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Isotopes and mass spectrometry - dawn of history to today

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Early History of Mass Spec

- 1897 Rutherford discovers the electron (cathode rays)



- 1919 Aston using a mass spectrograph shows that Neon with a non-integer MW (20.2 Da) is composed of two isotopes, ^{20}Ne and ^{22}Ne

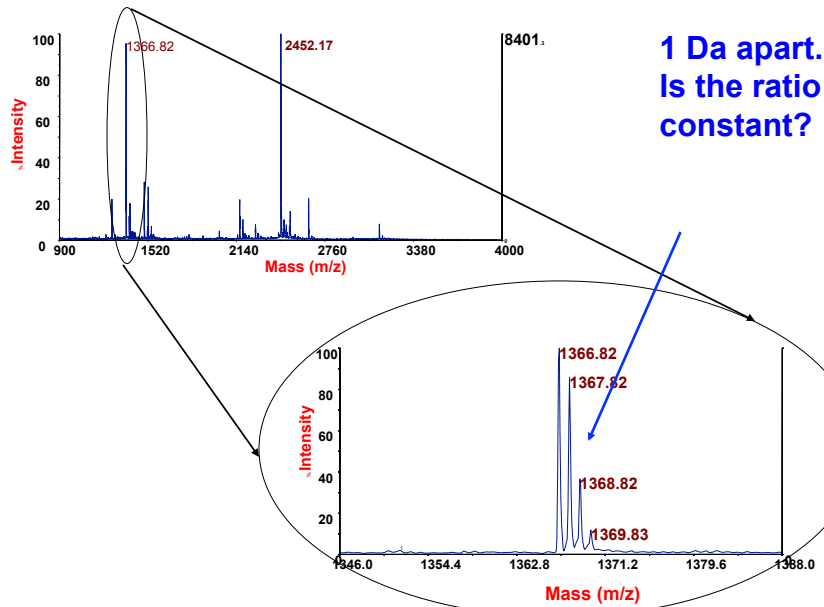


<http://www.asms.org/Publications/Historical/HistoryofMassSpectrometry/tabid/94/Default.aspx>

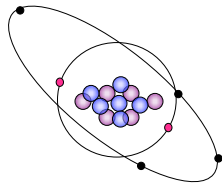
Mass spectrometry and nuclear war

- 1935 Dempster discovers ^{235}U , the uranium isotope first used for a nuclear fission (atom) bomb
- 1941-5 Manhattan project
 - Mass spectrometry, one of three methods used to isolate ^{235}U
- Bainbridge's careful measurement of masses of the elements revealed that hydrogen was heavier than predicted
 - This led to the concept that fusion of four H atoms to form He would result in the loss of mass in the form of energy - from Einstein, $E = mc^2$, i.e., a whole bunch!
 - Predicted mass for helium = 4.03298 vs actual of 4.02602
 - $\Delta = 0.00695$

Isotope profile of an individual peptide ion



Elements

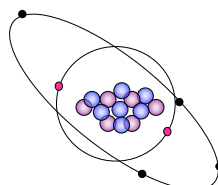


Carbon-12

6 protons
6 neutrons
6 electrons

Stable

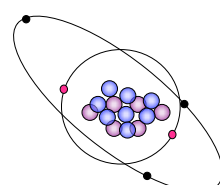
Naturally occurring isotopes



Carbon-13

6 protons
7 neutrons
6 electrons

Stable



Carbon-14

6 protons
8 neutrons
6 electrons

Unstable

Neutron decays to produce a proton, a β -particle and an anti-neutrino - ^{14}C becomes ^{14}N

Stable isotopes of the most abundant elements found in biological materials

Element	Mass	Abundance
H	1.0078	99.985%
	2.0141	0.015%
C	12.0000	98.89%*
	13.0034	1.11%*
N	14.0031	99.64%*
	15.0001	0.36%*
O	15.9949	99.76%*
	16.9991	0.04%*
	17.9992	0.20%*
S	31.9721	94.93%*
	32.9715	0.76%*
	33.9679	4.29%*
	35.9671	0.02%*

***Varies according to its source**

Formation of helium

The first step involves the fusion of two hydrogen nuclei ^1H (protons) into deuterium, releasing a positron and a neutrino as one proton changes into a neutron.

$$^1\text{H} + ^1\text{H} \rightarrow ^2_1\text{D} + e^+ + \nu_e + 0.42 \text{ MeV}$$

This first step is extremely slow, both because the protons have to tunnel through the Coulomb barrier and because it depends on weak interactions.

The positron immediately annihilates with an electron, and their mass energy is carried off by two gamma ray photons.

$$e^- + e^+ \rightarrow 2 \gamma + 1.02 \text{ MeV}$$

After this, the deuterium produced in the first stage can fuse with another hydrogen to produce a light isotope of helium, ^3He :

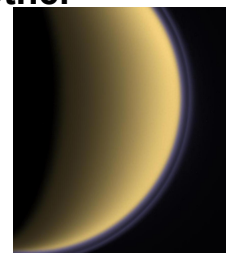
$$^2_1\text{D} + ^1\text{H} \rightarrow ^3_2\text{He} + \gamma + 5.49 \text{ MeV}$$

From here there are three possible paths to generate helium isotope ^4He . In pp I helium-4 comes from fusing two of the helium-3 nuclei produced; the pp II and pp III branches fuse ^3He with a pre-existing ^4He to make Be. In the Sun, branch pp I takes place with a frequency of 86%, pp II with 14% and pp III with 0.11%. There is also an extremely rare pp IV branch.

