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Supplemental Information

Structural Impact of Tau Phosphorylation at Threonine 231

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Inventory of Supplemental Information

Supplemental Tables

Table S1, related to Figure 4 | NMR acquisition parameters of Tau(225-246).

Experiment name	Field strength (MHz)	Spectral width F2 / F1 (Hz)	Total points F2 / F1 (Hz)	No. of transients	Carrier frequency F1 (ppm)[f]	Mixing time (ms)
^{1}H,^{1}H-TOCSY[a]	700	7002.8 / 7012.5	2048 / 800	16	4.7	40
^{1}H,^{1}H-TOCSY[a]	700	7002.8 / 7012.5	2048 / 800	32	4.7	70
^{1}H,^{1}H-NOESY[a]	700	7002.8 / 7012.5	2048 / 800	32	4.7	120
^{1}H,^{1}H-NOESY[a]	700	7002.8 / 7012.5	2048 / 800	32	4.7	200
^{1}H,^{1}H-NOESY[b]	700	7002.8 / 7011.5	2048 / 800	32	4.7	300
^{1}H,^{1}H-NOESY[c]	900	8992.8 / 9000.0	2048 / 672	24	4.7	100
^{1}H,^{13}C-HSQC[a]	700	7002.8 / 11285.5	2048 / 512	64	38	n.a.
J-modulated ^{1}H,^{13}C-CT-HSQC[a]	700	7002.8 / 5642.8	2048 / 270	56	53	n.a.
J-modulated ^{1}H,^{13}C-CT-HSQC[d]	700	7002.8 / 5642.8	2048 / 270	56	53	n.a.
^{1}H,^{15}N-HSQC[a]	700	7002.8 / 1986.7	2048 / 256	256	118	n.a.
^{1}H,^{15}N-BSD-IPAP-HSQC[b]	600	6009.6 / 1094.9	1024 / 512	256	122	n.a.
^{1}H,^{15}N-BSD-IPAP-HSQC[e]	600	6009.6 / 1094.9	1024 / 500	240	122	n.a.

[a] – The sample contained 2.9 mM peptide dissolved in 50 mM NaH_2PO_4/Na_2HPO_4, pH 6.8, 10% ($^{v}/_{v}$) D_2O.

[b] – The sample contained 4.0 mM peptide dissolved in 50 mM NaH_2PO_4/Na_2HPO_4, pH 6.8, 10% ($^{v}/_{v}$) D_2O.

[c] – The sample contained 1.0 mM peptide dissolved in 50 mM NaH_2PO_4/Na_2HPO_4, pH 6.8, 10% ($^{v}/_{v}$) D_2O.

[d] – The sample contained 4.2 mM peptide dissolved in 50 mM NaH_2PO_4/Na_2HPO_4, pH 6.8, 10% ($^{v}/_{v}$) D_2O and 5% ($^{w}/_{v}$) pentaethylene glycol monooctyl ether (C8E5)/n-octanol.

[e] – The sample contained 3.8 mM peptide dissolved in 50 mM NaH_2PO_4/Na_2HPO_4, pH 6.8, 10% ($^{v}/_{v}$) D_2O and 5% ($^{w}/_{v}$) C8E5/n-octanol.

[f] – Carrier frequencies in the direct dimension F2 were set to the water resonance.

Table S2, related to Figure 4 | NMR acquisition parameters of 2pTau(225-246).

