CONCEPT: SIZE EXCLUSION CHROMATOGRAPHY

●*Size Exclusion Chromatography*: purifies a protein based on its _____.

□ Also known as _____-*filtration chromatography*.

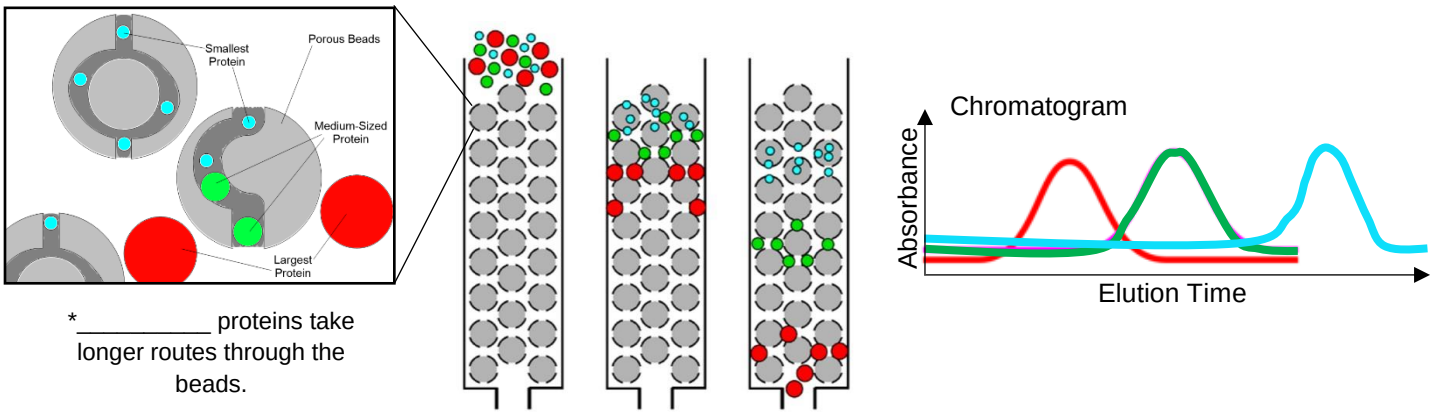
●Contrary to gel electrophoresis, _____ proteins elute *faster* & *earlier* from the column than smaller proteins.

□ Stationary phase consists of very _____ beads with cavities engineered to be a specific size.

□ Large proteins _____ enter the cavities of the beads & take a shorter, faster route through the column.

□ Small proteins enter the cavities of the beads & are _____ down with a longer route through the column.

EXAMPLE: Size Exclusion Chromatography.



*_____ proteins take longer routes through the beads.

*_____ proteins elute from column *first*.

PRACTICE: In a mixture of 5 proteins (listed below), which protein elutes 2nd in size-exclusion chromatography?

a) Cytochrome C (M_r = 13,000).

b) Immunoglobulin G (M_r = 145,000).

c) Ribonuclease A (M_r = 13,700).

d) RNA polymerase (M_r = 450,000).

e) Serum albumin (M_r = 68,500).

PRACTICE: Which of the following statements is false?

a) In ion exchange chromatography, the bound proteins are eluted using a salt solution.

b) Gel filtration chromatography can be used to determine an unknown protein's relative molecular size/mass.

c) In gel filtration chromatography, the smallest proteins are eluted from the column last.

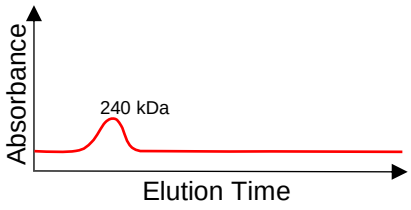
d) Separation of proteins in gel filtration chromatography is based on size & net charge of the proteins.

e) None of them. All above statements are true.

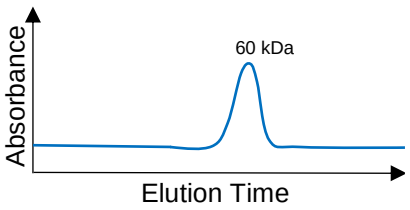
CONCEPT: SIZE EXCLUSION CHROMATOGRAPHY

PRACTICE: A new protein of unknown structure has been purified & gel filtration chromatography reveals that the native protein has a molecular weight of 240 kDa. Chromatography in the presence of 6 M guanidine hydrochloride (GuHCl), a chaotropic agent that has a similar effect on proteins as urea, yields a single absorbance peak corresponding to a protein of M_r 60 kDa. Chromatography in the presence both of 6 M guanidine hydrochloride and 10 mM β-mercaptoethanol (β-ME) yields peaks for proteins of M_r 34 kDa and 26 kDa. Using this data, which option best describes the structure of this protein? Hint: sketch a visual of the protein after each chemical treatment.

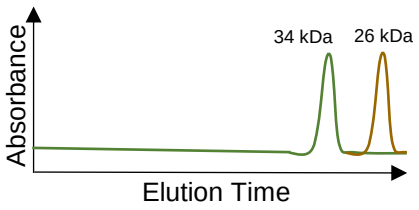
1) Native Protein



2) Protein + 6 M GuHCl



3) Protein + 6 M GuHCl + β-ME



a) A homotetramer (4 identical 60 kDa subunits); each subunit is a heterodimer of 2 disulfide-linked chains (34 & 26 kDa).

b) A heterooctomer (8 different subunits); four subunits each of 34-kDa & 26 kDa, all held together via disulfide bonds.

c) A homodimer (2 identical 120 kDa subunits); each subunit is a a homodimer of 2 disulfide-linked chains (60 kDa each).

d) A heterotetramer (4 different subunits); each subunit is a homodimer of 2 disulfide-linked chains (60 kDa each).

PRACTICE: To answer the questions A, B & C below, use the provided chart with the properties of the four proteins.

A) What is the order of elution of the proteins from a size-exclusion chromatography column?

a) A → B → C → D.

b) D → B → A → C.

c) B → D → A → C.

d) C → A → D → B.

Protein	MW	pI	Charge at:		
			pH = 6	pH= 7	pH = 8
A	62,457	8.1	32.2	10.9	0.7
B	115,471	5.69	-7.3	-29.6	-42
C	17,183	7.7	7.4	2.1	-0.7
D	69,366	6.29	3.4	-3.4	-20.2

B) Which pH is best for separating the proteins using anion-exchange chromatography?

a) pH = 6. b) pH = 7. c) pH = 8.

C) In what order would the proteins elute from the anion-exchange chromatography column?

a) A → C → D → B. b) D → A → B → C. c) B → D →C → A. d) C→ B → D → A.