Quantitative Biology Lectures, Part III

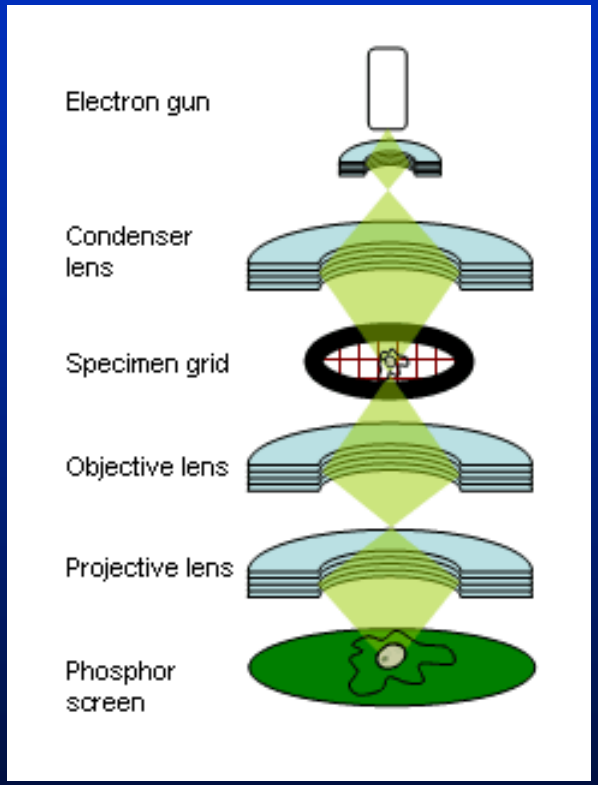
January 30, 2008

Single-Particle Electron Microscopy

EM Structural Analysis

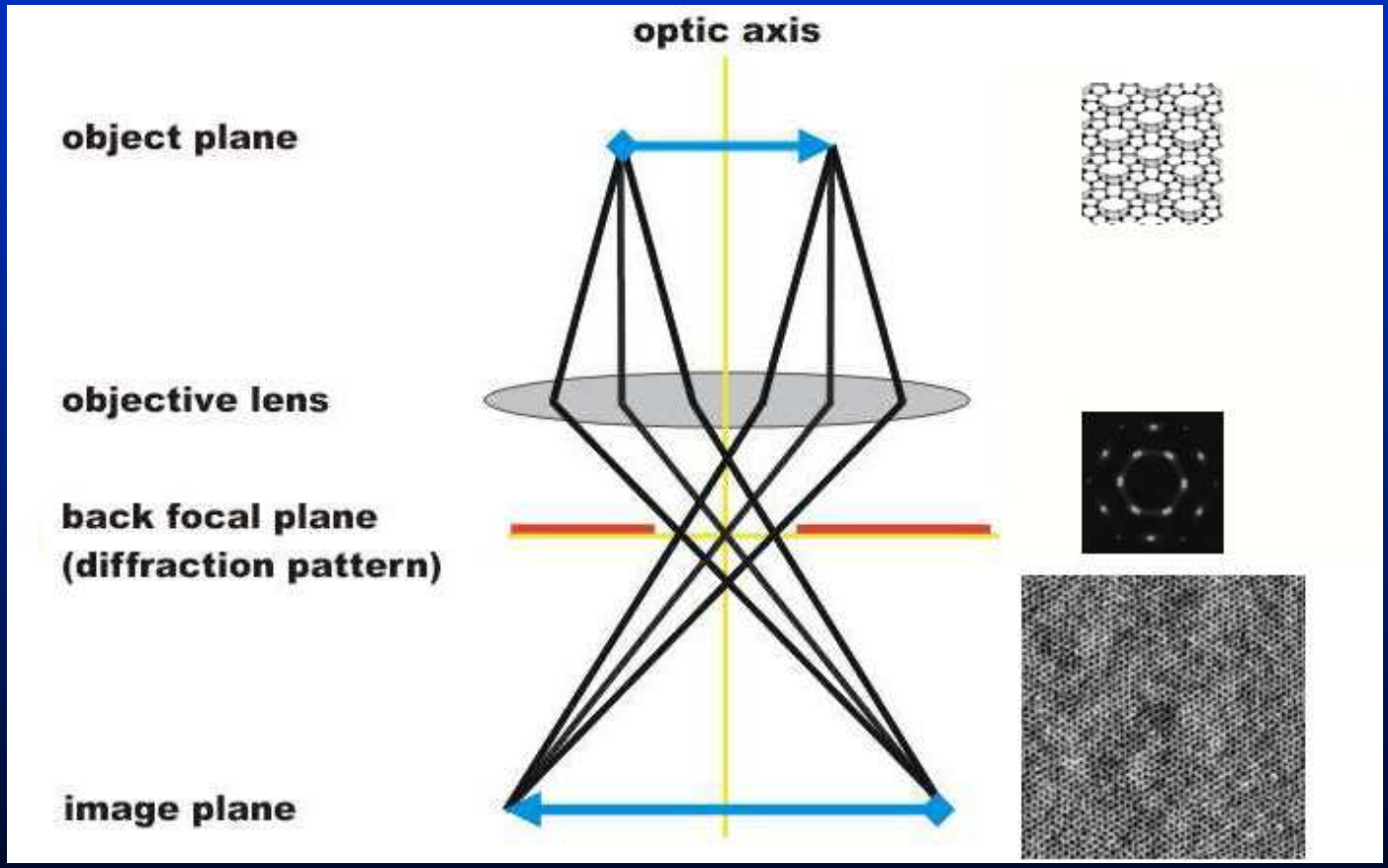
James Z. Chen

Electron Microscope

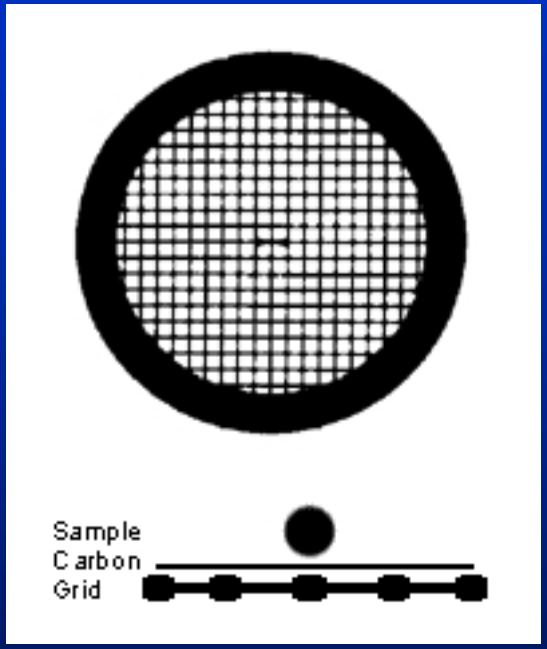


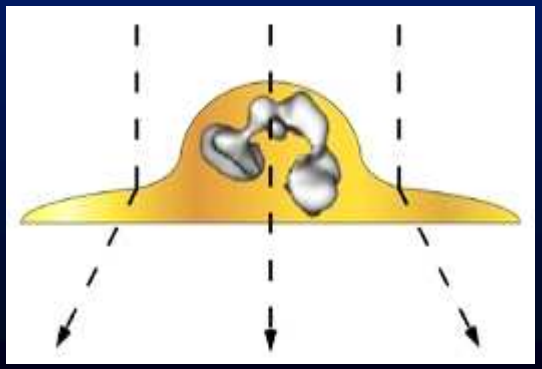
Transmission Electron Microscopy (TEM)

