Molecular basis for the R-type anion channel

QUAC1 activity in guard cells

Li Qin^{1,2#}, Ling-hui Tang^{1,2#}, Jia-shu Xu^{3,4#}, Xian-hui Zhang^{1,2#}, Yun Zhu^{3,4#}, Chun-rui Zhang^{1,2}, Xue-lei Liu^{1,2}, Mei-hua Wang^{1,2}, Fei Li⁵, Fei Sun^{3,4}, Min Su^{1,2*}, Yu-jia Zhai^{3,4*} & Yu-hang Chen^{1,2*}

¹ State Key Laboratory of Molecular Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China; Innovative Academy of Seed Design, Chinese Academy of Sciences, Beijing 100101, China

² College of Advanced Agricultural Sciences, University of Chinese Academy of Sciences, Beijing 100049, China

³ National Laboratory of Biomacromolecules, CAS Center for Excellence in Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China

⁴ School of Life Sciences, University of Chinese Academy of Sciences, Beijing 100049, China

⁵ Department of Biology, New York University, New York, NY 10003, USA

[#]These authors contributed equally to this work.

*Correspondence to:

Yu-hang Chen, E-mail: yuhang.chen@genetics.ac.cn; or

Yu-jia Zhai, E-mail: yujia@ibp.ac.cn; or

Min Su, E-mail: minsu@genetics.ac.cn.

SUMMARY

The rapid (R)-type anion channel plays a central role in controlling stomatal closure in plant guard cells, thus regulating the exchange of water and photosynthetic gas (CO₂) in response to environmental stimuli. The activity of the R- type anion channel is regulated by malate. However, the molecular basis of the R-type anion channel activity remains elusive. Here, we describe the first cryo-EM structure of the R-type anion channel QUAC1 at 3.5 Å resolution in the presence of malate. The structure reveals that the QUAC1 is a symmetrical dimer, forming a single electropositive T-shaped pore for passing anions across the membrane. The transmembrane and cytoplasmic domains are assembled into a twisted bi-layer architecture, with the associated dimeric interfaces nearly perpendicular. Our structural and functional analyses reveal that QUAC1 functions as an inward rectifying anion channel and suggests a mechanism for malate-mediated channel activation. Altogether, our study uncovers the molecular basis for a novel class of anion channels and provides insights into the gating and modulation of the R-type anion channel.

KEYWORDS

Guard cell, QUAC1, Channel, Cryo-EM, Electrophysiology

INTRODUCTION

Stomatal pore, formed by a pair of guard cells in leaf epidermis, plays a crucial role in regulating CO₂ assimilation and water evaporation in plants^{1,2}. Guard cells can integrate a wide range of environmental stimuli, such as drought, humidity, high CO₂, and ozone, and convert them into appropriate turgor pressure changes that regulate stomatal opening or closure^{3,4}.

It is well-established that anion effluxes are key events to initiate stomatal closure in response to environmental stimuli⁵⁻⁸. Previous studies showed that the guard cells harbour two distinct types of anion channels: rapid (R)-type and slow (S)-type⁹⁻¹². The R-type anion channel can be fully activated and rapidly inactivated within milliseconds, whereas the S-type anion channel takes up to several tens of seconds to be activated, followed by slow inactivation kinetics¹⁰. Recent works of SLow Anion Channel 1 (SLAC1), an S-type anion channel discovered in *Arabidopsis*¹³⁻¹⁵, have uncovered detailed mechanisms underlying S-type anion channel activity¹⁶⁻²¹. However, the molecular basis of the biological role of the R-type anion channel is still largely enigmatic due to the lack of structural information.

QUick Anion Channel 1 (QUAC1), initially named aluminium-activated malate transporter12 (ALMT12), represents a guard cell R-type anion channel in *Arabidopsis*²². The loss-of-function of *quac1* (*almt12*) mutation resulted in impaired stomatal closure^{22,23}. A double mutant lacking SLAC1 and QUAC1 was nearly unresponsive to various stimuli that were expected to cause stomatal closure²⁴. Apart from its role in stomatal regulation, QUAC1 also plays a vital role in cell polarity and the growth of pollen tubes via regulation of apical anion efflux^{25,26}. The activity of QUAC1 was reported to be modulated by malate^{22,27-29}, OST1 phosphorylation³⁰ and calmodulin³¹, but the underlying mechanisms remain elusive.

Here, we report the first cryo-EM structure of soybean QUAC1 from *Glycine max* (*GmQUAC1*, ~67% sequence identity to *Arabidopsis AtQUAC1*) at the resolution of 3.5 Å in the presence of malate. The structure revealed that the QUAC1 is a symmetrical dimer assembled into twisted bi-layer architecture with a central T-shaped pore across the membrane. The pore is lined with highly conserved positively charged residues, rendering the pore surface electropositive, consistent with the QUAC1 function as an anion channel. Further

electrophysiological analyses reveal that the QUAC1 displays rapid kinetics in channel gating during activation and inactivation.

Our truncation study reveals that the N-terminal juxtamembrane helix is crucial in maintaining structural integrity for proper channel function. Further mutagenesis analyses demonstrate that the C-terminal interaction locks the channel in a high-energy state, and that a domain-swapped helix plays an inhibitory role in channel regulation. Two pore-lining W90 residues, close to the lateral fenestration within the transmembrane domain, serve as toggle switches to regulate gating during channel activation. Therefore, the unique architecture endows QUAC1 function as an R-type anion channel, and our structural and functional analyses allow us to propose a novel mechanism for malate-mediated channel activation. Altogether, our study uncovers the molecular basis for a novel class of anion channels and provides insights into the gating and modulation of the R-type anion channel.

RESULTS

Bioinformatics analyses of plant ALMTs/QUACs

QUAC1 belongs to the ALMT family, which has diverse functions in plants, including stomatal function^{22,23,32–34}, pollen tube growth^{25,26}, Al³⁺ resistance^{35–38}, mineral nutrition^{39,40}, fruit acidity^{41,42}, microbe interactions^{43,44}, and seed development^{45,46}. There are thirteen members in the model plant *Arabidopsis thaliana*, except for one short sequence of a partial transmembrane domain (ALMT11). To better understand how QUAC (ALMT) proteins are represented in the plant kingdom, we clustered ~1,700 non-redundant sequences into a superfamily at the PSI-BLAST level $E \leq 5 \times 10^{-3}$, then into two distinct families at a threshold of $E \leq 10^{-160}$, and finally into subfamilies at a threshold of $E \leq 10^{-180}$, as detailed in [Extended Data Table 1](#).

Since more studies have shown that ALMT members function as anion channels rather than transporters, and the QUAC1 is now the best-characterized member, we adopted nomenclature for the QUAC superfamily, which was further divided into families QF1 and QF2. We further found that family QF1 has two subfamilies: QF1A comprises the *Arabidopsis* ALMT3, 4, 5, 6, 9 and their homologs, and QF1B has a distinct set of ALMT proteins. Family

QF2 has three subfamilies: the *Arabidopsis* ALMT1, 2, 7, 8, 10 proteins are in subfamily QF2A, the *Arabidopsis* ALMT12, 13, 14 proteins are in QF2B, and another distinct set of ALMT proteins are in QF2C (Extended Data Fig. 1).

The structure-based sequence alignment for the *GmQUAC1* and thirteen *Arabidopsis* QUAC/ALMTs is shown in Extended Data Fig. 2. Sequence analyses reveal that the ALMTs share relatively conserved transmembrane and cytosolic helical regions. Some conserved positively charged residues are distributed in the transmembrane regions and may be responsible for anion selectivity or voltage sensing. Some fingerprint motifs, including WEP (Trp-Glu-Pro) and PXWXG (Pro-X-Trp-X-Gly), are found in the cytosolic helical region. One of the most divergent regions (~50-100 aa in length) is found between H5 and H6 helices in the cytosolic portions, harbouring potential phosphorylation residues (Ser/Thr) for channel modulation. Further studies will provide mechanistic insight into the regulation of these characteristic motifs or distinct sets of QUACs.

