Supporting Information

for

Tryptophan probes of TDP-43 C-terminal domain amyloid formation

Sydney O. Shuster and Jennifer C. Lee*

Laboratory of Protein Conformation and Dynamics, Biochemistry and Biophysics Center, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland, 20892

*Corresponding author: Jennifer C. Lee

Email: leej4@nhlbi.nih.gov

Phone: 301-827-0723

This SI includes:

Extended Materials and Methods

Protein Expression. A single colony was chosen from the transformed plate, and inoculated into 500 mL LB medium with 100 mg/L kanamycin in a 2.8 L baffled shake flask. After overnight growth at 37 °C with a shaking speed of 220 rpm, the culture was used to inoculate 13-L medium in the fermenter. A 20-L BioFlo 4500 fermenter (New Brunswick Scientific, Edison, NJ) with a working volume of 13 L was used for large-scale fermentation. The fermenter was batched with 10 g/L tryptone, 5 g/L YE, 5 g/L NaCl, 3.5 g/L glucose, 1 g/L $MgSO_4 \cdot 7H_2O$, 12 g/L K_2HPO_4, with 50 mg/L kanamycin, pH 7. Temperature was controlled at 37 °C, pH was controlled at 7 with 30% NH_4OH, and dissolved oxygen was controlled at 20%. BioCommand Plus software from NBS was used for data collection. When $OD_{600\,nm}$ reached above 3.0, cells were induced with 0.8 mM IPTG. Addition of 3.0 g/L of glucose was added at the time of induction and 2 h post induction. Four hours after induction, cells were harvested using a CARR Pilot continuous centrifuge at a flow rate of 250 mL/min. Cell pellets were flash frozen with liquid nitrogen and stored at −80 ºC until protein purification (NHLBI Protein Expression Facility).

TEV protease purification. Cell pellet was resuspended in lysis buffer (1X PBS, 10 % glycerol, 25 mM imidazole, pH 8.0) and were lysed *via* 3 cycles of 30-s sonication on ice with a 3-mm tapered microtip attached to a Branson Sonifier 450 (50% duty cycle, output control = 5). DNA was precipitated by the addition of 0.1 % PEI. Precipitated DNA was removed by centrifugation at 18,000 ×*g* for 30 min at 4 °C. Protein in supernatant was bound to a preequilibrated HisTrap FF 16/10 column (GE Healthcare), and eluted with a linear gradient of elution buffer (lysis buffer with 500 mM imidazole, pH 8.0). Fractions containing TEV protease was buffer exchanged into 1X PBS with 1 mM DTT and 0.5 mM EDTA, concentrated to 1 mg/mL, snap frozen with liquid nitrogen, and stored at −80 ºC until use.

Limited Proteolysis. Reactions were prepared in glass vials (VWR International model no. 10803-890). Pre-formed TDP-43_{CTD} fibrils (50 μM) were mixed with either 2, 20, or 200 ng/mL of PK (Invitrogen) in 10 mM NaPi buffer at pH 7.4. Reactions were incubated overnight at 37 °C with shaking (600 rpm). To terminate the digestion, guanidine hydrochloride was added to a final concentration of 4 M. Trifluoroacetic acid was added to the samples to a final amount of 0.5% (w/v) and then subjected to LC-MS analysis using a Agilent 6200 series ESI-TOF LC-MS instrument (NHLBI Biochemistry Core). Deconvolution of the primary eluting species was then performed using MassHunter software (Agilent Technologies) to identify peptide fragments.

Table S1. Mass spectrometric analysis of PK digestion.