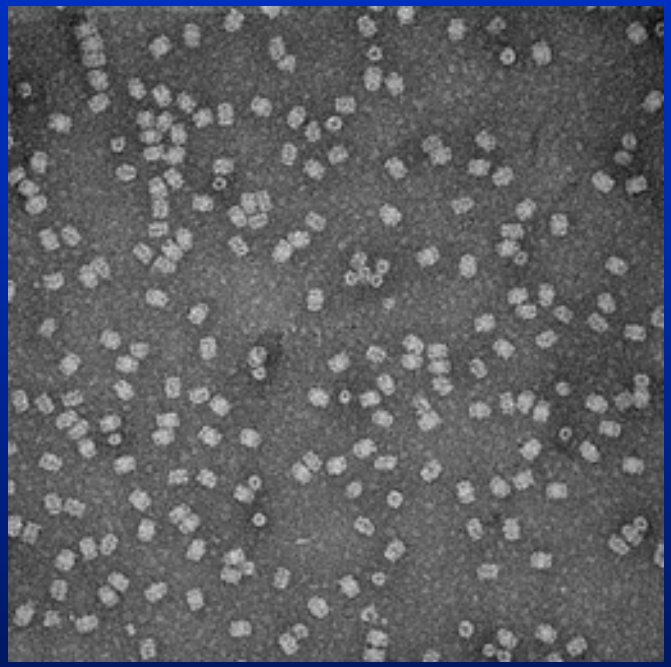
TEM Image Formation



Specimen Preparation: Negative Staining

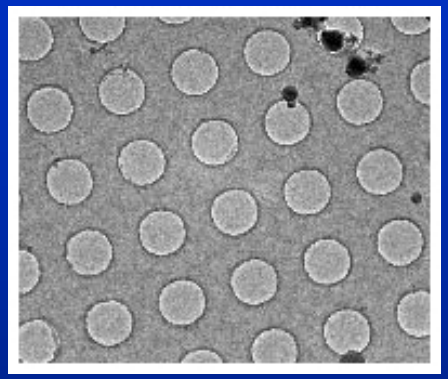


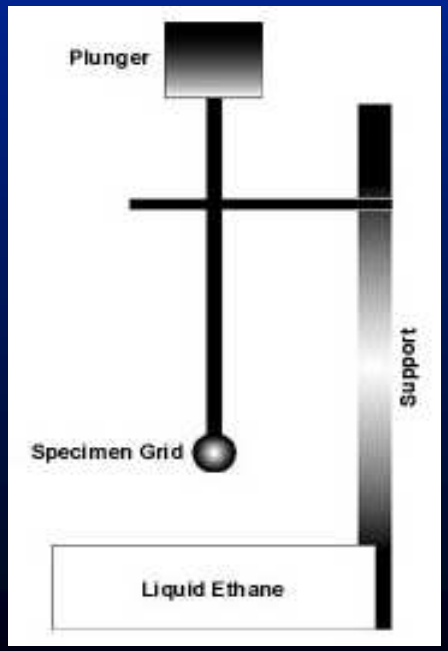


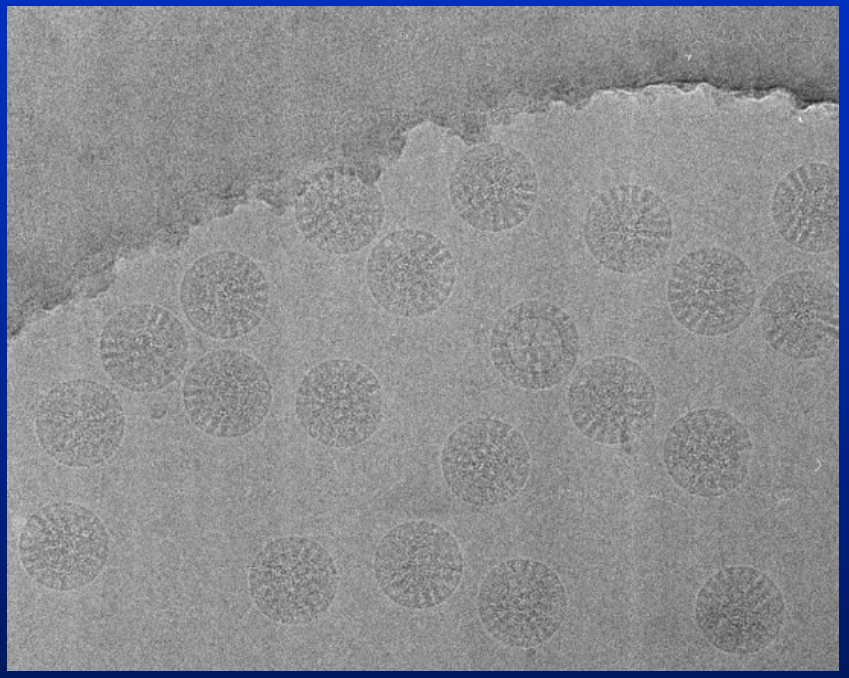


+ quick to make
+ high dose-tolerance
- preferred orientation
- lower resolution (nm)

Specimen Preparation: Cryo-plunging





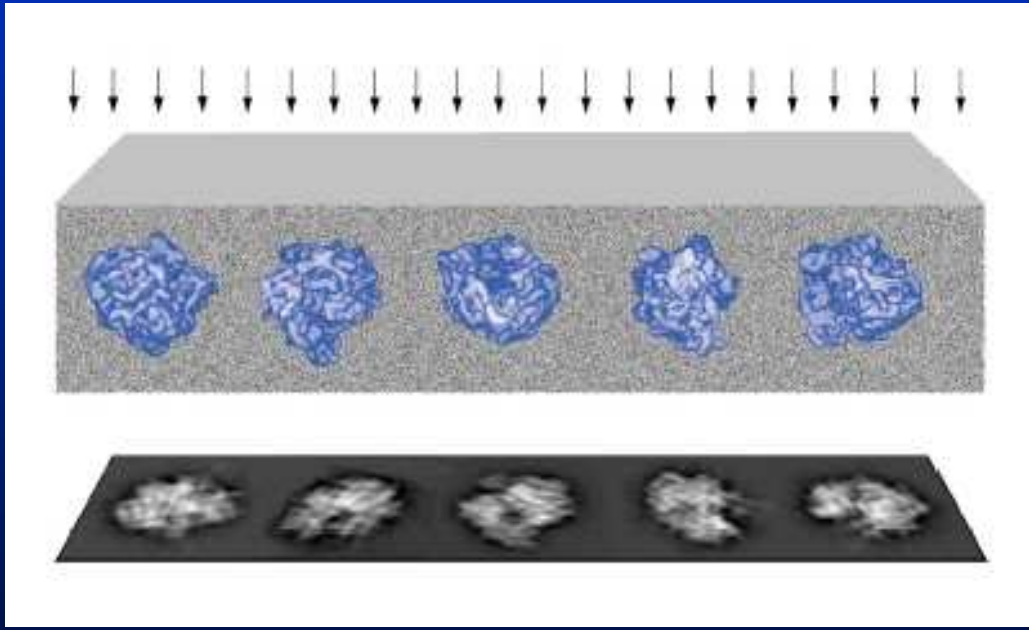


+ higher resolution (Å)

