Zn(II) binding site to the amyloid-β peptide: insights from spectroscopic studies with a wide series of modified peptides.

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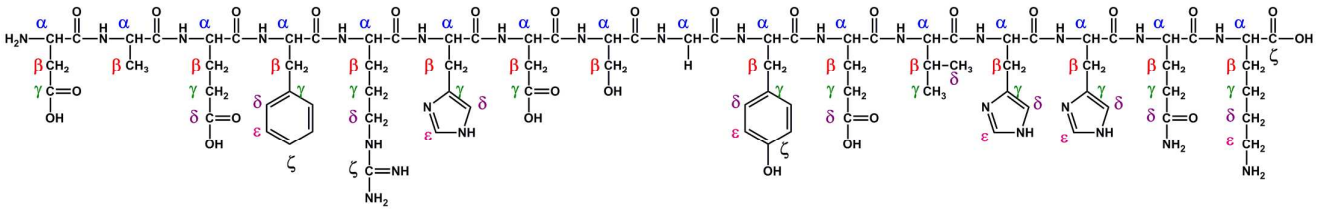
SUPPORTING INFORMATION



Scheme S1. Aβ16 (referred to as Aβ) peptide sequence with the atom identifiers of each amino-acid residue.

Table S1. Models of Zn(II) binding to Aβ previously proposed form NMR studies.

peptide used	conditions	Binding site proposed	Refs.	entry
Aβ16	1 mM in phosphate buffer 50 mM,[a] pH 7.1 (H_2O/D_2O, 9/1), 293 K.	His6, His13, His14, ?	[1]	**1**
Aβ16, Aβ28	~ 0.1 mM in D_2O, pH 7.8, 293 K.	NH_2[b], His6, His13, His14	[2]	**2**
Aβ40[c]	50 μM in phosphate buffer 10mM, pH 7.4 , 286 K.	NH_2[b], His6, His13, His14	[3]	**3**
Ac-Aβ16[d]	2 mM in phosphate buffer 50mM, pH 6.5 (H_2O/D_2O, 9/1).	His6, Glu11, His13, His14	[4]	**4**
Aβ28	0.4 mM, 100 mM SDS, pH 7.5, D_2O, 298 K.	NH_2[b], His6, Glu11, His13, His14	[5]	**5**
Aβ16-PEG[e]	2 mM pH 7.0, D_2O, 300 K.	NH_2, His6, His13, His14	[6]	**6**
Aβ16	300 μM in TRIS buffer, pH 7.4, D_2O, 318 K	His6, His13 **or** His14, Glu11, COO^-	this work	**7**

[a] Impact of zinc in phosphate buffer is weaker than in other buffer (or in absence of buffer) due to partial precipitation of $Zn_3(PO_4)_2$.

[b] N-terminal amine from Asp1 residue.

[c] ^{15}N and ^{13}C-labelled peptide.

[d] Acetylation was used to avoid precipitation at high peptide concentration.

[e] PEGylation was used to avoid precipitation at high peptide concentration.

Table S2. First coordination shell structural data obtained from R-space fits of Zn(Aβ) in solution EXAFS spectra. N = number of neighbours, R = absorber-neighbour distance, σ = Debye-Waller factor.

pH	N	σ^2 (Å^{-2})	R (Å)	ΔE (eV)	R factor
6.9	4.33	0.00694	1.98	-0.12	0.26 %
7.4	4.20	0.0067	1.99	2.31	0.38 %

Impact of pH on the XAS and NMR data.

The reason why the XAS and NMR experiments have been performed at two pH values is based on the fact that at pH 7.4 two species may coexist. Indeed, as shown in the full text, the N-terminal amine is bound to the Zn(II) at higher pH (pH 9.0). In addition, from literature data,[7, 8] it is known that the N-terminal amine is deprotonated around pH 8.0 in the apo-peptide. Hence we have anticipated that the coordination of the N-terminal amine occurs partially at pH 7.4. Hence we have performed all studies (XAS and NMR) at the two pH values. (i) simulations of the pH 6.9 and 7.4 EXAFS data show comparable simulation parameters, this is in line with the replacement of a coordinating group by the N-terminal amine, keeping a coordination number equals to 4 ; (ii) in contrast, impact of Zn(II) on the NMR data is better observed at pH 7.4. This may be due to a partial loss of solubility of the Zn(Aβ) complex when the pH is decreased to 6.9, leading to a less specific broadening. The effects are however very similar to those observed at pH 7.4 and the presence of a second minor species at pH 7.4 doesn't disturb the analysis of the NMR data. While some insights on the nature of this second minor species is given in the full text, its full identification is beyond the scope of the present paper that aims at describing the structure of the main species in physiological conditions.

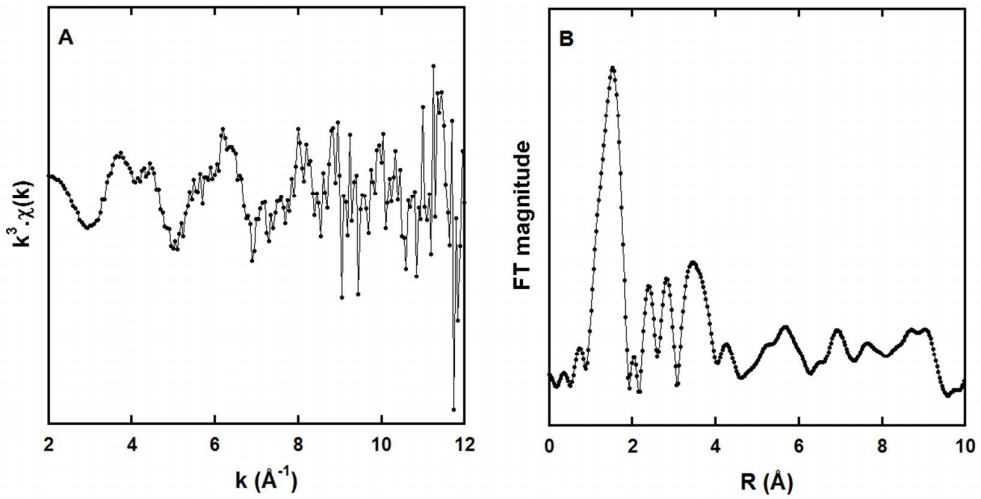


Figure S1. k^3-weighted experimental EXAFS spectrum of Zn(Aβ) at pH 6.9 and its corresponding non-phase-shift corrected Fourier transform. [Aβ] = 1.0 mM, [Zn(II)] = 0.9 mM in Hepes buffer 50 mM, T = 20 K.

