Use of isotopes in metabolomics

Stephen Barnes, PhD

2-22-17

Synopsis

- Natural abundance isotopes
- Tracing a metabolic pathway
  - Labeling a precursor for qualitative analysis
  - 95% isotope/5% unlabeled and 5% unlabeled/95% isotope
- Following individual carbon atoms
- Quantitative analysis of metabolic flux
- Post-extraction isotopic labeling

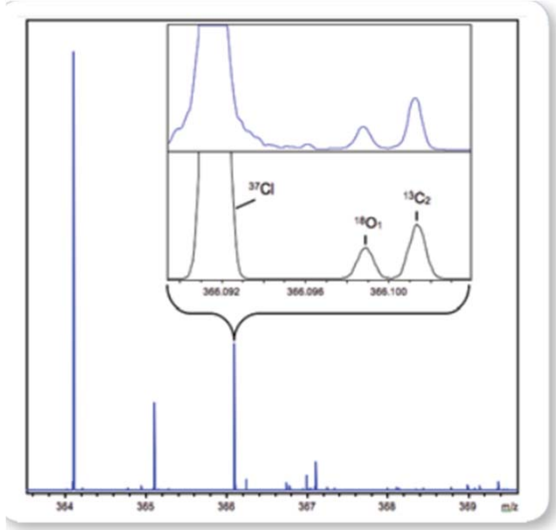
Value of natural isotopes

- **The natural abundance of isotopes enables the investigator to determine the charge state of an ion**
  - **The principal contribution to $[M+H]^+$ or $[M-H]^-$ isotope ions comes from ^{13}C (~1.1% of all carbon atoms)**
  - **The intensity of the ^{13}C isotope ion increases relative to the number of carbon atoms**
  - **There is often an observable $^{13}C_2$ isotope peak**

Value of the [M+/-H+2] peak

- **The mass difference due to a nominal increase in mass of 2 contains a lot of information**
  - **These are isotopic mass differences for each of the common elements**
    - **$^1H_2/^2H_2$ 2 x 1.006277 = 2.012554 (0.012%)**
    - **$^{12}C_2/^{13}C_2$ 2 x 1.003355 = 2.006710 (1.078%)**
    - **$^{14}N_2/^{15}N_2$ 2 x 0.997035 = 1.994079 (0.364%)**
    - **$^{16}O_2/^{17}O_2$ 2 x 1.004217 = 2.008434 (0.038%)**
    - **$^{16}O_2/^{18}O_1$ 1 x 2.004246 = 2.004246 (0.205%)**
    - **$^{32}S_2/^{33}S_2$ 2 x 0.999387 = 1.998774 (0.752%)**
    - **$^{32}S_2/^{34}S_1$ 1 x 1.995796 = 1.995796 (4.252%)**

The importance of the M+2 ion

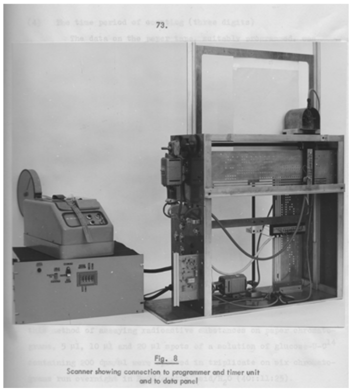


From Bruker

Using isotopes to trace a pathway

- Early studies (1930s) used ^{2}H, ^{13}C and ^{15}N labeling to map pathways
  - Limited to 1-200 *m/z* mass range
- 1950s/60s ^{14}C-radiotracers
  - 2D-Paper or thin layer chromatography
  - Radio gas chromatography
    - labeling of specific carbon atoms

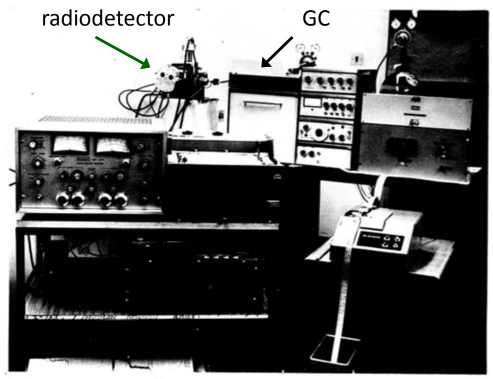
Origins of practical metabolomics
Imperial College 1967-1970



Radio 2D-paper chromatography scanner with digitization of collected data

The room had 20 of these scanners – data analyzed by a central computer (in 1968)

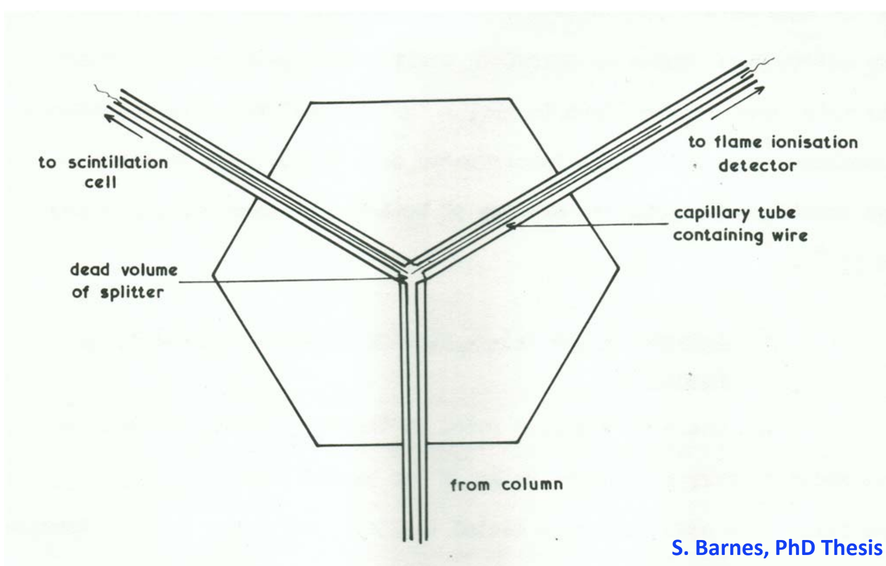
Courtesy of K.R. Mansford, PhD



Radio gas-liquid chromatography with digitization of collected data

Developed this for my PhD work (1967-1970) to study glucose metabolism in acellular slime moulds

