Structural and Mechanistic Insights into Symmetry Conversion in Plant GORK K^+ Channel Regulation

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Abstract

GORK is a shaker-like potassium channel in plants that contains ankyrin (ANK) repeats. In guard cells, activation of GORK causes K^+ efflux, reducing turgor pressure and closing stomata. However, how GORK is regulated remains largely elusive. Here, we solved the cryo-EM structure of *Arabidopsis* GORK, revealing an unusual symmetry reduction (from C4 to C2) feature within its tetrameric assembly. This symmetry reduction in GORK channel is driven by ANK dimerization, which disrupts the coupling between transmembrane helices and cytoplasmic domains, thus maintaining GORK in an autoinhibited state. Electrophysiological and structural analyses further confirmed that ANK dimerization inhibits GORK, and its removal restores C4 symmetry, converting GORK to an activatable state. This dynamic switching between C2 and C4 symmetry, mediated by ANK dimerization, presents a GORK target site that guard cells regulate to switch the plant K^+ channel between inhibited and activatable states, thus controlling stomatal movement in response to environmental stimuli.

35 **Main**

36 Potassium ions are essential for plant growth and development, playing roles in
37 osmoregulation, cell expansion, pH homeostasis, and establishing membrane potential
38 and proton motive force (*1, 2*). In *Arabidopsis thaliana*. 71 potassium channels and
39 transporters have been identified, among which 9 belongs to shaker-like channel family
40 (*3*). These channels can be categorized as outward-rectifying types, including GORK
41 and SKOR, or inward-rectifying types, including KAT1, KAT2, AKT1, and AKT2.
42 They sense changes in membrane potential (depolarization or hyperpolarization),
43 regulate potassium transport, and maintain ionic homeostasis, crucial for plant growth
44 and stress response (*2, 4-7*).

45 GORK, also known as Guard cell Outward-Rectifying K^+ channel, is the only
46 outward-rectifying potassium channel in guard cells. Since its discovery in 2000 (*8*),
47 extensive genetic studies have demonstrated its vital role in regulating stomatal
48 movement (*9-11*). Stomata, formed by paired guard cells, control water loss and CO_2
49 uptake in response to environmental stimuli, thereby regulating transpiration and
50 photosynthesis (*8, 12-17*). Ion channels in guard cells control turgor pressure by
51 mediating ion flux, which in turn regulates stomatal opening and closing (*2, 5, 18*).
52 KAT1 presents the major guard cell potassium uptake channel, required for increasing
53 turgor pressure and volume increase promoting stomatal opening. In contrast GORK
54 activation causes potassium efflux, reducing guard cell turgor and volume and in turn
55 stomatal closure (*3, 8, 9, 12, 19*).

56 Understanding the structure and function of these channels is critical for
57 unraveling their roles in ion homeostasis and stomatal movement. So far, the structural
58 study of plant KAT1 have provided important insights into its activation and role in
59 potassium influx control (*20, 21*). However, the structure and regulatory mechanisms
60 of GORK remain largely enigmatic. Unlike the inward-rectifying KAT1, the outward-
61 rectifying GORK harbors a unique ankyrin (ANK) repeat domain, whose functional
62 role in plant ion channel regulation remained yet unexplored.

63 In this study, we report the cryo-EM structures of *Arabidopsis* GORK (*At*GORK)
64 in both the full-length autoinhibited state and activatable states obtained by modelling
65 and structure-guided mutagenesis. The full-length *At*GORK^{FL} forms a tetramer with
66 elongated cytoplasmic regulatory domains, with its architecture showing a symmetry
67 reduction from four-fold (C4) to two-fold (C2). Remarkable, ANK from neighboring
68 protomers interact to form dimers, thus reducing symmetry of the tetramer and
69 rendering GORK in an autoinhibited state. Electrophysiological studies show that
70 mutations or truncations disrupting ANK interactions convert GORK into an
71 activatable state. Further structural analysis of ANK-truncated *At*GORK^{623} and
72 *At*GORK^{510} reveals that the cytoplasmic regulatory domains become highly dynamic,
73 while strict C4 symmetry is restored in the tetrameric assembly.
74 Thus, our study demonstrates that ANK acts as a molecular switch for symmetry
75 conversion (between C2 and C4), regulating GORK conformational transitions between
76 autoinhibited and activatable states. This research provides the first comprehensive
77 elucidation of the biological role of ANK in GORK, offering new insights into how
78 GORK mediates stomatal closure in response to environmental signals. Furthermore,
79 our findings broaden the understanding of ion channel regulation, particularly how
80 symmetry changes in structural assembly regulate ion channel activity.
81
82
83 **Cryo-EM structure determination of the *Arabidopsis* GORK**
84 To elucidate the structure of the GORK, we prepared *Arabidopsis* GORK (fused with
85 an N-terminal GFP tag) from HEK293 cells and conducted single particle cryo-EM
86 analysis. After solubilization in 1.0% n-Dodecyl-B-D-Maltoside (DDM) and 0.02%
87 cholesteryl hemisuccinate (CHS), the *At*GORK proteins underwent purification
88 through strep-tactin affinity chromatography, GFP removal via TEV enzyme cleavage,
89 and subsequent gel-filtration chromatography. The final solution contained 0.006%
90 detergent GDN, 150 mM NaCl, and 20 mM HEPES-Na pH 6.5. The most monodisperse

91 peak fractions were pooled and concentrated to approximately 3.5 mg/mL for cryo-EM
92 grid preparation.
93 We determined the cryo-EM structures of the full-length *At*GORK (*At*GORK^{FL},
94 residues 1 to 820) in an auto-inhibited state, along with two truncated versions
95 (*At*GORK^{623}, residues 1 to 623; *At*GORK^{510}, residues 1 to 510) in active states (Table
96 S1). Tetrameric reconstructions of *At*GORK were obtained in two conformational states
97 at resolutions of 3.4 Å (*At*GORK^{FL1}) and 4.3 Å (*At*GORK^{FL2}). allowing *de novo*
98 modeling of 662 of 820 amino acids (residues 58 to 720) for each protomer, while the
99 remaining regions were not resolved due to their intrinsic flexibility (fig. S1). For the
100 truncated *At*GORK^{510} and *At*GORK^{623}, cryo-EM maps at 3.4 Å and 3.2 Å resolution,
101 respectively, were obtained. Both maps showed high-quality density in their
102 transmembrane domain, allowing accurate modeling, while the flexible cytosolic
103 domains remained unmodeled due to their invisibility (fig. S2). Structure-based
104 sequence alignment for 24 representative GORKs from both monocots and dicots is
105 shown in fig. S3.
106
107 **Architecture of *Arabidopsis* GORK in symmetry-reduced conformation**
108 *Arabidopsis* GORK (*At*GORK) forms a homo-tetrameric structure around a central pore,
109 with a dimension of 110 Å (length) × 110 Å (width) × 170 Å (height) (Fig. 1A-C). Each
110 protomer has an N-terminal transmembrane domain (TMD, residues 58-313, containing
111 helices S1-S6), followed by three cytoplasmic domains: the C-linker (residues 314-365),
112 CNBDH (residues 366-510), and ANK (residues 511-724). The segment preceding the
113 TMD (residues 1-57) and the last portion (residues 725-824, containing a putative KHA
114 domain enriched in hydrophobic and acidic residues) are not visible in the structure,
115 likely due to their intrinsic flexibility or the flexibility of the connecting regions.
116 The *At*GORK structure, unlike other tetrameric ion channels such as KAT1,
117 exhibits a unique architectural assembly (*20*). Notably, the symmetry in the TMD
118 portion adopts a typical four-fold (C4), but reduces to a two-fold (C2) in their following

119 cytoplasmic domains, thus leading to a deformation within the tetrameric channel (Fig.
120 1D). Superimposition of its protomer reveals distinct conformations, with notable
121 rotations and displacements occurred in the C-linker, CNBDH, and ANK domains (Fig.
122 1E). This symmetry reduction within the tetrameric assembly appears to arise from
123 ANK dimerization (will discuss later). To the best of our knowledge, this symmetry
124 reduction in channel assembly is exceptionally rare and leads to deformation of the
125 tetrameric channel. This represents a unique structural feature of the GORK family and
126 likely serves as a unique mechanism for channel regulation. Thus, the current *At*GORK
127 structure likely represents an autoinhibited, pre-activation state.

128 The TMD consists of a voltage sensing domain (VSD, helices S1–S4) and a pore-
129 forming domain (PD, helices S5 and S6) (Fig. 2A). The VSD is connected to the PD
130 through a short linker, resulting in a "non-domain-swapped" configuration where the
131 VSD and PD of the same subunit form a cohesive bundle. This differs from the
132 canonical domain-swapped architecture in Shaker-like (*22*) and Na_V channels (*23*),
133 where the VSD and PD are separated by a long S4−S5 linker. The P-loop segment of
134 *At*GORK contains a highly conserved motif (^{271}TVGYG^{275}), similar to that of bacterial
135 KcsA (*24*), mammalian HCN (*25*), and plant outward-rectifying SKOR (*26*) and
136 inward-rectifying KAT1(*20*) and AKT1(*27*). Structural comparisons reveal that they
137 form a similar K^+ selectivity filter (fig. S4). Analysis using the HOLE (*28*) suggests
138 that the *At*GORK adopts a closed conformation. Below the selectivity filter lies the pore
139 gate region consists of residues on the S6 helix, with I303 and T307 creating narrowest
140 constrictions (radius of approximately 1 Å). The S4 helix contains conserved positively
141 charged residues (R174, R177, R179, and R187), and adopt a resting "up" conformation,
142 similar to that in plant inward-rectifying KAT1 (*20*) and AKT1 (*27*) (fig. S4B).

143 After the TMD, the C-linker is positioned next to the helix bundle crossing,
144 connecting the transmembrane helices to the cytoplasmic domains. It comprises four
145 helices (A'-D'), forming two "helix-turn-helix" motifs. These helices further assemble
146 into a gating ring architecture within the tetrameric channel through "elbow-on-

147 shoulder" inter-subunit interactions, where helix A'-turn-helix B' (elbow) of one
148 protomer interacts with helix C'-turn-helix D' (shoulder) of its neighboring protomer
149 (*29*) (Fig. 1C). Due to the reduced symmetry within the tetramer, the C-linkers exhibits
150 distinct conformations between adjacent protomers, either “flat” or “kinked” (Fig. 2B).
151 Consequently, the C-linker in *At*GORK deviates from C4 symmetry, and the presence
152 of the "flat" conformation disrupts the formation of a C4 symmetrical gating ring,
153 leading to decoupling between the transmembrane helices and the cytoplasmic domains,
154 ultimately affecting channel gating.

155 The CNBDH domain, connected to the C-linker, shares approximately 20%
156 sequence identity with canonical cyclic nucleotide-binding domains (CNBDs) (fig.
157 S5A), which play crucial roles in regulating channels, kinases and transcription factors
158 upon binding to secondary messengers (cAMP or cGMP). Despite low sequence
159 homology, the CNBDH share a similar structural fold to those of the CNBDs (fig. S5B).
160 The CNBDH of GORK consists of four short α-helices (A, P, B, and C) and an eight-
161 stranded β-roll (β1- β8) (Fig. 2C). Helix A is located before the β-roll, Helix P is inserted
162 within the β-roll (between β6 and β7), and Helices B and C are positioned afterward.
163 Helices A and B interact in an antiparallel manner, and the putative phosphate-binding
164 cassette comprises β6, Helix P, and β7.

165 Although the CNBDH shares overall structural similarity with animal CNBDs,
166 there are notable differences (fig. S5B). GORK lacks conserved residues for cAMP
167 binding, such as R561 in 7lfx or R620 in 2ptm, which is replaced by Q465. More
168 strikingly, alanine at the binding pocket entrance (A563 in 7lfx (*30*), or A622 in 2ptm
169 (*29*)) is replaced by bulky phenylalanine (F467 in *At*GORK), likely blocking nucleotide
170 access. Helix C also adopts a closed conformation, with its adjacent loop obstructing
171 the binding site entrance, suggesting that large conformational changes would be
172 required for cyclic nucleotide binding.

173 Following the CNBDH, six ankyrin repeats fold into a single ANK domain,
174 forming a slightly curved solenoid structure (Fig. 2D). The first three repeats (ANK1-

175 3) and the last three repeats (ANK4-6) are tandem units with ~34% sequence identity.
176 In each tandem unit, two repeats are canonical, consisting of a pair of anti-parallel α-
177 helices and an intervening "finger" loop, while the third is a degenerate repeat lacking
178 the "finger" loop. Remarkably, ANK repeats from two neighboring protomers forms a
179 stable dimer through specific interactions mediated by their protruding loops, creating
180 a buried area of ~1500 $Å^2$. This dimeric interaction is specific, corroborated by another
181 structure of the full-length of *At*GORK^{FL2}, which retains similar dimeric units despite
182 of variation in their tetrameric conformations (fig. S1E). A similar ANK dimerization
183 pattern has also been observed in SKOR (fig. S1F) (*26*), suggesting that this
184 dimerization is a common structural feature in outward rectifying plant ANK domains
185 K^+ channels.

186 As a result of ANK dimerization, positional deviations and conformational
187 changes are induced in the adjacent CNBDH and C-linker, leading to a reduction in
188 symmetry within the tetrameric channel. This reduction affects the communication and
189 coupling between cytoplasmic domains and transmembrane helices, ultimately
190 maintaining GORK in an autoinhibited, pre-activation state. The structures of GORK
191 and SKOR with reduced symmetry provide crucial insights into how the ANK domain
192 regulates these channels, maintaining them in an autoinhibited conformation under
193 resting conditions. This is supported by the observation of basal activity of full-length
194 *At*GORK.

195

196 **ANK dimerization maintains GORK channel in an auto-inhibited state**

197 The ANK repeats and their dimeric interactions are distinctive features of the GORK
198 channel, playing a key role in symmetry reduction within the tetrameric assembly. To
199 explore their function, we conducted functional analysis of the ankyrin repeats with
200 *At*GORK expressed in *Xenopus oocytes* and inspected via TEVC electrophysiology
201 (Fig. 3A). Removing the KHA domain but retaining the intact ANK domain in *At*GORK
202 (Δ737-820, residues 1-736), led to a slight increase in current. However, truncating half

203 (Δ624-820, residues 1-623) or the entire ANK domain (Δ511-820, residues 1-510)
204 significantly enhanced channel activity (Fig. 3B, C). These results indicate that the
205 ANK domain indeed plays an inhibitory role in maintaining the autoinhibited state of
206 GORK, as its disruption or removal markedly enhances channel activity.

207 The dimerization of ANK is driven by hydrophobic interactions involving the
208 hairpin loops protruding from ankyrin repeats. Inspired by this structural insight, we
209 replaced the hairpin loop with an unrelated short "SGGG" motif. Remarkably, replacing
210 of loops between repeats 1/2 and 2/3 significantly enhanced *At*GORK activity (Fig. 3D,
211 E). This aligns well with findings from ANK truncational studies (Fig. 3B, C),
212 confirming that disrupting ANK interactions leads to the auto-inhibition release in
213 *At*GORK, thereby allowing channel activation. In contrast, similar replacement of the
214 loops between repeats 4/5 and 5/6 impaired GORK function. These results indicate that
215 the role of ANK is more complex than initially anticipated. This protein-protein
216 interaction module in GORK appears crucial not only for maintaining the autoinhibited
217 state but also for supporting the open state during activation.

