# MALDI Activity 3

# MMass for MALDI-TOF Mass Spectrometry

## Model 1: Review of Ion Formation in MALDI-MS

Figure 1 below is a theoretical MALDI mass spectrum that shows several *m/z* signals in hemoglobin. Typically, when a scientist finds ions of interest, she will look to see if she can find associated peaks. If the protonated molecular ion, $[M+H]^+$, is found at a given *m/z*, then one may also find related peaks that indicate the attachment of metal cations, such as sodium and potassium, to the analyte molecule. Recall from MALDI Activity 2, if the molecular mass of a molecule is 125000 daltons, the $[M+H]^+$ would be found at 125001. If the sodiated molecular ion, $[M+Na]^+$, is present in the sample, it would be found at $[M + H]^+ + 22$. Similarly, the potassiated molecular ion, notated $[M+K]^+$, would be found at $[M + H]^+ + 38$.
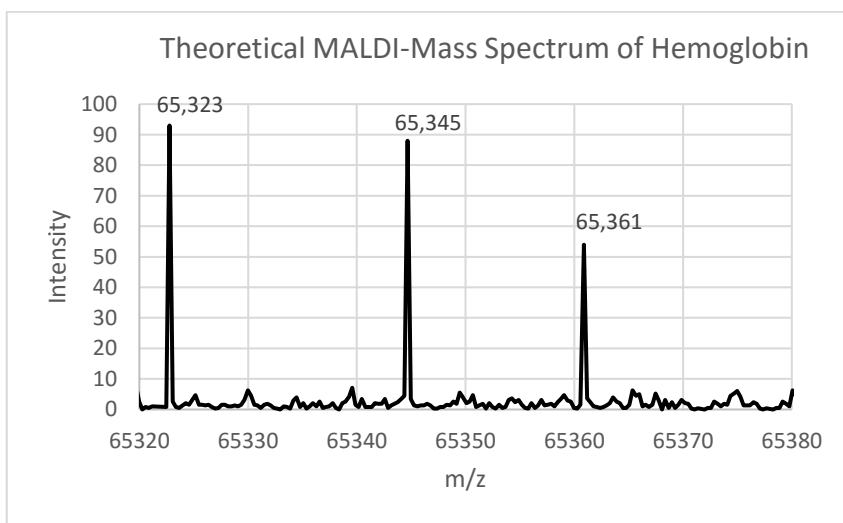


**Figure 1**: Theoretical MALDI-Mass Spectrum of Hemoglobin; *(MW$_{hemoglobin}$ = 65,322 daltons)*

The only naturally occurring isotope of sodium (Na) has a mass number of 23 amu, while the most abundant isotope of potassium (K) has a mass number of 39 amu. *(Recall that the mass number is the sum of the protons and neutrons in the nucleus of an isotope of a particular element).*

**Table 1**: Naturally occurring isotopes of several elements.[1]

| Element | Isotope | Mass (Da) | Natural abundance (%) |
|---|---|---|---|
| Sodium (Na) | $^{23}$Na | 22.9897677 | 100 |
| Potassium (K) | $^{39}$K | 38.9637074 | 93.2581 |
| | $^{40}$K | 39.9639992 | 0.0117 |
| | $^{41}$K | 40.9618254 | 6.7302 |
| Hydrogen (H) | $^{1}$H | 1.007825032 | 99.9885 |
| | $^{2}$H | 2.014101778 | 0.0115 |

| 1 |
|---|
| **H** |
| 1.008 |

| 3 |
|---|
| **Li** |
| 6.939 |

| 11 |
|---|
| **Na** |
| 22.99 |

| 19 |
|---|
| **K** |
| 39.10 |

1.  a)  In the spectrum above (Figure 1), identify any pairs of peaks that have the relationship
        X, X+22, and X, X+38?

    **List the masses of the <u>pairs</u> you identify on the lines below.**

    X and X+22       _____ and _____

    X and X+38       _____ and _____

    b)  What is a likely relationship between these pairs of peaks?
        (Use the terms $[M + H]^+$, $[M+ Na]^+$, $[M + K]^+$)

    X, X+22    _____    _____

    X, X+38    _____    _____

2. *Check your work:*  a) Is the mass of 65,323 Da used twice in part a and the symbol $[M+H]^+$ used twice
   in part b?  **Yes** or **No** (*Circle one*)

    b)  If you circled "Yes", move on to Model 2. If you circled "No", consult further with your
        group or ask another group or your instructor for help.

## Model 2: MMass, A MALDI-Mass Spectrometry Data Analysis Tool

Even the most accurate data is useless if it cannot be analyzed. For mass spectrometry data, proper
analysis requires being able to identify *m/z* ratios and the relative number of ions for each peak. The
MMass program is a tool that allows you to evaluate raw mass spectrometry data. As with any tool, you
must first learn to use it correctly before it is a benefit to you. This portion of the activity will take you
through how to acquire the MMass program, import raw mass spectrometry data, and introduce some
of the basic functions that can be used to carry out data analysis.

