

Cryo-EM Insights into Isoform-Specific Properties of the IP₃R2 Channel

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This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

• Manuscript: "Cryo-EM Reveals Isoform-Specific Structural Features of Type 2 IP3R"

Authors: Baker et al.

Summary

This manuscript reports cryo-EM structures of the inositol 1,4,5-trisphosphate receptor isoform 2 (IP3R2) in multiple conformational states, including ligand-bound and closed forms. As no prior high-resolution structure of IP3R2 has been published, this dataset fills an important gap in the structural biology of IP3 receptors. The work has potential significance for understanding isoform-specific regulation, ATP sensitivity, and disease-associated mutations.

However, in its current form, the manuscript does not clearly distinguish novel isoform-specific findings from previously established structural features of IP3R1 and IP3R3. Substantial restructuring and clarification are required before the work can be properly evaluated for publication.

I recommend major revision.

Major Concerns

1. Lack of Clear Delineation of Novelty

A significant portion of the Results section re-describes architectural features, ligand-binding sites, and domain arrangements that have already been established in prior IP3R structures. These descriptions are presented in a way that obscures what is genuinely new in IP3R2.

The manuscript would benefit from:

- Moving established structural descriptions to the Introduction or Supplementary Information.
- Explicitly stating, in each Results subsection, what differs in IP3R2 relative to other isoforms.
- Structuring the Results around isoform-specific differences rather than general receptor architecture, which is already known.

As written, the manuscript is substantially longer than necessary due to repetition of known features. As a result, the central contribution of the study is difficult to extract.

2. Isoform vs. Species Comparisons

Throughout the manuscript, sequence comparisons are performed across different isoforms taken from different species. This approach does not allow one to distinguish isoform-specific differences from species divergence.

If the authors wish to claim isoform-specific structural determinants:

- Comparisons must be performed between IP3R1, IP3R2, and IP3R3 within the same species.
- Any cross-species comparisons must be clearly justified and interpreted cautiously.

This issue substantially weakens conclusions regarding isoform specificity.

3. ARM2 "Auto-Inhibitory" Loop and Energy Landscape Claims

The proposed role of the ARM2 loop in modulating IP₃ sensitivity is intriguing. However:

- The loop is not fully resolved.
- Modeling relies on AlphaFold3 and Rosetta-based docking without reported validation metrics.

- No mutagenesis or functional data are provided to test the structural hypothesis.
- Claims that isoforms occupy distinct basins along an energy landscape defined by ARM2 mobility are speculative and not experimentally supported.

Mechanistic language should be tempered unless supported by quantitative or functional validation.

4. ATP Binding Pocket Interpretation

The manuscript proposes structural explanations for enhanced ATP sensitivity in IP3R2. However:

- Local resolution of the ATP-binding site is not reported.
- Differences in global and local resolution between compared structures are not accounted for despite their importance in the precision of the different models.
- Electrostatic comparisons and interaction diagrams lack methodological clarity.
- Sequence comparisons again mix isoform and species differences.

At moderate resolution, side-chain positioning and interaction networks must be interpreted cautiously. The current presentation does not sufficiently address modeling uncertainty.

Statements such as “these data establish that the ATP binding pocket is responsible for ATP regulation” are overly strong and circular.

5. Quantitative Claims (Table 2)

Table 2 presents qualitative comparisons without clear description of the source of the information. From what data was the table derived? Qualitative structural comparisons require transparent methodological description.

6. Ion Conduction Pathway and Disease Mutation

The localization of the G2498S mutation to the selectivity filter is potentially important. However, structural impact of the mutation is inferred rather than demonstrated.

The conduction pathway architecture largely recapitulates prior IP3R structures. Again, on Figure 10, no comparison is made with the other isoforms to contrast them with these new structures.

Overall, the structural determination of IP3R2 is an important and valuable contribution. However, the manuscript currently overstates novelty and advances mechanistic interpretations that are insufficiently supported by the presented data. With substantial revision and a focus on genuine isoform-specific insights, the work could make a meaningful contribution to the field. At present, major revision is required.

Reviewer #2

(Remarks to the Author)

Baker, Lin, Fan et al. present structures of the type 2 IP3 receptor in the ligand-free and ligand-bound states and describe in large detail important aspects of ligand-induced activation of the channel.

While structures of the other two types, IP3R1 and IP3R3, have been reported before, structures of IP3R2 have been missing so far. This alone makes this an important manuscript for the IP3R field and beyond, which mandates its publication in Nature Communications. However, the true value of this manuscript and the presented data is the possibility to analyze all the critical structure features side by side, and unfortunately this has been done insufficiently. Thus, before publications, the manuscript should be rewritten to focus more on this aspect, and also to put more emphasis on the technical limitations of the data.

Major points:

1. In large parts, the authors present structure features that have been observed before in the other IP3R types as if they were the first ones to see them, and use terminology such as “reveal”, “define” and “identify” for features and residues that have been known before (e.g. II.79-80). Some examples for such misleading representations are the descriptions of the IP3 binding site, the allosteric nexus and the ATP-binding site. Here it would be much more appropriate, and also easier for readers to follow, to present these features directly as comparisons with IP3R1 and IP3R3, instead of merely mentioning towards the ends of the respective paragraphs that similar observations have been made in these proteins as well.
2. The resolution of both structures is, with 3.3 Å and 3.6 Å, not excessively high and some of the claims the authors make with regard to hydrogen bonds and in general side chain interactions are a bit shaky, especially since it is not possible to judge the map quality in these regions from the figures. This applies, for example, to the position and coordination of IP3, as the density shown in Fig. 2b appears rather featureless and the viewing angle and zoom does not allow to assess the interactions described in the manuscript.
3. For conserved structure features like the IP3 binding site or the ATP-binding site, superposition figures would be very helpful and informative to discuss similarities and differences between the IP3R isoforms. Also, showing clear densities for ligands and relevant interacting residue side chains would be necessary in order to be able to discuss such interactions (e.g. Fig. 5d is a good start, but could be significantly improved).
4. I find it counter-intuitive to call the ARM2 loop “autoinhibitory” when it is only part of its function to stabilize the apo conformation, and it actually seems to prepare the IP3-binding site for more efficient binding. Maybe it would be possible to

find another, more appropriate name? In addition, I was rather surprised by the way how the authors introduce it in l. 128 where they write that it “partially occludes the IP3-binding pocket in the apo state” (which makes sense if it is autoinhibitory) and that this would lead to “higher IP3 binding affinity” (which does not make so much sense in the context of pure autoinhibition and gets explained only much later in the manuscript).