Experiment name	Field strength (MHz)	Spectral width F2 / F1 (Hz)	Total points F2 / F1 (Hz)	No. of transients	Carrier frequency F1 (ppm)[d]	Mixing time (ms)
$^{1}H,^{1}H$-TOCSY[a]	700	8417.5 / 8413.8	1024 / 800	32	4.7	40
$^{1}H,^{1}H$-TOCSY[a]	700	8417.5 / 8413.8	1024 / 800	32	4.7	70
$^{1}H,^{1}H$-NOESY[a]	600	7211.5 / 7203.0	2048 / 800	32	4.7	120
$^{1}H,^{1}H$-NOESY[a]	600	7211.5 / 7203.0	2048 / 800	32	4.7	200
$^{1}H,^{1}H$-NOESY[a]	600	7211.5 / 7203.0	2048 / 800	32	4.7	300
$^{1}H,^{1}H$-NOESY[b]	900	8992.8 / 9000.0	2048 / 672	24	4.7	100
$^{1}H,^{13}C$-HSQC[a]	700	8417.5 / 9873.5	1024 / 512	128	46	n.a.
J-modulated $^{1}H,^{13}C$-CT-HSQC[a]	600	6009.6 / 4981.1	2048 / 270	48	55	n.a.
J-modulated $^{1}H,^{13}C$-CT-HSQC[c]	600	6009.6 / 4981.1	2048 / 270	56	55	n.a.
$^{1}H,^{15}N$-HSQC[a]	600	6009.6 / 973.3	1024 / 256	208	122	n.a.
$^{1}H,^{15}N$-BSD-IPAP-HSQC[a]	600	6009.6 / 1094.9	1024 / 512	256	122	n.a.
$^{1}H,^{15}N$-BSD-IPAP-HSQC[d]	600	6009.6 / 1094.9	1024 / 512	256	122	n.a.

[a]– The sample contained 3.0 mM peptide dissolved in 50 mM NaH_2PO_4/Na_2HPO_4, pH 6.8, 10% ($^{v}/_{v}$) D_2O.

[b] – The sample contained 1.0 mM peptide dissolved in 50 mM NaH_2PO_4/Na_2HPO_4, pH 6.8, 10% ($^{v}/_{v}$) D_2O.

[c]– The sample contained 3.5 mM peptide dissolved in 50 mM NaH_2PO_4/Na_2HPO_4, pH 6.8, 10% ($^{v}/_{v}$) D_2O and 5% ($^{w}/_{v}$) C8E5/n-octanol.

[d]– Carrier frequencies in the direct dimension F2 were set to the water resonance.

Table S3, related to Figure 4 | NMR acquisition parameters of 4pTau(225-246).

Experiment name	Field strength (MHz)	Spectral width F2 / F1 (Hz)	Total points F2 / F1 (Hz)	No. of transients	Carrier frequency F1 (ppm)[d]	Mixing time (ms)
$^{1}H,^{1}H$-TOCSY[a]	700	7002.8 / 7011.5	2048 / 800	24	4.7	70
$^{1}H,^{1}H$-NOESY[a]	700	7002.8 / 7011.5	2048 / 800	32	4.7	200
$^{1}H,^{1}H$-NOESY[b]	700	8389.3 / 8401.6	2048 / 800	32	4.7	300
$^{1}H,^{13}C$-HSQC[b]	600	6009.6 / 9056.4	2048 / 512	16	41	n.a.
J-modulated $^{1}H,^{13}C$-CT-HSQC[b]	700	7002.8 / 5642.1	2048 / 270	40	56	n.a.
J-modulated $^{1}H,^{13}C$-CT-HSQC[c]	700	7002.8 / 5642.1	2048 / 270	40	56	n.a.
$^{1}H,^{15}N$-HSQC[b]	700	8389.3 / 1277.1	2048 / 128	120	122	n.a.
$^{1}H,^{15}N$-BSD-IPAP-HSQC[b]	600	6009.6 / 1094.9	1024 / 512	240	122	n.a.
$^{1}H,^{15}N$-BSD-IPAP-HSQC[c]	600	6009.6 / 1094.9	1024 / 512	224	122	n.a.

[a] – The sample contained 3.0 mM peptide dissolved in 50 mM NaH_2PO_4/Na_2HPO_4, pH 6.8, 10% ($^{v}/_{v}$) D_2O.

[b] – The sample contained 3.9 mM peptide dissolved in 50 mM NaH_2PO_4/Na_2HPO_4, pH 6.8, 10% ($^{v}/_{v}$) D_2O.

[c] – The sample contained 3.8 mM peptide dissolved in 50 mM NaH_2PO_4/Na_2HPO_4, pH 6.8, 10% ($^{v}/_{v}$) D_2O and 5% ($^{w}/_{v}$) C8E5/n-octanol.

