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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 $^{22}Ne$

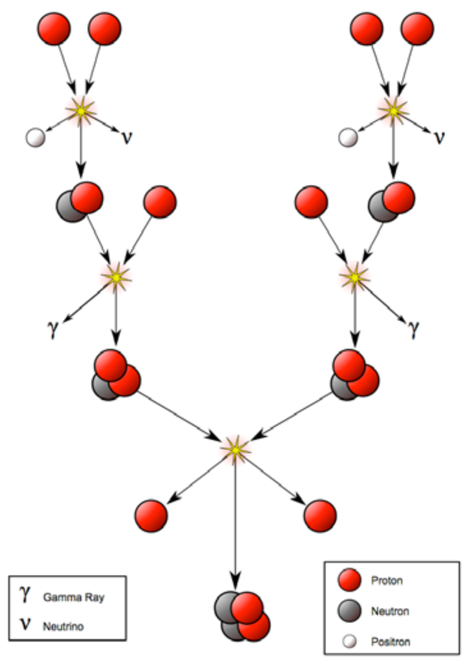
http://www.asms.org/Publications/Historical/HistoryofMassSpectrometry/tabid/94/Default.aspx

## 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 $^{235}U$
  - What were the other methods?
  - Richard Rhodes – *The making of the atomic bomb*

## Why we are depends on mass

- 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
  -  $\Delta m = 0.00695$ Da




## 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.

$$^1H + {}^1H \rightarrow {}^2_1D + e^+ + \nu_e + 0.42\ \text{MeV}$$

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, $^3He$:

$$^2_1D + {}^1H \rightarrow {}^3_2He + \gamma + 5.49\ \text{MeV}$$

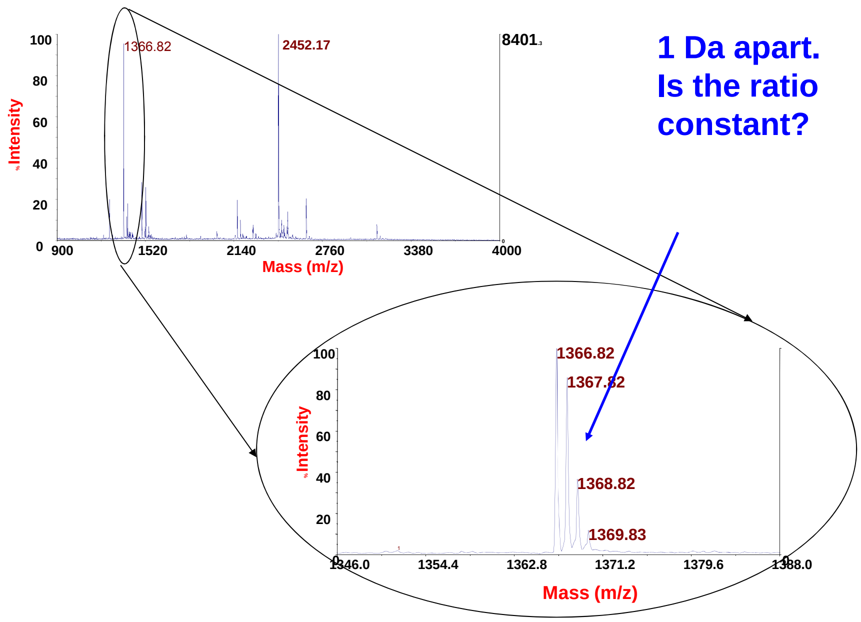
From here there are three possible paths to generate helium isotope $^4He$. In pp I helium-4 comes from fusing two of the helium-3 nuclei produced; the pp II and pp III branches fuse $^3He$ with a pre-existing $^4He$ to make Be. In the Sun, branch pp I takes place with a frequency of 86%, pp II with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

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

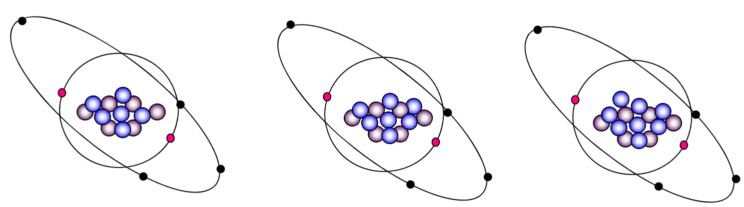
# Energy from hydrogen

- **Fusion of 4 g of hydrogen leads to the loss of 6.95 mDa**
- **$E = mc^2$**
- **$E = 6.95 \times 10^{-3} \times (3 \times 10^8 m/s)^2$ joules**
- **$E = 6.28 \times 10^{14}$ joules**
- **$E = 1.744 \times 10^{11}$ watt/hours**
  - **Let's suppose there are 100 million households in the USA and they each have five 100 watt bulbs that are on for 12 hours per day**
  - ***How long will the lights stay on?***