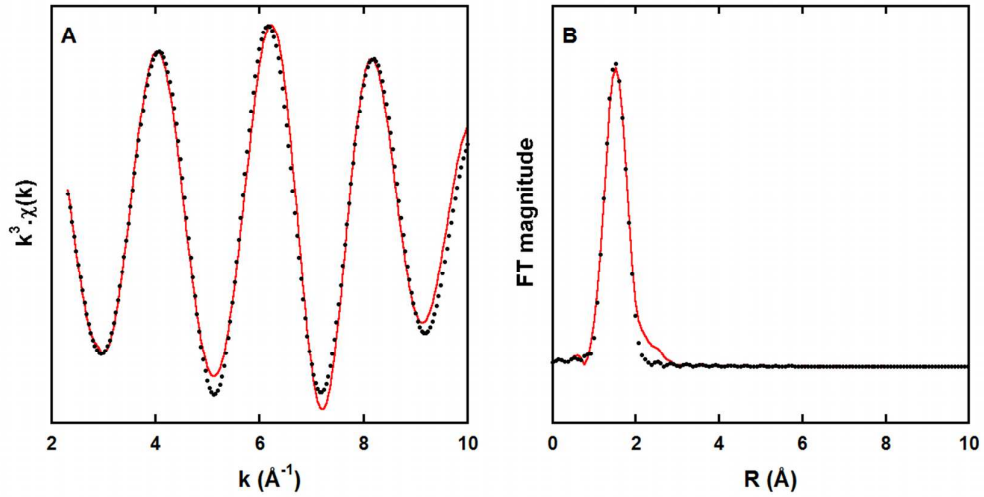


Figure S2. k^3-weighted experimental (black dots) and least-squares fitted (red line) first coordination shell EXAFS spectra of the Zn(Aβ) at pH 7.4 (A) and the corresponding non-phase-shift corrected Fourier transforms (B). Recording conditions: [Aβ] = 1 mM, [Zn(II)] = 0.9 mM in Hepes buffer 50 mM, T = 20 K.

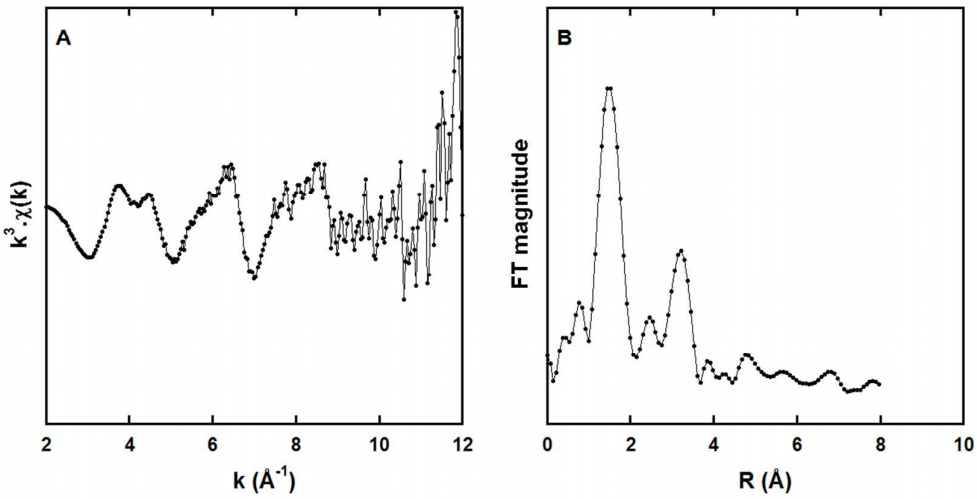


Figure S3. k^3-weighted experimental EXAFS spectrum of Zn(Aβ) at pH 7.4 and its corresponding non-phase-shift corrected Fourier transform. [Aβ] = 1.0 mM, [Zn(II)] = 0.9 mM in Hepes buffer 50 mM, T = 20 K.

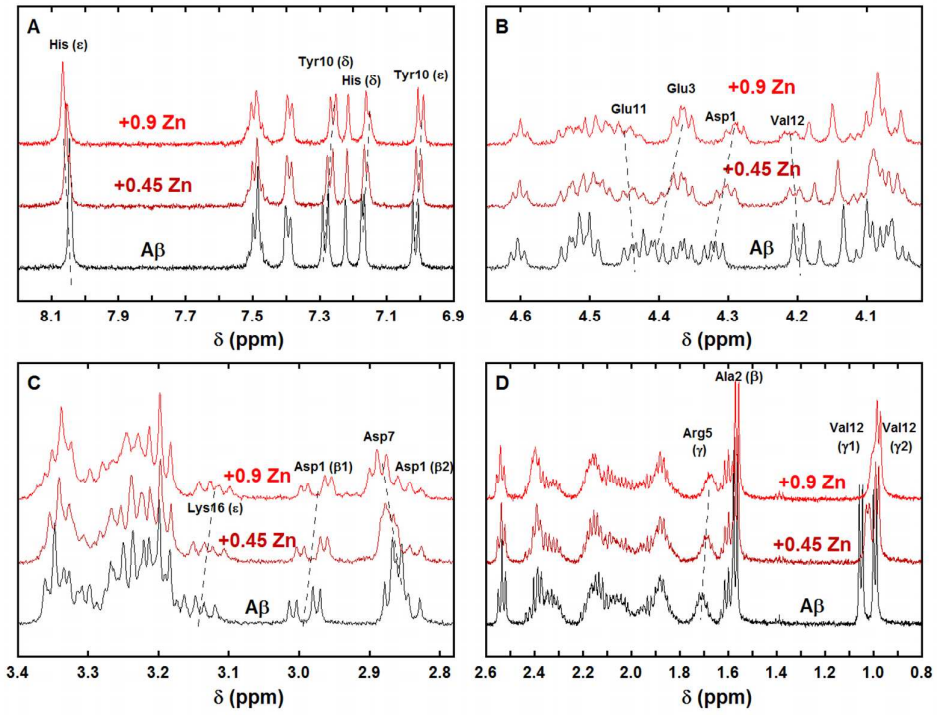


Figure S4. ^{1}H NMR spectra of Aβ (bottom black lines) and of Aβ in presence of 0.45 and 0.9 equiv. of Zn(II) in selected regions (A: aromatic, B: Hα, C: Hβ, D: Hβ and Hγ). [Aβ] = 300 µM, [Zn(II)] = 270 µM in d_{11}-TRIS buffer 50 mM, pH = 7.4, T = 318 K, v = 500 MHz. δ(His6 Hδ) > δ(His14 Hδ) > δ(His13 Hδ). For the details of the amino-acid residues nomenclature, see Scheme S1.

