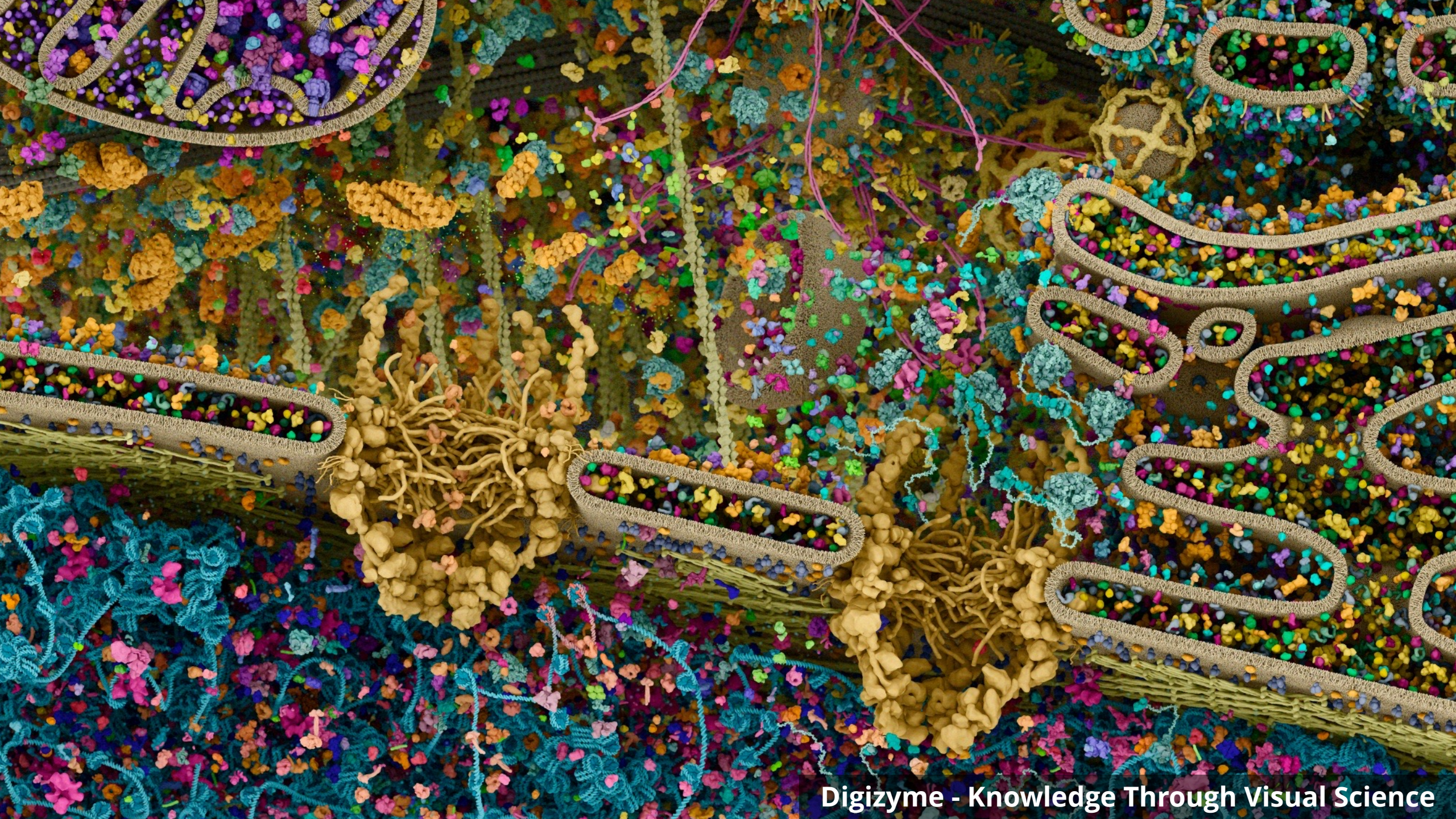
Introduction to mass spectrometry-based proteomics

