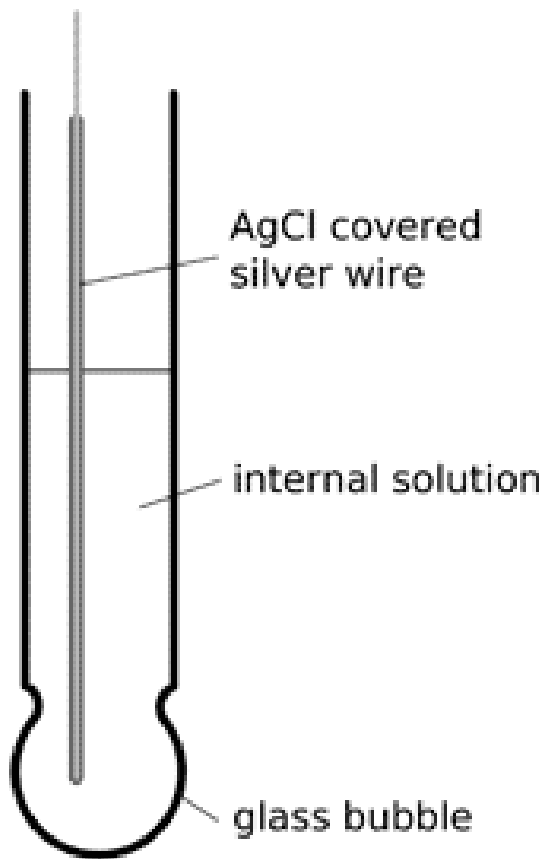# Chapter 2: Buffers and Titrations

Purpose:

1) Get to know your pH meter

2) Make a common buffer used in biochemistry and perform titrations of that buffer with acid or base to find the pK_a values for the buffer

3) Hydrolyze BSA with trypsin and calculate the number of Lys and Arg residues that BSA contains

pH Meter

- Glass-electrode sensitive to hydrogen ions
- Electrode somewhat sensitive to other alkali metals
- Complete system contains:
  - Electrometer – 5
  - Reference Electrode – 6
  - Solution to be measured – 1,4
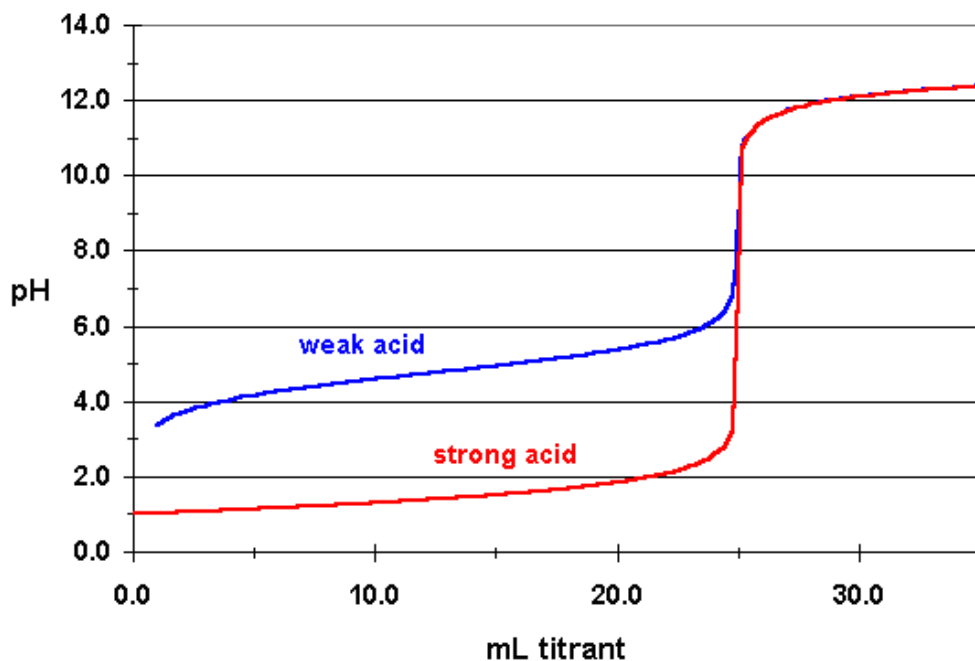 - Glass Electrode – 2,3
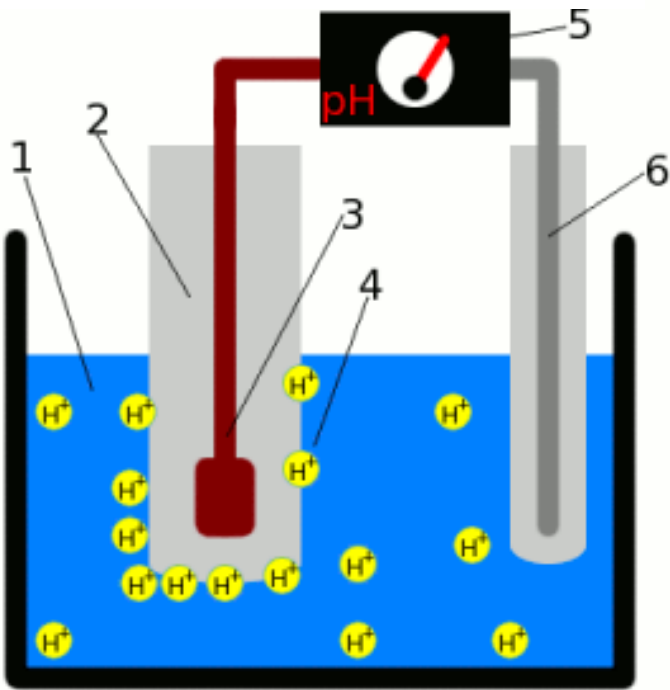
Titration Curves in Non-buffered Solutions

