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Toward a High-Resolution Structure of IP₃R Channel

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Abstract

The ability of cells to maintain low levels of Ca²⁺ under resting conditions and to create rapid and transient increases in Ca²⁺ upon stimulation is a fundamental property of cellular Ca²⁺ signaling mechanism. An increase of cytosolic Ca²⁺ level in response to diverse stimuli is largely accounted for by the inositol 1,4,5-trisphosphate receptor (IP₃R) present in the endoplasmic reticulum membranes of virtually all eukaryotic cells. Extensive information is currently available on the function of IP₃Rs and their interaction with modulators. Very little, however, is known about their molecular architecture and therefore most critical issues surrounding gating of IP₃R channels are still ambiguous, including the central question of how opening of the IP₃R pore is initiated by IP₃ and Ca²⁺. Membrane proteins such as IP₃R channels have proven to be exceptionally difficult targets for structural analysis due to their large size, their location in the membrane environment, and their dynamic nature. To date, a 3D structure of complete IP₃R channel is determined by single-particle cryo-EM at intermediate resolution, and the best crystal structures of IP₃R are limited to a soluble portion of the cytoplasmic region representing ~15% of the entire channel protein. Together these efforts provide the important structural information for this class of ion channels and serve as the basis for further studies aiming at understanding of the IP₃R function.

Keywords

IP₃R; calcium signaling; 3D structure; electron cryomicroscopy; single-particle reconstruction

1. Introduction

Intracellular Ca²⁺ signaling is a strictly controlled spatial and temporal process guided by the orchestrated mobilization of Ca²⁺ into the cytoplasm, *via* Ca²⁺ channels, either from the extracellular milieu (Ca²⁺ influx) or from intracellular stores (Ca²⁺ release). Ca²⁺ release is mediated by intracellular ligand-gated Ca²⁺ release channels present in the endoplasmic (ER) and sarcoplasmic (SR) reticulum membranes of all eukaryotic cells. Two closely related families of intracellular Ca²⁺ release channels have been identified: the ryanodine

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receptor (RyR) and the inositol 1,4,5-trisphosphate receptor (IP₃R). While the RyR represents primary Ca²⁺ release channel in striated muscle, IP₃R channels are detected in the ER of all cell types with the highest densities in the Purkinje cells of cerebellum. Both channels share 30-40 % sequence identity within their C-terminal regions, containing predicted membrane-spanning domains [1,2]. This structural homology accounts for many functional similarities between IP₃R and RyR channels and suggests a common molecular architecture for the ion-permission pathway. Functional Ca²⁺ release channels form large tetrameric structures with a molecular mass of ~1.3 MDa for IP₃Rs and ~2.3 MDa for RyRs.

Ca²⁺ release via IP₃R/RyR channels is one of the most ubiquitous and versatile cellular signaling mechanisms that regulates diverse physiological functions, including muscle contraction, fertilization, hormone secretion, gene transcription, metabolic regulation, immune responses, apoptosis, learning and memory. Dysfunction of these channels has been implicated in abnormal intracellular Ca²⁺ levels associated with many pathological conditions in humans such as cardiac hypertrophy, heart failure, hereditary ataxias, osteoporosis, atherosclerosis, hypertension, some migraines, Alzheimer's disease, Huntington's disease, Malignant Hyperthermia, Central Core and Multi-minicore diseases [3-8]. The focus of this review article is on structural studies of IP₃R channels with primary emphasis on structure determination of the tetrameric channel. The long-standing controversy about the 3D structure of complete IP₃R has been a critical obstacle substantially slowing progress of the research aiming to understand structure-functional aspects of these key membrane proteins. Recently, the 3D structure of the full-length tetrameric IP₃R channel has been unambiguously determined by single-particle electron cryo-microscopy [9,10]. To date, electron cryo-microscopy (cryo-EM) has emerged as the most effective and straightforward technique for the study of macromolecular membrane protein assemblies and their interactions [11]. While X-ray crystallography has recently made strides, only ~2% of PDB entries are related to membrane proteins, whereas they represent an estimated 20-30% of expressed proteins in the genome [12]. Additionally, most of these entries represent only soluble fragments rather than intact integral membrane proteins. Among these are the crystal structures of the N-terminal IP₃-binding domains of type 1 IP₃R [13-16]. However, a 3D structure of complete channel is critical to be able to trace the coordination of ligand-induced movements throughout the channel assembly and to establish a structural basis for the channel gating. This review summarizes the current knowledge of the 3D structure of IP₃R and discusses new insights gained into IP₃R channel function.

