Molecular Electron Microscopy

Cryo-Electron Microscopy

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Friday:  Introduction to Molecular Electron Microscopy
Monday:  Single-Particle EM
Monday:  High Resolution and Labeling

Methods, Samples & Symmetry

- 2D crystallography
  - membrane proteins: bacteriorhodopsin, LHC-II, aquaporin
  - other proteins: tubulin
- Helical structures
  - microtubules, bacterial flagellar filament
  - filamentous phages, filoviridae (eg, Ebola)
- Particles
  - Icosahedral virus capsids/complexes
  - Lower symmetry Clp-family of ATP-dependent proteases
  - No symmetry ribosome, phage particles
- Non-uniform structures
  - Virus capsids/complexes
  - Tomography
  - Clp-family of ATP-dependent proteases
  - Filamentous phages, filamentous phages
  - Non-uniform structures
  - Icosahedral virus capsids/complexes
  - Lower symmetry Clp-family of ATP-dependent proteases
  - No symmetry ribosome, phage particles

Image Reconstruction

- So we have some electron micrographs, what’s next?
- Image reconstruction: calculate a 3D density map
- Raw data are 2D projection images
- We can think of a projection image as a 2D density function representing a 3D volume
- Use the projection direction (orientation) for each image in a set images, back-project to simulate the original 3D volume
- "Single particle" and "tomography" are essentially the same:
  - SPR: many objects, orientations unknown
  - Tomo: one object, orientations ~known
Reconstructions - Central Section Theorem

Projection onto 2D plane (microscopy)

Fourier Transform

Fit through center of 3D volume

Reconstruction pathways

Fourier Transform

Need many images at different orientations to fill Fourier volume or Real Space volume

Back-Projection in 2D - 1. Projection images

Object

Back-Projection in 2D - 2. Projecting backwards

Object

Back-Projection in 2D - 3. Finer sampling

Object 30° 10° 5° 1°
Object! Back-Projection in 2D - 4. Details

Object! Back-Projection in 2D - 5. Another Example

Reconstructions - Central Section Theorem

Analysis of Single Particles

Single Particles means many isolated, or free-standing particles and preferably random views

Combine different views of identical objects

Resolution will be limited by:
- degree of identity
- accuracy of view estimations
- completeness of dataset (views)
- S/N and amount of data
- everything else!

Symmetry & size help, eg, icosahedral virus capsids

Contrast helps, eg cryo-negative stain
The icosahedron has 60-fold symmetry: 5x3x2x2
The simplest is T=1 (60 subunits)
More complex structures have more subunits: 60 x T
Selection rule:
\[ T = h^2 + hk + k^2 \]
\[ h, k = 0, 1, 2, \ldots \]
Allowed T-numbers:
1, 3, 4, 7, 9, 12, 13, 16, 19, ...
“Special” T-numbers:
2, 6, ???

\[ h^2 + h^*k + k^2 = T \]

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T-numbers

Image Reconstruction

Digitization
Initial orientations (common lines)
Reconstruction
Model-based Refinement
Selection

T = Triangulation

T = 1
T = 3
T = 4
**T=1: Adenovirus 3 Dodecahedral Particle**


**Structure: Visualization by cryoEM**

Dodecahedron Movie
"Extending Resolution" by Labeling

Conway et al, J Mol Biol 1997
Zlotnick et al, PNAS 1997
Conway et al, PNAS 1998

Confirmation by Xtallography

Wynne et al, Mol Cell, 1999; PDB 1qgt

Labeling with Fab's:
Linear and Conformational Epitopes

3105 F11A4
3120 Stain

HBcAg HBeAg
Cp147 Cp*150 Cpe

HBV; Fab-labelling

Fab 312 - model

HBV - Fab Complexes

Hepatitis B Virus: Capsid & Antibody
Feb 2002

Belnap et al. PNAS 2003

HBV - Fab Complexes

Residues in Epitope
78-83
77-80, 83-84
74-77
78-80, 83-84
22-26, 29-32, 126-127
50-52, 129-132

