MATERIALS AND METHODS

RPT Expression and Purification. Human RPT (amino acids 315–444) mutants were generated by site directed mutagenesis using Stratagene Quickchange kit (Agilent Technologies). Single point mutations were performed using the RPT pET21a(+) construct as a template.[1] Multiple mutations were generated sequentially.

List of primers used to design RPT mutants. For RPT truncation, sample name is displayed as amino acid (aa) numbers.

RPT mutant	5' Primer	3' Primer
E404A	ATGACACCTGCAGCGGTATCAATTGTG	CACAATTGATACCGCTGCAGGTGTCAT
E404Q	ATGACACCTGCACAGGTATCAATTGTG	CACAATTGATACCTGTGCAGGTGTCAT
E422A	GTAACAACTACAGCGTGGGTGGAGACC	GGTCTCCACCCACGCTGTAGTTGTTAC
E422Q	GTAACAACTACACAGTGGGTGGAGACC	GGTCTCCACCCACTGTGTAGTTGTTAC
E425A	ACAGAGTGGGTGGCGACCACAGCTAGAG	CTCTAGCTGTGGTCGCCACCCACTCTG
E425Q	ACAGAGTGGGTGCAGACCACAGCTAGAG	CTCTAGCTGTGGTCTGCACCCACTCTGT
E430A	ACCACAGCTAGAGCGCTACCTATCCCTG	CAGGGATAGGTAGCGCTCTAGCTGTGGT
E430Q	ACCACAGCTAGACAGCTACCTATCCCTG	CAGGGATAGGTAGCTGTCTAGCTGTGGT
E422A /E425A	GGTAACAACTACAGCGTGGGTGGCGACC	GGTCGCCACCCACGCTGTAGTTGTTAC
E422Q /E425Q	GGTAACAACTACACAGTGGGTGCAGACC	GGTCTGCACCCACTGTGTAGTTGTTACC
Δ404-410	GTATGACACCTGCAGAGTCTGGAACCACAGCTG	CAGCTGTGGTTCCAGACTCTGCAGGTGTCATAC

Recombinant RPT and mutants were expressed in BL21(DE3) RIPL cells and purified as previously described.[1] Protein concentrations were determined using a molar extinction coefficient estimated on the basis of amino-acid content: $\varepsilon_{280\ nm}$ = 5,500 M^{-1} cm^{-1}. Purity and homogeneity of samples were assessed by SDS PAGE. Protein molecular weights were confirmed by ESI-MS (NHLBI Biochemistry Core Facility). Purified protein was stored short term (less than 2 weeks) at 4 ^{o}C in pH 7.5 buffer (6M GuHCl, 100 mM Na_2HPO_4, 100 mM NaCl, 200 mM imidazole).

Fibril Aggregation/Disaggregation. Purified wild-type and mutant RPT were buffer exchanged twice using PD-10 columns (GE Healthcare) prior to aggregation by first desalting into pH 8.5 buffer (20 mM tris(hydroxymethyl)aminomethane (Tris), 100 mM NaCl) followed by the appropriate aggregation buffer (pH 5–5.5; 20 mM sodium acetate (NaOAc), 100 mM NaCl, pH 6–6.5; 20 mM 2-(N-morpholino)ethanesulfonic acid (MES), 100 mM NaCl and pH 7; 20 mM 3-(N-morpholino)propanesulfonic acid (MOPS), 100 mM NaCl). All buffers were filtered (0.2 μm). Final protein solutions (30 μM) were filtered through YM-100 filters (Millipore) to remove any preformed aggregates. Aggregation experiments were performed in sealed 96-well flat bottom plates (Corning Costar) containing 2-mm sterile glass beads to facilitate orbital shaking (1 mm, ca. 87.6 rpm) at 37 °C. A solution containing 200 μL protein supplemented with 10 μM thioflavin T (ThT) was measured by ThT (excitation and emission wavelengths were 415 and 480 nm, respectively) and Trp (excitation and emission wavelengths were 280 and 350 nm, respectively) fluorescence as a function of time using a Tecan Infinite M200 Pro microplate reader.