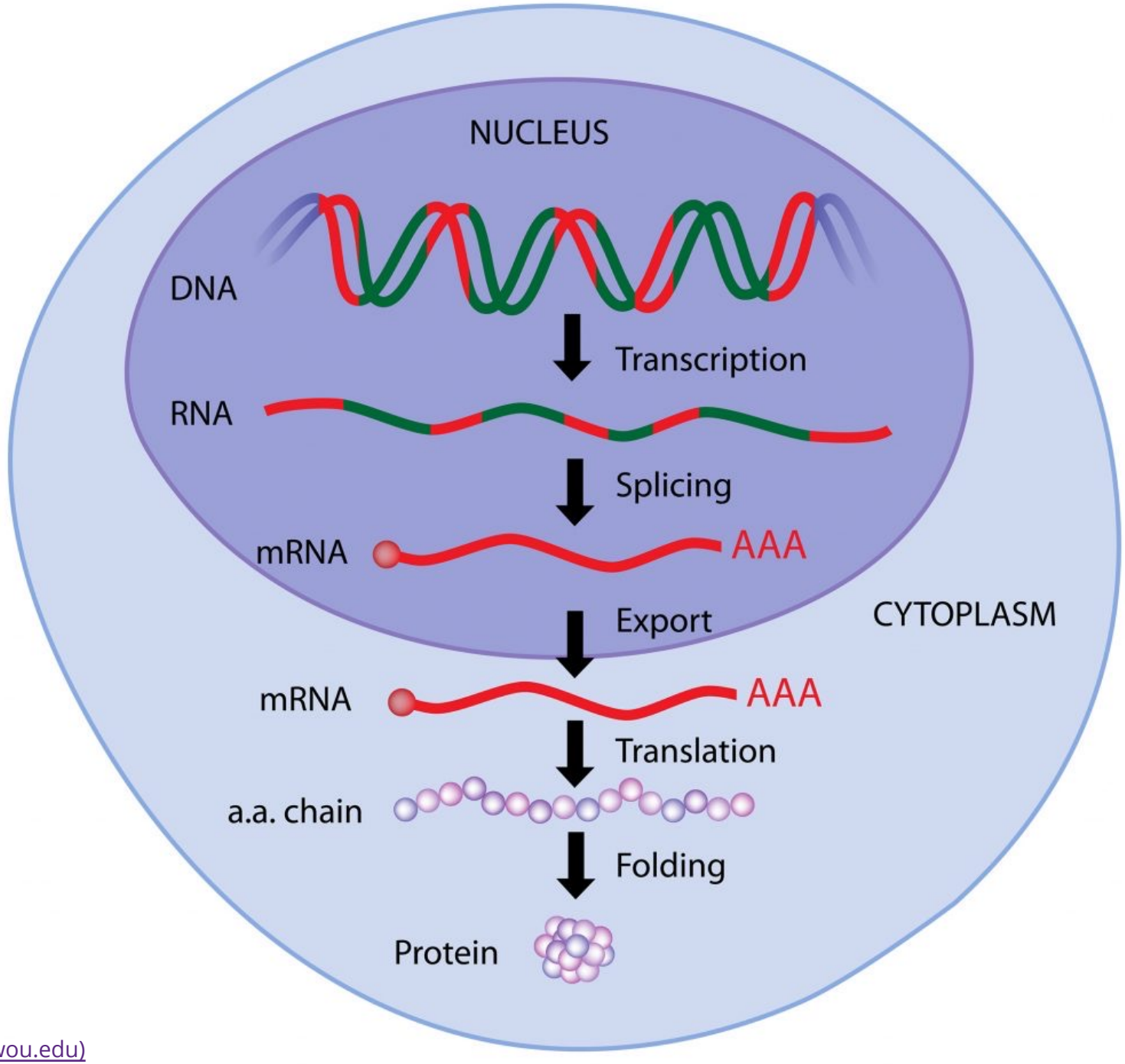
Robbin Bouwmeester

Lecture mass spectrometry basics - Part 1 of 7 - YouTube

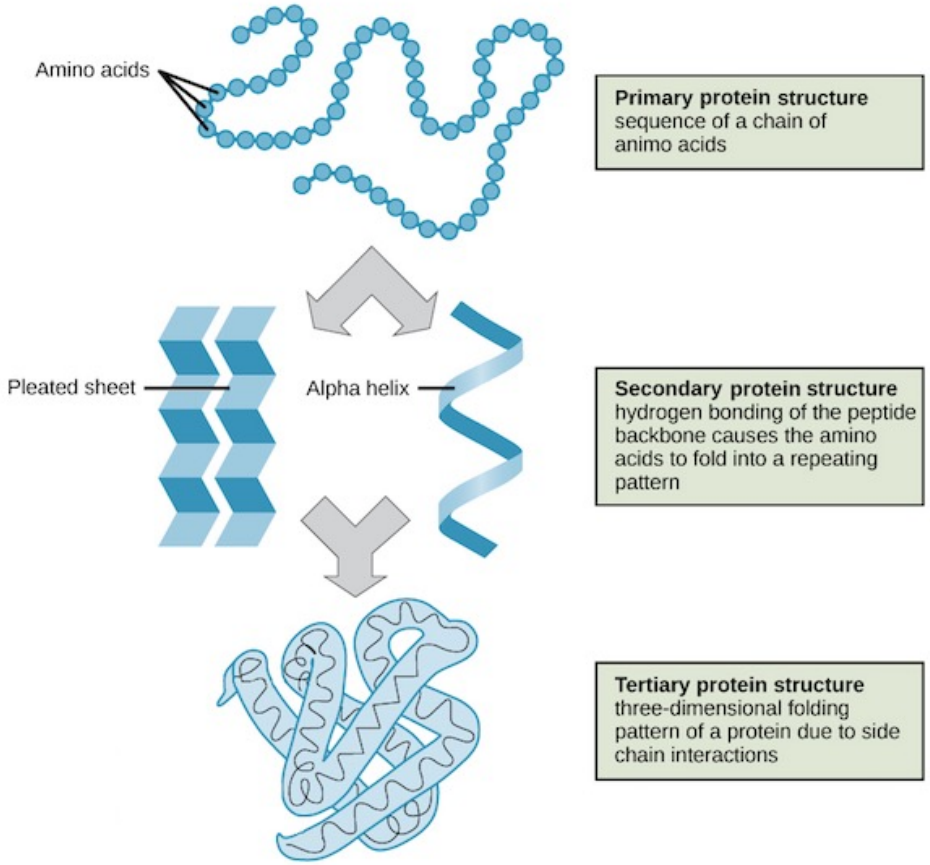
VIB-UGENT CENTER FOR MEDICAL BIOTECHNOLOGY
GHENT UNIVERSITY
Compomics
AGENTSCHAP INNOVEREN & ONDERNEMEN
Vlaanderen is ondernemen
Janssen PHARMACEUTICAL COMPANIES OF Johnson & Johnson

07/03/2023


Digizyme - Knowledge Through Visual Science



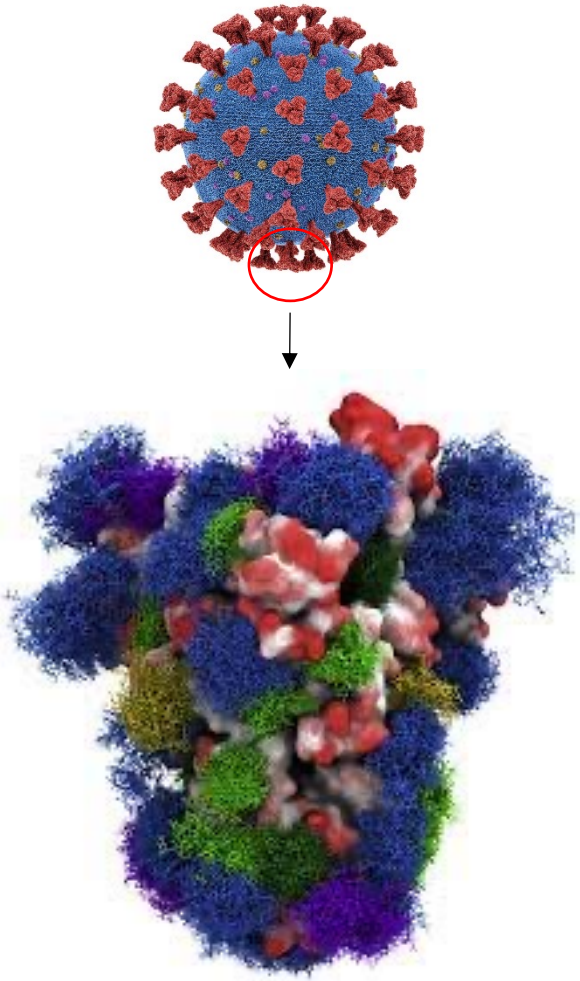
Chapter 11: Translation - Chemistry (wou.edu)



Primary protein structure
sequence of a chain of animo acids

Secondary protein structure
hydrogen bonding of the peptide backbone causes the amino acids to fold into a repeating pattern

Tertiary protein structure
three-dimensional folding pattern of a protein due to side chain interactions



[Protein structure: Primary, secondary, tertiary & quatrenary (article) | Khan Academy]

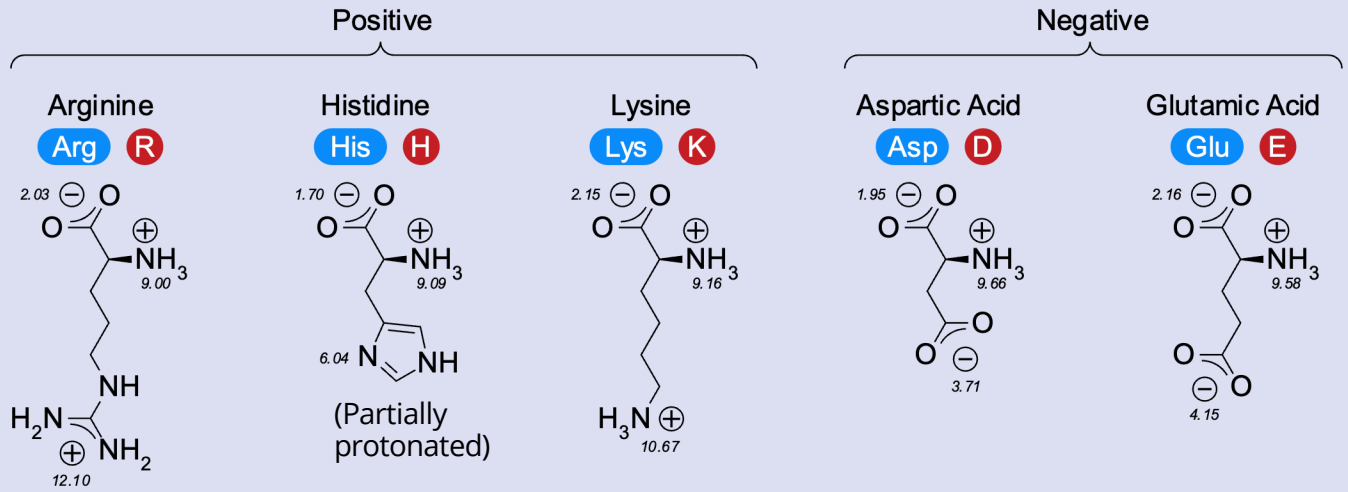
TWENTY-ONE PROTEINOGENIC α-AMINO ACIDS

Side chain charge at physiological pH 7.4

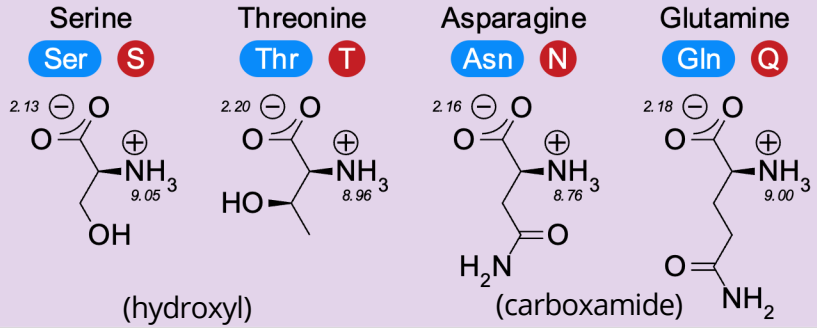
pK_a values shown italicized

⊕ Positive
⊖ Negative

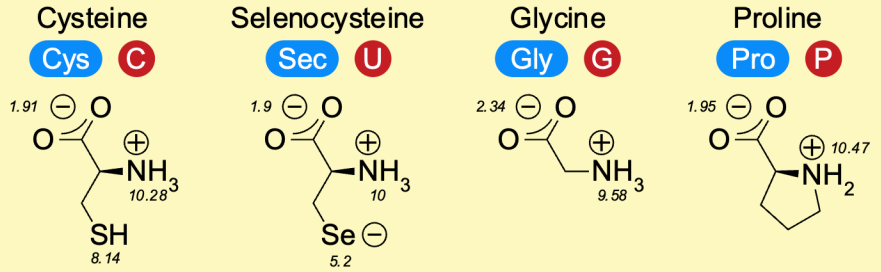
A. Amino Acids with Electrically Charged Side Chains