5. The results of 3D classification seem over-interpreted. The density presented in Fig. 4b is very featureless and displayed at a low contour level. Furthermore, the five 3D classes shown in Fig. S7a are overall rather divergent and not only in the ARM2 loop, which precludes a reliable interpretation. Please remove this or tone down in the manuscript.

6. In general, the authors adequately show the conformational flexibility of the “autoinhibitory” loop by MD simulations etc. However, the model they derive from that and from the observed structural differences in the IP3 binding sites between IP3Rs, namely that the IP3R2 loop would assist IP3 binding, is very speculative and should be clearly stated as such. Instead, the authors present this model in a wall of text on p.6 that reads as if it was based on solid experimental data. I would strongly encourage them to rework this part to make it more clear what evidence they have and where it is speculative, and also to make the text passage more concise and less redundant.

7. The discussion of the TM domain is rather lengthy, but lacks a clear and concise comparison with IP3R1 and IP3R3 regarding domain organization, pore-lining residues and activation mechanisms. In particular, I don't really understand why the authors discuss their structure more in the context of RyR1/2 than in the context of IP3R1/3.

8. Quality of the molecular models: Judging from the validation provided in Table1 and the PDB validation reports, it seems that the model building and refinement is not yet finished. In particular, both structures display elevated clash scores and suboptimal geometry values (bond lengths and angles, Ramachandran, Rotamers), resulting in overall rather high MolProbity scores. While I am aware that refining such large models is a significant task, I would urge the authors to be more stringent in this regard and further refine their models until they reach state-of-the-art quality. This will most likely not affect the conclusions that can be drawn from the structures, but rather reflects best practice in structural biology, especially for a manuscript that focuses so much on structures. Furthermore, as a minor comment related to this topic, there are some issues with the PDB validation reports, especially regarding the ligand-bound structure where MolProbity failed. Once the models are updated and re-uploaded, please also provide new validation reports without these issues, preferably those that are actually meant for manuscript review (which are usually obtained a few days after submission).

Minor points

- There are some discrepancies between the main manuscript, the Table1 and the PDB validation report regarding the map resolution and the number of initial particles of the ligand-bound structure. Please make this consistent.
- I would suggest to remove “high-resolution” from the abstract since I would not call 3.3 and 3.6 Å that.

Tobias Raisch
Max Planck Institute of Molecular Physiology, Dortmund, Germany

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The authors have addressed the most significant critiques appropriately. Presentation of the data is significantly improved.

Minor comment: Figure S8 is not readable in its current form.

Reviewer #2

(Remarks to the Author)

I appreciate the effort the authors have undertaken to improve the manuscript. It has indeed improved in many aspects and most of my points have been sufficiently answered. Especially, the manuscript is now much more easily digestible and focuses more on the novel points.

Still, there remain several points which I would like the authors to address before publication.

1. IP3 site: Fig. 2 has markedly improved and it seems obvious from the figure that IP3 and many of the coordinating amino acid side chains are sufficiently resolved and ordered. However, after having had the chance to look at the Cryo-EM maps

and molecular models provided by the authors, there remain some issues in both the figure and also the text describing the IP3 interaction:

- The IP3 density is not well enough resolved to be absolutely sure about the orientation of the molecule from the density alone. The authors should comment on how they guided the refinement of the compound, e.g. by using prior knowledge from IP3R1/IP3R3 structures. This needs to be made transparent in the manuscript and also that this limits the interpretability of interactions with surrounding amino acids (see also next point)
- Some of the residues they describe in the manuscript as being involved in IP3 binding are too far away in their model to contribute to the interaction. While there is some uncertainty about the placement of residues and IP3 due to the limited resolution, for some of the mentioned residues there is absolutely no way they interact with IP3 in the conformation that is observed in the structure, and the manuscript needs to be changed accordingly. The problematic residues are:
 - Q570 with a distance of ~5.8 Angstrom from the 3'-OH group
 - R503 with distances of ~4.7-5.3 Angstrom from 1'- and 5'-phosphate as well as 6'-OH group
 - R411 with a distance of ~5.5 Angstrom from the 4'-phosphate group
 - R265 with a distance of ~6.7 Angstrom from the 4'-phosphate group
 - K279 that is way too far and even points away from IP3
- I don't know what density map they show in Fig. 2b, but it is for sure not any of the ones they provided to this reviewer. Those are less well defined in this area.
- For some of the residues, i.e. R265, R269, R503, it is not possible in the current view to judge the quality of their density just from this single view, and residue K279 is missing altogether. It would be good to have additional views (most likely in a supplementary figure) that show the quality of the density of all residues mentioned in the text. Also some of the residue labels in Fig. 2b are not placed in unambiguous positions and some are missing.
- Next, there is a mismatch between residues coordinating 5-phosphate: In the text, K279 is mentioned, this is not shown in Fig. 2b, instead there is K507 that is not mentioned in the text.
- L.101: "while the orientations and geometries of the Y567 and R269 side chains are consistent with hydrogen-bonding interactions with the 2- and 5-hydroxyl groups of the inositol ring" does not make sense as the 5-OH is not free and the mentioned residues appear to interact with phosphate groups 5 and 4, respectively.

2. Fig. 5c shows a globular density surrounding the calcium ion. This is not in agreement with the density maps provided to me where there is continuous density with the neighbouring Gln1997

3. Conformational dynamics of ARM1 (p.5/6): This is not shown in any figure, Fig. 3 only shows one conformation and from the very small reconstruction images in Fig. S2, one cannot see anything.

Minor points:

- The authors use the term "near-atomic" throughout their manuscript, as a replacement for "high resolution". This is in general not a great term since it means nothing. I would suggest to remove it since it does not contain any information and might be misleading.
- L.80 should be "closer than in"
- L.217: "at sub-maximal IP3 concentration of" -> End of sentence is missing

Version 2:

Reviewer comments:

Reviewer #2

(Remarks to the Author)

The new version of the manuscript addresses all points that have been raised before. I especially appreciate the reworked IP3 binding site section.

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RESPONSE TO REVIEWERS

We thank reviewers for their overall positive comments and insightful suggestions. We have modified our manuscript (yellow highlight) in response to their suggestions by changes to the text, modification of figures and addition of new data. Our point-by-point responses are provided below.

