Flexible pivoting of dynamin PH-domain catalyzes fission: Insights into molecular degrees of freedom

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RE: Manuscript #E20-12-0794
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Dear Dr. Srivastava,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript is not acceptable for publication at this time, but may be deemed acceptable after specific revisions are made, as described in the reviewer comments below.

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Reviewer #1 (Remarks to the Author):

I found this paper very interesting and clearly written. The authors have employed a suite of coarse-grained tools and analysis methods to analyze dynamin interacting with the membrane. The authors provide all their configuration files on github. The authors have managed to obtain residue level insight by investigating specific mutants as postive or negative controls and made new predictions that can be tested in future experiments.

I have the following questions for the authors which perhaps they can make clear.

1. Was there any evidence of induction. of mean or gaussian curvature? If so can this be inferred from the undulation spectra? It appears that the snapshots for deformation show Gaussian curvature

2. The authors comment that dynamin reduces the bending modulus. How about Gaussian rigidity?

Reviewer #2 (Remarks to the Author):

Baratam et al.

This interesting paper describes a series of molecular dynamic simulations, anchored in the biochemical and structural literature, around the nature of dynamin pleckstrin homology domain (PDH) -membrane interactions. While this approach has been used in recent studies to study dynamin-membrane interactions and the mechanism of dynamin-mediated fission (e.g. by Pannuzzo et al. 2018, ref 29; Fuhrmans and Muller, 2015, ref 92; Matilla et al., 2015 ref 25) these studies have focused largely on the dynamin helix and not the PHD. The focus of this study is especially relevant to distinguish two predominant models for dynamin-mediated fission. The first, and still predominant model, focuses exclusively on GTP-driven conformational changes of the assembled dynamin helix to drive membrane constriction and fission, the second suggests an essential role for PHD-lipid interactions needed to destabilize the lipid bilayer and lower the energy for membrane fission. Indeed, in the most recent review on dynamin-catalyzed fission (Antonny et al., EMBOJ 2016) in which the two models were discussed there was considerable skepticism raised as to the role of the PHD, to quote
"It was shown that the PH domain of dynamin contains a rather short amphipathic loop that could wedge itself into the membrane to constrict it further (Ramachandran et al, 2009). Indeed, biochemistry experiments show that the residues of this helix insert deeper in the leaflet in a nucleotide-dependent manner (Mehrotra et al, 2014; Mattila et al, 2015). However, this hypothesis has received some skepticism, as the position of this loop, away from the PIP2 binding pocket in the PH structure, does not allow for insertion in the membrane without releasing its link to PIP2.

Moreover, the loop (a few amino acids) is so short that one can question the fact that it could generate enough curvature to constrict further the membrane.
A solution might come from the fact the PH domains would tilt when dynamin is constricted (Shnyrova et al, 2013) (see Fig 3A). In the super-constricted state, one PH domain per dimer seems tilted in the cryo-EM data, which could indeed push the helix further in the leaflet (Sundborger et al, 2014). However, the resolution of the currently available cryo-EM data is too limited in order to confirm tilting. Whether this loop insertion is sufficient to create curvature, and whether it keeps its link to PIP2 is still unclear."
Adding to this confusion was the paper by Dar and Pucadyil (ref 3), which despite its declarative title "The PHD of dynamin is dispensable for membrane constriction and fission"; nonetheless showed that the PHD greatly facilitated the rate of fission (i.e. like a catalyst?!).

The data presented here provides strong evidence in support of the second, less favored, two-stage model for dynamin-catalyzed fission and hence will be an important contribution to the field. While I am not qualified to assess the mathematics behind the molecular simulation, the data seem securely anchored in structural and biochemical findings of others. The analysis of point mutations previously studied and their effects on the modeled behaviors provide strong mechanistic insight into the functional consequences of these mutations. As for any good modelling paper, the results presented here also lead to new specific and testable hypotheses.

Major comments worth addressing:

• Lines 194-196. The two prevalent models are those stated above. I am not familiar with the 'Instability model' and not sure the two cited references (73 and 74) describe it well. Instead, I would cite Morlot and Roux (ref 2) as the most prevalent model and/or the review cited about that describes and juxtaposes both. Also, while you suggest that these will be 'discussed later in the text', I believe that your data merits a more thorough discussion of the two models and the now growing structural, biochemical and modeling evidence (including your work) supporting a role for PHD-lipid interactions through hydrophobic variable loops in driving fission by altering lipid conformation, membrane bending rigidity, etc.
• The results on VL3 and the Y600 residue are particularly interesting from a mechanistic standpoint. The Y600 was described as being essential for curvature generation/sensing, both in the context of full length dynamin (Liu et al., ref 11) and with the isolated PHD (Mehrotra et al., ref 18). I wonder if changes in lipid packing that occur in highly curved membranes might increase the exposure of the PIP2 headgroup for interactions with this residue or vice versa. In this regard, you state on lines 294-295, that the Y600L mutation 'is primarily attributed to the overall instability of the dynamin polymer on the membrane surface [18]." I didn't see data to support this conclusion in ref 18. Indeed, the isolated PHD Y600L showed defective curvature sensing independent of assembly and the assembly properties of the full length Y600L mutation was not studied.
• Lines 403-405. You suggest that the 'catalytic' role of dyn-PHD is more 'mechanical', but I find this a semantic argument. By definition a 'catalyst' lowers the energy barrier for a reaction to occur. The term does not infer the 'mechanism' of catalysis, for example many enzymes function by binding the substate in a stained conformation, hence 'mechanically' contributing to breaking of a bond. Others simply bind the transition state of the substrate more tightly (a feature used to design catalytic antibodies). I believe this distinction is important and the two prevailing models for dynamin-driven fission involve a) purely mechanical forces of twisting, torque, constriction or b) the need for catalysis through PHD mediated lipid interactions.
• While the authors have included a thorough citation list, one paper not cited (Srinivasan et al., EMBO J, 216, PMID 26783363) uses HDX-mass spec to identify changes in accessibility of dynamin residues upon nucleotide and/or membrane binding. Interestingly, and perhaps inconsistent with the

