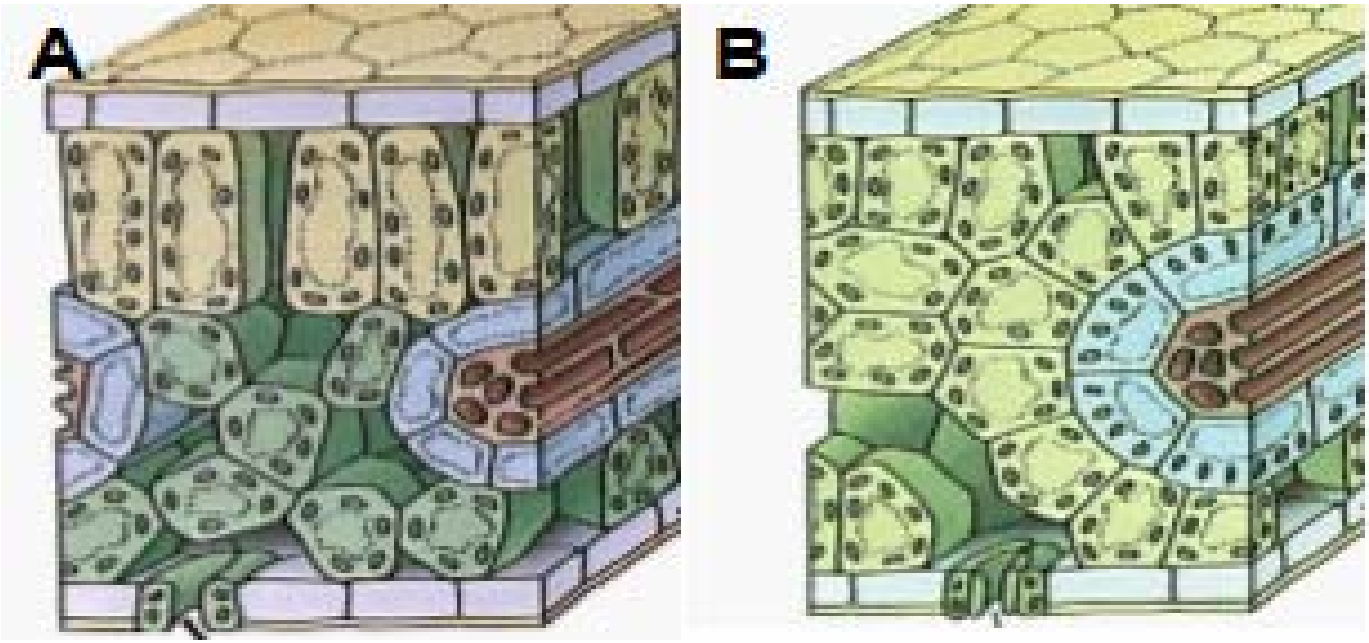
Biological question

Wheat (C3)

Corn (C4)

Biological question

Background:



Question:

What are the leaf proteins potentially responsible for the differences in photosynthetic efficiency ?

