A pivotal component of the calcium (Ca\(^{2+}\)) signaling toolbox in cells is the inositol 1,4,5-triphosphate (IP\(_3\)) receptor (IP\(_3\)R), which mediates Ca\(^{2+}\) release from the endoplasmic reticulum (ER), controlling cytoplasmic and organellar Ca\(^{2+}\) concentrations. IP\(_3\)Rs are co-activated by IP\(_3\) and Ca\(^{2+}\), inhibited by Ca\(^{2+}\) at high concentrations, and potentiated by ATP. However, the underlying molecular mechanisms are unclear. Here we report cryo-electron microscopy (cryo-EM) structures of human type-3 IP\(_3\)R obtained from a single dataset in multiple gating conformations: IP\(_3\)-ATP bound pre-active states with closed channels, IP\(_3\)-ATP-Ca\(^{2+}\) bound active state with an open channel, and IP\(_3\)-ATP-Ca\(^{2+}\) bound inactive state with a closed channel. The structures demonstrate how IP\(_3\)-induced conformational changes prime the receptor for activation by Ca\(^{2+}\), how Ca\(^{2+}\) binding leads to channel opening, and how ATP modulates the activity, providing insights into the long-sought questions regarding the molecular mechanism underpinning receptor activation and gating.
IP$_3$Rs are intracellular Ca$^{2+}$ channels, predominantly localized to the ER and activated by the binding of IP$_3$, generated in response to external stimulation of G-protein coupled receptors$^{1-3}$. Opening of the IP$_3$Rs results in the rapid release of Ca$^{2+}$ from the ER lumen into the cytoplasm, triggering diverse signaling cascades that regulate physiological processes such as learning, fertilization, gene expression, and apoptosis. Dysfunctional IP$_3$Rs cause abnormal Ca$^{2+}$ signaling and are associated with many diseases, including diabetes, cancer, and neurological disorders$^{4,5}$. There are three IP$_3$R subtypes (IP$_3$R-1, -2, and -3) that share 60% sequence identity, form homo- or hetero-tetramers, exhibit different spatial expression profiles, and are involved in different signaling pathways$^{1-3}$. Each IP$_3$R subunit is about 2700 amino acids in length and contains a transmembrane domain (TMD) and a large cytoplasmic region comprising two β-trefold domains (βTF1 and βTF2), three Armadillo repeat domains (ARM1, ARM2, and ARM3), a central linker domain (CLD), a juxtamembrane domain (JD), and a short C-terminal domain (CTD)$^{6-10}$ (Fig. 1).

In addition to IP$_3$, the receptor activation requires Ca$^{2+}$ at nanomolar concentrations, whereas Ca$^{2+}$ at higher concentrations is inhibitory, causing the receptor to be tightly regulated by Ca$^{2+}$.$^{11-15}$ The cryo-EM structure of human IP$_3$R-3 (hIP$_3$R-3) in the presence of the inhibitory Ca$^{2+}$ concentrations (2 mM) revealed two binding sites$^{6}$. However, their role in channel activation and inhibition has remained uncertain. Furthermore, although ATP binding potentiates the receptor by increasing the open probability and duration of the channel openings, the underlying molecular mechanism has not been uncovered$^{16,17}$. In this study, we illuminate the structural framework of receptor activation and channel opening by analyzing five cryo-EM structures of hIP$_3$R-3 in the closed-pre-activated, open-activated, and closed-inactivated conformations.

