Micro Electro Mechanical Systems for mechanical engineering applications

Lecture 12:
Detection Methods for BioMEMS (2)

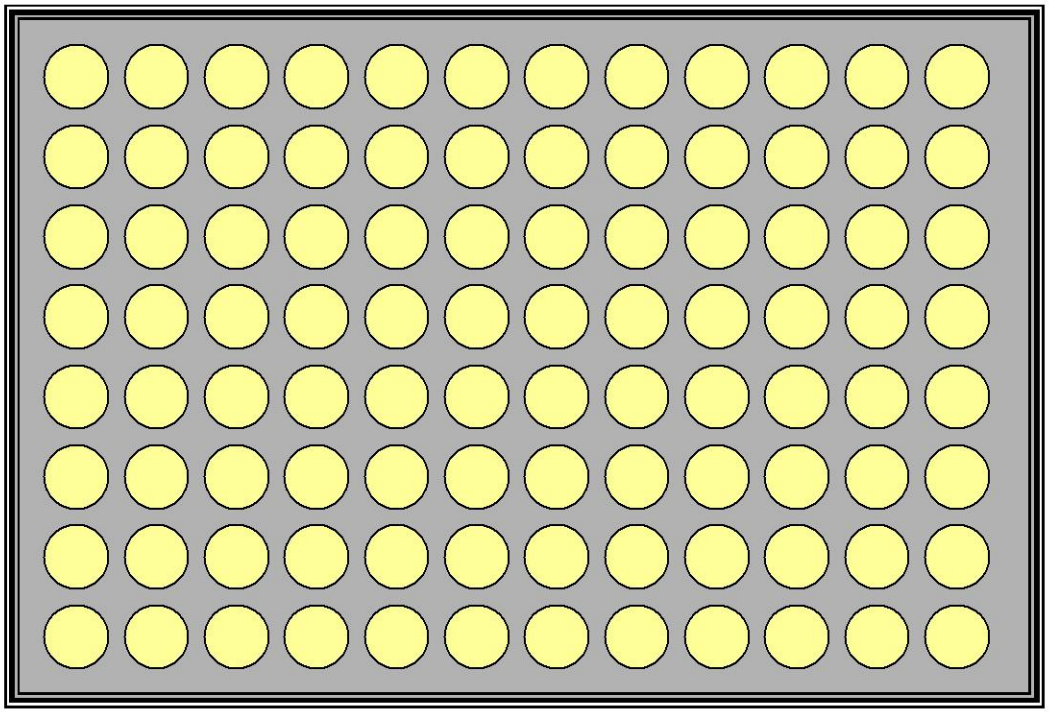
Kahp-Yang Suh

Assistant Professor
SNU MAE
sky4u@snu.ac.kr

1. ELISA (ENZYME-LINKED IMMUNOSORBENT ASSAY)

- valuable tools for use in clinical labs
- can measure antibodies or antigens
- inexpensive, rapid, quantitative, specific
- sensitive (pg/ml)
- expensive equipment not required (but helps!)
- can be automated

BASIC FORMAT



Solid phase = 96 / 384-well microplate

The five-step procedure: detection of the antigen

1) coat the microtiter plate wells with antigen

2) block all unbound sites to prevent false positive results

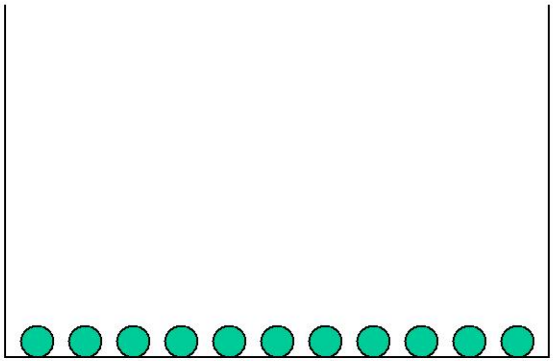
3) add antibody (from mouse) to the wells

4) add anti-mouse IgG conjugated to an enzyme that recognizes the primary antibody

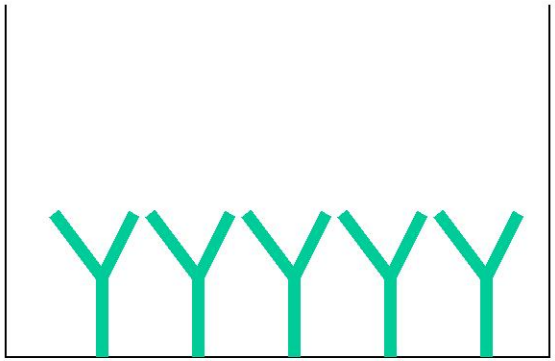
5) reaction of a substrate with the enzyme to produce a colored product, thus indicating a positive reaction

1) Coat solid phase with

antigen when analyzing antibody
antibody when analyzing antigen



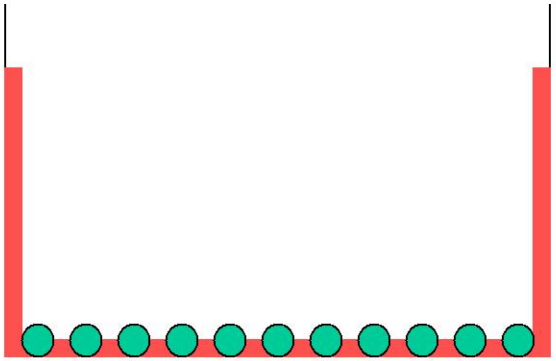
Analyte = antibody



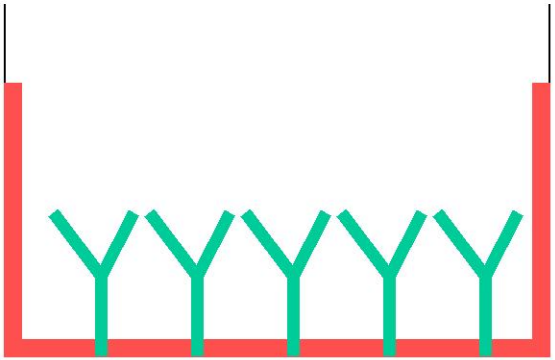
Analyte = antigen

Incubate, wash

2) Block free binding sites. Incubate. Wash.

