Supplementary Information for

The binding mode of a repeat-like Tau sequence with tubulin

Harindranath Kadavath, Yunior Cabrales Fontela, Mariusz Jaremko, Łukasz Jaremko, Kerstin Overkamp, Jacek Biernat, Eckhard Mandelkow, and Markus Zweckstetter

Supplementary Figures

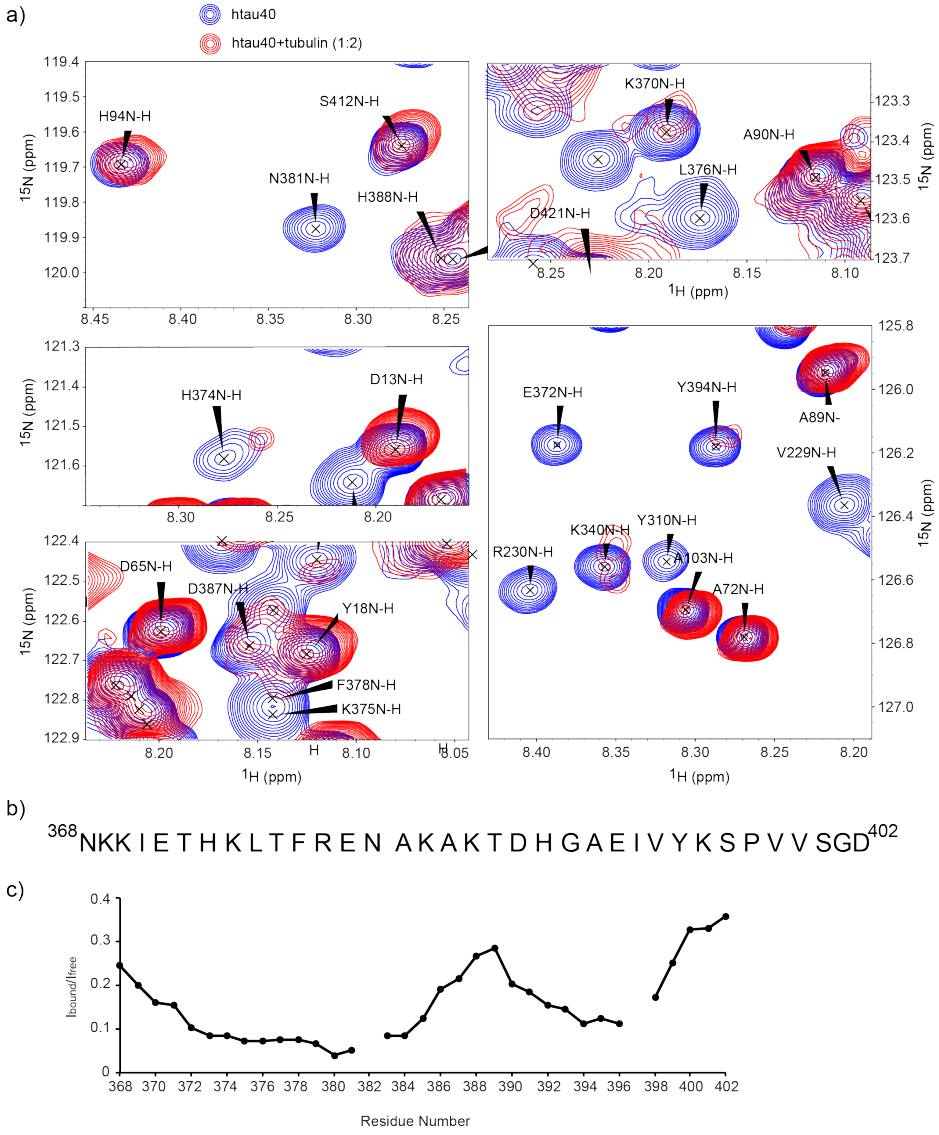

Figure S1. a) Selected regions of ^{1}H–^{15}N HSQC spectra of 2N4R Tau (20 µM) in the absence (blue) and presence of tubulin (red). Tau:tubulin heterodimer ratio was 1:2. Spectra were recorded at 700 MHz ^{1}H frequency, 5 °C. Attenuated resonances other than those of TauR' (e.g. that of Y310) arise from the interaction of the proline-rich and repeat regions of Tau. b) Amino acid sequence of TauR'. c) Residue-specific attenuation of ^{1}H-^{15}N cross-peaks within the TauR' region upon addition of soluble tubulin to 2N4R Tau (Tau:tubulin ratio of 1:2).

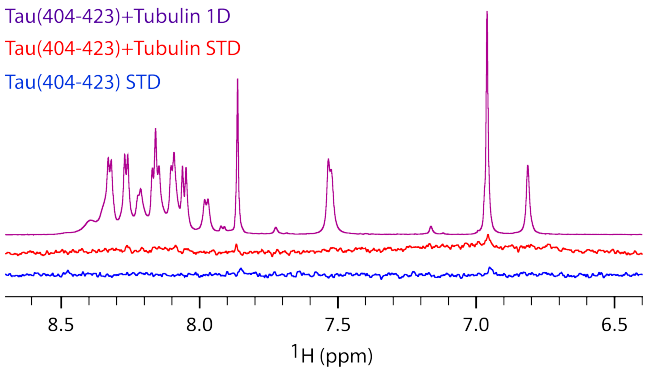

Figure S2. ^{1}H 1D (purple) and STD NMR spectra of Tau(404-423) in presence (red) and absence (blue) of tubulin. The Tau(404-423):tubulin heterodimer ratio was 40:1.

