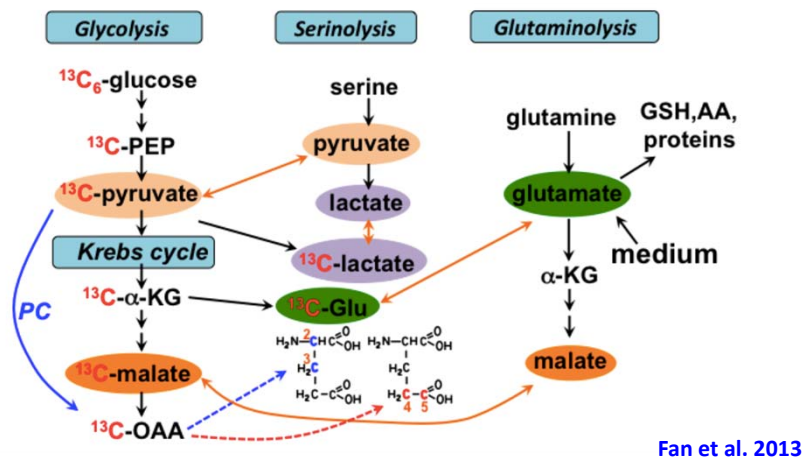


Following pathways with isotopes

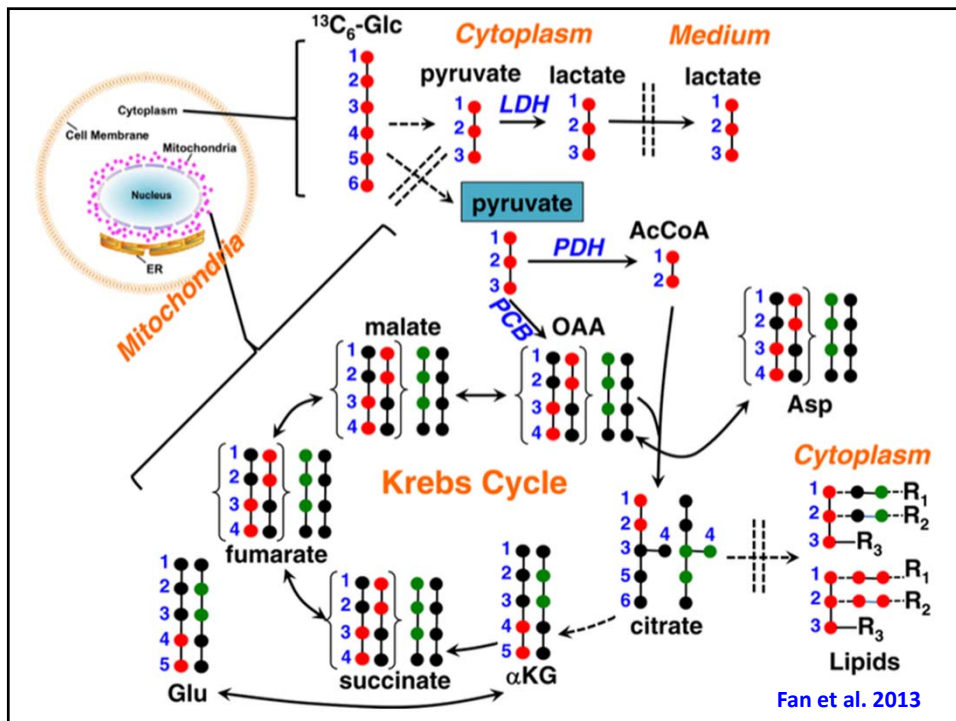
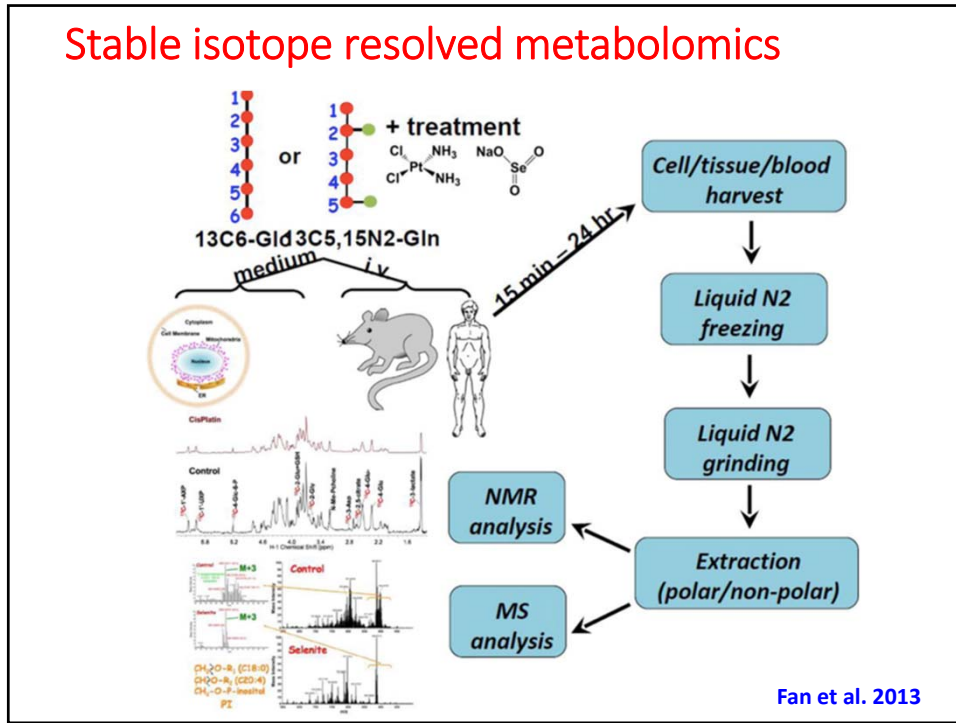
Stephen Barnes, PhD

