

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

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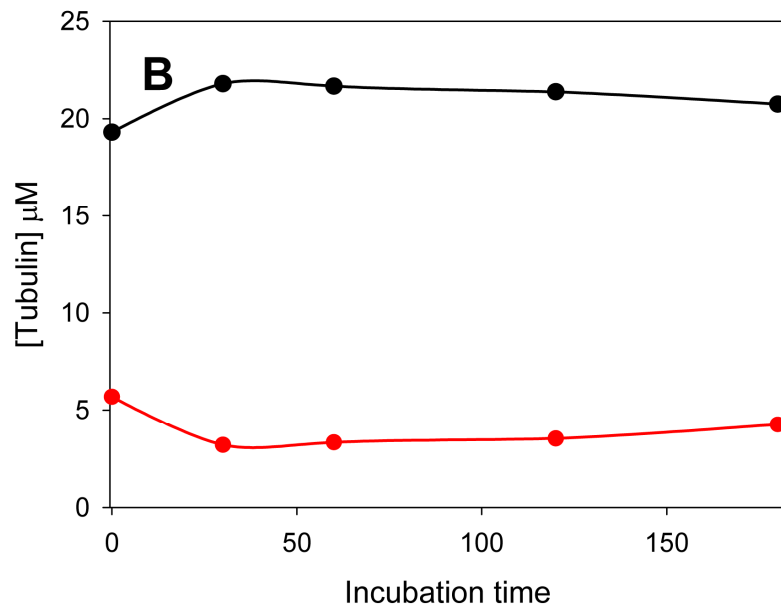
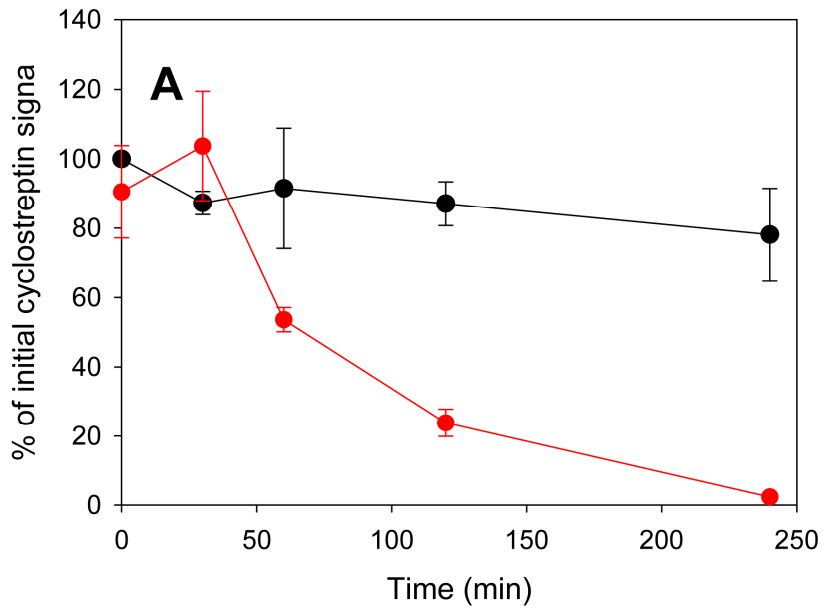


Figure S1. Cyclostreptin binding and stability of tubulin-cyclostreptin adduct : A). Time course of reaction of 25μM cyclostreptin with dimeric tubulin followed by HPLC-MS. Black circles and lines: Unreacted cyclostreptin in the absence of tubulin. Red circles and lines: Unreacted cyclostreptin in the 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).

