Dynein self-organizes while translocating the centrosome in T cells

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RE: Manuscript #E20-10-0668
TITLE: "Dynein self-organizes while translocating the centrosome in T cells"

Dear Dr. Berger:

both reviewers and I liked the manuscript, and it fits the quant bio issue of MBoC very well. Please take care of the reviewers' comments, and I will send the revised manuscript to one of the reviewers for the second look.

Sincerely,
Alexander Mogilner
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Berger,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript requires minor revisions before it can be published in Molecular Biology of the Cell, as described in the Monitoring Editor's decision letter above and the reviewer comments below.

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When submitting your revision include a rebuttal letter that details, point-by-point, how the Monitoring Editor's and reviewers' comments have been addressed. (The file type for this letter must be "rebuttal letter"; do not include your response to the Monitoring Editor and reviewers in a "cover letter.") Please bear in mind that your rebuttal letter will be published with your paper if it is accepted, unless you have opted out of publishing the review history.

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Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

Reviewer #1 (Remarks to the Author):

The paper "Dynein self-organizes while translocating the centrosome in T cells" by Gros et al. presents mainly Cytosim simulations and some experimental expansion microscopy results on centrosome translocation in T cells. The authors propose a mobile dynein anchoring in the actin-depleted circular zone in the IS.
imulations show that this model results in dynein clustering which helps to organize MTs into a stalk structure during translocation.

The proposed mobile anchoring of dyneins is motivated by the actin-depleted zone that is observed experimentally, which make it difficult to imagine fixed anchors. There are no direct experimental results presented on the anchoring itself, and some questions remain. First, a weaker mobile anchoring raises the question whether membrane anchors could eventually be pulled out by forces generated during translocation. I think this is unlikely as typical extraction forces that are reported are in the range of tens of pN, but I would recommend to actually measure the force on the anchor during the simulation in order to clarify this point.

Another important point is the clustering of dyneins, which is a main finding of the paper. Here, the authors stress that there is no interaction between dyneins. It is well known, however, that membrane inclusions will interact (identical inclusions should attract each other); therefore, the putative anchors in the membrane can give rise to an effective attraction between dyneins, which could be relevant for the translocation process. I would like to see a more detailed discussion of this issue.

Moreover, the authors seem to evade a concise definition of what they want to call a "cluster". In the main part of the paper they simply state that "we quantify it by using a density-based clustering algorithm" and in the "Methods" section they are only slightly more informative. The statement "The single hyperparameter 'epsilon' defines the maximum distance between samples to be considered as the same neighborhood. This neighborhood, however, does not define the maximum distance between points in a cluster." left me a little bit puzzled what the definition of a cluster actually is.
As this is a central part of the proposed stalk-forming mechanism the authors should be very clear here.
Moreover, there is no experimental information on dynein clustering, which could confirm the simulation data.

Also the notion of a stalk is a central concept but is dealt with in a very qualitative manner (not only in this paper but also in other papers in the literature). A concise criterion what one could call a stalk (for example, based on distances between MTs) would be very helpful in order to quantify whether an image (be it simulation or microscopy) actually displays a stalk or not. Maybe the authors could come up with some idea here. Otherwise, the discussion of this important issue will remain somewhat vague, unfortunately.

The simulation model is based on Cytosim. I find it important that the simulation model explicitly includes MT dynamics, which has been neglected in some previous approaches. MT dynamics should contribute to the dynamics restructuring during the translocation process.
Reading the main part of the manuscript first, I found the description of the model somewhat vague at several places: for example it was not completely clear to me at first, whether MT dynamics is contained or not. This important point was clarified only in the Methods section. Some questions remained, also after reading the Methods section:
1) Is there experimental motivation to choose 150 MTs? Is this a
typical number? In a previous paper the authors used 90 MTs.
2) What happens to dyneins in the sliding-dynein model if the MT shrinks past an attached dynein? Is the dynein simply lost?

