NMR
(isotopomer distributions)
in Biogeochemistry

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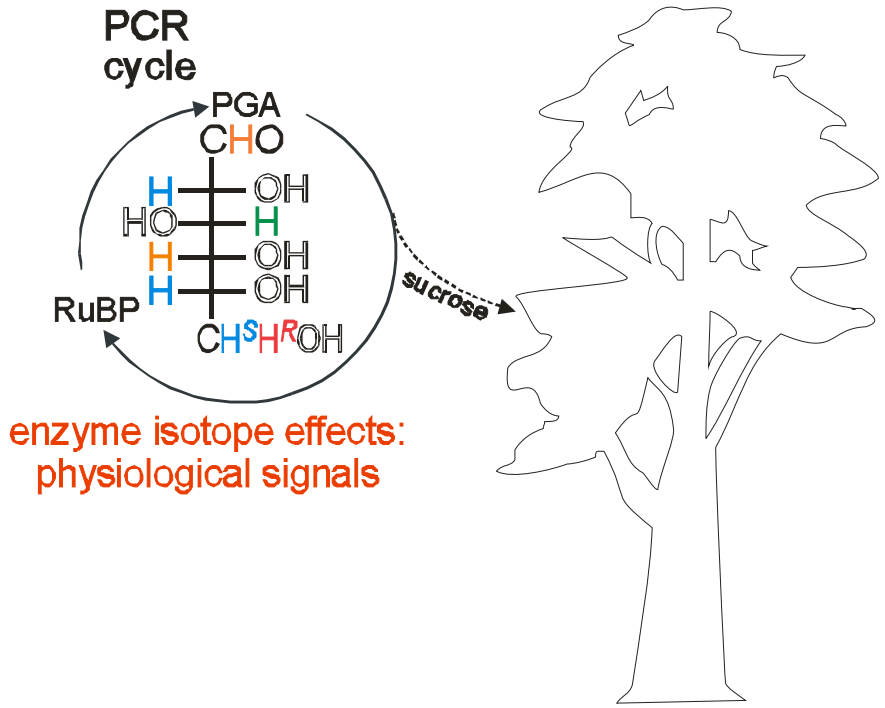
Outline

- Definition of Isotopomers
- Measurement techniques (principle, advantages)
  - off-line breakdown + IRMS
  - on-line breakdown-IRMS
  - light spectroscopy
- NMR
- Deuterium
  - climate and physiological signals from tree rings
  - persistent organochlorines
- Appetizers: ^{13}C: glucose, ^{15}N: N_2O
- Conclusions

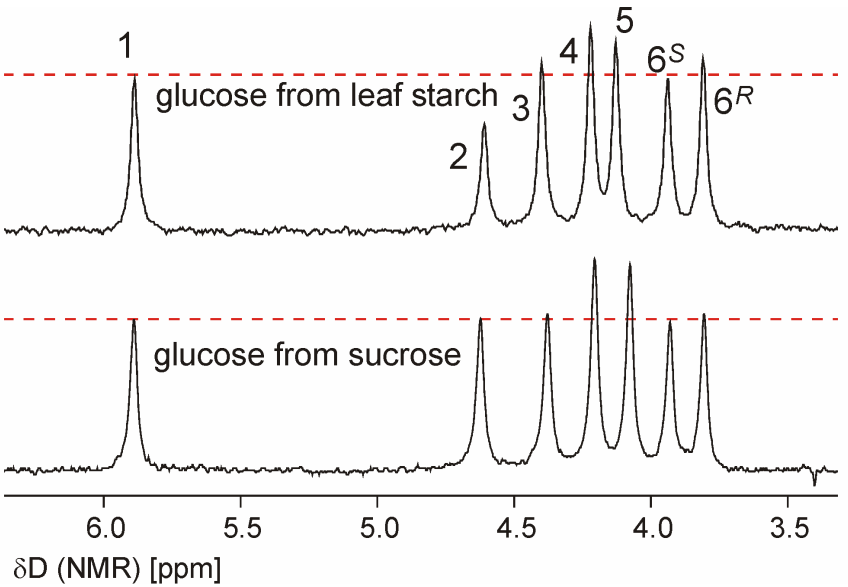
- Poster on liquid water / biological activity in frozen soils

Isotope effects cause isotopomer distributions



- Chemical isotope effects deplete isotopes in non-equivalent groups (isotopomers).
- The abundance of isotopomers can be described by isotopomer distributions (positional/site-specific δ value/isotope ratio).
- Wherever you look: Non-random isotopomer distributions.

Isotopomer distribution and δ



- ~40% depletion of C2-H in starch, linked to metabolic regulation (PGI).
- Depletes whole molecule by ~-60 ‰. δ difference cannot be interpreted alone.
- Express isotopomer distributions as relative abundance of isotopomer i A_i, so that the average is one. Then
- $\delta_i = (\delta_{\text{whole molecule}} + 1000) * A_i - 1000$
- No reference materials yet for isotopomer distributions.

Measuring Isotopomer Abundances

Normally, isotope ratio MS measures the average isotope abundance of a whole metabolite.

Techniques to measure Isotopomer abundances:

- off-line breakdown + IRMS
- breakdown coupled online to IRMS
- Light-spectroscopic techniques
- NMR

