Foundations in Biomedical Sciences

Gordon Rule
Department of Biological Sciences
Carnegie Mellon University

rule@andrew.cmu.edu

- Hydrogen bonds
  - Polar versus non-polar bonds
  - Hydrogen bonding in biochemistry
- Water
  - Structure and acid-base
- pH
  - Measurement of pKa by titrations
  - Generation of buffer solutions
- Amino acids
  - Calculation of protein concentration
  - Effect of pH on charge, calculation of charge.
  - Interpretation of fluorescence changes
  - UV absorption
- Protein Structure and stability
  - Primary & secondary structure

Polar Bonds & Molecules

Covalent Bond: Electron are shared between two atoms.

Polar Bond: A bond is polar if there is a *significant* difference in the electronegativities of the participating atoms, giving *appreciable partial charges on the atoms*.

The partial charges are proportional to the difference in the electronegativities of the two atoms: $\Delta e = e_A - e_B$

Electronegativities increase across the periodic table due to increased nuclear charge.

1 **H** 2.1							2 **He**
3 **Li** 1.0	4 **Be** 1.5	5 **B** 2.0	6 **C** 2.5	7 **N** 3.0	8 **O** 3.5	9 **F** 4.0	10 **Ne**

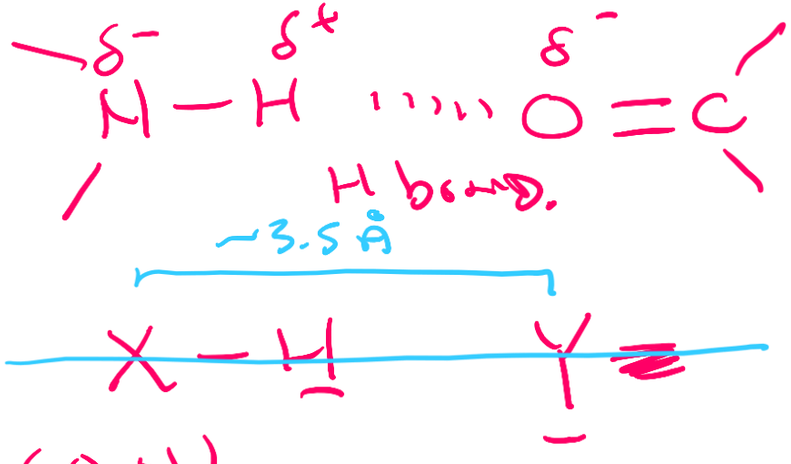
	$\lvert\Delta e\rvert$
C(2.5)—C(2.5)	0.0
C(2.5)—H(2.1)	0.4
C(2.5)—N(3.0)	0.5
C(2.5)—O(3.5)	1.0
O(3.5)—H(2.1)	1.4

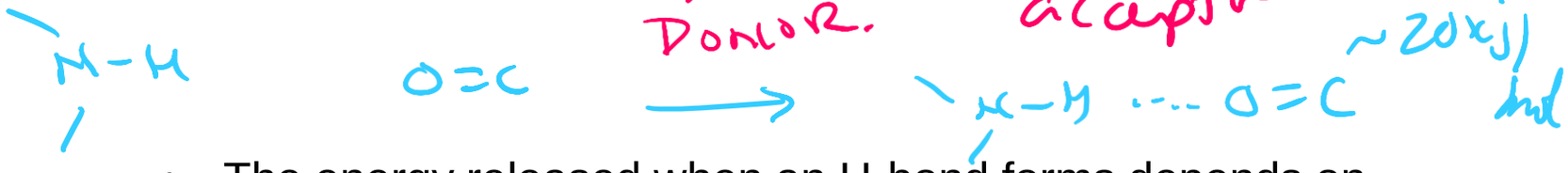
Reflection: *How do we know which bonds are polar?*

Hydrogen Bonds

- H-bonds are primarily (90%) an electrostatic attraction between:
  - Electropositive hydrogen, attached to an electronegative atom is the hydrogen bond donor (i.e. NH).
  - Electronegative hydrogen bond acceptor (e.g. the lone pairs of oxygen, or C=O group of an amide).

	$\lvert\Delta e\rvert$
N——H 3.0 2.1	0.9
O——H 3.5 2.1	1.4





- The energy released when an H-bond forms depends on the distance and angle of the bond.
  - Typical distance between electronegative atoms ~3.5 A
  - Favorable geometry when donor atoms and acceptor are linear (angle = 180°)
- Usually hydrogen bonds are exchanged, resulting in small ***net*** energy differences:

A "bond" implies electron sharing – about 10% of the electron is shared from one molecule to the next in the case of H-bonds

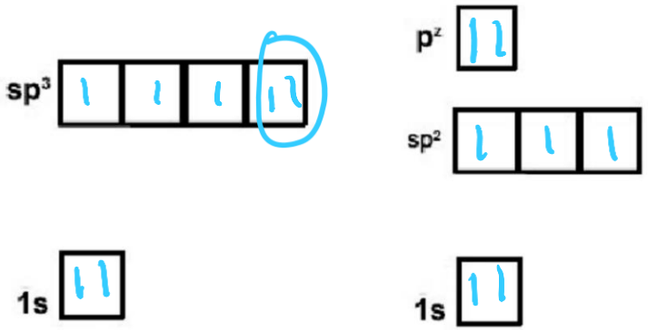
*Note that the proton is **NOT** transferred to the acceptor, it remains covalently bonded to the donor atom. The Hydrogen Bond is the **interaction** between the X-H donor and electronegative acceptor.*

How to Identify Hydrogen Bond Donor and Acceptors

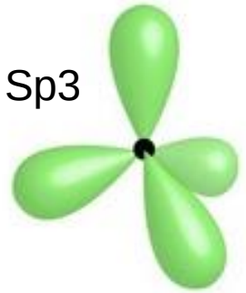
- O-H and N-H are always donors

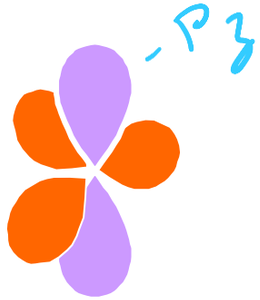
N in a delocalized system:

- Will not accept from above or below the plane of the system, because the lonepair is delocalized.
- Can accept in the plane of the ring if there is no attached hydrogen, via lone pair in sp2 orbital

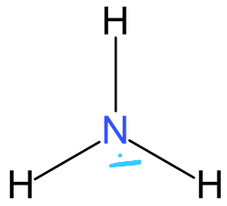


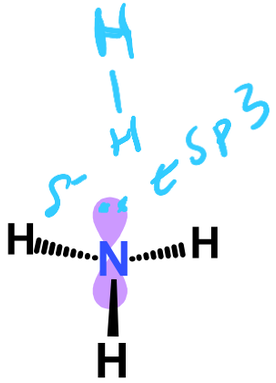
N – 7 electrons



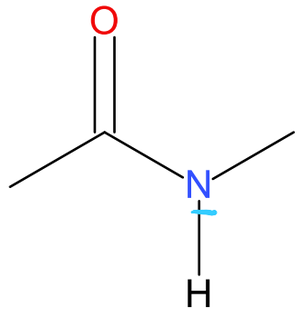


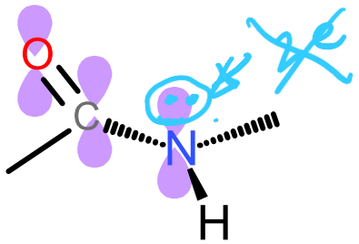
Nitrogen can form two types of hybrid orbitals, sp3 (tetrahedral geometry) or sp2 (planer) + pz





- Sp3 is used in ammonia, keeping the three hydrogen atoms as far from the full lonepair. The fourth sp3 orbital is full, with two electrons (lone pair).
- The lone pair is an excellent acceptor.



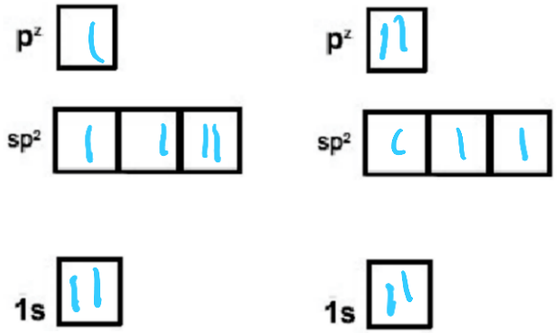


- Sp2 is used in amides, allowing favorable overlap of the full pz orbital with the pz on C and O
- The lone pair in the nitrogen pz is shared with the pz electrons on carbon and oxygen.
- Due to electron sharing, there is only a slight neg. charge and the group does not accept an H-bond.

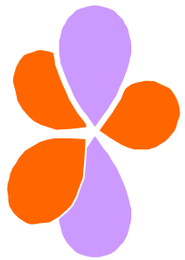
How to Identify Hydrogen Bond Donor and Acceptors

N in a delocalized system:

- Will not accept from above or below the plane of the system, because the lonepair is delocalized.
- Can accept in the plane of the ring if there is no attached hydrogen, via lone pair in sp2 orbital



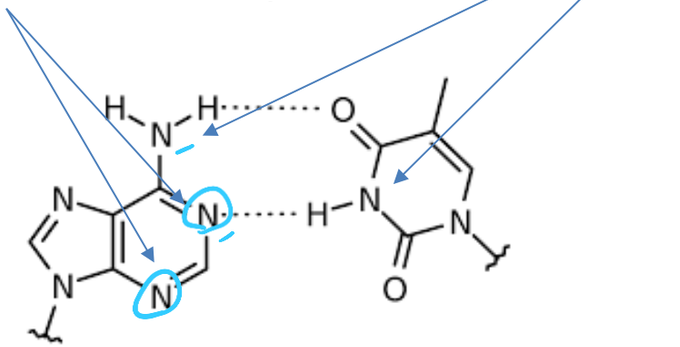
N – 7 electrons



Sp2

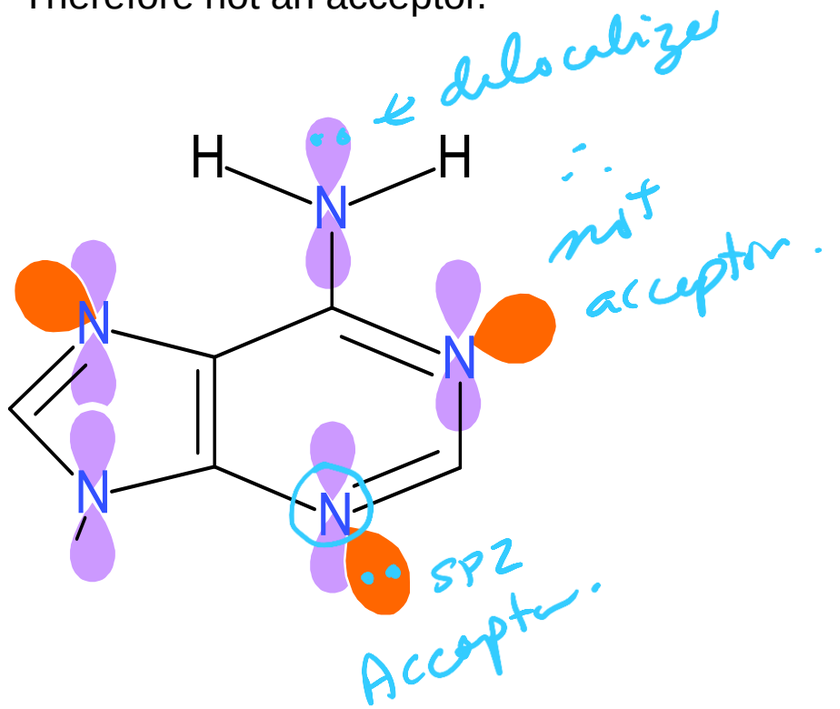
In planer aromatic rings the nitrogen must use sp2

- The pz orbital holds one electron, used to form double bonds
- The non-bonding sp2 contains the lone pair, an excellent electron acceptor.

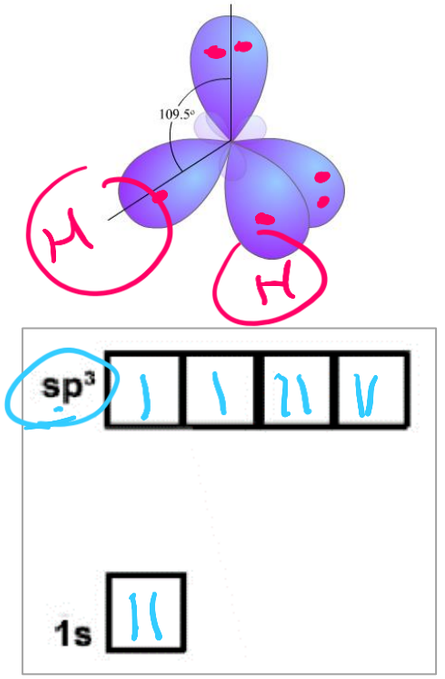


Adenine Thymine

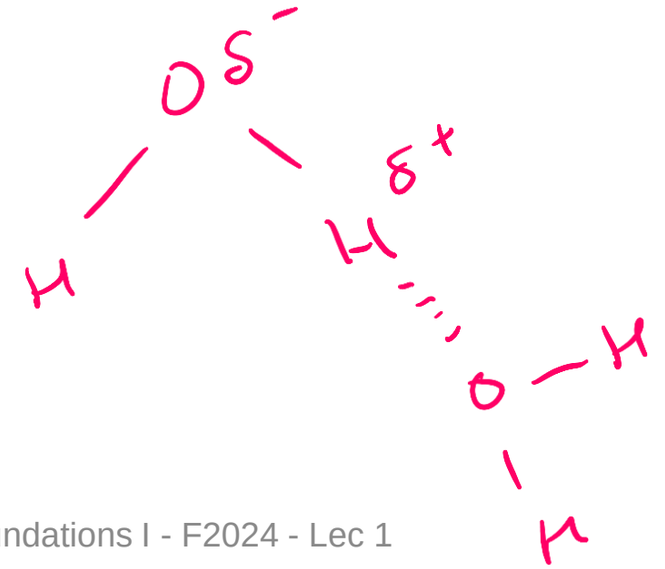
- The pz orbital holds two electrons since each sp2 has one to form the single bonds.
- Although the pz orbital contains two electrons (lone pair), these are delocalized (shared) over the rings. Therefore not an acceptor.



