Supplementary Information

The English (H6R) familial Alzheimer's disease mutation facilitates zinc-induced dimerization of the amyloid-β metal-binding domain

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MATERIALS

Chemicals and solvents were of HPLC-grade or better and were obtained from Sigma-Aldrich, USA. Reagents for SPR biosensor: HBS-EP+ buffer (150 mM NaCl, 10 mM HEPES, 3mM EDTA, 0.005% Surfactant P20, pH 7.4); 10 mM acetate buffers (pH 4.5); tiol coupling reagents kit; 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC); N-hydroxysuccinimide (NHS); 2-(2-pyridinyldithio)-ethaneamine (PDEA), were obtained from GE Healthcare Life Sciences, USA. Synthetic peptides (purity > 98%, checked by RP-HPLC) were purchased from Biopeptide Co., LLC, USA:

$^{Ac}A\beta_{1-16}$: Acetyl-DAEFRRDSGYEVHHQK-Amide (for ITC and NMR);

^{Ac}H6R-$A\beta_{1-16}$: Acetyl-DAEFRRDSGYEVHHQK-Amide (for SPR, ITC, NMR);

H6R-$A\beta_{1-16}$: DAEFRRDSGYEVHHQK-Amide (for ITC and NMR);

^{Ac}H6R-$A\beta_{1-16}$-G4-C: Acetyl-DAEFRRDSGYEVHHQKGGGGC-Amide (for SPR).

N- and C-termini of the peptides were protected with acetyl and amide, respectively. N-terminus of H6R-$A\beta_{1-16}$ was not protected. The amino acid sequence of peptides was confirmed on an ultra-high resolution Fourier transform ion cyclotron resonance mass-spectrometer Bruker 7T Apex-Qe (Bruker Daltonics, USA) using a de-novo sequencing approach based on collision induced dissociation (CID) fragmentation. The lyophilized peptides were dissolved in buffer before each experiment. The final peptide concentrations were determined by UV absorption spectroscopy using the extinction coefficient of 1450 M^{-1} cm^{-1} at 276 nm (from Tyr 10 of Aβ).

METHODS

Isothermal titration calorimetry (ITC)

Thermodynamic parameters of zinc binding to $^{Ac}A\beta_{1-16}$, ^{Ac}H6R-$A\beta_{1-16}$ and H6R-$A\beta_{1-16}$ (Fig. 1, Table 1) were measured using a MicroCal iTC200 System (GE Healthcare Life Sciences, USA) as described previously (*1*). Experiments were carried out at 25°C in 50 mM Tris buffer, pH 7.3. Aliquots of $ZnCl_2$ solution (2 μl) were injected into the 0.2 ml cell containing solution of peptides to achieve a complete binding isotherm (Fig. 1). Peptide concentration in the cell ranged from 0.25 to 0.75 mM and the $ZnCl_2$ concentration in the syringe ranged from 5 to 15 mM. Heat of dilution was measured by injecting the ligand ($ZnCl_2$) into the buffer solution; the values obtained were subtracted from the heat of reaction to obtain the effective heat of binding. The resulting titration curves were fitted using MicroCal Origin

software. The association constant (K_a) and binding stoichiometry (N) were determined by a non-linear regression fitting procedure.

Surface Plasmon Resonance (SPR) Biosensing

SPR measurements were performed with biosensor Biacore-T200 (GE Healthcare Life Sciences, USA) and the standard optical chips CM5 using Biacore Control v.3.1 and BiaEvaluation v.4.1 software for instrument operation. HBS-EP+ buffer was used for surface regeneration in SPR measurements. The running buffer was either 50 mM HEPES (pH 6.8) or the same buffer with 100 μM $ZnCl_2$. Molecular interactions were registered as real time records of biosensor signals in resonant units (RU). Peptide immobilization on the optical chip was carried out according to a standard Biacore protocol for tiol coupling reagents kit (Sensor surface handbook, GE Healthcare Life Sciences, USA). The peptide H6R-Aβ_{1-16}-G4-C was immobilized in FC2 channel of the biosensor. The channel FC1 has been left blank for the correction of nonspecific effects. The peptide ^{Ac}H6R-Aβ_{1-16} was dissolved in the running buffer at different concentrations (2, 5, 10, 15, 20 μM) and injected at a flow rate of 5 μl/min for 5 min. Additionally the control experiment was carried out for different peptide concentrations (2, 5, 10, 15, 20 μM) dissolved in the running buffer without $ZnCl_2$.

NMR experiments

Peptides at concentration 0.2-2 mM were dissolved in 10 mM bis-Tris-d_{19} **(**2,2-Bis(hydroxymethyl)-2,2′,2″-nitrilotriethanol-d_{19} with 98% ^{2}D enrichment) buffer solution (pH 6.8). Sodium salt of 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid (TSP) at concentration of 20 - 100 μM was added as a standard to all NMR samples. To avoid pH variation upon zinc addition in NMR titration experiments, both peptide and $ZnCl_2$ were dissolved in 20 mM bis-Tris-d_{19} solution followed by the adjustment of pH to 6.8. NMR spectra were measured at the temperature range between 274 K and 308K either in D_2O or in 90% H_2O/10% D_2O on a Bruker AVANCE 600 MHz spectrometer equipped with a triple resonance (^{1}H, ^{13}C and ^{15}N) pulsed field z gradient probe (Bruker Biospin, Germany). 1D NMR spectra were processed by TopSpin 2.0 and Mnova NMR (Mestrelab Research, Spain). 2D NMR spectra were processed by NMRPipe (3) and analyzed using SPARKY (4). Precision of the chemical shift measurement for well resolved signals in the 1D spectra is ~0.001 ppm, and in the 2D spectra ~0.005 ppm. Chemical shifts in Job's titration experiments were measured using the line shape NMR signal fitting by Mnova NMR program. Precision of the chemical shift measurement in this case was better than

~0.0005 ppm. Precision of the measurements of integral intensities in 1D spectra is better than 3%, and in 2D spectra is better than 10%.

NMR signal assignment. The ^{1}H, ^{15}N and ^{13}C signal assignments of the human ^{Ac}H6R-Aβ_{1-16} peptide and its complex with zinc were obtained using the following 2D spectra: DQF-COSY, TOCSY (mixing time of 70 ms), NOESY (mixing time of 200, 225 and 250 ms), ^{13}C-^{1}H HSQC and ^{15}N-^{1}H HSQC. Heteronuclear experiments were measured at the natural abundance of the ^{13}C and ^{15}N isotopes (Fig. S1). ^{15}N resonance assignments were obtained for free peptides only, as the substantial zinc-induced signal broadening complicates collection of the heteronuclear correlation spectra at natural abundance of ^{15}N. However resonance assignments for nearly all ^{1}H and ^{13}C nuclei were determined for all studied peptides and their complexes (Table S1-S3).