WT TDP-43 CTD (50 μM) + PK (200 ng/mL)		
Observed Mass (da)	Theoretical Mass (da)	Position in Sequence
14890.55	14889.93	*267-414
10568.67	10568.51	*267-368
9844.83	9844.7	270-368

WT TDP-43 CTD (50 μM) + PK (20 ng/mL)		
Observed Mass (da)	Theoretical Mass (da)	Position in Sequence
14890.24	14889.93	*267-414
12236.38	12236.18	*267-386
10712.76	10712.64	*267-371
10568.69	10568.51	*267-368

WT TDP-43 CTD (50 μM) + PK (2 ng/mL)		
Observed Mass (da)	Theoretical Mass (da)	Position in Sequence
14890.23	14889.93	*267-414
12236.55	12236.18	*267-386
10712.75	10712.64	*267-371
10568.67	10568.51	*267-368

W334 TDP-43 CTD (50 μM) + PK (20 ng/mL)		
Observed Mass (da)	Theoretical Mass (da)	Position in Sequence
14813.06	14811.85	*267-414
10712.85	10712.64	*267-371
10568.97	10568.51	*267-368

W385 TDP-43 CTD (50 μM) + PK (20 ng/mL)		
Observed Mass (da)	Theoretical Mass (da)	Position in Sequence
14813.14	14811.85	*267-414
12198.49	12197.14	*267-386
11441.19	11440.33	*267-378
10674.22	10673.6	*267-371

W412 TDP-43 CTD (50 μM) + PK (20 ng/mL)		
Observed Mass (da)	Theoretical Mass (da)	Position in Sequence
14812.45	14811.85	*267-414
12159.54	12158.1	*267-386
10674.05	10673.6	*267-371
10674.05	10568.51	*267-368

*indicates the fragment still contains the three-residue overhang GHM

Table S2. Trp Fit Parameters.[1]

Protein	λ_{max} (nm)	Γ (nm)	ρ
WT soluble	347 ± 0.2	61 ± 0.3	1.4 ± 0.0
fibrillar	336 ± 2.6	63 ± 2.5	1.4 ± 0.1
W334 soluble	347 ± 0.4	65 ± 0.9	1.3 ± 0.0
intermediate	338 ± 3.2	65 ± 0.5	1.3 ± 0.0
fibrillar	331 ± 2.3	63 ± 2.4	1.3 ± 0.1
W385 soluble	347 ± 0.3	64 ± 0.3	1.3 ± 0.0
fibrillar	323 ± 3.2	83 ± 3.9	1.2 ± 0.1
W412 soluble	348 ± 0.2	66 ± 0.1	1.3 ± 0.0
fibrillar	334 ± 2.6	75 ± 3.0	1.1 ± 0.1

[1]Data reported are averages and standard deviations from multiple protein preparations and at least 4 independent measurements.