218

219 **Mutational test on conserved acidic residues at the C-linker/TMD interface**

220 The C-linkers assemble into a disc-shaped gating ring via "elbow-on-shoulder"
221 interactions (*29*), directly contacting the TMD and playing a crucial role in channel
222 gating. Conserved negatively charged residues, including E317, D321, and D325, are
223 positioned on the C-linker A' helix, forming a negatively charged ring at the entrance
224 of the ion-conducting pathway (Fig. 4A) and engaging in an intricate interaction nexus
225 with conserved K/R from the TMD (Fig. 4B). Specifically, E317 and D321 (C-linker
226 of protomer A) form ionic interactions with K312 (Helix S6' of protomer D), while
227 R200 (Helix S5 of protomer A) forms a salt bridge with E317 from its adjacent
228 protomer B. In such a fashion, the C-linker-mediated gating ring is connected with the
229 VSD and PD within the TMD through their domain-swapped interfaces. These three

230 residues correspond to conserved R, D, and A in the inward K^+ channels KAT1(*20*) and
231 AKT1 (*27*), respectively.
232 To further validate their functional role, we introduced the mutations, E317R,
233 D321 (N and R), and D325A into the *At*GORK^{510} construct. Among these, D325A did
234 not cause significant changes, while E317R partially impaired GORK function.
235 Interestingly, both D321 mutants, either charge neutralization (D321N) or charge
236 reversal (D321R), displayed much larger currents compared to *At*GORK^{510}, lost voltage
237 dependence, and exhibited much slower deactivation kinetics (Fig. 4C, D). Remarkably,
238 the outward rectification characteristic was altered, allowing potassium influx. This
239 suggests that the gating ring of C-linker not only participates in channel activation, but
240 also its conserved negatively charged residue (D321) required for K^+ flux outward
241 rectification.
242
243 **Disrupting ANK-dimerization relieves constraints and restores C4 symmetry**
244 Our structural studies reveal that ANK dimerization in full-length *At*GORK is directly
245 responsible for reducing its tetrameric symmetry from C4 to C2, maintaining an
246 autoinhibited state. Under resting conditions, GORK shows minimal basal currents,
247 whereas ANK-truncated mutants, including *At*GORK^{623} and *At*GORK^{510}, show
248 significantly increased currents, suggesting that ANK truncations relieve autoinhibition
249 and enhances GORK activity. Structural analysis of *At*GORK^{623} and *At*GORK^{510} only
250 resolved the TMD portion, with their cytoplasmic regions being too flexible for
251 visualization (fig. S2). These findings suggest that ANK truncation relieves the
252 autoinhibitory constraints imposed by ANK dimerization, converting *At*GORK into an
253 activatable state.
254 Structural analysis also provided some clues regarding the restoration of C4
255 symmetry in the *At*GORK. Firstly, the structures of *At*GORK^{623} and *At*GORK^{510} are
256 highly similar, with a root-mean-square deviation (RMSD) of approximately 0.48 Å for
257 their superimposed tetramers (Fig. 5A). Importantly, the ANK-truncated protomers

258 exhibit strict C4 symmetry, with RMSDs of approximately 0.16 Å/256 superimposed
259 Cα. In contrast, the TMD of full-length *At*GORK^{FL1} displays quasi-C4 symmetry
260 (essentially C2 symmetry), with subtle yet distinct differences between protomers A/C
261 and B/D at the N-terminal end of helix S5, yielding an RMSD of approximately 0.75
262 Å/256 superimposed Cα (Fig. 5B, C). A comparison of protomers A and B in both
263 *At*GORK^{FL1} and *At*GORK^{623} reveals that the protomers in *At*GORK^{623} closely
264 resembles protomer A in *At*GORK^{FL1}, rather than protomer B (Fig. 5D). This suggests
265 that ANK dimerization results in C2 symmetry in the full-length *At*GORK^{FL1}, whereas
266 ANK truncation restores C4 symmetry in the *At*GORK^{623}.
267 We also performed AlphaFold3-based modeling on *At*GORK^{623}, showing that the
268 C-linker, CNBD, and ANK domains are in a flexible, loose state, with the overall
269 structure adopting C4 symmetry (fig. S6). The TMD in the AlphaFold3 predicted
270 *At*GORK^{623} structure is highly similar to that of cryo-EM structure of *At*GORK^{623}, with
271 an RMSD of 1.97 Å for the superimposed tetramers. Together with our structural and
272 electrophysiological data, these results support the role of ANK as a molecular switch
273 for symmetry conversion in GORK, revealing a novel mechanism for regulating ion
274 channel activity via symmetry conversions.
275
276 **A model for symmetry conversion in GORK regulation**
277 In summary, our analysis shows that full-length *At*GORK^{FL} adopts C2 symmetry due
278 to ANK dimerization in the cytoplasm. Cryo-EM and AlphaFold3 modeling of ANK-
279 truncated *At*GORK^{623} reveal a restoration of C4 symmetry within the tetrameric
280 assembly. This symmetry conversion, driven by ANK dimerization, regulates
281 intermodular communication and channel gating process. The transition between C2
282 and C4 symmetry governs GORK switching between autoinhibited and activatable
283 states. In short, ANK dimerization maintains GORK in C2 symmetry and an
284 autoinhibited state, while disrupting ANK dimerization restores C4 symmetry and

285 activates GORK (Fig. 6). Thus, the ANK domain acts as a molecular switch for
286 symmetry conversion, providing a finely tuned mechanism for GORK regulation.
287
288 **Discussion**
289 Plant potassium channels are crucial for K^+ transport and maintaining potassium
290 homeostasis (*2*). In *Arabidopsis*, nine Shaker-like potassium channels contain multiple
291 modular domains in their architectures, including the pore-forming TMD, the
292 regulatory C-linker, and CNBD, with some also containing ANK and KHA domains.
293 Based on the presence of the ANK domain, these channels can be divided into two
294 groups: ANK-free members (KAT1, KAT2, and KC1) and ANK-containing members
295 (GORK, SKOR, AKT1, AKT2, AKT5, and AKT6). Currently, structural information
296 are available for KAT1 (*20, 21*), SKOR (*26*), and AKT1 (*27, 31*), allowing us to
297 conduct systematic comparative analysis
298 In ANK-free KAT1, structural studies show that it forms a symmetric tetramer
299 with typical C4 symmetry throughout both the transmembrane and intracellular regions
300 (*20, 21*), similar to animal cyclic nucleotide-gated channels (CNGC) (*30*). In contrast,
301 GORK, which contains a unique ANK domain, exhibits a symmetry transition from C4
302 in the transmembrane region to C2 in the cytoplasmic region, resulting in distinct
303 structural features. A similar symmetry reduction is observed in other ANK-containing
304 potassium channels, such as AKT1 (*27*) and SKOR (*26*). This reduction in symmetry
305 is rare among ion channels, suggesting it may be a unique feature of ANK-containing
306 channels linked to their regulatory mechanisms.
307 Our study provides the first clear-cut evidence that ANK acts as a molecular switch
308 controlling GORK channel symmetry and activity. Structural analysis reveals that ANK
309 dimerization holds GORK in an autoinhibited state at rest. ANK dimerization mediates
310 interactions between protomers, reduces tetramer symmetry, and causes the
311 displacement of internal, highly mobile modules, with neighboring CNBD and C-linker
312 shifting from C4 symmetry to C2. The C-linker adopts two different conformations:

313 "kinked" and "flat" (Fig. 2B). The "kinked" conformation is common in C4 symmetric
314 channels, while the "flat" conformation is unique to ANK-containing channels with
315 reduced symmetry. Subtle yet distinct differences in the S5 helices between adjacent
316 protomers in *At*GORK^{FL1} renders a quasi-C4 symmetry in the TMD (Fig. 5B). The
317 reduction in GORK symmetry signifies an autoinhibited state, supported by the
318 observed basal electrophysiological activity.

319 Our ANK truncation experiments showed that ANK removal converts GORK
320 from an autoinhibited to an activatable state, as evidenced by enhanced activities in the
321 truncated *At*GORK^{510} and *At*GORK^{623} variants upon membrane depolarization (Fig.
322 3B-E). Structural analysis further revealed that the TMD restores strict C4 symmetry,
323 while the C-linker and CNBDH become highly mobile in these ANK-truncated mutants.
324 Additionally, AlphaFold3-based modeling confirmed that disrupting ANK
325 dimerization relieves constraints and restores C4 symmetry in the tetramer, enabling
326 GORK transition to an activatable state. Thus, ANK serves as a molecular switch to
327 regulate GORK activity by controlling the symmetry conversion of its tetrameric
328 assembly.

329 The activity of GORK channels is regulated by ANK dimerization, and the
330 dynamic formation and disruption of this dimerization may be controlled by kinase
331 phosphorylation. Previous studies showed that phosphorylation affects ANK-mediated
332 protein-protein interactions and channel activity (*32-36*). Several protein kinases,
333 including CPK21 (*37*) and CPK33 (*38*), have been identified as candidates for
334 regulating GORK activity, with phosphorylation sites, such as S649 in the ANK domain.
335 However, the molecular mechanisms of these kinases require further investigation.
336 Another similar example is AKT1, which is activated by CIPK23 and CBL1, with
337 multiple potential phosphorylation sites identified, including in the ANK domain. The
338 low-resolution structure of AKT1 co-expressed with CIPK23 and CBL1 shows C4
339 symmetry in the CNBDs, though it remains unclear whether this is due to ANK
340 phosphorylation (*27*).

Our study reveals that ANK regulates GORK channel activity through tetrameric symmetry conversion. Under resting conditions, ANK dimerization maintains GORK in a C2 symmetric conformation, maintaining it in a preactivated, autoinhibited state. In response to environmental stimuli, GORK undergoes a symmetry conversion and runs into an activatable state, enabling K^+ efflux and stomatal closure. This mechanism provides new molecular insights into stomatal regulation and offers potential avenues for developing improved crop varieties through ANK engineering.

Methods

Expression and purification of the GORK proteins

The genes of *Arabidopsis* full-length *At*GORK (AT5G37500) and truncated variants (*At*GORK^{623}, residues 1-623 and *At*GORK^{510}, residues 1-510) were cloned into a modified pEG BacMam vector, which included a PreScission protease cleavage site, GFP, an 8× His tag, twin-Strep tag, and Flag tag at the N-terminus. Recombinant proteins were expressed in HEK293F cells. In short, baculovirus was generated in *Sf9* insect cells, and P2 viruses were used to infect HEK293F cells. Cells were cultured in suspension, supplemented with 1% (v/v) fetal bovine serum, and maintained at 37 °C with 70% humidity and 6% CO_2. Cells were infected with P2 viruses at a density of approximately 2.6×10^6 cells/mL, and supplemented with 10 mM sodium butyrate upon 8 hours post-infection, and continued to grow at 30°C for another 50 hours. Cells were harvested, centrifuged, and stored at -80°C.

Harvested cells from a 300 mL of culture were resuspended in extraction buffer (20 mM HEPES-Na pH 8.0, 150 mM KCl, 10 mM DDM, 2 mM cholesteryl hemisuccinate (CHS) supplemented with protease inhibitors (2 μg/mL pepstatin, 2 μg/mL aprotinin, 2 μg/mL leupeptin and 1mM phenylmethylsulfonyl fluoride) and incubated at 4°C for 2 hours. Solubilized membranes were clarified by centrifugation at 41,000 rpm for 1 hour, and the supernatant was loaded onto a Streptactin Beads 4FF column. Following a 5-column volume buffer wash, proteins were eluted with solubilization buffer (20 mM HEPES-Na pH 8.0, 150 mM KCl, 0.02% GDN, and 2.5 mM desthiobiotin). After tag removal with TEV protease, the proteins were further purified using a Superose-6 column in solubilization buffer (20 mM HEPES-Na pH 8.0, 150 mM KCl, 0.006% GDN). The elution was analyzed by SDS-PAGE, and the peak fractions of the purified protein were pooled and concentrated to ~3.5 mg/mL.

For the preparation of truncated truncated versions (*At*GORK^{623} and *At*GORK^{510}), we followed similar procedures as above.

378 **Cryo-EM grid preparation and data acquisition**

379 The protein sample (4 µl, ~3.5 mg/ml) was applied to freshly glow-discharged holy
380 carbon film grids (Quantifoil, Cu, R1.2/1.3, 300 mesh) and blotted for 6.5 seconds at
381 100% humidity and 4°C using a Vitrobot Mark IV (Thermo Fisher Scientific), followed
382 by plunge freezing into liquid ethane.

383 All the films were collected on a Titan Krios transmission electron microscope
384 (Thermo Fisher Scientific, USA) operated at 300 kV equipped with a Gatan K2 or K3
385 Summit direct detection camera (Gatan Company, USA). Movies were recorded at
386 22,500× (for K3, corresponding 1.06 Å per pixel) or 130,000 × (for K2 summit,
387 corresponding 1.04 Å per pixel) nominal magnifications, using SerialEM software with
388 a beam-image shift method(*39, 40*), at a total dose of 50 e-/Å2 distributed over 32
389 frames, with defocus values between -1.2 and -2.2 µm.

390

391 **Data processing and model building**

392 All image processing steps were conducted using either CryoSPARC 3.1 (*41*) or
393 RELION 3.0 (*42*). CryoSPARC 3.1 was primarily used for reconstruction, while
394 RELION 3.0 was employed for alignment-free 3D classification to identify an alternate
395 conformation (*At*GORK^{FL2}) of the wild-type structure. Angular information was
396 converted using PyEM, and map analysis and adjustments were performed with UCSF
397 Chimera (*43*).

398 Raw movie frames were aligned using a dose-weighted patch alignment method,
399 followed by CTF estimation. Particle picking was performed an oval template (88 ×
400 168 Å), and coordinates were screened using normalized cross-correlation and local
401 power scores to eliminate false positives. Particles were then down-sampled to a quarter
402 size and classified based on shape (circular for top-view projections and T-shaped for
403 side-view projections) after initial 2D classification to improve classification efficacy.
404 A preliminary 3D reconstruction was generated from a small subset of particles.