NMR titration experiments. Zinc binding to ^{Ac}H6R-Aβ_{1-16} was elucidated using two types of NMR titration experiments. In the first series of experiments 1.1 mM peptide solution in 90% H_2O/10% D_2O and 10 mM bis-Tris-d_{19} (pH 6.88) was titrated by the 4 mM solution of $ZnCl_2$ dissolved in the same buffer with identical pH value. TSP at concentration of ~ 40 μM was added as a standard. Molar ratio of zinc/peptide in these experiments was successively increased from 0 to 10. For each titration point 1D proton NMR spectra were recorded at 283K.

RMSD for the chemical shifts changes between the free and zinc-bound peptides for residue k were calculated using the following equation (1):

$$RMSD_k = \sqrt{\frac{\sum_{i=0}^{N_k}\left[\frac{\delta^i_{complex}-\delta^i_{free}}{A}\right]^2}{N_k}} \qquad (1)$$

,where N_k – number of measured resonances for the residue k, A - normalization factor (1 for ^{1}H and 4 for ^{13}C shifts) to take into account the difference in chemical shift ranges between ^{1}H and ^{13}C resonances.

Titration experiments of the second type were carried out using the method of continuous variations (5) in order to determine stoichiometry of zinc binding to ^{Ac}H6R-Aβ_{1-16} peptide. Experiments involved preparation of the series of samples containing peptide and $ZnCl_2$ in varying proportions but at a fixed total concentration ([peptide] + [zinc] = 0.6 mM). Changes of the peptide chemical shifts or integral intensities of the signals were analyzed. Plot of the product of change of the measured parameter and portion of the peptide ($P_{peptide}\cdot\Delta\delta$) versus portion of zinc (P_{zinc}) for each sample forms a curve with a maximum at P_{zinc} which corresponds to the stoichiometry of the complex (5).

Figure S1. ^{1}H-^{15}N HSQC spectrum of ^{Ac}H6R-Aβ_{1-16} recorded at a natural abundance of the ^{15}N nuclei at 283K. The peptide concentration was ~2.5 mM. Spectrum was recorded in H_2O in the presence of 10 mM bis-Trid-d_{19} buffer, pH 6.8.

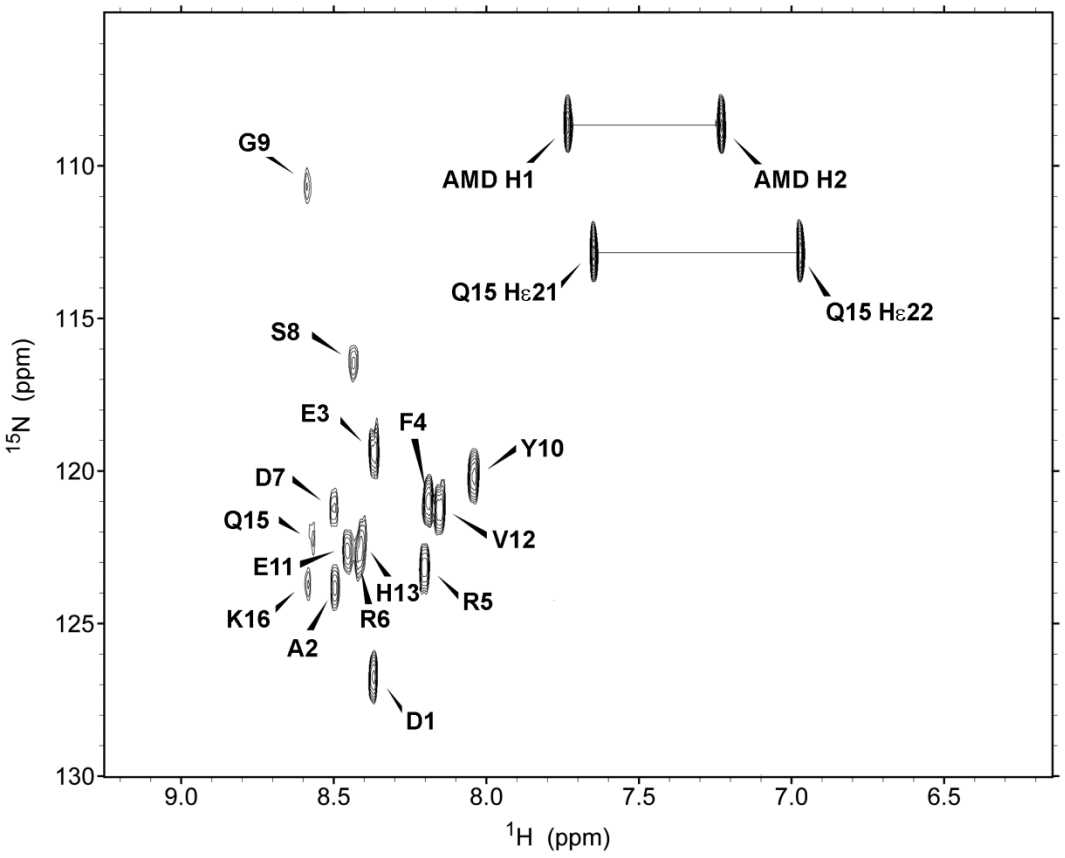

Figure S2. Aliphatic region (0.0 – 4.75 ppm) of the ^{1}H NMR spectra of the human ^{Ac}H6R-A$\beta_{1\text{-}16}$ peptide in the presence of half mole equivalent of $ZnCl_2$. Spectra shown in black, blue and red correspond to the peptide concentration of 0.6, 1.1 and 2.2 mM and TSP concentration of 30, 60 and 60 µM. Spectra were recorded in H_2O, 10 mM bis-Tris$_{d19}$, pH 6.8, 283K and 600 MHz. Intensity of the signal at ~0.2 ppm is proportional to the content of the dimeric form of the zinc-peptide complex Zn·(^{Ac}H6R-A$\beta_{1\text{-}16}$)$_2$.

