Science Afternoon at NIST: Separations by Mass
Monday, February 4, 2013

Mass Spectrometry is a powerful analytical technique used to identify molecules and characterize them based on mass and how the molecules fragment into pieces. A mass spectrometer is often used in conjunction with chromatography which separates mixtures based on the philosophy of "like dissolves like" in which molecules move from the liquid, or mobile, phase into the solid constituents of the column and back out as the mobile phase is altered. A mass spectrometer ionizes the molecules and they move through an area at different speeds based on their mass/charge ratio.

Maria Lorna DeLeoz and Ben Place, both NIST chemists, presented ways to teach about how a mixture of materials can be separated by mass, similar to how a mass spectrometer works.

After an overview of NIST, teachers were given the challenge of separating mixtures of different kinds of M&Ms, without even knowing what kinds they were starting with. Sure, it can be done by shape or color – but to separate the peanut from the almond M&Ms is a bit trickier. Time for separations by mass!

Discussion ensues as to the best way to separate the different M&Ms.

This group separated the candies by shape. One group came up with a unique method: the wobble test will separate M&Ms that wobble from those that don't.

And then it's time to take several of the "standard" M&Ms straight from the bag…

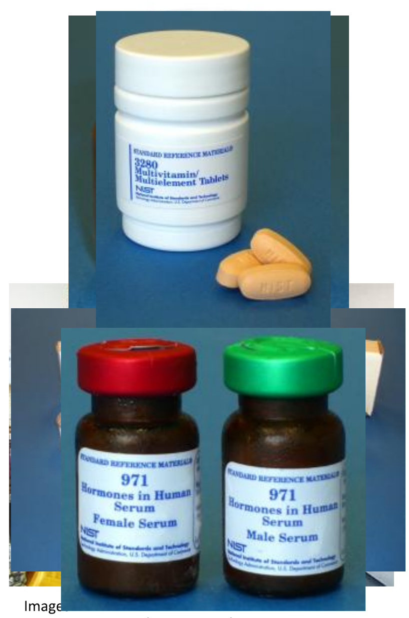
…and develop a range of masses possible for each particular type. For example, peanut M&Ms may have masses between 2.18 g -3.51 g whereas pretzel M&Ms may have masses between 2.84 g - 3.77 g. Yes, there is overlap between those two, but this information may be sufficient to predict the identity of many of the M&Ms in the mixture. Predict, yes, but how do you know for sure? You take the M&M and break it up, either with your teeth or with a knife!

One thing I learned from this activity: triple beam balances take much longer to use correctly than the electronic balances and would probably limit the number of samples measured. This is an example of the measuring tool affecting the experiment especially with the limited attention span of middle school students!

After this introductory activity, Ben Place gave an overview of mass spectrometry and began by explaining the kinds of questions mass spectrometry can be used to answer:

Questions Analytical (Bio)Chemists Ask

- Environmental
  - Is the water safe to drink?
  - Deepwater Horizon Oil Spill: Where did the oil go? When will the seafood be safe to eat again?
- Food
  - Why kinds of vitamins are in baby food?
  - Are there any toxic heavy metals in meat products?
- Medicinal
  - Are the vitamin levels on the label correct in a multivitamin?
  - What are the hormone levels in a patient's blood?



