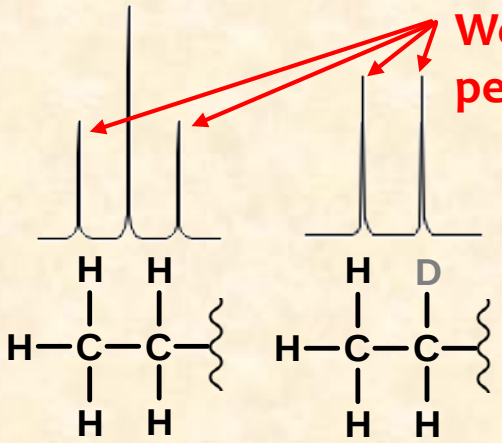
# Isotopomer Definitions

- **Isotopomer – molecules with identical isotopic composition but differing by the position of isotope.**
  - **Examples:**

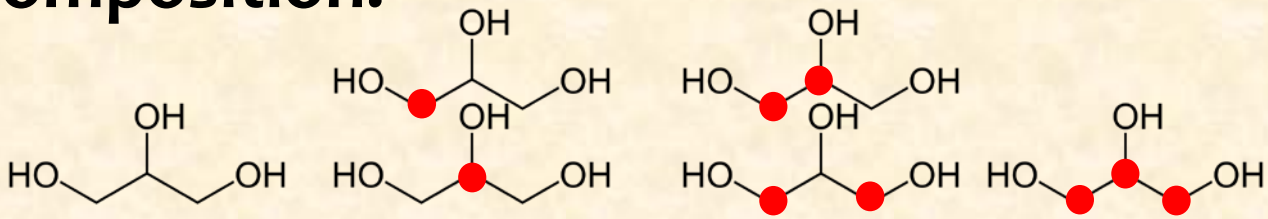

- **Partial Isotopomer - part of an isotopomer where the isotopic content of specific atoms is known.**
  - **In NMR, spin-spin coupling (J-coupling or scalar coupling) can produce peaks that indicate the isotopic status of neighboring atoms.**
  - **Example:**




**We need the ability to annotate these peaks in an isotope-informative manner.**

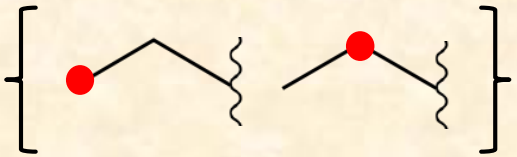
# Isotopologue Definitions

- **Isotopologue – set of molecules that differ *only* in their isotopic composition.**




  - Examples: $^{12}C_3{}^{1}H_8{}^{16}O_3$, $^{12}C_2{}^{13}C_1{}^{1}H_8{}^{16}O_3$, $^{12}C_1{}^{13}C_2{}^{1}H_8{}^{16}O_3$, $^{13}C_3{}^{1}H_8{}^{16}O_3$

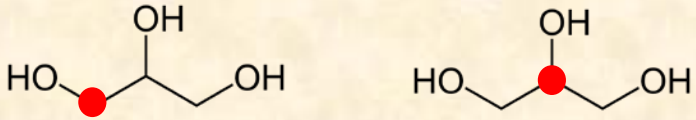
- **Isotopologue Fragment - a refined set of isotopomers where the ambiguity of isotope location is limited to a subset of the atoms.**
  - **Tandem mass spectrometry can produce spectral features that indicate where isotopes are localized within a chemical structure.**
  - **Example:**



# Detecting Metabolites Labeled with Stable Isotopes


## Nuclear Magnetic Resonance (NMR)

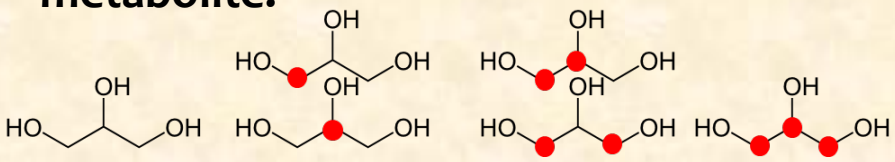
**Detected features identify the location of stable isotopes within the metabolite.**




