natureresearch

# Peer Review Information

**Journal:** Nature Microbiology
**Manuscript Title: Molecular structure of the intact bacterial flagellar basal body**
**Corresponding author name(s):** Susan Lea

**Decision Letter, initial version:**

Dear Susan,

Thank you for your patience while your manuscript "Molecular structure of the intact bacterial flagellar basal body" was under peer-review at Nature Microbiology. I'm sorry for the delay in getting back to you with a decision but unfortunately one of the referees took longer than anticipated to return their comments to us. Nonetheless, the manuscript has now been seen by 3 referees, whose expertise and comments you will find at the bottom of this email. As you will see from their comments, the referees are very positive and find your work of interest, and have only raised a few number that will need to be addressed before we can proceed. Should further experimental data or text modifications allow you to address these points, we would be very happy to look at a revised manuscript.

In particular, you will note that referee #2 asks that you rephrase some of the statements (for example, to replace the overall numbers of proteins instead with the "number of components and their oligomerization state [which] would be better in this case") and that you clarify some of the methods used (including those to identify YecR). Both referees #1 and #3 ask for additional information on FlhA and whether it may have been lost during purification or processing, with referee #3 further asking if FlhE is also present in the structures. The referees also made some suggestions for how to improve a few of the figures; and ask that you further discuss some of the limitations and how those impact the proposed models. For example, referee #2 asks that you discuss "why the flagella rod needs multiple proteins unlike the injectisome, which only has 2 proteins based on the structural information", if "the lower height of the P-ring is due to the loss of the L-ring and subsequent sliding down? or without the L-ring, the P-ring naturally assembles at a lower point", and "Does the knockout of the hook protein in any way interfere with the assembly of the rod in a similar manner?"; and referee #3 asks "Could the phenotype of LPS mutants be explained by the LPS interactions with FlgH?" and "How is this possible if the cap completely blocks the exit channel and the mechanism of cap function requires a new filament subunit to push the cap complex upwards?"

We are committed to providing a fair and constructive peer-review process. Please do not hesitate to contact us if there are specific requests from the reviewers that you believe are technically impossible or unlikely to yield a meaningful outcome.

We strongly support public availability of data. Please place the data used in your paper into a public

data repository, if one exists, or alternatively, present the data as Source Data or Supplementary Information. If data can only be shared on request, please explain why in your Data Availability Statement, and also in the correspondence with your editor. For some data types, deposition in a public repository is mandatory - more information on our data deposition policies and available repositories can be found at https://www.nature.com/nature-research/editorial-policies/reporting-standards#availability-of-data.

Please include a data availability statement as a separate section after Methods but before references, under the heading "Data Availability". This section should inform readers about the availability of the data used to support the conclusions of your study. This information includes accession codes to public repositories (data banks for protein, DNA or RNA sequences, microarray, proteomics data etc…), references to source data published alongside the paper, unique identifiers such as URLs to data repository entries, or data set DOIs, and any other statement about data availability. At a minimum, you should include the following statement: "The data that support the findings of this study are available from the corresponding author upon request", mentioning any restrictions on availability. If DOIs are provided, we also strongly encourage including these in the Reference list (authors, title, publisher (repository name), identifier, year). For more guidance on how to write this section please see:
http://www.nature.com/authors/policies/data/data-availability-statements-data-citations.pdf

If revising your manuscript:

* Include a "Response to referees" document detailing, point-by-point, how you addressed each referee comment. If no action was taken to address a point, you must provide a compelling argument. This response will be sent back to the referees along with the revised manuscript.

* If you have not done so already we suggest that you begin to revise your manuscript so that it conforms to our Letter format instructions at http://www.nature.com/nmicrobiol/info/final-submission. Refer also to any guidelines provided in this letter.

* Include a revised version of any required reporting checklist. It will be available to referees (and, potentially, statisticians) to aid in their evaluation if the manuscript goes back for peer review. A revised checklist is essential for re-review of the paper.

When submitting the revised version of your manuscript, please pay close attention to our href="https://www.nature.com/nature-research/editorial-policies/image-integrity">Digital Image Integrity Guidelines.</a> and to the following points below:

-- that unprocessed scans are clearly labelled and match the gels and western blots presented in figures.
-- that control panels for gels and western blots are appropriately described as loading on sample processing controls
-- all images in the paper are checked for duplication of panels and for splicing of gel lanes.

