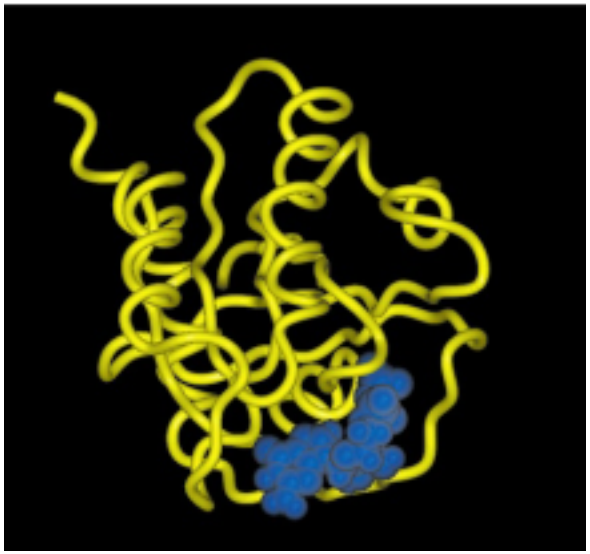
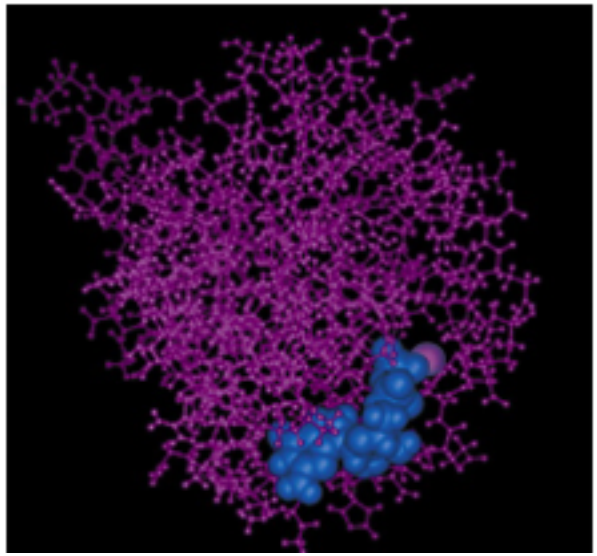
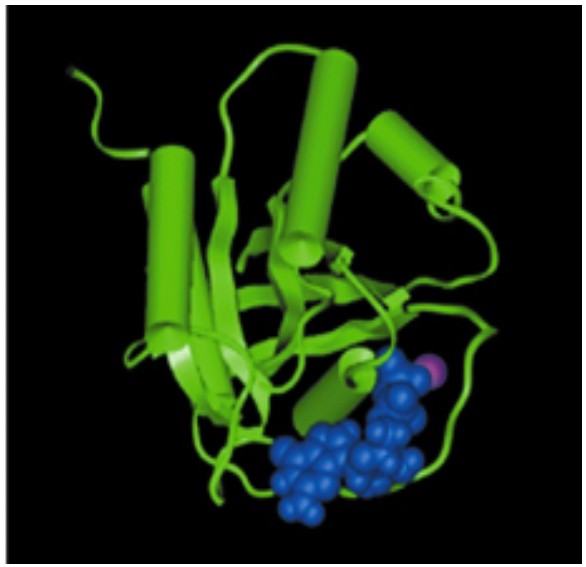
Structural Proteomics

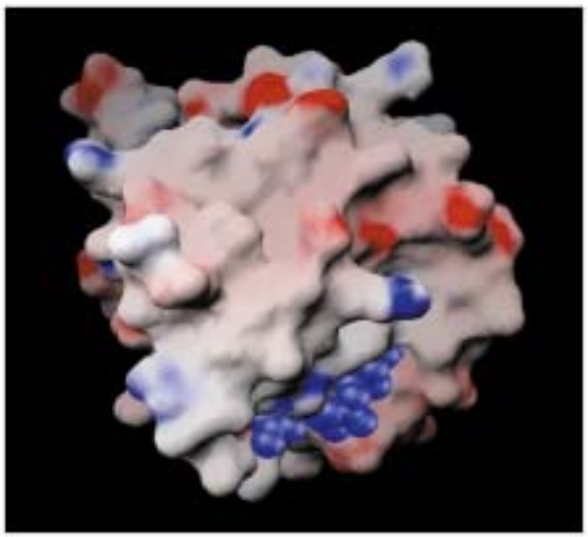
- Structural determination
  - X-ray crystallography
  - Nuclear magnetic resonance (NMR)
  - Cryoelectron microscopy
  - Fluorescence spectroscopy
  - Chemical crosslinking
  - etc…

