

## Peer Review Information

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**Journal:** Nature Microbiology

**Manuscript Title:** A two-track model for the spatiotemporal coordination of bacterial septal cell wall synthesis revealed by single-molecule imaging of FtsW

**Corresponding author name(s):** Piet de Boer, Jie Xiao

### Editorial Notes:

**Redactions – transferred manuscripts (mention of previous referee reports from elsewhere)** This manuscript has been previously reviewed at another journal. This document only contains reviewer comments, rebuttal and decision letters for versions considered at Nature Microbiology. Mentions of prior referee reports have been redacted.

### Reviewer Comments & Decisions:

**Decision Letter, initial version:**

Dear Jie,

Please accept once again my sincere apologies for the delay in getting back to you with a decision on your manuscript entitled "FtsW exhibits distinct processive motions driven by either septal cell wall synthesis or FtsZ treadmilling in *E. coli*", which was under peer review at Nature Microbiology. As I mentioned previously, the paper has now been seen by our referees, and in the light of their advice (see below) I am delighted to say that we can in principle offer to publish it. However, at this stage there were also several additional editorial checks that we needed to run on all files and figures, which is why the process was further delayed. In any case, we now completed all the required checks and below list all the points that need to be addressed during a final revision, before formal acceptance of the paper.

The referees' remaining comments are clear, and should not be difficult to implement. For example, referee #1 asks that you clarify some of the metrics used (such as "a % of number of cells and a % of fluorescence intensity", which should be left separate); that you include the treadmilling speed of the FtsZ mutants in Fig 2d or its legend; that you add a few more data points to the "M9-acetate" condition; and fix some small presentation issues. Similarly, referee #2 asks that you pay close attention during the revision to soften some of the claims made, so as to avoid "stronger and more

definitive interpretations than their data allow or are required for publication" and the "readers of their paper won't get distracted by over-interpretation and have greater confidence in the authors' impartiality and balanced interpretative skills." - the referee lists several points where this could be done. {REDACTED}

Editorially, we will also need you to make some changes so that the paper is as short as possible, follow our formatting rules and complies with our Guide to Authors at <http://www.nature.com/nmicrobiol/info/gta>.

Specific points:

In particular, while checking through the manuscript and associated files, we noticed the following specific points which we will need you to address:

1. Length. At over 5,000 words, your manuscript currently vastly exceeds our normal length limit for Articles of about 3,500 words. We have some flexibility, and can allow a revised manuscript at 3,800 words, but please consider this a firm upper limit. You could achieve some shortening by moving some details to the Methods section that should follow the main text (the length of the Methods section is unlimited and does not count towards the main text length) or by moving some of the discussion into a "Supplementary Discussion" section to be featured in SI.

2. Title. Titles should give an idea of the main finding of the paper and ideally not exceed 120 characters (including spaces). We discourage the use of active verbs and do not allow punctuation. In this case, we felt that the title should more explicitly mention single molecule imaging and the 2 track model, as we feel that this will encourage our more general readers to further engage with the manuscript. Therefore, we suggest revising the title along the lines of "Single-molecule imaging and genetic analyses of FtsW reveal a two-track model for spatiotemporal coordination of bacterial septal cell wall synthesis."

3. Abstract. Per journal guidelines, the abstract shouldn't exceed 200 words; at almost 300 words, the current abstract vastly exceeds this limit and needs to be revised. Please see below for additional information on how to revise this section.

