Multi-step Conformation Selection in Amyloid Assembly

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MATERIALS AND METHODS

Peptide solution preparation

[1-^{13}C]F19 Aβ(16-22) peptides are synthesized using FMOC-protected natural abundance (Anaspec, Fremont, CA) and [1-^{13}C]F19-enriched amino acids (Cambridge Isotopes, Tewksbury, MA) with solid state peptide synthesis using microwave assistance (Liberty CEM Microwave Automated Peptide Synthesizer, Matthews, NC, USA) on rink amide resin.[1-3] The synthetic peptides are purified with high-performance liquid chromatography (HPLC), Waters Delta 600 with a Waters Atlantis reverse phase C-18 preparative column (19 x 250 mm) in a water/acetonitrile gradient with 0.1% trifluoroacetic acid (TFA). The peptide solution from HPLC is then lyophilized and the resulting peptide powder is stored under -20 ^{o}C before use. Product mass is confirmed by MALDI-TOF on a Voyager-DETM STR Biospectrometry Workstation using α-cyano-4-hydroxycinnamic acid (CHCA) matrix.

To prepare the peptide solution for the kinetic measurements, the peptides are dissolved in hexafluoroisopropanol (HFIP) before sonication for two hours. HFIP is then evaporated with a Labconco CentriVap Concentrator-7970010, and the resulting peptide film is placed in a desiccator overnight to remove residual HFIP. To assemble the peptides, the peptide film is suspended again with HFIP, and sonicated for 15 min. For neutral solution, the peptides in HFIP are then diluted with 40% (v:v) acetonitrile in water to obtain a final HFIP concentration of 1% by volume and the desired peptide concentration. For acidic solution, 40% acetonitrile with 0.1% TFA is used, instead of pure 40% acetonitrile, to dilute the suspended peptides in HFIP. The peptide solution is then incubated at 22 ^{o}C and aliquots of the solution are removed at predetermined time points for kinetic measurements.

Transmission electron microscopy (TEM)

The morphologies of the peptide assemblies are reported with transmission electron microscopy (TEM). First, three water drops with an individual volume of 100 μL are loaded onto a piece of parafilm for sample dilution. Then, 5 μL of peptide solution is loaded onto the copper grid to deposit the assemblies. After one minute of deposition, the grid is put upside down to let the peptide solution contact the three water drops in sequence, which dilutes the peptide solution and washes away excess assemblies. The diluted peptide solution remaining on the grid is wicked away with a piece of filter paper and the grid is negatively stained with 1.5-wt % methylamine tungstate solution for 30 seconds. The stain solution is wicked away with filter paper and the grid is placed in a vacuum desiccator to dry overnight. TEM images are recorded with a Hitachi H-7500 transmission electron microscope at 75 kV.

Circular dichroism (CD)

The β-sheet content of the peptide solution is probed by circular dichroism (CD). CD measurement is performed with 23 μL peptide solution in a demountable window cell with a 0.1 mm path length. The spectrum is recorded using a Jasco J-810 spectropolarimeter with wavelengths from 185–260 nm, a resolution of 0.2 nm, a bandwidth of 2 nm, and a scanning rate of 200 nm per min. After background correction, the average of three consecutive scans is saved and the ellipticity at 217 nm is used to quantify the β-sheet content of the peptide solution.

Attenuated Total Reflectance Fourier Transform Infrared (FTIR)

Isotope-edited FTIR is used to probe peptide orientation (parallel, anti-parallel in- and out-of-register β-sheet). Eight μL of the peptide solution is loaded onto the diamond chip of a JASCO FT/IR-4100. After the sample dries, IR absorbance is recorded against a background spectrum over a wavenumber range of 1000 to 3600 cm^{-1} at room temperature. The average of 250 consecutive scans is saved and all spectra are normalized to the peak height of the ^{12}C band.

