Supporting Information

Separation of isomeric tau phosphopeptides from Alzheimer's disease brain by cyclic ion mobility mass spectrometry

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Figure S1. Western blot analysis of brain extract. The AD brains eluates after immuno-pulldown were analysed by Western blot using phosphorylation-dependent anti-tau antibodies against pThr212 and pThr217. Total tau was determined with a DC190 anti-tau antibody.

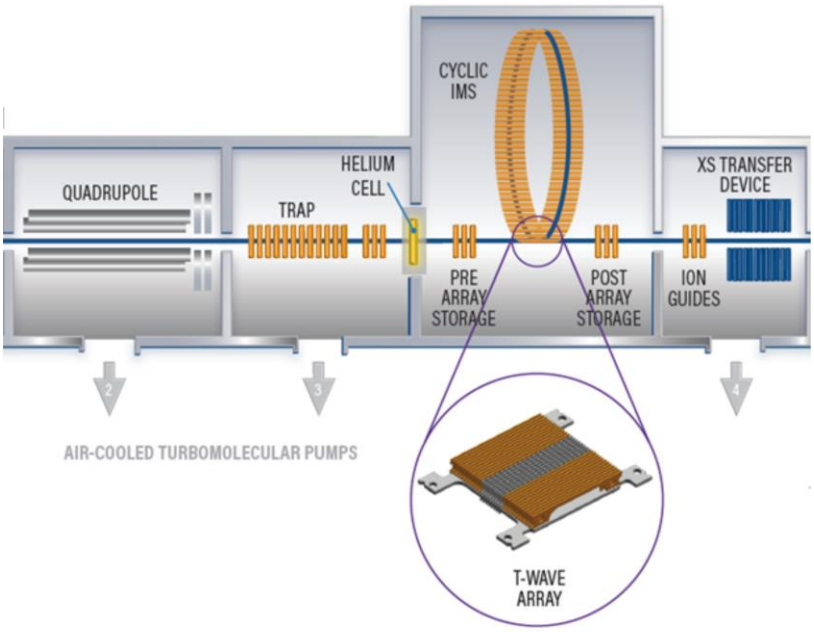

Figure S2. Schematic of the Waters Select Series Cyclic IM Spectrometer. After the initial trapping of ions in the first collision cell (trap), they are injected into the multifunctional array (T-wave array) where they are accelerated sidewise by a travelling wave into the cyclic separation IM cell.[1] Reprinted in part with permission of co-author Dale A. Cooper-Shepherd from "Application of cyclic ion mobility coupled to mass spectrometry for high peak capacity analysis of native and deuterated peptide mixtures", Martin Palmer; Malcolm Anderson; Dale A. Cooper-Shepherd; James I. Langridge; Robert Tonge; John R. Engen, poster nr. ThP285, Proceedings of 67th ASMS Conference on Mass Spectrometry and Allied Topics, Atlanta, Georgia, U.S.A., 2019.

