So $[H^+]$ is maintained at

$$[H^+] = K_{HA}\frac{[HA]}{[A^-]} = \frac{K_{HA}[HA][Na^+]}{K_{NaA}[NaA]}$$

even if some HCl or KOH is added. The new protons from the HCl combine with A^- ions from the Na_2A, forming HA. The new OH^- from the KOH absorb protons from HA which the OH^- force to dissociate into H^+ and A^-. This works as long as the amounts of HA and Na_2A are similar and sufficient.

In blood, a buffer of carbonic acid H_2CO_3 and bicarbonate HCO_3^- (with counterions Na^+, K^+, etc.) keeps $7.35 < pH < 7.45$.

Incidentally, since

$$K_{MA} = \frac{[M^+][A^-]}{[MA]}$$

it follows that the pM (8.26) is

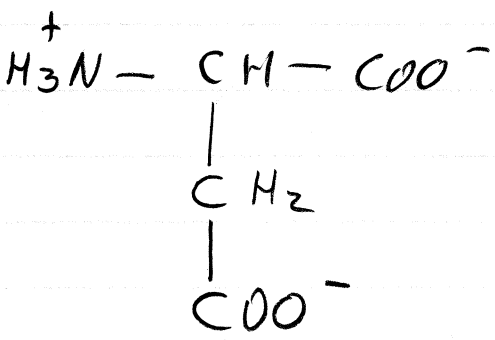
$$pM = -\log_{10}[M^+] = -\log_{10} \frac{K_{HA}[MA]}{[A^-]}$$

$$= pK_{HA} + \log_{10} \frac{[A^-]}{[MA]} \qquad (MM)$$

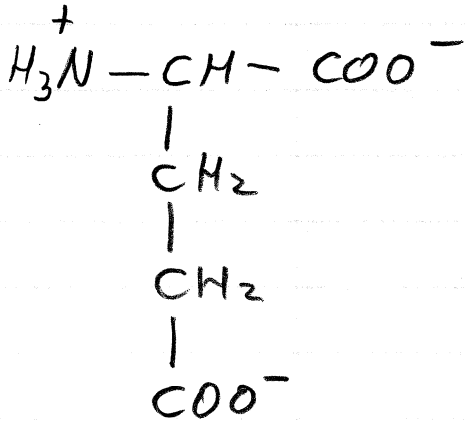
since $pK_{HA} = -\log_{10} K_{HA}$ (8.12). Chemists call (MM) the Henderson-Hasselbalch equation.

Proteins are chains of amino acids. There are 20 different amino acids in local living organisms — although a few of these can be modified.

The side chains of glutamic acid and of aspartic acid contain carboxylic groups that remain ionized at all physiological pH's.



Aspartic acid (asp) D

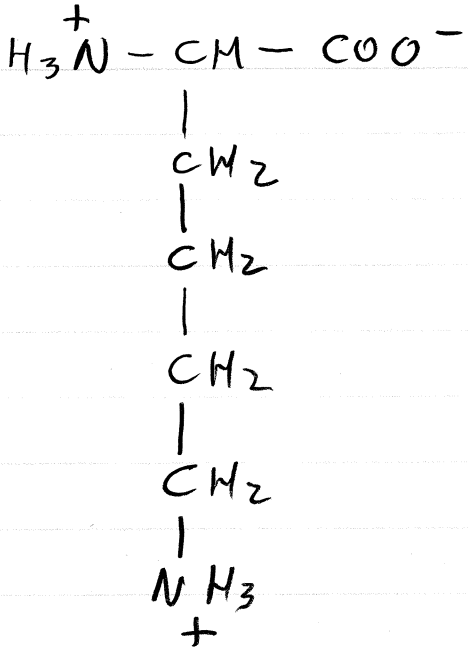


Glutamic acid (glu) E

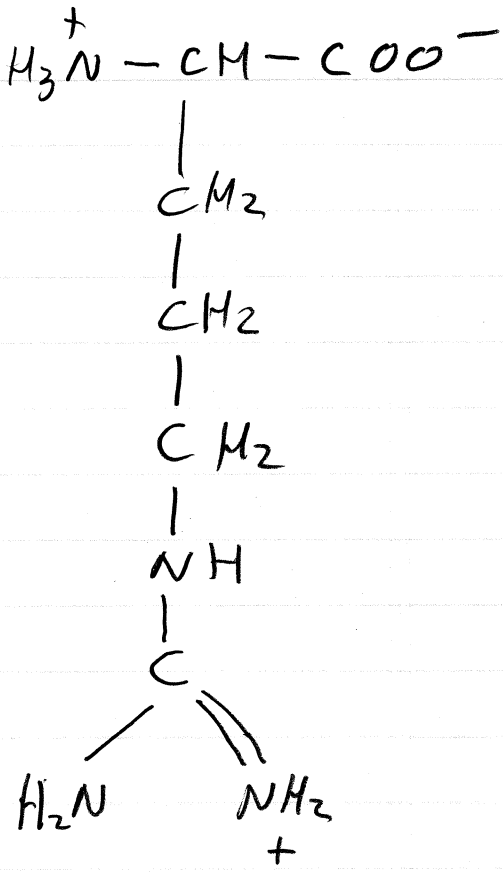
acidic side chain

$$-COOH \rightleftharpoons -COO^- + H^+$$

The side chains of lysine and arginine are protonated at physiological pH's. They are basic.



