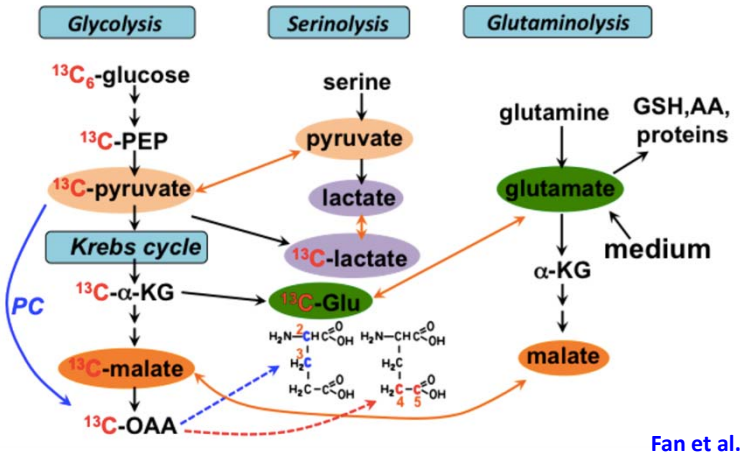
Following pathways with isotopes

Stephen Barnes

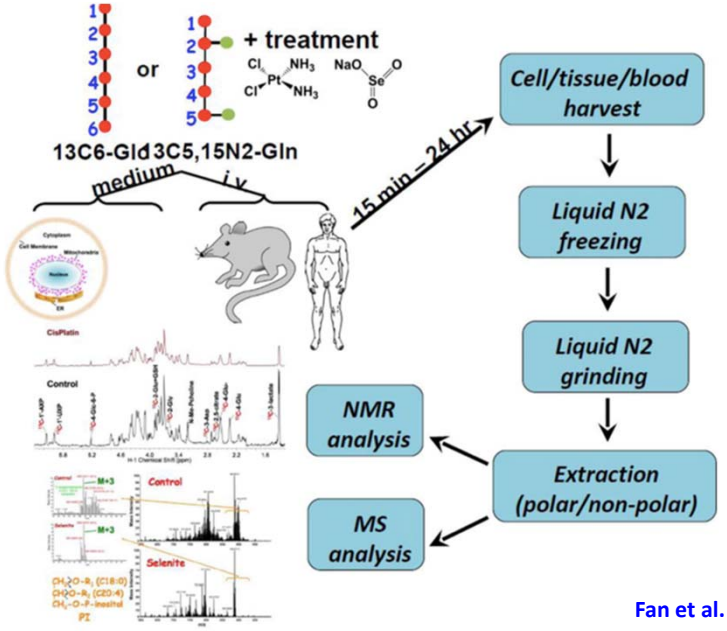
Fluxomics

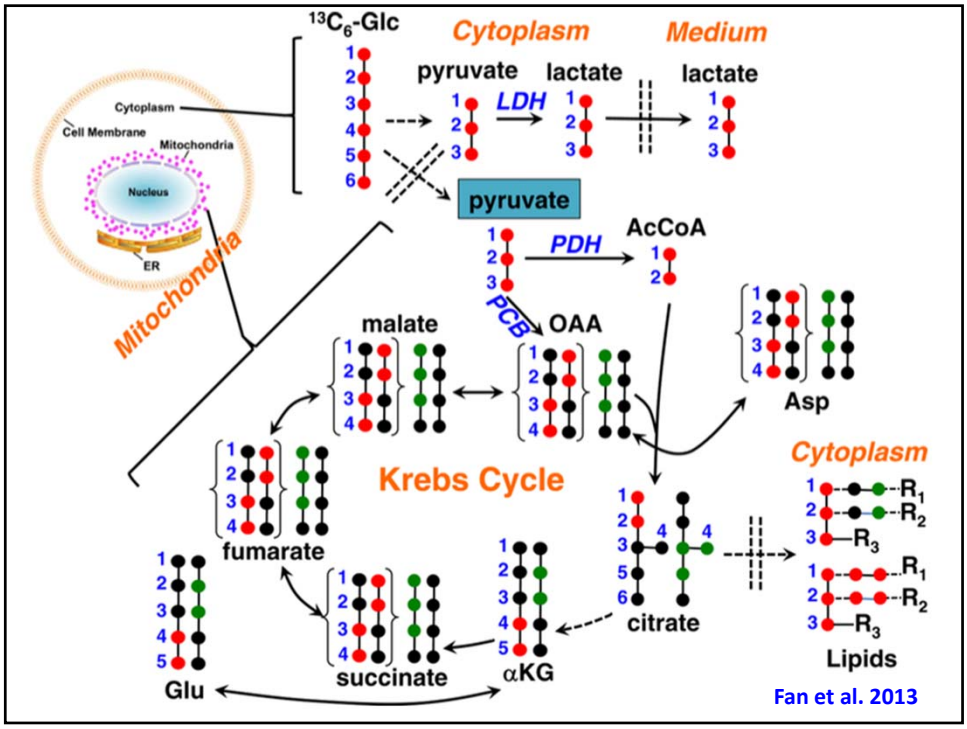
- **A feature of many metabolites is that they have multiple origins**

