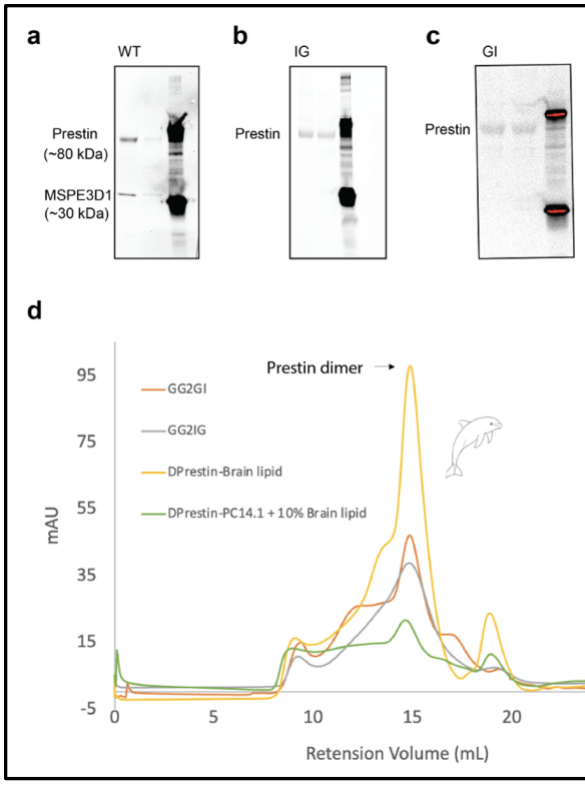


Fig. 1: SDS-PAGE and size-exclusion chromatography (SEC) HPLC of purified dolphin prestin mutant in detergent and nanodiscs. a-c) SDS-PAGE gel of purified prestin samples. Only representative purification samples are shown for clarity. Prestin monomer elutes at ~80 kDa (~160 kDa as a dimer). **d**) Purification in detergent and nanodiscs results in a monodisperse peak using a Superose 6 column, which was subsequently collected and concentrated for sample freezing (unpublished).

