# SUPPLEMENTAL MATERIALS

## Native Top-Down Mass Spectrometry and Ion Mobility Spectrometry of the Interaction of Tau Protein with a Molecular Tweezer Assembly Modulator

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**Tau expression and purification.** The 2N4R tau isoform (residues 1-441 of human MAPT) and the fragment tau(192–324) were produced in the *E. coli* BL21(DE3) strain carrying a pET15b recombinant plasmid (Novagen). The corresponding genes were inserted into NcoI/XhoI cloning sites. The induction phase was performed by addition of 0.5 mM isopropyl β-D-1-thiogalactopyranoside for 3 h at 37 °C. Cells were harvested by centrifugation at 5,000 × *g* for 30 min and the pellet was resuspended in 50 mM $NaH_2PO_4/Na_2HPO_4$, pH 6.2, 2.5 mM EDTA, 2 mM DTT and 0.5% Triton X-100 supplemented with a Complete™ protease-inhibitor cocktail (Roche). The lysate was obtained by homogenizing this suspension using a high-pressure homogenizer followed by centrifugation at 30,000 × *g* for 30 min. The extract was incubated at 75 °C for 15 min to precipitate most of the bacterial proteins from the soluble extract as a first purification step. The soluble proteins were separated from the precipitate by centrifugation at 4,000 × *g* for 20 min and the desired protein was purified by cation-exchange chromatography (HiTrap SP HP 1 ml, GE Healthcare). Elution fractions were analyzed by SDS-PAGE and fractions containing the pure protein were pooled together for buffer-exchange in 50 mM ammonium bicarbonate (HiPrep 26/10 desalting, GE Healthcare) prior to lyophilization. Lyophilized proteins were stored at -20 °C until further use.

***In-vitro*** **phosphorylation.** Recombinant ERK2 and MEK3-R4F kinases were produced as described previously [1]. Tau(192–324) was dissolved at 100 µM with 1 µM of ERK2 and ~0.1 µM of MEK3 in 400 µl of buffer containing 50 mM HEPES-KOH, pH 8.0, 12.5 mM $MgCl_2$, 50 mM NaCl, 2 mM EDTA, 1 mM DTT, 1 mM EGTA, and 12.5 mM ATP. The mixture was incubated at 37 °C overnight. Enzymatic reaction was stopped by incubating the reaction mixture at 75 °C for 15 min followed by centrifugation at 16,000 × *g* for 20 min. Then, the supernate was buffer-exchanged in 50 mM ammonium bicarbonate and lyophilized. Prior to further analyses, phosphorylation was qualitatively checked by a shift in the apparent mobility of the protein band on SDS-PAGE.

1. Qi, H., Despres, C., Prabakaran, S., Cantrelle, F. X., Chambraud, B., Gunawardena, J., Lippens, G., Smet-Nocca, C., Landrieu, I.: The Study of Posttranslational Modifications of Tau Protein by Nuclear Magnetic Resonance Spectroscopy: Phosphorylation of Tau Protein by ERK2 Recombinant Kinase and Rat Brain Extract, and Acetylation by Recombinant Creb-Binding Protein. Methods Mol. Biol. **1523**, 179-213 (2017)

## Tau isoforms analyzed and their amino acid sequences

**4-Repeat-tau (2N4R):** (441 aa) 45,900 Da

MAEPRQEFEV MEDHAGTYGL GDRKDQGGYT MHQDQEGDTD AGLKESPLQT PTEDGSEEPG SETSDAKSTP TAEDVTAPLV
DEGAPGKQAA AQPHTEIPEG TTAEEAGIGD TPSLEDEAAG HVTQARMVSK SKDGTGSDDK KAKGADGKTK IATPRGAAPP
GQKGQANATR IPAKTPPAPK TPPSSGEPPK SGDRSGYSSP GSPGTPGSRS RTPSLPTPPT REPKKVAVVR TPPKSPSSAK
SRLQTAPVPM PDLKNVKSKI GSTENLKHQP GGGKVQIINK KLDLSNVQSK CGSKDNIKHV PGGGSVQIVY KPVDLSKVTS
KCGSLGNIHH KPGGGQVEVK SEKLDFKDRV QSKIGSLDNI THVPGGGNKK IETHKLTFRE NAKAKTDHGA EIVYKSPVVS
GDTSPRHLSN VSSTGSIDMV DSPQLATLAD EVSASLAKQG L

