SUPPORTING INFORMATION

A structural ensemble of a tau-microtubule complex reveals regulatory tau phosphorylation and acetylation mechanisms

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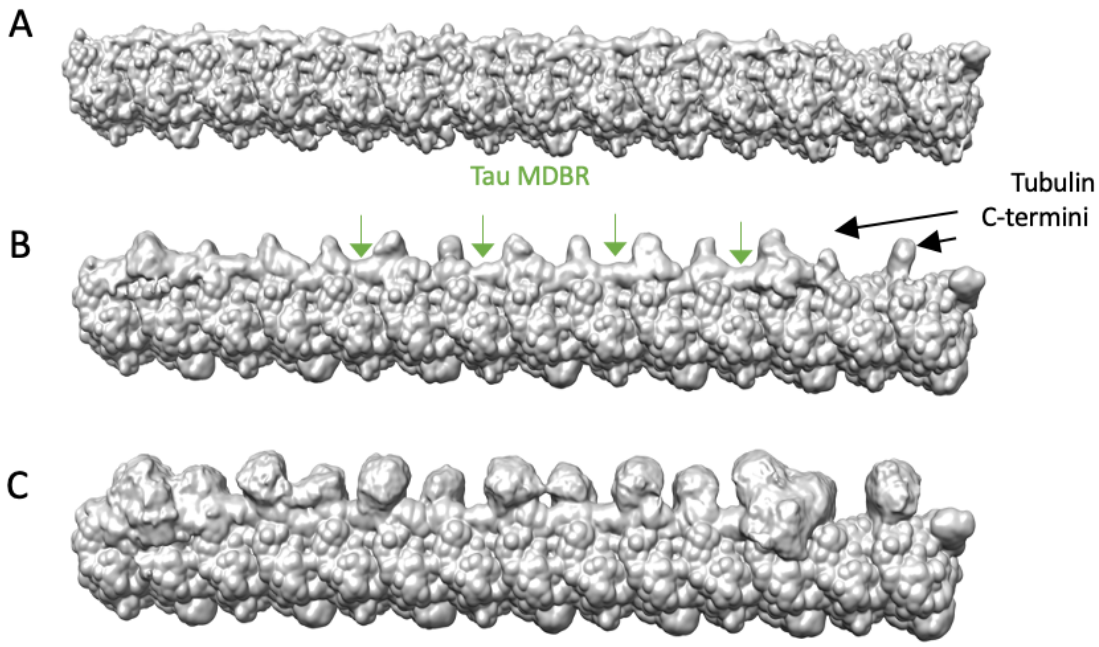


Figure S1. Back-calculated electron density maps from the EMMI ensemble of the tau-microtubule complex as a function of decreasing electron density thresholds: (A) threshold 1, (B) threshold 0.5, and (C) threshold 0.05.

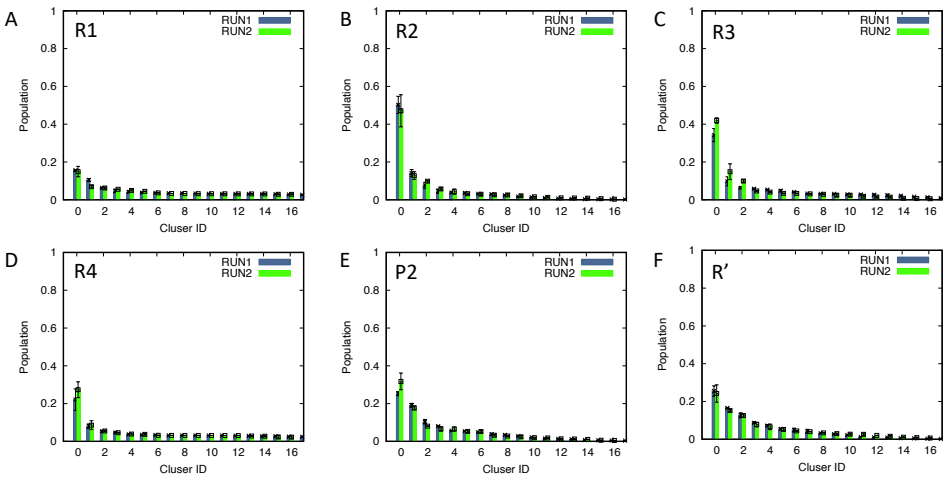


Figure S2. Identification of the ground (0) and excited states (1-16) of the R1 (A), R2 (B), R3 (C), R4 (D), P2 (E) and R' (F) regions of tau in the EMMI structural ensemble. A comparison of two different simulations (run1 and run2) demonstrates the convergence of the populations of the different states.