Finally, please ensure that you retain unprocessed data and metadata files after publication, ideally archiving data in perpetuity, as these may be requested during the peer review and production process or after publication if any issues arise.

Please use the link below to submit a revised paper:

[REDACTED]

<strong>Note:</strong> This url links to your confidential homepage and associated information about manuscripts you may have submitted or be reviewing for us. If you wish to forward this e-mail to co-authors, please delete this link to your homepage first.

Nature Microbiology is committed to improving transparency in authorship. As part of our efforts in this direction, we are now requesting that all authors identified as 'corresponding author' on published papers create and link their Open Researcher and Contributor Identifier (ORCID) with their account on the Manuscript Tracking System (MTS), prior to acceptance. This applies to primary research papers only. ORCID helps the scientific community achieve unambiguous attribution of all scholarly contributions. You can create and link your ORCID from the home page of the MTS by clicking on 'Modify my Springer Nature account'. For more information please visit please visit <a href="http://www.springernature.com/orcid">www.springernature.com/orcid</a>.

If you wish to submit a suitably revised manuscript we would hope to receive it within 2 months. If you cannot send it within this time, please let us know. We will be happy to consider your revision, even if a similar study has been accepted for publication at Nature Microbiology or published elsewhere (up to a maximum of 6 months).

In the meantime we hope that you find our referees' comments helpful and please let me know if you have any questions.

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Reviewer Comments:

Reviewer #1 (Remarks to the Author):

The authors present a comprehensive structural analysis of the flagellar basal body from Salmonella typhimurium. Their investigation includes a thorough modeling of LP-ring, rod and MS-ring components including embedded parts of the type-III-secretion system. Furthermore, the details of how the different proteins are connected at high-resolution allows to draw a model for basal body assembly and function. The presence of the hook-cap protein FlgD additionally provides first insights into hook assembly. The manuscript is a lovely piece of work and I can only recommend publication! It is clearly a hallmark in the field of flagella research.

There are some minor issues that require attention before publication:
-Line 71: Please refer to the individual panels of figure 1 (a, b and c in this case).
-Line 85: The right panel is rather confusing and lacks details. Maybe a detailed view of the hydrophobic interactions would help here?

-Line 113: The T3SS proteins in Fig 2C should be labeled (according to 2A). This will make it easier for the reader to see which component (mostly FliP) rearranges the most.
-Line 134: Where am I supposed to see residues 48-82 of FlgI in the two figures? A detailed picture might be helpful here. Furthermore, all components should be labeled accordingly in figure 3A. It complicates the readability of one has to always look on Fig 2 for the color code. Furthermore the “dashed white box” in ExFig 6 is barely visible. Maybe change the color?
-Lines 145 – 149: Where is this shown?
-Line 174: Fig. 3C appears after d-g. Should be rearranged.
-Line 188: „(6IEE)“ – please indicate that this is a PDB code
-Line 189: The residue range should be labeled in fig 4A. Also, Fig. 4C should be moved to the right side of the figure.
-Line 191: Not sure if it’s my version but ExFig 8D is a blank space?
-Materials and methods require some proofreading: e.g., Line 264: “0.5 % NaCl” should be “0.5% (w/v)”; amongst others

-In my feeling, the movie is not really intuitive for a non-expert. Labels and an orientation relative to the basal body would probable improve.

-I am wondering whether the authors could identify densities corresponding to FlhA during their classification? Or was the density information of too low quality as for the C-ring? Would be great of the authors could shortly comment.

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Reviewer #2 (Remarks to the Author):

Johnson et al present a paper entitled "Molecular structure of the intact bacterial flagellar basal body" describing the cryoEM structure of the flagellar basal body from Salmonella. The presented structures, solved to resolutions between 2.2 and 3.7 Å, reveal for the first time the molecular architecture of the dual membrane spanning flagella basal body encompassing 13 different protein types. The results represent a major breakthrough in the understanding of the molecular architecture of the flagellar apparatus providing detailed structural information about the LP-ring, rod proteins, MS-ring and export gate within the basal body, many of which are novel observations. The study also represents an outstanding example of data processing starting from a huge dataset (>60,000 movies) and using combined focussed refinements to extract information about different parts of the complex and ultimately the overall assembly. The manuscript is well written and presents the detailed structural data in a succinct manner. Given the historical interest in the bacterial flagellar, the manuscript should be of broad interest to readers of Nature Microbiology.