VL4 models described here, while these authors detected significant protection of residues in peptides derived from VL1 and VL3 upon lipid binding, there was no detectable change in protection of VL4 associated peptides. This should be discussed.

Minor comments/typos:

• Abstract. I recommend adding the disclaimer that "The PHD is dispensable for fission of model membranes, albeit a much slower rates..." As point mutations in the PHD clearly establish its critical role for CME/fission in living cells.
• Line 166. Should be Figure 3a
• Line 231-232. Figure 5c and 5d are swapped. It is also difficult to distinguish the colors and see the labels on the variable loops.
• Lines 275, "...our hypothesis is that mutation that keeps the ..." needs to be rewritten and clarified.
• Lines 284-286. This is an interesting hypothesis but needs clarification. The I533A mutation is more labile on the membrane but is severely defective in fission (ref 17). I imagine the same would be true for K539 in VL1, not studied in Fig 6E.
• Lines 294-295. I didn't see data to support this conclusion in ref 18. Indeed, the isolated PHD Y600L showed defective curvature sensing independent of assembly and its assembly properties were not studied.
• The paragraph starting on line 301 is very long and could be divided into 2 paragraphs, the second starting at line ~316 related to membrane interaction modes.

Response to reviewers comments (manuscript # E20-12-0794)

We thank the reviewers for careful inspection of our manuscript and for their insightful comments. In the following text, we have responded to their comments in a point-wise manner below. Whenever required, we have also incorporated suggested changes in the revised manuscript. Comments from the reviewers are marked in Red while the authors' response is in Blue.

Response to comments/suggestions from Reviewer #1

Comment #1: I found this paper very interesting and clearly written. The authors have employed a suite of coarse-grained tools and analysis methods to analyze dynamin interacting with the membrane. The authors provide all their configuration files on github. The authors have managed to obtain residue level insight by investigating specific mutants as positive or negative controls and made new predictions that can be tested in future experiments.

I have the following questions for the authors which perhaps they can make clear.

Thanks.
We provide pointwise response below.

Comment #2(a): Was there any evidence of induction of mean or gaussian curvature?

To address the above question, we evaluated the spatial distribution of mean and Gaussian curvature for our membrane system, which we describe below. We extracted the co-ordinates of phosphate headgroups of lipids corresponding to one of the leaflets from the coarse-grained simulation systems. And we applied a 2-D Delaunay triangulation algorithm to generate a polygonal mesh of vertices from the co-ordinates of phosphate headgroups. The surface, so generated, was used for evaluating the mean and Gaussian curvature at various vertices of the mesh. We performed these calculations using Python scripts where we used built-in subroutines from the VTK (Visualization ToolKit) library.[1] This same library is used as one of the core modules in Memsurfer.[2] The results were visualized using Paraview[3,4] and its plugins. The Python script developed for the purpose is deposited in the Github repository.

We applied our algorithm on pure membrane and membrane with randomly placed PH Domains. These two systems are discussed in the originally submitted manuscript. To further test for curvature induction, we also created a third system where the PH domains were arranged in double collar – this was done to crudely model the scaffold and with an intention to accentuate the effect of PH Domain on curvature induction. The results for the three system, both for the Gaussian and mean curvature analyses are shown in Fig. R1 and Fig. R2, respectively.

At individual snapshot level, we did not see any noticeable spatial correlation between position of induction of curvatures and position of PH domains. This was true for both mean and Gaussian curvature. It should be noted that the curvatures observed in these membrane systems are quite dynamic in nature. To observe curvatures that are persistent over time (and not simply thermal fluctuations), we performed time-averaging of curvature observed at each point of the membrane surface. Fig. R3 and Fig. R4 show the spatial distribution of time-averaged Gaussian and mean

curvatures observed in the CG simulations. We also generated movie files of the trajectories showing the time evolution of curvatures and they are included in in the supporting information as *gaussian-curvature.mp4* and *mean-curvature.mp4.*

