

Structural atlas of the intact jumbo phage phiKZ

Corresponding Author: Professor Hongrong Liu

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)

The manuscript by Xiao et al., entitled "Structural atlas of the intact jumbo phage phiKZ," reports the full structures of the jumbo phage phiKZ virion. Due to their large sizes, the structural characterization of jumbo phages has been challenging. PhiKZ is a prototypical jumbo phage, yet only its capsid structure had been previously resolved. This work describes high-resolution maps and models of the capsid, neck, tail, baseplate, and tail fibers. Overall, the study and methodology explained and presented well. In terms of the results, while generally presented well, I have suggestions to improve the clarity of some figures and to increase discussion of the biological significance.

Major points:

1. Lines 195-196: Can the authors elaborate on why is it highly probable that the tail tube organization seen in phiKZ will be conserved across all jumbo phage tail tubes? I see the mention of similarity with phiKp24, but I am not sure about other jumbo phages. Is this based on other structural studies? Predicted gene function? AlphaFold?
2. Some figures are a little difficult to follow in terms of color scheme. For example, Figure panels 2C and 2D are showing individual chains in the subunits highlighted in Figure 2A and 2B. But while one subunit in 2A is colored green and the other peach, there is a pink chain in 2C that corresponds to the green subunit and both purple and magenta chains in the peach subunit. Based on the color key at the top, it looks like these should correspond to portal, terminator, and stopper proteins. I had a similar problem with Extended Data Fig. 11, and Extended Data Fig. 12 A vs. 12B. I can appreciate that there are only limited colors and the authors want to emphasize each individual chain, so I am not sure what the best approach is. Maybe different shades of the same color.
3. Lines 225-227: Can the authors elaborate on the interactions between the pin ring and the spike? What types of interactions are these? Is there insight into the biology of spike penetration or the structure-function relationship of spike and pin ring? This is an interesting structure, but it felt glossed over.
4. In Extended Data Fig. 11, could the authors also include the color coding for proteins in panel A? This would make it easier to follow the individual chains in panels B through I and place them into the broader context. I know it is present on Fig. 12, but it is easier to follow when it is in the same figure.
5. Lines 326-327 and Extended Data Figs. 15B & 16: The distal end of the gp131 trimer is the region proposed to interact with bacteria, so biologically quite important. It would be interesting to have more discussion on this structure, homologous structures, etc. In 15B, is a little difficult to see how the gp131 trimer fits into the density of the fibers at their distal ends. Is it possible to include somewhere what this looks like with the density less opaque (maybe just as an outline)? I can see the trimer in Extended Data Fig. 16, but it's harder to visualize the oligomeric state and structure in Extended Data Fig. 15B.

Minor points:

1. Line 68: to be most accurate, please use "ejection" rather than "injection" for genome translocation
2. Line 76: italicize "*Pseudomonas aeruginosa*"
3. Line 123: "ModelAngelo" is misspelled
4. Figure 1B: It would be clearer to specify in the legend that this is the schematic of the structural region of the phiKZ genome. Otherwise, it reads as if the schematic is of the entire genome.
5. Lines 145, 168, 353: capitalize "Sf14"
6. Line 189: I think "stabile" should be "stabilize"
7. Line 378: remove "a" or make "strategies" singular

Reviewer #2

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reviewer #3

(Remarks to the Author)

The manuscript "Structural atlas of the intact jumbo phage phiKZ" by Xiao et al. investigates the structure of the large bacteriophage phiKZ using cryo-electron microscopy single particle analysis as well as a number of focused reconstructions to present a near complete view of the entire virion. This is important as very few (and much lower resolution) reconstructions are available for jumbo phages, and this helps the field understand the complex capsid, neck, tail and baseplates. It is essentially a deep dive into a structure. This paper is generally well written and the figures are absolutely gorgeous. I do have some comments that need to be addressed prior to publication.

One critical omission relates to the inner body, which is ignored in the text. This is a proteinaceous structure inside phiKZ capsids that may be important for genome ejection or DNA organization during packaging. The authors cited papers relevant to this but they do not discuss why in their work they are unable to visualize this structure. Despite the local refinement and the reported high resolution of their capsids, there is no hint of the inner body. Figure 1A shows some bizarre internal features, so I have some concerns over the alignments used to produce the final maps. See for example the low resolution reconstruction from the "bubblegram" paper. This should be able to be resolved with local refinement inside the capsid. These limitations need to be addressed. Either the authors need to solve the structure or state why they don't see this feature.

