SUPPORTING INFORMATION

Detection of Oligomers and Fibrils of α-Synuclein by AIE-gen with Strong Fluorescence

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Figure S7. Emission intensity of TPE-TPP (red, 15 μM) and ThT (blue, 15 μM) monitoring α–Syn (5 μM) fibrillation, λ_{ex} = 321 nm (for TPE-TPP), 430 nm (for ThT), λ_{em} = 480 nm (for TPE-TPP), 490 nm (for ThT) under (A) constant agitation at 500 rpm in 10 mM citrate buffer pH 6.2 with 0.2% NaN_3 at 37 ^oC,[4] and (B) constant agitation at 600 rpm in 25 mM Na-PO_4, pH 6.2, 0.02% NaN_3 at 37 ^oC.[5] S12

Experimental Section.

Materials and Methods

All the reagents (chemicals, solvents, etc.) used in this study were purchased from Sigma Aldrich unless otherwise specified. Water was purified using a Millipore filtration system. TPE-TPP was prepared according to our published procedures.[1] α-Synuclein (α-Syn) was recombined and purified by sequential centrifugation.

α-Syn powder was dissolved in Tris buffer (50mM Tris, 100mM NaCl) at pH 7.0. The solution was concentrated by centrifugation at 140,000× g for 5min in 10 kDa MWCO centrifugal filter (Amicon Ultra-0.5 mL, Millipore) and regenerated by centrifugation at 90,000× g for 5 min. Its concentration was determined by measuring its absorbance at 274 nm. Oligomeric and fibrillar α-Syn were aged from 200 μM recombinant α-Syn in an orbital thermomixer (Eppendorf) with different incubation conditions, i) constant agitation 1000 rpm in Tris buffer (50 mM Tris, 100 mM NaCl, pH 7.0) at 37 °C, ii) constant agitation 500 rpm in 10 mM citrate buffer pH 6.2 with 0.2% NaN_3 at 37 °C, and iii) constant agitation 600 rpm in 25 mM Na-PO_4, pH 6.2, 0.02% NaN_3 at 37 °C for 22 h and 5 days respectively.[2-5] The stock solutions of TPE-TPP and ThT with a concentration of 1.0 mM were prepared by dissolving an appropriate amount of the luminogen in DMSO and water respectively.

In the study of using TPE-TPP and Thioflavin T (ThT) as an ex situ probe, an aliquot of the α-Syn solution taken out from the incubation mixture at a defined time was diluted with Tris buffer, followed by the addition of the luminogen. The final concentrations of α-Syn and TPE-TPP or ThT were 5 μM and 15 μM respectively. TPE-TPP was used as in situ inhibitor, the luminogens were added to α-Syn solution prior to incubate at 37 °C with constant agitation at 1000 rpm.

Instrumentations

Steady-state fluorescence spectra were recorded on a Perkin-Elmer LS 55 spectrofluorometer with a Xenon discharge lamp excitation. Fluorescence micrographs were taken on an upright fluorescence microscope (Nikon TE2000-U) using a combination of excitation and emission filters for each dye: for TPE-TPP, excitation filter = 330–380 nm, dichroic mirror = 400 nm, and emission filter = 420 nm long pass; for ThT, excitation filter = 416–456 nm, dichroic mirror = 455 nm, and emission filter = 480 nm long pass. Samples were prepared by drop-casting the solutions containing α-Syn fibrils and TPE-TPP or ThT onto the microscope slides covered by microscope glasses. The fluorescence images were captured using a computer-controlled SPOT RT SE 18 Mono charge-coupled device (CCD) camera.