# Isotope profile of an individual peptide ion




# Elements



| | | |
|---|---|---|
| Carbon-12<br>6 protons<br>6 neutrons<br>6 electrons | Carbon-13<br>6 protons<br>7 neutrons<br>6 electrons | Carbon-14<br>6 protons<br>8 neutrons<br>6 electrons |
| Stable | Stable | Unstable<br>Neutron decays to produce a proton, a β-particle and an anti-neutrino - $^{14}C$ becomes $^{14}N$ |

**Naturally occurring isotopes**

## Stable isotopes of the most abundant elements found in biological materials

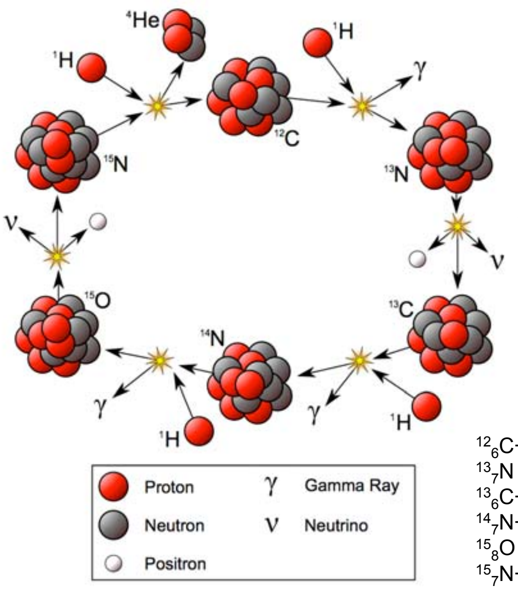
| Element | Mass | Abundance |
|---|---|---|
| H | 1.0078<br>2.0141 | 99.985%<br>0.015% |
| C | 12.0000<br>13.0034 | 98.89%*<br>1.11%* |
| N | 14.0031<br>15.0001 | 99.64%*<br>0.36%* |
| O | 15.9949<br>16.9991<br>17.9992 | 99.76%*<br>0.04%*<br>0.20%* |
| S | 31.9721<br>32.9715<br>33.9679<br>35.9671 |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

*Varies according to its source

# The $^{12}C/^{13}C$ ratio

- $^{12}C$ was born in the inferno of stars from the triple fusion of $^{4}He$ 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
  - Thaolins are in the atmosphere of the moon Titan - future source of life?

## The CNO cycle - Bethe-Weizsäcker-cycle




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

$^{12}_{6}C + ^{1}H \rightarrow ^{13}_{7}N + \gamma$ +1.95 MeV

$^{13}_{7}N \rightarrow ^{13}_{6}C + e^{+} + \nu_e$ +2.22 MeV

$^{13}_{6}C + ^{1}H \rightarrow ^{14}_{7}N + \gamma$ +7.54 MeV

$^{14}_{7}N + ^{1}H \rightarrow ^{15}_{8}O + \gamma$ +7.35 MeV

$^{15}_{8}O \rightarrow ^{15}_{7}N + e^{+} + \nu_e$ +2.75 MeV

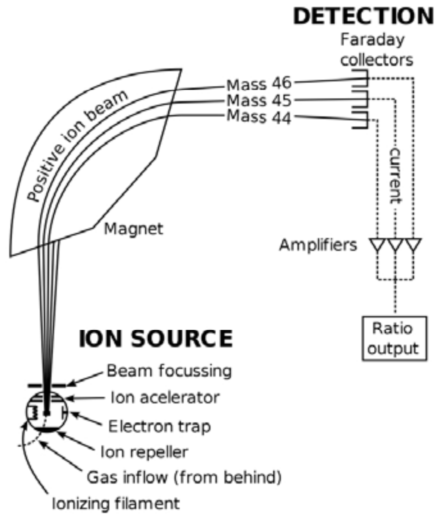
$^{15}_{7}N + ^{1}H \rightarrow ^{12}_{6}C + ^{4}_{2}He$ +4.96 MeV

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

## Terrestial $^{12}C/^{13}C$ ratio

- On average there is 1.11% of $^{13}C$ in 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
  - $^{12}C$ and $^{13}C$ partition differently in each of these environments due to physical effects
  - Compounds have different $^{12}C/^{13}C$ ratios

