Identification and function of conformational dynamics in the multidomain GTPase dynamin

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Editor: Andrea Leibfried

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.)

Thank you for submitting your manuscript entitled 'Identification and function of conformational dynamics in the multidomain GTPase dynamin'. I have now received the reports from all referees.

As you can see below, both referees recognize that there is interest in better understanding conformational changes occurring in dynamin. However, they think that some amendments and additional information are needed prior to publication of your work here.

Both referees provide constructive reports, and I would thus like to ask you to submit a revised version of the manuscript, addressing all concerns of the referees. Importantly, the HDX data need to be interpreted differently and with caution, and more information on them as well as on the FRET data are required (referee #2). All other concerns raised by referee #1 and #2 seem to be easily addressable.

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

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REFEREE REPORTS

Referee #1:

Srinivasan et al. use hydrogen deuterium exchange coupled with mass spectrometry, and a range of functional assays including FRET analyses, chemical cross-linking, GTPase, oligomerization, EM
and cell-based assays to describe conformational changes and domain rearrangements of dynamin during nucleotide (GMPPNP and GDP-AlF4) and membrane binding. With these approaches, the authors identify the main determinants of structural transitions in dynamin, which are the nucleotide-binding site, the bundle signaling element (BSE), helix alpha2 in the stalk, interface-3 of the stalk and the PH domain. Furthermore, their data confirm previous suggestions that the PH domain of dynamin acts in an auto-inhibitory fashion.

This is a detailed, comprehensive and very elegant manuscript describing the domain interplay of the key mechano-chemical enzyme. Results have direct implications for understanding the mechanism of dynamin in membrane fission and are therefore interesting for a wide range of researchers. So far, structural transitions in full-length dynamin have been mainly inferred based on comparisons of truncated dynamin structures (G domain-BSE constructs) or of tetrameric and oligomerized structures of dynamin. The current manuscript provides direct evidence how these domain transitions occur when full length dynamin is recruited from solution to membrane surfaces. Furthermore, the newly established assays will constitute useful tools for the community to perform time-resolved measurements of dynamin in action. A few points towards the end of the manuscript still need attention.

p11, middle: as Dyn1(closed) exhibits concentration-dependent GTPase activity in solution (Figure S4B). In this figure, it does not show concentration-dependent GTPase activity.

Figure 6: I was slightly confused about these data. Why is the S619L mutant less effectively recruited to CCPs when endogenous dynamin is present? Is the kinetics of recruitment different between wt and S619L, and therefore S619L is only slowly recruited when wt dynamin is competing? Or is the cytosolic concentration of S619L lower compared to wt since more of the S619L mutant remains bound to membranes, and hence the recruitment to CCPs occurs more slowly? Or still alternatively, could S619 directly act as binding site for cellular recruitment factors which would explain the differential recruitment? The authors should explain and discuss this point in more detail.

In agreement with the current data, it has previously been shown that the S619L mutation does not impair CME in myoblasts, but leads to T-tubule fragmentation in C2C12-derived myotubes and Drosophila body wall muscle (Chin et al, 2015). This information should be added.

Minor points:

p6, middle, switch II (136-153), cis stabilizing loop
Mention in text that these elements are also protected during nucleotide binding.

p7, helix alpha 2.
The close by location of Arg465 in helix alpha-3 mutated in CNM patients could be indicated as additional information.

p9, middle a role for the alpha2S helix as an allosteric relay.
It should be mentioned that the proposed allosteric relay does likely not occur within a peptide chain (as maybe expected for an allosteric relay), but rather via intermolecular contacts with the adjacent dimer.

Figure 4D: The Dyn1 closed (37 °C) trace is not referred to in the msc. What is the role of temperature here?

Figure 5F: Add scale/colour bar as in Figure 1.

Figure S3: Lettering is wrong in the figure

Typos:

End of abstract:
dynamin-catalyzed membrane fission
Referee #2:

This is a very comprehensive study with many different types of experiments extending all the way from FRET and HDX to in vivo studies to characterize the conformational changes in dynamin upon lipid and nucleotide binding. Overall the study is well-done and the experiments are appropriately interpreted. The system is really complicated and important, and the work greatly contributes to a deeper understanding of how the structure of dynamin causes its interesting functions. I have some suggestions to improve the manuscript, but I definitely think it is appropriate for publication in EMBO.

