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Anti-Doping: Drug Control Centre

KING'S College LONDON

"2nd IRMS Workshop
Drug Control Centre
King's Forensics
22nd and 23rd September, 2023"

# Advances in the IRMS & Concepts for the new IRMS Users

Rodrigo Aguilera Ph.D.

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# Endogenous steroids can be differentiated from the Administration of Synthetic Forms of Endogenous Anabolic Androgenic Steroids by GC/C/IRMS

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Becchi *et al 1994*

**SYNTHETIC TESTOSTERONE**
**(Testosterone Heptanoate)**

$\delta^{13}C = -29.63\ ‰$

**URINARY TESTOSTERONE BEFORE ADMINISTRATION**

$\delta^{13}C = -26.58\ ‰$

**URINARY TESTOSTERONE AFTER ADMINISTRATION**

$\delta^{13}C = -30.30\ ‰$

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# From the beginning
# IRMS Big Bang Theory

| | |
|---|---|
| GC | Gas Chromatography |
| C | Combustion |
| IRMS | Isotope Ratio Mas Spectrometry |

## Definition :

**The isotope ratio mass spectrometer technique allows the precise measureme of naturally occurring isotopes mixtures.**

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# From the beginning
# IRMS Big Bang Theory

**Isotopes** are atoms with the same atomic (proton) number that differ in atomic mass due to the number of neutrons they contain. I.e. $^{12}C$, $^{13}C$ and $^{14}C$.

The term **isotopic ratio** is a measure of the abundance of one isotope with respect to another.

It is usually given as a **percentage abundance** of the less abundant heavier isotope compared to the more abundant lighter isotope. I.e. $^{13}C$ / $^{12}C$.

**The isotopic abundances of these elements were fixed when the Earth was formed and, on a global scale, have not changed since.**

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# From the beginning
# IRMS Big Bang Theory

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## Commonly Measured Gases

| Element | | Gas | Abundance | Raw Material |
|---|---|---|---|---|
| H | $^{2}H/^{1}H$ | $H_2$ | 0.015% | Water, Methane, Cellulose |
| C | $^{13}C/^{12}C$ | $CO_2$ | 1.12% | $CO_2$, organics,carbonate |
| N | $^{15}N/^{14}N$ | $N_2$ | 0.3% | $N_2$, $NH_4$,nitrates |
| O | $^{18}O/^{16}O$ | $CO_2$,CO | 0.2% | $CO_2$,$H_2O$,Organics |

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# From the beginning IRMS Big Bang Theory

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|---|---|---|---|---|
| H | $^{2}H/^{1}H$ | $H_2$ | 0.015% | Water, Methane, Cellulose |
| C | $^{13}C/^{12}C$ | $CO_2$ | 1.1% | $CO_2$, organics,carbonate |
| N | $^{15}N/^{14}N$ | $N_2$ | 0.3% | $N_2$, $NH_4$,nitrates |
| O | $^{18}O/^{16}O$ | $CO_2$,CO | 0.2% | $CO_2$,$H_2O$,Organics |

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# From the beginning
# IRMS Big Bang Theory

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- **The isotope ratio of an element is not universal constant, but varies within different environments due to isotopic fractionation**

1. **Isotopic exchange reactions**
2. **Kinetic isotope processes**
3. **Radiogenic decay**

***This means that the origin of a material and the processes involved in its formation affect the isotopic ratios of the elements it contains***

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# From the beginning
# IRMS Big Bang Theory

## CARBON ISOTOPE RATIO

**Analytical technique**

**To obtain information of the source of the compounds**

**Carbon Origin**

**Vegetal,**

**Animal,**

**Synthetic**

**and Geographic Place**

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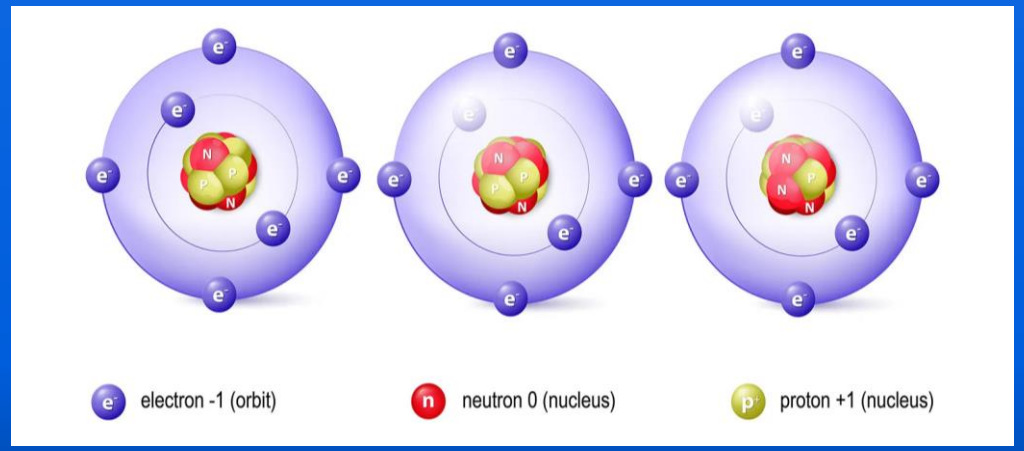
# From the beginning
# IRMS Big Bang Theory

**Carbon has two stable isotopes $^{12}C$ and $^{13}C$.**

**$^{12}C$ = 6 protons and 6 neutrons (around 98.9%).**
**$^{13}C$ = 6 protons and 7 neutrons (around 1.1%).**
**$^{14}C$ = 6 protons and 8 neutrons (Low abundance and radioactive).**

