

ME 411 / ME 511

Biological Frameworks for Engineers

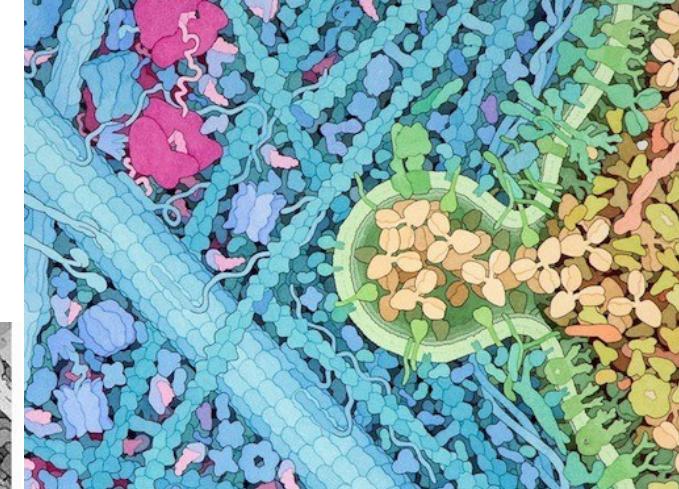
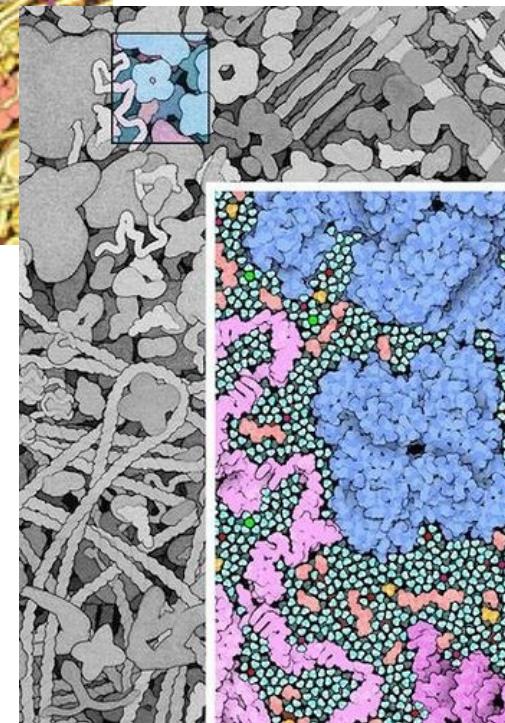
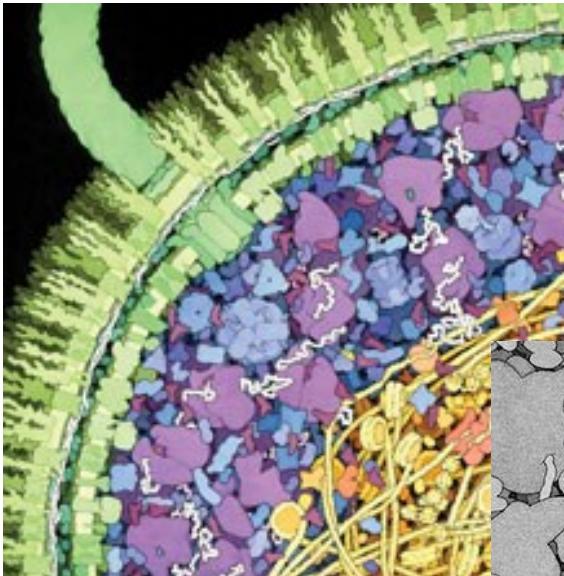
Class Organization

- Lab 1 worksheet due Mon, 10/15
- Lab 2 – Lab-on-a-Chip
 - Wed, More 320
 - Sign up for 1:30, 2:30, 3:30 slots
 - Max 12 people per slot

ME 411 / ME 511

Decoding Proteins and Protein Functions

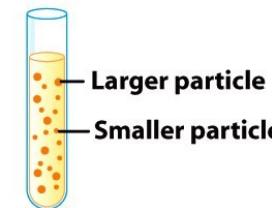
A cell is a crowded place...



