Advanced Cell Biology. Lecture 14

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Outline

How proteins are controlled

How proteins are studied

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How proteins are studied

Previous final question: the answer

How many amino acids are in the active site of lysozyme?

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- 2

Myosin movie

Myosin movie

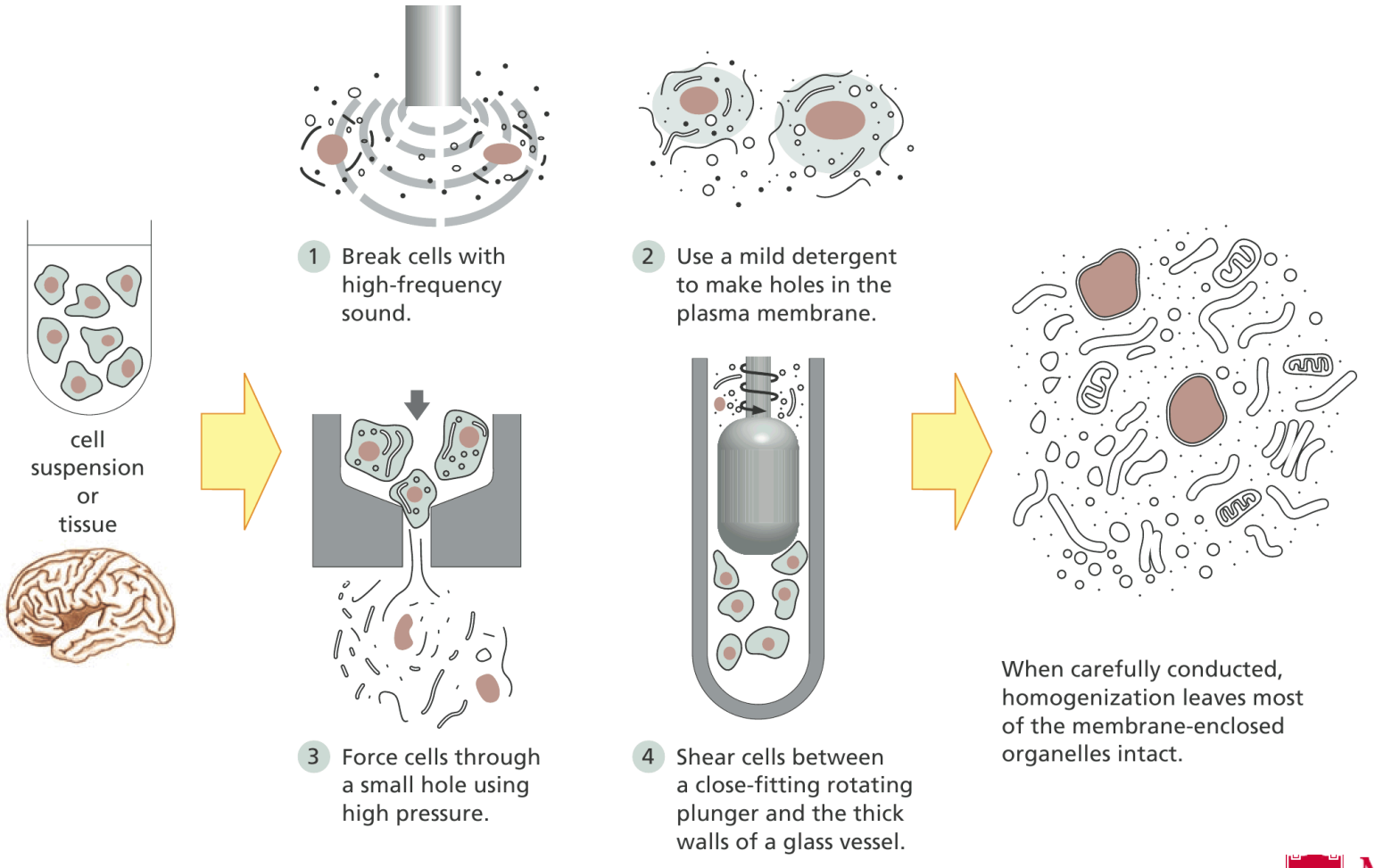
Protein machines

- Protein machine is a linked set of several proteins
- Hydrolysis of ATP or attached GTP drives conformational changes
- Covalent modifications of side chains work as a regulatory code for most protein machines