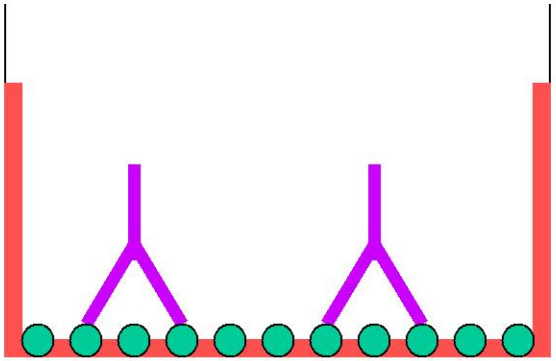


Analyte = antibody

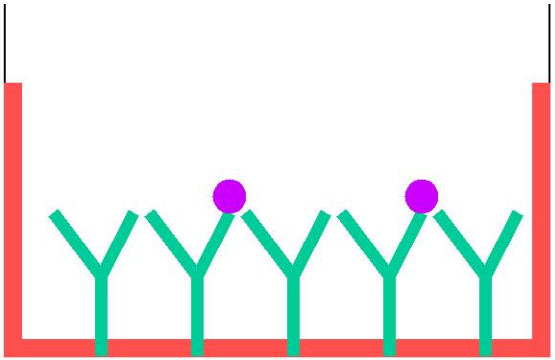


Analyte = antigen

3) Add sample. Incubate. Wash

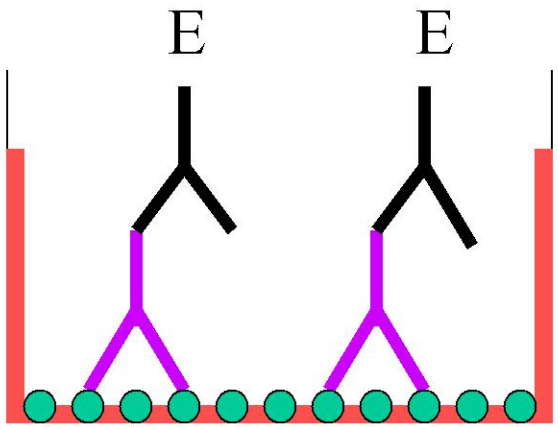


Analyte = antibody

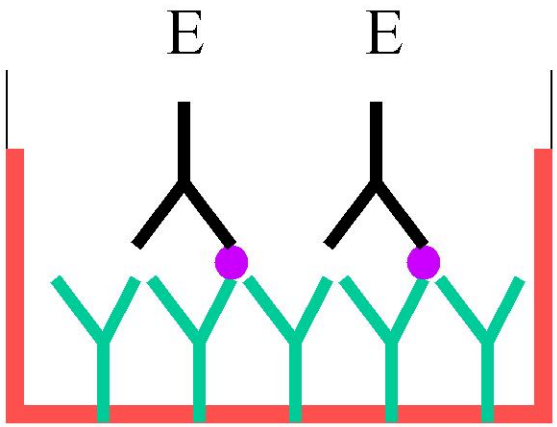


Analyte = antigen

4) Add conjugate. Incubate. Wash.



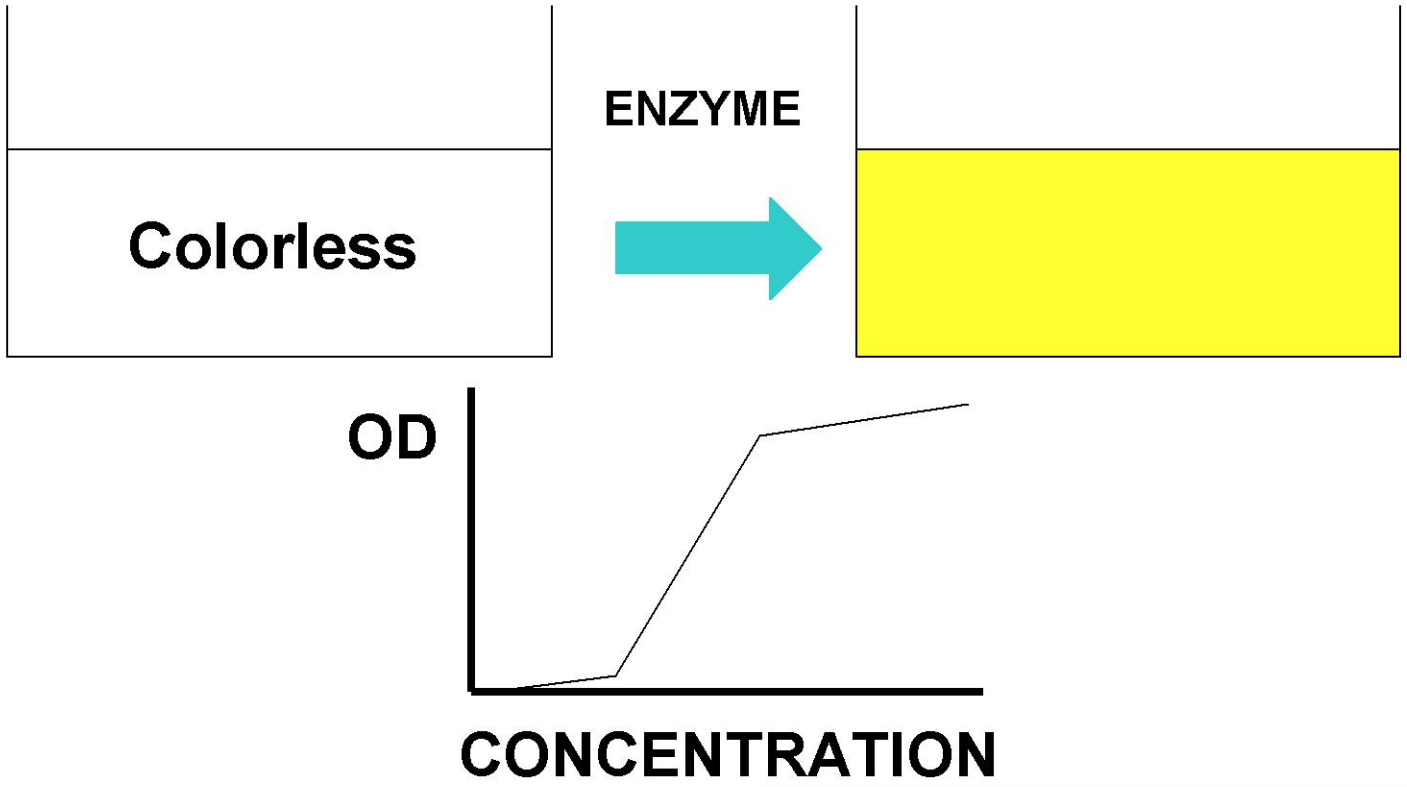
Analyte = antibody



Analyte = antigen

5) Add substrate

6) Incubate, stop, measure color change



Coating the plate

- protein-binding 96 (384)-well polystyrene plate
  eg Immulon-2 (Dynatech)

- buffer = 0.1M $Na_2CO_3/NaHCO_3$ pH 9.6
  0.1M tris-HCl pH 7.6
  0.01M PBS pH 7.3
  etc.

- antigen or antibody at 0.5 - 20 μg/ml

- 100 μl/well, 4°C overnight

Washing the plate

- buffer + 0.05% Tween 20
- 200 μl/well
- 3 - 6 washes with 1 minute soak
- automated washer
  or
- "flood and flick" (biohazard)
  or
- multichannel pipette for dispensing,
  manifold connected to vacuum pump
  (for safe disposal of wash fluid)

Blocking the plate

- 0.25% - 2% bovine serum albumin
  2% non-fat dried milk
  5 - 10% fetal calf serum
  in buffer + 0.05% Tween 20

- 100 μl/well, 37°C, $\geq$ 60 min

- Wash x3 with buffer-Tween 20

Sample

- Dilute in buffer-Tween 20
- include known positive and negative samples
- standards……. recombinant protein
  international standard antibody
  double-dilute from 10 pg/ml - 10 ng/ml
- 100 μl/well, duplicates
- 2 - 4 hours 20/37°C or overnight 4°C
- 3 - 6 washes with buffer-Tween 20

Conjugate

- For assays of (human) antibodies use :

  anti-(human) Ig-enzyme
  IgG / A / M / E / subclass-specific

- For assays of antigens use enzyme-conjugated antibody:

  against a *different epitope* to the one recognized by capture antibody

  often *monoclonal* capture antibody
  polyclonal detection antibody

Application of ELISA (1)

