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Peer Review Information

Journal: Nature Structural & Molecular Biology
Manuscript Title: Human PRPS1 filaments stabilize allosteric sites to regulate activity
Corresponding author name(s): Justin M. Kollman

Reviewer Comments & Decisions:

Decision Letter, initial version:

Message: 26th Oct 2022

Dear Justin,

Thank you again for submitting your manuscript "Human PRPS1 filaments stabilize allosteric sites to regulate activity". I apologize again for the delay in responding, which (as you know) resulted from the difficulty in obtaining suitable referee reports. Nevertheless, we now have comments (below) from the 3 reviewers 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. 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. If you have comments that are intended for editors only, please include those in a separate cover letter.

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.

We expect to see your revised manuscript within 6 weeks. If you cannot send it within this time, please contact us to discuss an extension; we would still consider your revision, provided that no similar work has been accepted for publication at NSMB or published elsewhere.

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.

Please follow the links below to download these files:

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-portfolio/editorial-policies/image-integrity">Digital Image Integrity Guidelines.

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.

Data availability: this journal strongly supports public availability of data. All data used in accepted papers should be available via a public data repository, or alternatively, as 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. Please note that for some data types, deposition in a public repository is mandatory - more information on our data deposition policies and available repositories can be found below: https://www.nature.com/nature-research/editorial-policies/reporting-standards#availability-of-data

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. Deposition of deep sequencing and microarray data is mandatory, and the datasets must be released prior to or upon publication. 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.

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

Nature Structural & Molecular Biology 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 www.springernature.com/orcid.

Please use the link below to submit your revised manuscript and related files:

[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

Dr Florian Ullrich
Associate Editor, Nature
Consulting Editor, Nature Structural & Molecular Biology
ORCID 0000-0002-1153-2040

Referee expertise:

Referee #1: PRPS, general biochemistry

Referee #2: cryo-EM, enzyme polymerization

Referee #3: cryo-EM

Reviewers' Comments:

Reviewer #1:
Remarks to the Author:
The manuscript by Hvorecny et al. reports an interesting study on human PRPS-1, a key metabolic enzyme. The topic itself is of relevance and the results presented by the Authors are innovative and made an impact in our understanding of PRSP-1 biochemistry and beyond. Indeed, the implication of their study in the broader context of highlighting and understanding the physio-pathological significance of protein large oligomers, is of relevance. The experimental approach and the data obtained are very solid and the conclusions drawn are entirely supported by the data. The manuscript is very well written, easy to follow and conveys clear messages also in term of hypothesis to be confirmed in future experiments. I consider the work presented a nice piece of Science that will

stimulate interesting discussions and further research in the specific field and beyond. However, before finally recommending publication in Nature Structural and Molecular Biology , would like to see the following points addressed in a revised version of the manuscript.

1. The Authors reports that the protein purified in absence of phosphate shows dimer only and no large oligomers could be observed until phosphate is added. This is a major point as one could imagine that filaments are formed even for the ligand-free enzyme. If it was the case then of course a structural analysis would be required for the "phosphate free" protein filaments. Therefore, the Authors are required to clearly state that protein concentration is not an issue in forming the large oligomers. Demonstration that increasing protein concentration of the phosphate free purified enzyme still does not result in oligomer formation is essential and should be reported. Of course if filaments are observed at higher protein concentration for the phosphate free purified enzyme, then structural characterization is required.
2. An interesting part of the manuscript is concerned with evolutionary considerations and conclusion that the highlighted features leading to filament formation have been conserved through billions of years. A more elaborated multi-sequence alignment needs to be produced and possibly included as Supplementary information. The inclusion of one prokaryotes only, Escherichia coli, is certainly not enough; moreover, at least one archaea enzyme should also be included and most importantly human PRPS-2 must also be added. The conservation of residues, that is currently presented in the manuscript in Figure 2 panel D, must be further expanded (and discussed) as suggest above and a new Figure/panel prepared. A complete sequence alignment should be carried out, briefly discussed in the manuscript and included as supplementary information.
3. The second and relevant PRPS isozyme, i.e. PRPS-2, is not mentioned in the manuscript. The Authors should add discussion on the implication their study could have of PRPS-2. Both the Introduction and Discussion sections should report references, considerations/analysis and possibly speculation, on oligomers significance/presence in PRPS-2. To be linked to sequence alignment as pointed out in point #1.
4. A potentially relevant development of the work presented here is of course in future medicine discovery projects. If oligomers are the most relevant active species in vivo and if mutations disrupting oligomer formation are associated with pathological states, one could expect that molecules capable of re-establishing/assisting oligomer formation in the enzyme variants, could have a therapeutic value. I perfectly understand that this is highly speculative consideration and I am not requesting any in silico design for example that will fall outside the scope of the current manuscript. I however feel that this perspective could be added to the conclusion also based on some of the structural observations reported (e.g, allosteric inhibitors)

Reviewer #2:
Remarks to the Author:
In their manuscript "Human PRPS1 filaments stabilize allosteric sites to regulate activity", the authors present the cryo-EM structures of PRPS1 filaments assembled in the presence of different ligands, and show how the assembly interface play a role in the activity by stabilizing allosteric activator binding sites. They show that disease associated mutations are linked to filament assembly and activity.
The novelty of the findings for human PRPS, the link to health and the technical quality of the study makes this paper an important contribution to the field.
Points of concern and possibilities for manuscript improvement are summarized below.

Major Remarks
A.1/ A study on E. coli PRPS filaments was published in June 2022 in eLife (https://doi.org/10.7554/eLife.79552), describing relatively similar ligand promoted assembly and stabilization. The authors cite the bioRxiv version of this work in the introduction and discussion, but do not refer to it while discussing their results. A comparison of the structures and interpretation would be beneficial for the presented work, in particular concerning the assembly interfaces, the general filament's geometry, and the ligand binding modes. Side by side figures (extended or main) comparing the features of PRPS filaments in procaryotes and eucaryotes would add to the completeness of the structural analysis.
A.2/ The high quality of the cryo-EM maps show that the author's single-particle image processing strategy was adequat, however the helical nature of the filaments seem poorly exploited. First, the authors do not describe in the method section how the filament's symmetry was determined, nor seem to have tried to impose and refine helical symmetry during 3D refinements. If the irregularity of the filaments is the reason for this choice, this should be stated and explained (do the authors have a comparison using helical refinement showing lower resolution ?). In this context of assembly interfaces playing a functional role, more details on the rigidity of the interfaces would be useful.

Minor Remarks :
B.1/ The authors show many negative staining EM images, but do not show any cryo-EM micrographs used for data processing. Showing raw data (e.g. in extended data figure 1 or 2) is useful for reproducibility or future studies on similar assemblies.
B.2/ Line 107-109 "Despite nearly identical filament assembly interfaces, the filaments have distinct helical symmetries, with 88º rotation and 63 Å rise per hexamer for the phosphate-bound structure and 94º/61 Å for the ADP-bound". First the authors do not precise whether these helical parameters are for a left- or right-handed helix. Second, the choice to express the helical twist with 88 and 94 degrees seem inappropriate for a D3 helix : the asymmetric "rotational window" being 120 degrees, smaller twist value (with inverse helical hand) of 120-88 and 120-94 degrees will better reflect what appears visually when looking at the filaments, and correspond to the elementary helix helical parameters.
B.3/ Ext data Fig 4 : the authors should correct the twist values as explained in B.2 and also check the direction of rotation of the circular arrows.