Principle, experimental setup, advantages / disadvantages

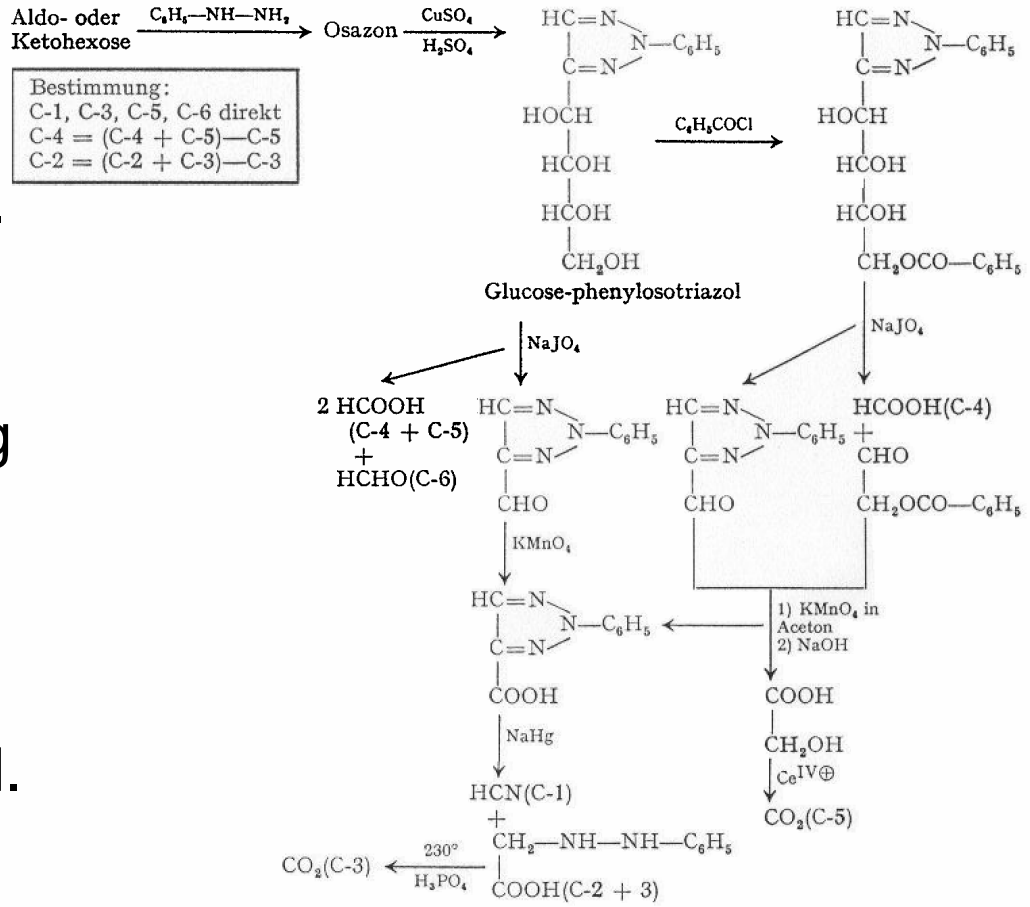
Off-line breakdown and IRMS

- Quantitative chemical breakdown, from isotope labelling (Floss and Simon).
- ^{13}C pattern of glucose reflects metabolic branching (Gleixner & Schmidt).

\+ Broad applicability.

\- Specific for each compound.

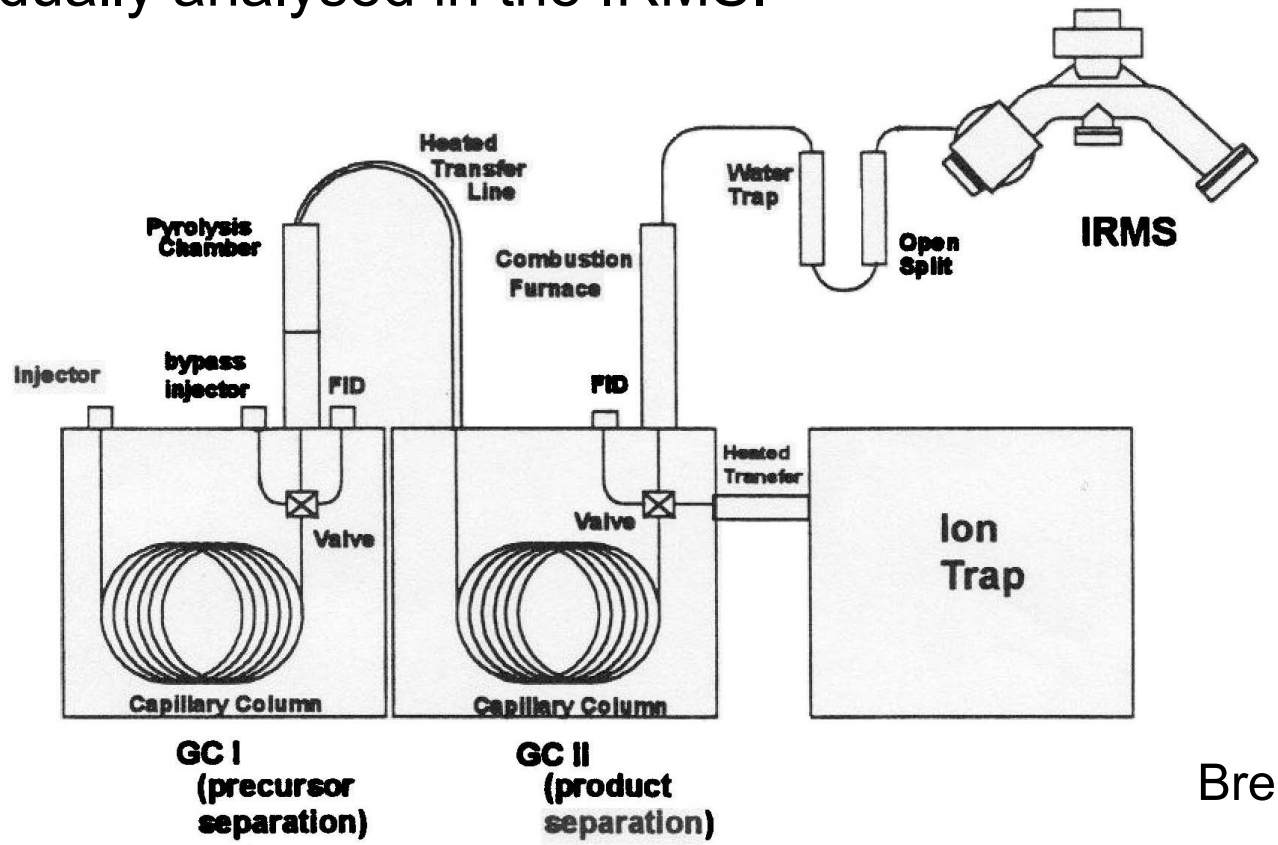
\- Extremely labor-intensive.



Schema 18. Abbau von Glucose und anderen Hexosen nach SIMON und STEFFENS (*678*)

Pyrolysis-GC-IRMS

Compound is fragmented, the fragments separated and on-line individually analysed in the IRMS.



Brenna 1997

FIG. 1. Experimental system for online PSIA. Capillary GC-I separates the target compound from the mixture, passes it into a hollow, deactivated fused-silica tube pyrolysis furnace held at about 550°C. Fragments pass through the heated transfer line to GC-II with the oven cooled to −40°C for collection and focusing. After separation, fragments can be directed by valving to (*i*) an FID, (*ii*) a Varian QISMS ion trap MS for structural analysis, or (*iii*) a combustion/water-trap/open-split interface to a Finnigan–MAT model 252 IRMS for high-precision isotopic analysis.

