Supporting information

The molecular recognition of epothilones by microtubules and tubulin dimers revealed by biochemical and NMR approaches

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Table S1. Aggregation state of tubulin in D_2O buffer at 25 °C determined by sedimentation velocity in the analytical ultracentrifuge

Tubulin preparation	$s^0_{20,w}$ (S)[1] (at age 2 h)	signal [fringes] (% of sedimenting protein analyzed)	$s_{20,w}$ (S) (at age 96 h)	signal [fringes] (% of sedimenting protein analyzed.)
W-TUB column (13 μM)	5.8 9.3 11.7 15.2	3.63 (94 %) 0.088 (2.3 %) 0.037 (0.9 %) 0.021 (0.5%)	5.8 8.7 11.2 13.7 15-30[2]	1.45 (38%) 0.099 (2.6%) 0.107 (2.8%) 0.369 (9.6%) nd[3]
W-TUB column (13 μM) + EpoA (0.5 mM)	5.8 9.3 11.7 14.3	3.47 (90 %) 0.124 (3.2%) 0.044 (1.1%) 0.021 (0.5%)	5.9 8.7 12.4 14.4 15-30[2]	1.03 (27%) 0.068 (1.8%) 0.051 (1.3%) 0.129 (3.3%) nd[3]
W-TUB column (130 μM)	5.8 7.9 11.8 14.6	37.6 (92%) 1.84 (4.5%) 0.84 (2.0%) 0.64 (1.6%)		
W-TUB column (130 μM) + EpoA (50 μM)	5.9 9.5 12.9 17.2	38.1 (92%) 1.6 (3.7%) 1.2 (2.9%) 0.43 (1.0%)		
W-TUB column (130 μM) + EpoA (150 μM)	5.9 9.4 12.3 15.4	35.6 (92%) 1.7 (4.5%) 0.84 (2.1%) 0.49 (1.3%)		
W-TUB column (130 μM) + EpoB (50 μM)	6.0 9.4 12.3 15.4	38.8 (94 %) 1.36 (3.3%) 0.74 (1.8%) 0.39 (0.9%)		
W-TUB Column (130 μM) + EpoA (150 μM)	6.0 9.7 12.7	30.2 (95%) 1.22 (3.8%) 0.50 (1.2%)		

[1]The sedimentation coefficient of tubulin was corrected by concentration employing the previously determined correction for the concentration dependence of the sedimentation coefficient of tubulin dimers, ($sC = s0(1 - gC)$, where C is the tubulin concentration (g/L) and g = 0.019 L/g) (*43*).

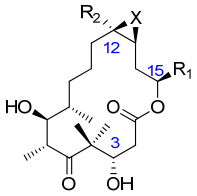
[2]broadly sedimenting zone

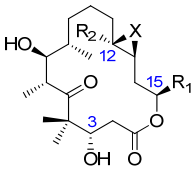
[3]not determined

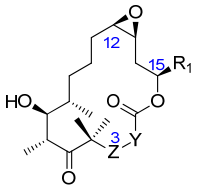
Table S2. Dihedrals angles of EpoA and EpoB in their respective microtubule-bound conformations. The values reported are calculated as the average of the three lowest energy conformers found in the conformational searches which are in agreement with the experimental NOE data. *Dihedral angle of the major conformer, syn conformation. The anti conformer is also present in solution and it is characterized by a C16C17C18N torsional close to twenty degrees. EpoA dihedrals reported by Carlomagno et al. (*14,20*) are given for comparison.

	EpoA **Carlomagno et al.**	**EpoA** (major conformer, syn)	**EpoB** (major conformer, syn)
C1C2C3C4	-152,5	-174,7	174,3
C2C3C4C5	-51,7	-58,6	-61,9
C3C4C5C6	-43	-74,6	-52,0
C4C5C6C7	156,4	147,1	152,7
C5C6C7C8	-70	-61,4	-64,2
C6C7C8C9	-74,8	-68,2	-74,8
C7C8C9C10	164,1	168,1	161,3
C8C9C10C11	-171,9	177,1	170,0
C9C10C11C12	-178	170,9	146,1
C10C11C12C13	-129,2	-105,7	-102,3
C11C12C13C14	4,1	-2,8	-2,0
C12C13C14C15	76,3	98,0	103,1
C13C14C15O1	-62,6	-75,4	-63,3
C14C15O1C1	179,5	149,7	112,6
C15O1C1C2	176,3	164,8	163,9
O1C1C2C3	-124,3	-47,1	-55,2
C14C15C16C17	-129,7	-116,7	-118,1
C15C16C17C18	178.9	176,3	176,6
C16C17C18N	137.9	151,8*	151,2*

Table S3. Chemical structures and thermodynamic binding data of the epothilones included in the SAR study.