Pg 5 The authors spend an entire paragraph discussing how "changes in the number of amide hydrogens exchanged with solvent deuterium between two states; (e.g. apo versus ligand bound) is indicative of conformational alterations." This paragraph is incorrect. In reality, comparison of bound states vs. unbound states show differences in amide exchange purely due to binding of the ligand to the surface of the protein. Most of the differences in exchange that are observed in the work presented in this paper correspond to these types of changes. In the HDX results section the authors sometimes allude to this (despite their introductory statements which explicitly ignore it) but they should be very clear. If the lipid-binding domain shows protection in the lipid-bound state as compared to the free state, this is undoubtedly due to the fact that the lipids are interacting with the surface of the protein and decreasing exchange. No conformational change need be hypothesized. The situation is similar for nucleotide binding. On the other hand, they do observe differences in exchange far from the known binding regions and these are likely the ones corresponding to conformational changes.

The supplementary heat maps need a much more thorough legend. As it is, these data could not be properly evaluated. It is not clear what is being depicted in these figures. Usually, heat maps show the change over time or the difference over time of the same peptides. In this case, according to the methods section, the authors state "deuterium uptake for each peptide is calculated as the average of % D2O for the 6 time points (10s, 30s, 60s, 300s, 900s and 3600s)". Are they really averaging deuterium uptake across such a wide range of times? Such an approach is completely unconscionable. They should choose a single time point to compare between states. There are numbers in the bars corresponding to the peptides that are also not defined. It's a little odd how (for example) the peptide corresponding to residues 80-94 shows increased exchange, but then two peptides below it do not show differences. This is also true for the peptide around residue 310. In Fig S2b, there are peptides that are nearly identical (residues 305-330 two of which are protected and one is not...It is also hard to reconcile the different peptides in Fig S2b corresponding to residues 170-210. There are many such cases in Fig S2d. These discrepancies made me think I may not have guessed correctly what is being depicted in these figures. In any case it is important to provide a clear legend so that readers can confidently identify discrepancies. Figure S2A has a box that is not defined near residue 430...

Pg 13 the authors use the word motility where I think they mean to use the word mobility. Regarding the bulk FRET data, the authors need to discuss somewhere how they have controlled for different oligomerization states in the bulk FRET data. Is it possible that the FRET is an average of different oligomeric states as well as differentially "open" or "closed" states? A few added sentences would be useful here.

1st Revision - authors' response 07 December 2015

Ms: EMBOJ-2015-93477 Response to Reviews

We thank both referees for their careful consideration of our manuscript and their overall enthusiastic response. Referee 1 commented that, “This is a detailed, comprehensive and very elegant manuscript describing the domain interplay of the key mechano-chemical enzyme. Results have direct implications for understanding the mechanism of dynamin in membrane fission and are
therefore interesting for a wide range of researchers”, and referee 2 stated that “The system is really complicated and important, and the work greatly contributes to a deeper understanding of how the structure of dynamin causes its interesting functions.” However, both referees made several helpful suggestions to improve the manuscript. We have addressed all of these concerns as described below.

Referee #1:

p11, middle: as Dyn1(closed) exhibits concentration-dependent GTPase activity in solution (Figure S4B). In this figure, it does not show concentration-dependent GTPase activity.

We thank the referee for pointing out this error. The figure legend was inadvertently reversed. The figure now correctly represents the legends and shows an increase in GTPase rate with increasing concentration.

Figure 6: I was slightly confused about these data. Why is the S619L mutant less effectively recruited to CCPs when endogenous dynamin is present? Is the kinetics of recruitment different between wt and S619L, and therefore S619L is only slowly recruited when wt dynamin is competing? Or is the cytosolic concentration of S619L lower compared to wt since more of the S619L mutant remains bound to membranes, and hence the recruitment to CCPs occurs more slowly? Or still alternatively, could S619 directly act as binding site for cellular recruitment factors which would explain the differential recruitment? The authors should explain and discuss this point in more detail.