REVIEWER #1:

This manuscript reports cryo-EM structures of the inositol 1,4,5-trisphosphate receptor isoform 2 (IP₃R2) in multiple conformational states, including ligand-bound and closed forms. As no prior high-resolution structure of IP₃R2 has been published, this dataset fills an important gap in the structural biology of IP₃ receptors. The work has potential significance for understanding isoform-specific regulation, ATP sensitivity, and disease-associated mutations. Overall, the structural determination of IP₃R2 is an important and valuable contribution.

Reviewer 1 – point 1: Lack of Clear Delineation of Novelty. A significant portion of the Results section re-describes architectural features, ligand-binding sites, and domain arrangements that have already been established in prior IP₃R structures. These descriptions present IP₃R2 in a way that obscures what is genuinely new about it.

Authors' response: We thank the reviewer for this thoughtful and constructive comment. As the first structural report of IP₃R2, a complete description of its overall architecture is essential to establish a structural framework for interpreting isoform-specific features. However, we agree that the original organization may not have sufficiently emphasized what is genuinely novel in IP₃R2 relative to previously reported IP₃R1 and IP₃R3 structures. To address this concern, we have substantially revised the manuscript to more clearly distinguish conserved architectural features from new, isoform-specific observations. Specifically:

- At the beginning of each Results subsection, we now explicitly state which features are conserved across IP₃R isoforms and which represent distinctions in IP₃R2.
- Comparative analyses with IP₃R1 and IP₃R3 are now integrated directly into each subsection and several new figures/panels were made or modified (Figs. 2c, 4c, 6c-d, 7d and Supplementary Figs. S6, S7, S8, S9, S10, S14) to highlight differences as they are introduced.
- Redundant descriptions of previously established architectural features have been condensed to sharpen focus on new findings.
- The Discussion is now presented as a separate section rather than being embedded within the Results. This separation clearly distinguishes structural observations from interpretation and allows the novel conceptual advances to be articulated more directly.

We believe these revisions substantially enhance clarity, improve readability, and more clearly define the central contribution of the work - namely, establishing the structural basis for isoform-specific properties of IP₃R2.

Reviewer 1 – point 2: Isoform vs. Species Comparisons. Throughout the manuscript, sequence comparisons are performed across different isoforms taken from different species. This approach does not allow one to distinguish isoform-specific differences from species divergence. Any cross-species comparisons must be clearly justified and interpreted cautiously. This issue substantially weakens conclusions regarding isoform specificity.

Authors' response: We agree that, in principle, isoform-specific conclusions are best supported by comparisons of IP₃R1, IP₃R2, and IP₃R3 from the same species, which would fully eliminate any contribution from species divergence. However, the sequences used here (rat IP₃R1, mouse IP₃R2, and human IP₃R3) were selected for specific, well-supported reasons. First, these correspond to the species of currently available cryo-EM structures for each isoform, and our analysis was intentionally designed to interpret structural features directly in the context of experimentally determined models. Second, mammalian IP₃R isoforms exhibit very high sequence identity across species, particularly within conserved functional domains, such that mammalian interspecies divergence is relatively minor compared with differences between isoforms, e.g., the sequence identity between IP₃R2 human and mouse is 95%. Notably, where comparisons have been performed, e.g. studies of the conserved Ca²⁺ binding site that is functionally important for activation of IP₃R, essentially identical results were obtained studying receptors across species (human and rat) and subtypes (IP₃R1 and IP₃R3) (PMID: 36122240). Finally, mouse IP₃R2 is the most widely used species in the physiological characterization for IP₃R2 (e.g., PMID:17327232, PMID: 27048566), including functional data from in this study, making it the most relevant reference sequence for interpreting known isoform-specific functional behavior.

Nonetheless, we agree that cross-species isoform comparisons should be interpreted cautiously, and we have revised the text and figures accordingly to include the rat, mouse and human IP₃R sequences (Figs. 4c, 6d, 7d, and Supplementary Figs. S6, S8, S9a).

Reviewer 1 – point 3: ARM2 “Auto-Inhibitory” Loop and Energy Landscape Claims
The proposed role of the ARM2 loop in modulating IP₃ sensitivity is intriguing. However:

- The loop is not fully resolved.
- Modeling relies on AlphaFold3 and Rosetta-based docking without reported validation metrics.
- No mutagenesis or functional data are provided to test the structural hypothesis.
- Claims that isoforms occupy distinct basins along an energy landscape defined by ARM2 mobility are speculative and not experimentally supported.

Mechanistic language should be tempered unless supported by quantitative or functional validation.

Authors' response: We agree that the proposed role of the ARM2 loop requires cautious interpretation. Although high-resolution features of the ARM2 loop were not well resolved in our reconstructions, 3D focused classification determined to 6 Å cutoff consistently yielded classes containing additional, albeit low-resolution, density in this region. It is of note that a long and flexible loop such as this might never be fully resolved in a full cryo-EM structure of the intact channel, due to the continuum of its intrinsic flexibility. However, a comparable structural feature had been observed previously in the cryo-EM structure of the IP₃R3 channel, and this region had been shown to be functionally important in IP₃R2 (PMID: 31915246, PMID: 40682657, PMID: 38240350).

We have now provided metrics for AlphaFold3 and Rosetta-based docking in the revised Methods section to better define the confidence and limitations of the predicted conformations.

In the revised manuscript, we have substantially moderated the mechanistic language describing the conformational landscape associated with ARM2 mobility. The ARM2 loop interpretation is now presented as a working model and has been moved to the Discussion section, where it is discussed more qualitatively without detailed mechanistic claims.

Reviewer 1 – point 4: ATP Binding Pocket Interpretation. The manuscript proposes structural explanations for enhanced ATP sensitivity in IP₃R2. However:

- Local resolution of the ATP-binding site is not reported.
- Differences in global and local resolution between compared structures are not accounted for despite their importance in the precision of the different models.
- Electrostatic comparisons and interaction diagrams lack methodological clarity.
- Sequence comparisons again mix isoform and species differences.

Authors' response:

- *Local resolution of the ATP-binding site is not reported.*

Response: We thank the reviewer for this suggestion and have now provided maps of locally refined regions colored by local resolution with corresponding FSC plots (Supplementary Figs. S3, S4). Local resolution maps were calculated using CryoSPARC and are now provided in the revised manuscript. The ATP-binding pocket is resolved at ~3.3 Å and ~2.9 Å in the CIA- and apo-IP₃R2 structures, respectively, supporting reliable modeling of side chain orientations and ligand coordination geometry in this region. These local resolution values are now explicitly reported in the Results section (lines 173-176).