For fibril disaggregation experiments, pre-aggregated RPT samples at pH 5 (500 μL containing 30 μM RPT and 10 μM ThT) were titrated with increments of 1 M NaOH (0.25–0.5 μL) to adjust the pH. Samples were measured by Trp fluorescence (λ_{ex} = 295 nm, λ_{obs} = 300−500 nm, 0.3 s integration time, 1 nm slits, 25 °C) in 1-cm quartz cuvettes using a Fluorolog 3 spectrofluorometer (Horiba Jobin Yvon). The

apparent extent or kinetics of dissolution at individual pH condition is pH dependent, *i.e*. higher the pH, faster the disassembly. In order to compare all data sets, an equilibration time of 10 minutes was chosen for practical purposes.

Circular Dichroism (CD) Spectroscopy. CD measurements (200 to 260 nm, 1 nm bandwidth, and continuous mode with a scanning rate of 100 nm/min) were performed in 1 mm cuvettes using a Jasco J-715 spectropolarimeter (Jasco Analytical Instruments). Each experiment is reported as an average of 3 accumulated scans. All measurements were performed at 25 °C. Data were analyzed using Igor 6.2 (Wavemetrics). The mean residue ellipticity was calculated using the equation: $[\Theta]=(100\theta)/(cln)$, where θ is the measured CD signal in mdeg, c is the protein concentration in mM, l is the path length in cm, and n is the number of amino acids.

Transmission Electron Microscopy. Samples (5 μL) taken after each aggregation run were deposited on TEM grids (400-mesh formvar and carbon coated copper, Electron Microscopy Sciences), incubated for 5 min, wicked with filter paper, followed by the addition of 5 μL of 1-2% (w/v) aqueous uranyl acetate solution. After incubating for 5 min, excess uranyl acetate was wicked away and grids were allowed to air dry. TEM was performed using a JEOL JEM 1200EX transmission electron microscope (accelerating voltage 80 keV) equipped with an AMT XR-60 digital camera (NHLBI TEM Core Facility).

Limited PK digestion. Proteinase K (Promega) digestion was performed by incubating 2 μg/ml proteinase K with 90 μM preformed RPT fibrils. Reactions were performed in 20 mM sodium acetate, 100 mM NaCl, pH 5.0, at room temperature in a total volume of 1.0 ml. The reactions were terminated by the addition of 4% TCA. Samples were lyophilized and resuspended in 5% acetic acid prior to LC-MS analysis (NIDDK, Advanced Mass Spectrometry Facility). For N-terminal sequencing, PK-resistant fragments were separated using a 10-20% Tricine gel and transferred to polyvinylidene difluoride (PVDF). Bands were excised from PVDF and submitted to the Keck Biotechnology Resource Laboratory.

[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.

Table S1. Proteinase K resistant fragments generated after limited digestion of RPT fibrils. Samples were analysed by SDS-PAGE and LC-MS. N-terminal sequencing was performed on bands excised from PVDF.

Amino acids	Sequence	Expected mass	Actual mass
371–444	STGMTPEKV PVSEVMGTTLAEMSTPEATGMTPAEV SIVVLSGTTAAQVTTTEWVETTAREL PIPEPEGPDASSIHHHHHH	8427.36	8426.51
390–444	AEMSTPEATGMTPAEV SIVVLSGTTAAQVTTTEWVETTAREL PIPEPEGPDASSIHHHHHH	6481.10	6480.10
393–444	STPEATGMTPAEV SIVVLSGTTAAQVTTTEWVETTAREL PIPEPEGPDASSIHHHHHH	6149.17	6149.71
400–444	MTPAEV SIVVLSGTTAAQVTTTEWVETTAREL PIPEPEGPDASSIHHHHHH	5506.06	5505.71

Note: Peptide sequences shown above were confirmed by N-terminal sequencing.

Figure S1. Disaggregation of wild-type RPT fibrils monitored by ThT emission. Samples (30 μM) were taken after aggregating at pH 5 and pH was carefully adjusted by adding small volumes of 1 M NaOH from pH 5–7 (black-to-light grey, respectively). Each scan was taken after a 10-min equilibration at the corresponding pH.

