Zinc stabilization of prefibrillar oligomers of human islet amyloid polypeptide

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Materials and Methods

Sample Preparation

Preformed aggregates from the samples were removed by dissolving the peptide in hexafluoroisopropanol followed by the removal of the solvent by lyophilization. To improve solubility, the lyophilized peptide was first dissolved in 0.1 mM HCl (pH 4.0) at 4 ºC followed by the addition of concentrated buffer to make 20 mM Tris-HCl solution with 50 mM NaCl at pH 7.3.

EXAFS measurements

Samples of $hIAPP_{1-37}$ and $hIAPP_{1-19}$ for EXAFS measurements were prepared from flash-frozen samples of either 250 μM $IAPP_{1-37}$ with 50 μM Zinc in sterile 20 mM Tris-HCl with 50 mM NaCl at pH 7.3 or 500 μM $IAPP_{1-19}$ with 100 μM Zinc in sterile 20 mM Tris-HCl with 50 mM NaCl at pH 7.3. The data were fit using EXAFSPAK[1] with theoretical amplitude and phase parameters calculated using FEFF 9[2] using two metal-ligand distances, the Debye-Waller factor for the Zn-N/O shell (2.3×10^{-3} Å^2), and ΔE_0 (-6.6 eV) as variable parameters.

AFM measurements

Roughness and morphology of $hIAPP_{1-37}$ aggregates at the nanometer scale were investigated by Atomic Force Microscopy (AFM) on a Multimode Nanoscope IIIA (Veeco) with a 10-μm scanner. Images of 10 μM $hIAPP_{1-37}$ were acquired in tapping mode with a fluid cell filled with 20 mM Tris buffer containing 50 mM NaCl and varying concentrations of $ZnCl_2$ as indicated. Sharpened silicon nitride probes with a radius of 30 nm, and a nominal spring constant, $k = 0.06$ N·m^{-1} were employed (Veeco probes). The roughness estimation was made on scan areas of 5x5 μm^2 using Nanoscope RIII software, in terms of standard deviation of heights (Rq) and arithmetic mean roughness (Ra).

NMR measurements

Spectra were collected on either a 900 MHz Bruker Avance NMR spectrometer equipped with a triple-resonance z-gradient cryogenic probe optimized for ^{1}H-detection (SOFAST-HMQC experiments) or a 500 MHz Bruker Avance II 500 MHz spectrometer equipped with a triple-resonance gradient probe (^{1}H experiments). SOFAST-HMQC spectra of 80 μM $hIAPP_{1-37}$ in solution (20 mM Tris, 50 mM NaCl, pH 7.3) were recorded at 4 °C with 128 t_1 experiments, 16 scans, and a 100 ms recycle delay.[3] 2D data was processed using TOPSPIN 2.1 (from Bruker). Resonance assignment was based on previous assignments.[4]