Reviewer #3:
Remarks to the Author:
Although initial observations of enzyme self-assembly have been made several decades ago, filamentous assemblies of enzymes have long been largely ignored and only recently seen renewed interest because advances in cryo-EM have enabled structural studies of such assemblies at high resolution, and because of emerging evidence that filamentation can serve as a mode of enzyme regulation. Filamentation may serve to stabilize the active or inactive state of the enzyme by cooperative activation or inhibition. However, the effect of filamentation on enzyme activity and regulation of catalysis has been studied in structural detail in only very few cases and our understanding of these effects, in particular regarding the mechanistic advantage of filamentation over other forms of allostery remains poorly characterised to date. In this paper, Hvorecny et al. investigate the structure of different structural states of human phosphoribosyl pyrophosphate

synthetase (PRPS) filaments during catalysis using cryo-EM. Their structures provide rare insight into how filamentation can regulate enzyme activity by affecting the structure of an allosteric site. In addition, the authors show how coupling of structural changes in the catalytic loop across protomers may facilitate catalysis by coordinating substrate turnover and product release. Together these data provide exciting new insight into the putative mechanistic workings of filamentous enzyme assemblies critical metabolic processes. The structural work and analysis are of high quality and mechanistically supported by biochemical and enzymatic experiments using engineered and disease-related mutations that affect filament stability. The study is concisely written and well-illustrated.

Comments:

- All enzymatic assays appear to have been performed in buffer containing phosphate. Could one perform the assay without phosphate? Since for the interface disrupting mutant E307A no phosphate was identified in the allosteric site from structural analysis, there should likely be no difference in catalytic activity for this (and possibly the other, R301A/S308*?) mutant, whereas there should be a measurable effect for the wild-type enzyme. This could provide additional support for the authors' conclusions.

- For all mutants and the wild-type enzyme, ADP binding seems to result in longer filaments suggesting additional stabilization, while the hexamer packing interface does barely change. Do the authors have additional clues explaining this observation?

- While I do not recommend additional experiments, investigating the effect of engineered mutants on filament assembly and activity of PRPS in a cellular context could provide stronger support to physiological implications and relevance of the mechanism proposed here. If this can be achieved without too much additional effort, the authors may wish to consider performing and including these experiments.

- Figure 2C: it would be helpful to indicate the interface in Figure 2B

- Figure 2 E/F: the authors may consider to employ the same color scheme in both panels for phosphate and ADP structures (i.e. use gray shades for the ADP structure in Figure 2E).

- Extended Data Figure 4; Figure legend: "F. Overlay of active sites shown in Main Text Figure B,C-D and also including PRPS1 + ADP", should be "F. Overlay of active sites shown in Main Text Figure 3, B,C-D and also including PRPS1 + ADP"?
-
- Extended Data Figure 9: Locally filtered or locally sharpened maps may be better conveying the differentially ordered density for the C terminus of protomer a.

- Extended Data Figure 10 and discussion: The authors mention that for the disease mutations S16P, I290T and V309F no filaments are formed and the authors relate the lack of self-assembly to reduced catalytic activity. However, Extended Data Figure 10 seems to show filaments for these mutants at levels not drastically different to wild-type enzyme for the condition containing ATP and R5P. Could the authors comment?

- Data availability: "upon reasonable request" sounds like a hollow statement to me. What request for providing data supporting the study would be considered unreasonable?

Author Rebuttal to Initial comments

Response to Reviewers

We thank the reviewers for their reading of the manuscript and their feedback. Below, we have addressed the comments and suggestions provided. Edits in the manuscript have been underlined and italicized within that document and also quoted in the text below.

Reviewers' Comments:

Reviewer #1:

Remarks to the Author:
The manuscript by Hvorecny et al. reports an interesting study on human PRPS-1, a key metabolic enzyme. The topic itself is of relevance and the results presented by the Authors are innovative and made an impact in our understanding of PRSP-1 biochemistry and beyond. Indeed, the implication of their study in the broader context of highlighting and understanding the physio-pathological significance of protein large oligomers, is of relevance. The experimental approach and the data obtained are very solid and the conclusions drawn are entirely supported by the data. The manuscript is very well written, easy to follow and conveys clear messages also in term of hypothesis to be confirmed in future experiments. I consider the work presented a nice piece of Science that will stimulate interesting discussions and further research in the specific field and beyond. However, before finally recommending publication in Nature Structural and Molecular Biology, I would like to see the following points addressed in a revised version of the manuscript.

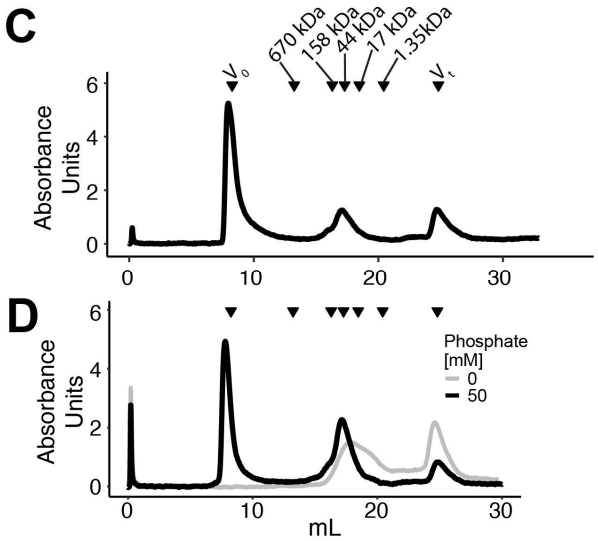
1. The Authors report that the protein purified in absence of phosphate shows dimer only and no large oligomers could be observed until phosphate is added. This is a major point as one could imagine that filaments are formed even for the ligand-free enzyme. If it was the case then of course a structural analysis would be required for the “phosphate free” protein filaments. Therefore, the Authors are required to clearly state that protein concentration is not an issue in forming the large oligomers. Demonstration that increasing protein concentration of the phosphate free purified enzyme still does not result in oligomer formation is essential and should be reported. Of course if filaments are observed at higher protein concentration for the phosphate free purified enzyme, then structural characterization is required.

   To address this question, we used size exclusion chromatography to identify filaments forming at high concentration. We used 30 μM PRPS1 (approximately 1mg/mL) to explore the possibility of filaments in the absence of phosphate, as this concentration corresponds to constraints in PRPS1 sample preparation for data

collection and analysis using cryo electron microscopy. While this concentration does not entirely preclude filament formation without phosphate at concentrations higher than 30 μM, a PRPS1 sample at higher concentration would not be amenable to structure determination by cryo electron microscopy.

The resulting chromatogram (shown below and in Ext. Data Figure 1D) demonstrates that no filaments are observed in the absence of phosphate at a protein concentration of 30 μM. In the phosphate condition (black line), we observe a large peak in the void volume, corresponding to filaments, and a minor peak at an elution volume corresponding to hexamers, dimers, and monomers. Conversely, only a hexamer/dimer/monomer peak is seen in the condition without phosphate (grey line), indicating that no filaments are present in the 30 μM sample without phosphate. We have updated the results to reflect this result.

