Supporting Information

Delineating the membrane-disrupting and seeding properties of the TDP-43 amyloidogenic core

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Supplementary Methods:

Reagents

Water was distilled and deionized using Milli-Q gradient A101 ultra filtration system. The purified water was used for all fibril incubations in the experiments. Fmoc-protected amino acids and Rink amide AM resin were purchased from Novabiochem and Merck respectively. The DMF was purchased from Sigma-Aldrich, EDT from Alfa Aesar, acetonitrile from C-ECHO, and Thioflavin-T (ThT) from ACROS. All other reagents were purchased from Merck.

Peptide Synthesis

The peptides, D1 (287-322), $TDP_{292-322}$, $TDP_{297-322}$, $TDP_{302-322}$, $TDP_{307-322}$, $TDP_{312-322}$, SC-$TDP_{307-322}$, $TDP_{307-322}$ G^3308P^3, were synthesized on a 0.4 mmol scale with a peptide synthesizer (Rainin instrument, PS3) utilizing 9-Fluorenylmethoxycarbonyl (Fmoc) chemistry. Use of a Rink amide AM resin (Merck) afforded an amidated C-terminus. The peptide $TDP_{312-322}$ was double-coupled and standard Fmoc reaction cycle was used as the rest of the peptides. All peptides were cleaved from resin with the cocktail containing 9.4 ml of TFA, 250 µL pure water, 250 µL EDT (1,2-ethanedithiol), and 100 µL TIS for 70 minutes at room temperature.

Peptide Purification and Identification

The resulting crude peptides were dissolved in 50% acetic acid(v/v), filtered through a 0.22µm Millipore filter and further analyzed and purified by reversed phase HPLC (Waters 2690 separation module with Waters 996 photodiode array detector and the reversed phase C-18 column). The molecular weight of the corresponding peak on HPLC was identified by MALDI mass spectrometry. The resulting pure peptides were lyophilized and stored at −80 °C before use.

Peptide Incubation

Concentrations of the peptide solutions were determined by UV spectrometry. Each peptide concentration was calculated using the following extinction coefficients: $\varepsilon_{256\ nm}$= 585 $M^{-1}cm^{-1}$ for 3 phenylalanine in D1; $\varepsilon_{256\ nm}$= 390 $M^{-1}cm^{-1}$ for 2 phenylalanine in $TDP_{292-322}$, $TDP_{297-322}$, $TDP_{302-322}$, $TDP_{307-322}$, $TDP_{312-322}$, SC-$TDP_{307-322}$, $TDP_{307-322}$ G^3308P^3. 50 µM of all the peptides were incubated in phosphate buffer (70 mM KCl, 20 mM sodium phosphate, pH=7.0) at 37 °C for several days without shaking.

Transmission Electron Microscopy

All the peptides (50 µM) were incubated in phosphate buffer for one week and an 5 μL aliquot of each solution was placed on a glow-charged, 300 mesh, formvar- and carbon-coated copper grid and negative stained with 2% uranylacetate (UA). After drying, all samples were recorded on a JEM-2011 electron microscope (JEOL, Japan) at the Institute of Cellular and Organism Biology in

Academia Sinica.

CD Measurements

Each of the peptides (50 μM) was dissolved in phosphate buffer and incubated at 37 °C. Before recording the spectrum, the solution was mixed by gently tapping the eppendorf tube 5 times. The spectra of the resulting fibril solutions were recorded using a J-815 CD spectrometer (JASCO, Japan) and corrected for the buffer signal over time with a 1 mm quartz cell in a series of incubational time frame. The CD spectra were collected at the bandwidth of 1 nm from 260 to 195 nm with the standard 4 sec response time. The scanning mode was continuous with the scanning speed 100 nm/min. Three scans were averaged for each sample.

Thioflavin T (ThT) Fluorescence Assays

2 mM concentrated dye solution of Thioflavin-T (ThT) was prepared in pH 8.5 buffer (140 mM KCl, 100 mM sodium phosphate) as the stock solution and filtered through a 0.22 μm Millipore filter. The fresh working solution was prepared by diluting the stock solution to 200 μM. A 25 μL aliquot of the fibril solution was mixed with 25 μL of the working solution at room temperature. The fluorescence emission spectrum between 460 and 600 nm was recorded in a 3 mm path-length rectangular fluorescence quartz cuvette on a ISS-PC1 spectrofluorometer (ISS, Champaign, IL, USA) with excitation at 442 nm. The emission intensity was measured at 482 nm, the maximum emission of the fluorescence intensity.

