Supporting information for

Rhodanine and thiohydantoin derivatives for detecting tau pathology in Alzheimer's brains

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Saturation assay with thioflavin-S using recombinant tau and $A\beta_{1-42}$ aggregates

The 441-aa isoform of human tau was expressed from a cDNA clone in *Escherichia coli* and purified as described previously(*1*). Tau aggregates were prepared by incubating tau protein (1 mg/mL in MES buffer, pH 6.8) at 37°C for 8 days with gentle and constant shaking in the presence of 0.1 mg/mL heparin(*2*). A solid form of $A\beta_{1-42}$ was purchased from Peptide Institute (Osaka, Japan). Aggregation was achieved by gently dissolving the peptide (0.25 mg/mL) in phosphate buffered-saline solution (pH 7.4). The solutions were incubated at 37 °C for 42 h with gentle and constant shaking. The binding experiments were carried out in Protein LoBind Tubes (Eppendorf). A mixture of tau aggregates (final conc., 0.2 μM) or $A\beta_{1-42}$ aggregates (final conc., 2.2 μM) were incubated at room temperature for 30 min in the presence of ThS (final conc., 0.2-15 μM), dispensed to MULTI WELL PLATE (0.4 mL × 96 wells flatbottom, SUMITOMO BAKELITE CO., LTD, Japan), and subjected to fluorescence spectroscopy (λ_{ex} = 440 nm; λ_{em} = 510 nm). The fluorescence intensity (λ_{ex} = 440 nm; λ_{em} = 510 nm) was plotted and K_d values of thioflavin-S for recombinant tau and $A\beta_{1-42}$ aggregates were calculated from saturation curves using GraphPad Prism software

(Graph Pad software, San Diego, CA) (Figure S1). The K_d value for tau and Aβ aggregates were 0.63 and 2.2 μM, respectively.

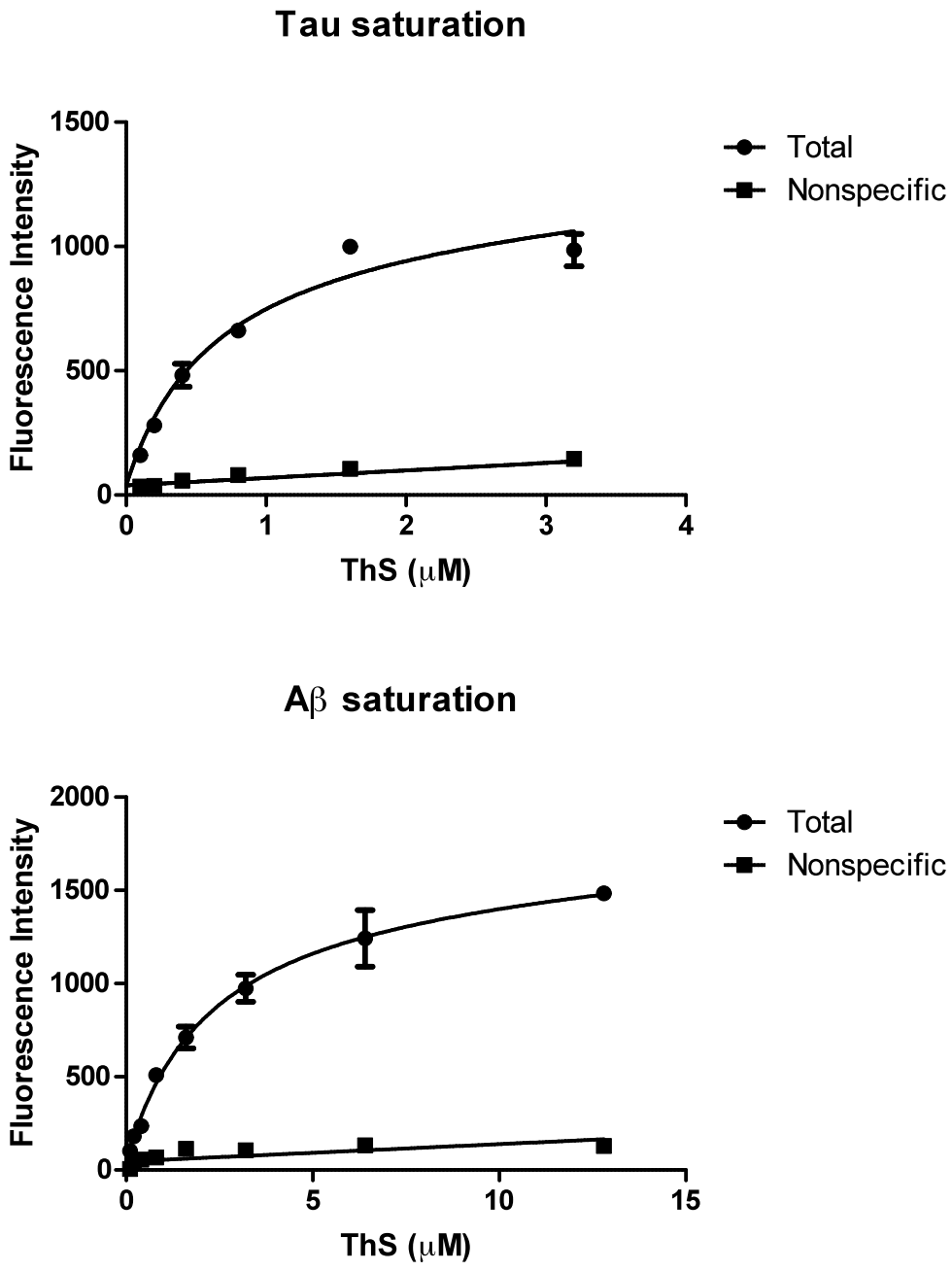

Figure S1. Binding of ThS to tau aggregates (upper panel) and Aβ aggregates (lower panel).

References

1. Han, D., Qureshi, H. Y., Lu, Y., and Paudel, H. K. (2009) Familial FTDP-17 missense mutations inhibit microtubule assembly-promoting activity of tau by increasing phosphorylation at Ser202 in vitro, *J Biol Chem 284*, 13422-13433.

2. Okamura, N., Suemoto, T., Furumoto, S., Suzuki, M., Shimadzu, H., Akatsu, H., Yamamoto, T., Fujiwara, H., Nemoto, M., Maruyama, M., Arai, H., Yanai, K., Sawada, T., and Kudo, Y. (2005) Quinoline and benzimidazole derivatives: candidate probes for *in vivo* imaging of tau pathology in Alzheimer's disease, *J Neurosci 25*, 10857-10862.