- low dose-tolerance

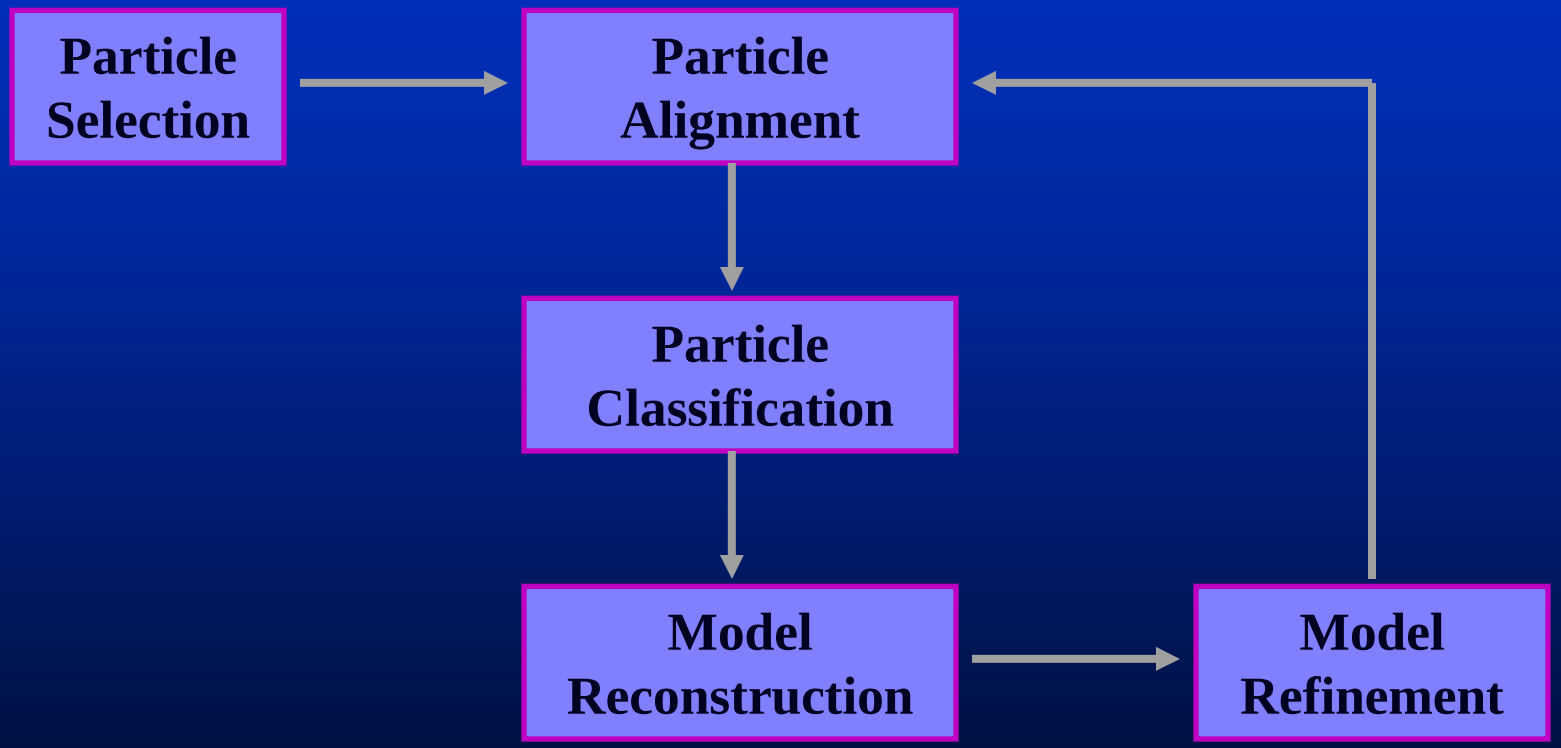
- must work under LN_2

Image Acquisition

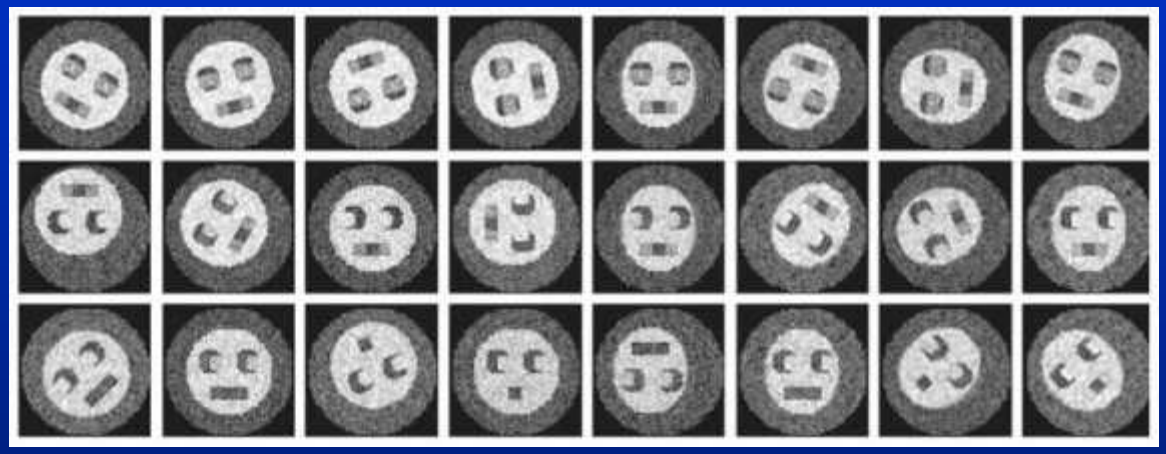


- **emulsion film**
- **CCD**
- **pixel detector**

Data Processing Pipeline

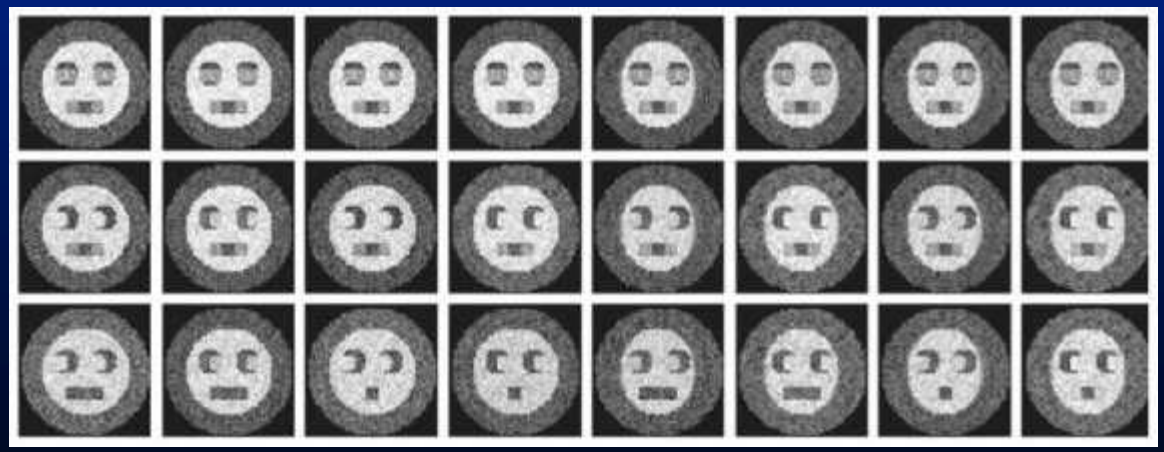


Particle Alignment



2D Alignment

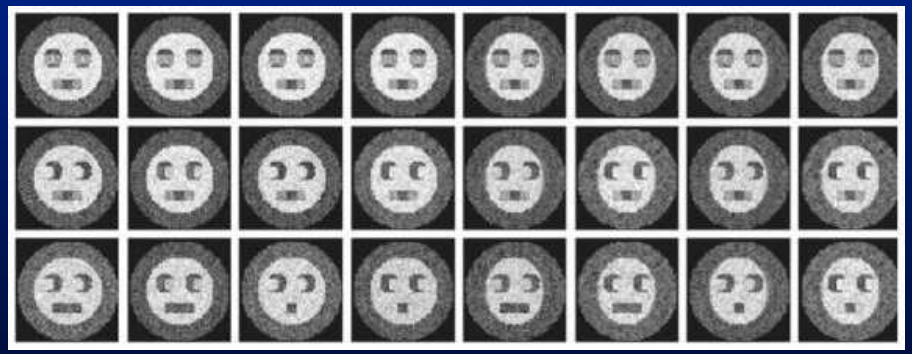
- **Translation**
- **Rotation**

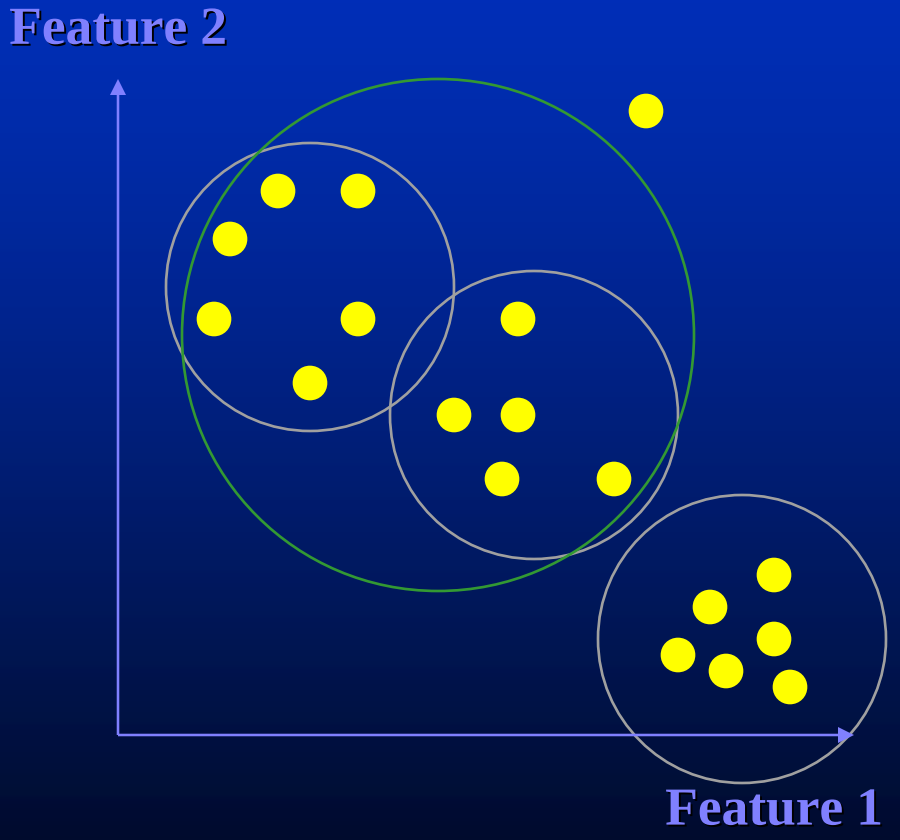


Particle Classification

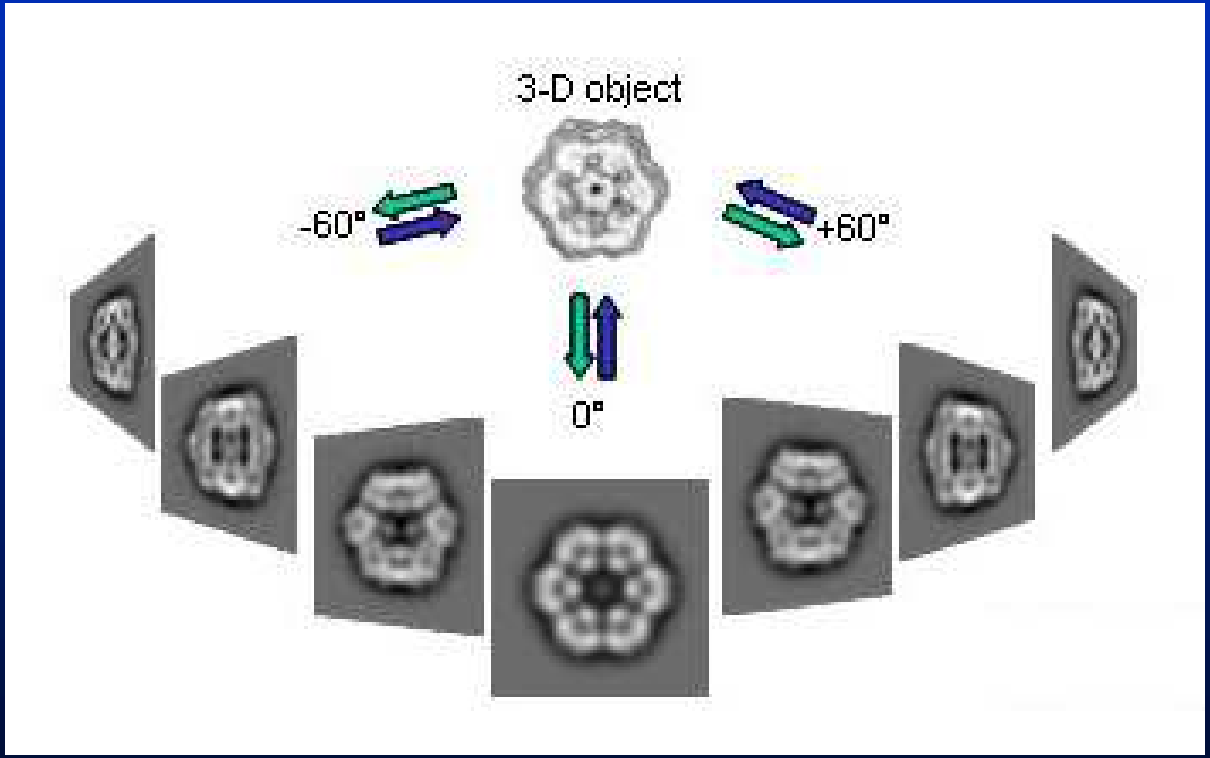
Goal: improve SNR by class averaging

- **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?**

M-M Force-Fields

$$U(\mathbf{R}) = \sum_{bonds} K_r\,(r - r_{eq})^2 \qquad \textit{bond}$$

$$+ \sum_{angles} K_\theta\,(\theta - \theta_{eq})^2 \qquad \textit{angle}$$

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

$$+ \sum_{i<j}^{atoms} \frac{A_{ij}}{R_{ij}^{12}} - \frac{B_{ij}}{R_{ij}^{6}} \qquad \textit{van der Waals}$$

$$+ \sum_{i<j}^{atoms} \frac{q_i q_j}{\varepsilon R_{ij}} \qquad \textit{electrostatic}$$