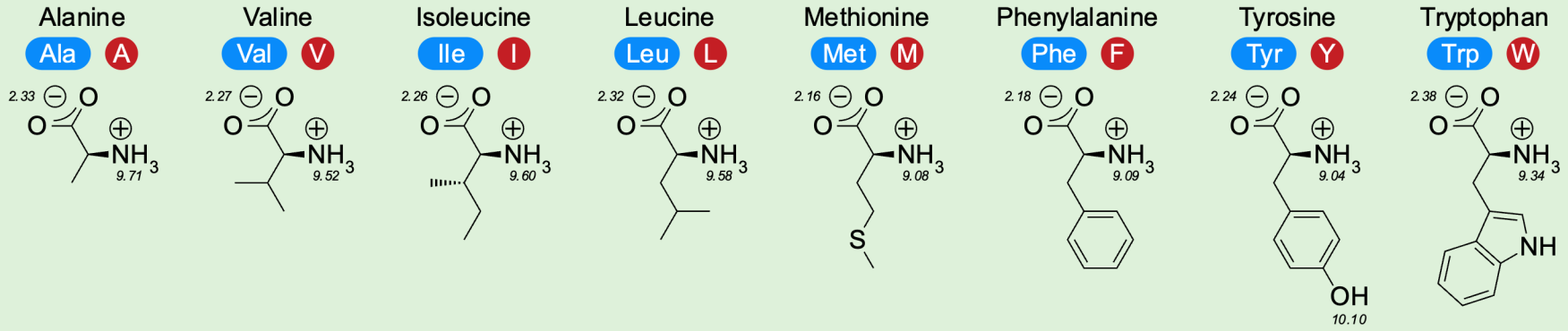
B. Amino Acids with Polar Uncharged Side Chains



C. Special Cases



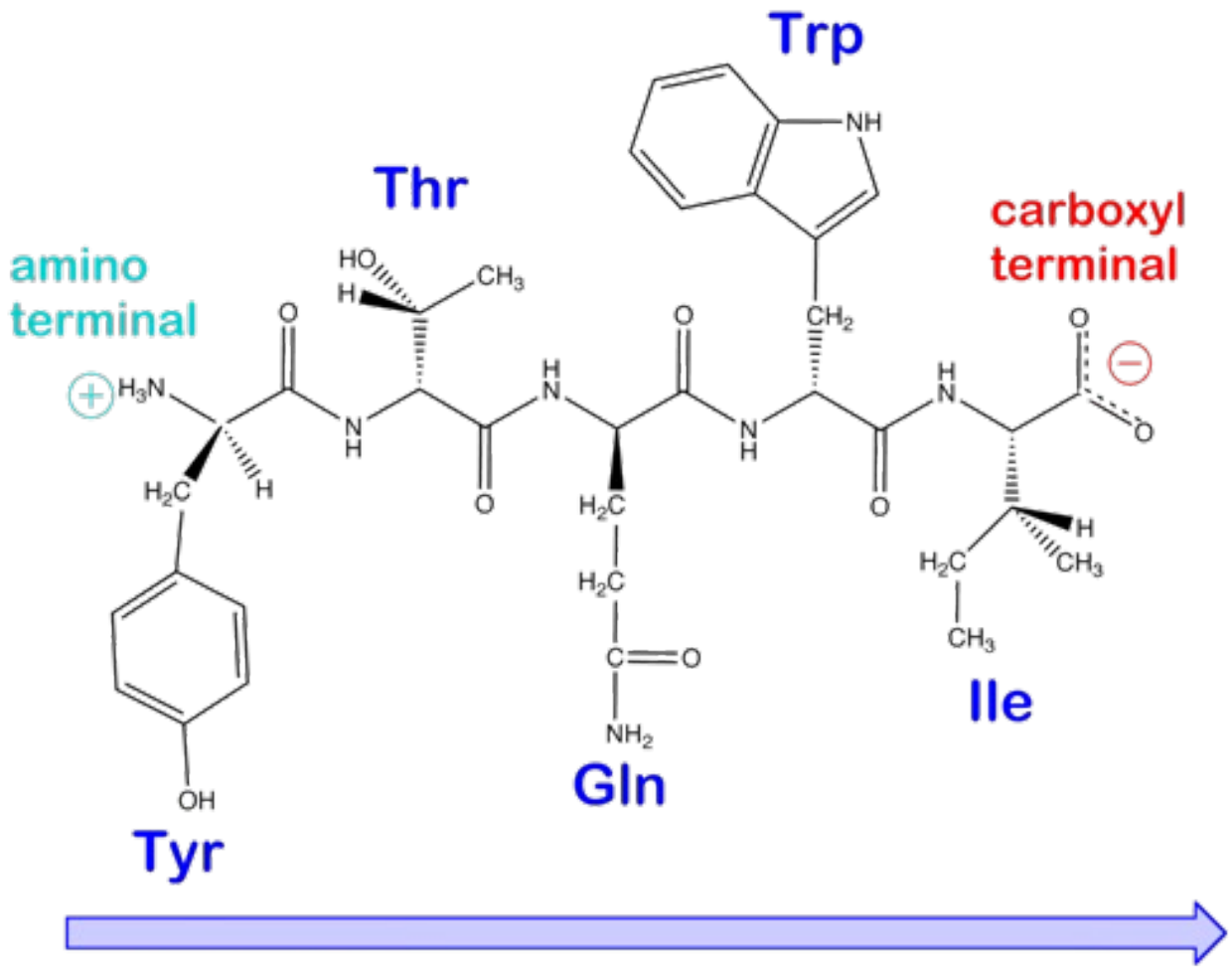
D. Amino Acids with Hydrophobic Side Chains



Amino acid - Wikipedia

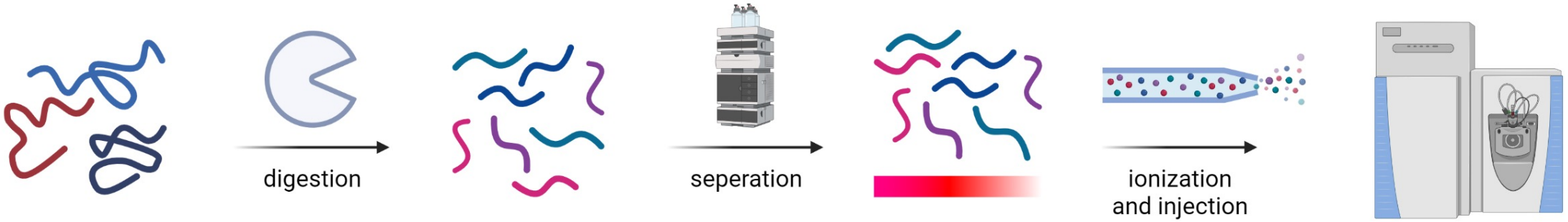
Amino acid	3-letter code	1-letter code	MW (Da)	Structure
Alanine	Ala	A	89.1	CH3-CH(NH2)-COOH
Arginine	Arg	R	174.2	HN=C(NH2)-NH-(CH2)3-CH(NH2)-COOH
Asparagine	Asn	N	132.1	H2N-CO-CH2-CH(NH2)-COOH
Aspartic Acid	Asp	D	133.1	HOOC-CH2-CH(NH2)-COOH
Cysteine	Cys	C	121.2	HS-CH2-CH(NH2)-COOH
Glutamic Acid	Glu	E	147.1	HOOC-(CH2)2-CH(NH2)-COOH
Glutamine	Gln	Q	146.1	H2N-CO-(CH2)2-CH(NH2)-COOH
Glycine	Gly	G	75.1	NH2-CH2-COOH
Histidine	His	H	155.2	NH-CH=N-CH=C-CH2-CH(NH2)-COOH |_____|
Isoleucine	Ile	I	131.2	CH3-CH2-CH(CH3)-CH(NH2)-COOH
Leucine	Leu	L	131.2	(CH3)2-CH-CH2-CH(NH2)-COOH
Lysine	Lys	K	146.2	H2N-(CH2)4-CH(NH2)-COOH
Methionine	Met	M	149.2	CH3-S-(CH2)2-CH(NH2)-COOH
Phenylalanine	Phe	F	165.2	Ph-CH2-CH(NH2)-COOH
Proline	Pro	P	115.1	NH-(CH2)3-CH-COOH |_____|
Serine	Ser	S	105.1	HO-CH2-CH(NH2)-COOH
Threonine	Thr	T	119.1	CH3-CH(OH)-CH(NH2)-COOH
Tryptophan	Trp	W	204.2	Ph-NH-CH=C-CH2-CH(NH2)-COOH |_____|
Tyrosine	Tyr	Y	181.2	HO-p-Ph-CH2-CH(NH2)-COOH
Valine	Val	V	117.1	(CH3)2-CH-CH(NH2)-COOH

Amino Acid Properties (geneinfinity.org)

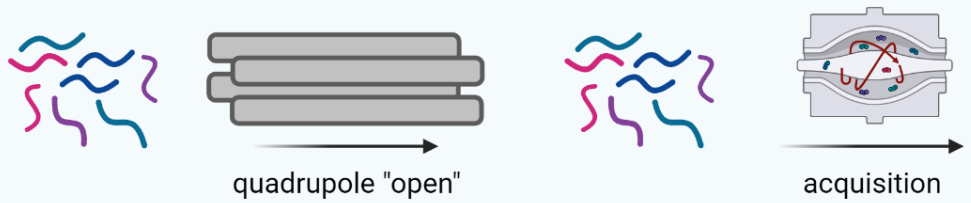


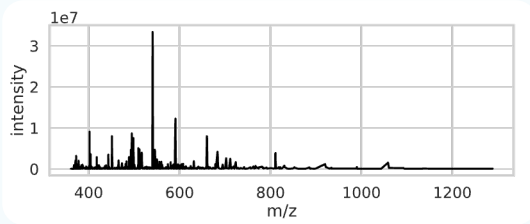
Levels of Protein Organization (uvm.edu)