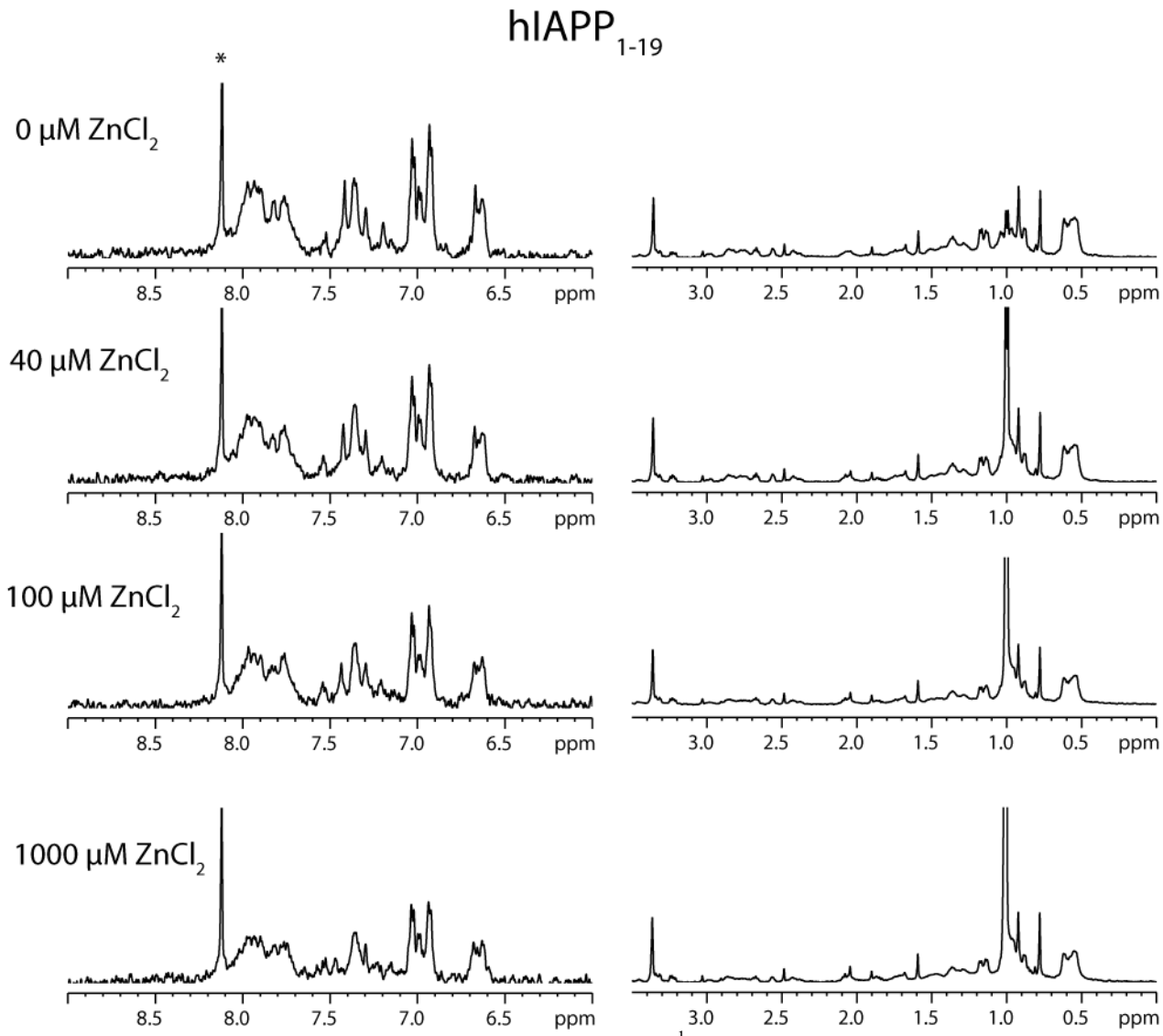

Figure S1. Amide (left) and aliphatic (right) regions of the ^{1}H spectra of 78 µM $hIAPP_{1-19}$ with the indicated concentrations of $ZnCl_{2\ 19}$ in 20 mM Tris-HCl with 50 mM NaCl at 4 ºC. The resonance corresponding to the imidazole side-chain is marked with an asterisk. Aliphatic and amide regions of the spectra are normalized separately.