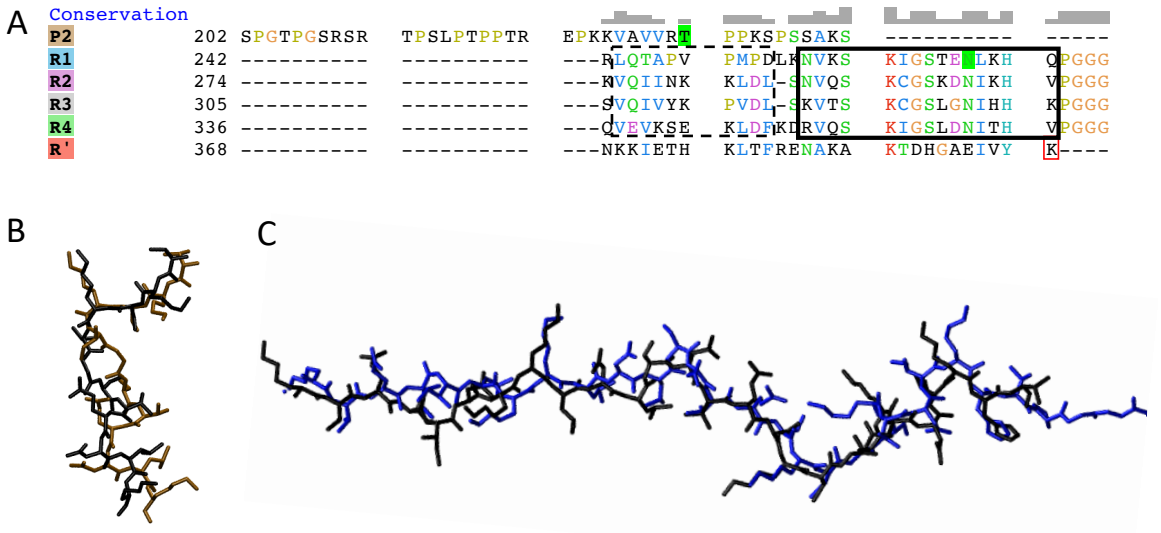
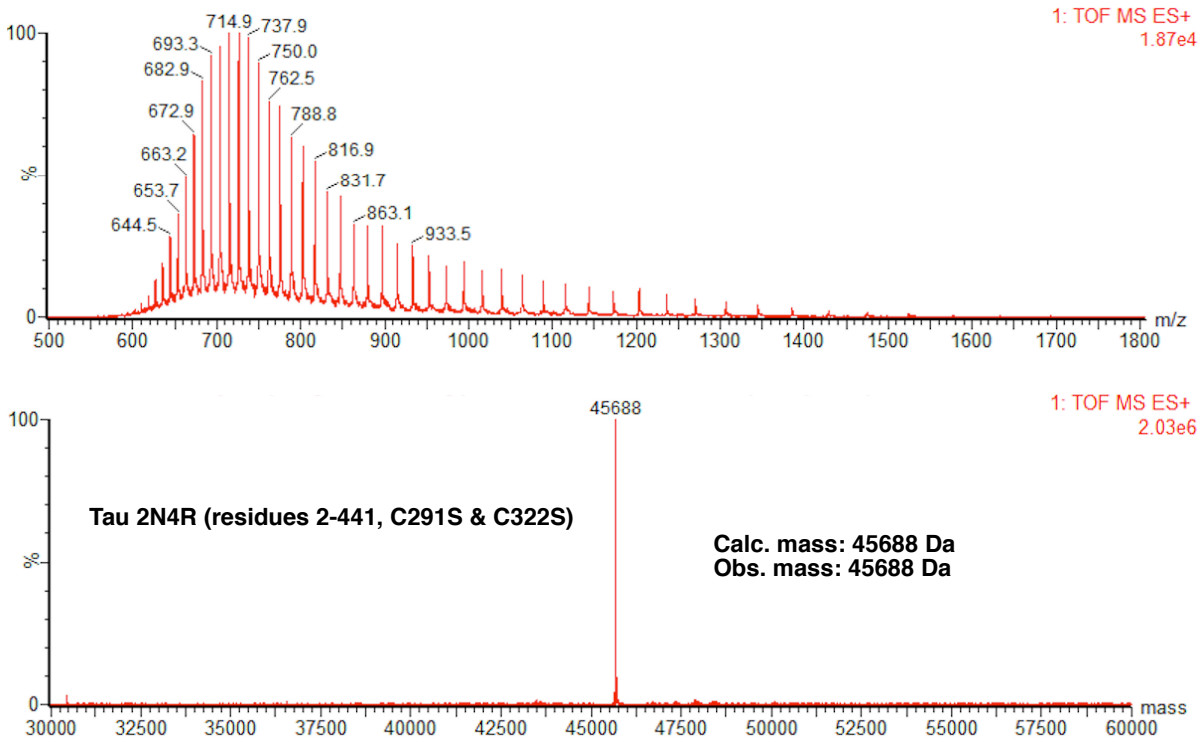


Figure S3. (A) Sequence alignment of the tau regions P2, R1, R2, R3, R4 and R'. Weak and strong interacting regions of tau with microtubules are shown with dashed and solid boxes, respectively. **(B)** Comparison between the structures of the R1 region obtained in this work (in brown) and previously reported in (PDB: 6CVJ)[6] (black). **(C)** Comparison of the structures of the R2 region obtained in this work (in blue) and previously reported in (PDB :6CVN)[6] (black).

A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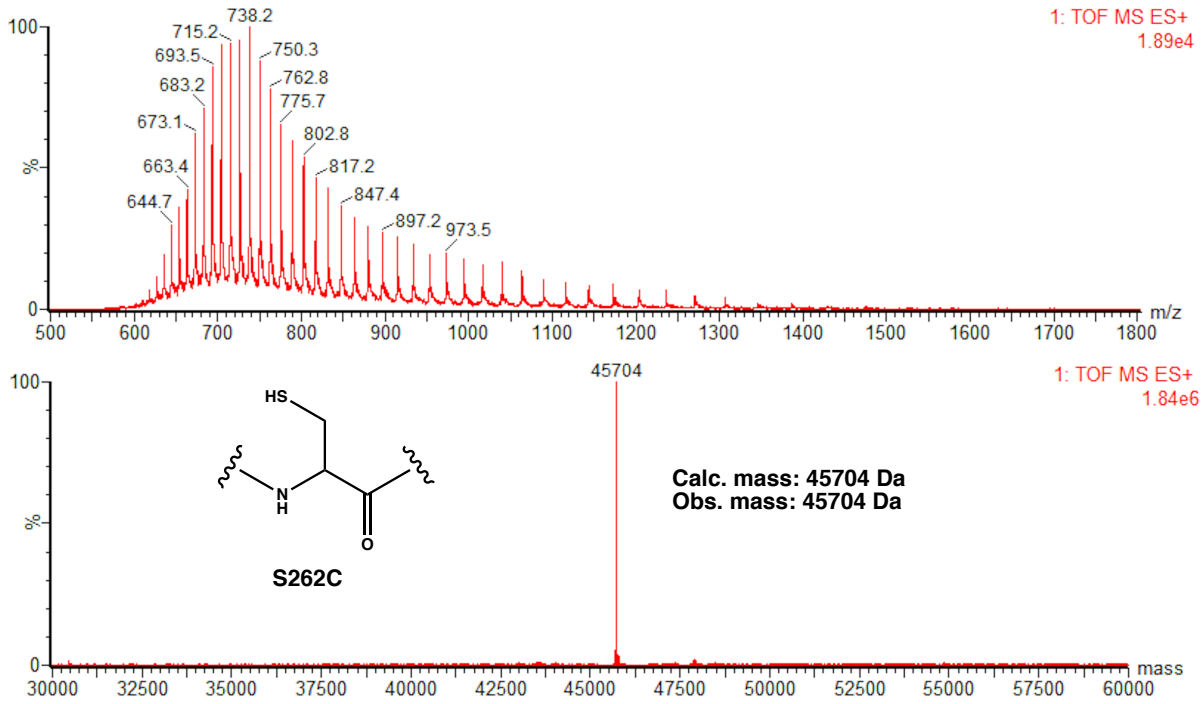
714.9
737.9
693.3
750.0
682.9
762.5
672.9
788.8
663.2
816.9
653.7
831.7
644.5
863.1
933.5
m/z
1: TOF MS ES+
2.03e6
45688
Tau 2N4R (residues 2-441, C291S & C322S)
Calc. mass: 45688 Da
Obs. mass: 45688 Da
mass

