

# Today's class:

## Purification of Biomolecules

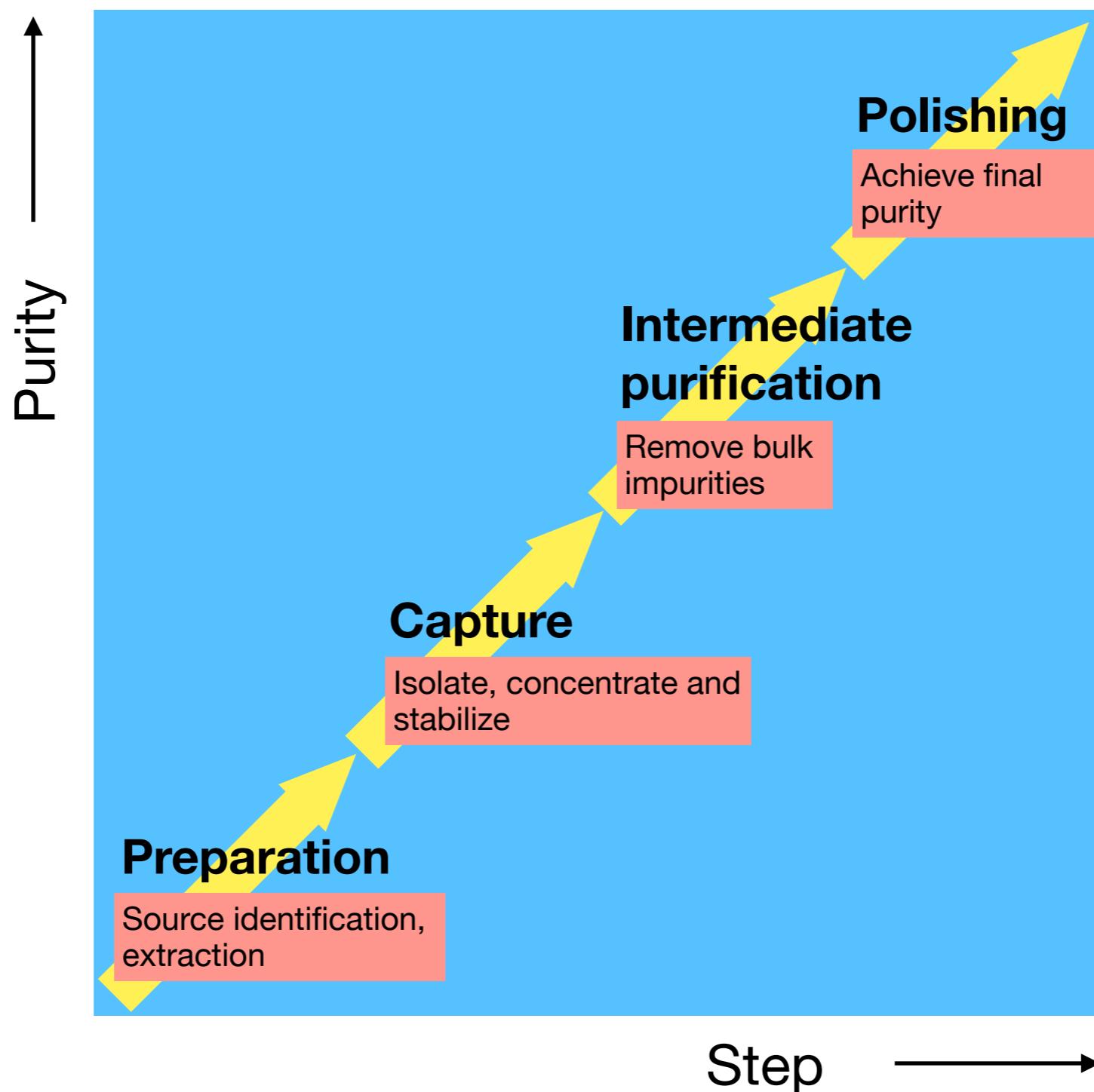
*This lecture follows the materials from different sources*

- *Fundamentals of Biochemistry by Voet, Voet and Pratt, 5th Ed, Wiley*
- *Proteins: Structure and Function by David Whitford, Wiley*

## Why do we need to purify biomolecules?

- To study functions in the cell
- To analyze physical properties
- To determine structural properties
- For uses in industrial or therapeutic applications

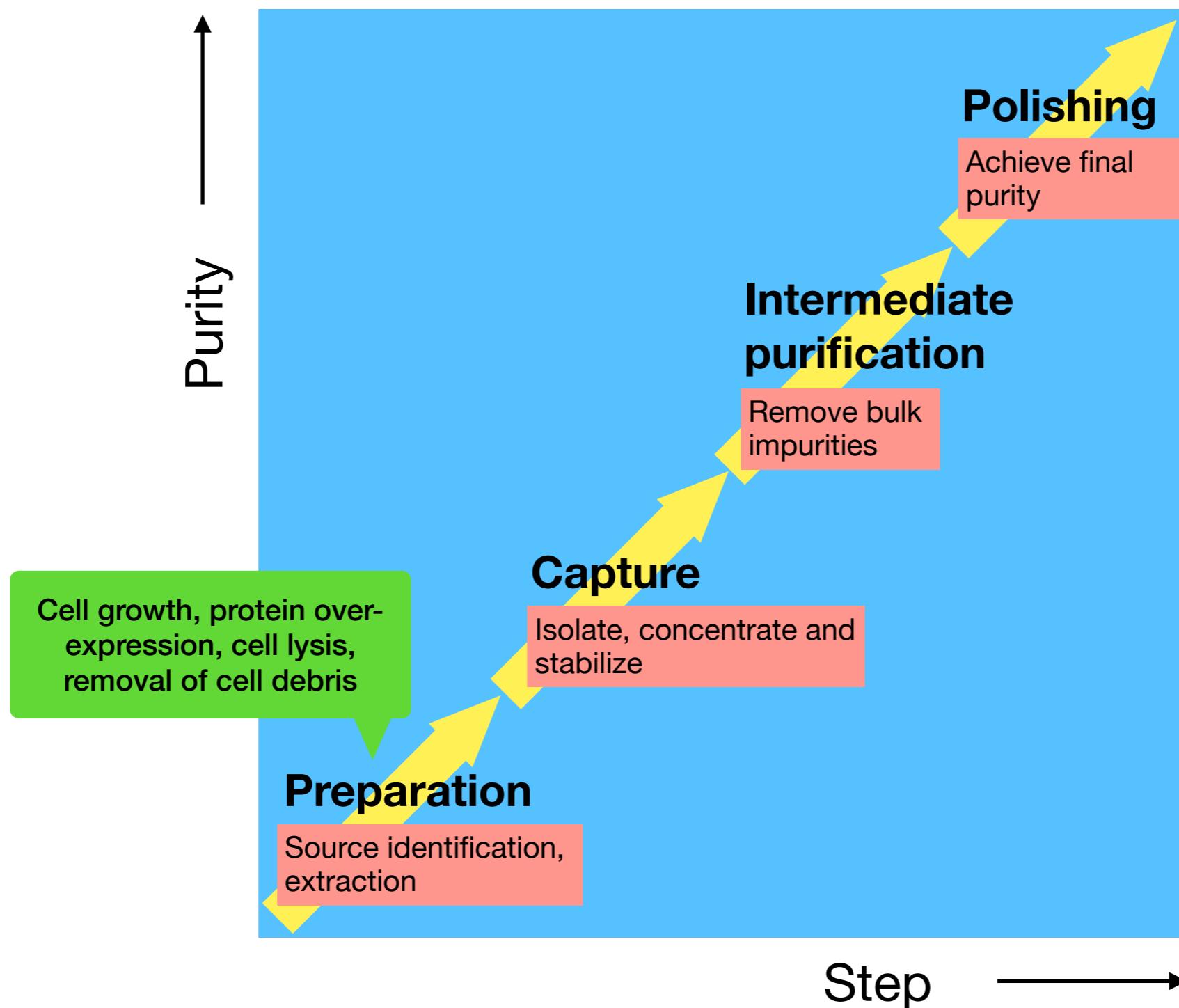
# Basic steps of any bimolecular purification process



