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Strain through the neck linker ensures processive runs: a DNA-kinesin hybrid-nanomachine study

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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 04 March 2009

Thank you for submitting your manuscript to the EMBOJ. Your study has now been seen by three experts in the field and their comments to the authors are provided below. As you can see, while referees #3 is not persuaded that the advance and insight provided is sufficient to consider publication in the EMBO Journal, referees #1 and 2 are more supportive of the analysis. However, referee #2 also raises significant concerns with the presentation of the findings and finds that a present it is difficult to fully evaluate the work and that a much better layout and explanation of the constructs used is needed for a full and proper evaluation. Given the support provided by both referees #1 and 2, we are willing to consider a revised manuscript. Referee #1 suggests the inclusion of an additional experiment and I would like you to address and incorporate this suggestion in the revised version. It will also be important to work on the presentation of the findings, along the lines suggested by referee #2, in order to make the findings more accessible to both experts in the field and a broader audience of molecular biologists. I should remind you that it is EMBO Journal policy to allow a single round of revision only and that it is therefore important to address the concerns raised at this stage.

Thank you for the opportunity to consider your work for publication. I look forward to your revision.

Yours sincerely,

Editor
The EMBO Journal

REFEREE REPORTS

Referee #1 (Remarks to the Author):

This paper reports a very clever new method to make processive motors by joining two expressed motor domains to DNA which can then be hybridized together. Remarkably, this works, although the motors are not as processive as wildtype kinesin and a bit slower. However, this might prove to be a very valuable method and it is certainly one of the more clever examples of protein engineering in the motor protein field. I think that their method alone merits publication in the EMBO J. However, they also use this DNA hybridization method to join the motor together in different configurations. The results from this work has allowed them to test the role of the neck linker in processive motility, which nicely demonstrate its plays an important role in biasing unidirectional motion. Overall, this is an interesting paper that should be published in your journal.

The one experiment that might useful to add is a "heterodimer" with an asymmetric kinesin. This seems to be a powerful use of this technology (e.g. making a motor domain with a mutation (e.g. a longer or shorter neck linker or a mutation that affects the ATPase) and another motor that is wild type. It might be nice to feature this in this paper and would not be very hard to do (this is the beauty of the DNA hybridization method), but I would leave the decision to the editors/authors.

Referee #2 (Remarks to the Author):

This is a potentially highly interesting paper using a novel approach to investigate the important issue of how kinesin motor domains in a dimer are able to coordinate their movement to generate processive movement. However, the presentation is not adequate to fully evaluate the work. The Results section is difficult to understand because it starts directly talking about results without clearly indicating which of the many constructs that are described is being use. Presumably the results of Fig. 1 and 2 are on a particular construct, but cannot unambiguously tell from the text or legend what construct is and how it is attached, especially as later figures in the supplement report on a number of different attachment points and linkers, etc. What is needed is a clear statement at the start of the results and throughout on just what construct is being used for each experiment. This should include a table or figure listing the constructs and giving a schematic of their key features including the point of attachment and the nature of the linker/coupling in each case. Also the results are presented in the text for many constructs and conditions and properties, but it is difficult to systematically organize them for comparison. A table(s) is needed that presents the constructs and their properties so that they can be more easily compared and so it can be more easily seen just what experiments were done with what range of constructs.

Note that the estimate of Rice et al for the free energy of ATP induced neck linker docking of 1-2 kT quoted on page 4 is for AMP-PNP that is likely a poor energetic analog of ATP. The true value for ATP itself may be as high as 10-14 kT (PNAS 102, 18338 (2005) and may in fact provide significant driving force.

Referee #3 (Remarks to the Author):

In this paper the authors assess the movement of pairs of kinesin monomers linked together by DNA rods. The kinesin molecules are the same in each case but the points at which the DNA is connected are varied, as is the separation of the heads due to the arrangement and length of the DNA. The results reaffirm the importance of the complete neck linker and its interaction with the motor domain for directed processive movement. Nothing that was tried made the motor work better than wild-type kinesin. I assume that the original motive for introducing a long stiff leverarm was to try to make kinesin more like myosin. The failure of this addition to produce a faster more efficient motor is put down to the effects of 'internal strain', whatever that means.
The lack of any really new insight into kinesin's mechanism makes me feel that the paper is unsuitable for a general interest journal like EMBO J. The combined use of protein and DNA is novel and potentially useful but should be demonstrated by making a motor with novel properties.