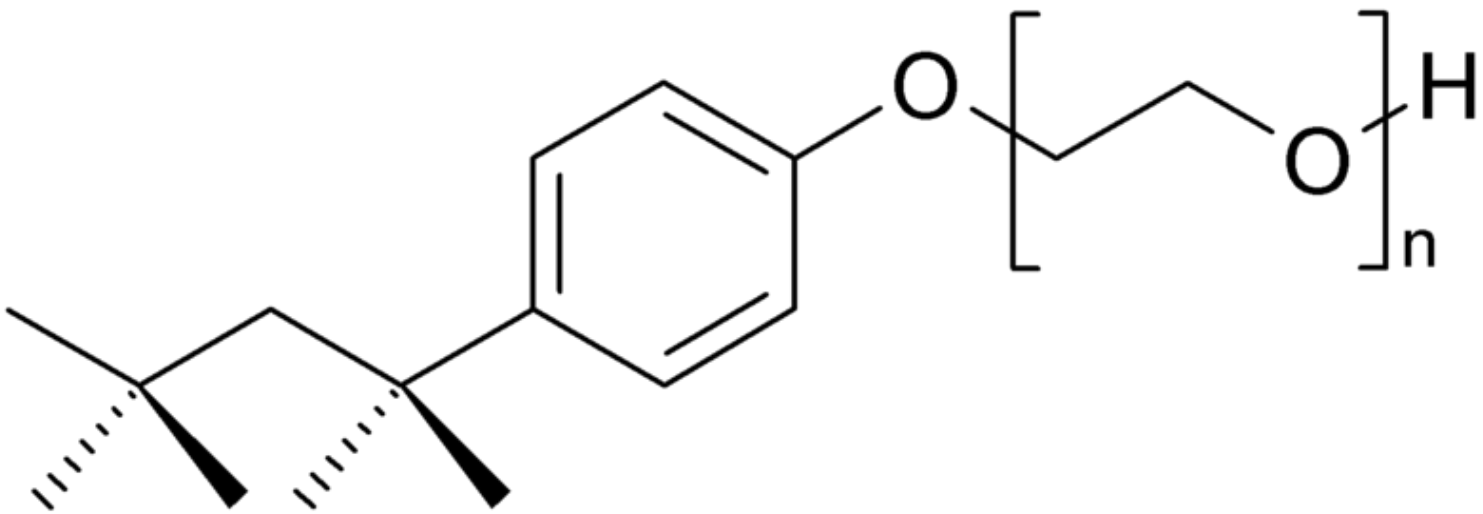
Homogenization

- Breaking cell contacts / cell walls
- Breaking cells: ultrasound, forcing through small holes, blending, using detergent (like Triton X-100) or osmosis
- Resulting product is a homogenate

Homogenization



Triton-X-100 molecule



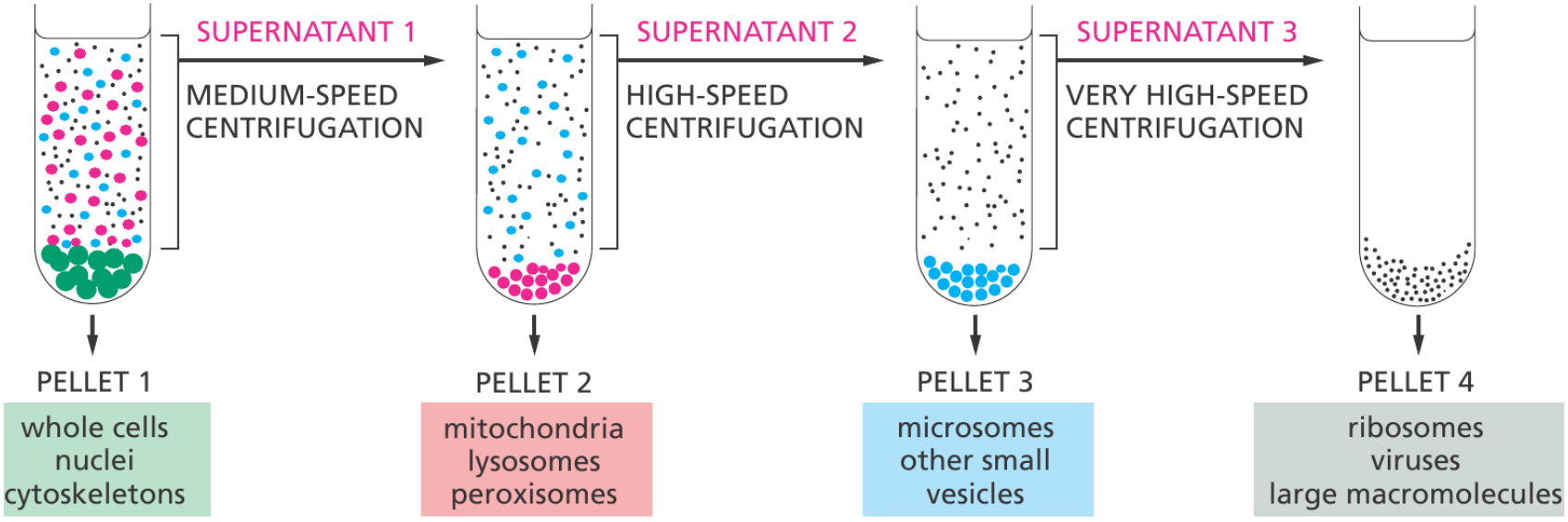
Differential centrifugation

- Step-by-step centrifugation where supernatant is used as a source of next step
- Typical sequence of sediments (pellets) is:

Differential centrifugation

- Step-by-step centrifugation where supernatant is used as a source of next step
- Typical sequence of sediments (pellets) is: nuclei $\rightarrow$ mitochondria, lysosomes $\rightarrow$ small vesicles $\rightarrow$ ribosomes, large macromolecules

