



It's Electrifying!-Danny Zucco (Grease)

# Electrophoresis

# Electrophoresis Factors

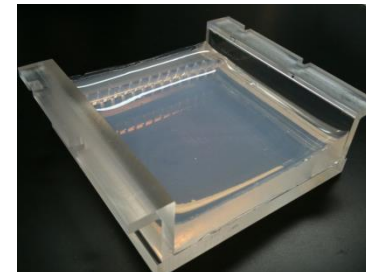
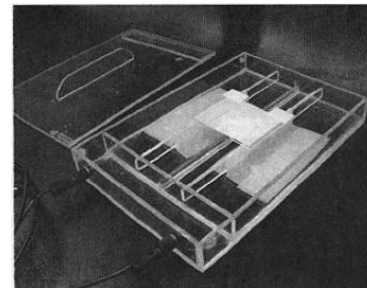
- pH of buffer
  - 8.6
  - Negative charged proteins
- **Anode attracts anions!** + Charged electrode
- Cathode attracts cations – Charged electrode

# Electrophoresis Factors

- Ionic Strength of Buffer
  - High Concentration = **S**low but **S**harp
  - Low concentration = **F**ast but **F**uzzy

# Electrophoresis Factors

- Support Medium
  - Paper
  - Cellulose Acetate
    - Flammable
  - Agarose gel
    - Most common
  - Capillary Electrophoresis?
    - Up and coming



# Electrophoresis Factors

- Voltage
  - Provides the pulling force on proteins
  - High voltage = harder pulling
  - Also generates heat, which must be cooled
    - Protein will denature at high temperatures, designed to work at 37°C



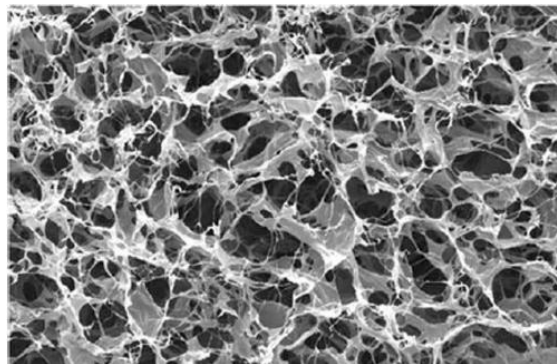
# Electrophoresis Factors

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- Time
  - Longer time running will give greater separation
    - Heat also builds up over time

# Electrophoresis Factors

- Size and Shape of Proteins
  - Agarose holds large proteins back
    - This slows the protein and may increase separation OR put it closer to another band of proteins