1st Revision - authors' response 01 April 2009

Referee 1's comment
The one experiment that might useful to add is a "heterodimer" with an asymmetric kinesin. This seems to be a powerful use of this technology (e.g. making a motor domain with a mutation (e.g. a longer or shorter neck linker or a mutation that affects the ATPase) and another motor that is wild type. It might be nice to feature this in this paper and would not be very hard to do (this is the beauty of the DNA hybridization method), but I would leave the decision to the editors/authors.

Our response
First of all, we greatly appreciate for your comment. As suggested, our conclusions would be strengthened if we examined the properties of a heterodimer. Thus, we added the results of heterodimer connected at "Mid" and "End of neck linker", which is impossible to create by protein-only construct, to the discussion section. We found that the neck linker's dual function by both heads is crucial for efficient movement. With our method, we could control the hetero dimeris layout (e.g. distance) and the number of molecules. Thus, our approach can also be a powerful tool to study the mechanism for multi-component coordination systems (e.g. Dynein-kinesin or NCD-kinesin).

Referee 2's comment
The presentation is not adequate to fully evaluate the work. What is needed is a clear statement at the start of the results and throughout on just what construct is being used for each experiment. This should include a table or figure listing the constructs and giving a schematic of their key features including the point of attachment and the nature of the linker/coupling in each case. Also the results are presented in the text for many constructs and conditions and properties, but it is difficult to systematically organize them for comparison. A table(s) is needed that presents the constructs and their properties so that they can be more easily compared and so it can be more easily seen just what experiments were done with what range of constructs.

Our response
We thank very much for your suggestion. We improved the beginning of the results section and figure legends. Furthermore we added a table to summarize the feature of the constructs. We hope that now it is easier for the reader to understand our work.

Referee 2's comment
Note that the estimate of Rice et al for the free energy of ATP induced neck linker docking of 1-2 kT quoted on page 4 is for AMP-PNP that is likely a poor energetic analog of ATP. The true value for ATP itself may be as high as 10-14 kT (PNAS 102, 18338 (2005) and may in fact provide significant driving force.

Our response
We greatly appreciate you for the suggestion of the reference. We add the sentence in the introduction and cited this article.

Referee 3's comment
The results reaffirm the importance of the complete neck linker and its interaction with the motor domain for directed processive movement. Nothing that was tried made the motor work better than wild-type kinesin. The lack of any really new insight into kinesin's mechanism makes me feel that the paper is unsuitable for a general interest journal like EMBO J. The combined use of protein and DNA is novel and potentially useful but should be demonstrated by making a motor with novel properties.

Our response
We thank very much for valuable comments and pointing out the potential of our method. As you suggested, we hope that we will be able to report another study of DNA-kinesin with novel properties in near future.

2nd Editorial Decision 24 April 2009

Thank you for submitting your revised manuscript to the EMBO Journal. I asked the original referee #2 to review the revised manuscript and I have now received the comments back from this referee. I did not ask referee #1 or referee #3 to review this version as they had few comments and were pretty much decided (positive and negative) during the initial round. As you can see below, referee #2 still has major issues with the presentation of the findings and the clarity of the text. Having read the revised manuscript, I agree with this opinion. The manuscript still needs a lot of work to make it more clear. The referee finds that the lack of clarity is severe enough that this referee no longer supports publication in The EMBO Journal. I have discussed the manuscript and the comments of referee #2 further with our executive editor. However, we do not feel that it would be fair to reject the manuscript at this point. But in order for us to consider publication of the study here in the EMBO Journal, a lot of work and effort has to be put into making the manuscript more clear. Lot of important information is found in the supplemental information, The EMBO journal publishes full-length articles and you have plenty of space left in the main text. The manuscript should be reorganized so that all the important information is found in the main body of the article. The clarity of the text also needs improvement. Perhaps a colleague can help you, as it can be helpful to have an outsider read and comment on the text. Finally you added an experiment to address a point brought up by referee #1, this results should be part of the result section and not in the discussion as it is at the moment.