## Isotope ratio mass spectrometer



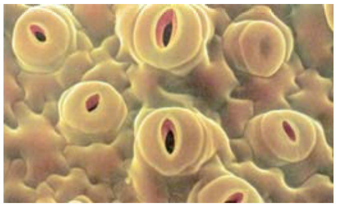

http://en.wikipedia.org/wiki/Isotope_ratio_mass_spectrometry

## Isotope ratio mass spectrometry

- Used for $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$
- Carbon compounds are converted to $CO_2$
- Low mass range 0-150 *m/z*
- Sector instrument
- Very sensitive
- Very accurate measurement of mass
- $^{13}C/^{12}C$ ratio can vary from 0.972% to 1.160%
- PeeDee belemnite (a fossil) has $^{13}C/^{12}C$ ratio of 0.0112372

## Fixation of $CO_2$ as organic carbon

- RuBisCO - enzyme complex in plants
  - Converts $CO_2$ to sugars
  - Prefers $^{12}C$ to $^{13}C$



- Plants take in $CO_2$ through stomata
  - Two models
    - Sponge divers (intermittent breathing)
      - These would sample all the isotopic forms of $CO_2$
    - Swimmers on surface (frequent breathing)
      - These would selectively take in $^{12}CO_2$

## $^{12}C/^{13}C$ ratio in plants

- The $^{13}C$ 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 $^{13}C/^{12}C$ ratio
  - This is a marker for selection of drought-resistant strains, important in the coming global warming

# Landis' backup doping test also positive

**Tour de France winner likely to lose crown, but says he'll appeal results**

Jasper Juinen / AP

Floyd Landis tested positive for a high ratio of testosterone and is likely to lose his Tour de France victory.

**NBC VIDEO**

Launch

**Landis on last leg?**

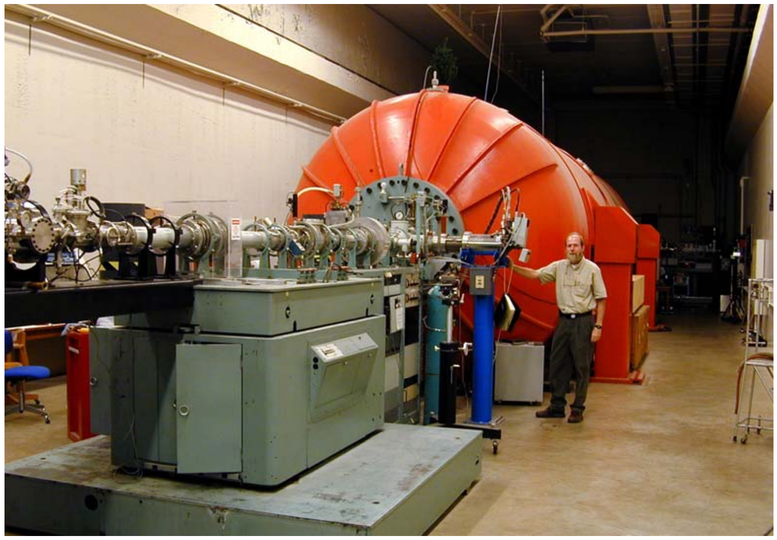
Aug. 5: The results of a second drug test for Tour De France winner Floyd Landis confirmed a level of synthetic testosterone. Tour officials say they no longer consider Landis the race winner. NBC's George Lewis reports.

Nightly News

# Athletes who use synthetic testosterone

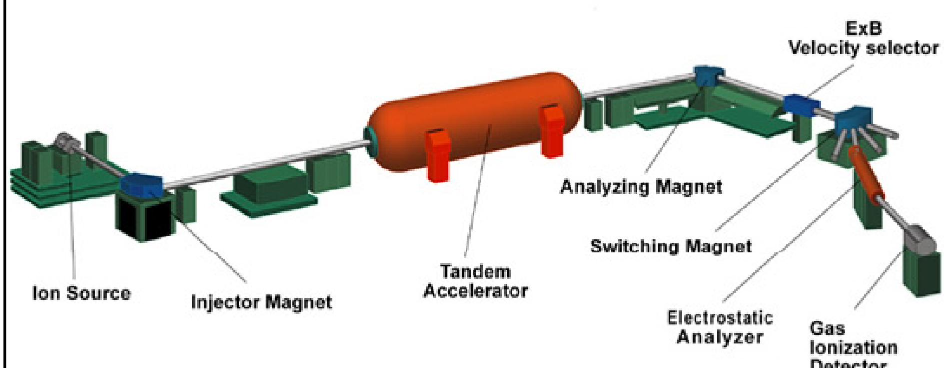
- **Synthetic testosterone is made from phytosterol percursors, typically derived from wild yams or soy.**
- **Those are both warm-climate C3 plants, which take up atmospheric carbon dioxide by a different route than temperate-zone C4 plants, leading to noticeably different isotope ratios.**
- **The 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

