Visualizing Biological Processes with CryoEM
"It is very easy to answer many of these fundamental biological questions; you just look at the thing!... Make the microscope one hundred times more powerful, and many problems of biology would be made very much easier. I exaggerate, of course, but the biologists would surely be very thankful to you .....”

Richard P. Feynman.

from: There's Plenty of Room at the Bottom, a lecture given to the American Physical Society in 1959.
The Scale of Biological Structures

http://www.jic.ac.uk/microscopy/images/scale.gif
Images by David Goodsell, Scripps Research Institute
The Transmission Electron Microscope
What distinguishes EM AND LM?

Resolution!
The Transmission Electron Microscope

Resolution: ~ 0.2 nm
So why can’t we just look at biological specimens and see details to 0.2 nm resolution?

TEM requires a thin specimen that can withstand a vacuum and high energy electrons.
CryoEM – embedding specimens in vitreous ice
CryoEM – embedding specimens in vitreous ice
What does a vitrified specimen look like?

3mm
The physical challenge of CryoEM - Radiation damage

“Low-dose” imaging
Plus... The mathematical challenge of cryoEM:
2D projections to 3D volumes
3D Structure of Macromolecular Machines: “electron crystallography” or “single particle” reconstruction methods
The challenge is:

How to we go from…. 

this  to…  that
The first step:
Improving Signal to Noise using Averaging

I copy
400 copies
600 copies
Plus the mathematics of tomography...

**CAT - scan**
- beam rotating
- patient stationary

**Single particle reconstruction**
- molecule "rotating"
- beam stationary

Slide courtesy Joachim Frank
The relation between averaging and resolution:

- **Low Resolution**
  - Size: 30 Å
  - Shape: Subunit

- **Intermediate Resolution**
  - Size: 10 Å
  - Shape: Domains, Helices

- **High Resolution**
  - Size: 5 Å
  - Shape: Beta sheets
  - Subunit: Backbone trace

2BTV Slide courtesy Wah Chiu
Single particle reconstruction: structure of macromolecular machines
The overall mission of NRA MM is to develop, test and apply technology aimed towards automating and streamlining cryo-electron microscopy (cryoEM) for structural biology.

**Automation goals**
- Facilitate the process
- Increase throughput
- Optimize resolution
- Expand the possibilities
- Open the technology to wider audience
Automated Pipeline for Molecular Microscopy

spec. prep.  spec. screening  data collection  processing  refinement

Adapted from a slide courtesy of: Peter Kuhn, Scripps-PARC Institute for Advanced Biomedical Sciences, TSRI
CryoEM Pipeline at NRAMM

Sample

Automated Data Collection (LEGIONON)

3D EM Density

Streamlined Processing (Appion)

Specimen Vitrification
CryoEM Pipeline at NRAMM

- Sample
- Specimen Vitrification
- Automated Data Collection (LEGINON)
- Streamlined Processing (Appion)
- 3D EM Density
CryoEM Pipeline at NRAMM

Sample

Automated Data Collection (LEGINON)

Specimen Vitrification

Streamlined Processing (Appion)

3D EM Density
CryoEM Pipeline at NRAMM

Sample

Automated Data Collection (LEGINON)

Specimen Vitrification

Streamlined Processing (Appion)

3D EM Density
Four case studies to illustrate the pipeline....
DNA packaging and delivery machines

P22

Lambda

Gabe Lander

In Collaboration with Alex Evilevitch, Sherwood Casjens, Peter Prevelige, and Jack Johnson.
Viruses vs. Bacteria: An Arms Race
Podoviridae Infection of Gram-Negative Cells
Podoviridae Infection of Cells

Phage
Podoviridae Infection of Cells
Virus Assembly and Maturation

maturation - gp3, gp2 package DNA

gp1

gp5

gp7, gp16, gp20

gp8

gp2

gp3

gp9

gp26

gp8

gp4

gp10
Lambda virus maturation
Virus Assembly and Maturation
4,899 images; 25,793 particles
P22 Tail Machine

- 2 megadaltons
- 5 types of proteins
- 51 subunits

12 x gp1
12 x gp4
6 x gp10
6 x 3 gp9
3 x gp26
Docked Crystal Structures

gp26 trimer


Steinbacher et al. JMB (1997)

gp9 trimer (tailspike)
Isolated P22 Tail Machines

~350,000 particles
resolution ~ 9 Å
Isolated P22 Portals

~50,000 particles
resolution ~ 10 Å
gp1 - 12 subunits

gp4 - 12 subunits

gp10 - 6 subunits

gp9 - 6 trimers

gp26 - 3 subunits
Case Study
Structure of COPII coats and cages

Scott Stagg

In Collaboration with
Paul LaPoint, Cemal Gurkan, Douglas Fowler, Ted Foss, Bill Balch.
Vesicular trafficking

