# *In Vitro* Models to Study Tau Aggregation, Phosphorylation and Uptake

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## BACKGROUND

The microtubule associated protein tau plays a crucial role in the pathology of several neurodegenerative diseases, especially Alzheimer's. Thus, the development of new compounds capable of inhibiting or preventing tau aggregation, hyperphosphorylation or uptake is moving in the focus of Alzheimer's disease treatment. Consequently, reliable and in an optimal case complementary *in vitro* and *in vivo* models that mirror tau pathology in human diseases are needed.

## MATERIALS and METHODS

We established and optimized several *in vitro* methods to monitor tau pathology: (1) a cell-free Thioflavin-T aggregation assay using recombinant 2N4R tau with P301L mutation, (2) stably transfected tau overexpressing SH-SY5Y cell lines to monitor tau phosphorylation, as well as (3) tau uptake assays using stably transfected tau overexpressing SH-SY5Y cell lines in combination with different tau seeds. Several positive controls like methylene blue, tau targeting antibodies or kinase inhibitors were tested with those assays to further validate them as drug screening tools.

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## SUMMARY and CONCLUSION

*In vitro* methods to screen for the activity of compounds are of high relevance for the early stages of drug development. The here presented assays were developed as complementary tools to our transgenic and induced *in vivo* tauopathy models TMHT and hTau as well as seed or virus injection, respectively.
Our results show the potency of several known tau interacting compounds within our models.

## RESULTS

### Cell-free aggregation

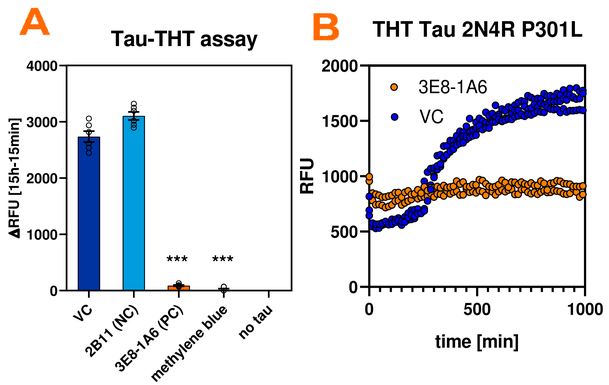


**Figure 1: Cell free THT-aggregation assay using recombinant 2N4R tau with P301L mutation. (A)** Results of aggregation assay shown as ΔRFU values. The antibodies 2B11 (negative control NC, specific for phoyphorylated tau), 3E8-1A6 (positive control PC, targeting microtubule binding domain) as well as methylene blue as inhibitor of aggregation were assessed. One-Way ANOVA with Dunnett's multiple comparisons test vs. vehicle control (VC). Mean ± SEM; n = 6. ***p<0.001. (B) monitoring tau aggregation over time using the THT assay; blue = vehicle control (VC), orange = antibody 3E8-1A6 as aggregation inhibitor.

## RESULTS

### Tau uptake and seeding

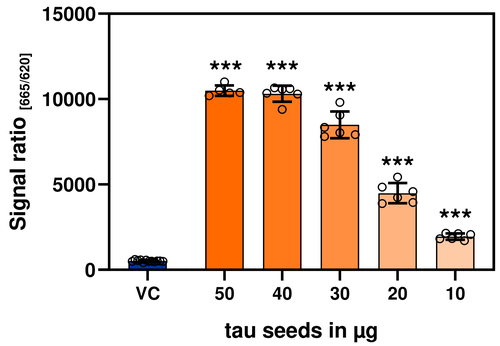


**Figure 2: Tau uptake in retinoic acid (RA) differentiated, stably transfected SH-SY5Y cell line overexpressing 2N4R tau with P301L mutation.** After differentiation with 10 µM RA for 5 days, cells were incubated for 48 h with different amounts of tau seeds (sarcosyl extracted from human AD brain according to Julian et al., 2012). Tau aggregation was assessed using HTRF based *Tau Aggregation Kit* from Cisbio. Vehicle treated cells serve as control. One-Way ANOVA with Dunnett's multiple comparisons test vs. vehicle control (VC). Mean ± SEM; n = 6. ***p<0.001.