Summary: Pyrolysis GC-IRMS

+ Small sample amounts.
+ Fast!
- Conditions for each compound needed.
- Isotope effects during breakdown.
- Isotope scrambling is a big problem, especially for D.
- Not isotope-specific, but mass-specific.
  Cross-talk, for example between *isotopologes*
  $^{15}N^{16}O$ and $^{14}N^{17}O$.

FTIR: experimental setup

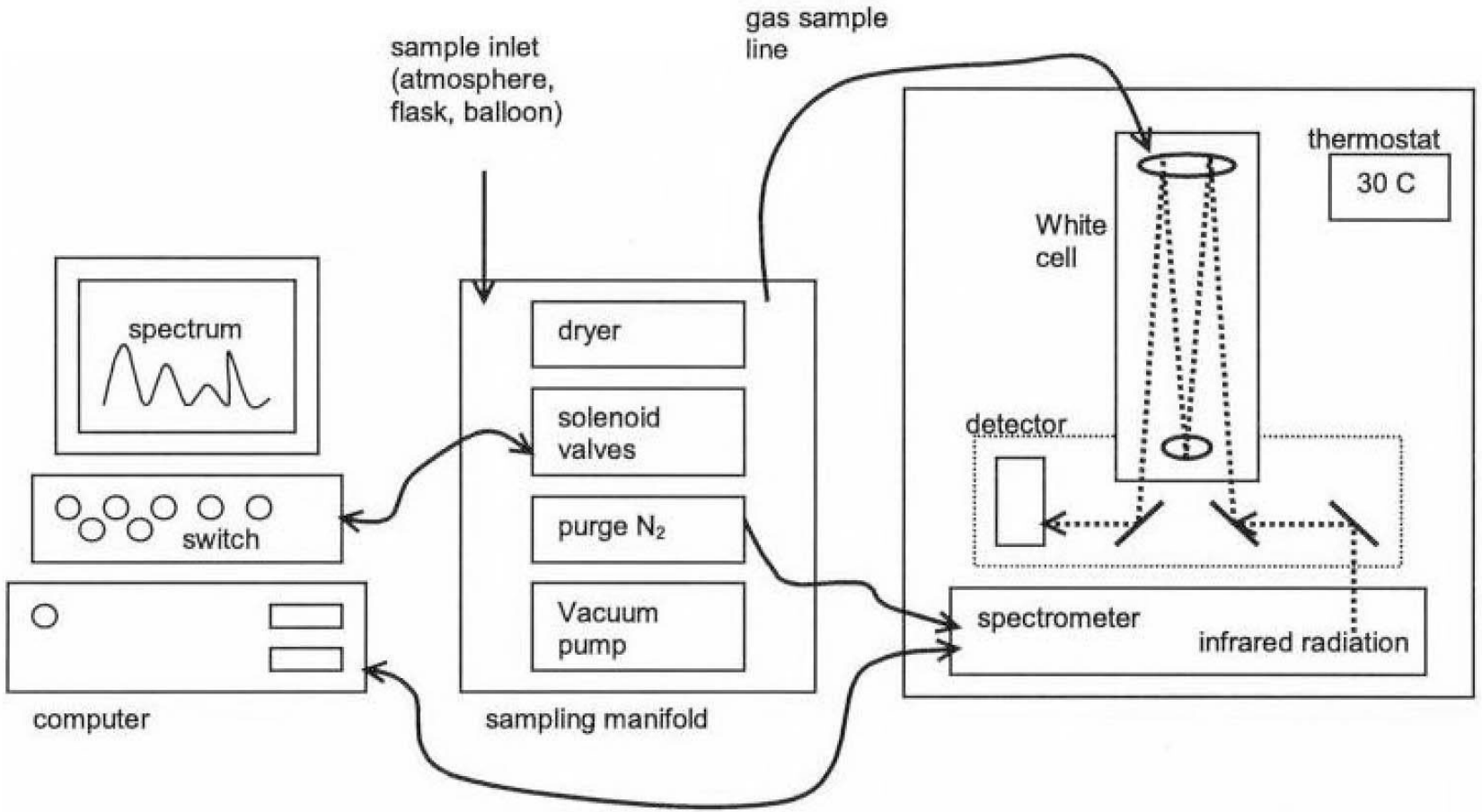


Fig. 1. Schematic of the low resolution FTIR instrument for trace gas analysis.

