Conformational plasticity in the selectivity filter of the TRPV2 ion channel

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Transient receptor potential vanilloid (TRPV) channels are activated by ligands and heat and are involved in various physiological processes. In contrast to the architecturally related voltage-gated cation channels, TRPV1 and TRPV2 subtypes possess another activation gate at the selectivity filter that can open widely enough to permeate large organic cations. Despite recent structural advances, the mechanism of selectivity filter gating and permeation for both metal ions and large molecules by TRPV1 or TRPV2 is not well known. Here, we determined two crystal structures of rabbit TRPV2 in its Ca2+-bound and resiniferatoxin (RTx)- and Ca2+-bound forms, to 3.9 Å and 3.1 Å, respectively. Notably, our structures show that RTx binding leads to two-fold symmetric opening of the selectivity filter of TRPV2 that is wide enough for large organic cation permeation. Combined with functional characterizations, our studies reveal a structural basis for permeation of Ca2+ and large organic cations in TRPV2.

The TRPV channel family, part of the TRP channel superfamily, is composed of six members, TRPV1–TRPV6, that are involved in various physiological processes including nociception, osmosensation, and Ca2+ regulation1–4. Two members of this family, TRPV1 and TRPV2, are activated by natural products including capsaicin and cannabinoids, as well as heat5–9. The question of how TRPV1 and TRPV2 increase their Ca2+ conductance in response to physical and chemical stimuli has been at the center of the field since their discovery7. Although they are architecturally related to canonical voltage-gated cation channels, a crucial distinction exists in that TRPV1 and TRPV2 possess two activation gates: one at the selectivity filter (the SF gate) and the other at the S6 helical bundle crossing at the intracellular mouth of the pore (the common gate). This is in stark contrast with canonical voltage-gated cation channels, in which the common gate is the only activation gate8–13. A recent structure of the TRPV1 channel in complex with the double-knot toxin (DkTx) and RTx has provided us with a better understanding of how binding of these toxins in the vicinity of the SF gate and the common gate, respectively, leads to the opening of both gates14.

Despite such progress, many questions still remain unanswered. First, several TRPV channels (TRPV1, TRPV2, TRPA1) have been shown to permeate large organic cationic molecules (up to ~400 Da), which are much larger than metal ions15–22. This phenomenon, initially termed ‘pore dilation’, was thought to occur through gradual change in the selectivity filter upon repeated stimulation12,19. Although this model of dynamic changes in the selectivity during gating has since been challenged17–20, the ability of some TRPV channels to permeate large organic cations is well established and has been exploited as a mechanism for delivery of small-molecule drugs17,20,21,27. However, the structural basis for this phenomenon remains elusive, because a structure of a fully open SF gate has not been captured to date. Common gate opening in several ion channels has been shown to be large enough to pass large organic cations4,23–30. Second, the mechanism of Ca2+ recognition in the SF of TRPV1 and TRPV2 is not known, as ions were not resolved in any of the structures reported to date21–33. Third, because previous studies of TRPV1 used both DkTx and RTx to open the SF gate and the common gate, it is not clear how binding of the vanilloid toxin RTx at the inner leaflet of the membrane opens the SF gate of TRPV1 in the absence of DkTx. Finally, until now, all of the structural studies of the TRP channels have had four-fold (C4) symmetric conformations, but whether the channel gating involves less symmetric or asymmetric (C2 or C1) arrangements within the tetramer has not been clear, as asymmetric TRPV channel structures have not been captured.

To address these issues and study the mechanism of ligand-dependent TRPV2 activation, we have performed structural studies of TRPV2. TRPV2 shares a high sequence identity with TRPV1 (~50%) and is activated by heat, cannabinoids and 2-aminoethoxydiphenyl borate (2-APB), but not by RTx. Recently, two groups independently showed that introduction of four point mutations rendered TRPV2 sensitive to RTx, thereby demonstrating the conserved mechanism of ligand-dependent activation of TRPV1 and TRPV2 (refs 34,35).

Here, we present two crystal structures of TRPV2, one bound to Ca2+ and the other bound to RTx and Ca2+, at 3.9 Å and 3.1 Å, respectively (Table 1 and Fig. 1a). Through structural analysis combined with functional studies, we have identified novel structural arrangements of the SF gate of TRPV2 elicited by RTx binding that enables permeation of large cations.

Results

Crystal structures of TRPV2 with and without RTx. For structure determination, we used the minimal TRPV2 construct (miTRPV2) from the rabbit, Oryctolagus cuniculus, similar to those used for cryo-EM studies of rabbit TRPV2 and rat TRPV1 (refs 31–33) (48% sequence identity to rat TRPV1; Methods and Supplementary Fig. 1). We previously showed that both wild-type (wt) TRPV2 and miTRPV2 are activated by 2-APB using Ca2+ imaging and whole-cell patch-clamp recording experiments32. We introduced four point mutations (F470S, L505M, L508T and Q528E; TRPV2 QM) into wtTRPV2 and miTRPV2, respectively (Table 1 and Fig. 1a). Through structural analysis combined with functional studies, we have identified novel structural arrangements of the SF gate of TRPV2 elicited by RTx binding that enables permeation of large cations.

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Both structures exhibit the well-established TRPV channel fold in which the four protomers with six transmembrane helices (S1–S6) form a domain-swapped homotetramer, with each voltage-sensor like domain (VSLD, S1–S4) interacting with the pore domain (S5–S6) of its neighboring protomer and with the S4–S5 helix (S4–S5 linker) mediating this domain-swapped configuration (Fig. 1). The higher-resolution crystal structure of miTRPV2<sub>RTx,Ca<sup>2+</sup></sub> enabled a more complete model building than the miTRPV2<sub>EM</sub> structure<sup>a</sup>. Notably, both the N-terminal ankyrin repeat domain (ARD) and the C-terminal domain (CTD), which were not well resolved in previous TRPV1 and TRPV2 structures<sup>14,31–33,36</sup>, are almost fully resolved in the electron density map (Supplementary Figs. 3 and 4). All four RTx-binding sites are occupied in the structure of miTRPV2<sub>RTx,Ca<sup>2+</sup></sub>, as shown in the F<sub>o</sub> − F<sub>c</sub> simulated annealing omit map (Supplementary Fig. 5). Interestingly, the top-down view shows that both miTRPV2<sub>Ca<sup>2+</sup></sub> and miTRPV2<sub>RTx,Ca<sup>2+</sup></sub> structures assume a two-fold symmetric arrangement within the homotetramer, a marked contrast to the four-fold symmetric miTRPV2<sub>EM</sub> structure (Fig. 1b).