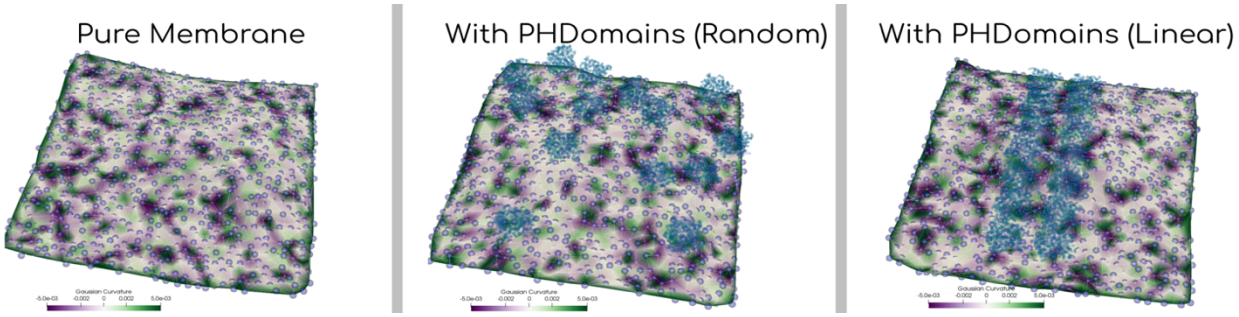


Fig. R1 : Single snapshot of Gaussian curvature observed in (a) pure membrane, (b) membrane with randomly placed PH Domains and, (c) membrane with PH Domains arranged in a collar formation.

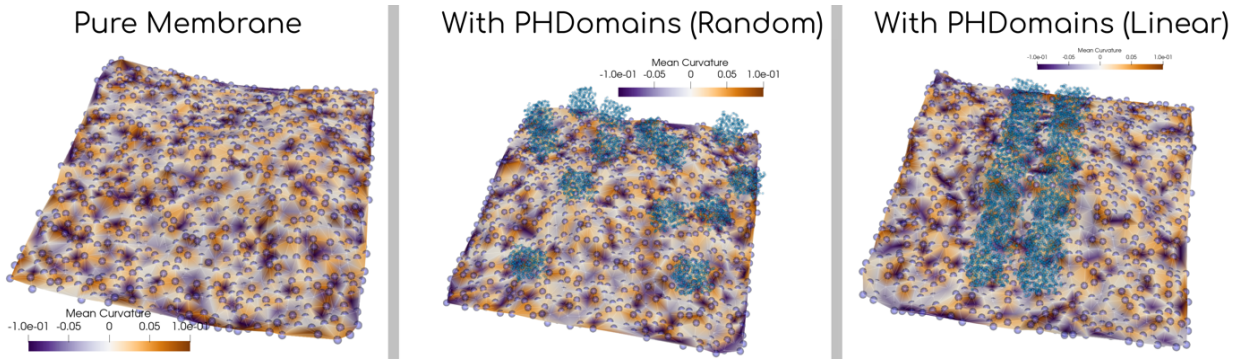


Fig. R2 : Single snapshot of mean curvature observed in (a) pure membrane, (b) membrane with randomly placed PH Domains and, (c) membrane with PH Domains arranged in a collar formation.

As seen in Fig. R3, the randomly arranged PH Domain system does exhibit small scattered patches of both positive (Green) and negative (Purple) Gaussian curvatures (though small in magnitude) while pure membrane exhibits predominantly zero curvature (White color). In particular, there is one particular region on the membrane where noticeable Purple patch is seen and this corresponds to the region where a few PH domains are closely positioned. To some extent, this observation suggests that PH domain may be inducing a negative Gaussian curvature (Saddle regions) on the membrane. For the new hypothetical system with PH Domains clustered in a linear arrangement, we can clearly see the presence of persistent patches of both positive and negative Gaussian curvature in systems with PH Domain. We see similar trends for mean curvatures as well (Fig. R4) where most significant changes in curvatures are seen in collar formation. However, we do not find this surprising as the system was designed for an amplified induction of curvatures. Of note, in physiological conditions, the distance between two PH Domains in a dynamin collar is much larger than what we have here. Nevertheless, this analyses sheds some important insights into the curvature generation role of PH Domain.

In our revised manuscript, we have included a separate section in the supporting information where we report the above-discussed curvature analyses.

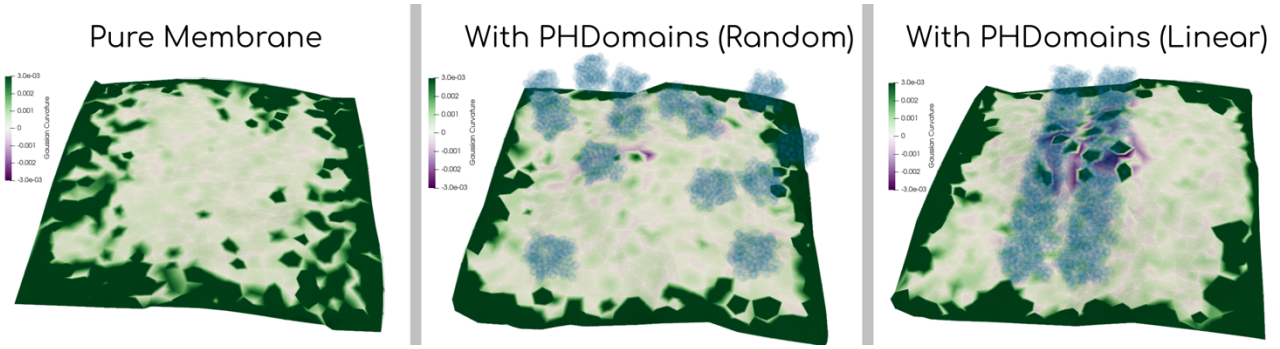


Fig. R3 : Time-averaged Gaussian curvature observed in (a) pure membrane, (b) membrane with randomly placed PH Domains and, (c) membrane with PH Domains arranged in a collar formation. The high positive Gaussian on the four edges are artefact of boundary effects and should be ignored.

