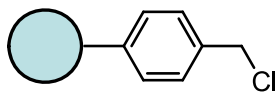


Solid-Phase Peptide Synthesis

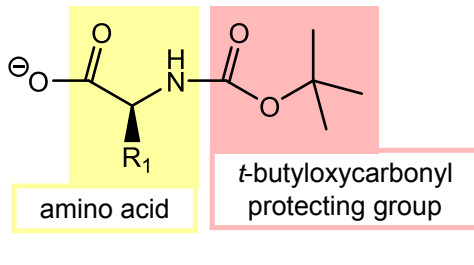


chloromethylated
polystyrene
bead

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

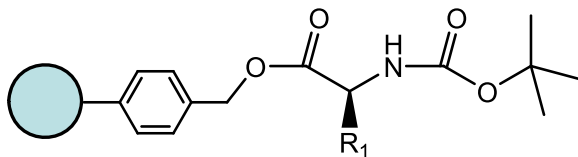
As with DNA, oligopeptide synthesis works best on solid phase.

(Minimizes purification steps, maximizes yield.)



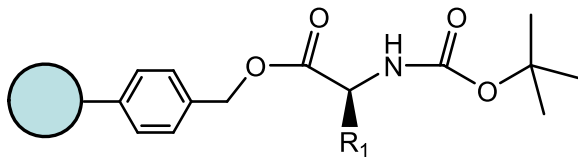
amino acid

t-butyloxycarbonyl
protecting group



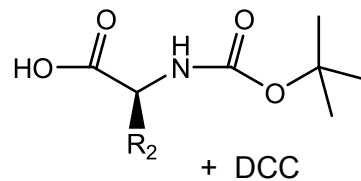
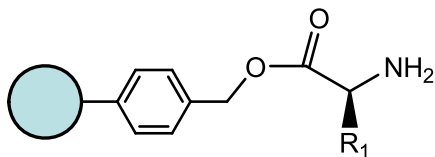
Protecting group prevents $-NH_2$ from reacting with resin.

