nature research

Peer Review Information

Journal: Nature Structural and Molecular Biology
Manuscript Title: Cryo-EM structure of disease-related prion fibrils provides insights into seeding barriers
Corresponding author name(s): Professor Witold Surewicz

Editorial Notes:

Reviewer Comments & Decisions:

Decision Letter, initial version:

7th Sep 2021

Dear Dr. Surewicz,

Thank you again for submitting your manuscript "Cryo-EM structure of disease-related prion fibrils provides insights into seeding barriers". I apologize for the delay in responding, which resulted from the difficulty in obtaining suitable referee reports. Nevertheless, we now have comments (below) from the 2 reviewers (both experts in cryo-EM and prions) who evaluated your paper. In light of those reports, we remain interested in your study and would like to see your response to the comments of the referees, in the form of a revised manuscript.

You will see that the reviewers are positive about the interest and quality of the structures. However, reviewer 1 is concerned about differences to previously published structural models and the novelty of

some of the conclusions. Reviewer 2 suggests to add data on the infectivity of human 23-144 fibrils and feels some effort should be made to show that synthetic and disease-associated PrP23-144 fibrils are indeed structurally similar. Please be sure to address/respond to all concerns of the referees in full in a point-by-point response and highlight all changes in the revised manuscript text file.

We appreciate the requested revisions are extensive. We thus expect to see your revised manuscript within 6 months. If you cannot send it within this time, please let us know. We will be happy to consider your revision as long as nothing similar has been accepted for publication at NSMB or published elsewhere. Should your manuscript be substantially delayed without notifying us in advance and your article is eventually published, the received date would be that of the revised, not the original, version.

We are committed to providing a fair and constructive peer-review process. 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.

As you already know, we put great emphasis on ensuring that the methods and statistics reported in our papers are correct and accurate. As such, if there are any changes that should be reported, please submit an updated version of the Reporting Summary along with your revision.

Reporting Summary:
https://www.nature.com/documents/nr-reporting-summary.pdf

Please note that the form is a dynamic 'smart pdf' and must therefore be downloaded and completed in Adobe Reader.

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.

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.

If there are additional or modified structures presented in the final revision, please submit the corresponding PDB validation reports.

SOURCE DATA: we urge authors to provide, in tabular form, the data underlying the graphical representations used in figures. This is to further increase transparency in data reporting, as detailed in this editorial (http://www.nature.com/nsmb/journal/v22/n10/full/nsmb.3110.html). Spreadsheets can be submitted in excel format. Only one (1) file per figure is permitted; thus, for multi-paneled figures, the source data for each panel should be clearly labeled in the Excel file; alternately the data can be provided as multiple, clearly labeled sheets in an Excel file. When submitting files, the title field should indicate which figure the source data pertains to. We encourage our authors to provide source data at the revision stage, so that they are part of the peer-review process.

While we encourage the use of color in preparing figures, please note that this will incur a charge to partially defray the cost of printing. Information about color charges can be found at

http://www.nature.com/nsmb/authors/submit/index.html#costs

We require deposition of coordinates (and, in the case of crystal structures, structure factors) into the Protein Data Bank with the designation of immediate release upon publication (HPUB). Electron microscopy-derived density maps and coordinate data must be deposited in EMDB and released upon publication. Deposition and immediate release of NMR chemical shift assignments are highly encouraged. To avoid delays in publication, dataset accession numbers must be supplied with the final accepted manuscript and appropriate release dates must be indicated at the galley proof stage. Please find the complete NRG policies on data availability at http://www.nature.com/authors/policies/availability.html.

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[Redacted]

Note: This URL links to your confidential home page and associated information about manuscripts you may have submitted, or that you are reviewing for us. If you wish to forward this email to co-authors, please delete the link to your homepage.

We look forward to seeing the revised manuscript and thank you for the opportunity to review your work.