Subunit arrangement of miTRPV2<sub>Ca<sup>2+</sup></sub> is determined by a π-helix in the S4–S5 linker. We first compared the structures of miTRPV2<sub>Ca<sup>2+</sup></sub> and miTRPV2<sub>EM</sub> and found that while the dimensions of the cytoplasmic ARDs remain unchanged, the dimensions of the two membrane channel regions are different: miTRPV2<sub>Ca<sup>2+</sup></sub> is contracted at the extracellular side of the membrane by ~13 Å along one direction (y axis) and ~6 Å along the other direction (x axis) compared to miTRPV2<sub>EM</sub> (measured using Ca of N429 at the top of opposing S2 helices), leading to a two-fold symmetric structural arrangement in the channel region of miTRPV2<sub>Ca<sup>2+</sup></sub> (Fig. 2a). Such two-fold symmetric arrangements of TRP channels have never been observed before. Because of the domain-swapped configuration, each subunit is composed of VSLD of one polypeptide chain and the pore domain of the neighboring chain, and the S4–S5 linker connects one subunit to the adjacent subunit. Structural analyses revealed that the contraction of miTRPV2<sub>Ca<sup>2+</sup></sub> along the y axis with respect to miTRPV2<sub>EM</sub> is caused by an ~7° rigid-body rotation of the entire subunit around an axis formed by the S4–S5 linker and the 3<sup>α</sup>-helical part of S4 (S4b) (Fig. 2b). Superposition of each subunit of miTRPV2<sub>Ca<sup>2+</sup></sub> with the subunit of miTRPV2<sub>EM</sub> reveals that the VSLD (S1–S4) and the pore domain (S5–S6) align well (Ca r.m.s.d. of 0.75 Å), whereas the S4–S5 linker and the pore helix exhibit large conformational changes (Fig. 2b–d).

On the basis of structural comparisons of the TRPV1 and TRPV2 cryo-EM structures, it has been proposed that a π-helix, a rare helix with a wider turn than an α-helix (4.1 versus 3.6 residues per turn), plays an important role in TRPV channel gating by introducing a transient hinge in the key helices<sup>15</sup>. Recent structural studies have shown that this α-to-π helical transition indeed occurs in S6 of TRPV6 (ref.<sup>19</sup>). While the S4–S5 linker and S5 in miTRPV2<sub>EM</sub> form a continuous π-helical structure, miTRPV2<sub>Ca<sup>2+</sup></sub> contains a π-helix between the S4–S5 linker and S5 helices (termed π-hinge<sub>S4,S5</sub>). Notably, the two-fold symmetric miTRPV2<sub>Ca<sup>2+</sup></sub> contains π-hinges at two different positions. Whereas the more contracted subunit has a π-hinge<sub>S4,S5</sub> beginning at D534, the less contracted subunit contains a π-hinge<sub>S4,S5</sub> that begins at I531. This difference in the π-hinge positions results in different angles between the S4–S5 linker and S5 and, therefore, in different degrees of subunit rotation, which is responsible for the two-fold symmetric rearrangements (Fig. 2d). The two-fold symmetric contraction of miTRPV2<sub>Ca<sup>2+</sup></sub> near the extracellular region leads to pore-helix rearrangements with ~7.5° angle tilt and ~3.5-Å outward displacement compared to that of miTRPV2<sub>EM</sub> (Fig. 2c).

RTx binding leads to two-fold symmetric rearrangement of TRPV2. Although all four subunits are bound to RTx in

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**Table 1 | Data collection and refinement statistics**

|                      | miTRPV2<sub>Ca<sup>2+</sup></sub><sup>(a)</sup> (PDB 6BWJ) | miTRPV2<sub>RTx,Ca<sup>2+</sup></sub><sup>(b)</sup> (PDB 6BWJ) | miTRPV2<sub>EM</sub><sup>(c)</sup> SeMet<sup>(d)</sup> |
|----------------------|--------------------------------------------------------|--------------------------------------------------------|--------------------------------------------------------|
| **Data collection**  |                                                        |                                                        |                                                        |
| Space group          | P2<sub>2</sub>,<sub>1</sub>, P2<sub>2</sub>,<sub>1</sub>, P2<sub>2</sub>,<sub>1</sub> |                                                        |                                                        |
| Cell dimensions      |                                                        |                                                        |                                                        |
| a, b, c (Å)          | 92.03, 122.36, 90.05, 121.22, 187.21                   | 93.5, 125.39, 185.56                                   | 190.9                                                 |
| α, β, γ (%)          | 90, 90, 90, 90, 90, 90, 90, 90                         | 90, 90, 90                                           |                                                        |
| Resolution (Å)       | 39.85–3.9                                              | 45.03–3.3                                            | 48.51–5.0                                            |
| R<sub>r</sub> / R<sub>free</sub> | 0.05 (0.29)                                            | 0.24 (0.27)                                          | 0.24 (0.27)                                          |
| R<sub>ext</sub> / R<sub>max</sub> | 8.8 (2.7)                                             | 8.6 (1.0)                                            | 11.4 (3.8)                                           |
| Completeness (%)     | 99.3 (99.6)                                            | 100 (100)                                            | 99.9 (100)                                           |
| Redundancy           | 175 (17.8)                                             | 13.7 (13.7)                                         | 28.1 (28.2)                                          |
| **Refinement**       |                                                        |                                                        |                                                        |
| Resolution (Å)       | 39.85–3.9                                              | 40.04–3.9                                            | 32.21–4.75                                           |
| No. reflections       | 19,867 (1,930)                                         | 37,513 (3,639)                                        | 45,03–3.1                                           |
| F<sub>o</sub> − F<sub>c</sub> | 0.02 (0.05)                                            | 0.24 (0.27)                                          | 0.24 (0.27)                                          |
| Protein              | 8,514                                                  | 18,363                                               | 18,189                                               |
| Ligand/ion           | 1 (Ca<sup>2+</sup>)                                    | 172 (RTx), 2 (Ca<sup>2+</sup>)                       | 172 (RTx), 2 (Ca<sup>2+</sup>)                       |
| B factors            | 103.37                                                 | 86.58                                                | 106.07                                               |
| R.m.s. deviations    | 147.30                                                 | 106.07                                               |                                                        |
| Bond lengths (Å)     | 0.002                                                  | 0.005                                                | 0.55                                                 |
| Bond angles (°)      | 0.73                                                   |                                                      |                                                      |

*aMerged from four native miTRPV2<sub>Ca<sup>2+</sup></sub> crystals. *Merged from two native miTRPV2<sub>RTx,Ca<sup>2+</sup></sub> crystals. *Merged from four native SeMet-miTRPV2<sub>Ca<sup>2+</sup></sub> crystals.