2. Diversity within the IP₃ R channel family

Since it was discovered over two decades ago that specific proteins tightly bound to ER membranes function as Ca²⁺-permeable ion channels activated by selective ligand inositol 1, 4, 5-trisphosphate (IP₃) [17-19], substantial efforts have been made to understand the mechanism of action of these ion channels (Fig. 1). Cloning of receptor proteins established that IP₃R is an unusually large membrane protein, comprising four subunits of ~2,700 amino acid residues each (Fig. 2) [1,2]. Furthermore, mammalian genes have been identified encoding three homologous isoforms (types 1-3) of the receptor that share ~70% overall sequence identity which increases to ~90% within the predicted transmembrane (TM)

region. Each type shows distinct properties in terms of modulation by endogenous and exogenous ligands and tissue distribution. IP₃R diversity is further expanded by alternative gene splicing within the cytoplasmic region (Fig. 2) as well as by the homo- and hetero-tetrameric assembly of the IP₃R subunits into functional channels. These gene sub-families correspond to sub-types of Ca²⁺ release channels that are primarily defined based on the functional and pharmacological criteria rather than rigorous analysis of structure-function relationships (reviewed in [20,21]). However, IP₃R protein can be subdivided into four functional regions (Fig. 2): an IP₃-binding region comprising ~600 residues at the N-terminus of the receptor protein; a central modulatory region with sites for interaction with regulatory molecules, a C-terminal channel-forming region (residues 2276-2589) containing six putative transmembrane (TM) domains, and the C-terminal tail including the last ~160 residues [22,23]. Predicted membrane topology of IP₃R suggests that both the N- and C-termini are intracellular and form a large cytoplasmic portion of the IP₃R channel that includes ~90% of the protein mass.

A functional hallmark of IP₃R channels is that their ion channel activity is regulated by coupled interplay between the binding of its primary ligands, IP₃ and Ca²⁺. Inositol 1,4,5-trisphosphate (IP₃) is a second messenger produced through phosphoinositide turnover in response to many extracellular stimuli such as hormones, growth factors, neurotransmitters, neurotrophins, odorants and light [20,21]. The changes in IP₃R pore structure that allow it to open are initiated by IP₃ binding to the N-terminal IP₃-binding region (Fig. 2A) that contains a ligand-binding suppressor domain (SD, residues 1-224). This domain fine-tunes IP₃-binding affinity for a particular receptor subtype and for the IP₃-binding core (IBC, residues 225-604) comprising two distinct domains and representing the minimum region required for specific IP₃ binding. In addition, IP₃R channel activity is closely regulated by a wide array of intracellular regulatory molecules that interact with the channel complex in a dynamic manner to provide functional feedback. Through functional analysis and co-immunoprecipitation studies, some of these sites for protein-protein interactions were mapped to the primary structure of IP₃R protein (Fig. 2B) (reviewed in [20,21]). While only a single IP₃ binding site exists on a single IP₃R subunit, there are multiple Ca²⁺ binding sites (Fig. 2). Thus, the diversity in distribution of regulatory proteins and IP₃R isoforms is essential for the versatility of Ca²⁺ signaling mechanism via IP₃R channels. These channels can conceptually be considered as modular scaffold proteins, where binding of IP₃ and regulatory components can change the scaffold structure and direct changes in subsequent events leading to generation of intracellular Ca²⁺ signals. The fact that Ca²⁺ serves multiple roles raises the possibility of crosstalk between processes, which should otherwise be independent. More information, however, is required for detailed mechanistic understanding of the diverse functions of IP₃R.