## Variations




## Mass

## Volume

## Energy

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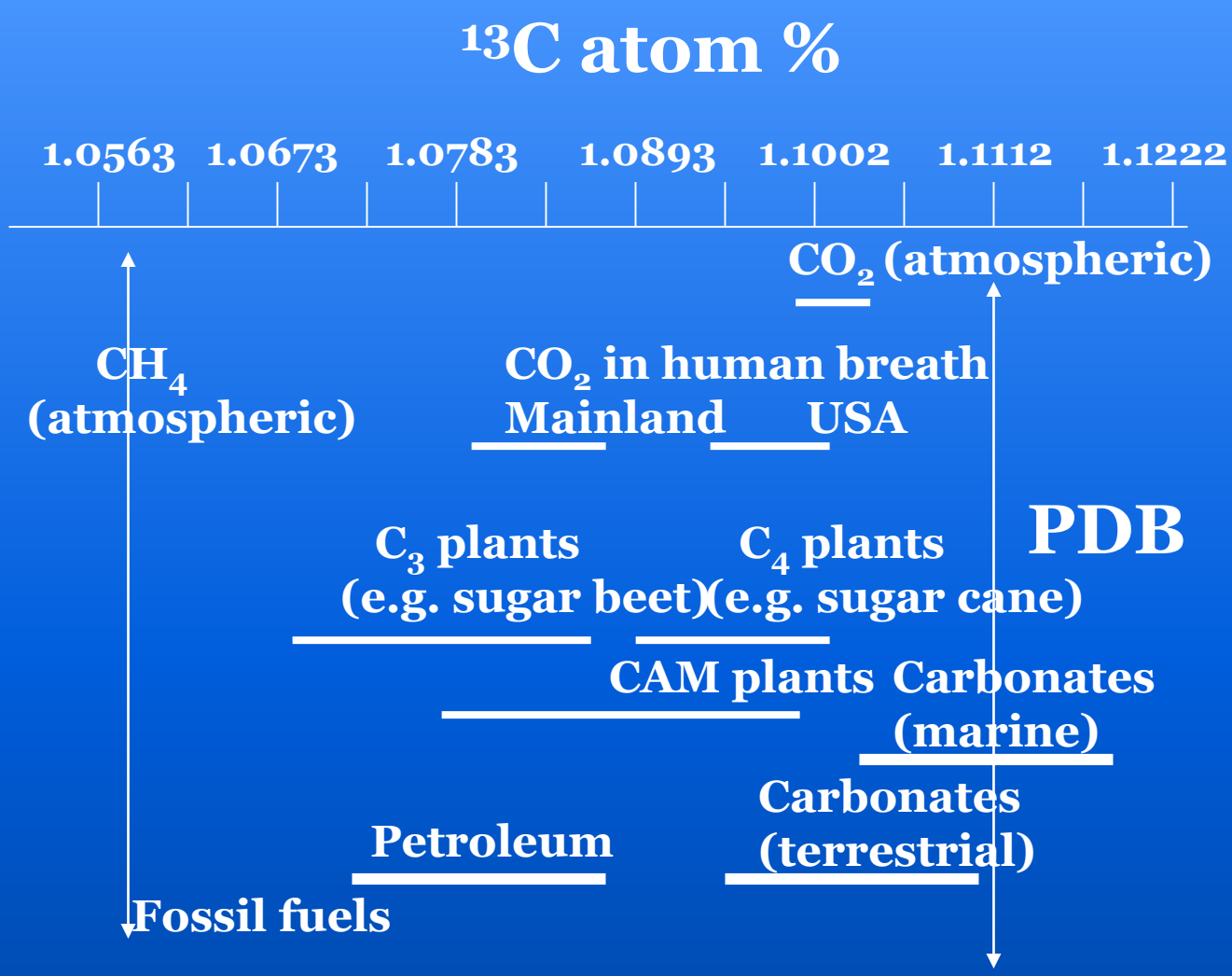

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# Endogenous steroids can be differentiate from the Administration of Synthetic Forms of Endogenous Anabolic Androgenic Steroids by GC/C/IRMS

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Becchi *et al 1994*

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# The Delta Value Notation

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**Studies examining stable isotopes at or near natural abundance levels are usually reported as delta value**

**Delta values are not absolute isotope abundances but differences between sample readings and a recognised standard**

**Standard: Vienna Pee Dee Belemnite ($CaCO_3$)**

**Results given as a delta value:**

$$\delta\ ^{13}C = \frac{(^{13}C / ^{12}C)_{sample} - (^{13}C / ^{12}C)_{standard(PBD)}}{(^{13}C / ^{12}C)_{standard\ (PBD)}} \times 1000$$

**The Peedee Formation is a geologic formation in North and South Carolina. A marine deposit, named for exposures along the Great Peedee River, it preserves belemnites and foraminifera fossils dating from the Late Cretaceous.[1] The formation is notable for its occurrence of Belemnitella Americana, known as the Pee Dee Belemnite (PDB), a long-standing standard in stable carbon isotope research.**

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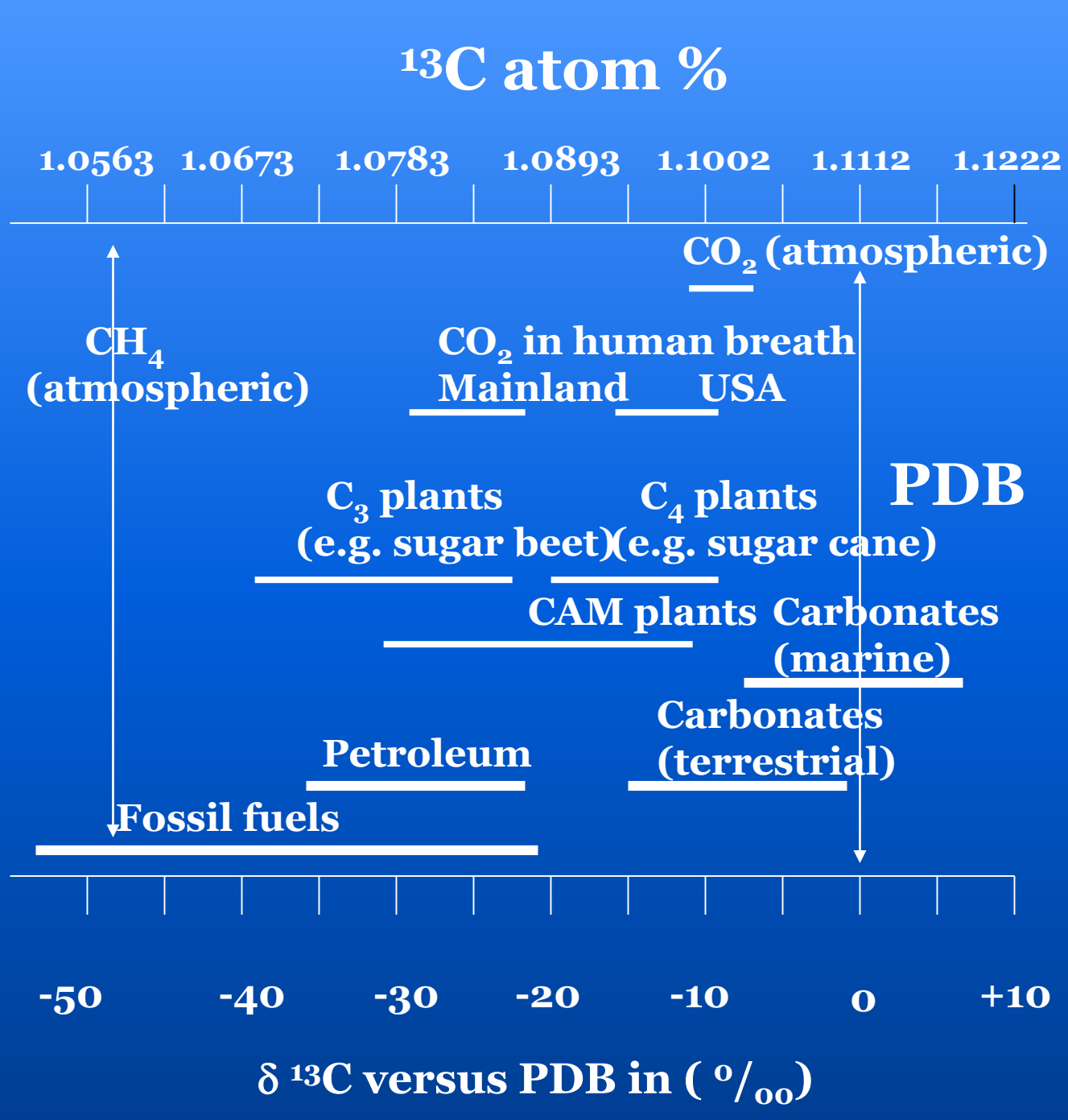