Lysine (lys)
K

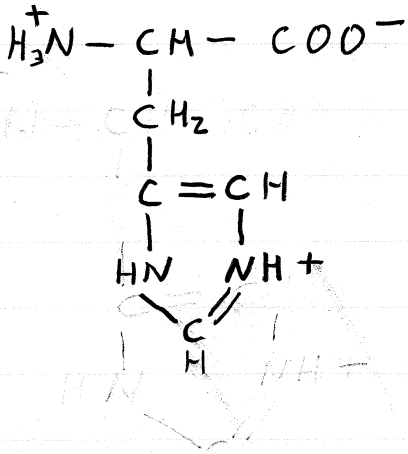


Arginine (arg) R

basic side chain

$$-NH_3^+ \rightleftharpoons -NH_2 + H^+$$

Histidine is protonated below about pH 6.



Histidine (his)
H

For each of these five amino acids

$$10^{-12.5} < K_{eq} = \frac{[H^+][R]}{[H^+R]} < 10^{-3.7}$$

↑ arginine ↑ aspartic acid

$$P_\alpha = \frac{[H^+R]}{[H^+R] + [R]}$$

P_α is the probability that the side chain is protonated. So

$$P_\alpha = \frac{1}{1 + \frac{[R]}{[H^+R]}} = \frac{1}{1 + \frac{K_{eq}}{[H^+]}}$$

Since $pH = -\log [H^+]$, it follows that

$$10^{pH} = \frac{1}{[H^+]}$$

so in an environment of acidity pH,

$$P_\alpha = \frac{1}{1 + 10^{pH} K_{eq}}$$

is the probability the side chain will be protonated.

Now by (8.12)

$$pK = -\log_{10} K_{eq}$$

$$10^{-pK} = K_{eq}$$

so

$$P_\alpha = \frac{1}{1 + 10^{pH - pK}}$$

$$= \frac{1}{1 + 10^{x}}$$

(where $x = pH - pK$) is the probability that a side chain of pK will be protonated at pH. Now the average charge $\langle q \rangle$ on an acidic residue is

$$\langle q \rangle = (-e)(1 - P_\alpha) \qquad (-COO^-)$$

and on a basic residue is

$$\langle q \rangle = e P_\alpha \qquad (-NH_3^+) \text{ where } e > 0.$$

Note that if $pH = pK$,

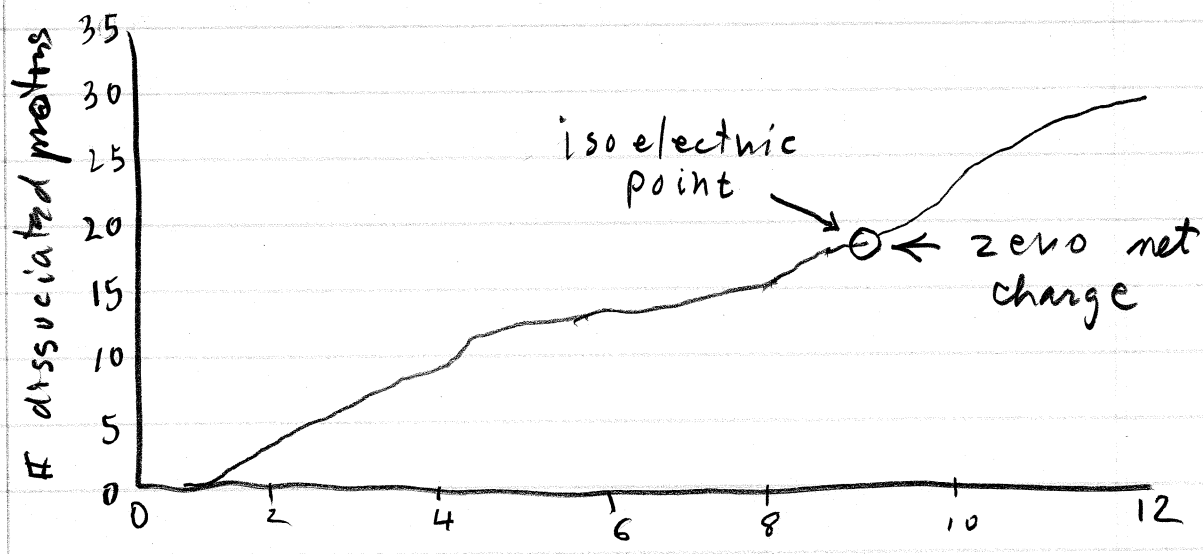
then $x = 0$ and

$$P = \frac{1}{1+1} = \frac{1}{2}$$

so the probability of protonation is 50%.