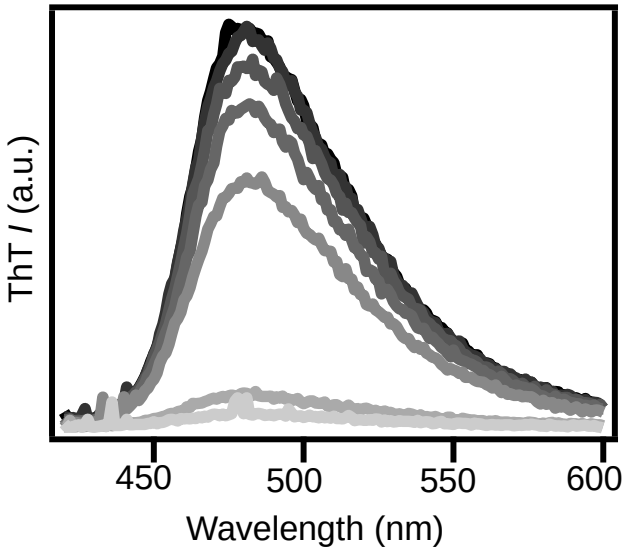

Figure S2. Representative TEM images for single Ala- and Gln-mutants (**A**) E404A, (**B**) E422Q, (**C**) E425Q and (**D**) E430Q taken after aggregation at pH 5. Scale bar is 100 nm.

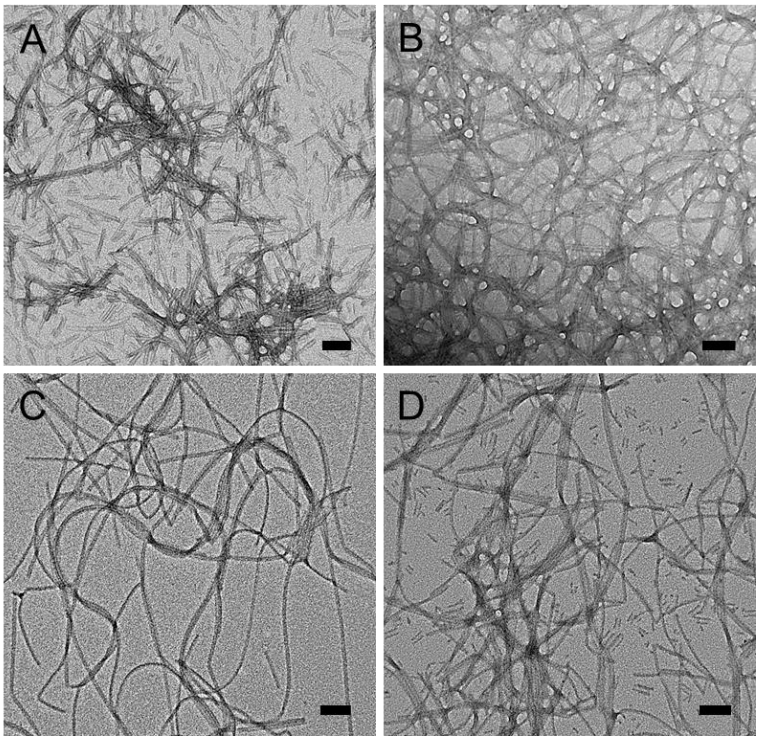

Figure S3. Aggregation kinetics of E422A (left) and E422Q (right) monitored by W423 emission at pH 5 (red), 5.5 (blue), 6 (green), 6.5 (pink) and 7 (purple). Graphs are plotted on the same scale (15000– 27000 a.u.). Note incubation times were continued beyond 170 h to show no aggregation occured at pH 7.