**Isotopomer – molecules with identical isotopic composition but differing by the position of isotope.**

## Mass Spectrometry (MS)

**Detected features identify the number of stable isotopes within the metabolite.**

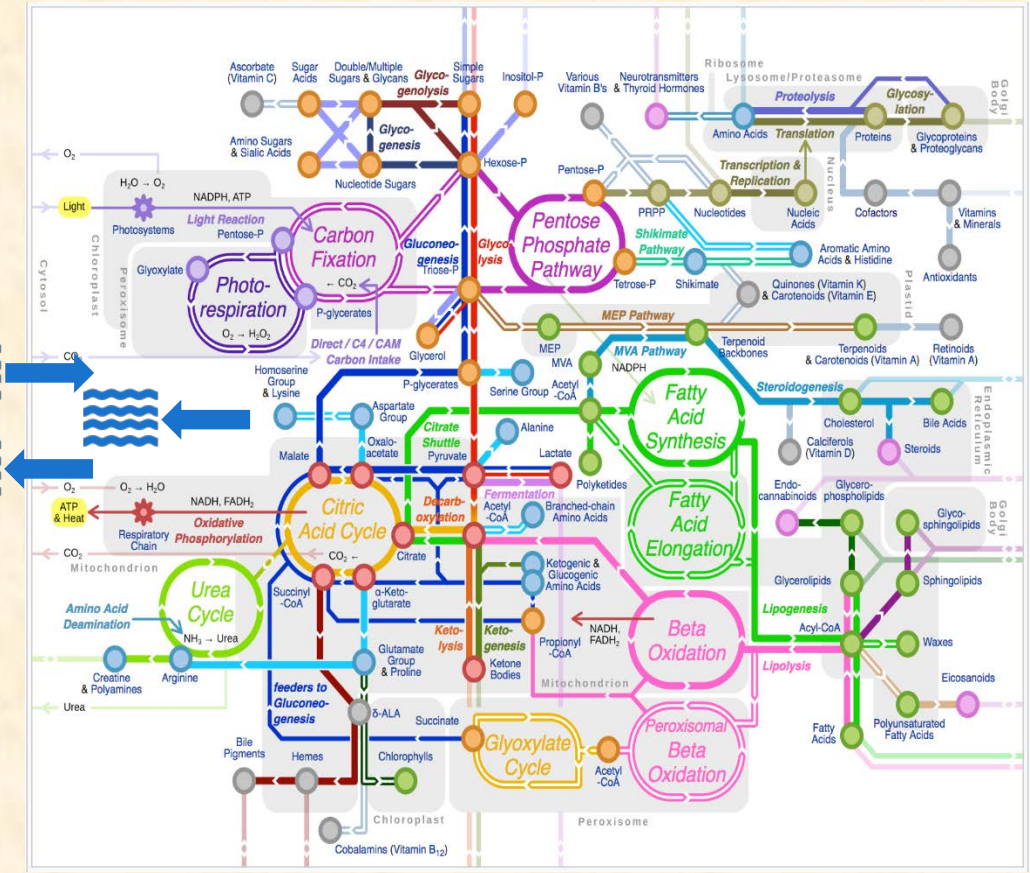



**Isotopologue – set of molecules that differ *only* in their isotopic composition.**

# Why is isotopic labeling useful in metabolomics?

**Isotopic labeling allows the detection of isotope accumulation within metabolites that are under homeostatic control.**

- **Homeostasis keeps many intermediate metabolites within tightly-controlled concentration ranges.**
- **While metabolites that accumulate in reservoirs can change significantly, intermediates of metabolism may not change much.**
- **However, isotopes from an enriched metabolite source can accumulate to detectable levels within intermediate metabolites of interest.**