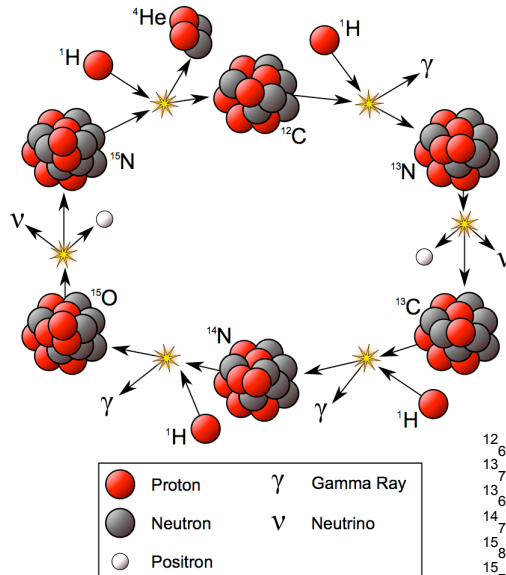
http://en.wikipedia.org/wiki/Nuclear_fusion

The $^{12}\text{C}/^{13}\text{C}$ ratio

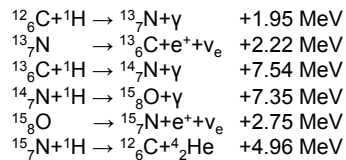
- ^{12}C was born in the inferno of stars from the triple fusion of ^4He nuclei
- ^{13}C is present in varying ratios to ^{12}C among different stars and galaxies
- Carbon is present in interstellar space as CN and CO, and as methane and other hydrocarbons in planets
 - Tholins are in the atmosphere of the moon Titan - future source of life?



The CNO cycle - Bethe-Weizsäcker-cycle



The CNO chain starts occurring at $\sim 13 \times 10^6$ K, but its energy output rises much faster with increasing temperatures. At $\sim 17 \times 10^6$ K, the CNO cycle starts becoming the dominant source of energy. The sun has a temperature of around $\sim 15.7 \times 10^6$ K and only 1.7% of ${}^4\text{He}$ nuclei being produced in the Sun are born in the CNO cycle.

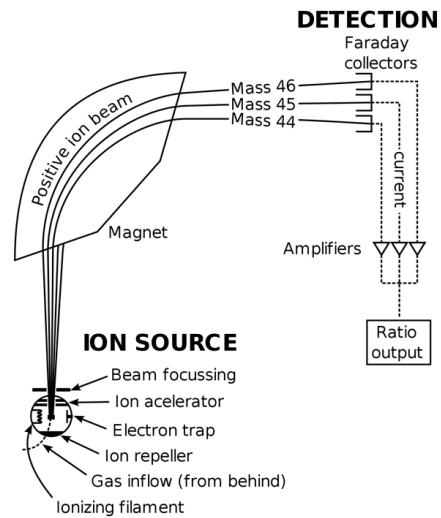


http://en.wikipedia.org/wiki/CNO_cycle

Terrestrial ${}^{12}\text{C}/{}^{13}\text{C}$ ratio

- On average there is 1.11% of ${}^{13}\text{C}$ amongst the total carbon on Earth
- Carbon is present in many forms accessible to synthetic and biosynthetic processes - mostly starting from CO_2
- CO_2 is in the atmosphere, in the sea as HCO_3^- , in the soil as carbonates, and as organic intermediates
 - ${}^{12}\text{C}$ and ${}^{13}\text{C}$ partition differently in each of these environments due to physical effects
 - Compounds have different ${}^{12}\text{C}/{}^{13}\text{C}$ ratios

Isotope ratio mass spectrometer



http://en.wikipedia.org/wiki/Isotope_ratio_mass_spectrometry

Isotope ratio mass spectrometry

- Used for $^{13}\text{C}/^{12}\text{C}$, $^{15}\text{N}/^{14}\text{N}$, $^{18}\text{O}/^{16}\text{O}$
- Carbon compounds are converted to CO_2
- Low mass range 0-150 m/z
- Sector instrument
- Very sensitive
- Very accurate measurement of mass
- $^{13}\text{C}/^{12}\text{C}$ ratio can vary from 0.972‰ to 1.160‰
- PeeDee standard has $^{13}\text{C}/^{12}\text{C}$ ratio of 1.12372‰

Fixation of CO₂ as organic carbon

- **RuBisCO - enzyme complex in plants**
 - Converts CO₂ to sugars
 - Prefers ¹²C to ¹³C
- **Plants take in CO₂ through stomata**
 - Two models
 - **Sponge divers (intermittent breathing)**
 - These would sample all the isotopic forms of CO₂
 - **Swimmers on surface (frequent breathing)**
 - These would selectively take in ¹²CO₂

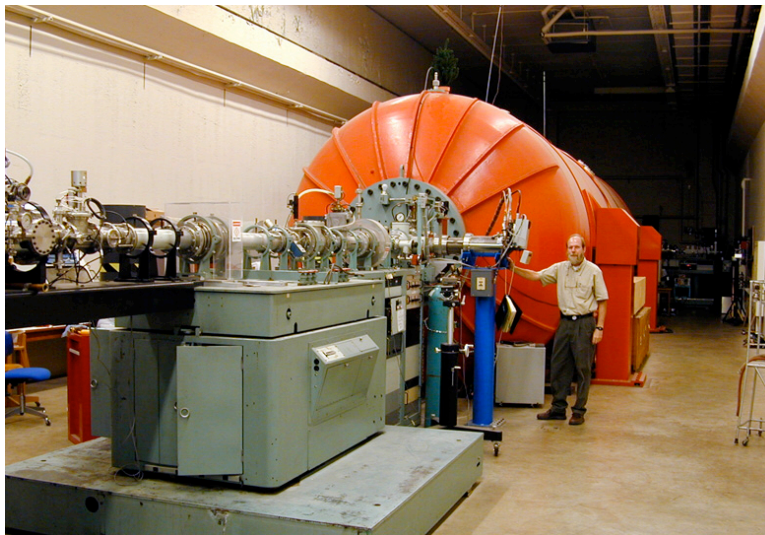
¹²C/¹³C ratio in plants

- **The ¹³C content would be higher in plants that held their breath like the divers**
 - i.e., the stomata were open less frequently
- **Drought-resistant wheat strains have a higher ¹³C/¹²C ratio**
 - This is a marker for selection of drought-resistant strains, important in the coming global warming