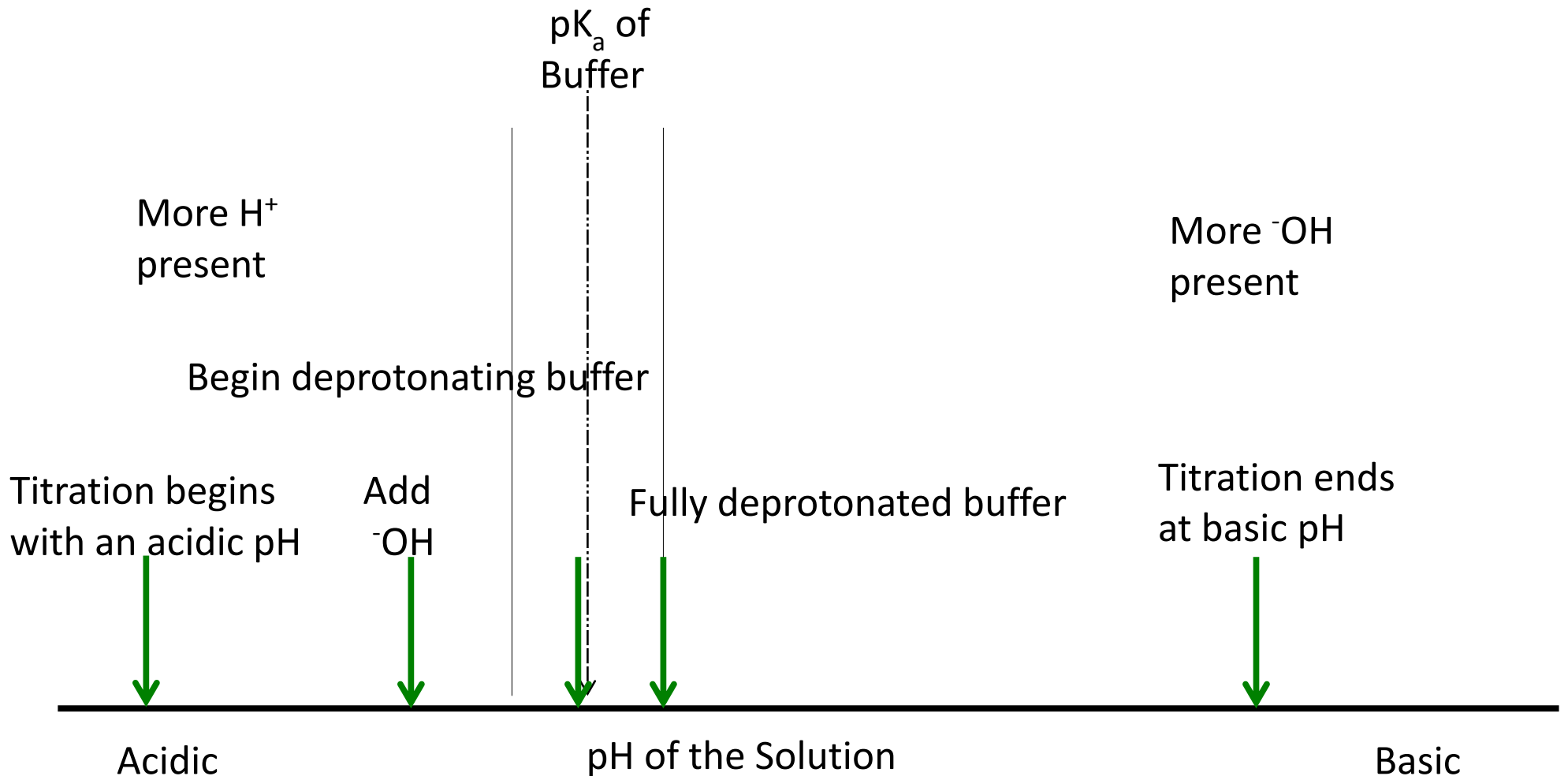Weak Acid =
0.1 M Acetic Acid
