Supplemental Figure Legends

FIGURE S1. **Alignment of chicken erythrocyte βVI tubulin (CβVI) sequence with major bovine brain βII tubulin (BβII) and major human βI tubulin (HβI) sequences.** All sequence divergences are highlighted in grey. Sequence differences that result in most significant changes in amino acid side chain are marked with an asterisk. Secondary structure designations are shown below the alignment, with Sn representing β-sheets and Hn representing α-helices. The positions of loops T1-T7, which are involved in nucleotide binding, are indicated. The H1-S2 and the M-loops, which form lateral contacts between MT protofilaments, are also labeled.

FIGURE S2. **Representative mass spectra of isobaric α- and β-tubulin peptides.** Sequence coverage was determined based on FT-MS data with a mass accuracy threshold of ≤2 ppm. Isobaric peptides, those whose masses are identical, but whose sequences differ, were identified by MS/MS analysis. Shown here are representative LTQ-MS (I), MS/MS (II), FT-MS at 0 min HDX (III), and FT-MS at 30 min HDX in the presence of peloruside A (IV) for one α-tubulin peptide, α287-294 (a), and one β-tubulin peptide, β341-353 (b).

FIGURE S3. **Map of peptides for chicken erythrocyte α-tubulin (grey, UniProt P02552 fragment) and β-tubulin (black, UniProt P09207) obtained from a peptic digest.** Each line represents a unique peptide identified from a combination of ProteinProspector MSNonSpecific search with mass error threshold of <2.0 ppm and subsequent MS/MS analysis of the isobaric peaks. Only those peptides that remained detectable after deuterium incorporation are included in the map: 30 α-tubulin peptides (74% sequence coverage) and 24 β-tubulin peptides (73% sequence coverage).

FIGURE S4. **Representative Gaussian fits for the determination of the centroid mass values.** The centroid m/z value (x_c) for each peptide was determined from a Gaussian curve fit to the corresponding isotopic peak distribution using OriginPro 8. Shown here are the Gaussian fits for an α-tubulin peptide, α287-294 (H9). Note a distinct shift to the left of the centroid value in the peloruside A sample (b) as compared to control (a), indicating a reduction in mass associated with decreased deuterium incorporation in response to drug binding.

FIGURE S5. **FT-MS spectra of the drugs extracted from the bovine brain and chicken erythrocyte MT pellets.** Epothilone B (a), peloruside A (b), and laulimalide (c) were extracted from the Taxol-MT pellets as described in Experimental Procedures. Briefly, BBT or CET was polymerized in the presence of GTP, equimolar Taxol and equimolar epothilone B, peloruside A, or laulimalide. The supernatant was removed, the pellet was resuspended in 0.1 M MEM buffer, and the drugs were extracted with CH_2Cl_2 three times. The organic layers were combined, dried overnight, and resuspended in 70% (v/v) methanol. Drug content was determined by mass spectrometry using direct infusion into a 12-T Varian IonSpec FT-ICR MS (Varian Inc.) The peaks corresponding to the detected drugs are highlighted in bold and labeled with drug names (Tx, EpoB, PelA, or LML) and appropriate mass designations.

FIGURE S6. **Effect of F270V mutation on epothilone binding energy (a) and pose (b).** Table in (a) lists the average computed ΔG_{bind} for the taxane site of epothilone B and ixabepilone, obtained for wild type CET and the F270V mutant. In (b), the β-tubulin components of the taxane site are shown in gray, and the secondary structure designations are labeled according to Löwe *et al.* (6). The epothilone B pose is shown as dark blue sticks in the WT CET, while that shown as light blue sticks is the pose in the F270V mutant. Phe270 is shown in green.

Table S1. Non-redundant peptides of α- and β-tubulin identified by ProteinProspector prior to H/D exchange. The mass error threshold was set at 2 ppm, and the actual error for each peptide is reported in the "Delta ppm" column.

Submitted	MH$^+$	MH$^+$	Delta	Start	End	Sequence	RT (min)	
	Equivalent	Matched	ppm				START	END
Alpha Tubulin								
737.3498	737.3498	737.3498	-0.0221	377	383	(C) MLSNTTA (I)	1.5	2
640.2437^{+2}	1279.4801	1279.4808	-0.549	439	450	(D) SVEGEGEEEGEE (I)	2	2.5
657.3669	657.3669	657.3678	-1.44	173	178	(Y) PARQVS (T)	2	2.5
631.3308^{+2}	1261.6543	1261.6535	0.632	319	329	(L) YRGDVVPKDVN(A)	2.5	3
503.2702^{+2}	1005.5331	1005.5332	-0.0931	369	377	(L) AKVQRAVCM(L)	2.5	3
1005.5326	1005.5326	1005.5332	-0.614	369	377	(L) AKVQRAVCM(L)	2.5	3
738.3269	738.3269	738.3273	-0.551	1	6	(T) MRECIS(V)	2.5	3
500.2560^{+2}	999.5047	999.504	0.698	2	10	(M) RECISVHVG(Q)	3.5	4
808.3292	808.3292	808.3294	-0.266	295	301	(A) CFEPANQ(M)	4.5	5
619.3665	619.3665	619.3661	0.616	234	239	(Q) IVSSIT(A)	5.5	6
819.8729^{+2}	1638.7385	1638.7372	0.835	302	315	(Q) MVKCDPRHGKYMAC(C)	6	6.5
728.3895^{+2}	1455.7717	1455.7703	0.986	53	66	(F) FSETGAGKHVPRAV(F)	6	6.5
723.3903^{+2}	1445.7733	1445.7747	-0.955	318	330	(L) LYRGDVVPKDVNA(A)	6	6.5
687.8722^{+2}	1374.7371	1374.7376	-0.34	318	329	(L) LYRGDVVPKDVN(A)	6	6.5
666.8490^{+2}	1332.6907	1332.6906	0.0624	319	330	(L) YRGDVVPKDVNA(A)	6	6.5
581.2529^{+3}	1741.7441	1741.7463	-1.26	302	316	(Q) MVKCDPRHGKYMACC(L)	6	6.5
560.8454^{+2}	1120.6835	1120.6837	-0.161	336	344	(I) KTKRTIQFV(D)	6	6.5
546.9168^{+3}	1638.7358	1638.7372	-0.798	302	315	(Q) MVKCDPRHGKYMAC(C)	6	6.5
990.5103	990.5103	990.5102	0.0844	378	387	(M) LSNTTAIAEA(W)	6	6.5
804.4089	804.4089	804.4098	-1.09	287	294	(L) SVAEITNA(C)	6	6.5
690.3302	690.3302	690.3305	-0.378	249	254	(L) NVDLTE(F)	6	6.5
690.3309	690.3309	690.3305	0.636	249	254	(L) NVDLTE(F)	6.5	7
801.9215^{+2}	1602.8357	1602.8387	-1.86	53	67	(F) FSETGAGKHVPRAVF(V)	7	7.5
731.8156^{+2}	1462.6239	1462.6267	-1.91	190	202	(L) TTHTTLEHSDCAF(M)	7	7.5
700.8745^{+2}	1400.7417	1400.742	-0.203	158	169	(L) SVDYGKKSKLEF(S)	7	7.5
577.9686^{+3}	1731.8912	1731.8925	-0.74	78	91	(E) VRTGTYRQLFHPEQ(L)	7	7.5
534.9509^{+3}	1602.8381	1602.8387	-0.346	53	67	(F) FSETGAGKHVPRAVF(V)	7	7.5
678.3564	678.3564	678.357	-0.812	243	248	(L) RFDGAL(N)	7	7.5
921.4766^{+3}	2762.4152	2762.4108	1.62	92	116	(Q) LITGKEDAANNYARGHYTIGKEIID(L)	8	8.5
837.4420^{+2}	1673.8767	1673.8752	0.932	120	134	(L) DRIRKLADQCTGLQG(F)	8	8.5
587.6518^{+3}	1760.9408	1760.9402	0.364	214	227	(C) RRNLDIERPTYTNL(N)	8	8.5
719.3407^{+2}	1437.6741	1437.6744	-0.179	426	438	(M) AALEKDYEEVGVD(S)	8.5	9
691.3577^{+4}	2762.409	2762.4108	-0.656	92	116	(Q) LITGKEDAANNYARGHYTIGKEIID(L)	8.5	9
644.0088^{+3}	1930.0118	1930.0103	0.822	154	169	(L) MERLSVDYGKKSKLEF(S)	8.5	9
589.3175^{+2}	1177.6277	1177.6265	1.02	400	408	(Y) AKRAFVHWY(V)	8.5	9
859.9414^{+2}	1718.8755	1718.8748	0.414	272	286	(T) YAPVISAEKAYHEQL(S)	9	9.5
740.8804^{+4}	2960.4998	2960.5056	-1.96	307	333	(D) PRHGKYMACCLLYRGDVVPKDVNAAIA(T)	9	9.5

