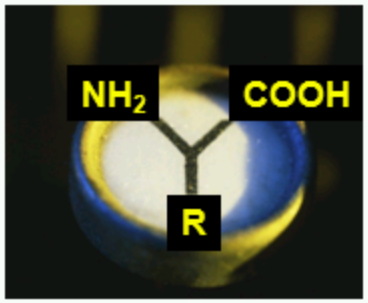
To butylate or Not to butylate – Should that even be a question?



The Historical and Present Day Basis for Derivatization in the MS/MS Analysis of DBS in Newborn Screening

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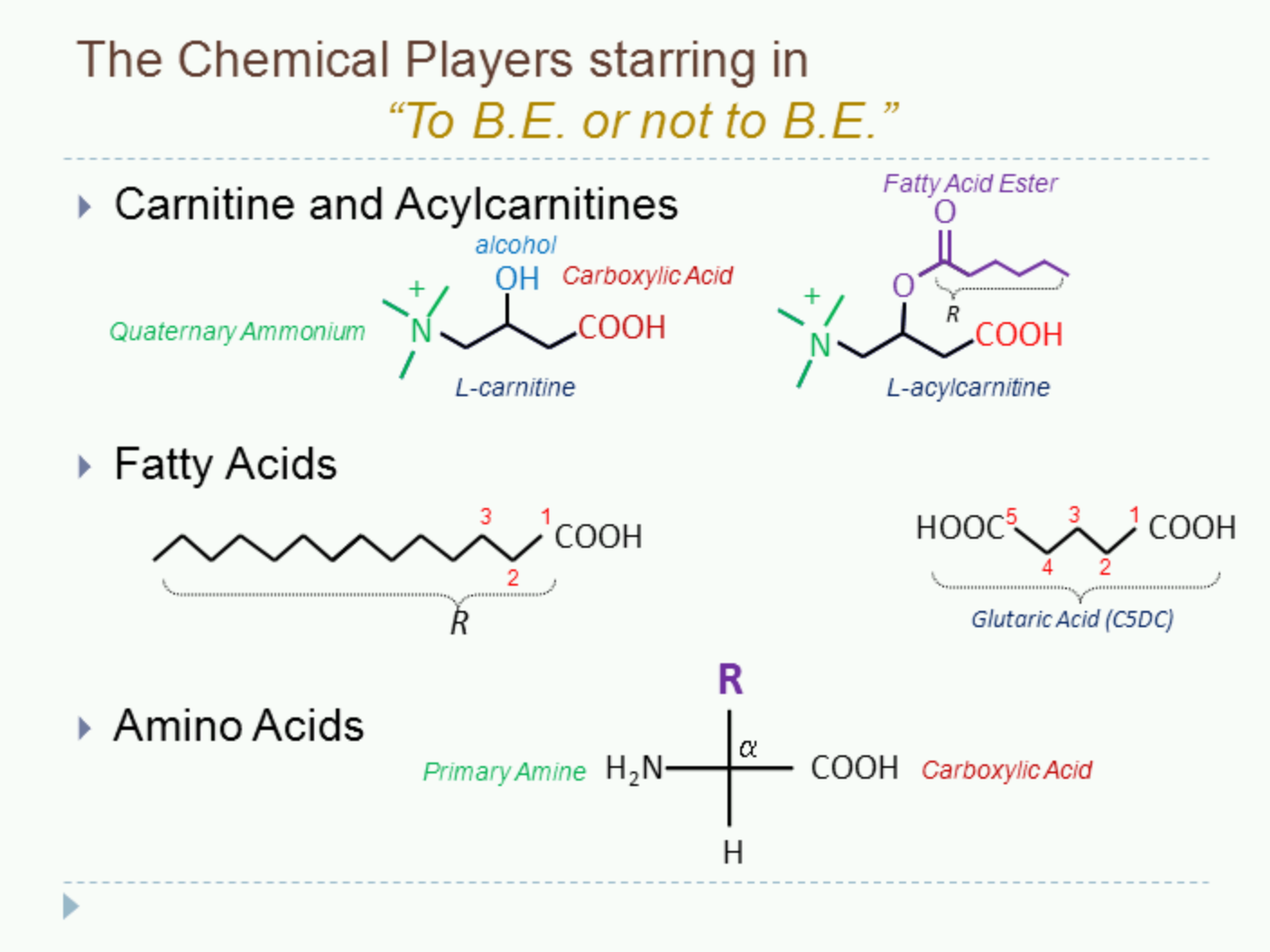


