Supporting Information for:

A Fluorescent Molecular Imaging Probe with Selectivity for Soluble Tau Aggregated Protein

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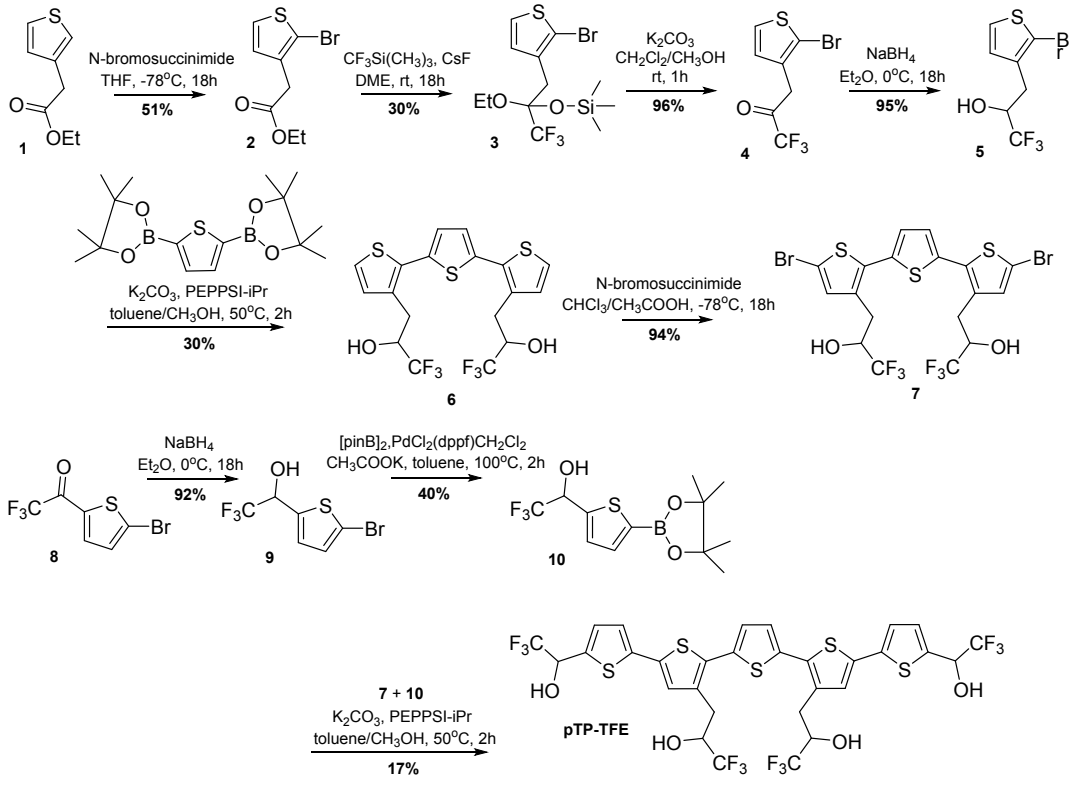
Experimental Procedures

S1. Experimental materials and instruments

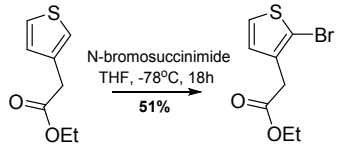
All reagents and solvents were obtained from Sigma-Aldrich and Alfa Aesar, unless otherwise stated and used without further purification. Preparative chromatographic separations were performed on Aldrich Science silica gel 60 (35-75 μm) and reactions followed by TLC analysis using Sigma-Aldrich silica gel 60 plates (2-25 μm) with fluorescent indicator (254 nm) and visualized with UV. 1H, 13C and 19F Nuclear magnetic resonance spectra were recorded in Fourier transform mode at the field strength specified on a Bruker Avance 300, 400 or 500 MHz spectrometer. Spectra were obtained from the specified deuterated solvents in 5 mm diameter tubes. Chemical shift in ppm is quoted relative to residual solvent signals calibrated as follows: **$CDCl_3$** δ_H ($CH Cl_3$) = 7.26 ppm, δ_C = 77.2 ppm; **$(CD_3)_2SO$** δ_H (CD_3SOCHD_2) = 2.50 ppm, δ_C = 39.5 ppm; **MeOD-d_4** δ_H (CD_2HOD) = 3.31 ppm, δ_C = 49.0 ppm; **$(CD_3)_2CO$** δ_H (CD_2COCHD_2) = 2.09 ppm, δ_C (($CD_3)_2CO$) = 30.6 ppm. 19F NMR chemical shifts are recorded in ppm relative to trichlorofluoromethane. Multiplicities in the ^{1}H NMR spectra are described as: s = singlet, d = doublet, t = triplet, q = quartet, quint. = quintet, m = multiplet, b = broad; coupling constants are reported in Hz. Mass spectra were recorded on a Waters Micromass LCT instrument, or from the EPSRC Mass Spectrometry Service at the University of Swansea.

S2. Synthesis and characterisation of pTP-TFE

The synthesis of *penta*thiophene-trifluoroethanol (**pTP-TFE**) is summarized in the scheme below:



S2.1. Compound 2

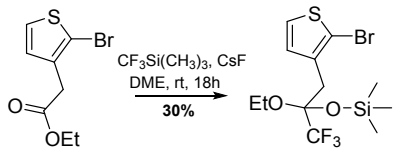


1
$C_8H_{10}O_2S$
(170.04)

2
$C_8H_9BrO_2S$
(247.95)

Ethyl 2-(2-bromothiophen-3-yl)acetate (2).
Ethyl 3-thiopheneacetate (**1**) (18.7 g, 110 mmol) was dissolved in THF (50 mL) and cooled to -78°C. N-bromosuccinimide (18.9 g, 106 mmol) was added portion wise over one hour. The solution was allowed to warm up to room temperature and left to stir overnight. Solvent was removed under vacuum and the reaction mixture dissolved in 50 ml ethyl acetate. The organic layer was washed with brine (3 x 50 mL) and dried over anhydrous Na_2SO_4. Solvent was removed in vacuo and the product purified by column chromatography using a gradient of EtOAc/hexane running from 1 % EtOAc to 5 %. The product was obtained as a colourless oil (13.5 g, 54.2 mmol, 51 % yield).
^{1}H-NMR (300 MHz, $CDCl_3$): 1.29 (t, J=7.1Hz, 3H, OCH_2CH_3); 3.64 (s, 2H, CH_2); 4.20 (q, J=7.1Hz, 2H, OCH_2CH_3); 6.96 (d, J=5.6Hz, 1H, Ar-*H*); 7.26 (d, J=5.6Hz, 1H, Ar-*H*); ^{13}C-NMR (75 MHz, d_6-DMSO)): 14.2, 35.1, 61.1, 111.6, 125.7, 128.7, 133.6, 170.2; LCMS: 249.2 [ES+].

S2.2. Compound 3

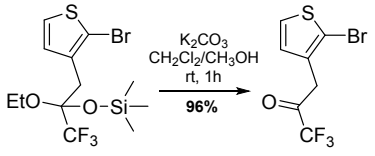


2
$C_8H_9BrO_2S$
(247.95)

3
$C_{12}H_{18}BrF_3O_2SSi$
(389.99)

((3-(2-bromothiophen-3-yl)-2-ethoxy-1,1,1-trifluoropropan-2-yl)oxy)trimethylsilane (3).
Ethyl 2-(2-bromothiophen-3-yl)acetate (**2**) (13.5 g, 54.2 mmol) was dissolved in 1,2-dimethoxyethane (40 ml). CsF was dried under high vacuum at 120°C and a catalytic amount added to the reaction mixture. The reaction was stirred at room temperature overnight. Upon completion, solvent was reduced under vacuum and the reaction mixture dissolved in Et_2O (200 ml). The organic layer was washed with H_2O (3 x 200 mL), brine (200 mL) and dried over anhydrous Na_2SO_4. Solvent was removed in vacuo and the product purified by column chromatography using 1 % EtOAc/hexane. The product was obtained as a colourless oil (6.39 g, 16.4 mmol, 30% yield).
^{1}H-NMR (300 MHz, $CDCl_3$): 0.00 (s, 9H, $Si(CH_3)_3$); 1.12 (t, J=7.0Hz, 3H, OCH_2CH_3); 3.00 (m, diastereotopic, 2H, CH_2); 3.64 (m, 2H, OCH_2CH_3); 6.87 (d, J=5.8Hz, 1H, Ar-*H*); 7.09 (d, J=5.8Hz, 1H, Ar-*H*); ^{13}C-NMR (75 MHz, $CDCl_3$): 0.0, 13.9, 32.4, 58.3, 97.7 (q, ^{2}J = 30.1 Hz), 112.3 (q, ^{1}J = 282.4 Hz), 123.1, 123.3, 128.6, 132.6; ^{19}F-NMR (280 MHz, $CDCl_3$): -79.4 (s, 3F, CF_3).

S2.3. Compound 4

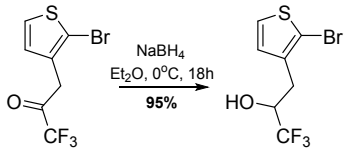


3
$C_{12}H_{18}BrF_3O_2SSi$
(389.99)

4
$C_7H_4BrF_3OS$
(271.91)

3-(2-bromothiophen-3-yl)-1,1,1-trifluoropropan-2-one (4)
3-(2-bromothiophen-3-yl)-2-ethoxy-1,1,1-trifluoropropan-2-yl)oxy)trimethylsilane (**3**) (6.39 g, 16.3 mmol) was dissolved in 1:1 dichloromethane / methanol (100 mL) and 9.1 g of K_2CO_3 added (65.4 mmol, 4eq). The reaction was stirred at room temperature for one hour, diluted in CH_2Cl_2 and washed with 1N HCl (2 x 200 mL) and brine (200 mL). The organic phase was dried over anhydrous Na_2SO_4 and solvent removed in vacuo. The product was used without further purification and obtained as a light brown oil (4.27 g, 15.7 mmol, 96 % yield).
^{1}H-NMR (300 MHz, $CDCl_3$): 4.06 (s, 2H, CH_2); 6.89 (d, J=5.6Hz, 1H, Ar-*H*); 7.33 (d, J=5.6Hz, 1H, Ar-*H*); ^{13}C-NMR (75 MHz, $CDCl_3$): 36.9, 115.6 (q, ^{1}J = 292.3 Hz); 126.0, 126.4, 128.4, 130.1, 187.2 (q, ^{2}J = 35.7 Hz); ^{19}F-NMR (280 MHz, $CDCl_3$): -78.5 (s, 3F, CF_3); LCMS: 271.1 [ES-].