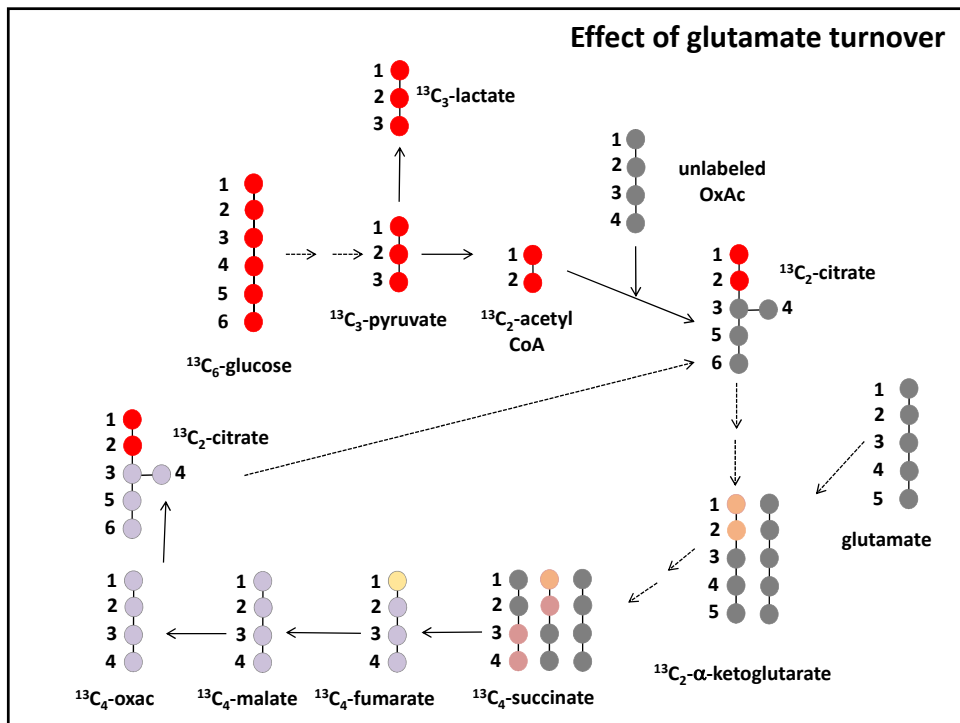
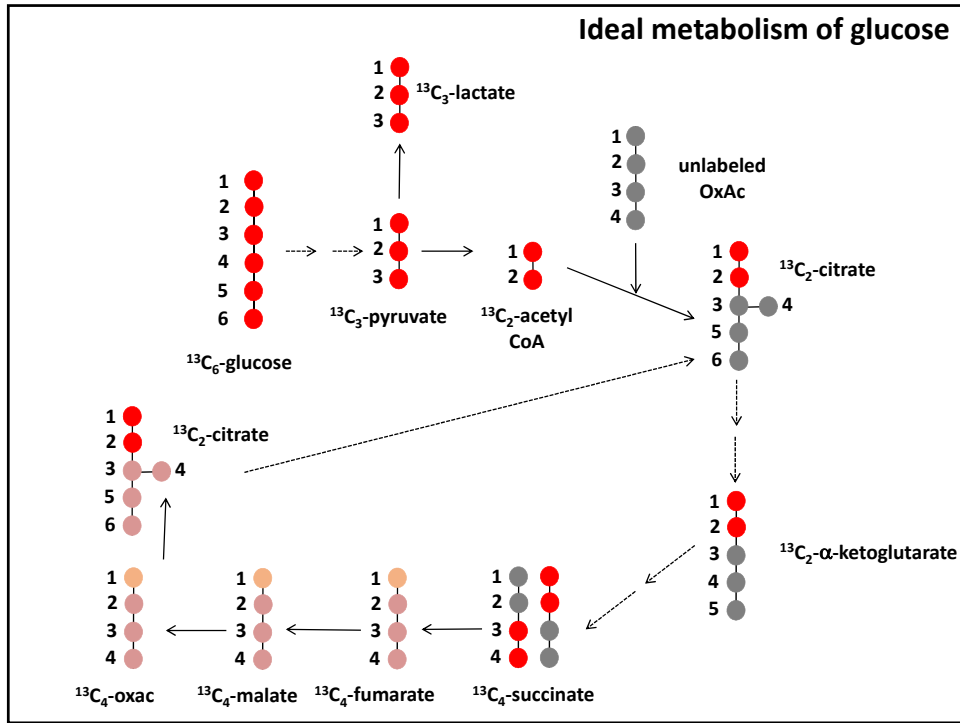
Fluxomics

