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- Specific credits via citations at bottom of slides
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Outline

- Stable-Isotope Resolved Metabolomics
  - Intro
  - Metabolism is substructures
  - “Sufficient” Resolution in MS
  - Forward to mSIRM
Metabolites are the “nuts & bolts” of biology: Like real nuts & bolts, the same parts are used in many places for different reasons.

Main Strategy: 

Looking for HAY STRAW in haystack! But we still need needles!

Converts certain straws ➔ needles
Chemical ID ≠ Biochemical ID
Analyte ≠ “Metabolite”

Fan et al., Pharmacology & Therapeutics 133 (2012) 366–391
Metabolic fate of $^{13}$C$_6$-glucose in human NSCLC

**Glycolysis**
- Lactate → Pyruvate → CO$_2$ → 3-PGA → 3-C$_6$-Glc → Cancer
- Gly → Ser → Normal

**Krebs Cycle**
- Aspartate → OAA → citrate → c-Aconitate → CO$_2$ → succinyl CoA
- Malate → CO$_2$ → succinate → γKetoglutarate → oxaloacetate → Fumarate → CO$_2$ → acetyl CoA

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- Stable-Isotope Resolved Metabolism
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**High Information Throughput (HIT)**
- Control
- Selenite
- CH$_2$-O-R$_1$ (C18:0)
- CH-O-R$_2$ (C20:4)
- CH$_2$-O-P-inositol PI

**Control**
- 13C$_6$ Glucose A549

**Selenite**
- 13C$_6$ Glucose A549
- 5 min Nanospray LTQ-FT @ 200K Resolution
Metabolism is Substructures

**13C Dispersal into Substructures**

**Glycolysis**
- 1-C metabolism
- Lactate → Oxaloacetate
- α-Ketoglutarate
- Pyruvate → CO₂
- 3-PGA
- Glc

**Krebs Cycle**
- Aspartate → Oxaloacetate
- Malate → Fumarate
- Succinate → CO₂

**UDP-GlcNAC 13C Convergence**

- Uridine-diphosphate-N-acetylglucosamine

- N-Linked GlcNAcylation:
  - Posttranslational modification of proteins at N residues.
  - Involved in protein targeting via the ER and Golgi complex.
- O-Linked GlcNAcylation:
  - Posttranslational modification of proteins at S and T residues.

**UDP-GlcNac Labeling has Complex Time Course**

- UDP-GlcNAc labeling FT-MS

**Known UDP-GlcNac Biosynthesis**

- Carbohydrate metabolism
- NADH production

- Glycolysis
- Pyruvate dehydrogenase complex

- Pentose phosphate pathway
- Glucose-6-phosphate
- Fructose-6-phosphate

- Glucose-6-phosphate
- Fructose-6-phosphate
- Glucosamine-6-phosphate
- UDP-glucosamine-6-P

- Pyrimidine biosynthesis
- NADH production

- Carbamoyl phosphate synthetase II
- Pyrimidine biosynthesis

- UMP
- CMP
- UDP-glucose

Moiety Model of Isotopologue Intensities

\[ I_0 = g_0 r_0 a_0 u_0 \]
\[ I_1 = g_0 r_0 a_0 u_1 \]
\[ I_2 = g_0 r_0 a_0 u_2 + g_0 r_0 a_2 u_0 \]
\[ I_3 = g_0 r_0 a_0 u_3 + g_0 r_0 a_2 u_1 \]
\[ I_4 = g_0 r_0 a_0 u_4 \]
\[ I_5 = g_0 r_0 a_0 u_5 + g_0 r_5 a_0 u_1 \]
\[ I_6 = g_0 r_0 a_0 u_6 + g_0 r_5 a_0 u_2 \]
\[ I_7 = g_0 r_0 a_0 u_7 + g_0 r_5 a_0 u_3 + g_0 r_5 a_2 u_1 \]
\[ I_8 = g_0 r_0 a_0 u_8 + g_0 r_5 a_0 u_3 + g_0 r_5 a_2 u_2 \]
\[ I_9 = g_0 r_0 a_0 u_9 + g_0 r_5 a_2 u_1 \]
\[ I_{10} = g_0 r_0 a_0 u_{10} + g_0 r_5 a_2 u_3 \]
\[ I_{11} = g_0 r_0 a_0 u_{11} + g_0 r_5 a_2 u_3 \]
\[ I_{12} = g_0 r_0 a_0 u_{12} \]
\[ I_{13} = g_0 r_0 a_0 u_{13} + g_0 r_5 a_2 u_0 \]
\[ I_{14} = g_0 r_0 a_0 u_{14} + g_0 r_5 a_2 u_1 \]
\[ I_{15} = g_0 r_0 a_0 u_{15} + g_0 r_5 a_2 u_2 \]
\[ I_{16} = g_0 r_0 a_0 u_{16} + g_0 r_5 a_2 u_3 \]
\[ I_{17} = \text{NA contribution only} \]

Solving these parameter values estimates \(^{13}\text{C}\) incorporation into UDP-GlcNAc biosynthesis.