S2.4. Compound 5

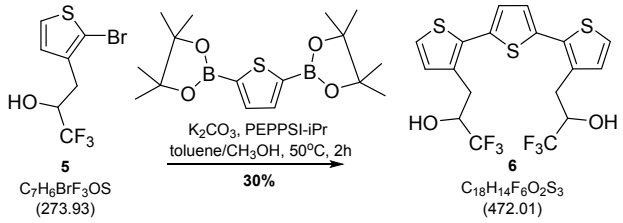


3-(2-bromothiophen-3-yl)-1,1,1-trifluoropropan-2-ol (5)

3-(2-bromothiophen-3-yl)-1,1,1-trifluoropropan-2-one (**4**) (3.0 g, 11.0 mmol) was dissolved in 75 mL of dry Et_2O and cooled to 0°C. $NaBH_4$ (460 mg, 12.1 mmol, 1.1 eq) was added portion wise and the mixture stirred at room temperature overnight. 2M HCl (100 mL) was added and the mixture extracted with Et_2O (100 mL) the organic phase was washed with 2M HCl (2 x 100 mL), brine (100 mL) and dried over anhydrous Na_2SO_4. Solvent was reduced in vacuo and the product purified on a Gilson prep module, eluting on a gradient from 5 % EtOAc / Hexanes to 20 % EtOAc / Hexanes over 20 min. The product was obtained as a colourless oil (1.1 g, 4.0 mmol, 36 % yield).

^{1}H-NMR (300 MHz, $CDCl_3$): 2.23 (d, J=5.6Hz, 1H, O*H*); 2.90 (m, doublet of diastereotopic protons, 2H, CH2); 4.11 (m, 1H, C*H*); 6.84 (d, J=5.6Hz, 1H, Ar-*H*); 7.19 (d, J=5.6Hz, 1H, Ar-*H*); ^{13}C-NMR (75 MHz, $CDCl_3$): 29.9, 70.2 (q, ^{2}J = 30.8 Hz), 111.5, 124.9 (q, ^{1}J = 292.3 Hz), 126.0, 128.9, 135.2; ^{19}F-NMR (280 MHz, $CDCl_3$): -79.9 (d, J=6.4Hz, 3F, CF_3); HRMS (ASAP+) *m/z* 274.9347 (calcd. 247.9353 for $C_7H_7OF_3SBr$).

S2.5. Compound 6

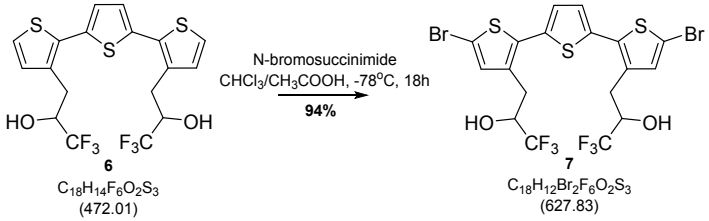


3,3'-([2,2':5',2''-terthiophene]-3,3''-diyl)bis(1,1,1-trifluoropropan-2-ol) (6)

K_2CO_3 (1.3 g, 9.4 mmol, 5eq) and 2,5-Bis-thiopheneboronic acid pinacol ester (616 mg, 1.84 mmol, 1eq) were dried under high vacuum for 5 min. 3-(2-bromothiophen-3-yl)-1,1,1-trifluoropropan-2-ol (**5**) (1.06 g, 3.85 mmol, 2eq) was added to the reaction vessel and all three reagents dissolved in a 1:1 mixture of toluene and methanol (60 mL). The mixture was degassed by bubbling nitrogen through the solution for 10 min. PEPPSI-iPr (124 mg, 0.2 mmol, 0.1eq) was added and the mixture heated at 50°C for one hour, followed by another hour at 70°C. Upon completion, the reaction mixture was diluted in $CHCl_3$ (150 mL) and treated with 2M HCl (150mL). The organic phase was washed with 2M HCl (2 x 150 mL), brine (150 mL) and dried over anhydrous Na_2SO_4. Solvent was reduced in vacuo and the product purified on a silica column, eluting at 20 % Et_2O / Hexanes and rising to 20 % Et_2O / Hexanes. The product was obtained as a yellow oil (544 mg, 1.15 mmol, 62 % yield).

^{1}H-NMR (300 MHz, $CDCl_3$): 2.30 (d, J=4.6Hz, 2H, O*H*); 3.19 (m, doublet of diastereotopic protons, 4H, CH_2); 4.24 (m, 2H, C*H*); 7.07 (d, J=5.3Hz, 2H, Ar-*H*); 7.16 (s, 2H, Ar-*H*); 7.30 (d, J=5.3Hz, 2H, Ar-*H*); ^{13}C-NMR (75 MHz, $CDCl_3$): 29.4, 70.7 (q, ^{2}J = 30.8 Hz), 124.8 (q, ^{1}J = 292.3 Hz), 124.9, 127.5, 130.4, 132.6, 132.9, 135.6; ^{19}F-NMR (280 MHz, $CDCl_3$): -79.7 (d, J=6.4Hz, 3F, CF_3); LCMS: 472.0 [ES-]; HRMS (ASAP+) *m/z* 473.0136 (calcd. 473.0138 for $C_{18}H_{14}F_6O_2S_3$).

S2.6. Compound 7

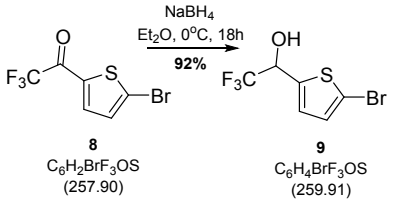


3,3'-(5,5''-dibromo-[2,2':5',2''-terthiophene]-3,3''-diyl)bis(1,1,1-trifluoropropan-2-ol) (7).

3,3'-([2,2':5',2''-terthiophene]-3,3''-diyl)bis(1,1,1-trifluoropropan-2-ol) (**6**) (200 mg, 0.42 mmol) was dissolved in 1:1 $CHCl_3$ / CH_3COOH (40 mL) and N-bromosuccinimide (150 mg, 0.84 mmol, 2eq) was added slowly. The solution was left to stir at room temperature overnight. The reaction mixture was diluted in H_2O (100 mL) and extracted with $CHCl_3$ (100 mL). The organic layer was treated with 10% KOH (2 x 100 mL), H_2O (2 x 100 mL) and brine (100 mL). The organic phase was dried over Na_2SO_4 and solvent was removed

under vacuum. The product was purified by filtration through a silica pad and obtained as a yellow solid (250 mg, 0.40 mmol, 94 % yield).
^{1}H-NMR (300 MHz, $CDCl_3$): 2.24 (d, J=5.7Hz, 2H, O*H*); 3.00 (m, doublet of diastereotopic protons, 4H, CH_2); 4.10 (m, 2H, C*H*); 6.97 (s, 2H, Ar-*H*); 7.01 (s, 2H, Ar-*H*); ^{13}C-NMR (75 MHz, $CDCl_3$): 29.1, 70.7 (q, ^{2}J = 31.4 Hz), 111.9, 124.8 (q, ^{1}J = 284.5 Hz), 127.7, 133.0, 133.5, 134.0, 134.8; ^{19}F-NMR (280 MHz, $CDCl_3$): -79.7 (d, J=6.4Hz, 3F, CF_3); LCMS: 630.2 [ES-]; HRMS (ASAP+) *m/z* 630.8336 (calcd. 630.8328 for $C_{18}H_{12}F_6O_2S_3Br_2$).

S2.7. Compound 9

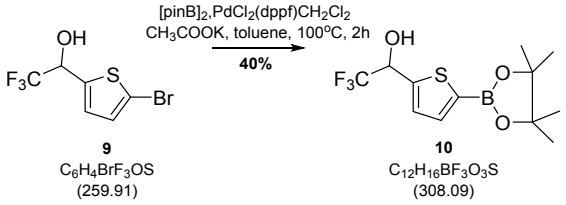


8
$C_6H_2BrF_3OS$
(257.90)

9
$C_6H_4BrF_3OS$
(259.91)

1-(5-bromothiophen-2-yl)-2,2,2-trifluoroethan-1-ol (9).
2-bromo-5-trifluoroacetylthiophene (obtained from Fluorochem Ltd., Hadfield, UK) (**7**) (260 mg, 1.0 mmol) was dissolved in dry Et_2O (2.5 mL) and cooled to 0°C. $NaBH_4$ (40 mg, 1.1 mmol, 1.1 eq) was added portion wise and the mixture stirred at room temperature overnight. 2M HCl (10 mL) was added and the mixture extracted with Et_2O (10 mL) the organic phase was washed with 2M HCl (2 x 10 mL), brine (10 mL) and dried over anhydrous Na_2SO_4. Solvent was reduced in vacuo and the product used without further purification. The product was obtained as a colourless oil (240 mg, 0.9 mmol, 92 % yield).
^{1}H-NMR (300 MHz, $CDCl_3$): 2.80 (s (broad), 1H, OH); 5.12 (q, J=6.2Hz, 1H, C*H*); 6.87 (d, J=3.8Hz, 1H, Ar-*H*); 6.92 (d, J=3.8Hz, 1H, Ar-*H*); ^{13}C-NMR (75 MHz, $CDCl_3$): 69.4 (q, ^{2}J = 34.3 Hz), 114.3, 123.5 (q, ^{1}J = 284.5 Hz), 127.8, 129.8, 137.6; ^{19}F-NMR (280 MHz, $CDCl_3$): -78.9 (d, J=6.2Hz, 3F, CF_3); HRMS (ASAP+) *m/z* 242.9089 (calcd. 242.9091 for $C_6H_4BrF_3OS$).