The alternative mechanisms of dynein sliding or capture shrinkage are also investigated and discussed as in many publications before. Here, the conclusion is that dynein-sliding is more robust and to be favored. What is not discussed is a possible cooperation of both mechanisms, which has been advertised by Hornak/Rieger. If capture shrinkage does not lead to proper translocation for larger unbinding rates >0.04/s. On the other hand, they observe that the translocated state is less stable for higher unbinding rates for dynein sliding. This suggests that
cooperation of both mechanisms could actually lead to robust translocation over a wider range of unbinding rates.

Overall, I find the proposed model very reasonable, the simulation work is sound, and there is general agreement with the expansion microscopy results although the comparison between experiment and simulation is very qualitative. Therefore, I recommend publication if the remaining issues that I raised above are discussed.

Reviewer #2 (Remarks to the Author):

Review on the manuscript: "Dynein self-organizes while translocating the centrosome in T cells" submitted to MBoC by OJ Gros, HGH Damstra, LC Kapitein, A. Akhmanova and F. Berger.

This, mostly computational, study deals with the structural reorganisation of T-cells when approaching an antigen presenting cells, namely with the translocation of the centrosome towards the immunological synapse (IS). Its goal is to investigate the effect of the mobility of IS-bound dynein-I molecular motor proteins in the context of this restructuring.

Using the software cytosim the authors perform particle-based simulations of the centrosome, the nucleus, the microtubules

originating at the centrosome and their interaction with IS-bound, though otherwise mobile, dynein-I motor proteins (the IS is the spatially bounded section of the T-cell membrane interfacing with the antigen presenting cell).

The central findings are that the interplay of these agents is sufficient to explain the restructuring and that the membrane-bound mobility of MT-sliding dynein motors increases the robustness of centrosome translocation as compared to dynein motors which engage in MT-capture and shrinkage. The simulations support the emergence of a microtubule stalk linking the centrosome to the IS which has been observed in recent experimental studies. Furthermore the simulations predict the emergence of a dynein cluster at the centre of the IS. This suggests that recently discovered T-cell receptor-microclusters in the IS of T-cells play the role of mobile, membrane-bound dynein anchors.

The computational research presented in this manuscript has been carried out very thoroughly and the manuscript is very well written - indeed I've enjoyed reading it a lot.

One issue I identified is merely about wording. The authors classify the mechanism by which dynein forms clusters in the context of this model as self-organization (e.g. title, abstract, page 3, bottom, results-section: "Dynein self-organizes into clusters over time", etc.). At the beginning of the section "discussion" they state that "These clusters emerge without any attractive interactions between the dynein molecules and thus arise from self-organization."
It think that wording might be misleading as - for example - the wikipedia page on "self-organisation" defines it as " a process where some form of overall order arises from local interactions between parts of an initially disordered system."

For this reason I definitely suggest to omit the statement cited above.

It seems to me that the formation of the dynein clusters in this model is caused by the interplay of their high mobility and the fact that dynein upon binding to microtubules loses much of its mobility. Therefore the microtubules approaching the IS act as traps for the highly diffusive dynein motors and cause their apparent cluster formation. This happens as soon as the centrosome is close to the centre of the IS such that many MT approach the IS right where the centrosome is located. As reported in the manuscript in some simulations it even happens earlier, I believe because single MTs align in parallel to the IS. Yet, since in both cases the cluster formation is caused by MTs acting as traps for the mobile dynein motors I wouldn't use the term self-organisation in this context.
I admit that to some extent this is also a matter of personal taste. Therefore I suggest that during the revision the authors not necessarily change their wording, but rethink it at least.

One typo/inconsistency: in the section "Dynein motors" as well as in Table 1 the unloaded speed of dynein is stated as 1 um/s, whereas according to Figure 1B it is 0.5 um/s.

Dynein self-organizes while translocating the centrosome in T cells

Point-by-point response to the comments of the reviewers

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  Simulations show that this model results in dynein clustering which helps to organize MTs into a stalk structure during translocation.

