Initial Model Generation

Workshop on Advanced Topics in EM Structure Determination

The Scripps Research Institute
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The issue: Structures of the IP3 receptor as determined by single particle EM

- Jiang et al., 2002
- Serysheva et al., 2003
- Jiang et al., 2003
- Sato et al., 2004
Structure determination by single particle EM

Sample

Specimen preparation

EM specimen

Data collection

Set of 2D images

3D reconstruction

Initial 3D model

3D reconstruction

Verification

Refinement

Refinement of orientation parameters, magnification, and CTF parameters

Angular distribution

Comparison with known structures

Refinement of orientation parameters, magnification, and CTF parameters

Comparison with 2D data set

Comparison with known structures

Sample preparation

– Negative staining

– Cryo-negative staining

– Vitrification

Data collection

– Images of untilted samples

– Tilt pairs

– Tilt series

3D reconstruction

– Random conical tilt

– Ab initio angle assignment

– Tomographic reconstruction

Verification

– Angular distribution

– Comparison with 2D data set

– Comparison with known structures
Sample

Images that are used for a 3D reconstruction
have to be of identical molecules!

Sample can be heterogeneous
even if it is biochemically homogeneous
– conformational heterogeneity
– unstable complexes

Before attempting any 3D reconstruction:
Understand your sample!
We always prepare negatively stained specimens first

good contrast & often preferred orientations
(dependes somewhat on preparation method)

We always calculate class averages (0° images)
different averages = different conformations
(but can also be different orientations)

We usually calculate 3D reconstructions (RCT)
different 3D maps = different conformations
(but can also be deformations)
Sample - Negative staining

Many preparation artifacts (incomplete stain embedding, adsorption deformations, specimen flattening upon drying)

Limitation of the resolution to about 20 Å

Particles adopt preferred orientations on the continuous carbon film!

Need to record images of tilted specimens

**BUT:**

Very useful for heterogeneous samples

3D reconstruction by RCT is very reliable
Random conical tilt reconstruction

Tiltaxis

Radermacher et al. (1987)
Random conical tilt reconstruction

Backprojection of specimen at different tilt angles

IMAGING

Different projection views

ASSIGN ORIENTATIONAL PARAMETERS $x$, $y$ and $\Phi$

BACKPROJECTION

3D reconstruction of specimen
RCT - AMPA receptor

Heterogeneity due to different conformations
RCT - AMPA receptor

Heterogeneity due to different conformations
RCT - AMPA receptor

Heterogeneity due to different conformations
RCT - Sec23/Sec24 complex

Heterogeneity due to different orientations
Negligible deformations
RCT - Sec23/Sec24 complex
Heterogeneity due to different orientations

Lederkremer et al., 2001

Bi et al., 2002
RCT - Tf-TfR complex

Heterogeneity due to different orientations
Severe deformations

Face view  Top view

Face view  Top view
RCT - Tf-TfR complex

Heterogeneity due to different orientations
Severe deformations

Conventional negative staining
Addition of glycerol
minimizes adsorption artifacts
prevents specimen flattening
serves as cryo-protectant

Carbon sandwich
reduces incomplete stain embedding

Freezing
prevents specimen flattening
RCT - Tf-TfR complex

Heterogeneity due to different orientations
Severe deformations

Conventional negative staining

0° tilt

60° tilt

Cryo-negative staining

0° tilt

60° tilt
RCT - Tf-TfR complex

Heterogeneity due to different orientations
Severe deformations

Face view

Top view

Cryo-negative staining
Single particles in ice

5 parameters to determine
Angular reconstitution (Imagic)

1. Chose 3 projection images that are perpendicular views of the particle (anchor set)
2. Add in further projections and keep refining

van Heel, 1987

Serysheva et al., 1995
Angular reconstitution - Ryanodine receptor

Serysheva et al., 1995
Angular reconstitution - Tf-TfR complex

Vitrified ice
Angular reconstitution - Tf-TfR complex

Angular reconstitution

Atomic model
Angular reconstitution (IMAGIC)

Structure depends critically on the anchor set
(these should ideally be 3 perpendicular views,
which is hard to know with an unknown molecule)

