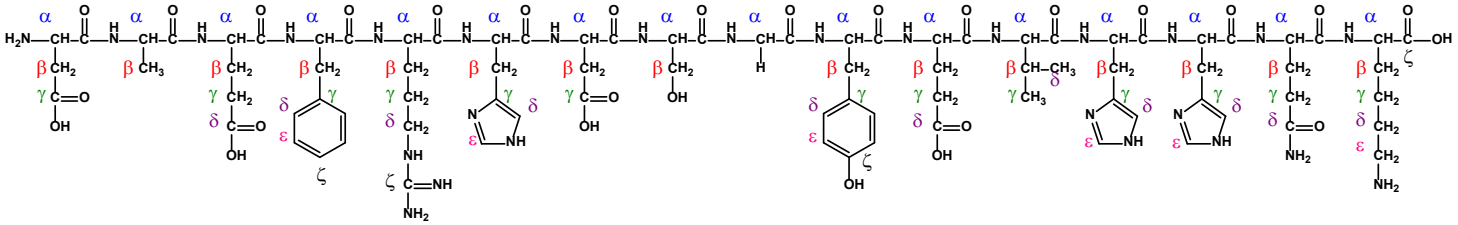
pH-dependent Cu(II) Coordination to Amyloid-β Peptide: Impact of Sequence Alterations, Including the H6R and D7N Familial Mutations.

Bruno Alies, Hélène Eury, Christian Bijani, Lionel Rechignat, Peter Faller and Christelle Hureau

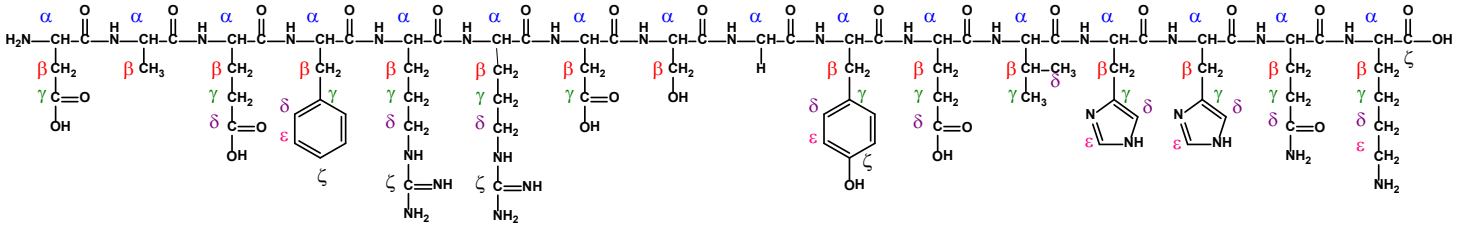
CNRS ; LCC (Laboratoire de Chimie de Coordination) ; 205, route de Narbonne, F-31077 Toulouse, France. Université de Toulouse; UPS, INPT ; LCC ; F-31077 Toulouse, France

Supporting Information.

Table of contents.



Scheme S1. Aβ16 peptide sequence with the atom identifiers of each amino-acid residue.



Scheme S2. H6R-Aβ peptide sequence with the atom identifiers of each amino-acid residue.

