the presence of ATP[25]. Arg352 sits within the subunit interface of our p97 D1 homology model, but is exposed at the edge of Interface 6 in the different Vps4 crystal structures. Thus, W388A and R352A are examples of mutations that allow formation of one Vps4 state but inhibit formation of the other, and reflect the use of overlapping but distinct interfaces for dimer and dodecamer formation.

A Functional Role for Pore Loop 2

Our model for the p97 D1 homology model for the hexameric ring of Vps4 places two different loops near the center of the narrow pore (Fig. 7). Analogous loops are also present in other AAA ATPases, and they have been termed Pore Loop 1 ([206]WMG[208], green in Figures 7a, b) and Pore Loop 2 ([241]RGEGESEASRR[251], blue) [48; 49; 50]. A section of Pore Loop 2 is also known as the “Arginine Collar”, owing to the presence of three conserved arginine residues (Arg241, Arg250, and Arg251 in Vps4)[51; 52; 53]. Unlike previous Vps4 structures, Pore Loops 1 and 2 are both well ordered in the three crystallographically independent molecules of Vps4 crystal form 2. Pore Loop 1 forms a short two turn helix between strand 2 and helix 3, and the conserved Trp206/Met207 dipeptide forms a hydrophobic patch that sits above the pore in the hexamer model, with Met207 projecting into the central cavity (green in Figs. 7a, b). Pore Loop 2 forms an extended loop between strand 3 and helix 4, and the conserved Pore Loop 2 Arg residues at positions 250 and 251 form a positively charged “collar” that sits immediately beneath the hydrophobic Pore 1 residues (blue in Fig.7a, upper panel). Charged Pore 2 residues also serve to restrict the diameter of the pore itself to ~10 Å, with Glu243 and 245 (red), forming a negatively charged ring