Supporting information for "Tau isoform-specific stabilization of intermediate states during microtubule assembly and disassembly"

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Supporting Information

Statistical analysis:

Microtubule end morphology data. Count data were grouped into the binary categories of either "projection" or "not projection" (which includes blunt and splayed ends) and were then summed by condition across all experiments. We used a generalized linear model (GLM) with β-binomial error term and “identity" link (JMP Pro 14) to test for a significant effect of experimental condition on projection frequencies. Regression with β-binomial error terms provided a way to negate the potential pitfalls (reduced accuracy) of using a t-test-based or traditional linear model on data that do not fulfill the basic assumptions of these approaches due to their non-normal distribution, unequal variances across conditions (and resulting overdispersion), and strictly bounded nature of the response variable (projection frequency) to values falling between 0 and 1.

The identity link was chosen over the more common logit because of the categorical nature of the predictor (experimental condition).

After the beta binomial regression, pairwise comparisons were made using the “contrast” function (JMP), which uses a joint F test to test for significant differences between the projection frequencies of the desired conditions. P-values were corrected for multiple comparisons using the sequential Bonferroni correction. 95% confidence intervals on projection frequencies were generated using the Wilson score (JMP), which provides an "approximate conservative" estimate of confidence intervals, although it assumes the data are normally distributed (which is true of binomial data at high enough n). Wilson score predictions were adjusted for multiple comparisons by dividing the input alpha (0.05) by N, where N was the number of comparisons.

Length and abundance data. Unless noted otherwise, length data, as well as abundance data, were first analyzed by Levene's test to determine if variances were equal across conditions (homoscedasticity), an important assumption of normal ANOVA tests. If data fulfilled this assumption, a type II ANOVA with a sequential Bonferroni correction for multiple comparisons was used to compare group means. In cases when the variances were unequal, Welch's ANOVA, which does not assume equal variances, followed by the sequential Bonferroni, was used to compare group means (RStudio, car package).

For the dependence of *de novo* spiral length and abundance on tau and tubulin concentrations, the family-wide ANOVA test (see above), if significant, was followed by an ordered heterogeneity test (JMP) to test for correlation between protein concentration (tau:tubulin molar ratio or tubulin concentration) and spiral length. This approach is particularly useful for data in which the predictor (in this case tau or tubulin concentration) is ordered and is predicted to result in a response (spiral length or abundance) with a unidirectional trend (120-122).

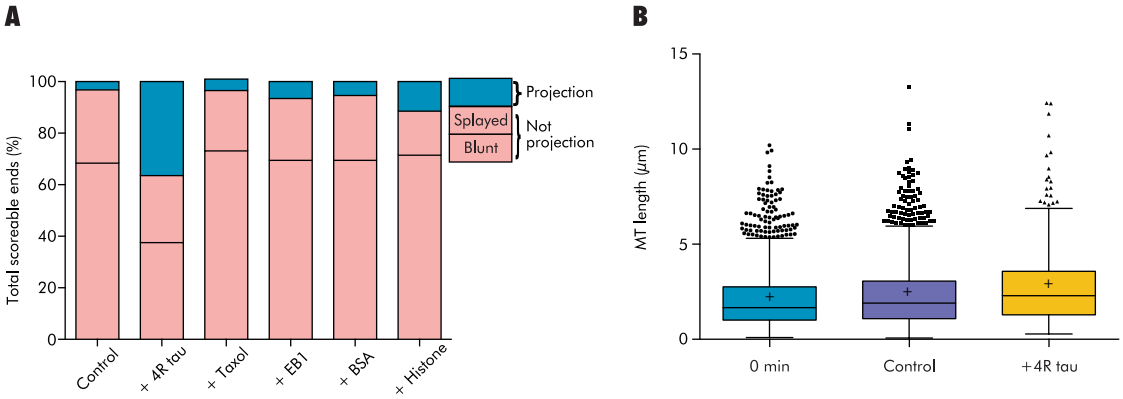
Lastly, projection length and MT length data shown in Fig. 5H and Supp. Fig. 3C were analyzed to compare mean lengths within each condition over the 60 min incubation period. Because neither dataset met the assumptions for an ANOVA or other parametric test, nonparametric approaches were used. In cases when $n \geq 20$, where n represents the number of random, independent fields of view quantified (at either 2500X (MT length) or 30,000X (projection length)), a 2-sample bootstrapping approach was used to compare groups (n = 1,000,000 iterations, Statistics101). In cases when $n < 20$, we used a low power nonparametric test, the difference in confidence intervals (DCI) test (JMP), to test for differences in projection length in controls. For projection abundance data in Fig. 5G, because $n < 10$ and variances were unequal, we used a 2-sample Alexander Govern test to test for differences in group means between control and 4R tau samples at 60 min.

