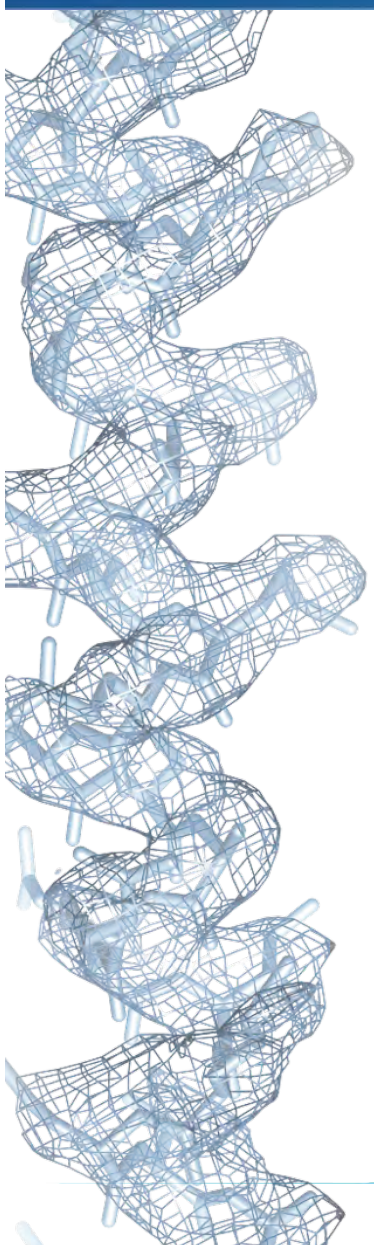
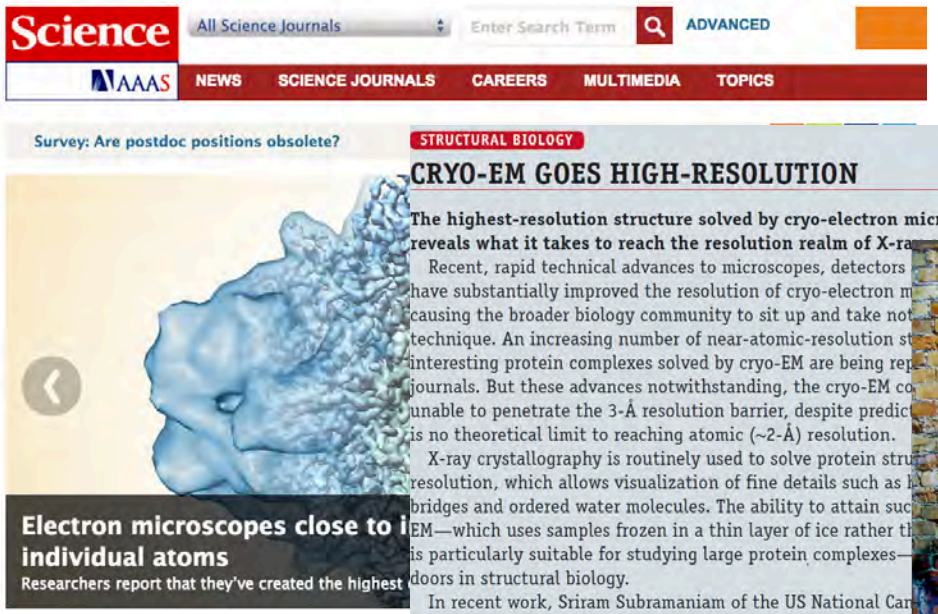


Proposal to Launch a National Cryo-EM User Facility at FNL

Sriram Subramaniam
National Cancer Institute
NIH, Bethesda, MD

September 30, 2015

Protein structure determination by cryo-EM



Science
June 2015

STRUCTURAL BIOLOGY

CRYO-EM GOES HIGH-RESOLUTION

The highest-resolution structure solved by cryo-electron microscopy to date reveals what it takes to reach the resolution realm of X-ra

