nature portfolio

Peer Review File

An integrative modelling approach to the mitochondrial cristae

Corresponding Author: Dr Tsjerk Wassenaar

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
In this work, the authors introduce an integrative modeling approach to represent the human mitochondrial cristae junction. While many molecular dynamics studies have focused on specific components or properties of mitochondria, such as the structure of the inner and outer membranes, the effect of cardiolipin on membrane elasticity, protein-induced membrane curvature, and more recently, a lipid-only model of the entire mitochondrion, the model presented here is the first complete representation of the mitochondrial cristae. It stands as the most comprehensive and representative model to date, utilizing integrative modeling techniques.

To construct this model, the authors employ a range of computational techniques, combining experimental data, AI predictions, and the high-level Martini3 force field. The reviewer acknowledges the challenges involved in building such models, which require advanced computational skills. This model could serve as a foundation for future work, enabling the development of more detailed and realistic models as additional experimental data becomes available.

The paper is well-written, and the results are clearly presented. I suggest that the label "D)" in Fig. 1 be replaced with "B)", as Fig. 1D is discussed prior to Fig. 1C in the text.

Despite the advanced techniques and novel results, the model does not provide fundamentally new insights into the biological mechanisms of mitochondrial cristae. Therefore, the reviewer believes that the manuscript may not be suitable for the high-level journal Communications Biology. It may be more appropriate for Methodological/Computational journals.

Reviewer #2

(Remarks to the Author)
The authors present a molecular model of a mitochondrial cristae and adjacent portion of the mitochondrial outer membrane, the most complete and complex model of the mitochondrial membranes to date. The model incorporates 13 of the most important and abundant mitochondrial membrane protein complexes, embedded in either the outer or inner mitochondrial membranes, the latter of which has realistic cristae geometry. Both membranes have a physiologically accurate lipid composition. The final model constructed, the 'MitoSlice' allows for compartmentalisation between the mitochondrial matrix, the inter membrane space, and the cytosol.

The principal result is the construction of the model, in itself an important step. The authors also present data emerging from the construction and simulation of the model, regarding protein-lipid interactions, protein-protein interactions, and lipid localisation to the three geometric regions of the cristae.

There are a number of interesting observations re protein-lipid interactions (eg. difference in protein-lipid interactions between ANT1 and ANT2, enrichment of PI around most of the proteins simulated) and eg. enrichment of PI at the cristae ridges, which could be followed up in much more depth, but this is perhaps best kept as the focus for other publications.

While there are clearly limitations to the current model (lack of full MICOS complex, OPA1, correct relative abundance of inner mitochondrial membrane proteins) the authors are careful to point these out and stress that this is a 'living model' to be updated as new data becomes available.

Given then, that the principal impact of the paper is as a careful proof of concept, it would be helpful to note the pathway to inclusion of new components to the model. The material found at the github link reportedly includes all the scripts needed to completely construct the model presented - it would be helpful to note this in the paper itself.

Conceptually, the idea to build such a model is not new, the analyses performed are not particularly novel, and the resulting data (eg concerning protein-lipid interactions) could be analysed in greater depth. However the construction of the model is clearly technically very challenging and an excellent achievement in itself, and the authors are to be commended for making scripts for the model construction publically available - the idea of the work being a 'living model' is really exciting and makes the impact of the work much greater. Since new experimental techniques are developing and data is rapidly becoming available regarding mitochondrial structure and dynamics, the establishment of such a model is timely and an important contribution to the field.