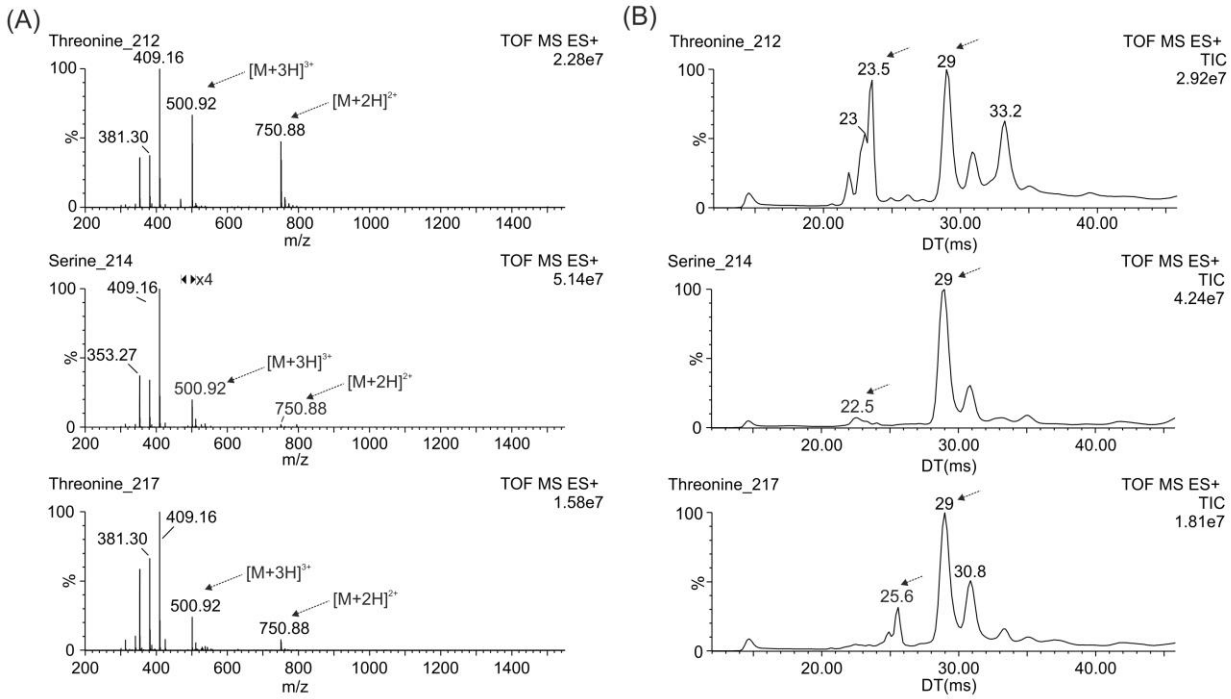

Figure S3. Separation of studied tau phosphopeptides by cIM. The full scan MS spectra (A) and single pass total ion mobilograms (B) of infused standards of isomeric phosphopeptides (T212, S214 and T217). Arrows mark mobility peaks related to $[M+3H]^{3+}$ (around 23 ms) and $[M+2H]^{2+}$ (cca 29 ms).

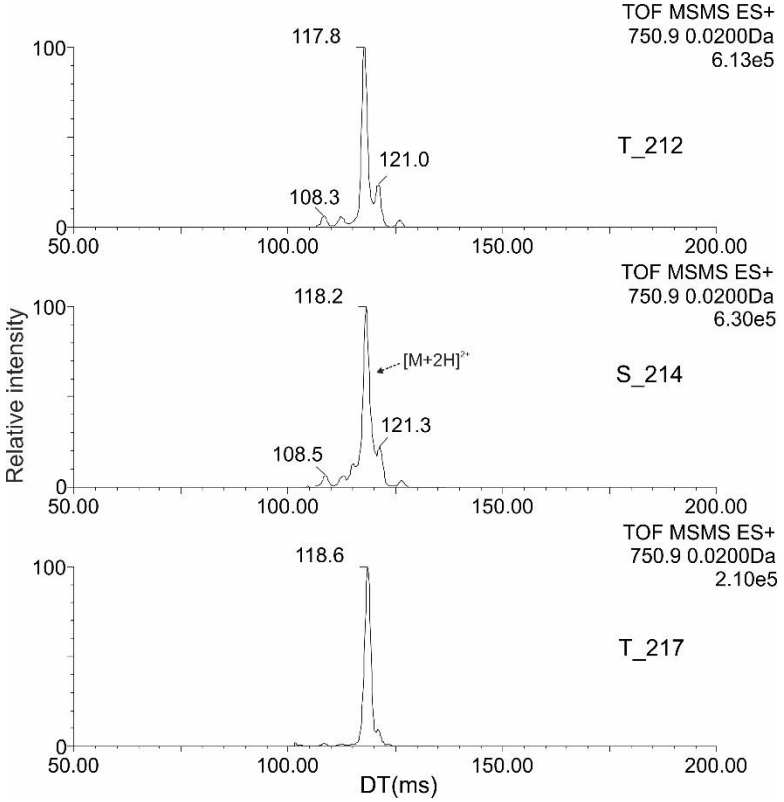

Figure S4. Ion mobilograms of $[M+2H]^{2+}$ after 5 pass separation. Extracted ion mobilograms of precursor ion at m/z 750.9.