Athletes who use synthetic testosterone

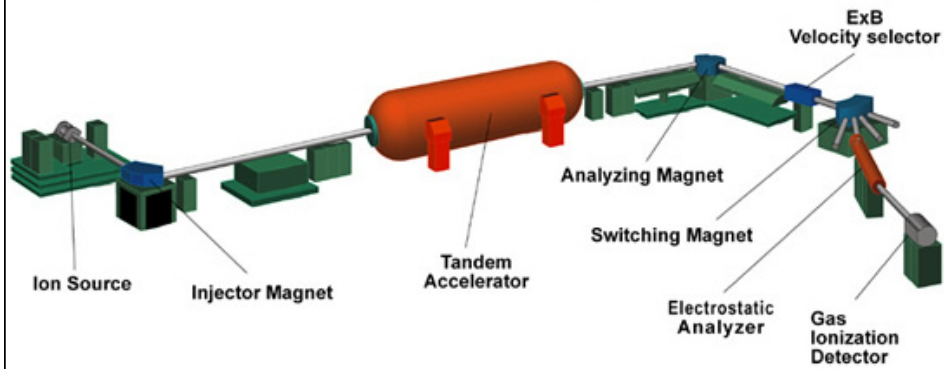
- Synthetic testosterone is made from phytosterol precursors, typically derived from wild yams or soy.
- Those are both warm-climate C3 plants, which take up atmospheric carbon dioxide by a different route than temperate-zone C4 plants, leading to noticeably different isotope ratios.
- The typical Western industrial-country diet is derived from a mixture of C3 and C4 stocks, so the appearance of testosterone with a C3-plant isotopic profile is usually diagnostic.

The ultimate mass spectrometer



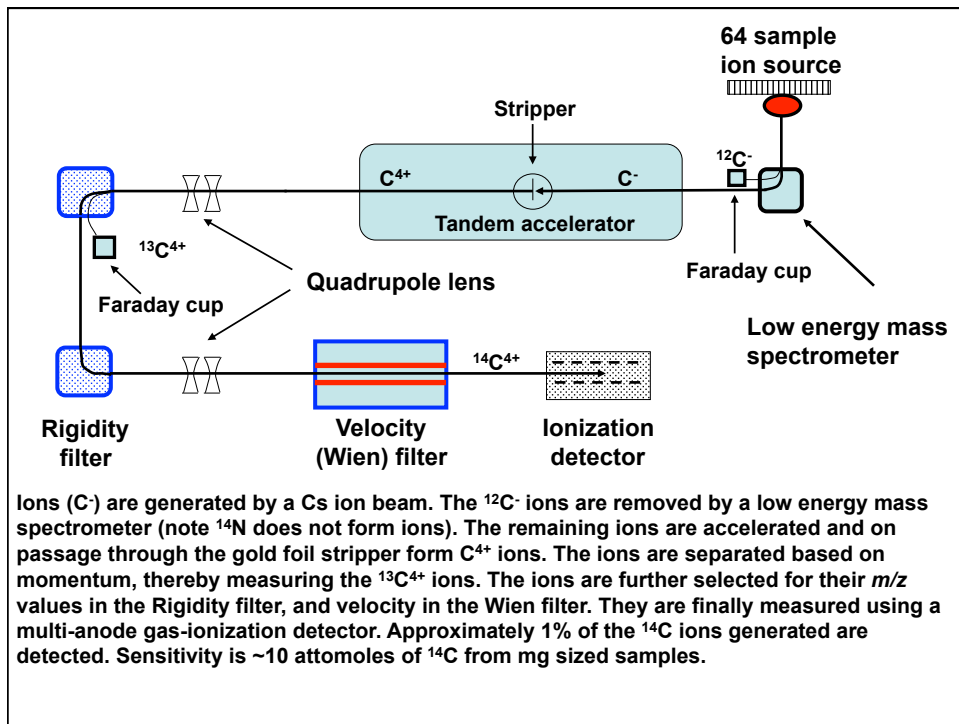
Accelerator mass spectrometer

Set up of AMS at Purdue



10 GeV AMS at LLNL

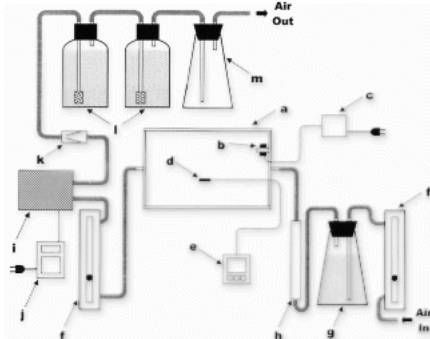




Sensitivity of ^{14}C -AMS

- If one carbon atom is incorporated into a compound, then the specific activity is $\sim 50 \mu C_i/\mu mol$
- The human body naturally contains 50-90 nCi of radioactivity – therefore, a 50 nCi dose is reasonable and small compared to most clinical studies
- 50 nCi is 1 nmol (10^{-9} mole)
- AMS can measure 1 ^{14}C atom in 10^{15} carbon atoms, or 10 attomoles (10^{-17} moles) – 10^{-8} of the dose
- For a 70 kg human, 1 mg of tissue represents 1.4×10^{-5} .
- If distributed evenly, the S/N is 700:1

