Crystal structure of the cyclostreptin-tubulin adduct: Implications for tubulin activation by taxane-site ligands.

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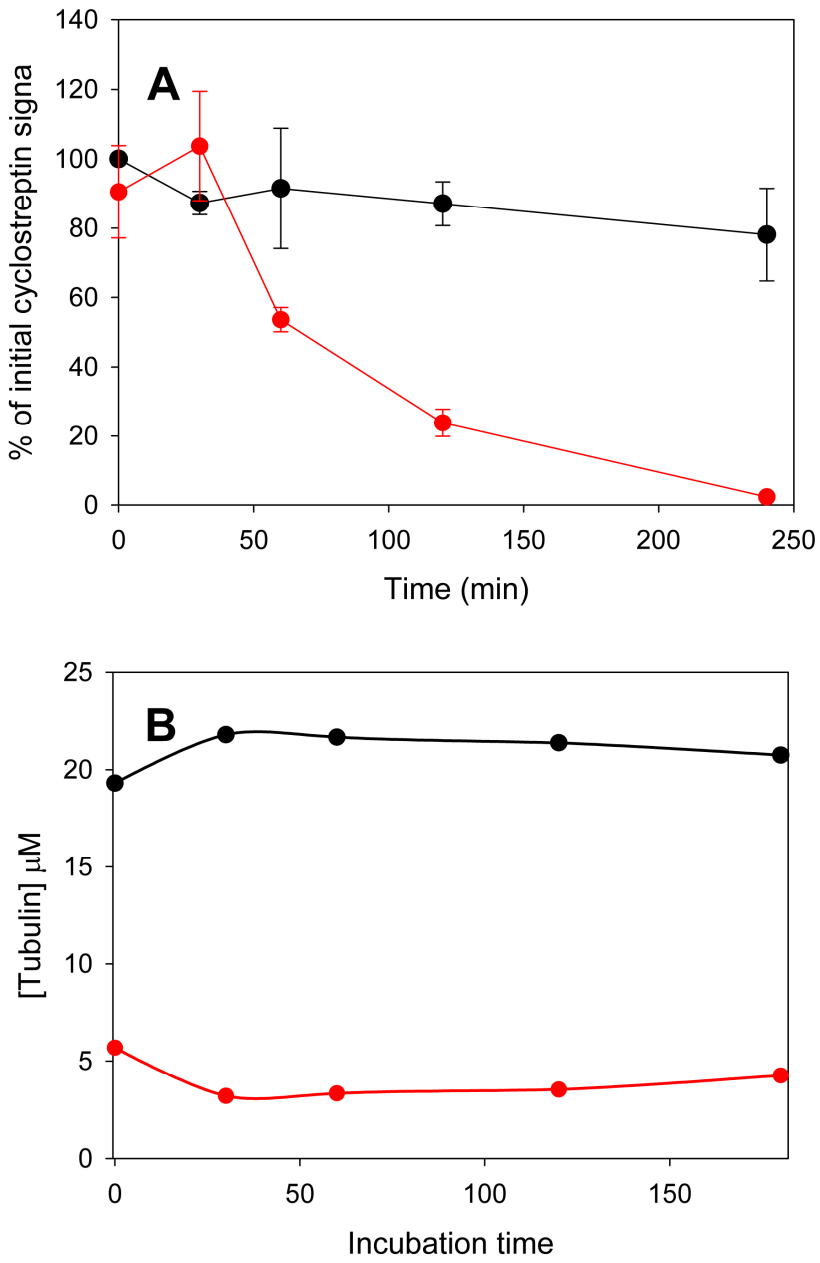

Figure S1. Cyclostreptin binding and stability of tubulin-cyclostreptin adduct : A). Time course of reaction of 25µM cyclostreptin with dimeric tubulin followed by HPLC-MS. Black circles and lines: Unreacted cyclostreptin in the absence of tubulin. Red circles and lines: Unreacted cyclostreptin in the presence of 20 µM tubulin. B) Quantification of the assembly of the tubulin-cyclostreptin complex incubated at different times. Black circles and lines pelleted tubulin (microtubules), red circles and lines supernatant tubulin (not assembled dimers).

