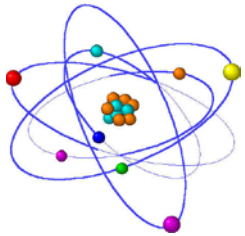
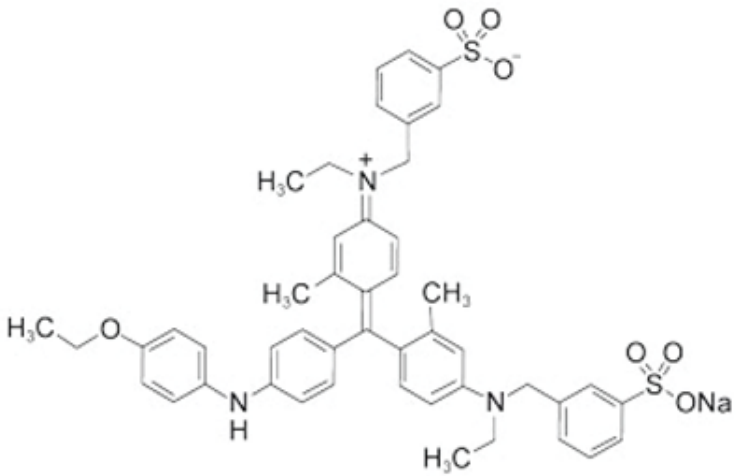
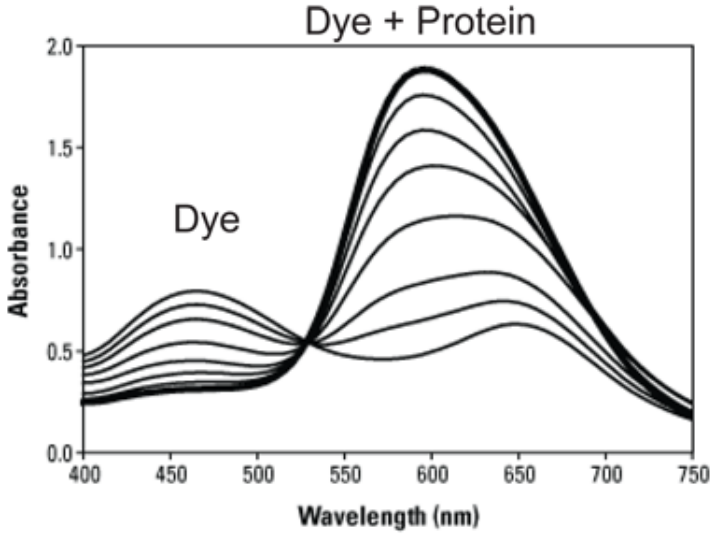
Biochemistry Lab



Protein Purity Assessment and Identification

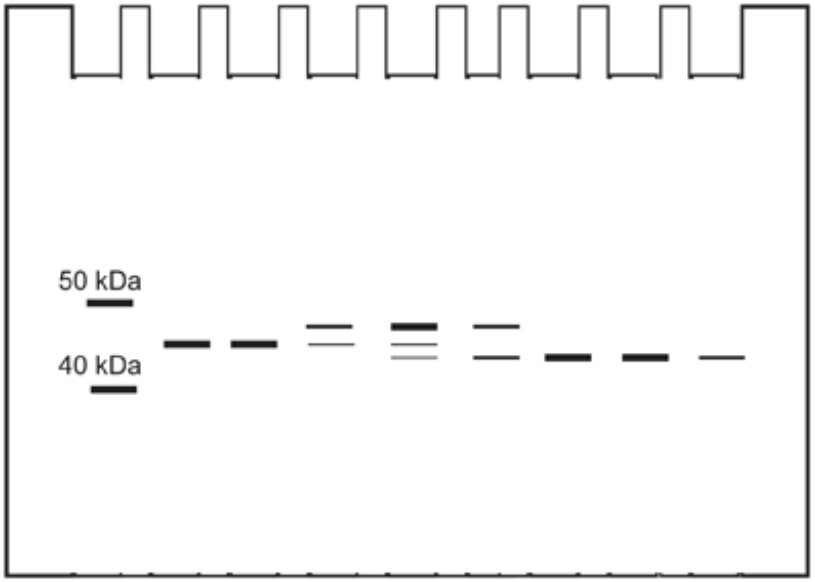
Coomassie Staining





How does Coomassie interact with proteins?