[Diagram showing vesicular trafficking pathways involving COP I, COP II, Clathrin, ER, Golgi, and surface membrane.]
Sec13/31 self-assembles into cages

Sec13/31 and Sec23/24 together assemble into a variety of structures of varying sizes and shapes
Sec13/31 self-assembles into cages

Sec13/31 and Sec23/24 together assemble into a variety of structures of varying sizes and shapes


Sec13/31 cage

Sec13/31 and Sec23/24 coat
Case Study
30S ribosome assembly

Craig Yoshioka

In Collaboration with
Megan Talkington and
James Williamson
Ribosome Assembly

Small Subunit (30S)

16S rRNA

21 Proteins (S1-S21)

Large Subunit (50S)

5S rRNA

23S rRNA

33 Proteins (L1-L36)
Ribosome Assembly, Time: ~42 sec
Ribosome Assembly, Time: 3 min
Ribosome Assembly, Time: 3 min
A potential folding pathway?
Automated Molecular Imaging Group at TSRI:

Jim Pulokas
Denis Fellmann
Joel Quispe
Anchi Cheng
Mark Palmer
Gabriel Lander
Pick-Wei Lau
Anke Mulder
Dmitry Lyumkis
Craig Yoshioka
Neil Voss
Christopher Irving
Lorraine Lathrop
Clint Potter
Bridget Carragher

National Resource for Automated Molecular Microscopy
http://nramm.scripps.edu
National Resource for Automated Molecular Microscopy
http://nramm.scripps.edu
Workshop on Advanced Topics in EM Structure Determination
November 10 -16, 2007
National Resource for Automated Molecular Microscopy
The Scripps Research Institute, La Jolla, CA

Participants
Xabier Agirrezabala; Jussi Aittoniemi; Teddy Ajero; Cinzia Ambrosi; Chris Arthur; Francisco Asturias; Tim Baker; Albion Baucom; David Belnap; Stefan Bohn; Chris Booth; Edward Brignole; Bridget Carragher; Joshua Chappie; James Chen; Shaoxia Chen; Songye Chen; Xiaocheng Chen; Anchi Cheng; Wah Chiu; So-Hye Cho; Jack Coats; Julia Cope; Pierre-Damien Coureux; Alexandra Deaconescu; Erik Debler; Frank Depoix; David DeRosier; Amedee des Georges; Kenneth Downing; Edward Egelman; Mary Evans; Denis Fellmann; Joachim Frank; Yoshinori Fujiyoshi; Carrie Gabaldon; Lu Gan; Christoph Gerle; Robert Glaeser; Thomas Goddard; Niko Grigorieff; Irina Gutsche; Stephen Harrison; Richard Henderson; Christopher Irving; Grant Jensen; Qiu-xing Jiang; Jack Johnson; Debbie Kelly; Christopher Kennaway; Reza Khayat; Kevin Koehntop; Justin Kollman; Anselm Kusser; Meindert Lamers; Gabriel Lander; Lorraine Lathrop; Pick-Wei Lau; Catherine Lawson; Jyh-Yeuan Lee; Kelly Lee; Kyung Eun Lee; Sukyeong Lee; Andres Leschziner; Hazel Levy; Yen-Chywan Liaw; Steven Ludtke; Jason Mears; Ulrich Meissner; Linda Melanson; Stephen Mick; Anna-Clare Milazzo; Ron Milligan; Crystal Moran; Anke Mulder; David Nackashi; Sunita Nayak; Eva Nogales; Pawel Penczek; Steven Pfeiffer; Clint Potter; Jim Pulokas; Joel Quispe; Ludovic Renault; Klaus Schulten; Paul Shao; Fred Sigworth; Thomas Smith; Daniel Southworth; Scott Stagg; Raymond Stevens; Min Su; Haixin Sui; Dongyan Tan; Kenneth Taylor; Dennis Thomas; Vinzent Unger; Nigel Unwin; Auke van Balen; Sangita Venkataraman; Neil Voss; Thomas Walz; Hongwei Wang; Andrew Ward; Liz Wilson-Kubalek; Chuan Xiao; Yi Xing; Fei Xu; Alevtyna Yakushevska; Mark Yeager; Craig Yoshioka;