Expression and Purification of the Recombinant α-Syn Protein

Full-length of human α-Syn structural gene was subcloned into the pET11d vector (kindly from Dr Poul Henning Jensen), expressed in BL21 (DE3) pLysS competent cells (Invitrogen). The overnight culture was

induced at an A600 of OD 0.7–0.9 for 12 hours with 1mM isopropyl 1-thio-β-D-galactopyranoside (Astral Scientific). The pellet were then harvested by centrifugation at 7,000 rpm (J2-21, Beckman) for 15 min and rinsed by 1:10 w/v of buffer A solution(50 mM Tris-HCl pH 7.6 / 5 mM EDTA /1 mM DTT) with protease inhibitors. Cells were sonicated at 90W for 6× 10 sec by a microtip sonifier (Branson) and spun at 14, 000 rpm for 10 min. Supernatant were boiled for 10 min and further spun at 18, 000× g for 10 min. Supernatant then were filtered (0.22 μm) and applied to an anion-exchange Mono Q 5/50GL column (GE) on FPLC system (ÄKTAFPLC, GE) at 1 ml / min buffer A and eluted with 0 – 100 % buffer B (50 mM Tris-HCl pH 7.6 / 5 mM EDTA / 1 mM DTT / 1 M NaCl) in 20 ml. Fractions were monitored by absorbance at 280 nm. Collected fractions were analyzed by 12.5% SDS-PAGE and Coomassie Blue staining. Target fractions were concentrated by centrifugation at 3000× g for 15 min in 3 kDa MWCO centrifugal filters (Amicon Ultra-4, Millipore).

Separation of α-Syn Monomers, Oligomers and Fibrils

Fibrils were sedimented at 120,000× g by a Optima TLX Ultracentrifuge (Beckman Instruments) for 60 min. 200 μL supernatant were injected into a Tricorn Superdex 200 10/300 GL column (GE Healthcare) and elute with 50 mM Sodium phosphate, containing 150 mM NaCl (pH 7.0) at 0.8 ml/min. Fractions were collected around the elution volume from 7 to 15 mL. Target fractions were again concentrated by centrifugation at 3000× g for 15 min in 3 kDa MWCO centrifugal filters (Amicon Ultra-0.5 mL, Millipore). Freshly made samples were further analyzed by dot blot immunostaining and Atomic force microscopy (AFM).

α-Syn Binding Affinity Test of ThT and TPE-TPP

An aliquot of the α-Syn solution taken out from the incubation mixture at a defined time was diluted with Tris buffer, followed by the addition of the ThT. The final concentrations of α-Syn and ThT were 5 μM and 15 μM respectively. After measuring the fluorescence intensity of ThT, TPE-TPP (15 μM) was then added to the same solution mixture. The fluorescence intensity of ThT was measured again after 1 min incubation under room temperature.

TPE-TPP and ThT Fluorescence Binding Assay

Assays were performed in Tris buffer using a fixed concentration of α-Syn (5 μM). Reactions were incubated for 1 hr under room temperature before measurement on a Perkin-Elmer LS 55 spectrofluorometer with excitation wavelength 321 nm and 430 nm for TPE-TPP and ThT respectively. All data points were performed at least three times and were analyzed using OriginPro 8 to obtain K_d values using the one site specific binding module.

Electrophoresis, Western and Dot Blotting

Gel electrophoresis was performed under denaturing conditions by using 12.5% SDS-PAGE gels to analyze α-Syn monomers. Samples were mixed with SDS sample buffer and boiled for 5 min prior applying to SDS-PAGE. Coomassie Brilliant Blue G-250 (Bio-Rad) was used for gel staining.

Proteins were then electrophoretically transferred onto PVDF membranes (#162-0264, Bio-Rad) and blocked with 5% skim milk in Tris-buffered saline (TBS) containing 0.05% Tween 20 (TBST) for 1 hr. Membranes were then washed with wash buffer and probed with rabbit anti-pan Syn antibody (1.5 μg/ml) at 4 °C for overnight. After washed with 0.1% skim milk powder in TBST, membranes were further probed with goat anti-rabbit IgG conjugated with horseradish peroxidase (HRP) (1:10,000, A-0545, sigma) for 1 hr at room temperature. Membranes were washed three times with 0.1% skim milk powder in TBST and developed with SuperSignal HRP substrate (Thermo Scientific). Then chemiluminescence was visualized under Cool Fuji Film LAS4000 Imager.