4. Main text display items and supplementary information. Please note that manuscripts in the Article format are limited to 6 display items in the main text (figures and tables), while the rest must be presented as supplementary information. All Supplementary Information must be submitted in accordance with the instructions in the attached Inventory of Supporting Information, and should fit into one of three categories: EXTENDED DATA (ED); SUPPLEMENTARY INFORMATION (SI); and SOURCE DATA. Below are detailed instructions on how to format each category. For your paper, we suggest that you do the following:

a. Main figures: We suggest keeping the 6 current main figures as the main display items. Please see below for additional information on how to properly format main figures.

b. Extended data (ED): ED figures are more prominently featured than SI Figures, since these are featured in-line on the HTML and added at the end of the main article. These should be multi-panel figures, very much like main figures – you can think of them almost as additional "main figures". We can accommodate up to 10 ED figures. In this case, the article currently includes 15 ED items, which will need to be revised accordingly to a maximum of 10. For example, some figures like ED1, ED4 or

ED5 can easily be moved to SI; whereas others like ED3, ED9, ED10, ED12, ED13 can be combined into larger, multi-panel figures. Please see below for additional information on how to properly format ED figures.

c. Supplementary information (SI): the remaining figures and tables can be included as SI, which will mostly be featured in a single, flattened PDF (except for some additional files that may be too large for the PDF). We suggest that you keep the current tables and any remaining SI figures (that exceed the limit of 10 ED figures) in a single PDF. This PDF can also include the aforementioned "Supplementary Discussion" section, if needed. Please see below for additional information on how to properly format SI files.

d. Source data: we now ask authors to provide as much source data as possible. This includes, when applicable, raw versions of all gels shown, and the numerical data used to generate graphs and statistics. For gels, these should be raw, uncropped, unmanipulated versions of all gels, ideally showing the original molecular weight markers and using text boxes to indicate which sections of the full gels were cropped to generate the figures shown. Raw data will be linked to specific main figures and ED figures. Therefore, please include:

- i) full numerical and statistical data for Fig 1, 2, 3, 4, 5, ED2, ED3, ED7, ED8, ED9, ED11, ED13, ED14 and ED15;
- ii) full length gels for Figs ED2g and ED15b.

Please see below for additional information on how to provide source data.

5. Priority claims. Per journal guidelines, we recommend that you avoid the use of terms like 'new', 'newly', 'novel', 'first' and other priority claims throughout the text in order to avoid any perception of grandstanding and so that the reader can focus on the significance, rather than the novelty, of the findings. Therefore, please revise lines 370 ('novel'), 19 and 36 ('new'), and any other relevant sections accordingly.

6. Scale bars. Per style guidelines, all microscopy figures should include a scale bar, which should be defined in the legend, rather than in the figure itself. Please define the length of the bars in the legends to figures 5a, ED10, ED12 and ED15.

7. Molecular weight markers. Per style guidelines, MW markers must be displayed in all panels in which this information is relevant. Please add this information to ED Fig 15.

8. Replicates and statistics. While carefully checking the figures, we noted a few things that need to be revised so that they comply with our style guidelines for data presentation and accurately report on the number of replicates, statistical testing, etc. Please find these points detailed in the attached "NMICROBIOL-20093043-T\_ExtendedComments" file and revise the highlighted figure legends accordingly.

9. Competing interests. Per journal guidelines, please add a competing interest statement to the text. Note that this statement should include both financial and non-financial interests. See below for additional information on how to format this section.

10. Data availability. Please include a detailed data availability section at the end of the methods. Note that this section should refer to all source data and include all accession codes for relevant data deposited to databases. See below for additional details on how to format this section and some useful

examples can be found here (<https://www.nature.com/articles/s41564-019-0614-3#data-availability> or <https://www.nature.com/articles/s41564-019-0609-0#data-availability>).

11. Code availability. Please include a code availability section at the end of the methods. Note that this section should accession details for customized scripts, which should be deposited to relevant databases (such as GitHub). All accession codes must be live by the time of publication of the piece. Please describe access to the ImageJ macro and MATLAB codes for single molecule tracking script in this section.

12. Author contributions statement. Please provide a detailed author contributions statement (see below for additional information on how to format this section). A good example can be found at the end of the following article: <http://www.nature.com/nature/journal/v532/n7599/full/nature17433.html>

13. Reporting checklist. Note that a final version of the reporting checklist will be published with your manuscript. Therefore, please revise this document according to the instructions found in the annotated PDF attached to this message (NMICROBIOL-20093043-T\_ReportingSummary.pdf).