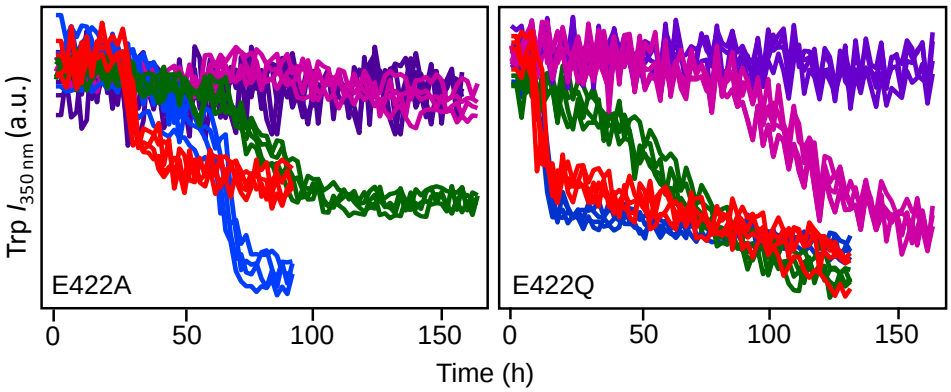

Figure S4. Disaggregation of E422Q fibrils monitored by W423 and ThT emission. Samples (30 µM) were taken after aggregating at pH 5 and pH was carefully adjusted by adding small volumes of 1 M NaOH. Each scan was taken after a 10-min equilibration at the corresponding pH (see legend).

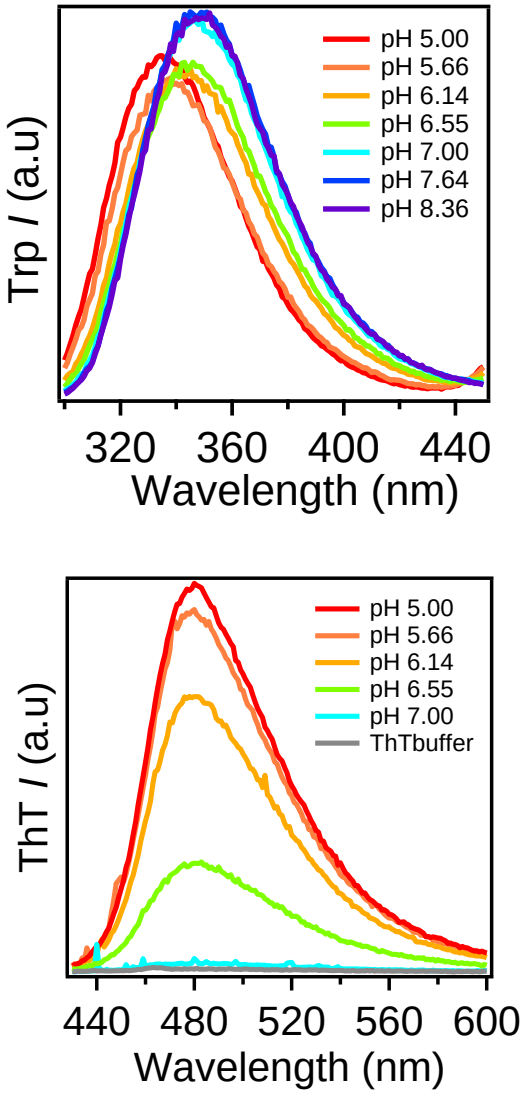

Figure S5. Aggregation kinetics of single Ala/Gln mutants at E404, E425 and E430 at pH 6 monitored by ThT emission. Continued incubation showed no changes in fluorescence for E425A/Q and E430A/Q mutants. ThT intensity scale is (0–20000 a.u.).

