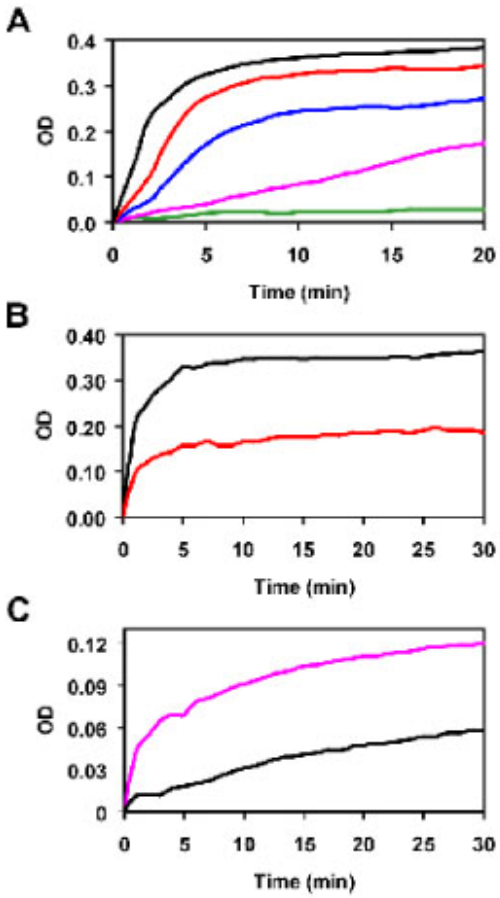
Tubulins from different tissue vary in the relative abundance of specific isoforms and the nature of post-translational modifications. These tissue type specific variants of tubulin have different biological and biochemical properties. It follows that the development of anti-tubulin ligands would benefit from the use of tubulin species purified from tissues that are relevant to the pathology under investigation. The specificity of ligands for a particular tubulin variant can be determined by performing comparative studies with both fungal and neuronal tubulins. We have advanced this concept by developing the Tubulin Ligand Index (TLI) system. In this system, IC50 values for inhibitory compounds or EC50 values for stabilizing molecules are determined in polymerization assays using plant and neuronal tubulins. The IC50 or EC50 values for each tubulin variant are analyzed as a ratio (neuronal/fungal) and allow for determinations of the relative specificity for each tested compound. TLI values greater than 1.0 indicate that a particular compound is more active on fungal tubulin. Conversely, TLI values less than 1.0 suggest that a compound is more specific for neuronal tubulin. Table 1 summarizes data from a study comparing the affects of several tubulin ligands on Agaricus bisporus tubulin in comparison to bovine neuronal tubulin using the TLI system.

Table 1: Comparison of IC50's of four compounds acting on neuronal and Agaricus bisporus tubulin polymerization.

Ligand	EC50* or IC50* neuronal tubulin (µM)	EC50* or IC50* A. bisporus tubulin (µM)	Tubulin Ligand Index Ratio (Neuronal/A.bisporus)
Paclitaxel	0.48	6	0.08
Rhizoxin	3.40	10	0.34

* = Values based on the rate of microtubule polymerization, CV +/- 24%.

Figure 1: Example raw data from Agaricus bisporus tubulin undergoing polymerization.



Legend to Figure 1: Fungal Tubulin Polymerization Assays.

A. PEG Dose-Response.
Polymerizations were carried out in G-PEM (1 mM GTP in PEM) supplemented with 5% glycerol and decreasing concentrations of PEG; 4.0% (black), 3.5% (red), 3.0% (blue), 2.5% (pink), 0% (green). Reactions were allowed to progress for 20 min at 30°C, with optical density readings (OD340) taken every min.

B. Decreased Polymerization by Rhizoxin In the Presence of 3.5% PEG. Polymerizations were performed the absence (black) or presence (red) of 10 µM Rhizoxin using 3.5% PEG in G-PEM+%5 glycerol as the buffer. Reactions were allowed to progress for 30 min at 30°C, with optical density readings (OD340) taken every min.

C. Enhanced Polymerization by Taxol in the Presence of 2.5% PEG.
Polymerizations were carried out in the presence (pink) or absence (black) of 10 µM taxol in G-PEM+%5 glycerol supplemented with 2.5% PEG. Reactions were allowed to progress for 30 min at 30°C, with optical density readings (OD340) taken every min.