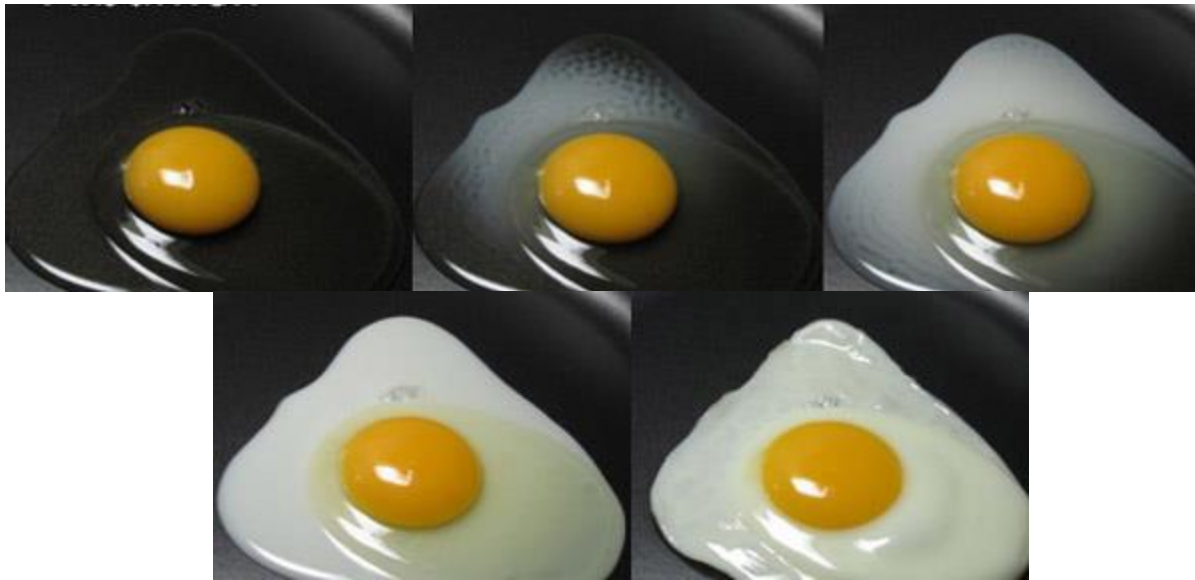
# Electrophoresis Factors

- Diffusion
  - Some diffusion is inevitable
    - Leads to lack of resolution, definition
  - Causes:
    - Increased thickness of medium
    - Increased time of separation
    - Increased temperatures



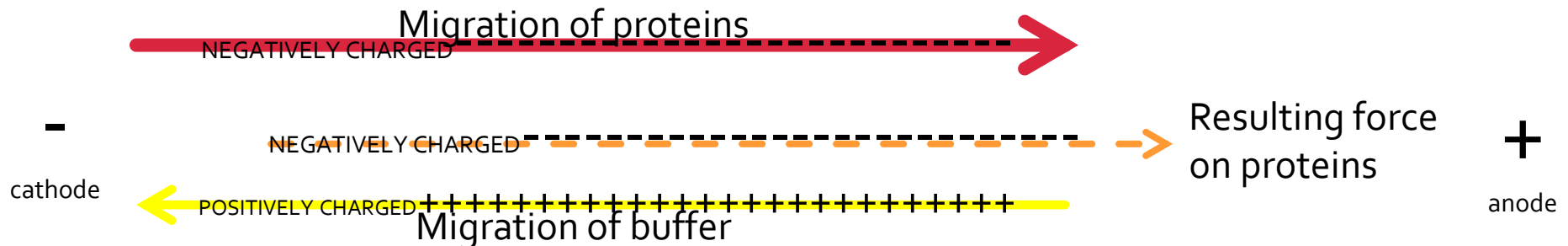
# Electrophoresis Factors

- Temperature
  - Mobility of proteins is increased with higher temperatures
  - At some point proteins will denature



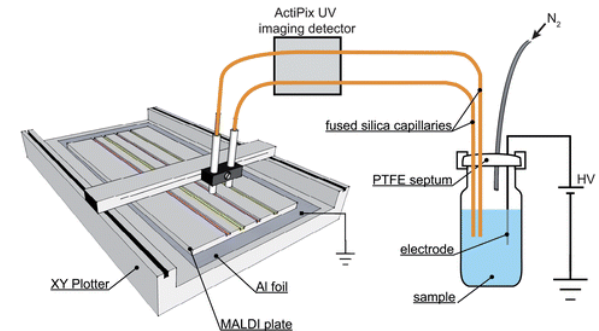
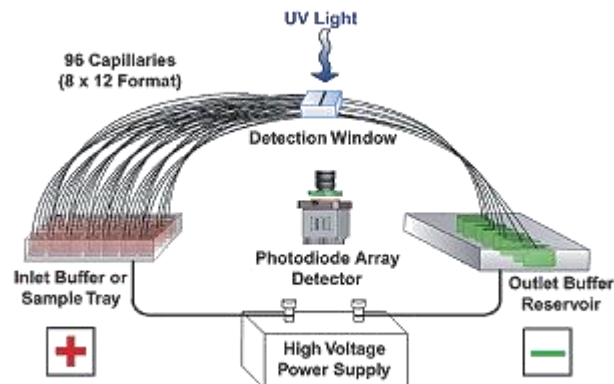
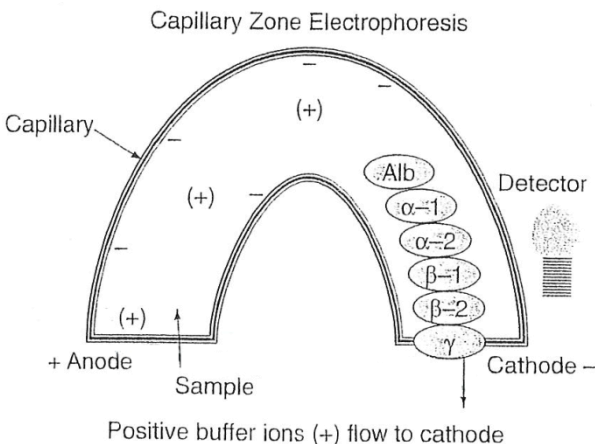
# Electrophoresis Factors