Extended Data Figure 1C-D:



Extended Data Figure 1. (Figure 2) Filament formation in PRPS1. […] C. Elution profile from a size exclusion column (Superose 6) of PRPS1 in 50 mM phosphate buffer, pH 7.6. D. Elution profile from a size exclusion column (Superose 6) of PRPS1 in 100 mM KCl, 50 mM HEPES, pH 7.6 in the presence
(black) or absence (grey) of 50 mM potassium phosphate, pH 7.6. PRPS1: monomer, 35 kDa; dimer,
70 kDa; hexamer, 210 kDa; filament, ≧420 kDa. […]

Results: "Consistent with these earlier studies, we found that purifying recombinant PRPS1 in a phosphate-free buffer yields mostly dimers and few hexamers as observed by negative stain EM, with no filaments observed in size exclusion chromatography up to 30 μM protein concentration (Ext. Data Figure 1A, C-D). Addition of PRPS1 to

phosphate buffer creates a mixed population of species, including short linear polymers (Fig. 2A, Ext. Data Fig. 1).”

2. An interesting part of the manuscript is concerned with evolutionary considerations and conclusion that the highlighted features leading to filament formation have been conserved through billions of years. A more elaborated multi-sequence alignment needs to be produced and possibly included as Supplementary information. The inclusion of one prokaryotes only, Escherichia coli, is certainly not enough; moreover, at least one archaea enzyme should also be included and most importantly human PRPS-2 must also be added. The conservation of residues, that is currently presented in the manuscript in Figure 2 panel D, must be further expanded (and discussed) as suggest above and a new Figure/panel prepared. A complete sequence alignment should be carried out, briefly discussed in the manuscript and included as supplementary information.

We now include a more diverse sequence alignment in Ext. Data Figure 3D, with a subset of sequences presented in Fig. 2D (and are reproduced below). We discuss the alignments and their implications within the results, discussion, and figure legends:

Figure 2:

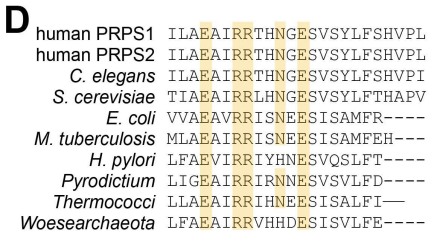
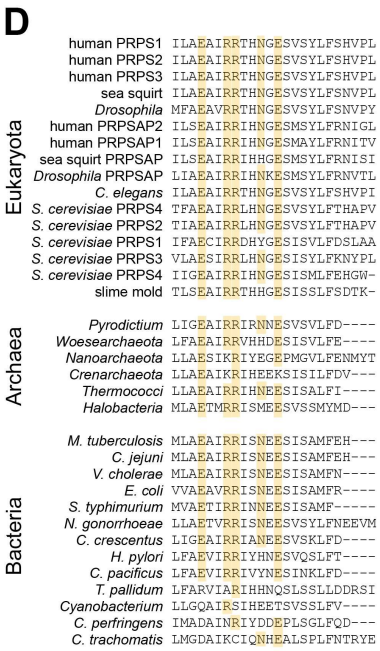


Figure 2. Presence of Phosphate or ADP dictate filament structure of PRPS1. […] D. The Cterminal residues from a sequence alignment of the amino acids of a subset of PRPS proteins showing conservation among kingdoms; identical filament interface residues are highlighted in orange. A more extensive alignment can be found in Ext. Data Figure 3D. […]

Ext Data Figure 3D:



Extended Data Figure 3: (Figure 2,3,5) Volumes and models of filament interface residues. […] D.
The C-terminal portion of a protein sequence alignment comparing PRPS across kingdoms. Identical residues are highlighted in orange. […]

Results: "These residues are deeply conserved and are found in PRPS from eukaryotes, archaea, and prokaryotes (Figure 2D, Ext. Data Fig 3D)."

Discussion: "The deep evolutionary conservation of the residues that mediate PRPS1 filament assembly suggests that PRPS likely assembles filaments in many species. Two recent studies, of human PRPS2 and *E. coli* PRPS, demonstrate that the filament assembly interface is indeed conserved."

Methods: "Sequence Alignments: Amino acid sequences of PRPS from a variety of organisms across kingdoms were identified by the NCBI online portal for BLAST [Redacted] manually curated, and aligned using the EMBL-EBI online portal for MAFFT (https://www.ebi.ac.uk/Tools/msa/mafft/) [65]. PRPS protein sequences lacking a classical Class I PRPS C-terminus[1] were excluded and sequence curation targeted organism representation across kingdoms and was not exhaustive."

3. The second and relevant PRPS isozyme, i.e. PRPS-2, is not mentioned in the manuscript. The Authors should add discussion on the implication their study could have of PRPS-2. Both the Introduction and Discussion sections should report references,

considerations/analysis and possibly speculation, on oligomers significance/presence in PRPS-2. To be linked to sequence alignment as pointed out in point #1.

Human PRPS isoforms and associated proteins have been added to the alignments in Ext. Data Figure 3D and PRPS2 is included in the main text Figure 2D, as shown above. We have also introduced the set of proteins with references in the introduction and discussed the implications of the conserved interface residues with additional references in the discussion.

Introduction: "Many eukaryotes have more than one PRPS gene with high sequence similarity and there is evidence that the isoforms interact [11,12]. Humans contain three isoforms of PRPS (1,2, and 3/1L1) as well as two associated proteins with conserved amino acid sequences and structures [13–16]. While PRPS1 is expressed ubiquitously within the human body, both PRPS2 and PRPS3 show tissue specific expression, and the PRPS associate proteins may inhibit PRPS activity [4,17]."

Results: "The interface residues are also broadly conserved amongst the eukaryotic isozymes; for example, these residues are all conserved in human PRPS2, PRPS3, and the associated proteins PRPSAP1 and PRPSAP2."

Discussion: "The filament architecture of E. coli PRPS changes depending on ligand state, but the assembly interfaces and filament geometries of inhibited E. coli PRPS and human PRPS2 appear to be nearly identical to the human PRPS1 structures we report here[27,50] (Ext. Data Fig. 3H,I). Fluorescence imaging has shown that PRPS assembles into micron scale filamentous structures in human, rat, drosophila, budding yeast, and E. coli cells, further supporting conservation of PRPS filament structure and function[27–29]. Moreover, the conservation of the assembly interface in multiple isoforms from a single species, such as PRPS1-3 from human or PRPS1-5 from S. cerevisiae (Figure 2D and Ext Data Figure 3D), suggests that the isoforms all form filaments and that mixed filaments containing multiple isoforms may assemble within the cell, yet another potential level of regulation. Immunoprecipitation, colocalization, and genetic experiments support some level of coassembly[11,12,28]."

4. A potentially relevant development of the work presented here is of course in future medicine discovery projects. If oligomers are the most relevant active species in vivo and if mutations disrupting oligomer formation are associated with pathological states, one could expect that molecules capable of reestablishing/assisting oligomer formation in the enzyme variants, could have a therapeutic value. I perfectly understand that this is highly speculative consideration and I am not requesting any in silico design for example that will fall outside the scope of the current manuscript. I however feel that this perspective could be added to

the conclusion also based on some of the structural observations reported (e.g, allosteric inhibitors).

We have added discussion points about how this work may contribute to future development of small-molecule therapies that would help those patients suffering from disorders caused by mutations in PRPS1:

Discussion: "As assembly is regulated by allosteric ligands, there is potential for the development of therapeutic approaches that target the allosteric site or that pattern the C-terminal α-helix to promote assembly. This may also be an avenue for cancer-related therapies, as PRPS2 regulates biosynthesis in Myc-driven tumors[49]. However, as the allosteric site supports both activation and inhibition, increase assembly may have unintended effects, both on PRPS1 and on the highly similar isoforms."