B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715.2
693.5
750.3
683.2
762.8
775.7
673.1
802.8
663.4
817.2
644.7
847.4
897.2
973.5
m/z
1: TOF MS ES+
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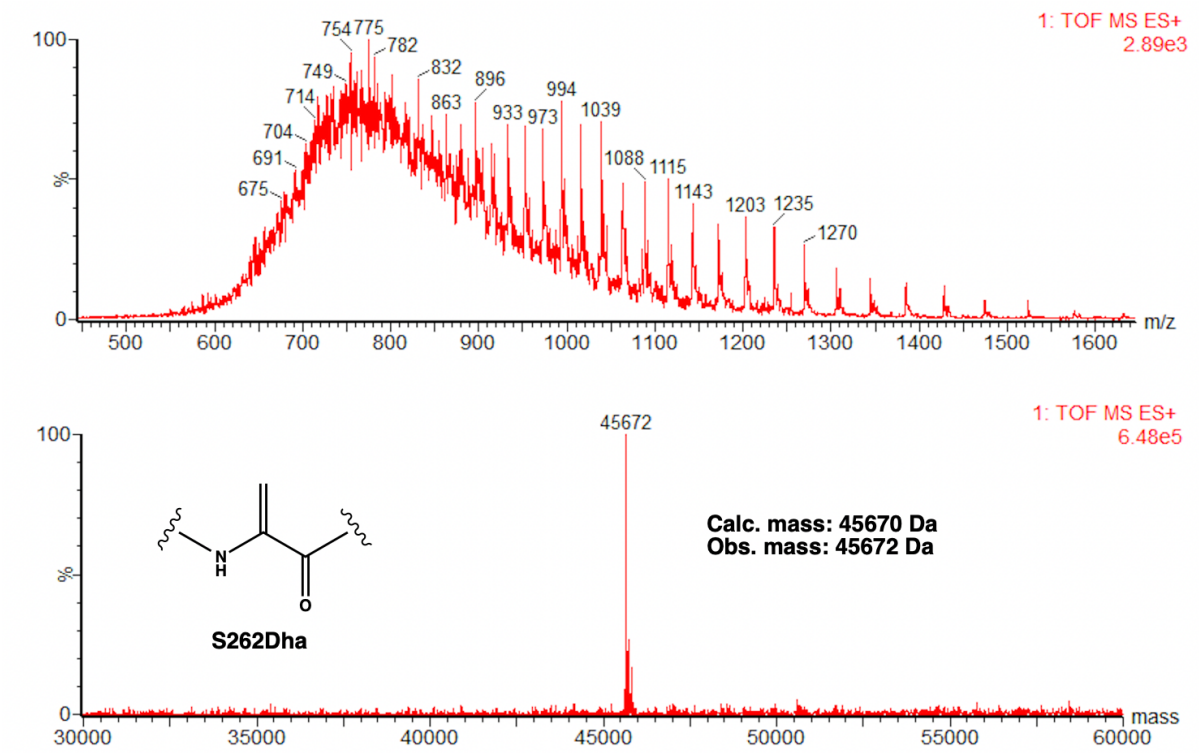
45704
HS
N
H
O
S262C
Calc. mass: 45704 Da
Obs. mass: 45704 Da
mass

C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749 714 704 691 675 863 933 973
m/z
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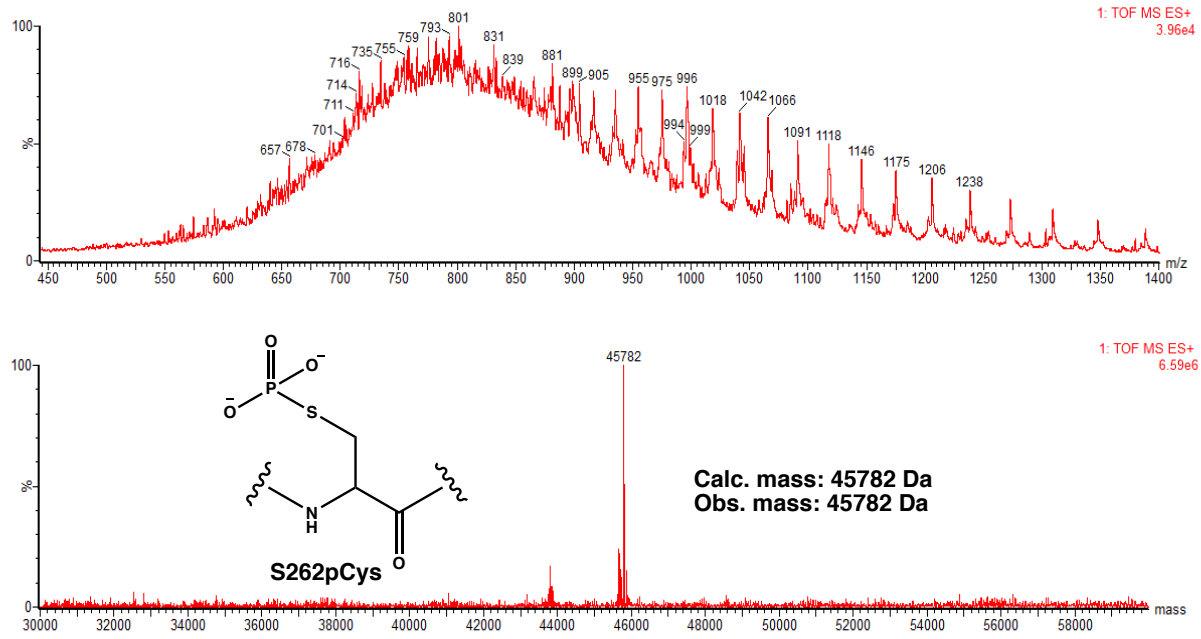
6.48e5
45672
Calc. mass: 45670 Da
Obs. mass: 45672 Da
S262Dha
mass

D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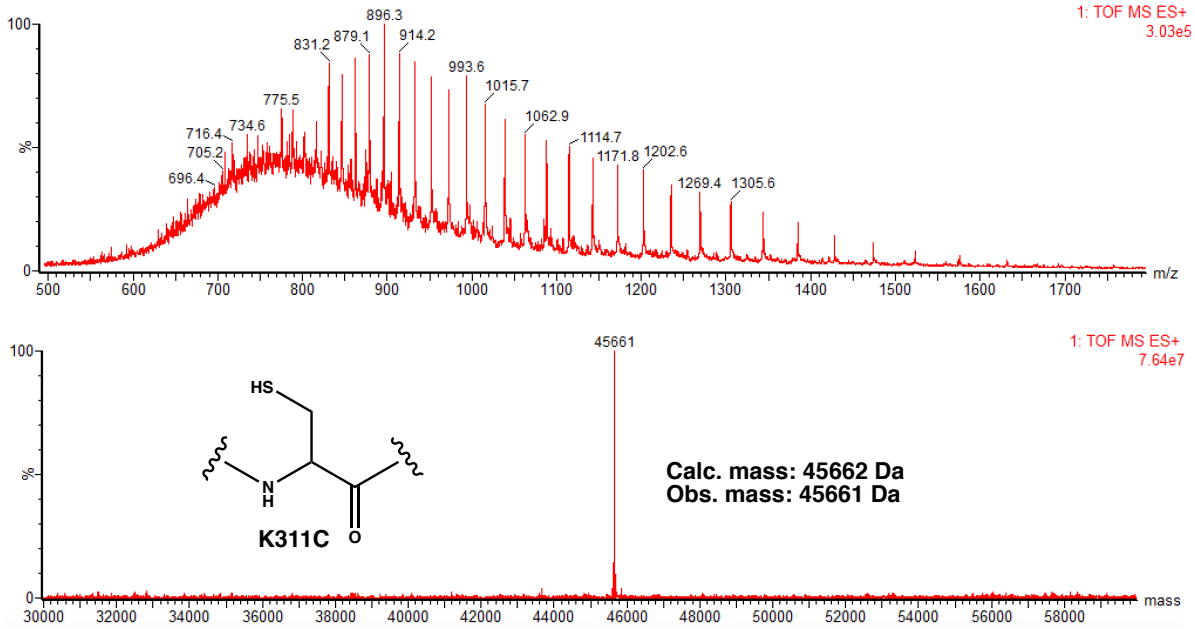
m/z
1: TOF MS ES+
6.59e6
45782
Calc. mass: 45782 Da
Obs. mass: 45782 Da
S262pCys
mass

E

Figure S4. LC-MS spectra of tau and its phosphorylated chemical mutant at S262 and its intermediates. **(A)** LC-MS spectrum of 2N4R tau (residues 2-441, C291S & C322S), all tau variants are residues 2-441. **(B)** LC-MS spectrum of tau(S262C). **(C)** LC-MS spectrum of tau(S262Dha). **(D)** LC-MS spectrum of tau(S262pC). We note that differences of 1 or 2 Daltons between observed and calculated masses rather commonly arise from errors from the deconvolution of the spectra or from slight changes in protonation states.

