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Supporting Information

Site-Specific Dynamics of Amyloid Formation and Fibrillar Configuration of $A\beta_{1-23}$ Using an Unnatural Amino Acid

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Experimental Methods

Materials. All chemical reagents were purchased from commercial suppliers and used without further purification. Fmoc-*p*-cyano-phenylalanine (Fmoc-Phe_{CN}) was purchased from Chem-Impex International Inc. (Wood Dale, IL). Other Fmoc-group protected natural amino acids and Rink amide resin were purchased from Novabiochem (Gibbstown, NJ).

Synthesis of $A\beta_{1-23}$ Peptide and Mutants. The peptides were synthesized on a PS3 automated peptide synthesizer (Protein Technologies Inc., Woburn, MA) using standard Fmoc protocol. Peptides were cleaved from resin using a cleavage solution containing 95% trifluoroacetic acid (TFA), 2.5% triisopropylsilane (TIS) and 2.5% Milli-Q H_2O. The crude peptide was purified by HPLC using a C18 reverse phase column, and verified by matrix-assisted laser desorption ionization (MALDI) mass spectrometry. The purity of the peptides was examined using analytical reverse phase HPLC with C18 column, and the purity of the peptides used is $\geq$ 95%.

Preparation of Fresh Peptide Solution. For $A\beta_{1-23}$ or the mutants, ~1.0 mg of lyophilized peptide was dissolved in 1 mL of Milli-Q H_2O. The solution was filtered through 0.22 μm filter (MILLEX®-GV) and used immediately in subsequent experiments. The peptide concentration was determined optically using either Tyr absorbance at 280 nm (ε_{280} = 1280 cm^{-1} M^{-1}) or Phe_{CN} absorbance at 240 nm (ε_{240} = 13,000 cm^{-1} M^{-1}).[1]

ThT Kinetic Assay Using a Fluorescence Plate Reader. The fresh peptide solution was diluted in 50 mM phosphate buffer (150 mM NaCl) of pH 7.4. The solution also contained a final ThT concentration of 20 μM. Then 100 μL of the solution was transferred into a well of a 96-well microplate (Costar black, clear bottom). The plate was sealed with a microplate cover to minimize evaporation and then loaded into a Gemini SpectraMax EM fluorescence plate reader (Molecular Devices, Sunnyvale, CA), where it was incubated at 37 ℃. The fluorescence

(excitation at 440 nm, emission at 485 nm) was measured from the bottom of the plate at 10 min intervals, with 5 s of agitation before each reading. The experiment usually lasted 50-70 h to ensure the full aggregation of the peptides.

Phe_{CN} Fluorescence and ThT Fluorescence Measurement. The fresh peptide solution was diluted in 50 mM phosphate buffer (150 mM NaCl) of pH 7.4 containing a final ThT concentration of 20 μM. The sample was incubated at 37 ℃ in a 1.5 mL reaction tube on a rotating shaker (20 rpm) in an incubator (VWR™). At desired time points, 100 μL of the incubated sample was taken out, briefly vortexed, and then transferred into a 0.1 mL quartz sample cuvette. Phe_{CN} fluorescence spectra were collected on a FluoroMax-4 spectrofluorometer (HORIBA Jobin Yvon) from 260 nm to 500 nm (slit 5 nm) with an excitation wavelength of 240 nm (slit 5 nm). ThT fluorescence spectra of the same peptide solution were also measured from 460 nm to 700 nm (slit 5 nm) with an excitation wavelength of 440 nm (slit 5 nm). To minimize the fluorescence intensity uncertainty measured at different time, a solution of 20 μM free Phe_{CN} unnatural amino acid in the same buffer was also scanned at the same time when the samples were measured, and the spectra were used as a reference for sample spectrum normalization.

The aggregation kinetics of the peptides probed by ThT fluorescence were fitted by a sigmoidal curve using the following equation:[2]

$$y = y_0 + \frac{y_{max} - y_0}{1 + e^{-k(t-t_{50})}}$$

where y is the fluorescence intensity at time t, y_0 and y_{max} are the initial and maximum fluorescence intensities, respectively, t_{50} is the time at which the fluorescence intensity reaches 50% of maximum, and k is the apparent aggregation rate constant.