Solid-Phase Peptide Synthesis

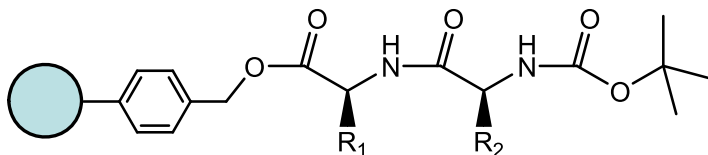


deprotect

CF_3COOH
 CH_2Cl_2

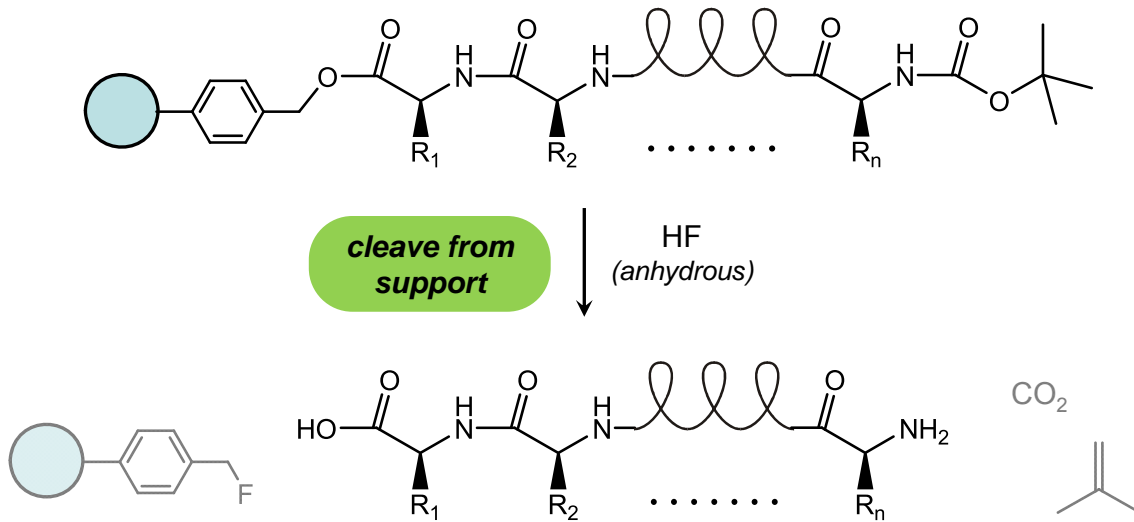


← is equivalent to



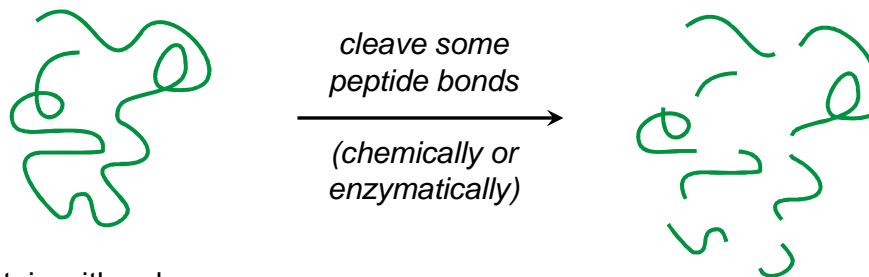
R_1, R_2 etc. may require their own protecting groups.