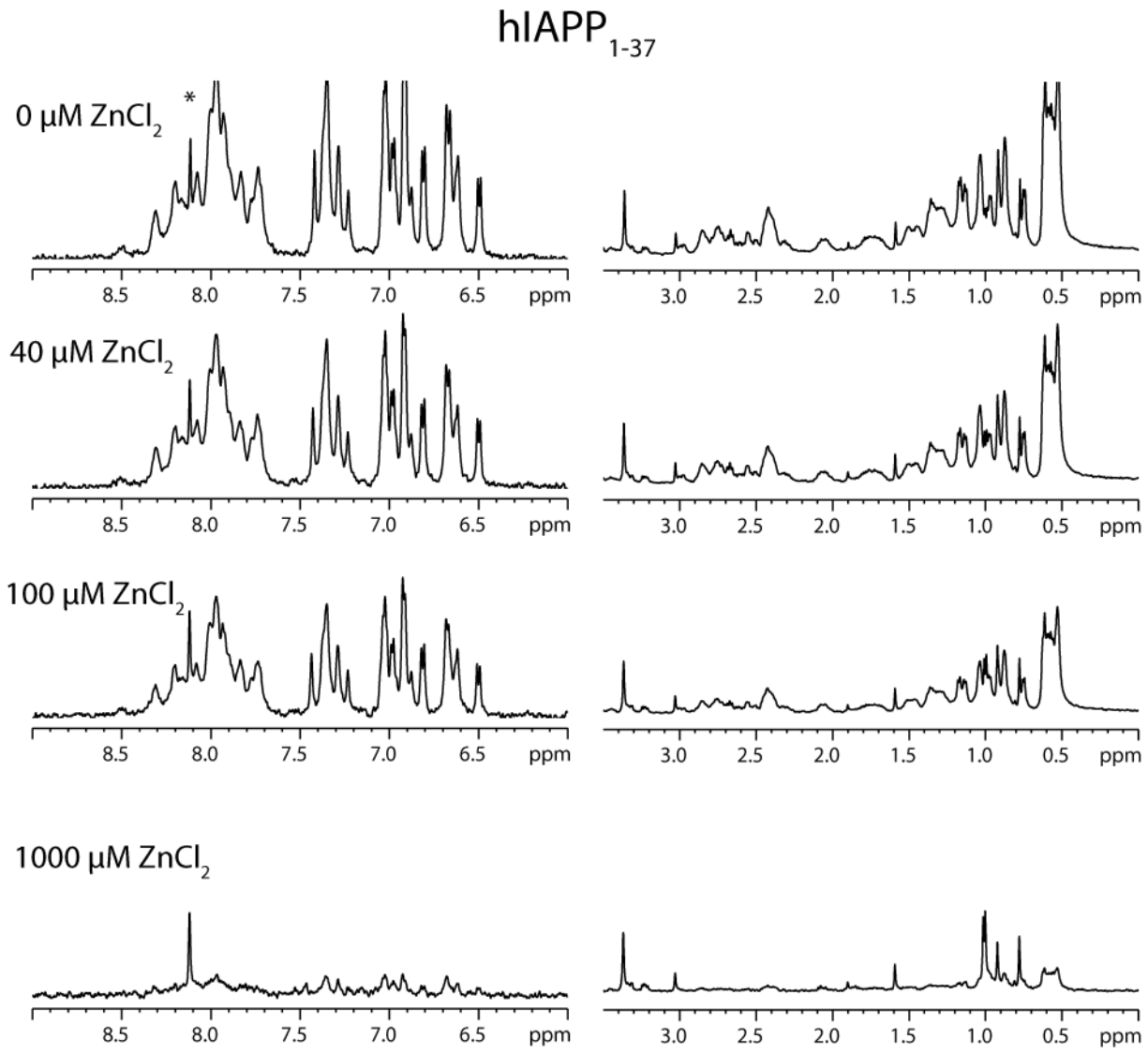

Figure S2. Amide (left) and aliphatic (right) regions of the ^{1}H spectra of 78 μM $hIAPP_{1-37}$ with the indicated concentrations of $ZnCl_2$ in 20 mM Tris-HCl with 50 mM NaCl at 4 ºC. The resonance corresponding to the imidazole side-chain is marked with an asterisk. Aliphatic and amide regions of the spectra are normalized separately.

