Electronic Supplementary Material (ESI) for Molecular BioSystems.

Supplemental Information

Phosphorylation of Ser8 promotes a novel topology of zinc-induced dimerization of amyloid-β metal-binding domain

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Fig. S1. Fragment of NOESY spectrum (200 ms mixing time) of pAβ(1-16) at concentration 1.4 mM, recorded at 10˚C in 10 mM Bis-Tris-d_{19}, 90% H_2O/10% D_2O, pH 6.9. The figure shows assignments of sequential correlations between backbone amide protons.

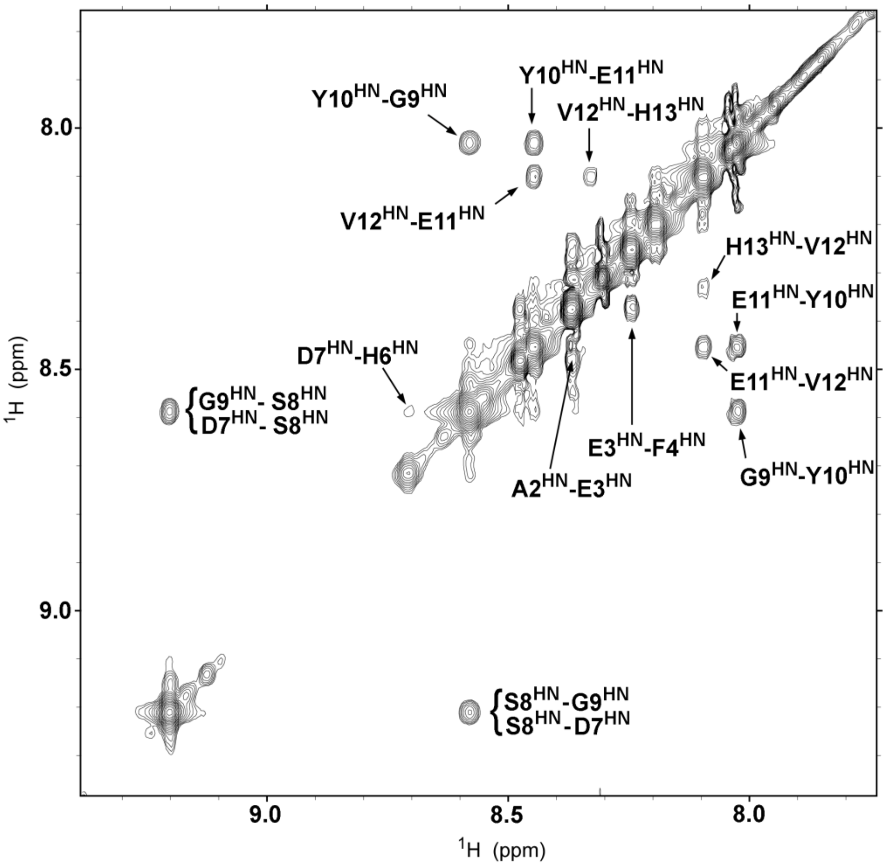

Figure S2. 600 MHz [1]H NMR spectra of pAβ(1-16) at concentration 1.8 mM in its free state and after addition of twofold molar excess of $ZnCl_2$. Spectra were collected at 10˚C in 10 mM Bis-Tris-d_{19}, 90% H_2O/10% D_2O, pH 6.9.