Kind regards,
Florian

Florian Ullrich, Ph.D.
Associate Editor
Nature Structural & Molecular Biology
ORCID 0000-0002-1153-2040

Reviewers' Comments:

Reviewer #1:
Remarks to the Author:
This work determined the first cryo-EM structure of fibrils formed by familial prion disease-related Y145Stop mutant human prion. Unlike previous determined fibril structures of prion, this fibril structure is composed of four protofilaments intertwined with a left-handed helix. Two clusters of hydrophobic residues are essential in stabilizing the S-shape fold. This cryo-EM structure provides

evidence for understanding the prion transmissibility barriers between human and hamster PrP23-144. It also highlights that the residues outside the core region may play an important role in determining the fibril polymorphism.

Major concerns:

1.The authors previously reported the ssNMR model of the same Y145Stop fibril (PMID: 28963458). However, the fibril model is very different from the cryo-EM structure reported in this work. In previous paper, the fibril is formed by two protofilament and prion protein adopts R-shape but not S-shape in each protofilament. The authors need to address this issue. Does the second-round seeding (which was used for fibril preparation in this study) dramatically alter the fibril structure? Is there a minor species of R-shape fibril in the cryo-EM fibril sample? The AFM images (Extended data Fig.1c) show heterogeneity of fibril samples.

2.The author mentioned the Y145Stop fibril is distinct from the prion fibril structures reported previously. They need to do detailed structure comparison of the U145Stop fibril with the other prion structures, including several cryo-EM structures and the ssNMR structure.

3.As for the structural basis of prion conformational adaptability, Sigurdson and Eisenberg provided several direct structural evidences in their previous work (PMID: 24596090, PMID: 21323366). Thus, the author shouldn't emphasis the novelty of their newly determined fibril structure in explaining the conformational adaptability.

Page 5, line 5, "huPrP90-178 fragment" should be "huPrP94-178 fragment"

Reviewer #2:
Remarks to the Author:
Li et al provide a high-resolution cryo-EM-based structure of synthetic fibrils formed with a C-terminally truncated human PrP fragment of residues 23-144. Importantly, this same fragment occurs naturally in humans with the Y145Stop mutation, which leads to a form of genetic prion disease. It remains unclear if these synthetic fibrils share the same structure as those formed in vivo, but these findings help significantly to frame detailed near-atomic level considerations of structural possibilities for the bona fide disease associated amyloid fibrils that appear to be pathogenic in humans. Their new structure, when considered with these groups' previous studies, also helps to rationalize potential seeding barrier mechanisms. An earlier study reported the high-resolution structure of fibrils of human PrP 90-178 (a non-physiological fragment) and although these fibrils have ordered cores that are formed by a similar span of residues as the PrP23-144 fibrils described here, the respective core structures are quite different. As the authors point out, the difference in these structures highlights the likely influence of flanking residues in these physiological vs non-physiological human PrP fragments in fibrillization. Overall, the current studies are novel, important for the prion field, well performed, and well described. I have only a few questions and suggestions for improvement. In my opinion, this study is excellent and laudable as it is, but if the authors can offer further insights into the issues that I raise below would add real value and context to this work.

Major:
1. Although this group has shown previously that fibrils of mouse 23-144 are infectious, this cannot be

assumed to be true of the human 23-144 fibrils studied here. Have the authors inoculated these human fibrils into humanized mice to test their infectivity? Do they any evidence that the human fibrils share structural characteristics with the infectious mouse fibrils?

2. The other major uncertainty is whether the authors' synthetic human PrP23-144 fibrils are similar in structure to those formed in humans with the Y145Stop mutation. I would say that it is beyond the scope of this study to determine the structure of the latter fibrils, even if there were enough tissue available from such rare patients to purify them (which I would guess is doubtful). However, it would be relevant to this issue if the authors could offer any lower resolution data (biochemical, H/D exchange, ultrastructural, etc.), and/or salient arguments, that point to either similarities or differences between the synthetic and disease-associated PrP23-144 fibrils.

3. Relevant to the previous point, have the authors tried seeding the growth of human 23-144 fibrillization with Y145Stop GSS brain homogenate? If so, do those fibrils appear to be similar to those seeded with synthetic fibrils as was done for the current study, as judged, for example, by ultrastructure, partial protease resistance, FTIR, H/D exchange, ss-NMR, etc.? Any such data might help to address the comparability, or potential lack thereof, of the synthetic vs natural 23-144 fibrils, and hence, the biological relevance of the synthetic fibrils studied here.

