Supplemental data

Polymorphism of β_2-microglobulin amyloid fibrils manifested by ultrasonication-enhanced fibril formation in trifluoroethanol

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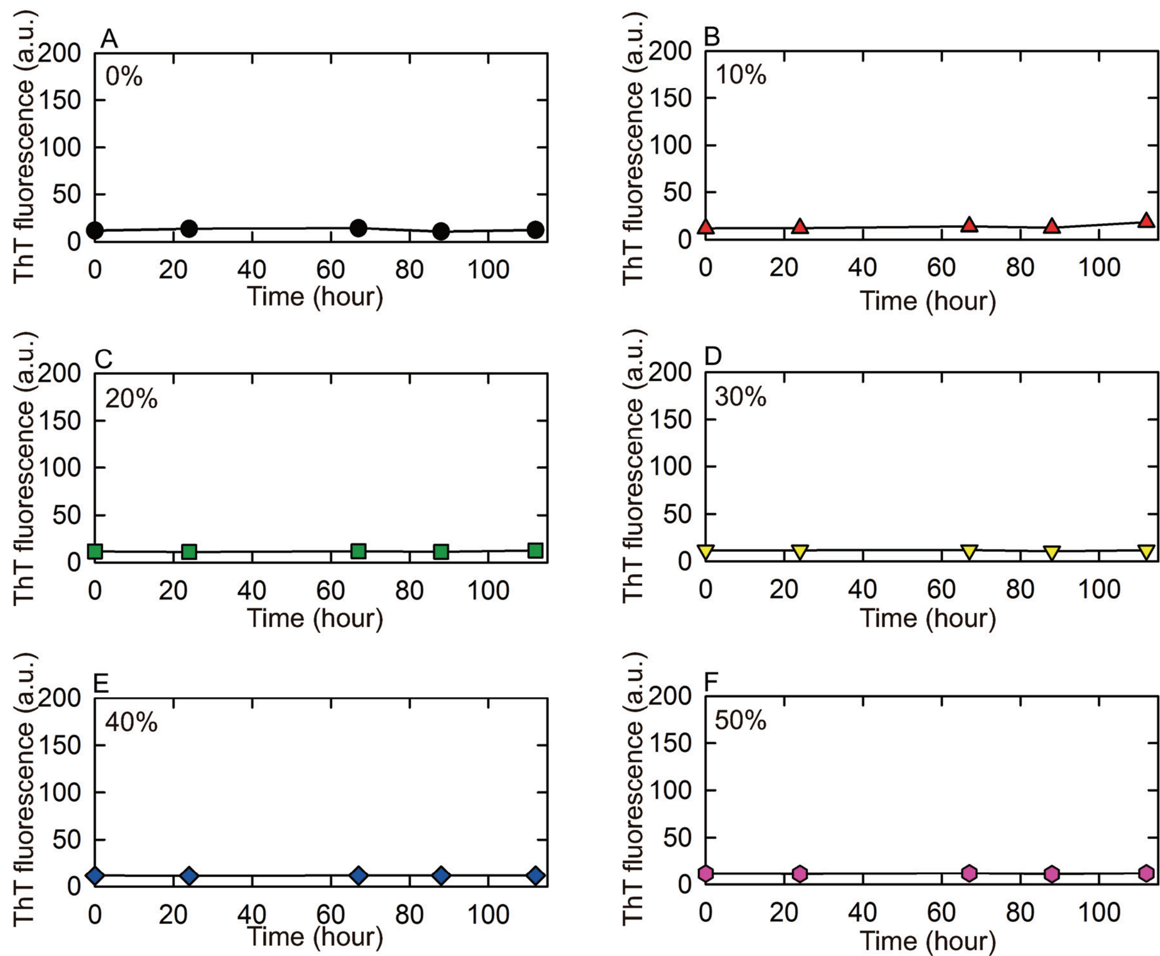
SUPPLEMENTAL EXPERIMENTAL PROCEDURES

