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High-resolution views of TRPV1 and their implications for the TRP channel superfamily

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Abstract

The first high-resolution structures of a near-full-length TRP channel were recently described, structures of the noxious heat receptor TRPV1 in the absence or presence of vanilloid agonists and a spider toxin. Here we briefly review the salient features, including the overall architecture, agonist binding sites, and conformational changes related to channel pore gating. We also discuss some of the structures' implications for the TRP channel family and a few of the many questions still left unanswered.

Keywords

TRPV1; cryoelectron microscopy; resiniferatoxin; gating; double-knot toxin; TRP box

The painful sensation evoked by noxious hot temperatures and pungent vanilloid compounds such as capsaicin (found in chili peppers) and resiniferatoxin (RTX; found in *Euphorbia resinifera* cacti-like plants) has been attributed to the activation of the transient receptor potential of subtype vanilloid 1 (TRPV1) ion channel, making TRPV1 an important therapeutic pharmacological target (Szallasi and Sheta, 2012). TRPV1 is expressed in peripheral nociceptor neurons, where its activation initiates action potentials leading to an eventual burning pain sensation. TRPV1 is not only activated by natural plant vanilloids, but also by endogenous compounds related to arachidonic acid metabolites (Pingle et al., 2007; Starowicz et al., 2007), and by animal venom toxins including the vanillotoxin or double-knot toxin (DkTx) from *Psalmopoeus cambridgei* spiders ((Siemens et al., 2006); this book). Three recent structures of TRPV1 (Cao et al., 2013b; Liao et al., 2013), one in the absence of ligands – the “apo” state, one in the presence of both DkTx and RTX, and one in the presence of capsaicin, reveal molecular mechanisms for channel gating and provide a 3D scaffold onto which to interpret or reinterpret decades of accumulated physiological, pharmacological and biochemical data on TRPV1 and other TRP channels. Here we provide a brief introduction to these structures.

Three-dimensional structure determination of TRPV1 by cryo-electron microscopy

TRPV1 is the namesake member of the vanilloid or TRPV subfamily of TRP channels. The homotetrameric TRPV channels contain a highly variable N-terminal cytoplasmic region that precedes six N-terminal ankyrin repeats, followed by a ~70-residue highly conserved linker region, a transmembrane domain formed by six membrane-spanning segments and a long pore-forming loop between the fifth and sixth segments, and a ~150-residue cytoplasmic C-terminal region.

To determine high-resolution structures of rat TRPV1, some modifications were introduced within its primary sequence, deleting regions predicted to be largely unstructured. Such protein engineering is commonly used in structural biology to improve sample homogeneity. Both the very N- and C-termini were removed, residues 1–109 and 765–838, respectively. In addition, the extracellular turret between the S5 and pore helices (residues 604–626), which includes the only glycosylation site (Rosenbaum et al., 2002), was deleted. Engineered TRPV1 was expressed in HEK293 cells, extracted in dodecylmaltoside detergent, purified, and stabilized by exchanging the detergent with amphipols (Popot et al., 2011), before structure determination. Importantly, this construct retained sensitivity to heat, capsaicin, pH and DkTx, as assessed in HEK293 cells by electrophysiology (Liao et al., 2013).

