The Vermont Center for Immunology and Infectious Diseases (VCIID) COBRE **Mass Spectrometry, Proteomics & Metabolomics Core**

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Purpose of the VCIID-COBRE Mass Spectrometry Core

To provide support to investigators needing **mass spectrometry**-based analyses in the areas of **proteomics** and **metabolomics**

Also provide support for

- experimental design (prior to beginning studies)
- data analysis and interpretation of data

History of the Vermont Center for Immunology and Infectious Diseases (VCIID) COBRE

- Vermont Genetics Network (VGN INBRE) Proteomics Core (established 2005)
  - Thermo-Finnigan Deca XP LCQ ion trap LCMS (NIH SIG 2003)
  - VGN funded additional LCMS instrumentation

VCIID COBRE (2006) Proteomics Core:

- Phase I (P20: Aug 2006 – June 2011)
  - *Phase I funds new investigator projects; limited core support*
  - Layered upon the VGN Proteomics Core
  - ABI-SCIEX 4000-QTrap TSQ/LIT ESI-LCMS/MS (NSF MRI 2008)
- Phase II (P20: July 2011 – June 2016)
  - Added small molecule and metabolomics; now separate from the VGN
  - Waters Xevo G2-XS QTOF ESI-UPLCMS/MS (NIH SIG 2014)
- Phase III (P30: Aug 2016 – July 2021)
  - *Phase III focuses on core labs and other investigator support (e.g. pilot & feasibility funds)*
  - Renamed *Mass Spectrometry Core* that includes proteomics and metabolomics
  - COBRE sunsets July 2021 – convert to full fee-for-service core

VCIID Mass Spectrometry Core Services

Proteomics

- Vermont Genetics Network (VGN) INBRE Proteomic Core
  - Protein IDs
  - PTM searches
  - Protein quantification
- VCIID Core
  - Specialized problems
  - Specialized quantification
    - Posttranslational modifications
  - Protein turnover: rates of synthesis & breakdown

Small molecule

- **Untargeted global metabolomics**
  - Lipodomics → Univ. of Maine Core
  - Organic acids, sugars, alcohols, amines
- **Targeted metabolomics**
  - Quantification
    - using stable isotope labeled (SIL) standards
  - Isotope labeling and flux analysis
  - Kinetics
- Drug & other metabolite quantification
- Exact mass analysis
  - Elemental composition

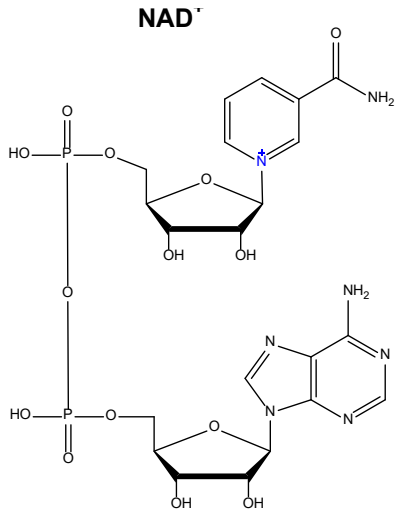
Investigation of MCJ Protein

- Background:
  - Recently identified MCJ (methylation-controlled J) protein
    - also called DnaJ homolog subfamily C member 15 (DnaJC15) – Uniprot Q9Y5T4
    - 150 amino acids (AA), 16,383 Da
  - MCJ (DnaJC15) is an endogenous negative regulator of Complex I of the electron transport chain
    - The 1st 35 AAs form the key mitochondrial intermembrane domain
  - MCJ is abundantly expressed in CD8 cells, relative to other immune cells
  - Mercedes Rincón (Univ. of Colo., Denver):
    - *Proposes:* MCJ alters CD8-mediated immunity to influenza
    - *Developed* an MCJ peptide analogue to be used as a drug
    - *Hypothesis:* NAD^+ binding is be required for MCJ-mediated inhibition of mitochondrial respiration