Stream splitter for radio GC



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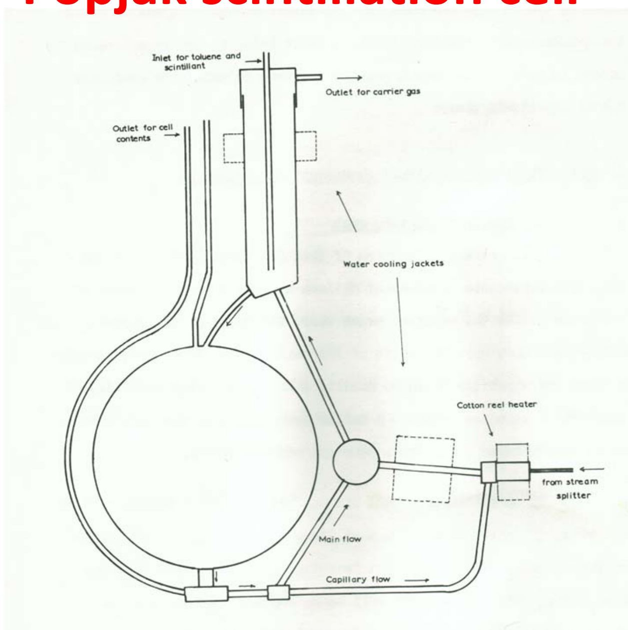
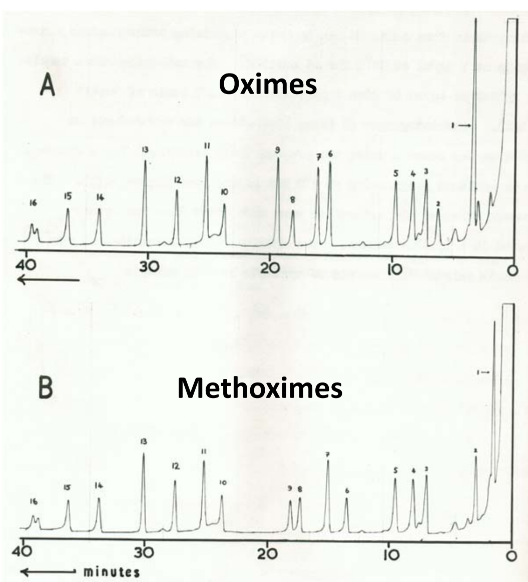
Popjak scintillation cell



GC of glycolytic and Krebs cycle intermediates



Temperature programming of TMS ester/ethers on a 5' x ¼ inch packed column of Chromosorb W coated with OV-1 liquid phase

1=pyruvate , 2=?? , 3=phosphate , 4=succinate , 5=fumarate, 6=oxaloacetate, 7=malate, 8=αKG, 9=hexadecane, 10=αGP, 11=citrate, 12=α–D-glucose, 13=β–D-glucose, 14=docosane, 15=F6P, 16=G6P

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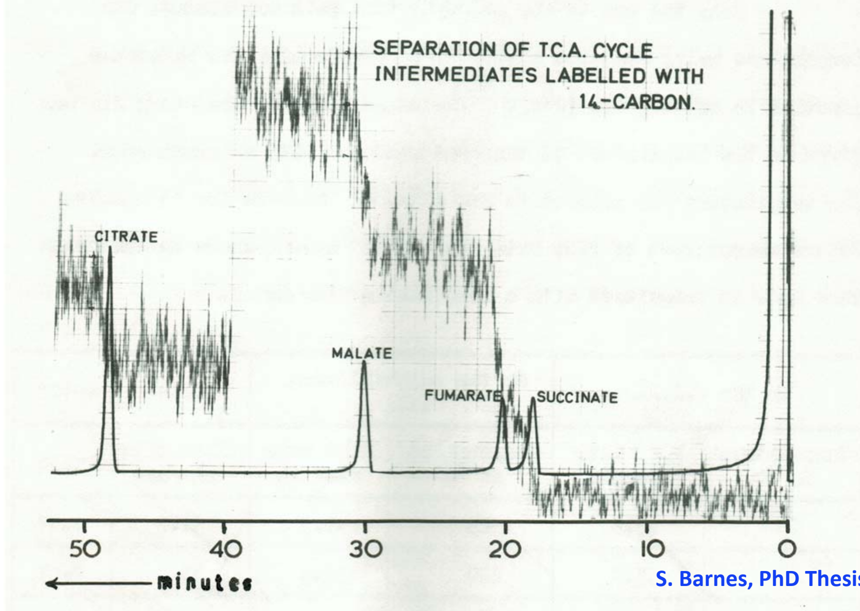
Radio-GC of Krebs Cycle intermediates