405 *At*GORK^{FL1} was constructed with Nu-refinement using C2 symmetry with
406 156,313 particles, while *At*GORK^{FL2} used 39,551 particles with C1 symmetry. The
407 resolutions were 3.4 Å for *At*GORK^{FL1} and 4.3 Å for *At*GORK^{FL2}, respectively. The
408 alphafold2-predicted *Arabidopsis* GORK structure was used as the initial model, fitted
409 by individual domains in UCSF ChimeraX (*44*), and refined iteratively in Coot (*45*)
410 and PHENIX (*46*), and corrected iteratively. For the truncated *At*GORK^{623} and
411 *At*GORK^{510}, the TMD structure from *At*GORK^{FL1} was used as the initial model,
412 followed by iterative refinement and iterative adjustments in Coot and PHENIX. The
413 resolutions were 3.2 Å for *At*GORK^{623} and 3.4 Å for *At*GORK^{510}, respectively. All
414 figures were generated using UCSF ChimeraX.
415
416 **Electrophysiology**
417 The electrophysiological experiments of GORK and its mutants were conducted as
418 previously described (*47, 48*). Briefly, all coding DNA sequence (cDNAs) of *At*GORK
419 wild type or mutated variants were cloned into a pGHME2 plasmid for expression in
420 *Xenopus* oocyte. cRNAs were synthesized using T7 polymerase with linear plasmids.
421 Oocyte sacs were extracted and digested with 0.2 mg/ml collagenase (Sigma) in OR2
422 buffer (82.5 mM NaCl, 2.5 mM KCl, 1 mM $MgCl_2$, and 5 mM HEPES-Na pH 7.5). 36
423 ng of cRNAs were injected into isolated oocytes for the GORK wild type, and the
424 cRNA doses adjusted to be of equal number of moles with the wild type were injected
425 for the mutants. Injected oocytes were incubated at 18°C in ND96 buffer (96 mM NaCl,
426 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 2 mM KCl, and 5 mM HEPES-Na pH 7.5) for 48 hours
427 before use.
428 TEVC recordings were carried out using an OC-725C amplifier (Warner
429 Instruments) and Digidata 1550B (Molecular Devices) with pClamp software.
430 Recordings were performed in a bath solution containing 10 mM KCl, 90 mM NaCl, 1
431 mM $CaCl_2$, 2 mM $MgCl_2$, and 10 mM Tris/MES pH 7.4, with osmolarity adjusted to
432 ~220 mOsmol/kg using D-mannitol. Microelectrodes were filled with 3 M KCl (0.5-1

433 MΩ resistance), and agar bridges served as bath electrodes. Currents were recorded in
434 response to 2500 ms voltage steps ranging from -110 mV to +70 mV, in 20-mV
435 increments. The membrane potential was held at -110 mV for 50 ms before each voltage
436 step and 450 ms following each voltage step. Currents at 50 ms before the end of each
437 step were used for statistical analysis. Data were processed using Clampfit 10.6
438 (Molecular Devices) and Prism (ver. 8.0, GraphPad).
439
440 **Data availability:**
441 All data generated or analysed in this paper are presented in the main text, figures and
442 the extended data figures and supplementary videos, or are available from the
443 corresponding author upon request. The cryo-EM maps of the *At*GORK full-length
444 (*At*GORK^{FL1} and *At*GORK^{FL2}) and truncated version (*A*GORK^{623} and *A*GORK^{510}) have
445 been deposited in the Electron Microscopy Data Bank with accession codes EMD-
446 62338, EMD-37500, EMD-62337 and EMD-62339, respectively, and their structural
447 models have been deposited in the PDB with accession codes 9KHF, 8WFZ, 9KHE and
448 9KHG, respectively (Table 1).
449
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453
454 **Author contributions:** Q.-Y.L. performed protein purification, Cryo-EM data
455 collection, data analysis and mutant design; L.-H.T. and C.-R.Z performed Cryo-EM
456 data collection, structural determination and analysis; L. Q. performed
457 electrophysiology and data analysis, K.-W. and G.-H.Z. performed experiments; S.-
458 G.H. and M.S. analyzed data; R.H., analyzed data and wrote the manuscript, Y.-H.C.
459 initiated the project, planned and analyzed experiments, supervised the research, and
460 wrote the manuscript with input from all authors.

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Fig.1

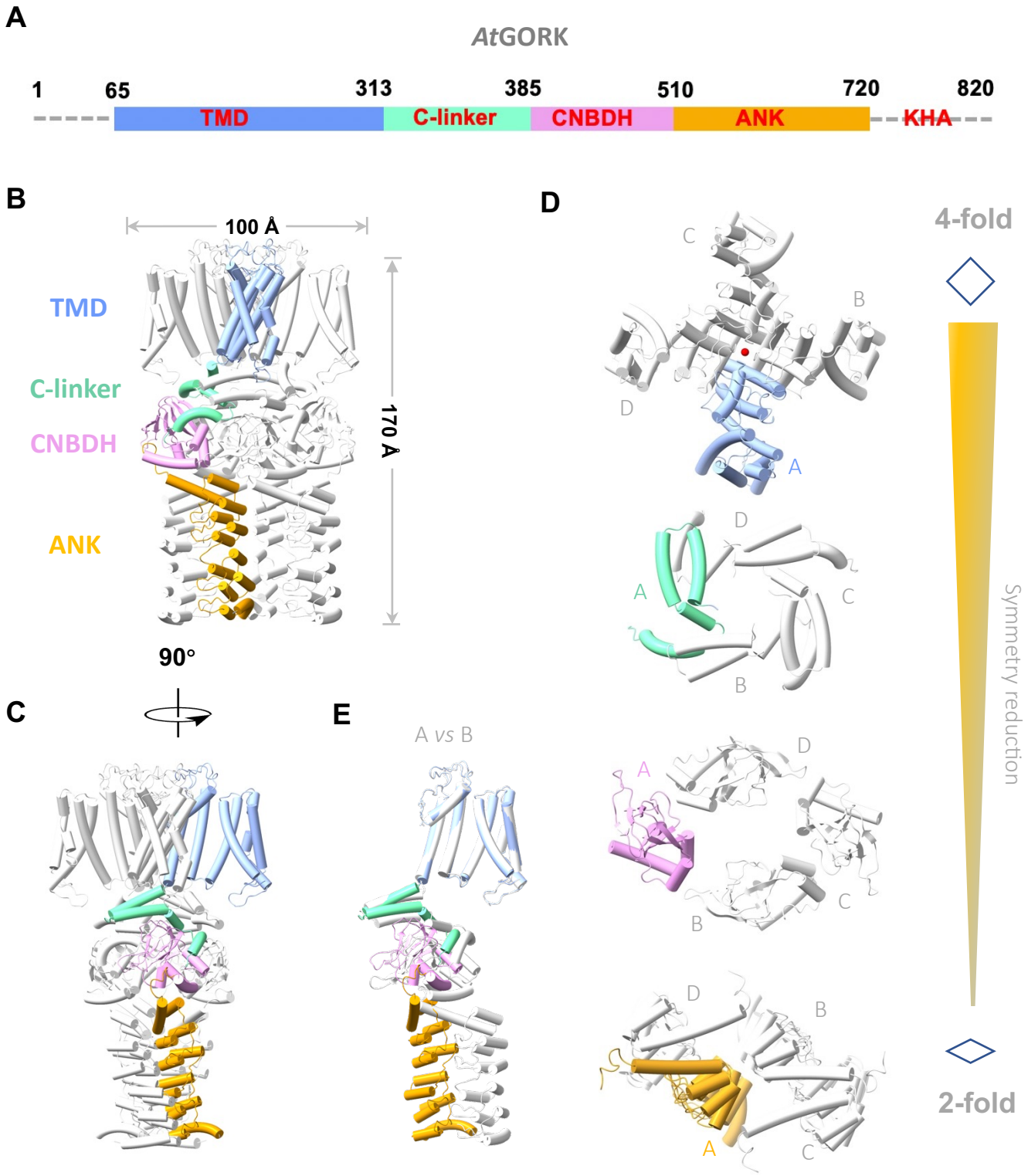


Fig. 1 | Architechture of the *Arabidopsis* GORK.

(**A**) Schematic diagram of the domain architecture in *Arabidopsis* GORK (*At*GORK). The transmembrane domain (TMD, blue), the C-linker domain (C-linker, Cyan), the cyclic nucleotide-binding domain homolog (CNBDH, purple) and the Ankyrin domain (ANK, orange) are shown. (**B and C**) The cartoon drawing of the *At*GORK tetramer. One protomer is colored as indicated in (**A**), and other protomers are colored in grey. (**D**) Symmetry reduction (from C4 to C2) within the tetrameric assembly. This represents a unique structural feature of the GORK family and likely serves as a unique mechanism for channel regulation. The current *At*GORK structure likely represents an autoinhibited, pre-activation state. Different protomers are indicated with letter A-D. (**D**) Structural superimposition of protomer A with its neighboring protomer B.

Fig.2

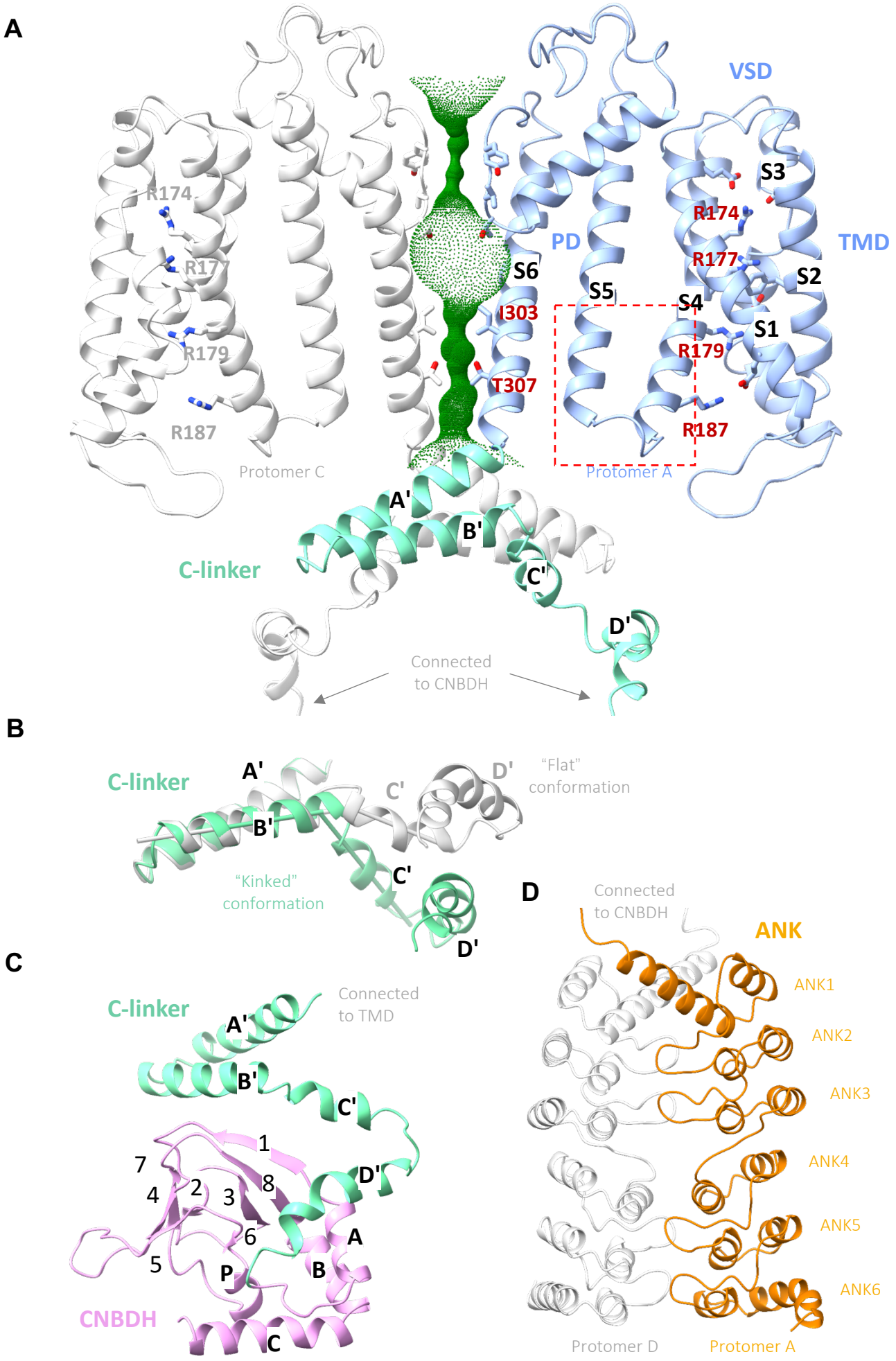

A
VSD
S3
R174
PD
TMD
R177
S6
S2
S5
S4
I303
S1
R179
T307
R187
R174
R177
R179
R187
Protomer C
Protomer A
A'
B'
C-linker
C'
D'
Connected
to CNBDH
B
C-linker
A'
C'
D'
"Flat"
conformation
B'
"Kinked"
conformation
C'
D'
D
Connected
to CNBDH
ANK
ANK1
ANK2
ANK3
ANK4
ANK5
ANK6
Protomer D
Protomer A
C
C-linker
A'
Connected
to TMD
B'
C'
1
7
D'
4
2
3
8
6
A
5
P
B
CNBDH
C

Fig. 2 | Structural domains of *At*GORK: TMD, C-linker, CNBDH and ANK

(**A**) Ribbons drawing of the TMD/C-linker portion, with two protomers (A and C) are shown, colored and oriented as in Fig. 1C. The VSD is connected to the PD through a short linker, resulting in a "non-domain-swapped" configuration. The pore-lining surface was computed by the program HOLE and drawn into a cartoon model of the GORK channel pore in a closed conformation. Critical residue in VSD (R174, R177, R179, and R187), selectivity filter (^{271}TVGYG^{275}) and the narrowest constrictions (I303 and T307) are shown in sticks. The S4 helix adopt a resting "up" conformation, similar to that in plant inward-rectifying KAT1 and AKT1.

(B) Structural comparison of the C-linker of protomer A ("Kinked" conformation) with protomer B ("flat" conformation). The "flat" conformation of the C-linker actually disrupts the formation of a C4 symmetrical gating ring, resulting in decoupling between the transmembrane helices with the cytoplasmic domains, ultimately affecting channel gating.

(C) Ribbons drawing of the C-linker/CNBDH portion.

(D) Ribbons drawing of the dimeric ANK portion. ANKs from two neighboring protomers tightly interact, creating a buried area of ~1500 Å^2. The first three and the last three repeats are tandem units. Repeats 1-2/4-5 are canonical α-helix/α-helix/β-hairpin motifs, while repeat 3/6 lacks the β-hairpin. The presence of the ANK domain and its dimerization are unique features of the GORK family. ANK dimerization leads to symmetry reduction in the tetrameric channel. This, in turn, affects the coupling between the transmembrane helices and cytoplasmic domains, ultimately holding the ion channel in an autoinhibited, pre-activation state.

Fig.3

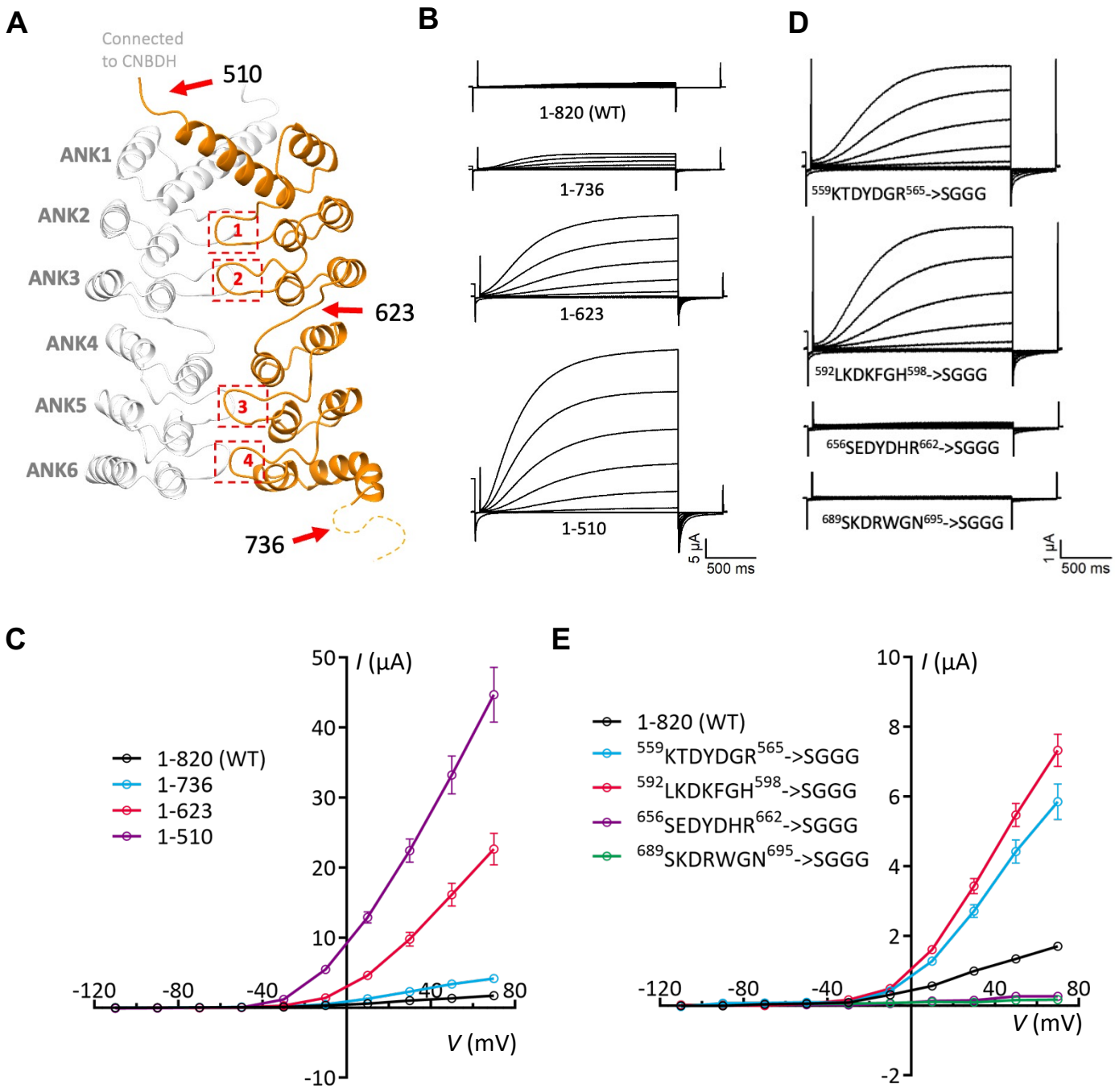


Fig. 3 | ANK play an inhibitory in regulating *At*GORK

(**A**) Ribbons drawing of the ANK dimeric domain, with truncated points or mutated loops are highlighted with red arrow or in red box. (**B, C**) The C-terminal truncation of *At*GORK, with representative current traces in (**B**) and current-voltage (*I-V*) relations in (**C**). Truncating half ANK domain (Δ624-820, residues 1-623) or the entire ANK domain (Δ511-820, residues 1-510) significantly increased currents. These results indicate that the ANK domain plays an inhibitory role in maintaining the autoinhibited state of GORK, as disrupting or removing the ANK domain substantially enhances channel activity.