Protein extraction

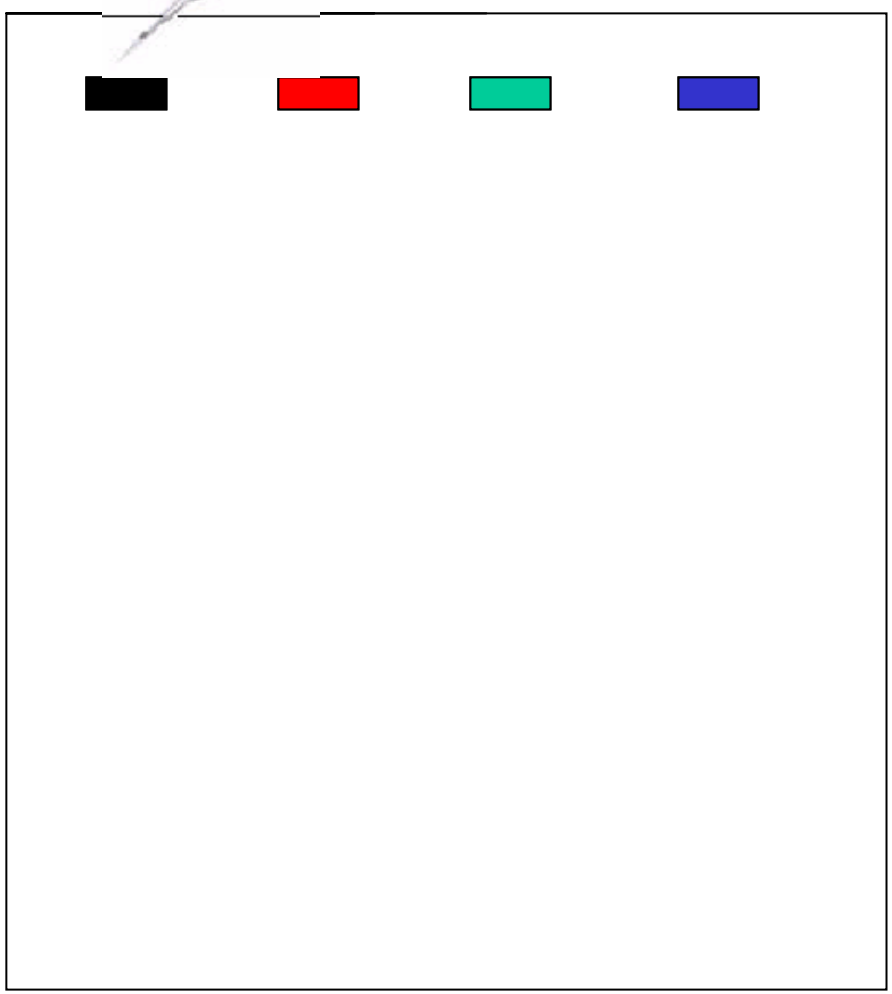
Wheat (C3) and Corn (C4)