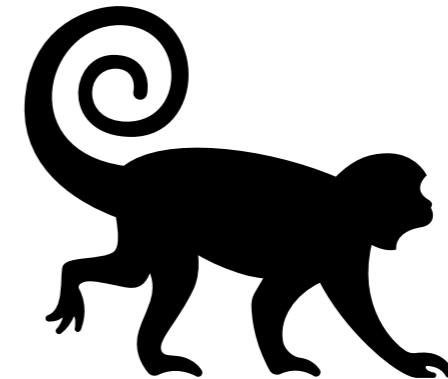
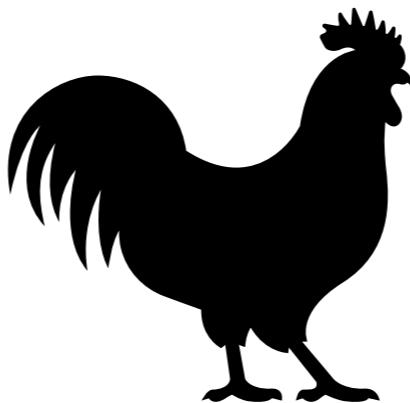
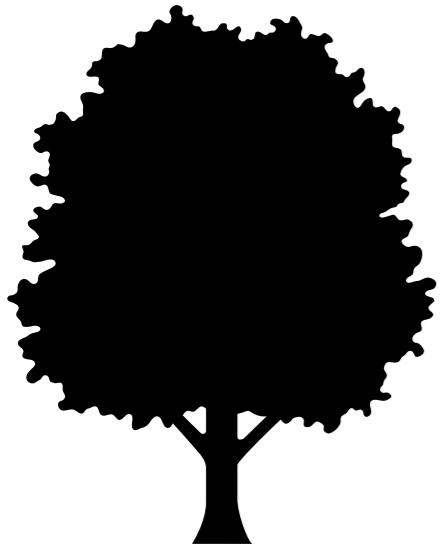
# Protein Purification based on physical and chemical properties

Protein Characteristic	Purification Procedure
Solubility	Salting out
Ionic Charge	Ion exchange chromatography Electrophoresis Isoelectric focusing
Polarity	Hydrophobic interaction chromatography
Size	Gel filtration chromatography SDS-PAGE
Binding Specificity	Affinity chromatography

# Steps of protein purification



# Choosing sources for protein isolation



Two strategies

- Directly isolate from the host organism - animals or plant tissues
- Use molecular biology to express the protein of interest in a host such as *E.coli*.

## Criteria for choosing a particular source

- Ease of obtaining sufficient quantities of the tissue - *blood is easy to get!*
- Amount of protein in the tissue - *high vs low conc*
- Any properties specific to the protein that stands out - *secreted, muscle etc*

