Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

Folding of the Tau Protein on Microtubules**

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Supporting Methods

Sample Preparation. 441-residue Tau was expressed in *Escherichia coli* and purified as described previously.[1] For ^{15}N-labeling, E.coli bacteria were grown in minimal medium containing 1g/liter of $^{15}NH_4Cl$. Synthetic peptides were purchased from EZBiolab, USA, or synthesized in house on ABI 433A (Applied Biosystems) and Liberty 1 (CEM) machines. Peptides were synthesized with acetyl- and amide protection groups at the N- and C-termini, respectively. Peptides were purified by reversed-phase HPLC and the pure product was lyophilized.

Microtubule assembly. Porcine brain tubulin was purified as described.[2] Tubulin polymerization was performed in microtubule assembly buffer containing 100 mM Na-PIPES, pH 6.9, 1 mM EGTA, 1 mM MgSO4, 1 mM GTP and 1 mM DTT. To induce formation of microtubules, fixed concentrations of tubulin (20-50 µM) were incubated with equal concentrations of paclitaxel at 37°C for 20-30 min. The suspensions of the samples were fractionated by ultracentrifugation at 40,000 x g for 20 min. For NMR measurements, the microtubule pellet was suspended in 50 mM phosphate buffer.

NMR Spectroscopy. NMR measurements were performed in 50 mM sodium phosphate buffer, pH 6.8, 10% D_2O. 2D ^{1}H-^{15}N HSQC experiments [3] of 15N-labeled full-length Tau were recorded at 5 °C on Bruker spectrometers equipped with cryoprobes. Unlabeled Tau peptides were sequence-specifically assigned using 2D homonuclear DQF-COSY, TOCSY and NOESY spectra[4] and supported by 2D ^{13}C and ^{15}N natural abundance HSQC spectra recorded at 5 °C on Bruker 600 and 700 MHz spectrometers (Supporting Tables S1-S3). Tr-NOESY spectra[5] (Supporting Tables S1-S3) were performed at 5 °C on a Bruker 900 MHz spectrometer equipped with a cryoprobe. Samples were prepared with a concentration of 0.7 mM peptide and 35 µM microtubules at a molar ratio of 20:1. The buffer contained 50 mM NaH_2PO_4/Na_2HPO_4, pH 6.8, 10% D_2O. NOE mixing times of 30, 50, 80, 100, 150 and 250 ms were used. All spectra were processed using Topspin 3.1(Bruker) and NMRPipe[6] and analyzed using Sparky 3.114.

Structure Calculation. Distance restraints were obtained from tr-NOE contacts observed in 2D ^{1}H-^{1}H NOESY spectra acquired with either 80 or 100 ms mixing time. For structure calculations, only medium- and long-range contacts were taken into account and were enforced as distance restraints with a lower bound of 1.8 Å and an upper bound of 6.0 Å (Supporting Table S4). Initial structure calculations were performed using CYANA 3.0 and 200 conformers were calculated using the standard simulated annealing schedule with 10000 torsion angle dynamics steps per conformer. Subsequently, the structures derived from CYANA were refined in XPLOR-NIH using a restrained simulated annealing protocol[7]. Default values were used for force constants and molecular parameters unless otherwise indicated. 200 conformers were calculated and the 20 lowest-energy conformers were selected for further analysis using iCing (https://nmr.le.ac.uk/icing/#file). Visualization was performed using MOLMOL[8] and PYMOL (The PyMOL Molecular Graphics System, Version 1.5.0.4 Schrödinger, LLC).

Electron Microscopy. Electron microscopy grids were directly prepared after the turbidity assay and all steps carried out at 37°C. Formvar carbon coated copper grids (200 mesh) were glow-discharged for 30sec in a Pelco easiGlow instrument. 5µl samples were mixed with a final concentration of 2% (v/v) glycerol and incubated for 3 minutes on top of the grid. The solution was removed by filter paper, the grid washed 3 times with assembly buffer (supplemented with 2% (v/v) glycerol) and negatively stained by incubating for 1 minute with 2% uranyl acetate, followed by one washing step with ddH_2O. The grids were analyzed at 200 kV in a JEOL JEM-2200FS transmission electron microscope.

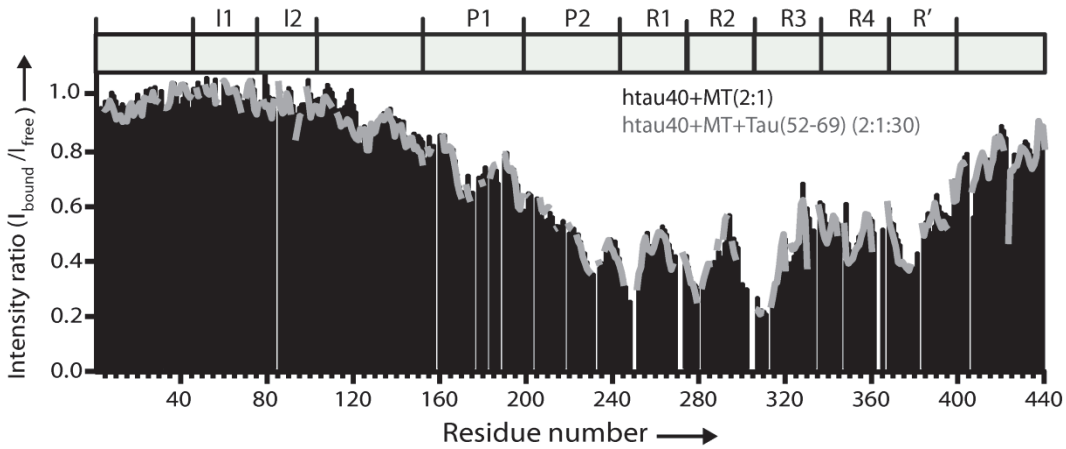


Figure S1. Competition between Tau(52-69) and full-length Tau for binding to microtubules. Black bars show the ratio of ^{1}H-^{15}N NMR signal intensities of Tau bound to microtubules, I_{bound} and free Tau, I_{free}, and grey line represents the ratio of signal intensities after addition of a 15-fold excess of Tau(52-69). Signal intensities remain unchanged.