S2.8. Compound 10

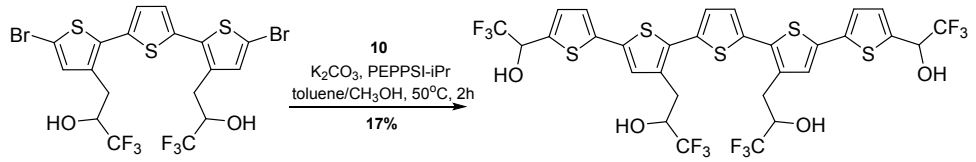


9
$C_6H_4BrF_3OS$
(259.91)

10
$C_{12}H_{16}BF_3O_3S$
(308.09)

2,2,2-trifluoro-1-(5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiophen-2-yl)ethan-1-ol (10)
Potassium acetate (150 mg, 1.54 mmol, 4eq) and bis(pinacol)diborane (194 mg, 0.77 mmol, 2eq) were dried under high vacuum for 5 min. 1-(5-bromothiophen-2-yl)-2,2,2-trifluoroethan-1-ol (**9**) (100 mg, 0.38 mmol, 1eq) was added and the three reagents dissolved in 4 mL 1,4-dioxane. The mixture was degassed by bubbling nitrogen through the solution for 10 min. $PdCl_2(dppf)Ch_2Cl_2$ (31 mg, 0.04 mmol, 0.1eq) was added and the mixture heated at 100°C for 2 hours. Upon completion, the reaction mixture was diluted in $CHCl_3$ (15 mL) and treated with 2M HCl (15 mL). The organic phase was washed with 2M HCl (2 x 15 mL), brine (15 mL) and dried over anhydrous Na_2SO_4. Solvent was reduced in vacuo and the product purified on a silica column, eluting at 20 % EtOAc / Hexanes. The product was obtained as a colourless oil (47 mg, 0.15 mmol, 40 % yield).
^{1}H-NMR (300 MHz, $CDCl_3$): 1.27 (s, 12H, $(CH_3)_2$); 2.95 (s (broad), 1H, OH); 5.22 (q, J=6.2Hz, 1H, C*H*); 7.18 (d, J=3.8Hz, 1H, Ar-*H*); 7.48 (d, J=3.8Hz, 1H, Ar-*H*); ^{13}C-NMR (75 MHz, $CDCl_3$): 24.7, 29.7, 69.4 (q, ^{2}J = 34.3 Hz), 119.4, 122.5 (q, ^{1}J = 292.1 Hz), 128.5, 137.0, 143.1; ^{19}F-NMR (280 MHz, $CDCl_3$): -78.6 (d, J=6.2Hz, 3F, CF_3); LCMS: 309.4 [ES+]; HRMS (ASAP+) *m/z* 306.0815 (calcd. 306.0823 for $C_{12}H_{15}BO_3F_3S$).

S2.9. Compound pTP-TFE



7
$C_{18}H_{12}Br_2F_6O_2S_3$
(627.83)

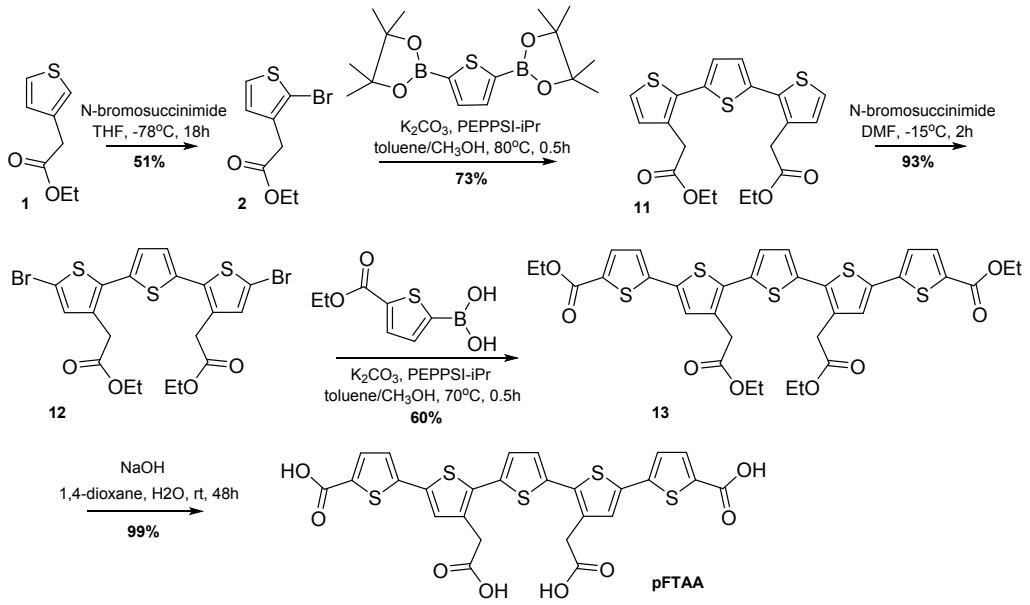
pTp-TFE
$C_{30}H_{20}F_{12}O_4S_5$
(831.98)

3,3'-(5,5''''-bis(2,2,2-trifluoro-1-hydroxyethyl)-[2,2':5',2'':5'',2''':5''',2''''-quinquethiophene]-3''',4'-diyl)bis(1,1,1-trifluoropropan-2-ol) (pTP-TFE).

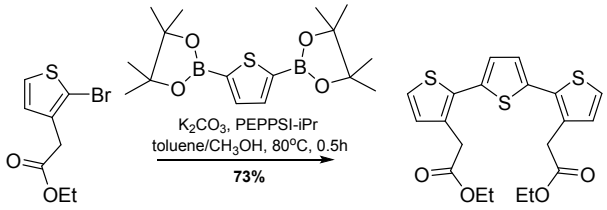
K_2CO_3 (32 mg, 0.24 mmol, 5eq) was dried under high vacuum for 5 min. 3,3'-(5,5''-dibromo-[2,2':5',2''-terthiophene]-3,3''-diyl)bis(1,1,1-trifluoropropan-2-ol) (**7**)
(30 mg, 0.047 mmol, 1eq) and 2,2,2-trifluoro-1-(5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiophen-2-yl)ethan-1-ol (**10**) (29 mg, 0.095 mmol, 2eq) were added to the reaction vessel and all three reagents dissolved in a 1:1 mixture of toluene and methanol (4 mL). The mixture was degassed by bubbling nitrogen through the solution for 10 min. PEPPSI-iPr (4 mg, 0.005 mmol, 0.1eq) was added and the mixture heated at 50°C for one hour, followed by another hour at 70°C. Upon completion, the reaction mixture was diluted in CH_2Cl_2 (10 mL) and treated with 2M HCl (10 mL). The organic phase was washed with 2M HCl (2 x 10 mL), brine (10 mL) and dried over anhydrous Na_2SO_4. Solvent was reduced in vacuo and the product purified on a silica column, eluting at 5 % CH_3OH / CH_2Cl_2. The product was obtained as an orange solid (6.7 mg, 0.008 mmol, 17 % yield).
^{1}H-NMR (300 MHz, $(CD_3)_2CO$): 3.14 (m, doublet of diastereotopic protons, 4H, C*H*$_2$); 4.51 (m, 2H, C*H*); 5.58 (q, J=6.8, 2H, CH); 5.64 (d, J=6.4Hz, 2H, OH); 6.36 (d, J=5.8Hz, 2H, OH); 7.23 (d, J=3.8Hz, 2H, Ar-*H*); 7.27 (d, J=3.8Hz), 2H, Ar-*H*); 7.41 (s, 2H, Ar-*H*); 7.44 (s, 2H, Ar-*H*); ^{13}C-NMR (125 MHz, $(CD_3)_2CO$): 29.1, 68.3 (q, ^{2}J = 32.8 Hz), 69.5 (q, ^{2}J = 30.0 Hz), 124.4 (q, ^{1}J = 279.8 Hz), 125.5 (q, ^{1}J = 283.2 Hz), 123.6, 127.3, 127.7, 128.1, 131.2, 135.0, 135.3, 136.9, 137.4, 137.9; ^{19}F-NMR (280 MHz, $CDCl_3$): -79.2 (d, J=6.7Hz, 3F, CF_3); -80.0 (d, J=6.7Hz, 3F, CF_3); HRMS (ASAP+) *m/z* 832.9852 (calcd. 832.9852 for $C_{30}H_{20}F_{12}O_4S_5$).

S3. Synthesis and characterisation of pFTAA.

The synthesis of pentamer formyl thiophene acetic acid (**pFTAA**) followed the synthetic strategy described by Aslund and co-workers [1] with slight modifications; the method here described uses ethyl-esters as building blocks instead of methyl-esters. The synthesis of **pFTAA** is summarized in the scheme below:

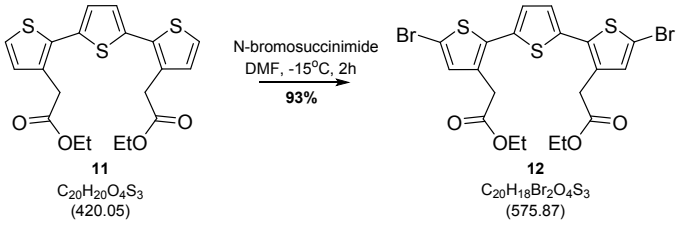


S3.1. Compound 11



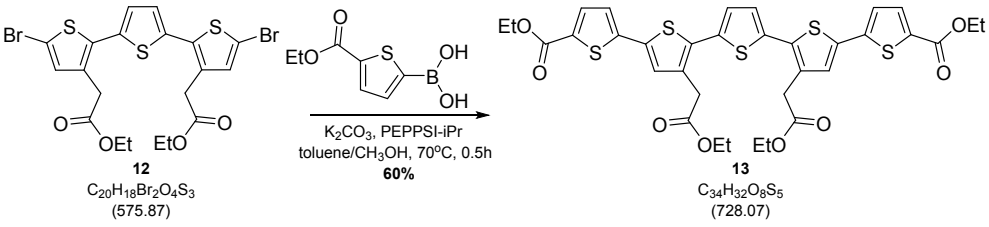
Diethyl 2,2'-([2,2':5',2''-terthiophene]-3,3''-diyl)diacetate (11).
K_2CO_3 (1.38 g, 10 mmol) was briefly dried under high vacuum and ethyl 2-(2-bromothiophen-3-yl)acetate (**2**) (2.0 g, 8 mmol), as well as 2,5-Bis-thiopheneboronic acid pinacol ester (1.34 g, 4 mmol) added to the reaction vessel. The reagents were dissolved in 4 mL toluene and 6 mL methanol and the mixture degassed for 10 min using nitrogen. PEPPSI-iPr (67.9 mg, 0.1 mmol) was added and the mixture heated at 80°C for 30 min. Upon cooling, the reaction mixture was dissolved in ethyl acetate (20 mL) and washed with 2N HCl (2 x 20 mL) and brine (20 mL). The organic layer was dried over anhydrous Na_2SO_4, solvent removed in vacuo and the product purified by column chromatography using 33 % Et_2O/Petroleum ether. The product was obtained as a pale-yellow oil (1.09 g, 73 % yield).
^{1}H-NMR (300 MHz, $CDCl_3$): 1.29 (t, J=7.1Hz, 6H, OCH_2CH_3); 3.80 (s, 4H, CH_2); 4.21 (q, J=7.1Hz, 4H, OCH_2CH_3); 7.08 (d, J=5.4Hz, 2H, Ar-*H*); 7.18 (s, 2H, Ar-*H*); 7.28 (d, J=5.4Hz, 2H, Ar-*H*); ^{13}C-NMR (75 MHz, d_6-DMSO)): 14.2, 34.9, 61.1, 124.7, 127.3, 130.3, 130.6, 132.8, 135.8, 170.9.

