SUPPORTING INFORMATION

for

Probing Fibril Dissolution of a Functional Amyloid on the Microscopic and Residue Level

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MATERIALS AND METHODS

Protein expression, purification, labeling and fibril formation. His-tagged RPT was expressed in *E. coli* and purified as previously described, [1] except where cells were grown in media containing 98% ^{13}C-glucose and 99% ^{15}N-amonium chloride for NMR spectroscopy.[2] Fibrils were prepared by incubating and shaking (600 rpm) 100 μM RPT in 20 mM sodium acetate buffer with 100 mM NaCl, pH 5.0 at 37 °C. For solution state NMR, fibrils were produced from seeds derived from fibrils that were grown under the conditions shown above. The seeds were broken into fragments with lengths below 100 nm by probe sonication (Branson Sonifier Model 250, lowest power, 1 min) and added to a monomeric 100 μM RPT solution. Fibril growth was monitored by transmission electron microscopy and thioflavin T fluorescence and found to be complete after several days.

Atomic force microscopy and kymography. RPT fibrils (final conc. ~ 5μM) were deposited on freshly cleaved mica (Ted Pella, CA). After 10 min of incubation the surface was thoroughly washed with either (20 mM sodium acetate buffer with 100 mM NaCl, pH 5.0) or (20 mM MES buffer with 100 mM NaCl, pH 6.5). The topography of the mica surface was imaged in buffer using non-contact mode atomic force microscopy (Multimode with NanoScope V, scanning head J, Bruker/Veeco, Santa Barbara, CA; pyrex-nitride probe, PNP-TR-20, typical resonance frequency 67 kHz tip, radius <10 nm; NanoAndMore GmbH, Wetzler, Germany). One and two-micrometer images were typically acquired at 2 Hz scanning rate with 1024x1024 resolution which corresponds to ~ 1/2 nm/pixel and ~ 500 ms spatial and temporal resolution. During the scanning high setpoint and a low amplitude values were chosen to minimize the pushing force of the AFM tip. At pH 5.0 the appearance (shape, length and height) of the fibrils remained unchanged after multiple repeated scanning cycles. The disassembly of the fibrils was initiated by flushing the assay chamber with (20 mM MES, pH 6.5 and 100 mM NaCl). The process was followed by repeated scans of the same area. In a separate set of experiments a method called scanning force kymography was used. The amyloid fibrils were not oriented on the mica surface and the long (<1 μm) fibrils were usually bent (Figure S2). The shorter fibrils, due to their high persistence length were usually not bent. By carefully adjusting the angle of the fast scanning axis, we were able to follow the dynamics of the disassembly of these short (< 500 nm) fibrils. During the recording of kymographs (Figure S3), first we scanned the surface and then adjusted the scan axis according to filament orientation. When the cantilever tip reachs the amyloid fibril, in the consecutive scan we disabled the slow scan axis and repetitively scanned the amyloid fibril along its axis. To alleviate the problem of thermal drift we usually carried out thermal relaxation of the piezoelectric stage, typically for 1 h. Images were analyzed using NanoScope Software (Bruker/Veeco) and OriginPro 8.5 (OriginLab, Northampton, MA).

NMR spectroscopy. ^{15}N HSQC spectra were acquired on an 800 MHz Bruker spectrometer with cryoprobe at 22 °C at pH 5.0 and pH 6.5 in the same buffers used for AFM measurements. NMR resonance assignments of ^{13}C/^{15}N labeled RPT in monomer form at pH 6.5 were done by standard procedure using CBCA(CO)NH and HNCACB experiments, supplemented by an ^{15}N-edited NOESY experiment to aid assignment of closely overlapped resonances. The backbone amide resonances of 87 of the 119 non-proline residues were unambiguously assigned, while 29 more were conditionally assigned to degenerate resonances where individual amide signals could not be resolved (Figure S5). Resonances for only three of the six C-terminal histidines were observed. All C^{α} and C^{β} resonances of the assigned residues were consistent with flexible random coil.[3] For the ^{15}N HSQC spectrum of the RPT construct in fibril form at pH 5.0, assignments for the observed N-terminal residues, 316-377, were done by analogy with no assigned resonances differing by more than 0.03 ppm in the ^{1}H dimension from those in the monomer form (Figure S5).