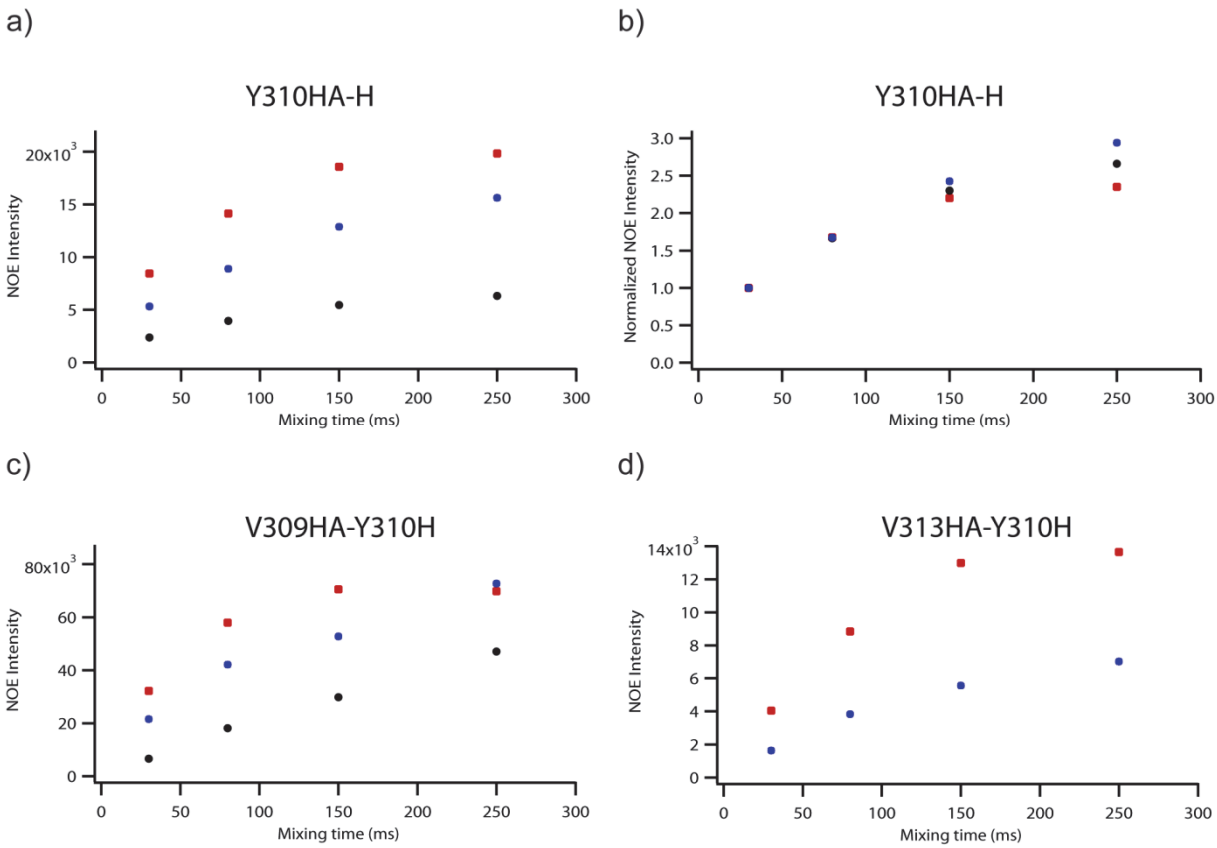


Figure S2. NOE build up curves of Tau(296-321) in the absence (black) and presence of microtubules (red) or unpolymerized tubulin (blue).

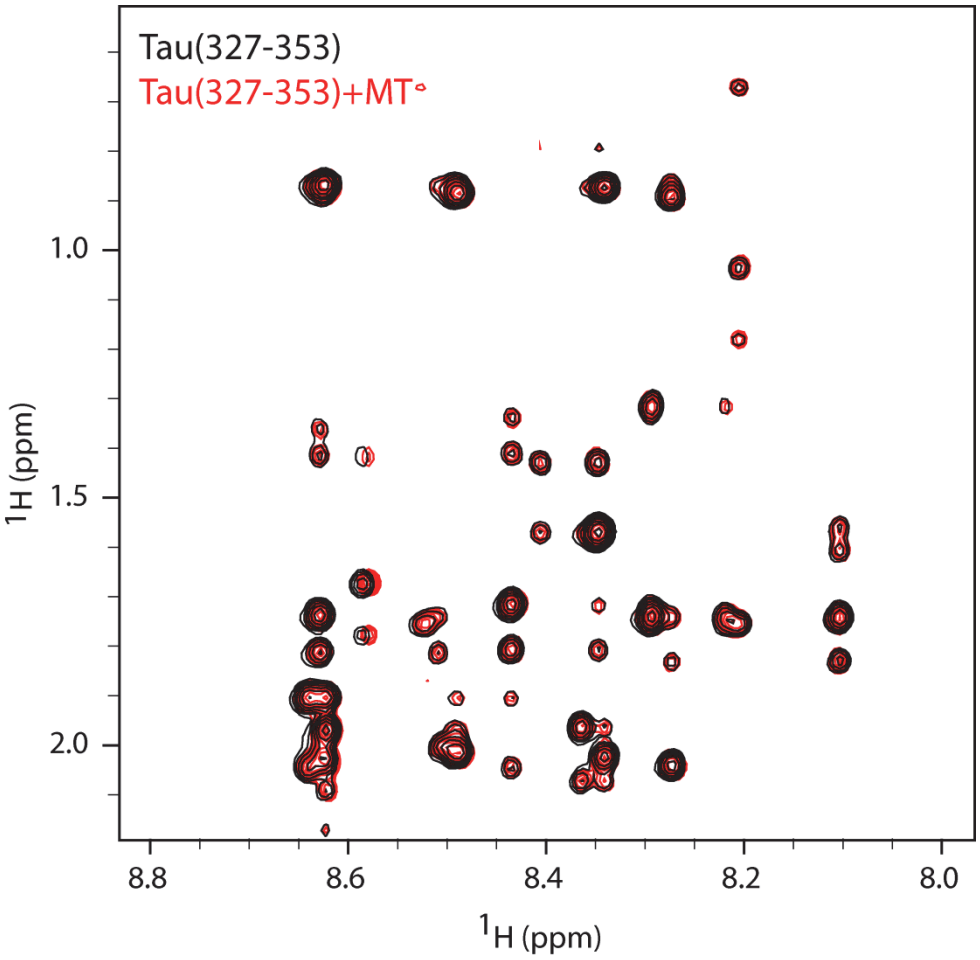


Figure S3. Selected regions of 2D ^{1}H-^{1}H NOESY spectra recorded on samples containing 1 mM Tau(327-353) alone (black) or in the presence of 50 µM microtubules (red). The peptide:tubulin heterodimer ratio was 20:1. Spectra were recorded at 278K and 900 MHz proton Larmor frequency.

