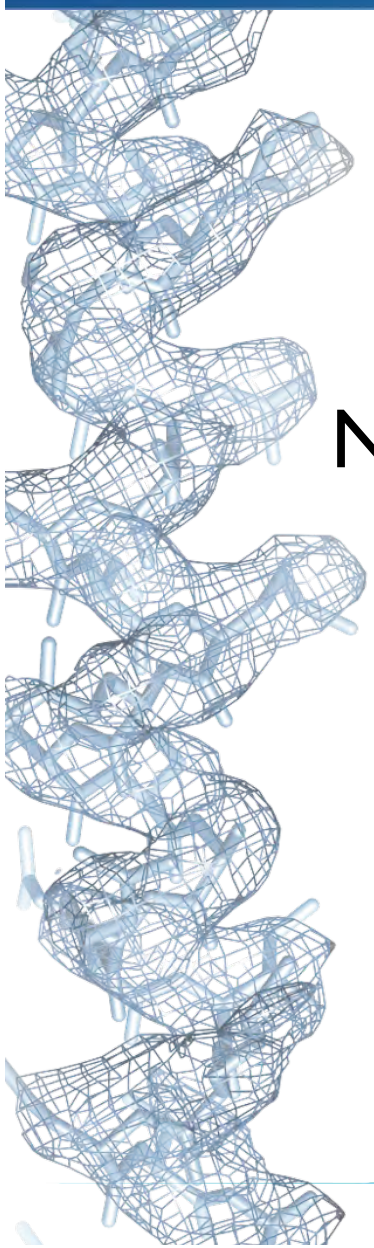


Discussions for a

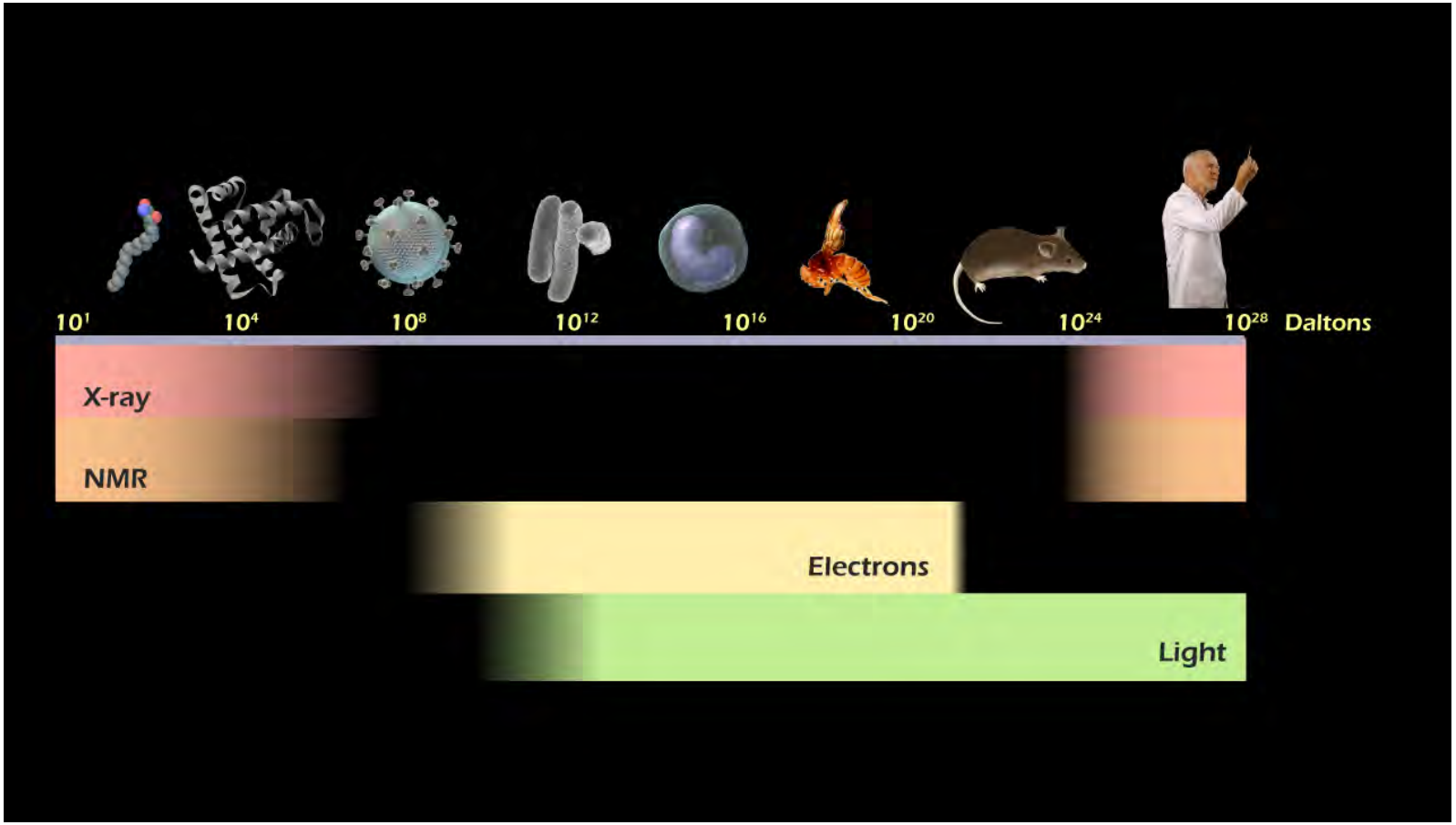
National Molecular Microscopy Laboratory

Sriram Subramaniam, Ph.D.
Laboratory of Cell Biology
Center for Cancer Research
National Cancer Institute

September 2014

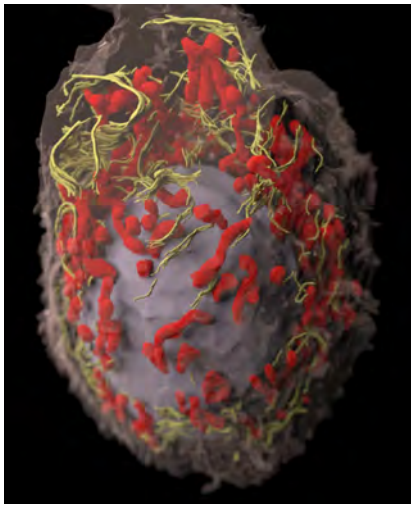
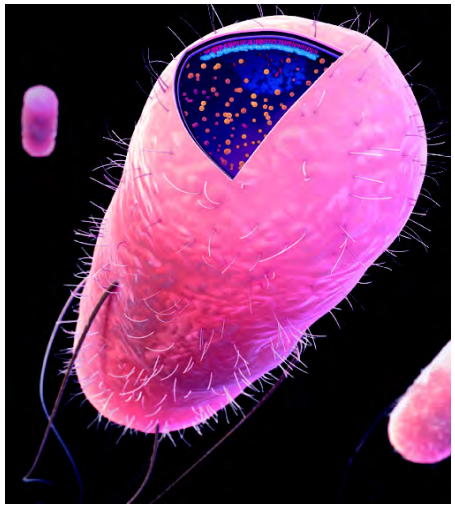
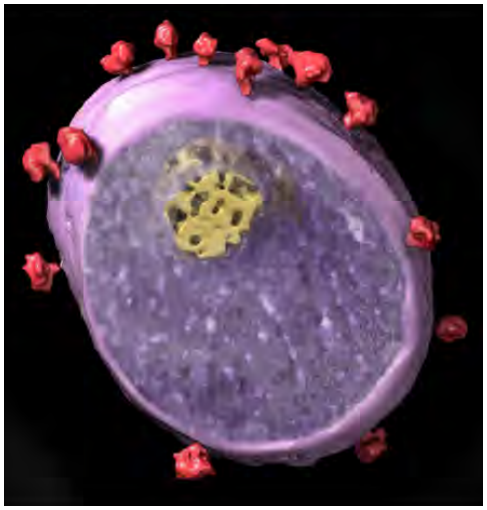
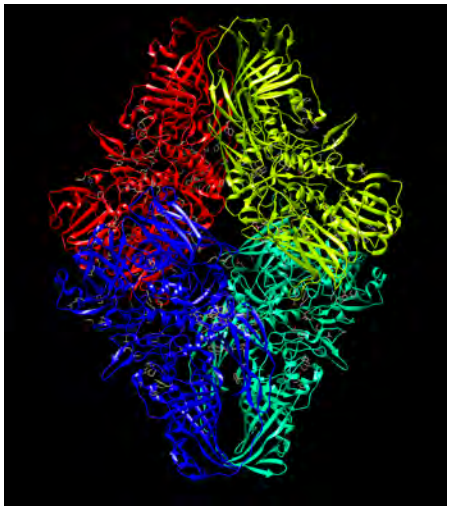
Outline of presentation

Imaging gaps in biology and medicine

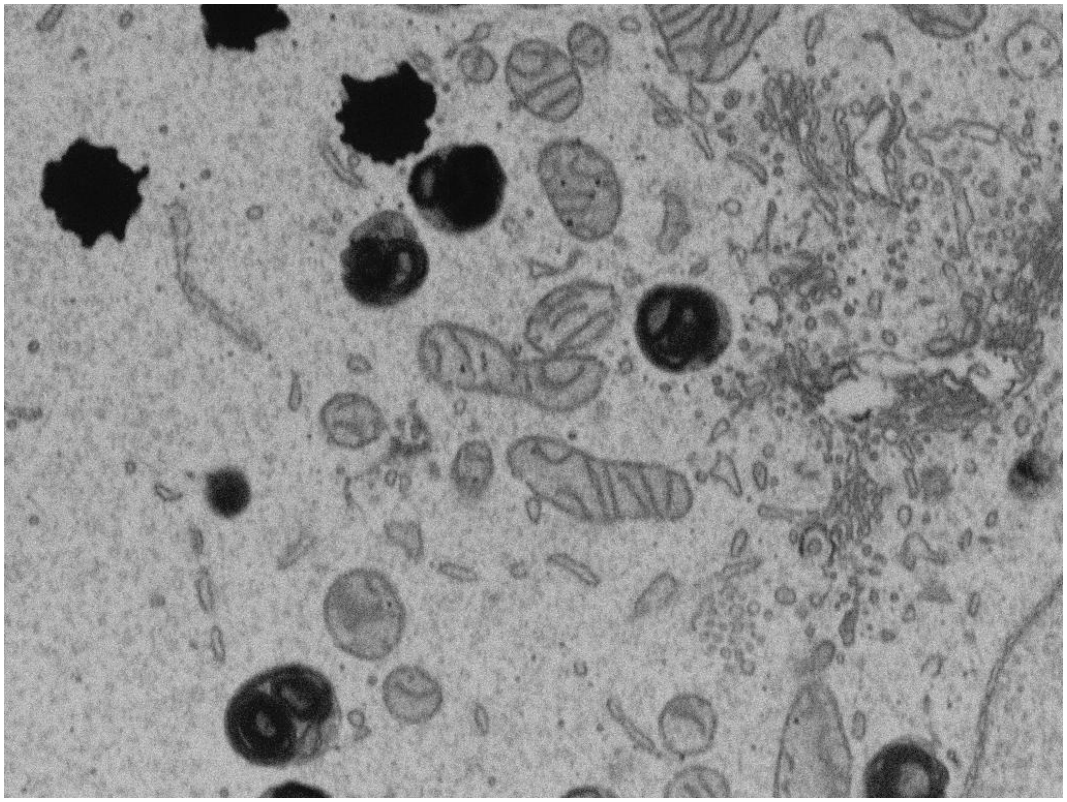


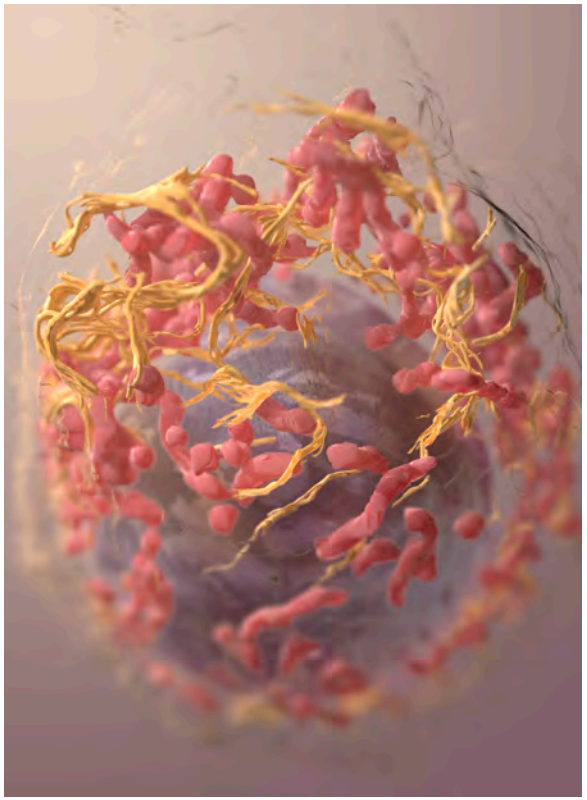
Subramaniam, *Curr. Opin. Microbiol.* (2005)