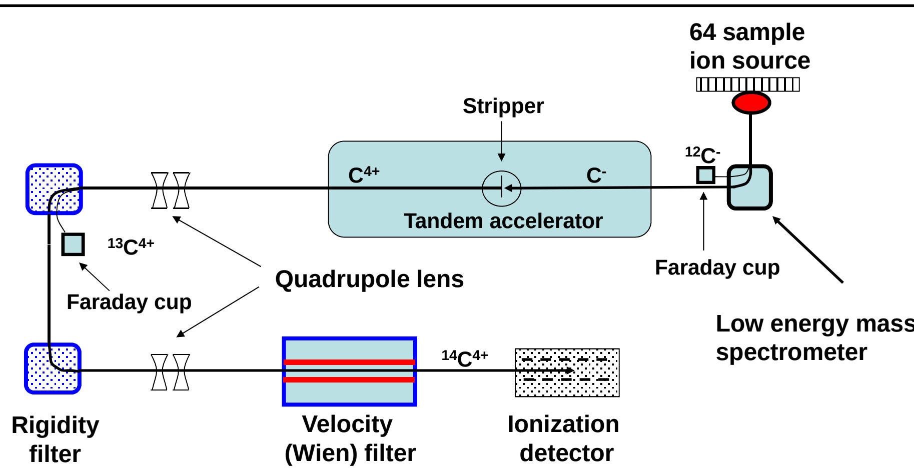


Accelerator mass spectrometer

## Set up of AMS at Purdue



# 10 GeV AMS at LLNL

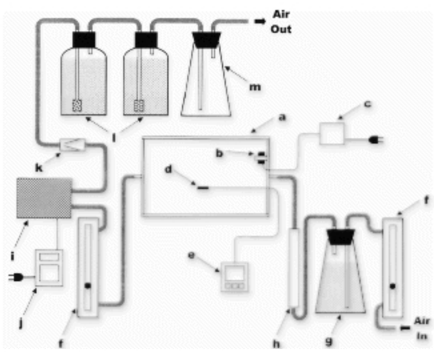



**Ions ($C^-$) are generated by a Cs ion beam. The $^{12}C^-$ ions are removed by a low energy mass spectrometer (note $^{14}N$ does not form ions). The remaining ions are accelerated and on passage through the gold foil stripper form $C^{4+}$ ions. The ions are separated based on momentum, thereby measuring the $^{13}C^{4+}$ ions. The ions are further selected for their *m/z* values in the Rigidity filter, and velocity in the Wien filter. They are finally measured using a multi-anode gas-ionization detector. Approximately 1% of the $^{14}C$ ions generated are detected. Sensitivity is ~10 attomoles of $^{14}C$ from mg sized samples.**

# Sensitivity of $^{14}C$-AMS

- If one carbon atom is incorporated into a compound, then the specific activity is ~50 $\mu C_i/\mu mol$
- The human body naturally contains 50-90 nCi of radioactivity – therefore, a 50 $nC_i$ 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 x $10^{-5}$.
- If distributed evenly, the S/N is 700:1

# $^{14}C$-polyphenols by metabolic labeling




Plant cells incubated with $^{14}C$-labeled sucrose in a closed system

$^{14}C$-labeled polyphenols extracted and fractionated

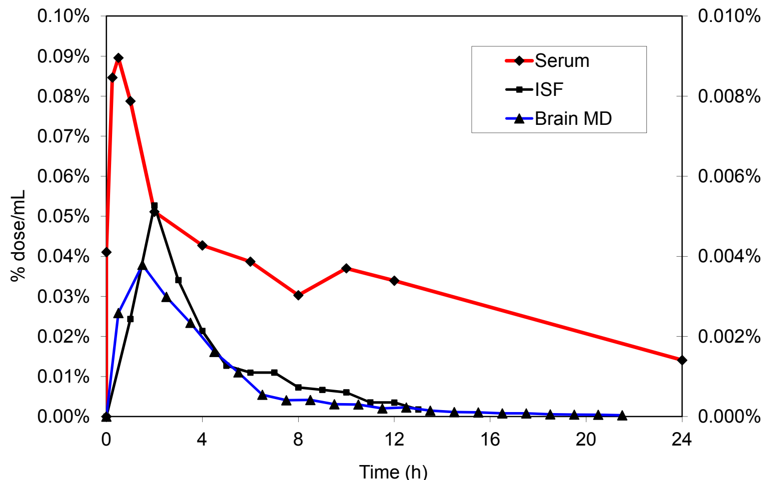
50 nCi of $^{14}C$-labeled polyphenol(s) is 1.11 x $10^5$ dpm or 1 x $10^{-9}$ mol (0.3 μg)

