# Basic Ideas on Structure

Biological macromolecules = biopolymers

repeating subunits – 4 general types:

1. Proteins – linear chains of aa (~ 20 types)

   masses of 20 KDa – $10^6$ Da (1 Da = 1 g/mol)

2. Nucleic Acids – DNA/RNA – 4 bases each

   1+2 make up viruses - typical structure

3. Lipids – group of smaller organic molecules insoluble in water – includes triglycerides = neutral fats (most abundant) and steroids (e.g. cholesterol)

4. (poly)saccharides – (mono- di- or poly) – carbohydrates - some are structural or metabolic

# Cells are compartmentalized

- Cell size = 1 – 100 um – 1 m (nerve cell)
- E coli example
- Careful about thinking of these images as static – there is lots of motion going on
- For example, a 160,000 Da typical protein would diffuse its own size of ~10 nm in about 2 us in water, but in concentrated cytoplasm it takes about 1000x longer or 2 ms.

outer

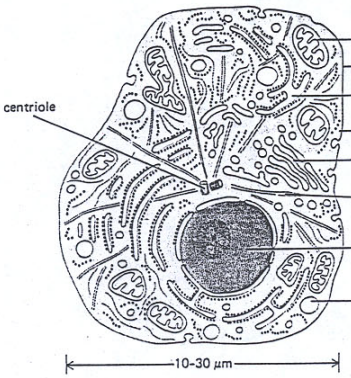
inner

Membrane production

UPS packaging

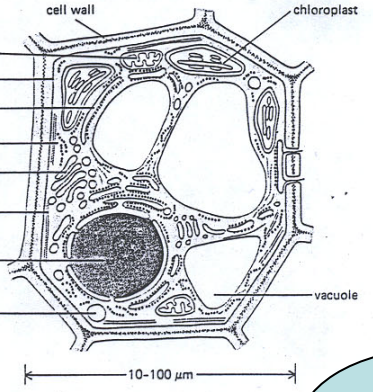
## ANIMAL CELL

thin section of a generalized animal cell




## PLANT CELL

thin section of a generalized cell from a higher plant

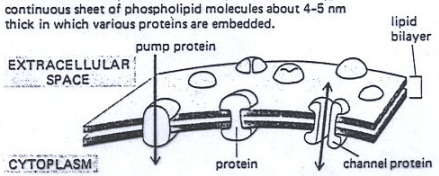



mitochondria
plasma membrane
endoplasmic reticulum
cytosol
Golgi apparatus
filamentous cytoskeleton
nucleus
lysosomes peroxisomes

# THE MEMBRANE SYSTEM OF THE CELL

## PLASMA MEMBRANE

The outer boundary of the cell is the plasma membrane, a continuous sheet of phospholipid molecules about 4–5 nm thick in which various proteins are embedded.



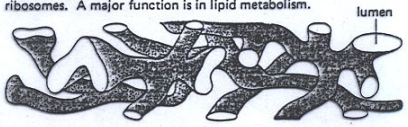

Some of these proteins serve as pumps and channels for transporting specific molecules into and out of the cell.

## ENDOPLASMIC RETICULUM

Flattened sheets, sacs, and tubes of membrane extend throughout the cytoplasm of eucaryotic cells, enclosing a large intracellular space. The ER membrane is in structural continuity with the outer membrane of the nuclear envelope and it specializes in the synthesis and transport of lipids and membrane proteins.

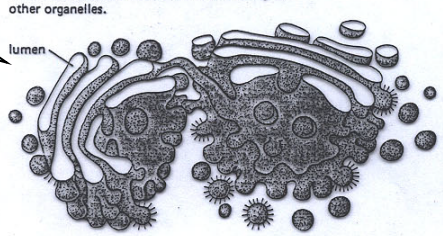
The rough endoplasmic reticulum (rough ER) generally occurs as flattened sheets and is studded on its outer face with ribosomes engaged in protein synthesis.


The smooth endoplasmic reticulum (smooth ER) is generally more tubular and lacks attached ribosomes. A major function is in lipid metabolism.