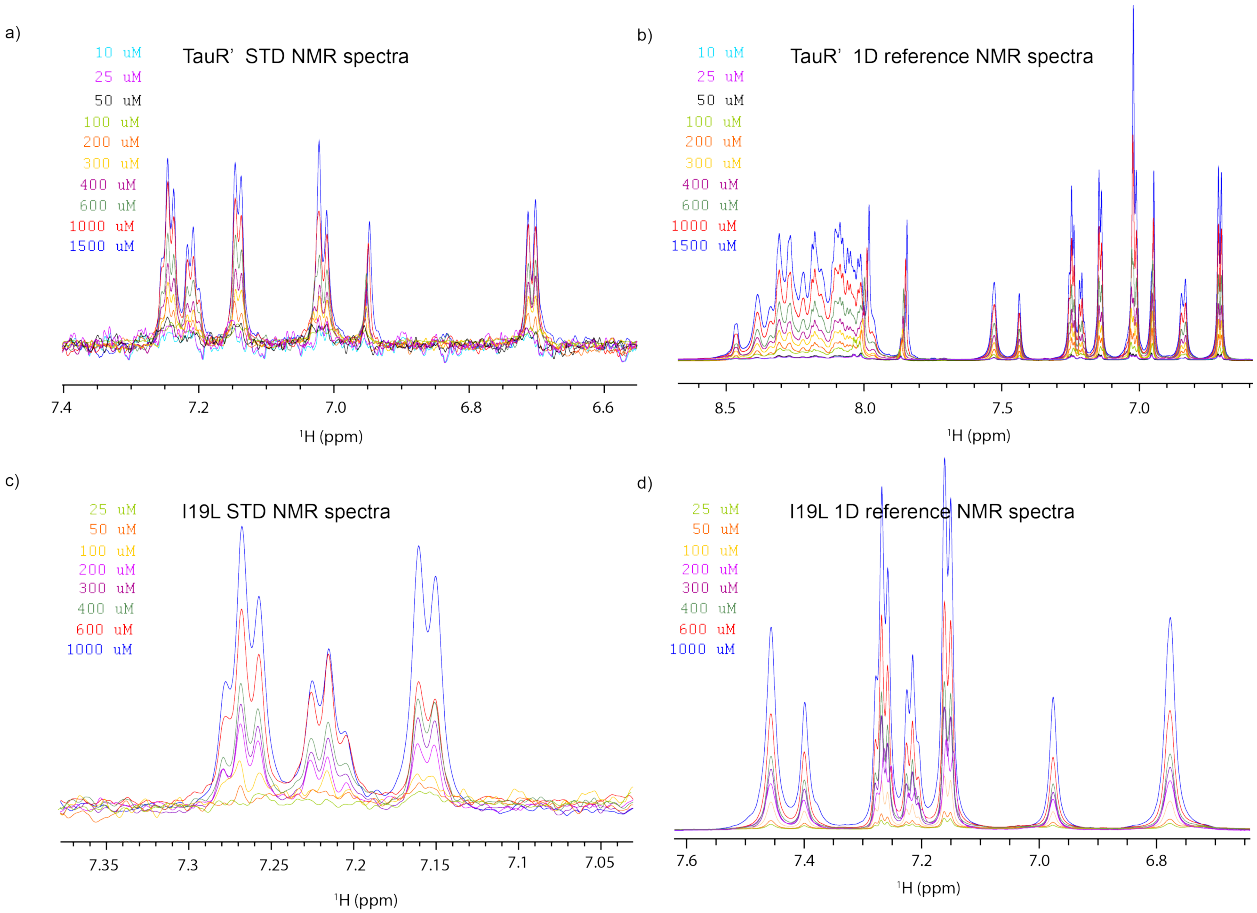

Figure S3. STD NMR spectra of TauR' and I19L. a, b) Stacked plot of the aromatic spectral region of 1D STD NMR spectra recorded at a single saturation time (t_{sat}=2 s, 25 °C) and the 1D reference spectra (b) during the titration of a sample of 10 μM tubulin with increasing concentrations of TauR' (as indicated in the panel). (c, d) Aromatic spectral region of 1D STD and reference NMR spectra (d) of I19L in presence of 5 μM tubulin. I19L concentrations are indicated.