Some minor comments below:

I find quoting overall numbers of proteins in this highly oligomerized structure odd and not informative. The number of components and their oligomerization state would be better in this case.

Line 92: Proteomic analysis was used to identify YecR, although this is not referenced anywhere in figures or supplementary information.

Line 120-125: can the author comment/elaborate on why the flagella rod needs multiple proteins

unlike the injectisome, which only has 2 proteins based on the structural information? Also, the sentence on line 125 "But each protein has a different domain inserted between the helices to drive correct assembly of the helix." is a little difficult to understand and should be clarified.

Line 143: do the authors suggest the lower height of the P-ring is due to the loss of the L-ring and subsequent sliding down? or without the L-ring, the P-ring naturally assembles at a lower point? The rod seems thinner on the bottom, so why does the P-ring stop at that particular point?

Line 180: In the assembly of the injectisome (Wagner 2020), in absence of needle protein PrgI, the PrgJ rod does not assemble as well. Does the knockout of the hook protein in any way interfere with the assembly of the rod in a similar manner?

Line 200: Given the lower local resolution of the non-helical region, it would be appropriate to clarify any limitations of the proposed model/interpretation of the data therein.

Please clarify if FlgD was identified in the proteomic analysis that identified YecR.

Figures and Extended Data Figs - with the exception of Extended Data Fig 2, the map mesh is difficult to see.

Extended Data Fig. 1: the flowchart could benefit from tidying up

Extended Data Fig. 8: panel d is missing.

**********************************************************
Reviewer #3 (Remarks to the Author):

In the present manuscript, Johnson and colleagues present for the first time a high-resolution molecular structure of an intact flagellar basal body. The flagellum is a remarkably complex macromolecular machine and the primary motility device of many bacteria. How 174 molecules of more than a dozen different proteins come together to form the basal body of the flagellum is simply astonishing. I am amazed that this structure is not in Nature itself as an article.

The manuscript itself has been a pleasure to read and I can only congratulate the authors on this exciting work. The reported structure constitutes a break-through in the field and will surely find its way into many textbooks.

Some minor comments that the authors might consider discussing:

1) LPS mutants are known to affect flagella assembly. Previously, it was suggested that LPS pertubations results in downregulation of the flagellar master regulator flhDC via the Rcs system. Could the phenotype of LPS mutants be explained by the LPS interactions with FlgH?

2) What is the phenotype of a yecR mutant? Strain TH26465 (DyecR::tetRA) is mentioned in the Materials and Methods, but nowhere else in the manuscript. How conserved is YecR (STM1934 in Salmonella)?

3) Is there any density that might correspond to FlhE? FlhE was previously suggested to be part of the basal body (Lee, J., and Harshey, R.M. (2012) Mol Microbiol 84: 550-565).

4) I was surprised by the finding that the hook cap FlgD completely blocks the secretion channel. If I understand the proposed mechanism correctly, any cap movement requires secretion and assembly of a new filament subunit to push the cap upwards. However, a hook mutant still secretes rod substrates (and non-structural proteins such as the molecular ruler FliK) into the supernatant. In addition, flagellin subunits are known to be 'spilled-over' during filament assembly.
How is this possible if the cap completely blocks the exit channel and the mechanism of cap function requires a new filament subunit to push the cap complex upwards?

5) The manuscript does not mention FlhA, which is a major component of the export apparatus. I assume FlhA was lost during basal body purification. Considering the myriads of interactions between the MS ring and the export gate, is there enough space inside the MS ring for the FlhA nonamer?