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# Endogenous Pathway

↓

**CHOLESTEROL**
**Steroid Biosynthesis**

↓

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## Endogenous Pathway

↓

**CHOLESTEROL**

**Steroid Biosynthesis**

↓

**PREGNANEDIOLS (ERC)**

**Precursors**

**DHEA**

**5 - ANDROSTENEDIOL**

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**Endogenous Pathway**

↓

**CHOLESTEROL**

**Steroid Biosynthesis**

↓

**PREGNANEDIOLS (ERC)**

**Precursors**

**DHEA**

**5 - ANDROSTENEDIOL**

**TESTOSTERONE**

**METABOLITES**

**Natural**

$^{13}C / ^{12}C$

$\delta \sim -20$ to $-23$ ‰

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**Endogenous Pathway**

**Oral, Intramuscular Subcutaneous**

**TESTOSTERONE**

**CHOLESTEROL**

**Steroid Biosynthesis**

**Natural**

$^{13}C / ^{12}C$

$\delta \sim -20$ to $-23$ ‰

**PREGNANEDIOLS (ERC)**

**Precursors**

**DHEA**

**5 - ANDROSTENEDIOL**

**Modified**

$^{13}C / ^{12}C$

$\delta \sim -27$- $30$ ‰

**TESTOSTERONE**

**METABOLITES**

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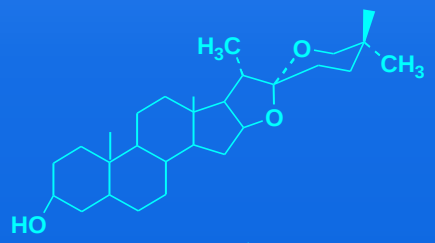
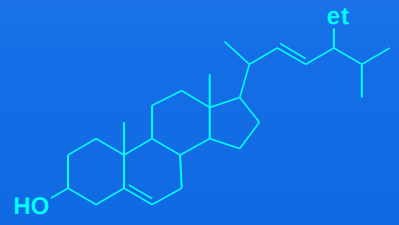
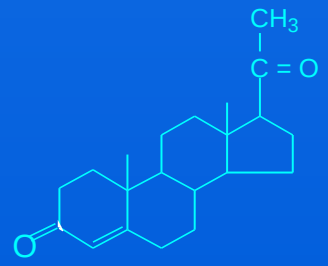
# PLANTS $C_3$

**DIOSGENIN**
**MEXICAN YAM ROOTS**




**STIGMASTEROL**
**SOY BEAN**




**ESTROGENS**

**ANDROGENS**

**CORTICOSTEROIDS**

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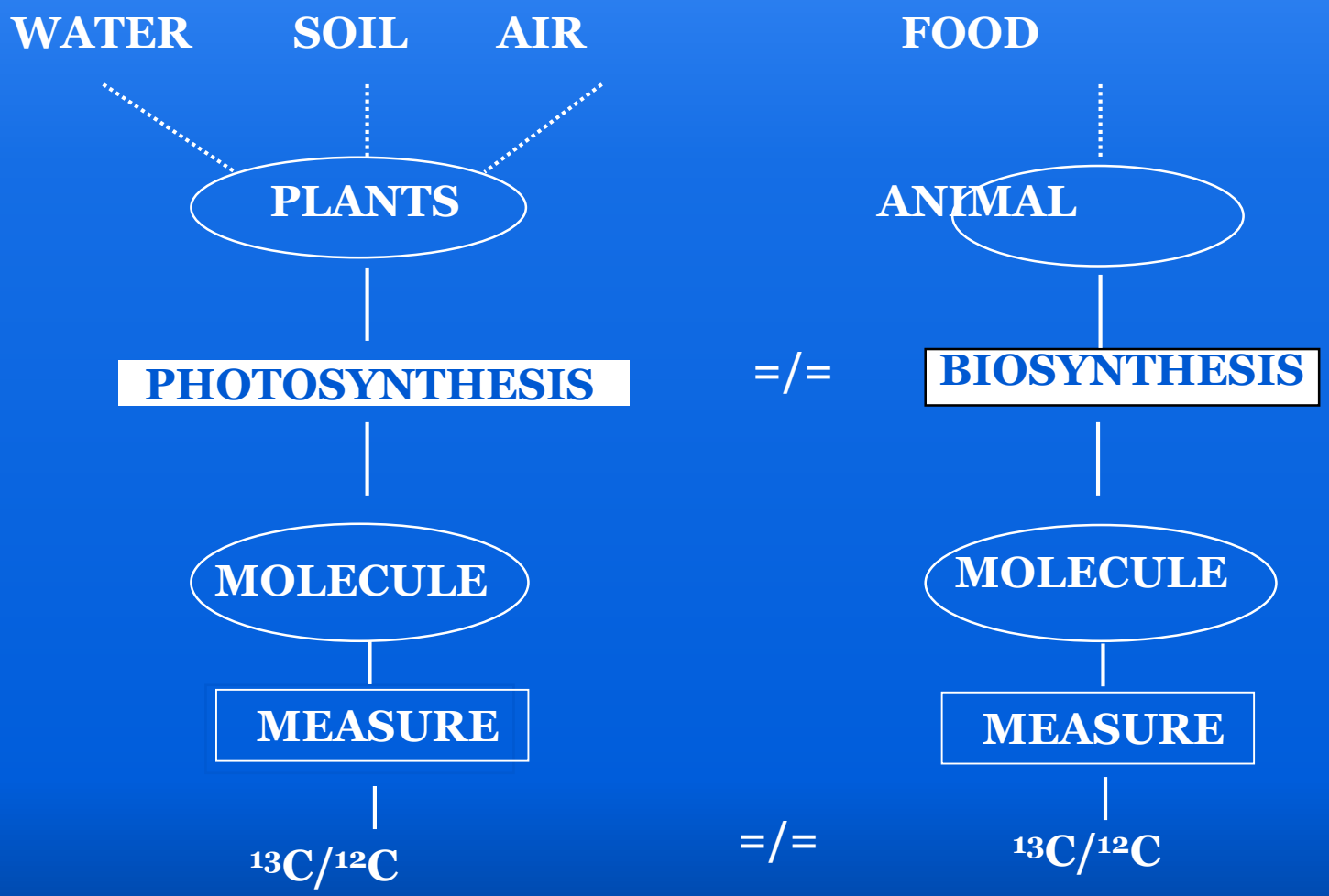
# DIFFERENCES BETWEEN ANIMAL AND VEGETABLE MOLECULES



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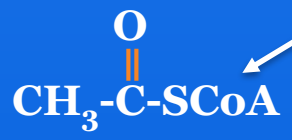
# Biosynthesis and Metabolism of Cholesterol