The TRPV1 structures were determined using single-particle reconstruction from cryoelectron microscopy (cryoEM) images. CryoEM has always had the theoretical ability to reach atomic resolution, but has been plagued by technical challenges that render images much “fuzzier” than their theoretical limit, greatly limiting the resolution of the resulting structures except in rare special cases such as very well ordered specimens of highly symmetric viral particles (Grigorieff and Harrison, 2011). However, the cryoEM technique has seen dramatic technical developments in the last few years (Li et al., 2013), and the TRPV1 structures are some of the first structures demonstrating how these improvements can yield essentially atomic resolution for large molecular complexes with low symmetry for which little-to-no structural information was previously available (Henderson, 2013). The technical developments can be summarized in two major advances. The new direct electron detectors have very high sensitivity, low noise and very fast readout, allowing the accumulation of images as movies (i.e. with a time component) rather than as still images (time-averaged). This then enables corrections for the electron beam-induced motion of the sample during image accumulation, generating images that are now exceptionally sharp. The new data format also enables clever processing at different steps in the structure-determination process. At first, averaging all frames of the “movies” yields high signal-to-noise to facilitate accurate semi-automated particle selection. Then eliminating the early frames which have the most beam-induced motion and later frames with too much sample damage produces the most accurate images for classification, averaging and structure determination. Finally, an important point is that the TRPV1 structure determination was a multistep iterative process with very conservative assessments of data quality, following the strictest standards in the field. Ultimately, fewer than 37% of the TRPV1 particles from the high-resolution dataset were selected to calculate the high-resolution maps. The published

structures therefore likely represent only one of multiple conformational states present in the sample. However, this should not be viewed as “cherry-picking” data, it is instead a judicious choice to use only the most similar and best defined particles to yield the highest resolution map – analogous to “crystallization without crystals” (Frank, 2006).

Overall Architecture of TRPV1

The TRPV1 structures are tetrameric (Fig. 1), as expected from biochemistry and sequence similarity to the ligand-gated and voltage-gated channel superfamily (Kedei et al., 2001). Notably, the four-fold symmetry was strictly enforced during data processing to obtain the TRPV1 structures, providing four-fold averaging to increase signal intensity. The fact that averaging improved the overall resolution indicates that the four subunits within each TRPV1 tetramer are indeed largely structurally equivalent. However, averaging would also conceal any potential local asymmetries.

The transmembrane domain of each TRPV1 subunit includes six transmembrane helices as well as a shorter pore helix. Similarly to voltage-gated ion channels such as Kv1.2 (Long et al., 2005), the transmembrane helices of each subunit form two distinct “bundles”. Viewed from the top, the S1–S4 bundles are located on the periphery of the channel, while the four S5–S6 bundles tetramerize to form the central ion pore (Fig. 1C). Importantly, S4 from one subunit interacts with S5 and S6 of the anti-clockwise adjacent subunit; in other words, each peripheral S1–S4 bundle is in close proximity to the S5–S6 bundle of another subunit. Similarly, the S1 helix of one subunit abuts the S5 and pore helices of the anti-clockwise neighbor. This same intertwined subunit organization of the transmembrane domain was first observed in the Shaker voltage-gated channel (Long et al., 2005).

As in many tetrameric ion channels, the S5–S6 region of TRPV1, including the intervening pore helix and reentrant loop, forms an “inverted teepee”-shaped pore (Fig. 2C). In contrast to the tall selectivity filter of potassium channels first observed in KcsA (Doyle et al., 1998), the TRPV1 pore contains a rather short constricted region that likely acts as the selectivity filter (Fig. 2C), consistent with the fact that TRPV1 is a rather non-selective cation channel (Caterina et al., 1997). The TRPV1 pore features two constrictions at glycine 643 and isoleucine 679, which have been assigned as the upper and lower gates, respectively (Fig. 2; more on gating below). In the extracellular (or “upper”) half of the membrane, the short pore helices form a funnel-like shape that tapers from the extracellular face of the channel into the membrane center. At the closest approach of the pore helix C-termini, the four glycine 643 residues point their carbonyl groups towards the center of the pore, forming the upper gate constriction and most likely contributing to cation selectivity. S6 residue tyrosine 671 sits just below glycine 643, consistent with its influence on ion selectivity (Mohapatra et al., 2003). The lower gate is located in the middle of the S6 helix, which lines the pore. The tilt of the S6 helices relative to the plane of the membrane means that they have a single crossing point corresponding to the lower gate, about 15 Å below the upper gate (Fig. 2B). This lower gate is formed by the four hydrophobic isoleucine 679 sidechains. Of note, the results of a cysteine-accessibility scan of the TRPV1 S6 helix (Salazar et al., 2009) are in partial, but not complete agreement with the cryoEM structure. Overall, the two gates of the TRPV1 pore are placed well within the center of the membrane plane and the tilted

arrangement of the four S5–S6 bundles allows for an aperture-like widening and narrowing of the two gates in response to stimuli.