S3.2. Compound 12



Diethyl 2,2'-(5,5''-dibromo-[2,2':5',2''-terthiophene]-3,3''-diyl)diacetate (12).
Diethyl 2,2'-([2,2':5',2''-terthiophene]-3,3''-diyl)diacetate (**11**) (800 mg, 1.9 mmol) was dissolved in DMF (8 mL) and cooled to -15°C. N-bromosuccinimide (354 mg, 2.0 mmol) was added slowly. The solution was allowed to warm up to room temperature and left to stir for 2 hours. Solvent was removed under vacuum and the reaction mixture dissolve in 20 mL ethyl acetate. The organic layer was washed with 1N HCl (2 x 20 mL), brine (20 mL) and dried over anhydrous Na_2SO_4. Solvent was removed in vacuo and the product purified by column chromatography using 33 % Et_2O/petroleum ether. The product was obtained as a yellow solid (1.07 g, 93 % yield).
^{1}H-NMR (300 MHz, $CDCl_3$): 1.30 (t, J=7.1Hz, 6H, OCH_2CH_3); 3.71 (s, 4H, CH_2); 4.21 (q, J=7.1Hz, 4H, OCH_2CH_3); 7.06 (s, 2H, Ar-*H*); 7.12 (s, 2H, Ar-*H*); ^{13}C-NMR (75 MHz, d_6-DMSO)): 14.2, 34.7, 61.3, 111.7, 127.8, 131.3, 133.0, 134.0, 134.9, 170.4.

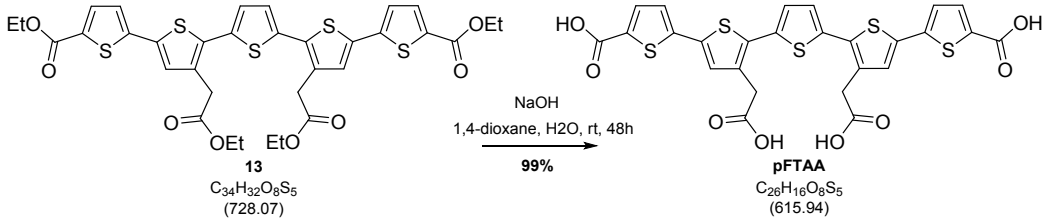
S3.3. Compound 13



Diethyl 3''',4'-bis(2-ethoxy-2-oxoethyl)-[2,2':5',2'':5'',2''':5''',2''''-quinquethiophene]-5,5''''-dicarboxylate (13).
K_2CO_3 (765 mg, 5.55 mmol) was briefly dried under high vacuum and diethyl 2,2'-(5,5''-dibromo-[2,2':5',2''-terthiophene]-3,3''-diyl)diacetate (**12**) (1.07 g, 1.85 mmol), as well as (5-(ethoxycarbonyl)thiophene-2-yl)boronic acid (760 mg, 3.8 mmol) added to the reaction vessel. The reagents were dissolved in 4 mL toluene and 6 mL methanol and the mixture degassed for 10 min using nitrogen. PEPPSI-iPr (67.9 mg, 0.1 mmol) was added and the mixture heated at 70°C for 15 min. Upon cooling, the reaction mixture was dissolved in ethyl acetate (20 mL) and washed with 2N HCl (2 x 20 mL) and brine (20 mL). The organic layer was dried over anhydrous Na_2SO_4, solvent removed in vacuo and the product purified by column chromatography using 100 % CH_2Cl_2. The product was obtained as a bright orange solid (811 mg, 60 % yield).

^{1}H-NMR (300 MHz, $CDCl_3$): 1.23 (t, J=7.1Hz, 6H, OCH_2CH_3); 1.32 (t, J=7.1Hz, 6H, OCH_2CH_3); 3.70 (s, 4H, CH_2); 4.15 (q, J=7.1Hz, 4H, OCH_2CH_3); 4.29 (q, J=7.1Hz, 4H, OCH_2CH_3); 7.08 (d, J=3.9Hz, 2H, Ar-*H*); 7.15 (s, 2H, Ar-*H*); 7.19 (s, 2H, Ar-*H*); 7.63 (d, J=3.9Hz, 2H, Ar-*H*); LR-MS: 728.3 [ES+].

S3.4. Compound pFTAA



3''',4'-bis(carboxymethyl)-[2,2':5',2'':5'',2''':5''',2''''-quinquethiophene]-5,5''''-dicarboxylic acid (pFTAA).
Diethyl 3''',4'-bis(2-ethoxy-2-oxoethyl)-[2,2':5',2'':5'',2''':5''',2''''-quinquethiophene]-5,5''''-dicarboxylate (**13**) (105 mg, 0.14 mmol) was dissolved in 4 mL 1,4-Dioxane and 1 mL of 1N NaOH added. After approximately 3 hours a precipitate was starting to form and 4 mL of H_2O was added to the reaction mixture. The solution was left stirring at room temperature for 48 hours, reaction progress was monitored via LCMS. Upon completion solvent was evaporated and the product dried under high vacuum. The product was obtained as an orange solid (88 mg, 99 % yield).
^{1}H-NMR (300 MHz, CD_3OD): 3.66 (s, 4H, CH_2); 7.16 (d, J=3.8Hz, 2H, Ar-*H*); 7.29 (s, 2H, Ar-*H*); 7.32 (s, 2H, Ar-*H*); 7.47 (d, J=3.9Hz, 2H, Ar-*H*); ^{13}C-NMR (75 MHz, CD_3OD)): 38.4, 123.1, 126.7, 128.0, 130.2, 130.6, 134.9, 135.7, 135.8, 140.1, 141.9, 168.4, 177.4; LR-MS: 617.2 [ES+]; 615.2 [ES-].

S4. Quantum yield measurement of pTP-TFE

The quantum yield of pTP-TFE (Φ_X) was calculated by the equation below.[1] Fluorescein in 0.1 M NaOH was used as the standard reference compound (excited at 490nm, Φ_{ST}=0.9). $Grad_x$ and $Grad_{ST}$ are the slopes of the linear fit for the integrated fluorescence intensity of pTP-TFE and fluorescein as a function of absorbance. UV-Vis spectrophotometer (Carey 60) and fluorescence spectrophotometer (Carey Eclipse) were used for absorbance and fluorescence measurements, respectively. The integrated area of the fluorescence intensity was analysed by GraphPad Prism 8.0. η_x and η_{ST} are the refractive indices of pTP-TFE and fluorescein solutions, respectively. The index of the refection for DMSO is 1.49 and 1.33 for 0.1M NaOH.

$$\Phi_X = \Phi_{ST}\left(\frac{Grad_x}{Grad_{ST}}\right)\left(\frac{\eta_X^2}{\eta_{ST}^2}\right)$$

S5. Binding of compounds to Aβ aggregates

S5.1. Protein preparation
Aβ(1-40) monomer protein (1 mg, rPeptide), lyophilized from hexafluoroisopropanol, was dissolved in 16.5 mL PBS buffer (10 mM; pH 7.4) and then sonicated (30 sec) to produce a 14 μM Aβ (1-40) solution.

S5.2. Compound stock solution preparation
4 mM stock chemical solutions were prepared by dissolving the compounds, ThT (1.28mg) and pTP-TFE (3.3 mg) in DMSO (1 mL) or for pFTAA (2.46 mg) in PBS buffer (1 mL). 5 µL of the stock solutions were then diluted to 10 µM with PBS buffer (2 mL).

S5.3. Aggregation of Aβ
S5.3.1 Aggregation of Aβ40
200 μL of 14 μM Aβ (1-40) solution was added to wells in a 96-well half area plate (black, µclear with lid, Greiner Bio-one). The protein solutions were then incubated at 37°C with 70 repetitive cycles of agitation (5 min agitation, 5 min standing).

S5.3.2 Aggregation of Aβ40:Aβ42 (9:1) mixture
86.5 μL of 230 μM Aβ (1-40) monomer solution was mixed with 10 μL of 220 μM Aβ (1-42) monomer solution then diluted to 1100 μL with PBS buffer, resulting in a solution of 18 μM Aβ (1-40) and 2 μM Aβ (1-42) (molar ratio: 9:1). 100 μL of stock solution was added to each well in a 96-well half-area plate (black, µclear with lid, Greiner Bio-one). The protein solutions were then incubated at 37°C with 100 repetitive cycles of agitation (5 min agitation, 5 min standing).

S5.4. Aggregation of Aβ with pFTAA, pTP-TFE and ThT

S5.4.1 Aggregation of Aβ40

2 µL of the chemical stock solutions of pFTAA, pTP-TFE and ThT (10 µM in PBS buffer) were added to separate wells in a 96-well plate containing 200 µL of 14 µM Aβ (1-40) monomers. In addition, for control experiments 2 µL of pFTAA, pTP-TFE and ThT (10 µM in PBS buffer) was added to separate wells containing 200 µL PBS buffer. All solutions were then incubated at 37°C with 70 repetitive cycles of agitation (5 min agitation, 5 min standing). POLARstar Omega plated reader (BMG LABTECH) was then used to measure the fluorescence intensity of pFTAA, pTP-TFE and ThT (excitation λ 450 nm; emission λ 520 nm; for all three compounds) from the bottom of the plate. The gain was adjusted to 80 % to obtain a good read out of fluorescent intensity. The data was normalized to the maximum fluorescence intensity for each compound and represents the average of three experiments for each compound.

S5.4.2 Aggregation of Aβ40:Aβ42 (9:1) mixture

1 µL of the chemical stock solutions of pFTAA, pTP-TFE and ThT (10 µM in PBS buffer) were added to separate wells in a 96-well half area plate containing 100 µL of 18 µM Aβ (1-40) and 2 µM Aβ (1-42) monomers mixture. In addition, for control experiments 1 µL of pFTAA, pTP-TFE and ThT (10 µM in PBS buffer) was added to separate wells containing 100 µL PBS buffer. All solutions were then incubated at 37°C with 100 repetitive cycles of agitation (5 min agitation, 5 min standing). POLARstar Omega plated reader (BMG LABTECH) was then used to measure the fluorescence intensity of pFTAA, pTP-TFE and ThT (excitation λ 450 nm; emission λ 520 nm; for all three compounds) from the bottom of the plate. The gain was then adjusted to 80 % to obtain a good read out of fluorescent intensity. The data was normalized to the maximum fluorescence intensity for each compound and represents the average of three experiments for each compound.