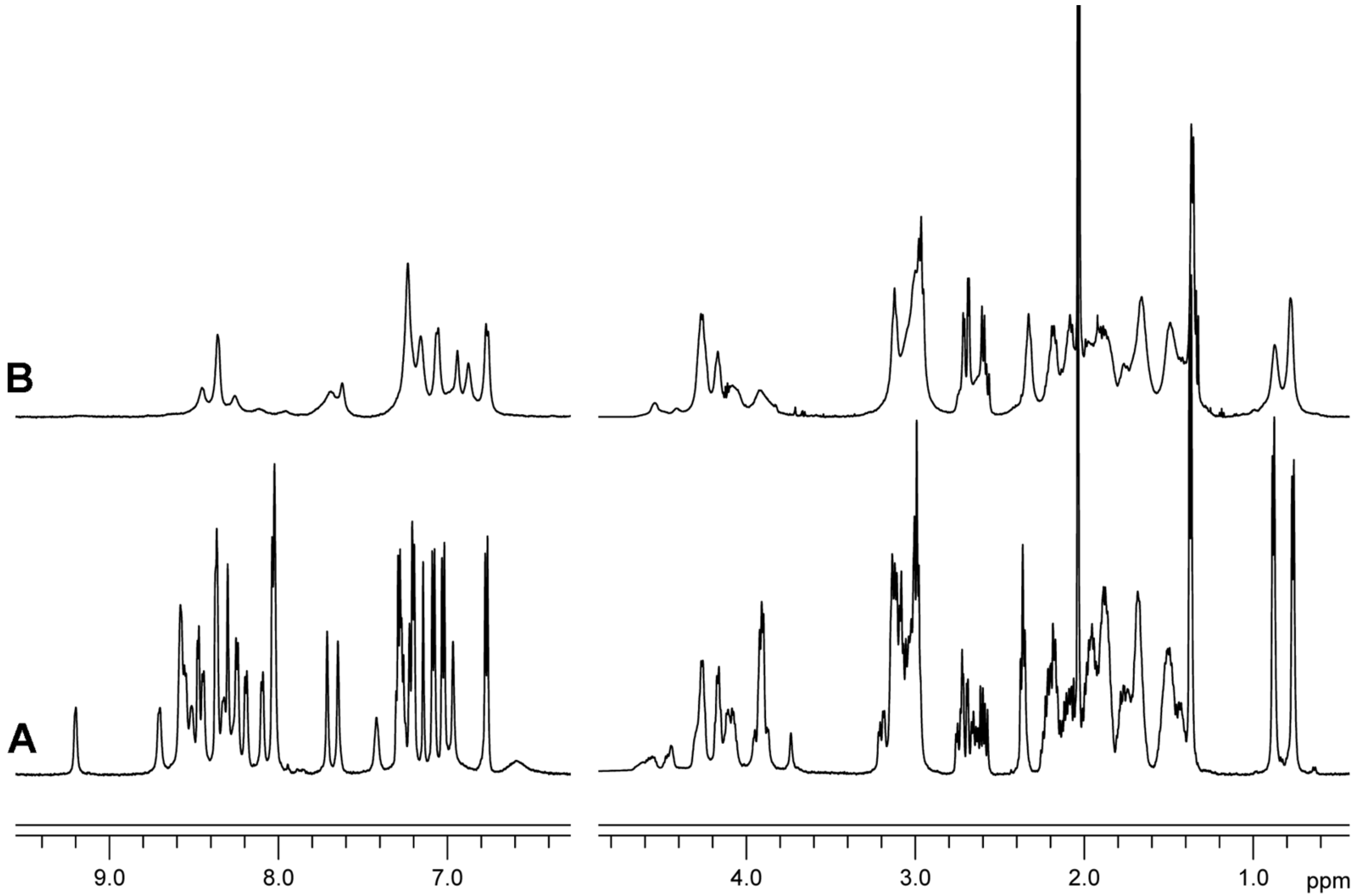

Figure S3. Amide region (6.7 – 9.2 ppm) of the NMR spectra of pAβ(1-10) at concentration 0.5 mM for the free peptide and series of samples with increased concentration of Zn^{2+}. Spectra were collected in 10 mM Bis-Tris-d_{19}, 90% H_2O/10% D_2O, pH 6.8. Labels on the right-hand side represent molar ratio of [peptide]:[Zn^{2+}] in each sample. Assignments of the representative resonances are shown.