To monitor fibril dissolution, fibrils were first pelleted at 50,000 rpm (Beckman TLA 100) for 30 min and then resuspended in pH 6.5 buffer to a final conc. ~100 μM. The experiment was repeated twice. The NMR spectrometer was pre-tuned and pre-shimmed on a blank sample containing only the sample buffer. ^{15}N HSQC spectra were acquired every 30 min for at least 18 h and subsequently for every hour for an addition 24 h. The quality of tuning was monitored at the beginning and end of the measurement, showing only slight degradation during the kinetic runs, while auto-shimming during the disassembly maintained magnetic field homogeneity, with no significant drop in the deuterium lock signal.

Size exclusion chromatography. RPT fibils (final conc. ~ 100 μM) were re-suspended in (20 mM MES buffer with 100 mM NaCl, pH 6.5) for various lengths of time (30 min – 24 h) (Figure S7). Samples were filtered through a 0.45 micron filter before being applied to a Superdex 200 10/300 GL column (GE Healthcare, separation range 10 – 600 kDa) equilibrated with pH 6.5 buffer. Fractions were collected (0.5 ml) and checked by SDS-PAGE. To show the presence of monomer during fibril dissolution, purified RPT in 6M GuHCl was run as a control experiment, since under these conditions RPT has been shown to be monomeric.

[1] R. P. McGlinchey, F. Shewmaker, P. McPhie, B. Monterroso, K. Thurber, R. B. Wickner, *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 13731-13736.
[2] F. Shewmaker, R. P. McGlinchey, K. R. Thurber, P. McPhie, F. Dyda, R. Tycko, R. B. Wickner, *J. Biol. Chem.* **2009**, *284*, 25065-25076.
[3] D. S. Wishart, B. D. Sykes, *J. Biomol. NMR* **1994**, *4*, 171-180.

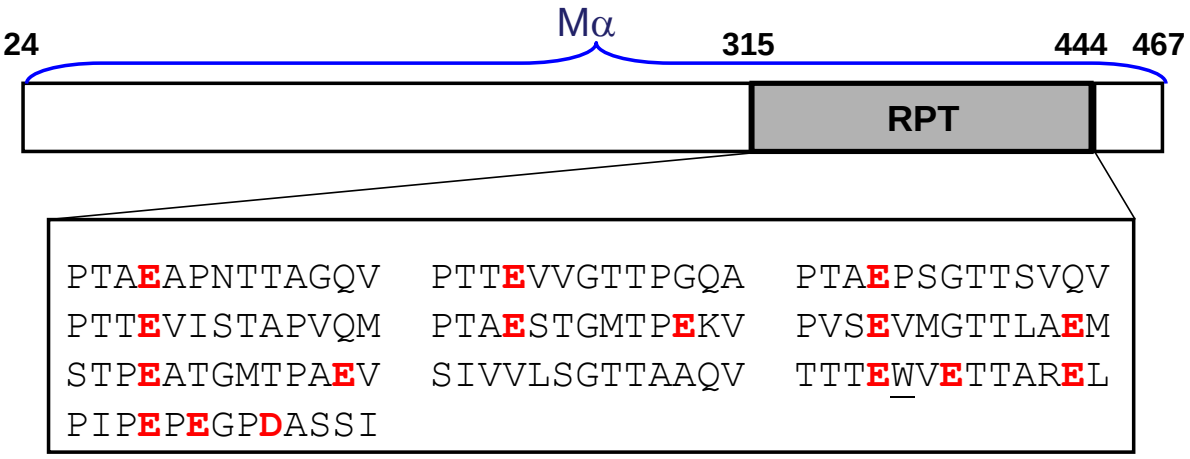


Figure S1. Amino acid sequence of RPT. Acidic residues are colored in red. Native Trp is underlined.