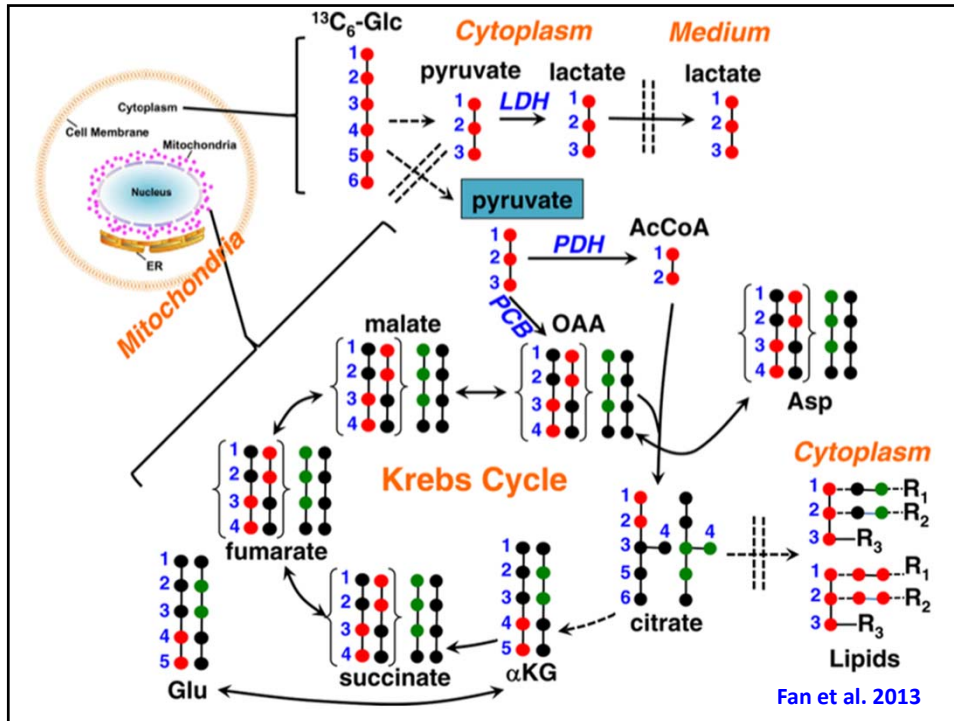
- A feature of many metabolites is that they have multiple origins



