A Functional Role for Vps4 Pore Loop 2

The central pores of AAA ATPases are typically defined by two adjacent loops that project into the central cavity. We have previously shown that hydrophobic Pore Loop 1 residues are required for VPS4A function, as judged by the ability of Pore Loop 1 mutants to dominantly inhibit HIV-1 release and infectivity[25]. In the current study, we used the same approach to show that Pore Loop 2 residues that help create the arginine collar are also required for human VPS4A function. Specifically, we found that overexpression of either $VPS4A_{R236A}$ or $VPS4A_{R246A}$ inhibited HIV-1 vector release and reduced viral titers more than 40-fold, showing that these mutant proteins were potent dominant negative inhibitors of HIV budding. As illustrated in Fig. 7, the two arginine residues appear to perform different functions, with VPS4A Arg236 (equivalent to yeast Vps4 Arg241) forming part of the positively charged collar that sits beneath the Pore 1 loop, and VPS4A Arg246 (equivalent to yeast Vps4 Arg251) lining the opposite side of the pore itself. Equivalent arginine residues are also important for the activity of other AAA ATPases, including the two closest relatives of VPS4; p97[51], and spastin[52; 53]. The functional importance of these conserved residues highlights the similarities between Vps4 and the other two related AAA ATPases, and suggests that conserved residues within the arginine collar may perform analogous mechanistic functions, possibly by binding the substrate and/or by assisting in protein denaturation[51] as polypeptide substrates are transferred through the central pore.