EMBO reports

Remodeling and activation mechanisms of outer arm dyneins revealed by cryo-EM

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1st Editorial Decision 29th Mar 2021

Dear Dr. Bui,

Thank you for transferring your manuscript to EMBO Reports, which was previously reviewed at The EMBO Journal.

Having looked at the referee reports, I would like to invite you to submit a revised manuscript. As mentioned in my colleague Ieva's previous letter, MD simulations should be described and discussed in more depth as indicated by reviewer #1. Moreover, the structure provided should be compared and contrasted to the one recently published (PMID: 33473120) as suggested by referee #2. Lastly, citations of the relevant literature should be expanded as pointed out by the expert advisor.

Considering the amount of work required to address these concerns, we believe that four weeks should be sufficient to revise the manuscript. Please let me know if you anticipate problems meeting this deadline.

Please revise your manuscript with the understanding that the referee concerns (as in their reports) must be fully addressed and their suggestions taken on board. Please address all referee concerns in a complete point-by-point response. Acceptance of the manuscript will depend on a positive outcome of a second round of review. It is EMBO reports policy to allow a single round of revision only and acceptance or rejection of the manuscript will therefore depend on the completeness of your responses included in the next, final version of the manuscript.

As a matter of policy, competing manuscripts published during the revision period will not negatively impact on our assessment of the conceptual advance presented by your study. However, we request that you contact the editor as soon as possible upon publication of any related work, to discuss how to proceed. Should you foresee a problem in meeting this three-month deadline, please let us know in advance and we may be able to grant an extension.

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We are aware that many laboratories cannot function at full efficiency during the current COVID-19/SARS-CoV-2 pandemic and have therefore extended our 'scooping protection policy' to cover the period required for a full revision to address the experimental issues highlighted in the editorial decision letter. Please contact the scientific editor handling your manuscript to discuss a revision plan should you need additional time, and also if you see a paper with related content published elsewhere.***

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2. Your manuscript contains statistics and error bars based on n=2 or on technical replicates. Please use scatter plots in these cases.
You can submit the revision either as a Scientific Report or as a Research Article. For Scientific Reports, the revised manuscript can contain up to 5 main figures and 5 Expanded View figures. If the revision leads to a manuscript with more than 5 main figures it will be published as a Research Article. In this case the Results and Discussion section should be separate. If a Scientific Report is submitted, these sections have to be combined. This will help to shorten the manuscript text by eliminating some redundancy that is inevitable when discussing the same experiments twice. In

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Supplementary/additional data: The Expanded View format, which will be displayed in the main HTML of the paper in a collapsible format, has replaced the Supplementary information. You can submit up to 5 images as Expanded View. Please follow the nomenclature Figure EV1, Figure EV2 etc. The figure legend for these should be included in the main manuscript document file in a section called Expanded View Figure Legends after the main Figure Legends section. Additional Supplementary material should be supplied as a single pdf labeled Appendix. The Appendix includes a table of content on the first page with page numbers, all figures and their legends. Please follow the nomenclature Appendix Figure Sx throughout the text and also label the figures according to this nomenclature. For more details please refer to our guide to authors.

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When submitting your revised manuscript, please carefully review the instructions that follow below. Failure to include requested items will delay the evaluation of your revision.

1) a .docx formatted version of the manuscript text (including legends for main figures, EV figures and tables). Please make sure that the changes are highlighted to be clearly visible.

2) individual production quality figure files as .eps, .tif, .jpg (one file per figure).

3) a .docx formatted letter INCLUDING the reviewers' reports and your detailed point-by-point responses to their comments. As part of the EMBO Press transparent editorial process, the point-by-point response is part of the Review Process File (RPF), which will be published alongside your paper. For more details on our Transparent Editorial Process, please visit our website:
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You are able to opt out of this by letting the editorial office know (emboreports@embo.org). If you do opt out, the Review Process File link will point to the following statement: "No Review Process File is available with this article, as the authors have chosen not to make the review process public in this case."

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5) Please note that all corresponding authors are required to supply an ORCID ID for their name upon submission of a revised manuscript (<https://orcid.org/>). Please find instructions on how to link your ORCID ID to your account in our manuscript tracking system in our Author guidelines (<http://embor.embopress.org/authorguide>).

6) We replaced Supplementary Information with Expanded View (EV) Figures and Tables that are collapsible/expandable online. A maximum of 5 EV Figures can be typeset. EV Figures should be cited as 'Figure EV1, Figure EV2" etc... in the text and their respective legends should be included in the main text after the legends of regular figures.

- For the figures that you do NOT wish to display as Expanded View figures, they should be bundled together with their legends in a single PDF file called *Appendix*, which should start with a

short Table of Content. Appendix figures should be referred to in the main text as: "Appendix Figure S1, Appendix Figure S2" etc. See detailed instructions regarding expanded view here: <http://embor.embopress.org/authorguide#expandedview>.

- Additional Tables/Datasets should be labeled and referred to as Table EV1, Dataset EV1, etc. Legends have to be provided in a separate tab in case of .xls files. Alternatively, the legend can be supplied as a separate text file (README) and zipped together with the Table/Dataset file.

7) We would also encourage you to include the source data for figure panels that show essential data.

Numerical data should be provided as individual .xls or .csv files (including a tab describing the data). For blots or microscopy, uncropped images should be submitted (using a zip archive if multiple images need to be supplied for one panel). Additional information on source data and instruction on how to label the files are available <http://embor.embopress.org/authorguide#sourcedata>.

8) Our journal encourages inclusion of *data citations in the reference list* to directly cite datasets that were re-used and obtained from public databases. Data citations in the article text are distinct from normal bibliographical citations and should directly link to the database records from which the data can be accessed. In the main text, data citations are formatted as follows: "Data ref: Smith et al, 2001" or "Data ref: NCBI Sequence Read Archive PRJNA342805, 2017". In the Reference list, data citations must be labeled with "[DATASET]". A data reference must provide the database name, accession number/identifiers and a resolvable link to the landing page from which the data can be accessed at the end of the reference. Further instructions are available at <http://embor.embopress.org/authorguide#datacitation>.