irreversible, whereas the control TRPV2 constructs were not sensitive to RTx (Fig. 1c,d and Supplementary Fig. 2). Both TRPV2<sub>EM</sub> and miTRPV2<sub>EM</sub> were blocked by 200μM Ruthenium red (RuR) from both sides of the membrane in the inside-out configuration; RuR completely blocked outward currents of TRPV2<sub>EM</sub> when introduced to the cytosolic side of the excised patch (Fig. 1c,d) and blocked inward currents when introduced into the pipette solution (Supplementary Fig. 2). In the whole-cell configuration, RuR substantially reduced TRPV2 currents when introduced into the extracellular solution (Supplementary Fig. 2). Both TRPV2<sub>EM</sub> and miTRPV2<sub>EM</sub> could still be activated by 2-APB (Supplementary Fig. 2), suggesting that introducing a separate RTx-binding site does not interfere with 2-APB binding to the TRPV2 channels.

Both miTRPV2 alone and miTRPV2<sub>EM</sub> in complex with RTx were crystallized under similar conditions in the presence of Ca<sup>2+</sup> (Methods). The crystal structures of miTRPV2 in the presence of Ca<sup>2+</sup> (referred to as miTRPV2<sub>Ca<sup>2+</sup></sub>) and miTRPV2<sub>EM</sub> in complex with RTx in the presence of Ca<sup>2+</sup> (referred to as miTRPV2<sub>RTx,Ca<sup>2+</sup></sub>) were determined to 3.9 Å and 3.1 Å, respectively. Molecular replacement was employed for phasing, using fragments of the cryo-EM structure of miTRPV2<sub>EM</sub> (PDB 5AN8) as search models. The model building was guided by anomalous difference Fourier maps obtained from the structure of selenomethionine (SeMet)-labeled miTRPV2<sub>Ca<sup>2+</sup></sub> (Supplementary Fig. 3). The models were built and refined to a good overall geometry (Table 1).

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the structure of miTRPV2_{RTx,Ca}^{2+} (Fig. 1a and Supplementary Fig. 5), the channel region of miTRPV2_{RTx,Ca}^{2+} exhibits a widening along only one dimension (the x axis), near the extracellular side by ~7 Å with respect to miTRPV2_{Ca}^{2+} (Fig. 2e). Notably, the widening in subunits A and C results in a novel arrangement of the SF gate. Upon a rigid-body rotation around the π-hinge S4–S5, the subunits of miTRPV2_{RTx,Ca}^{2+} and miTRPV2_{Ca}^{2+} aligned well (Ca r.m.s.d. = 0.99 Å), with only the S4–S5 linker and the pore helix deviating considerably (Supplementary Fig. 5). In the structure of miTRPV2_{RTx,Ca}^{2+}, the position of π-hinge_{S4–S5} in all four subunits is the same, but two diagonally positioned subunits (A and C) assume a hinge orientation and angle between the S4–S5 linker and
Fig. 2 | \(\pi\)-helices in the S4–S5 linker determine the quaternary structure of subunits and conformation of the pore helices. a, Alignment of \(\text{miTRPV2}_{\text{Ca}^{2+}}\) (blue) and \(\text{miTRPV2}_{\text{EM}}\) (green). The ARDs were removed for clarity. Top view of the channel (left) illustrates the overall differences in dimensions in the two structures. A side view of subunits A and C (middle) illustrates a contraction measured to 6 Å. The contraction between subunits B and D (right) was measured to 13 Å (Cα distance between residues N429 in opposing subunits). b, A 7° rotation of the \(\text{miTRPV2}_{\text{Ca}^{2+}}\) subunit B around the S4–S5 linker is sufficient to achieve alignment with the subunit B of the \(\text{miTRPV2}_{\text{EM}}\) (Cα r.m.s.d. ~0.75 Å). The dashed line represents the rotation axis. PH, pore helix. c, Pore helices of the aligned subunits of \(\text{miTRPV2}_{\text{Ca}^{2+}}\) and \(\text{miTRPV2}_{\text{EM}}\) are positioned at different angles. d, Alignment of S5 helices of subunits A and B of the \(\text{miTRPV2}_{\text{Ca}^{2+}}\) structure illustrates that their S4–S5 linkers diverge significantly from each other. e, Alignment of \(\text{miTRPV2}_{\text{RTx,Ca}^{2+}}\) (red) and \(\text{miTRPV2}_{\text{Ca}^{2+}}\) (blue). Top view of the alignment (left) shows that subunits A and C of the \(\text{miTRPV2}_{\text{RTx,Ca}^{2+}}\) are widened with respect to \(\text{miTRPV2}_{\text{Ca}^{2+}}\), and that their pore helices are significantly displaced. Side view of subunits A and C (middle) show the extent of the widening (7 Å difference in Cα distance from N429 of subunit A to subunit C). f, Overlay of the subunits A (red) and B (pale cyan) S5 helices of \(\text{miTRPV2}_{\text{RTx,Ca}^{2+}}\) indicates a substantial difference in the S4–S5 linker conformations in the two subunits.
S5 that is distinct from the other two subunits (B and D), giving rise to the two-fold symmetric pore conformation (Fig. 2e,f). The RTx binds in a pocket formed by S3, S4b, the S4–S5 linker, S5 and S6. However, its interactions with the pocket are different in the two adjacent subunits of $^{+}\text{miTRPV}_{2}\text{RTx,Ca}^{2+}$, and this difference in RTx binding appears to induce different hinge orientations and subunit arrangements and ultimately result in the two-fold symmetric widening of the SF gate in the structure of $^{+}\text{miTRPV}_{2}\text{RTx,Ca}^{2+}$ (Supplementary Fig. 5).