Minor:
4. A peer-reviewed extension of Ref 17 (a preprint) has now been published and should be updated.

Byron Caughey

Author Rebuttal to Initial comments

Response to the comments of the reviewers

We wish to thank both reviewers for their insightful comments. Below is our point-by-point response to these comments and a description of revisions made in the manuscript. These revision include additions to the text (marked in red) as well as three Supplementary Figures.

Reviewer #1

This work determined the first cryo-EM structure of fibrils formed by familial prion disease-related Y145Stop mutant human prion. Unlike previous determined fibril structures of prion, this fibril structure is composed of four protofilaments intertwined with a left-handed helix. Two clusters of hydrophobic residues are essential in stabilizing the S-shape fold. This cryo-EM structure provides evidence for understanding the prion transmissibility barriers between human and hamster PrP23-144. It also highlights that the residues outside the core region may play an important role in determining the fibril polymorphism.

Major concerns:

1. The authors previously reported the ssNMR model of the same Y145Stop fibril (PMID: 28963458). However, the fibril model is very different from the cryo-EM structure reported in this work. In previous paper, the fibril is formed by two protofilament and prion protein adopts R-shape but not S-shape in each protofilament. The authors need to address this issue. Does the second-round seeding (which was used for fibril preparation in this study) dramatically alter the fibril structure? Is there a minor species of R-shape fibril in the cryo-EM fibril sample? The AFM images (Extended data Fig.1c) show heterogeneity of fibril samples.

The previously proposed structural model for huPrP23-144 fibrils based on limited number of ssNMR constraints is indeed different from that derived herein from high-resolution cryo-EM data. Even though the former model is preliminary in nature, we agree with the reviewer that this issue needs to be addressed.

With regard to reviewer's specific questions, the possibility that the fibril structure could be affected by second-round seeding used in preparation of samples for cryo-EM analysis is, in our opinion, rather unlikely, as seeded reactions typically faithfully propagate the structures of the template seed. A more plausible (even though still hypothetical) scenario is that there might be a second structural polymorph in the original cryo-EM sample that is preferentially propagated during the seeded reaction. Regardless which of these scenarios is correct and still preliminary nature of the ssNMR-based model, it is worth noting that the same sets of hydrophobic residues that are involved in the *intermolecular* interactions in the cryo-EM structure were detected in the fibril sample analyzed by solid-state NMR, even though these interactions were interpreted as *intramolecular* in solid-state NMR experiments. Thus, if the preliminary ssNMR-based model is confirmed (these studies are still ongoing to unambiguously discriminate between inter- and intra-molecular interactions between certain residues, which for this system is technically very challenging), this would suggest that there might be two closely related polymorphic forms of the huPrP23-144 fibril core that are stabilized by key interactions between the same residues.

We now address this issue in a first paragraph on p. 4 and new Supplementary Figure S1.

2. The author mentioned the Y145Stop fibril is distinct from the prion fibril structures reported previously. They need to do detailed structure comparison of the U145Stop fibril with the other prion structures, including several cryo-EM structures and the ssNMR structure.

As recommended by the reviewer, we now include an additional figure (Supplementary Fig. S2) which provides a comparison of different types of PrP fibrils for which high-resolution structures have been determined by cryo-EM. With regard to the comparison with the structure suggested based on solid-state NMR constraints, we now discuss this issue on p. 4 and in Supplementary Fig. S1 (see above).

3. As for the structural basis of prion conformational adaptability, Sigurdson and Eisenberg provided several direct structural evidences in their previous work (PMID: 24596090, PMID: 21323366). Thus, the author shouldn't emphasis the novelty of their newly determined fibril structure in explaining the conformational adaptability.