#### General points:

We will also need you to check through all of the following general points when preparing the final version of your manuscript:

The paper's summary paragraph (about 150-200 words; no references) should serve both as a general introduction to the topic, and as a brief, non-technical summary of your main results and their implications. It should start by outlining the background to your work (why the topic is important) and the main question you have addressed (the specific problem that initiated your research), before going on to describe your new observations, main conclusions and their general implications. Because we hope that scientists across the wider microbiology community will be interested in your work, the first paragraph should be as accessible as possible, explaining essential but specialised terms concisely. We suggest you show your summary paragraph to colleagues in other fields to uncover any problematic concepts.

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>

Choosing the right electronic format for your figures at this stage will speed up the processing of your paper. We would like the figures to be supplied as vector files - EPS, PDF, AI or postscript (PS) file formats (not raster or bitmap files), preferably generated with vector-graphics software (Adobe Illustrator for example). Please try to ensure that all figures are non-flattened and fully editable. All

images should be at least 300 dpi resolution (when figures are scaled to approximately the size that they are to be printed at) and in RGB colour format. Please do not submit Jpeg or flattened TIFF files. Please see also 'Guidelines for Electronic Submission of Figures' at the end of this letter for further detail.

Please view [http://www.nature.com/authors/editorial\\_policies/image.html](http://www.nature.com/authors/editorial_policies/image.html) for more detailed guidelines.

We will edit your figures/tables electronically so they conform to Nature Microbiology style. If necessary, we will re-size figures to fit single or double column width. If your figures contain several parts, the parts should be labelled lower case a, b, and so on, and form a neat rectangle when assembled.

Please check the PDF of the whole paper and figures (on our manuscript tracking system) VERY CAREFULLY when you submit the revised manuscript. This will be used as the 'reference copy' to make sure no details (such as Greek letters or symbols) have gone missing during file-transfer/conversion and re-drawing.

All Supplementary Information must be submitted in accordance with the instructions in the attached Inventory of Supporting Information, and should fit into one of three categories:

1. **EXTENDED DATA:** Extended Data are an integral part of the paper and only data that directly contribute to the main message should be presented. These figures will be integrated into the full-text HTML version of your paper and will be appended to the online PDF. There is a limit of 10 Extended Data figures, and each must be referred to in the main text. Each Extended Data figure should be of the same quality as the main figures, and should be supplied at a size that will allow both the figure and legend to be presented on a single legal-sized page. Each figure should be submitted as an individual .jpg, .tif or .eps file with a maximum size of 10 MB each. All Extended Data figure legends must be provided in the attached Inventory of Accessory Information, not in the figure files themselves.

2. **SUPPLEMENTARY INFORMATION:** Supplementary Information is material that is essential background to the study but which is not practical to include in the printed version of the paper (for example, video files, large data sets and calculations). Each item must be referred to in the main manuscript and detailed in the attached Inventory of Accessory Information. Tables containing large data sets should be in Excel format, with the table number and title included within the body of the table. All textual information and any additional Supplementary Figures (which should be presented with the legends directly below each figure) should be provided as a single, combined PDF. Please note that we cannot accept resupplies of Supplementary Information after the paper has been formally accepted unless there has been a critical scientific error.

All Extended Data must be called out in your manuscript and cited as Extended Data 1, Extended Data 2, etc. Additional Supplementary Figures (if permitted) and other items are not required to be called out in your manuscript text, but should be numerically numbered, starting at one, as Supplementary Figure 1, not SI1, etc.

