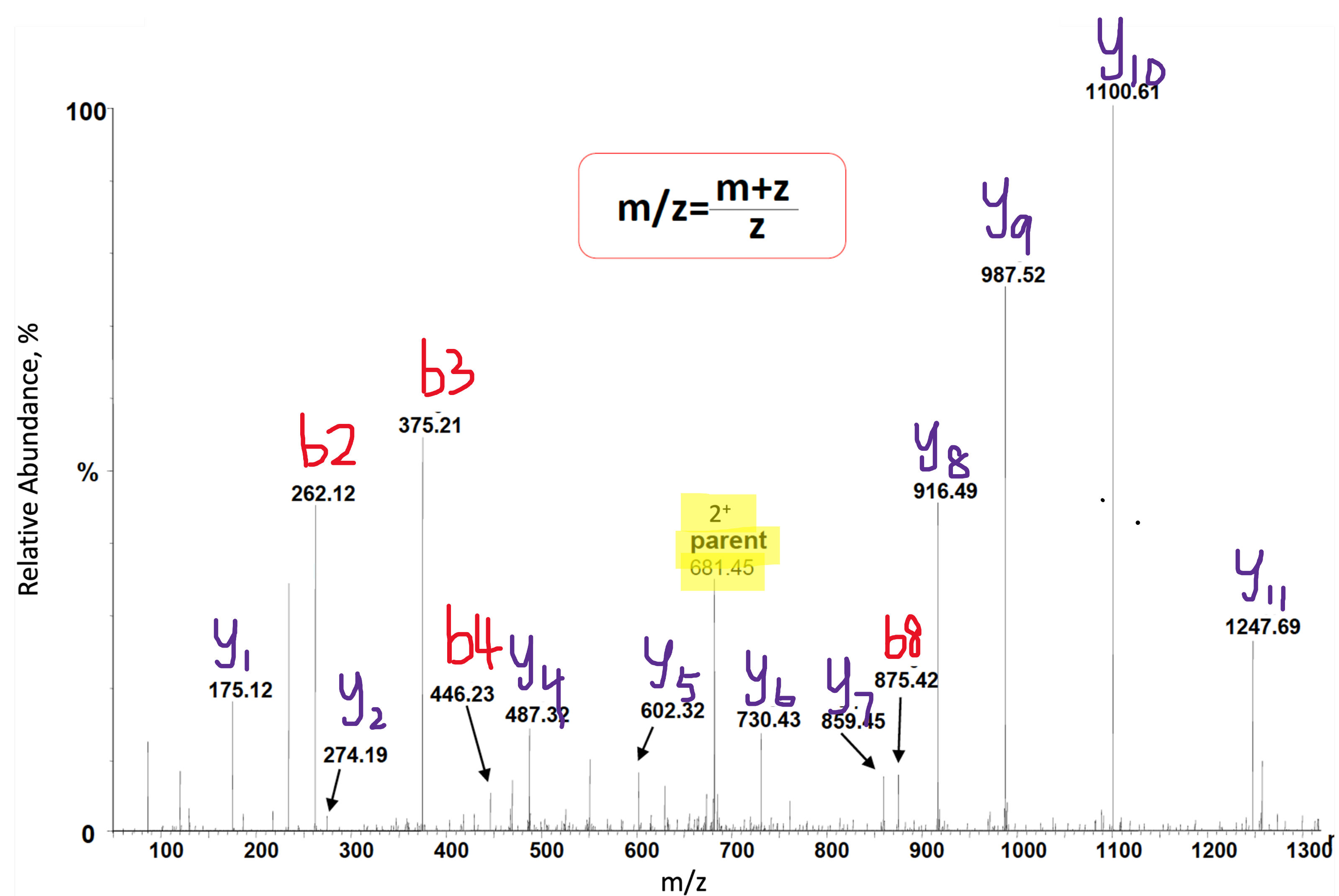


N F I/L A G E Q/K D X X V R

XX could be NV, VN, RG, GR

Notes for this question only: As long as your answer includes the correct sequence, no penalty applied on the wrong sequence.