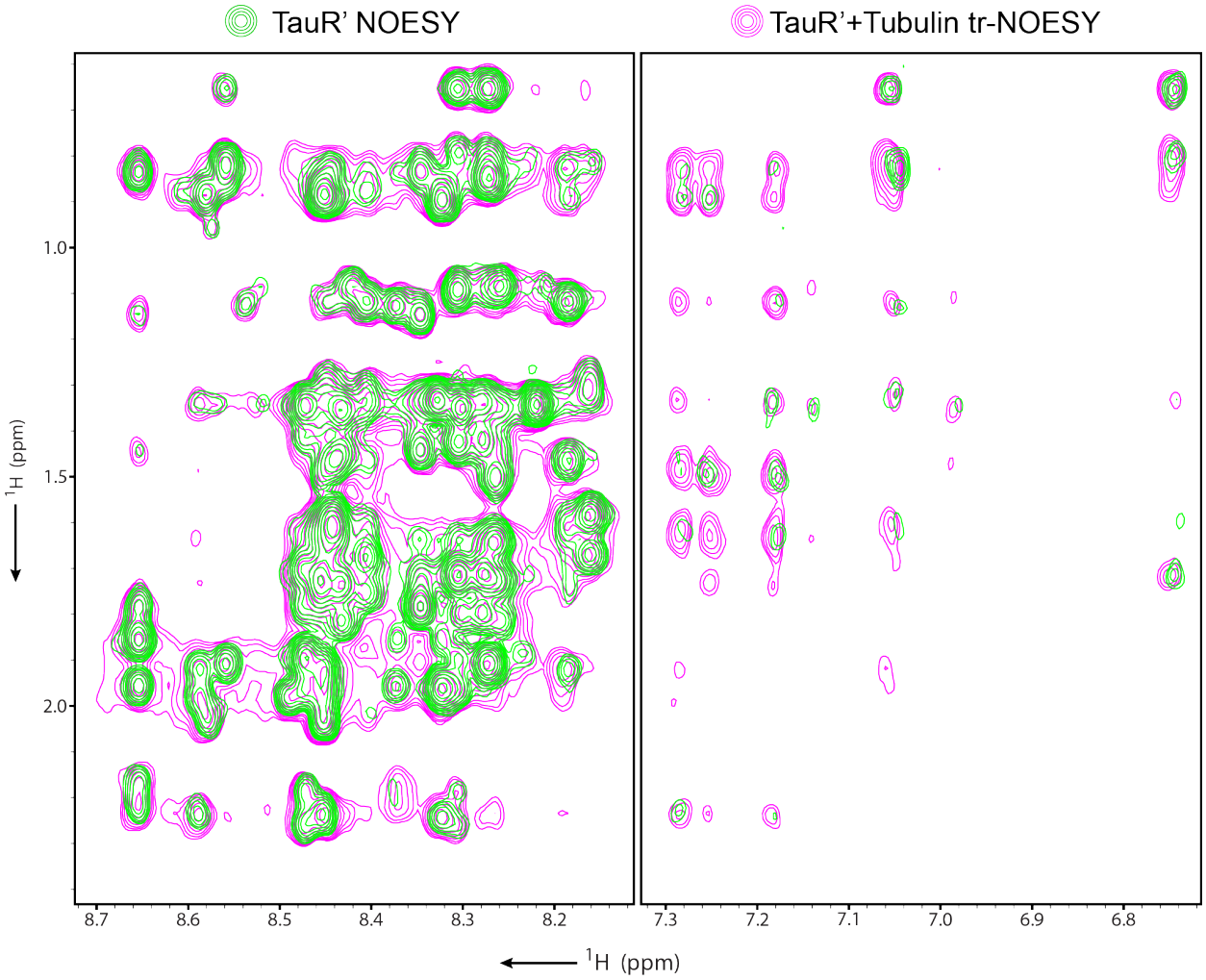

Figure S4. Superposition of selected regions of ^{1}H-^{1}H NOESY spectra recorded on TauR' in the absence (green) and presence (magenta) of tubulin, with NOESY mixing time of 100 ms.

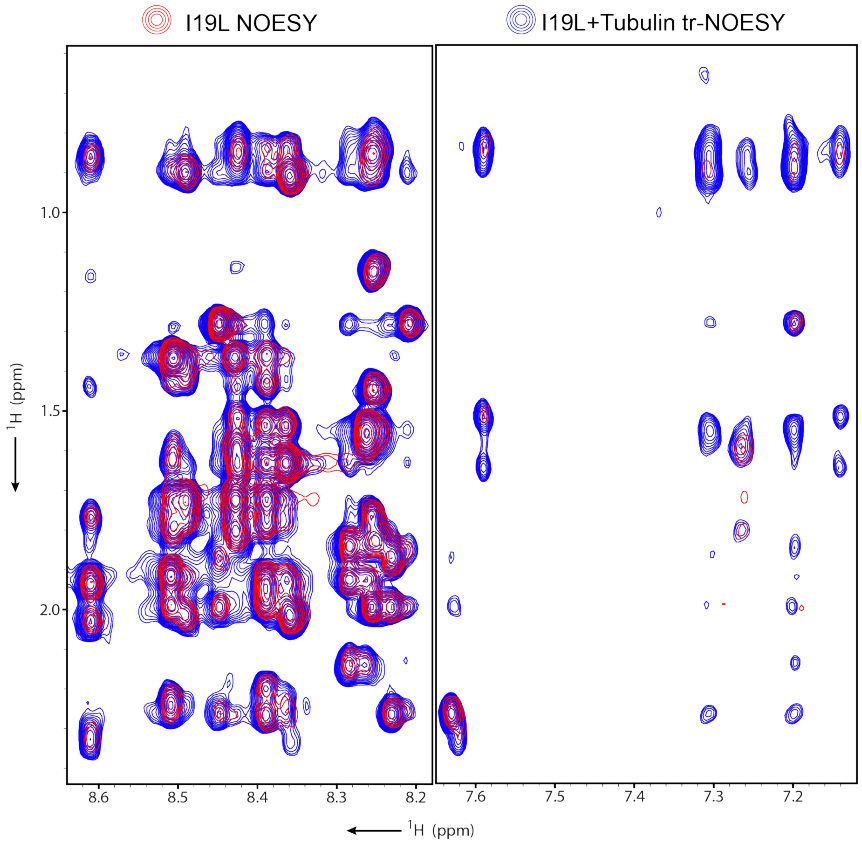

Figure S5. Superposition of selected regions of ^{1}H-^{1}H NOESY spectra recorded on I19L in the absence (red) and presence (blue) of tubulin, with NOESY mixing time of 100 ms.