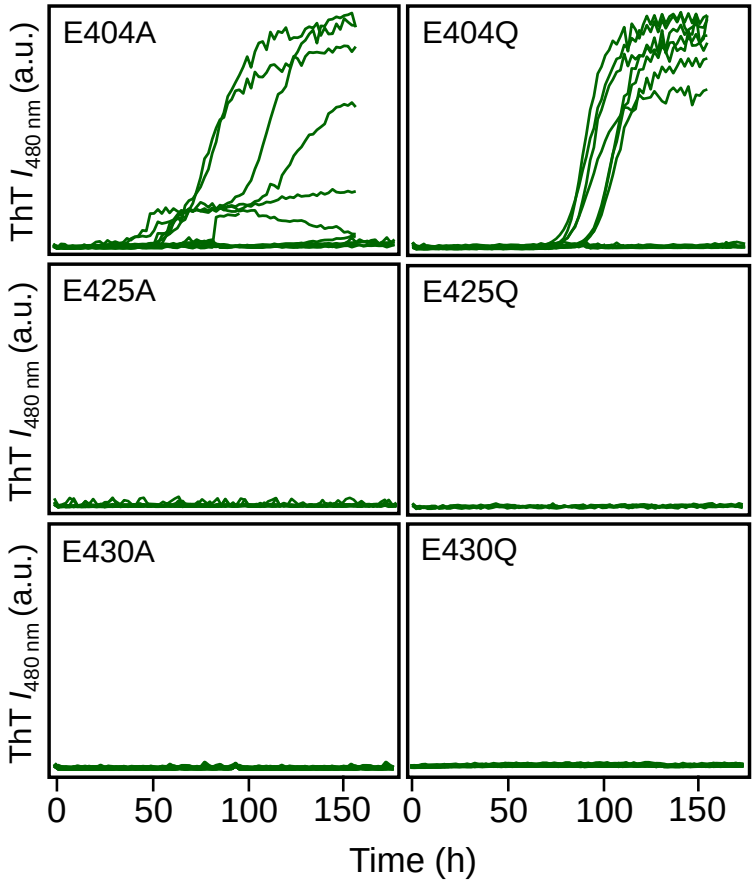

Figure S6. Aggregation kinetics of E404A/E422A (top) and E422Q/E422Q (bottom) monitored by W423 emission at pH 5 (red), 6 (green), and 7 (purple). Graphs are plotted on the same scale (50000–15000 a.u.).

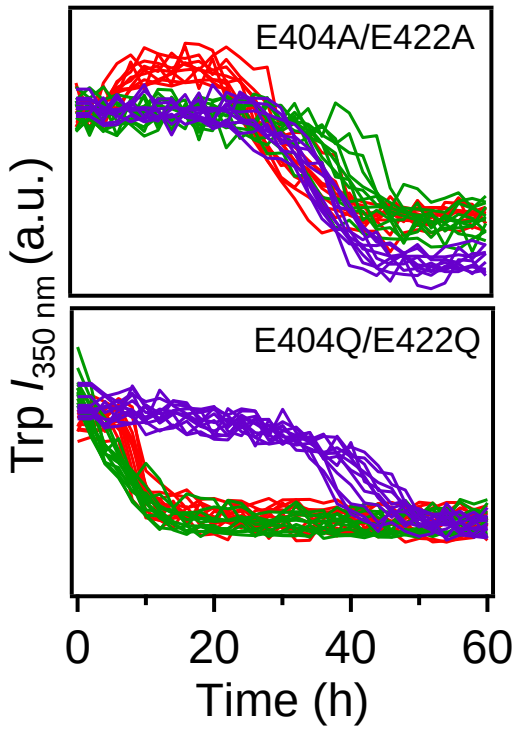

Figure S7. Far-UV CD spectra of (**A**) E404A/E422A and (**B**) E404Q/E422Q mutants aggregated at pH 5 (red), 6 (blue) and 7 (green). Start (T=0) and end (T=End) of aggregation were recorded.

