Advancing Structural Biology by Driving Cryo-EM in New Directions

***Strategic Plan Theme*: Global Impact, Global Impact**

***Funding Level*: More than $5 million, More than $5 million**

***Facility Needs*: Adjustments to existing facilities will be needed, Adjustments to existing facilities will be needed**

***Submitting Unit*: Biochemistry and Molecular Biology, Biochemistry and Molecular Biology**

Collaborating colleges/departments/units involved with this proposal.

Departments: Biochemistry and Molecular Biology, Chemistry, IQ, Microbiology and Molecular Genetics, Plant Biology, Plant Research Laboratories, Plant Resilience Institute, Physiology, Colleges: CHM, COM, CNS, RTSF Core facility (therefore benefits BMB, Chem, MMG, Pharm Tox, Physiology, Plant Biology, IQ, and more)

What is the proposal's big theme or idea?

Michigan State University (MSU) has a strong, internationally recognized structural biology research program shared between the Colleges of Engineering, Human Medicine, Osteopathic Medicine, and Natural Science. MSU recently implemented the Global Impact Initiative ("GII"), which recruited ~75 faculty with the goal of finding solutions to "grand challenges", of which Structural Biology is a main research priority. MSU has also recently established an Institute for Quantitative ("IQ") Heath Sciences and Engineering with an additional 23 faculty positions. One of the focus areas in IQ is the "Structure of Life". MSU has also recently established a department of Computational Mathematics, Science and Engineering (CMSE) to implement high performance computing, and applications towards scientific and engineering modeling and data science. This proposal seeks to expand our established RTSF Core Cryo-EM Facility to provide direct support for the growing needs of these faculty hires and ongoing Structural Biology initiatives, and to set the stage for expansion and recruitment. Currently our core services over 20 laboratories from five departments and three colleges. With our proposed expansion we will be able to broaden the impact of this technology campus-wide.

In response to MSU's Strategic Plan and building on a growing Structural Biology emphasis, a core RTSF facility was established in 2018 to enable cryo-EM on campus, which was the first of its kind dedicated to imaging biological specimens. Part of MSU's initial commitment was a ~$5M investment in basic infrastructure that included the purchase of a single workhorse instrument (FEI Talos Arctica equipped with a Falcon 3 camera), along with auxiliary, support equipment (glow discharging unit, robotic freezing device, carbon coater, etc.), computational storage (>1 peta byte), and renovations to existing space on campus (ERC East room D122). *Importantly, and as part of a long-term vision, the renovations and building designs as well as the computational storage system housed at MSU's Data Center were forward-thinking, allowing us to ultimately expand our facility to accommodate up to three microscopes and additional hires. This included design of the HVAC system, fire suppression and oxygen sensor systems, a liquid nitrogen delivery system, water chiller system, and building plans that could be installed immediately without a need for redesign/contracting. In addition, MSU had included a plan for supporting the facility through new positions. This included two GII tenure track faculty hires, hiring of a senior staff member, and implementing a Director to oversee the facility. The big idea of this proposal is to expand our facility to enable new research directions., Michigan State University (MSU) has a strong, internationally recognized structural biology research program shared between Colleges of Engineering, Human Medicine, Osteopathic Medicine, and Natural Science. Seven new structural biology faculty members have been hired in the last 10 years in the department of Biochemistry and Molecular Biology (BMB) to complement an already strong program. At the university level, MSU

recently implemented the Global Impact Initiative ("GII"), which recruited ~75 faculty with the goal of finding solutions to "grand challenges", of which Structural Biology is a main research priority. MSU has also recently established an Institute for Quantitative ("IQ") Heath Sciences and Engineering with an additional 23 faculty positions. One of the focus areas in IQ is the "Structure of Life". MSU has also recently established a department of Computational Mathematics, Science and Engineering (CMSE) to implement high performance computing, and applications towards scientific and engineering modeling and data science. This proposal seeks to expand our established RTSF Core Cryo-EM Facility to provide direct support for the growing needs of these faculty hires and ongoing Structural Biology initiatives, and set the stage for expansion and recruitment.

What is the proposal's goal?