Helix S6 leads C-terminally into the TRP box, a ~25-residue conserved sequence feature of TRPV, TRPC and TRPM channels (as well as TRPN channels) implicated in channel gating and regulation (Venkatachalam and Montell, 2007; Wu et al., 2010). Residues 690–711 of the TRPV1 TRP box form a long helix parallel to the membrane plane that emanates from the channel center and points towards the periphery, like a spoke on a wheel (Fig. 2D). Interestingly, this means that the N-terminal half of the TRP box helix sits just under the S4–S5 linker, while its C-terminal half lies under the S1–S4 bundle of its own subunit. The S4–S5 linker crosses the TRP box helix approximately perpendicularly, suggesting that movement of the TRP box will influence the S4–S5 linker and thus the pore, and vice versa (Fig. 2A–D). Factors acting on the S4–S5 linker – such as capsaicin or RTX (see below) – will therefore influence the orientation of the TRP box.

The S1–S4 bundle straddles the TRP box helix, with helices S1 and S4 on one side and S2 and S3 on the other (Fig. 2). In voltage-gated channels, the S1–S4 bundle forms the voltage sensor domain, with four highly conserved voltage-sensing arginines on the S4 helix that move across the membrane in response to a voltage stimulus (Catterall, 2010). TRP channels are much less voltage-sensitive than voltage-gated channels. Accordingly, few if any basic residues are present in the S4 helix of TRP channels. In TRPV1, the S1–S4 bundle adopts a structure analogous to that of a voltage sensor domain in a depolarized (activated) state (Long et al., 2005), both in the absence or presence of agonists, suggesting that the S1–S4 bundle is essentially static (Cao et al., 2013b). Moreover, its core packs many aromatic residues, further suggesting that it is not a dynamic structure. However, slight rigid-body motions of the entire bundle could readily be transmitted to the channel gates, with the TRP box helix acting as a lever to affect the S4–S5 linker and channel pore.

The TRPV1 intracellular regions, which are both N- and C-terminal to the transmembrane domain in the primary sequence, come together to form an upside-down bowl-shaped structure below the ion channel pore, resembling the “hanging gondola” (Kobertz et al., 2000) described for a large number of ion channels. This bowl opens a large cavity that is accessible to the cytoplasm (Fig. 1D). Although the importance of this intracellular shape is not yet clear, TRP channels interact with a large number of accessory molecules and proteins which may access their binding sites through this opening.

The N- and C-termini interact with each other through small interfaces (Fig. 1E) and connect to the transmembrane domain mainly through the TRP box helix (Fig. 1 and 2). In fact, the ~70-residue linker region between the N-terminal ankyrin repeat domain (ARD) and the S1 helix, or “ARD-S1 linker”, is clearly the hub of these interactions. The ARD-S1 linker, comprised of several short helices and a β -hairpin, wraps the TRP box helix from below, with a short “pre-S1” helix well-positioned for allosteric communications between the intracellular and transmembrane domains (Fig. 2). The ARD-S1-linker effectively extends the ARD, forming a docking site for the exposed face of the sixth ankyrin repeat. In addition a β -sheet formed from the ARD-S1-linker and the C-terminus is in contact with the ARD of an adjacent subunit (Fig. 1E).

The overall structure of the TRPV1 intracellular domains, when viewed from the intracellular side, resembles a right-handed four-bladed pinwheel with each blade corresponding to one ARD (Fig. 1D). Of note, the recently published low-resolution cryoEM structure of TRPV2 shows a left-handed pinwheel-shaped volume (Huynh et al., 2013). However, the structure determination method used for TRPV2, angular reconstitution, arbitrarily assigns handedness, requiring a separate test to determine absolute handedness (Rosenthal and Henderson, 2003). Therefore angular reconstitution can lead to an inadvertent inversion of the resulting 3D volume, particularly for low-resolution symmetric structures where chiral features such as right-handed α -helices are not distinguishable. From our own simple docking we conclude that the TRPV1 atomic model (Liao et al., 2013) indeed fits very well into an inverted TRPV2 volume, somewhat better than it fits in the published TRPV2 volume. Most importantly, this does suggest that TRPV1 and TRPV2 have very similar structures.