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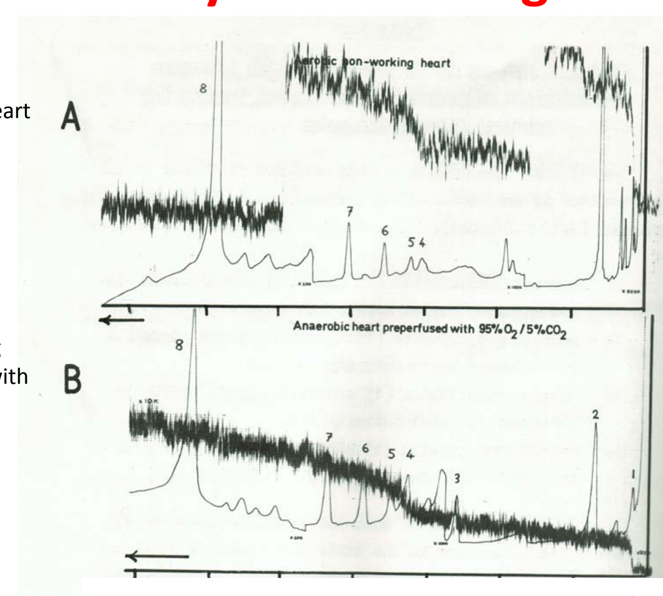
Radio GC analysis of beating heart

Aerobic beating heart

Anerobic beating heart perfused with 95% O_2/5% CO_2



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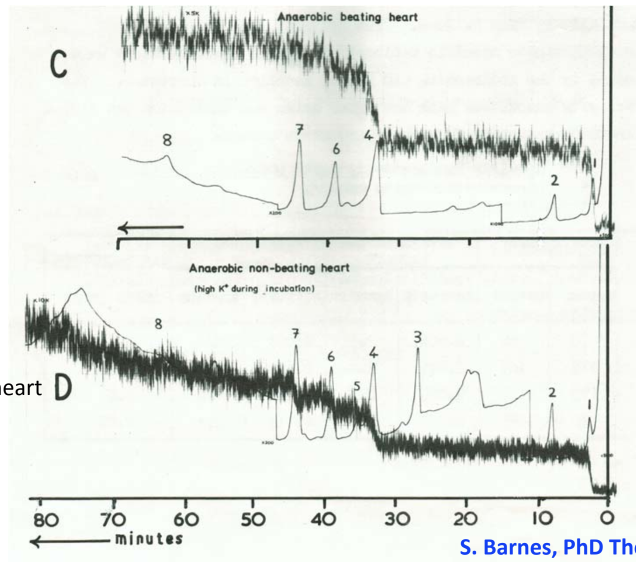
Radio GC analysis of anerobic heart

Anerobic beating heart

Anerobic non-beating heart



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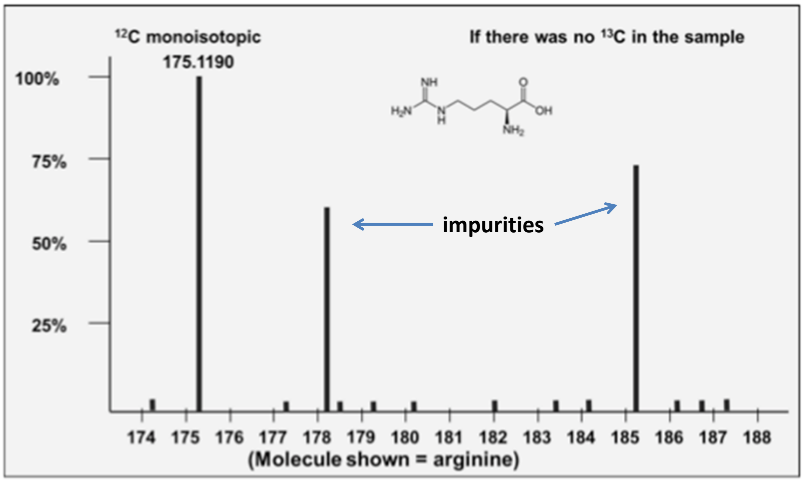
Tracking metabolites with IROA

- Isotope ratio outlier analysis (IROA)
  - Not used for flux analysis, but rather to create a unique signal for metabolites
  - Used for LC-MS (and possibly GC-MS)
  - Designed to distinguish between metabolites of interest and background signals
  - Requires uniform labeling at the 95% and 5% ^{13}C-enrichment levels

All ^{12}C in arginine $[M+H]^+$



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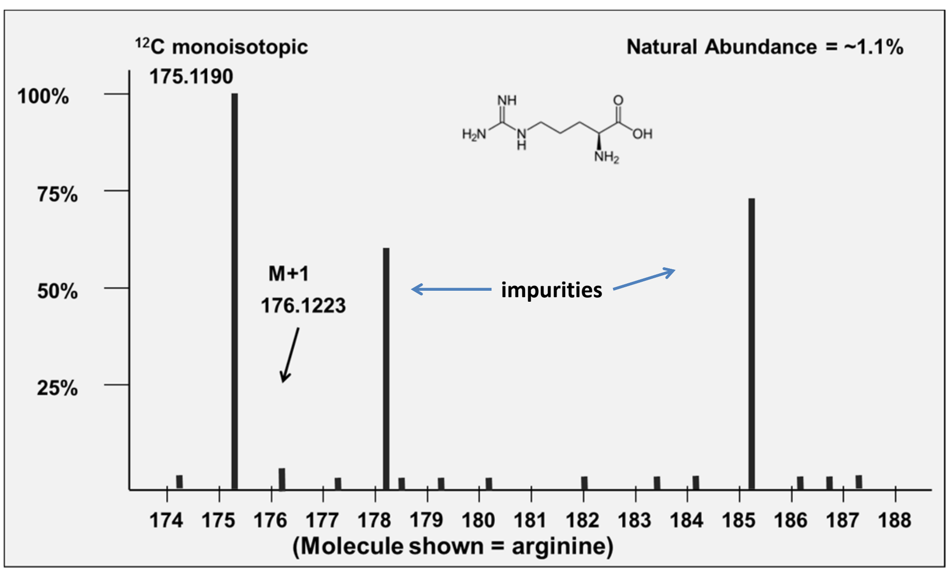
Natural abundance of ^{13}C in arginine