Major comments

1. The therapeutic potential of this phage is overstated. Given the large genome and questions remaining about the functions of all of the genes, which is necessary for use in clinical trials, this phage is not really an ideal candidate for therapeutics.
2. Page 8, Line 195-199. The authors claim that tail tubes of all jumbo phages will have the same organization. Why not do some bioinformatics? Genomes are publicly available for many jumbos. Do some basic alignments/analysis to support these claims.
3. Line 400. The bacterial inner vesicle is unique to phiKZ. This needs more explanation and to include references.

Minor comments:

1. Not all figures have scale bars. Figures 1 & 2 are much easier to interpret than other figures. I suggest being consistent with scale bars.
2. Check species names throughout—should be italicized.
3. "Near atomic resolution" should be removed throughout. You provide the resolutions in quantitative terms (Å) so this description is meaningless.
4. Line 420: grammar issue: "these structural provide"
5. Extended data Figure 1A—that is a very tight box around the extracted particle. Perhaps there is a better one to highlight?
6. Extended data Figure 7 D&E—why is the color coding different?
7. Extended data Table 1: needs a legend to explain the color coding.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

All prior issues have been addressed. The text has been revised to answer my remaining questions, and the figures are now much clearer.

Reviewer #2

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reviewer #3

(Remarks to the Author)

My comments have been addressed.

Open Access This Peer Review File is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

In cases where reviewers are anonymous, credit should be given to 'Anonymous Referee' and the source.

The images or other third party material in this Peer Review File are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder.

To view a copy of this license, visit <https://creativecommons.org/licenses/by/4.0/>

We would like to express our gratitude to the reviewers for their critical review of our work and for their constructive suggestions on how to improve the manuscript. The manuscript has undergone a thorough revision process to address all comments and suggestions. Specifically, we have added Figs. S11, S12, S14, S18c and S18d to the updated manuscript. For ease of reading, we have copied the original comments in black and marked our responses in blue. The page and line numbers provided correspond to those in the revised manuscript file with colour highlighting.

Reviewer #1 (Remarks to the Author):

The manuscript by Xiao et al., entitled “Structural atlas of the intact jumbo phage phiKZ,” reports the full structures of the jumbo phage phiKZ virion. Due to their large sizes, the structural characterization of jumbo phages has been challenging. PhiKZ is a prototypical jumbo phage, yet only its capsid structure had been previously resolved. This work describes high-resolution maps and models of the capsid, neck, tail, baseplate, and tail fibers. Overall, the study and methodology explained and presented well. In terms of the results, while generally presented well, I have suggestions to improve the clarity of some figures and to increase discussion of the biological significance.

We thank the reviewer for the positive evaluation of our manuscript and the suggestions for improvement.

Major points:

1. Lines 195-196: Can the authors elaborate on why is it highly probable that the tail tube organization seen in phiKZ will be conserved across all jumbo phage tail tubes? I see the mention of similarity with phiKp24, but I am not sure about other jumbo phages. Is this based on other structural studies? Predicted gene function? AlphaFold?

We agree that our initial statement was overly broad. In order to address this issue, a BLASTp search was performed, with the phiKZ tail tube protein as the query. This finding indicates that while a subset of jumbo phages exhibit clear homologs (>30% identity), many others do not. Structural predictions using AlphaFold3 have confirmed that the phiKZ-like tube architecture is present only in those phages with sequence homology. Consequently, we have

revised the text (lines 197-200) to state that this organization is conserved specifically among jumbo phages encoding homologous tail tube proteins, not all jumbo phages. The supporting sequence alignments and structural superpositions are now provided in Figures S11 and S12.

2. Some figures are a little difficult to follow in terms of color scheme. For example, Figure panels 2C and 2D are showing individual chains in the subunits highlighted in Figure 2A and 2B. But while one subunit in 2A is colored green and the other peach, there is a pink chain in 2C that corresponds to the green subunit and both purple and magenta chains in the peach subunit. Based on the color key at the top, it looks like these should correspond to portal, terminator, and stopper proteins. I had a similar problem with Extended Data Fig. 11, and Extended Data Fig. 12 A vs. 12B. I can appreciate that there are only limited colors and the authors want to emphasize each individual chain, so I am not sure what the best approach is. Maybe different shades of the same color.

