reduced the gel filtration mobility of $Vps4_{\Delta MIT}$ to that expected for a monomer (compare red and black curves in Fig. 5, panels 3 (L151D) and 4 (W388A), and similar data not shown for the I351A, I354D mutants). Analytical ultracentrifugation experiments confirmed that $Vps4_{\Delta MIT,L151D}$ was indeed monomeric in solution (Fig. 4, panel 4, MW_{obs} = 38,815 g/mol, $MW_{obs}/MW_{monomer}$ = 1.03)). Thus, residues used to create the asymmetric Interface 6 seen in all known $Vps4_{\Delta MIT}$ crystal forms are also required for solution dimerization. As discussed below, we propose that the Vps4 solution dimer interface is likely to be a variant of Interface 6 in which the subunit conformations are altered so that the assembly does not propagate into a polymer.

Requirements for Vps4 Dodecamerization

Mutant $Vps4_{\Delta MIT}$ constructs that can bind but not hydrolyze ATP (e.g., $VPS4_{\Delta MIT,E233Q}$) form stable higher order assemblies that have been modeled as double rings, with six[31] or seven[27] subunits per ring. Conversion of $Vps4_{\Delta MIT}$ dimers into higher order structures (here termed dodecamers) can also be analyzed by gel filtration chromatography (Fig. 6, panel 1), which provides a convenient assay for mutations that inhibit Vps4 ring formation.

We previously proposed a homology model for one of the two rings of the $Vps4_{\Delta MIT}$ dodecamer. This model was created by substituting Vps4 subunits in place of the crystallographically characterized p97 D1 subunits in their hexameric ring conformation[25; 47]. This model for the Vps4 ring generates subunit interfaces that are similar, but not identical to the crystallographically defined Vps4 Interface 6. Differences between the crystallographic interface and the homology model are