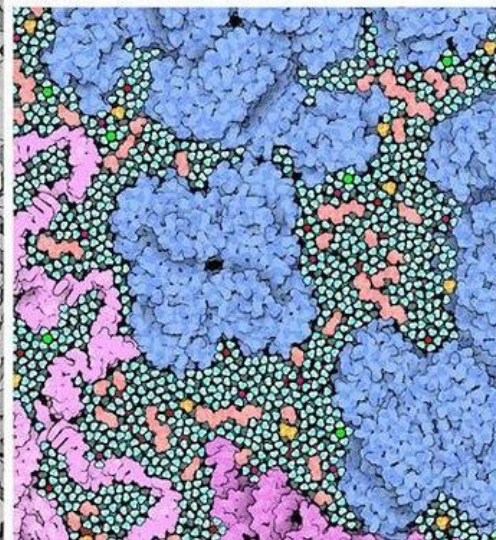ME 498 / ME 599

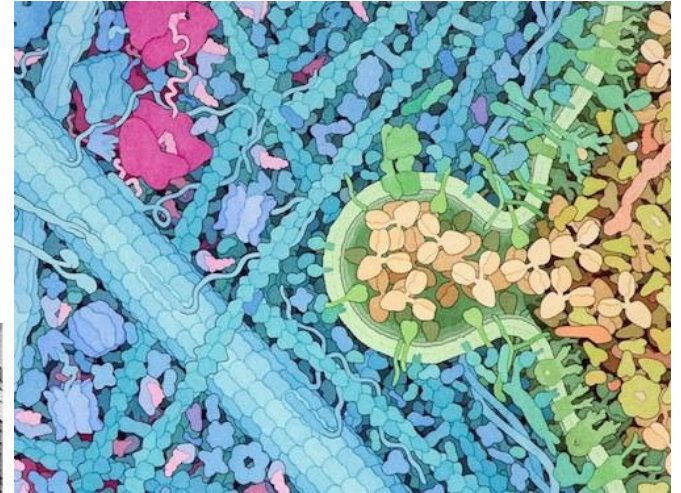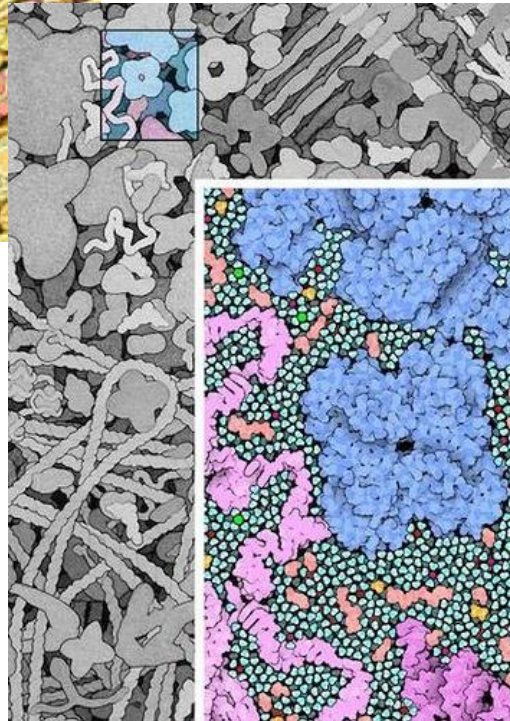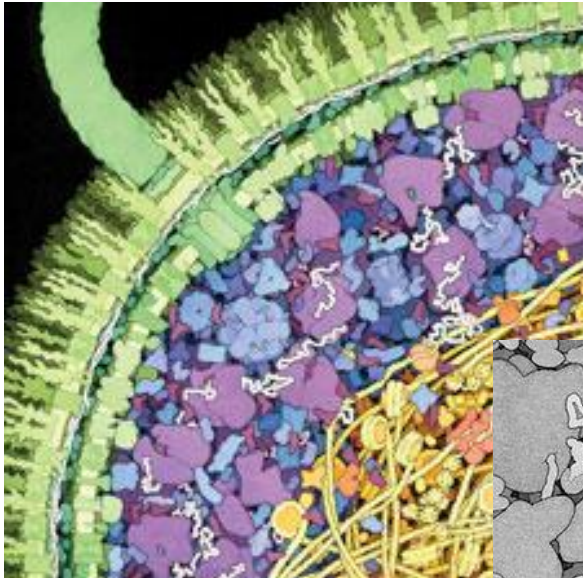# Biological Frameworks for Engineers

Class Organization

- Session 9 – Immunology
  - Shirin Feghhi

- Lab 2 – Lab-on-a-Chip
  - Friday 2:30-4pm
  - More 320

ME 498 / ME 599

Decoding Proteins and Protein Functions

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A cell is a crowded place…

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Protein Purification

(a) Differential centrifugation
1 Sample is poured into tube
- Larger particle
- Smaller particle

2 Centrifuge **Particles settle according to mass**
Centrifugal force →

3 Stop centrifuge **Decant liquid into container**
Supernatant — Pellet

(b) Rate-zonal centrifugation
1 Sample is layered on top of density gradient
- Larger particle
- Smaller particle