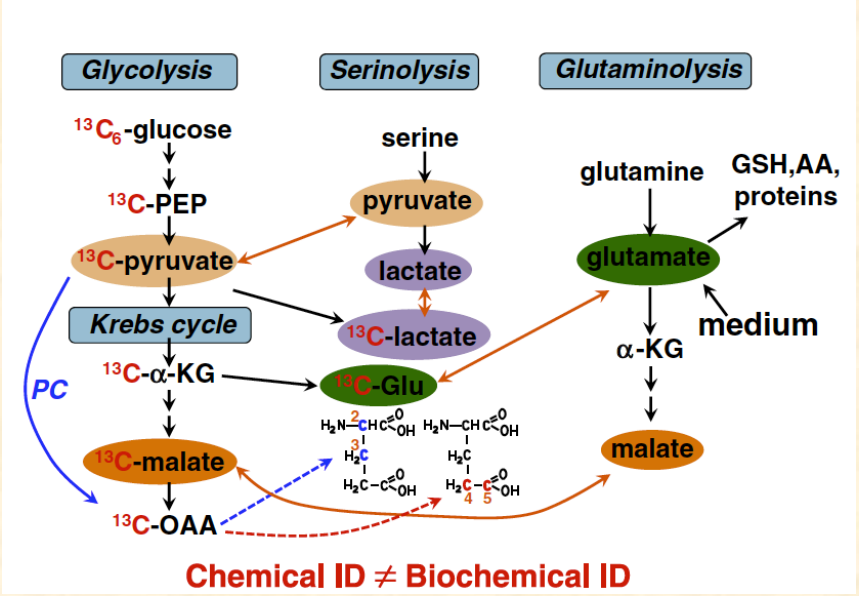



https://en.wikipedia.org/wiki/Metabolic_network

# Why is isotopic labeling useful in metabolomics?

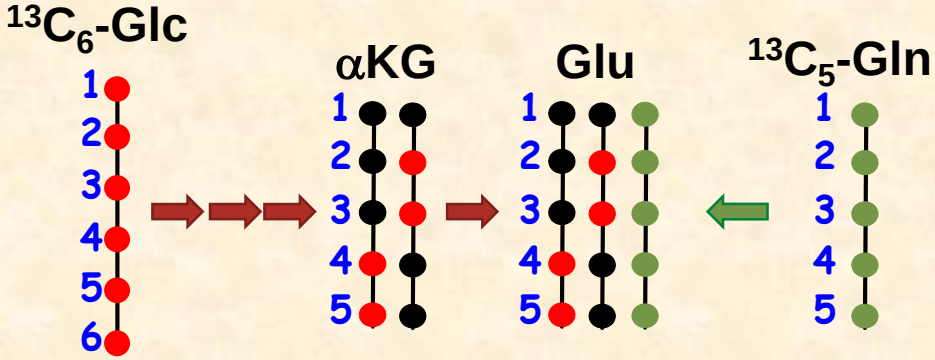
**Isotopic labeling facilitates placement of measured metabolite abundances within a metabolic network.**

- **Chemical identification of a metabolite may not help determine what part of the metabolic network is being perturbed.**




Fan et al, *Pharmacology & Therapeutics*, 133, 366 (2012).

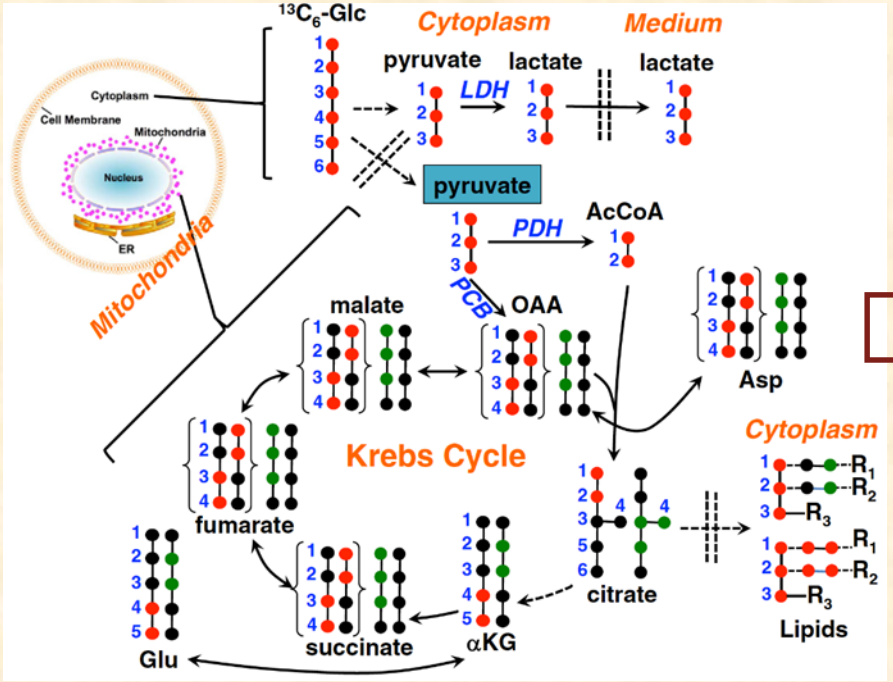
- **Detection of labeled metabolite substructure can help place isotope accumulation within a metabolic path context.**



# Why is isotopic labeling useful in metabolomics?

**Isotopic labeling facilitates the measurement of isotopic flux, which can be interpreted in terms of metabolic flux.**

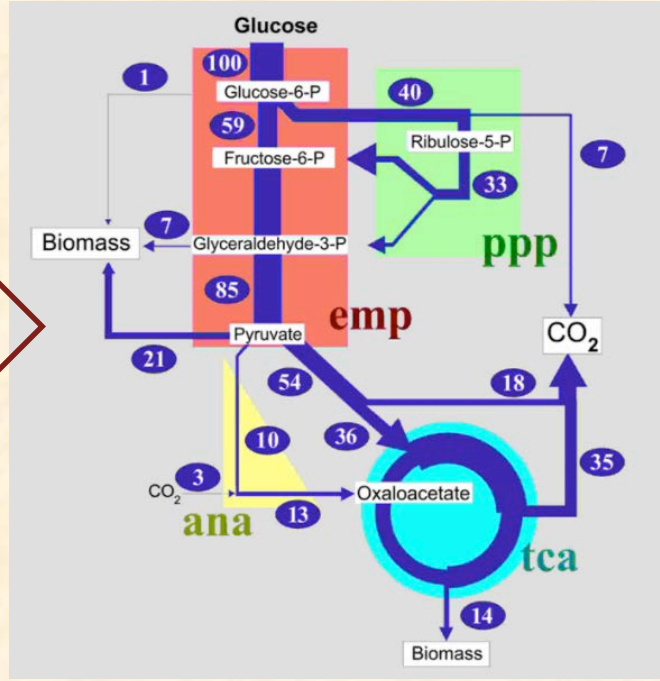
## Isotopic Flux




Fan et al, *Pharmacology & Therapeutics*, 133, 366 (2012).