**Food**

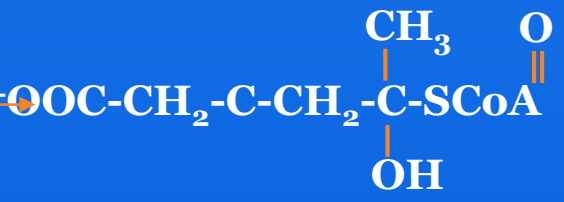
**Protein** **Carbohydrate** **Fat**

**Digestion and Absorption**

**Fatty Acids, Glycerol**
**Amino Acids and Glucose**

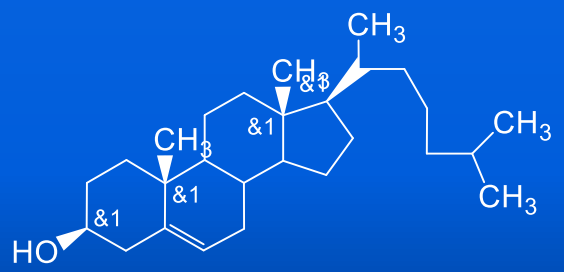



**acetyl coenzyme A**

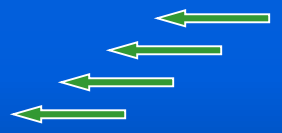


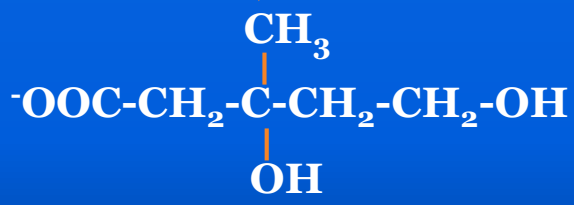

**3-hydroxy-3-methyl-glutaryl-CoA**

***HMG CoA reductase***



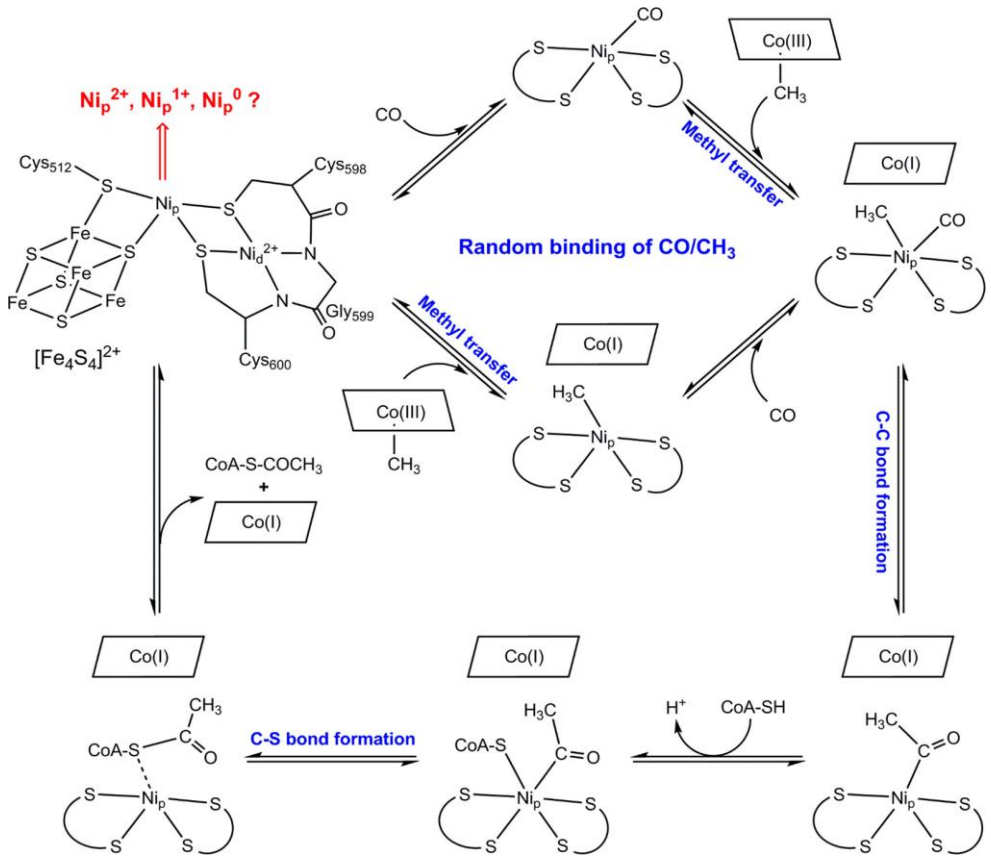

**Cholesterol**





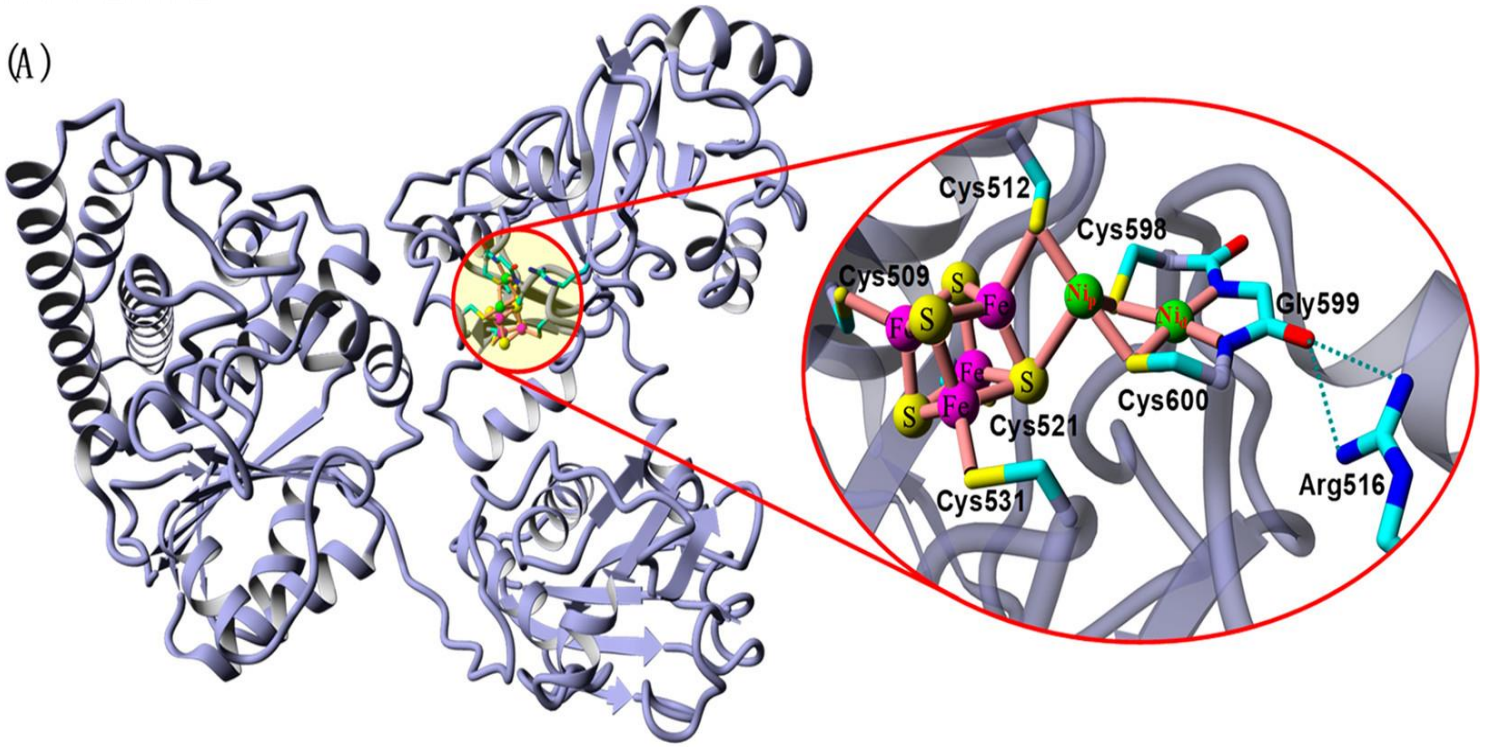

**mevalonate**

# Isotope fractionation with $^{13}C$ depletion



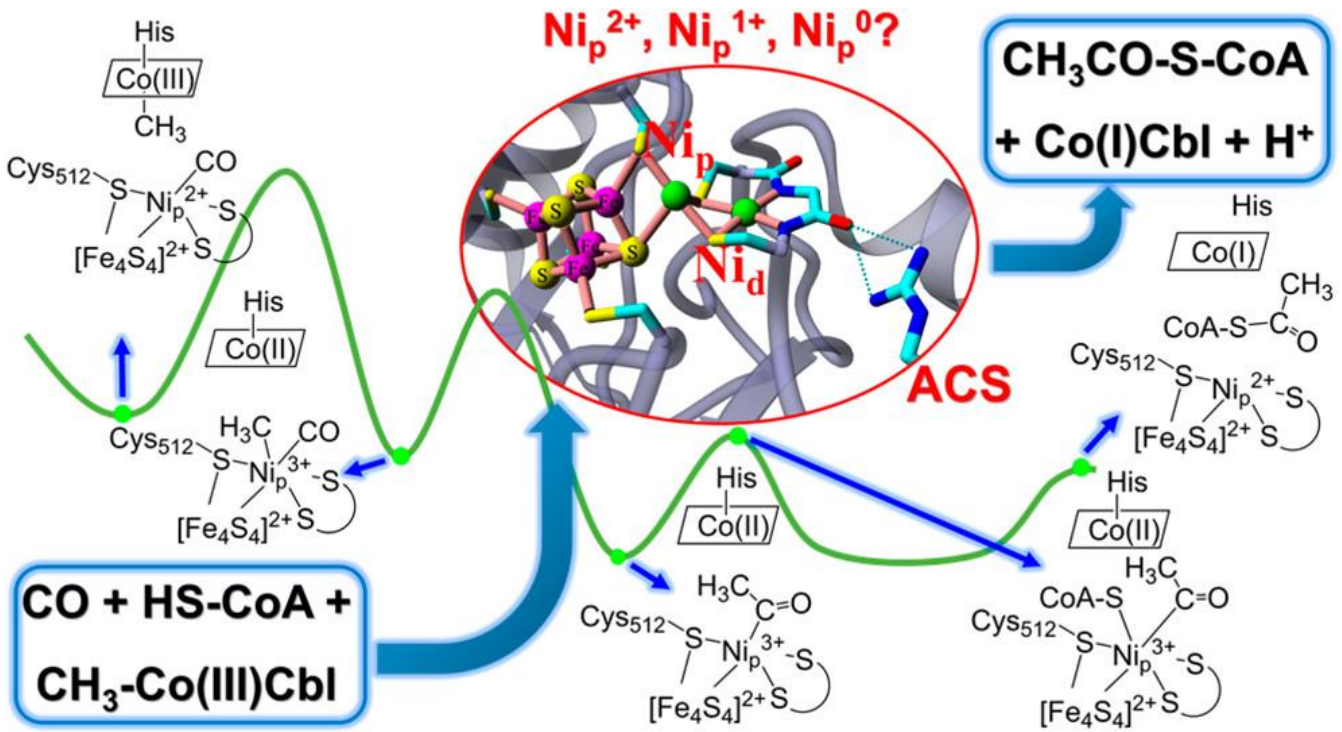

Mechanism of Acetyl-CoA synthesis.