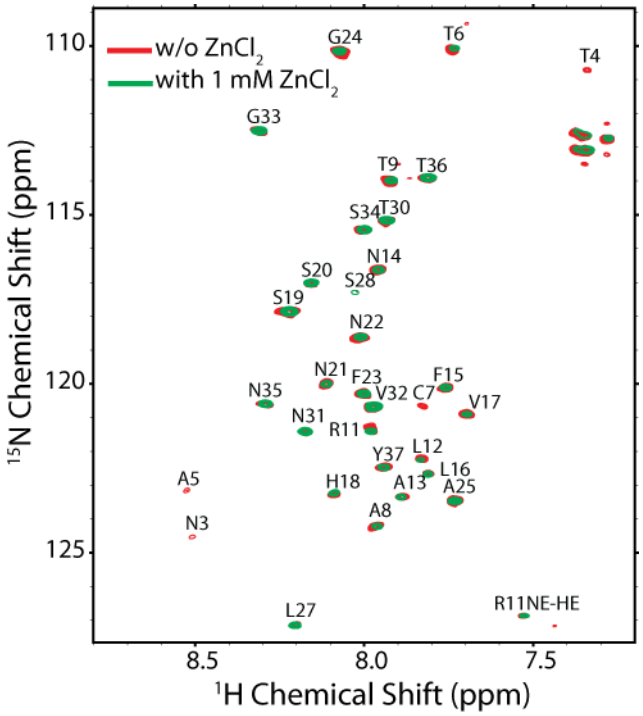

Figure S3. Comparison of the ^{1}H-^{15}N SOFAST spectra of 78 μM $hIAPP_{1-37}$ with and without 1 mM $ZnCl_2$ in 20 mM Tris-HCl with 50 mM NaCl at 4 ºC at the initial time point.

