Biochemistry 9001
“Protein structure determination by 3D Electron Microscopy”

Tommi A. White, Ph.D.
Director, Electron Microscopy Core Facility
Assistant Research Professor, Biochemistry
Goals of this course

• Introduce and familiarize you to concepts related to 3D Electron Microscopy and protein structure determination
• Provide hands on training and techniques to assist you in protein structure determination using electron microscopy
Biochem 9001 alumni pursuing cryoEM:

Bassem Shebl - 2015
Tom Walz Lab, Rockefeller University Manhattan, New York

Tara Marcink - 2017
Amadee des Georges, CUNY Manhattan, New York
Instructors

• Tommi A. White
  • Director, EMC & Research Asst Prof, Biochemistry

• Jacob Gotberg
  • Cyberinfrastructure Engineer, Research Computing Support Services

• Filiz Bunyak
  • Research Asst Prof, Computer Science

TEACHING ASSISTANTS

• Nguyen Phuoc Nguyen ("Wen") – Computer Science
• Ashley Campbell - Biochemistry
Course structure

• 8 lectures
• 5 labs & lab reports
• 3 weeks for individual projects
  • Project Abstract
  • Project Presentation
  • Project report
Lectures

• Mondays/Wed: 10:30 am – noon, Vet Med W116
  • Theory, Literature review, examples from literature and EMC
Labs

• 3 hours: in EMC, W125-W137 Veterinary Medicine
• Pairs (sign up!)
  • Tuesday/Thursday: 9-Noon
  • No labs 6/4-6/5
• Hands on applications, demonstration and Labs
• Register in “Facility Online Manager”
  • http://emc.missouri.edu/policies-info/
  • http://emc.missouri.edu/reservations/
  • Use MoCode F0146 (“zero”)
Hosted by Office of Research

Early 90's had electron microscopes in
- Geological Sciences
- CAFNR
- School of Medicine
- Veterinary medicine

1995 combined into Electron Microscopy Core
- Hosted by Veterinary Pathology
- Located in Veterinary Medicine Building

Electron Microscopy Core (EMC)

Electron Microscopy Core (EMC)
Projects

• before June 29th – Project consultation
• July 2nd– Project Abstract, 3 slides
• July 2-20 – Project Data Collection/Processing
• July 25th – Project Presentation, 30 min
• July 27th – Project Report, Microscopy & Microanalysis Paper format
High Performance Computation

Jacob Gotberg

- Generate ssh keys
  - [http://docs.rnet.missouri.edu/HowTo/ssh](http://docs.rnet.missouri.edu/HowTo/ssh)
  - Share only your PUBLIC key, never share your private one

- Tutorials

<table>
<thead>
<tr>
<th>Training</th>
<th>When</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>New User</td>
<td>Wednesdays 10:00 - 11:00</td>
<td>4F51A Ellis Library</td>
</tr>
<tr>
<td>HPC</td>
<td>Wednesdays 11:00 - 12:00</td>
<td>4F51A Ellis Library</td>
</tr>
</tbody>
</table>

- Nguyen Nguyen
Why do we need 3D?
What is 3DEM?
What is 3DEM?

- **3-Dimensional Electron Microscopy**
  - Using electron microscopy to determine a structure in 3-dimensions
    - Cellular
    - Subcellular (organelles/membranes)
    - Proteins
  - Proteins are embedded to maintain their shape
    - Heavy metal stain (negative staining)
    - Sugar (trehalose)
    - Ice (non-crystalline)
- **Examples:**
  - Single Particle Reconstruction (Single Particle Analysis, Cheng Lab)
  - Random Conical Tilt (RCT, Radermacher Lab)
  - Electron Tomography (Subvolume Averaging, Subramaniam)
  - Electron Crystallography (MicroED, Gonen Lab)
  - Serial Block Face Imaging (FIBSEM Tomography, Denk Lab)
Single Particle Reconstruction