Structure of Water



i. Oxygen has 8 electrons. The molecular orbitals in water are complex, however much of the behavior of water can be understood by assuming that oxygen form sp3 hybrid orbitals.
ii. Sp3 hybrid orbitals are generated from the 2s and the three 2p orbitals, the orbitals point towards the corner of a tetrahydron.
iii. The orbitals in oxygen are populated such that two orbitals are filled and two contain one electron each.
iv. The filled orbitals cannot form bonds and are often called *lone pairs*.
v. The half-filled orbitals participate in the formation of a *sigma* bond between oxygen and hydrogen.



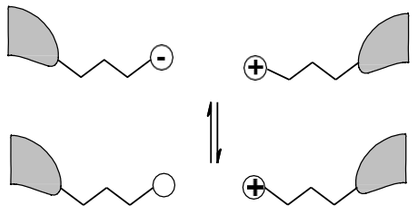
Acid-Base Chemistry

Learning Goals:

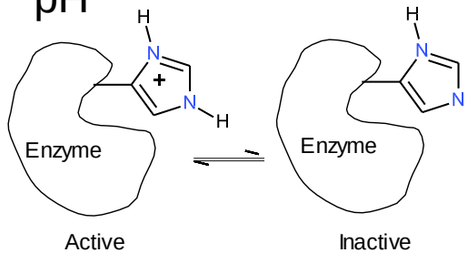
- Compare relative acid strength based on pK_a values of weak acids.
- Predict protonation state given pH of the solution and the pKa of the acid.
- Understand effect of chemical structure on pK_a values of acids in biochemical molecules

Why pH is important in Biochemistry.

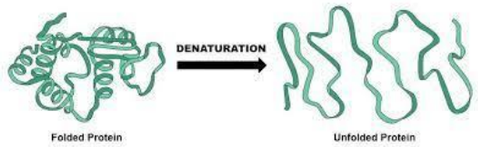
i) Molecular interactions can be sensitive to pH.



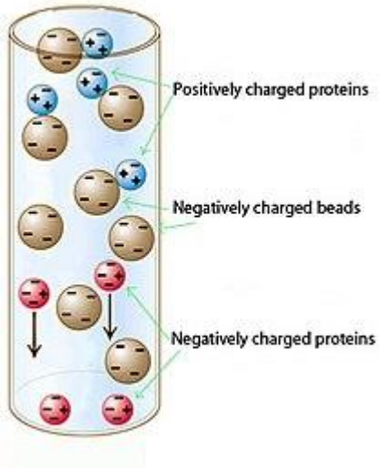
ii) Biological activity can be sensitive to pH



iii) Proteins can unfold at extremes of pH.



iv) Protein purification by ion exchange is affected by pH

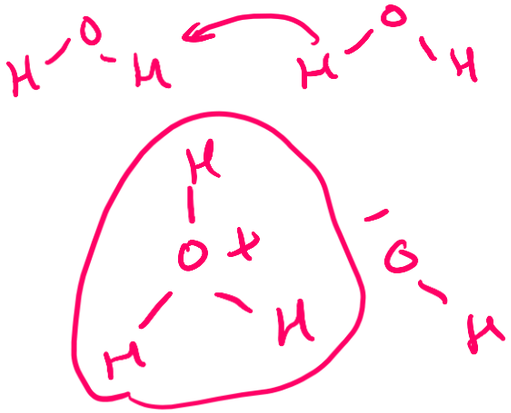


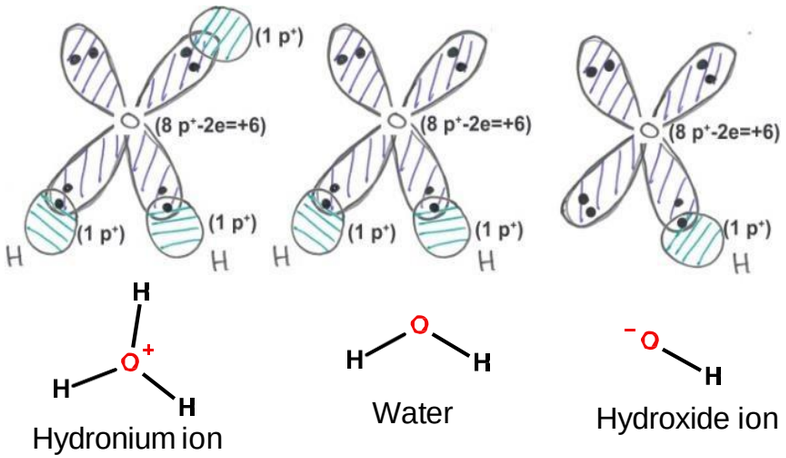
Changing the pH can change the charge on ionized groups.

Ionization Properties of Water:

Water can gain a proton on one of its lone pair orbitals to become a hydronium ion H_3O^+, or it can lose a proton to become a hydroxide ion (OH^-).

The hydronium ion is usually abbreviated H^+ or "proton".





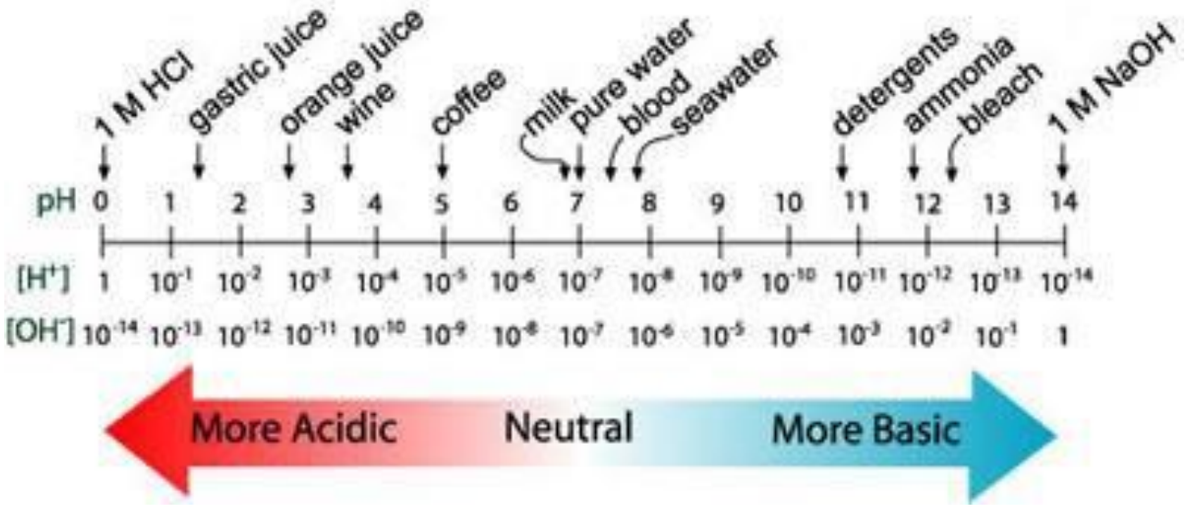
How to represent the hydronium ion concentration?

pH: pH is measured as the -log[H^+],

- smaller pH, more acidic the solution, higher [H^+].

Neutral pH is 7.0. At this pH there are an equal number of H^+ and OH^- ions in solution. [H^+]=10^{-7} M.

pH is a property of the solution, and can be changed by the addition of acids (e.g. HCl) or bases (e.g. NaOH) to any desired value.



Acids and Bases:

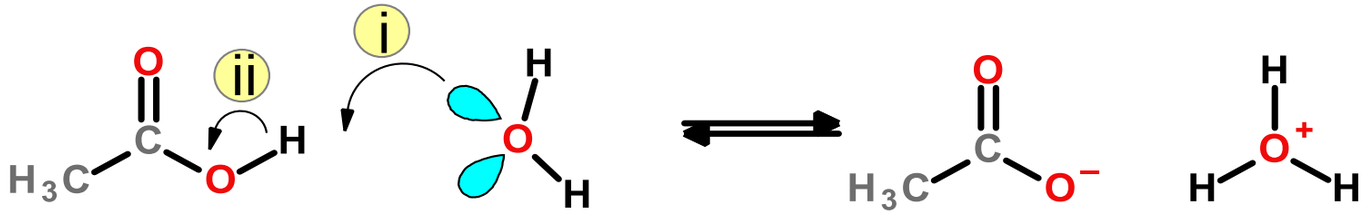
- Acid: can donate protons to water, forming its conjugate base and a hydronium ion.
- Strong acid – always completely ionized/deprotonated (pKa <= 2).
- Weak acid – mixture of protonated and deprotonated species exist over some pH range.
- Base: can accept protons (*we will refer to bases as weak acid*)

Ionization or *dissociation* of the proton from the acid:

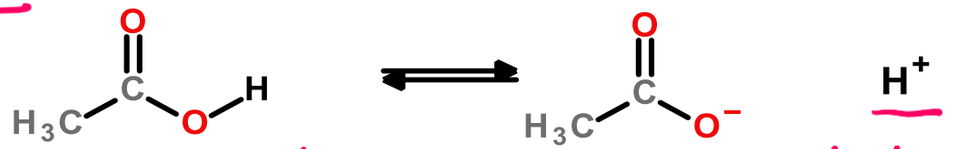
i) Lone pair electron on O (water) forms bond with hydrogen

ii) Electron in O-H bond on acid is transferred to oxygen

Overall - hydrogen is transferred to water. Acid – , Hydronium +



A simpler notation, by removing water from each side:

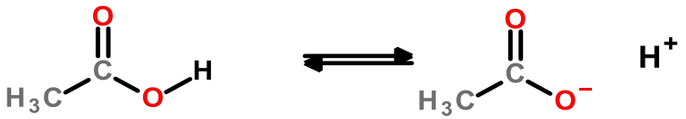


Nomenclature: Protonated deprotonated.

Key Concepts:

- We (or the cell) set the pH!
- The weak acid does not affect the pH of the solution by deprotonation or protonation.
- The weak acid responds to the pH (hydrogen ion concentration) by changing its protonation state to reflect the pH of the solution

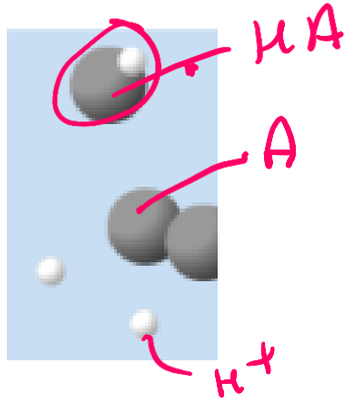
Le Chatelier's Principle

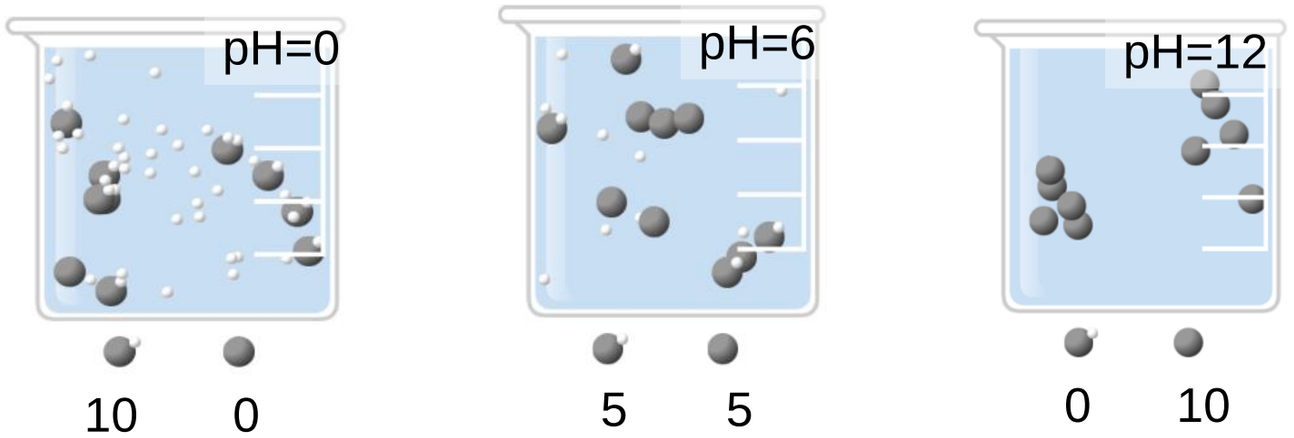


A system responds to excess reactant (product) by creating more product (reactant) to maintain equilibrium.

How does pH affect Protonation?

$$f_{HA} = \frac{[HA]}{[HA] + [A]}$$





# Collisions	many ⇒ f_{HA} = 1	$[HA] = [A]$	few f_{HA} = 0

Characterization of Acid Strength Using pK_a.

$$HA \leftrightarrow A^- + H^+$$

The equilibrium constant for that dissociation:

$$K_{EQ} = K_a = \frac{[A][H^+]}{[HA]}$$

Why?

- *Compare acids*
- *Predict ionization, given pH of solution*

The equilibrium constant for acid dissociation is given a special name, the 'k-a', or 'k-acidity'. The acidity constant, K_a is a *fundamental* property of the acid, it does **not** depend on the pH of the solution.

The pKa is experimentally measured (more later).

The pKa is a constant for a group, but depends on (more later):

- the chemical structure of the group.
- the electrostatic environment of the acidic group.