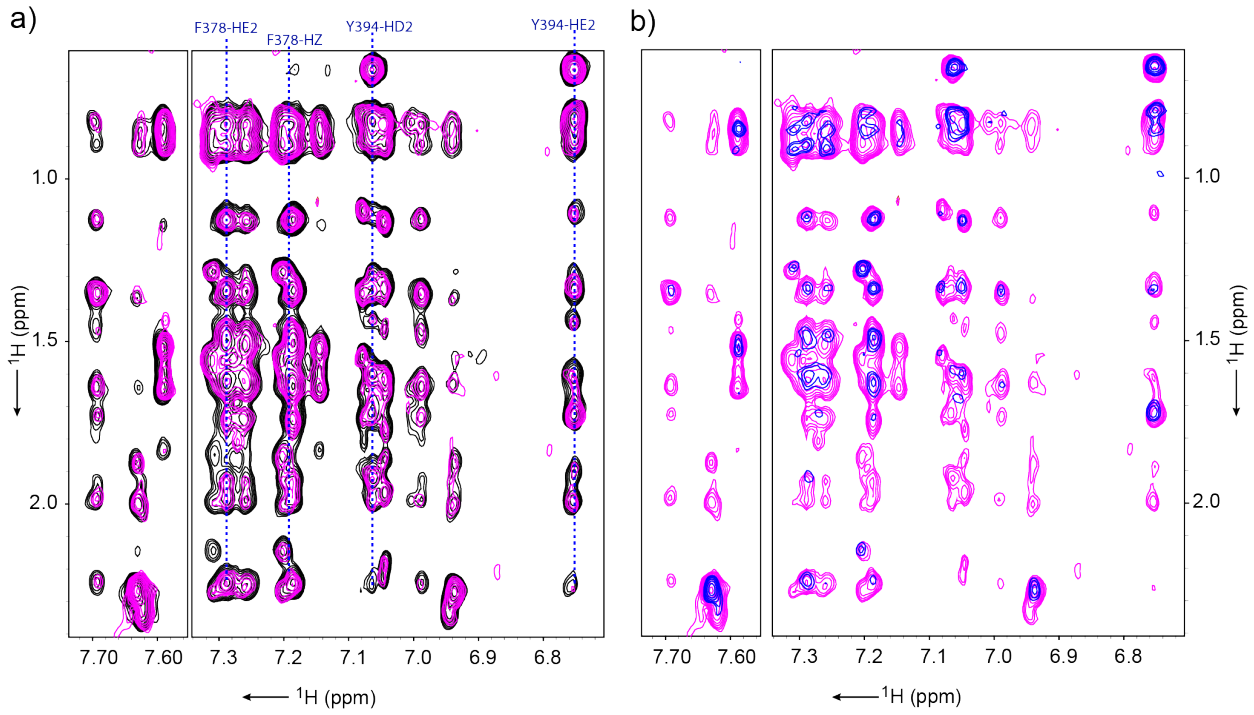

Figure S6. (a) Superposition of a selected region of ^{1}H-^{1}H NOESY spectra recorded with NOE mixing times of 100 ms (magenta) and 200 ms (black) for the ternary mixture of TauR' (1.1 mM), I19L (1.1 mM) and tubulin (35 μM). Selected resonances of TauR' are labeled. **(b)** Comparison of the same region as in ***a*** of ^{1}H-^{1}H NOESY spectra (mixing time of 100 ms) of the ternary mixture of TauR' (1.1 mM), I19L (1.1 mM) and tubulin (35 μM) (magenta) and a mixture of TauR' (1.1 mM), I19L (1.1 mM) (blue) in the absence of tubulin.

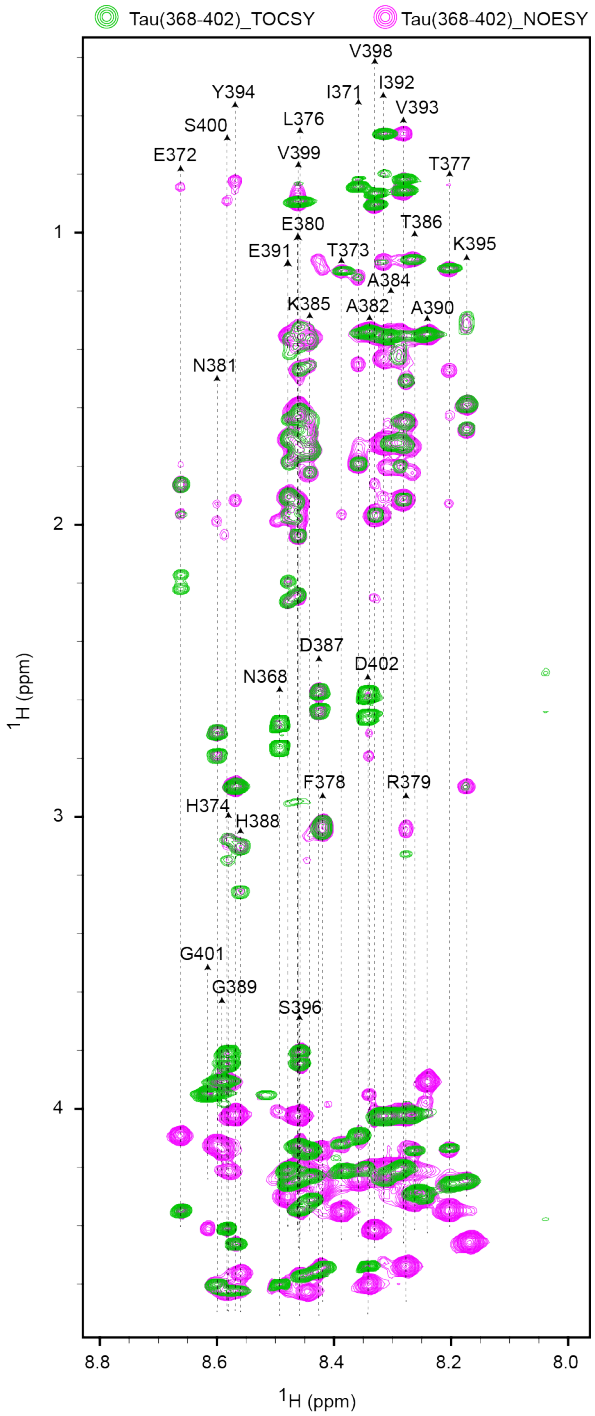

Figure S7. Superposition of a selected region of ^{1}H-^{1}H NOESY (magenta) and ^{1}H-^{1}H TOCSY (green) spectra of TauR'. The sample contained 1 mM TauR' in 50 mM sodium phosphate, pH 6.8. HN chemical shifts of TauR' residues are marked by dashed lines.