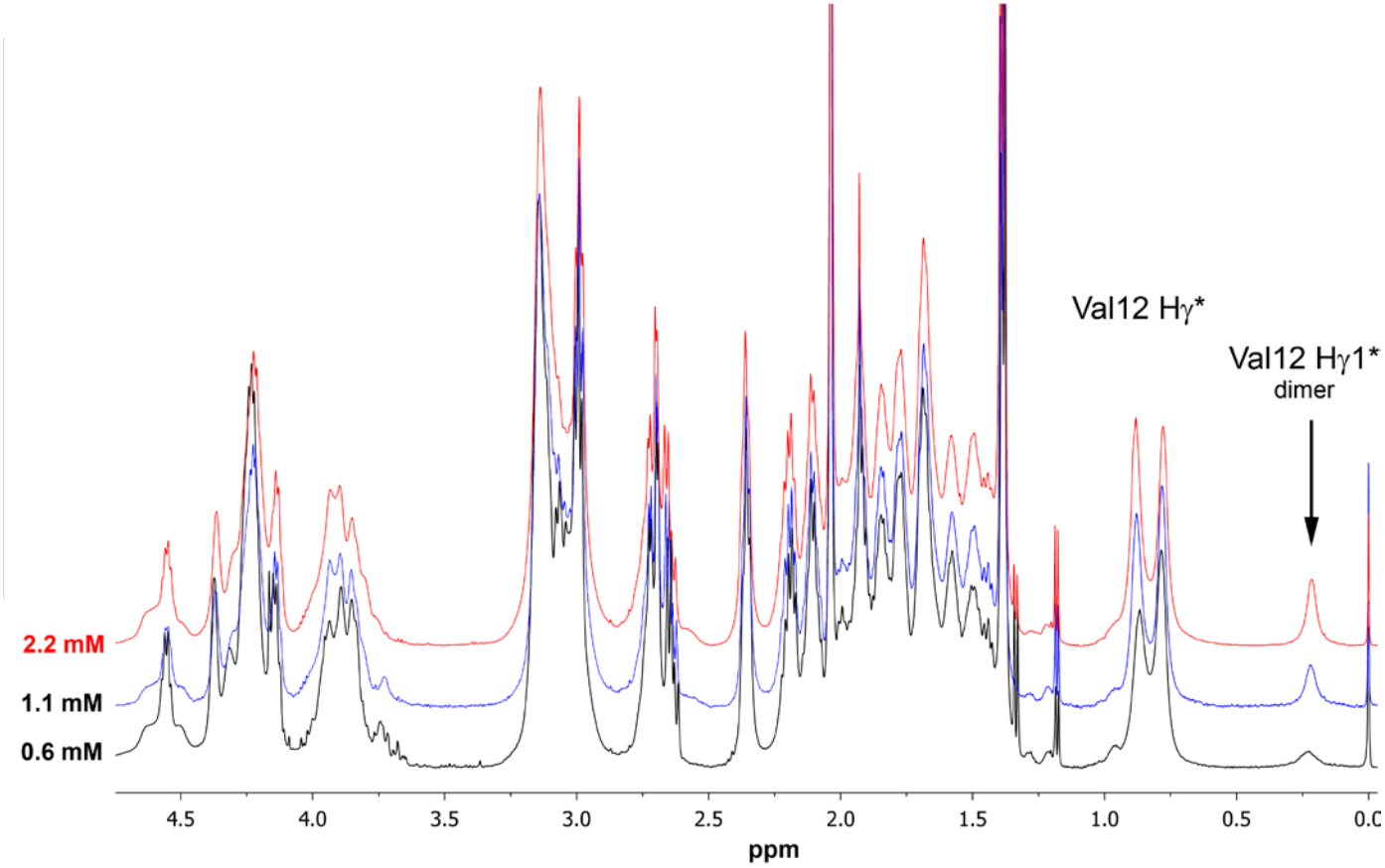

Figure S3. Titration of the human ^{Ac}H6R-A$\beta_{1\text{-}16}$ peptide (initial concentration ~1 mM) by $ZnCl_2$ in H_2O, 10 mM bis-Tris$_{d19}$, 40 µM TSP, pH 6.8. Spectra were recorded at 283K and 600 MHz. Molar ratio of the peptide and Zn^{2+} is shown for each spectrum.

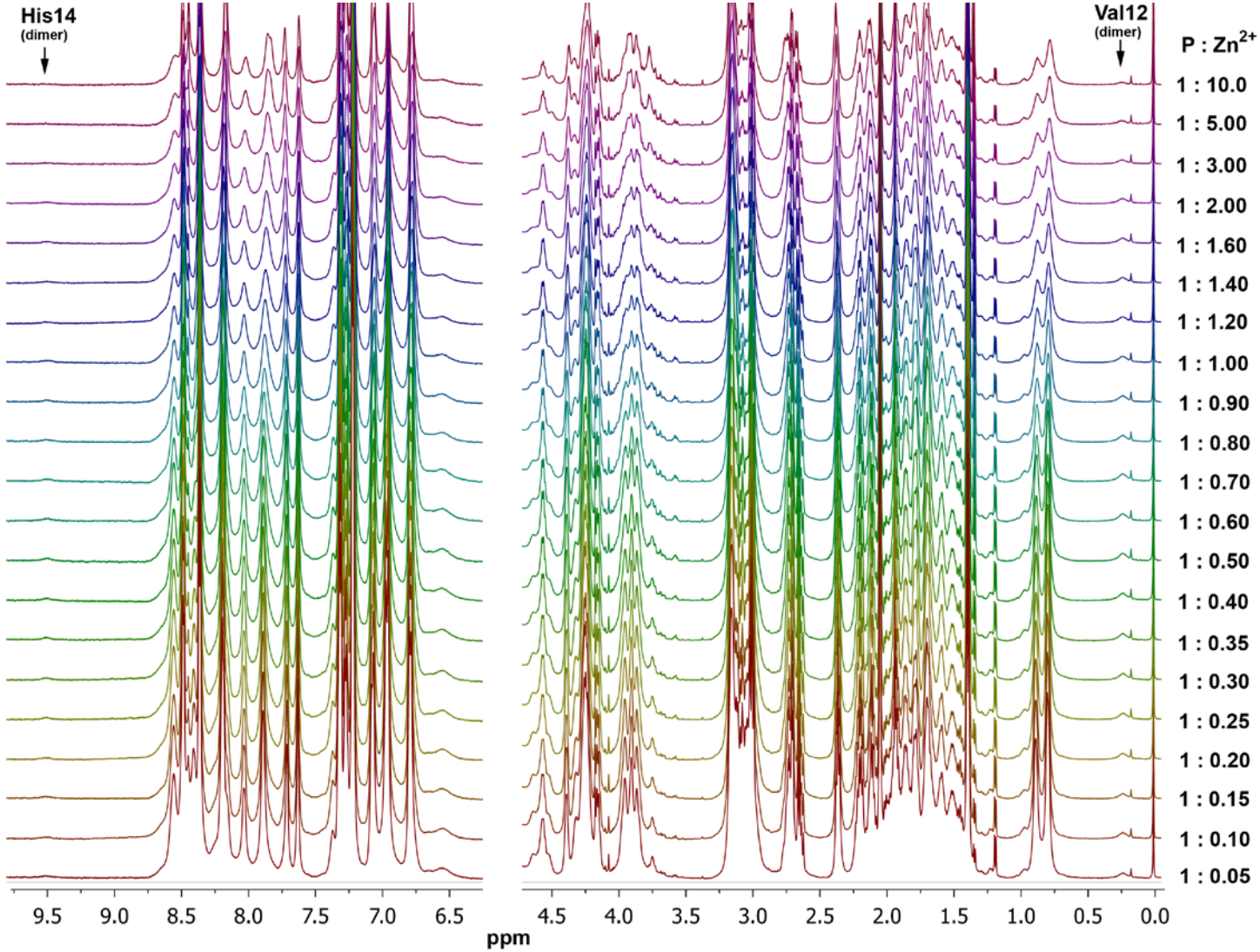

Figure S4. Titration of the human ^{Ac}H6R-Aβ_{1-16} peptide (initial concentration ~1 mM) by $ZnCl_2$ in H_2O, 10 mM bis-Tris$_{d19}$, pH 6.8. Spectra were recorded at 283K and 600 MHz. Figure shows aromatic and amide proton regions with the reduced intensity compared to Fig. S3.