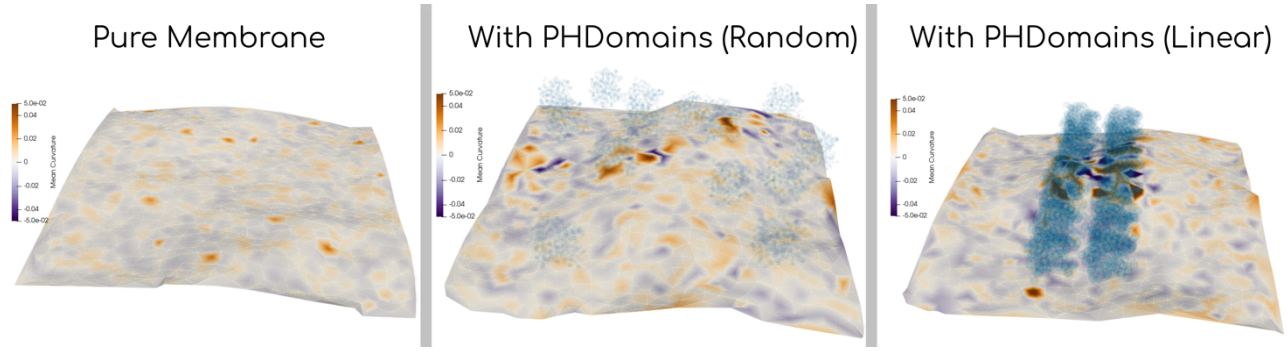


Fig. R4 : Time-averaged mean curvature observed in (a) pure membrane, (b) membrane with randomly placed PH Domains and, (c) membrane with PH Domains arranged in a collar formation.

Comment #2(b): If so, can this be inferred from the undulation spectra? It appears that the snapshots for deformation show Gaussian curvature.

In the response to Comment #2(a) above, we have reported the mean and Gaussian curvatures by direct calculations and we hope that suffices. In the following text, we address the issues of curvature calculations from undulation spectra.

In our experience, making inferences about curvatures using undulation spectra is replete with non-trivial factors. The classical Helfrich theory assumes pure undulations (thermal fluctuations) and for a membrane exhibiting curvatures, the Hamiltonian has to be augmented with terms that accounts for curvature-undulation coupling. An attempt in that direction was made by Ravi Radhakrishnan's laboratory at UPenn[5] and we tried that algorithm for our studies to see if we could capture the curvature using the spectra. The augmented Helfrich Hamiltonian is give in the following equation where $C_{0,q}$ is the additional terms entering the spectrum and one that couples thermal fluctuations with curvatures.

$$\mathcal{H} = A\sum_{q}\left\{\langle|h_q|^2\rangle\left(\frac{1}{2}k_c q^4 + \frac{1}{2}\gamma q^2\right) + \left(k_c\{\langle h_q C_{0,q}\rangle q^2 + \langle {C_{0,q}}^2\rangle\right)\right\}$$

We chose to run the algorithm on the newly created collar system since the curvatures are supposed to be most accentuated in that system. Please see Fig. R5 below.

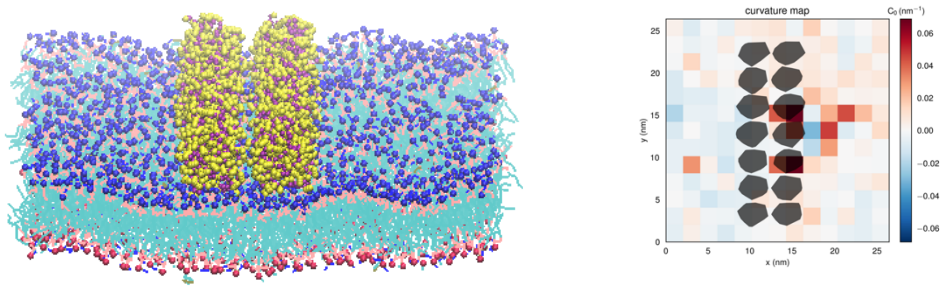


Fig. R5: Curvature analysis of the collar system trajectories by fitting the spectra to the modified Helfrich formulation wherein the curvature and undulations are coupled.[5] (Left) The Martini system with 2048 lipids and 14 PH Domains arranged in a scaffold formation. (Right) Ensembled average curvature field obtained from the spectra analysis of the augmented Hamiltonian.

The curvature values we obtained are of similar range as shown in the direct calculations above using fundamental forms of the surface. However, two things needs to be considered here. Both considerations make the curvature calculations using undulation spectra tenuous and hence we have avoided this approach in our manuscript. Firstly, do note that the augmented Hamiltonian actually couples **spontaneous** curvature ($C_{0,q}$) [something not directly/easily measurable in experiments] and the term entering the spectrum are not the Fourier components of the bilayer curvature per se. Secondly, the curvature reported from the spectra analysis is always ensemble averaged and limits the insights we can obtain from simulations as compared to direct calculations using fundamental forms of the surfaces where we can frame-wise track the spatial evolution of curvature.

