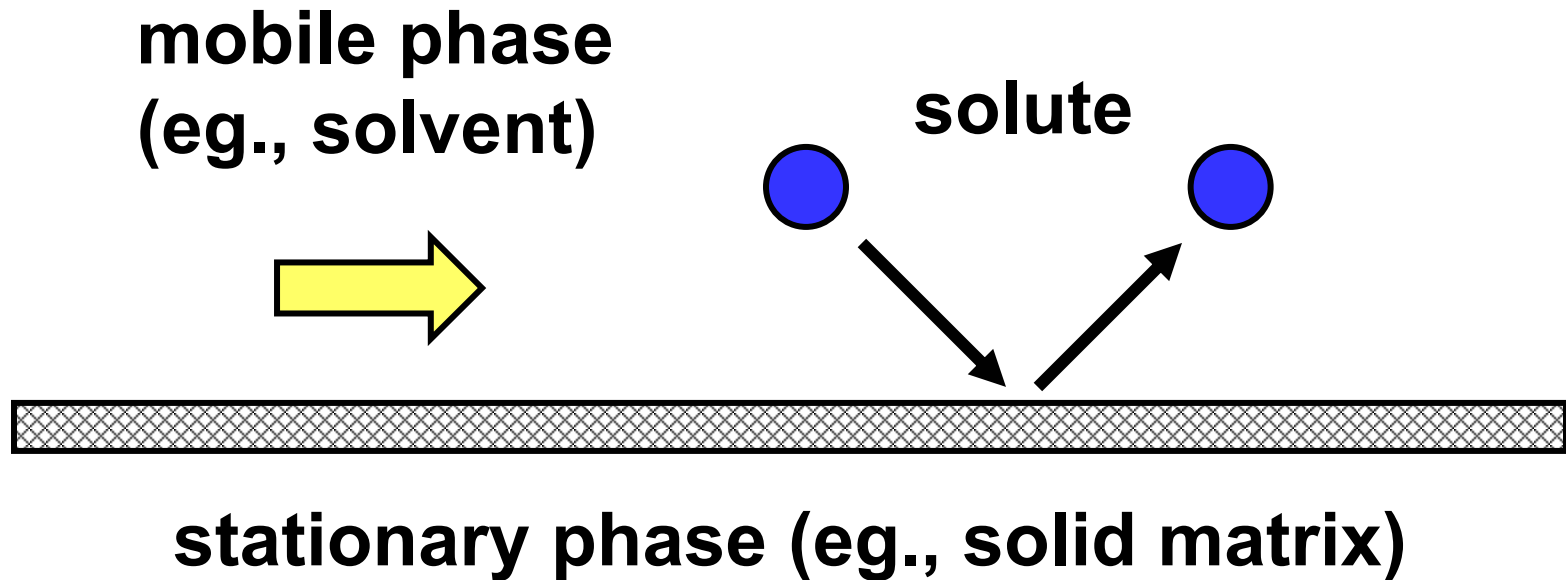
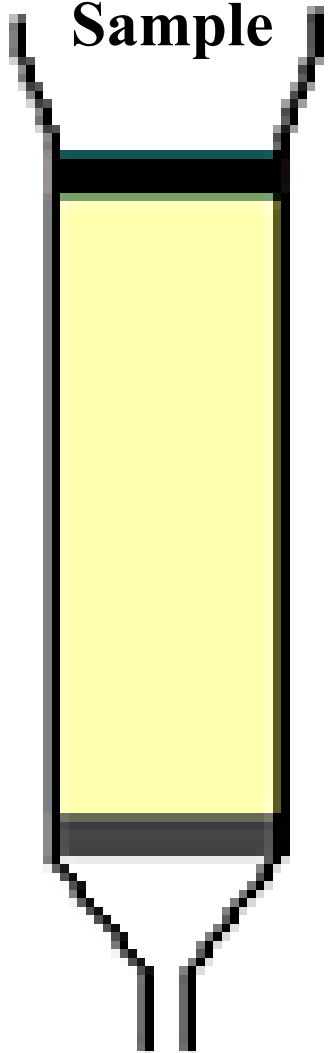