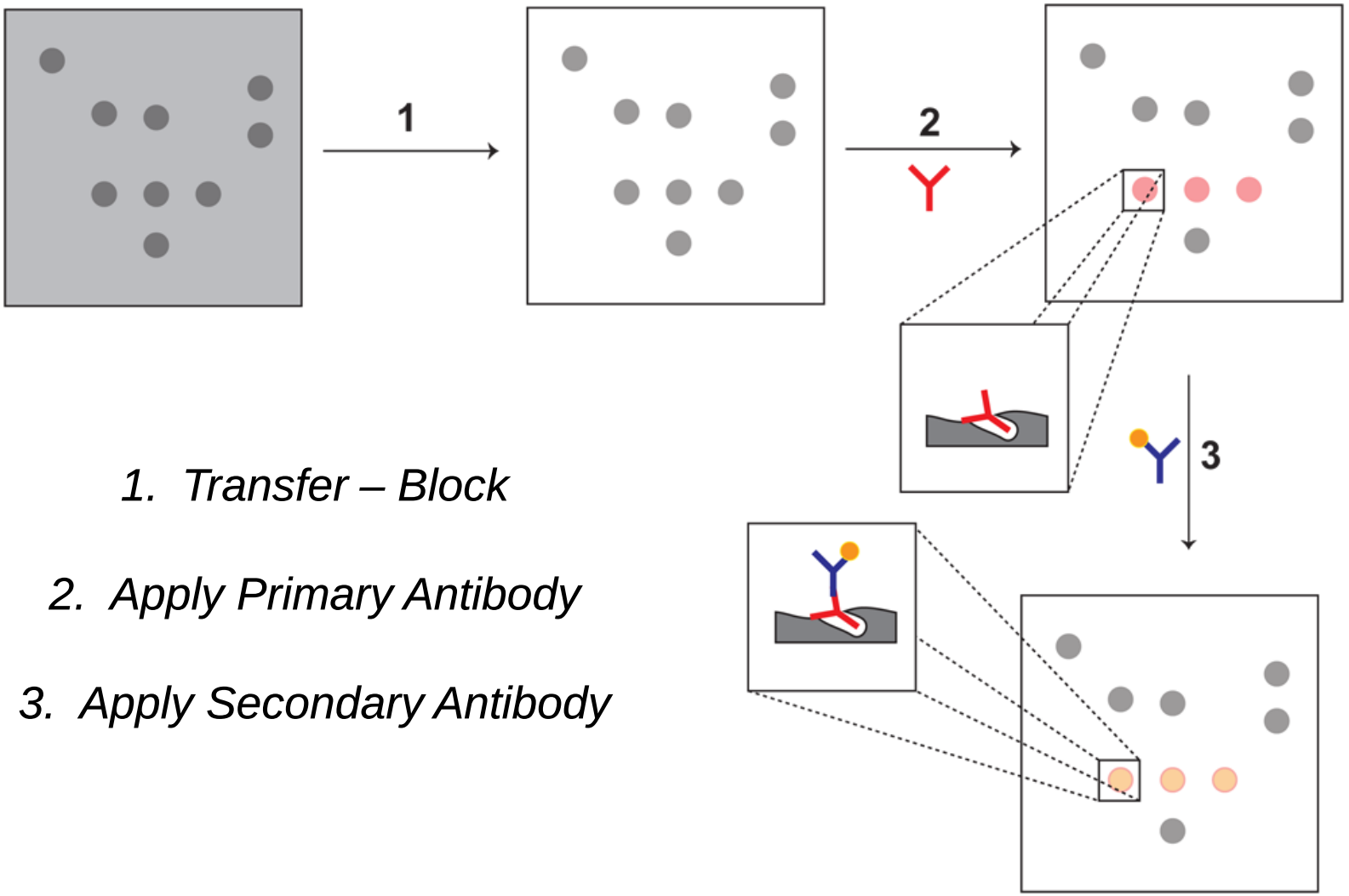
Coomassie Staining



Benefits

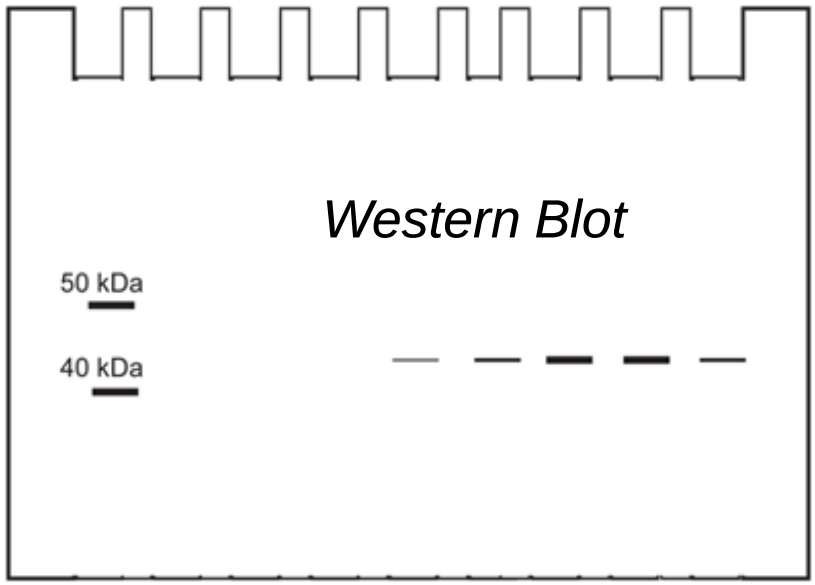
Limitations

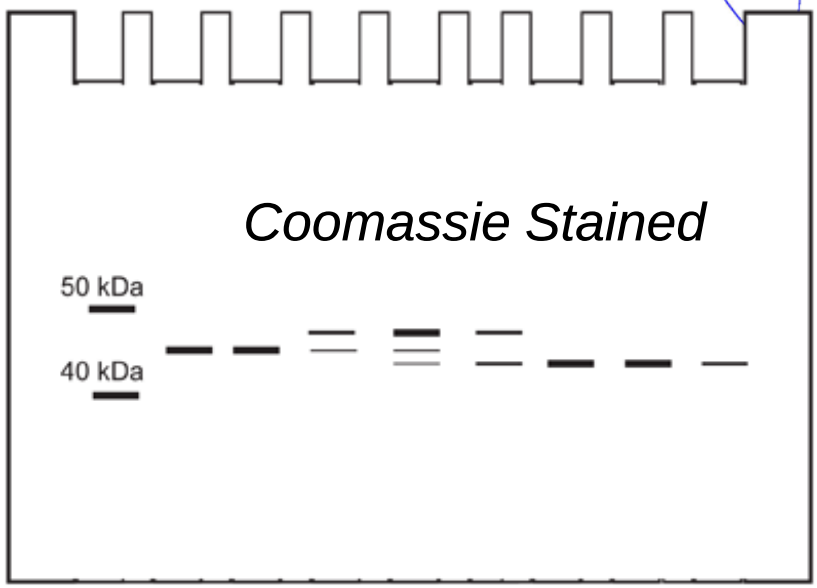
Western Blotting



1. *Transfer – Block*
2. *Apply Primary Antibody*
3. *Apply Secondary Antibody*

Western Blots

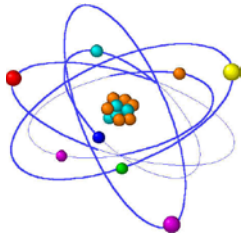




Benefits

Limitations

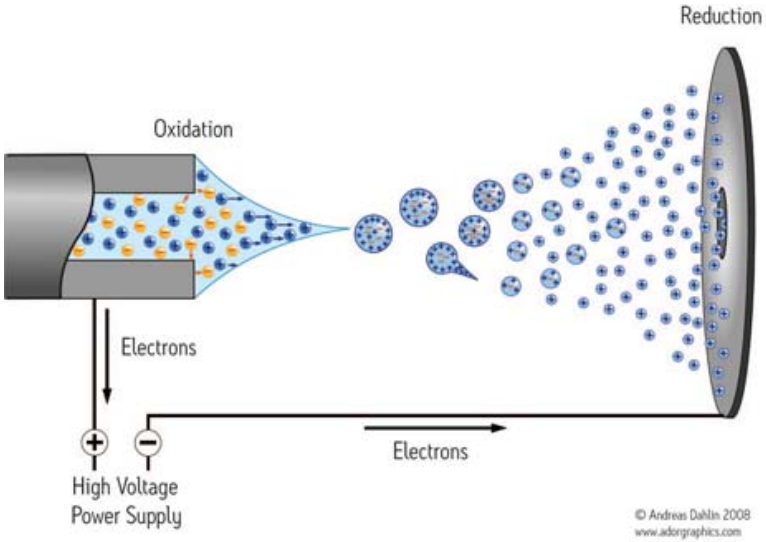
Mass Spectrometry