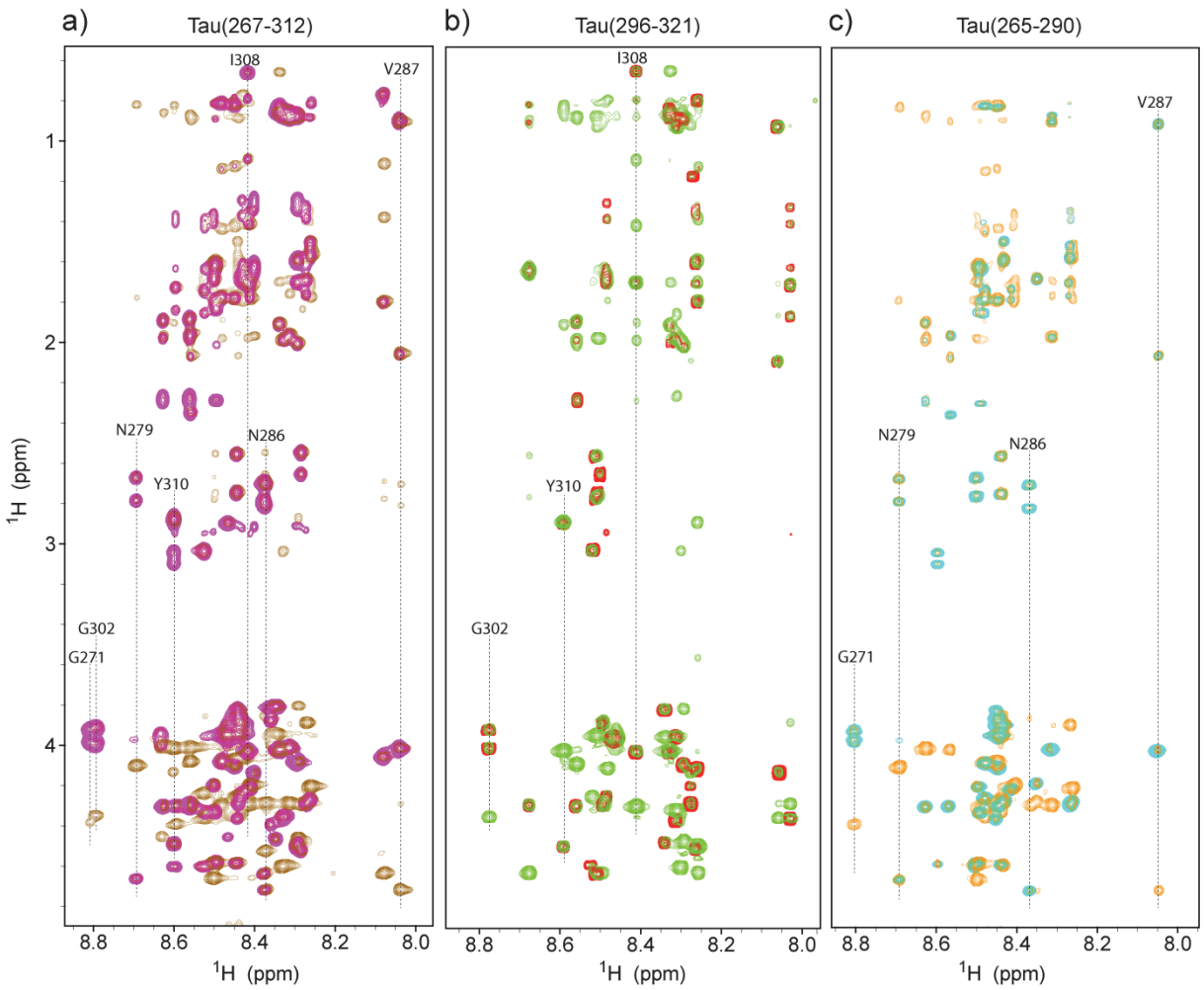
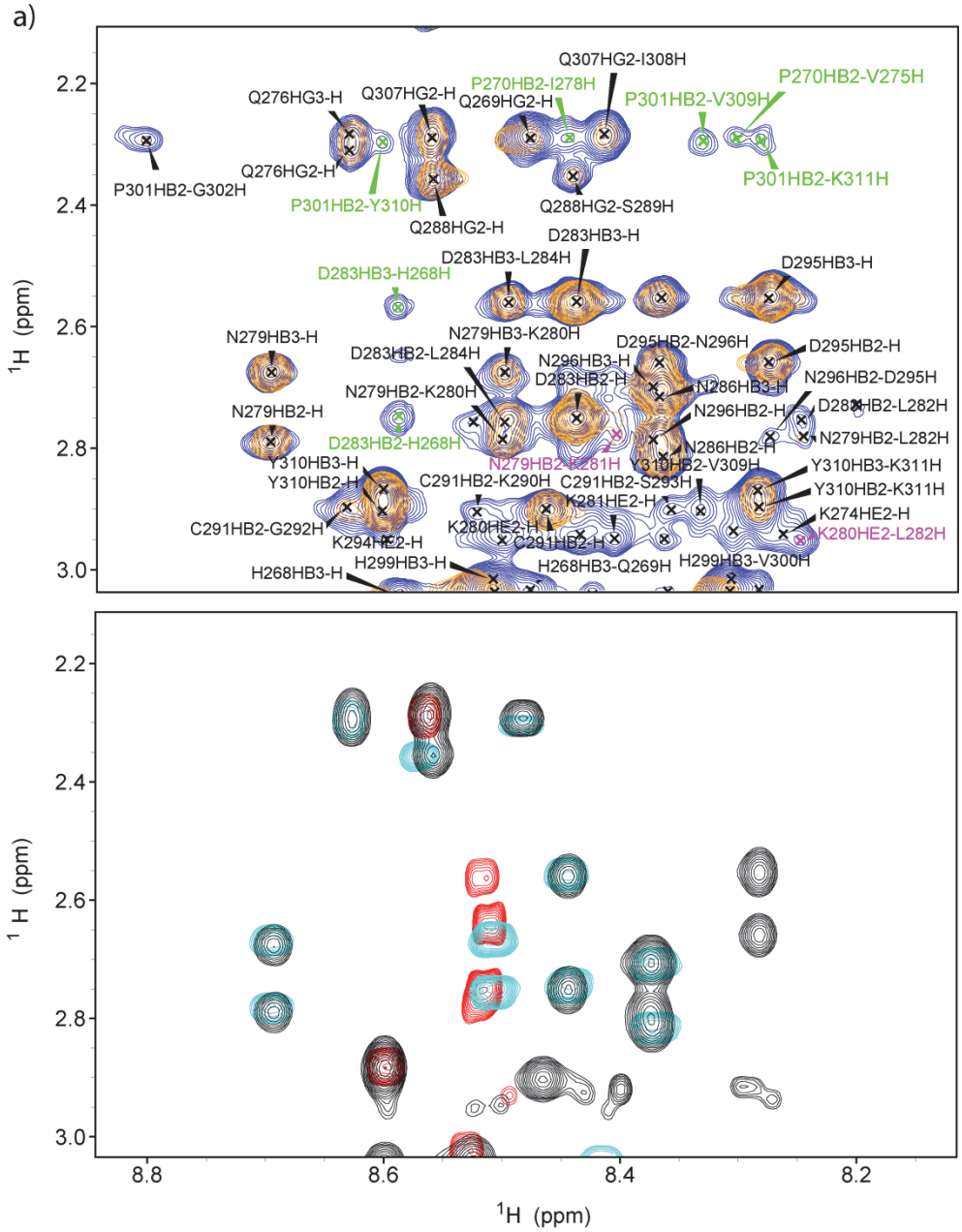


Figure S4. Superposition of the fingerprint region of TOCSY and NOESY spectra used for the resonance assignment of the Tau peptides. (a) TOCSY (magenta) and NOESY (brown) of Tau(267-312), (b) TOCSY (red) and NOESY (green) of Tau(296-321) and (c) TOCSY (cyan) and NOESY (orange) of Tau(265-290). Selected spin systems are labeled. The absence and presence of a few spin systems in Tau(267-312) in comparison to shorter peptides is due to the difference in the length of the sequences.

a)