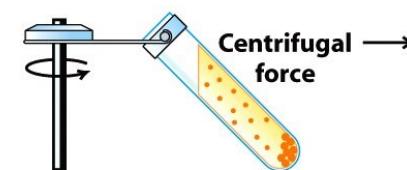
Protein Purification

(a) Differential centrifugation

1 Sample is poured into tube



2 Centrifuge
Particles settle according to mass



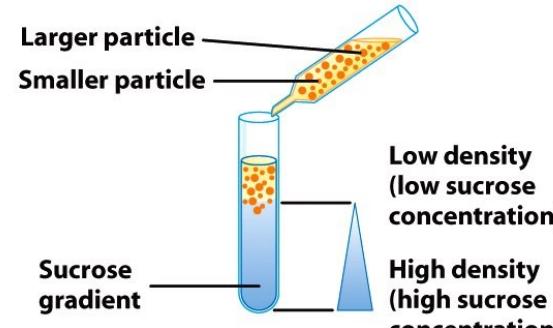
3 Stop centrifuge
Decant liquid into container

Supernatant

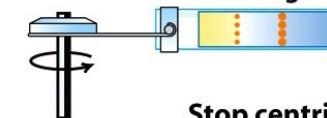
Pellet

(b) Rate-zonal centrifugation

1 Sample is layered on top of density gradient



2 Centrifuge
Particles settle according to mass
Centrifugal force →



3 Stop centrifuge
Collect fractions and do assay

Decreasing mass of particles

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Gel Electrophoresis

- Separation by charge-to-mass ratio
- Small proteins migrate faster than large ones
- SDS-PAGE
 - Sodium dodecylsulfate
 - Polyacrylamide Gel Electrophoresis