# Isotope fractionation with $^{13}C$ depletion




(B) $CO + HS\text{-}CoA + CH_3\text{-}Co(III)Cbl\text{-}CFeSP \rightarrow CH_3CO\text{-}S\text{-}CoA + Co(I)Cbl\text{-}CFeSP + H^+$

# Isotope fractionation with $^{13}C$ depletion



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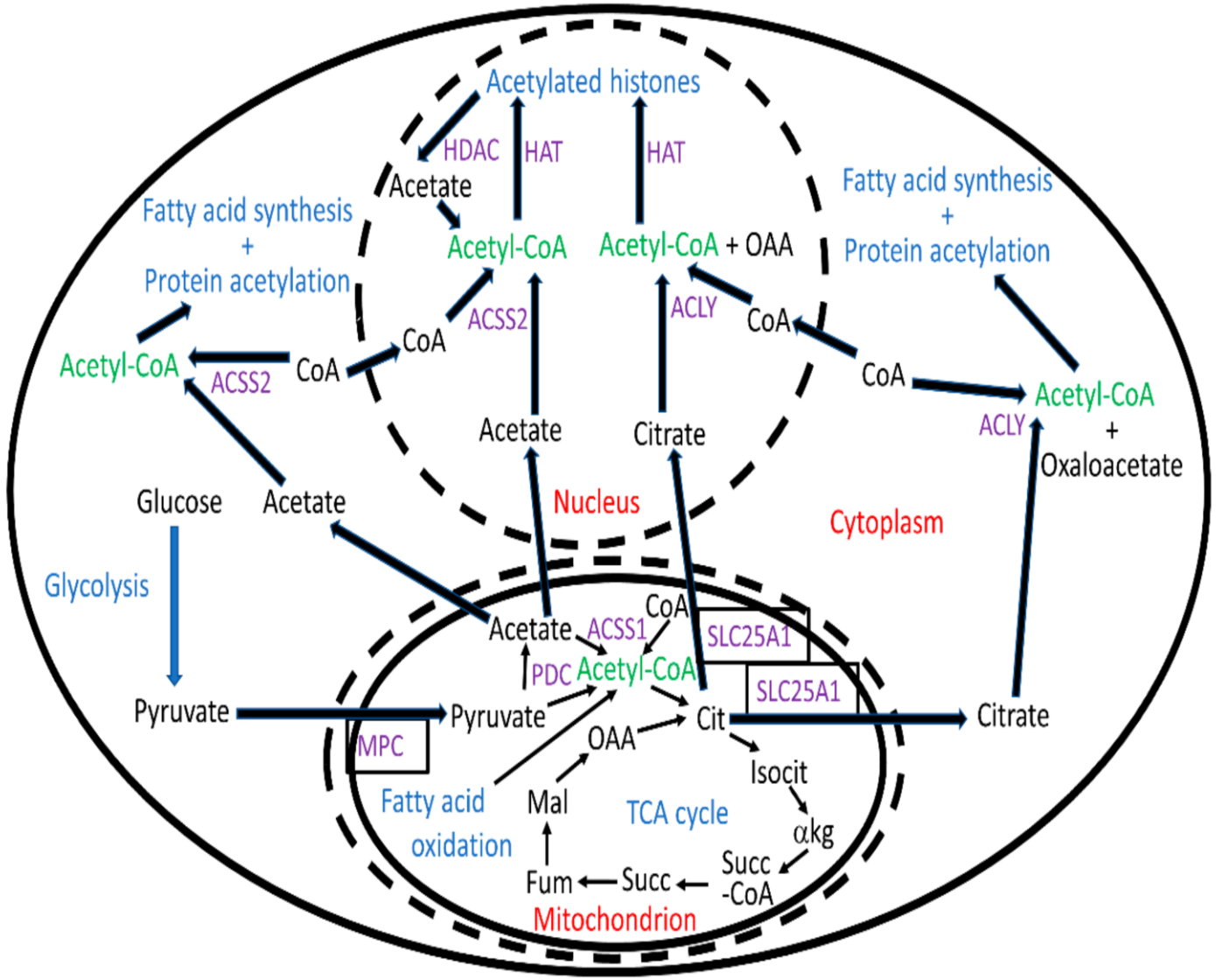

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# Isotope fractionation with $^{13}C$ depletion

| Carbon Source | $\delta^{13}C$ (‰) | |
|---|---|---|
| | Carbon Source | Lipid Fraction |
| Glucose | -9.5 | -15.7<br>-16.3 |
| Sodium Pyruvate | -20.5 | -28.9<br>-28.9 |
| Sodium Acetate | -20.1 | -21.5<br>-20.7 |