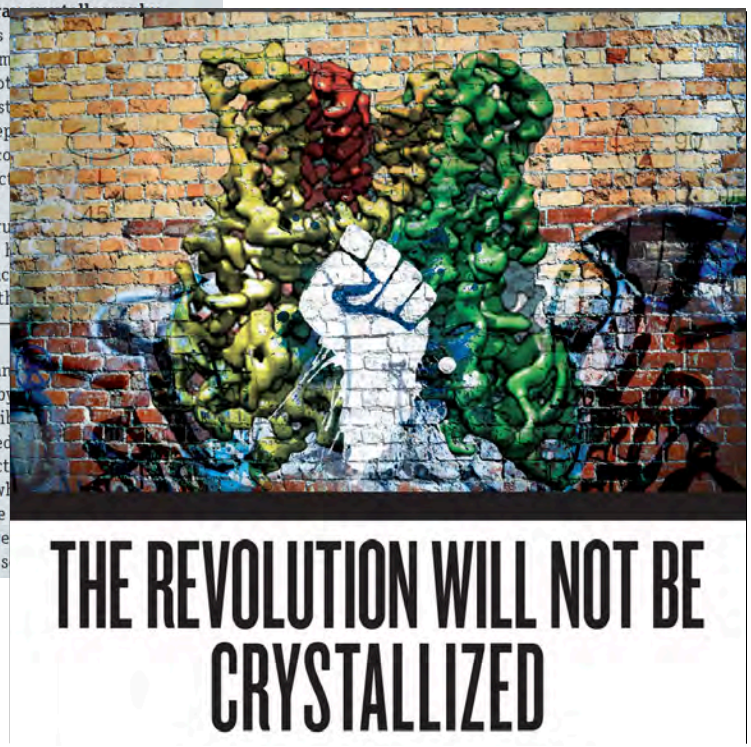
Recent, rapid technical advances to microscopes, detectors have substantially improved the resolution of cryo-electron m causing the broader biology community to sit up and take not technique. An increasing number of near-atomic-resolution st interesting protein complexes solved by cryo-EM are being rep journals. But these advances notwithstanding, the cryo-EM co unable to penetrate the 3-Å resolution barrier, despite predict is no theoretical limit to reaching atomic (~2-Å) resolution.

X-ray crystallography is routinely used to solve protein stru resolution, which allows visualization of fine details such as h bridges and ordered water molecules. The ability to attain suc EM—which uses samples frozen in a thin layer of ice rather th is particularly suitable for studying large protein complexes— doors in structural biology.

In recent work, Sriram Subramaniam of the US National Can colleagues reported the highest-resolution structure solved by complex between *Escherichia coli* β-galactosidase and an inhib thiogalactopyranoside) (Bartesaghi *et al.*, 2015). The reported

Just last year, Subramaniam's group reported a 3.2-Å struct β-galactosidase, a fairly ordinary enzyme of about 460 kDa wh solved by crystallography, allowing the researchers to vet the Reaching 3.2 Å was commendable, but Subramaniam was eage group might do to break through the 3-Å barrier. "There are s