Supp. Figure 1. Decoration of pre-assembled GMPCPP microtubules with 4R tau. (A) Specificity of end projections. GMPCPP MTs (0.5 μM) were incubated with 4R tau (10:1 tau:tubulin molar ratio), MT-stabilizing drug taxol (5 μM), MT-binding protein EB1 (5 μM), negatively charged BSA (5 μM), or positively charged histone (1.8 μM) in a decoration assay. (B) GMPCPP MT length over time (10 min) after decoration with buffer or 4R tau (1:5 tau:tubulin molar ratio).

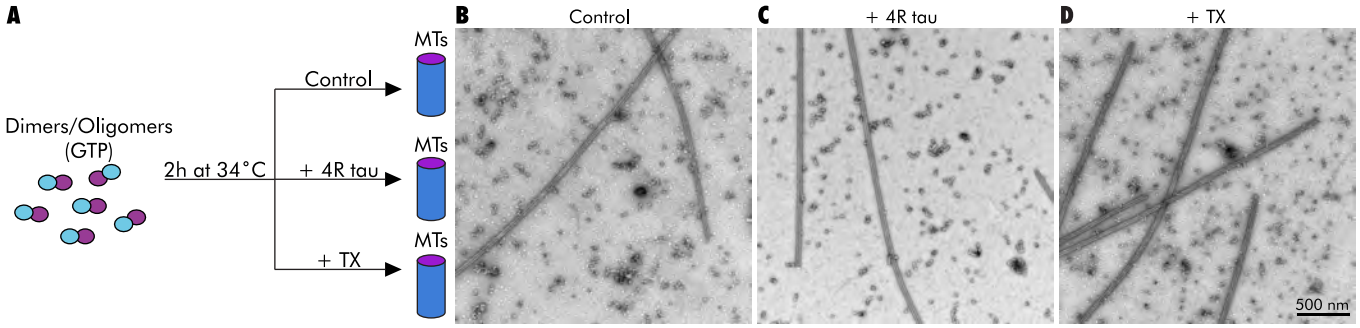
Supp. Figure 2. Microtubules co-assembled with 4R tau in the presence of GTP are morphologically normal. (A) Schematic of a GTP co-assembly experiment. (B-D) Tubulin (26 μM) was assembled at 34°C for 2h in the presence of 2 mM GTP either alone (B), with 4R tau (1:5 tau:tubulin molar ratio) (C), or with 10 μM taxol (TX) (D). All three assembly conditions resulted in morphologically normal MTs.

Supp. Figure 3. Microtubule length decreases as a result of cold-induced disassembly. (A) Time course lengths of GMPCPP MTs (6.1 μM) cooled to 4°C in the presence or absence of 4R tau (1:4 tau:tubulin molar ratio). Note that while MT length decreases an identical amount over the 60 min incubation period in control and 4R tau-containing samples, the 4R tau-containing samples exhibit numerous projections. **** $p<0.0001$, *** $p < 0.001$, ** $p <0.01$, * $p <0.05$.

Supp. Figure 1



Supp. Figure 2



Supp. Figure 3

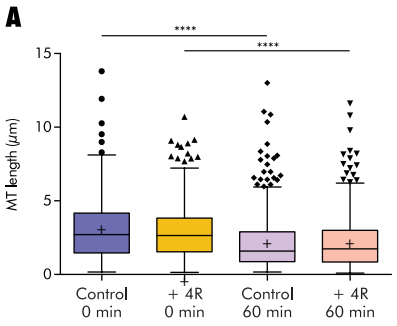


Table S1. Effects of 3R and 4R tau on pre-assembled GMPCPP MT projection frequency and length

Condition	Total MT ends (#)	Ends with projections (#)	Projection frequency	CI(L)	CI(U)	Mean projection length (nm)	SEM	CI(L)	CI(U)	Total projections (#)	# expts
CTR	828	52	0.0628	0.045	0.086	138.0	13.0	112.6	164.0	92	19
3R tau	256	148	0.5781	0.503	0.650	275.0	8.9	257.8	293.0	254	2
4R tau	293	169	0.5768	0.507	0.644	245.0	9.3	226.6	263.4	298	3

*all at a constant [tubulin] of 0.5 μM and 1:5 tau:tubulin molar ratio

**values for 95% confidence intervals (CI(L) - CI(U)) of count data were generated by Wilson score

Table S2. Tubulin concentration dependence of de novo spirals assembled with 4R tau