Q307HG2-I308H
P270HB2-I278H
Q269HG2-H
P301HB2-V309H
P270HB2-V275H
Q276HG3-H
Q307HG2-H
Q276HG2-H
P301HB2-G302H
P301HB2-Y310H
Q288HG2-H
Q288HG2-S289H
P301HB2-K311H
D283HB3-H
D283HB3-L284H
D283HB3-H268H
D295HB3-H
N279HB3-H
N279HB3-K280H
D295HB2-N296H
D295HB2-H
D283HB2-L284H
N296HB3-H
D283HB2-H
N286HB3-H
N296HB2-D295H
N279HB2-K280H
N279HB2-H
N296HB2-H
D283HB2-L282H
D283HB2-H268H
N279HB2-L282H
N279HB2-K281H
N286HB2-H
Y310HB3-H
Y310HB2-V309H
Y310HB3-K311H
Y310HB2-H
C291HB2-K290H
C291HB2-S293H
Y310HB2-K311H
K281HE2-H
K274HE2-H
C291HB2-G292H
K280HE2-H
K280HE2-L282H
K294HE2-H
C291HB2-H
H299HB3-H
H268HB3-Q269H
H299HB3-V300H
H268HB3-H
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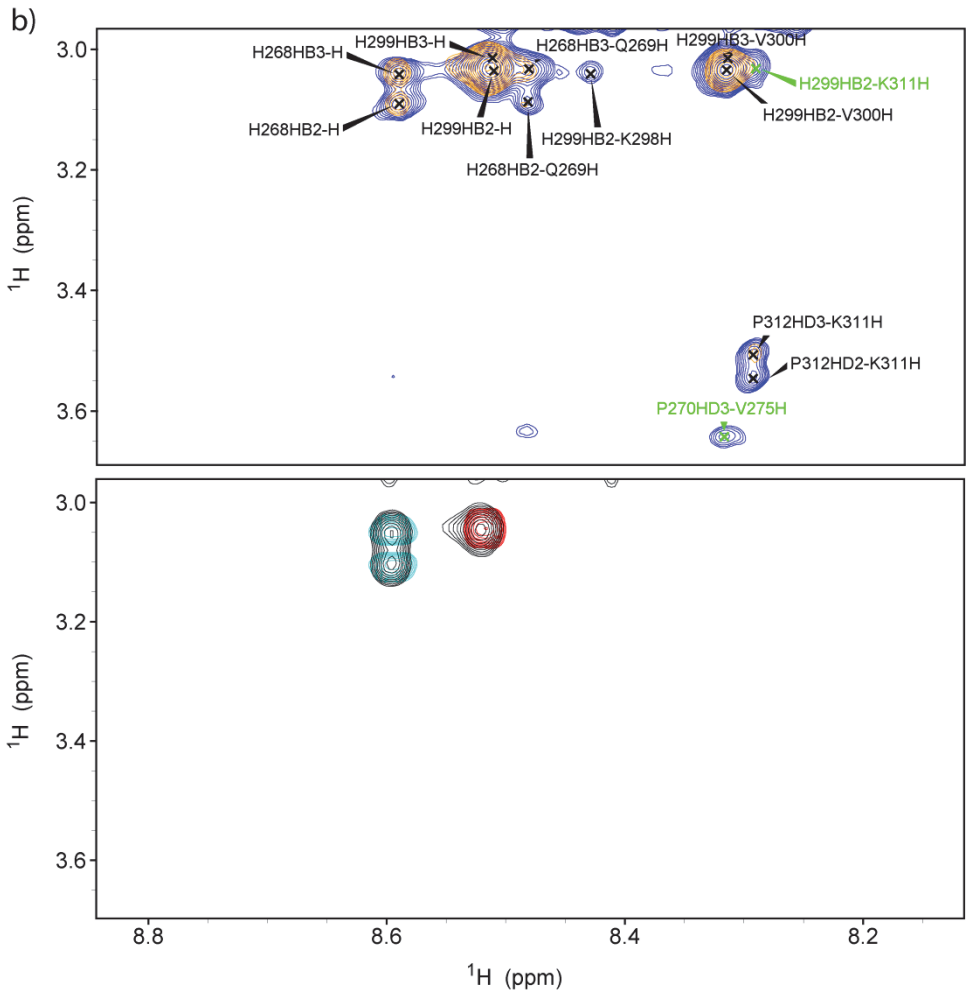
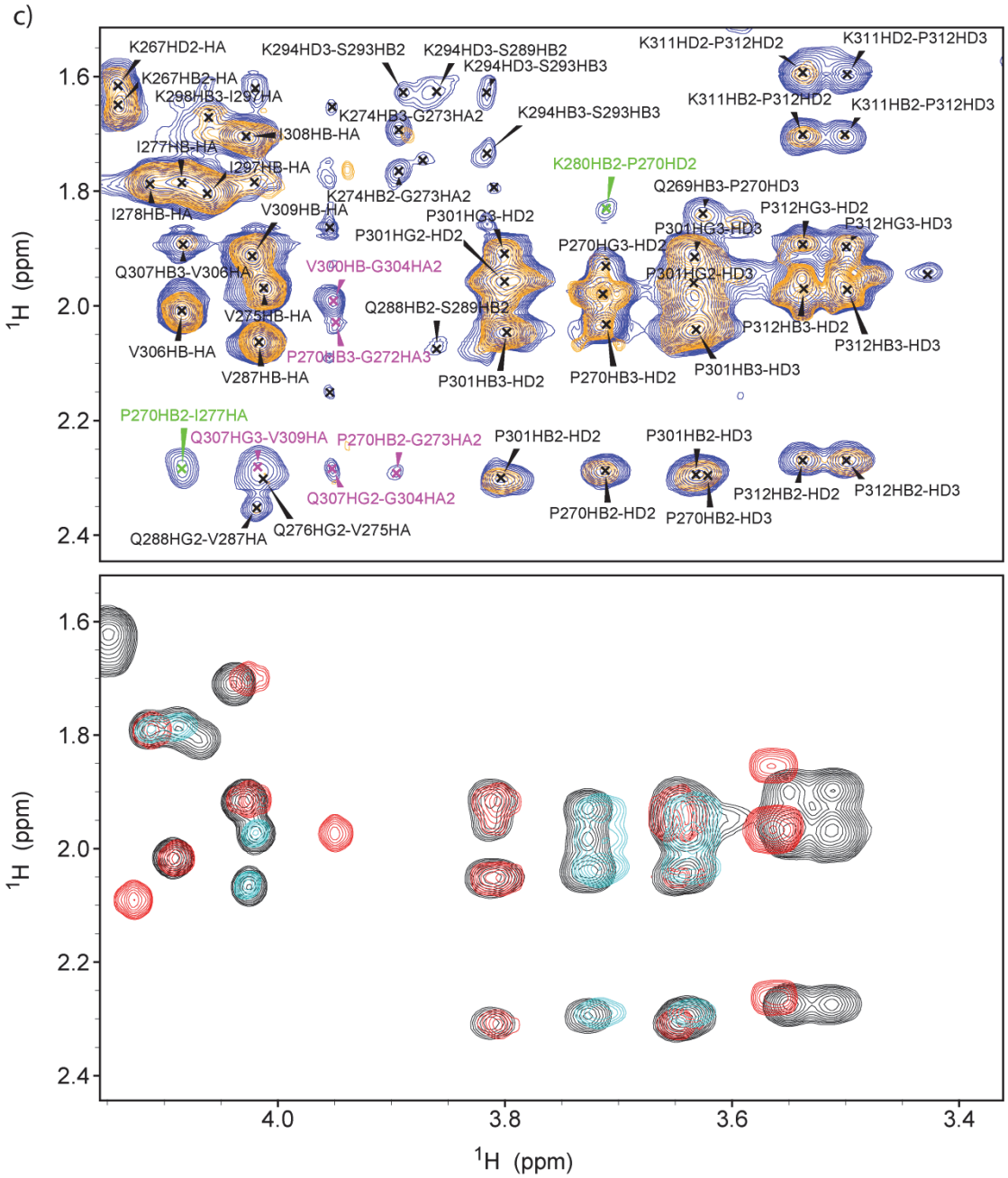
2.8
3.0
8.8
8.6
8.4
8.2


b)
H268HB3-H
H299HB3-H
H268HB3-Q269H
H299HB3-V300H
H299HB2-K311H
H268HB2-H
H299HB2-H
H299HB2-K298H
H299HB2-V300H
H268HB2-Q269H
P312HD3-K311H
P312HD2-K311H
P270HD3-V275H
3.0
3.2
3.4
3.6
^{1}H (ppm)
8.8
8.6
8.4
8.2
^{1}H (ppm)

c)


K267HD2-HA
K267HB2-HA
K298HB3-I297HA
I277HB-HA
I297HB-HA
I278HB-HA
I308HB-HA
K294HD3-S293HB2
K294HD3-S289HB2
K294HD3-S293HB3
K274HB3-G273HA2
K294HB3-S293HB3
K274HB2-G273HA2
V309HB-HA
K311HD2-P312HD2
K311HD2-P312HD3
K311HB2-P312HD2
K311HB2-P312HD3
K280HB2-P270HD2
Q269HB3-P270HD3
P312HG3-HD2
P312HG3-HD3
P301HG3-HD2
P301HG2-HD2
P301HG3-HD3
P270HG3-HD2
P301HG2-HD3
Q307HB3-V306HA
V300HB-G304HA2
V275HB-HA
Q288HB2-S289HB2
P312HB3-HD2
P312HB3-HD3
V306HB-HA
P270HB3-G272HA3
V287HB-HA
P301HB3-HD2
P270HB3-HD2
P301HB3-HD3
P270HB2-I277HA
Q307HG3-V309HA
P270HB2-G273HA2
P301HB2-HD2
P301HB2-HD3
Q307HG2-G304HA2
P312HB2-HD2
P312HB2-HD3
P270HB2-HD2
P270HB2-HD3
Q288HG2-V287HA
Q276HG2-V275HA
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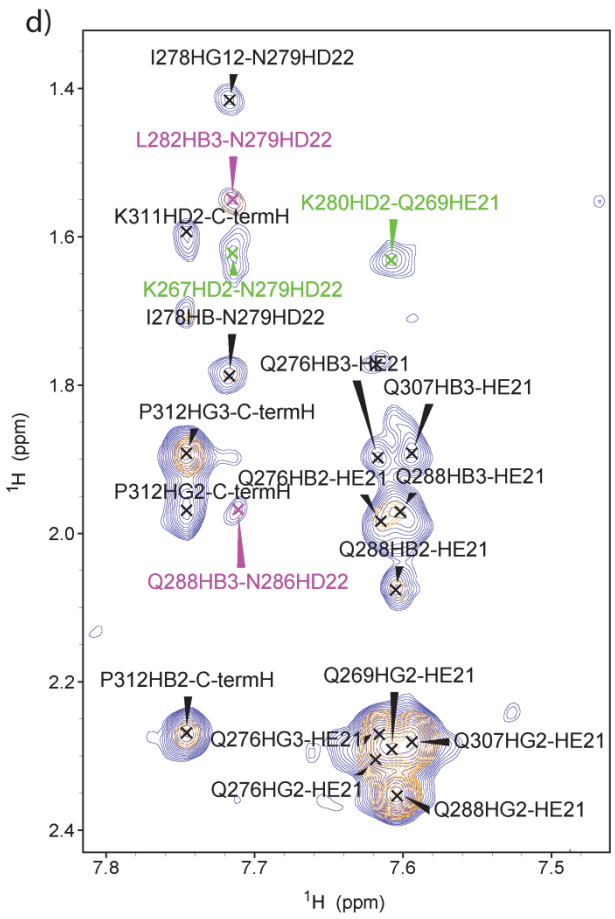
3.8
3.6
3.4
^{1}H (ppm)