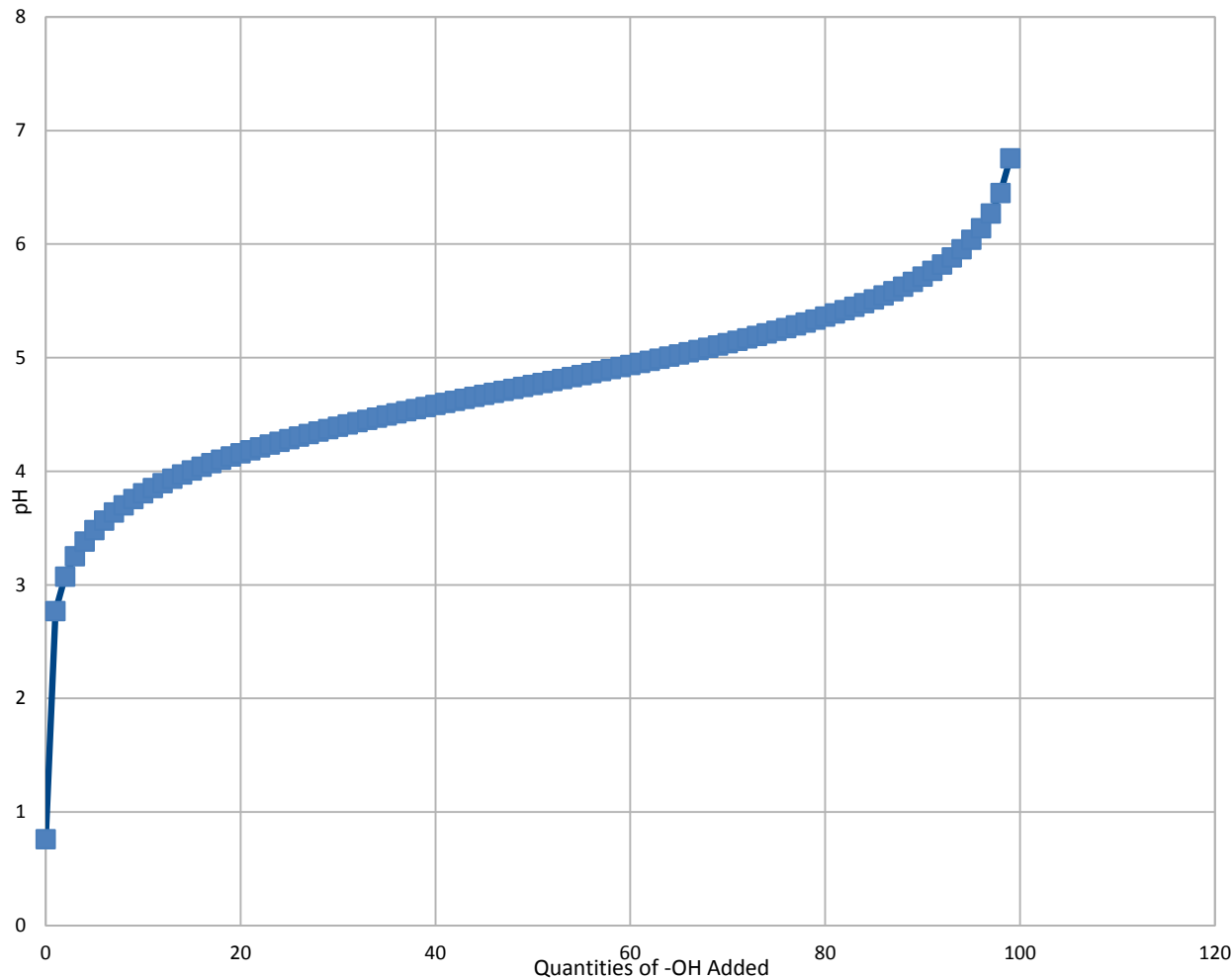Strong Acid =
0.1 M Hydrochloric Acid

