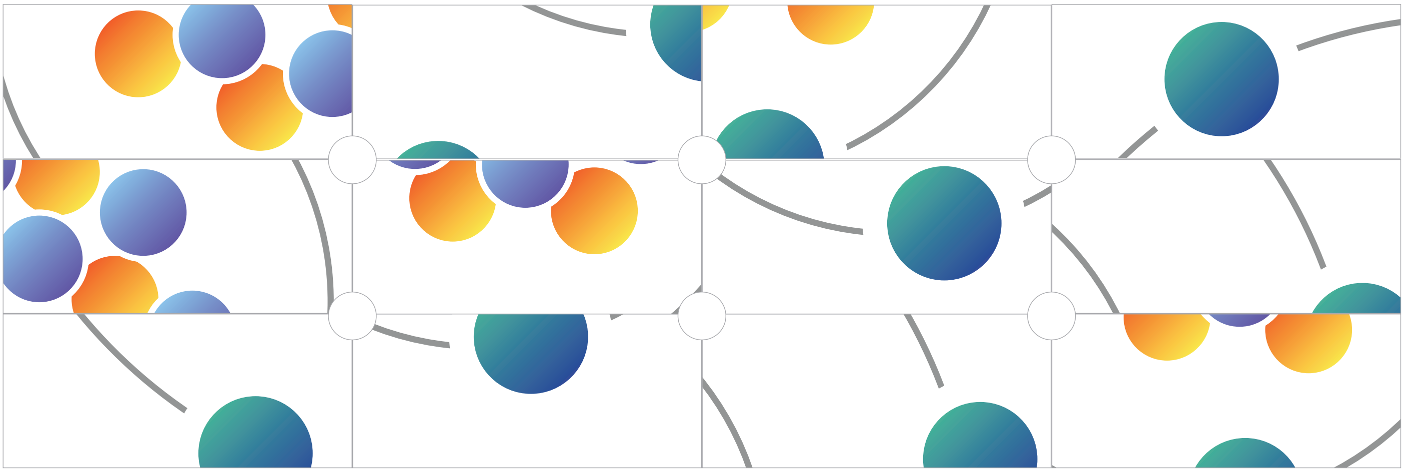

Isotope profiling

An authority on isotope analysis methods, **Professor Gérald Remaud** explains the importance of isotope ratios in nature, and discusses new nuclear magnetic resonance-based tools to measure them, which have applications for both human and environmental health

What is isotope fractionation and how can knowing the intramolecular ratio of two isotopes help trace the origin of the molecule?

In nature, chemical elements may exist with different numbers of neutrons in the nucleus of the atom. We refer to these as isotopes. For example, carbon exists as carbon-12 (^{12}C) and carbon-13 (^{13}C), with a respective average distribution of 98.9 per cent and 1.1 per cent. ^{13}C has one additional neutron than ^{12}C. Therefore, we can describe chemical elements in terms of their heavy and light isotopes.

During a physical, chemical, biochemical or physiological process, the heavy atom may display different behaviour to the lighter one, and the process may favour one over the other, leading to a non-statistical distribution of the heavy isotope in a given molecule. This discrimination is named isotope fractionation. If a process generates isotope fractionation, it gives way to a characteristic isotope ratio. Measuring it can provide information on the process, and therefore on the history of the molecule (its raw material, synthesis pathway or purification techniques, for example).

Can you provide a brief overview of your research aims and the motivations driving them?

The aims of our work are encompassed by the research domains of the Elucidation of Biosynthesis by Isotopic Spectrometry (EBSI) team within the University-French National Centre for Scientific Research (CNRS) unit, CEISAM. The overarching goal of our research is to describe and understand metabolism from the point of view of isotopes.

To achieve this goal, we need new analytical methodologies. Thus, our focus is on isotope profiling methods with our unique capability – which is the first in the world – to use nuclear magnetic resonance (NMR) spectrometry as a tool for ^{13}C position-specific isotope analysis.

Our motivations lie in the application of these new tools for addressing issues in several domains, such as health and nutrition, the environment (eg. pollution and changes to plant physiology associated with climate change) and forensic studies (eg. authenticity, counterfeiting and traceability).

What limitations have previous methods had in terms of following isotope fractionation phenomena?

Isotope ratios are usually measured by mass spectrometry. The mass spectrometer is very simple; it is always in the same configuration, and because of this, the same type of gaseous molecule must always be introduced into the source. Therefore, it is necessary to convert an organic molecule into a gas before it can be analysed. For example, to determine the $^{13}C/^{12}C$ ratio, the product is first combusted into carbon dioxide (CO_2), which is then introduced into the source of the spectrometer. It is then easy to measure the signal corresponding to $^{13}CO_2$ over $^{12}CO_2$. Using this approach, it is clear that there is only one analytical parameter: the global ^{13}C content of the given molecule.

How are you breaking through these limits with the use of NMR?

