

## **MALDI-TOF MS Analysis of Proteins (Code B):**

### **1. General Procedure**

A saturated sinnapinic acid in 60% acetonitril / 1% acetic acid is used as the matrix solution. 1 µl of protein sample is spotted on the sample target, and then 1 µl of saturated matrix solution is added on the top. After the crystal of protein and matrix is formed, the sample target is inserted into the mass spectrometer.

MALDI MS is acquired in linear mode at positive mode on Applied Biosystems Voyager-DE STR MALDI-TOF mass spectrometer equipped with a 337 nm laser. Acceleration voltage is set at 25 kV, grid voltage at 90%, guide wire at 0.02%, and delay time at 175 nsec. The mass spectra are externally calibrated by the molecular weights of two standard proteins.

### **2. Mass Accuracy and Resolution**

The mass accuracy is 0.1%. The resolution is around 100.  $\text{Resolution} = M / \Delta M$ .

### **3. How to Read Spectrum?**

During the MALDI process, one proton binds the protein resulting in one additional positive charge. Two or three protons can also bind to the protein. Therefore, the protein can have one, two, or three positive charges. The mass spectrometer measures the mass  $[M+H]^+$  for single charges,  $[M+2H]^2+$  for double charges, and  $[M+3H]^3+$  for triple charges. The corresponding molecular weight can be calculated:

$$\text{MW1} = (m/z) \times 1 - 1$$

$$\text{MW2} = (m/z) \times 2 - 2$$

$$\text{MW3} = (m/z) \times 3 - 3$$

$$\text{The average MW} = (\text{MW1} + \text{MW2} + \text{MW3}) / 3$$

[See a representative MALDI spectrum of a protein](#)