We would like to express our gratitude for the constructive comments provided by the reviewer. In order to enhance clarity, we have revised the figures accordingly. For Figure 2c and 2d, we have adopted the recommendation of utilizing different shades of the same color for the constituent chains within each subunit. For Figure S13 (previously Extended Data Fig. 11), we have added a clear color key to Panel a to facilitate the contextualization of the individual components. Finally, we have revised Figure S15b (previously Extended Data Fig. 12b) to ensure color consistency between its panels. It is believed that these changes will significantly improve the interpretability of the figures.

3. Lines 225-227: Can the authors elaborate on the interactions between the pin ring and the spike? What types of interactions are these? Is there insight into the biology of spike penetration or the structure-function relationship of spike and pin ring? This is an interesting structure, but it felt glossed over.

We thank the reviewer for highlighting this interesting feature. To elaborate on the interactions between the pin ring and the spike, we have now analyzed the interface and found that the pin ring (gp174) and the central spike (gp164) are linked primarily by weak electrostatic interactions (lines 229-231), as demonstrated in a new panel (Fig. S14). This type of weak,

releasable contact is analogous to the interaction between the gp44 pin domain and the tail tip in phage E217, where it is thought to retain the tip in the pre-infection state. This new detail provides a plausible mechanistic basis for the large-scale baseplate conformational changes and spike penetration that were previously proposed. We have now expanded the Discussion section to incorporate these crucial functional insights, thereby significantly strengthening the proposed infection model (lines 423-429).

4. In Extended Data Fig. 11, could the authors also include the color coding for proteins in panel A? This would make it easier to follow the individual chains in panels B through I and place them into the broader context. I know it is present on Fig. 12, but it is easier to follow when it is in the same figure.

We would like to express our gratitude for the constructive comments provided by the reviewer. Accordingly, we have updated Fig. S13 (previously Extended Data Figure 11) and added a clear color coding for proteins in Panel a.

5. Lines 326-327 and Extended Data Figs. 15B & 16: The distal end of the gp131 trimer is the region proposed to interact with bacteria, so biologically quite important. It would be interesting to have more discussion on this structure, homologous structures, etc. In 15B, is a little difficult to see how the gp131 trimer fits into the density of the fibers at their distal ends. Is it possible to include somewhere what this looks like with the density less opaque (maybe just as an outline)? I can see the trimer in Extended Data Fig. 16, but it's harder to visualize the oligomeric state and structure in Extended Data Fig. 15B.

We have expanded the discussion of this area in the revised manuscript (lines 327–336). Specifically, we highlight that structural modeling reveals the C-terminus of gp131 to be located at the very distal end of the tail fiber, a positioning that is compatible with a potential role in host recognition or initial binding events (Fig. S18b-c).

A comparison was made of the structural characteristics of the C-terminus of gp131 with AlphaFold3-predicted homologs from other jumbo phages, including possible tail fiber or tailspike proteins: gp184 from phage psa21, gp216 from phage 201phi2-1, and gp150 from phage phiPA3. In conjunction with the observed structural similarity and the conserved

sequence features reported in previous studies (Virology 2012, 434: 257-64), these findings suggest that the C-terminus of the gp131 trimer may contribute to host recognition or facilitate the initial stages of bacterial infection (Fig. S18d).

In order to improve the visualization of how the gp131 trimer fits into the cryo-EM density at the distal fiber ends, a new panel has been incorporated, as illustrated in Figure S18c. In this new figure, the density map is displayed as a transparent outline, which more clearly reveals the docked gp131 trimer model within it (line 327). It is hoped that this new illustration will resolve the ambiguity noted in the original Extended Data Fig. 15B.

Minor points:

1. Line 68: to be most accurate, please use “ejection” rather than “injection” for genome translocation

We have revised it (line 63).

2. Line 76: italicize “*Pseudomonas aeruginosa*”

We have revised it (line 71).

3. Line 123: “ModelAngelo” is misspelled

We have revised it (line 131).

4. Figure 1B: It would be clearer to specify in the legend that this is the schematic of the structural region of the phiKZ genome. Otherwise, it reads as if the schematic is of the entire genome.

We have revised it (line 802).

5. Lines 145, 168, 353: capitalize “Sf14”

We have revised it (lines 148, 170, and 361).

6. Line 189: I think “stabile” should be “stabilize”

We have revised it (line 191).

7. Line 378: remove “a” or make “strategies” singular

We have revised it (line 386).

Reviewer #2 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

We thank the reviewer for the note regarding the co-review process. Nature Communications' initiative to support and train Early Career Researchers in the peer review process is to be commended.