**Results and discussion**

**Cryo-EM structures of hIP$_3$R-3 gating conformations.** Bimodal regulation of IP$_3$R activity by Ca$^{2+}$ complicates sample preparation because of the requirement for fine adjustment of Ca$^{2+}$ concentration to trap the channel in the open conformation. Although free Ca$^{2+}$ concentrations in solutions can easily be controlled by using Ca$^{2+}$ buffers such as EGTA or BAPTA, it becomes challenging during sample preparation for cryo-EM due to the small volumes used. Typically, a 2–3 µl protein sample is applied to a cryo-grid, but more than 99% of the sample volume is lost during grid preparation due to extensive blotting with filter paper$^{18}$. During this time, the samples contact filter paper and cryo-grids, containing various amounts of Ca$^{2+}$. Small sample volumes and short time frames may reduce these buffers’ efficiency, causing the free Ca$^{2+}$ concentration to increase to inhibitory levels prior to sample freezing. In order to maximize the chances of obtaining particles in the active state, we prepared the sample in: (1) EDTA, which has ~200 fold faster binding kinetics to Ca$^{2+}$ than EGTA$^{19}$, a common Ca$^{2+}$ chelator, and is more likely to chelate excess Ca$^{2+}$ and other divalent cations within the short period prior to the sample plunging, (2) ATP, which increases the open probability of IP$_3$Rs and dampens the inhibitory effect of Ca$^{2+}$,$^{16,17}$, and (3) high concentrations of IP$_3$.

The final hIP$_3$R-3 sample was purified in the presence of 1 mM EDTA and supplemented with 0.5 mM IP$_3$, 5 mM ATP, and 0.1 mM CaCl$_2$ before preparing cryo-grids. Although the free Ca$^{2+}$ concentration was calculated around 100 nM under these conditions using Maxchelator$^{20}$, the actual free Ca$^{2+}$

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**Fig. 1 Cryo-EM structures of hIP$_3$R-3 in multiple conformations.** a Domain boundaries of hIP$_3$R-3. b-d Composite maps of hIP$_3$R-3 in pre-active A (b), active (c), inactive (d) conformations. Each domain in one of the subunits is colored as in (a). Maps within the boxes, shown transparent, are close-up views of the Ca$^{2+}$ binding site (red) and the pore (blue) with ribbon representation of hIP$_3$R-3. Select residues are shown in the sticks. Dashed circles indicate opening through the gate.
concentration may be higher due to potential leakage of Ca\(^{2+}\) during the cryo-grid preparation as mentioned above. We performed a cryo-EM analysis on a large dataset by employing exhaustive 3D classification strategies to separate particles belonging to different functional states resulting in five high resolution (3.2–3.8 Å) structures (Supplementary Figs. 1–6; Supplementary Table 1). The pore region in all structures resolved to 3.5 Å or better, allowing us to build side chains and determine if the channel was open or closed (Fig. 1; Supplementary Figs. 2–6).

Three structures have closed pores with well-resolved densities for IP\(_3\) and ATP and are referred to as pre-active A, B, and C (Fig. 1b; Supplementary Figs. 1–7). The structure named “active” displays drastic conformational changes at the TMD, leading to pore opening (Fig. 1c). In addition to the well-resolved densities for IP\(_3\) and ATP, the active structure reveals substantial density, interpreted as Ca\(^{2+}\), at the ARM3-JD interface, referred to as the activatory Ca\(^{2+}\) binding site (Fig. 1c; Supplementary Figs. 5, 7). In the fifth structure, the channel is closed, the activatory Ca\(^{2+}\) binding site is occupied, and the intersubunit interactions of the cytoplasmic domains are lost (Fig. 1d; Supplementary Fig. 6). The structure is highly similar to the hIP\(_3\)R-3 structures obtained in the presence of inhibitory Ca\(^{2+}\) concentrations\(^6\), except for βTF1, which moves closer to the ARM1 (Supplementary Fig. 8). Most notably, ARM2 adopted the same conformation relative to ARM1 and CLD, creating the binding site for the second Ca\(^{2+}\) observed at high Ca\(^{2+}\) concentrations (Supplementary Fig. 8). While these similarities suggest a Ca\(^{2+}\) ion occupies this site and the structure represents the Ca\(^{2+}\) inhibited state, the quality of the map around the region did not allow accurate inspection of the presence of Ca\(^{2+}\) (Supplementary Fig. 8). Therefore, we refer to the structure as “inactive” while it remains unclear if it represents a desensitized state that hIP\(_3\)R-3 adopts without additional Ca\(^{2+}\) binding or an inhibited state forced by binding of additional Ca\(^{2+}\) to an inhibitory site.