Animations courtesy of Max Planck Institute of Biochemistry, Martinsried, Germany
Ribosome structures to near-atomic (4.1 Å) resolution from thirty thousand cryo-EM particles (2013)
Xiao-chen Bai, Israel S Fernandez, Greg McMullan, Sjors HW Scheres
2.2 Å resolution cryo-EM structure of β-galactosidase in complex with a cell-permeant inhibitor.
Cryo-Electron Tomography

Animations courtesy of Max Planck Institute of Biochemistry, Martinsried, Germany
3DEM Examples: Cryo-electron Tomography: SIV virions

T.A. White, A. Bartesaghi...S. Subramaniam (2011) PLoS Pathogens
3DEM Examples: Cryo-electron Tomography: Chlamydomonas

3DEM Examples: Cryo-electron Tomography: Chlamydomonas

3DEM Examples: Cryo-electron Tomography: HeLa Cell

J. Mahamid...Baumeister, (2016) Science
3DEM Examples: Serial Block Face imaging
Nature’s Method of the Year 2015: Cryo-EM
Nobel Prize in Chemistry 2017
cryo-electron microscopy

Dubochet
Frank
Henderson
Structural Biology in the Post Genomics Era

Table 2  Success rates for major steps in structure determination

<table>
<thead>
<tr>
<th>Status</th>
<th>Total number of targets</th>
<th>% Success (step)</th>
<th>% Success (overall)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cloned</td>
<td>125,316</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Expressed</td>
<td>83,115</td>
<td>66.3</td>
<td>66.3</td>
</tr>
<tr>
<td>Purified</td>
<td>29,409</td>
<td>35.4</td>
<td></td>
</tr>
<tr>
<td>Diffraction-quality crystals or NMR spectrum</td>
<td>8,690</td>
<td>29.5</td>
<td></td>
</tr>
<tr>
<td>In PDB</td>
<td>5,811</td>
<td>66.9</td>
<td></td>
</tr>
</tbody>
</table>
Structures solved: XPC

X-ray Protein Crystallography

Graph from the Protein Databank (PDB) June 2018
Structures solved: NMR

Nuclear Magnetic Resonance

Graph from the Protein Databank (PDB) June 2018
Structures solved: CryoEM

DRAMATIC GROWTH of structures determined by CryoEM

Cryo-electron Microscopy

Graph from the Protein Databank (PDB) June 2018
Comparison to X-ray Crystallography

• To determine the structure factors, need both
  • amplitudes
  • phase

• “Phase problem”
  • In diffraction patterns, you can only measure the intensity/amplitudes of the planes

• “Phase measuring diffractometer” - W. Hoppe, 1983
  • With TEM images, we measure both amplitude and phase

Callaway (2015) Science
Cryo-TEM is Unique

- protein complexes and molecular machines/assemblies
- molecular motions and dynamic processes
- heterogeneous samples
- no crystallization needed
Proteins Act in Complexes

A Human Protein-Protein Interaction Network

- automated Yeast two-hybrid system
- 3186 mostly novel protein interactions among 1705 proteins
- large highly connected network
- resulted in 911 high-confidence interactions among 401 proteins

The vast majority of these interactions involve dynamic protein complexes with sizes between 50 - 500kD.
Added Value of Cryo-TEM

99% of all PDB XRC structures

Success rate

XRC/NMR

New Era for Structural Biology

from protein monomer to complexes

New biological Insights

Statistical analysis of PDB entries by end 2012

Success rate

99% of all PDB XRC structures

New Era for Structural Biology

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New biological Insights

Statistical analysis of PDB entries by end 2012
Complementarity of XPC, NMR and Cryo-TEM

Towards an integrative structural biology approach!!
Increasing numbers of high resolution structures

Resolution trends of released EMDB entries

Conformational insights: In-silico purification (still quite tricky...multi-body refinement)

6.6Å Eukaryotic Translational Initiation Complex.
Low population of complex prevents (<3%) crystallization