**Downloading MMass:** To do this portion of the activity, you will need to go to http://www.mmass.org/
and download the MMass data tool.  MMass is an open source tool used to view, analyze and share
mass spectrometry data in a simple user-friendly manner. Figure 2 shows the link you need to click on to
choose the download for your computer operating system. After extracting all files from the
downloaded zip file **you should save the files to a location you will remember.** You do not need to
install the MMass program, it is a simple executable file you can open using the icon pictured here:
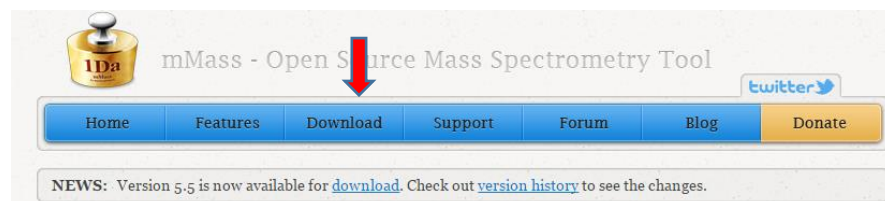




**Figure 2:**  The heading of the MMass.org web page with download highlighted.

**Sample Data:** Sample hemoglobin data used in figure 1 is available from the implementation section of project website. We will import the data to practice using MMass to analyze data. This practice data can be found at the direct link below.

http://webs.anokaramsey.edu/MALDIEducation/maldi-tof-implementation-materials/

Right click on the link called "HemoglobinExampleData" and choose "Save file {or link} as" to save a text file with all the data. You can now open the hemoglobin data file in the MMass program ( 🍯 mmass ).

Choose "Open" from the file menu of MMass or choose the following icon: 🖼️ from the menu bar and select the hemoglobin data you saved.

**Peak Picking:** Click on the peak picking icon ( 🔬 ) at the bottom of the window to allow you to confirm the labeled peaks from figure 1 are correct. To do this, you need to **left-click and drag** across the peak you are interested in as is shown at the right. The program will automatically create a data table of the peaks you label on the upper right-hand side of the program window. Figure 3 shows what you should observe if you have done this correctly.
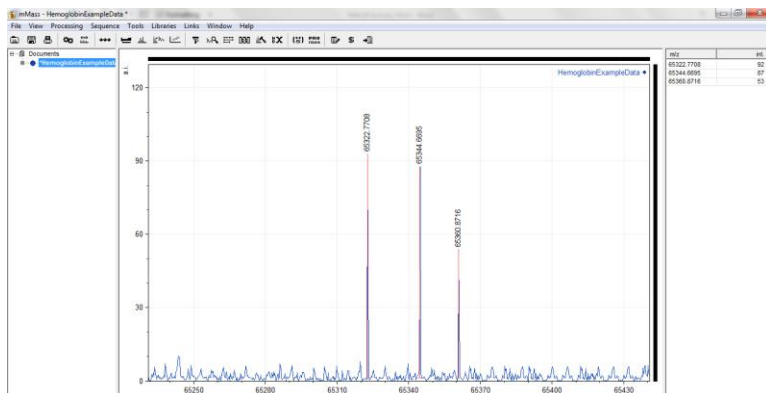


**Figure 3:** Analyzed hemoglobin sample data using the MMass data analysis tool.



Peak selection example

**Zooming:** A **right-click and drag** allows you to zoom in for a closer look at part of the spectrum. See the spectrum view below. Double clicking on the screen will zoom back out to the normal view.
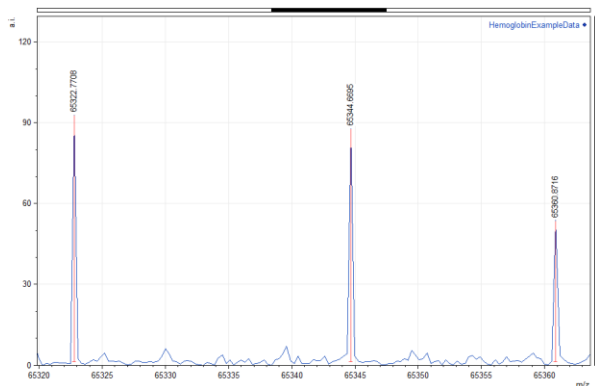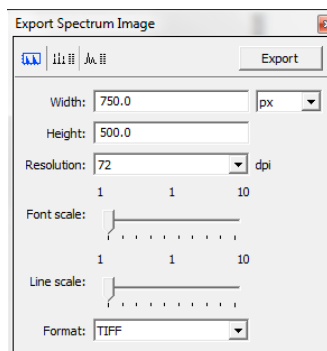


**Figure 4:** Zoomed spectrum.



Figure 5: The export menu

**Saving Spectra:** Use the "Export" option under the "File" menu.  Figure 5 above shows the export menu. You can choose the file type (PNG, TIFF, JPG) in the format drop-down menu.  You can keep the default options for most situations. Click "Export" to save your file with an appropriate name to keep track of what the picture represents.

References:
1. http://www.webelements.com/ accessed 1/18/2016
2. Chapagain, B., P.; Wiesman, Z. *J. Agric. Food Chem.* **2009**, *57,* 1135–1142