Solid-Phase Peptide Synthesis



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

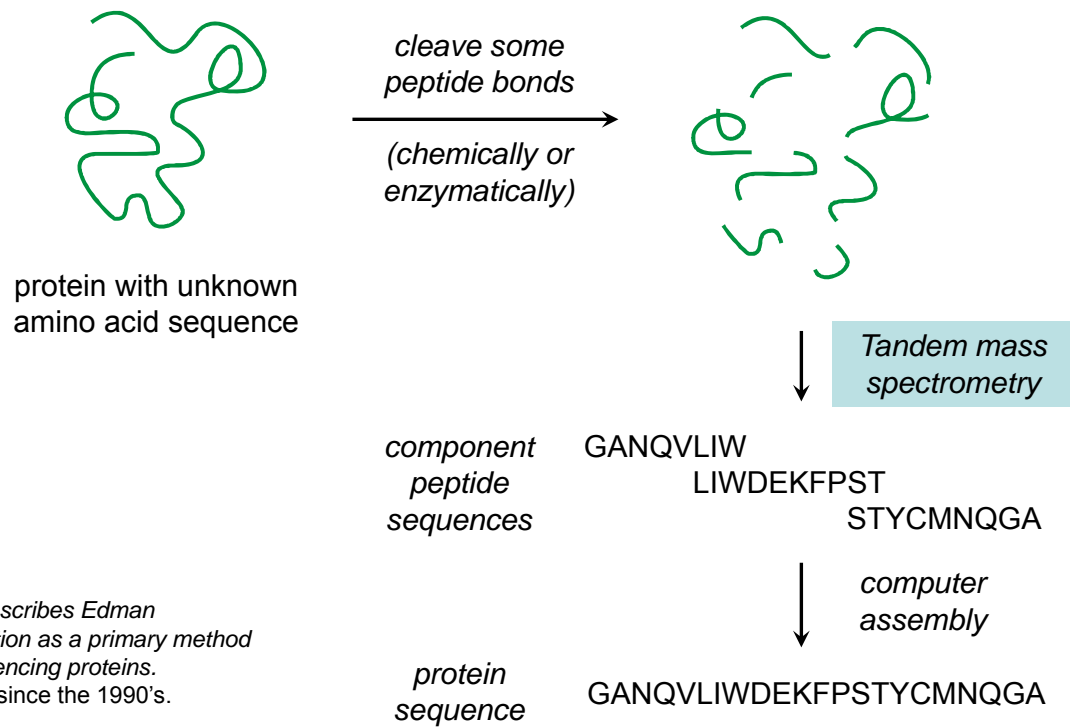
Protein Sequencing



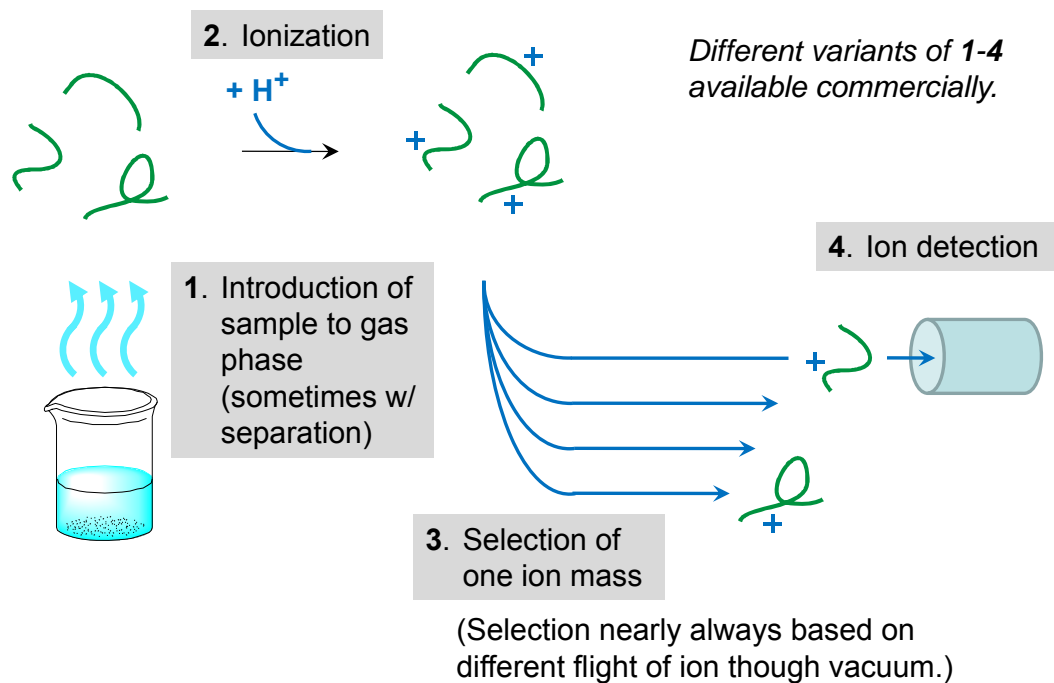
protein with unknown amino acid sequence

Cleaving Agent	Specificity
Cyanogen bromide	Hydrolyzes on C-side of Met
Trypsin	Hydrolyzes on C-side of Arg/Lys
Chymotrypsin	Hydrolyzes on C-side of aromatics (Phe, Tyr, Trp)