There is extensive accumulated knowledge about the structure of the TRPV ARDs ((Gaudet, 2008); see also Chapter X (Hellmich and Gaudet, 2014)). The TRPV1 ARD consists of six ankyrin repeats, each containing a pair of anti-parallel α -helices, the “inner” and “outer” helices, followed by a “finger” loop extending at a $\sim 90^\circ$ angle relative to the helical axes. The repeats stack side-by-side such that the inner helices and fingers form a concave surface (Lishko et al., 2007). This concave surface is often a site of ligand interactions in other ankyrin repeat-containing proteins (Gaudet, 2008). As predicted by a number of biochemical experiments (Gaudet, 2008, 2009), the TRPV1 ARDs do not interact directly with each other. Rather, as mentioned above, ARD interactions are mediated by a three-stranded β -sheet that connects the convex face of ankyrin repeat 6 from one subunit at one end and the concave ARD face at Finger 3 and ankyrin repeats 3 and 4 of the adjacent subunit at the other end. Two β -strands were assigned to the ARD-S1 linker and the third β -strand to the C-terminus (Fig. 2A–B; (Liao et al., 2013)).

However, the density in the β -sheet region is not as well defined as in the transmembrane region, preventing the unequivocal assignment of the sequence register of these strands, and from our own inspection of the publicly available density maps (Lawson et al., 2011), we believe that the current assignment should be considered tentative. Except for this tentative C-terminal β -strand, most of the 53 residues C-terminal to the TRP box could not be unambiguously resolved in the EM density map. Furthermore, the construct used for the structure determination of TRPV1 lacks the most C-terminal 73 residues, including a region that can interact with calmodulin (Lau et al., 2012; Numazaki et al., 2003) and is also important for the regulation of TRPV1 by phosphoinositides (Cao et al., 2013a; Prescott and Julius, 2003).

The N-terminal 109 residues were also deleted from the construct and replaced by a maltose-binding protein affinity tag that was then removed by proteolysis before TRPV1 structure determination (Liao et al., 2013). Surprisingly, the EM map shows no density for residues 110–195, corresponding to the first two ankyrin repeats, although the other four repeats are quite well resolved (Fig. 1A). This is unexpected because isolated TRPV channel ankyrin repeats have been very amenable to crystallographic trials and form stable, compact domains without much discernable intrinsic dynamics (Gaudet, 2008). It is unclear why no

density was observed for the first two ankyrin repeats, and the purification protocol suggests the possibility that they were proteolysed and therefore missing from the EM sample. Interestingly, the low-resolution TRPV2 3D volume does accommodate all six ankyrin repeats, both in the published model (Huynh et al., 2013) and in our own docking into the inverted volume.

The intracellular domains of TRPV1, while well-conserved within the TRPVs, are not common to the other TRP subfamilies. More specifically, while some elements, such as ankyrin repeats, recur in other TRP subfamilies, the TRPV1 sequence regions forming connections between intracellular domains and connecting them to the transmembrane domains (aside from the TRP box) are unique to the TRPV subfamily. Therefore, while the specific arrangement of the TRPV1 intracellular domains can serve as a scaffold to model other TRPVs and interpret biochemical and functional data, it provides only conceptual suggestions of how the other TRP channel intracellular domains are arranged. For example, the ARD-S1 linker region of TRPA1 harbors highly conserved cysteine residues that are covalently modified by electrophilic agonists to activate the channel (Hinman et al., 2006; Kang et al., 2010; Macpherson et al., 2007). The TRPA1 linker has no significant sequence similarity to TRPV1 and its architecture may not follow that of TRPV1, but could similarly form a complex docking structure for the large 17-repeat TRPA1 ARD.