Mass of b-ions = Σ (residue masses) + 1 (H^+)

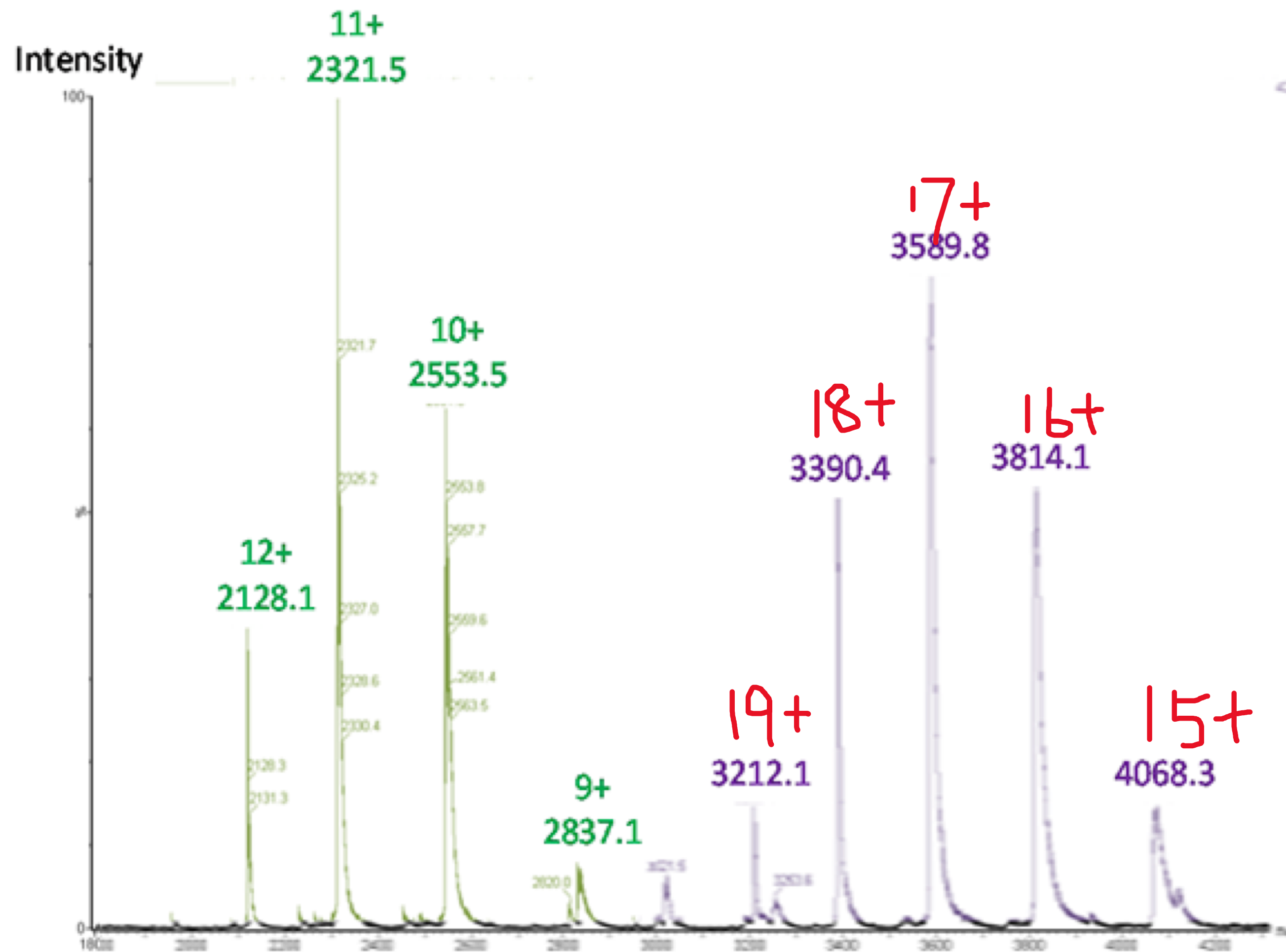
Mass of y-ions = Σ (residue masses) + 19 (OH + H + H^+)

M - y_{n-1} ion + 1 = mass of 1st residue on N terminus

M- b_{n-1} ion - 17 = mass of 1st residue on C terminus

The b ions and y ions are complementary,
So, **Mass of peptide = $b_m + y_{n-m} - 2$**

n is the number of residues;
m is the number of residues on a fragment ion.



