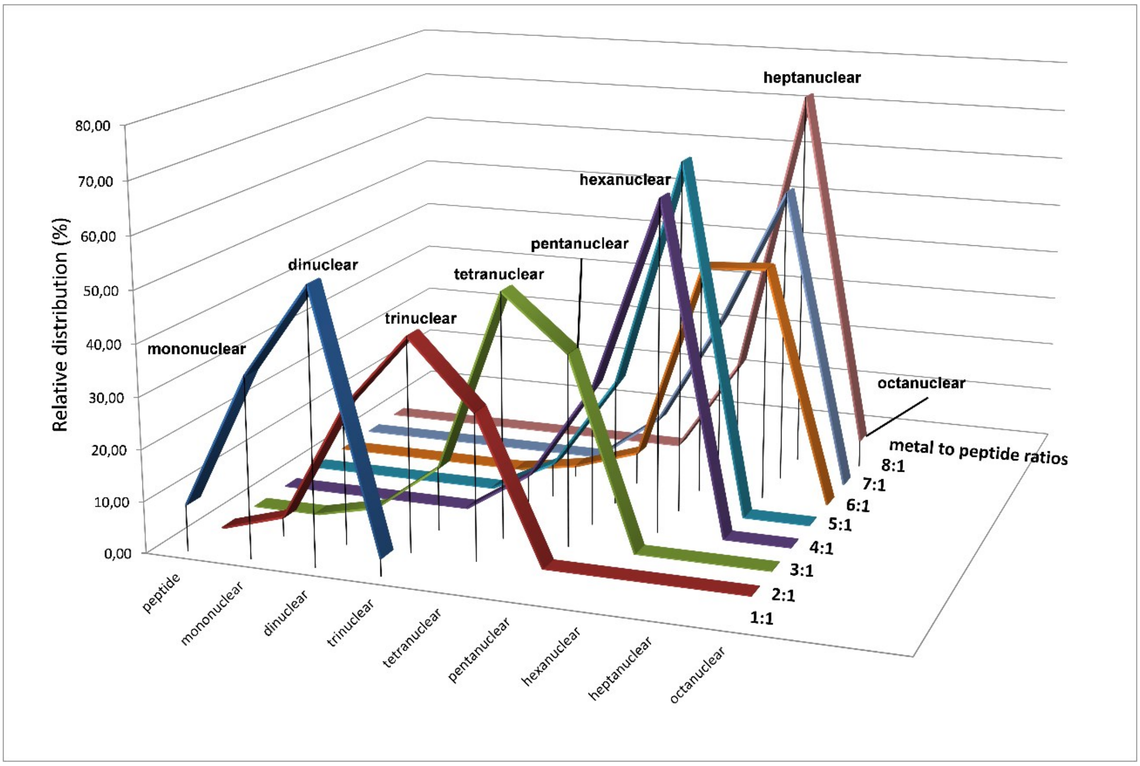

Fig. S3. Relative distribution of oligonuclear copper(II) complex species of the Cu(II)–Aβ(1-16)$_2$ system at different metal to ligand ratios and pH 8.5.