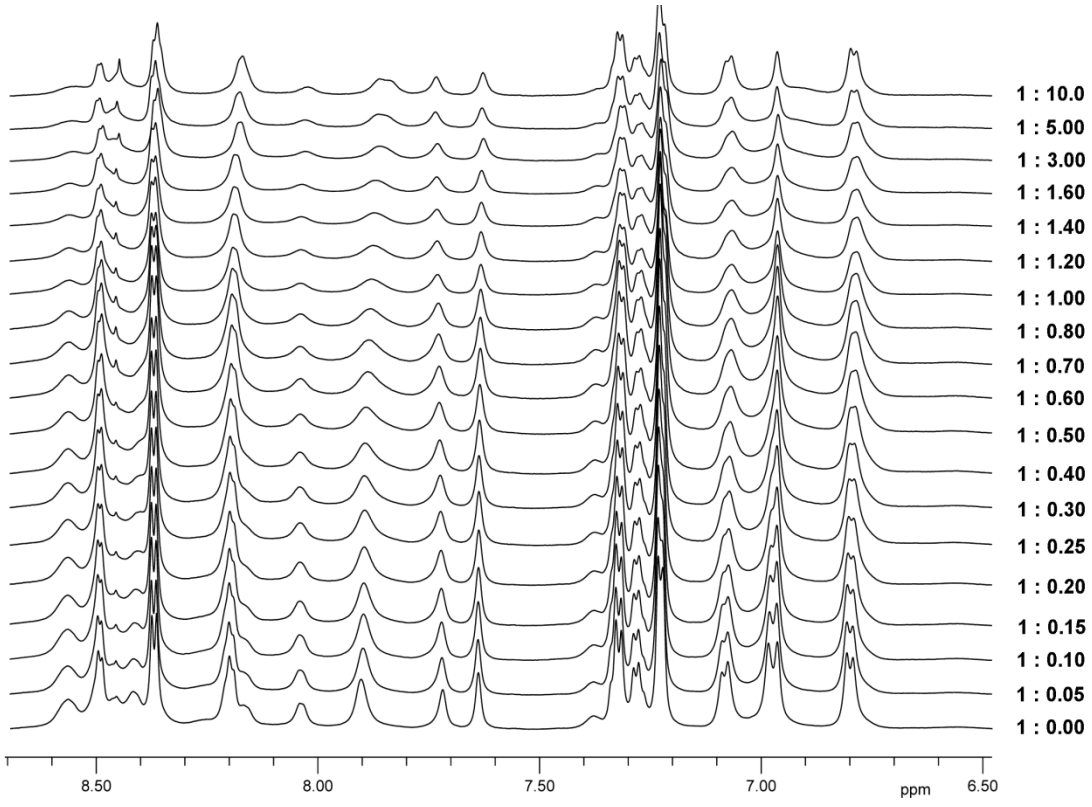

Figure S5. Titration of the human ^{Ac}H6R-A$\beta_{1\text{-}16}$ peptide (initial concentration ~1 mM) by $ZnCl_2$ in D_2O, 10 mM bis-Trisd19, measured at pH 6.85. Spectra were recorded at 283K and 600 MHz.

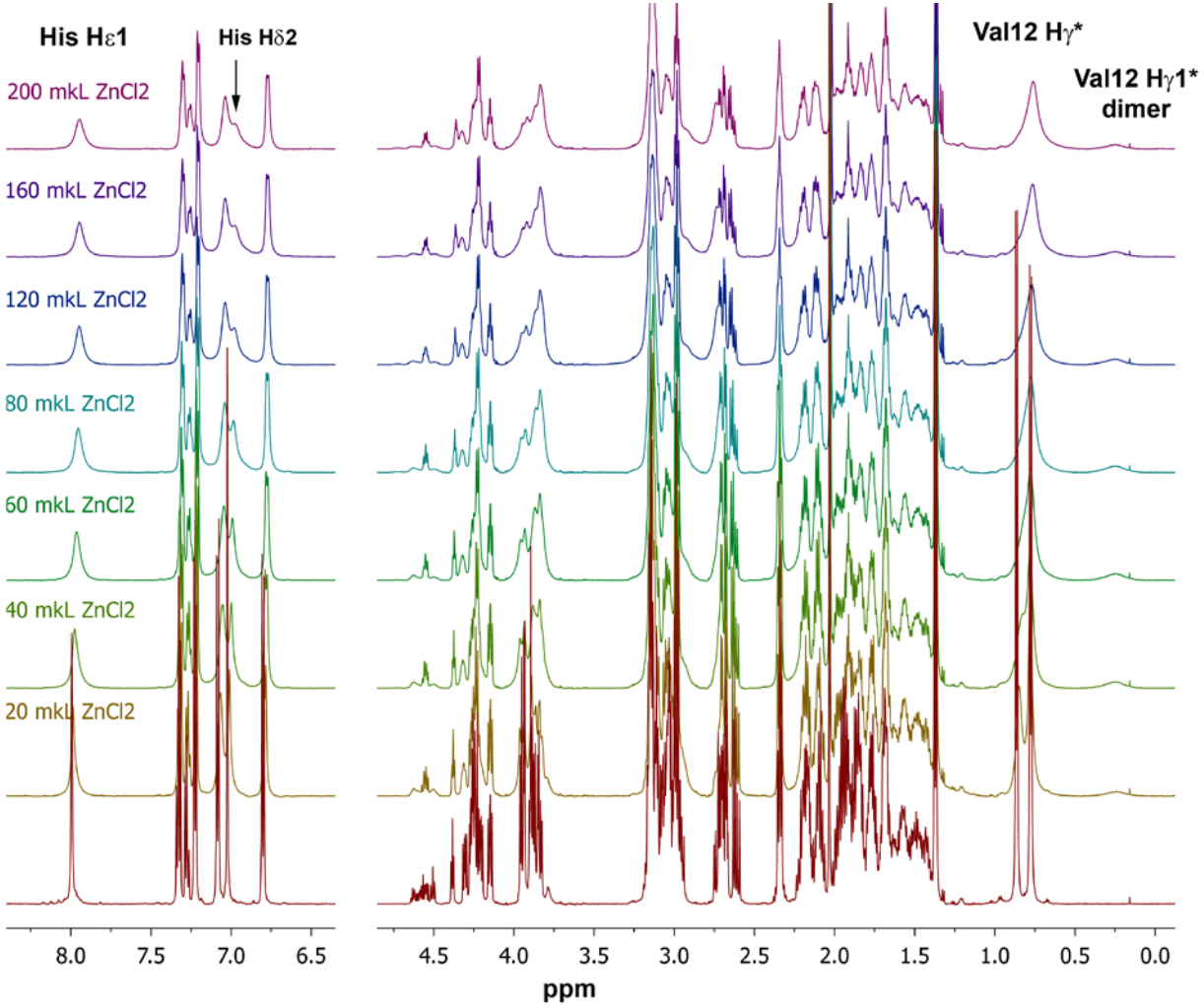

Figure S6. Changes of the chemical shifts of the signals Hε1 and Hδ2 of His13 and His14 residues during titration of the ^{Ac}H6R-Aβ_{1-16} peptide by $ZnCl_2$. Initial concentration of the peptide was 1.1 mM. Final ratio [Zn^{2+}]/[peptide] = 10.