We note that local resolution estimates can be influenced by specific algorithmic parameters (e.g., window size, step size, and masking) and local heterogeneity in the cryo-EM map and therefore should be interpreted together with the underlying cryo-EM density. In practice, the interpretability of structural features – such as well-resolved side chain density – provides an important indicator of map quality in a given region. Accordingly, we provide focused views of the nucleotide-binding region to allow direct visual assessment of density quality and side chain resolvability (Fig. 6 a, b). Together, these additions clarify the reliability of the structural interpretation of the ATP-binding pocket.

- *Differences in global and local resolution between compared structures are not accounted for despite their importance in the precision of the different models.*

Response: We agree that resolution differences must be carefully considered in structural comparisons. In the revised manuscript, we explicitly report both global and local resolution values for the structures being compared. The global resolutions of the compared structures are 3.3 Å (apo-IP₃R2) and 3.6 Å (CIA-IP₃R2), and the local resolutions for these maps were provided in the original manuscript – Supplementary Figs. S1, S2. We have now performed masked local refinements for multiple domains (Supplementary Figs. S3, S4) and generated composite maps for both ligand-free and ligand-bound states. Local refinements for the ATP binding domains (Supplementary Figs. S3, S4) resulted in ~3.3 Å and ~2.9 Å resolution maps for CIA- and apo-IP₃R2 structures, respectively.

The global resolutions of the structures of compared isoforms are 3.6 Å (CIA-IP₃R2) and 3.3 Å (apo-IP₃R2); 3.5 Å (8EAR) and 3.0 Å (7LHF) for rat IP₃R1; and 3.8 Å (7T3T) and 3.5 Å (6DQJ) for human IP₃R3. These values are now explicitly stated in the manuscript (see legend for Supplementary Fig. S7). Importantly, structural comparisons were performed in areas with comparable local resolution and our interpretations rely on features clearly resolved by the EM densities. Side chain conformations discussed in the manuscript are supported by clearly resolved cryo-EM density, and interpretations are limited to features that are confidently resolved in the maps.

- *Electrostatic comparisons and interaction diagrams lack methodological clarity.*

Response: The Methods section has been expanded to clarify these analyses. Electrostatic potentials were calculated in UCSF ChimeraX using AMBER-assigned atomic charges and radii. Identical parameters were applied to all structures to ensure consistency. Potentials were mapped onto molecular surfaces and visualized using the same color scale.

Ligand-protein interaction diagrams were generated in Maestro (Schrödinger) using the Ligand Interaction Diagram tool, which automatically identifies and classifies noncovalent contacts - including hydrogen bonds, hydrophobic interactions, π - π stacking, salt bridges, and water-mediated interactions based on geometric criteria. The resulting interaction networks were visualized as two-dimensional schematics summarizing key ligand-binding site contacts. Default geometric cutoffs were used and applied uniformly across all models. These methodological details are now explicitly described to ensure transparency and reproducibility.

- *Sequence comparisons again mix isoform and species differences.*

Response: Please see our response above to “Reviewer 1 – point 2”.

Briefly, structural conservation across species is high, whereas comparison among IP₃R subtypes is more informative for understanding isoform-specific functional properties. A new Supplementary Figure S6 has been included to clearly distinguish species conservation from isoform divergence.

In summary, we have:

- Reported and visualized local resolution at the ATP-binding site.
- Explicitly addressed global versus local resolution differences.
- Clarified electrostatic calculation parameters and visualization methods.
- Specified software tools and interaction criteria for ligand analysis.
- Distinguished species conservation from isoform-specific sequence variation.

We believe these additions substantially strengthen the structural interpretation of the ATP-binding pocket and reinforce the mechanistic basis for ATP-dependent regulation in IP₃R2.

Reviewer 1 – point 5: At moderate resolution, side-chain positioning and interaction networks must be interpreted cautiously. The current presentation does not sufficiently address modeling uncertainty. Statements such as “these data establish that the ATP binding pocket is responsible for ATP regulation” are overly strong and circular.

Authors’ response: We thank the reviewer for this important comment and agree that structural interpretation at moderate resolution requires careful qualification.

We have revised the manuscript to more explicitly acknowledge the limitations imposed by the maps' moderate resolution. In particular:

- Side chains within the ATP-binding pocket were interpreted only where supported by well-defined EM density.
- Residues with weaker or ambiguous density are described more conservatively.
- Local resolution values for the ATP-binding region are now explicitly reported and discussed in the context of model reliability.
- We explicitly state that hydrogen bonds and electrostatic interactions are inferred based on the best-fit model within the constraints of the density and should be interpreted with appropriate caution.
- Throughout the manuscript, we have replaced definitive language ("establish," "demonstrate conclusively") with more appropriate terms such as "support," "suggest," and "are consistent with."

Importantly, our conclusions do not rely solely on structural inference. Rather, the structural model, interpreted within the limits of the map resolution, is rigorously supported by independent functional data (Figs. 8-10 and Supplementary Fig. S13). Together, these complementary approaches provide convergent evidence that the identified ATP-binding pocket mediates ATP-dependent regulation of channel activity.

We believe these revisions more accurately reflect the level of certainty supported by the data and improve the rigor and transparency of the manuscript.

Reviewer 1 – point 6: Quantitative Claims (Table 2). Table 2 presents qualitative comparisons without clear description of the source of the information. From what data was the table derived? Qualitative structural comparisons require transparent methodological description.

Authors' response: We thank the reviewer for this important comment regarding transparency and methodological clarity.

Upon careful reconsideration, we agree that the presentation of Table 2 could be interpreted as implying quantitative conclusions beyond what is directly supported by the structural data. Because these comparisons were derived from structural analysis of cryo-EM density maps and refined atomic models - rather than from independent energetic or biochemical measurements - we believe that retaining the table in a quantitative format could lead to overinterpretation. Thus, Table 2 is now not presented in the revised manuscript.

We have toned down the corresponding statements in the Results and Discussion sections. Comparative descriptions are now presented explicitly as qualitative structural observations based on model inspection within the limits of map resolution without implying measured energetic or functional parameters.

We believe these revisions improve clarity, eliminate potential ambiguity, and strengthen the overall rigor of the manuscript.