Table S3. Zn(II) induced shifts and broadening of protons from Aβ peptide and modified counterparts. For nomenclature of protons, see Scheme S1.

	D1	A2	E3	F4	R5	H6	D7	E11	V12	H13	H14	entry
Aβ	α -33 2 β1 -16 2 β2 +12 2	α 3 2 β 6 1	α -42 2	δ -5 1 ε 1 ζ 1	α -9 1 γ -37 2	δ -8 1 ε >9 1	β +22 1	α +15 2	α +12 4 γ1 -60 4 γ2 -15 1	δ -16 3 ε > 1	δ -13 1 ε >9 1	**1**
Ac-Aβ	α β1 ~ 2 1 β2 +22 1	α 0 1 β -6 1	α β γ	δ -6 1 ε 0 2 ζ -5 2	α γ -20 1	δ 2 1 ε 2		α β γ	α 22 4 γ1 -55 5 γ2 -10 2	δ -18 3 ε 1	δ -14 1 ε 1	**2**
H6A-Aβ	α β1 +16 2 β2 +22 3	α β	α β γ	δ -13 0 ε -8 0 ζ -9 0	α γ -7 1		β +17 1	α β γ	α 4 γ1 -26 5 γ2 +2 1	δ 3 ε ~ 28 2	δ 1 ε ~ 28 2	**3**
H13A-Aβ	α β1 -9 3 β2 +8 1	α β -6 1	α -38 β γ	δ -1 1 ε 1 ζ 1	α γ -37 2	δ < 5 1 ε + 42 1	β +21 2	α >11 β γ	α -10 1 γ1 -9 1 γ2 0 1		δ <5 1 ε +42 1	**4**
H14A-Aβ	α +24 3 β1 -11 2 β2 +5 3	α β	α -50 > 3 β γ	δ -1 1 ε +10 1 ζ +4 1	α γ -35 1	δ +5 1 ε ~30 1	β +20 3	α β γ	α +39 4 γ1 -78 5 γ2 -26 1	δ +20 2 ε ~30 1		**5**
D1N-Aβ	α β1 0 1 β2 0 1	α +2 1 β -4 1	α -8 2 β γ	δ -5 1 ε -5 2 ζ 0 1	α 0 2 γ -23 2	δ +3 1 ε +6 1	β +29 2	α +21 3 β γ	α +2 4 γ1 -56 5 γ2 -10 2	δ -15 3 ε +6 1	δ -10 1 ε +6 1	**6**
E3Q-Aβ	α 3 β1 -4 2 β2 +14 3	α 3 β 0 1	α 3	δ -14 2 ε 0 2 ζ 3	α 3 γ -34 2	δ +2 2 ε >19 3	β +29 4	α 3 β γ	α 5 γ1 +54 5 γ2 -17 2	δ -10 4 ε >19 3	δ -7 2 ε >19 3	**7**
D7N-Aβ	α -30 1 β1 +23 1 β2 +18 3	α 0 1 β	α β γ	δ -2 1 ε +9 1 ζ +4 2	α γ -52 1	δ -13 1 ε >16 1		α β γ	α 0 4 γ1 -39 4 γ2 -17 1	δ -16 3 ε >16 1	δ -10 1 ε >16 1	**8**

E11Q-Aβ	α	+29	2	α	0	1	α	-75	5	δ	-5	1	α		δ	-18	1			α	-13	2	δ	-18	1	δ	-18	1	
	β1	-18	1	β	-10	1	β			ε	+6	1	γ	-48	2	ε	>3	2	β	26	1		γ1	-18	1	ε	>3	2	ε	>3	2	**9**
	β2	-1	3				γ			ζ		2											γ2	-18	2						
Y10F-Aβ	α	-14	2	α			α	-50 >	3	δ			α			δ	-30	1			α	+8	3	α	+10	3	δ	-8	3	δ	-7	1	
	β1	-12	1	β	-8	2	β			ε			γ	-35	2	ε	>20	2	β	+28	2	β			γ1	-46	4	ε	>20	2	ε	>20	2	**10**
	β2	+1	2				γ			ζ												γ			γ2	-16	2						
entry	**A**			**B**			**C**			**D**			**E**			**F**			**G**			**H**			**I**			**J**			**K**		

Chemical shifts are given in 10^{-3} ppm. Recording frequency is 500 MHz.
Broadening is characterized by a number ranging from 0 to 5, 0 corresponding to no change and 5 to complete disappearance of the peak.