Comment #2(c): The authors comment that dynamin reduces the bending modulus. How about Gaussian rigidity?

The Gaussian rigidity term in the membrane free energy is topologically invariant. Hence, unless the membrane remodelling involves a topology or boundary change (such as fission or fusion or a phase interface), the Gaussian rigidity has no effect on the total free energy of the membrane.[6,7] While presence of PH domains does create local curvatures, the total free energy contribution from the saddle splay (Gaussian) curvatures is a constant for our flat bilayer simulations and it should not change for our system since no topology changes are involved in our simulations.

References:

1. Schroeder, Will J., Bill Lorensen, and Ken Martin. *The visualization toolkit: an object-oriented approach to 3D graphics*. Kitware, 2004.

2. Bhatia, Harsh, et al. "MemSurfer: a tool for robust computation and characterization of curved membranes." *Journal of chemical theory and computation* 15.11 (2019): 6411-6421.

3. Ahrens, James, Berk Geveci, and Charles Law. "Paraview: An end-user tool for large data visualization." *The visualization handbook* 717.8 (2005).

4. Bethel, E. Wes, Hank Childs, and Charles Hansen, eds. *High performance visualization: Enabling extreme-scale scientific insight*. CRC Press, 2012.

5. Bradley, Ryan P., and Ravi Radhakrishnan. "Curvature–undulation coupling as a basis for curvature sensing and generation in bilayer membranes." *Proceedings of the National Academy of Sciences* 113.35 (2016): E5117-E5124.

6. Hu, Mingyang, John J. Briguglio, and Markus Deserno. "Determining the Gaussian curvature modulus of lipid membranes in simulations." *Biophysical journal* 102.6 (2012): 1403-1410.

7. Fonda, Piermarco, et al. "Measuring Gaussian rigidity using curved substrates." *Physical Review Letters* 125.18 (2020): 188002.

Response to comments/suggestions from Reviewer #2

This interesting paper describes a series of molecular dynamic simulations, anchored in the biochemical and structural literature, around the nature of dynamin pleckstrin homology domain (PHD)-membrane interactions. While this approach has been used in recent studies to study dynamin-membrane interactions and the mechanism of dynamin-mediated fission (e.g. by Pannuzzo et al. 2018, ref 29; Fuhrmans and Muller, 2015, ref 92; Matilla et al., 2015 ref 25) these studies have focused largely on the dynamin helix and not the PHD. The focus of this study is especially relevant to distinguish two predominant models for dynamin-mediated fission. The first, and still predominant model, focuses exclusively on GTP-driven conformational changes of the assembled dynamin helix to drive membrane constriction and fission, the second suggests an essential role for PHD-lipid interactions needed to destabilize the lipid bilayer and lower the energy for membrane fission. Indeed, in the most recent review on dynamin-catalyzed fission (Antonny et al., EMBOJ 2016) in which the two models were discussed there was considerable scepticism raised as to the role of the PHD, to quote "*It was shown that the PH domain of dynamin contains a rather short amphipathic loop that could wedge itself into the membrane to constrict it further (Ramachandran et al, 2009). Indeed, biochemistry experiments show that the residues of this helix insert deeper in the leaflet in a nucleotide-dependent manner (Mehrotra et al, 2014; Mattila et al, 2015). However, this hypothesis has received some scepticism, as the position of this loop, away from the PIP2 binding pocket in the PH structure, does not allow for insertion in the membrane without releasing its link to PIP2. Moreover, the loop (a few amino acids) is so short that one can question the fact that it could generate enough curvature to constrict further the membrane. A solution might come from the fact the PH domains would tilt when dynamin is constricted (Shnyrova et al, 2013) (see Fig 3A). In the super-constricted state, one PH domain per dimer seems tilted in the cryo-EM data, which could indeed push the helix further in the leaflet (Sundborger et al, 2014). However, the resolution of the currently available cryo-EM data is too limited in order to confirm tilting. Whether this loop insertion is sufficient to create curvature, and whether it keeps its link to PIP2 is still unclear.*"

Adding to this confusion was the paper by Dar and Pucadyil (ref 3), which despite its declarative title "*The PHD of dynamin is dispensable for membrane constriction and fission*"; nonetheless showed that the PHD greatly facilitated the rate of fission (i.e. like a catalyst?!).

The data presented here provides strong evidence in support of the second, less favoured, two-stage model for dynamin-catalyzed fission and hence will be an important contribution to the field. While I am not qualified to assess the mathematics behind the molecular simulation, the data seem securely anchored in structural and biochemical findings of others. The analysis of point mutations previously studied and their effects on the modelled behaviours provide strong mechanistic insight into the functional consequences of these mutations. As for any good modelling paper, the results presented here also lead to new specific and testable hypotheses.

Thanks. Yes, our results do lend credence to the model that emphasizes the role of PHD-membrane interactions in mediating/expediting the fission activity.