  The proposed mobile anchoring of dyneins is motivated by the actin-depleted zone that is observed experimentally, which make it difficult to imagine fixed anchors. There are no direct experimental results presented on the anchoring itself, and some questions remain. First, a weaker mobile anchoring raises the question whether membrane anchors could eventually be pulled out by forces generated during translocation. I think this is unlikely as typical extraction forces that are reported are in the range of tens of pN, but I would recommend to actually measure the force on the anchor during the simulation in order to clarify this point."

  As the reviewer points out, a direct anchoring of dynein to the membrane may not be strong enough to sustain the force generation during MTOC translocation. To follow the recommendation by the referee, we quantified the forces that are exerted on the dynein-membrane anchors and added a supplemental figure S2A. In this figure we display the forces on dynein in the Y direction (perpendicular to the synapse) at every position of dynein within the first 300 s of phase III. We see here that the forces can increase to values higher than 10 pN, with the maximum measured as 17.7 pN. Such a large force is likely quite short-lived due to the force-dependent unbinding of dynein from the MT. In our model, we use a characteristic unbinding force of 4 pN for the dynein motors to detach from the MTs. Whether this short-lived strong force is sufficient to extract the anchor from the membrane is hard to say with the current knowledge of the system. In several studies, investigators have reported membrane invaginations in the center of the immunological synapse, within the first minutes after T-cell activation (Singleton et al., 2006; Yi et al., 2013), suggesting the existence of pulling forces on the membrane.

  Now, we refer to the new data on page 8:

  > Analyzing the force on the dynein motors perpendicular to the IS plane indicates that the more mobile, clustering dynein molecules are under a larger, perpendicular force on the order of 10 pN (Figure S2).

  We have added the following text to the discussion (page 13) to clarify this point and to discuss the data presented in Fig S2:

  > These anchors have to sustain the forces generated by dynein without getting extracted from the membrane. In addition to the dynein-generated forces, rearrangements of the MT network can stretch these MT-dynein-membrane linkers and result in forces on the anchors which are above dynein's stall force. We measure some of the forces on dynein to be above 10 pN, however, because we define the characteristic detachment force of dynein from the MT as 4 pN, the membrane anchor will not be exposed to these forces for an extended period of time. Dynein thus probably detaches from the MT before a substantial force could pull its from the anchor, or the anchor out of the membrane. From several experimental studies, the existence of dynein molecules strongly anchored to the membrane seems plausible. Different studies have reported membrane invaginations in the center of the

immunological synapse within the first minutes after T cell activation (Singleton et al., 2006; Yi et al., 2013). These invaginations suggest that a force is strongly coupled to the membrane and pulls it towards the center of the cell. Most likely this force is generated by multiple membrane-anchored dynein molecules pulling on the MT network. Stable dynein anchoring to the membrane during force production has been reported in other biological systems (Kotak *et al.*, 2012; Schmidt *et al.*, 2017). Contrasting these models, however, we assume that the dynein anchors in the T cell are very mobile, because of the highly dynamic environment of the IS.

In conclusion, we are confident that our model in which dynein is anchored to the membrane is realistic, because the occuring forces are not unreasonable and experimental evidence indicates the possibility of strongly bound dyneins to the membrane.

- *"Another important point is the clustering of dyneins, which is a main finding of the paper. Here, the authors stress that there is no interaction between dyneins. It is well known, however, that membrane inclusions will interact (identical inclusions should attract each other); therefore, the putative anchors in the membrane can give rise to an effective attraction between dyneins, which could be relevant for the translocation process. I would like to see a more detailed discussion of this issue."*

Multiple processes in the IS may contribute to dynein clustering, such as interaction between membrane inclusions and the concurrent formation of SMACs. In this study, we wanted to show that even without predefined aggregation mechanisms, in our model, dynein will cluster purely through its dynamic interaction with the MT network. We agree with the referee that other possible attractive forces, although not implemented in our simulations, leading to clustering should be discussed in detail in the manuscript.

