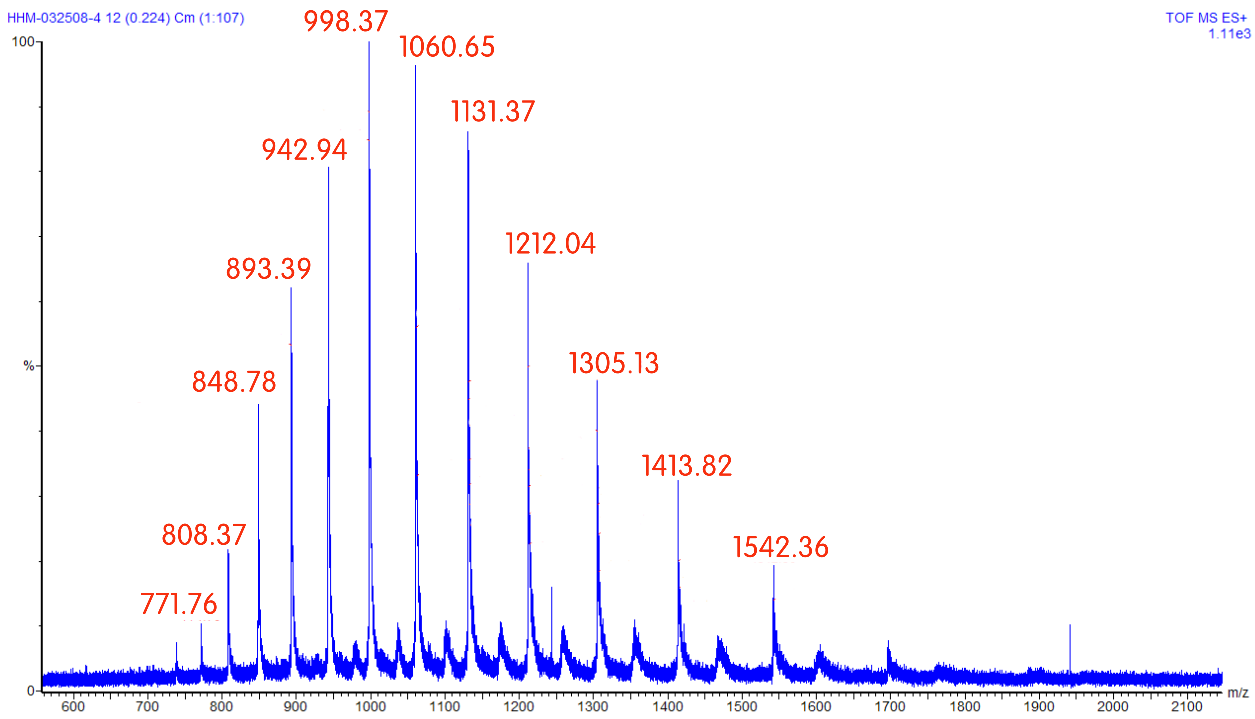
Electrospray Ionization MS Problem Set #1

1. Consider the ESI-MS spectrum for horse heart myoglobin (HHM)



The peaks you see here are are not like the peaks you've seen in electron impact (EI) or chemical ionization (CI) methods. Those spectra also featured many peaks, but they came from many fragments each with a different mass. In ESI-MS the peaks, as seen above, all represent the *same mass*, but *different charges*.

(a) *If* the MW of HHM is about 17,000 Dalton,

what is the charge on the peak labelled m/z = 1542.36?

17000 Da / 1542.36 = +11.02

what is the charge on the peak labelled m/z = 1212.04?

17000 Da / 1212.04 = +14.03

what is the charge on the peak labelled m/z = 1060.65?

17000 Da / 1060.65 = +16.03

what is the charge on the peak labelled m/z = 893.39?

17000 Da / 893.39 = +19.03

Do you see a pattern here? What is it?

Yes. For each new peak the charge increases by +1 and the m/z decreases by MW/(n+1)n.

(b) What if you didn't know what the mass of HHM was? Could you figure it out?

Yes, you could. You need to go through a process of **deconvolution**. It works as follows. Open an Excel spread sheet. In a list enter $(m/z)_{obs}$ for all the peaks shown in the spectrum above. Then assign to each peak a charge using the pattern you discovered above, with the peak at 1542.36 as being 11+. Make a list of those charges. Multiply your two columns to get an approximate MW derived from each peak. What range of MW do you get?

m/z range from +22 to +11 = 16,979 – 16,966 = 13 amu
st. dev = ± 4.21

Repeat this exercise, by building a new table (don't throw the first table away, cut and paste to make new one), but start with a different charge, say make the peak at 1542.36 as having a 12+. What range of MW do you get?

m/z range from +23 to +12 = 17,750 – 18,508 = –758 amu
st. dev = ± 244

Repeat again, but with the peak at 1542.36 having a charge of 10+. What range of MW do you get?

m/z range from +21 to +10 = 16,207 – 15,424 = 783 amu
st. dev = ± 252

Which of the guesses (hypotheses) gave you the *most consistent* value of MW for HHM?

The first one with +11 corresponding to 1542.36 and +22 corresponding to 771.76, which give masses 16,979 – 16,966 amu

By this method we can use inductive reasoning to hone in a probable MW.

(c) You may have noticed that in (b) even the most consistent MW still gave a range of MWs. Can we do better? Yes, we can.

We've been taking shortcuts. Specifically, we've said that the *m* determined from *m/z* is equal to the MW. That's not quite true. Remember the HHM cations get their charge from added H+. Each time we up the charge by 1, we are also increasing the mass by 1 amu. For a species with charge = *z*, we must have a mass $m = MW + z^*m_H$., where MW is the (constant) molecular weight of the unprotonated molecule and m_H = mass of the (added) proton. For example, if the species has a charge of 14+, then it would have a mass of MW + 14 (assuming the mass of H is 1.00). Thus, the m/z can be expressed as

$$(m/z)_{obs} = (MW + z) / z$$

Algebraically rearrange this equation to find the MW.

$MW = z^* (m/z)_{obs} - z$ *or* $MW = z^* [(m/z)_{obs} - 1]$

Now for every m/z value in your Excel table calculate a MW using the formula given above.

Once you have a list of more accurate MW, use Excel to calculate an average MW and a standard deviation (those are both built-in functions in Excel). Report your average MW and its standard deviation.

m/z	z	MW
771.76	22	16956.7
808.37	21	16954.8
848.78	20	16955.6
893.39	19	16955.4
942.94	18	16954.9
998.37	17	16955.3
1060.65	16	16954.4
1131.37	15	16955.6
1212.04	14	16954.6
1305.13	13	16953.7
1413.82	12	16953.8
1542.36	11	16955.0
	average	16954.98
	st. dev	0.83

Are you impressed that this MS technique can give a highly accurate MW for such an enormous molecule? *YES!*