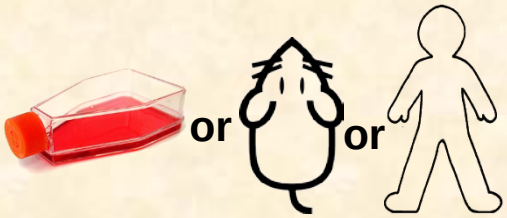
Interpreted

## Metabolic Flux




https://www.13cflux.net/13cflux2/mfa.jsp%3Bjsessionid=1FC4F4B2D7F8ADF775F0314689B71C7A

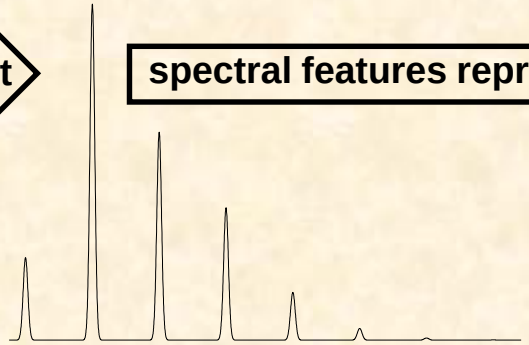
# The Spectral Feature Annotation Problem



or or or ???

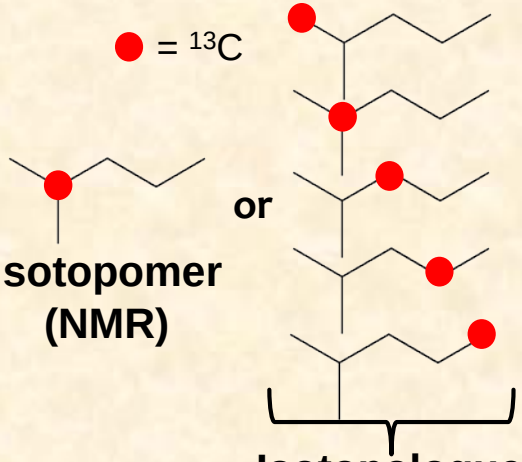
Stable Isotope Tracing Experiment

collect



Analytical Data

spectral features represent




Isotopomer (NMR)

or

Isotopologue (MS)

annotate

What should this annotation representation be?

Compact Annotation Representation

download

Metabolomics Workbench
MetaboLights
another public repository

deposit

Compact Annotation Representation

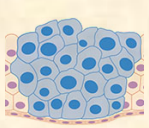
convert

```
C 1 0 0 0 0
1 C 1 1 0 0
0 1 C 0 0 0
0 1 0 C 1 0
0 0 0 1 C 1
0 0 0 0 1 C
```

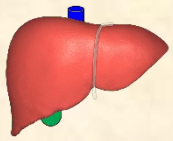
Isotope-Resolved Molecular Representation

reanalyze

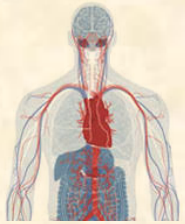
derive



or



or



New Biological Interpretation

# Restating the Problem

**How do we annotate spectral features so that computers can accurately represent them as isotopically-resolved chemical entities?**

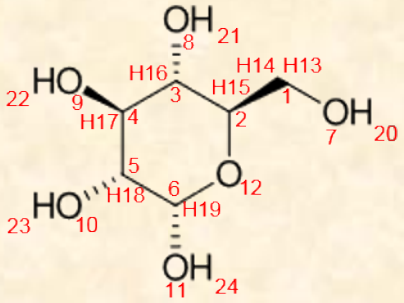
# A Solution

**Extend the current International Chemical Identifier (InChI) standard for this purpose.**

# Justification

- **InChI is an open IUPAC standard for representing chemical entities that is widely used, with support from multiple software packages.**
- **Software exists that can convert between InChI and molecular representations like CT/MOL/SDF format.**

# The InChI Isotopologue and Isotopomer Proposal



alpha-D-glucopyranose

**The proposal can represent isotope-specific InChI for both NMR and MS spectral feature annotation:**

- **Full isotopomers**
  - **Example: full isotopomer with respect to carbon for alpha-D-glucopyranose with $^{13}C$ at the 4th carbon.**
    - InChI=1/C6H12O6/c7-1-2-3(8)4(9)5(10)6(11)12-2/h2-11H,1H2/t2-,3-,4+,5-,6+/m1/s1/i1+0,2+0,3+0,4+1,5+0,6+0
- **Partial Isotopomers (for NMR)**
  - **Example: partial isotopomer of alpha-D-glucopyranose with $^{13}C$ at the 1st and 2nd carbons:**
    - InChI=1S/C6H12O6/c7-1-2-3(8)4(9)5(10)6(11)12-2/h2-11H,1H2/t2-,3-,4+,5-,6+/m1/s1/i1+1,2+1
- **Isotopologues (for MS)**
  - **Example: $^{13}C_2{}^{2}H_3$ isotopologue of alpha-D-glucopyranose.**
    - InChI=1/C6H12O6/c7-1-2-3(8)4(9)5(10)6(11)12-2/h2-11H,1H2/t2-,3-,4+,5-,6+/m1/s1/a(C2+1)
- **Isotopologue Fragments (for Tandem MS)**
  - **Example: $^{13}C_2$ limited to atoms 4,5,6 isotopologue fragment of alpha-D-glucopyranose.**
    - InChI=1/C6H12O6/c7-1-2-3(8)4(9)5(10)6(11)12-2/h2-11H,1H2/t2-,3-,4+,5-,6+/m1/s1/a(C2+1,4,5,6)