It is important to note that our initial 3D classification runs resulted in two major classes grouping the pre-active and active structures into one class and the inactive structure into another (Supplementary Fig. 1). It was essential to perform another round of 3D classification focusing only on the core of the protein to separate the particles in the active state from the pre-active states, potentially due to subtle differences in the overall structures and the much fewer number of particles in the active state (20,039 particles compared to 346,684 particles in the pre-active states) (Supplementary Fig. 1; Supplementary Table 1).

Priming of hIP\(_3\)R-3 for activation. To compare the structures presented here, we aligned their selectivity filters and pore helices (residues 2460–2481), which reside at the luminal side of the TMD and are virtually identical in all classes. The pre-active A structure is almost identical to the previously published IP\(_3\)-bound hIP\(_3\)R-3 structure\(^6\) (Supplementary Fig. 9a) and reveals that IP\(_3\) binding causes the ARM1 to rotate about 23° relative to the βTF-2, causing global conformational changes within the cytoplasmic domains, as observed in previous cryo-EM and X-ray crystallography experiments\(^6,8,21–24\) (Supplementary Fig. 9b, c).

The pre-active B and C structures adopt distinct conformations that are intermediates between the pre-active A and open state structures. Based on these conformational changes, we propose a sequential transition from pre-active A to B, then C, although the alternative transitions cannot be ruled out entirely. During the transition to the pre-active B state, the N-terminal domain (NTD) of each protomer comprising βTF1, βTF2, ARM1, ARM2, and CLD rotates about 4° counter-clockwise relative to the TMD and moves about 2 Å closer to the membrane plane (Fig. 2a; Supplementary Movie 1). In the pre-active C state, the NTDs remain primarily unchanged compared to the pre-active B state, while the ARM3 and JD are rotated by 7°, causing mild distortions at the cytoplasmic side of the TMD without opening the channel (Fig. 2b; Supplementary Movie 1). Compared to the ligand-free conformation, the βTFs move about 7 Å closer to the membrane plane, and ARM3-JD rotates about 11° in the pre-active C conformation.

Ca\(^{2+}\)-mediated conformational changes leading to pore opening. In the absence of Ca\(^{2+}\), the ARM3 and JD act as a rigid body, where there are no significant conformational changes relative to each other (Fig. 2a, b). When Ca\(^{2+}\) is bound, the JD rotates about 11° relative to the ARM3 (Fig. 3a), resembling a claspshell closure, which leads to global conformational changes in the whole receptor, including the movement of the NTD closer to the membrane plane by 2 Å (Fig. 3b; Supplementary Movie 1). In contrast to the limited rotation of the ARM3 (about 5°), the JD rotates about 14° around an axis roughly perpendicular to the membrane plane, leading to conformational changes at the TMD and resulting in pore opening in the active state (Fig. 3b).

Ca\(^{2+}\) is coordinated by E1882 and E1946 on the ARM3 and the main-chain carboxyl group of T2581 on the JD (Fig. 3c). H1884 and Q1949 are also close and may interact with Ca\(^{2+}\) through
ATP binding site. Within the JD, we observed a well-resolved cryo-EM density for ATP in all the structures (Supplementary Fig. 7). The quality of the maps obtained through local refinement allowed unambiguous modeling of ATP, revealing its key interactions with the protein residues (Fig. 4a, b). The adenosine base intercalates into a cavity surrounded by F2538, the backbone amide group of F2539, and the carbonyl groups of H2563 and I2559 (Fig. 4b). The phosphate moieties interact with K2560, located on the opposite side of the binding site. A leucine residue (L4980) occupies this position in RyR-1s. The differences in the number and location of the basic residues likely force the phosphate moieties of ATP to adopt different conformations. Furthermore, F2156 in hIP3R-3 points toward the adenosine binding pocket, prohibiting ATP from adopting the conformations observed in RyR-1s due to steric clash in hIP3R-3s (Supplementary Fig. 10c, d).