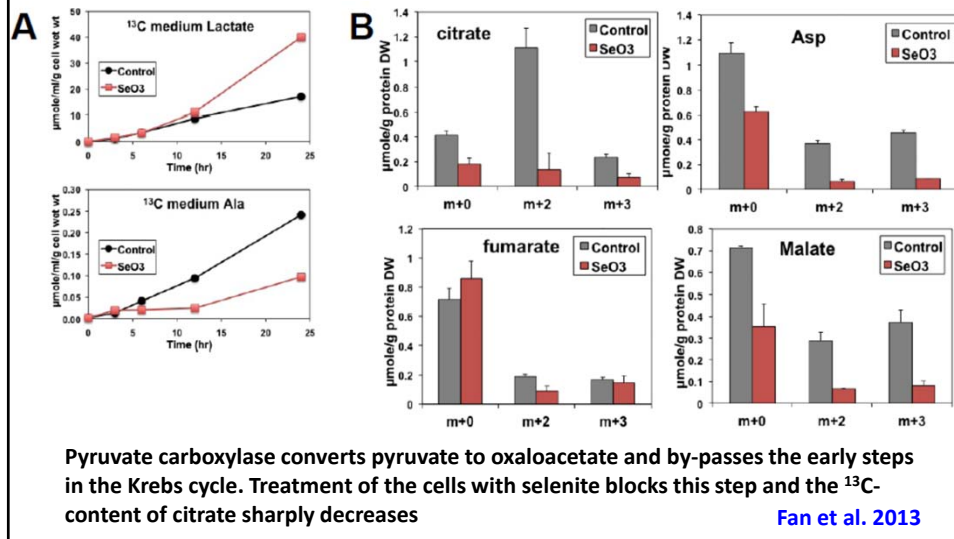
Stable isotope resolved metabolomics



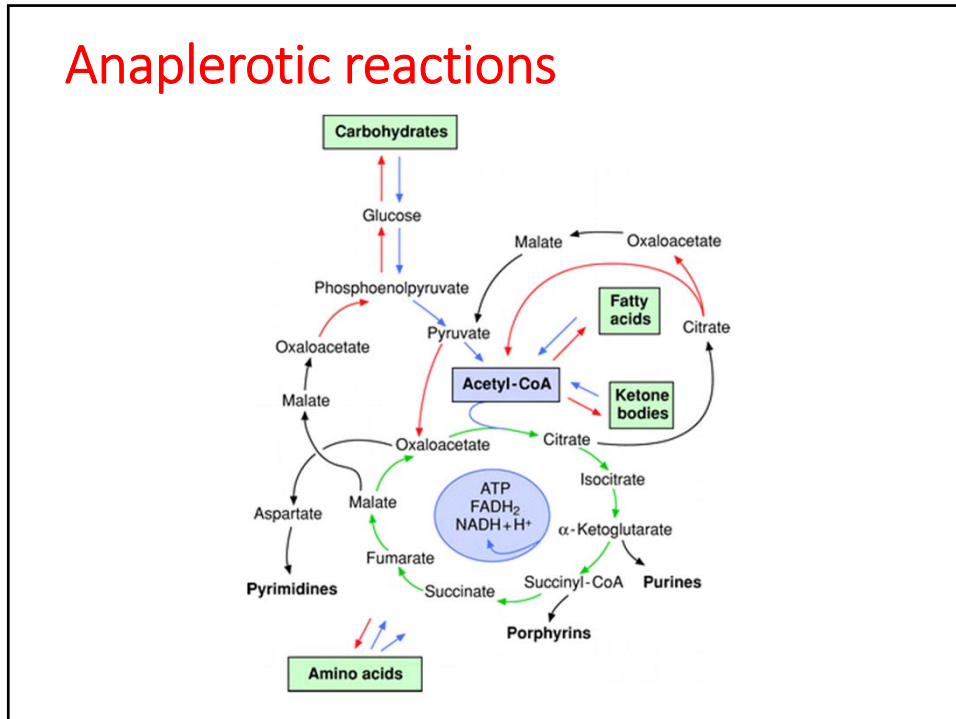




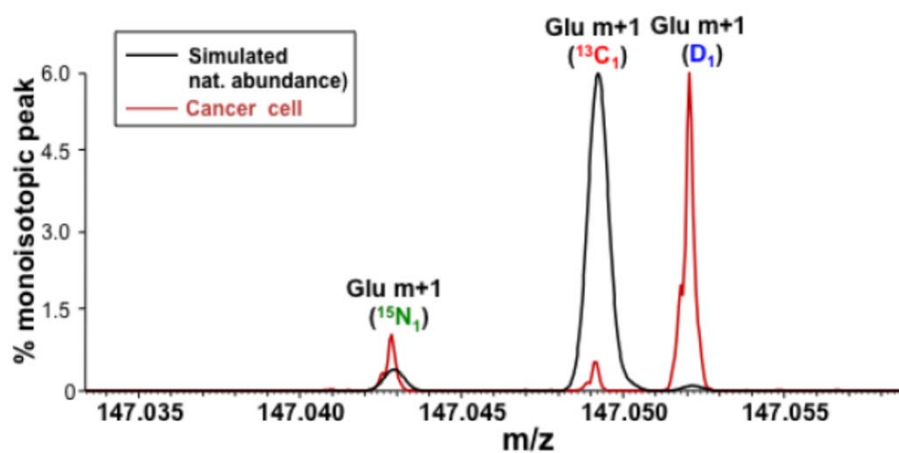
Effect of selenite on pools of intermediates