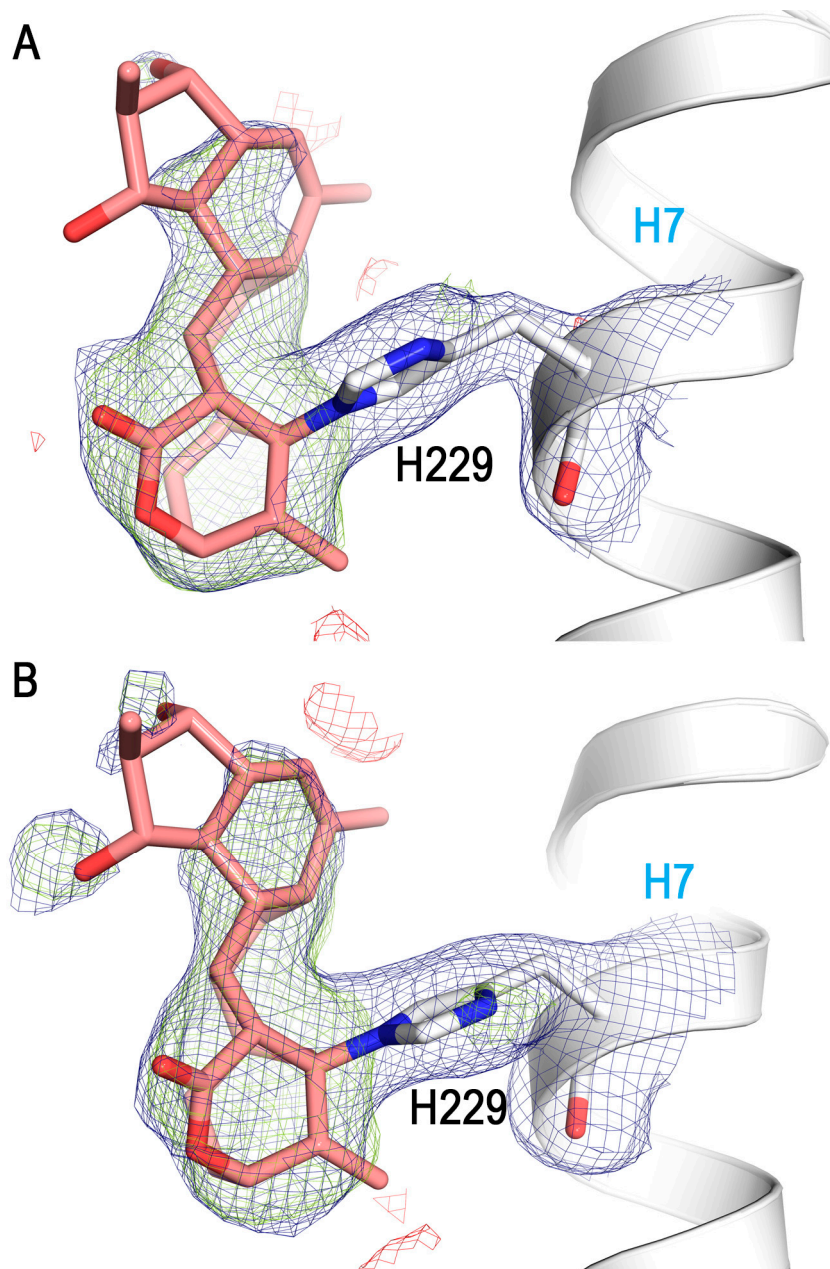


Figure S2. Electron-density maps of both the cyclostreptin molecules bound to tubulin in chain B (panel A) and chain D (panel B) of the  $T_2R$ -TTL complex. The SigmaA-weighted  $2mFo-DFc$  (dark blue mesh contoured at  $+0.7\sigma$ ) and  $mFo-DFc$  (light green and red mesh contoured at  $\pm 2.5\sigma$ , 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<sub>2</sub>R-TTL-cyclostreptin complex

	T2R-TTL- cyclostreptin
<b>Data collection</b>	
Space group	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>
Cell dimensions	
<i>a</i> , <i>b</i> , <i>c</i> (Å)	104.6, 158.4, 179.95
Resolution (Å)	49.7 – 1.9 (1.95-1.90)
<i>R</i> <sub>merge</sub> (%)	10.2 (448.5)
<i>R</i> <sub>meas</sub> (%)	10.4 (457.2)
<i>R</i> <sub>pim</sub> (%)	2.5 (84.3)
<i>I</i> / $\sigma I$	22.7 (0.9)
CChalf	100 (32.2)
Completeness (%)	100 (100)
Redundancy	26.8 (27.0)
<b>Refinement</b>	
Resolution (Å)	49.7 – 1.9
No. unique reflections	234314
<i>R</i> <sub>work</sub> / <i>R</i> <sub>free</sub>	18.7 / 21.3
No. atoms	
Protein	17404
Ligand	58
Water	681
Average <i>B</i> -factors (Å <sup>2</sup> )	
Protein	62.3
Ligand (chain B / D)	85.1 / 91.5
Water	55.7
Wilson <i>B</i> -factor	42.4
R.m.s. deviations	
Bond lengths (Å)	0.004
Bond angles (°)	0.652
Ramachandran statistics <sup>c</sup>	
Favored regions (%)	98.0
Allowed regions (%)	2.0
Outliers (%)	0

\*Values in parentheses are for highest-resolution shell.