Reviewer #2:

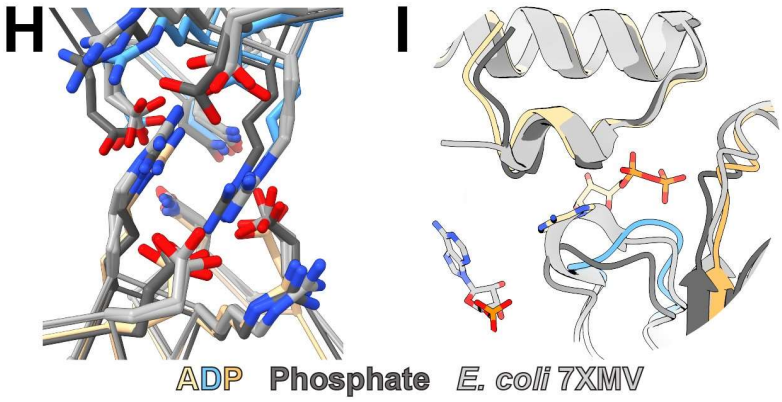
Remarks to the Author:
In their manuscript "Human PRPS1 filaments stabilize allosteric sites to regulate activity", the authors present the cryo-EM structures of PRPS1 filaments assembled in the presence of different ligands, and show how the assembly interface play a role in the activity by stabilizing allosteric activator binding sites. They show that disease associated mutations are linked to filament assembly and activity. The novelty of the findings for human PRPS, the link to health and the technical quality of the study makes this paper an important contribution to the field. Points of concern and possibilities for manuscript improvement are summarized below.

Major Remarks

A.1/ A study on E. coli PRPS filaments was published in June 2022 in eLife (https://doi.org/10.7554/eLife.79552 [doi.org]), describing relatively similar ligand promoted assembly and stabilization. The authors cite the bioRxiv version of this work in the introduction and discussion, but do not refer to it while discussing their results. A comparison of the structures and interpretation would be beneficial for the presented work, in particular concerning the assembly interfaces, the general filament's geometry, and the ligand binding modes. Side by side figures (extended or main) comparing the features of PRPS filaments in procaryotes and eucaryotes would add to the completeness of the structural analysis.

With the published *E. coli* PRPS structures now available, we have added Ext. Data Figure 3H and 3I, which directly compare the interface and ligand binding modes, and have discussed the structures in the text:

Ext Data Figure 3H and 3I:



Extended Data Figure 3: (Figure 2,3,5) Volumes and models of filament interface residues. […] H. Overlay of the phosphate- (dark grey) and ADP-bound (orange/blue) filament interfaces with the E. coli PRPS filament interfaces (light grey, PDB ID 7XMU, 7XMV) I. Comparison of phosphate- (dark grey) and ADP-bound (orange/blue) structures to E. coli PRPS filament structures (light grey, PDB ID 7XMV). Structures have been aligned on the allosteric domain of protomer a, and phosphates from the phosphate-bound human and E. coli structures have been omitted for clarity.

Results: Comparison of both phosphate-bound and ADP-bound human PRPS1 to recently published filament structures of PRPS from E. coli demonstrate structural conservation[27]. The interface residues identified in the nucleotide-bound structures of PRPS from E. coli (PDB IDs 7xmu and 7xmv) adopt similar positions to those found in the interfaces of the human PRPS1 filament (Ext. Data Fig 3H). The helical rise and twist of the nucleotide-bound filament from E. coli (-27º and 63 Å, respectively) are very similar to the ADP-bound human filament (-26º and 62 Å). However, the allosteric ADP and AMP found in PRPS from E. coli are bound in an entirely different allosteric site as compared to human PRPS1 (Ext. Data Fig 3I). Thus, while the binding site of inhibitory nucleotides appears to have evolved differently over time, filament assembly has remained.
Additionally, the residues that comprise the second interface described for PRPS from E. coli (PDB ID 7xn3) are not full conserved; notably, Tyr24 from E. coli corresponds to Gly24 in humans.

Discussion: The deep evolutionary conservation of the residues that mediate PRPS1 filament assembly suggests that PRPS likely assembles filaments in many species. Two recent studies, of human PRPS2 and E. coli PRPS, demonstrate that the filament assembly interface is indeed conserved. The filament architecture of E. coli PRPS changes depending on ligand state, but the assembly interfaces and filament

geometries of inhibited E. coli PRPS and human PRPS2 appear to be nearly identical to the human PRPS1 structures we report here[27,50] (Ext. Data Fig. 3H,I).

A.2/ The high quality of the cryo-EM maps show that the author's single-particle image processing strategy was adequate, however the helical nature of the filaments seem poorly exploited. First, the authors do not describe in the method section how the filament's symmetry was determined, nor seem to have tried to impose and refine helical symmetry during 3D refinements. If the irregularity of the filaments is the reason for this choice, this should be stated and explained (do the authors have a comparison using helical refinement showing lower resolution ?). In this context of assembly interfaces playing a functional role, more details on the rigidity of the interfaces would be useful.

A description of initial symmetry determination has been added to the ***Methods*** section: "The initial *ab initio* reconstruction and 3D refinement for a subset of datasets (PRPS1 + ADP) was completed without symmetry imposed and compared to previously published PRPS hexameric crystal structures to confirm the presence of D3 symmetry in the oligomer[18,19]."

A description of the calculation of helical rise and twist has been added to the ***Methods*** section:
"Helical twist and rise were calculated with the final refined and sharpened interface maps in Chimera (v1.15), using the "measure symmetry" command with optimization and the estimated helical parameters of a -30º twist and a 62 Å rise."

In short, we took a strictly single-particle approach to the reconstruction, and helical symmetry was directly measured from the D3-symmetrized high resolution maps by measuring the rotation/translation between adjacent hexameric helical protomers. As the PRPS1 oligomers that comprise the filaments are relatively large (210 kDa) and symmetrical, we chose to focus our refinement on a single repeating subunit, which has proven to be an effective strategy employed by our group and others[36–38]. We found that as we reduced our refinement masks from 3-5 hexameric helical protomers to 1 that the resolution improved significantly (~ 0.5 Å global resolution), suggesting that the slight curvature we see in PRPS1 filaments over longer lengths imposes a limit on resolution. Imposition of helical symmetry restraints to this reconstruction and refinement strategy would therefore likely lower the global resolution. In other words, like most filamentous polymers, PRPS filaments include some degree of flexibility, such that the smaller the helical segment the reconstruction is focused on the less flexibility degrades the resolution. Because reconstructions containing two oligomers centered on the interface achieved resolution and map quality very close to the single hexamer reconstruction, it is hard to say whether the flexibility arises within the hexamers or at the helical assembly interfaces. We have added this explanation to the results section.

