Supporting Information

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SI Text

Purification of Mα (P1216). The protein expression and purification procedure followed that described by Fowler et al. (1). Cells carrying p1216 [derived from pET21a(+) expressing the Mα fragment studied by Fowler et al. (1)] were collected by centrifugation (10,000 rpm) at 4°C and resuspended in 125 mM Tris-HCl, pH 7.4. The resuspended pellet was thawed and the cells were lysed by probe sonication. P1216 (Mα fragment) formed inclusion bodies that were collected by centrifugation (30,000 rpm), and washed twice in washing buffer (1.5 M NaCl, 50 mM KH_2PO_4/K_2HPO_4, pH 7.4, and 1% Triton X-100) and then in 125 mM Tris-HCl, pH 7.4. The pellet was dissolved in extraction buffer (8 M GdmCl, 50 mM KH_2PO_4/K_2HPO_4, pH 7.4, 100 mM KCl, and 5.0 mM EDTA) by magnetic stirring at 4°C for 2 days. The resulting solution was centrifuged at 30,000 rpm for 60 min. The solution containing P1216 was purified by gel filtration using a Superdex 200 26/60 column equilibrated with extraction buffer. Fractions containing P1216 were verified via SDS/PAGE and concentrated using a 3-kDa MWCO Centricon filter. Concentrated P1216 fractions purified via gel filtration chromatography were used in all experiments. A portion of P1216 was also further purified by anion exchange chromatography (5 mL, HiTrap Q HP). Attempts to form amyloid with this material also failed.

Amyloid-Specific Dye Assays. Thioflavin T (ThT) fluorescence of RPT, HET-s, Ure2p, and Sup35NM (10 μg/mL) in 125 mM K acetate, pH 5.0, and 0.01 mg/mL ThT with constant stirring at room temperature in a quartz cuvette was measured with a PTI QuantaMaster Fluorimeter (2). Excitation was at 440 nm and data collected between 450–550 nm.

For Congo red birefringence, RPT fiber suspensions were mixed with 0.1 volume of 2% (wt/vol) Congo red (sigma), and after 60 min incubation at room temperature, were centrifuged at 13,200 rpm for 60 s. The aggregates were washed several times with 500 μL water. Aggregates were re-suspended in an equal volume of water and 10 μL was placed on a glass slide and allowed to dry for 60 min. Excess CR was removed by washing with 90% ethanol. Samples were viewed by polarization microscopy using a Zeiss Axiophot with a Photometrics Coolsnap HQ camera.

Proteinase K Assay. Proteinase K digestion was performed by incubating 20 ng proteinase K with 20 μg soluble or fibrous RPT for 0, 5, 10, or 20 min. Reactions were performed in 125 mM K acetate buffer, pH 5.0, at room temperature in a total volume of 20 μL. The reactions were terminated by the addition of 50 μL of 88% formic acid. Samples were lyophilized and re-suspended in 125 mM K acetate, pH 5.0, with 2% SDS before SDS/PAGE analysis.

Light Scattering. After overnight dialysis against 125 mM Tris Cl, pH 7.5, proteins were filtered with 0.02-μm Anotop 10 Plus filters (Whatman), centrifuged 30 min at 66,700 × *g* at 4°C, and protein concentration measured by A_{280}. Experiments were performed as described (3). Composition gradients of Pmel17:RPT were made by simultaneously varying the rates of injection of protein solution and buffer from 2 syringes into the flow cell of a 690 nm laser light scattering detector which provides simultaneous measurement of static scattering and protein concentration. The variation of the static light scattering signal, R/K_o, with composition and the autocorrelation functions (ACFs) from quasi-elastic light scattering of the protein solutions were analyzed as described (4).