A few very minor comments:
'extensive literature search' - it would be helpful at this point to reference the principal eg proteomics papers used to decipher major cristae components

'upper' and 'lower', when referring to leaflets is not clear - this should be changed to eg inner and outer, ie line 669-671

SI Figure 16
The caption should be extended here - it is not clear why the matrix is not symmetric - eg the interaction between ATP synthase and ANT1 is either 0.53 or 1.00. Presumably this is because eg all ATP synthase interact with ANT1, but for instance only proportion of all ANT1s interact with ATP synthase? It should also be made clear which are the numbers being used in Fig 6D

Reviewer #3

(Remarks to the Author)
In this manuscript, the authors set up and perform large-scale coarse-grained molecular dynamics simulations of mitochondrial crista. I do not have expertise with molecular dynamics, so I cannot evaluate the technical aspects of this work. I will give my opinion as an outsider/non-expert.
First, I appreciate the authors' contribution of providing a consensus set of complex structures for human mitochondrial proteins along with a draft model of how they behave in the inner and outer membranes.
Second, I believe that readers would benefit from the authors elaborating on a number of points:
1) More explanation should be given on precisely how the authors selected the 13 proteins/complexes.
2) I had difficulty understanding the boundary conditions that were used in the simulations and their impact on the behavior of the simulated membrane. Is the size of the simulated volume adjusted to set the pressure? Doesn't the size of the box and the initial number of lipid molecules set the tension in the membrane? Do the periodic boundary conditions effectively force the membrane to be flat at large scales? This as well as the absence of the MICOS complex in the simulation and the gradual opening of the cristae calls into question the idea that the simulation recapitulates relevant structural features. One might expect that in the MitoSlice model (depicted but not simulated) the cristae would almost completely flatten since they have less excess lipid for the provided area. It would be helpful if these issues were discussed in more detail.
3) I would guess that the results of these simulations would depend strongly on the surface tension in the membrane. What was the surface tension in the membrane in these simulations and how did the authors decide to use the value they did?
4) Some mitochondria lack cristae, such as those in early development or subpopulations dedicated to proline biosynthesis (Ryu KW, Fung TS, Baker DC, Saoi M, Park J, Febres-Aldana CA, Aly RG, Cui R, Sharma A, Fu Y, Jones OL, Cai X, Pasolli HA, Cross JR, Rudin CM, Thompson CB. Cellular ATP demand creates metabolically distinct subpopulations of mitochondria. Nature. 2024 Nov;635(8039):746-754. doi: 10.1038/s41586-024-08146-w. Epub 2024 Nov 6. PMID: 39506109). It would be interesting if the authors could speculate about how the methods they use could be adopted to those circumstances.
5) It would be very exciting if the authors could attempt to use their simulations to make predictions that could be tested experimentally. For example, it would be very interesting if the authors could attempt to use their simulations to make predictions about the frequency and/or spatial organization of different respiratory supercomplexes, which could be compared with the experimental measurement presented in (Zheng W, Chai P, Zhu J, Zhang K. High-resolution in situ structures of mammalian respiratory supercomplexes. Nature. 2024 Jul;631(8019):232-239. doi: 10.1038/s41586-024-07488-9. Epub 2024 May 29. PMID: 38811722; PMCID: PMC11222160).
6) The authors find that the spacing between the IM and OM remains roughly constant at 20 nm over the course of 4 μs. Is that also true in the absence of MIC60 tethering the IM and OM? If so, are both copies required or can you get away with one?

Version 1:

Reviewer comments:

Reviewer #2

(Remarks to the Author)
Points made in the review of the initial submission have been addressed adequately, in my opinioin, and no further revisions

are required.

Reviewer #4

(Remarks to the Author)
In this work, the authors address the specific challenge of building and simulating the most comprehensive model of the mitochondrial cristae junction to date. The model composition was carefully chosen, with particular attention to membrane details. Both the models and tools are freely available to the community. This work is timely, as it provides a valuable foundation for larger-scale simulations in which cooperative dynamics can only be captured within such complex systems. The authors have also addressed previous concerns related to the molecular dynamics analysis. I recommend this work for publication.

Response to Reviewers

Brown *et al*. "An integrative modelling approach to the mitochondrial cristae"

We are thankful for the overall positive and constructive feedback from the Reviewers. Here, we provide a point-by-point response to their comments.

Reviewer #1 (Remarks to the Author):

In this work, the authors introduce an integrative modeling approach to represent the human mitochondrial cristae junction. While many molecular dynamics studies have focused on specific components or properties of mitochondria, such as the structure of the inner and outer membranes, the effect of cardiolipin on membrane elasticity, protein-induced membrane curvature, and more recently, a lipid-only model of the entire mitochondrion, the model presented here is the first complete representation of the mitochondrial cristae. It stands as the most comprehensive and representative model to date, utilizing integrative modeling techniques.

