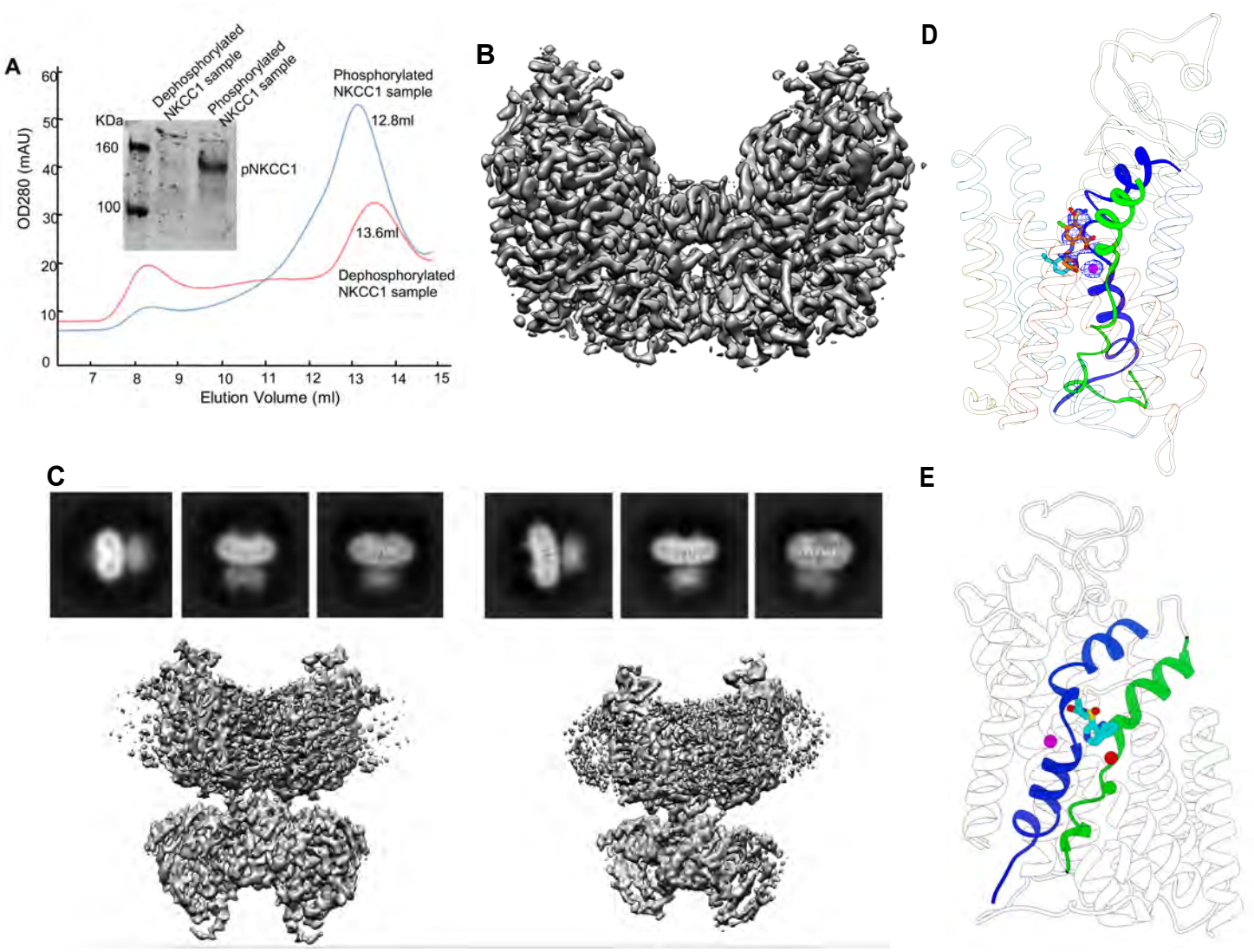

Cryo-EM structures of phosphorylated NKCC1. (**A**) Co-expression of NKCC1 with the upstream WNK-SPAK kinases yielded phosphorylated transporter sample as confirmed by a phospho-specific NKCC1 antibody. Phosphorylated NKCC1 also migrates as a larger protein than the untreated NKCC1. (**B**) Untreated human NKCC1 transporter shows no density for the cytosolic domains. (**C**) Phosphorylated human NKCC1 transporter yielded two cryo-EM maps, both of which show well-resolved cytosolic domains. One NKCC1 dimer exhibits an overall 2-fold symmetry, while the other shows mismatch of 2-fold symmetry axis of the transmembrane and cytosolic domains. (**D-E**) Active phosphorylated NKCC1 enabled determination of co-structures of NKCC1 bound with the loop diuretic drug furosemide (D) and torsemide (E), which are both unpublished. Only the transmembrane region of one NKCC1 subnit is shown, highlighting torsemide and furosemide (stick), Na+ and K+ (magenta sphere), water molecule (red sphere), and Cl- (green sphere).