Structure of the TMD in the open conformation. The TMD of IP3Rs has the same overall architecture of voltage-gated ion channels with a central pore domain, consisting of S5, S6, and pore (P) helix, surrounded by pseudo-voltage-sensor domains (pVSDs), consisting of S1, S2, S3, and S4 helices along with two IP3/RyR specific TM helices (S1’ and S1”) (Fig. 5). In the closed channel, F2513 and I2517 of the S6 helix form two layers of hydrophobic constriction at the pore, blocking the path for the permeation of hydrated ions (Fig. 5). JD’s rotation upon Ca2+ binding pushes the pVSD’s cytoplasmic side away from the pore domain by about 7 Å and tilts the cytoplasmic side of the S6 helix (S6cyt) by 12° (Fig. 5a; Supplementary Movie 1). Concurrently, the S4-S linker and S5 helix move away from the S6 helix, thereby inducing a distortion of S6 around the constriction site and moving F2513 and I2517 away from the pore. As a result, the diameter of the water-accessible pore increases to 8 Å, large enough to permeate hydrated cations (Fig. 5b). The flexibility introduced by the neighboring glycine residue (G2514), mutation of which to alanine in IP3R-1 is associated with spinocerebellar ataxia 29 (SCA29)31, is likely critical to the movement of F2513. The tilting of the S6cyt breaks the salt bridge between D2518 and N2510 extends from a different position on the S6 helix (Supplementary Fig. 10c, d). In hIP3R-3, there is only a single lysine residue (K2152) on the corresponding helix, and the phosphate moieties interact with K2560, located on the opposite side of the binding pocket. A leucine residue (L4980) occupies this position in RyR-1s. The differences in the number and location of the basic residues likely force the phosphate moieties of ATP to adopt different conformations. Furthermore, F2156 in hIP3R-3 points toward the adenosine binding pocket, prohibiting ATP from adopting the conformations observed in RyR-1s due to steric clash in hIP3R-3s (Supplementary Fig. 10c, d).
**Fig. 4** ATP binds to the JD. 

**a** The cryo-EM density of ATP (red mesh) from the composite map of the pre-active A state and the modeled ATP molecule. 

**b** Close-up view of the ATP binding site in the pre-active A state. Dashed lines indicate hydrogen bonding. 

**c** Sequence alignment of hIP3R subtypes around the residues forming the ATP binding site. Residues shown in (b) are highlighted. Select residues are indicated by arrows, and E2149 is labeled in red.

**Fig. 5** Structure of the IP3R-3 in the open conformation. 

**a** Comparison of the hIP3R-3 structures in the pre-active C and active conformations aligned as in Fig. 2. 

**b** Ion permeation pathways of hIP3R-3 in pre-active C and active conformations (radii coloring: red, <0.8 Å; green, 0.8-4.0 Å; gray, >4.0 Å) along with the 1D graph of the pore radius. The JD and TMD of the active state are shown in cyan and violet, respectively. The pre-active C structure is colored orange.
RyRs, which lack the extended CTD, did not diminish receptor conformational changes during channel opening or undergoes conformational changes at the NTD, ARM3, and JD, without any restriction of the pore (e.g., mutation of G251431). Two of the mutated gating S4-S5 linker, which couples the tilting of the pVSD to the bulging I251739) or restrict conformational changes required for dilation of the pore (e.g., mutation of N251038 or F252041) interact with the residues on the S4-S5 linker, which couples the tilting of the pVSD to the bulging of the constriction site (Supplementary Fig. 12b). Mutations of these residues are likely to impair this coupling and thus hinder gating.

The flexibility of the CTD. The CTD, extending from the JD along the symmetry axis, forms a left-handed coiled-coil motif before interacting with the BTF2 of the neighboring subunit. The density for the CTD was poorly resolved in all of the states (Supplementary Fig. 13a, b). However, the coiled-coil motifs were visible in the unsharpened maps in the pre-active and active states, enabling us to model poly-alanine peptides without assigned registries (Supplementary Fig. 13a, b). The densities for the extensions from the coiled-coil motif towards the βTF2 become visible when viewed at lower thresholds, whereas the linkers between the JD and the coiled-coil motif remain invisible, indicating higher flexibility for this region (Supplementary Fig. 13a, b). We did not observe any interpretable density for the CTD in the inactive state (Supplementary Fig. 13a, b).