Anaplerotic reactions

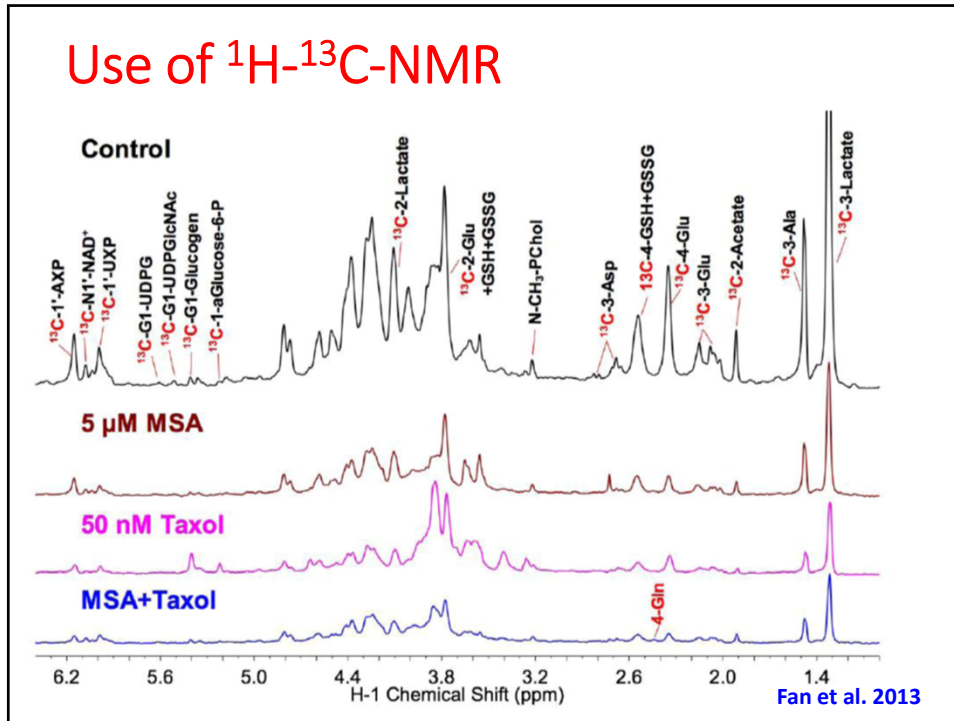


High resolution FT-ICR-MS

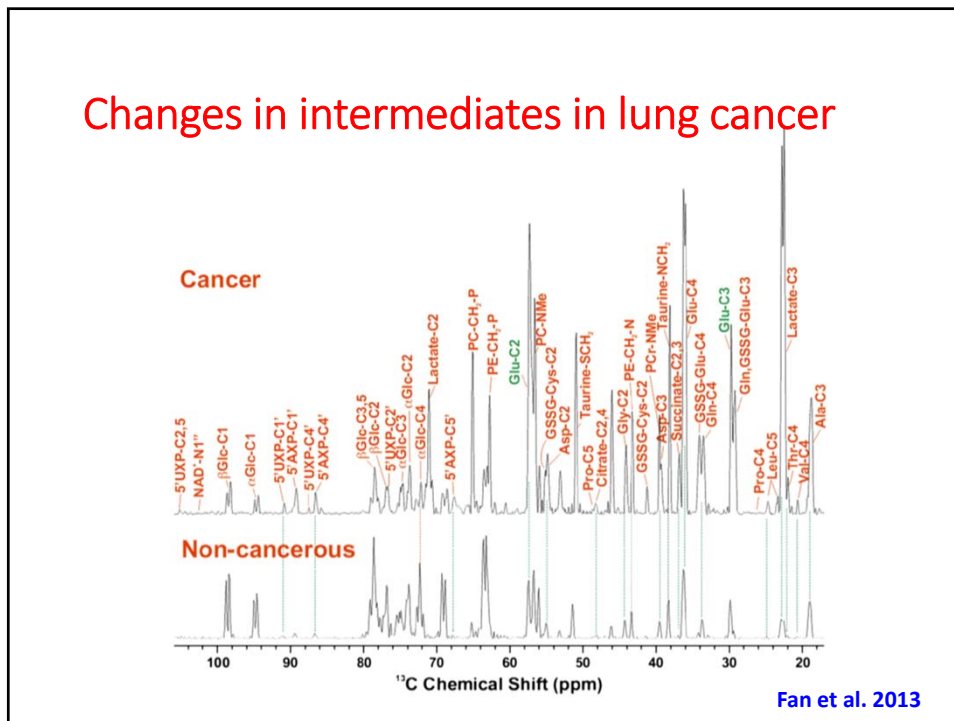


Fan et al. 2013

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



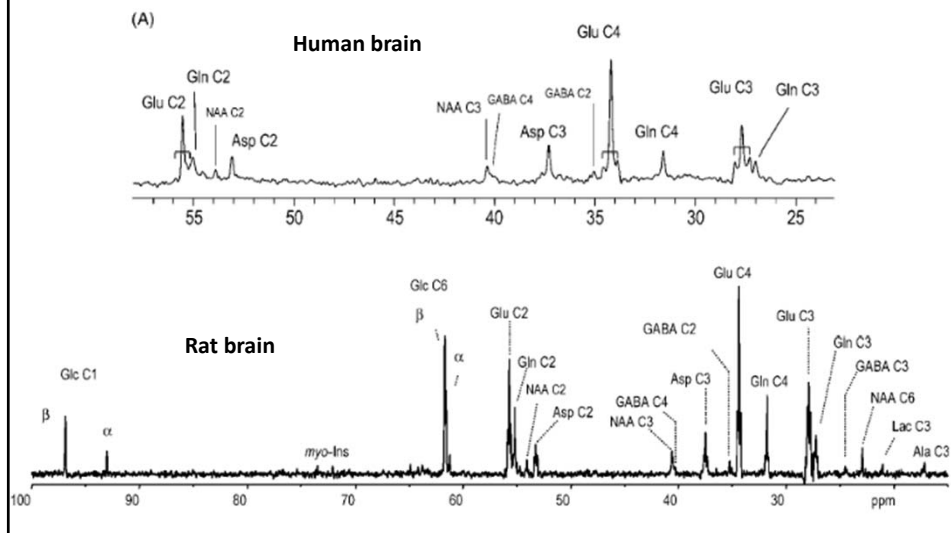
Changes in intermediates in lung cancer



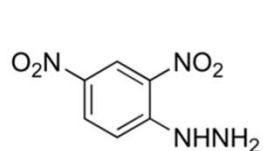
Biological NMR

- 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 (see talk by Dr. Krishna) on a piece of tissue

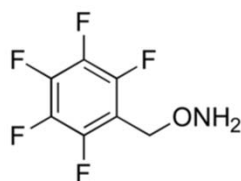
NMR analysis of metabolites from ^{13}C -labeled precursors using pulse sequences



