Unlocking the Secrets of Proteins:
*The Rise of Cryo Electron Microscopy*

Caitlin Howell
Professor of Bioengineering
University of Maine
Acknowledgement

Ernesto Arias-Palomo
What are proteins?
All organisms are made of cells

10 trillion cells

1 cell
Complex Molecular Tools

Vision: Rhodopsin
Complex Molecular Tools

Cell transport: Kinesin
Why Study Protein Structure?

“If you want to understand function, study structure.”

- Francis Crick
<table>
<thead>
<tr>
<th>Primary Structure</th>
<th>Secondary and Tertiary Structures</th>
<th>Quaternary Structure</th>
<th>Function</th>
<th>Red Blood Cell Shape</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normal hemoglobin</strong></td>
<td><img src="image" alt="β subunit" /></td>
<td><img src="image" alt="Normal hemoglobin" /></td>
<td>Molecules do not associate with one another; each carries oxygen.</td>
<td><img src="image" alt="Red Blood Cell" /></td>
</tr>
<tr>
<td>1 Val</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>2 His</td>
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<td>3 Leu</td>
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<td>4 Thr</td>
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<td>5 Pro</td>
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<td>6 Glu</td>
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<tr>
<td>7 Glu</td>
<td></td>
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</tbody>
</table>

| **Sickle-cell hemoglobin** | ![Exposed hydrophobic region](image) | ![Sickle-cell hemoglobin](image) | Molecules crystallize into a fiber; capacity to carry oxygen is reduced. | ![Red Blood Cell](image) |
| 1 Val | | | | |
| 2 His | | | | |
| 3 Leu | | | | |
| 4 Thr | | | | |
| 5 Pro | | | | |
| 6 Val | | | | |
| 7 Glu | | | | |
Alzheimer’s Disease
Still a mystery

1. Tau tangles

2. Beta-amyloid plaques
How can we look at proteins?

- FTIR
- XPS
- X-rays
- Electron microscopy
- Light microscope
- Unaided human eye
X-Ray Crystallography

Salt (NaCl)
X-Ray Crystallography

Nobel Prize Watson and Crick 1962

Nobel Prize Ramakrishnan, Steitz, and Yonath 2009
Electron Microscopy

**Light Microscopy**
- Resolution: \( \approx 200 \text{nm} / 2000 \AA \)

**Electron Microscopy**
- Resolution: \( \approx 0.0019 \text{nm} / 0.019 \AA \)
Electron Microscopy

Bacterial Rhodopsin
Henderson 1974
First use of Cryo EM: Viruses

Debochet 1984

Cryo-electron microscopy of viruses

Marc Adrian, Jacques Dubochet, Jean Lepault & Alasdair W. McDowall
European Molecular Biology Laboratory, Postfach 10.2209, D-6900 Heidelberg, FRG

Thin vitrified layers of unfixed, unstained and unsupported virus suspensions can be prepared for observation by cryo-electron microscopy in easily controlled conditions. The viral particles appear free from the kind of damage caused by dehydration, freezing or adsorption to a support that is encountered in preparing biological samples for conventional electron microscopy. Cryo-electron microscopy of vitrified specimens offers possibilities for high resolution observations that compare favourably with any other electron microscopic method.

Quarterly Review of Biophysics 21, 2 (1988), pp. 139-228
Printed in Great Britain

Cryo-electron microscopy of vitrified specimens

Jacques Dubochet, Marc Adrian, Jiin-Ju Chang, Jean-Claude Homo, Jean Lepault, Alasdair W. McDowall and Patrick Schultz
European Molecular Biology Laboratory (EMBL), Postfach 10.2209, D-6900 Heidelberg, FRG
Freezing versus Negative Staining

Negative staining

staining agent

20Å

Cryo-EM

vitreous ice (buffer)

0.019Å
Improvements: Detectors

Film

CCD

-25°C

Scintillator

Fiber optics

CCD array

Peltier cooler
Cryo EM

Titan Krios  ~$5-7,000,000
Image processing
Image processing

Individual Particles → Projections → Reconstruction

Arias-Palomo, E
Ways to View Proteins
Bacterial DNA replication

DnaB

DnaC
≈2/3 of the particles
≈1/3 of the particles

Complete DnaBC
DnaB
DNA C breaks the ring of the helix

Dome: DnaBC

Arias-Palomo E, O’Shea VL, Hood IV, Berger JM. Cell. 2013
DNA BC

Arias-Palomo E, O’Shea VL, Hood IV, Berger JM. Cell. 2013
DNA BC

Arias-Palomo E, O’Shea VL, Hood IV, Berger JM. Cell. 2013
DNA BC in action

Movie: Bailey-EM
Another Improvement: Detectors

CCD

Direct Detection
Another Improvement: Detectors
Drug Discovery

Isocitrate Dehydrogenase

Membrane Channel

TRPA1 – Wasabi receptor

Gamma-secretase

Beta-amyloid plaques

Alzheimer’s Disease
Still a mystery

ARTICLE

Three-dimensional structure of human γ-secretase

ARTICLE

An atomic structure of human γ-secretase
Future Directions

- Ability to study small proteins (<100 kDa)
- Improve sample preparation
- Better Automation
- Develop more reliable validation tools