Major comments worth addressing:

• Lines 194-196. The two prevalent models are those stated above. I am not familiar with the 'Instability model' and not sure the two cited references (73 and 74) describe it well. Instead, I would cite Morlot and Roux (ref 2) as the most prevalent model and/or the review cited about that describes and juxtaposes both. Also, while you suggest that these will be 'discussed later in the text', I believe that your data merits a more thorough discussion of the two models and the now growing structural, biochemical and modeling evidence (including your work) supporting a role for PHD-lipid interactions through hydrophobic variable loops in driving fission by altering lipid conformation, membrane bending rigidity, etc.

Thanks. In our revised manuscript, we rephrase the sentence to "*The implications of these molecular degrees of freedom in terms of the two paradigmatic models of membrane fission, namely the "constriction/ratchet model" (refs) and the "catalytic model" [23, 26] are discussed in detail later in the text.*" Instability model (or more appropriately Curvature Instability Model proposed by Prof. Stanislas Leibler) does not account for motor/GTP activity and is an equilibrium model where small absorbed molecules may cause spontaneous curvatures and possible instability in the curvature as in the case with echinocytosis in the human red blood cells. We realize that it is not suitable reference here and have removed it. We regret the confusion caused due to the usage of the term "instability model".

To juxtapose our study with the two prevalent model (as discussed by Antonny et al. in EMBO2016 paper), we have added the following paragraph in the Conclusion section.

"With a growing structural, biochemical and modeling evidence supporting the role for PHD-lipid interactions, it is important to put our results in context of the two prevalent models for dynamin-induced fission mechanism [25]. The mechano-chemical (*constriction/ratchet)* model and the catalytic activities *(constriction/stochastic-crossover*) model may not be mutually exclusive and could together constitute the underlying fission mechanism. This aspect is very elegantly presented in the contribution from Frolov and Bashkirov in a recently published topical review [96]. In our work, through molecular scale modeling, we also try to clearly bring out the catalytic aspect of PHD and our work provides molecular insights into how the various variable loops may mediate membrane association, assembly, membrane mechanical properties and pre-fission lipid conformations. Role of membrane rigidity in dynamin-mediated fission is established firmly by experiments [97, 98] and our data on the role of PHDs in inducing local curvatures and enhanced membrane fluctuations (reducing rigidity) lends further credence to the mechanism that proposes stochastic cross over to fission once the constriction reaches a reversible hemifission state [3, 18, 24, 26, 92, 99]. The mechano-chemical *constrictase/ratchet* model for fission [1, 19, 84, 100, 101] treats dynamin as a pure GTP-driven motor protein that triggers sliding of the helical turn leading to membrane constriction and eventual fission. The stalk domain in dynamin, which mediates dimerisation/assembly as well the power-stroke sliding motion, is connected to the activity-providing GTPase on one end and to the membrane-associating PHD on the other end. The x-ray diffraction derived structural data on dynamin constructs [[1, 19, 84]] provides a strong basis for the existence of the above-mentioned model. However, for the stalk domain in particular, which is central to this mechanism, the structural data does not reconcile with the later cryo-EM data [28, 29]. This inconsistency could be due to the possible conformational

rearrangements in the stalk domain particularly during the membrane-bound assembly process. If that is the case, for the stalk domain to function effectively in the proposed mechanism, the role of PHDs as a highly-regulated flexible pivots further comes to the fore and may be used to further reconcile the two prevalent proposed mechanisms in the literature."

• The results on VL3 and the Y600 residue are particularly interesting from a mechanistic standpoint. The Y600 was described as being essential for curvature generation/sensing, both in the context of full length dynamin (Liu et al., ref 11) and with the isolated PHD (Mehrotra et al., ref 18). I wonder if changes in lipid packing that occur in highly curved membranes might increase the exposure of the PIP2 headgroup for interactions with this residue or vice versa. In this regard, you state on lines 294-295, that the Y600L mutation 'is primarily attributed to the overall instability of the dynamin polymer on the membrane surface [18]." I didn't see data to support this conclusion in ref 18. Indeed, the isolated PHD Y600L showed defective curvature sensing independent of assembly and the assembly properties of the full length Y600L mutation was not studied.

Our statement that defects with the Y600L mutation "*are primarily attributed to the overall instability of the dynamin polymer on the membrane surface [18].*" is supported by data in ref. 18 (https://doi.org/10.1091/mbc.e13-09-0548). In their paper, under the section titled "**Evidence for an alternate PH-domain membrane orientation**" (page no. 883, right column) Mehrotra et al., describe spin-sedimentation and FRET (BODIPY-rhodamine) studies of dynamin mutants M534A and Y600L with lipid nanotube and liposomes of varying curvature. The authors observed significantly lower FRET efficiencies in these mutants relative to wild type dynamin system. However, they do not observe any difference in the spin-sedimentation profiles of mutants and wild type as the spin-sedimentation "assay does not distinguish between membrane-associated and membrane-dissociated polymeric dynamin species". With these observations in hand, the authors "attribute the loss of function in Y600L and M534A mutants to an overall instability of the dynamin polymer on the membrane surface". The plots corresponding to these observations were provided in the supporting information (figure S6) of Ref. 18.