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# Major Steroid Biosynthetic Pathways In Human Beings

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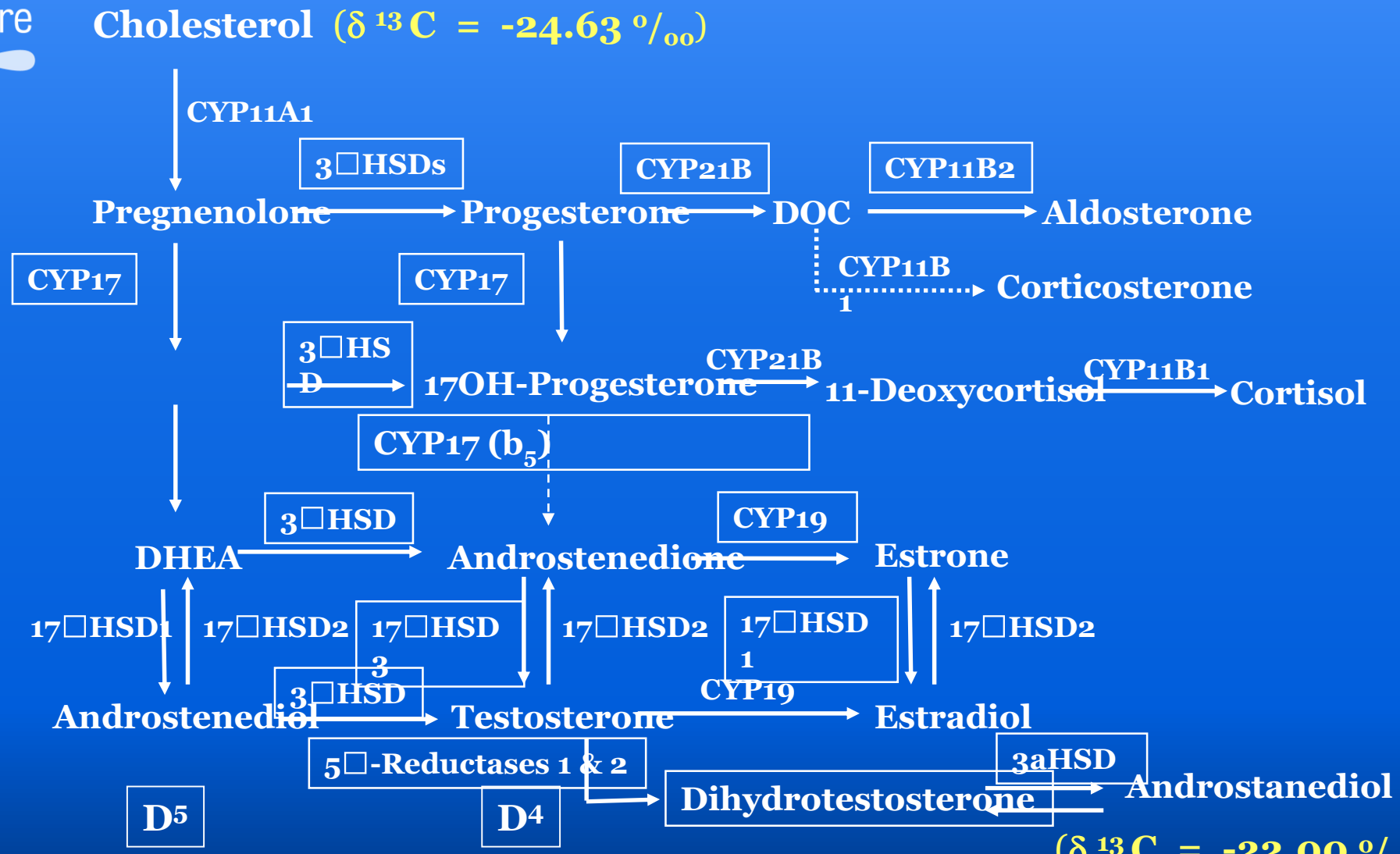

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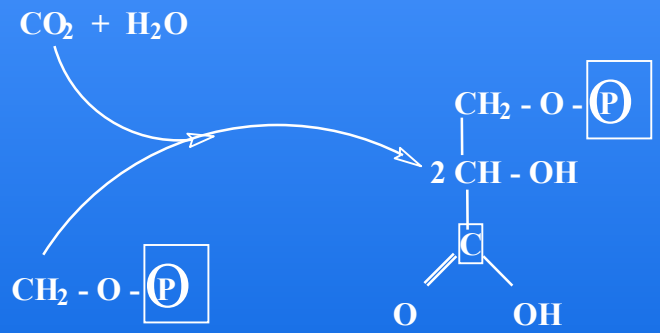
# PHOTOSYNTHESIS OF THE PLANTS $C_3$




Acid 3-phosphoglyceric

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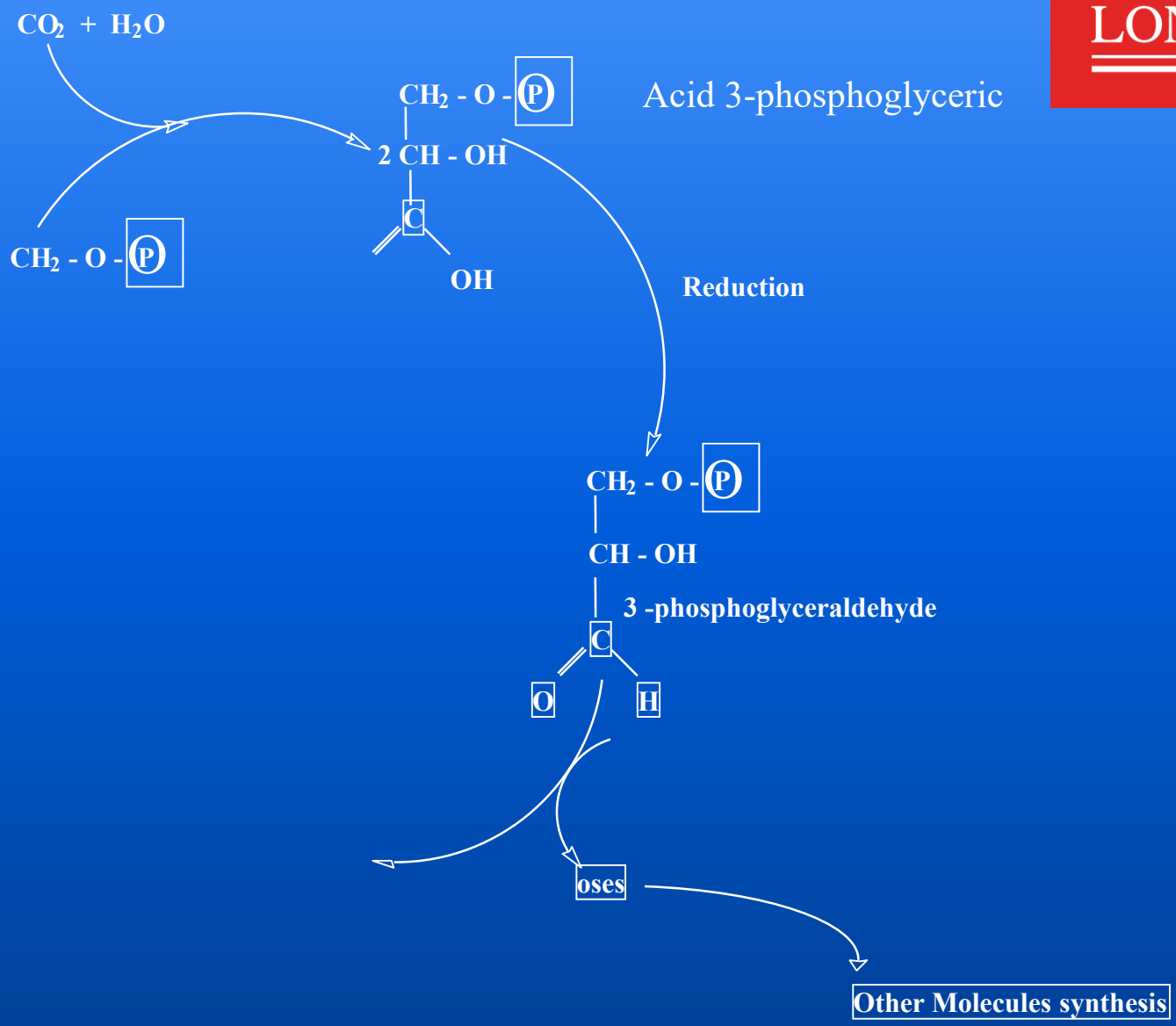
# PHOTOSYNTHESIS OF THE PLANTS $C_3$