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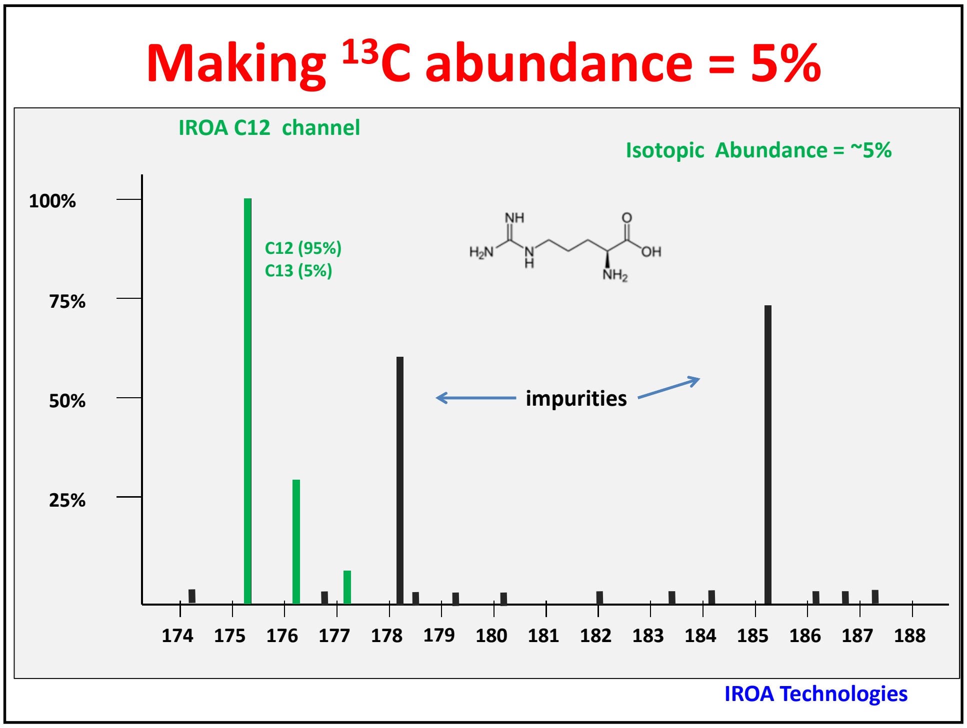
Making ¹³C abundance = 5%
IROA C12 channel
Isotopic Abundance = ~5%
100%
75%
50%
25%
C12 (95%)
C13 (5%)
impurities
174 175 176 177 178 179 180 181 182 183 184 185 186 187 188
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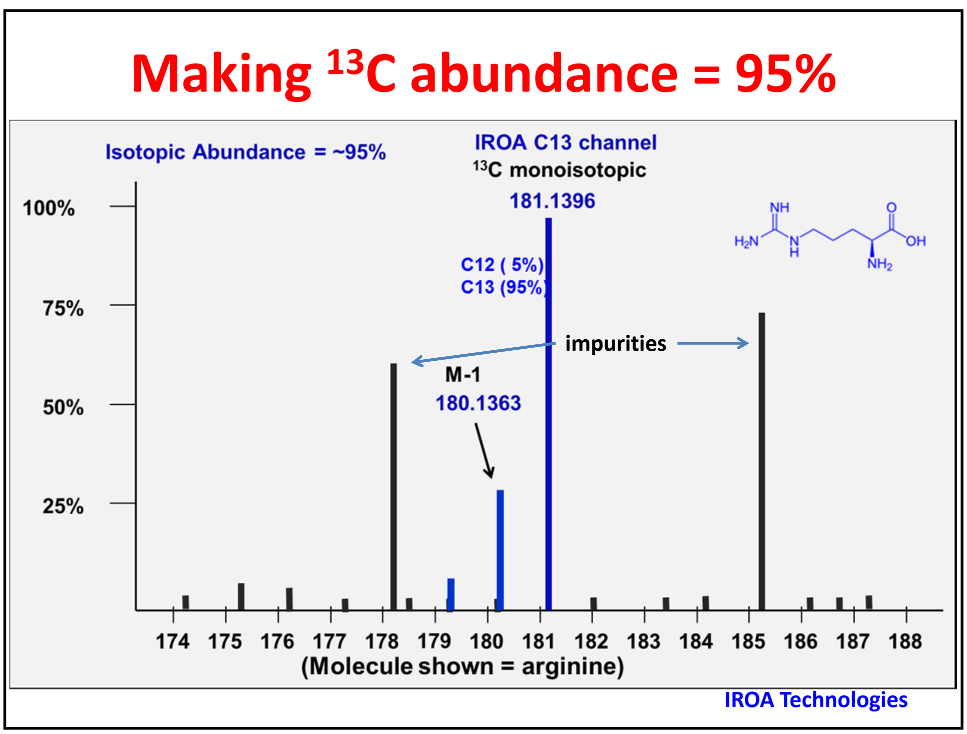
Making ¹³C abundance = 95%
Isotopic Abundance = ~95%
IROA C13 channel
¹³C monoisotopic
181.1396
100%
75%
50%
25%
C12 (5%)
C13 (95%)
impurities
M-1
180.1363
174 175 176 177 178 179 180 181 182 183 184 185 186 187 188
(Molecule shown = arginine)
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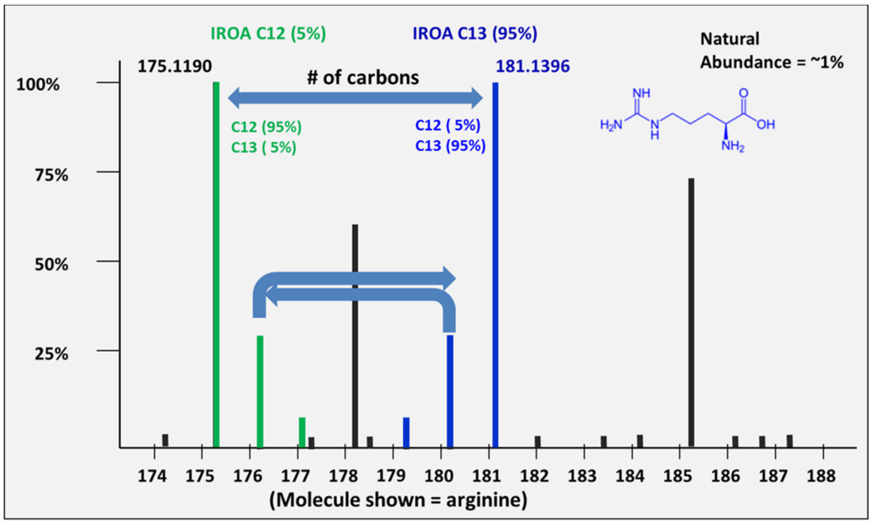
Pairing the 5% and 95% ^{13}C-labeling