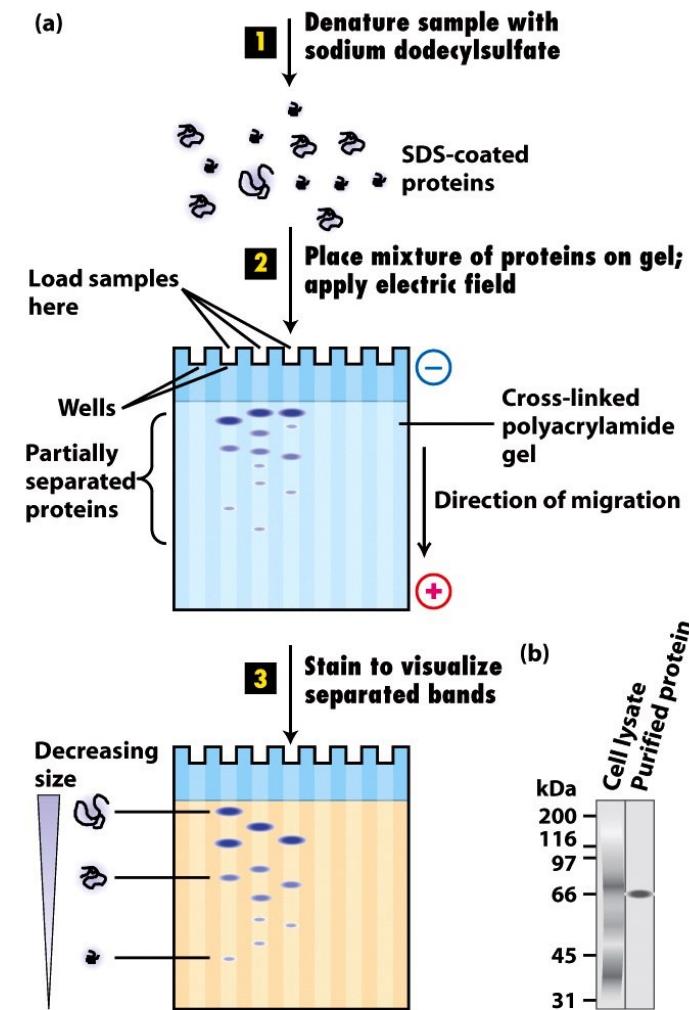


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2-D Gel Electrophoresis

Separate in first dimension by charge

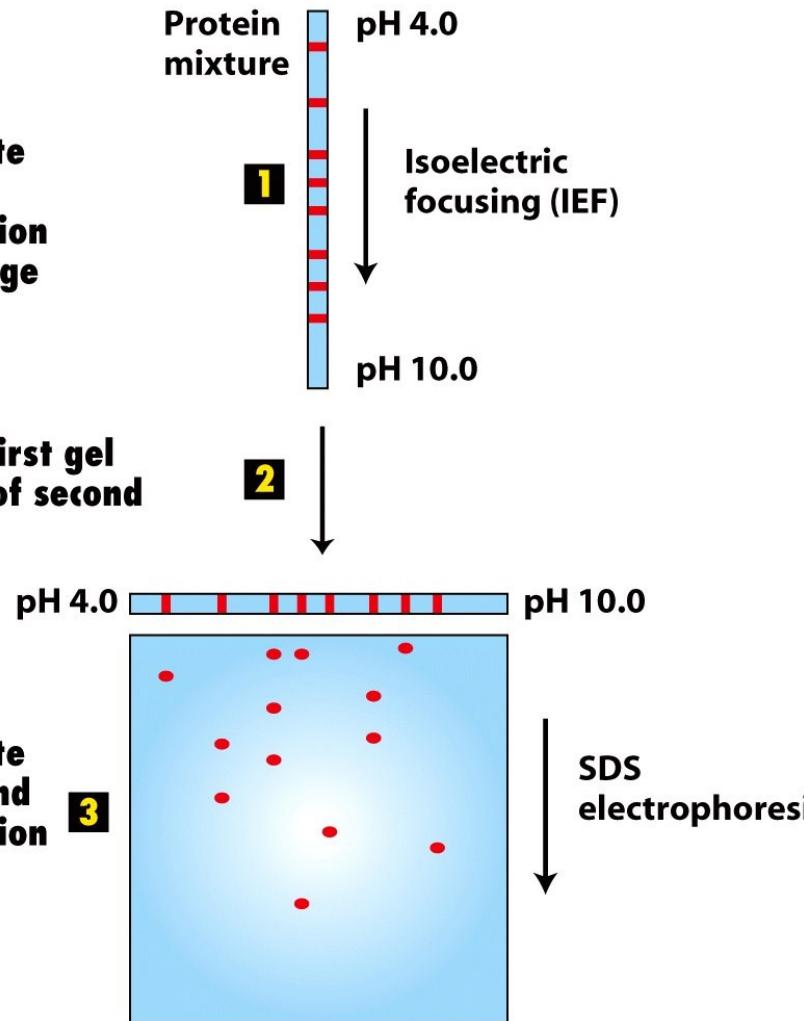
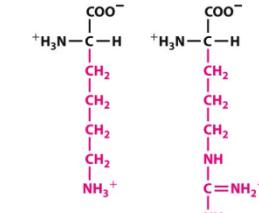


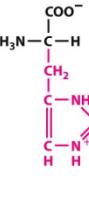
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HYDROPHILIC AMINO ACIDS

Basic amino acids

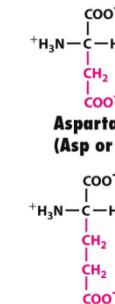


Lysine (Lys or K)

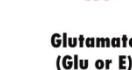


Arginine (Arg or R)

Acidic amino acids



Aspartate (Asp or D)



Glutamate (Glu or E)