S5.5. Binding affinity of pTP-TFE and pFTAA to Aβ aggregates

S5.5.1 Binding to Aβ40

Aβ40 protein aggregates at 90 min, 200 min and 24 hours from S5.3.1 were obtained by removing a sample from the aggregating mixture. After been flash frozen by liquid nitrogen the samples were then stored at -80 °C until usage. For the binding experiments serial dilutions of pFTAA was performed to give solutions with concentrations of 5µM to 19nM and with pTP-TFE 20µM to 78nM. POLARstar Omega plated reader was then used to measure the fluorescence intensity from the bottom of the plate (excitation 450 nm; emission 520 nm) after 5 minutes of Aβ (1-40) aggregates being added to pFTAA and pTP-TFE separately. The final concentration of Aβ (1-40) was 7µM in each 96-well. The *K*d value for each compound was fitted by GraphPad Prism 8.0. Data points and error bars in Figure 1 represent the average ± standard error of the mean (n=3).

S5.5.2 Binding to Aβ40:Aβ42 (9:1) mixture

Aβ40:Aβ42(9:1) protein aggregates at 200 min and 24 hours from S5.3.2 were obtained by removing a sample from the aggregating mixture. After been flash frozen by liquid nitrogen the samples were then stored at -80 °C until usage. For the binding experiments serial dilutions of pTP-TFE was performed to give solutions with concentrations of 9µM to 35nM. POLARstar Omega plated reader was then used to measure the fluorescence intensity from the bottom of the plate (excitation 450 nm; emission 520 nm) after 5 minutes of Aβ40:Aβ42(9:1) aggregates being added to pTP-TFE separately. The *K*d value for each compound was fitted by GraphPad Prism 8.0. Data points and error bars in Figure S6 represent the average ± standard error of the mean (n=3).

S6. Binding of compounds to tau aggregates

S6.1 Tau aggregates incubation assay

100 µg of tau monomers lyophilized powder (Tau-441 human; Eurogentec LTD) was dissolved in 100 µL 20 mM BES buffer (pH 7.4 with 25 mM NaCl and 2 mM Dithiothreitol (DTT). Three samples were prepared. To eliminate any intramolecular disulfide bridges of compact monomers the solutions were then incubated at 56°C for 10 min. After cooling to room temperature in a water bath, heparin (molar ratio of 4:1 Tau: heparin) and protease-inhibitor-mix (leupeptin {1 µg/ml}, aprotinin {1 µg/ml}, pepstatin {1 µg/ml}, benzamidin {1 mM}) were added and the mixture incubated at 37°C for 10 days without shaking. Every 24h DTT (2µL, 100mM) was added to the tau mixture (100 µL) to a final concentration of 2 mM.

S6.2. Compound preparation

2 µM pFTAA and 2 µM pTP-TFE were prepared as described under S4.2, but diluted in BES buffer (pH 7.4, with 25 mM NaCl) instead of PBS buffer before use.

S6.3. Fluorimeter assay

A fluorimeter was used to monitor changes in fluorescent intensity upon adding pFTAA and pTP-TFE to tau protein at different stages in the aggregation process. This consisted of 20 µL of 2.7 µM tau protein being mixed with 20 µL of 2 µM pFTAA or pTP-TFE. Hitachi F-4500 Fluorescence spectrophotometer was used to measure fluorescent intensity (pFTAA: excitation λ 488 nm; emission λ 520 nm; pTP-TFE: excitation λ 420 nm; emission λ 520 nm). Aliquots of the tau protein aggregation were taken at specific time points after initiation of the incubation (0 h, 5 h, 24 h, 48 h, 80 h, 96 h, 7 days and 10 days) and their fluorescence with pFTAA and pTP-TFE were measured.

The data (Figure 2) was background corrected and normalized to the maximum fluorescence intensity for each compound and represents the average (n=3). ± standard error of the mean. The ratio of pTP-TFE emission (Figure S4A) was calculated by dividing the normalized fluorescence emission at λ 495 nm by the normalized fluorescence emission at λ 560 nm.

S6.4. Binding affinity of pTP-TFE with tau aggregates taken from incubation

Tau aggregates samples prepared from S5.1 were taken at 96 hours and 240 hours of the incubation time. A serial dilution of pTP-TFE was from 2 μM to 39 nM for the 96 hour tau aggregates binding test and 10μM to 20 nM for 240 hours tau aggregates binding test. The final concentration of tau was 2.3 μM in each 96-well for the binding assay. POLARstar Omega plated reader was used to measure the fluorescence intensity from the bottom of the plate (excitation 450 nm; emission 520 nm). The K_d value in Figure 2 was fitted by GraphPad Prism 8.0. The data points and error bars in Figure 2 represent the average ± standard error of the mean (n=3).

S7. Dot blot analysis

3μL of 20 μM solutions of tau or Aβ40 aggregates were spotted onto nitrocellulose membrane (Bio-Rad) at selected time points.[2] For tau, the selected time points were 0, 48 hours and 10 days. For Aβ40, the selected time points were 0, 200min and 24 hours. The membranes were blocked with 10% (w/v) fat-free milk in 50 mM Tris,150 mM NaCl, pH 7.4 and 0.05% (v/v) Tween-20 (TBST buffer) solution for 1 h at room temperature. Then incubated with polyclonal anti-oligomer A11 antibody (1:1000 dilution; Invitrogen) in 5% (w/v) fat-free milk and TBST buffer overnight at 4 °C. The membranes were then washed 3 times in TBST before being incubated with anti-rabbit secondary antibody (1:50000 dilution) in 5% (w/v) fat-free milk and TBST buffer at room temperature for 1 h.

After the first step dot blot imaging, the membranes were then washed with mild stripping buffer (15 g glycine, 1 g SDS, 10 mL Tween 20, pH to 2.2, in 1 L distilled water) twice for 1 hour and then followed with 3 TBST buffer washes. The membrane was then restained with $A\beta_{1\text{-}16}$ 6E10 monoclonal antibody (1:1000 dilution, BioLegand UK Ltd) for amyloid aggregates and HT7 tau monoclonal antibody (1:1000, Stratech Science Ltd) for tau aggregates, in 5% (w/v) fat free milk and TBST buffer overnight at 4 °C. The membranes were washed 3 times in TBST before being incubated with anti-mouse secondary antibody (1:50000 dilution) in 5% (w/v) fat-free milk and TBST buffer at room temperature for 1 h.

S8. Transmission electron microscopy (TEM) of Aβ and tau protein

Aliquots of Aβ (1-40) and tau protein were collected at different time points during fibril formation and all tau fractions after sucrose gradient ultracentrifuge. 5 μL of each sample was applied to a carbon film coated 400 mesh copper (Cu) grid for 2 min. The grids were washed and negatively stained with uranyl acetate (1%, w/v) and water and air-dried before being examined with transmission electron microscopy (TEM, Tecnai G2 200kv)). TEM imaging photos were taken at 90 min, 200 min and 700 min after initiation of the experiment for Aβ (1-40) and 24 hours, 96 hours and 10 days for tau protein.

S9. Sucrose gradient ultracentrifuge method

The hTau IsoformD (0N4R) monomer was dissolved in 100 mM Tris, 150 mM NaCl, 0.1 mM EDTA, pH 7.5 buffer condition. Then using a PD MiniTrap G-10 column (GE healthcare Life sciences) the buffer was changed to 20 mM BES buffer pH 7.4. Finally, 1mg of tau monomer is dissolved in 1 mL BES buffer. Heparin was then added at molar ratio of 1:4 heparin:tau. The tau monomer (1mg/mL, 25μM) was then incubated at 37°C for 10 day, during which DTT was added every 24 h to a final concentration of 2 mM. Over the 10 day incubation period aliquots (100 μL) were taken every 24 hrs to generate 10 samples of tau aggregate solution. Each collected sample was flash frozen using liquid nitrogen and stored at -80°C.

50%, 40%, 30%, 20%, 10%, 5% sucrose PBS (10 mM pH 7.4) solution were then prepared. Then into an ultracentrifuge tube (14 ml, thin wall, polypropylene, clear, from Beckman Coulter) was added 2 mL of each sucrose PBS solution, starting with 50% at the bottom to finish with the 5% solution at the top of the tube to create the sucrose gradient. Then the combined tau aggregate solutions (totalling 1 mL) was added to the top of the sucrose gradient. The sucrose gradient tube was then loaded on to an ultracentrifuge rotor (SW 40 Ti Rotor, Swinging Bucket, Titanium, 6 x 14 mL). An additional tube containing a control sucrose gradient without any tau protein (but of equal weight with PBS buffer on top) was also added to the rotor. The sucrose gradients were then centrifuged (284,570 *g*-force, Optima™ XE) for 4 hours at 4°C. After centrifuge, using a syringe, 2mL samples were taken from each of the 6 layers in the sucrose gradient from both the tau and control tubes. 100 μL was taken from each sample and then flash frozen using liquid nitrogen and stored at -80 °C to be used for binding affinity measurements (S8) and TEM imaging. To confirm tau protein was present in the 6 samples from the sucrose gradient process their absorbance at 280nm were measured by Nanodrop.

S10. Binding affinity of pTP-TFE and pFTAA to tau soluble aggregate fractions

For control experiments pTP-TFE with 50%, 40%, 30%, 20%, 10%, 5% sucrose PBS (10 mM pH 7.4) buffer were first prepared. A serial dilution of pTP-TFE (from 10 μM to 1 nM) in PBS was then prepared for the binding test with tau samples from the 6 sucrose gradient layers. . For each binding test 8 μL of tau soluble aggregates fractions from the sucrose gradient aliquots were added to 20 μL of pTP-TFE. Then the mixture of tau aggregates and pTP-TFE were incubated for 5 minutes at 37°C in a POLARstar Omega plate reader before measurement of the fluorescent intensity with the fluorescence data acquired from the bottom of the plate at excitation 450 nm; emission 520 nm. Each group of experiments was repeated at least three times. The binding affinity data was analysed and background fluorescence intensity from control experiments (50% - 5% fractions in PBS only) subtracted. K_d values was fitted by GraphPad Prism 8.0 software. To determine the binding affinity of pFTAA to the tau soluble fractions (with 50%, 40%, 30%, 20%, 10%) the same procedure was performed as described for pTP-TFE. The 5% sucrose tau fraction was not tested with pFTAA since the binding affinity of 10% sucrose layer is already low (30μM) with pFTAA. The data points and error bars showed in Figure S3 and S4 represent the average ± standard error of the mean (n=3).

S11. Cellular uptake of pTP-TFE on primary human fetal neurons

S11.1 Ethics

Human fetal tissue was collected from routine termination of pregnancies at the Cambridge University Hospitals (Addenbrooke's) and experiments performed at the John van Geest Centre for Brain Repair (University of Cambridge). All human tissue was collected under full ethical approval in accordance with the United Kingdom's Department of Health guidelines and local ethical approval (NRES Committee East of England - Cambridge Central, reference no. 96/085). Postmortem brain material was obtained from the Cambridge Brain Bank with full ethical committee approval and the tissue stored under a license from the Human Tissue Authority (reference no. 10/H0308/56). Tissue was donated from registered prospective donors with informed consents. The postmortem human tissue analysis was conducted at the John van Geest Centre for Brain Repair, under full ethical approval in accordance with the United Kingdom's Department of Health guidelines and local ethical approval (Local Research Ethics Committee, reference no. 01/177).