At Michigan State University (MSU), we have invested in the building blocks to begin solving the needs of the Structural Biology community as a whole. However, we have grown substantially in recent months and are now beyond our initial capability. Thinking ahead to accommodate planned future hires and sustaining the growth rate of biomedical research on campus and beyond (e.g., Henry Ford Institute and others), we have a major need to expand our facility. Here, we propose a five-year strategic plan that will help fill several of MSU's Missions and Strategic Plans. First, we propose to increase efficiency 6-fold through the purchase of a high throughput camera (Falcon 4i), which will allow more users to gain access of our current equipment and make existing projects more efficient. This next generation of cameras has become the norm across national cryo-EM facilities. Second, we propose purchasing and installation of additional equipment and hiring of new faculty to expand our current capabilities to drive research in new directions (e.g., Ceta-D camera, Titan Krios, and Aquilos). The Ceta-D camera adds the capability of solving extremely small molecules (e.g., compounds & metabolites). The Titan Krios in tandem with an Aquilos would add the capability of Cryo-EM FIB milling to allow us to look at extremely large structures such as whole cells. Third, we propose that the new facility will provide ample opportunities for expanding our education and training missions. One aspect would be installation of a Glacios microscope to increase the number of students we can accommodate in our existing courses—so we can dedicate the higher end instrument time for high resolution data collection for more advanced training/users. Another goal is to apply for training grants (such as NIH T32 and NSF NRT programs). I can imagine several innovative training programs that would cover the entire scope of the facility such as "understanding how structural biology mediates life, from atoms to tissues"., At Michigan State University (MSU), we have invested in the building blocks to begin solving the needs of the Structural Biology community as a whole. However, we have grown substantially in recent months and are now beyond our initial capability. Thinking ahead to accommodate planned future hires and sustaining the growth rate of biomedical research on campus and beyond (e.g. Henry Ford Institute and others), we have a major need to expand our facility. Here, we propose a five year strategic plan that will help fill several of MSU's Missions and Strategic Plans (See Figure 3, Table 2). First, we propose to increase efficiency 6-fold through the purchase of a high throughput camera (Falcon 4i), which will allow more users to gain access of our current equipment, and makes existing projects more efficient. This next generation of cameras has become the norm across national cryo-EM facilities. Second, we propose purchasing and installation of additional equipment and hiring of new faculty to expand our current capabilities to drive research in new directions (e.g. Ceta-D camera, Titan Krios, and Aquilos). The Ceta-D camera add the capability of solving extremely small molecules (e.g. compounds/metabolites). The Titan Krios in tandem with an Aquilos would add the capability of Cryo-EM FIB milling to allow us to look at extremely large structures such as whole cells. Third, we propose that the new facility will provide ample opportunities for expanding our education and training missions. One aspect would be installation of a Glacios microscope to increase the number of students we can accommodate in our existing courses—so we can dedicate the higher end instrument time for high resolution data collection for more advanced training/users. Another goal is to apply for training grants (such as NIH T32 and NSF NRT programs). I can imagine a number of innovative training programs that would cover the entire scope of the facility such as "understanding how structural biology mediates life, from atoms to tissues".

Define the significance, or impact of your big idea.

Cryo-electron microscopy (cryo-EM) has emerged as an enormously powerful method for visualizing a variety of specimens [1, 2], permitting biological structures to be preserved in a near native state. High-resolution cryo-EM imaging with "Direct Detection Device" (DDD) technology (developed ~2012) and three dimensional image reconstruction (3DR) methods has resulted in structures at atomic resolution [3, 4] and is revolutionizing the field of cryo-EM [5]. Cryo-EM has already had a profound impact on biomedical research. The cryo-EM "revolution", which has taken place over the past few years has been highly transformative, allowing such applications as high-resolution imaging of complex macromolecules. Several examples of biomedically important structures include the 26S proteasome [6], CRISPR/RNA complexes [7], drug binding to proteins like p97 that are cancer targets [8], antibody interactions with the emerging Zika virus [9], and countless others. Some examples directly from MSU include: understanding mechanisms of virus infection [10], how membrane transporters contribute to antibiotic-resistance [11], understanding calcium sequestering in mitochondria during cardiac events [12, 13], and many others. As such, cryo-EM has a profound impact in many broad disciplines, including but not limited to: Biochemistry, Chemistry, Computational Sciences, Microbiology, Physiology, etc. Cryo-EM is now becoming a standard tool and is democratizing structural biology: anyone that can purify a protein, a compound, or a cell, can effectively solve a structure. Therefore, our efforts to expand this technology on campus will broadly and significantly increase research funding across all biochemical/biological disciplines.

