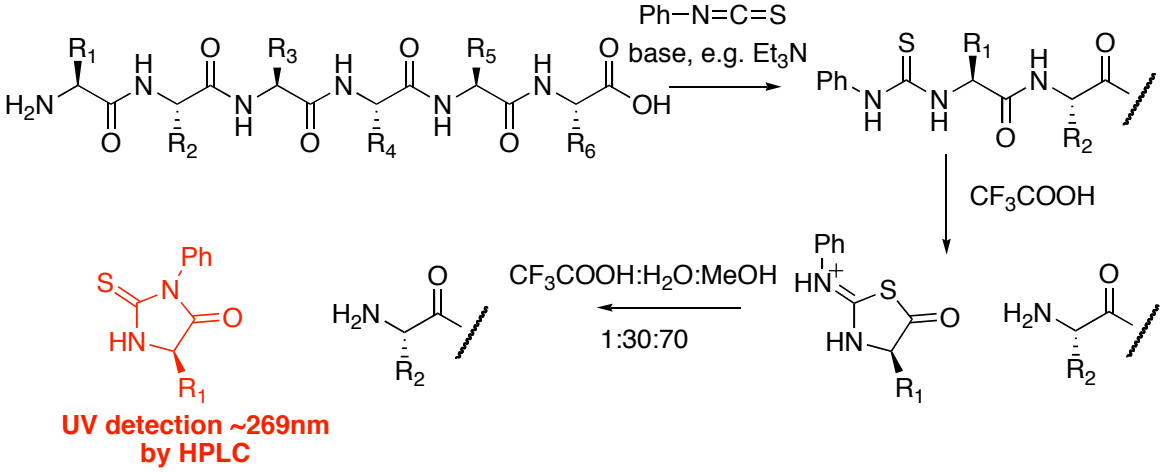
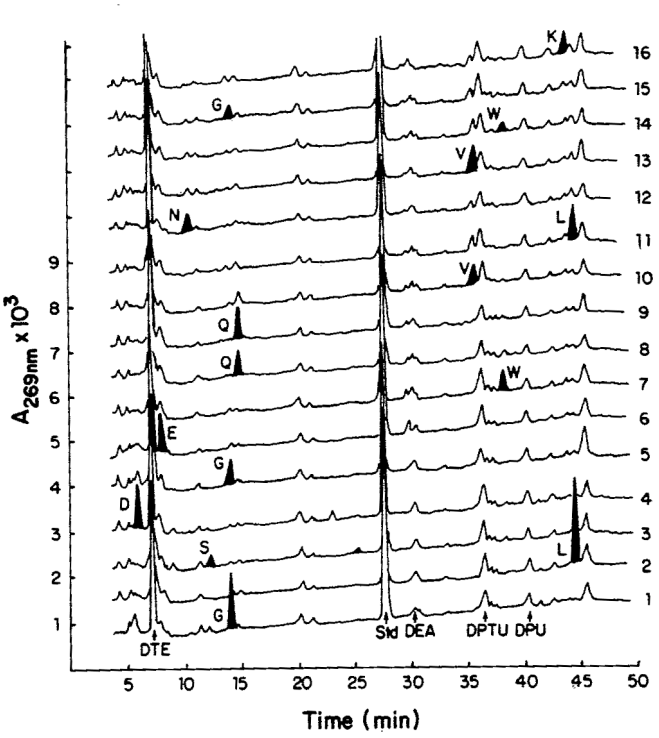
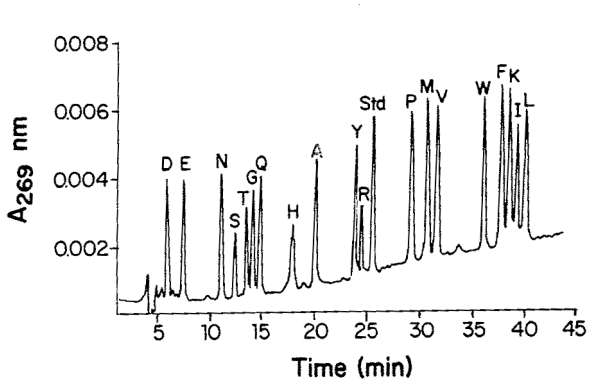
Edman Degradation

