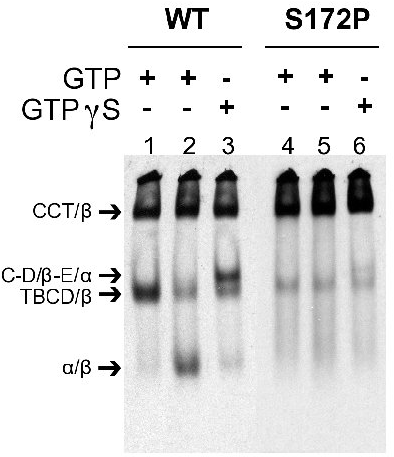
Supplementary Figure 11: Lowered Efficiency of Supercomplex Formation in p.S172P.



Supplementary Figure 11: Lowered Efficiency of Supercomplex Formation in p.S172P. Analysis on a non-denaturing gel of the products of *in vitro* folding reactions done in which ^{35}S-methionine-labeled, unfolded wild type (WT) or mutant (S172P) proteins were presented by sudden dilution from denaturant into reactions containing CCT, ATP, TBCD and GTP (lanes 1 and 4), or CCT, ATP, TBCD, TBCE, TBCC, native tubulin and either GTP or the slowly hydrolysable GTP analog GTP-γ-S (respectively lanes 2, 5 and lanes 3, 6). Arrows (top to bottom) denote the migration positions of the CCT/β-tubulin binary complex (CCT/β), the frozen TBCC-containing supercomplex (C-D/β-E/α)[2], the TBCD/β-tubulin co-complex, and the native tubulin heterodimer (α/β). Note that GTP promotes the discharge of heterodimers in presence of native tubulin and TBCC while in the presence of GTP-γ-S, the discharge is severely inhibited, with the appearance of the "frozen" supercomplex (lanes 2-3). This frozen supercomplex contains about 70% of the radioactivity discharged from TBCD (lane 3). In the case of p.S172P, however, there is no clear evidence of the discharge of label from the TBCD/β-tubulin intermediate (lane 5). In addition, the formation of the "frozen" supercomplex in the presence of GTP-γ-S is correspondingly weak (lane 6). See Supplementary Note for more details.