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

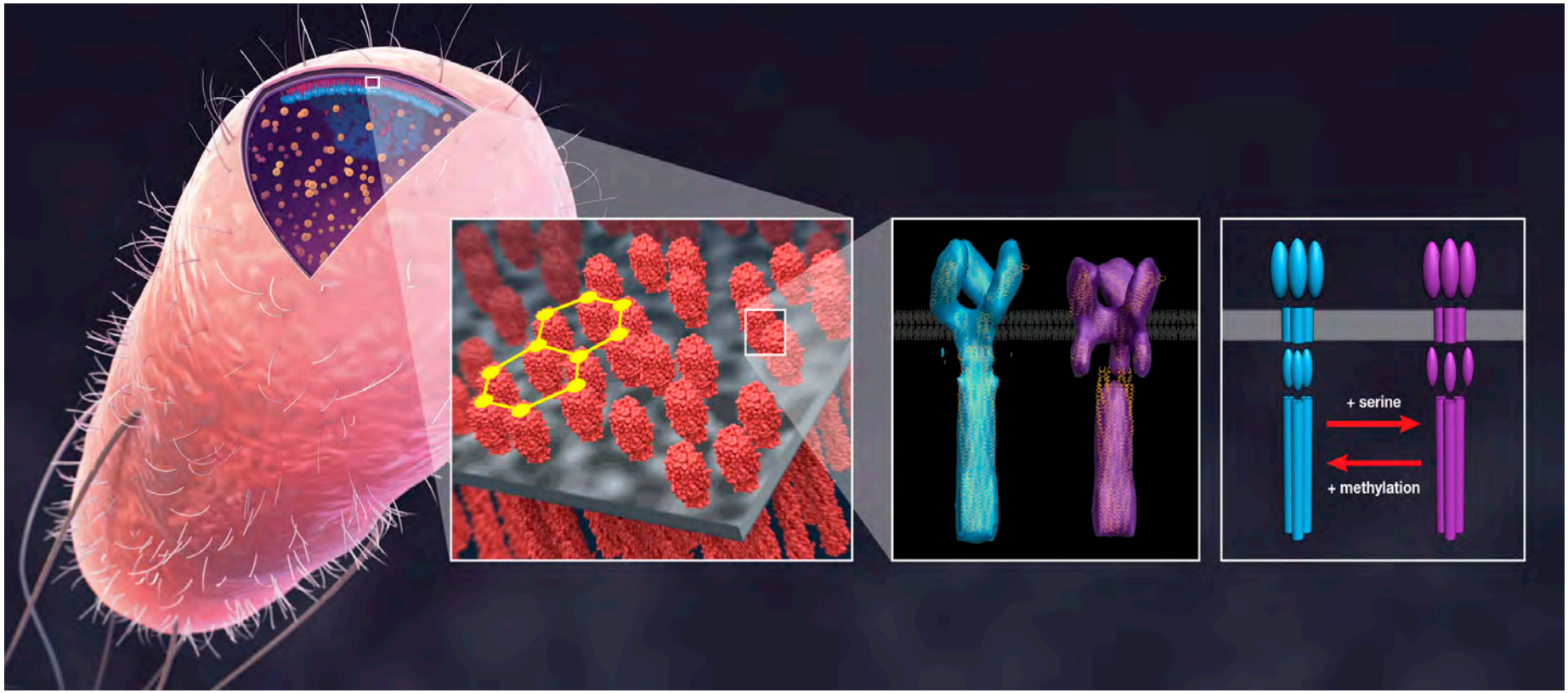
3D mapping of cancer cells	Spatial architecture of signal transduction	Mechanisms of HIV entry	Protein complexes in metabolism
			
~ 15000 nm	~ 1500 nm	~ 150 nm	~ 15 nm

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

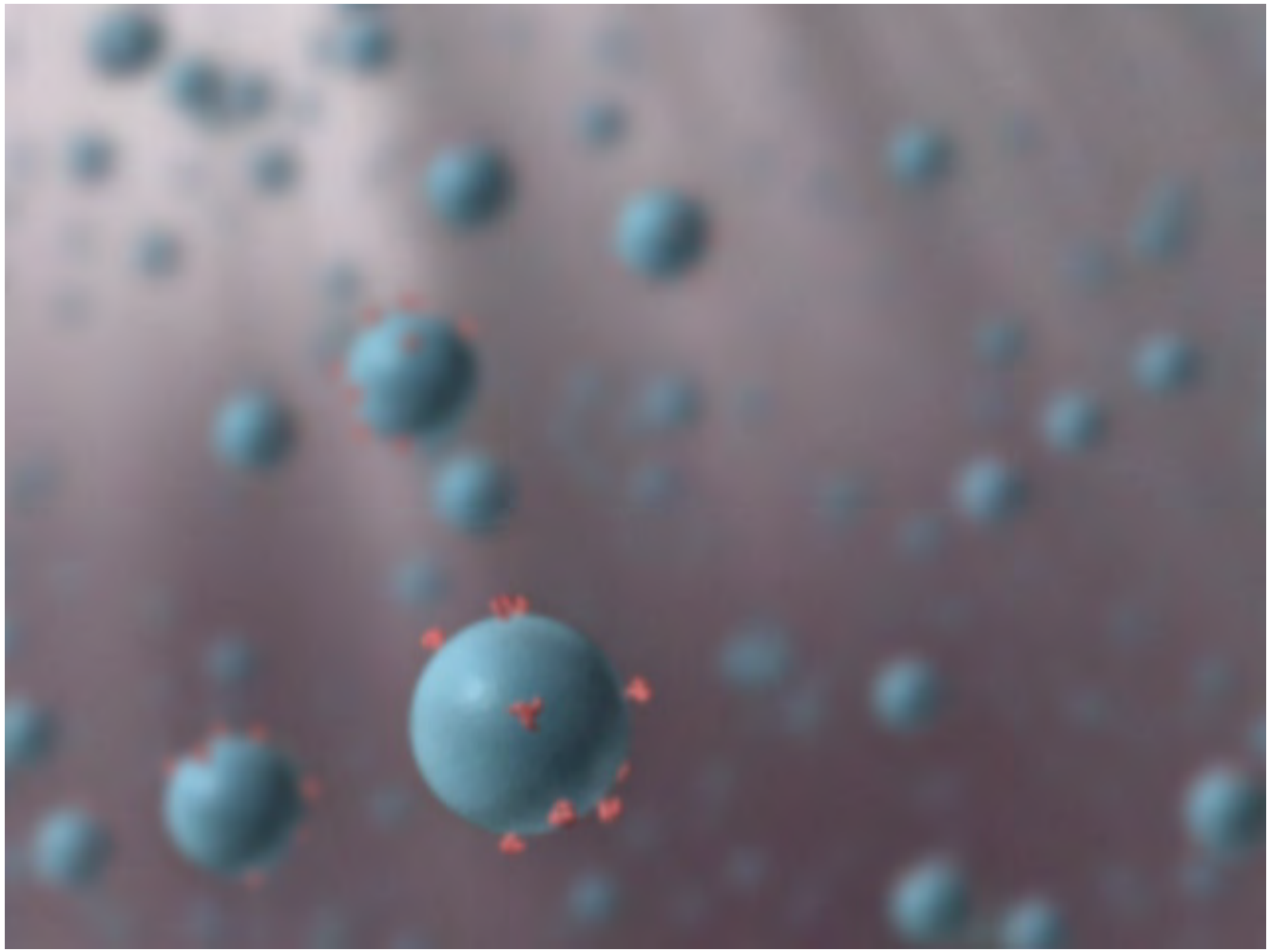




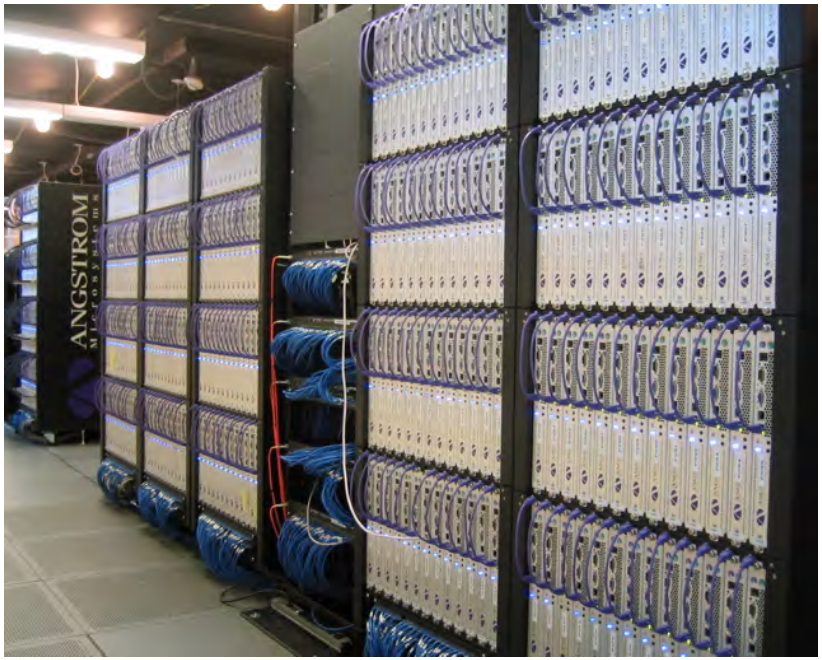
Imaging receptor arrays and signaling complexes in intact cells



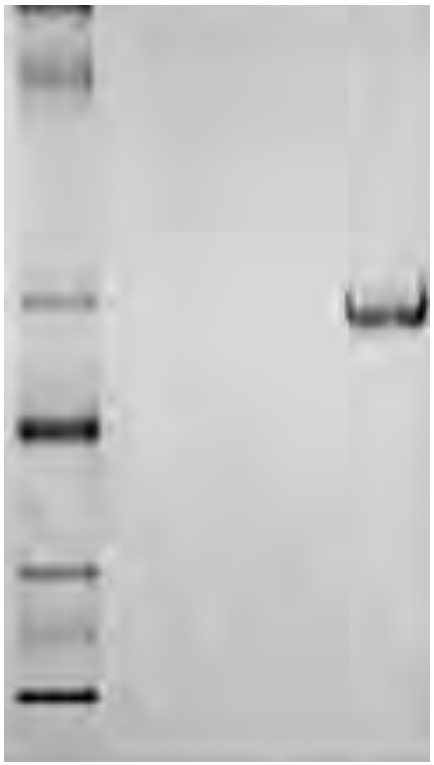
Cryo-electron tomography of HIV



From spikes to structure



Structure determination without crystallography: A biochemist's dream

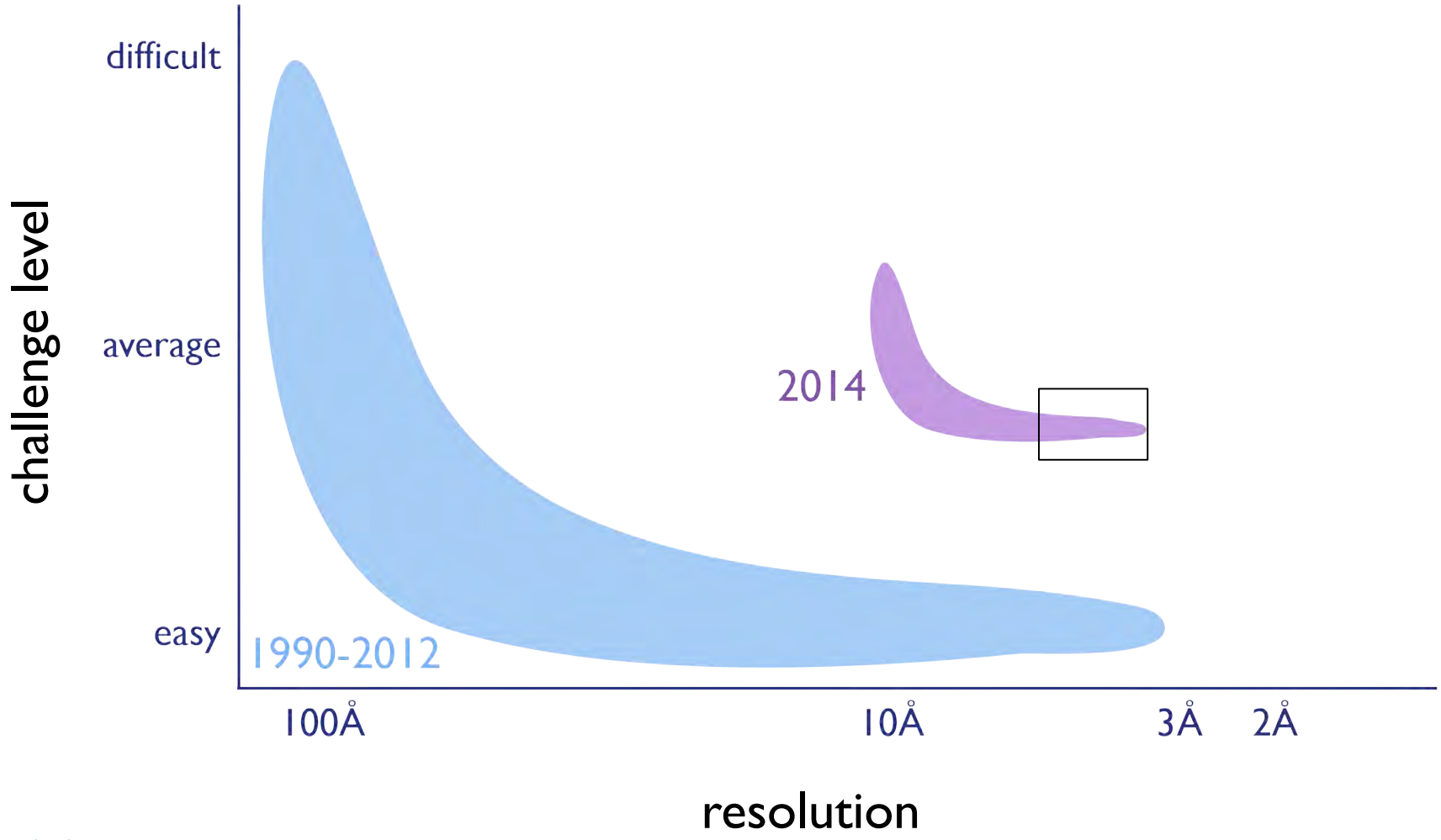


Purified protein complex

Vision for a structure determination machine

S. Subramaniam, U.S. Patent No. 6,987,266 B2 (issued Jan 2006)

Recent progress in cryo-EM field



The emergence of atomic resolution cryo-EM

Henderson, Quart. Rev. Biophys. (1995)