**3-Repeat-tau (0N3R)/PHP-Tau (0N3R):** (352 aa) 36,800 Da

MAEPRQEFEV MEDHAGTYGL GDRKDQGGYT MHQDQEGDTD AGLKAEEAGI GDTPSLEDEA AGHVTQARMV SKSKDGTGSD
DKKAKGADGK TKIATPRGAA PPGQKGQANA TRIPAKTPPA PKTPPSSGEP PKSGDRSGYS SPGSPGTPGS RSRTPSLPTP
PTREPKKVAV VRTPPKSPSS AKSRLQTAPV PMPDLKNVKS KIGSTENLKH QPGGGKVQIV YKPVDLSKVT SKCGSLGNIH
HKPGGGQVEV KSEKLDFKDR VQSKIGSLDN ITHVPGGGNK KIETHKLTFR ENAKAKTDHG AEIVYKSPVV SGDTSPRHLS
NVSSTGSIDM VDSPQLATLA DEVSASLAKQ GL

**4-Repeat tau fragment F3:** (257-360) 10,880 Da

ASKIGSTENL KHQPGGGKVQ IINKLDLSNV QSKCGSKDNI KHVPGGGSVQ IVYKPVDLSK VTSKCGSLGN IHHKPGGGQV
EVKSEKLDFK DRVQSKIGSL DNI

**tau fragment F8 with C291A mutation:** (192-324) 13,800 Da

GDRSGYSSPG SPGTPGSRSR TPSLPTPPTR EPKKVAVVRT PPKSPSSAKS RLQTAPVPMP DLKNVKSKIG STENLKHQPG
GGKVQIINKK LDLSNVQSKA GSKDNIKHVP GGGSVQIVYK PVDLSKVTSK CGS

| | | | |
|---|---|---|---|
| phospho-tau-F8 frag | 192-324 | ERK2 | Phosphorylation sites S199, S202, T205, T217, T231, S235 |

