Nystrom et al Supplementary information

Supplementary information contains:

Supplementary methods

Staining solutions: *LCOs*: qFTAA and hFTAA were synthesized as described previously (*1*). 1 mg ml^{-1} stock solutions of the LCO were prepared in 2 mM NaOH and stored at 4 °C until needed. Fresh staining solutions were prepared for each staining by diluting the stock 1:500 in PBS. For simultaneous double staining the staining solutions were mixed at a ratio of 2 qFTAA:1 hFTAA giving a final concentration of 2.4 µM qFTAA and 0.77 µM hFTAA. *ThT:* 2 mM ThT stock solution was prepared in milliQ water and stored at 4 °C. 20 µM ThT working solution was prepared by dilution stock in PBS pH 7.4. *CN-PiB:* 5 µM working solution was prepared by diluting a stock of 5 mM CN-PiB in dimethyl sulfoxide into PBS pH 7.4, *Congo red:* 3 mg/ml stock solution was prepared by mixing 0.3 g Congo red, 0.3 g NaCl and 100 ml 80% (v/v) EtOH. The stock was stored at room temperature. Working solution was prepared by mixing 100 µl CR stock with 9.9 ml 1% (w/v) NaOH. The working solution was filtered and used within 20 minutes.

***Ex situ* kinetics of recombinant Aβ fibrillation:** Lyophilized recombinant Aβ1-40 and Aβ1-42 (rPeptide) was resuspended in 2 mM NaOH at a concentration of 1 mg ml^{-1} and frozen in aliquots at -20 °C. Adequate amounts of peptide were thawed and 10 µM assay solution was prepared in PBS, pH 7.4 immediately prior to use. Staining solution was prepared at 10x concentration (described above) and 5 µl aliquots were dispensed in 96-well plates kept on ice. 45 µl samples were withdrawn from the fibrillation reaction at different time points and placed in the staining solution. The stained samples were left to sediment for 48 h on ice. 5 µl samples were withdrawn from the bottom of each well and placed on superfrost ultra plus microscope glass. When the liquid had evaporated, Dako fluorescence mounting medium (Dako Cytomation) and cover glass was applied. The slides were left at room temperature in the dark overnight. Slides were sealed with nail polish and analyzed the next day.

***In situ* kinetics of recombinant Aβ fibrillation**: Fibrillation of Aβ for *in situ* monitoring was performed by mixing 10 µM Aβ in PBS with 300 nM qFTAA or hFTAA and 600 nM ThT respectivaly. qFTAA, hFTAA and ThT were used both each by themselves and in combinations. The reaction samples were aliquoted in 96 well plates and fibrillation was performed and monitored *in situ* in a Tecan Saphire2 microplate reader (Tecan, Männerdorf, Switzerland) at 37 °C. Spectra were collected every 30 minutes after excitation at 430 nm. Kinetic traces were retrieved by extracting fluorescence intensity at 490 nm for ThT, 500 nm for qFTAA and 585 nm for hFTAA. The intensities were plotted against time and the kinetic traces were normalized.

Preparation of tissue sections: Transgenic APP/PS1 mice and APP23 mice were sacrificed at different ages, whereby one brain hemisphere was gently snap frozen by immersion in a 2-methylbutane bath surrounded by dry ice. 10 µm cryosections were fixed in 96% (v/v) ethanol for 10 minutes and further rehydrated in 70% (v/v)ethanol, dH_2O and PBS, pH 7.4, 10 min for each step. LCO and ThT staining solution was applied to the cryosections and left for 30 minutes. The sections were washed 3x5 minutes with PBS and mounted with Dako fluorescence mounting medium. For step wise co-staining of qFTAA or hFTAA with CN-PiB, 5 µM CN-PiB was applied as an additional step between washing and mounting and the slides were washed again before mounting. The slides were left in room temperature in the dark overnight, sealed with nail polish and analyzed the next day. For Congo red staining, cryosections were fixed and rehydrated as above and further stained with Mayer´s hematoxylin for 2 minutes, rinsed in tap water and stained with Congo red working solution for 10 minutes before rinsing in dH_2O and differentiation in alkaline ethanol. The slides were mounted with Dako mounting medium and sealed with nail polish. For antibody labeling, fixed and rehydrated cryosections were incubated in block solution (PBS, 0,1% (v/v) Triton X-100, 2% (w/v) BSA) for 30 minutes followed by incubation overnight at 4 °C with 6E10 antibody diluted 1:100 in block solution. After washing 3X10 minutes with PBS, Alexa Fluor 488 goat anti-mouse antibody was added and incubated 30 minutes. Final washing with PBS 3X10 minutes was performed prior to mounting with Vectashield mounting medium (Vector laboratories).