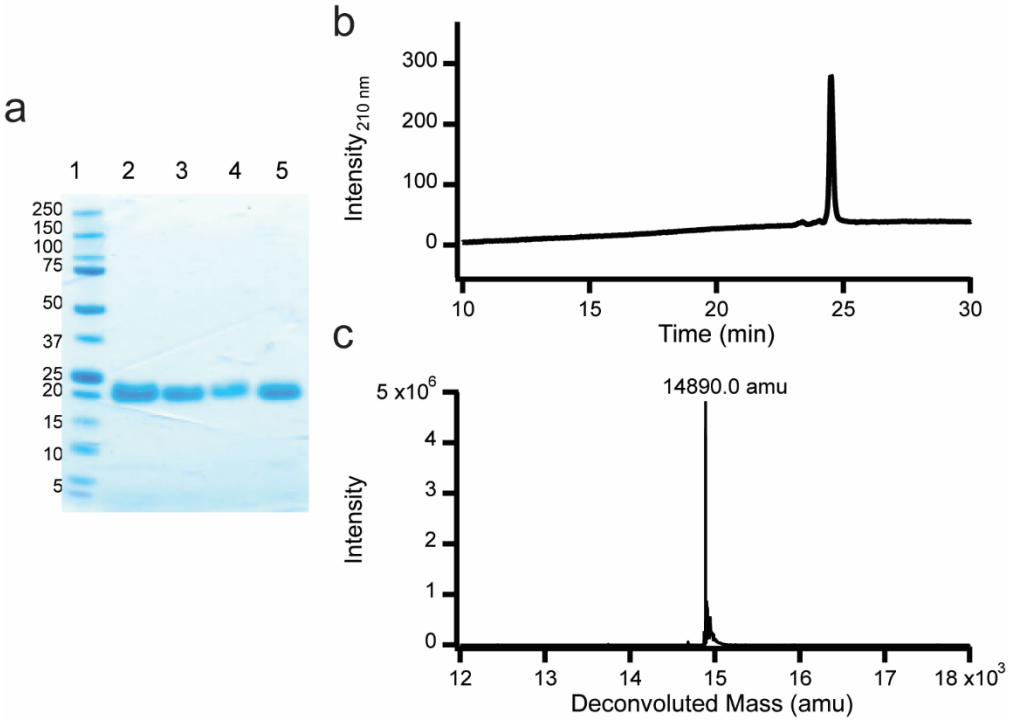

Figure S1. Characterization of purified TDP-43$_{CTD}$ proteins. (a) SDS-PAGE of purified WT and mutant TDP-43$_{CTD}$. Lanes are as follows, (1) Ladder (2) WT (3) W334 (4) W385 (5) W412. Representative LC trace (b) and MS analysis (c) of purified WT TDP-43$_{CTD}$.