When the $[H^+] = K_a$, then exactly ½ of the acid is protonated.

$$K_a = \frac{[A][H^+]}{[HA]}$$

If $K_a = \frac{[A]}{[HA]} K_a$, then $[HA] = [A]$

pK_a: Since the pH scale is used to characterize $[H^+]$, it is useful to express the acidity constant on the same scale, by taking its negative log, giving the "p-K-a": $pK_a = -\log K_a$

When the pH = pK_a, then exactly ½ of the acid is protonated.

A lower pKa indicates a stronger acid

Equilibrium Constants: Why is the ratio of products to reactants a constant when a system is at equilibrium?

Consider a simple reaction: $A \underset{k_{Rev}}{\overset{k_{For}}{\rightleftharpoons}} B$

Equations that describe the rate of change of [A]:

$$\frac{d[A]}{dt} = -k_{For}[A] + k_{Rev}[B]$$

[A] *decreases* at a rate $k_{For}[A]$
[A] *increases* at a rate of $k_{Rev}[B]$

at equilibrium d[A]/dt=0 (d[B]/dt=0)

$$0 = -k_{For}[A]_{eq} + k_{Rev}[B]_{eq}$$

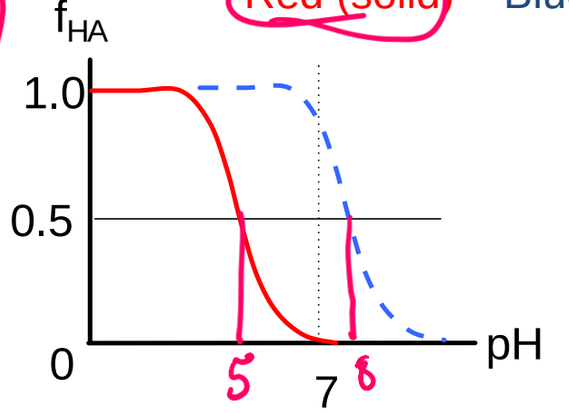
$$\frac{k_{For}}{k_{Rev}} = \frac{[B]_{eq}}{[A]_{eq}} = K_{eq}$$

Which acid has the lower pKa?

Red (solid) Blue (dashed)

Which acid is more deprotonated at pH 7 (stronger acid)?

Red (solid) Blue (dashed)

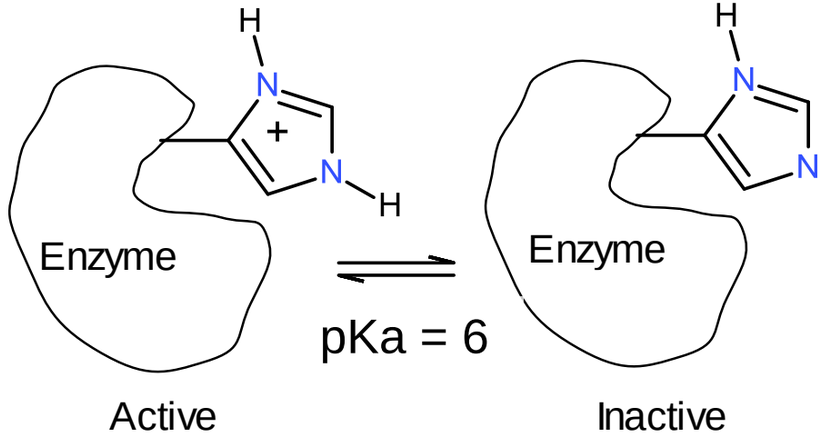


Prediction of Protonation State at any pH:

In many cases only one of the two species (protonated or deprotonated) may be biologically active.
Given the pK_a of the ionizable group, and the pH of the solution, we would like to determine how pH affects the activity of the molecule.

We want to calculate the following:

- The fraction that is protonated: f_{HA}.
- The fraction that is deprotonated: f_{A^-}



Activity ∝ f_{HA}

Defining Fraction Protonated (f_{HA})/Deprotonated(f_A)

$$f_{HA} = \frac{[HA]}{[HA]+[A]} = \frac{1}{1+\frac{[A]}{[HA]}} = \frac{1}{1+R}$$

$$R = \frac{[A]}{[HA]}$$

$$f_A = \frac{[A]}{[HA]+[A]} = \frac{[A]/[HA]}{1+\frac{[A]}{[HA]}} = \frac{R}{1+R}$$

We need to know R in terms of pH and pK_a.

Beginning with the equilibrium constant for ionization:

$$K_a = \frac{[A][H^+]}{[HA]}$$

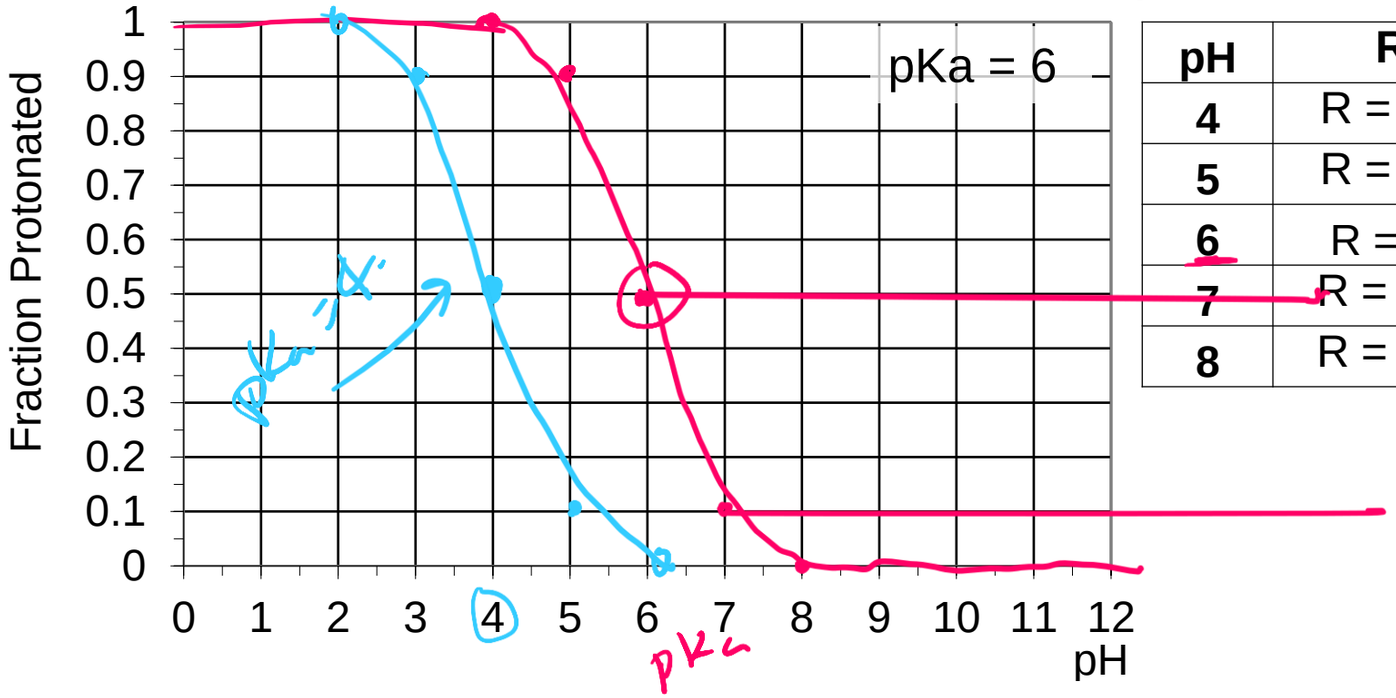
$$-\log(K_a) = -\log\left(\frac{[A][H^+]}{[HA]}\right) = -\log\left(\frac{[A]}{[HA]}\right) - \log[H^+]$$

$$pKa = -\log\left(\frac{[A]}{[HA]}\right) + pH$$

$$\log\left(\frac{[A]}{[HA]}\right) = pH - \text{pKa}$$

$$\frac{[A]}{[HA]} = R = 10^{(pH-pKa)}$$

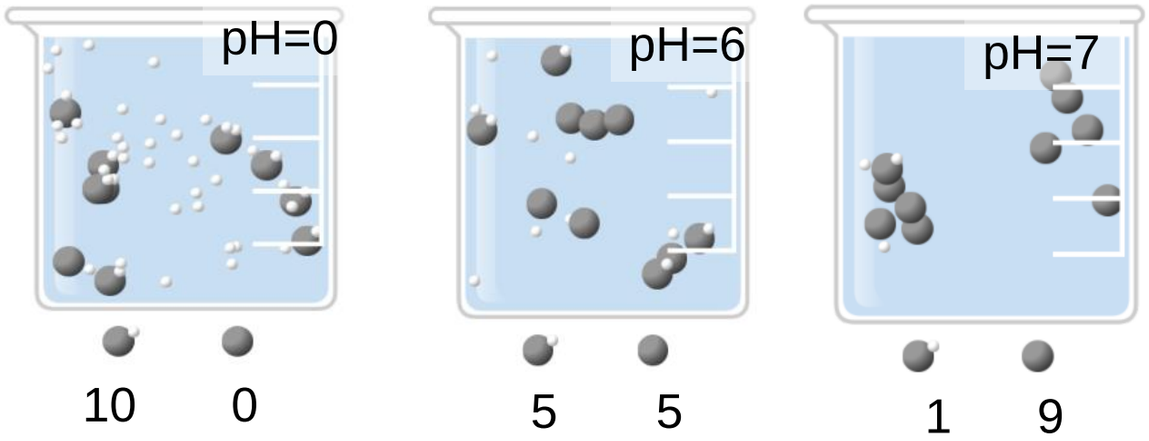
How does pH affect f_{HA} (& Activity)

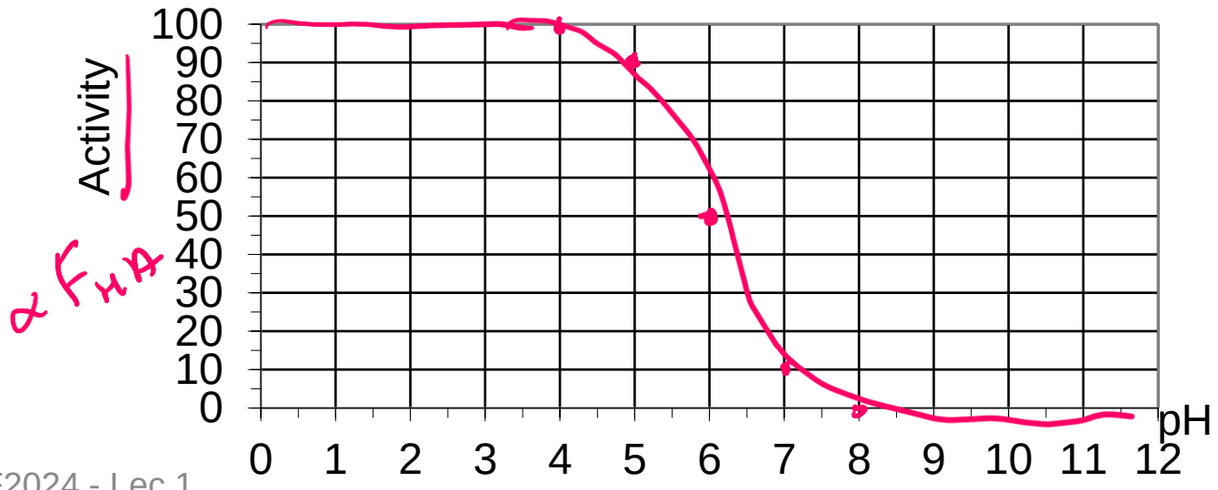


pH	$R=10^{(pH-pKa)}$	$F_{HA}=1/(1+R)$	$\Delta=pH-pK_a$
4	$R = 10^{(4-6)} = 10^{-2}$	$F_{HA} = 1/(1 + 0.01) = 0.99$	-2
5	$R = 10^{(5-6)} = 10^{-1}$	$F_{HA} = 1/(1 + 0.10) = 0.91$	-1
6	$R = 10^{(6-6)} = 10^{0}$	$F_{HA} = 1/(1 + 1) = 0.5$	0
7	$R = 10^{(7-6)} = 10^{+1}$	$F_{HA} = 1/(1 + 10) = 0.091$	+1
8	$R = 10^{(8-6)} = 10^{+2}$	$F_{HA} = 1/(1 + 100) = 0.01$	+2

Important general features to note:

i) when pH = pK_a - 2, $f_{HA} = 0.99$
ii) when pH = pK_a -1, $f_{HA} = 0.91$
iii) when pH = pK_a $f_{HA} = f_{A^-} = 0.5$
iv) when pH = pK_a + 1 $f_{HA} = 0.09$
v) When pH = pK_a + 2 $f_{HA} = 0.01$





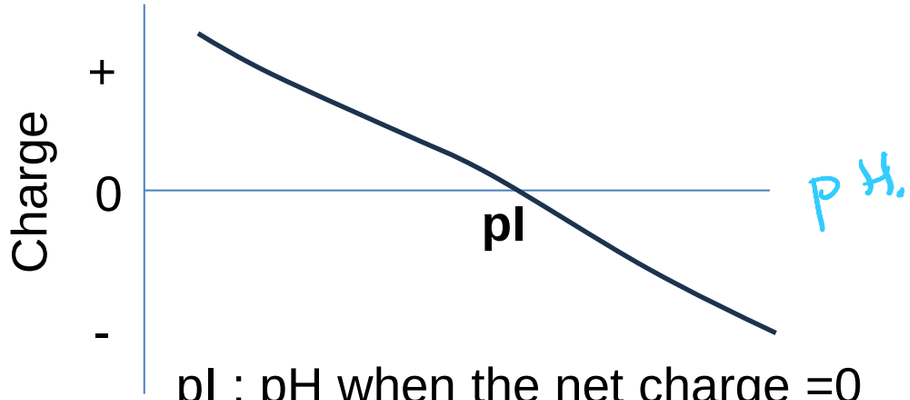
Charge Calculations:

The overall charge on a molecule as a function of pH can be calculated by summing the contribution from each ionizable group, as indicated in the equation on the right.

$$q_{total} = \sum (f_{HA} \cdot q_{HA} + f_{A-} \cdot q_{A-})$$

Knowledge of the overall charge on proteins is important:

- *For designing protein purification schemes.*
- *Predicting inter-molecular interactions*



pI : pH when the net charge =0

Approach:

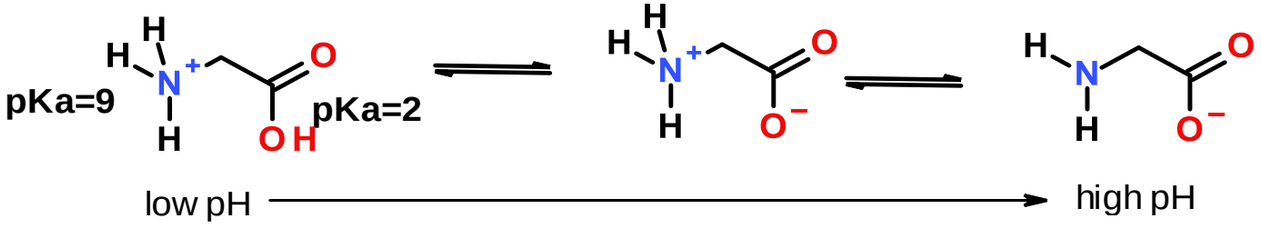
i) Identify all ionizable groups on the molecule & their charge when protonated and deprotonated (number of terms in the sum).

ii) Use the known pK_a of each group to determine the fraction protonated (f_{HA}) and deprotonated (f_{A-}) at the required pH.