- Equivalence Point
  - Point at which reaction is neutralized
  - Inflection point in titration curve

- Strong Acid – pH 7.0

- Weak Acid – pH 8.8

- Buffered solutions behave as weak acids

- Table of pK_a values – Lab Manual p. 36

pH Changes in Buffered Solutions

pK_a of
Buffer

More H^+
present

More ^-OH
present

Begin deprotonating buffer

Titration begins
with an acidic pH

Add
^-OH

Fully deprotonated buffer

Titration ends
at basic pH

Acidic

pH of the Solution

Basic

Buffered Titration Curve

Titration of a Buffer

- Modeled on Henderson-Hasselbach equation

$$pH = pK_a + \log\frac{[A^-]}{[HA]}$$

$$HA \rightleftharpoons A^{-1} + H^+$$

Buffered Titration Curve

Titration of a Buffer
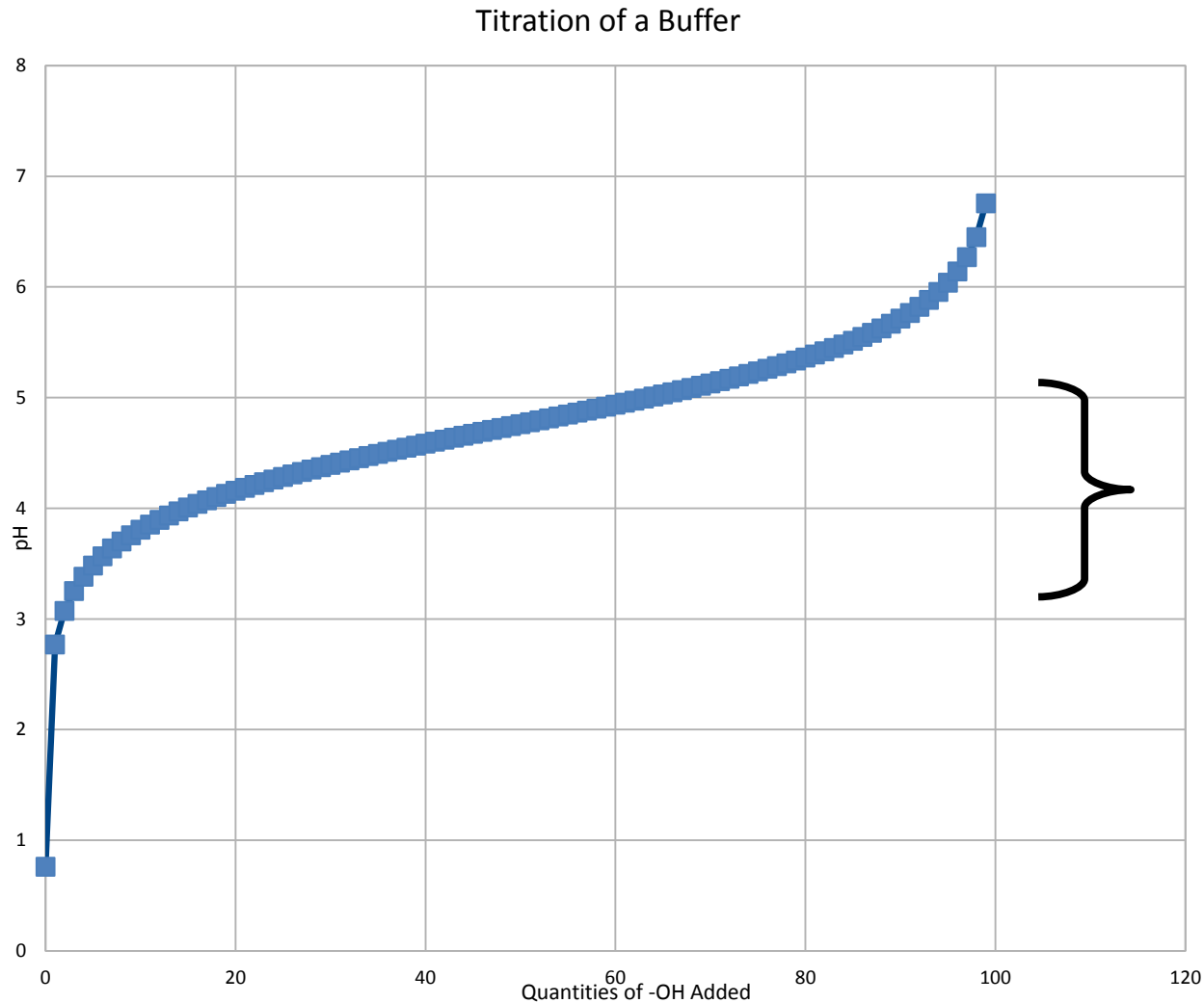