(**D, E**) The loop-substitution in ANK of *At*GORK, with representative current traces in (**D**) and current-voltage (*I-V*) relations in (**E**). Substitution of loops between repeats 1/2 and 2/3 with "SGGG" significantly enhanced GORK activity. However, similar replacement of the loops between repeats 4/5 and 5/6 impaired GORK function. This suggests that ANK's role is complex - essential not only for maintaining an autoinhibited state but also for supporting the open state during activation.

Data are mean ± SEM, $n \geq 8$.

Fig.4

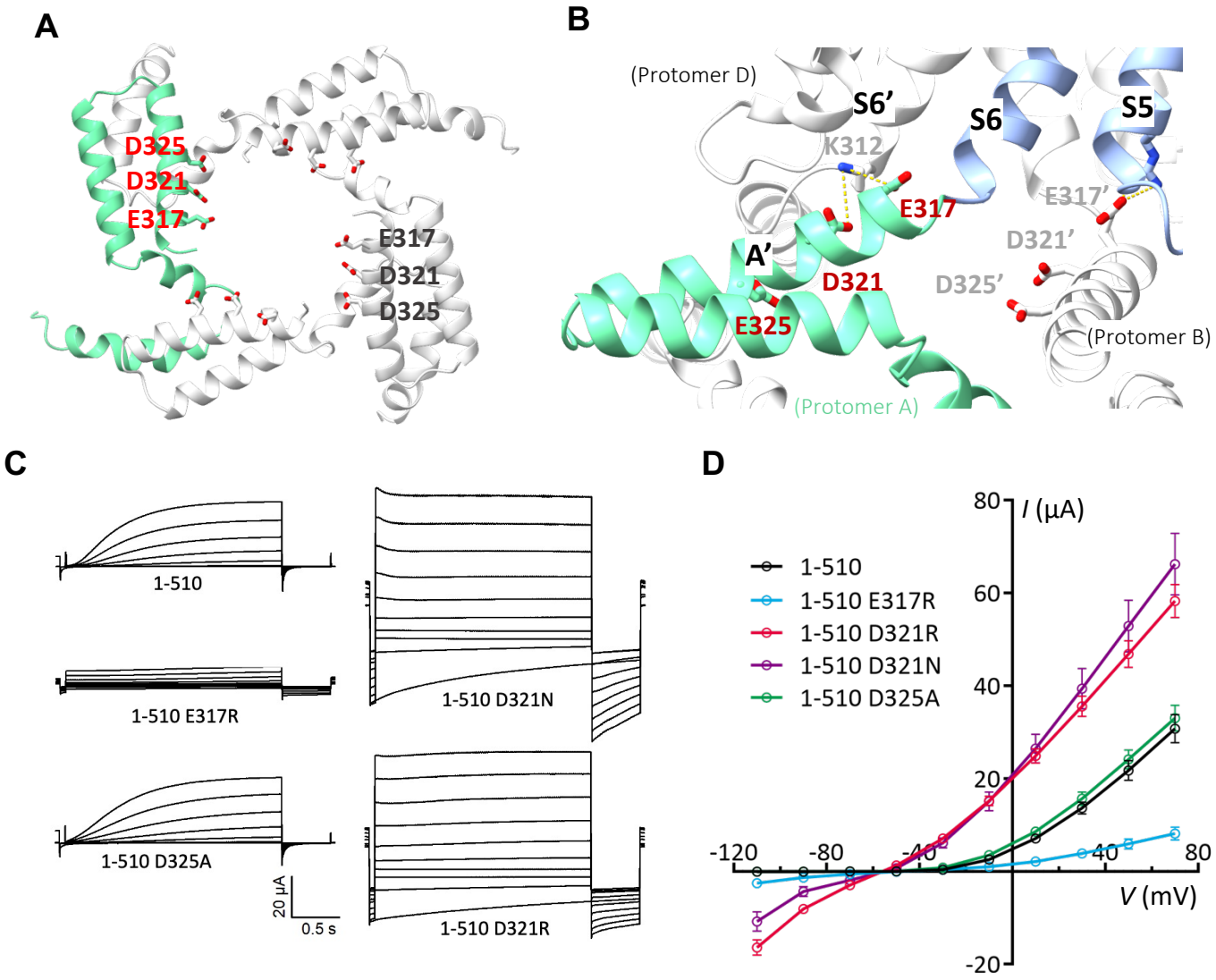


Fig. 4 | Mutational tests Mutational test on conserved acidic residues at the C-linker/TMD interface.

(**A**) Ribbons drawing of the C-linkers in tetrameric assembly, highlighting negatively charged residues (E317, D321, D325) in sticks. (**B**) Close-up view of the interface between the C-linker and TMD. E317 and D321 (C-linker of protomer A) form ionic interactions with K312 (Helix S6' of protomer D), while R200 (Helix S5 of protomer A) forms a salt bridge with E317 from its adjacent protomer B.

(**C, D**) Mutational analyses of E317, D321 and D325 in *At*GORK$^{1\text{-}510}$. The representative current traces (**C**) and the current-voltage (*I-V*) relations (**D**) are shown. Either charge neutralization (D321N) or reversal (D321R), displayed much larger currents compared to *At*GORK$^{1\text{-}510}$, lost voltage dependence, and exhibited much slower deactivation kinetics. Remarkably, the outward rectification characteristic was altered, allowing potassium influx. This suggests that the gating ring of C-linker not only participates in channel activation, but also its conserved negatively charged residue (D321) plays a role in regulating K^+ flux direction.

Data are mean ± SEM, $n \geq 8$.

Fig.5

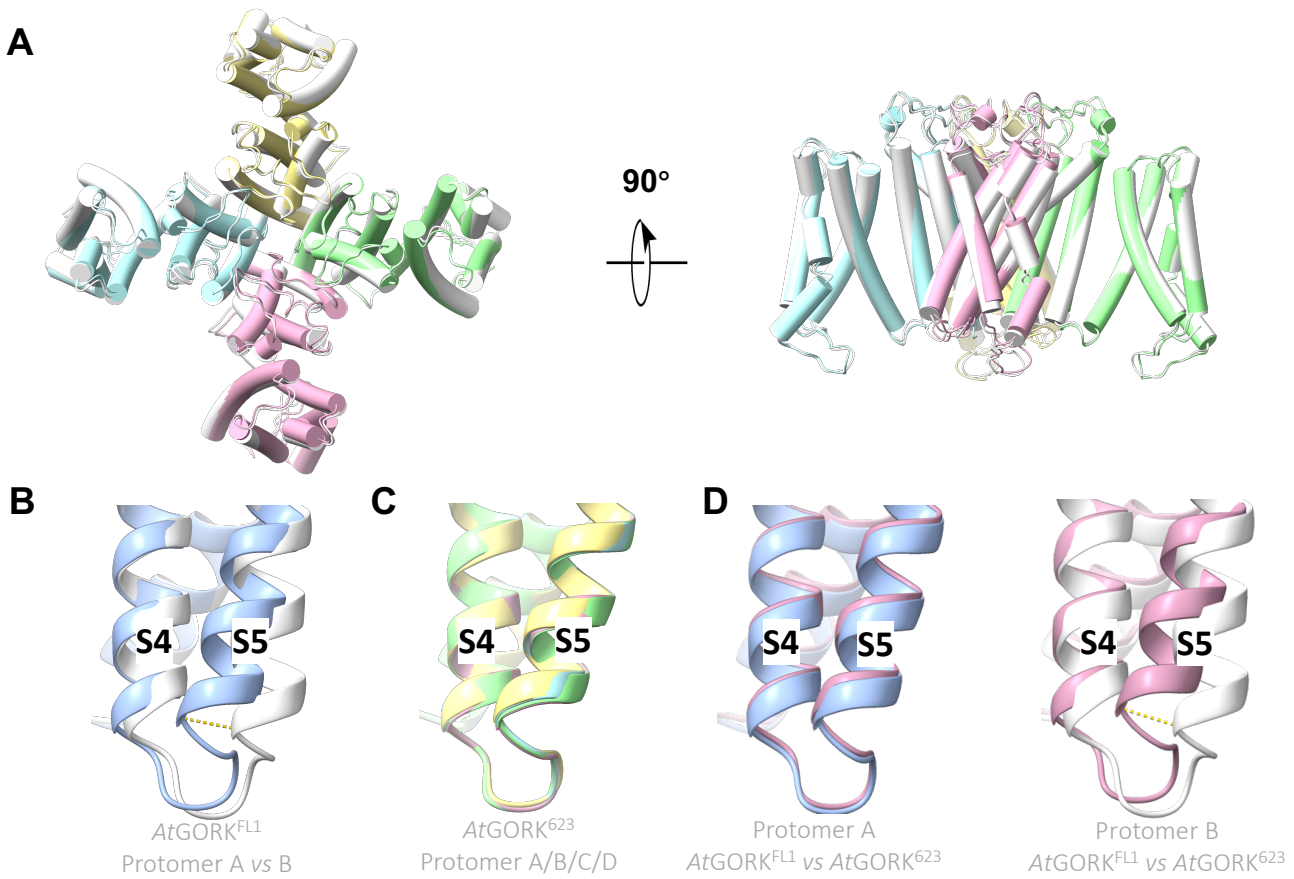


Fig. 5 | A working model of symmetry conversion in plant GORK regulation

(**A**) Superimposition of *At*GORK^{623} with *At*GORK^{510}, with each protomer of *At*GORK^{623} shown in different color, while all protomers in *At*GORK^{510} are displayed in grey. Both truncated mutants showed nearly identical structures, with a root-mean-square deviation (RMSD) of approximately 0.48 Å for their superimposed tetramers. (**B-D**) Close-up views of S4-S5 helix regions in the superimposed protomers of *At*GORK^{FL1} and *At*GORK^{623}. (B) compares protomer A (blue) and protomer B (grey) of *At*GORK^{FL1}, while (C) shows all four protomers of *At*GORK^{623}. These comparisons reveal that *At*GORK^{FL1} exhibits quasi-C4 symmetry, whereas *At*GORK^{623} has strict C4 symmetry. (D) further compares protomers A and B of both *At*GORK^{FL1} and *At*GORK^{623}, showing that the protomers in *At*GORK^{623} closely resembles protomer A in *At*GORK^{FL1}, rather than protomer B. ANK truncation in *At*GORK^{623} restores C4 symmetry in the tetrameric assemblies.

