1. Project title

Multiscale spatiotemporal modeling of cardiac mitochondria

2. A one/two sentence summary of the project topic with a hyperlink to more detailed information via the IMAG wiki.

This project seeks to combine multiscale modeling with experiments to understand the dynamic regulation of cardiac muscle mitochondria. Specifically, it seeks to investigate how mitochondrial function is linked to the organelle's inner membrane (crista) nanoarchitecture. A second challenge was to develop an automated method to extract 3D surfaces of membranes (needed for modeling) from electron microscopic tomograms of mitochondria which previously has required much manual intervention.

https://www.imagwiki.nibib.nih.gov/sites/default/files/Lederer_2018%20IMAG%20Futures%20Pre-Meeting%20Abstract.docx

3. Details regarding Model Credibility plan following the CPMS Ten Simple Rules (TSR) format. It is requested that these details be presented as deemed appropriate for each modeling approach. This will be used to help define best practices for future reporting activities. These details should include:

    A. List of planned actions outlined in Model Credibility plan

We have developed our model of mitochondria with observed cristae architectures using the Virtual Cell Modeling Platform to enable sharing and usability by both modelers and biologists.

We are considering the creation of a visual tutorial on how to use our model, possibly using JOVE (Journal of Visual Experiments) to make the model easier for potential users to use.

We will reach out to colleagues in the Virtual Cell group and bioenergetics communities, at the annual MSM and Biophysical Society meetings to test and provide feedback on the model.

We will be publishing journal articles based on the results of the model demonstrating its scientific utility.

    B. A brief description of information gained by each credibility action

In the following paragraphs, information gained by each plan is explained:

The publicly available Virtual Cell (VCell) platform enables sharing the model with some or all users. While developing the model we easily could share it with experts, especially the VCell

team and colleagues in the bioenergetics community and have their comments about our model and our assumptions. In the future, the model(s) will be shared with the public. In this way, scientists from various backgrounds will have access to the model and can use it in their own projects. Availability of the model should also encourage experimental biologists to generate new data to fill in gaps and test different conditions. This model will benefit researchers and educators with backgrounds from Math to Biology.

In order to facilitate future external use of the model, we are working on a video tutorial. Hands-on experience with Virtual Cell is required to understand how it works. Thus, the tutorial on the use of our complex model within the VCell environment should encourage non-specialist scientists to use it. Having the model widely used for different purposes will open new doorways for future collaborations and projects.

By presenting our results in local, national, and international meetings, now and in the near future, we test our assumptions and hypotheses during the developmental stage of the model. Also presenting our results extends the horizon on potential applications of our model, which should be possible with minor modifications.

When the model is published soon as a series of related papers, it will reach experts from all over the world. This step is vital for future utility of the model.

With regard to the development of automated segmentation for application to 3D tomographic volumes, mitochondria represent a special challenge due to the complex topology of the inner membrane, which vary from tightly apposed lamellar stacks with tubular interconnections and fenestrations, to intertwined purely tubular compartments. We have created a database of over 50 mitochondrial tomograms that has allowed us to discover and quantify structural details not previously described. This is allowing more realistic simulations of mitochondrial functions. When complete, the mitochondrial structure database will be made publicly available, so it can be mined by others working in related fields. We intend to provide the automated segmentation tools with the database.

C. Actions and activities classified within the CPMS TSR framework (item-by-item summary table). If any of the TSR items are not being implemented/considered or additional items are being implemented, this information should also be explicitly stated

Task	Actions and Activities
1. Define context clearly	The goal is to develop a model that is useful to both modelers and experimental scientists. The Virtual Cell platform was chosen because it provides a platform for enabling scientists

	from backgrounds ranging from mathematical to biological to use the model. Also, it has several features required for the model such as the ability to read in a stack of 2D images to define a 3D structure. A second challenge was to create a method to develop an automated method to extract 3D surfaces of membranes from electron microscopic tomograms of mitochondria. For this we wanted to leverage existing image processing methods combined into a pipeline using a commonly used platform such as MATLAB to make it useable and modifiable by a wide user base.
2. Use appropriate data	Model dynamic equations were constrained by experimental studies at multiple scales. Model geometries come directly from electron tomograms or idealizations of such structures. Biochemical kinetic parameters and associated data were extracted from primary literature, in some cases with advice and guidance by colleagues in the biophysics community. The membrane segmentation was performed on mitochondrial electron tomograms (used as 3D stacks) generated by the Wadsworth 3DEM Group.
3. Evaluate within context	Specific test cases were chosen to verify model behavior. Studies varying specific parameters representing biological quantities of interest were performed. Numerical experiments simulating various experimental studies and physiological conditions were analyzed. Studies changing distributions of model components to explore different hypotheses were also performed. The results of the automated segmentation procedure were compared to membrane

