Limitations in Structural Biology:

- **X-ray Crystallography**: requires crystals! But size of complex amenable to such techniques has now reached several million Da. Crystal packing can also be an issue.
- **NMR Spectroscopy**: still limited in size of macromolecule that can be solved (~40kDa)
- **Optical Microscopy**: conventional imaging limited by $\lambda$ (~5,000 Å). As $\lambda$ decreased, phototoxicity (radiation damage) becomes problem.
- **Scanning Probe (AFM, STM)**: only provides surface, images have never achieved expected potential due to specimen preparation problems.
- **Electron Microscopy**: Radiation damage main problem, electron optical limitations ~ 1 Å

History of EM

- 1897 - Thomson describes electrons
- 1926 - magnetic lenses built to focus electrons
- 1931 - Ruska built first transmission EM
- 1945 - Porter et al. use EM to look at cultured cells
- 1968 - DeRosier and Klug generate 3D reconstruction from 2D EM images of helical bacteriophage tail
- 1975 - Unwin and Henderson show that near-atomic resolution of membrane protein crystal possible

Comparison of LM, TEM, SEM

Scanning Electron Microscopy (SEM)

- Very useful for looking at cell surfaces
- In general, requires metal coating, at best 50 Å resolution
Transmission Electron Microscopy (TEM)

Very useful for looking at macromolecular assemblies
Capable (for crystals) of better than 2 Å resolution
fast-freeze/deep-etch metal replica shown

Why use electrons?
To image (and not just diffract), need lenses to focus
Radiation damage is actually worse for x-rays than it is for electrons:

\[
\frac{\sigma_{\text{inelastic}}}{\sigma_{\text{elastic}}} = 3 \text{ for electrons} \\
\frac{\sigma_{\text{inelastic}}}{\sigma_{\text{elastic}}} = 10 \text{ for x-rays}
\]

but energy deposited by x-rays is 1,000 times greater than e−!

Resolution of non-biological specimens not limited by radiation damage

Actual EM of thin layer from gold crystal, spacing = 2.0 Å

Radiation damage from e− can be easily measured

Transmission Electron Microscopy (TEM)

- Metal shadowed specimen (myosin) shown
- Negative stain shown
- Cryo-EM of unstained, frozen-hydrated sample- pili
- Cryo-EM of unstained, frozen-hydrated sample- GroEL
Mechanism of image formation

- In light microscopy, can use either amplitude or phase contrast.
  - Amplitude contrast arises from differential transmission (absorption) of light.
  - Phase contrast arises from retardation of wavefront by greater density.
- In EM, can use either amplitude (e.g., negative stain) or phase contrast (cryo-EM).

Overcoming poor signal-to-noise ratio (due to low dose) is frequently main problem.

Average from 2,053 images

Several sources of noise, as dose decreases e- counting statistics dominates (low dose ~ 2-5 e/Å², 10⁹ rad).

To overcome poor Signal to Noise Ratio, must average.

Crystals versus "single particles".
Advantages of using two-D crystals:
- High homogeneity.
- Well-developed theory of reconstruction.
- Atomic resolution demonstrated for a small number of specimens.

Disadvantages:
- Only a single conformation or binding state, out of several potentially important ones, is realized as a result of crystal constraints.
- Computation becomes difficult in the presence of disorder.
- Difficulty of growing crystals.
- Must tilt crystal, physical limit to tilts.

2D Crystals
SINGLE PARTICLE RECONSTRUCTION is the term used for the reconstruction of a macromolecule from images of a specimen in which the molecule exists in many realizations in the form of single, isolated particles, i.e., without contact with neighboring molecules.

Advantages of using single-particle reconstruction:
- all solution states can be captured in principle
- disorder is no longer an issue, since each molecule is treated separately
- no need for crystallization

Disadvantages:
- separating out homogeneous states can be daunting
- computationally challenging
“single particles”

Computer averaging of single particles to overcome noise

- six degree of freedom for any particle
- but only five after projection
- must find three Euler angles, x-, y-shifts
- What are the obstacles? What limits resolution?

Transmission Electron Microscopy (TEM)

Combining x-ray and EM maps of E. coli ribosome is extremely powerful

Many macromolecular complexes have internal symmetry
Transmission Electron Microscopy (TEM)

empty procapsid, mature virus (phage P22)  

Helical symmetry can be exploited

First 3D reconstruction by EM was from a helical filament (bacteriophage tail) - DeRosier and Klug (1968)

In principle, all views can be obtained from a single filament

Therefore, tilts are not needed (as for crystals), nor is determination of orientation

Bacterial pili: example of weakly scattering object

Unambiguous fit of monomeric crystal subunit from Neisseria gonorrhoeae

~ 3.6 subunits per turn of a 37 Å pitch helix

Unambiguous fit of monomeric crystal subunit from *Neisseria gonorrhoeae*