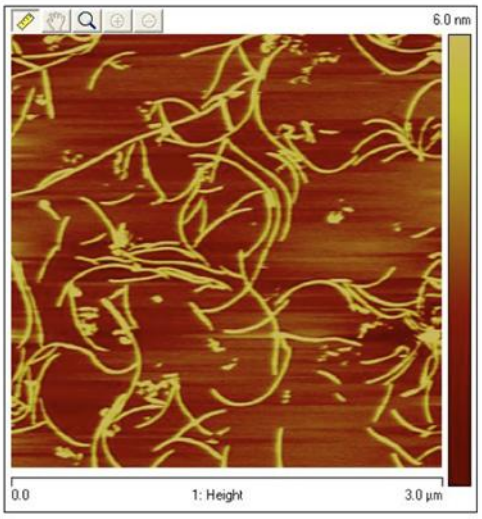
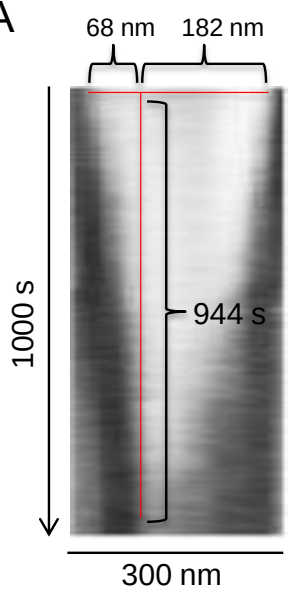


Figure S2. AFM image of RPT fibrils (5 µM in 20 mM sodium acetate, 100 mM NaCl, pH 5.0) on mica. Scale on right axis indicates fibril height.

A



B

slow end (nm/s)	fast end (nm/s)	slow end (nm/s)	fast end (nm/s)
0.056	0.213	0.045	0.222
0.023	0.098	0.006	0.045
0.067	0.078	0.045	0.125
0.015	0.276	0.018	0.078
0.018	0.066	0.012	0.076
0.032	0.139	0.045	0.156
0.056	0.175	0.035	0.169
0.112	0.132	0.066	0.165
0.089	0.127	0.112	0.088