Nature Methods
July 2015

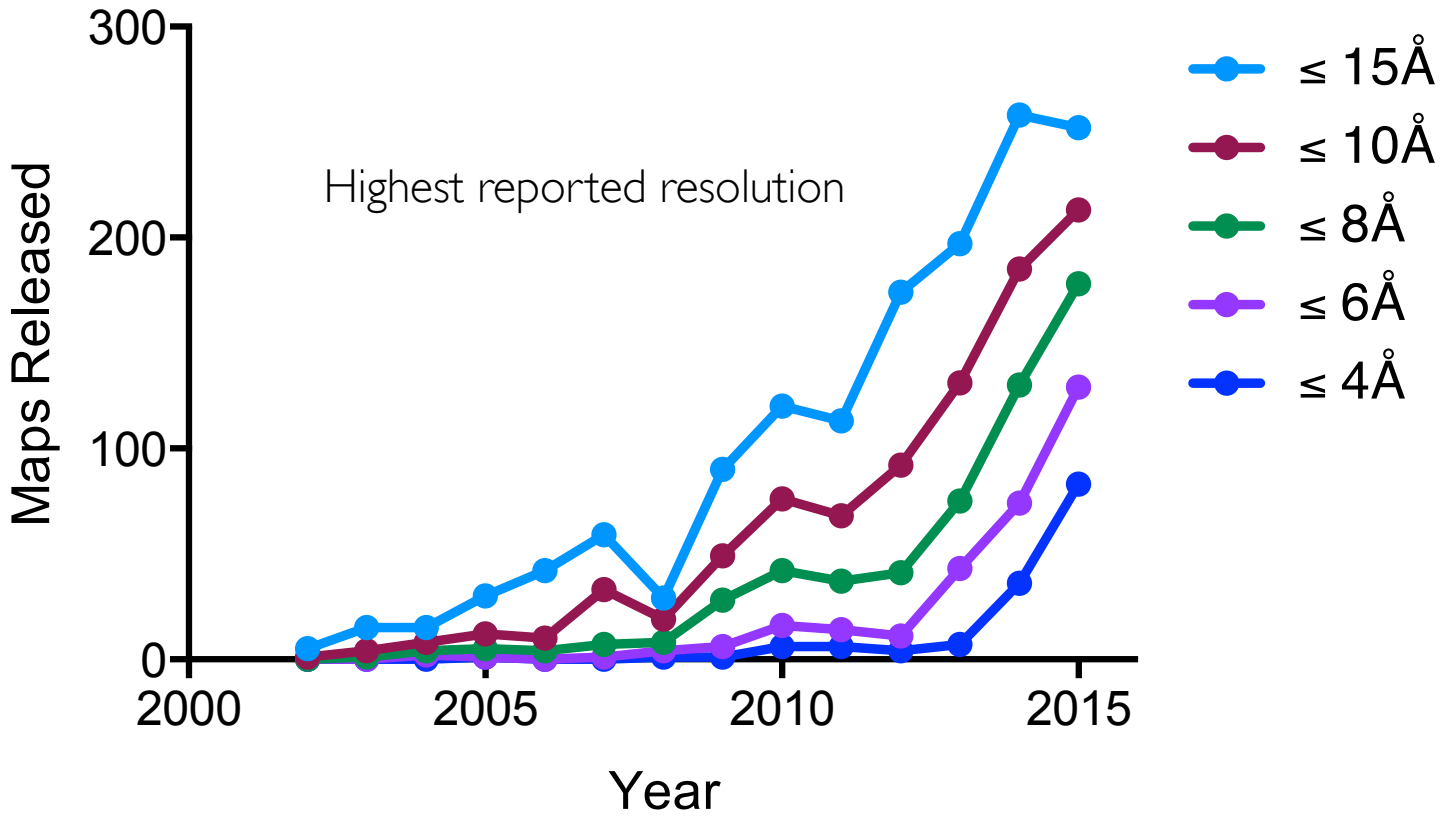


Nature
September 2015

The rise of cryo-EM

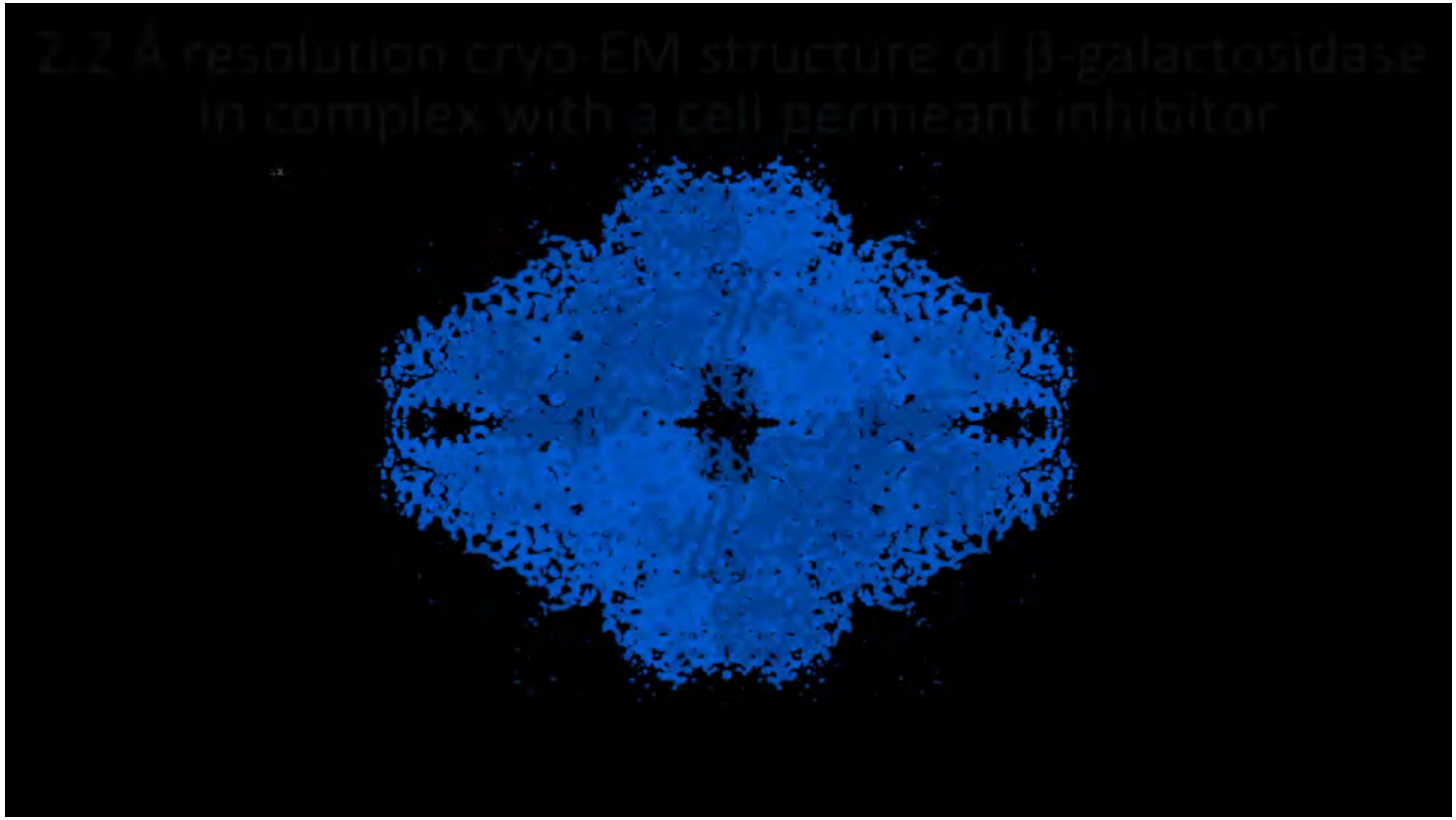
- Structure determination at high resolution without 3D crystals
- Structural analysis of dynamic protein assemblies
- Mapping conformational states of integral membrane proteins
- Localization of drug binding sites
- High degree of automation in data collection and processing