Reviewer 1 – point 7: Ion Conduction Pathway and Disease Mutation. The localization of the G2498S mutation to the selectivity filter is potentially important. However, the mutation's structural impact is inferred rather than demonstrated. The conduction pathway architecture largely recapitulates prior IP₃R structures. Again, on Figure 10, no comparison is made with the other isoforms to contrast them with these new structures.

Authors' response: We thank the reviewer for this thoughtful comment.

We agree that the structural impact of the G2498S mutation is inferred from its position within the selectivity filter rather than directly demonstrated. The manuscript has been revised to explicitly state that the proposed effect on ion conduction represents a structural interpretation based on localization within the pore architecture. Language implying direct functional validation has been removed, and the discussion of this mutation has been appropriately qualified.

Consistent with this refinement, the G2498S mutation has been de-emphasized in the Abstract. The revised Abstract now centers on the overall structural determination of IP₃R2 and the identification of isoform-specific features, rather than on mechanistic implications of a single variant.

We also acknowledge that the overall architecture of the ion conduction pathway is highly conserved and largely recapitulates previously reported IP₃R structures. The revised manuscript now explicitly states this and frames the findings within the context of prior structural work.

In response to the reviewer's suggestion, we have incorporated additional direct comparisons with other IP₃R isoforms in Supplementary Figs. S6, S7, S9, S14 and in the corresponding text. These comparisons clarify both the strong conservation of the conduction pathway and the subtle distinctions observed in IP₃R2, rather than implying substantial architectural divergence.

More broadly, we have carefully revised the manuscript to avoid overstating novelty. Mechanistic interpretations are now framed as structural insights consistent with and extending prior work. We believe these revisions more accurately reflect the scope of the data while preserving the principal contribution of the study - the first structural definition of IP₃R2 and the identification of its isoform-specific features within a conserved channel architecture.

REVIEWER 2.

Baker, Lin, Fan et al. present structures of the type 2 IP₃ receptor in the ligand-free and ligand-bound states and describe in large detail important aspects of ligand-induced activation of the channel. While structures of the other two types, IP₃R1 and IP₃R3, have been reported before, structures of IP₃R2 have been missing so far. This alone makes this an important manuscript for the IP₃R field and beyond, which mandates its publication in Nature Communications. However, the true value of this manuscript and the presented data is the possibility to analyze all the critical structural features side by side, and unfortunately, this has been done insufficiently. Thus, before publication, the manuscript should be rewritten to focus more on this aspect and also to put more emphasis on the technical limitations of the data.

Reviewer 2 – point 1: In large parts, the authors present structure features that have been observed before in the other IP₃R types as if they were the first ones to see them, and use terminology such as “reveal”, “define” and “identify” for features and residues that have been known before (e.g. II.79-80). Some examples for such misleading representations are the descriptions of the IP₃ binding site, the allosteric nexus and the ATP-binding site. Here it would

be much more appropriate, and also easier for readers to follow, to present these features directly as comparisons with IP₃R1 and IP₃R3, instead of merely mentioning towards the ends of the respective paragraphs that similar observations have been made in these proteins as well.

Authors' response: We thank the reviewer for this comment and agree that aspects of the wording in the original manuscript may have unintentionally overstated novelty.

Structural elements such as the IP₃-binding site, the allosteric nexus, and the ATP-binding site have indeed been well characterized in IP₃R1 and IP₃R3. Our intent was to describe their structural manifestation within IP₃R2, whose structure is determined here for the first time, rather than to imply their initial discovery in the receptor family.

To address this concern, we have revised the manuscript throughout to:

- Replace terminology such as “reveal,” “define,” and “identify” with more precise language where appropriate.
- Introduce conserved architectural elements explicitly in the context of prior IP₃R1 and IP₃R3 structures.
- Integrate direct comparisons with other isoforms at the outset of each relevant section, rather than deferring them to the end of paragraphs.

The revised presentation clearly distinguishes conserved structural features from isoform-specific differences observed in IP₃R2. We believe this restructuring improves precision, enhances readability, and more accurately conveys the novelty of the study - namely, defining the IP₃R2 structure within the established architectural framework of the IP₃R family while highlighting its isoform-specific characteristics.

Reviewer 2 – point 2. The resolution of both structures is, with 3.3 Å and 3.6 Å, not excessively high and some of the claims the authors make with regard to hydrogen bonds and in general side chain interactions are a bit shaky, especially since it is not possible to judge the map quality in these regions from the figures. This applies, for example, to the position and coordination of IP₃, as the density shown in Fig. 2b appears rather featureless and the viewing angle and zoom does not allow for assessing the interactions described in the manuscript.

Authors' response: We thank the reviewer for this important comment and agree that structural interpretations at 3.3-3.6 Å resolution require careful qualification.

Although the overall resolutions of the apo and CIA structures are 3.3 Å and 3.6 Å, respectively, the local resolution within the IP₃-binding pocket is higher (2.9 Å and 3.5 Å). This local resolution permits reliable placement of side chains in this region. In the revised manuscript, we explicitly state that only residues supported by well-defined local cryo-EM densities are interpreted structurally. Regions with weaker or ambiguous density are not described in terms of specific hydrogen bonds or precise interaction geometries.

To address the concern regarding visualization, we have added focused density maps of the IP₃-binding pocket (Fig. 2, Supplementary Figs. S3, S4, S10), that show both the ligand and coordinating residues. These maps allow direct assessment of density quality in the region discussed. In addition, we have revised the language throughout to avoid overinterpretation. Interactions are now described as being consistent with coordination or potential hydrogen bonding rather than being presented as definitive assignments.

For Fig. 2b specifically, we have adjusted the viewing angle, zoom level, and map representation to more clearly display the density supporting IP₃ placement. The accompanying text has been revised accordingly to reflect the level of certainty supported by the local map quality.

We believe these revisions improve transparency, appropriately calibrate the interpretation to the map resolution, and allow readers to directly evaluate the structural basis for ligand coordination.

See also our response to **Reviewer 1 – point 4** regarding local resolution reporting and modeling constraints.

Reviewer 2 – point 3: For conserved structure features like the IP₃ binding site or the ATP-binding site, superposition figures would be very helpful and informative to discuss similarities and differences between the IP₃R isoforms. Also, showing clear densities for ligands and relevant interacting residue side chains would be necessary in order to be able to discuss such interactions (e.g. Fig. 5d is a good start, but could be significantly improved).

Authors' response: We thank the reviewer for this constructive suggestion and agree that direct structural comparisons substantially improve clarity.