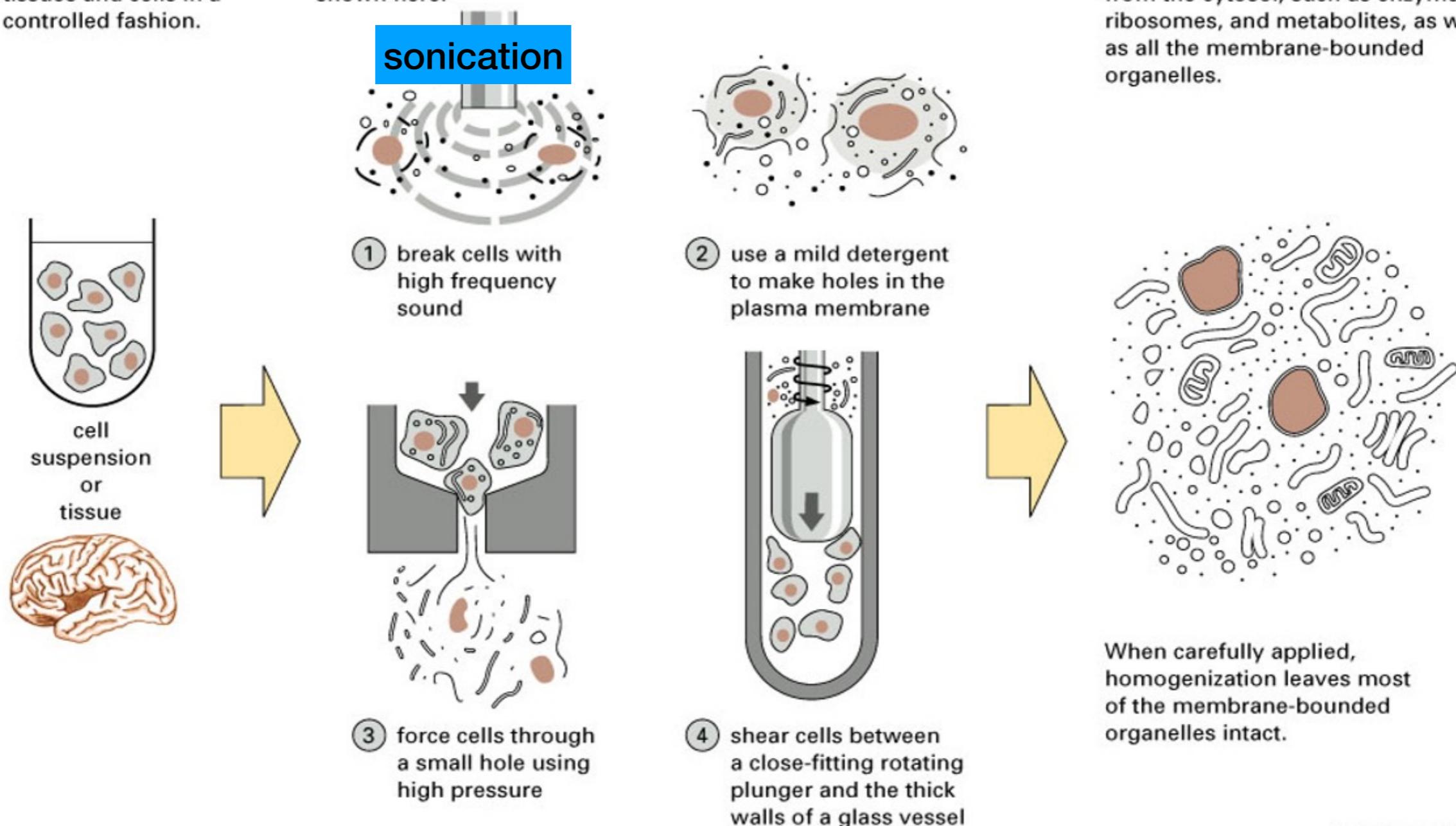
# Extraction of the protein

## BREAKING CELLS AND TISSUES

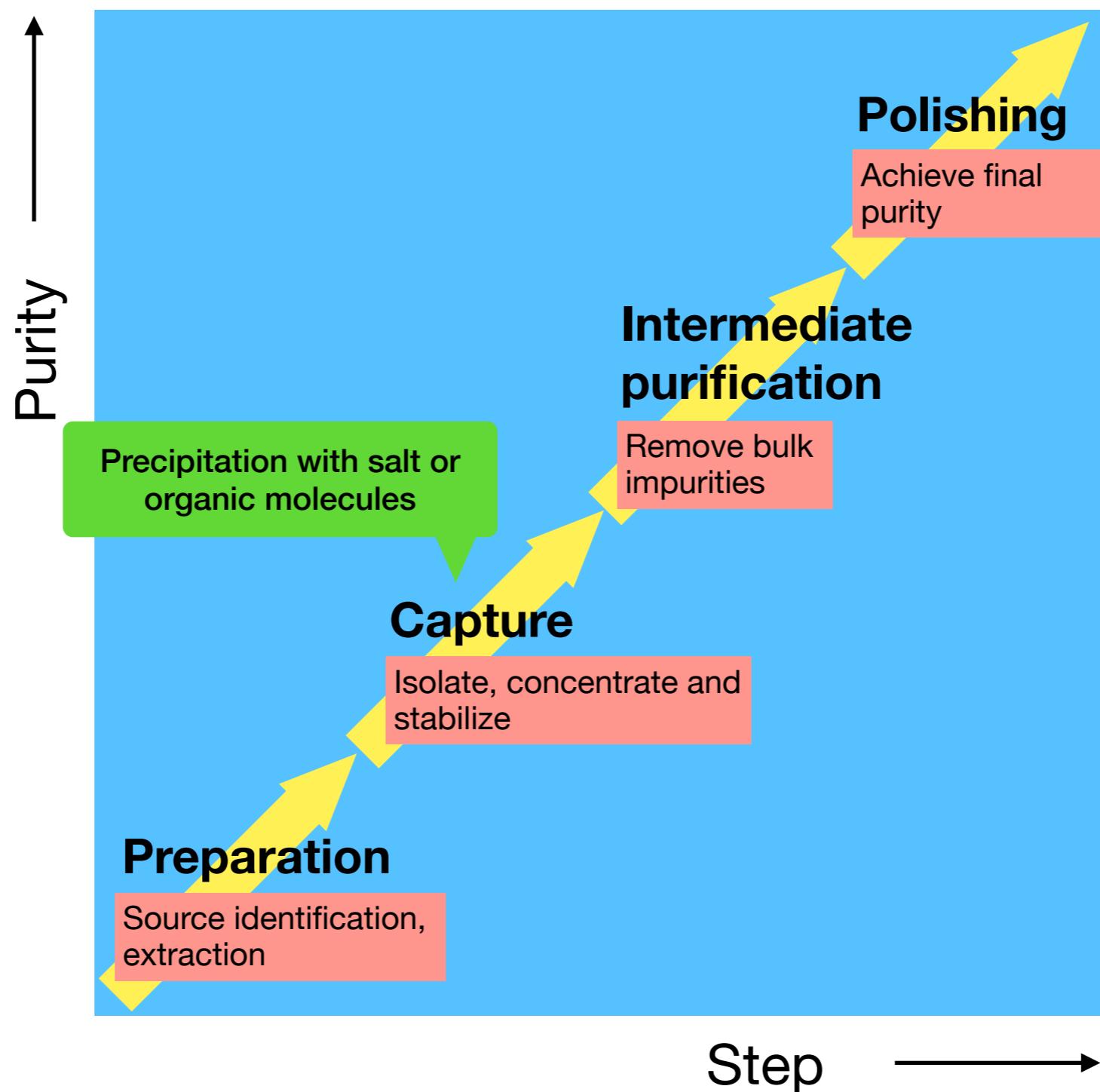
The first step in the purification of most proteins is to disrupt tissues and cells in a controlled fashion.

Using gentle mechanical procedures, called homogenization, the plasma membranes of cells can be ruptured so that the cell contents are released. Four commonly used procedures are shown here.

The resulting thick soup (called a homogenate or an extract) contains large and small molecules from the cytosol, such as enzymes, ribosomes, and metabolites, as well as all the membrane-bounded organelles.



