

REQUEST FOR PROPOSALS: ARNOLD AND MABEL BECKMAN FOUNDATION INSTRUMENTATION GRANT FOR FOCUSED ION BEAM MILLING SAMPLE PREPARATION FOR CELLULAR CRYO-ELECTRON TOMOGRAPHY

Single particle cryo-electron microscopy (Cryo-EM) has revolutionized structural biology, as recognized by the Nobel Prize in Chemistry in 2017. A limitation of single particle Cryo-EM, however, is that the molecule of interest must be separated from the cell, and thus it is removed from its natural environment. A breakthrough method, focused ion beam (FIB) milling of cellular samples, has been developed to overcome this limitation by cutting thin lamellas of cell interiors such that they are accessible to high-resolution cryo-electron tomography (Cryo-ET) imaging *in situ*. This breakthrough enables high-resolution 3-D structure determination for organelles and proteins in their natural environments.

A new technical challenge has emerged in being able to realize the full potential of FIB-milling by identifying exactly which cells in a large sample, and which organelles within these cells, are of particular interest to answering fundamental structural biology questions prior to FIB-milling of the sample. One solution to this problem is in the coupling of high-resolution cryogenic light microscopy techniques with FIB-milling in preparing cellular samples for Cryo-ET imaging.

The Arnold and Mabel Beckman Foundation's mission is to support leading edge research, in the fields of chemistry and life sciences, and particularly to foster the invention of methods, instruments, and materials that open up new avenues of research and application in these sciences and related disciplines. In support of this mission, the Foundation is requesting proposals for a one-time grant opportunity for the procurement of cryogenic light microscopes, FIB-milling sample preparation equipment, appropriate Cryo-EM energy filters or detectors, and other freezing and grid preparation equipment as part of a larger cellular Cryo-ET workflow that will enable advanced research projects. In addition, applicants may include technology development efforts in one or more of the steps in the overall cellular Cryo-ET sample preparation workflow.

The Foundation will provide support of up to \$1.5 million per Institution, which can be used flexibly for instrumentation acquisition, development, and maintenance; support for personnel, junior scientists and training programs; support for data science collaborations within the research teams; and/or costs for the proposed research programs. Applicant institution(s) must demonstrate their commitment for additional funding beyond the \$1.5 million support from the Arnold and Mabel Beckman Foundation, if necessary, to complete the objectives of the program described below. Additional information on the institutional support requirements can be found in the template in the online application portal.

PROGRAM OBJECTIVES

Proposals should be centered around core biological problems and a research program that is enabled by the high-resolution, *in-situ* imaging capabilities of the cryogenic light microscopy guided FIB-milling for cryo-electron tomography of cellular interiors. Additional consideration will be given to proposals that include other imaging, sensing, or analysis technologies as an integral part of one or more of the proposed research project(s). Applicants shall identify the configuration of each element of the complete imaging workflow, identify those elements that will be purchased in the program, and justify why that level of capability is necessary and appropriate for the proposed research program.

Proposals for procurement of commercial systems and/or development or improvement of elements of the cellular Cryo-ET sample preparation workflow will be considered. Proposals should also address any required energy filter or detector upgrades, if necessary, in their existing Cryo-EM system for tomography imaging. If any instrumentation development work is proposed, then the proposal must also address the engineering approach and plans to complete the technology development, and how

these developments will further the specific biological research aims. The Foundation recognizes that these development approaches may be higher risk, and so Full Proposals shall also address alternative strategies that will be pursued should one or more aspects of the new technological developments fail to achieve their proposed performance metrics.

Successful proposals must also include a detailed plan on how the data generated from the system will be stored and analyzed. These plans should include how the data scientists will be incorporated into the research teams and how any new analysis techniques, along with representative data sets to demonstrate these techniques, will be shared with the broader research community. All costs for incorporating the data analysis expertise and data storage infrastructure in the proposed program must be included in the budget for the overall program.

Inclusion and support of junior faculty and scientists is also a priority for the Arnold and Mabel Beckman Foundation, and successful proposals shall include interdisciplinary teams that include junior faculty and junior scientists, such as staff scientists, postdoctoral fellows, and graduate students, as integral components of the team. A training plan shall also be developed that specifically addresses how junior scientists will benefit from new training opportunities, especially training programs that will extend beyond the individual Institution or consortium.

ELIGIBILITY CRITERIA

The Foundation will consider proposals from individual institutions or from consortia of institutions whose combined resources will address the program requirements. For any consortium application, there must be a “Lead Institution”, defined as the institute that will maintain the purchased equipment on their property and will accept the entire grant award on behalf of the consortium. The Lead Institution submitting the proposal must be a nonprofit US university or research institution.

Universities or Institutions interested in applying must either already have the required cryo-electron tomography capability in place and operational, or have definitive plans to develop this capability in the near term (e.g. a commitment for a junior faculty hire in tomography), or have a track-record of successfully utilizing Cryo-EM Centers with tomography capabilities. The Foundation will require interested applicants to first complete an eligibility form in the online application system (<https://beckman-foundation.smapply.io/>) with the following questions:

1. What are your current and/or planned near future capabilities in Cryo-ET instrumentation and personnel, and any existing FIB-Milling systems?
2. Explain how your Institution will manage the additional instrument time requirements for this Cryo-ET program. If you will be utilizing a CryoEM Center for the final imaging, indicate which Center and briefly describe your history of coordinating with them.
3. Provide a brief description of your planned research program that will be enabled by the addition of this instrumentation.

The Foundation will provide the Full Proposal documentation to applicants determined to be eligible for the program based on the responses above. Questions regarding eligibility can be directed to Anne Hultgren at InstrumentGrants@beckman-foundation.org. Decisions on eligibility by the Foundation will be final.

TIMELINE

Eligibility Determination Submission Deadline: February 26, 2021 by 1 pm Pacific/4 pm Eastern
Eligibility submissions will be reviewed on a rolling basis. Eligible applicants will be provided access to the Full Proposal submission portal as soon as the review of their submission is complete, and no later than March 5, 2021.

Full Proposals Due: June 11, 2021 by 1 pm Pacific/4 pm Eastern

Award announcements anticipated in late 2021

APPLICATION SUBMISSION

Online submission portal: <https://beckman-foundation.smapply.io/>

One co-PI must be identified as the Lead PI for the team to create an account on the submission portal and submit the eligibility form and final Full Proposal on behalf of the full team. The submission portal allows for the addition of collaborators to access and upload documents into the account. Instructions for collaborators are provided in the submission portal.

SELECTION & REVIEW PROCESS

Applications will be reviewed by a scientific review committee appointed by the Foundation. The Foundation reserves the right to use a review panel composed of internal and external reviewers, subject to a non-disclosure agreement and without conflicts of interest. Due to the administrative requirements involved, the Arnold and Mabel Beckman Foundation is unable to provide critical commentary on unsuccessful proposals.