$$m/z = \frac{m+z}{z}$$

Calculate the charge

$$z_n = \frac{m_{n+1} - 1.0078}{m_n - m_{n+1}}$$

Mass of Protein A = 25525.15 $\pm$ 0.26 Da

Mass of AB complex = 61009.76 $\pm$ 0.66 Da

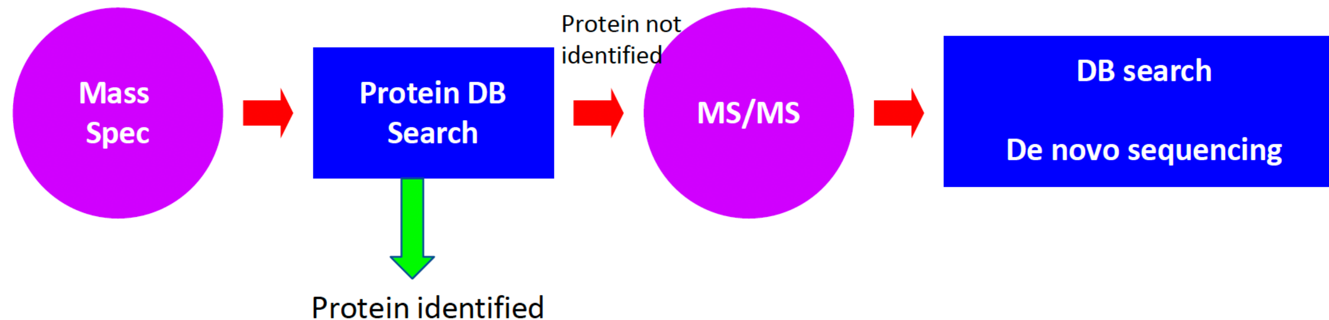
- Two single nucleotide polymorphisms (SNPs) on a protein but not sure whether the corresponding mutated protein(s) is also expressed. He decided to isolate this protein using an immunoprecipitation approach and hope to pull down both the WT and the mutant(s) (if it's expressed at all) together.

MILVDLEPGTMDSVRSGPFGQIFRPDNFVFGQSGAGNNWAKGHYTEGAELVDSVLD
VVRKEAESCDCLQGFQLTHSLGGGTGSGMGTLLISKIREEYPDRIMNTFSVVPSPKVS
DTVVEPYNATLSVHQLVENT**D**ETYCIDNEALYDICFRTLRLTTPTYGDLNHLVSGTM
ECVTTCLRFPGQLNADLRKLAVNMVPFPRLHFFMPGFAPLTSRGSQQYRALTVPDLT
QQVFDAKNMMAACDPRHGRYLTVAAVFRGRMSMKEVDEQMLNVQNKNSSYFVE
WIPNNV**K**TAVCDIPPRGLKMAVTFIGNSTAIQELFKRISEQFTAMFRRKAFLHWYTGE
GMDEMEFTEAESNMNDLVSEYQQYQDATAEEEEDFGEEAEEEA

(potential mutations suggested by the SNP analysis:

"D" to "N" and "K" to "Q")

Question: Find out if the mutant(s) was expressed.



MILVDLEPGTMDSVRSGPFGQIFRPDNFVFGQSGAGNNWAKGHYTEGAELVDSVLD
VVRKEAESCDCLQGFQLTHSLGGGTGSGMGTLLISKIREEYPDRIMNTFSVVPSPKVS
DTVVEPYNATLSVHQLVENT**D**ETYCIDNEALYDICFRTLRLTTPTYGDLNHLVSGTM
ECVTTCLRFPGQLNADLRKLAVNMVPFPRLHFFMPGFAPLTSRGSQQYRALTVPDLT
QQVFDAKNMMAACDPRHGRYLTVAAVFRGRMSMKEVDEQMLNVQNKNSSYFVE
WIPNNV**K**TAVCDIPPRGLKMAVTFIGNSTAIQELFKRISEQFTAMFRRKAFLHWYTGE
GMDEMEFTEAESNMNDLVSEYQQYQDATAEEEEDFGEEAEEEA

(potential mutations suggested by the SNP analysis:

"D" to "N" and "K" to "Q")

Question: Find out if the mutant(s) was expressed.