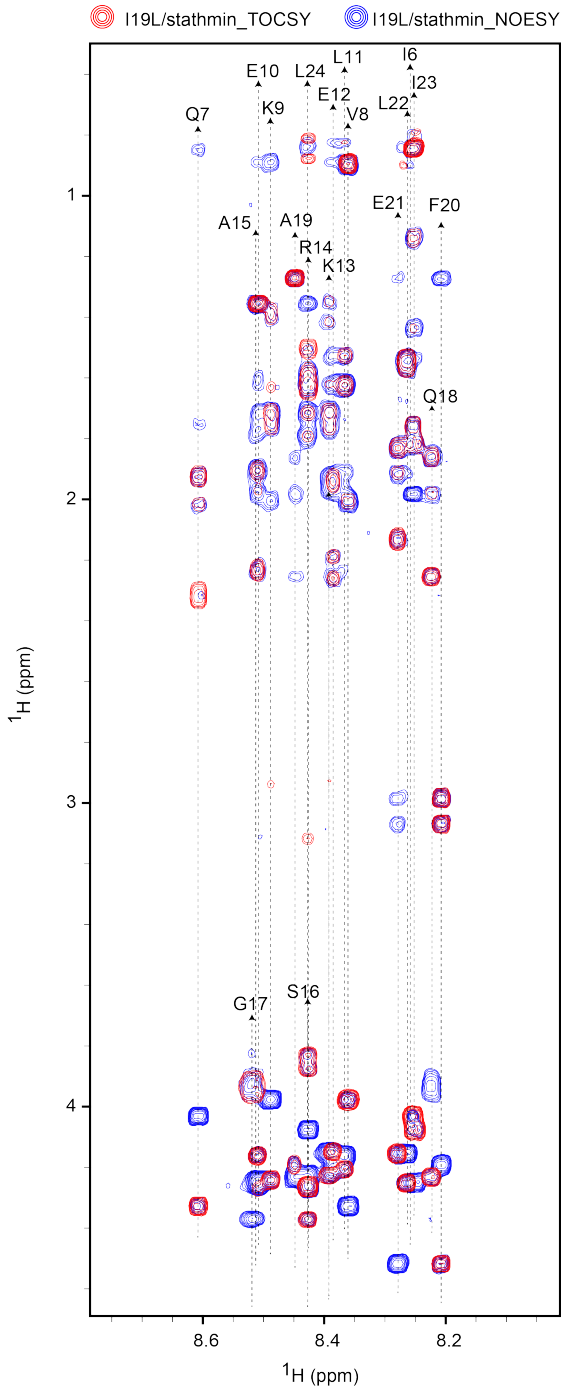

Figure S8. Superposition of a selected region of ^{1}H-^{1}H TOCSY (red) and NOESY (blue) spectra of I19L. The sample contained 1.0 mM I19L in 50 mM sodium phosphate, pH 6.8. HN chemical shifts of I19L residues are marked by dashed lines.