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

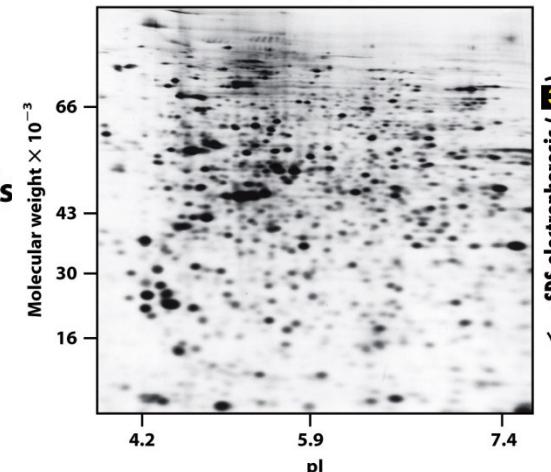


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Chromatography

Gel filtration chromatography

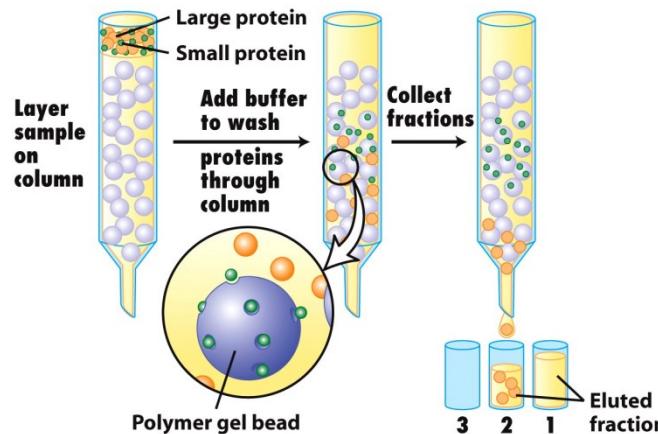
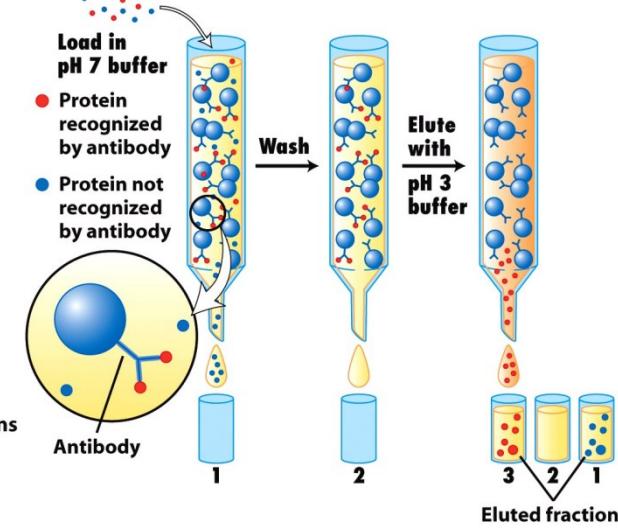


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Antibody-affinity chromatography