9) Please make sure to include a Data Availability Section before submitting your revision - if it is not applicable, make a statement that no data were deposited in a public database. Primary datasets (and computer code, where appropriate) produced in this study need to be deposited in an appropriate public database (see <http://embor.embopress.org/authorguide#dataavailability>).

Please remember to provide a reviewer password if the datasets are not yet public.

The accession numbers and database should be listed in a formal "Data Availability " section (placed after Materials & Method) that follows the model below. Please note that the Data Availability Section is restricted to new primary data that are part of this study.

Data availability

The datasets (and computer code) produced in this study are available in the following databases:

- RNA-Seq data: Gene Expression Omnibus GSE46843 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE46843)
- [data type]: [name of the resource] [accession number/identifier/doi] ([URL or identifiers.org/DATABASE:ACCESSION])

*** Note - All links should resolve to a page where the data can be accessed. ***

10) Regarding data quantification, please ensure to specify the name of the statistical test used to generate error bars and P values, the number (n) of independent experiments underlying each data point (not replicate measures of one sample), and the test used to calculate p-values in each figure legend. Discussion of statistical methodology can be reported in the materials and methods section, but figure legends should contain a basic description of n, P and the test applied.
Please note that error bars and statistical comparisons may only be applied to data obtained from at least three independent biological replicates.
Please also include scale bars in all microscopy images.

We would also welcome the submission of cover suggestions, or motifs to be used by our Graphics Illustrator in designing a cover.

I look forward to seeing a revised version of your manuscript when it is ready. Please let me know if you have questions or comments regarding the revision.

Kind regards,

Deniz Senyilmaz Tiebe

Deniz Senyilmaz Tiebe, PhD
Editor
EMBO Reports

REFEREE REPORTS transferred from The EMBO Journal

Referee #1:

GENERAL SUMMARY AND OPINION ABOUT THE PRINCIPAL SIGNIFICANCE OF THE STUDY, ITS QUESTIONS AND FINDINGS:

I was informed by EMBO that part of the submitted manuscript has been scooped by a paper published in Nature Communications on Jan 2021 (Pubmed ID 33473120) and since main findings by the authors were posted on the BioRxiv website before the publication of the Nat Comms paper the study is covered by our preprint scooping protection policy. Therefore, the Nature Communications paper was not included in my revisions.

The manuscript is well written. The topic covered is interesting and timely. The study provides useful insight into the mechanism comprising attachment of the ODA to the doublet microtubule and the remodeling and activation of the ODA complex. I think it would be of the interest of the readers of the EMBO journal.

My main concern is regarding the MD simulations, which are actually coarse-grained MD simulations not all-atom MD simulations.

Since coarse-grained MD simulations lack atomic details, they have limitations on what conclusions can be drawn for them. This, on the other hand, further depends on how these coarse-grained simulations were performed. In my opinion, in the current state of the coarse-grained MD section of the manuscript, there are not enough details provided for me to perform an in-depth evaluation.

Furthermore, in half of the coarse-grained MD simulations external forces were applied. In other word, steered molecular dynamics simulations were performed. Details regarding these simulations are not mentioned at all.

However, my main issue is that the attractive force between Shulin and other proteins was decreased 0.3 times the default in order to observe shulin detachment in short time. This is a major bias and was not indicated in the main text while presenting and discussing the data. This is described only in a single sentence in the Materials and Methods section. This should be clearly stated in the main text and figure 5. The authors did not observe Shulin detachment in coarse-grained MD simulations with default parameters. They observed Shulin detachment by significantly weakening Shulin's interaction with the proteins. This needs to be clearly stated and discussion should clearly indicate under which conditions detachment was observed.

Despite my critics above, I think that the coarse grained MD simulations still provide useful data for this study. They suggest that Shulin binding is weakened upon applying external force. This finding is complimentary to their experimental findings.

I have to emphasize that the coarse-grained MD simulations only comprise a small part of the study. If the coarse-grained MD simulations performed in this study would have performed as all-atom MD simulations, then this would require an extensive amount of computational resources. It would probably not be feasible to ask for all-atom MD simulations at this stage. Furthermore, there is extensive experimental data in the study. As long as the reader is made

aware of the limitations and what was performed in detail, I believe it shouldn't be a problem.

The authors may consider including coarse-grained MD details in the supplementary data if they feel that it will affect the conciseness of their manuscript.

Taken all together, it is my opinion that this study can be published after performing the revisions provided below. Therefore, my recommendation will be minor revision.

SPECIFIC MAJOR CONCERNS ESSENTIAL TO BE ADDRESSED TO SUPPORT THE CONCLUSIONS

The authors perform coarse-grained MD simulations. Each amino acid is represented by a single bead. Whether implicit solvent is included in the simulations is not indicated. Thus, I have to conclude that solvent effect was not included. This would affect the results significantly.
Since coarse-grained MD simulations do not comprise any atomic detail, the way they are performed and to what extend a conclusion can be drawn should be treated very carefully. Dyneins have a very complex machinery. The machinery correlates atomic events to global molecular motions. To what extend coarse-grained MD simulations can capture them is debatable. Yet, we know for fact that due to the missing atomic detail they cannot catch all of them. However, coarse-grained MD simulations have the advantage over all-atom MD simulations that they are computationally much less expensive thus allowing for longer MD simulation lengths. However, this study does not make use of them. In the study it is indicated that 5x10^7 MD steps were performed for each of the 10 simulations. This is not a large number. Even all-atom MD studies are able to go easily several orders more steps than this.

The MD simulations section lacks much detail and should be extensively revised. For example

"The force field for observing dynamics used AICG2+ (Li, W., et al., 2012; Li, W. et al., 2014)."

It seems there is something missing in this sentence. AICG2+ is a coarse-grained model. Why does it fit this problem and in which related applications were it successfully applied?