LC-MS/MS recap

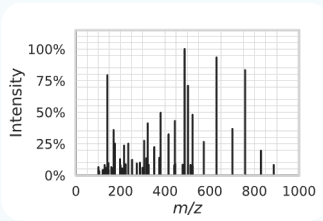


MS1 survey scan:
Find precursors (= full peptides)

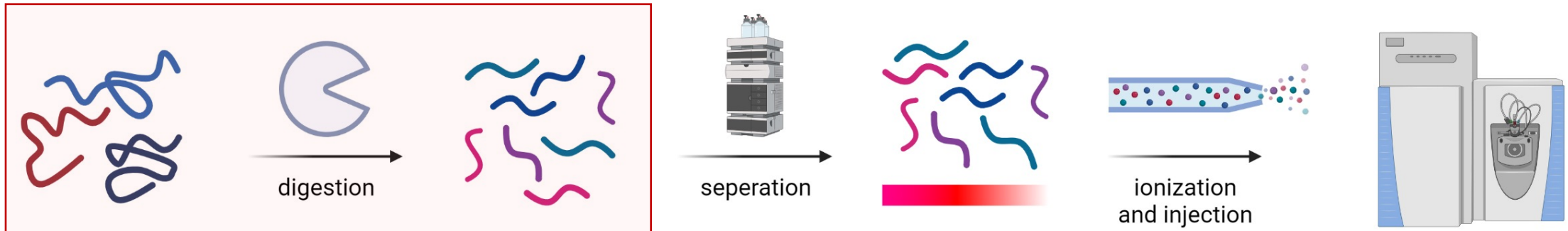




MS2 scan:
Get fragmentation spectrum for peptide at selected m/z window



LC-MS/MS recap



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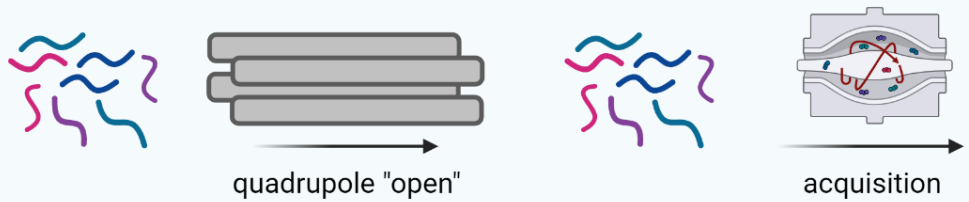

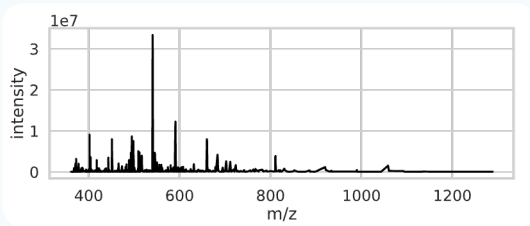

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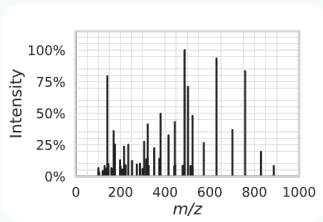


NRRPCHSHTKECESAWKNRPCHSHTKKPCHSHTKKNRKVWKIPPFFW

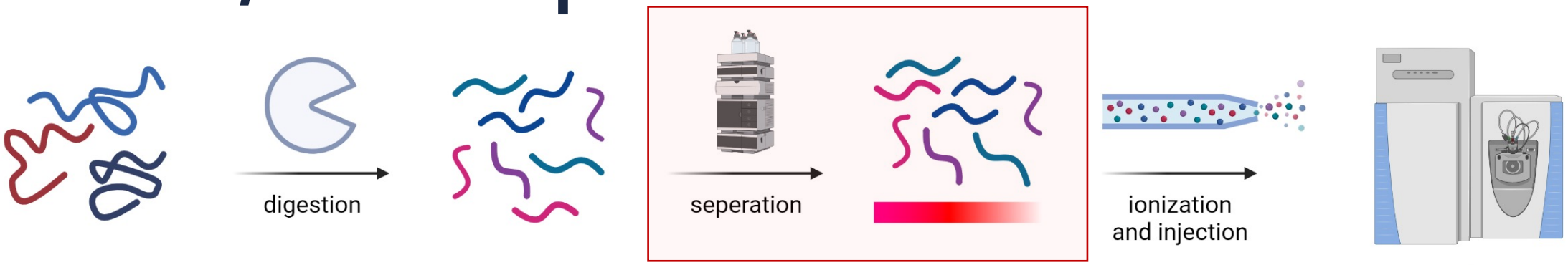
trypsin digest

NR PCHSHTK NR K K K IPPFFW

R ECESAWK PCHSHTK PCHSHTK NR VWK

PCHSHTKECESAWK

LC-MS/MS recap



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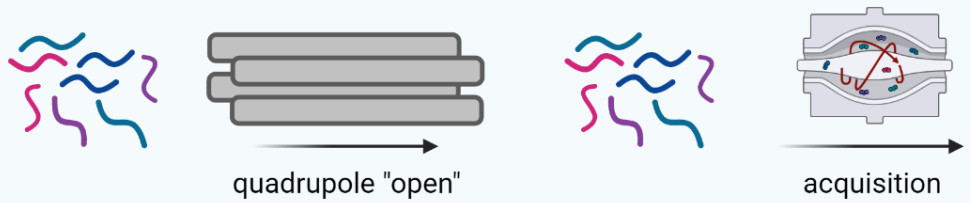

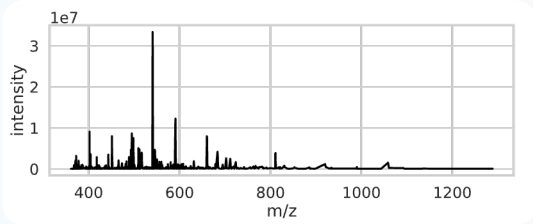

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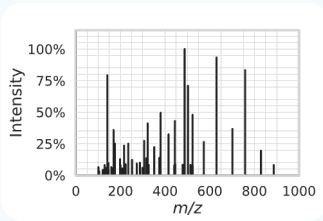
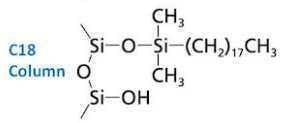
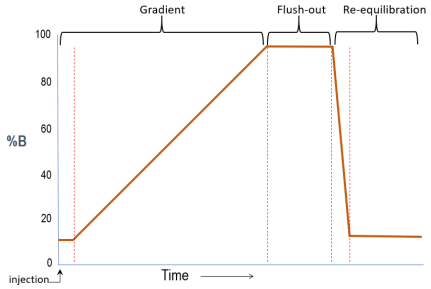


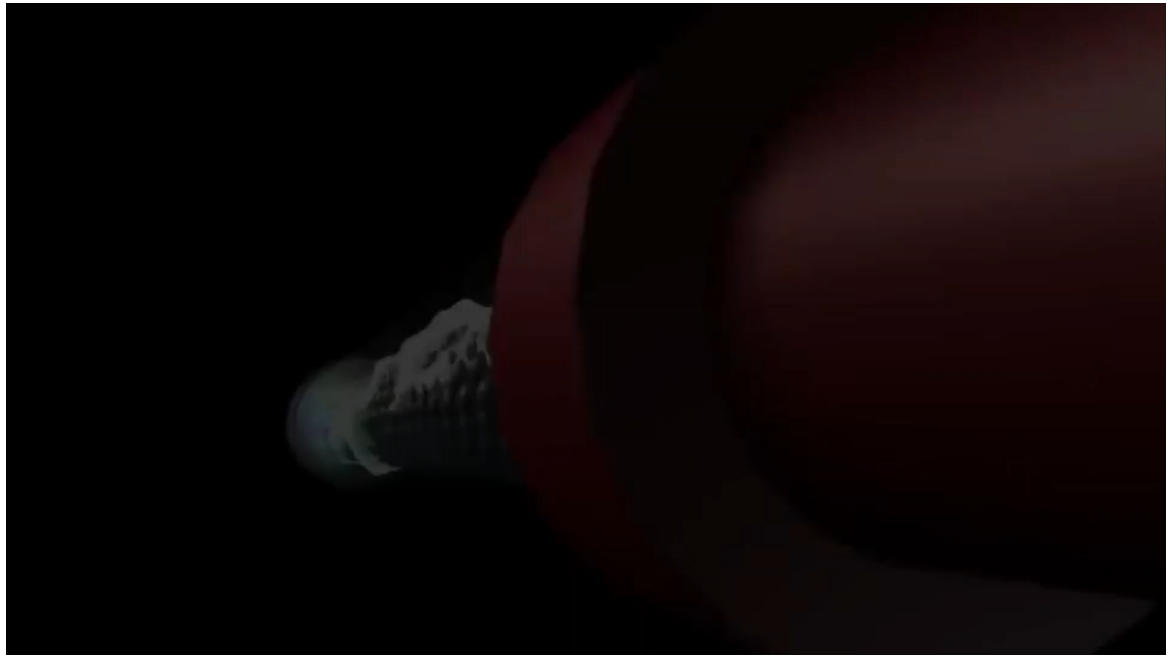
Liquid Chromatography (LC) separates based on, for example, hydrophobicity of peptides

