nature portfolio

Peer Review File

Asymmetric architecture and adaptation of *Treponema* flagella

Corresponding Author: Professor Jun 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)
Compared to canonical external bacterial flagella, the composition and structure of the cell-internal flagellar filaments of spirochetes are considerably more intricate, reflecting their distinct functional modes. Over the past decades, several structural components of these filaments have been identified, and their roles in flagellar synthesis and function have been partially characterized. However, comprehensive structural information has remained incomplete. In this manuscript, the authors identify the proteins constituting the filament and employ a combination of cryo-electron microscopy and cryo-electron tomography to analyze Treponema denticola flagellar filaments both in vivo and in vitro, thereby generating a near-atomic-resolution structural model. Furthermore, they generate substitution mutants to validate key interactions among the different structural components. Overall, the authors present an impressive and comprehensive model of the complex flagellar filament that integrates previously published observations and substantially refines earlier models of flagellar function and assembly.

I find the study convincing and the outcome truly impressive. I have a few minor suggestions (outlined below), most of which concern the presentation. However, I would like to emphasize two points in particular.
First, in the paragraph starting at line 194, the authors describe the generation of substitution mutants to validate the critical residues predicted by the model to be required for FlaB1–FlaA interactions. I wonder why a similar approach was not used to characterize the FlaA1/FlaA2 interactions.
Second, the authors mention a study on the structure of the Leptospira flagellar filament (Brady et al, reference 27). Although this work has been published as a preprint, it reports several interesting findings that appear to overlap with the results presented here. I am therefore surprised that these similarities are not discussed in greater detail in the manuscript.

Minor points:

Figure 1a: I suggest to use more contrasting colors to show the two different types of filament, also the outline font is not well readable.

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This manuscript provides a deep understanding of the assembly and properties of the internal flagella from a disease-causing spirochete. Detailed data on the composition and structure of the flagella provides a mechanism of action for motility of this spirochete that causes periodontitis.

This work is of significance to advancing structural biology, cryo-electron microscopy and tomography, genetics of spirochetes, mass spectrometry, and the interface between protein structure prediction and protein structure determination, including the interface between AI tools, experimental tools, and computer image processing.

The experiments described support the conclusions and claims, other than one minor point stated below.
The methodologies, data analysis, interpretation, and conclusions are all rigorous.
There is enough detail for the results to be reproduced.

Is the methodology sound? Does the work meet the expected standards in your field?
Is there enough detail provided in the methods for the work to be reproduced?

Minor issues to be addressed:

Figure 1 - add the pink arrow to the legend

132-133 -- Fig 2d,g show the diameter and inner core rather than the rise and twist of the filament.

150 "These observations suggest that FlaB1 is the major flagellin and is critical for assembly of the sheathed flagellar filament core"
This statement is not supported by the data currently presented; in the absence of FlaB3, FlaB1 is better able to complensate for the loss than FlaB2.

Figure 2f - please state in the legend that light and dark pink are both FlaA1, and that light and dark purple are both FlaA2

Figure 3 - it is not clear why seeing both FlaB1 and FlaB3 are needed, if not to discuss the distinctions.

-- what happens if you overexpress flaA - fully sheathed? change in motility?

Figure 4e -- this is not a cross-section view, but a cut-away view.

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flagella anchored at opposite poles converts motor torque into whole-cell corkscrew motion"

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is the minimum inter-particle distance along, or perpendicular to, the filament axis?

Figure 6 - Where is FlaAP2 in panel L?

Reviewer #3

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This manuscript describes the flagellar structure of a Treponeme, with a core of a flagellin analog, and several sheath proteins. The data is adequate and properly processed to create 3 maps in the 2-3Å range. Important local interactions are described in detail.
I have some questions about the modeling, and a possible solution.

1. Based on the PDB Validation Reports, which I consider to be critical in evaluating structures, all the geometry parameters are poor with respect to other EM structures. Clash scores (10, 20 and 22) are higher than what are considered good (<10). This indicates that non-bonded atoms are closer together than they should be.
2. The highest-Z-score atomic bond distance differences from ideal values are also predominantly shorter than the ideal bond distance.
3. Ramachandran and Sidechain outliers are also indications of distortion of ideal geometry.
4. The structure with the highest resolution (10PP) has the worst geometry, so the better the map, the harder it is to get the structure to fit.
5. More remotely related, but relevant, the Q-score is more comparable to structures with a lower resolution.

