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, $^{20}\text{Ne}$ and $^{22}\text{Ne}$

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 \(^1\)H (protons) into deuterium, releasing a positron and a neutrino as one proton changes into a neutron.

\[ ^1\text{H} + ^1\text{H} \rightarrow ^2\text{D} + e^+ + \nu_e + 0.42 \text{ MeV} \]

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, \(^{3}\)He:

\[ ^2\text{D} + ^1\text{H} \rightarrow ^3\text{He} + \gamma + 5.49 \text{ MeV} \]

From here there are three possible paths to generate helium isotope \(^4\)He. In pp I helium-4 comes from fusing two of the helium-3 nuclei produced; the pp II and pp III branches fuse \(^3\)He with a pre-existing \(^4\)He 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.


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Energy from hydrogen

- Fusion of 4 hydrogen atoms leads to the loss of 6.95 mDa
- \( E = mc^2 \)
- \( E = 6.95 \times 10^{-3} \times (3 \times 10^8 \text{m/s})^2 \) 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 many days will the lights stay on?*
Isotope profile of an individual peptide ion

1 Da apart. Is the ratio constant?

Elements

Carbon-12
6 protons
6 neutrons
6 electrons
Stable

Carbon-13
6 protons
7 neutrons
6 electrons
Stable

Carbon-14
6 protons
8 neutrons
6 electrons
Unstable

Naturally occurring isotopes

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

Carbons-12, 13, 14
Stable isotopes of the most abundant elements found in biological materials

<table>
<thead>
<tr>
<th>Element</th>
<th>Mass</th>
<th>Abundance</th>
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<td>99.985%</td>
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<tr>
<td></td>
<td>2.0141</td>
<td>0.015%</td>
</tr>
<tr>
<td>C</td>
<td>12.0000</td>
<td>98.89%*</td>
</tr>
<tr>
<td></td>
<td>13.0034</td>
<td>1.11%*</td>
</tr>
<tr>
<td>N</td>
<td>14.0031</td>
<td>99.64%*</td>
</tr>
<tr>
<td></td>
<td>15.0001</td>
<td>0.36%*</td>
</tr>
<tr>
<td>O</td>
<td>15.9949</td>
<td>99.76%*</td>
</tr>
<tr>
<td></td>
<td>16.9991</td>
<td>0.04%*</td>
</tr>
<tr>
<td></td>
<td>17.9992</td>
<td>0.20%*</td>
</tr>
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*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 cycle 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 \(^4\)He nuclei being produced in the Sun are born in the CNO cycle.

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

Terrestrial \(^{12}\)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 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 (a rock) 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
Ions (C) are generated by a Cs ion beam. The $^{12}\text{C}^-$ ions are removed by a low energy mass spectrometer (note $^{14}\text{N}$ does not form ions). The remaining ions are accelerated and on passage through the gold foil stripper form $^{13}\text{C}^{4+}$ ions. The ions are separated based on momentum, thereby measuring the $^{12}\text{C}^{4+}$ 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 $^{14}\text{C}$ ions generated are detected. Sensitivity is ~10 attomoles of $^{14}\text{C}$ from mg sized samples.
Sensitivity of $^{14}$C-AMS

- If one carbon atom is incorporated into a compound, then the specific activity is $\sim 50 \ \muCi/\mumol$
- 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 $1.4 \times 10^{-5}$.
- 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 $1.11 \times 10^5$ dpm or $1 \times 10^{-9}$ 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 $1 \times 10^{-12}$ mol

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

The detection limit for $^{14}$C using AMS is $1 \times 10^{-17}$ mol, i.e., S/N is 100:1
Differential retention of $^{14}$C-labeled proanthocyanidin by AMS

\[ \begin{align*} 
\text{Serum} & : 0.10\% \\
\text{ISF} & : 0.08\% \\
\text{Brain MD} & : 0.06\% 
\end{align*} \]

\[ \begin{align*} 
0 & : 0.00\% \\
4 & : 0.01\% \\
8 & : 0.02\% \\
12 & : 0.03\% \\
16 & : 0.04\% \\
20 & : 0.05\% \\
24 & : 0.06\% 
\end{align*} \]

Time (h)

\[ \begin{align*} 
0.000\% & : 0.000\% \\
0.002\% & : 0.004\% \\
0.008\% & : 0.010\% \\
0.010\% & : 0.010\% 
\end{align*} \]

% dose/mL

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

Whale of a story!

Heather Koopman
UNC Wilmington

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?

George Jackson
Purdue U

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, $x^n; nx^{n-1}y; (\Sigma n-1)*x^{(n-2)}y^2; \ldots$
  - $x^n$ 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 $r\cdot ry=ny$
  - 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

Total ion current

Selected ion chromatogram for m/z 653.313

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

Total ion current

Selected ion chromatogram for $m/z$ 461.667
Isotope peaks for AEFVEVTK

Mass differences for H, C, N and O isotopes

- $^2\text{H} - ^1\text{H} = 1.0063$ Da
- $^{13}\text{C} - ^{12}\text{C} = 1.0031$ Da
- $^{15}\text{N} - ^{14}\text{N} = 0.9970$ Da
- $^{17}\text{O} - ^{16}\text{O} = 1.0042$ Da
- Therefore, $^{13}\text{C}$ and $^{15}\text{N}$ will be different by $-0.0061$ Da and $^{13}\text{C}$ and $^{17}\text{O}$ by $0.0011$ Da
- The $^2\text{H}$ and $^{13}\text{C}$ difference would be $0.0032$ Da, but only accounts for 1% of the apparent $^{13}\text{C}$ difference
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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 $2 \times \frac{653}{0.0064} = 204,063$
  
  * Achievable on 7T FT-ICR instrument if the FID is observed for several seconds
Mass resolution on different instruments

$\text{RP} = 491.2594 / 0.0055 \text{ amu} = 89,319$

$\text{RP} = 491.45 / 0.69 \text{ amu} = 712$

$^{15}\text{N}/^{14}\text{N}$ 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 $92^\circ\text{C}$
Processes leading to changing $^{15}\text{N}/^{14}\text{N}$ ratio

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.

\textit{Garvin et al., Science 323, 1045 (2009)}

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

- Evaporation of $\text{H}_2^{18}\text{O}$ requires more energy than $\text{H}_2^{16}\text{O}$
  - Water vapor is enriched in $^{16}\text{O}$ whereas ice is enriched in $^{18}\text{O}$
  - 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 $^{18}\text{O}$ – this can be detected in trees
  - Calcite (shells) takes one O from water and parallels the ice record
18O/16O isotope ratio and climate change


Association of δ18O changes with tropical storms

The bulk time-dependent change in 18O 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 18O.

Miller et al., PNAS 103:14294, 2006