"In the AICG2+, the reference structure was assumed as the most stable conformation, and their parameters are defined from the reference of all-atom structures."

Which parameters do the authors refer to?

"Here, by comparing the inactive Shulin-ODA model with the active ODA structure on the doublet, we identified chains that keep their binding and chains which change their binding schemes"

How did the authors do this?

"Based on this information, we adjusted the parameters that determine the attraction between the chains. First, since Dyh3, 4, and 5 clearly have a different contact style, we set the

attraction force between these chains to be 0.5 times of the default. Next, since the contacts between Dyh3, Dic2, and the LC tower were not significantly changed, we increased the attraction by a factor of 10 in order to treat this chain as a rigid body"

What is the attraction factor? Most of the readers won't know it. There should be a description of the coarse grained model provided and which parameters are changed in this model. Even the main equations of the coarse-grained model could be provided.

"Lastly, the attractive force between Shulin and others was decreased by 0.3 times the default in order to observe the Shulin detachment in a reasonable simulation time"

What is a reasonable simulation time? This is a bit vague statement. These type of parameters are going to strongly affect the outcome and observation of the coarse-grained MD simulations. Why they are selected should be rigorously stated. Furthermore, this bias in the simulations should be clearly indicated in the main text (Docking of the ODA complex to the DC induces the remodeling) and figure 5.

What is the time step used? How many fs, ps, or ns?

In the discussion section in the following sentence

"Based on our structure and MD simulation, we present a model of how the ODA attachment induces remodeling and activation of the ODA complex (Fig. 6)."

It should be clearly stated that these are coarse-grained MD simulations with weakened Shulin interaction.

MINOR CONCERNS THAT SHOULD BE ADDRESSED

1) In the abstract it is written that

"Combined with molecular dynamics simulations, we present a model of how the attachment of the ODA to the doublet microtubule induces remodeling and activation of the ODA complex."

Here it should be clearly indicated that those MD simulations are coarse-grained molecular dynamics simulations.

2) Same issue exists in the in the final sentence of the introduction

"Combined with molecular dynamics (MD) simulations, we have revealed how the ODA complex undergoes an activating rearrangement when it is docked onto the doublet microtubule."

It should read combined with "coarse-grained molecular dynamics (MD) simulations"

3) Similarly in the "Docking of the ODA complex to the DC induces the remodeling" section it is written "To test if the remodeling of the Dyh3 HC causes the detachment of Shulin, we

performed a MD simulation.".
Here it should read "coarse-grained MD simulations"

4) In the figures Shuilin is neither tagged nor its color is indicated.

5) It is also a bit unusual to have MD steps instead of simulation time. I would strongly suggest the reader to change it to time.

6) In the section "Docking complex modelling" usage of BLASTP, MODELLER and Coot is indicated. The authors may prefer to include a bit more regarding the search and modelling parameters used.

7) The authors wrote "When we blasted CCDC151, we found Q22T00 as a homolog". This sounds a bit odd. Maybe something like "Using BLAST, Q22T00 was as identified as a homolog for CCDC151". Again as indicated in the previous comment, the parameters used in the BLAST search would be beneficial for the readers.

8) Similarly, in the MD simulations sections "The inactive Shulin-ODA structure had some missing residues. For MD simulation, we modelled loops for missing regions by MODELLER (Šali, A. and Blundell., T. L., 1993)." Which PDB structure did MODELLER use as a template? As a reader, I would be interested in this information.

9) Considering that there are only a couple of all-atom MD studies on dynein and a limited number of coarse-grained MD studies, it is my opinion that the presence should at least be acknowledged.

The reader should know that all-atom MD and coarse-grained MD are performed in the literature successfully for dyneins. A sentence or maybe two summarizing MD studies in the literature would suffice.

Here are a couple of all-atom MD studies

Can, S., Lacey, S., Gur, M., Carter, A. P., and Yildiz, A. (2019). Directionality of dynein is controlled by the angle and length of its stalk. Nature, 566(7744), 407-410.
Kamiya, N., Mashimo, T., Takano, Y., Kon, T., Kurisu, G., and Nakamura, H. (2016). Elastic properties of dynein motor domain obtained from all-atom molecular dynamics simulations. Protein Engineering, Design and Selection, 29(8), 317-325.

Here are a few coarse-grained MD studies

Dutta, M., and Jana, B. (2019). Role of AAA3 Domain in Allosteric Communication of Dynein Motor Proteins. ACS omega, 4(26), 21921-21930.
Dutta, M., and Jana, B. (2021). Computational modeling of dynein motor proteins at work. Chemical Communications.
Goldtzvik, Y., Mugnai, M. L., & Thirumalai, D. (2018). Dynamics of allosteric transitions in dynein. Structure, 26(12), 1664-1677. e1665.
Kubo, S., Li, W., and Takada, S. (2017). Allosteric conformational change cascade in cytoplasmic dynein revealed by structure-based molecular simulations. PLoS computational biology, 13(9), e1005748. (This is already in the references so citing it again won't increase

the total number of references)
Wang, Q., Jana, B., Diehl, M. R., Cheung, M. S., Kolomeisky, A. B., and Onuchic, J. N. (2018). Molecular mechanisms of the interhead coordination by interhead tension in cytoplasmic dyneins. Proceedings of the National Academy of Sciences, 115(40), 10052-10057.
Zheng, W. (2012). Coarse-grained modeling of the structural states and transition underlying the powerstroke of dynein motor domain. The Journal of chemical physics, 136(15), 04B617.

Please note that these are suggestions. There are already 59 citations in the manuscript. Maybe the authors prefer only to cite the most recent ones. I leave it up to them.

Referee #2:

The authors seek to understand the interaction of ODAs and doublet microtubules using tetrahymena cilia extract. Using cryo-EM and modified non-helical single particle analysis, the authors obtained the structure of the ODA-doublet microtubule complex at a resolution between 5.5-7 A. In addition, the authors made a comparison to a recently published structure of ODA in the inactive form in complex with the protein shulin. The authors discussed how the structural change occurs upon engagement of doublet-microtubules within cilia, and further how shulin release, i.e. activation of ODA, may happen in the presence of force using MD simulation.