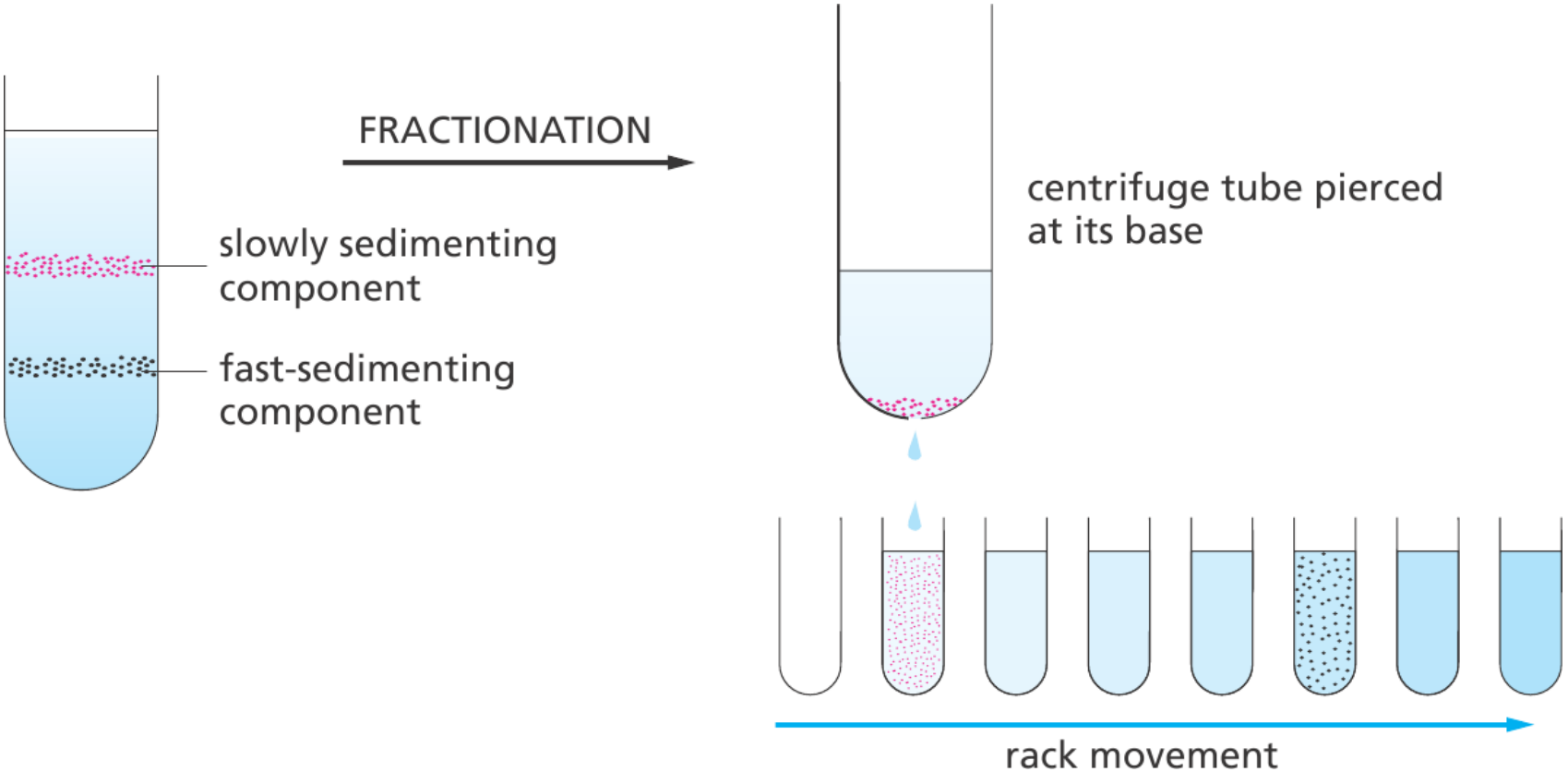
Differential centrifugation



Velocity sedimentation

- When a gradient of something (e.g., sucrose) present inside a tube, different types of molecules have different sedimentation speed depending on their size (larger proteins sediment **faster**)
- Fractionation may be used to remove parts of this gradient: pipetting (usually with cut pipette tip) or puncturing the bottom of a tube