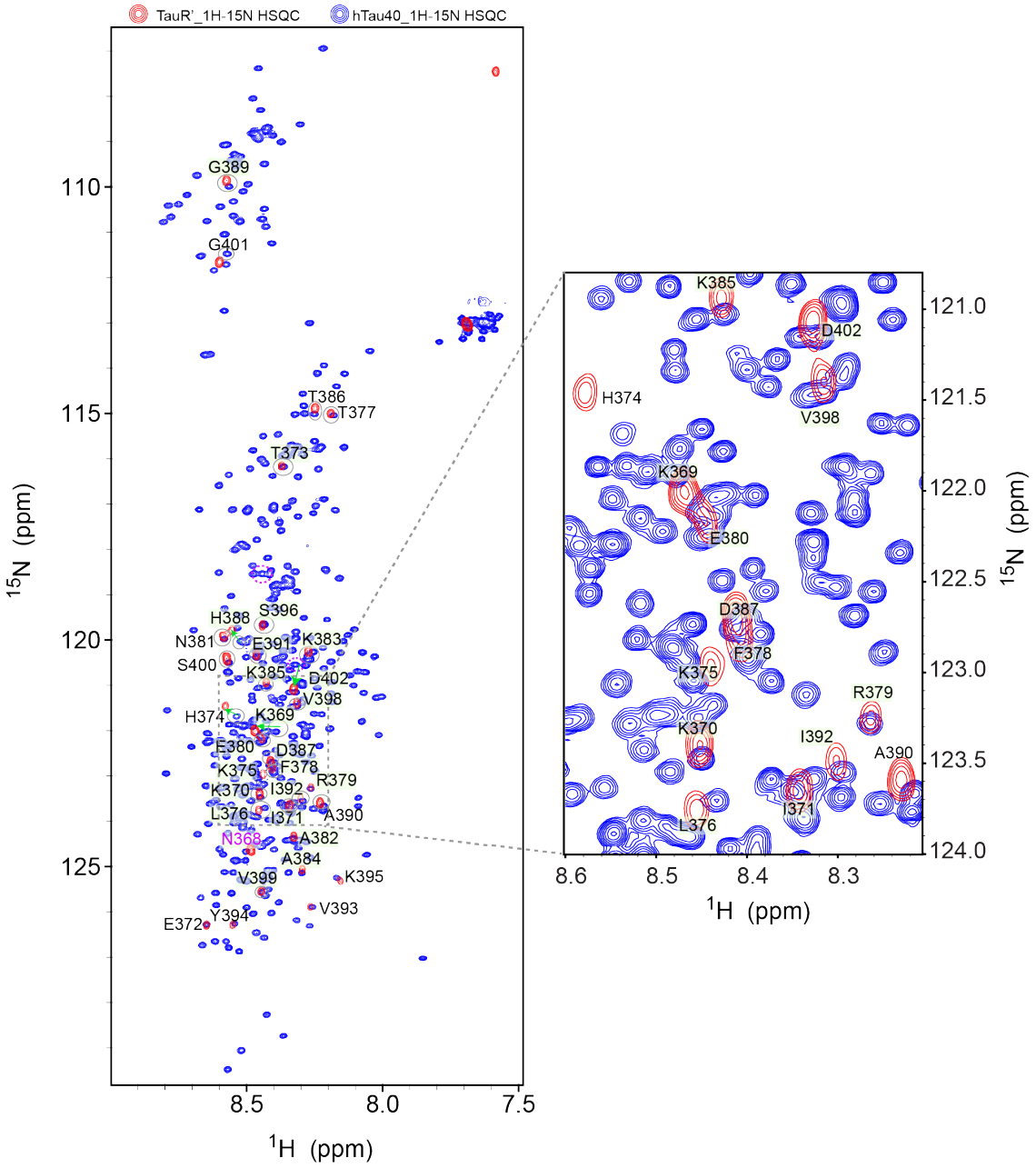

Figure S9. Superposition of ^{1}H–^{15}N HSQC spectra of ^{15}N-labelled full-length 2N4R Tau (25 μM; blue) and natural abundance spectra of TauR' (1.0 mM; red). Spectra were recorded at 278 K.

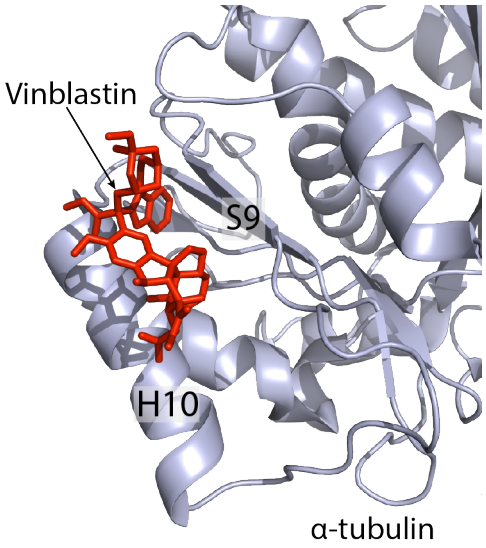

Figure S10. Vinblastine bound to α-Tubulin in the 3D structure of the (Tc)2R complex (PDB ID 1Z2B; [1]).

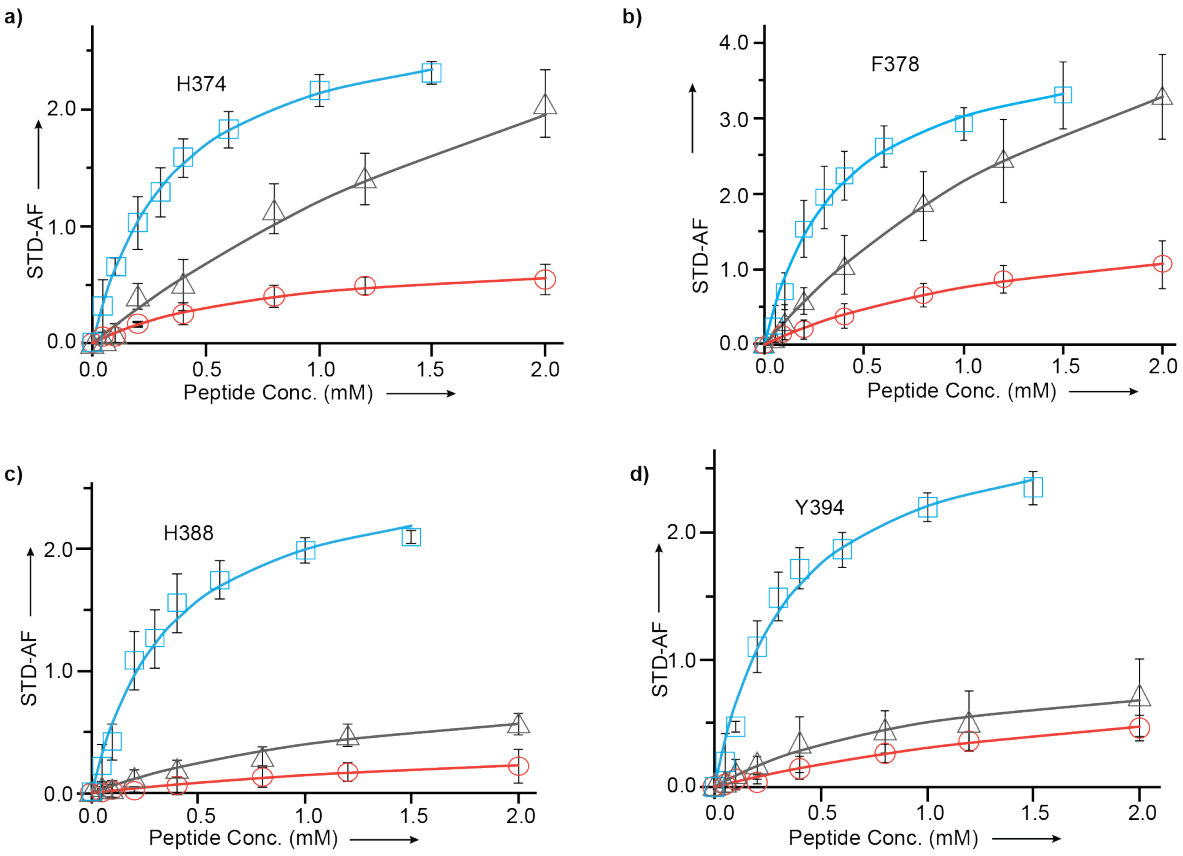

Figure S11. Phosphorylation modulates the TauR'/tubulin interaction. **a-d)** STD-AF values of selected atoms of TauR' (blue), TauR'-Y394p (grey) and TauR'-S396p (red) for increasing peptide concentrations in presence of 10 µM tubulin. Lines represent best fits, from which K_d values were derived.