Pmel17:RPT showed a linear dependence of the static light scattering with the concentration, indicating the absence of self-association under these conditions {Attri, 2005 #2223}. The average apparent molecular weight, M_{app}, was 14.2 kDa, in good agreement with the monomer size (13,917 Da). Provided that the estimated concentration is accurate (Pmel contains a single aromatic residue), this clearly indicates that the protein in solution as purified is in the monomer state. Pmel17:RPT fibers assembled at pH 5.0 were disassembled by overnight dialysis shifting the pH from 5.0 to 7.5. The M_{app} obtained for this sample was 24.0 ± 0.5 kDa.

The analysis of the autocorrelation functions by fitting a single exponential function to monomeric Pmel17:RPT showed a slight deviation, within uncertainty, and the apparent diffusion coefficient obtained, D_{app}, is smaller than expected for a protein of this size. This strongly suggests that the monomeric protein presents a nonspherical, extended geometry in solution, which produces an anisotropic diffusion and the observed deviation from the behavior expected for solutions containing a single species (5). The clear deviation of the ACFs of the dissociated fibers from a single exponential model indicates that the fiber disaggregation produced by the change in pH yields an ensemble of species, in which the monomer may be present together with higher molecular weight aggregates.

1. Fowler DM, *et al.* (2006) Functional amyloid formation within mammalian tissue. *Plos Biology* 4:e6.
2. Kryndushkin D, Shewmaker F, Wickner RB (2008) Curing of the [URE3] prion by Btn2p, a Batten disease-related protein. *EMBO J* 27:2725–2735.
3. Attri A, Minton AP (2005) New methods for measuring macromolecular interactions in solutions via static light scattering: Basic methodology and application to nonassociating and self-associating proteins. *Anal Biochem* 337:103–110.
4. Kameyama K, Minton AP (2006) Rapid quantitative characterization of protein interactions by compositiion gradient static light scattering. *Biophys J* 90:2164–2169.
5. Wilcoxon J, Schurr M (1983) Dynamic light scattering from thin rigid rods: Anisotropy of translational diffusion of tobacco mosaic virus. *Biopolymers* 22:849–867.