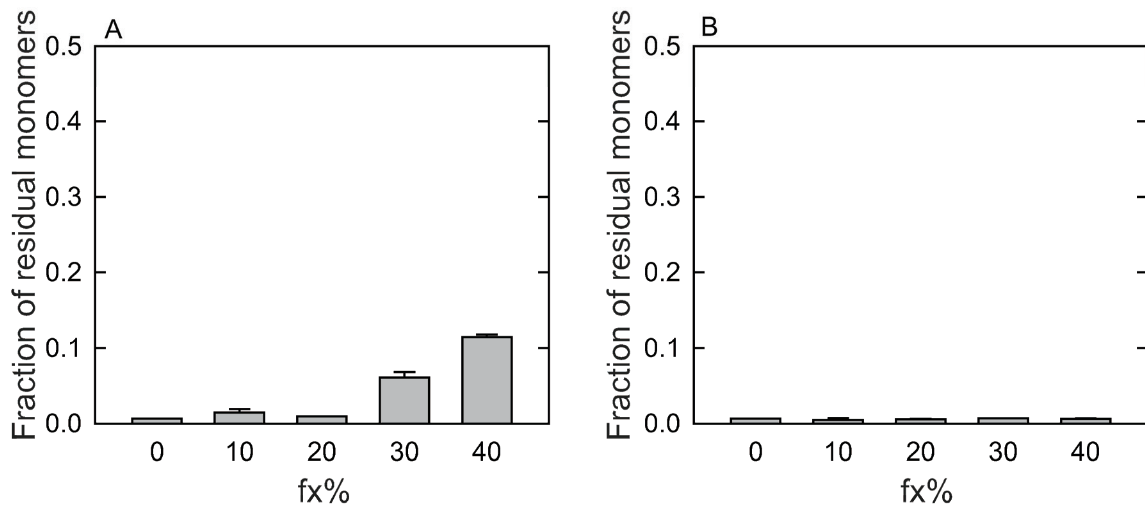
Measurement of Critical Monomer Concentration (CMC) of f0%-f40% Fibrils− The concentration of β2-m monomers remaining inside the supernatant was determined by BCA assay to measure the extent of fibrillation. After reaching constant ThT fluorescence intensity with fibril-monomer equilibrium, the f0%-f40% amyloid fibrils were centrifuged at 14,000 rpm (18,700 g) for 1 hour at 25 ºC to separate the monomers from the fibrils. In the case of f0% fibrils grown in the absence of TFE and f0%-f40% fibrils grown in the presence of 5% TFE, the sedimentation of which hardly occurred because of stable dispersion of β2-m amyloid fibrils with the aid of positive charge repulsion (1), NaCl was added to the final concentration of 0.5 M to accelerate coagulation of fibrils resulting in easier precipitation, and the centrifugation started within several minutes. The supernatant was then collected and the concentration of residual monomer component was determined with a Micro BCA™ Protein Assay Reagent kit (Pierce, IL, USA) using calibration curves of β2-m monomer dissolved in the same solvent as that of reaction mixture.

Responses of Amyloid Fibrils to Pressure− The effects of pressure on the conformation of β2-m amyloid fibrils were analyzed using tryptophan fluorescence spectra and light scattering intensity with a modified Hitachi F-4500 fluorescence spectrophotometer equipped with a pressure optical bomb with three sapphire windows and a water circulating system connected to a thermoregulated water bath (Teramecs Co., Kyoto, Japan). β2-m amyloid fibrils dissolved at a concentration of 0.3 mg/ml in 10 mM HCl containing 0.1 M NaCl and 5 % TFE were sealed with a quartz inner cell embedded inside the pressure bomb, as described previously (2). The temperature of the sample liquid was maintained at 37 ºC. Pressure was increased in steps of 25 MPa up to 400 MPa, and after each change of pressure, the sample was incubated for approximately 5 min and spectroscopic data were collected. After reaching 400 MPa, the pressure was released to atmospheric pressure in steps of 100 MPa, and the reversibility was examined after incubation for 10 min at each pressure. The intensities of light scattering were corrected for temperature- and pressure-dependent changes in water volume, and the tryptophan spectra were quantified by the center of spectral mass $\langle \nu \rangle$ using equation 1 in the main text.