Ion-exchange chromatography

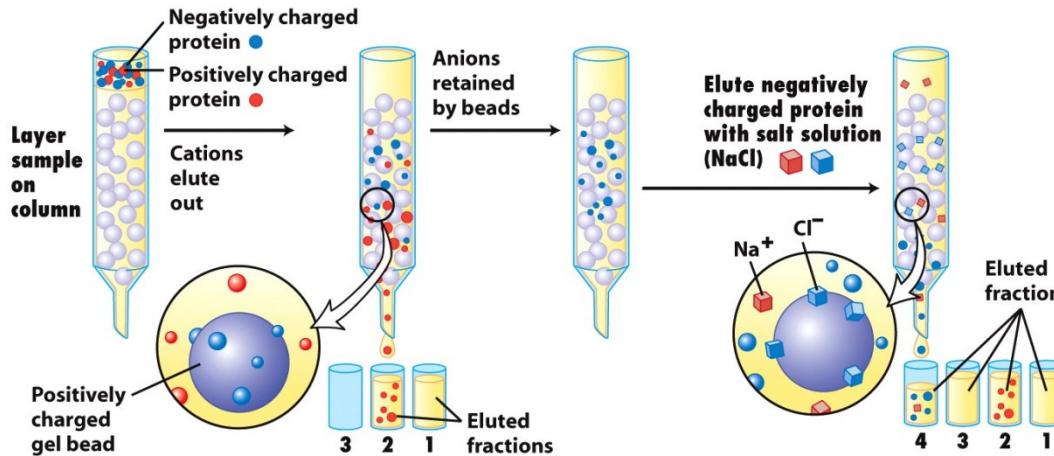


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Western Blot

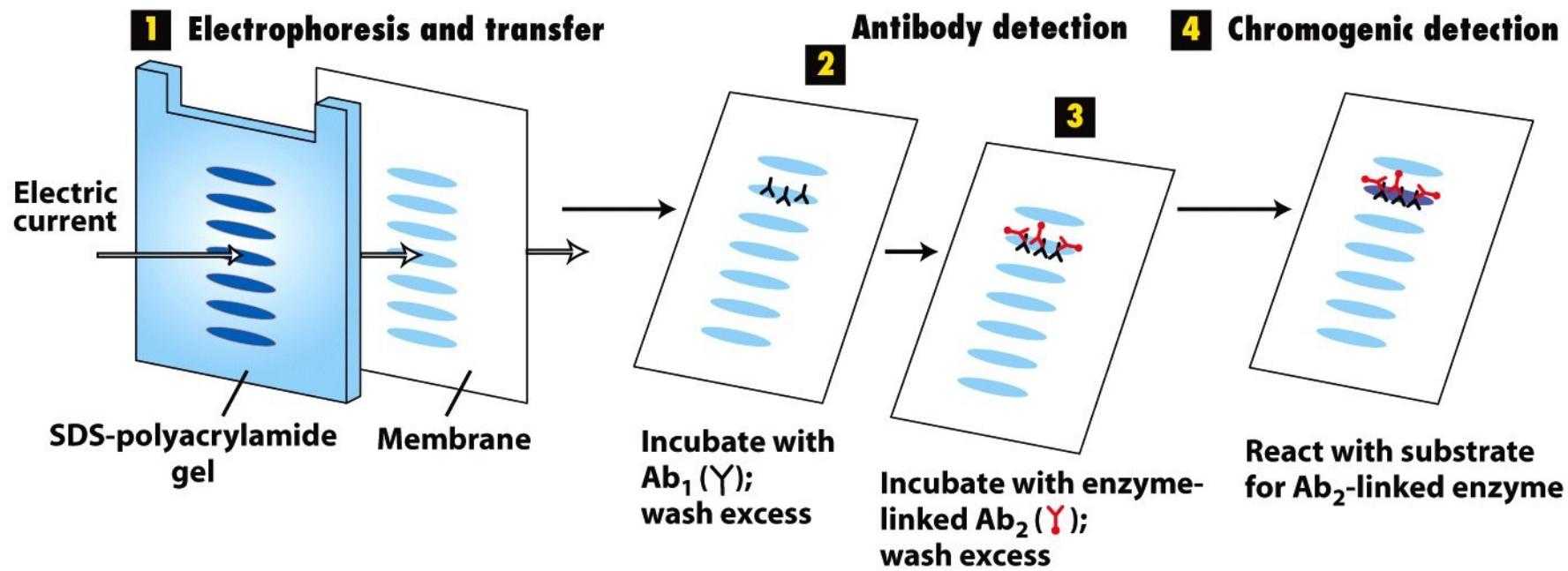


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Mass Spectrometry

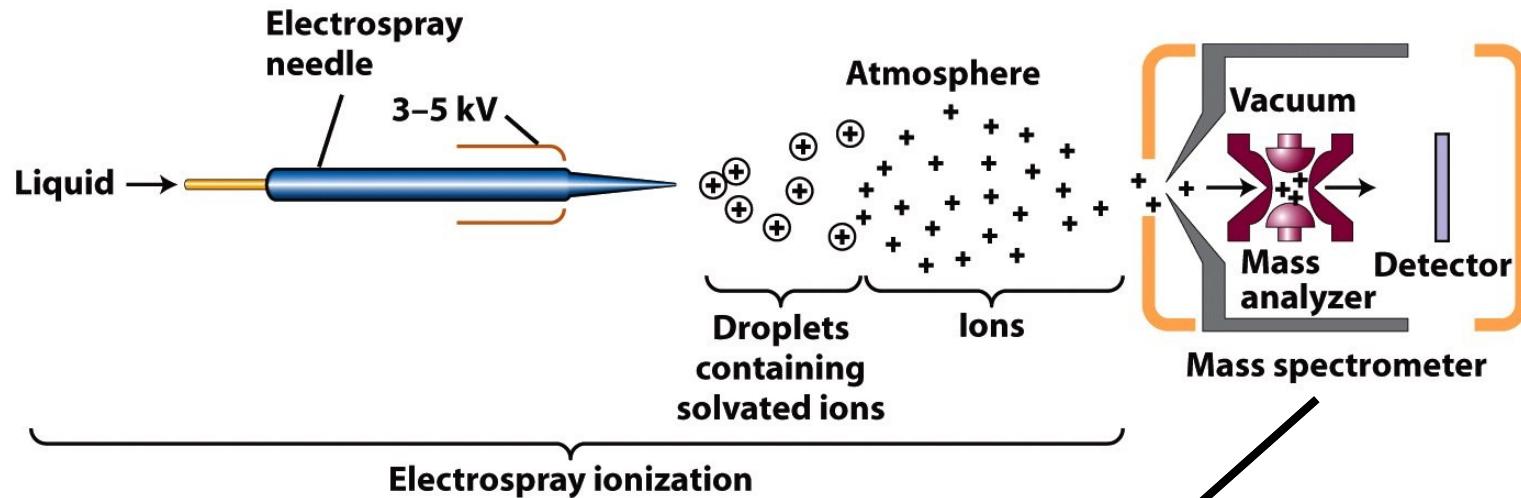
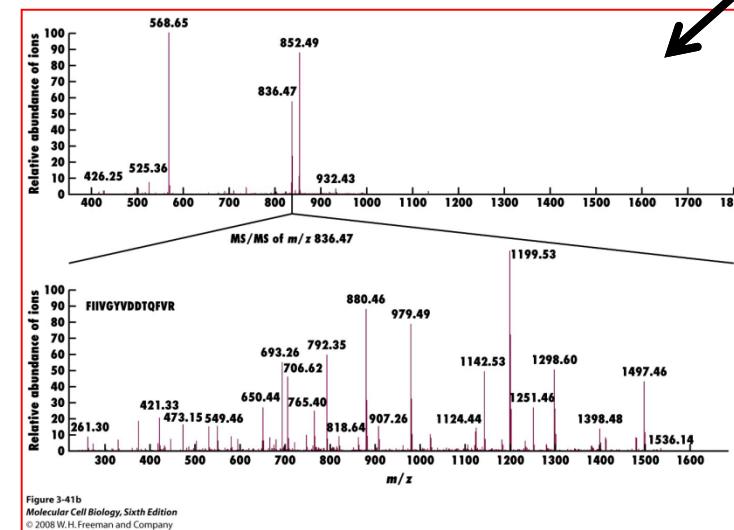