Circular Dichroism (CD) Measurement. The fresh peptide samples were prepared in 50 mM phosphate buffer (150 mM NaCl) of pH 7.4 with a total volume of 1 mL and incubated

quiescently at 37 ℃ in an incubator (VWR^{TM}). At desired time points, 300 μL of the incubated solution was transferred into a 1 mm path length quartz sample cuvette. CD spectra were collected on a J-810 spectropolarimeter (JASCO). Five scans were averaged for each sample.

Atomic Force Microscopy (AFM) Measurement. An aliquot of the peptide solution (20 μL) was absorbed onto the surface of freshly cleaved mica (8×8 mm) for 2 min. The liquid was then wicked off by absorption into filter paper. Salts and unbound materials were removed by three washes with 20 μL of Milli-Q water. The samples were dried overnight before measurement. AFM images were acquired in tapping mode using an Asylum Research MFP 3D Bio AFM system with MikroMasch NSC15/AI BS cantilevers.

Fourier Transform Infrared Spectroscopy (FTIR) Measurement. The residual trifluoroacetic acid (TFA) from peptide synthesis and purification was removed by lyophilization against 0.1 M DCl/D_2O for three times. Static FTIR spectra of the peptides were collected on a Nexus 870 spectrometer (Nicolet, WI) equipped with a DTGS detector using 1 cm^{-1} resolution. The sample and buffer reference were injected to CaF_2 cells with teflon spacer. The path length of the IR liquid cells (108 μm) was determined by measuring the interference fringes when the cell was empty. The cells were mounted on a programmable thermostated translation stage which allows measurements of the sample and the reference under identical conditions. The temperature was controlled within ±0.5 ℃ precision with a water circulator. Typically, 256 scans were averaged to generate one spectrum. The spectra were baseline corrected using the Omnic spectra software.

The attenuated total reflection FTIR (ATR-FTIR) spectra were measured using a Perkin Elmer-Spectrum 100. A drop of each peptide solution (50 mM phosphate buffer of pD* 7.4, 150 mM NaCl) was placed on the ATR crystal and a spectrum measured after evaporation of the solvent. Each spectrum corresponds to an average of 16 scans.

Raman Spectroscopy Measurement. Lyophilized peptide powder was dissolved in 50 mM non-saline phosphate buffer of pH 7.4, with a final peptide concentration of 2 mM. The sample solution was incubated at 25 ℃ quiescently in a 1.5 mL reaction tube. At desired time points, the incubated sample solution was gently vortexed, and 2 μL of solution was pipetted onto a glass microscope slide and allowed to dry (30-60 min). Raman spectra were recorded with an XploRA Raman microscope (Horiba Scientific, Edison, NJ) using ~10 mW of 532 nm laser light for excitation. Laser focusing and light collection was performed with a 100 x dry microscope objective. Other measurement parameters included 2400 g/mm grating, 500 μm confocal hole diameter, and 200 μm slit width. A minimum of 14 spectra were recorded from randomly chosen points within each sample, with 30 s data accumulation per point. Individual Raman spectra were then averaged together to produce the averaged spectra that are presented.

Anisotropy and Fluorescence Lifetime Measurement. Steady-state anisotropy experiment was performed on a ChronosFD (ISS, Illinois) spectrofluorometer with excitation at 240 nm and emission collected through a 305 nm cutoff filter. The peptide at a concentration of 100 μM in 50 mM phosphate buffer (150mM NaCl) of pH 7.4 was held in a 2 mm × 10 mm path length quartz cuvette with excitation along the 10mm path. Fluorescence lifetime measurements were performed also using a ChronosFD (ISS, Illinois) spectrofluorometer. The peptide was excited with a 288 nm diode and emission was collected through a 305 nm long pass filter. 2,5-Diphenyl-oxazole (PPO) in ethanol (τ = 2.5 ns) was used as a lifetime reference. Frequency domain data were analyzed using the GlobalsWE software and the χ^2 parameter and residuals plots were used as a criterion for goodness of fit. Fluorescence decays were modeled using a multiple-exponential decay function.