If D mutated into N,

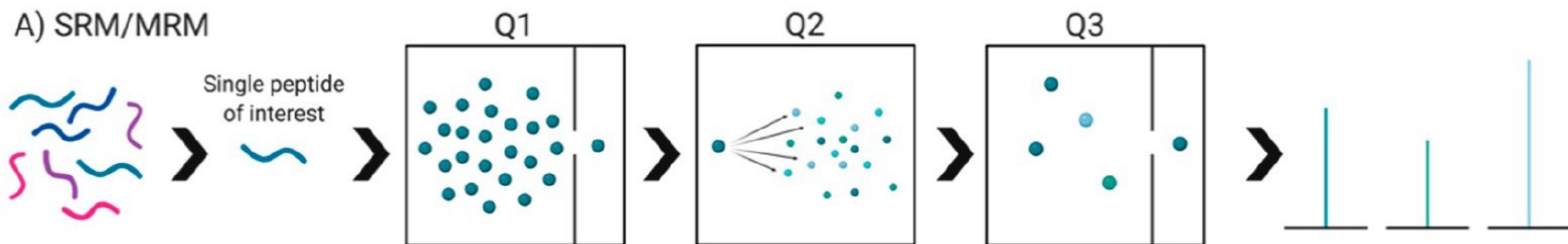
Mass	Position	Peptide sequence
4478.0802	113-151	VSDTVVEPYNATLSVHQLVE NTNETYCIDNEALYDICFR

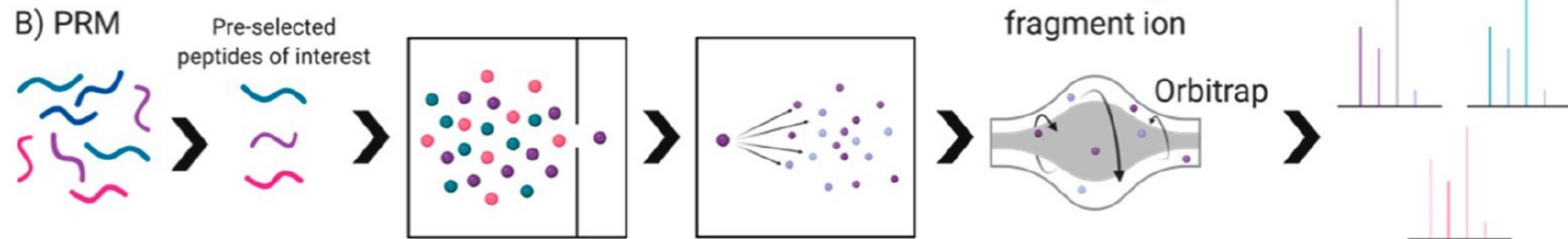
If K mutated into Q,

Mass	Position	Peptide sequence
2649.2766	275-297	NSSYFVEWIPNNVQTAVCDI PPR

targeted proteomics techniques could be used.

mass	position	#MC	modifications	peptide sequence
6073.4116	331-382	0		AFLHWYTGEGMDEMEFTEAE SNMNDLVSEYQQYQDATAEE EEDFGEEAEEEA
4479.0642	113-151	0		VSDTVVEPYNATLSVHQLVE NTDETYCIDNEALYDICFR
3197.4911	61-92	0		EAESCDCLQGFQLTHSLGGG TGSGMGTLLISK
2798.3433	16-41	0		SGPFGQIFRPDNFVFGQSGA GNNWAK
2725.2994	155-179	0		LTTPTYGDLNHLVSGTMECV TTCLR
1958.9817	42-59	0		GHYTEGAELVDSVLDVVR
1869.9779	301-317	0		MAVTFIGNSTAIQELFK
1696.8329	275-288	0		NSSYFVEWIPNNVK
1675.8393	1-15	0		MILVDLEPGTMDSVR
1645.8795	221-235	0		ALTVPDLTQQVFDAK
1620.8355	201-214	0		LHFFMPGFAPLTSR
1446.6893	263-274	0		EVDEQMLNVQNK
1319.7028	101-112	0		IMNTFSVVPSPK
1229.5983	319-328	0		ISEQFTAMFR
1143.6343	191-200	0		LAVNMVPFPR
1130.5952	180-189	0		FPGQLNADLR
1039.5935	248-256	0		YLTVAAVFR
1008.4060	236-244	0		NMMAACDPR
971.4978	289-297	0		TAVCDIPPR
808.3471	95-100	0		EEYPDR
738.3529	215-220	0		GSQQYR