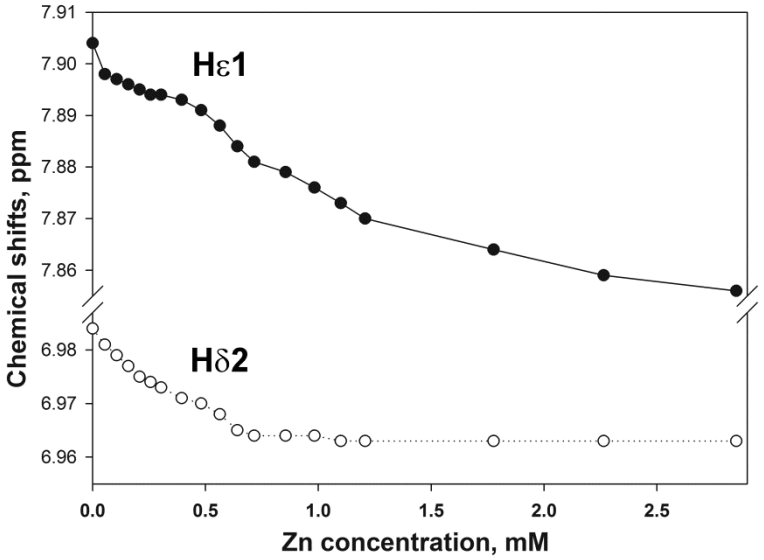

Figure S7. Fragment of ROESY spectrum of ^{Ac}H6R-A$\beta_{1\text{-}16}$ in the presence of $ZnCl_2$. Spectrum was recorded at the peptide concentration 2.25 mM at 274K in H_2O, in the presence of 1.15 mM $ZnCl_2$, 10 mM bis-Tris$_{d19}$, pH 6.86. Signals of Val12 are shown. ROESY mixing time is 100 ms. Positive cross-peaks (red) correspond to chemical exchange, negative cross-peaks (blue) arise from through-space dipole-dipole interactions of nuclei.

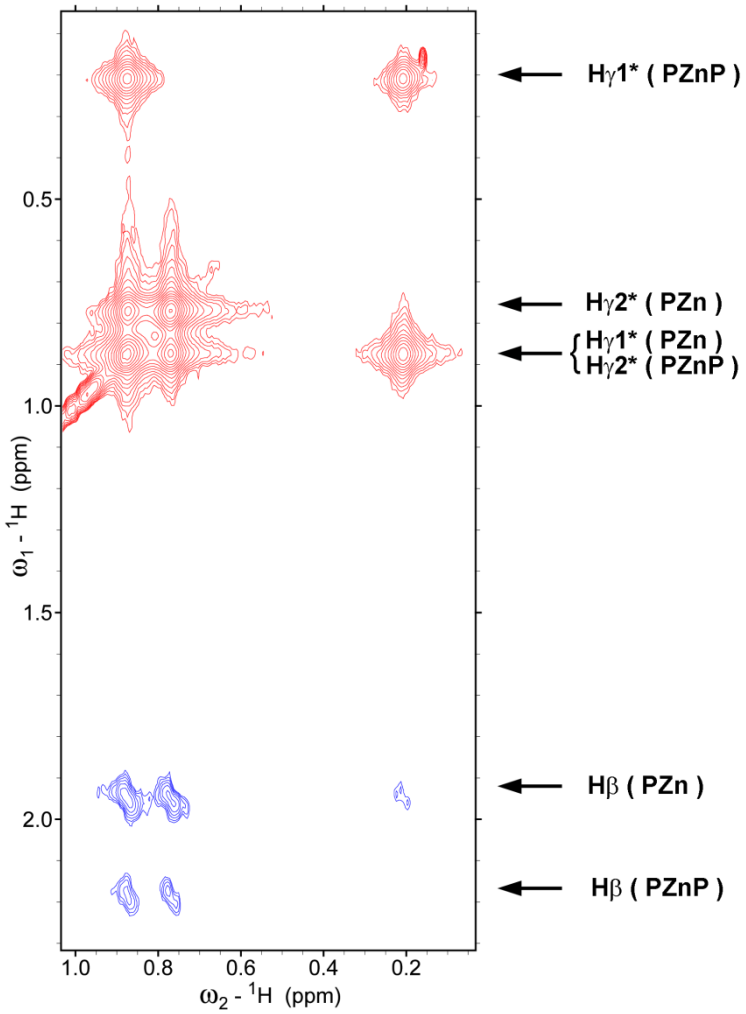

Figure S8. Comparison of 1D spectra of H6R-Aβ_{1-16} (A) and ^{Ac}H6R-Aβ_{1-16} (B) in the presence of half mole equivalent of $ZnCl_2$. Spectra were recorded at the peptide concentration of 1.4mM at 278K in H_2O, in the presence of 10 mM bis-Tris$_{d19}$, pH 6.8. TSP was used as internal standard at the concentration of 20 µM (A) and 60 µM (B).