Cryo-EM structural determination of QUAC1

We screened six plant QUAC1s for expression and found that the *GmQUAC1* was suitable for further structural and functional studies (see method for details). Upon solubilization in 1.0% n-dodecyl- β -D-maltopyranoside (DDM) and 0.02% cholesteryl hemisuccinate (CHS), the *GmQUAC1* proteins were purified by Ni²⁺-affinity chromatography, and the resulting peak-fractions were pooled and loaded onto a gel-filtration column for further purification, buffer and detergent exchange. The final solution contained 0.005% detergent LMNG, with either 150 mM NaCl or 75 mM L-malate.

We further determined its cryo-EM structure in the presence of 75 mM L-malate (Extended Data Fig. 3, 4 and Extended Data Table 2). The dimeric reconstruction at 3.5 Å resolution allowed *de novo* modelling of 410 of the 537 amino acids (residues 36-393 and 455-506) per protomer chain. Other regions are not resolved in the density map due to their intrinsic flexibility in the protein. The search in the Dali server⁴⁷ for similar structures returned no significant hit. To the best of our knowledge, the QUAC1 protomer has a unique protein fold and architecture, representing a novel class of ion channels.

The QUAC1 channel forms a twisted bi-layer architecture

The QUAC1 channel is a flat vase-shaped homodimer with a two-fold axis perpendicular to the plasma membrane (Fig. 1a and Supplementary Video 1). The overall molecule forms a bi-layer architecture, divided into two portions of the transmembrane domain (TMD) and the cytoplasmic helical domain (CHD). The domain topology for each protomer is shown in Fig. 1b. The TMD comprises 6 TM helices, arranging as three pairs of V-shaped helical hairpins stacked against each other, whereas the CHD has 7 helices, forming a helical bundle with a short domain-swapped finger helix. The N-terminal pre-TM region contains a juxtamembrane helix bent at a conserved proline before the TMD. A disorder region between helix H5 and H6 in the CHD enriches with Ser/Thr residues (Extended Data Fig. 2), raising an intriguing possibility of containing phosphorylation sites for channel regulation.

The dimer formation is mediated by interactions from both TMDs and CHDs, which bury surface areas of $\sim 3,500 \text{ \AA}^2$ and $\sim 3,300 \text{ \AA}^2$, respectively. When viewed from the extracellular side, the TM helices trace the circumference of an ellipse with TM1-TM6 from one protomer followed by another set of TM1-TM6 from the second protomer in an antiparallel manner (Fig. 1c). At the membrane, TM1 and TM2 directly interact with TM4 and TM5 from another protomer, respectively. In the cytoplasm, the CHD of each protomer unites together at the distal end of the molecule, mediated by intensive interactions. A domain-swapped finger helix (H6) also participates in C-terminal interactions (Fig. 1d). However, the functional role of these interactions remains unclear.

In the bi-layer structure of QUAC1, the TMDs connect to the CHDs via a highly conserved PXWXG motif (Pro-X-Trp-X-Gly). They interact with each other in a twisted manner, with their dimeric interfaces nearly perpendicular to each other (forming a dihedral angle of $\sim 83^\circ$) (Fig. 2a). Interestingly, another characteristic motif WEP (Trp-Glu-Pro), located in the connecting loop between helix H2 and H3, is found in the vicinity of the PXWXG motif at the layer interface between TMD and CHD. These two motifs, together with other surrounding charged residues (Arg/Lys: R56, K63, R291, and R294; Asp/Glu: D54, E223, D224, and E289),

form intensive interactions at the layer interface, and thus may play a vital role in coupling channel regulation to gating (Fig. 2b). Mutations in the WEP motif (E286Q in *TaALMT1* and E276Q in *AtQUAC1*)^{27,48} completely abolish their channel activities, possibly due to impaired coupling between the TMD and CHD caused by the altered interaction at the layer interface. Thus, the unique architecture design causes the channel in a high-energy state, providing a structural basis for QUAC1 function as an R-type channel. These observations also imply that the intramolecular domain rearrangements may occur upon channel activation.

Structural features of the ion conduction pathway in QUAC1

Unlike the trimeric SLAC1, which has three independent pores²⁰, the dimeric QUAC1 forms a single T-shaped tunnel with a bifurcated entrance in the cytoplasm (Fig. 3a). The TM2, TM3, TM5, and TM6 from both protomers create a pore with a radius of ~4-6 Å within the membrane (Fig. 3b), as estimated by HOLE⁴⁹. Overall, the QUAC1 pore is lined with highly conserved and general hydrophobic residues (Fig. 3c). Nevertheless, some highly conserved positively charged residues are distributed along the pore, thus rendering the surface electropositive (Fig. 3d).

Two arginines (R187 and R198) form a positively charged ring facing the pore entrance at the extracellular side. Three positively charged residues from TM3 and TM4 (K109, R113, and R158) protrude into the pore, making a second positively charged ring within the membrane. They are associated with other conserved residues from TM2 and TM5 (E100, D168, and Y169), mediating an inter-helices network within the pore. Another two lysines (K164 and K165) form additional constrictions at the bottom of the T-shaped pore (Fig. 3e). Altogether, the structural feature of QUAC1 likely contributes to its function as an anion-conducting channel. The above findings suggest that permeable anions interact with the pore-lining charged residues, thus participating in channel regulation.

Remarkably, a central kink (~24°) in TM6 causes a lateral fenestration from the membrane to the lumen of the channel pore within each protomer (Fig. 4a). The fenestration measures ~6 Å × 20 Å, and its dimension depends upon the conformation of pore-forming TMs, especially

the kinked TM6. The presence of kinked helix and lateral fenestration in the structure implies that the channel in 75 mM malate is in a constrained conformation. Interestingly, several unmodeled densities and a highly conserved pore-lining W90 residue are found within or near the fenestration (Fig. 4b), raising an interesting question if the bulky W90 residue serves as a toggle switch to regulate channel gating during QUAC1 activation.

Functional characterization of QUAC1 by TEVC

A total of 34 ALMTs were found in soybean⁵⁰, but none of them has yet been functionally characterized by electrophysiology. We expressed the *GmQUAC1*/ALMT12 in *Xenopus laevis* oocytes and measured channel conductance by applying the two-electrode voltage-clamp (TEVC) technique. In the recordings, the application of voltage pulses elicited rapidly activating currents in the bath solution of malate, resembling the *AtQUAC1*²². We also found that the steady-state currents mediated by *GmQUAC1* displayed a bell-shaped current-voltage curve (Extended Data Fig. 5). This electrophysiological behaviour points to a strong voltage dependence for the QUAC1 channel activation, a hallmark feature of the R-type anion channel in guard cells^{10,22}.

We also measured the conductance of *GmQUAC1* in other bath solutions. Surprisingly, we found that the activation/inactivation kinetics and voltage-dependency of *GmQUAC1* in NaNO₃ or NaCl solutions are distinguished from those in malate solution (Extended Data Fig. 5). In contrast to substantial instantaneous currents, only small steady-state currents can be seen in the recording of *GmQUAC1* in the bath solution of NaNO₃ or NaCl (Extended Data Fig. 5). Overall, our TEVC recordings demonstrate that external anions play a crucial role in regulating QUAC1 channel properties, especially the activation/inactivation kinetics and steady-state currents. We hypothesize that the anions interplay with the pore-lining residues, such as the inter-helices charged residues interacting network within the pore (Fig. 3e), and act as door stopper wedges to regulate channel gating.