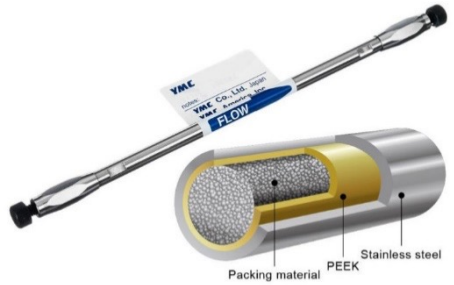
Stationary phase



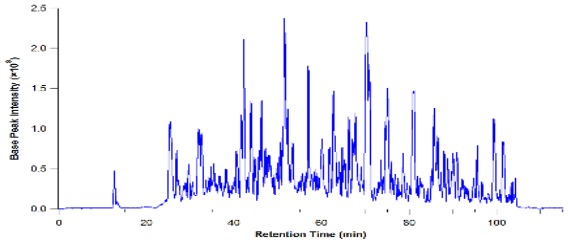
Mobile phase



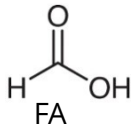




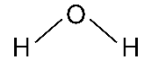
Chromatogram



Solvent A:

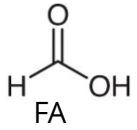


FA

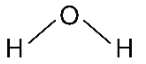


H20 (LC grade)

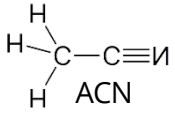
Solvent B:



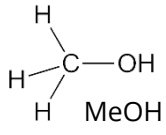
FA



H20 (LC grade)

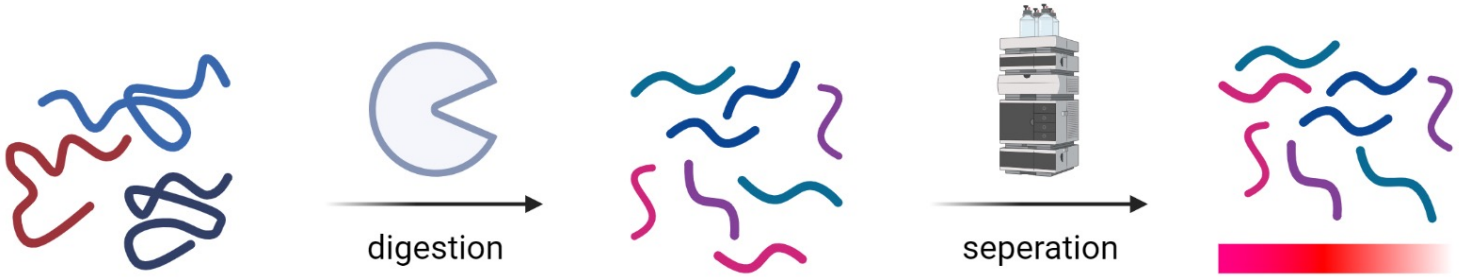


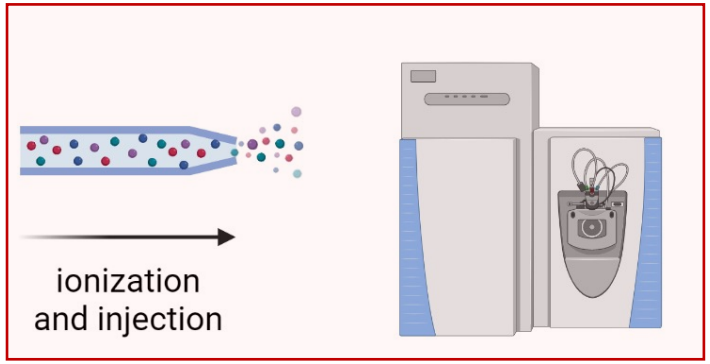
ACN



MeOH

LC-MS/MS recap





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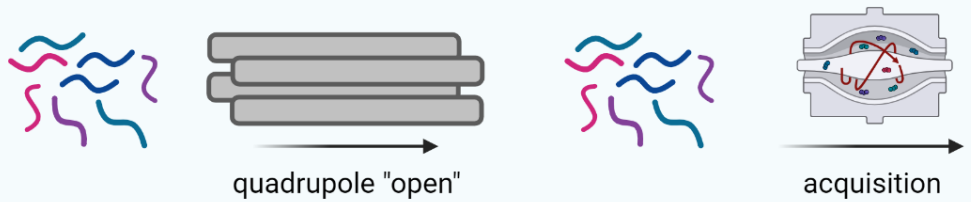