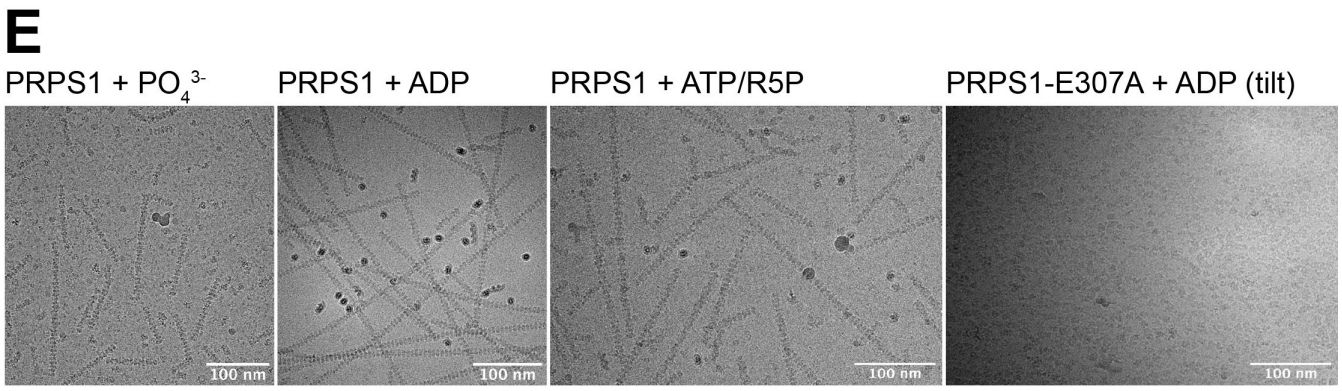
Results: “We determined two maps for each filament dataset, centered on the hexamer or the interface (Data Table 1). By masking the oligomers outside of the central hexamer or interface (Ext Data Figure 2), we improved our map quality and resolution; however, both hexamer and interface maps achieved similar resolutions, suggesting that individual interfaces are relatively rigid with some filament flexing occurring over longer distances.”

Minor Remarks :

B.1/ The authors show many negative staining EM images, but do not show any cryo-EM micrographs used for data processing. Showing raw data (e.g. in extended data figure 1 or 2) is useful for reproducibility or future studies on similar assemblies.

We have added motion-corrected, summed micrographs filtered for visualization for a subset of the datasets (Ext. Data Figure 1E), representing the combination of microscopes, cameras, and tilts used in this manuscript:

Ext. Data Figure 1E:



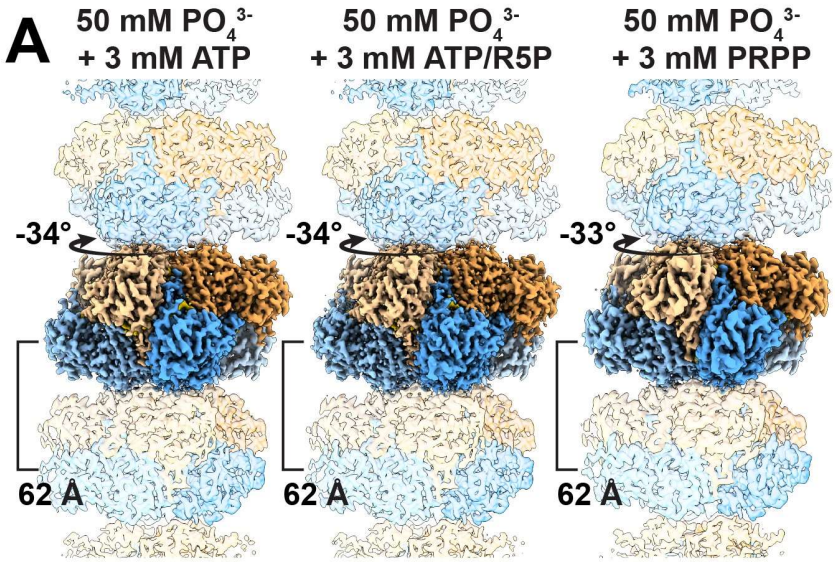
Extended Data Figure 1. (Figure 2) Filament formation in PRPS1. […] E. Motion-corrected and summed cryo electron micrographs, Gaussian blurred and contrast adjusted for visualization, from four datasets presented in this manuscript, representing the cameras and tilts used in data collection. Microscopes, cameras, and stage tilts are listed in Data Table 1.

B.2/ Line 107-109 “Despite nearly identical filament assembly interfaces, the filaments have distinct helical symmetries, with 88º rotation and 63 Å rise per hexamer for the phosphate-bound structure and 94º/61 Å for the ADP-bound”. First the authors do not precise whether these helical parameters are for a left- or right-handed helix. Second, the choice to express the helical twist with 88 and 94 degrees seem inappropriate for a D3 helix : the

asymmetric “rotational window” being 120 degrees, smaller twist value (with inverse helical hand) of 120-88 and 120-94 degrees will better reflect what appears visually when looking at the filaments, and correspond to the elementary helix helical parameters.

We have redefined the helix of PRPS1 as left-handed, corresponding to the smaller twist value, and indicate left-handed twist with negative values With these changes, the helical parameters have been recalculated and updated in the text and figures:

Ext. Data Figure 4A:



Extended Data Figure 4. (Figure 3) Substrate- and product-bound filaments. A. Volume of PRPS1 filaments bound to phosphate/ATP (left), phosphate/ATP/R5P (middle), or phosphate/PRPP (right); protomers colored in blue or orange. […]

Results: “Despite nearly identical filament assembly interfaces, the filaments have distinct helical symmetries, with a left-handed rotation of -32º and a 62 Å rise per hexamer for the phosphatebound structure and -26º/62 Å for the ADP-bound (Figure 2B).”

B.3/ Ext data Fig 4: the authors should correct the twist values as explained in B.2 and also check the direction of rotation of the circular arrows.

As shown above (B.2/), we have updated the twist values which now match the direction of the arrows in the figure.

Reviewer #3:

Remarks to the Author:
Although initial observations of enzyme self-assembly have been made several decades ago, filamentous assemblies of enzymes have long been largely ignored and only recently seen renewed interest because advances in cryo-EM have enabled structural studies of such assemblies at high resolution, and because of emerging evidence that filamentation can serve as a mode of enzyme regulation. Filamentation may serve to stabilize the active or inactive state of the enzyme by cooperative activation or inhibition. However, the effect of filamentation on enzyme activity and regulation of catalysis has been studied in structural detail in only very few cases and our understanding of these effects, in particular regarding the mechanistic advantage of filamentation over other forms of allostery remains poorly characterised to date. In this paper, Hvorecny et al. investigate the structure of different structural states of human phosphoribosyl pyrophosphate synthetase (PRPS) filaments during catalysis using cryo-EM. Their structures provide rare insight into how filamentation can regulate enzyme activity by affecting the structure of an allosteric site. In addition, the authors show how coupling of structural changes in the catalytic loop across protomers may facilitate catalysis by coordinating substrate turnover and product release. Together these data provide exciting new insight into the putative mechanistic workings of filamentous enzyme assemblies critical metabolic processes. The structural work and analysis are of high quality and mechanistically supported by biochemical and enzymatic experiments using engineered and disease-related mutations that affect filament stability. The study is concisely written and wellillustrated.

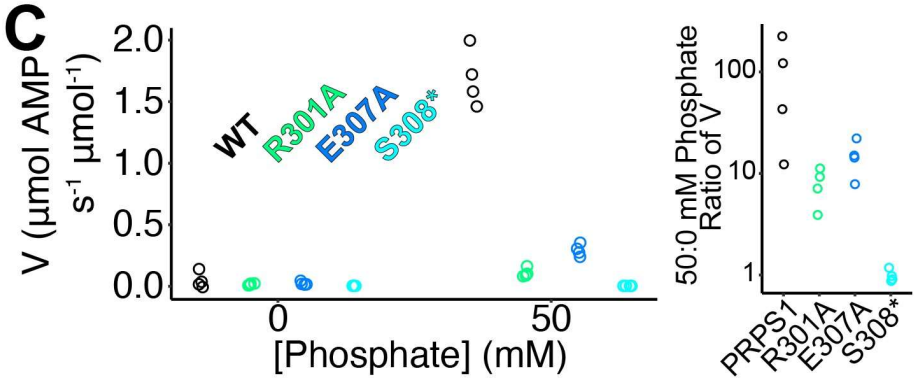
Comments:

- All enzymatic assays appear to have been performed in buffer containing phosphate. Could one perform the assay without phosphate? Since for the interface disrupting mutant E307A no phosphate was identified in the allosteric site from structural analysis, there should likely be no difference in catalytic activity for this (and possibly the other, R301A/S308*?) mutant, whereas there should be a measurable effect for the wild-type enzyme. This could provide additional support for the authors' conclusions.