615.6646^{+3}	1844.9792	1844.9766	1.44	78	92	(E) VRTGTYRQLFHPEQL(I)	9	9.5
573.6303^{+3}	1718.8763	1718.8748	0.893	272	286	(T) YAPVISAEKAYHEQL(S)	9	9.5
720.0057^{+3}	2158.0025	2158.0056	-1.41	201	218	(C) AFMVDNEAIYDICRRNLD(I)	9.5	10
665.8482^{+2}	1330.6891	1330.6902	-0.844	83	92	(T) YRQLFHPEQL(I)	9.5	10
659.3416^{+3}	1976.0102	1976.0124	-1.07	272	289	(T) YAPVISAEKAYHEQLSVA(E)	9.5	10
656.3010^{+2}	1311.5947	1311.5964	-1.28	139	152	(F) HSFGGGTGSGFTSL(L)	9.5	10
540.2561^{+4}	2158.0026	2158.0056	-1.4	201	218	(C) AFMVDNEAIYDICRRNLD(I)	9.5	10
1129.5602	1129.5602	1129.5623	-1.87	68	77	(F) VDLEPTVIDE(V)	9.5	10
589.2985	589.2985	589.298	0.783	384	388	(A) IAEAW(A)	9.5	10
877.4775^{+2}	1753.9477	1753.9483	-0.335	352	368	(F) KVGINYQPPTVVPGGDL(A)	10	10.5
629.3473^{+3}	1886.0273	1886.0276	-0.156	118	134	(L) VLDRIRKLADQCTGLQG(F)	10	10.5
834.3808^{+3}	2501.1278	2501.1249	1.17	26	49	(C) LEHGIQPDGQMPSDKTIGGGDDSF(N)	11	11.5
880.9738^{+2}	1760.9403	1760.9402	0.0665	214	227	(C) RRNLDIERPTYTNL(N)	11.5	12
672.0342^{+3}	2014.088	2014.0909	-1.41	255	271	(E) FQTNLVPYPRIHFPLAT(Y)	11.5	12
1033.5546	1033.5546	1033.5564	-1.78	181	189	(A) VVEPYNSIL(T)	11.5	12
705.8981^{+2}	1410.7889	1410.7892	-0.219	260	271	(L) VPYPRIHFPLAT(Y)	12	12.5
623.0124^{+3}	1867.0226	1867.0225	0.0897	256	271	(F) QTNLVPYPRIHFPLAT(Y)	12	12.5
615.3356^{+3}	1843.9922	1843.9912	0.547	173	189	(Y) PARQVSTAVVEPYNSIL(T)	12	12.5
924.3915	924.3915	924.392	-0.558	344	351	(F) VDWCPTGF(K)	12	12.5
934.0164^{+2}	1867.0255	1867.0225	1.63	256	271	(F) QTNLVPYPRIHFPLAT(Y)	12.5	13
650.8833^{+2}	1300.7593	1300.7583	0.767	228	239	(L) NRLIGQIVSSIT(A)	13.5	14
835.9128^{+2}	1670.8183	1670.8173	0.614	137	153	(S) VFHSFGGGTGSGFTSLL(M)	14.5	15
903.2605^{+5}	4512.2734	4512.2679	1.23	371	408	(K) VQRAVCMLSNTTAIAEAWARLDHKFDLMYAKRAFVHWY(V)	15	15.5
775.4319^{+2}	1549.8565	1549.8584	-1.23	234	248	(Q) IVSSITASLRFDGAL(N)	15	15.5
726.6667^{+4}	2903.645	2903.6465	-0.54	319	344	(L) YRGDVVPKDVNAAIATIKTKRTIQFV(D)	15.5	16
Beta Tubulin								
834.4091^{+2}	1667.8109	1667.8098	0.692	158	171	(E) EYPDRIMNTFSVVP (S)	0	0.5
691.8018^{+2}	1382.5963	1382.5958	0.381	435	446	(E) YEEAEASPEKET (M)	0.5	1
967.5133	967.5133	967.5142	-0.926	316	323	(C) IFRGRMST(R)	2.5	3
484.2607^{+2}	967.5141	967.5142	-0.0752	316	323	(C) IFRGRMST(R)	2.5	3
808.3437	808.3437	808.344	-0.395	295	301	(F) DARNMMA(A)	5	5.5
959.4786	959.4786	959.4793	-0.678	369	377	(I) GNNTAIQEL(F)	5	5.5
1126.5754	1126.5754	1126.5739	1.35	331	340	(L) LSVQTKNSSY(F)	5.5	6
563.7915^{+2}	1126.5757	1126.5739	1.64	331	340	(L) LSVQTKNSSY(F)	5.5	6
493.2605^{+3}	1477.7669	1477.7692	-1.55	301	313	(M) AACDPRRGRYLTV(A)	6	6.5
739.3887^{+2}	1477.7701	1477.7692	0.597	301	313	(M) AACDPRRGRYLTV(A)	6	6.5
888.4413	888.4413	888.4421	-0.942	324	330	(T) REVDEQL(L)	6	6.5
870.3785^{+2}	1739.7497	1739.7494	0.188	432	446	(D) VEEYEEAEASPEKET(M)	6	6.5
720.3227	720.3227	720.3233	-0.787	113	118	(L) IENVMD(V)	6.5	7
631.3284^{+3}	1891.9706	1891.9695	0.629	152	166	(L) INKIREEYPDRIMNT(F)	6.5	7
946.4889^{+2}	1891.9705	1891.9695	0.563	152	166	(L) INKIREEYPDRIMNT(F)	6.5	7
674.336	674.336	674.3355	0.673	202	207	(C) IDNEAL(Y)	6.5	7