Chromatography Components

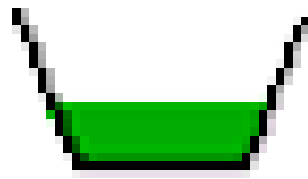
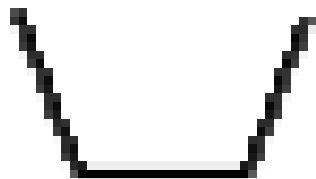
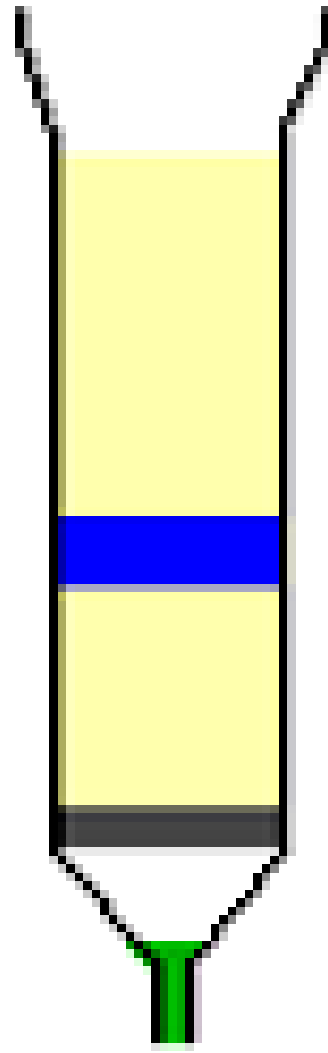
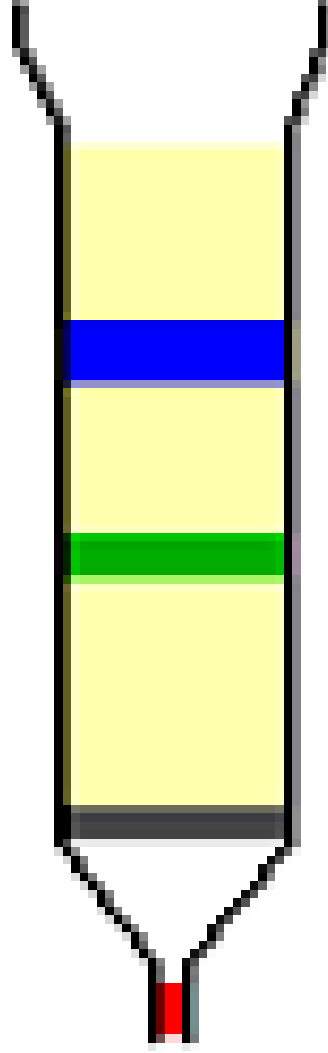
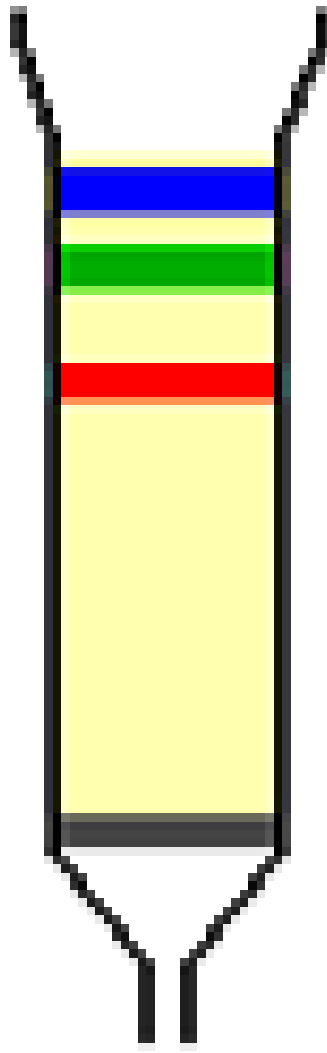


Solutes which interact differently with the stationary phase can be separated.