Ionization	→	Separation	→	Detection
Chemical Ionization		Time of Flight		Electron Multiplier
Electron Ionization		Quadrupole		
Inductively Coupled Plasma		Ion Trap		
Glow Discharge				
Fast Atom Bombardment				
Electrospray				
MALDI				

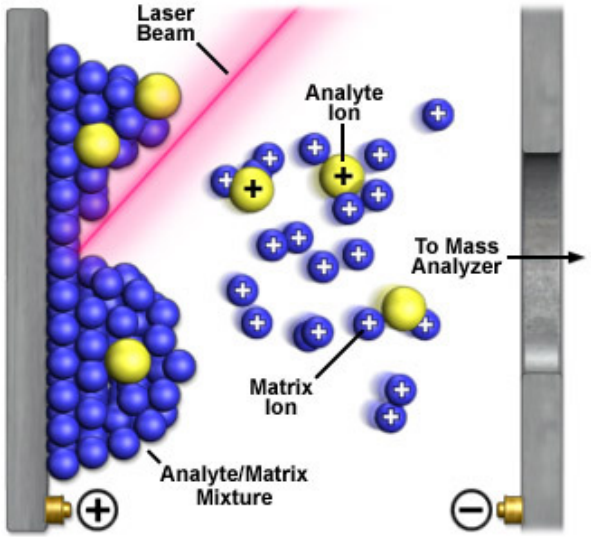
Soft Ionization Techniques

Electrospray Ionization (ESI)



- Aqueous sample introduced to metal capillary
- High voltage (2000-4000 V) applied