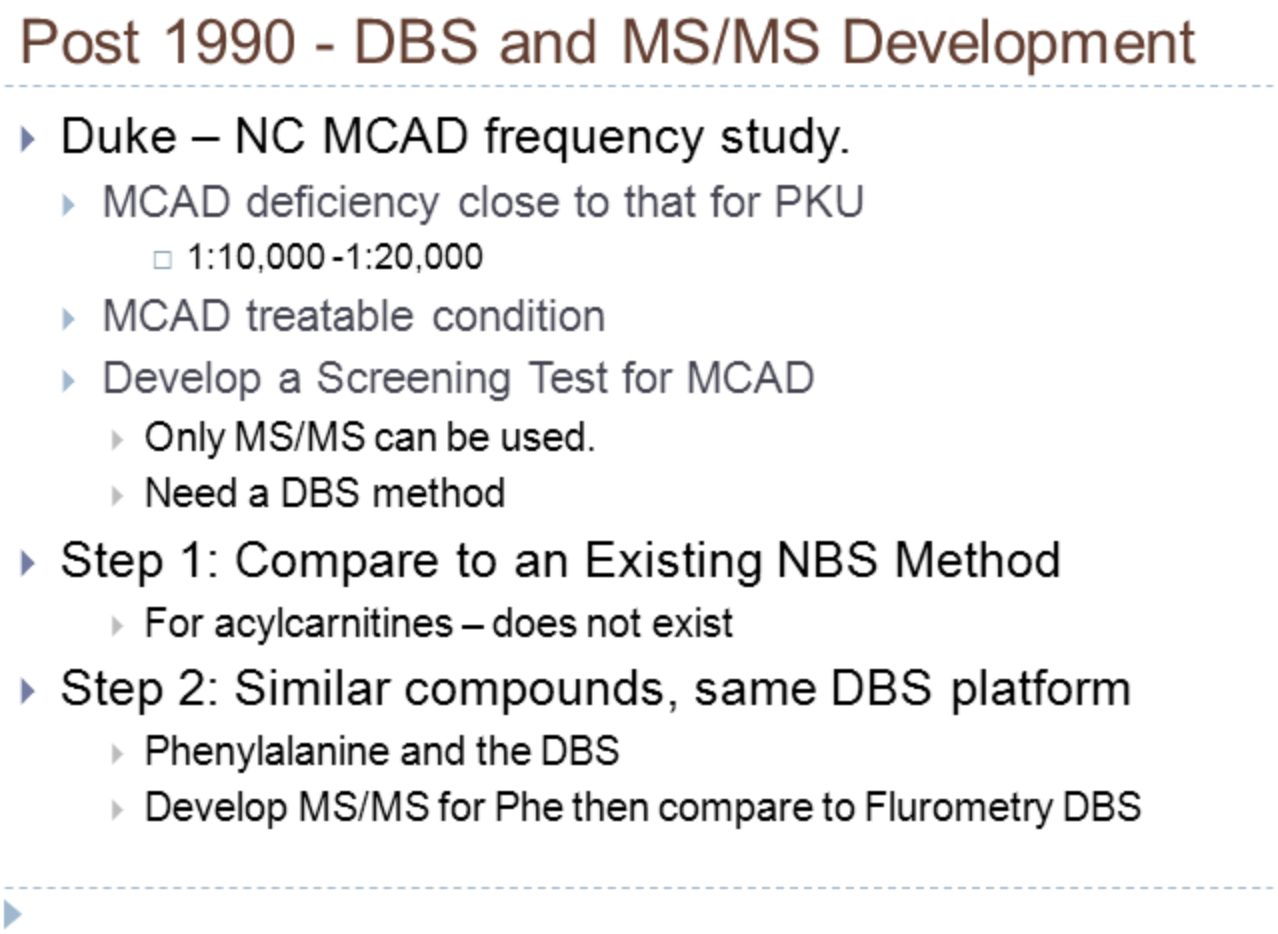
The Chemical Players starring in "To B.E. or not to B.E."

Pre–DBS History of MS/MS and NBS

- Carnitine and Acylcarnitines Analysis before 1990
  - GC/MS (~1985)
    - Pre-formed cation of carnitine made it involatile. Required removal of "quat", extensive prep.
  - HPLC only (no MS)
    - Free Carnitine and short chain acylcarnitines
  - Immunoassays and Radio-immunoassays
    - Free Carnitine measured before and after hydrolysis with a strong base (NaOH)
      - FC: Free Carnitine (FC before hydrolysis)
      - TC: Total Carnitine (FC after hydrolysis)
      - AC: Total Acylcarnitine (TC – FC)
- *LC-MS of Carnitine and Acylcarnitines (>1985)*
  - *Fast Atom Bombardment Ionization (FAB)*
    - *Manual Analysis, Drop of glycerol on probe*
  - *Precursors of 99 (Methyl Esters) -*
    - *Derivatization using MeOH + 6 N HCl*