516.9407^{+3}	1548.8075	1548.8064	0.769	301	314	(M) AACDPRRGRYLTVA(C)	6.5	7
535.7657^{+2}	1070.5241	1070.5234	0.694	315	323	(A) CIFRGRMST(R)	6.5	7
807.3994	807.3994	807.3995	-0.178	364	371	(M) AATFIGNN(T)	6.5	7
559.6294^{+3}	1676.8736	1676.8755	-1.09	52	65	(F) NEAYSHKYVPRSIL(V)	7	7.5
777.3446	777.3446	777.3447	-0.168	201	207	(F) CIDNEAL(Y)	7	7.5
756.3279^{+2}	1511.6485	1511.6496	-0.727	408	421	(E) FSEAEGNTNDLVSE(Y)	8	8.5
861.4017	861.4017	861.4022	-0.634	66	73	(L) VDLEPGTM(D)	8.5	9
700.3380^{+2}	1399.6687	1399.67	-0.887	188	199	(L) SIHQLIENTDET(F)	8.5	9
647.3199^{+3}	1939.9451	1939.9477	-1.3	315	330	(A) CIFRGRMSTREVDEQL(L)	8.5	9
637.3245^{+2}	1273.6417	1273.6423	-0.447	331	341	(L) LSVQTKNSSYF(V)	8.5	9
663.0082^{+3}	1987.01	1987.0072	1.42	50	65	(V) YFNEAYSHKYVPRSIL(V)	9	9.5
609.3291^{+3}	1825.9727	1825.9741	-0.768	4	20	(E) IVHLQIGQCGNQIGAKF(W)	10	10.5
650.2963^{+2}	1299.5853	1299.5852	0.116	21	31	(F) WEVISDEHGID(I)	10	10.5
650.3416^{+4}	2598.3446	2598.3463	-0.678	44	64	(Q) LERINVYFNEAYSHKYVPRSI(L)	10	10.5
714.8578^{+4}	2856.4094	2856.4136	-1.47	74	100	(M) DSVRSSKIGPLFRPDNFIHGNSGAGNN(W)	10	10.5
952.8097^{+3}	2856.4145	2856.4136	0.339	74	100	(M) DSVRSSKIGPLFRPDNFIHGNSGAGNN(W)	10	10.5
700.6813^{+6}	4199.0514	4199.0442	1.72	74	112	(M) DSVRSSKIGPLFRPDNFIHGNSGAGNNWAKGHYTEGAEL(I)	10	10.5
840.6155^{+5}	4199.0484	4199.0442	1	74	112	(M) DSVRSSKIGPLFRPDNFIHGNSGAGNNWAKGHYTEGAEL(I)	10	10.5
668.3618^{+3}	2003.0708	2003.0709	-0.0196	212	228	(C) FRTLKLTNPTYGDLNHL(V)	10	10.5
730.3946^{+3}	2189.1692	2189.1713	-0.951	212	230	(C) FRTLKLTNPTYGDLNHLVS(L)	10	10.5
804.4042^{+2}	1607.8011	1607.7999	0.778	266	280	(F) FMPGFAPLTARGSQQ(Y)	10	10.5
877.9793^{+2}	1754.9513	1754.9483	1.74	379	392	(F) IRVSEQFSAMFRRK(A)	11	11.5
565.2962^{+2}	1129.5851	1129.5856	-0.463	354	363	(V) CDIPPRGLKM(A)	11.5	12
660.2702	660.2702	660.2698	0.644	208	212	(L) YDICF(R)	12	12.5
874.9364^{+2}	1748.8655	1748.8636	1.1	284	298	(A) LSVPELTQQMFDARN(M)	12	12.5
684.3885^{+2}	1367.7697	1367.7682	1.14	342	353	(F) VEWIPNNVKVAV(C)	12	12.5
577.2343^{+2}	1153.4613	1153.4612	0.0776	119	128	(D) VVRNECESCD(C)	13	13.5
535.2185	535.2185	535.2181	0.796	128	132	(C) DCLQG (F)	13	13.5
785.9170^{+2}	1570.8267	1570.8258	0.617	136	152	(L) IHSLGGGTGSGMGTLLI(N)	13	13.5
923.4812^{+2}	1845.9551	1845.9568	-0.891	341	356	(Y) FVEWIPNNVKVAVCDI(P)	13	13.5
662.3221^{+2}	1323.6369	1323.6362	0.575	32	44	(D) IAGNYCGNASLQL(E)	13.5	14
866.9365^{+2}	1732.8657	1732.8687	-1.71	133	150	(G) FQLIHSLGGGTGSGMGTL(L)	13.5	14
552.0039^{+3}	1653.9971	1653.9985	-0.826	250	263	(D) LRKLAVNMVPFPRL(H)	13.5	14
646.7147^{+3}	1938.1295	1938.1258	1.91	250	265	(D) LRKLAVNMVPFPRLHF(F)	13.5	14
609.0190^{+3}	1825.0424	1825.0418	0.368	251	265	(L) RKLAVNMVPFPRLHF(F)	13.5	14
768.3982^{+2}	1535.7891	1535.7886	0.315	281	293	(Q) YRALSVPELTQQM(F)	13.5	14
1057.5707^{+2}	2114.1341	2114.138	-1.82	168	187	(F) SVVPSPKVSDTVVEPYNAIL(S)	14.5	15
757.9226^{+2}	1514.8379	1514.8366	0.889	341	353	(Y) FVEWIPNNVKVAV(C)	14.5	15
650.3415^{+4}	2598.3442	2598.3463	-0.832	44	64	(Q) LERINVYFNEAYSHKYVPRSI(L)	15	15.5
866.7866^{+3}	2598.3452	2598.3463	-0.418	44	64	(Q) LERINVYFNEAYSHKYVPRSI(L)	15	15.5
854.4601^{+2}	1707.9129	1707.9098	1.82	231	246	(S) LTMSGVTTSLRFPGQL(N)	15	15.5
657.8288^{+2}	1314.6503	1314.6511	-0.578	378	388	(L) FIRVSEQFSAM(F)	15	15.5

Table S2. Drug-induced average ΔHDX for each detected peptide in α- and β-tubulin, with corresponding standard deviations, and P-values.