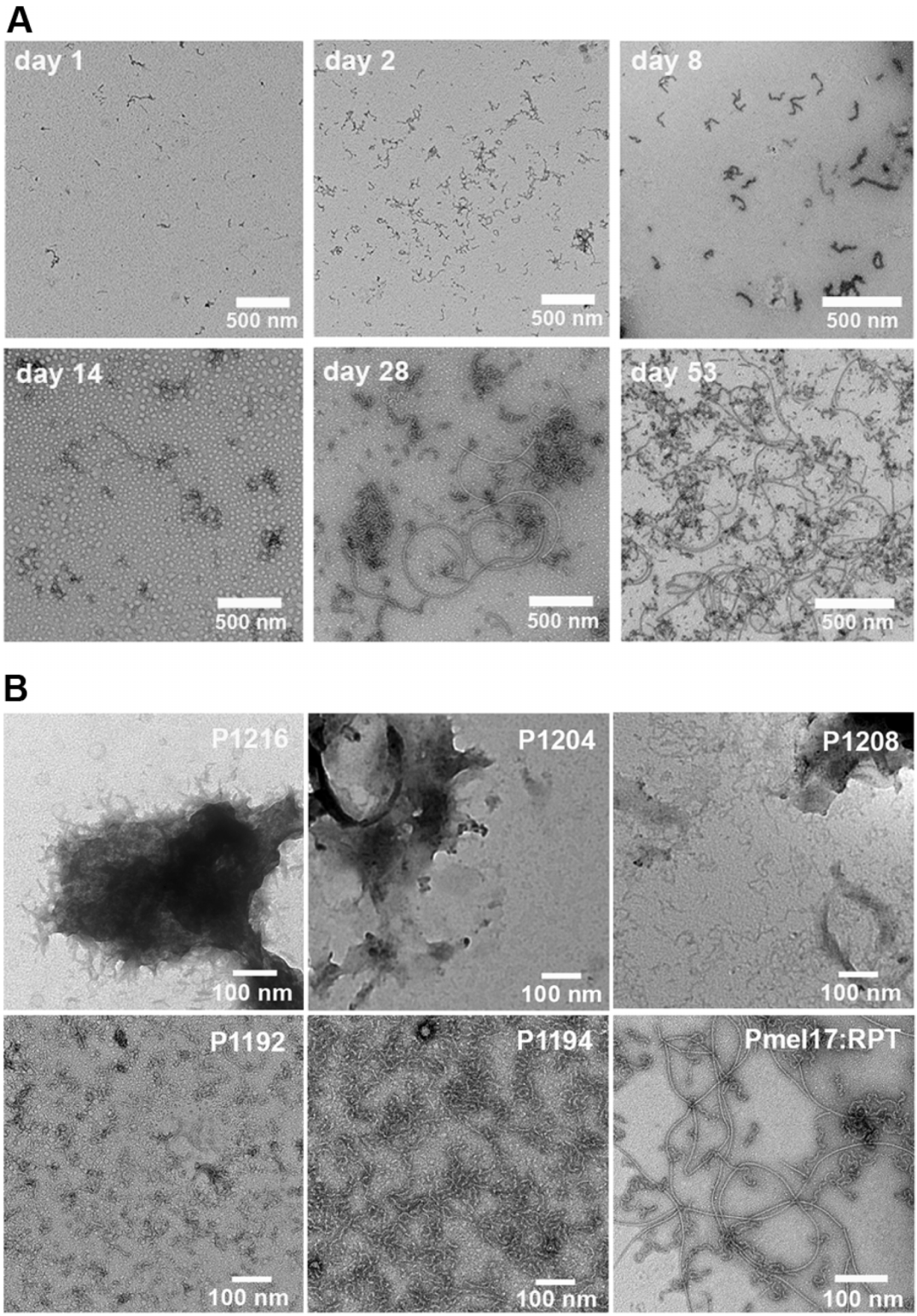

Fig. S1. (*A*) Transmission electron micrographs of purified recombinant RPT monitored over time at pH 5.0 with no agitation. Spacing between arrowheads is approximately 6–10 nm. (*B*) Transmission electron micrographs of Pmel17 fragments (P1216, P1204, P1208. P1194, and P1192) negatively stained with uranyl acetate. Pmel17:RPT fibers are shown for comparison.

repeat1 PTAEAPNTTAGQV
repeat2 PTTEVVGTTPGQA
repeat3 PTAEPSGTTSVQV
repeat4 PTTEVISTAPVQM
repeat5 PTAESTGMTPEKV
repeat6 PVSEVMGTTLAEM
repeat7 STPEATGMTPAEV
repeat8 SIVVLSGTTAAQV
repeat9 TTTEWVETTAREL
repeat10 PIPEPEGPDASSIHHHHHH

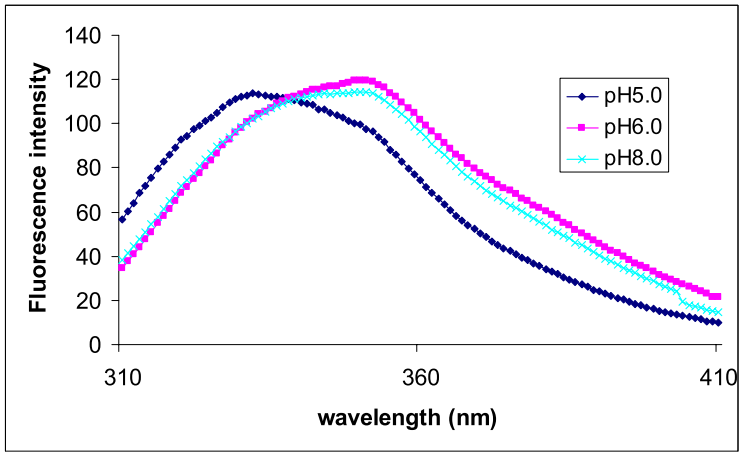

Fig. S2. Intrinsic tryptophan fluorescence assay (excitation 295 nm) of Pmel17:RPT fibers were measured between pH 5.0 and 8.0. Pmel17:RPT tryptophan emission at pH 5.0 is blue-shifted by 20 nm with respect to Pmel17:RPT at pHs 6.0 and 8.0. This shift is indicative of burial and shielding of the tryptophan residue from the aqueous buffer. The red-shift at pHs 6.0 and 8.0 suggests that at least the C-terminal end of Pmel17:RPT is exposed.