3. **SOURCE DATA:** We encourage you to provide source data for your figures whenever possible. Full-length, unprocessed gels and blots must be provided as source data for any relevant figures, and should be provided as individual PDF files for each figure containing all supporting blots and/or gels

with the linked figure noted directly in the file. Statistics source data should be provided in Excel format, one file for each relevant figure, with the linked figure noted directly in the file. For imaging source data, we encourage deposition to a relevant repository, such as figshare (<https://figshare.com/>) or the Image Data Resource (<https://idr.openmicroscopy.org>).

Nature Research journals [encourage authors to share their step-by-step experimental protocols](https://www.nature.com/nature-research/editorial-policies/reporting-standards#protocols) on a protocol sharing platform of their choice. Nature Research's Protocol Exchange is a free-to-use and open resource for protocols; protocols deposited in Protocol Exchange are citable and can be linked from the published article. More details can found at [www.nature.com/protocolexchange/about](https://www.nature.com/protocolexchange/about).

Please note that after the paper has been formally accepted you can only provide amended Supplementary Information files for critical changes to the scientific content, not for style. You should clearly explain what changes have been made if you do resupply any such files.

Figure legends must provide a brief description of the figure and the symbols used, within 350 words. This must include definitions of any error bars employed in the figures.

It is a condition of publication that you include a statement before the acknowledgements naming the author to whom correspondence and requests for materials should be addressed.

Finally, we require authors to include a statement of their individual contributions to the paper -- such as experimental work, project planning, data analysis, etc. -- immediately after the acknowledgements. The statement should be short, and refer to authors by their initials. For details please see the Authorship section of our joint Editorial policies at [http://www.nature.com/authors/editorial\\_policies/authorship.html](http://www.nature.com/authors/editorial_policies/authorship.html)

We will not send your revised paper for further review if, in the editors' judgement, the referees' comments on the present version have been addressed. If the revised paper is in Nature Microbiology format, in accessible style and of appropriate length, we shall accept it for publication immediately.

Please resubmit electronically

- \* the final version of the text (not including the figures) in either Word or Latex.
- \* publication-quality figures. For more details, please refer to our Figure Guidelines, which is available here: [https://mts-nmicrobiol.nature.com/letters/Figure\\_guidelines.pdf](https://mts-nmicrobiol.nature.com/letters/Figure_guidelines.pdf)
- \* Extended Data & Supplementary Information, as instructed
- \* a point-by-point response to any issues raised by our referees and to any editorial suggestions.
- \* any suggestions for cover illustrations, which should be provided at high resolution as electronic files. Please note that such pictures should be selected more for their aesthetic appeal than for their scientific content. I am sure you will understand that we cannot make any promise as to whether any of your suggestions might be selected for the cover of Nature Microbiology.

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We hope that you will support this initiative and supply the required information. Should you have any query or comments, please do not hesitate to contact me.

I think that's it. Sorry for sending you such a long list of points to address, but resolving these issues at this stage should help to ensure that everything runs smoothly once the papers is passed on to our production team, which should help to speed up publication of the article.

We hope to hear from you within four weeks but please let us know if the revision process is likely to take longer or if you have any questions.

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Reviewer Expertise:

- Referee #1: Bacterial cell division
- Referee #2: Bacterial cell division
- Referee #3: Bacterial cell division; single molecule imaging

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Reviewer Comments:

Reviewer #1 (Remarks to the Author):

The manuscript by Yang and colleagues is now considerably improved, due to additional experiments that strengthen the proposed model. The authors have also looked deeper into the nature of the stationary FtsW molecules, which was missing in the initial submission.

Authors have adequately answered my comments, so I have only minor points at this stage:

Line 65 – The % given results from a % of number of cells and a % of fluorescence intensity. It is difficult to assess the exact meaning of 55%. I would leave the two measures separate, as the data is in Fig 1 b and c. The combined number doesn't add useful information to the reader.

Line 145 - It would be easier to have the treadmilling speed of the FtsZ mutants available in Fig 2d or



its legend, so that the reader does not have to immediately go the SI table 4. This information is important for the conclusion that is taken from this Figure.