Taken together, this suggests that the pixel spacing value may be too low. The effect of having inaccurate Å/pixel is more pronounced and obvious for structures spanning and fitted across hundreds of Å. This might not invalidate the authors' conclusions about atom-atom interactions at the local level, but higher-quality models would make these conclusions more accurate and solid. The data were measured on an FEI Tecnai F30 microscope with a K3 camera.

I would suggest refitting (from scratch so as to not be trapped into a local minimum) the starting atomic coordinates used here into maps whose Å/pixel is larger, and then refining them in the same way as was done here. If the data were collected close together in time and in the same way, a global trend may emerge. The image processing does not have to be redone, since the effect of a wrong Å/pixel has only a second-order effect on the CTF and other image processing procedures. It is the bond-lengths, etc. which are the best rulers to use at this point. Ideally the geometry, fit to map, Q-score and other parameters would be optimum at a certain Å/pixel, and would fall off again beyond that.

It is not clear whether the filament core, composed of 11 protofilaments, is itself asymmetric and thus supercoiled in the absence of sheath proteins, and if so, how that asymmetry arises from identical subunits, or whether the asymmetry is induced by the binding of the sheath proteins. Are the authors calling the side of the flagella facing the major sheath protein the Symmetric side, and the side facing the concave-surface the Asymmetric side, since the entire flagella is asymmetric (i.e. supercoiled)? It would be better to refer to the "convex" or concave" side of the asymmetric flagella, I think.

After receiving a companion manuscript from researchers using the same data collection facility, and studying a similar macromolecular complex, I have these further observations:
Reading the PDB validation report for the second manuscript (BUT see further below), I noticed that the two data sets were collected on different microscopes. This is not a problem, of course. However, the pixel spacing deposited with the PDB validation reports for the two microscopes was exactly the same. This is highly unlikely. Even the same microscope calibrated twice will probably not have exactly the same Å/pixel.
I had a problem with the first manuscript that suggested to me that the Å/pixel was not accurate. Perhaps the authors used a different Å/pixel for the first manuscript, and simply transcribed the data incorrectly during deposition. An alternative explanation is that they used the same Å/pixel for the two microscopes during data processing, and only one was the correct one. The second manuscript's data collection and modeling statistics seem acceptable so far.
I emphasize that I am not judging these manuscripts against each other. The second manuscript simply conforms to the expectations I would have of any well-determined structure. It is convenient for this example that the data collection facility and the characteristics of the specimen are similar.

After this observation, I reread this manuscript again and found these further points:
I found an additional anomaly or two with this Treponema manuscript.
I noted in my review (above) that the PDB Validation report indicated that the data was collected on a Tecnai microscope. After reading the manuscript again, the Methods section of the manuscript itself says that the relevant data was collected on a Krios (but not necessarily the same one), with the same Å/pixel as the other manuscript. This makes the difference between the quality of the models for the two manuscripts even more puzzling. I still suspect that the volume of the Treponema maps is scaled too small, leading to unusually large clash scores and short bond lengths.
The FSC of the maps also do not fall to zero for the Treponema manuscript (as it usually does), but it does for the maps in the other manuscript.
I have put all these observations into this revised version of the Treponema review, acknowledging my acquaintance with the other manuscript.

Version 2:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
My issues have been appropriately addressed by the authors. Congratulations on this great work.

Reviewer #2

(Remarks to the Author)
The authors have answered the reviewers' comments satisfactorily.

Reviewer #3

(Remarks to the Author)
I acknowledge and deeply appreciate the authors' efforts to answer my concerns. They have created new maps that have improved FSC behavior and realistic resolution estimates with different numbers of particles. Their model-building and real-space refinement has yielded much better validation statistics that have fewer clashes. Finally, their Q-scores (map-to-model statistics) are now solidly in the range that is consistent with these new resolution estimates. I acknowledge that my hypothesis regarding an error in Å/pixel spacing turned out not to be correct one, but I'm glad that the authors found the right solution.
Please update all the figures in the text and supplementary material using the new maps and models that are or will be deposited with the EMDB and PDB, since they appear identical to the previous figures.