# Status of the Proposal

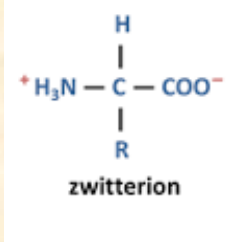
- **The proposal has been accepted by the IUPAC InChI subcommittee for implementation.**
- **Most of the isotopomer representation is implemented.**
- **No timetable yet for when the rest of the proposal will be implemented.**

# Proposal Development Team

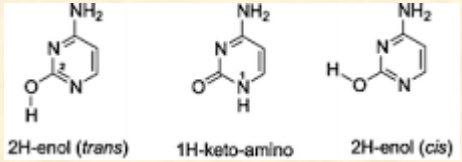
- **Hunter Moseley**
- **Philippe Rocca-Serra**
- **Reza Salek**
- **Masanori Arita**
- **Emma Schymanski**

# Issues with Using InChI for Unambiguous NMR Metabolite Feature Annotation

- **Zwitterions (having both positive and negative charges) cannot be represented by the standard InChI string.**
  - **Example: amino acids under physiological conditions.**
  - **Use the fixed hydrogen extension of InChI to represent zwitterions.**




- **Standard InChI does not always represent the major tautomer form of the metabolite.**
  - **Example: nucleobases have multiple tautomeric forms.**
  - **Use the fixed hydrogen extension of InChI to represent the major tautomeric form.**