Line 264 – N=5 for M9-acetate is a quite low. Can authors analyse at least 10 cells?

SI – in the methods section the symbol for degrees appears as a square (I'm seeing the PDF in mac)

Extended Data Figure 3 – each data point in this graph is independent, so why connecting them with a line?

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Reviewer #2 (Remarks to the Author):

The authors have addressed my principal concerns with additional data, increased sample size, and data presentation. I think this study represents an important contribution to the field and provides a mechanistic framework to dissect the regulation of septal cell wall synthesis in the (near) future.

I am supportive of publication in Nature Microbiology.

I do however think that the authors tend to gravitate toward stronger and more definitive interpretations than their data allow or are required for publication. I would encourage them to soften/qualify their descriptors. By making these changes, readers of their paper won't get distracted by over-interpretation and have greater confidence in the authors' impartiality and balanced interpretative skills.

I've highlighted examples in addition to specific requested changes below:

Line 21: "may constitute the essential, biofunction sPG synthase specific for new sPG synthesis" can be changed to "an essential"

Lines 29 "is driven by" and Line 30: "is driven by"  
Change to: "A fast-moving population that correlates with treadmilling dynamics and a slow moving population that depends on sPG synthesis -or- that is likely to be engaged in sPG synthesis."

Figure 2 Title: change to "differentially correlate with"

Line 119: "Hereafter, we only focus on dynamic of molecules in the septum where septal cell wall constriction takes place."

How are the authors monitoring constriction? (What is the basis for determining constricting vs. non-constricting septa? - More to the point: How do the authors decide which traces to keep vs. throw out?) Please clarify or change wording.

Line 137 "cognate complex" and "indistinguishable" change to:  
"FtsW's cognate transpeptidase FtsI exhibited a statistically similar speed distribution to that of FtsW"

Line 177: "Given our results so far" implies the results in the current manuscript, but I think the authors are referring to their previous work. Change to "Our previous work indicates that sPG

synthesis activity is not affected in FtsZ GTPase mutants in E. coli. Accordingly, it is possible that the fast-moving population of ...."

I am not sure why the section on FtsW(E289G) provides the % change of the fast -moving population and the average speed of all directionally moving particles. The most relevant comparison is between the % of slow-moving particles. This is buried in Table S6. This paragraph could be distilled to 3-4 sentences by simply stating the change in percentage of slow-moving FtsW molecules. "The slow-moving population shifted from 63.6+/-7.6% to 79.1+/- 5.6%." I don't think this is the authors' intent but it reads like they are trying to inflate the effect by reporting the smaller numbers so the fold-change looks larger. This has the potential to undermine confidence . . .

In this same paragraph the authors use the term "significantly" twice. (no P-values are presented in text or Table S6). The shift seems real but modest. Unless the changes are statistically significant and documented, please change wording to something like modest but reproducible changes (?)

More generally:

Since the central conclusion of this study is that there are two distinct populations of directionally moving FtsW (or FtsI) molecules, I am not sure why the average speed of all directionally moving particles is so often cited in the paper. Please consider removing most of these.

Line 207:

"Since MTSES specifically blocks FtsWI302C-dependent sPG synthesis, we reasoned that the remaining slow-moving population of FtsW could be driven by sPG synthesis from PBP1A, 1B, 1C, and MtgA (Fig. 1)."

Insert "in the context of the sPG synthesis complex" after MtgA.

Line 238 - This would be a good place to highlight that these findings are similar to what has been observed for the dynamic movement of the Rod complex.

Line 301 - consider changing "poised" to "stalled". Poised implies ready to begin.

Line 318:

Our results so far support a model in which . . .

Change to:

Our results so far strongly suggest that two processive moving populations of FtsW exist in vivo. Our analysis of these populations supports a model in which the fast-moving population is driven by FtsZ's treadmilling dynamics but are not engaged in sPG synthesis, whereas the slow-moving population is driven by sPG synthesis.