- Used for sequencing from *N*-terminus towards C-terminus.
- Success of this method is the high yields that are obtained (typically >93%).
- Mechanism is based on intramolecular cleavage of thiourea





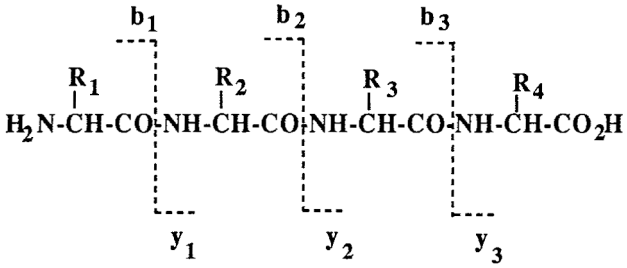
- Iterative release allows repeated residue determination
- Protein can be attached to solid phase via C-terminus and carbodiimide coupling to improve yields



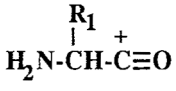
Mass Spectrometry

- Two main ionization methods: Electrospray Ionization (ESI) and Matrix-Assisted Laser Desorption Ionization (MALDI)
- MALDI is popular with crude samples e.g. cell fractions or lysates but ESI often gives better precision
- Sample are often cleaved beforehand (see Peptide Mapping) and MS is a complementary method in many cases
- Ions can be selected on the basis of m/z (mass/charge) and then diverted into a separate chamber or second MS. Thus this technique is known as MS-MS. The first MS to measure all ions, the second to measure the 'mass selected ions'

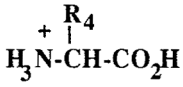
• Sequencing can be achieved by Collision Induced Dissociation (CID) of peptide linkages.
• This can be achieved by varying the potential on the ions formed to accelerate them with good control when exposed to a collision gas introduced into the spectrometer.
• Cleavage can result in an acylium (C=O+) – so-called Y series ions – or ammonium (NH+) – so called B-series ions.



Examples

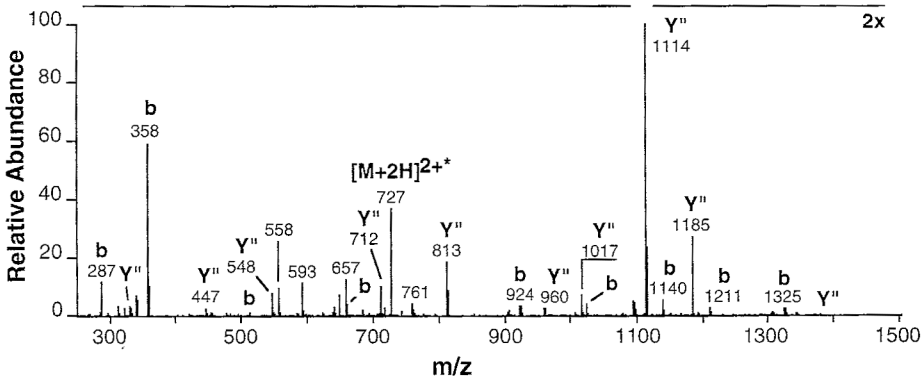


b_1 ion



y_3 ion

• Databases now exist of common fragmentations linked with known sequences such that most modern sequencing spectrometers will not only sequence but also identify your protein from genome information.