To better highlight similarities and isoform-specific features, we have added structural superposition figures of the IP₃-binding and ATP-binding sites across the relevant isoforms. These superpositions (new Figs. 2c, 6, 7d and Supplementary Fig. S9) allow direct visual comparison of backbone architecture, side chain positioning, and ligand coordination geometry within a consistent structural framework.

In addition, ligand densities for IP₃ and ATP are now shown at orientations to enable clear assessment of map quality in these regions (Figs 2b, 6a, b; Supplementary Fig. 10b, c). Close-up views explicitly show both the cryo-EM densities and the corresponding refined side chains involved in ligand coordination.

Fig. 5 has been revised accordingly and a new Fig. 6 focused on the ATP-binding site was added. Only residues supported by well-defined local density are highlighted as coordinating interactions; regions of weaker density are described more conservatively.

We believe these additions substantially improve transparency, allow direct visual comparison among isoforms, and strengthen the structural basis for the interpretations presented.

Reviewer 2 – point 4: I find it counter-intuitive to call the ARM2 loop “autoinhibitory” when it is only part of its function to stabilize the apo conformation, and it actually seems to prepare the IP₃-binding site for more efficient binding. Maybe it would be possible to find another, more appropriate name? In addition, I was rather surprised by the way how the authors introduce it in l. 128 where they write that it “partially occludes the IP₃-binding pocket in the apo state” (which makes sense if it is autoinhibitory) and that this would lead to “higher IP₃ binding affinity” (which does not make so much sense in the context of pure autoinhibition and gets explained only much later in the manuscript).

Authors' response: We agree that the term “*autoinhibitory*” does not fully capture the functional role of the ARM2 loop. Previous functional studies identified an inhibitory sequence within the ARM2 region and described it as “*autoinhibitory*” based on its role in suppressing channel activation (PMID: 38240350). However, our structural analysis suggests a more nuanced mechanism. Rather than directly competing with IP₃ for binding, the ARM2 loop undergoes conformational rearrangement that modulates access to the IP₃-binding cleft. In this sense, it acts as a regulatory element that dynamically controls the structural state of the binding pocket, rather than serving as a simple steric inhibitor.

To avoid conceptual ambiguity, we have replaced the term “*autoinhibitory*” with “*ARM2 regulatory loop*” throughout the manuscript. We have also revised the introductory description (lines 127–128) to clarify that partial occlusion in the apo state reflects conformational modulation of the binding cleft, rather than direct antagonism of IP₃ binding. This revision resolves the apparent contradiction between partial occlusion and enhanced binding affinity and provides a more accurate mechanistic description consistent with both structural and functional observations.

Reviewer 2 – point 5: The results of 3D classification seem over-interpreted. Furthermore, the five 3D classes shown in Fig. S7a are overall rather divergent and not only in the ARM2 loop, which precludes a reliable interpretation. Please remove this or tone down in the manuscript.

Authors' response: We thank the reviewer for this careful assessment and agree that the original presentation may have overstated the interpretability of the 3D classification results. The original figure showed focused 3D classification maps filtered to low resolution, which likely exaggerated apparent differences between classes. Importantly, the intent of this analysis was not to imply global structural divergence, but rather to assess the presence of additional density near the IP₃-binding cleft.

To address this concern, we have performed additional rounds of focused 3D classification and added additional refinements. The updated results are now presented in new Supplementary Fig. S11a. These analyses confirm that the extra density near the binding cleft is present, but its precise localization varies among classes due to the intrinsic flexibility of the ARM2 loop. In the consensus cryo-EM map, this density appears weak and poorly defined, with low statistical significance, again consistent with conformational heterogeneity in this region.

As noted in the original manuscript, we were not able to reliably model this flexible segment. Following the reviewer's suggestion, we have toned down the interpretation of the 3D classification results in the revised manuscript to avoid over-interpretation and describe these findings more cautiously as evidence of local conformational variability rather than discrete structural states. We believe the revised analysis and moderated interpretation more accurately reflect the limitations of the data.

Reviewer 2 – point 6: In general, the authors adequately show the conformational flexibility of the “*autoinhibitory*” loop by MD simulations etc. However, the model they derive from that and from the observed structural differences in the IP₃ binding sites between IP₃Rs, namely that the IP₃R2 loop would assist IP₃ binding, is very speculative and should be clearly stated as such.

Instead, the authors present this model in a wall of text on p.6 that reads as if it was based on solid experimental data. I would strongly encourage them to rework this part to make it more clear what evidence they have and where it is speculative, and also to make the text passage more concise and less redundant.

Authors' response: We thank the reviewer for this comment and agree that the original version did not sufficiently distinguish between experimentally supported observations and speculative interpretation.

In the revised manuscript, we have substantially reworked this section to improve clarity and concision. Redundant text has been removed, and the presentation now clearly separates: (i) Observations directly supported by structural comparisons and MD simulations, and (ii) Our proposed mechanistic model, which is explicitly framed as a hypothesis.

We now state clearly that the idea that the IP₃R2 loop may facilitate or stabilize IP₃ binding is a working model derived from comparative structural analysis and simulation data, rather than a directly experimentally validated mechanism. The revised wording emphasizes that this interpretation is consistent with the available data but represents a model rather than a proven mechanism.

We believe these changes improve transparency, appropriately qualify the interpretation, and provide a clearer distinction between evidence and model.

Please also see our response to “Reviewer 2 – point 4” for related clarification.

Reviewer 2 – point 7: The discussion of the TM domain is rather lengthy, but lacks a clear and concise comparison with IP₃R1 and IP₃ R3 regarding domain organization, pore-lining residues, and activation mechanisms. In particular, I don't really understand why the authors discuss their structure more in the context of RyR1/2 than in the context of IP₃R1/3.

Authors' response: We thank the reviewer for this constructive comment. In the revised manuscript, we have substantially tightened and reorganized the TM domain discussion to provide a clearer and focused comparison of IP₃R2 with IP₃R1 and IP₃R3, explicitly addressing overall TM domain organization, pore-lining residues, and structural features relevant to channel gating and activation. New Supplementary Figs. S9 and S14 present a detailed structural comparison of the TM domains across the IP₃R family, highlighting its conservation and isoform-specific differences

We have retained a brief comparison with RyR1/2 but clarified its purpose. Although IP₃Rs and RyRs belong to distinct channel families, they share a conserved tetrameric architecture and common principles of intracellular Ca²⁺ release channel gating. Comparing the gate architecture with other six-transmembrane tetrameric channels provides a broader structural perspective and helps distinguish conserved architectural features from IP₃R-specific adaptations. In the revised version (lines 279-285; 361-364), we explicitly state this rationale and reduce redundancy to ensure that the discussion remains focused while preserving the conceptual relevance of the broader comparison.