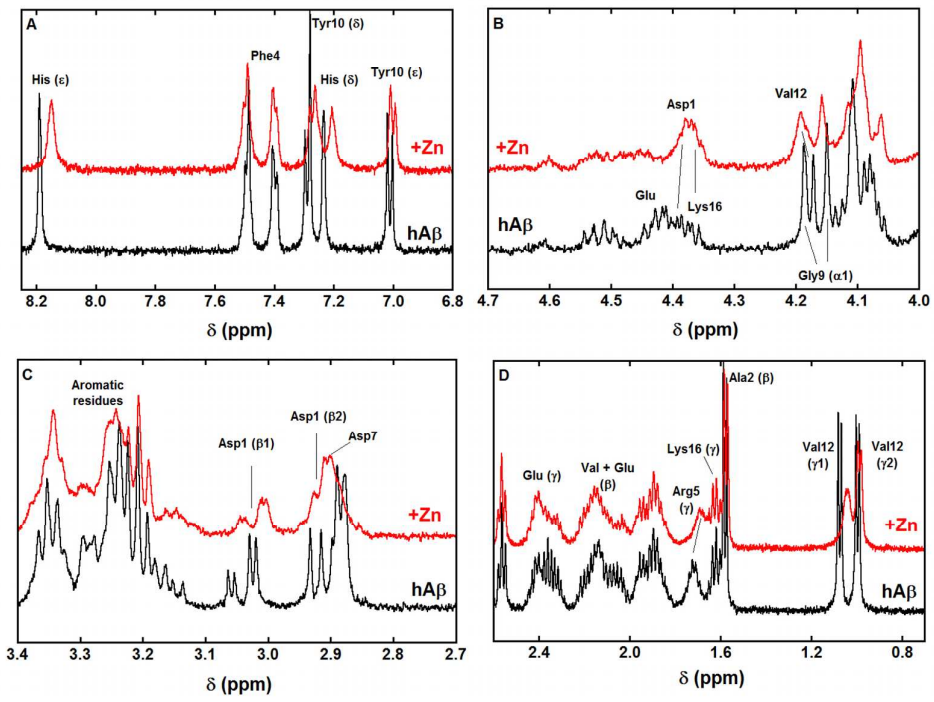


Figure S5. ^{1}H NMR spectra of Aβ (bottom black lines) and of Aβ in presence of 0.9 equiv. of Zn(II) (top red lines) in selected regions (A: aromatic, B: Hα, C: Hβ, D: Hβ and Hγ). [Aβ] = 300 μM, [Zn(II)] = 270 μM in d_{19}-BisTRIS buffer 50 mM, pH = 6.9, T = 318 K; v = 500 MHz. For the details of the amino-acid residues nomenclature, see Scheme S1.

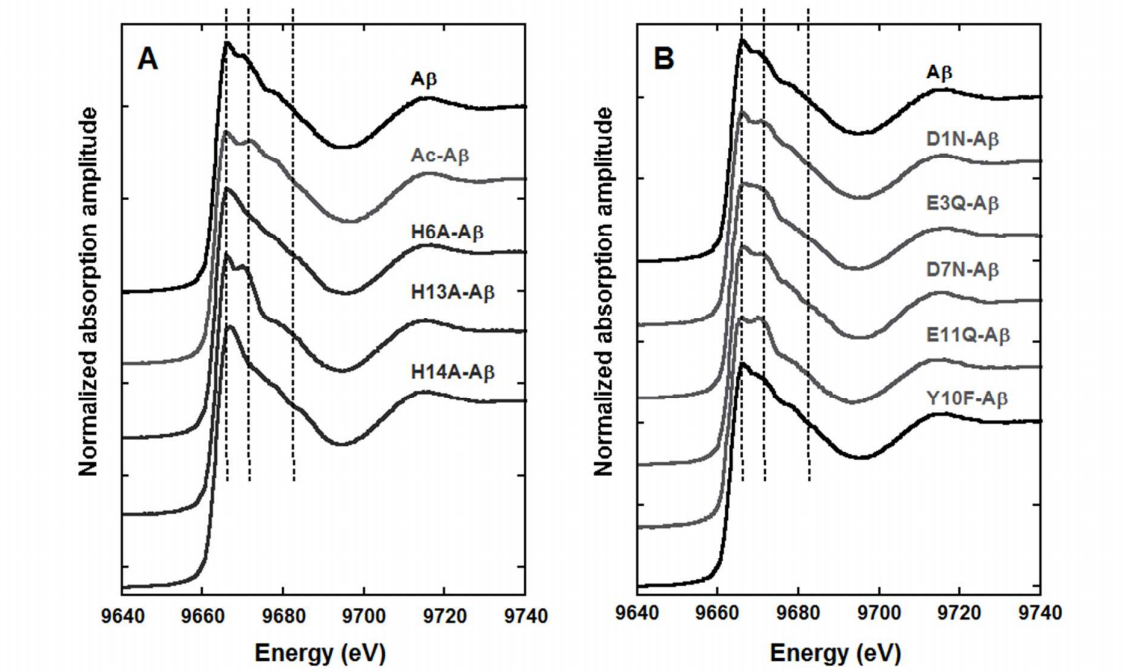


Figure S6. Zn(II) k-edge XANES spectra of Zn(II) bound to Aβ (black line) and to N mutants (panel A) and O mutants (panel B), hepes buffer 50 mM pH 7.4 [Zn(II)] = 1 mM, [peptide] = 1.1 mM. T = 20 K.

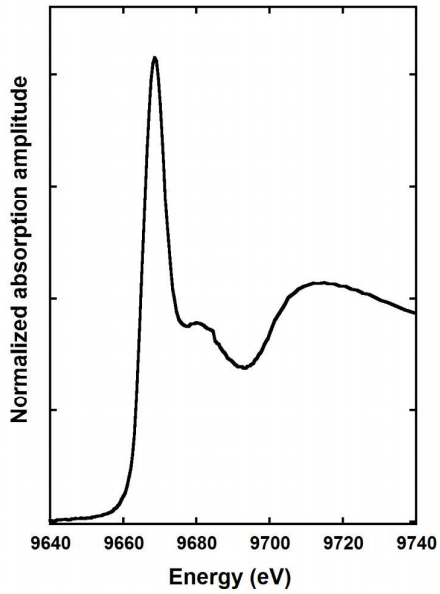


Figure S7. Zn(II) k-edge XANES spectra of Zn(II) in hepes buffer 50 mM pH 7.1 [Zn(II)] = 1 mM, T = 20 K.