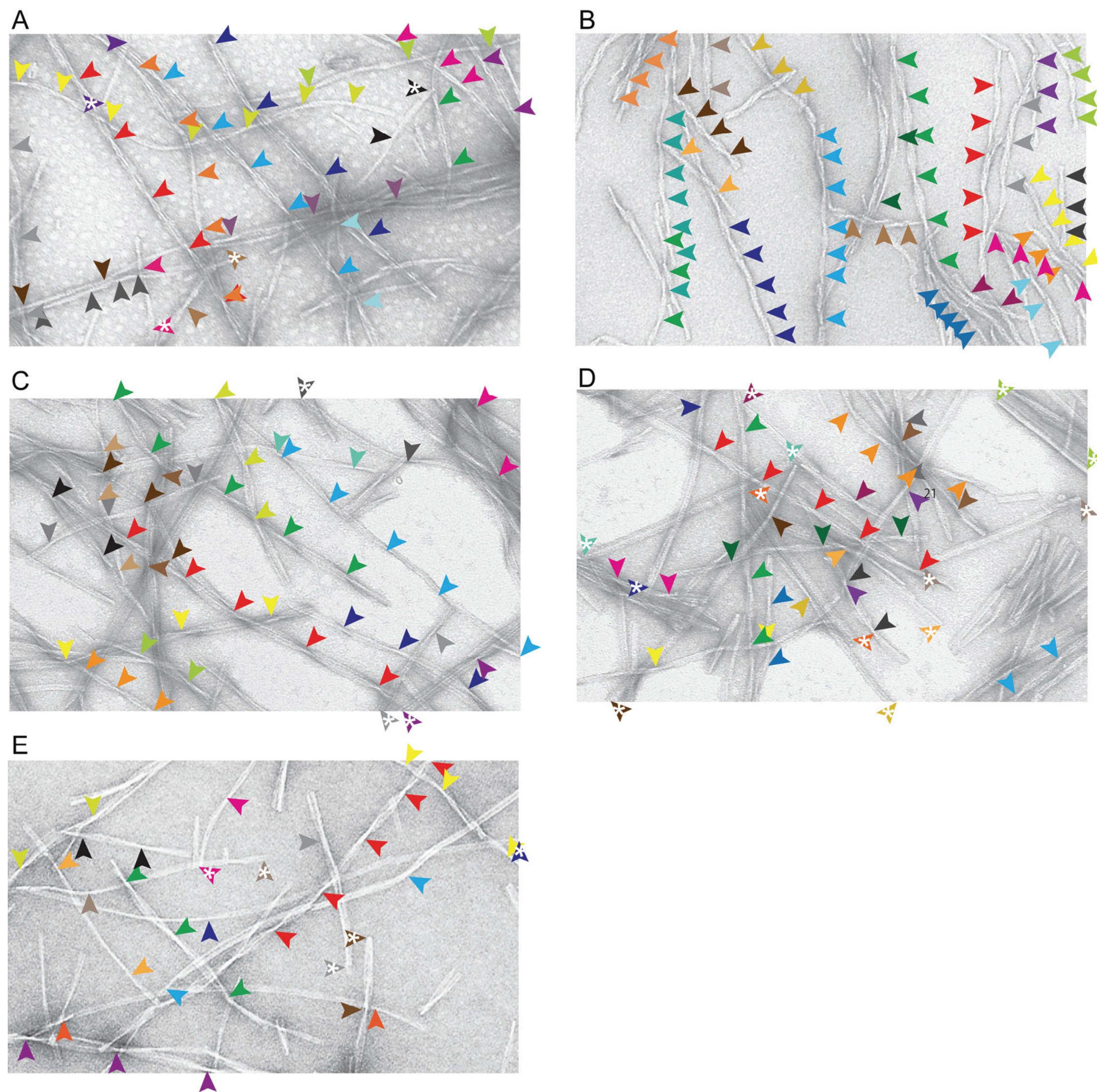
SUPPLEMENTAL FIGURES



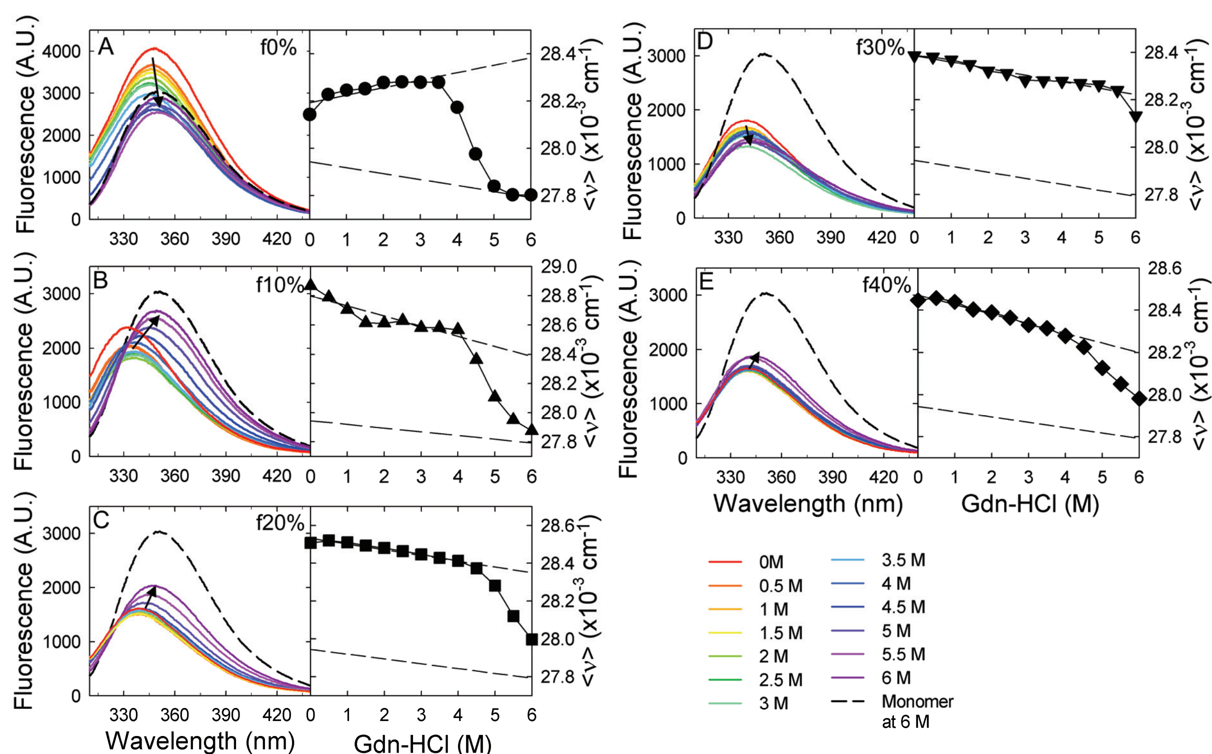
Supplemental Fig. S1. Incubation of β2-m solution at 37 ºC under quiescent conditions. Fibril formation in the absence (0%) or presence of TFE (10-50%) was monitored by ThT fluorescence. Concentrations of TFE are 0 (A), 10 (B), 20 (C), 30 (D), 40 (E), and 50% (F). In the absence of ultrasonication pulses, all samples exhibited no significant increase in ThT fluorescence within the experimental period (112 hours).



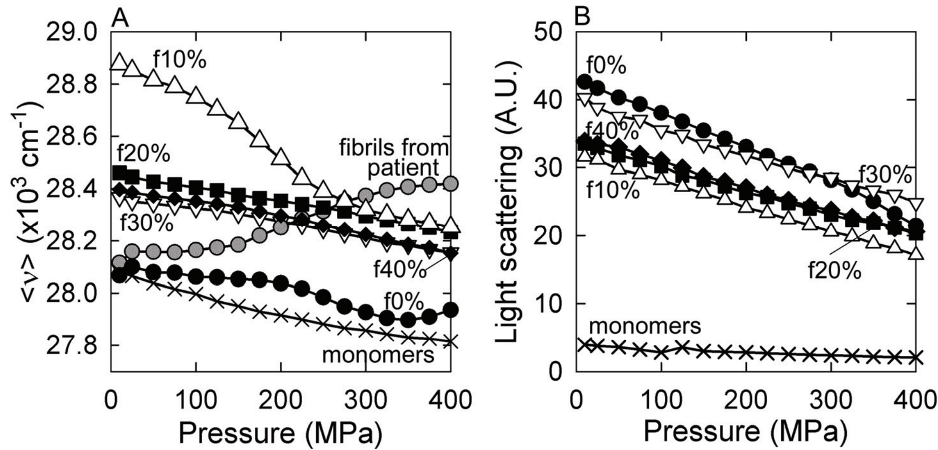
Supplemental Fig. S2. Concentration of residual monomers in f0%-f40% fibrils. The concentration of residual monomer β2-m in F2 (for f0% and f20%-40%) or F3 (for f10%) fibrils was measured by BCA assay. Both of fibrils formed by the seed-dependent extension under the solvent conditions containing the same TFE concentration as that present during the nucleation (A) and under the aqueous condition in the presence of 5% TFE (B) were analyzed. Fractions of residual monomers were calculated by dividing the measured CMC by the total protein concentration of the reaction mixture (i.e., 0.33 mg/ml, which includes proteins added as seeds), which are represented in this figure.