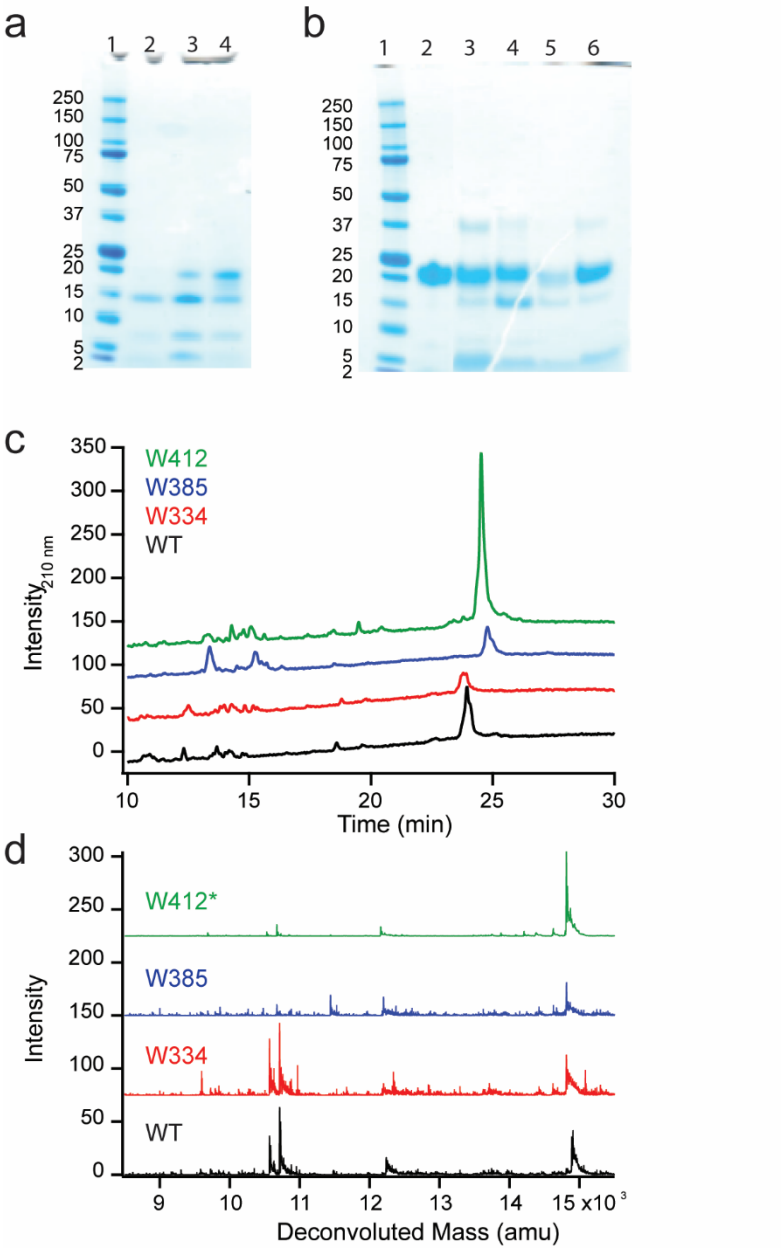

Figure S2. Comparison of PK digestion of mutants to WT. (a) SDS-PAGE of limited-PK digestion of WT TDP-43_{CTD} (~ 50 µM) as a function of decreasing PK. Lanes: (1) ladder, (2) 200 ng/mL PK, (3) 20 ng/mL PK, and (4) 2 ng/mL PK. (b) Comparison of SDS-PAGE analysis of PK digestion of fibrils (~ 50 µM) at 20 ng/mL. Lanes (1) Ladder, (2) Soluble WT Control (−PK), (3) WT + PK, (4) W334 + PK, (5) W385 + PK, and (6) W412 +PK. (c) LC traces monitored at 210 nm and (d) MS analysis for limited-PK digestion of WT (black), W334 (red), W385 (blue), W412 (green) TDP-43_{CTD}. W412 has been scaled down by a factor of 10 for ease of comparison. Spectra are off-set. Masses are reported in **Table S1**. Incubated with 20 ng/mL PK for 18 h at 37 °C in pH 7.4 buffer with shaking.

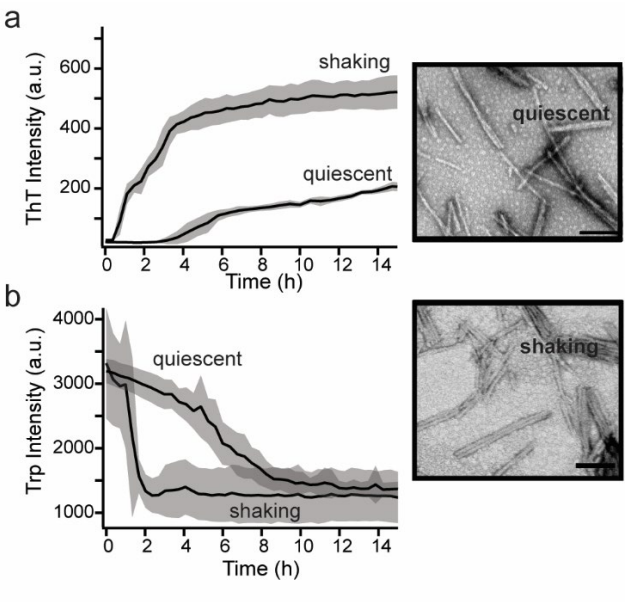

Fig S3. Quiescent *vs*. shaking conditions for aggregation. ThT (a) and Trp (b) aggregation kinetics of WT TDP-43_{CTD} in shaking and quiescent conditions ([TDP-43_{CTD}] = 10 μM, [ThT] = 5 μM in 10 mM NaPi, pH 7.4 at 37 ºC, final [GuHCl] is 135 and 160 mM for shaking and quiescent conditions, respectively). Lines and shading represent the mean and standard deviation, respectively (n = 5). (Right) TEM images of shaking and quiescent conditions. Scale bars are 100 nm.

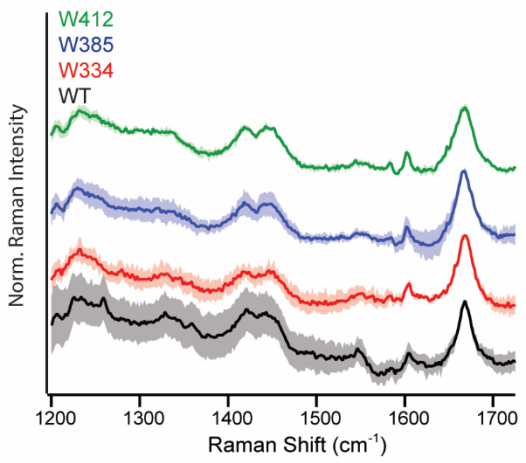

Figure S4. Full Raman spectra of WT and mutants. Comparison of WT (black), W334 (red), W385 (blue), and W412 (green) fibrils. Spectra are normalized to the amide-I (1668 cm^{-1}) and offset for clarity. Lines and shading represent the mean and standard deviation, respectively (n = 3).