EPR data

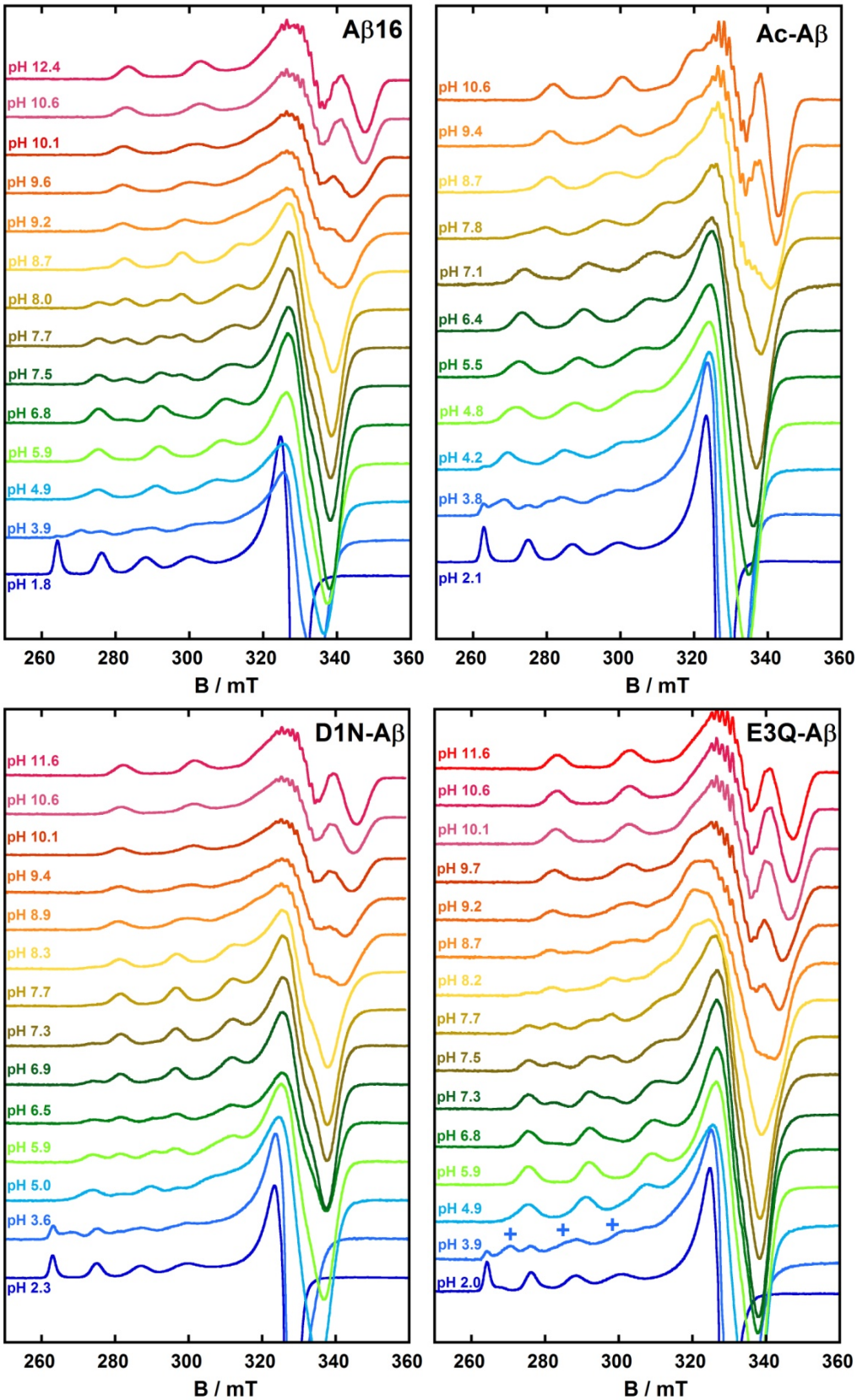


Figure S1. EPR spectra of [Cu^{II}(peptide)] complexes as a function of pH; peptide = Aβ, Ac-Aβ, D1N-Aβ and E3Q-Aβ. [Cu^{II}(peptide)] = 1 mM in D_2O. ν = 9.5 GHz, amplitude modulation = 0.5 mT, microwave power = 20 mW. T = 110 K.