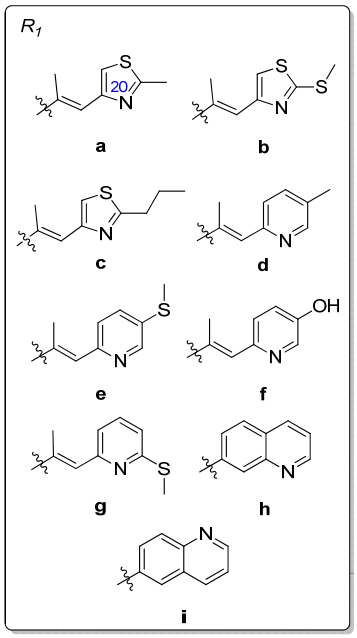






Compound	R_1	R_2	X	ΔG [kJ·mol^{-1}]
EPA	**a**	H	O	-44.5 ± 0.3
EPB	**a**	Me	O	-52.6 ± 0.5
EP3	**b**	Me	O	-55.4 ± 0.6
EP4	**d**	H	CH_2	-49.4 ± 0.3
EP14	**a**	H	CH_2	-49.2 ± 0.4
EP19	**b**	Me	CH_2	-60.8 ± 0.5
EP20	**d**	Me	CH_2	-52.2 ± 0.4
EPKT4	**h**	Me	O	-52.8 ± 0.3
EPKT5	**i**	Me	O	-46.2 ± 0.3
EPKT6	**c**	H	O	-43.3 ± 0.5
EP5	**a**	H	CH_2	-48.6 ± 0.1
EP6	**a**	Me	O	-48.2 ± 0.2
EP7	**d**	H	O	-51.5 ± 0.2
EP8	**d**	Me	CH_2	-50.3 ± 0.1
EP9	**g**	Me	CH_2	-48.9 ± 0.3
EP10	**b**	H	CH_2	-54.5 ± 0.6
EP11	**b**	Me	CH_2	-51.8 ± 0.3
EP12	**e**	Me	O	-47.7 ± 0.4
EP13	**f**	Me	CH_2	-43.6 ± 0.3
EP15	**a**	H	$(CH_2)_2$	-44.5 ± 0.3

Compound	R_1	Z-Y	ΔG [kJ·mol^{-1}]
EPKT2	**a**	CH_2-CH_2	-39.9 ± 0.2
EPKT3	**a**	CH=CH	-42.6 ± 0.6



Data for EpoA, EpoB and EP3–EP20 from ref. 9. Data for EPKT2– EPKT6 from ref. 19.

Table S4. Selected projection to latent structure (PLS) pseudo-coefficients (absolute value ≥ |0.01|) for the amino acid residues (numbering as in PDB entry 4I50) that contribute the most to explaining the predicted binding free energy differences.

Residue	van der Waals	residue	electrostatic
Leu 217	0.035	Lys 19	-0.212
Asp 226	0.115	Asp 26	0.224
His 229	0.720	Glu 27	0.254
Leu 230	0.554	Asp 226	0.215
Ala 233	-0.522	Pro 274	-0.138
Phe 272	0.784	Thr 276	0.010
Pro 274	0.392	Arg 278	-0.035
Leu 275	-0.213	Gln 281	0.598
Thr 276	0.229	Arg 284	0.566
Arg 278	0.066	Glu 290	-0.310
Gln 281	0.568	Asp 297	-0.121
Arg 284	-0.047	Arg 320	-0.166
Ala 285	0.036	Arg 369	-0.203
Leu 286	-0.135	Lys 372	-0.009
Glu 290	0.111		
Leu 371	0.165	water molecule	0.018
Lys 372	0.031		