S11.2 Cell Cultures

For primary human neuronal cultures, human fetal cortical tissue from gestational age 61–70day old aborted fetuses were dissected under sterile condition as published previously.[3] In short, the tissue was then mechanically triturated into a single cell suspension, before seeding onto PDL-coated glass coverslips treated with laminin (50 µg/mL, Sigma L2020) for 30 min, at a density of 2,000,000 viable cells per T25 flasks, in differentiation media composed of DMEM, 4.5 g/L glucose (Life Technologies 11965-084) supplemented with 10% FBS, 2% B-27 (Gibco 17504044), and 1% antibiotics.

S11.3 Cell uptake of pTP-TFE procedure

5 µL of 4 mM pTP-TFE DMSO solution was added to 5mL of cell medium in four flasks containing primary human neuronal cultures and left to incubate at 37°C, 5% CO_2 for 15min, 30min, 60min and 120min respectively. At each time point, the cell medium was removed and cells washed three times with 1 mL PBS. PBS fractions were then retained for analysis as P1, P2 and P3. 0.5 mL acetonitrile was then added to each flask of the cells to lyse cells and extract pTP-TFE accumulated in the intracellular compartment; acetonitrile fraction was retained for analysis as A1. This was followed by two more 0.5 mL acetonitrile extractions, retained as A2 and A3. P1, P2, P3, A1, A2 and A3 samples were homogenized for 3 x 10 seconds and centrifuged for 5 min at 30130 *rcf* 4°C. The supernatant was then used for analysis by LC-MS/MS.

S11.4 LC-MS/MS Analysis

LC-MS/MS analysis was performed using a Waters Acquity-H/Xevo TQD LC–MS equipped with an electrospray ion source. Instrument control, data acquisition and data processing were performed using Masslynx V4.1 software.
Appropriate MS-MS transitions at unit resolution for pTP-TFE were developed using the IntelliStart® program in Masslynx software, by infusion of a 1.2 µM solution of **pTP-TFE** in MeCN/H_2O (85:15) at 60 µL/min in combined mode with LC flow set to 85% B at 0.6 $ml.min^{-1}$. Three transitions were developed in ES+ mode:

832.07 → 619.99 (cone voltage 90 V; collision energy 56 V, dwell time 25ms)
832.07 → 763.08 (cone voltage 90 V; collision energy 42 V, dwell time 25ms)
832.07 → 551.05 (cone voltage 90 V; collision energy 56 V, dwell time 25ms)

For analysis of samples, the three transitions developed above in MRM mode in combination with the following LC conditions for separation of **pTP-TFE**: Column: Waters Acquity UPLC® BEH C_{18} 1.7 µm, 2.1×50 mm. Method: Solvent A: H_2O containing 0.1 % (v/v) formic acid; Solvent B: MeCN containing 0.01% (v/v) formic acid. Linear gradient: 0 min: 95% solvent A; 2 min: 5% solvent A; 3 min: 5% solvent A; 3.1 min: 95% solvent A; 4 min: 95% solvent A. Flow rate: 0.6 $ml.min^{-1}$, Injection volume: 1 µL. Under these conditions **pTP-TFE** had a retention time of 2.4 minutes (Figure S9).
A standard curve was constructed with standard solutions of pTP-TFE by plotting the peak area of the total MRM response over a concentration range of 0.625 nM to 80 nM (Figure S9). Samples were quantified against this standard curve. If sample concentrations were found to lie outside this linear range, they were diluted as appropriate before being re-analysed.

S12. Confocal microscopy of post-mortem brain slice

Frozen brain slices were thawed and fixed using escalating level of ethanol, and rehydrated with decreasing level of ethanol. After blocking with 2.5% Bovine Serum Albumin (BSA) for 1 h, slides were incubated overnight with primary antibodies including monoclonal phospho-Tau (Ser202, Thr205) antibody (AT8, ThermoFisher MN1020, 1:1000), monoclonal phospho-Tau (Thr212, Ser214) antibody (AT100, ThermoFisher MN1060, 1:1000), and **pTP-TFE** (1µM). Slides were then stained with secondary antibodies (Alexa 647, Molecular Probes, 1:1000), and blocked with 0.2% Sudan black B (Sigma) in 70% ethanol for 30 sec, before visualization using a TCS SP2 confocal microscope (Leica).

Results

S13. Additional figures

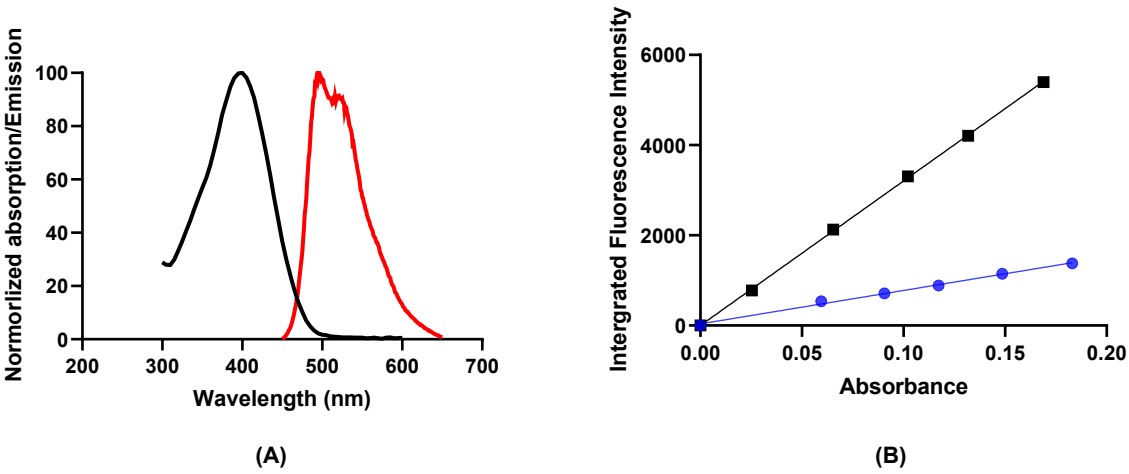


Figure S1: Fluorescence characteristics of pTP-TFE. (A) Absorption and emission spectra of **pTP-TFE** in DMSO. (B) Relative fluorescence quantum yields. Absolute quantum yield of **pTP-TFE** in DMSO solution was found to be 0.27 (blue dots), using Fluorescein in 0.1M NaOH as reference with quantum yield 0.90 (black squares).

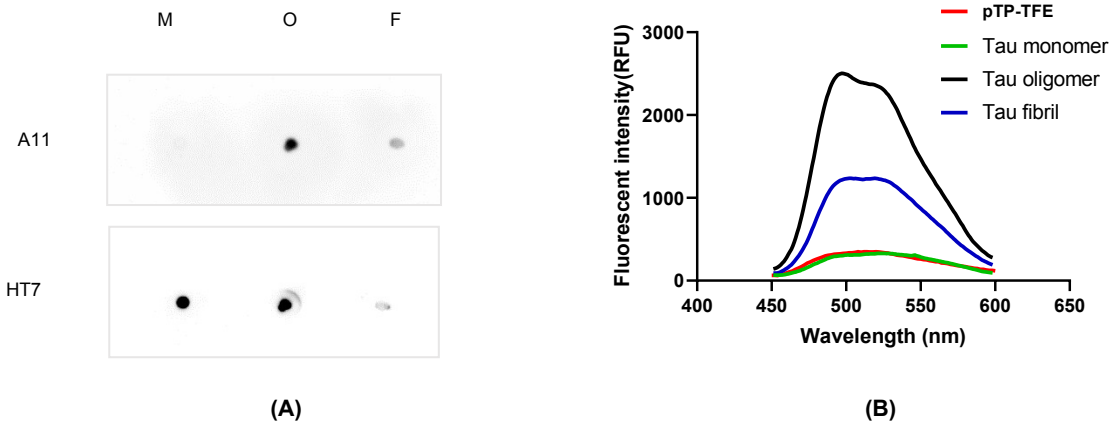


Figure S2: Characterization of monomers, oligomers and fibrils from human recombinant tau-441 (2N4R). (A) Dot blot of tau probed by oligomer specific A11 and tau HT7 antibodies. (B) Emission spectra of **pTP-TFE** alone and when incubated with monomer, oligomer and fibrils of tau (excitation wavelength at 420nm, dye 1 μM, Tau 2 μM).

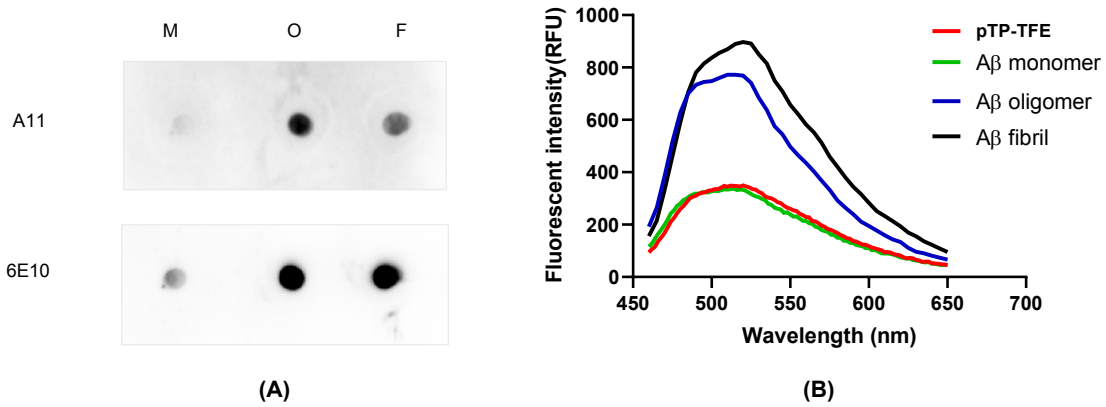


Figure S3: Characterization of monomers, oligomers and fibrils from human recombinant Beta-amyloid (1-40). (A) Dot blot of Aβ probed by oligomer specific A11 and Aβ 6E10 antibodies. (B) Emission spectra of **pTP-TFE** alone and when incubated with monomer, oligomer and fibrils of Aβ (excitation wavelength at 420nm, dye 1 μM, Aβ 2 μM).