To address this point, we add the following text in the discussion (page 11):

> In the real biological system, the clustering of dynein molecules in the membrane could be enhanced by several additional mechanisms. Anchoring proteins in the membrane could attract each other because of membrane-induced interactions (Dan *et al.*, 1993; Aranda-Espinoza *et al.*, 1996). Through these attractive forces, microdomains in the membrane could emerge and contribute to the overall organization concurrent with the SMAC formation (Simons and Ikonen, 1997). Although these processes may contribute to the molecular organization in the IS, we did not include them in our study to be able to focus on how the system self-organizes without any explicitly defined clustering mechanism.

- *"Moreover, the authors seem to evade a concise definition of what they want to call a "cluster". In the main part of the paper they simply state that "we quantify it by using a density-based clustering algorithm" and in the "Methods" section they are only slightly more informative. The statement "The single hyperparameter 'epsilon' defines the maximum distance between samples to be considered as the same neighborhood. This neighborhood, however, does not define the maximum distance between points in a cluster." left me a little bit puzzled what the definition of a cluster actually is.*

As this is a central part of the proposed stalk-forming mechanism the authors should be very clear here."

To identify clusters, we assume that a cluster is a collection of dynein motors that are densely packed in some subspace of the 2D synapse plane. This definition purposely avoids defining a shape of the cluster or very tight density thresholds. A suitable clustering algorithm that meets these criteria is the widely-used DBSCAN density based method, which searches for 'core points' that are deemed dense enough to belong to a cluster. Because the amount of core points can grow, for this method it is not necessary to specify the cluster shape or size a priori (i.e. the maximum distance between all points of the cluster). Another

advantage of this method is that the number of clusters is a free parameter, and we thus do not have to assume that there will be a single cluster, even though we only report the biggest cluster.

We added the following text on page 8 in the main section to clarify the method:

> In short, this algorithm identifies regions in which points are closely packed together by assigning a point to a cluster if it is close to many points of that cluster. We choose this algorithm because no prior knowledge of the number of clusters is necessary, it identifies arbitrarily shaped clusters, and accounts for noise in the data. Applying this method to the position of dynein molecules for every timestep reveals how clusters develop during the simulation.

To describe the DBSCAN clustering method in detail, we added the following text to the method section on page 17:

> The DBSCAN algorithm depends on two parameters: a distance 'epsilon' and 'min_samp' the minimum number of points defining a cluster. Epsilon was set to 0.5 µm, as this reproduced clusters visually, and for 'min_samp' we used the SciPy default value 5. A point is considered a core point of a cluster if within a circle with radius epsilon around this point at least 'min_samp' points including the point itself are found. We searched for dense clusters of dynein positions in every separate time frame (0.4 s) in each run. The traces, shown in Figure 3B take only the largest cluster that is found into account and are averaged over four time frames (a total of 1.6 s) with a shaded area as the confidence interval. This averaging procedure reduces the noise of separate clustering attempts and shows the general trend more clearly.

- *"Moreover, there is no experimental information on dynein clustering, which could confirm the simulation data."*

As remarked by the reviewer, we do not provide direct experimental data to support evidence for the clustering of dynein molecules during centrosome translocation. Indeed, this would be a very nice validation of our simulation data. However, from a molecular-biological point of view there are several technical problems that we have to overcome first to visualize dynein dynamics in these cells. To acquire such data, extensive experimental efforts are necessary which are beyond the scope of this project. However, multiple previous studies indirectly support the idea of clusters of dynein: either by showing a cluster of MT-force generation in the synapse center (Yi et al.) or by demonstrating a clustering of TCRs by dynein (Hashimoto-Tane et al).