3. Overall 3D structure of tetrameric IP₃R

Historically, 3D structure determination of Ca²⁺ release channels did not start until IP₃R protein was successfully purified in detergent-solubilized form (Fig. 1) [24,25]. Structural studies have been focused primary on type 1 IP₃R (IP₃R1), which is the predominant isoform in the ER of cerebellar Purkinje cells and the best characterized of the mammalian isoforms. The structural analysis of the full-length tetrameric IP₃R channels has been

hampered by their enormous size (over 1.2 MDa), interaction with lipid membranes in their native state and their dynamic nature, making X-ray or NMR techniques difficult to apply. Single-particle cryo-EM has been a powerful method providing the capabilities to determine reliable structures in 3-5 Å range and to evaluate the dynamic aspects of macromolecular assemblies [26-36]. It is noteworthy, however, that the quaternary structure of IP₃R1 channel has remained controversial (reviewed in [37]) and not until recently has a 3D structure of tetrameric functional IP₃R1 channel in the closed state been unambiguously determined [9]. Its accuracy was to ~17 Å resolution clearly identifying structural flexibility, rather than imaging or specimen problems, as the resolution-limiting factor (Fig. 3)[10]. This implies that higher resolutions should, indeed, be possible with sufficiently large data set while also subclassifying particles. Moreover, these recent cryo-EM studies [9,10] demonstrated that the root of controversy between earlier cryo-EM reconstructions of IP₃R1 [38-40] is likely due to insufficient contrast in the cryo-EM raw data underlying these structures. The EM community is now beginning to establish tools and standards for better identifying such errors [10,41,42], and if best practices were followed now, it is doubtful that any of the earlier cryo-EM structures of IP₃R1 [38-40] would have been published.

We have currently reasonable knowledge about the quaternary structure of entire IP₃R1 channel that is based solely on single-particle cryo-EM studies. The 3D structure of the tetrameric IP₃R1 channel exhibits an overall characteristic mushroom shape: the large cytoplasmic (CY) region is connected via stalk-like densities with the smaller transmembrane (TM) region (Fig. 3). The delineated subunit boundaries confirm common architectural arrangement of Ca²⁺ release channels where the four identical or highly homologous subunits form a single central ion conduction pathway arranged around the four-fold symmetry axis normal to the membrane plane (Fig. 3). The TM regions of RyR1 and IP₃R1 channels are quite similar, although the cytoplasmic portions, aside from the difference in their overall dimensions, have quite different architectural arrangements with the most notable features such as a central plug in the IP₃R1 structure prominent enough to be visible directly in 2D images [9]. These observations are consistent with the idea that functional diversity of Ca²⁺ release channels and their ability to respond to various cellular stimuli are encoded in the CY region of the channel protein. The CY domains are targets of protein-protein interactions and interactions with a large array of intracellular small molecules that are linked to the channel gating activity (Fig. 2B)(reviewed in [20,21,43]).

4. Architecture of channel gating machinery

Understanding the channel gating machinery at an atomic level remains the most challenging issue in ion channel biology fascinating structural biologists for many years. Recent cryo-EM structure of IP₃R1 provides initial insights into the 3D arrangement of the Ca²⁺-permeation pathway across the membrane (Fig. 4)[9]. The TM structure reveals six putative α-helices. Four helices (one from each subunit) form a twisted bundle around the central axis. These helices are long enough to span the lipid bilayer shaping the ion-conduction pathway like a teepee-like structure with the apex pointing toward the cytoplasmic region. The position and geometry of these helices is strikingly similar to that of the inner (pore-lining) helices in closed-state K⁺ channels (Fig. 4) [44-46]. One α-helix is identified at the interface between the TM and CY structures and runs nearly parallel to the

membrane plane (Fig. 4). This helix is similarly placed to the interfacial helix identified earlier in RyR1 [47] and Kir channels [45,46]. Other short helices are most likely represent small segments of longer helices that were not yet entirely resolved due to the resolution limits in this cryo-EM map [9]. The position of one of these helices is similar to that of the outer helix in the K⁺ channels [44-46]. Moreover, the luminal face of IP₃R1 has prominent densities that surround the pore entrance similar to the highly structured turrets of Kir channels [46]. While such overall similarity between the TM structure of Ca²⁺ release channels and that of certain K⁺ channels can be observed at the current resolution (12-17 Å) [9,47,48], it is evident based on cryo-EM studies of other ion channels, such as the nicotinic acetylcholine receptor [49,50], that precise geometry of the current secondary structure elements will be further refined and adjusted with improving resolution.