Nakane (2018) BioXrv
Why/How is cryoEM gaining so many adopters now?
Ultrastable Microscopes: Autoloaders

- 24/7 operation without operator on site
- Fully digital microscopy
- Automated sample handling
- Fully automated data collection (SPA and Tomography)
- Easy-to-use
Direct Detectors: super-resolution

Brilot, et al. 2012, JSB
Technology Breakthrough Meets Market Demands

Direct Electron Detection

Added Value of Cryo-TEM

The Resolution Revolution

Werner Kühlbrandt

SCIENCE VOL 343 28 MARCH 2014
Why electrons?
Serial Block Face Imaging

Tomography

Single Particle Analysis

Daltons

X-ray

NMR

Light

Electrons

Subramaniam 2005 *Curr Opin Microbiol.*, modified TAW
Electrons

- Involved in electricity, magnetism, thermal conductivity
- A moving electron creates a magnetic field
- Wave-particle duality
- Small particle
  - $9 \times 10^{-28}$ grams
- Negatively charged
  - $1.6 \times 10^{-19}$ Coulomb
- Higher wavelength
  - $\sim 2$ picometers @ 200 keV
- 90% the speed of light
  - 270,000 km/s
- Easily accelerated
  - resting energy 0.511 MeV

Williams & Carter, 1996
Transmission Electron Microscopy (TEM)
Transmission Electron Microscopy (TEM)
Filament (LaB6, FEG)

Condenser Lenses (C1 – beam size, C2 – intensity)
Condenser Aperture (reduces scattering)

Objective Lens (upper/lower pole piece)
Objective Aperture (contrast)

Projection Lens (3)

Fluorescent Screen

Digital Camera (CCD)
Vacuum

• Electron microscopes have high vacuums...why?
  • Electrons are very easily scattered
  • Want to control their trajectories

• Challenge for biological specimens
  • Most are hydrated (esp. proteins)
  • How to work around?
<table>
<thead>
<tr>
<th>Type</th>
<th>Range (mbar)</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanical</td>
<td>atm - $10^{-2}$</td>
<td>roughing, backing</td>
</tr>
<tr>
<td>Oil Diffusion</td>
<td>$10^{-1} - 10^{-9}$</td>
<td>main, column</td>
</tr>
<tr>
<td>Turbomolecular</td>
<td>$10^{-1} - 10^{-9}$</td>
<td>main, column</td>
</tr>
<tr>
<td>Ion</td>
<td>$10^{-5} - 10^{-12}$</td>
<td>column, gun</td>
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</table>
Filaments

Lanthanum Hexaboride

Schottky Field Emitter
Field Emission Gun: Types

<table>
<thead>
<tr>
<th>TABLE 5.1 Characteristics of the Principal Electron Sources</th>
</tr>
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<tbody>
<tr>
<td>Units</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>Work function, $\Phi$</td>
</tr>
<tr>
<td>Richardson’s constant</td>
</tr>
<tr>
<td>Operating temperature</td>
</tr>
<tr>
<td>Current density (at 100 kV)</td>
</tr>
<tr>
<td>Crossover size</td>
</tr>
<tr>
<td>Brightness (at 100 kV)</td>
</tr>
<tr>
<td>Energy spread (at 100 kV)</td>
</tr>
<tr>
<td>Emission current stability</td>
</tr>
<tr>
<td>Vacuum</td>
</tr>
<tr>
<td>Lifetime</td>
</tr>
</tbody>
</table>

**LaB₆**
- JEOL JEM 1400
- Lanthanum Hexaboride
- Vacuum ($10^{-4}$)
- Crossover = 10 micron
- $1K$

**Schottky FEG**
- FEI Tecnai F30 Twin
- Zirconium Oxide
- High Vacuum $<10^{-6}$
- 0.015 micron
- $12K$
Spatial Coherence

• Perfect spatial coherence would be all electrons emit from the same point

• Smaller tips
  • more coherent
  • Better phase contrast
  • Better resolution

• Which tip has better spatial coherence?
Field Emission Gun (FEG)

• Source of electrons = the “gun”
  • Provides large stable current in a small electron beam
  • Source determines resolution
  • Tungsten (W) or ZrO₂

W&C p. 80
Field Emission Gun: How does it work?