¹⁴C-polyphenols by metabolic labeling



Plant cells incubated with ¹⁴C-labeled sucrose in a closed system

¹⁴C-labeled polyphenols extracted and fractionated

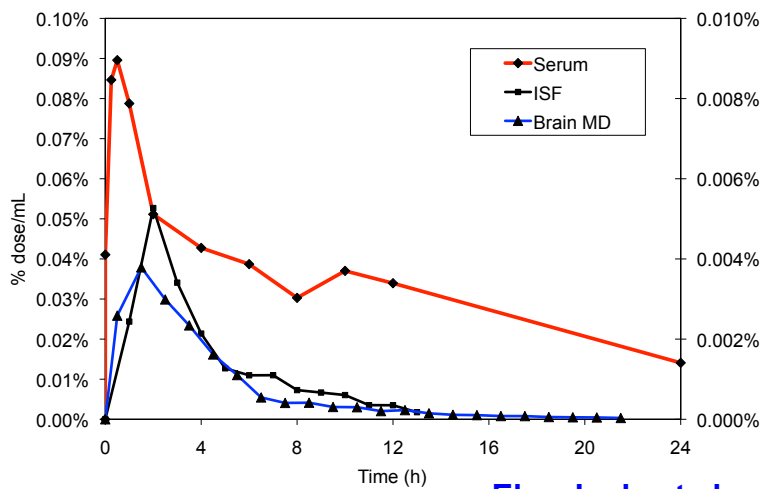
50 nCi of ¹⁴C-labeled polyphenol(s) is 1.11×10^5 dpm or 1×10^{-9} mol (0.3 μg)

Let's suppose that 0.1% of the dose is absorbed into the brain, then that is 111 dpm, 0.3 ng or 1×10^{-12} mol

For a 3 g rat brain, then 3 mg is 0.11 dpm, 0.3 pg or 1×10^{-15} mol

The detection limit for ¹⁴C using AMS is 1×10^{-17} mol, i.e., S/N is 100:1

Differential retention of ¹⁴C-labeled proanthocyanidin by AMS

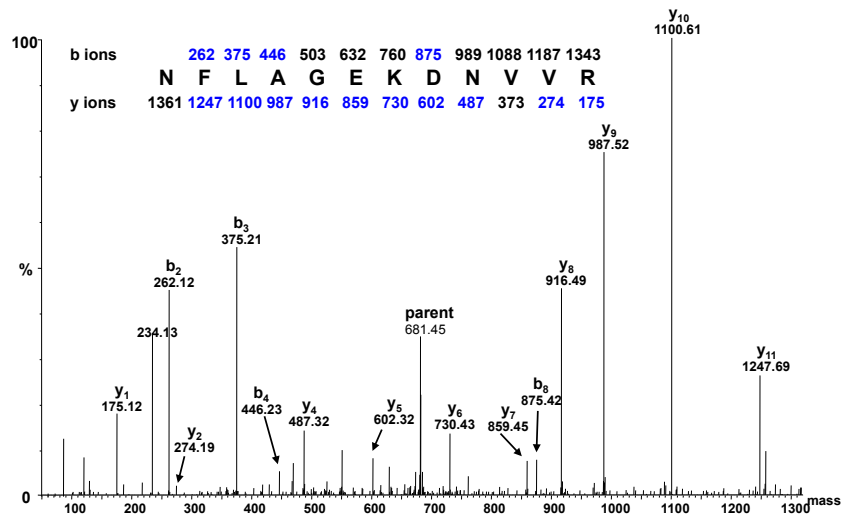


Elsa Janle et al.

Can isotope ratios be ascertained for compounds?

- Conventional isotope ratio measurements are based on converting the carbon-containing compounds to CO₂
- Given the improvements in mass spectrometry, could we investigate the isotope ratios of peptides and deduce the underlying ¹³C/¹²C ratio?