Alpha Tubulin

Peptide	Sequence	Ixabepilone			Epothilone B			Peloruside A			Laulimalide		
		ΔHDX	σ	P-value*	ΔHDX	σ	P-value	ΔHDX	σ	P-value	ΔHDX	σ	P-value
26-49	(C) LEHGIQPDGQMPSDKTIGGGDDSF(N)	-168.01	59.82	0.190	-1051.6	93.56	0.001	-412.61	301.5	0.110	-521.6	162.77	0.019
53-66	(F) FSETGAGKHVPRAV(F)	-409.67	67.36	0.005	-646.16	48.15	0.002	-734.06	60.38	0.001	-574.11	120.21	0.017
68-77	(F) VDLEPTVIDE(V)	-53.05	26.76	0.150	-637.73	28.46	0.000	21.72	233.96	0.900	-20.37	21.28	0.690
78-91	(E) VRTGTYRQLFHPEQ(L)	-230.82	41.61	0.004	-566.4	117.09	0.001	-653.12	15.2	0.000	-798.39	41.13	0.000
92-116	(Q) LITGKEDAANNYARGHYTIGKEIID(L)	-321.81	190.03	0.120	-959.35	143.07	0.000	-680.16	41.9	0.001	-706.32	496.84	0.090
120-134	(L) DRIRKLADQCTGLQG(F)	-515.32	94.34	0.001	-377.34	41.84	0.001	-357.71	166.24	0.026	-618.18	59.51	0.000
137-153	(S) VFHSFGGGTGSGFTSLL(M)	-596.17	34.14	0.003	-705.08	84.69	0.009	-772.74	17.42	0.003	-694.69	90.42	0.003
154-169	(L) MERLSVDYGKKSKLEF(S)	-499.87	78.02	0.014	-501.52	62.21	0.002	-390.48	75.09	0.011	-574.77	71.72	0.002
181-189	(A) VVEPYNSIL(T)	-113.03	41.58	0.051	-36.72	35.76	0.610	205.15	19.67	0.011	235.76	25.2	0.008
190-202	(L) TTHTTLEHSDCAF(M)	-996.79	125.61	0.000	-959.11	71.2	0.000	-519.3	157.32	0.007	-677.69	209.73	0.006
214-227	(C) RRNLDIERPTYTNL(N)	-206.77	61.2	0.088	-389.93	85.5	0.023	-394.91	135.49	0.029	-576.73	87.12	0.003
225-230	(Y) TNLNRL(I)	-271.45	30.17	0.003	-208.68	31.72	0.004	-917.07	39.03	0.000	-835.61	11.89	0.000
234-239	(Q) IVSSIT(A)	-179.25	27.25	0.006	-176.89	14.61	0.003	-361.6	8.33	0.000	-319.6	19.39	0.000
243-248	(L) RFDGAL(N)	-173.44	3.14	0.001	-173.13	8.93	0.002	-215.65	27.97	0.003	-235.62	14.17	0.001
249-254	(L) NVDLTE(F)	-243.26	36.64	0.003	-180.55	55.41	0.030	-285.44	28.63	0.002	-285.42	4.56	0.001
260-271	(L) VPYPRIHFPLAT(Y)	-166.35	55.79	0.500	-200.99	39.36	0.960	-186.92	45.93	0.410	-203.33	79.96	0.270
272-289	(T) YAPVISAEKAYHEQLSVA(E)	-256.59	36.8	0.034	-407.51	45.01	0.007	-346.95	50.21	0.038	-696.02	47.62	0.003
287-294	(L) SVAEITNA(C)	-420.63	84.14	0.006	-351.76	29.92	0.002	-668.31	22.09	0.000	-679.95	29.09	0.000
295-301	(A) CFEPANQ(M)	-235.61	53.59	0.002	-168.1	38.91	0.010	-454.87	14.05	0.001	-594.34	81.45	0.002
302-315	(Q) MVKCDPRHGKYMAC(C)	-972.82	128.57	0.001	-937.14	126.68	0.001	-811.67	25.65	0.000	-778.69	37.75	0.000
319-329	(L) YRGDVVPKDVN(A)	-452.91	115.75	0.006	-474.9	82.42	0.024	-514.5	111.35	0.008	-463.58	76.51	0.007
319-344	(L) YRGDVVPKDVNAAIATIKTKRTIQFV(D)	-1751.88	174.44	0.001	-1304.8	154.36	0.002	-1216.09	112.9	0.001	-1764.91	101.49	0.000
336-344	(I) KTKRTIQFV(D)	-51.66	42.05	0.640	-23.49	54.84	0.820	88.43	21.79	0.460	-144.11	42.09	0.310
344-351	(F) VDWCPTGF(K)	-1233.56	66.75	0.000	-974.04	8.34	0.000	-1731.03	533.14	0.005	-1413.82	337.75	0.003
352-368	(F) KVGINYQPPTVVPGGDL(A)	-475.91	53.88	0.001	-546.85	28.43	0.002	-730.41	84.54	0.002	-825.69	165.26	0.003
369-377	(L) AKVQRAVCM(L)	-438.96	33.89	0.003	-401.47	81.79	0.002	-508.19	63.76	0.001	-485.48	85.4	0.001
378-387	(M) LSNTTAIAEA(W)	-583.18	11.58	0.002	-587.01	42.61	0.001	-477.32	60.01	0.001	-551.96	46.75	0.001
400-408	(Y) AKRAFVHWY(V)	-298.48	28.49	0.001	-84.9	15.31	0.077	8.56	0.27	0.530	-105.23	59.38	0.058
426-438	(M) AALEKDYEEVGVD(S)	-1528.2	179.43	0.006	-1818.3	84.69	0.002	-1595.27	216.86	0.001	-1630.51	156.53	0.002

Beta Tubulin

Peptide	Sequence	Ixabepilone			Epothilone B			Peloruside A			Laulimalide		
		ΔHDX	σ	P-value	ΔHDX	σ	P-value	ΔHDX	σ	P-value	ΔHDX	σ	P-value
4-20	(E) IVHLQIGQCGNQIGAKF(W)	-1154.3	138.02	0.001	-512.98	97.01	0.014	-1026.01	35.50	0.002	-1110.72	252.07	0.014
21-31	(F) WEVISDEHGID(I)	-359.81	36.31	0.000	-211.83	53.77	0.037	-274.08	56.35	0.017	-510.28	294.02	0.062
44-64	(Q) LERINVYFNEAYSHKYVPRSI(L)	-365.83	42.55	0.001	-299.7	46.35	0.002	-393.4	89.51	0.007	-697.68	8.08	0.000
52-65	(F) NEAYSHKYVPRSIL(V)	-557.84	50.03	0.035	-508.13	21.54	0.050	-789.72	35.45	0.013	-696.2	138.07	0.017
66-73	(L) VDLEPGTM(D)	-36.32	41.13	0.610	-143.45	68.71	0.039	-260.36	29.43	0.014	-335.2	49.12	0.003
74-100	(M) DSVRSSKIGPLFRPDNFIHGNSGAGNN(W)	-534.63	51.46	0.015	-1037.82	302.79	0.002	-947.34	87.36	0.004	-1427.31	73.32	0.000
74-112	(M)DSVRSSKIGPLFRPDNFIHGNSGAGNNWAKGHYTEGAEL(I)	-1424.07	188.21	0.003	-2231.42	94.12	0.000	-2096.93	84.23	0.001	-2642.13	109.72	0.000
113-118	(L) IENVMD(V)	-119.85	21.9	0.018	-53.14	30.32	0.210	-796.4	21.78	0.000	-509.14	23.4	0.000
133-150	(G) FQLIHSLGGGTGSGMGTL(L)	-660.4	85.16	0.000	-893.54	251.16	0.000	-256.46	134.35	0.075	-496.46	165.92	0.460
152-166	(L) INKIREEYPDRIMNT(F)	-341.77	22.56	0.001	-319.25	33.31	0.003	-204.97	34.03	0.006	-206.69	39.84	0.029
168-187	(F) SVVPSPKVSDTVVEPYNAIL(S)	478.29	212.67	0.021	222.41	52.8	0.280	199.32	44.21	0.360	154.03	26	0.470
188-199	(L) SIHQLIENTDET(F)	-539.33	77.99	0.001	-281.41	48.63	0.033	-543.72	81.95	0.003	-738.29	46.74	0.001
202-207	(C) IDNEAL(Y)	-23.26	17.25	0.290	-11.28	5.15	0.420	-3.19	8.11	0.850	4.67	14.02	0.820
208-212	(L) YDICF(R)	-345.35	29.94	0.000	-328.1	54.05	0.000	-316.39	10.25	0.001	-332.16	16.06	0.000
212-230	(C) FRTLKLTNPTYGDLNHLVS(L)	-1345.5	131.88	0.000	-1369.29	138.72	0.000	-1318.95	99.37	0.000	-1407.96	190.52	0.000
231-246	(S) LTMSGVTTSLRFPGQL(N)	-1136.9	153.86	0.014	-1107.25	215.58	0.012	-1221.33	317.1	0.007	-1373.4	188.12	0.008
251-265	(L) RKLAVNMVPFPRLHF(F)	486.17	102.04	0.023	389.47	113.55	0.046	409.92	128.29	0.028	449.9	25.17	0.045
266-280	(F) FMPGFAPLTARGSQQ(Y)	36.89	12.71	0.003	-264.92	45.45	0.007	-332.95	22.34	0.001	-427.65	88.76	0.007
281-293	(Q) YRALSVPELTQQM(F)	-400.52	56.04	0.007	-600.72	17.11	0.003	-1766.35	78.12	0.000	-2214.04	94.23	0.000
301-313	(M) AACDPRRGRYLTV(A)	-865.29	72.89	0.000	-604.77	43.42	0.000	-2152.03	34.9	0.000	-1882.09	149.05	0.000
316-323	(C) IFRGRMST(R)	-255.33	102.93	0.026	-375.1	77.82	0.009	-250.64	44.47	0.007	-301.79	47.25	0.005
331-340	(L) LSVQTKNSSY(F)	-519.93	80.93	0.013	-562.59	67.87	0.006	-1748.22	84.81	0.000	-1702.27	35.69	0.000
341-353	(Y) FVEWIPNNVKVAV(C)	-1283.58	59.18	0.002	-978.4	294.94	0.002	-1962.03	209.65	0.001	-2027.09	133.15	0.001
435-446	(D) VEEYEEAEASPEKET(M)	-136.52	34.94	0.015	-303.54	126.28	0.022	-215.04	11.12	0.000	-427.47	68.3	0.001