The focus of the paper by Sigurdson and Eisenberg is on the structure of short peptides encompassing residues ~166-175, i.e., within the β2-α2 loop. This part of the protein is not present in the PrP23-144 variant. Perhaps a more relevant paper would be another study from the Eisenberg group that describes species-dependent differences in crystal structures of peptides encompassing residues 138-144. This study focuses exclusively on differences in the backbone conformation and, as such, does not provide any information regarding effects due to steric clashes between side chains of residues 139 and 112 in the neighboring PrP subunits. Those are the latter effects that appear to be a major determinant of seeding compatibility/incompatibility between PrP23-144 from different species. Nevertheless, this early study is certainly worth citing in our paper, and we are grateful to the reviewer for bringing it to our attention.

Page 5, line 5, "huPrP90-178 fragment" should be "huPrP94-178 fragment"

The typo on p. 5 has been corrected.

Reviewer #2

Li et al provide a high-resolution cryo-EM-based structure of synthetic fibrils formed with a C-terminally truncated human PrP fragment of residues 23-144. Importantly, this same fragment occurs naturally in humans with the Y145Stop mutation, which leads to a form of genetic prion disease. It remains unclear if these synthetic fibrils share the same structure as those formed in vivo, but these findings help significantly to frame detailed near-atomic level considerations of structural possibilities for the bona fide disease associated amyloid fibrils that appear to be pathogenic in humans. Their new structure, when considered with these groups' previous studies, also helps to rationalize potential seeding barrier mechanisms. An earlier study reported the high-resolution structure of fibrils of human PrP 90-178 (a non-physiological fragment) and although these fibrils have ordered cores that are formed by a similar span of residues as the PrP23-144 fibrils described here, the respective core structures are quite different. As the authors point out, the difference in these structures highlights the likely influence of flanking residues in these physiological vs non-physiological human PrP fragments in fibrillization. Overall, the current studies are novel, important for the prion field, well performed, and well described. I have only a few questions and suggestions for improvement. In my opinion, this study is excellent and laudable as it is, but if the authors can offer further insights into the issues that I raise below would add real value and context to this work.

While this reviewer was very positive about the overall quality and significance of our study, he made a few excellent suggestions for further improvements.

1. Although this group has shown previously that fibrils of mouse 23-144 are infectious, this cannot be assumed to be true of the human 23-144 fibrils studied here. Have the authors

inoculated these human fibrils into humanized mice to test their infectivity? Do they any evidence that the human fibrils share structural characteristics with the infectious mouse fibrils?

This is a very good point that wasn't properly addressed in the original manuscript. Unfortunately, for technical reasons (exceedingly long incubation times of prions in currently available "humanized" mice models), infectivity experiments with human PrP23-144 fibrils (that have very long incubation times even in mice overexpressing mouse PrP) are not practical. Therefore, we focused our efforts during the past 10 months on structural comparison between human PrP23-144 fibrils and their mouse counterparts that have been previously shown to be infectious. Even though the latter fibrils can exist in two distinct polymorphic forms, the structure of one of them was found to be identical to that of human PrP23-144 fibrils. We now include these new data in Supplementary Fig. S3 and discuss them briefly on p. 4.

2. The other major uncertainty is whether the authors' synthetic human PrP23-144 fibrils are similar in structure to those formed in humans with the Y145Stop mutation. I would say that it is beyond the scope of this study to determine the structure of the latter fibrils, even if there were enough tissue available from such rare patients to purify them (which I would guess is doubtful). However, it would be relevant to this issue if the authors could offer any lower resolution data (biochemical, H/D exchange, ultrastructural, etc.), and/or salient arguments, that point to either similarities or differences between the synthetic and disease-associated PrP23-144 fibrils.

3. Relevant to the previous point, have the authors tried seeding the growth of human 23-144 fibrillization with Y145Stop GSS brain homogenate? If so, do those fibrils appear to be similar to those seeded with synthetic fibrils as was done for the current study, as judged, for example, by ultrastructure, partial protease resistance, FTIR, H/D exchange, ss-NMR, etc.? Any such data might help to address the comparability, or potential lack thereof, of the synthetic vs natural 23-144 fibrils, and hence, the biological relevance of the synthetic fibrils studied here.