For dot blots, 0.1 μg of monomer and oligomer was spotted onto Hybond-C Extra nitrocellulose membranes (0.45 μm, Amersham). The membranes were blocked with 5% skim milk in TBST for 1 hr at room temperature and probed with primary antibodies, sheep anti-α-Syn antibody (116-133, 0.5 μg/mL, lab made) and Mouse anti-α-Syn oligomer monoclonal antibody (0.7 μg/ml, kindly from Dr. Omar El-Agnaf) respectively for 2 hrs at room temperature. The membranes were washed and further incubated with donkey anti-sheep and goat anti mouse IgG conjugated with HRP (1:10,000, # 713-035-003, Jackson and # 172-1011, Bio-Rad) for 1 hr at room temperature. The blots were developed with Super signal West pico chemiluminescence kit (Thermo Scientific) and visualized under Cool Fuji Film LAS4000 Imager.

Atomic Force Microscope

α-Syn monomers, oligomers and fibrils were diluted to 20 μM and 4 μL of each sample were deposited on freshly cleaved mica (Alfa Aesar). Samples were allowed to sit for 2 min, then gently rinsed with 200 μL of MilliQ water and dried with a gentle stream of nitrogen gas.

AFM height images were acquired using a Multimode AFM with Nanoscope V controller (Digital instruments/Bruker) using tapping mode with scanning probe microscopy system and incorporating custom made ultra-high vacuum STM. The images were obtained in air under ambient conditions with tapping mode set-point between 70 to 90% of the free amplitude. All feedback parameters were optimized to produce the best quality images with scan rates typically between 1 to 2 Hz. The probes used were NSC15 Mikromasch Silicon tapping mode probes. The manufacturer quotes these probes as having nominal spring constant of 40 N/m, resonant frequency of 325 kHz and tip radius equal to 10 nm. The height of protein particles was measured to estimate the size. Multiple areas were observed in order to avoid experimental error.

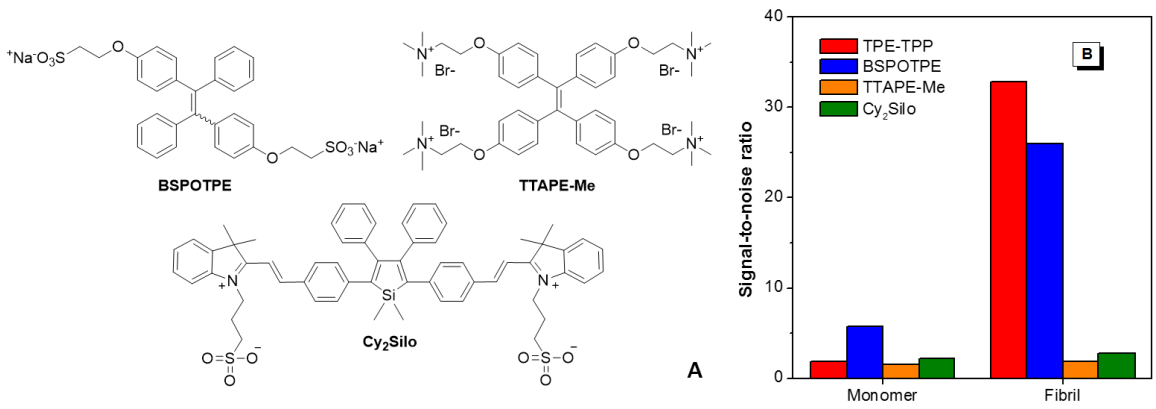


Figure S1. (A) Chemical structures of BSPOTPE, TTAPE-Me and Cy_2Silo; (B) Signal-to-noise ratio of TPE-TPP (red, 15 µM), BSPOTPE (blue, 15 µM), TTAPE-Me (orange, 15 µM) and Cy_2Silo (green, 15 µM) dyed α–Syn (5 µM) species, monomer (t= 0 hr) and fibril (t= 97 hr), λ_{ex} = 321 nm (for TPE- TPP), 350 nm (for BSPOTPE & TTPAE-Me), 480 nm (for Cy_2Silo), λ_{em} = 480 nm (for TPE-TPP, BSPOTPE & TTPAE-Me), 690 nm (for Cy_2Silo).