3120
312
3120
312
F11A4

78-83
74-77
78-80, 83-84
22-26, 29-32, 126-127
50-52, 129-132
HBV & Fabs: Conclusions

In General:
- 9Å maps, localize specific amino acids by tagging
- Identify conformational epitopes by cryoEM & modeling
- Occupancy: steric blocking & epitope conformation
- Diversity: 4 Fabs Þ 4 epitopes

HBV-Specific:
- Distinction between core-antigen and e-antigen
  - Accessible surfaces different
  - Fabs sensitive to small changes in structure (eg, 3120)
- Structural basis for literature on competition assays
  - Crowding: almost any pair of Fabs will compete
  - Competition does not imply overlapping epitopes

T = Triangulation

T = 7d

T = 7l

Generalized Capsid Assembly Pathway

Human papillomavirus

T = ???

12 pentavalent, 60 hexavalent sites Þ 2^d + 2^e + 1 Þ T = 7
72 pentamers: 72 x 5 = 360 subunits, but 360 / 60 Þ T = 6!

HK97 Capsid Assembly Pathway


Phage HK97 capsid structures by cryoEM

Conway et al, J Mol Biol 1995

Prohead

Head

500 Å

200 Å

T=7

Phage HK97 capsid at atomic resolution

• isopeptide bond Asn356-Lys169
  • auto-catalyzed by Glu363
  • branched protein backbone
  • catenated rings: protein chainmail

Phage HK97 procapsids

Prohead I

Prohead II

Phage HK97 capsid expansion

Prohead

EXPANSION

Head

Phage HK97 capsid expansion models

Prohead II

Phage HK97 capsid expansion models

Prohead II
Other phage capsids share the HK97 fold

- HK97 gp5
- T4 gp24
- P22 gp5
- ε29 gp8

Jiang et al., 2003, PNAS
Fokine et al., 2005, PNAS
Jiang et al., 2003, PNAS
Moraes et al., 2005, Molecular Cell

Herpesvirus capsids also exploit the HK97 fold?

- HSV1 VP5
- HK97 gp5

Baker et al., J Virol 2006, Figure 1
The HK97 fold is numerous and diverse

There are other capsid protein fold families

Adenovirus

Phage PRD1

PBCV-1

Capsid protein fold families

Domain-spanning families of capsid morphology:
- Ad/PRD1/STIV: pseudo-T=25 or trimeric "hexon"
- Reovirus/Φ6: T=2, asymmetric dimer, multi-layered, dsRNA
- Herpesvirus/SP01: T=16, asymmetric "triplexes"

Emerging lineages of capsid protein fold:
- Double-barrel-roll: Ad, PRD1, PBCV-1, STIV
- Asymmetric-dimer: Φ6, reovirus, rotavirus, L-A
- HK97: T4 / phi29 / P22 (T7/T5/gifsy-2) → HSV-1

Review of 2nd Lecture

- TEM images are projection images and include the entire structure (they are not slices)
- Central Section Theorem is the basis for image reconstruction
- Alternatively, back-projection methods in real space
- Decay of high frequencies may be compensated by R-weighting (ie, according to radius) or more sophisticated (but computationally expensive) methods.
- Single Particle Analysis: many particles oriented randomly
- Reconstruction procedure: starting model, model-based refinement of orientations
- Example 1 (T=3) adenovirus penton base particles
- Example 2 (T=3 & T=4) Hepatitis B virus capsid: 9 nm resolution structure by cryoEM extend resolution by labeling specific residues and visualizing label position
- Human papillomavirus: morphology to inform on structural details: forbidden T=4 icosahedral geometry
- Phage HK97: capsid dynamics during assembly, including proteolysis and expansion
- Hybrid cryoEM-Xray crystallography approach for pseudo-atomic resolution