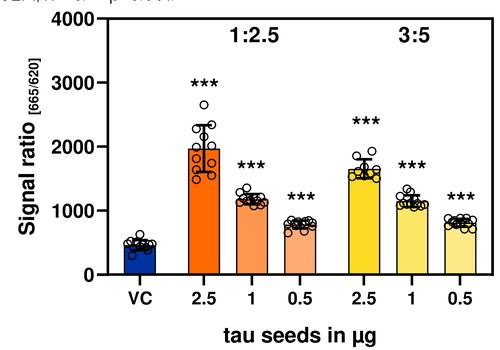


**Figure 3: Tau seeding in differentiated, stably transfected SH-SY5Y cell line overexpressing 2N4R tau with P301L mutation.** After differentiation with 10 µM RA for 5 days, cells were incubated for 48 h with different amounts of tau seeds co-incubated with lipofectamin at different ratios. Cells treated with lipofectamin without AD seeds serve as control. Tau aggregation was assessed using HTRF based *Tau Aggregation Kit* from Cisbio. One-Way ANOVA with Dunnett's multiple comparisons test vs. vehicle control (VC). Mean ± SEM; n = 6. ***p<0.001.

## RESULTS

### Tau phosphorylation

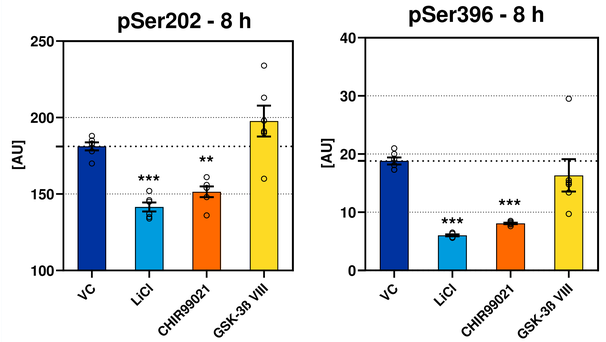


**Figure 4: Tau phosphorylation in differentiated stably transfected SH-SY5Y cell line overexpressing 2N4R tau with V337M/R406W mutations after 8 h of incubation with compounds.** Tau phosphorylated at Ser202 as well as Ser396 was assessed with MSD immunosorbent assay in RIPA extracts of the cells, harvested 8 h after treatment with 20 mM LiCl, 1 µM CHIR99021 and 1 µM GSK-3β VIII. One-Way ANOVA with Dunnett's multiple comparisons test vs. vehicle control (VC). Mean ± SEM; n = 6. **p<0.01; ***p<0.001.

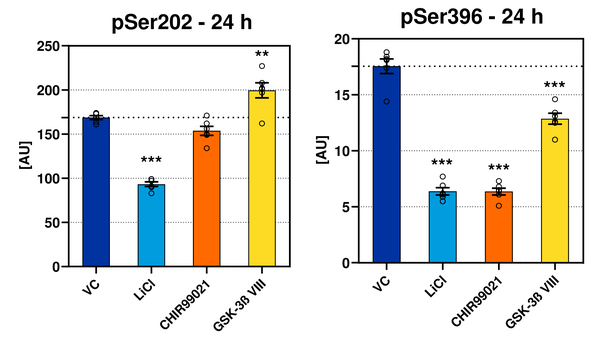


**Figure 5: Tau phosphorylation in differentiated stably transfected SH-SY5Y cell line overexpressing 2N4R tau with V337M/R406W mutations after 24 h of incubation with compounds.** Tau phosphorylated at Ser202 as well as Ser396 was assessed with MSD immunosorbent assay in RIPA extracts of the cells, harvested 24 h after treatment with 20 mM LiCl, 1 µM CHIR99021 and 1 µM GSK-3β VIII. One-Way ANOVA with Dunnett's multiple comparisons test vs. vehicle control (VC). Mean ± SEM; n = 6. **p<0.01; ***p<0.001.