Refernces: 1. Baker TS, et al Microbiology & Molecular Biology Reviews. 1999;63(4):862-922. 2. Eisenstein M. Nature methods. 2016;13(1):19-22. 3. Smith MT, Rubinstein JL. Science. 2014;345(6197):617-9. 4. Binshtein E, Ohi MD. Biochemistry. 2015;54(20):3133-41. 5. Egelman EH. Biophysical journal. 2016;110(5):1008-12. 6. Dambacher CM, Worden EJ, Herzik MA, Martin A, Lander GC. eLife. 2016;5:e13027. 7. Chowdhury S, et al. Cell. 2017;169(1):47-57 e11. 8. Merk A, et al. Cell. 2016;165(7):1698-707. 9. Hasan SS, et al. Nat Commun. 2017;8:14722. 10. Schrad JR, et al. Cell. 2020;181(5):1046-61 e6. 11. George NL, Schilmiller AL, Orlando BJ. Proceedings of the National Academy of Sciences of the United States of America. 2022;119(14):e2123268119. 12. Strubbe-Rivera JO, et al. Appl Sci (Basel). 2021;11(5). 13. Strubbe-Rivera JO, et al. Sci Rep. 2021;11(1):1037. , Cryo-electron microscopy (cryo-EM) has emerged as an enormously powerful method for visualizing a variety of specimens (1, 2), permitting biological structures to be preserved in a near native state. High-resolution cryo-EM imaging with "Direct Detection Device" (DDD) technology (developed ~2012) and three dimensional image reconstruction (3DR) methods has resulted in structures at atomic resolution (3, 4) and is revolutionizing the field of cryo-EM (5). Cryo-EM has already had a profound impact on biomedical research. The cryo-EM "revolution", which has taken place over the past few years has been highly transformative, allowing such applications as high-resolution imaging of complex macromolecules. Several examples of biomedically important structures include the 26S proteasome (6), CRISPR/RNA complexes (7), drug binding to proteins like p97 that are cancer targets (8), antibody interactions with the emerging Zika virus (9), and countless others such as antibiotic-resistance (Figure 1). As such, cryo-EM has a profound impact in many broad disciplines, including but not limited to: Biochemistry, Chemistry, Computational Sciences, Microbiology, Physiology, etc. Cryo-EM is now becoming a standard tool for biochemists and democratizing structural biology: anyone that can purify a protein, a compound, or a cell, can effectively solve a structure. Therefore our efforts to expand this technology on campus will broadly and significantly increase research funding across all biochemical/biological disciplines.

Who will be impacted?

Since the installation of the facility (instrument purchased in Feb 2018, installation fully complete by Sept 2019), we have been wildly successful. Both tenure-track faculty positions were filled with researchers focusing on small membrane protein cryo-EM: Dr. Kelly Kim (joined MSU in March 2020), and Dr. Ben Orlando (joined MSU in Aug 2020). Despite these two junior faculty members joining during a global pandemic, they are off to a great start. Dr. Kim recently scored favorably on an NIH R35 (MIRA) application, Dr. Orlando was scored in a range that will result in immediate funding for an NIH R35 (MIRA) application, and just published ground-breaking results in Proceedings of the National Academy of Sciences (PNAS). His success has recently been featured in MSU Today: https://msutoday.msu.edu/news/2022/peering-into-antibiotic-resistance. In addition, we hired a staff scientist to oversee the facility. Dr. Sundharraman Subramanian has done an

excellent job at this position and was a recipient of the 2022 BMB Outstanding Scientific Staff Award. Lastly, Dr. Kristin Parent was appointed as interim Director for the initial year of operation, and then appointed Director for an additional three-year term. Two new courses were developed as part of MSU's Educational Mission. These include a course related to computational Image Processing Methods (BMB961 SS), and a hands-on microscopy laboratory course (BMB961, FS).

As of April 2022, the Cryo-EM facility on campus services 20 labs across five departments and three colleges, fostering new collaborations campus wide, and also off campus. In addition, several contacts off campus have been made, including collaborations with local companies (Avomeen, Zoetis, etc) and partnerships with the Henry Ford Institute indicating strong ties to the greater Michigan community and beyond. As such, we are now operating at full capacity for March and April 2022 (28/31 days booked each month; 3 days intentionally left open for preventative maintenance). The next two months are already fully booked as well. The recommendations for our proposed 5 year Strategic Plan (described below) are a result of the advice of the Oversight Board in reviewing our productivity/trajectory for the past two and a half years.