Single-channel analysis of QUAC1 channel in planar lipid bilayer

To further understand the single-channel properties, we first fused the *GmQUAC1*^{NaCl} proteins (purified in 150 mM NaCl) into a lipid bilayer and measured channel conductance under different symmetrical solutions (Fig. 5a). When using symmetrical NaNO₃ solutions, the application of a series of transmembrane voltages to the *trans* chamber resulted in frequent openings and closings of the *GmQUAC1* channel, as evidenced by the current fluctuation in the recording traces at different applied voltages (Extended Data Fig. 6a). We also found that the channel gating transits between two predominant full open and closed states, and sometimes to a sub-conductance that is ~50% level of the full conductance (Fig. 5b). This observation is consistent with the two-entrance T-shaped tunnel presented in the QUAC1 channel (Fig. 3a). At ~50% of the full level, the intermediate sub-conductance suggested one of two entrances was blocked or closed. In addition, the QUAC1 channel gating displays a flickering feature that represents the rapid character of the activation/inactivation kinetics for R-type anion channels.

In contrast, we observed much reduced channel activity in the measurements in the solution of 150 mM NaCl (Extended Data Fig. 6b). The mean single-channel conductance for the *GmQUAC1*^{NaCl} is 75.5 ± 4.5 pS (n=5) and 58.5 ± 7.0 pS (n=5) in the solutions of NaNO₃ and NaCl, respectively (Fig. 5c). Intriguingly, the channel open probability displays a strong voltage dependency, much higher at positive voltage than at negative one (Fig. 5d). As in our experimental setup, positive currents mean anions flow from the *cis* (representing cytoplasm) to the *trans*-chamber (representing extracellular side), and *vice versa*. Therefore, these results demonstrate that the QUAC1 channel functions as an R-type anion channel, favouring efflux (corresponding to anions flow from *cis*- to *trans*-chamber).

Malate regulates QUAC1 channel gating.

To investigate the effect of malate on the channel activity, we reproduced the same experiments with the *GmQUAC1*^{malate} (purified in 75 mM L-malate) as above. Interestingly, we observed that *GmQUAC1*^{malate} exhibits a similar current amplitude but a much higher open probability than those with *GmQUAC1*^{NaCl} in both solutions of NaNO₃ and NaCl (Fig. 5c and Extended

Data Fig. 6). The mean single-channel conductance for the *GmQUAC1*^{malate} is 88.5 ± 2.4 pS (n=4) and 62.7 ± 4.1 pS (n=4) in the solutions of NaNO₃ and NaCl, respectively. The open probabilities of *GmQUAC1*^{malate} also display a similar voltage dependency as observed for *GmQUAC1*^{NaCl} (Figure 5D and S6). Taken together, our results suggest that malate regulates QUAC1 activation by modulating channel opening probability.

To further examine malate regulation on the channel gating, 2 mM L-malate was added to the solutions upon the *GmQUAC1*^{NaCl} fused into a lipid bilayer. Interestingly, the channels became more active after adding 2 mM L-malate to either the *trans*- or *cis*-chamber (Fig. 6a and 6b). The current amplitude slightly increases, whereas the open probability becomes much higher (Fig. 6c and 6d). These observations confirm that malate can stimulate channel activity via enhanced open probability. Our findings also suggest that the malate regulation site may be located within the pore and can be accessed from either the extracellular or intracellular side of the membrane. We speculate that the *GmQUAC1* structure in the presence of 75 mM L-malate may represent an open state, although the malate-binding site remained unclear due to insufficient information.

The N-terminal pre-TM helix is required for channel activity.

The sequence analysis shows that the N-terminal segment before TMD is one of the most divergent regions in the QUAC family (Extended Data Fig. 2). The QUAC1 structure reveals that this region contains a juxtamembrane pre-TM helix (Fig. 1b), enriching positively charged residues. To examine its functional role, we designed a truncation construct ($\Delta 1-53$). Surprisingly, we found that the $\Delta 1-53$ mutant resulted in nearly null currents in the TEVC recordings (Extended Data Fig. 7). The observation demonstrates that the pre-TM helix is indispensable for channel activity. Considering its association with the inner leaflet of the membrane, we propose that the pre-TM helix may serve as a lever to regulate the proper conformation of the QUAC1 channel.

The C-terminal dimeric interaction is crucial for channel function.

The cytosolic C-terminal portion is mainly formed by α -helices, except that a large divergent region between helix H5 and H6 is disordered or missing in the QUAC family (Extended Data Fig. 2). The *GmQUAC1* structure revealed extensive interactions between two CHDs at the distal end of the dimer (Fig. 7a). Two highly conserved hydrophobic residues (F470 and L474 from helix H7) protrude into the hydrophobic pocket formed by another protomer. Together with their antiparallel counterparts from another protomer, these bulky hydrophobic residues form a zipper-like interaction at the dimeric interface (Fig. 7b). In addition, a short finger helix (H6), whose sequence is conserved in the subfamily QF2B (Extended Data Fig. 2), also participates in the dimeric interaction in a domain-swapped manner (Fig. 7c). These interactions unite the two CHDs together and thus creates an anchor point for mechanical force transduction between TMD and CHD.

To investigate the functional role of the dimeric interactions in channel regulation, we generated mutations, aiming to disrupt the dimer-interface (A477E and F470A/L474A), or to break the finger helix interaction in H6 (Δ 460-467 and S461D), in both the CHD (residues 221-537) bacteria-expression constructs for gel-filtration analysis and the full-length oocyte-expression constructs for conductance measurement. In contrast to the wild type, both dimer-interface disrupting mutants exhibited backwards-shifted elution peaks (from 9.2 ml to 10.2 ml) in the gel filtration profile and showed almost null currents in TEVC recording. In another experiment, the two altered helix H6 mutants cause loosened dimers but unexpectedly enhanced channel activities (Fig. 7d-f and Extended Data Fig. 8). Altogether, our results show that the C-terminal interaction has two important functions: (1) joins the two CHD together and locks the channel in a high-energy state; (2) a domain-swapped helix (H6) plays an inhibitory role, and its release enhances the channel activity. Our results demonstrated that the C-terminal interaction provides a basis for fast regulation of the R-type anion channel.

Gating of the QUAC1 channel

Our electrophysiological experiments have demonstrated that malate plays a vital role in modulating *GmQUAC1* activity; however, we wonder how malate regulation is coupled to channel activation. The *GmQUAC1* structure reveals that the conserved pore-lining tryptophan W90 is in close contact with an unmodelled density within the fenestration (Fig. 4b). To understand the functional role of W90, we made mutations of W90A and W90F. Compared to wild-type, the removal of indole ring in W90A mutant causes ~5 times larger conductance in the presence of external malate. A similar aromatic amino acid substitution in the W90F mutant can stimulate channel currents even larger (Extended Data Fig. 9a and 9b). These observations imply that the conserved W90 may interact with surrounding residues and function as a toggle switch to regulate channel gating.

This notion is confirmed in the measurement of combined mutants of W90F with other two loss-of-function mutants, Δ 1-53 and A477E. We observed that both null mutants restore channel activity to a moderate level after the addition of W90F (Extended Data Fig. 9c and 9d). We speculate that malate promotes QUAC1 activation probably via regulating the conformation of tryptophan W90. Distinct from the S-type SLAC1 regulation controlled by phosphorylation/ dephosphorylation¹⁶⁻²⁰, our study reveals that the R-type QUAC1 gating can be directly mediated by malate, which is much faster. These findings may explain the fast kinetics observed in the R-type channel, compared with the slow activity found in the S-type channel.