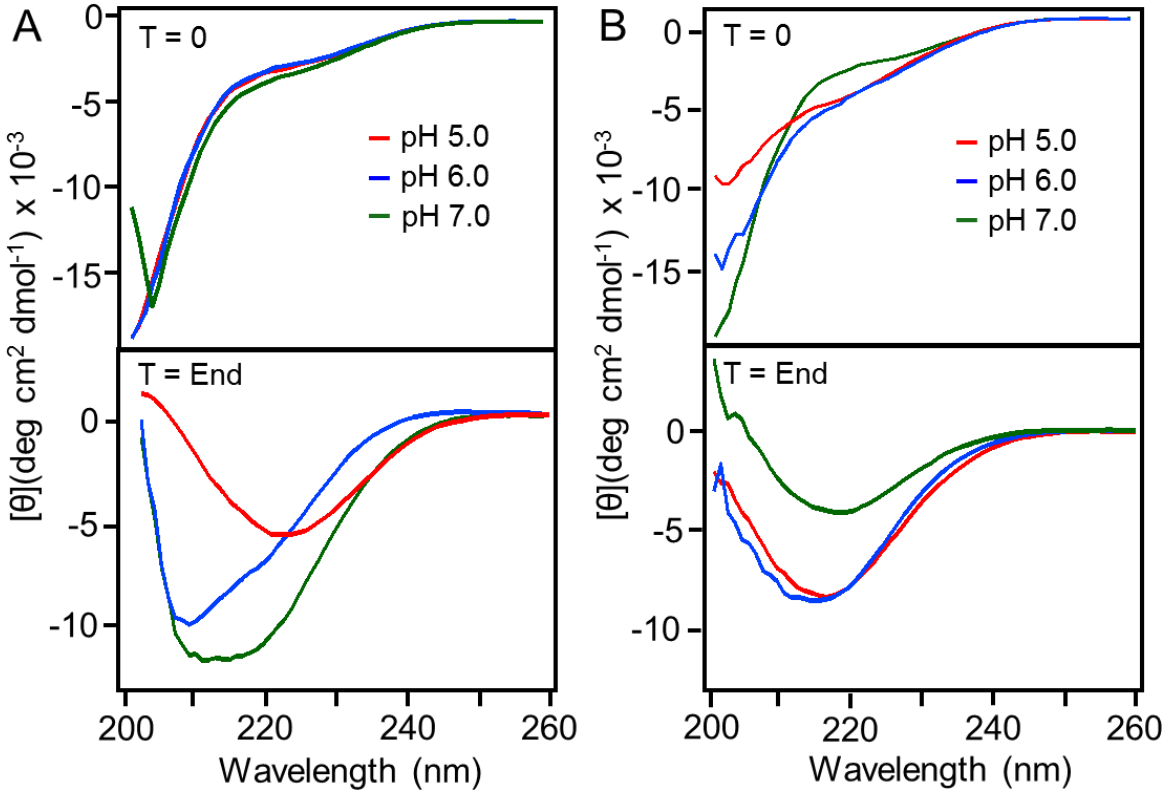

Figure S8. Disaggregation of E404Q/E422Q fibrils monitored by W423 and ThT emission. Samples (30 µM) were taken after aggregating at pH 5 and pH was carefully adjusted by adding small volumes of 1 M NaOH. Each scan was taken after a 10-min equilibration at the corresponding pH (see legend).