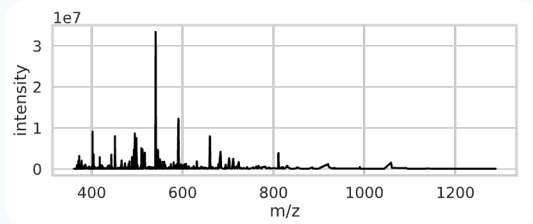

MS2 scan:
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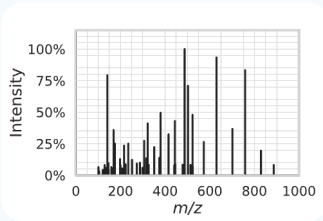
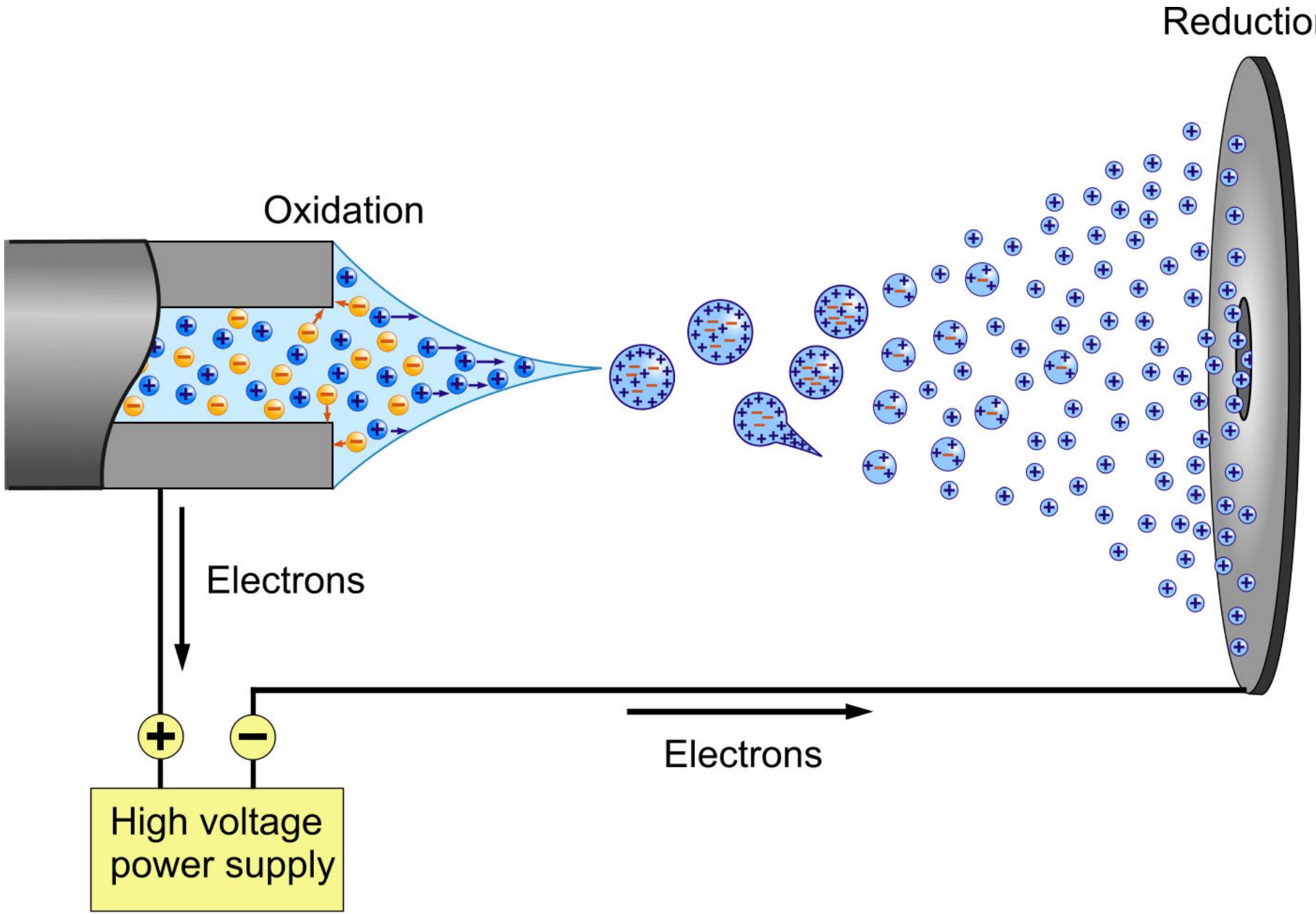
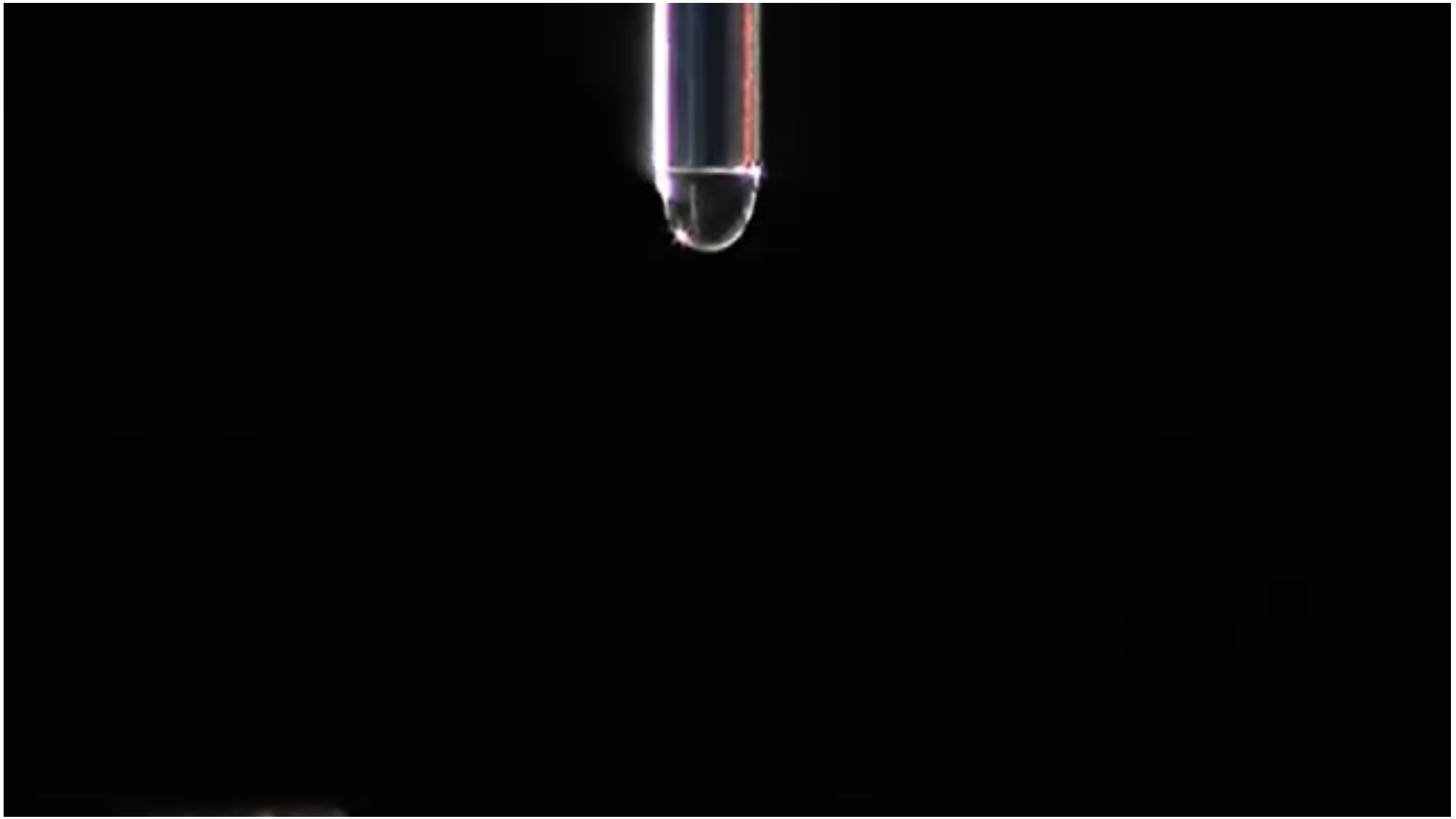


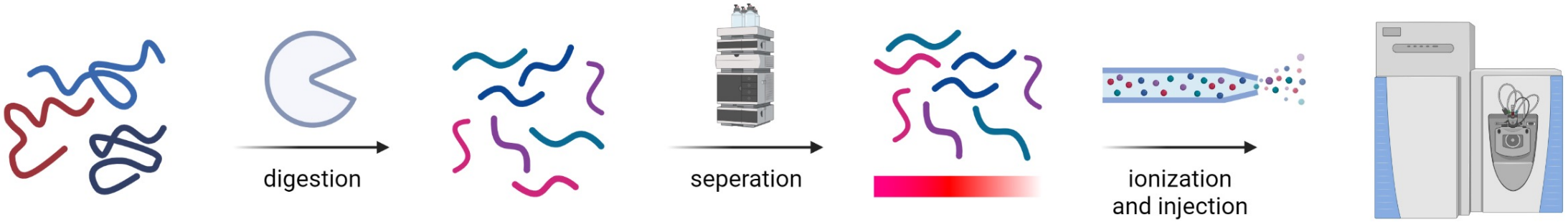


Electrospray ionization - Wikipedia



electrospray process - YouTube

LC-MS/MS recap



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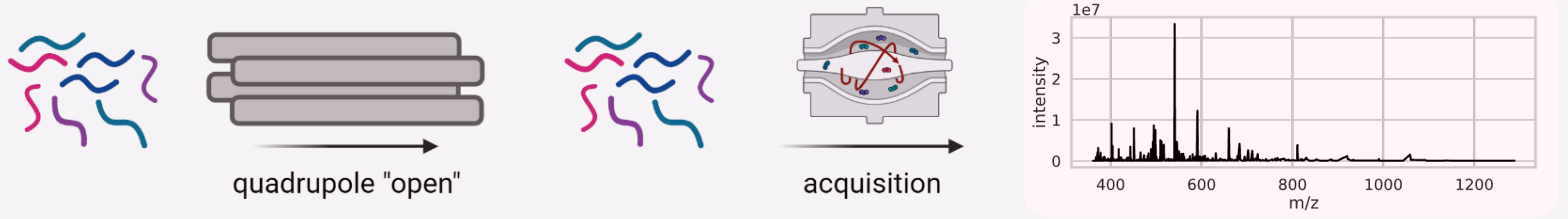
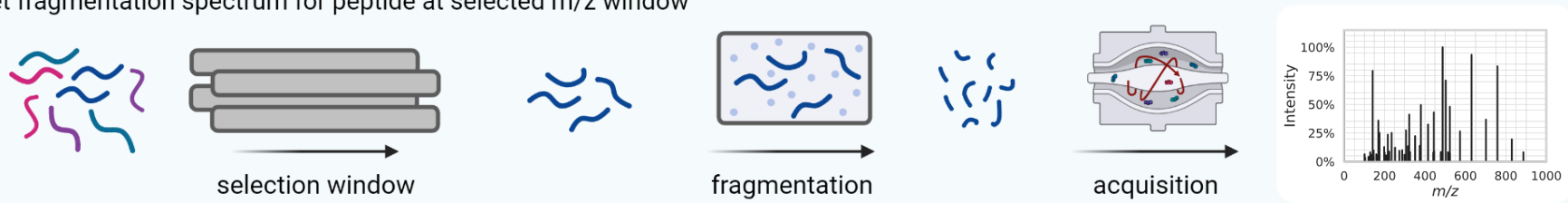


