



Computing the molecular structures of cells and viruses using 3D electron microscopy

Sriram Subramaniam



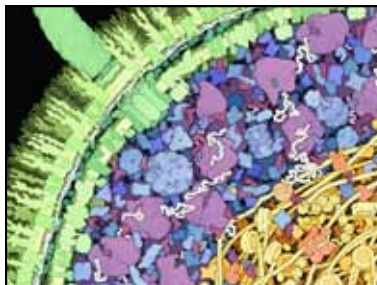
Imaging gaps in biology and medicine




Subramaniam, Curr. Opin. Microbiol. (2005)

A CLEAR PICTURE of the interior of a living cell that shows the precise distribution of molecules at the proper scale, the proper concentration and with no missing parts, seems to us to be essential to the understanding of the workings of life. However, this type of picture is virtually absent from the popular and technical literature. The reason for the paucity of comprehensive pictures is simple: there is no single experimental method to determine the information needed for their construction. Electron microscopy gives a view that is too narrow, subcellular structure is studied, but individual molecules are not seen. X-ray crystallography and X-ray diffraction, at the other extreme, see the finer, individual molecules in great detail, but information on how cellular environment is lost in their production. The intermediate level— the molecular structure of cells— must be synthesized from information from these two extremes. Merging many individual molecular pictures together to form a complete overall view.

David Goodsell TIBS (1991)

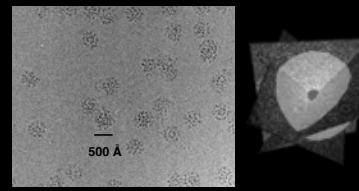


From molecules to cells



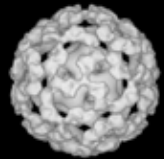
Dynamic multiprotein complexes
Signaling assemblies in intact cells
HIV structure and cell entry mechanisms
Subcellular architecture of melanoma cells

Images of single multi-enzyme complexes

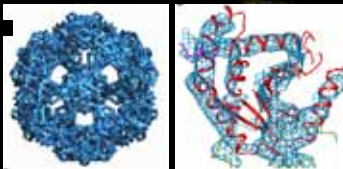


500 Å

Reconstructed 3D structure....

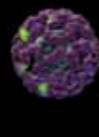


Reconstructed 3D structure....



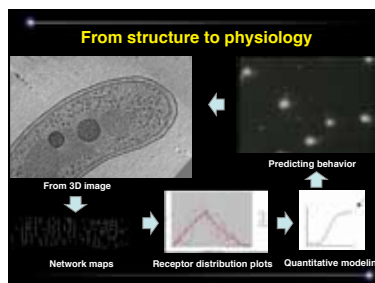
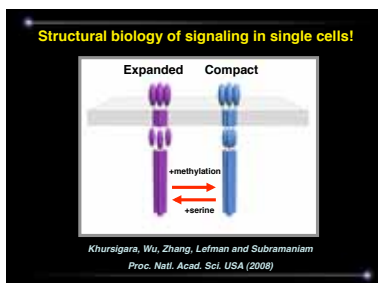
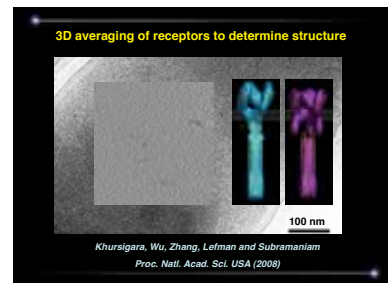
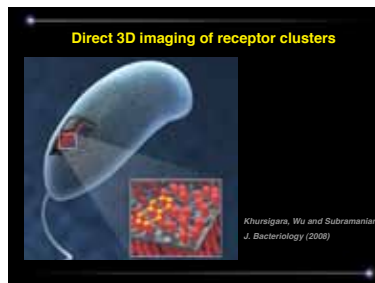
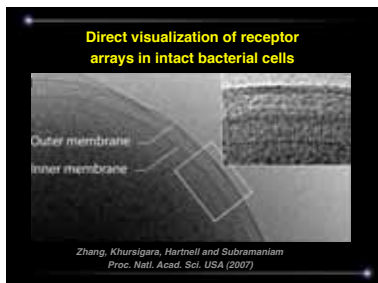
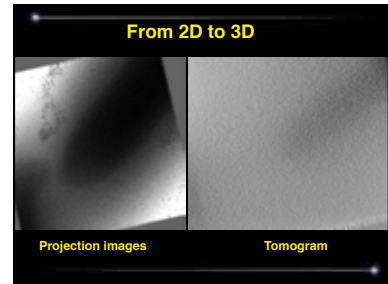
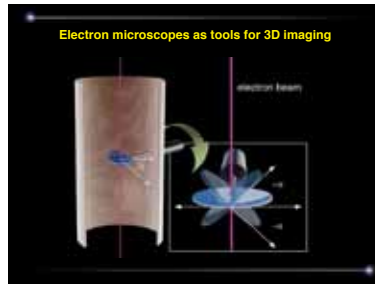
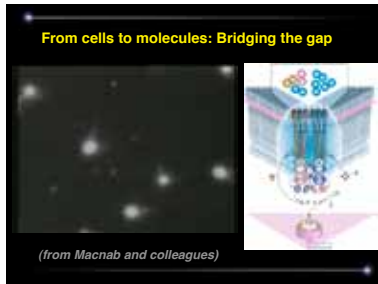
....and atomic interpretation