# Steps of protein purification



# Fractional precipitation of proteins

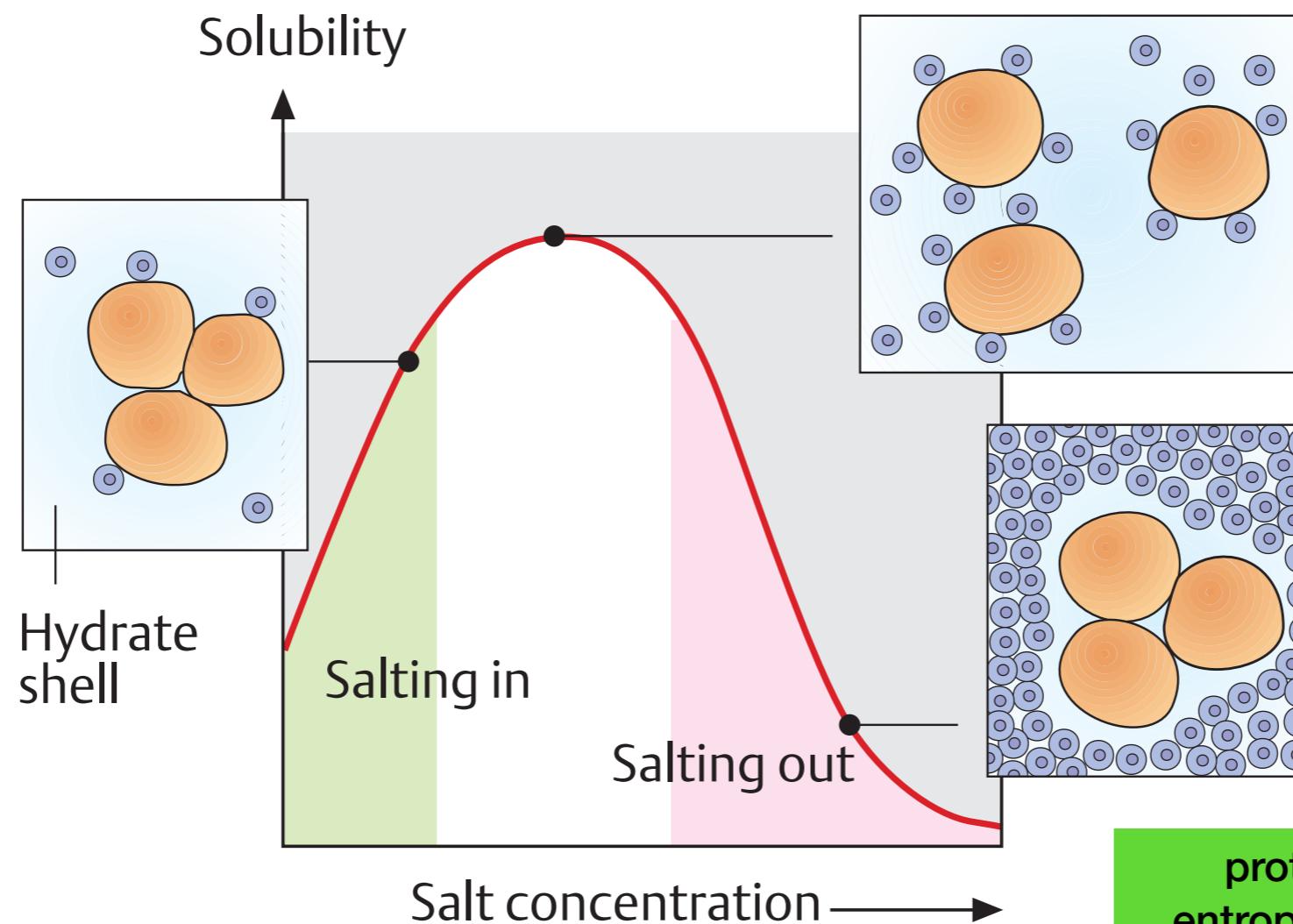
These methods alter solubility to separate proteins

Protein solubility can be altered via two main strategies

- Changing the ionic strength of the medium - *Salt precipitation*
- Tuning pH of the medium - *Isoelectric precipitation*

# Salt precipitation of proteins

As ionic strength of the medium increases  
proteins become more soluble



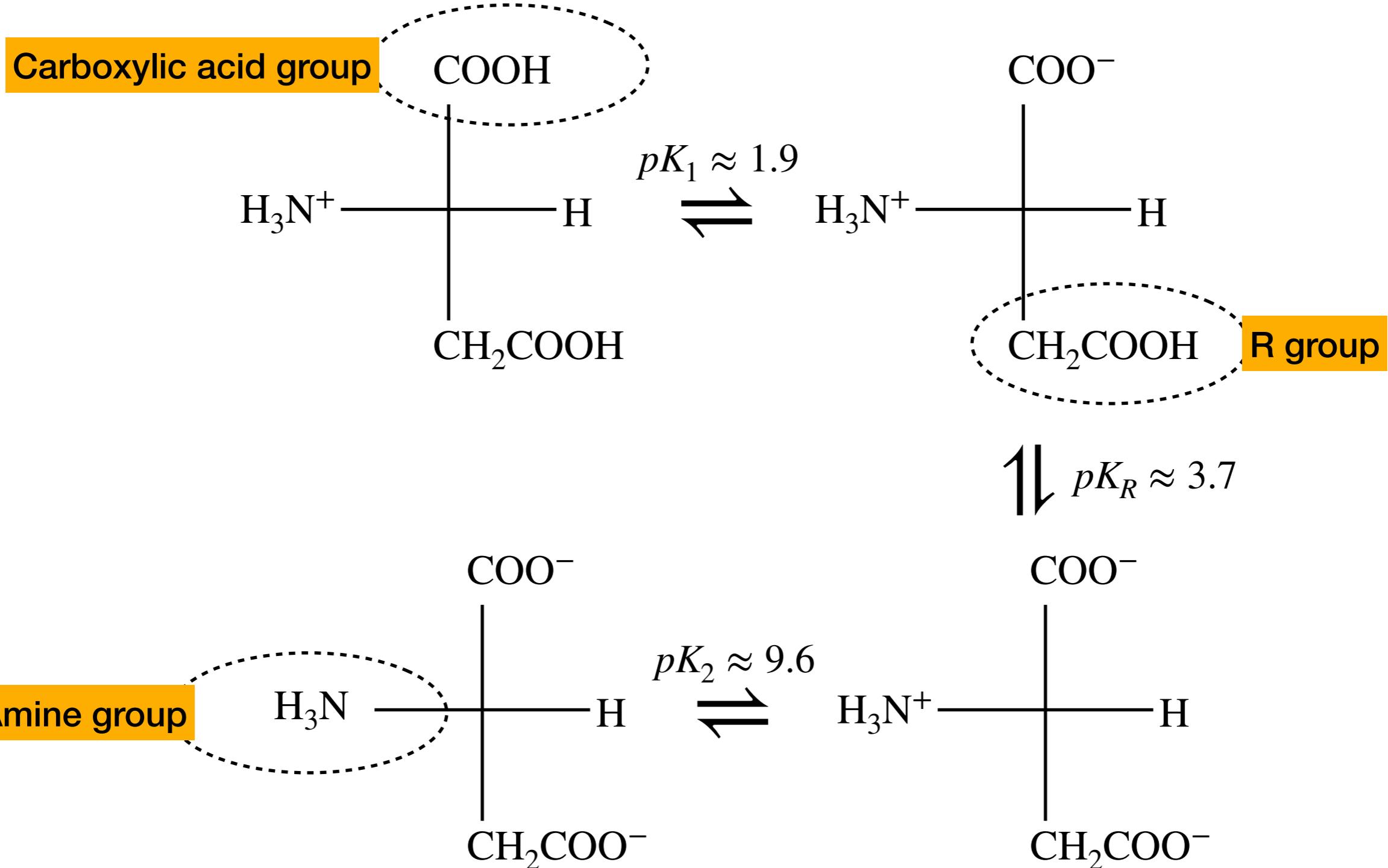
As ionic strength becomes very high too many ions compete with proteins for hydration