Structural comparison of $^{+}\text{miTRPV}_{2}\text{RTx,Ca}^{2+}$ with $^{+}\text{miTRPV}_{2}\text{EM}$ shows that two subunits are widened by $\sim 2\AA$ along one direction (the x axis), and two subunits are contracted by $\sim 7\AA$ along the other direction (the y axis), near the extracellular side with respect to $^{+}\text{miTRPV}_{2}\text{EM}$ (Supplementary Fig. 6). When the subunits of the $^{+}\text{miTRPV}_{2}\text{RTx,Ca}^{2+}$ were superposed to the subunit of $^{+}\text{miTRPV}_{2}\text{EM}$, the VSLD and the S5 and S6 aligned well (Cα r.m.s.d. $= 0.61\AA$), but there was a substantial divergence in the pore helix and the S4–S5 linker (Supplementary Fig. 6). There is an $\sim 19^\circ$ difference

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**Fig. 3 | Comparison of $^{+}\text{miTRPV}_{2}\text{EM}$, $^{+}\text{miTRPV}_{2}\text{Ca}^{2+}$ and $^{+}\text{miTRPV}_{2}\text{RTx,Ca}^{2+}$ pores.**

**a.** A close up of the top view of the $^{+}\text{miTRPV}_{2}\text{EM}$ (green), $^{+}\text{miTRPV}_{2}\text{Ca}^{2+}$ (blue) and $^{+}\text{miTRPV}_{2}\text{RTx,Ca}^{2+}$ (red) channels, showing S5, S6 and pore helices. The pore helices are drawn in both helix and cylinder representation. A black box indicates subunits B and D, and a green box indicates subunits A and C. **b.** S6 and pore helices of subunits B and D. S6 is shown in cartoon representation, and pore helices are shown in both cylinder and cartoon representation. The cylinders are colored to indicate the helical dipoles. Dashes and values represent distances across the selectivity filter. $\text{Ca}^{2+}$ ions are shown as green spheres with $2F_o - F$ density contoured at $1-1.3\sigma$. **c.** S6 and pore helices of subunits A and C. **d.** Top view of the pore in $^{+}\text{miTRPV}_{2}\text{EM}$ (left, green), $^{+}\text{miTRPV}_{2}\text{Ca}^{2+}$ (middle, blue) and $^{+}\text{miTRPV}_{2}\text{RTx,Ca}^{2+}$ (right, red). Residues 601-605 are shown in stick representation. The dashed lines show $\text{Ca}^{2+}$ ion coordination in the selectivity filter of $^{+}\text{miTRPV}_{2}\text{RTx,Ca}^{2+}$. 

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are ~6 Å away from the cavity through concerted rear-structure, the distance and difficult, the carbonyls of I603 appear, struc-
ture shows that although the pore helices in miTRPV2EM are arranged in a four-fold
ion permeation path (Fig. 3a).

positioned in a distinctly two-fold symmetric manner around the
pore helices of miTRPV2EM, A structural comparison of the channel regions
of miTRPV2 EM, (A and C) of the
s4–s5 linker and the resulting subunit arrangement within the pore
(Supplementary Fig. 6).

RTx binding leads to two-fold symmetric wide opening of select-
ivity filter gate. A structural comparison of the channel regions of
miTRPV2QM, miTRPV2Ca and TRPV2QM with 50 μM extracellular RuR (a) and their peak fluorescence intensities (b), YO-PRO-1 uptake was triggered by the addition of 250 nM RTx (black arrow). TRPV2-expressing cells (identified by cotransfected mCherry) from ≥3 repeats of imaging experiments were chosen for analysis; the total number of cells analyzed are noted in parentheses in b, which also shows individual data points. a.u., arbitrary units. Representative RTx-evoked TRPV2QM current traces from inside-out patches in the presence of extracellular 0 μM, 10 μM or 50 μM YO-PRO-1 in the pipette solution. Currents were normalized to the peak amplitudes at +120 mV. d, Extracellular YO-PRO-1 attenuates the TRPV2QM inward currents as quantified by the ratios of peak current amplitudes at -120 mV and +120 mV. The number of patches is noted in parentheses. Data in a, b, d are presented as mean ± s.e.m. P values in d were calculated using two-tailed unpaired Student’s t test; ***P = 0.0003 and ****P < 0.0001.

in the angle of the two pore helices as well as an ~3.5-Å outward
shift in the pore helix of miTRPV2RTx,Ca+, suggesting that the
pore helix conformation is dependent on the conformation of the
S4–S5 linker and the resulting subunit arrangement within the pore
(Supplementary Fig. 6).

A closer look at the pore-lining components, S6 and the pore
helix, of the widened subunits (A and C) and the contracted sub-
units (B and D) further illustrates the conformational changes of the
pore upon RTx binding (Fig. 3b,c). In the miTRPV2QM structure, the SF gate is tightly closed, as the distance between diagon-
ally positioned G604 carbonyl oxygens is 5.2 Å. In the structure of
miTRPV2Ca2+, the corresponding distances between G604 carbonyl
oxygens are 6.1–6.4 Å. Here, we observed an electron density peak, which we tentatively assigned as Ca2+, given that Na+ is difficult to observe at this resolution. Although the quality of the electron density map around the pore made unambiguous assignment of the pore helices in miTRPV2Ca2+ difficult, the carbonyls of I603 appear to coordinate the Ca2+ ion via water molecules. In the contracted subunits (B and D) of the miTRPV2RTx,Ca2+ structure, the distance between the G604 carbonyl oxygens increased to ~7.1 Å, and two putative Ca2+ ions are present in the pore: one in the SF and the
other in the cavity. Notably, the high-resolution structure reveals a
novel mode of coordination of the Ca2+ ion in the SF: two pore heli-
ces are aligned in a way that allows the helical dipole to be used in
coordinating the ion in the SF, as carbonyls of F601, T602 and I603, located at the C termini of the pore helices from the two contracted
subunits, are in a position to make water-mediated coordination of
the Ca2+ ion in the SF (Fig. 3b,d). Strikingly, in widened subunits (A and C) of the miTRPV2Ca2+, the distance between the I603 carbonyls increases to ~12.3 Å (Fig. 3c). Thus, these two subunits are not involved in ion coordination. Although only two subunits are involved, six carbonyl atoms are within range to participate in coordination of the semihydrated Ca2+ ion in the SF (Fig. 3d). The cavity of miTRPV2RTx,Ca2+ is hydrophobic, and Y632 is the only polar amino acid in the cavity. Hydroxyl groups of Y632 from the two contracted subunits of miTRPV2RTx,Ca2+ are ~6 Å away from the cavity ion, making these two residues likely candidates for water-mediated coordination of ions at this site (Fig. 3b). An additional density peak was observed in the vicinity of the cavity ion in miTRPV2RTx,Ca2+, which could not be assigned to polypeptide or ions (Supplementary Fig. 4). The common gate of the miTRPV2RTx,Ca2+ is slightly more open than that of miTRPV2QM, but its opening is not wide enough to permeate hydrated Ca2+ ions, suggesting that only the SF gate is open in the miTRPV2RTx,Ca2+ structure (Supplementary Fig. 6).