References:

(1) M. J. Tucker, R. Oyola, R and F. Gai *Biopolymers* 2006, **83**, 571-576.

(2) C. Cabaleiro-Lago, F. Quinlan-Pluck, I. Lynch, S. Lindman, A. M. Minogue, E. Thulin, D. M. Walsh, K. A. Dawson and S. Linse *J. Am. Chem. Soc.* 2008, **130**, 15437-15443.

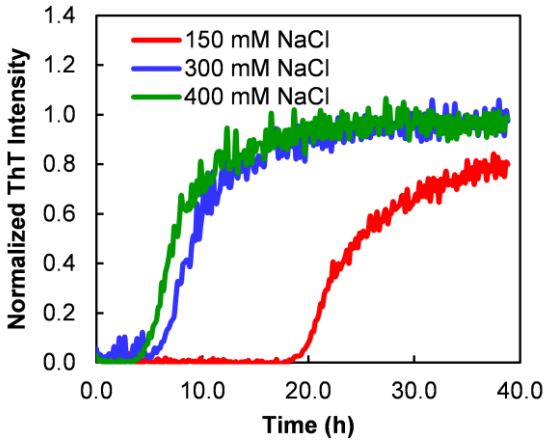

Figure S1. Effect of ionic strength on $A\beta_{1-23}$ amyloid formation. Aggregation kinetics of $A\beta_{1-23}$ peptide (50 μM) with agitation (5s of shaking every 10 min) in 50 mM phosphate buffer of pH 7.4 with different concentration of NaCl at 37 ^{o}C were followed by ThT fluorescence on a fluorescence plate reader. Different amount of NaCl was used in the assay to change the ionic strength. The results suggest that stronger ionic strength accelerates the aggregation rate of $A\beta_{1-23}$ peptide.

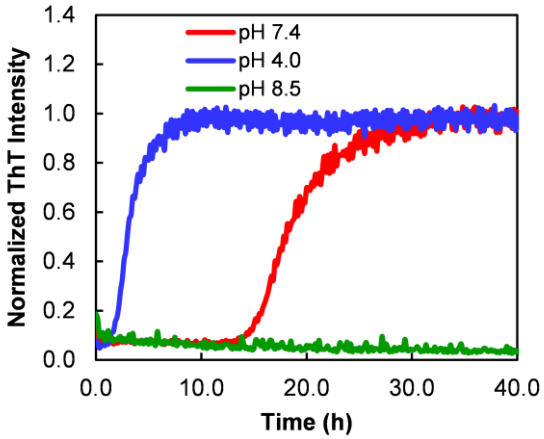

Figure S2. Effect of pH on $A\beta_{1-23}$ amyloid formation. Aggregation kinetics of $A\beta_{1-23}$ peptide (50 μM) with agitation (5s of shaking every 10 min) in 50 mM acetate buffer of pH 4.0, 50 mM phosphate buffer of pH 7.4, and 50 mM phosphate buffer of pH 8.5 at 37 ^{o}C were followed by ThT fluorescence on a fluorescence plate reader. The results suggest that $A\beta_{1-23}$ aggregates faster at pH 4.0 compared to that at pH 7.4, while the aggregation is interfered significantly at pH 8.5.

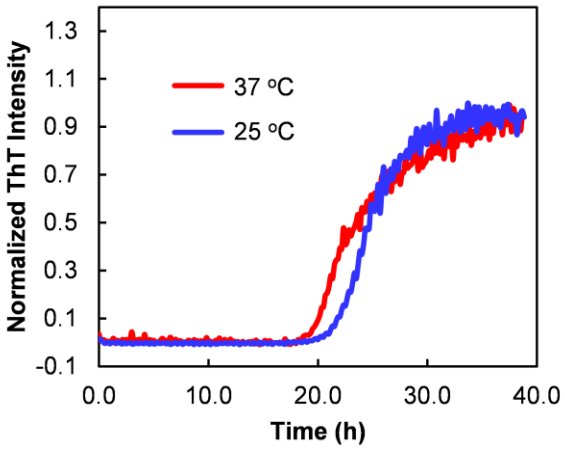

Figure S3. Effect of temperature on $A\beta_{1-23}$ amyloid formation. Aggregation kinetics of $A\beta_{1-23}$ peptide (50 μM) in 50 mM phosphate buffer of pH 7.4 (150 mM NaCl) with agitation (5s of shaking every 10 min) were followed by ThT fluorescence on a fluorescence plate reader. The results suggest that $A\beta_{1-23}$ aggregates slightly faster at 37 ^{o}C compared to that at 25 ^{o}C.