IR spectra of N_2O isotopomers and isotopologes

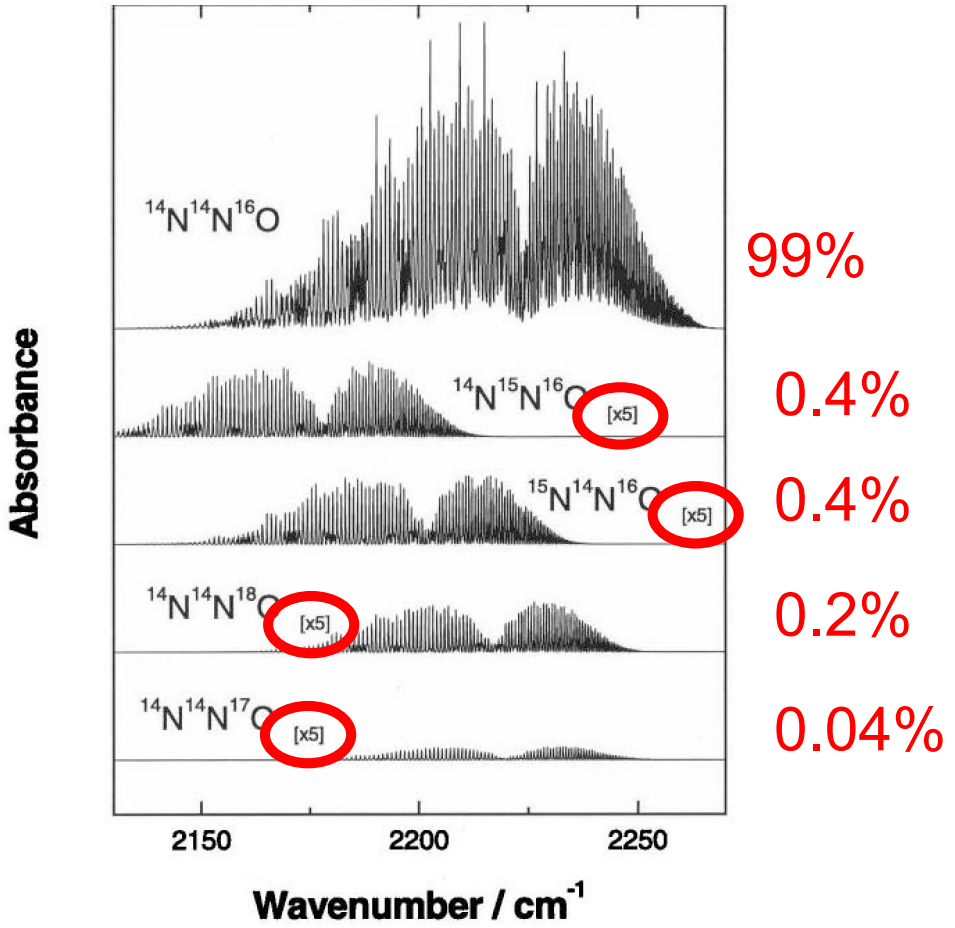


Fig. 7. High resolution (0.005 cm^{-1}) FTIR absorbance spectra of the individual isotopomers of N_2O, $^{14}N^{14}N^{16}O$, $^{14}N^{15}N^{16}O$, $^{15}N^{14}N^{16}O$, $^{14}N^{14}N^{18}O$, $^{14}N^{14}N^{17}O$, calculated from the HITRAN database using the MALT program.

Light spectroscopy

- Each isotopomer has a distinct IR spectrum.
- Spectra overlap, but can be resolved at high resolution.

\+ No fragmentation of molecule
\+ Sensitive
\- Only small molecules, because measurement in gas phase and spectral overlap.

- Used to measure $OO^{18}O$ / $O^{18}OO$ and ^{15}NNO / $N^{15}NO$

NMR

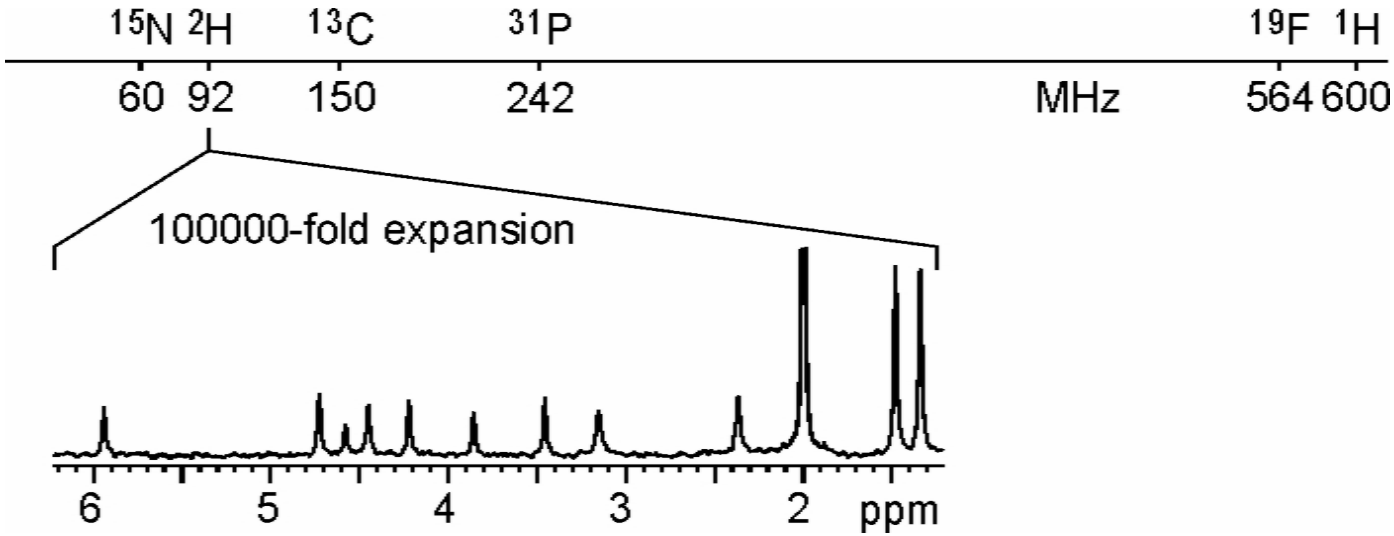
- Nuclei of many isotopes behave like induced magnets in an external magnetic field.
- Radiofrequency pulses „rotate magnets“, which then oscillate and emit radiofrequency.
- Detect radiofrequency, Fourier transform > spectrum.

NMR setup

NMR electronics and magnet.

Sample in 500 µl solution in glass tube.