Fluorescence microscopy: A 436 nm long pass excitation filter (436/10 (LP475)), was used for the hyperspectral imaging. Images of recombinant fibrils were collected using 10x magnification. 3 representative images from each timepoint were analyzed using 9 large region of interest (ROIs) per image. The 500/540 ratio from the resulting 27 spectra per time point were averaged. To generate the age transition curve of APP/PS1 mice, 5 overview images of the cortex at 10x magnification were collected. For ratiomeric calculation 9 plaques from each image (45 plaques from each animal) were analyzed in the same manner as for the fibrillation kinetic samples, using a small ROI from the center of each plaque. The correlation coefficients between the sample spectra and a reference spectrum of qFTAA, acquired by hyperspectral imaging of a section stained with qFTAA only, were calculated for every pixel in an image. ROIs on plaques were then extracted from each image and the center was defined from the intensity image (sum over the spectrum) by finding the maximum intensity in the image after being passed through a 3x3 median filter (*2*). Spectra displayed are selected representative spectra from single ROIs. Imaging of Congo Red stained sections was performed using a 560 nm long pass filter for excitation and antibody stained sections were imaged using a 480 nm long pass filter. Imaging of CN-PiB samples and their references was done using 405 nm long pass filter for excitation.

Atomic Force Microscopy (AFM): As substrate for AFM samples, mica disks were modified to optimize fibril binding. Freshly cleaved mica was modified with 1% (v/v) trimethoxysilylpropyldiethylenetriamine in 1 mM acetic acid for 30 min following by 30 min incubation with 0.5% (v/v) glutaraldehyde in deionized water. After each step of modification, mica sheets were rinsed with deionized water and dried with a flow of compressed nitrogen. Subsequently, a droplet of 12 µL of 10 µM recombinant Aβ fibrils in PBS was deposited onto a substrate,

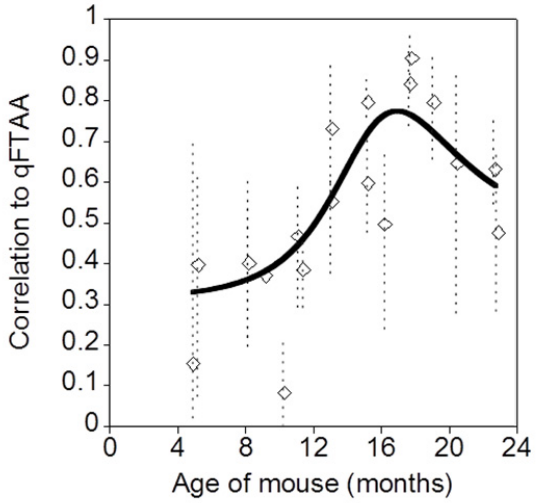
incubated for 15 min. and then rinsed with deionized water to remove all unbound material. Rinsed mica sheets were dried in vacuum (~1–0.1 mPa). Immobilized dried Aβ fibrils were imaged using AFM (Digital Instruments Multimode) operated in tapping mode under ambient conditions. Silicon nitride cantilevers PPP-NCH (Nanosensors) were used (nominal resonance frequency: 204–497 kHz). Overlapping of trace and retrace signals were used as a prerequisite for adequate image acquisition.

Statistical Analysis of Spectral Differences Among Plaques. Statistical analysis was performed using a One-way ANOVA (nonparametric) test (GraphPad prism 6.0) carrying out Kruskal-Wallis and Dunns´ multiple comparison tests. This results was also verified with an unpaired t test with Welch´s correction.