- Electroendosmosis
  - Force-Counterforce
  - Especially in poor quality agars buffer will travel towards the cathode

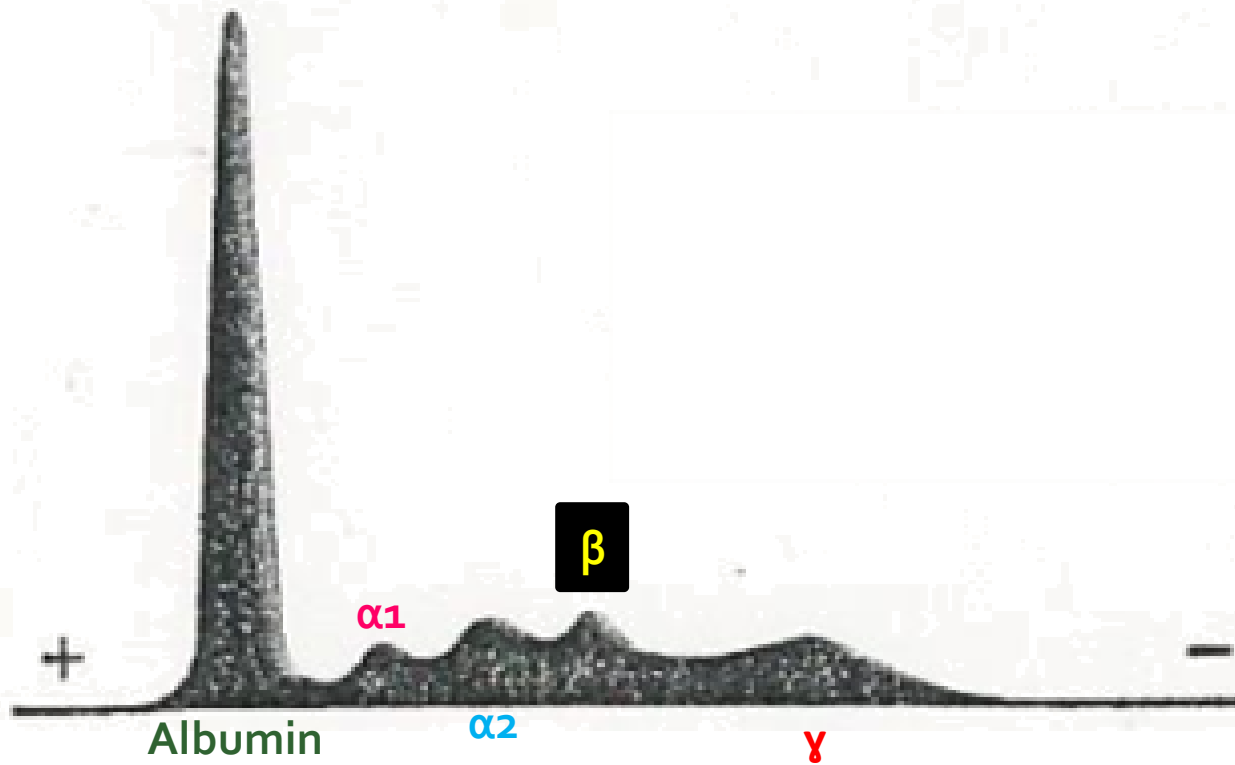


# Capillary Electrophoresis

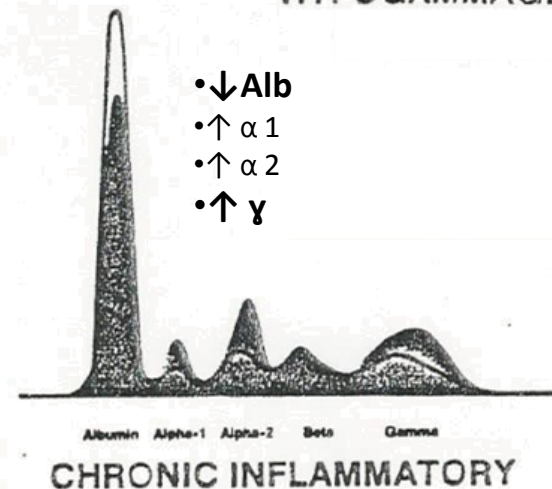
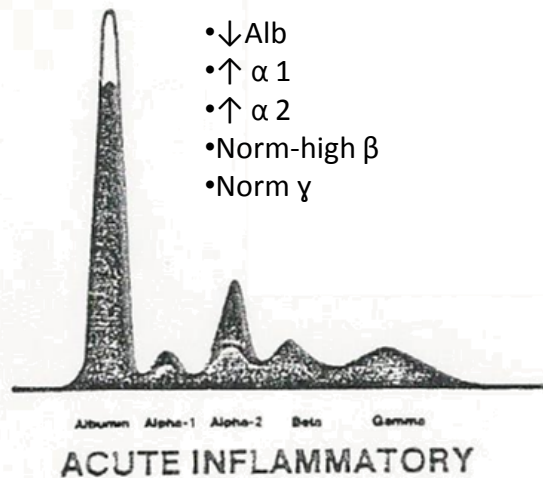