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All together now...

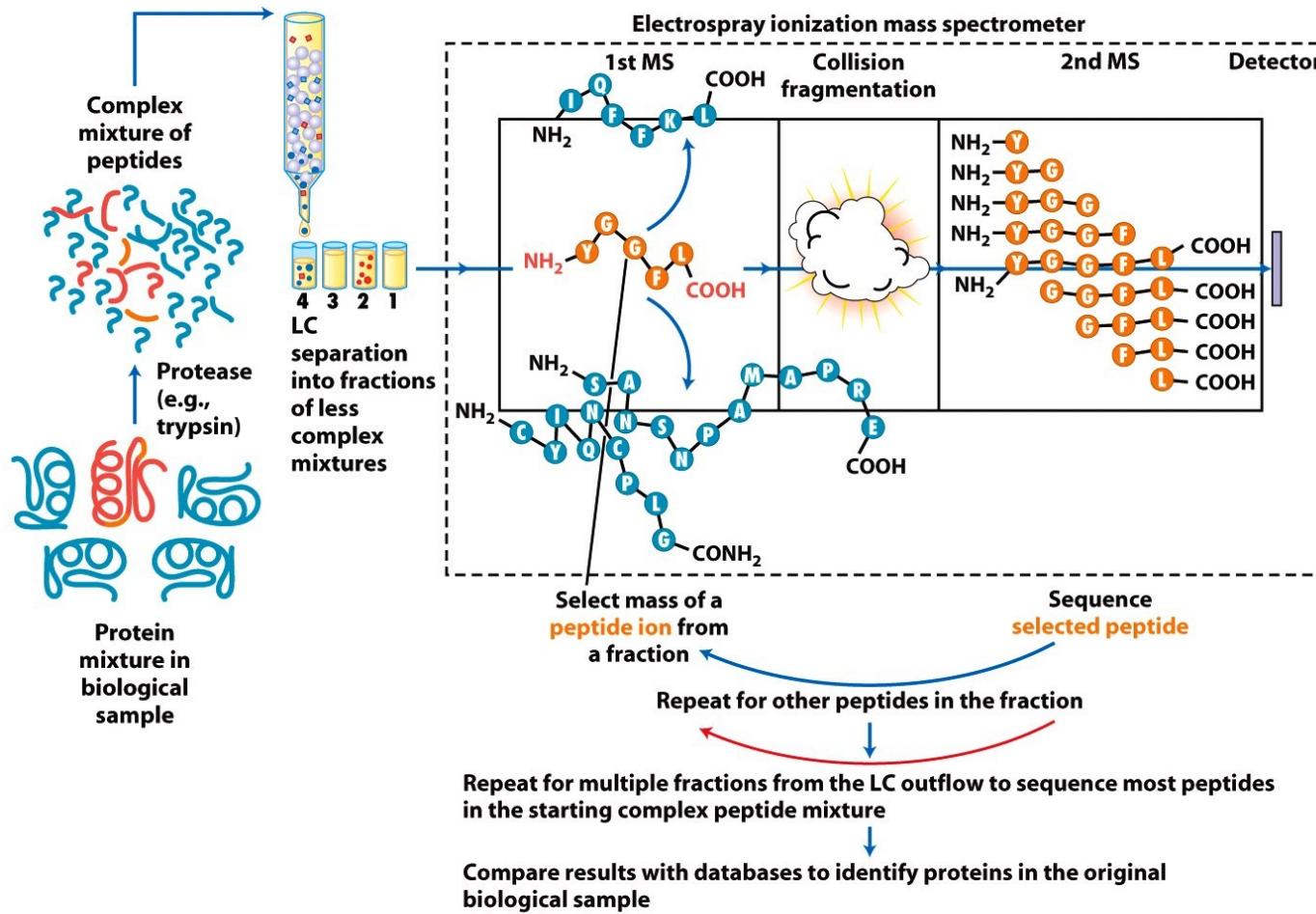


Figure 3-43

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X-Ray Crystallography