The structure of this paper has much lower resolution (5.5-7 A) compared to a recently published report by Alan Brown and colleagues (3.8 A), therefore the structure itself does not give much additional information as is. The structures resemble each other and the authors much descriptively explain the structures. There are slightly different observations, for example, the presence of LC3 in this paper, or the linker region for b-HC or Dyh4 connected to AAA5 in post power stroke reported by Alan Brown and colleagues, whereas in this paper, in tetrahymena, it was slightly leaning towards the AAA4. This could be due to the interaction of LC3.
The most interesting part of this paper is the comparison of the structure with the recently published inactive form of ODA(ODA-shulin) and molecular modelling to enable the discussion of how the conformational change of ODA occurs form the inactive to the active form. As the authors make extensive analysis of the structures, this paper makes a distinction from the report of Alan Brown.

Overall, it is an interesting story enough to consider because of the comparison of active/inactive form of ODA. For the structure itself, the quality is not as stellar as the one from Alan Brown, but it would be beneficial if the authors compare their structure with Alan Brown's more side-by-side, mention differences and discuss if that is due to the different biological source (Tetrahymena vs Chlamydomonas) or due to the resolution difference.

Scientific advisor's comments:

1. The authors do not do a very good job in citing the literature. They often omit the original papers and instead cite their own, much more recent publications. Just two examples:

- "Unlike cytoplasmic dyneins which walk on microtubules while carrying cargos, axonemal dyneins are anchored firmly on the doublet microtubules (Bui, K. H. et al., 2008; Bui, K. H. et al., 2009)." That axonemal dyneins are anchored firmly to the doublet is know from the '80, the authors should take the time to look for the original publications.

- "By cryo-electron tomography (cryo-ET) work, ODAs were shown to form a 24-nm repeating row on the doublet microtubules (Bui, K. H. et al., 2012; Lin J. and Nicastro, D. 2018)." Also in this case it is know from the '80 that ODA bind every 24 nm from standard EM images. Additionally, Nicastro published a paper in 2006 where she shows ODA in cryo-ET for the first time... it is strange that the authors cite themselves with a paper from 2012 and then Nicastro with a paper from 2018.

2. The statement: " However, the interactions among the ODA and DC complexes and the doublet have not been revealed in subnanometer resolution." is wrong. The sentence should be removed and the authors should cite the paper from Brown's lab (Pubmed ID 33473120).

3. About novelty, unluckily the high resolution structure of the ODA docked on the microtubule doublet is not novel anymore. I say unluckily, because this is a big part of the paper, it is technically very well done, and it must have been a considerable investment of time and efforts for the authors.
The only novel contribution is in the very last chapter of the manuscript results: "Docking of the ODA complex to the DC induces the remodeling". This aspect was only briefly mentioned at the end of the Discussion in the Brown's paper, but not addressed experimentally.

So, there is an aspect of novelty in the submitted manuscript. Unluckily, I am not an expert in molecular dynamics and it is difficult for me to provide rigorous technical evaluation of this part of the manuscript.

Rebuttal of the original manuscript's comments

First, we would like to thank the reviewers and editors for the constructive suggestions and criticisms. We appreciate all the comments and try to improve the manuscripts according to the suggestions. In this revision, here are the main things that we improve:

- Adding Supplementary figure 3 to compare between our model & PDB 7kzm (ODA model from Chlamydomonas by Brown lab)
- Adding more detail regarding methods for our coarse-grained molecular dynamics and other analyses.
- The deposited PDB, composite map & validation report are downloadable for the reviewers from https://www.dropbox.com/sh/suzzrtfwpaoo90f/AAAUIMYPY_XQaOdiRRlClpPPa?dl=0
-

Below is our rebuttal to the reviews.

>Referee #1:

>GENERAL SUMMARY AND OPINION ABOUT THE PRINCIPAL SIGNIFICANCE OF THE
>STUDY, ITS QUESTIONS AND FINDINGS:

>I was informed by EMBO that part of the submitted manuscript has been scooped by a
>paper published in Nature Communications on Jan 2021 (Pubmed ID 33473120) and since
>main findings by the authors were posted on the BioRxiv website before the publication of
>the Nat Comms paper the study is covered by our preprint scooping protection policy.
>Therefore, the Nature Communications paper was not included in my revisions.

>The manuscript is well written. The topic covered is interesting and timely. The study
>provides useful insight into the mechanism comprising attachment of the ODA to the doublet
>microtubule and the remodeling and activation of the ODA complex. I think it would be of the
>interest of the readers of the EMBO journal.

We appreciate that the referee #1 has agreed that our work has shown useful insight into the dynein activation mechanisms.

>My main concern is regarding the MD simulations, which are actually coarse-grained MD
>simulations not all-atom MD simulations.

>Since coarse-grained MD simulations lack atomic details, they have limitations on what
>conclusions can be drawn for them. This, on the other hand, further depends on how these
>coarse-grained simulations were performed. In my opinion, in the current state of the
>coarse-grained MD section of the manuscript, there are not enough details provided for me
>to perform an in-depth evaluation.

>Furthermore, in half of the coarse-grained MD simulations external forces were applied. In
>other word, steered molecular dynamics simulations were performed. Details regarding
>these simulations are not mentioned at all.

>However, my main issue is that the attractive force between Shulin and other proteins was >decreased 0.3 times the default in order to observe shulin detachment in short time. This is a >major bias and was not indicated in the main text while presenting and discussing the data. >This is described only in a single sentence in the Materials and Methods section. This should >be clearly stated in the main text and figure 5. The authors did not observe Shulin >detachment in coarse-grained MD simulations with default parameters. They observed >Shulin detachment by significantly weakening Shulin's interaction with the proteins. This >needs to be clearly stated and discussion should clearly indicate under which conditions >detachment was observed.