Users (Listed Alphabetically By Department) BMB (Erich Grotewold, Eric Hegg, Jian Hu, Jon Kaguni, Kelly Kim, Lee Kroos, Ben Orlando, Kristin Parent, Michaela TerAvest)

Chem (Mary Andorfer, Heedeok Hong, Xuefei Huang, Xiangshu Jin, Jetze Tepe, David Weliky); potential new recruits: Jonesy Jones & Tuo Wang

IQ (Danny Woldring)

MMG (Sean Crosson, Gemma Reguera, Chris Waters) PB/PRL/PRI (Bjoern Hamberger, Peter Lundquist, Josh Vermaas) Phys (Jason Bazil, Julia Busik)

, Community/stakeholder impact & Collaborators and Additional Inputs In addition to the core MSU faculty and staff mentioned above, the RTSF Cryo-EM Core Facility has an Oversight Board (see Table 1) that serves to review the success of the Cryo-EM facility annually, provide advice and a strategic plan moving forward that is reported to Dr. Doug Gage in the Office of Research and Innovation annually. As of April 2022, the Cryo-EM facility on campus services 20 labs across 5 departments and 3 colleges, fostering new collaborations campus wide, and also off campus (see Figure 2). In addition, several contacts off campus have been made, including collaborations with local companies (Avomeen, Zoetis, etc) and partnerships with the Henry Ford Institute indicating strong ties to the greater Michigan community and beyond. As such, we are now operating at full capacity for March and April 2022 (28/31 days booked each month; 3 days intentionally left open for preventative maintenance). The next two months are already fully booked as well. The recommendations for our proposed 5 year Strategic Plan (described below) are a result of the advice of the Oversight Board in reviewing our productivity/trajectory for the past two and a half years.

What does sustainability for your proposal look like?

The RTSF Cryo-EM Core Facility has an Oversight Board that serves to review the success of the Cryo-EM facility annually, provide advice and a strategic plan moving forward that is reported to Dr. Doug Gage in the Office of Research and Innovation annually.

Oversight Board: James Conway (U of Pittsburgh, external board member, runs a cryo-EM core facility) Dr. Gongpu Zhao (Van Andel Research Institute, external board member, runs a cryo-EM core facility) Elizabeth Wright (U of Wisconsin, Madison, external board member, runs a cryo-EM core facility) Melanie Ohi (U of Michigan, external board member, runs a cryo-EM core facility) Assaf Gilad (MSU, internal board member, Director within IQ, non-user of the facility) Robert Hausinger (MSU, internal board member, member of MMG and BMB, user of facility)

The Oversight Board, so far has been helpful in reviewing our operations, annual budget, and as a result of these annual meetings, they have provided advice for our future needs. In terms of sustainability, we will continue to utilize the Oversight Board and adjust our operations as needed, annually. In terms of costs, the major drivers are the initial investment of new equipment. User fees should result in a self-sufficient RTSF facility (pays for the service contracts and consumables) after the initial expansion period. We have these tools currently in place. We are a non-profit facility, and aim to "break even" even year.

Potential Risks: None anticipated. Given the success of the initial stage of the RTSF Cryo-EM Facility as well as the established infrastructure, including help from the Oversight Board, we have all the tools in place to "hit the ground running". Rewards and benchmarks of success will include research papers (e.g., 2020 Cell, Parent lab, and 2022 PNAS, Orlando lab, etc.) federally funded grants, (e.g. MIRA to both Parent and Orlando and Burroughs Wellcome to Parent, etc.) and student success in terms of SIRS evaluations, graduations and securing future placement will be the basic benchmarks of success. Additionally, we conduct a user survey annually to make sure we are meeting the needs of the community., Potential Risks None anticipated. Given the success of the initial stage of the RTSF Cryo-EM Facility as well as the established infrastructure including help from the Oversight Board, we have all the tools in place to "hit the ground running". Rewards and Benchmarks of Success Research papers (e.g. 2020 Cell, Parent lab, and 2022 PNAS, Orlando lab) federally funded grants, MIRA to both Parent and Orlando and Burroughs Wellcome to Parent, etc. and student success in terms of graduations and securing future placement will be the basic benchmarks of success. Sustainability and Resources Needed The major cost drivers are the initial investment. User fees should result in a self-sufficient RTSF facility after the initial expansion period. We have these tools currently in place.