**Figure S1**. Closed state profilin:β-actin with nonexchanging-closed state actin. (a) Classic view of the two molecules that were overlayed by superposition of their large domains (grey). Closed state profilin:actin is colored blue and nonexchanging-closed actin is colored green. The two structures aligned with an rmsd of 1.1 Å. A zoomed-in view of the active site ATPs with associated metals showing a close agreement in their atomic positions is on the right. The metals lie on top of each other (b) Top view of the overlay looking down from the bulk solvent. Zoomed-in view of active site ATPs from the top is on the right. Note the agreement between the loop at residues 11-19.

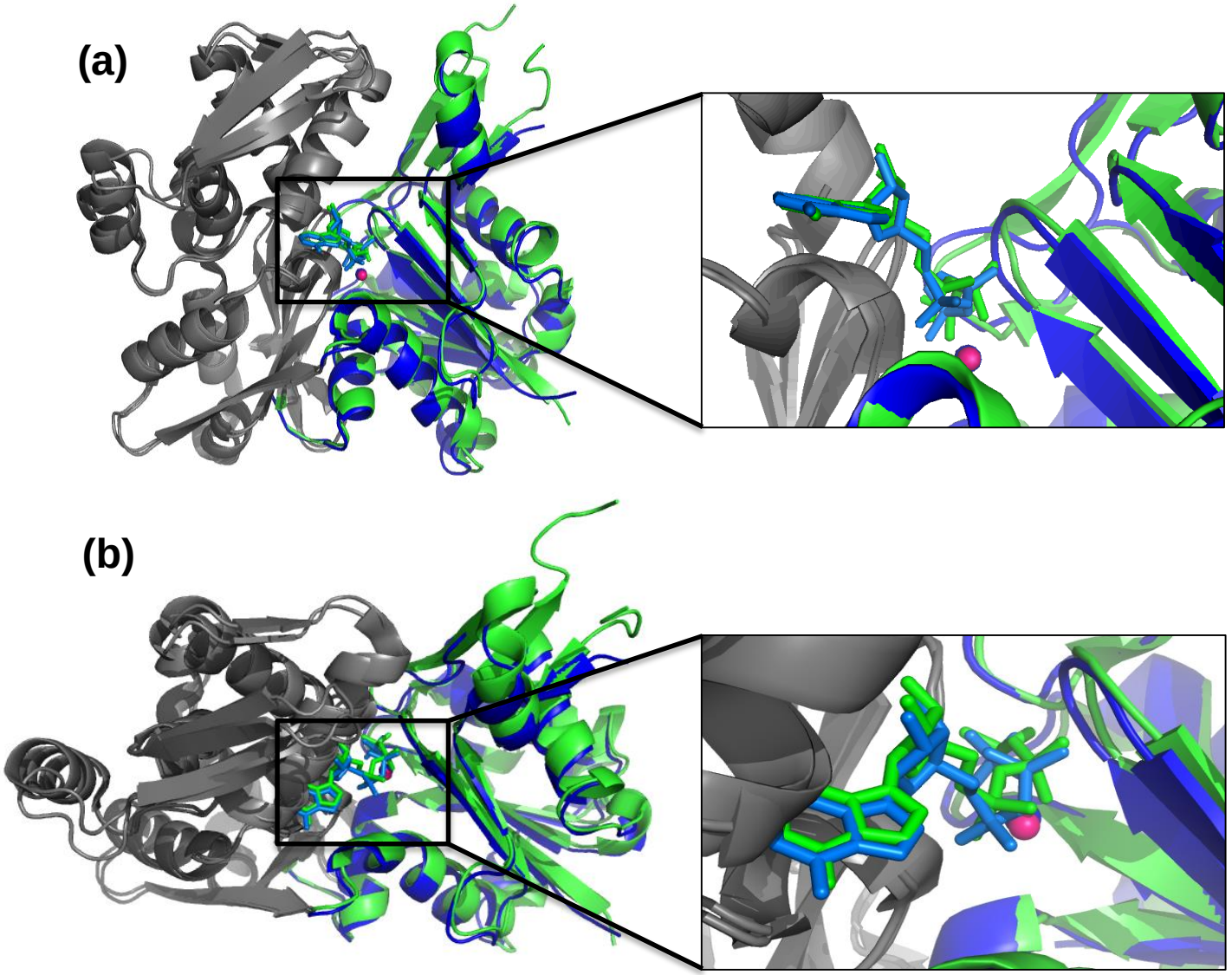
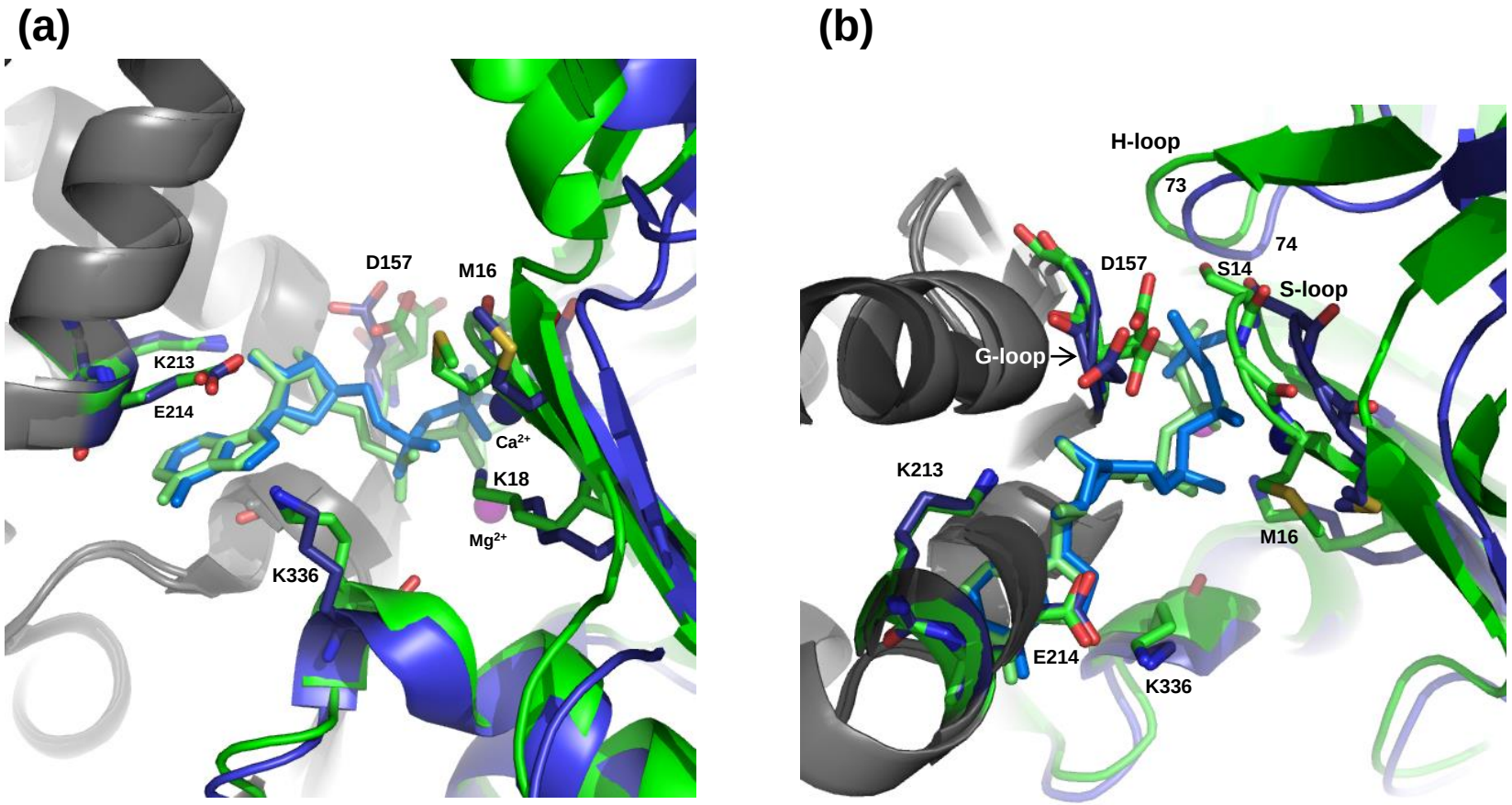