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

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

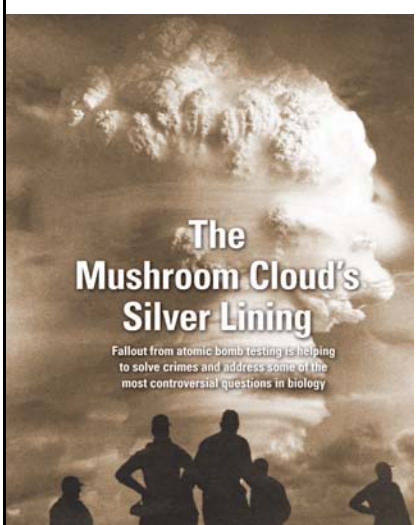
The detection limit for $^{14}C$ using AMS is 1 x $10^{-17}$ mol, i.e., S/N is 100:1

## Differential retention of $^{14}$C-labeled proanthocyanidin by AMS




Elsa Janle et al.

## The lens and its $^{14}$C-content

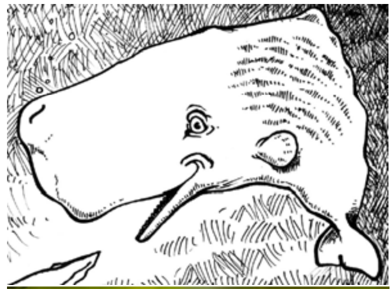



- The $^{14}$C-content of a cellular or tissue sample is a reflection of when the compounds therein were synthesized
- The half-life of $^{14}$C is 5,730 years
- The atmospheric $^{14}$C was doubled by the above ground atomic weapon testing in the 1950s and early 60s and is steadily declining
- The $^{14}$C content allows calculation of the age of the sample
- Used to determine the age of the victims of the 2004 Indonesian tsunami
- Is being used to determine the age of fat cells in the body

Science 321:1434 (2008)

# Whale of a story!

Heather Koopman
UNC Wilmington



George
Jackson
Purdue U

Whale blubber is laid down slowly and may be related to the marine $^{14}C/^{12}C$ composition over time

Is the less solid fat around the echo sensing organ laid down early? Does it turnover?

Applying AMS analysis of whale bone to calibrate the year of deposition and the marine $^{14}C$-content. This will be used to assess the age of the whale blubber.

# Can isotope ratios be ascertained for compounds?

- **Conventional isotope ratio measurements are based on converting the carbon-containing compounds to $CO_2$**
- **Given the improvements in mass spectrometry, could we investigate the isotope ratios of peptides and deduce the underlying $^{13}C/^{12}C$ ratio?**

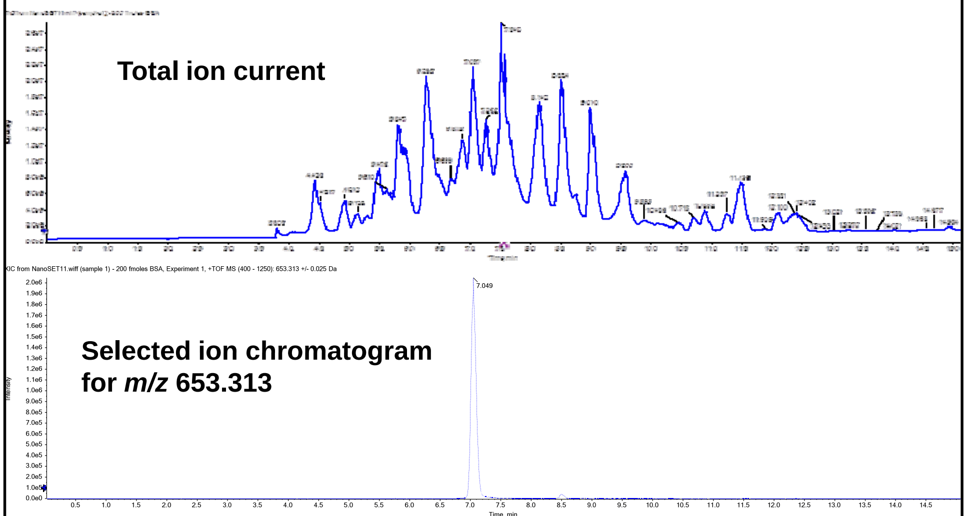
## Expected isotope abundances

- 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, $x^n$; $nx^{(n-1)}y$; $(\Sigma n-1)*x^{(n-2)}y^2$,.....
  - $x^n$ 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)