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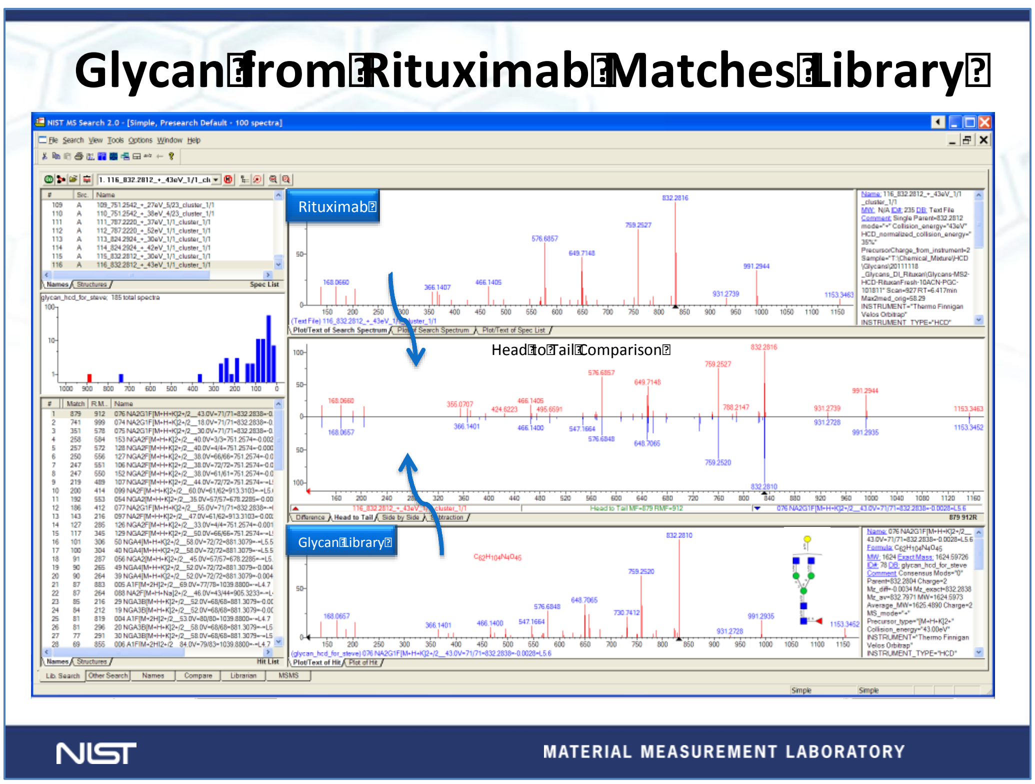
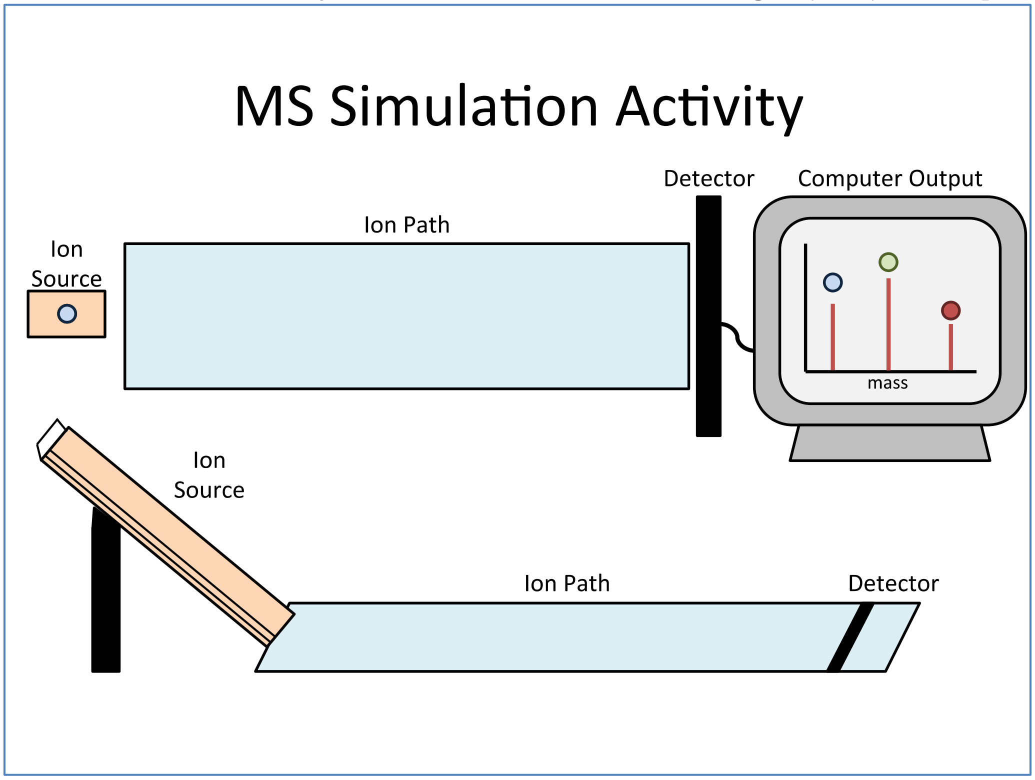
Before moving on to another activity, Lorna talked about her work at NIST developing a mass spectral database of glycans (the sugar molecules that are normally found attached to proteins) and glycopeptides (the sugar molecules still attached to small proteins) in therapeutic drugs.