A) SRM/MRM
Single peptide
of interest
Q1
Q2
Q3
Detect single
fragment ion


B) PRM
Pre-selected
peptides of interest
Orbitrap

VSDTVVEPYNATLSVHQLVENTNETYCIDNEALYDICFR

Fragment Ion Table, monoisotopic masses

Seq	#	B	Y	# (+1)
V	1	100.07574	4478.08031	39
S	2	187.10776	4379.01190	38
D	3	302.13471	4291.97987	37
T	4	403.18238	4176.95292	36
V	5	502.25080	4075.90525	35
V	6	601.31921	3976.83683	34
E	7	730.36180	3877.76842	33
P	8	827.41457	3748.72583	32
Y	9	990.47790	3651.67306	31
N	10	1104.52082	3488.60973	30
A	11	1175.55794	3374.56681	29
T	12	1276.60562	3303.52969	28
L	13	1389.68968	3202.48202	27
S	14	1476.72171	3089.39795	26
V	15	1575.79012	3002.36592	25
H	16	1712.84903	2903.29751	24
Q	17	1840.90761	2766.23860	23
L	18	1953.99167	2638.18002	22
V	19	2053.06009	2525.09596	21
E	20	2182.10268	2426.02754	20
N	21	2296.14561	2296.98495	19
T	22	2397.19328	2182.94202	18
N	23	2511.23621	2081.89435	17
E	24	2640.27880	1967.85142	16
T	25	2741.32648	1838.80883	15
Y	26	2904.38981	1737.76115	14
C	27	3007.39900	1574.69782	13
I	28	3120.48306	1471.68863	12
D	29	3235.51000	1358.60457	11
N	30	3349.55293	1243.57763	10
E	31	3478.59552	1129.53470	9
A	32	3549.63264	1000.49211	8
L	33	3662.71670	929.45499	7
Y	34	3825.78003	816.37093	6
D	35	3940.80697	653.30760	5
I	36	4053.89103	538.28066	4
C	37	4156.90022	425.19660	3
F	38	4303.96863	322.18741	2
R	39	4460.06974	175.11900	1

NSSYFVEWIPNNVQTAVCDI PPR

Fragment Ion Table, monoisotopic masses

Seq	#	B	Y	# (+1)
N	1	115.05025	2649.27664	23
S	2	202.08228	2535.23371	22
S	3	289.11431	2448.20168	21
Y	4	452.17763	2361.16965	20
F	5	599.24605	2198.10633	19
V	6	698.31446	2051.03791	18
E	7	827.35705	1951.96950	17
W	8	1013.43637	1822.92691	16
I	9	1126.52043	1636.84759	15
P	10	1223.57319	1523.76353	14
N	11	1337.61612	1426.71077	13
N	12	1451.65905	1312.66784	12
V	13	1550.72746	1198.62491	11
Q	14	1678.78604	1099.55650	10
T	15	1779.83372	971.49792	9
A	16	1850.87083	870.45024	8
V	17	1949.93924	799.41313	7
C	18	2052.94843	700.34472	6
D	19	2167.97537	597.33553	5
I	20	2281.05944	482.30859	4
P	21	2378.11220	369.22452	3
P	22	2475.16496	272.17176	2
R	23	2631.26607	175.11900	1

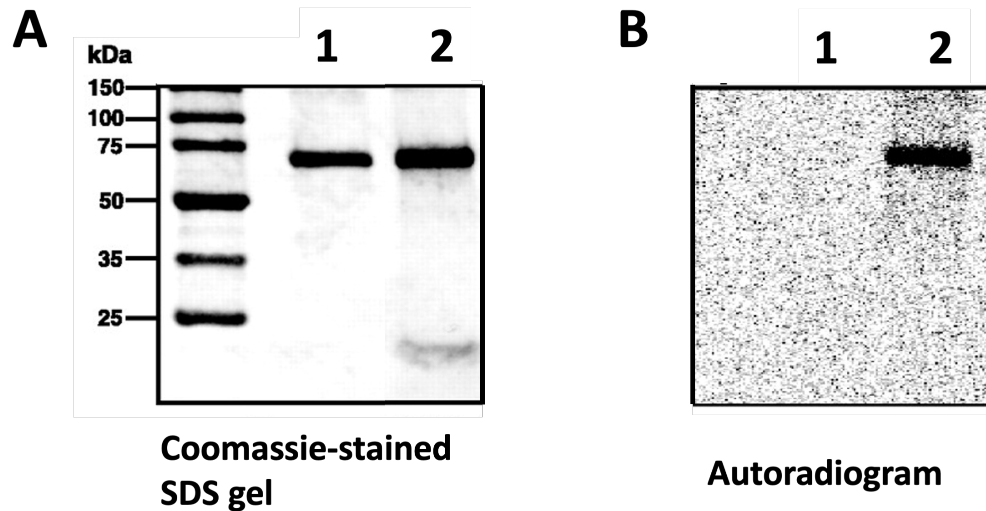


Figure 1. Results of incubating AcuA proteins with radioactive [1-^{14}C]acetyl-CoA. A. Lane 1, *Protein X*; lane 2*, Protein X mixed with* AcuA; B. Autoradiogram of the left panel.