* Highlighted in pink are P values for peptides significantly protected from deuterium incorporation, with P<0.01; in blue, P<0.05; in light blue are residues with ΔHDX values slightly outside the assigned significance, with 0.05<P<0.09; and in green are the P values for peptides significantly <u>de</u>protected, with P<0.05.

Table S3. Summary of computational docking simulations for MSAs in native (wild-type) and mutated tubulin. (a) The binding energies (ΔG_{bind}) for each drug in the corresponding site are in kcal/mol (at 298 K). (b) For every drug, the difference between the binding energy for each site and for the taxane site of native tubulin is shown. The differences that are greater than 1 unit are highlighted in bold, as they represent the more significant changes in binding affinities.

(a) Computational Binding Energies (ΔG_{bind})

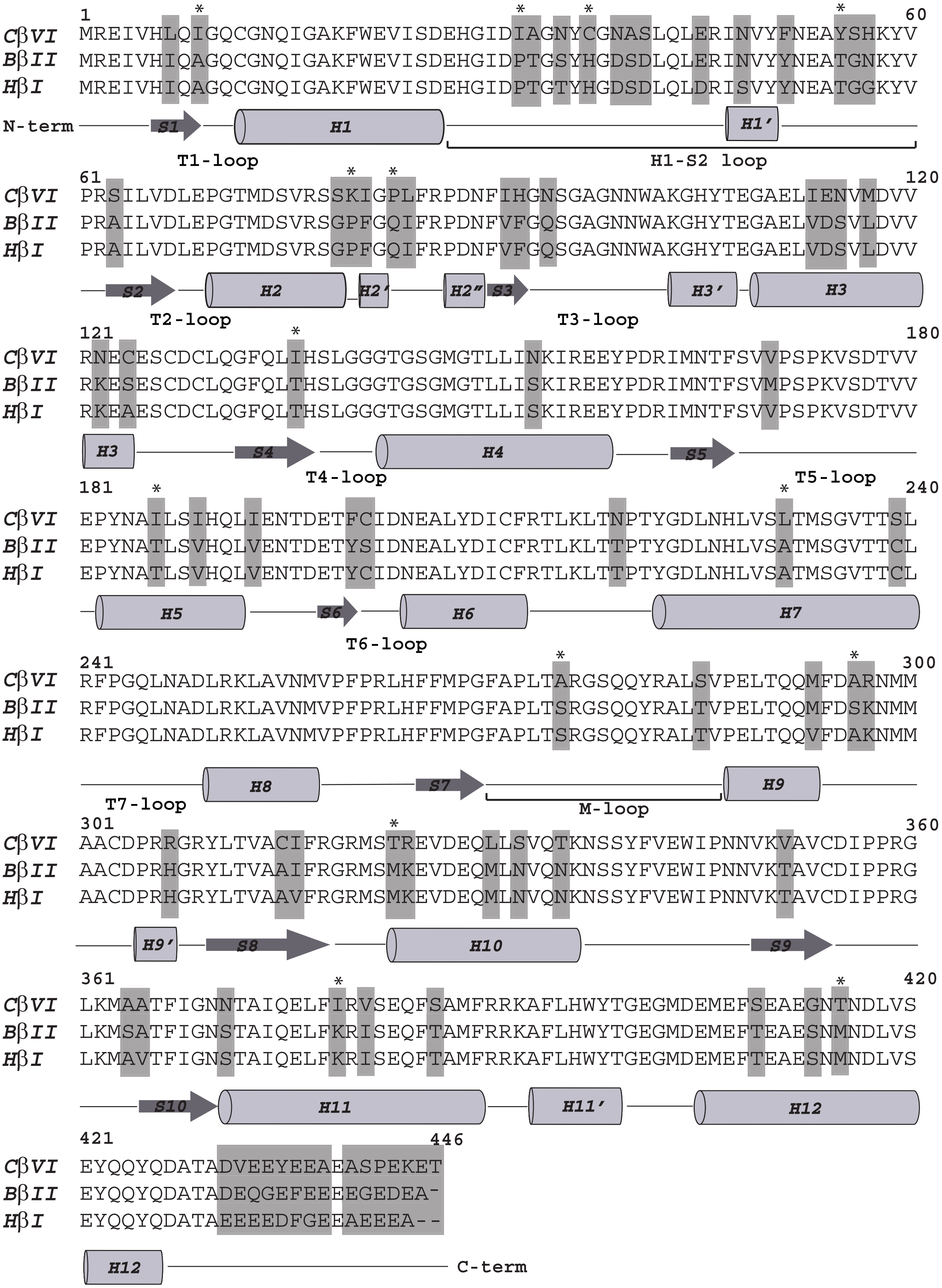
Tubulin Source	**Chicken Erythrocyte Tubulin (CET)**														**Bovine Brain Tubulin(BBT)**		
Binding Site	**Taxane site**								**Alternative site**					**α-tubulin**	**Taxane site**	**Alternative site**	**α-tubulin**
Drug/Mutation	**Native**	**F270V**	**L231A**	**A275S**	**A275SL231A**	**A296SA275S**	**A296SL231A**	**A296SA275SL231A**	**Native**	**A296S**	**A296SA275S**	**A296SL231A**	**A296SA275SL231A**	**Native**	**Native**		
EpoB average	-11.34	-11.64	-10.54	-10.2	-10.42	-10.5	-10.57	-10.64	-9.11	-9.19	-9.13	-9.12	-8.32	-8.12			
EpoB most common	-11.3	-11.5	-10.6	-10.3	-10.65	-10.5	-10.7	-10.55	-9.25	-9.25	-9.25	-9.22	-7.8	-8.15			
EpoB minumum	-11.59	-11.95	-10.82	-10.52	-10.77	-10.58	-10.77	-10.76	-9.32	-9.33	-9.29	-9.34	-9.08	-8.45			
Ixa average	-11.88	-11.44	-11.87	-10.59	-11.71	-10.48	-11.72	-12.09	-9.85	-9.77	-9.02	-9.8	-8.58	-9.19			
Ixa most common	-11.8	-11.15	-11.9	-10.65	-11.8	-10.55	-11.8	-12	-9.8	-9.8	-9.05	-9.8	-8.5	-9.25			
Ixa minimum	-12.04	-11.94	-11.95	-10.91	-11.85	-10.85	-11.82	-12.1	-9.94	-9.89	-9.09	-9.89	-8.88	-9.58			
LML average	-9.52	-9.65	-9.41	-8.49	-9.21	-8.34	-9.03	-9.13	-7.49	-7.88	-7.71	-7.89	-7.95	-7.14	-8.62	-9.99	-7.2
LML most common	-9.65	-9.8	-9.45	-8.65	-9.3	-8.3	-9.15	-9.55	-7	-7.5	-7.4	-7.8	-7.8	-7.35	-8.9	-10	-7.4
LML minimum	-9.95	-10.09	-9.51	-8.85	-9.38	-8.55	-9.29	-9.6	-7.83	-8.32	-8.49	-8.26	-7.99	-7.38	-9.08	-10.18	-7.62
PelA average	-9.52	-9.74	-9.05	-9.09	-8.65	-9.24	-8.84	-8.76	-9.68	-8.82	-9.12	-8.65	-8.76	-7.29	-8.62	-8.45	-5.92
PelA most common	-9.45	-9.8	-9.2	-9.1	-8.4	-9.3	-8.85	-8.6	-9.65	-8.65	-9.3	-8.75	-8.65	-7.4	-8.7	-8.3	-5.9
PelA minimum	-9.88	-10.14	-9.34	-9.28	-8.81	-9.48	-9.25	-8.94	-9.79	-9.02	-9.84	-9.54	-8.99	-7.59	-9.1	-9.04	-6.54
Taxol average	-13.54	-11.43	-11.96	-12.73	-11.83	-12.69	-11.81	-11.96	-8.7	-8.59	-8.8	-8.48	-8.37	-8.38			
Taxol most common	-13.8	-11.95	-12.55	-12.5	-12.3	-13.35	-11.85	-11.7	-8.15	-8.85	-8.4	-8.25	-8.05	-8.8			
Taxol minimum	-14.07	-12.24	-12.6	-12.94	-12.45	-13.47	-12.98	-12.14	-9.79	-9.7	-9.74	-9.44	-9.14	-8.85			