## GOLGI APPARATUS

A system of stacked, membrane-bounded, flattened sacs involved in modifying, sorting, and packaging macromolecules for secretion or for delivery to other organelles.




Around the Golgi apparatus are numerous small membrane-bounded vesicles (50 nm and larger). These are thought to carry material between the Golgi apparatus and different compartments of the cell.

## LYSOSOMES

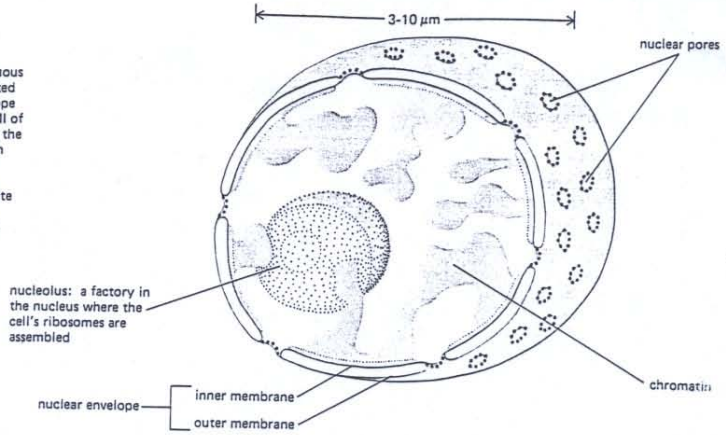
membrane-bounded vesicles that contain hydrolytic enzymes involved in intracellular digestions


## PEROXISOMES

membrane-bounded vesicles containing oxidative enzymes that generate and destroy hydrogen peroxide

## NUCLEUS

The nucleus is the most conspicuous organelle in the cell. It is separated from the cytoplasm by an envelope consisting of two membranes. All of the chromosomal DNA is held in the nucleus, packaged into chromatin fibers by its association with an equal mass of histone proteins. The nuclear contents communicate with the cytosol by means of openings in the nuclear envelope called nuclear pores.



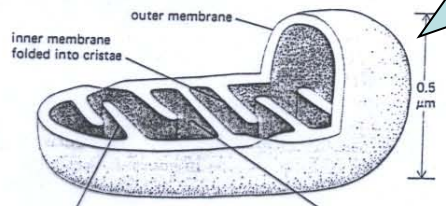

## CYTOSKELETON

In the cytosol, arrays of protein filaments form networks that give the cell its shape and provide a basis for its movements. In animal cells the cytoskeleton is often organized from an area near the nucleus that contains the cell's pair of centrioles. Three main kinds of cytoskeletal filaments are:

1. microtubules — 25-nm diameter
2. actin filaments — 8-nm diameter
3. intermediate filaments — 10-nm diameter

## MITOCHONDRIA

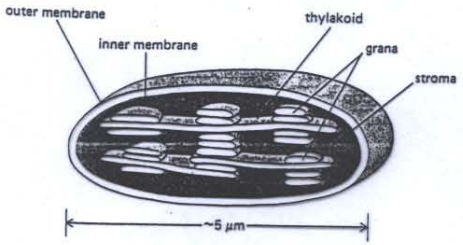
About the size of bacteria, mitochondria are the power plants of all eucaryotic cells, harnessing energy obtained by combining oxygen with food molecules to make ATP.

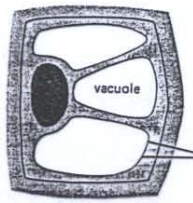



Energy production

## SPECIAL PLANT CELL ORGANELLES

**chloroplasts**—These chlorophyll-containing plastids are double-membrane-bounded organelles found in all higher plants. An elaborate membrane system in the interior of the chloroplast contains the photosynthetic apparatus.




vacuole—A very large single-membrane-bounded vesicle occupying up to 90% of the cell volume, the vacuole functions in space-filling and also in intracellular digestion.

vacuole membrane (tonoplast)

cell wall—Plant cells are surrounded by a rigid wall composed of tough fibrils of cellulose laid down in a matrix of other polysaccharides.