protein aggregation is the entropically favorable outcome which precipitates the protein

## Ammonium sulfate is usually the chosen salt for protein precipitation

- It is cheap
- It is highly soluble
- It produces ions that interact with much more water molecules than nearly any salt
- Does not react with the proteins

## Isoelectric precipitation of proteins uses zwitterionic nature of amino acids



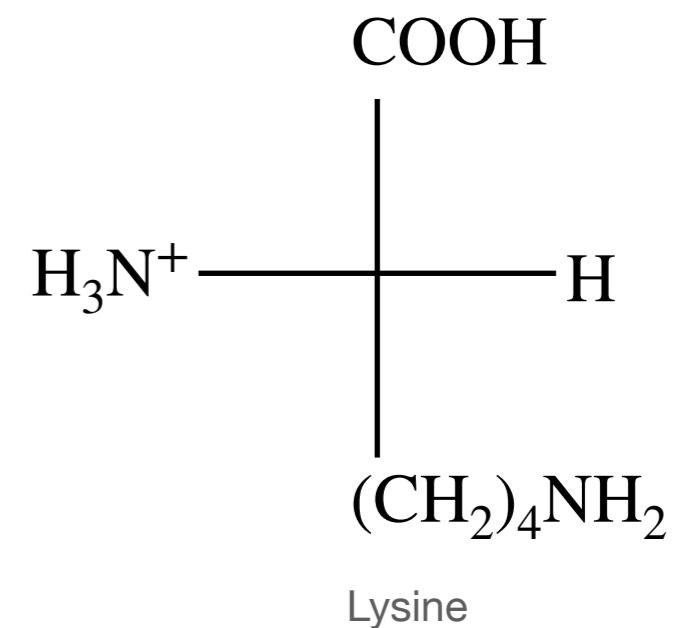
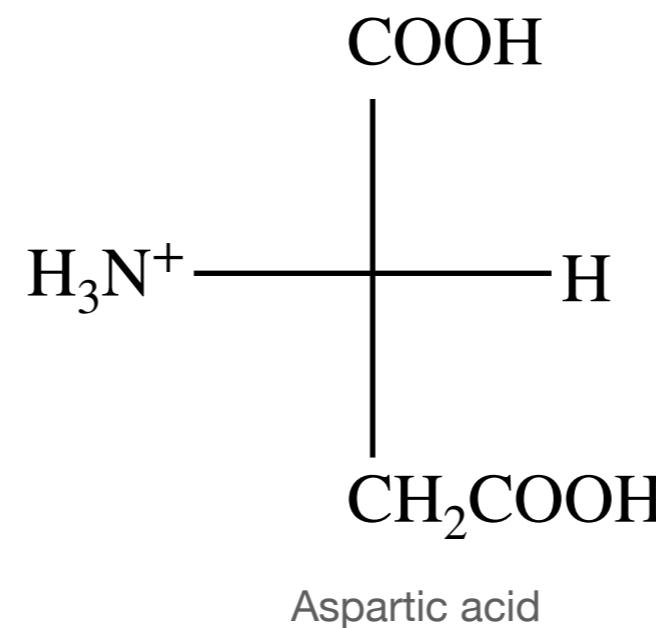
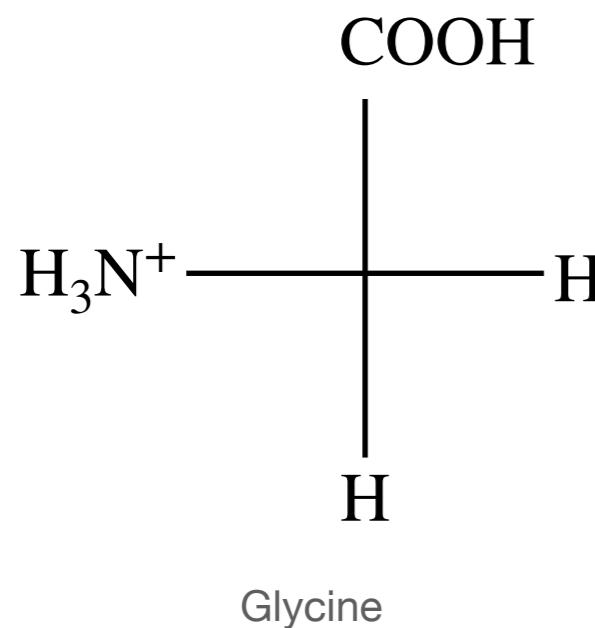
## Isoelectric point of amino acids

$pI$  = Isoelectronic point of the protein, pH at which the protein is exactly neutral