> As suggested, we tested enzyme activity with and without phosphate for wildtype PRPS1 and the engineered mutations. The results are shown below and have been added to Ext. Data Figure 7 (panel C) and addressed in the results and discussion sections. WT and mutant proteins all have very low activity in the absence of phosphate. In these conditions adding phosphate increases activity in wildtype PRPS1 about 100x, but only increases activity in R301A and E307A about 10x, and has no effect on S308*. This is consistent with the phosphate binding site being intact in the two point mutants, suggesting that R301A and E307A still bind phosphate with dramatically reduced affinity, while S308* only retains the intrinsic unregulated activity of the enzyme. Note that we had to adapt our enzyme assay to use an alternative to phosphate buffer for this assay,

which leads to a decrease in the absolute level of WT PRPS1 activity (compared, for example, to the Vmax in fig. 4C).

Ext. Data Figure 7C:



Extended Data Figure 7. (Figure 4) Mutation of filament interface residues. […] C. Assay performed in buffer containing: 50 mM Potassium HEPES pH 7.6, 6 mM $MgCl_2$, 0.1 mM EDTA, 1 mM DTT, 0.1 mg/mL bovine serum albumin. Left: Activity assay of the three engineered mutations with or without 50 mM potassium phosphate, pH 7.6 (N = 4). Right: Ratio of 50 mM phosphate: 0 mM phosphate activities (V) from the panel to the left. […]

Results: "We also tested the effects of the allosteric activator phosphate, and found that in its absence the wildtype and non-assembly mutant enzymes all have very low activity. While the activity of wildtype enzyme increases about 100-fold in the presence of phosphate, this response is reduced to ~10-fold in the point mutants, and eliminated in the truncation mutant. This is consistent with reduced affinity for phosphate in the point mutants, where the phosphate binding site remains intact, and complete loss of the binding site in the truncation mutant (Ext. Data Figure 7C)"

Discussion: "Increased activity in the filament likely arises from stabilization of the allosteric regulatory site by filament assembly contacts; in free hexamers the allosteric site is disordered, decreasing the binding of the essential activator phosphate and the consequent conformational changes that increase catalytic activity."

- For all mutants and the wild-type enzyme, ADP binding seems to result in longer filaments suggesting additional stabilization, while the hexamer packing interface does barely change. Do the authors have additional clues explaining this observation?

Our structural data shows that ADP directly engages with more amino acid residues lining the allosteric site than phosphate, suggesting that ADP interacts more strongly with that site than phosphate, leading to higher occupancy of the allosteric site in ADP conditions and therefore promoting longer filaments. Supporting this idea, our sample preparation for both negative stain and cryo grids was completed in a 50 mM phosphate buffer and supplemented with 3 mM ADP, suggesting that the ADP displaces the phosphate. Additionally, kinetic parameters in the literature support this idea (1974a Roth et al, *J. Biol. Chem.;* 1974b Roth et al, *J. Biol. Chem.*; 1995 Becker et al *J. Clin. Investig.*), though direct comparisons are difficult as ADP can also bind in the active site and all biochemical experiments are completed in the presence of phosphate as phosphate is a required activator.

- While I do not recommend additional experiments, investigating the effect of engineered mutants on filament assembly and activity of PRPS in a cellular context could provide stronger support to physiological implications and relevance of the mechanism proposed here. If this can be achieved without too much additional effort, the authors may wish to consider performing and including these experiments.

We also believe exploring the effects of the engineered mutants in a cellular context would be extremely valuable. However, these experiments are outside of the scope of this current study.

- Figure 2C: it would be helpful to indicate the interface in Figure 2B

We have indicated the filament interface shown in 2C with a box in 2B and have updated the figure legend:

Figure 2B:

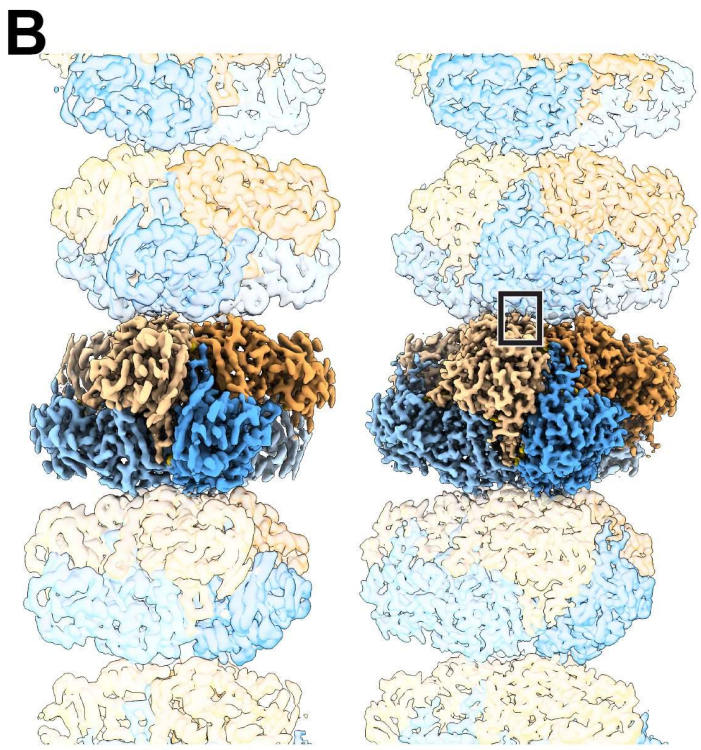


Figure 2. Presence of Phosphate or ADP dictate filament structure of PRPS1. […] B. Cryo-EM structure of PRPS1 filaments bound to phosphate (left) or ADP (right); protomers colored in blue or orange. Boxed area indicates the location of the interface in (C). […]

- Figure 2 E/F: the authors may consider to employ the same color scheme in both panels for phosphate and ADP structures (i.e. use gray shades for the ADP structure in Figure 2E).

We have update Figure 2 to unify the color scheme. The ADP-bound structure is now shown in blue/orange and the phosphate-bound structure is shown in shades of grey:

Figure 2C,E-F:

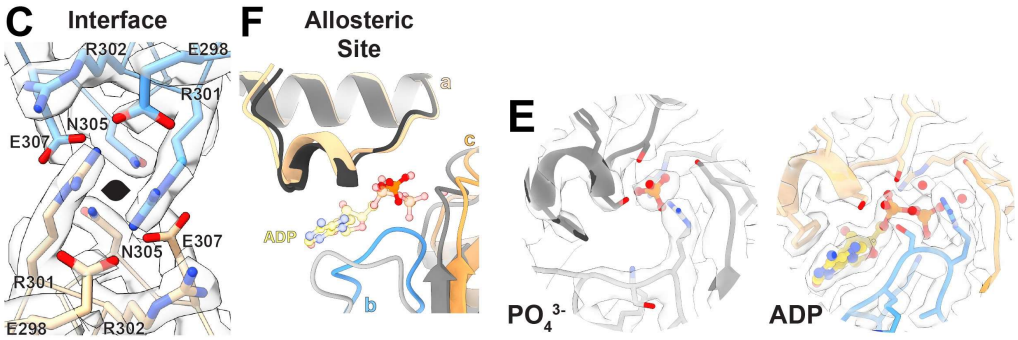


Figure 2. Presence of Phosphate or ADP dictate filament structure of PRPS1. […] C. Model and map of the primary interface residues in the ADP-bound structure, with two-fold symmetry axis indicated. Boxed area indicates the location of the interface in (C). […] E. Model and map of the allosteric sites in the phosphate- and ADP-bound filaments. F. Ribbon diagram and ligands in the allosteric site show that when aligned on the allosteric domain of protomer a, the ligand present dictates the positioning of protomers b and c (phosphate-bound in greys; ADP-bound in orange/blue).