DISCUSSION

Recently, AlphaFold has made significant progress in 3D structure prediction from protein sequence⁵¹. By taking advantage of this advanced technology, we generated an AlphaFold predicted model for the *GmQUAC1* protein that is the focus of our current study. Surprisingly, the AlphaFold predicted *GmQUAC1* structure (AF-structure) is surprisingly similar to that determined by cryo-EM (cryo-EM structure), with an r.m.s.d. of 2.1 Å/160 C $_{\alpha}$ and 1.4 Å/207 C $_{\alpha}$ for the TMD and CHD, respectively. However, further superimposition reveals a noticeable

difference in the orientation of TMD vs CHD in QUAC1, with an r.m.s.d. of 3.1 Å/388 C α and 3.2 Å/776 C α for the monomer and dimer, respectively (Extended Data Fig. 10a).

Unlike the kinked TM6 observed in the cryo-EM structure, the TM6 in the AF-structure adopts a straight conformation, eliminating the lateral fenestration in TMD (Extended Data Fig. 10b). As the malate regulation effect on the channel is not taken into account, the resulting predicted model may represent an apo-structure in a low energy state. The cryo-EM structure determined in the presence of malate reveals a high-energy twisted conformation, thus representing a malate-activated structure. These two structures allow us to make comparisons and gain insight into the gating mechanism of the QUAC1 channel. It seems that the pore-forming helices move slightly outward, as suggested by the porcupine plot on the C α comparison between the AF-structure and the cryo-EM structure (Extended Data Fig. 10c). Another noticeable difference is the dihedral angle between the TMD and CHD interfaces, which is 86.5° and 83.0° for the AF-structure and cryo-EM structure, respectively (Extended Data Fig. 10d). This observation shows a slight rotation (~3.5°) between these two states, suggesting that domain reorganization occurs during the conformational conversion. We speculate that the domain reorganization is associated with malate regulation, and subsequently triggers conformational changes of the toggle switch in the pore to activate the channel (Extended Data Fig. 10e and Supplementary Video 1).

Our work provides the first glimpse of the molecular structures of the R-type anion channel. Our study showed that the TMD and CHD portions in QUAC1 interact in a twisted manner, rendering the channel in a high-energy state. Based on our data, we propose the following mechanism for QUAC1 activation: upon malate binding, the QUAC1 undergoes intramolecular domain reorganization to form a twisted bi-layer architecture, which subsequently induces the conformational change of the toggle switch W90. These changes promote the channel conversion from the basal to the activated state. The malate-mediated activation is further enhanced by other modulations, such as kinase phosphorylation or calmodulin-binding, probably via releasing its inhibitory domain-swapped helix from the inter-protomer interaction (Fig. 7g and Supplementary Video 2). Our study provides mechanistic insights into the gating

activation of the R-type channel and offers a plausible explanation for fast kinetics in the R-type currents.

As both SLAC1 and QUAC1 represent two essential distinct channels that mediate osmotic active anions efflux from guard cell, one remaining puzzle is how they interplay with each other to initiate stomatal closure in response to various environmental stimuli. Further investigation to address this issue will shed light on the understanding of guard cell stomatal signaling and provide critical information for engineering drought-resistant or water-use efficiency crops.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at XXX

ACKNOWLEDGMENTS

We thank the staff at the Center for Biological Imaging (CBI), Institute of Biophysics, Chinese Academy of Sciences. This project is financially supported by the National Key Research and Development Program of China (2020YFA0509903 and 2016YFA0500503 to Y.-h.C., and 2017YFA0504703 to Y.-j.Z.), the Strategic Priority Research Program of the Chinese Academy of Sciences (XDA24020305 to Y.-h.C., and XDB37040102 to F.S.), and the National Natural Science Foundation of China (31872721 to Y.-h.C., and 31771566 to Y.-j.Z.).

DECLARATION OF INTERESTS

The authors declare no competing financial interests.

AUTHOR CONTRIBUTIONS

L.Q. performed protein purification, Cryo-EM data collection, TEVC experiments, and data analysis; L.-h.T., J.-s.X., Y.Z. performed Cryo-EM data collection and structural determination; X.-h.Z. performed mutagenesis and protein purification; M.S. performed PLB experiments and data analysis; C.-r.Z., X.-l.L., M.-h.W. performed experiments; F.L., F.S., Y.-j.Z. analyzed data; Y.-h.C. initiated the project, planned and analyzed experiments, supervised the research and wrote the manuscript with input from all authors.

REFERENCES

1. Hetherington, A & Woodward, F. The role of stomata in sensing and driving environmental change. *Nature* **424**, 901–908 (2003).
2. Sirichandra, C., Wasilewska, A., Vlad, F., Valon, C. & Leung, J. The guard cell as a single-cell model towards understanding drought tolerance and abscisic acid action. *J. Exp. Bot.* **60**, 1439–1463 (2009).
3. Kim, T.-H., Böhmer, M., Hu, H., Nishimura, N. & Schroeder, J. I. Guard cell signal transduction network: advances in understanding abscisic acid, CO₂, and Ca²⁺ signaling. *Annu. Rev. Plant Biol.* **61**, 561–591 (2010).

- 401 4. Murata, Y., Mori, I. C. & Munemasa, S. Diverse stomatal signaling and the signal integration mechanism.
402 *Annu. Rev. Plant Biol.* **66**, 369–392 (2015).
- 403 5. Kollist, H., Nuhkat, M. & Roelfsema, M. R. G. Closing gaps: linking elements that control stomatal
404 movement. *New Phytol.* **203**, 44–62 (2014).
- 405 6. Roelfsema, M. R. G., Hedrich, R. & Geiger, D. Anion channels: master switches of stress responses.
406 *Trends Plant Sci.* **17**, 221–229 (2012).
- 407 7. Pantoja, O. Recent advances in the physiology of ion channels in plants. *Annu. Rev. Plant Biol.* **72**, 463–
408 495 (2021).
- 409 8. Hedrich, R. Ion channels in plants. *Physiol. Rev.* **92**, 1777–1811 (2012).
- 410 9. Keller, B. U., Hedrich, R. & Raschke, K. Voltage-dependent anion channels in the plasma membrane of
411 guard cells. *Nature* **341**, 450–453 (1989).
- 412 10. Schroeder, J. I. & Keller, B. U. Two types of anion channel currents in guard cells with distinct voltage
413 regulation. *Proc. Natl. Acad. Sci. USA* **89**, 5025–5029 (1992).
- 414 11. Schroeder, J. I. *et al.* Cytosolic calcium regulates ion channels in the plasma membrane of *Vicia faba*
415 guard cells. *Nature* **338**, 427–430 (1989).
- 416 12. Hedrich, R., Busch, H. & Raschke, K. Ca²⁺ and nucleotide dependent regulation of voltage dependent
417 anion channels in the plasma membrane of guard cells. *EMBO J.* **9**, 3889–3892 (1990).
- 418 13. Vahisalu, T. *et al.* SLAC1 is required for plant guard cell S-type anion channel function in stomatal
419 signalling. *Nature* **452**, 487–491 (2008).
- 420 14. Negi, J. *et al.* CO₂ regulator SLAC1 and its homologues are essential for anion homeostasis in plant cells.
421 *Nature* **452**, 483–486 (2008).
- 422 15. Saji, S. *et al.* Disruption of a gene encoding C4-dicarboxylate transporter-like protein increases ozone
423 sensitivity through deregulation of the stomatal response in *Arabidopsis thaliana*. *Plant Cell Physiol.* **49**,
424 2–10 (2008).
- 425 16. Geiger, D. *et al.* Activity of guard cell anion channel SLAC1 is controlled by drought-stress signaling
426 kinase-phosphatase pair. *Proc. Natl. Acad. Sci. USA* **106**, 21425–21430 (2009).
- 427 17. Lee, S. C., Lan, W., Buchanan, B. B. & Luan, S. A protein kinase-phosphatase pair interacts with an ion
428 channel to regulate ABA signaling in plant guard cells. *Proc. Natl. Acad. Sci. USA* **106**, 21419–21424