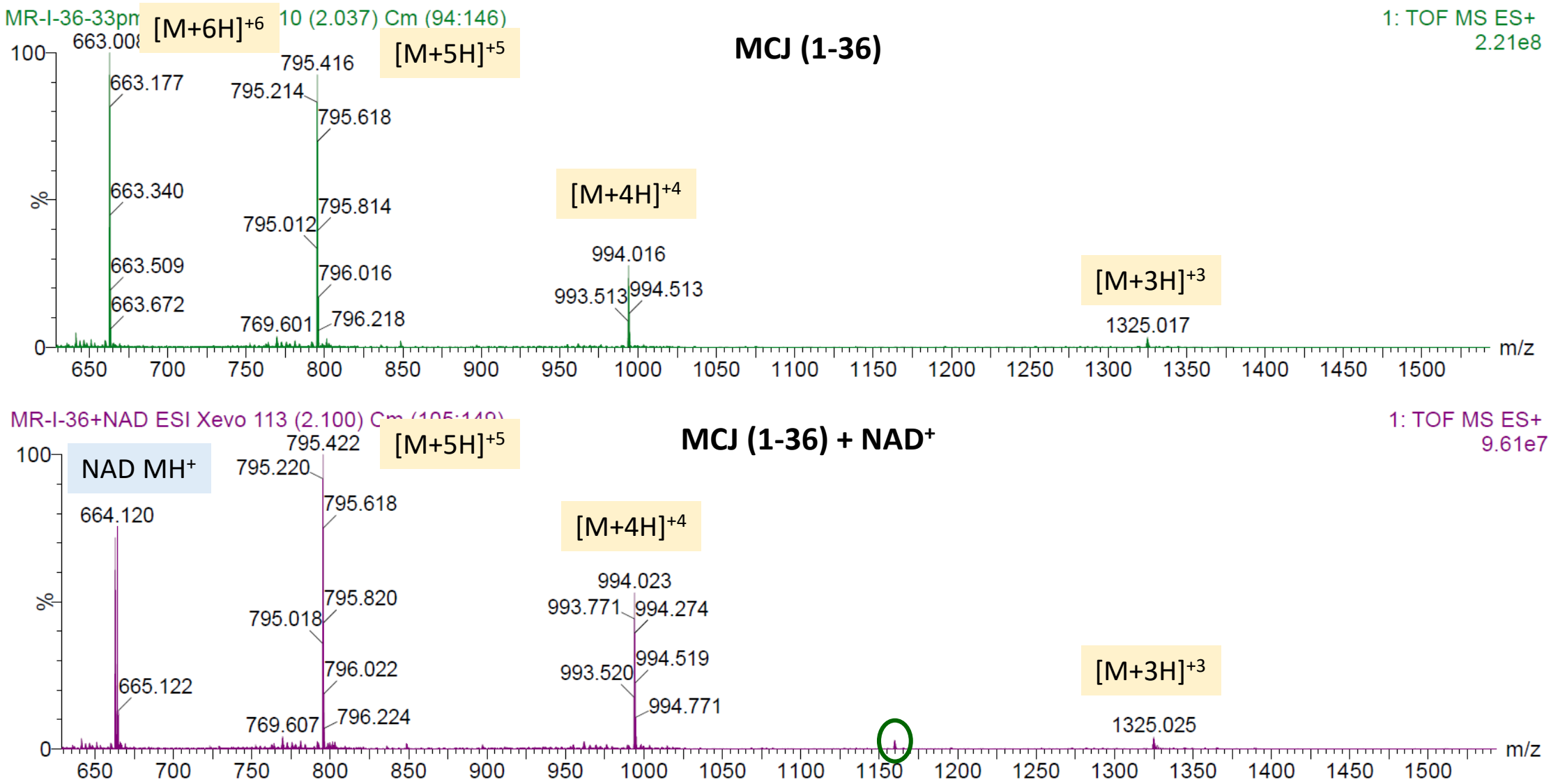
Can We Measure MCJ–NAD$^+$ Noncovalent Complex Binding by Mass Spectrometry?