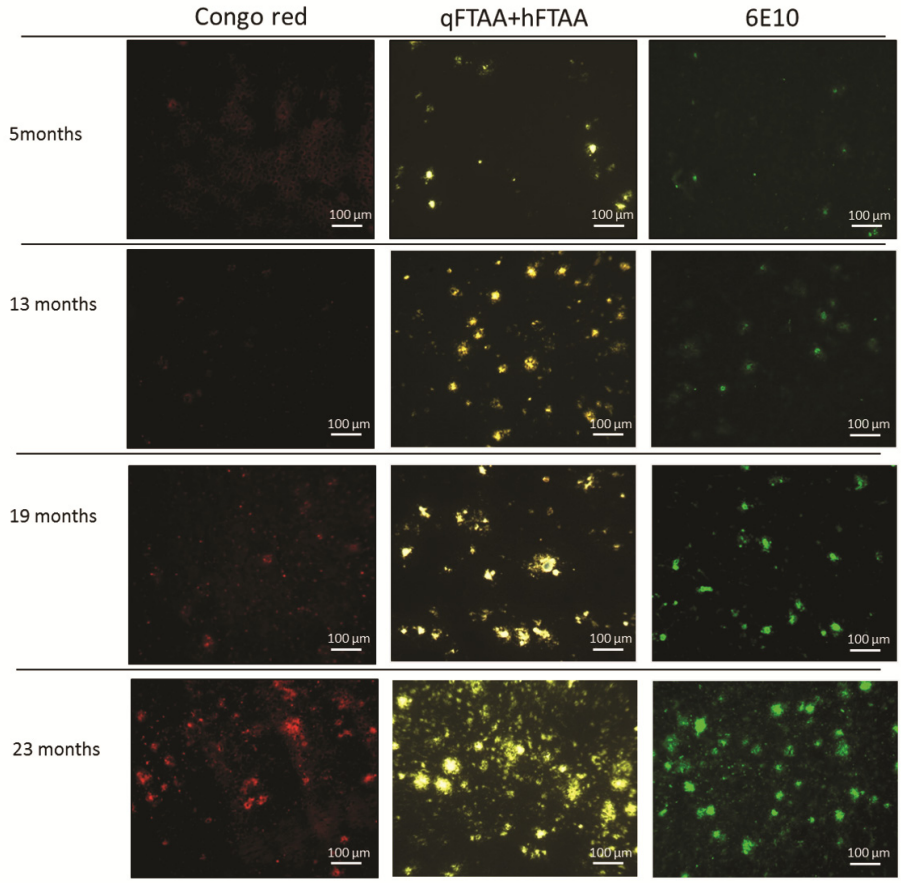
1. Klingstedt, T., Aslund, A., Simon, R. A., Johansson, L. B., Mason, J. J., Nystrom, S., Hammarstrom, P., and Nilsson, K. P. (2011) Synthesis of a library of oligothiophenes and their utilization as fluorescent ligands for spectral assignment of protein aggregates, *Organic & biomolecular chemistry 9*, 8356-8370.

2. Ellingsen, P. G., Reitan, N. K., Pedersen, B. D., and Lindgren, M. (2013) Hyperspectral analysis using the correlation between image and reference, *J. Biomed. Opt. 18*.