Supporting Figures

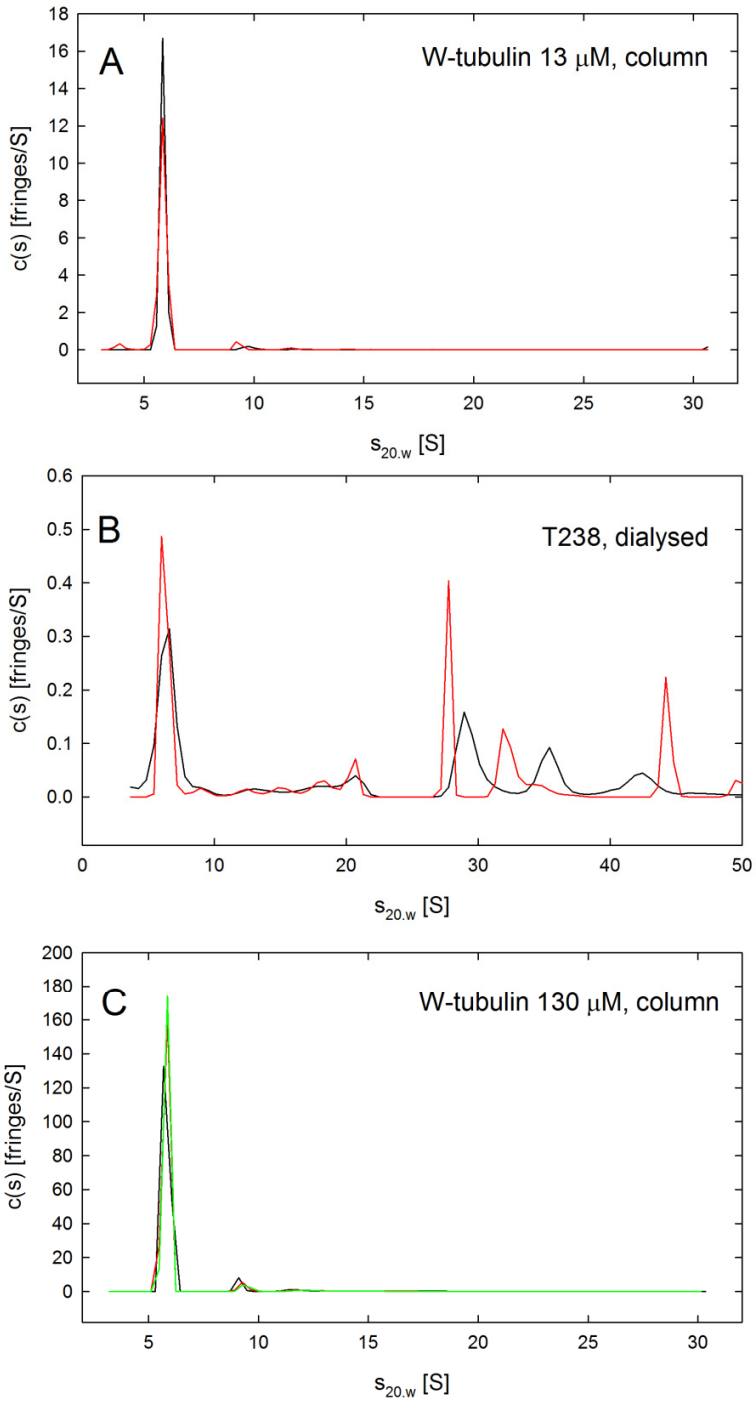


Figure S1.- Aggregation state of tubulin samples equilibrated in different D_2O buffers for NMR experiments. Sedimentation velocity experiments at 25 ºC in an analytical ultracentrifuge equipped with interference optics, data analysis to determine the sedimentation coefficient distribution *c(s)* and correction of *s* values for solvent composition and temperature to standard conditions (H_2O, 20 ºC) were as described before *(6)*. A. For sample preparation, tubulin purified in large scale in our laboratory and stored in liquid nitrogen (W-tubulin (*40*)) was equilibrated immediately before use in 10 mM sodium phosphate buffer, 0.1 mM GTP in 99.9 % D_2O, pH* 7.0 by chromatography through a Sephadex G-25 (medium) column (0.9x20 cm, 30ml/h). The tubulin concentration (13 μM W-tubulin) was measured spectrophotometrically employing an extinction coefficient of 116,000 $M^{-1}cm^{-1}$ at 276 nm (*40*). The D_2O concentration of the column effluent was ~ 99%, determined by gravimetric measurements. The c(s) distributions for samples without (black lines) or with 0.5 mM epopthilone A (red lines) are shown. B. For comparison with previous NMR studies (*14,15,19*), commercial lyophilized tubulin (T238 from Cytoskeleton, Denver, CO, USA) was dissolved and dialyzed (2 x 20h, 4 °C) against 2.5 mM PO_4H_3/NaOH, 1.5 mM $Ca(OH)_2$ made in 99.9 % D_2O (the pD of the resulting