We agree with the reviewer about the increased exposure of the PIP2 headgroup and the correlated interactions with residues of VL3. Our data (Fig. 9 (e)) confirms this as well. Along with Y600, R601 and R594 also display long-lived contacts/interactions with PIP2. The AAMD movie file also corroborates the observation that VL4 interacts with PIP2 strongly though insertion of VL4 in the membrane, but is always transient unlike contacts made by VL1 and VL3. Whether the local induction of curvature in membrane makes the PIP2 stick out and favours interaction with VL4 non-transiently or vice versa is something that we have also wondered about. Since our work is on a flat bilayer (and the role of arginine residues on the VL3 is noticeable in interacting with PIP2), we believe that irrespective of the origin, once the PIP2 makes contact with VL3, the interaction would be strong and persistent. We do provide a testable hypothesis to this end: "*While Y600L mutation has been shown to inhibit fission, our data show that R594 and R601 as critical residues on VL3 (Fig. 9(d)) for PIP2 interactions and could be tested experimentally to provide further insights into dynamin's membrane association mechanisms.*"

Also, we do agree with the reviewer's comment that "the assembly properties of the full length Y600L mutation" has not been studied. And our data (in Fig. 7) shows that the mutation causes the PH domain

to become orientationally more labile, which may affect the assembly and formation of the collar. This can be again be tested experimentally.

• Lines 403-405. You suggest that the 'catalytic' role of dyn-PHD is more 'mechanical', but I find this a semantic argument. By definition a 'catalyst' lowers the energy barrier for a reaction to occur. The term does not infer the 'mechanism' of catalysis, for example many enzymes function by binding the substrate in a stained conformation, hence 'mechanically' contributing to breaking of a bond. Others simply bind the transition state of the substrate more tightly (a feature used to design catalytic antibodies). I believe this distinction is important and the two prevailing models for dynamin-driven fission involve a) purely mechanical forces of twisting, torque, constriction or b) the need for catalysis through PHD mediated lipid interactions.

We agree. The argument does come across as purely semantic in nature and in our revised manuscript, we have rephrased the sentence to accommodate the suggestion made by the reviewer. The sentence now reads as: "*The "catalytic" role of dyn-PHD is evident in our work due to its role in lowering the barrier to fission. Multiple binding loops around a PIP_2 may explain how the dyn-PHD is able to "dynamically" keep itself anchored to the membrane while undergoing very rapid shape changes in the midst of fission process.*"

• While the authors have included a thorough citation list, one paper not cited (Srinivasan et al., EMBO J, 216, PMID 26783363) uses HDX-mass spec to identify changes in accessibility of dynamin residues upon nucleotide and/or membrane binding. Interestingly, and perhaps inconsistent with the VL4 models described here, while these authors detected significant protection of residues in peptides derived from VL1 and VL3 upon lipid binding, there was no detectable change in protection of VL4 associated peptides. This should be discussed.

Thanks for bringing this to our notice. Unfortunately, we missed this important work. We have gone through the work and have put it in the context of our work. Srinivasan et al., used HDX-mass spectroscopy to identify the protected and the deprotected residues based on decreased or increased solvent exchange, respectively. We noticed that the results are reported relative to apo dynamin in solution. The residues that show differences in their solvent accessibility, with respect to the apo dynamin in solution, show up in the heat map as either protected or deprotected residues. If it so happens that the residue is inaccessible to the solvent in both the apo state and the membrane bound state, then the residue might not be identified in this given experiment. This is a possible scenario that may explain our observations with VL4. This also seems likely given the fact that dynamin1 exist as dimers and tetramers in solution. If the residues in VL4 remain protected in ensemble of conformations that dynamin takes in solution (involved in interface interactions between monomers) as well as in the membrane bound system (association with membrane) then these residues would neither turn up as protected nor deprotected regions in the heat maps.

To put the work by Srinivasan and co-workers in context of our work, we have added the following towards the end of our Discussion section where we discuss VL4 residues. "*In a HDX-mass spectroscopy based study, Srinivasan et al. explored the accessibility of dynamin residues upon nucleotide and/or membrane binding. Unlike our findings, they did not see any detectable change in protection of VL4 associated peptides, which can be misinterpreted as absence of membrane association for VL4. It should be noted that the membrane accessibility of a given residue was*

reported based on the difference in solvent exchange behaviour of the residue when the dynamin is membrane bound and when it is in apo state (existing as dimer or tetramer in solution). It is possible that VL4 residues may be inaccessible to solvent even in apo state due to the conformation that dynamin dimers/tetramers takes in solution. In that case, the membrane bound state and apo state of the residue will show no difference in solvent exchange, which may likely reconcile the inconsistency with our results that clearly shows that VL4 is membrane bound."

Minor comments/typos:

Thanks for pointing out all the typographical errors. We have corrected them in our revised version and also followed your suggestions for improving the readability of manuscript.

• Abstract. I recommend adding the disclaimer that "The PHD is dispensable for fission of model membranes, albeit a much slower rates..." As point mutations in the PHD clearly establish its critical role for CME/fission in living cells.

Thanks. Since this statement is written based on the work of Dar et al (Ref 3) which is a reconstitution *in vitro* study, it is indeed very appropriate to mention this. We have made this change to our revised version.

• Line 166. Should be Figure 3a

Thanks. This is corrected in our revised version.