A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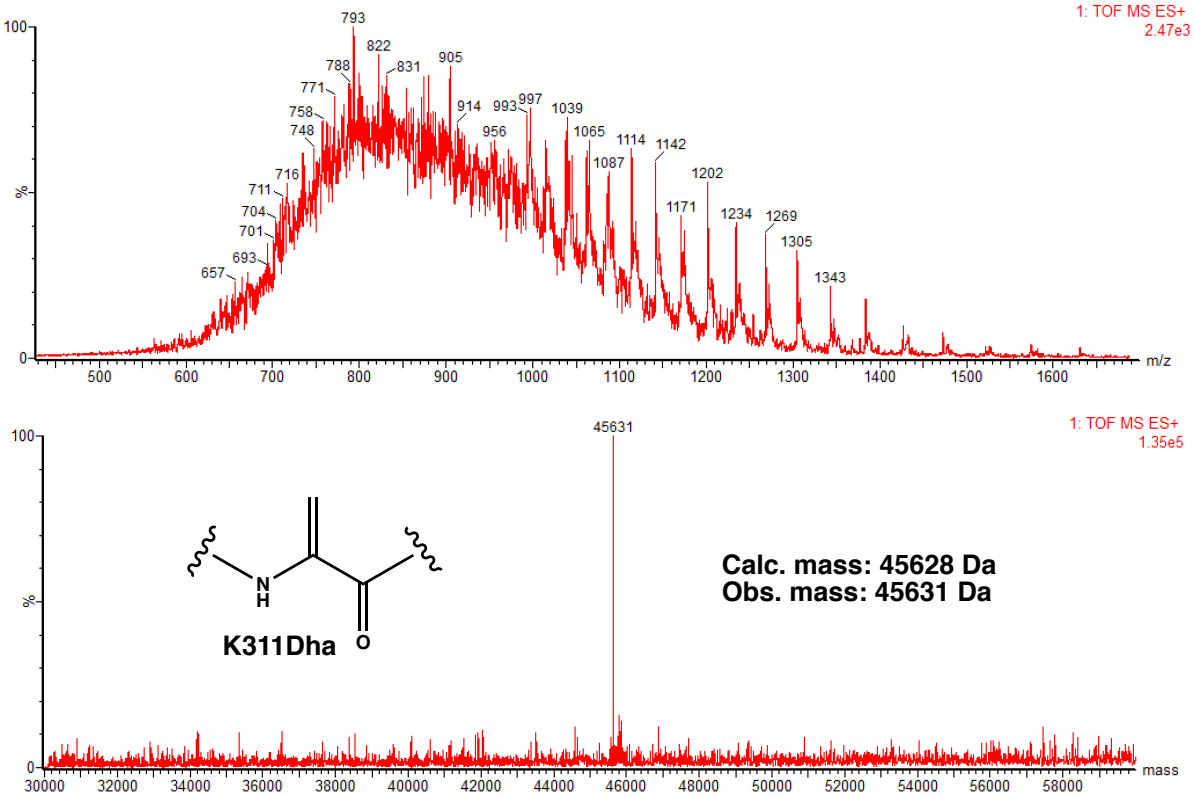
3.03e5
896.3
831.2
879.1
914.2
993.6
1015.7
775.5
1062.9
716.4
734.6
1114.7
705.2
1171.8
1202.6
696.4
1269.4
1305.6
m/z
1: TOF MS ES+
7.64e7
45661
HS
N
H
K311C
O
Calc. mass: 45662 Da
Obs. mass: 45661 Da
mass

B


1: TOF MS ES+
2.47e3
793
822
788
831
905
771
758
914
993
997
748
956
1039
1065
1114
1142
711
716
1087
1202
704
1171
1234
1269
701
1305
693
657
1343
m/z
1: TOF MS ES+
1.35e5
45631
N
H
K311Dha
O
Calc. mass: 45628 Da
Obs. mass: 45631 Da
mass

