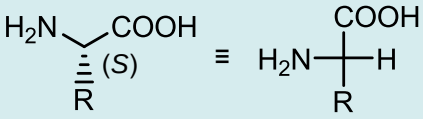
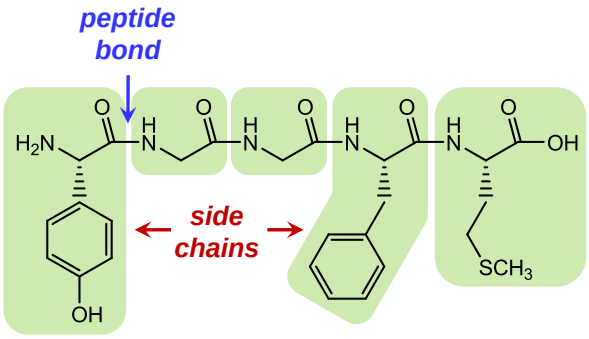
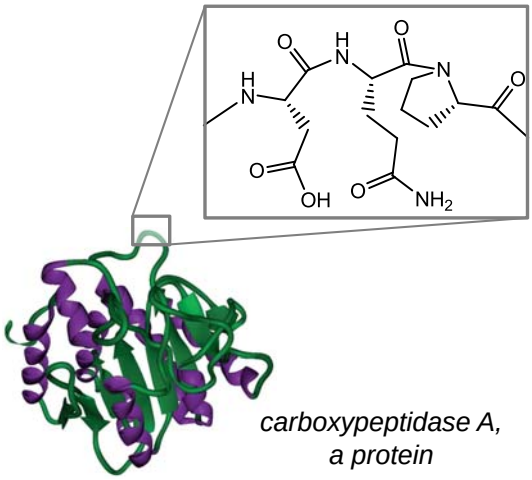
Amino Acids: Constituents of Peptides and Proteins

General structure: 

Natural amino acids are L-amino acids.



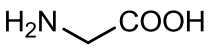
met-enkephalin, a peptide



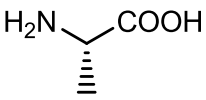
carboxypeptidase A, a protein

The Amino Acids

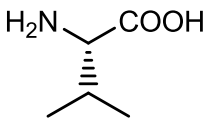
Nonpolar



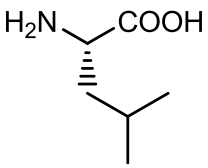
glycine (Gly, G)



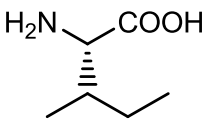
alanine (Ala, A)



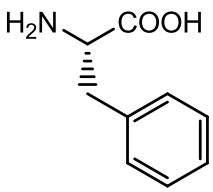
valine* (Val, V)



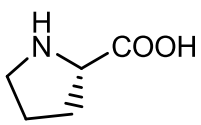
leucine* (Leu, L)



isoleucine* (Ile, I)



phenylalanine* (Phe, F)



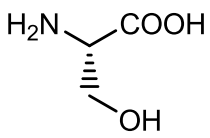
proline (Pro, P)

Pro is a bit unusual; side chain cycle with amine

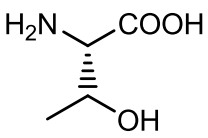
*essential

The Amino Acids

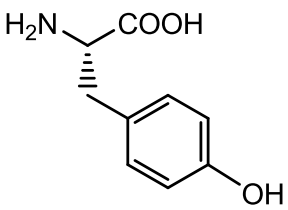
Neutral side chains containing N,O,S



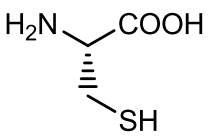
serine
(Ser, S)



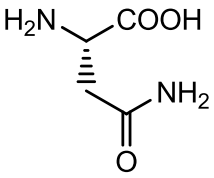
threonine*
(Thr, T)



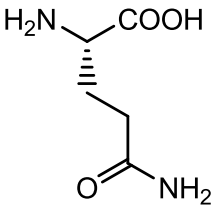
tyrosine
(Tyr, Y)



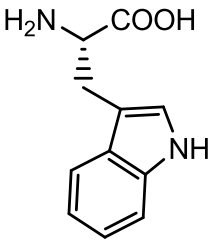
cysteine
(Cys, C)



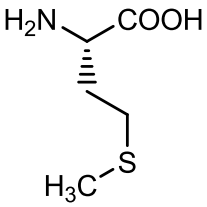
asparagine
(Asn, N)



glutamine
(Gln, Q)