(b) Differences from native Taxane site energy

EpoB average	0	-0.3	0.8	**1.14**	0.92	0.84	0.77	0.7	**2.23**	**2.15**	**2.21**	**2.22**	**3.02**	**3.22**			
EpoB most common	0	-0.2	0.7	**1**	0.65	0.8	0.6	0.75	**2.05**	**2.05**	**2.05**	**2.08**	**3.5**	**3.15**			
EpoB minumum	0	-0.36	0.77	**1.07**	0.82	**1.01**	0.82	0.83	**2.27**	**2.26**	**2.3**	**2.25**	**2.51**	**3.14**			
Ixa average	0	0.44	0.01	**1.29**	0.17	**1.4**	0.16	-0.21	**2.03**	**2.11**	**2.86**	**2.08**	**3.3**	**2.69**			
Ixa most common	0	0.65	-0.1	**1.15**	0	**1.25**	0	-0.2	**2**	**2**	**2.75**	**2**	**3.3**	**2.55**			
Ixa minimum	0	0.1	0.09	**1.13**	0.19	**1.19**	0.22	-0.06	**2.1**	**2.15**	**2.95**	**2.15**	**3.16**	**2.46**			
LML average	0	-0.13	0.11	**1.03**	0.31	**1.18**	0.49	0.39	**2.03**	**1.64**	**1.81**	**1.63**	**1.57**	**2.38**	0	**-1.37**	**1.42**
LML most common	0	-0.15	0.2	**1**	0.35	**1.35**	0.5	0.1	**2.65**	**2.15**	**2.25**	**1.85**	**1.85**	**2.3**	0	**-1.1**	**1.5**
LML minimum	0	-0.14	0.44	**1.1**	0.57	**1.4**	0.66	0.35	**2.12**	**1.63**	**1.46**	**1.69**	**1.96**	**2.57**	0	**-1.1**	**1.46**
PelA average	0	-0.22	0.47	0.43	0.87	0.28	0.68	0.76	-0.16	0.7	0.4	0.87	0.76	**2.23**	0	0.17	**2.7**
PelA most common	0	-0.35	0.25	0.35	**1.05**	0.15	0.6	0.85	-0.2	0.8	0.15	0.7	0.8	**2.05**	0	0.4	**2.8**
PelA minimum	0	-0.26	0.54	0.6	**1.07**	0.4	0.63	0.94	0.09	0.86	0.04	0.34	0.89	**2.29**	0	0.06	**2.56**
Taxol average	0	**2.11**	**1.58**	0.81	**1.71**	0.85	**1.73**	**1.58**	**4.84**	**4.95**	**4.74**	**5.06**	**5.17**	**5.16**			
Taxol most common	0	**1.85**	**1.25**	**1.3**	**1.5**	0.45	**1.95**	**2.1**	**5.65**	**4.95**	**5.4**	**5.55**	**5.75**	**5**			
Taxol minimum	0	**1.83**	**1.47**	**1.13**	**1.62**	0.6	**1.09**	**1.93**	**4.28**	**4.37**	**4.33**	**4.63**	**4.93**	**5.22**			

Table S4. Effect of amino acid composition in the binding sites on the ΔG_{bind} of laulimalide.