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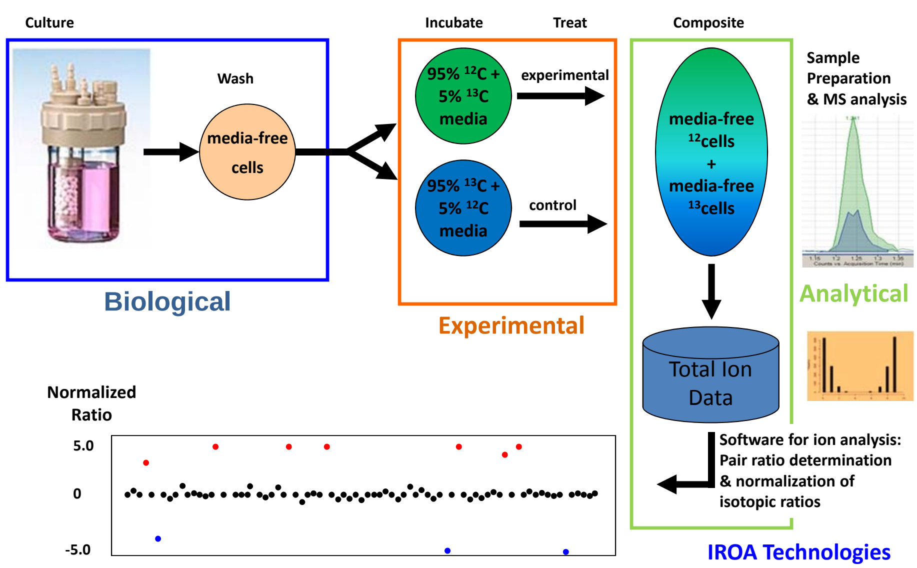
The IROA approach



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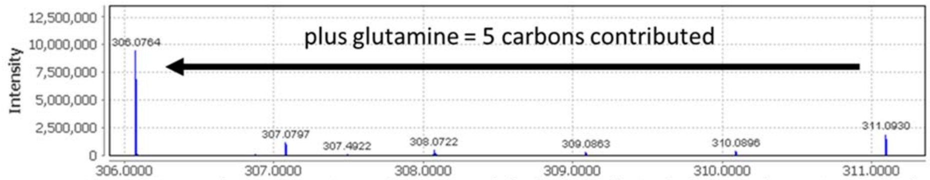
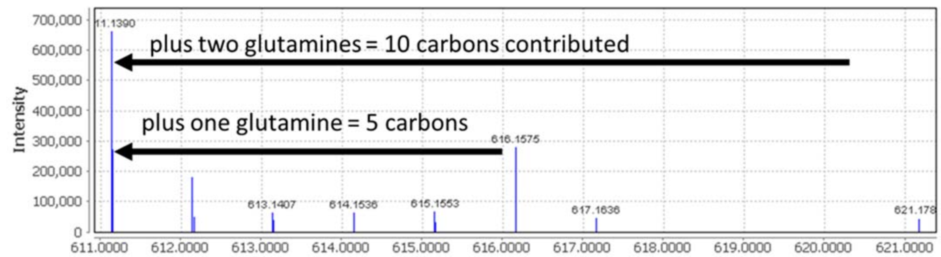
The span of isotopes = # carbon atoms



Glutathione shows the sizes of the non-labeled, and singly labeled species, which inherits a five carbon fragment from glutamine.



Oxidized glutathione shows the sizes of the non-labeled, singly labeled, and doubly labeled species. Each inheriting a five carbon fragment from glutamine.

