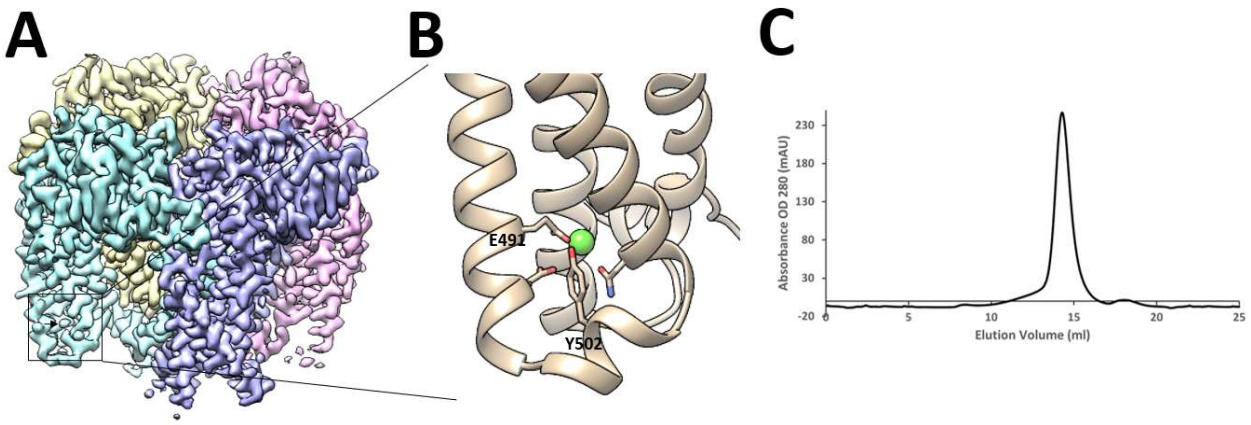

Figure 1. A novel calcium binding site on PC-2. A) A side view of the tetrameric PC-2 channel with an EM density corresponding to a coordinated Ca2+; B) An enlarged view of the Ca2+ binding site showing several coordinating residues. Ca2+ density is shown as mesh. C) Size-exclusion chromatogram of a representative VSLD Ca2+ site mutant (E491A/Y502A), indicative of excellent biochemical tractability and suitability for cryo-EM studies.

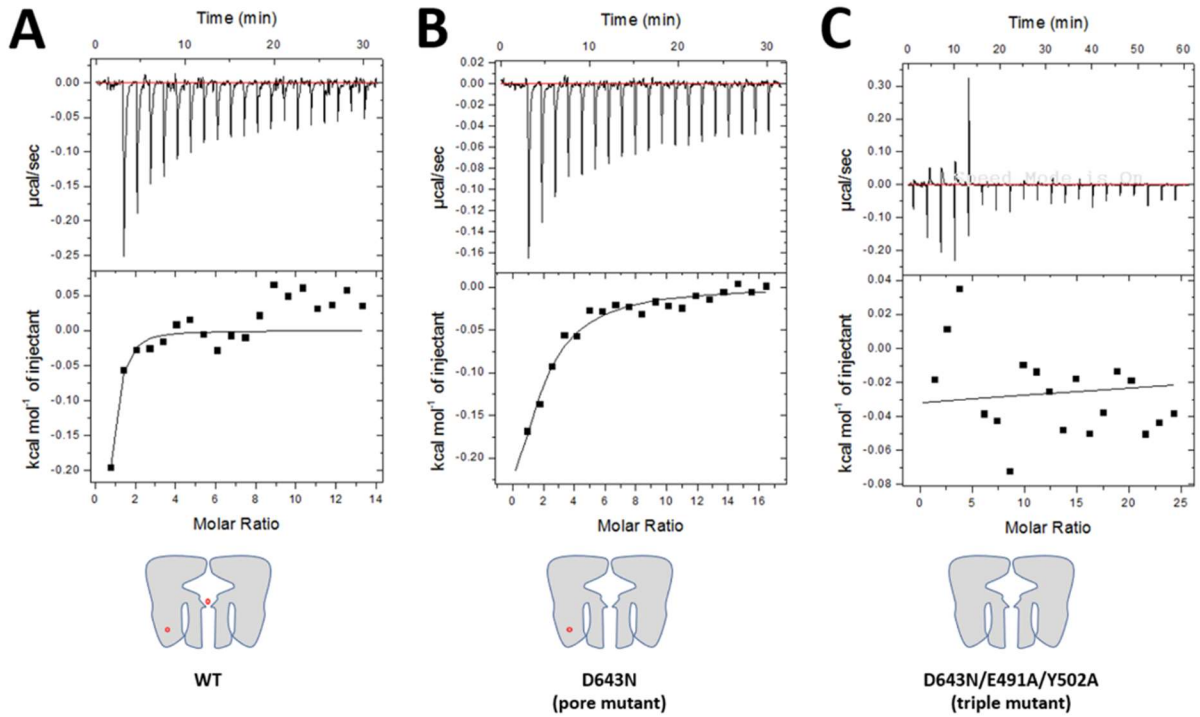

Figure 2. Ca2+ ion binds to the novel calcium binding site within the VSLD domain. PC-2 WT also contains a calcium binding site at the selectivity filter region of the pore. D643N mutation was introduced to deconvolute simultaneous binding of Ca2+ to the pore and VSLD. While Ca2+ binding events were detected in both PC-2 WT (A) and the pore mutant with an intact VSLD Ca2+ site (B), no Ca2+ binding was detected when both sites were mutated.