**Apply
Sample**



Continue Developing with Solvent

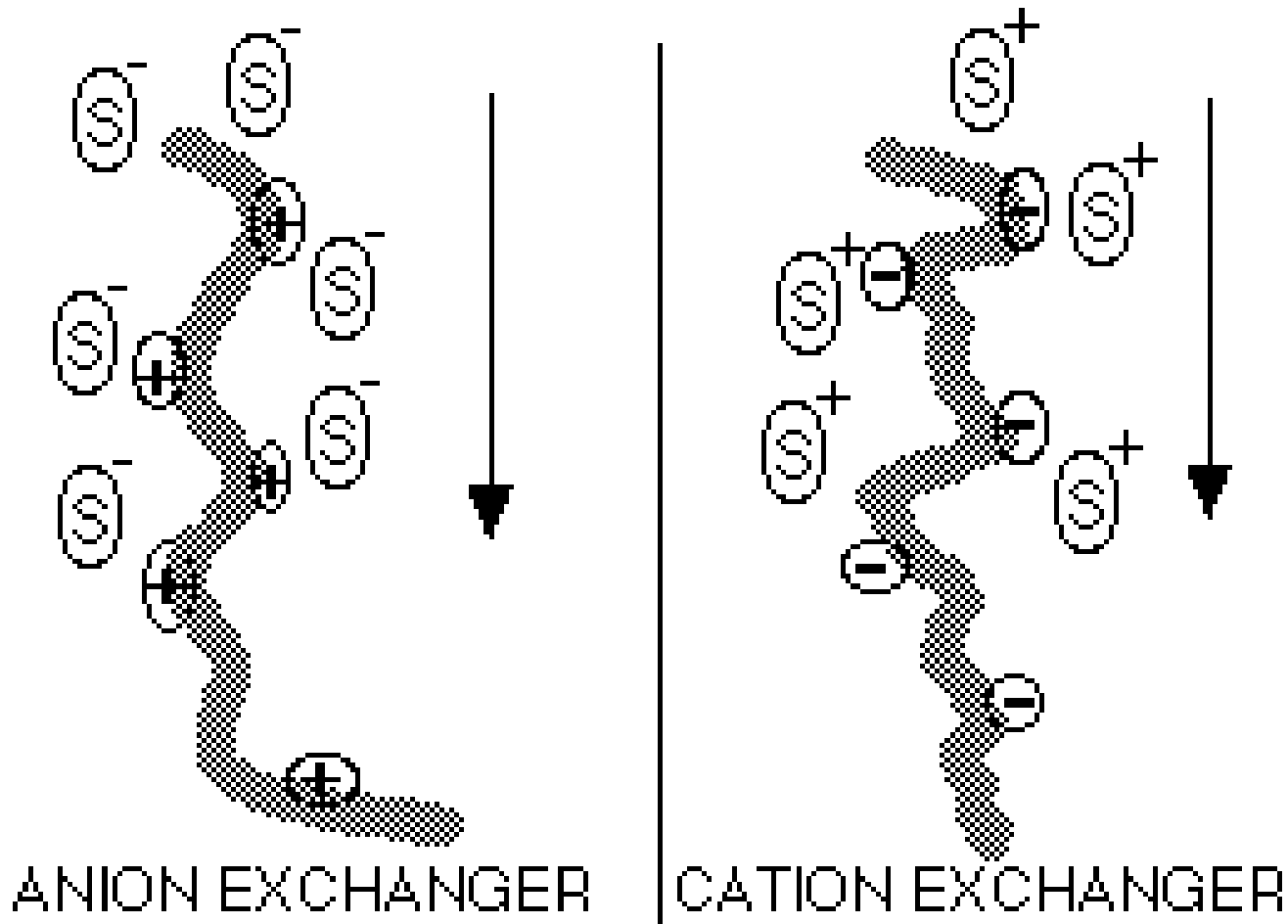


Common Media Used in Liquid Protein Chromatography

Media Type	Discrimination
Ion Exchange	Charge
Gel Filtration	Size and Shape
Hydrophobic	Surface Hydrophobicity
Reverse Phase	Total Hydrophobicity
Affinity	Specific Amino Acids
Adsorption	Amino Groups?

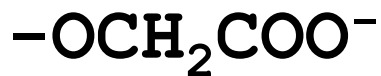
Ion Exchange Chromatography (IEC)

- based on charge-charge interactions between solid matrix and solute



IEC Stationary Phase

- matrix composed of cross-linked polymers (eg., cellulose, sepharose, dextran, etc.)
- fixed charged groups are acids (cation exchanger) or bases (anion exchanger)
- exchangers can be 'strong' or 'weak'
- use highly ionizable exchangers (ie, strong) to separate weakly ionizable solutes and visa versa



Carboxymethyl (CM)

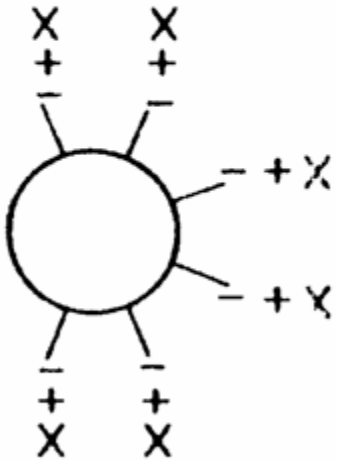


Diethylaminoethyl (DEAE)