Fig.6

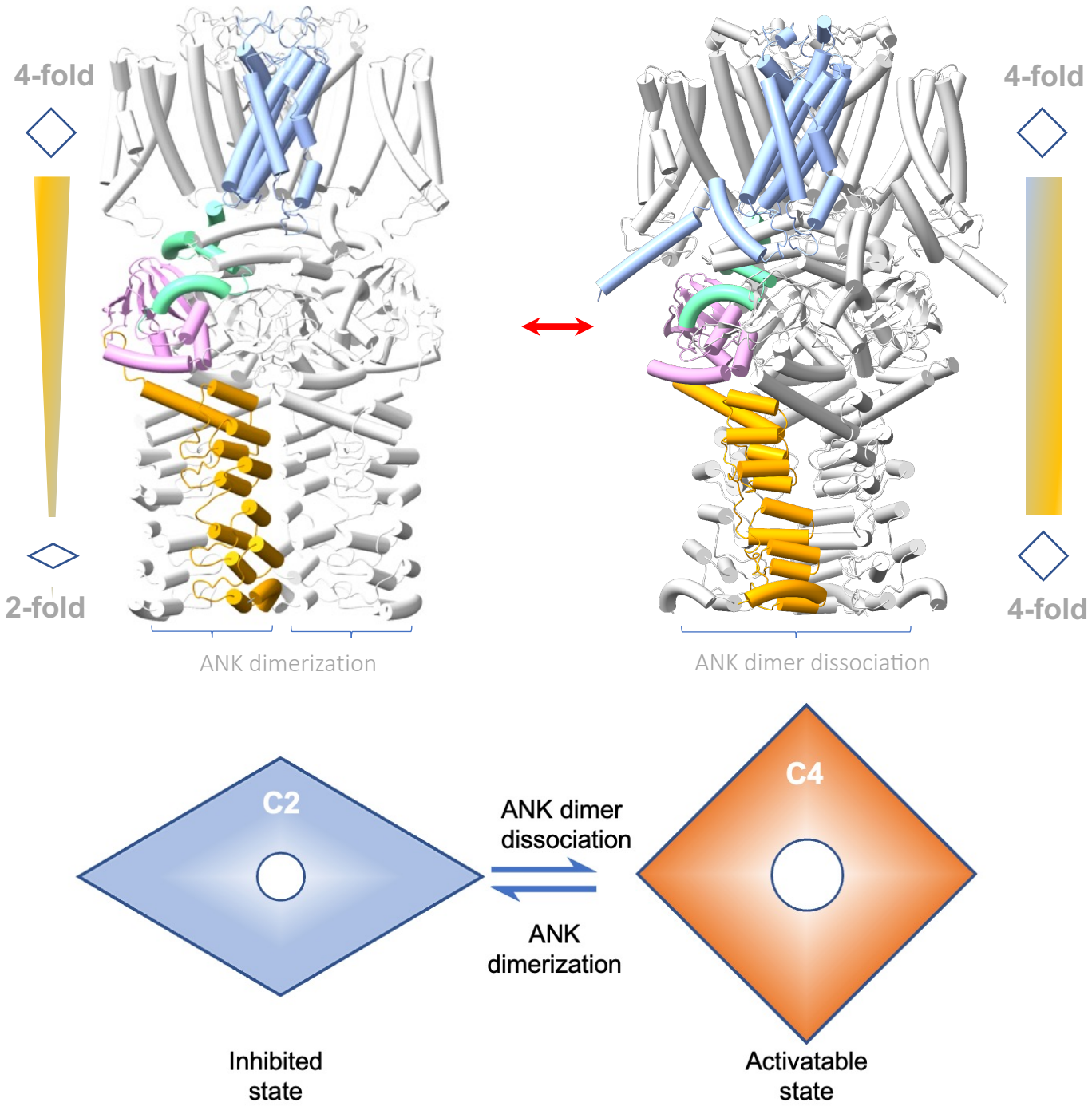
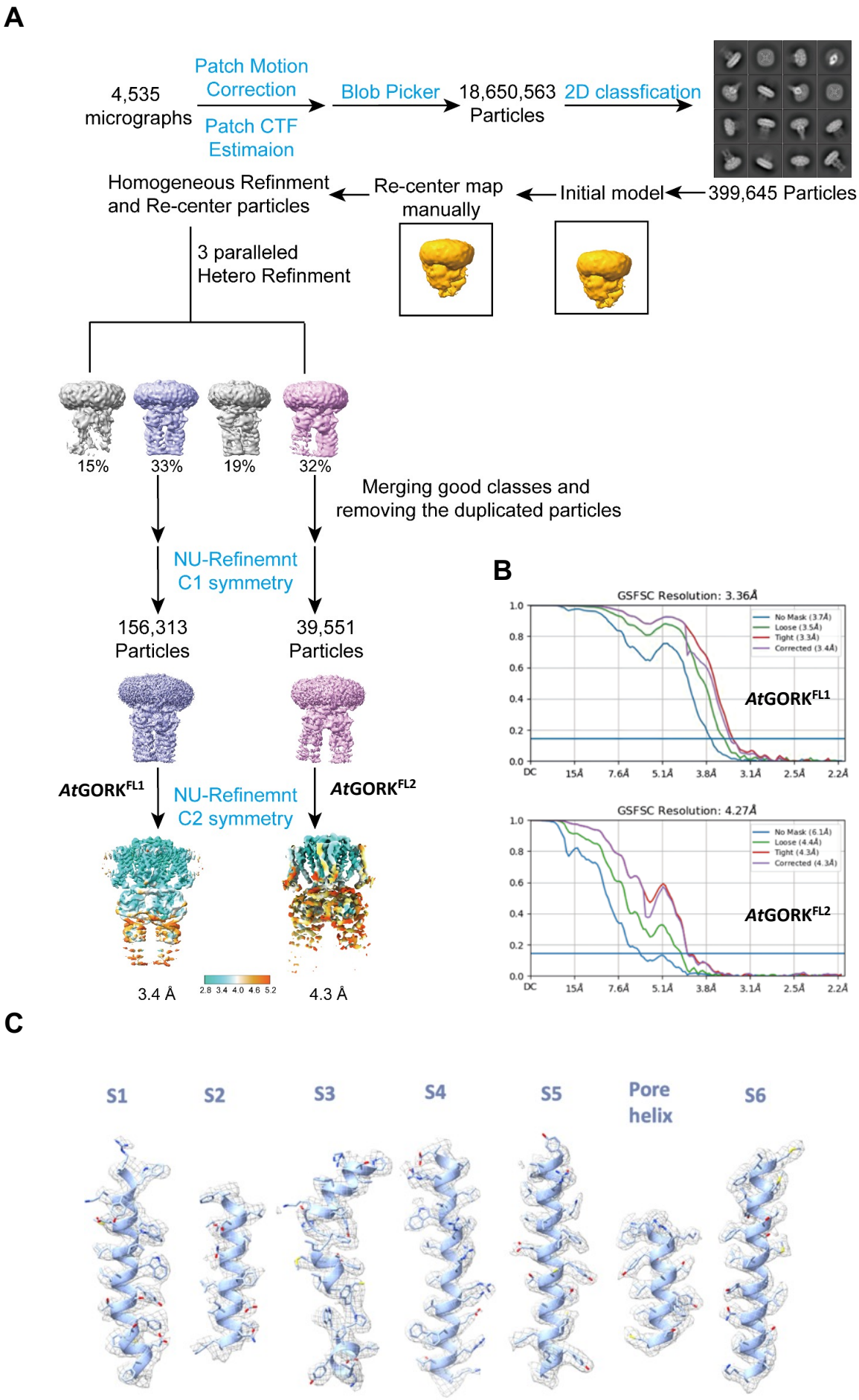


Fig. 6 | A model of symmetry conversion in plant GORK regulation

The GORK structure in the autoinhibited state (left, Cryo-EM) exhibits C2 symmetry, while in the activatable state (right, Alphafold3 prediction), it exhibits C4 symmetry. In brief, ANK dimerization reduces the symmetry of the GORK tetramer from C4 to C2, resulting in an autoinhibited state. Disrupting dimerization restores C4 symmetry, converting GORK to an activatable state. This dynamic symmetry conversion in the GORK tetramer provides a unique mechanism for regulating channel activity, enabling transitions between inhibited and activatable states - a distinctive feature of GORK family proteins containing the ANK domain. In such a way, ANK functions as a molecular switch, regulating the GORK channel in guard cells and controlling stomatal movement in response to environmental stimuli.

Fig. S1


A
4,535 micrographs
Patch Motion Correction
Patch CTF Estimaion
Blob Picker
18,650,563 Particles
2D classfication
399,645 Particles
Initial model
Re-center map manually
Homogeneous Refinment and Re-center particles
3 paralleled Hetero Refinment
15%
33%
19%
32%
Merging good classes and removing the duplicated particles
NU-Refinemnt C1 symmetry
156,313 Particles
39,551 Particles
AtGORKFL1
AtGORKFL2
NU-Refinemnt C2 symmetry
3.4 Å
4.3 Å
B
GSFSC Resolution: 3.36Å
No Mask (3.7Å)
Loose (3.5Å)
Tight (3.3Å)
Corrected (3.4Å)
AtGORKFL1
GSFSC Resolution: 4.27Å
No Mask (6.1Å)
Loose (4.4Å)
Tight (4.3Å)
Corrected (4.3Å)
AtGORKFL2
C
S1
S2
S3
S4
S5
Pore helix
S6

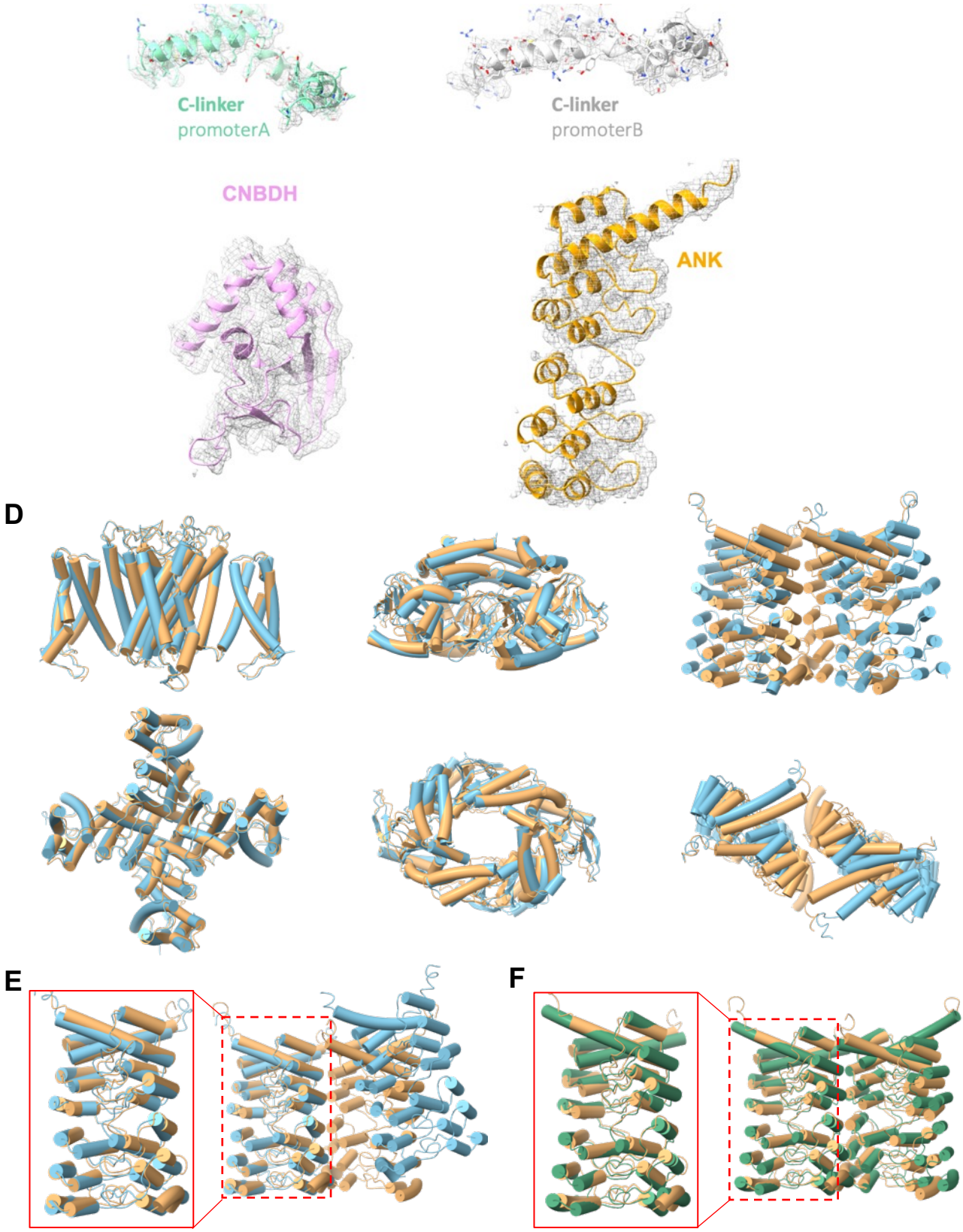


Fig. S1 | Structural determination of full-length *Arabidopsis* GORK (*At*GORK^{FL})

(**A**) Workflow for image processing of full-length *Arabidopsis* GORK. (**B**) Fourier shell correlation (FSC) curve indicating overall resolution of 3.4 Å for *At*GORK^{FL1} and 4.3 Å for *At*GORK^{FL2}, as estimated using the 0.143 cut-off criterion (dotted line). (**C**) Representative cryo-EM density map showing TM segments (S1-S6) and domains (C-linker, CNBDH and ANK).

(**D**) Structural comparison of domains in *At*GORK^{FL1} (light orange) and *At*GORK^{FL2} (light blue).

(**E**) Superimposition of ANK dimers in *At*GORK^{FL1} (light orange) and *At*GORK^{FL2} (light blue), with a RMSD of 1.21 Å/dimer.

(**F**) Superimposition of ANK dimers in *At*GORK^{FL1} (light orange) and *At*SKORK (purple, 8jet), with a RMSD of 1.33 Å/dimer. Despite variations in ANK conformation across the tetramer, the dimeric interaction is conserved, indicating that ANK dimerization is a fundamental structural feature of potassium channels with ANK domains.

Fig. S2

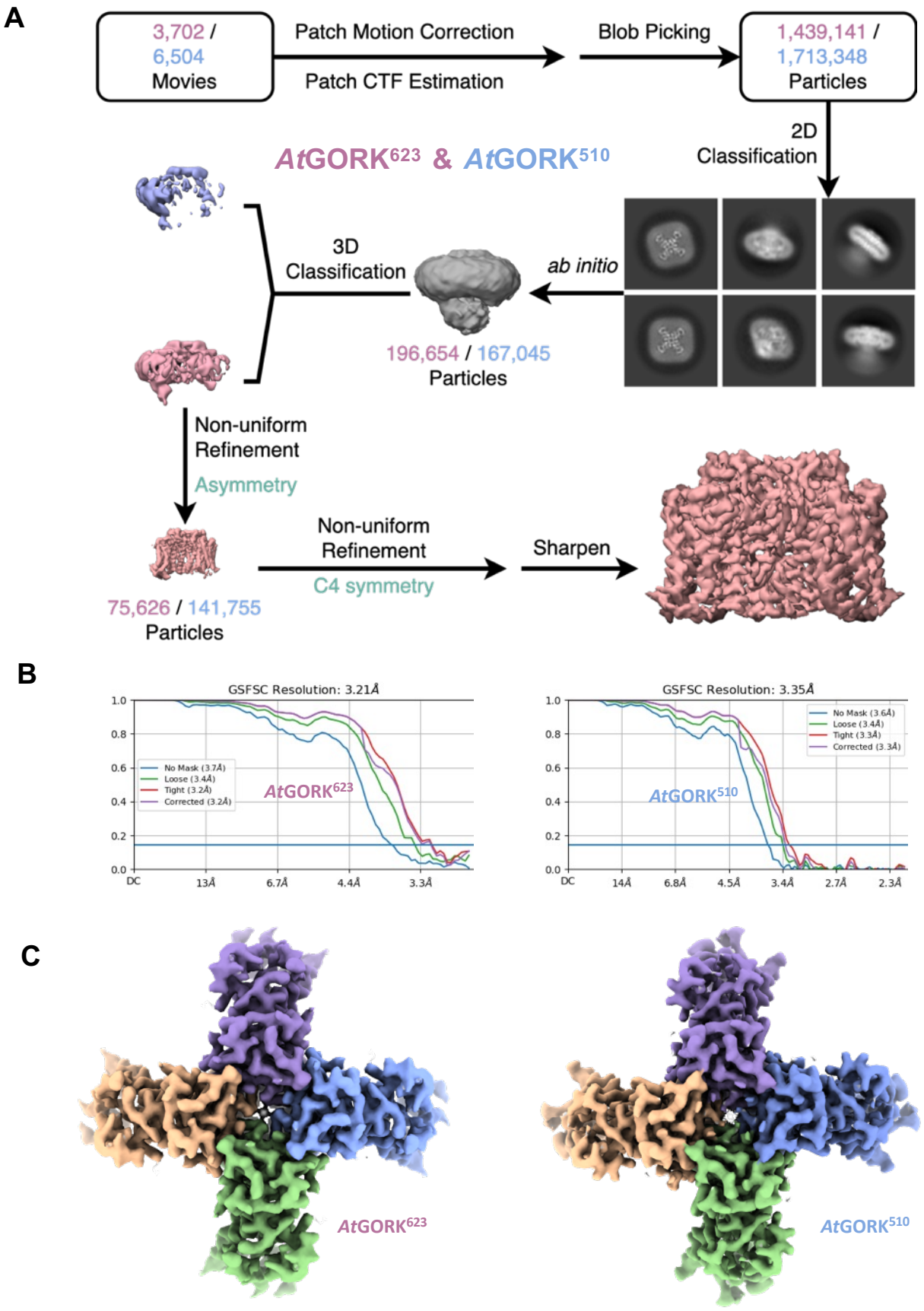


Fig. S2 | Structural determination of the truncated *At*GORK^{623} and *At*GORK^{510}

(**A**) Workflow for image processing of the truncated *At*GORK^{623} (residues 1-623) and *At*GORK^{510} (residues 1-510) .

(**B**) Fourier shell correlation (FSC) curve indicating overall resolution of 3.2 Å for *At*GORK^{623} and 3.4 Å for *At*GORK^{510}, as estimated using the 0.143 cut-off criterion (dotted line).

(**C**) Cryo-EM structures of *At*GORK^{623} (3.2 Å, left) and *At*GORK^{510} (3.4 Å, left).