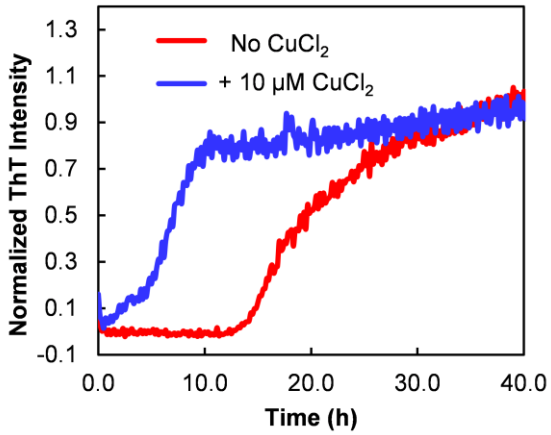

Figure S4. Effect of Cu^{2+} metal ion on $A\beta_{1-23}$ amyloid formation. Aggregation kinetics of $A\beta_{1-23}$ peptide (50 μM) in 50 mM phosphate buffer of pH 7.4 (150 mM NaCl) with agitation (5s of shaking every 10 min) were followed by ThT fluorescence on a fluorescence plate reader. The results suggest that the presence of Cu^{2+} ion significantly accelerates the $A\beta_{1-23}$ aggregation rate.

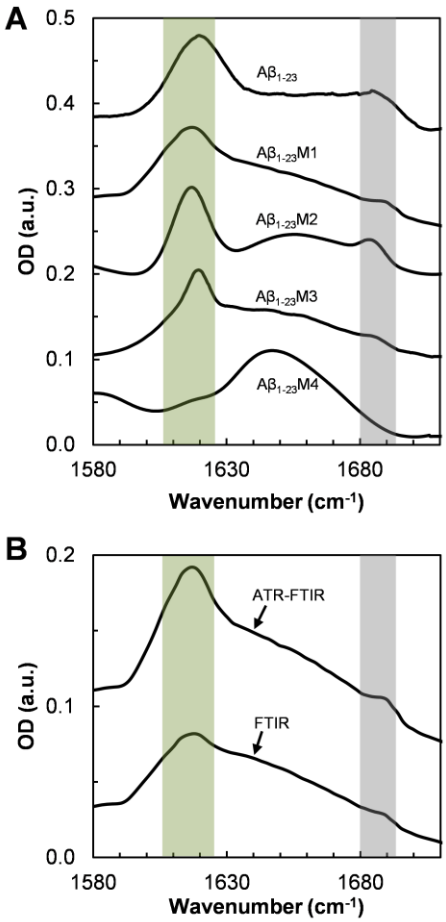

Figure S5. (A) Amide I′ band of $A\beta_{1-23}$ and the mutants. The spectra were taken after incubation of the peptide samples (1 mM) in 50 mM phosphate buffer of pD* 7.4 (150 mM NaCl) at 25 ^{o}C for 4 d. The spectra of $A\beta_{1-23}$, $A\beta_{1-23}$M1 and $A\beta_{1-23}$M3 were measured using ATR-FTIR, and the spectra of $A\beta_{1-23}$M2 and $A\beta_{1-23}$M4 were measured in solution using FTIR. The colored regions indicate IR frequencies characteristic of antiparallel β-sheet structucture. The data are normalized and offset for clarity. (B) Comparison of the FTIR and ATR-FTIR spectra of $A\beta_{1-23}$M1 (1 mM). The spectra were measured after 4 d of incubation in 50 mM phosphate buffer of pD* 7.4 (150 mM NaCl) at 25 ^{o}C. The data are normalized and offset for clarity. The FTIR and ATR-FTIR spectra show high similarity, both containing the pair of low and high frequency bands characteristic for antiparallel β-sheet struture.

