CONCEPT: AFFINITY CHROMATOGRAPHY

●*Affinity Chromatography*: effectively purifies a protein based on its _____ (specific binding capabilities).

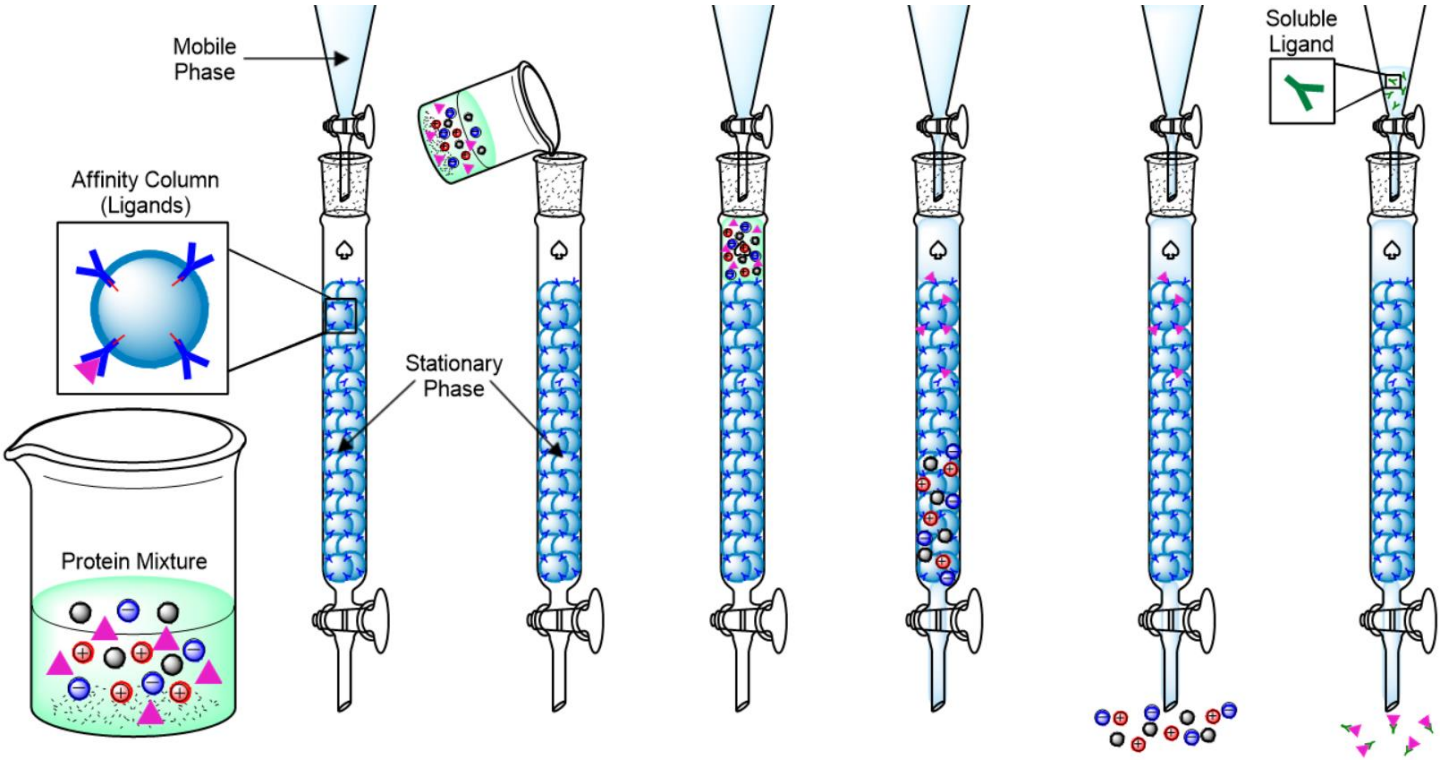
□ Stationary phase material in the chromatography column is covalently linked to a _____.

●*Ligand*: a small substance that specifically _____ to a biomolecule to form a complex with it.

□ Target protein binds to the ligand that's _____ to the stationary phase & _____ in the column.

□ All other proteins do *not* bind the ligand & are _____ out of the column with the mobile phase.

EXAMPLE: Affinity Chromatography.



●Bound target protein can be later _____ from the column by adding a soluble ligand or salt to the mobile phase.

PRACTICE: In your own words, describe the principles involved in protein purification by affinity chromatography.

PRACTICE: The target protein to be purified is likely eluted from the affinity chromatography column by _____.

Explain potential advantages & disadvantages of the elution strategies.

a) Altering the pH of the mobile phase.

b) Addition of a chaotropic agent such as urea.

c) Addition of salt and/or free ligand.

d) Raising the temperature in the column.

CONCEPT: AFFINITY CHROMATOGRAPHY

PRACTICE: A biochemist is attempting to separate a DNA-binding protein (protein X) from other proteins in solution (proteins A, B & C). Consider the chart & answer the questions below about what type of technique is best for separation.

	pI (Isoelectric Point)	Molar Mass	Binds to DNA?
Protein A	7.4	82,000	Yes
Protein B	3.8	21,500	Yes
Protein C	7.9	23,000	No
Protein X	7.8	22,000	Yes

A) What type of chromatography is best for separating protein X from protein A? _____

B) What type of chromatography is best for separating protein X from protein B? _____

C) What type of chromatography is best for separating protein X from protein C? _____