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

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

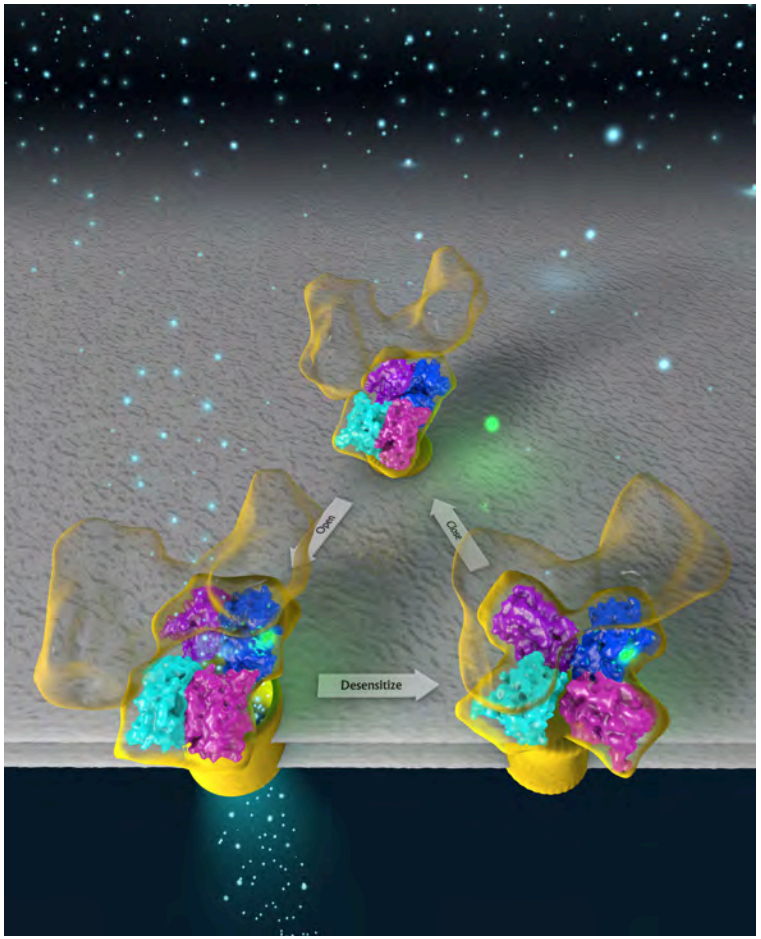
1995: Articulation of prospects of obtaining atomic resolution protein structures *without* crystals

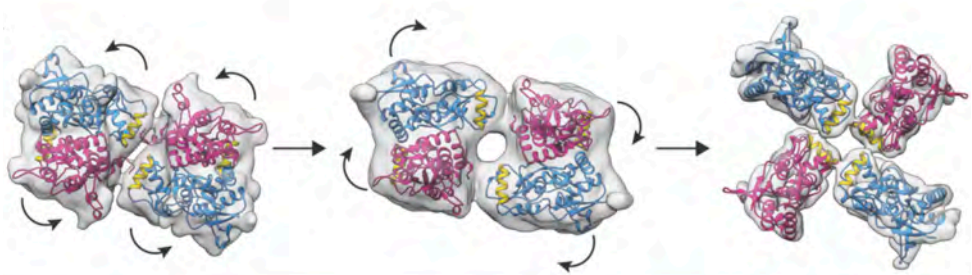
2008: First near-atomic resolution icosahedral viral structures (3.9 Å)

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

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

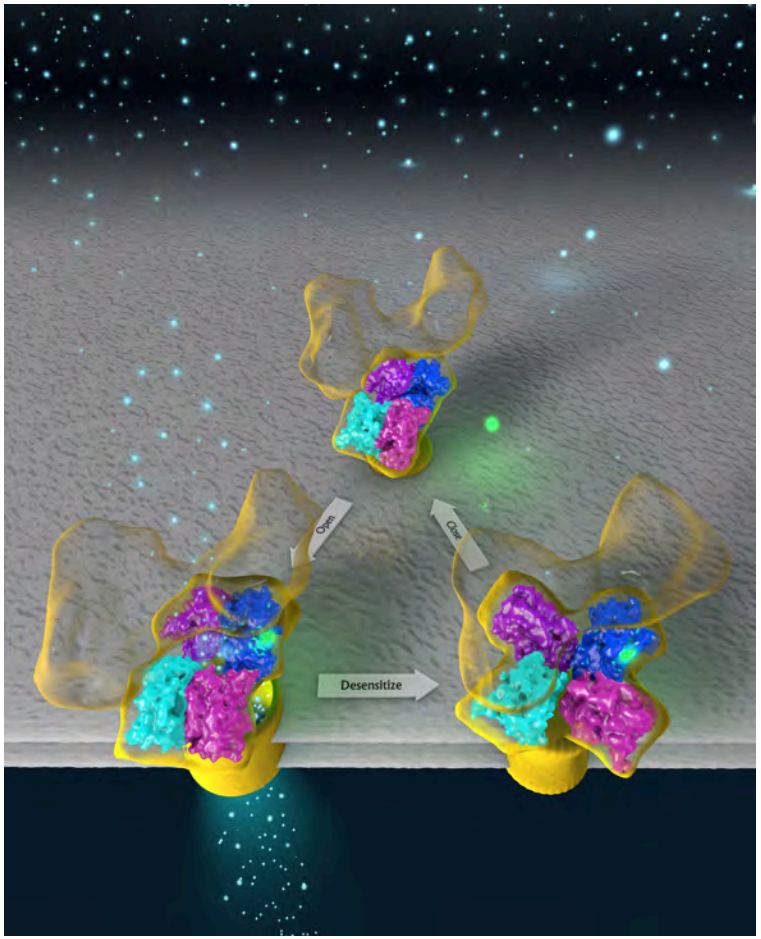
Glutamate receptor gating cycle

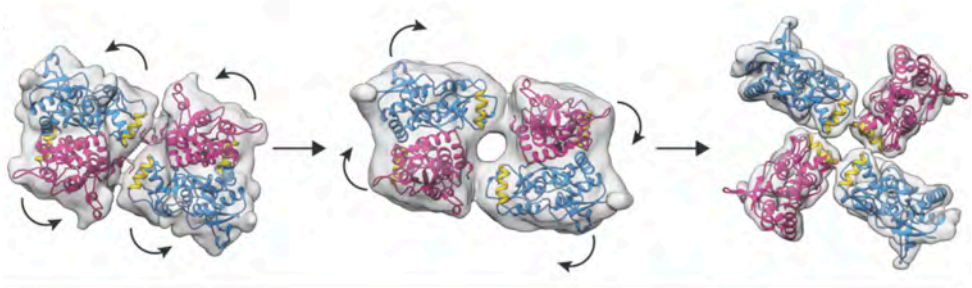


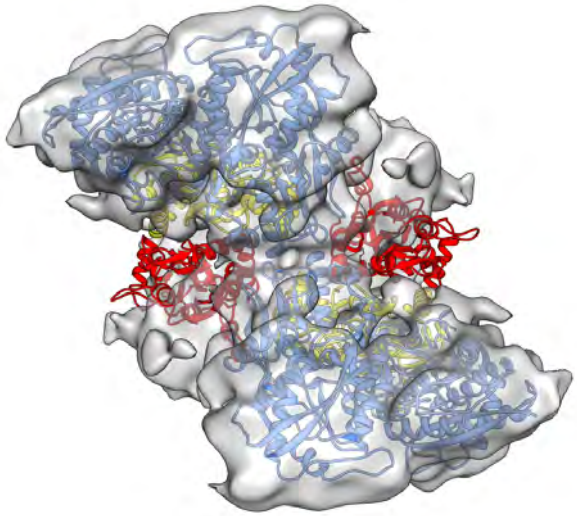


Meyerson et al *Nature* (2014)

Glutamate receptor gating cycle

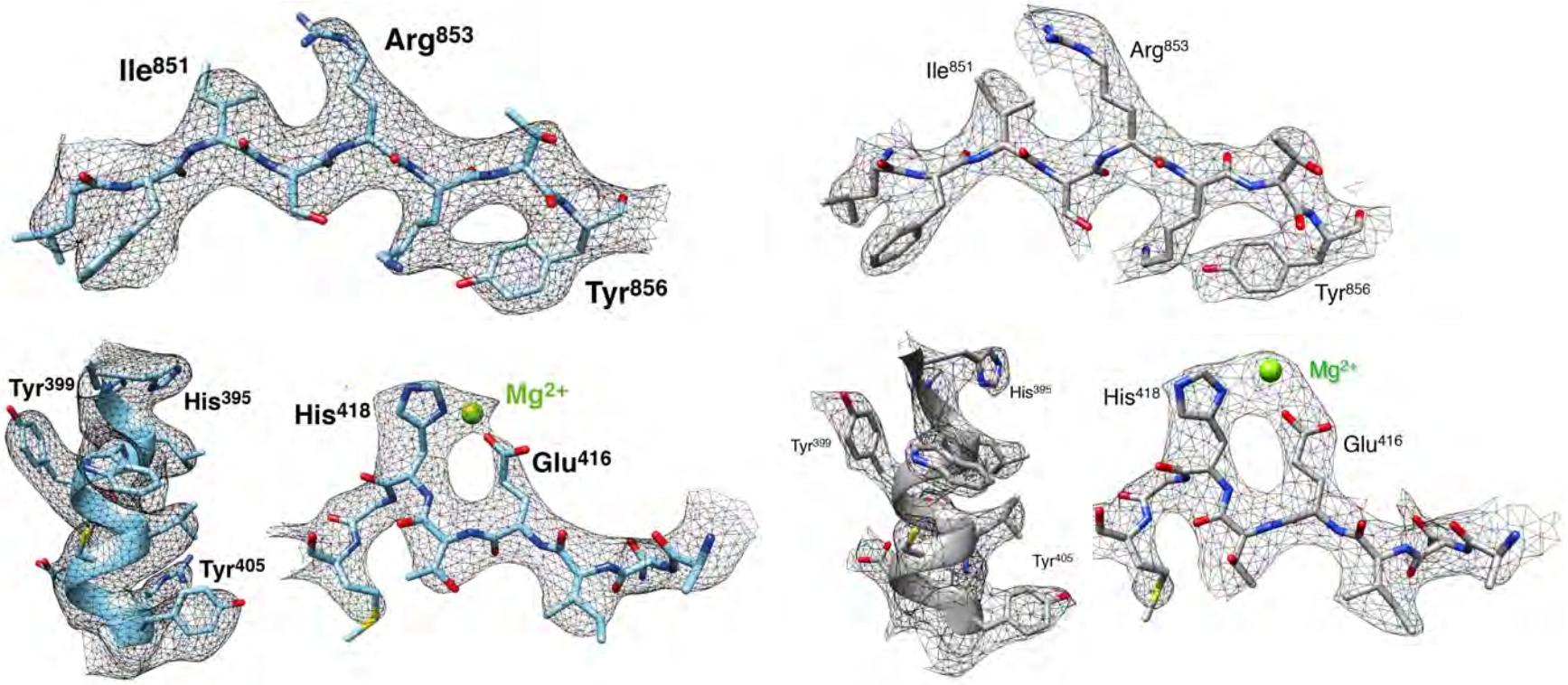






Precise knowledge of protein movements will enable design of drugs that trap distinct functional states