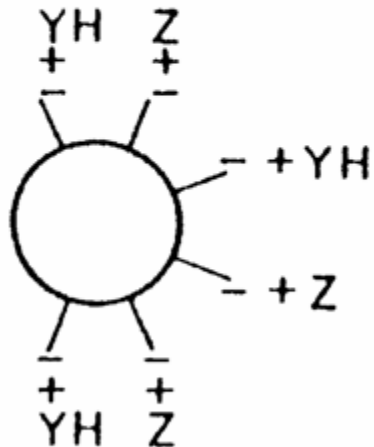
Basic Principal of IEC

Apply Solute

YH^+ & Z^+

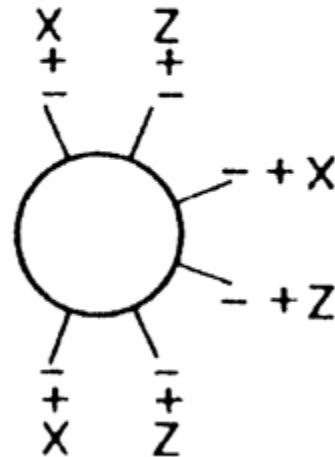


Solute Bound



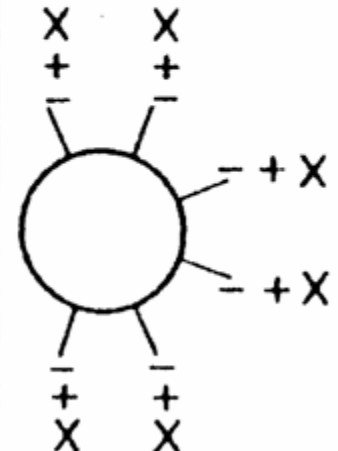
X^+ X^+ X^+ X^+

Add Counter-ion

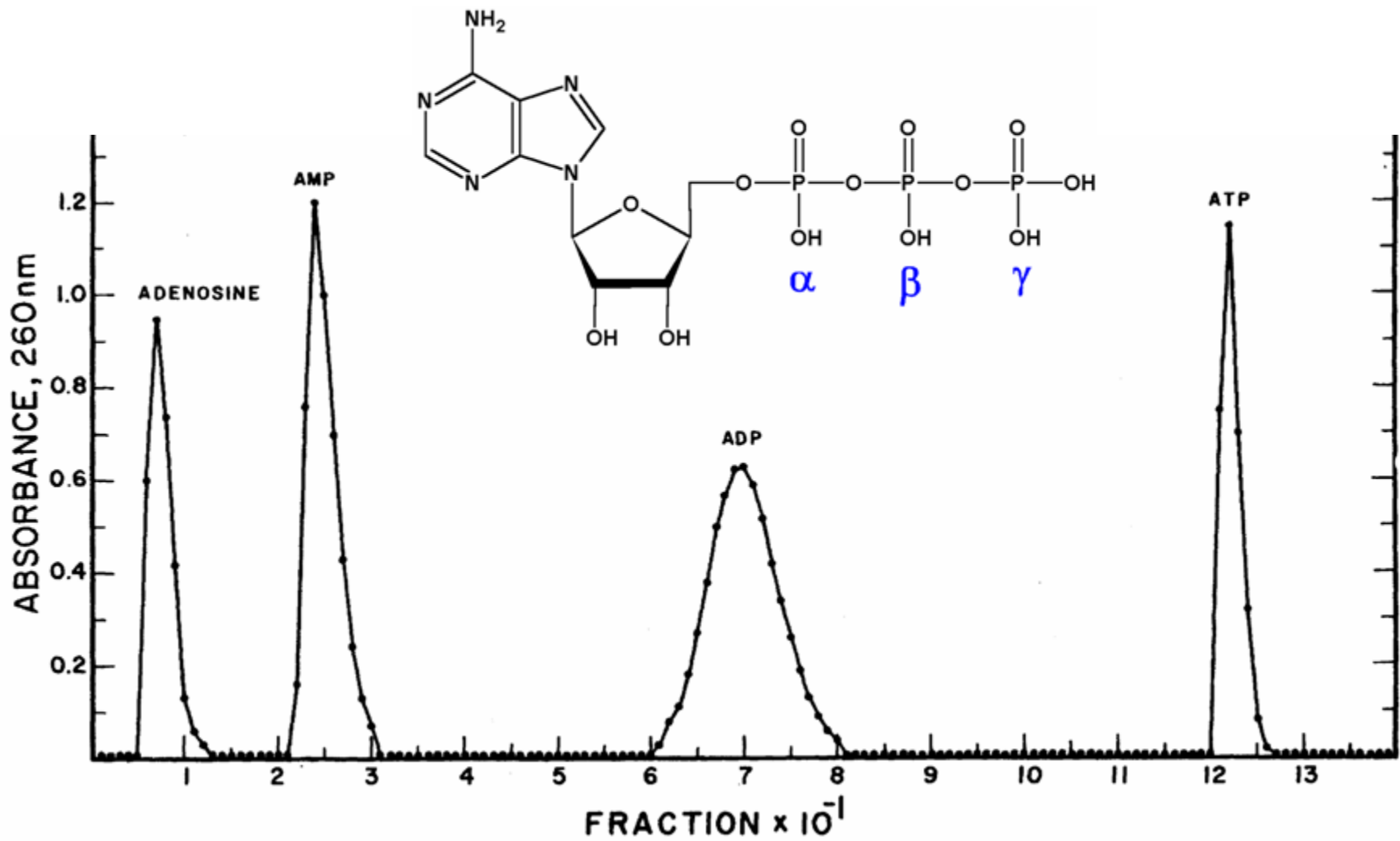


YH^+ YH^+ YH^+ YH^+

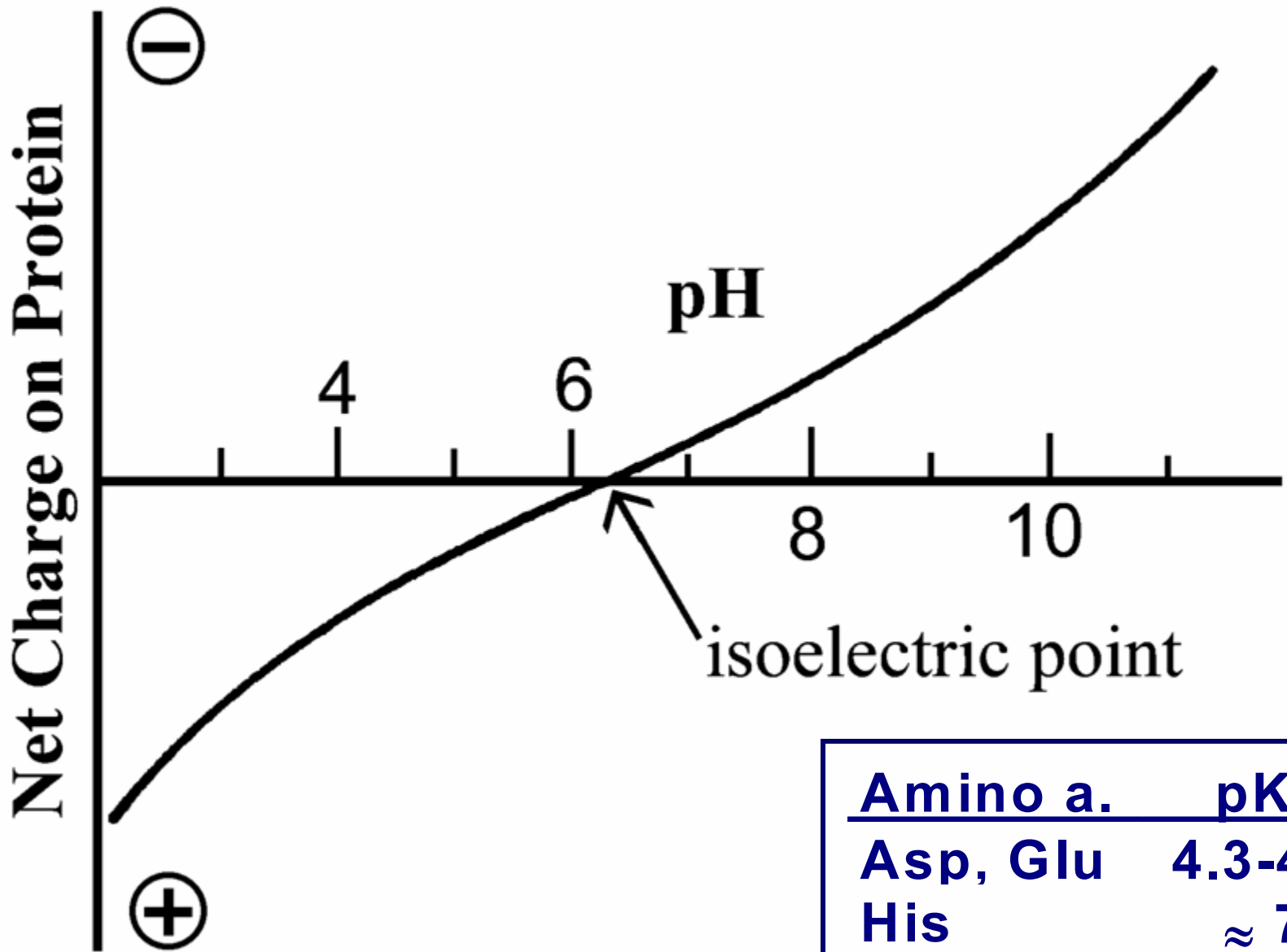
Add More Counter-ion



Z^+ Z^+ Z^+ Z^+ Z^+



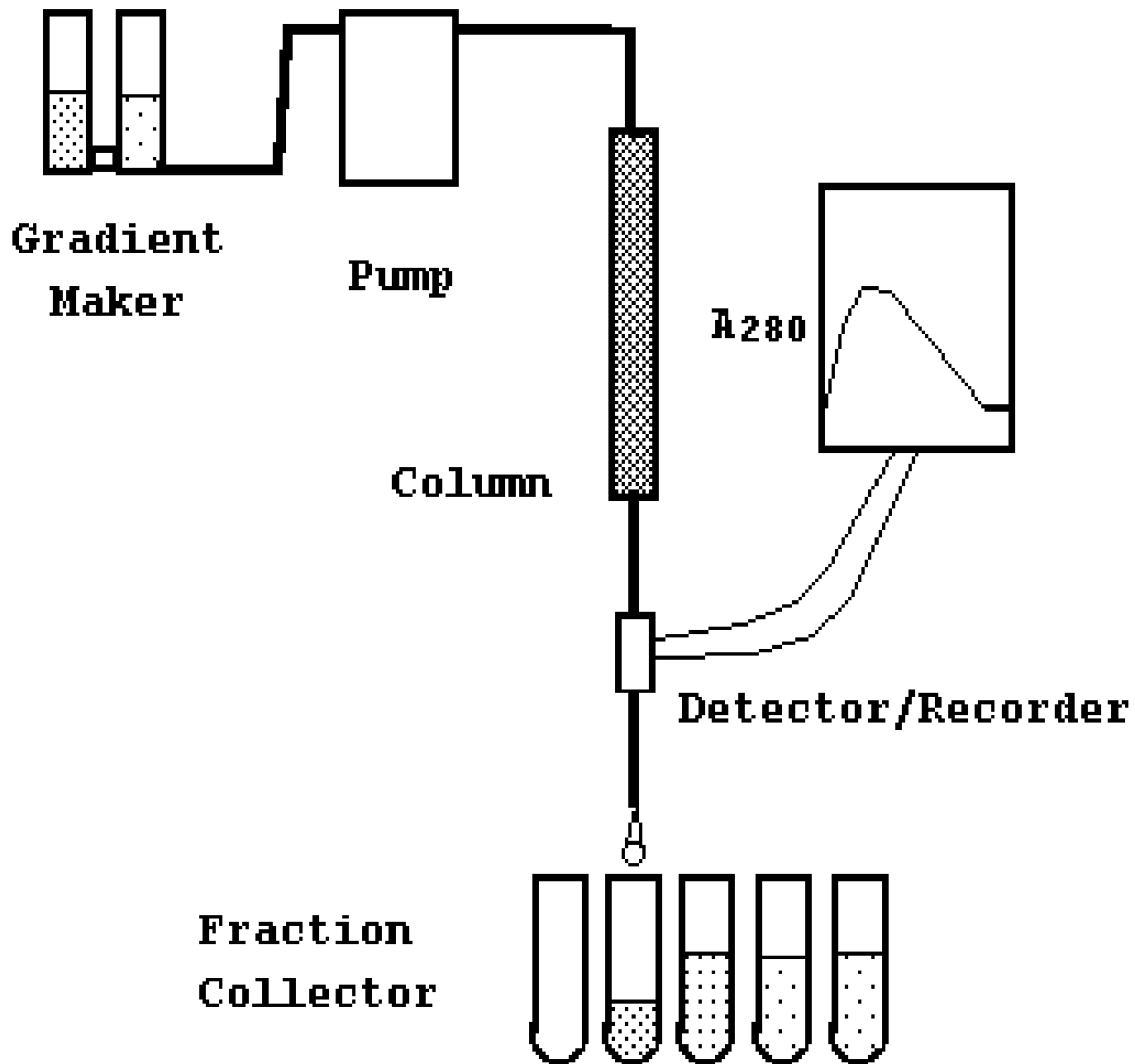
increasing formate ion concentration →



<u>Amino a.</u>	<u>pK_a</u>
Asp, Glu	4.3-4.7
His	≈ 7
Lys, Arg	> 10

IEC General Protocol

- **Prepare or purchase column**
 - matrix
 - anion vs cation
 - strong vs weak
 - size (ie, capacity) and shape
 - equipment
- **Adjust pH and initial counter ion**
- **Apply sample**
 - typically low ionic strength
- **Wash**
- **Elute**
- **Collect and analyze fractions**



HPLC/FPLC

- **compression of matrix material limits flow rate**
- **low flow rates result in diffusion and loss of resolution**
- **advances associated with HPLC:**
 - **↑ sorbent mechanical strength**
 - **↓ sorbent particle size**
 - **pumps with greater flow rates**