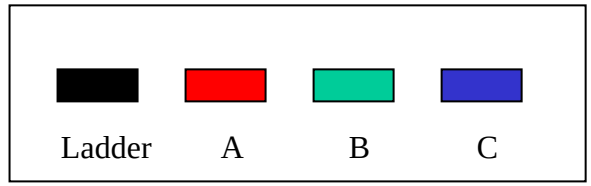
Leaves: ~ 0.2 g fresh weight

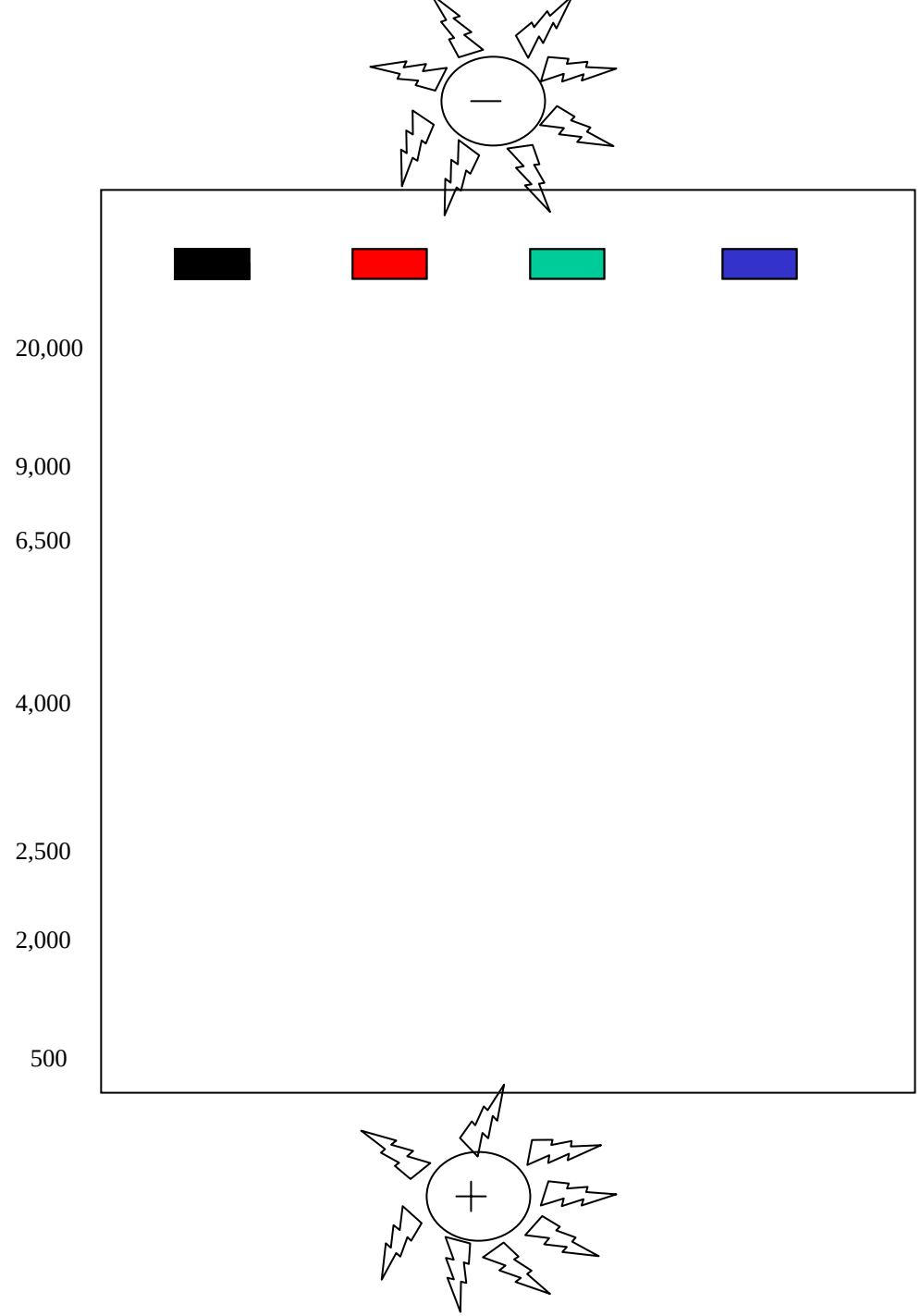
Grind in gel buffer ,
heat it up, and
quick spin.

- Glycerol – high density for loading
- SDS – denature protein and put on charge
- ß-Mercaptoethanol – reduce S-S bond
- Bromophenol blue – monitor process of electrophoresis

