Understanding macromolecular structure with transmission electron microscopy

Electron microscopy for structural biology

Why visualize macromolecular structures?

- Seeing subunit conformations and interactions can reveal mechanisms of activity, regulation, and assembly

What is transmission electron microscopy?

- Light and electron microscopes use lenses to focus and magnify a wave reflected from or transmitted through a specimen
- In transmission imaging the wave passes through the specimen and provides internal details
- Electrons could provide 100,000x better resolution than visible light
  - Electrons accelerated at 100 - 300 kV have wavelength (λ) ~= 0.04 - 0.02 Å
- Point resolution of conventional TEM is limited to ~1 Å by:
  - Lens aberrations,
  - Electron source size, and
  - Electron energy coherence (wavelength distribution)
- Magnification range over 4-orders: ~50x - ~500,000x

What are the boundaries of macromolecular TEM?

- Specimen thickness: < 200 nm
  - Electrons strongly interact with matter so specimen must be thin.
- Size of macromolecule: >100 kDa
  - Big enough to distinguish molecule from background noise
  - Up to large macromolecular assemblies like viruses and microtubules, … whole cells, tissue sections
- Radiation sensitivity of biological specimens
  - Damage is minimized by limiting electron dose (< 30 $e^-/Å^2$) → low signal-to-noise
- Imaging must be done in a vacuum so specimen must be preserved
  - frozen “cryo-EM”: near native structure, but cumbersome and low contrast
  - stained: drying can cause deformations, but quick and better contrast than cryo

Why is TEM a useful method for visualizing the structure of macromolecules?

- Direct visualization = instant gratification (compared to NMR and X-ray crystallography)
- Various kinds macromolecular assemblies can be examined:
  - In situ structures (e.g., nuclear pores)
  - 2D crystals (especially membrane proteins)
  - Filamentous assemblies (e.g., flagella, microtubules, phage tails)
  - Single particles (e.g., viruses, GroEL, ribosomes, FAS)

How are 3D structures reconstructed from noisy images of single particles?

1. Identify particles in each micrograph
   a. Datasets usually have hundreds of micrographs each containing hundreds of particles.
2. Combine similar particles to boost signal-to-noise (SNR)
   a. Particles are aligned and sorted so that similar particles can be averaged
   b. Accuracy of alignment and clustering depends on size of molecule and contrast
3. Determine relative orientations of averages and/or particles
   a. Each 2D particle image has 5 variables that define its relationship to the 3D reconstruction (longitude, latitude, rotation, x-center, y-center)
4. Compensate for imaging aberrations either by correction of 2D particle images, 2D averages, or the final 3D reconstruction
5. More particles and higher symmetry lead to higher resolution structures

Why don't reconstructions of biological molecules from TEM images achieve resolutions that are limited by the instrument?

Recent and future developments in macromolecular TEM

- Specimen preparation and handling:
  - graphene oxide specimen support
  - gold specimen support to reduce beam induced specimen movement
  - specimen autoloader cassettes
- Microscopy:
  - energy filter
  - aberration correctors
  - phase plate
  - brighter, more coherent electron sources
  - more stable optics
- Image acquisition:
  - direct electron detectors
  - automated imaging and data storage
- Image analysis and structure interpretation:
  - new algorithms and software at all steps of data processing

Evaluating a 3D-EM paper

- Data quality
  - Can you see particles in their micrographs?
  - What features can you see in 2D class averages?
- Particle identification
  - Was selection biased by searching for correlation with an initial model?
- Method for calculating 3D reconstruction
  - Was reconstruction biased by initial model?
- Do reconstructions have sufficient detail to justify interpretation?
  - Resolution is typically quantified by "Fourier shell correlation" (FSC)
  - FSC is a measure of self-consistency (precision)
  - A structure can be precisely wrong

Example 1: Mammalian fatty acid synthase structure studied by 3D-EM
(Brignole, Smith, Asturias. NSMB 2009)

Introduction to FAS structure

The overall reaction is:

Acetyl-CoA + 7 Malonyl-CoA + 14 NADPH + 14 H^+ → Palmitate + 8 CoA + 14 $NADP^+$

FAS occurs in several different architectural flavors

- **Type II**: Bacteria, mitochondria, and chloroplast have free-standing enzymes
  - Ketoacyl synthase (KS)
  - Malonyl transferase (MT)
  - Ketoacyl reductase (KR)
  - Dehydratase (DH)
  - Enoyl reductase (ER)
  - Acyl carrier protein (ACP)
  - Thioesterase (TE)
- **Type Ib**: Fungi have enzymes clustered on two polypeptides that form a barrel shaped $\alpha_6\beta_6$ hetero-dodecamer
- **Type Ia**: Metazoan (animal and slime mold) cytosolic FAS
  - All enzymes needed for de novo fatty acid biosynthesis on a single giant polypeptide
  - In metazoans the enzymes involved are arranged:
    KS --- MAT (malonyl and acetyl substrates) --- DH --- MeT --- ER --- KR --- ACP --- TE
  - Homodimeric, 2 x 275kDa = 550 kDa
- Mycobacteria and some related soil bacteria have all three types of FAS
- The different FAS systems also have some differences in enzyme specialization and fold
- Crystal structures and EM structures have been determined for all free-standing Type II enzymes, a couple Type Ib complexes, and a Type Ia complex
- The crystal structure of mammalian Type Ia FAS showed that the chain processing enzymes (KR, DH, and ER) form the “upper body”, while chain elongation enzymes (MAT, KS) form the “lower body”
- The ACP cannot reach far enough to shuttle intermediates between catalytic domains without dramatic conformational changes.

Discussion of results

Why might EM be a useful method for studying conformational changes of FAS?

How were specimens prepared and imaged? What are the advantages and limitations of this approach?

How were 2D and 3D structures determined? Are the structures reliable? How might they be improved?

How were the 3D structures analyzed and interpreted? Are the models correct? How might the models be improved?

Do the observed conformations allow all of the necessary interactions with ACP?

Faced with a conformational continuum, how were different mutants compared?

Example 2: Pikromycin synthase structure studied by 3D-EM
(Dutta *et al*., Nature 2014)

How do the approaches differ that were used to determine the structures of module 5 and FAS? Why was the reconstruction of module 5 higher resolution than the FAS structures?

Can FAS structures be extrapolated to polyketide synthase modules that are FAS-like, but lack enoyl reductase, dehydratase, and/or methyltransferase domains?

Why would organisms evolve giant multifunctional enzymes to synthesize polyketides and fatty acids?

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