- 429 (2009).
- 430 18. Brandt, B. *et al.* Reconstitution of abscisic acid activation of SLAC1 anion channel by CPK6 and OST1
431 kinases and branched ABI1 PP2C phosphatase action. *Proc. Natl. Acad. Sci. USA* **109**, 10593–10598
432 (2012).
- 433 19. Maierhofer, T. *et al.* Site-and kinase-specific phosphorylation-mediated activation of SLAC1, a guard cell
434 anion channel stimulated by abscisic acid. *Sci. Signal.* **7**, 1–12 (2014).
- 435 20. Deng, Y. N. *et al.* Structure and activity of SLAC1 channels for stomatal signaling in leaves. *Proc. Natl.*
436 *Acad. Sci. USA* **118**, 1–8 (2021).
- 437 21. Chen, Y.-H. *et al.* Homologue structure of the SLAC1 anion channel for closing stomata in leaves. *Nature*
438 **467**, 1074–1080 (2010).
- 439 22. Meyer, S. *et al.* AtALMT12 represents an R-type anion channel required for stomatal movement in
440 *Arabidopsis* guard cells. *Plant J.* **63**, 1054–1062 (2010).
- 441 23. Sasaki, T. *et al.* Closing plant stomata requires a homolog of an aluminum-activated malate transporter.
442 *Plant Cell Physiol.* **51**, 354–365 (2010).
- 443 24. Jalakas, P. *et al.* Combined action of guard cell plasma membrane rapid- and slow-type anion channels in
444 stomatal regulation. *Plant Physiol.* (2021) doi:10.1093/plphys/kiab202.
- 445 25. Gutermuth, T. *et al.* Tip-localized Ca²⁺-permeable channels control pollen tube growth via kinase-
446 dependent R- and S-type anion channel regulation. *New Phytol.* **218**, 1089–1105 (2018).
- 447 26. Herbell, S., Gutermuth, T. & Konrad, K. R. An interconnection between tip-focused Ca²⁺ and anion
448 homeostasis controls pollen tube growth. *Plant Signal. Behav.* **13**, 1559–2324 (2018).
- 449 27. Mumm, P. *et al.* C-terminus-mediated voltage gating of arabidopsis guard cell anion channel QUAC1.
450 *Mol. Plant* **6**, 1550–1563 (2013).
- 451 28. Hedrich, R. *et al.* Malate-sensitive anion channels enable guard cells to sense changes in the ambient CO₂
452 concentration. *Plant J.* **6**, 741–748 (1994).
- 453 29. Raschke, K. Alternation of the slow with the quick anion conductance in whole guard cells effected by
454 external malate. *Planta* **217**, 651–657 (2003).
- 455 30. Imes, D. *et al.* Open stomata 1 (OST1) kinase controls R-type anion channel QUAC1 in *Arabidopsis*
456 guard cells. *Plant J.* **74**, 372–382 (2013).

- 457 31. Luu, K., Rajagopalan, N., Ching, J. C. H., Loewen, M. C. & Loewen, M. E. the malate-activated
458 ALMT12 anion channel in the grass *Brachypodium distachyon* is co-activated by Ca^{2+} /calmodulin. *J.*
459 *Biol. Chem.* **294**, 6142-6156 (2019).
- 460 32. Meyer, S. *et al.* Malate transport by the vacuolar *AtALMT6* channel in guard cells is subject to multiple
461 regulation. *Plant J.* **67**, 247–257 (2011).
- 462 33. De Angeli, A., Zhang, J., Meyer, S. & Martinoia, E. *AtALMT9* is a malate-activated vacuolar chloride
463 channel required for stomatal opening in *Arabidopsis*. *Nat. Commun.* **4**, 1–10 (2013).
- 464 34. Kovermann, P. *et al.* The *Arabidopsis* vacuolar malate channel is a member of the ALMT family. *Plant J.*
465 **52**, 1169–1180 (2007).
- 466 35. Sasaki, T. *et al.* A wheat gene encoding an aluminum-activated malate transporter. *Plant J.* **37**, 645–653
467 (2004).
- 468 36. Hoekenga, O. A. *et al.* *AtALMT1*, which encodes a malate transporter, is identified as one of several
469 genes critical for aluminum tolerance in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* **103**, 9738–9743 (2006).
- 470 37. Collins, N. C., Shirley, N. J., Saeed, M., Pallotta, M. & Gustafson, J. P. An ALMT1 gene cluster
471 controlling aluminum tolerance at the Alt4 Locus of Rye (*Secale cereale* L.). *Genetics* **179**, 669–682
472 (2008).
- 473 38. Ligaba, A., Katsuhara, M., Ryan, P. R., Shibasaka, M. & Matsumoto, H. The *BnALMT1* and *BnALMT2*
474 genes from rape encode aluminum-activated malate transporters that enhance the aluminum resistance of
475 plant cells. *Plant Physiol.* **142**, 1294-1303 (2006).
- 476 39. Piñeros, M. A. *et al.* Not all ALMT1-type transporters mediate aluminum-activated organic acid
477 responses: The case of *ZmALMT1* - An anion-selective transporter. *Plant J.* **53**, 352–367 (2008).
- 478 40. Ligaba, A., Maron, L., Shaff, J., Kochian, L. & Piñeros, M. Maize *ZmALMT2* is a root anion transporter
479 that mediates constitutive root malate efflux. *Plant, Cell Environ.* **35**, 1185-1200 (2012).
- 480 41. Bai, Y. *et al.* A natural mutation-led truncation in one of the two aluminum-activated malate transporter-
481 like genes at the Ma locus is associated with low fruit acidity in apple. *Mol. Genet. Genomics* **287**, 663–
482 678. (2012).
- 483 42. De Angeli, A. *et al.* The vacuolar channel *VvALMT9* mediates malate and tartrate accumulation in berries
484 of *Vitis vinifera*. *Planta* **238**, 283-291 (2013).

485 43. Rudrappa, T., Czymmek, K. J., Paré, P. W. & Bais, H. P. Root-secreted malic acid recruits beneficial soil
486 bacteria. *Plant Physiol.* **148**, 1547-1556 (2008).

487 44. Takanashi, K. *et al.* A dicarboxylate transporter, *LjALMT4*, mainly expressed in nodules of lotus
488 japonicus. *Mol. Plant-Microbe Interact.* **29**, 584–592 (2016).

489 45. Xu, M. *et al.* The barley anion channel, *HvALMT1*, has multiple roles in guard cell physiology and grain
490 metabolism. *Physiol. Plant.* **153**, 183-193 (2015).

491 46. Heng, Y. *et al.* *OsALMT7* maintains panicle size and grain yield in rice by mediating malate transport.
492 *Plant Cell* **30**, 889–906 (2018).

493 47. Holm, L. DALI and the persistence of protein shape. *Protein Sci.* **29**, 128–140 (2020).

494 48. Furuichi, T. *et al.* An extracellular hydrophilic carboxy-terminal domain regulates the activity of
495 *TaALMT1*, the aluminium-activated malate transport protein of wheat. *Plant J.* **64**, 47-55 (2010).