- Empirically, H-H equation useful for buffering range

- Buffers most effective near pK_a

$$pH = pK_a \text{ when } [A^-] = [HA]$$

Buffering Capacity

- Ability of buffer to resist changes in pH with addition of acid or base

$$\text{Buffer Capacity} = \frac{-dH^+}{dpH} = 2.303 \left[\frac{[A^-][HA]}{[A^-]+[HA]} \right]$$

- Highest buffering capacity obtained when $[A^-] = [HA]$

Procedure: Titration

- Make His Buffer

  - Starting pH?

- Four Titrations

  - Titrate Acid Group of His

  - Titrate the Two Basic Groups of His

  - Titrate Water with Acid

  - Titrate Water with Base

- Subtract Water Values from His to Get Pure His Curve
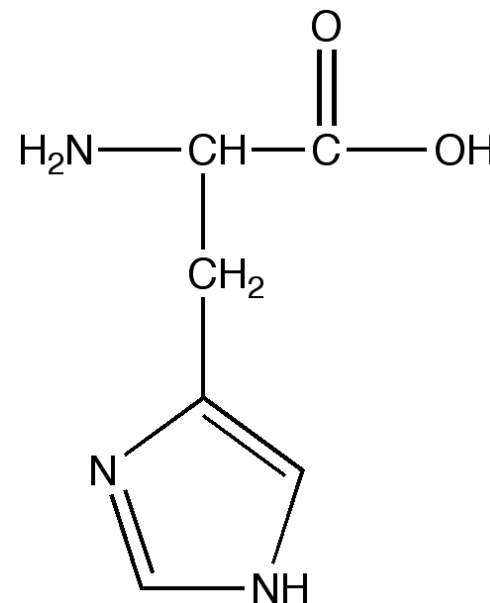
Procedure: Titration