The wide opening at the SF gate in the miTRPV2RTx,Ca2+ structure suggests that, in this state, the channel could permeate molecules much larger than small metal ions such as Na+, K+ and Ca2+. Therefore, this finding might represent the first observation of a fully open SF gate that is permeable to large organic cations. To test whether full-length TRPV2QM and miTRPV2QM are able to permeate large organic cations, we expressed the constructs in HEK293 cells and measured the uptake of the fluorescent

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Fig. 5 | The importance of the hydrogen bond triad between PH and S5 in mTRPV2<sub>Ca<sup>2+</sup></sub> in YO-PRO-1 permeation. a, Side view of the overlay of the mTRPV2<sub>Ca<sup>2+</sup></sub> (blue) and mTRPV2<sub>Ca<sup>2+</sup></sub> (red) subunits D and A, with RTx shown in stick and sphere representation and colored magenta. Binding of RTx to the mTRPV2<sub>Ca<sup>2+</sup></sub> would push down on the S4–S5 linker π-hinge (rotation plane indicated by dashed line) and force subunit A to rotate. b, A side view of the hydrogen bond triad in the widened subunit A. c, Side view of the hydrogen bond triad in the contracted subunit B. In addition, residue Y632 from the neighboring subunit forms a hydrogen bond with the backbone carbonyl of I603 in the pore helix. d, e, YO-PRO-1 dye uptake by HEK293T cells expressing TRPV2<sub>QM</sub>, Y542A, T602A and Y632A (d) and their peak fluorescence intensities (e). YO-PRO-1 uptake was triggered by addition of 250 nM RTx (black arrow). TRPV2-expressing cells (identified by cotransfected mCherry) from ≥3 repeats of imaging experiments were chosen for analysis; the total number of cells analyzed are noted in parentheses in e. a.u., arbitrary units. f, g, Representative inside-out patch recordings from HEK293T cells expressing TRPV2<sub>QM</sub>, Y542A, T602A and Y632A before and after intracellular application of 10 µM YO-PRO-1 (green trace in f) or 250 µM TBA (purple trace in g). h, i, Quantification of current inhibition by YO-PRO-1 (h) or TBA (i) of patches from cells expressing TRPV2<sub>QM</sub>, Y542A, T602A and Y632A. The number of patches is noted in parentheses. Data in d, e, h, i are presented as mean ± s.e.m.; bar graphs also show individual data points. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, two-tailed unpaired Student's t test. For YO-PRO-1 experiments, P = 0.0035 for Y542A, P < 0.0001 for T602A, P < 0.0001 for Y632A. For TBA experiments, P = 0.0481 for T602A, P < 0.0001 for Y632A, P = 0.2834 for Y542A and P = 0.355 for Y632F.
molecule YO-PRO-1 (M.W. 376 Da) upon application of RTx. We observed that YO-PRO-1 was rapidly taken up by the full-length TRPV2QM- and miTRPV2 QM-expressing cells, which is consistent with previous reports on YO-PRO-1 permeation in TRPV1 (refs 15,22) (Fig. 4a,b). We also observed YO-PRO-1 uptake by wtTRPV2- and TRPV2 QM-expressing cells upon application of 2-APB (Supplementary Fig. 2h), indicating that 2-APB can also trigger similar rearrangement of the SF gate to allow YO-PRO-1 ions to go through. However, the 2-APB-facilitated YO-PRO-1 uptake was slower than the RTx-induced YO-PRO-1 uptake (Fig. 4a), probably because 2-APB is a weaker agonist for TRPV2 than RTx is for TRPV2 QM. The YO-PRO-1 uptake was completely blocked by 50 μM extracellular RuR (Fig. 4a,b). As RuR is a pore blocker for TRPV2 channels (Fig. 1d), this result indicates that YO-PRO-1 enters the cells through the SF gate of the TRPV2 channels. To further test whether YO-PRO-1 directly permeates through the TRPV2 SF gate, we examined the effects of YO-PRO-1 on Na⁺ permeation through the TRPV2 channel. When YO-PRO-1 was applied from the extracellular side, inward Na⁺ currents were reduced in a dose-dependent manner, with minimal effect on the outward Na⁺ currents, and vice versa (Figs. 4c,d and 5f). It is worth noting that the blockade effect of YO-PRO-1 depends on membrane voltage: the larger the driving force, the stronger the blockade effect. Considering the dose- and voltage-dependence of YO-PRO-1 blockade of Na⁺ currents through TRPV2 from both sides of the membrane (Figs. 4c,d and 5f) and its uptake through the TRPV2 channel pore (Fig. 4a,b), we conclude that the large cation YO-PRO-1 serves as a permeant pore blocker that can go through the SF gate and compete with smaller metal ions for the permeation pathway of TRPV2. Owing to its large size, the permeability of YO-PRO-1 must be low, but it is sufficient to limit Na⁺ permeation in our patch-clamp recordings and to enable fluorescent labeling of nucleic acids inside the cell in our dye-uptake experiment. Taking these results together, we conclude that large

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**Fig. 6 | Functional characterization of the rat TRPV1 T641A mutant.**

a, Hydrogen bond networks around the pore of the closed TRPV1 channel (PDB 5IRZ). A tight network of hydrogen bonds is present at the Y584-T641-Y666 triad and between Y671 and I642. Hydrogen-bonding is absent at the subunit interface of the DkTx/RTx-bound TRPV1 channel (PDB 5IRX). b,c, YO-PRO-1 dye uptake by HEK293T cells expressing rat TRPV1 (b) and their peak fluorescence intensities (c). YO-PRO-1 uptake was triggered by the addition of 250 nM RTx (black arrow). TRPV1-expressing cells (identified by cotransfected mCherry) from ≥3 repeats of imaging experiments were chosen for analysis, and the total numbers are noted in parentheses. d,e, Representative inside-out recordings of TRPV1 WT (d) and TRPV1 T641A (e) (equivalent to the TRPV2 T602A) before (black trace) and after (green trace) intracellular application of 10 μM YO-PRO-1. f, Quantification of current inhibition by YO-PRO-1 of TRPV1 WT and TRPV1 T641A. Data are presented as mean ± s.e.m.; in bar graphs, individual data points are also shown. P < 0.0001 for T641A, two-tailed unpaired Student's t test.

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suggests that binding of RTx in this mode is RPV2, as it would clash with L535 at and the corresponding subunit. In the contracted subunits, this residue lines the miTRPV2,Ca-hingeS4–S5, which results in a different conformation via the π to push down on the base of S5 and force the S4–S5 linker to bend.