Structure also depends on the order in which
additional projections are included

Angular reconstitution is best for:
large specimens with symmetry,
and available structural information
(can be obtained from
random conical tilt)
OP command (SPIDER)

Penczek et al., 1996

A common-lines based method for determining orientations for $N > 3$ particle projections simultaneously

Essentially the opposite of standard common lines approach

(Instead of trying to determine the Euler angles based on pair-wise angles of common lines in the projections’ planes, one assumes that rotation matrices are known, finds set of angles of common lines and computes the overall discrepancy along these lines)

Applied to 70S ribosome from *E. coli*
OP command - Tf-TfR complex

OP reconstructions

OP reconstruction (33 classes)

Atomic model

OP reconstruction (160 classes)
3D reconstruction in EMAN

Also uses a set of projections to generate an initial model
Baumeister et al. (1999)

Electron tomographic reconstruction

± 90° 2° steps
± 60° 2° steps
± 90° 5° steps
± 60° 5° steps
Electron tomography - Clathrin-coated vesicles

QuickTime™ and a Cinepak decompressor are needed to see this picture.
Electron tomography - Clathrin-coated vesicles
Model refinement - Tf-TfR complex

Angular reconstitution

Refined density map (FREALIGN)

Atomic model
Model refinement - Tf-TfR complex

Reference model (face view in cryo-negative stain)

Alignment of 500 class averages

Atomic model filtered to 40 Å
Model refinement - Tf-TfR complex

Reference model (top view in cryo-negative stain)

Alignment of 500 class averages

Atomic model filtered to 40 Å
Model refinement - Tf-TfR complex

Reference model (face view in conventional negative stain)

Alignment of 500 class averages

Atomic model filtered to 40 Å
Model refinement - APC

Anaphase promoting complex (S. pombe) in negative stain
Model refinement - APC

RCT reconstruction in negative stain

RCT reconstruction in cryo-negative stain

Reconstruction with vitrified ice data using CNS RCT as reference model
Model refinement - APC

RCT reconstruction in negative stain

Reconstruction by aligning raw images to RCT map in negative stain

Reconstruction by aligning class averages to RCT map in negative stain

Reconstruction by aligning ice data to RCT map in cryo-negative stain
Model verification - APC
Model verification - APC

Reconstruction of vitrified ice data using CNS RCT as reference model

Reconstruction of vitrified ice data using OP command

3D variance map
Penczek et al., 2006
Model verification - Clathrin cages

Clathrin cages in vitrified ice
Model verification - Clathrin cages
Model verification - Clathrin cages
Model verification - AMPA receptor

mGluR1
Kunishima et al. 2000
(K. Morikawa)

GluR2
Armstrong et al. 2000
(E. Gouaux)

KcsA
Doyle et al. 1998
(R. MacKinnon)
Model verification - AMPA receptor

Options:
- NTD
- LBD
- TMD
Model verification - Spliceosome

Mammalian C complex  (Jurica et al., 2004)

S. pombe Cdc5p complex
Model verification - Spliceosome

S. pombe Cdc5p complex in vitrified ice using cryo-negative stain reconstruction as initial model
Model verification - Spliceosome
Conclusions

Never just believe your initial model!

There is currently no general way to generate a reliable initial model.

Calculate random conical tilt reconstructions of (cryo-)negatively stained specimens!

The density map is probably distorted, but it is a good basis to interpret subsequent reconstructions.

Use your “biological intelligence”!
Some Literature

Oxford University Press, Inc.

reconstruction from a single-exposure, random conical tilt series applied to the 50S ribosomal
subunit of Escherichia coli. *J. Microsc.* **146**: 113-136

3D reconstruction. *Ultramicroscopy* **21**: 111-123

Serysheva, I. I., Orlova, E. V., Chiu, W., Sherman, M. B., Hamilton, S. L. and
van Heel, M. (1995) Electron cryomicroscopy and angular reconstitution used to
visualize the skeletal muscle calcium release channel. *Nat. Struct. Biol.* **2**: 18-24

orientations for N > 3 particle projections simultaneously. *Ultramicroscopy* **63**: 205-218