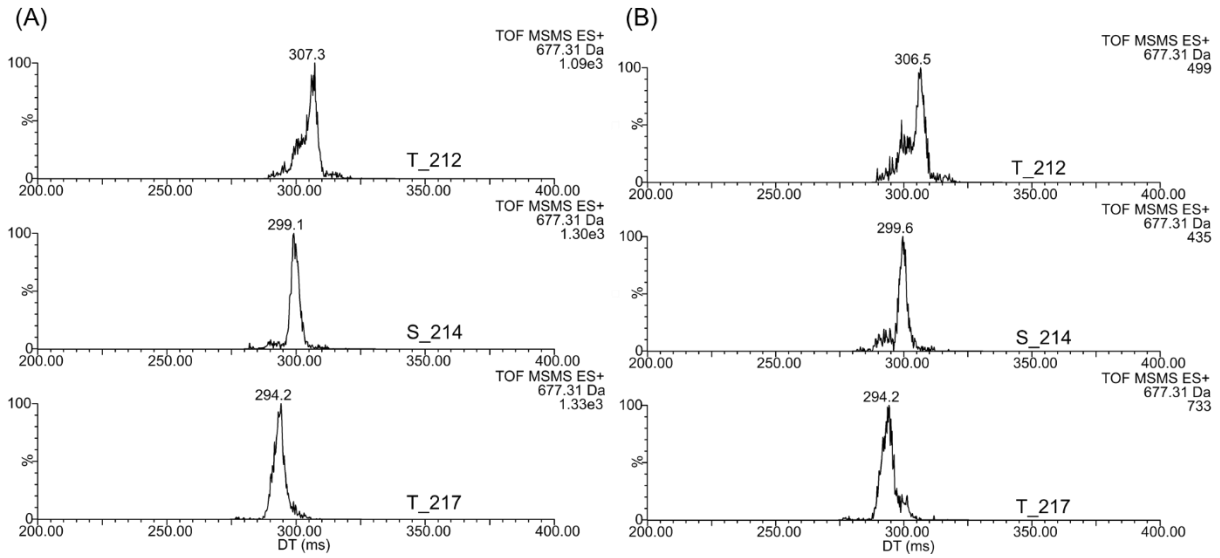

Figure S5. Ten pass ion mobilograms of phosphopeptides at low concentrations. Extracted ion mobilograms of N terminal b6 fragment ion at m/z 677 generated by CID in trap (24 V) from $[M+3H]^{3+}$ for T212, S214, T217, the concentration of standards: 1 nmol/L (A); 0.1 nmol/L (B); without smoothing, drift time aligned to Figure 3 by subtracting 4 ms.

Table S1. Characteristic fragment ions for precursor $[M+3H]^{3+}$. A unique fragment ion is in bold; for common fragment ions differing significantly in signal intensity, s stands for stronger, w for weaker.

phosphopeptides	fragment ions (m/z)
T212	279.1 (s); 286.1 (w); 1116.6 (s); 1135.7 (s); 1222.7 (s)
S214	
T217	279.1 (w); 286.1 (s); **1102.5**; 1116.6 (w); 1135.7 (w); 1222.7 (w)

Reference

(1) Giles, K.; Ujma, J.; Wildgoose, J.; Pringle, S.; Richardson, K.; Langridge, D.; Green, M. A Cyclic Ion Mobility-Mass Spectrometry System. *Anal. Chem.* **2019**, *91* (13), 8564-8573.