Comparison of flexible domains in KV and TRPV2 channels. To further probe the importance of a two-fold symmetric SF gate widening in large organic cation permeation, we tested whether TRPV2QM can permeate tetrabutylammonium (TBA) ions. TBA is smaller than YO-PRO-1 (M.W. 242 Da versus 376 Da), but unlike YO-PRO-1, it is a symmetric molecule. We found that TBA blocks TRPV2QM in a manner similar to RuR (Fig. 5g,i). This finding suggests that the pore might open in a two-fold symmetric manner during gating and that the TBA ion, despite its smaller size, cannot permeate, because its symmetric shape is incompatible with the asymmetric opening at the SF gate. The Y632A mutation significantly impaired the ability of TBA to block the Na+ current, which is consistent with the impaired blocking of currents by YO-PRO-1 in this mutant (Fig. 5g,i).

In the TRPV1 structures, we found that the corresponding hydro- gen-bond triad exists in the closed state of TRPV1 (apoTRPV1), whereas the hydrogen-bond triad is broken in the open state of TRPV1 (DkTx and RTX-bound TRPV1) (Fig. 6a). The DkTx- and RTX-bound TRPV1 pore is slightly expanded compared to that of apoTRPV1, analogous to the widened subunit of miTRPV2_{RTX,Ca}^{+}. In order to test whether these interactions are also important for permeation of large organic cationic molecules in TRPV1, we generated point mutations T641A and Y584A and performed the same set of electrophysiological experiments. While Y584A did not generate appreciable Na+ currents, we found that T641A produced robust Na+ currents. Notably, the uptake of YO-PRO-1 by the

Two-fold symmetric SF gate widening for YO-PRO-1 permeation. Previous structural studies of TRPV1 have associated RTX primarily with opening of the common gate and DkTx with opening of the SF gate because of the proximity of these toxin-bindings sites to the respective gates. Because our structure only contains RTx, it offers an opportunity to gain insight into the allosteric coupling between RTx binding and gating at the SF. Alignment of the widened subunit of miTRPV2_{RTX,Ca}^{+} and the corresponding subunit of miTRPV2_{Ca}^{+} suggests that binding of RTx in this mode is not compatible with miTRPV2_{Ca}^{+}, as it would clash with L535 at the base of S5 adjacent to π-hingeS4–S5 (Fig. 5a). RTx binding appears to push down on the base of S5 and force the S4–S5 linker to bend into a different conformation via the π-hingeS4–S5, which results in a rotation and consequent widening of the entire subunit. The RTx molecule in the contracted subunits is bound in a different mode and does not appear to exert the same pressure on the π-hingeS4–S5, which would explain why it does not elicit widening of the subunit (Supplementary Fig. 7).

Interestingly, the pore helix of the contracted subunits is supported by a hydrogen bond triad that consists of Y542 in S5, T602 in the pore helix, and Y627 in S6. By contrast, the hydrogen bond network at the Y542-T602-Y627 triad is broken in the widened subunit (Figs. 3b,c). This hydrogen bond triad is also broken in the structure of miTRPV2_{QMM}, which might account for its distinct pore helix position (Supplementary Fig. 7). Away from the hydrogen bond triad, Y632 assumes two distinct conformations in the two subunits of miTRPV2_{RTX,Ca}^{+}. In the contracted subunits, this residue lines the cavity and provides an apparent ion coordination site (Figs. 3b and 5b), and in the widened subunit, it interacts with the pore helix of the neighboring contracted subunit (Figs. 3c and 5c).

To test whether the conformation observed in the miTRPV2_{RTX,Ca}^{+} is important for permeation of large molecules, we introduced point mutations to residues Y542, T602, Y627, which form the hydrogen bond triad, and Y632, which lines the cavity, in full-length TRPV2_{QMM}. We tested the effects of the point mutations on the permeation of YO-PRO-1, on the RTX-induced Na+ currents, and on the reduction of Na+ currents by YO-PRO-1 (Fig. 5d–i). Because we observed that the Y627A mutant does not exhibit any appreciable currents, we excluded it from our subsequent analyses. Intriguingly, the uptake of YO-PRO-1 by the T602A mutant was significantly reduced and slowed, while its apparent Na+ conductance was preserved. Furthermore, the reduction of Na+ currents by YO-PRO-1 was substantially impaired in this mutant (Fig. 5d–f,h). This effect may be due to an increase in the size or width of the cavity in Y632A, resulting from removal of a bulky aromatic residue.

The Y632A mutation exhibited a robust YO-PRO-1 uptake and large sodium currents. However, YO-PRO-1-mediated reduction of Na+ currents was substantially impaired in this mutant (Fig. 5d–j). This result may be due to an increase in the size or width of the cavity in Y632A, resulting from removal of a bulky aromatic residue.

Uptake of Na+ by YO-PRO-1 and reduction of Na+ conductance, and increased permeation of large organic cationic molecules. In order to test whether these interactions are also important for permeation of large organic cationic molecules in TRPV1, we-generated point mutations T641A and Y584A and performed the same set of electrophysiological experiments. While Y584A did not generate appreciable Na+ currents, we found that T641A produced robust Na+ currents. Notably, the uptake of YO-PRO-1 by the

Fig. 7 | Comparison of flexible domains in Kv and TRPV2 channels. Kᵥ (left), (Kᵥ1.2–2.1 paddle chimera, PDB 2R9R) with the mobile regions, S3b, S4 and S4–S5 linker, colored in red. The arrow shows the motion of the mobile regions. The voltage-sensing domain is able to move freely, as it is not impeded by extensive interactions with the pore-forming domains. In TRPV2 (right) the VSLD is tightly coupled to the pore-forming domains. This makes independent movement of the VSLD unlikely, and instead, we observed rotation of the entire subunit (dotted oval), which is dictated by the mobile regions (colored in red).
T641A mutant was significantly reduced and slowed, but the reduction of Na\(^+\) currents by YO-PRO-1 was enhanced by the mutation, consistent with the effects of the T602A mutation in TRPV2\(_{9\alpha}\) (Fig. 6b–f). These data suggest that, like in TRPV2, a two-fold symmetric arrangement of subunits in TRPV1 plays a role in permeation of large cationic molecules.

**Discussion**

Here, we have shown that the SF gate of TRPV2 can adopt multiple conformations through different symmetrical arrangements in the pore and that these distinct conformations are associated with differential Ca\(^{2+}\) binding in the pore, suggesting that the ion permeation and channel gating is coupled in the TRPV1 and TRPV2 channels through the SF gate.