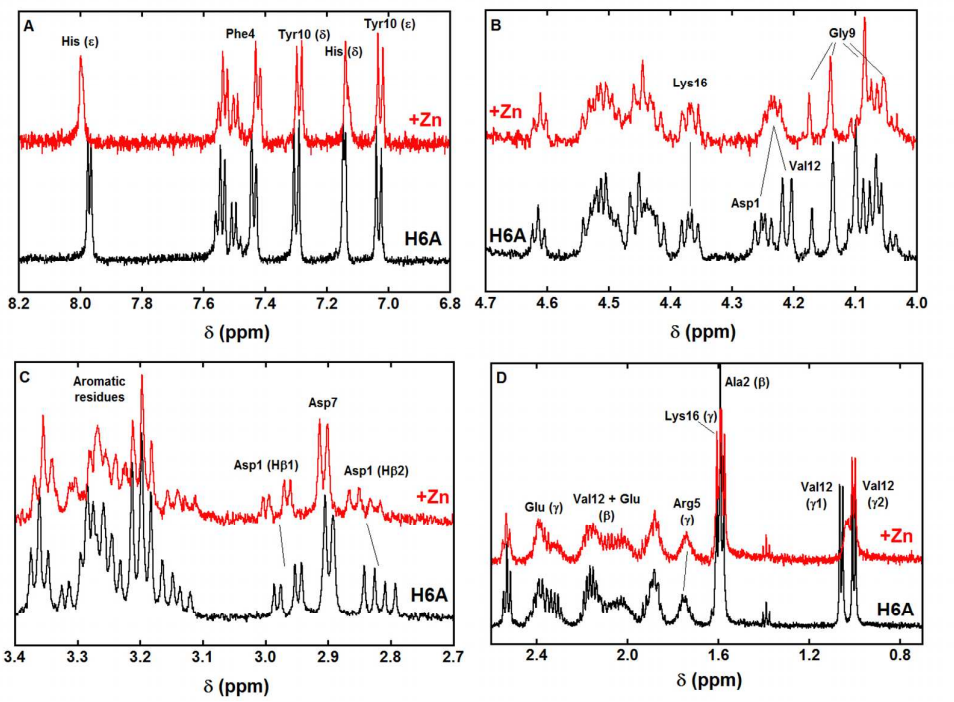


Figure S8. ^{1}H NMR spectra of H6A-Aβ (bottom black lines) and of H6A-Aβ in presence of 0.9 equiv. of Zn(II) (top red lines) in selected regions (A: aromatic, B: Hα, C: Hβ, D: Hβ and Hγ). [Aβ] = 300 µM, [Zn(II)] = 270 µM in d_{11}-TRIS buffer 50 mM, pH = 7.4, T = 318 K, v = 500 MHz. For the details of the amino-acid residues nomenclature, see Scheme S1.

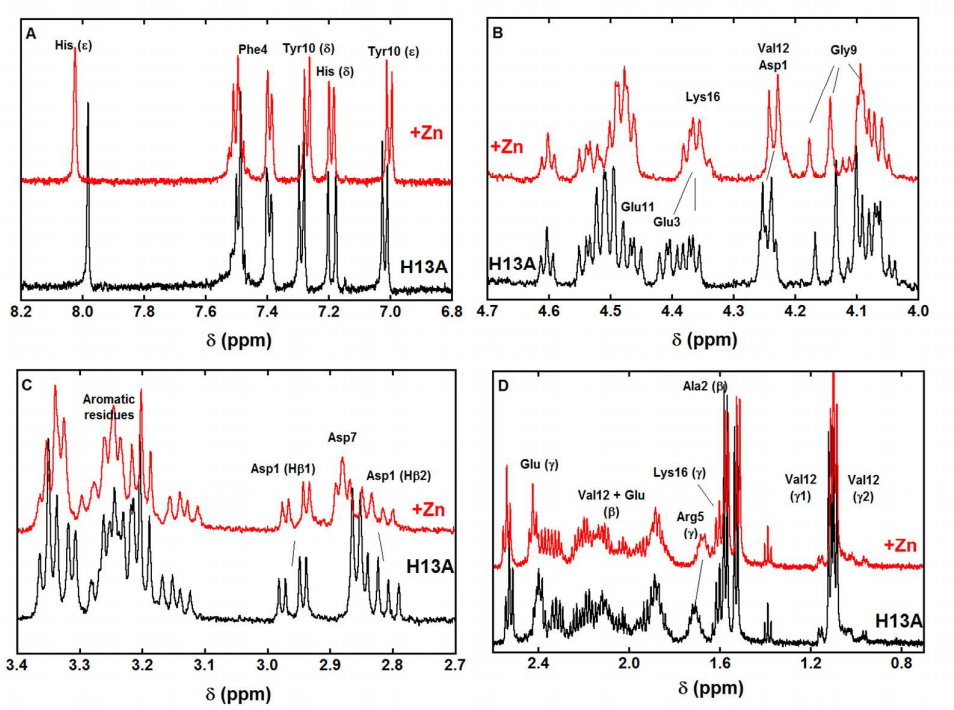


Figure S9. ^{1}H NMR spectra of H13A-Aβ (bottom black lines) and of H13A-Aβ in presence of 0.9 equiv. of Zn(II) (top red lines) in selected regions (A: aromatic, B: Hα, C: Hβ, D: Hβ and Hγ). [Aβ] = 300 μM, [Zn(II)] = 270 μM in d_{11}-TRIS buffer 50 mM, pH = 7.4, T = 318 K, v = 500 MHz. For the details of the amino-acid residues nomenclature, see Scheme S1.

