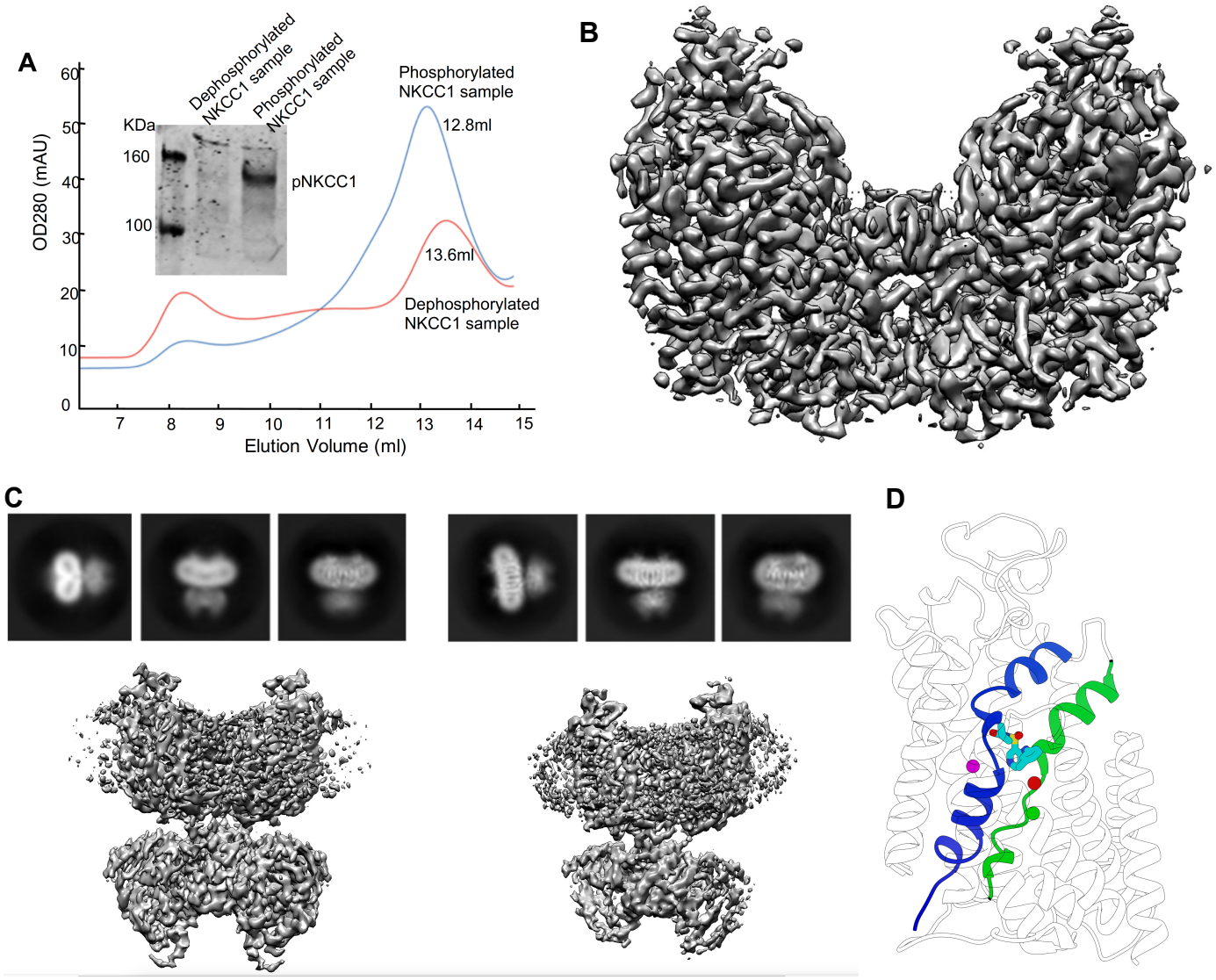

Cryo-EM structures of phosphorylated NKCC1. (**A**) Co-expression of NKCC1 with the upstream WNK-SPAK kinases yielded phosphorylated transporter sample as confirmed by a phospho-specific NKCC1 antibody. Phosphorylated NKCC1 also migrates as a larger protein than the untreated NKCC1. (**B**) Untreated 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**) Active phosphorylated NKCC1 enabled determination of a co-structure of NKCC1 bound with the loop diuretic drug torsemide (unpublished). Only the transmembrane region of one NKCC1 subnit is shown, highlighting torsemide (stick), Na+ (magenta sphere), a water molecule (red sphere), and Cl- (green sphere).