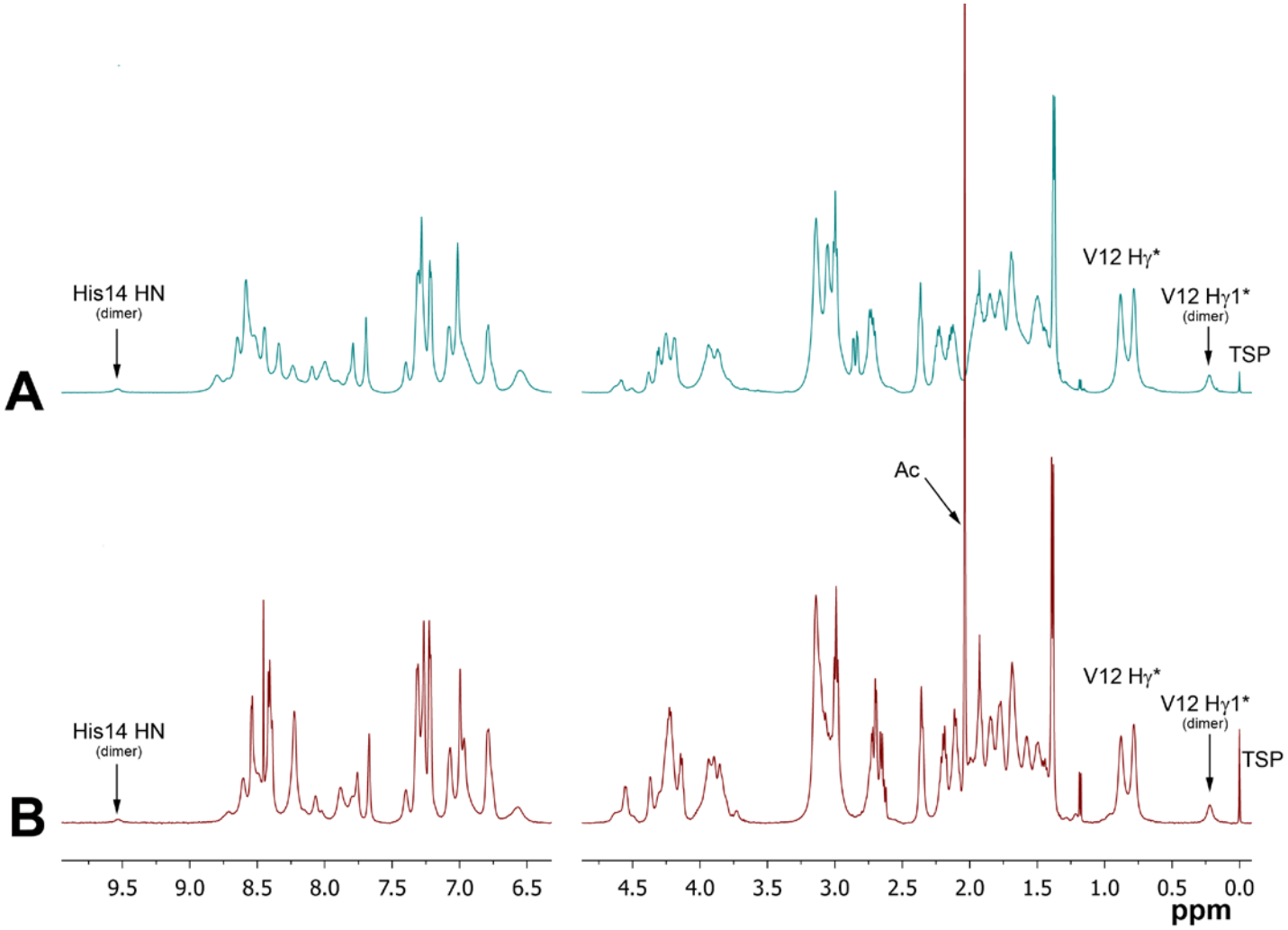

Figure S9. Fragments of 2D NOESY spectra of H6R-Aβ$_{1-16}$ (A) and ^{Ac}H6R-Aβ$_{1-16}$ (B) in the presence of half mole equivalent of $ZnCl_2$. Concentration of the H6R-Aβ$_{1-16}$ peptide is 1.1 mM, concentration of ^{Ac}H6R-Aβ$_{1-16}$ is 2.25 mM. Spectra were recorded at 600 MHz, 274K in H_2O, in the presence of 1.15 mM $ZnCl_2$, 10 mM bis-Tris$_{d19}$, pH 6.80 (A) and 6.86 (B). Regions of His14 HN resonance which belongs to the dimeric forms are shown. Identical NOE patterns indicate identical properties of N-Ac and non-acylated peptides.