[d] – Carrier frequencies in the direct dimension F2 were set to the water resonance.

Table S4, related to Figure 4 | NMR acquisition parameters of Tau(211-242) phosphorylated at T231.

[1]Experiment name	Field strength (MHz)	Spectral width F2 / F1 (Hz)	Total points F2 / F1 (Hz)	No. of transients	Carrier frequency F1 (ppm)[d]	Mixing time (ms)
$^{1}H,^{1}H$-NOESY[a]	800	8802.8 / 8792.9	2048 / 600	40	4.7	200
$^{1}H,^{1}H$-NOESY[a]	800	8802.8 / 8792.9	2048 / 543	40	4.7	300
$^{1}H,^{13}C$-HSQC[b]	600	7183.9/ 9051.1	2048 / 512	88	42	n.a.
$^{1}H,^{15}N$-HSQC[b]	600	7183.9 / 1094.3	2048 / 128	200	122	n.a.

[a] – The sample contained 1.0 mM peptide dissolved in 50 mM NaH_2PO_4/Na_2HPO_4, pH 6.8, 10% ($^{v}/_{v}$) D_2O.

[b] – The sample contained 1.0 mM peptide dissolved in 50 mM NaH_2PO_4/Na_2HPO_4, pH 6.8, 10% ($^{v}/_{v}$) D_2O.