Key: Waters & SCIEX ESI-interfaces do not have heated-capillary inlets

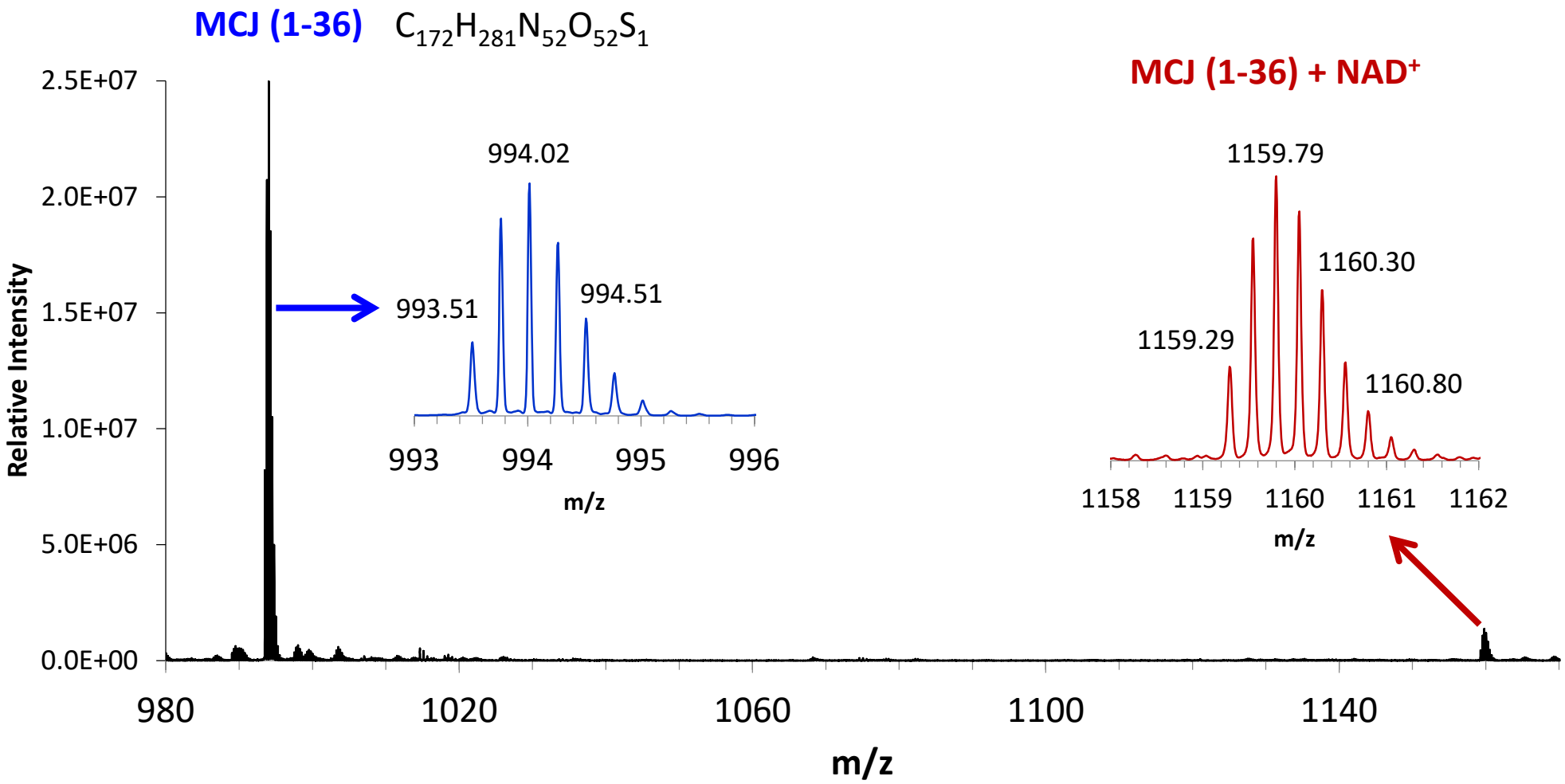
- MCJ key intermembrane domain (AA 1-36):
  MAARGVIAPVGESLRYAEYLQPSAKRPDADVDQQRL
  - MW: 3970 Da
- Nicotinamide adenine dinucleotide (NAD$^{+)}$
  - MW: 664 Da
- MCJ-NAD$^+$ complex: MW: 4634 Da