Structure and function of pyruvate dehydrogenase



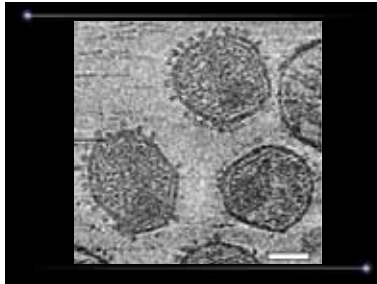
50 nm

Milne et al 2002, 2004, 2006; Borghia et al 2004; Lengyel et al 2008



Yet, the major goal of HIV/AIDS research eludes us: the development of a safe and effective HIV vaccine, our best hope for ultimately ending the pandemic. The search for a vaccine has been made extremely difficult by the nature of the virus, particularly its ability to integrate into the genome of host cells, to mutate readily and to conceal that part of its outer coat that would induce protective antibodies.

Dr. Anthony Fauci in *Nature*, May 2008



Structural analysis of viral spikes by electron tomography

2D image

tomogram

3D volume

Cryo-electron microscopy of "symmetric" vs. "asymmetric viruses"

Each viral particle identical; can achieve resolutions better than ~4 Å by 3D averaging

Each viral particle different; missing data distorts feature depending on orientation

From Subramaniam et al. *Curr Opin Struct Biol* (2007)

Classification and 3D averaging with missing wedge correction in biological electron tomography

Journal of Structural Biology

Classification and 3D averaging with missing wedge correction in biological electron tomography

© Subramaniam et al. 2007

HIV-1 surface spike in complex with neutralizing antibody

gp41

Map at 20 Å resolution

HIV-1 surface spike in complex with CD4 and 17b

V1/V2

Map at 20 Å resolution

Catching HIV in the act

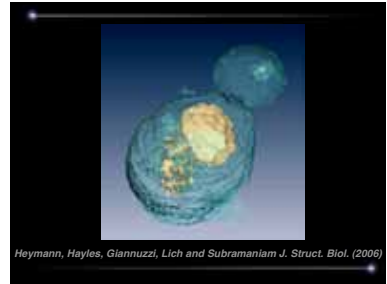
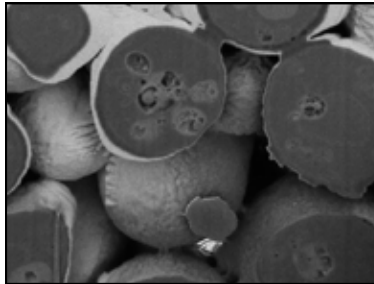
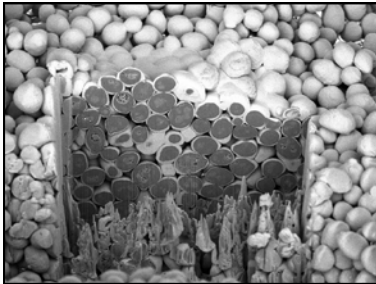
Liu, Barlesaghi, Borgnia, Sapiro and Subramaniam
Nature (2008)

Most objects of biological interest are thicker than 500 nm...

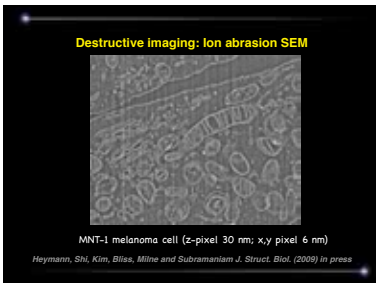
We therefore need methods that extend beyond conventional transmission electron microscopy

Destructive imaging: Ion abrasion SEM

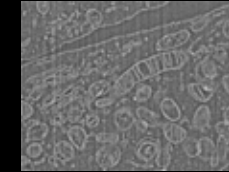
"Slice and View"



Heymann, Hayles, Giannuzzi, Lich and Subramaniam J. Struct. Biol. (2009)

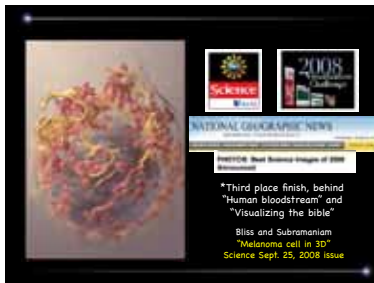


Destructive imaging: Ion abrasion SEM



MNT-1 melanoma cell (z-pixel 30 nm; x,y pixel 6 nm)

Heymann, Shi, Kim, Bliss, Milne and Subramaniam J. Struct. Biol. (2009) in press



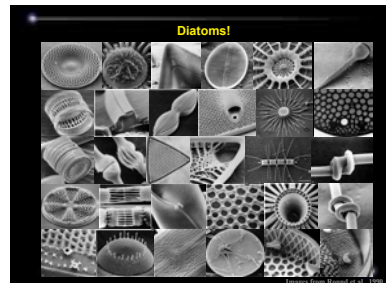
2008
SCIENCE

NATIONAL GEOGRAPHIC NEWS
Special Double Issue

2008
Best Science Images of 2008
Melanoma

*Third place finish, behind
"Human bloodstream" and
"Visualizing the bible"

Bliss and Subramaniam
"Melanoma cell in 3D"
Science Sept. 26, 2008 issue



Diatoms!

Images from Rosset et al., 2008



Walking into dividing diatoms



Thank you Biowulf!