(a) Please analyze the results. **[5 marks]**

^{14}C acetyl group gets transferred from acetyl-CoA to protein X. This post-translational modification is catalysed by AcuA, suggesting that AcuA is an acetyltransferase.

(b) Briefly describe what technique can be used to confirm the results. **[5 marks]**

Acetyl group increases the mass of 42 Da following acetylation of the peptide. So MS-based technique.

Excise the **two** protein "spots" → In-gel trypsin Digest → Recover tryptic peptides → RP-HPLC-MALDI-TOF.

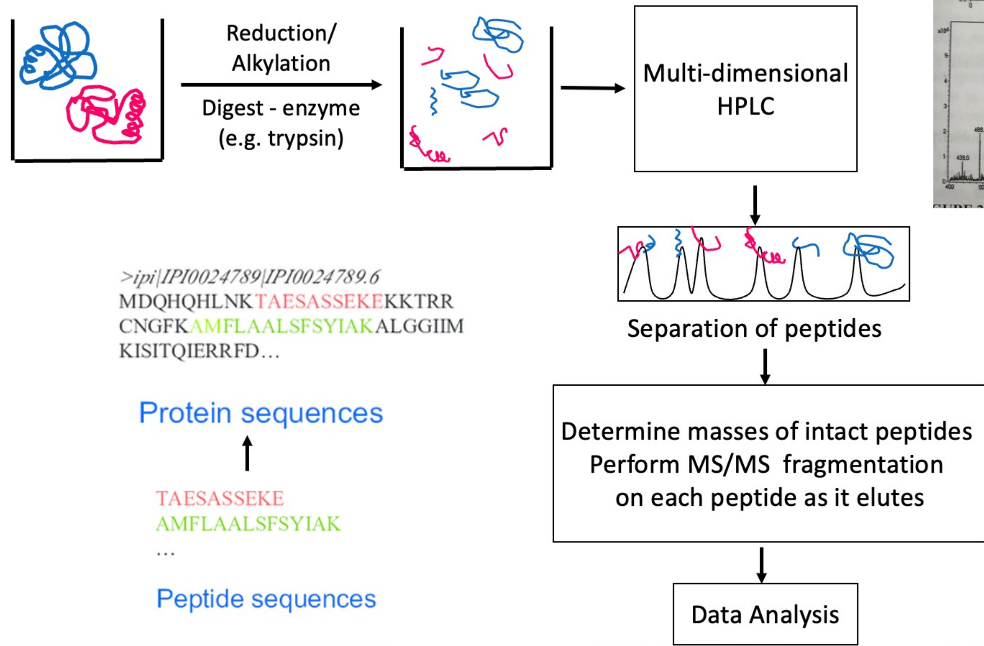
(Note： MS1 spectra comparison probably be good enough, if you do MS/MS is also OK).

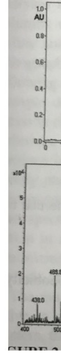
(c) Previous MS studies found that a single peptide of protein X, with the sequence SGKIMR and a mass of 690.5 Da, was affected when the protein X was incubated with AcuA. Which amino acid residue in the peptide is likely to be affected? Please draw a diagram of the steps used to locate the modification. **[10 marks]**

Most likely to be lysine - ε-amino group is known to be susceptible to acetylation.

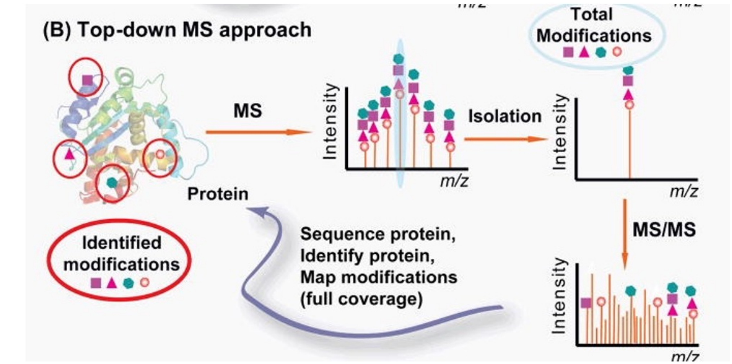
Bottom-up or Top-down….