Line 364: Change to: Our data further suggest that FtsN promotes the release of inactive sPG synthase from treadmilling FtsZ polymers to pursue the sPG-track for active synthesis.

Acknowledgements: Berhardt should be Bernhardt

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Reviewer #3 (Remarks to the Author):

**Author Rebuttal to Initial comments**

Reviewer #1 (Remarks to the Author):

The manuscript by Yang and colleagues is now considerably improved, due to additional experiments that strengthen the proposed model. The authors have also looked deeper into the nature of the stationary FtsW molecules, which was missing in the initial submission.

Authors have adequately answered my comments, so I have only minor points at this stage:

Line 65 – The % given results from a % of number of cells and a % of fluorescence intensity. It is difficult to assess the exact meaning of 55%. I would leave the two measures separate, as the data is in Fig 1 b and c. The combined number doesn't add useful information to the reader.

We respectfully disagree with the reviewer's suggestion. The meaning of the 55% is the reduction of the total sPG amount considering both the lower percentage of cells with labeled septa and the lower percentage of septal fluorescence intensity in this labeled population of cells. The equation for the calculation of the total 55% reduction using the two percentages was described in the main text and the results were plotted in Figure 1c. Therefore, Figure 1c should be kept as it is.

Line 145 - It would be easier to have the treadmilling speed of the FtsZ mutants available in Fig 2d or its legend, so that the reader does not have to immediately go the SI table 4. This information is important for the conclusion that is taken from this Figure.

We thank the reviewer for the suggestion. We added the average FtsZ treadmilling speeds in Fig. 2d.

Line 264 – N=5 for M9-acetate is a quite low. Can authors analyse at least 10 cells?

We performed another experiment that resulted 8 more cells (13 total). The average constriction rate is similar to the previous measurement. The data in Supplementary Table 8 and Extended Data Fig. 6 are updated including the new results.

SI – in the methods section the symbol for degrees appears as a square (I'm seeing the PDF in mac)

The degree symbol looks correct on my PC.

Extended Data Figure 3 – each data point in this graph is independent, so why connecting them with a line?

We replot the graph in bar graph in Extended Data Fig. 1c.

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Reviewer #2 (Remarks to the Author):

The authors have addressed my principal concerns with additional data, increased sample size, and data presentation. I think this study represents an important contribution to the field and provides a mechanistic framework to dissect the regulation of septal cell wall synthesis in the (near) future.

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I've highlighted examples in addition to specific requested changes below:

Line 21: "may constitute the essential, biofunction sPG synthase specific for new sPG synthesis" can be changed to "an essential"

We changed the description of FtsW to "an essential".

Lines 29 "is driven by" and Line 30: "is driven by"

Change to: "A fast-moving population that correlates with treadmilling dynamics and a slow moving population that depends on sPG synthesis -or- that is likely to be engaged in sPG synthesis."

We changed the sentence to "a fast-moving population **correlated with** the treadmilling dynamics of the essential cytoskeletal FtsZ protein and a slow-moving population **dependent on** active sPG synthesis.". (Line 23-24)

Figure 2 Title: change to "differentially correlate with"

We changed the legend of Fig. 2 to “FtsW exhibits two processive moving populations that are differentially correlated with FtsZ’s treadmilling dynamics.”.

Line 119: "Hereafter, we only focus on dynamic of molecules in the septum where septal cell wall constriction takes place."

How are the authors monitoring constriction? (What is the basis for determining constricting vs. non-constricting septa? - More to the point: How do the authors decide which traces to keep vs. throw out?) Please clarify or change wording.