Sucrose gradient
Low density (low sucrose concentration)
High density (high sucrose concentration)

2 Centrifuge **Particles settle according to mass**
Centrifugal force →

3 Stop centrifuge **Collect fractions and do assay**

Decreasing mass of particles

Figure 3-34
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Gel Electrophoresis

- Separation by charge-to-mass ratio
- Small proteins migrate faster than large ones
- SDS-PAGE
  - <u>S</u>odium <u>d</u>odecyl<u>s</u>ulfate
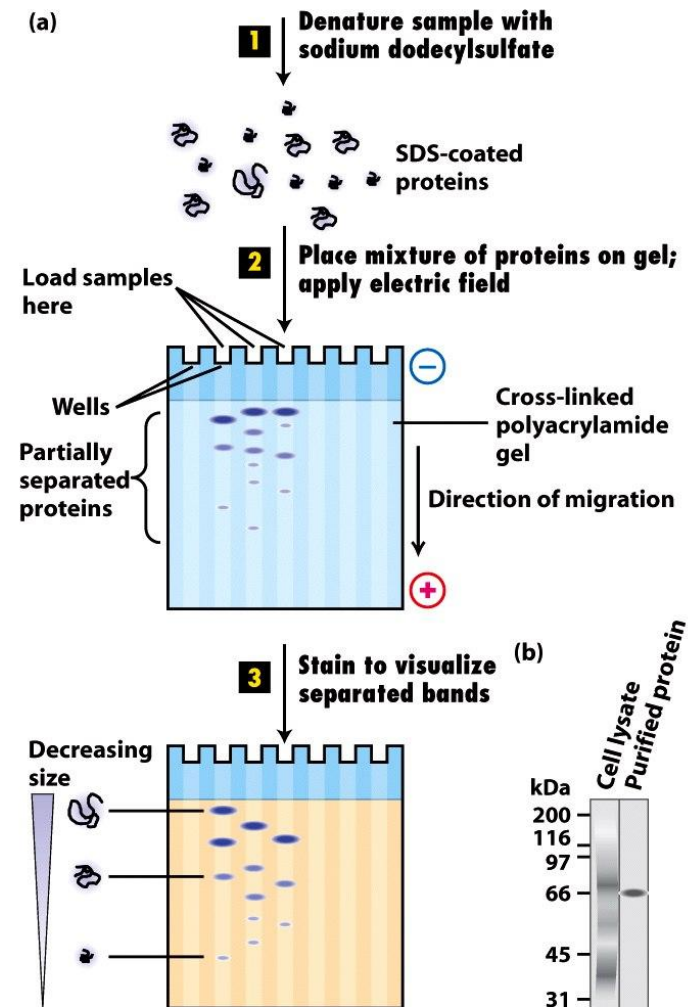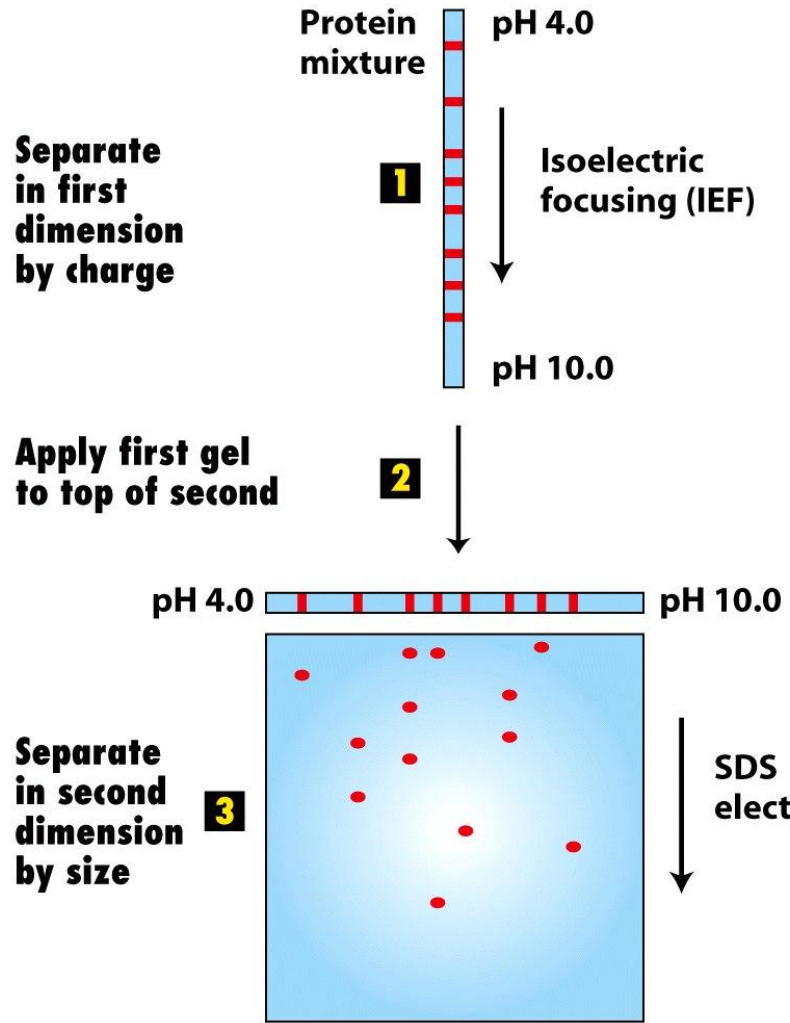 - <u>P</u>oly<u>a</u>crylamide <u>G</u>el <u>E</u>lectrophoresis