Bottom-up Proteomics





Top-down Proteomics



- Characterize intact proteins from complex biological systems
- Most useful for single proteins or relatively simple mixture
- Proteins are typically ionized by ESI and trapped in FT-ICR or orbit trap mass spectrometer
- Fragmentation for tandem MS is accomplished by electron-capture dissociation (ECD) or electron-transfer dissociation (ETD)
  - ECD and ETD typically provide more uniform dissociation than conventional CID (collision induced dissociation), while preserving the labile modifications

(d) When protein X was incubated with acetate, ATP and CoA, it produced AMP with a specific activity of 3.3 ± 0.41 μmol of AMP released per min per mg of protein. However, when it was incubated under the same conditions but additionally with AcuA, there was no detectable activity. What implications do these results have for the *in vivo* regulation of the AcsA acetyl-coenzyme A synthetase? **[5 marks]**

- Acetylation of protein X (by AcuA) inhibits its catalytic activity *in vitro*.

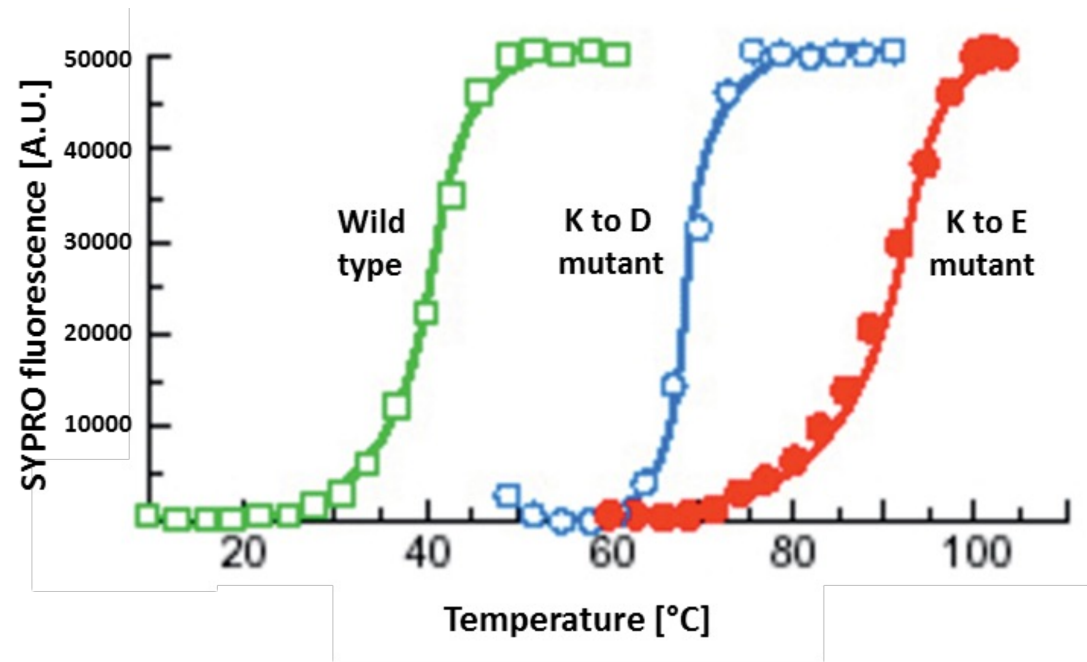
acetyl-coenzyme A synthetase AcsA or X

AcuA

- If this also occurs *in vivo,* then it's an example of product inhibition.

(e) Diagram the process to monitor the level of protein X in *Bacillus subtilis*. **[10 marks]**

Many techniques work. But the answer must include identification of protein X and time course for "monitoring".



(a) What is the impact of the mutations on the polymerase? **[5 marks]**

K to D and K to E mutations increase the heat tolerance of the polymerase.

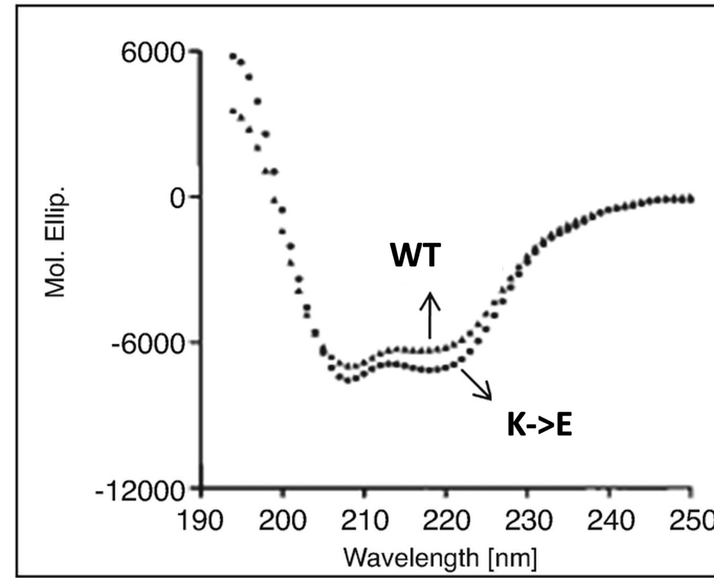
Reason: higher temperature to denature: from 40°C to 70 °C and 85°C