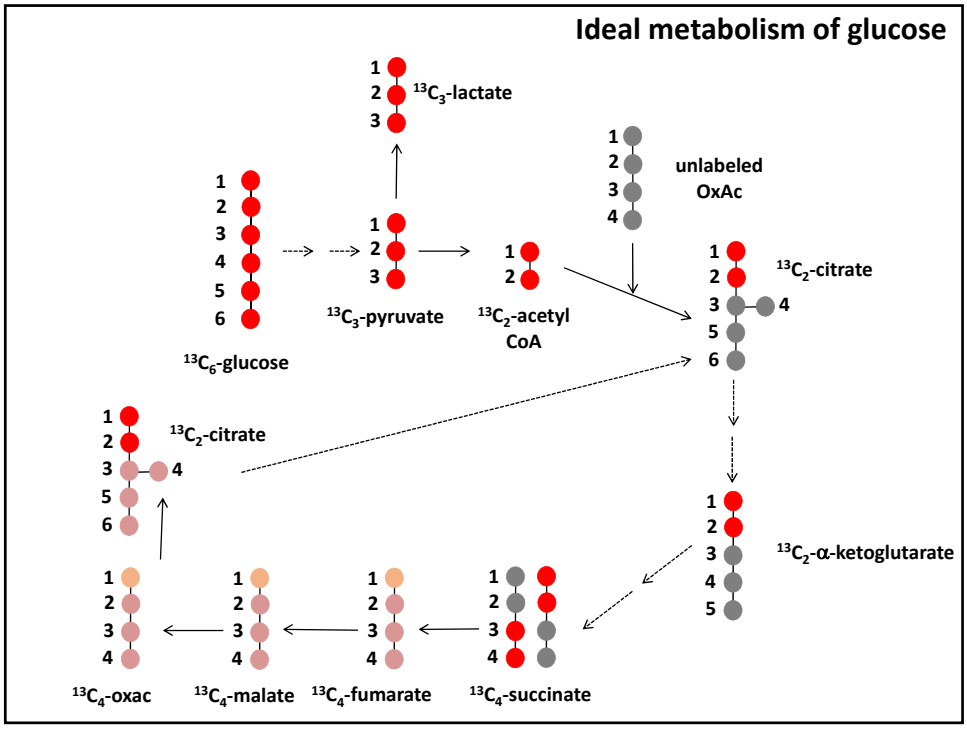


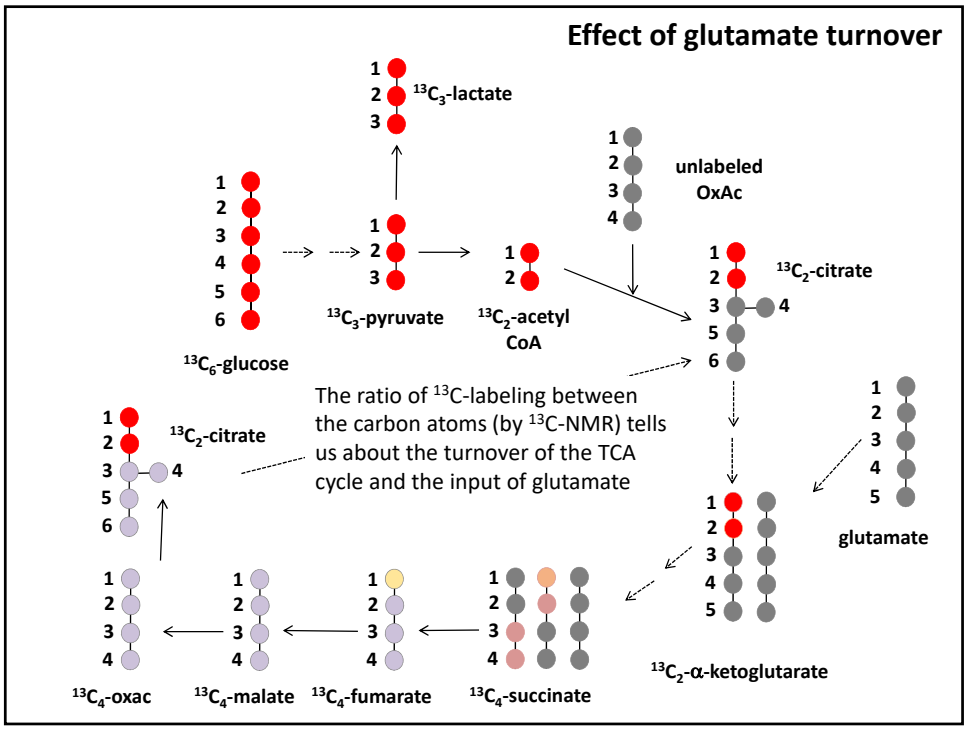
Fan et al. 2013

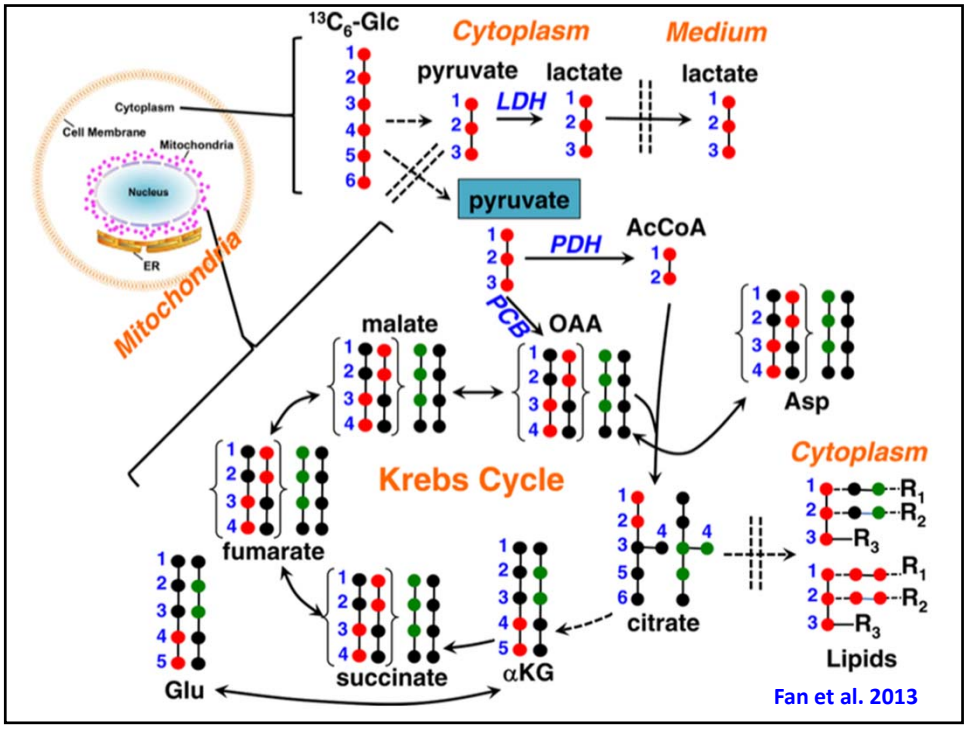
Stable isotope resolved metabolomics





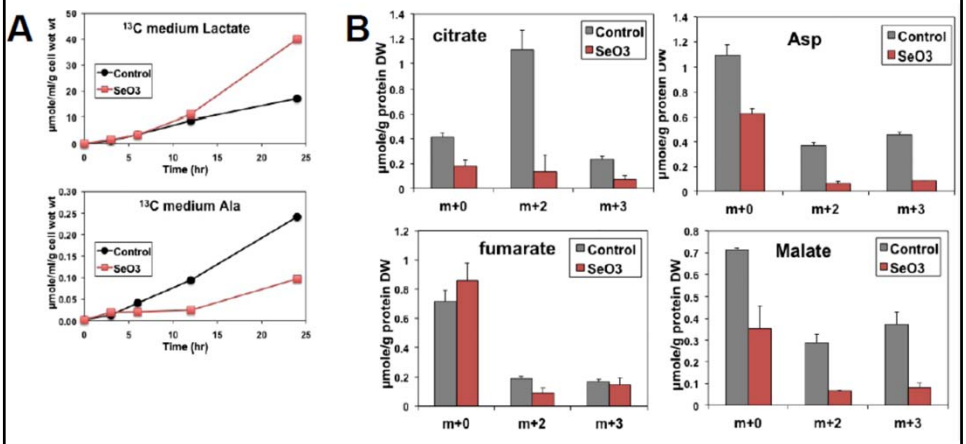

Ideal metabolism of glucose
$^{13}C_3$-lactate
unlabeled OxAc
$^{13}C_6$-glucose
$^{13}C_3$-pyruvate
$^{13}C_2$-acetyl CoA
$^{13}C_2$-citrate
$^{13}C_2$-citrate