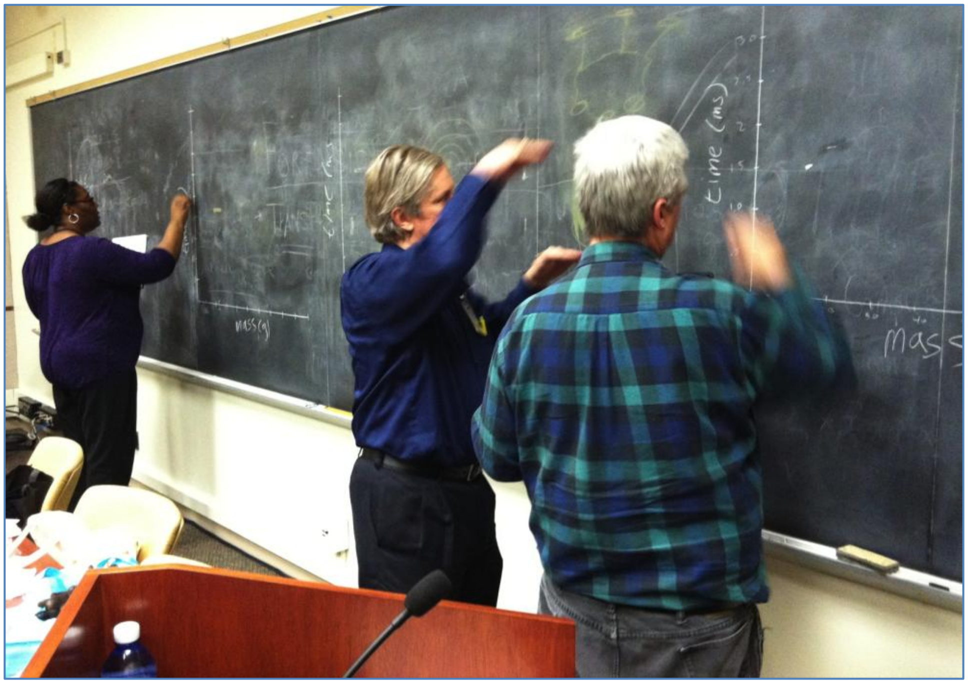
Lorna's work enables scientists in their own labs to identify types of glycans based on the use of NIST software and NIST data gathered on many different types of mass spectrometers under different conditions. The middle panel above shows a butterfly plot comparing two spectra: a known from NIST and an unknown. A quality score is also given as an assessment of how closely the two spectra match.

Then it was on to another activity which simulated a Time-of-Flight (TOF) mass spectrometer.



Teachers carried out an activity in which they used different size and mass balls to simulate ions and measured how long it took the balls of particular masses to travel a certain distance.

Here teachers time with their smart phones as several groups work side by side to measure the "time of flight" of different balls.



After constructing a calibration curve teachers took a ball with an unknown mass and measured how long it took the ball to traverse the set distance, and calculated the mass of the ball. How'd it work? Not very well, which led to discussion of possible improvements such as using balls of different sizes, increasing the distance traveled, spreading out the graph to include just the range of results observed, and better controlling the variables. A couple of teachers noticed that the results with the same kind of ball varied from group to group: a great example of the variability associated with the equipment such as the ramp or the ball itself – or even the person releasing the ball! Sources of uncertainty are everywhere.

Then it was off to visit the lab and see mass spectrometers in action. Half the teachers went with Ben and the other half with Lorna, while the teachers participating virtually watched the video of the visit to Lorna's lab. Here Lorna shows the Time-of-Flight mass spectrometer which is connected to a liquid chromatograph. First one kind of separation, then another kind of separation yielding greater and greater specificity.

This mass spectrometer is typically used to examine mixtures of small molecules, such as creatinine in urine.

And then over to see one of the instruments that Lorna uses all the time…

… the Orbitrap, for examination of glycans and glycopeptides. Here Lorna shows how peaks from the chromatogram are taken and the material fragmented to provide more information about the contents.

Teachers left for the evening with a better understanding of how mixtures can be separated by mass and how mass spectrometry is used in the lab, including ways to teach these concepts to their students. Plus they now have connections with additional NIST scientists! Besides Lorna and Ben, Richard Steiner and Fatima Sequeira participated in the Science Afternoon at NIST: Separations by Mass. All four, plus Mary Satterfield, are available for further contact:

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