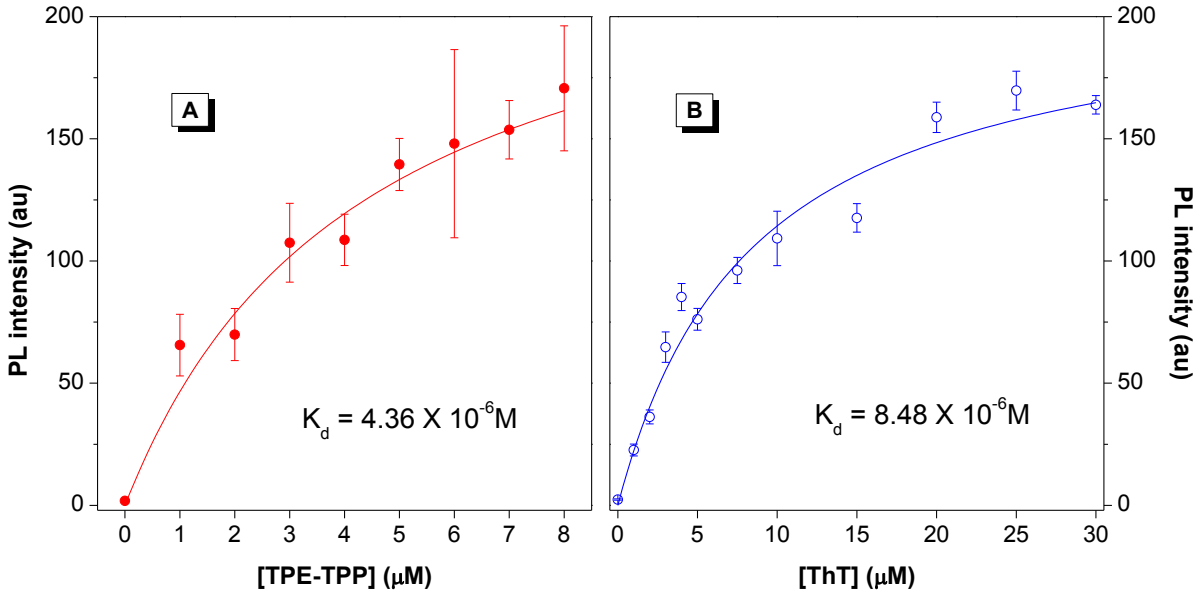


Figure S2. Saturation binding of (A) TPE-TPP and (B) ThT to fibrillar form of α–Syn (5 μM).

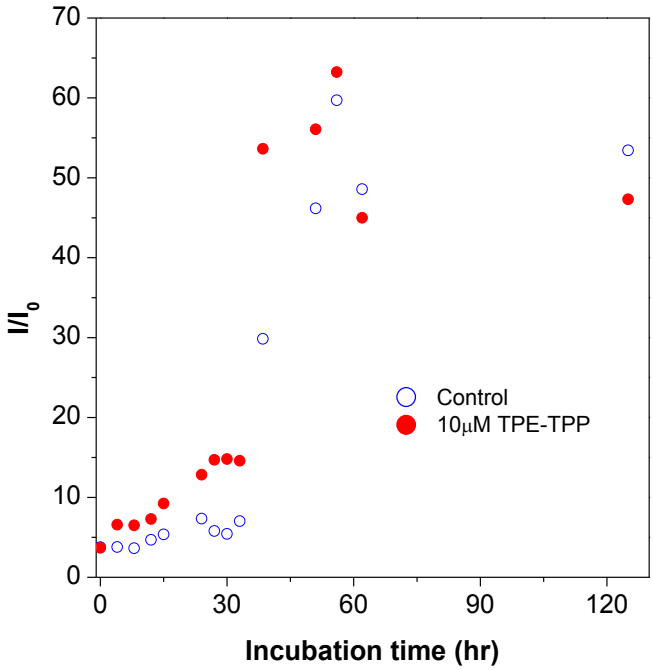


Figure S3. Plot of I/I_0 of TPE-TPP (15 μM) monitoring α–Syn (5 μM) fibrillation without (control) and with the addition of TPE-TPP (10 μM) at the start of the fibrillation process, λ_{ex} = 321 nm, λ_{em} = 480 nm.