To construct this model, the authors employ a range of computational techniques, combining experimental data, AI predictions, and the high-level Martini3 force field. The reviewer acknowledges the challenges involved in building such models, which require advanced computational skills. This model could serve as a foundation for future work, enabling the development of more detailed and realistic models as additional experimental data becomes available.

The paper is well-written, and the results are clearly presented.

We are grateful to the Reviewer for their appreciation of our work and for acknowledging the future scope.

I suggest that the label "D)" in Fig. 1 be replaced with "B)", as Fig. 1D is discussed prior to Fig. 1C in the text.

Agreed; this has now been changed in the manuscript.

Despite the advanced techniques and novel results, the model does not provide fundamentally new insights into the biological mechanisms of mitochondrial cristae. Therefore, the reviewer believes that the manuscript may not be suitable for the high-level journal Communications Biology. It may be more appropriate for Methodological/Computational journals.

We appreciate the recognition of the advanced techniques and novel results that are the foundation of the manuscript. However, we believe there are fundamentally new insights presented, such as the structure of the MIC60 subcomplex, which is widely reported to be critical for cristae formation and maintenance, of which there are no reported complete structures.
We have expanded upon the new knowledge in response to Reviewer 3, question 5.

Reviewer #2 (Remarks to the Author):

The authors present a molecular model of a mitochondrial cristae and adjacent portion of the mitochondrial outer membrane, the most complete and complex model of the mitochondrial membranes to date. The model incorporates 13 of the most important and abundant mitochondrial membrane protein complexes, embedded in either the outer or inner mitochondrial membranes, the latter of which has realistic cristae geometry. Both membranes have a physiologically accurate lipid composition. The final model constructed, the 'MitoSlice' allows for compartmentalisation between the mitochondrial matrix, the inter membrane space, and the cytosol.

The principal result is the construction of the model, in itself an important step. The authors also present data emerging from the construction and simulation of the model, regarding protein-lipid interactions, protein-protein interactions, and lipid localisation to the three geometric regions of the cristae.

We are thankful to the Reviewer for their kind comments regarding the construction of the system and the impact a 'living model' can have.

There are a number of interesting observations re protein-lipid interactions (eg. difference in protein-lipid interactions between ANT1 and ANT2, enrichment of PI around most of the proteins simulated) and eg. enrichment of PI at the cristae ridges, which could be followed up in much more depth, but this is perhaps best kept as the focus for other publications.

We appreciate this comment, and while in-depth analysis will be best for other publications, we have added further analysis about selected protein-lipid interaction in lines 265-269 and 277-285 for high-throughput simulations and lines 484-489 for the crista model.

While there are clearly limitations to the current model (lack of full MICOS complex, OPA1, correct relative abundance of inner mitochondrial membrane proteins) the authors are careful to point these out and stress that this is a 'living model' to be updated as new data becomes available.

Given then, that the principal impact of the paper is as a careful proof of concept, it would be helpful to note the pathway to inclusion of new components to the model. The material found at the github link reportedly includes all the scripts needed to completely construct the model presented - it would be helpful to note this in the paper itself.

We are grateful for the suggestion to direct readers to the GitHub repository, and we have included this in lines 88-89 ('*Efficient tools necessary to build the system are shared in an online repository*') and 629-631 ('*The required scripts and structures are freely shared so others can construct and add to the current representation*').
To make it as easy as possible for users to add new proteins of interest, we have included a new section on the GitHub page instructing users how to add new structures into the entire model (https://github.com/chelsea-brown/Mitochondrial_cristae/tree/main?tab=readme-ov-file#how-to-add-tochange-the-system).