We believe these revisions improve the balance, clarity, and structural logic of the TM domain discussion and better align it with the reviewer's expectations.

Reviewer 2 – point 8: Quality of the molecular models: Judging from the validation provided in Table1 and the PDB validation reports, it seems that the model building and refinement is not yet finished. In particular, both structures display elevated clash scores and suboptimal geometry values (bond lengths and angles, Ramachandran, Rotamers), resulting in overall rather high MolProbity scores. While I am aware that refining such large models is a significant task, I urge the authors to be more stringent in this regard and continue refining their models until they reach state-of-the-art quality. This will most likely not affect the conclusions that can be drawn from the structures, but rather reflects best practice in structural biology, especially for a manuscript that focuses so much on structures.

Furthermore, as a minor comment related to this topic, there are some issues with the PDB validation reports, especially regarding the ligand-bound structure where MolProbity failed. Once the models are updated and re-uploaded, please also provide new validation reports without these issues, preferably those intended for manuscript review (which are usually obtained a few days after submission).

Authors' response: We thank the reviewer for this comment and fully agree that stringent stereochemical quality is essential, particularly for a structure-focused manuscript.

During the initial submission, the models were refined to maintain careful balance between stereochemical quality and agreement with the experimental cryo-EM density, without evidence of overfitting. While MolProbity score itself does not directly scale with map resolution, models exhibiting unusually idealized geometry and very low outlier statistics at this resolution may reflect over-interpretation relative to the experimental data.

In response to the reviewer's suggestion, we have undertaken additional rounds of refinement, taking advantage of improved maps generated during the revision process. The updated models now show improved validation statistics, including reduced clash scores (7.52 / 9.32), improved rotamer outliers (2.36% / 3.25%), improved Ramachandran statistics (93.84% favored, 0.52% outliers / 90.43% favored, 0.39% outliers), and correspondingly improved MolProbity scores (2.11 / 2.38) for apo- and CIA-IP₃R2, respectively (Table 1). Importantly, these improvements were achieved while preserving close agreement with the experimental density and maintaining conservative interpretation consistent with map resolution.

The refined models have been re-deposited, and the final curated PDB validation reports are now provided.

We believe these refinements bring the models in line with current best practices for cryo-EM structures at this resolution and further strengthen the structural foundation of the manuscript.

Reviewer 2 - minor points:

1. *There are some discrepancies between the main manuscript, the Table1 and the PDB validation report regarding the map resolution and the number of initial particles of the ligand-bound structure. Please make this consistent.*

Response: Thank you. This has been corrected.

2. *I would suggest to remove "high-resolution" from the abstract since I would not call 3.3 and 3.6 Å that.*

Response: We have removed this.

RESPONSE TO REVIEWERS

We thank the reviewers for their careful evaluation, constructive feedback and overall positive assessment of our revised manuscript. We are pleased that the majority of the reviewers' concerns have been satisfactorily addressed, and we have responded to the few remaining points in detail below.

Reviewer 1 - minor comment: Figure S8 is not readable in its current form.

Authors' response: We thank the reviewer for pointing this out. We have replaced Figure S8 with a higher-resolution version and improved the formatting to enhance readability.

Reviewer 2 - point 1: IP₃ site: Fig. 2 has markedly improved and it seems obvious from the figure that IP₃ and many of the coordinating amino acid side chains are sufficiently resolved and ordered. However, after having had the chance to look at the Cryo-EM maps and molecular models provided by the authors, there remain some issues in both the figure and also the text describing the IP₃ interaction:

1-1. The IP₃ density is not well enough resolved to be absolutely sure about the orientation of the molecule from the density alone.

Authors' response: We thank the reviewer for this important comment. At the resolution of the ligand-bound map (3.6 Å), the cryo-EM density defines the overall position and orientation of IP₃ within the binding pocket, although precise placement of individual phosphate groups cannot be assigned from density alone with atomic-level certainty. To clarify this point, we have revised both the Methods (lines 510–520) and the Results (lines 1010–1020). The IP₃ model was built using multiple complementary constraints, including the experimentally observed cryo-EM density within the ligand-binding pocket, the conserved architecture of the binding site established in previous IP₃R1 structures, and extensive biochemical and mutagenesis studies identifying residues critical for IP₃ coordination (PMID: 36376291; PMID: 22683260; PMID: 22286060; PMID: 8663526). Given the strong sequence and structural conservation of the ligand-binding core across IP₃R isoforms, these features provide a robust framework for modeling ligand placement in IP₃R2.

In terms of actual fitting, a single IP₃ molecule was initially positioned into the density observed at the interface between the ARM1 and β TF2 domains, corresponding to the canonical IP₃ - binding site identified in previous IP₃R structures and the aforementioned mutagenesis studies. Ligand poses generated with Rosetta were consistent with the experimental density, and the best-fitting model was selected for iterative real-space refinement in Phenix, yielding a stable, well-converged ligand fit within the binding pocket.

1-2. Some of the residues they describe in the manuscript as being involved in IP₃ binding are too far away in their model to contribute to the interaction. The problematic residues are:

- Q570 with a distance of ~5.8 Angstrom from the 3'-OH group
- R503 with distances of ~4.7-5.3 Angstrom from 1'- and 5'-phosphate as well as 6'-OH group

- R411 with a distance of ~5.5 Angstrom from the 4'-phosphate group
- R265 with a distance of ~6.7 Angstrom from the 4'-phosphate group
- K279 that is way too far and even points away from IP₃

Authors' response: We thank the reviewer for this careful evaluation of the IP₃ binding site. In response to this comment, we re-examined all residues previously described as interacting with IP₃ and revised the manuscript accordingly. In the updated version, only residues located within conservative interaction distances (generally ≤ 4.0 Å) and supported by the cryo-EM density are described as potential direct ligand-coordinating residues.

Residues positioned farther from the ligand are no longer assigned as making direct contacts with IP₃. Instead, they are described as contributing to the broader electrostatic and structural environment of the binding pocket, which may indirectly influence ligand stabilization and binding. These revisions have been applied consistently throughout the text, Fig. 2, and Supplementary Fig. S10.