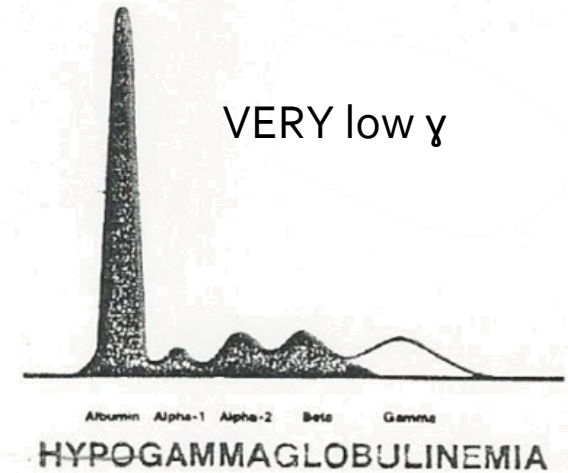
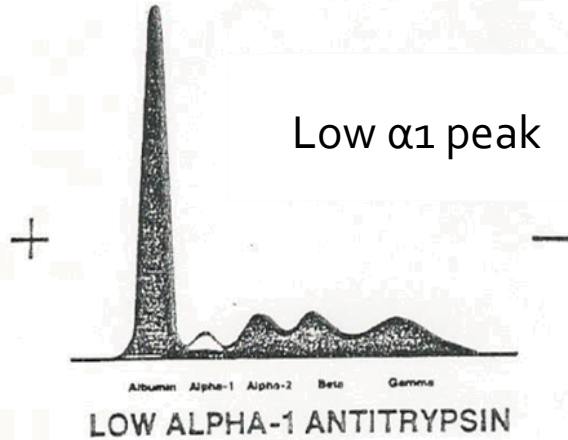
- Force entirely generated by electro-osmotic flow
  - Result is movement towards cathode
  - Detection by UV, fluorescence, laser, chemiluminescence, mass spec



