CHEM523 Protein Sequencing Problem

**Problem 1**

1) You are in a South American rain forest looking for naturally occuring peptides with potential as drugs. You have a mobile biochemistry lab with common reagents and enzymes, an amino-acid analyzer, gel-filtration and ion-exchange chromatography, and electrophoresis. You also have an Edman Sequenator, but you have contaminated one or more of your reagents, and as a result, you cannot sequence peptides longer than about 12 residues before contaminants obscure the results. While screening extracts from the ovaries of an tropical orchid, you find a peptide with potential as an antiviral. Deduce its amino-acid sequence using the available tools.

1) MW by electrophoresis can tell you how big a sequencing problem you are up against.

Result: about 4000

This doesn’t really tell us much, does it? Sometimes extra information is just that: extra.

2) Amino-acid analysis can help you decide how to fragment the peptide for sequencing:

Result: A2C2D2E4FG3HKLMN2P2Q2R4S4T3W

3) How many peptides expected from each of these possible cleavage reagents?

* Cyanogen bromide (C-side of M).
	+ There’s 1 Met, so you would expect a single cleavage unless it is the last amino acid
* Staph. aureus V8 protease (C-side of D and E).
	+ Since there are 6 Asp and Glu residues, you’d expect 6 cuts unless any of them are at the carboxy-terminusof the peptide.
* Trypsin (C-side of K and R).
	+ There are 4 Arg and 1 Lys, so you’d expect 5 cuts unless any of them are on the carboxy terminus of the protein.

4) Cleavage by trypsin followed by gel-filtration chromatography gives the expected 6 products, which you sequence (shown in order of emergence from column):

T-1 ETMESSAGEFGR

T-2 SQTWALDHSECR

T-3 GPQDNK

T-4 TCR

T-5 NP

T-6 R

Five possible cuts would generate 6 peptides, so we know that Arg and Lys aren’t at the carboxy-termnius. This tells us a lot. Most importantly, we know the sequence of the tryptic peptides.

5) Cleavage by Staph. aureus V8 protease followed by gel-filtration chromatography gives the expected 7 products, which you sequence (shown in order of emergence from column):

S-1 RSQTWALD

S-2 FGRGPQD

S-3 NKTCRNP

S-4 SSAGE

S-5 TME

S-6 CRE

S-7 HSE

Deduce the primary structure of this polypeptide.

To do this, you need to overlap the peptides. I’ll write the Tryptic peptides in Purple and the V8 peptides in Green.



**Additional questions that could be asked as part of this problem:**

Why would cyanogen bromide not be a good choice as a cleavage reagent?

Can you account for the order of elution of peptides from the two chromatographies?

Predict the order of elution of the tryptic peptides from a cation-exchange column eluted with pH-8.5 buffer and a salt gradient.

Predict the order of elution of the V8 protease peptides from an anion exchange chromatography column eluted with a pH-6.5 buffer and a salt gradient.