Supplemetal Figures and Legends

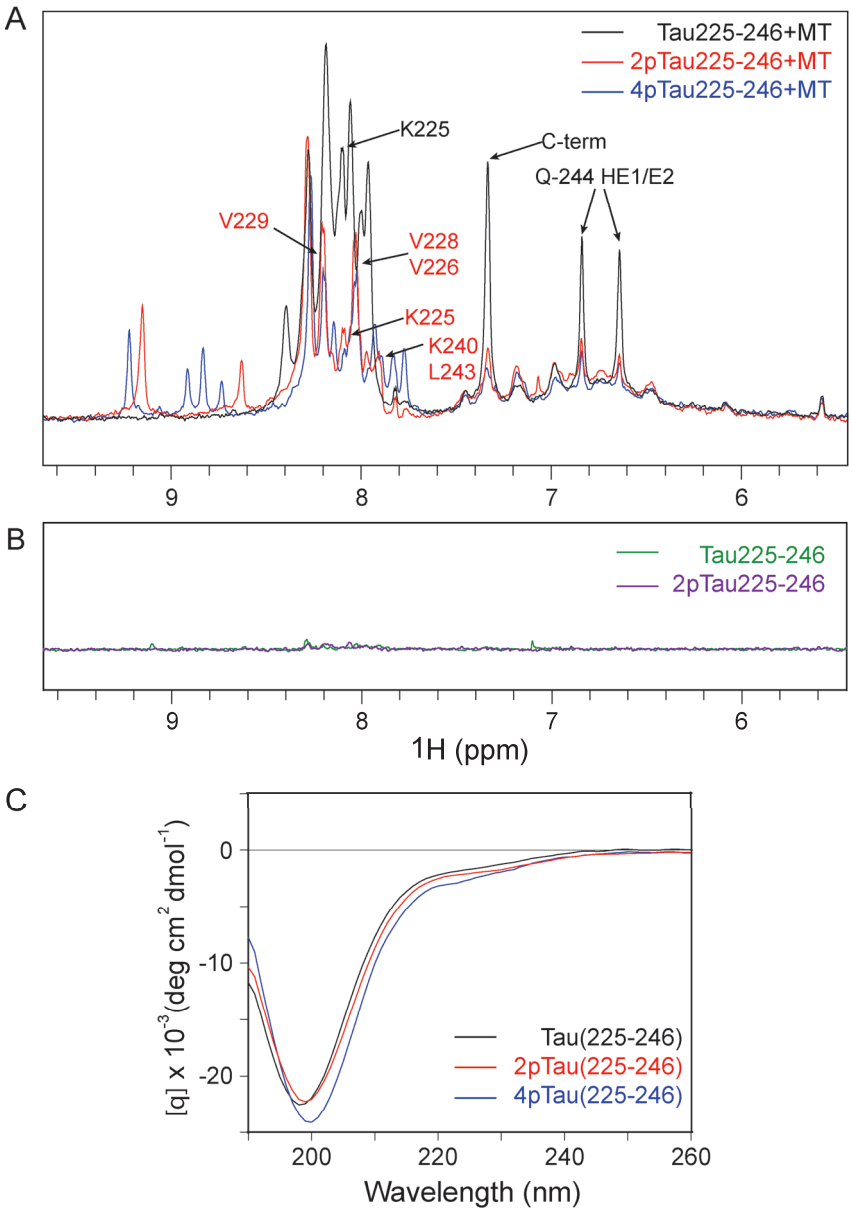

Figure S1, related to Figure 2. Influence of phosphorylation on the interaction of the proline-rich region P2 of Tau with microtubules. (A) Overlay of STD spectra of non-phosphorylated Tau(225-246) (black), T231/S235-phosphorylated Tau(225-246) (red) and T231/S235/S237/S238-phosphorylated Tau(225-246) (blue) in the presence of microtubules (molar ratio of 40:1). Arrows mark selected resonances. (B) Superposition of control STD spectra of Tau(225-246) (green) and T231/S235-phosphorylated Tau(225-246) (purple) in the absence of microtubules, with identical experimental conditions as in (A). (C) CD spectra of

Tau(225-246) (black), T231/S235-phosphorylated Tau(225-246) (red) and T231/S235/S237/S238-phosphorylated Tau(225-246) (blue).

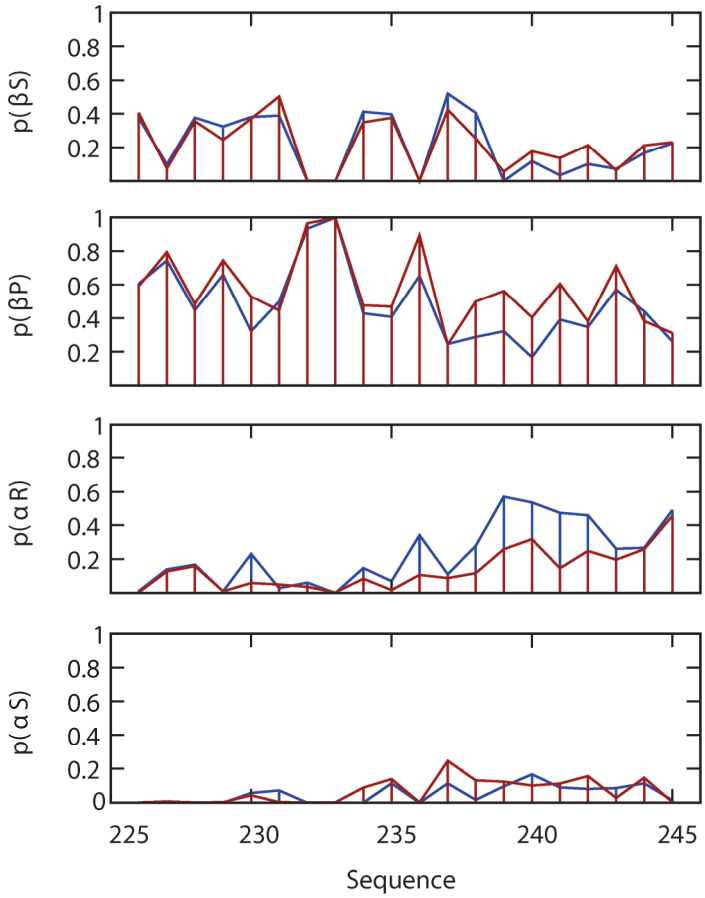

Figure S2, related to Figure 4. The Flexible Meccano/Asteroids analysis of Tau(225-246) (brown) and T231/S235/S237/S238-phosphorylated Tau(225-246) (blue). The Ramachandran space was divided into four quadrants and the populations are denoted as p(αL), p(αR), p(βP) and p(βS).