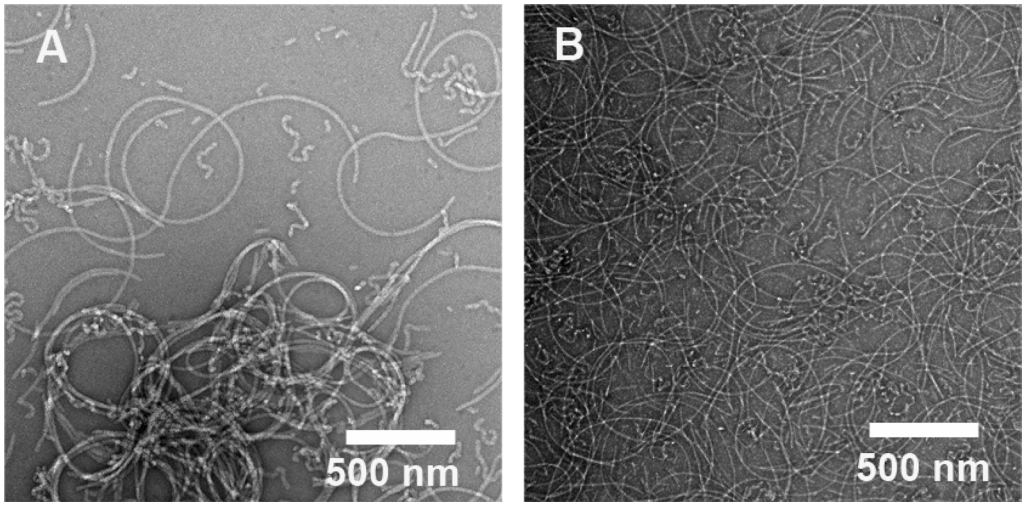

Fig. S3. Electron micrograph images of Pmel17:RPT fibers incubated in the presence (*A*) or absence (*B*) of 2% SDS. A solution containing 2% SDS was added to Pmel17:RPT fibers and incubated for 60 min at room temperature. The TEM images indicate that the generated fibrils show SDS-resistance, consistent with that observed for other amyloids (ref).