## Author Rebuttal to Initial comments

Reviewer #1 (Remarks to the Author):

The authors present a comprehensive structural analysis of the flagellar basal body from Salmonella typhimurium. Their investigation includes a thorough modeling of LP-ring, rod and MS-ring components including embedded parts of the type-III-secretion system. Furthermore, the details of how the different proteins are connected at high-resolution allows to draw a model for basal body assembly and function. The presence of the hook-cap protein FlgD additionally provides first insights into hook assembly. The manuscript is a lovely piece of work and I can only recommend publication! It is clearly a hallmark in the field of flagella research.

We thank the reviewer for their appreciation of our work.

There are some minor issues that require attention before publication:
-Line 71: Please refer to the individual panels of figure 1 (a, b and c in this case).

We have amended the figure references to cite individual panels

-Line 85: The right panel is rather confusing and lacks details. Maybe a detailed view of the hydrophobic interactions would help here?

We have tried several different representations and, given the space constraints, have not been able to improve on the original panel. We hope that the interested reader will download the coordinates and inspect the details for themselves.

-Line 113: The T3SS proteins in Fig 2C should be labeled (according to 2A). This will make it easier for the reader to see which component (mostly FliP) rearranges the most.

This figure is already rather busy and given the labels for the export gate components are just above panel (c) and that the legend specifically points to them being coloured in the same fashion as panels (a) and (b) we find it unhelpful to add further clutter to the figure by duplicating the labels 1 cm below.

-Line 134: Where am I supposed to see residues 48-82 of FlgI in the two figures? A detailed picture might be helpful here.

This is the region highlighted in ExFig6, now further highlighted by boxing on ExtFig6(a), and explicitly pointed to by the text. While the residues discussed are within suitable Cα - Cα distances to form salt bridges, the maps lack sufficient density at the end of the sidechains to unambiguously assign rotamer conformations. We therefore feel that showing specific details of one set of sidechains is inappropriate. We have revisited the language used in discussing this and hope it is acceptable without an additional illustration.

Furthermore, all components should be labeled accordingly in figure 3A. It complicates the readability of one has to always look on Fig 2 for the color code.

We have added further labels to Figure 3A and hope this aids comprehension

Furthermore the “dashed white box” in ExFig 6 is barely visible. Maybe change the color?

Changed to green for improved visibility

-Lines 145 – 149: Where is this shown?

This is the region highlighted in ExFig6(b) – now further highlighted by boxing on ExtFig6(a) and explicitly pointed to by the text

-Line 174: Fig. 3C appears after d-g. Should be rearranged.

Rearranged

-Line 188: „(6IEE)“ – please indicate that this is a PDB code

So indicated

-Line 189: The residue range should be labeled in fig 4A.

Added

Also, Fig. 4C should be moved to the right side of the figure.

Rearranged

-Line 191: Not sure if it’s my version but ExFig 8D is a blank space?

Apologies – it should be a co-variation plot, hopefully now visible.

-Materials and methods require some proofreading: e.g., Line 264: “0.5 % NaCl” should be “0.5% (w/v)”; amongst others

Apologies for our errors – we have carefully proof-read and added details as requested.

-In my feeling, the movie is not really intuitive for a non-expert. Labels and an orientation relative to the basal body would probable improve.

The main purpose of this movie is to show that the model we describe does not imply rotation of the cap (the previously accepted model). We have added a specific statement to the legend that the cap is coloured and oriented as in Figure 4e with the basal body toward the bottom of the figure and hope this is sufficient to aid understanding.

-I am wondering whether the authors could identify densities corresponding to FlhA during their classification? Or was the density information of too low quality as for the C-ring? Would be great of the authors could shortly comment.
No density is observed for FlhA we assume it is largely lost during purification or grid preparation and it therefore cannot be reconstructed. We now explicitly note this in the discussion.

**********************************************************
Reviewer #2 (Remarks to the Author):

Johnson et al present a paper entitled "Molecular structure of the intact bacterial flagellar basal body" describing the cryoEM structure of the flagellar basal body from Salmonella. The presented structures, solved to resolutions between 2.2 and 3.7 Å, reveal for the first time the molecular architecture of the dual membrane spanning flagella basal body encompassing 13 different protein types. The results represent a major breakthrough in the understanding of the molecular architecture of the flagellar apparatus providing detailed structural information about the LP-ring, rod proteins, MS-ring and export gate within the basal body, many of which are novel observations. The study also represents an outstanding example of data processing starting from a huge dataset (>60,000 movies) and using combined focussed refinements to extract information about different parts of the complex and ultimately the overall assembly. The manuscript is well written and presents the detailed
structural data in a succinct manner. Given the historical interest in the bacterial flagellar, the manuscript should be of broad interest to readers of Nature Microbiology.