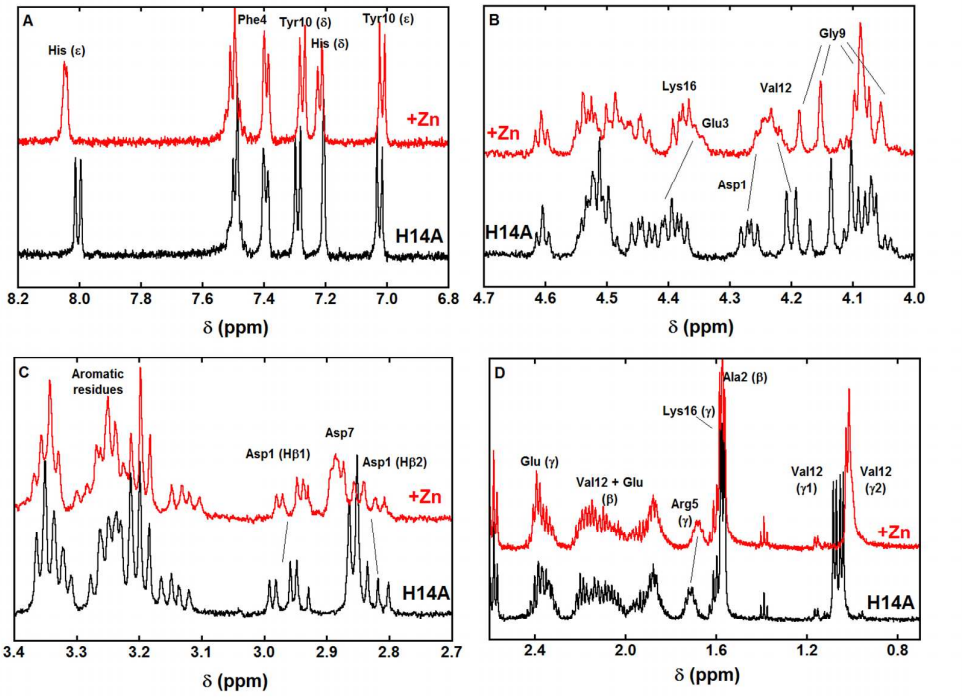


Figure S10. ^{1}H NMR spectra of H14A-Aβ (bottom black lines) and of H14A-Aβ in presence of 0.9 equiv. of Zn(II) (top red lines) in selected regions (A: aromatic, B: Hα, C: Hβ, D: Hβ and Hγ). [Aβ] = 300 μM, [Zn(II)] = 270 μM in d_{11}-TRIS buffer 50 mM, pH = 7.4, T = 318 K, v = 500 MHz. For the details of the amino-acid residues nomenclature, see Scheme S1.

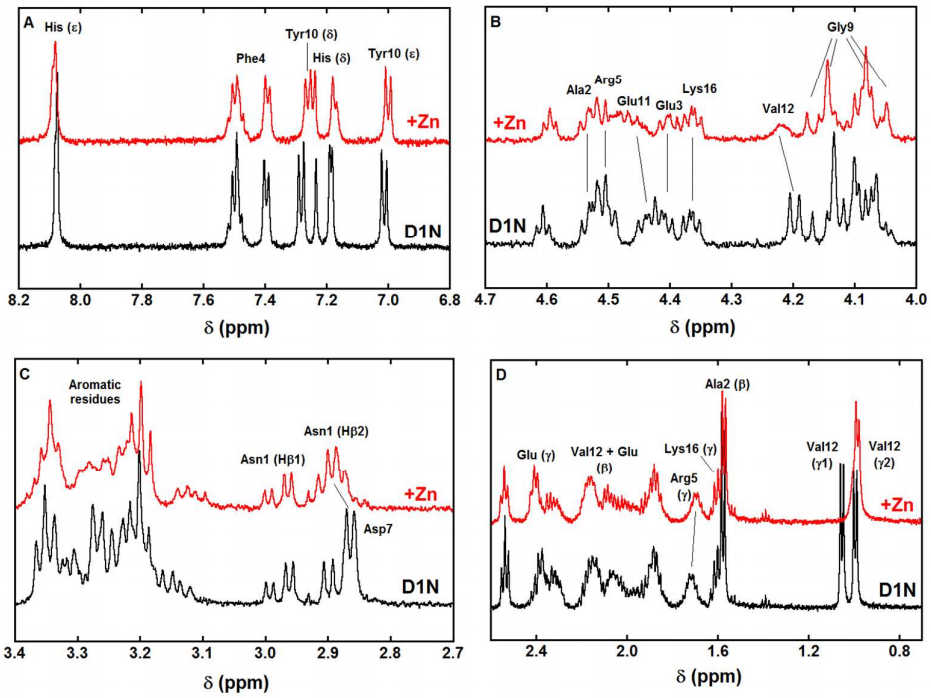


Figure S11. ^{1}H NMR spectra of D1N-Aβ (bottom black lines) and of D1N-Aβ in presence of 0.9 equiv. of Zn(II) (top red lines) in selected regions (A: aromatic, B: Hα, C: Hβ, D: Hβ and Hγ). [Aβ] = 300 μM, [Zn(II)] = 270 μM in d_{11}-TRIS buffer 50 mM, pH = 7.4,T = 318 K, v = 500 MHz. For the details of the amino-acid residues nomenclature, see Scheme S1.

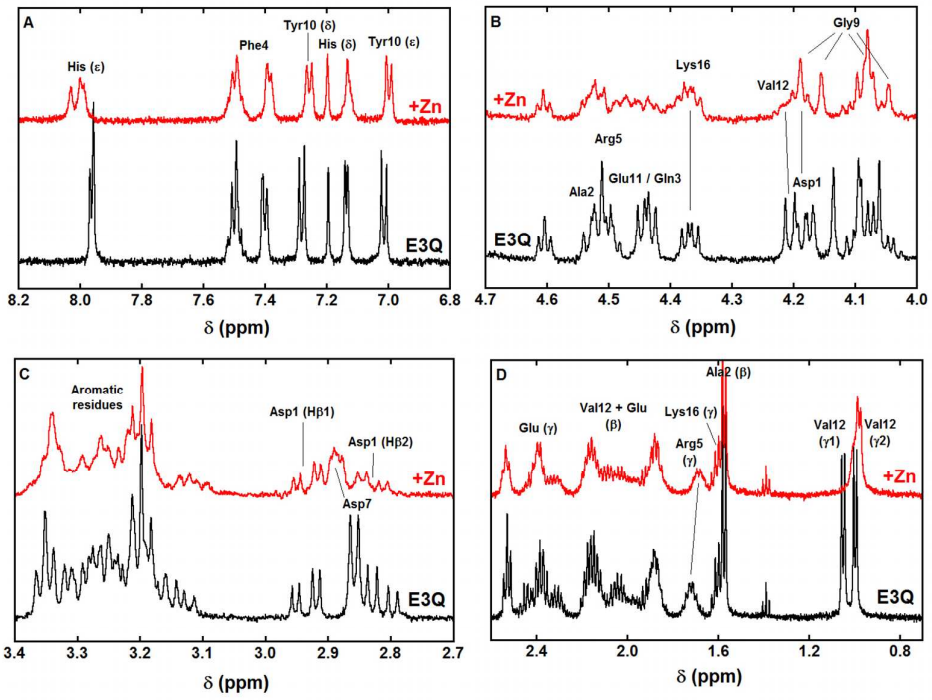


Figure S12. ^{1}H NMR spectra of E3Q-Aβ (bottom black lines) and of E3Q-Aβ in presence of 0.9 equiv. of Zn(II) (top red lines) in selected regions (A: aromatic, B: Hα, C: Hβ, D: Hβ and Hγ). [Aβ] = 300 μM, [Zn(II)] = 270 μM in d_{11}-TRIS buffer 50 mM, pH = 7.4, T = 318 K, v = 500 MHz. For the details of the amino-acid residues nomenclature, see Scheme S1.