Comparison between cryo-EM and X-ray maps of β-galactosidase

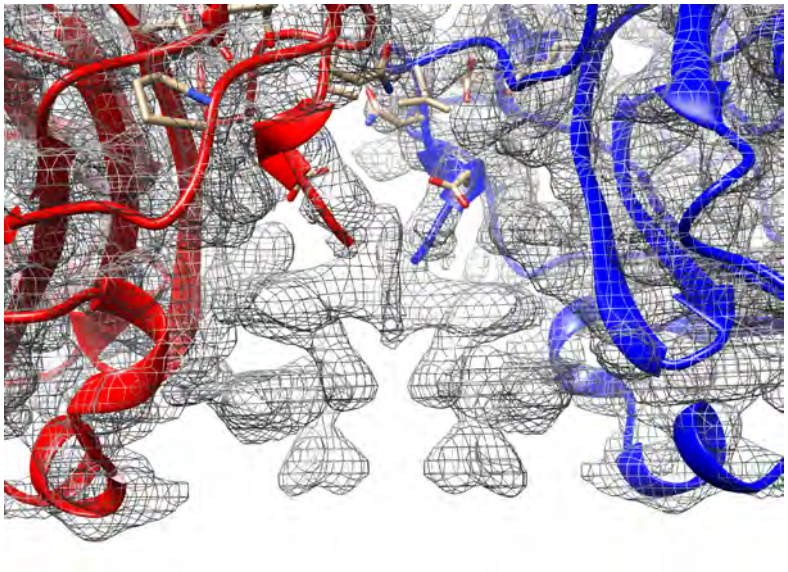


cryo-EM
3.2 Å

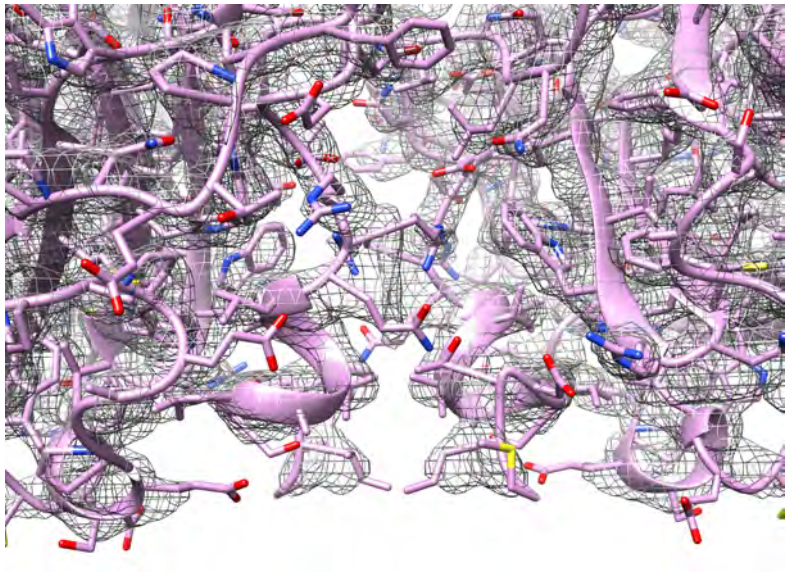
X-ray
3.0 Å

Bartesaghi et al *Proc. Natl. Acad. Sci. USA* (2014)

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



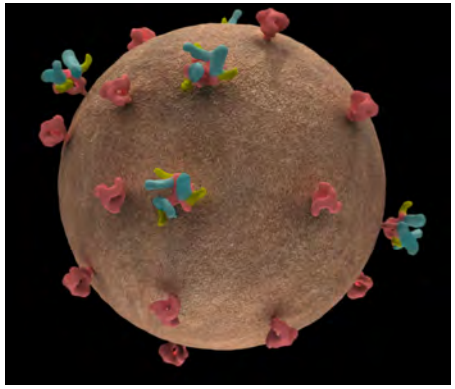
Fitted with X-ray coordinates



Fitted with cryo-EM coordinates

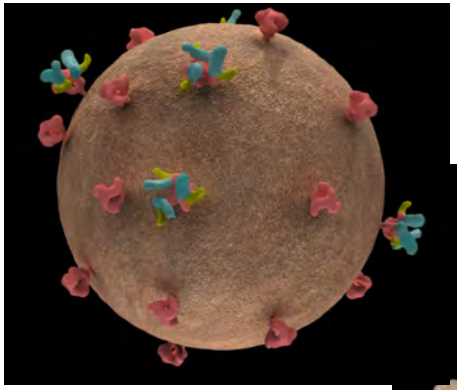
Cryo-EM can provide atomic resolution structures of full-length proteins under native conditions

Understanding HIV entry: A multifaceted challenge

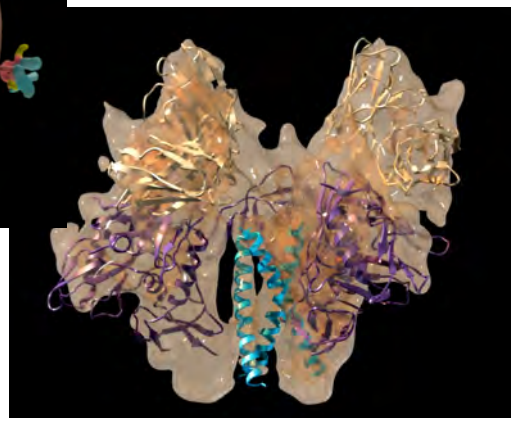


Antibody neutralization

Understanding HIV entry: A multifaceted challenge

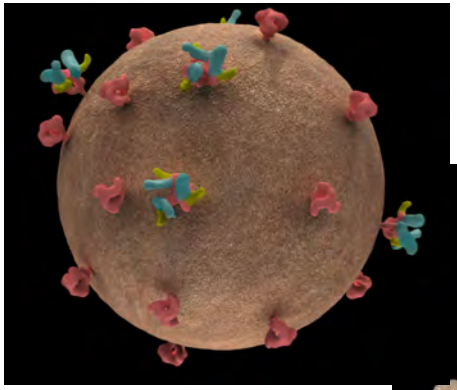


Antibody neutralization

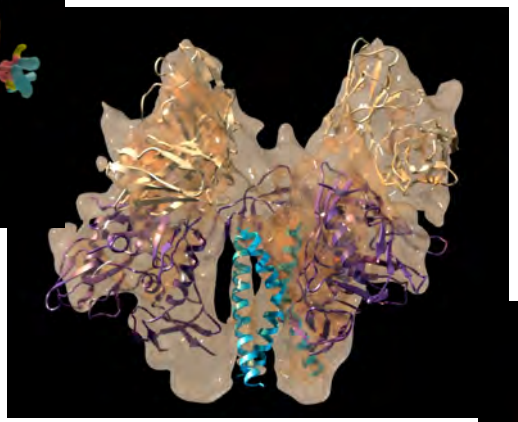


3D structure of trimeric Env

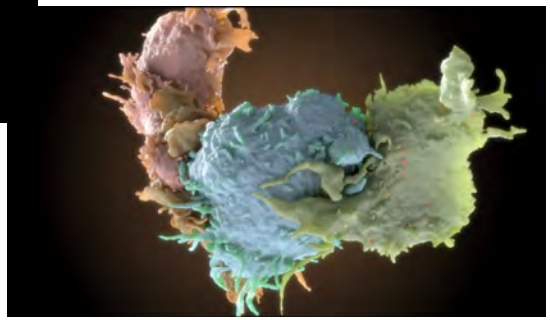
Understanding HIV entry: A multifaceted challenge



Antibody neutralization

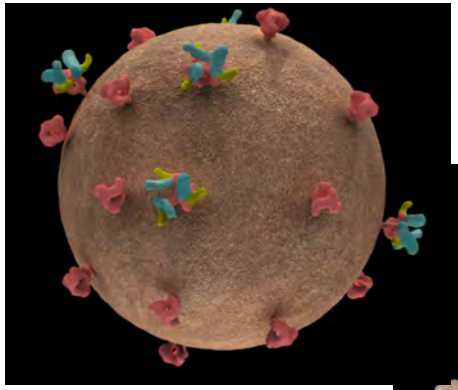


3D structure of trimeric Env

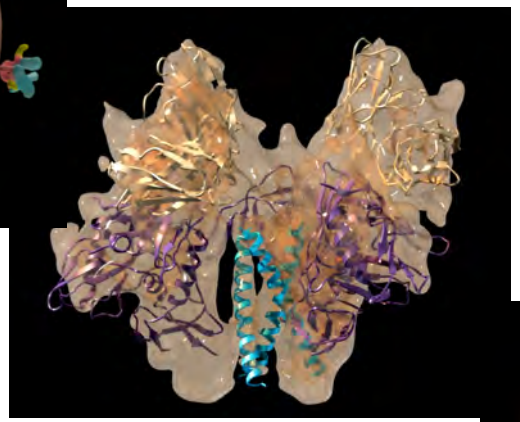


Cell-cell transmission

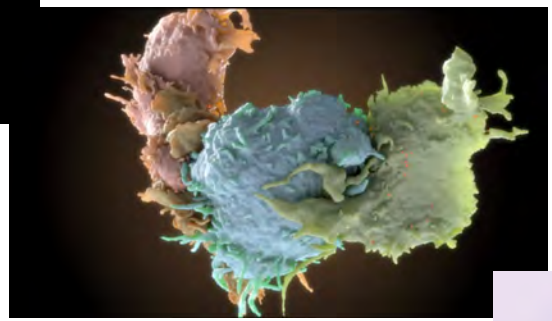
Understanding HIV entry: A multifaceted challenge



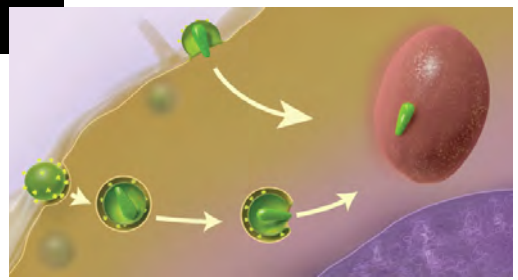
Antibody neutralization



3D structure of trimeric Env



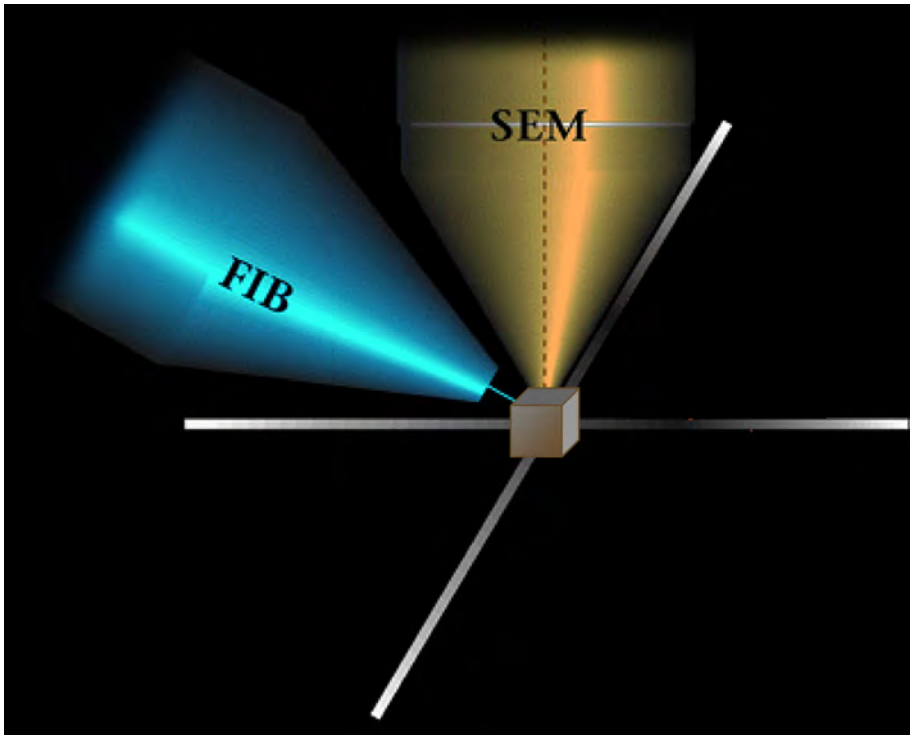
Cell-cell transmission

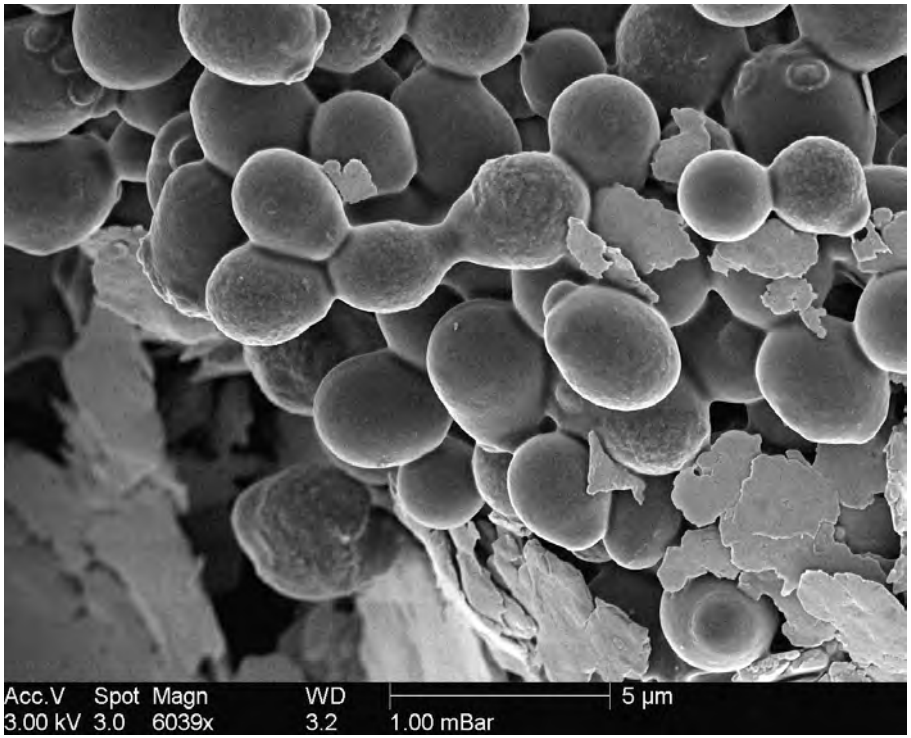


Pathway to the nucleus

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

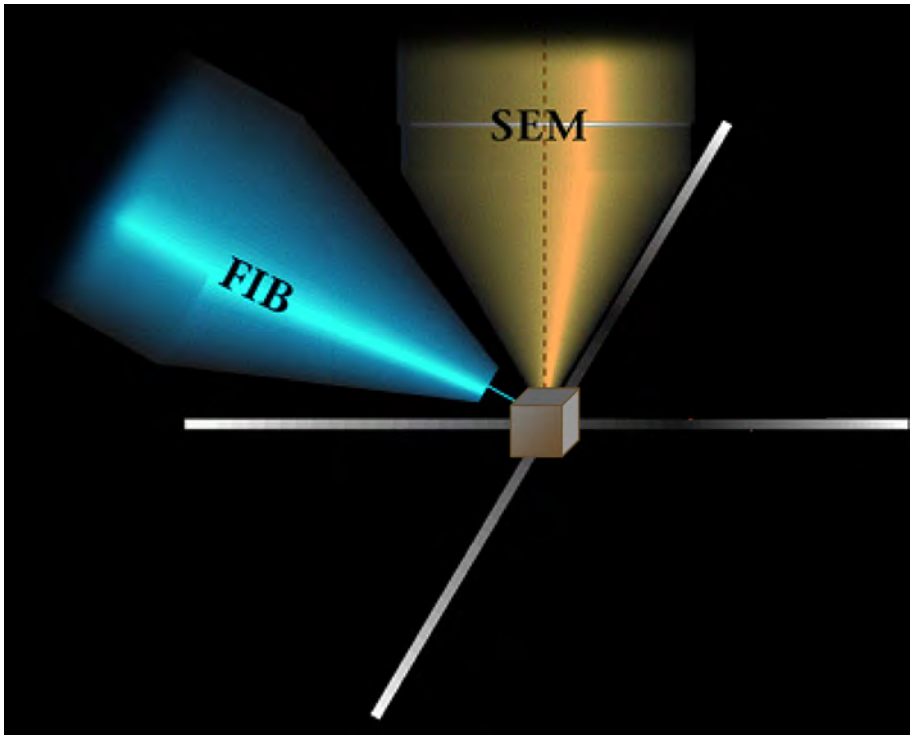
Focused ion beam Scanning Electron Microscopy for rapid 3D imaging of cells and tissue