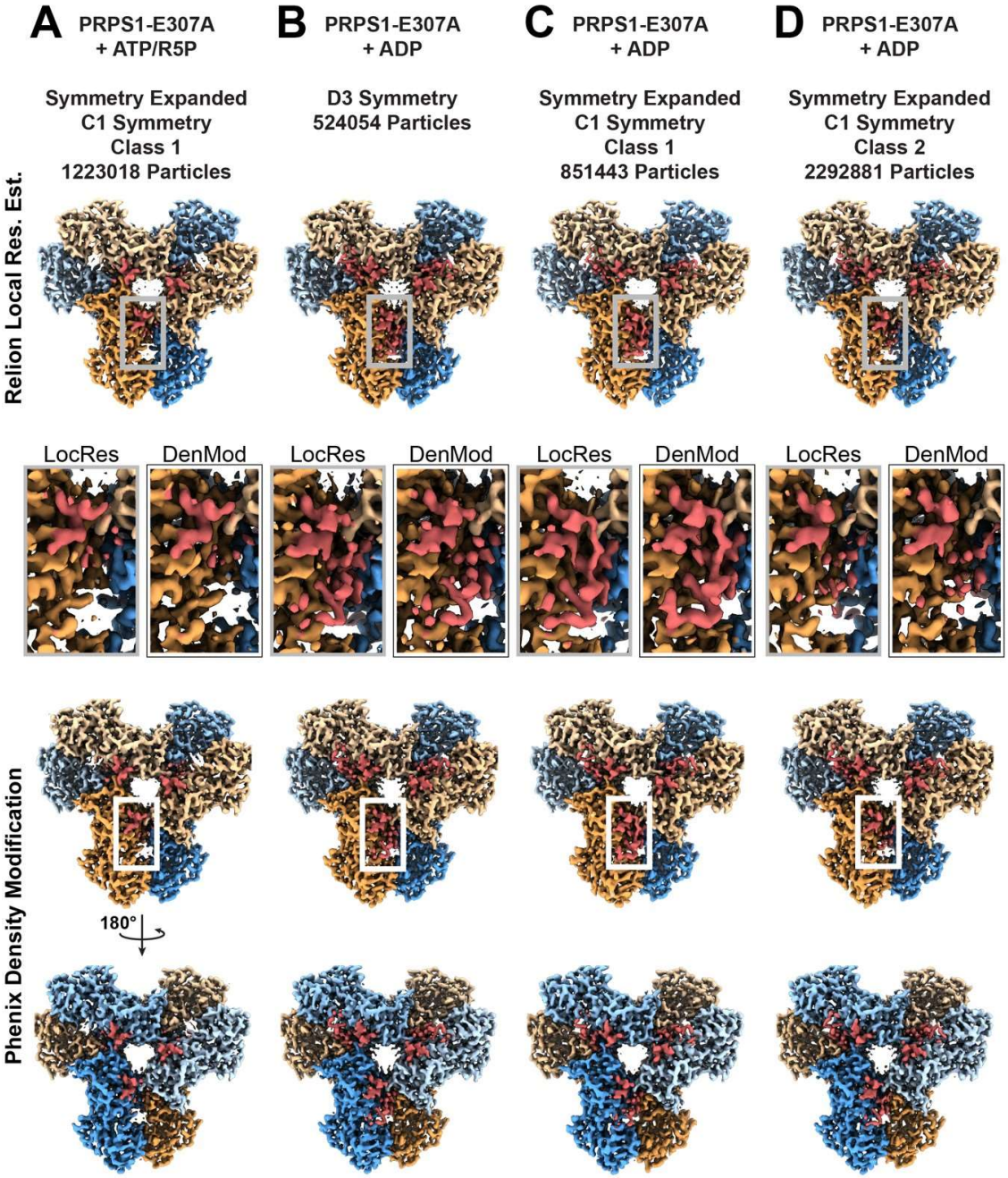
- Extended Data Figure 4; Figure legend: “F. Overlay of active sites shown in Main Text Figure B,C-D and also including PRPS1 + ADP”, should be “F. Overlay of active sites shown in Main Text Figure 3, B,C-D and also including PRPS1 + ADP”?

  Correct. This has been updated in the text.

- Extended Data Figure 9: Locally filtered or locally sharpened maps may be better conveying the differentially ordered density for the C terminus of protomer a.

  The maps shown in the original version of Ext. Data Figure 9 included Density Modification in Phenix as the final processing step. We also explored the use of local resolution filtering using Relion’s implementation of ResMap and have added those steps to the methods and the maps to the figure. This method produces maps that look equivalent to the density modified maps.

  Ext Data Figure 9:



Extended Data Figure 9. (Figure 5) Volumes for C-termini of PRPS1-E307A mutations. A-D. Panel detailing PRPS1-E307A maps and models, with protomers in blue/orange and C-termini highlighted in red. *Row 1*: Dataset, symmetry, and number of particles included in the map. *Row 2*: View of one face of the ResMap filtered volumes from PRPS1-E307A datasets. *Row 3*: Insert showing volume of C-

termini of protomer a from Relion's implementation of ResMap (grey box) or Phenix's Density Modification (black/white box). *Rows 4 & 5*: View of both faces of the density modified volumes from PRPS1-E307A datasets. C-termini are highlighted in red.

Methods: "For a subset of datasets (PRPS1-E307A + ATP/R5P and PRPS1-E307A + ADP), local resolution estimation was also performed using Relion's implementation of ResMap[59]."

- Extended Data Figure 10 and discussion: The authors mention that for the disease mutations S16P, I290T and V309F no filaments are formed and the authors relate the lack of self-assembly to reduced catalytic activity. However, Extended Data Figure 10 seems to show filaments for these mutants at levels not drastically different to wild-type enzyme for the condition containing ATP and R5P. Could the authors comment?

In the results section, we previously said, "the loss of function mutations are defective in filament assembly." In the discussion, we suggested, "as filament formation is crucial for efficient catalysis, even a slight shift in the propensity to assemble could have serious consequences for catalytic activity." None of the disease mutations tested have completely lost their ability to form filaments, as demonstrated in the ADP condition. While we see a striking decrease of filaments in the phosphate only condition, addition of ATP and R5P causes short filaments to assemble. We have added the panel of negative stain images containing phosphate, ATP, and R5P to main text Figure 6 and have edited the results section to state these results more clearly.