We are glad the reviewer appreciates the vast amount of work represented in this paper in addition to the importance of the result.

Some minor comments below:

I find quoting overall numbers of proteins in this highly oligomerized structure odd and not informative. The number of components and their oligomerization state would be better in this case.

Whilst we appreciate the reviewer's point, the number of protein chains in total is given as a simple way for the reader to appreciate the complexity of the object. We feel that space and readability would preclude an enumeration of the precise composition in the abstract.

Line 92: Proteomic analysis was used to identify YecR, although this is not referenced anywhere in figures or supplementary information.

We apologise and have added the excel sheet containing the proteomics data that identified YecR. This is now referenced in the text.

Line 120-125: can the author comment/elaborate on why the flagella rod needs multiple proteins unlike the injectisome, which only has 2 proteins based on the structural information?

We agree that it is fascinating and would speculate that it relates to the likely need for great structural stability in the flagellar system particularly in coupling the axial components to the FliF MS Ring for rotation. We have added some additional discussion of this fascinating aspect of the structure.

Also, the sentence on line 125 "But each protein has a different domain inserted between the helices to drive correct assembly of the helix." is a little difficult to understand and should be clarified.

We have reworded to hopefully clarify.

Line 143: do the authors suggest the lower height of the P-ring is due to the loss of the L-ring and subsequent sliding down? or without the L-ring, the P-ring naturally assembles at a lower point? The rod seems thinner on the bottom, so why does the P-ring stop at that particular point?

Unfortunately, our data do not allow us to distinguish between these hypotheses, the earlier work on poly-rod and poly-P-ring assemblies (cited in the text) would suggest that the P-ring can assemble at any point on the distal FlgG structure, and presumably the thinner diameter of the proximal rod prevents stable assembly at this point. The direction of assembly and data from the cited papers would suggest that the P-ring then imprints the L-ring, but as we lack data to further support this idea, we do not discuss at this time.

Line 180: In the assembly of the injectisome (Wagner 2020), in absence of needle protein PrgI, the PrgJ rod does not assemble as well. Does the knockout of the hook protein in any way interfere with the assembly of the rod in a similar manner?

From the assembly we observe it does not appear to so do, probably because the injectisome needle protein is not a true analogue of the hook, but rather an analogue of one of the rod components. A more appropriate equivalence would be to look at FliE assembly in a knockout of the FlgB protein.

Line 200: Given the lower local resolution of the non-helical region, it would be appropriate to clarify any limitations of the proposed model/interpretation of the data therein.

Reworded to make clear.

Please clarify if FlgD was identified in the proteomic analysis that identified YecR.

FlgD was observed (see new excel spreadsheet of proteomic data) – we now note this in the text

Figures and Extended Data Figs - with the exception of Extended Data Fig 2, the map mesh is difficult to see.

We have tweaked the parameters for the mesh trying to balance map visibility with visibility of the structures underlying. Given the complexity of the figures we have largely optimized them to show these details when zoomed in on a screen rather than at the size shown when printed.

Extended Data Fig. 1: the flowchart could benefit from tidying up

We apologise and have tidied this figure.

Extended Data Fig. 8: panel d is missing.
Apologies – clearly the co-variation panel was lost in file conversion as it was present in the uploaded figures. We will check more carefully on this iteration

**********************************************************
Reviewer #3 (Remarks to the Author):

In the present manuscript, Johnson and colleagues present for the first time a high-resolution molecular structure of an intact flagellar basal body. The flagellum is a remarkably complex macromolecular machine and the primary motility device of many bacteria. How 174 molecules of more than a dozen different proteins come together to form the basal body of the flagellum is simply astonishing. I am amazed that this structure is not in Nature itself as an article.

We thank the reviewer for their excitement and share their amazement that ***Nature*** is not interested in such structures.

