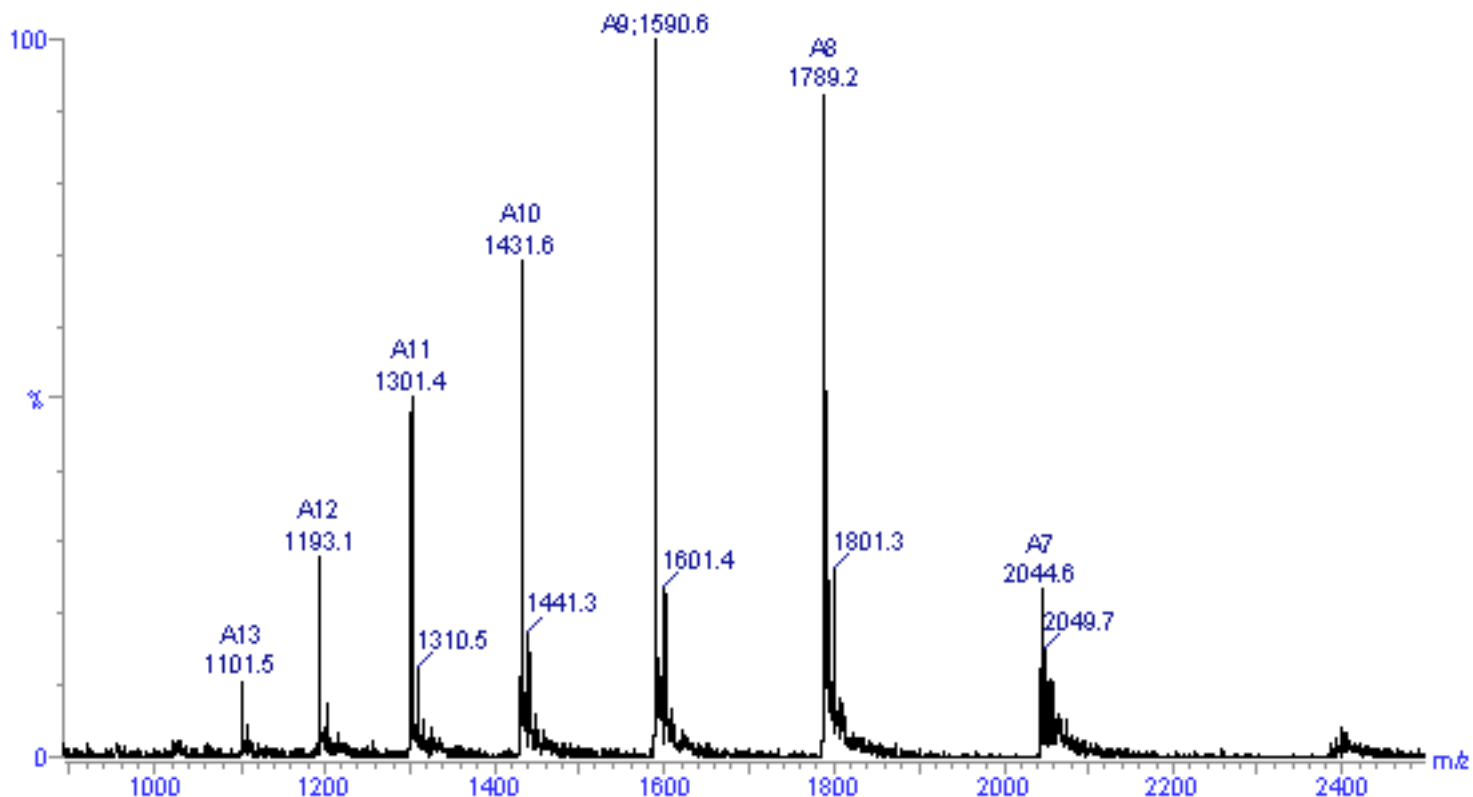


8. (1.0 points) The following spectrum was found in an old laboratory notebook in the Arriaga Lab. Unfortunately, this spectrum was not properly annotated. Please help out by answering the questions below.



- What type of ionization was used to obtain this spectrum? MALDI or ESI?
- If one assumes that the number after the letter 'A' on top of each cluster represents the charged state, what is the original MW of this molecule?
- If one 'zooms in' on one of the clusters, what would you expect to observe for the m/z values of each ion in the cluster?
- Draw a hypothetical spectrum that would be obtained if instead of using the ionization technique selected in (a), one uses the other ionization technique (i.e. MALDI or ESI).
(There is more space for this answer in the following page)

9. (2.0 points). By submitting the sequence of P08833 (Precursor of human insulin-like growth factor-binding protein 1) to a BLASTA sequence alignment, one of the resulting matches is: Q5SVY8 (Precursor of mouse insulin-like growth factor-binding protein 1).

