Following pathways with isotopes
Stephen Barnes

Fluxomics

- A feature of many metabolites is that they have multiple origins

![Diagram showing metabolic pathways with isotopes](image-url)

Fan et al. 2013
The ratio of $^{13}$C-labeling between the carbon atoms (by $^{13}$C-NMR) tells us about the turnover of the TCA cycle and the input of glutamate.
Effect of selenite on pools of intermediates

Pyrurate carboxylase converts pyruvate to oxaloacetate and by-passes the early steps in the Krebs cycle. Treatment of the cells with selenite blocks this step and the $^{13}$C-content of citrate sharply decreases. 

Fan et al. 2013
Anaplerotic reactions

High resolution FT-ICR-MS

Fan et al. 2013
Use of $^1$H-$^{13}$C-NMR

Changes in intermediates in lung cancer
Biological NMR

• If $^{13}$C-labeled precursors are used, there is a very much enhanced set of $^{13}$C NMR resonances
• You have a choice between analysis of a biological extract (have all the time you need)
• And direct analysis in tissue:
  • Surface coil technology in the living animal
  • Magic Angle Spinning on a piece of tissue

NMR analysis of metabolites from $^{13}$C-labeled precursors using pulse sequences
Carbonyl derivatization reagents

- DNPH
- PFBHA
- TMPP-PrG
- Girard-P reagent
- 4-APEBA

Isotopic carbonyl reagents

- QDA
- *QDA
Thiol derivatization reagents

IAA

IAM

R = CH₃, MMTS

NEM

VP

Detectable thio-metabolites

L-glutathione

L-cysteinylglycine

L-homocysteine

L-cysteine

hypotaurine
Thiol metabolites in A459 cell extract

$^{15}$N-labeled derivatization reagent

Gori et al., 2014

Lane et al., 2014
\textbf{15N-NMR of derivatized metabolites}

1D-HSQC spectrum of stds

1D-HSQC spectrum of polar extract of A549 cells

1D-presat spectrum of A549 cells

\textbf{2D-1H, 15N-NMR of standards}

Lane et al., 2014
2D-$^1$H, $^{15}$N-NMR of A459 cell extract

References