MD Simulation

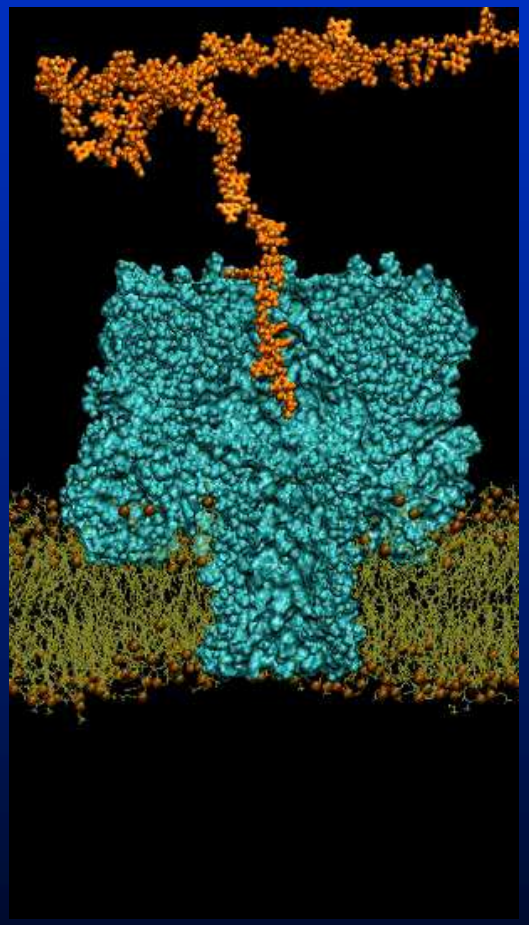
For each atom:

$$\boldsymbol{r}(t+\delta t) = \boldsymbol{r}(t) + \boldsymbol{v}(t)\delta t$$

$$\boldsymbol{v}(t+\delta t) = \boldsymbol{v}(t) + \boldsymbol{a}(t)\delta t$$

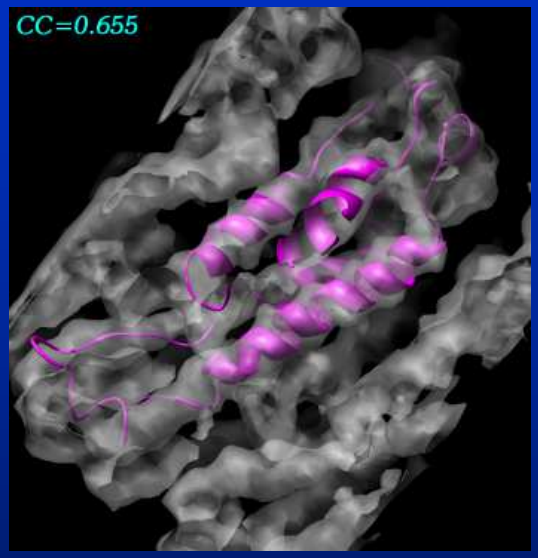
$$\boldsymbol{a}(t) = \boldsymbol{F}(t)/m$$

$$\boldsymbol{F} = -\frac{d}{d\boldsymbol{r}}U(\boldsymbol{r})$$



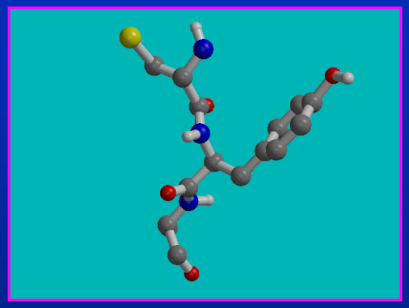
Electrophoretically-driven translocation of a DNA strand through the transmembrane pore of alpha-hemolysin. TCB, UIUC.

PDB-Map Docking

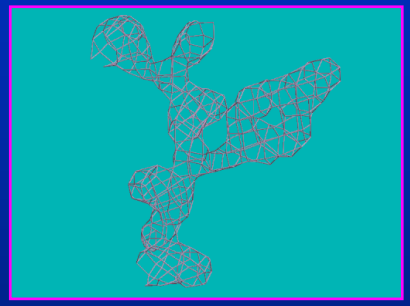


- Why force-fields?
  - Stereo-chemically correct structure
  - Reduce DOF in low-resolution EM density map
- Structural analysis / interpretation
  - Protein/complex structure refinement
- EM structure prediction
  - Molecular conformation prediction

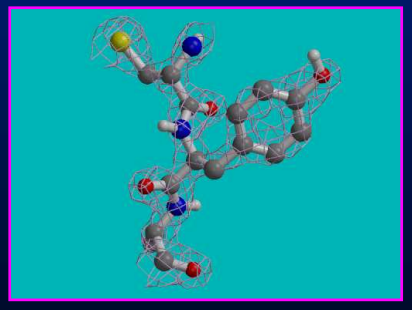
Restrained MD Refinement



Dynamics



$$E_{TOTAL} = E_{CHEM.} + E_{\oplus}$$



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

Refinement Methodology

A resolution-dependant density calculation

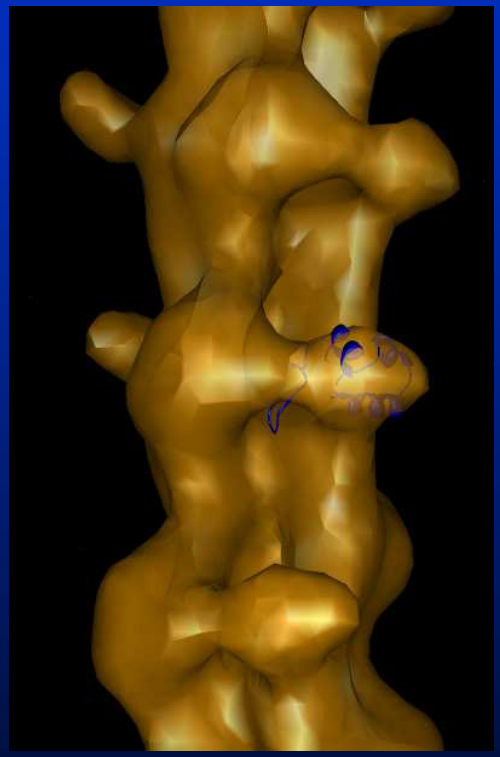
$$\rho_{calc}(r) = 4\pi Q \int_{d^*_{low}}^{d^*_{high}} g(d^*)\, d^{*2}\, \frac{\sin(2\pi r d^*)}{2\pi r d^*}\, dd^*$$