Direct Infusion of MCJ and MCJ+NAD⁺ by ESI-LCMS



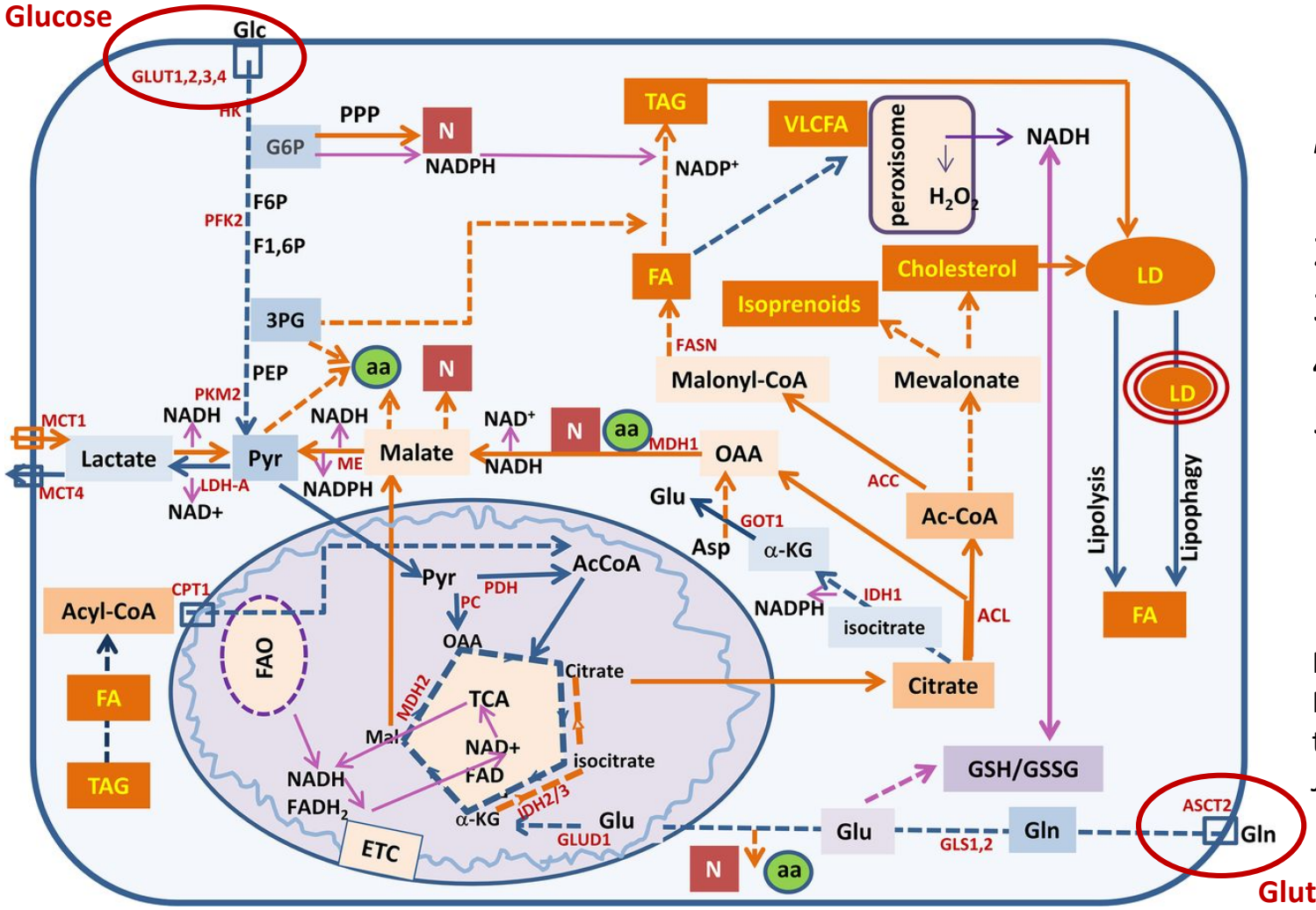
Direct Infusion of MCJ and MCJ+NAD$^+$ by ESI-LCMS: MCJ z=4 Charge State



MCJ Protein Intermembrane Domain Binding with NAD^+

- Demonstrated:
  - Presence of NAD^+ - MCJ (1-36) complex
  - MCJ-NAD^+ complex dependent upon NAD^+ concentration
- Further experiments with truncated forms
  - MCJ (11-36): NAD^+ complex found
  - MCJ (11-20): NAD^+ complex *not* found
  - Results agree with computational modeling studies
- Results became basis for
  - An R21 application