FT-Raman Measurements

Raman spectra were obtained as previously described.[1] FT-Raman spectra of each TDP-43 C-terminal fragment fibril samples were obtained by using a Bruker RFS-100 FT-spectrophotometer (Bruker Optik GmbH, Lubeck, Germany). Sample was placed into the tiny hole of a stainless steel holder for the Raman measurements. A continuous wave Nd-YAG laser (Coherent Lubeck GmbH, Lubeck, Germany) with wavelength 1064 nm, pumped by diode laser, was used as the near infrared Raman excitation source. A He-Ne laser beam was overlaid with the 1064 nm laser beam in order to visualize the Raman sampling spot. The laser light with power of 100 mW was introduced and focused on the sample. The scattered radiation was collected at 180 °C with an ellipsoidal mirror and was filtered, modulated and reflected back into the highly sensitive GaAs detector that was cooled by liquid nitrogen. Raman spectra were produced over the Raman shift 0-3500 cm^{-1}. Typically, 500 interferograms were co-added at 4 cm^{-1} resolution with a sampling period of about 15 min. The spectra in the 1520-1720 cm^{-1} region were subjected to numerical curve fitting (Grams/386; Galactic Ind. Co.). The band shapes were approximated by a Lorentz function. The baseline was approximated by a straight line between two points, 1520 and 1720 cm^{-1}, on the two sides of the band envelope. Each numerical calculation of the Raman intensity ratio was based on the average of triplicate measurements at least. FT-Raman spectra reported in this study are all originals, and have

not been smoothed, normalized, and baseline corrected through data manipulation.

Preparation of Liposomes

Lipids, 1,2-Dimyristoyl-snglycero-3-phosphocholine (DMPC) and 1,2-ditetradecanoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol) (sodium salt) (DMPG) were purchased from Avanti Polar Lipid, Inc. (Alabaster, AL, US), and cholesterol (95%) was obtained from Sigma-Aldrich Company Ltd. (Dorset, UK). These chemicals were used without further purification. DMPG (3.3mg), DMPC (5.0mg), and cholesterol (2.5mg) were dissolved in 1:1 chloroform/methanol. Nitrogen gas was used to remove the solvents from the sample, and the flask containing the lipid film was then stored in a lyophilizer for 2 hrs to remove the solvents more completely. The dried lipid was rehydrated by 1 ml of 50 mM calcein PBS (phosphate buffered saline) titrated with potassium hydroxide to pH 7.0. The solution was sonicated for 1 hr. Freeze-thaw cycles in liquid nitrogen and a 70 ℃ hot plate were processed for 7 times. The suspension was passed tens of times continuously through an Avanti Mini-Extruder with two stacked 100 nm polycarbonate membranes (Avanti Polar Lipid, Inc). Unencapsulated calcein was removed by self-packed size-exclusion column with Sepharose CL-4B gel filtration medium (Sigma).

Calcein Leakage Assays

Each peptide (100 μM) was preincubated for 30 mins first and then added into the liposome solution to a final concentration of 0.7 mM lipid and 50 μM peptide. The liposome and peptide mixtures were incubated at 37 ℃ with 1400 rpm shaking in an Eppendorf Thermomixer® mixer/ incubator. Fluorescence was measured at EX/EM 490/520 nm. The percentage of fluorescence intensity is defined as $(F_P-F_L)/(F_T-F_L)$, where F_P is the fluorescence signal after the peptide is added, F_L is the fluorescence for liposome only, and F_T is the fluorescence signal obtained after 5% of Triton X-100 added.

Seeding experiments

We have incubated different peptides with TDP-43 generated from the eukaryotic cell free system. We chose rabbit reticulocyte lysate as our eukaryotic cell-free system, and sonicated fiber fragments as our seeds toward full-length protein. Fiber solutions of WT-$TDP_{307-322}$ and SC-$TDP_{307-322}$ were sonicated with amplitude 100 and cycle 0.6 to generate short, fragmented fiber fragments. An approximated final concentration ratio of seeds and protein is about 1:1 (2 μM). The solution was maintained at 30 °C with shaking at 1400 rpm. The solution was centrifuged at 13.2 krpm to divide supernatant and pellet after 24 hrs. Using SDS-PAGE and western-blot, the protein amount in supernatant and pellet were quantified. The insolubility ratio was calculated as pellet/(pellet + supernatant). The immunogold electron microscopy (IEM) was done by replacing the second anti-body in western blot with immunogold linked antibody.