(a)

1 Denature sample with sodium dodecylsulfate

SDS-coated proteins

Load samples here

2 Place mixture of proteins on gel; apply electric field

Wells

Partially separated proteins

Cross-linked polyacrylamide gel

Direction of migration

3 Stain to visualize separated bands

(b)

Decreasing size

Cell lysate Purified protein

kDa		
200		
116		
97		
66		
45		
31		

Figure 3-35
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2-D Gel Electrophoresis

Protein mixture

pH 4.0

1 Separate in first dimension by charge

Isoelectric focusing (IEF)

pH 10.0

2 Apply first gel to top of second

pH 4.0 — pH 10.0

3 Separate in second dimension by size

SDS electrophoresis

Figure 3-36a
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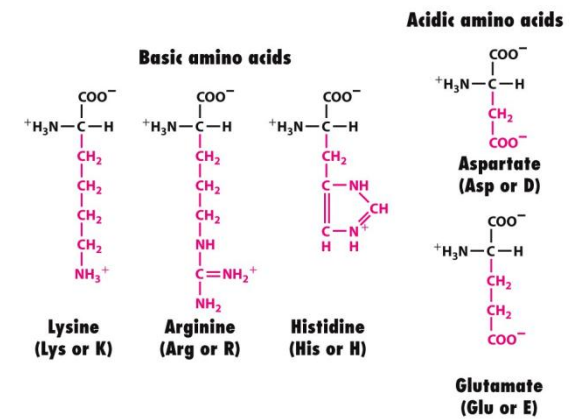© 2008 W. H. Freeman and Company

HYDROPHILIC AMINO ACIDS

Basic amino acids

Acidic amino acids

Lysine (Lys or K)

Arginine (Arg or R)

Histidine (His or H)

Aspartate (Asp or D)

Glutamate (Glu or E)

Figure 2-14 part 2
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Isoelectric focusing (**1**) →

Molecular weight $\times 10^{-3}$

SDS electrophoresis (**3**)

66

43

30

16

4.2   5.9   7.4

pI

TY OF
GTON
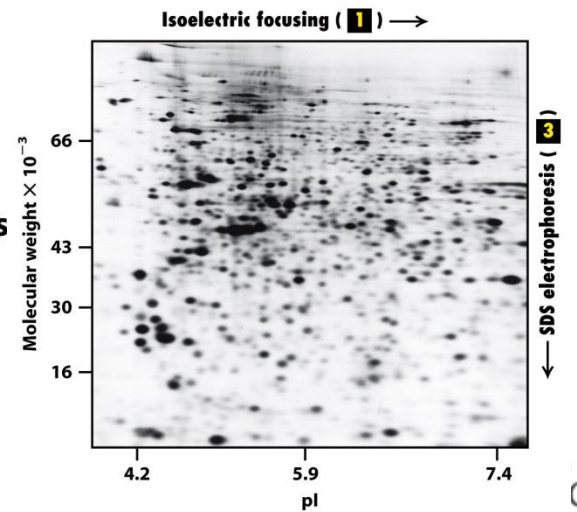
Figure 3-36b
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Chromatography
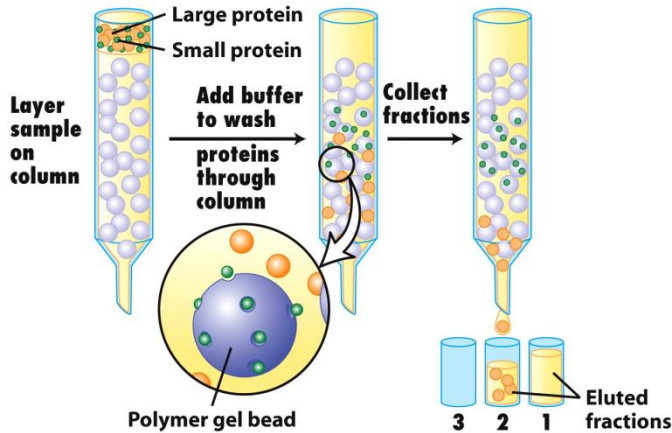
Western Blot