Metabolic Pathways Altered in Cancer



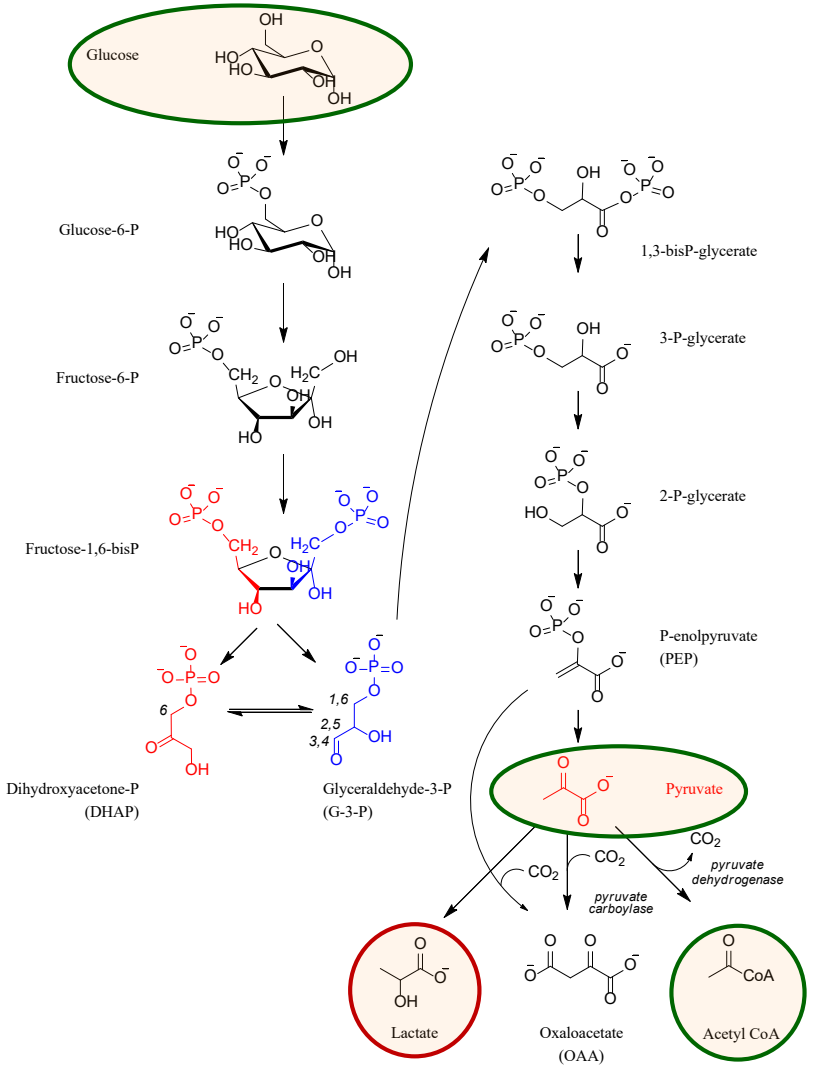
Increases in

1. De Novo Lipogenesis
2. Cholesterogenesis
3. Glycolysis
4. Pentose Phosphate Pathway (PPP)
5. Glutaminolysis