Fragmentation of a peptide

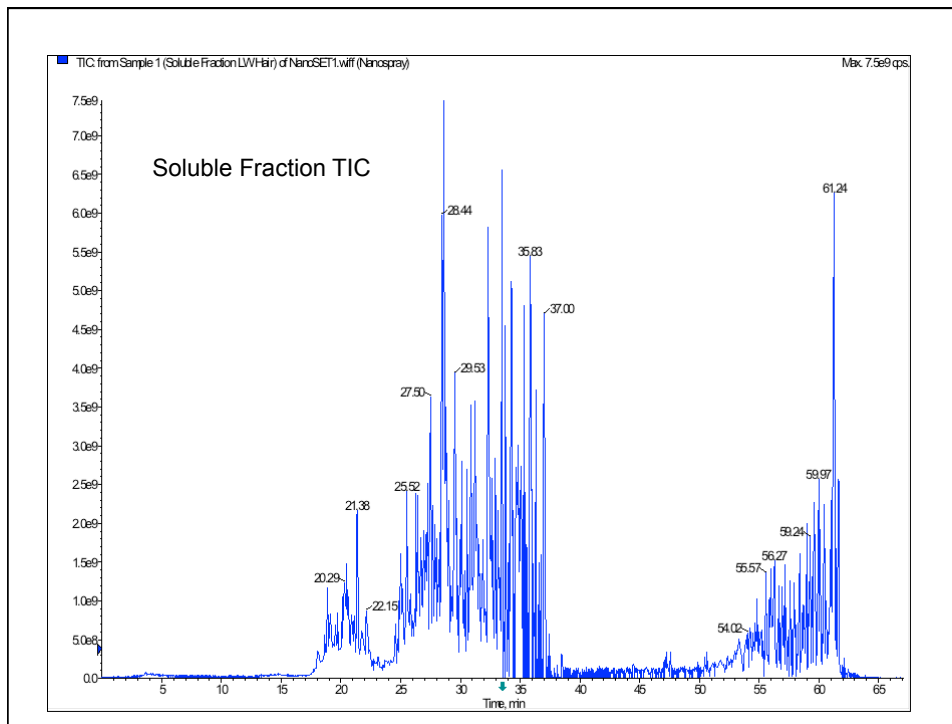


Expected isotope abundances

- x is the fraction of carbon atoms that are ^{12}C
- y is the fraction of carbon atoms that are ^{13}C
- For 1 carbon, the distribution is $x;y$
- For 2 carbons, $x^2;2xy;y^2$
- Using the binomial expansion
 - For n carbons, $x^n; nx^{(n-1)}y; (\sum n-1)x^{(n-2)}y^2, \dots$
 - x^n are all ^{12}C ; for the next isotope peak there is one ^{13}C
 - The ratio (r) of those first two peaks = ny/x
 - But $x+y=1$, so $x=1-y$, hence $r = ny/(1-y)$ and $r-ry=ny$
 - Further, $y(n+r)=r$, and therefore $y = r/(n+r)$

Calculating the $^{13}\text{C}/^{12}\text{C}$ ratio in keratin peptides

- Observe peptides with clean isotope profiles
- Identify the peptide from their MS/MS spectra
 - Determine the number (n) of carbon atoms in the peptide
- Calculate the areas under the observable isotope peaks
- Estimate the $^{13}\text{C}/^{12}\text{C}$ ratio using the correction for n