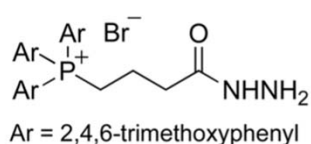
Carbonyl derivatization reagents



DNPH

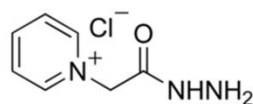


PFBHA

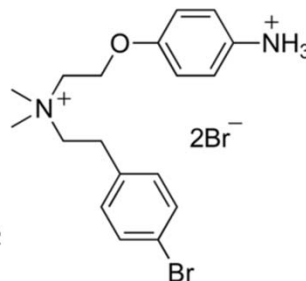


Ar = 2,4,6-trimethoxyphenyl

TMPP-PrG

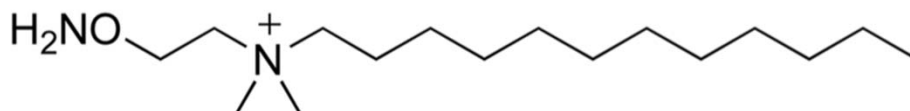


Girard-P reagent

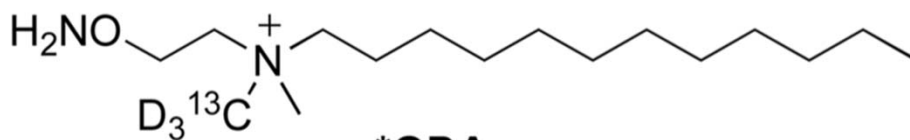


4-APEBA

Isotopic carbonyl reagents

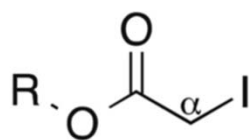


QDA

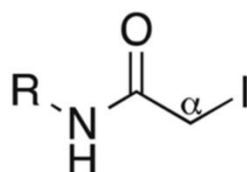


$^*\text{QDA}$

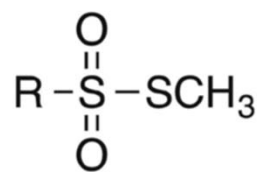
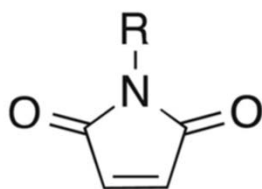
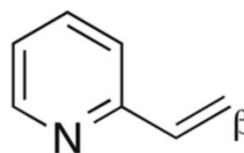
Thiol derivatization reagents



