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Supporting Information

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The tubulin-bound conformation of discodermolide derived by NMR in solution supports a common pharmacophore model for epothilone and discodermolide.

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Structure calculation protocol. The simulated annealing protocol comprised one high temperature phase, two cooling phases and a final minimization. Force constants were kept low during the high temperature phase and increased gradually to their full value during the first cooling phase. The only exception is the force constant K_{RELA} of the E_{RELA} potential (defining the deviation of experimental from back-calculated NOEs), whose initial value was set to 6% of its final value. During the high temperature phase of the simulated annealing, the value of K_{RELA} was successively doubled up to its full value. 6.5 ps dynamics (timestep = 5 fs) were run at each value of K_{RELA}, totaling 32.5 ps dynamics at 2000 K. This was followed by a first cooling phase from 2000 K to 1000 K in steps of 50 K and a second cooling phase from 1000 K to 100 K. Final structures were subjected to 200 steps of energy minimization.

The same weight was applied to all experimental peak intensities. Protons in methyl groups were averaged as $\langle r^{-3} \rangle^{-1/2}$. From the initial rates of NOE buildups of those proton pairs whose distance is independent of the conformation, a value of approximately 3.5 ns was estimated for the effective rotational correlation time ($t_{c,eff}$). All adjustable parameters of the relaxation potential, including the rotational correlation time were optimized by a systematic grid search.

A z-leakage term of 1 s^{-1} is often used to account both for non-NOE relaxation effects and for the fact that the protein acts as a relaxation sink.[1] Grid search between 0 and 10 s^{-1} showed that the distribution of conformers and the relative difference of their energies remained unchanged, and that the main effect of larger z-leakage values was to reduce the absolute value of the energy. A uniform value of the generalized order parameter S^2 was set for the whole molecule. Initially, a reasonable value of 0.85 was assumed. Subsequent tests showed that the resulting conformations were rather insensitive to variations of S^2 in the 0.5 - 0.95 range.

The quality of the fit of the refined structure to the experimental NOE data was determined with the generalized R-factor R^n with n=1/6.[2] The R factor measures the deviation of computed data from experimental data, normalized to the experimental values. The $R^{1/6}$ factor decreased from 0.302 for the initial extended structure to 0.078 for the family of structures of Fig. 2A. The good correlation between geometry, E_{TOTAL} and $R^{1/6}$ (Fig. S2) further illustrates the good quality of the structures.

To test the consistency of experimental data, the NOE information from some mixing times was completely omitted from the experimental restraint tables. Calculations run with reduced sets of only three, four or five mixing times in several combinations converged consistently to the same result, while calculations with no experimental NOE restraints did not converge to any well-defined conformation. All our tests prove that the tubulin-bound DDM conformation of Fig. 2 is exclusively determined by the experimental NMR data.

[1] R. E. London, M. E. Perlman, D. G. Davis, *J. Magn. Reson.* **1992**, *97*, 79-98.

[2] T. L. James, M. Gochin, D. J. Kerwood, U. Schmitz, P. D. Thomas, *J.C. Hoch, ed. New York: Plenum Press* **1991**, 331-347.

A B C

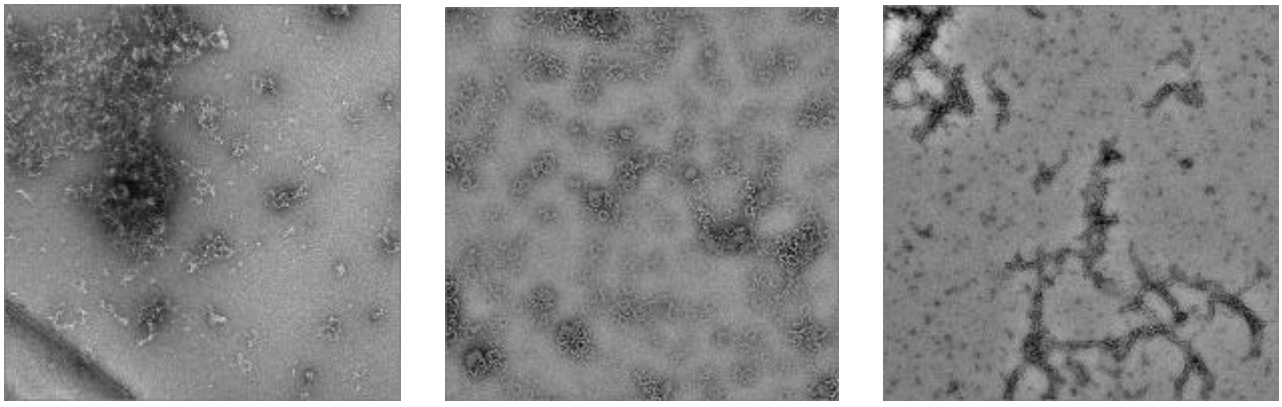

Fig. S1. (A) Electron microscopy (EM) image of tubulin (10μM) dissolved in the same D_2O buffer used for the NMR experiments (3 mM phosphate, 1.5 mM calcium and *ca.* 0.7 mM sodium at pH 7.0). No MTs are present under these conditions. (B) EM image of tubulin (10μM) dissolved in 95/5 buffer/DMSO. The presence of 5% DMSO as cosolvent induces the formation of protofilament rings. (C) The same tubulin sample as in (B) was incubated for 1 hour with 0.5 mM Discodermolide, leading to the formation of ordered polymers (microtubule sheets or open microtubules).