272 AA; align

Score = 350 bits (899), Expect = 3e-95

Identities = 162/247 (65%), Positives = 185/247 (74%), Gaps = 11/247 (4%)

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Query: 24  AGAPWQCAPCSAEKLALCPPVSASCSEVTRSAGCGCCPMCALPLGAACGVATARCAGLS 83
          A  PW CAPC+AE+L LCPPV ASC E++R AGCGCCP CALP+GAACGVATARCA+GLS
Sbjct: 26  APQPWHCAPCTAERLGLCPPVPASCPEISRPAAGCGCCPTCALPMGAACGVATARCAOGLS 85

Query: 84  CRALPGEQQPLHALTRGQACVQESDASAPH-----AAEAGXXXXXXXXXXXXX 132
          CRALPGE +PLHALTRGQACV E  A A                      AA
Sbjct: 86  CRALPGEPRPLHALTRGQACVPEPAAPATSTLFSSQHHEEAKAAVVSADELSEPEMTEE 145

Query: 133 XLLDNFHLMAPSEEDHSILWDAISTYDGSKALHVTNIKKWKEPCRIELYRVVESLAKAQE 192
          LLD+FHLMAPS ED  ILW+AISTY  +A  + ++KKWKEPC+ ELY+V+E LA AQ+
Sbjct: 146 QLLDSFHLMAPSREDQPILWNAISTYSSMRAREIADLKKWKEPCQRELYKVLERLAAAQQ 205

Query: 193 TSGEEISKFYLPNCNKNNGFYHSRQCETSMDGEAGLCWCVYPWNGKRIPGSPEIRGDPNCQ 252
          +G+EI KFYLPNCNKNNGFYHS+QCETS+DGEAGLCWCVYPW+GK+IPGS E RGDPMC
Sbjct: 206 KAGDEIYKFYLPNCNKNNGFYHSKQCETS LDGEAGLCWCVYPWWSGKKIPGSLETRGDPNCH 265

Query: 253 IYFNVQN 259
          YFNV N
Sbjct: 266 QYFNVHN 272

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- (a) What are identities, positives, and gaps?
- (b) Give an example of a 'positive' (i.e. provide the amino acid position and the amino acid residue(s)).
- (c) If you believe that a spot in a 2D gel corresponds to either P08833 or Q5SVY8, can you use a peptide mass fingerprint experiment to distinguish between the two proteins? Explain your answer
- (d) If your answer to (c) is YES, give the sequence of one tryptic peptide in each protein that is unique and that would help you unequivocally identify the protein matching the spot. If the answer is no, what would you do to further elucidate the identity of the spot?
- (e) Explain why not all the predicted peptides are experimentally observed in peptide mass fingerprint experiments.

10. (1.5 points) Design a SERS experiment. Using the query sequence in question 9, identify a peptide that may have an acetylation. Outline an experiment to determine whether this peptide carries this modification in a small sample. (Points to consider are: (i) Peptide isolation, (ii) treatment to make it evoke a SERS response, (iii) standards, (iv) the possibility that only a fraction of the peptide is modified, (v) a sketch of the expected SERS spectra).

11.(0.5 points) What is the difference between ‘top down and ‘bottom up’ proteomics? What approach requires better accuracy? Explain your answer.

- 12. (1.0 point)** (i) Explain how methylation of acidic amino acid residues can help us identify phosphopeptides in a phosphoproteomics experiment. (ii) Explain how could one enrich for non-methylated phosphopeptides. (iii) Explain how neutral-ion loss can help us identify phosphorylated amino acid residues; include a scheme that describe the relevant instrumental details of a neutral-ion loss experiment.

13. (2.0 points) Design a quantitative proteomics experiment based on iTRAQ labeling. In this experiment, the investigator is interested in determining the time dependence of the abundance of Thioredoxin (a protein involved in oxidative stress protection) in rat muscle after treatment with Menadione (oxidative stress agent). (Points to consider are: (i) The isoform of interest localizes to mitochondria, (ii) at least three time points are required, (iii) trypsin is used to produce peptides, (iv) sketches of MS and MS/MS spectra are required, (v) clear indication on how one obtains the quantitative information from the spectra.