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Arabidopsis_GORK      ---------------MGRL-----RR---R-------QEI-------IDHEEEESNDDVSSRRGKLSLAETFRWLDSSEHRRIET--------DGH--NDYKYIIHPK-NRWYKAWEMFIL 73
Brassica_GORK         ---------------MGCL-----RR---R-------QES-------IAE-EDDINDDVSRRRGRFSLAESFRWLDSPEHLKDDS--------DGH--NEYPWIIKPS-IRWYKAWELFIL 72
Zingiber_GORK         MERDSSRRK-WKRTMSAVMAPRPSEAEEEAVGLASDGGSGGGDEYTVEEVHDLIG---SSRGSRQSVLADESRR------------------ASGDELLLRFVFRPD-NGWYETWTQFIM 97
Elaeis_GORK           ------------------------------MSPRAKNGSPEIEYEL---EEPIA---SSRGIRLFLLTSEFALGPLRRRRAT----------SQEKLLERFVIEPD-NRWYQLWTRFIL 72
Phoenix_GORK          ------------------------------MSPHAKNESQEIEYEL---EEPMG---SSRGIRWSLITSDFAIGPLRRR-AH----------SHEKLLERFVIEPD-NKWYQLWTRFIL 71
Oryza_GORK            MGRGIGSKRRVEDDDGENMPGRK---KKE--EEEEEEDDDGEEEYEVDVVRDRIG---SSRGSRLALFGSDLRLGRFRPRRRRV--APV---DGDDGIFQDFVIDPD-NKWYRLWTRFIL 106
Brachypodium_GORK     MGRGLGSKSRVES-------------------------EVEEEEYEVEVVPDRLK---SSRNSRLALFGSELRLDRFRPRRRRRRRAAA---DGEDGFFHDLIIHPE-NKWYRIWSRFIL 88
Panicum_GORK          MGRGLGSKRRVAEGDTK----------------PEEEEEGSSEEYEVEVVRDHIA---SSRGSRLALFGSDLRLGRFRPRRRRA---------AAEGFFQDLVIHPD-NRWYRLWTKFIL 91
Papaver_GORK          MPD----------HLHHL-----HRHHHERERDKREEDEDETEEFKVEKLKDRIL---SSRGSRFDLITNELGLDNQ-TRRKISREDFI---NGIR----GFVIHPD-NRWYLIWTRIIL 93
Amborella_GORK        ------------------------------MCPGSEERDMQEEYQVEEFRERIN---SSRGSRFALLSELTKR---------GGGSQERLIQGFRGCTQSVIIHPD-SRWYQVWTFIIL 76
Vulgaris_GORK         ---------MTPKQLGRIISGKTMSRIREEGGSTSSSSSNRIEDFVVEKPRERLT---SSRGSRLDLLVNELNDES--TRRQFSREGFF---LGVKDLYHGLVIHPEDNRWYKAWDKFIL 103
Chenopodium_GORK      ---------MKPKQIARIMMTRG--REERMEGSTSSSSSNREEDFMVERPRERLY---ASVGSRFNLLVTEFSHES--SQRQFTREGFF---VGVKDLYHGLVIHPEHNRWYKAWDNIIL 101
Lactuca_GORK          ------------MRIGST---------ADERRRKAVEDEVVGEEYVVEDVRDHDHGRLTKRRSRFTLLAGDLDLDP--SRRDFNTEK---FIDGIRGCSPALFIHPQ-NSWYKAWEKFIL 93
Daucus_GORK           ----------------MSNRRRMEEEKRRRRKGQDSEDSSNDEEYEVEDVREDVR---ESRGHHMARIASEFEAATEAYRRIYSREHIF---KGLTDLSRGCSIHPD-NRWYHAWEKFIL 97
Medicago_GORK         ---M---KMKEEEDEGEKNRINNQTATCSGSSSLTPSSSSDEREYQVQDLRDKLK---SSRGSRFNLIEKELGLKI--GWRKFSR----------RTLLHEFVINPL-NRWYRAWLKFIL 98
Prosopis_GORK         MPGN---RLTEEAVTEEREEKRKQMLSTSRSSSSSSSSSSDDREYELEDLRDRLK---SSRGSRFQLIENELKE-L--NQKKFSRHV----IDGIRGLSKDFIIHPE-NRWYRAWTKFIL 106
Nicotiana_GORK        ------------------------MTRVAEEVLETMNMNGGVEYEVEDVRDKIE---SSRGSRFQLIEDDL-GLNL-FRRRISRL--------KINLSQVFIIHPE-NRWYRMWENFIL 81
Solanum_GORK          -------------------------MTRGEEVLEIDINGGRAEYEIEDMRDKME---SSRGSRFKLIENDLVGADM-FSRRRRKFSRESLLNGLKDLSQGFVIHPE-NRWYRMWENFIL 89
Coffea_GORK           ------------------------MGRGNSHREEGESRNGEGEYEVEDLRDRIK---SSRGSRFELIEHELGL-DS-THRRFSRQSVI---NGLKDLSQGLVIHPD-NRWYRAWEKFIL 86
Sesamum_GORK          --MG---SFKKSSF--RN-----TDESSANGEESSLNGVNGGEEYMVEDLRERIK---SSRGSRFTLIENELGLIDS-ARRRVSRQTVI---DGLKDLSQGLFIHPD-NRWYRAWEKFIL 100
Cucumis_GORK          -----------MAVSG-------GREKEEEAGTHRRSDSDGEVEYRVESVRERIS---SSRGSRFNLIQRQLRID--QSRRRFSRENLI---NGIK----CLVILPD-SRWYRAWTKFIL 89
Vitis_GORK            --MN---RFMY--LVGRG----------------QVVDDEDDGDFQMPDLRERIK---GSSASVLNMFNGDLGLR------RFSGGSCF---NGIK----GFIILPD-NWWYRTWTKFIL 80
Cannabis_GORK         ---M---SFAAANGIGRV-----DQNDERDHDMVSSSSDGDEEEFELQDIRDRIK---SSRGSRFNLIANEFGLASAI--KKFSRENVM---NGIRDLSNGVVIHPD-NRWYRAWTKFIL 100
Gossypium_GORK        -----------MGDIGRS-----RRV-----RDERCATMANEEEYEVEDFREGNK---SSRGSRFNLIANELGLVAARARMNLSRQSVL---NGIKDLSDGP-IHPD-NRWYRAWTKFIL 91
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                                  S5                                                  Pore-Helix                                  S6
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                                                H5                      H6          H7                                                ANK1
Arabidopsis_GORK     EALVTKTDGSEESVTLLGPHTSFGDISIICNISQPFTVRVCELCHLLRLDKQSFSNILEIYFHDGRTILNNIMEEKE---SNDRIKKLESDIVIHIGKQEAELALKVNSAAFQGDFYQLK   548
Brassica_GORK        EALETKTDGTEDLVELLEPHTSFGDISIICNISQPFTIRVRSLCHLLRLDKQSFSNILEIYFHDGRKILNNLMEGKE---SNERIKKLESDIMIHIGKQEAELALKVNSAAFQGDIYQLK   547
Zingiber_GORK        EGITIGEDGSEETLLLLEPNSSFGEISILCNIPQPYAVRVRELCRLLRLDKQLFANIMEIYFVDGRTILSNLIEEN-E--SNGRIKHLESDITFHIGKQEAALALQVNSAAYYGDLLQLK   572
Elaeis_GORK          EGVGIGEDGP----LELEPNSSFGEIAILCNIPQPYTVRVCELCRLLRIDKQIFTNILQIYFVDGRKILSNLLEGN-D---SIQIKQLEGDIIFHIGKQEAELALRVNNAAFYGDLHYLK   542
Phoenix_GORK         EGVGIGEDGSKDTVLTLQPNSSFGEIAILCNIPQPFTVRVCELCRLLRIDKQVFTNILQIYFVDGRRILNNLLEGN-D---SNRIKQLESDITFHITKQEAELALRVNSDAFYGDLHHLK   545
Oryza_GORK           EGVGIGEDGQEETILMLEPESSFGEIAVLCNIPQPFTVRVCELCRLLRLDKQSFTNILEIFFVDGRRILSNLSESS-E--YGSRIKQLESDITFHIGKQEAELTLRVNNAAFYGDMHQLK   581
Brachypodium_GORK    EGVGIGEDGQEETLLMLEPESSFGEIAILCNIPQPYSVRVCELCRLLRLDKQSFTNILEIYFVDGRKILSNLTENN-E--YGGRVKQLESDITFHIGKQEAELTLRVNSAAFYGDLHQLK   563
Panicum_GORK         EGVGIGEDGQEETLLMLEPESSFGEISILCNIPQPYTVRVCELCRLLRLDKQSFTNILEIYFVDGRRILSNLSE-S-E--YGGRVKQLESDITFHIGKQEAELILRVNSAAFYGDLHQLK   565
Papaver_GORK         EEMGIGEEGSEVTVSLLEPNSSFGELSILCNMPQPYTIRVCELCRLLRLDKESFISILEICSSDERIILTNLLEGKESNKDFHRMKQLESDVMFHIGKQEGELALKVNSAACHGDLYHLK   571
Amborella_GORK       EEVGIGEDGLEETIVQLEHDNSFGEIAILCNIPQPYTVRVCELCRLLRLDKQSFSNILQIYFIDARTVLNNLLEGK-E--SSLRLKQLESDIIFHINKQEAELALRVNSAAYYGDLYHLK   551
Vulgaris_GORK        EEVGIGQDGSEETVSRLEPGSTFGQISIFCNIPQPTTVRVLELCRLLRIDKESLSNIIDIYFYDGKKIFDNLLEGN-D--GKYNLKQVESDIASHIGKQESELALKVNNAAYHGDLYQIK   578
Chenopodium_GORK     EEVGIGQDGSEQIISRLEPNNTFGQISIFCNVPQPSIIRVIDLCRLLRIDKESLSNIIDIYFYDGKKIFDNLLEGNNN--GKYNLKQVESDIASHIAKQESELALKVNNAAHHGDLYQLK   577
Lactuca_GORK         EEIGICEDGSEETVSILKPHSSFGEVSILCNIPQPYTVRVCELCRLLRLDKQSFSNILEIYFHDGRKILNNLVEFN-A--SDARMKQLETDIILHIGKHEAELALRVNSSAFYGDLYQLK   568
Daucus_GORK          EEVGICEDGTEETVSVLRPNSLFGEVSILCNIPQPYTVKVSELCRLLRLDKQSFSNILEIYFHDGRKILNNLLEGK-E--SNLRLKQLESDITFHINKQEAELALRVNSAAYYGDLYQLK   574
Medicago_GORK        EEVGIADDGSEETVALLERNSSFGEISILCNIPQPHTVRVCELCRVLRIDKQSFSNILDIYFYDGKKVLDNLLEGK-E--S-IRGKQLESDITFHIGKLESELALKVNRTAFDGDLYQLK   572
Prosopis_GORK        EEVGPGENGSEETVSLLEPNSSFGQISILCNIPQPYTVRVCELCRLLRLDKQSFTDILDIYFYDGRKVLNNLLEGK-D--S-SRGKQLESDITFHIGKQEAELALKVNNAAFNGDLNHIK   580
Nicotiana_GORK       EEVGIAKDGSEETVALLEPNSSFGDISIVCNIPQPYTVRVCELCRLIRIDKQSFSNILEIYFHDGRRILSNLLQGK-E--SNLRVKQLESDIAFHIGKHEAELALKVNSAAYHGDLHQLK   556
Solanum_GORK         EEVGIAKDGLEETVSLLEPNSSFGDISIVCNIPQPYTVRVCELCRLLRIDKQSFANILEIYFHDGRRILSNLLQGK-E--SNLRVKQLESDIALHIGKHEAELALKVNSAAYHGDLHQLK   564
Coffea_GORK          EEVGTAKDGSEETLSLLEPNSVFGIVSILCNIPQPYTVRVCELCRLLRIDKQSFSNILEVYFHDGRTVLTNLLEEK-D--SAFSLKQLESDITLHIVKQEAELALKVNSAAYYGDLQQLK   561
Sesamum_GORK         EEVGIAADGSEDTVSLLEPNSLFGEISILCNIPQPYTVRVCELCRLLRIDKQSFSNILEIYFHDGRKVLTNLLEVI-T--D-----STISDITFHIGKQEAELALRVNSAAYYGDLYQLK   570
Cucumis_GORK         EELGIGQDSSEETIDLLQPNSSFGEISILCNIPQPYTVRVCELCRLLRIDKQSFTNILDIYFYDGRKILNNLLEGK-E--TNLRVKQLESDITFHIGKQEAELALKVNSAAYHGDLYQLK   564
Vitis_GORK           EEIGIGADGSEETVLPLQPNSSFGEISILCNIPQPYTVRVLELCRLLRLDKQSFTDILEIYFYDGRRILNNLLEGK-E--SNLRVKQLESDITFHIGRQEAELALRVNSASYHGDLYQLK   555
Cannabis_GORK        EEVGIGEDGSEETVSLLQPSSSFGEISILCNIPQPYTVRVCELCRLLRLDKQSFTNILDIFFYDGRKILNNLLEVK-E--SNLRVKQLESDITFHIGKQEAELALKVNSSAFHGDLHQLK   575
Gossypium_GORK       EGIAIGEDGSEETVSLLEPNSSFGEISILCNIPQPYTVRVCELCRLLRLDKQSFSNILEIYFYDGRKVLNNLLEGK-E--SNLRVKQLESDISFHIGRQEAELALRVNCAAYNGDFYQLK   566
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                                                ANK2                    ANK3                    ANK4
Arabidopsis_GORK     SLIRSGADPNKTDYDGRSPLHLAACRGYEDITLFLIQEGVDVNLKDKFGHTPLFEAVKAGQEGVIGLLVKEGASFNLEDSGNFLCTTVAKGDSDFLKRLLSSGMNPNSEDYDHRTPLHVA   668
Brassica_GORK        SLVRSGADPNKTDYDGRAPLHLAASRGYEDITLFLIQEGVDINQKDKFGNTPLLEAVKAGQDRVIDLLVKEGASFDLEDAGNFLCTVVVKGDSDFLKRLLSSGMDPNTEDYDHRTPLHVA   667
Zingiber_GORK        GLVKAGADPKNTDYDGRSPLHLAASRGFEDITSFLIQECVDINVSDNFGNTPLFEAVKNGHDKVASLLYNNGAKLNLKDPSSHLCSAVARGDSDFIKRLLAYGIDLNLKDYDSRTPLHVA   692
Elaeis_GORK          GLIQAGADPNKTDYDGRSPLHLAAAKGHEDITLFLIQEGVNINLSDNFGNTPLFEAVKVGHDRVASLLFSRGAQLNLKDAGSHLCTAAAKGDSDFIRRALSYGVDPNSKDYDHRTALHIA   662
Phoenix_GORK         GLIQAGADPNKTDYDGRSPLHLAAARGHEDITLFLIQEGVDINLSDNFGNTPLFEAIKDGHDRVASLLFSRGAQLSLKDAGSQLCAAAAEGDSDFIKRALSYGVDPNSKDYDHRTALHIA   665
Oryza_GORK           SLIRAGADPKNTDYDGRSPLHLAACKGFEDVVQFLLHEGVDIDLSDKFGNTPLLEAVKQGHDRVATLLFSKGAKLSLENAGSHLCTAVARGDTDFVRRALAYGGDPNARDYDHRAPLHIA   701
Brachypodium_GORK    GLIRAGADPKNTDYDGRSPLHLAASKGYEDVAQFLIHEGADINLIDKFGNTPLLEAVKQGHDRVATLLFKKGAILNLQNAGSHLCSAVSKGDSDFIRRALACGADPDSKDYDHRSPLHIA   683
Panicum_GORK         SLIRAGADPKNTDYDGRTPLHLAACRGYEDVVQFLLAEGVDINLTDQFGNTPLLEAVKQGHERVAALLFAKGAKLSLKNAGSHLCTAVAKGDSDFIRRALAFGADPNCGDYDHRTPLHIA   685
Papaver_GORK         RLIRAGADPNKTDYDGRTPLHIAASEGFEHIASFLIQEGVGVNIADKFGNTPLFEAVKRGNDRVASLLVKEGASLNIVDGGSCLCQAVSRGDSDFVKRLLSNGLDPNSKDYDQRTALHVS   691
Amborella_GORK       GLIRAGADPKKTDYDGRSALHLAASKGFEDITTFLIRETVDVNLTDKFGNTPLLEAIRYGHDRVASLLVKEGASLDIDDAGGFLCKAVASGNTDFLKRLLVYGMDPNSRDYDYRTALHKA   671
Vulgaris_GORK        NLLNAGADMNKTDYNGRSPLHIAAARGCEEIILLLVKEGVRINEKDNFGNTPLLEAVKNGNDKAAALLYREGARLDVKDVGSFLCTAVLGGDVDYLRRILANGIDPNSKDYDYRTPLHVA   698
Chenopodium_GORK     SLLNAGADLKKTDYNGRTPLHIAASGGCEDIVLFLIEEGADVNRTDNFGNTPLLEAVKNGHDKVAALLYKEKARIDVKDVGSFLCTVVLSGDVEFLKRILTNGMDPNSKDYDHRTPLHVA   697
Lactuca_GORK         SLIRAGADPNKKDYDGRTPLHLAASKGYEDITLFLIQEKVEVNVLDNFGNTPLLEAIKNGHDKVASILVKEGGSLKIDDAGWFLCTSVARGDIDYIRRILSNGVDPNSKDYDFRTPLHVA   688
Daucus_GORK          GLVRAGADPNKTDYDGRSPLHLAASKGYEDIILFLTQEGVDINISDNFGNTPLFEAIKSGHDQVVSLLIKEGAILKIGDSGPFLCTAVARGDYDLIKRVLSAGIDPNSSDYDHRTPLHVA   694
Medicago_GORK        SLIRAGADPKKTDYDGRSPLHLAACRGYEDITLLLIHERVDMNVKDNFGNTPLLEAVKNGHDRIASLLVREGASLKIDDGGSFLCSAVARGDSDYLKRLLSNGMDANLKDYDYRTPLHVA   692
Prosopis_GORK        GLIRAGADPNKADYDGRSALHLAASRGFEDITIFLIQEGVEIDLRDNFGNTPLLEAVKNGHDRVASLLVGKGASMKIEDAGSVLCNAVARGDSDYLKRLLSYGMDPNTKDYDYRSPLHVA   700
Nicotiana_GORK       SLIRAGADPNKKDYDGRSPLHLAASRGYEDITLFLIQEGVDINAPDKFGNTPLLEAIKSGHDRVASLLVKEGAFLKIENAGSFLCMVIAKGDSDLLRRLLSNGVDPNSKDYDQRTPLHVA   676
Solanum_GORK         SLIRAGADPKKKDYDGRSPLHLAASRGYEDITLFLIQEGIDINAPDKFGNTPLLEAIKIGHDRVASLLVKEGALLNIENAGSFLCMVIARGDSDLLRRLLSNGVDPNTKDYDQRTPLHVA   684
Coffea_GORK          NLIRAGADPNKNDYDGRSPLHLAASRGYEDIAQFLIQEGVDINVTDNFGNTPLLEAIKSGHEHVASLLVKEGASLQIENAGNFLCTVVAKGDSDLLRRFLTRGIDPNSKDYDQRTPLHVA   681
Sesamum_GORK         SLIRSGADPNKKDYDGRSALHLAASRGYEDVTLFLIQEGVDINAEDNFGNTPLLEAIKSGHDRVASLLSREGALLKIDNPGAYLCTVVSKGDTDLLRRVLANGIDPNSKDYDHRTPLHLA   690
Cucumis_GORK         GLVRAGADPNKTDYDGRSPLHLAASRGFEDIVVFLIQEGVNIDLKDNFGNTPLMEAIKNGNDQVAVLLSKEGASLKVDNPGSFLCTAVSRGDSDLVKRLLCYGIDPNSKDYDSRTPLHIA   684
Vitis_GORK           SLIRAGADPNKTDYDGRSPLHLASARGFEDIVTFLIQEGVDVNISDNFGNTPLLEAIKNAHDRVASLLVNKGALLKIDDAGGFLCATIARGDSDFLKRILSNGIDPNSKDYDHRTPLHVA   675
Cannabis_GORK        SLIRAGADLNKTDYDGRSPLHLAASRGYEDITRFLIQQGVNVNAIDNFGNTPLLEALKNGHDRVSSLLVQEGASLMIDNAGSFLCTAVSRGDSDFLKRVLSNGVDPNSKDYDHRSPLHVA   695
Gossypium_GORK       SLIRAGADPNKTDYDGRSPLHLAASKGYEDITSFLIRHPVDINLKDKFGNTPLLEAIKNGHDNLAALLIKEGASLNIDDAGSYLCTAVAKGDSDFLRRLLSNGVDPNSRDYDHRTPLHVA   686
                      *:.:*** :: **:**: **:*:. * *.:  :*  .   ::  *:**:***:**:: .::    :*  . . : : : .  **     *: : ::* *  * : :  *** *: ** :
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                                ANK5                                ANK6                                                                                                                                KHA
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Arabidopsis GORK        ASEGLFLMAKMLVEAGASVISKDRWGNSPLDEARLCGNKKLIKLLEDVKNAQSSIYPSSLRE-------LQ-EERIERRKCTVFPFHPQEAK----EERSRKHGVVVWIPSNLEKLIVTA          776
Brassica_GORK           ASEGLFLMAKMLVEAGASVVAKDRWGNSPLDEARMCGNKKLIKLLEDADTSQPYIRPSSFHE-------PQ-DEKFERRKCTVFPFHPHE-------EPSRKHGVMVWLPRDLQKLVETA          772
Zingiber GORK           ASVGFYAIAKMLSEAGASVFSVDRWGNTPLDEAMKCGSESMVMLLEDAKSQELSKFPERAQE-------LQ-EKMQSRRRCTVFPYHPRDL-----IESR-KEGVMLWIPHTLEELINSS          798
Elaeis_GORK             ASEGLYFIAKLLLEAGASVFAMDRWGATPLDEGRKSGNQSLMLLLEGAKSDELSKFPEHARE-------VQ-DKMHP-RRCTVFPFHPWDP-----KEAR-REGVVLWIPCTIEELIRSS          767
Phoenix GORK            AAEGLYFIAKLLLDAGASVFAMDRWGATPLDEARKSGNKSLMLLLEGAKSDELSKFPERARE-------VQ-DKMHP-RRCTVFPFHPWDS-----KEER-REGVMLWIPCTMEKLIRSS          770
Oryza_GORK              AAEGLYLMAKLLVDAGASVFATDRWGTTPLDEGRRCGSRTMVQLLEAAKSGELSRYPERGEE-------VR-DKMHP-RRCSVFPHHPWDG-----GERR-REGVVVWIPHTIEGLVSSA          806
Brachypodium GORK       AAEGLYMMAKLLVEAGASVFATDRWGTTPLDEGRKSGSKPLMMLLEQAKAEELSKFPARSEE-------VR-DKMHP-RRCSVFPNHPWDT-----DGKR-KEGVTLWIPHTIDWLIRSA          788
Panicum_GORK            AAEGLYLIAQMLVEAGASVFATDRWGTTPLDEARKCGGRTLLALLEQARAEELSKFPERGDE-------VR-DKMHP-RRCSVFPYHPWRAAAGTGAGRR-KEGVVLWIPHTIEGLVASA          795
Papaver GORK            VSQGSYMIAKVLIEGGASVLTRDRWGNTPVDEGRTSGNKKLIKLLEEAESAELSTASTSGSAIDNTSELQVTNKANP-RKCTVFPFHPWDP-----KEKR-KEGVVLWVPPSVDELISMA          804
Amborella_GORK          AAEGLYSLAKNLLEAGASVFSKDRWGNTPLDEGHRSGSKLVINLLEEAKIDQLSRYPNYSQE-------IQ-GKIEA-KKCTVFPFHPWGP-----REER-KEGVVMWIPPTLDQLLSSS          776
Vulgaris GORK           CSQGLYLMAKVLVEAGAYVTLKDRWGNTALDEAWMCGNKNLIKLLETAKSAQLSQSSGNIEE-------LS-DKKLQ-KKCTVFPFNPWGS-----KENR-RPGIVLWVPRTIDDLAKLA          803
Chenopodium_GORK        CSQGLYLMAKLLVEAGAYVTLKDRWGNTPLDEAWMCGNKNLIKLLEAAKAAQLAQSSDHIEE-------ML-DKKLH-KKCTVFPFHPWGT-----KENR-KPGIVLWLPRTIDELIRLA          802
Lactuca GORK            ASQGSYIIVKLLVEAGASVLSKDRWGNTPLDEGRMSGNKMLIKLLEEAKDVQLSELPEGSQE-------IT-DKMHP-RKCTVYAFHPWEE-----KDKI-TCGVVLWIPRTTEQLIKTA          793
Daucus_GORK             ASQGLYLLAKLLLESGASVISRDRWGNTPLDEGRMSGNKNLIKLLEIAKSSQLAEISNQSPE-------IT-DKPQP-KKCRVYPFHPDKP-----KENKPSYGVMLWVPETMEELIKKA          800
Medicago GORK           ASEGLIFMAKLLLEAGASVFTKDRWGNTPLDEARMSGNKNLIKLLEDAKSAQLTEFPF-PQE-------IT-DKVHP-KKCTVFPFHPWDP-----KEQR-RNGIVLWIPHTIQELIITA          796
Prosopis_GORK           AAEGLFLMAKLLIEAGASVFIQDRWGNTPLDEARMCGNKNLIELLEDARSAQLSRFPHLSSE-------LT-VKLHP-KKCTVFPFHPWGP-----KEHR-RHGIVLWVPHTIEELIETA          805
Nicotiana GORK          ASQGLYSMAKLLLGAGASVFTKDRWGNTPVDEARVSGNKQMIGLLEEAKSAQLSEFPDVPHE-------IS-EKLRP-RKCTVFPFHPWES-----KDVR-KHGVVMWIPQTIEELVTTA          781
Solanum_GORK            ASQGQYSMAKLLLGAGASVFSKDRWGNTPVDEARVSGNKQMISLLEEAKSAQLCEFPDVPHE-------IS-DKLRP-RKCTVLPFHPWES-----KDLR-KHGVVLWIPQTIEELVTTA          789
Coffea GORK             ASQGQYFMAKLLLDAGASVFARDRWGNTPLDEGRISGNKNMMDLLEEAESAQSSELSDLSQE-------STADKMLR-RKCTVFPFHPWEP-----KDAR-KCGVVLWVPDSIEELIREA          787
Sesamum_GORK            ASQGLYLMAKLLVEAGASVFSKDRWGNTPLDEGRLCGNKNMIRLLEEAKKAQLSEYPECSQE-------VT-DRKHT-KKCTVFPFHPWDP-----KEGR-KHGIVMWVPNTIDELIKAA          795
Cucumis GORK            VSEGLTLMAKLLLESGASVFSKDRWGNTPLDEGRICGNKNMLKLLEEAKASQLSESPYSSRE-------FT-DKKPT-KKCTVFPFHPWDP-----EENK-RPGIMLWVPLTIEELIKES          789
Vitis_GORK              ASEGLYFMAKLLLEARASVFSKDRWGNTPLDEGWKCGNKNLMKLLEDAKVAQLSEFPDCSRE-------IT-DKMHP-RKCTVFPFHPWDP-----KEHK-RPGIMLWVPQTIEELIKTA          780
Cannabis GORK           ASEGLYLMAKLLIEAGANVFSKDRWGNTPLDEGRMCGNKNLIKLLEDAKTAQLLDFPNSGRE-------IT-EKIHR-RKCTVFPFHPWDP-----KELR-RPGIVLWVPNSIEQLINTA          800
Gossypium_GORK          ASEGLYIMAKLLIEAGASVFSKDRWGNTPLDEARMCGNKNLIKLLEDAKSTQLSELPHCSKE-------FT-DKIHP-KKCTVFPFHPWDA-----KDQR-RHGIVLWVPHTMEALVTTA          791
                         : *   :.: *  . * *   **** : :**.  .*.. :: *** .   :                      .    ::* *   :*               *: :*:*   : *   :