d)
I278HG12-N279HD22
L282HB3-N279HD22
K280HD2-Q269HE21
K311HD2-C-termH
K267HD2-N279HD22
I278HB-N279HD22
Q276HB3-HE21
Q307HB3-HE21
P312HG3-C-termH
Q276HB2-HE21
Q288HB3-HE21
P312HG2-C-termH
Q288HB2-HE21
Q288HB3-N286HD22
P312HB2-C-termH
Q269HG2-HE21
Q276HG3-HE21
Q307HG2-HE21
Q276HG2-HE21
Q288HG2-HE21
1.4
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2.4
7.8
7.7
7.6
7.5
1H (ppm)
1H (ppm)

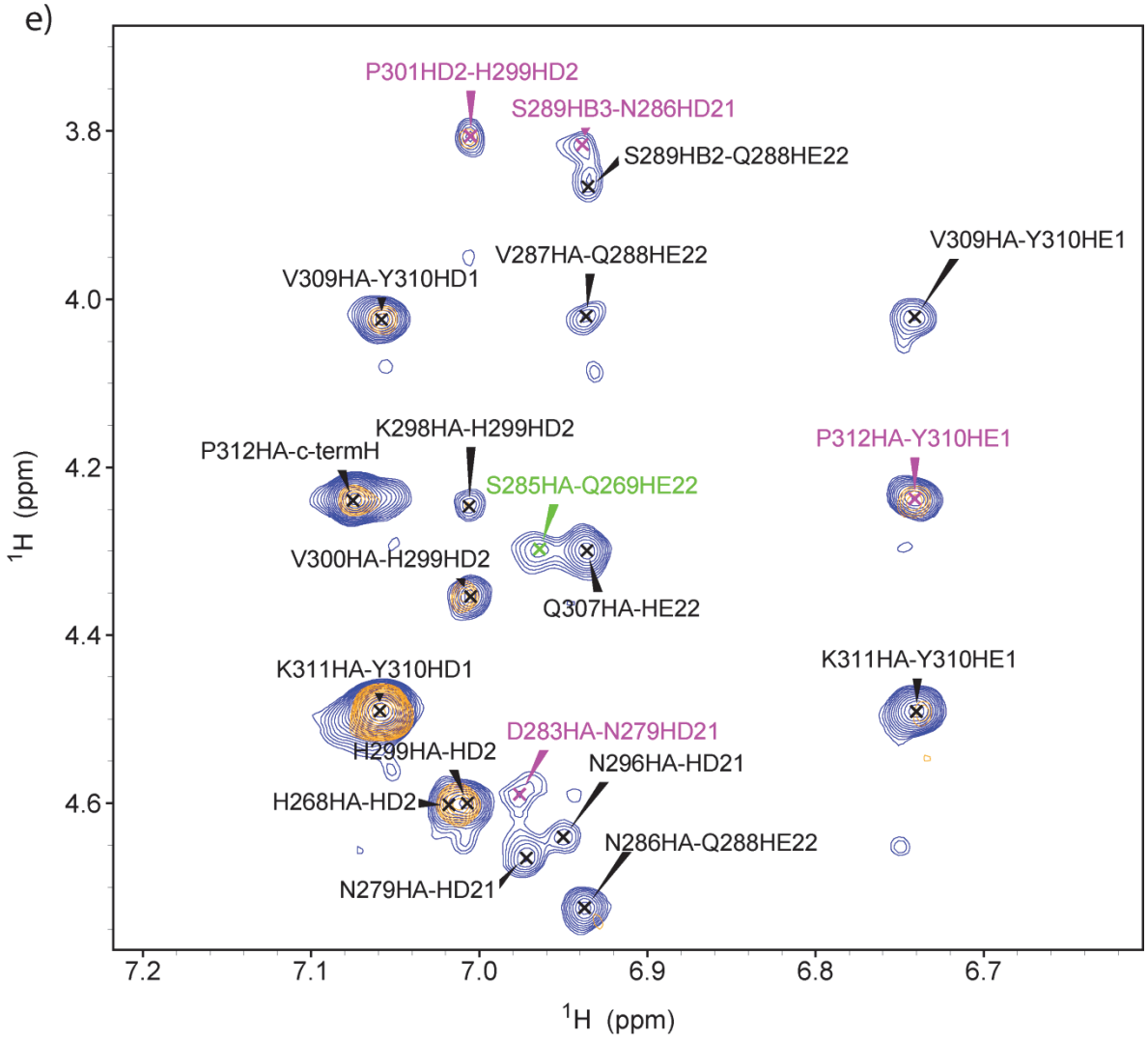
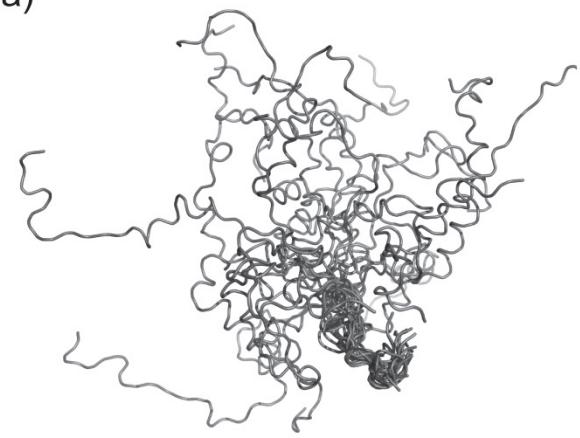


Figure S5. Selected regions of NOESY and TOCSY spectra used for sequence-specific assignment and identification of transferred NOE contacts. In (a-e) the superposition of the NOESY spectrum of MT-bound (blue) and free (orange) Tau(267-312) with the corresponding assignments is shown. Long- and medium-range NOEs are highlighted in green and magenta, respectively. In (a)-(c) an additional panel is shown below, which shows a superposition of the TOCSY spectra of Tau(267-312) (black), Tau(265-290) (cyan) and Tau(296-321) (red). Additional TOCSY peaks in the lower panels are due to the difference in the residue ranges in each peptide. There are no TOCSY peaks in the regions shown in (d) and (e).

a)

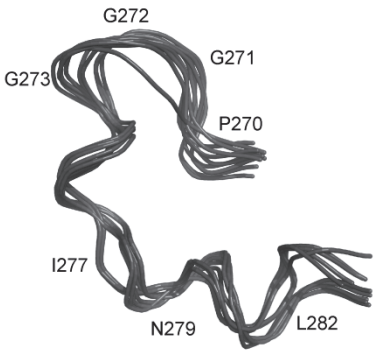


b)