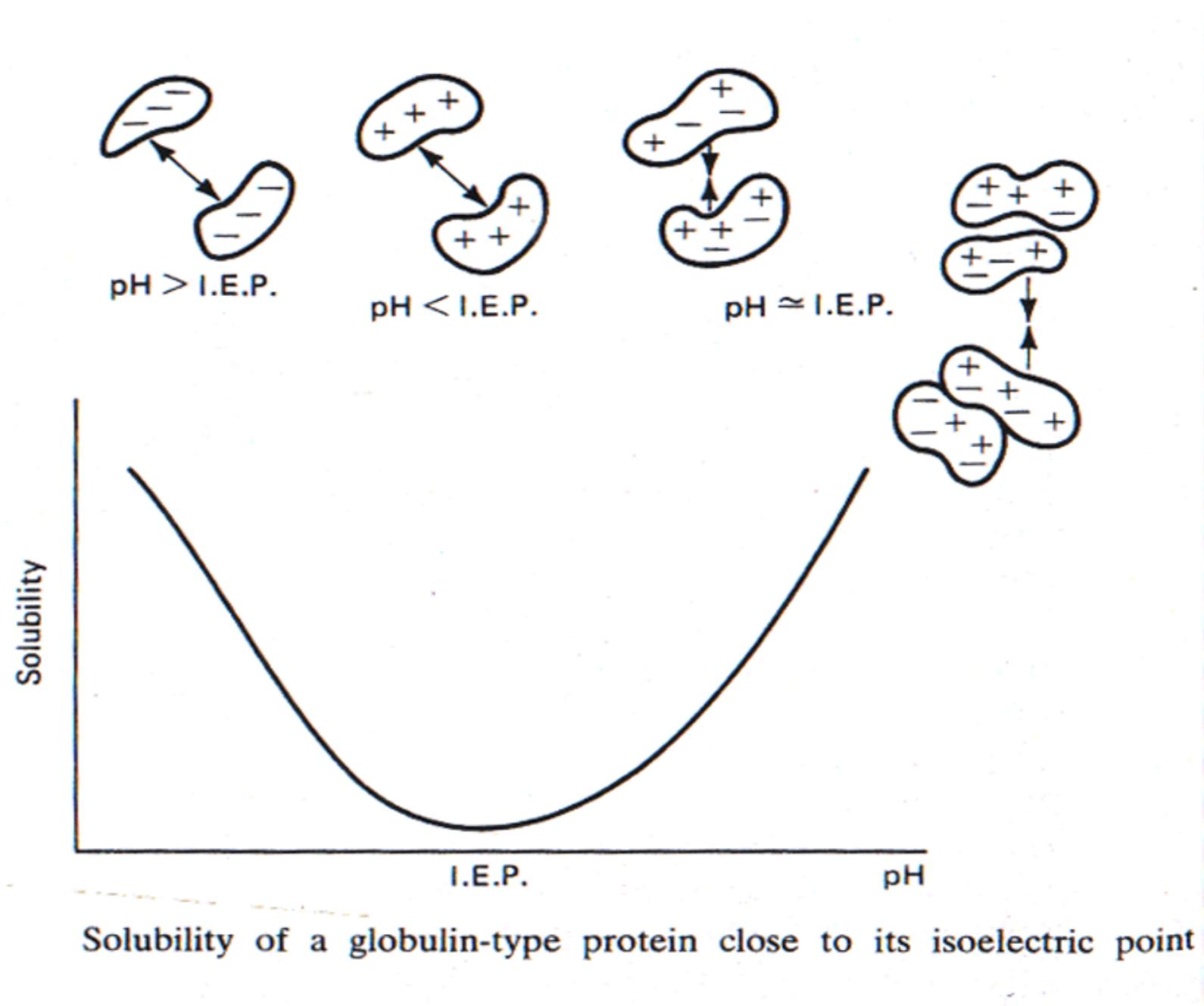
$$pI = \frac{1}{2}(pK_i + pK_j)$$

where  $K_i$  and  $K_j$  are the dissociation constants of the two ionizations involving the neutral species.

- For monoamino, monocarboxylic acids such as glycine,  $K_i$  and  $K_j$  represent  $K_1$  and  $K_2$ .
- For aspartic and glutamic acids,  $K_i$  and  $K_j$  are  $K_1$  and  $K_R$
- For arginine, histidine, and lysine,  $K_i$  and  $K_j$  are  $K_R$  and  $K_2$