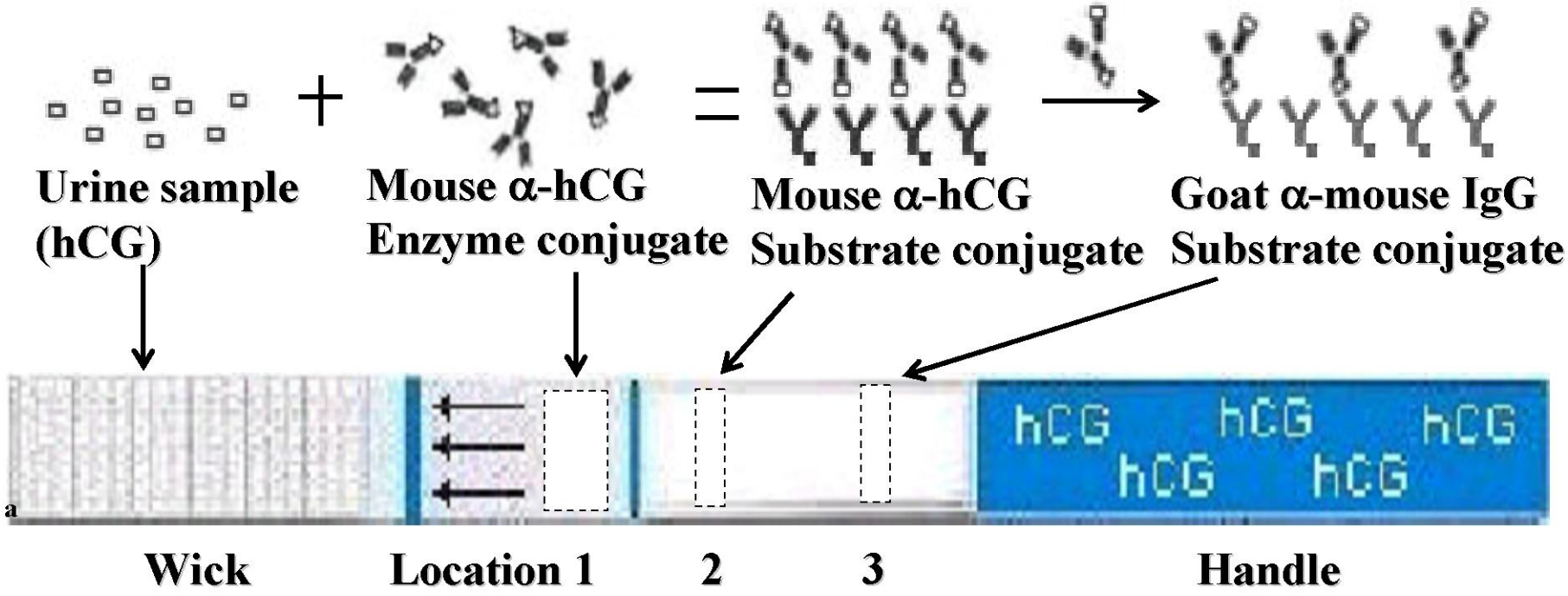
- **Diagnostic applications (i.e.)**
  - Diagnosing diseases: Herpes, Syphilis, Lyme disease, SARs
  - Vaccination titers: Chicken pox, Hepatitis
  - The ↑ the amount of antibody ↑ the chance of exposure and/or immunity

- **Research applications (i.e.)**
  - Specificity of an antibody for an antigen
  - Determining antigenic determinants
  - Vaccine development

Application of ELISA (2)

- Pregnancy Test

- ELISA-based test
- Checks a woman's urine for human chorionic gonadotropin (hCG)



Limitations of ELISA

- **Only detects antibody/antigen, does not tell you if an infection is ongoing**
- **People may be poor producers of antibody or recently exposed to an antigen = false negative**
  - For SARs can only detect 21 days after the onset of clinical symptoms
- **Unrelated antibodies or antigens may react in an unspecific way = false positive**

2. Mass Spectrometry

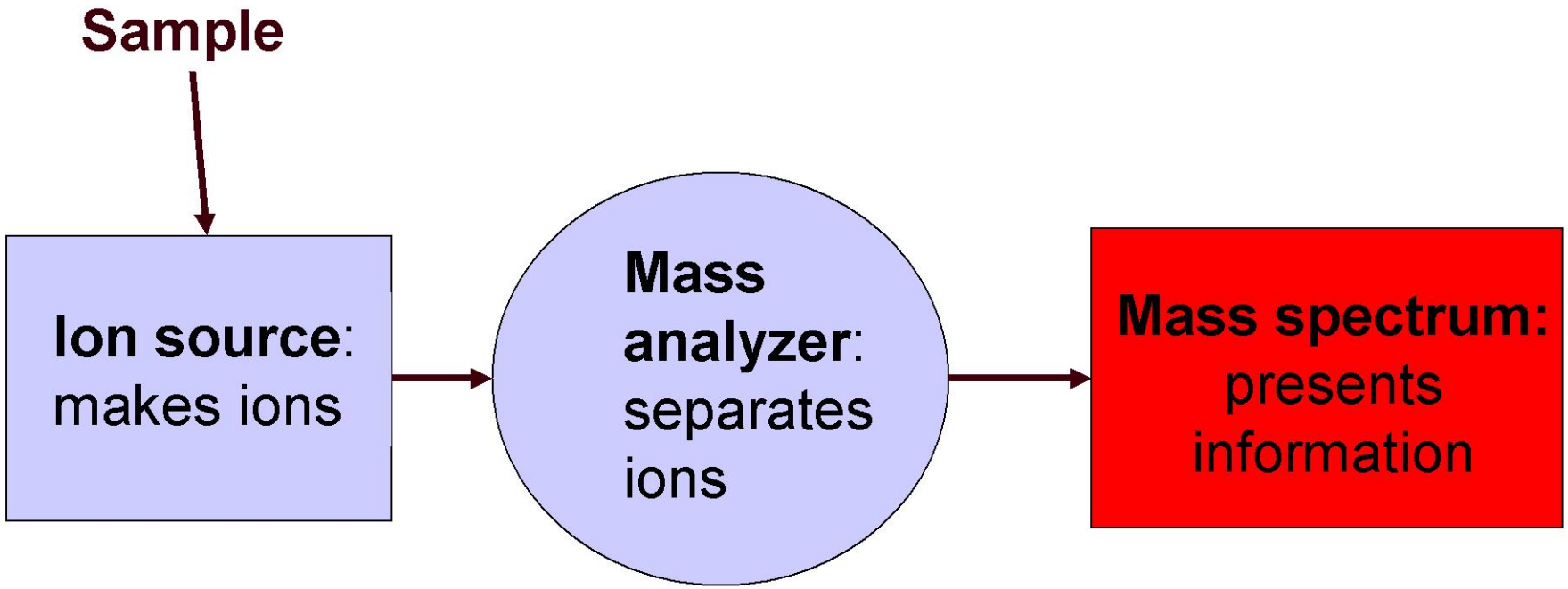
What does a mass spectrometer do?

1. It measures mass better than any other technique.
2. It can give information about chemical structures.

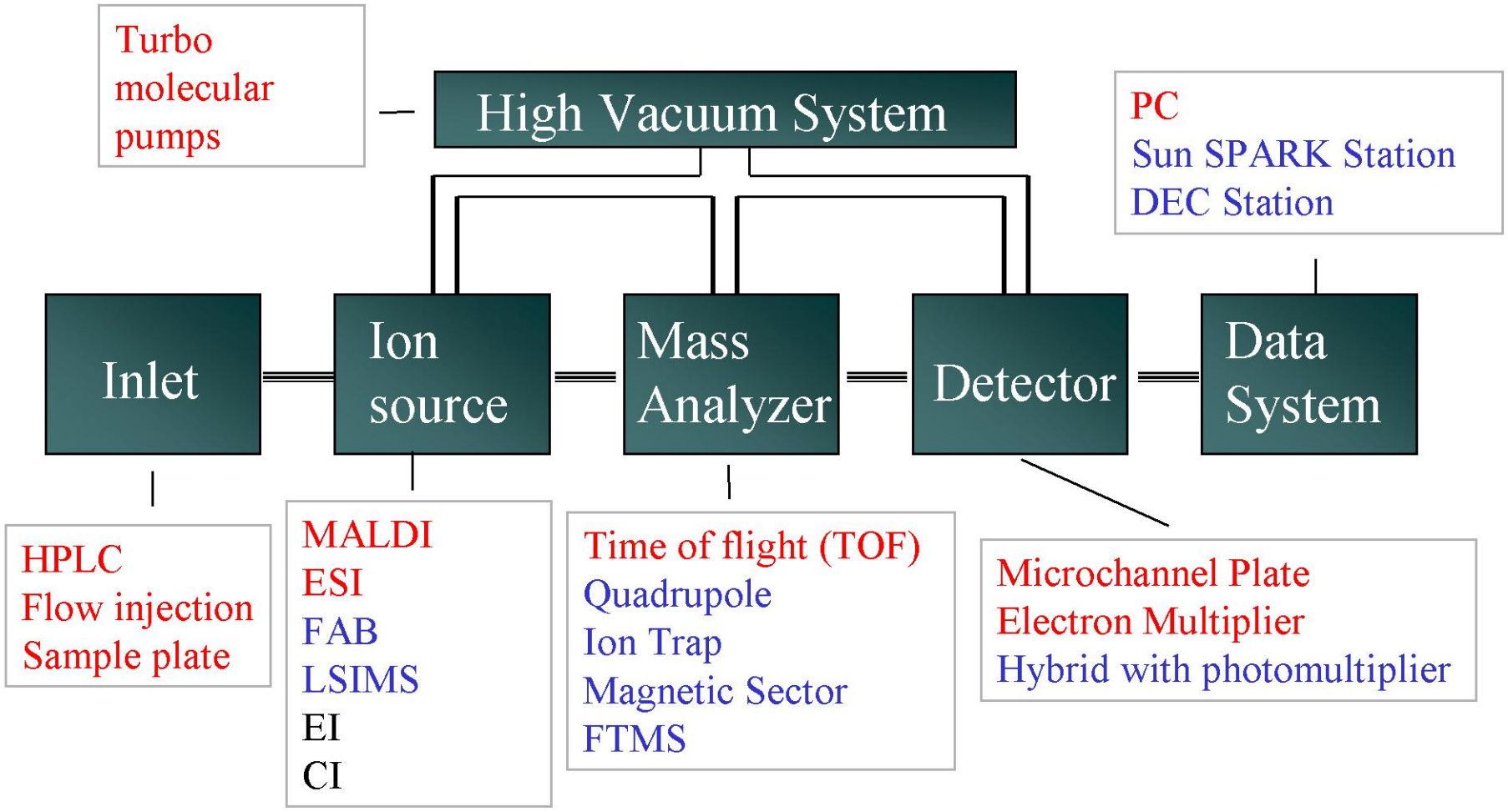
What are mass measurements good for?