1 **Electrophoresis and transfer**

Electric current

SDS-polyacrylamide gel

Membrane

2 **Antibody detection**

Incubate with Ab_1 (Y); wash excess

3

Incubate with enzyme-linked Ab_2 (Y); wash excess

4 **Chromogenic detection**

React with substrate for Ab_2-linked enzyme
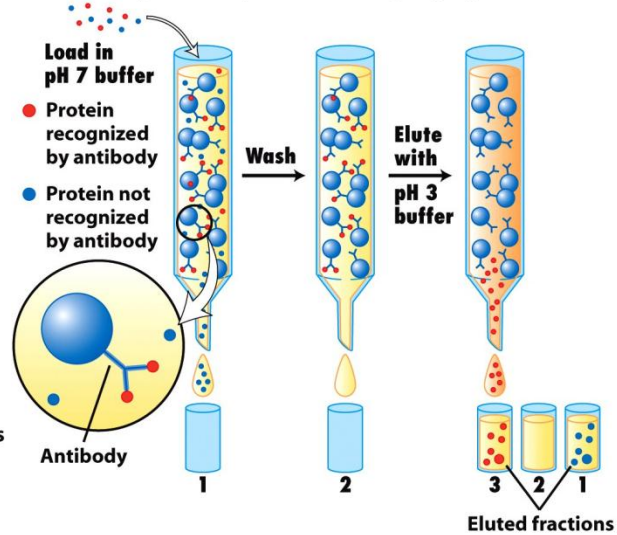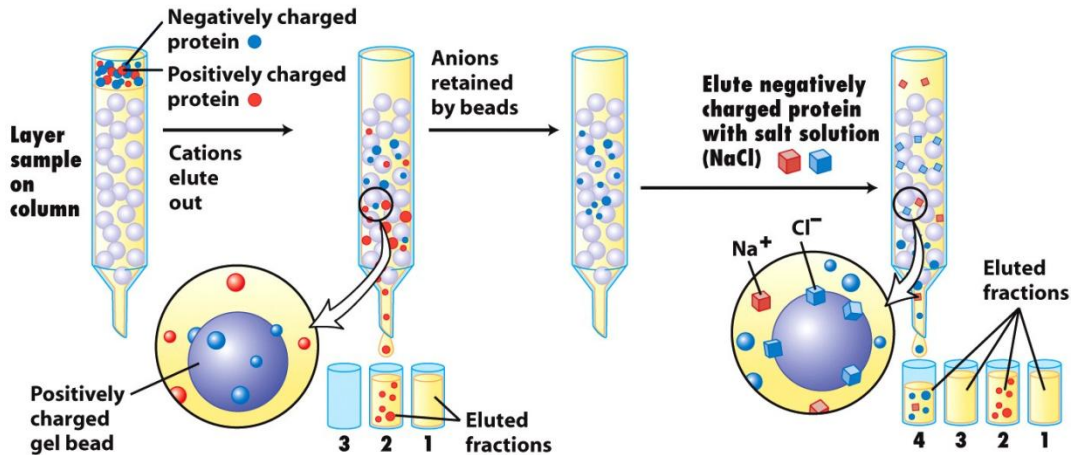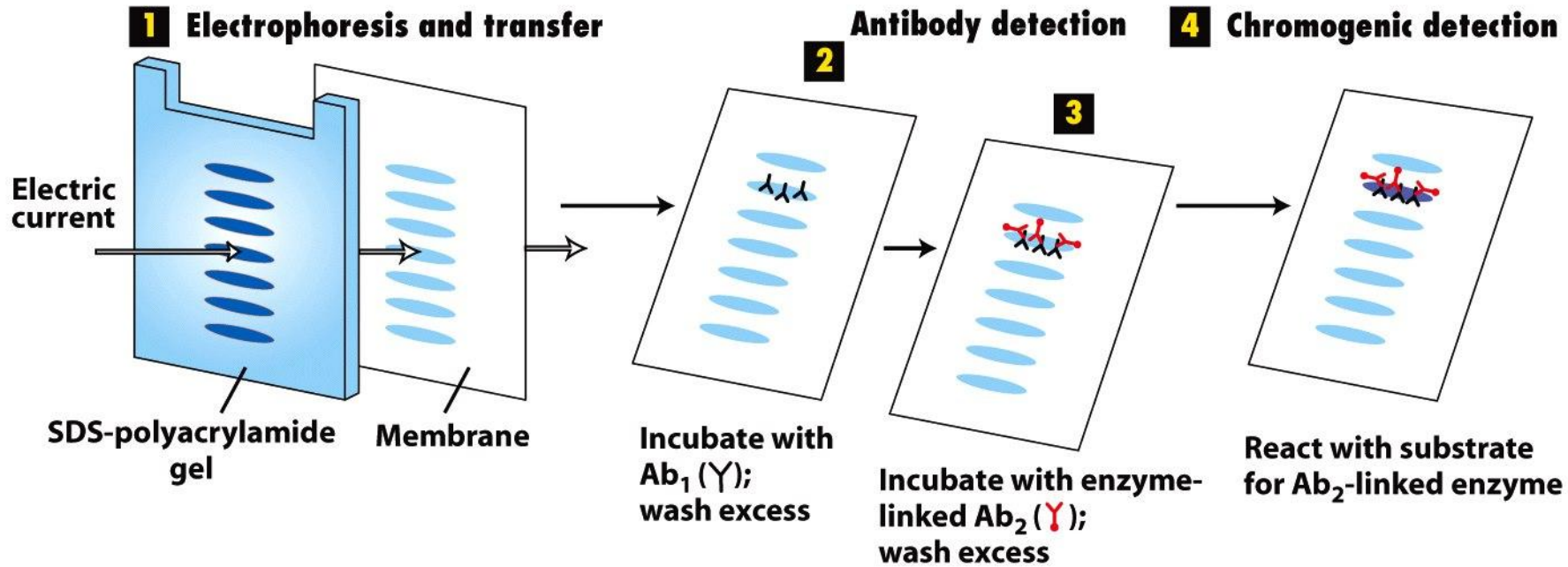
Figure 3-38
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Mass Spectrometry