- Make His Buffer – 0.4 M His-HCl = 0.4 M HA

- Deprotonated His (His^0) = $[A^-]$ = $[H^+]$

$$pH = pK_a + \log\frac{[A^-]}{[HA]}$$

$$pH = pK_a + \log\frac{[H^+]}{[HA]}$$

$$-\log[H^+] = -\log[K_a] + \log[H^+] - \log[HA]$$

$$2(-\log[H^+]) = -\log[K_a] - \log[HA]$$

$$2pH = pK_a - \log[HA]$$

- Substituting pK_{a2} of His = 6.04

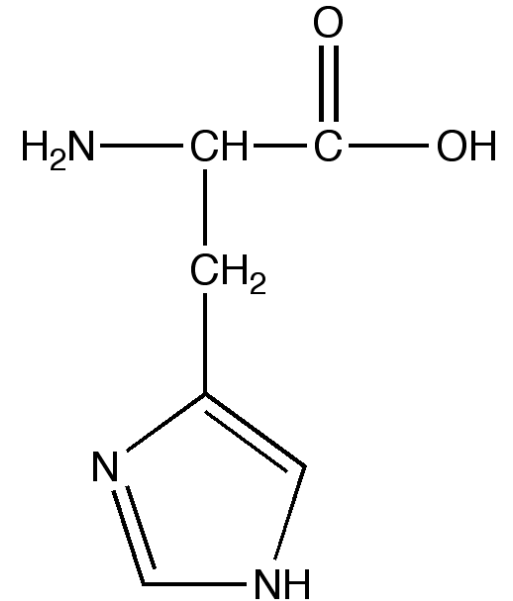$$pH = (6.04 - \log[0.4])/2 = 3.22$$
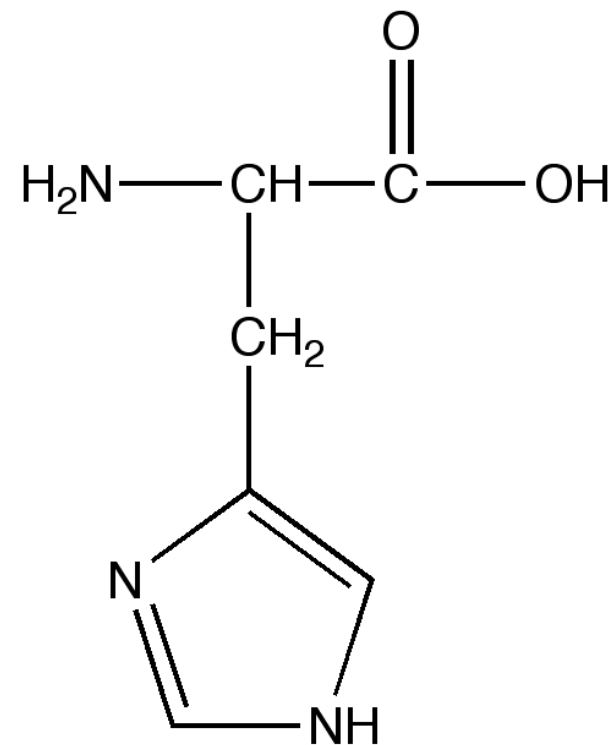
Derivation p. 54
of Lab Manual