The reviewer raises some interesting possibilities, but we think the simplest interpretation is that WT endogenous Dyn2 competes more effectively for membrane binding sites than the S619L mutant. In the absence of competition from the endogenous, the mutant is recruited. This interpretation is supported by our biochemical data with purified proteins (Fig S5B), where we show that WT-Dyn2 binds liposomes more effectively than S619L. The mutation may also impair binding to partners responsible for membrane recruitment. We have now described this interpretation the text (top of page 15).

In agreement with the current data, it has previously been shown that the S619L mutation does not impair CME in myoblasts, but leads to T-tubule fragmentation in C2C12-derived myotubes and Drosophila body wall muscle (Chin et al, 2015). This information should be added.

Thanks for pointing out this omission. We have now included this information (from one of my former postdocs!) in the main manuscript (bottom of page 14).

Minor points:

p6, middle, switch II (136-153), cis stabilizing loop
Mention in text that these elements are also protected during nucleotide binding.

Thank you for pointing this out, we have now added this information to the main manuscript.

p7, helix alpha 2.
The close by location of Arg465 in helix alpha-3 mutated in CNM patients could be indicated as additional information.

Thanks for suggesting this. We have now discussed this interesting finding on page 16 in the first paragraph of the Conclusion.

p9, middle a role for the alpha2S helix as an allosteric relay.
It should be mentioned that the proposed allosteric relay does likely not occur within a peptide chain (as maybe expected for an allosteric relay), but rather via intermolecular contacts with the adjacent dimer.

The referee’s point is well taken. While we mentioned this briefly in the original submission, we have now added the following statement on page 7 (1st paragraph) to emphasize this important point, “The obligate oligomeric nature of dynamin and the position of the relay helix at the oligomeric
interface suggests that it is the core component of an intermolecular network that allows conformational transmission across seemingly autonomous domains."

Figure 4D: The Dyn1 closed (37°C) trace is not referred to in the msc. What is the role of temperature here?

We apologize for not referring to this data. The referee is correct that at 37°C the cross-linked protein elutes in the void volume at 37°C, indicating instability, as by EM these structures are amorphous aggregates. This aggregation is also reflected in increased basal GTPase activity, which was not apparent when basal and assembly stimulated GTPase activity was reported on the same scale. Our data on liposome binding was performed at room temperature and the defects in fission were observed both at room temperature and at 37°C with similar effect. We have now discussed this in the text (on page 11) and redrawn Figure 4B to better illustrate the enhanced basal GTPase activity of Dyn1 closed.

Figure 5F: Add scale/colour bar as in Figure 1.

We have now added heat map scale bar to describe the figure 5F.

Figure S3: Lettering is wrong in the figure

Corrected. Thank you.

Typos:

End of abstract:
dynamin-catalyzed membrane fission

P12 top, ...provide direct support the evidence that ...

The typographic errors are corrected.

Referee #2:

Pg 5 The authors spend an entire paragraph discussing how "changes in the number of amide hydrogens exchanged with solvent deuterium between two states; (e.g. apo versus ligand bound) is indicative of conformational alterations." This paragraph is incorrect. In reality, comparison of bound states vs. unbound states show differences in amide exchange purely due to binding of the ligand to the surface of the protein. Most of the differences in exchange that are observed in the work presented in this paper correspond to these types of changes. In the HDX results section the authors sometimes allude to this (despite their introductory statements which explicitly ignore it) but they should be very clear. If the lipid-binding domain shows protection in the lipid-bound state as compared to the free state, this is undoubtedly due to the fact that the lipids are interacting with the surface of the protein and decreasing exchange. No conformational change need be hypothesized.

The situation is similar for nucleotide binding. On the other hand, they do observe differences in exchange far from the known binding regions and these are likely the ones corresponding to conformational changes.

The referee is correct and we apologize for the lack of clarity around the interpretation of the obtained HDX data. It was our intent to use this technique to dissect conformational changes from perturbation in exchange behavior resulting from ligand interactions at known binding sites. We have edited the results section to be more in line with the observations.