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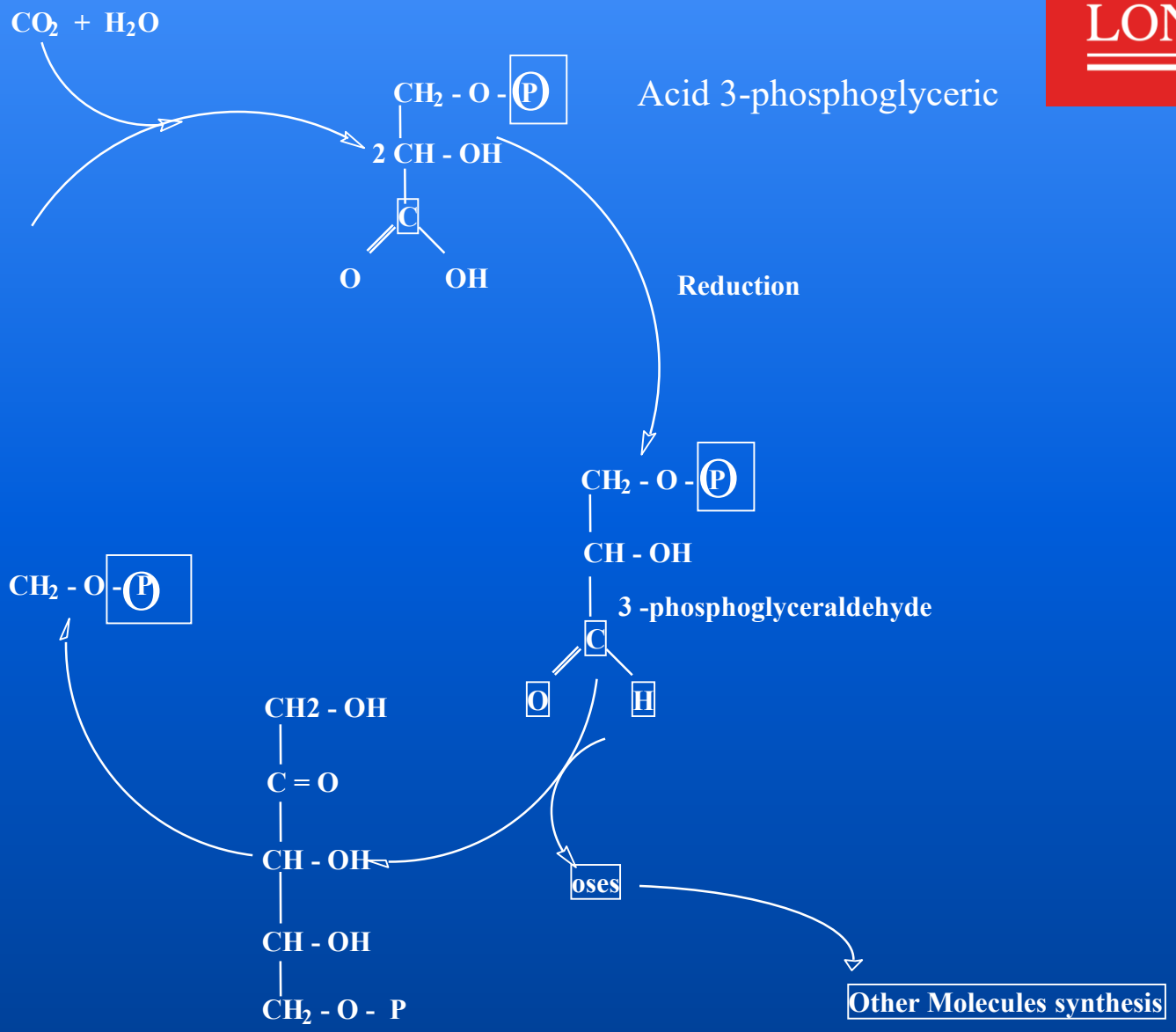

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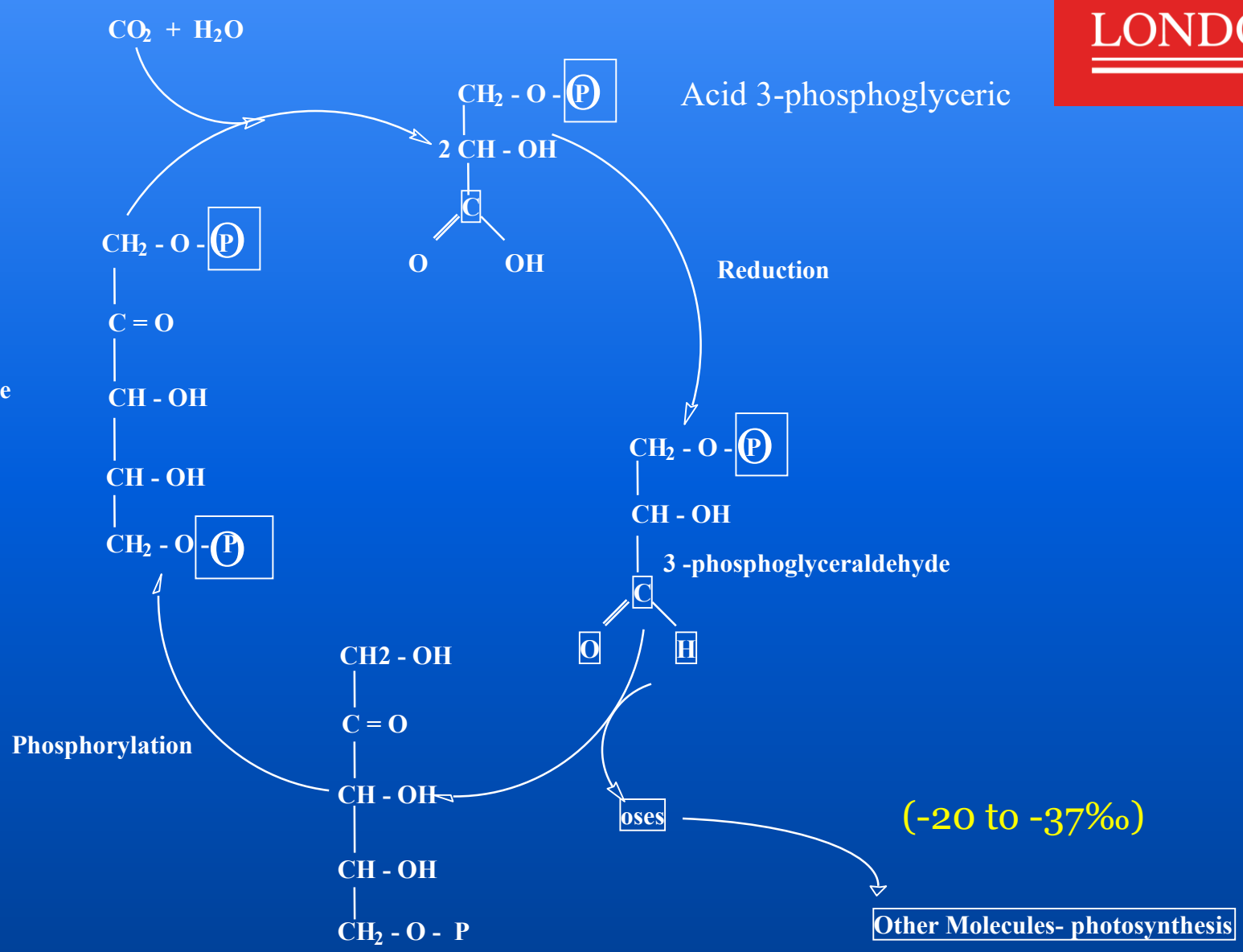