We apologize for insufficient details for the coarse-grained MD simulation. We have included more details for the coarse-grained MD simulation in the main text as well as Materials and Methods section, especially about the external forces applied. Sentences describing the weakened interaction of Shulin were also added so that readers can clearly understand the condition. We believe that there is enough information for readers to evaluate our coarse-grained MD simulation.

>Despite my critics above, I think that the coarse grained MD simulations still provide useful >data for this study. They suggest that Shulin binding is weakened upon applying external >force. This finding is complementary to their experimental findings.

We appreciate that referee #1 understands the importance of our study.

>I have to emphasize that the coarse-grained MD simulations only comprise a small part of >the study. If the coarse-grained MD simulations performed in this study would have >performed as all-atom MD simulations, then this would require an extensive amount of >computational resources. It would probably not be feasible to ask for all-atom MD >simulations at this stage. Furthermore, there is extensive experimental data in the study. As >long as the reader is made aware of the limitations and what was performed in detail, I >believe it shouldn't be a problem.

We appreciate that the referee #1 agrees that our coarse-grained MD simulation is still meaningful for this project. More details of our coarse-grained MD simulation is incorporated into the manuscript so that the readers can understand what kind of conclusions can be drawn as well as the limitations of our method.

>The authors may consider including coarse-grained MD details in the supplementary data if >they feel that it will affect the conciseness of their manuscript.

We think that our coarse-grained MD simulation is important for our model of activation of the ODA complex, and therefore, we wish to keep these results in the main figure. We have put details of our coarse-grained MD simulation like equations in Supplementary text.

>Taken all together, it is my opinion that this study can be published after performing the >revisions provided below. Therefore, my recommendation will be a minor revision.

We appreciate the positive response of the referee and we believe that our revised manuscript is suitable for the publication now.

SPECIFIC MAJOR CONCERNS ESSENTIAL TO BE ADDRESSED TO SUPPORT THE CONCLUSIONS

>The authors perform coarse-grained MD simulations. Each amino acid is represented by a >single bead. Whether implicit solvent is included in the simulations is not indicated. Thus, I >have to conclude that solvent effect was not included. This would affect the results >significantly.

The effect of water solvent is treated implicitly via Langevin dynamics. This point is also mentioned in the Materials and Method section.

>Since coarse-grained MD simulations do not comprise any atomic detail, the way they are >performed and to what extend a conclusion can be drawn should be treated very carefully. >Dyneins have a very complex machinery. The machinery correlates atomic events to global >molecular motions. To what extend coarse-grained MD simulations can capture them is >debatable. Yet, we know for fact that due to the missing atomic detail they cannot catch all of >them. However, coarse-grained MD simulations have the advantage over all-atom MD >simulations that they are computationally much less expensive thus allowing for longer MD >simulation lengths. However, this study does not make use of them. In the study it is >indicated that 5x10^7 MD steps were performed for each of the 10 simulations. This is not a >large number. Even all-atom MD studies are able to go easily several orders more steps >than this.

In the previous MD simulation using AICG2+, the allosteric conformational change of dynein was simulated with 10^7 MD steps and it succeeded to observe their mechanism (Kubo et al., PLoS Comp 2017), therefore, we believe that the 5x10^7 MD steps are enough to simulate the dynein conformational change. This point was included in the manuscript so that the reason we chose this time scale would be clearer.

>The MD simulations section lacks much detail and should be extensively revised.
>For example

>"The force field for observing dynamics used AICG2+ (Li, W., et al., 2012; Li, W. et al., >2014)."

>It seems there is something missing in this sentence. AICG2+ is a coarse-grained model. >Why does it fit this problem and in which related applications were it successfully applied?

We have fixed the sentence. Here, we do not try to obtain the detailed information of the residues. Rather, we want to gain insights into the relationship between global conformational change and detachment of Shulin. These points were also included in the main text.

>"In the AICG2+, the reference structure was assumed as the most stable conformation, and >their parameters are defined from the reference of all-atom structures."

>Which parameters do the authors refer to?

For example, native bond, native angle, native dihd, native contact. Here is the equation.

$$V_{AICG2+}(R|R_0) = \sum_i K_{b,i}\left(b_i - b_{i,0}\right)^2 + V_{loc}^{flp}$$
$$+ \sum_{j=i+2} \varepsilon_{loc,ij}\, exp\left(-\frac{\left(r_{ij} - r_{ij0}\right)^2}{2W_{ij}^2}\right)$$
$$+ \sum_{j=i+3} \varepsilon_{loc,ij}\, exp\left(-\frac{\left(\phi_{ij} - \phi_{ij0}\right)^2}{2W_{\phi,ij}^2}\right)$$
$$+ \sum_{i<j-3}^{nat\ contact} \varepsilon_{go,ij}\left[5\left(\frac{r_{ij0}}{r_{ij}}\right)^{12} - 6\left(\frac{r_{ij0}}{r_{ij}}\right)^{10}\right] + \sum_{i<j-3}^{non-native} \varepsilon_{ev}\left(\frac{d}{r_{ij}}\right)^{12}$$

Each term represents the elasticity of the virtual bond, the sequence-dependent angle- and dihedral-angle potential, the structure-based local potential between i-th and i+2-th residues, the structure-based local potential for dihedral angles, the Go potential for non-local natively interacting pairs, and the generic repulsion for the rest of the non-local pairs. The vector R represents the $3n_{aa}$-dimensional Cartesian coordinates of the simulated protein where n_{aa} is the number of the protein amino acids. R_0 is the corresponding coordinate in the reference structure. All variables with the subscript 0 refer to parameters defined by the reference structure (initial structure).

b_i: bond length beween i-th and i+1-th residues.
V_{loc}^{flp}: (Terakawa et al., Biophys J 2011)
r_{ij}: distance between i-th and j-th residues.
ϕ_{ij}: dihedral angle defined as i-th, i+1-th, i+2-th, and i+3-th residues.
$W_{ij}^2, W_{\phi,ij}^2$: widths of the attractive interaction
$K_{b,i}, \varepsilon_{loc,ij}, \varepsilon_{go,ij}$: determined by AMBER force field
ε_{ev}, d: determined from a structural servay.