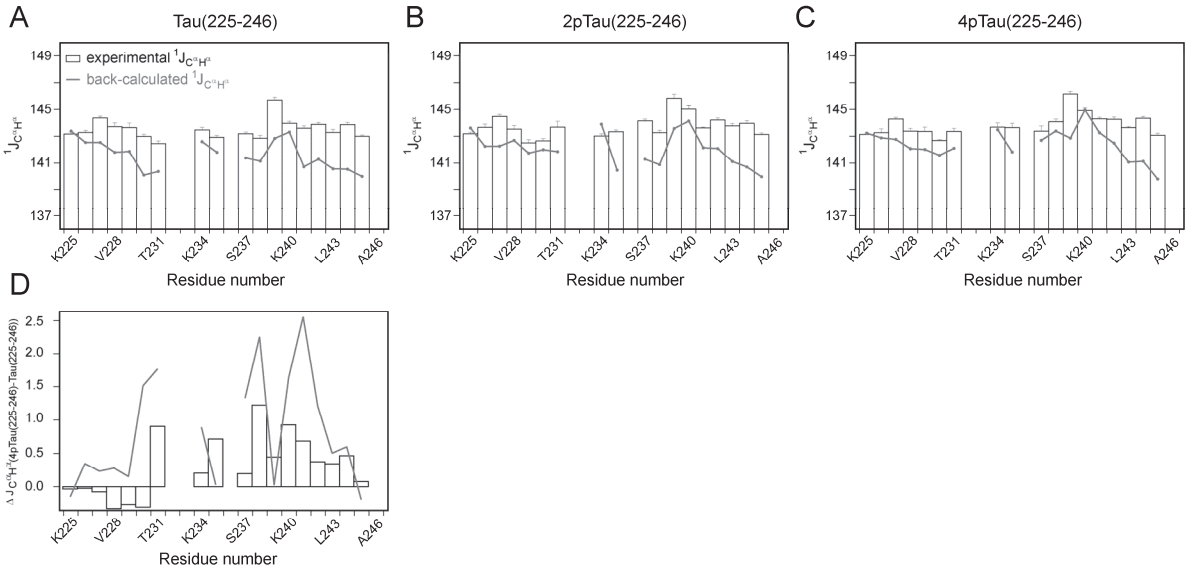

Figure S3, related to Figure 5. Cross-validation of molecular ensembles. (A-C) Comparison of the experimentally determined $^1J_{H\alpha C\alpha}$ couplings (open bars), which were not used in the ensemble calculations, with values back-calculated from the molecular ensembles (grey line) for non-phosphorylated Tau(225-246) (A), T231/S235-phosphorylated Tau(225-246) (B) and T231/S235/S237/S238-phosphorylated Tau(225-246) (C). (D) Residue-specific differences between $^1J_{H\alpha C\alpha}$ couplings of T231/S235/S237/S238-phosphorylated Tau(225-246) and non-phosphorylated Tau(225-246). Experimental (open bars) as well as back-calculated (grey line) $^1J_{H\alpha C\alpha}$ couplings were increased for residues 231-244.