The supplementary heat maps need a much more thorough legend. As it is, these data could not be properly evaluated. It is not clear what is being depicted in these figures.

We have changed the heat maps to add grey (no statistically significant change) and described this scale more clearly in Figure legend 1, where it first appears.

Usually, heat maps show the change over time or the difference over time of the same peptides. In this case, according to the methods section, the authors state "deuterium uptake for each peptide is
calculated as the average of %D2O for the 6 time points (10s, 30s, 60s, 300s, 900s and 3600s)". Are they really averaging deuterium uptake across such a wide range of times? Such an approach is completely unconscionable. They should choose a single time point to compare between states.

We apologize for the lack of clarity in describing the HDX data in the methods and results. The reviewer suggests it would be better to compare %D2O values at a single time point as supposed to an average across multiple time points to better observe the differences between the Apo and ligand bound states. This may be true if the analysis was done manually, however, the software used in these experiments (HDX Workbench) is performing statistical analysis at all time points in the large dataset. Any peptide that shows a statistically significant difference between the Apo and ligand bound states at any single time point is highlighted (colored) as statistically significantly different even when average %D2O values are used because the software uses p values at individual time points to color code peptides. To consolidate the enormous amount of data, we have simplified the plots by showing the average over all time points. This is now more clearly explained in the text.

It is also important to note that presenting the HDX data as an average across all time points in the data set is a standard practice in the HDX field and we (the Griffin lab) have published several manuscripts using the same approach. Moreover, picking an individual time point to depict the HDX data has the caveat of missing perturbation differences that might be unique to shorter or longer time points. Hence we have kept the current approach of averaging the HDX data across various time points but have included a clearer description of the approach and data processing in the methods section. Peptides that show statistically significant differences even at only one time point can be the focus of additional figures, plots, graphs. Overall this approach helps consolidate a very large amount of information without losing important information.

There are numbers in the bars corresponding to the peptides that are also not defined. It's a little odd how (for example) the peptide corresponding to residues 80-94 shows increased exchange, but then two peptides below it do not show differences. This is also true for the peptide around residue 310.

We thank the reviewer for pointing this out as we needed to add additional background information to avoid ambiguity in the interpretation of the HDX data. This observation is expected given what the experiment is measuring: amide exchange at the peptide level, not single amide level. Assuming the reviewer is referring to figure S2a, yes there are indeed three peptides covering the regions 80-94 one of which is colored yellow (80-94) (meaning deprotection of one or more amides somewhere in residues 80-94) and two shorter peptides (84-94) that are colored grey (no statistically significant change in exchange behavior in residues 84-94). The conventional approach in the field of bottom up HDX-MS is to use overlapping peptic peptides in the heat map to help narrow down the perturbation differences to specific regions of proteins (within a few amino acids) using a subtractive analysis approach. Using that strategy, the way to interpret the HDX data in this region is to subtract the two shorter grey peptides (84-94) from the longer yellow peptide (80-94) and the deprotection differences can be localized to residues 80-83, whereas in residues 84-94 there is no statistically significant change in protection. The same situation holds true for figures S2b and S2d. An explanation for this has been included in the methods section.

In Fig S2b, there are peptides that are nearly identical (residues 305-330 two of which are protected and one is not...It is also hard to reconcile the different peptides in Fig S2b corresponding to residues 170-210.

See response above. While there are three peptides corresponding to the region 305-332 in figure S2b two of which are colored green (protection) and one is colored grey, all three peptides show the same trend of protection to exchange (difference in D2O value of -5 to -6). The HDX workbench software colors peptides green/blue only if they show a difference in average D2O value of -5 or greater and if at least one of the time point shows a statistically significant difference between the two states with a p-value of < 0.05. The peptide that is colored grey did not show the statistically significant difference at the two time points based of the software and hence colored grey even though it showed a trend towards deprotection. However, to avoid confusion to the readers this peptide has been removed in the current version of figure S2b.
There are many such cases in Fig S2d. These discrepancies made me think I may not have guessed correctly what is being depicted in these figures. In any case it is important to provide a clear legend so that readers can confidently identify discrepancies.