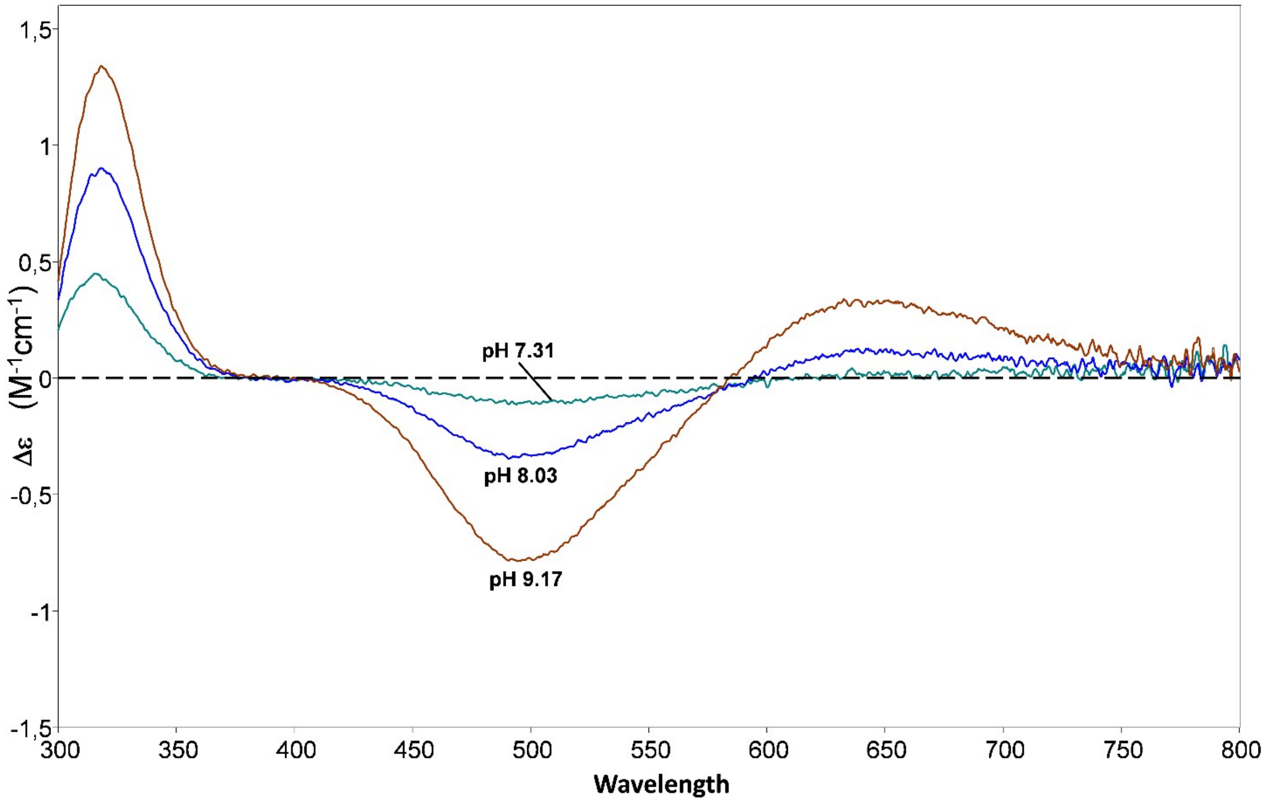

Fig. S4. CD spectra of the copper(II)–Aβ(1-16) system at 1:1 metal to peptide ratios and different pH values (see main text).