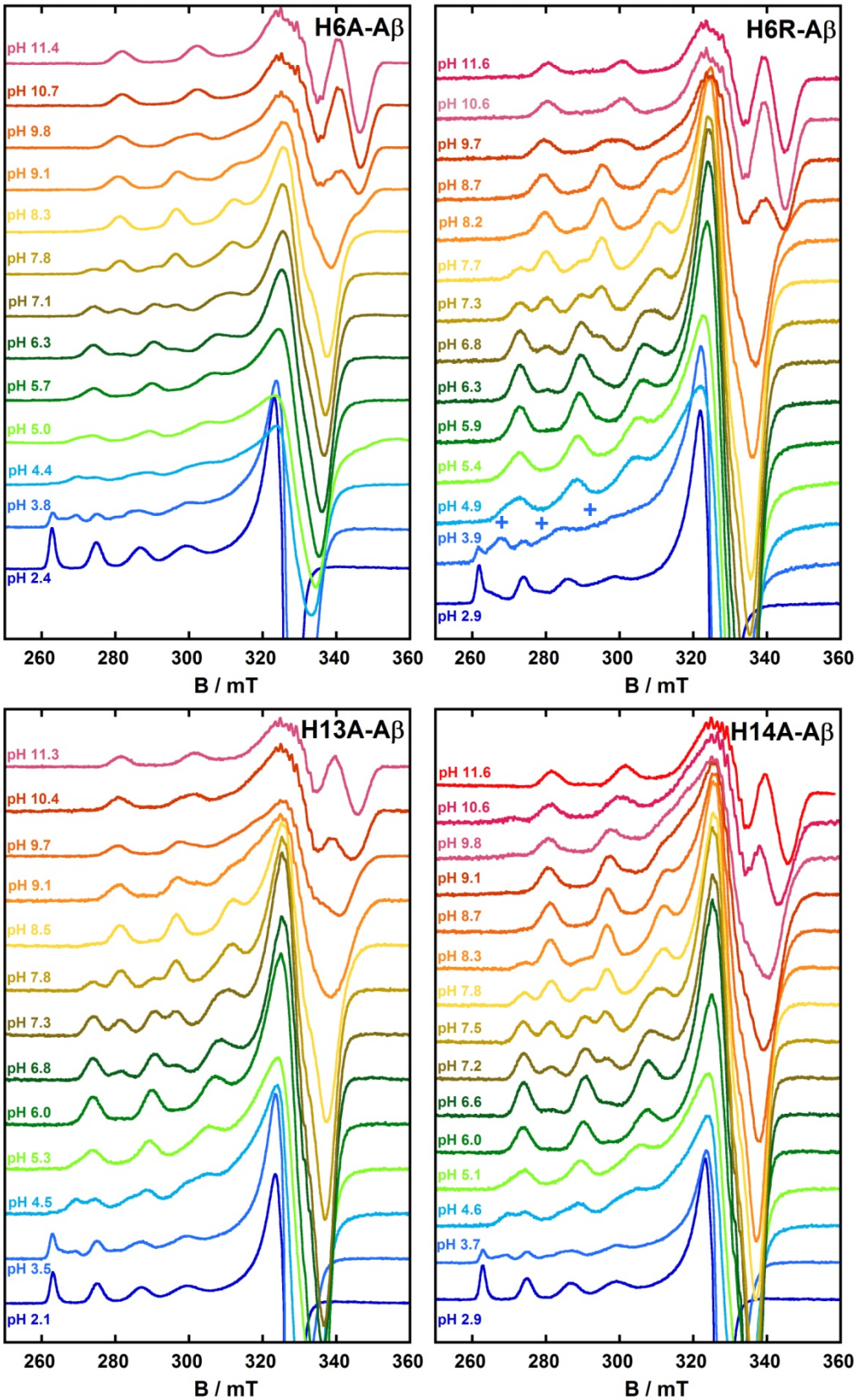


Figure S2. EPR spectra of [Cu^{II}(peptide)] complexes as a function of pH; peptide = H6A-Aβ, H6R-Aβ, H13A-Aβ and H14A-Aβ. [Cu^{II}(peptide)] = 1 mM in D_2O. ν = 9.5 GHz, amplitude modulation = 0.5 mT, microwave power = 20 mW. T = 110 K.

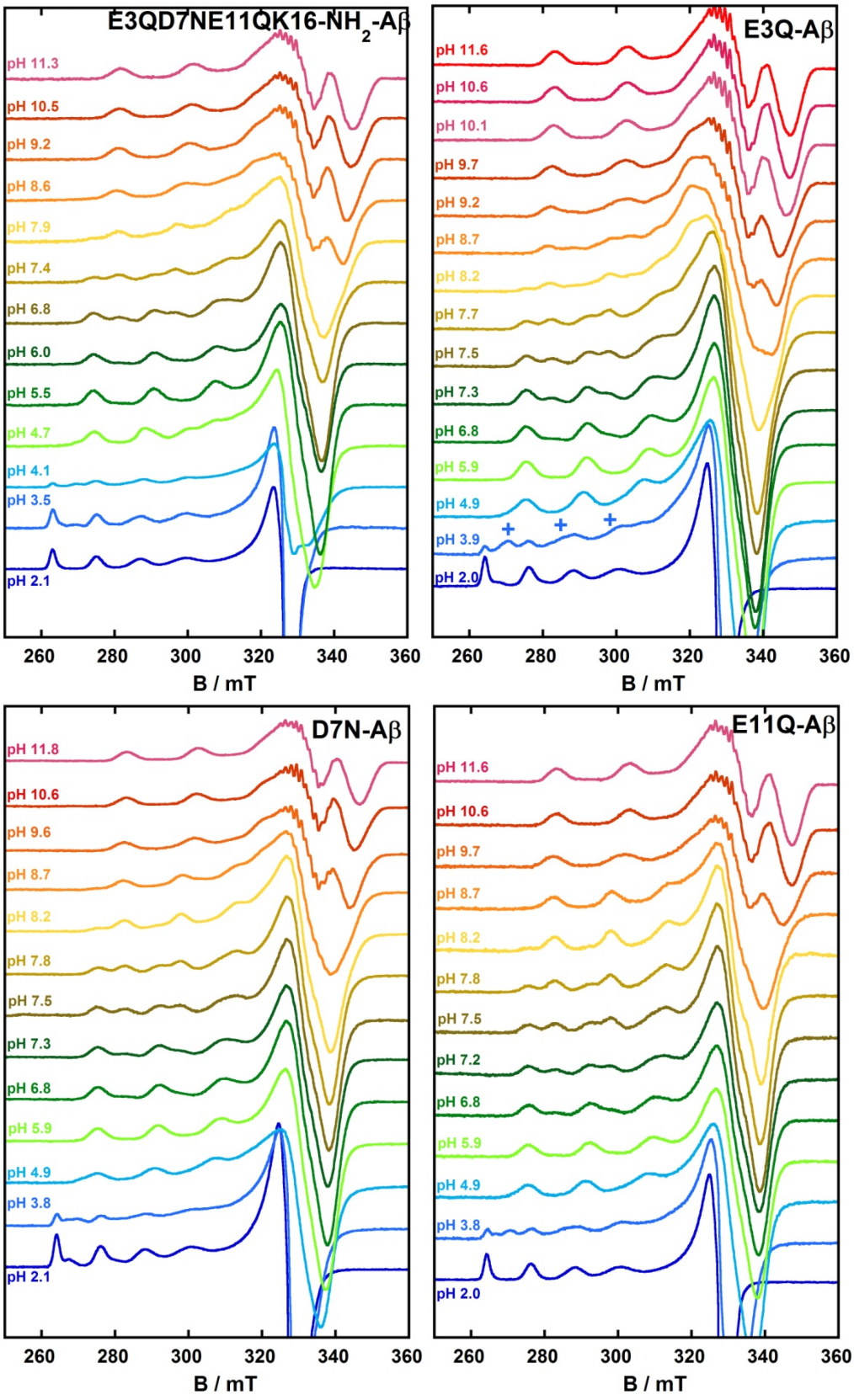


Figure S3. EPR spectra of [Cu^{II}(peptide)] complexes as a function of pH; peptide = E3QD7NE11QK16-NH_2-Aβ, E3Q-Aβ, D7N-Aβ and E11Q-Aβ. [Cu^{II}(peptide)] = 1 mM in D_2O. ν = 9.5 GHz, amplitude modulation = 0.5 mT, microwave power = 20 mW. T = 110 K.

