Supplementary Materials and Methods

Materials

Truncated 297-391 dGAE tau monomers (SPR-444) and pre-formed fibrils (SPR-461) were acquired from StressMarq Biosciences. The dGAE tau monomers served as the negative controls. Rabbit anti-dGAE antibody (SPC-806) was obtained from StressMarq Biosciences. Nitrocellulose membranes with pore sizes of 0.2 µm and 0.45 µm, as well as Polyvinylidene fluoride (PVDF) and cellulose acetate membranes with a 0.45 µm pore size, were obtained from GE Healthcare.

Filter Trap Assay and Western blotting

Tau protein samples were diluted in either 2 % (w/v) SDS, 1 % (w/v) sarkosyl, PBS pH 7.4 or assembly buffer in 0.6 ml or 1.5 ml Protein LoBind Tubes (Eppendorf). A volume of 50 µl per well was vacuum-filtered in duplicate using a pre-wet membrane in the indicated solutions in a 96-well Dot-Blot apparatus (Bio-Rad). In Figure 1A, proteins were loaded at a concentration of 2 µg/ml, using a total volume of 100 ng per 50 µl. For Figure 1B, protein concentrations ranged from 0.156 to 2 µg/ml, with amounts varying from 7.81 to 1000 ng per 50 µl. The membrane was washed twice with TBST (Tris-buffered saline with 0.1 % Tween-20) and blocked with 1 % skim milk in TBST for 1 hour. It was then incubated overnight at 4 °C with a rabbit anti-dGAE polyclonal antibody (1 : 2000) in 1 % skim milk in TBST. Following three TBST washes, the membrane was incubated with anti-rabbit IgG-HRP (1 : 5000) (Cell Signaling) for 1 hour at room temperature. After three additional TBST washes, detection was performed using ECL SuperSignal West Pico PLUS Chemiluminescent Substrate (Thermo Fisher Scientific), and the chemiluminescent signal was captured using ChemiDoc Touch (Bio-Rad). Signal intensity was quantified with Bio-Rad Image Lab 6.1 software.

SDS-PAGE and Coomassie Brilliant Blue staining

Tau protein samples (100 ng/lane) were diluted in Laemmli buffer containing either SDS or LDS with or without 100 mM dithiothreitol (DTT). Samples underwent processing in two distinct conditions: non-boiled, and boiled for 10 minutes. SDS-PAGE was performed using 4 – 20 % Mini-PROTEAN TGX Precast Protein Gels (Bio-Rad) in Tris-glycine-SDS running buffer (pH 8.3) at 150 V for 45 minutes. After electrophoresis, gels were stained with Coomassie Brilliant Blue using Imperial Protein Stain (Thermo Fisher Scientific) and imaged with ChemiDoc Touch (Bio-Rad) for documentation.

Native PAGE and Western blotting

Protein samples (100 ng/lane) including PBS, 2 % SDS or 1 % sarkosyl with 10 % (v/v) glycerol and 0.01 % (w/v) bromophenol blue, were subjected to electrophoresis using 4 – 20 % Mini-PROTEAN TGX Precast Protein Gels in Tris-glycine buffer at 150 V for 45 minutes. Following electrophoresis, proteins were transferred onto nitrocellulose membranes for an hour. Tau proteins were then detected using a rabbit anti-dGAE tau antibody according to the aforementioned Western blotting protocol.

Statistical Analysis

All quantitative data are expressed as mean ± SEM. A two-tailed, unpaired Student's *t*-test was used for statistical determinations with GraphPad Prism 10 software. Values of $p < 0.05$ are considered statistically significant (*$p < 0.05$, **$p < 0.01$ or ***$p < 0.001$).