Supplementary material for Snyder *et al*. (April 17, 2001) *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas.051309398

Computational Procedures

Twenty-six paclitaxel (PTX) conformers were derived from x-ray crystal structures or from NMR nuclear Overhauser effect data of PTX and its derivatives (1-10). Side-chain conformations of the taxane structures were retained, but each set of coordinates was modified to give the corresponding PTX structure. Individual conformers were subsequently docked as a rigid body into the experimental density map of the tubulin (TB)-PTX complex. The best fit for each conformation was determined by visual inspection on an SGI O2 workstation by using SYBYL 6.4.2 (http://www.tripos.com) and O 6.1.0 (http://www.imsb.au.dk/~mok/o/). In the complex of $\alpha\beta$-TB fitted with the best conformation of PTX, hydrogens were added to the ligand, and atoms were typed and charged for use with the Kollman all-atom AMBER force field. All subsequent molecular dynamics (MD) and mechanics calculations, with the exception of the FLEXX searches, were run on an SGI POWER CHALLENGE with SYBYL 6.4.1 at the BioMolecular Computing Resource at Emory University. The FLEXX work was performed with SYBYL 6.6.2.

The refined electron crystallography (EC) structure (3.5 Å)* lacks numerous high-energy features found in the unrefined coordinates deposited in the Protein Data Bank (1TUB.pdb). Nonetheless, we desired to produce a relatively strain-free model of the protein--ligand complex. In a first step, all side-chain atoms of the α and β monomers and the PTX ligand (5,275 atoms) were defined as an aggregate, fixing these atoms in coordinate space. In the presence of this aggregate, the remainder of the protein--ligand complex was subjected to 1,000 fs of MD at 20 K with a time step of 0.5 fs, a distance-dependent dielectric of 4.5, and an NTV ensemble [i.e., canonical ensemble; constant number of atoms (particles), temperature, and volume]. In a second step, constraints were placed on all main-chain atoms, and the remainder of the protein was allowed to relax by MD for 500 fs. This expedient removes a great majority of nonbonded steric contacts. Contrary to the situation for 1TUB.pdb, no energy-rich hot spots were observed when the MD trajectory was visualized as a video stream. One structure on the latter trajectory around 200 fs was selected for molecular mechanics optimization. The energy of this structure is near the constant low-energy plateau of the trajectory, and it still matches the EC density very well. All atoms outside a 10-Å sphere around PTX in the β-TB-binding site for this complex were defined as an immobile aggregate. Geometry optimization for atoms within the sphere, using the Kollman all-atom force field, was carried to convergence by using a distance-dependent dielectric of 4.5 and an rms gradient energy cutoff of 0.3 kcal. The overall process, which began with a large positive energy, now yields a large negative energy. It should be noted that these MD simulations have been performed at low temperature, in the absence of solvent, and over a time span to prevent a significant rearrangement of the system. Higher temperatures and longer times, without solvation, evolve tubulin structures incompatible with the crystal structure. By using the former conditions, we achieve a strain-free structural model that remains fully faithful to the EC density and the refined coordinates. One measure of the latter is that the final optimized regions of the protein exhibit an excellent fit within the experimental density

map. Furthermore, a superposition of the EC-refined β-tubulin and the final optimized subunit (all heavy atoms in the main and side chains) leads to the diminutive rms deviation of 0.47 Å for a total of 3,402 atoms. A similar MD exercise was performed for the α-subunit. We believe the resulting strain-free αβ-TB dimer to be suitable for further modeling studies such as docking and solvated dynamics.

To examine the possible existence of other PTX conformers in the β-TB-binding site, both DOCK (http://www.cmpharm.ucsf.edu/kuntz/ kuntz.html) (11) and FLEXX (12) flexible docking procedures were applied to randomly generate conformations of the ligand. Thus, all heavy-atom torsions within the C-2, C-4, and C-13 side chains of PTX (with the exception of the ester and amide bonds) were explored in the search protocols. For DOCK, the search was performed three times, each run seeded with a different random number. On average, an individual search involves many thousand "tries." A "try" consists of the generation of a new conformation and an attempt to fit it to the binding site. In each PTX case, 2,500 fits were ultimately scored, and the highest-scored 100 docked structures were saved for graphical examination. Only two conformers of PTX were sited in the pocket; the remainder were docked outside or on the periphery of the binding cleft. The lowest energy was the T-form, whereas the other can be described as an uncollapsed "polar" Taxol conformation exhibiting torsional exchange of C-3′ phenyl and C-3′ NHCOPh relative to T-Taxol. However, the latter clearly falls outside the EC ligand density, while simultaneously scoring 45 kcal/mol higher in energy. The FLEXX searches were performed on a TB structure that was allowed to relax for an additional 800 fs of low-temperature MD and allowed to proceed until 200 solutions were achieved. As in the DOCK exercise, the vast majority of the PTX-TB complexes find the ligand outside the binding pocket. In only four cases was Taxol flexibly and successfully fitted to the pocket. The two lowest-energy forms (within 5 kcal/mol of one another) correspond to the T-Taxol conformation. The other two conformers not only did not fit the EC density, but the ligand--protein complexes were ranked with D-score (13) and G-score (14) to be ≈+50 and +100 kcal/mol less stable, respectively, than the lowest energy T-Taxol complex.

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*The structure of the $\alpha\beta$-tubulin dimer was refined to 3.5 Å by using simulated annealing torsion angle refinement and phase information from experimental images; *R* factor 0.23 and free *R* factor 0.30. (J. Lowe, H. Li, K.H.D., and E.N., unpublished work.)