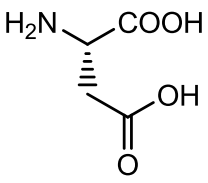
tryptophan*
(Trp, W)



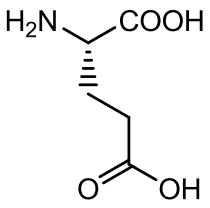
methionine*
(Met, M)

The Amino Acids

Acidic side chains



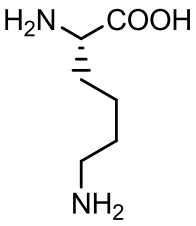
aspartic acid
(Asp, D)



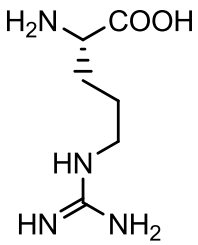
glutamic acid
(Glu, E)

Side chain deprotonates at physiological pH (~7), becomes negatively charged.

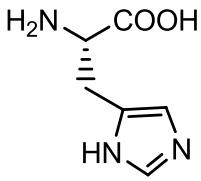
Basic side chains



lysine*
(Lys, K)



arginine*
(Arg, R)



histidine*
(His, H)

Side chain protonates at physiological pH (~7), becomes positively charged.

Amino Acids Are Zwitterions at Neutral pH

Typical pK_a's:

—COOH

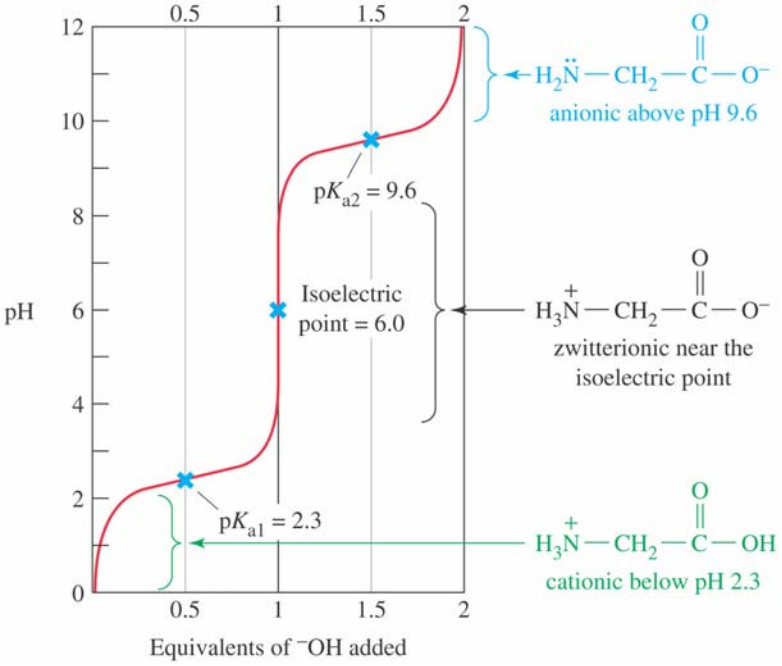
p$K_a \approx 2.3$

—$NH_3^{\oplus}$

p$K_a \approx 9.6$

So between pH = 2.3 and 9.6, molecule has both + and – charge.

Halfway between is pI.



Isoelectric point (pI): pH at which average molecular charge is perfectly neutral.

Some Amino Acids Are Charged at Neutral pH

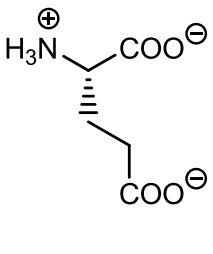
Typical pK_a's:

—COOH

p$K_a \approx 2.3$

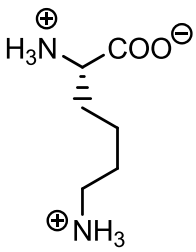
—$NH_3^{\oplus}$

p$K_a \approx 9.6$



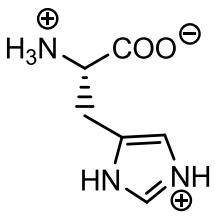
glutamate
(from glutamic acid)

pI = 3.2



lysine

pI = 9.7



histidine

pI = 7.6

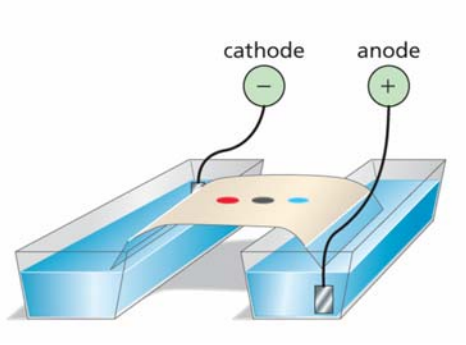
Isoelectric point (pI): pH at which average molecular charge is perfectly neutral.