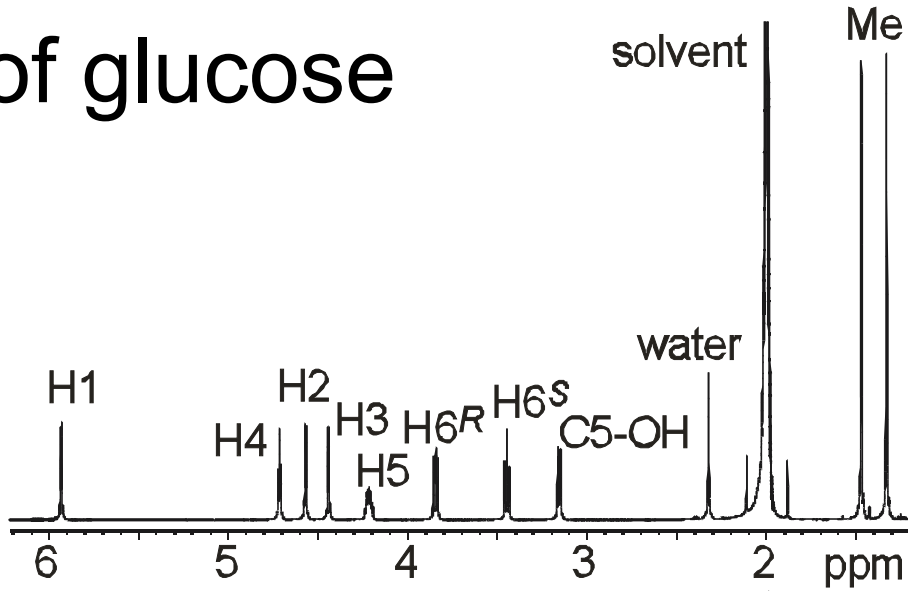
Spectra of NMR isotopes



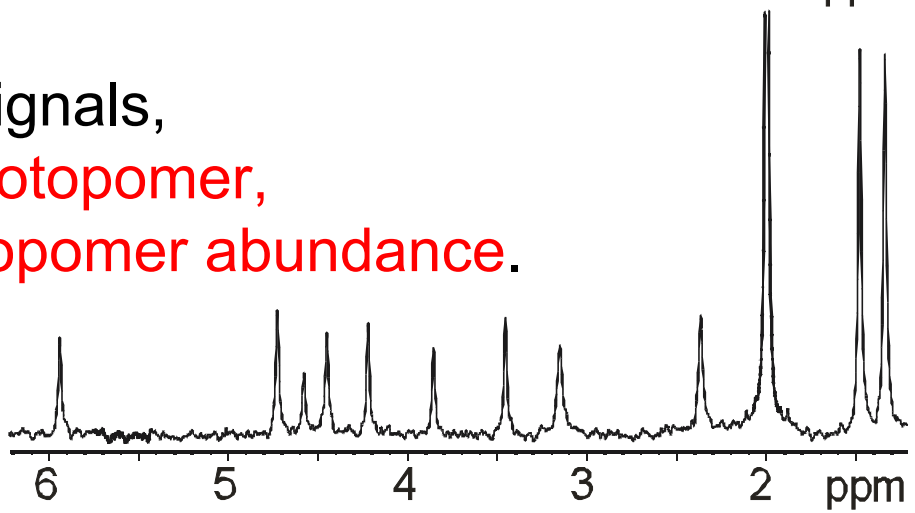
- Sensitivity, quality of spectra given by nuclear properties: $^{1}H > (^{19}F, ^{31}P) > ^{2}H > ^{13}C > ^{15}N >>> ^{17}O$.
- 100000-fold expansion: ^{2}H spectrum.
- One signal per isotopomer.
- Integrals reflect isotopomer abundance.

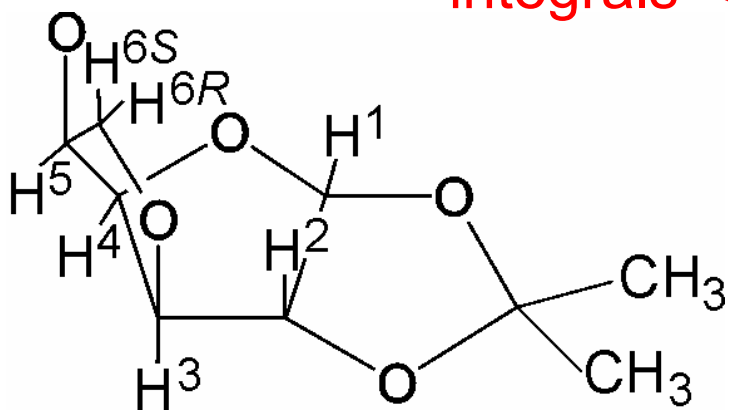
^{1}H and ^{2}H NMR of glucose

^{1}H: Equal integrals,
who is who?



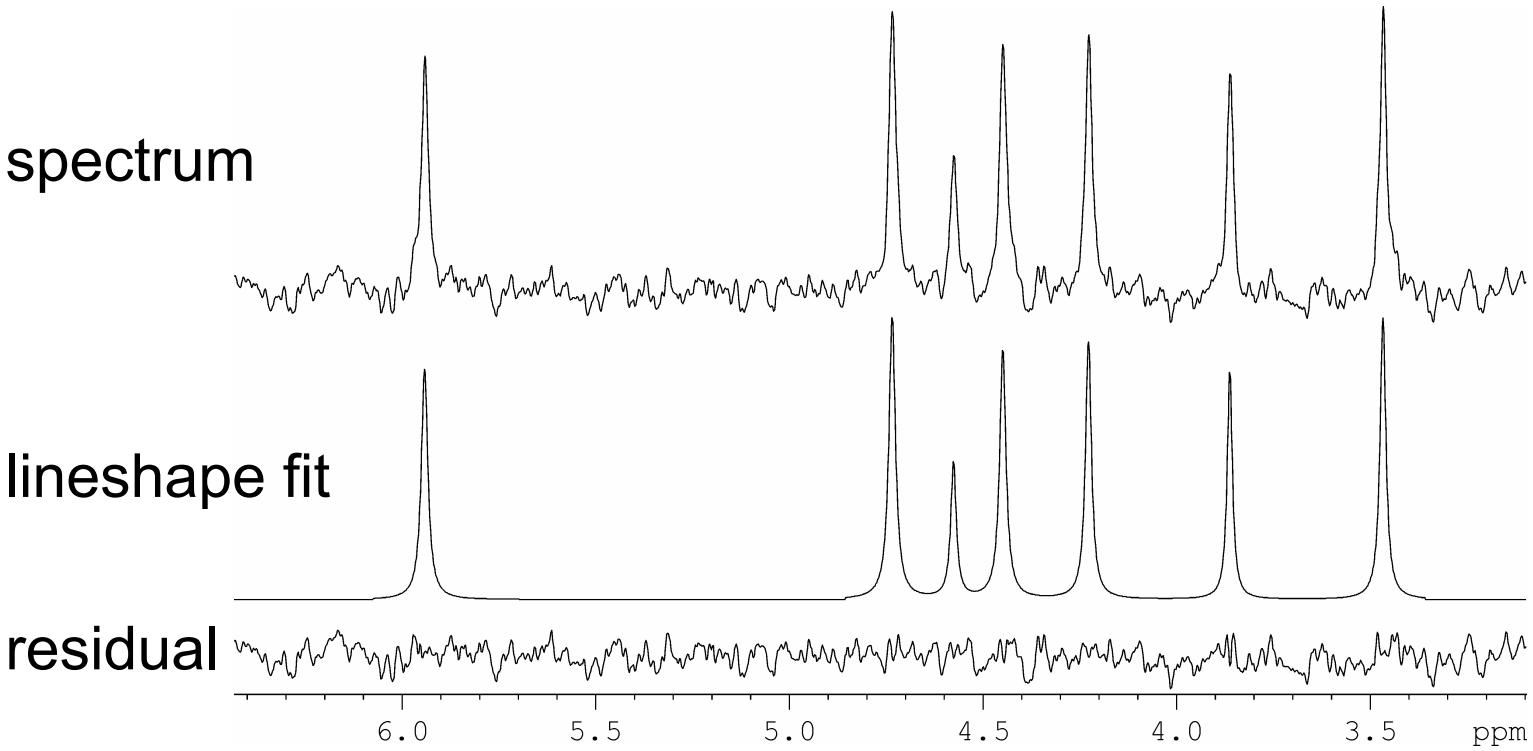
^{2}H: Corresponding signals,
one signal per isotopomer,
integrals <> isotopomer abundance.





ppm scale: **Position** of signals!