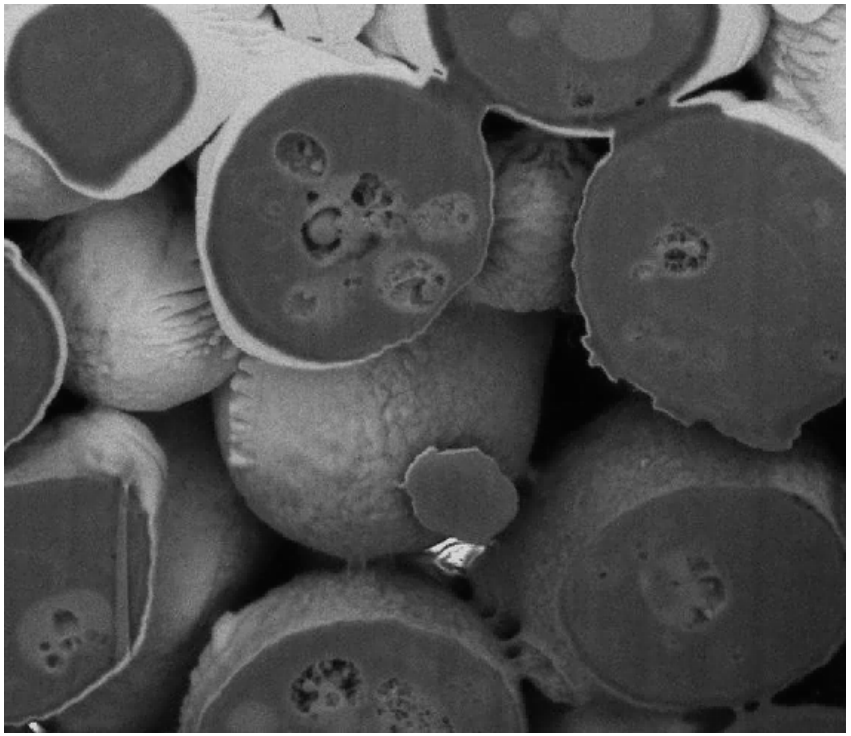




Iteration of slicing and imaging

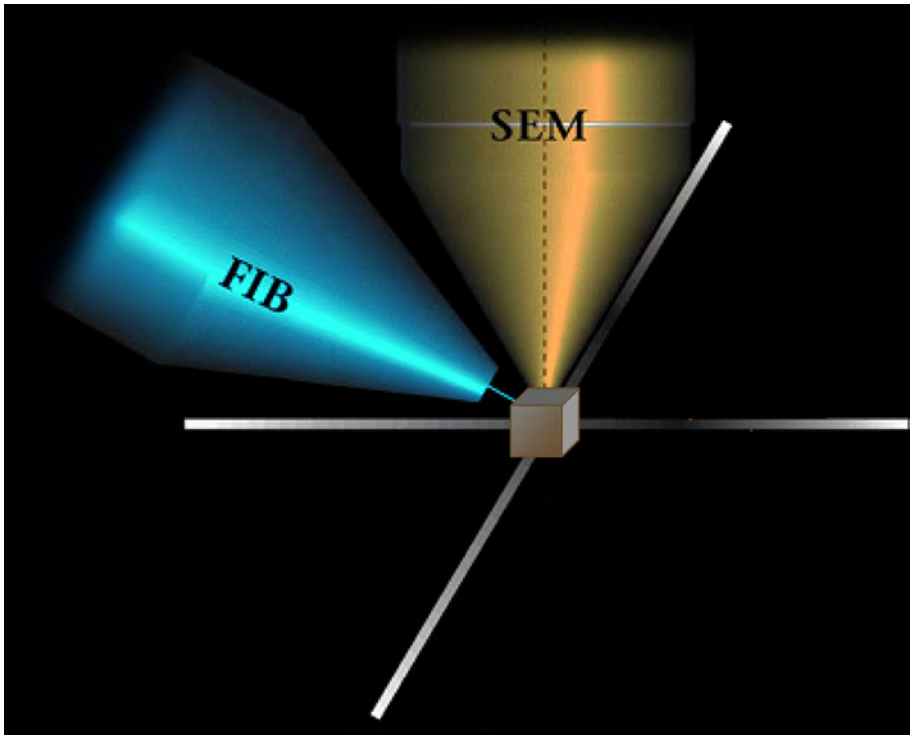
Focused ion beam Scanning Electron Microscopy for rapid 3D imaging of cells and tissue



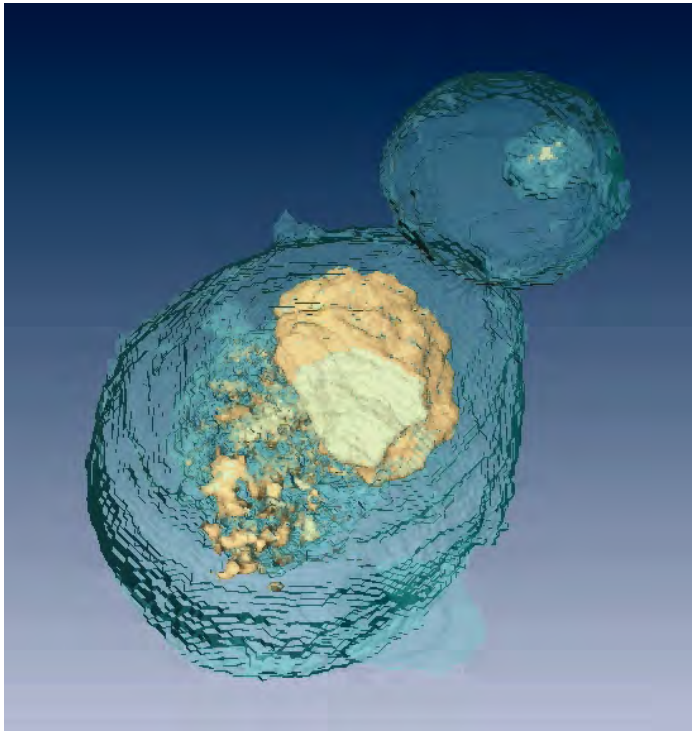


Iteration of slicing and imaging

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

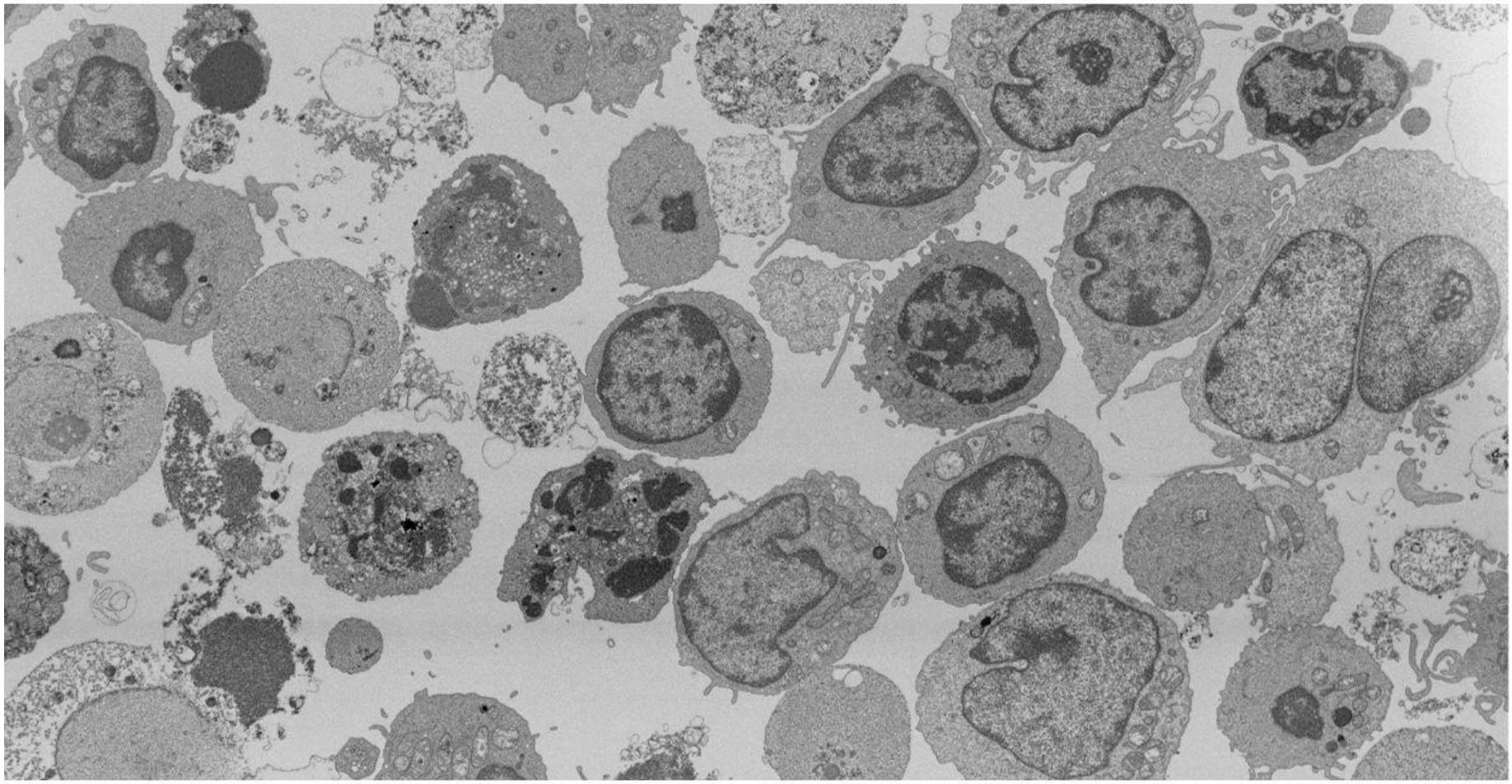


Iteration of slicing and imaging

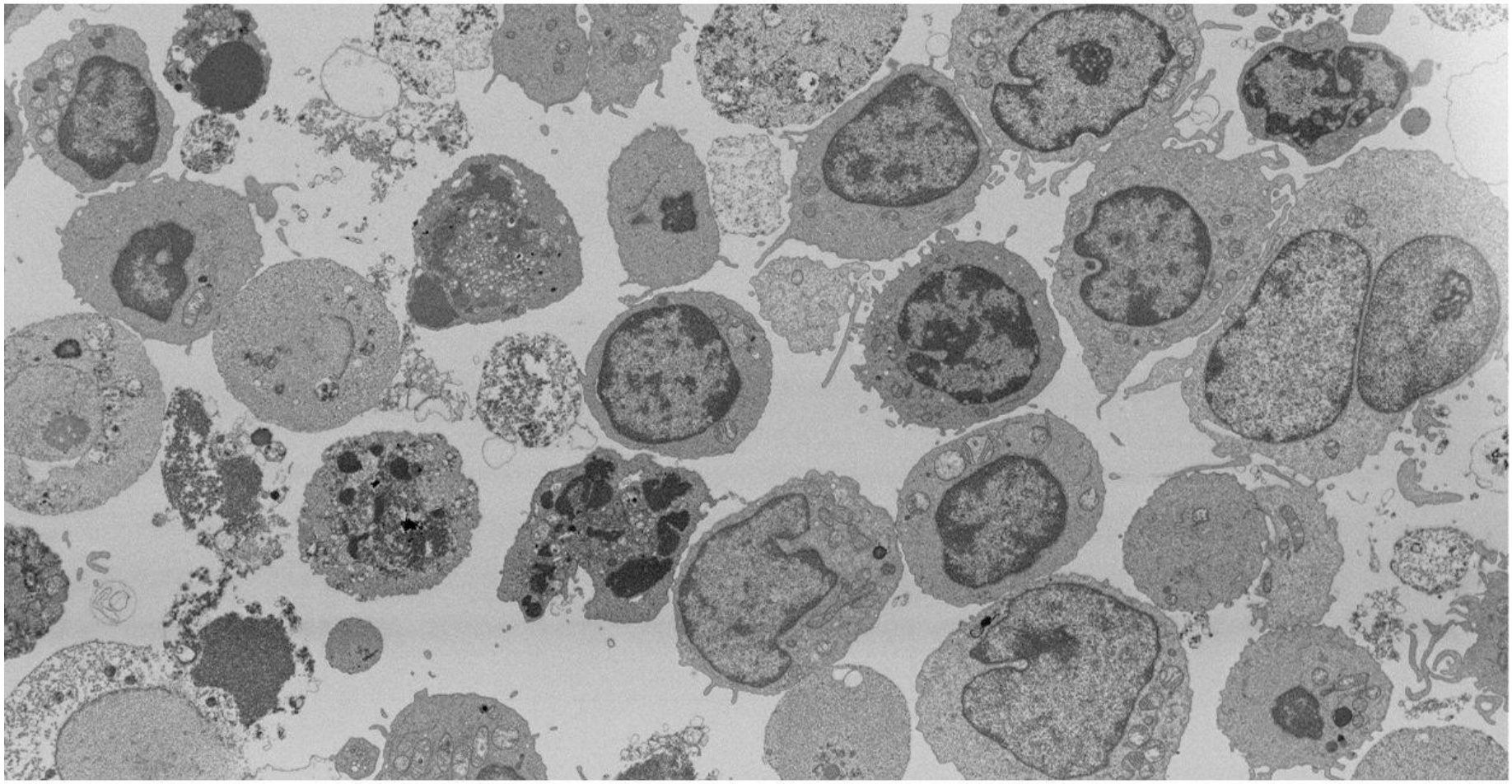


Heymann et al (2006)

