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Title: "Microtubules and Tubulin-binding Drugs Studied by Cryo-EM and Electron Crystallography"

The electron crystallographic structure of tubulin bound to the anti-cancer drug Taxol (1,2) answered a number of questions about the dynamic behavior of microtubules and revealed the nature of the Taxol binding site. A number of other compounds with widely differing structures are now known that, like Taxol, stabilize microtubules. We have also resolved the native microtubule structure by cryo-EM to a resolution of 8 Angstroms, which is sufficient to dock the crystal structure of the protein to a very high degree of precision. This then allows us to understand the protein-protein interactions involved in microtubule formation and gain further insights on dynamics. We are now studying several of the drugs known to stabilize microtubules, including epothilone, eleutherobin and discodermolide, and have collected 3-D electron diffraction data sets of each to around 2.8 Å. Difference maps show that they all bind in the same area as Taxol. However, the resolution of the tubulin-Taxol crystal structure, 3.5 Å, has required development of refinement and modeling procedures in order to dock the drugs accurately enough to make chemical sense of the interactions. This approach converged to a stable solution for the epothilone-tubulin structure (3) that is consistent with a wealth of SAR data and shows that each drug interacts in a specific way with the protein but suggests a common mechanism for the stabilizing effects..

- (1) E. Nogales et al., *Nature* **391** 199 (1998)
- (2) J. Löwe et al., *J. Mol. Biol.* **313** 1045 (2001)
- (3) J.H. Nettles et al., *Science* **305** 866 (2004)