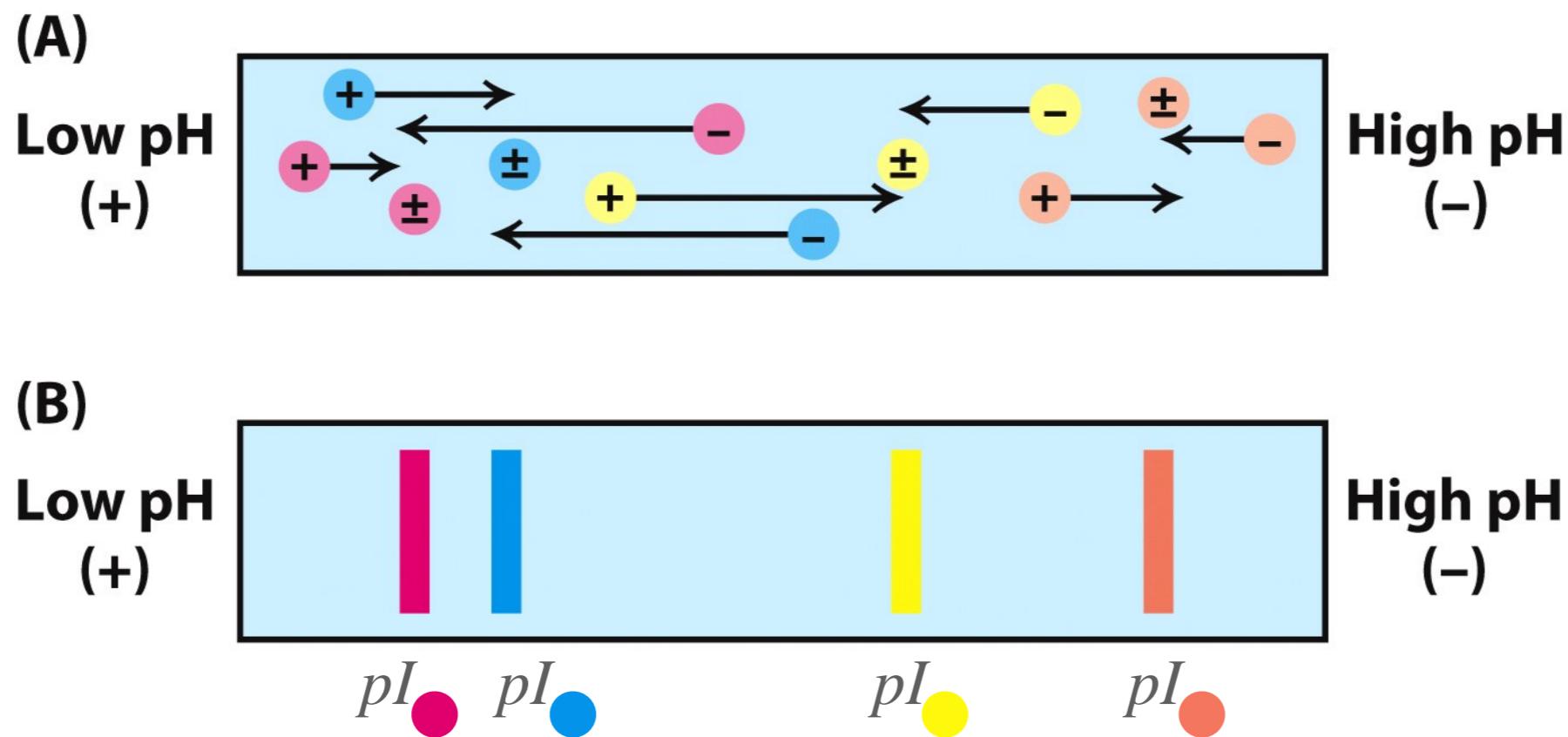
# Isoelectric precipitation of proteins



Here the pH of the protein mixture is adjusted to the pI of the protein to be isolated to selectively minimize its solubility

# Isoelectric focusing of proteins depends on pH gradients and electrophoresis

A protein is immobile in an electric field at  $\text{pH} = \text{pI}$

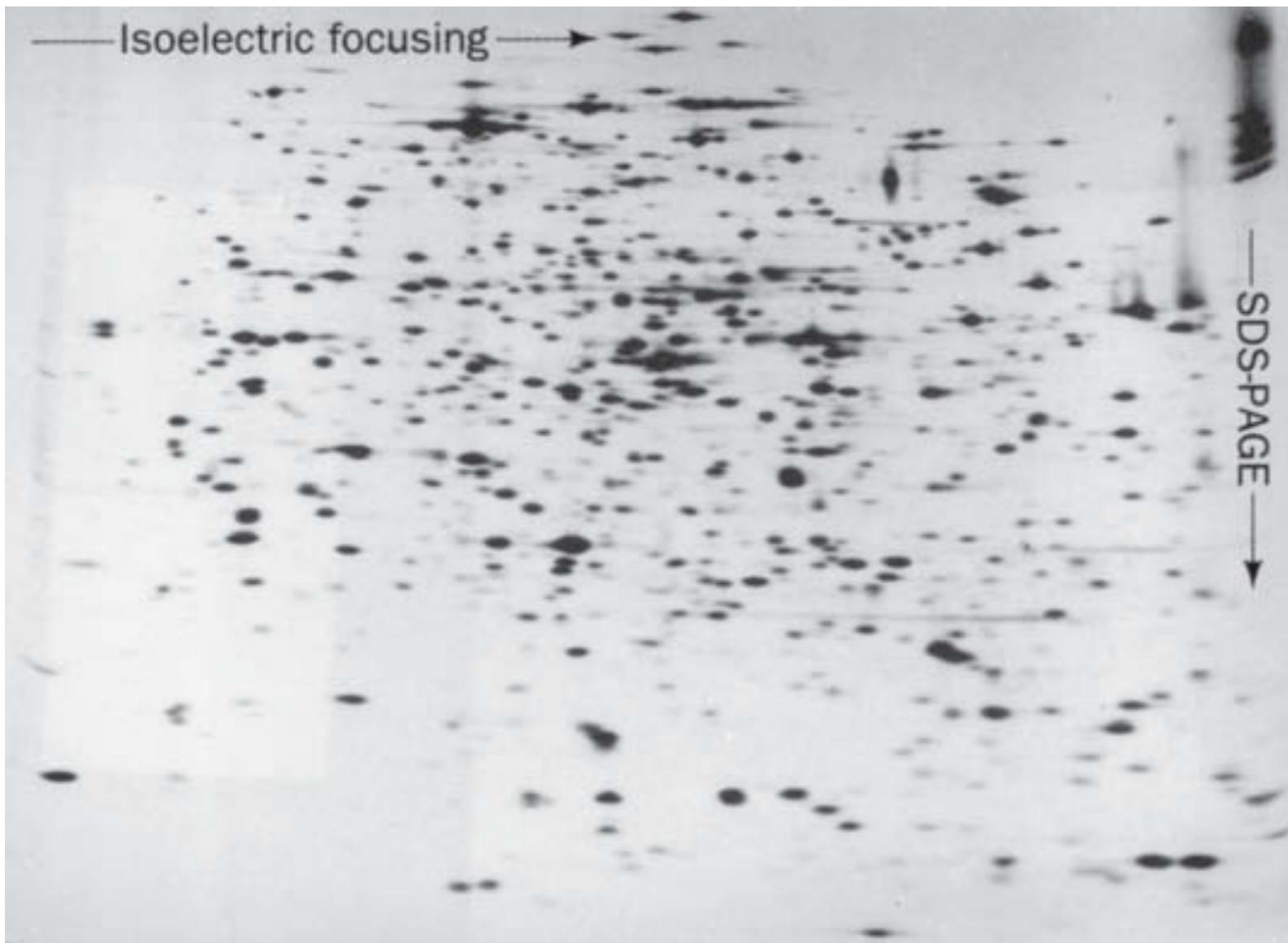


Any movement away from their isoelectric position, alters the net charge on the protein and the resulting electrophoretic forces moves it back to its isoelectric position.

Each species of protein is thus “focused” into a narrow band about its  $\text{pI}$