C

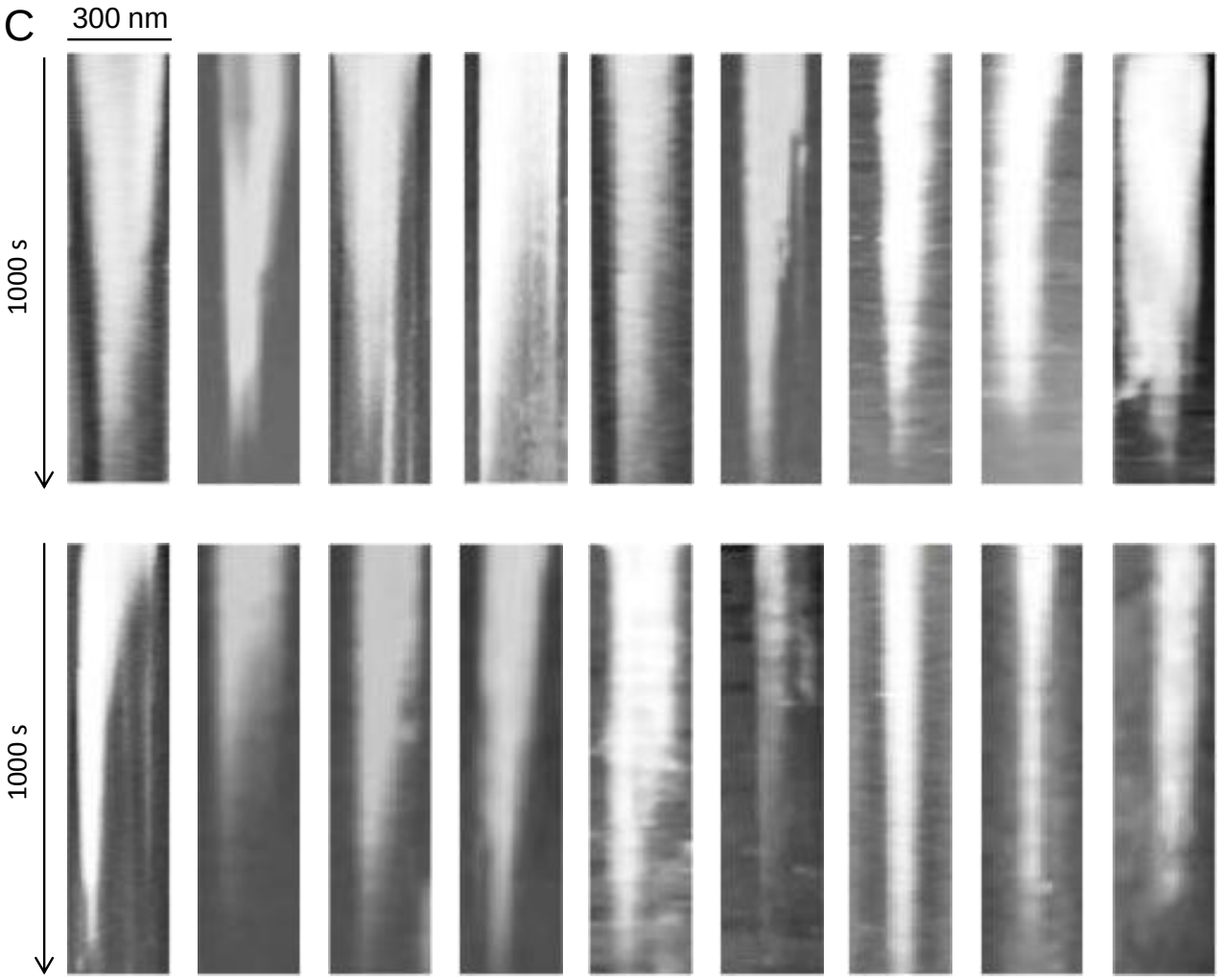


Figure S3. Scanning force kymograph (SFK) images of RPT fibrils on mica. (**A)**. Representative kymograph showing fast and slow dissolving fibril ends. (**B** and **C).** A collection of 18 kymographs showing dissolution of individual fibrils along with rates coresponding to their fast and slow dissolving ends. Rates of fibril dissasembly were calculated by measuring the total distance (nm), in which the fibril ends dissolved over the total time recorded for each kymograph.

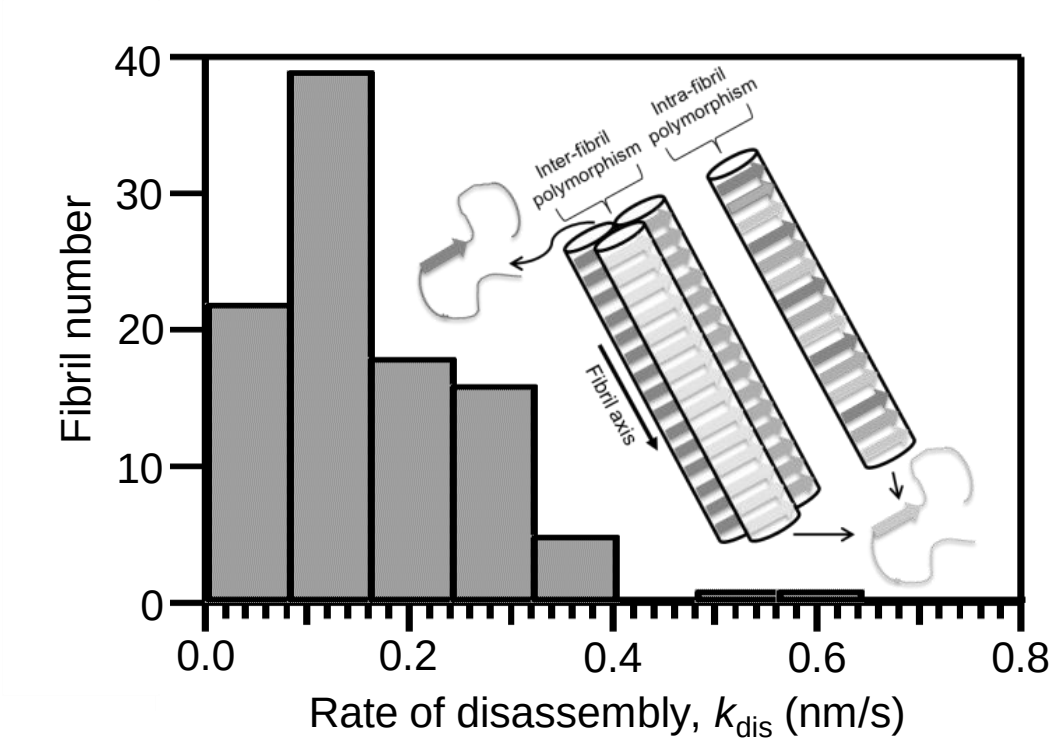


Figure S4. Histogram showing rates of disassembly (k_{dis}) for individual fibrils (n = 102, bin width = 0.08 nm/s, 10 bins). (*Inset*) Schematic representation of RPT fibril dissolution showing inter/intra fibril polymorphism. With each image taken every 128 s, 42–43 monomers would be released after each scanning cycle.