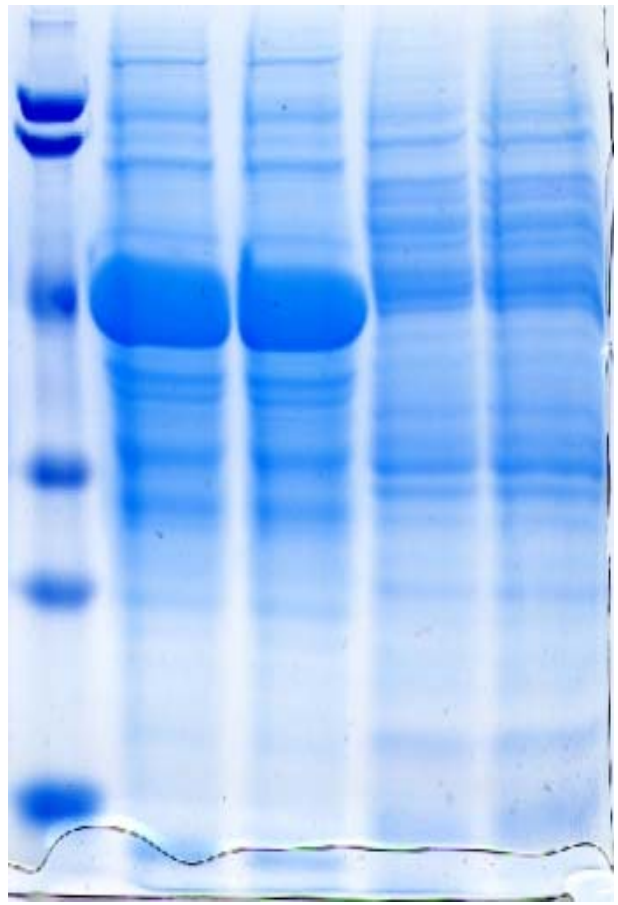

marker



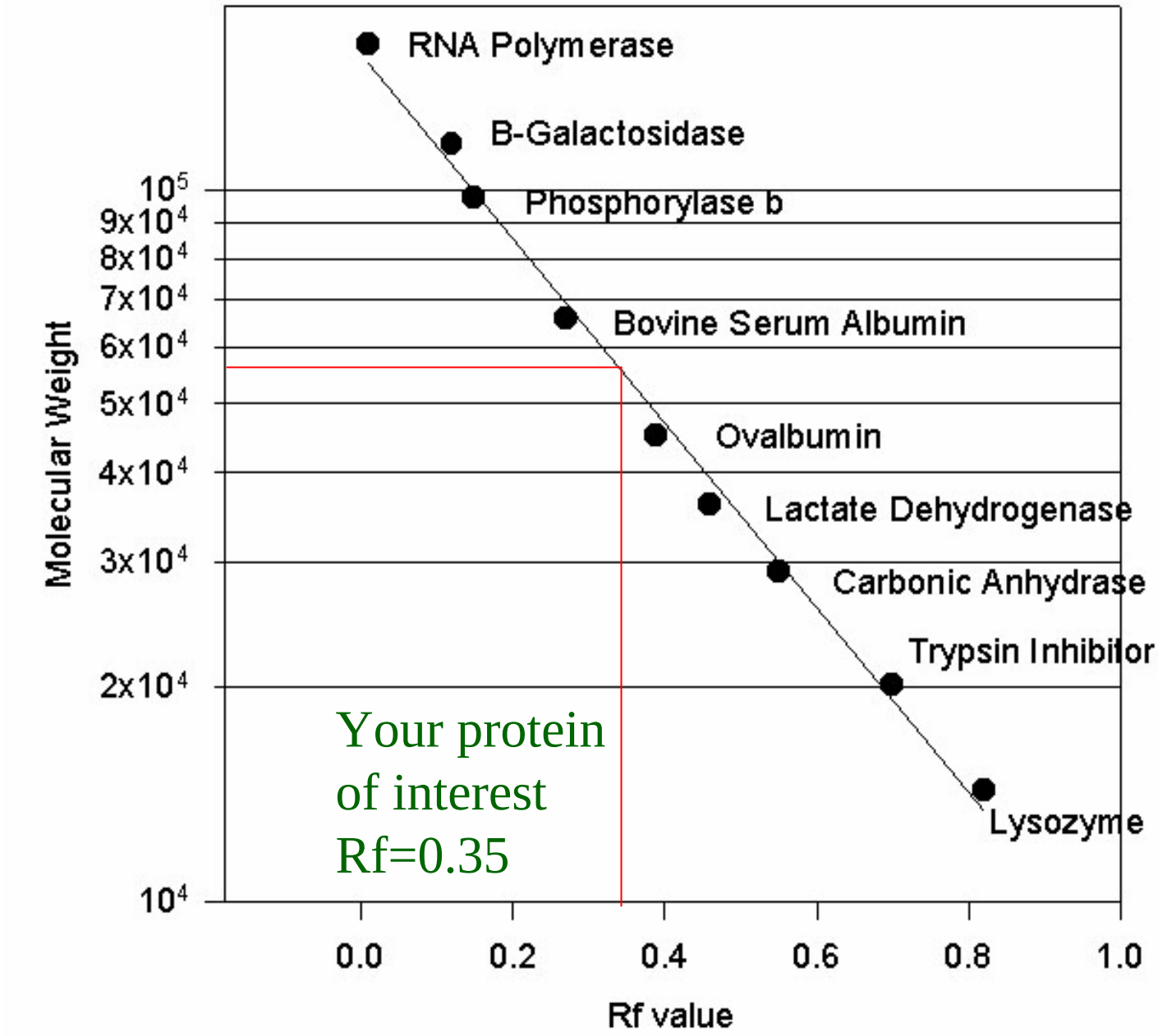

Ladder
A
B
C



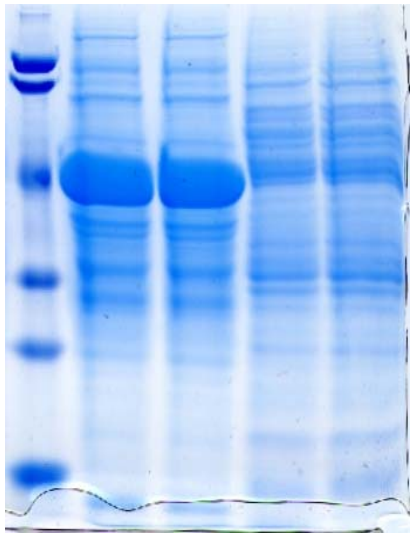
Coomassie staining



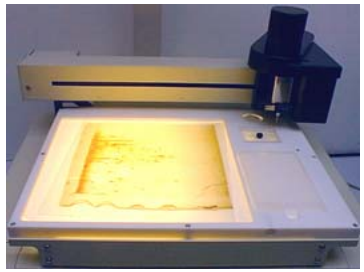
Calculate protein MW


RNA Polymerase
B-Galactosidase
Phosphorylase b
Bovine Serum Albumin
Ovalbumin
Lactate Dehydrogenase
Carbonic Anhydrase
Trypsin Inhibitor
Lysozyme
Molecular Weight
10^5
9x10^4
8x10^4
7x10^4
6x10^4
5x10^4
4x10^4
3x10^4
2x10^4
10^4
0.0
0.2
0.4
0.6
0.8
1.0
Rf value
Your protein of interest Rf=0.35

Peptide Mass Fingerprinting (PMF)

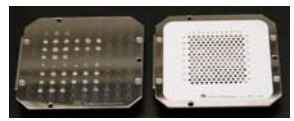


SDS Gel



Pick proteins

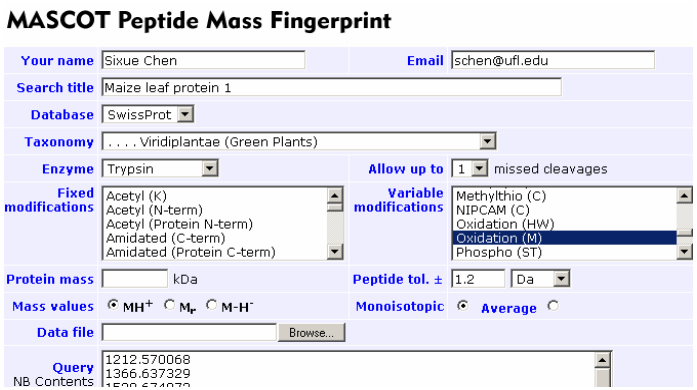
Digest *



Spot on target *

Collect mass data *

Search databases



* performed by teachers with student help.

Suitcase TOF: A Man-Portable Time-of-Flight Mass Spectrometer

Scott A. Ecelberger, Timothy J. Cornish, Bernard F. Collins, Douglas L. Lewis, and Wayne A. Bryden

The need for man-portable analytical instrumentation to detect and identify potential chemical and biological hazards in the environment is growing as the number of natural and man-made threats increases. To that end, APL has been developing the Suitcase TOF, a small suitcase-sized Time-of-Flight Mass Spectrometer, and testing it extensively on known chemical and biological agents. This article introduces the reader to TOF mass spectrometry, describes the features of the Suitcase TOF that make it portable and rugged, and touches on the results of agent testing. Application areas include first-responder and special operations spot checks, clinical medicine, medical research, infrastructure and environmental monitoring, law enforcement, and military troop protection.