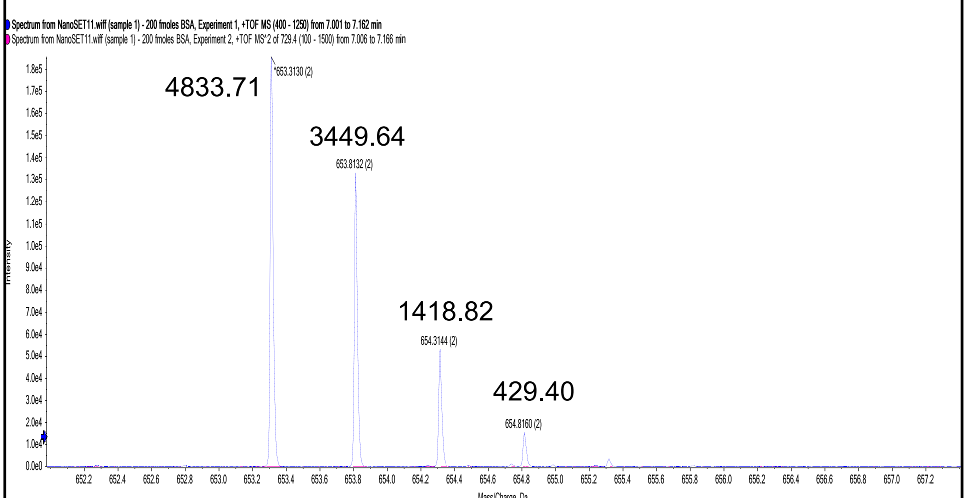
## Calculating the $^{13}C/^{12}C$ ratio in peptides

- 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 $^{13}C/^{12}C$ ratio using the correction for n