**Figure S1.** Sequences of tau proteins measured for this study.

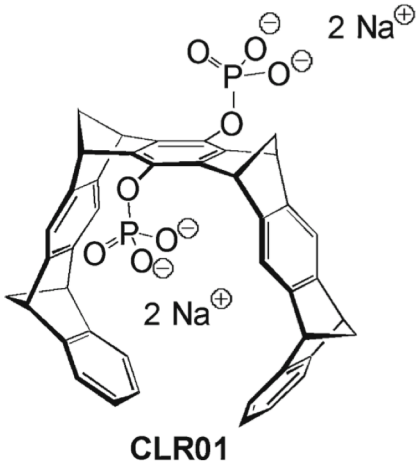


**Figure S2.** Structure of CLR01 tweezer compound.


**Figure S3.** ECD-MS fragmentation of 17+ charge state of 4R-repeat domain C291A tau fragment.

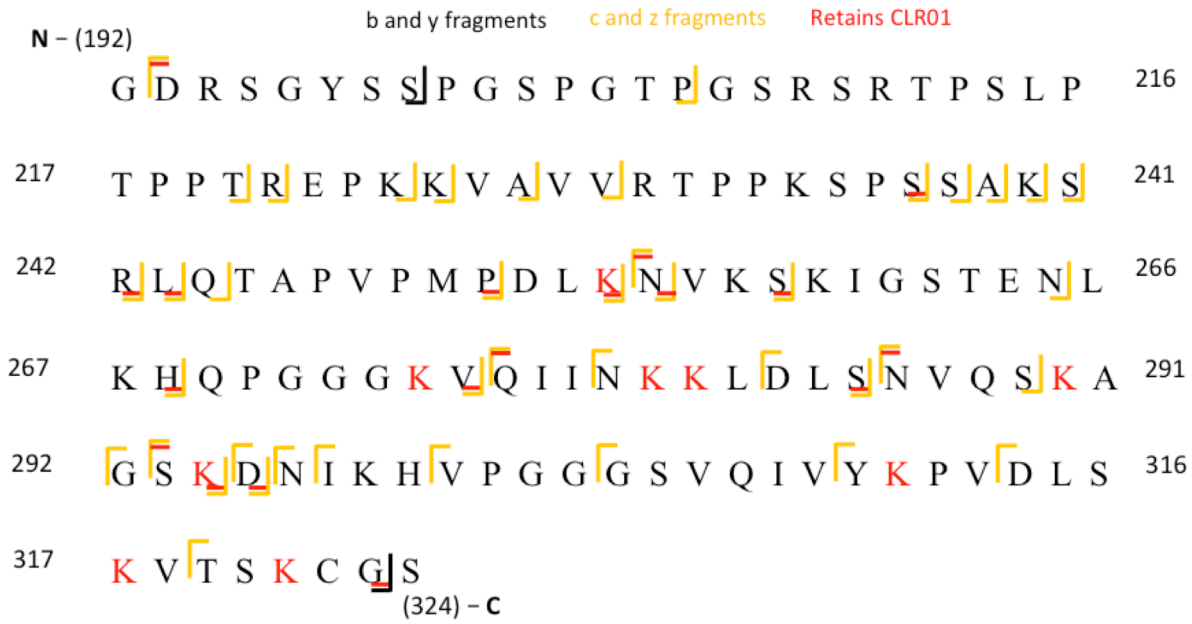


**Figure S4.** ECD-MS fragmentation of 17+ charge state of 4R-repeat domain C291A tau fragment bound to CLR01 (1:1 complex). Red lines indicate CLR01-bound fragment ions.


**Figure S5.** ECD-MS of 4R-repeat domain phosphorylated 4R-tau fragment C291A. (Red lines indicate phosphorylated fragment ions).

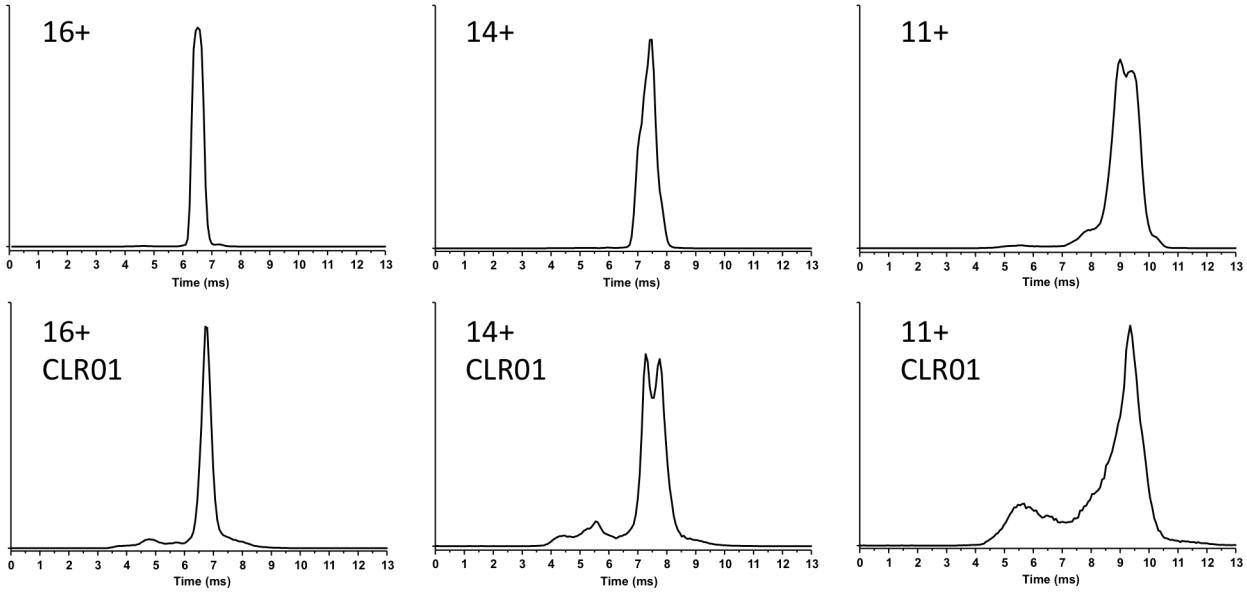


**Figure S6.** IM-MS of 16+, 14+, and 11+ charge state of 4R-tau C291A fragment with and without CLR01 bound (1:1 complex).