- *"Also the notion of a stalk is a central concept but is dealt with in a very qualitative manner (not only in this paper but also in other papers in the literature). A concise criterion what one could call a stalk (for example, based on distances between MTs) would be very helpful in order to quantify whether an image (be it simulation or microscopy) actually displays a stalk or not. Maybe the authors could come up with some idea here. Otherwise, the discussion of this important issue will remain somewhat vague, unfortunately."*

As acknowledged by the reviewer the notion of a stalk is a rather opaque concept, only qualitatively defined in our study and also in the literature. We agree that a quantitative definition of the stalk would be beneficial for the field. Unfortunately, we cannot give a quantitative definition at the moment that is useful for the experimental data, as well as for our simulation results. The definition that we use in the manuscript is based on the morphology of stalks, as a bundle of MTs that align towards the synapse along the shortest distance between the centrosome and the synapse. As reported before, the structure needs to also shrink during centrosome translocation. We cannot apply this dynamic property to our experimental data, because we do not obtain live images. Nonetheless, We do find these forms of structures in our simulations (Figure

5B,C), and we find similar, however static, structures in the microscopy images (Figure 5F,G). A simple quantitative approach would be to identify MT bundles as structures consisting of multiple filaments which are aligned and relatively straight. While this definition seems relatively easy, applying such a criterion to experimental images is difficult, because we do not have single filament resolution and ways of tracing the filaments automatically. Additionally, the stalks in the biological system often appear to be more bundled than in the simulations. This discrepancy is apparent in both our experiments and the T-cell images that were reported previously (Yi et al., 2013). While our simulations do describe a clear way how the attachment of dynein at the synapse of stalks can work, the bundling of the stalk in the T cells was not completely replicated. This bundling can probably be attributed to either a geometric force constraining the space (such as a deformable nucleus) or active bundling by MT-MT binding proteins. Lacking the clear bundling phenomenon, we did not feel confident to apply a criterion based on bundles to quantitatively identify stalks in our simulations.

To clarify this point we added the following text to the first paragraph of the discussion on page 11:

> We defined a 'stalk' as a qualitative morphological feature, because we were unable to quantitatively analyze MT bundles in the experimental data and in the simulations. Our simulation showed only a relatively small number of MT filaments contributing to the stalk, making a quantitative criterion based on such a small number very difficult. Because of experimental limitations, we were not able to draw significant quantitative conclusions on the formation of MT bundles. Stalks can only appear in the model if enough dynein molecules converge before the centrosome translocates. Some factors of our model definition may hinder the stalk formation in our current model, such as the rigid attachment at the centrosome and relatively short MT network. Additionally, in cells, it is also likely that MT-bundling proteins can stabilize stalks, which we ignored in our simulations.

- *"The simulation model is based on Cytosim. I find it important that the simulation model explicitly includes MT dynamics, which has been neglected in some previous approaches. MT dynamics should contribute to the dynamics restructuring during the translocation process.*

Reading the main part of the manuscript first, I found the description of the model somewhat vague at several places: for example it was not completely clear to me at first, whether MT dynamics is contained or not. This important point was clarified only in the Methods section. Some questions remained, also after reading the Methods section:

1) Is there experimental motivation to choose 150 MTs? Is this a typical number? In a previous paper the authors used 90 MTs.

2) What happens to dyneins in the sliding-dynein model if the MT shrinks past an attached dynein? Is the dynein simply lost?"

To address the broad readership of MBoC, we decided to only briefly describe our modelling approach in the main text and give specific details in the method section. We agree with the referee that we could have been more explicit about the force-dependent MT dynamics. To clarify this general point and the first specific point raised by the referee, we added the following text on page 4 to further explain how we model MT dynamic instability:

> The numerical values of the parameters describing the MT dynamics were estimated from TIRF microscopy data of EB3-GFP comets in polarized T cells (Hooikaas *et al.*, 2020), using a classic two-state MT dynamics model, in which the MT can be in a growing state and in a shrinking state. In the growing state, the MT polymerization rate decays exponentially with the force. The transition from the growing state to the shrinking state is characterized by a catastrophe rate, which is different for a freely growing end and for an end exposed to a large force (see Methods). We use 150 MTs which is in the range of the number of MTs previously estimated in these cells (Hooikaas *et al.*, 2020).