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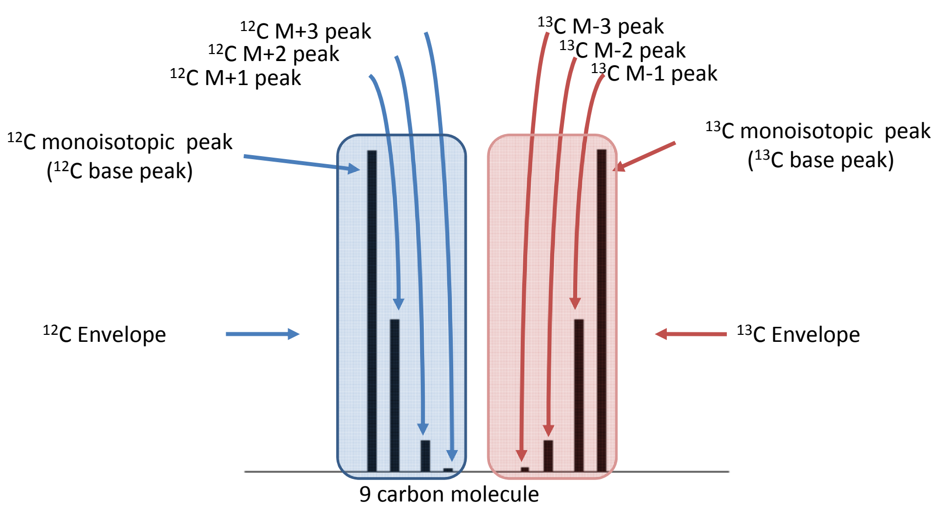
The IROA peak pattern



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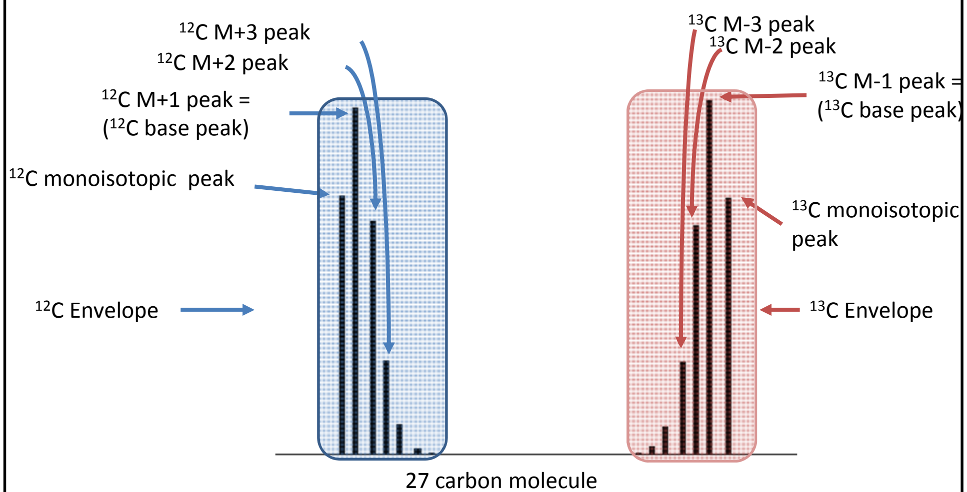
IROA profile of a bigger metabolite



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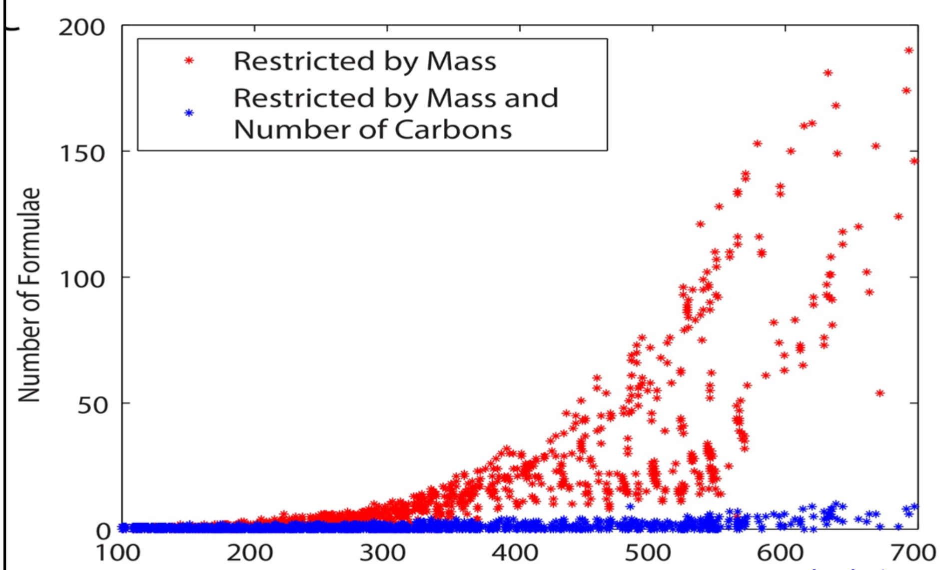
Value of knowing the carbon