Figure 6:

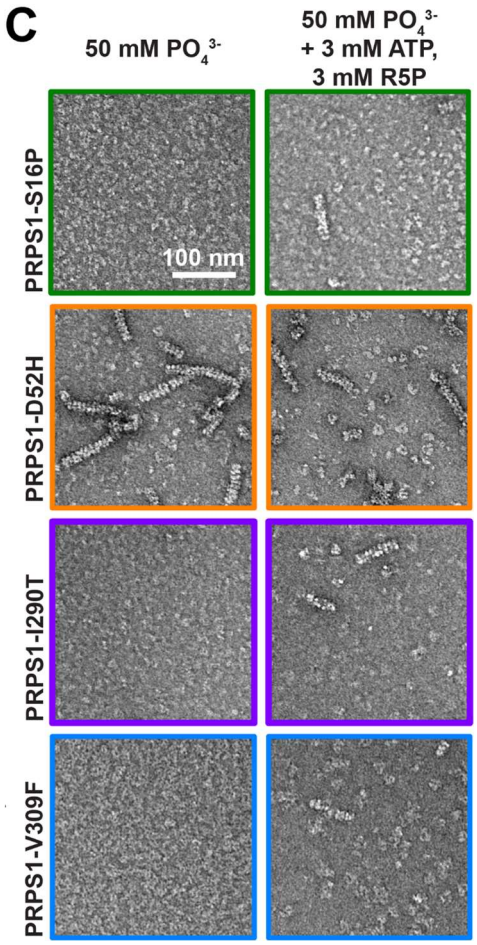
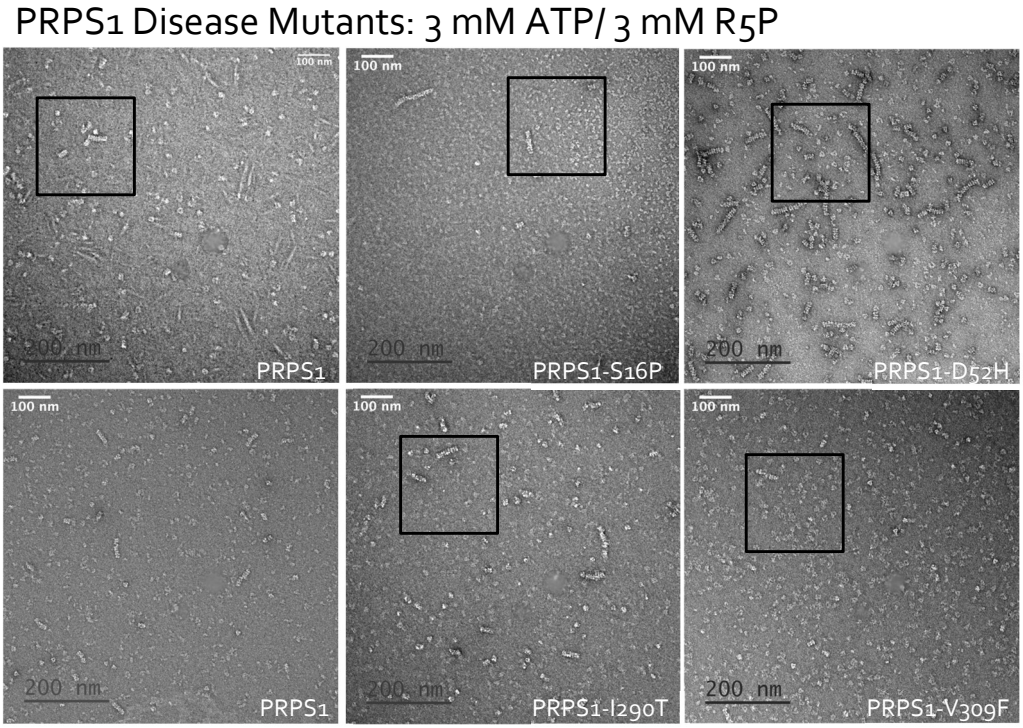


Figure 6. Mutations near the N- and C-termini alter filament formation which correlates with catalysis. […] C. Negative stain EM images of the four disease mutations in the presence of phosphate (*left*) and in the presence of phosphate, ATP, and ribose-5-phosphate (*right*).

Results: "The pattern of activity displayed by the mutations is paralleled in their assembly into filaments. The D52H mutation retains filament assembly similar to or increased from the wild type enzyme (Figure 6C, Ext. Data Figure 10 B-C). Conversely, the loss of function mutations show decreases in filament assembly in some of the conditions tested. This is most striking in the phosphate only condition, where very few or no filaments are seen (Figure 6C, Ext. Data Figure 10 C). Upon addition of ATP and R5P, short filaments assemble, suggesting that ATP and ribose-5phosphate can also promote filament formation (Figure 6C, Ext. Data Figure 10 C). While the effects of the disease mutations are not as dramatic as the engineered, filament-disrupting mutations described above, the data are consistent with the

hypothesis that disruption of filament formation decreases the activity of the enzyme."

Below, we have also included the full micrographs of the phosphate/ATP/R5P condition, plus an additional micrograph of the wildtype enzyme in this condition, for the reviewer to examine. While I290T appears roughly equivalent to wildtype PRPS1 in the phosphate/ATP/R5P condition, fewer filaments are seen in the micrographs containing either S16P or V309F. However, these results are qualitative and therefore we do not draw strong conclusions from them.



Response to Reviewers figure only. Micrographs acquired on an FEI Morgagni using Digital
Micrograph. Images contrast adjusted and scale bars added in Fiji. Note: 200 nm scale bars are inaccurate; please refer to the 100 nm scale bar at the top of the micrograph.

- Data availability: "upon reasonable request" sounds like a hollow statement to me. What request for providing data supporting the study would be considered unreasonable?

We have revised the data availability statement:

"[…] Other data supporting this study are available from the corresponding author upon request."

Decision Letter, first revision:

Message: Our ref: NSMB-A46483A

16th Nov 2022

Dear Justin,

Thank you for submitting your revised manuscript "Human PRPS1 filaments stabilize allosteric sites to regulate activity" (NSMB-A46483A). 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.

To facilitate our work at this stage, we would appreciate if you could send us the main text as a word file. Please make sure to copy the NSMB account (cc'ed above).

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

Dr Florian Ullrich
Associate Editor, Nature
Consulting Editor, Nature Structural & Molecular Biology
ORCID 0000-0002-1153-2040

Reviewer #1 (Remarks to the Author):

The Authors satisfactorily addressed all my requests and implemented new data/figures and comments in the revised version of the manuscript which I recommend for publication.

Reviewer #2 (Remarks to the Author):

In their revised version of the manuscript "Human PRPS1 filaments stabilize allosteric sites to regulate activity", the authors have adequately addressed the points of concern, adding text and figures when necessary. No other revision seem necessary, the manuscript is appropriate for publication.

Reviewer #3 (Remarks to the Author):

The authors have thoroughly addressed all my initial comments.

Arjen Jakobi

Final Decision Letter:

Message : 10th Jan 2023

Dear Dr. Kollman,

We are now happy to accept your revised paper "Human PRPS1 filaments stabilize allosteric sites to regulate activity" for publication as a Article 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.

Over the next few weeks, your paper will be copyedited to ensure that it conforms to Nature Structural & Molecular Biology style. Once your paper is typeset, you will receive an email with a link to choose the appropriate publishing options for your paper and our Author Services team will be in touch regarding any additional information that may be required.

After the grant of rights is completed, 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.

You will not receive your proofs until the publishing agreement has been received through our system.

Due to the importance of these deadlines, we ask that you please let us know now whether you will be difficult to contact over the next month. If this is the case, we ask you provide us with the contact information (email, phone and fax) of someone who will be able to check the proofs on your behalf, and who will be available to address any last-minute problems.

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Note the policy of the journal on data deposition: http://www.nature.com/authors/policies/availability.html.

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Sincerely,

Katarzyna Ciazynska
(she/her)
Associate Editor
Nature Structural & Molecular Biology
https://orcid.org/0000-0002-9899-2428

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