To identify, verify, and quantitate: metabolites, recombinant proteins, proteins isolated from natural sources, oligonucleotides, drug candidates, peptides, synthetic organic chemicals, polymers

How does a mass spectrometer work?



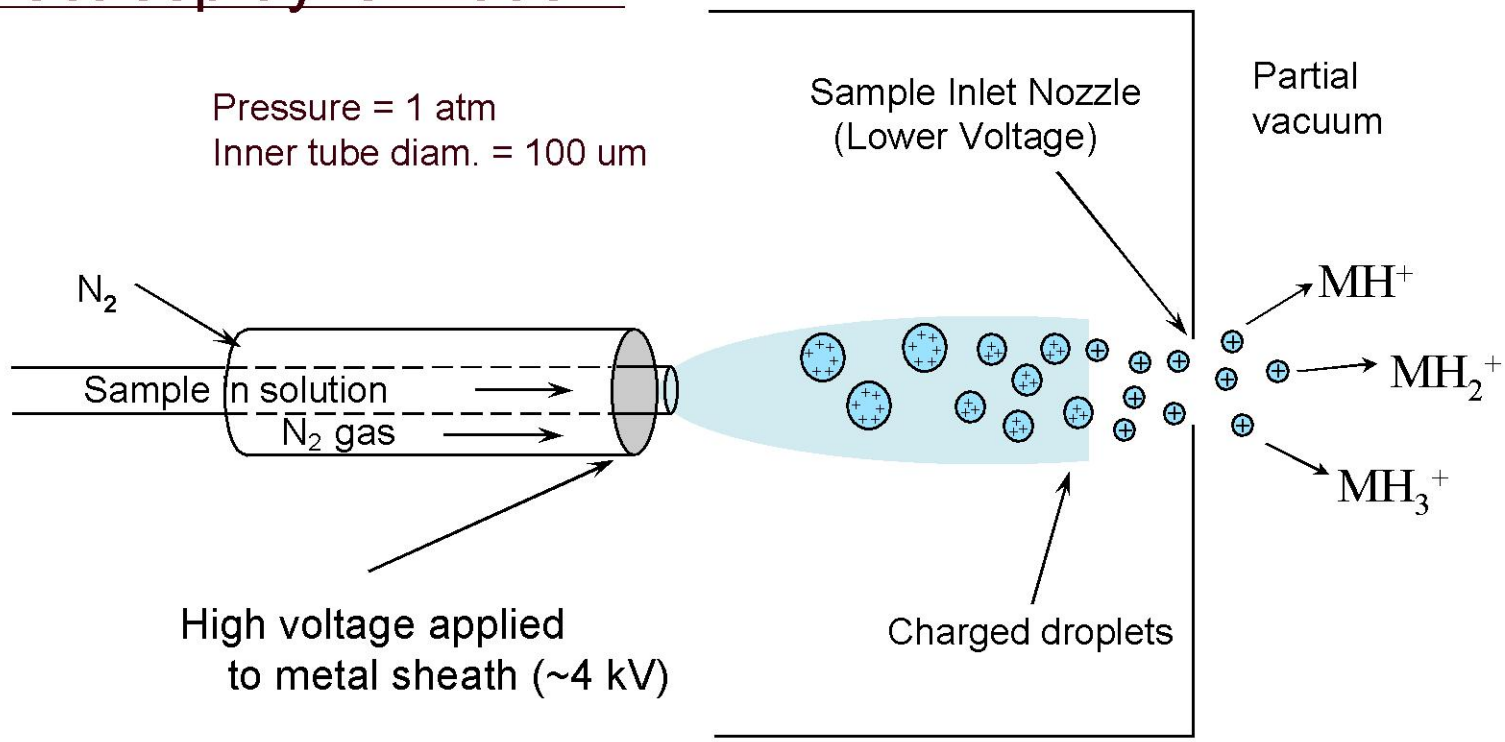
Mass Spectrometer Block Diagram



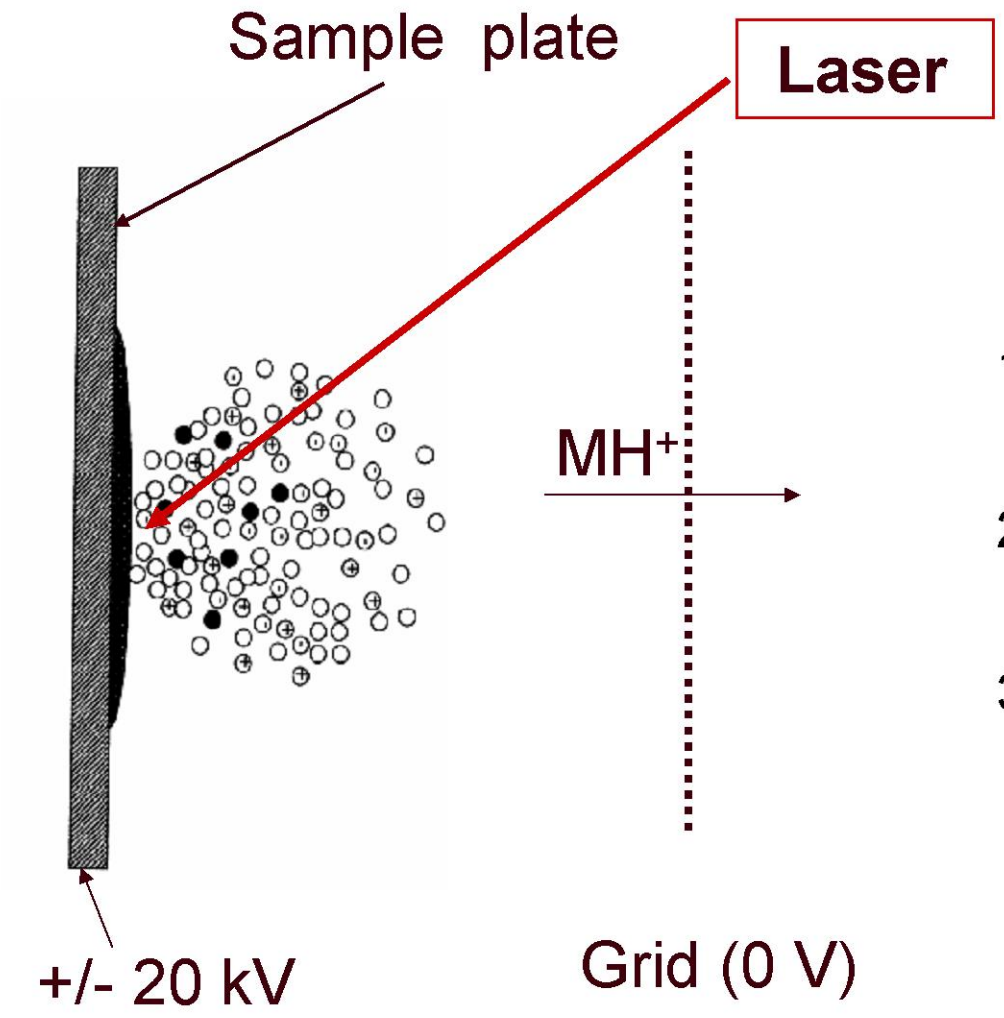
Ion Sources make ions from sample molecules

(Ions are easier to detect than neutral molecules.)