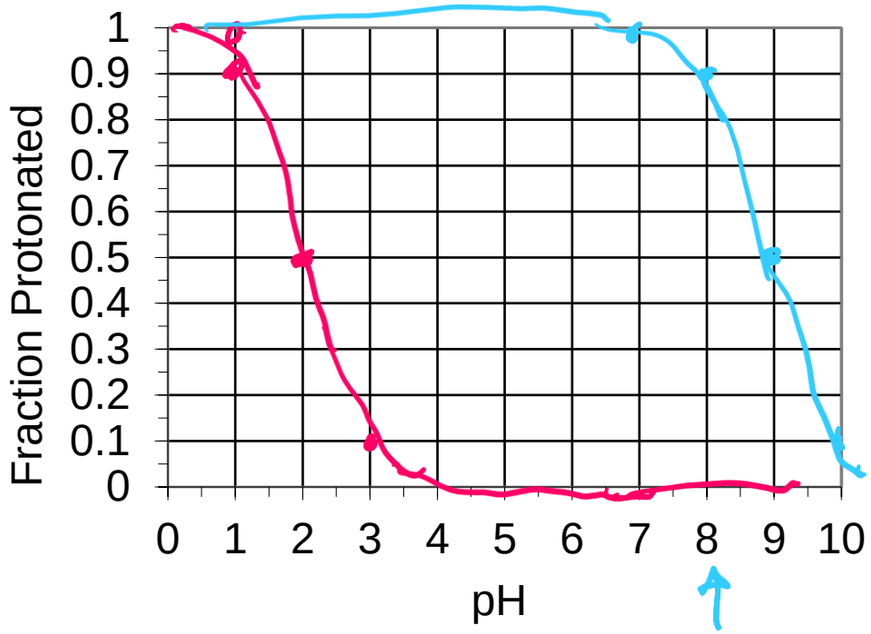
iii) Calculate the overall charge by summing the contribution of each group.

Charge Calculations

Example: What is the net (average) charge on glycine at **pH=8**?

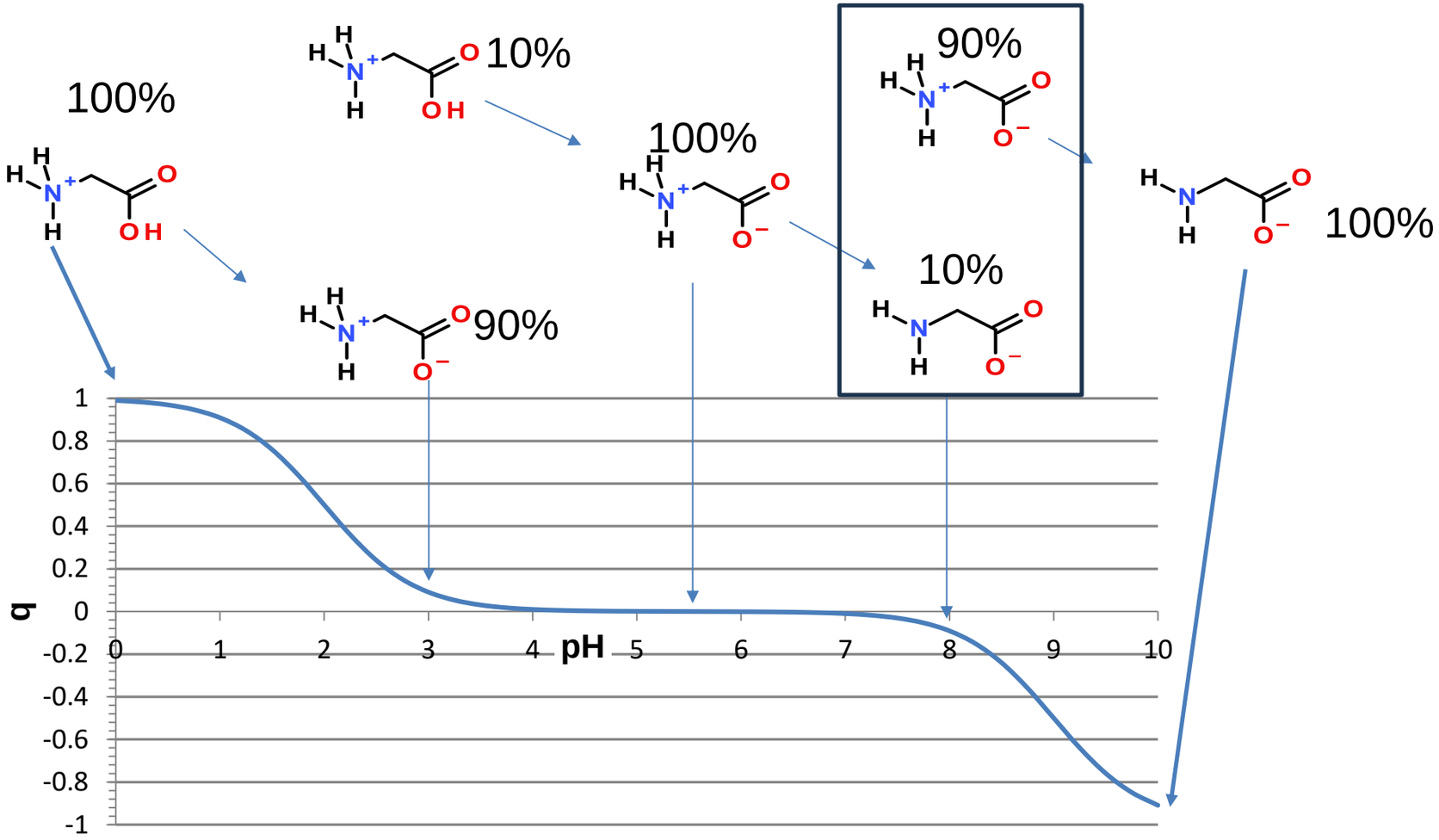


$$q_{total} = \sum (f_{HA} \cdot q_{HA} + f_{A-} \cdot q_{A-})$$



Group	f_{HA}	q_{HA}	f_A	q_A	q
-COOH pKa=2	( ) x	(0) +	(1) x	(-1) =	-1
-NH3 pKa = 9	(0.9) x	(+1) +	( ) x	(0) =	+0.9

-0.1



Important Definition

Isoelectric pH (pI) pH where the net charge is zero (pH = 5.5 in this case).

Titration Curves – pKa Measurement & pH Buffers

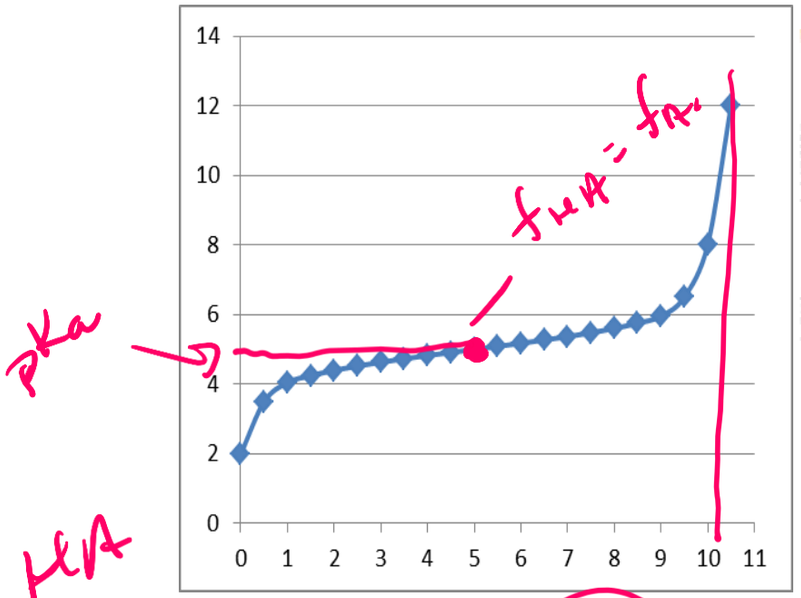
K_a values, or acidity constants, must be measured by direct experiment - with a pH titration. Known amounts of a strong base are added to a solution of the **weak acid.** The response of the weak acid to the added base can be measured by:
i) Changes in the pH of the solution for simple compounds,
ii) Spectroscopic methods for molecules with many ionizable groups.

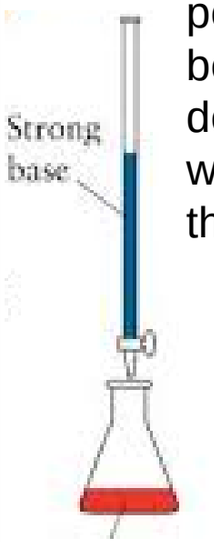
Titration Curve:
As the base is added it removes the proton from the acid and the change in protonation of the weak acid affects the pH.

At the beginning of the titration all of the weak acid is protonated ($f_{HA} = 1$)

At the equivalence point enough base has been added to fully deprotonate the weak acid, i.e. $f_{HA} = 0$.

½ through the titration f_{HA}=0.5 and f_A =0.5, pH = pK_a

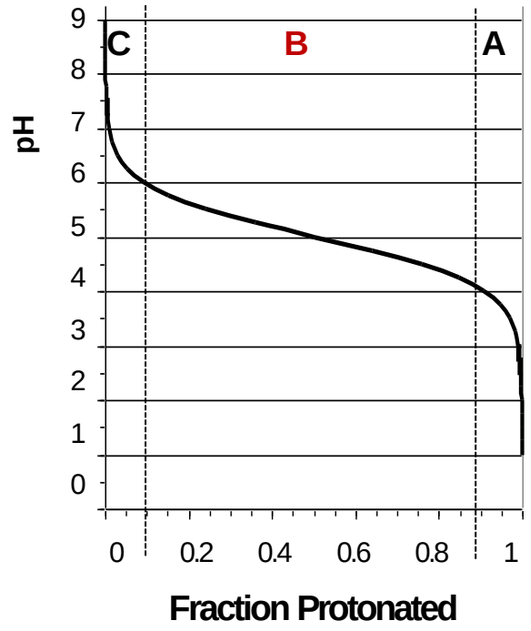


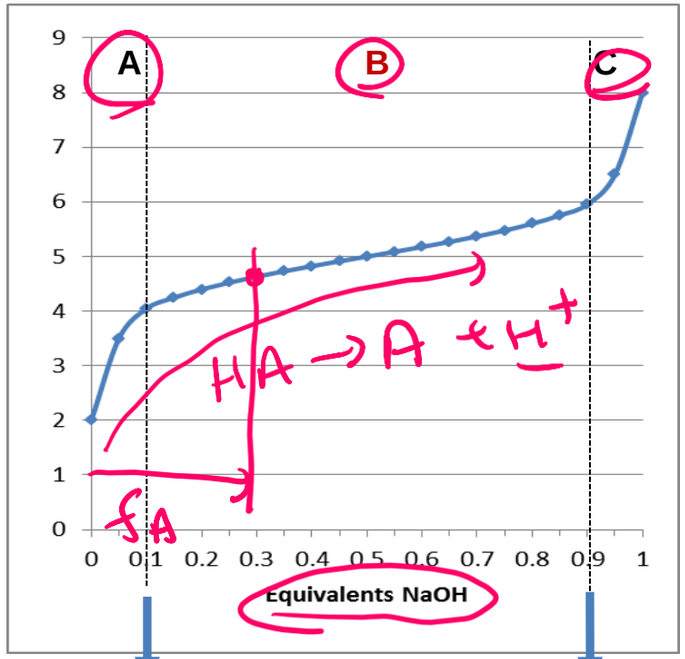


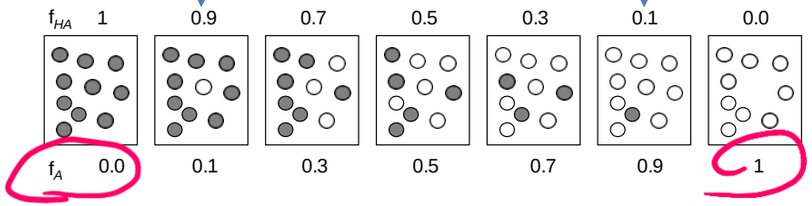
At the half-equivalence point enough base has been added to deprotonate ½ of the weak acid ([HA]=[A]), therefore.

Buffers: A pH buffer is an acid that resists changes in the solution pH by absorbing or releasing protons.

