Protein semi-synthetic approach to probe C-terminal hyper-phosphorylation of the Alzheimer-relevant protein Tau

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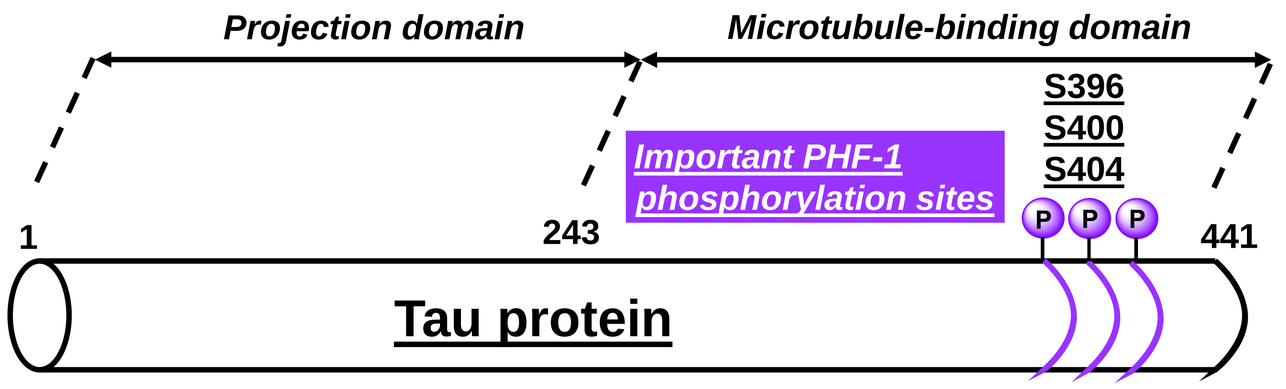
Background

- Microtubule-associated Tau plays an important role in Alzheimer's disease (AD)[1].
- PTMs play a major role in the regulation of tubulin polymerization and/or stabilization of microtubule assembly[2-4].
- The PHF-1 epitope (Ser396, Ser400, and Ser404) of Tau comprising three phosphorylation sites is believed to be essential in the progression of AD[5].