C

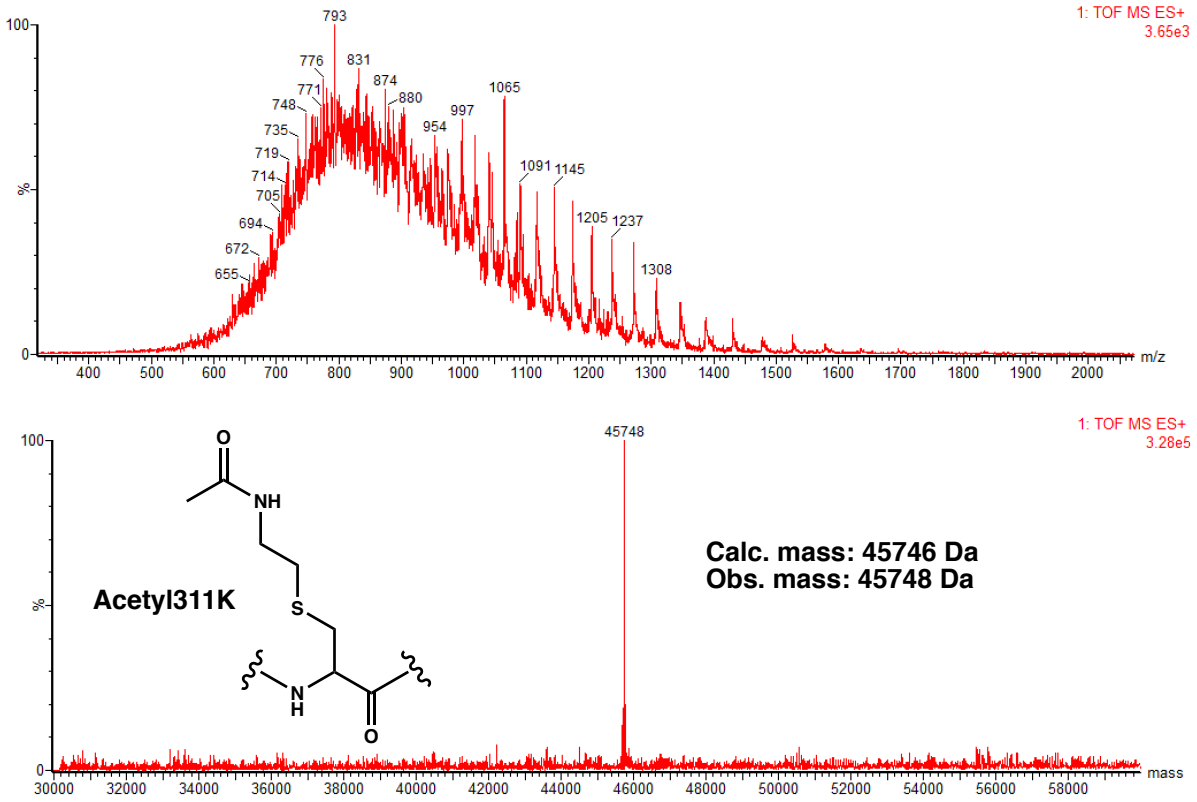
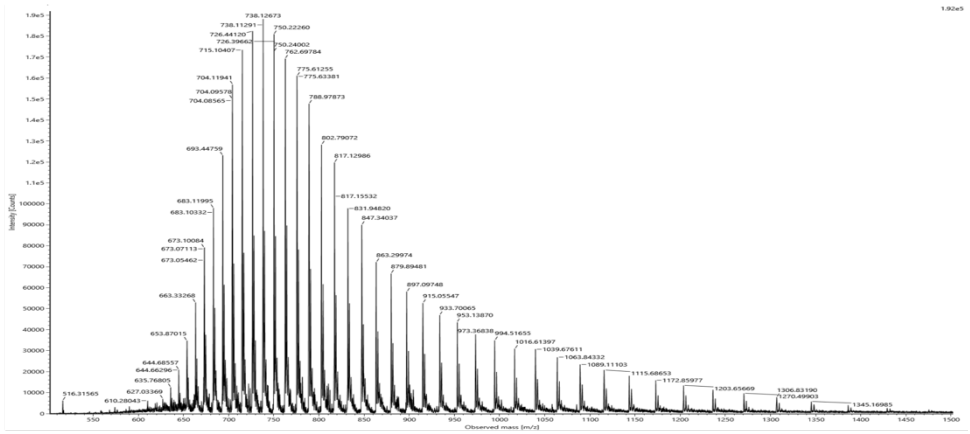
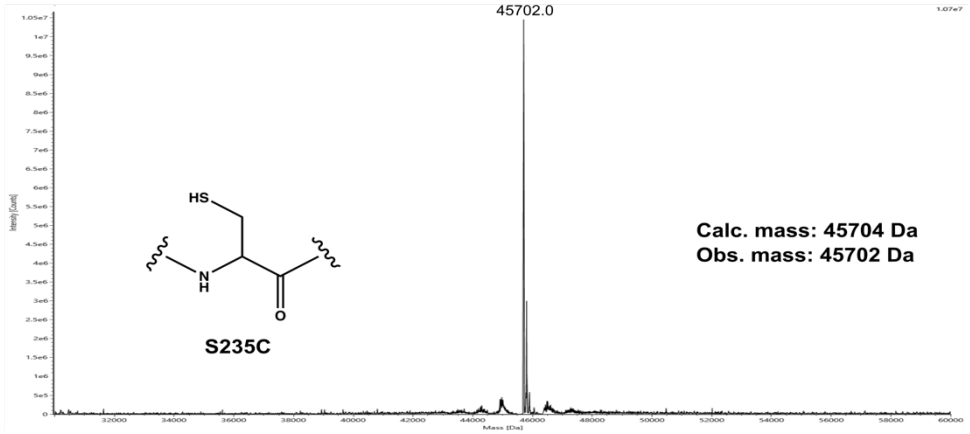


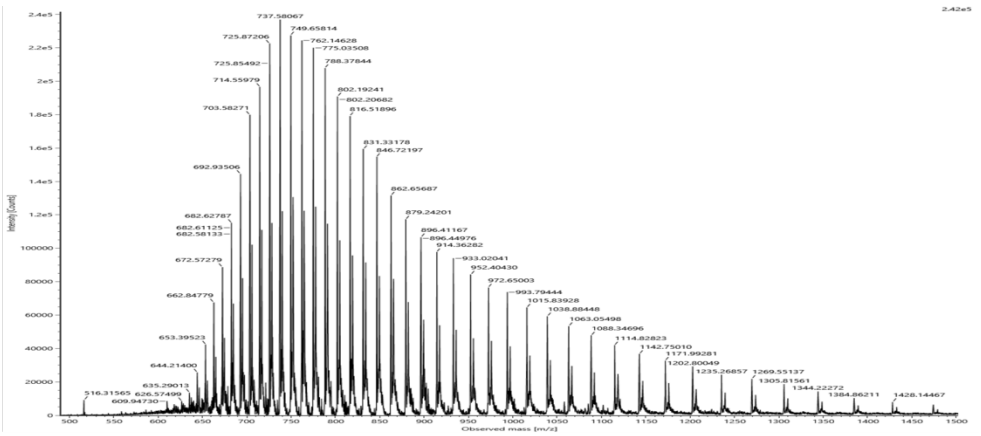
Figure S5. LC-MS spectra of the acetylated K311 (aK311) tau chemical mutant and its intermediates. **(A)** LC-MS spectrum of tau(K311C). **(B)** LC-MS spectrum of tau(K311Dha). **(C)** LC-MS spectrum of tau(aK311).