An IR basis set is established to probe the structural transition of the assemblies with spectrum deconvolution.[2] The peptide orientation of mature Aβ(16-22) assemblies have been

previously defined by solid-state NMR as antiparallel in-register at neutral pH (Figure 1a – main text) and out-of-register (Figure 1b – main text) at acidic pH.[4] Peptide solutions of [1-^{13}C]F19 Aβ(16-22), independent from those for kinetic measurements, are incubated under either neutral or acidic pH. Their IR spectra are assigned as the basis set for their corresponding registries after the spectra remain constant for more than a month, when the assembly morphology and corresponding IR spectra do not change significantly. For spectrum deconvolution with the basis set, the kinetic IR spectra with the range from 1575 cm^{-1} to 1725 cm^{-1} are fit with the linear combination of the basis set spectra using the NonlinearModelFit module of *Mathematica 10.0* (Wolfram Research, Inc., 2014).

Residue F19 of Aβ(16-22) is chosen for ^{13}C-enrichment because it provides the most significant difference in IR bands between different registries.[4] To obtain the IR signatures of the in-register and out-of-register structures, [1-^{13}C]F19 Aβ(16-22) solutions with a concentration of 1.0 mM are incubated under neutral and acidic pH, respectively. Their IR spectra are assigned as the basis set after the spectra remain constant for at least one month, as shown in Figure S1.

Also, to make the basis set robust enough to analyze other possible structures, both the parallel β-sheet signature from [1-^{13}C]F19 Aβ(16-22)E22Q (Figure S1, black line)[3] and the unassembled peptide signature from [1-^{13}C]F19 Aβ(16-22) (Figure S1, blue line)[3] are added to the basis set. It should be noticed that [1-^{13}C]F19 Aβ(16-22)E22Q also forms a particle-assembly equilibrium at the end of the incubation, which is consistent with the final equilibrium of [1-^{13}C]F19 Aβ(16-22).

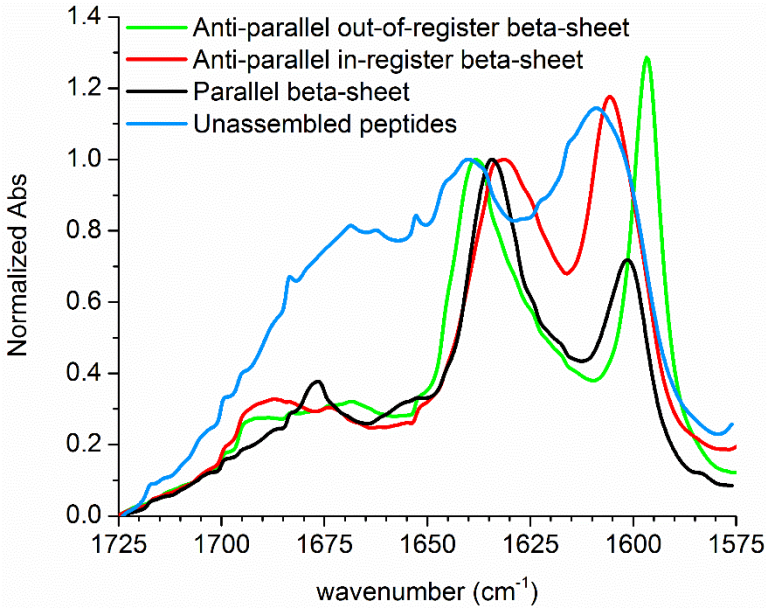

Figure S1. The IR basis set for spectra deconvolution. Parallel β-sheet spectrum (black) is obtained with [1-^{13}C]F19 Aβ(16-22)E22Q assemblies.[3] The antiparallel in-register β-sheet signature (red) is obtained from [1-^{13}C]F19 Aβ(16-22) assemblies incubated at neutral pH, while the antiparallel out-of-register signature (green) comes from the same peptide incubated at acidic pH. The [1-^{13}C]F19 Aβ(16-22) peptide is dissolved in HFIP to disassemble the possible assemblies, resulting in the unassembled peptide signature (blue).