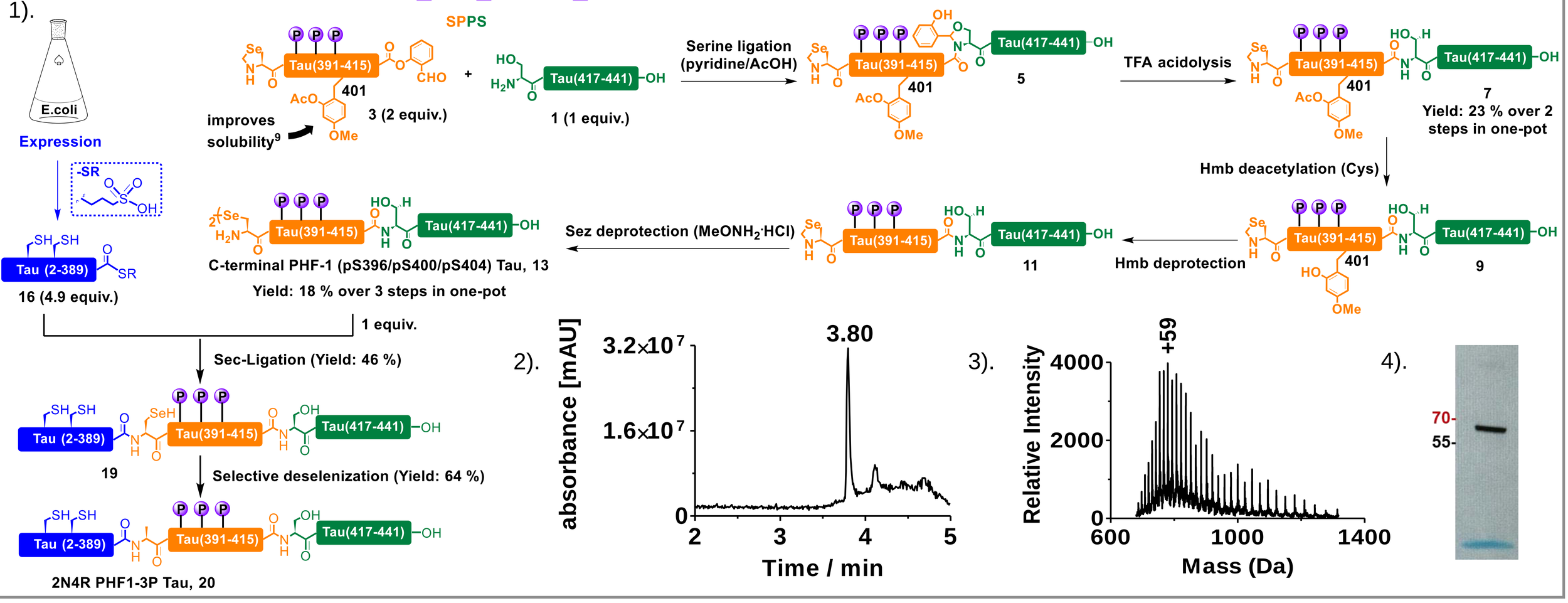


Aim: To obtain homogeneous PTM-patterns in C-terminus of Tau by Expressed Protein Ligation → **EPL strategy[6-8]**

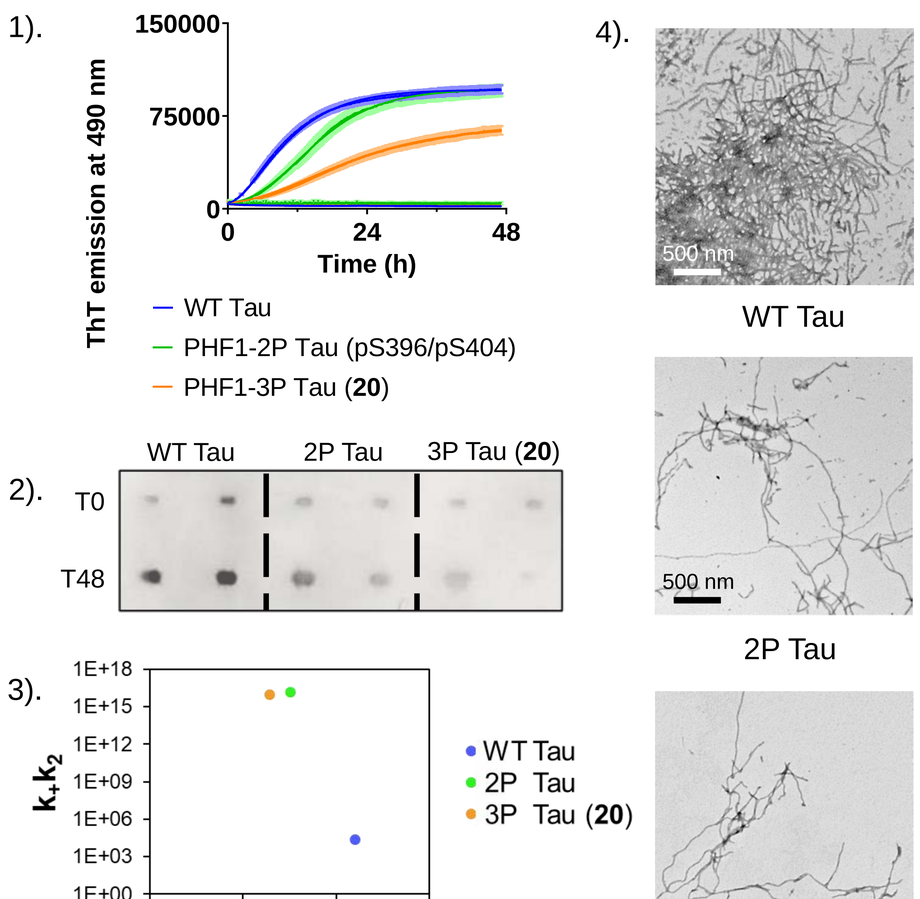
Semi-synthetic strategy to prepare homogeneous site-specific 2N4R PHF1-3P Tau protein

Concerns: 1. Impossible to synthesize **multiple-phosphorylated** C-terminal Tau peptides (390-441) *via* a single SPPS run.
2. Low ligation efficiency of NCL in preparation of C-terminal Tau peptides (390-441).