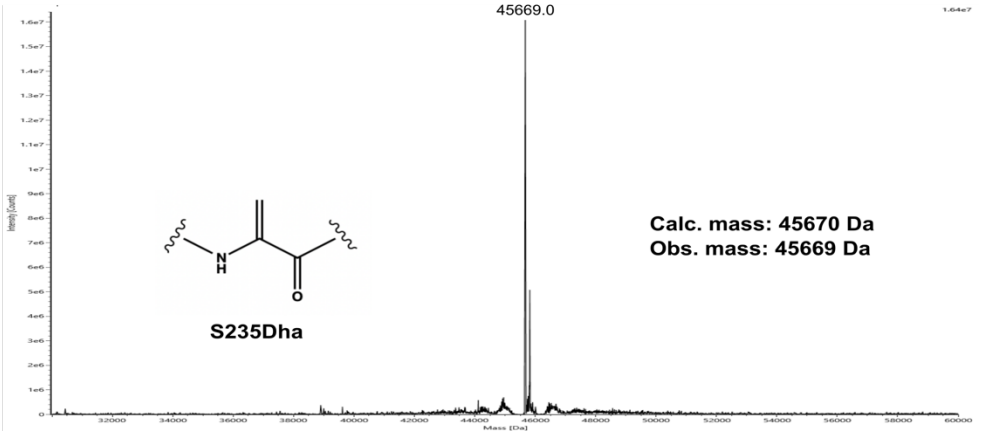
A




45702.0
HS
N
H
O
S235C
Calc. mass: 45704 Da
Obs. mass: 45702 Da

B




45669.0
N
H
O
S235Dha
Calc. mass: 45670 Da
Obs. mass: 45669 Da

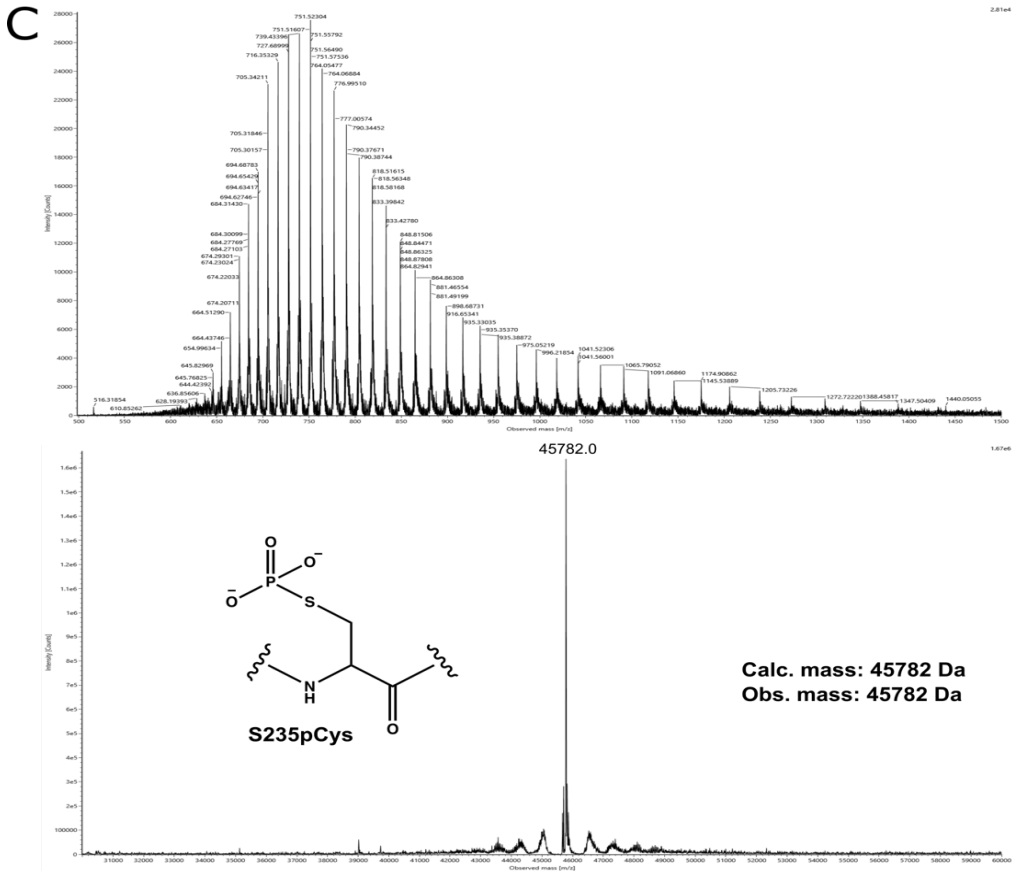
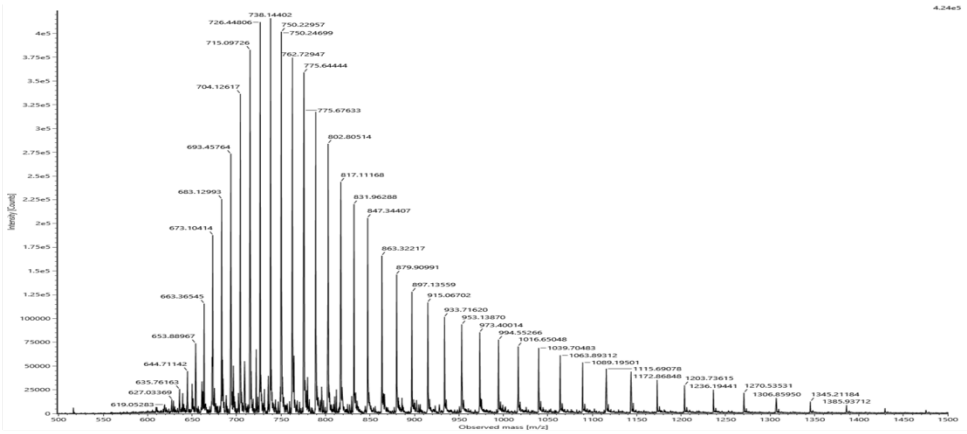
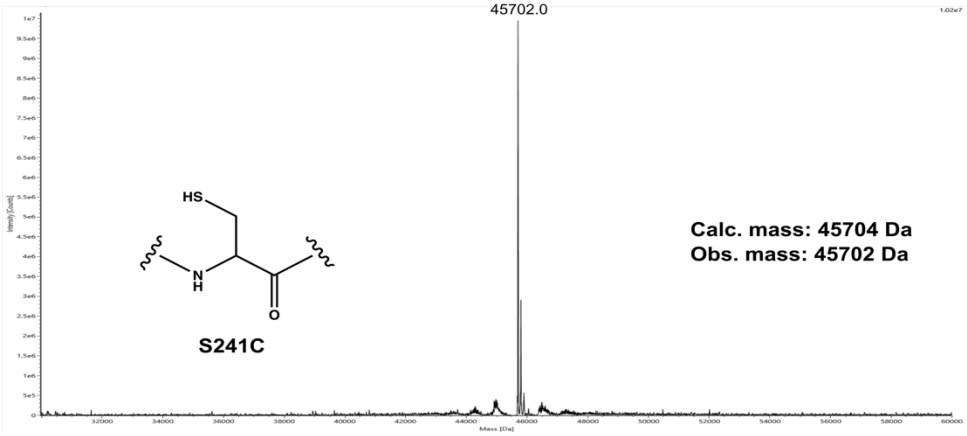


Figure S6. LC-MS spectra of the phosphorylated chemical mutant of tau at S235 and its intermediates. **(A)** LC-MS spectrum of tau(S235C). **(B)** LC-MS spectrum of tau(S235Dha). **(C)** LC-MS spectrum of tau(S235pC).