Graphical representations









MCB Figure 3-5

- Ras
  - Guanine nucleotide binding protein
- Coordinates deposited in PDB

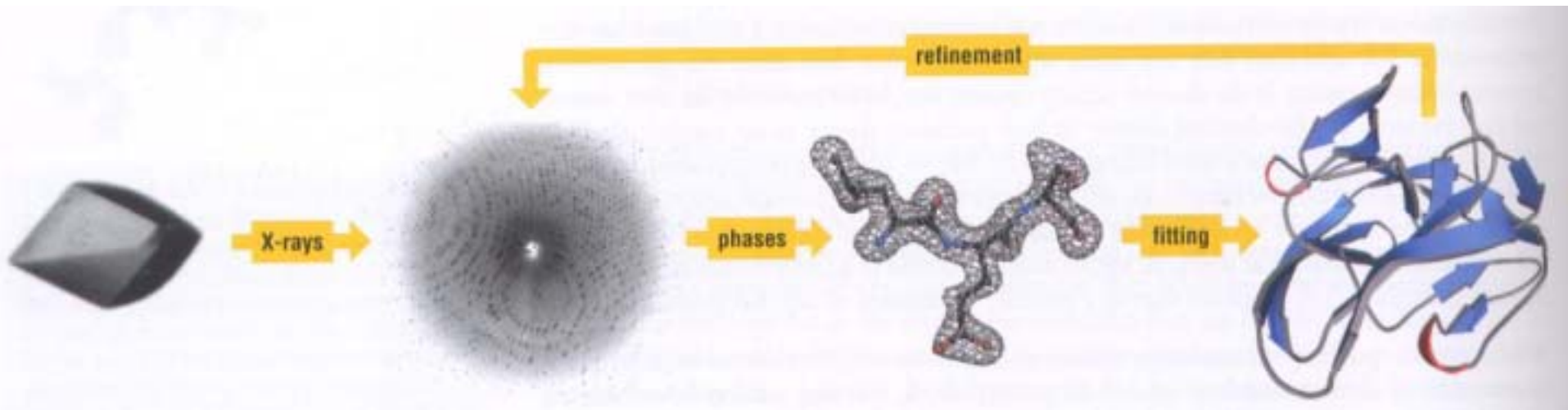
Resolution

- 6 Å density map
  - “Blubs” of electron density (not backbone)
  - Overall molecular shape (crevices, subunits)
  - A helices will appear as rods
  - Heavy atom binding sites
- 3 Å resolution
  - Possible to trace polypeptide backbone
  - Large amino acid side chain
- 2.5 Å resolution
  - All side chains visible
  - Protrusion of the carbonyl group from the peptide plane