We detailed how we chose and excluded trajectories in the Methods part: “Only trajectories near the midpoint of the cell's long axis or near visible constriction sites (from the bright field image as our previous work<sup>24</sup>) where cell division takes place were used in the analysis to ensure the molecules are cell division and sPG related.” (Line 475-478)

Line 137 "cognate complex" and "indistinguishable" change to:

"FtsW's cognate transpeptidase FtsI exhibited a statistically similar speed distribution to that of FtsW"

We changed the sentence to “FtsW's cognate transpeptidase FtsI exhibited a statistically similar, two-population speed distribution ( $p = 0.32$ , K-S test,”. (line 104-106)

Line 177: "Given our results so far" implies the results in the current manuscript, but I think the authors are referring to their previous work. Change to "Our previous work indicates that sPG synthesis activity is not affected in FtsZ GTPase mutants in E. coli. Accordingly, it is possible that the fast-moving population of ...."

I am not sure why the section on FtsW(E289G) provides the % change of the fast -moving population and the average speed of all directionally moving particles. The most relevant comparison is between the % of slow-moving particles. This is buried in Table S6. This paragraph could be distilled to 3-4 sentences by simply stating the change in percentage of slow-moving FtsW molecules. "The slow-moving population shifted from 63.6+/-7.6% to 79.1+/- 5.6%." I don't think this is the authors' intent but it reads like they are trying to inflate the effect by reporting the smaller numbers so the fold-change looks larger. This has the potential to undermine confidence . . .

We thank the referee for the suggestion. The comparisons are all based on the slow-moving population now. (Line 139-140)

In this same paragraph the authors use the term "significantly" twice. (no P-values are presented in text or Table S6). The shift seems real but modest. Unless the changes are statistically significant and documented, please change wording to something like modest but reproducible changes (?)

We have removed all unnecessary “significantly” or “significant” from the manuscript.

More generally:

Since the central conclusion of this study is that there are two distinct populations of directionally moving FtsW (or FtsI) molecules, I am not sure why the average speed of all directionally moving particles is so often cited in the paper. Please consider removing most of these.

We thank the referee for the suggestion and removed many places where we compared the average speeds. However, this information is important to support our two-population fit was not from imperfect non-linear regression. Therefore, we still kept the average speeds in the Figures and some places to confirm the changes of two-populations.

Line 207:

"Since MTSES specifically blocks FtsWI302C-dependent sPG synthesis, we reasoned that the remaining slow-moving population of FtsW could be driven by sPG synthesis from PBP1A, 1B, 1C, and MtgA (Fig. 1)."

Insert "in the context of the sPG synthesis complex" after MtgA.

We added this to the sentence. (Line 148)

Line 238 - This would be a good place to highlight that these findings are similar to what has been observed for the dynamic movement of the Rod complex.

We thank the referee for the suggestion and cited two studies of the Rod complex dynamics. (lin166-168)

Line 301 - consider changing "poised" to "stalled". Poised implies ready to begin.

We changed it to “stalled”.(Line 214)

Line 318:

Our results so far support a model in which . . .

Change to:

Our results so far strongly suggest that two processive moving populations of FtsW exist in vivo. Our analysis of these populations supports a model in which the fast-moving population is driven by FtsZ’s treadmilling dynamics but are not engaged in sPG synthesis, whereas the slow-moving population is driven by sPG synthesis.

Considering the length limit of main text, we changed the sentence to “Our results so far suggest that two processive moving populations of FtsW exist *in vivo*.” (Line 227-228)

Line 364: Change to: Our data further suggest that FtsN promotes the release of inactive sPG synthase from treadmilling FtsZ polymers to pursue the sPG-track for active synthesis.

We changed the sentence as the referee suggested (Line 260-262).

Acknowledgements: Berhardt should be Bernhardt

We thank the referee for the careful reading, and we corrected then name.

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Reviewer #3 (Remarks to the Author):

None

**Final Decision Letter:**

Dear Jie,

I am delighted to accept your Article "A two-track model for the spatiotemporal coordination of bacterial septal cell wall synthesis revealed by single-molecule imaging of FtsW" for publication in Nature Microbiology. Thank you for having chosen to submit your work to us and many congratulations.

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.

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