Two gates to the TRPV1 pore

In addition to the TRPV1 structure in its apo state, two structures in the presence of potent agonists provide insights into ligand gating of the channel (Cao et al., 2013b). A comparison of all three available structures suggests the presence of two gates, an upper and a lower gate (Fig. 2; (Cao et al., 2013b; Liao et al., 2013)). A structure of TRPV1 bound to both RTX and DkTx, two strong TRPV1 agonists, has the widest pore, which appears to be wide enough to represent a conducting state. Another structure of TRPV1 in the presence of 50 μ M capsaicin showed different conformational changes that suggest a shift to a semi-open, possibly corresponding to the previously observed flickering open state (Cao et al., 2013b; Hui et al., 2003).

DkTx is a toxin from *Ornithoctonus huwena* tarantulas that irreversibly activates TRPV1 and leads to enhanced Ca^{2+} influx through the pore thereby eliciting a pain response (Siemens et al., 2006). This peptide toxin belongs to the family of inhibitor cysteine knot (ICK) toxins and consists of two ICKs with nearly identical sequences connected by a short linker to form a potent bifunctional molecule. Four knots – thought to be from two DkTx molecules – are bound to one TRPV1 tetramer (Fig. 2E–F; (Cao et al., 2013b)). Each knot is docked at the extracellular membrane interface, interacting with the S4 and pore helices of one subunit and the S6 helix of a second subunit. DkTx is thus perfectly placed to act upon the ion channel pore. Interestingly, DkTx is in close proximity to the deleted extracellular turret (residues 604–626) raising the possibility that it can interact with these residues in wildtype TRPV1. This is consistent with the moderately reduced EC_{50} of DkTx for the deletion construct over wildtype TRPV1 (Liao et al., 2013).

Residues that affect TRPV1 sensitivity to vanilloid compounds such as capsaicin, RTX and the antagonist capsazepine, had been identified primarily in transmembrane segments S3–S4 (e.g. (Chou et al., 2004; Gavva et al., 2004; Jordt et al., 2000; Phillips et al., 2004)), leading to an expectation that vanilloids would bind to the S1–S4 bundle. EM density consistent with RTX was found in a hydrophobic, membrane-embedded cavity formed by helices S3 and S4 and the S4–S5 linker of one subunit, as well as helices S5 and S6 of the adjacent subunit (Fig. 2E). When viewing TRPV1 from the extracellular site, this vanilloid-binding pocket sits roughly below the DkTx-binding site. The structure determined in the presence of capsaicin showed a smaller EM density feature in the same pocket, consistent with one common binding site for vanilloids (Cao et al., 2013b). This is consistent with previous data indicating that capsaicin approaches its binding site from the cytoplasmic side (Jung et al., 1999). Of note, neither the RTX or capsaicin density was well-defined enough to distinguish the orientation of the ligands in the pocket. Additionally, the authors also observed some density in the same site in the apo structure, possibly corresponding to a lipid or detergent molecule (Cao et al., 2013b), suggesting that this site is in exchange with lipid molecules. TRPV1 is also activated by lipids and lipophilic molecules such as anandamide and diacylglycerols. Consistently, previous studies already indicated a shared binding site for diacylglycerols and capsaicin, and anandamide and RTX, respectively (Woo et al., 2008; Zygmunt et al., 1999).