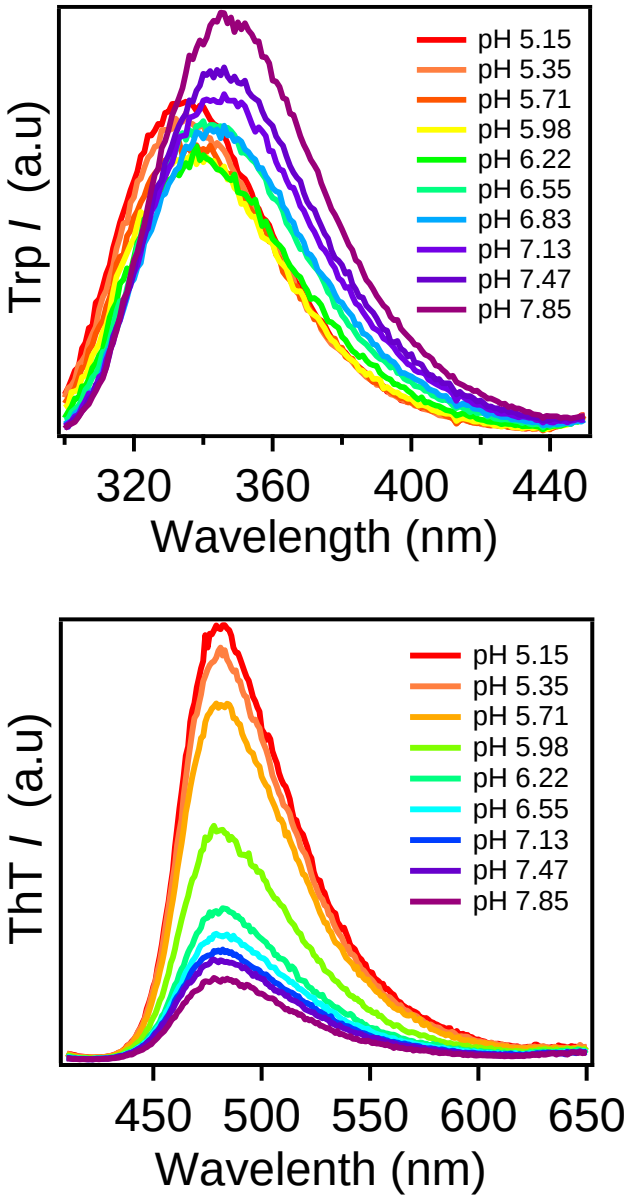

Figure S9. Aggregation kinetics of E404/E422/E425/E430Q (Quad Q) mutant at pH 5 (red), 5.5 (blue), 6 (green) 6.5 (pink) and 7 (purple). Samples were monitored by ThT (**A**) and Trp (**B**) fluorescence. ThT and Trp intensity scales are (0–38000 a.u.) and (10000–16000 a.u.), respectively. Representative TEM images of Quad Q fibrils formed from aggregating at pH 5–7. Scale bar is 100 nm.