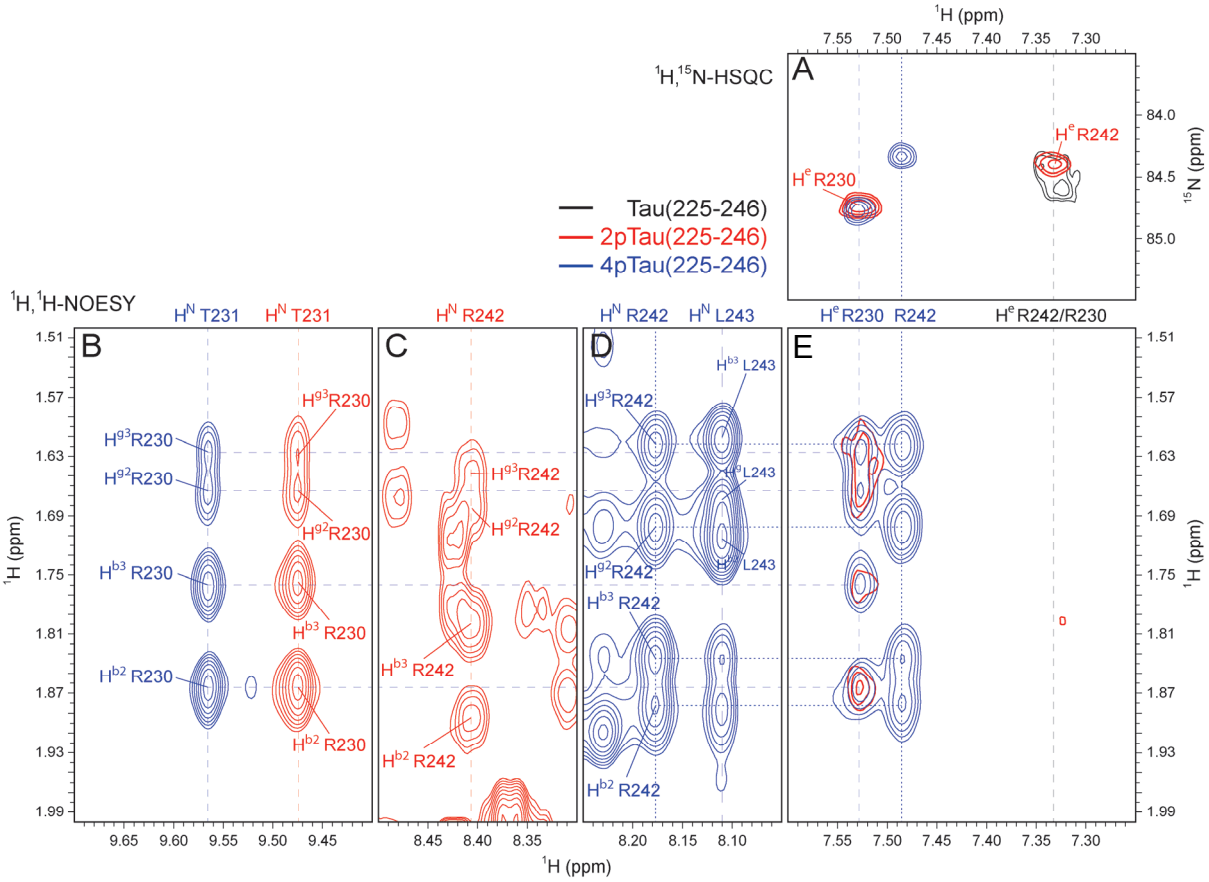

Figure S4, related to Figure 6. Assignment of arginine H^{ε} side chain resonances. (A) The ^{1}H,^{15}N-HSQC spectrum shows a downfield-shift of the H^{ε} frequencies of specific arginine residues in T231/S235-phosphorylated Tau(225-246) (red contours) and T231/S235/S237/S238-phosphorylated Tau(225-246) (blue contours) when compared to non-phosphorylated Tau(225-246) (black). (B-E) H^{ε} frequencies were assigned to particular arginine residues through the use of intra-residual cross peaks (E) in ^{1}H,^{1}H NOESY spectra. While panel (B) shows sequential cross peaks between the amide proton of T231 and the side chain protons of R230, panels C and D display intra-residual cross peaks of R242. Dashed lines mark the frequencies of selected amide and side chain proton resonances.