Growth of cryo-EM structures



From the Protein Data Bank website: pdbe.org/emstats

Cryo-EM of beta-galactosidase at 2.2 Å resolution



Bartesaghi, Merk, Banerjee, Matthies, Wu, Milne and Subramaniam, ***Science (2015)***

Chronology of efforts to launch cryo-EM at FNL

Spring-Summer 2014: Initial discussions between Subramaniam and NFAC leadership (Joe Gray, Dave Heimbrook and Harold Varmus)

September 2014: Subramaniam presentation at NFAC meeting proposing creation of National lab for cryo-EM at Frederick

December 2014: Subramaniam and Varmus organize workshop with leading structural biologists, institutional heads and other NIH IC representatives

Workshop results in recommendation of an urgent need for national user facilities similar to the synchrotron facilities available for X-ray crystallography

February 2015: Subramaniam presentation at the FNLAC meeting with a revised plan for the National Microscopy Laboratory

March 2015: Subramaniam presentation to Francis Collins and all NIH IC Directors on rapid growth of cryo-EM field and national needs

Defining user communities

- **Research groups already experienced in cryo-EM technology**
  - have some access to local screening microscopes
  - Inadequate access to high-end instrumentation
  - are key drivers of growth of cryo-EM in the US

- **Structural biologists in adjacent disciplines (X-ray, NMR)**
  - see value in using cryo-EM
  - have expertise in protein biochemistry
  - need training in cryo-EM specimen preparation, data collection and processing

- **Biologists with interest in important biomedical problems**
  - Interested in adding cryo-EM methods to their toolkit
  - need training and collaboration in all aspects of the workflow from protein purification to the final interpretation of the structures

New Titan Krios arrives: September 25, 2015

Proposed Budget FY2016 - FY2019

Initial equipment investment:

- NCI intramural program loans a new Titan Krios microscope for 4 years to FNL cryo-EM facility (value: $4M)
- FNL provides funding for microscope upgrades: $3M

Annual running costs:

- $2M ($1M personnel (not counting Director; $1M service contract/IT /consumables)

FY2016: $5M

Equipment addition:

- $3M for screening microscope

Annual running costs:

- $2.5M ($1.5M personnel; $1M service contract/IT /consumables)

FY2017: $5.5M

Equipment addition:

- $3.5M for next generation electron microscope

Annual running costs:

- $5.5M ($3.5M personnel; $2M service contract/IT /consumables)

FY2018 and FY2019: $4.5M/year

A possible model for user access

Proposed Cost

- NCI-funded user: $1000/slot
- Non-NCI, NIH-funded user: $1500/slot
- Non-NIH funded, academic user: $2000/slot

Instrument time

- Each slot is 24 hours (cryo-EM and cryo-electron tomography)
- Maximum 2-3 slots/turn and two turns/month
- Users expected to arrive with samples ready to load
- Must provide evidence of sample quality from prior cryo-EM screening

Overall capacity

- First-come, first-serve, at least initially, to assess demand
- Projected capacity of 5 slots/week and 20 slots/month

Administration: Cryo-EM FNL initiative

Organizational structure

- Director
- Executive Assistant
- Lead microscopist
- Microscopists for 24/7 operation (2x)
- IT support

Steering Committee

- Selected from key representatives of user community and institutions
- Provide advice and guidance on mission and evaluate performance

Funding and evaluation

- Reports provided to FNLAC and NCI leadership every six months

Cryo-EM at the FNLCR: Proposed Execution Plan