Integrating ^{2}H NMR spectra



- Determine parameters for integrateable spectra.
- Assure pure Lorentzian lineshapes.
- Lineshape fit, accuracy limited by S/N

NMR: advantages and disadvantages

+ Intact molecule is analysed $\Rightarrow$ no fractionation.
+ Molecule-specific setup relatively simple.
+ NMR can resolve CHD and CDH isotopomers.
+ NMR is isotope-specific, ^{2}H and ^{13}C abundances do not interfere with each other.

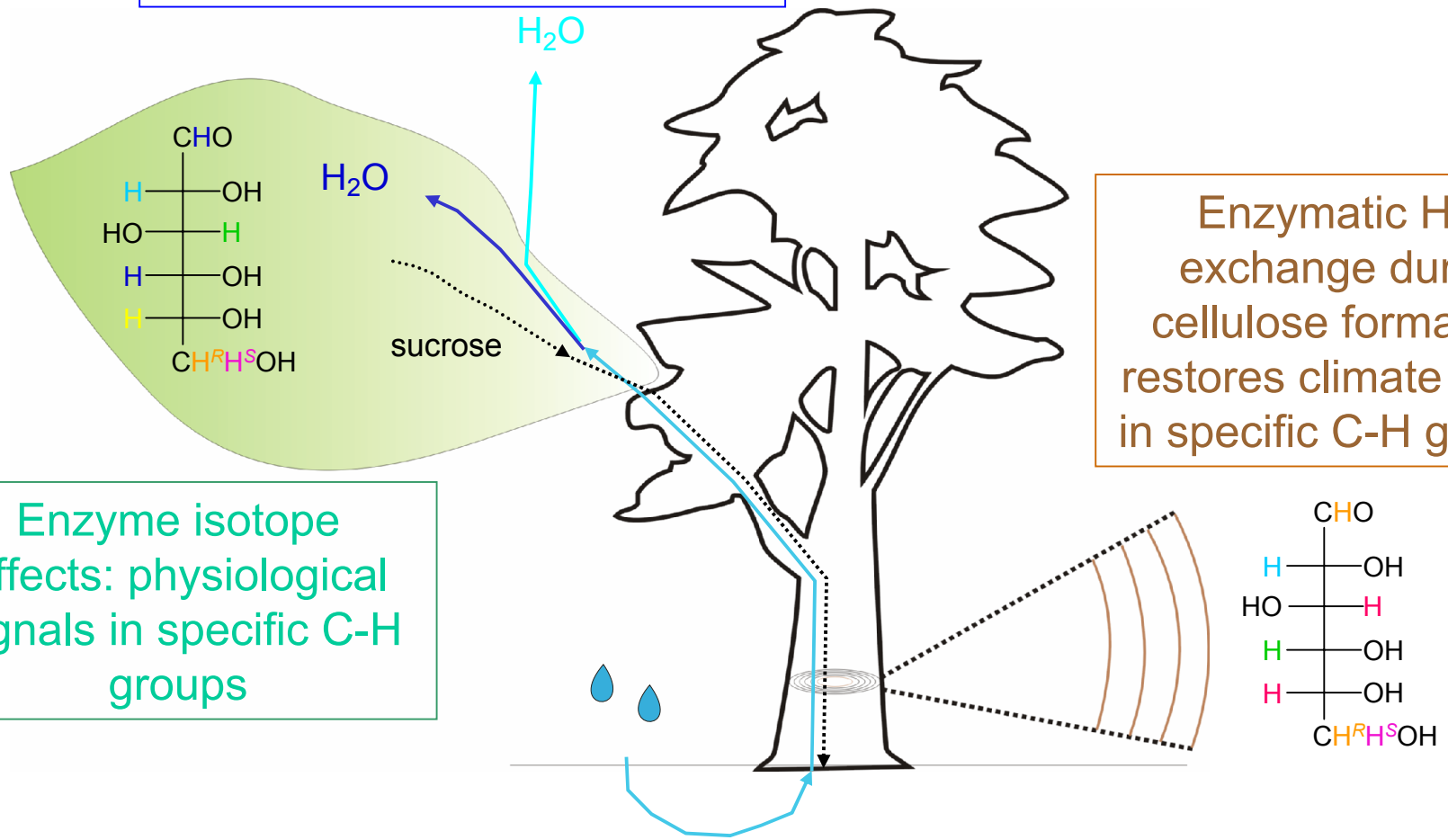
- Low sensitivity
- Measures ONLY intramolecular distribution, no difference method SAMPLE-STANDARD available.

Isotopomer distributions: ^{2}H

- Variation ≈ 500‰
- NMR, breakdown + IRMS
- Alcohols, amino acids, terpenes / aromes, fatty acids, sugars, alkaloids, organochlorines.
- Several Mechanisms: Source water δ, evapotranspiration, kinetic isotope effects, enzyme-catalysed exchange.