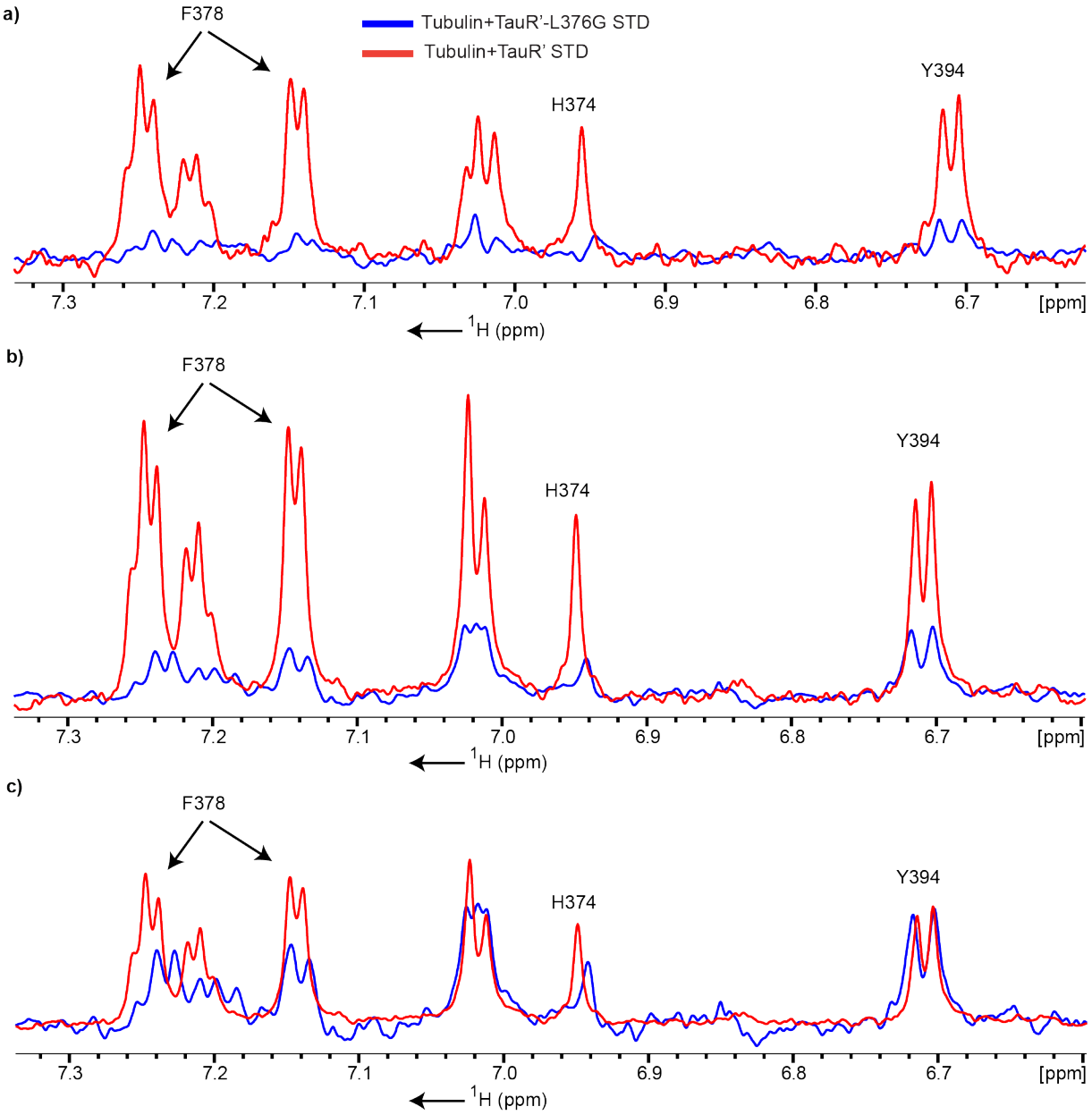

Figure S12. Superposition of STD spectra of TauR' (red) and TauR'-L376G (blue). a) STD spectra from 10 μM tubulin and 400 μM peptide. b) 10 μM tubulin with 2 mM TauR'-L376G (blue) and with 1.5 mM TauR' (red). c) same as in (b) but normalized against the intensity of Y394.

Supplementary Tables

Table S1. Acquisition parameters of NMR spectra recorded for TauR'.

Experiment name	Field strength (MHz)	Spectral width F2 / F1 (Hz)	Total points F2 / F1 (Hz)	No. of transients	Carrier frequency F1 (ppm)[b]	Mixing time (ms)
$^{1}H,^{1}H$-TOCSY[a]	700	8389.2/ 8401.6	2048 / 800	56	4.7	80
$^{1}H,^{1}H$-TOCSY[a]	800	8802.8/ 8793.9	2048 / 800	40	4.7	80
$^{1}H,^{1}H$-NOESY[a]	700	8389.2/ 8401.6	2048 / 800	56	4.7	150
$^{1}H,^{1}H$-NOESY[a]	900	10775.8/ 10797.6	2048 / 750	56	4.7	50/100
$^{1}H,^{13}C$-HSQC[a]	900	9920.6 / 14028.3	1024 / 256	40	37	n.a.
$^{1}H,^{15}N$-HSQC[a]	900	9920.6/ 2370.8	1024 / 128	256	118	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] – Carrier frequencies in the direct dimension F2 were set to the water resonance.