Arabidopsis GORK        AKELGLSDG-ASFVLLSEDQGRITDIDMISDGHKLYMISDTTDQT-------      820
Brassica_GORK           AQELGISNE-VPFVILSEEGGRITDIDMISDGQKLYLISDSTDQST------      817
Zingiber GORK           QELLNCSVS----RLLSEEGGRILPAGLITDNLAPSVHAQNTTSTSDKELN-      845
Elaeis_GORK             REQLKSSGS----CILSEDGGRILDVDMISDGQKLYLVADQETTAHNNQHT-      814
Phoenix GORK            REQLKSSGS----RILSEDGARILDVDMISDGQKLYLVADQETSTHNNQHT-      817
Oryza_GORK              QEKLGLAGSGEGLRLLGEDGARVLDVDMVHDGQKLYLVVGGGGDDGGTEARQ      858
Brachypodium GORK       QEKLGLSGS--CLRLLGEDGARVQDVDMVNDGQKLYLVGDEDVGRSE-----      833
Panicum_GORK            QEKLGVPGPGARLRLLCEDGARVLDVDMVNDGQKLYLVGGDDDDQEDGE---      844
Papaver GORK            SEHLQFYD--GS-RILSEDGGKILYIEMIHDGQKLHLV----------         839
Amborella_GORK          SELLGCF---CT-CILSEDAGKILSVDMISDGQKLYAISDDSHIRDSL----      820
Vulgaris GORK           SEKLNFPS--GS-CILSDDGGKILDIDMIENGQKLYLISETE----------      842
Chenopodium_GORK        SEKLNFLT--GS-CILSEDGGKILDVDMIENGQKLYLISETE----------      841
Lactuca GORK            AEQLKLENYDTC-CIVTEDAGKILDVDMILDGQKLYLISEE----------      833
Daucus_GORK             AEQLNCPQ--NS-CILSEDAAKILDADMISDGQKLYLIDETQ----------      839
Medicago GORK           AEQIGFSS--DA-CILSEDAGKIIDISMIKDDQKLYLVNETH----------      835
Prosopis_GORK           AEQLNVSS--DC-SILSEDAGKITDANLIMDGQKLHLVHETH----------      844
Nicotiana GORK          SEKLNFPS-D-S-CILSEDAGKILDIDMIADGQKLYLINDQST---------      821
Solanum GORK            SEQLDFPS-GTS-CILSEDAGKILDVDMIVDGQKLYLINEST----------      829
Coffea GORK             SKQLNFAS--CT-CILSEDAGKIIDVDMISNNQKLYLMNETD----------      826
Sesamum GORK            SDQLESPN--GS-CILSEDGGKIFDVDMITDGQRLYMINETD----------      834
Cucumis GORK            SEQLQVSG--EC-CILSEDGGKILDVHMIDESQKLYLVPDTH----------      828
Vitis GORK              TEGLQFSS--ES-CILSEDGGKILDVDMISDGQKLYLLCETLDI--------      821
Cannabis GORK           ADQLEISS--AA-CILSEDGGKILEEDMINDGQKLYLVGDTYSA--------      841
Gossypium GORK          AEQLDLAG--AS-CMLTENGGKILDVDMINDGEKLYLIGGTH----------      830
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The cryo-EM structure of *At*GORK is used to restrict sequence gaps to inter-helical segments, with the superior coils and arrows defining extents of the secondary elements. The sequences include representative members from dicots and monocots: Arabidopsis thaliana (NP_198566.2), Brassica rapa (XP_009102317.1), Zingiber officinale (XP_042469382.1), Elaeis guineensis (XP_010905454.2), Phoenix dactylifera (XP_008795354.1), Oryza sativa Japonica Group (NP_001408448.1), Brachypodium distachyon (XP_003560852.1), Panicum virgatum (XP_039805885.1), Papaver somniferum (XP_026377173.1), Amborella trichopoda (XP_011629401.1), Beta vulgaris subsp. vulgaris (XP_010693307.2), Chenopodium quinoa (XP_021754949.1), Lactuca sativa (XP_023769988.1), Daucus carota subsp. sativus (XP_017218838.1), Medicago truncatula (XP_003616247.2), Prosopis alba (XP_028768743.1), Nicotiana tabacum (XP_016460239.1), Solanum lycopersicum (XP_004250206.1), Coffea arabica (XP_027077850.1), Sesamum indicum (XP_020551503.1), Cucumis sativus (XP_004140369.2), Vitis vinifera (XP_010660282.1), Cannabis sativa (XP_030505721.2), Gossypium hirsutum (XP_016749823.2).