Electrospray needle

3–5 kV

Liquid →

Atmosphere

Vacuum

Mass analyzer   **Detector**

Mass spectrometer

Droplets containing solvated ions   **Ions**

Electrospray ionization
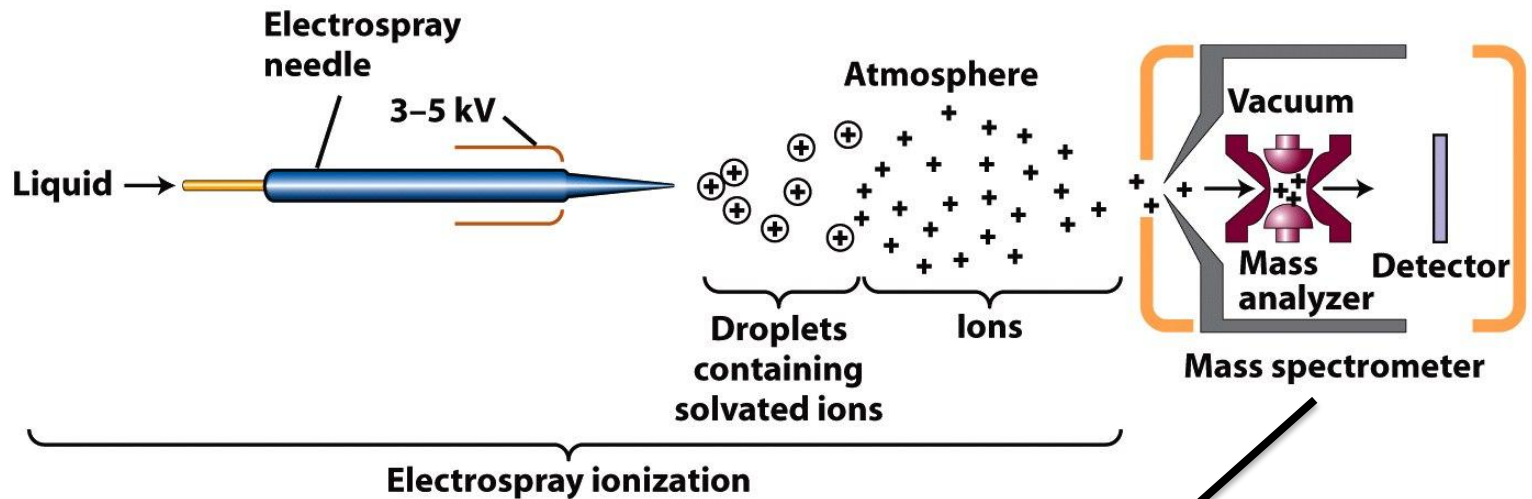
Figure 3-41a
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MS/MS of m/z 836.47