Electrospray ionization:



MALDI: Matrix Assisted Laser Desorption Ionization

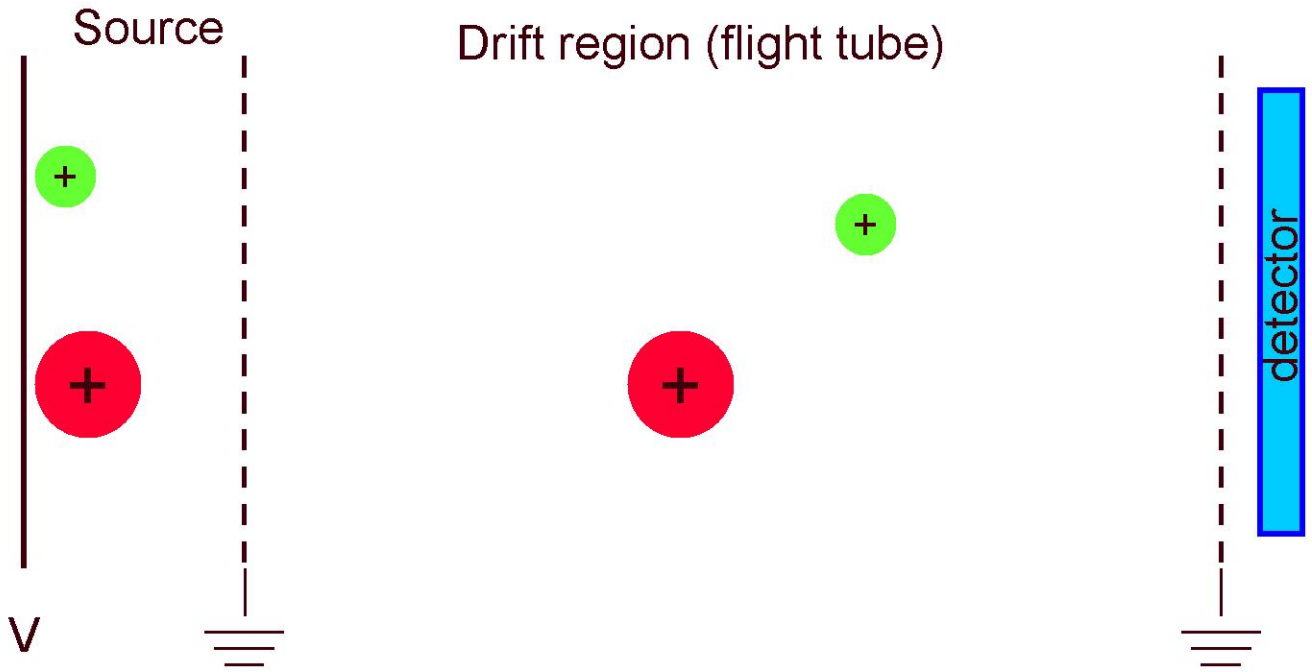


1. Sample is mixed with matrix (X) and dried on plate.
2. Laser flash ionizes matrix molecules.
3. Sample molecules (M) are ionized by proton transfer: $XH^+ + M \rightarrow MH^+ + X$.

Mass analyzers separate ions based on their mass-to-charge ratio (m/z)

- Operate under high vacuum (keeps ions from bumping into gas molecules)
- Actually measure mass-to-charge ratio of ions (m/z)
- Key specifications are resolution, mass measurement accuracy, and sensitivity.
- Several kinds exist: for bioanalysis, quadrupole, time-of-flight and ion traps are most used.

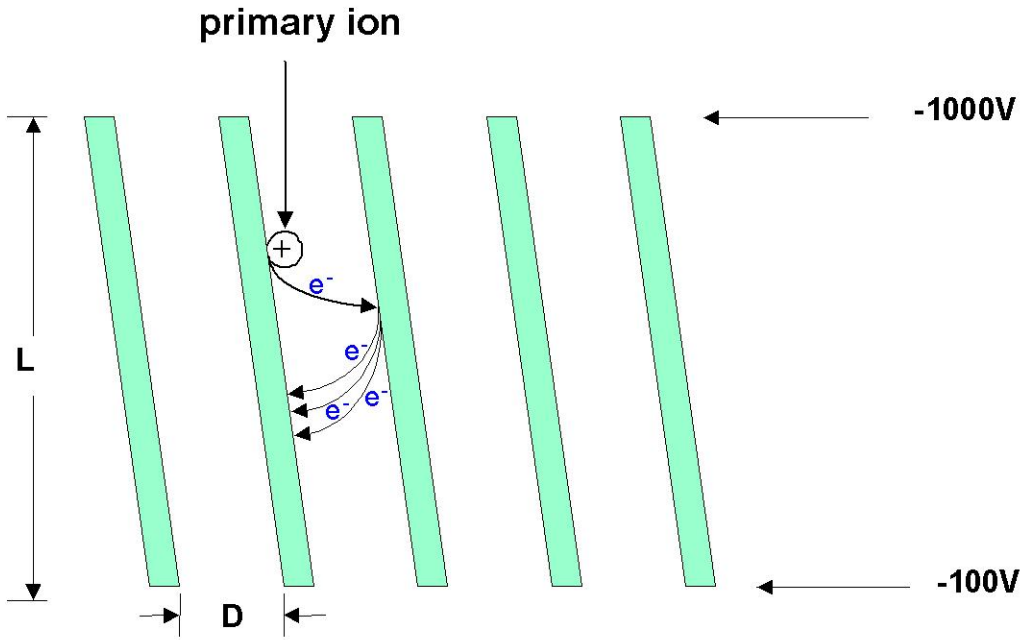
Time-of-flight (TOF) Mass Analyzer



- Ions are formed in pulses.
- The drift region is field free.
- Measures the time for ions to reach the detector.
- Small ions reach the detector before large ones.

