Single-Particle Electron Microscopy

EM Structural Analysis

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Electron Microscope

Transmission Electron Microscopy (TEM)
TEM Image Formation

- Object plane
- Objective lens
- Back focal plane (diffraction pattern)
- Image plane

Optic axis
Specimen Preparation: Negative Staining

- quick to make
- high dose-tolerance
- preferred orientation
- lower resolution (nm)
Specimen Preparation: **Cryo-plunging**

+ higher resolution (Å)
- low dose-tolerance
- must work under LN$_2$
Image Acquisition

- emulsion film
- CCD
- pixel detector
Data Processing Pipeline

- Particle Selection
- Particle Alignment
- Particle Classification
- Model Reconstruction
- Model Refinement
Particle Alignment

2D Alignment
- Translation
- Rotation
Particle Classification

Goal: improve SNR by class averaging

• PCA to identify eigen images (modes)
• decompose images in the eigen-space
• find point clusters ⇒ classes
Model Reconstruction
Topics

- Molecular Mechanics Force-Fields
- Docking via MD simulation
- Structure refinement/prediction
- What will be?
$U(R) = \sum_{\text{bonds}} K_r (r - r_{eq})^2$

$+ \sum_{\text{angles}} K_\theta (\theta - \theta_{eq})^2$

$+ \sum_{\text{dihedrals}} \frac{V_n}{2} (1 + \cos[n\phi - \gamma])$

$+ \sum_{i<j} \frac{A_{ij}}{R_{ij}^{12}} - \frac{B_{ij}}{R_{ij}^6}$

$+ \sum_{i<j} \frac{q_i q_j}{\epsilon R_{ij}}$

\text{bond} \quad \quad \text{angle} \quad \quad \text{dihedral} \quad \quad \text{van der Waals} \quad \quad \text{electrostatic}$
**MD Simulation**

For each atom:

\[
\begin{align*}
r(t + \delta t) &= r(t) + v(t)\delta t \\
v(t + \delta t) &= v(t) + a(t)\delta t \\
a(t) &= \frac{F(t)}{m} \\
F &= -\frac{d}{dr}U(r)
\end{align*}
\]

Electrophoretically-driven translocation of a DNA strand through the transmembrane pore of alpha-hemolysin. TCB, UIUC.
PDB-Map Docking

Why force-fields?

- Stereo-chemically correct structure
- Reduce DOF in low-resolution EM density map

Structural analysis / interpretation

- Protein/complex structure refinement

EM structure prediction

- Molecular conformation prediction
Restrained MD Refinement

Dynamics

- Rigid-body MD
- Torsion-angle MD
- Cartesian-space MD
- Energy minimization

\[ E_{TOTAL} = E_{CHEM.} + E_{\Theta} \]
Refinement Methodology

A resolution-dependant density calculation

\[ \rho_{\text{calc}}(r) = 4\pi Q \int_{d^*_{\text{low}}}^{d^*_{\text{high}}} g(d^*) d^* \sin\left(\frac{2\pi rd^*}{2\pi rd^*}\right) dd^* \]

\[ E_{\oplus} = \sum_{\text{atoms}} \left(k\rho_{\text{obs}} + b - \rho_{\text{calc}}\right)^2 \]
Refinement Protocol

1. Rigid-body annotation
2. Torsion-angle degree of freedom
3. Symmetry enforcement (optional)
4. Refinement via simulated annealing
Actin-DHP Docking

CC = 0.49

CC = 0.67
Helical Structure of Flagella Hook
**Optimal Conformation**

**Crystal Structure**
- \( E(\text{bond}) = 0.990 \)
- \( E(\text{angl}) = 10.353 \)
- \( E(\text{tors}) = 3.161 \)
- \( E(\text{VDW}) = -208 \)
- \( E(\text{PVDW}) = 0.3E+8 \)
- \( E(\text{res}) = 167.0 \)

**Manual Adjustment**
- \( E(\text{bond}) = 0.975 \)
- \( E(\text{angl}) = 10.397 \)
- \( E(\text{tors}) = 3.169 \)
- \( E(\text{VDW}) = 0.5E+7 \)
- \( E(\text{PVDW}) = 0.3E+5 \)
- \( E(\text{res}) = 161.0 \)

**RSMD Refinement**
- \( E(\text{bond}) = 0.923 \)
- \( E(\text{angl}) = 10.351 \)
- \( E(\text{tors}) = 3.138 \)
- \( E(\text{VDW}) = -191 \)
- \( E(\text{PVDW}) = -14.38 \)
- \( E(\text{res}) = 156.0 \)
TMV Conformational Change

By courtesy of Carsten Saches.
Rotavirus TLP

- dsRNA virus
- 11 RNA segments
- triple-layered capsid
- 6 structural proteins
- diameter ~800 Å
- weight ~60 MD

Cryo-EM Structural Analysis

- TF30: 300 kV
- dose: 15 – 20 e/Å²
- defocus: 1.0 – 3.0 μm
- ~ 5,000 single particles

- projection matching
- CTF correction
- FREALIGN refinement
- resolution: ~ 4Å
EM Density Map

VP6 + VP7

α-helix
The Road Ahead …

- High-resolution single-particle EM
- Tackle dynamic, heterogeneous systems
- EM & computational structural biology
Conclusion

Quantitative Biology

Biology
Physics
Mathematics
Computer Science
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