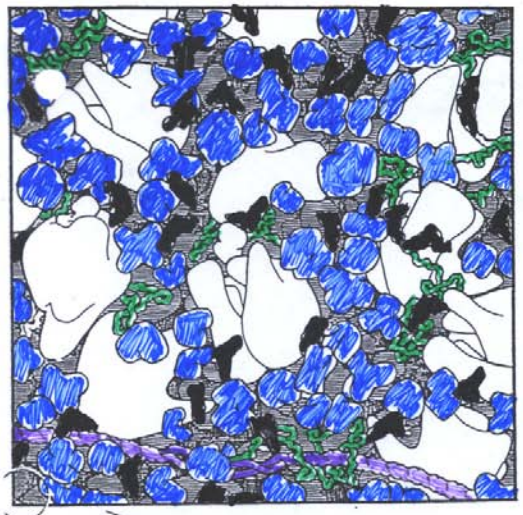
# Cytoplasm components

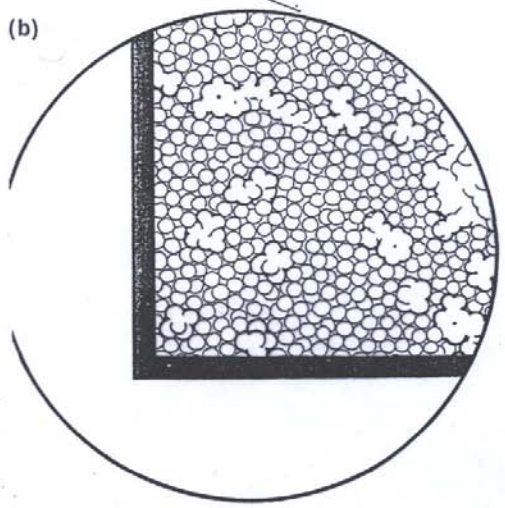
1. Structural proteins –

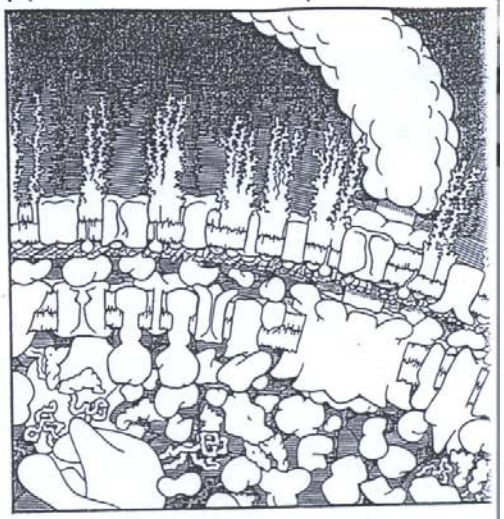
   microfilaments (actin), microtubules (tubulin) and intermediate filaments (diverse)
2. Ribosomes – site of protein synthesis
3. Mitochodria – site of energy production
4. Golgi apparatus – stacked membrane protein packaging assembly
5. Other small organelles + small molecules + proteins