Table S2. Acquisition parameters of NMR spectra recorded for I19L.

Experiment name	Field strength (MHz)	Spectral width F2 / F1 (Hz)	Total points F2 / F1 (Hz)	No. of transients	Carrier frequency F1 (ppm)[b]	Mixing time (ms)
$^{1}H,^{1}H$-TOCSY[a]	600	6147.5/ 6148.6	2048 / 600	80	4.7	75
$^{1}H,^{1}H$-TOCSY[a]	700	8389.2/ 8401.6	2048 / 512	72	4.7	60
$^{1}H,^{1}H$-NOESY[a]	600	6147.5/ 6148.6	2048 / 600	80	4.7	250
$^{1}H,^{1}H$-NOESY[a]	700	8389.2/ 8401.6	2048 / 512	80	4.7	200

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

Table S3. NMR acquisition parameters for INPHARMA experiments.

Sample content	Experiment name	Field strength (MHz)	Spectral width F2 / F1 (Hz)	Total points F2 / F1 (Hz)	No. of transients	Carrier frequency F1 (ppm)[e]	Mixing time (ms)
TauR'	^{1}H,^{1}H-NOESY[a]	900	10775.862 / 10798.851	2048 / 512	96	4.7	100
TauR'+tubulin	^{1}H,^{1}H-tr-NOESY[b]	900	10775.862 / 10798.851	2048 / 512	96	4.7	100
I19L	^{1}H,^{1}H-NOESY[a]	900	10775.862 / 10798.851	2048 / 512	96	4.7	100
I19L+tubulin	^{1}H,^{1}H-tr-NOESY[b]	900	10775.862 / 10798.851	2048 / 512	96	4.7	100
TauR'+I19L	^{1}H,^{1}H-NOESY[c]	900	10775.862 / 10798.851	2048 / 512	96	4.7	100, 200
TauR'+I19L+tubulin	^{1}H,^{1}H-tr-NOESY[d]	900	10775.862 / 10798.851	2048 / 512	96	4.7	100, 200

[a] – The sample contained 0.7 mM peptide dissolved in 50 mM NaH_2PO_4/Na_2HPO_4, pH 6.8, 10% ($^v/_v$) D_2O.
[b] – The sample contained 0.7 mM peptide and 35 µM tubulin dissolved in 50 mM NaH_2PO_4/Na_2HPO_4, pH 6.8, 10% ($^v/_v$) D_2O.
[c] – The sample contained 1.1 mM of each peptide dissolved in 50 mM NaH_2PO_4/Na_2HPO_4, pH 6.8, 10% ($^v/_v$) D_2O.
[d] – The sample contained 1.1 mM of each peptide and 35 µM tubulin dissolved in 50 mM NaH_2PO_4/Na_2HPO_4, pH 6.8, 10% ($^v/_v$) D_2O.
[e] – Carrier frequencies in the direct dimension F2 were set to the water resonance.

Supplementary Experimental Section

STD NMR spectra [2] of TauR' and I19L with and without tubulin heterodimers were recorded on a 700 MHz spectrometer at 25 °C on samples containing 0.6 mM peptide and 15 µM tubulin (molar ratio 40:1). The magnetization of tubulin was saturated using a series of equally spaced 50 ms Gaussian-shaped pulses (40 pulses give a total saturation time of 1.5 s). On- and off-resonance frequencies were set to -0.5 ppm and 60 ppm, respectively. STD buildup curves were obtained with saturation times of 0.5, 1, 1.5, 2, 3, 4, 5 and 7 s. STD amplification factors (STD-AF) were calculated as the product of ηSTD (ratio of STD intensity to reference intensity) and the excess of ligand.[2] STD-AFs at different saturation times (t_{sat}) were fitted to the equation STD-AF(t_{sat}) = STD^{max} (1–exp(–k_{sat} t_{sat})), where STD^{max} represents the asymptotic maximum of the STD buildup curve and k_{sat} is a rate constant, which measures the speed of STD buildup. Binding affinities were determined using STD experiments at increasing peptide concentrations (TauR' and I19L) for constant tubulin concentrations of 10 µM and 5 µM, respectively. STD-AF values (at a fixed saturation time) of a peptide proton as a function of peptide concentration follows a hyperbolic dose-response curve [3] and were fitted according to STD-AF=(α_{STD}*[L])/(K_d+[L]), where α_{STD} represents the maximum STD amplification factor for a given proton signal. INPHARMA experiments [4] were performed by acquiring ^{1}H-^{1}H NOESY (NOE

mixing times of 100 and 200 ms) spectra on a sample containing 1.1 mM TauR', 1.1 mM I19L and 35 μM Tubulin in NMR buffer. To distinguish intra-peptide NOEs from INPHARMA contacts, NOESY spectra of TauR' and I19L n the presence of unpolymerized tubulin (35 μM) were acquired separately.

Supplementary References

[1] B. Gigant, C. Wang, R. B. G. Ravelli, F. Roussi, M. O. Steinmetz, P. A. Curmi, A. Sobel, M. Knossow, *Nature* **2005**, *435*, 519-522.

[2] M. Mayer, B. Meyer, *Angew Chem Int Edit* **1999**, *38*, 1784-1788.

[3] C. A. Lepre, J. M. Moore, J. W. Peng, *Chem Rev* **2004**, *104*, 3641-3676.

[4] V. M. Sanchez-Pedregal, M. Reese, J. Meiler, M. J. Blommers, C. Griesinger, T. Carlomagno, *Angew Chem Int Ed Engl* **2005**, *44*, 4172-4175.