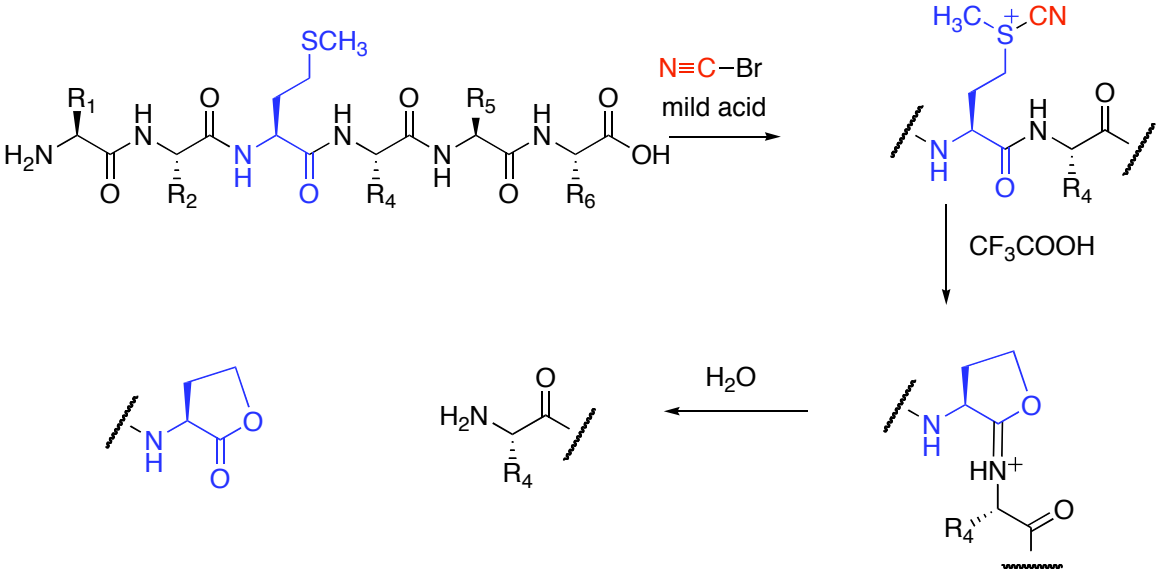
T G Q A P G F T Y D A N K

n	b	Y"	n
0	1.0	**1470.7**	14
1	102.1	**1369.6**	13
2	159.1	**1312.6**	12
3	**287.1**	**1184.6**	11
4	**358.2**	**1113.5**	10
5	**455.2**	**1016.5**	9
6	**512.2**	**959.4**	8
7	**659.3**	**812.4**	7
8	**760.4**	**711.3**	6
9	**923.5**	**548.3**	5
10	**1024.5**	**447.2**	4
11	**1139.5**	**333.2**	3
12	**1210.5**	261.2	2
13	**1324.6**	147.1	1
14	1452.7	19.0	0

This fragment came from an EndoLys cleavage.
The MH_2^{2+} peak (m/z 727) was 'mass selected'

Peptide Mapping

- Sequence specific cleavage methods allow the protein chain to be chopped.
- One common chemical method is cyanogen bromide cleavage.



- The other common method is to use highly specific peptidase enzymes.

Common proteases and their preferred cleavage sites.

Protease	Type	Preferred Cleavage Sites
α-chymotrypsin and subtilisins	Ser	-Trp(Tyr,Phe,Leu,Met) ↓ Xaa-
elastase	Ser	-Ala(Ser,Met,Phe) ↓ Xaa-
pepsin	Asp	-Phe(Tyr,Leu) ↓ Leu(Phe)-
thermolysin	metallo	-Phe(Gly,Asp,Leu) ↓ Leu(Phe)-
papain	Cys	-Phe(Leu,Val)-Xaa ↓ Xaa-
trypsin	Ser	-Arg(Lys) ↓ Xaa-
clostripain	Cys	-Arg ↓ Xaa-
endoprotease Lys-C (*Achromobacter*)	Ser	-Lys ↓ Xaa-
endoprotease Glu-C (V8 protease)	Ser	-Glu (Asp) ↓ Xaa-
carboxypeptidase Y	Ser	-Xaa ↓ Xaa-OH
carboxypeptidase B	metallo	-Xaa ↓ [Arg,Lys]-OH
carboxypeptidase A	metallo	-Xaa ↓ [Asp,Glu,Phe,Leu]-OH
aminopeptidase M	metallo	H_2N-Xaa ↓ Xaa-
pyroglutamate-aminopeptidase	Cys	pGlu ↓ Xaa-
cathepsin C	Cys	H_2N-Xaa-Xaa ↓ Xaa-
proline iminopeptidase	Ser	Pro ↓ Xaa-