Crystal and X-ray Diffraction

- Crystal
- X-ray diffraction
- Electron density map
- Structural refinement

Fig 5-3, Protein Structure and Function 2004



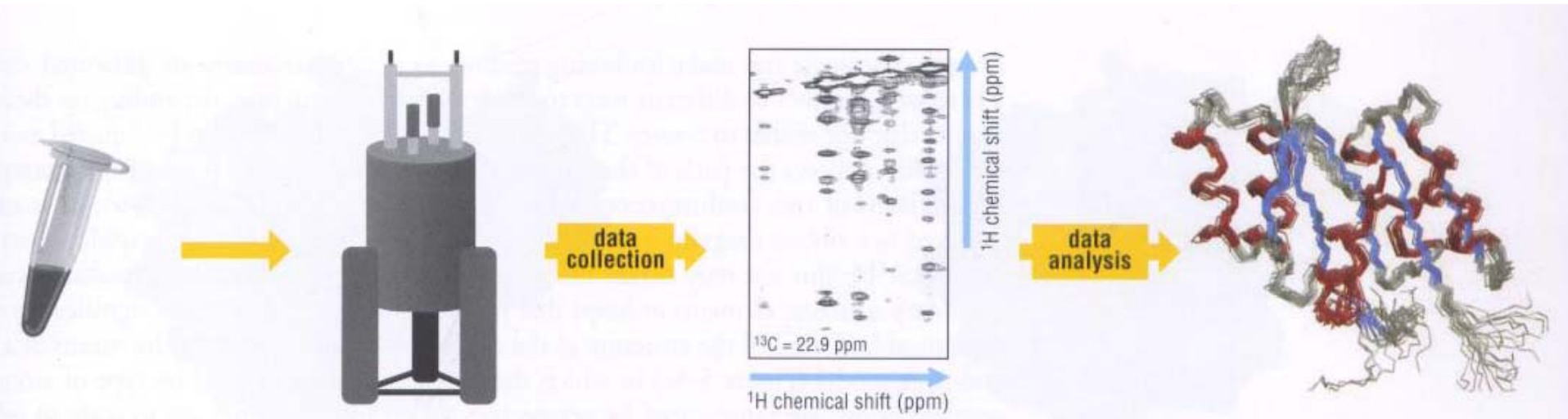
X-ray Crystallography

- Advantage:
  - High resolution structure
- Limitation:
  - Membrane protein
  - Large protein complex (heterogeneous)
  - Disordered
  - Phase problem
  - Heavy computational work
  - Need other structural information

Nuclear Magnetic Resonance

- Nucleus resonance
  - Magnetic field
  - Nuclear resonance at matched frequency
  - Chemical shift
- Effect of neighboring atoms
  - Distances calculated