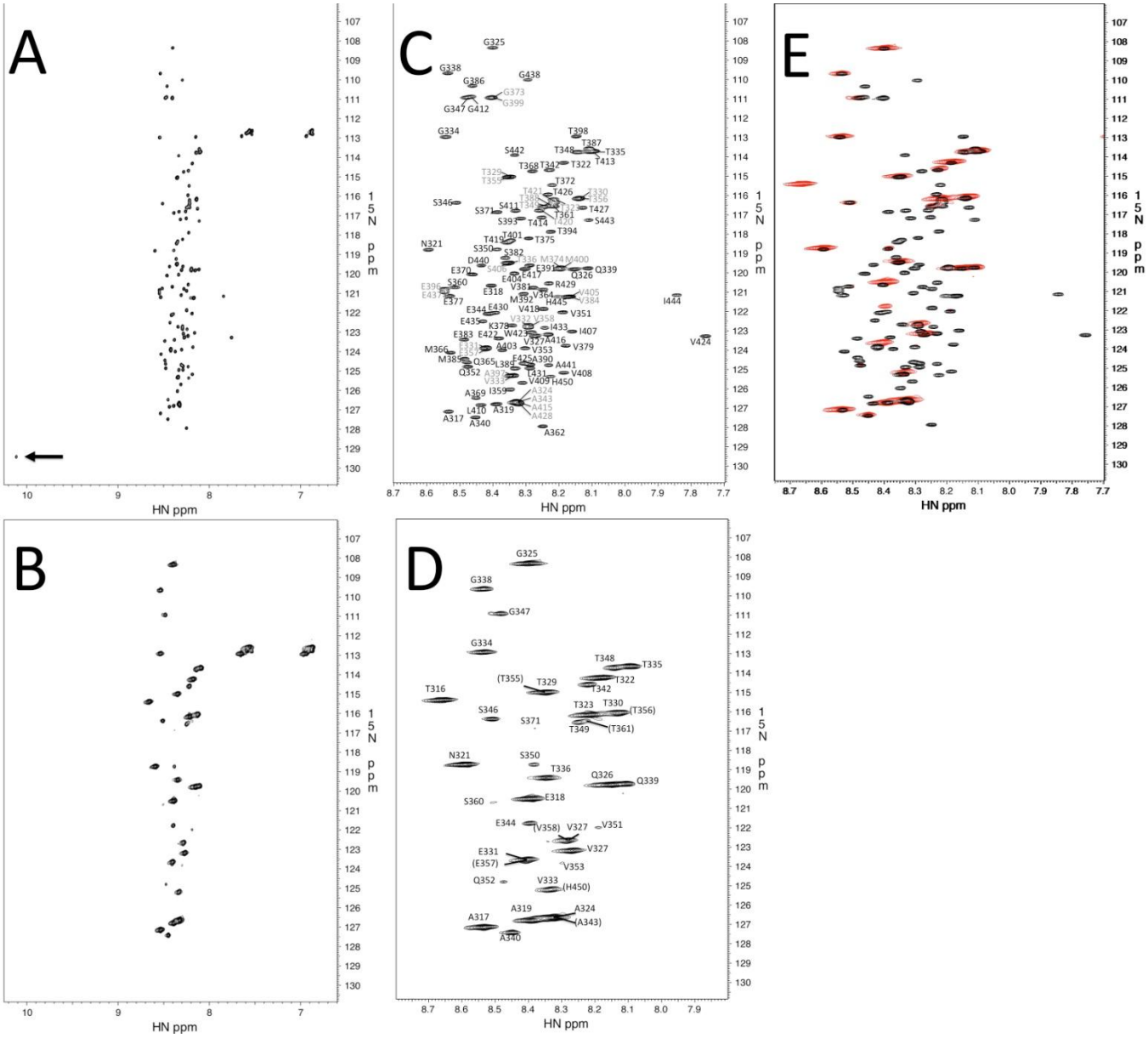


Figure S5. ^{15}N HSQC spectra of Pmel17 RPT monomer (**A**) at pH 6.5 and fibril (**B**) at pH 5.0, both at 22 °C. In (**A**) the arrow indicates the side chain amide resonance of W423. Close-up view of the backbone amide region with residue assignments for monomer (**C**) and fibil (**D**). In (**C**) ambiguous assignments are shown in gray, and in (**D**) residues whose signals are likely obscured by stronger resonances are in parentheses. (**E**) Overlay of monomer spectrum (black) and fibril spectrum (red).