In the EPR spectra of the [Cu^{II}(E3Q-Aβ)] complexes, a new signal with with $g_{//}$ = 2.17(8) and $A_{//}$ = 160 10^{-4} cm^{-1} EPR parameters is detected near pH 8.5-9.0. To ensure that this newly detected species is not an artifact due to impurity in the peptide batch we double purified it on fplc column. Complexes obtained with the doubly purified peptide display the same EPR signatures. Hence, it was concluded that this new species corresponds to a slightly distorted component **III**, the EPR parameters of which are characteristic of distorted Cu(II) binding site,[1] a feature rarely encountered in Cu(II) peptidic complexes. Such unusual Cu(II) geometry maybe due to constraints caused by the E3Q mutation. We conjecture that the probable Glu3-Arg5 salt bridge is broken in the E3Q mutant and replaced by the Arg5-Asp7 salt brige that would induce constraint regarding the binding of His6 residue in component **III**. This assumption is reinforced by the fact that this new species is not detected with the triple E3QD7NE11QK16-NH_2 mutant, in which the Arg5-Asp7 bridge cannot be formed. Further confirmation of this assumption is needed but beyond the scope of the present paper. It is worth noting that pKa(**II**/**III**) values is not affected by the presence of this new species as confirmed by the similar pKa(**II**/**III**) value found for the [Cu^{II}(E3QD7NE11QK16-NH_2-Aβ)] species.

CD.