In response to the first specific question, we chose 150 MTs as this was in the lower range of the number of MTs counted in Hooikaas-Damstra et al. Jurkat cells, as we wanted to be close to the in vivo range, but simultaneously limit computational complexity. The previous modeling work was limited to 90 MTs because of spatial restrictions in the 2D modeling space. However, this limitation was overcome when we switched to the 3D system. Concerning the second question, dynein in the sliding-dynein model unbinds when a MT shrinks past the motor.

To address the referee's second specific question and also to clarify our model, we revised the methods part on page 15:

> If a dynein molecule is attached to a shrinking MT, and the MT's end reaches the dynein molecule, it immediately unbinds from the filament.

And on page 16:

> We use a classical two-state model to describe the growth and shrinkage of MTs, as implemented in *Cytosim* and used for previous studies (Dogterom and Yurke, 1997; Janson *et al.*, 2003; Letort *et al.*, 2016; Lacroix *et al.*, 2018). This model includes force-dependent MT growth and catastrophe. The MT can be in a shrinking state with a constant depolymerization speed and in a growing state with a force-dependent polymerization speed. The polymerization speed decreases exponentially with force, $v_{growth} = v_0 e^{-F/F_{stall}}$, in which v_0 is the force-free polymerization speed and F_{stall} is the characteristic stall force (Dogterom and Yurke, 1997). The transition from the growing state to the shrinking state is described by the force-dependent catastrophe rate, $k_{cat} = k_{cat,stall}\left(1 + \left(\left(\frac{k_{cat,stall}}{k_{cat,0}}\right) - 1\right) e^{-\frac{F}{F_{stall}}}\right)^{-1}$ (Janson *et al.*, 2003), in which $k_{cat,stall}$ is the catastrophe rate under stall and $k_{cat,0}$ is the catastrophe rate for a freely growing MT. We estimated the numerical values of the parameters from data of MT dynamics from (Hooikaas *et al.*, 2020).

- *"The alternative mechanisms of dynein sliding or capture shrinkage are also investigated and discussed as in many publications before. Here, the conclusion is that dynein-sliding is more robust and to be favored. What is not discussed is a possible cooperation of both mechanisms, which has been advertised by Hornak/Rieger. If capture shrinkage does not lead to proper translocation for larger unbinding rates >0.04/s. On the other hand, they observe that the translocated state is less stable for higher unbinding rates for dynein sliding. This suggests that cooperation of both mechanisms could actually lead to robust translocation over a wider range of unbinding rates."*

We agree with the referee that our results certainly do not invalidate a model with both MT-sliding and MT-capture-shrinkage dynein, as proposed by Hornak and Rieger, and that this model may cause more robustness of the translocated state. However, the MT-capture-shrinkage model was proposed mostly to explain the MT morphology and centered force generation during centrosome translocation, while we can explain these phenomena in a model with only the mobile MT-sliding dynein. Because we consider the MT-sliding mechanism a much more well-described phenomenon, and find that it functions as a minimal model for the observed system, we did not look further into combinations of the two mechanisms.

We now discuss the point raised by the referee in the discussion on page 12:

> Our results suggest that in the MT-sliding model the MTOC can be translocated for a larger range of parameter values for the unbinding rate of dynein from the MTs, compared to the MT-capture shrinkage model. It is possible that both mechanisms work together, where the MTOC is robustly

translocated through MT-sliding and the translocated state is stabilized by a MT-capture shrinkage mechanism. However, we also believe it may be possible that there is no MT-capture shrinkage dynein in the T cell.

- *“Overall, I find the proposed model very reasonable, the simulation work is sound, and there is general agreement with the expansion microscopy results although the comparison between experiment and simulation is very qualitative. Therefore, I recommend publication if the remaining issues that I raised above are discussed.”*

We thank the referee for the positive evaluation of our work and we hope that we discussed the raised issues adequately in the revised manuscript.