	Mean Binding Energies (ΔG_{bind}) at 298 K (kcal/mol)				
	Wild Type CET	A275S	A296S	A275S/A296S	Wild Type BBT
Alternative Site	-7.49	-	-7.88	-8.34	-9.99
Taxane Site	-9.52	-8.49	-	-7.71	-8.62
$\Delta\Delta G_{bind}$ (Alt-Tx)	2.03	-	-	-0.63	-1.37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*
*
*
60
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BβII MREIVHIQAGQCGNQIGAKFWEVISDEHGIDPTGSYHGDSDLQLERINVYYNEATGNKYV
HβI MREIVHIQAGQCGNQIGAKFWEVISDEHGIDPTGTYHGDSDLQLDRISVYYNEATGGKYV
N-term
S1
H1
H1'
T1-loop
H1-S2 loop
61
*
*
120
CβVI PRSILVDLEPGTMDSVRSSKIGPLFRPDNFIHGNSGAGNNWAKGHYTEGAELIENVMDVV
BβII PRAILVDLEPGTMDSVRSGPFGQIFRPDNFVFGQSGAGNNWAKGHYTEGAELVDSVLDVV
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S2
H2
H2'
H2"
S3
H3'
H3
T2-loop
T3-loop
121
*
180
CβVI RNECESCDCLQGFQLIHSLGGGTGSGMGTLLINKIREEYPDRIMNTFSVVPSPKVSDTVV
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HβI RKEAESCDCLQGFQLTHSLGGGTGSGMGTLLISKIREEYPDRIMNTFSVVPSPKVSDTVV
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S4
H4
S5
T4-loop
T5-loop
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*
*
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S6
H6
H7
T6-loop
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*
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HβI RFPGQLNADLRKLAVNMVPFPRLHFFMPGFAPLTSRGSQQYRALTVPELTQQVFDAKNMM
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S7
H9
T7-loop
M-loop
301
*
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*
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S10
H11
H11'
H12
421
446
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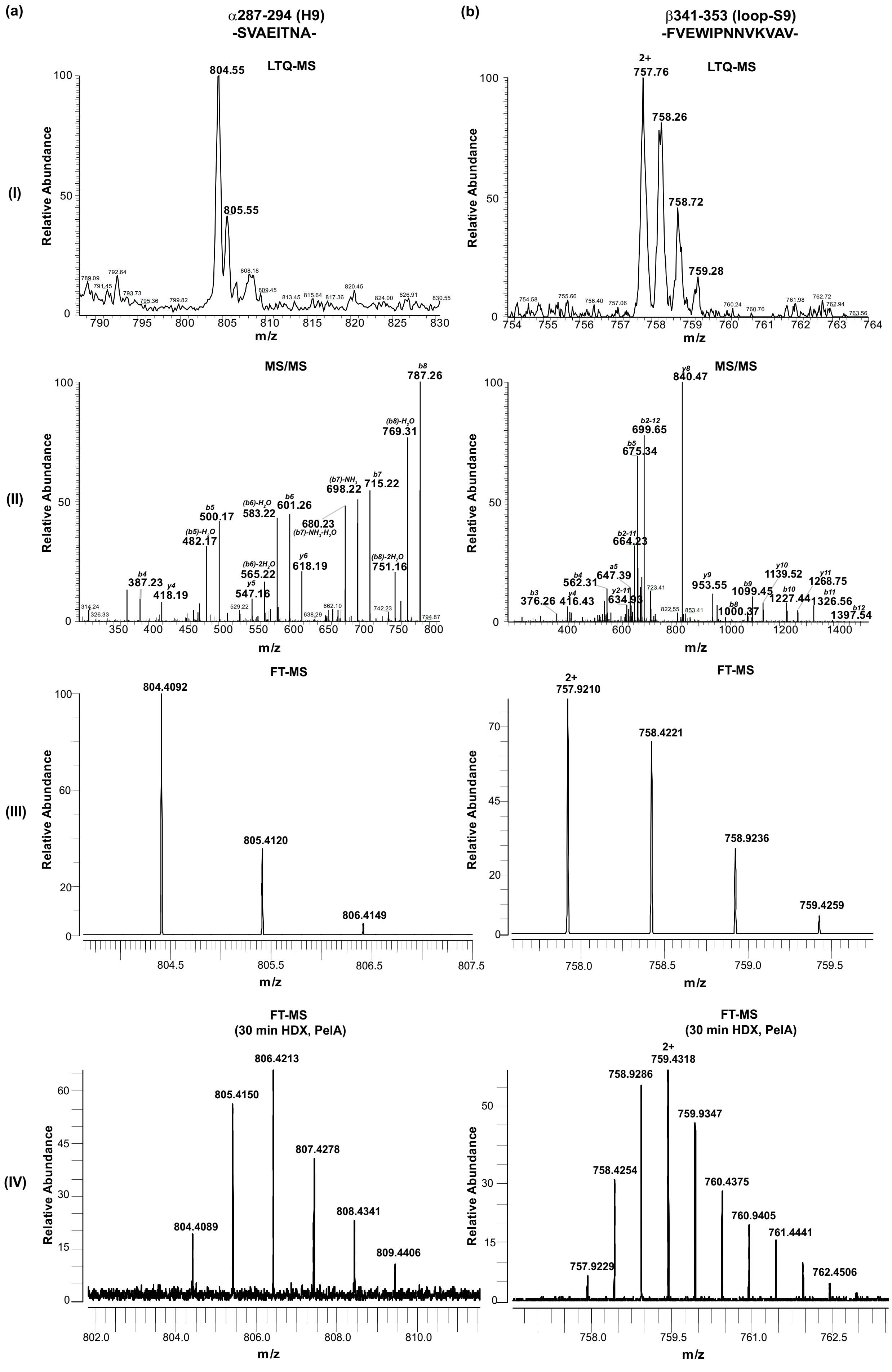
HβI EYQQYQDATAEEEEDFGEEAEEEA--
H12
C-term

Figure S1



(a)
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(b)
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(I)
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793.73
795.36
799.82
808.18
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813.45
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817.36
820.45
824.00
826.91
830.55
2+
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758.26
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754.58
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(II)
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y5
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y10
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807.4278
808.4341
804.4089
809.4406
2+
759.4318
758.9286
759.9347
758.4254
760.4375
760.9405
761.4441
757.9229
762.4506
Relative Abundance
m/z

Figure S2

```
α-TUB  MRECISVHVGQAGVQIGNACWELYCLEHGIQPDGQMPSDKTIGGGDDSFNTFFSETGAGK
               10        20        30        40        50        60
β-TUB  MREIVHLQIGQCGNQIGAKFWEVISDEHGIDIAGNYCGNASLQLERINVYFNEAYSHKYV

α-TUB  QPDGQMPSDKTIGGGDDSFNTFFSETGAGKQLITGKEDAANNYARGHYTIGKEIIDLVLD
               70        80        90       100       110       120
β-TUB  PRSILVDLEPGTMDSVRSSKIGPLFRPDNFIHGNSGAGNNWAKGHYTEGAELIENVMDVV

α-TUB  RIRKLADQCTGLQGFSVFHSFGGGTGSGFTSLLMERLSVDYGKKSKLEFSIYPARQVSTA
              130       140       150       160       170       180
β-TUB  RNECESCDCLQGFQLIHSLGGGTGSGMGTLLINKIREEYPDRIMNTFSVVPSPKVSDTVV

α-TUB  VVEPYNSILTTHTTLEHSDCAFMVDNEAIYDICRRNLDIERPTYTNLNRLIGQIVSSITA
              190       200       210       220       230       240
β-TUB  EPYNAILSIHQLIENTDETFCIDNEALYDICFRTLKLTNPTYGDLNHLVSLTMSGVTTSL

α-TUB  SLRFDGALNVDLTEFQTNLVPYPRIHFPLATYAPVISAEKAYHEQLSVAEITNACFEPAN
              250       260       270       280       290       300
β-TUB  RFPGQLNADLRKLAVNMVPFPRLHFFMPGFAPLTARGSQQYRALSVPELTQQMFDARNMM

α-TUB  QMVKCDPRHGKYMACCLLYRGDVVPKDVNAAIATIKTKRTIQFVDWCPTGFKVGINYQPP
              310       320       330       340       350       360
β-TUB  AACDPRRGRYLTVACIFRGRMSTREVDEQLLSVQTKNSSYFVEWIPNNVKVAVCDIPPRG

α-TUB  TVVPGGDLAKVQRAVCMLSNTTAIAEAWARLDHKFDLMYAKRAFVHWYVGEGMEEGEFSE
              370       380       390       400       410       420
β-TUB  LKMAATFIGNNTAIQELFIRVSEQFSAMFRRKAFLHWYTGEGMDEMEFSEAEGNTNDLVS

α-TUB  AREDMAALEKDYEEVGVDSVEGEGEEEGEE
              430       440       450
β-TUB  EYQQYQDATADVEEYEEAEASPEKET
```