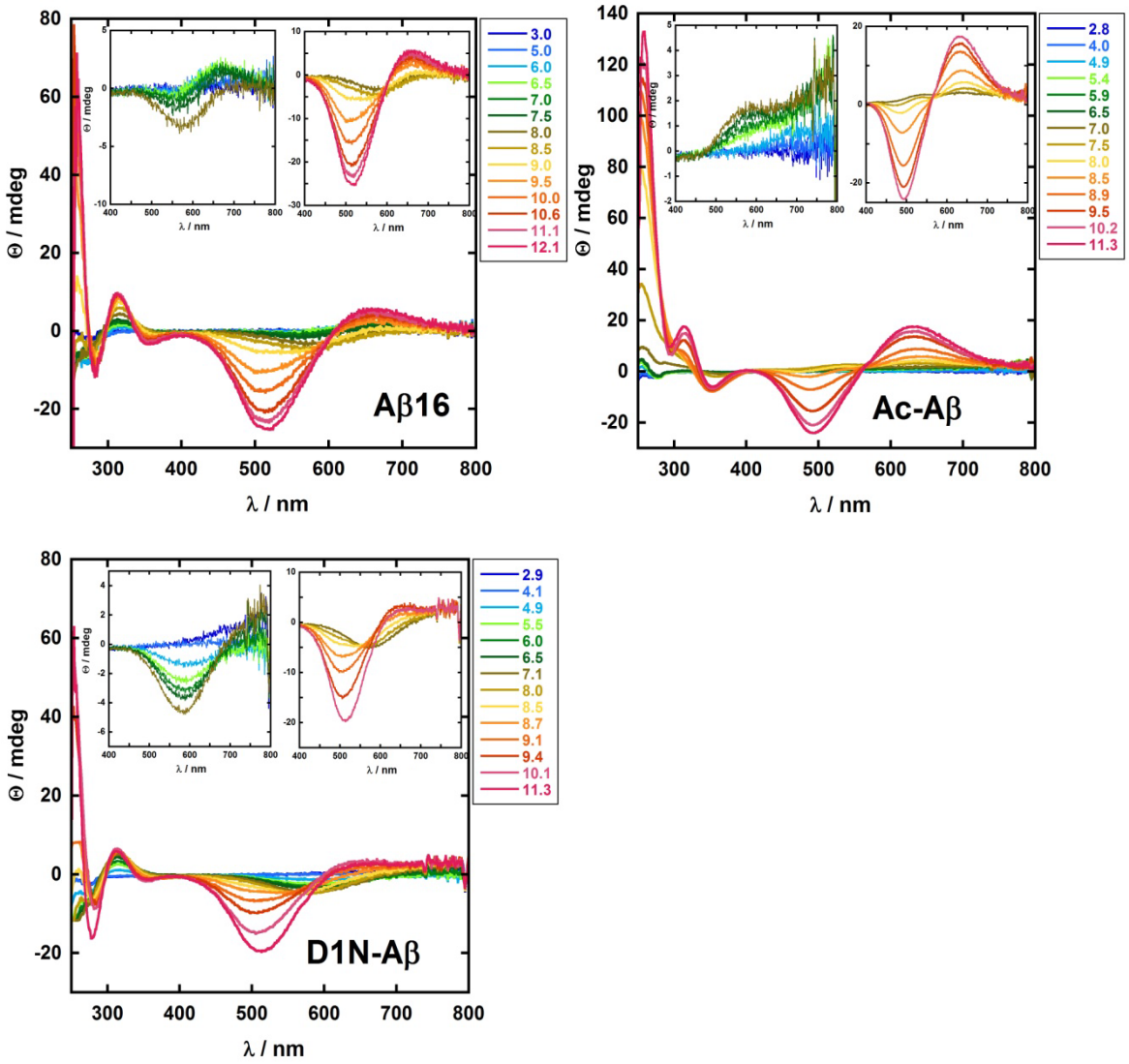


Figure S4. Effect of pH on the CD spectra of [Cu^{II}(peptide)] complexes, peptide: Aβ16, Ac-Aβ and D1N-Aβ. Left insets: pH from approx. 3 to Approx. 7.5 and right insets: pH from approx. 7.5 to approx. 12. [Cu^{II}(peptide)] = 0.5 mM, ℓ =1 cm, T = 20°C.

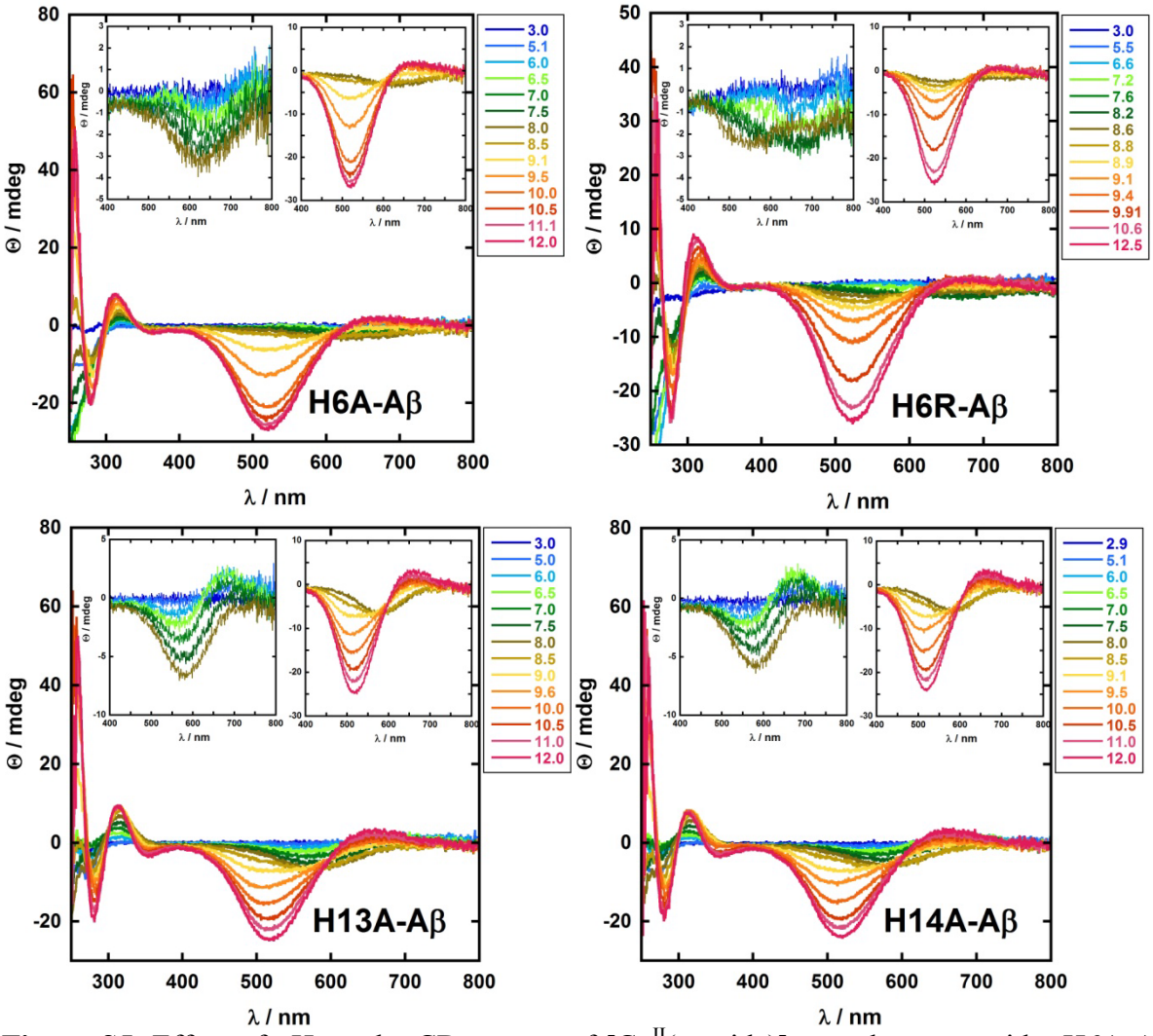


Figure S5. Effect of pH on the CD spectra of [Cu^{II}(peptide)] complexes, peptide: H6A-Aβ, H6R-Aβ, H13A-Aβ and H14A-Aβ. Left insets: pH from approx. 3 to Approx. 7.5 and right insets: pH from approx. 7.5 to approx. 12. [Cu^{II}(peptide)] = 0.5 mM, ℓ =1 cm, T = 20°C.