Four fractionation mechanisms in plants

D enrichment by transpiration: humidity signal

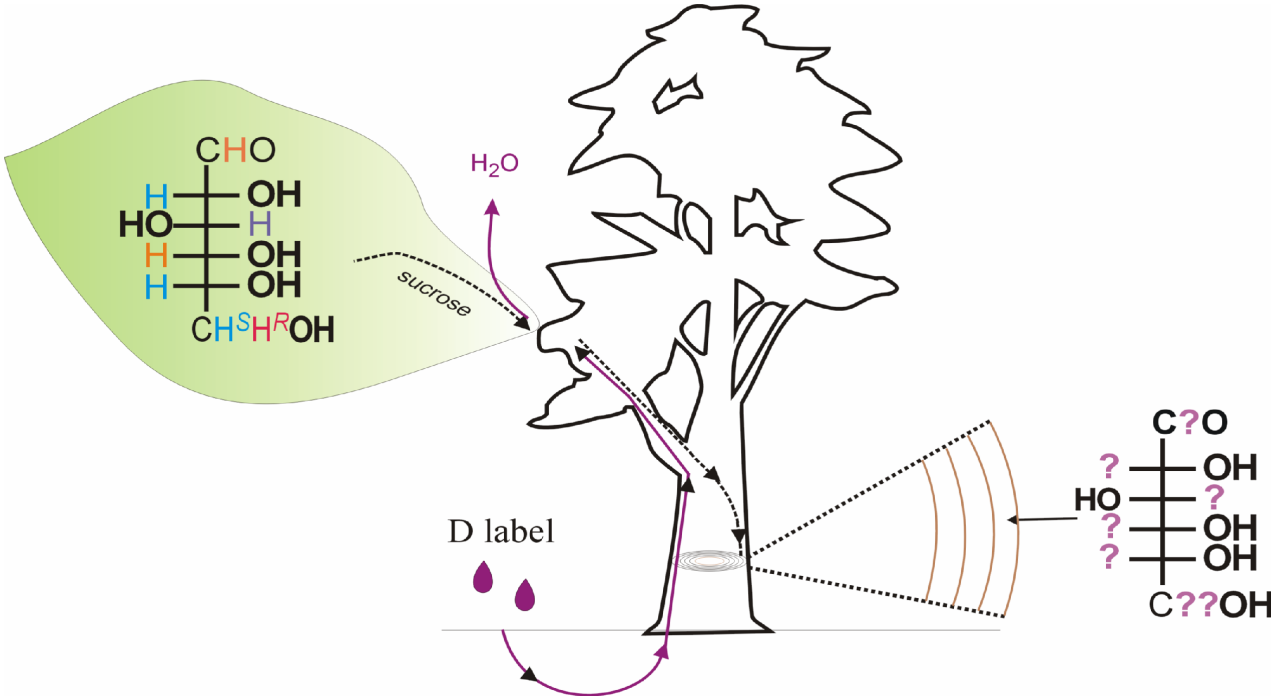


Enzymatic H/D exchange during cellulose formation: restores climate signal in specific C-H groups.

Enzyme isotope effects: physiological signals in specific C-H groups

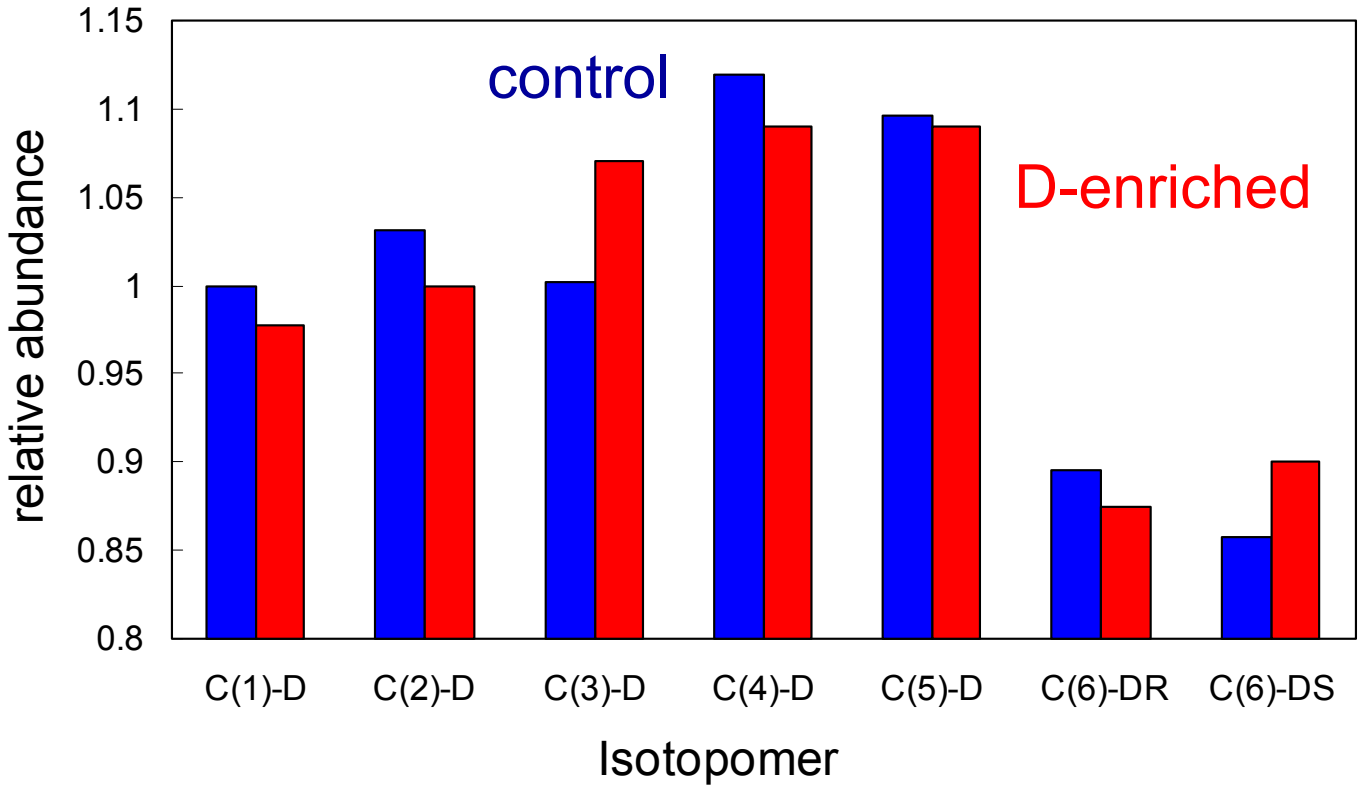
D abundance of precipitation: climate signal

H/D exchange experiment on trees



- Grow oak and spruce with D-enriched water.
- Create D gradient in plants.
- Compare labelling of soluble sugars and tree rings.

Leaf-level isotopomer distribution



Conclusion 1. Leaf water ~+1000‰, leaf glucose D distribution not affected, reflects ONLY biochemistry.