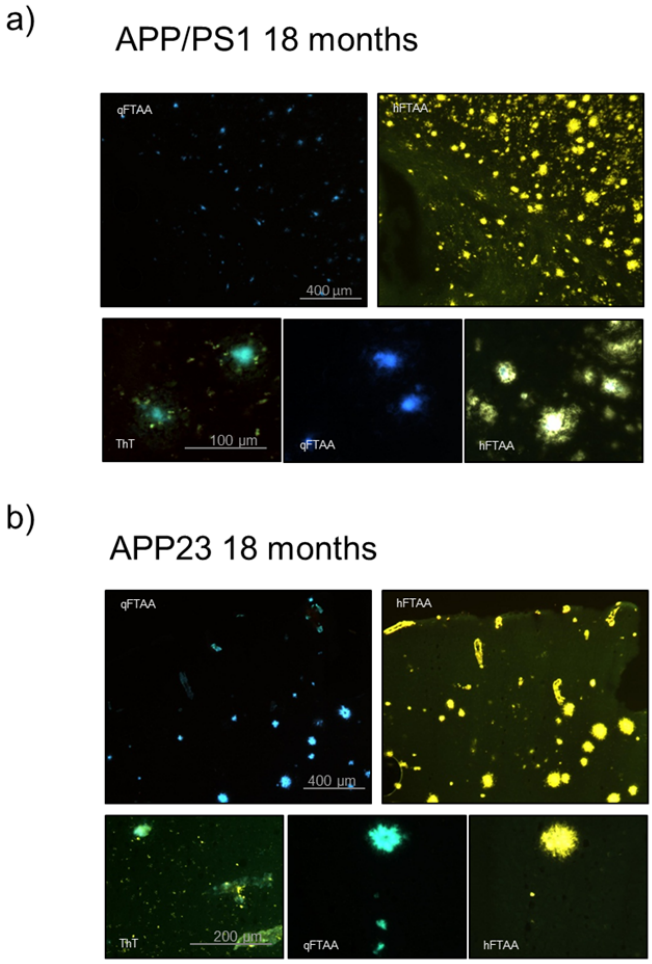
Supplementary figures



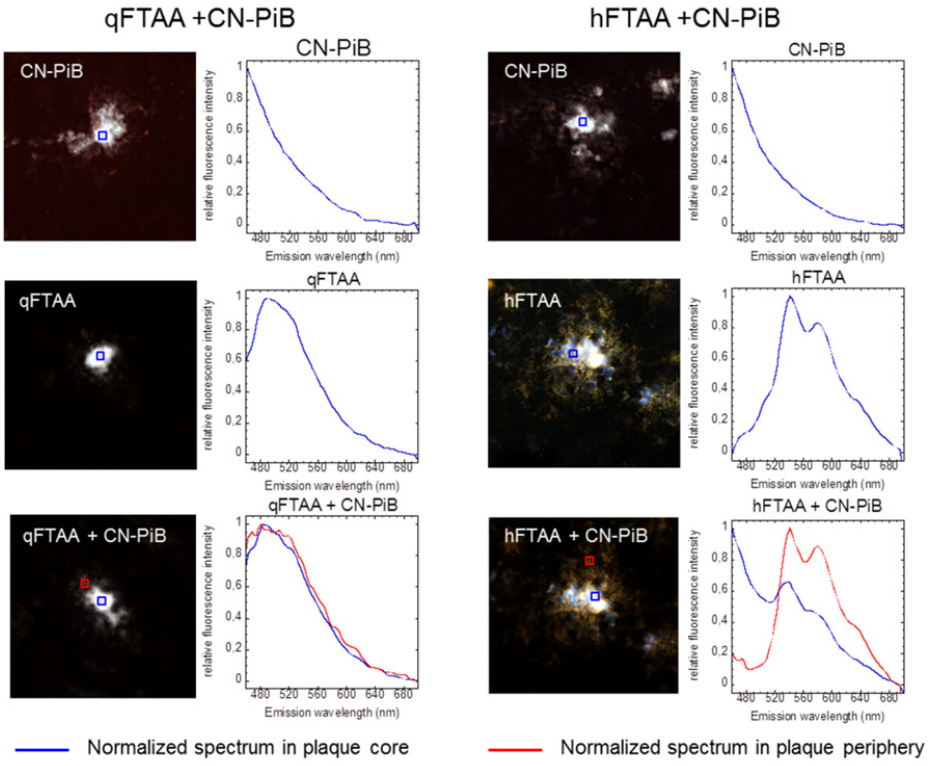
Suppl figure 1: Age dependent variation of plaque staining in APP/PS1 mice. Correlation coefficients for spectral correlation for pure qFTAA emission from the center of randomly selected plaques (n=10-25 per mouse). The mean values (◊) were plotted against age. The vertical dotted lines represent the 50th percentile of the correlation coefficients.