A

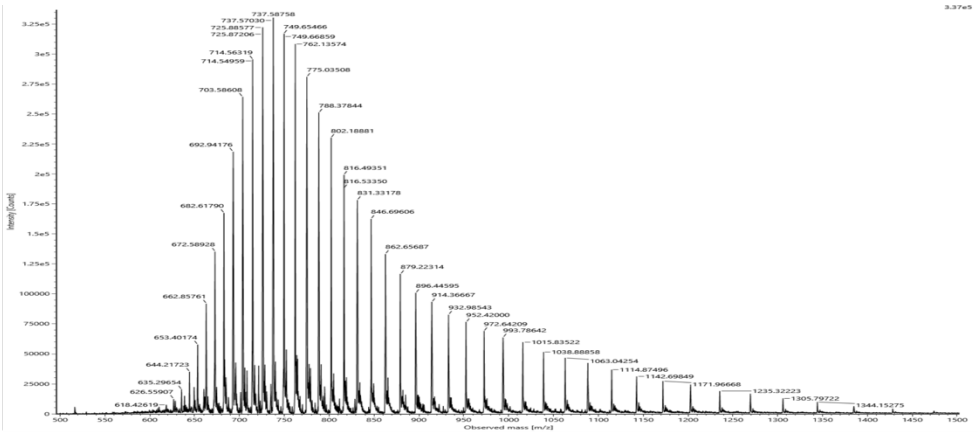


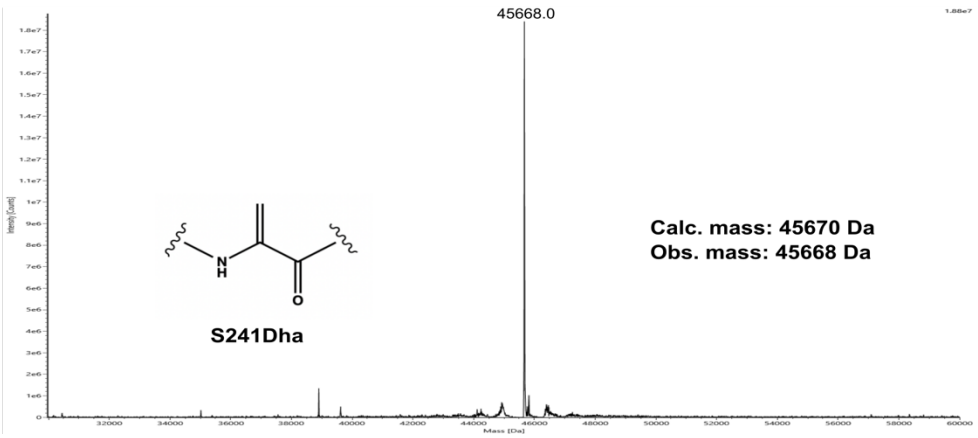


S241C

Calc. mass: 45704 Da
Obs. mass: 45702 Da

B





S241Dha

Calc. mass: 45670 Da
Obs. mass: 45668 Da

C

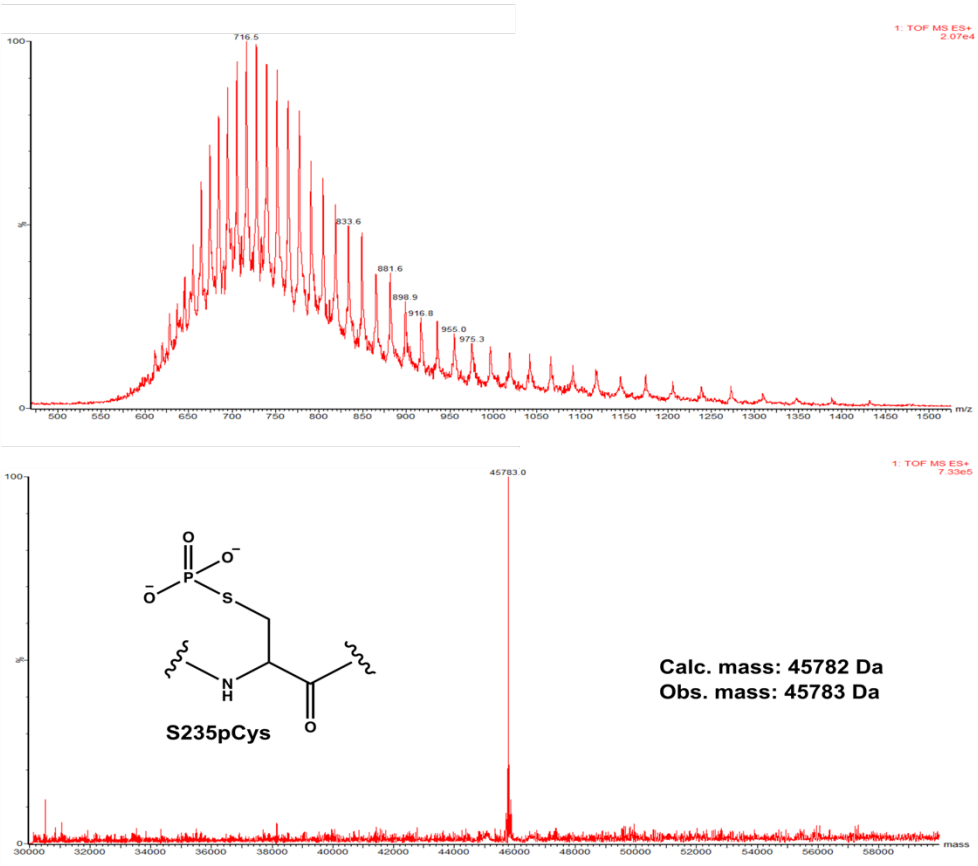
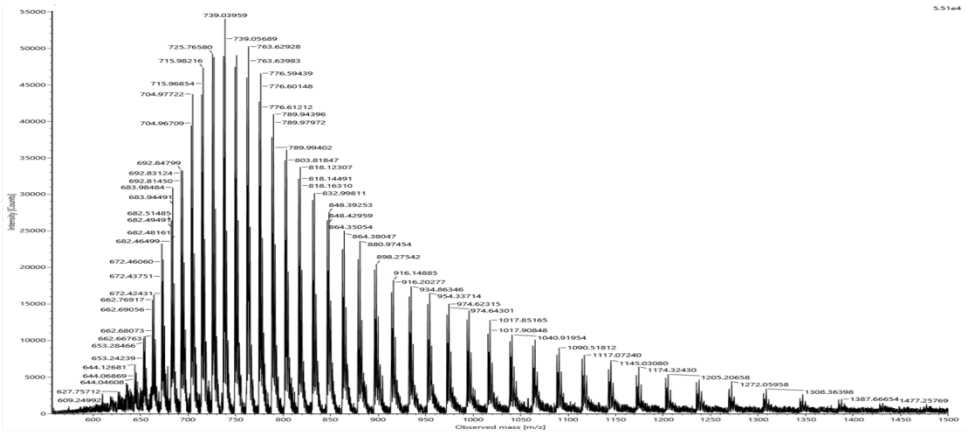
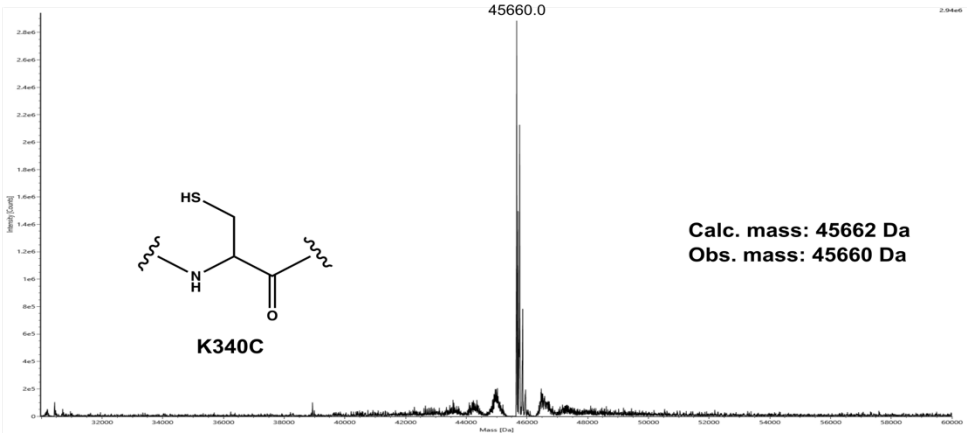