ACME = Acyl Carnitine Methyl Esters

Pre–DBS History of MS/MS and NBS



Post 1990 - DBS and MS/MS Development

Chance Favors the Prepared Mind… *or the lucky!*

- **Use existing MS/MS method for acylcarnitines**
  - FAB MS/MS and Pre 99 scans
  - Methyl Ester analysis for AA did not work (Interference for Phe)
  - Alternative Derivative: Butyl Ester
    - interference disappeared
- **Validation Phase**
  - In development of analysis of Phe – discovered many other compounds – amino acids in a full scan profile.
- **Eureka Moment**
  - …Leu, Tyr and Met – Oh My!
- **AC's adapted from ME to BE for NBS**
  - FIB and improved sensitivity
    - Better long chain AC sensitivity
  - Better separation of C5DC from C8
  - Clinical Method = ME, NBS = BE

Chance Favors the Prepared Mind… or the lucky!

Basis for Derivatization – Improved Ionization

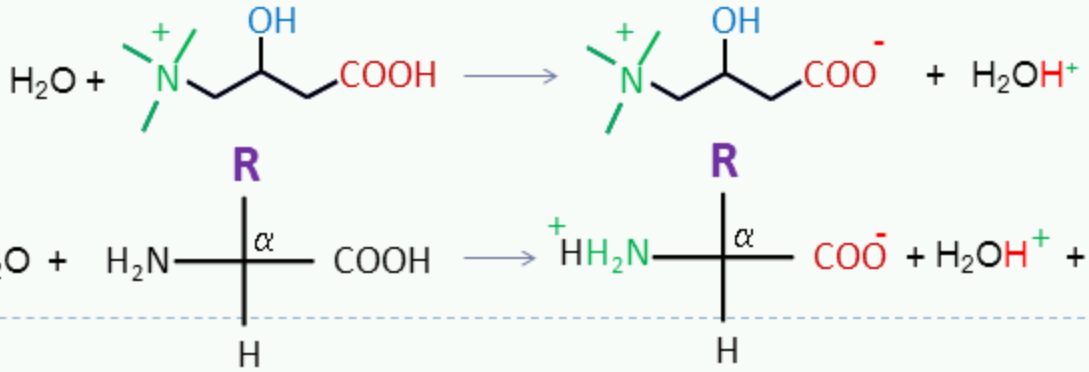
- What is **ionization**?
  - The process in which a molecule becomes an ion.
  - Ion = charged molecule.
  - Two charges: Positive (+) and Negative (-)
  - Neutral equals no net charge.

- Mass Spectrometers require Ions
  - Ions give molecules **wings** and fly through a vacuum.
  - Mass spec works by repelling, attracting with the same and opposite charges to measure mass.

