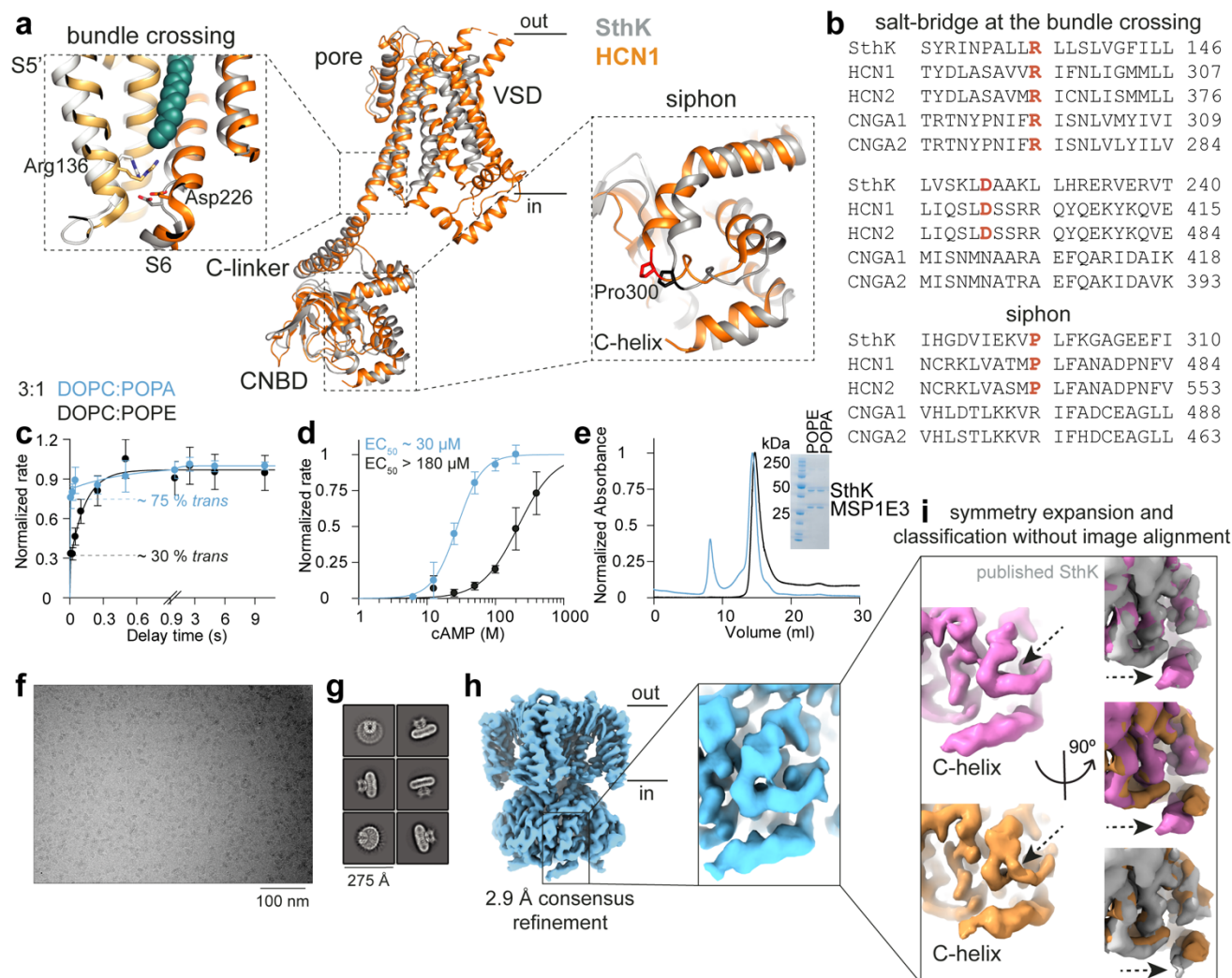




Supplementary Figure: **a**) overlay of SthK (PDB: 6CJQ³) and human HCN1 (PDB: 5U6O⁵), emphasis on the bundle crossing and the siphon. **b**) sequence alignment of the bundle crossing and proline in the siphon. **c**) activation kinetics of SthK from a stopped-flow flux assay, fast activation is a measure for molecules with *trans* Pro300, slow activation for *cis* Pro300. **d**) activation constant EC_{50} for SthK. *trans* Pro300 leads to high affinity, *cis* Pro300 to low affinity for cAMP. **e**) gel filtration and SDS-PAGE of SthK in nanodiscs. **f**) representative micrograph of SthK in 3:1 DOPC:POPA nanodiscs (0.1 lowpass filter applied in crYOLO⁶). **g**) representative 2D classes generated in Relion 3.1⁷ from particles as shown in (f). **h**) 2.9 Å reconstruction of apo SthK in 3:1 DOPC:POPA nanodiscs from particles as in (g). **i**) structural heterogeneity in the siphon and the C-helix region after symmetry expansion of particles shown in (h) followed by classification without image alignment, published density of SthK shown as reference (grey, EMD-7482³).

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