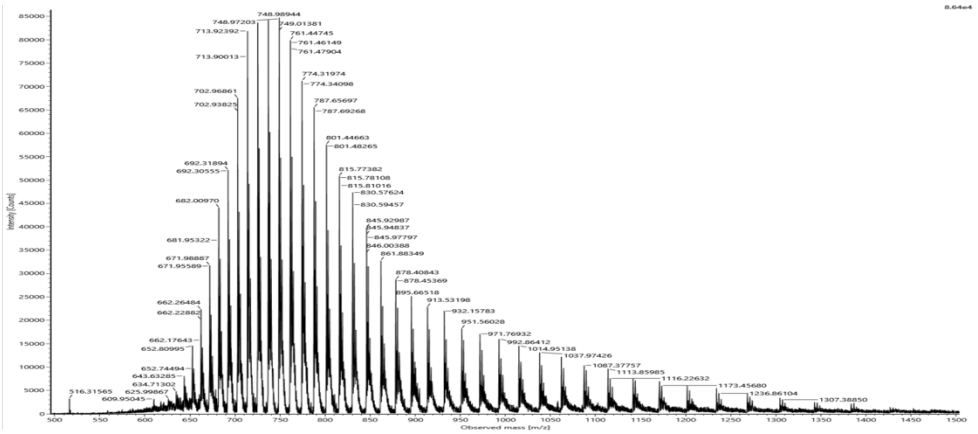
Figure S7. LC-MS spectra of the phosphorylated chemical mutant of tau at S241 and their intermediates. **(A)** LC-MS spectrum of tau(S241C). **(B)** LC-MS spectrum of tau(S241Dha). **(C)** LC-MS spectrum of tau(S241pC).

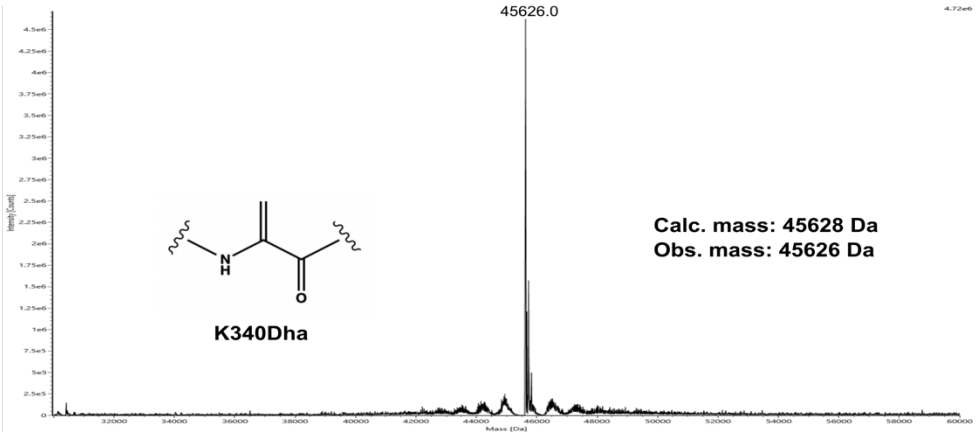
A




45660.0
HS
N
H
O
K340C
Calc. mass: 45662 Da
Obs. mass: 45660 Da

B




45626.0
N
H
O
K340Dha
Calc. mass: 45628 Da
Obs. mass: 45626 Da

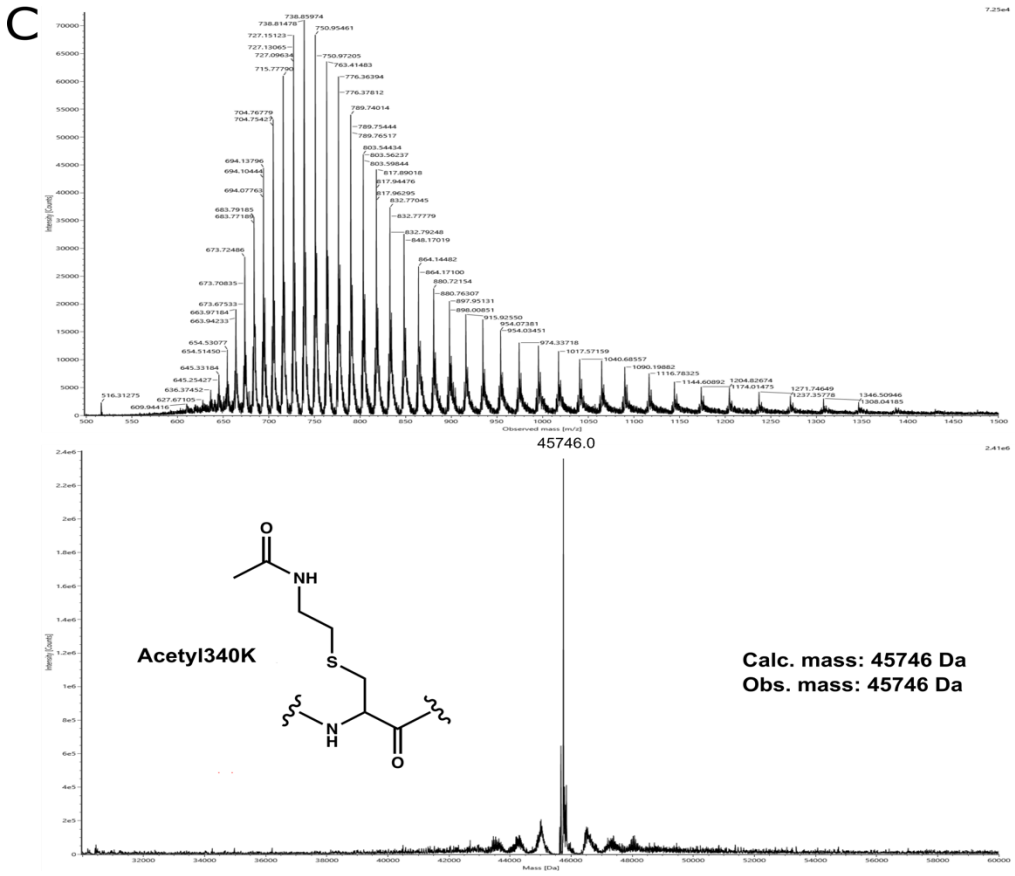


Figure S8. LC-MS spectrum of the acetylated K340 (aK340) tau chemical mutant and its intermediates. **(A)** LC-MS spectrum of tau(K340C). **(B)** LC-MS spectrum of tau(K340Dha). **(C)** LC-MS spectrum of tau(aK340).