# Two dimensional gel electrophoresis

Isoelectric focusing + gel electrophoresis



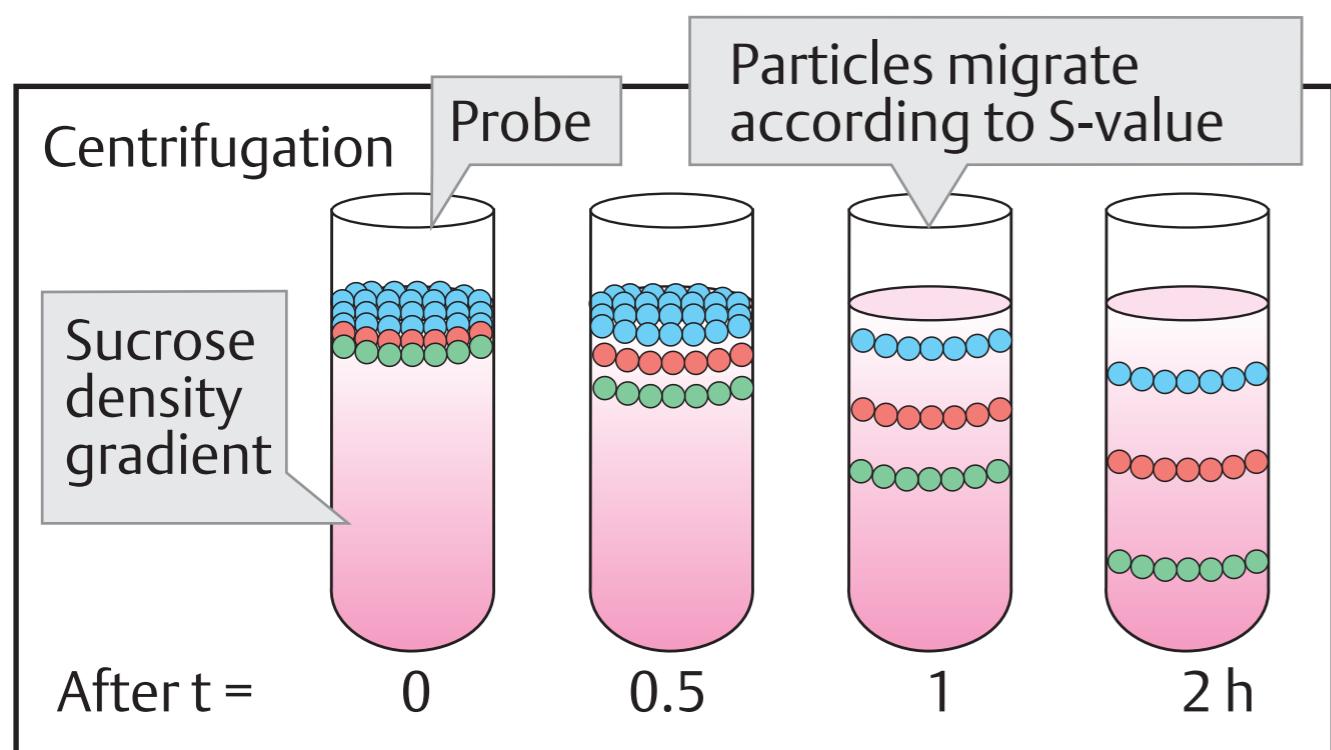
Courtesy of Patrick O'Farrell, University of California at San Francisco

**FIG. 5-11 Two-dimensional gel electrophoresis.**

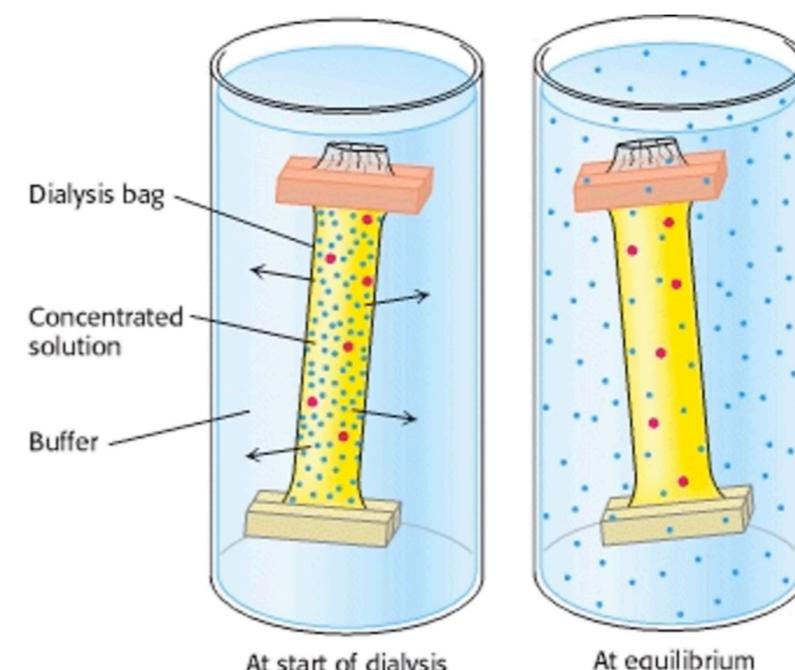
In this example, *E. coli* proteins that had been labeled with  $^{14}\text{C}$ -amino acids were subjected to isoelectric focusing (horizontally) followed by SDS-PAGE (vertically). More than 1000 spots can be resolved in the autoradiogram shown here.

# Proteins of similar solubility can be discriminated by molecular size

## Zonal centrifugation



## Dialysis



None of these processes can fully distinguish between proteins

That's why we need more sophisticated methods like

Liquid chromatography

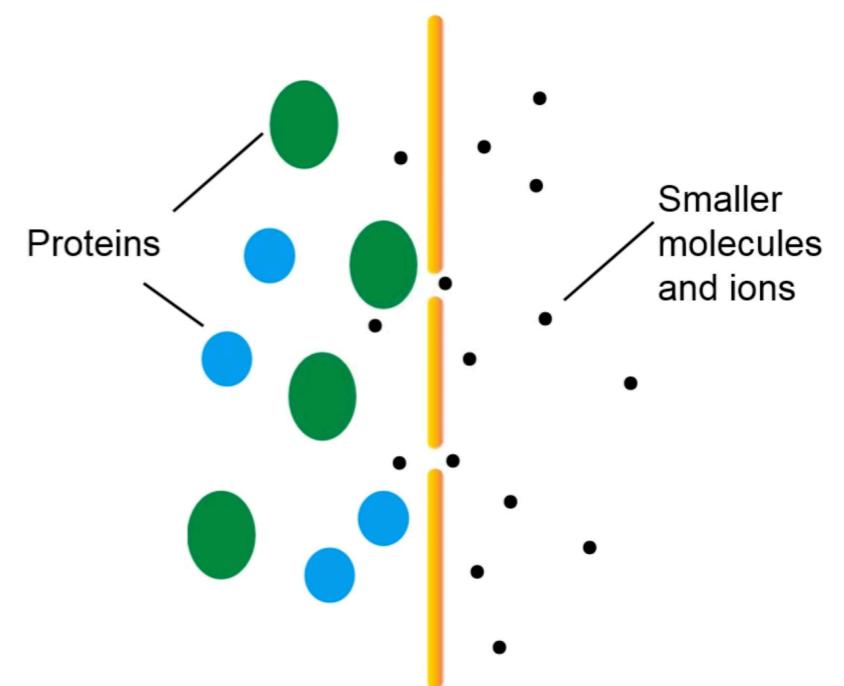


Image courtesy: Creative Biomart