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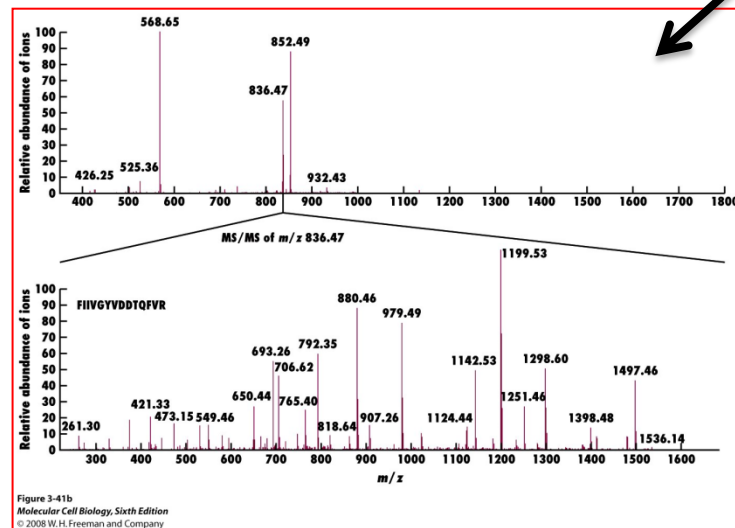
Figure 3-41b
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All together now…
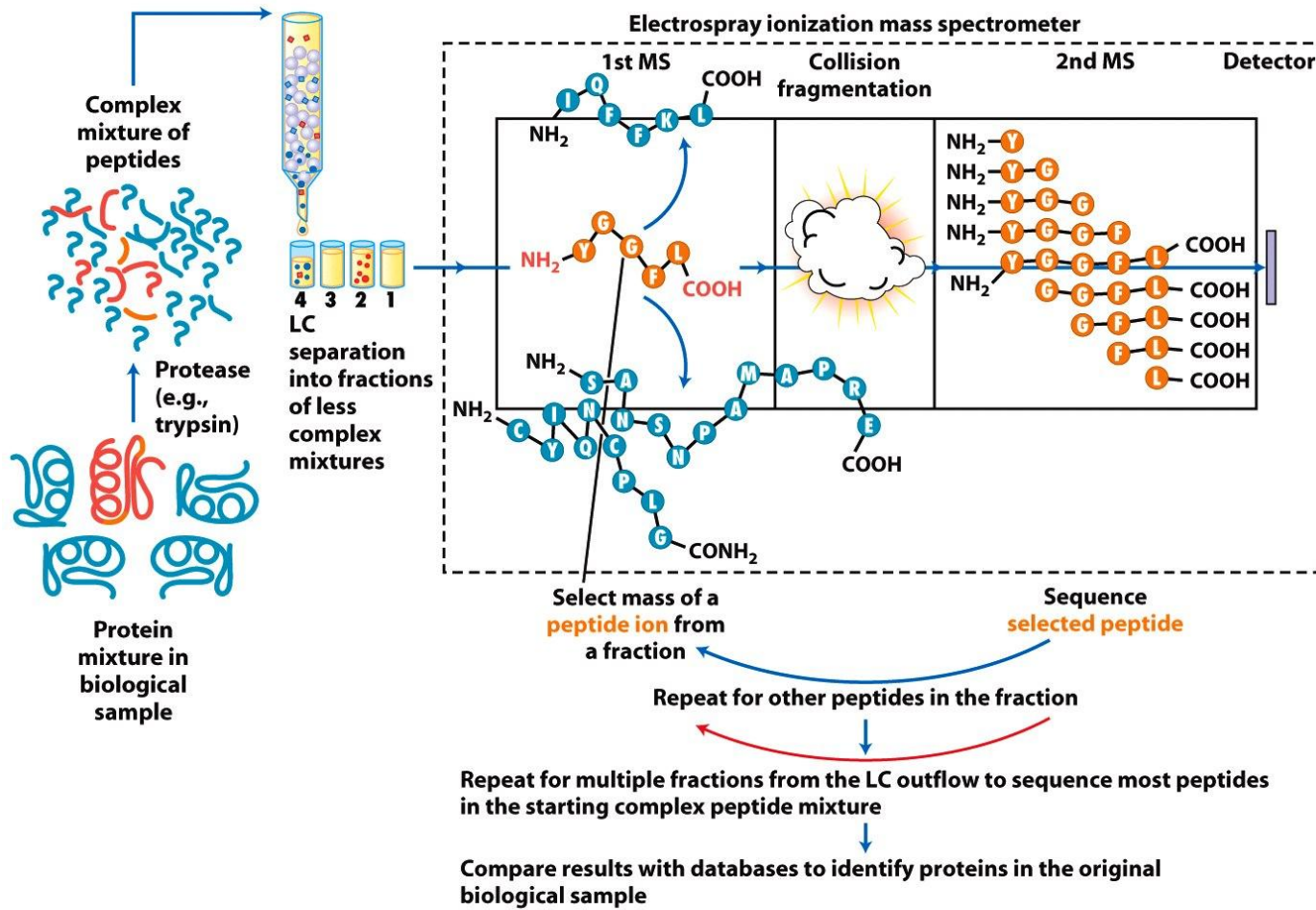
Figure 3-43
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X-Ray Crystalography
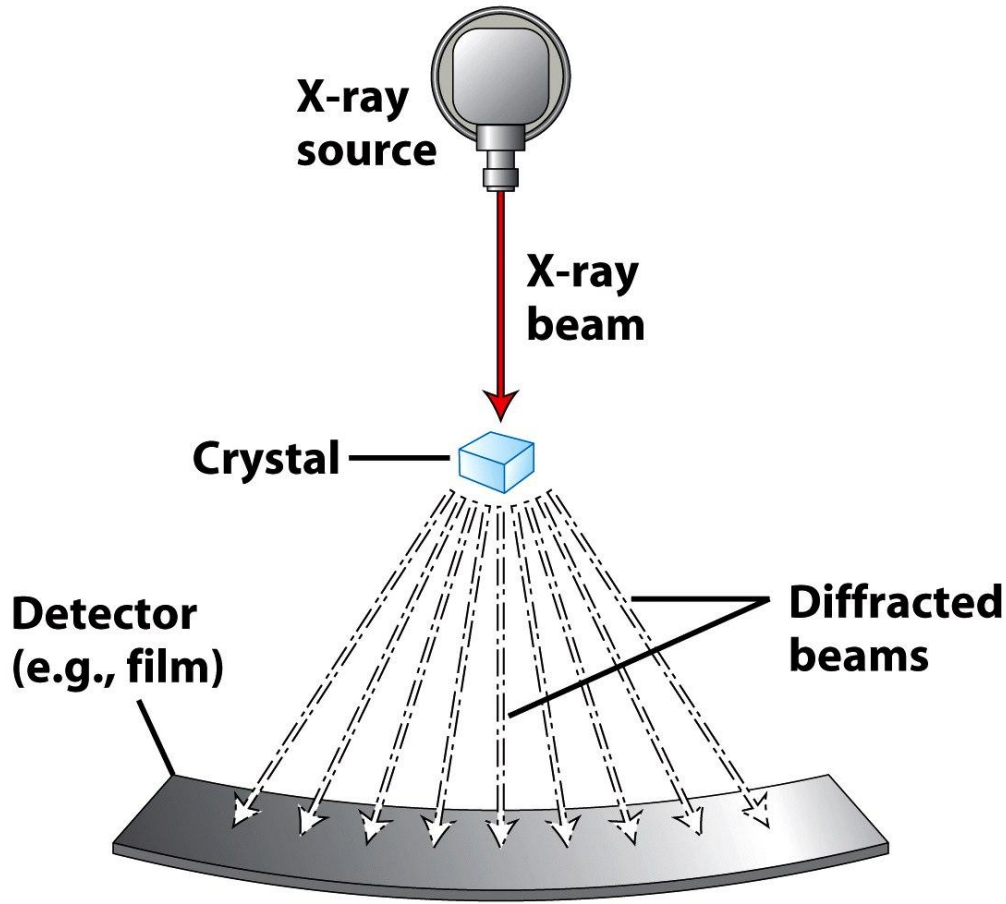
Figure 3-42a
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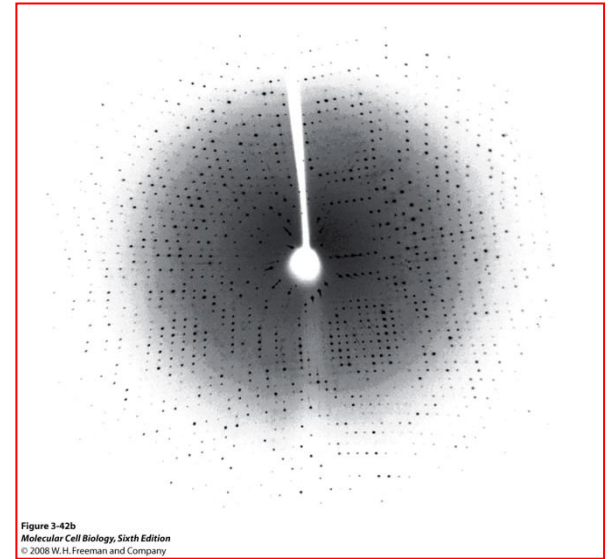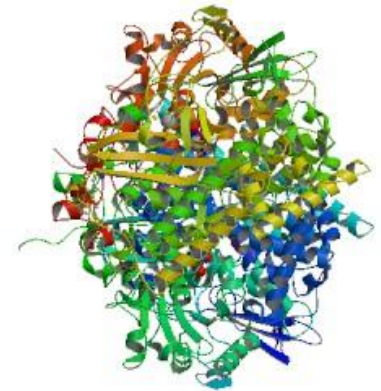© 2008 W. H. Freeman and Company