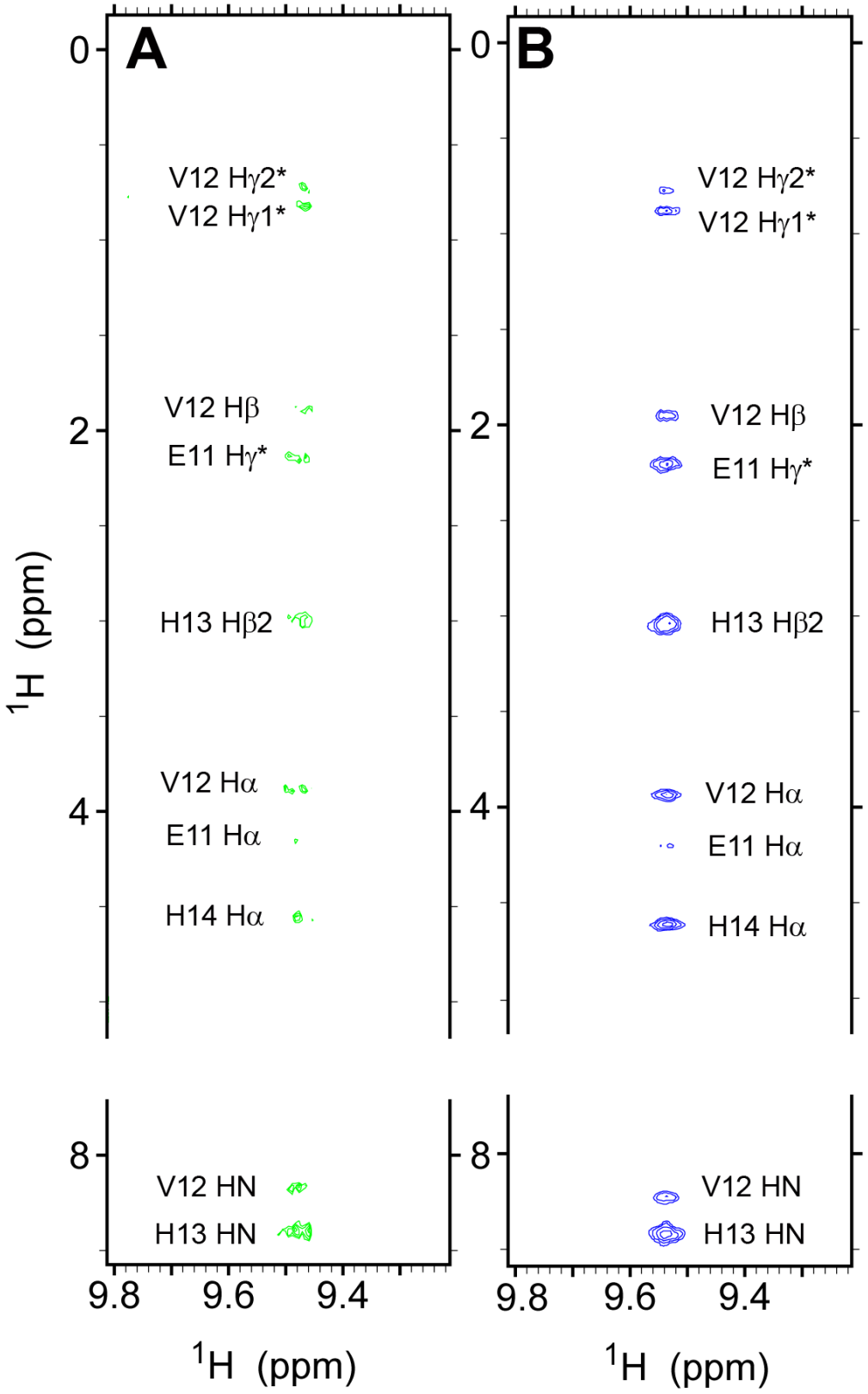

Figure S10. Comparison of 1D spectra of ^{Ac}H6R-Aβ_{1-16} (A) and wild-type human ^{Ac}Aβ_{1-16} (B) in the presence of half mole equivalent of $ZnCl_2$. Spectra were recorded at the peptide concentration of 1.4mM at 278K in H_2O, in the presence of 10 mM bis-Tris$_{d19}$, pH 6.8. TSP was used as internal standard at the concentration of 60 µM (A) and 40 µM (B).

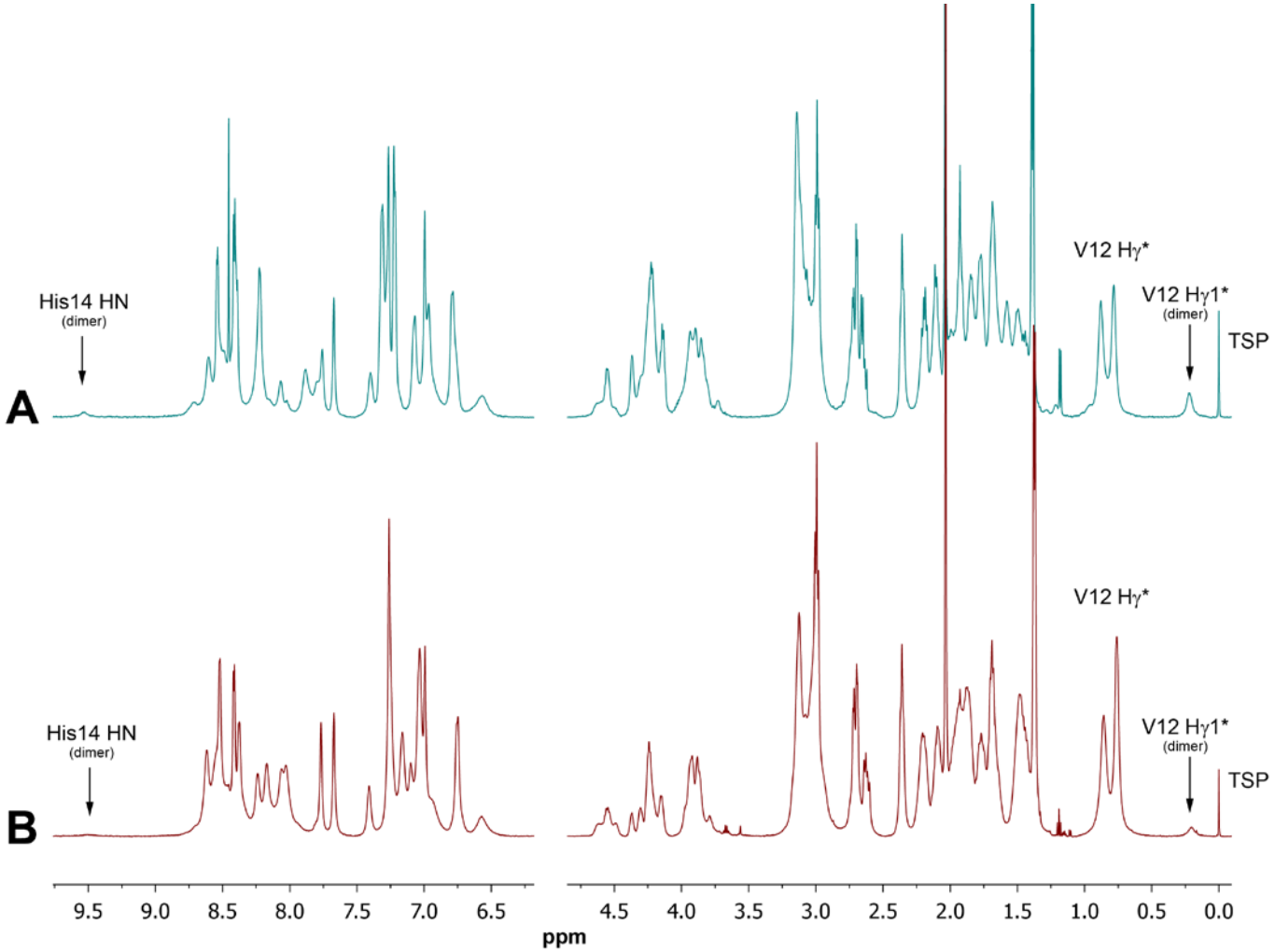

Table S1. Thermodynamic parameters of zinc ions binding to ^{Ac}H6R-Aβ_{1-16} and H6R-Aβ_{1-16} obtained by ITC at 25°C in 50 mM Tris buffer, pH 7.3.

Peptide	N^a	K_a ^{b}×10^{-4} (M^{-2}) ^{c}	ΔH ^{a} (kcal M^{-1})	$T\Delta S$ (kcal M^{-1})
^{Ac}H6R-Aβ_{1-16}	0.5	0.24	-5.6	-1
H6R-Aβ_{1-16}	0.5	0.23	-3.1	1.5

[a]Standard deviation does not exceed ±10%.

[b]Standard deviation does not exceed ±20%.

[c]Due to peptide dimerization, K_a dimension is equal to M^{-2}.

Table S2. Chemical shifts (ppm) of the ^{1}H, ^{13}C and ^{15}N signals of free peptide ^{Ac}H6R-Aβ_{1-16}.