In both agonist-bound TRPV1 structures the pore appears open. Capsaicin mainly increased the diameter of the lower gate, while the interplay of DkTx and RTX increased the diameter of both upper and lower gates (Cao et al., 2013b). Because the structural effects on TRPV1 of either DkTx or RTX on their own have not yet been investigated, some assumptions are required to develop a model of allosteric gating by TRPV1 ligands. The effects on the upper gate were assigned to DkTx based on its extracellular position. Similarly, vanilloids were inferred to act mainly through the lower gate. A simplified gating model is illustrated in Fig. 3. In the apo state, the TRPV1 pore is closed at both the upper and lower gates (G643 and I679, see also Fig. 2C). Vanilloid binding between the S1–S4 and S5–S6 bundles affects the S4–S5 linker, leading to a slight tilt of the TRP box helix and iris-like opening of the connecting S6 helix lining the pore, opening the lower gate. In contrast, DkTx approaches from the extracellular side, leading to opening of the upper gate through binding and slight displacement of the pore helix (Fig. 2C,F). Because DkTx also interacts with helix S6, it may affect the lower gate as well.

The current high-resolution structures have yet to elucidate the physiologically important mechanism of heat sensing by TRPV1. Mutagenesis experiments have indicated the involvement of a number of regions in TRPV1 channel gating by heat, including the pore (Grandl et al., 2010), the extracellular turrets ((Cui et al., 2012; Yang et al., 2010); results which have been controversial (Yao et al., 2010)), as well as the ankyrin repeats and ARD-S1 linker (Yao et al., 2011). These results suggest that either or both gates could be modulated by the temperature-induced conformational changes.

Intracellular signaling mechanisms

The intracellular domains of TRP channels are the most variable regions within the TRP protein family. They are also known to be important for channel function – deletions and mutations are often deleterious to channel function, even causing various channelopathies (Nilius and Owsianik, 2010; Nilius and Voets, 2013). The lack of similarity of these domains and their arrangements to that of other proteins has largely prevented the reliable prediction of their 3D connections and arrangements, although such connections were anticipated.

The structures of TRPV1 do indeed show that the N-terminal region of one subunit is connected to the C-terminal region of the adjacent subunit, with the whole intracellular assembly forming a large inverted-bowl shaped structure below the channel pore (Fig. 1D). However, the connections between the subunits are relatively small in surface area, leaving open the possibility that they are transient rather than permanent. Also, the density of the intracellular domains is less well defined than the transmembrane domain, suggesting that it is either less symmetric or more flexible, or both. The highly conserved concave surface of the TRPV1 ARD, which has been implicated in ligand interactions (Gaudet, 2008; Lishko et al., 2007; Phelps et al., 2007; Phelps et al., 2010), is largely exposed to the inside of the bowl, including several of the residues that cause constitutive activity and/or lack of desensitization. However, some of the concave surface is involved in the intracellular assembly, interacting with the three-stranded β -sheet (Fig. 1D). It is possible that the main role of the TRPV1 ARD is to interact with other regions within the channel. Alternatively, there may be physiological states of the channel in which the ARD detaches from the linker to interact with other cellular partners.

The C-terminal region of TRPV1 implicated in PIP₂ regulation (Cao et al., 2013a; Prescott and Julius, 2003) and calmodulin binding (Lau et al., 2012; Numazaki et al., 2003) was not included in the construct used to determine these first structures. The only structural element assigned to the C-terminal region is a single β -strand. There is also unassigned density on the outside of the bowl near the ARD-S1 linker (Fig. 1A). Therefore, there are many possible locations for the remaining ~100 C-terminal residues, which could either form a membrane-proximal collar on the outside of the currently available structure, or form a plug underneath the intracellular pore opening. Similar speculations can be made for the ~110 missing N-terminal residues, although in contrast to the C-terminus, their removal has little consequence in channel function (Jung et al., 2002; Vlachova et al., 2003).

Conclusion

In summary, the high-resolution TRPV1 structures determined by cryoEM represent a huge leap in our understanding of the TRPV channel architecture. The TRP box seems perfectly positioned nexus to communicate information about stimuli that interact with TRPV1's many domains to the two gates that guard the pore. The TRPV1 intracellular domains form an upside-down bowl structure underneath the pore, with the ARD-S1 linker connecting ARDs to each other and to the TRP box helix. Based on sequence conservation in the TRP channel family, a similar TRP box helix is most likely present at least in the TRPC, TRPM and TRPN family members. However, the structure of the TRPV1 intracellular domains are

likely to only be representative of the TRPV subfamily, and visualizing the intracellular domain architecture of the other subfamilies will require additional structural studies. Finally, it is worth emphasizing that although crystallography will still play a part in discovering atomic-level details crucial for pharmacology, the TRPV1 structures also represent a breakthrough in high-resolution structure determination by single-particle cryoEM, which will likely play a transformative role in future TRP channel structural biology.