Overall, we are willing to provide you with another chance to improve the clarity and presentation of the findings, but the manuscript really has to be significantly reorganized and improved in order for publication here.

I hope that you find these comments helpful and I thank you for the opportunity to consider your work for publication.

Yours sincerely,

Editor
The EMBO Journal

REFEREE REPORT

Referee #2 (Remarks to the Author):

This is a potentially interesting paper using a novel approach to investigate the important issue of how kinesin motor domains in a dimer are able to coordinate their movement to generate processive movement. The authors state as their principal premise that the role of neck linker docking is not well established and still controversial. The importance of neck linker docking however is now fairly well established. The types of novel constructs developed here, though, have the potential of providing detailed information on how the heads communicate. It is amazing that these and other previous constructs with large changes in communication between the heads are as processive and motile as they are and this paper contributes extensively to that base of knowledge. However, the constructs presented here have not produced the type of unambiguous results that allow new conclusions to be reached. An additional consideration is that the arguments and reasoning is very long, complex and not clearly stated. Many aspects that are critical to understanding what was done and the results are easily lost in a long supplement. This makes the work less suitable for a general interest publication such as EMBO J.

One issue on the presentation and interpretation is the need to incorporate the expanded structural part of figure S8a into figure 2A to insure that the reader understands that the construct being used has a very long flexible (non-protein) linker between the end of the DS DNA and the protein. Although this is described in the supplement, this is a critical point that is not mentioned directly on pages 6-7 of the Results. This leaves the reader thinking on reading the Results that the construct in figure 2A is like wild type except that the neck coil is replaced with DS DNA, when in fact the primary change from wild type is the insertion of a long flexible linker between the neck linker and the dimerization domain (whether coiled coil or DS DNA).

Motile probability is not a substitute for run length, dwell time and landing rate. What is the justification for normalizing to the number of stuck non-moving spots? Motor/fluorescent spots can become fixed on a microtubule for a number of reasons. Of course if large numbers of spots were not motile, then that would be of concern as it would indicate that the motile ones were an abnormal subpopulation. But if the purpose is to determine run length of the motile species, why is that not reported directly? Why not just replace figure 3E with Figure S4A for all lengths? One issue comparing them is that even at 5.1 nm, the residence time in S4a is extremely short. Does S4A not contain the data for the longer constructs of up to 14 nm because the residence times become too short to accurately quantify the time and velocities? If so, what does a motile probability mean if they are only motile for a couple of frames?

Although shown correctly by the position of the square in Fig. 3B, position 342 is not at end of neck linker as stated on page 8. It is well past that and almost a full turn into the coiled coil neck. The first residue of the neck coil is A337 as shown by both sequence prediction and by its position at the start of the neck coil in X-ray structures of dimers. W340 is a key hydrophobic residue that studies have shown plays a critical role in stabilizing the start of the coiled coil. These complexities are addressed in the long supplement, but should be stated in the results. Also another major difference between the constructs here and previous linker inserts is that most are done with heads that extend to 349 that is half way into the neck coil. Constructs of this length are monomers when expressed alone, but only slightly longer ones are dimerized.

2nd Revision - authors' response 31 August 2009

Referee 2's comment
The constructs presented here have not produced the type of unambiguous results. Although shown correctly by the position of the square in Fig. 3B, position 342 is not at end of neck linker as stated on page 8. It is well past that and almost a full turn into the coiled coil neck. The first residue of the neck coil is A337 as shown by both sequence prediction and by its position at the start of the neck coil in X-ray structures of dimers. Most experiments are done with heads that extend to 349 that is half way into the neck coil. Constructs of this length are monomers when expressed alone, but only slightly longer ones are dimerized.