	Spiral length					Spiral abundance					
[Tubulin] (μM)	Mean length (nm)	SEM	CI(L)	CI(U)	n	Mean per field (#)	SEM	CI(L)	CI(U)	n	# expts
0.9	351.2	10.7	330.2	372.3	374	14.2	1.56	10.97	17.38	28	3
4.5	452.1	27.9	397.1	507.0	200	29.0	2.49	22.91	35.09	7	3
9.1	539.2	26.8	486.4	592.0	209	32.4	2.38	26.74	38.01	8	3

*all at 1:5 tau:tubulin molar ratio

**CI(L) and CI(U) show lower and upper bounds of 95% confidence interval

Table S3. 4R tau concentration dependence of de novo spirals

	Spiral length					Spiral abundance					
Tau:tu molar ratio	Mean length (nm)	SEM	CI(L)	CI(U)	n	Mean per field (#)	SEM	CI(L)	CI(U)	n	# expts
1:60	466.6	24.3	418.4	514.9	99	11	0.62	9.56	12.44	9	2
1:20	404.1	16.3	371.8	436.5	117	16.71	1.61	12.76	20.66	7	2
1:5	351.2	10.7	330.2	372.3	374	14.18	1.56	10.97	17.38	28	3
1:1	209.9	12.0	186.1	233.7	143	14.4	1.60	10.78	18.02	10	3
5:1	230.2	12.8	204.7	245.1	85	5.73	0.68	4.28	7.191	15	3

*all at a constant [tubulin] of 0.9 μM

**CI(L) and CI(U) show lower and upper bounds of 95% confidence interval

Table S4. 3R and 4R tau-stabilized de novo spiral length and abundance

	Spiral length					Spiral abundance					
Condition	Mean length (nm)	SEM	CI(L)	CI(U)	n	Mean per field (#)	SEM	CI(L)	CI(U)	n	# expts
CTR	319.0	20.1	278.9	359.0	76	4.12	0.84	2.34	5.90	17	4
3R tau	449.8	14.2	421.8	477.8	181	10.06	0.97	8.02	12.09	18	2
4R tau	402.0	10.7	381.0	423.0	484	13.32	1.26	10.76	15.88	37	4

*all at a constant [tubulin] of 0.9 μM and 1:5 tau:tubulin molar ratio

**CI(L) and CI(U) show lower and upper bounds of 95% confidence interval

Table S5. 3R and 4R tau-stabilized disassembly spiral length and abundance

	Spiral length					Spiral abundance					
Condition	Mean length (nm)	SEM	CI(L)	CI(U)	n	Mean per field (#)	SEM	CI(L)	CI(U)	n	# expts
CTR	296.1	22.9	250.6	341.6	93	2.02	0.31	1.39	2.64	52	8
3R tau	324.0	15.7	334.0	396.3	129	3.67	0.74	2.12	5.17	39	6
4R tau	497.0	14.0	601.0	655.9	920	19.17	1.36	16.4	21.9	48	3

*all at a constant [tubulin] of 0.5 μM and 1:5 tau:tubulin molar ratio

**CI(L) and CI(U) show lower and upper bounds of 95% confidence interval

Table S6. Projection frequency and length of pre-assembled GMPCPP MTs decorated with FTDP-17 mutant tau

Condition	Total MT ends (#)	Ends with projections (#)	Projection frequency	CI(L)	CI(U)	Mean projection length (nm)	SEM	CI(L)	CI(U)	# expts
4R/wt	212	78	0.3679	0.306	0.435	252.0	24.0	203.8	299.8	4
K280	136	36	0.2647	0.198	0.345	248.0	37.9	170.6	324.8	4
P301L	150	37	0.2467	0.185	0.321	171.0	23.0	124.3	217.7	4
R406W	104	30	0.2885	0.210	0.382	191.0	21.3	148.1	233.8	3

*all at a constant [tubulin] of 0.5 μM and 1:5 tau:tubulin molar ratio

**values for 95% confidence intervals (CI(L) - CI(U)) of count data were generated by Wilson score

Table S7. FTDP-17 mutant tau-stabilized disassembly spiral length and abundance

	Spiral length					Spiral abundance					
Condition	Mean length (nm)	SEM	CI(L)	CI(U)	n	Mean per field (#)	SEM	CI(L)	CI(U)	n	# expts
4R/wt	569.7	18.4	533.5	605.9	383	7.938	0.59	6.74	9.13	48	5
P301L	441.8	27.4	387.5	496.2	113	3.929	0.95	1.98	5.88	28	3
K280	297.4	24.3	249.4	345.4	128	4.357	0.89	2.54	6.18	28	3
R406W	578.8	29.8	520.0	637.7	163	5.258	0.54	4.15	6.36	31	3

*all at a constant [tubulin] of 0.5 μM and 1:5 tau:tubulin molar ratio

**CI(L) and CI(U) show lower and upper bounds of 95% confidence interval

Supporting References

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