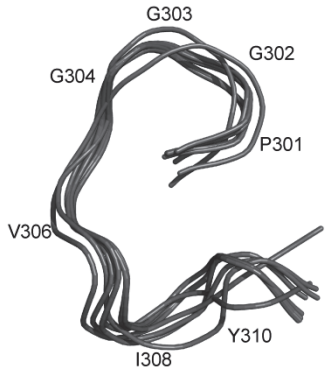
Structural statistics for the 20 final conformers of free Tau(267-312)	
Number of restraints	66
Medium range NOE (1< |i-j| < 5)	66
Long range NOE (|i-j| ≥ 5)	0
NOE violations >0.5 Å/structure	0
Ramachandran plot statistics	
Residues in most favored regions	83.7 %
Residues in additionally allowed regions	12.4 %
Residues in generously allowed regions	2.7 %
Residues in disallowed regions	1.1 %

Figure S6. In the absence of microtubules Tau(267-312) does not fold into a stable conformation. (a) Conformations of Tau(267-312) obtained by structure calculations, in which only NOE peaks of the peptide in the free state in solution were used. Intraresidual and sequential NOEs were excluded and the lower and upper limit was 1.8 and 6.0 Å, respectively. Conformations were aligned within the residue range 300-310. (b) Structural statistics of the 20 lowest-energy conformers of free Tau(267-312) in solution.

a)



b)



c)

Structural statistics for the 20 final conformers of MT-bound Tau(267-312)	
Number of restraints	182
Medium range NOE (1< |i-j| < 5)	141
Long range NOE (|i-j| ≥ 5)	41
NOE violations >0.5 Å/structure	0
Ramachandran plot statistics	
Residues in most favored regions	73.4 %
Residues in additionally allowed regions	23.9 %
Residues in generously allowed regions	1.7 %
Residues in disallowed regions	1.0 %
RMS deviations from the average structure	
Backbone atoms (Å)	1.80
Heavy atoms (Å)	1.87
(Residue range)	(269-284)
	1.49
	1.50
	(300-310)

Figure S7. Ensemble of the 10 lowest-energy conformers of Tau(267-312) determined on the basis of medium- and long-range contacts, which were enforced as distance restraints with a common lower and upper limit of 1.8 and 6.0 Å, respectively. Compared to the ensembles shown in Figure 3c and 3d the force constant for the XPLOR RAMA potential was increased. (a) Residues 269-284 of Tau(267-312) were aligned. (b) Residues 300-310 of Tau(267-312) were aligned. (c) Structural statistics.

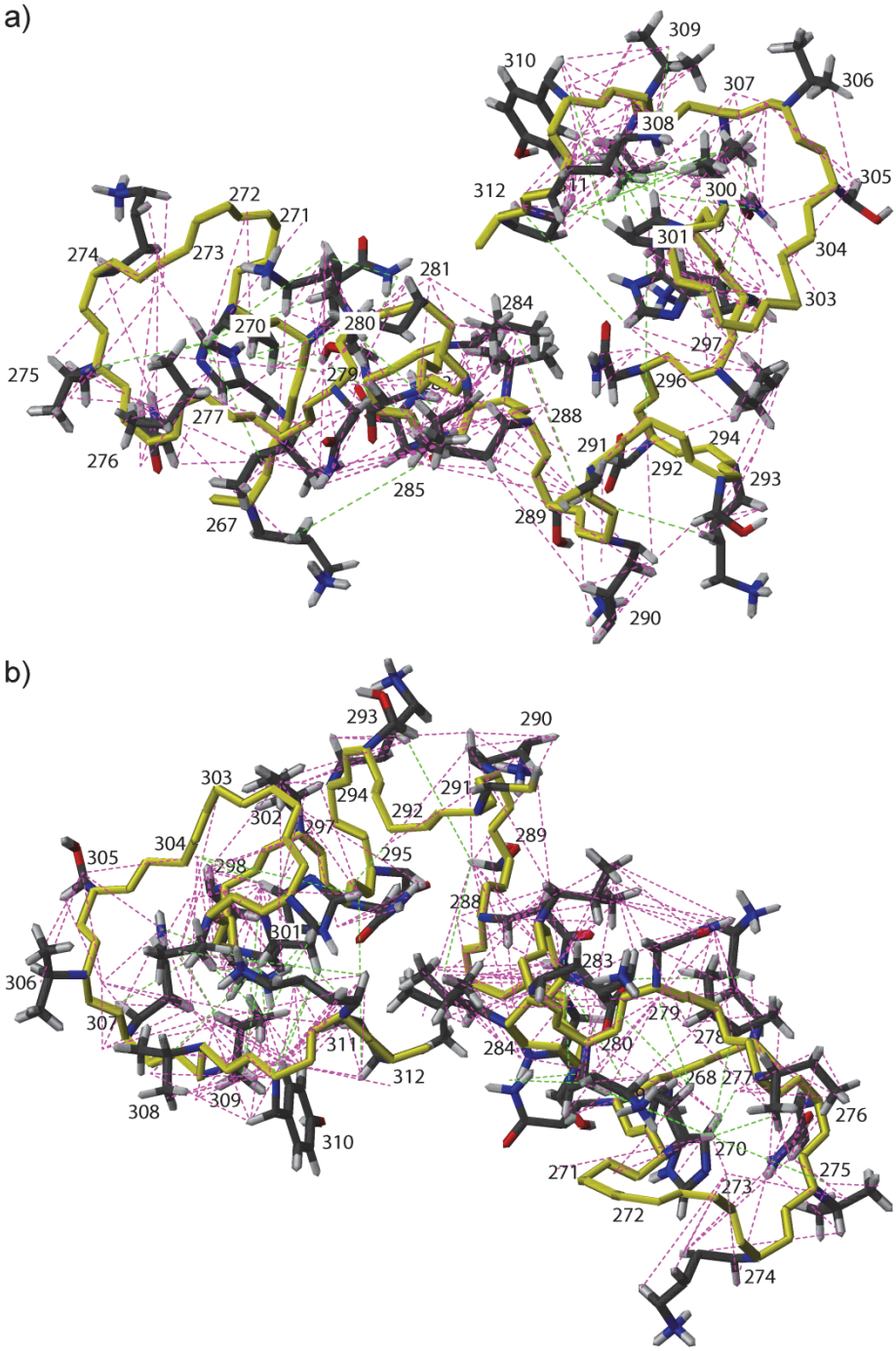


Figure S8. Ribbon representation of the lowest-energy conformer of Tau(267-312) in the presence of microtubules with NOE-derived distance restraints. Side chains are depicted with bonds. Long-range and medium-range contacts are shown by green and magenta dotted lines, respectively. (a) and (b) present two different views (similar to Figure 3).