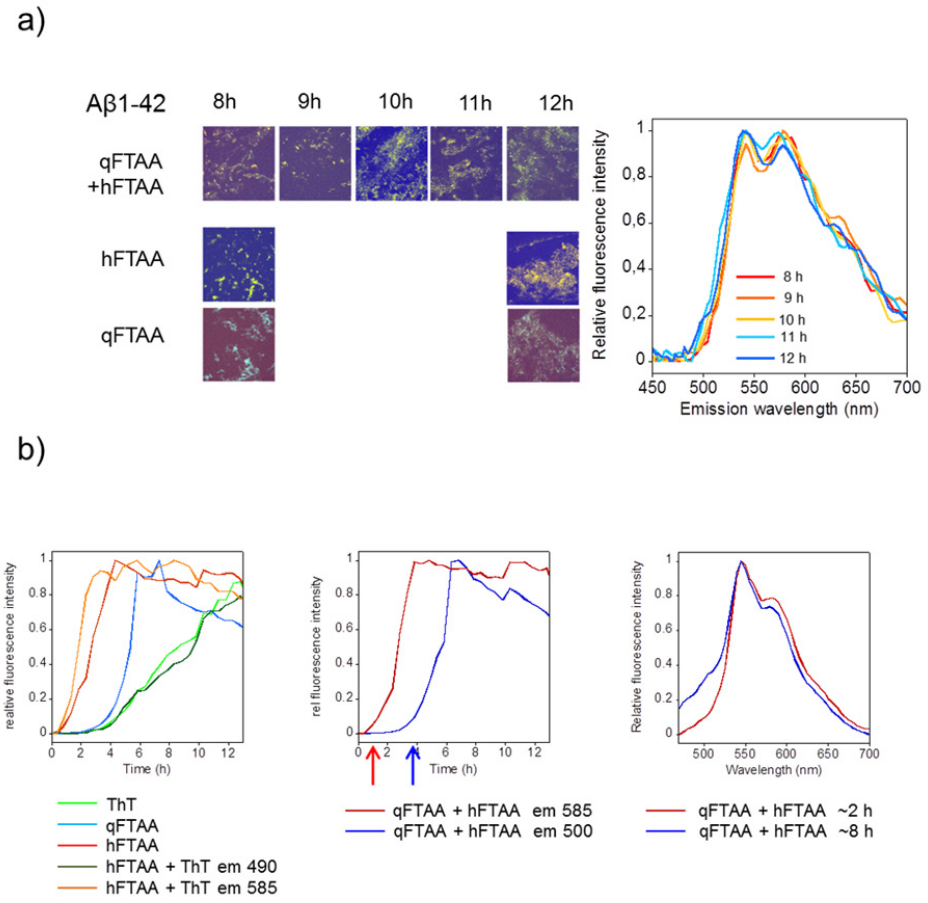
Suppl figure 2: Comparison of staining propensity of different probes Congo red, qFTAA+hFTAA co-staining, and 6E10 antibody staining of corresponding sections from mice of different ages.



Suppl figure 3: Micrographs of 18 months old APP/PS1 and APP23 mice using different probes**.** Serial sections of age matched APP/PS1 and APP23 mice were stained with ThT, qFTAA and hFTAA and respectively to demonstrate the variability in staining capability between qFTAA and hFTAA and the similarity in staining pattern between qFTAA and ThT. (a) APP/PS1 (b) APP23



Suppl figure 4: Comparison of staining propensity of different probes CN-PiB, qFTAA and hFTAA in 18 months old APP/PS1 mouse. The left panel shows co-localization of qFTAA and CN-PiB in the plaque core. The right panel shows co-localization of hFTAA and CN-PiB in the plaque core while the periphery of the plaque is dominated by hFTAA fluorescence.



Suppl figure 5: Fibrillation and staining of recombinant Aβ1-42 (a) In vitro formed fibrils of recAβ1-42 were collected at different timepoints and were stained with a mixture of qFTAA and hFTAA. The sedimented aggregates were placed on microscope glass and imaged using hyperspectral imaging. The ratio between peak intensities at 500 nm and 540 nm was plotted against time. The kinetics discloses an increase in qFTAA fluorescence over time but show weaker intensity compared to recAβ 1-40. (b) To deduce differentiated kinetic probing from qFTAA and hFTAA fluorescence, in situ kinetics using different combination of qFTAA, hFTAA and ThT was performed. The kinetic trajectories reveal that qFTAA and ThT respond to fibril formation at the same timepoint whereas hFTAA responds with a strong fluorescence signal in the qFTAA and ThT silent lag phase. The red arrow indicates the starting point for hFTAA fluorescence and the blue arrow indicates the starting point for qFTAA fluorescence. Note that for recAβ 1-42 a low qFTAA signal compared to hFTAA is also evident at time points where qFTAA fluorescence is prominent in recAβ 1-40 fibrillation kinetics.