496 49. Smart, O. S., Neduvélil, J. G., Wang, X., Wallace, B. A. & Sansom, M. S. P. HOLE: A program for the
497 analysis of the pore dimensions of ion channel structural models. *J. Mol. Graph.* **14**, 354–360 (1996).

498 50. Peng, W. *et al.* Characterization of the soybean *GmALMT* family genes and the function of *GmALMT5*
499 in response to phosphate starvation. *J. Integr. Plant Biol.* **60**, 216–231 (2018).

500 51. Jumper, J. *et al.* Highly accurate protein structure prediction with AlphaFold. *Nature* (2021)
501 doi:10.1038/s41586-021-03819-2.

502 52. Altschul, S. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs.
503 *Nucleic Acids Res.* **25**, 3389–3402 (1997).

504 53. Wang, X.-H. *et al.* Structural basis for the activity of TRIC counter-ion channels in calcium release. *Proc.*
505 *Natl. Acad. Sci. USA* **116**, 4238–4243 (2019).

506 54. Mastronarde, D. N. Automated electron microscope tomography using robust prediction of specimen
507 movements. *J. Struct. Biol.* **152**, 36-51. (2005).

508 55. Wu, C., Huang, X., Cheng, J., Zhu, D. & Zhang, X. High-quality, high-throughput cryo-electron
509 microscopy data collection via beam tilt and astigmatism-free beam-image shift. *J. Struct. Biol.* **208**,
510 107396 (2019).

511 56. Zivanov, J. *et al.* New tools for automated high-resolution cryo-EM structure determination in RELION-
512 3. *Elife* **7**, 1–22 (2018).

513 57. Punjani, A., Rubinstein, J. L., Fleet, D. J. & Brubaker, M. A. cryoSPARC: algorithms for rapid
514 unsupervised cryo-EM structure determination. *Nat. Methods* **14**, 290–296 (2017).

515 58. Asarnow, D., Palovcak, E. & Cheng, Y. UCSF pyem. (2021).

516 59. Pettersen, E. F. *et al.* UCSF Chimera--a visualization system for exploratory research and analysis. *J.*
517 *Comput. Chem.* **25**, 1605–1612 (2004).

518 60. Zheng, S. Q. *et al.* MotionCor2: anisotropic correction of beam-induced motion for improved cryo-
519 electron microscopy. *Nat. Methods* **14**, 331–332 (2017).

520 61. Zhang, K. Gctf: Real-time CTF determination and correction. *J. Struct. Biol.* **193**, 1–12 (2016).

521 62. Zhang, K., and Sun, F. Gautomatch: an efficient and convenient gpu-based automatic particle selection
522 program. <https://www2.mrc-lmb.cam.ac.uk/download/gautomatch-056>. (2011).

523 63. Liebschner, D. *et al.* Macromolecular structure determination using X-rays, neutrons and electrons: recent
524 developments in it Phenix. *Acta Crystallogr. Sect. D* **75**, 861–877 (2019).

525 64. Kucukelbir, A., Sigworth, F. J. & Tagare, H. D. Quantifying the local resolution of cryo-EM density
526 maps. *Nat. Methods* **11**, 63–65 (2014).

527 65. Emsley, P., Lohkamp, B., Scott, W. G. & Cowtan, K. Features and development of Coot. *Acta*
528 *Crystallogr. Sect. D* **66**, 486–501 (2010).

529 66. Gabler, F. *et al.* Protein sequence analysis using the MPI bioinformatics toolkit. *Curr. Protoc.*
530 *Bioinforma.* **72**, e108 (2020).

531 67. Barad, B. A. *et al.* EMRinger: Side chain-directed model and map validation for 3D cryo-electron
532 microscopy. *Nat. Methods* **12**, 943–946 (2015).

533 68. Pettersen, E. F. *et al.* UCSF ChimeraX: Structure visualization for researchers, educators, and developers.
534 *Protein Sci. A Publ. Protein Soc.* **30**, 70–82 (2021).

535 69. Bi, G. *et al.* The ZAR1 resistosome is a calcium-permeable channel triggering plant immune signaling.
536 *Cell* **184**, 3358–3360 (2021).

METHODS

Bioinformatics analysis of ALMT proteins

Sequences related to QUAC1/ALMTs were searched and analyzed by using PSI-BLAST⁵². Searches at $E < 5 \times 10^{-3}$ starting with thirteen individual *Arabidopsis* ALMTs identified a common pool of over 1,700 plant ALMT-related protein sequences, which were pooled together and used for sub-classification into families and subfamilies. Detailed information is reported in the footnotes to the Extended Data Table 1.

Cloning and expression of QUAC1/ALMT12 in yeast *S. pombe*

Full-length coding sequences of six plant QUAC1s from rice, wheat, corn, soybean, tomato and cotton, were cloned into a modified pREP1 vector with a C-terminal 10× His tag. The resulting constructs were transformed into a leucine auxotrophic *S. pombe* strain, and the transformants were selected on the standard EMM plates without leucine, as previously described⁵³. To prevent protein expression during strain growth, 25 μM thiamine was added to inhibit the promoter.

Scaled-up production and purification of *Gm*QUAC1

To prepare the seed, transformed cells were inoculated into 100 ml EMM culture medium supplemented with 25 μM thiamine and were shaken at 200 rpm and 30°C for 24 hr. For protein expression and scaled-up production, the seed cells were collected by centrifugation and washed with sterile water twice before inoculation to a culture of 500 ml. After 12 hr growth, 500 ml of fresh medium was supplemented, and the culture continued to grow for an additional 24 hr. Cells were harvested through centrifugation for 20 min at 4,500 rpm.

For protein purification, cells were re-suspended in lysis buffer (50 mM Tris-HCl pH 8.0, 200 mM NaCl, 2.5% glycerol, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 1 μg/ml pepstatin, 2 mM PMSF and 2 mM DTT) and lysed using a high-pressure cell disrupter (JNBIO) with 4 passes at ~18,000 psi. Cell debris was removed by centrifugation at 12,000 rpm for 15 min, and the supernatant was subjected to a further ultra-centrifugation at 41,000 rpm for 1 hr. The

membrane was collected and homogenized in a solubilization buffer (50 mM Tris-HCl pH 8.0, 200 mM NaCl and 2.5% glycerol) and incubated with a final concentration of 1.0% DDM and 0.02% CHS by gentle stirring for 1 hr at 4°C. After ultra-centrifugation at 35,000 rpm for 50 min, the resulting supernatant was purified by Ni²⁺-affinity column pre-equilibrated with the same solubilization buffer supplemented with 0.05% DDM and 2 mM TCEP. After 20 columns volume of buffer wash, the protein was eluted with 350 mM imidazole in the solubilization buffer. The resulting *GmQUAC1* protein, without removal of His-tag, was concentrated to ~10 mg/ml and loaded onto a Superose6 (10/300) gel-filtration column for further purification and detergent exchange. The gel-filtration buffer contained 20 mM Tris, 150 mM NaCl (pH 8.0, adjusted with HCl) or 75 mM L-malate (pH 8.0, adjusted with NaOH), and 0.005% LMNG. The elution fractions were analyzed by SDS-PAGE, and the peak fractions were collected and concentrated for functional analysis (~2 mg/ml) or making cryo-EM grids (~5 mg/ml).

Cryo-EM grid preparation and data acquisition

4 µl of *GmQUAC1*^{malate} proteins (purified in 75 mM L-malate) were applied to newly glow-discharged holy carbon film grids (Au R1.2/1.3, 300 meshes, Quantifoil, Germany). The grids were blotted with force 3 and blotting time of 9.0 s at 100% humidity and 4°C and were vitrified by plunge freezing into liquid ethane using Vitrobot Mark IV (Thermo Fisher Scientific, USA). All movies were collected on a Titan Krios G2 transmission electron microscope (Thermo Fisher Scientific, USA) operated at 300 KV, equipped with a Gatan K2 Summit direct detection camera (Gatan Company, USA) and a post-column GIF energy filter.