The <u>local</u> pH near each amino acid (aa) determines P for that a.a.

One may <u>Titrate</u> a solution of a given protein as in Fig. 8.1 on page 313 for the number of protons dissociated from each ribonuclease molecule as the pH rises:



Put an electric field $\mathcal{E}$ across a solution of a given protein solution at a given pH. There will be a force $q\mathcal{E}$ on the protein. By Stokes's law (4.14) the viscous-friction coefficient is

$$\zeta = 6\pi\eta R$$

where η is the viscosity of the fluid and R the radius of the protein. The migration of the protein is <u>electrophoresis</u>. Its speed is more complex than $v = q\mathcal{E}/\zeta$.

The probability P_α of the protonation of residue α

$$P_\alpha = \frac{1}{1 + 10^{x_\alpha}}$$

changes rapidly from 1 to 0 as $x_\alpha = pH - pK_\alpha$ passes 0.

So as the pH rises, the charge on each protein will jump down in steps of e from some positive value Ne to zero and then to negative values. At low pH, the protein will move with E, then will stop when the pH is at the protein's isoelectric point, and then will move against E as the pH increases further.

Linus Pauling (et al.) used this technique in 1949 to separate the β-globin chains of normal (wild-type) hemoglobin from those of sickle-cell hemoglobin. These 146 aa proteins

differ only by the mutation of glutamic acid to valine at position 6. Glu is negatively charged for $pH > pK_{glu} = 4.25$, so it carries a negative charge at all physiological pH's. But valine is neutral and hydrophobic. The mutant proteins clump in fibers of 14 interwound helical strands that give the red blood cell a sickle shape. These deformed, stiff red blood cells get stuck in capillaries and are destroyed, causing anemia. At $pH = 6.9$, the wild-type and sickle-cell hemoglobins have opposite charges.

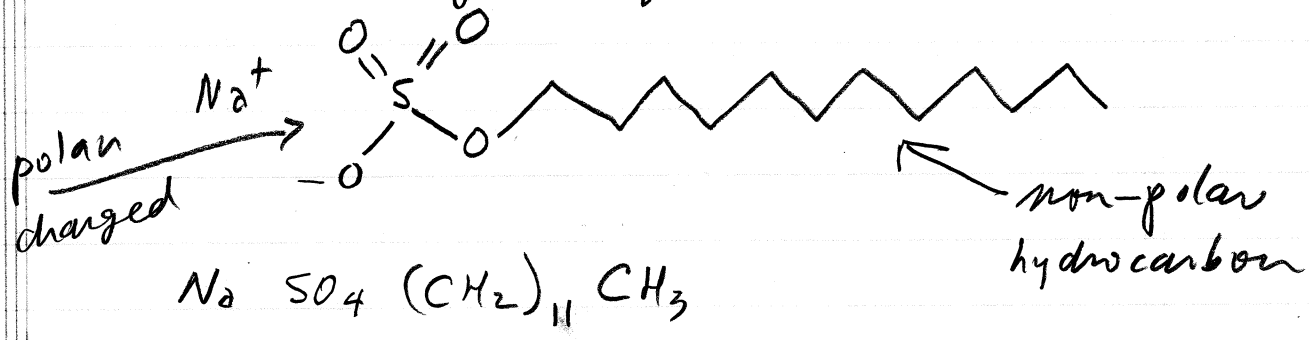
A different mutation, also at

position 6 of the β chain, causes hemoglobin-C disease. Here glutamic acid, which has charge $-e$, is replaced by lysine, with charge $+e$.

There also are three kinds of hemoglobin-M disease caused by histidine 58α → tyrosine or his 63β → tyrosine or valine 67β → glutamic acid. These mutations are on residues near a heme where O_2 binds; they cause cyanosis — incomplete oxygenation of hemoglobin.