Fig 5-4, Protein Structure and Function 2004



Purified, labeled protein

NMR spectrometer

Resonance assignment, internuclear distance measurement

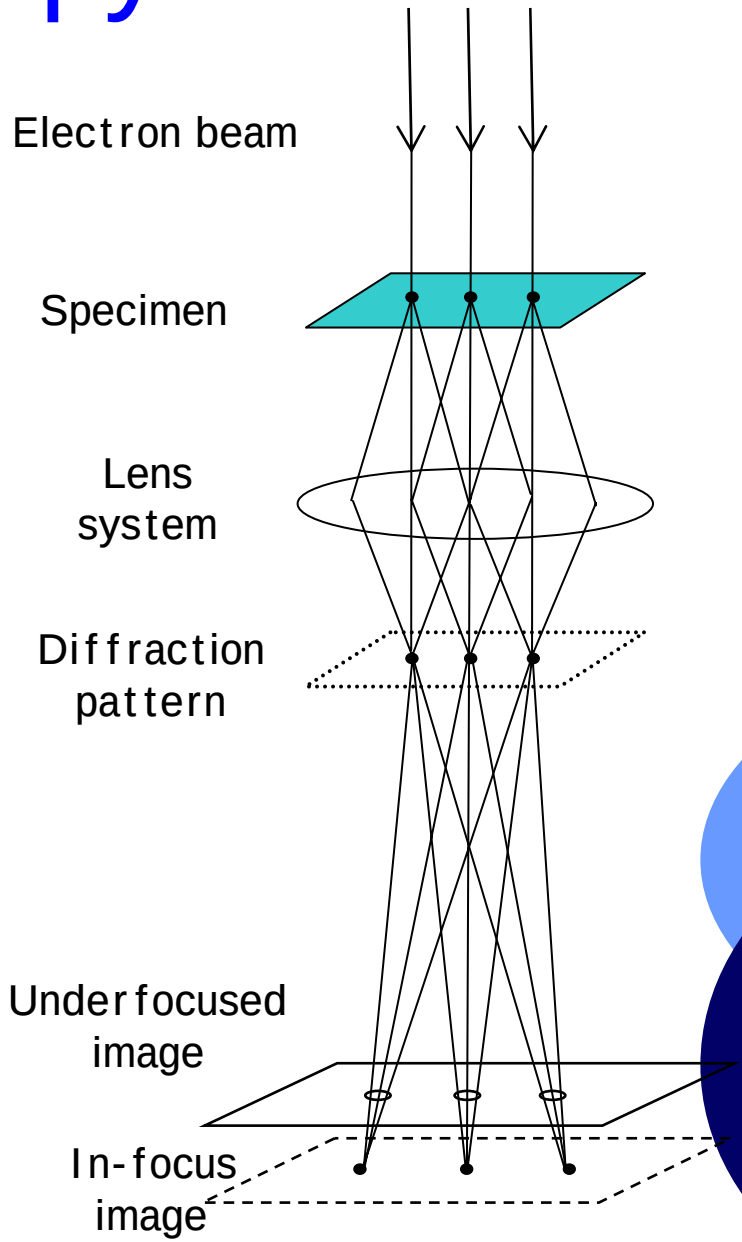
Protein structure

Nuclear Magnetic Resonance

- Advantage
  - Solution structure
  - Dynamic information
- Limitation
  - Concentrated sample (aggregation ?)
  - Isotope labeling
  - Up to 50 kD (high-field, multi-dimensional NMR)
  - Membrane protein (solid state NMR)

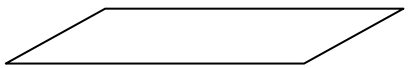
Electron Microscopy

- Advantages
  - Native and hydrated sample
    - Unstained, unfixed
  - No upper size limit
    - Macromolecular assembly
  - Membrane proteins
  - Little sample needed
- Limitation:
  - Instruments
  - Computational techniques
  - Low resolution structure
    - 3 ~ 0.3 nm
- Basic principles

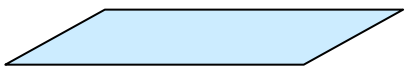


CryoEM

Purified protein
(complex) solution



Quick freeze



Frozen hydrated sample

TEM

Photographs on film
(3D object → 2D projections)

Digitize

Image analysis

Refinement

Image reconstruction
(2D projections → 3D structure)