- Released to vacuum
- Desolvation of aerosol leaving highly charged ions

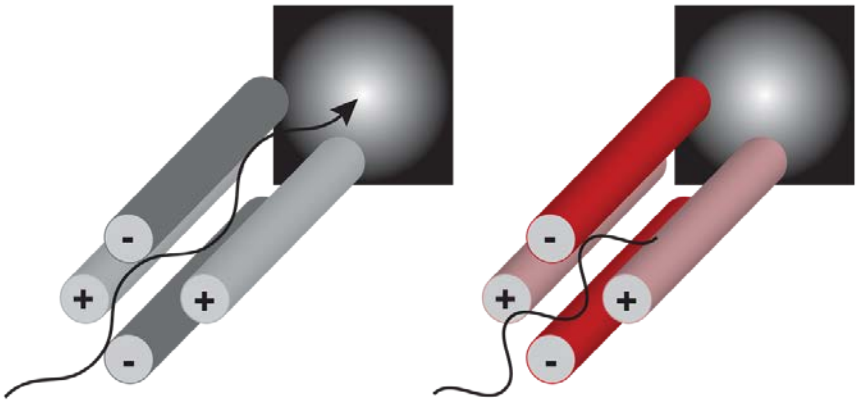
Matrix Assisted Laser Desorption Ionization (MALDI)



- Aqueous sample is cocrystallized on a metal surface with a Matrix
- Intense Laser beam is directed toward sample/matrix mixture - desorption
- Matrix absorbs the energy and is ionized
- Some of the charge is transferred to the analyte