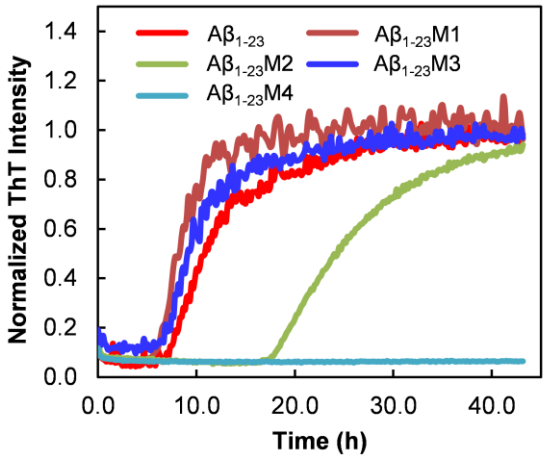

Figure S6. Aggregation kinetics (pH 7.4, 37 ^{o}C) of the wild type $A\beta_{1-23}$ peptide and the four mutants (70 μM) with agitation (5 s of shaking every 10 min). The amyloidogenesis reaction was followed by ThT fluorescence on a fluorescence plate reader. Fluorescence is normalized to the value of the plateau phase in a given reaction.

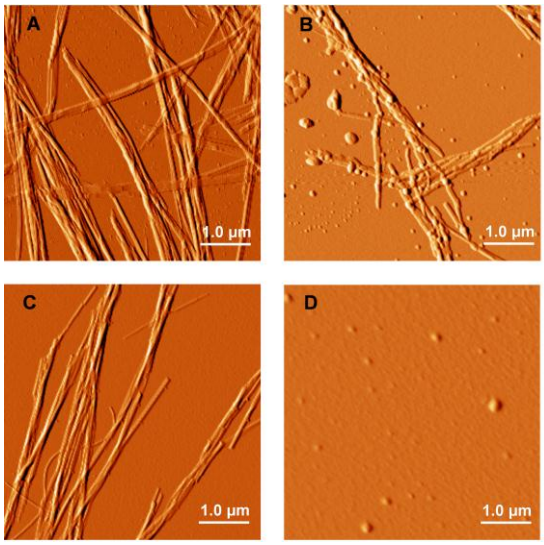

Figure S7. Tapping mode atomic force microscopy image of $A\beta_{1-23}$M1 (A), $A\beta_{1-23}$M2 (B), $A\beta_{1-23}$M3 (C), and $A\beta_{1-23}$M4 (D). The AFM images were acquired after incubating the samples (100 μM) in 50 mM phosphate buffer (150 mM NaCl) of pH 7.4 for 6 d at 37 ^{o}C.

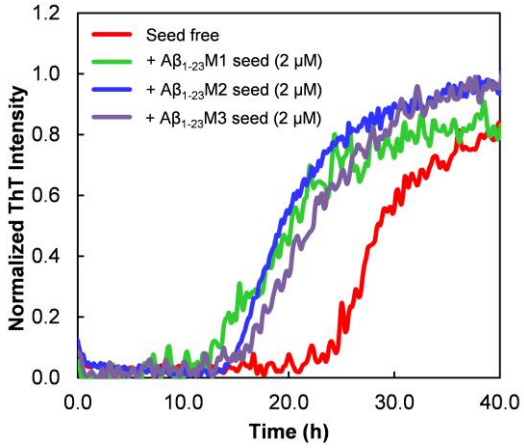

Figure S8. Aggregation kinetics (pH 7.4, 37 ^{o}C) of $A\beta_{1-23}$ peptide (50 μM) with agitation (5s of shaking every 10 min) in the absence or presence of the preformed amyloid seeds of $A\beta_{1-23}$ mutants (2 μM equivalent monomeric concentration). $A\beta_{1-23}$M1-M3 amyloid seeds were prepared by incubating monomeric peptide solution (100 μM) in PBS (50 mM Na-phosphate, 150 mM NaCl, pH 7.4) at 37 ℃ in a 1.5 mL reaction tube on a rotating shaker (20 rpm) for 7 days. Seeds were sonicated in a water bath sonicator for 20 min before being added to the $A\beta_{1-23}$ solution for kinetic measurement.