C-terminal Tau: A^{390}EIVYK**S**PVV**S** G^{401}DT**S**PRHLSN VSSTGSIDMV DSPQLATLAD EVSASLAKQGL^{441}-OH

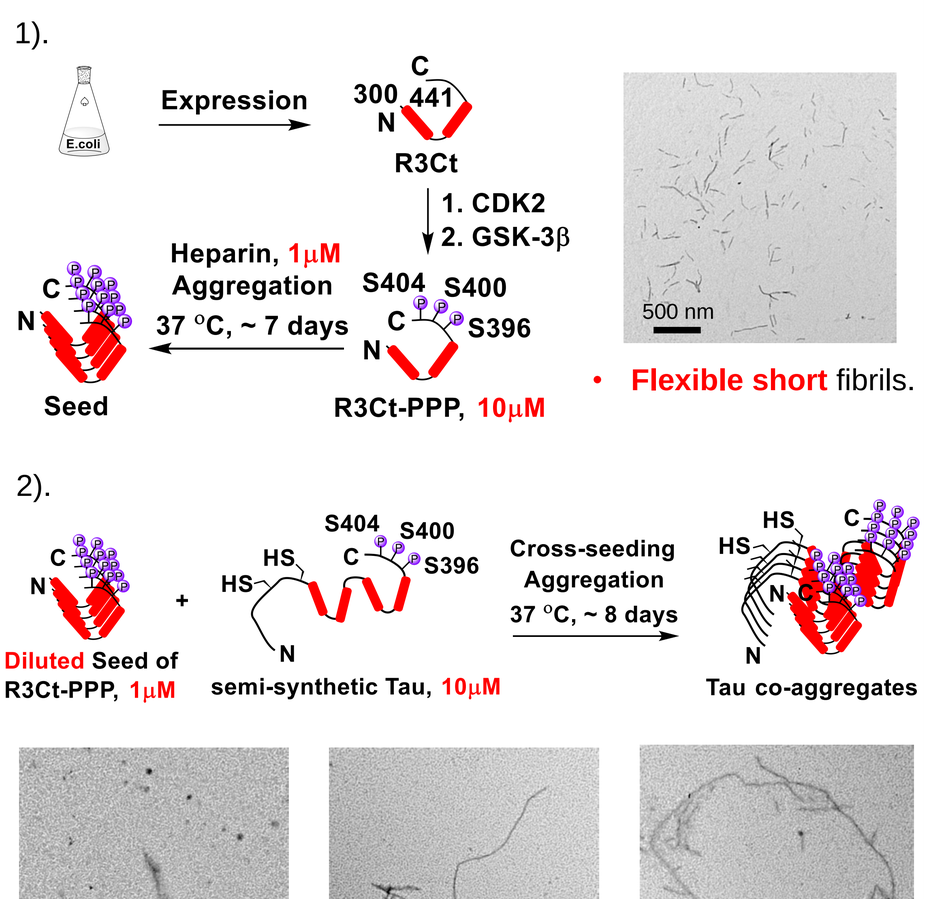


Heparin-induced *in vitro* Tau aggregation assay



- 10 μM Tau: 2.5 μM Heparin.
- PHF1-3P Tau (**20**, orange) exhibits a **slowest** onset of aggregation.
- PHF1-3P Tau (**20**) involves both **primary** (k_n) and **secondary** nucleation pathways (k_2).

Tau seed-induced *in vitro* Tau cross-seeding aggregation assay



- **Flexible short** fibrils.
- **Clusters** of Tau co-aggregates in R3Ct-PPP with 3P Tau (**20**).

Conclusion and future work

- **Combination of Sec- and Ser-Ligation** in preparation of homogeneous site-specific 2N4R PHF1-3P Tau.
- PHF1-3P Tau **inhibits** heparin induced aggregation.
- PHF1-3P Tau **facilitates** the development of elongated PHF-like fibrillar aggregates in cross-seeding aggregation.
- ❑ **cryo-EM** analysis of the generated Tau filaments.
- ❑ **Liquid-liquid phase separation (LLPS)** of semi-synthetic Tau proteins.
- ❑ *ex-vivo* Tau filaments **delivery** assay on neuronal cell line.
- ❑ **Seeding** of Tau aggregation in biosensor cells (HEK293T).

@PhosphorusFive

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