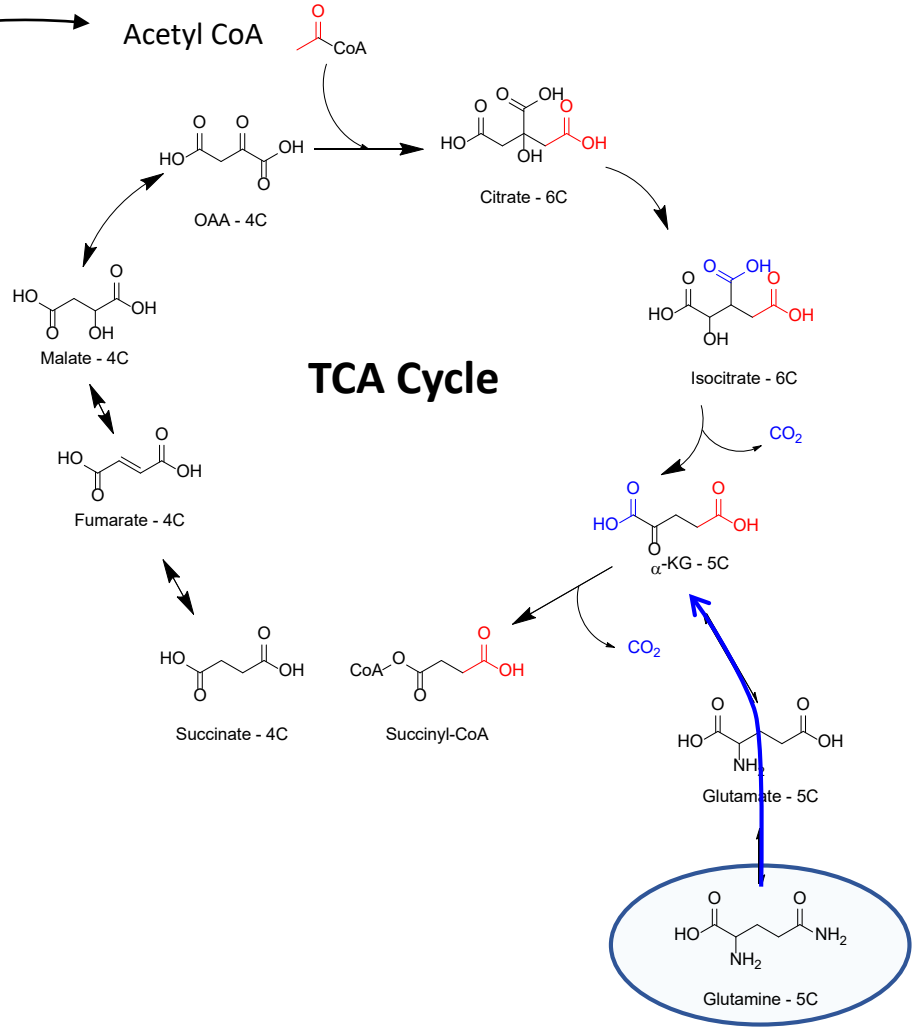
M Gómez de Cedrón & A Ramírez de Molina. Microtargeting cancer metabolism: opening new therapeutic windows based on lipid metabolism. *J. Lipid Res.* 57: 193-206, **2016**