Data collection was performed on EF-TEM mode with a slit width of 20 e⁻·V. The magnification was set to a nominal 130,000x, corresponding to a calibrated pixel size of 1.04 Å/pixel at the specimen level (0.52 Å/pixel in super-resolution mode). Images were recorded using SerialEM (version 3.8.4)⁵⁴ with a beam-image shift method⁵⁵. During the 8s exposure, 32 frames were collected with a dose of around 60 e⁻/Å². A total of 6,189 movie stacks were collected, with defocus ranging from -1.2 µm to -2.2 µm.

Image processing and model building

For cryo-EM image processing, all main steps were performed using RELION 3.0⁵⁶ or cryoSPARC 3.1⁵⁷. Pyem⁵⁸ and UCSF Chimera⁵⁹ were used for format conversion of data files and reconstructions analysis, respectively.

In brief, 6,189 movie stacks were aligned by 5×5 patches with dose weighting and binned to 1.04 Å using Motioncor2⁶⁰. The contrast transfer function parameters were estimated using GCTF⁶¹. Micrographs with defects in the thon rings were discarded. 2,987,283 candidate particles were initially picked using Gautomatch without template⁶², and followed by several rounds of 2D classification in cryoSPARC, giving a set of 2,136,754 high-quality particles. A rough initial model was generated by a subset of 100,000 particles. Subsequently, six rounds of 3D classification were performed at C1 symmetry. Three parallel 3D classifications were performed within each round, and the resulting good particles were combined for sequential round classification. After that, a set of 428,433 particles that generated a map of 4.0 Å using NU-Refinement in cryoSPARC were subjected to Bayesian polishing. Three rounds of 3D classification were further performed as described below. Each round contains an NU-Refinement at C2 symmetry in cryoSPARC followed by an alignment-free 3D classification with a micelle-free mask in Relion. Finally, NU-refinement of 169,576 particles yielded a reconstruction with a resolution of 3.5 Å, based on the gold-standard Fourier Shell Correlation (FSC) using the 0.143 criterion. Density modification was performed using PHENIX⁶³. Local resolution was determined using ResMap⁶⁴.

A *de novo* atomic model was built manually in Coot⁶⁵ using the predicted secondary structure from MPI quick2d⁶⁶. The model was further refined using PHENIX⁶³. The final model was validated using EMRinger⁶⁷. All structure figures were prepared in UCSF ChimeraX⁶⁸.

The two-electrode voltage-clamp recordings

The TEVC recordings were conducted as previously described²⁰. In brief, all cDNAs for *GmQUAC1*, including wild-type or mutants, were cloned into plasmid pGHME2 for expression

in *Xenopus laevis* oocyte. Linearised plasmids were used to generate cRNAs using T7 polymerase. 36 nanograms of cRNA of each construct were injected into isolated oocytes. Oocytes were then incubated at 18°C for ~48 hr in ND96 buffer (96 mM NaCl, 1.8 mM CaCl₂, 1 mM MgCl₂, 2 mM KCl, 5 mM HEPES-Na, pH 7.5).

Using a Oocyte Clamp OC-725C amplifier (Warner Instruments) and a Digidata 1550 B low-noise data acquisition system with pClamp software (Molecular Devices), TEVC recordings were performed in the bath solutions: 10 mM MES/Tris pH 5.6, 1mM Ca(gluconate)₂, 1 mM Mg(gluconate)₂, 70 mM Na(gluconate), 1 mM LaCl₃, 30 mM NaCl or NaNO₃, or 30 mM L-malate (pH 5.6, prepared from L-malic acid with NaOH). Osmolarity was adjusted to ~220 mOsmol*kg⁻¹ with D-Sorbitol. The microelectrode solutions contained 3 M KCl, and the bath electrode was a 3 M KCl agar bridge. Voltage-clamp currents were measured in response to 200 ms-long voltage steps to test potentials that ranged from +60 mV to -180 mV in 20 mV decrement. Prior to each voltage step, the membrane was held at +60 mV for 50 ms, and following each voltage step, the membrane was returned to -180 mV for 50 ms. I-V relations for *GmQUAC1* channels were generated from currents measured at 5 ms after the beginning (Instantaneous currents, *I*_{inst}) or before the end (Steady-state currents, *I*_{ss}) of each test voltage step. Three independent batches of oocytes were investigated and showed consistent findings. Data from one representative oocyte batch were shown. The recordings were analyzed using Clampfit 10.6 (Molecular Devices) and Prism (ver. 5.0, GraphPad).

The planar lipid bilayer recordings

Lipid bilayer experiments were conducted as previously described in our study of the ZAR1 channel⁶⁹. The purified *GmQUAC1* (in 150 mM NaCl or 75 mM L-malate), at a concentration of 1.5~2.5 µg/ml, was fused into planar lipid bilayers formed by painting a lipid mixture of phosphatidylethanolamine (DOPE) and phosphatidylcholine (DOPC) (Avanti Polar Lipids) in a 5:3 ratio in decane across a 200 µm hole in a polystyrene partition separating the internal and external solutions in polysulfonate cups (Warner Instruments). The *trans* chamber (1.0 ml), representing the extracellular compartment, was connected to the head stage input of a bilayer

voltage-clamp amplifier. The *cis* chamber (1.0 ml), representing the cytoplasmic compartment, was held at virtual ground. Solutions used for I-V relationship analysis were as follows: 150 mM NaCl or 150 mM NaNO₃. All solutions were buffered with 20 mM Tris (pH 8.0). Purified proteins were added to the *cis* side and fused with the lipid bilayer.

Currents were recorded every 1~2 min after application of the voltage to the *trans* side. Single-channel currents were recorded using a Bilayer Clamp BC-525D (Warner Instruments), filtered at 800 Hz using a Low-Pass Bessel Filter 8 Pole (Warner Instruments) and digitized at 4 kHz. All experiments were performed at room temperature (23 ± 2°C). The recordings were analyzed using Clampfit 10.6 (Molecular Devices) and Prism (ver. 5.0, GraphPad).

Biochemical characterization of CHD of *GmQUAC1*

cDNAs encoding the C-terminal cytoplasmic helical domain of *GmQUAC1* (wild-type or mutants) were subcloned into a modified bacterial expression vector pET-24a, with a C-terminal 10× His tag. Transformed *E. coli* BL21 (DE3) cells were grown in a TB medium containing Kanamycin (50 µg/ml). Protein expression was induced in cells grown to an optical density at OD₆₀₀ of ~0.8 with 0.4 mM isopropyl β-D-thiogalactoside (IPTG) and followed by overnight cell growth at 16°C. Cells were collected and subsequently lysed using a high-pressure cell disrupter (JNBIO) with 3 passes at ~15,000 psi. The target protein was purified by using Ni²⁺ affinity chromatography and further analyzed by Superdex75 (10/300) gel filtration chromatography and SDS-PAGE.

The three-dimensional structure modelling using AlphaFold2

Briefly, the sequence for AlphaFold prediction was generated by two individual *GmQUAC1* sequences, connecting by a linker of tandemly repeated sequence of (GSGS)₅₀. Running with the local installed AlphaFold2 program and the CASP14 preset and databases (as of July 23, 2021), five dimer models were generated, with their pLDDT (predicted IDDT-Cα) scores ranging from 64.6 to 68.8. All the AlphaFold predicted structures (AF-structure) are nearly identical, with apparent two-fold symmetry. The two protomers in the top-rank dimer

model (scored 68.8) display an r.m.s.d of 0.92 Å, was selected for further structural comparison with the cryo-EM structure. For a better view, the disordered (residues 1-35, 394-454 and 507-537) regions were removed in the final model.

