

9. What “semi-quantitative” methods were used to measure changes in protein abundance with fly age in this work?

10. The bottom part of Figure 2 shows the base peak chromatograms for three separations. Define base peak. What is a base peak chromatogram?

11. How effective was the SCX-LC-MS/MS method at identifying peptides compared to the LC-MS/MS method? What do you think is the reason for this difference in performance when an SCX step is added?

12. Which proteins in Figure 6 change with the flies’ age? What biological processes are associated with these proteins?

13. How do the authors’ results compare to results obtained by more traditional methods used to study proteins?

In-Class Questions

1. Consider your answer to out-of-class question 7c. How are tandem MS experiments achieved on the LC-MS-MS system? How does fragmentation occur, and what types of fragments are formed? How do these fragments yield information about the peptides in the sample?
2. Proteomics experiments often produce vast quantities of data.
 - a. In Figure 4, what is being shown in the panels on the far left of the figure? How does this data relate to the data in the six panels to the right?
 - b. In your opinion, should authors be required to share all of the raw data used to construct figures in published articles? Why or why not?
 - c. When data is shared, who should bear the cost of the servers, web development and other infrastructure needed to do so?

Some possibilities include individual researchers through their grants, government organizations like the National Science Foundation and National Institutes of Health, the publishers of the journals the data appears in, or other researchers who choose to access the data. You may think of others.

3. Quantitation by mass spectrometry in proteomics is challenging because the wide variety of peptide chemistries result in variable MS signal between peptides, and ionization efficiency can also change over the course of an LC run.
 - a. Why didn't the authors use internal standards to account for these differences? How were they able to compare the results from the different samples in the absence of an internal standard?
 - b. In Figure 6, what is the difference between the open symbols and the filled symbols?
 - c. Consider the Figure 6 data and your answer to out-of-class question 13. Do you think the two "semi-quantitative" methods used for analysis are reliable? What limitations do these techniques face?