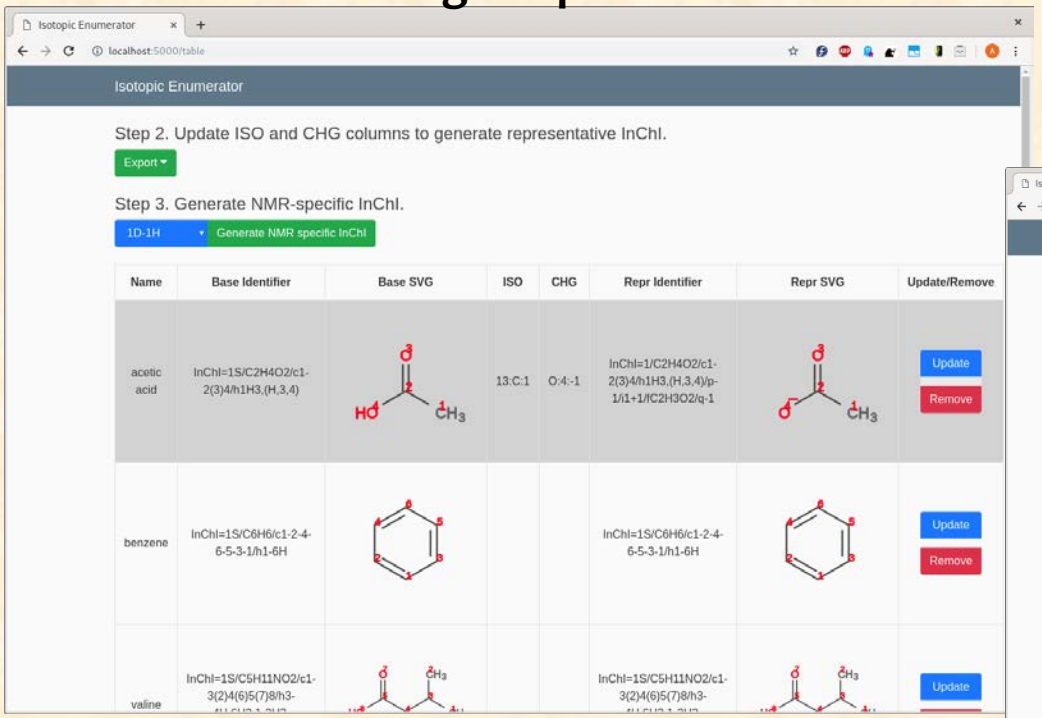


# Isotopic Enumerator

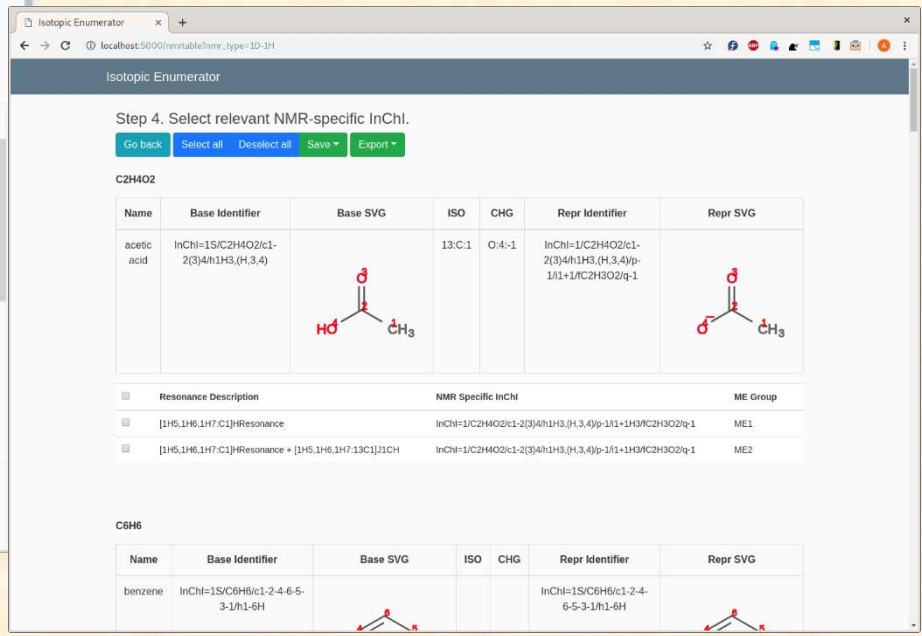
**Facilitates generation of InChI strings useful for unambiguous metabolite feature annotation:**

- **A starting "Base InChI" for database lookup.**
- **"Representation InChI" which has the correct charge state and tautomeric form.**
- **"Isotopic InChI" which is isotope-specific.**
  - **"NMR-specific InChI" for each possible spectral feature for a specific metabolite and experiment.**
- **Available on GitHub and the Python Package Index (PyPI):**
  - https://github.com/MoseleyBioinformaticsLab/isoenum
  - https://github.com/MoseleyBioinformaticsLab/isoenum-webgui

**View for Generating "Representative InChI"**




**View for Generating "NMR-specific InChI"**



# Acknowledgements

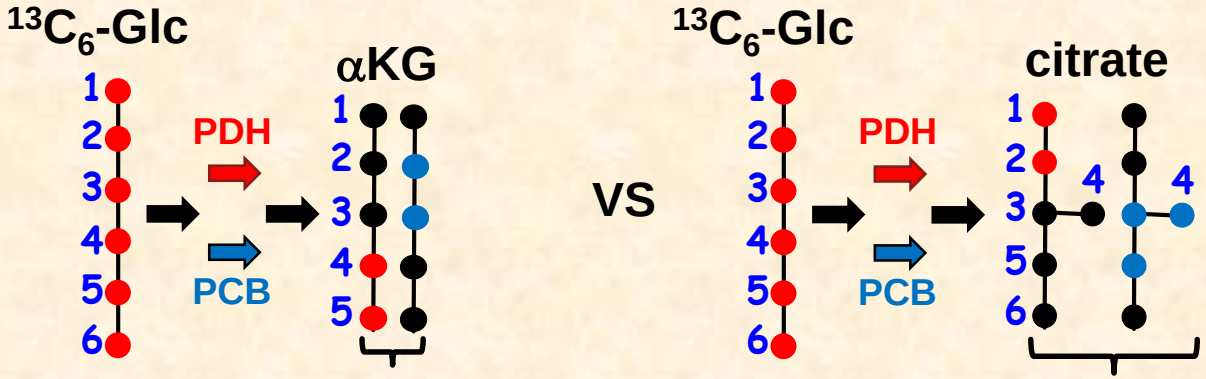

- Steve Heller
- Igor Pletnev
- Dmitrii Tchekhovskoi
- Leah Rae McEwen


# The InChI Isotopologue and Isotopomer Proposal Development Team


- Hunter Moseley
- Philippe Rocca-Serra
- Reza Salek
- Masanori Arita
- Emma Schymanski

# Other SIRM-Specific Experimental Design Questions

- **Can isotopologue and/or isotopomer data enable the answering of the main biological questions?**
  - **For example:**