$^{13}C_2$-α-ketoglutarate
$^{13}C_4$-oxac
$^{13}C_4$-malate
$^{13}C_4$-fumarate
$^{13}C_4$-succinate


Effect of glutamate turnover
$^{13}C_3$-lactate
unlabeled OxAc
$^{13}C_6$-glucose
$^{13}C_3$-pyruvate
$^{13}C_2$-acetyl CoA
$^{13}C_2$-citrate
$^{13}C_2$-citrate
glutamate
$^{13}C_2$-α-ketoglutarate
$^{13}C_4$-oxac
$^{13}C_4$-malate
$^{13}C_4$-fumarate
$^{13}C_4$-succinate
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



Fan et al. 2013

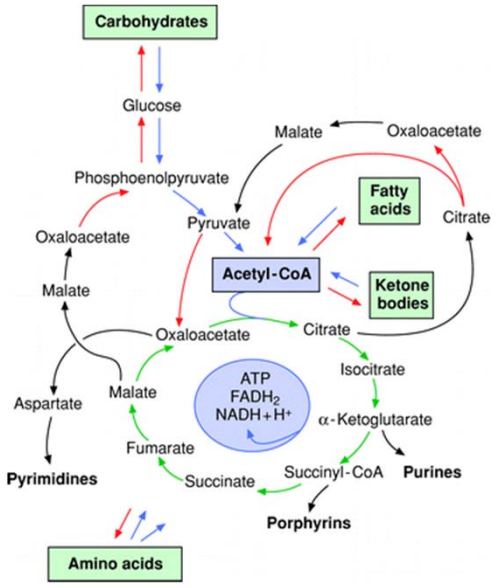
Effect of selenite on pools of intermediates



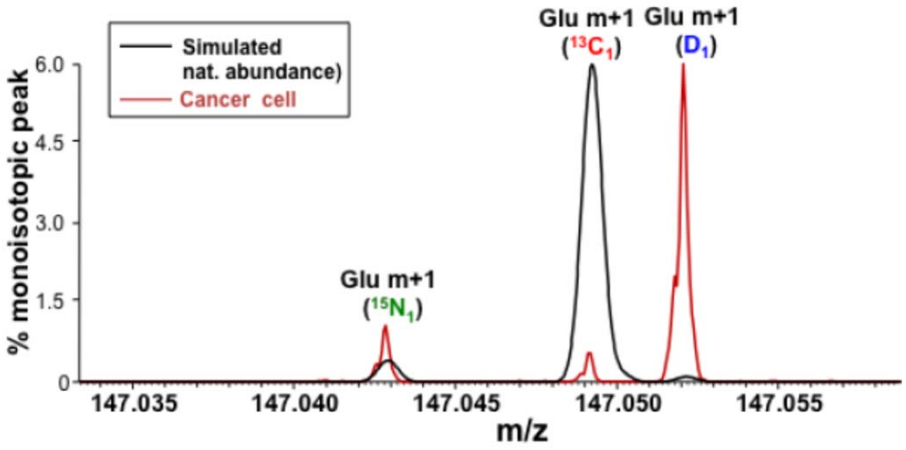
Pyruvate carboxylase converts pyruvate to oxaloacetate and by-passes the early steps in the Krebs cycle. Treatment of the cells with selenite blocks this step and the ^{13}C-content of citrate sharply decreases