Why Weak Acids are Buffers







Equivalents NaOH = moles NaOH/mole of weak acid

Buffering range/region

$pH = pK_a \pm 1$

Change in weak acid protonation through titration:

Predominant reactions in:

Region A: *Added OH- removes H+ from solution, raising pH*

Region B: *As OH- is added, weak acid deprotonated, released proton contributes to the pH, reducing the change in pH*

Region C: *Added OH- removes H+ from solution, raising pH*

Buffering capacity: Total moles of a strong acid or base that can be absorbed by a buffer solution and keep the pH within the buffer region. It depends on the concentration of the weak acid, and where the pH is relative to the edges of the buffer region. The higher concentration of weak acid, the higher the capacity.

Buffers Construction: Need to determine the ratio of [A-] to [HA] (=R) to give desired pH of the solution.

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

adj solution this ratio to give desired pH.

Typical Problems - Monoprotic Buffer:

- concentration [A_T] , [A_T] = [HA] + [A]
- volume V, pH
- List of weak acids and their pKa values.

Method:

1. Select a weak acid whose pK_a is within one pH unit of the desired pH.
2. *Calculate* the fraction protonated and deprotonated at the desired pH, f_{HA} & f_{A^-}.
3. Obtain this ratio of [HA] to [A^-] in solution by one of the following methods:

i) Mix the indicated concentration of the weak acid (HA) and its conjugate base (NaA) to give the desired pH:

moles (HA)= $f_{HA} \times [A_T] \times V$ moles (A) = $f_{A^-} \times [A_T] \times V$

ii) Use [A_T] amount of the *acid form* of the weak acid and add sufficient *strong base* (e.g. NaOH) to make the required concentration of [A^-] to attain the desired pH. You are titrating starting from the left side and converting enough of the fully protonated acid to give the correct amount of the deprotonated acid. The added base converts HA to A^-.

The amount of strong base to add is f_{A^-} equivalents. *moles NaOH = $f_{A^-} \times [A_T] \times V$*

iii) Use [A_T] amount of the *conjugate base* form of the weak acid (e.g. Na salt) and add sufficient *strong acid* (e.g. HCl) to make the required concentration of [HA] to attain the desired pH. You are protonated the fully deprotonated acid by just the right amount to give the correct amount of the protonated acid. The added strong acid converts A to HA. ***The amount of strong acid to add is f_{HA} equivalents.*** *moles HCl = $f_{HA} \times [A_T] \times V$*

Example: Make 1L of 1 M buffer solution at pH 5.0 using either imidazole (pKa~6), or pyruvate (pKa~2.5). You have both the protonated and deprotonated species (e.g. Na salt) in hand.

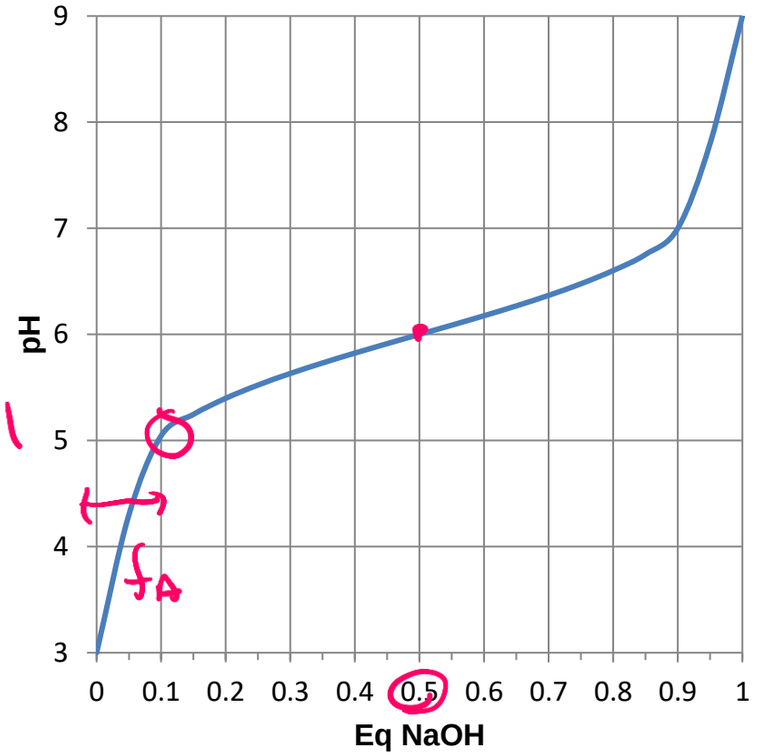
1. Which buffer would you use, why?

Im pKa with 1 unit.

2. Determine fraction protonated and deprotonated at the desired pH:

$$R = 10^{(pH-pKa)} \quad = 10^{5-6} = 10^{-1} = 0.1$$

$$f_{HA} = \frac{1}{1+R} = \frac{1}{1.1} \approx 0.9 \qquad f_A = \frac{R}{1+R} \approx 0.1$$



3. Since we have both forms (HA), (A) we can use any of the three methods to make the buffer:

Method i: Mix the acid and the conjugate base.

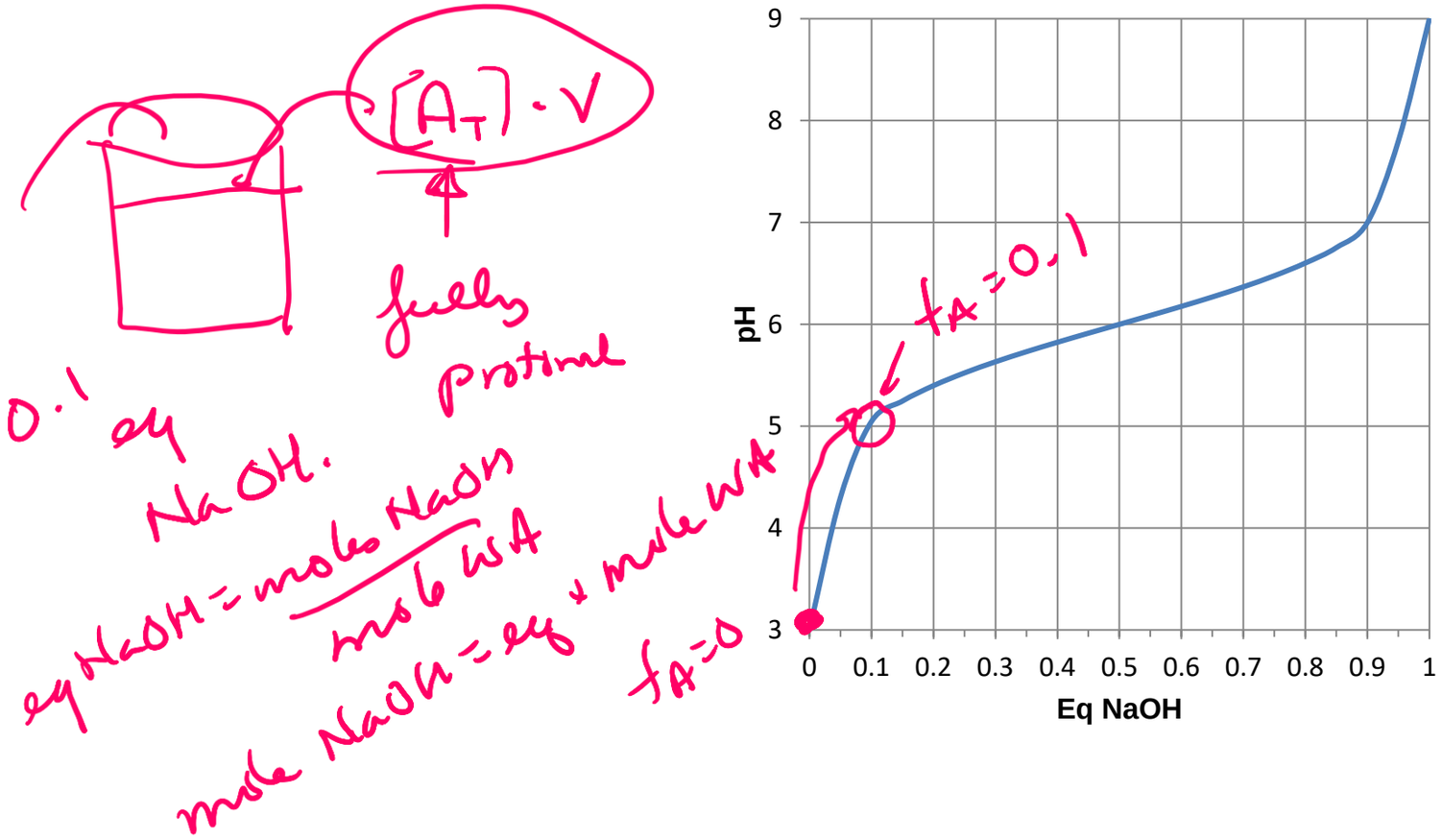
moles (HA)= $f_{HA} \times [A_T] \times V$ moles (A) = $f_{A^-} \times [A_T] \times V$

total conc

Method ii: Start with the fully protonated acid and add NaOH.

Use [A_T] amount of the *acid form* of the weak acid and add sufficient *strong base* (e.g. NaOH) to make the required concentration of [A^-] to attain the desired pH.
You are titrating starting from the left side and converting enough of the fully protonated acid to give the correct amount of the deprotonated acid. The added base converts HA to A^-.

The amount of strong base to add is f_{A^-} equivalents. *moles NaOH = $f_{A^-} \times [A_T] \times V$*

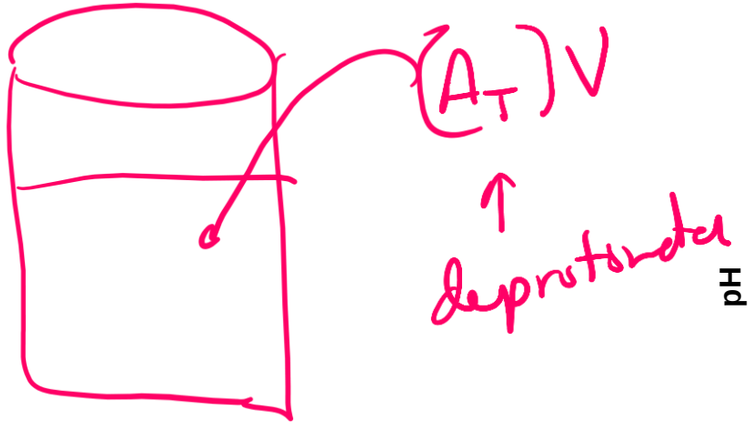


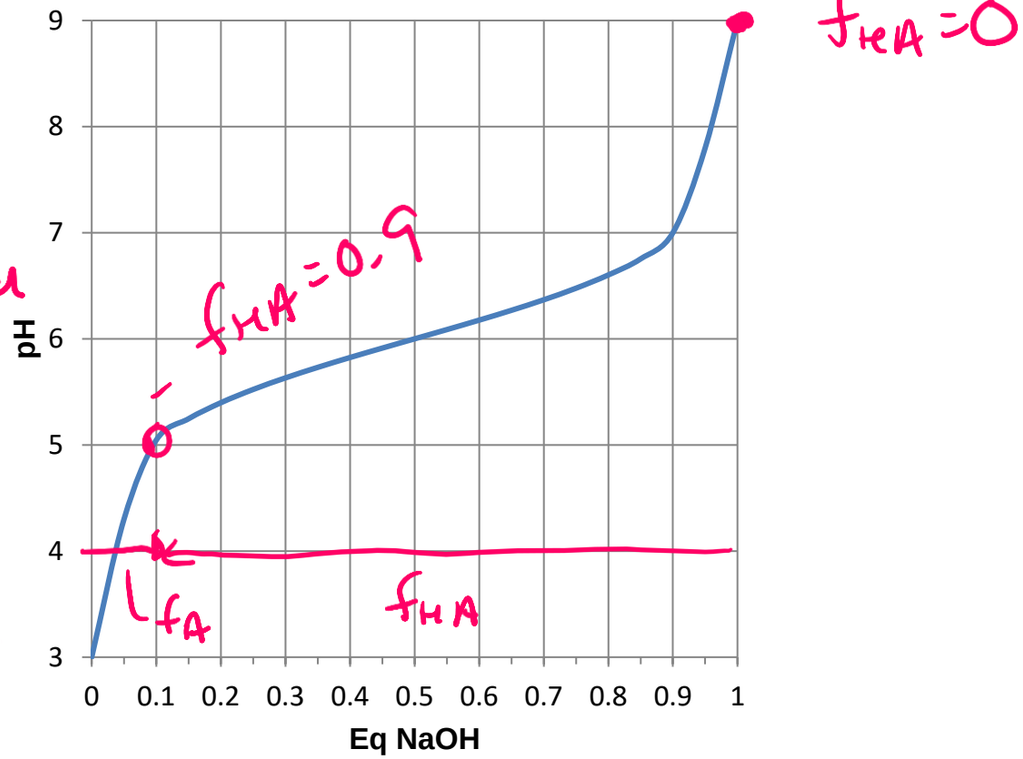
Method iii: Start with the full deprotonated acid (conjugate base) and add HCl.

Use [A_T] amount of the *conjugate base* form of the weak acid and add sufficient *strong acid* (e.g. HCl) to make the required concentration of [HA] to attain the desired pH.

You are protonated the fully deprotonated acid by just the right amount to give the correct amount of the protonated acid. The added strong acid converts A to HA.

The amount of strong acid to add is f_{HA} equivalents. *moles HCl = $f_{HA} \times [A_T] \times V$*





0.9 eq HCl

moles HCl = eq - A_T.V

Polyprotic Buffers:

Any of the pKas in a poly-protic acid can be used to buffer the solution. For example, phosphate has three pKas and therefore three buffer regions.

The overall approach in generating buffers using polyprotic acids is:

1. Select the pKa that corresponds to the required buffer region
2. Do all of the calculations using that pKa, giving f_{HA} and f_A
3. Make the buffer using one of three ways:

Example: *Assuming pH is in the 2nd buffer region.*

Method i:

Use the form of the compound that is present at the beginning of the buffer region as "HA", e.g. $H_2PO_4^-$.