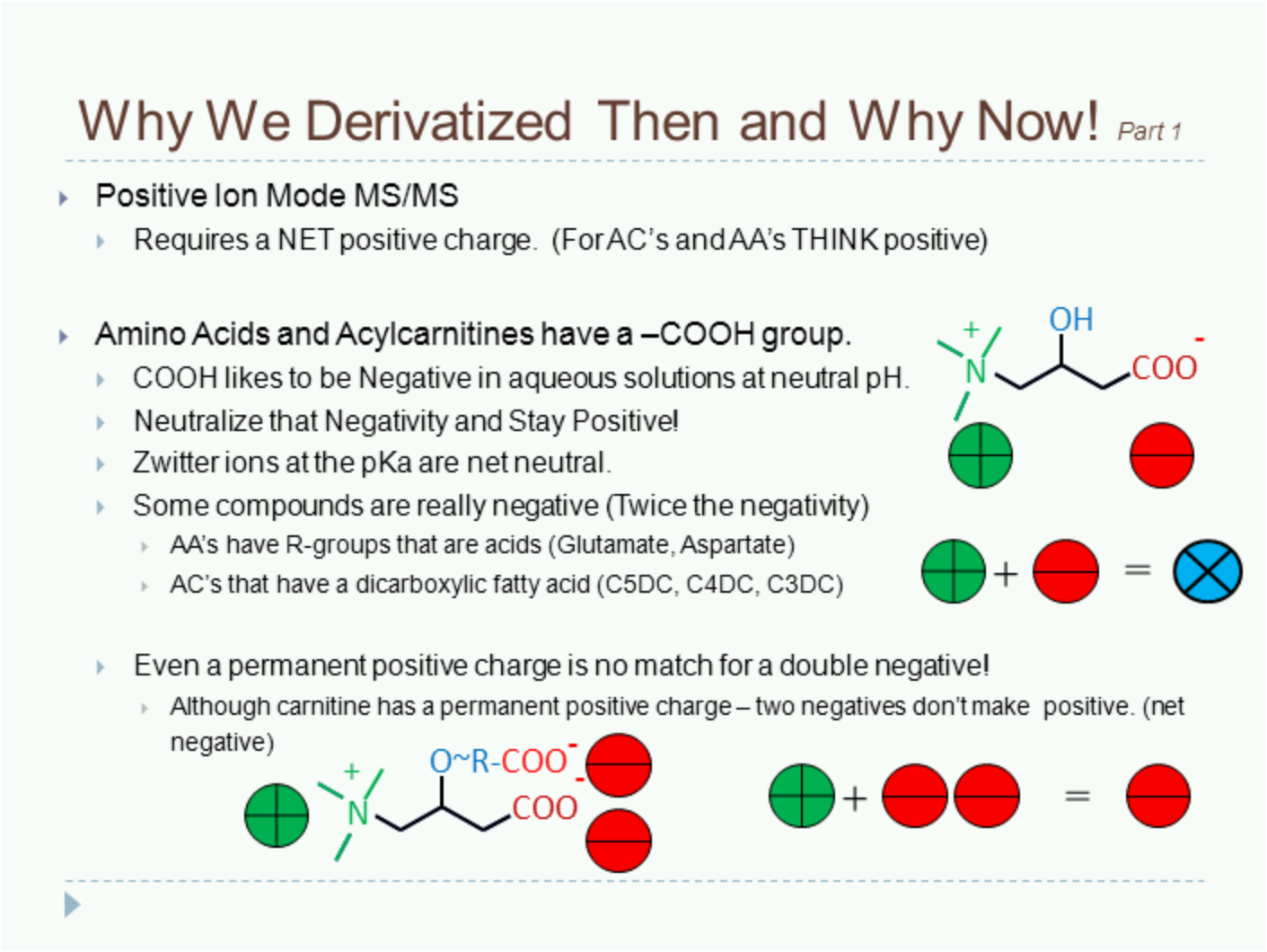
Basis for Derivatization – Improved Ionization

Ionization Efficiency

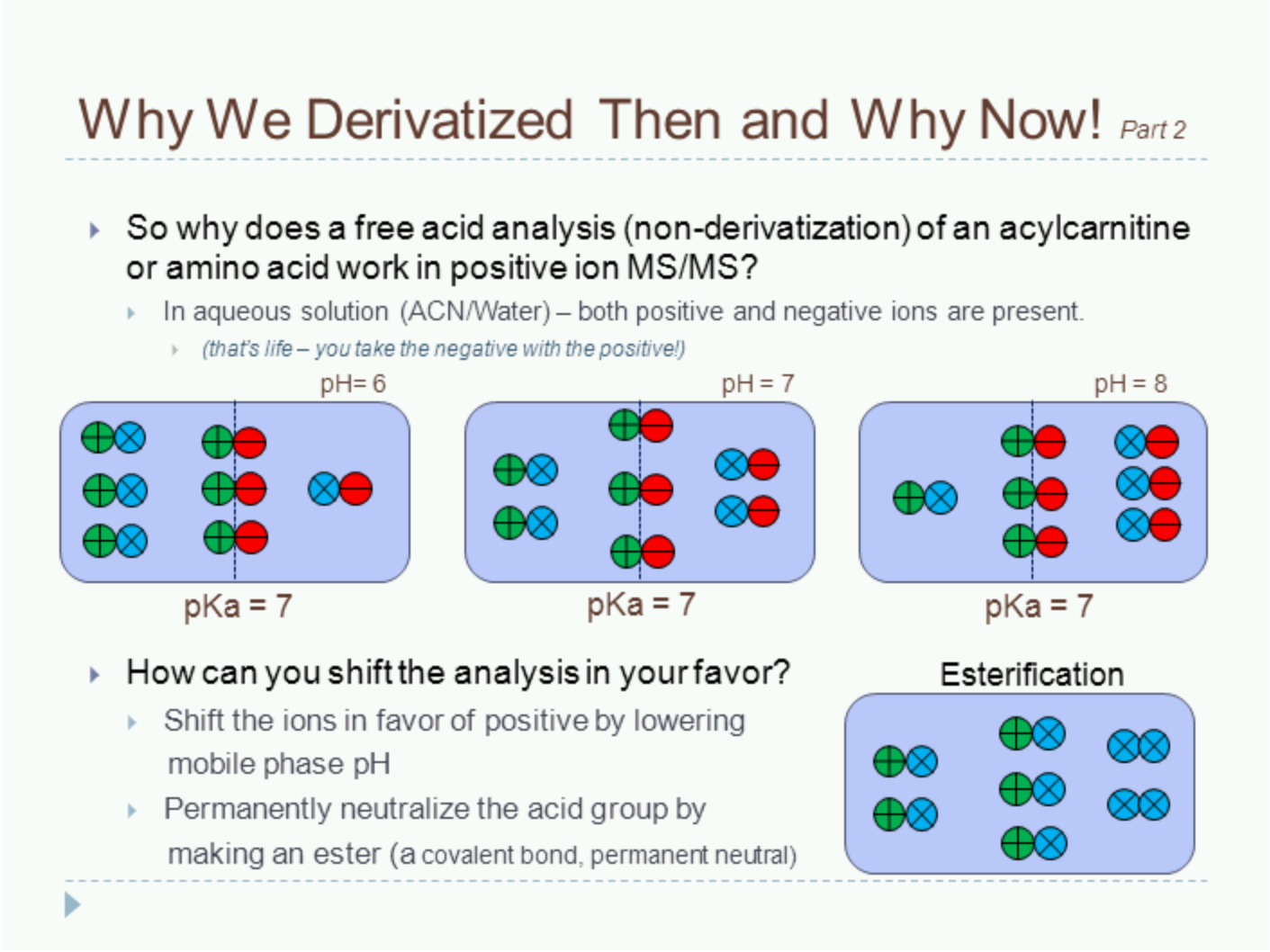
- The relative amount of + or - charge of a specific type relative to the total quantity of molecules.
  - Detection efficiency = total number of charge molecules detected = analytical sensitivity.
    - Includes ionization efficiency as part of detection efficiency.
- Molecules must be polar and/or ionizable to be detected by MS/MS.
  - Must have groups that can be charged (NH_3, COOH, OH, SH) in a polar solution (water plus polar organic solvents)