Fan et al. 2013

Anaplerotic reactions

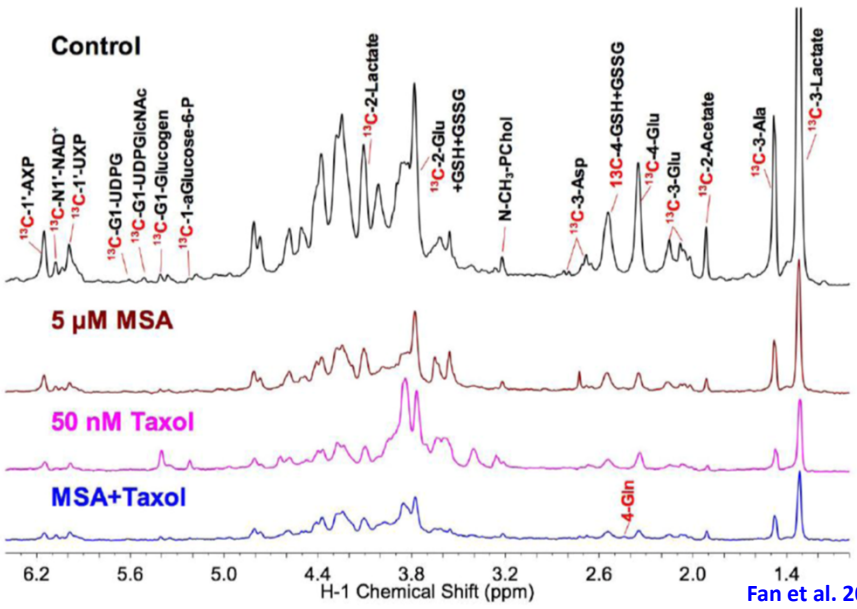


High resolution FT-ICR-MS



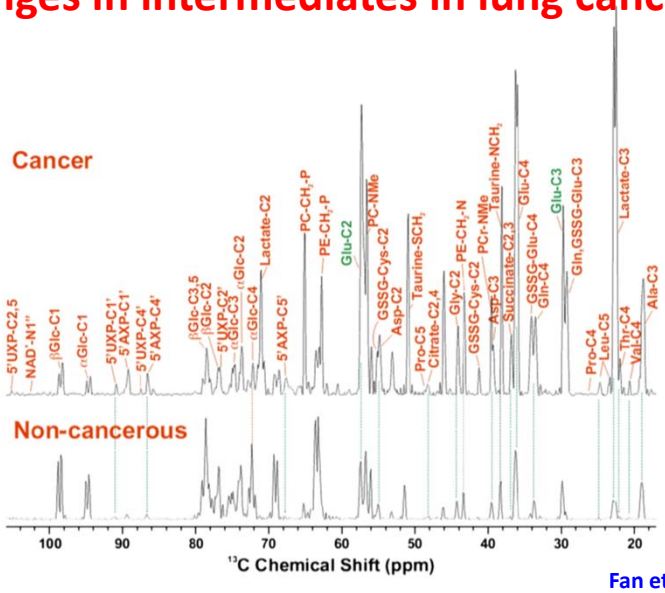
Fan et al. 2013

Use of ^{1}H-^{13}C-NMR



Fan et al. 2013

Changes in intermediates in lung cancer

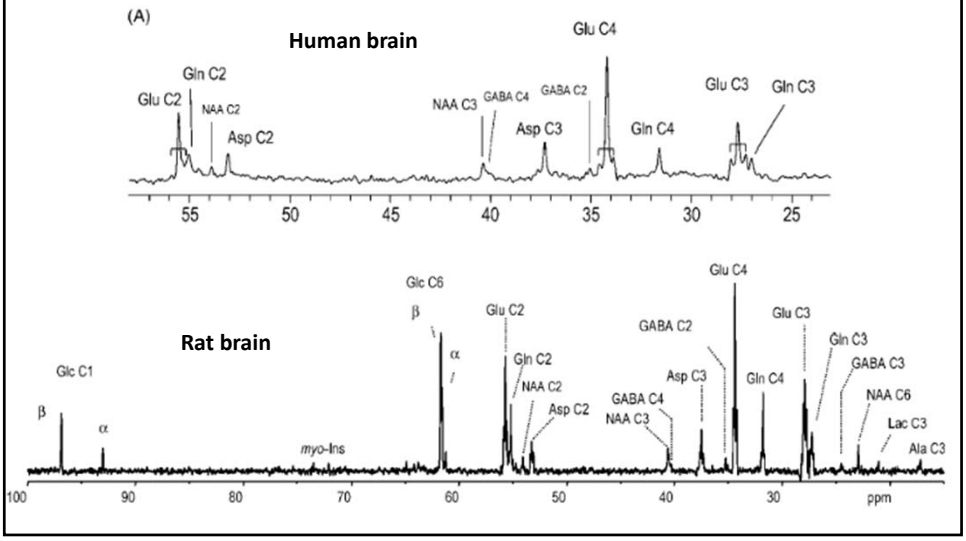


Fan et al. 2013

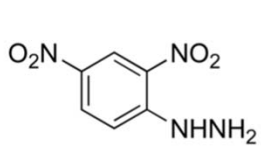
Biological NMR

- 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

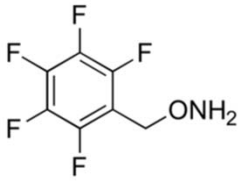
NMR analysis of metabolites from ^{13}C-labeled precursors using pulse sequences