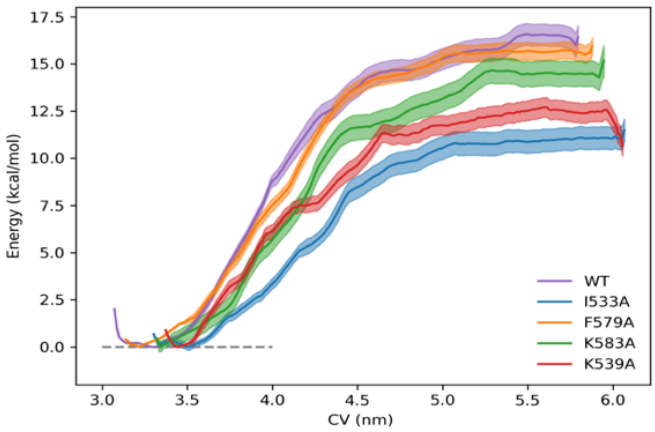
• Line 231-232. Figure 5c and 5d are swapped. It is also difficult to distinguish the colors and see the labels on the variable loops.
We have incorporated the correction and the figures have been updated with darker labels. Please note that the loops VL-1 and VL-4 are buried inside the lipid membrane in figure 5B (converged state of PHD on membrane).

• Lines 275, "...our hypothesis is that mutation that keeps the ..." needs to be rewritten and clarified.

We have written the sentence as below for more clarity. "In our AAMD simulations and free energy calculations, we find that mutants have weaker membrane binding free energy and many of them increase the orientation fluctuations of the PHD. We hypothesize that mutation that makes the PHD highly labile (orientationally) adversely affects collar assembly process leading to compromised fission behavior."

• Lines 284-286. This is an interesting hypothesis but needs clarification. The I533A mutation is more labile on the membrane but is severely defective in fission (ref 17). I imagine the same would be true for K539 in VL1, not studied in Fig 6E.

We calculated the free energy profile of K539A mutant (reported as red line in the following figure.) and we indeed find a similar profile as observed in the case of I533A i.e. significant difference in binding free energy with respect to wild type. In our revised manuscript, we have included the result from K539A data in Fig. 6.



• Lines 294-295. I didn't see data to support this conclusion in ref 18. Indeed, the isolated PHD Y600L showed defective curvature sensing independent of assembly and its assembly properties were not studied.

This is addressed in the major comments above.

• The paragraph starting on line 301 is very long and could be divided into 2 paragraphs, the second starting at line ~316 related to membrane interaction modes.

Thanks. We have separated them into two paragraphs in our revised manuscript.

RE: Manuscript #E20-12-0794R
TITLE: "Flexible pivoting of dynamin PH-domain catalyzes fission: Insights into molecular degrees of freedom"

Dear Dr. Srivastava:

Please, follow the recommendations from Reviewer #2 about a careful checking of English and of the references. It should be quick.

Sincerely,
Patricia Bassereau
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Srivastava,

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 (if any) below.

A reminder: Please do not contact the Monitoring Editor directly regarding your manuscript. If you have any questions regarding the review process or the decision, please contact the MBoC Editorial Office (mboc@ascb.org).

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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In preparing your revised manuscript, please follow the instruction in the Information for Authors (www.molbiolcell.org/info-for-authors). In particular, to prepare for the possible acceptance of your revised manuscript, submit final, publication-quality figures with your revision as described.

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Authors of Articles and Brief Communications whose manuscripts have returned for minor revision ("revise only") are encouraged to create a short video abstract to accompany their article when it is published. These video abstracts, known as Science Sketches, are up to 2 minutes long and will be published on YouTube and then embedded in the article abstract. Science Sketch Editors on the

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Thank you for submitting your manuscript to Molecular Biology of the Cell. Please do not hesitate to contact this office if you have any questions.

Sincerely,

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

Reviewer #1 (Remarks to the Author):

all my comments were addressed

Reviewer #2 (Remarks to the Author):

I thank the authors for their thorough and constructive response to the reviews and trust they agree that the revised manuscript is improved. The modeling results are rigorous and thoughtfully interpreted and will hopefully encourage more experiments probing the mechanisms underlying dynamin-catalyzed fission. Before publication the authors should have a native english-speaker carefully read the manuscript for small grammatical (e.g. agreement between plural nouns and verbs) and other errors (e.g. lines 194-199 are direct repeats). More importantly, the authors should very carefully check their cited references. For example the new reference (96) to a Frolov and Bashkirov review is missing, indeed I could not find it on Pubmed. I did not check, but I suspect there are other errors.

RE: Manuscript #E20-12-0794RR
TITLE: "Flexible pivoting of dynamin PH-domain catalyzes fission: Insights into molecular degrees of freedom"

Dear Dr. Srivastava:

I am pleased to accept your manuscript for publication in Molecular Biology of the Cell. Congratulations!

Sincerely,
Patricia Bassereau
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Srivastava:

Congratulations on the acceptance of your manuscript.

A PDF of your manuscript will be published on MBoC in Press, an early release version of the journal, within 10 days. The date your manuscript appears at www.molbiolcell.org/toc/mboc/0/0 is the official publication date. Your manuscript will also be scheduled for publication in the next available issue of MBoC.

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