QUANTIFICATION AND STATISTICAL ANALYSIS

In all figure legends, n represents the number of independent biological replicates. All quantitative data were presented as mean ± SEM.

DATA AND CODE AVAILABILITY

All data are available in the manuscript or the supplementary material. The accession number for the 3D cryo-EM density map reported in this paper is XXX, and the Protein Data Bank (PDB) accession code for the coordinate is XXX.

ADDITIONAL RESOURCES

This study did not generate any additional resources.

Figure 1

Fig. 1 Architecture of the QUAC1 channel

a, Overall structure of the *GmQUAC1* channel. The cryo-EM density maps (top) and ribbons drawings (bottom) are shown, with the pre-TM helix in pink, the transmembrane domain (TMD) in green and the cytoplasmic helical domain (CHD) in blue in one protomer. Another protomer is coloured in grey.

b, Topology of the *GmQUAC1* protomer. The six helices in TMD are marked by TM1-6, and the seven helices in CHD are marked as H1-7. The membrane boundary is shown as grey lines. The disordered regions are indicated as dashed lines.

c, Top view of the cross-section of the transmembrane layer at the indicated positions by the arrow lines in (**a**). The elliptical dashed line marks the pore, and the TMs are indicated.

d, Bottom view of the C-terminal dimeric domains, colored as in (**a**). The domain-swapped finger helix H6 is shown as cylinder cartoon, and a red dash line marks the dimeric interface of the CHD.

Figure 2

Fig. 2 The twisted bi-layer architecture of the QUAC1 channel

a, The dimeric interfaces of the TMD and CHD of the *Gm*QUAC1 channel. These dimeric interfaces are nearly perpendicular to each other, forming a dihedral angle of $\sim 83^\circ$. The ribbon model is colored as in Fig. 1a.

b, The layer interface between the TMD and CHD. The TMD portion connects to the CHD portion via a highly conserved PXWXG motif (P218-N219-W220-S221-G222, in salmon), which is interact with another characteristic motif WEP (W288-E289-P290, located between helix H2 and H3, in blue). Other surrounding charged residues at the interface are also shown in sticks, including R56, R291, R294, D54, E223, D224 and E289. The ribbon model is colored as in (a).

Figure 3

Fig. 3 Structural feature of the QUAC1 channel pore

a, The pore-lining surface was computed by the program HOLE and drawn into a ribbon model of the *Gm*QUAC1 pore. We used a simple van der Waals surface for the protein and the program default probe radius of 1.15 Å. The T-shaped pore is shown in salmon dots.

b, Plot of the pore radius as a function of the pore axis.

c and d, Side views of the cross-section through the ion-conducting pore. The molecular surface colored with sequence conservation for 137 non-redundant proteins of the QF2B subfamily is shown in (c) and the molecular surface colored with electrostatic potential is shown in (d).

e, Top views of the cross-section through the ion-conducting pore at three indicated positions by the arrow in (c) and (d). The ribbon is colored as in (a). The conserved pore-lining charged residues are shown in sticks, coloured with the sequence conservation as in (c).

Figure 4

Fig. 4 The lateral fenestration in the QUAC1 channel

a, The lateral fenestration, with a dimension of $\sim 6 \text{ \AA} \times 20 \text{ \AA}$, is shown in transparent red, which is formed by TM2, TM3, TM5 and TM6 from one protomer. A kink ($\sim 24^\circ$) in TM6 is indicated.

b, The unidentified densities (in yellow) within the fenestration (indicated by arrows). The side view (right) and top view (left) are shown. The W90 residues are shown as red sticks. The locations of fenestration are indicated with a blue stars in the top view.

Figure 5

Fig. 5 Single channel analysis of the QUAC1 channel in planar lipid bilayer

a, The schematic illustration of single-channel recording of *GmQUAC1* channel in a planar lipid bilayer. The chambers were filled with 1.0 ml of symmetrical solutions of 150 mM NaCl or 150 mM NaNO₃, and the *GmQUAC1*^{NaCl} or *GmQUAC1*^{malate} (purified in 150 mM NaCl or 75 mM L-malate, respectively) were added to the *cis*-side. The *trans*-chamber, representing the extracellular compartment, was connected to the head stage input of a bilayer voltage-clamp amplifier. The *cis*-chamber, representing the cytoplasmic compartment, was held at virtual ground.

b, Representative current traces for single channel analysis of *GmQUAC1*^{NaCl} are shown at +60 mV or -60 mV holding potentials in the NaNO₃ solution. The closed (C) and full-open (O) states are indicated.

c and **d**, The current-voltage relationships (**c**) and open probabilities (**d**) for the single-channel recordings of *GmQUAC1*. The chamber solutions (NaNO₃ or NaCl) and protein (*GmQUAC1*^{NaCl} or *GmQUAC1*^{malate}) are indicated in the inset (Data are mean ± SEM, n≥4).

Figure 6

Fig. 6 Single channel analysis of malate regulation on QUAC1

a and **b**, The chambers were filled with symmetrical solutions of 150 mM NaCl, and the purified *Gm*QUAC1^{NaCl} proteins were added to the *cis*-side. The representative current traces of 60 s at +60 mV, before and after adding 2 mM L-malate to the *trans*- (**a**) or *cis*- (**b**) chamber, are shown.

c and **d**, The regulation effect of malate on the *Gm*QUAC1^{NaCl} in the NaCl solutions. The current amplitude (left) and open probability (right) analysis for the recordings, before (-) or after (+) addition of 2 mM L-malate to the *trans*- (**c**) or *cis*- (**d**) chamber (Data are mean $\pm$ SEM, n=3 for each group).

Figure 7

Fig. 7 Effect of dimeric interactions on channel activity and the proposed model for QUAC1 regulation

a, The cartoon of the *GmQUAC1* channel is shown, with one protomer in grey and one in white. The domain-swapped helix (H6, residues 460-467) is colored in orange, and key residues at the dimeric interface are highlighted: S461 in green, A477 in magenta and F470/L474 in blue.

b and **c**, Close-up views of the cytoplasmic dimer interface. Residues at the dimeric interface are shown as sticks and colored as in (**a**).

d and **e**, The purification of the CHD proteins (residues 221-537, wild-type or mutants) on a Superdex75 (10/300) column. Compared to the wild-type (peak at ~9.2 ml), the eluted peaks for the dimer-disrupting mutants (A477E and F470A/L474A) shift backwards to ~10.2 ml, suggesting a disassociation of dimer into monomer. The elution peaks at ~9.7 ml for the two finger helix altered mutants (Δ 460-467 and S461D) suggest loosened conformations upon the removal of finger helix interaction. The eluted fractions were also analyzed by SDS-PAGE, as shown in (**e**).

f, The TEVC recording of *GmQUAC1* channel (wild-type and mutants) in *Xenopus laevis* oocytes. The same set of mutations, as in (**d**), were generated into full-length constructs for conductance measurement in the bath solution of 30 mM L-malate (Data are mean $\pm$ SEM, $n \geq 8$).

g, Model for the QUAC1 channel regulation. Upon malate binding, the QUAC1 undergoes intramolecular domain reorganization to form a twisted bi-layer architecture, and subsequently induces the conformational change of the toggle switch W90. These changes promote the channel conversion from closed to open state. Other modulations, such as phosphorylation or calmodulin binding, can further enhance the malate-mediated activation, probably via the release of its inhibitory domain-swapped helix from the inter-protomer interaction.