HPLC = High Performance (Pressure) Liquid Chromatography

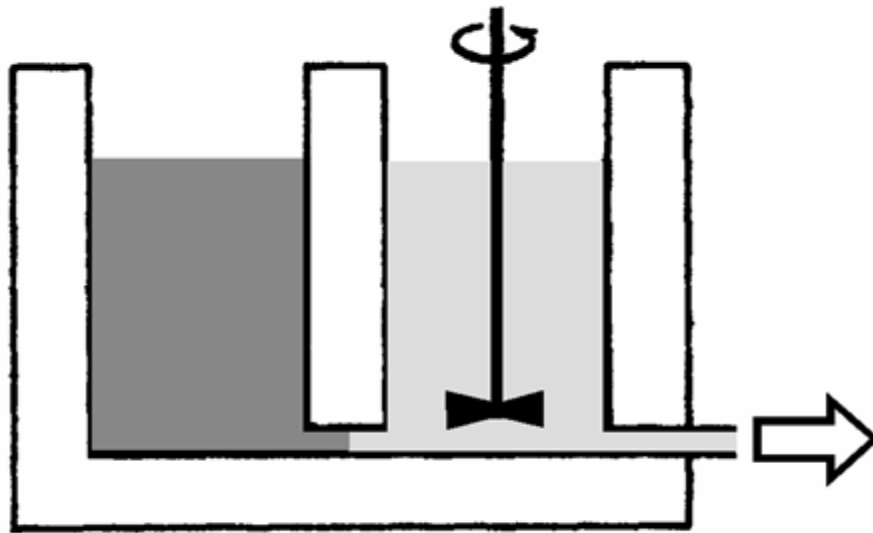
FPLC = Fast Protein Liquid Chromatography

IEC General Protocol

- **Prepare or purchase column**
 - matrix
 - anion vs cation
 - strong vs weak
 - size (ie, capacity) and shape
 - equipment
- **Adjust pH and initial counter ion**
- **Apply sample**
 - typically low ionic strength
- **Wash**
- **Elute**
- **Collect and analyze fractions**