# Isolation of a BSA tryptic peptide




# Isotope peaks for LGEYGFQNALIVR

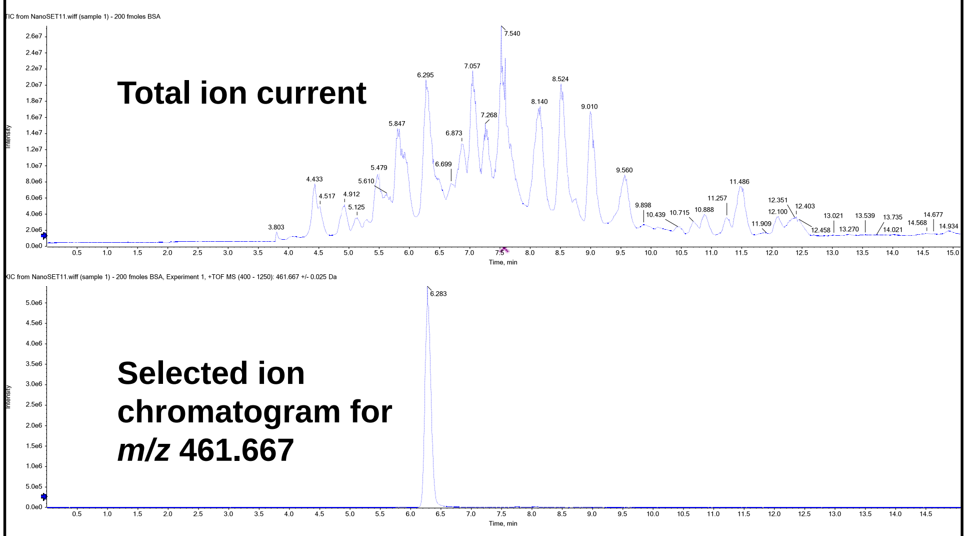



C68 H107 N18 O19

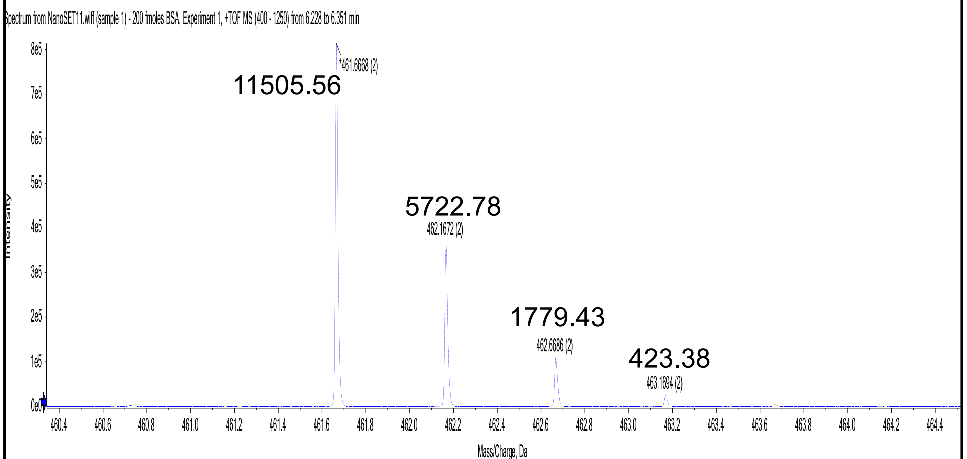
# Estimating $^{13}C$ content

- The ratio of the 1-$^{13}C$ and $^{12}C$ isotope peak heights is 3449.64/4833.71,
  - i.e., r = 0.713663
- Y, the fraction of the carbons that are $^{13}C$, is r/(n+r)
- Y = 0.713663/(68 + 0.713663)
- Y = 0.01038, i.e. 1.038%

# Isolation of a BSA tryptic peptide



# Isotope peaks for AEFVEVTK




# Mass differences for H, C, N and O isotopes

- **$^{2}H$ - $^{1}H$ = 1.0063 Da**
- **$^{13}C$ - $^{12}C$ = 1.0031 Da**
- **$^{15}N$ - $^{14}N$ = 0.9970 Da**
- **$^{17}O$ - $^{16}O$ = 1.0042 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 $^{2}H$ 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

| Element | Mass | Abundance |
|---|---|---|
| H | 1.0078<br>2.0141 | 99.985%<br>0.015% |
| C | 12.0000<br>13.0034 | 98.89%*<br>1.11%* |
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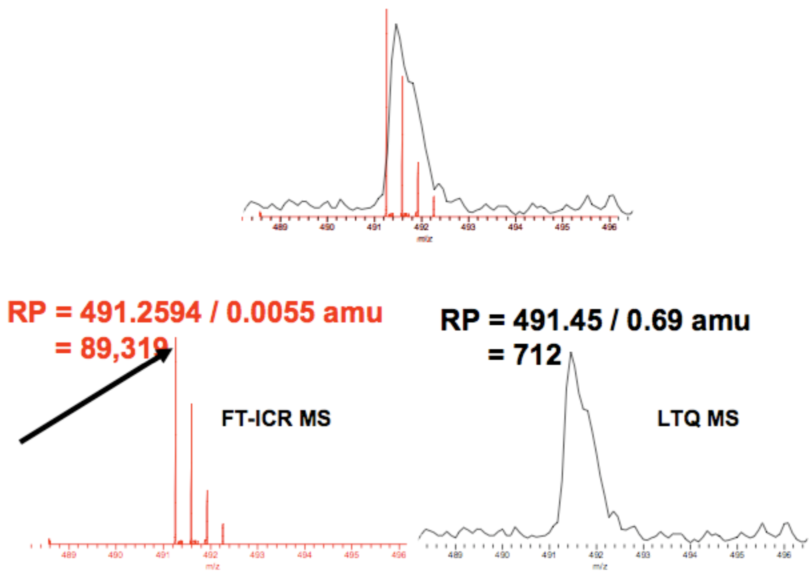
| S | 31.9721<br>32.9715<br>33.9679<br>35.9671 | 94.93%*<br>0.76%*<br>4.29%*<br>0.02%* |

*Varies according to its source

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

- $\Delta$ $^{13}C$-$^{12}C$ = 1.0034 Da
- $\Delta$ $^{15}N$-$^{14}N$ = 0.9970 Da
- Therefore, the difference between a $^{13}C$ and $^{15}N$ contribution is 0.0064 Da
- The *m/z* of the doubly charged BSA peptide is 733
- Resolution needed is 2 x 653/0.0064 = 204,063
- Achievable on 7T FT-ICR instrument if the FID is observed for several seconds

## Mass resolution on different instruments




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

- Earth did not start with an oxygen atmosphere
  - Dominated by a $N_2/NH_3/CN^-$ anerobic 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 $^{15}N/^{14}N$ ratio