Reviewer #2 (Remarks to the Author):

- *“Review on the manuscript: "Dynein self-organizes while translocating the centrosome in T cells" submitted to MBoC by OJ Gros, HGH Damstra, LC Kapitein, A. Akhmanova and F. Berger.*

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The central findings are that the interplay of these agents is sufficient to explain the restructuring and that the membrane-bound mobility of MT-sliding dynein motors increases the robustness of centrosome translocation as compared to dynein motors which engage in MT-capture and shrinkage. The simulations support the emergence of a microtubule stalk linking the centrosome to the IS which has been observed in recent experimental studies. Furthermore the simulations predict the emergence of a dynein cluster at the centre of the IS. This suggests that recently discovered T-cell receptor-microclusters in the IS of T-cells play the role of mobile, membrane-bound dynein anchors.

The computational research presented in this manuscript has been carried out very thoroughly and the manuscript is very well written - indeed I've enjoyed reading it a lot.

One issue I identified is merely about wording. The authors classify the mechanism by which dynein forms clusters in the context of this model as self-organization (e.g. title, abstract, page 3, bottom, results-section: "Dynein self-organizes into clusters over time", etc.). At the beginning of the section "discussion" they state that "These clusters emerge without any attractive interactions between the dynein molecules and thus arise from self-organization."

It think that wording might be misleading as - for example - the wikipedia page on "self-organisation" defines it as " a process where some form of overall order arises from local interactions between parts of an initially disordered system."

For this reason I definitely suggest to omit the statement cited above.”

We agree with the referee that we can be more specific about what we mean by “self-organization”. We omitted the statement as suggested by the reviewer and carefully changed the wording in some parts in the manuscript. Furthermore we now include a more detailed definition of “self-organization” in the discussion and state precisely which process we classify accordingly. Following this definition we still classify the cluster formation of dynein before centrosome translocation as a self-organizing process and therefore did not change the title of the manuscript.

We added the following text to the discussion on page 11:

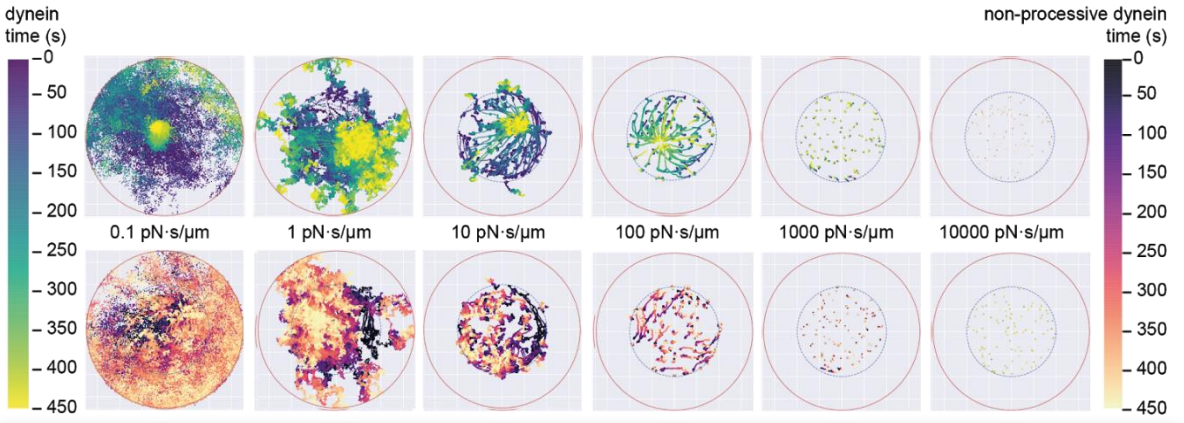
> The dynein clusters form without any explicitly defined attractive forces between dynein motors and are caused by the mobility of dynein and the organization of the MT network. The accumulation of dynein motors at a translocated centrosome is a relatively intuitive process: the minus-end directed motion of dynein along the MT propagates the predefined MT organization to the arrangement of dyneins on the molecular scale. Strikingly, we also observe that dynein molecules accumulate before the centrosome is fully translocated. This process happens when dynein molecules bound to MTs are dragged in the membrane to a point in which they mostly experience forces perpendicular to the IS. At this point, dynein either stalls or is about to unbind from the MT. The location where this process happens is defined by the initial angle of the MT anchor on the centrosome, the filament length, its rigidity and dynein mechanics, but is likely under the centrosome as the MT is pulled taut. We term this pre-translocation clustering as a self-organization process because this organization cannot directly be predicted from the properties of the individual parts without considering all their interactions and dissipative dynamics in the system as a whole (Karsenti, 2008). In the real biological system, the clustering of dynein molecules in the membrane could be enhanced by several additional mechanisms. Anchoring proteins in the membrane could attract each other because of membrane-induced interactions (Dan *et al.*, 1993; Aranda-Espinoza *et al.*, 1996). Through these attractive forces, microdomains in the membrane could emerge and contribute to the overall organization concurrent with the SMAC formation (Simons and Ikonen, 1997). Although these processes may contribute to the molecular organization in the IS, we did not include them in our study to be able to focus on how the system self-organizes without any explicitly defined clustering mechanism.