Procedure: Titration

- Make His Buffer
  - Starting pH = 3.22
- Four Titrations
  - Titrate Acid Group of His
  - Titrate the Two Basic Groups of His
  - Titrate Water with Acid
  - Titrate Water with Base
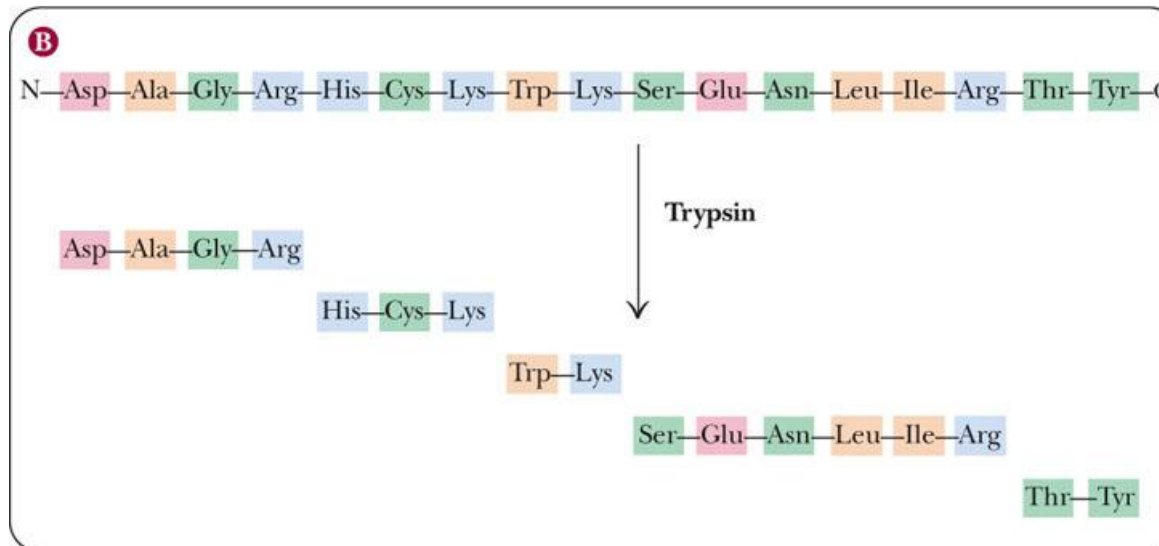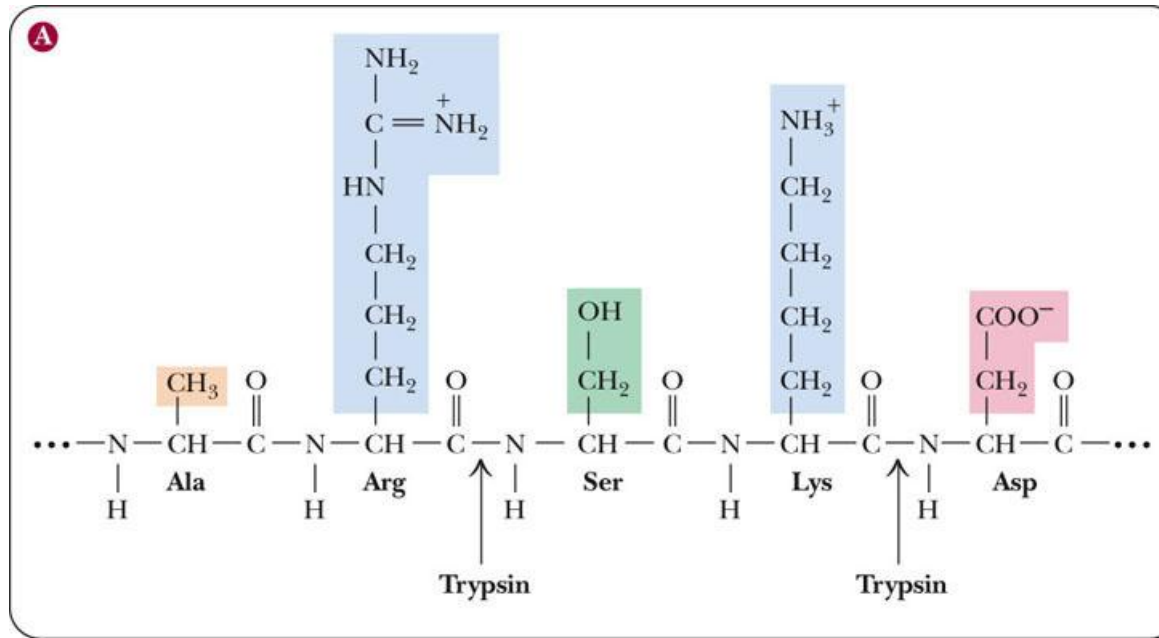- Subtract Water Values from His to Get Pure His Curve