Supplemental Fig. S3. Crossover points of amyloid fibrils used for the quantification of repeat distance. (A) f0%, (B) f10%, (C) f20%, (D) f30%, and (E) f40%. In each panel, amyloid fibrils marked with several arrowheads represent specimens used for the calculation of repeat distances shown in Fig. 3F. In each fibril, two or more crossover points are indicated by arrowheads and the repeat distance was determined by averaging all the spaces sandwiched by the arrowheads for each fibril. When no or only one crossover point was found within the visual field, tentative repeat distance, i.e., distance between the two fibril ends or between the twist and fibril end was measured. In this case, the ends of fibrils are indicated by arrowheads with asterisks. Fibrils without any labels are not included in the analysis because of their short length less than 100 nm or ambiguous twists.



Supplemental Fig. S4. Tryptophan fluorescence spectra (left of each panel) and <*ν*> values (right of each panel) during the unfolding transitions of f0%−f40% amyloid fibrils. (left) The concentration of Gdn-HCl increases from 0 M (red) to 6 M (purple) in steps of 0.5 M, as guided by an arrow. The spectra of monomeric β2-m in 6 M Gdn-HCl are also shown with dashed lines. (right) Plots of <*ν*> values calculated from the tryptophan fluorescence spectra against the concentration of Gdn-HCl. The baselines for fibrillar and monomeric states with dashed lines were used for calculating the fraction of fibrils in FIGURE 5A. The baseline for the monomer was obtained experimentally by measuring the fluorescence spectra of monomeric β2-m at different concentrations of Gdn-HCl.



Supplemental Fig. S5. Pressure-induced responses of TFE-derived β2-m amyloid fibrils. Changes in (A) <*v*> values and (B) light scattering intensity are plotted against pressure. Fibrils used are f0% (closed circles), f10% (open triangles), f20% (closed squares), f30% (open inverted triangles), and f40% (closed diamonds), which were formed by seed-dependent extension in an aqueous solution. The gray circles in panel A represent the result for amyloid fibrils formed by the seeding of originally *ex vivo* β2-m amyloid fibrils from patients (2). The plots of monomeric β2-m under the same conditions (×) are also shown as a reference. As pressure increased, the fluorescence spectra of f0% and f10% showed a cooperative red shift resulting in sigmoidal curves with transitions at between 200 MPa and 300 MPa towards smaller <*v*> values (closed circles and open triangles, respectively in panel A). Both patterns of these transitions were similar, with respect to the presence of cooperative transitions, to that observed in the conventional β2-m amyloid fibrils formed by the seeding of the fibrils originally from patients under acidic pH (gray circles in panel A), indicative of the pressure-induced cooperative transition of fibril structures. Although the direction of the change in <*v*> in f0% was unexpectedly opposite to that of the conventional fibrils, this might be caused by reflecting the microscopic differences present between the fibrils from patients and those originating spontaneously under the acidic conditions. By contrast, the fluorescence spectra of f20%−f40% did not show any cooperative transitions, only linear red shifts, indicating that the structural transition was no longer induced by pressure (closed squares, open inverted triangles, and closed diamonds, respectively in panel A). The disappearance of the pressure response in f20%−f40% possibly indicates the elimination of packing defects. All results were accompanied by a similar gradual decrease in light scattering intensity (panel B), confirming that neither depolymerization nor dissociation of fibril aggregations occurred during the pressurization.

REFERENCES

1. Chatani, E., Lee, Y. H., Yagi, H., Yoshimura, Y., Naiki, H., and Goto, Y. (2009) *Proc. Natl. Acad. Sci. U.S.A.* **106**, 11119-11124
2. Chatani, E., Kato, M., Kawai, T., Naiki, H., and Goto, Y. (2005) *J. Mol. Biol.* **352**, 941-951