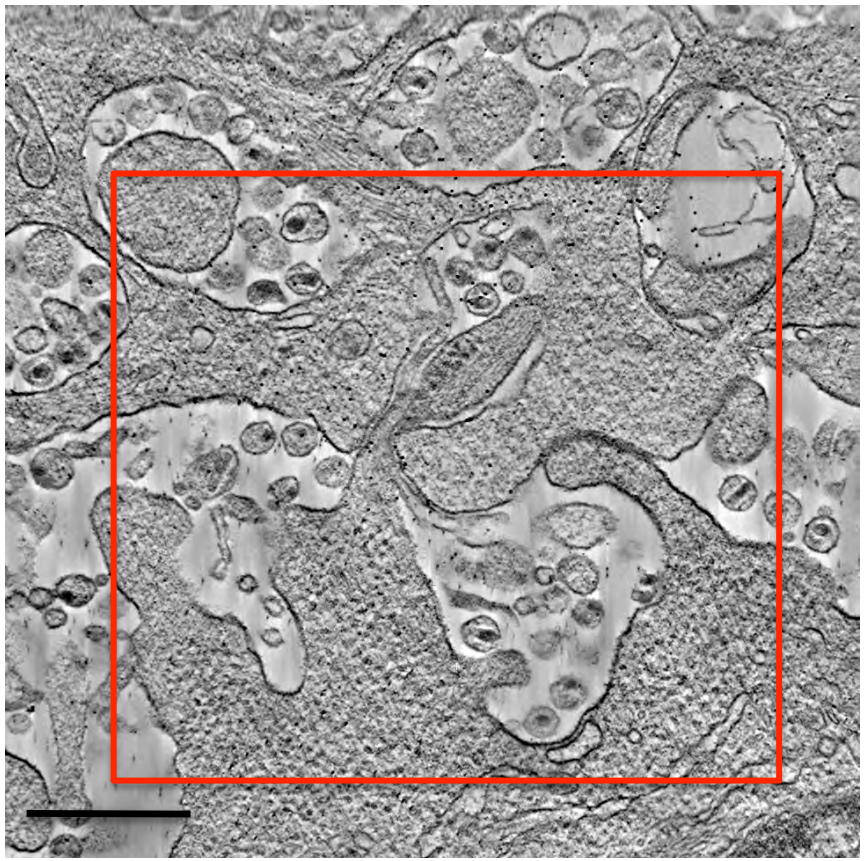
A journey into T-cell synapses

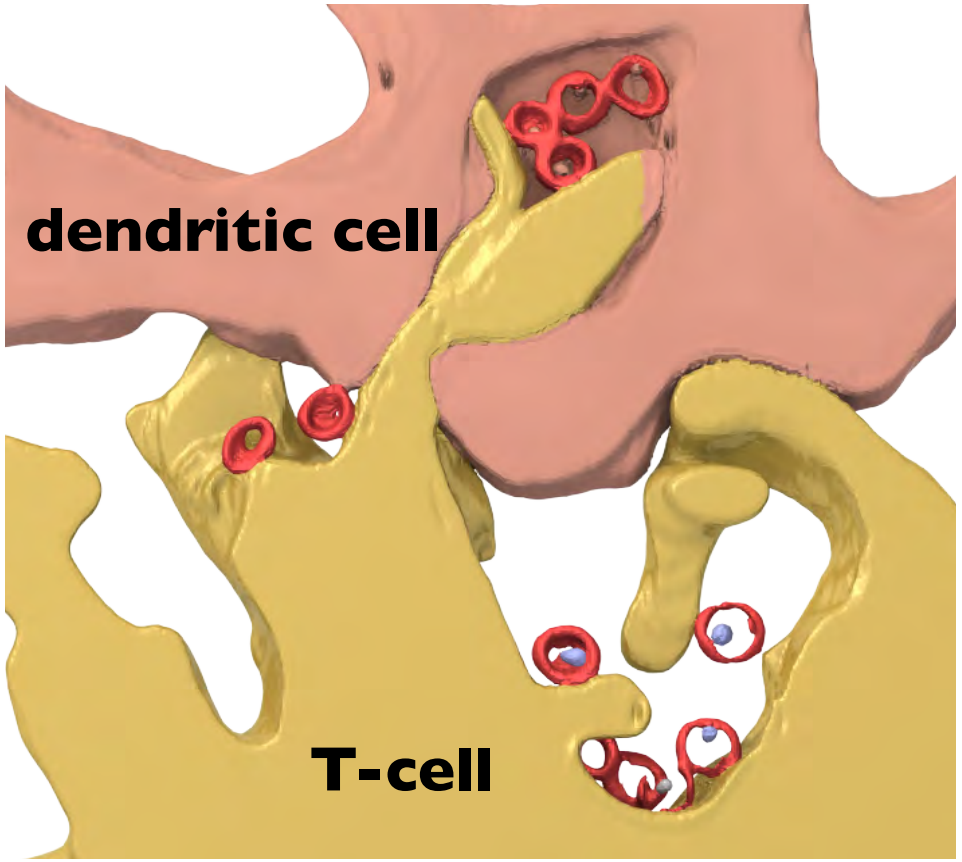


A journey into T-cell synapses

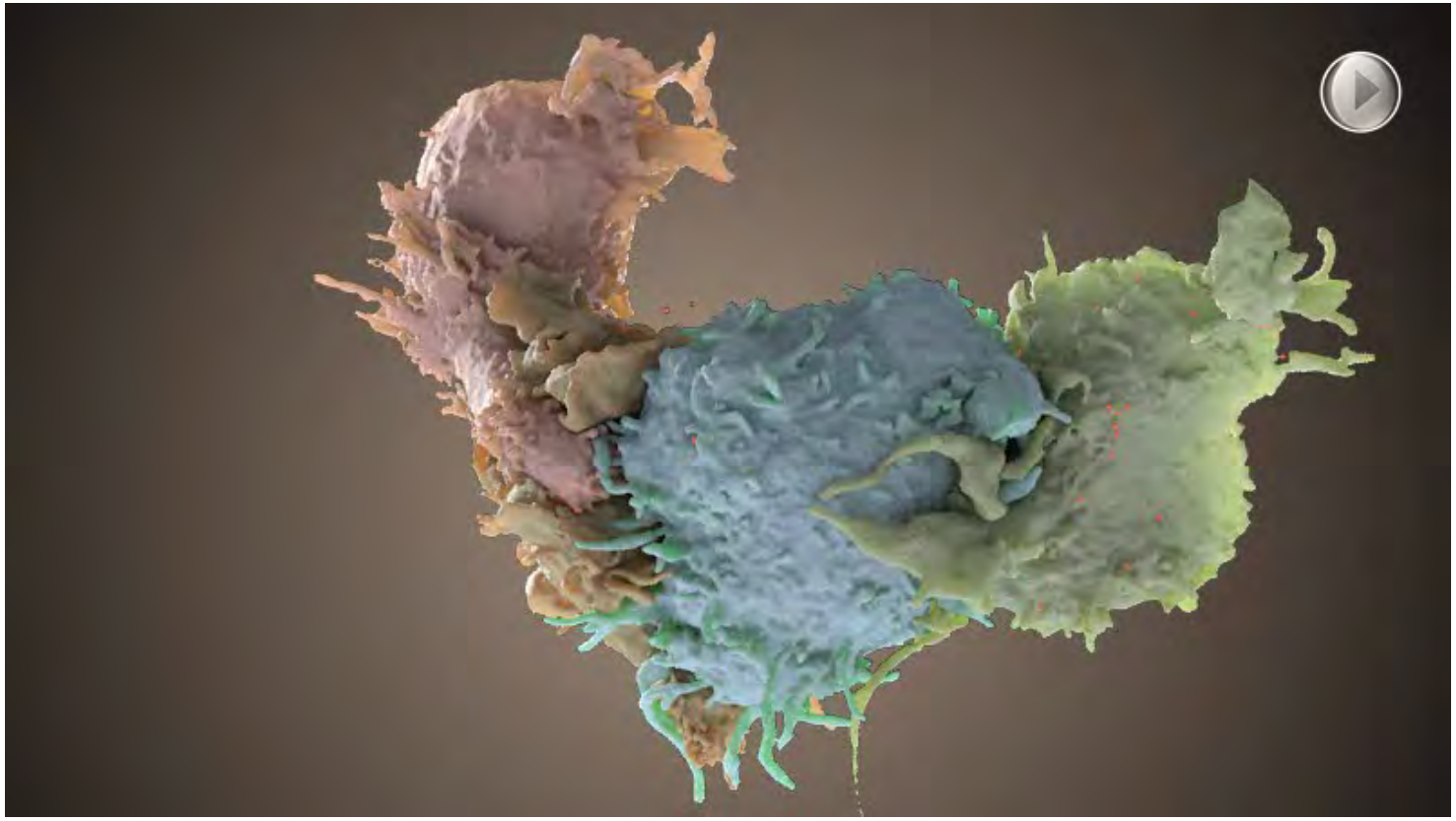