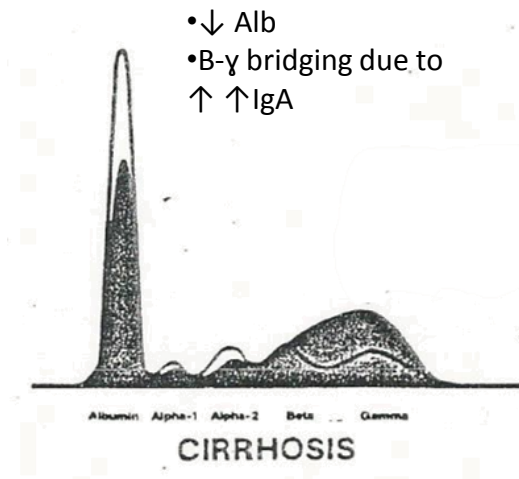
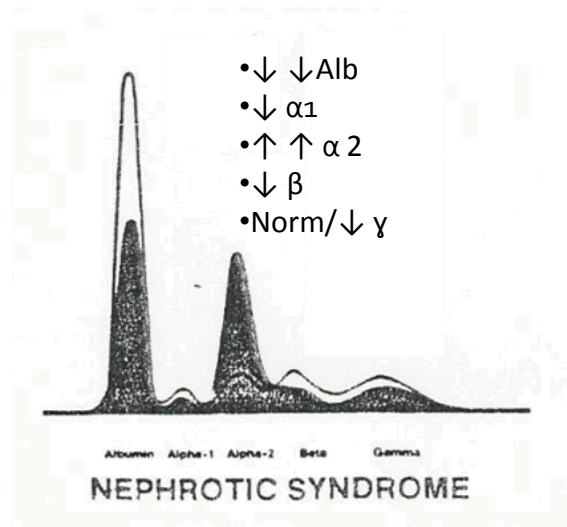
# Electrophoresis Pattern



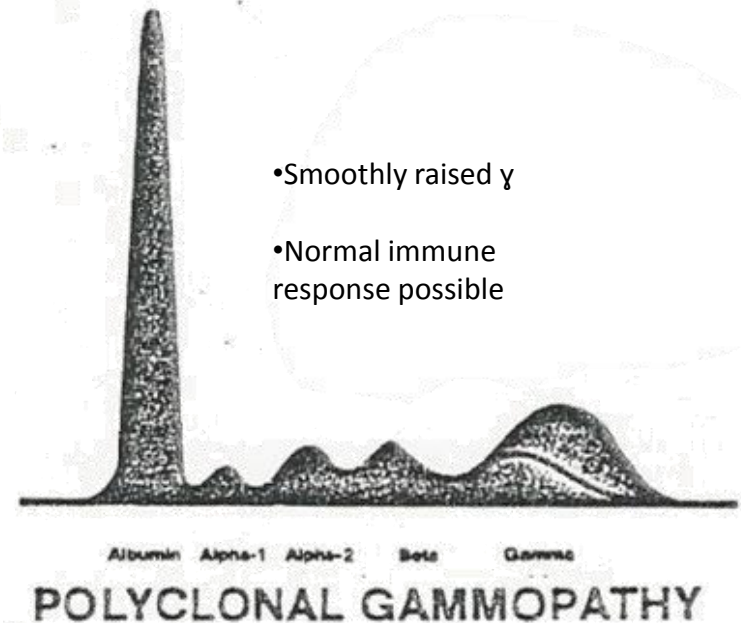
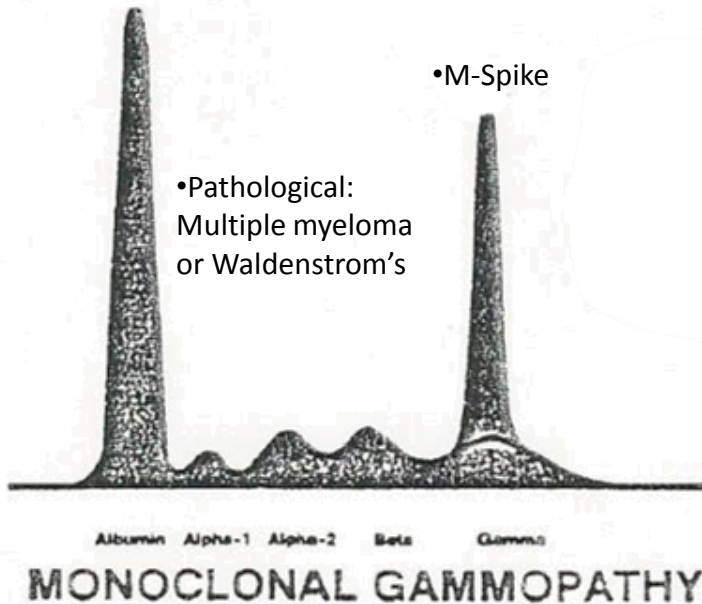
# Pathologies