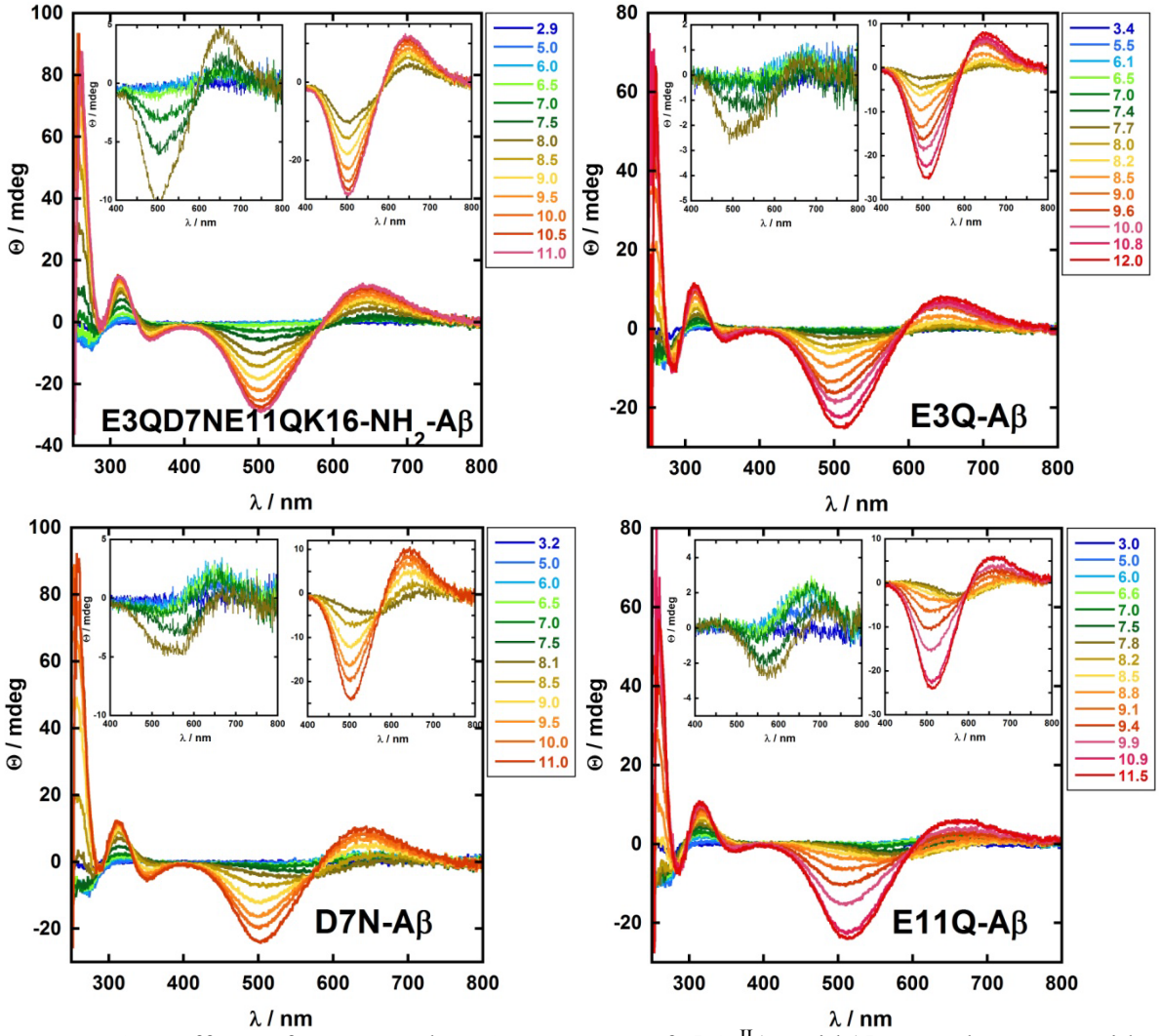


Figure S6. Effect of pH on the CD spectra of [Cu^{II}(peptide)] complexes, peptide = E3QD7NE11QK16-NH_2-Aβ, E3Q-Aβ, D7N-Aβ and E11Q-Aβ. Left insets: pH from approx. 3 to Approx. 7.5 and right insets: pH from approx. 7.5 to approx. 12. [Cu^{II}(peptide)] = 0.5 mM, ℓ =1 cm, T = 20°C.

Assignments of the ^{1}H and ^{13}C signals

All the ^{1}H and ^{13}C signals were assigned on the basis of chemical shifts, spin-spin coupling constants, splitting patterns and signal intensities, and by using ^{1}H-^{1}H TOCSY, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC experiments (Tables S1 and S2).

It was not possible to attribute some signals unambiguously. More precisely, it was not possible to fully differentiate signals corresponding to His13 and His14 residues.

