2). These data confirm that Vps4 Interface 2, like Interface 1, does not mediate Vps4 dimerization in solution.

These observations indicate that none of the symmetric dimer interfaces seen in more than one Vps4 crystal form are likely to mediate Vps4 dimerization in solution. We therefore considered the possibility that Vps4 might form an asymmetric dimer in solution. In particular, all known Vps4 crystal forms contain an asymmetric sixth interface that is larger than any of the two-fold symmetric interfaces (760±80 $Å^2$). Interface 6 connects the small domain of one subunit with the large domain of an adjacent subunit, and is used to create the continuous helix of Vps4 molecules. The interface is complex, and involves contacts between helix 1 and the helix 1/strand 1 loop on one side of the interface, and helices 7-9 and the C-terminal edge of the β domain on the other. Additional contacts between adjacent large domains are also made between the two molecules that bind ATP-γS in our crystal form 2 (between helix 5 on one side of the interface and the strand 2-helix 3 loop on the other). These additional contacts result from closure of the interdomain angle upon ATPγS binding (see Fig. 3).

The requirement for Interface 6 in Vps4 dimerization was tested using four different point mutations (L151D, I351A, I354D, and W388A). We initially changed the highly buried L151, I351 and I354 residues to aspartate in order to robustly disrupt the interface. However, the I351D protein was only partially soluble, and we therefore replaced this residue with alanine. W388 was changed to alanine rather than aspartate because this dramatic reduction in side chain volume was expected to yield a sufficient change in interface stability. Strikingly, every one of these mutations