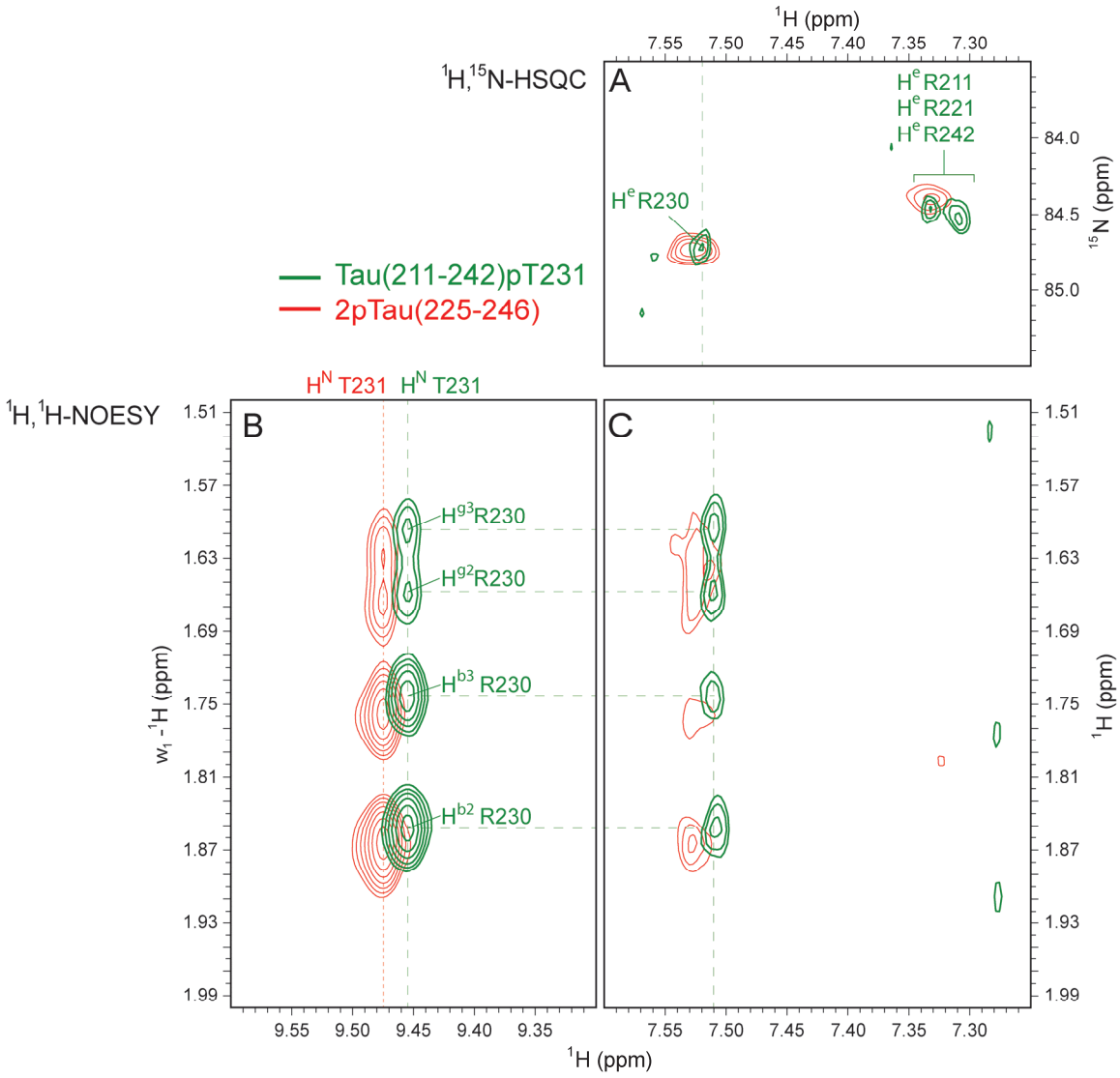

Figure S5, related to Figure 6. Assignment of arginine Hε side chain resonances in T231-phosphorylated Tau(211-242). (A) Analogous to Figure S3, the ^{1}H,^{15}N-HSQC spectrum shows a downfield-shift of the Hε frequency of R230 in T231-phosphorylated Tau(211-242) (green contours) similar to the one observed for T231/S235-phosphorylated Tau(225-246) (red contours). (B,C) Similar inter- and intra-residual cross peaks for both peptides in ^{1}H,^{1}H NOESY spectra.