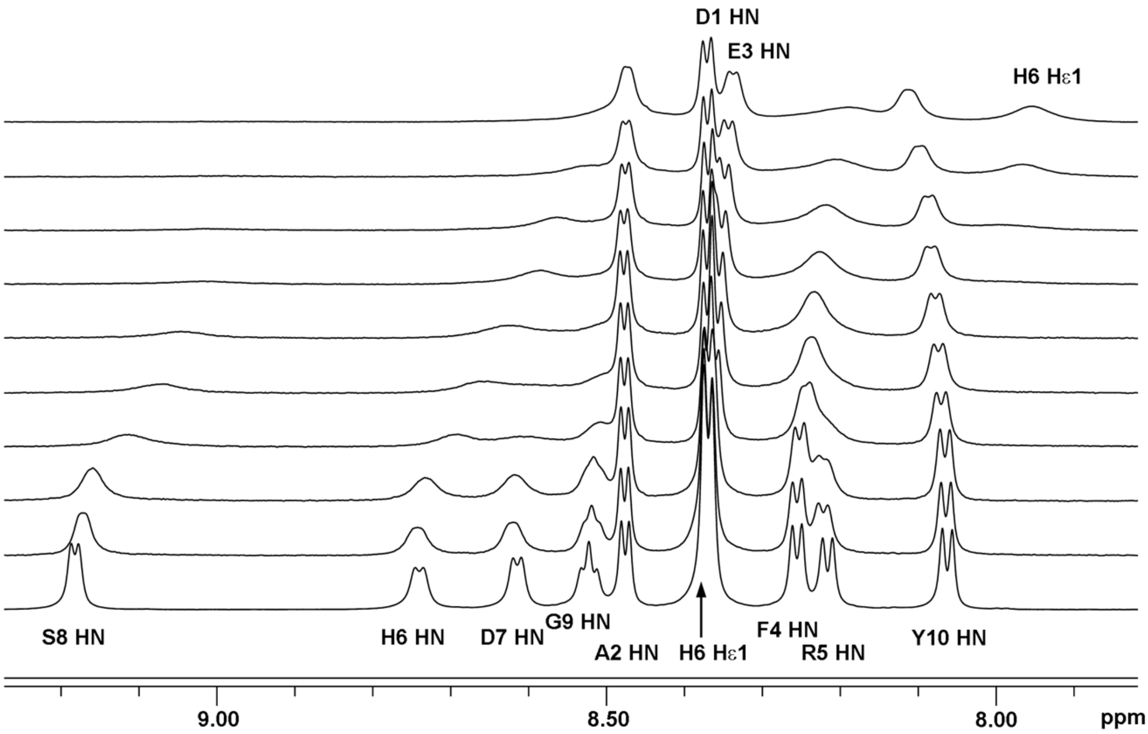

Figure S4. Representative regions of the NMR spectra of Aβ(11-16) at concentration 0.5 mM for the free peptide and series of samples with increased concentration of Zn^{2+}. Spectra were collected in 10 mM Bis-Tris-d_{19}, 100% D_2O, at measured pD 7.2. Labels on the right-hand side represent molar ratio of [peptide]:[Zn^{2+}] in each sample. Assignments of the representative resonances are shown.