The default values of these parameters in CafeMol manual (http://www.cafemol.org).

We have included these equations and parameters in the supplementary text. We hope that the referee finds the information sufficient.

>"Here, by comparing the inactive Shulin-ODA model with the active ODA structure on the >doublet, we identified chains that keep their binding and chains which change their binding >schemes"

>How did the authors do this?

Here, we compared the structures of Shulin-ODA and our active ODA complex and checked which interactions are kept after the conformational change by visual inspection. We have added the sentences describing this point.

>"Based on this information, we adjusted the parameters that determine the attraction >between the chains. First, since Dyh3, 4, and 5 clearly have a different contact style, we set >the attraction force between these chains to be 0.5 times of the default. Next, since the >contacts between Dyh3, Dic2, and the LC tower were not significantly changed, we >increased the attraction by a factor of 10 in order to treat this chain as a rigid body"

>What is the attraction factor? Most of the readers won't know it. There should be a >description of the coarse grained model provided and which parameters are changed in this >model. Even the main equations of the coarse-grained model could be provided.

We have included the main equation in Supplementary text and cited it. The attraction factor that we mentioned here is “The Go potential for non-local natively interacting pairs” in the AICG2+ equation. The Go potential is already well estimated for the intra-chain interactions, but it needs to be edited for the inter-chain interactions, so we changed these parameters. This point was also included in the Supplementary text.

>"Lastly, the attractive force between Shulin and others was decreased by 0.3 times the >default in order to observe the Shulin detachment in a reasonable simulation time"

>What is a reasonable simulation time? This is a bit vague statement. These type of >parameters are going to strongly affect the outcome and observation of the coarse-grained >MD simulations. Why they are selected should be rigorously stated. Furthermore, this bias in >the simulations should be clearly indicated in the main text (Docking of the ODA complex to >the DC induces the remodeling) and figure 5.

Here, we aimed to see if the conformational change of ODA complex induces the Shulin detachment and we are not trying to obtain insights into the time scale of the detachment. With these simulation times, we were able to see the difference in Shulin detachment rate with and without applied force. We revised our sentences carefully so that our intentions would be clearer for the readers.

>What is the time step used? How many fs, ps, or ns?

One MD step roughly corresponds to ~1 ps. Thus, each trajectory corresponds to approximately 50 μs, but the correspondence with the real time is weak. In addition, the event might not really occur in this time scale since the external force values are only

parameters, which are not obtained from the experiment. However, it is still possible to confirm that the existence of the external force promotes the dissociation itself. These points are now included in the manuscript.

>In the discussion section in the following sentence
>"Based on our structure and MD simulation, we present a model of how the ODA attachment
>induces remodeling and activation of the ODA complex (Fig. 6)."

>It should be clearly stated that these are coarse-grained MD simulations with weakened
>Shulin interaction.

We mentioned these points so that the readers can evaluate our condition clearly.

MINOR CONCERNS THAT SHOULD BE ADDRESSED

>1) In the abstract it is written that

>"Combined with molecular dynamics simulations, we present a model of how the attachment
>of the ODA to the doublet microtubule induces remodeling and activation of the ODA
>complex."

>Here it should be clearly indicated that those MD simulations are coarse-grained molecular
>dynamics simulations.

The expression in the abstract was changed accordingly.

>2) Same issue exists in the in the final sentence of the introduction

>"Combined with molecular dynamics (MD) simulations, we have revealed how the ODA
>complex undergoes an activating rearrangement when it is docked onto the doublet
>microtubule."

>It should read combined with "coarse-grained molecular dynamics (MD) simulations"

We changed the expression to show clearly that it is coarse-grained molecular dynamis (MD) simulations.

>3) Similarly in the "Docking of the ODA complex to the DC induces the remodeling" section it
>is written "To test if the remodeling of the Dyh3 HC causes the detachment of Shulin, we
>performed a MD simulation.".
>Here it should read "coarse-grained MD simulations"

The expression was fixed.

>4) In the figures Shuilin is neither tagged nor its color is indicated.

We have labeled Shulin in the figure and mention that Shulin is shown green in the figure legend.

>5) It is also a bit unusual to have MD steps instead of simulation time. I would strongly >suggest the reader to change it to time.

We included the sentence about the relationship between MD steps and the time in the main text. However, it is a rough correlation and showing them as time might cause the misunderstanding. Therefore, we wish to keep the label of the figure as MD steps. We mentioned the information about simulation time in the figure legend instead.

6) In the section "Docking complex modelling" usage of BLASTP, MODELLER and Coot is indicated. The authors may prefer to include a bit more regarding the search and modelling parameters used.

We have incorporated the details of parameters for these parts.

7) The authors wrote "When we blasted CCDC151, we found Q22T00 as a homolog". This sounds a bit odd. Maybe something like "Using BLAST, Q22T00 was as identified as a homolog for CCDC151". Again as indicated in the previous comment, the parameters used in the BLAST search would be beneficial for the readers.

We have modified the sentences so that it would be clearer for the readers.

8) Similarly, in the MD simulations sections "The inactive Shulin-ODA structure had some missing residues. For MD simulation, we modelled loops for missing regions by MODELLER (Šali, A. and Blundell., T. L., 1993)." Which PDB structure did MODELLER use as a template? As a reader, I would be interested in this information.

We have used the PDB 6ZYW structure for the template and this information was incorporated into the manuscript.

>9) Considering that there are only a couple of all-atom MD studies on dynein and a limited >number of coarse-grained MD studies, it is my opinion that the presence should at least be >acknowledged.

>The reader should know that all-atom MD and coarse-grained MD are performed in the >literature successfully for dyneins. A sentence or maybe two summarizing MD studies in the >literature would suffice.