MS2 scan:
Get fragmentation spectrum for peptide at selected m/z window

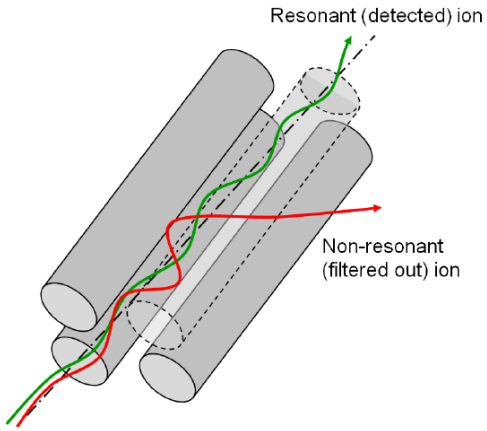


amino acid	code	abbrev	composition	mono mass	avg mass
glycine	G	GLY	C_2H_3NO	57.021463735	57.05132
alanine	A	ALA	C_3H_5NO	71.037113805	71.0779
serine	S	SER	$C_3H_5NO_2$	87.032028435	87.0773
proline	P	PRO	C_5H_7NO	97.052763875	97.11518
valine	V	VAL	C_5H_9NO	99.068413945	99.13106
threonine	T	THR	$C_4H_7NO_2$	101.047678505	101.10388
cysteine	C	CYS	C_3H_5NOS	103.009184505	103.1429
leucine	L	LEU	$C_6H_{11}NO$	113.084064015	113.15764
isoleucine	I	ILE	$C_6H_{11}NO$	113.084064015	113.15764
asparagine	N	ASN	$C_4H_6N_2O_2$	114.042927470	114.10264
aspartic acid	D	ASP	$C_4H_5NO_3$	115.026943065	115.0874
glutamine	Q	GLN	$C_5H_8N_2O_2$	128.058577540	128.12922
lysine	K	LYS	$C_6H_{12}N_2O$	128.094963050	128.17228
glutamic acid	E	GLU	$C_5H_7NO_3$	129.042593135	129.11398
methionine	M	MET	C_5H_9NOS	131.040484645	131.19606
histidine	H	HIS	$C_6H_7N_3O$	137.058911875	137.13928
phenylalanine	F	PHE	C_9H_9NO	147.068413945	147.17386
selenocysteine	U	SEC	C_3H_5NOSe	150.953633405	150.3079
arginine	R	ARG	$C_6H_{12}N_4O$	156.101111050	156.18568
tyrosine	Y	TYR	$C_9H_9NO_2$	163.063328575	163.17326
tryptophan	W	TRP	$C_{11}H_{10}N_2O$	186.079312980	186.2099
pyrrolysine	O	PYL	$C_{12}H_{19}N_3O_2$	237.147726925	237.29816

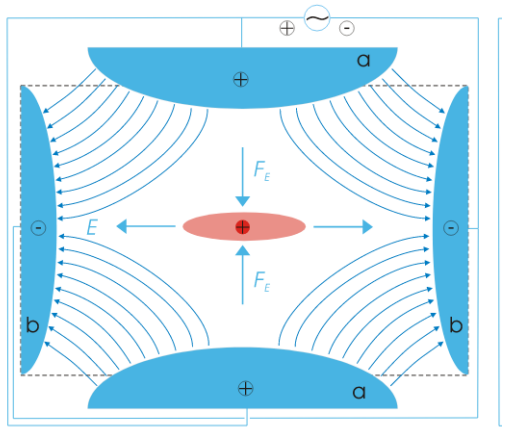
Element	Isotope	mass	Mass difference	Abundance (%)
Hydrogen	^{1}H	1.007825		99.985
	^{2}H	2.014102	+1.006277	0.015
Carbon	^{12}C	12.0		98.890
	^{13}C	13.003355	+1.003355	1.110
Nitrogen	^{14}N	14.003074		99.634
	^{15}N	15.000109	+0.997035	0.366
Oxygen	^{16}O	15.994915		99.762
	^{17}O	16.999132	+1.004217	0.038
	^{18}O	17.999161	+2.004246	0.200
Phosphor	^{31}P	30.973762		100
Sulfur	^{32}S	31.972071		95.020
	^{33}S	32.971459	+0.999388	0.750
	^{34}S	33.967867	+1.995796	4.210
	^{36}S	35.967081	+3.995010	0.020

Natural isotopic distribution: relative abundance of isotopes and their masses in Dalton