Moles of HA = f_{HA} x [A_T] x V

Use the form of the compound that is present at the end of the buffer region as "A", e.g. HPO_4^{2-}.

Moles of A = f_A x [A_T] x V

Method ii:

Use the fully protonated form of the acid (H_3PO_4) to start.

Moles of H_3A = [A_T] x V

Equivalents of NaOH = 1 + f_A

Moles of NaOH = eq NaOH x [A_T] x V

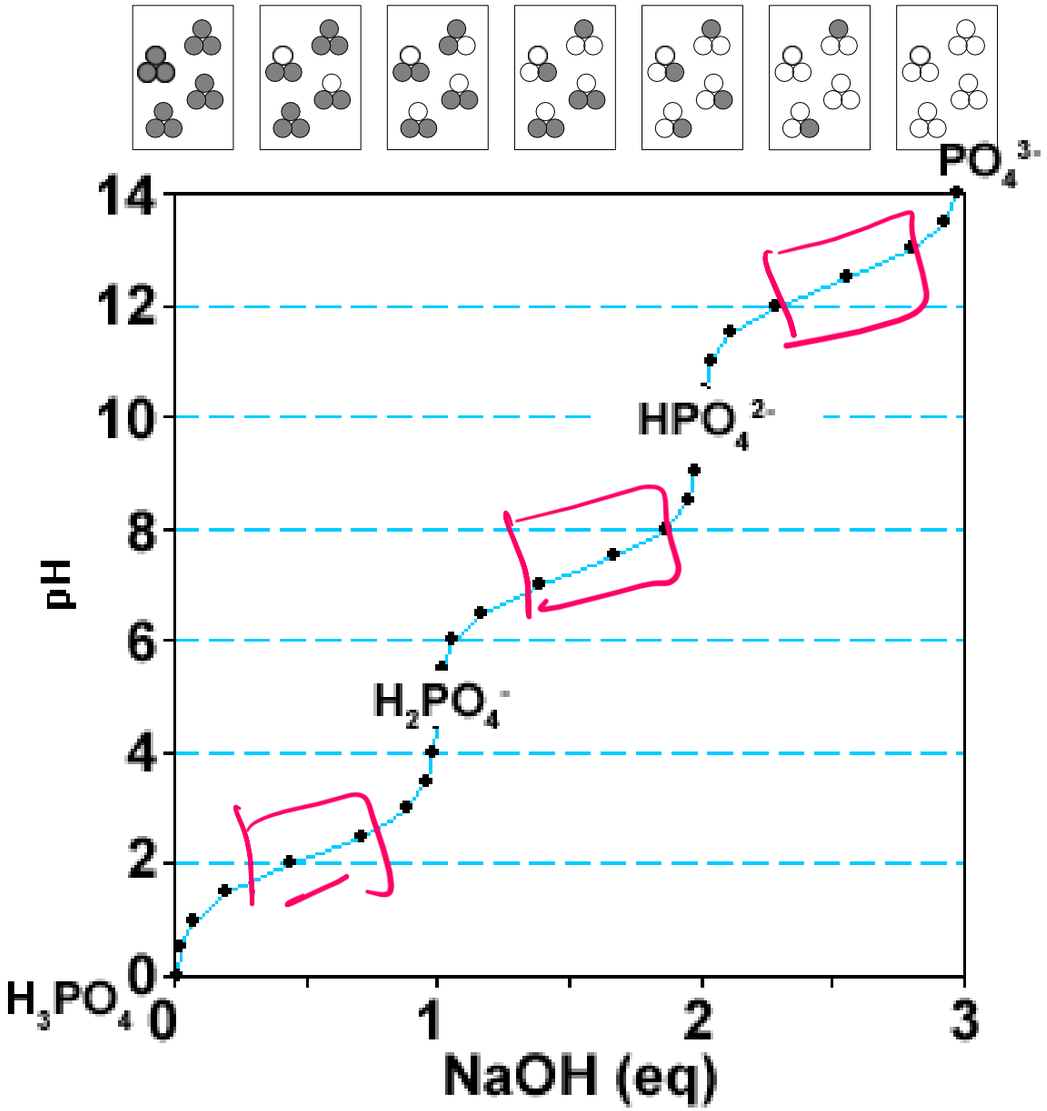
Method iii:

Use the fully deprotonated form of the acid (Na3PO4) to start.

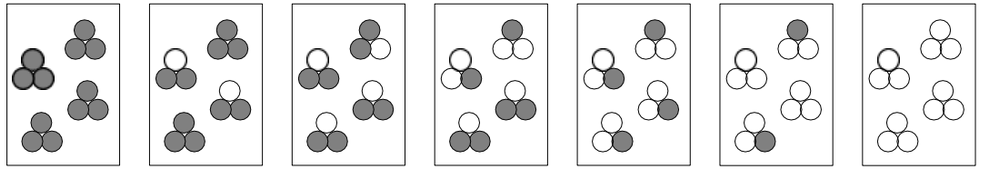
Moles of Na_3A = [A_T] x V

Equivalents of HCl = 1 + f_{HA}

Moles of HCl = eq HCl x [A_T] x V



Example: Make 1L of a 0.2M phosphate buffer with a pH = 8.0. pKa values are 2.1, 7.2, and 12.7 for phosphoric acid.



1. Use pKa closest to desired pH.

   pK_2 should be used.

2. Calculate f_{HA} and f_{A^-}. *(Note: "HA" = H_2PO_4, "A"=HPO_4)*

   $R = 10^{(pH-pKa)} = 10^{(8.0-7.2)} = 10^{0.8} = 6.31$

   $f_{HA} = 1/(1+6.31) = 1/7.31 = 0.137$ $\quad f_A = (1-f_{HA}) = 0.863$

3. Select one of the following three methods:

   i) Use the chemical forms of "(HA)" and "(A-)" that represent the species present at the pKa you used, in this case:

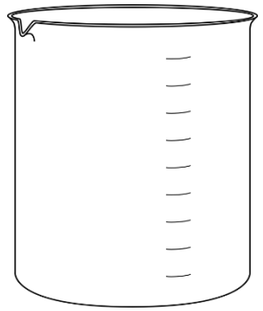
   NaH_2PO_4 = "HA" , Na_2HPO_4="A".

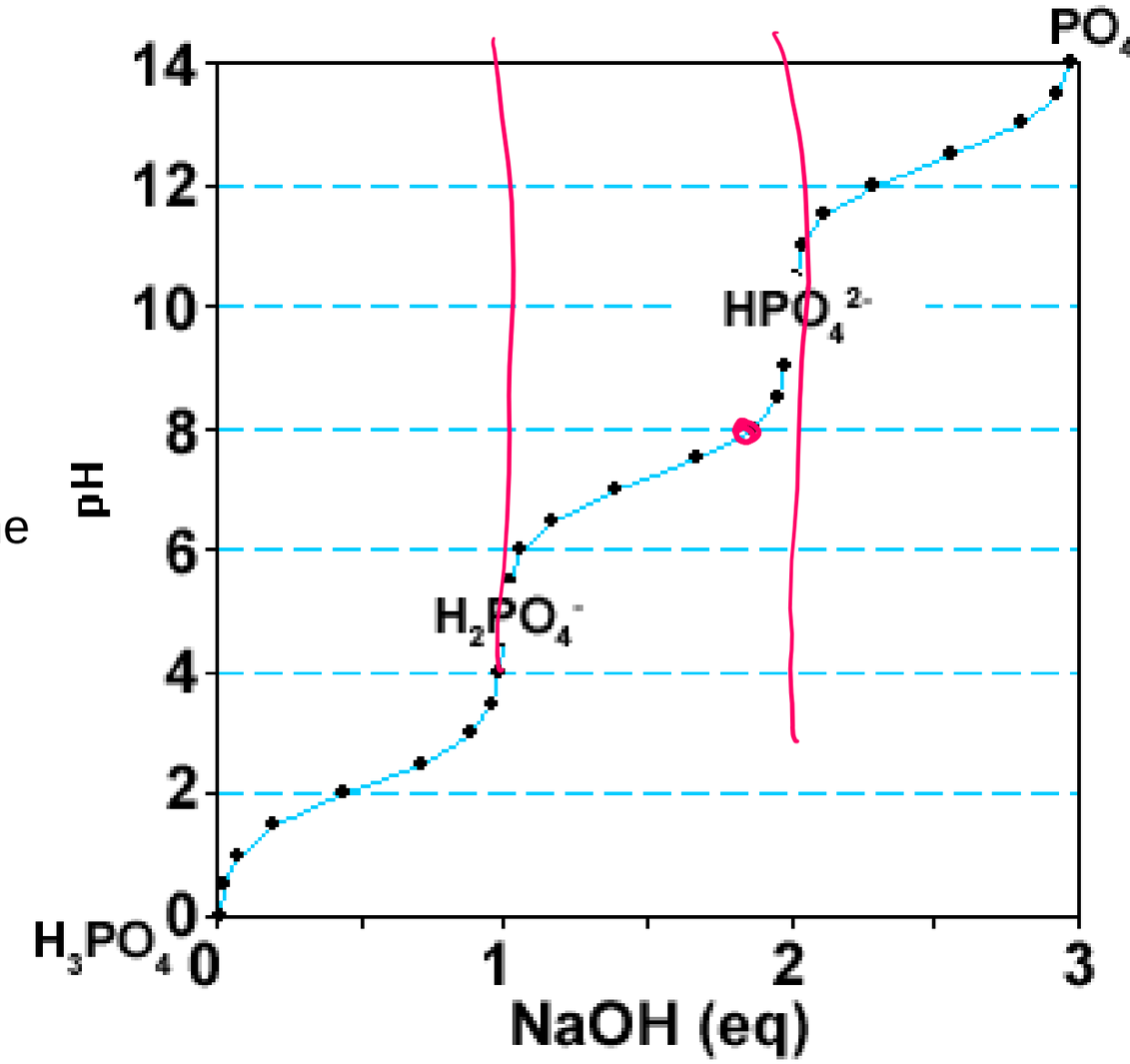
moles NaH_2PO_4=

$f_{HA} \times [A_T] \times V = 0.137 \times 0.2$ mole/L x 1 L

moles Na_2HPO_4=

$f_A \times [A_T] \times V = 0.863 \times 0.2$ mole/L x 1 L





Methods ii & iii may require adjustments to equivalents due to additional equivalence points/buffer regions that have to be crossed to get to the desired pH.

f_{HA} = 0.137 f_A = 0.863 at desired pH = 8

ii) Starting from completely protonated form (**H_3PO_4**). Add sufficient whole equivalents (n) to reach the buffer region you are using, plus an additional f_{A^-} to reach the pH within that buffer region.

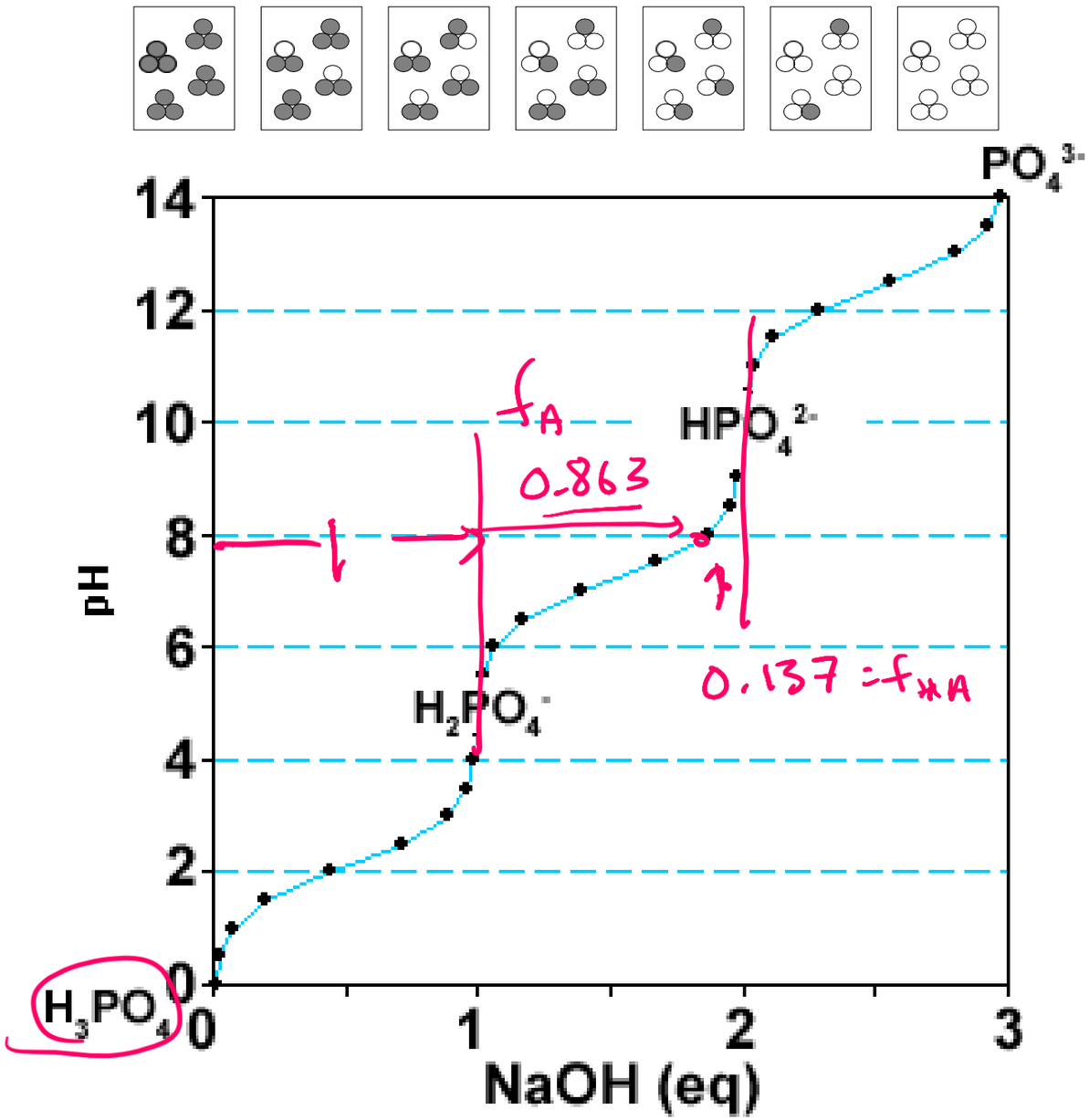
eq NaOH = 1 + f_A = 1+ 0.863 moles NaOH = eq x V x A_T