100 $nm^3$ 600 of these in E coli









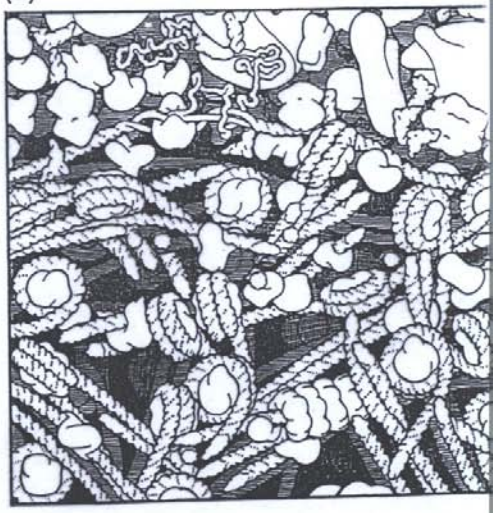
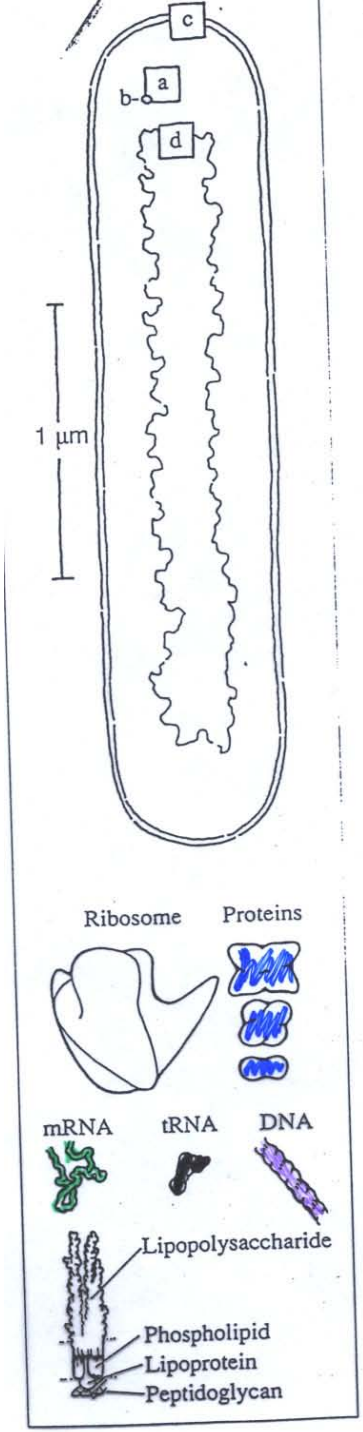


**Figure 1**

we ... d magnify a cell one million times, making molecules the size of every-
y objects, what would we see? Three portions of a typical *E. coli* cell are mag-
fied one million times. A schematic of the cell at 50 000 times magnification
ows the location and size of each 100 nm window with respect to the whole
ll and the key identifies the macromolecular components. Although only three
examples are shown in the key, proteins come in many shapes and siz
(a) The cytoplasm, showing all macromolecular components. (b) Close-up
one portion of the cytoplasm, showing all molecules, including water (circle
small molecules (dark outlines) and a small portion of a protein. (c) The
wall, showing all macromolecular components. (d) The nuclear region, show
all macromolecular components.



# Some Central Question in Biophysics

1. What is detailed Structure?
2. Structure/Function Relations
3. Role of flexibility/ motions
4. Structural Motifs – calculate possible numbers
5. Protein folding problem
6. Effect of single-site specific genetic changes
7. Ligand-macromolecule interactions
8. Regulation/Control of Structure/Function processes

# Key areas of Current Study

1. Structure/Function relations in proteins/DNA/complexes
2. Membranes + Channels
3. Motor proteins/molecular machines
4. Photo-biophysics
5. Imaging/Microscopy/New techniques