For IP₃R-1, the CTD was proposed to transmit the conformational changes induced by IP₃ at the NTD to the JD⁴⁵. In IP₃R-3, there are no apparent changes on the coiled-coil motif in the pre-active states, but the coiled-coil motif rotates about 20° around the symmetry axis and moves closer to the TMD by 6 Å in the active state (Supplementary Fig. 13c, d). However, the linker between the coiled-coil motif and JD remains flexible, suggesting that the structural rearrangements of this domain are not directly enforcing the channel opening (Supplementary Fig. 13). In line with these observations, removing CTD residues interacting with the βTF2 or swapping the C-terminal region of IP₃R-1 with the RyRs, which lack the extended CTD, did not diminish receptor activation⁴¹,⁴²,⁴³.

Mechanism of hIP₃R-3 activation and gating. It has been long recognized that IP₃ binding primes the receptor for activation by Ca²⁺,⁴³ but how the priming is achieved has remained elusive. Our structures reveal that IP₃ binding leads to several conformational changes at the NTD, ARM3, and JD, without any apparent structural changes at the activatory Ca²⁺ binding site, and that the ARM3 and JD adopt a new pre-gating conformation relative to the TMD with modest changes at the intersubunit interface between the JDs at the cytoplasmic side of the TMD (Fig. 6; Supplementary Fig. 14; Supplementary Movie 1). In addition, ARM3s are constrained in their pre-gating conformation by the tetrameric cage-like assembly of the NTDs, forcing the JDs to rotate upon Ca²⁺ binding. The NTD assembly is maintained by the βTF1-βTF2 intrasubunit interactions (βTF ring), which remains intact in the pre-active and active states (Fig. 6; Supplementary Fig. 15; Supplementary Movie 1) and acts as a pivot for the conformational changes that stabilize the ARM3. On the other hand, its disruption in the inactive state leads to the loosening of the tetrameric assembly of the NTDs, relieving the ARM3 constraints and causing the JD and TMD to adopt the closed channel conformation despite the bound Ca²⁺ to the activatory site. Supporting this hypothesis, the removal of βTF1 or mutation of W168, which resides at the βTF1-βTF2 interface (Supplementary Fig. 15), was shown to abolish IP₃R activity⁴⁴,⁴⁵.

In conclusion, the ensemble of structures obtained from the same sample demonstrates structural heterogeneity of IP₃Rs in the presence of IP₃, ATP, and Ca²⁺. Our ability to correlate these structures with their plausible functional states allowed us to define the conformational changes at different gating states, revealing the structural features that underpin IP₃R activation and gating. These structures will likely serve as foundations for future experiments addressing biophysical and functional questions related to IP₃Rs. Furthermore, our study reinforces the power of cryo-EM in analyzing heterogeneous samples and highlights the importance of a thorough investigation of the data to identify physiologically relevant conformations, even when they constitute only a tiny fraction of the sample.

Methods
Protein expression and purification. Expression and purification of hiP3R-3 were performed as previously described with minor modifications⁴⁶. Briefly, hiP3R-3 (residues 4-2671) with a C-terminal OneStep tag was expressed using the Multibac expression system⁴⁶. SF9 cells (4 × 10⁶ cells/mL) were harvested by centrifugation (4000 × g 48 h after infection with the baculovirus). Cells resuspended in a lysis buffer of 200 mM NaCl, 40 mM Tris-HCl pH 8.0, 2 mM EDTA pH 8.0, 10 mM β-mercaptoethanol (BME), and 1 mM Phenylmethylsulfonyl fluoride (PMSF) were lysed using Avastin EmulsiFlex-C3. After centrifugation of the lysate at 7000 × g for 20 min to remove large debris, the membrane was pelleted by centrifugation at 185,000 × g (Type Ti45 rotor) for 1 h. Membrane pellets were homogenized in ice-cold resuspension buffer (200 mM NaCl, 40 mM Tris-HCl pH 8.0, 2 mM EDTA pH 8.0, 10 mM BME) using a Dounce homogenizer, and solubilized using 0.5% Lauryl maltose nonylferyl glycol (LMNG) and 0.1% glycodeiosgenin (GDN) at a membrane concentration of 100 mg/mL. After 4 h of gentle mixing in the cold room, the insoluble material was pelleted by centrifugation at 185,000 × g (Type Ti45 rotor) for 1 h, and the supernatant was passed through an IRA Biogel column by gravity flow. The resin was washed with 5 column volume (CV) of wash buffer composed of 200 mM NaCl, 20 mM Tris-HCl pH 8.0, 10 mM BME, 0.005% GDN, 0.005% LMNG, followed by 5 CV of wash buffer supplemented with 5 mM ATP and 20 mM MgCl₂ to remove any bound chaperone proteins, and finally with SCV of wash buffer supplemented with 1 mM EDTA. The protein was eluted using wash buffer supplemented with 1 mM EDTA and 100 mM D-Biotin (pH 8.2). The protein was further purified by size exclusion chromatography (SEC) using a Superose 6 Increase column (10/300 GL, GE Healthcare) equilibrated with the SEC buffer composed of 200 mM NaCl, 20 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0, 2 mM TCEP, 0.005% LMNG, and 0.005% GDN. The fractions corresponding to hIP3R-3 were combined and concentrated to 4 mg/mL using a 100 kDa centrifugal filter (Sartorius). The concentrated sample was then centrifuged at 260,000 × g using an S100AT rotor (ThermoFisher Scientific). The concentration dropped to 1.8 mg/mL.