Moseley et al., BMC Biol 2011;9:37

Metabolism is Substructures

Moiety Model Output: Deconvoluted Time Course of UDP-GlcNAc

There is no: "Biosynthetic rate" or "Flux thru" UDP-GlcNAc

Metabolism is substructures

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

Without MSA

With MSA

Earlier example of “global” lipid analysis was regarding substructures.

Control

Selenite

CH₂-O-R₁ (C18:0)

CH₂-O-R₂ (C20:4)

CH₂-O-P-inositol

PI

“Sufficient” MS Resolution

FT-MS of Lipids:
5000 real peaks in 5 min
A549 cells U-[13C]-glucose

How do we know it’s [13C]-labeled?

FT-MS of Lipids:
5000 real peaks in 5 min
A549 cells U-[13C]-glucose

Need Sub-atomic Resolving Power!
**“Sufficient” Resolution Unlocks Untargeted Analysis with Nanospray Sensitivity in 10 min**

- **A** (12C): FTMS 12C
- **B** (13C): FTMS 13C

**How Much is “Sufficient” Resolution?**

- +2 sits on top of +0, same intensity
- R=100K is required theoretically
- Let’s increase abundance of [13C2]PC34:3 by 10x

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**How Much is “Sufficient” Resolution?**

- Increased abundance of [13C2]PC34:3 by 10x
- Abundance changes the resolution requirement !!!
- R ≥ 200K is required actually

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Lorkiewicz et al., Metabolomics DOI: 10.1007/s11306-011-0388-y
“Sufficient” Resolution Unlocks Untargeted Analysis with $^{13}$C

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<thead>
<tr>
<th>m/z</th>
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Unlabeled

Increase “Sufficient” Resolution to >350K Enables MS1 = Untargeted Multiplexing of Isotopic Substructures Ex: $^{13}$C$^{15}$N Nucleotides

Wait! Earlier +5 was $^{13}$C$_2$ : ribose ring. What gives?


“Sufficient” Resolution Unlocks Untargeted Analysis with $^{13}$C – Substructure with MS1

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Increase “Sufficient” Resolution to >350K Enables MS1 = Untargeted Multiplexing of Isotopic Substructures Ex: $^{13}$C$^{15}$N Nucleotides

From $^{13}$C$_5$N$_2$ Gln

Key Concepts of “Sufficient” MS Resolution

- Biochemical Information Content
- Structural Data Content

Decimal Places of Resolution

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HUMAN SUBJECTS

13C Dispersal into Substructures

HUMAN SUBJECTS

13C 15N SIRM in Tissue Slice Expts

Prep

blood sample

Tissue resection: tumor x non-tumor

Lab

Sample medium at 0 and other timed intervals

Insulate 37°C 4 h

Freeze

T h retrieve slices, part into formalin, freeze remainder

Extract metabolites

Extract into polar + non-polar + protein fractions

Quantitative isotopomer and isotopologue analysis by NMR and MS

1-C metabolism

Gly

SHMT

Ser

CO2

Oxaloacetate

Citrate

c-Aconitate

CO2

Succinate

Glu

Lactate

Aspartate

Malate

Fumarate

Succinyl CoA

Glu

NC

CA

Warburg slices

Sellers et al. JCI http://dx.doi.org/10.1172/JCI72873

Fan et al, Bioprotocols, in press

Fan et al, Cold Spring Harbor Molecular Case Studies, accepted
SIRM provides atom-resolved sources of nucleotide synthesis
Lesson in using double-label

Cancer cells use biosynthesized Gly for nucleotide biosynthesis
vs abundant exogenous Gly
Lesson in double-label

Other isotopes help track $^{13}\text{C}$ & vice versa

Data from Sellers et al. JCI http://dx.doi.org/10.1172/JCI72873
Cancer cells use biosynthesized Gly for nucleotide biosynthesis vs abundant exogenous Gly

Lesson in double-label

Summary

• Stable-Isotope Resolved Metabolism
  ➢ Metabolism is substructures
  ➢ “Sufficient” Resolution in MS
  ➢ Forward to mSIRM

13C 15N labeling = biologically relevant substructures with untargeted MS1

13C 15N labeling = biologically relevant substructures with untargeted MS1

….. revealing key dynamics of single-label by using double-label
Summary

• Stable-Isotope Resolved Metabolism
  ➢ Metabolism is substructures
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Untargeted MS1: Simultaneous multiple pools with $^{13}$C $^{15}$N labeling

HIT enables human patient Cancer vs Non-cancer tissues