# Sample Preparation Overview

A. Organisms

   1. Bacteria – most studied; easy, fast to grow large quantities; genetic engineering

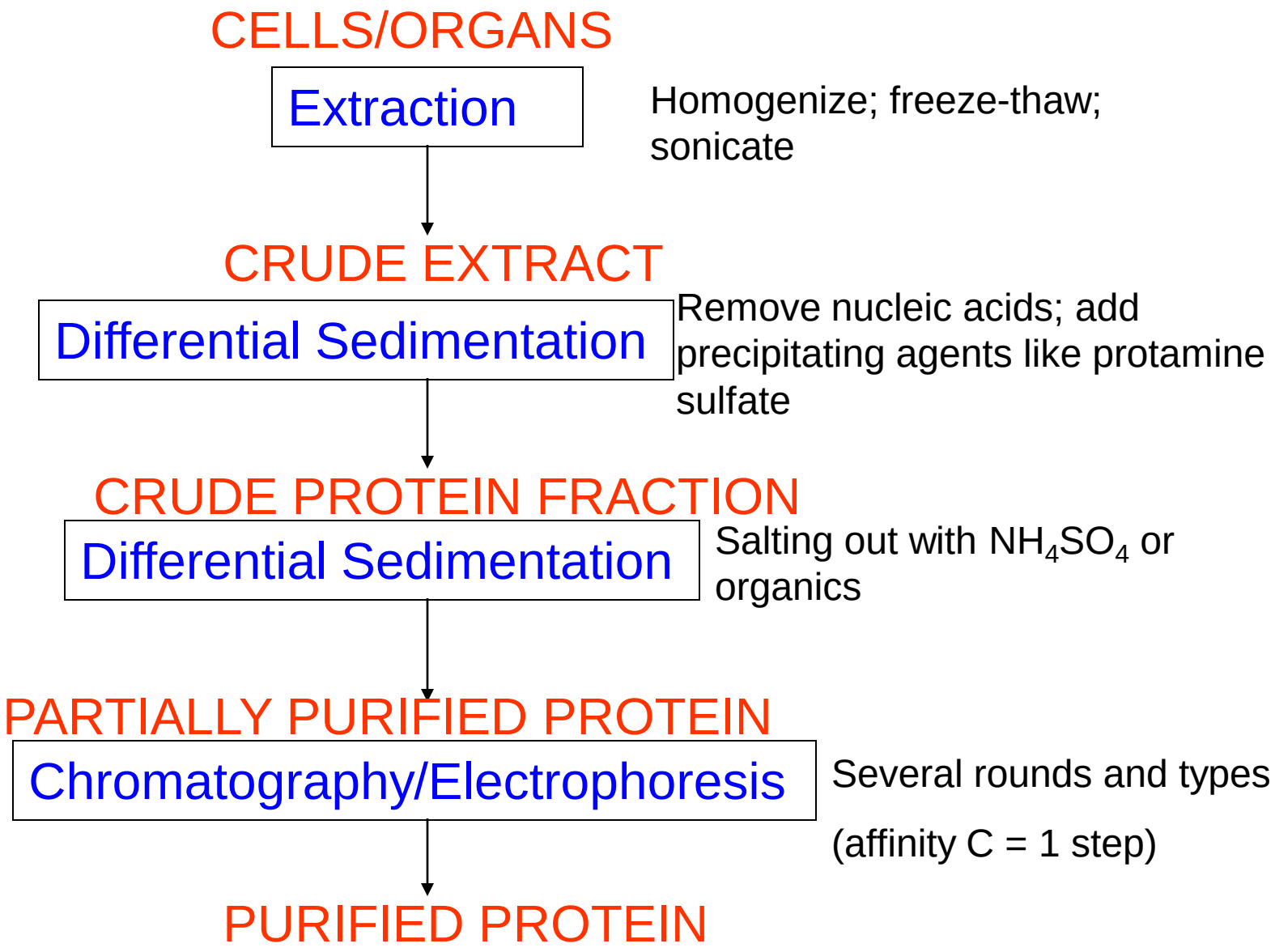
   2. Complex – mammalian whole body, organ (heart, kidneys), medical applications

B. Components

   1. Cells – tissue culture – stem cell lines!; motility, growth, communication

   2. Isolated macromolecules – typical prep

# Typical Protein Prep



# Tests of Purity

A. For purity of Protein:
   1. Does it crystallize?
   2. Analytical ultracentrifuagation
   3. SDS gel electrophoresis
   4. Can specific activity (if an enzyme) be increased?

B. For purity of “Form”
   1. Analytical ultracentrifugation
   2. Light scattering
   3. EM
   4. Others

Precautions: Keep Cold, Work Fast, Beware of Surface Denaturation

# How do we learn about DNA/Proteins?

1. Look at them – EM, laser manipulation
2. Watch them move – light & hydrodynamic methods
3. Measure a signal from them – NMR, ESR, Fluorescence, …
4. Attach a label to them and monitor a signal

   Fluorescence,…
5. “Look” at them with x-rays when a crystal is available