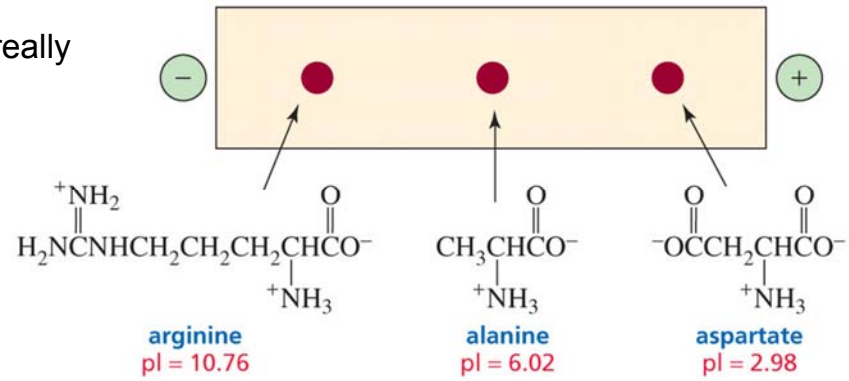
Amino Acid Analysis: Electrophoresis

Amino acids can be identified based on electrophoretic mobility.

Amino acids stained with ninhydrin for visualization.

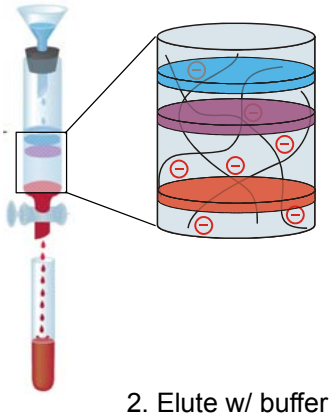
This method not really used any more.

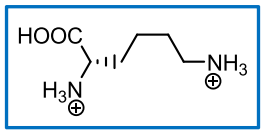




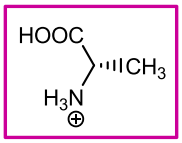
Amino Acid Analysis: Ion-Exchange Chromatography

1. Load negatively charged matrix with amino acid mixture at low pH.

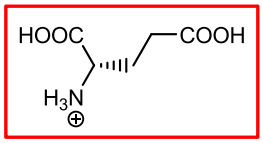




Lys, pI = 9.7



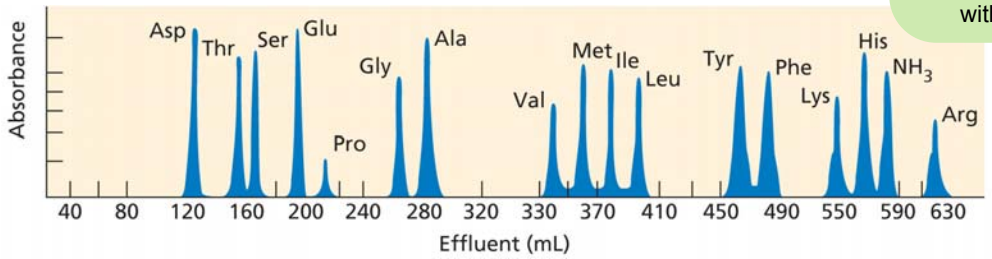
Ala, pI = 6.0



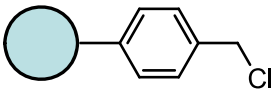
Glu, pI = 3.2

2. Elute w/ buffer of increasing pH.

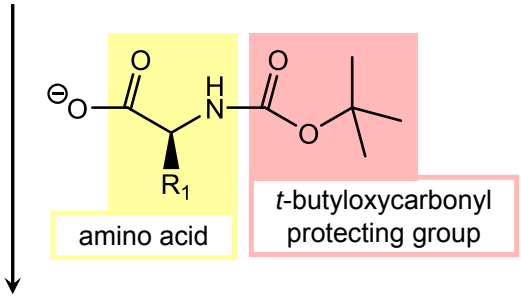
Elution depends both on ionization and on hydrophobic interaction with column.