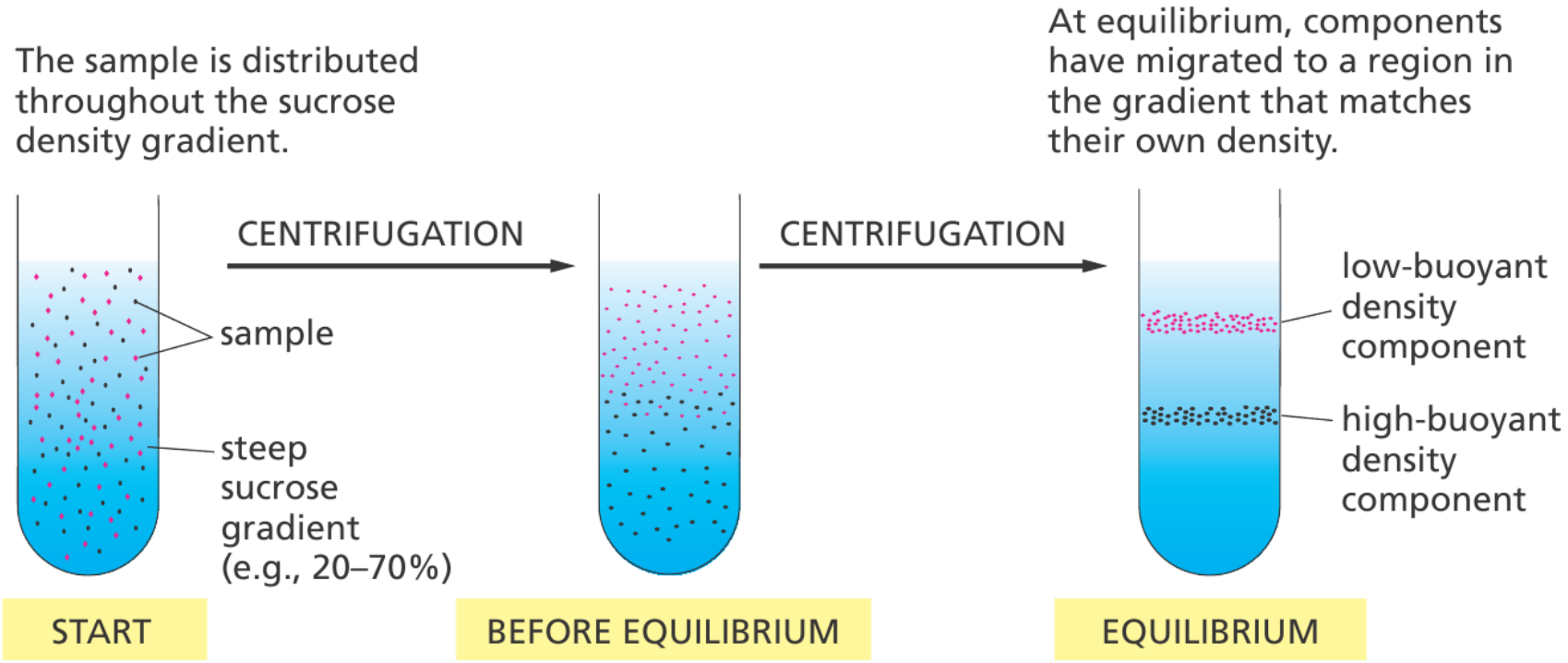
Velocity sedimentation



Equilibrium sedimentation

- Based on buoyant density (floating ability of molecules)
- More thick solution of sucrose or $CsCl_2$ (20–70%) with a gradient is used
- Every component of cell will move down until it reaches some density of surrounding liquid

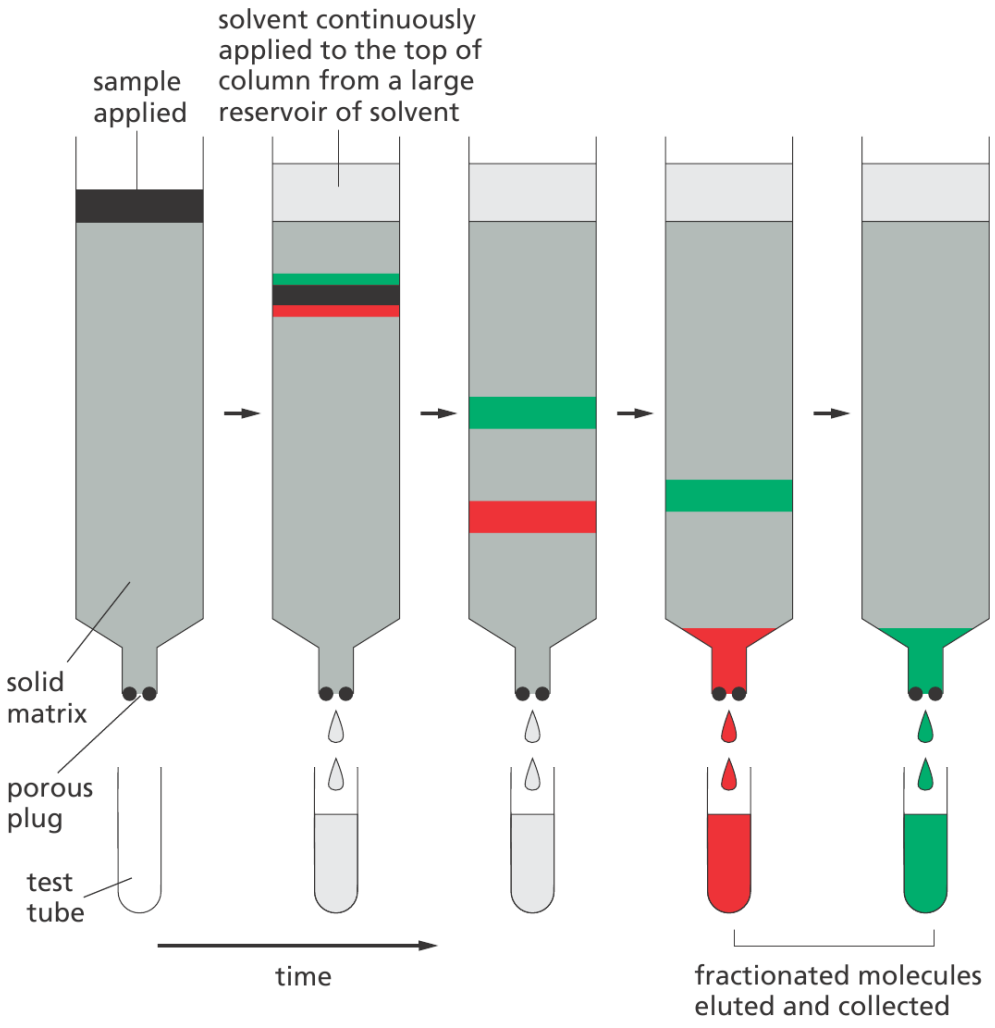
Equilibrium sedimentation



Column chromatography

- Mixture of proteins is pumped through chromatographic column
- There is a matrix inside column which contains chemicals with different affinities to different proteins
- As a result, some proteins will be attached to the matrix
- Then, we wash attached proteins out and therefore separate them from initial mixture
- There are numerous different variants of chromatography