See above two comments. We apologize for not providing sufficient explanation.

Figure S2A has a box that is not defined near residue 430...

The box around residues 430-435 in figures S2a-d represents the helix $\alpha^2$ and a note for the same has been included in the figure legends.

Pg 13 the authors use the word motility where I think they mean to use the word mobility

Thank you for pointing this out. We have now changed motility to mobility throughout the manuscript to unambiguously represent PHD motion relative to the stalk.

Regarding the bulk FRET data, the authors need to discuss somewhere how they have controlled for different oligomerization states in the bulk FRET data. Is it possible that the FRET is an average of different oligomeric states as well as differentially "open" or "closed" states? A few added sentences would be useful here.

IAEDANS excitation is strongly dependent on neighboring tryptophan residues (when excited at 295 nm) and the loss of FRET is a clear indicator of the perturbed interaction between PHD and stalk, but does not report oligomerization. Thus, dynamin oligomerizes in low salt buffers (50mM NaCl) as reflected by changes in light scattering, however this assembly does not involve PHD mobility nor alter the FRET signal. This is an important point that we now show in Supplemental Fig S3E and describe in the text (page 10).

2nd Editorial Decision 09 December 2015

Thank you for submitting the revised version of your manuscript to us. Your manuscript has now been seen once more by the original referees (see comments below), and I am happy to inform you that they are both broadly in favor of publication, pending satisfactory minor revision.

I would therefore like to ask you to address referee #1’s remaining concerns and to provide a final version of your manuscript.

REFEREE REPORTS

Referee #1:

Almost there, but there is still a mistake in Figure S4b. In this figure, one of the dynamin variants exhibits a concentration-dependent GTPase increase and the other not. Since dynamin CC is essentially behaving as wt in the biochemical assays in Figure 4, this variant should show concentration-dependent GTPase activity (it does not in the modified figure). I therefore assume it is the closed variant that does not have a concentration-dependent GTPase activity? The authors should carefully recheck what they have measured here. It could be an interesting point if the closed dynamin variant does not display concentration-dependent GTPase rates.

Typos:
p11, bottom: As Dyn1closed forms higher order oligomers in the presence of GDP-ALF4 (Fig. S4A) (NOT S4B).

Figure Legend S3: GTPase activity of Dyn1wt, IAEDANS labelled (*) and un-labelled Dyn1Y354C (ADD (*))
Figure S3: Y354C Dyn1 (Sup) (5 is missing)

Referee #2:

The authors have addressed all of my concerns. The manuscript is now acceptable.

Response to Referee #1:

Almost there, but there is still a mistake in Figure S4b. In this figure, one of the dynamin variants exhibits a concentration-dependent GTPase increase and the other not. Since dynamin CC is essentially behaving as wt in the biochemical assays in Figure 4, this variant should show concentration-dependent GTPase activity (it does not in the modified figure). I therefore assume it is the closed variant that does not have a concentration-dependent GTPase activity? The authors should carefully recheck what they have measured here. It could be an interesting point if the closed dynamin variant does not display concentration-dependent GTPase rates.

Typos:
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Figure Legend S3: GTPase activity of Dyn1wt, IAEDANS labelled (*) and un-labelled Dyn1Y354C (ADD (*))

Figure S3: Y354C Dyn1 (Sup) (5 is missing)

Once again our labeling of the Y-axis of now Extended View figure 3C, was unclear. The data is presented as Specific GTPase activity (we had labeled the Y axis ‘GTPase rate’, which was ambiguous); hence the uncrosslinked control, which like WT does not aggregate at these concentrations, shows no concentration-dependent increase in basal specific GTPase activity; whereas the Dyn1closed construct does. We have also rewritten the text (bottom, page 11) to make this much clearer, and of course, fixed the typos.

3rd Editorial Decision

Thank you very much for sending your revised manuscript. I appreciate the introduced changes and I am happy to accept your manuscript for publication in The EMBO Journal.