Reviewer #3 (Remarks to the Author):

The manuscript “Structural atlas of the intact jumbo phage phiKZ” by Xiao et al. investigates the structure of the large bacteriophage phiKZ using cryo-electron microscopy single particle analysis as well as a number of focused reconstructions to present a near complete view of the entire virion. This is important as very few (and much lower resolution) reconstructions are available for jumbo phages, and this helps the field understand the complex capsid, neck, tail and baseplates. It is essentially a deep dive into a structure. This paper is generally well written and the figures are absolutely gorgeous. I do have some comments that need to be addressed prior to publication.

We thank the reviewer for the positive evaluation of our manuscript and the suggestions for improvement of the manuscript.

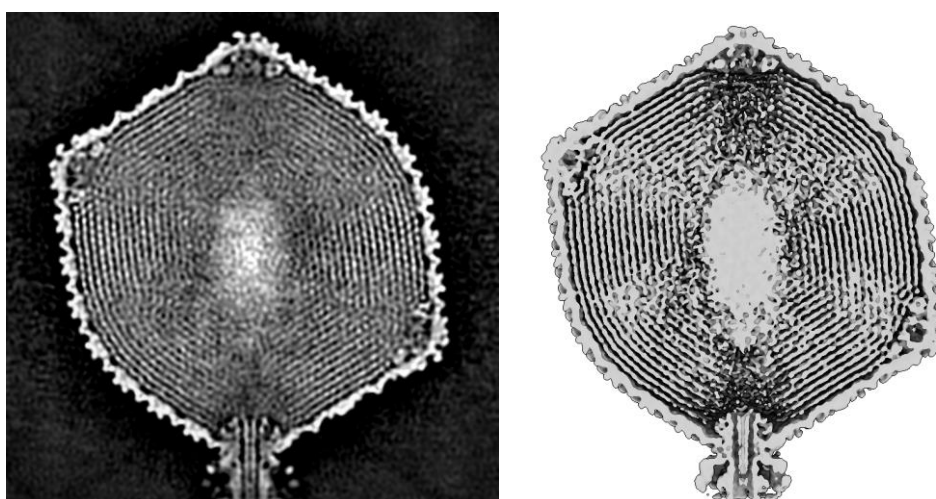
One critical omission relates to the inner body, which is ignored in the text. This is a proteinaceous structure inside phiKZ capsids that may be important for genome ejection or DNA organization during packaging. The authors cited papers relevant to this but they do not discuss why in their work they are unable to visualize this structure. Despite the local refinement and the reported high resolution of their capsids, there is no hint of the inner body. Figure 1A shows some bizarre internal features, so I have some concerns over the alignments used to produce the final maps. See for example the low resolution reconstruction from the “bubblegram” paper. This should be able to be resolved with local refinement inside the capsid. These limitations need to be addressed. Either the authors need to solve the structure or state

why they don't see this feature.

We thank the reviewer for this critical question regarding the inner body. Indeed, we attempt to visualize this structure during the process of data processing. Following the icosahedral reconstruction, the asymmetric structure of phiKZ was reconstructed, incorporating the unique portal-neck complex to a resolution of approximately 10 Å using our symmetry-mismatch reconstruction method (see the figures below, which display a central section (left) and cut-open views (right) of the asymmetric map). However, despite the successful reconstruction of the head with its portal-neck, no discernible density corresponding to the proteinaceous inner body was observed within the capsid interior in the asymmetric structure (see the figures below). Consequently, this finding precluded any further attempts at local refinement on this specific feature. The absence of the inner body signal is likely a consequence of positional heterogeneity relative to the portal-neck-focused alignment.

It is acknowledged that resolving the inner body would likely require a more optimized asymmetric reconstruction strategy, enabling effective 3D classification of the capsid interior to isolate particles with a consistently positioned inner body.

In order to address this issue, a paragraph has been incorporated into both the Results section (lines 109-118) and the Methods section (lines 472-497). These paragraphs provide detailed descriptions of the attempts made, as well as a thorough account of the technical reasons underlying our inability to resolve the inner body.



Major comments

1. The therapeutic potential of this phage is overstated. Given the large genome and questions remaining about the functions of all of the genes, which is necessary for use in clinical trials, this phage is not really an ideal candidate for therapeutics.

A thorough revision of the manuscript has been conducted, with the objective of tempering the assertions made with regard to the therapeutic potential. The revised version employs more conservative language (e.g., "may inform," "potential applications") and emphasizes the role of our findings in inspiring future research (lines 34-35; 47-48; 71; 99-101; 439-440).