Our response
As suggested, we changed construct K349CLM 342 to K336CLM 337 in Figures 3 and 4 (originally Supplementary Figure S4). Similar results were obtained. One difference was the position of the second peak (from DNA length of 12nm (K349CLM 342) to 8nm (K336CLM 337)). However, the new results are more reasonable since 8nm is equal to the difference between a two-step distance (16nm) and the native neck linker length (8nm). Note: We continuously used K336CLM base constructs in Figures 6-8 since we used them in the figures from the original version.

Referee 2's comment
Motile probability is not a substitute for run length, dwell time and landing rate. What is the justification for normalizing to the number of stuck non-moving spots? Motor/fluorescent spots can become fixed on a microtubule for a number of reasons. Of course if large numbers of spots were not motile, then that would be of concern as it would indicate that the motile ones were an abnormal subpopulation. But if the purpose is to determine run length of the motile species, why is that not reported directly? Why not just replace figure 3E with Figure S4A for all lengths?

Our response
While the author agrees with the referee's concerns, we sought to make very straightforward comparisons between constructs while also being able to minimize data selection artifacts. Thus, we used motile probability, as this parameter is a very simple indicator. To a large extent motile probability reflects run length (see Figure 4). Furthermore, according to your suggestion, we investigated run length in Figures 4 and 7 and discussed these results.

Referee 2's comment
One issue on the presentation and interpretation is the need to incorporate the expanded structural

part of figure S8a into figure 2A to insure that the reader understands that the construct being used has a very long flexible (non-protein) linker between the end of the DS DNA and the protein. Although this is described in the supplement, this is a critical point that is not mentioned directly on pages 6-7 of the Results. This leaves the reader thinking on reading the Results that the construct in figure 2A is like wild type except that the neck coil is replaced with DS DNA, when in fact the primary change from wild type is the insertion of a long flexible linker between the neck linker and the dimerization domain (whether coiled coil or DS DNA).

Our response
Thank you very much for your constructive suggestion. According to your suggestion, we added a sentence in the results section associated with Figure 2 and presented the expanded structural part of the DNA-protein connection in Figure 7C. We hope that it is now easier for the reader to understand our work.

3rd Editorial Decision 28 September 2009

Thank you for submitting your revised manuscript to the EMBO Journal. I asked the original referee #2 to evaluate the revised version and I have now heard back from this referee. I am very pleased to say that this referee finds the revised manuscript significantly improved and recommends publication here. The referee has just one minor remaining issue that has to be clarified before publication here and that concerns the control experiment for fig 1B,C. I would like to ask you to respond to this issue in a final revision. Once we receive the revised version we will proceed with the acceptance of the paper for publication here.

I am looking forward to seeing the final version!

Yours sincerely,

Editor
The EMBO Journal

REFEREE REPORT

Referee #2 (Remarks to the Author):

The presentation in the revised manuscript is greatly improved. One remaining issue that was not noted before is how can a 6 bp DNA be stably dimerized even at the low concentrations used for motility assays? The controls of fig. 1B,C were done with a 20 bp version and it is not clear that would have gotten similar results with a 6 bp version. Some comment on the reasons for believing it is dimerized or similar gel filtration data seems required.

3rd Revision - authors' response 05 October 2009

Referee 2's comment
The presentation in the revised manuscript is greatly improved. One remaining issue that was not noted before is how can a 6 bp DNA be stably dimerized even at the low concentrations used for motility assays? The controls of fig. 1B,C were done with a 20 bp version and it is not clear that would have gotten similar results with a 6 bp version. Some comment on the reasons for believing it is dimerized or similar gel filtration data seems required.

Our response
As suggested, we added the following sentence regarding 6 bp constructs to the end of the Figure 1 legend, "Note: we obtained similar results using 6 bp constructs (data not shown)." From the following results, we conclude that the 6 bp constructs were dimerized. First, we obtained similar

results as the 20 bp constructs from gel filtration data. Second, as we wrote in the Supplemental results, we observed the expected FRET signal at the single molecule level for the sense - antisense DNA-kinesin pair, but not for the sense - sense or antisense - antisense pairs (Supplemental Figure S3 and 6 bp version of Supplemental Figure S2).