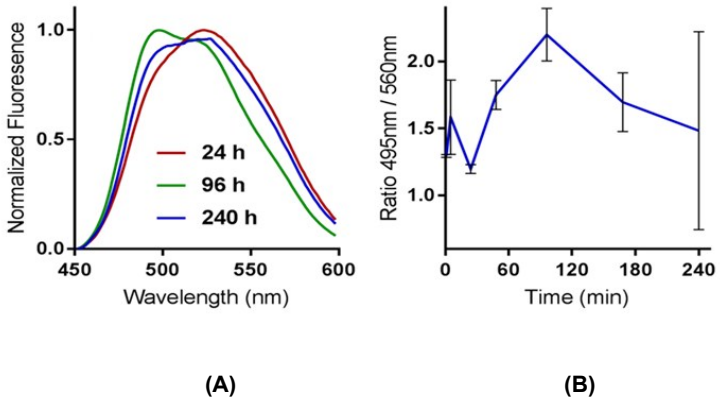


Figure S4: (A) Emission spectroscopy shift of **pTP-TFE** binding to tau aggregates at 24, 96 and 240 h. (B) Ratio of **pTP-TFE** emission at 495 nm / 560 nm.

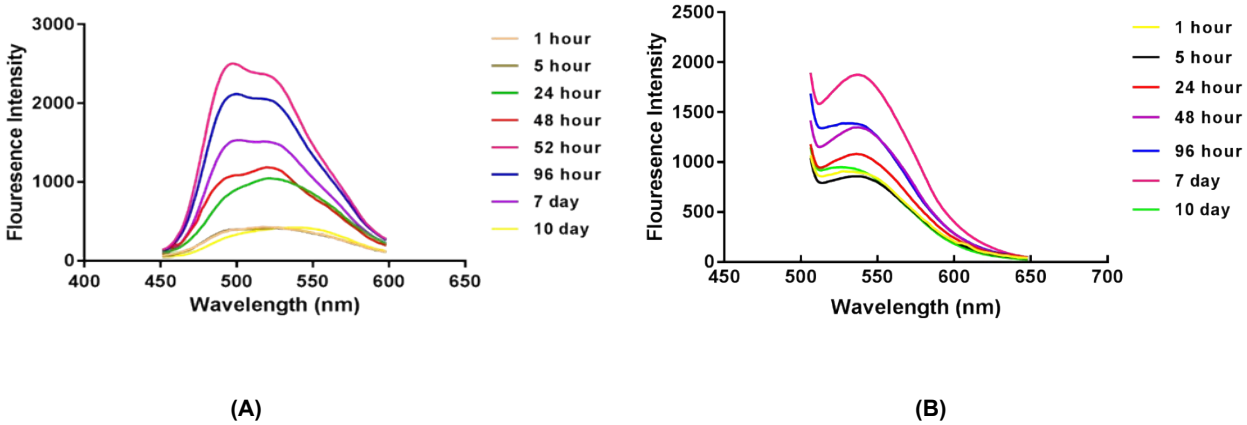


Figure S5. Representative fluorescence spectroscopy data of **pTP-TFE and pFTAA** incubated with tau protein at different time points post-initiation of the experiment (**pTP-TFE** excitation λ 420 nm, emission λ 520 nm; **pFTAA** excitation λ 488 nm, emission λ 520 nm)

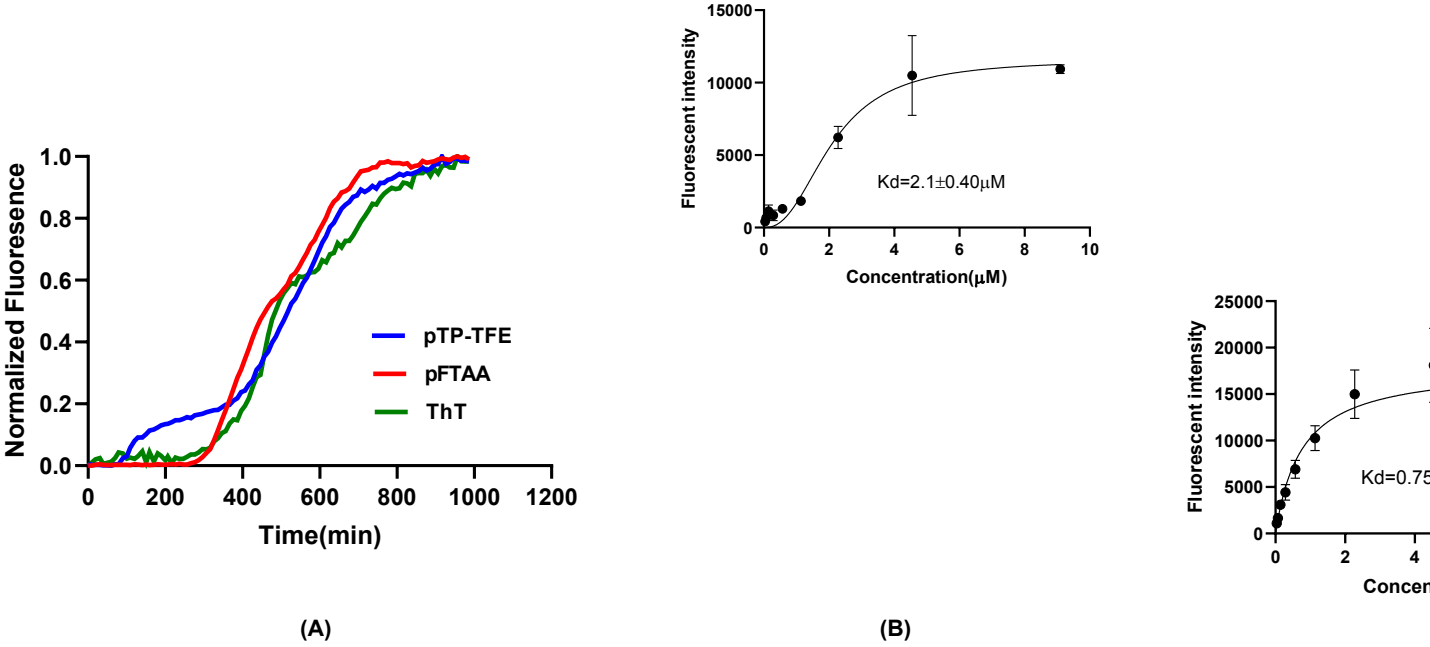


Figure S6. (A) Normalized fluorescence intensities of **pTP-TFE**, **pFTAA** and **ThT** in Aβ aggregation assay starting with Aβ40: Aβ42 = 9:1 monomers (n=3). (B) Top: Fluorescence intensity binding curve and calculated binding affinity of **pTP-TFE** to Aβ40:Aβ42 (9:1) mixture at 200 minutes of aggregation; Bottom: Binding affinity of **pTP-TFE** to Aβ40:Aβ42 (9:1) mixture fibrils.

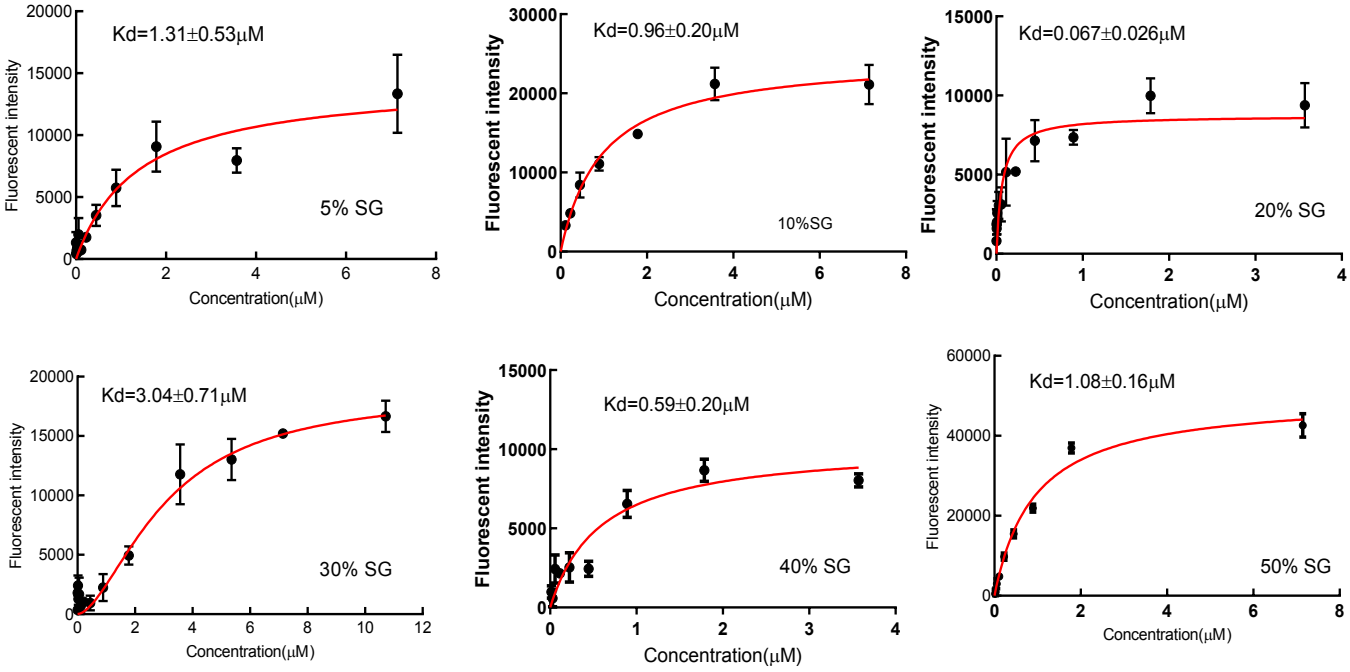


Figure S7: Fluorescence intensity binding curve and calculated binding affinity of **pTP-TFE** to tau soluble aggregates at 5%, 10%, 20%, 30%, 40% and 50% sucrose gradient fractions.