Conceptually, the idea to build such a model is not new, the analyses performed are not particularly novel, and the resulting data (eg concerning protein-lipid interactions) could be analysed in greater depth. However the construction of the model is clearly technically very challenging and an excellent achievement in itself, and the authors are to be commended for making scripts for the model construction publically available - the idea of the work being a 'living model' is really exciting and makes the impact of the work much greater. Since new experimental techniques are developing and data is rapidly becoming available regarding

mitochondrial structure and dynamics, the establishment of such a model is timely and an important contribution to the field.

A few very minor comments:
'extensive literature search' - it would be helpful at this point to reference the principal eg proteomics papers used to decipher major cristae components

We appreciate the suggestion and have added references in lines 99-101.

'upper' and 'lower', when referring to leaflets is not clear - this should be changed to eg inner and outer, ie line 669-671

We value the suggestion, but the convention (as used in *insane* [PMID: 26574417] and other tools) is to assign the leaflets as ‘upper’ and ‘lower’. This also prevents confusion when discussing the ‘inner’ and ‘outer’ membranes, which each have ‘upper’ and ‘lower’ leaflets. To make this clearer, we have added labels in Figure 2B and added a reference to this in lines 252 and 254 when describing the leaflets.

SI Figure 16
The caption should be extended here - it is not clear why the matrix is not symmetric - eg the interaction between ATP synthase and ANT1 is either 0.53 or 1.00. Presumably this is because eg all ATP synthase interact with ANT1, but for instance only proportion of all ANT1s interact with ATP synthase? It should also be made clear which are the numbers being used in Fig 6D

We acknowledge the helpful advice and have altered SI Figure 19 and the captions. As is rightfully stated, the discrepancy between the corresponding matrix positions was due to A having contact with B but not all of B having contact with A. As general contacts are displayed here, we have changed Figure SI 19, averaging the corresponding matrix values and only showing half of the symmetric matrix. The numbers used in Figure 6D should also be more representative of the values seen in SI Figure 16, an average of all protein-protein contacts between that protein pair. The figure caption has been modified to clarify this.

Reviewer #3 (Remarks to the Author):

In this manuscript, the authors set up and perform large-scale coarse-grained molecular dynamics simulations of mitochondrial crista. I do not have expertise with molecular dynamics, so I cannot evaluate the technical aspects of this work. I will give my opinion as an outsider/non-expert.
First, I appreciate the authors' contribution of providing a consensus set of complex structures for human mitochondrial proteins along with a draft model of how they behave in the inner and outer membranes.

We appreciate the Reviewer's assessment and perspective as a 'non-expert' in molecular dynamics and their thoughtful insights, particularly given the audience of this biologically focused journal.

Second, I believe that readers would benefit from the authors elaborating on a number of points:
1) More explanation should be given on precisely how the authors selected the 13 proteins/complexes.

We are grateful for the suggestion, and we have added references to lines 99-101 to clarify the selections. The MitoCop database [PMID: 34800366] informed the highly abundant proteins/protein chains for inclusion, which showed that VDAC1 and ANT1/ANT2 were highly abundant and therefore warranted inclusion. The ATPase and MICOS subcomplex were selected as they are necessary for membrane curvature, l-OPA1 and the rest of the MICOS complex were attempted to be modelled for these reasons, but we were unsuccessful. Protein import and folding in mitochondria have been well studied and reported, meaning there was a substantial amount of information on the TIM, TOM and SAM complexes, which gave us confidence in their inclusion in our model. This is also true for the respiratory complexes, which are also fundamental in the functioning of mitochondria.

2) I had difficulty understanding the boundary conditions that were used in the simulations and their impact on the behavior of the simulated membrane. Is the size of the simulated volume adjusted to set the pressure? Doesn't the size of the box and the initial number of lipid molecules set the tension in the membrane? Do the periodic boundary conditions effectively force the membrane to be flat at large scales? This as well as the absence of the MICOS complex in the simulation and the gradual opening of the cristae calls into question the idea that the simulation recapitulates relevant structural features. One might expect that in the MitoSlice model (depicted but not simulated) the cristae would almost completely flatten since they have less excess lipid for the provided area. It would be helpful if these issues were discussed in more detail.