Cryo-EM sample preparation and data collection. Purified hiP3R-3 in the SEC buffer containing 1 mM EDTA was supplemented with 500 µM IP₃ (from 10 mM stock in water), 0.1 mM CaCl₂, and 5 mM ATP (from 100 mM stock, pH 7.2). 2.0 µL of the protein sample was applied to 300 mesh Cu Quantifoil 1.2/1.3 grids (Quantifoil Microtools) that were glow discharged for 20 s at 25 mA. The grids were blotted for 7 s at force 10 using single-layer Whatman ashless filter papers (Cat. #: 1442-055, GE Healthcare) and were plunged into liquid ethane using an FEI MarkIV Vitrobot at 8 °C and 100% humidity. The filter papers were not pre-treated with Ca²⁺ chelators or any other chemicals. Four grids prepared using the same sample were imaged using a 300 kV FEI Krios G3i microscope equipped with a Gatan K3 direct electron camera in four different data collection sessions at Case Western Reserve University. Movies containing 40–50 frames were collected at a magnification of ×105,000 in super-resolution mode with a physical pixel size of 0.828 Å/pixel and defocus values at a range of ~0.8 to ~1.6 µm using the automated imaging software SerialEM⁴⁷ and EPU (ThermoFisher Scientific).

Cryo-EM data processing. Datasets from four sessions were initially processed separately using RELION 3.0⁴⁸. We used MotionCor2⁴⁹ and Gic⁵⁰ to perform beam-induced motion correction and CTF estimations, respectively. We performed auto pick with the Laplacian-of-Gaussian option of RELION. The auto-picked particles binned 4 × 4, and performed 2D class classification. Using the class averages with apparent features, we performed another round of particle picking. We cleaned the particles, extracted as 4 × 4 binned, through 2D classification and performed 3D classification using the hiP3R-3 map (EMD-20849⁵¹), which was converted to the EMD-7355 format and pixel size was rescaled to match 4×4. Two of the maps had a compact NTD and tight interactions between subunits as in previously published IP₃R structures in the absence of Ca²⁺, hereafter called “compact”
conformation (Supplementary Fig. 1). The other one had the NTD of each subunit tilted away from the central symmetry axis resembling hIP3R-3 structures obtained in the presence of high Ca\(^{2+}\) concentrations, hereafter called “loose” conformation (Supplementary Fig. 1). These particles were separately selected and reextracted using a box size of 480 x 480 pixels at the physical pixel size. After 3D refinements, we performed CTF refinement and Bayesian polishing.