Structural characterization of fibrils isolated from brain tissue of individuals with Y145Stop mutation has been our objective for a long time. Unfortunately, this mutation is a very rare, with only a handful of well-characterized cases worldwide. Despite our best efforts, we were not able to obtain even small quantities of brain tissue from these cases for our studies.

4. A peer-reviewed extension of Ref 17 (a preprint) has now been published and should be updated.

This reference has now been updated.

Decision Letter, first revision:

Our ref: NSMB-BC45265A

8th Jul 2022

Dear Witold,

Thank you for submitting your revised manuscript "Cryo-EM structure of disease-related prion fibrils provides insights into seeding barriers" (NSMB-BC45265A). It has now been seen by the original referees and their comments are below. The reviewers find that the paper has improved in revision, and therefore we'll be happy in principle to publish it in Nature Structural & Molecular Biology, pending minor revisions to comply with our editorial and formatting guidelines.

We are now performing detailed checks on your paper and will send you a checklist detailing our editorial and formatting requirements in about a week. Please do not upload the final materials and make any revisions until you receive this additional information from us.

Thank you again for your interest in Nature Structural & Molecular Biology Please do not hesitate to contact me if you have any questions.

Kind regards,
Florian

Florian Ullrich, Ph.D.
Associate Editor
Nature Structural & Molecular Biology
ORCID 0000-0002-1153-2040

Reviewer #1 (Remarks to the Author):

My concerns were addressed by the authors. I don't have further question. I support publication of the work in NSMB.

Reviewer #2 (Remarks to the Author):

The authors have addressed my concerns to the extent that seems humanly possible at the present time.

Decision Letter, final checks:

Our ref: NSMB-BC45265A

28th Jul 2022

Dear Dr. Surewicz,

Thank you for your patience as we've prepared the guidelines for final submission of your Nature Structural & Molecular Biology manuscript, "Cryo-EM structure of disease-related prion fibrils provides insights into seeding barriers" (NSMB-BC45265A). Our sincere apologies for the delay regarding this

while we've been experiencing severe and unexpected staffing shortages Nature Structural and Molecular Biology.

Please carefully follow the step-by-step instructions provided in the attached file, and add a response in each row of the table to indicate the changes that you have made. Please also check and comment on any additional marked-up edits we have proposed within the text. Ensuring that each point is addressed will help to ensure that your revised manuscript can be swiftly handed over to our production team.

We would like to start working on your revised paper, with all of the requested files and forms, as soon as possible (preferably within two weeks). Please get in contact with us if you anticipate delays.

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In recognition of the time and expertise our reviewers provide to Nature Structural & Molecular Biology's editorial process, we would like to formally acknowledge their contribution to the external peer review of your manuscript entitled "Cryo-EM structure of disease-related prion fibrils provides insights into seeding barriers". For those reviewers who give their assent, we will be publishing their names alongside the published article.

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If you have any further questions, please feel free to contact me.

Best regards,

Sophia Frank
Editorial Assistant
Nature Structural & Molecular Biology
nsmb@us.nature.com

On behalf of

Florian Ullrich, Ph.D.
Associate Editor
Nature Structural & Molecular Biology
ORCID 0000-0002-1153-2040

Reviewer #1:
Remarks to the Author:
My concerns were addressed by the authors. I don't have further question. I support publication of the work in NSMB.

Reviewer #2:
Remarks to the Author:
The authors have addressed my concerns to the extent that seems humanly possible at the present time.

Final Decision Letter:

3rd Aug 2022

Dear Witold,

We are now happy to accept your revised paper "Cryo-EM structure of disease-related prion fibrils provides insights into seeding barriers" for publication as a Brief Communication in Nature Structural & Molecular Biology.

Acceptance is conditional on the manuscript's not being published elsewhere and on there being no announcement of this work to the newspapers, magazines, radio or television until the publication date in Nature Structural & Molecular Biology.

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Kind regards,
Florian

Florian Ullrich, Ph.D.
Associate Editor
Nature Structural & Molecular Biology
ORCID 0000-0002-1153-2040