$$E_{\oplus} = \sum_{atoms} \left(k\rho_{obs} + b - \rho_{calc}\right)^2$$

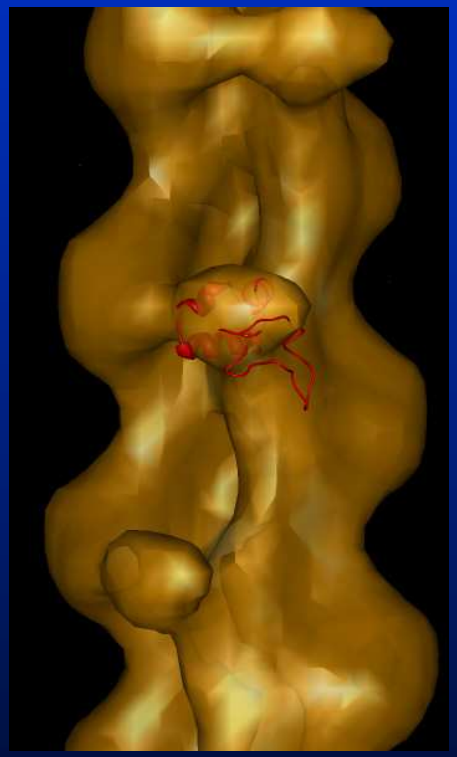
Refinement Protocol

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

Actin-DHP Docking

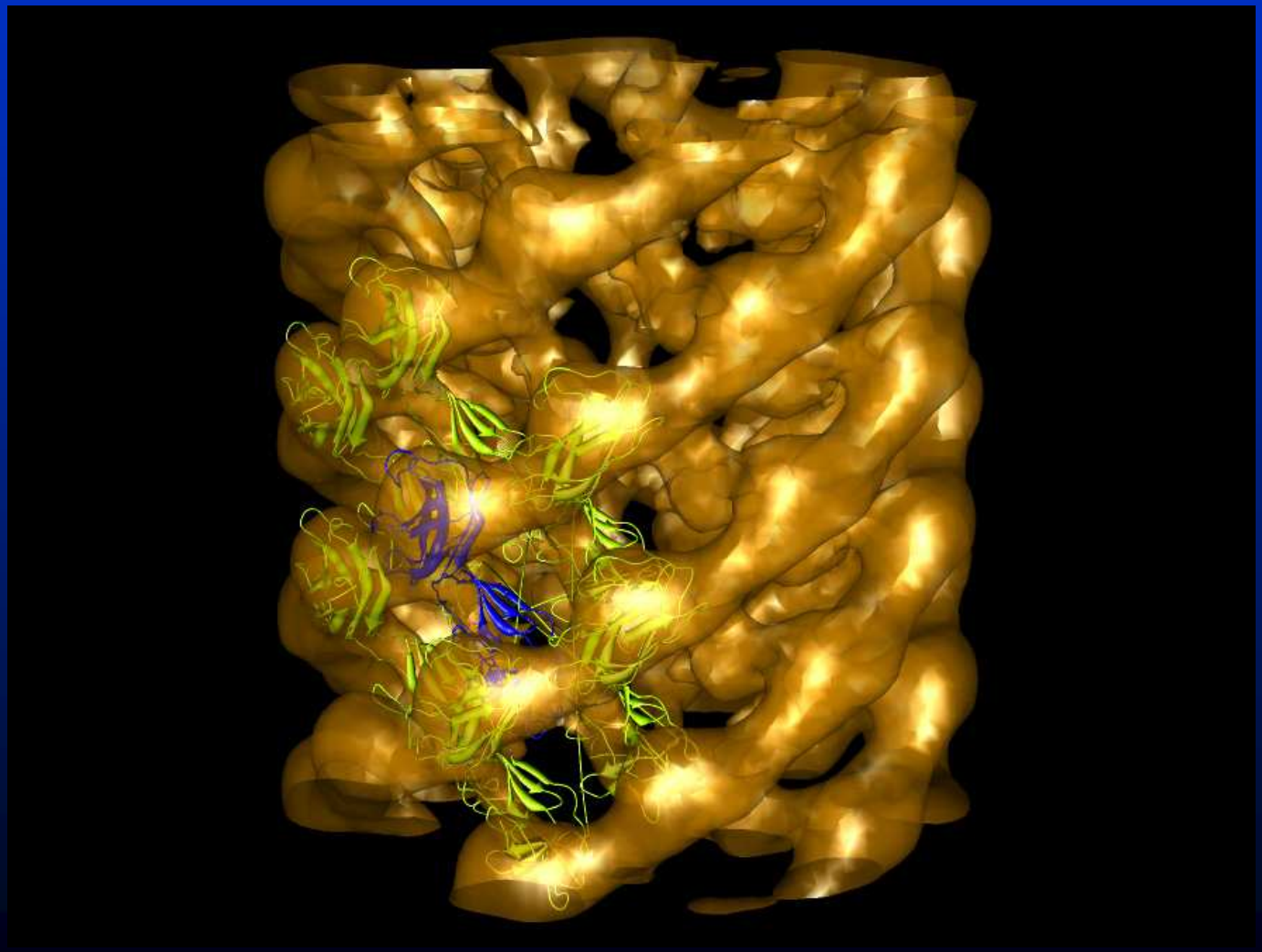


CC = 0.49

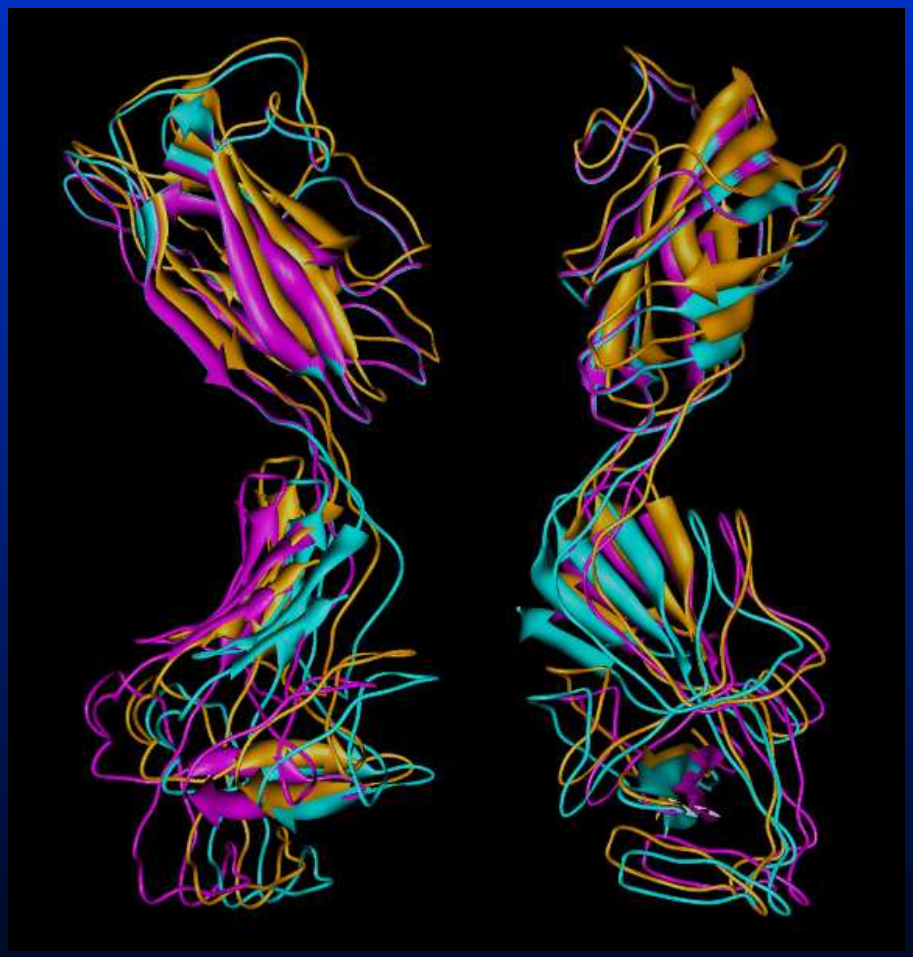


CC = 0.67

Helical Structure of Flagella Hook



Optimal Conformation



Crystal Structure

E(bond)=0.990 E(angl)=10.353 E(tors)=3.161
E(VDW)=-208. E(PVDW)=0.3E+8 E(rres)=167.0