Digestion of BSA with Trypsin

Proteolytic Cleavage of Proteins

Trypsin

Cleaves C-terminal of (+) charged side chains

Trypsin

© Cengage Learning

# Procedure: Determining the Number of Lys and Arg (combined) in BSA

- Denature BSA at 80-90 °C until cloudy

- Digest BSA with Trypsin

  - Titrate during reaction to maintain pH value 8.5

  - Indicate volume KOH added and the time elapsed

- Calculate the Number of Peptide Bonds Cleaved When Reaction is Complete

  - Calculate mmols KOH added at endpoint

  - Calculate number of Arg + Lys per molecule BSA

Relating the Titration to Arg + Lys Residues

Denatured BSA, M_r = 66,000 g/mol

Trypsin Cleavage

H⁺   H⁺   H⁺   H⁺   H⁺
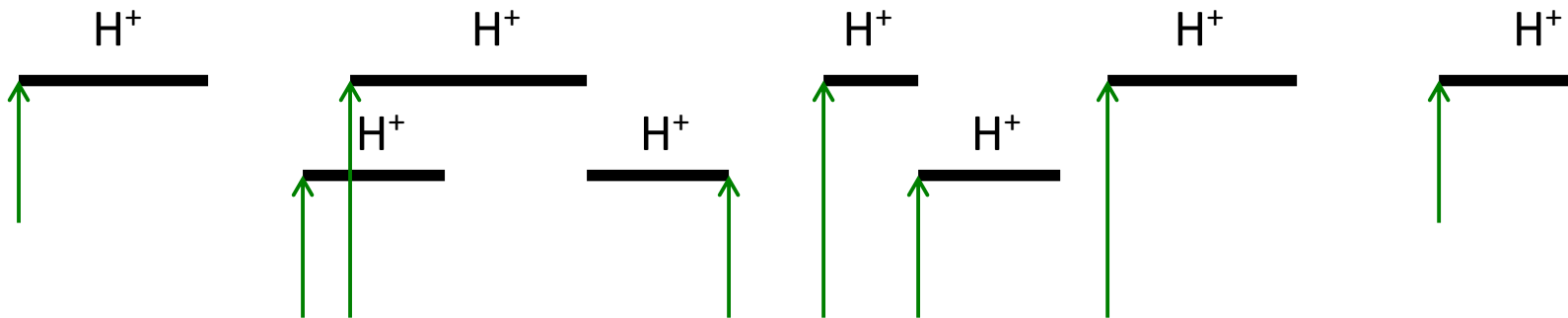
New N-Termini Add to Buffer Capacity

Relating the Titration to Arg + Lys Residues

- Since pH is only slightly greater than the pK$_a$ of N-terminus

  - Each new N-terminus will buffer the new H$^+$ released from the reaction

    - Not every amino group will gain a proton

- How much H$^+$ is actually produced?

  - Depends on ratio of [A$^-$]/[HA]

  - If pH is constant, [A$^-$]/[HA] must remain constant

Relating the Titration to Arg + Lys Residues

- **Problem 10, p. 43**: What is ratio of $[A^-]/[HA]$ for the protonation of an amine with a $pK_a = 8.2$, at pH 8.5?

$$R\text{-}NH_2 + H^+ \rightleftharpoons R\text{-}NH_3^+$$

$$pH = pK_a + \log\frac{[A^-]}{[HA]}$$

$$8.5 = 8.2 + \log [R\text{-}NH_2]/[R\text{-}NH_3] = 8.2 + \log [A^-]/[HA]$$

pH of reaction   Amino group pK_a

$$0.3 = \log [A^-]/[HA]$$

$$\boxed{[A^-]/[HA] = 10^{0.3} = 2/1}$$

2/3 depronated $[A^-]$, 1/3 protonated $[HA]$

Relating the Titration to Arg + Lys Residues

- The trypsin digestion alters the **buffer capacity** of the solution

    - As more amino groups are formed, some accept a proton

    - Other protons are neutralized by KOH titration

- **Total # of peptide bonds cleaved** = (mmol of KOH added)(3 peptide bonds cleaved/2 mmol KOH added)

- **Total # of Lys + Arg per molecule of BSA** =
(# of peptide bonds cleaved)/(mmol of BSA used)

    - Calculate mmol of BSA using MW (66,000 g/mol)