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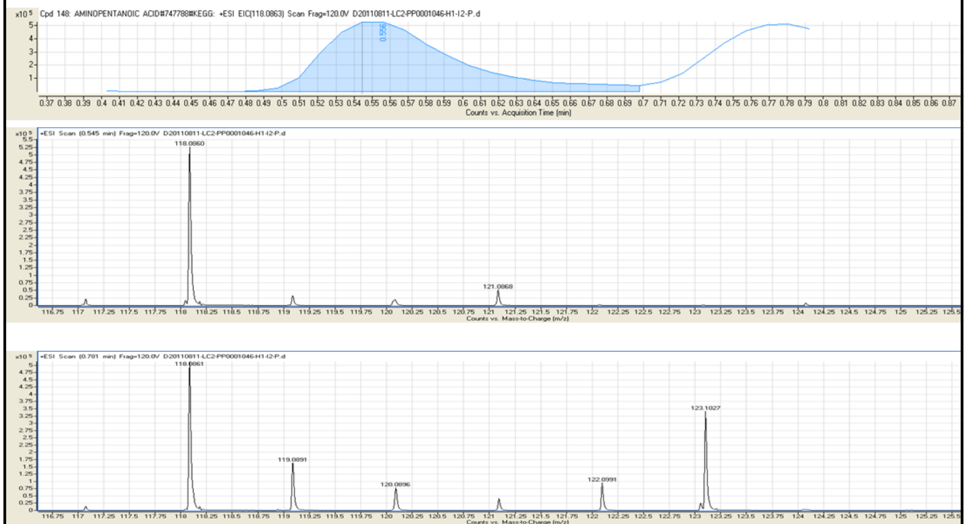
Avoiding metabolite artifacts with IROA



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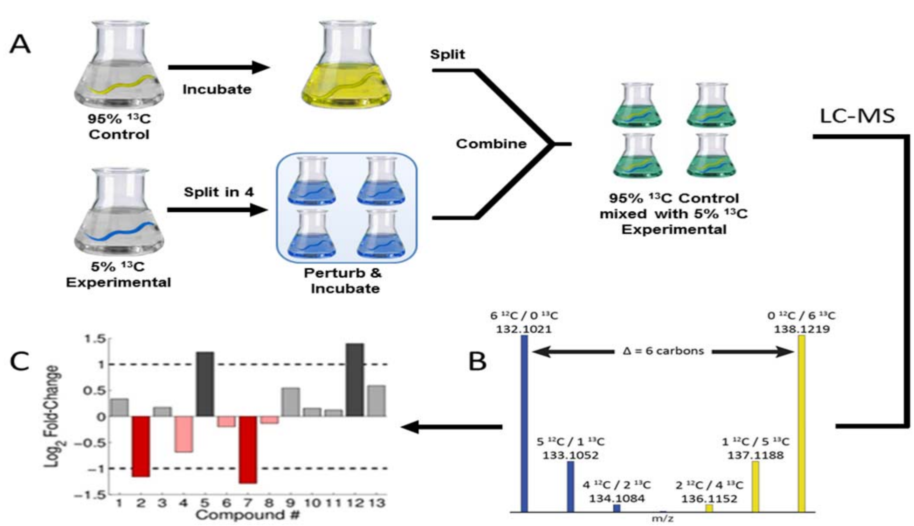
IROA with *C. elegans*



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Effect of a toxin on *C. elegans*

- **742 strong IROA peak pairs were found**
  - **314 named / 428 formula determined**
  - **Positive and negative mode LC**
  - **Thermo Orbi-trap @ 70K resolution**

- **Strong response signature determined**
  - **Basic statistics, PCA, Random Forest, NMF, SOM**
  - **74 compounds were considered significant by at least 3 of these methods.**

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Ions significantly affected by the toxin

Name		p.value	F-Value treatment^2
L-LYSINE	P000009	7.89E-05	89.71
Possibly $C_5H_7N_3O_9S$	P000018	3.06E-05	124.99
L-ARGININE	P000019	0.000131	74.84
Possibly $C_5H_9NO_{11}$	P000025	0.000182	66.63
UNK *m/z* 369.2215 RT 0.58	P000040	2.19E-05	140.24
SACCHAROPINE	P000046	7.23E-05	92.51
L-THREONINE	P000051	2.64E-05	131.52
L-GLUTAMIC ACID	P000053	1.09E-06	389.79
4-OXOPROLINE	P000054	1.74E-05	151.81
Possibly C_4H_5NO	P000058	1.8E-05	150.26
L-VALINE	P000060	0.000262	58.37
CITRULLINE	P000061	3.15E-05	123.67
4-METHYLENE-L-GLUTAMINE	P000062	0.000169	68.40
L-METHIONINE S-OXIDE	P000065	7.55E-06	202.32
L-PROLINAMIDE	P000085	0.000227	61.56
STACHYDRINE	P000102	4.75E-05	107.19
UNK *m/z* 206.0368 RT 0.71	P000114	0.000251	59.35
N-ACETYLPUTRESCINE	P000122	8.96E-07	417.06
EPSILON-CAPROLACTAM	P000123	1.29E-08	1731.72
2-AMINO-OCTANOIC ACID	P000131	0.000213	62.99
UNK m/z 345.1258 RT 0.97	P000141	0.000111	79.36
Possibly $C_{10}H_{19}N_2O_5P_2$	P000151	0.000154	70.78
CYS-GLY	P000152	0.000116	78.29
URATE	P000156	0.000222	62.02
Possibly $C_{13}H_{16}N_5OPS$	P000218	1.1E-05	177.82

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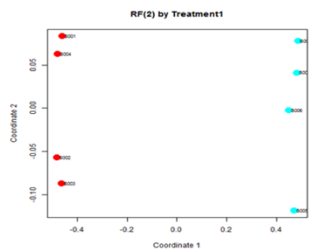
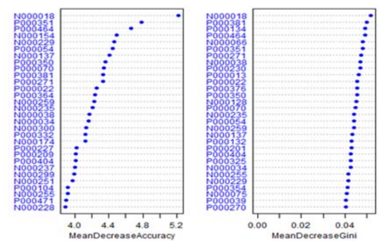
Multivariate statistics

