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that the $Vps4_{\Delta MIT,Q216A}$ protein remained dimeric over the entire concentration range tested (Fig. 4, panel 3, MW_{obs} = 71,668 g/mol, $MW_{obs}/MW_{monomer}$ = 1.90). The $Vps4_{\Delta MIT}$ mobility was similarly unaffected by two other Vps4 Interface 1 mutations (N224A and M220D, data not shown), and we therefore conclude that Interface 1 does not mediate Vps4 dimerization in solution.

Four other classes of two-fold symmetric subunit contacts were seen in at least two different $Vps4_{\Delta MIT}$ crystal forms (Interfaces 2-5). However, these interfaces were approximately half the size of Interface 1, with average buried surface areas of: 350±70, 280±130 Å^2, 320±150 Å^2, and 360±120 Å^2, respectively. None of these interfaces occurred in all of the reported crystal forms, and the residues within Interfaces 2, 4, and 5 are not well conserved across species. Interface 3 residues are well conserved, but this is likely explained by the fact that they form the "Rossmann fold" element of the nucleotide binding site. These observations imply that Interfaces 2-5 are also unlikely to mediate Vps4 dimerization in solution.

Interface 2 connects the two small domains of Vps4 and primarily involves contacts between residues in helices 7 and 9 in adjacent small domains. This interface is present in our $Vps4_{\Delta MIT}$-SO_4 crystal structure (buried surface area, 330Å^2), and also in $Vps4_{\Delta MIT}$ crystals with a bound sulfate (2QP9) and without a bound ligand (2RKO) (430Å^2, and 290Å^2, respectively). Xiao et al. reported that two mutations within this interface (M330D and L407D) did not alter the gel filtration mobility of $Vps4_{\Delta MIT}$[28]. In good agreement with this report, we also found that the L407D mutation had no effect on the gel filtration mobility of $Vps4_{\Delta MIT}$ (Fig. 4b, panel