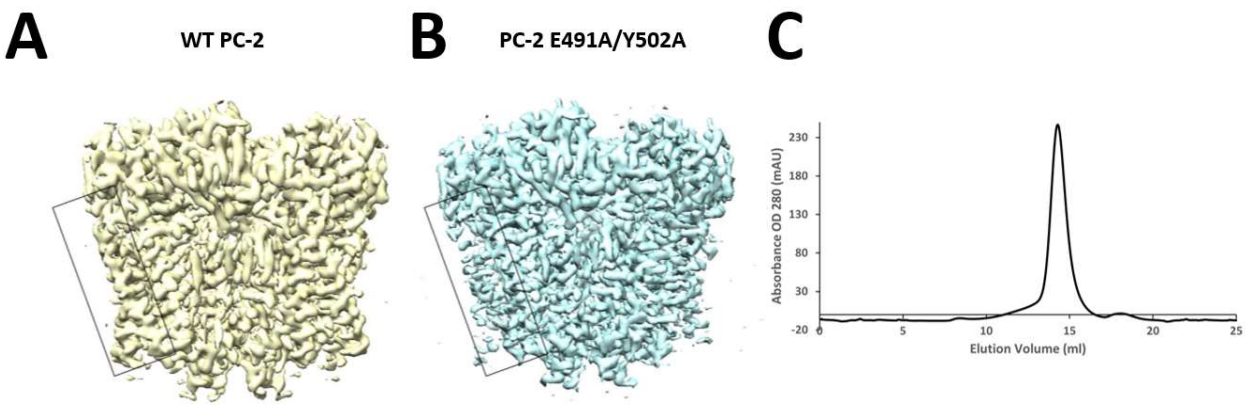

Figure 1. Structure of a PC-2 VSLD Ca2+ site mutant. A) A side view of the tetrameric PC-2 wild type channel; B) A side view of the VSLD Ca2+ site mutant (E491A/Y502A). C) Size-exclusion chromatogram of a representative VSLD Ca2+ site mutant (E491A/Y502A), indicative of excellent biochemical tractability and suitability for cryo-EM studies.

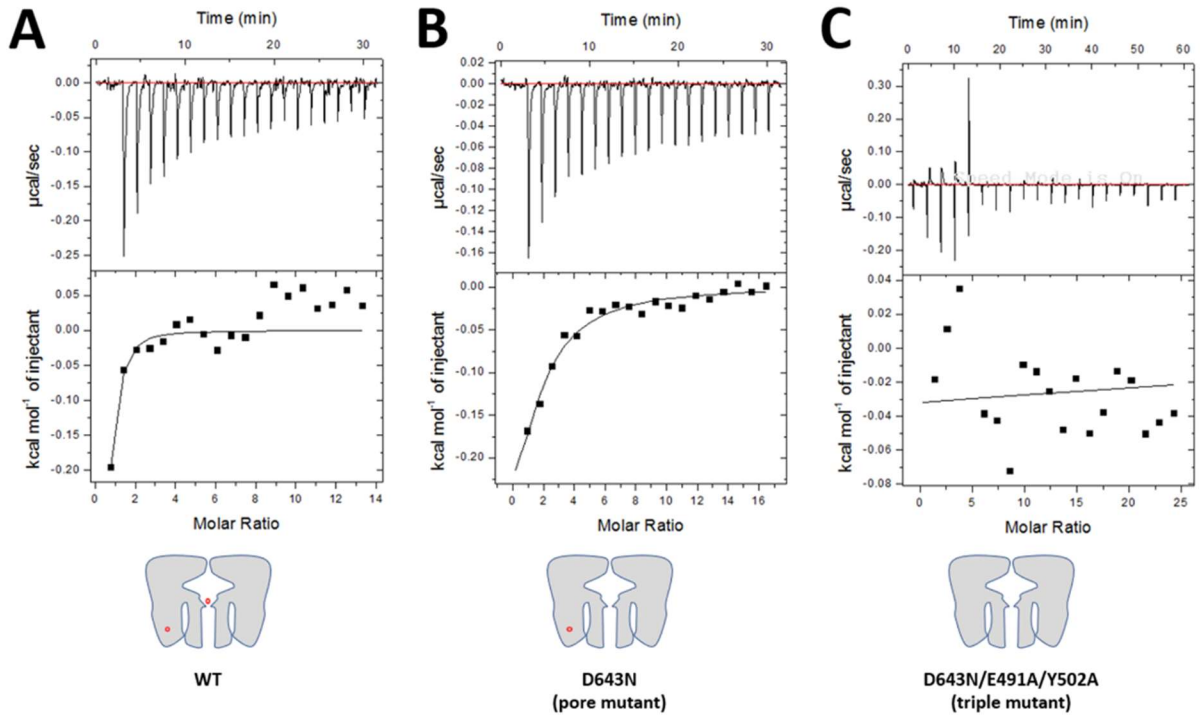

Figure 2. Ca2+ ion binds to the novel calcium binding site within the VSLD domain. PC-2 WT also contains a calcium binding site at the selectivity filter region of the pore. D643N mutation was introduced to deconvolute simultaneous binding of Ca2+ to the pore and VSLD. While Ca2+ binding events were 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.