Table S1: ^{1}H and ^{13}C chemical shifts of H6R-Aβ peptide at pH = 6.4. Color code used in scheme S2.

position		α		β		γ		δ		ε		ζ		CO peptide bond
amino-acid		δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	
Asp	1	4.18	**50.7**	2.79/2.67	**37.2**		**176.0**							**169.6**
Ala	2	4.26	**49.8**	1.31	**16.3**									**174.5**
Glu	3	4.17	**53.6**	1.82	**27.5**	2.15	**33.4**		**181.3**					**173.2**
Phe	4	4.54	**54.8**	3.00	**36.7**		**135.9**	7.15	**129.1**	7.25	**128.7**	7.20	**127.1**	**172.5**
Arg	5	4.21	**52.9**	1.72/1.62	**28.2**	1.47	**24.3**	3.10	**40.4**				**156.6**	**172.9**
Arg	6	4.21	**53.3**	1.78	**28.0**	1.56	**24.3**	3.08	**40.4**				**156.6**	**173.3**
Asp	7	4.59	**51.4**	2.67	**38.4**		**177.6**							**173.5**
Ser	8	4.34	**56.1**	3.84/3.81	**60.9**									**172.3**
Gly	9	3.90/3.83	**42.5**											**171.2**
Tyr	10	4.46	**55.4**	2.98/2.90	**36.0**		**127.8**	7.02	**130.4**	6.73	**115.4**		**154.4**	**172.9**
Glu	11	4.15	**53.8**	1.87	**27.5**	2.09	**33.4**		**181.3**					**173.2**
Val	12	3.90	**59.7**	1.88	**29.8**	0.82	**17.9**	0.72	**18.1**					**173.1**
His	13	or 4.62 / 4.60	**52.6 / 52.8**	3.00/3.12	**27.1 or 27.2**		**129.8 or 130.0**	7.06	**117.0 or 117.1**	8.18 or 8.19	**134.4**			**171.6**
His	14	or 4.62 / 4.60	**52.6 / 52.8**	3.00/3.12	**27.1 or 27.2**		**129.8 or 130.0**	7.06	**117.0 or 117.1**	8.18 or 8.19	**134.4**			**171.6**
Gln	15	4.26	**53.1**	2.06/1.94	**26.8**	2.31	**30.9**		**177.9**					**172.1**
Lys	16	4.09	**55.1**	1.77/1.76	**30.8**	1.36	**22.1**	1.63	**26.3**	2.94	39.16		**178.5**	

Table S2: ^{1}H and ^{13}C chemical shifts of H6R-Aβ peptide at pH = 8.6. Color code used in scheme S2.

position		α		β		γ		δ		ε		ζ		CO peptide bond
amino-acid		δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	
Asp	1	3.79	**52.0**	2.66/2.52	**41.3**		**178.3**							**175.8**
Ala	2	4.27	**50.1**	1.39	**16.5**									**175.3**
Glu	3	4.19	**54.1**	1.92	**27.4**	2.18	**33.5 or 33.6**		**181.3**					**173.6**
Phe	4	4.60	**55.0**	3.11/3.07	**36.7**		**136.1**	7.24	**129.1**	7.34	**128.7**	7.30	**127.2**	**172.7**
Arg	5	4.29	**53.1**	1.70	**28.2**	1.54	**24.4**	3.16	**40.5**				**156.7**	**173.1**
Arg	6	4.29	**53.4**	1.76/1.84	**28.1**	1.62	**24.4**	3.16	**40.5**				**156.7**	**173.3**
Asp	7	4.64	**51.6**	2.72	**38.5**		**177.6**							**173.6**
Ser	8	4.41	**56.1**	3.90/3.86	**61.0**									**172.3**
Gly	9	3.96/3.88	**42.6**											**171.2**
Tyr	10	4.56	**55.2**	3.06/2.94	**36.1**		**127.8**	7.10	**130.5**	6.82	**115.5**		**154.6**	**172.9**
Glu	11	4.24	**53.8**	1.92/1.83	**27.6**	2.10	**33.5/33.6**		**181.3**					**173.4**
Val	12	4.00	**59.8**	1.97	**30.0**	0.87	**17.8**	0.80	**18.2**					**173.1**
His	13	4.60	**53.5 or 53.6**	3.06/3.00	**28.7**		**132.9**	6.89 or 6.90	**116.7**	7.66 or 7.68	**136.0**			**172.6**
His	14	4.60	**53.5 or 53.6**	3.06/3.00	**28.7**		**132.9**	6.89 or 6.90	**116.7**	7.66 or 7.68	**136.0**			**172.4**
Gln	15	4.32	**53.1**	2.11/1.97	**26.7**	2.33	**31.0**		**177.9**					**172.0**
Lys	16	4.16	**55.0**	1.83/1.72	**31.0**	1.40	**22.1**	1.68	**26.3**	2.99	**39.3**		**178.6**	

NMR data.