**Figure S2**. Wide-open state profilin:β-actin overlaid with nonexchanging-closed state actin. The ATP binding site (a) view of the nucleotide base interactions and (b) view of the triphosphate interactions.



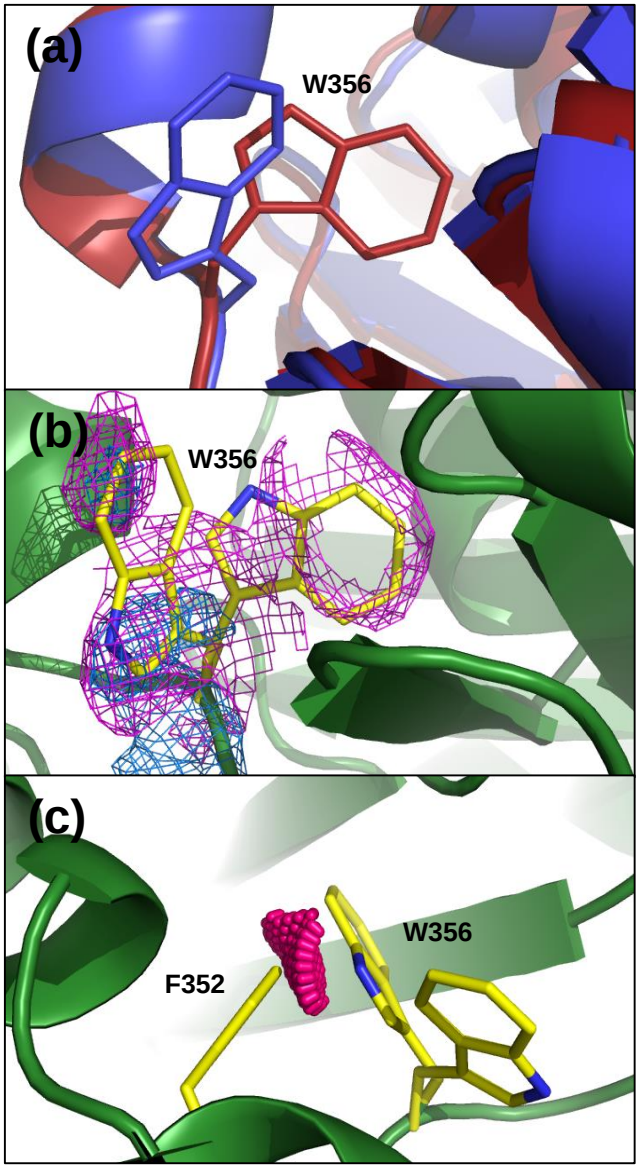



**Figure S3.** Dual conformations of Trp-356 **(a)** Overlay of wide-open state profilin:actin (blue) with partially-open state profilin:actin (red). **(b)** Closed-state profilin:actin showing $F_O$-$F_C$ omit density (pink) for both Trp-356 conformations. **(c)** Closed-state profilin:actin showing MolProbity clash probes. The probes represent steric clashes between the buried Trp-356 with Phe-352.

Only one position was modeled for Trp-356 and Phe352 for the following reasons. Omit $F_O$-$F_C$ difference maps of a 4 Å spherical region around (and including) Trp-356 showed density for a second, and buried Trp-356 conformation further confirmed this (Fig. 6(d)). However, when this second position was modeled, it was seen that are major clashes with the adjacent Phe-352 side-chain. In our closed state actin however, we found no evidence in the electron density for a second Phe-352 conformation. This could be due to the resolution limit of the closed actin data (2.4 Å).