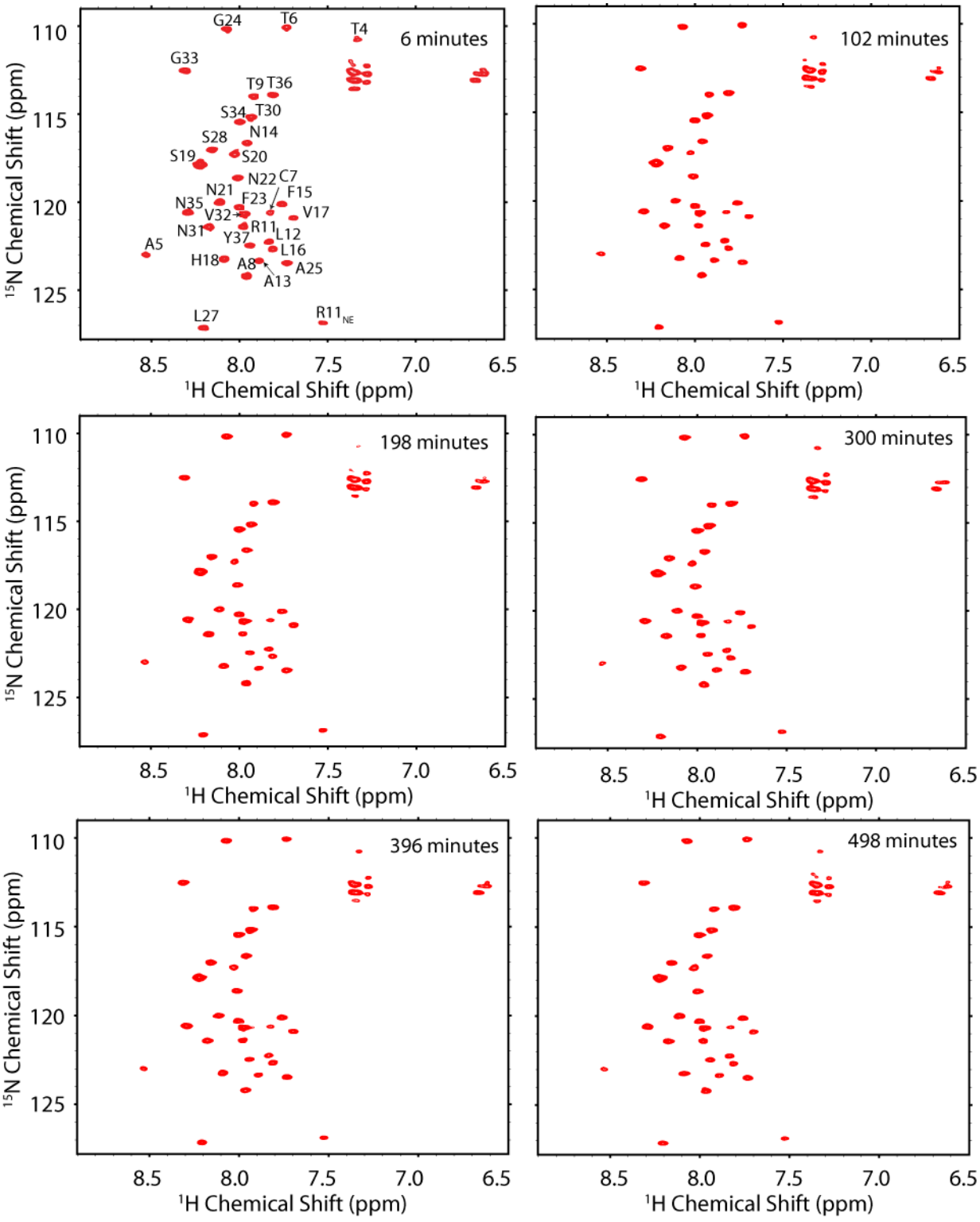

Figure S4. 2D ^{1}H-^{15}N SOFAST spectra of 78 µM $hIAPP_{1-37}$ without $ZnCl_2$ in 20 mM Tris-HCl with 50 mM NaCl at 4 ºC at the indicated time points.