Produces electrons upon application of a large electric potential

- FEG = cathode (-300 kV)
- Anode 1 = “extraction voltage”
  - Positive by a few kV
  - Intense electric field
  - Electrons tunnel out
- Anode 2 = accelerates electrons
  - TEM = 50-300 kV

Produces a “refined” electrostatic lens for crossover
Lenses:

• Magnify or demagnify
• Illuminate
• Focus
Lenses: what are they?

Electromagnets

- Vary...
  - current using a coil around a soft iron core
  - Affects resultant magnetic field
Lenses are finite

All lenses are imperfect insofar as they cannot gather all the radiation emitted by an object and so can never create a perfect image.
Chromatic Lens Abberations

Spherical

Chromatic
Lens Abberations: Astigmatism

Circular Cross Section Of The Electron Beam

Elliptical, Astigmatic Cross Section Of The Electron Beam

Weak Magnetic Field

Top View Of Electromagnetic Lens

Strong Magnetic Field

Astigmatic Electron Beam
Lens aberrations: Astigmatism

Astigmatism

Corrected Astigmatism
Apertures

• Strip of metal
• 4 Holes drilled with decreasing size
• Limits undesirable scattering, leading to noise in images.
Digital Camera
Detective Quantum Efficiency

\[
DQE = \left( \frac{\frac{S_{\text{out}}}{N_{\text{out}}}}{\frac{S_{\text{in}}}{N_{\text{in}}}} \right)^2
\]

• Metric to compare efficiency of detection

• Where...
  • \( S = \) signal (electrons)
  • \( N = \) Noise (background)

• A perfect detector would have a DQE of 1

• Detectors have <1
Charge Coupled Device (CCD)

- Metal-insulator-silicon devices that store charge
- Each pixel in an array
  - is an individual capacitor
  - Isolated from each other
  - Collect charge in the well
  - Proportional to incident radiation intensity
- Our CCDs
  - JEOL = 2K x 2K
  - F30 = 4K x 4K
Renaissance in imaging...

- Direct electron Detection
- Revolutionizing cryo-EM
- Take movies and compensate for drift over course of acquisition
Dose

• TEM is Electro-magnetic Radiation

• Damage to sample
  • “knock-on” – incoming electron interacts with atomic nucleus
  • Mass loss (decrease in sample thickness)
  • Bubbling (hydrogen bonds broken)

• Limit doses given to sample
  • “Low Dose”
  • 20-80 e-/Å²
TEM Contrast mechanisms

Thickness

Mass

Diffraction
Beam-specimen interactions

**Elastic Scattering**
- No Energy loss
- High angle (>5°)
- Interactions with nucleus or inner valence electrons

**Inelastic Scattering**
- Energy Loss
- Low angle <5°
- Interactions with outer valence electrons
- Lost energy as
  - X-rays
  - Plasmons
  - Beam damage
  - Heat/bubbling
Reading/Watching

• E. Callaway 2015 “Revolution will not be crystallized” Nature News Feature


• Y. Cheng, et al. 2015 “A Primer to Single-Particle Cryo-Electron Microscopy” Cell

• A. Merk et al. 2016 “Breaking Cryo-EM Resolution Barriers to Facilitate Drug Discovery” Cell

• Grant Jensen’s CryoEM Videos 1&2
Single Particle Reconstruction

• “quantitative way of determining the structure of macromolecules from micrographs, showing them as a collection of isolated, unattached particles”
• Uses not one, but thousands of macromolecules
• Multiple orientations or “Views” in 2D projections
• Group same views together, align and average
• Reconstruct 3D from multiple 2D views

Frank, 2009, QrevBiophys