Figure S3

α287-294 (H9)
-SVAEITNA-

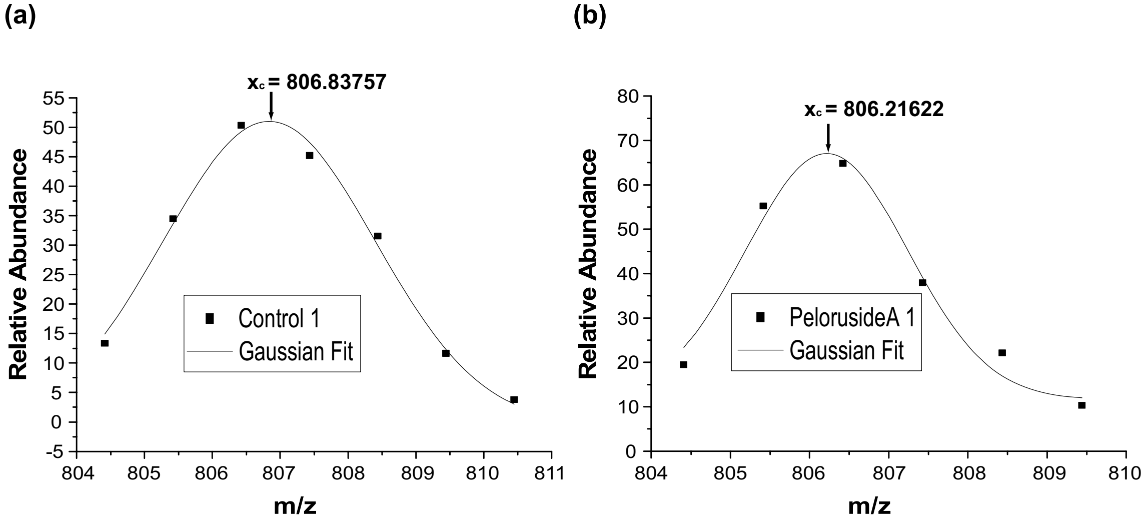

Figure S4

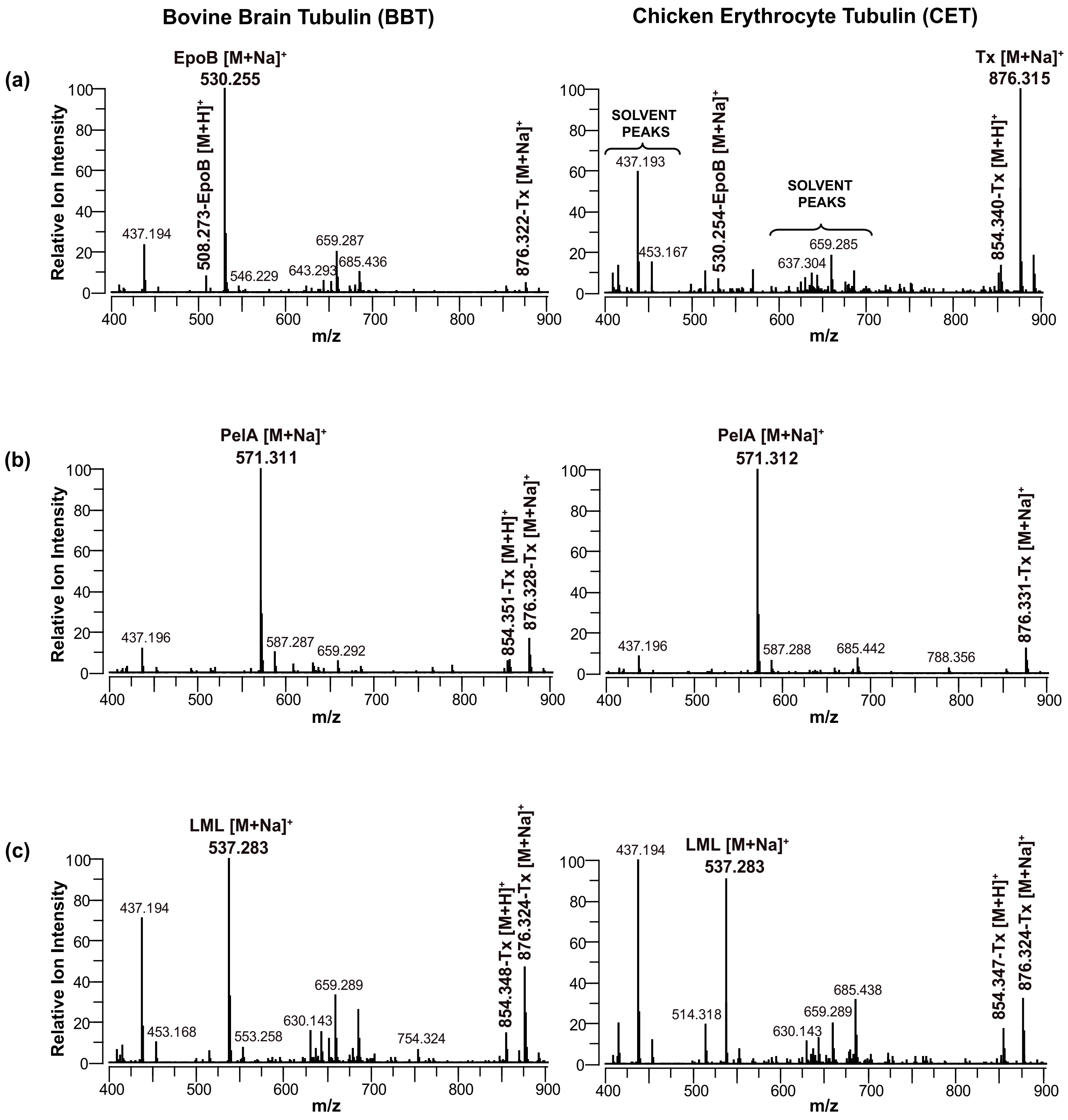
Bovine Brain Tubulin (BBT)
Chicken Erythrocyte Tubulin (CET)
(a)
EpoB [M+Na]⁺
530.255
508.273-EpoB [M+H]⁺
876.322-Tx [M+Na]⁺
437.194
546.229
643.293
659.287
685.436
Tx [M+Na]⁺
876.315
SOLVENT PEAKS
437.193
453.167
530.254-EpoB [M+Na]⁺
SOLVENT PEAKS
637.304
659.285
854.340-Tx [M+H]⁺
(b)
PelA [M+Na]⁺
571.311
437.196
587.287
659.292
854.351-Tx [M+H]⁺
876.328-Tx [M+Na]⁺
PelA [M+Na]⁺
571.312
437.196
587.288
685.442
788.356
876.331-Tx [M+Na]⁺
(c)
LML [M+Na]⁺
537.283
437.194
453.168
553.258
630.143
659.289
754.324
854.348-Tx [M+H]⁺
876.324-Tx [M+Na]⁺
437.194
LML [M+Na]⁺
537.283
514.318
630.143
659.289
685.438
854.347-Tx [M+H]⁺
876.324-Tx [M+Na]⁺
Relative Ion Intensity
0
20
40
60
80
100
m/z
400
500
600
700
800
900

Figure S5

(a)

ΔG_{bind} at 298 K (kcal/mol)		
	Wild Type	F270V
Epothilone B	-11.34	-11.64
Ixabepilone	-11.88	-11.44

(b)

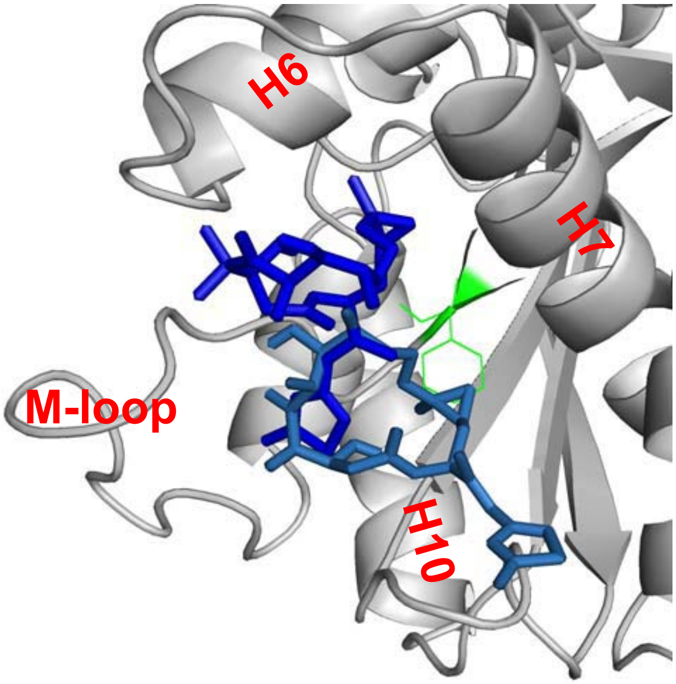

Figure S6