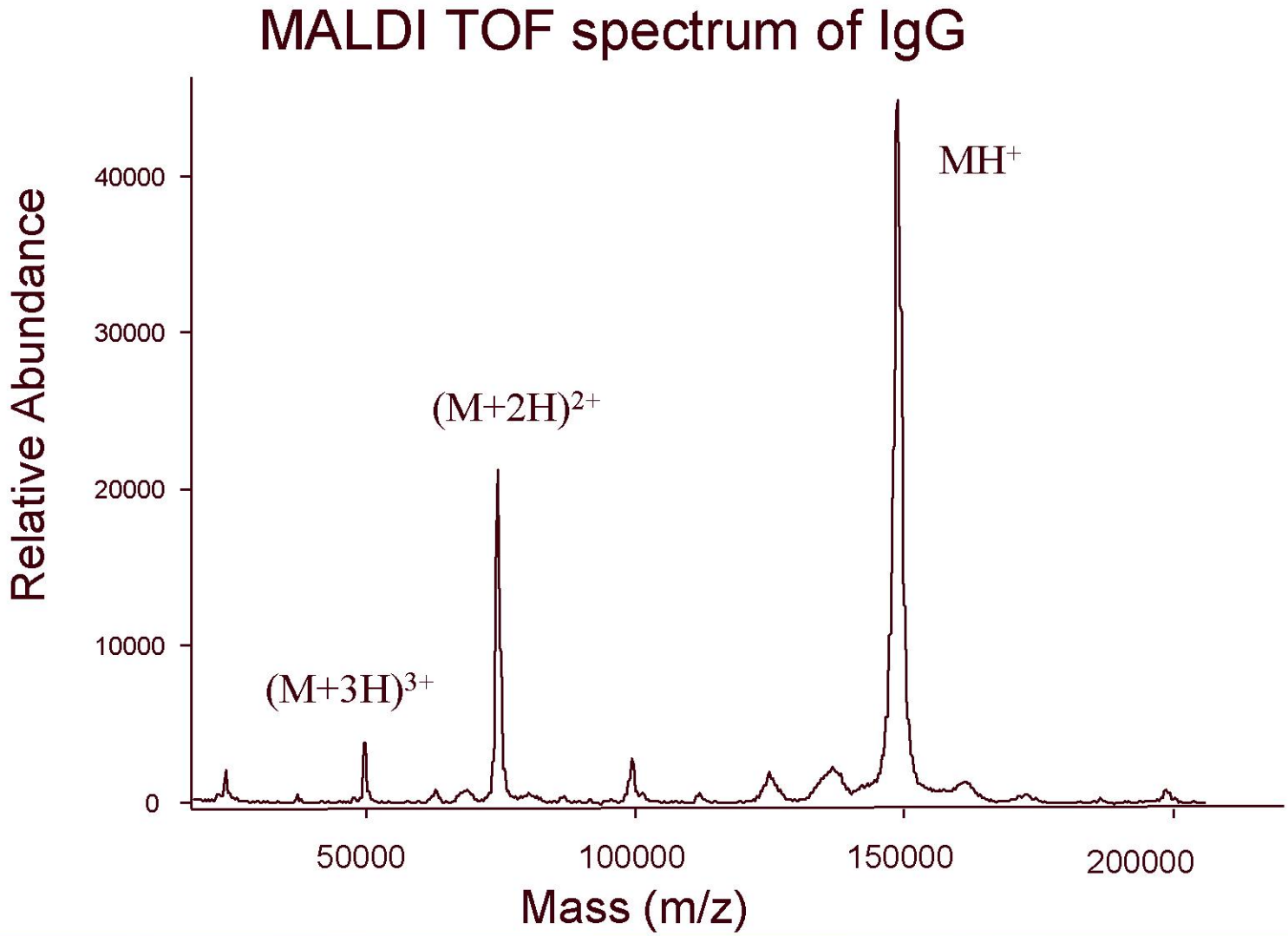
Microchannel Detector Plate

Ions are detected with a microchannel plate

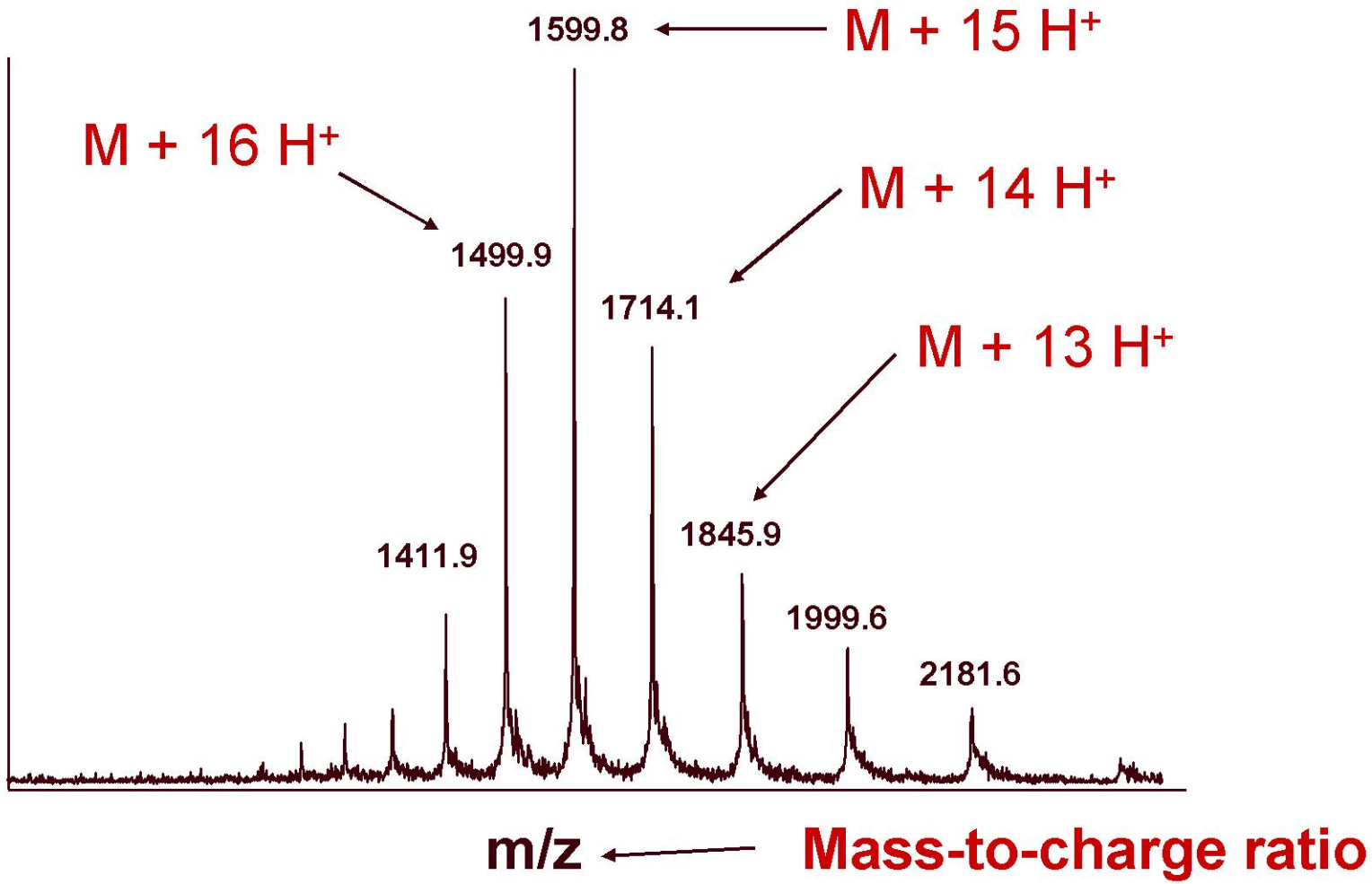


L >> D

Mass spectrum



ESI Spectrum of Trypsinogen (MW 23983)



How do mass spectrometers get their names?

- Types of ion sources:

- Electrospray (ESI)
- Matrix Assisted Laser Desorption Ionization (MALDI)

- Types of mass analyzers:

- Quadrupole (Quad, Q)
- Ion Trap
- Time-of-Flight (TOF)

- Either source type can work with either analyzer type: "MALDI-TOF," "ESI-Quad."