Separation Techniques

Quadrupole

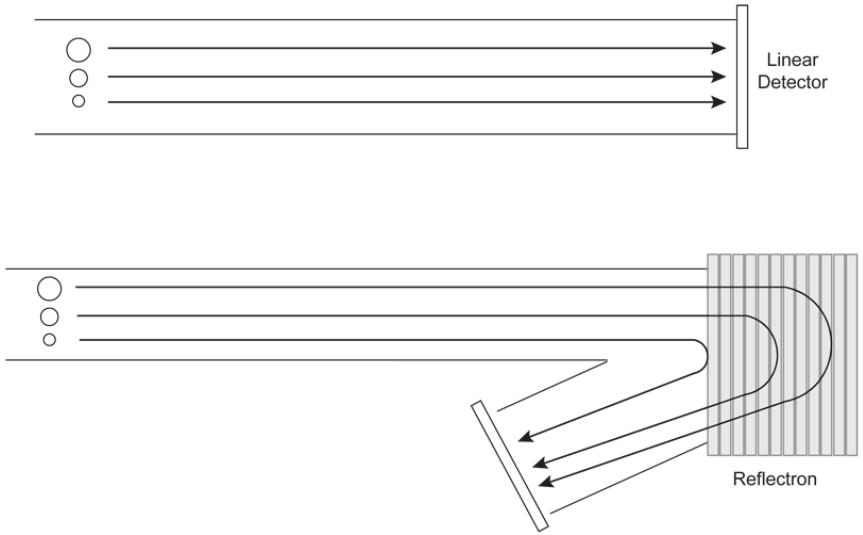


•Four rods are arranged opposite each other and connected electronically

•Voltage applied to each rod is carefully regulated

•The trajectory of a charged particle is influenced by the electric field

Flight Tube

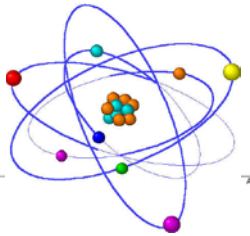


•Molecules separate by the time it takes for them to travel from the ion source to the detector

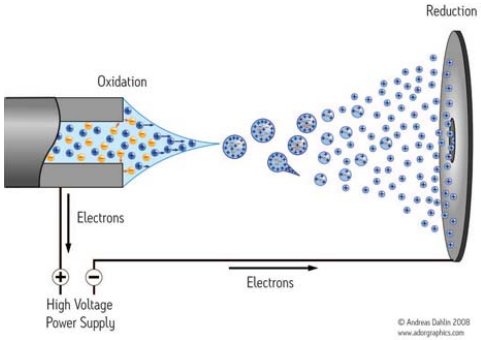
•Resolution is dependent on tube length (limits resolving power)