Intimate contact at the cell-cell interface



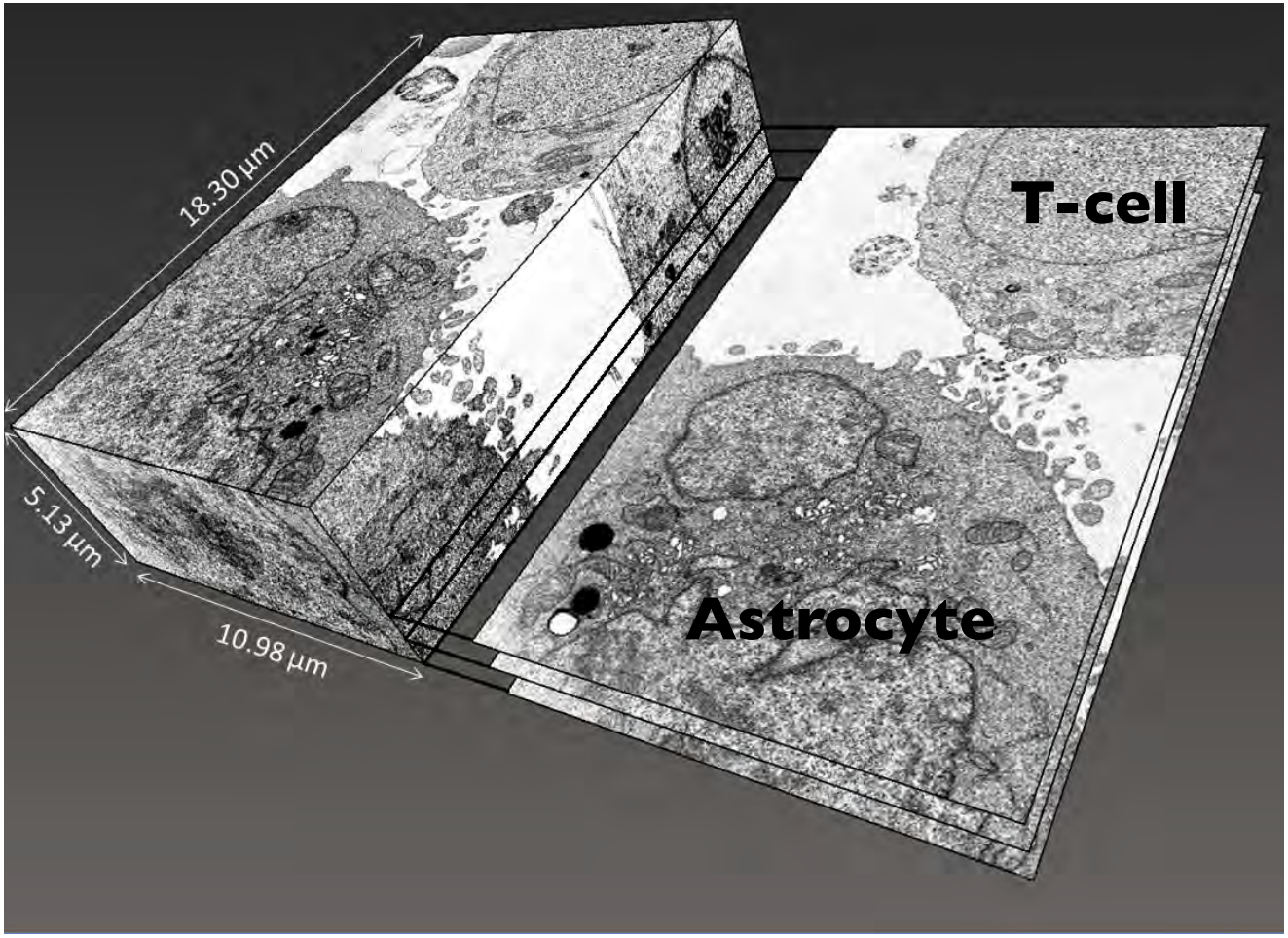


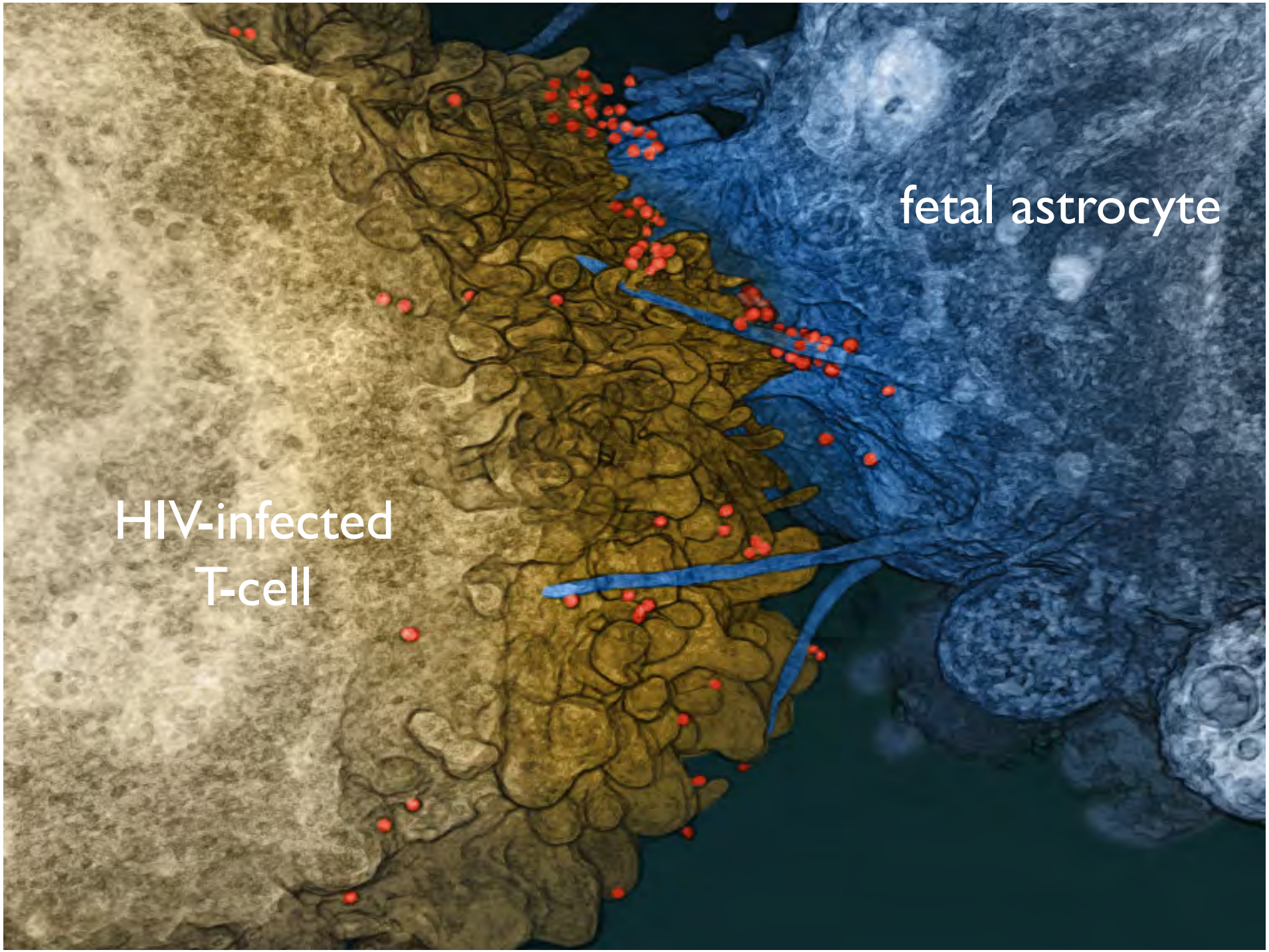
Synapses between primary T-cells



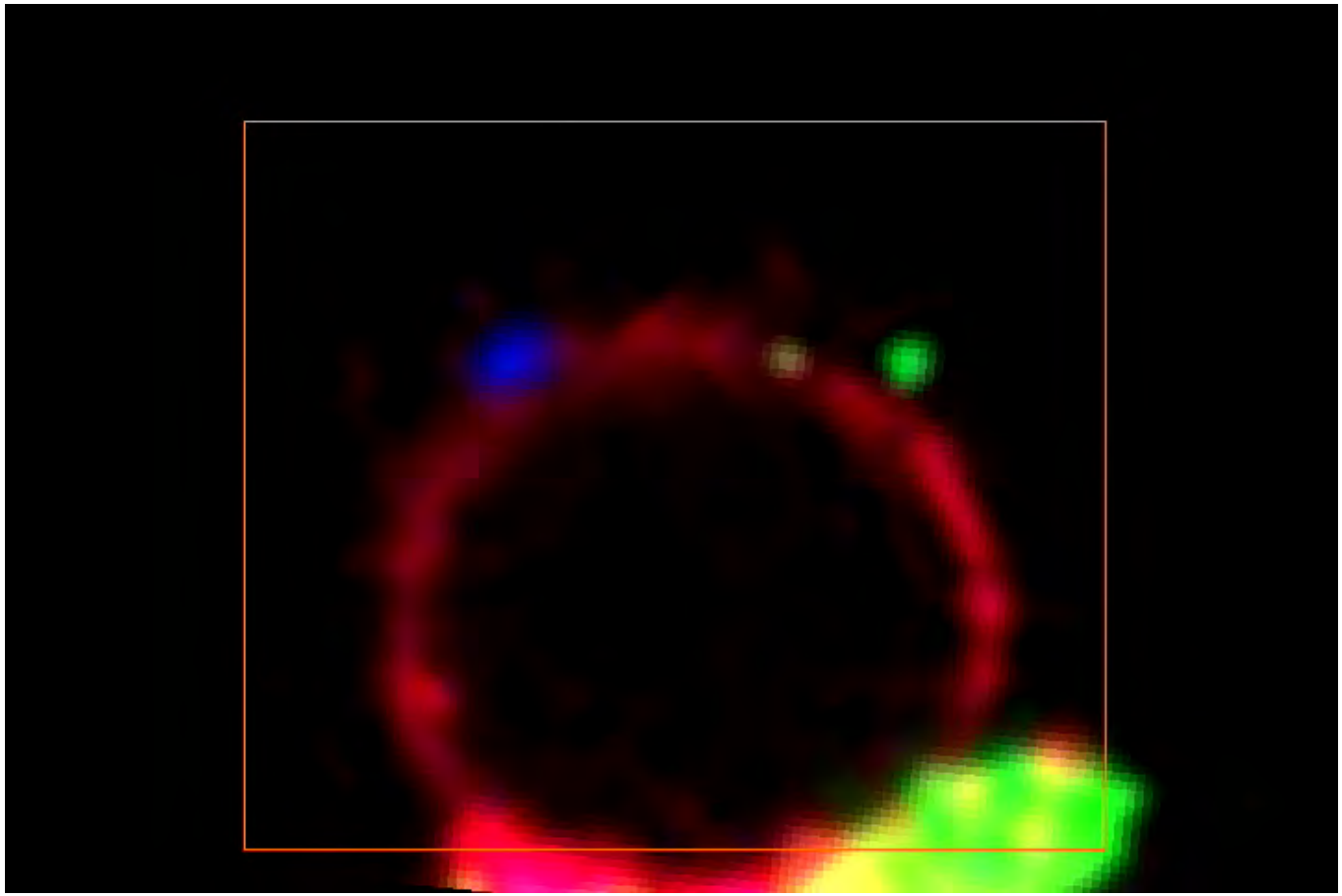
Do et al (2014)