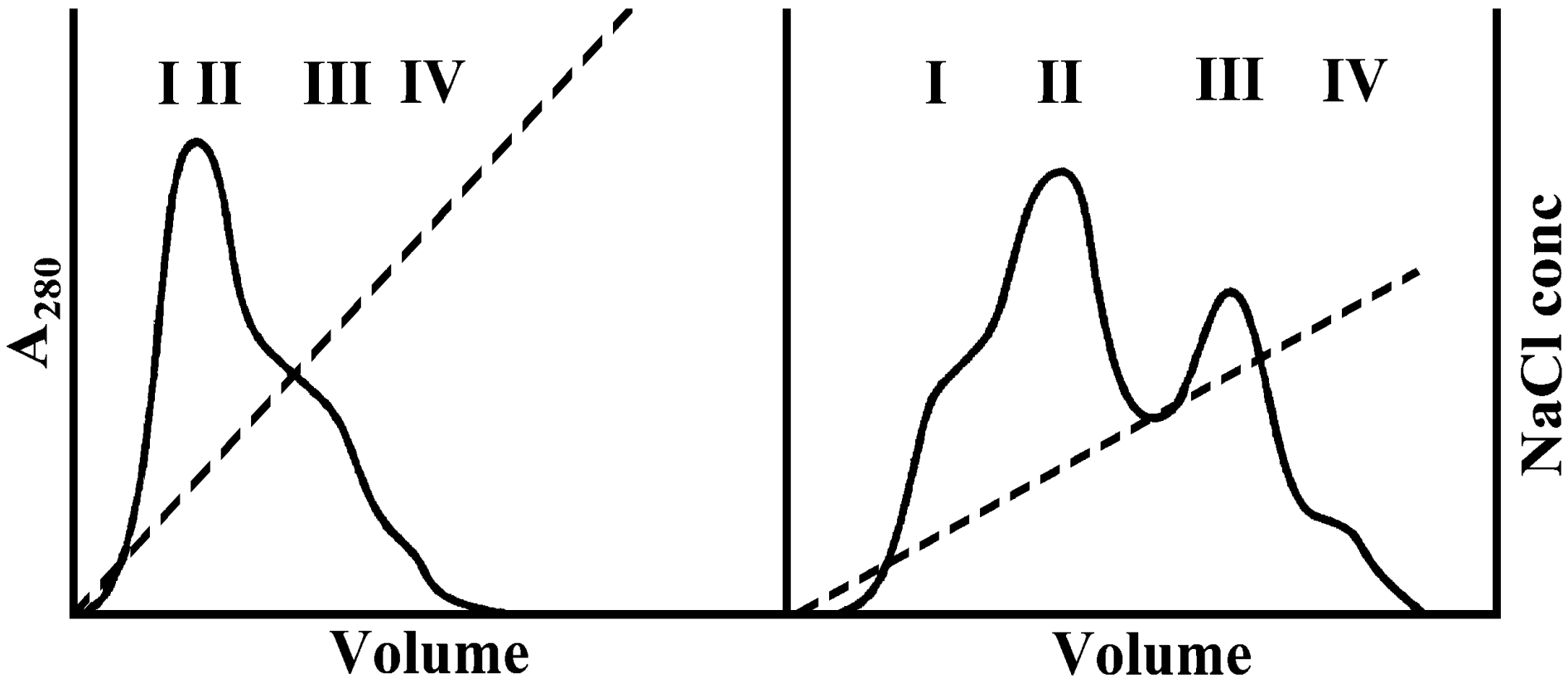
Elution from IEC Column

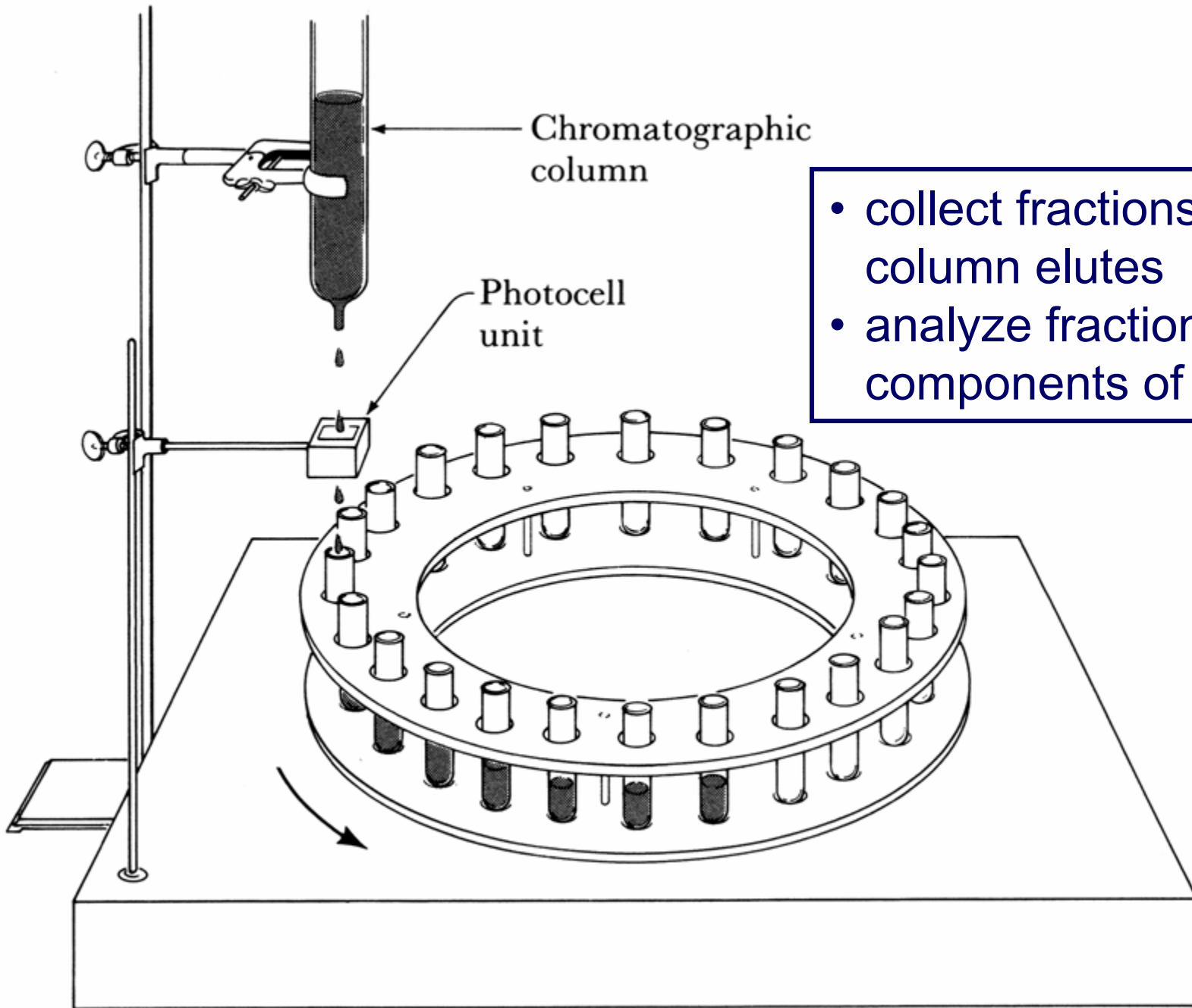
- change pH
- increase counter-ion (ie, salt) concentration
 - in steps (eg, 0.1, 0.2, 0.3, 0.4 M NaCl)
 - gradually (eg, 0→0.4 M NaCl) with gradient maker
- elution volume (or gradient slope)