2. Page 8, Line 195-199. The authors claim that tail tubes of all jumbo phages will have the same organization. Why not do some bioinformatics? Genomes are publicly available for many jumbos. Do some basic alignments/analysis to support these claims.

We concur with the reviewer's assertion that our initial claim was excessively broad in scope. As suggested, bioinformatics analysis (BLASTp) and AlphaFold3 predictions were performed, revealing that the tail tube organization observed in phiKZ is conserved among other jumbo phages sharing sequence homology with the phiKZ tail tube protein, though not in all jumbo phages. Accordingly, we have refined the text to limit this conclusion to homologous jumbo phages and added Figures S11 and S12 to display the sequence alignments and structural superpositions that support this finding (lines 197-200).

3. Line 400. The bacterial inner vesicle is unique to phiKZ. This needs more explanation and to include references.

We thank the reviewer for this insightful comment. The "inner vesicle" is unique to phiKZ-like jumbo phages, now recognized as the Early Phage Infection (EPI) vesicle. The formation of this structure occurs subsequent to the injection of phage DNA into the cytoplasm, as opposed to its genesis as a pre-existing structure penetrated by the phage tail (Curr Opin Microbiol 2025, 88: 102676; Cell Host Microbe 2024, 32: 1050-1058.e7; J Virol 2024, 98: e0020524.).

In order to address this issue, we have revised the manuscript (lines 406-411). The revised sentence now clarifies: This contraction drives the spike complex and tail tube through both the outer and inner membranes, delivering the phage genome into the cytoplasm. In phiKZ-like

jumbo phages, the ejected genome is then rapidly encapsulated within a specialized compartment known as the early phage infection (EPI) vesicle, which is derived from the host's inner membrane. Conversely, for phages such as T4, the genome is released directly into the cytoplasm without the formation of such a protective structure. In addition, we have also added several references [39-41] to support this updated description.

Minor comments:

1. Not all figures have scale bars. Figures 1 & 2 are much easier to interpret than other figures. I suggest being consistent with scale bars.

We have added scale bars to all figures depicting structural models or electron micrographs where they were previously missing (Figs 3, 4, 5, S1, S13, S15, and S18).

2. Check species names throughout—should be italicized.

We thank the reviewer for pointing this out. We have checked the manuscript thoroughly and have italicized all species names accordingly (line 71).

3. “Near atomic resolution” should be removed throughout. You provide the resolutions in quantitative terms (Å) so this description is meaningless.

We agree with the reviewer and have removed the phrase “near-atomic resolution” throughout the manuscript (lines 57, 96, and 125).

4. Line 420: grammar issue: “these structural provide”

We have revised it (line 436).

5. Extended data Figure 1A—that is a very tight box around the extracted particle. Perhaps there is a better one to highlight?

In order to enhance the clarity and precision of the figure, a comprehensive revision of Figure S1a has been undertaken. A new micrograph has been selected, which features a better-defined particle. Furthermore, a more generous annotation box has been employed, encompassing the entire virion, including the distal tail fibers. This provides a much clearer and more accurate representation of the intact phage morphology. The corresponding zoomed-in panel has been updated accordingly.

6. Extended data Figure 7 D&E—why is the color coding different?

Indeed, the legend did not provide a sufficient explanation regarding the coloring scheme.

The two panels illustrate different levels of organization: Panel d employs alternating colors (green and salmon) to distinguish adjacent gp34 tetramers in the ring assembly, while Panel e utilizes distinct colors to highlight the different domains within a single gp34 monomer. In order to elucidate this matter, we have now revised the figure legend for Figure S7 to explicitly describe the logic behind the coloring of both panels, specifying the purpose of the colors in the assembly view (d) and detailing which color corresponds to which domain in the monomer view (e).

7. Extended data Table 1: needs a legend to explain the color coding.

We have added a legend to Table S1 to explain the color coding scheme.

We would like to express our gratitude to the reviewers for their critical review of our work and for their constructive suggestions on how to improve the manuscript. We have copied the original comments in **black** and marked our responses in **blue**.

Reviewer #1 (Remarks to the Author):

All prior issues have been addressed. The text has been revised to answer my remaining questions, and the figures are now much clearer.

We thank the reviewer for the detailed comments.

Reviewer #2 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

We thank the reviewer for the note regarding the co-review process. Nature Communications' initiative to support and train Early Career Researchers in the peer review process is to be commended.

Reviewer #3 (Remarks to the Author):

My comments have been addressed.

We thank the reviewer for their detailed review.