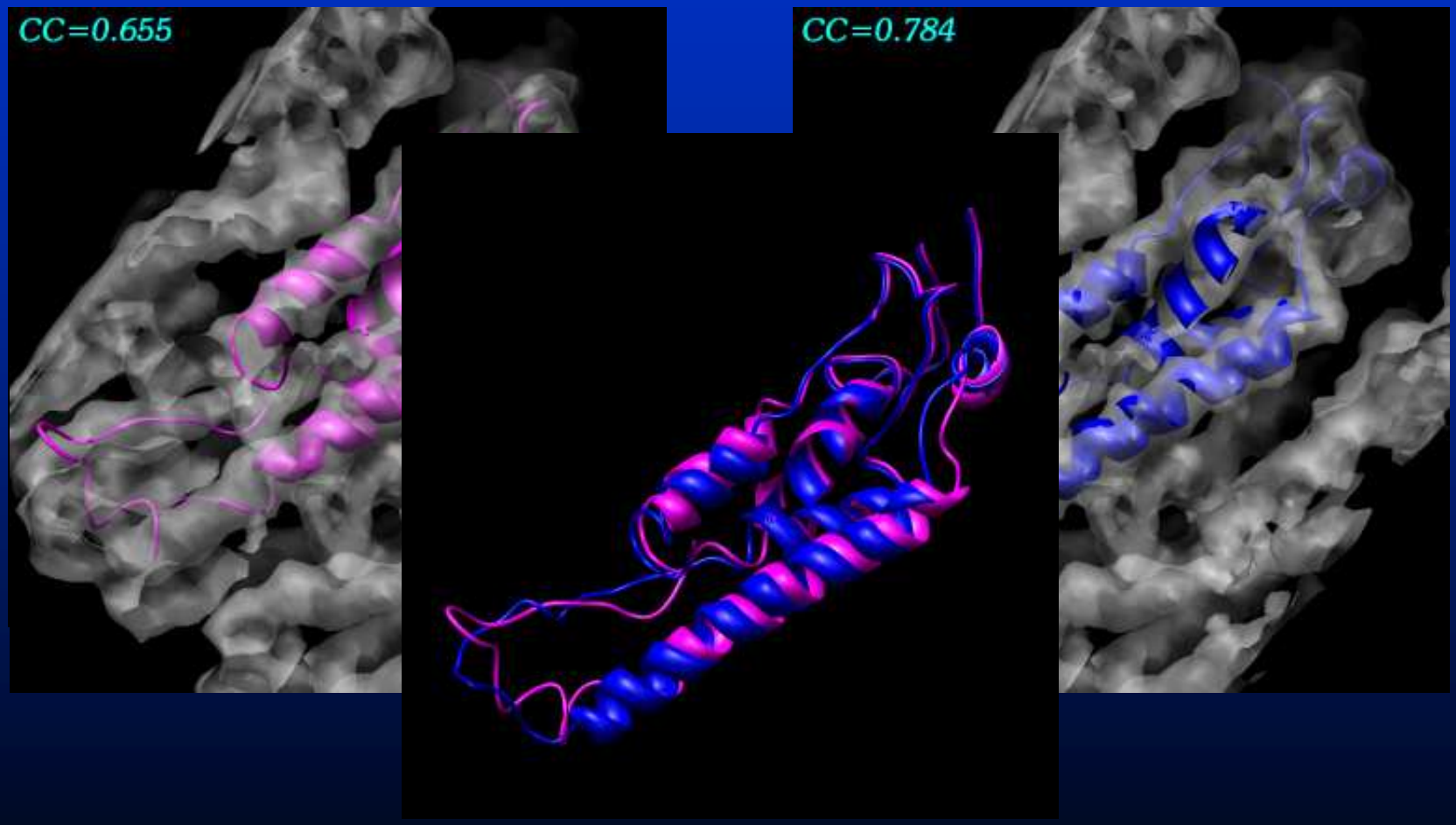
Manual Adjustment

E(bond)=0.975 E(angl)=10.397 E(tors)=3.169
E(VDW)=0.5E+7 E(PVDW)=0.3E+5 E(rres)=161.0

RSMD Refinement

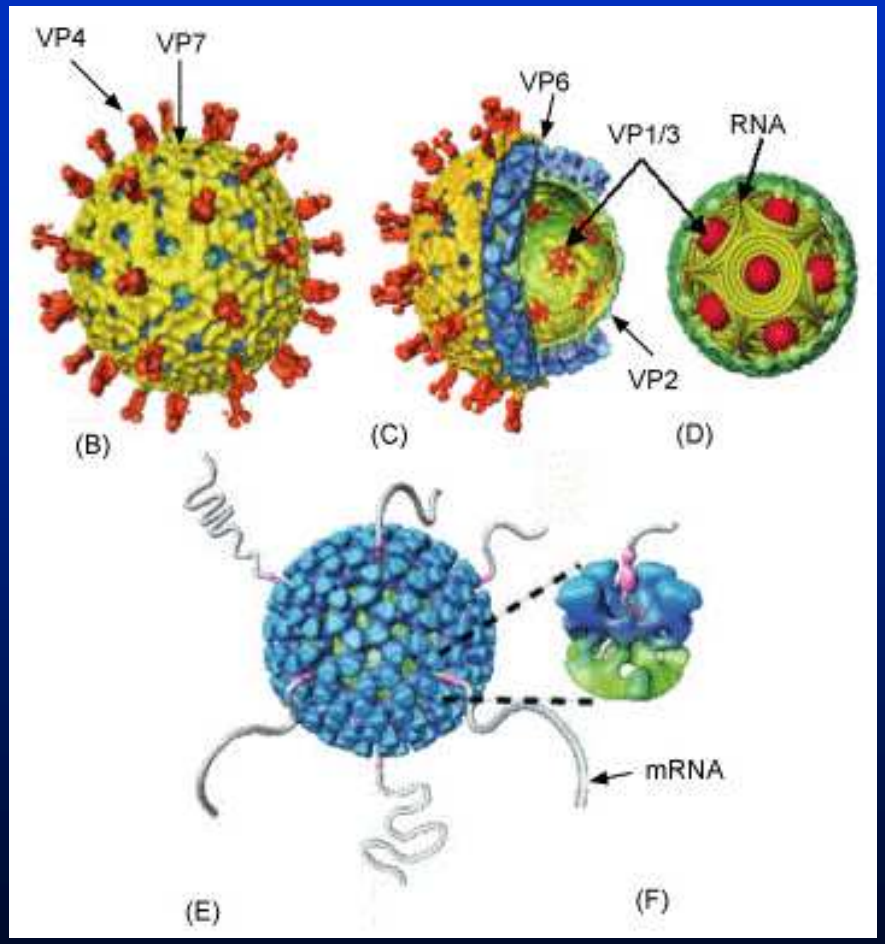
E(bond)=0.923 E(angl)=10.351 E(tors)=3.138
E(VDW)=-191. E(PVDW)=-14.38 E(rres)=156.0

TMV Conformational Change



By courtesy of Carsten Saches.