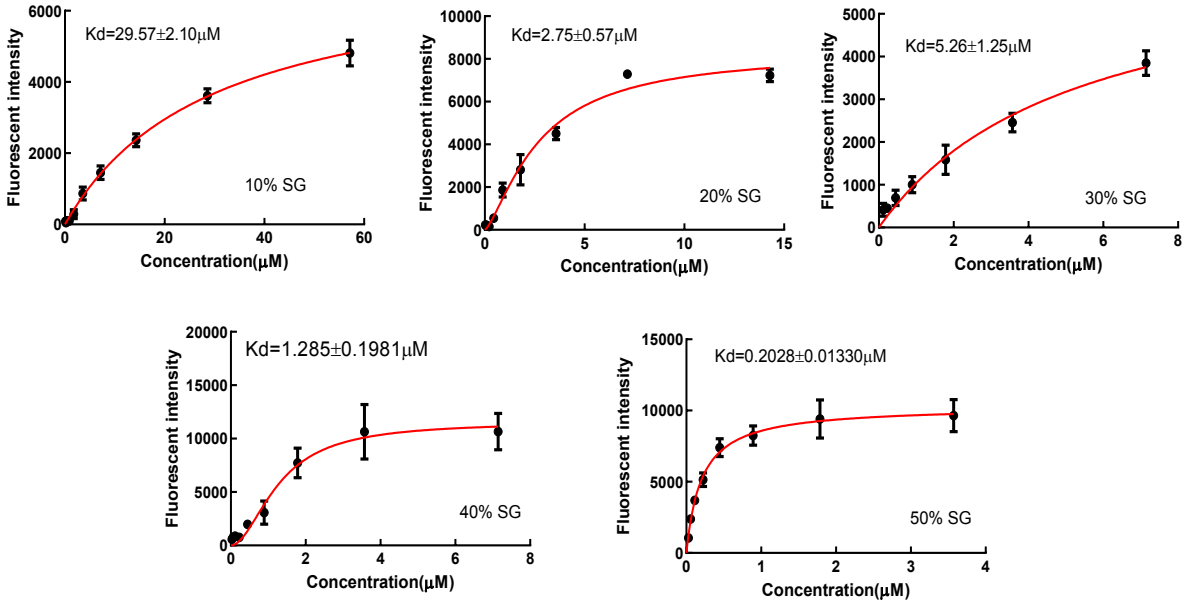


Figure S8: Fluorescence intensity binding curve and calculated binding affinity of **pFTAA** to tau soluble aggregates at 10%, 20%, 30%, 40% and 50% sucrose gradient fractions.

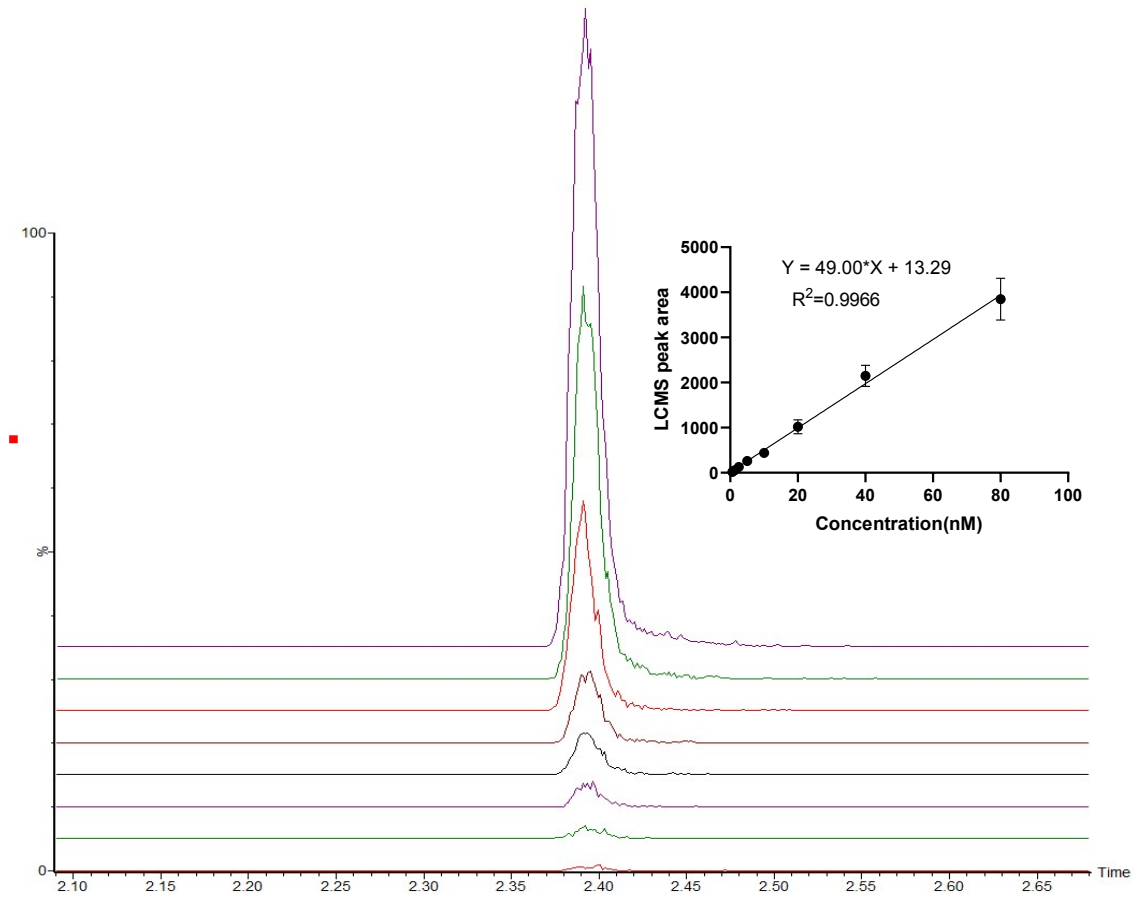


Figure S9. LC-MS/MS chromatogram of **pTP-TFE**. LCMS-MS peak for **pTP-TFE** showing retention time of 2.4 minutes. Standard curve for **pTP-TFE** showing total peak area for all three transition plotted against concentration.

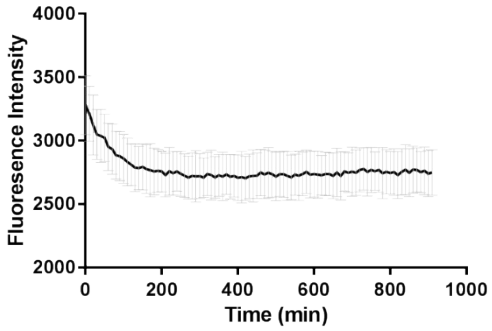


Figure S10. Fluorescence intensity of 2 µL, 0.1µM pTP-TFE in 200µL PBS buffer over the course of the Aβ plate reader experiment as described S4.3 (excitation λ 450 nm; emission λ 520 nm).

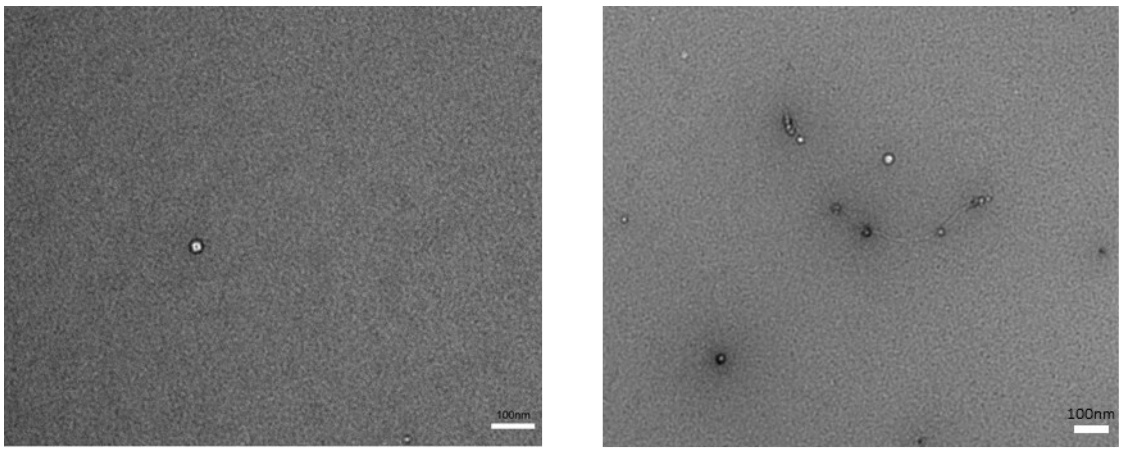


Figure S11. TEM imaging of tau oligomer with high resolution showed size around 10-20nm diameter.

S14. Additional Table

S14.1 Cellular uptake of pTP-TFE

The proportion of intracellular pTP-TFE is calculated by:

$$y = \left\{ \frac{A1 + A2 + A3}{4\mu M \times 5mL} \right\} x100\%$$

A1, A2 and A3 are the amounts of the pTP-TFE extracted after each 0.5mL acetonitrile extraction of the cells. P1, P2 and P3 are three extracellular PBS washes after the cell medium was removed.

Group	Time (min)	P1(nmol)	P2(nmol)	P3(nmol)	A1(nmol)	A2(nmol)	A3(nmol)
1	15	0.04	0.07	0.06	0.63	0.04	0.00
1	30	0.06	0.02	0.01	1.04	0.10	0.01
1	60	0.10	0.04	0.01	1.29	0.06	0.01
1	120	0.05	0.02	0.01	1.56	0.13	0.03
2	15	0.03	0.01	0.00	0.47	0.06	0.01
2	30	0.07	0.01	0.00	0.88	0.13	0.02
2	60	0.13	0.05	0.02	1.53	0.12	0.02
2	120	0.18	0.06	0.02	2.00	0.17	0.03
3	15	0.07	0.01	0.00	0.36	0.03	0.01
3	30	0.07	0.01	0.00	0.46	0.06	0.01
3	60	0.10	0.02	0.01	1.32	0.14	0.02
3	120	0.11	0.02	0.01	1.78	0.15	0.02

Table S1. Amount of pTP-TFE in samples asdetermined by LC-MS/MS.

S14.2 Information on human brain slides

Brain slides name	Type	Regions of brain	Age	Disease length
PT57	AD	Frontal cortex	97	Alzheimer's braak 6
PT96	AD	Frontal cortex	83	Alzheimer's braak 5
PT93	AD	Frontal cortex	81	Alzheimer's braak 5
PT103	AD	Frontal cortex	78	Alzheimer's braak 4
JR69	PSP	Globus pallidus/putamen	74	5 years
JR73	PSP	Globus pallidus/putamen	73	6 years
PN3	PSP	Globus pallidus/putamen	75	6 years
NP16.258	Healthy	Frontal cortex	49	/
PT154	Healthy	Frontal cortex	72	/

Table S2. Information of the human brain slide

References:

1. E. Austin, M. Gouterman, *Bioinorg. Chem.*, 1978, **9**, 281.

2. C. L. Teoh, D. Su, S. Sahu, S. W. Yun, E. Drummond, F. Prelli, S. Lim, S. Cho, S. Ham, T. Wisniewski, Y. T. Chang, *JACS*, 2015, **137**, 13503.

3. W. Kuan, K. Stott, X. He, T. Wood, S. Yang, J. C. F. Kwok, K. Hall, Y. Zhao, O. Tietz, F. I. Aigbirhio, A. C. Vernon, R. A. Barker, *Mol Psychiatry*, 2019.