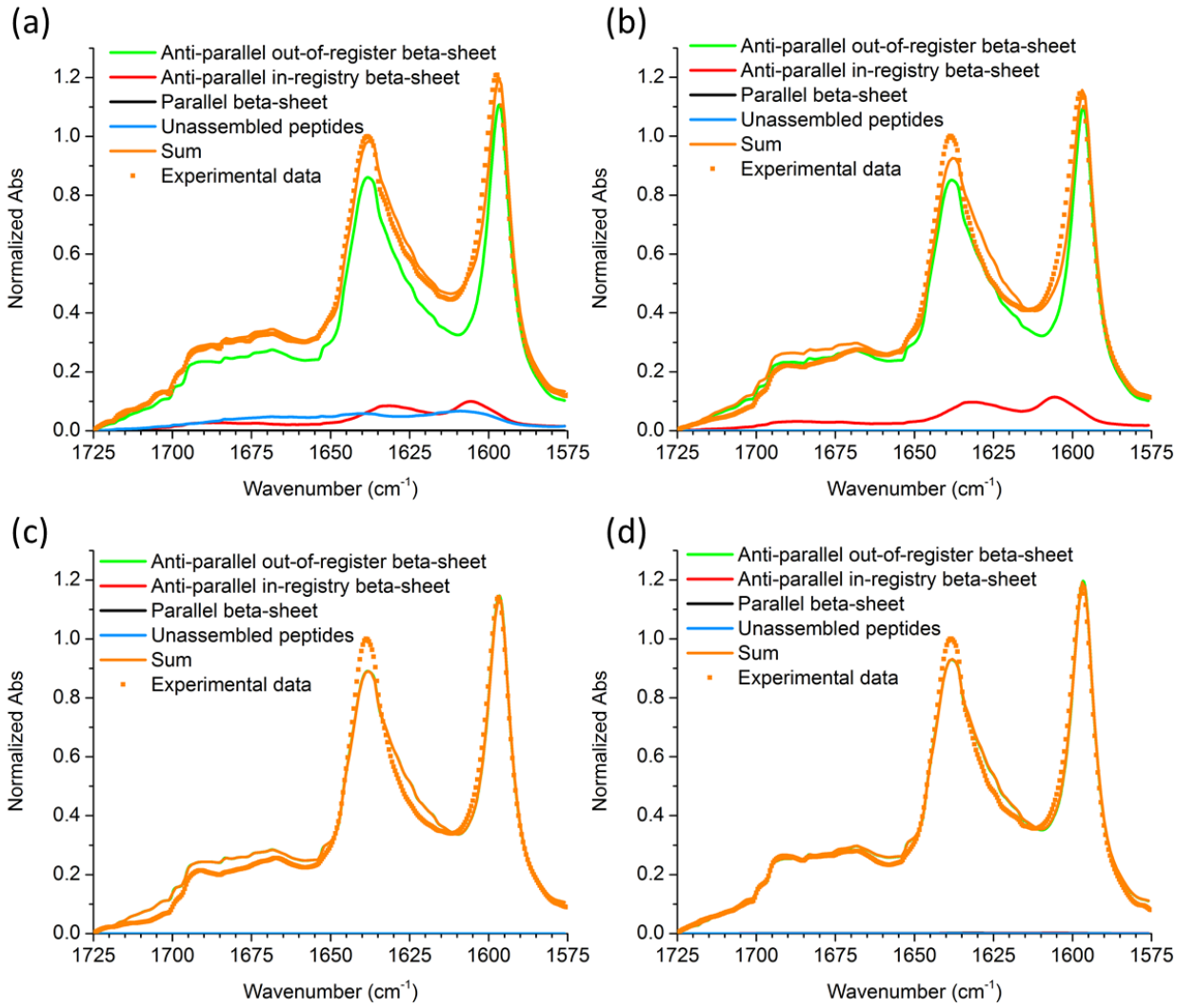

Figure S2. Representative fits for the IR spectrum of 1 mM Aβ(16-22) at acidic pH after incubated for (a) 1 hr (b) 7 days (c) 12 days and (d) 17 days. For (c) and (d), the orange fit sum overlays on the green anti-parallel out-of-register component, which is the only component for the fits.