We combined all the polished particles and performed another round of 3D classification, using one of the compact structures as a reference map. We grouped particles into “compact” and “loose” classes (Supplementary Fig. 1). Refinement of the particles in the “compact” conformation yielded a 3D reconstruction with an average resolution of 3.9 Å. Although there were slight changes at the TMD compared to the structure in the closed state, these changes were not significant enough to suggest that the channel was open. To more clearly resolve the density around the TMD, we performed another round of 3D classification using a mask that only covers the ARM3, JD, and TMD and without performing an angular or translational alignment in Relion3 (Supplementary Fig. 1)52.3. 3D refinements of the particles in each class were performed using non-uniform refinement in CryoSPARC, enforcing C4 symmetry and local CTF refinements (Supplementary Fig. 1)53. Five classes led to four high-resolution (better than 4 Å) 3D reconstructions, whereas the 3D refinement of the other three classes resulted in poorly resolved maps. The particles in the “loose” conformation were processed using non-uniform refinement in CryoSPARC, but without enforcing any symmetry. Local resolution estimates were calculated using CryoSPARC53 (Supplementary Figs. 2–6). Some of the data processing and refinement software was supported by SBGrid54.

To improve the quality of the maps, we performed local refinements using masks covering parts of the original cryo-EM maps (Supplementary Figs. 2–6). We prepared five masks that cover distinct domains of one of the subunits for the protomers A, B, C, and active conformations. After symmetry expansion using C4 symmetry, we performed local refinement using CryoSpac (Supplementary Figs. 2–5). For the inactive state, we prepared four masks that cover the cytoplasmic domains of each subunit and another mask that covers the tetratricopeptide ARM3, JD, and TMD (Supplementary Fig. 6). The local refinements were performed using C1 symmetry for the cytoplasmic domains and C4 symmetry for the tetraticopeptide ARM3, JD, and TMD. The resulting local refinement maps were aligned onto the original maps using Chimera55 and merged using the “VOP maximum” command of Chimera55 to prepare the composite maps (Supplementary Figs. 2–6).

**Model building.** Model building was performed using Coot56. We first placed the hIP3R-3 structure in ligand-free conformations (PDB ID: 6UQK)57 into the composite map of Pre-active A, and performed rigid-body fitting of individual domains of one of the protomers. We then manually fit the residues into the density and expanded the protomer structure into a tetramer using the C4 symmetry. We performed real-space refinement using Phenix57. We repeated build-refine iterations till a satisfactory model was obtained. This model was used as a starting model for the other structures following the same workflow. Regions without interpretable densities were not built into the model. Residues without apparent density for their side chains were built without their side chains (i.e., as alanines) while maintaining their correct labeling for the amino acid type. The coiled-coil regions were modeled as poly-alanines without residue assignment using the unsharpened maps. Validations of the structural models were performed using MolProbity58, implemented in Phenix57.

**Figure preparation.** Figures were prepared using Chimera55, ChimeRX59, and The PyMOL Molecular Graphics System (Version 2.0, Schrödinger, LLC). Calculation of the pore radii was performed using the software HOLE60.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

The data that support this study are available from the corresponding author upon reasonable request. Cryo-EM maps and atomic coordinates are deposited to the Electron Microscopy Data Bank (EMDB) and Protein Data Bank (PDB) databases, respectively. The accession codes are EMD-25667 and 7T3P for pre-active A, EMD-25668 and 7T3Q for pre-active B, EMD-25669 and 7T3R for pre-active C, EMD-25670 and 7T3T for active, and EMD-25671 and 7T3U for inactive states, respectively. The following previously published datasets were used: EMD-20849, Cryo-EM structure of type 3 IP3 receptor revealing presence of a self-binding peptide10. 6UQK, Cryo-EM structure of type 3 IP3 receptor revealing presence of a self-binding peptide10. 6DRC, High IP3 Ca\(^{2+}\) human type 3 1,4,5-inositol triphosphate receptor6. 6DQV Class 2 IP3-bound human type 3 1,4,5-inositol triphosphate receptor2. 5TAL, Structure of rabbit RyR1 (Caffeine/ATP/Ca\(^{2+}\) dataset, class 1 and 2)25. 7M6A, High resolution structure of the membrane embedded skeletal muscle ryanodine receptor29. Reagents and other materials will be available upon request from E.K. with a completed materials transfer agreement.

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**Author contributions**
E.K. conceived the project and performed cryo-EM data analysis; E.A.S. optimized and performed protein expression and purification; H.T. performed grid preparation for cryo-EM. All authors contributed to the preparation of the manuscript.

**Competing interests**
The authors declare no competing interests.

**Additional information**

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