	surfaces manually curated from tomograms by experts in the Wadsworth Group and examined by experts.
4. List limitations explicitly	The Virtual Cell platform has a strict limitation on the number of grid points in the model that limit simulation size at the resolution needed. This problem is more severe for 3D simulations, which are the most realistic. The crowding and juxtaposition of membranes in the interior of mitochondria and the inherently noisy nature of the tomograms, combined with directional resolution loss in the tomograms, often has made parts of the organelles more difficult to distinguish. Therefore, the automation must be combined with manual segmentation for accuracy.
5. Use version control	Different versions of the model were retained to maintain a manual version control using the Virtual Cell revision control system. The original 3D volume for each tomogram, has been retained, along with the iterations of postprocessing (noise reduction) and segmentation that followed.
6. Document accurately	Documentation for each simulation is being kept offline in lab notebooks and online in spreadsheets associated with VCell. The documentation will be added to annotate the model once production versions are completed. The 3D volumes associated with each electron tomogram (original, progressively denoised versions) are stored in DropBox folders and various media in our labs, readily available with associated documentation on file.
7. Disseminate broadly	We have presented the model and simulations at the Biophysical Society Meetings and NIH

	Consortium PI Meeting. We will continue to present results of both efforts at conferences and lab meetings with outside colleagues. We are planning the creation of a visual tutorial on how to use our software, possibly using JOVE (Journal of Visual Experiments), to assist potential users.
8. Get independent reviews	We will reach out to colleagues in the Virtual Cell group and community, at the MSM consortium meeting, and the annual Biophysical Society meeting to test and comment on the model. We will reach out to the 3D electron microscopy community to test the automated methods.
9. Test competing implementations	There are no competing implementations of mitochondrial function at this level of complexity to our knowledge. We will compare our 3D membrane segmentation methods to existing computational methods, such as those available in IMOD (Univ Colorado, Boulder). These have not worked satisfactorily in the past with structures as complex as the mitochondrial inner membrane.
10. Conform to standards	We are conforming to the Virtual Cell requirements and standards. We have had to push the envelope creating new constructs to accomplish model goals. We are using standard image processing routines that are proven to work for applications like ours and combining them into a unique pipeline that produces reproducible results.

D. Description of how the planned activities will lead to a credible model

A whole reconstruction of the mitochondria will be produced, processed, and saved in a format that will be compatible with a wide range of platforms in order to create a product that is accessible and customizable within the community. The initial credibility of the model will be achieved when it is able to predict behaviors, such as changes in mitochondrial ATP production in response to energy demand and calcium release events, consistent with experiment.

E. Progress to-date and plans for the next reporting cycle (6 months). What has been achieved since last reporting?

During this reporting period, we have been refining our simulations involving the response of mitochondria to ADP depletion and to calcium release events. We have been further exploring the effects of changes in cristae geometry (corresponding to observations in tomograms) and extending the 2D simulations to 3D (not done before). We have implemented and run 3D simulations using both idealized 3D geometry and individual crista compartments extracted from electron tomograms.

4. Issues/concerns identified as critical or problematic to achieve the standard of credibility set by MSM Consortium.

   This project was awarded before a model credibility was required. We have since tried to conform with the standards of credibility. An important concern is buy-in by the bioenergetics and calcium communities. The credibility of the project would be greatly enhanced if experienced colleagues have the time and interest to critically evaluate our results. We will make every effort to achieve this in the time remaining, and expect some success, given the potential applicability of the model to a range of important questions in mitochondrial function.

5. What other factors, if any, contribute to credibility but cannot be reported within the TSR structure? In requesting this information, we seek to identify credibility activities/issues and appropriate ways to report them at upcoming IMAG/MSM meetings.

   We are currently computation-limited, in terms of the size and number of simulations we can run through VCell home resources. This is important to credibility because we have not been able to run all the control and parameter-varying simulations needed for full confidence in the modeling at every stage. Porting the software to available supercomputers requires reprogramming at a level small, specialized groups like ours cannot support. It is likely other projects are facing the same problem.