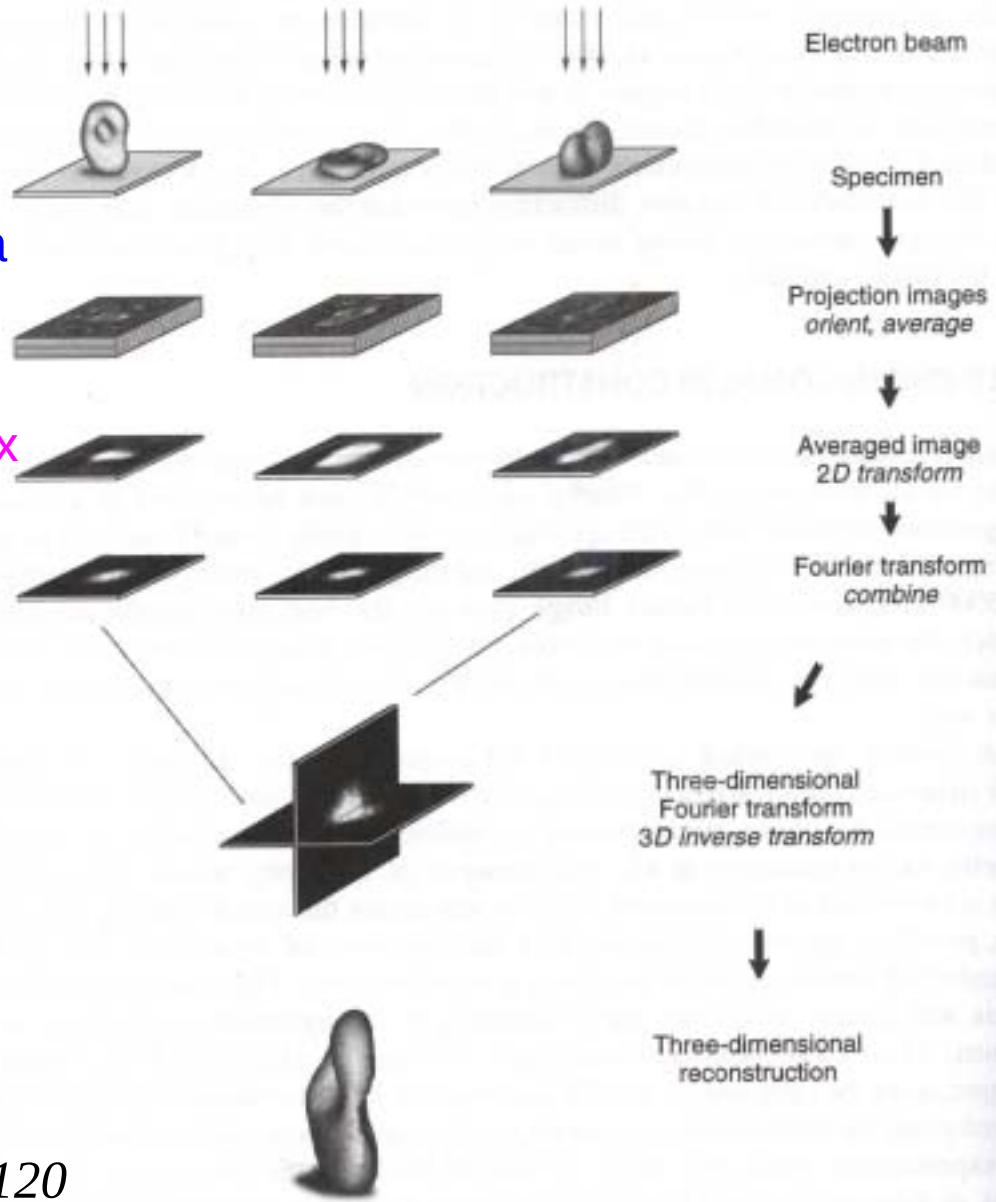
3D density map

From other techniques

Final 3D structure

Electron Crystallography

- Image reconstruction
  - 2D projection → 3D structure
  - Intrinsic membrane protein in a lipid bilayer
    - Bacteriorhodopsin
    - Plant light-harvesting complex
    - Aquaporin
  - Helical assemblies
    - Tubulin