Figure 3-42b
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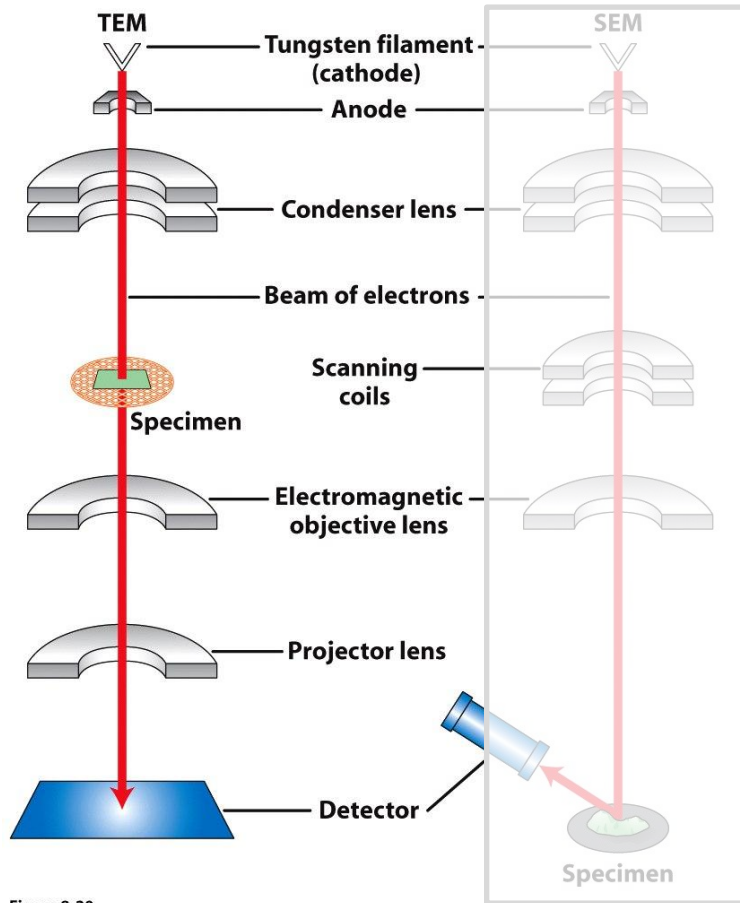© 2008 W.H. Freeman and Company