It is also worth noting that during our crystallization efforts, we encountered crystals with two-fold symmetric arrangements of the channel in the absence of Ca\(^{2+}\), as well as crystals with a tetragonal space group (a four-fold symmetric arrangement) in the presence of Ca\(^{2+}\) (L.Z. and S.-Y.L., unpublished observations), suggesting that Ca\(^{2+}\) does not play a role in the two-fold symmetric arrangement of the channel and that the channel can adopt a four-fold symmetric conformation in the crystal. Furthermore, the Y542-T602-Y627 triad interactions that we showed are important in the permeation of large cations in TRPV2 have only been observed in the crystal structures of TRPV2, further supporting the physiological relevance of the two-fold symmetric arrangements in TRPV2 permeation and gating. However, we cannot distinguish whether the observed two-fold symmetric arrangement of the SF within the TRPV2 tetramer upon RTX binding represents an open state or an important intermediate state on the conformational path toward a fully open symmetric state for large organic cation permeation. Interestingly, asymmetric gating at the SF has been observed in MD simulations of the TRPV1 channel19.

Symmetry breaking in channel gating has been reported in structural studies of the homo-oligomeric Mg\(^{2+}\) ion channel CorA\(^{20}\). However, whereas CorA assumes an asymmetric open conformation in the absence of its ligand, Mg\(^{2+}\), symmetry breaking in TRPV2 appears to be elicited by ligand binding. How does ligand-induced break (or reduction) in the symmetrical quaternary structure arrangement in TRPV2 occur? Because TRPV channels adopt a domain-swapped arrangement with the S4–S5 linker joining neighboring subunits, we reasoned that ligand binding to the S4–S5 linker influences the rearrangement of subunits. In the recent cryo-EM structural studies of the Na\(^{+}\)-activated K\(^{+}\) channel Slo2.2, which is not domain swapped, MacKinnon and colleagues tracked the structural changes in the conformational ensemble of the Slo2.2 channel upon increasing the concentration of Na\(^{+}\) (ref. 40). However, they observed no apparent symmetric arrangements during the structural transitions, consistent with the idea of the importance of the domain-swapped configuration for reduced symmetric conformational transitions. However, this raises the question of why reduced symmetric quaternary structural arrangements during channel gating have never been observed in voltage-gated K\(^{+}\) (K\(\alpha\)) channels that do adopt a domain-swapped arrangement. We speculate that this distinction between TRPV1 and TRPV2 and K\(\alpha\) channels arises from the differences in design of the dynamic and static regions within these channels. First, the interactions between S5 and S6 (pore domain) and the VSLD within the subunit are tight in TRPV1 and TRPV2 channels so that each subunit (VSLD and pore) can move as an independent unit. In K\(\alpha\), the mobile S4 of the voltage-sensing domain does not interact tightly with the pore domain (Fig. 7). Second, in order to maintain the high K\(^{+}\) selectivity, K\(\alpha\) channels are rigidly structured around the SF, the pore helix, pore loop, S5 and S6, which prevents them from entering conformations with reduced symmetry. Similarly, the crystal structure of the Ca\(^{2+}\)-selective TRPV6 channel, in which the SF does not act as a gate, revealed a tight network of interactions around the SF, which could explain why TRPV6 has only been captured in C4-symmetric arrangements\(^{41}\). By contrast, the nonselective TRPV1 and TRPV2 channels possess more flexibility around the SF gate which renders them capable of adopting two-fold symmetric pore arrangements (Fig. 7).

Our structural and functional studies illustrate the plasticity within the TRPV2 channel that enables permeation of large cations and show that this plasticity originates from the diverse conformations of the S4–S5 linker that dictates the quaternary structure of the channel.

**Methods**

Methods, including statements of data availability and any associated accession codes and references, are available at https://doi.org/10.1038/s41594-018-0059-z.

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Author contributions
L.Z. crystallized the protein and solved the structures under the guidance of S.-Y.L. S.L. carried out all electrophysiological experiments under the guidance of H.Y., S.-Y.L., L.Z., S.L. and H.Y. wrote the paper. All authors discussed the results and commented on the manuscript.

Competing interests
The authors declare no competing interests.

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Methods

Expression and purification. The miTRPV2 construct was prepared as previously described. In brief, the codon-optimized gene for the full-length rabbit TRPV2 was synthesized, and the truncated construct, miTRPV2, containing residues 57–561 and 583–722 was cloned into a pBluescript vector with a C-terminal FLAG affinity tag. Baculovirus was produced according to manufacturer’s protocol (Invitrogen, Bac-to-Bac). To obtain the miTRPV2 construct, point mutations F470S, L505M, and F470H were introduced into the miTRPV2 construct. For protein expression, Sf9 insect cells were infected with baculovirus at a density of 1.3 × 10^6 cells ml⁻¹ and grown at 27 °C for 72 h in an orbital shaker. After 72 h, cell pellets were collected, resuspended in buffer A (50 mM TRIS, pH 8, 150 mM NaCl, 2 mM CaCl₂, 1 mM MgCl₂, leupeptin, 1.5 µg ml⁻¹ pepstatin, 0.84 µg ml⁻¹ aprotinin, 0.3 mM PMSF, 14.3 mM β-mercaptoethanol, and DNase) and broken by sonication (3×30 pulses).

miTRPV2 was prepared for crystallization as follows: 40 mM dodecyl β-maltoside (DDM, Anacrace) and 4 mM cholesteryl hemimucin tris salt (CHS, Sigma) were added to the lysate for extraction at 4 °C for 1 h. Insoluble material was removed by centrifugation (8,000g, 30 min), and anti-FLAG resin was added to the supernatant for 1 h at 4 °C.

After incubation, the resin was loaded onto a Bio-Rad column and washed with Buffer B (50 mM TRIS, pH 8, 150 mM NaCl, 2 mM CaCl₂, 0.1 mM dodecyl maltoside neopentyl glycol (DMNG, Anacrace), and 0.1 mM CHS, 0.1 mg ml⁻¹ 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC, Avanti Polar Lipids), and 10 mM DTT) before being eluted in five column volumes of buffer C (50 mM TRIS, pH 8, 150 mM NaCl, 0.1 mM DMNG, 0.1 mM CHS, 0.1 mg ml⁻¹ DMPC, 10 mM DTT, and 0.1 mg ml⁻¹ FLAG peptide). The eluate was incubated overnight at 4 °C with 3.3 mg ml⁻¹ Tris (2-carboxyethyl) phosphine hydrochloride (TCEP, Sigma Aldrich) and PreScission protease. PreScission protease and 3 mM TCEP were added to the resin, and the resin was incubated for 18 h at 4 °C. The cell pellets were harvested by centrifugation, and the protein was prepared as described for miTRPV2 except without β-mercaptoethanol and DTT addition.