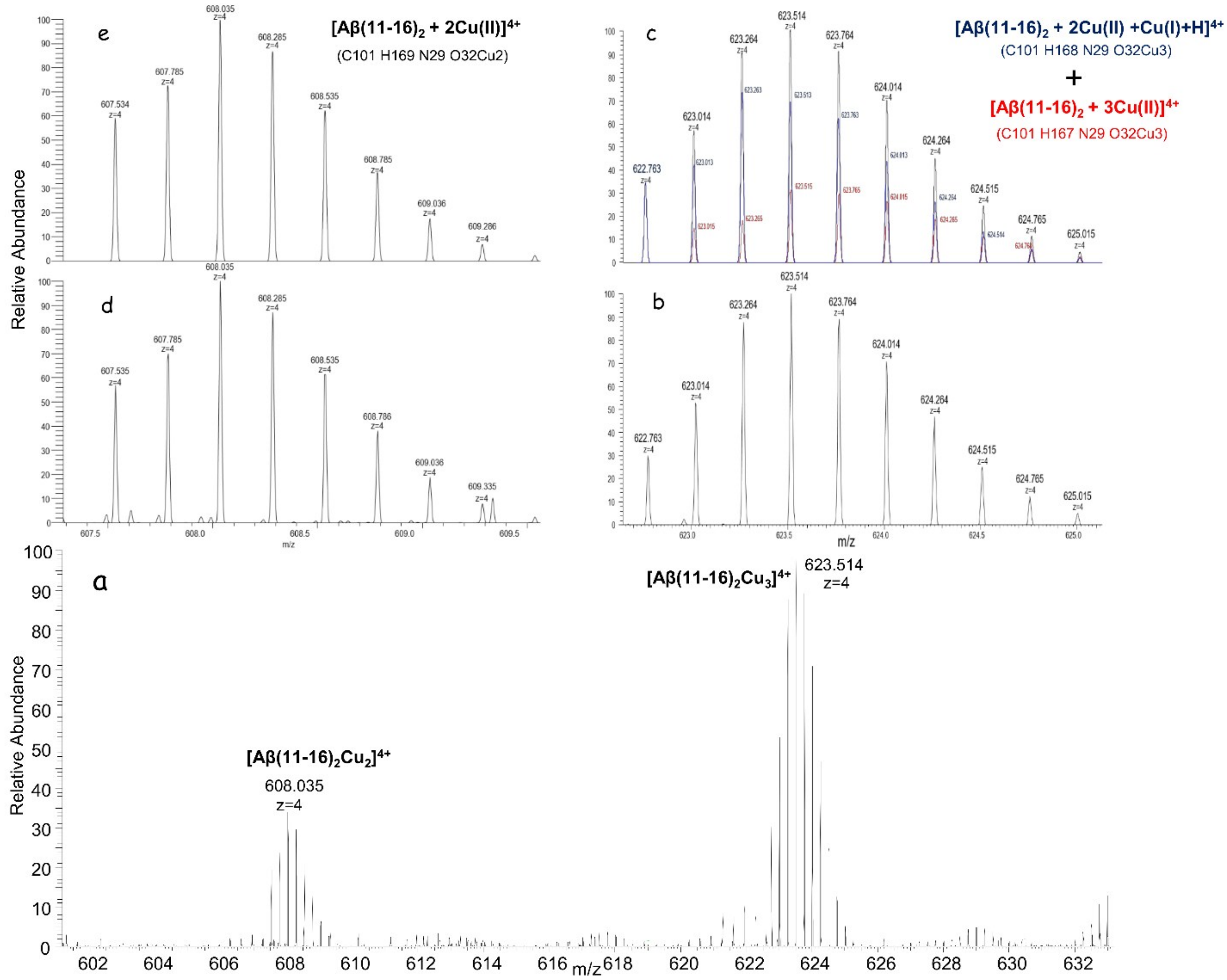

Fig. S5. ESI Mass spectrum of Cu(II)–Aβ(11-16)$_2$ system; **a)** isotopic distribution of peak corresponding to dinuclear (m/z 608.035) and trinuclear (m/z 623.514) specie; **b)** enlargement of the isotopic distribution of peak at m/z 623.514; **c)** sum of the theoretical isotopic distributions of [Aβ(11-16)$_2$ +2Cu(II)+Cu(I)+H]$^{4+}$ and [Aβ(11-16)$_2$+3Cu(II)]$^{4+}$ complexes; **d)** enlargement of isotopic distribution of peak at m/z 608.035; **e)** theoretical isotopic distribution of [Aβ(11-16)$_2$ + 2Cu(II)]$^{4+}$.

Table S1. Part of peaks list extracted by ESI-MS spectrum of Cu(II)–Aβ(1-16)$_2$ system recorded at 1:1 metal to ligand ratios and pH=7.0.

Peaks list spectrum of Cu–Aβ(1-16)$_2$ 1:1 pH=7.0		
m/z	**Abs. Intensity**	**Mass formula**
945.039	1848149.0	
945.239	4464235.5	
945.440	6656572.0	
945.640	7357012.0	**($C_{203}H_{305}N_{59}O_{68}Cu$)** [Aβ(1-16)$_2$ + Cu(II)+3H]$^{5+}$
945.840	6452420.0	
946.041	4683431.0	
946.241	2887001.8	
-	-	
957.822	7751906.5	**($C_{203}H_{303}N_{59}O_{68}Cu_2$)**

		$[A\beta(1\text{-}16)_2 + 2Cu(II){+}H]^{5+}$
958.023	7698726.0	
958.223	6603920.5	
958.423	4738633.5	
958.623	3106493.8	
958.823	1745409.9	
-	-	

Table S2. Absolute intensity values of m/z signals corresponding to the complex species identified in the Cu(II)–$A\beta(1\text{-}16)_2$ system at pH=7.0 and at different metal to ligand ratios.