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Fig. 1. High-resolution TRPV1 cryoEM structure

(A) The cryoEM density (transparent surface) is superimposed on the TRPV1 molecular model (pdb: 3j5p). Note that no density was observed for the first two ankyrin repeats (bracket). An arrow points to unmodeled cryoEM density near the ARD-S1 linker. (B) One subunit is shown as colored cartoon, while the other three are shown as white surface. The view is rotated 60° on the vertical axis relative to (A). Both (A) and (B) represent “side” views, parallel to the membrane plane. The approximate location of the membrane is indicated by black bars. (C) View of the TRPV1 tetramer from the extracellular “top” face. Two subunits are colored and two are white, to highlight the intertwining of the

transmembrane domains. (D) Intracellular “bottom” face of the TRPV1 tetramer as a surface representation, emphasizing the “bowl” shape of the intracellular domains. One subunit is colored. (E) Inset shows the relatively small interface between an ARD and the β -sheet of the adjacent subunit. All panels follow the same color scheme: ARD, cyan; ARD-S1 linker, yellow; S1–S4 bundle, lilac; S5–S6 pore region, blue; TRP box helix, red; C-terminus, green.

Fig. 2. Details of the TRPV1 structure

(A–B) The TRP box is straddled by the S1–S4 helix bundle from above and buttressed by the ARD-S1-linker from below, placing it in a perfect position to communicate peripheral signals to the ion channel pore. The S4–S5 linker sits approximately perpendicularly above the TRP box and leads to the S5-pore helix-S6 pore region. The ARD was omitted for clarity. Only one (A) or two (B) subunits are shown for simplicity, viewed from the membrane plane. (C) The arrangement of the pore formed by constrictions at residues G643 at the C-terminus of the pore helix and I679 in helix S6 leading into the TRP box helix. (D) Cartoon representation of the transmembrane domain tetramer (residues 429 to 711) viewed from the intracellular side shows the TRP box helices arranged as spokes on a wheel. Gate residues 643 and 679 are shown as spheres. (E) The transmembrane region of the RTX and DkTx-bound TRPV1 tetramer. The RTX density, shown as a surface, is nudged in a crevasse formed by the S1–S4 bundle of one subunit and the pore region of the adjacent subunit. Each DkTx knot, shown as a mesh surface, binds the top of the S4 and pore helices from one subunit and the top of S6 from the adjacent subunit. (F) The open pore of the RTX and DkTx-bound TRPV1 structure (compare to panel C). Color coding is the same as in Fig. 1, with RTX in red and DkTx in green. Panels A–D represent the apo structure (pdb: 3j5p) and panels E–F the RTX and DkTx-bound structure (pdb: 3j5q).

Fig. 3. Model of TRPV1 channel gating by capsaicin, RTX and DkTx. (A) The TRPV1 pore is closed at both the upper and lower gates in the absence of ligands. (B–C) Upon agonist binding, the pore expands to allow ion flux. Different gates are affected depending on ligand type. (B) Capsaicin (chili pepper) acts upon the S4–S5 linker that lies on top of the TRP box. The TRP box thus communicates capsaicin binding to the pore and leads to opening of the lower gate in S6. (C) Upon binding of both RTX (chili pepper) and DkTx (spider), both the upper and lower gates open. RTX is presumed to have the same effect as capsaicin, thus opening the lower gate. DkTx binds from the extracellular side and could thus be responsible for the opening of the outer pore. Because it also interacts with the S6 helix, DkTx could also affect the lower gate. Color coding follows Fig. 1 and 2.