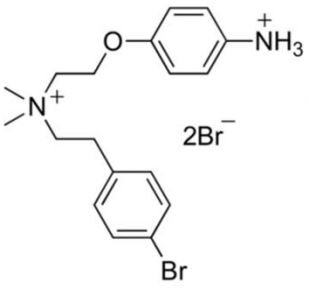
Carbonyl derivatization reagents



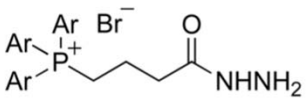
DNPH



PFBHA

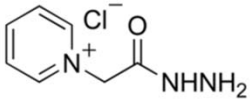


4-APEBA



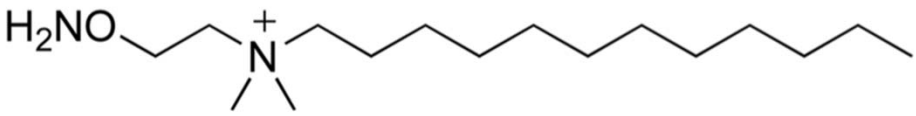
Ar = 2,4,6-trimethoxyphenyl

TMPP-PrG

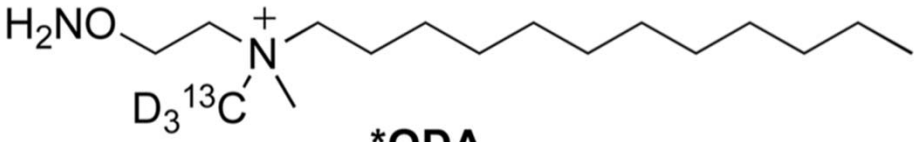


Girard-P reagent

Isotopic carbonyl reagents

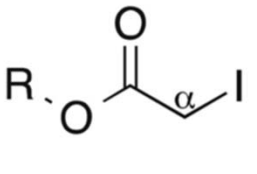


QDA

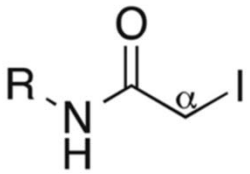


*QDA

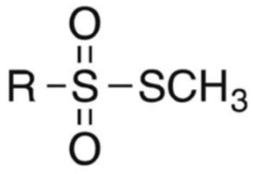
Thiol derivatization reagents



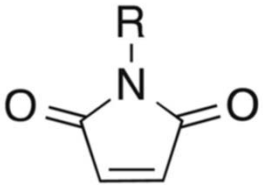
IAA



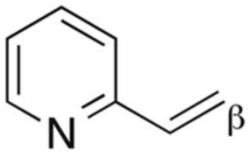
IAM



R = CH_3, **MMTS**

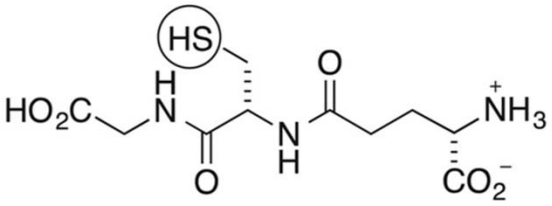


R = CH_3CH_2, **NEM**