	Metal to peptide ratios							
Species [$P{=}A\beta(1\text{-}16)_2$]	**1:1**	**2:1**	**3:1**	**4:1**	**5:1**	**6:1**	**7:1**	**8:1**
P	2660867							
P+Na	585871,3							
P+K	1066661							
P+2Na								
P+Cu	7357012							
P+Cu+Na	1978162,1							
P+Cu+K	3613334,3							
P+Cu+2Na								
P+2Cu	7751906.0	4073753,5	1651967,6	1420040,6	482334,6	342973,8	298689,5	338410,7
P+Cu+2K	1022971							
P+2Cu+Na	2033766,9	1017496,1	376892	278764,6				
P+2Cu+K	3663851,8	1782727,5	634028,1	457532,1				
P+2Cu+2Na								
P+2Cu+Na+K	575625,52							
P+2Cu+2K		658657,1						
P+2Cu+2Na+K								
P+3Cu+2Na								
P+3Cu+3Na								
P+3Cu+K		3643295,8	2382080,3	2269656,5	1133305,5	757033,6	685856,6	599045
P+3Cu+K+Na								
P+3Cu+K+2Na								
P+3Cu+2K		734502,3	499285,9	494954,8	279155,3	197129,8	197067,3	166123,8
P+3Cu+K+3Na								
P+4Cu+K+Na			295101,4					
P+4Cu+K		978025,6	1135267,8	1630316	1223313,5	947410,9	951426,3	766854,8
P+4Cu+2K			435370,2	521005,3	285923,7	214700,6	205867,8	211390,1
P+4Cu+K+3Na								
P+4Cu+2K+2Na								
P+4Cu+2K+3Na								
P+3Cu	863438,28	8491568	6145182	6824471	3525234,3	2805991	2720311,8	2916233,3

P+3Cu +Na		2032708,5	1378199,1	1284595,5	678581,8	427733,3	388399,7	349526,5
P+4Cu		2195972,5	3014149,3	4789161,5	3800798,3	3462684	3749622,5	3638685,3
P+4Cu+Na		473494,5	582171,3	776400,7	632010,4	459375,1	447233,8	349137,1
P+4Cu+2Na								
P+4Cu+3Na								
P+5Cu			513611,6	1104123,6	1252665,6	1302812,9	1463009	1159976,6
P+5Cu+Na					214489,5	175088,1	177186,9	119560,1
P+5Cu+K								
P+5Cu+2(NH4)			591698,4	1110758,1	919765	920462,9	1022919,3	1244888,5
P+5Cu+2K					427232,8	386764,4	398473,2	421694,2
P+5Cu+K+Na								
P+5Cu+2Na+K								
P+5Cu+3Na+K								
P+6Cu+2(NH4)					545290,3	654878,3	771382,8	922796,2
P+6Cu					282418,6	237974	234249,5	252562,4

Table S3. Relative percentage of the oligonuclear species of Cu(II)–Aβ(1-16)$_2$ system formed at pH=7.0 and at different metal to peptide ratios.

	Metal to peptide ratios							
Oligonuclear species	**1:1**	**2:1**	**3:1**	**4:1**	**5:1**	**6:1**	**7:1**	**8:1**
Apo-peptide	5,49%	0,00%	0,00%	0,00%	0,00%	0,00%	0,00%	0,00%
mononuclear	46,12%	0,00%	0,00%	0,00%	0,00%	0,00%	0,00%	0,00%
dinuclear	45,56%	28,88%	13,56%	9,08%	3,16%	2,58%	2,18%	2,51%
trinuclear	2,83%	57,14%	52,99%	45,81%	36,82%	31,50%	29,11%	29,95%
tetranuclear		13,98%	27,82%	35,78%	38,95%	38,25%	39,05%	36,90%
pentanuclear		0,00%	5,63%	9,33%	19,22%	20,95%	22,33%	21,89%
hexanuclear		0,00%	0,00%	0,00%	1,85%	6,72%	7,33%	8,73%

Table S4. Absolute intensity values of m/z signals corresponding to the complex species identified in the Cu(II)–Aβ(1-16)$_2$ system at pH=8.5 and at different metal to ligand ratios.