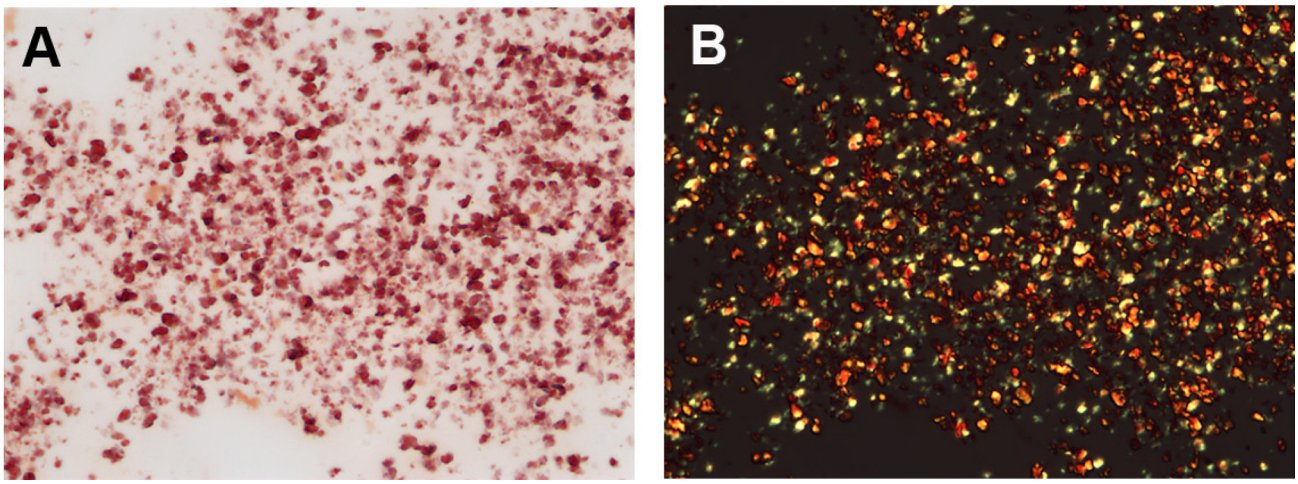

Fig. S4. Congo red birefringence of Pmel17:RPT fibers. Filaments were stained with CR and observed at 100× magnification under bright field (*A*) or by polarization microscopy (*B*). Pmel17:RPT fibers stained with Congo red under polarizing light show a characteristic yellow/apple-green birefringence, which is indicative of the presence of amyloid.