The manuscript itself has been a pleasure to read and I can only congratulate the authors on this exciting work. The reported structure constitutes a break-through in the field and will surely find its way into many textbooks.
We certainly hope that more detailed figures will be needed in future text books!

Some minor comments that the authors might consider discussing:

1) LPS mutants are known to affect flagella assembly. Previously, it was suggested that LPS pertubations results in downregulation of the flagellar master regulator flhDC via the Rcs system. Could the phenotype of LPS mutants be explained by the LPS interactions with FlgH?

It is entirely possible that loss of this close interaction leads to the phenotypes observed in these backgrounds. However, from our data we cannot distinguish between the suggested regulator driven phenotype and one caused by a more direct mechanism.

2) What is the phenotype of a yecR mutant? Strain TH26465 (DyecR::tetRA) is mentioned in the Materials and Methods, but nowhere else in the manuscript. How conserved is YecR (STM1934 in Salmonella)?

Using standard assays, the yecR mutant has no discernible phenotype (new supplemental figure 1). We assume this is likely due to it assisting in efficient insertion of the LP ring, rather than playing an essential role, perhaps via mechanisms related to other cell stress responses alluded to in the previous point. YecR is very highly conserved

being 100% identical across most *E. coli* and *Salmonella* strains.

3) Is there any density that might correspond to FlhE? FlhE was previously suggested to be part of the basal body (Lee, J., and Harshey, R.M. (2012) Mol Microbiol 84: 550-565).

We see no evidence for FlhE in the volumes and the manuscript mentioned provides no evidence for it being part of the assembled structure, just evidence that its removal perturbs flagellar activity and assembly. It is possible that it is involved at an earlier stage of assembly but is lost by the stages we capture in our structures.

4) I was surprised by the finding that the hook cap FlgD completely blocks the secretion channel. If I understand the proposed mechanism correctly, any cap movement requires secretion and assembly of a new filament subunit to push the cap upwards. However, a hook mutant still secretes rod substrates (and non-structural proteins such as the molecular ruler FliK) into the supernatant. In addition, flagellin subunits are known to be 'spilled-over' during filament assembly.
How is this possible if the cap completely blocks the exit channel and the mechanism of cap function requires a new filament subunit to push the cap complex upwards?

We have reviewed the text to clarify our explanation – but out hypothesis is that the movement of the new subunit (of any sequence, not just the filament) to be inserted displaces the cap subunit that is most loosely packed, starting the movement up of the cap assembly and allowing access to the assembly point. We assume that subunits that 'spill-over' into the supernatant reflect subunits that do not correctly insert in the assembly point – perhaps because they have the incorrect sequence for insertion in that context. Abortive assembly in this fashion would likely result in the cap falling back to the stable position occupied before the subunit was secreted.

5) The manuscript does not mention FlhA, which is a major component of the export apparatus. I assume FlhA was lost during basal body purification. Considering the myriads of interactions between the MS ring and the export gate, is there enough space inside the MS ring for the FlhA nonamer?

As noted for reviewer 1 we see no density for FlhA and must assume it has fallen out during purification or vitrification. There would be room for an object the size of the FlhA nonamer, as deduced from tomograms, below the export gate.

## Decision Letter, first revision:

Dear Susan,

Thank you for submitting your revised manuscript "Molecular structure of the intact bacterial flagellar basal body" (NMICROBIOL-20123743A). We've now had the chance to carefully assess the revisions made and your rebuttal to the original comments from the referees, and are satisfied that the changes made sufficiently address their original concerns. Therefore, we'll be happy in principle to publish the manuscript in Nature Microbiology, pending minor revisions to comply with our editorial and formatting guidelines.

We are now performing detailed checks on your paper and all associated files and will soon send you a

checklist detailing all our final editorial and formatting requirements. Please do not upload any final materials nor make any revisions until you receive this additional information from us.

Thank you again for your interest in Nature Microbiology and please do not hesitate to contact me in the meantime if you have any questions.

## Decision Letter, final requests

Dear Susan,

Thank you for your patience as we've prepared the guidelines for final submission of your Nature Microbiology manuscript, "Molecular structure of the intact bacterial flagellar basal body" (NMICROBIOL-20123743A). Please carefully follow the step-by-step instructions provided in the personalised checklist attached, to ensure that your revised manuscript can be swiftly handed over to our production team.