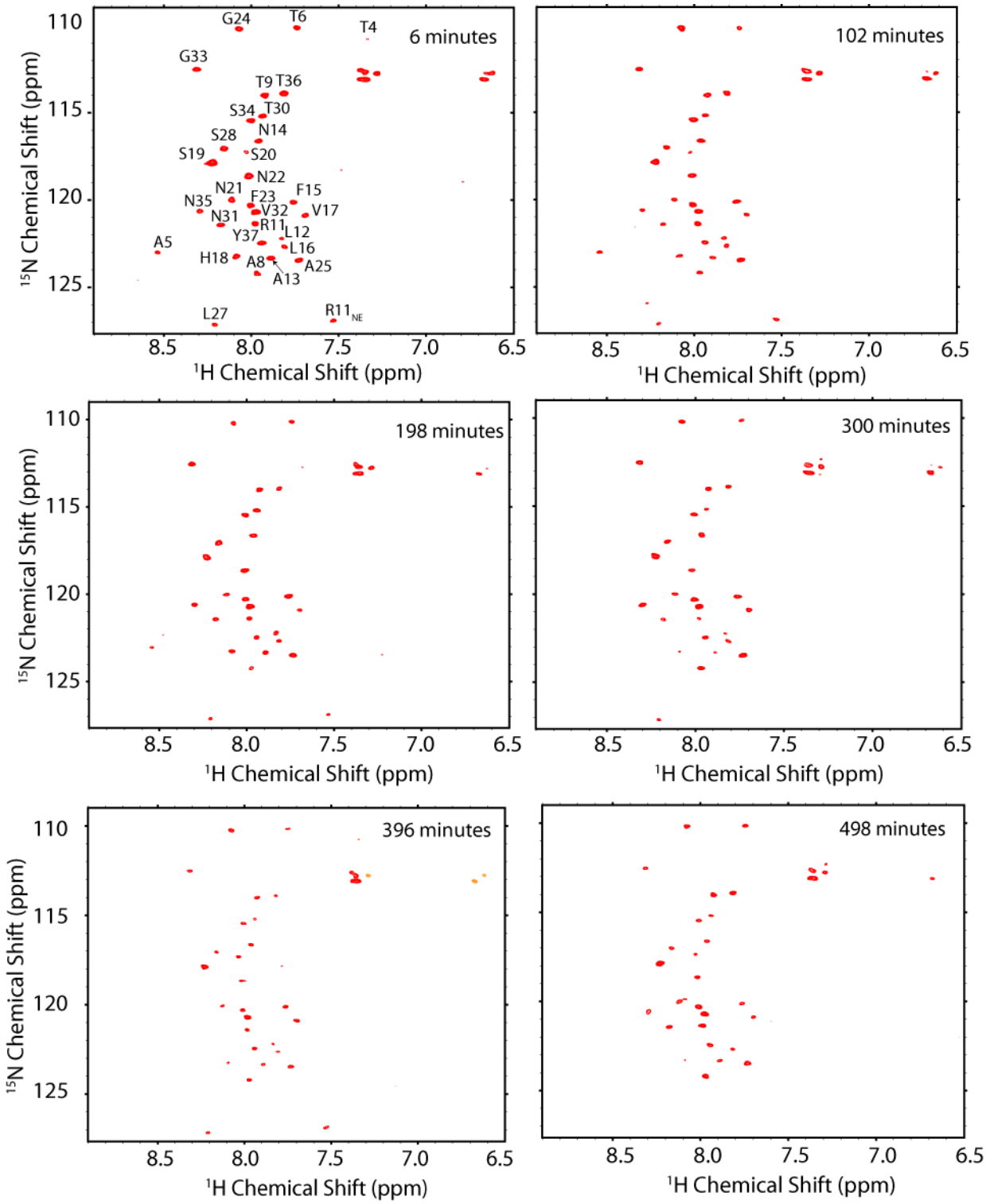

Figure S5. 2D ^{1}H-^{15}N SOFAST spectra of 78 μM $hIAPP_{1-37}$ with 1mM $ZnCl_2$ in 20 mM Tris-HCl with 50 mM NaCl at 4 ºC at the indicated time points.