The Warburg Effect: Glycolysis Versus Oxidative Phosphorylation

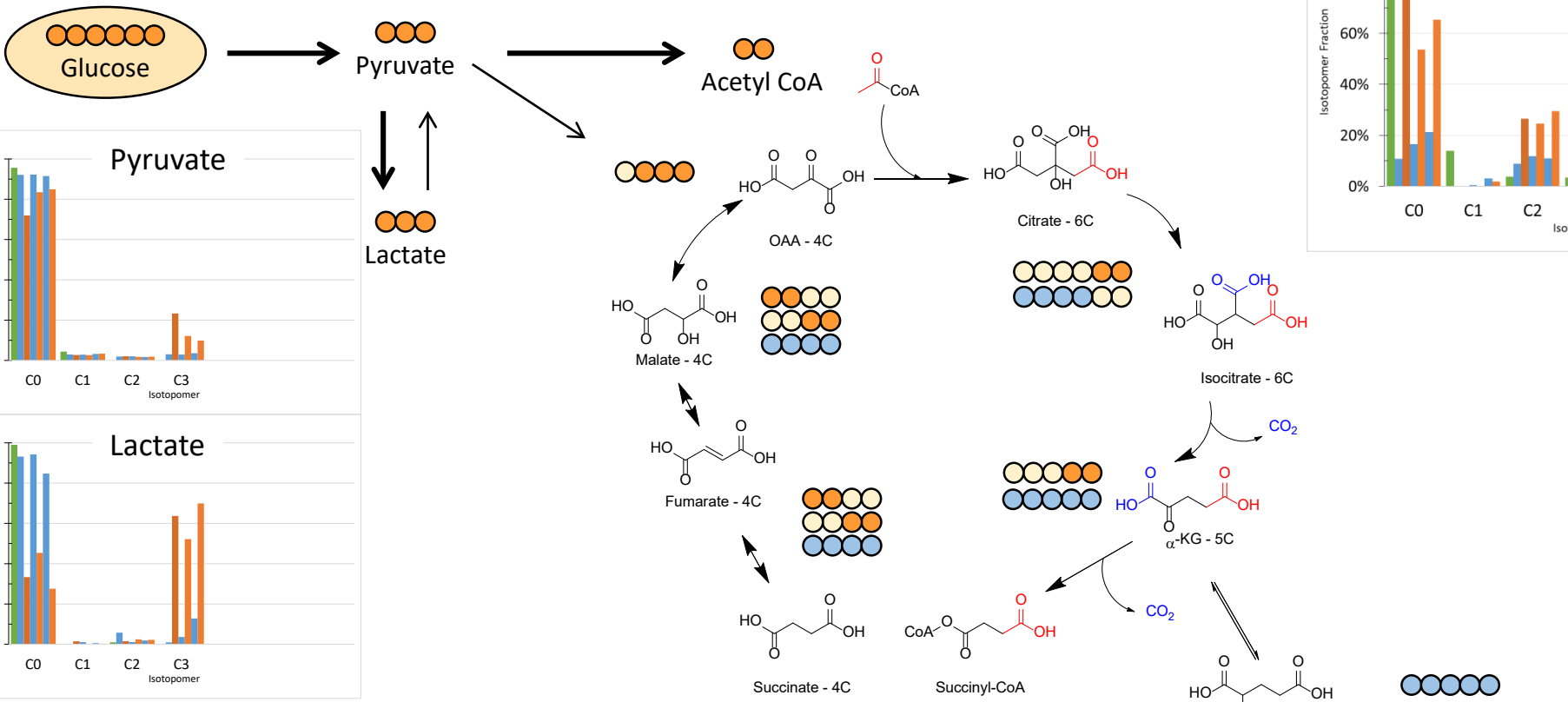
Glycolysis

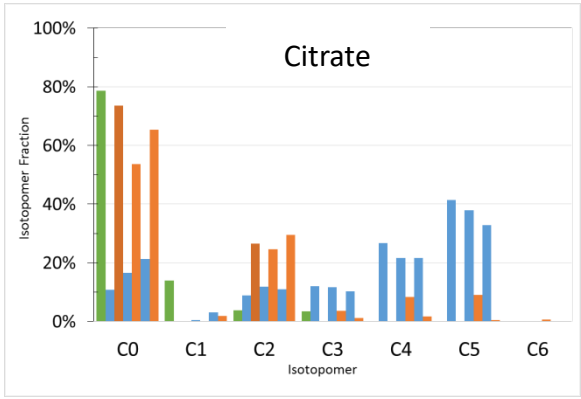


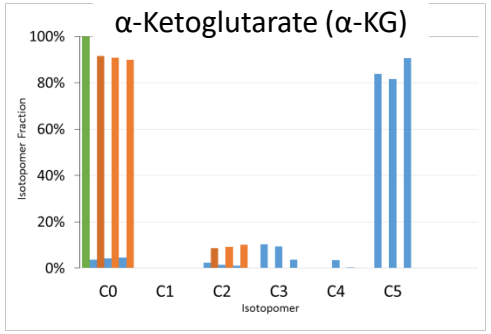
Oxidative Phosphorylation

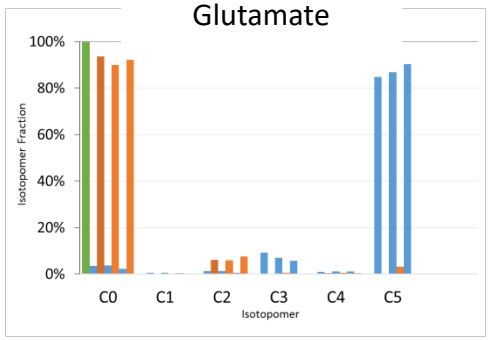


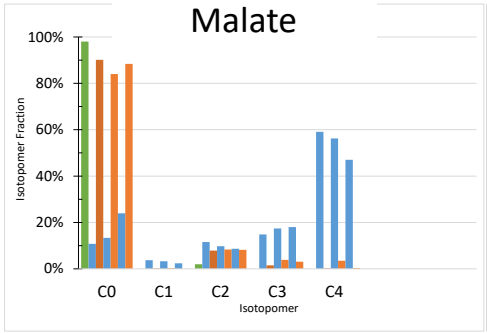
Determining Rates of Glycolysis Vs. Oxidative Phosphorylation (TCA Cycle)