Point-by-point response to the reviewers' comments

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

Compared to canonical external bacterial flagella, the composition and structure of the cell-internal flagellar filaments of spirochetes are considerably more intricate, reflecting their distinct functional modes. Over the past decades, several structural components of these filaments have been identified, and their roles in flagellar synthesis and function have been partially characterized. However, comprehensive structural information has remained incomplete. In this manuscript, the authors identify the proteins constituting the filament and employ a combination of cryo-electron microscopy and cryo-electron tomography to analyze Treponema denticola flagellar filaments both in vivo and in vitro, thereby generating a near-atomic-resolution structural model. Furthermore, they generate substitution mutants to validate key interactions among the different structural components. Overall, the authors present an impressive and comprehensive model of the complex flagellar filament that integrates previously published observations and substantially refines earlier models of flagellar function and assembly.

We thank the reviewer for the positive assessment of our work.

I find the study convincing and the outcome truly impressive. I have a few minor suggestions (outlined below), most of which concern the presentation. However, I would like to emphasize two points in particular. First, in the paragraph starting at line 194, the authors describe the generation of substitution mutants to validate the critical residues predicted by the model to be required for FlaB1–FlaA interactions. I wonder why a similar approach was not used to characterize the FlaA1/FlaA2 interactions.

We thank the reviewer for this helpful suggestion and fully agree that substitution mutants are important for validating the critical FlaB1–FlaA interactions, which are a central finding of our study. In addition to these interactions, our work identifies multiple protein–protein interfaces including FlaA1/FlaA2 that are relatively extensive and involve numerous residues as presented in the manuscript. In such cases, site-directed mutagenesis of individual residues may have limited ability to resolve their specific contributions.

We appreciate this point and will consider incorporating targeted mutagenesis, guided by bioinformatic analyses, to further validate and refine these interactions predicted by our periplasmic flagellar model in future studies.

Second, the authors mention a study on the structure of the Leptospira flagellar filament (Brady et al, reference 27). Although this work has been published as a preprint, it reports several interesting findings that appear to overlap with the results presented here. I am therefore surprised that these similarities are not discussed in greater detail in the manuscript.

We thank the reviewer for this helpful suggestion, particularly in light of two recent cryo-EM studies of the Leptospira flagellar filament (Kawamoto et al., 2026; San Martin et al., 2026). We have now expanded the discussion to compare the similarities and differences between the flagellar filaments of Leptospira biflexa and Treponema denticola.

In both systems, the filament consists of a multi-protein core and a multi-protein sheath. The core proteins are secreted through the flagellar type III secretion system, whereas the sheath proteins are exported via the general Sec pathway. However, an important distinction lies in the experimental approaches: the Leptospira studies are based on cryo-EM analyses of purified flagella, whereas our work on T. denticola integrates both purified filaments and in situ structures within intact cells.

Our analysis reveals that, in T. denticola, the filament core is fully enclosed by a sheath composed of five proteins at the proximal end, while the distal region remains non-sheathed. In addition, we

demonstrate that interactions between the core protein FlaB1 and the major sheath protein FlaA are critical for flagellar assembly and motility. In contrast, the Leptospira studies resolve only partial sheath structures that do not completely enclose the core.

Together, our findings provide, to our knowledge, the first complete structural model of a periplasmic flagellar filament in situ and offer new insights into how the filament assembles and functions to enable distinct spirochetal motility. We appreciate the reviewer's suggestion, which has helped us better contextualize our findings within this rapidly advancing field.

Minor points:

Figure 1a: I suggest to use more contrasting colors to show the two different types of filament, also the outline font is not well readable.
92/93, I am sorry, and this is likely due to my untrained eye, but I can't see this in Fig. 1b.

We have revised the figure. We have also replaced all "unsheathed" with "non-sheathed" in this figure as well as in the main text to ensure consistent descriptions throughout the manuscript. In addition, Fig. 1b has been replaced with a clearer and more representative micrograph to improve visibility of the periplasmic flagellar filaments in the intact cell.

Figure 1d, in wt lane there is a prominent band larger than that of FlaA (closer to the 50 kDa(?) marker) that is not occurring in the flaA mutant. Any idea what this protein is?

We characterized this band using LC-MS/MS and found it is the major surface protein (Msp) of T. denticola, which is abundant and often associated with purified PFs.

121, the author may mention the difference in spreading zone appearance on swim plates.

*We included the following description: "*but also displayed spreading zones with indistinct boundaries on swimming plates*".*

133, here it is not clear for me, how the authors deduce the symmetry, please elaborate in one or two sentences.