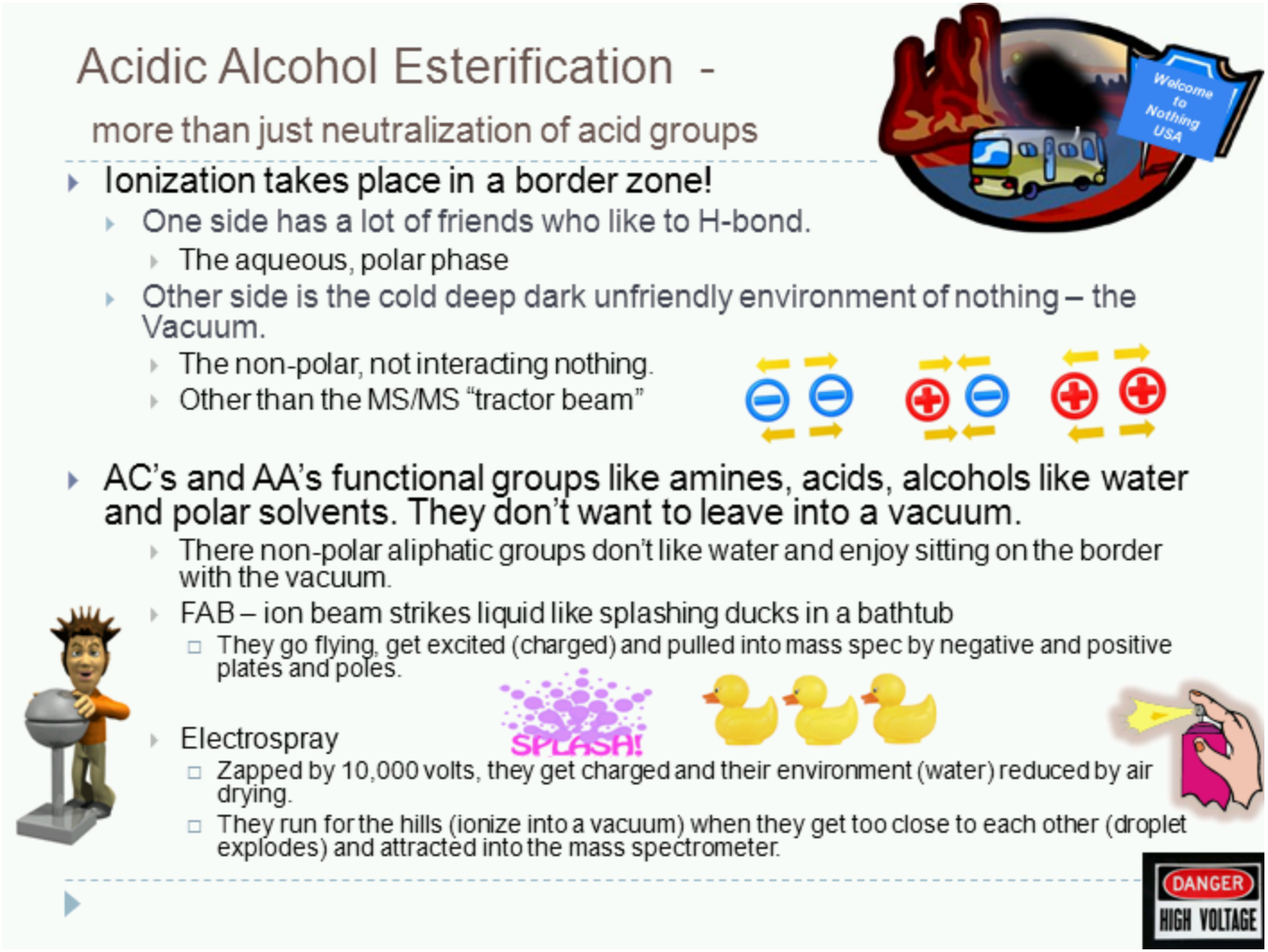
Ionization Efficiency



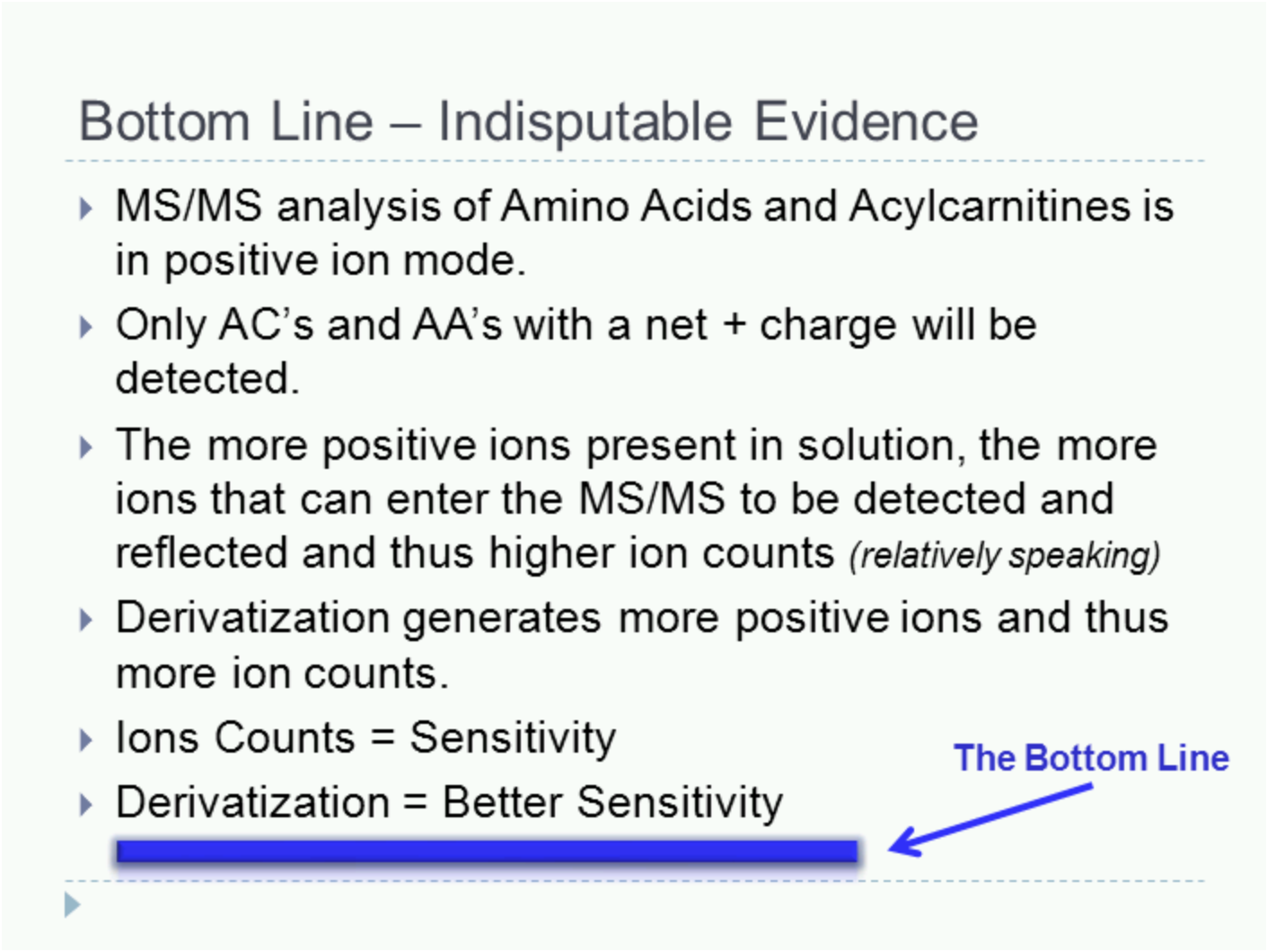
Why We Derivatized Then and Why Now! Part 1