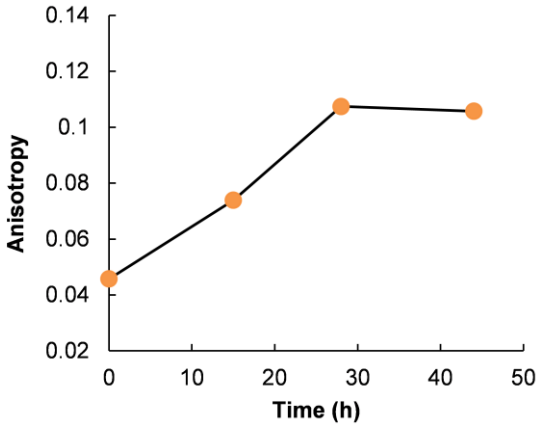

Figure S9. The increase of anisotropy of $Phe_{CN}10$ residue in $A\beta_{1-23}$M1 over a period of quiescent incubation in 50 mM phosphate buffer of pH 7.4 (150 mM NaCl).

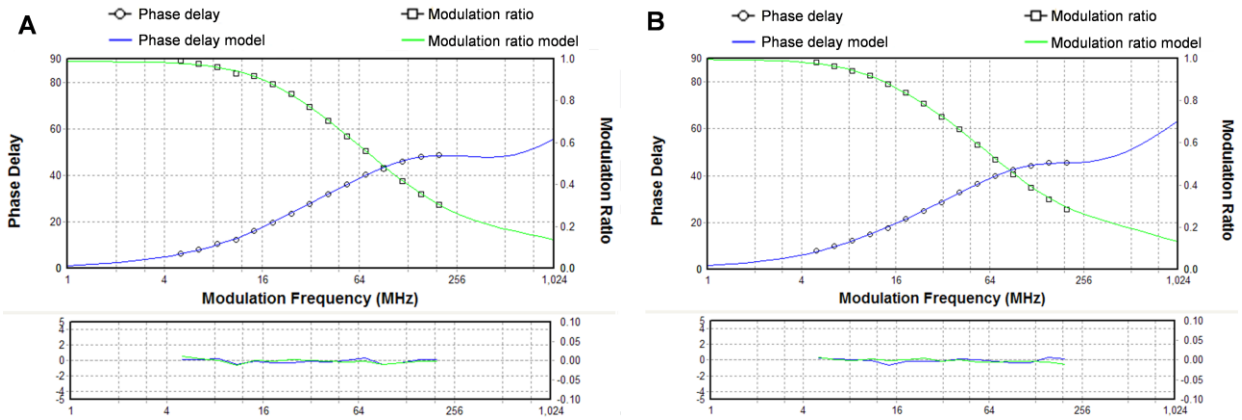

Figure S10. Frequency responses (phase and modulation) of the $Phe_{CN}10$ residue in an $A\beta_{1\text{-}23}M1$ fresh sample (A) and after 46 h of incubation (B) in 50 mM phosphate buffer of pH 7.4 (150 mM NaCl). A satisfactory fit is obtained using a triple-exponential function, with residual plots shown in the lower panels and fitting parameters listed in the table below:

Sample	τ_1 (ns) (f_1)[a]	τ_2 (ns) (f_2)	τ_3 (ns) (f_3)	χ^2	Average lifetime τ (ns)
Fresh	0.14 ± 0.03 (0.138 ± 0.008)	1.93 ± 0.1 (0.40 ± 0.02)	5.80 ± 0.2 (0.45 ± 0.02)	2.22	3.39
After 46 h	0.23 ± 0.02 (0.176 ± 0.005)	2.66 ± 0.08 (0.49 ± 0.01)	9.20 ± 0.4 (0.32 ± 0.07)	1.82	4.36

a. Fractional contribution of each component to the total emission intensity.