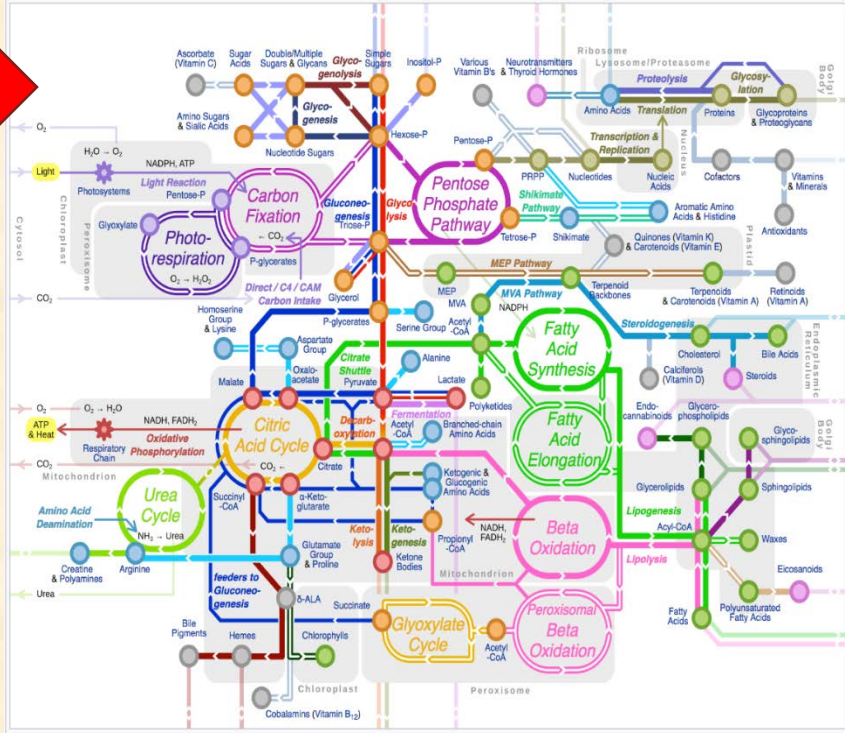



**Requires isotopomer-specific or isotopologue-fragment information to distinguish.**

**Isotopologue-specific information is adequate to distinguish.**

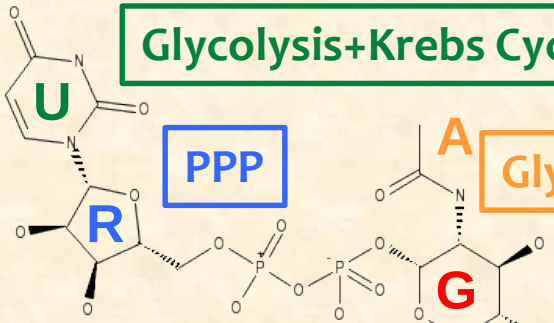
- **Can the metabolites of interest be detected and accurately assigned by the analytical technique that will provide the isotope-specific data that is needed?**

# How do I get the isotope label into the metabolites I am interested in?




1. **What source metabolites will be taken up by the system I am studying? How will it be taken up?**
   - **Know thy biological system or consult with someone that knows.**
2. **Will a source metabolite trace through the pathways of interest to the metabolites of interest?**
   - **Study the relevant metabolic network.**
3. **Can I get the labeled version of this source metabolite at a reasonable price?**
   - **Negotiate with the vendors.**
4. **How long and much of the source metabolite is needed for my experiments?**
   - **Draw from previous experiments, but also test how long it takes for isotope to enter metabolites of interest.**

# Pathway Contributions to $^{13}C$ Labeling of UDP-GlcNAc from $^{13}C$-Glucose




UDP-GlcNAc

Glycolysis+Krebs Cycle

PPP

Glycolysis

Hexosamine

$^{13}C_6$-Glucose

Glycolysis

$^{13}C_3$-Pyruvate

*pyruvate dehydrogenase complex*

$^{13}C_2$-Acetyl-CoA

Krebs Cycle

$^{13}C_?$-Oxaloacetate

*aspartate aminotransferase*

$^{13}C_?$-Aspartate

+

Carbamoyl phosphate

+

PRPP

($^{13}C_5$-Ribose)

*carbamoyl phosphate synthetase II*

$CO_2$

$^{13}C_6$-Glucose

PPP

Pyrimidine Biosynthesis

$^{13}C_6$-Glucose

*hexokinase*

$^{13}C_6$-Glucose-6-P

*phosphohexose isomerase*

$^{13}C_6$-Fructose-6-P

*glucosamine -fructose 6-P aminotransferase*

$^{13}C_6$-Glucosamine-6-P

$^{13}C_2$-Acetyl-CoA

CoA

*glucosamine 6-P N-acetyltransferase*

$^{13}C_8$-N-acetylglucosamine-6-P

*P acetyl glucosamine mutase*

$^{13}C_8$-N-acetylglucosamine-1-P

*UDP-N-acetylglucosamine pyrophosphorylase*

$^{13}C_?$-UDP-N-acetylglucosamine

(UDP-GlcNAc)

*UDP-glucosamine epimerase*

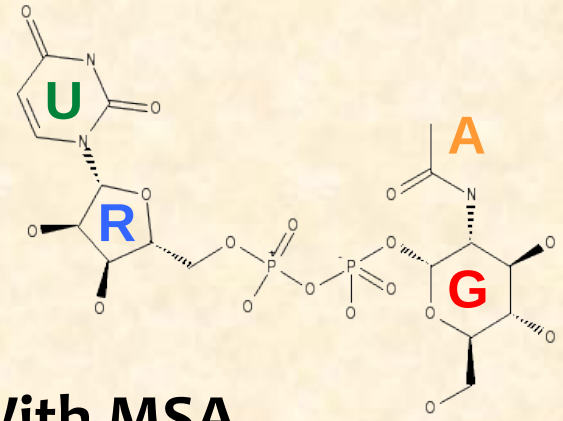
$^{13}C_?$-UDP-N-acetylgalactosamine

(UDP-GalNAc)