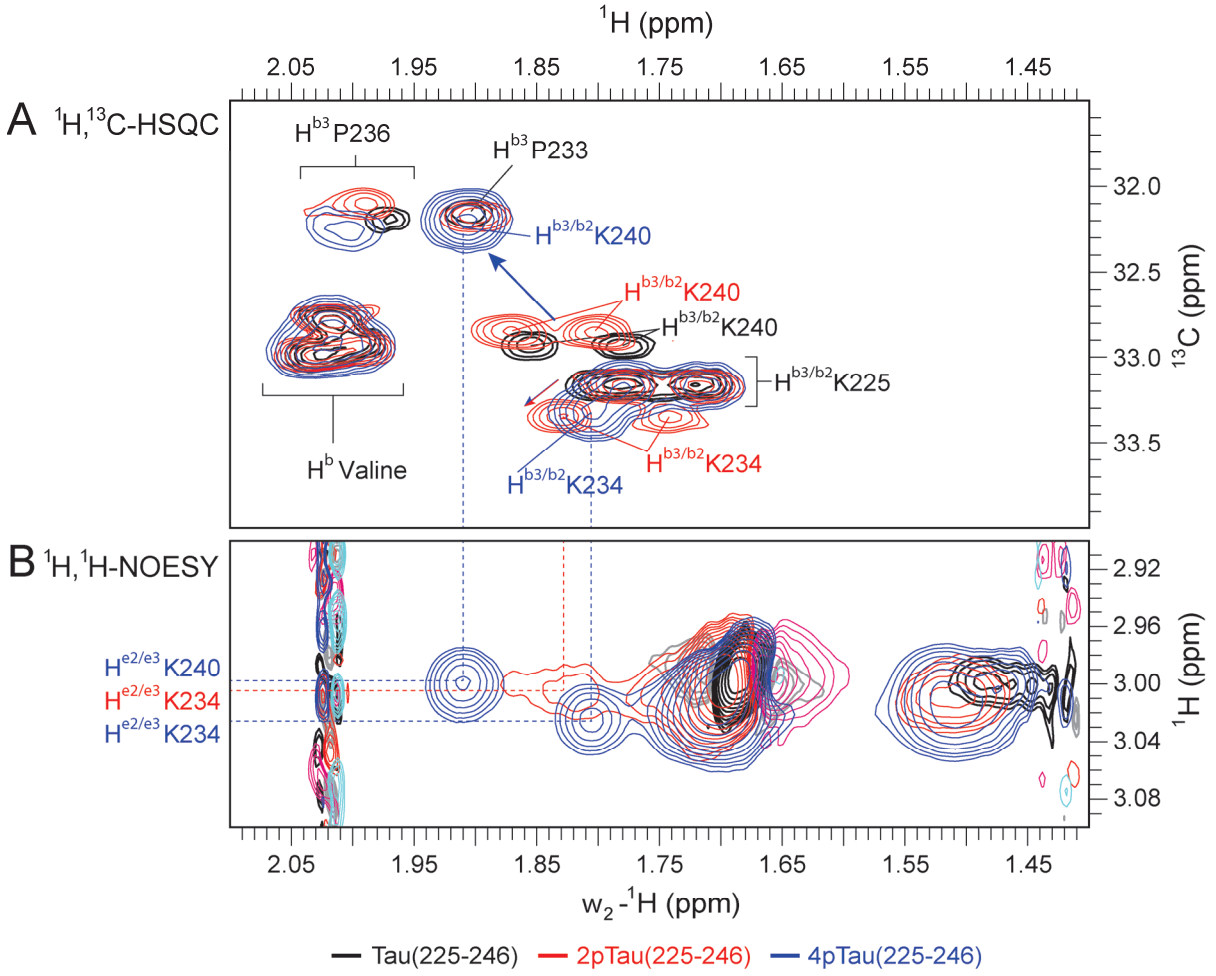

Figure S6, related to Figure 7. Phosphorylation of Tau(225-246) affects the chemical environment of specific lysine side chains. (A) Superposition of a selected region from ^{1}H,^{13}C-HSQC spectra of Tau(225-246) in the non-phosphorylated (black), T231/S235-phosphorylated (red) and T231/S235/S237/S238-phosphorylated state (blue). The Hβ resonances of K234 and K240 were most strongly shifted upon phosphorylation. (B) Superposition of a selected region from ^{1}H,^{1}H-NOESY spectra of Tau(225-246) in the non-phosphorylated (black), T231/S235-phosphorylated (red) and T231/S235/S237/S238-phosphorylated state (blue). Phosphorylation results in line narrowing and signal enhancement of intra-residual NOEs between the Hβ and He protons of K234 and K240.