- *“It seems to me that the formation of the dynein clusters in this model is caused by the interplay of their high mobility and the fact that dynein upon binding to microtubules loses much of its mobility. Therefore the microtubules approaching the IS act as traps for the highly diffusive dynein motors and cause their apparent cluster formation. This happens as soon as the centrosome is close to the centre of the IS such that many MT approach the IS right where the centrosome is located. As reported in the manuscript in some simulations it even happens earlier, I believe because single MTs align in parallel to the IS. Yet, since in both cases the cluster formation is caused by MTs acting as traps for the mobile dynein motors I wouldn't use the term self-organisation in this context.*

I admit that to some extent this is also a matter of personal taste. Therefore I suggest that during the revision the authors not necessarily change their wording, but rethink it at least.”

We do not fully agree with the assessment that the term cannot be applied to the clustering process we describe. As pointed out by the referee, there are two separate processes that result in clustering of dynein: one that takes place after the centrosome has translocated and the other before the centrosome has translocated. Both of these processes cause clustering of dynein, and the first is more intuitive. However, both of these clustering processes are not just a trapping phenomena. The organization of the MTs is pre-defined as having a central nucleating point, and the spatial organization of the MT density does change with centrosome translocation. However, the process of clustering and thus the propagation of the MT

organization to the dynein level is only due to the processivity of dynein on the fiber (be it MT-sliding or shrinking) causing it to organize itself based on the MT organization. We further studied this process by running a simulation with added non-processive dynein, a motor that cannot walk and only binds to the MT (Extra Figure 1). These non-processive binders are still mobile in the synapse and translocated along with its captured filament during centrosome translocation, leading to a peripheral distribution and not to centralized clustering. Although these results are interesting, they do not add much to the conclusions presented in our manuscript and therefore we did not include them in the main text.



Extra Figure 1. *Dynein movement causes cllustering.* Simulations were run with 100 processive dynein motors and 100 non-processive dynein motors. Both rows show the two fractions of the same run for different drag condtions. The upper row shows processive dynein, bottom row shows non-processive dynein. The colors indicate the time point in the run. The blue dashed line indicates the approximate end of the MT-binding region.

In conclusion, we took the advice from the referee seriously and rethought our terminology and distinguished the different clustering processes in more detail, as presented in the revised version of the manuscript.

- *One typo/inconsistency: in the section "Dynein motors" as well as in Table 1 the unloaded speed of dynein is stated as 1 um/s, whereas according to Figure 1B it is 0.5 um/s.*

  We thank the referee for pointing out this error, which was a relic of a previous version. we have changed the values of 1 µm/s to 0.5 µm/s.

RE: Manuscript #E20-10-0668R
TITLE: "Dynein self-organizes while translocating the centrosome in T cells"

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Monitoring Editor
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Reviewer #1 (Remarks to the Author):

In their rebuttal and the modified manuscript, the authors addressed all of the issues that I raised (at least the ones that do not require new experiments). Therefore, I recommend publication in its present form.