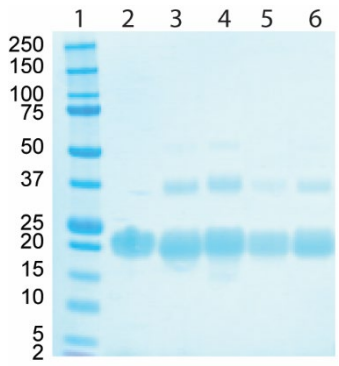

Figure S5. Comparison of amounts of fibrils formed by WT and mutant TDP-43_{CTD}. SDS-PAGE analysis of the pellet fraction of aggregated material. Lanes: (1) ladder, (2) Soluble WT control, (3) WT, (4) W334, (5) W385, and (6) W412. 10 µM proteins were aggregated in 10 mM NaPi, pH 7.4 under shaking conditions at 37 ºC. We attribute the band at 37 kDa to an SDS-insoluble dimer formed during aggregation.

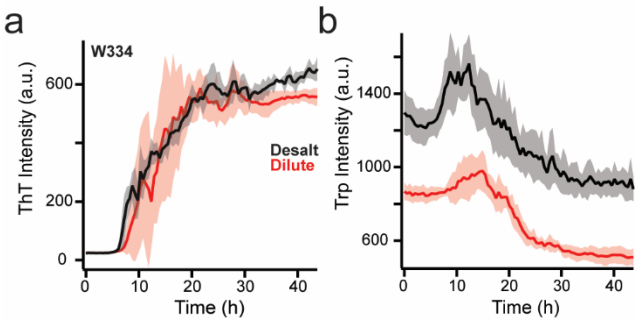

Figure S6. Effect of guanidine on W334 aggregation kinetics. Comparison of W334 TDP-43$_{CTD}$ (a) Trp and (b) ThT kinetics at 10 μM in desalted (black, 0 mM GuHCl) and diluted (red, 170 mM GuHCl) conditions (10 mM NaPi, pH 7.4 at 37 °C, under quiescent conditions). Lines and shading represent the mean and standard deviation ($n \geq 5$).

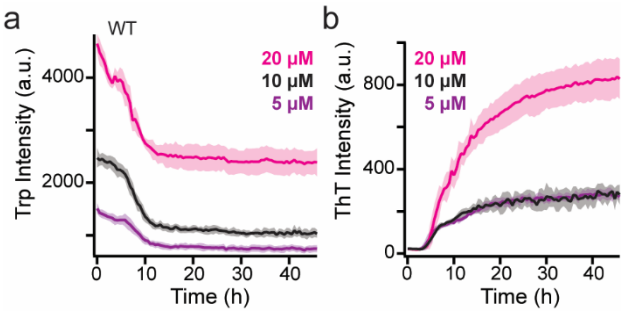

Figure S7. Protein concentration dependence of WT TDP-43$_{CTD}$ aggregation. Comparison of (a) Trp and (b) ThT kinetics at 5, 10, and 20 µM (10 mM NaPi, 180 mM GuHCl, pH 7.4 at 37 °C, under quiescent conditions). Lines and shading represent the mean and standard deviation ($n \geq 5$). Note that the ThT curves for 5 and 10 µM overlay.