INTRODUCTION

The mass spectrometer is a powerful analytical device that has the capability of detecting a wide range of chemical and biological substances. Mass spectrometry is a method of measuring the masses and fragmentation patterns of those substances to determine the composition of the original sample. Coupled with the soft ionization technique called Matrix-Assisted Laser Desorption and Ionization (MALDI),[1] the Time-of-Flight Mass Spectrometer (TOFMS) can measure very large, intact molecules. For example, biological toxins with masses greater than 50 kDa have been readily detected in our portable MALDI TOFMS.

The ability to deploy mass spectrometers for the field detection of chemical and biological threats has been hindered by the size, weight, and power requirements of typical instruments. APL has been developing a small suitcase-sized TOFMS[2,3] that has undergone extensive testing on known chemical and biological agents. This article gives an overview of the components of the Suitcase TOF and their operation, presents encouraging results of field testing on actual agents, and touches on future improvements toward fielding a man-portable TOF instrument.

INSTRUMENT DESCRIPTION AND OPERATION

The Suitcase TOFMS (Fig. 1) has four major subsystems—the vacuum system, optical system, source/analyzer, and electronics/data system—each with unique and innovative features.[4]

You are a Bio-Sleuth

Police/Detective

Who robbed the bank?

Biologist

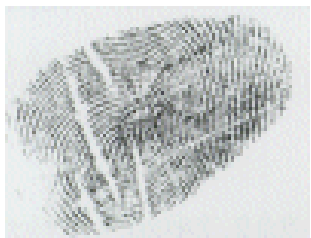
What is this protein ?

GATHER EVIDENCE

Police/Detective

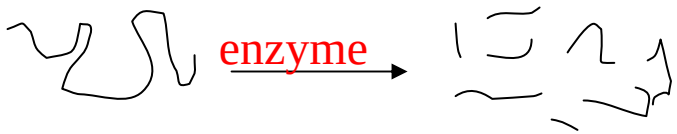
1. Interview witnesses

2. Dust for fingerprints

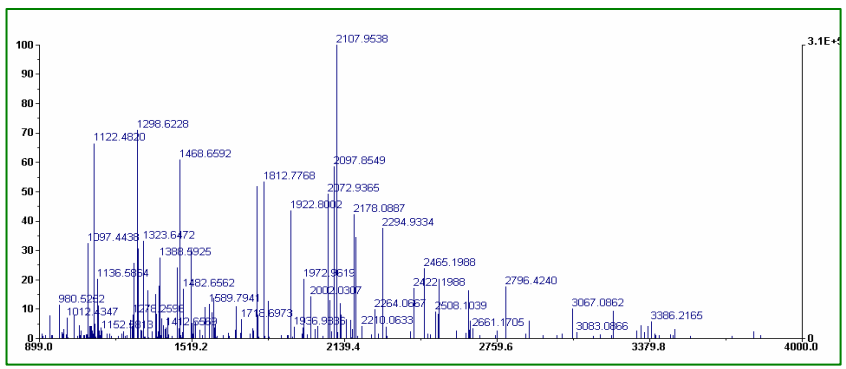


Biologist

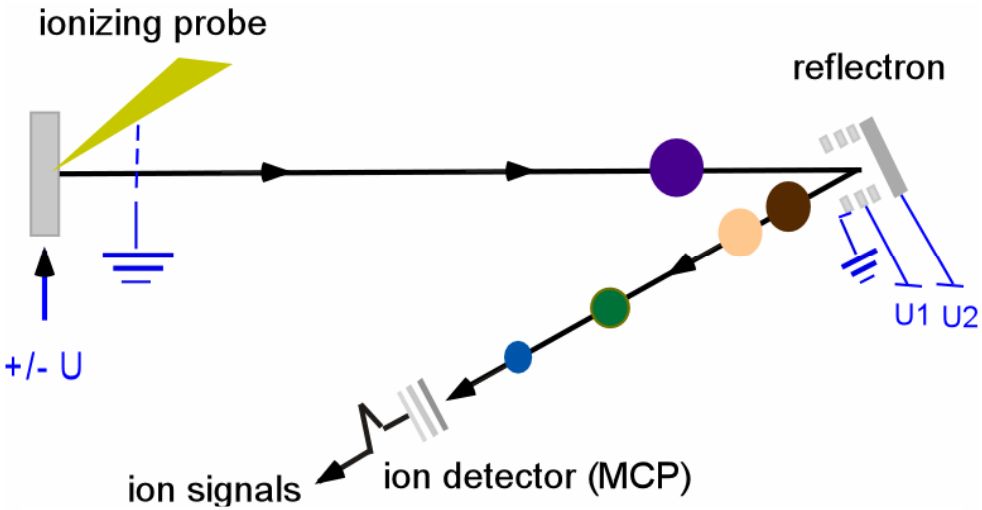
1. Interview biologist to find out information about the protein
2. Cleave protein to obtain peptide mixture