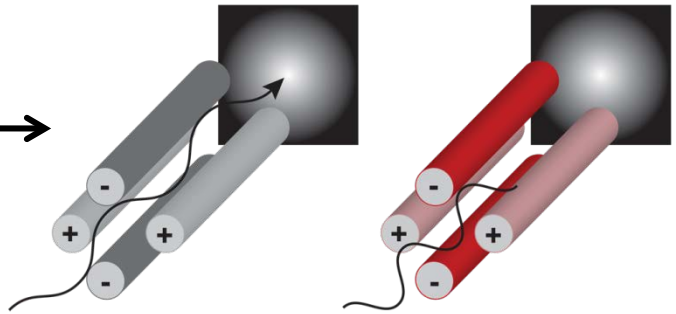
•Reflectron enhances the resolution

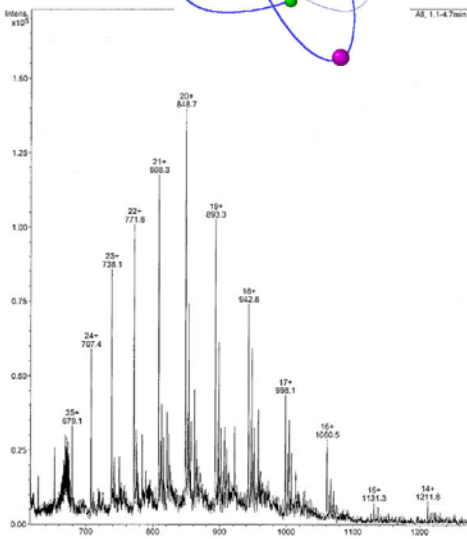
Ideal Pairs



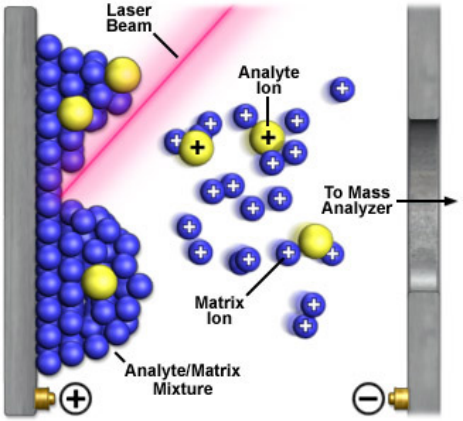
ESI-QMS

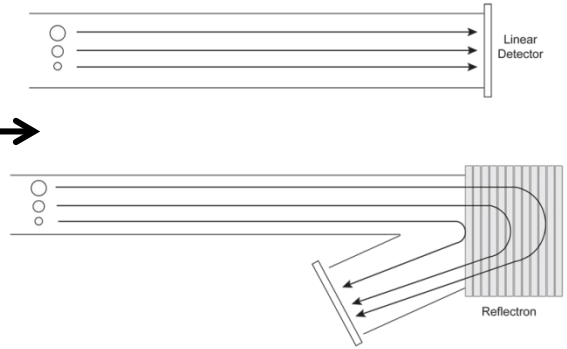


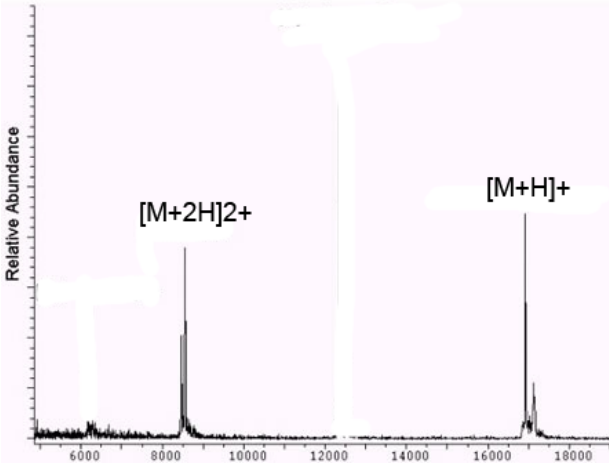




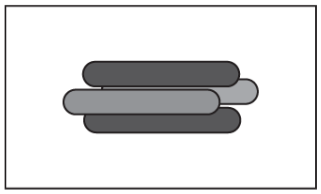
MALDI-TOF MS



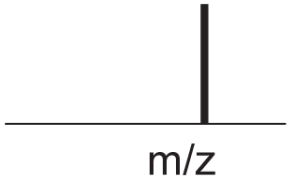


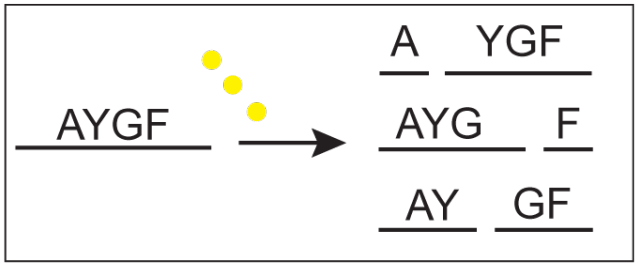


Other Applications of MS

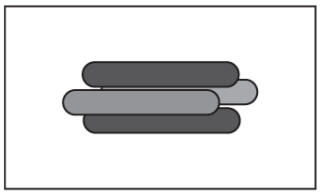


Chamber 1



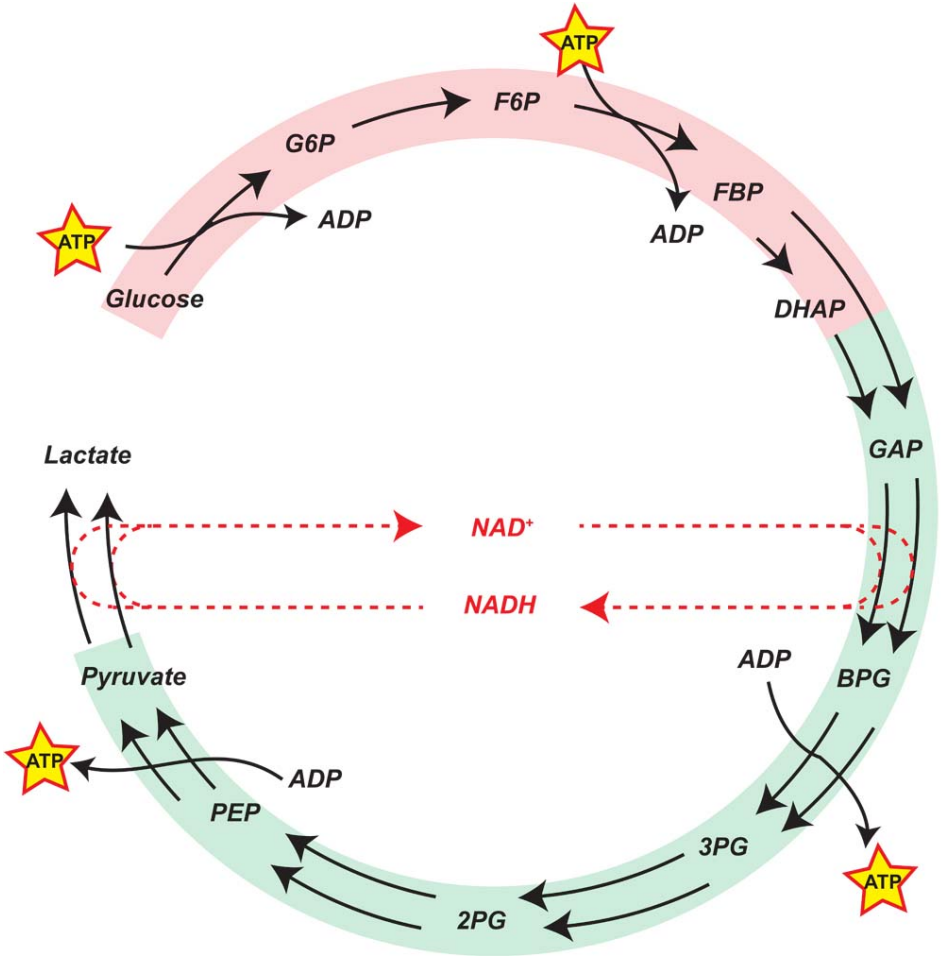


Chamber 2

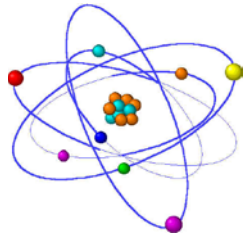


Chamber 3

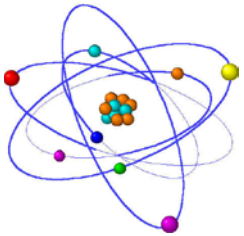
Lactate Dehydrogenase



Lactate Dehydrogenase



In lab this week



1. Determine protein concentration two ways:
   - Bradford Assay (coomassie staining)
   - Abs_{280}
2. Quick Enzyme Activity Assay
   - Prepare 3 dilutions of your enzyme:
     - 1:2, 1:3, 1:4
   - In 4 separate tubes, mix together:
     - 900 μL Assay Buffer (this is your lysis buffer)
     - 33 μL NADH
     - 33 μL Pyruvate
     - 33 μL Enzyme (undiluted, 1:2, 1:3, or 1:4)
   - Monitor Abs_{340} over 2 minutes
   - Pick the dilution that gives you a D Abs_{340} ~ 0.5 over 1 minute – you'll use this dilution in subsequent weeks