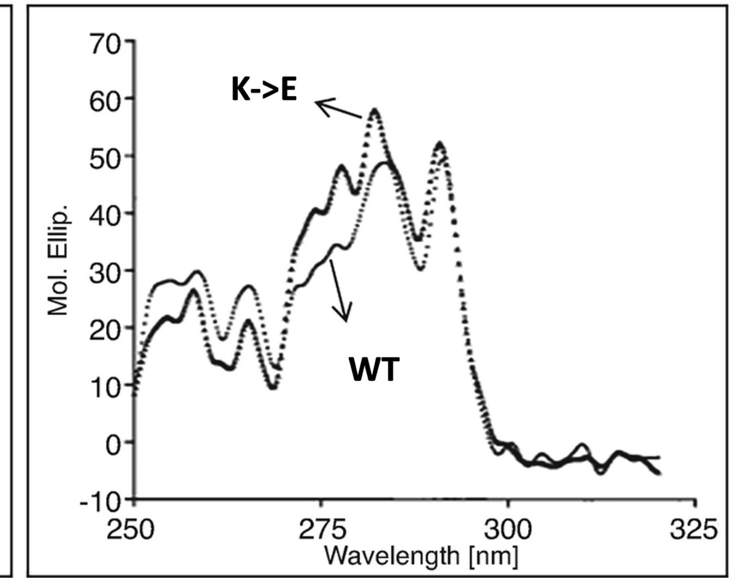
(b) You are aiming to develop the enzyme for use in polymerase chain reaction (PCR). Which enzyme is most likely to survive twenty repeated PCR cycles? Explain your answer. **[5 marks]**

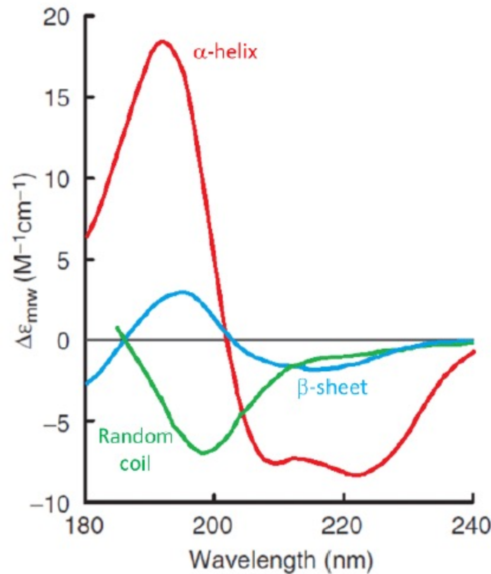
K to E mutant: as the denaturing temperature is the highest.

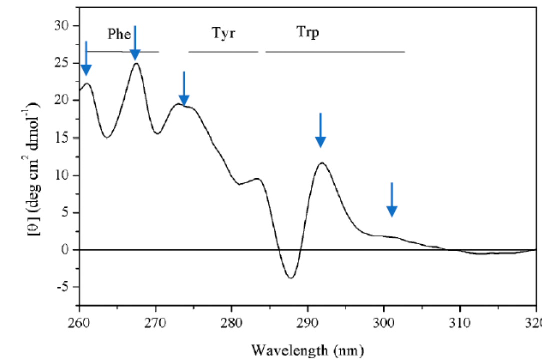
Thus, most likely to survive a PCR condition: 94°C, 60°C, 72°C,

(c) The circular dichroism (CD) results of the wild type and the K->E mutant are shown in Figure 3 (right). Please analyze the CD results. **[5 marks]**









- Trp shows a peak close to 290 nm with fine structure between 290 and 305 nm
- Tyr shows a peak between 275 and 282 nm
- Phe shows weaker but sharper bands with fine structure between 255 and 270 nm

- α helix : negative bands at 208 and 222 nm and positive band at 193 nm
- β sheet : negative band at 218 nm and positive band at 195 nm
- Random coil: negative band at 195 nm and low ellipticity above 210 nm