A



B



C

Name		X1.HP	heatShock	MeanDecreaseAccuracy	MeanDecreaseGini
Possibly C8H18N6PS2	N000018	5.165779	5.091283	5.219694	0.051833
UNK MZ 197.0968 RT 0.55	P000351	4.61306	4.651274	4.787018	0.047917
Possibly C11H14N3O6	P000464	4.527905	4.623332	4.660738	0.04925
Possibly C11H23N5PS	N000154	4.310867	4.345237	4.49079	0.038917
UNK MZ 373.1256 RT 0.60	N000229	4.384942	4.313313	4.461684	0.0415
4-OXOPROLINE	P000054	4.307818	4.380554	4.447013	0.044083
D-ALANYL-D-ALANINE	N000137	4.357447	4.298196	4.403362	0.043417
UNK MZ 160.0958 RT 0.55	P000350	4.194825	4.331486	4.35738	0.045333
S-ADENOSYLMETHIONINE	P000070	4.247356	4.247356	4.345614	0.04475
UNK MZ 224.1142 RT 0.78	P000381	4.245944	4.309512	4.333807	0.049917
Possibly C6H13N2S2	P000271	4.286066	4.238801	4.330957	0.047167
Possibly C5H10N4O5P	P000022	4.237845	4.104634	4.253873	0.045583
Possibly C14H29N11O3S	P000364	4.207787	4.123607	4.236865	0.036583
Possibly C18H18N11S3	N000259	4.115656	4.119522	4.230318	0.044
N-FORMIMINO-L-GLUTAMATE	N000235	4.115656	4.091016	4.208769	0.044167

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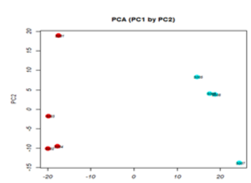
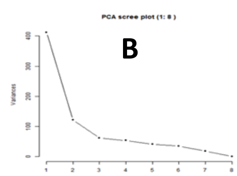
PCA analysis of toxin's effect

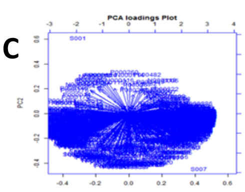
A



B



C



D

	PC1	PC2	PC3	PC4	PC5
P000123	-0.04867	-0.00122	-0.0028	0.002634	-0.00263
P000305	-0.04864	-0.01333	0.003947	-0.00022	0.000461
P000328	-0.04854	-0.01474	-0.00285	0.001186	0.002235
P000065	-0.04839	-0.01076	-0.00949	0.016892	0.002798
N000034	-0.04838	-0.00625	0.004756	-0.00207	-0.00469
P000268	-0.04834	-0.01365	0.013402	-0.00273	-0.00146
P000122	-0.04832	-0.00499	0.000535	0.0041	0.008176
P000485	-0.04814	-0.01292	0.013305	-0.00659	0.01036
N000006	-0.04792	-0.00964	-0.02169	0.005208	0.003092
N000068	-0.04775	-0.00849	0.026794	-0.00377	-0.00419
P000304	-0.04768	-0.02207	0.008128	-0.00055	0.004545
N000057	-0.04759	-0.00101	0.027516	-0.00376	-0.00763
N000174	-0.04753	0.005826	0.00295	0.003997	-0.0041
P000152	-0.04751	-0.01938	0.01513	-0.00054	0.003671
P000141	-0.04749	-0.00612	0.024997	-0.00648	-0.00814

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Summary of most likely metabolites

Name		Stats1	RFTop1	RFTop2	NMF3	NMF4	NMF5	NMF6	Count
UNK *m/z* 160.0958 RT 0.55	P000350	1	1	1	1	1	1	1	7
UNK *m/z* 197.0968 RT 0.55	P000351	1	1	1	1	1	1	1	7
UNK *m/z* 216.0852 RT 0.61	N000034	1	1	1	1	1	1	1	7
D-ALANYL-D-ALANINE	N000137	1	1	1	1	1	1	1	7
Possibly $C_{25}H_{34}N_4O_5$	N000174	1	1	1	1	1	1	1	7
UNK *m/z* 373.1256 RT 0.60	N000229	1	1	1	1	1	1	1	7
2-AMINO-OCTANOIC ACID	P000131	1	1	0	1	1	1	1	6
Possibly $C_6H_8N_4O_3$	P000354	1	1	0	1	1	1	1	6
UNK *m/z* 510.2122 RT 0.68	P000373	1	1	0	1	1	1	1	6
UNK *m/z* 224.1142 RT 0.78	P000381	0	1	1	1	1	1	1	6
Possibly $C_6H_9NO_6P$	P000410	1	1	0	1	1	1	1	6
Possibly $C_{11}H_{14}N_3O_6$	P000464	0	1	1	1	1	1	1	6
Possibly $C_6H_4N_2O_6P$	P000471	1	1	0	1	1	1	1	6
Possibly $C_5H_{12}N_2O_7PS$	N000006	1	1	0	1	1	1	1	6
Possibly $C_{11}H_{23}N_5PS$	N000154	1	1	1	0	1	1	1	6
D-GLUCOSE	N000228	1	1	0	1	1	1	1	6
UNK *m/z* 548.2037 RT 0.63	N000232	1	1	0	1	1	1	1	6
GLYCERATE	N000237	1	1	0	1	1	1	1	6

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