**To speed things up with regards to publication, we would like to ideally receive your revised paper (with all of the requested files and forms) within 5 business days, by 15th March 2021. Please let me know if you need a little more time though.**

If you have not done so already, please alert us to any related manuscripts from your group that are under consideration or in press at other journals, or are being written up for submission to other journals (see: https://www.nature.com/nature-research/editorial-policies/plagiarism#policy-on-duplicate-publication for details).

In recognition of the time and expertise our reviewers provide to Nature Microbiology's editorial process, we would like to formally acknowledge their contribution to the external peer review of your manuscript entitled "Molecular structure of the intact bacterial flagellar basal body". For those reviewers who give their assent, we will be publishing their names alongside the published article.

Nature Microbiology offers a Transparent Peer Review option for new original research manuscripts submitted after December 1st, 2019. As part of this initiative, we encourage our authors to support increased transparency into the peer review process by agreeing to have the reviewer comments, author rebuttal letters, and editorial decision letters published as a Supplementary item. When you submit your final files please clearly state in your cover letter whether or not you would like to participate in this initiative. Please note that failure to state your preference will result in delays in accepting your manuscript for publication.

<b>Cover suggestions</b>

As you prepare your final files we encourage you to consider whether you have any images or illustrations that may be appropriate for use on the cover of Nature Microbiology.

Covers should be both aesthetically appealing and scientifically relevant, and should be supplied at the best quality available. Due to the prominence of these images, we do not generally select images featuring faces, children, text, graphs, schematic drawings, or collages on our covers.

We accept TIFF, JPEG, PNG or PSD file formats (a layered PSD file would be ideal), and the image should be at least 300ppi resolution (preferably 600-1200 ppi), in CMYK colour mode.

If your image is selected, we may also use it on the journal website as a banner image, and may need to make artistic alterations to fit our journal style.

Please submit your suggestions, clearly labeled, along with your final files. We'll be in touch if more information is needed.

Nature Microbiology has now transitioned to a unified Rights Collection system which will allow our Author Services team to quickly and easily collect the rights and permissions required to publish your work. Approximately 10 days after your paper is formally accepted, you will receive an email in providing you with a link to complete the grant of rights. If your paper is eligible for Open Access, our Author Services team will also be in touch regarding any additional information that may be required to arrange payment for your article.

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Please use the following link for uploading these materials:

[REDACTED]

I look forward to receiving the final version of your manuscript and please don't hesitate to contact me if you have any questions.

**Final Decision Letter:**

Dear Susan,

I am delighted to accept your Letter "Molecular structure of the intact bacterial flagellar basal body" for publication in Nature Microbiology. Thank you for having chosen to submit your work to us and many congratulations to you and your co-authors.

Before your manuscript is typeset, we will edit the text to ensure it is intelligible to our wide readership and conforms to house style. We look particularly carefully at the titles of all papers to ensure that they are relatively brief and understandable.

Once your manuscript is typeset and you have completed the appropriate grant of rights, you will receive a link to your electronic proof via email with a request to make any corrections within 48 hours. If, when you receive your proof, you cannot meet this deadline, please inform us at rjsproduction@springernature.com immediately. Once your paper has been scheduled for online publication, the Nature press office will be in touch to confirm the details.

Acceptance of your manuscript is conditional on all authors' agreement with our publication policies (see www.nature.com/nmicrobiolate/authors/gta/content-type/index.html). In particular your manuscript must not be published elsewhere and there must be no announcement of the work to any media outlet until the publication date (the day on which it is uploaded onto our website).

Nature Microbiology is a Transformative journal and offers an immediate open access option through payment of an article-processing charge (APC) for papers submitted after 1 January, 2021 . In the event that authors choose to publish under the subscription model, Nature Research allows authors to self-archive the accepted manuscript (the version post-peer review, but prior to copy-editing and typesetting) on their own personal website and/or in an institutional or funder repository where it can be made publicly accessible 6 months after first publication, in accordance with our self-archiving policy. <a href="https://www.nature.com/nature-research/editorial-policies/self-archiving-and-license-to-publish">Please review our self-archving policy</a> for more information.

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