4. Gating by conformational coupling

A central mechanistic question of IP₃R gating is how IP₃ binding in the *N*-terminal sequence of the channel protein is communicated through the membrane in order to open the pore formed near the *C*-terminus (Fig. 2). Furthermore, how does the channel change its conformation to allow Ca²⁺ translocation across the membrane? IP₃R channels open transiently upon stimulation by IP₃ and Ca²⁺ that allows Ca²⁺ ions to flow through them from the ER, ultimately leading to a change in intracellular Ca²⁺ levels. How this activation is accomplished is not known, yet it is fundamental to our understanding of Ca²⁺ signaling mechanisms via IP₃Rs. A number of functional models have been proposed to describe the interplay between IP₃ and Ca²⁺ in the modulation of IP₃R gating (reviewed in [20,21,51]). These models differ in terms of consequences of IP₃ and Ca²⁺ binding and interdependence (or lack thereof) in agonist binding. However, lack of high-resolution 3D structure of the entire functional IP₃R channel limits our ability to discriminate between these models.

Several lines of evidence support a model of IP₃R gating by conformational coupling between the IP₃-binding and TM domains. Changes in the pore structure that allow it to open are initiated by IP₃ binding to the IBC region (Fig. 2). However, IP₃ fails to open the pore without the SD domain, although it still binds to IP₃R [52,53]. Moreover, the SD decreases the affinity of IP₃R1 for IP₃ [53,54]. The *N*-terminal ligand-binding domains (*i.e.* SD and IBC domains) of IP₃R1 have been expressed as soluble proteins and crystallized in Apo- and IP₃-bound states [13-16]. Some studies demonstrated that the IBC adopts a more constrained structure when it binds IP₃. Moreover, IP₃ binding causes twisting of the SD around the flexible hinge connecting it to the IBC (Fig. 5A, B) [15,16]. Other evidence for interactions between the ligand-binding domains and IP₃-induced conformational changes comes from NMR and small-angle X-ray scattering studies performed when these domains are expressed as independent entities [55]. It has, therefore, been suggested that the SD is the essential link between IP₃ binding to the IBC and the subsequent conformational changes that lead to opening of the pore in IP₃R1. However, it is not yet clear whether similar rearrangements take place in the full-length protein, and if they are related to channel opening. Although low-resolution negative staining EM studies showed two distinct structures of IP₃R1 - one is square-shaped and the other is pinwheel-like, the relative abundance of each seems to be Ca²⁺-dependent [56] and the precise nature and structural basis of these differences were not established due to the fundamental resolution limit. It is

clear that higher-resolution structures of the entire IP₃R1 in the Apo- and ligand-bound states are urgently needed to assess conformational changes underlying IP₃-induced gating of the channel.

Ultimately, channel opening requires a structural re-arrangement of the ion conduction pathway. However, the precise molecular nature of such conformational changes and how ligand-binding is communicated to the channel pore remain largely speculative at the moment without a sufficiently high-resolution reconstruction of the full-length tetrameric IP₃R [57].

5. Building a quasi-atomic model by putting pieces together

The atomic-resolution structure of the complete IP₃R Ca²⁺ release channel is not yet available, but recent crystallographic studies have begun to provide high-resolution structures for soluble cytoplasmic domains of the IP₃R1 [13-16]. It has become common practice to build pseudoatomic models for the quaternary structure by fitting crystal structures of molecular fragments into lower-resolution cryo-EM reconstruction. A recently solved crystal structure of the *N*-terminal ligand-binding region of IP₃R1 was localized into the central cytoplasmic domains of the tetrameric IP₃R by fitting into the cryo-EM density map of IP₃R1 resolved at intermediate resolution by single-particle cryo-EM [9,16]. The top solutions derived by several docking programs (unpublished results from our group) place the *N*-terminal IP₃-binding domains in the same position within the channel tetramer: domains are arranged around the central plug and form the apical surface of the CY region distal to the TM domains (Fig. 5 C, Graphical abstract). Furthermore, this location is consistent with subunit boundaries identified in the cryo-EM structure of IP₃R1 and with the accessibility of binding sites for IP₃ within the tetrameric structure (Fig. 5C) [9]. Docking studies suggest that inter-subunit contacts between the *N*-terminal ligand-binding domains within the tetrameric channel are likely formed by the SD loop (residues 165-180) and a flexible loop of the IBC (residues 421-429) (Fig. 5D). It is important to note that Y167A mutation within the SD loop completely impairs IP₃-induced Ca²⁺ release but does not affect the IP₃-binding activity [58]. This implies that this region is critical for the functional coupling between ligand binding and channel opening. However, the precise molecular mechanism of this coupling remains unclear. The consensus view that has emerged from co-immunoprecipitation and cross-linking studies suggests that the SD of one IP₃R subunit may interact directly with the cytoplasmic TM4-5 loop of another subunit [54,59,60]. However, in light of the docking studies, it is difficult to imagine that the SD and TM domains can interact directly since they are located at least 70 Å apart in the tetrameric IP₃R1 (Graphical abstract). Another attractive possibility is that the C-terminal tail may mediate interactions between the pore and IP₃-binding domains [58,61]. It is clear that these models have to be verified by direct structural analysis of the entire IP₃R1 at resolutions sufficient to unambiguously establish spatial relationships between the structure-functional domains.