The first equivalent of NaOH that is added converts all of the H_3PO_4 to H_2PO_4

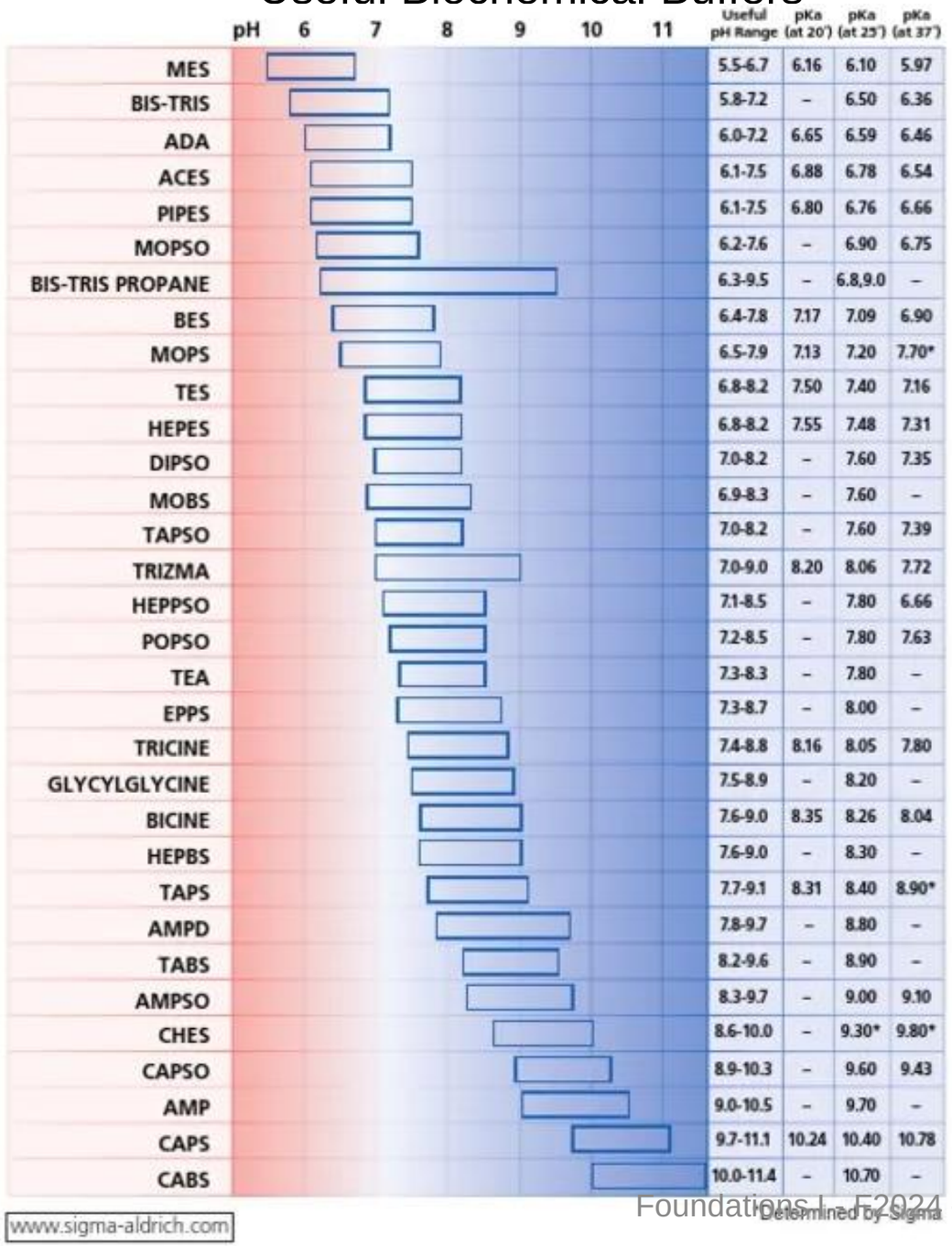
iii)Starting from completely ionized form (**Na_3PO_4**). Add sufficient whole equivalents (*n*) to reach the buffer region you are using, plus and additional f_{AH} equivalents *of* HCl to get to the desired pH in that buffer region.

eq HCl = 1 + f_{AH} =1+0.137 moles HCl = eq HCl x V x A_T

The first equivalent of HCl that you add protonates all of the PO_4 to HPO_4



Useful Biochemical Buffers



	Useful pH Range	pKa (at 20°)	pKa (at 25°)	pKa (at 37°)
MES	5.5-6.7	6.16	6.10	5.97
BIS-TRIS	5.8-7.2	–	6.50	6.36
ADA	6.0-7.2	6.65	6.59	6.46
ACES	6.1-7.5	6.88	6.78	6.54
PIPES	6.1-7.5	6.80	6.76	6.66
MOPSO	6.2-7.6	–	6.90	6.75
BIS-TRIS PROPANE	6.3-9.5	–	6.8,9.0	–
BES	6.4-7.8	7.17	7.09	6.90
MOPS	6.5-7.9	7.13	7.20	7.70*
TES	6.8-8.2	7.50	7.40	7.16
HEPES	6.8-8.2	7.55	7.48	7.31
DIPSO	7.0-8.2	–	7.60	7.35
MOBS	6.9-8.3	–	7.60	–
TAPSO	7.0-8.2	–	7.60	7.39
TRIZMA	7.0-9.0	8.20	8.06	7.72
HEPPSO	7.1-8.5	–	7.80	6.66
POPSO	7.2-8.5	–	7.80	7.63
TEA	7.3-8.3	–	7.80	–
EPPS	7.3-8.7	–	8.00	–
TRICINE	7.4-8.8	8.16	8.05	7.80
GLYCYLGLYCINE	7.5-8.9	–	8.20	–
BICINE	7.6-9.0	8.35	8.26	8.04
HEPBS	7.6-9.0	–	8.30	–
TAPS	7.7-9.1	8.31	8.40	8.90*
AMPD	7.8-9.7	–	8.80	–
TABS	8.2-9.6	–	8.90	–
AMPSO	8.3-9.7	–	9.00	9.10
CHES	8.6-10.0	–	9.30*	9.80*
CAPSO	8.9-10.3	–	9.60	9.43
AMP	9.0-10.5	–	9.70	–
CAPS	9.7-11.1	10.24	10.40	10.78
CABS	10.0-11.4	–	10.70	–

www.sigma-aldrich.com

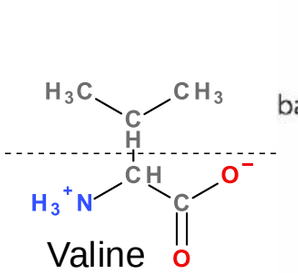
Determined by Sigma

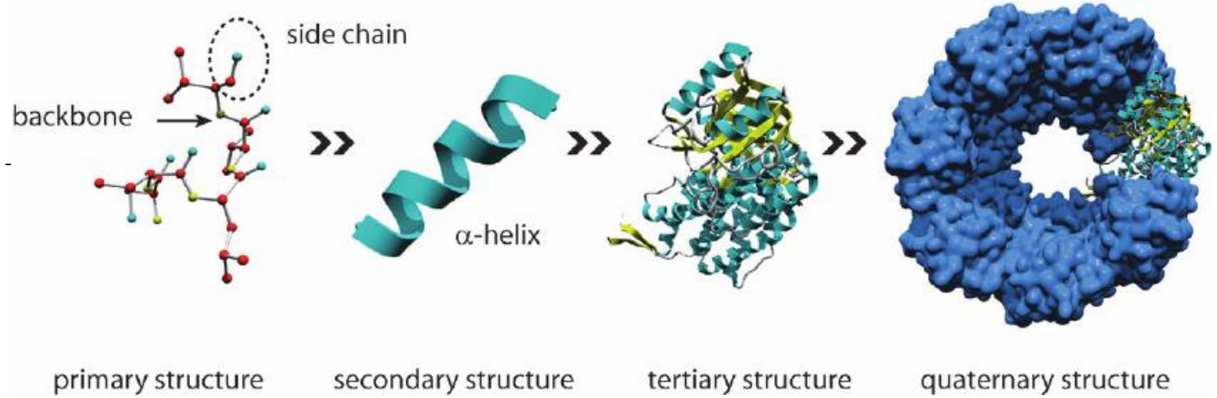
Protein friendly organic buffers that span a large pH range are available.

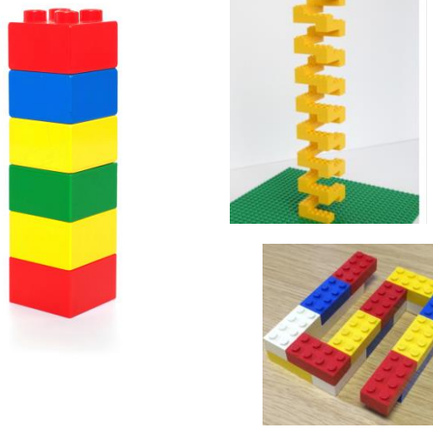
Note many show a large temperature effect on their pKa

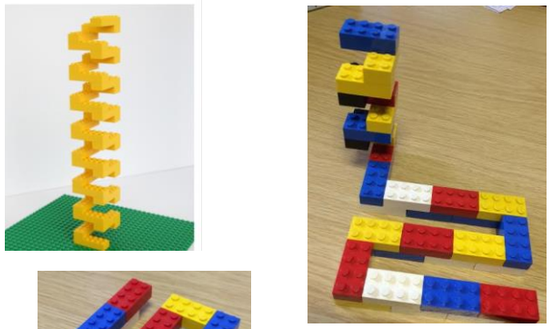
Structural Hierarchy of Proteins

- Primary - sequence of amino acids, no 3D structural information
- Secondary - local structural elements, only mainchain atoms involved
- Tertiary - 3D position of ***all*** atoms, functional form of many proteins.
- Quaternary - multiple chains – multiple chains often required for function.







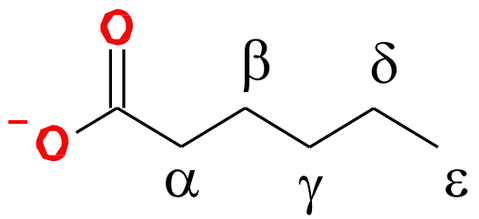


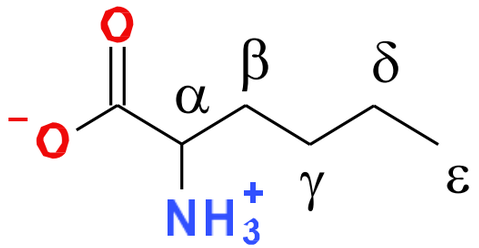
(White and black represent two different chains)

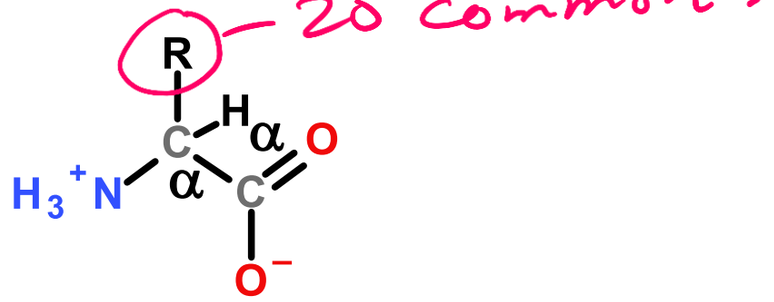
Biological Function

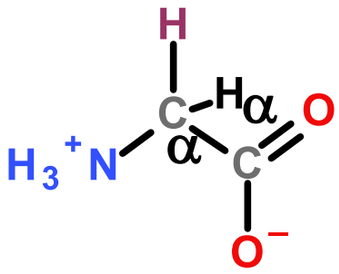
Amino Acids: Structure and Properties

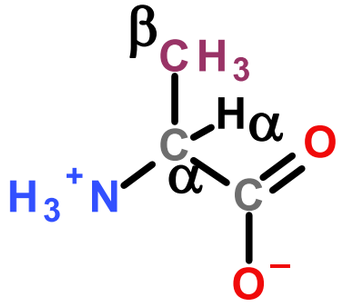
- An amino acid is a carboxylic acid with an amino group. The first carbon after the carbonyl carbon is the α-carbon. Most biological amino acids are α-amino acids - the amino group is attached to the α - carbon.
- There are 20 amino acids that are common, they differ only in the group (sidechain) attached to the Cα carbon.
- The N, Cα, Hα, C=O, C-OH atoms are the same in each of the 20 commonly found amino acids. The N, Cα, Hα, C=O will be the repeating unit in the protein polymer, making the "**mainchain**" or "**backbone**".
- The **sidechain** atoms are unique to each amino acid and give rise to the unique properties of that amino acid.
- The sidechain atoms are designated with Greek letters, based on the nomenclature for carboxylic acids.