Supplementary Figures:

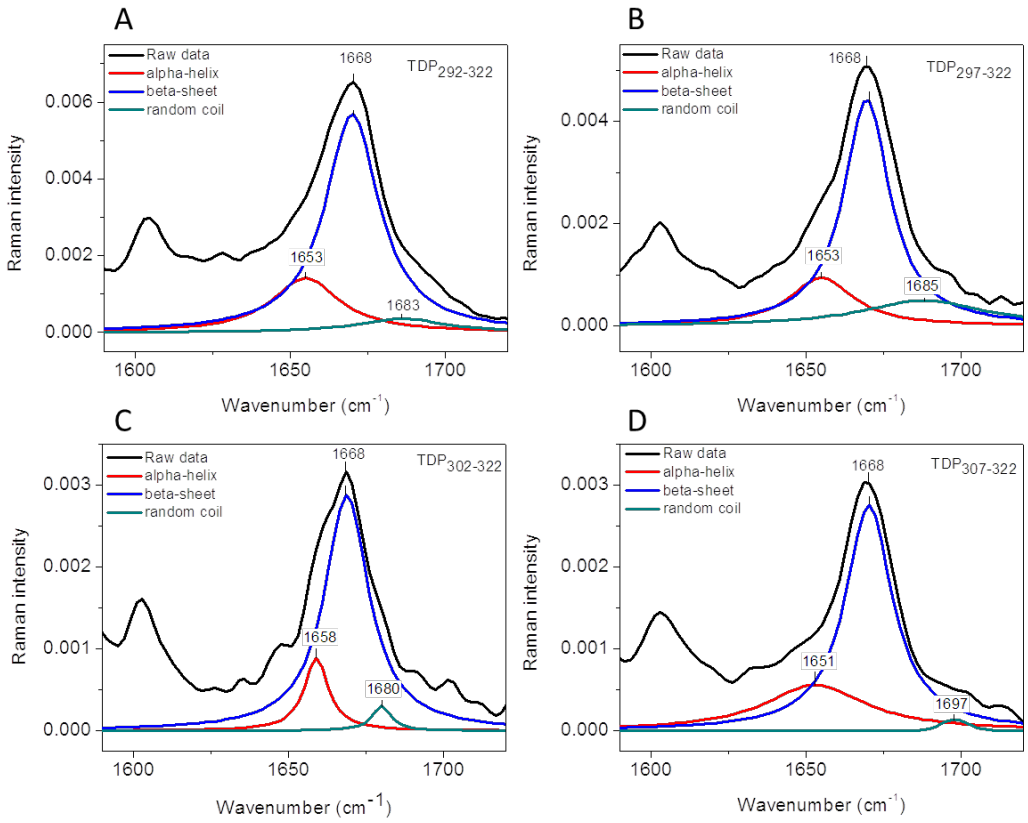

Figure S1. Raman spectra revealed dominance of β-sheet formation of truncated peptides. (A) $TDP_{292\text{-}322}$. (B) $TDP_{297\text{-}322}$. (C) $TDP_{302\text{-}322}$. (D) $TDP_{307\text{-}322}$.

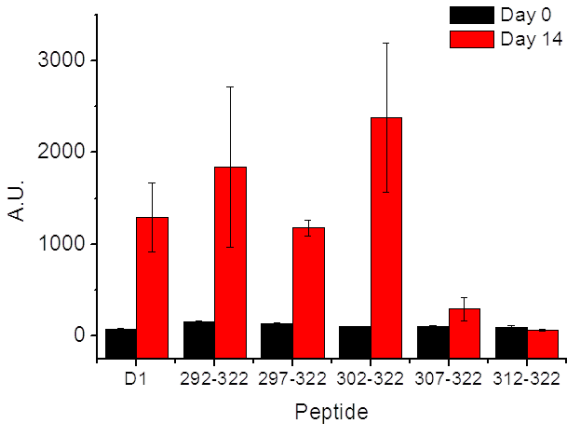

Figure S2. ThT-fluorescence assay compared the emission intensity for each truncated peptides from initial (day 0) to final stage (day 14).