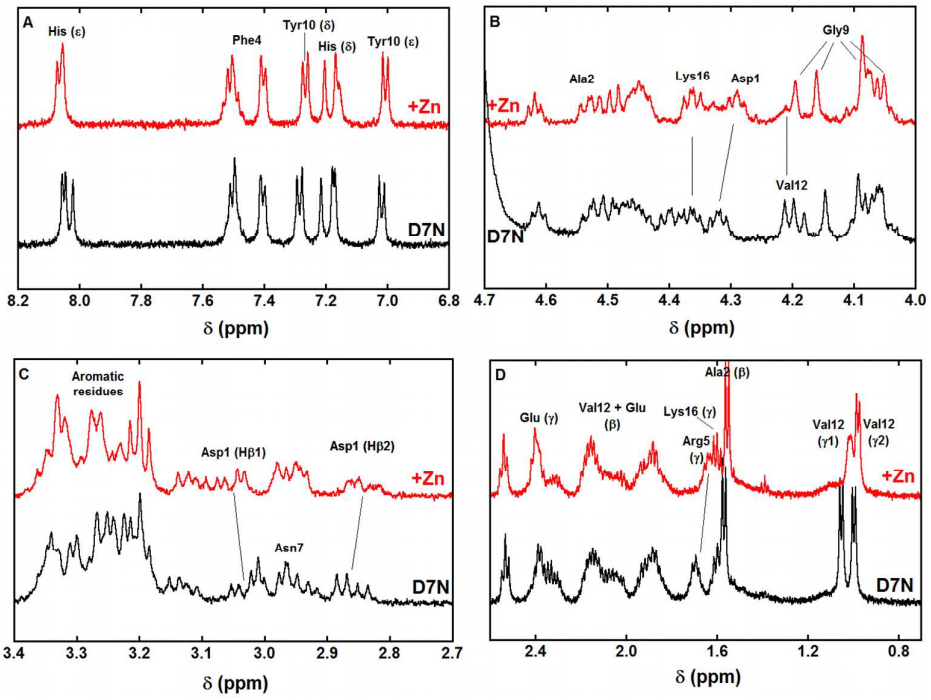


Figure S13. ^{1}H NMR spectra of D7N-Aβ (bottom black lines) and of D7N-Aβ in presence of 0.9 equiv. of Zn(II) (top red lines) in selected regions (A: aromatic, B: Hα, C: Hβ, D: Hβ and Hγ). [Aβ] = 300 μM, [Zn(II)] = 270 μM in d_{11}-TRIS buffer 50 mM, pH = 7.4, T = 318 K, v = 500 MHz. For the details of the amino-acid residues nomenclature, see Scheme S1.

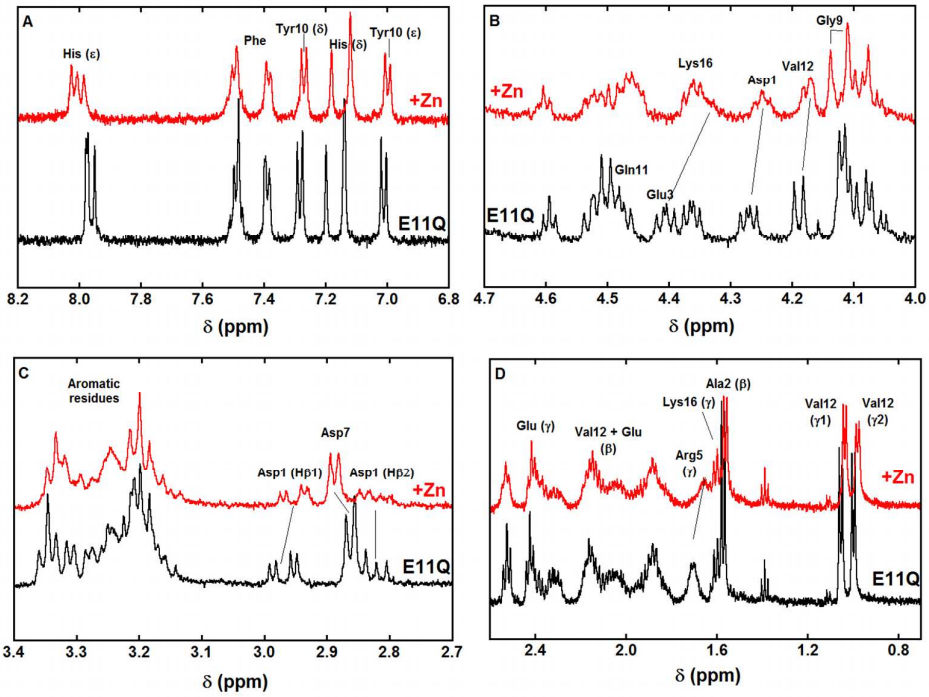


Figure S14. ^{1}H NMR spectra of E11Q-Aβ (bottom black lines) and of E11Q-Aβ in presence of 0.9 equiv. of Zn(II) (top red lines) in selected regions (A: aromatic, B: Hα, C: Hβ, D: Hβ and Hγ). [Aβ] = 300 μM, [Zn(II)] = 270 μM in d_{11}-TRIS buffer 50 mM, pH = 7.4, T = 318 K, v = 500 MHz. For the details of the amino-acid residues nomenclature, see Scheme S1.