We thank the Reviewer for the questions and thoughts. A rectangular periodic boundary condition (PBC) was used, with the pressure set at 1 bar. The NpT ensemble was used in these simulations, where the number of particles, pressure and temperature are kept constant. The box size is set prior to the simulation, and the number of particles (solvent, lipids, ions and protein) are added to reflect those found at these pressures. The lipids are added to a given area at a set ratio based on their area-per-lipid, meaning that the surface tension should be the same, given different box sizes for the same lipid ratio.
The pressure is controlled using semi-isotropic pressure coupling, where the x and y dimensions are linked but independent of the z-dimension of the box. The semi-isotropic coupling means the membrane would be tensionless if it were not for the lipid asymmetry and proteins inducing curvature. The membrane behaviour follows from the lipids and proteins and their interplay, and this determines the overall lateral pressure.
In the setup with a double membrane, the intrinsic coupling between the membranes adds a further factor, requiring the expansion of the outer membrane to accommodate the flattening

of the inner membrane. The stiffness of the former will prevent this from happening, rather than forcing the inner membrane to fold, thus supporting the cristae. The overall changes in area over the simulations for the individual membranes and the overall system can be seen in SI Figures 15,16.
The periodic boundary conditions for the membrane mean that at each side of the box, the membrane must be in the same z-position, but the PBC will not force the flattening of the membrane. The fact that membranes can go from flat to curved (and be maintained) can be seen in Movie S1 with the simulated ATPase dimers in this manuscript. Other recent studies [PMID: 39453744] on different systems show how curvature can be induced and maintained when using periodic boundary conditions.
We have added some clarifying sections into the manuscript in lines 600-604 ('*although this could be a feature of the two membrane's areas being intrinsically coupled in these systems. While in vivo, the mitochondrial membranes will not be independent, it is difficult to measure how this will compare to simulation. Removing the periodicity issues can only be achieved by simulating an entire mitochondrion'*) to avoid any future confusion. Further clarifying lines (759-760, 776-777 and 784-785) have been added to the methods in addition.

3) I would guess that the results of these simulations would depend strongly on the surface tension in the membrane. What was the surface tension in the membrane in these simulations and how did the authors decide to use the value they did?

The simulations are performed using a semi-isotropic pressure coupling method, which results in tensionless membranes. However, there will still be leaflet tension in the IMM due to the asymmetric lipids and proteins – reflecting the biological reasons that this membrane is buckled (PMID: 32071438, PMID: 38735128).

4) Some mitochondria lack cristae, such as those in early development or subpopulations dedicated to proline biosynthesis (Ryu KW, Fung TS, Baker DC, Saoi M, Park J, Febres-Aldana CA, Aly RG, Cui R, Sharma A, Fu Y, Jones OL, Cai X, Pasolli HA, Cross JR, Rudin CM, Thompson CB. Cellular ATP demand creates metabolically distinct subpopulations of mitochondria. Nature. 2024 Nov;635(8039):746-754. doi: 10.1038/s41586-024-08146-w. Epub 2024 Nov 6. PMID: 39506109). It would be interesting if the authors could speculate about how the methods they use could be adopted to those circumstances.

We are grateful for the suggestion to highlight how these methods can be applied to exciting new results, such as the Ryu *et al.* 2024 paper highlighted. In short, these methods can be applied to any mitochondrial type, as long as there is data about the membrane morphology and their protein concentrations. The same workflow can be applied to differently shaped membranes and protein concentration by using a different triangulated surface and altering the bentopy/TS2CG input, otherwise performing the same steps. This has been highlighted in the manuscript in lines 621-624 ('*This approach can also be expanded to other mitochondria of interest, as the morphology varies greatly between species and even between mitochondria in the same cell under different metabolic conditions. To this end, different membrane shapes, proteins and protein concentrations can be used as input'*) and an extra section in the GitHub repository (https://github.com/chelsea-brown/Mitochondrial_cristae/tree/main?tab=readme-ov-file#how-to-add-tochange-the-system).