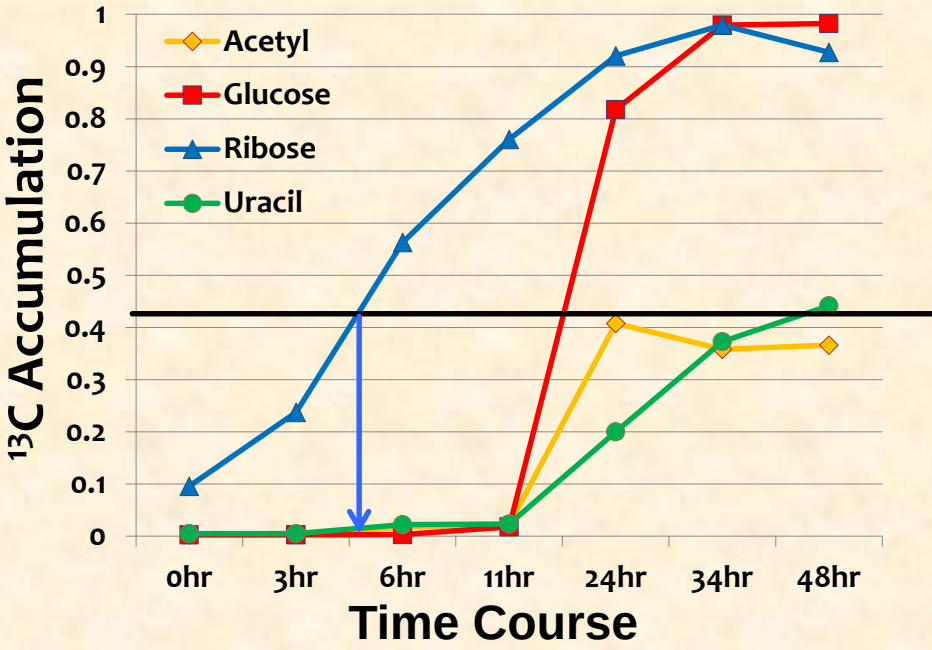
Moseley et al. *BMC Biology* 9, 37 (2011).

# Comparison of UDP-GlcNAc Derived Pathway-Specific Relative Metabolic Flux Biomarkers

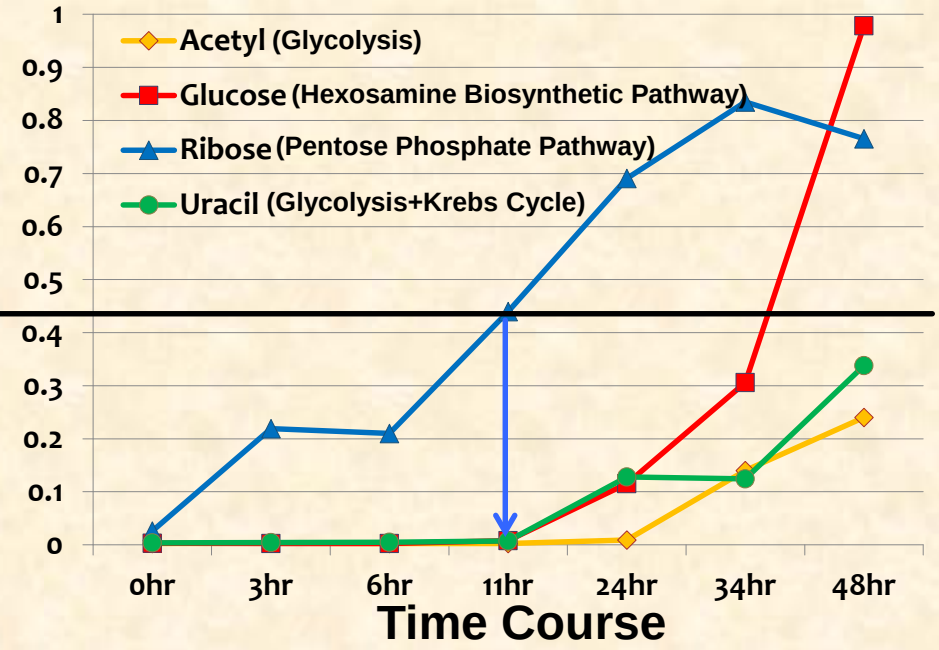
**Example: LNCaP-LN3 prostate cancer cells with and without MSA (methylselenic acid).**




## Without MSA




## With MSA




**This is an interpretation of isotopic accumulation within chemical functional groups in terms of pathway-specific relative flux.**