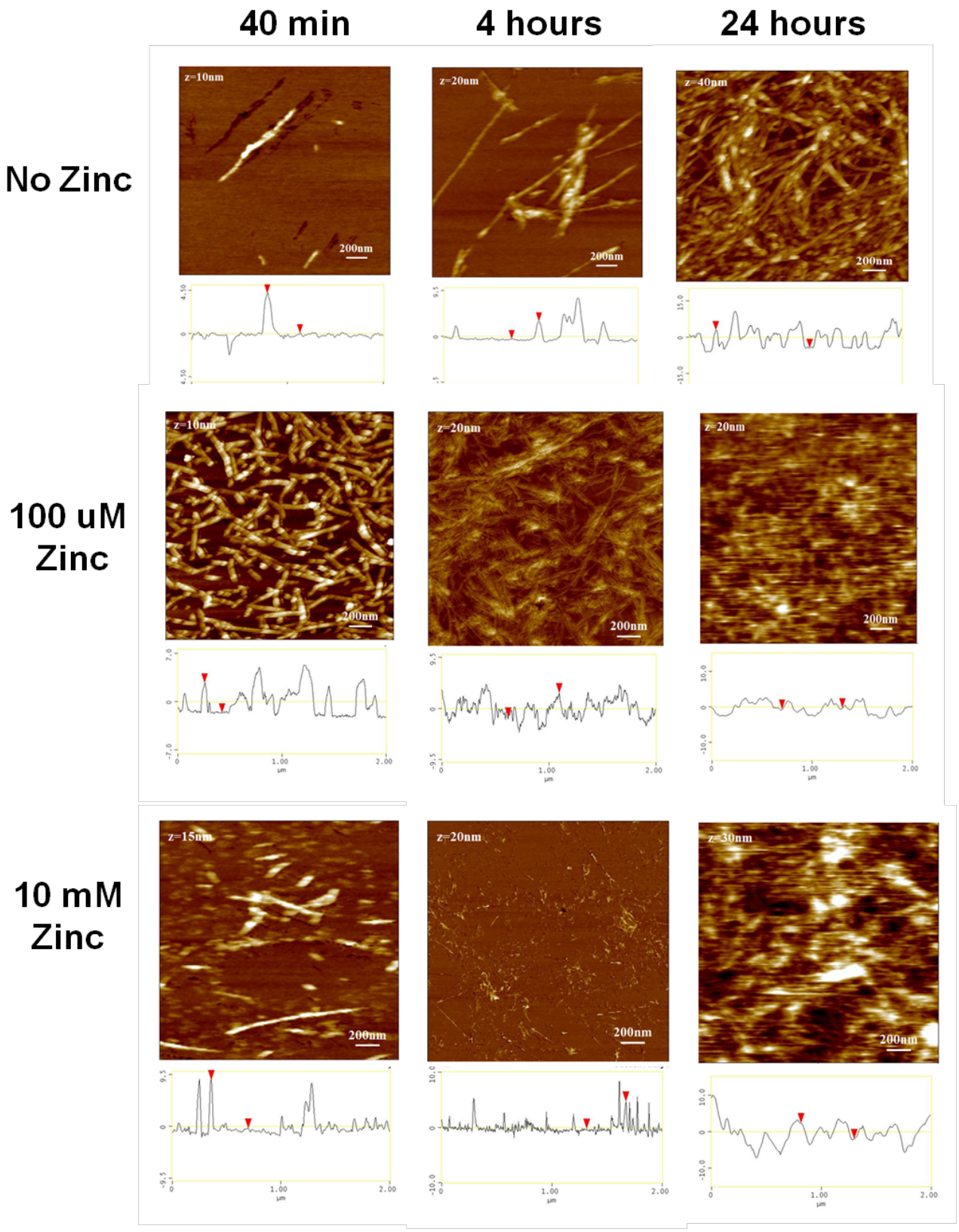

Figure S6. Time lapse tapping mode AFM images and cross-sectional analysis of 10 μM $hIAPP_{1-37}$ incubated with the indicated concentrations of $ZnCl_2$.

Table S1. Cross sectional analysis of AFM data.

	Height(nm)	*Width(nm)*
No Zn^{2+} 40 min	5.86±1.98	81.38±7.97
No Zn^{2+} 4 hrs	3.73±2.26	48.46±8.09
No Zn^{2+} 24 hrs	8.90±2.19	50.29±7.65
100µM Zn^{2+} 40 min	3.96±0.59	58.04±8.86
100µM Zn^{2+} 4 hrs	4.49±1.31	/
100µM Zn^{2+} 24 hrs	/	/
10 mM Zn^{2+} 40 min	8.00±0.82	66.01±23.43
10mM Zn^{2+} 4hrs	5.29±0.97	/
10mM Zn^{2+} 24hrs	/	/

References

1. G. N. George and I. J. Pickering, *EXAFSPAK: A Suite of Computer Programs for the Analysis of X-ray Absorption Spectra*, Technical Report, Stanford Synchrotron Radiation Laboratory, Stanford, CA (1995).
2. J. J. Rehr, J. J. Kas, F. D. Vila, M. P. Prange and K. Jorissen, *Phys. Chem. Chem. Phys.* 2010, **12**, 5503-5513.
3. P. Schanda, V. Forge and B. Brutscher, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 11257-11262.
4. S. Salamekh, J. R. Brender, S. J. Hyung, R. P. R. Nanga, S. Vivekanandan, B. T. Ruotolo and A. Ramamoorthy, *J. Mol. Biol.*, 2011, **410**, 294-306.