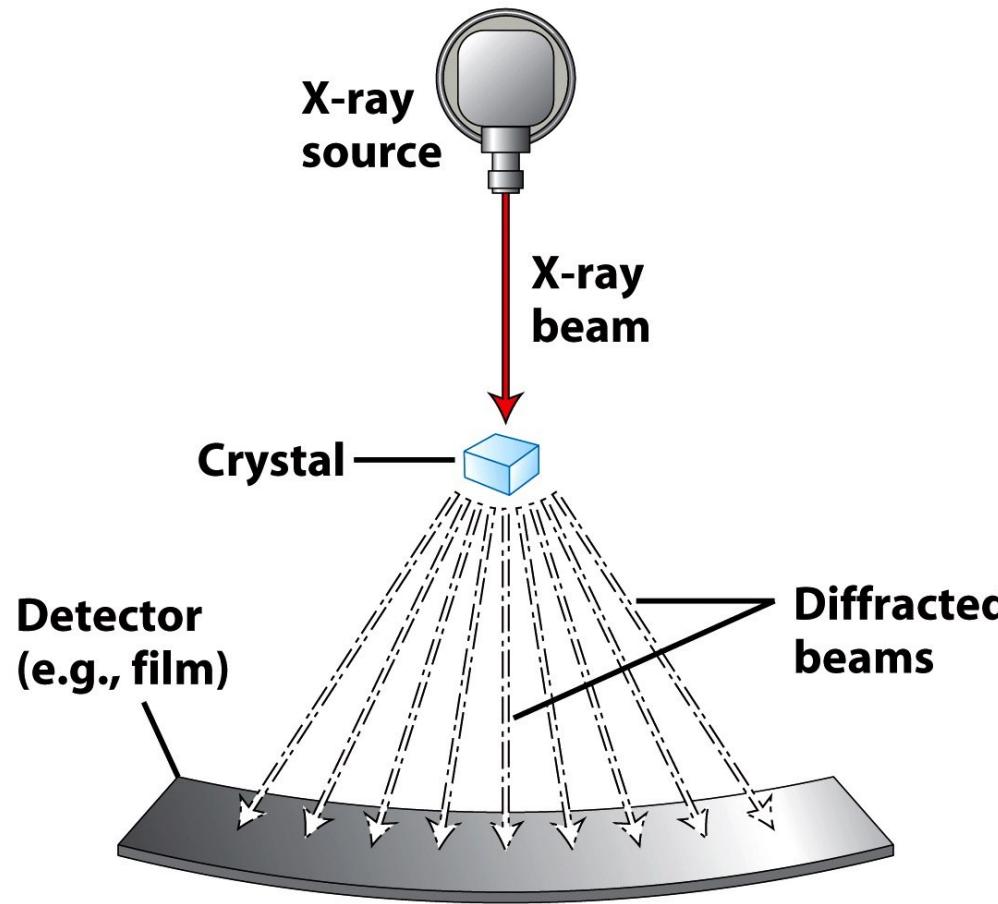


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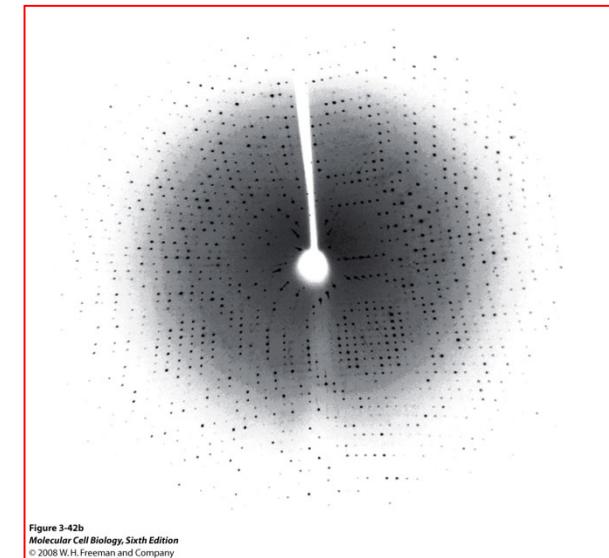
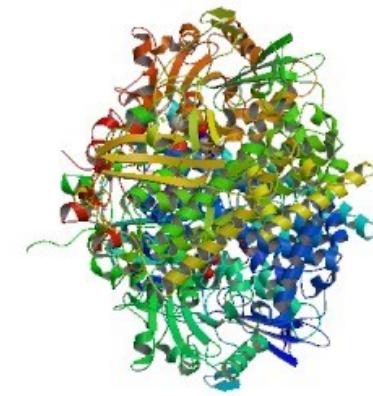