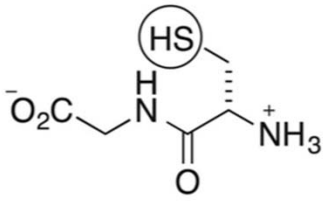


VP

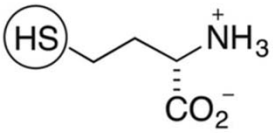
Detectable thio-metabolites



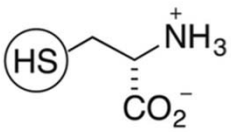
L-glutathione



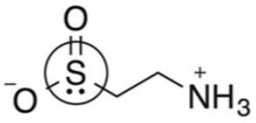
L-cysteinylglycine



L-homocysteine

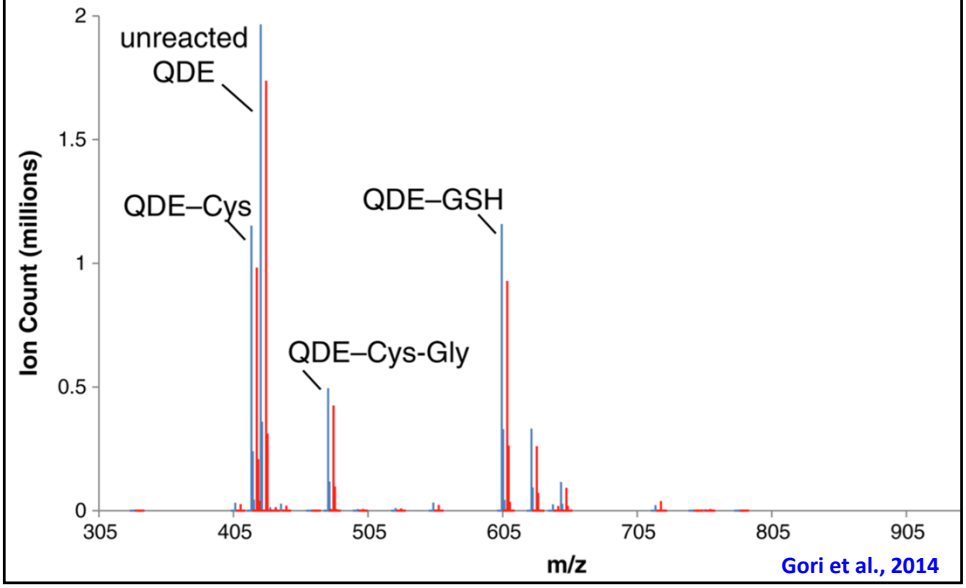


L-cysteine



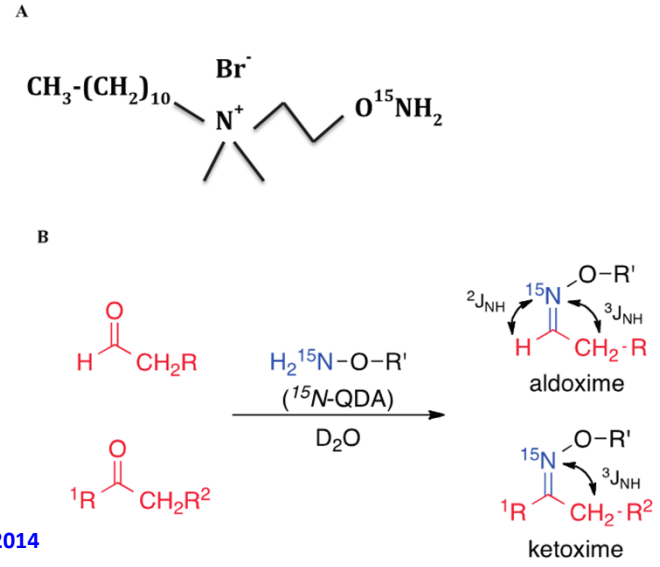
hypotaurine

Thiol metabolites in A459 cell extract



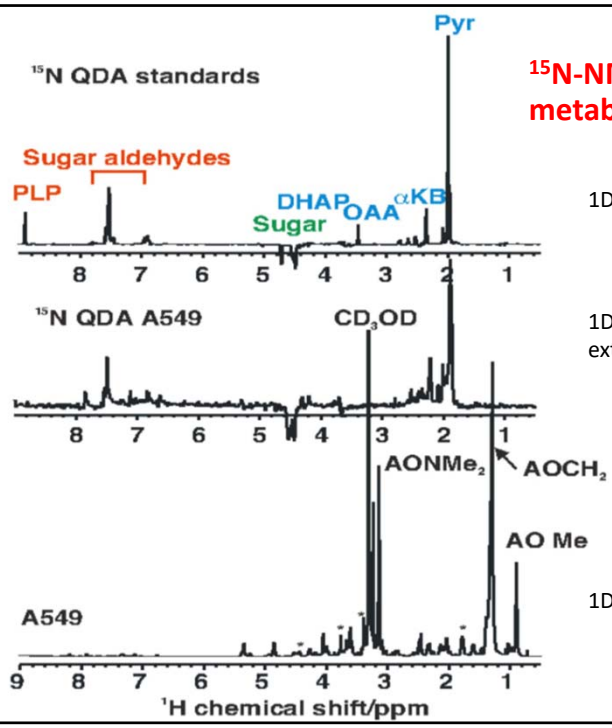
Gori et al., 2014

^{15}N-labeled derivatization reagent



Lane et al., 2014

^{15}N-NMR of derivatized metabolites



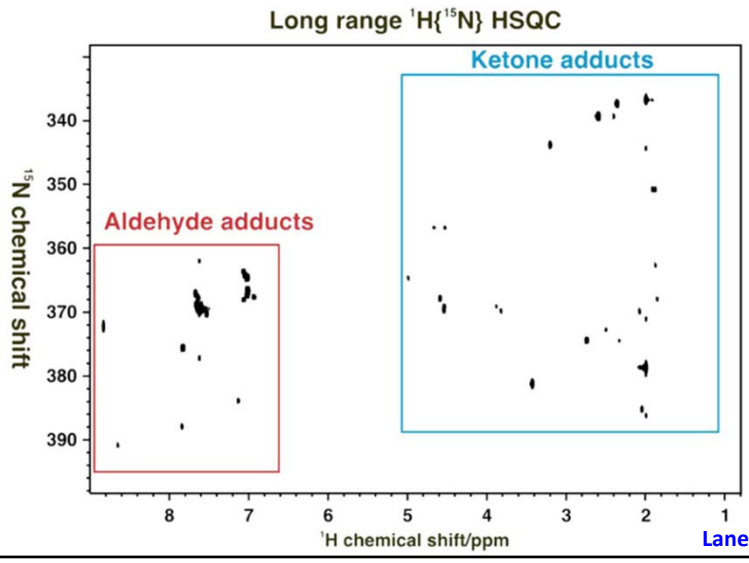
1D-HSQC spectrum of stds

1D-HSQC spectrum of polar extract of A549 cells

1D-presat spectrum of A549 cells

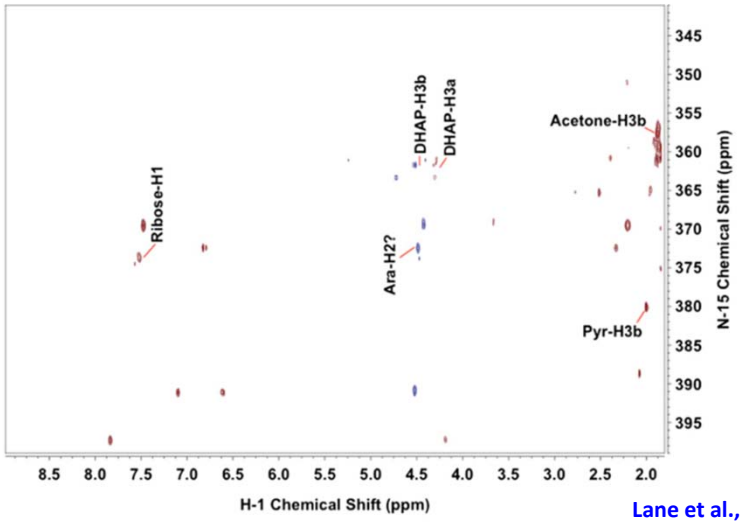
Lane et al., 2014

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



Lane et al., 2014

2D-^{1}H, ^{15}N-NMR of A459 cell extract



Lane et al., 2014

References

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- Qiu Y, Moir R, Willis IM, Beecher C, Tsai YH, Garrett TJ, Yost RA, Kurland IJ. Isotopic Ratio Outlier Analysis (IROA) of the S. cerevisiae metabolome using accurate mass GC-TOF/MS: A new method for discovery. Anal Chem. 2016 Jan 28. [Epub ahead of print]
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- Fan TW, Lorkiewicz PK, Sellers K, Moseley HN, Higashi RM, Lane AN. Stable isotope-resolved metabolomics and applications for drug development. Pharmacol Ther. 2012 Mar;133(3):366-91.