Why We Derivatized Then and Why Now! Part 2



Acidic Alcohol Esterification - more than just neutralization of acid groups

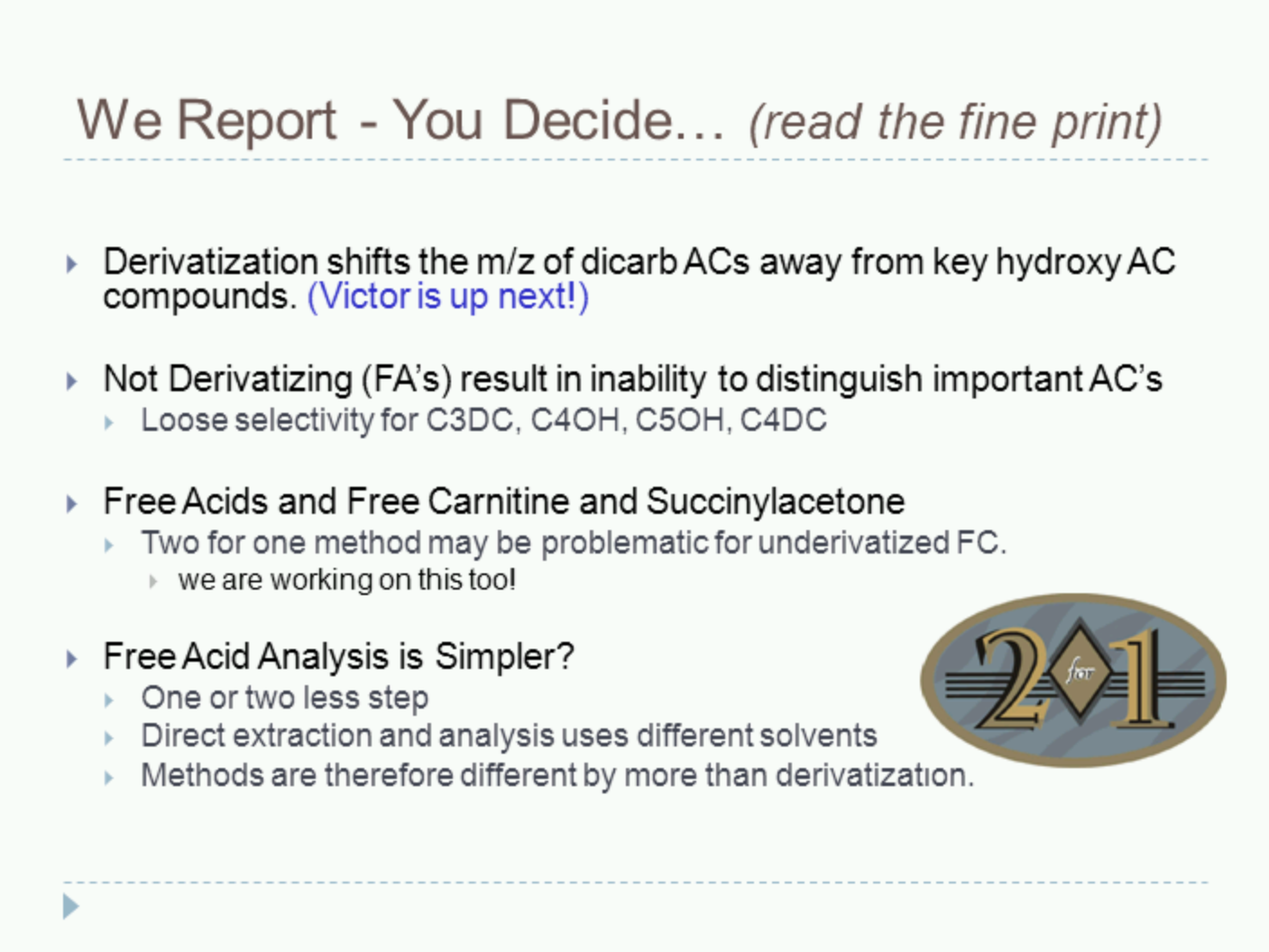


Bottom Line – Indisputable Evidence

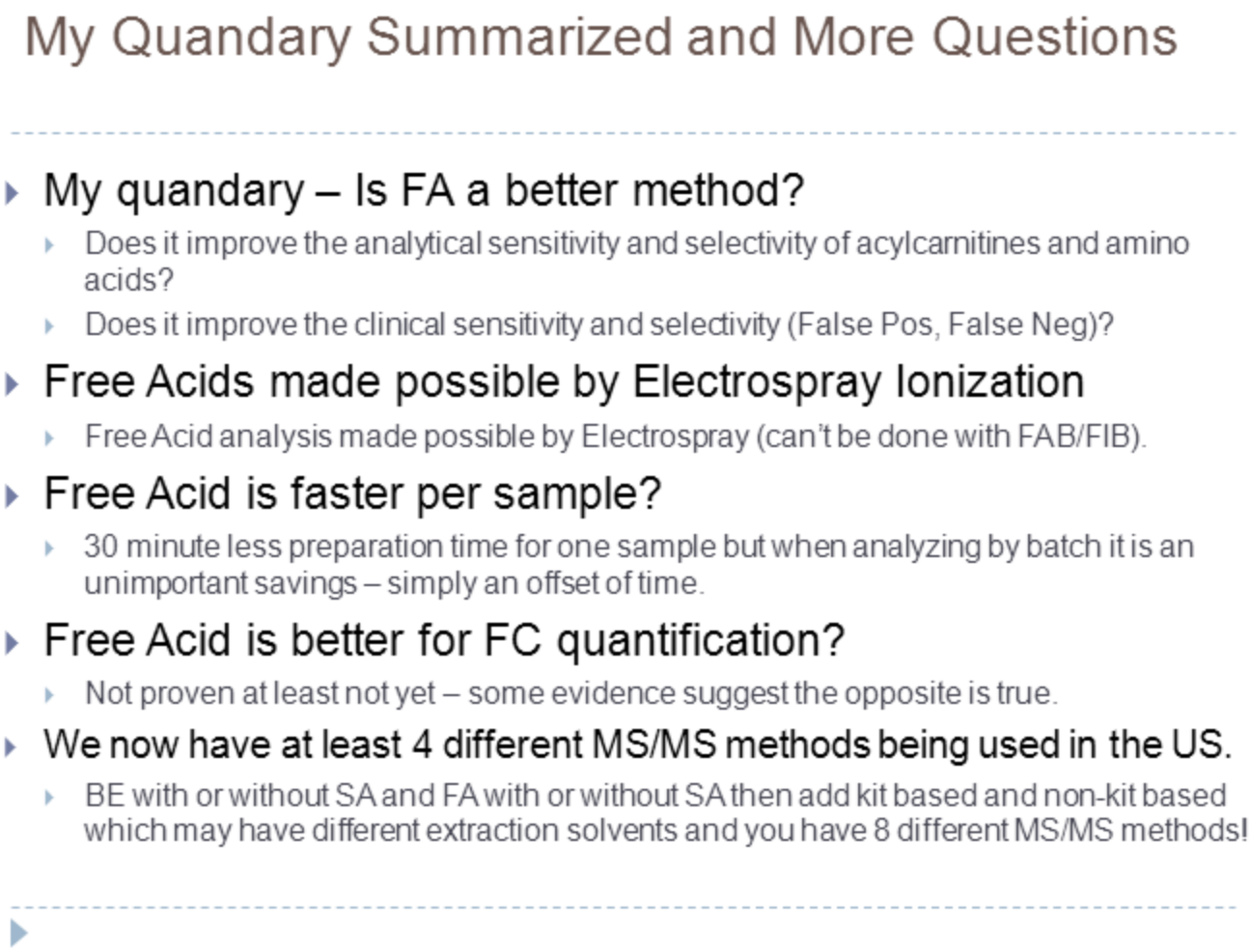
Butyl Esters are not Picture Perfect …

- Derivatization does convert AC to FC.
  - Quantification of FC tends to be higher.
    - Can be corrected – we are working on it!
  - Glutamate = 260, C2 = 260
    - Lots of Glutamate! – contributes to C2!
    - Can be corrected – we are working on that too!
- Derivatization ruins the lab dryers over time.
  - Need all teflon dryers *(or all fiber glass Corvettes).*
- Derivatization is an MS cleaner!
  - Because of acidic environment
    - Fewer LC clogs
    - Cleaner source
    - Paper fibers dissolved.
    - (note – base is use to hydrolyze AC)

Butyl Esters are not Picture Perfect …



We Report - You Decide… (read the fine print)



My Quandary Summarized and More Questions