

Dempsey et al, 2013
Supplementary figure legends

Fig. S1. Analysis of co-polymerisation of BSA (red arrow) with bovine brain tubulin (black arrow). (A–C) Various concentrations of BSA alone or BSA and bovine brain tubulin were incubated at 37 °C in the presence of 30 μ M taxol and centrifuged through a glycerol cushion. Concentrations used were: A, 12 μ M BSA \pm ~24 μ M bovine brain tubulin; B, 16 μ M BSA \pm ~16 μ M bovine brain tubulin; C, 18 μ M BSA \pm ~12 μ M bovine brain tubulin. The resulting pellets were resuspended in SDS gel loading buffer and equal proportions from the pellet fractions of BSA alone (X) and bovine brain tubulin + BSA mixture (Y) were resolved by SDS-PAGE (10%) and stained with Coomassie Blue. D: Equal proportions from the supernatant (S) and pellet (P) fractions for experiment A only (BSA + bovine brain tubulin) are also shown. No BSA incorporation into polymerised bovine brain tubulin for experiments A, B, and C could be detected by densitometry. The running positions of molecular weight markers are shown in kDa on the left of each gel. The data shown are from single, representative experiments.

Fig. S2. Determination of bovine brain tubulin monomer displacement by (A) MBP- α -tubulin (panel A) and MBP- β -tubulin (panel B) using a modified SDS-PAGE as described by Banerjee et al. Lanes 1: bovine brain tubulin (5 μ g) was resolved on the modified SDS-PAGE into two distinct monomers (black arrows), α -tubulin (above) and β -tubulin (below). Lanes 2: pellet samples from the co-sedimentation assay using a mixture of bovine brain tubulin (16 μ M) [black arrows] and the MBP-*P. falciparum* tubulins (16 μ M) [red arrows] were resolved. Densitometric analysis (data not shown) determined that the bovine tubulin monomers were similar in concentration in all lanes, indicating that no detectable displacement had occurred in the presence of the recombinant *P. falciparum* tubulins.

Fig S3. α -Tubulin alignment with the putative herbicide binding sites highlighted. The α -tubulin genes are grouped as herbicide resistant (*Homo sapiens*, *Bos taurus*, *Rattus norvegicus*, *Sus scrofa*, *Corydoras griseus*, *Ovis aries*, *Drosophila melanogaster*, *Saccharomyces cerevisiae*, *Acanthamoeba castellanii*) or herbicide sensitive (*Plasmodium falciparum*, *Daucus carota*, *Pisum sativum*, *Arabidopsis thaliana*, *Setaria viridis*, *Eleusine indica*, *Oryza sativa*, *Zea mays*, *Chlamydomonas reinhardtii*, *Tetrahymena thermophila*, *Giardia lamblia*, *Naegleria gruberi*, *Toxoplasma gondii*, *Leishmania tarentolae*, *Trypanosoma brucei*). Gene accession numbers can be found in supplementary material (Supplementary Table S2). The refined, putative herbicide sites (see text) have been highlighted.

Fig. S4. SDS-PAGE (A) and western blot (B) analysis of wild type and altered MBP- α -tubulin. All the samples represent 3 μ g of MBP- α -tubulin with or without amino acid alterations. A, SDS-PAGE; B, Western blot of a similar gel probed with an anti- α -tubulin serum; 1, wild type; 2, Val4Cys; 3, Phe24His; 4, Cys65Ala; 5, Leu136Phe; 6, Thr239Ile; 7, Arg243Ser. All of the α -tubulins were relatively pure and no obvious breakdown was observed on either the polyacrylamide gel or the western blot. The black arrow represents MBP- α -tubulin.