Slope of Gradient

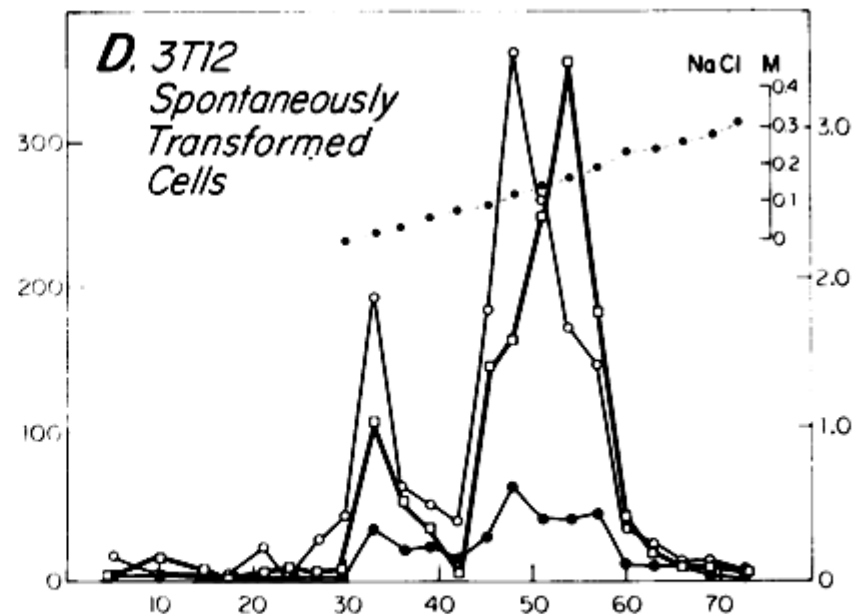
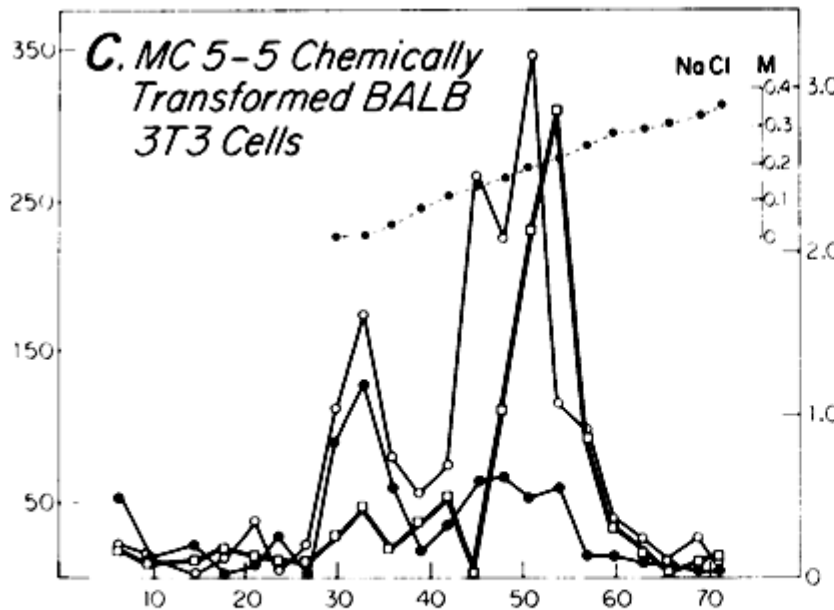
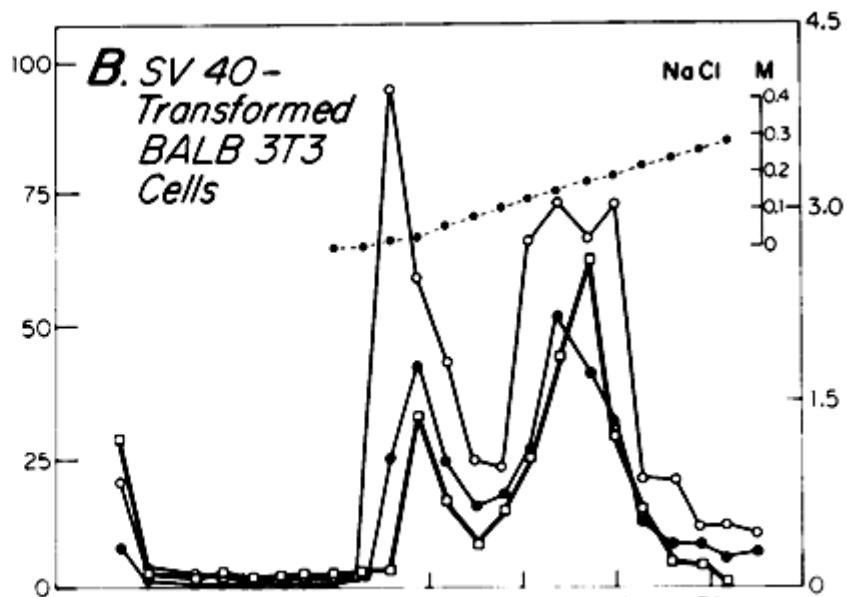
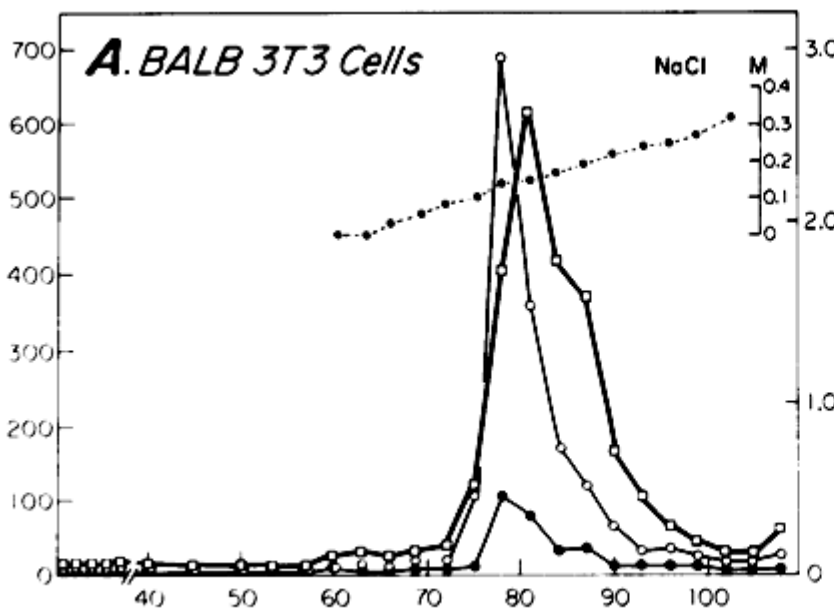
- increasing elution volume improves resolution, but dilutes sample





- collect fractions as column elutes
- analyze fractions for components of interest

Kinase Units/ml



pmole [³H]-cAMP/ml

Fraction Number