	Metal to peptide ratios							
Species [P=Aβ(1-16)$_2$]	**1:1**	**2:1**	**3:1**	**4:1**	**5:1**	**6:1**	**7:1**	**8:1**
P	446332,90							
P+Na	537226,20							
P+K	185862,20							
P+2Na	366662,50							
P+Cu	1476230,90	82735,2						
P+Cu+Na	2285311,00	132565,5						
P+Cu+K	649757,20	126982,2						
P+Cu+2Na	1610747,60	110556,1						

P+2Cu	1827496,60	338577,5	43139,8					
P+2Cu+Na	2115700,00	465258,5	74398,8					
P+2Cu+K	646812,30	423620,9	37638,3					
P+2Cu+2Na	1792428,30	414155,5	69504,8					
P+2Cu+Na+K	973907,00	759901,9						
P+2Cu+3Na	963047,60							
P+2Cu+2Na+K	795099,60	867173,5						
P+3Cu+2Na	363882,20	750751,1	322153					
P+3Cu+3Na	198920,40							
P+3Cu+K		731324,2	165380,5					
P+3Cu+K+Na		1044711,8						
P+3Cu+K+2Na		989165,4						
P+3Cu+2K+Na		784315,9						
P+3Cu+K+3Na		760772,8						
P+4Cu+K+Na		867110,5	637419,8					
P+4Cu+K+2Na		714188,4	771582,6					
P+4Cu+2K+Na		544392,9						
P+4Cu+K+3Na		522130				196732,7		
P+4Cu+2K+2Na		475720,8						
P+4Cu+2K+3Na		340364,2						
P+3Cu			175046,2					
P+3Cu +Na			320581,5					
P+4Cu			435426,2	81519,9	155495,9			
P+4Cu+Na			668001,9	147492,3	267757,4			
P+4Cu+2Na			687758,7	140035,4	240334,3			
P+4Cu+3Na			600128,4					
P+5Cu				270800,2	615576,9	310566,8	180109,1	
P+5Cu+Na				478421,7	1083403,5	270151,8	269762,2	
P+5Cu+K								
P+5Cu+2Na			711498,8	479901,8	821524,3		217925,3	
P+5Cu+3Na			550607,4					
P+5Cu+K+Na			518999,8					
P+5Cu+2Na+K			509534,4					
P+5Cu+3Na+K			398734,7					
P+5Cu+4Na+K			266374,6					
P+6Cu				454941,5	1110251	1636637	598289,7	164898,2
P+6Cu+Na				589561,9	1659970	1504961	873598,6	316479,8
P+6Cu+K								
P+6Cu+2Na				556598,9	1625305,8	1076592	763996,7	323498,1
P+6Cu+3Na				421549,2	1200009,1			
P+6Cu+Na+K				345666,3				
P+6Cu+2Na+K				310103,8	840720,6			
P+6Cu+3Na+K				222743,9	603936,9			
P+7Cu						1369541	839399,7	448278,5
P+7Cu+Na						934940,4	853377,8	580427,5
P+7Cu+K						641197,5		

P+7Cu+2Na						568045,9	676027,1	541476,7
P+7Cu+Na+K						500301,6	537576,4	
P+7Cu+3Na							511504,8	497939,8
P+7Cu+2Na+K						275522,2	423742,4	471980,3
P+7Cu+3Na+K							288904,6	364161,5
P+7Cu+4Na+K								300149,4
P+8Cu								
P+8Cu+Na								
P+8Cu+K								
P+8Cu+2Na								
P+8Cu+Na+K								226627,9

Table S5. Relative percentage of the oligonuclear species of Cu(II)–Aβ(1-16)$_2$ system formed at pH=8.5 and at different metal to peptide ratios.

	Metal to peptide ratios							
Oligonuclear species	**1:1**	**2:1**	**3:1**	**4:1**	**5:1**	**6:1**	**7:1**	**8:1**
peptide	8,91%	0,00%	0,00%	0,00%	0,00%	0,00%	0,00%	0,00%
mononuclear	34,94%	3,70%	0,00%	0,00%	0,00%	0,00%	0,00%	0,00%
dinuclear	52,88%	26,69%	2,82%	0,00%	0,00%	0,00%	0,00%	0,00%
trinuclear	3,27%	41,33%	12,35%	0,00%	0,00%	0,00%	0,00%	0,00%
tetranuclear		28,28%	47,72%	8,20%	6,49%	2,12%	0,00%	0,00%
pentanuclear			37,11%	27,32%	24,65%	6,25%	9,49%	0,00%
esanuclear				64,48%	68,86%	45,43%	31,79%	19,00%
eptanuclear						46,20%	58,72%	75,65%
ottanuclear								5,35%