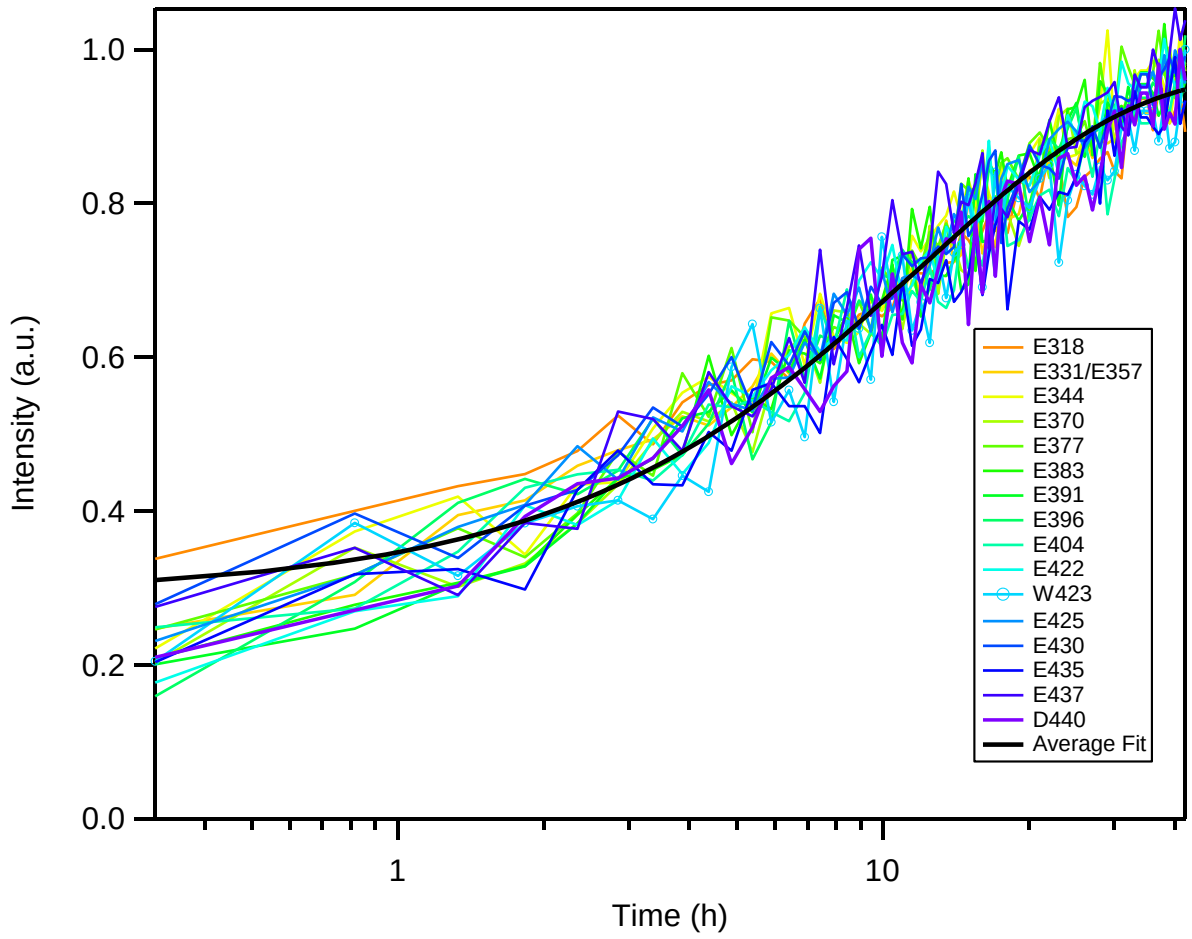


Figure S6. Dissolution kinetics of acidic residues and W423 signal intensity (peak height) in ^{15}N HSQC spectra (20 mM MES, 100 mM NaCl, 22 °C). The black line is a single-exponential fit curve (k = (12 h)$^{-1}$).