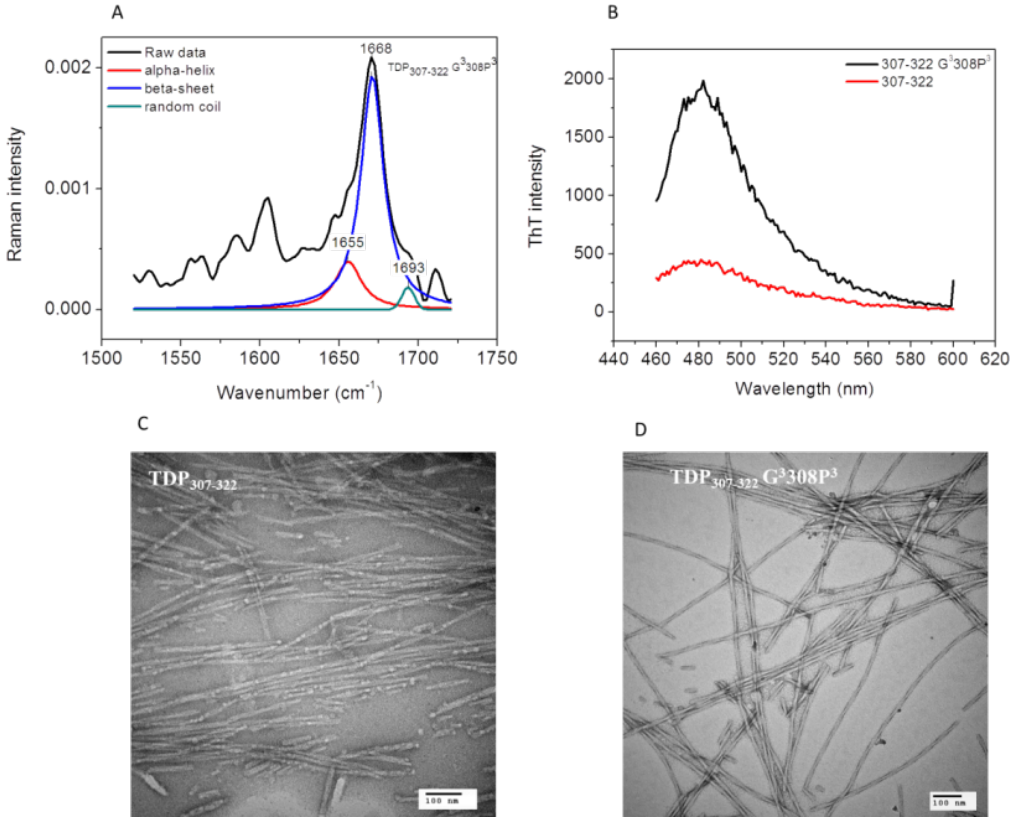

Figure S3. Supporting evidence for the amyloid nature of the core sequence $TDP_{307\text{-}322}$ was uncovered from a study of $TDP_{307\text{-}322}$ G^3308P^3. (A) The Raman spectra revealed that $TDP_{307\text{-}322}$ G^3308P^3 was β-sheet dominant. (B) Comparison of $TDP_{307\text{-}322}$ G^3308P^3 and $TDP_{307\text{-}322}$ showed that low ThT binding ability was an intrinsic property of $TDP_{307\text{-}322}$. However, replacements of the glycines by prolines in $TDP_{307\text{-}322}$ restored the ThT binding ability and changed the morphology of the fiber. (C) TEM of $TDP_{307\text{-}322}$. (D) TEM of $TDP_{307\text{-}322}$ G^3308P^3.

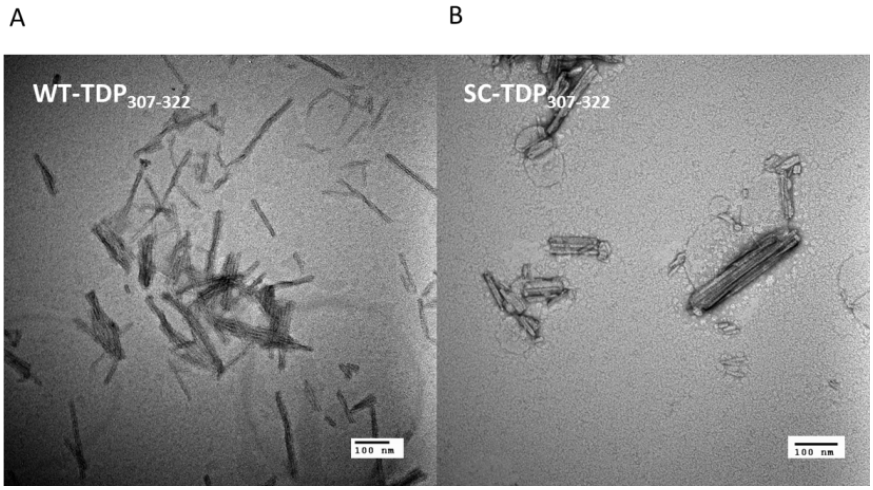

Figure S4. TEM images revealed short fragments of the sonicated fibers of $TDP_{307-322}$ and $SC\text{-}TDP_{307-322}$.

References:

1. A. K. Chen, R. Y. Lin, E. Z. Hsieh, P. H. Tu, R. P. Chen, T. Y. Liao, W. Chen, C. H. Wang and J. J. Huang, *Journal of the American Chemical Society*, 2010, **132**, 1186-1187.