*We have revised the main text**:** "The core structure resembles a filament with a helical symmetry of a 4.80 Å rise and a 65.29° twist, as estimated by cryoSPARC."*

In figure 4b, the labeling of the insets is barely visible.

Corrected.

224, it is probably due to my ingnorance, but it is clear for all others what a „Tai-chi-like longitudinal dimer" is?

*We have revised the corresponding text**:** "Notably, two FlaA1 protofilaments assemble into a centrosymmetric architecture consisting of two opposing lobes arranged around the center as longitudinal dimers, flanked bilaterally by two FlaA2 protofilaments (Fig. 5a)."*

241, is the reference to Figure 5a correct?

Should be Fig. 6a. *We have revised Fig. 6a to highlight FlaAP1 and FlaAP2 while highlighting FlaA1 and FlaA2 in Fig. 5a.*

241, please define the HEAT repeat.

We have revised the text in the Results as follows: "FlaAP1 is a HEAT-repeat–containing protein composed of tandem repeats of two antiparallel α-helices connected by turns and arranged along a common axis, with adjacent repeats linked by flexible inter-repeat loops."

Reviewer #2 (Remarks to the Author):

This manuscript provides a deep understanding of the assembly and properties of the internal flagella from a disease-causing spirochete. Detailed data on the composition and structure of the flagella provides a mechanism of action for motility of this spirochete that causes periodontitis.

This work is of significance to advancing structural biology, cryo-electron microscopy and tomography, genetics of spirochetes, mass spectrometry, and the interface between protein structure prediction and protein structure determination, including the interface between AI tools, experimental tools, and computer image processing.

The experiments described support the conclusions and claims, other than one minor point stated below. The methodologies, data analysis, interpretation, and conclusions are all rigorous.

We thank the reviewer's positive comments.

There is enough detail for the results to be reproduced.

Is the methodology sound? Does the work meet the expected standards in your field?
Is there enough detail provided in the methods for the work to be reproduced?

We have included details in methods and results to make sure that the study is rigorous and reproducible.

Minor issues to be addressed:
Figure 1 - add the pink arrow to the legend

Corrected.

132-133 -- Fig 2d,g show the diameter and inner core rather than the rise and twist of the filament.

Corrected.

150 "These observations suggest that FlaB1 is the major flagellin and is critical for assembly of the sheathed flagellar filament core"

This statement is not supported by the data currently presented; in the absence of FlaB3, FlaB1 is better able to complensate for the loss than FlaB2.

We agree with the reviewer and have revised the sentence to: "These observations suggest that, while FlaB1 is the major flagellin protein, there may be functional redundancy among the three FlaB proteins."

Figure 2f - please state in the legend that light and dark pink are both FlaA1, and that light and dark purple are both FlaA2

Corrected.

Figure 3 - it is not clear why seeing both FlaB1 and FlaB3 are needed, if not to discuss the distinctions.

FlaB1 is structurally similar to FlaB3; however, we observed notable differences between them, which motivated us to present both proteins in the same figure. To highlight these distinctions, we have revised the text as follows: "The glycan located between flexible loop 2 and loop 3 at the interface of the FlaB1 longitudinal dimer likely contributes to filament stabilization. Its absence in the corresponding region of the FlaB3 longitudinal dimer may reduce the stability of this interface and the FlaB1 filament."

-- what happens if you overexpress flaA - fully sheathed? change in motility?

This is an interesting question. We have not yet explored the effects of FlaA overexpression, but we agree that this would be a valuable direction for future investigation.

Figure 4e -- this is not a cross-section view, but a cut-away view.

Corrected.

215-219 It is unclear that the data shown to this point "demonstrate that FlaB1 residues N147 and D162 are critical for robust interactions"; there are no data regarding whether WT also has protofilaments (they are present in preparations of isolated flagella), and it is not certain (to me) whether the protofilaments are flaB1 or FlaA.

We agree with the reviewer's concerns. In the original manuscript, our description of these data was ambiguous. Our intention was to indicate that mutations at these two specific sites lead to reduced motility; however, the available evidence is insufficient to support a definitive role for these residues in mediating robust interactions. To improve clarity, we have revised the relevant text as follows: "Together, these results suggest that mutations at FlaB1 residues N147 and D162 impair motility, consistent with their potential role in core–sheath interactions and in maintaining the assembly and function of sheathed filaments."