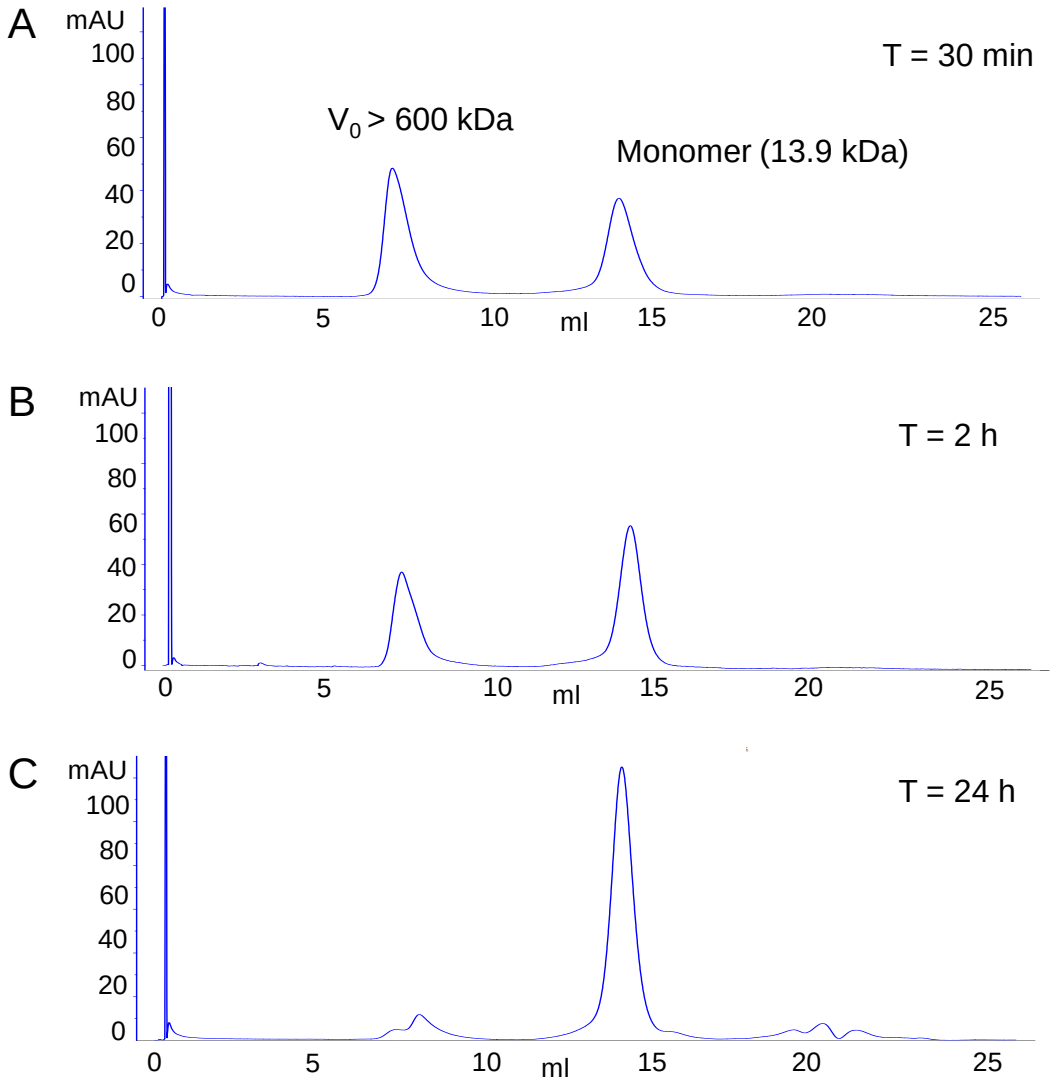


Figure S7. Size exclusion chromatography (SEC) traces showing dissolution of RPT fibrils in pH 6.5 buffer. RPT fibrils were resuspended at 6.5 and incubated for (**A**) 30 min, (**B**) 2 h and (**C**) 24 h before being applied to a pre-equilibrated column. A control experminent (not shown) containing RPT purified in 6M GuHCl was pre-mixed with RPT taken after 24 h in pH 6.5 buffer. The resulting chromatogram showed an increase in the peak corresponding to monomer (~14.5 ml eluted).