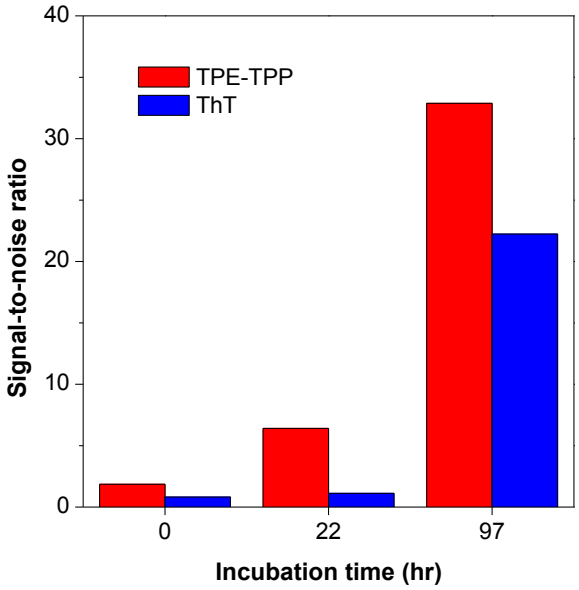


Figure S4. Signal-to-noise ratio of TPE-TPP (red, 15 µM) and ThT (blue, 15 µM) dyed α–Syn (5 µM) fibrillation at different incubation time of t= 0, 22 & 97 hr, λ_{ex} = 321 nm (for TPE- TPP), 430 nm (for ThT), λ_{em} = 480 nm (for TPE-TPP), 490 nm (for ThT).

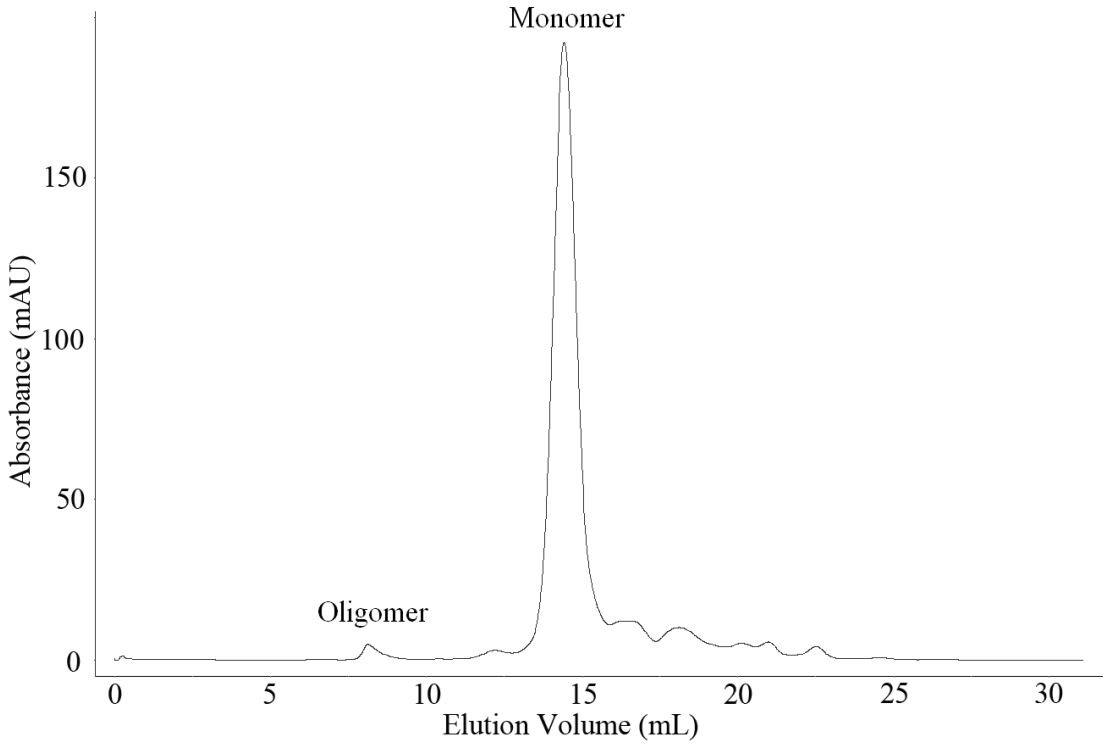


Figure S5. Size-exclusion chromatography of α-Syn oligomers and monomers collected at t = 22 hr.

