Discussions for a

National Molecular Microscopy Laboratory

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Outline of presentation

SUMMARY

Why now?

Why FNL?

Workflows

Innovation

Leadership

Scope

Training

Partnerships

Milestones
Imaging gaps in biology and medicine

### 2005-2014: A 10-year plan in molecular microscopy

<table>
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<tr>
<th>Study Area</th>
<th>Spatial Resolution</th>
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<tbody>
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<td>3D mapping of cancer cells</td>
<td>~ 15000 nm</td>
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<tr>
<td>Spatial architecture of signal transduction</td>
<td>~ 1500 nm</td>
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<tr>
<td>Mechanisms of HIV entry</td>
<td>~ 150 nm</td>
</tr>
<tr>
<td>Protein complexes in metabolism</td>
<td>~ 15 nm</td>
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</table>
Focused ion beam Scanning Electron Microscopy for rapid 3D imaging of cells and tissue
Imaging receptor arrays and signaling complexes in intact cells
Cryo-electron tomography of HIV
From spikes to structure
Structure determination without crystallography: A biochemist’s dream

Purified protein complex

Vision for a structure determination machine

Recent progress in cryo-EM field
The emergence of atomic resolution cryo-EM

1990: First atomic resolution model from electron crystallography of 2D protein crystals (3.5 Å)

1995: Articulation of prospects of obtaining atomic resolution protein structures without crystals

2008: First near-atomic resolution icosahedral viral structures (3.9 Å)

2013: First near-atomic resolution membrane protein structure (3.4 Å)

2014: Structure of a dynamic metabolic enzyme implicated in cancer (3.0 Å)


The potential and limitations of neutrons, electrons and X-rays for atomic resolution microscopy of unstained biological molecules

RICHARD HENDERSON
MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

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Glutamate receptor gating cycle

Glutamate receptor gating cycle

Precise knowledge of protein movements will enable design of drugs that trap distinct functional states.
Comparison between cryo-EM and X-ray maps of β-galactosidase

Cryo-EM structure of β-galactosidase at 3.2 Å resolution

Cryo-EM can provide atomic resolution structures of full-length proteins under native conditions
Understanding HIV entry: A multifaceted challenge

Antibody neutralization
Understanding HIV entry: A multifaceted challenge

Antibody neutralization

3D structure of trimeric Env
Understanding HIV entry: A multifaceted challenge

Antibody neutralization

3D structure of trimeric Env

Cell-cell transmission
Understanding HIV entry: A multifaceted challenge

A complete understanding of the problem requires integration of information across cellular and molecular scales

Antibody neutralization

3D structure of trimeric Env

Cell-cell transmission

Pathway to the nucleus
Focused ion beam Scanning Electron Microscopy for rapid 3D imaging of cells and tissue

Iteration of slicing and imaging
Focused ion beam Scanning Electron Microscopy for rapid 3D imaging of cells and tissue

Iteration of slicing and imaging
Focused ion beam Scanning Electron Microscopy for rapid 3D imaging of cells and tissue

Iteration of slicing and imaging

A journey into T-cell synapses
A journey into T-cell synapses
Intimate contact at the cell-cell interface

dendritic cell

T-cell
Synapses between primary T-cells

Do et al (2014)
HIV transfer to fetal astrocytes

Do et al (2014)
HIV-infected T-cell

fetal astrocyte
Correlative live confocal and ion-abrasion SEM imaging: A cell biologist’s dream

3D image of entire T-cell
Correlative live confocal and ion-abrasion SEM imaging: A cell biologist’s dream

The dynamic HIV spike
Molecular architecture of trimeric HIV envelope glycoproteins

Subtomogram averages at ~ 20 Å resolution
Catching HIV in the act with electron tomography

Tran et al *PLos Path.* (2012)
Meyerson et al *PNAS* (2013)
Structures of soluble HIV-1 Env immunogens at ~6Å - 9Å resolution

Bartesaghi et al (2013)
Why FNL?

- c-CRADA mechanism for facile collaborations with industrial and academic collaborators

- Strong infrastructure can be established at FNL for collaborations requiring support for pre-microscopy (biochemistry) and post-microscopy (computing) applications

- CCR/NCI cryo-EM program already has footprint at ATRF

- Proximity to many leading institutions along East Coast with strong structural biology programs
Scope

- Similarities and differences with DOE national laboratories that support high resolution electron microscopy
- National laboratory versus local academic user facilities
- Private sector and NIH-wide participation
- Synergy between components that provide user access to existing technologies versus those that develop breakthrough technologies
- Budget considerations
Leadership

• Set clear long-term vision for laboratory

• Important to maintain both technology development and routine user access components of laboratory

• Nucleation of highly motivated multi-disciplinary teams that can identify and tackle difficult challenges

• Effective strategies to stay at forefront of new developments in structural and cell biology

• Opportunity to establish internationally unique center
Training

• Mechanisms to host long-term and short-term visits from extramural researchers

• Core team of specialists to provide support in all aspects of structural investigation from biochemistry to computation

• Resident scholar program

• Strong training partnerships with neighboring institutions

• Peer review and competitive award by extramural study section panel