H/D exchange during cellulose synthesis: results

- Comparing D enrichment of leaf glucose, tree ring glucose and soil water shows:
  - all D isotopomers are more abundant in cellulose
  - H/D exchange is strongly variable among isotopomers.
  - Exchange reflects enzymology of cellulose synthesis.
- C(2)-H exchanges most, adopts climate signal of source water.
- C(6)-H^RH^S exchange least, retain leaf-level signals
- Biochemical conclusions:
  - 75% exchange of C(2)-H by PGI: 50% sucrose breakdown by invertase.
  - 38, 47, 48% exchange of (3,4,5)-H: 50% of all hexose cycle through trioses before cellulose synthesis

Physiological signal: CO_2 fertilisation

- The ratio of the C6-D^S/C6-D^R isotopomers depends on [CO_2] during plant growth.
- General observation in C3 species, absent in C4.
- C3-$D^{S/R}$ of PGA passed on to glucose.
- C6-$D^{S/R}$ do not exchange during cellulose synthesis, can therefore transfer leaf level signal into tree rings.
- This is observed in spruce tree rings.

- Possibility to measures photorespiration from tree ring D isotopomers.

Conclusions

- Identifiable exchanging / nonexchanging C-H groups

- Conclusion 1:
  Ratios of non-exchanging C-H (e.g. C(6)-$D^{R/S}$): Leaf level isot enzyme isotope effects > metabolic regulation.
  Abundance of non-exchanging C-H: Possible humidity signal from leaf water enrichment.
- Conclusion 2: Exchanging C-H: Precipitation signal: Temperature.
- Correlated climate and physiological information.

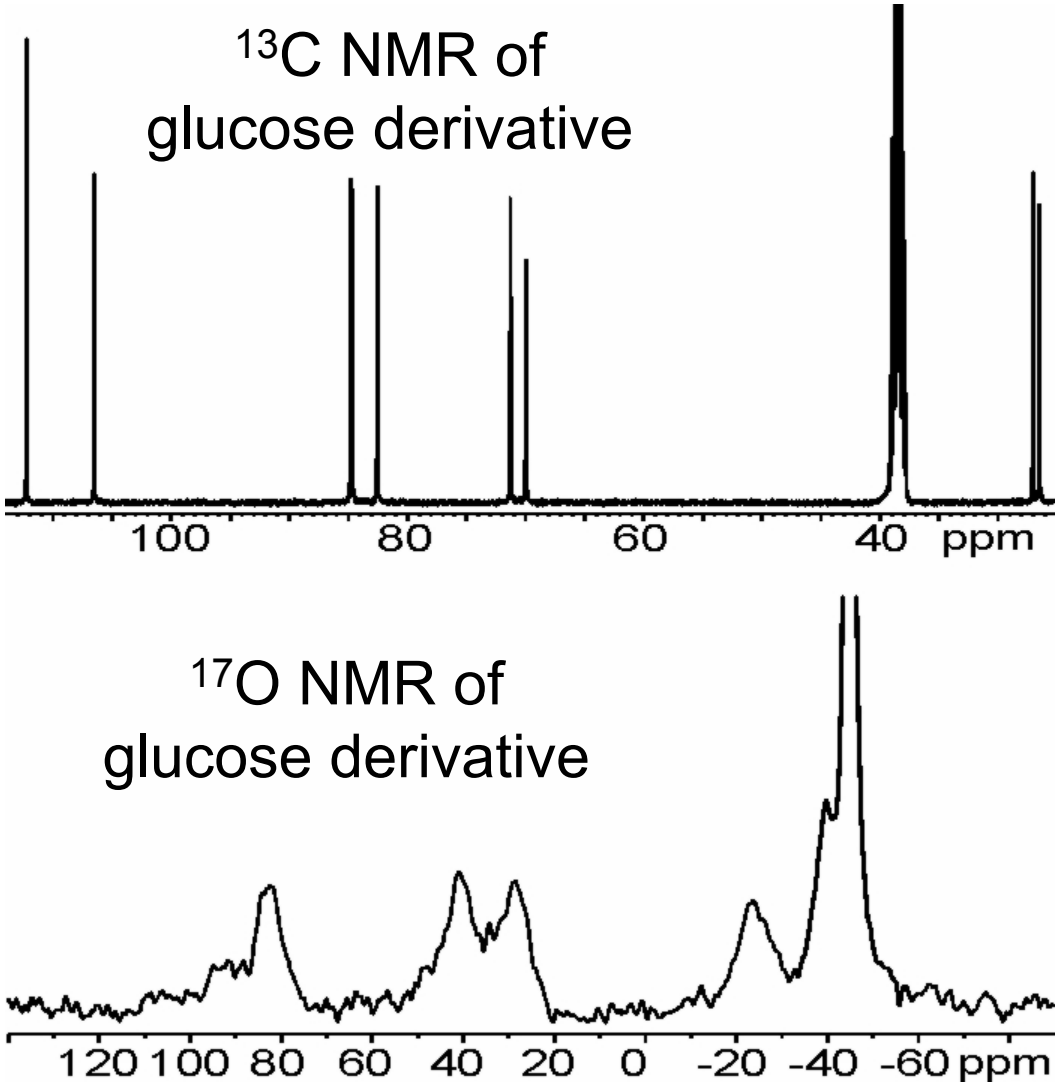
? Adaptation / fertilisation of trees due to increasing CO_2?

? Tree adaptations during deglaciation?

Appetizer: Deuterium distributions of DDT derivatives

- DDT and by-product have very different δD (-7 vs. 75 ‰), although they supposedly come from the same source.
- Deuterium NMR shows that one hydrogen of the by-product is enriched to $\delta D = 800$ ‰
- This enrichment explains the positive δ of the by-product, both compounds can come from same source

Other nuclei



- ^{13}C:
  - very nice resolution
  - Detecting position of label is very easy.
  - Quantification is technically challenging, but 1% precision reached (difference between C3 and C4 glucose visible, but not variation in isotopomer abundance).
- ^{15}N is similar to ^{13}C:
  - ^{15}N isotopomers of N_2O can be studied.
  - Can complement IRMS for reference compounds.
- ^{17}O: lost cause, the large linewidth is an intrinsic property of the ^{17}O nucleus.

Carbon, oxygen and deuterium in plants

^{13}C:

- Rubisco/diffusion fractionation affects all isotopomers, captured by δ
- Downstream metabolism: isotopomer abundances, difficult, interesting!

^{18}O:

- Temperature signal from precipitation
- Leaf ^{18}O enrichment, affects all isotopomers, captured by δ
- CONSTANT fractionation during $C{=}O/H_2O$ exchange
- tree ring $\delta^{18}O$ can be modeled

^{2}H:

- Temperature signal from precipitation
- Leaf ^{2}H enrichment, affects all isotopomers, affects δ
- tree ring $\delta^{2}H$ CANNOT be modeled
- Enzyme isotope effects decisive for individual isotopomers, most information is in the isotopomer distribution

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