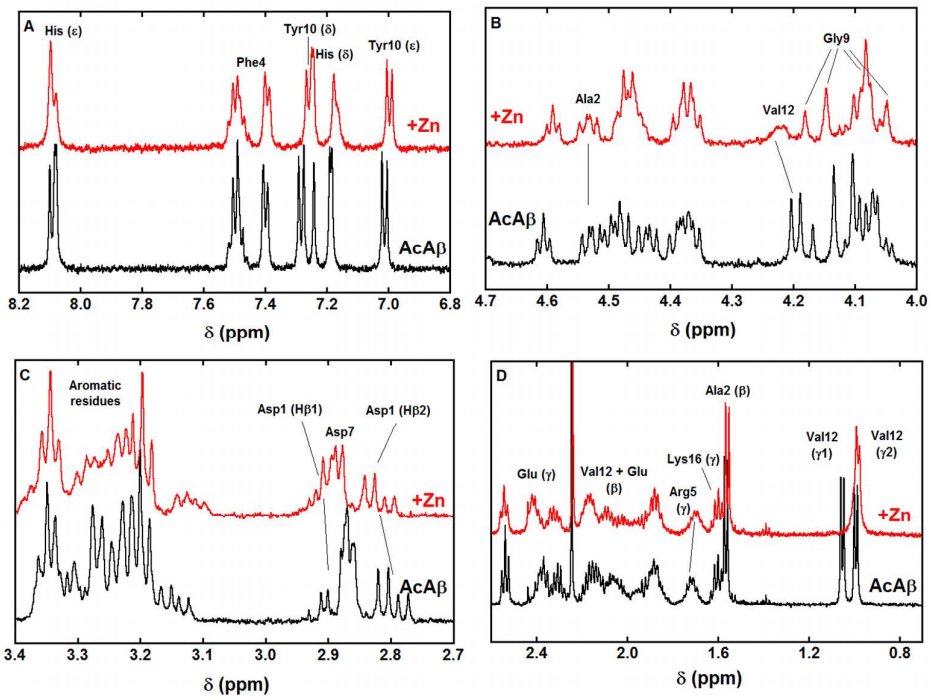


Figure S15. ^{1}H NMR spectra of Ac-Aβ (bottom black lines) and of Ac-Aβ in presence of 0.9 equiv. of Zn(II) (top red lines) in selected regions (A: aromatic, B: Hα, C: Hβ, D: Hβ and Hγ). [Aβ] = 300 μM, [Zn(II)] = 270 μM in d_{11}-TRIS buffer 50 mM, pH = 7.4, T = 318 K, v = 500 MHz. For the details of the amino-acid residues nomenclature, see Scheme S1.

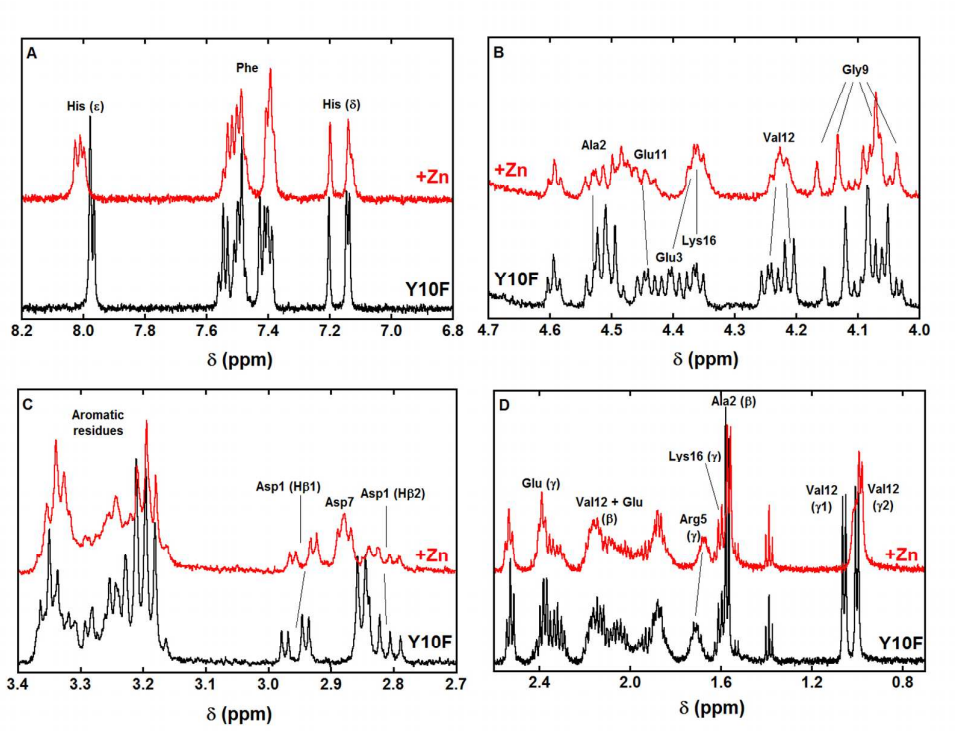


Figure S16. ^{1}H NMR spectra of Y10F-Aβ (bottom black lines) and of Y10F-Aβ in presence of 0.9 equiv. of Zn(II) (top red lines) in selected regions (A: aromatic, B: Hα, C: Hβ, D: Hβ and Hγ). [Aβ] = 300 μM, [Zn(II)] = 270 μM in d_{11}-TRIS buffer 50 mM, pH =7.4, T = 318 K, v = 500 MHz. For the details of the amino-acid residues nomenclature, see Scheme S1.

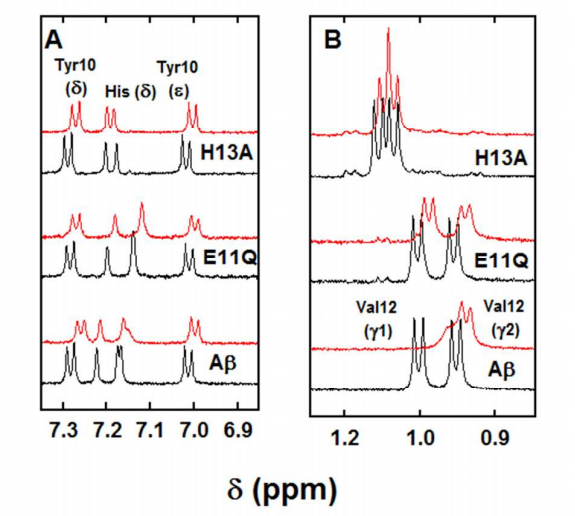


Figure S17. ^{1}H NMR spectra of Aβ, E11Q-Aβ and H13A-Aβ peptides (bottom black lines) and of Aβ, E11Q-Aβ and H13A-Aβ peptides in presence of 0.9 equiv. of Zn(II) (top red lines) in selected regions (A: aromatic, B: Val12 Hβ). [Aβ] = 300 μM, [Zn(II)] = 270 μM in d_{11}-TRIS buffer 50 mM, pH = 7.4, T = 318 K, v = 500 MHz. For the details of the amino-acid residues nomenclature, see Scheme S1.

References

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