IAA

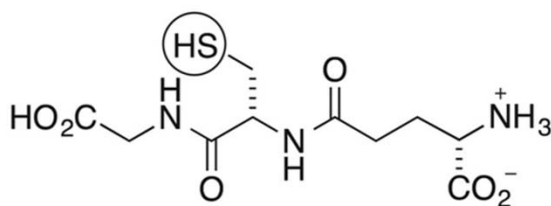


IAM

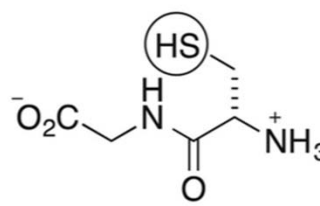
R = CH₃, MMTSR = CH₃CH₂, NEM

VP

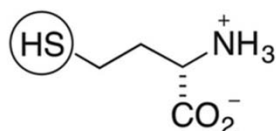
Detectable thio-metabolites



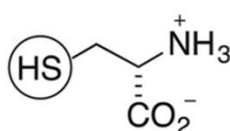
L-glutathione



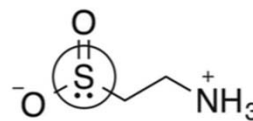
L-cysteinylglycine



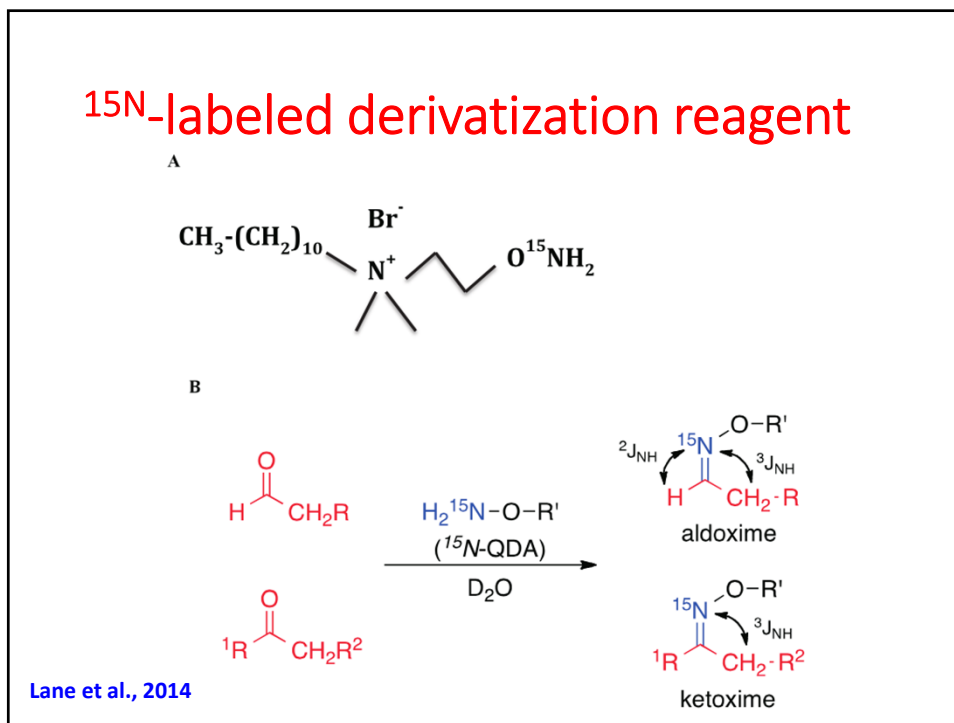
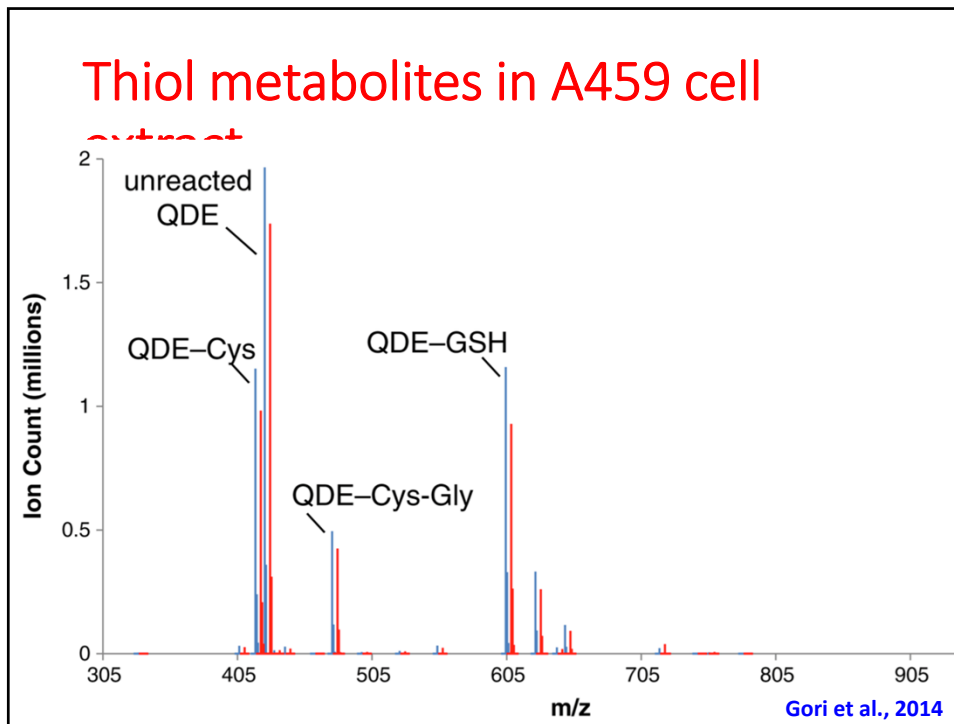
L-homocysteine