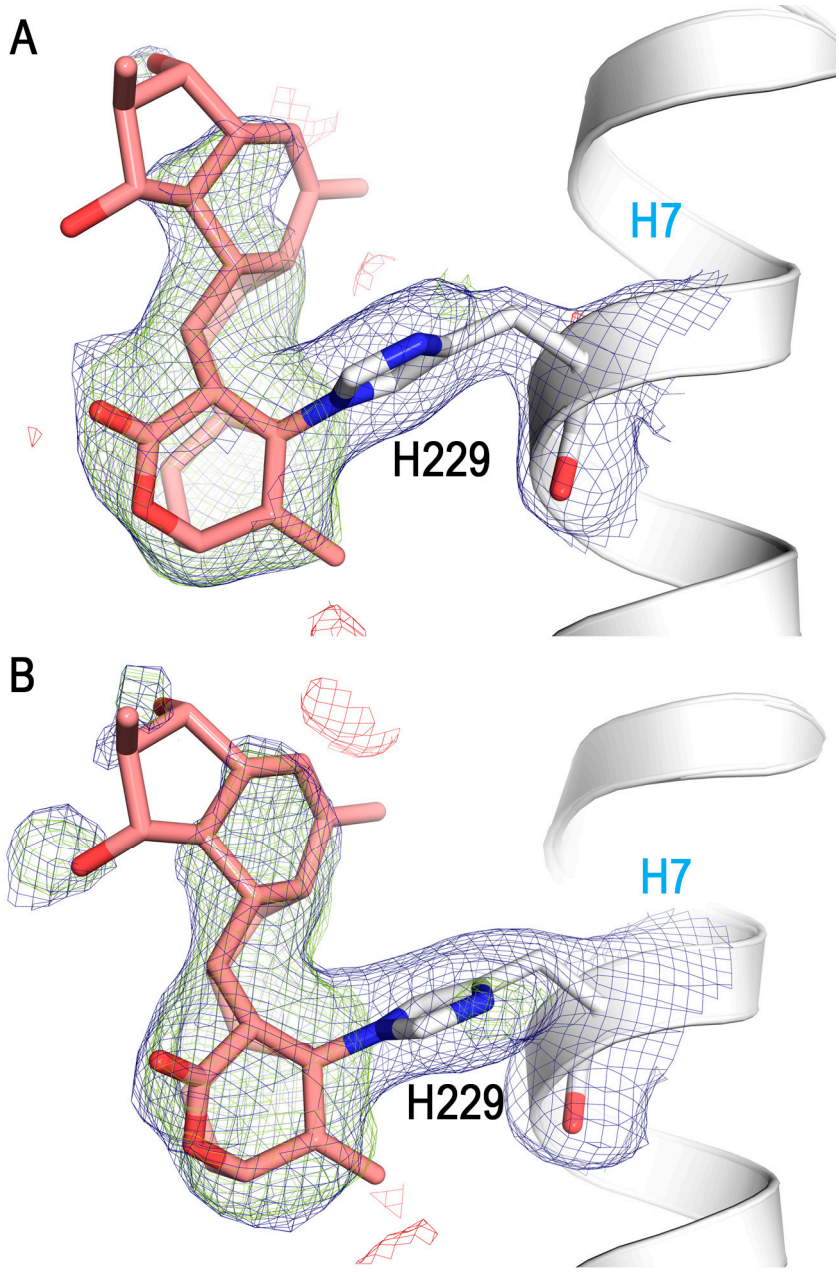

Figure S2. Electron-density maps of both the cyclostreptin molecules bound to tubulin in chain B (panel A) and chain D (panel B) of the T_2R-TTL complex. The SigmaA-weighted 2mFo-DFc (dark blue mesh contoured at +0.7σ) and mFo-DFc (light green and red mesh contoured at +/- 2.5σ, respectively) simulated annealing omit maps were calculated by excluding the atoms of the cyclostreptin molecules. Both the covalently bound cyclostreptin molecules (salmon) and His229 residues are depicted in stick representation.

Table S1. Data collection and refinement statistics for the T_2R-TTL-cyclostreptin complex

	T2R-TTL-cyclostreptin
Data collection	
Space group	$P2_12_12_1$
Cell dimensions	
a, *b*, *c* (Å)	104.6, 158.4, 179.95
Resolution (Å)	49.7 – 1.9 (1.95-1.90)
R_{merge} *(%)*	10.2 (448.5)
R_{meas} *(%)*	10.4 (457.2)
R_{pim} *(%)*	2.5 (84.3)
$I / \sigma I$	22.7 (0.9)
CChalf	100 (32.2)
Completeness (%)	100 (100)
Redundancy	26.8 (27.0)
Refinement	
Resolution (Å)	49.7 – 1.9
No. unique reflections	234314
R_{work} / R_{free}	18.7 / 21.3
No. atoms	
Protein	17404
Ligand	58
Water	681
Average *B*-factors (Å^2)	
Protein	62.3
Ligand (chain B / D)	85.1 / 91.5
Water	55.7
Wilson *B*-factor	42.4
R.m.s. deviations	
Bond lengths (Å)	0.004
Bond angles (°)	0.652
Ramachandran statistics[c]	
Favored regions (%)	98.0
Allowed regions (%)	2.0
Outliers (%)	0

*Values in parentheses are for highest-resolution shell.