Moreover, while commonplace, it is important to note that the success and accuracy of docking attempts are limited by the size and shape of the crystal structure domain as well as by the resolution of the EM map. Although this approach can definitely provide valuable structural insights and can expand horizons for functional interpretations, establishing the

uniqueness and quality of the fit using only computational operations remains problematic [62]. At the marginal resolution limit (12-15 Å), when no secondary structure elements can be identified in the majority of the density map as in the case of IP₃R1 Ca²⁺ release channel, no mechanism exists to validate fits of relatively small domains into the large cryo-EM map without additional supporting data such as specific labelling and these limitations can further lead to ambiguities in fitting.

6. Conclusions

Understanding molecular machinery of ion channels at the atomic level remains a major challenge that attracts many structural biologists. The current round of cryo-EM structural data [9,10] in combination with recent crystallographic studies of small soluble domains [13-16], has provided an important structural basis for posing new hypotheses that can be tested in experiments aiming to understand how IP₃-gated Ca²⁺ release channels work at the molecular level. However, lack of atomic details for the entire Ca²⁺ release channel molecule limits the conclusions that can currently be drawn leaving significant obscurity of functional and structural nature.

Integral membrane proteins are the most difficult targets for structural analysis due to their tight association with the lipid membranes *in vivo* and their intrinsic dynamic nature. Despite recent strides made by x-ray crystallography, it remains unclear to what extent the 3D structure of detergent-solubilized channels represents the structure of the physiologically active channel in a biological membrane environment. To overcome limitations present in structural studies of membrane proteins in detergent-solubilized state, new cryo-EM approaches are currently being developed. These include structural analysis of ion channels reconstituted into a lipid membrane environment [63] and substitution of detergents with new surfactants such as ‘amphipols’, for better structural preservation in the absence of a bilayer, while keeping the membrane proteins soluble and in functional form in aqueous solution [64,65]. Notably, recent technical advances in the cryo-EM field that include the use of the direct electron detection cameras and improved image-processing software packages [36,66-69], allowed to resolve the structure of the heat-sensitive TRPV1 channel at 3.4 Å resolution [65,70]. Thus, research aiming at detailed understanding of IP₃R function is now poised to move forward toward structure determination of the entire IP₃R channel at atomic resolution.

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Highlights

- The 3D structure of the tetrameric IP₃R is unambiguously determined by single-particle cryo-EM
- Structural evidence for allosteric coupling between the ligand-binding and channel domains of IP₃R
- Advances in the cryo-EM field allow for near-atomic resolution structures of ion channels

Figure 1. Timeline of IP₃R research highlights

In the 1970s it was found that receptor-activated hydrolysis of PIP₂ increases intracellular Ca²⁺ mobilization [71], and that IP₃ is the second messenger stimulating Ca²⁺ release from intracellular stores [72,73]. It was subsequently demonstrated that Ca²⁺ regulates IP₃-induced Ca²⁺ release [19,58]. These findings paved the road for intensive research of molecular mechanism underlying this messenger pathway. In 1988, IP₃-binding protein was purified from brain [24,25] and was identified as Ca²⁺ release channel by reconstitution into lipid vesicles [17,18]. The receptor protein was cloned by two research groups in 1989 [1,2]. In the next two decades, much was learned about the structure-function of IP₃R: first images of purified IP₃R1 [74]; IP₃-binding site is identified in the primary sequence of IP₃R [75]; involvement of C-terminus in channel gating [76]; native arrangement of IP₃R in cerebellum [77]; direct association of IP₃-binding and channel domains [59]; first 3D map of IP₃R1 by cryo-EM [38]; Ca²⁺-induced conformational changes in IP₃R1 [78]; crystal structure of IP₃-binding core domains [13]; 3D structures of IP₃R1 by negative stain EM [79] and by cryo-EM [40]; Ca²⁺-induced re-arrangements of IP₃R1 by EM [56]; another 3D structure of IP₃R1 by cryo-EM [39]; crystal structure of IP₃-binding suppressor domain [14]; ligand-induced conformational changes in the N-terminal region by NMR and small-angle X-ray scattering [55]; 3D cryo-EM structure of IP₃R1 in the closed state at subnanometer resolution [9]; crystal structure of N-terminal ligand binding domains in Apo- and IP₃-bound forms [15]; Apo and ligand-bound crystal structures of Cys-less forms of the N-terminal ligand-binding domains [16]; validation of IP₃R1 cryo-EM structure [10].