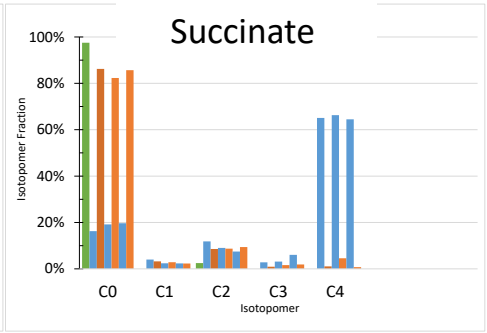






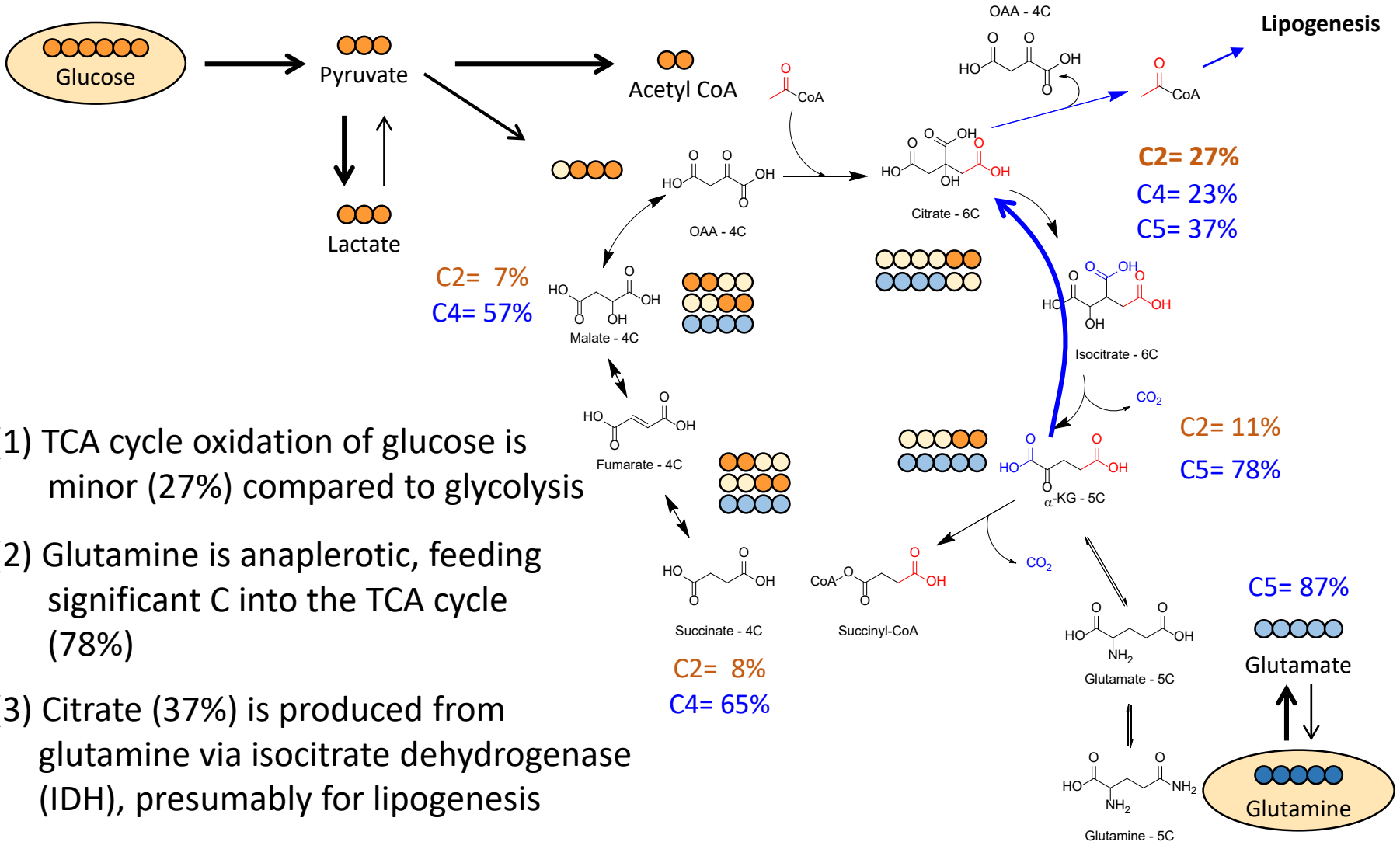


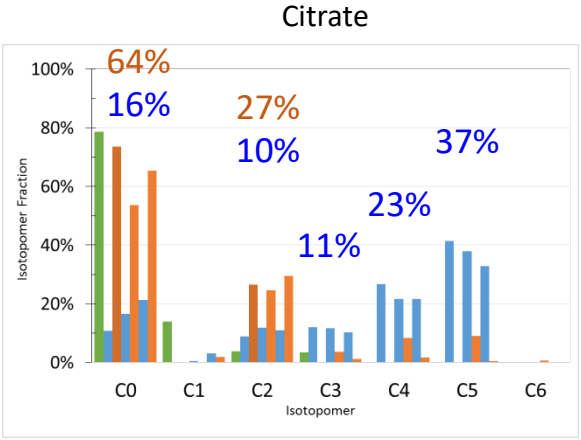




Time points: 0, 2, 4, 8 h

Results of D-[U-^{13}C]Glucose and L-[U-^{13}C]Glutamine Labeling





(1) TCA cycle oxidation of glucose is minor (27%) compared to glycolysis

(2) Glutamine is anaplerotic, feeding significant C into the TCA cycle (78%)

(3) Citrate (37%) is produced from glutamine via isocitrate dehydrogenase (IDH), presumably for lipogenesis

Time points: 0, 2, 4, 8 h

Metabolomics in the VCIID-COBRE Mass Spectrometry Core

- **Untargeted metabolomics –** *you don't have an hypothesis*
  - Identification and relative quantification of metabolites
  - Useful for defining changes between two or more states/conditions

- **Targeted metabolomics –** *you do have an hypothesis*
  - Absolute quantification of specific metabolites using SIL standards

- **Metabolic flux analysis using SIL tracers**
  - Definition of precursor-product relationships defining metabolic pathways
  - Kinetic rate measurements using multiple time points

Stable isotope labels (SIL): ^{2}H, ^{13}C, & ^{15}N