Partial list of proteins in hair soluble fraction by LC-tandem MS

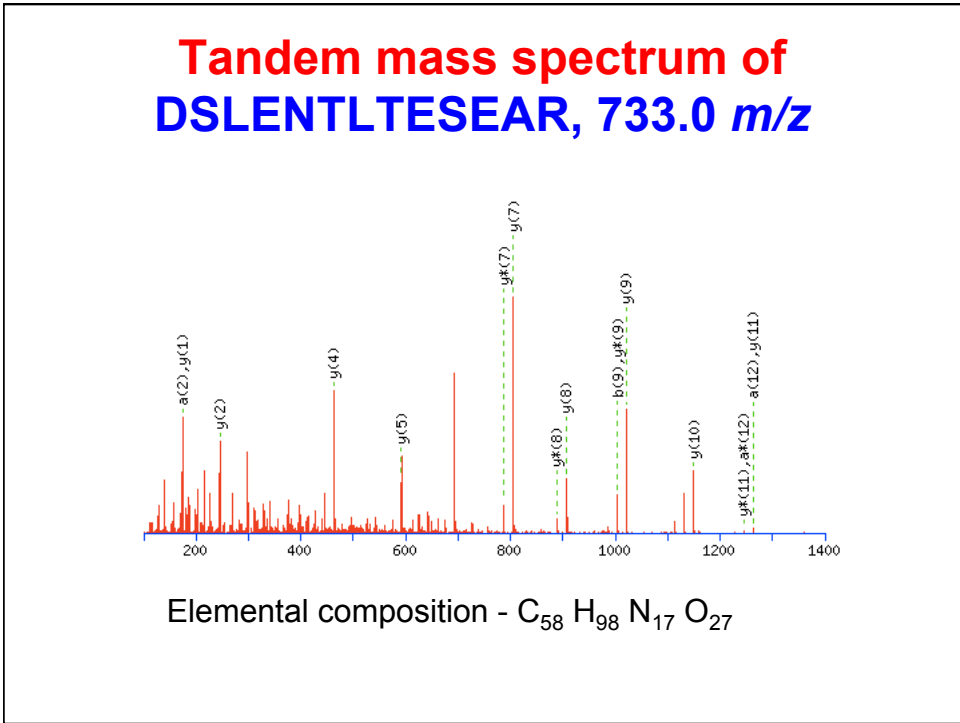
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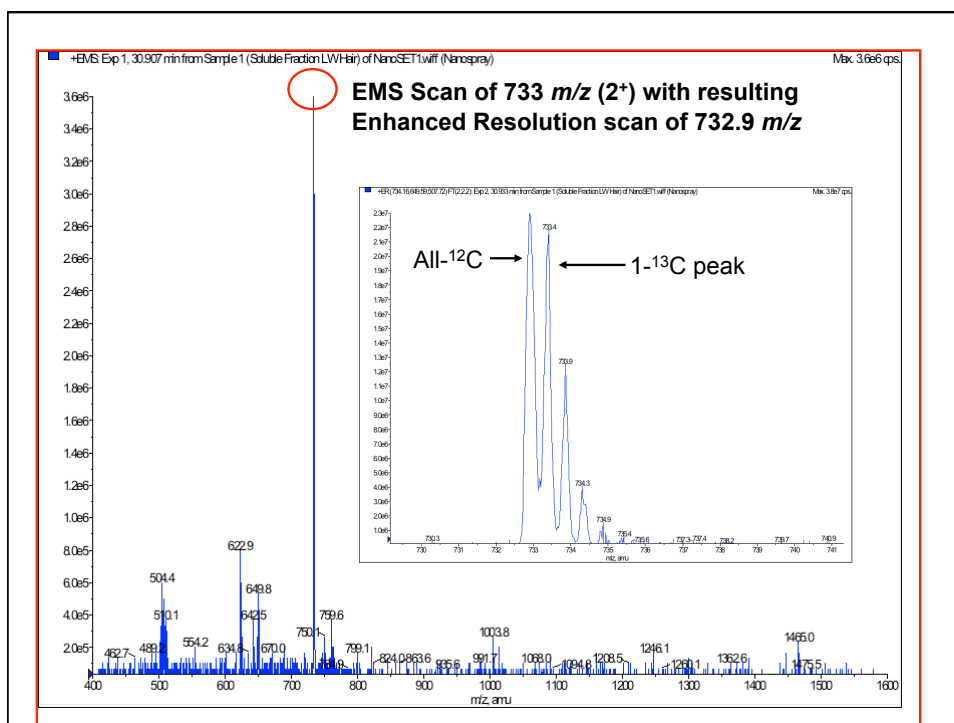
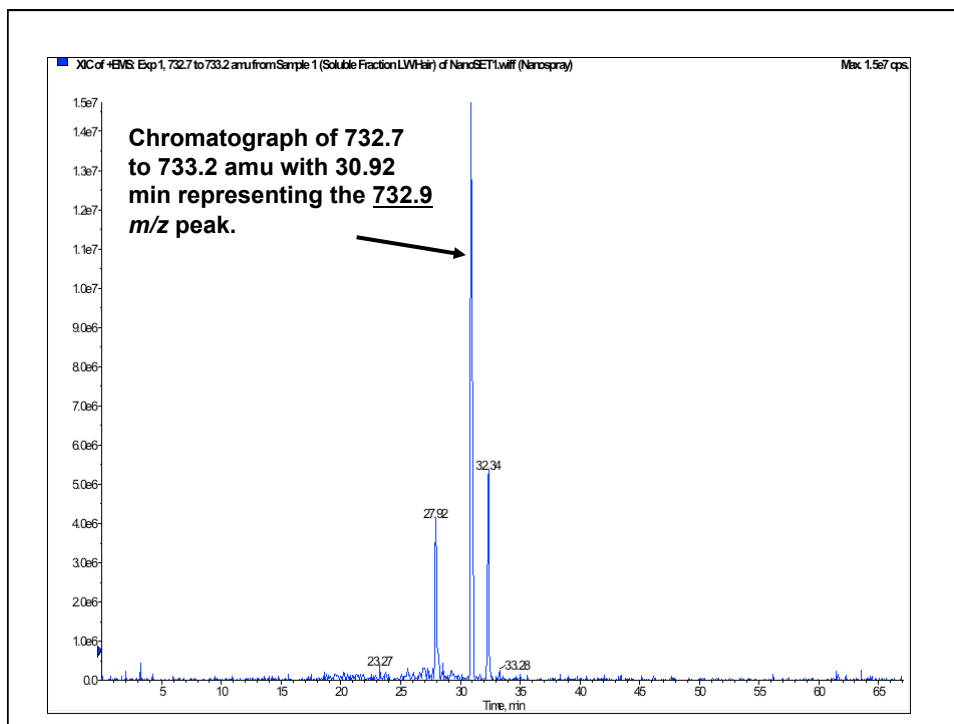
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Email         :
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MS data file  : C:\DOCUME~1\MASSSP-1\LOCALS~1\Temp\mas1916.tmp
Database      : NCBIInr 20070927 (5519594 sequences; 1911975371 residues)
Taxonomy      : Homo sapiens (human) (194674 sequences)
Timestamp     : 2 Jan 2009 at 23:09:26 GMT
Significant hits:
gi|14917117| keratin 33A [Homo sapiens]
gi|46575688| Keratin 33A [Homo sapiens]
gi|14917115| keratin 31 [Homo sapiens]
gi|2950209| hair keratin acidic 3-II [Homo sapiens]
gi|119581133| keratin, hair, acidic, 4 [Homo sapiens]
gi|27371164| Keratin 34 [Homo sapiens]
gi|1181996| hair type II basic keratin [Homo sapiens]
gi|4504935| keratin 85 [Homo sapiens]
gi|7161767| type II hair keratin 3 [Homo sapiens]
gi|34810822| Chain B, Non-Covalent Complex Between Alpha-1-Pi-Pittsburgh And S195a Trypsin
gi|1668744| HhA5 hair keratin type I intermediate filament [Homo sapiens]
gi|116488398| keratin 32 [Homo sapiens]
gi|27694303| KRT36 protein [Homo sapiens]
gi|12329925| keratin associated protein [Homo sapiens]
gi|186629| keratin 10
gi|623409| keratin 10
gi|13994362| keratin associated protein 3.1 [Homo sapiens]
gi|11935049| keratin 1 [Homo sapiens]
gi|50949256| hHa7 protein [Homo sapiens]
gi|15055511| keratin associated protein 3-3 [Homo sapiens]
gi|3901030| cytokeratin type II [Homo sapiens]
gi|22798968| type II hair keratin 2 [Homo sapiens]
gi|131412225| keratin 13 isoform a [Homo sapiens]
gi|5030431| vimentin [Homo sapiens]
gi|34535209| unnamed protein product [Homo sapiens]
gi|181573| cytokeratin 8
gi|311882| high sulfur keratin [Homo sapiens]
gi|119609073| CD163 antigen-like 1, isoform CRA_a [Homo sapiens]

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gi|14917117 Mass: 47166 Score: 1035 Queries matched: 30
 Keratin 33A [Homo sapiens]
 Check to include this hit in error tolerant search or archive report