Figure 2. Topology of structure-functional domains in primary sequence of IP₃R

(A) Four key regions defined in the primary structure of IP₃R are highlighted: the N-terminal ligand-binding region, the large central modulatory region, the membrane-spanning/pore-forming, and the C-terminal ‘gate-keeping’ region; SI, SII and SIII refer to the three splice sites. Lower panel shows detailed topology of domains within the N- and C-terminal regions (see also Figure 5). (B) Putative binding sites for several modulatory molecules of IP₃R are indicated in the primary sequence of the receptor protein: CaM – calmodulin, IRBIT- IP₃R binding protein released with IP₃, FKBP12 - 12-kDa FK506-binding protein, PKA – cAMP-dependent protein kinase A, Cyt c – cytochrome c; Htt – huntingtin; RIH - RyR/IP₃R homology regions. Amino acid residue numbering is the same as for the mouse IP₃R1 (GI: 313104120) [1].

Figure 3. 3D structure of tetrameric IP₃R1 channel determined by single-particle Cryo-EM Surface representation of cryo-EM density map of IP₃R1 determined by single-particle cryo-EM at intermediate resolution (EMD-5278) [9,10]. IP₃R1 protein has been visualized under conditions that favor the closed state of the channel, *i.e.* in the absence of its primary ligands, IP₃ and Ca²⁺ [9]. Surface representation of the structure is shown in three orthogonal views: from the cytoplasmic side (A), from the luminal side (B) and along membrane plane (C, D). One putative subunit is depicted in color; (D) two opposing subunits are shown to allow visualization of internal features in the channel structure. Scale bar is 100 Å.

Figure 4. Close-up view of the TM region of IP₃R1

(A) Cryo-EM densities of two opposing subunits of IP₃R1 are shown as a side view. Identified α -helices are annotated with cylinders and labeled as in [9]. Scale bar is 50 Å. (B-D) X-ray structure of the eukaryotic Kir2.2 (PDB ID:3JYC) is shown as a ribbon and superimposed with the secondary structure elements identified in the TM region of IP₃R1. (B) Two subunits are viewed along the membrane plane. Four subunits are viewed along the channel central axis from the luminal side (C) and from the cytoplasmic side (D).

Figure 5. Structure of the N-terminal ligand-binding region of IP₃R1

(A-B) Ribbon representations of the Apo- and IP₃-bound crystal structures of the ligand-binding domains of IP₃R1 channel (PDB ID: 3T8S): β trefoil fold of the IP₃-binding suppressor domain (β SD), β and armadillo repeat folds of the IP₃-binding core domains (β IBC and α IBC) are shown in cyan, yellow and red, respectively; IP₃ molecule is green; N- and C-termini are shown with spheres and labeled. The cleft between β IBC and α IBC domains undergoes conformational changes upon IP₃-binding (indicated with arrows in B) [15,16]. (C-E) Rigid body fitting of the crystal structure of N-terminal ligand binding region in the Apo conformation (PDB ID: 3TBS) into the cryo-EM density map of tetrameric IP₃R1 channel (EMD-5278) [9]. (C) The tetrameric CY region is viewed from the cytoplasmic side along the four-fold axis. Green color indicates a putative IP₃-binding pocket within the tetrameric channel assembly. (D) Zoomed-in view of the region marked by dashed line in (C). EM densities are shown only for one subunit; the loops making contacts between neighboring subunits (purple arrows) are colored with purple for the SD and with orange for the IBC. Position of Y167 'hotspot' residue is indicated with purple sphere within the SD loop. Residues involved in IP₃-binding are shown with green, green arrow indicates IP₃-binding pocket.