Supporting Information

T-Taxol and the Electron Crystallographic Density in β-Tubulin

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Conformations

A conformation of PTX was modified by replacing the *para*-H of the C-2 benzoyl side chain phenyl with *para*-F to give 2-FB-PT. Then, four C-13 side chain angles obtained from **Figure 5** of the Geney et al. paper[1] were incorporated to generate the New York conformation (**PTX-NY**): C12-C13-O13-C1' 155°, C13-O13-C1'-O1' 4°, O13-C1'-C2'-O2' 22°, and O2'-C2'-C3'-N3' 178°. The structure was then superimposed onto T-Taxol to provide the relative orientation of the two structures as depicted in **Figure 3A** of the Geney work.[1] Each was then docked into β-tubulin (1JFF)[2] as illustrated in the main text **Figure 2A** of the main text, the O-C1'-C2'-C3' train of atoms taking different pathways in the binding pocket. Prior to molecular dynamics (MD) treatment, the C-C[(=O)NH_2] bond of Asp26 was rotated to give the benzamide phenyl room to reside in the pocket without severe steric conflict. MD was subsequently performed on a 10 Å sphere around the binding site at 20 °K with a time step of 0.5 fs and dielectric constant of 4.5 for 5 ps. Within this sphere the ligand and protein side chains were allowed to move, all atoms outside the sphere frozen in order to maintain the overall integrity of the protein. Throughout the simulations, the C2'-OH---N-His227 distance was constrained to 2.5-3.5 Å in accord with the observation that **PTX-NY** enjoys a hydrogen bond between these centers. As the molecular dynamic simulations proceeded, the binding pocket of tubulin expanded slightly to better accommodate the ligand, although the only significant change observed was the position of the His227 side chain. Since, the unprotonated N of the imidazole ring attempted to form a hydrogen bond with the C2'-OH of the ligand (~3.0 Å), the plane of the ring rotated relative to its position in 1JFF to accommodate the interaction. The hydrogen bond length varied between 2.5 and 3.4 Å throughout the entire simulation, although for 90% of the trajectory it oscillated evenly between 2.5 and 3.0 Å.

The PTX RMSD of the simulation from start to finish was 0.7 Å assuring that the ligand did not reorder conformationally or depart the pocket. In accord, the energy varied within only 3 kcal/mol throughout the last ps of the short trajectory. With this model in hand, the constraints were removed and full optimization of the complex with the backbone atoms held fixed was performed with the MMFF94 force field. The resulting structure sustained the following atomic separations: d1 = 11.0 Å and d2 = 10.4 Å.

The above treatment of the complex between tubulin and **PTX-NY** does not constitute a full MD study of the system. This would require longer simulation times and higher temperatures. Our purpose here is not to perform such a study but to generate a reasonable model of the New York conformer for comparison with the electron crystallographic structure of tubulin bound to T-Taxol. Examination of Figure 2a in the

main text and Figure 3a in the Geney-Ojima investigation[1] makes it clear we have accomplished this goal. A more vigorous treatment would most likely not do so. For example, optimization of the T-Taxol structure of Figure 2a with either the MM3* or MMFF force fields causes a very slight reorientation of the terminal C3' phenyl rings (i.e. around the C-C_6H_5 bonds), but no other significant changes. T-Taxol is clearly a minimum on these two potential energy surfaces. The same treatment for the New York conformer, however, causes the C3'-OH group to reorganize within the C-13 side chain conformation such that the optimized conformer is no longer in a position to make a hydrogen bond to His227. Since this is a key feature of the Geney et al. model, we have made every effort to retain it in the corresponding protein complex. The gentle 20 °K treatment permits this while eliminating close interatomic contacts.

The behavior of the bridged compound **1** in the New York conformation **(1-NY)** was described in the main text in terms of its reorganization upon optimization as an unbound ligand. A similar evaluation was performed within the β-tubulin binding site by subjecting **1-NY** to an MMFF94 force field MD treatment similar to that described above for **PTX-NY**. That is, MD was performed within a 10 Å sphere around the binding site at 20 °K with a time step of 0.5 fs and dielectric constant of 4.5 for 5 ps. During the last 3 ps, the overall RMS deviation of the ligand was 0.35 Å and the energy varied by less than 3 kcal/mol. This was followed by optimization of the ligand and all residues within 10 Å of the binding site. The resulting model displays the ligand as attempting to depart from the **1-NY** conformer as shown in Figure 3 of the main text, but to a considerably lesser extent. Namely, the C-2' OH and the C-3' phenyl rings have adjusted in response to the steric contacts, but the binding site constrains a full expression of the effect. In addition, unlike the T-Taxol conformer of **1**, the bridged PTX structure **1-NY** recedes from deep occupation of the binding pocket so as to avoid a steric clash between the tether and β-tubulin's Phe272. The situation is reminiscent of that for bridged taxanes with extended C-4 OAc to C-3' phenyl linkers determined to have reduced activity based on a very similar unfavorable ligand-Phe272 steric interaction.[3] The outcome for **1-NY** is inconsistent with the exceptional activity of taxane **1**.[4]

EC Density Maps

For all maps,[5] the 1JFF protein structure and corresponding structure factors were employed with b-factors flattened to 30.0 during the map generation process. The T-Taxol structure was taken from the computationally refined tubulin-PTX complex,[6] while the New York conformation was obtained as described above. Both $2F_{obs}$-F_{calc} and difference maps were generated using CNS 1.1[7] and compared to maps generated from CCP4[8] for consistency. It should be noted that Fourier difference maps are capable of visualizing changes at low noise levels between two closely related ligands in a common binding site that correspond to only a few carbon atoms.[9] CNS topology and parameter files were obtained from the HIC-UP (Hetero-compound Information Centre - Uppsala) server hosted by Uppsala Universitet.[10] For easier visualization of the CNS $2F_{obs}$-F_{calc} omit map, a solvent mask was generated to hide solvent and protein density farther than 1.5 Å from either ligand's coordinates. The maps were compared in 'O'[11] and the publication images were generated with Pymol. Cut-off values for the difference maps were determined visually to maximize image quality and did not exceed a difference cut-off of +/- 2.50 sigma as determined by 'O'. Due to scaling inconsistencies between the

masked $2F_{obs}$-F_{calc} omit map and the original map, the two maps were visually compared in 'O' at 1.00 sigma and the masked cut-off was adjusted accordingly

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