

*The following are unedited comments provided anonymously by study participants in response to the Research Group's appeal for feedback:*

- Too much time was expended on this project. However this sample may cause us to look into procedures to remove traces of surfactants.
- This survey will be very informative for us. However I was not asked the type of instrument or a kind of software for database search. I think these data would be very important for this kind of survey. I am looking forward to including these kinds of information in the next survey. Thank you.
- Data return on-line is powerful but takes a lot of time. Please advise participants next year that this could take time (so we can enter this with our morning coffee!)
- The challenging part of the study was the limited sample amount.
- I have not used up the sample. There is enough left to sequence up to ~ 10 peptides but I feel that would be shouting around in the dark. I would prefer at this point in the analysis to start with fresh intact protein and go for its mass using electrospray and MALDI. There was keratin in the sample. For some peptide spectra I cannot come up with an assignment (ion trap data).
- Analyses were done with nanospray manual sequencing. If I had more time I might try HPLC-MS.
- SAMPLE CAN NOT DISSOLVE IN 50mM NH<sub>4</sub>HCO<sub>3</sub>. SO I COULDN'T DO IN SOLUTION DIGESTION.
- We decided to choose carbonic anhydrases as a target of this study although number of more abundant human keratins were confidently identified in this sample as well. Amount of carbonic anhydrases in PRG04 was found much lower (at least 100 times see supporting data) than proposed 3 pmol which may indicate that our assumption was incorrect.
- The strategy was to go first for identification. Therefore the sample was digested immediately. The sample was not used up. There is enough left to sequence about 10 peptides. However I would prefer to look at the intact protein now because I feel that the third protein is one of the identified with a slight modification (likely bovine CAII). Also I probably would try protein separation first. There are spectra I cannot assign and database search does not help. There was keratin in the sample.
- In my point of view this survey did not really address proteomic core facilities. It was a mass spectrometry task. Usually proteomic core facilities cover a much wider spectrum of protein analysis techniques. I didn't care that the protein was intact. So I injected it with no digestion. It appears that some degradations occurred during mailing or storage at room temperature in my laboratory.
- Experimental Details. 1/3 of sample was run on SDS page for MW determination and to be used for in gel digestion. No stained bands were evident. remaining 2/3 were digested in solution after reduction and carbamidomethylation in the presence of Rapigest reagent (WATERS). 2LCMSMS runs of 500 fmol with a 75 uM C18 column onto a qTOF
- GLOBAL were used to obtain protein IDs. Attempt at peptide mass fingerprint with 500fmol using a MALDI-TOF was unsuccessful. Data-mining using MASCOT and proteinlynx Global

Server 2 with error tolerant searching was used to identify N-terminal acetylation and R to N substitution. A place at the beginning of this survey to describe the experimental protocol would have been a good addition to this survey as well as more initial explanation of the types of questions that would be asked.

- I am nearly certain that the proteins arrived in degraded conditions (although I cannot be certain and cannot prove this). Our nanoLC MS/MS instrumentation (Deca XP+ with LCP nanoLC) was benchmarked for adequate performance prior to analyzing the sample by injection a 10 fmol in-solution digest (10 ul of 1 fmol/ul) of BSA and adequate coverage was found (> 25% aa). Therefore 3 x 3 pmol of total protein should without any doubt lead to better identifications. I first ran 3 runs of 1/10 of the sample (3 different digests) and observed nearly no peptides. 3 x 300 fmol injected should be sufficient to identify proteins. I suggest in any further similar studies to add a possible benchmark that allows to check the quality of the sample (independent of the study) prior to analysis so that no bias is introduced by longer shipping or shipping to warmer countries.
- We attempted LCMS of an intact sample aliquot LCMS of a trypsin digest and AP-MALDI of a trypsin digest but due to instrument failure we didn't have enough sample to do further work.
- Survey was confusing. Database searched was Swissprot so I do not have the gi number's handy. I accidentally used a 6 digit ID number on the previous survey( sorry!!) I have now resubmitted the survey results with a 5 digit number. old 6 digit number = 115963 new 5 digit number = 11596. A couple of good questions for the survey might have been how much of the sample did you consume in the analysis. And what specific instrument did you use for the analysis?
- Sample appeared to stick to sample tube and not dissolve in aqueous. Only after washing with SDS did we see any proteins. As a result about 80% of sample was lost running gels that yielded nothing.
- It would be interesting to see what post-mass spec tools labs used to identify and differentiate their data. Our lab for instance has several post search algorithm programs and scripts written in house that were key to our identifications. We are always interested in the developments on this end of the data as I am sure many labs in the proteomics area are.
- We used approximately 15% of the sample to obtain intact Maws and run ESI and MALDI LC/MS/MS. This submission was cumbersome several entries were redundant. From the intact MW measurements and information on Swiss rot we tentatively identified 2 of the 3 proteins correctly (carbonic anhydrase is a common standard!). We would have liked to see questions about the database search strategy used in the questionnaire.
- Other than Maldi peptide mass mapping we also tried nLC/MS/MS but failed due to the system problem.
- The amount of protein provided did not appear to be as much as specified. A semi-tryptic peptide was found in bovine carbonic anhydrase II.
- It would be helpful to know any information on the samples regarding the presence/absence of salts and detergents. be
- Good job setting this up. This is a good trial for us in distinguishing closely related proteins
- Some of these questions will probably result in ambiguity. Greater care with the wording of the questions may be necessary.

- I can not find any peptide from a major stretch close to the C term so I guess the last iso form is hidden there. I do not have an HPLC so the 'missing' peptides may be due to MALDI suppression.
- We found ubiquitin in the sample but were unable to find any ubiquitinated peptides. We also found Human keratin type 1 and type 2
- This was a useful and informative study!
- We had a difficult time distinguishing between the two human isoforms of CAII. The one peptide that differed was observed primarily in the +3 form and was very large therefore fragmentation was somewhat limited. If time permitted we could have gotten more information by making the methyl ester of the mixture and re-running.
- I have injected 25% of the sample two times in our LCQ to see if I could improve coverage. The other 50% I tried double digest with Glu-C and trypsin (25%) and elastase and trypsin (25%) both double digest did not work I think they were too long. For this analysis I spent more time on analyzing the data than in manipulation trying to find a different peptide. I look forward to see the results!
- The cover letter stated can be dissolved in most common aqueous solutions so we first tried 25 mM Ambic and saw nothing using 1/3 of the total sample. We then made that solution 10% in MeCN and redigested and got great signal.