229-230 The lower magnification of Fig 5h makes it unclear what interactions occur between FlaB1 and FlaA1, so it is not shown explicitly that there is 'no direct interaction'.

We thank the reviewer for the comment. Our intention was to convey that the contacts between FlaB1 and FlaA1 are likely mediated by van der Waals interactions. They are relatively weak compared to covalent bonds, hydrogen bonds, or salt bridges. We acknowledge that this point was not clearly described in the original manuscript and have revised the text as follows: "In contrast to the stronger FlaA–FlaB1 interactions, interactions between FlaA1 and FlaB1 are markedly weaker, and no significant hydrogen bonds or salt bridges were observed at the FlaA1–FlaB1 interface (Fig. 5h), indicating reduced core–sheath coupling along the concave side of the supercoiled sheathed filament."

240-241 FlaAP1 and FlaAP2 are not labeled in Fig 5a.

To avoid potential confusion, we have revised Fig. 5a to highlight only FlaA1 and FlaA2 by selectively coloring these two proteins. Similarly, in Fig. 6a, we focus on FlaAP1 and FlaAP2 by coloring only these proteins.

300-301 It is not clear how Movie S4 shows that "Coordinated rotation of periplasmic flagella anchored at opposite poles converts motor torque into whole-cell corkscrew motion"

We appreciate the reviewer's concern and have revised part of the paragraph to: "Rather than functioning as an isolated propeller, rotation of the periplasmic filament drives deformation and counter-rotation of the entire cell body (Movie S4), providing a mechanistic basis for efficient spirochetal motility in highly viscous host environments.

304 the title of this section is misleading -- the data provided show how genetics and assembly govern the biophysical properties of the flagella, but there is no information on how rotation of the flagella is regulated

We appreciate the reviewer's suggestion and have revised the subtitle to: "How are the assembly and biophysical properties of the flagellar filament regulated?"

486-487 Is it correct that the minimum filament length was 1 Angstrom? is the minimum inter-particle distance along, or perpendicular to, the filament axis?

We thank the reviewer for pointing out the typo in our description. We have corrected the corresponding units throughout the main text. The revised text is as follows: "Filament particles were automatically picked using the Filament Tracer[52] in cryoSPARC with a filament diameter of 240 Å, a minimum inter-particle distance of 36 Å, and a minimum filament length of 240 Å.

Figure 6 - Where is FlaAP2 in panel L?

FlaAP2 is not visible in the panel L. To improve clarity, we have revised the labels accordingly in Fig. 6.

Reviewer #3 (Remarks to the Author):

This manuscript describes the flagellar structure of a Treponeme, with a core of a flagellin analog, and several sheath proteins. The data is adequate and properly processed to create 3 maps in the 2-3Å range. Important local interactions are described in detail. I have some questions about the modeling, and a possible solution.
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3. Ramachandran and Sidechain outliers are also indications of distortion of ideal geometry.
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5. More remotely related, but relevant, the Q-score is more comparable to structures with a lower resolution.
Taken together, this suggests that the pixel spacing value may be too low. The effect of having inaccurate Å/pixel is more pronounced and obvious for structures spanning and fitted across hundreds of Å. This might not invalidate the authors' conclusions about atom-atom interactions at the local level, but higher-quality models would make these conclusions more accurate and solid. The data were measured on an FEI Tecnai F30 microscope with a K3 camera.

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best rulers to use at this point. Ideally the geometry, fit to map, Q-score and other parameters would be optimum at a certain Å/pixel, and would fall off again beyond that.

We thank the reviewer for the constructive suggestions regarding model building. We have confirmed that the pixel spacing of our cryo-EM data was properly calibrated; thus, any limitations in modeling are likely attributable to lower resolution in some regions of the asymmetric cryo-EM maps. To improve model fitting, we performed additional model building and real-space refinements, and the corresponding validation results are now included.

It is not clear whether the filament core, composed of 11 protofilaments, is itself asymmetric and thus supercoiled in the absence of sheath proteins, and if so, how that asymmetry arises from identical subunits, or whether the asymmetry is induced by the binding of the sheath proteins. Are the authors calling the side of the flagella facing the major sheath protein the Symmetric side, and the side facing the concave-surface the Asymmetric side, since the entire flagella is asymmetric (i.e. supercoiled)? It would be better to refer to the "convex" or concave" side of the asymmetric flagella, I think.