>Here are a couple of all-atom MD studies

>Can, S., Lacey, S., Gur, M., Carter, A. P., and Yildiz, A. (2019). Directionality of dynein is >controlled by the angle and length of its stalk. Nature, 566(7744), 407-410.
>Kamiya, N., Mashimo, T., Takano, Y., Kon, T., Kurisu, G., and Nakamura, H. (2016). Elastic >properties of dynein motor domain obtained from all-atom molecular dynamics simulations.

>Protein Engineering, Design and Selection, 29(8), 317-325.

>Here are a few coarse-grained MD studies

>Dutta, M., and Jana, B. (2019). Role of AAA3 Domain in Allosteric Communication of Dynein >Motor Proteins. ACS omega, 4(26), 21921-21930.
>Dutta, M., and Jana, B. (2021). Computational modeling of dynein motor proteins at work. >Chemical Communications.
>Goldtzvik, Y., Mugnai, M. L., & Thirumalai, D. (2018). Dynamics of allosteric transitions in >dynein. Structure, 26(12), 1664-1677. e1665.
>Kubo, S., Li, W., and Takada, S. (2017). Allosteric conformational change cascade in >cytoplasmic dynein revealed by structure-based molecular simulations. PLoS computational >biology, 13(9), e1005748. (This is already in the references so citing it again won't increase >the total number of references)
>Wang, Q., Jana, B., Diehl, M. R., Cheung, M. S., Kolomeisky, A. B., and Onuchic, J. N. >(2018). Molecular mechanisms of the interhead coordination by interhead tension in >cytoplasmic dyneins. Proceedings of the National Academy of Sciences, 115(40), >10052-10057.
>Zheng, W. (2012). Coarse-grained modeling of the structural states and transition underlying >the powerstroke of dynein motor domain. The Journal of chemical physics, 136(15), 04B617.

>Please note that these are suggestions. There are already 59 citations in the manuscript. >Maybe the authors prefer only to cite the most recent ones. I leave it up to them.

We appreciate the reviewer's valuable suggestion. We have cited several papers utilizing MD simulation for dynein molecules.

Referee #2:

The authors seek to understand the interaction of ODAs and doublet microtubules using tetrahymena cilia extract. Using cryo-EM and modified non-helical single particle analysis, the authors obtained the structure of the ODA-doublet microtubule complex at a resolution between 5.5-7 A. In addition, the authors made a comparison to a recently published structure of ODA in the inactive form in complex with the protein shulin. The authors discussed how the structural change occurs upon engagement of doublet-microtubules within cilia, and further how shulin release, i.e. activation of ODA, may happen in the presence of force using MD simulation.

>The structure of this paper has much lower resolution (5.5-7 A) compared to a recently >published report by Alan Brown and colleagues (3.8 A), therefore the structure itself does >not give much additional information as is. The structures resemble each other and the >authors much descriptively explain the structures. There are slightly different observations, >for example, the presence of LC3 in this paper, or the linker region for b-HC or Dyh4 >connected to AAA5 in post power stroke reported by Alan Brown and colleagues, whereas in >this paper, in tetrahymena, it was slightly leaning towards the AAA4. This could be due to the >interaction of LC3.
>The most interesting part of this paper is the comparison of the structure with the recently >published inactive form of ODA(ODA-shulin) and molecular modelling to enable the >discussion of how the conformational change of ODA occurs form the inactive to the active >form. As the authors make extensive analysis of the structures, this paper makes a >distinction from the report of Alan Brown.

We appreciate that the reviewer understands the novelty of our report.

>Overall, it is an interesting story enough to consider because of the comparison of >active/inactive form of ODA. For the structure itself, the quality is not as stellar as the one >from Alan Brown, but it would be beneficial if the authors compare their structure with Alan >Brown's more side-by-side, mention differences and discuss if that is due to the different >biological source (Tetrahymena vs Chlamydomonas) or due to the resolution difference.

We appreciate that the referee #2 understands the different focuses of our work and Alan Brown lab's. Now we have cited Brown lab's recent Nature Communication paper and discussed the similarity and difference of the two structures with a new supplementary figure (Fig. S3).

Scientific advisor's comments:

1. The authors do not do a very good job in citing the literature. They often omit the original papers and instead cite their own, much more recent publications. Just two examples:

- "Unlike cytoplasmic dyneins which walk on microtubules while carrying cargos, axonemal dyneins are anchored firmly on the doublet microtubules (Bui, K. H. et al., 2008; Bui, K. H. et al., 2009)." That axonemal dyneins are anchored firmly to the doublet is know from the '80, the authors should take the time to look for the original publications.

- "By cryo-electron tomography (cryo-ET) work, ODAs were shown to form a 24-nm repeating row on the doublet microtubules (Bui, K. H. et al., 2012; Lin J. and Nicastro, D. 2018)." Also in this case it is know from the '80 that ODA bind every 24 nm from standard EM images. Additionally, Nicastro published a paper in 2006 where she shows ODA in cryo-ET for the first time... it is strange that the authors cite themselves with a paper from 2012 and then Nicastro with a paper from 2018.

We apologize for the poor choices for the literature. We have now cited more traditional papers for these points.

>2. The statement: " However, the interactions among the ODA and DC complexes and the >doublet have not been revealed in subnanometer resolution." is wrong. The sentence should >be removed and the authors should cite the paper from Brown's lab (Pubmed ID 33473120).

The sentence is removed and we have cited Brown lab's work and discussed the differences. We have included a supplementary figure (Fig. S3) for the comparison.

>3. About novelty, unluckily the high resolution structure of the ODA docked on the >microtubule doublet is not novel anymore. I say unluckily, because this is a big part of the >paper, it is technically very well done, and it must have been a considerable investment of >time and efforts for the authors.
>The only novel contribution is in the very last chapter of the manuscript results:
>"Docking of the ODA complex to the DC induces the remodeling". This aspect was only >briefly mentioned at the end of the Discussion in the Brown's paper, but not addressed >experimentally.