Column chromatography

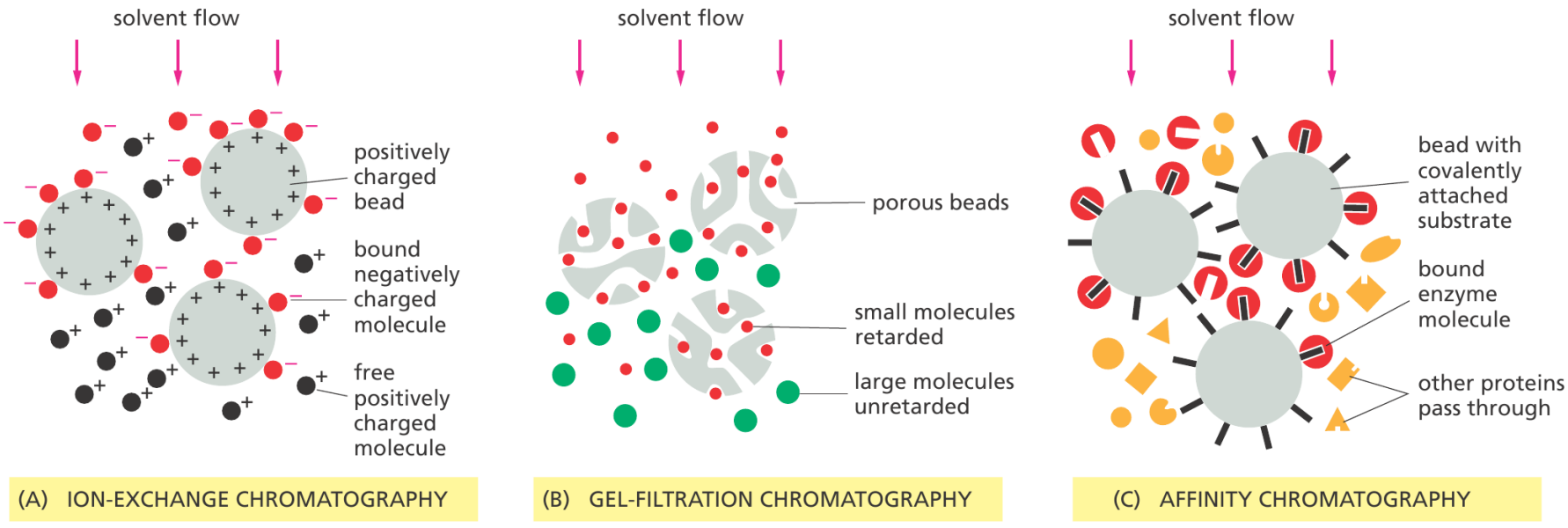


Different kinds of chromatography

Chromatography may be based on:

- Electric charge: ion-exchange chromatography
- Size: gel-filtration chromatography
- Binding: affinity chromatography

Variants of chromatography



Affinity chromatography

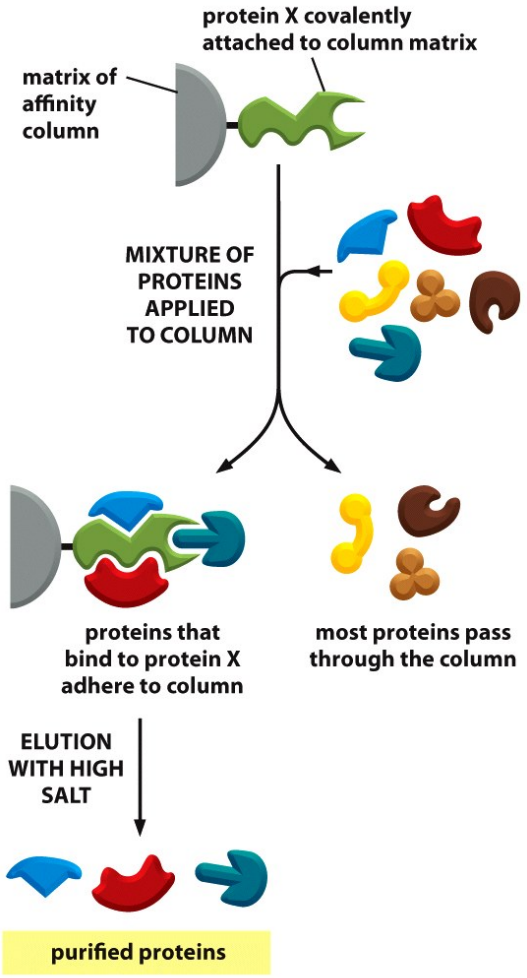
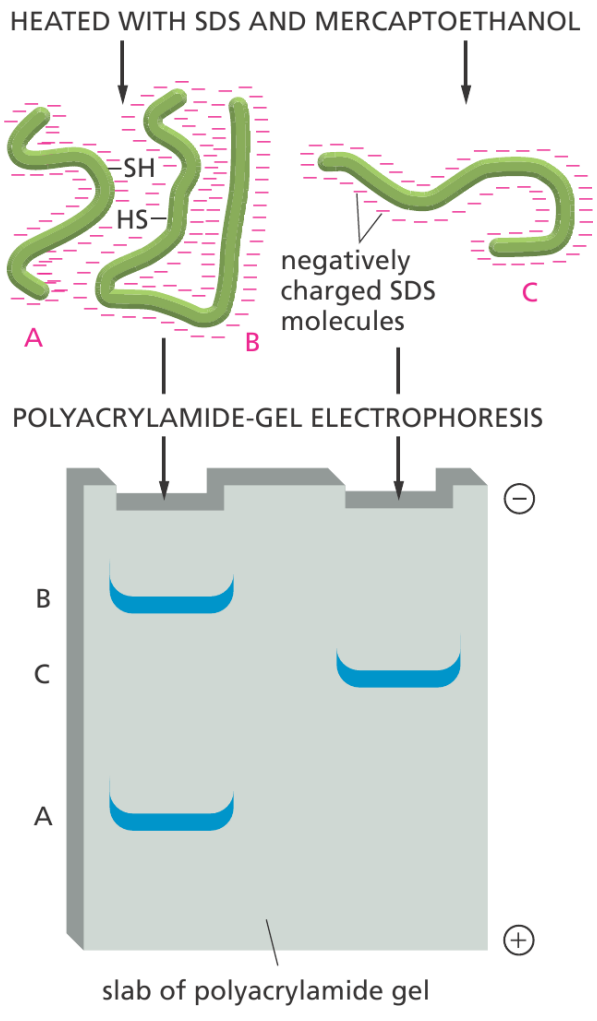