Solid-Phase Peptide Synthesis



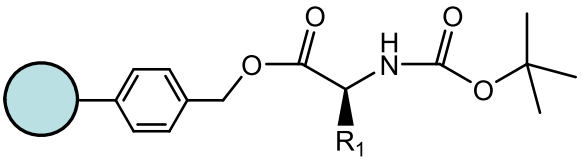
chloromethylated polystyrene bead



Peptides must be synthesized by repetitive addition of amino acid building blocks.

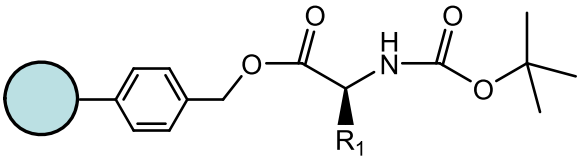
As with DNA, oligopeptide synthesis works best on solid phase.

(Minimizes purification steps, maximizes yield.)



Protecting group prevents –NH_2 from reacting with resin.

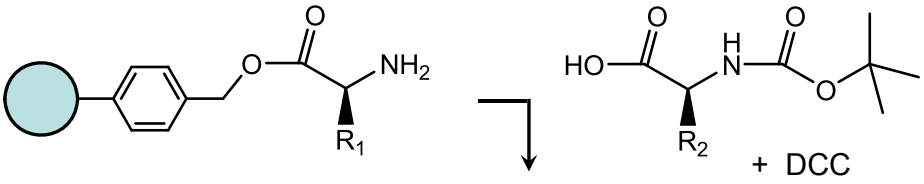
Solid-Phase Peptide Synthesis

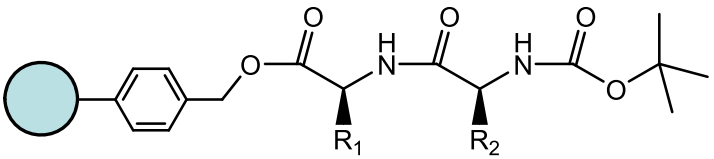


is equivalent to

deprotect

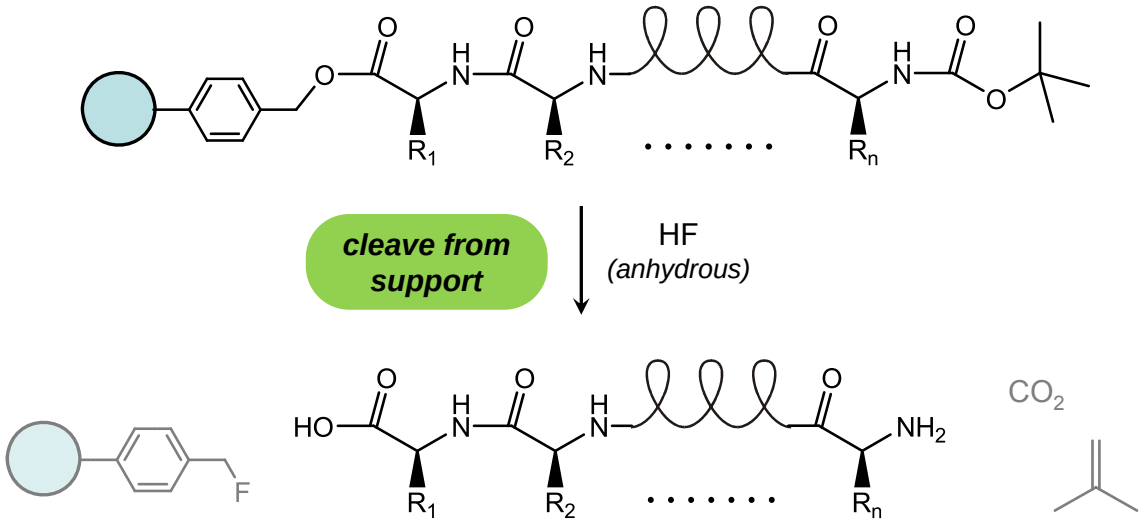
CF_3COOH
CH_2Cl_2





R_1, R_2 etc. may require their own protecting groups.

Solid-Phase Peptide Synthesis



Per cycle yield > 99%;
Capable of routinely synthesizing 60-amino acid peptides.
Fmoc protecting groups now more common (Workshop 23).