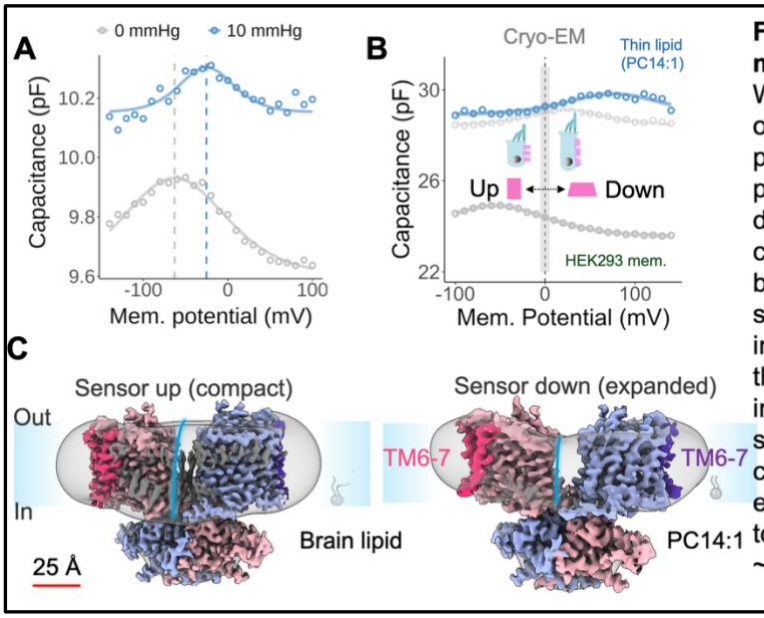
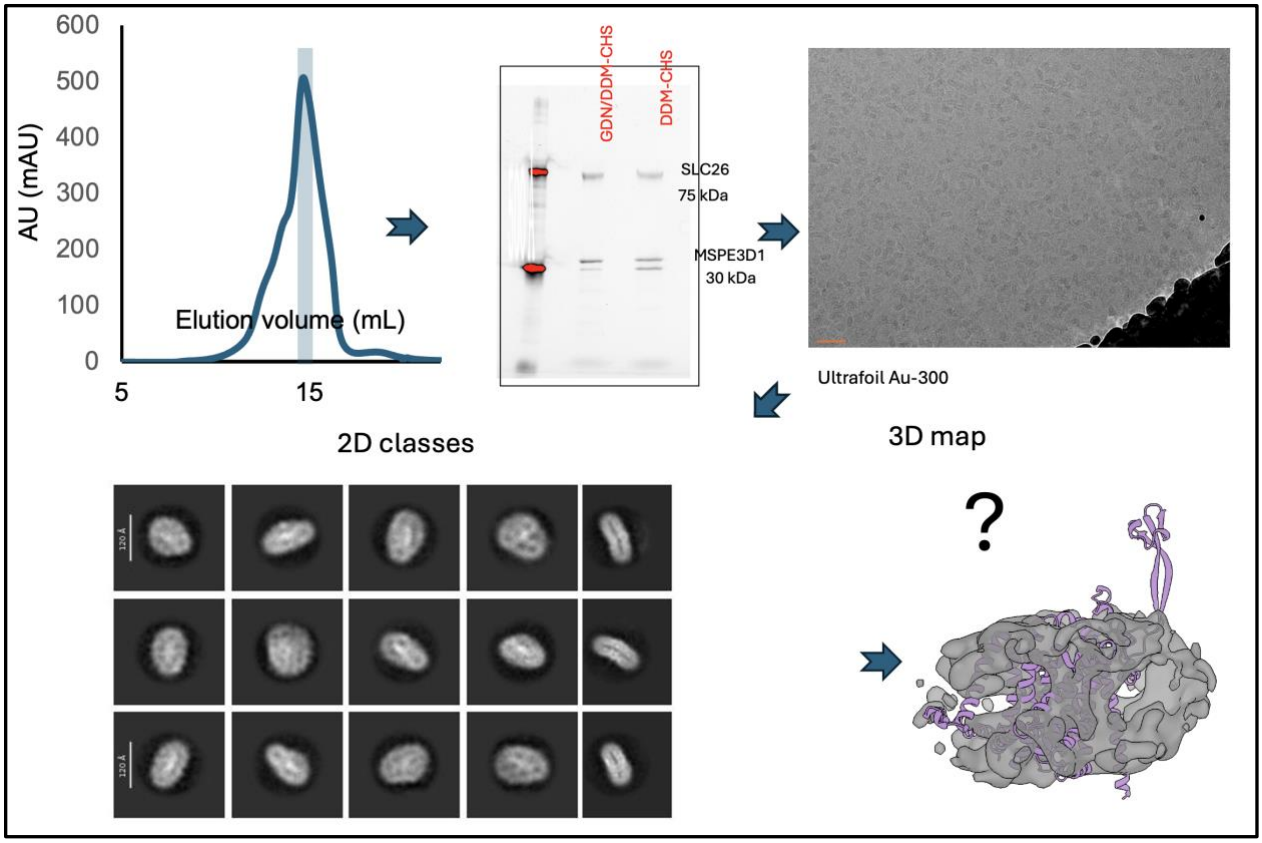


Fig. 2: Prestin responds to changes in membrane tension and thickness. A) Whole-cell patch-clamp electrophysiology on HEK293 cells overexpressing dolphin prestin demonstrates that increasing plasma membrane tension induces depolarizing shifts in prestin's nonlinear capacitance (NLC). B) Membrane thinning by PC14:1 lipids similarly causes significant depolarizing shifts in NLC, indicating prestin's sensitivity to membrane thickness. C) Cryo-EM structures of prestin in nanodiscs reveal distinct conformational states influenced by different lipid compositions, providing structural evidence for prestin's mechanosensitivity to membrane thickness at high resolution (~ 2.8 & 3.28 Å; unpublished).


600
500
400
300
200
100
0
AU (mAU)
5
15
Elution volume (mL)
GDN/DDM-CHS
DDM-CHS
SLC26
75 kDa
MSPE3D1
30 kDa
Ultrafoil Au-300
2D classes
3D map
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