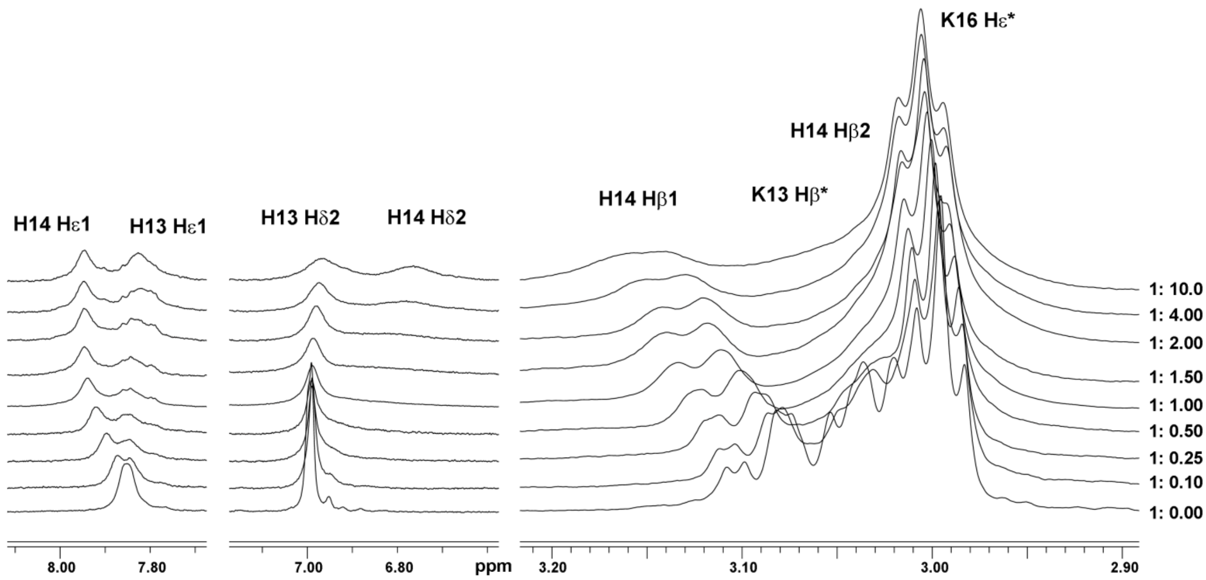

Table S1. Chemical shifts of pAβ(1-16) at concentration 1.8 mM in the free state (black) and in the presence of 5-fold molar excess of $ZnCl_2$ (red). Significant line broadening of the signals in the presence of zinc ions does not allow to detect many signals in NMR spectra of the Zn^{2+}- pAβ(1-16) complex.

Resid.	Chemical shift, ppm											
	^{15}N	HN	Hα	Hβ1	Hβ2	Hγ1	Hγ2	Other ^{1}H	Cα	Cβ	Cγ	Other ^{13}C
D 1	126.9	8.371 8.405	4.550 4.545	2.702 2.702	2.594 2.592	-	-	-	54.492	41.314	-	-
A 2	124.1	8.475 8.504	4.265 4.265	1.369 1.369	-	-	-	-	52.590	19.056	-	-
E 3	119.7	8.367 8.405	4.167 4.171	1.888 1.893	1.888 1.893	2.180 2.185	2.056 2.072	-	56.570	30.135	36.105	-
F 4	121.2	8.245 8.310	4.582 4.541	3.081 3.007	3.009 3.007	-	-	Hδ* 7.199 7.155 Hε* 7.283 7.235 Hζ 7.252	57.450	39.431	-	Cδ 131.722 Cε 131.313 Cζ 129.774
R 5	123.8 Nε 84.33	8.193 8.129	4.264 4.267	1.735 1.702	1.659 1.627	1.518 1.498	1.518 1.498	Hδ* 3.135 3.124 Hε 7.421	55.397	31.082	26.914	Cδ 43.019
H 6	122.0	8.706 NA	4.617 NA	3.194 NA	3.100 NA	-	-	Hδ$_2$ 7.138 Hε$_1$ 8.253	55.569	29.880	-	Cδ$_2$ 119.808 Cε$_1$ 136.834
D 7	123.6	8.581 NA	4.651 NA	2.733 NA	2.654 NA	-	-	-	54.091	41.422	-	
S 8	118.7	9.203 NA	4.440 NA	4.093 NA	4.093 NA	-	-	-	58.593	65.362	-	-
G 9	110.6	8.582 8.685	3.943, 3.879 3.885, 3.885	-	-	-	-	-	45.383	-	-	-
Y 10	120.4	8.026 7.979	4.470 4.490	3.040 3.025	3.040 3.025	-		Hδ* 7.084 7.063 Hε* 6.769 6.773	58.606	38.769	-	Cδ 133.139 Cε 131.313
E 11	121.9	8.446 NA	4.177 NA	1.956 NA	1.885 NA	2.217 NA	2.175 NA	-	56.182	30.138	36.146	-
V 12	120.6	8.098 8.144	3.910 3.912	1.950 1.960	-	0.881 0.879	0.766 0.778	-	63.027	32.344	20.789 20.756	-
H 13	121.4	8.327 NA	4.608 NA	3.081 NA	3.032 NA	-	-	Hδ$_2$ 7.025 NA Hε$_1$ 8.023 NA	55.989	30.302	-	NA
H 14	120.6	8.268 NA	4.567 NA	3.115 NA	2.990 NA	-	-	Hδ$_2$ 7.011 NA Hε$_1$ 7.997 NA	56.204	30.444	-	NA
Q 15	121.7 Nε$_2$ 112.8	8.515 NA	4.297 4.280	2.107 NA	1.991 NA	2.357 2.330	2.357 2.330	Hε$_{21}$ 7.648 7.689 Hε$_{22}$ 6.968 6.879	55.763	29.094	33.568	-
K 16	123.6	8.551 NA	4.261 4.241	1.856 1.848	1.778 1.768	1.487 1.474	1.433 1.427	Hδ* 1.686 1.666 Hε* 2.990 2.970	56.060	32.914	24.859	Cδ 28.917 Cε 41.750

