pI = 9.14

pI = 3.9

1. You have two small proteins (really small):

NH_2– DCGEATQHQMLHTTIKPRKIEKIFIT –COOH

NH_3 – SFQGGEDELTVYGPK –COOH

a. Determine the pI of each protein.

$H_{12}A^{+7} - H_{11}A^{+6} - H_{10}A^{+5} - H_9A^{+4} - H_8A^{+3} - H_7A^{+2} - H_6A^{+1} - H_5A^{0} - H_4A^{-1}$

9.135

$H_7A^{+2} - H_6A^{+1} - H_5A - H_4A^{-1}$

3.9

b. You want to separate these proteins using ion exchange chromatography.

i. Which type of ion exchange would you use (cation exchange or anion exchange)? Why did you select that type?

cation exchange – at neutral pH, the big peptide is very (+), so it will be attracted to a (–). The small peptide will be (–)

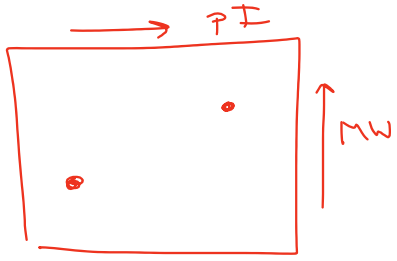
ii. What pH would you select for your experiment? Why?

pH = 7 . see above

iii. What would you use as a competition molecule to ensure that the proteins elute?

Salt – NaCl

c. Sketch a 2D gel that would result if these two proteins were run together. Label the axes appropriately. Perhaps you would like to use the average MW of an amino acid to give you a rough estimate of the size.



2. A tandem MS experiment results in peaks at the following m/z ratios. Determine the sequence of this peptide.

128.2	185.2	313.3	460.5	623.7	736.9	851	980.1
	57	128.1	147.2	163.2	113.2	114.1	129.1
K or Q	G	Q or K	F	Y	L/I	N	E

Glu Asn Leu Tyr Phe Gln Gly Lys Ile

3. Here is the sequence of a small protein. Identify all sites that would be hydrolyzed by elastase.

MQDPYVKEAENLKKYFNAGHSDVADNGTLFLGILKNWKEESDRKIMQSQIVSFYFKLFKNFKDDQSIQKSVETI

KEDMNVKFFNSNKKKRDDFEKLTNYSVTDLNVQRKAIHELIQVMAELSPAAKTGKRKRSQMLFRG

I did not count single amino acids

How many peptides would be produced? 24

If you have a mixture of these peptides, is there any way of knowing how they should be ordered?

Nope!

4. A team of researchers identify a human protein through 2D electrophoresis that they are interested in identifying. This protein is independently digested with Trypsin and Asp-N and sequenced by mass spectrometry. The resulting sequences are shown below. What is the sequence of this protein?

Asp-N Digested	Trypsin Digested
DHVYGLPGLLGSRSFQGGE	IEK
DQFIVTAVSVIHGVEAFGYRVQEK	GIK
DCGEATQHQMLHTTIKPRKIEKIFITHMAG	VQEK
MELLFLGTGAGIPAKARNVTSVALKLLEERRSVWLF	LLEER
DELTVYGPKGIKAFIETSLAVTKTHLTYPLAIQEIEEGIVFE	NVTSVALK
	AFIETSLAVTK
	MELLFLGTGAGIPAK
	SFQGGEDELTVYGPK
	IFITHMAGDHVYGLPGLLGSR
	SVWLFDCGEATQHQMLHTTIKPR
	THLTYPLAIQEIEEGIVFEDDQFIVTAVSVIHGVEAFGYR

MELLFLGTGAGIPAKARNVTSVALKLLEERRSVWLFDCGEATQHQMLHTTIKPRKIEKIFITHMAGDHVYGLPGLLGSRS
FQGGEDELTVYGPK