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# Expected values of isotope fractionation in various components of the $CO_2$ fixation process

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| Step | Isotope Discrimination $\delta^{13}C$ ($^0/_{00}$) |
|---|---|
| Gas-phase diffusion of $CO_2$ | 4.4 |
| Dissolution of $CO_2$ | -0.9 |
| Liquid-phase diffusion of $CO_2$ or $HCO_3^-$ | 0 |
| $CO_2$ hydration | -7 |
| Carboxylation of phosphoenolpyruvate | |
| Relative to $HCO_3^-$ | 2 |
| Relative to $CO_2$ | -5 |
| Carboxylation of Ribulose bisphosphate | 30 |
| Respiratory decarboxylation | 0-20 |

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# Conclusion

## *Isotopic fractionation*

**Isotopic exchange** involves the redistribution of an element's isotopes among different chemical states or phases, such as oxygen in liquid water and water vapour. In this manner isotopic changes can occur without any net change in chemical distribution.

**Kinetic isotope** effects are observed in unidirectional processes such as chemical reactions, where the rate of reaction is dependent on the masses of the molecules involved. Fractionation occurs, as a consequence of the relationship between mass a kinetic energy. As such molecules of a lighter mass will react quicker and thus the kinetic isotope effect generally results in the depletion of the heavier isotope in the product molecule.

**Radioactive** decay refers to the process where over time the number of radioactive parent isotopes decrease as they decay into daughter products.

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# Conclusion

***Isotopic fractionation of carbon by plants***

**Natural differences in the carbon isotope ratio of plants occur as a result of differing 13C discrimination in the photosynthetic pathways used for carbon fixation. Depending on the method of carbon fixation used, plants can be placed into 3 categories, C3 fixing plants (Calvin cycle), C4 fixing plants as well as a less common third type, CAM plants.**

**C4 plants are capable of surviving in the type of hot dry climates which C3 plants generally find difficult. Plants using his method of carbon fixation include corn, sugar cane and members of the grass family, which produce glucose with a delta value of around -11 ‰.**

**The Crassulacean acid metabolism (CAM) plants use both methods of carbon fixation (C3 during daylight and C4 at night) and as such display an intermediate carbon ratio.**

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# Advances in the IRMS

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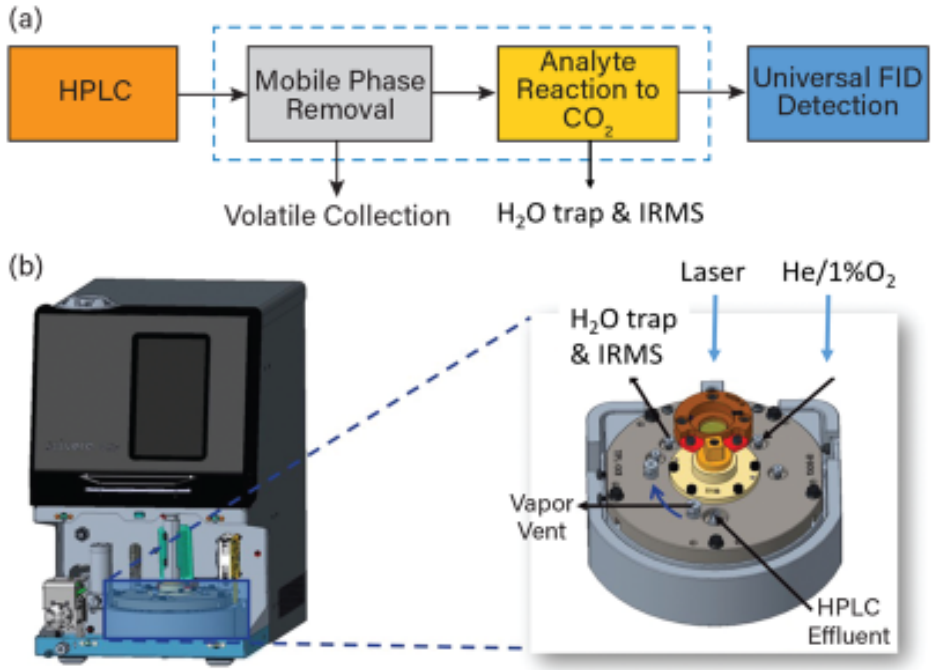
## Rotating-catalytic disc interface for LC-IRMS: Expansion to Include Organic Mobile Phases




(a) The process of HPLC solvent removal and dried analyte combustion to $CO_2$, then reduction to $CH_4$ for carbon selective detection by FID. On the transition metal catalyst coated rotating disc, the analyte is converted by laser-activated photo-catalytic oxidation to $CO_2$.

(b) A CAD model of Solvere™ shows the reaction cell with inlets and outlets, a laser window, and an internal rotating disc (not pictured) where analytes are deposited and reacted. The coupling to IRMS involved disc transition metal catalyst modification and redirected sampling of the $CO_2$ without use of methanizer/FID.

*Based on a modified Solvere™ carbon selective detector by Activated Research Company.

- Initial results were the limit of quantification (LOQ) of $\delta^{13}C_{VPDB}$ was 1 µg for sucrose and 10 µg for androsterone with average precisions of SD($\delta^{13}C$) ± 0.8‰. Using methanol and water mobiles phases.
- With further development to improve sensitivity and application to chromatography, the prototype proof-of-principle LC-IRMS shows promise to resolve a major drawback in current LC-IRMS systems.

*Tobias, H; Jones, A; Saunders, T; Brenna, J.T. Manuscript submitted for publication.*

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# Acknowledgement


**Kim Wolff, MBE**
**Professor of Analytical, Forensic and Addiction Science**
**Head of the Drug Control Centre (DCC)**
**Director of King's Forensics**
**Principal Fellow of the Higher Education Academy (PFHEA)**
**King's College London**

**Dr. Osquel Barroso**
**WADA**
**Senior Associate Director**
**Science & Medicine, Laboratories.**


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# Thank you for listening to The IRMS Big Bang Theory