Details of quantum-mechanical/molecular-mechanical calculations

The model[1] of the structure of the complex 2Aβ(11-14)-Zn^{2+} described earlier was used to build the 2pAβ(1-16)-Zn^{+2} model, using the PyMol program (The PyMOL Molecular Graphics System, Version 1.5.0.4 Schrödinger, LLC.). The model was constructed in the parm99sb force field.[2] The model contained parameters corresponding to the geometry of a complex in which zinc atom is coordinated by the Glu11 and His14 residues of both peptides, and the pSer8 and His6 residues. The dimer was put into the center of a triclinic cell with the distance to the borders of 20 Å. Zinc coordination was modeled with corresponding distance restraint. Model was optimized in implicit solvent treatment in cyclic manner. Optimized conformation was subjected to MD simulation in explicit solvent for 100 ns. The GROMACS 4.6 software package[3] was used for simulation and analysis of the trajectories. The simulations in explicit solvent were carried out at 300 K under the control of a velocity rescaling thermostat[4] at constant pressure and using the PME[5] to take into account the electrostatic interactions. The cell was filled with TIP4P water[6] and the negative charge of the system was compensated by sodium ions. The concentration of monovalent ions was set to 0.15 M. The most represented conformations around zinc cation near pSer8 over the last 10ns of the trajectory were selected and their geometry was optimized using the QM/MM method as described by Biswas and Gogonea[7]. The QM system was described in terms of the plane-wave density functional theory (PW-DFT)[8] with a spin polarized formalism and PW91[9] functional. The interactions between valence electrons and the ionic cores are described by ultrasoft VDB pseudopotential. All atoms from His6, Asp7 and pSer8 were included in the QM system. QM/MM geometry optimization was performed with the GROMACS/CPMD package. Since we applied ultrasoft pseudopotentials, the basis set for the valence electrons consists of plane waves expanded up to a cutoff of 30 Ry. The QM subcell had a cubic shape with 40 Ry side length, resulting in about 90.000 plane waves for wavefunction.

References

1 S. A. Kozin, Y. V. Mezentsev, A. A. Kulikova, M. I. Indeykina, A. V. Golovin, A. S. Ivanov, P. O. Tsvetkov and A. A. Makarov, *Mol Biosyst*, 2011, **7**, 1053-1055.

2 K. Lindorff-Larsen, S. Piana, K. Palmo, P. Maragakis, J. L. Klepeis, R. O. Dror and D. E. Shaw, *Proteins*, 2010, **78**, 1950-1958.

3 S. Pronk, S. Pall, R. Schulz, P. Larsson, P. Bjelkmar, R. Apostolov, M. R. Shirts, J. C. Smith, P. M. Kasson, D. van der Spoel, B. Hess and E. Lindahl, *Bioinformatics*, 2013, **29**, 845-854.

4 G. Bussi, D. Donadio and M. Parrinello, *J. Chem. Phys.*, 2007, **126**, 014101.

5 T. Darden, D. York and L. Pedersen, *J. Chem. Phys.*, 1993, **98**, 10089-10092.

6 W. L. Jorgensen, J. Chandrasekhar, J. D. Madura, R. W. Impey and M. L. Klein, *J. Chem. Phys.*, 1983, **79**, 926-935.

7 P. K. Biswas, *J. Chem. Phys.*, 2005, **123**, 164114.

8 M. M. Pant and Rajagopa.Ak, *Solid State Commun*, 1972, **10**, 1157-1160.

9 J. P. Perdew, J. A. Chevary, S. H. Vosko, K. A. Jackson, M. R. Pederson, D. J. Singh and C. Fiolhais, *Physical review. B, Condensed matter*, 1992, **46**, 6671-6687.