3. Obtain peptide mass fingerprints !

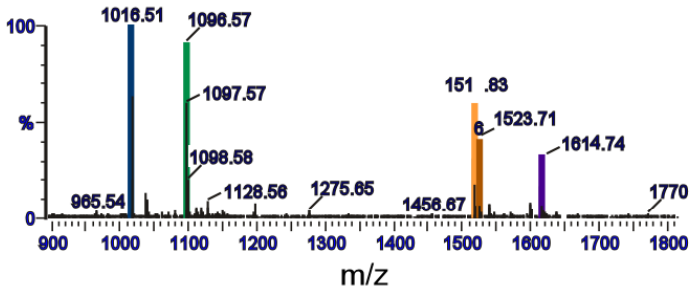


Peptide mass fingerprinting (PMF)



Sequence database

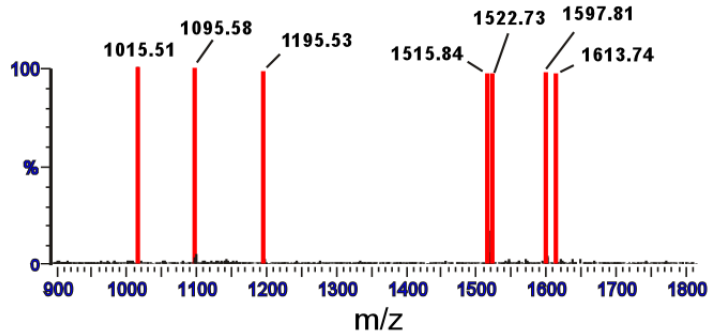
```
MARTFFVGGNFKLNGSKQSIKEIVERLNTASIPENVEVVICPPA
TYLDYSVSLVKKPQVTVGAQNAYLKASGAFTGENSVDQIKDVGA
KWVILGHSERRSYFHEDDKFIADKTKFALGQGVGVILCIGETLE
EKKAGKTLDVVERQLNAVLEEVKDWTNVVVAYEPVWAIGTGLAA
TPEDAQDIHASIRKFLASKLGDKAASELRILYGGSANGSNAVTF
KDKADVDGFLVGGASLKPEFVDIINSRN
```



Peptide masses

1016.5095
1096.5690
1516.8315
1523.7136
1614.7263

"*in silicon*" digestion



Peptide masses	Sequences
1015.5130	(R)TFFVGGNFK(L)
1095.5830	(K)WVILGHSER(R)
1195.5260	(R)RSYFHEDDK(F)
1515.8410	(K)KPQVTVGAQNAYLK(A)
1522.7260	(K)ASGAFTGENSVDQIK(D)
1597.8100	(R)ILYGGSANGSNAVTFK(D)
1613.7360	(R)SYFHEDDKFIADK(T)

Match ?

DATABASE SEARCH RESULTS

Police/Detective	Mass Spectrometrist
FBI fingerprint database	Translated protein database
Identifies the robber	Identifies the protein
Anthony J. Felon	Phosphoenolpyruvate decarboxylase

Plants Are Diverse, Available in Nature, Lots of Questions to Ask and Address






www.moleculardetective.org