- Analyzers can be combined to create "hybrid" instruments. ESI-QQQ, MALDI QQ TOF, Q Trap

3. Quartz Crystal Microbalance (QCM)

- The quartz crystal microbalance (QCM) is a variant of acoustic wave microsenseors that are capable of ultrasensitive mass measurements. Under favorable conditions, a typical QCM can measure a mass change of 0.1-1 ng/cm^2.
- The QCM oscillates in a mechanically resonant shear mode (determined by the dimensions of the crystal and the mass loading) under the influence of a high frequency AC electric field which is applied across the thickness of the crystal. A change in the mass of the working electrode causes a change in the resonant frequency of the piezoelectric device, which can then be related directly to the quantity of added mass via the Sauerbrey equation:

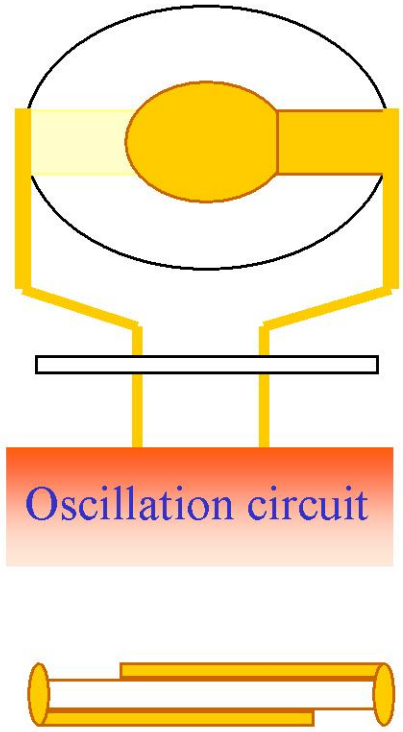
$$\Delta f = -C_f \Delta m \qquad C_f = \frac{2 f_0^2}{\sqrt{\rho \mu}\, A}$$

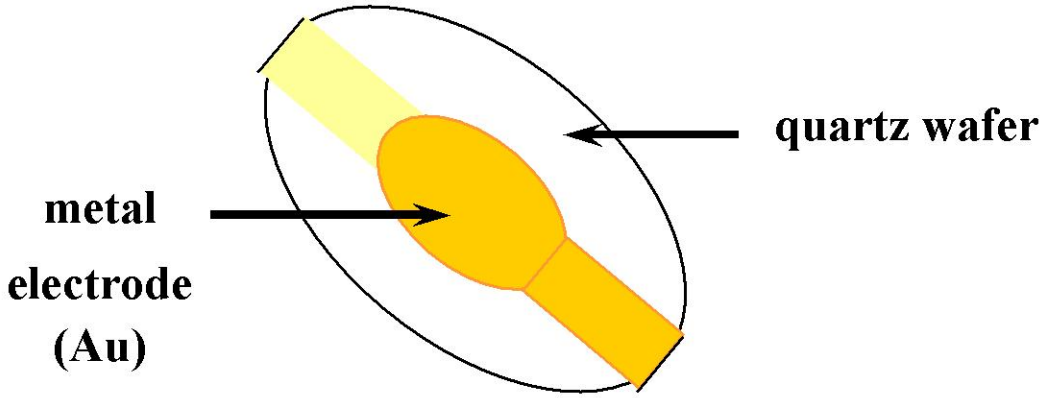
where C_f is a constant which depends on the density ρ of the crystal, μ the shear modulus of quartz , the area A of the gold coated quartz disc , and f_0 the resonant frequency of the fundamental mode of the crystal.

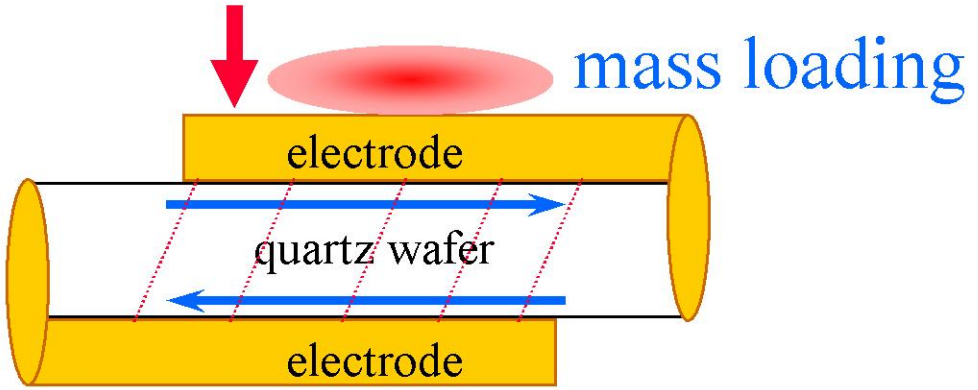
- Hence an increase in mass implies a decrease in frequency and one can use small changes in frequency to monitor very small changes in mass in a very accurate manner.

Quartz Crystal Microbalance (Con't)

piezoelectricity



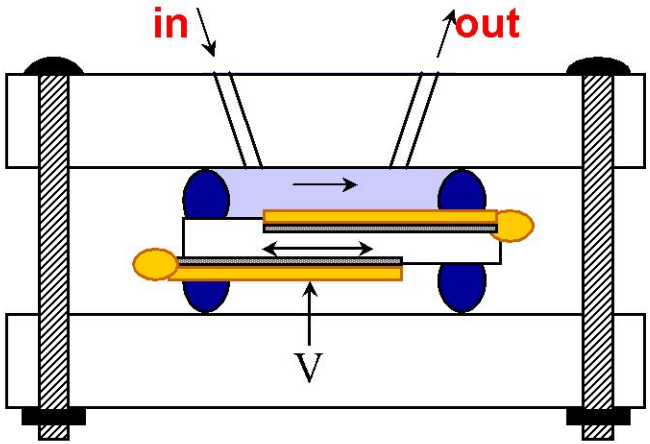


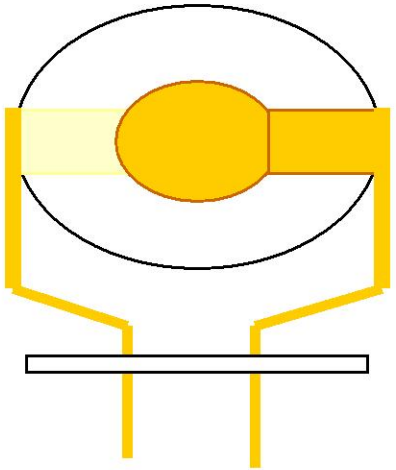


Sauerbrey equation $\Delta f = K^* \Delta m/A$ (in air)

Quartz Crystal Microbalance (Con't)

Flow Injected Analysis system (FIA)





Liquid $\Delta f = C f_0^2 \Delta m + C f_1^{2/3} (\Delta \eta_L \Delta \rho_L)^{1/2}$

$C = -2.26 \times 10^{-6}$ $cm^2/Hz\text{-}g$

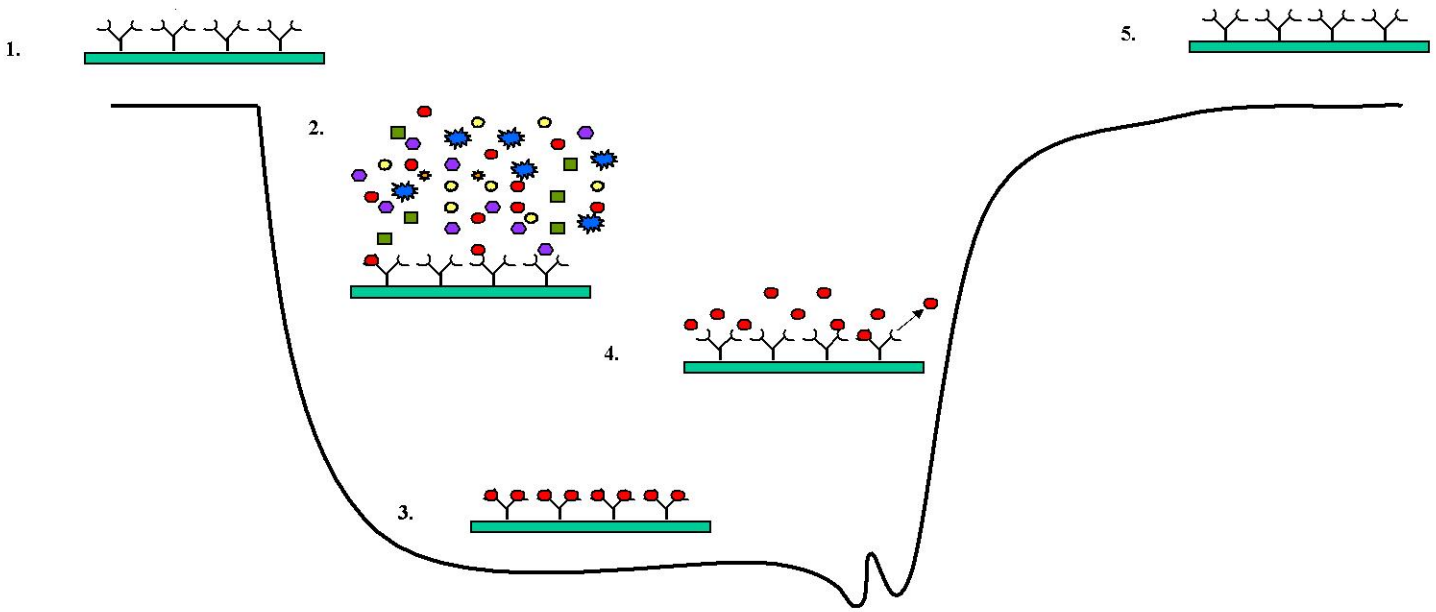
η_L: viscosity of liquid, ρ_L: density of liquid