Figure 4-49 Essential Cell Biology 3/e (© Garland Science 2010)

Gel electrophoresis

- Since proteins have different weight and electric charge, they will move at different speed if we apply electric field to initial mixture
- To make speed slower, one needs to use gels (e.g., polyacrylamide) instead of liquids
- Protein molecules usually processed with sodium dodecyl sulfate (SDS) to make charge equal and mercaptoethanol is used to break disulfide bridges. As a result, they will move only on the basis of their molecular weight.

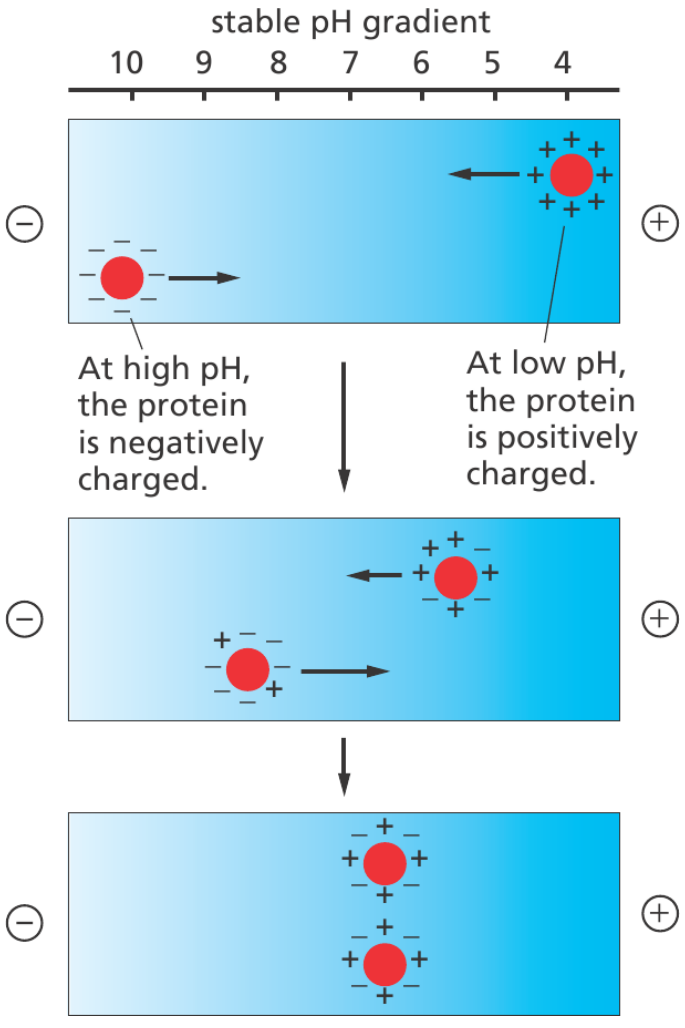
Polyacrylamide gel electrophoresis



Isoelectric focusing

- This is a variant of electrophoresis in narrow tubes with pH gradient
- Proteins will move until it charge will become 0. This is an isoelectric point of protein.