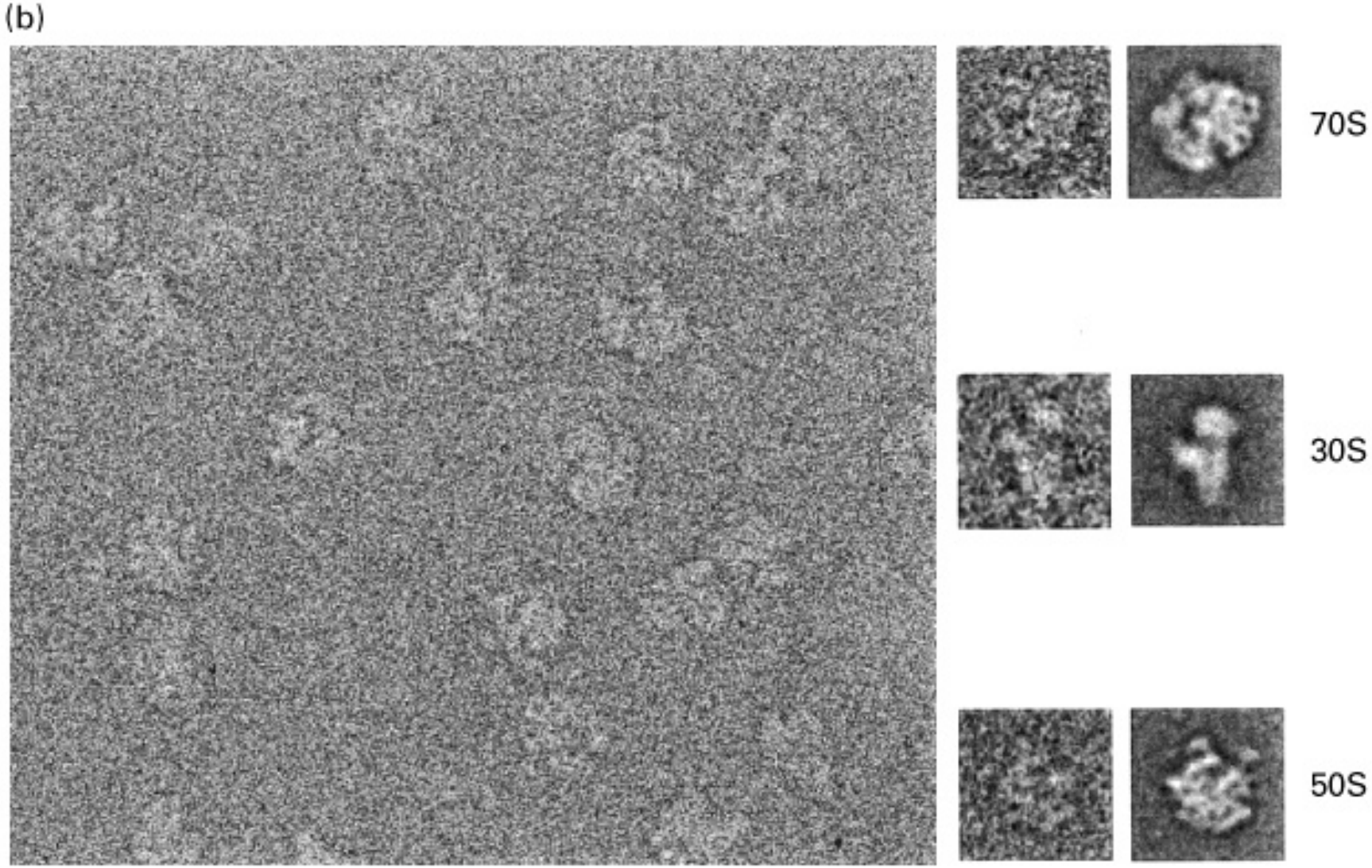


Structural Bioinformatics, p. 120

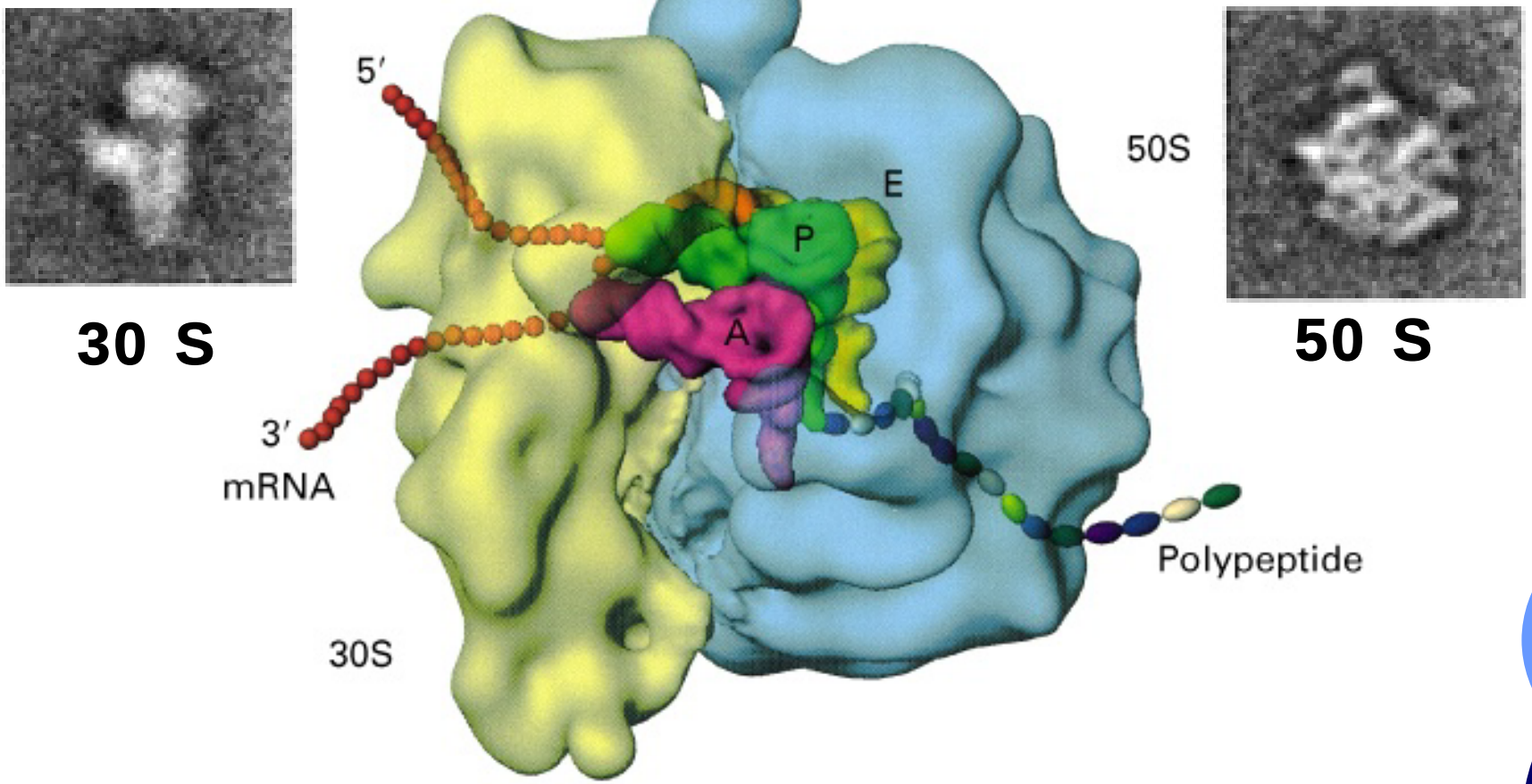
Electron Tomography

- Single particle analysis
- Protein dynamics and intermediate states