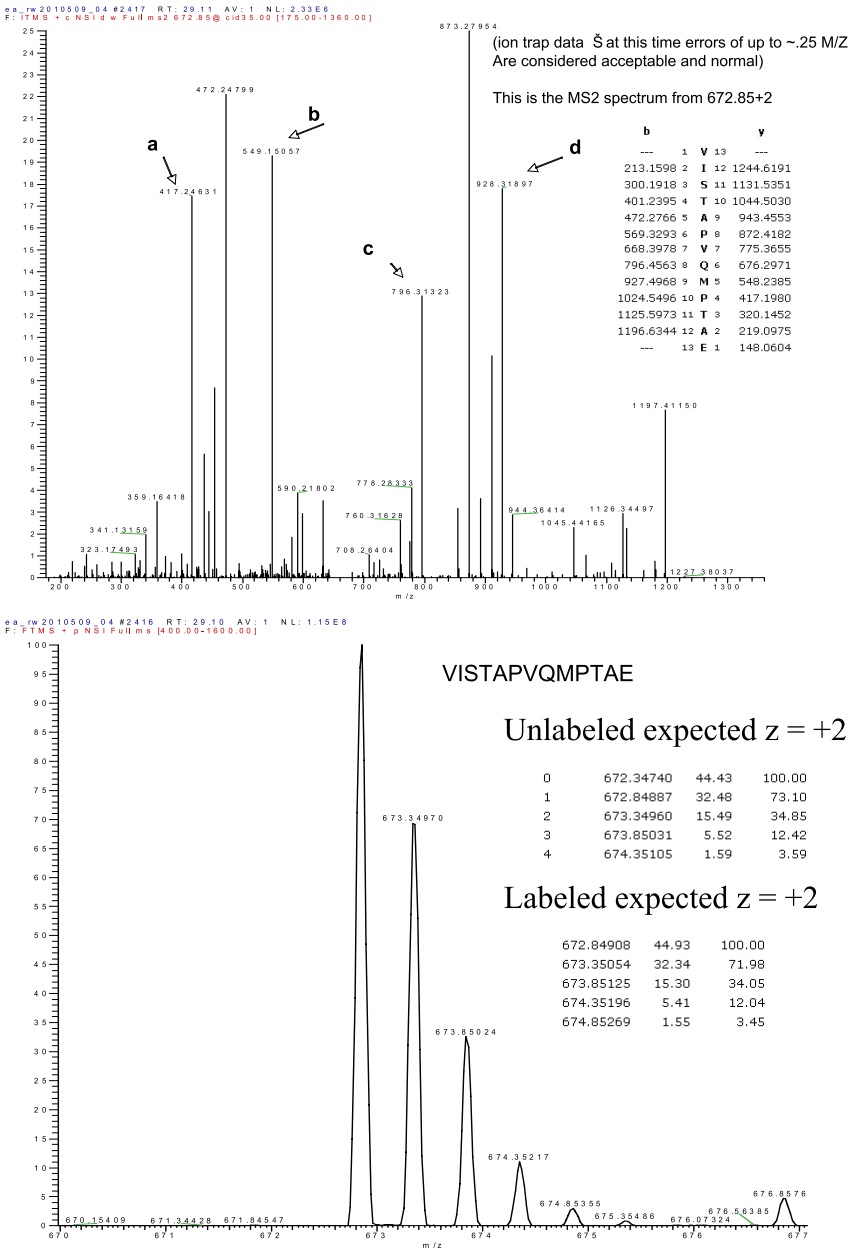

Fig. S5. Assessing incorporation of 1-^{13}C-methionine. The purified Met-1-^{13}C Pmel17:RPT sample was analyzed on a NanoAcquity(Waters)/LTQ-FT*ultra*(Thermo) LC/MS system with the mass spectrometer set up to collect a sequence of spectra; MS1 at high resolution by the FTICR between 300 and 1,600 *m/z*, followed by collection of MS2 data using the ion-trap from the 3 top peptides of charge + 2 or higher (with dynamic exclusion). MS2 data were collected for the peptide VISTAPVQMPTAE identified from the high resolution (±5 ppm) MS1, medium resolution (±0.25 *m/z*) data set by searching using Mascot search engine with broad parent and child mass windows against an *in silico* digested MSDB database (with no species specification). The top figure shows the MS2 mass spectrum assigned to the peptide by Mascot as well as the expected masses of fragment ions of the *unlabeled* peptide (generated using ProteinProspector, UCSF). There are 2 observations of the methionine residue fragment mass. Peak a appears to have the expected mass for y4 (PTAE) but peak b has a mass 1 Da higher than expected for y5 (MPTAE). Moreover there is a mass + 1 peak clearly seen for y7-y11. Then from the b-series peak c has the mass expected for b8 (VISTAPVQ) but then there is a peak 1 Da higher than the mass expected for b9 (VISTAPVQM). Moreover there are peaks for b2, b3, and b5 and for mass + 1 of b11 and b12. Coincidence of the mass of b8 with the correct fragment mass is also important because it indicates that the 1 Da mass shift does not originate from deamidation of the glutamine. The bottom figure shows detail from the MS1 data collected just before the MS2 data were collected and shows the area near the mass range collected for the MS2 fragment spectrum. Inset tables for the expected masses and relative intensities of the isotopmers for the unlabeled as well as labeled peptides are also shown (generated using ProteinProspector, UCSF). There is very close agreement between the masses and intensities expected for the labeled peptide and those observed in the spectrum, in particular, the absence of a major peak at 672.3474. The labeling efficiency appears nearly perfect, but there are types of space charge effect present in the ICR that could suppress very weak populations close to the 672.84 so it is most conservative to note that the only observed species was the + 1 Da form.

Table S1. Oligonucleotides used

pET21a(+) derivative	Pmel17 fragment (amino acid)	5′ Primer	3′ Primer
p1216	(25–467)	aaaacatatgaaagta cccagaaaccaggact	tggctcgagtttacaccagccttaaggt ggc
p1204	(25–457)+His6	aaaacatatgaaagta cccagaaaccaggact	tggctcgagtttaatggtgatgatggtg atgcaggggcccagggaacctgtaa
p1208	(204–457)+His6	aaaacatatgagctca gccttcaccattactga	tggctcgagtttaatggtgatgatggtg atgcaggggcccagggaacctgtaa
p1192	(293–469)+His6	aaaacatatgcaggct gccattcctctcacct	tggctcgagtttaatggtgatgatggtg atgtctcttcaccagccttaaggt
p1194	(293–456)+His6	aaaacatatgcaggct gccattcctctcacct	tggctcgagtttaatggtgatgatggtg atgggggcccagggaacctgtaata
pmel17:RPT	(315–444)+His6	aaaacatatgccaactg cagaggcc	tggctcgagtttaatggtgatgatggtg atggattgagctaacatctgg