Specifically, K507, R510, Y567, and K569 are positioned near the 5-phosphate group (~3.0–3.9 Å), R269 is located near the 4-phosphate group (~2.9 Å), and R568 is proximal to the 1-phosphate (~4.3 Å) and 2-hydroxyl group (~3.1 Å). K569 is additionally positioned near the 6-OH group (~3.1 Å). By contrast, residues such as R503, R411, and R265 are located beyond typical hydrogen-bonding distances and are therefore no longer interpreted as direct ligand-contact residues, although they may contribute to the positively charged environment of the binding pocket. K279 has been removed from the interaction analysis.

Finally, Q570 is not present in IP₃R2 and is not discussed in the manuscript as an interacting residue. The reviewer may have intended to refer to Q270 or N570, which appear in Supplementary Fig. S10 as nearby polar residues but are not assigned as direct IP₃-coordinating residues. Residue numbering throughout the revised manuscript has been verified against the mouse IP₃R2 sequence.

1-3. I don't know what density map they show in Fig. 2b but it is for sure not any of the ones they provided to this reviewer. Those are less well defined in this area.

Authors' response: Figure 2b was prepared using the focused local refinement, and the figure legend has been updated to reflect this.

1-4. For some of the residues, i.e. R265, R269, R503, it is not possible in the current view to judge the quality of their density just from this single view, and residue K279 is missing altogether. It would be good to have additional views (most likely in a supplementary figure). Also some of the residue labels in Fig. 2b are not placed in unambiguous positions and some are missing.

Authors' response:

Additional views are provided in the Supplemental Figure S10 and labels in Fig 2b are shown with arrows to improve clarity in residue identification. As previously mentioned,

K279 does indeed point away from the ligand binding pocket and is now not included in our description of the IP₃ binding pocket.

1-5. Next, there is a mismatch between residues coordinating 5-phosphate: In the text, K279 is mentioned, this is not shown in Fig. 2b, instead there is K507 that is not mentioned in the text.

Authors' response: We thank the reviewer for identifying this inconsistency between the text and Fig. 2b. The discrepancy resulted from an earlier annotation that was not fully updated during figure preparation. We have corrected the manuscript and now consistently identify K507 as one of the residues positioned near the 5-phosphate group of IP₃. The corresponding text, figure labels, and legend have been revised to ensure consistency throughout the manuscript.

1-6. L.101: “while the orientations and geometries of the Y567 and R269 side chains are consistent with hydrogen-bonding interactions with the 2- and 5-hydroxyl groups of the inositol ring” does not make sense as the 5-OH is not free and the mentioned residues appear to interact with phosphate groups 5 and 4, respectively.

Authors' response: We thank the reviewer for pointing out this error in our original description. We agree that the previous wording incorrectly referred to interactions with the 5-hydroxyl group, which is not present in IP₃, and did not accurately reflect the geometry of the modeled interactions.

The text has been revised to correctly describe the positions of Y567 and R269 relative to the phosphate groups of IP₃, based on the structural model and distance analysis. In the revised manuscript, interaction assignments are limited to residues whose positions and orientations are consistent with the cryo-EM density and plausible hydrogen-bonding geometry. Residues located beyond typical interaction distances are no longer described as making direct ligand contacts (see also response to point 1-2).

Reviewer 2 - point 2: Fig. 5c shows a globular density surrounding the calcium ion. This is not in agreement with the density maps provided to me where there is continuous density with the neighbouring Gln1997

Authors' response: We thank the reviewer for this comment. The appearance of Ca²⁺ density and its continuity with coordinating residues depend on the contour level used for map visualization. At lower thresholds, the density can appear continuous with the surrounding coordination environment, whereas at higher thresholds, it appears more localized within the binding site. Such contour-dependent variation in density appearance is commonly observed in cryo-EM maps, particularly for ligand and ion densities.

In the revised Figure 5c, we show Ca²⁺, Zn²⁺, and ATP densities with explicitly stated σ cutoff values to ensure full transparency of visualization parameters. The atomic model is consistent with the cryo-EM density across the displayed range.

To further illustrate the effect of contour level on map appearance, we have added a supplementary figure S13 showing the Ca^{2+} , Zn^{2+} , and ATP binding sites at several representative contour levels. This illustrates that the apparent continuity or localization of ion density relative to coordinating residues depends on the chosen threshold and does not affect the underlying structural interpretation.

Reviewer 2 - point 3: Conformational dynamics of ARM1 (p.5/6): This is not shown in any figure, Fig. 3 only shows one conformation, and from the very small reconstruction images in Fig. S2, one cannot see anything.

Authors' response: We note that the conformational dynamics discussed in this section primarily involve the ARM2 domain, and we assume this is what the reviewer is referring to.

To address this point, we have added a supplementary movie 3 that visualizes the range of ARM2 conformations observed in our dataset. This movie highlights the continuous nature of the domain movements and provides a clearer representation of the structural variability that is not readily apparent from static images.

We have also updated the Fig. S2 presentation and accompanying description to more clearly indicate the observed conformational differences.

Reviewer 2 - minor points:

1. The authors use the term “near-atomic” throughout their manuscript, as a replacement for “high resolution”. This is in general not a great term since it means nothing. I would suggest to remove it since it does not contain any information and might be misleading.

Authors' response: We thank the reviewer for this helpful suggestion and agree that the term “near-atomic resolution” is not strictly defined and can be ambiguous. Although it is commonly used in cryo-EM to describe maps in the ~2.5–4 Å range, we recognize that it is qualitative and may be misleading when used in place of explicit resolution reporting. In the revised manuscript, we have therefore removed or minimized the use of this terminology and instead report the achieved map resolutions explicitly (3.3 Å and 3.6 Å). Where relevant, we additionally describe structural features supported by the maps, including side-chain density and local resolution variation, to provide a clearer and more quantitative assessment of map quality and interpretability.

2. L.80 should be “closer than in” - **thank you, we have fixed it.**

3. L.217: “at sub-maximal IP3 concentration of” -> End of sentence is missing - **thank you, it has been fixed.**

RESPONSE TO REVIEWERS

We thank the reviewers for their positive evaluation of our revised manuscript.

Reviewer #2 (Remarks to the Author):

The new version of the manuscript addresses all points that have been raised before. I especially appreciate the reworked IP3 binding site section.

Authors' response: Thank you. We appreciate the evaluation and feedback through this process.