f_0: resonant frequency of the fundamental mode in air

f_1: resonant frequency of the fundamental mode in liquid

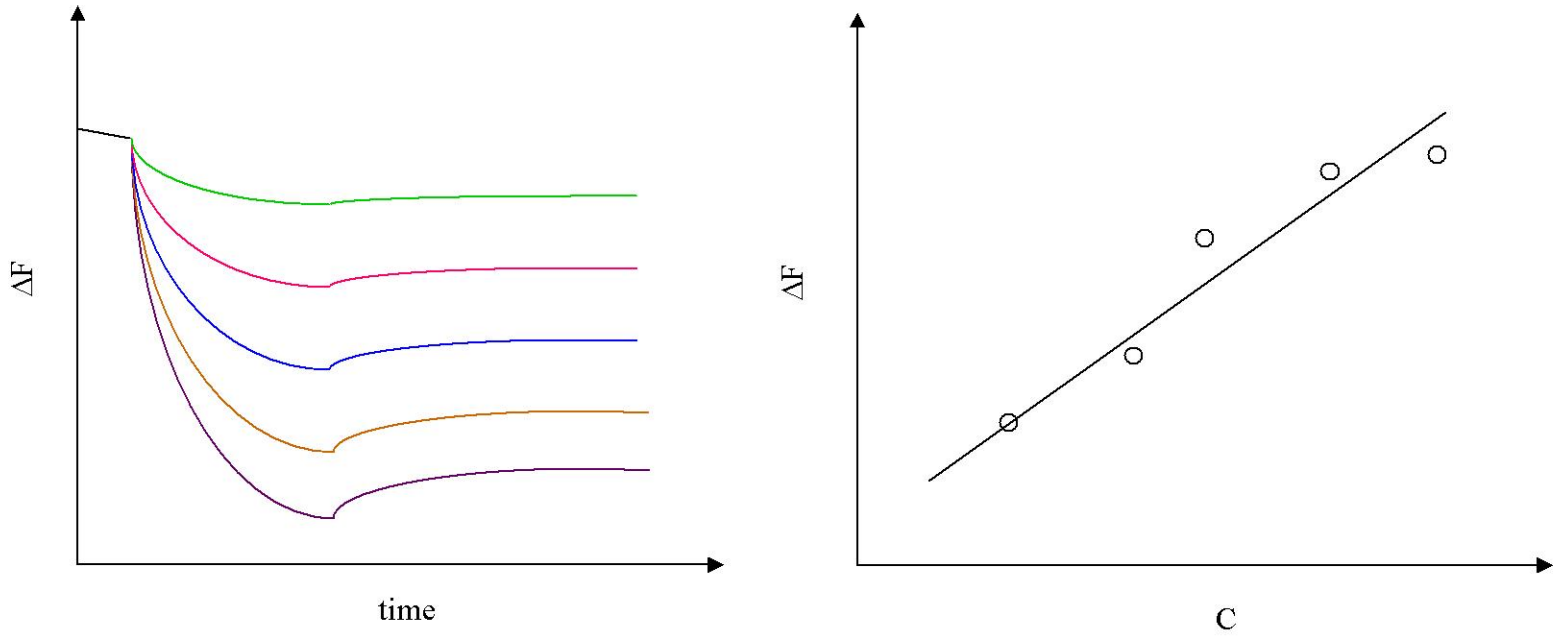
Typical reaction curve of chip

1. Immobilization of Ab on chip surfaces
2. Injection of analyte
3. Binding of Ab and Ag
4. Regeneration of the Ab on chip
5. Reuse of the Ab on chip



Concentration Measurement

$$\Delta f = mC + a$$



4. Surface Plasmon Resonance (SPR)

- **SPR technology enables real-time detection and monitoring of biomolecular events and provides quantitative information on:**
  1. Specificity – how specific is the binding between two molecules?
  2. Concentration – how much of a given molecule is present and active?
  3. Kinetics – what is the rate of association/dissociation?
  4. Affinity – how strong is the binding?
  5. Binding partners - provide identification of binding targets by linking SPR to molecular species

What is SPR?

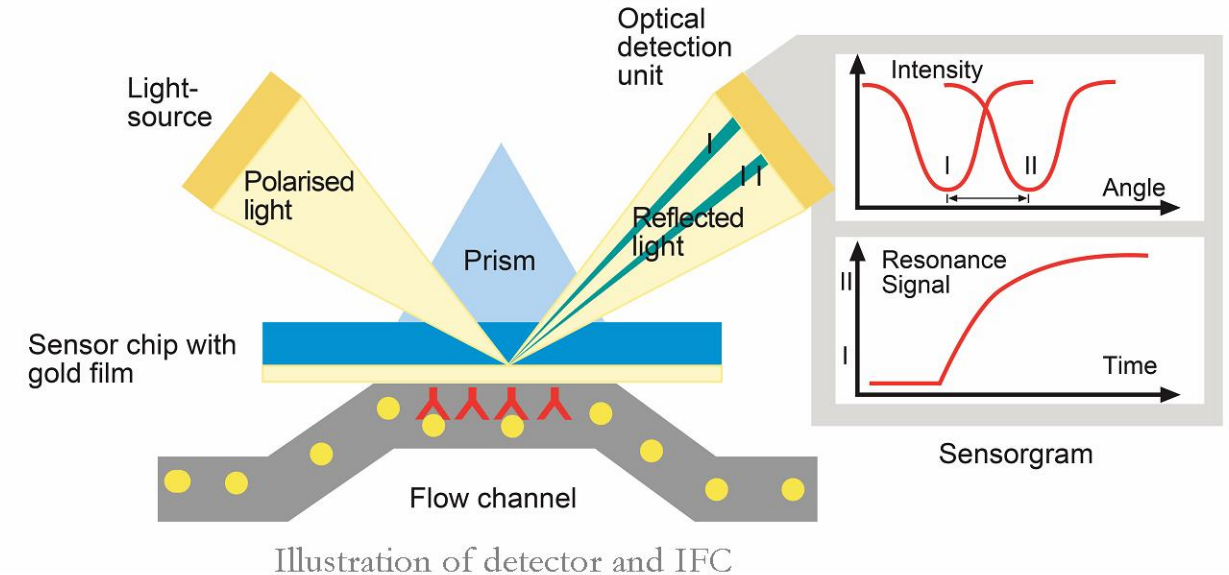


Illustration of detector and IFC

- SPR arises when light is reflected under certain conditions from a conducting film at the interface between 2 media of different refractive index.
- The media are sample and glass of the sensor chip, and the conducting film is a thin layer of gold on the chip surface.
- SPR causes a reduction in the intensity of reflected light at a specific angle of reflection. This angle varies with the refractive index close to the surface on the side opposite from the reflected light (sample side).
- SPR response values are expressed in resonance units (RU). One RU = 0.0001°. For most proteins this is about a change in conc. of 1pg/mm^2 on the sensor surface.

The sensorgram provides quantitative information in real-time on specificity of binding, active concentration of molecule in a sample, kinetics and affinity.