Amphiphiles are molecules that have a hydrophobic part and a hydrophilic part. The detergent

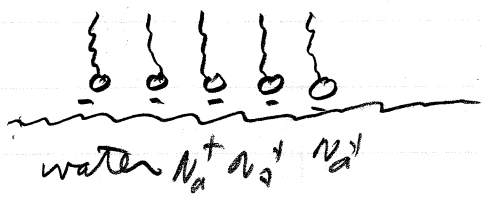
sodium dodecyl sulfate (SDS)



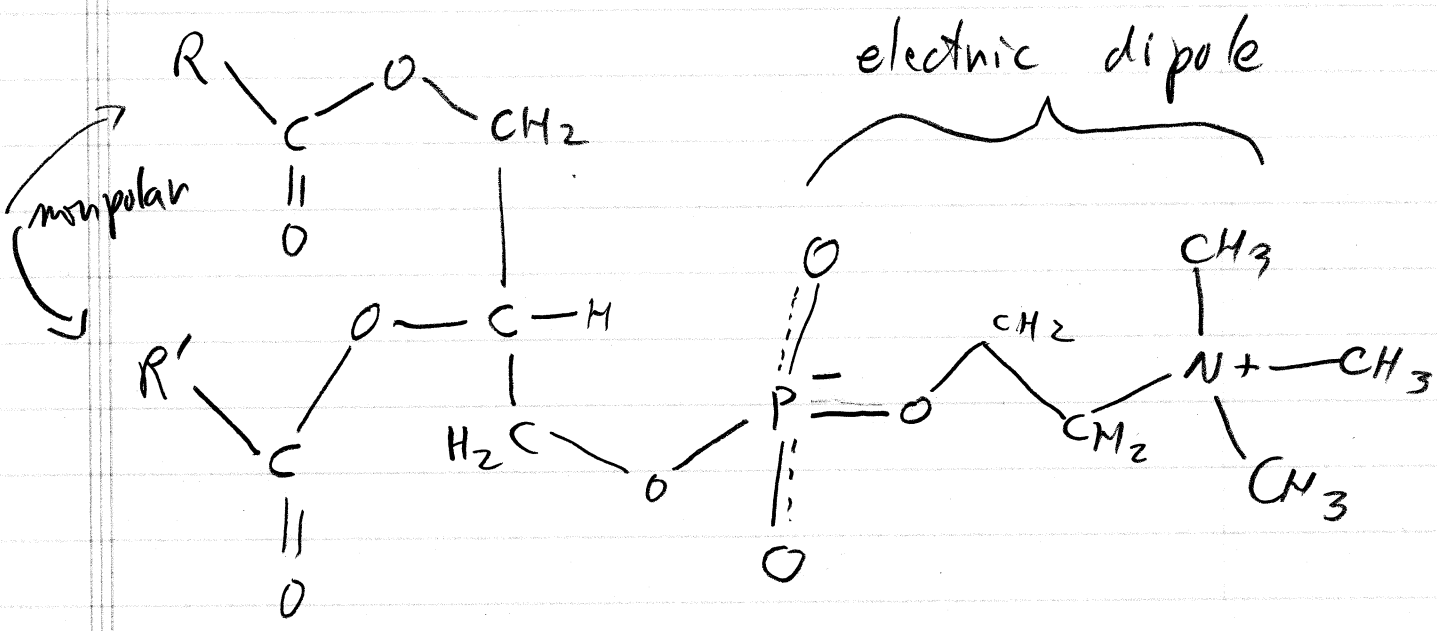
$Na\ SO_4\ (CH_2)_{11}\ CH_3$

(aka sodium lauryl sulfate) is an ionic surfactant used in toothpaste, shampoo, shaving cream, and in SDS-PAGE (SDS polyacrylamide gel electrophoresis).

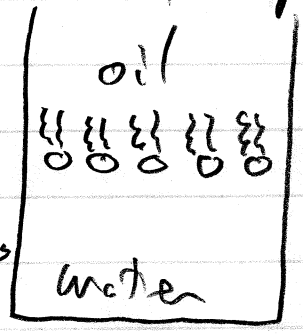
Surfactants reduce surface tension by forming a monomolecular layer on the surface of the water with the hydrocarbon tails in the air, the negatively charged sulfate groups in the water, and the Na^+ in a layer of counterions.



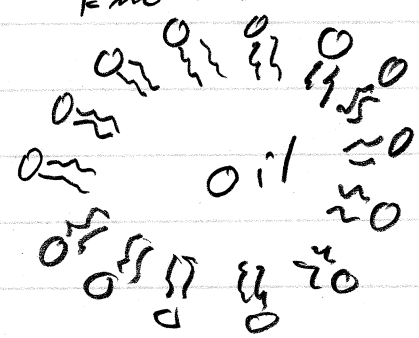
Phosphatidylcholine, a phospholipid,



is another example of an amphiphile. It occurs in the phospholipid bilayer of a cell's membrane. Lecithin is a phospholipid found in yolks of eggs, which with olive oil makes the <u>emulsion</u> known as mayonnaise.



The two phases need not touch.



water, acetic acid = vinegar