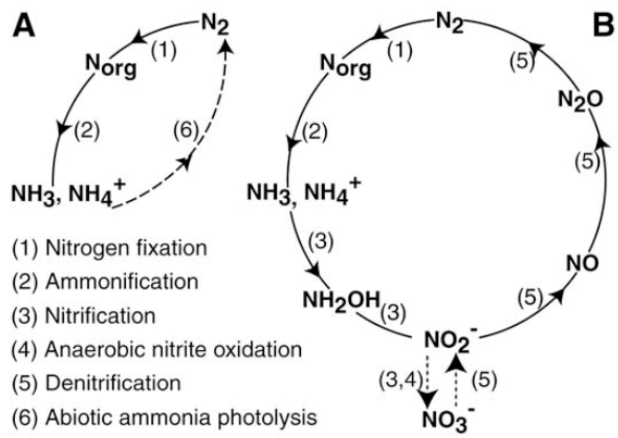



**Fig. 2.** Nitrogen cycle transformations. **(A)** Hypothesized anaerobic N cycle before Mount McRae $\delta^{15}N$ excursion and **(B)** hypothesized suboxic aerobic N cycle at peak of Mount McRae $\delta^{15}N$ excursion. The broken line indicates abiotic processes, and the dotted line indicates plausible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

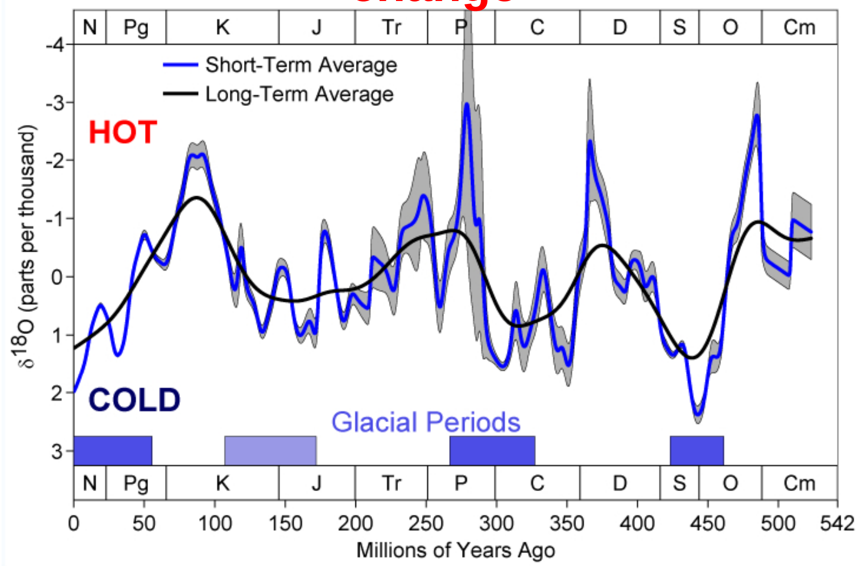
**Examination of the $^{15}N/^{14}N$ ratio in 2.5 billion year old Mount McRae Shale in W. Australia reveals a transient period of nitrification and implies that nitrifying and denitrifying bacteria were already present**

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

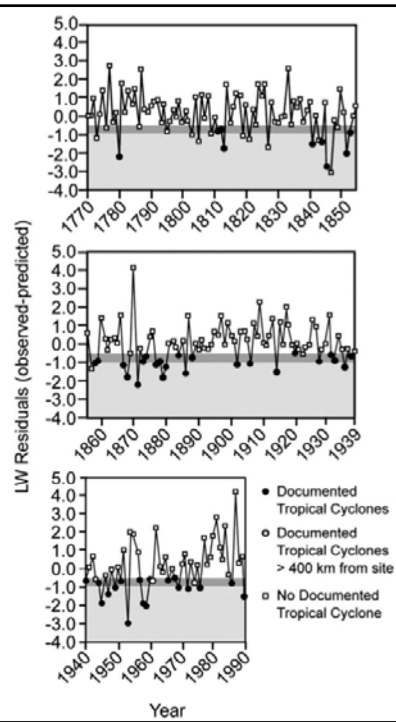
## $^{18}O/^{16}O$ isotope ratio

- **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 $^{18}O/^{16}O$ ratio in ice cores allows scientists to estimate the temperature over the past millions of years**
  - **As the temperature decreases, the $^{18}O/^{16}O$ 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**

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




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




## Association of $\delta^{18}O$ 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