Single Particle Reconstruction

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The Plan

(Monday and Tuesday)

 This afternoon and all day tomorrow will be in the computer classroom. No lectures in the lecture hall tomorrow morning.

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 Prasad, BVV, and Ludtke, S.J. Advances in Protein Chemistry and Structural Biology. Volume 82.
 Recent Advances in Electron Cryomicroscopy, Part A. Elsevier, Inc. Dec 2010.

 Prasad, BVV, and Ludtke, S.J. Advances in Protein Chemistry and Structural Biology. Volume 82.
 Recent Advances in Electron Cryomicroscopy, Part B. Elsevier, Inc. May 2011.

Unit Refresher

(orders of magnitude)

- 1 Angstrom (Å) = 0.1 nm ~ size of an atom (C-C bond ~1.4 Å)
- 10 Å = 1 nm ~ diameter of an alpha helix
- 100 Å = 10 nm ~ size of typical proteins
- 1000 Å = 100 nm \sim size of a typical virus particle
- 10,000 Å = 1 μm ~ size of prokaryotic cell
- 100,000 Å = 10 μ m ~ size of a eukaryotic cell
- <3 Å resolution required in x-ray crystallography for a protein backbone trace





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TEM Techniques

- @ 2-D Crystallography
- Helical <u>Reconstruction</u>
- Electron Tomography
- Single Particle Reconstruction

TEM Techniques

Single Particle Reconstruction

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- <4 Å resolution possible (~8-12 Å more common)

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Single Particle Processing

- Image Acquisition
- Particle Picking
- @ 2-D Analysis
- Symmetry/Low Resolution Model
- Determine CTF Parameters
- High Resolution Refinement
- Post-processing
- Oynamics Analysis

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Particle Picking

@ manual or semi-automated process

False positives are very dangerous, but also beware of excluding views you weren't expecting

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2-D Analysis

Seven if you know the quaternary structure, still worthwhile. May be surprises.

At least 1000–2000 particles with uniform orientation distribution, perhaps fewer if symmetry or preferred orientation







2-D Refinement

Particles

0.000

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2-D Refinement

Particles

Align Particles

(1) 秋秋 (1) (1) SVD/PCA

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Dynamics/Heterogeneity

- After separating particles into different views:
- Statistical analysis (bootstrapping, MSA) to locate regions of motion/heterogeneity
- Classify particles in a single orientation to make pseudo-time movies
- Multiple model 3-D refinement

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Fatty Acid Synthase

- Synthesizes saturated hydrocarbon chains
- <u>⌀ Homo-dimer,</u> ~500 kDa
- Ø 7 Enzymatic activities
- Obesity
- O Cancer
 - Highly expressed in most tumors
 - Particularly important in breast and prostate cancer
 - @ FAS inhibitors can prevent tumor growth

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Symmetry / Initial Model

- Biochemical clues to symmetry ?
- May be obvious from 2-D refinement
- Double-check with quick, low resolution 3-D refinements
- If still ambiguous, may need better data or tomography

3-D Reconstruction

- Start with particles and an initial 'guess' at the 3-D structure

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GroEL

- @~800 kDA
- ⊘homo 14-mer
- ☞ Type 1 chaperonin (GroES co-chaperonin)
- Several crystal structures available



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Postprocessing

ø10 − 20 Å

- ødock Xtal structures / homology models of components
- 👁 5 10 Å
 - Secondary structure analysis
- ø< 5 Å
 - Backbone tracing, atomistic models











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MMCPN

- Type 2 Chaperonin (built in lid)
- Archaeal analog of (mammalian)TriC/CCT
- → Homo 16-mer
 - (Mammalian TriC has 8 different subunits)





