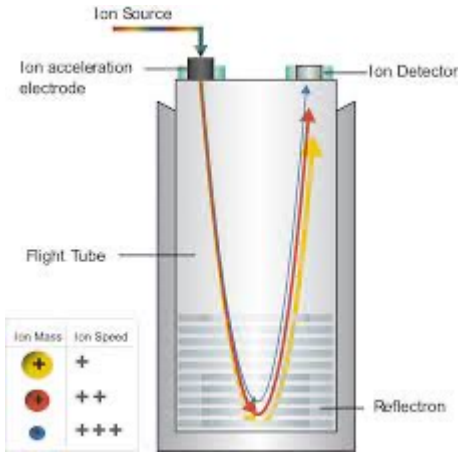
Quadrupole



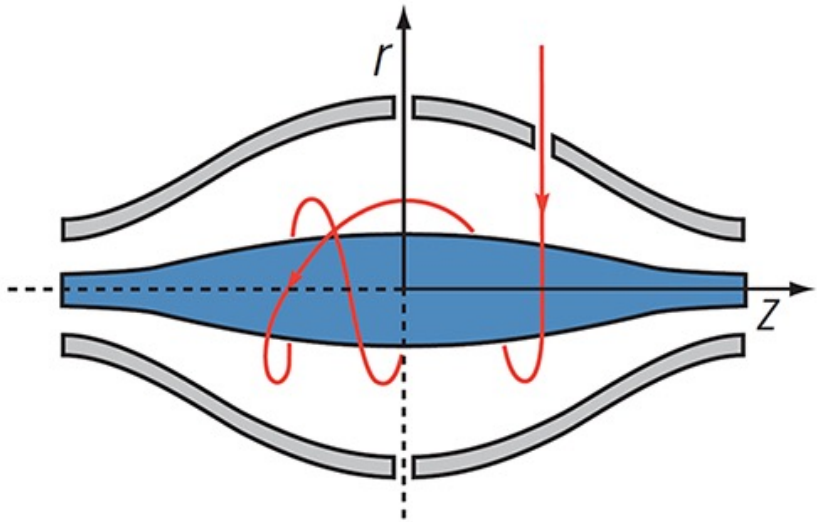
Ion trap



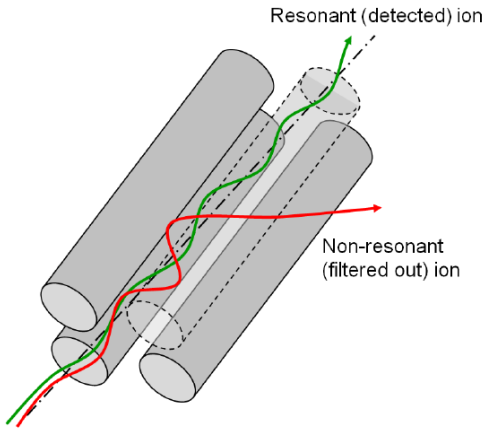
Time of flight



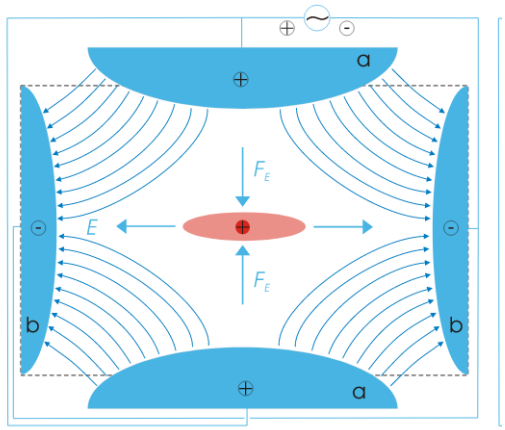
Orbitrap



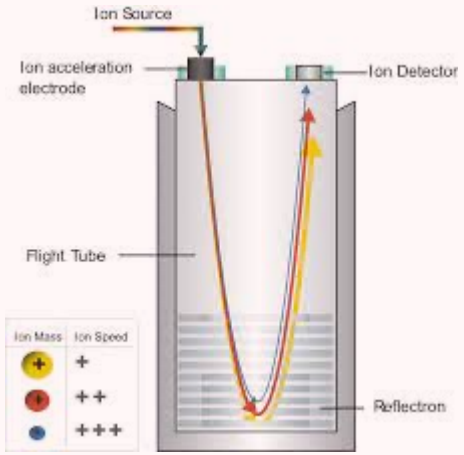
Quadrupole



Ion trap



Time of flight



Orbitrap

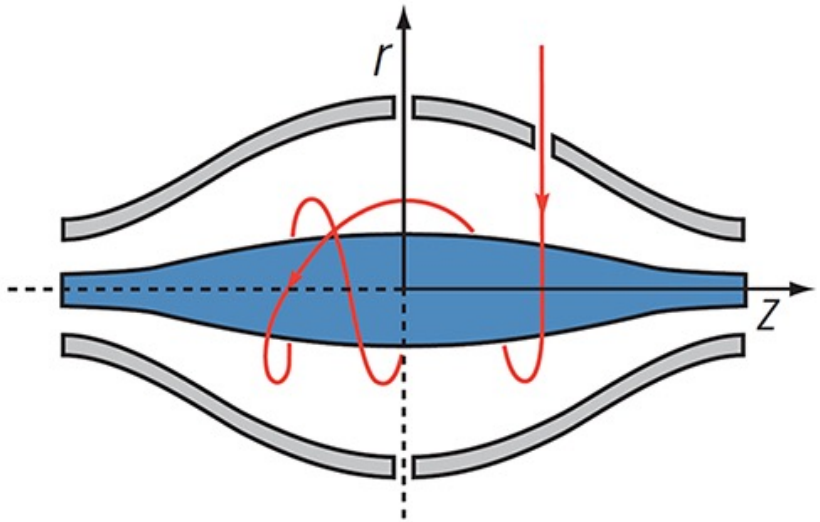
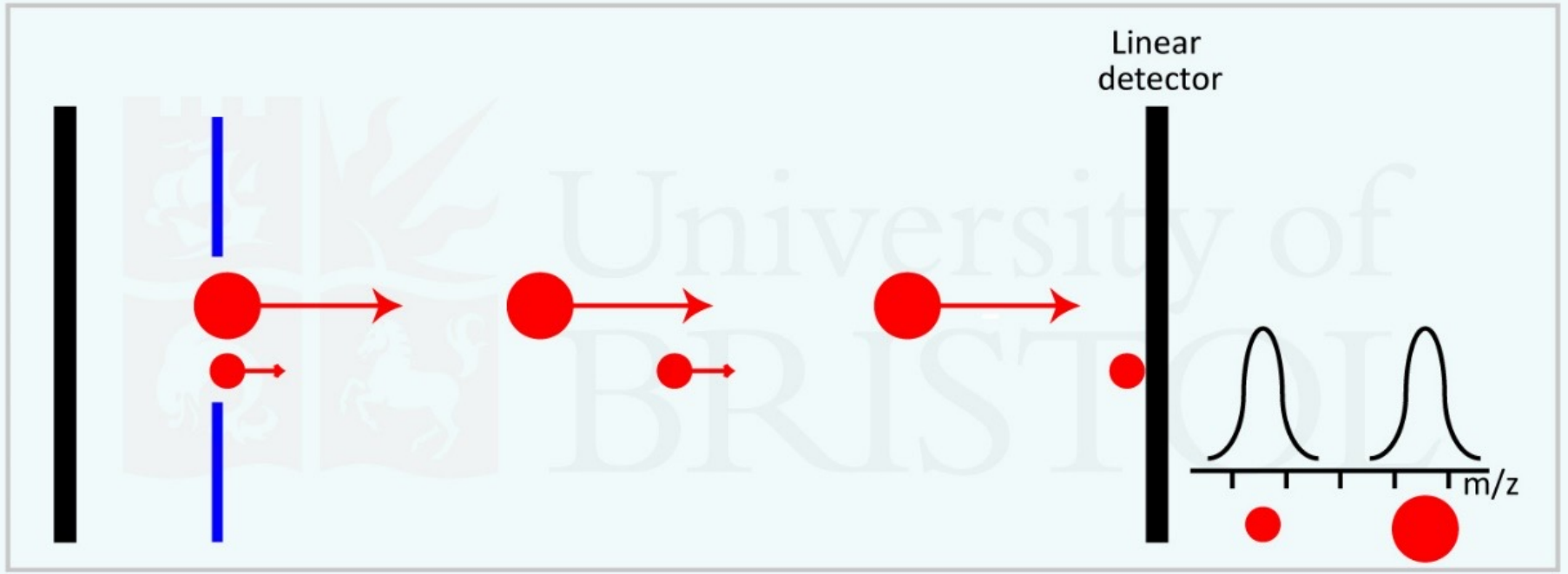


Figure 2.



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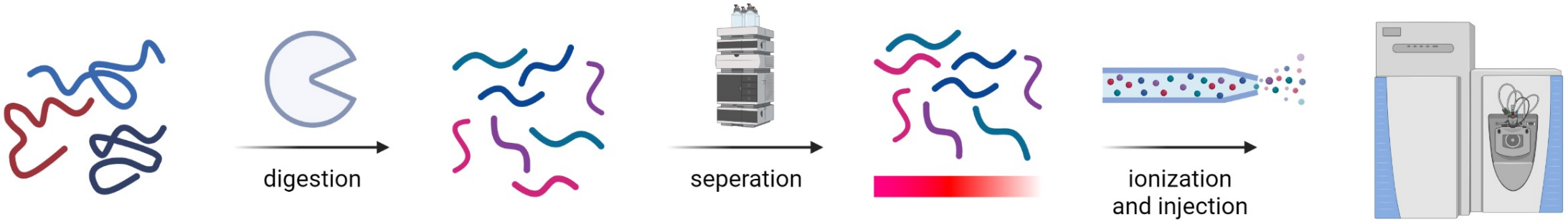
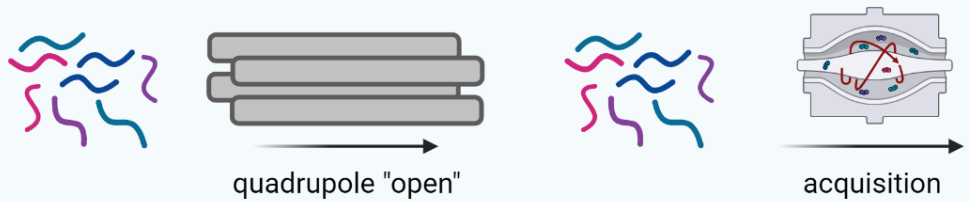
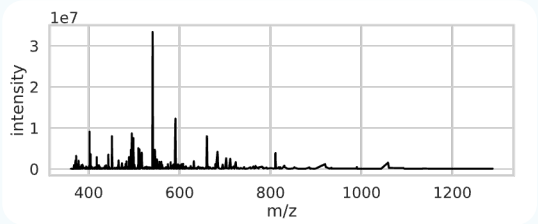
LC-MS/MS recap

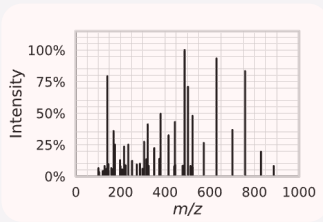


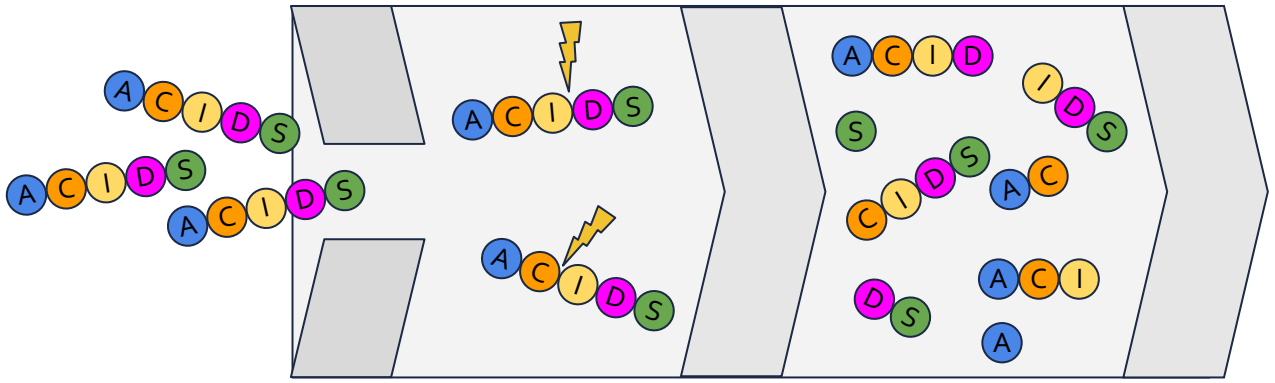
MS1 survey scan:
Find precursors (= full peptides)

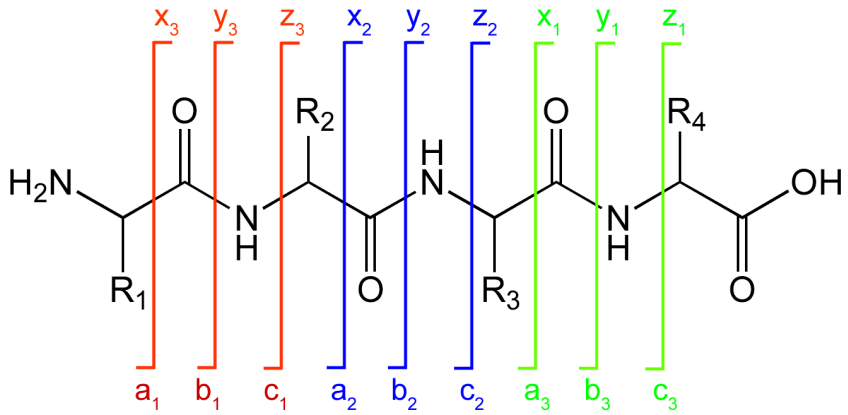


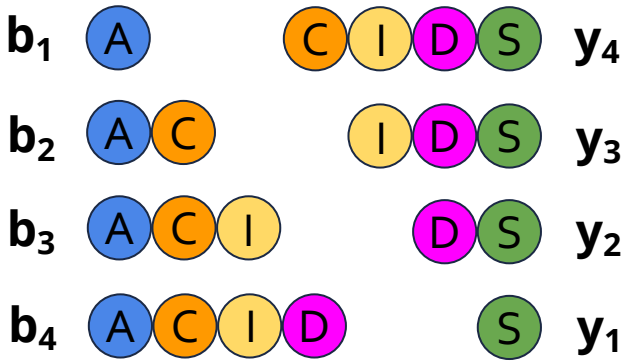


MS2 scan:
Get fragmentation spectrum for peptide at selected m/z window









Mass calculation