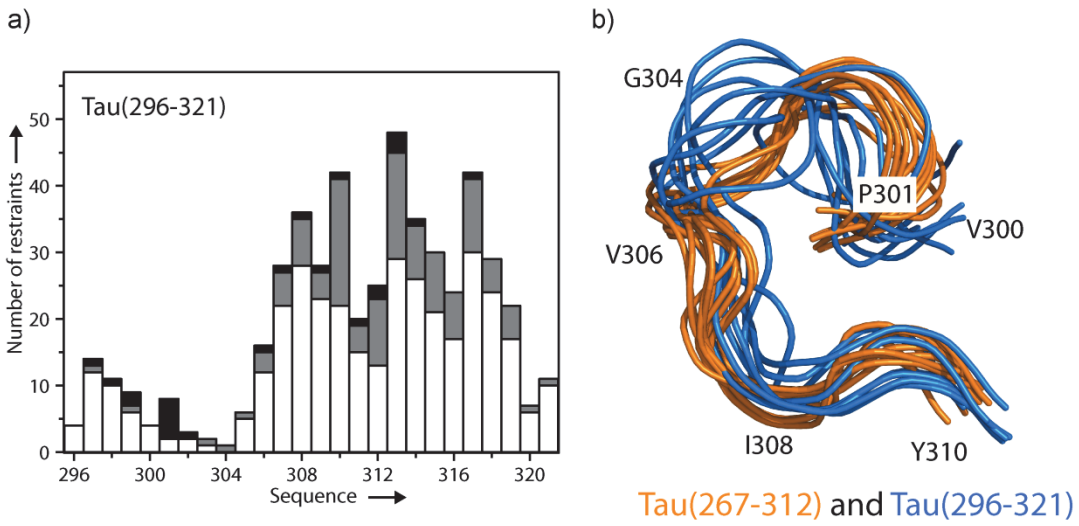


Figure S9. Conformation of Tau(296-321) induced upon binding to microtubules. (a) Distribution of distance constraints as a function of residue number. Distance constraints were classified as intraresidual and sequential (|i-j| ≤ 1; white), medium-range (1 < |i-j| ≤ 4; grey) and long-range (|i-j| ≥ 5; black), respectively. (b) Superposition of the 10 lowest-energy conformers of the structured region of Tau(296-321) (blue) with the corresponding region of Tau(267-312) (orange). The superposition of the two different ensembles was done using the align command in PyMOL (The PyMOL Molecular Graphics System, Version 1.5.0.4 Schrödinger, LLC). The structures were determined on the basis of medium- and long-range transferred NOE contacts, which were induced by binding to microtubules. All NOE contacts were enforced as distance restraints with lower and upper limits of 1.8 Å and 6.0 Å, respectively.

Table S1. NMR acquisition parameters of Tau(267-312).

Experiment name	Field strength (MHz)	Spectral width F2 / F1 (Hz)	Total points F2 / F1 (Hz)	No. of transients	Carrier frequency F1 (ppm)[c]	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 / 800	56	4.7	50/100/200/300
$^{1}H,^{1}H$-transferred NOESY[b]	900	10775.8/ 10797.6	2048 / 800	56	4.7	50/100/200/300
$^{1}H,^{13}C$-HSQC[a]	700	7716.1 / 12323.8	2048 / 256	48	40	n.a.
$^{1}H,^{15}N$-HSQC[a]	700	7716.1/ 1702.8	2048 / 128	320	118	n.a.

[a] – The sample contained 1 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 and 50 µM microtubules dissolved in 50 mM NaH_2PO_4/Na_2HPO_4, pH 6.8, 10% ($^{v}/_{v}$) D_2O.

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

Table S2. NMR acquisition parameters of Tau(265-290).

Experiment name	Field strength (MHz)	Spectral width F2 / F1 (Hz)	Total points F2 / F1 (Hz)	No. of transients	Carrier frequency F1 (ppm)[c]	Mixing time (ms)
$^{1}H,^{1}H$-TOCSY[a]	700	7716.1/ 7713.7	2048 / 1024	80	4.7	60
$^{1}H,^{1}H$-TOCSY[a]	600	6602.1/ 6602.7	2048 / 512	72	4.7	75
$^{1}H,^{1}H$-NOESY[a]	700	7716.1/ 7713.7	2048 / 1024	80	4.7	200
$^{1}H,^{1}H$-NOESY[a]	900	9920.6/ 9901.1	2048 / 512	64	4.7	50/100/200/300
$^{1}H,^{1}H$-transferred NOESY[b]	900	9920.6/ 9901.1	2048 / 512	64	4.7	50/100/200/300
$^{1}H,^{13}C$-HSQC[a]	700	7716.1 / 12323.8	2048 / 256	144	40	n.a.
$^{1}H,^{15}N$-HSQC[a]	700	7716.1/ 1847.7	2048 / 128	320	118	n.a.

[a] – The sample contained 1 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 and 50 µM microtubules dissolved in 50 mM NaH_2PO_4/Na_2HPO_4, pH 6.8, 10% ($^{v}/_{v}$) D_2O.

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

Table S3. NMR acquisition parameters of Tau(296-321).

Experiment name	Field strength (MHz)	Spectral width F2 / F1 (Hz)	Total points F2 / F1 (Hz)	No. of transients	Carrier frequency F1 (ppm)[c]	Mixing time (ms)
$^{1}H,^{1}H$-TOCSY[a]	700	7716.1/ 7713.7	2048 / 1024	72	4.7	60
$^{1}H,^{1}H$-TOCSY[a]	600	6602.1/ 6602.7	2048 / 512	56	4.7	75
$^{1}H,^{1}H$-NOESY[a]	700	7716.1/ 7713.7	2048 / 1024	80	4.7	300
$^{1}H,^{1}H$-NOESY[a]	900	9920.6/ 9901.1	2048 / 512	40	4.7	30/80/150/250
$^{1}H,^{1}H$-transferred NOESY[b]	900	9920.6/ 9901.1	2048 / 512	40	4.7	30/80/150/250
$^{1}H,^{13}C$-HSQC[a]	700	7716.1 / 12323.8	2048 / 256	104	40	n.a.
$^{1}H,^{15}N$-HSQC[a]	700	7716.1/ 1702.8	2048 / 128	320	118	n.a.

[a] – The sample contained 1 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 and 50 µM microtubules dissolved in 50 mM NaH_2PO_4/Na_2HPO_4, pH 6.8, 10% ($^{v}/_{v}$) D_2O.

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

Table S4. Distance restraint file used for the structure calculation of microtubule-bound Tau(267-312). Only medium- and long-range NOEs were used with lower and upper limits of 1.8 and 6.0 Å, respectively.

Residue/Atom		Residue/Atom		Lower [Å]	Upper [Å]
295	HA	297	HN	1.80	6.00
281	HA	283	HN	1.80	6.00
306	HA	308	HN	1.80	6.00
279	HA	281	HN	1.80	6.00
278	HA	280	HN	1.80	6.00
295	HB1	297	HN	1.80	6.00
295	HB2	297	HN	1.80	6.00
308	HD1#	310	HN	1.80	6.00
308	HD1#	310	HA	1.80	6.00
310	HD1	312	HD1	1.80	6.00
310	HE1	312	HD1	1.80	6.00
310	HE1	312	HA	1.80	6.00
271	HN	273	HN	1.80	6.00
293	HA	295	HN	1.80	6.00
285	HA	287	HN	1.80	6.00
297	HD1#	299	HN	1.80	6.00
306	HN	308	HD1#	1.80	6.00
308	HD1#	310	HD1	1.80	6.00
308	HB	310	HE1	1.80	6.00
308	HD1#	310	HE1	1.80	6.00
286	HA	288	HN	1.80	6.00
302	HN	304	HN	1.80	6.00
285	HN	287	HN	1.80	6.00
305	HA	307	HN	1.80	6.00
309	HB	311	HN	1.80	6.00
299	HD2	301	HD2	1.80	6.00
299	HD2	301	HD1	1.80	6.00
268	HD2	270	HA	1.80	6.00
308	HG12	310	HE1	1.80	6.00
299	HD2	301	HG1	1.80	6.00
299	HD2	301	HG2	1.80	6.00
297	HN	299	HN	1.80	6.00
308	HG11	310	HE1	1.80	6.00
306	HA	308	HD1#	1.80	6.00
296	HA	298	HN	1.80	6.00
310	HD1	312	HB2	1.80	6.00
310	HD1	312	HB1	1.80	6.00
310	HE1	312	HD2	1.80	6.00
310	HD1	312	HD2	1.80	6.00
299	HE1	301	HA	1.80	6.00