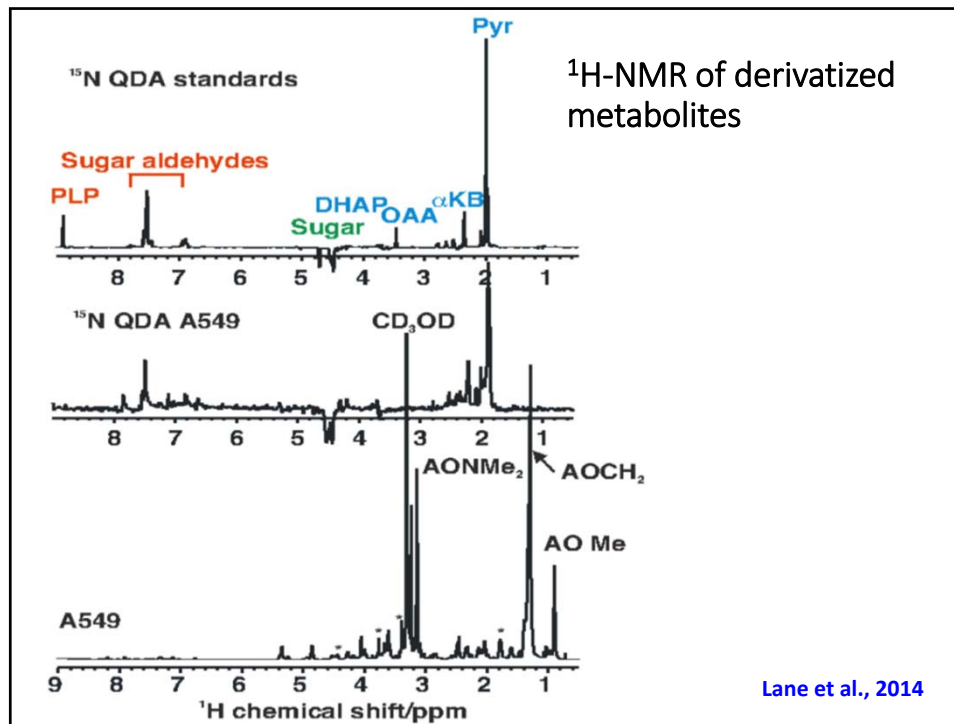


L-cysteine

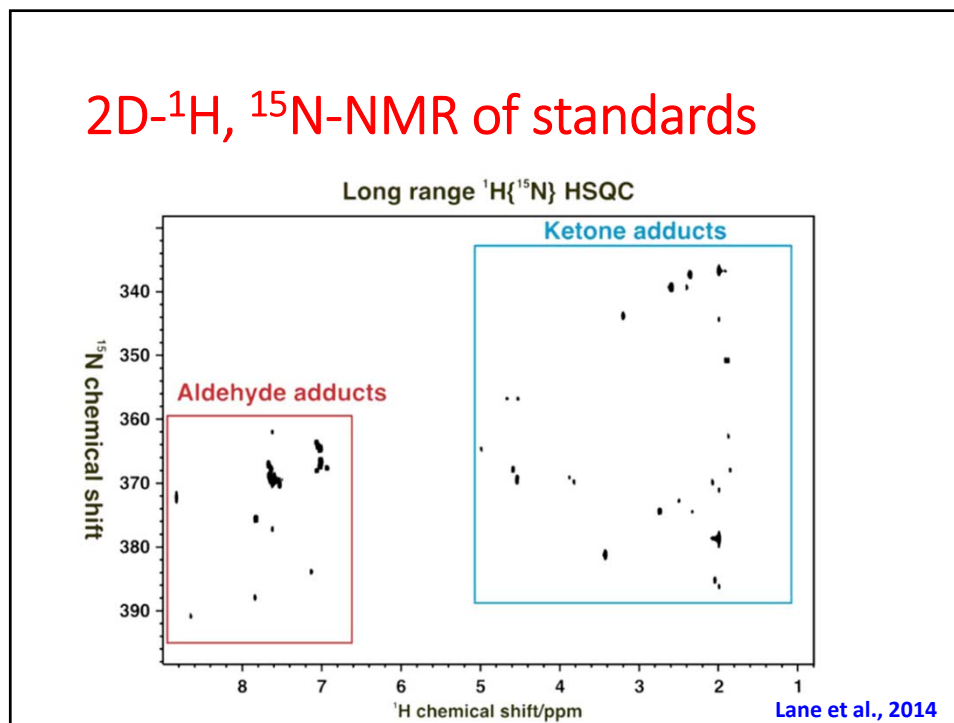


hypotaurine

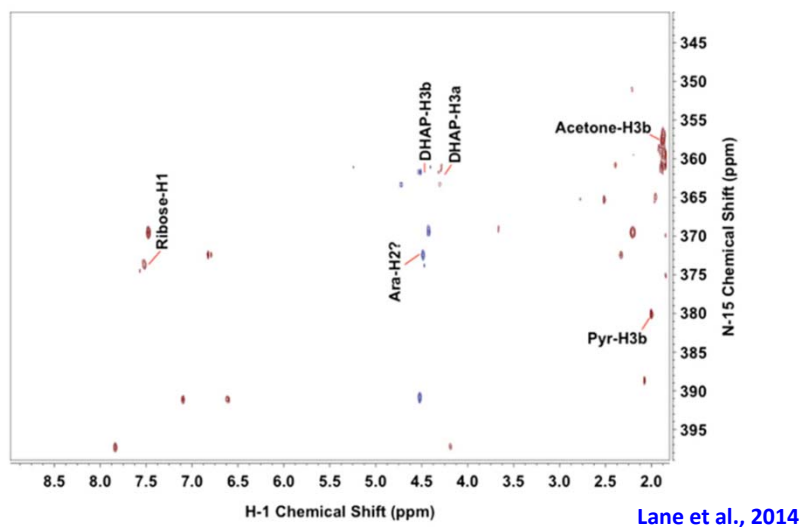




2D-¹H, ¹⁵N-NMR of standards



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



References

- Stupp GS, Clendinen CS, Ajredini R, Szewc MA, Garrett T, Menger RF, Yost RA, Beecher C, Edison AS. Isotopic ratio outlier analysis global metabolomics of *Caenorhabditis elegans*. [Anal Chem. 2013 Dec 17;85\(24\):11858-65.](#)
- Lane AN, Arumugam S, Lorkiewicz PK, Higashi RM, Lauhé S, Nantz MH, Moseley HN, Fan TW. Chemosensitive detection and discrimination of carbonyl-containing compounds in metabolite mixtures by ^1H -detected ^{15}N nuclear magnetic resonance. [Magn Reson Chem. 2015 Jan 23. doi: 10.1002/mrc.4199.](#)
- 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.](#)