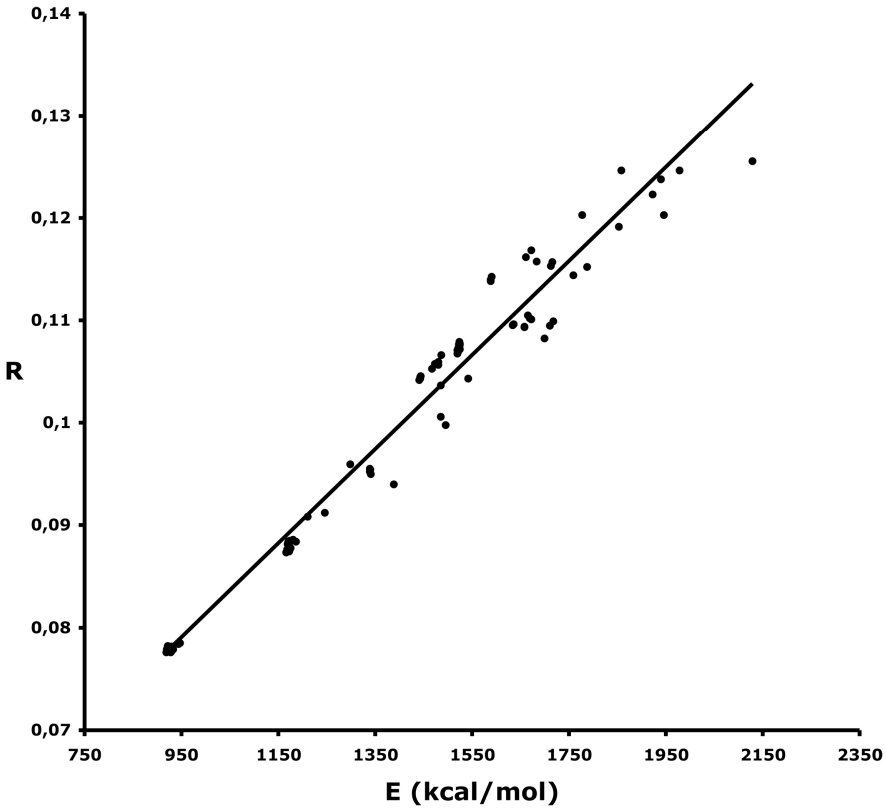

Fig. S2 Plot of the generalized R factor $R^{1/6}$ *versus* the total energy of the ensemble of 100 calculated structures. The values of $R^{1/6}$ correlate with the total energy, thus proving that the total energy is determined by the experimental NMR data. Inspection of the structure geometries show that each cluster of points in the plot corresponds to one different conformation of DDM. The 12 lowest-energy structures that represent the NMR-derived conformation of DDM correspond to the lowest energy cluster.

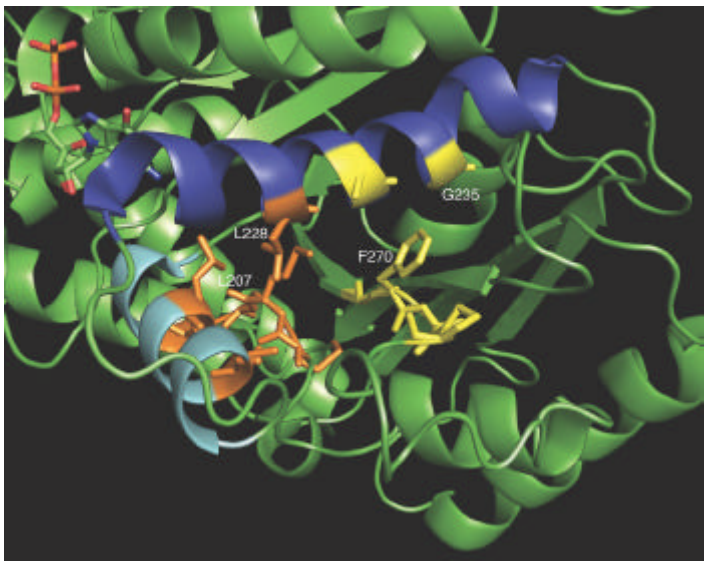

Fig. S3 Taxane binding pocket on the β subunit of tubulin from the pdb entry 1JFF.[10] Helix H6 and H7 are shown in cyan and dark blue, respectively. Two hydrophobic subpockets are identified in the taxane binding site: the first (in orange) is formed by A206, L207 and I210 of H6, L228 of H7 and M299, M300 and A301 of the H9-B8 loop; the second (in yellow) is formed by G269, F270, A271 and P272 of the M-loop and by A231 and G235 of H7.