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
228	639.38	638.37	638.33	0.04	0	17	2.9e+002	4	A.SYLEK.V
249	823.47	822.46	822.45	0.01	0	27		44	R.LASYLEK.V
296	486.60	971.19	970.54	0.65	0	72	0.0012	1	Y.QAEIIELR.R
307	494.82	987.62	987.52	0.10	0	27		59	K.TIEELQQK.I
314	999.73	998.72	998.58	0.14	0	(24)		75	R.LVVQIDNAK.L
315	500.64	999.26	998.58	0.69	0	41	1.7	1	R.LVVQIDNAK.L
325	505.32	1008.62	1009.51	-0.88	0	32		16	K.YETELSLR.Q
386	566.90	1131.79	1131.60	0.19	0	52	0.14	1	R.ILDELTLCR.S
397	577.43	1152.84	1152.52	0.32	0	(21)	1.5e+002	3	K.ETMQFLNDR.L
404	585.39	1168.77	1168.52	0.25	0	54	0.084	1	K.ETMQFLNDR.L + Oxidation (M)
411	1186.64	1185.64	1185.60	0.04	0	(34)		11	R.DNAELENLIR.E
412	593.86	1185.71	1185.60	0.11	0	39	3.2	1	R.DNAELENLIR.E
417	598.40	1194.79	1194.60	0.19	0	70	0.0024	1	R.YSSQLSQVQR.L
419	599.28	1196.56	1196.55	0.01	0	29		35	R.LECEINTYR.S
427	609.83	1217.64	1217.61	0.03	0	52	0.19	1	R.SDLEAQVESLK.E
435	619.95	1237.89	1238.65	-0.76	1	47	0.43	1	R.TKYEETLSLR.Q
438	620.87	1239.73	1238.60	1.13	0	22	1.7e+002	2	Q.NHEQEVNTLR.C
439	622.92	1243.82	1242.66	1.16	0	34		9.2	R.QLVESDINGLR.R
457	644.95	1287.88	1287.70	0.19	1	44	0.85	1	R.RILDELTLCR.S
485	684.38	1366.74	1366.66	0.08	0	(33)		12	K.QNHEQEVNTLR.C
486	456.67	1366.98	1366.66	0.32	0	54	0.078	1	K.QNHEQEVNTLR.C
507	709.96	1417.91	1417.64	0.27	0	63	0.013	1	K.STGPCISNPCGLR.A
521	733.01	1464.00	1463.67	0.33	0	62	0.013	1	R.DSLENTLTSEAR.Y
522	733.39	1464.77	1464.73	0.04	1	52	0.17	1	R.SQYEALVETNRR.E
523	489.32	1464.95	1464.73	0.22	1	(16)	5.5e+002	1	R.SQYEALVETNRR.E
538	752.92	1503.83	1503.77	0.06	0	65	0.0083	1	R.QNQEYQVLLDVR.A
558	767.64	1533.27	1533.83	-0.56	0	(43)	0.91	1	N.ALEIELQAQHNLR.D





Estimating ^{13}C content

- The ratio of the ^{13}C and ^{12}C isotope peak heights is 2.18/2.28, i.e., $r = 0.95614$
- Y , the fraction of the carbons that are ^{13}C , is $r/(n+r)$
- $Y = 0.95614/(58 + 0.95614)$
- $Y = 0.015947$, i.e. 1.59%

Mass differences for H, C, N and O isotopes

- $^2\text{H} - ^1\text{H} = 1.0063 \text{ Da}$
- $^{13}\text{C} - ^{12}\text{C} = 1.0031 \text{ Da}$
- $^{15}\text{N} - ^{14}\text{N} = 0.9970 \text{ Da}$
- $^{17}\text{O} - ^{16}\text{O} = 1.0042 \text{ Da}$
- Therefore, ^{13}C and ^{15}N will be different by -0.0061 Da and ^{13}C and ^{17}O by 0.0011 Da
- The ^2H and ^{13}C difference would be 0.0032 Da , but only accounts for 1% of the apparent ^{13}C difference

Stable isotopes of the most abundant elements found in biological materials

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	33.9679	4.29%*
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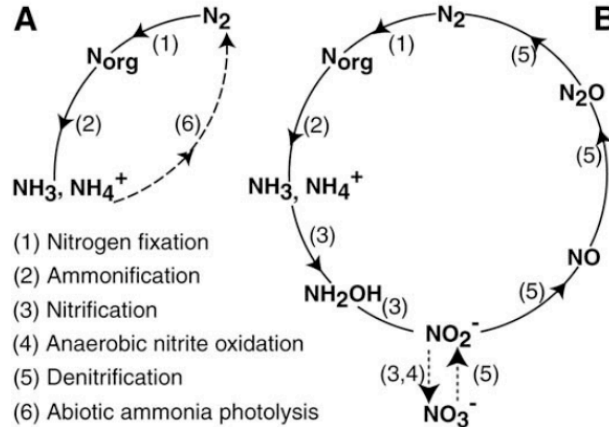
*Varies according to its source

$^{15}\text{N}/^{14}\text{N}$ ratio over the archaea periods

- Earth did not start with an oxygen atmosphere
 - Dominated by a $\text{N}_2/\text{NH}_3/\text{CN}^-$ anaerobic environment for first several billion years
 - First settlers on Earth were archaea bacteria
 - A nitrogen-fixing archaeon from a deep sea volcanic vent operates at 92°C

Processes leading to changing $^{15}\text{N}/^{14}\text{N}$ ratio

Fig. 2. Nitrogen cycle transformations. **(A)** Hypothesized anaerobic N cycle before Mount McRae $\delta^{15}\text{N}$ excursion and **(B)** hypothesized suboxic aerobic N cycle at peak of Mount McRae $\delta^{15}\text{N}$ excursion. The broken line indicates abiotic processes, and the dotted line indicates plausible but unproven processes.