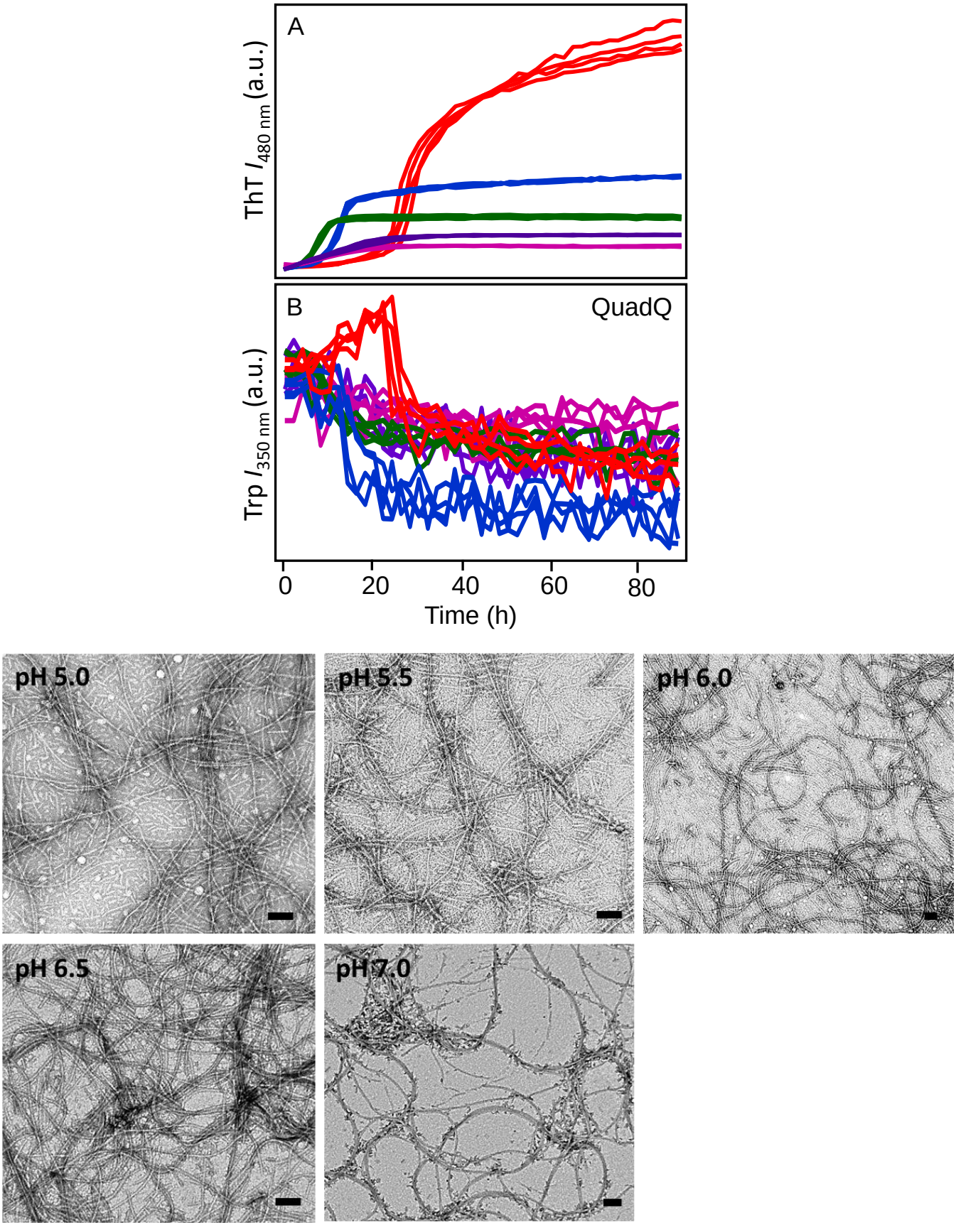

Figure S10. Aggregation kinetics of RPT truncation mutant Δ405–410 monitored by (**A**) ThT and (**B**) W423 fluorescence at pH 5. ThT and Trp intensity scales are (0–1000 a.u.) and (20000–29000 a.u.), respectively. Incubation times were continued beyond 100 h and no changes were observed.

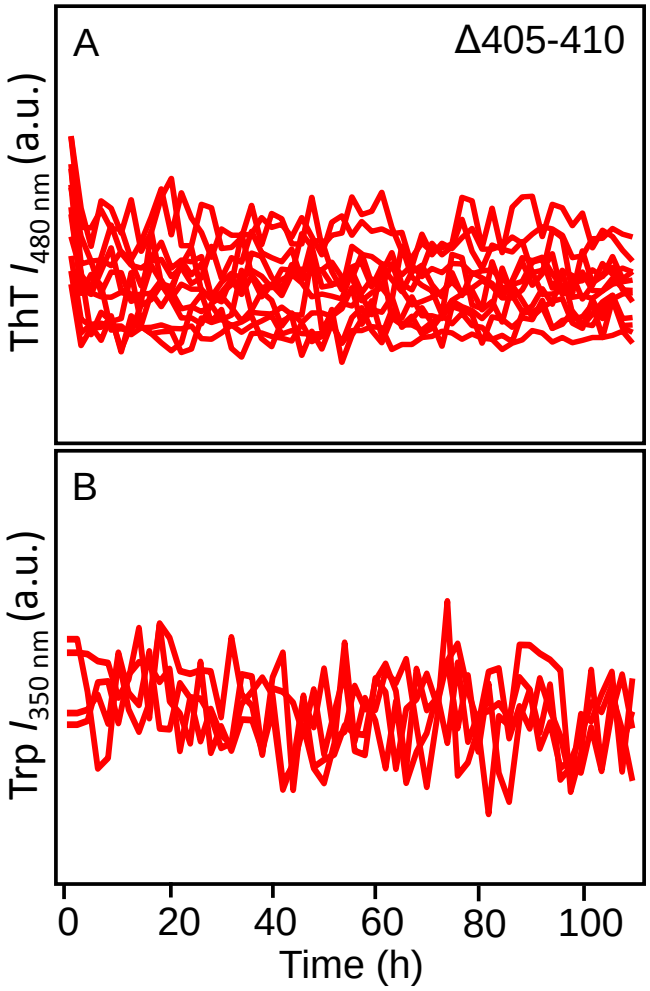

Figure S11. Schematic diagram depicting possible side chain orientations for E404 (green) and E422 (blue) as either outside or inside the β-strands of RPT filaments (**A**–**D**). Note only several residues are shown for each of the two proposed β-strands (403–411) and (415–423). G412 likely forms a β-turn linking the two strands and is not shown. Part of the critical amyloidogenic region, VSIVVL (cyan) is shown. Fibril axis is coming out of the page.