We thank the reviewer for the constructive suggestions regarding our descriptions. As the filament is intrinsically asymmetric, we have revised the manuscript to ensure consistent definitions of the "convex" and "concave" sides of the flagellum. Furthermore, both the proximal and distal regions of the flagellar filaments wrap smoothly around the cell body, indicating a consistent curvature in both the sheathed and non-sheathed regions. Based on our observations from in situ and in vitro micrographs, the non-sheathed filaments are also asymmetric and supercoiled.

After receiving a companion manuscript from researchers using the same data collection facility, and studying a similar macromolecular complex, I have these further observations: Reading the PDB validation report for the second manuscript (BUT see further below), I noticed that the two data sets were collected on different microscopes. This is not a problem, of course. However, the pixel spacing deposited with the PDB validation reports for the two microscopes was exactly the same. This is highly unlikely. Even the same microscope calibrated twice will probably not have exactly the same Å/pixel.

I had a problem with the first manuscript that suggested to me that the Å/pixel was not accurate. Perhaps the authors used a different Å/pixel for the first manuscript, and simply transcribed the data incorrectly during deposition. An alternative explanation is that they used the same Å/pixel for the two microscopes during data processing, and only one was the correct one. The second manuscript's data collection and modeling statistics seem acceptable so far.
I emphasize that I am not judging these manuscripts against each other. The second manuscript simply conforms to the expectations I would have of any well-determined structure. It is convenient for this example that the data collection facility and the characteristics of the specimen are similar.

We thank the reviewer for this helpful comment. The datasets in the two manuscripts were collected on the same 300 kV Titan Krios microscope at Yale. Although our dataset was acquired in super-resolution mode at a nominal pixel size of 0.534 Å/pixel (81,000× magnification), an F-crop factor of 1/2 was applied during Patch Motion Correction, yielding an effective pixel size of 1.068 Å/pixel. We consider this pixel size sufficient to support the reported near-atomic resolution structure determination. This processing step likely explains the discrepancy between the pixel size reported in our manuscript and that reported by Drs. Alejandro Buschiazzo and Charles Sindelar. We also note that Dr. Charles Sindelar was involved in both studies.

To clarify this point, we have revised the relevant text in the Methods section as follows:

Lines 490–492:
"…The total electron dose was ~70 $e^-/Å^2$. The movies were collected in super-resolution mode. An F-crop factor of 1/2 was applied during Patch Motion Correction, resulting in an effective pixel size of 1.068 Å/pixel. A total of 6,672 micrographs were collected…"

Lines 529–530:
"…All micrographs were first motion-corrected with an F-crop factor of 1/2 applied and then subjected to Patch CTF Estimation in cryoSPARC. Filament particles…"

In addition, we have updated the map and model statistics, including the PDB validation reports. The models were further refined to improve their quality as much as possible. Please refer to the attached files for details.

After this observation, I reread this manuscript again and found these further points:
I found an additional anomaly or two with this Treponema manuscript.
I noted in my review (above) that the PDB Validation report indicated that the data was collected on a Tecnai microscope. After reading the manuscript again, the Methods section of the manuscript itself says that the relevant data was collected on a Krios (but not necessarily the same one), with the same Å/pixel as the other manuscript. This makes the difference between the quality of the models for the two manuscripts even more puzzling.

We thank the reviewer for pointing out this error in the PDB deposition. Although all data were collected on a 300 kV Titan Krios microscope, we inadvertently selected the Tecnai model during deposition. This has now been corrected in the PDB entry.

I still suspect that the volume of the Treponema maps is scaled too small, leading to unusually large clash scores and short bond lengths.
The FSC of the maps also do not fall to zero for the Treponema manuscript (as it usually does), but it does for the maps in the other manuscript.
I have put all these observations into this revised version of the Treponema review, acknowledging my acquaintance with the other manuscript.

We thank the reviewer for raising this important concern regarding the FSC. After carefully re-examining the data, we determined that the issue did not arise from an incorrect scaling factor, but rather from the inclusion of duplicated particles during filament tracing. After removing these duplicated particles and reprocessing the dataset, the FSC curves now properly decay to zero. Accordingly, the particle numbers, resolution estimates, and FSC curves have been updated in the manuscript and figures, as reflected in the main text and Figs. S4 and S5.

Following additional real-space refinement, we obtained models of improved quality with more favorable validation statistics. The updated models and corresponding validation reports are provided in the attached files.