  - Rapid freezing (less than 1 msec)
- Reconstruction of specimen with unique structure
  - Whole cell

CryoEM of *E. coli* ribosome

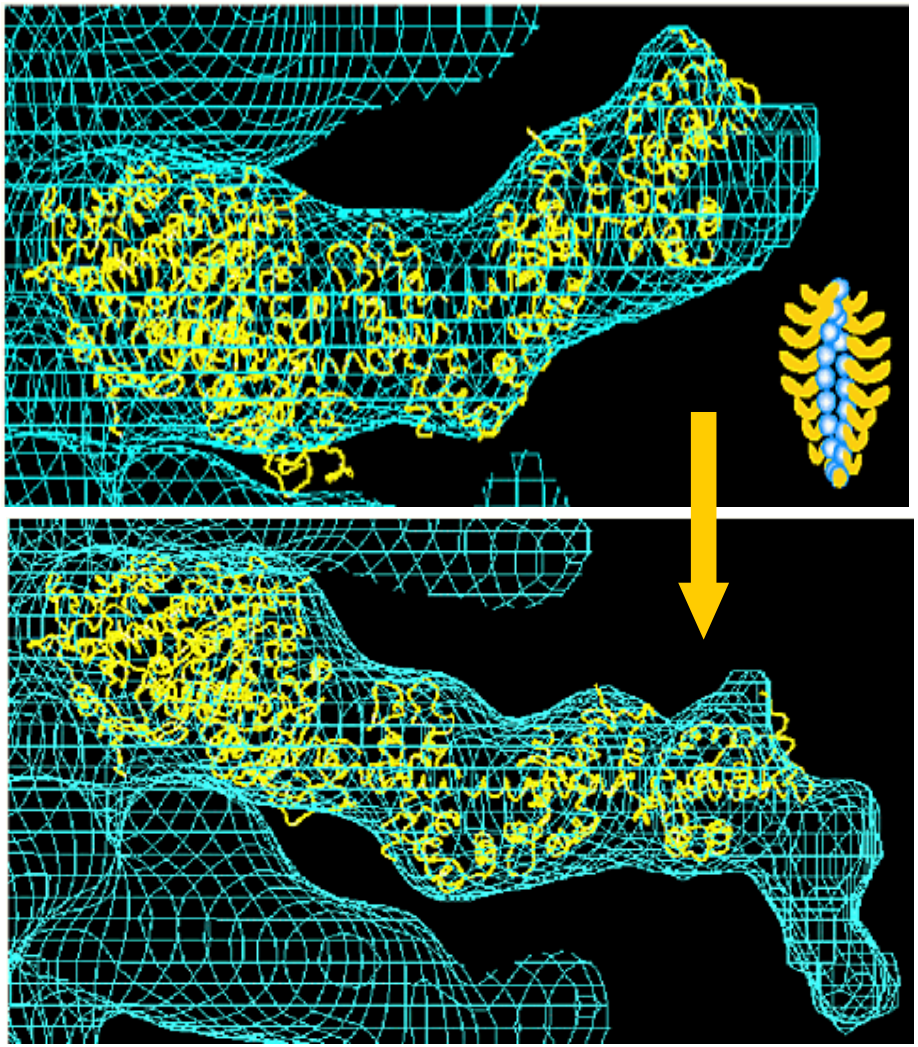


Structural model (25 Å)