Cryo-EM

Figure 9-20
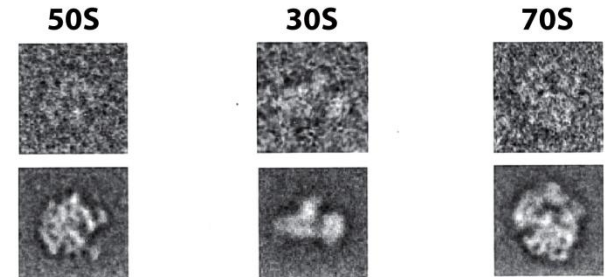*Molecular Cell Biology, Sixth Edition*
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50S   30S   70S

Figure 4-26a
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Figure 4-26b
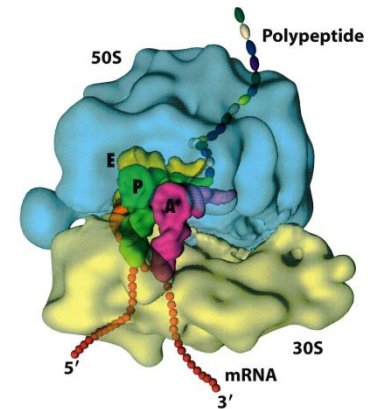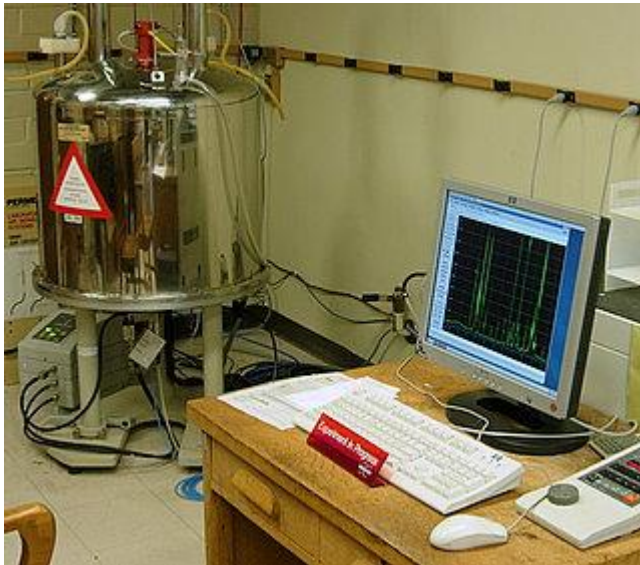*Molecular Cell Biology, Sixth Edition*
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E. coli Ribosome

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NMR Spectroscopy

David Baker, Howard Hughes Institute, University of Washington.

Decoding proteins

TeraGrid 2007
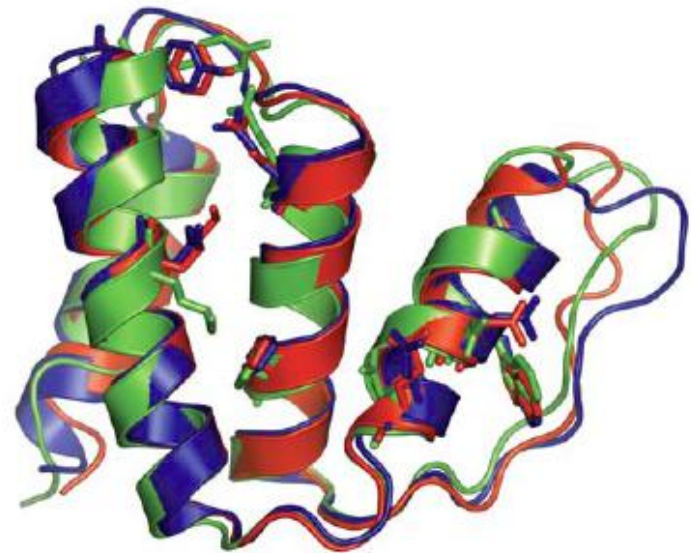
Protein structure prediction by the Rosetta code, showing the unknown structure (blue), the X-ray structure (red, unknown when the prediction was calculated), and a low-resolution NMR structure (green).

Image courtesy of Ross Walker, SDSC, and Srivatsan Raman, University of Washington.

Protein Function

- Pharmacological inhibitors
- Recombinant DNA
- Knock-out mice
- RNA Interference

Questions ?