residue	^{15}N	Cα	Cβ	other ^{13}C,^{15} N	HN	Hα	Hβ	Hγ	other ^{1}H
D1	126.72	54.58	41.28		8.37	4.55	2.70 2.63		
A2	123.82	53.02	18.91		8.50	4.23	1.38		
E3	119.34	56.89	29.89	36.15 (Cγ)	8.37	4.14	1.91	2.19 2.10	
F4	121.00	57.91	39.27	131.74 (Cδ*) 131.32 (Cε*) 129.84 (Cζ)	8.19	4.56	3.11 3.07		7.23 (Hδ*) 7.33 (Hε*) 7.28 (Hζ)
R5	123.14	55.94	30.73	27.10 (Cγ) 43.11 (Cδ) 84.62 (Nε)	8.20	4.22	1.78 1.69	1.57 1.52	3.15 (Hδ*) 7.37 (Hε)
R6	122.83	56.04	30.60	27.03 (Cγ) 43.11 (Cδ) 84.55 (Nε)	8.42	4.25	1.84 1.77	1.64 1.59	3.15 (Hδ*) 7.28 (Hε)
D7	121.19	54.22	41.18		8.50	4.64	2.74 2.70		
S8	116.37	58.96	63.53		8.44	4.38	3.90 3.85		
G9	110.69	45.25			8.58	3.95 3.90			
Y10	120.18	58.25	38.75	133.13 (Cδ*) 118.02 (Cε*)	8.04	4.50	3.04 2.97		7.09 (Hδ*) 6.80 (Hε*)
E11	122.60	56.47	30.24	36.15 (Cγ)	8.46	4.20	1.93 1.87	2.21 2.17	
V12	121.21	62.73	32.46	20.69 (Cγ1) 20.78 (Cγ2)	8.15	3.93	1.95	0.88 0.78	
H13	122.23	55.81	30.25	119.54 (Cδ2) 137.64 (Cε1)	8.41	4.63	3.10 3.04		7.01 (Hδ2) 7.95 (Hε1)
H14	121.11	56.01	30.37	119.54 (Cδ2) 137.64 (Cε1)	8.35	4.59	3.12 3.02		7.01 (Hδ2) 7.95 (Hε1)
Q15	122.17	55.73	29.22	33.54 (Cγ) 112.86 (Nε2)	8.57	4.31	2.11 1.99	2.36	7.65 (Hε21) 6.97 (Hε22)
K16	123.71	56.19	32.95	24.84 (Cγ) 28.93 (Cδ) 41.77 (Cε)	8.59	4.26	1.86 1.79	1.49 1.44	1.69 (Hδ*) 2.99 (Hε*)

Table S3. Chemical shifts (ppm) of the ^{1}H, ^{13}C and ^{15}N signals of the binary complex of peptide ^{Ac}H6R-Aβ_{1-16} with Zn^{2+}.

residue	^{15}N	Cα	Cβ	other ^{13}C	HN	Hα	Hβ	Hγ	other ^{1}H
D1	126.92	54.51	41.17		8.41	4.56	2.71 2.65		
A2	123.84	53.21	18.92		8.54	4.22	1.38		
E3	119.47	56.95	29.79	35.95 (Cγ)	8.39	4.14	1.92	2.20 2.12	
F4	121.12	58.16	39.18	131.72 (Cδ*) 131.37 (Cε*) 129.80 (Cζ)	8.21	4.54	3.12 3.07		7.21 (Hδ*) 7.30 (Hε*) 7.26 (Hζ)
R5	123.11	56.36	30.66	27.32 (Cγ) 43.10 (Cδ) 84.42 (Nε)	8.20	4.19	1.79 1.70	1.58 1.51	3.14 (Hδ*) 7.39 (Hε)
R6	122.67	56.11	30.55	27.10 (Cγ) 43.10 (Cδ) 84.35 (Nε)	8.39	4.23	1.85 1.78	1.65 1.58	3.15 (Hδ*) 7.29 (Hε)
D7	121.31	54.13	40.77		8.49	4.65	2.76 2.70		
S8	116.90	58.90	63.45		8.45	4.35	3.91 3.84		
G9	110.80	45.12			8.57	3.92			
Y10	120.23	58.24	38.92	133.11 (Cδ*) 117.97 (Cε*)	8.06	4.49	3.04 2.95		7.07 (Hδ*) 6.78 (Hε*)
E11		55.98	29.92	35.78 (Cγ)	8.47	4.20	1.92 1.88	2.20	
V12		62.62		20.63 (Cγ1) 20.69 (Cγ2)	8.21	3.93	1.95	0.87 0.76	
H13		55.94			8.41	4.61			6.97 (Hδ2) 7.89 (Hε1)
H14		55.96			8.19	4.57	3.15 3.10		6.97 (Hδ2) 7.89 (Hε1)
Q15		55.69	29.44	33.55 (Cγ) 113.17 (Nε2)	8.54	4.32	2.11 2.00	2.37	7.67 (Hε21) 7.00 (Hε22)
K16	123.83	56.28	32.94	24.85 (Cγ) 28.98 (Cδ) 41.73 (Cε)	8.63	4.25	1.84 1.79	1.49 1.44	1.69 (Hδ*) 3.00 (Hε*)

Table S4. Chemical shifts (ppm) of the ^{1}H, ^{13}C and ^{15}N signals of the ternary complex of human peptide ^{Ac}H6R-Aβ_{1-16} dimer with Zn^{2+}. Only the signals which have chemical shift values different from the monomeric zinc-peptide complex are shown in the table.

residue	Cα	Cβ	other ^{13}C	HN	Hα	Hβ	Hγ	other ^{1}H
G9	44.92			8.57	4.01			
Y10	57.04	38.97	132.69 (Cδ*) 118.05 (Cε*)	8.18	4.75	2.79		6.98 (Hδ*) 6.76 (Hε*)
E11	56.37			8.23	3.99			
V12	60.90		16.34 (Cγ1)	7.78	4.61	2.19	0.23 0.87	
H13	57.04			9.54	4.76	3.03		8.02 (Hε1)
H14								8.02 (Hε1)
Q15			33.34 (Cγ)	8.79	4.36	2.12 2.00	2.27 2.20	
K16		32.32		8.74	4.25	1.96		

Table S5. The data used for Job's plot (Fig. 4). Total concentration of [peptide] and [Zn^{2+}] was kept at 0.6 mM in all samples. Spectra were recorded in D_2O solution of 10 mM bis-$Tris_{d19}$, with pH 6.9. TSP was used as internal standard for measurement of chemical shits. The values of chemical shifts were determined by line shape fitting of the signals using Mnova NMR software (Mestrelab Research, Spain). Relative $Val12^{dimer}$ intensity is the ratio of the well-resolved integral intensity of the signal at ~0.2 ppm to the integral intensity of all signals Val12 for the methyl groups (0.2 – 0.9 ppm).

Sample	**Zn portion (pZn)**	**Peptide portion (pP)**	**Chemical shift of His Hδ, ppm**	**Δδ(His Hδ), ppm**	**10·Δδ·pP**	**Relative $Val12^{dimer}$ intensity (I^{Val12})**	**ΔI^{Val12}·pP**
1	0.000	1.000	7.0060	0.0000	0.0000	0.000	0.0000
2	0.125	0.875	7.0017	0.0043	0.0375	0.050	0.0438
3	0.250	0.750	6.9950	0.0110	0.0825	0.082	0.0615
4	0.334	0.666	6.9880	0.0180	0.1199	0.103	0.0686
5	0.375	0.625	6.9850	0.0210	0.1312	0.106	0.0663
6	0.500	0.500	6.9780	0.0280	0.1400	0.110	0.0550
7	0.625	0.375	6.9750	0.0310	0.1163	0.100	0.0375
8	0.750	0.250	6.9718	0.0342	0.0855	0.080	0.0200

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