Figure 3-42b
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Cryo-EM

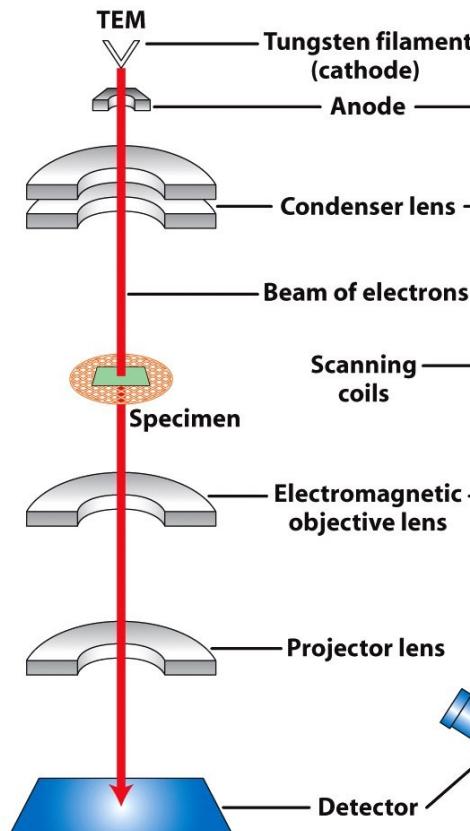


Figure 9-20
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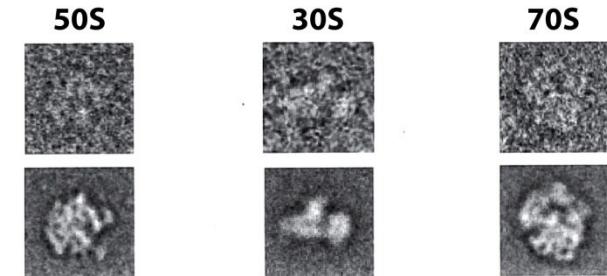


Figure 4-26a
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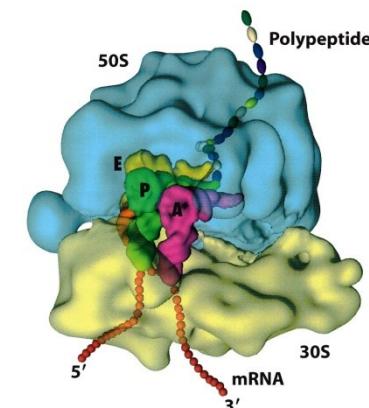


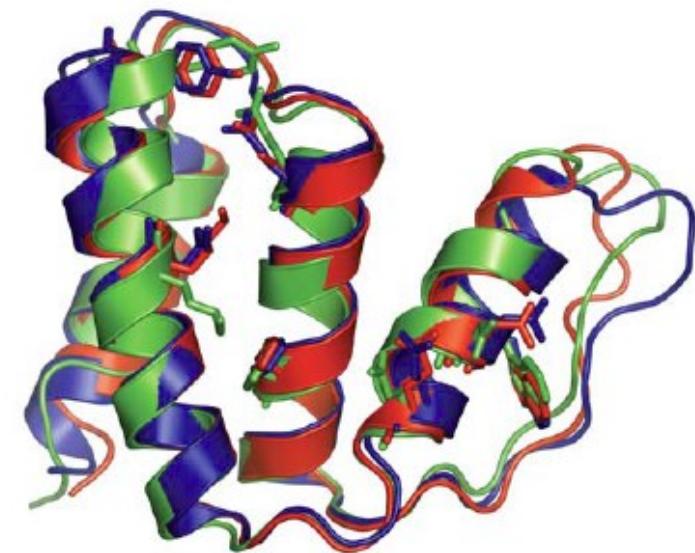
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E. coli Ribosome

NMR Spectroscopy



David Baker, Howard Hughes Institute, University of Washington.



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 ?