HIV transfer to fetal astrocytes



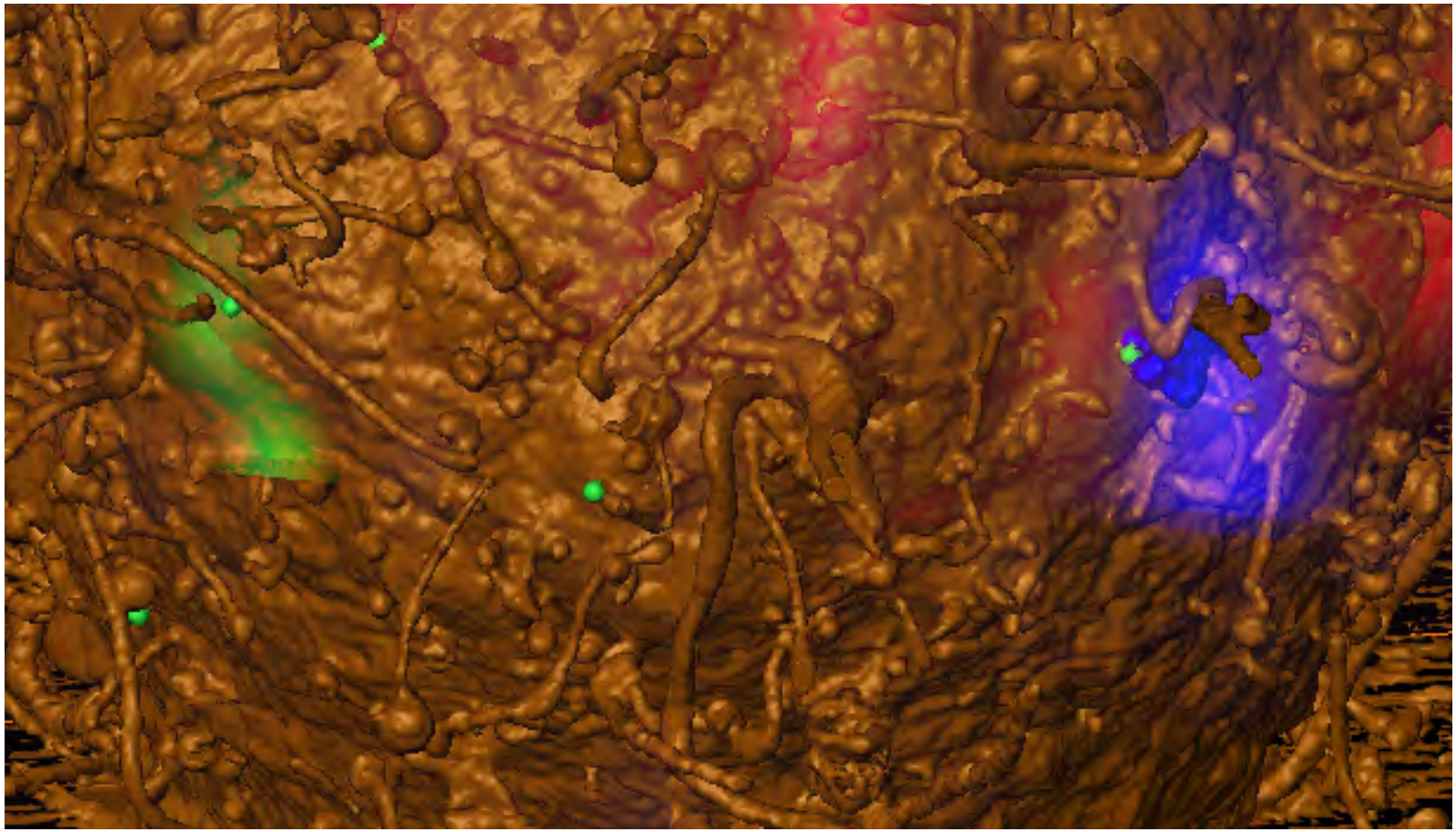

fetal astrocyte
HIV-infected
T-cell

Correlative live confocal and ion-abrasion SEM imaging: A cell biologist's dream



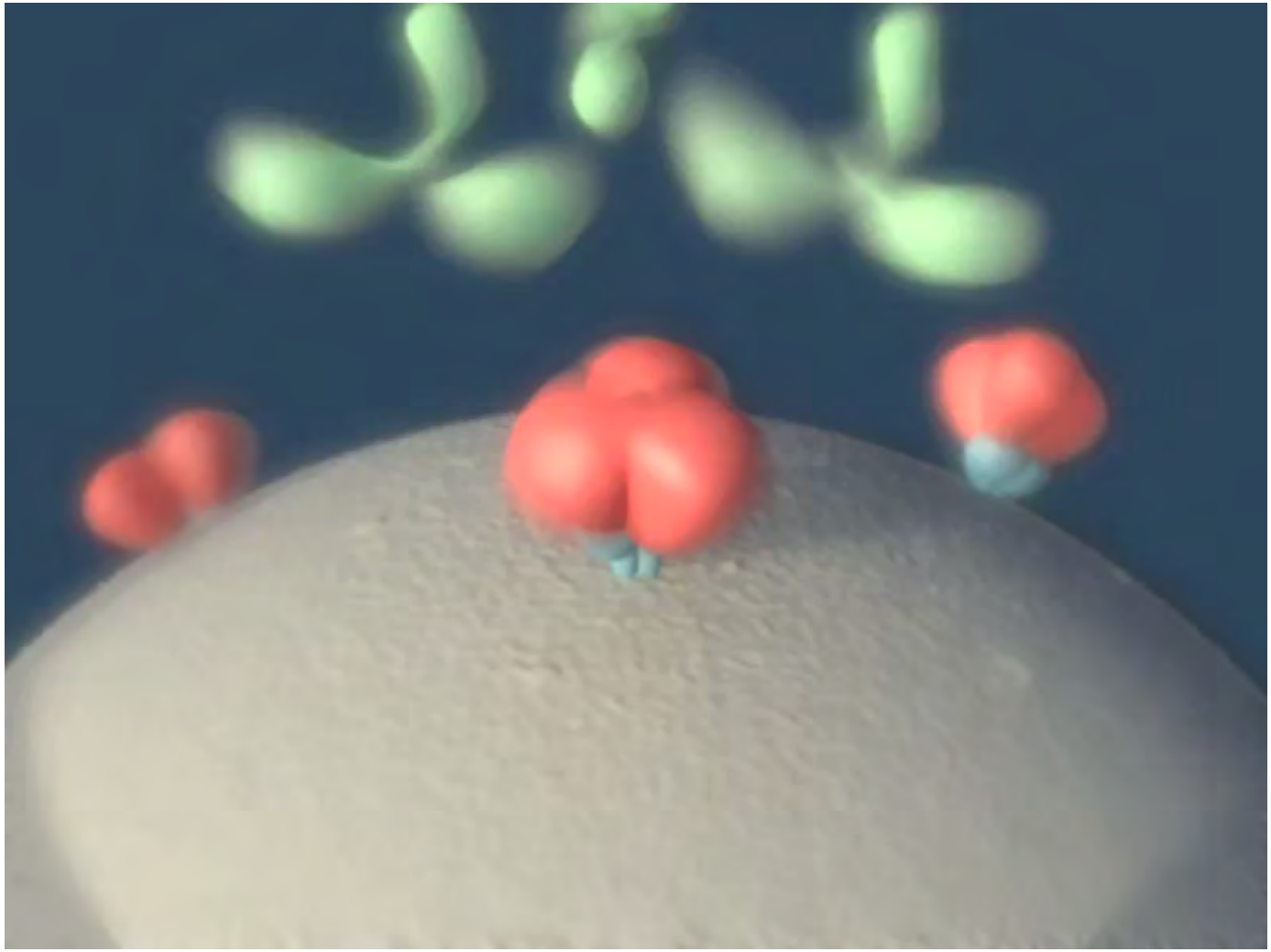
3D image of entire T-cell

Correlative live confocal and ion-abrasion SEM imaging: A cell biologist's dream

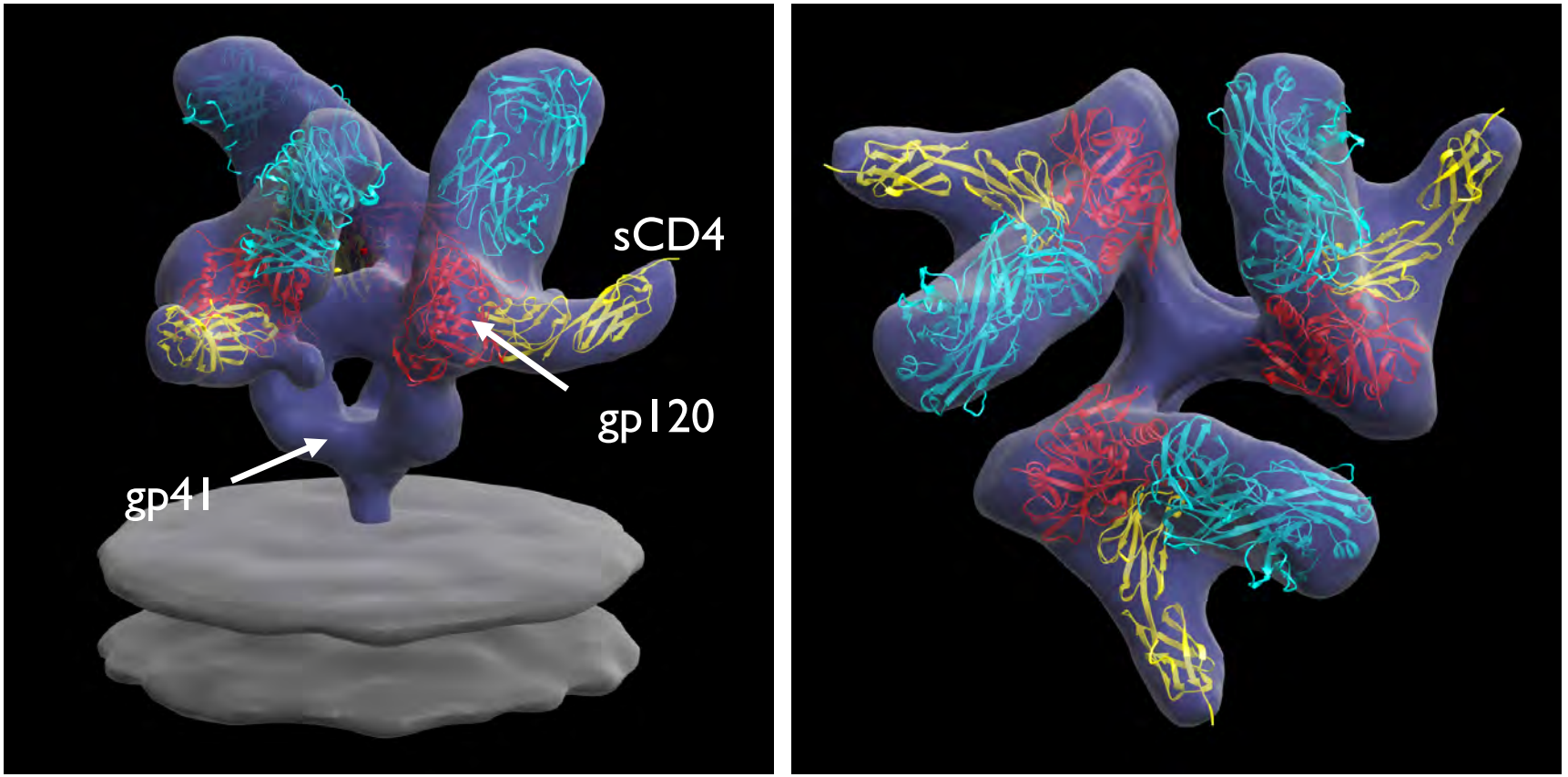


Narayan et al J. Struct. Biol. (2014)

The dynamic HIV spike

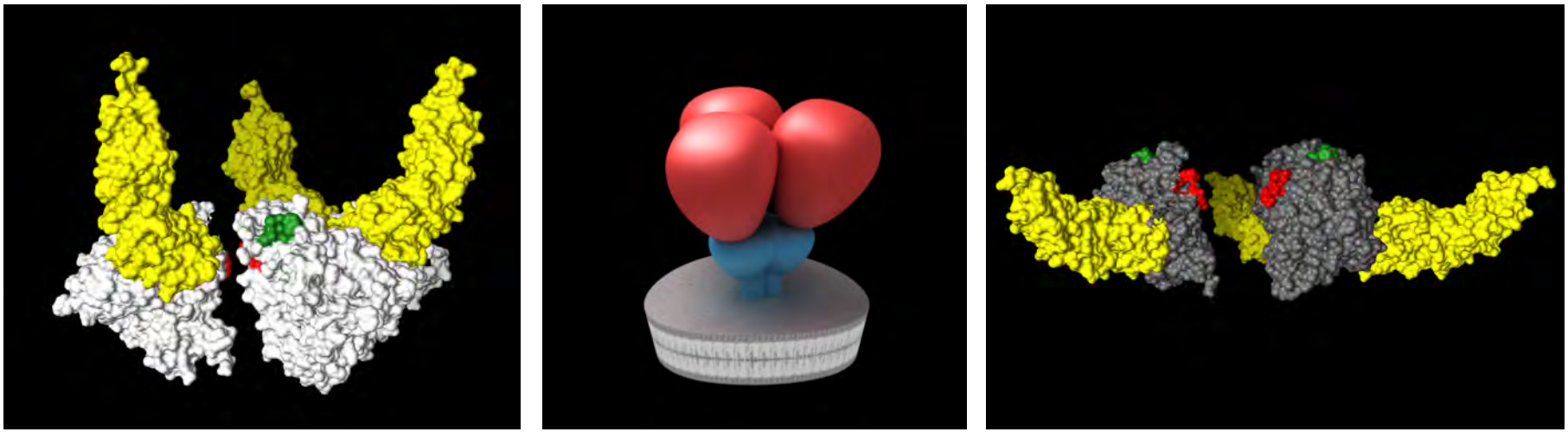


Molecular architecture of trimeric HIV envelope glycoproteins



Subtomogram averages at ~ 20 Å resolution

Catching HIV in the act with electron tomography



closed

open

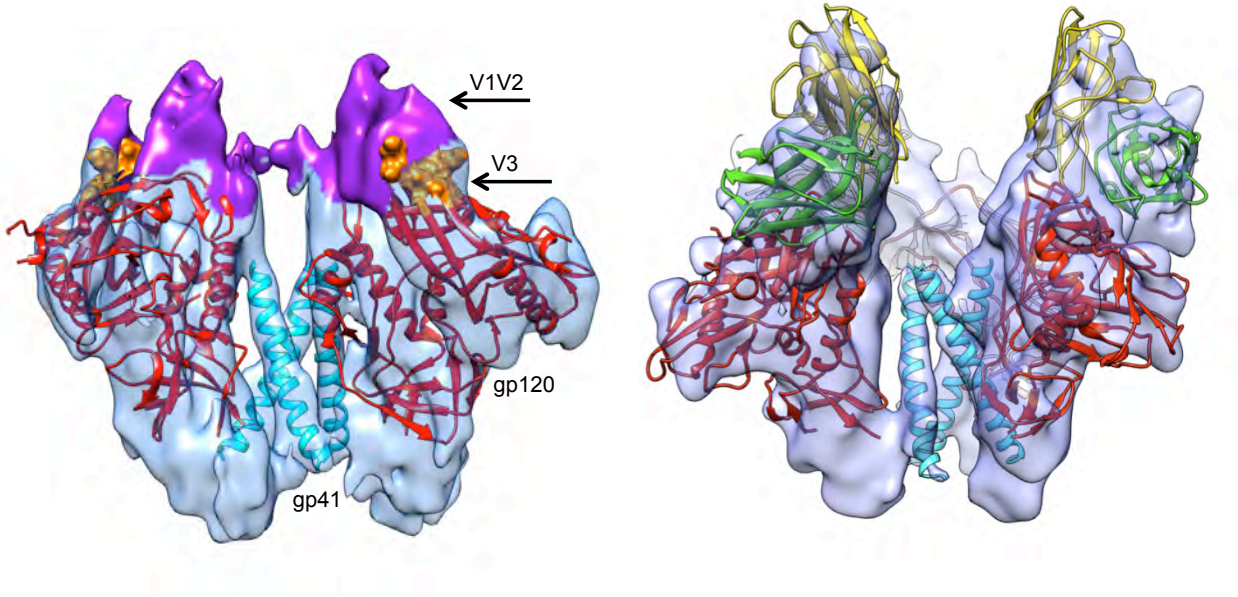
Liu et al, *Nature* (2008)

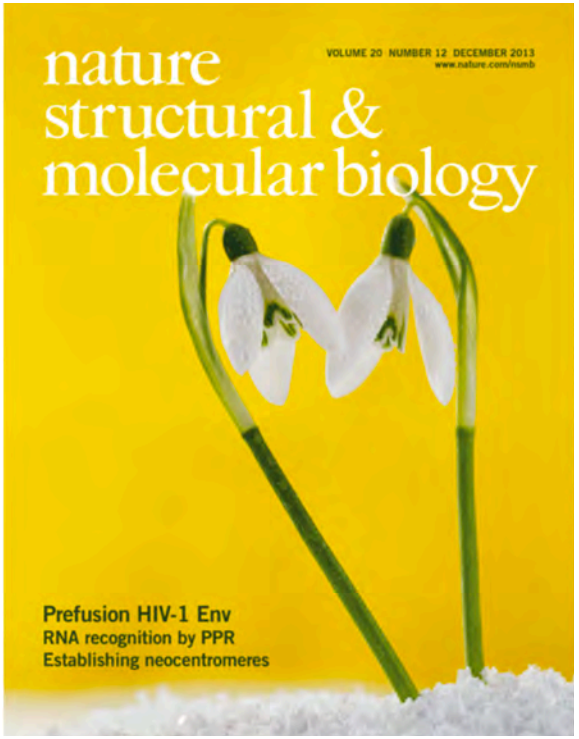
White et al *PLoS Path.* (2010)

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