Figure S3. Sequence alignments for the representative GORKs from both dicots and monocots.

Fig.S4

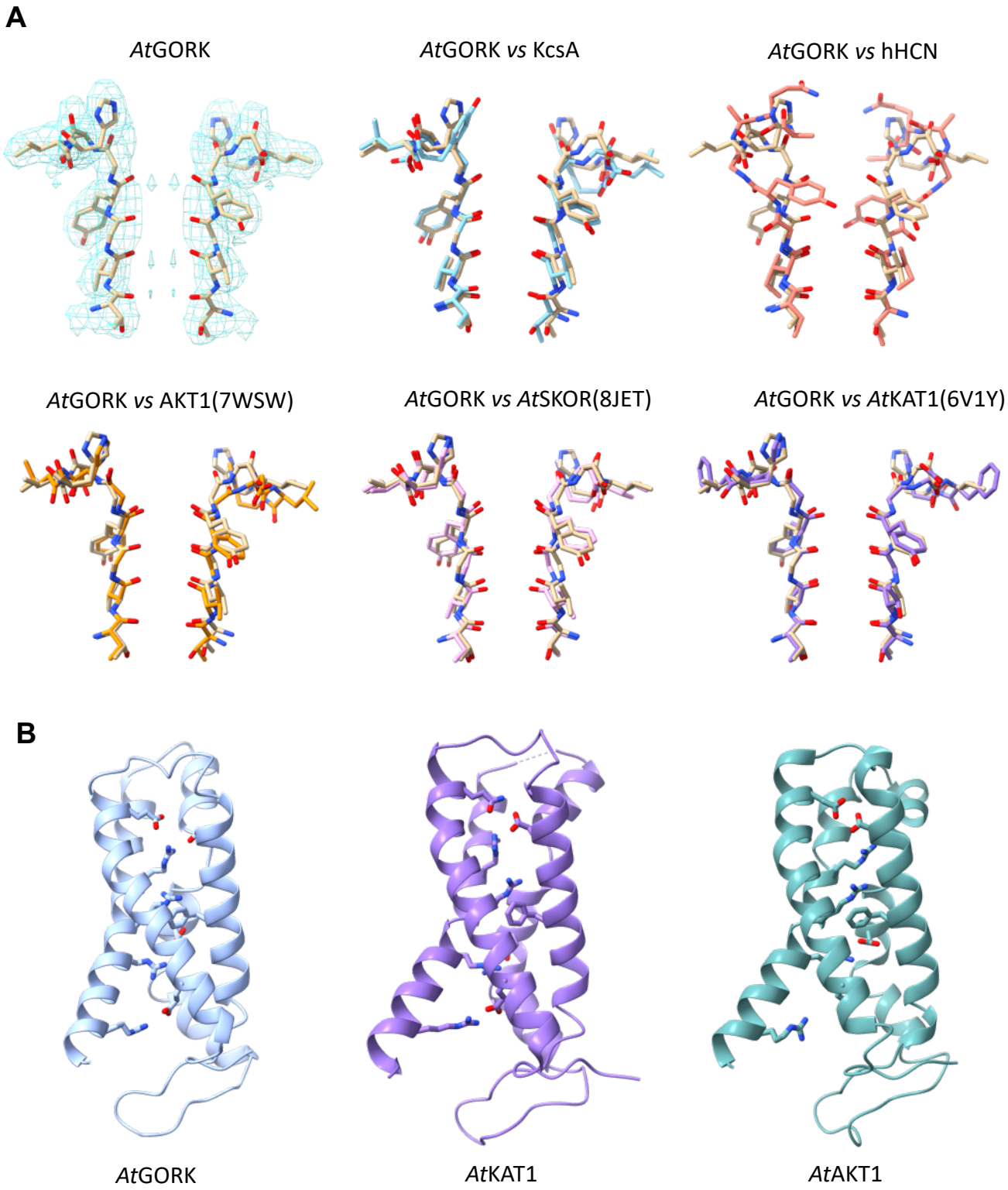

Fig. S4 | Structural comparison of K^+-selectivity filter and VSD of *At*GORK with other K^+ channels

(**A**) Comparison of K^+-selectivity filters from *At*GORK with other K^+ channels. (**B**) Comparisons of voltage sensing domain (S1-S4) from *At*GORK (light-blue), AtKAT1(purple, 6v1y) and AtAKT1 (dark-green, 8wsw). The S4 helix in these three K^+ channel contains conserved positively charged residues, as shown in sticks, and adopt a resting "up" conformation.

Fig.S5

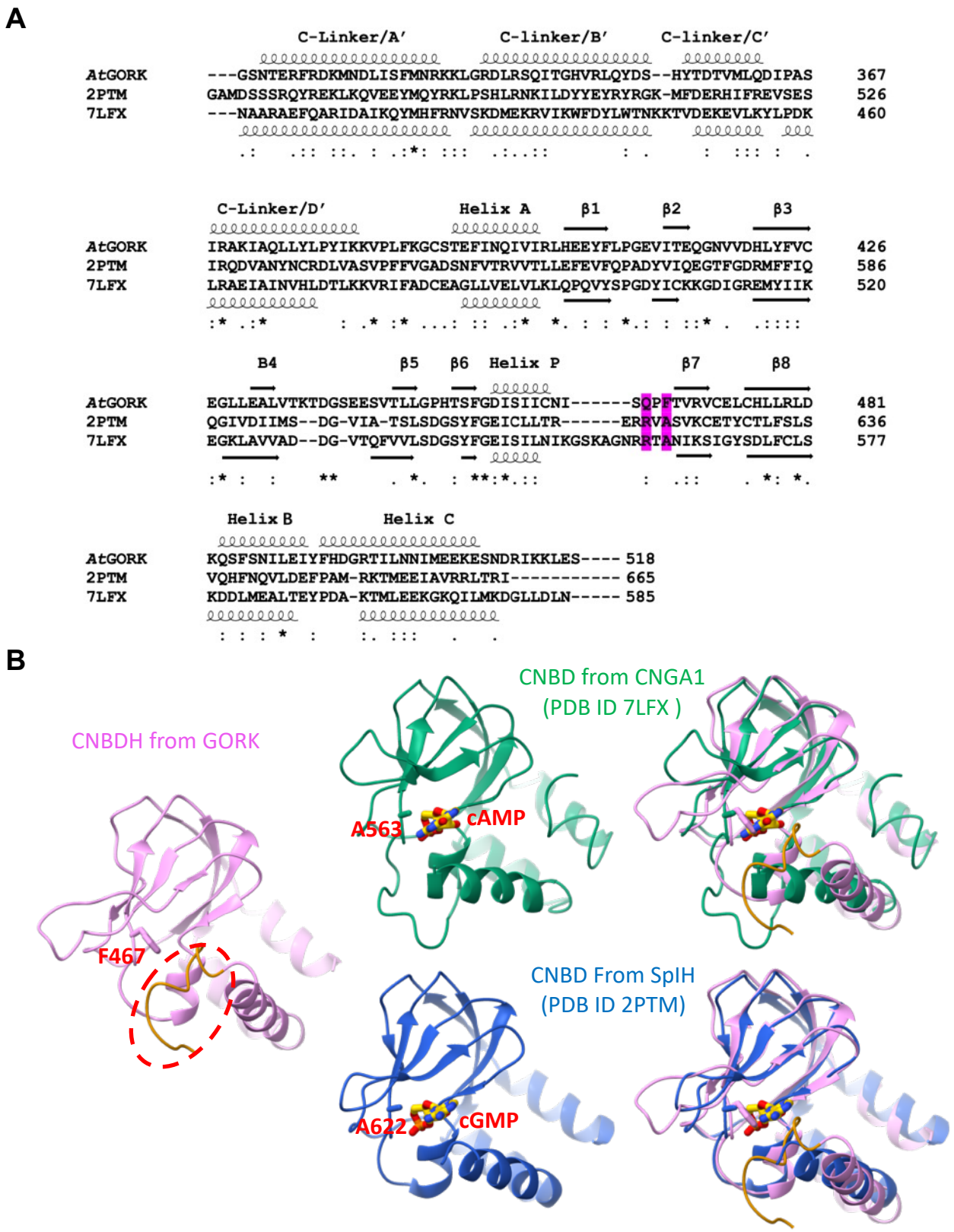

Fig. S5 | Structural comparison of CNBD domain of *At*GORK with other K⁺ channels

(**A**) Structure-based sequence alignment of CNBDH of *At*GORK with CNBD from other K⁺ channels. (**B**) Comparisons of CNBDH of *At*GORK with CNBDs from CNGA1 (7lfx, green) and SpIH (2ptm, blue). A small side-chain residue alanine (A563 in 7lfx, or A622 in 2ptm), located at the entrance of the cAMP/cGMP binding pocket, is replaced by bulky phenylalanine (F467 in *At*GORK), which likely blocks access for the secondary messenger molecules. Helix C adopts a closed conformation, with its adjacent connecting loop (red circle) to the next domain positioned at the entrance of the cAMP/cGMP binding site, indicating that large conformational changes would be required for cAMP/cGMP binding.

Fig.S6

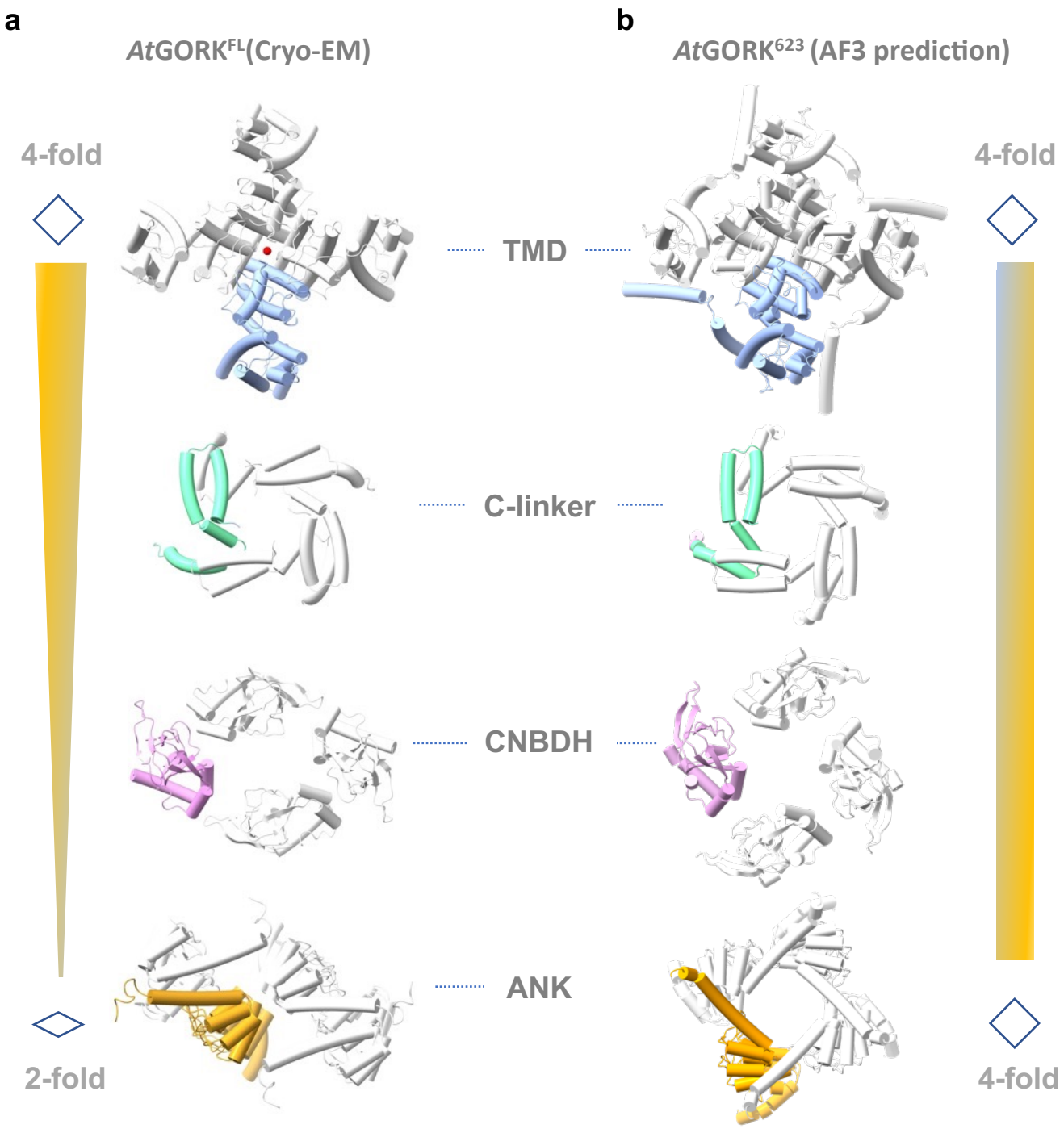

Fig. S6 | Symmetry analysis of domain assembly in *At*GORK^{FL}(Cryo-EM) and *At*GORK^{623}(AF3 prediction)

Table S1: Statistics of data collection, image processing, and model building

Sample	AtGORK^{FL1}	AtGORK^{FL2}	AtGORK^{623}	AtGORK^{510}
PDB	9KHF	8WFZ	9KHE	9KHG
EMDB	62338	37500	62337	62339
Data collection and processing				
Microscope	Titan Krios	Titan Krios	Titan Krios	Titan Krios
Detector	Gatan K3	Gatan K3	Gatan K2	Gatan K3
Magnification	22,500 x	22,500 x	130,000 x	22,500 x
Voltage (kV)	300	300	300	300
Electron exposure (e-/Å^2)	50	50	50	50
Nominal defocus range (μm)	-1.2 to -2.0	-1.2to -2.0	-1.2 to -2.0	-1.2 to -2.0
Frames per movie	32	32	32	32
Pixel size (Å)	1.06	1.06	1.04	1.06
Reconstruction				
Software	cryoSPARC 3.2	cryoSPARC 3.2 & Relion 3.0	cryoSPARC 3.2	cryoSPARC 3.2
Symmetry imposed	C2	C1	C4	C4
Initial particle images (no.)	18,650,563	18,650,563	1,139,470	2,840,921
Final particle images (no.)	156,313	39,551	75,626	141,755
Map resolution (Å)	3.4	4.3	3.2	3.3
Refinement and model validation				
CC (mask)	0.7	0.76	0.82	0.84
CC (box)	0.63	0.66	0.58	0.64
CC (peaks)	0.55	0.45	0.54	0.62
CC (volume)	0.66	0.76	0.81	0.80
RMSD Bond Length (Å)	0.003	0.003	0.003	0.003
RMSD Bond angles (degrees)	0.632	0.701	0.627	0.572
Favored (%)	94.96	96.52	95.47	97.83
Allowed (%)	4.85	3.48	4.53	2.17
Ramachandran plot outliers (%)	0.19	0.00	0.00	0.00
Molprobity score	2.01	1.97	1.91	1.57
Clash score	14.33	17.44	11.95	10.17