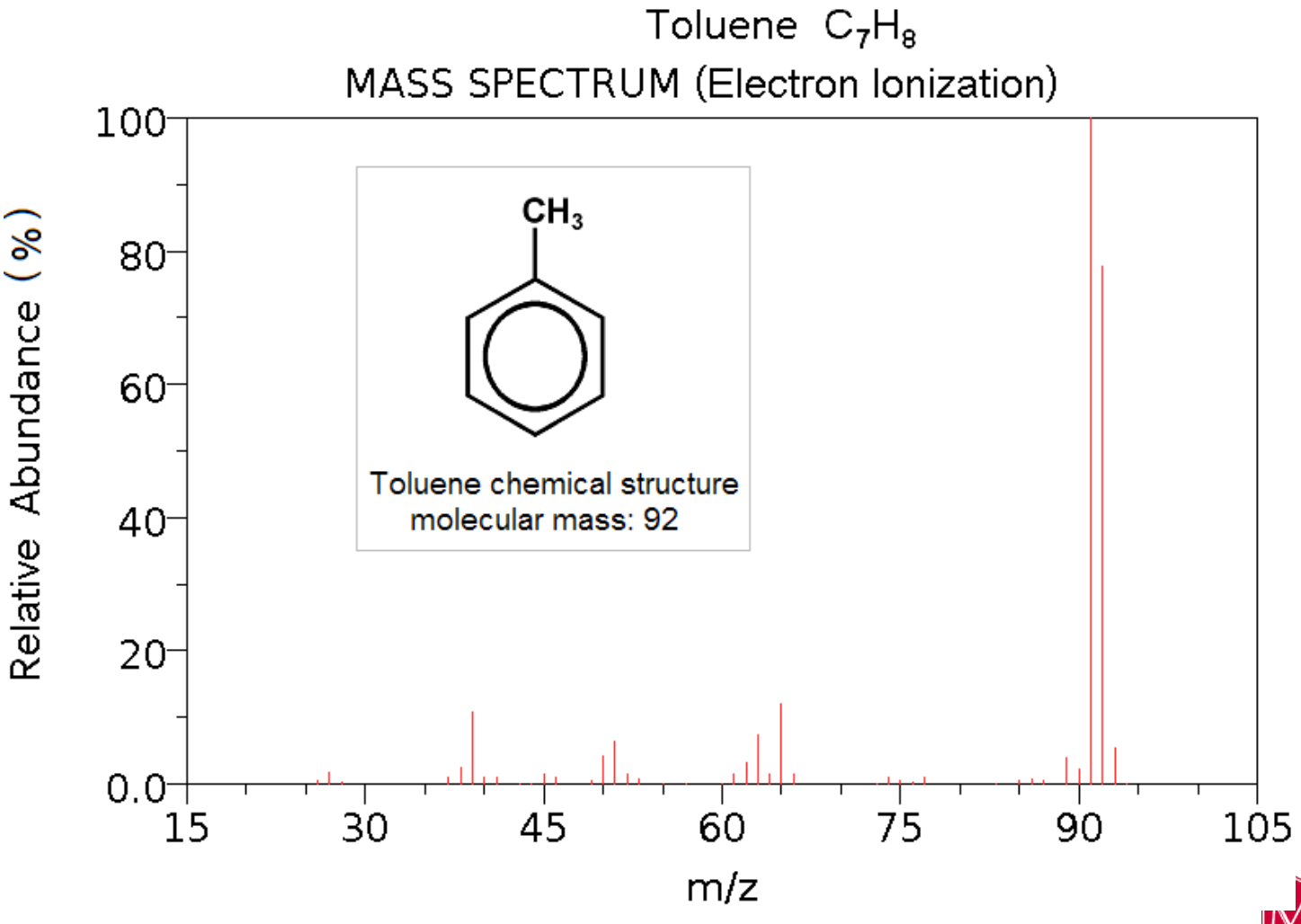
Isoelectric focusing



Mass spectrometry

- Based on measuring mass of protein fragments
- Protein is fragmented, then placed into the engine which calculates mass of every fragment
- This will produce a kind of fingerprint image (mass spectrum) for every protein fragment
- Combination of mass spectra from fragments will give a protein sequence

Mass spectrometry



Mass spectrometry real life example (*Rubisco* protein)

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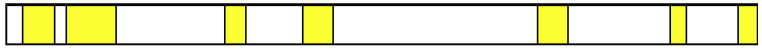
Reference: gi|16326265|dbj|BAB70576.1| ribulose 1,5-bisphosphate carboxylase
Database: C:\Xcalibur\database\ACE.fasta
Number of Amino Acids: 464 Monoisotopic MW: 51406.9 pI: 6.00



Protein:

GFKAGVKDYK **LTYYTPDYET KDTDILAAFR** VTPQPGV**PPE EAGAAVAAES**
STGTWTTVWT DGLTSLDRYK GRCYHIEPVA GEESQFIAYV AYPLDLFEEG
SVTNMFTSIV GNVFGFKALR ALRLEDLRIP PAYSK**TFQGP PHGIQVER**DK
LNKYGRPLLG CTIKPKLGLS AKNYGRAVYE CLR**GGLDFTK DDENVNSQPF**
MRWRDRFLFC AEAIYKAQAE TGEIKGHYLN ATAGTCEEMM KRAVFARELG
VPIVMHDYLT GGFTANTSLA HYCRDNGLLL HIHRAMHAVI DRQKNHGIHF
RVLAKALRMS GGDHIHAGTV VGKLEGER**EI TLGFVDLLRD DYIEKDR**SRG
IYFTQDWVSL PGVLPVASGG IHVWHMPALT EIFGDDSVLQ FGGGTLGHPW
GNAPGAVANR **VALEACVQAR** NEGRDLAREG NEIIREAGKW SPELAAACEV
WK**EIKFEFPA MDTL**

Protein Coverage:

Sequence	MH+	% Mass	AA	% AA
FKAGVKDYKL	1168.67	2.27	2 - 11	2.16
FKAGVKDYKLTYYTPDYET	2302.13	4.48	2 - 20	4.09
KAGVKDYKLTYYTPDYET	2155.06	4.19	3 - 20	3.88
AGVKDYKLTYY	1320.68	2.57	4 - 14	2.37
AGVKDYKLTYYTPDYETK	2155.06	4.19	4 - 21	3.88
GVKDYKLTY	1086.58	2.11	5 - 13	1.94
YKLTYYTP	1048.53	2.04	9 - 16	1.72
KLTYYTPDYETKDTDILAAF	2368.16	4.61	10 - 29	4.31
LTYYTPDYETK	1393.65	2.71	11 - 21	2.37
LTYYTPDYETKDTDILAA	2093.00	4.07	11 - 28	3.88
LTYYTPDYETKDTDILAAFRV	2495.23	4.85	11 - 31	4.53
LTYYTPDYETKDTDILAAFRVTPQPGVPP	3268.64	6.36	11 - 39	6.25
PDYETKDT	968.42	1.88	16 - 23	1.72
PDYETKDTDILAA	1451.69	2.82	16 - 28	2.80
DYETKDTDILAAFRVTP	1954.98	3.80	17 - 33	3.66
ETKDTDILAAFRV	1478.78	2.88	19 - 31	2.80
KDTDILAAFRVTPQPGVPPEEAGAAVAAESST	3195.62	6.22	21 - 52	6.90
DTDILAAFR	1021.53	1.99	22 - 30	1.94
DTDILAAFRVT	1221.65	2.38	22 - 32	2.37
DTDILAAFRVTPQPGVPPEEAGAAVAA	2663.37	5.18	22 - 48	5.82
DILAAFRV	904.53	1.76	24 - 31	1.72
DILAAFRVTPQP	1327.74	2.58	24 - 35	2.59
DILAAFRVTPQPGVPPE	1806.97	3.52	24 - 40	3.66
ILAAFRVTPQP	1212.71	2.36	25 - 35	2.37
ILAAFRVTPQPGVPPE	1691.95	3.29	25 - 40	3.45
LAAFR	577.35	1.12	26 - 30	1.08
LAAFRVTPQPGV	1255.72	2.44	26 - 37	2.59
AAFRVTPQPGVPPEEAGAAVAAESSTG	2567.27	4.99	27 - 53	5.82
AFRVTPQPGVPPEEAGAAVAAESSTGTWTTVWT	3371.65	6.56	28 - 60	7.11
FRVTPQPGVPPEEAGAAVAAES	2180.10	4.24	29 - 50	4.74
RVTPQPGV	853.49	1.66	30 - 37	1.72
RVTPQPGVPPEEAG	1433.74	2.79	30 - 43	3.02
TPQPGVPPEEAGAAVAAESSTGTWTTVWTD	3013.41	5.86	32 - 61	6.47
PQPGVPPEEAGAAVAAESS	1763.84	3.43	33 - 51	4.09
QPGVPPEEAGAA	1122.54	2.18	34 - 45	2.59
QPGVPPEEAGAAVAAESSTGT	1925.91	3.75	34 - 54	4.53
GVPPEEAGAAVAAESSTGTWTT	2088.97	4.06	36 - 57	4.74
GVPPEEAGAAVAAESSTGTWTTVWTD	2590.19	5.04	36 - 61	5.60
VPPEEAGAAVAA	1081.55	2.10	37 - 48	2.59
VPPEEAGAAVAAE	1210.60	2.35	37 - 49	2.80
VPPEEAGAAVAAESSTGTWT	1930.90	3.76	37 - 56	4.31
VPPEEAGAAVAAESSTGTWTTVWTDGLT	2804.33	5.46	37 - 64	6.03
PPEEAGAAVAAESSTGTWTTVWTDGLTSLDR	3176.50	6.18	38 - 68	6.68
PEEAGAAVAAESSTGTWTTV	1934.90	3.76	39 - 58	4.31
PEEAGAAVAAESSTGTWTTVW	2120.98	4.13	39 - 59	4.53
EAGAAVAAESSTGTWTTVWTDGLT	2382.11	4.63	41 - 64	5.17

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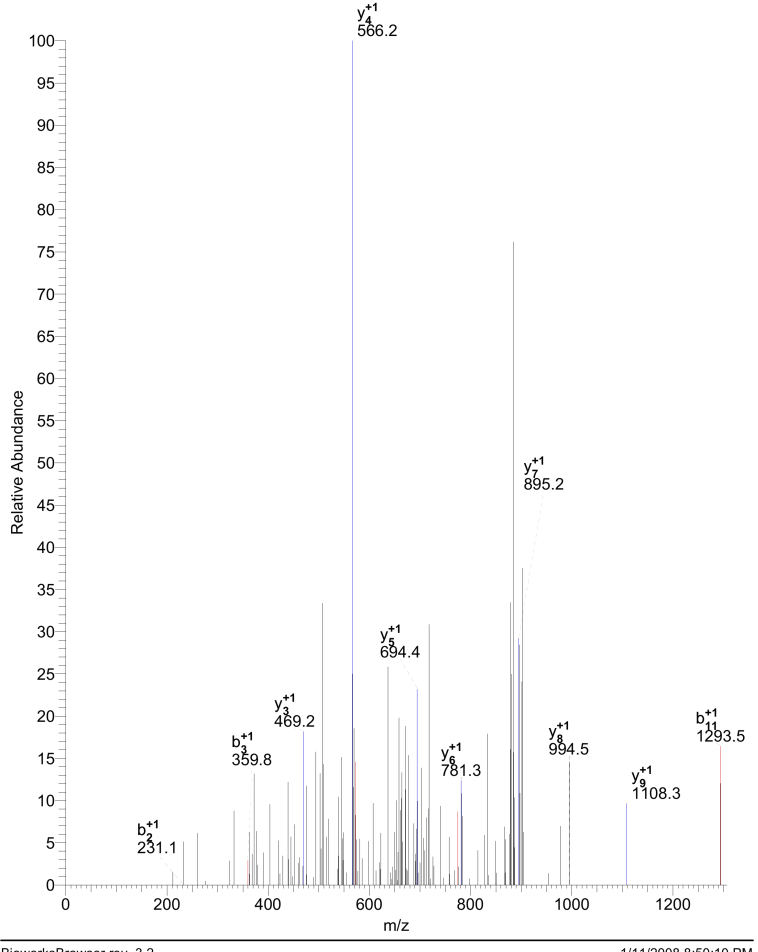

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Final question (1 point)

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Which way of sedimentation employs differences between molecular sizes?

Summary

- Homogenization produce the initial mixture of proteins
- Separation of this mixture could be done through centrifugation, sedimentation, electrophoresis and chromatography
- Mass spectrometry + software similarity analysis is used for protein sequencing
- X-rays, NMR and/or software folding are used for understanding 3D protein structures
- Results of analyses are depositing into on-line protein databases

For Further Reading

A. Shipunov.
Advanced Cell Biology [Electronic resource].
2011—onwards.
Mode of access: `http://ashipunov.info/shipunov/school/biol_250`

B. Alberts et al.
Essential Cell Biology. 3rd edition.
Garland Science, 2009.
Chapter 4.