297	HB	299	HE1	1.80	6.00
297	HD1#	299	HE1	1.80	6.00
277	HG2#	279	HA	1.80	6.00
268	HD2	270	HD#	1.80	6.00
270	HB#	272	HA#	1.80	6.00
270	HG#	272	HA#	1.80	6.00
273	HA#	275	HN	1.80	6.00
273	HA#	275	HG#	1.80	6.00
274	HB#	276	HN	1.80	6.00
274	HB#	276	HE2#	1.80	6.00
274	HG#	276	HG#	1.80	6.00
274	HG#	276	HE2#	1.80	6.00
276	HB#	278	HG1#	1.80	6.00
276	HG#	278	HG1#	1.80	6.00
276	HE2#	278	HD1#	1.80	6.00
277	HB	279	HD2#	1.80	6.00
277	HG1#	279	HN	1.80	6.00
278	HA	280	HE#	1.80	6.00
279	HA	281	HB#	1.80	6.00
279	HB#	281	HN	1.80	6.00
279	HD2#	281	HE#	1.80	6.00
280	HA	282	HD#	1.80	6.00
280	HE#	282	HN	1.80	6.00
284	HB#	286	HN	1.80	6.00
284	HD#	286	HN	1.80	6.00
285	HB#	287	HN	1.80	6.00
286	HA	288	HG#	1.80	6.00
286	HA	288	HE2#	1.80	6.00
286	HD2#	288	HB#	1.80	6.00
287	HG#	289	HB#	1.80	6.00
288	HG#	290	HG#	1.80	6.00
288	HG#	290	HD#	1.80	6.00
290	HG#	292	HA#	1.80	6.00
291	HB#	293	HN	1.80	6.00
292	HA#	294	HN	1.80	6.00
295	HB#	297	HG2#	1.80	6.00
296	HN	298	HG#	1.80	6.00
297	HN	299	HB#	1.80	6.00
297	HD1#	299	HB#	1.80	6.00
298	HA	300	HG#	1.80	6.00
299	HA	301	HD#	1.80	6.00
299	HB#	301	HA	1.80	6.00
299	HB#	301	HD#	1.80	6.00
299	HD2	301	HD#	1.80	6.00
300	HG#	302	HN	1.80	6.00

304	HA#	306	HN	1.80	6.00
305	HA	307	HE2#	1.80	6.00
306	HG#	308	HN	1.80	6.00
307	HG#	309	HA	1.80	6.00
308	HG1#	310	HD1	1.80	6.00
308	HD1#	310	HB#	1.80	6.00
309	HA	311	HG#	1.80	6.00
310	HN	312	HD#	1.80	6.00
310	HD1	312	HG#	1.80	6.00
310	HD1	312	HD#	1.80	6.00
310	HE1	312	HB#	1.80	6.00
310	HE1	312	HG#	1.80	6.00
310	HE1	312	HD#	1.80	6.00
284	HA	287	HN	1.80	6.00
279	HD22	282	HD1#	1.80	6.00
279	HD22	282	HD2#	1.80	6.00
279	HD21	282	HD1#	1.80	6.00
279	HD21	282	HD2#	1.80	6.00
294	HA	297	HN	1.80	6.00
286	HA	289	HN	1.80	6.00
285	HB1	288	HN	1.80	6.00
285	HB2	288	HN	1.80	6.00
296	HN	299	HD2	1.80	6.00
284	HN	287	HN	1.80	6.00
295	HA	298	HN	1.80	6.00
270	HB#	273	HA#	1.80	6.00
279	HB#	282	HN	1.80	6.00
279	HB#	282	HD#	1.80	6.00
279	HD2#	282	HB#	1.80	6.00
279	HD2#	282	HD#	1.80	6.00
280	HA	283	HB#	1.80	6.00
281	HA	284	HD#	1.80	6.00
284	HB#	287	HN	1.80	6.00
284	HB#	287	HB	1.80	6.00
284	HD#	287	HN	1.80	6.00
284	HD#	287	HB	1.80	6.00
285	HB#	288	HN	1.80	6.00
286	HA	289	HB#	1.80	6.00
286	HD2#	289	HB#	1.80	6.00
288	HB#	291	HA	1.80	6.00
290	HB#	293	HB#	1.80	6.00
294	HA	297	HG1#	1.80	6.00
294	HD#	297	HG1#	1.80	6.00
300	HG#	303	HN	1.80	6.00
300	HG#	303	HA#	1.80	6.00

301	HD#	304	HN	1.80	6.00
304	HA#	307	HN	1.80	6.00
304	HA#	307	HG#	1.80	6.00
284	HD2#	288	HE22	1.80	6.00
284	HD1#	288	HE22	1.80	6.00
284	HD1#	288	HE21	1.80	6.00
284	HD2#	288	HE21	1.80	6.00
308	HB	312	HD2	1.80	6.00
308	HB	312	HD1	1.80	6.00
284	HB#	288	HE2#	1.80	6.00
284	HD#	288	HG#	1.80	6.00
284	HD#	288	HE2#	1.80	6.00
293	HB#	297	HN	1.80	6.00
294	HN	298	HG#	1.80	6.00
300	HB	304	HA#	1.80	6.00
308	HB	312	HD#	1.80	6.00
308	HD1#	312	HG#	1.80	6.00
270	HB#	275	HN	1.80	6.00
270	HB#	275	HA	1.80	6.00
270	HD#	275	HN	1.80	6.00
284	HD#	289	HB#	1.80	6.00
289	HB#	294	HD#	1.80	6.00
299	HD2	304	HA#	1.80	6.00
299	HE1	304	HA#	1.80	6.00
290	HB#	296	HN	1.80	6.00
301	HD#	307	HE2#	1.80	6.00
270	HB#	277	HA	1.80	6.00
270	HB#	277	HG2#	1.80	6.00
292	HA#	299	HB#	1.80	6.00
300	HG#	307	HN	1.80	6.00
301	HB#	308	HD1#	1.80	6.00
301	HD#	308	HG2#	1.80	6.00
301	HD#	308	HD1#	1.80	6.00
300	HB	308	HD1#	1.80	6.00
270	HB#	278	HN	1.80	6.00
270	HB#	278	HG1#	1.80	6.00
300	HG#	308	HG1#	1.80	6.00
301	HB#	309	HN	1.80	6.00
301	HD#	309	HN	1.80	6.00
301	HD#	309	HG#	1.80	6.00
267	HN	276	HB#	1.80	6.00
270	HG#	279	HA	1.80	6.00
298	HD#	307	HB#	1.80	6.00
301	HB#	310	HN	1.80	6.00
301	HG#	310	HA	1.80	6.00

301	HG#	310	HD1	1.80	6.00
270	HB#	280	HB#	1.80	6.00
270	HB#	280	HD#	1.80	6.00
298	HG#	308	HG2#	1.80	6.00
301	HB#	311	HN	1.80	6.00
301	HG#	311	HA	1.80	6.00
300	HB	311	HA	1.80	6.00
269	HG#	280	HG#	1.80	6.00
269	HE2#	280	HD#	1.80	6.00
270	HG#	281	HD#	1.80	6.00
300	HG#	311	HB#	1.80	6.00
299	HB#	311	HN	1.80	6.00
284	HD#	297	HN	1.80	6.00
299	HE1	312	HB#	1.80	6.00
269	HB#	283	HA	1.80	6.00
268	HN	283	HB#	1.80	6.00
269	HE2#	285	HN	1.80	6.00
269	HE2#	285	HA	1.80	6.00
268	HB#	286	HN	1.80	6.00
267	HD#	286	HA	1.80	6.00
269	HA	288	HE2#	1.80	6.00

Table S5. NMR constraints and structural statistics for the ensemble of lowest-energy structures of the microtubule-bound structures of Tau(296-321) and Tau(267-312).

Structural statistics for the 20 final conformers of MT-bound Tau peptides	**Tau(296-321)** (26 residues)	**Tau(267-312)** (46 residues)
Number of restraints	73	182
Medium range NOE (1< |i-j| < 5)	60	141
Long range NOE (|i-j| ≥ 5)	13	41
NOE violations > 0.5 Å/structure	0	0
Ramachandran plot statistics		
Residues in most favored regions	70.5%	57.3%
Residues in additionally allowed regions	17.9%	23.1%
Residues in generously allowed regions	8.7%	15.3%
Residues in disallowed regions	2.9%	4.3%
RMS deviations from the average structure		
Backbone atoms (Å)	2.69	2.92
Heavy atoms (Å)	2.70	3.22
(Residue range)	(300-313)	(269-284)
		2.18
		2. 20
		(300-310)

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