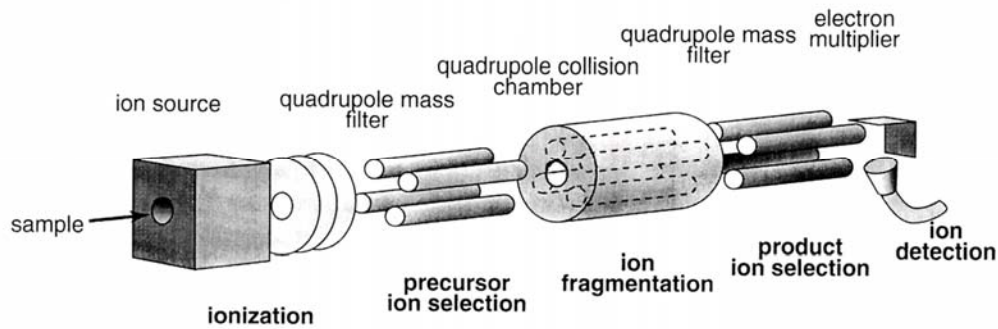
Protein Sequencing



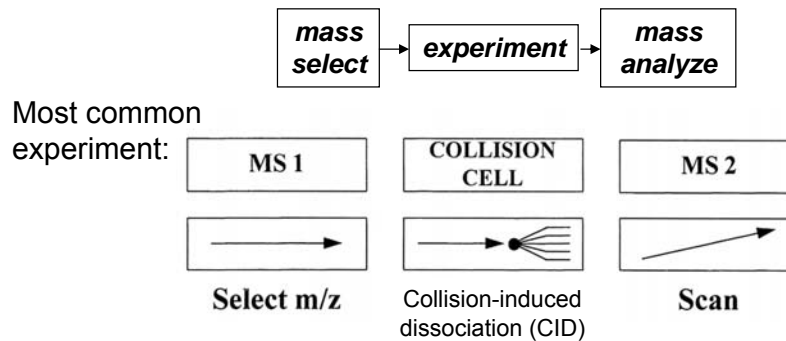
Reminder: General Characteristics of Mass Spectrometry



Tandem Mass Spectrometry (MS/MS)

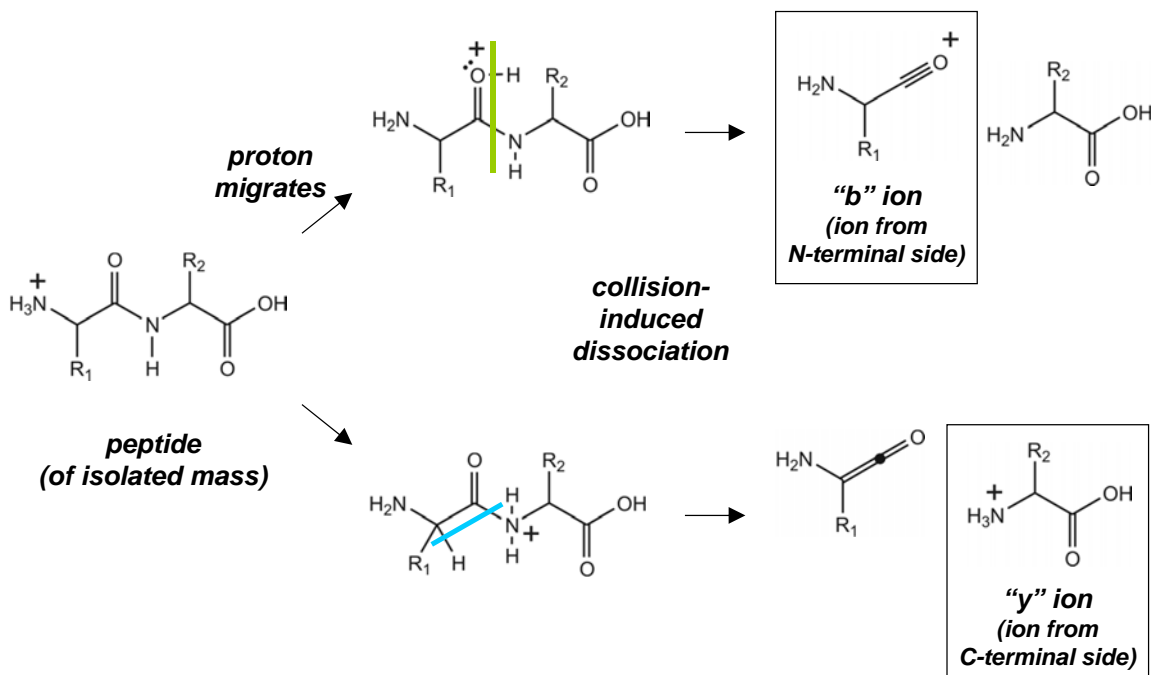


Method for “experimenting” on isolated ions, to learn about their structure (instead of just mass).



Tandem Mass Spectrometry (MS/MS)

- For macromolecules, CID allows molecule to be “sequenced”.



Computational Analysis of MS/MS Data

Computer searches for an amino acid sequence that could be responsible for particular pattern of ion fragments.

Example: Two *b*-ions differ by $272-171 = 101$.

Only Thr has $m = 101$. So it must be in position 3. Computer analyzes all possible peak pairs, searching for consistencies.