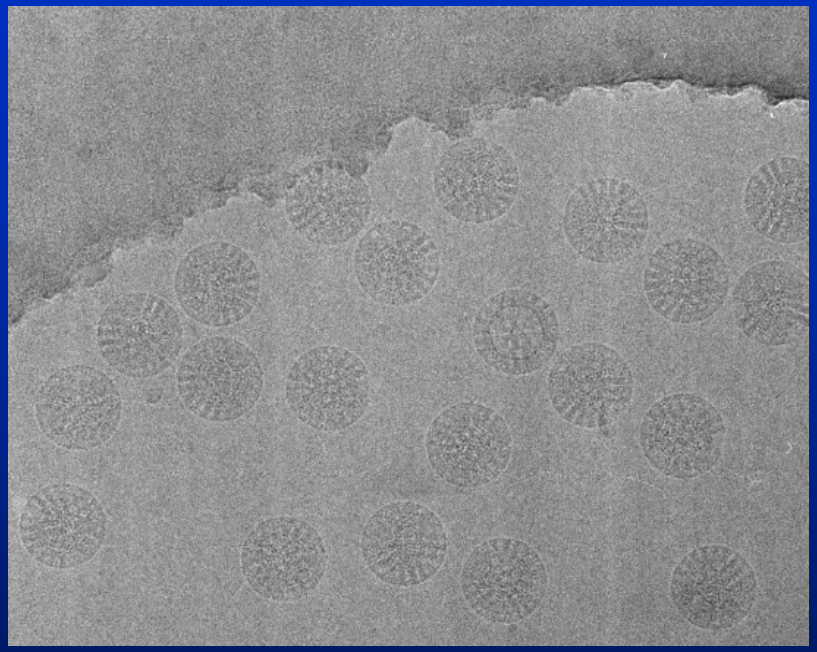
Rotavirus TLP



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

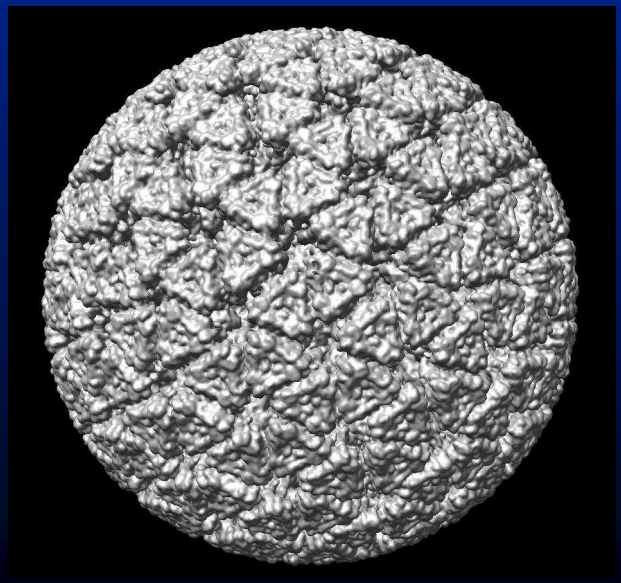
Jayaram *et al*, Virus Research, **101**:67-8, 2004.

Cryo-EM Structural Analysis

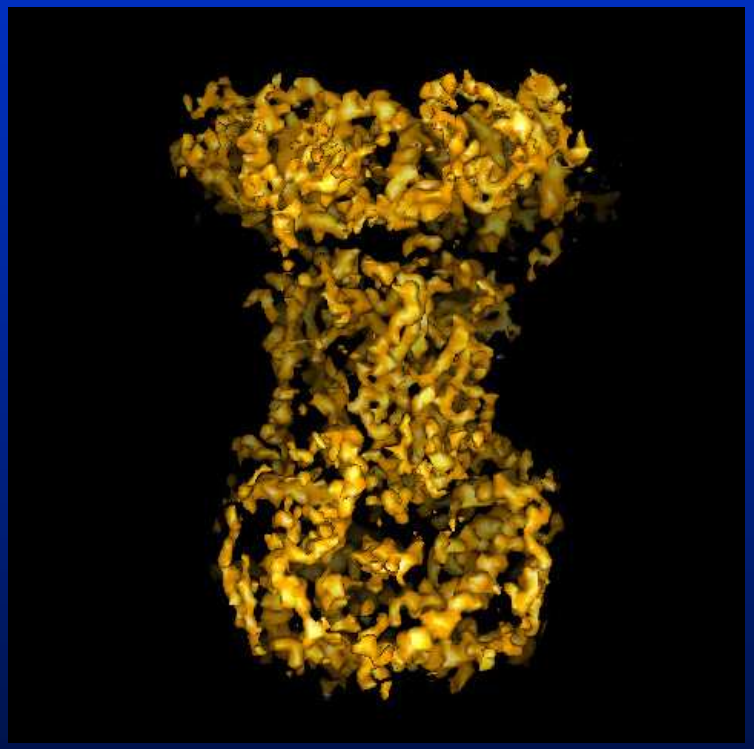


- **TF30: 300 kV**
- **dose: 15 – 20 $e^-/Å^2$**
- **defocus: 1.0 – 3.0 µm**
- **~ 5,000 single particles**

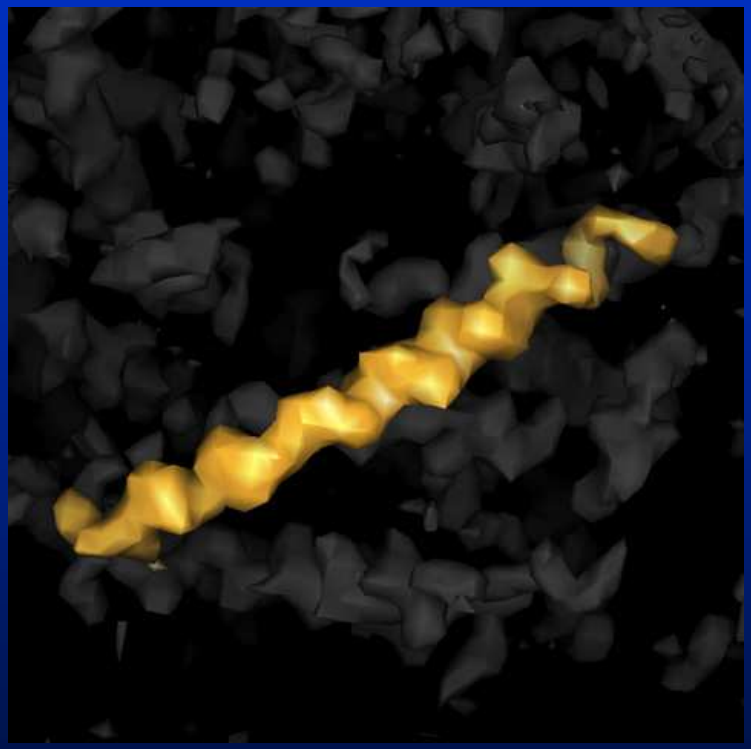
- **projection matching**
- **CTF correction**
- **FREALIGN refinement**
- **resolution: ~ 4Å**



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 …