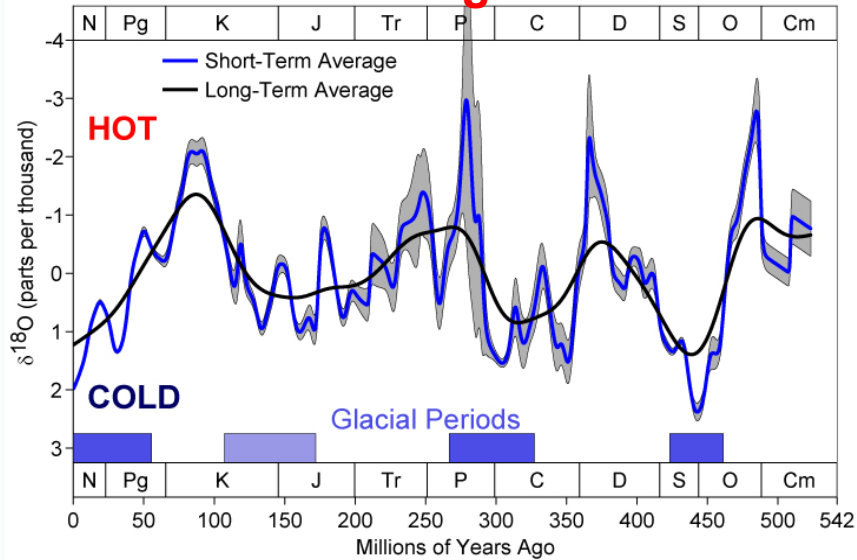
Examination of the $^{15}\text{N}/^{14}\text{N}$ ratio in 2.5 billion year old Mount McRae Shale in W. Australia reveals a transient period of nitrification and implies that nitrifying and denitrifying bacteria were already present

Garvin et al., Science 323, 1045 (2009)

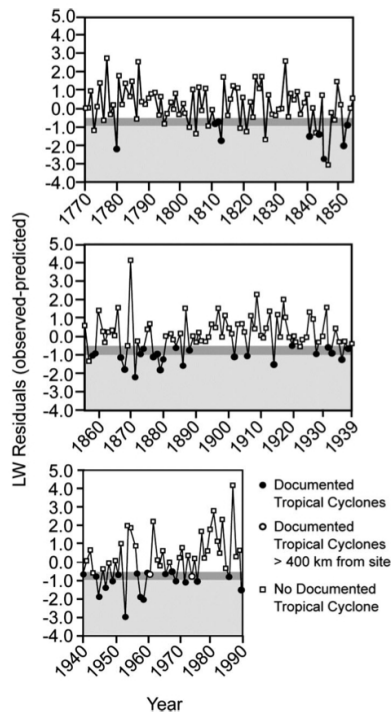
$^{18}\text{O}/^{16}\text{O}$ isotope ratio

- **Evaporation of H_2^{18}O requires more energy than H_2^{16}O**
 - Water vapor is enriched in ^{16}O whereas ice is enriched in ^{18}O
 - Measurement of $^{18}\text{O}/^{16}\text{O}$ ratio in ice cores allows scientists to estimate the temperature over the past millions of years
 - As the temperature decreases, the $^{18}\text{O}/^{16}\text{O}$ ratio falls
 - Hurricanes cause a severe depletion of ^{18}O – this can be detected in trees
 - Calcite (shells) takes one O from water and parallels the ice record

$^{18}\text{O}/^{16}\text{O}$ isotope ratio and climate change



http://en.wikipedia.org/wiki/Oxygen_isotope_ratio_cycle



Association of $\delta^{18}\text{O}$ changes with tropical storms

The bulk time-dependent change in ^{18}O is modeled and then fitted to the isotope record as measured in tree rings.

The known tropical cyclones are associated with significant deviation to lower amounts of ^{18}O .

Miller et al., PNAS 103:14294, 2006

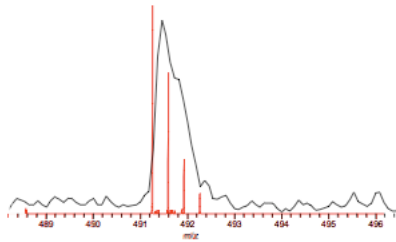
^{15}N and the $^{13}\text{C}_1$ isotope abundance

- It appears that there is a contribution from the ^{15}N and ^{17}O isotopes
 - For ^{15}N it is estimated as $(0.36/1.11)/(58/17) = 0.09506$ in r
 - Thus $r^* = 0.86108$ and $y^* = 0.86106/(58 + 0.86104)$, i.e., 0.014629, or 1.4629%

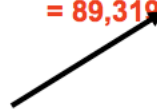
Conclusions so far

- The method using comparison of the isotope peaks of a peptide is not sufficiently good (yet)
- The calculations used in this first approach employed peak heights
 - It would be better if we could deconvolute the overlapping peaks to obtain estimate of the peak areas
 - Better still to resolve the peaks entirely and separate the isotopic contaminants

Mass resolution on different instruments

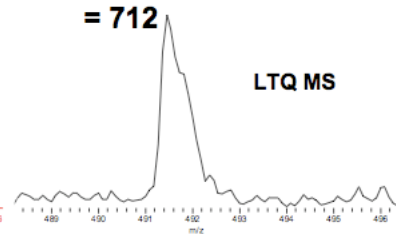


RP = 491.2594 / 0.0055 amu
= 89,319



FT-ICR MS

RP = 491.45 / 0.69 amu
= 712



LTQ MS

Can high resolution FT-ICR-MS resolve the ^{13}C , ^{15}N isotope contributions?

- $\Delta ^{13}\text{C}-^{12}\text{C} = 1.0034 \text{ Da}$
- $\Delta ^{15}\text{N}-^{14}\text{N} = 0.9970 \text{ Da}$
- Therefore, the difference between a ^{13}C and ^{15}N contribution is 0.0064 Da
- The m/z of the doubly charged keratin peptide is 733
- Resolution needed is $2 \times 733 / 0.0064 = 229,063$
- Achievable on 7T FT-ICR instrument if the FID is observed for several seconds