buffer was 6.85), employing washed CelluSep dialysis membrane (4-6 kDa cutoff) in a QuixSep micro dialysis device (Membrane Filtration Products Inc., San Antonio, Texas, USA). A theoretical 10.7 μM tubulin concentration was dissolved, from which 6 μM tubulin was recovered. Commercial tubulin T238 was also column equilibrated in the same buffer as W-tubulin for comparison, it behaves essentially identically as W-tubulin. The c(s) distributions for samples without (black lines) or with 0.5 mM epothilone A (red lines) are shown. C. As control for the oligomerization state of tubulin in the binding experiments shown in Figure 1 W-tubulin was equilibrated immediately before use in 10 mM sodium

phosphate buffer, 0.1 mM GTP in 99.9 % D_2O, pH* 7.0 by chromatography through Sephadex G-25 (medium). The tubulin concentration (130 μM W-tubulin) was measured spectrophotometrically employing an extinction coefficient of 116,000 $M^{-1}cm^{-1}$ at 276 nm (*40*). The c(s) distributions for samples without (black lines), with 50 μM epothilone A (red lines) or with 50 μM epothilone B (green lines) are shown.

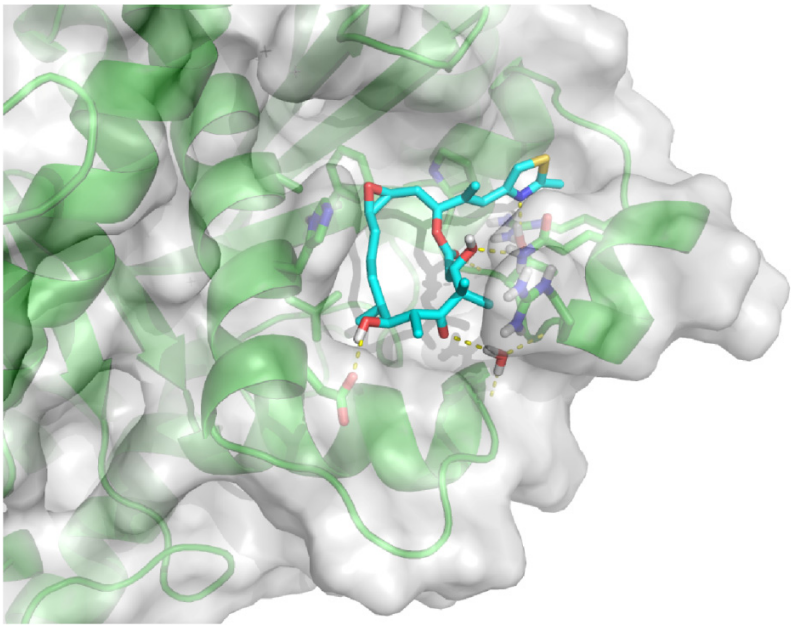


Figure S2.- Close-up view of EpoA bound to β-tubulin, as present in PDB entry 4I50. Note the extra water molecule that is proposed to bridge good hydrogen-bonding interactions between the carbonyl oxygen at position 5 of EpoA and both the main-chain NH of Arg278 and the main-chain CO of Leu217.