>So, there is an aspect of novelty in the submitted manuscript. Unluckily, I am not an expert in >molecular dynamics and it is difficult for me to provide rigorous technical evaluation of this >part of the manuscript.

We appreciate that the Scientific advisor finds our work meaningful in the sense that we have shown the activation mechanisms of the dynein.
Since referee #1 was the specialist of the molecular dynamics, we have fixed the manuscript according to referee #1's comments. We believe that the change we have made improved our manuscript in the part of MD simulation.

Apart from referees' comments, there was a mistake in the label of the X-axis of Fig. 4E and F, so the values were corrected. In the previous version, it was labeled as 10^5 MD steps, but it was actually 10^4 MD steps. The mistakes of the values were corrected now.

1st Revision - Editorial Decision 2nd Jun 2021

Dear Huy,

Thank you for submitting your revised manuscript. It has now been seen by one of the original referees.

As you can see, the referee finds that the study is significantly improved during revision and recommends publication. However, I need you to address the editorial points below before I can accept the manuscript.

• Please address the remaining minor concern of referee #1.
• As previously discussed, please add links that resolve to the Cryo-EM map and corresponding PDB datasets listed in the Data Availability section. Also, please deposit the mass spec dataset generated in this study to a public database, and provide its link in the Data Available section, too.
• As per our format requirements, in the reference list, citations should be listed in alphabetical order and then chronologically, with the authors' surnames and initials inverted; where there are more than 10 authors on a paper, 10 will be listed, followed by 'et al.'. We note that the reference list currently numerical. Please see https://www.embopress.org/page/journal/14693178/authorguide#referencesformat
• We note the following regarding the figure callouts:
o There is a callout to Fig EV3H, which doesn't exist.
o Fig EV4B-K callouts are missing.
o Fig EV5C callout is missing.
o Appendix Fig S1 panels need calling out.
o There is a callout to Figure S3, which doesn't exist.
• We note that there is a movie legend in the manuscript file, but the movie file is missing. Please provide the movie file ZIPped with its legend, and remove the movie legend from the manuscript text.
• We note that the source data is in ALN format, which we cannot open. Please provide it in a different format if possible.
• We notice that Fig EV4J panel is missing, jumps from I to K.
• We note that there is a supplementary table in the Manuscript file. This could be added to the Appendix.
• Please consider combining the supplementary references and the main references, as there are no length restrictions with the references.
• Papers published in EMBO Reports include a 'synopsis' and 'bullet points' to further enhance discoverability. Both are displayed on the html version of the paper and are freely accessible to all readers. The synopsis includes a short standfirst summarizing the study in 1 or 2 sentences that summarize the paper and are provided by the authors and streamlined by the handling editor. I would therefore ask you to include your synopsis blurb and 3-5 bullet points listing the key experimental findings.
• In addition, please provide an image for the synopsis. This image should provide a rapid overview of the question addressed in the study but still needs to be kept fairly modest since the image size cannot exceed 550x400 pixels.
• Our production/data editors have asked you to clarify several points in the figure legends (see attached document). Please incorporate these changes in the attached word document and return it with track changes activated.

Thank you again for giving us to consider your manuscript for EMBO Reports, I look forward to your minor revision.

Kind regards,

Deniz

--
Deniz Senyilmaz Tiebe, PhD
Editor
EMBO Reports

Referee #1:

The authors addressed most of the issues and critics.

The most recent all-atom MD simulations study was not cited in the revised manuscript. I think it should be cited. Please find the reference below

Can, S., Lacey, S., Gur, M., Carter, A. P., and Yildiz, A. (2019). Directionality of dynein is controlled by the angle and length of its stalk. Nature, 566(7744), 407-410.

The authors have addressed all minor editorial requests.

2nd Revision - Editorial Decision 30th Jun 2021

Dear Huy,

Thank you for submitting your revised manuscript. I have now looked at everything and all is fine. Therefore, I am very pleased to accept your manuscript for publication in EMBO Reports.

Congratulations on a nice work!

Kind regards,

Deniz
--
Deniz Senyilmaz Tiebe, PhD
Editor
EMBO Reports

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Corresponding Author Name: Khanh Huy Bui, Muneyoshi Ichikawa
Journal Submitted to: EMBO Reports
Manuscript Number: EMBOR-2021-52911V2

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- ➔ the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- ➔ figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- ➔ graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- ➔ if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be iustified
- ➔ Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- ➔ a specification of the experimental system investigated (eg cell line, species name).
- ➔ the assay(s) and method(s) used to carry out the reported observations and measurements
- ➔ an explicit mention of the biological and chemical entity(ies) that are being measured.
- ➔ an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- ➔ the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- ➔ a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- ➔ a statement of how many times the experiment shown was independently replicated in the laboratory.
- ➔ definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	Sample-size calculation or pre-specification of sample size were not performed in our experimental design.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	N/A
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	No samples were excluded from our analysis.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	N/A
For animal studies, include a statement about randomization even if no randomization was used.	N/A
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	N/A
4.b. For animal studies, include a statement about blinding even if no blinding was done	N/A
5. For every figure, are statistical tests justified as appropriate?	No statistical tests were performed in this study.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	No statistical tests were performed in this study.
Is there an estimate of variation within each group of data?	N/A

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Is the variance similar between the groups that are being statistically compared?	N/A

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	N/A
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	N/A

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D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	N/A
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	N/A
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	N/A

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	N/A
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	N/A
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	N/A
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	N/A
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	N/A
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	N/A
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	N/A

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	Yes
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	Yes. The data produced in this study are available in the following databases: ・Cryo-EM map: EMDB EMD-23926 (https://www.emdataresource.org /EMD-23926) ・Model coordinates: PDB 7MOQ (https://www.rcsb.org/structure/7MOQ) ・Mass spectrometry data: DataDryad: doi:10.5061/dryad.p2ngf1vqv (https://doi.org/10.5061/dryad .p2ngf1vqv)
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	N/A
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	N/A

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	N/A