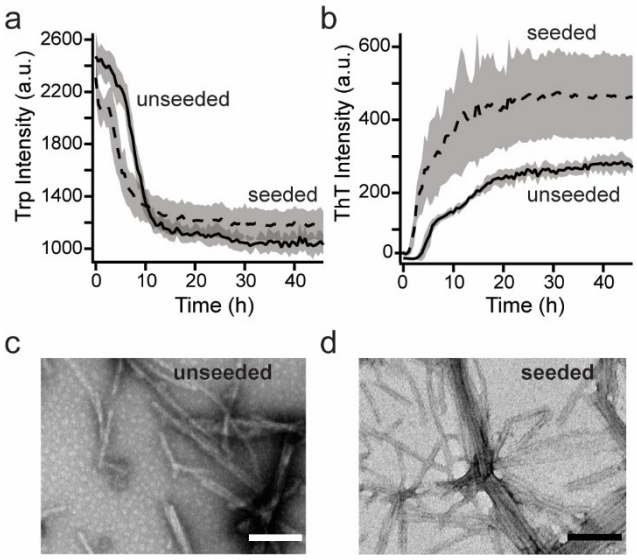

Figure S8. Characterization of seeded WT TDP-43_{CTD} aggregation. Comparison of (a) Trp and (b) ThT aggregation kinetics in the absence (solid) and presence (dashed) of 10% v/v pre-formed WT seeds ([WT] = 10 μM, in 10 mM NaPi, 180 mM GuHCl, pH 7.4 at 37 °C, under quiescent conditions). Lines and shading represent the mean and standard deviation ($n \geq 5$). Resulting TEM from (c) unseeded and (d) seeded reactions. Scale bars are 100 nm.

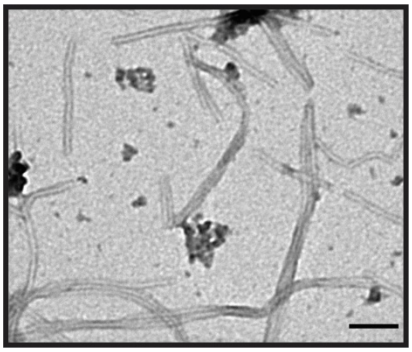

Figure S9. WT TDP-43$_{CTD}$ forms fibrils at intermediate time-point in seeded reaction. TEM taken at intermediate time point (~4.5 h) from seeding of WT TDP-43CTD with 10% v/v preformed WT seeds ([WT] = 10 μM, in 10 mM NaPi, pH 7.4 at 37 °C, under quiescent conditions). Scale bar is 100 nm.

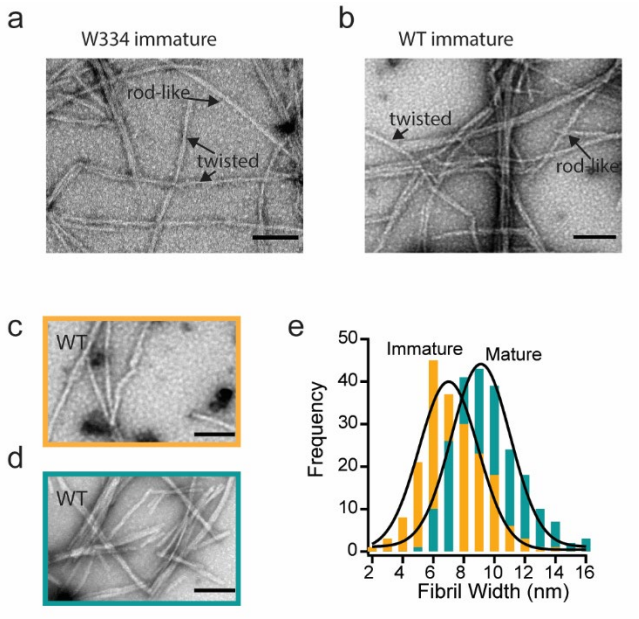

Figure S10. TEM characterization of WT at intermediate time-point. Comparison of TEM images of (a) W334 and (b) WT TDP-43_{CTD} taken after ~1 h of incubation. Rod-like and twisted fibrils are indicated by arrows. TEM images of WT TDP-43_{CTD} at (c) ~1 h of incubation (orange, immature) and after (d) ≥ 24 h of incubation (teal, mature) ([protein] = 10 μM, in 10 mM NaPi, pH 7.4 at 37 °C, under shaking conditions). Scale bars are 100 nm. (e) Histograms of immature (orange) and mature (teal) fibril width. n = 185 measurements for each. Gaussian fits are shown as black lines.