5) It would be very exciting if the authors could attempt to use their simulations to make predictions that could be tested experimentally. For example, it would be very interesting if the authors could attempt to use their simulations to make predictions about the frequency and/or spatial organization of different respiratory supercomplexes, which could be compared with the experimental measurement presented in (Zheng W, Chai P, Zhu J, Zhang K. High-resolution in situ structures of mammalian respiratory supercomplexes. Nature. 2024 Jul;631(8019):232-239. doi: 10.1038/s41586-024-07488-9. Epub 2024 May 29. PMID: 38811722; PMCID: PMC11222160).

We thank the reviewer for the comment and agree that it is very exciting to use these techniques to make predictions that can be tested experimentally. We believe we have already made a number of these in this body of work. Namely, the prediction of the structure of the MIC60 subcomplex, which is yet to be resolved fully. These subcomplexes are critical for cristae maintenance, so having an understanding of their structure and function is of value to the community. It is also interesting that we cannot pack the inner membrane proteins to the amounts reported experimentally [PMID: 32916174]. Considering that many membrane protein components are missing, it could indicate that these values are too high. Experimentally determining protein concentrations for a wider range of mitochondrial types (ie from different cell types) would shine more light on this issue.
The opening of the crista junction in simulations without the OMM adds further evidence to the requirement of l-OPA1 and/or the small-MICOS subunit to maintain the curvature and junction width. When experimentally elucidated or confident models for these proteins are resolved, they can then be included into these systems and the results compared to this body of work. This can also help resolve a finer level of detail into how these protein complexes perform their reported functions.
Contrasting the lipid sorting in a protein-free membrane from a previously published *Communications Biology* paper [PMID: 38182788], we see unexpected lipid sorting in this crowded membrane. The distribution of lipids in such membranes could also be tested experimentally.
In the original manuscript, we briefly investigated protein-lipid interaction but performed some further analysis to predict experimentally testable predictions for this. Protein-lipid interactions are incredibly important to membrane protein structure, function and oligomerisation [PMID: 30758191]. We performed further analysis and identified some interesting differences between ANT1 and ANT2 (SI Figure 11), which could indicate differences in their functionality. Measuring the POPC density around the TOM complex (SI Figure 13) aligned well with experimentally resolved POPC density [PMID: 35733257]. This offers more confidence to the PAPI density measured (SI Figure 12), which could play an important role in the stabilisation of the complex or interaction with other proteins in the outer mitochondrial membrane. This will be interesting to test experimentally and further computationally. The information about these interactions has been added to the manuscript in lines 265-269 and 277-285.
We also measured protein-lipid interaction in the crista system (SI Figure 21), which showed similar behaviour to those measured in the high-throughput simulations. This shows the importance of including the annular lipids and could reflect their importance for function. As longer simulations and larger systems are studied, measuring the distance between monomers and proteins in contact could reveal key functional lipids in protein-protein interfaces. This has been added to the manuscript in lines 484-489.
Concerning the predictions regarding the supercomplexes, larger-scale simulations containing different arrangements and proportions of these would be the intended method to include the respiratory supercomplex in the ratios measured in the Zheng *et al*. paper mentioned. Over the entire (or large patch of) mitochondrion, the frequency and spatial organisation would be able to be measured with the statistics and sampling to be confident in the conclusions drawn. However, the Reviewer highlights the possibility of answering such exciting questions with work that will be built on the foundations presented here.

6) The authors find that the spacing between the IM and OM remains roughly constant at 20 nm over the course of 4 μs. Is that also true in the absence of MIC60 tethering the IM and OM? If so, are both copies required or can you get away with one?

We are thankful for the interesting question. We performed two further independent replicates of the system, simulating each for 1 μs (SI Figures 16,18). In these simulations, the MICOS subunits did not make contact with the outer membrane but still stayed at approximately the same distance. Over longer simulation times, however, this might change, especially when soluble components in the intermembrane space are introduced.

From the simulations performed, it is difficult to say how many MIC60 complexes would be needed, but the numbers used in these simulations were set to reflect the protein concentration measured in other studies [PMID: 34800366].
As larger and more complex versions of this system are assembled and simulated, it would be a very interesting question to investigate how many of the various protein complexes are needed to perform the functions experimentally observed.