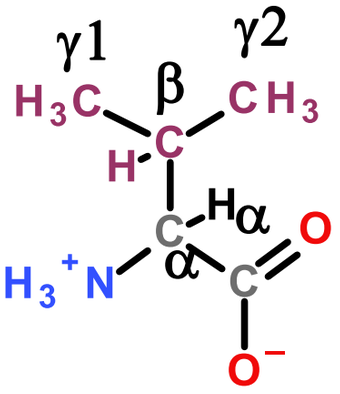






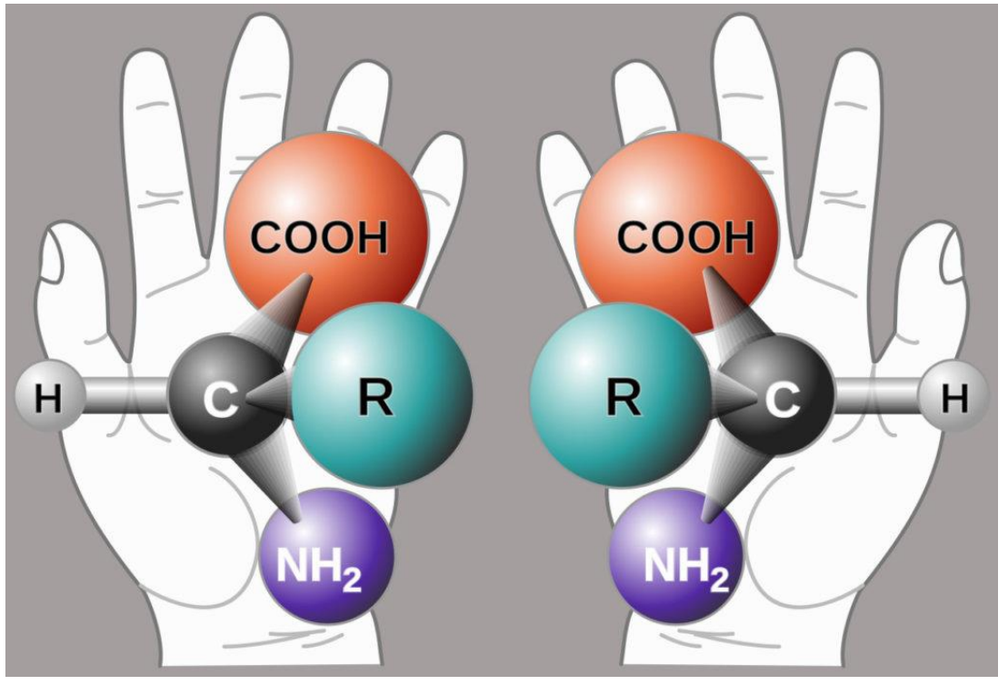






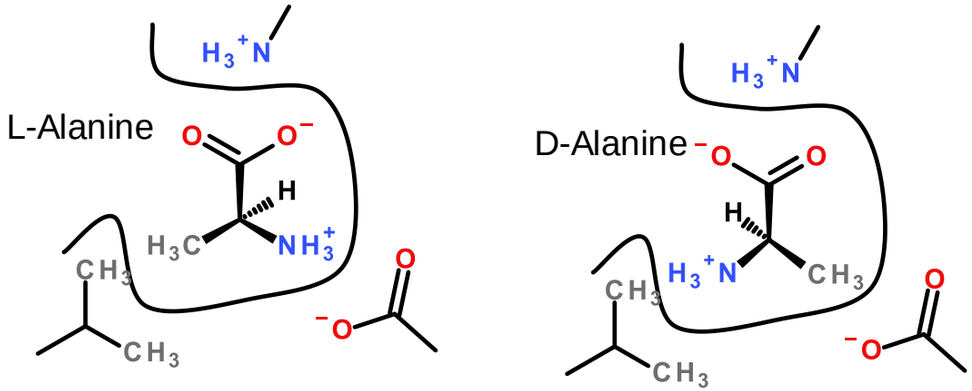
Chirality & Optical Activity:
In all amino acids (except glycine) the α-carbon is chiral because it is attached to four different groups.

This means that the mirror images of these compounds cannot be superimposed. The two mirror images are called ***enantiomers***.



- Most common amino acids have an S configuration. An older, but very much used, notation is D and L. This notation is based on the chirality of a reference compound and **all amino acids that are found in proteins that are ribosomal in origin are L.**

Importance of chirality in Biology: Usually only one enantiomer is active in biological systems. As indicated above, only L-amino acids are used to make proteins on the ribosome. Amino acids of the other enantiomer (D) are generally harmless.



A binding pocket for Alanine – which will bind better L or D? Why?

Primary Structure

- Amino acids are joined together to form linear polymers by the formation of a **peptide bond** between the carboxyl of one amino acids and the amino group of the next.
- This reaction releases water: a **dehydration** reaction.
- The peptide bond can be broken (*lysis*) by the addition of water = **hydrolysis**.

Incorporated amino acid = ***residue*** (atoms are lost when the peptide bond is formed).

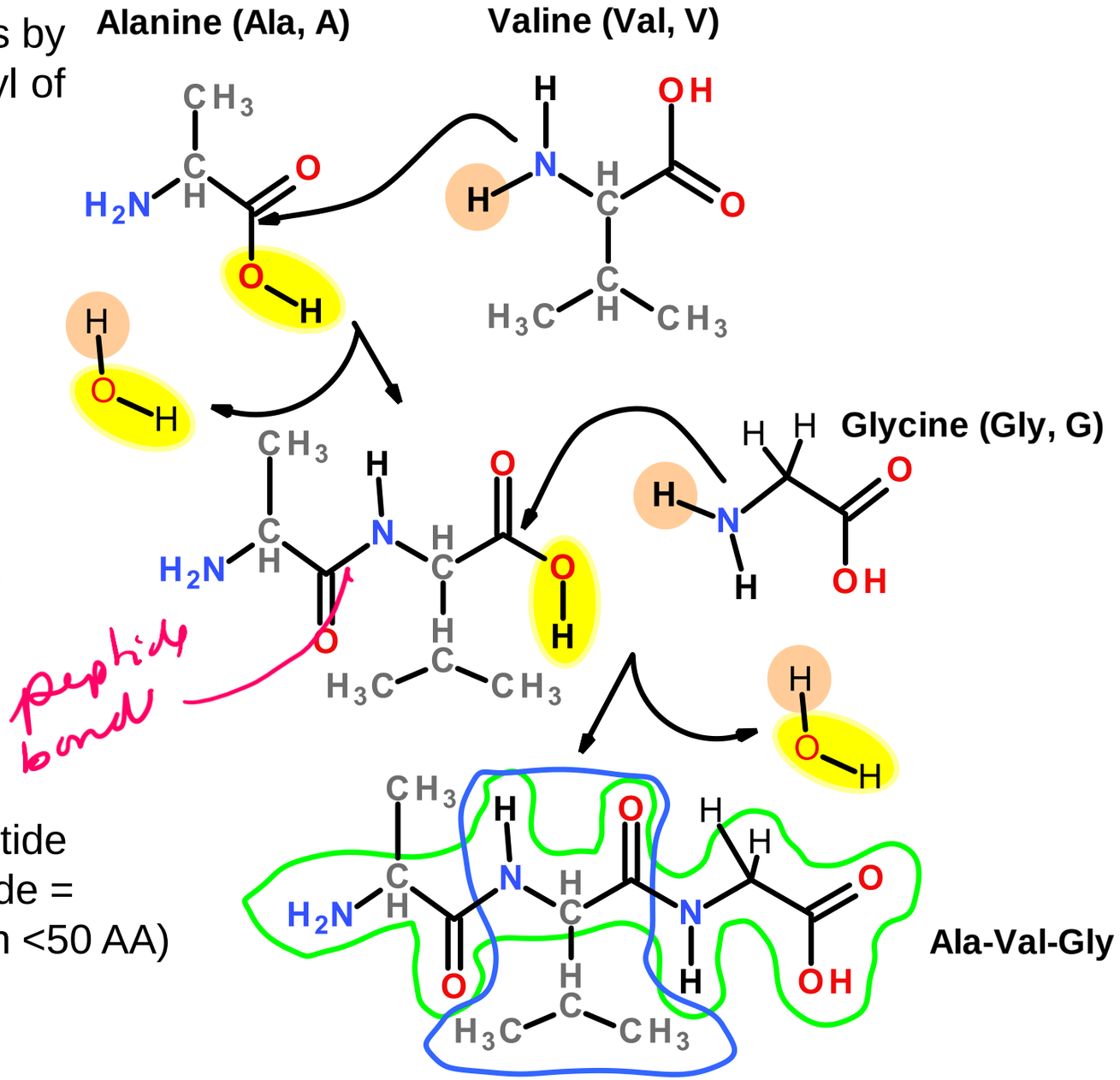
Polarity of chain direction – amino (N) terminus to carboxy(C) terminus = order of amino acids = *sequence* = *primary structure*

Mainchain (or backbone) – linear atoms of the polymer

Sidechain – atoms off the Ca carbon

– ***Primary Structure – Expectations***

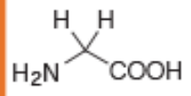
- Draw chemical structure from sequence.
- Determine the seq. from structure.
- Distinguish/identify:
  - **Mainchain** & **Sidechain** atoms,
  - **Residue** = aa in polymer,
  - N & C terminus,
  - Peptide bond(s).



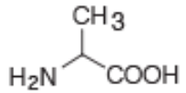
Amino Acids – Structure and Properties

Polar sidechains

SMALL

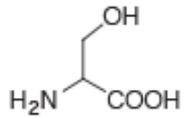


Glycine (Gly, G)
MW: 75.07

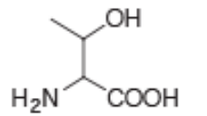


Alanine (Ala, A)
MW: 89.09

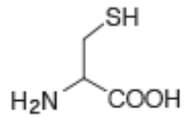
NUCLEOPHILIC



Serine (Ser, S)
MW: 105.09, $pK_a \sim 16$



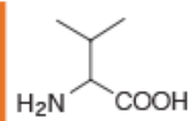
Threonine (Thr, T)
MW: 119.1, $pK_a \sim 16$



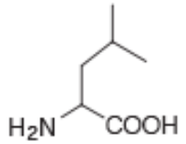
Cysteine (Cys, C)
MW: 121.2, $pK_a = 8.18$

Non-polar sidechains

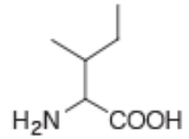
HYDROPHOBIC



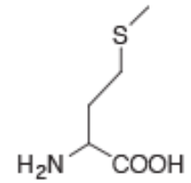
Valine (Val, V)
MW: 117.1



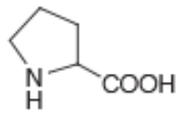
Leucine (Leu, L)
MW: 131.2



Isoleucine (Ile, I)
MW: 131.2

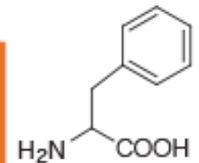


Methionine (Met, M)
MW: 149.2

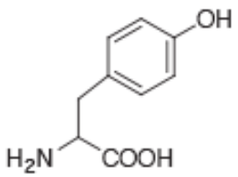


Proline (Pro, P)
MW: 115.1

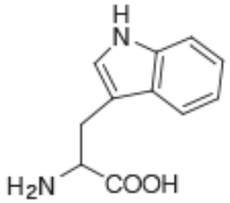
AROMATIC



Phenylalanine (Phe, F)
MW: 165.2

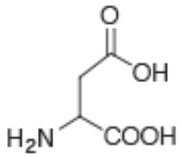


Tyrosine (Tyr, Y)
MW: 181.2, $pK_a = 10.46$

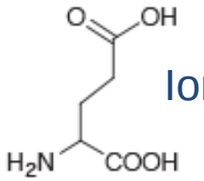


Tryptophan (Trp, W)
MW: 204.2

ACIDIC



Aspartic Acid (Asp, D)
MW: 133.1, $pK_a = 3.9$

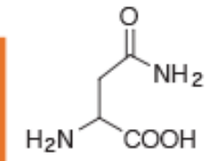


Glutamic Acid (Glu, E)
MW: 147.1, $pK_a = 4.07$

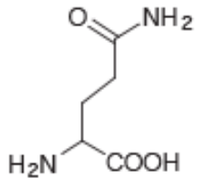
Ionizable sidechains

Polar sidechains

AMIDE

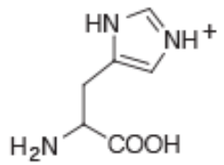


Asparagine (Asn, N)
MW: 132.1

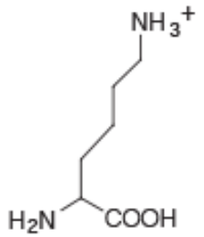


Glutamine (Gln, Q)
MW: 146.1

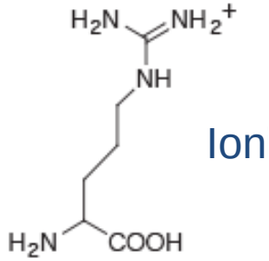
BASIC



Histidine (His, H)
MW: 155.2, $pK_a = 6.04$



Lysine (Lys, K)
MW: 146.2, $pK_a = 10.79$



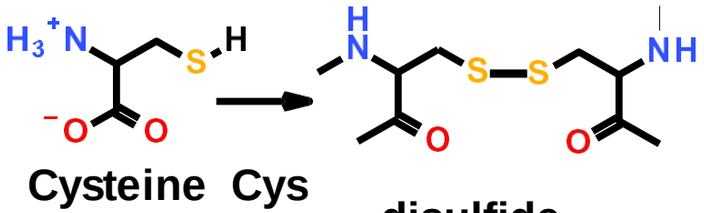
Arginine (Arg, R)
MW: 174.2, $pK_a = 12.48$

Ionizable sidechains

Amino Acids with Unique Structural Properties

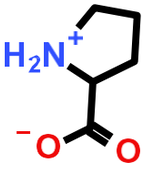
Cysteine:

- Forms disulfide bond by oxidation of the sulfur – covalent crosslink stabilizes proteins.

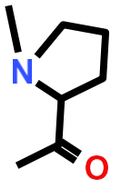


Cysteine Cys

disulfide bond



Proline Pro



no NH in protein

Proline:

- No H-bond donor when incorporated into proteins.
- Ring reduces flexibility of sidechain and the mainchain atoms.