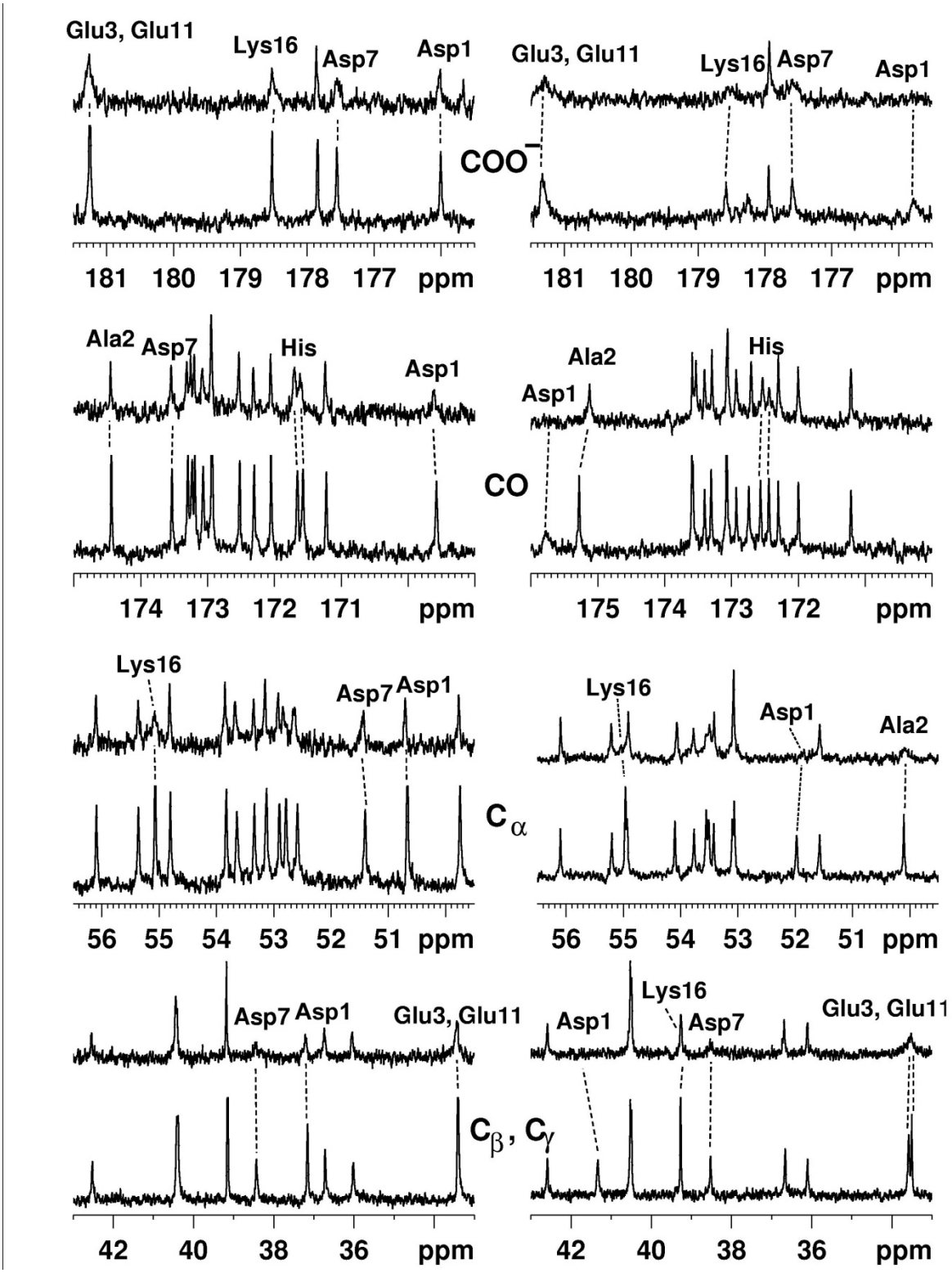


Figure S7. $^{13}C\{^{1}H\}$ NMR spectra of 5 mM H6R-Aβ peptide in D_2O (bottom spectra) and in presence of 0.02 equiv. of Cu(II) (top spectra) at pH 6.4 (left) and of 0.05 equiv. of Cu(II) (top spectra) at pH 8.6 (right). T = 25°C, ν = 125.8MHz. Shift of some peaks is due to slight modification in the pH value induced by Cu(II) addition.

Comparison of the ^{13}C NMR data of the H6R-Aβ mutant in presence of Cu(II) with those of the Aβ16 peptide[2] lead to two comments : i) in component **I**, Asp7 residue is more broadened in the former case. This may be due to possible electrostatic interactions with the positively charged Arg6 adjacent residue. ii) in component **I**, Asp1 C_α atom is less affected in the H6R mutant. This is in line with the absence of a metallacycle between the terminal $-NH_2$ and the CO from Asp1-Ala2 in case of the H6R mutant (see Scheme 3 in full text), thus leading to a weaker $-NH_2$ binding than in case of the Aβ16 peptide.

(1) Peisach, J.; Blumberg, W. E. *Arch. Biochem. Biophys.* **1974**, *165*, 691-708.
(2) Hureau, C.; Coppel, Y.; Dorlet, P.; Solari, P. L.; Sayen, S.; Guillon, E.; Sabater, L.; Faller, P. *Angew. Chem. Int. Ed.* **2009**, *48*, 9522-9525.