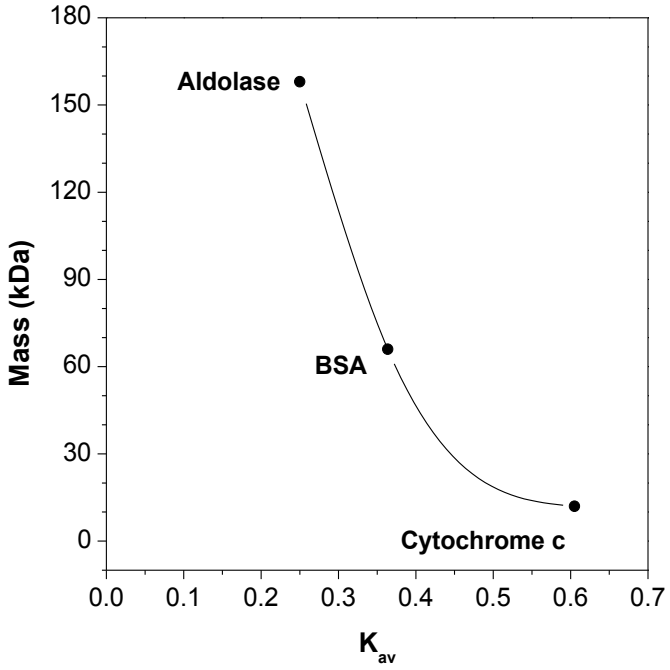
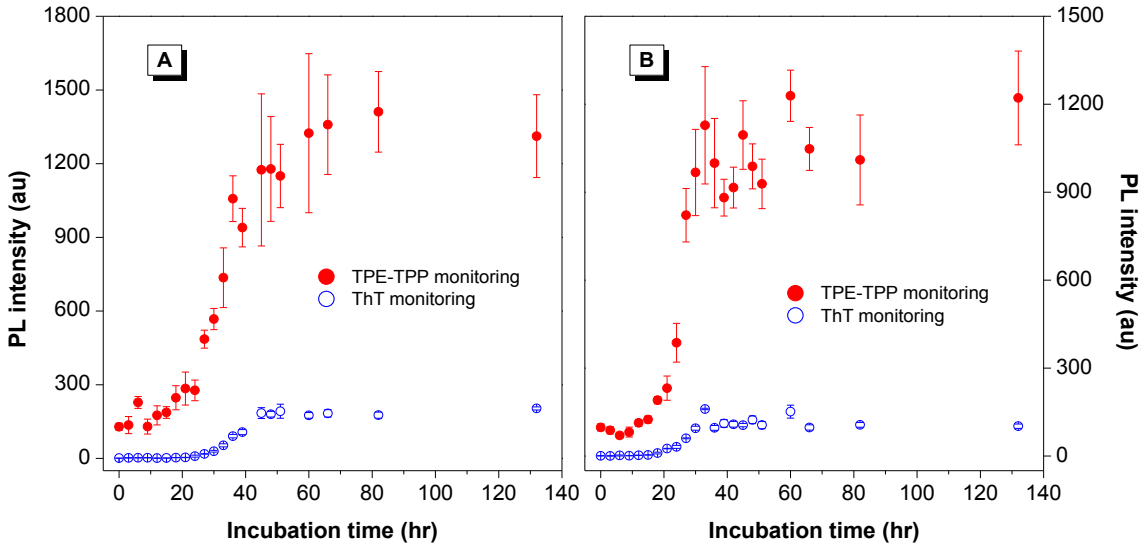


Figure S6. Calibration curve for the standard proteins and size of α-Syn monomer on Tricorn™ Superdex 200 10/300 GL column. (Molecular weight of Aldolase, BSA and cytochrome c are 158, 66 and 12 kDa respectively.)



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