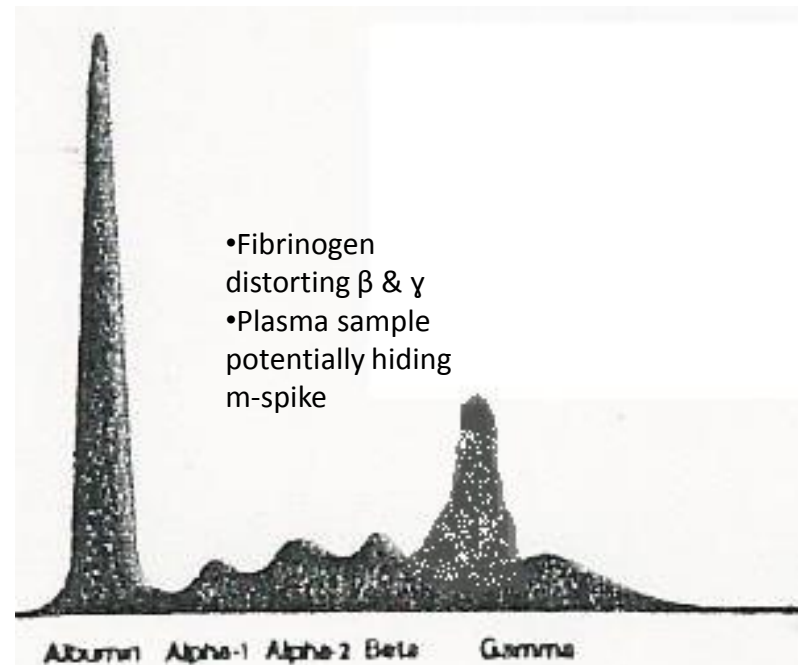
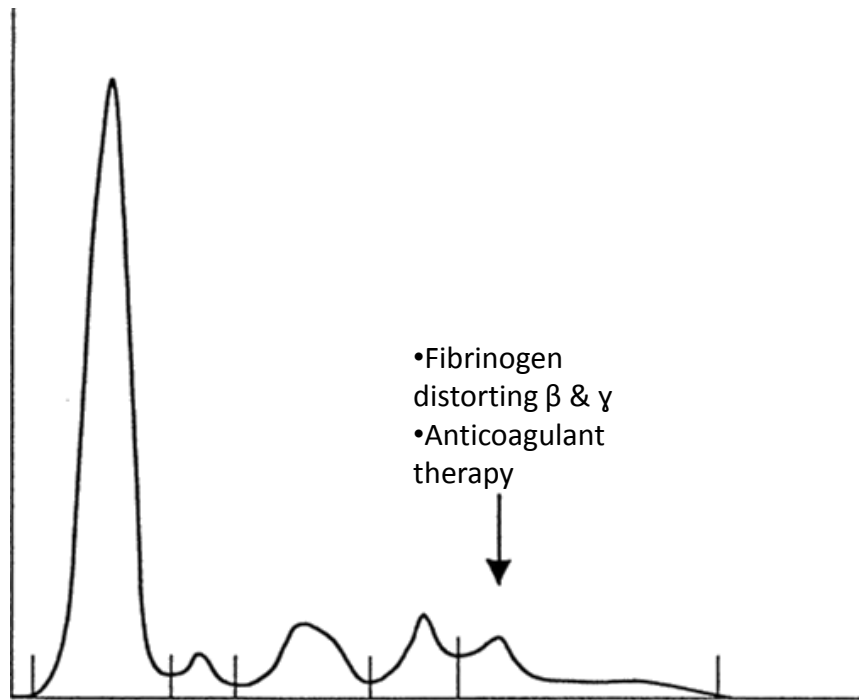
# Pathologies



# Pathologies



# Mistakes Happen





# Now Let's Try It