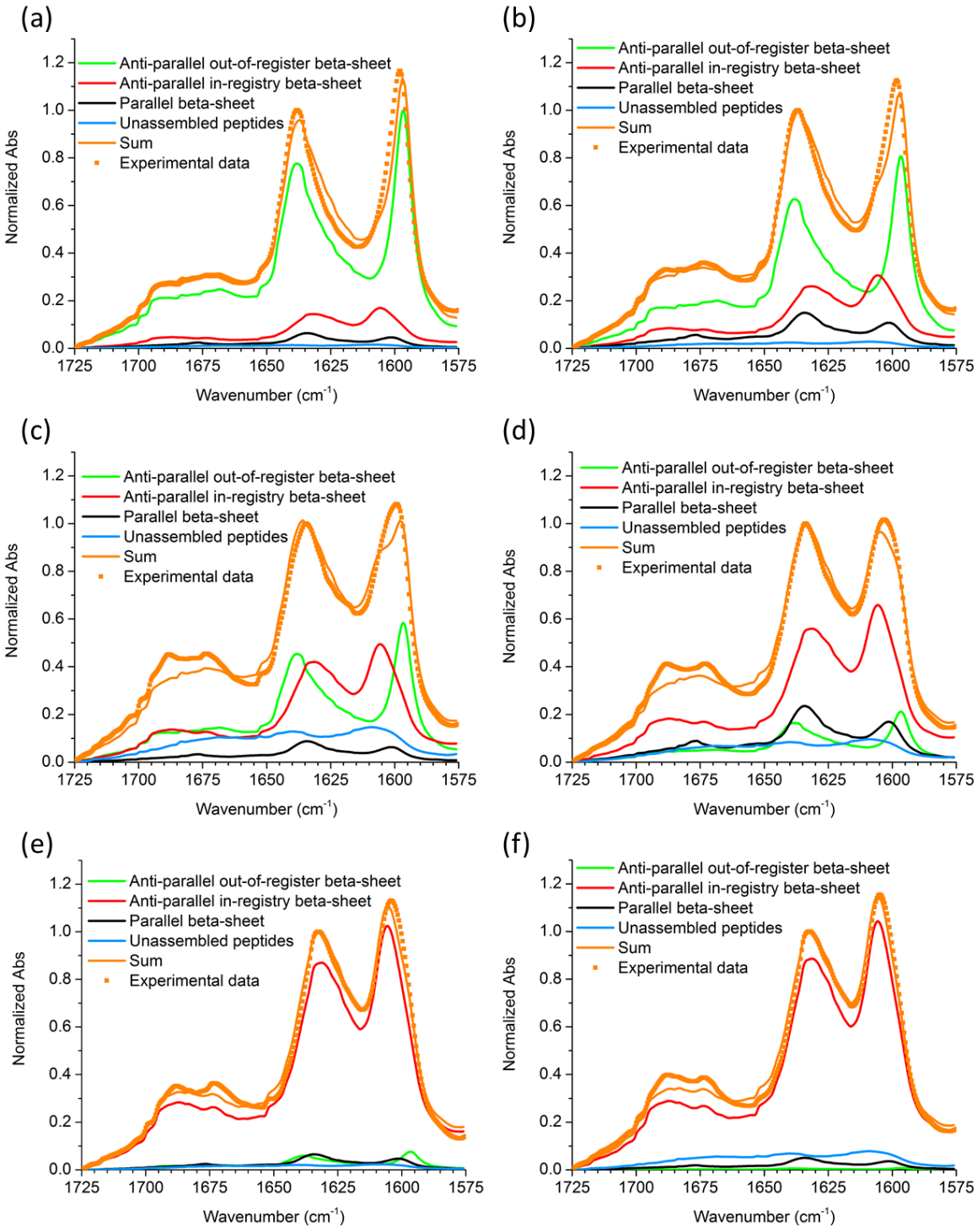

Figure S3. Representative fits for the IR spectrum of 1 mM Aβ(16-22) at neutral pH after incubated for (a) 1 hr (b) 1 days (c) 5 days (d) 9 days, (e) 14 days, and (f) 21 days.

Reference

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(4) Mehta, A. K.; Lu, K.; Childers, W. S.; Liang, Y.; Dublin, S. N.; Dong, J.; Snyder, J. P.; Pingali, S. V.; Thiyagarajan, P.; Lynn, D. G. *J. Am. Chem. Soc.* **2008**, *130*, 9829.