Transformative Potential of High Resolution Cryo-Electron Microscopy

Sponsoring ICOs: NIGMS, NEI, NHLBI, NIDDK, NINDS, ORIP

Interested ICOs: NCI, NIAID, NIDA
Why Now? New Technological Breakthroughs in Cryo-EM

1) New electron microscopy technology dramatically improves our ability to see biological molecules

   Old Methods

   New Methods

   TRPV1 Ion Channel: Mediates burn sensation, Yifan Cheng UCSF

2) New motion correction methods resolve blurring of images due to movement of particles in electron beam

   Rotavirus Particles
   Niko Grigorieff, Janelia Farms
Scientific Opportunities through Cryo-EM

- **Determine structures more rapidly and easily**
  

- **Direct visualization of subcellular structures, *in situ***
  
  Richard Henderson: “If it carries on, and all the technical problems are solved, cryo-EM could indeed become, not just a first choice, but a dominant technology. We are probably halfway there.” *Nature* (2015)

**Impacts on Research:** Structures of hard to crystallize and complex molecules, such as channels and receptors; elucidating conformational changes in complexes; rapid determination of effects of mutations on structure; structural basis of drug action; structures of molecules determined inside of (or on) cells.
“Another major advance toward developing an effective HIV vaccine came in 2013 when a team of researchers led by John Moore at Weill Cornell Medical College in New York City and Ian Wilson at the Scripps Research Institute in La Jolla, California, obtained an atomic-level image of the HIV envelope trimer, the principal target for broadly neutralizing antibodies.”

-Wayne Koff, *The Scientist*, May 1, 2015
The U.S. Is Falling Behind Asia and Europe in Cryo-EM

- **Initial Investment**, 1-2 Cryo-EM microscopes, shared facility
- **Moderate Investment**, 3-4 Cryo-EM microscopes, regional facility
- **Significant Investment**, 5+ Cryo-EM microscopes, HTP user facility
Challenges for Researchers Today

Infrastructure
- Current technology only available to a few experts
- Inadequate to take advantage of scientific opportunity

Investigator base
- Workforce bottleneck: major training need
- Crystallographers want to move to EM

Equipment
- Expensive, limited numbers
- Inaccessible to most potential users
- Highly inefficient for each institution to buy and maintain its own cryo-EM

Technology Development Needed for Tomography

- Reconstruction of the structures of molecules inside of cells
- Recognition of molecules in tomograms is still done largely by eye
- More sensitive, automated, better resolution methods for tomography are needed

Short-term Strategy: NIGMS Regional Consortia

NIGMS Regional Consortia Program (RFA- GM-16-001)

• Supports only equipment upgrades for expert laboratories

• No research assistance for screening or computational analysis

• No training

Unambiguous establishment of the rotameric conformation of an isoleucine residue in a 2.8 Å structure of *Thermoplasma acidophilum* 20S proteasome, Campbel et al., *eLife* (2015)
The Synchrotron Model for Cryo-EM

- State of the art regional user facilities
- Access open to all through peer review process
- Training for users
- Professional and technical staff to assist with data collection and analysis; maintain and upgrade equipment; provide training
- Wet lab facilities & lodging
- High-throughput and mail-in services
Goals, Deliverables, Impact

- Move U.S. to the forefront of cryo-EM research
- Provide efficient and economical access to cryo-EM technologies and training: create economies of scale
- Develop new technologies and computational methods to lower cost, improve resolution, and increase throughput and ease of use
- Push the frontiers of *in situ* Cryo-EM (tomography)

**BETTER RESOLUTION**
This composite image of the protein β-galactosidase shows how cryo-EM has progressed over the years, from the indistinct blobs once obtained with the technique (left) to the nearly 2-Å-resolution structures possible today (right).

*Credit:* Sriram Subramaniam/NCI
## Draft Proposed Budget

### 3 Comprehensive Centers

<table>
<thead>
<tr>
<th></th>
<th>Year 1</th>
<th>Year 2</th>
<th>Year 3</th>
<th>Years 4-5</th>
<th>5 Year Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Equipment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 microscopes @ 3 centers</td>
<td>$22M</td>
<td>$22M</td>
<td>$22M</td>
<td>0</td>
<td>$66M</td>
</tr>
<tr>
<td><strong>Operating Cost</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staff, facilities, maintenance</td>
<td>$4M</td>
<td>$6.4M</td>
<td>$8.7M</td>
<td>$7.1M</td>
<td>$33.3M</td>
</tr>
<tr>
<td><strong>Training Cost</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 FTEs @ 3 centers</td>
<td>$0.6M</td>
<td>$1.2M</td>
<td>$1.8M</td>
<td>$1.8M</td>
<td>$7.2M</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>$26.6M</td>
<td>$29.6M</td>
<td>$32.5M</td>
<td>$8.9M</td>
<td>$106.5M</td>
</tr>
</tbody>
</table>

### Investigator-Initiated Research

<table>
<thead>
<tr>
<th>Activity</th>
<th>TC yearly</th>
<th>5 Year Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryoelectron Tomography TR&amp;D</td>
<td>R21, R01</td>
<td>$5M</td>
</tr>
<tr>
<td>Single Particle Analysis CryoEM TR&amp;D</td>
<td>R21, R01</td>
<td>$2.5M</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$7.5M</td>
</tr>
</tbody>
</table>
Sustainability Plan

• Depending on future needs and technological developments, we could enhance or expand the number of regional facilities in a second phase of Common Fund support.

• Support for regional facility operations and maintenance would shift from the Common Fund to ICs, other federal agencies (e.g., NSF, DoE, DoD), other funders (e.g., HHMI) and industry.

  ➢ Analogous to current model for supporting synchrotrons
Thank You!

Questions?
Technology Development for Tomography

**Single Particle Reconstruction**

For molecules in ice. Many particles, one orientation and image per particle, low electron dose, high resolution. Particles must all be the same. Ultimate achievable resolution 2Å or better.

**Tomographic Reconstruction**

For frozen hydrated cells. All images (~100) are recorded from the same specimen. One-of-a-kind objects. High electron dose. Ultimate achievable resolution will be limited by radiation damage (15-20Å?).