The miTRPV2 protein was extracted with 20 mM acetyl glucose neopentyl glycol (OGNG, Anacrace) and 2 mM CHS in the presence of 2 μM RTx (Sigma Aldrich) for 1 h at 4 °C. Following removal of insoluble matter by centrifugation (8,000g, 30 min), the extract was loaded onto anti-FLAG resin and incubated for 1 h at 4 °C.

The resin was washed with 10 × CV buffer B, (50 mM TRIS, pH 8, 150 mM NaCl, 2 mM CaCl₂, 2 mM OGNG, 0.2 mM CHS, 0.1 mg ml⁻¹ DMPC, 2 μM RTx, and 10 mM DTT) and the protein was eluted in buffer C, (50 mM TRIS, pH 8, 150 mM NaCl, 2 mM CaCl₂, 0.1 mM DMNG, 0.1 mM CHS, 0.1 mg ml⁻¹ DMPC, 2 μM RTx, 10 mM DTT, and 0.1 mg ml⁻¹ FLAG peptide). The eluate was incubated over night at 4 °C with PreScission protease and 3 μM TCEP before being subjected to gel filtration on a Superose 6 column.

Crystallization, structure determination and refinement. Following size exclusion chromatography on a Superose 6 column (GE Healthcare), the miTRPV2 protein peak was collected and concentrated to 8–10 mg ml⁻¹, supplemented with 5 mM TCEP and mixed with crystallization solution at a 1:1 ratio in 96-well sitting drop plates. The miTRPV2 protein data were low-pass filtered at 5000 Å (Apollonios), and the resin was removed. The crystals of transmembrane helices and ligands. The quality of the map for the final miTRPV2 structure was determined by measuring the voltage at which the current was zero, and five consecutive NMCD+ current traces were averaged for each recording. The shift in Eᵥ was calculated by subtracting the Eᵥ of NMCD+ from the Eᵥ measured in symmetrical Na+.

The permeability ratio P_H2O/P_Ca was calculated using the equation:

\[ P_{H2O}/P_{Ca} = [Na⁺]_{out}/[Na⁺]_{in} \times \exp(\Delta \Gamma_{on}/R/T) \]

where \( \Delta \Gamma_{on} \) is the measured reversal potential shift, \( F \) is the Faraday’s constant (96,485 C mol⁻¹), \( R \) is the gas constant (8.314 J mol⁻¹ K⁻¹), \( I \) is the absolute temperature (298.15 K at 25 °C), and \([Na⁺]_{in}\) and \([Na⁺]_{out}\) are the concentrations (in mM) of extracellular Na+ and intracellular Na+, respectively. Source data for electrophysiology experiments are available in Supplementary Dataset 1.
YO-PRO-1 dye uptake assay. YO-PRO-1 dye uptake experiments were done at room temperature. Transfected cells reseeded on PDL, and laminin-coated glass coverslips were washed twice with Dulbecco’s PBS (Sigma) containing 0.2 mM KCl, 0.2 mM KH$_2$PO$_4$, 8 mM NaCl and 1.15 mM Na$_2$HPO$_4$. Washed cells were incubated for 5 min at 37°C in 10 μM YO-PRO-1 imaging solution made from diluting 1 mM YO-PRO-1 DMSO stock solution (ThermoFisher) in Dulbecco’s PBS. YO-PRO-1 uptake was triggered by the addition of 250 nM RTx. Time-dependent YO-PRO-1 uptake by mCherry-positive cells was monitored by an inverted fluorescence microscope (IX73, Olympus America Inc.) using a FITC channel (excitation wavelength 470/40 nm; emission wavelength 525/50 nm). Images were acquired by a CMOS camera (Prime 95b, Photometrics) at 5 s intervals. MetaMorph software (Molecular Devices) was used to control the fluorophore excitation and image acquisition. MATLAB software (MathWorks) was used to analyze the time-dependent increase in YO-PRO-1 uptake. Source data for fluorescence experiments are available in Supplementary Dataset 1.

Reporting Summary. Further information on experimental design is available in the Nature Research Reporting Summary.

Data availability. The sequence of rabbit TRPV2 can be found in the National Center for Biotechnology Information (accession code XM_017349044). Coordinates and structure factors are deposited in the Protein Data Bank with accession codes PDB 6BWJ and PDB 6BWM. Source data for all electrophysiology and fluorescence imaging experiments (Figs. 1c,d, 4a–d, 5d–i, 6b–f and Supplementary Figs. 2a–h and 8a–d) are available in Supplementary Dataset 1.

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Life Sciences Reporting Summary

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- **Experimental design**

1. **Sample size**
   - Describe how sample size was determined.
   - For electrophysiology and imaging, sample sizes represent the number of cells used for recordings. The sizes were chosen based on past experiences and on the numbers to reach statistic significance.

2. **Data exclusions**
   - Describe any data exclusions.
   - For electrophysiology and imaging, no data was excluded unless the recording quality was poor due to factors such as large noise or instability.

3. **Replication**
   - Describe the measures taken to verify the reproducibility of the experimental findings.
   - Experiments were reproduced according to the sample size as indicated in each figure.

4. **Randomization**
   - Describe how samples/organisms/participants were allocated into experimental groups.
   - For electrophysiology and imaging, samples were grouped based on genes of interest transfected into the cells.

5. **Blinding**
   - Describe whether the investigators were blinded to group allocation during data collection and/or analysis.
   - Investigators were not blinded to group allocation.

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.

6. **Statistical parameters**
   - For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

   - n/a
   - Confirmed

   - The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)

   - A statement indicating how many times each experiment was replicated

   - Only common tests should be described solely by name; describe more complex techniques in the Methods section.

   - A description of any assumptions or corrections, such as an adjustment for multiple comparisons

   - Test values indicating whether an effect is present

   - A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)

   - Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation)

**See the web collection on statistics for biologists for further resources and guidance.**
Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

For electrophysiology: Clampfit 10 (Molecular Devices) and Microsoft Excel (Microsoft).
For imaging: MetaMorph (Molecular Devices), MATLAB (MathWorks) and Microsoft Excel (Microsoft).
For crystallography: XDS, CCP4, Phenix.
For model building, refinement and structural analysis: Coot, Phenix, PYMOL.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository [e.g. GitHub]. Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.

No restrictions

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

N/A

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

HEK293T cells were purchased from ATCC.

b. Describe the method of cell line authentication used.

The cell line was authenticated by ATCC and no further authentication was performed.
c. Report whether the cell lines were tested for mycoplasma contamination.

Cells were tested negative for mycoplasma at Duke Cell Culture Facility

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

N/A

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide all relevant details on animals and/or animal-derived materials used in the study.

N/A

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

N/A