- Image reconstruction based on 4300 projections

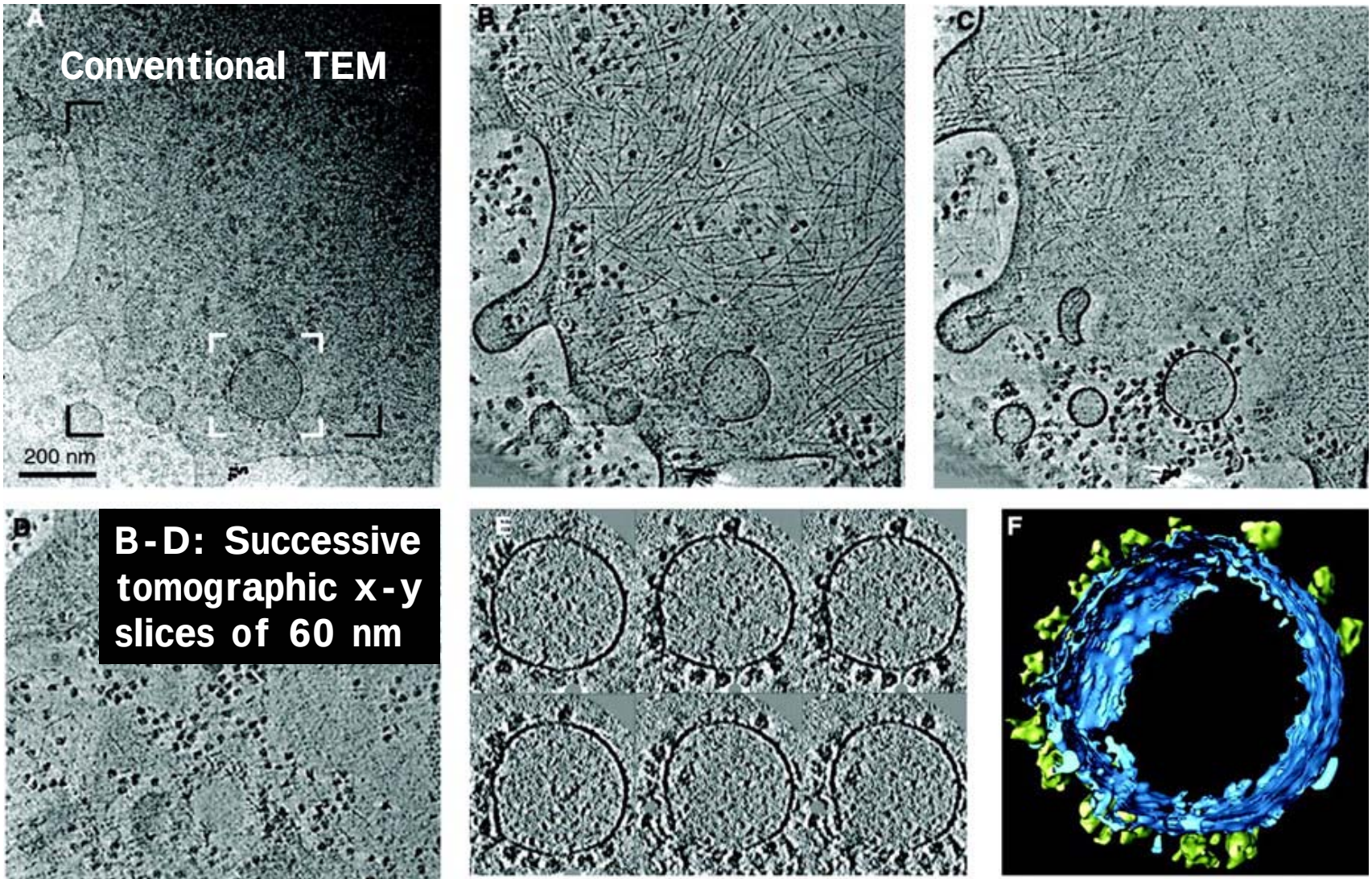
CryoEM + Crystallography



- Myosin S1 decorated actin filament
- Upon ADP binding:
  - 30º inter-domain rotation
  - ~60Å movement

Nature Struct. Biol. 2000. 7, 711-714.

Cryoelectron Tomography



X-y slices through a RER

Science 2002. 298, 1029-1213.

Cytoplasmic macromolecular complex