Using NMR, a more refined approach is accessible because of its double intrinsic property: separation of the signal at each site of the molecule and quantification of the amount of resonating nuclei under the peak. Thus, if the molecule contains five carbon atoms, there will be five parameters instead of one – discrimination is much higher. However, to implement this approach for ^{13}C requires establishing ^{13}C NMR conditions to attain target precision – the level needed for isotopic applications at natural abundance. This presents a real challenge for an NMR spectroscopist: to distinguish a variation of the peak intensity of just 0.1 per cent. However, our research has shown that it is possible after appropriate refinements.

Do you support budding scientists in this field?

My team regularly hosts Master's students for their internships. Some are from the Analytical Chemistry Master's Programme (M2 A3M), for which I am responsible. Furthermore, we award PhD grants to some of the students working in our lab, many of whom come from outside France.

Discrimination and detection

A group of isotopic spectrometrists at the **University of Nantes**, France, is the first in the world to use NMR as a tool for carbon-13 position-specific isotope analysis. This sophisticated approach provides a unique means to model the fate of pollutants

THERE IS HUGE diversity on Earth, even at the level of atoms. Isotopes are a great illustration of this, as atoms of the same element that contain different numbers of neutrons. For example, the most common form of hydrogen contains no neutrons at all, but the isotope deuterium has one, while tritium has two. About two-thirds of stable elements occur naturally on Earth in multiple stable isotopes, but many human-made applications exploit their properties.

What makes isotopes so valuable is the fact that they redistribute themselves in different proportions, according to various processes. For example, water, food and, ultimately, even our bodies contain both hydrogen and deuterium in a specific proportion.

The determination of the relative abundances of isotopes of a given element in a particular sample (isotopic signature) is termed isotope analysis, and is surprisingly pervasive. Biomolecules often have significant variations of carbon, nitrogen and oxygen isotopes, and discovering these variations can enable scientists to detect if a food product is adulterated or uncover the geographic origins of a sample. It can also be used to determine where an individual has been living using a sample of hair, track down counterfeit materials or even understand how planets formed.

ISOTOPIC NMR SPECTROMETRY

The conventional means to access the isotopic signature of a sample is isotope ratio measurement by mass spectrometry (irm-MS). However, it is a method that only provides either the mean or the global amount of the heavy isotope in a given molecule; therefore, only one parameter is exploitable. This is due to the way that it proceeds: samples must be introduced to the mass spectrometer as pure gases. As a result, organic matter must be converted into a gas, which can be achieved using an elemental analyser via an oxidation furnace. This can transform any carbon species into carbon dioxide. Similar to many techniques, a pure compound must be used.

Professor Gérald Remaud, leader of the Elucidation of Biosynthesis by Isotopic Spectrometry (EBSI) group at the University of Nantes, is an expert in isotope ratio analysis. With his team, he has developed a new method to assess the isotope ratio for a given element by NMR spectrometry, which overcomes irm-MS's limitations.

A MORE REFINED APPROACH

The potential of NMR spectrometry comes from what is called its 'double intrinsic property'; it can separate the signal coming from an isotope at each individual site in a molecule and can quantify the separated signals, thereby providing access to numerous isotopic parameters simultaneously, instead of only one – an irm-MS drawback. The technique is more refined, and provides more valuable information.

Remaud is applying this approach for carbon-13 (^{13}C) – a stable, environmental isotope that makes up 1.1 per cent of all natural carbon on Earth. To date, isotope fractionation in ^{13}C has been the only method used, with the isotope content being measured by irm-MS, which loses valuable site-related information. NMR spectrometry, however, promises the ability to measure – site by site – the ratio of $^{13}C/^{12}C$, revealing information hidden from irm-MS.

However, implementing this approach is a difficult process. Developing a method suitable for most natural products requires an extremely high precision level of 0.1 per cent, which requires overcoming problems fundamental to ^{13}C NMR.

IN THE ETHER

To demonstrate the ability of isotopic ^{13}C NMR, Remaud is currently applying the technique to environmental pollution in French National Research Agency (ANR)-funded project 'ISOTO-POL'. Isotopic analysis is particularly important in this context, as it can detect the source of environmental pollutants and the processes involved in their migration, biodegradation and removal.

ISOTO-POL will use the technique to detect soil contaminants – specifically ethers used as performance enhancers in gasoline, including methyl tert-butyl ether (MTBE). "MTBE is frequently detected in water and one of the most common contaminants in the US," Remaud explains. "To demonstrate the capability of isotopic ^{13}C NMR, we needed to apply it to a molecule that is a

One of the mass spectrometers used for measuring isotope ratios in the CEISAM lab. It is equipped with an elemental analyser and gas chromatograph.

MONITORING SOIL CONTAMINATION WITH A POLLUTANT FROM FUEL

OBJECTIVE

To develop tools for position-specific carbon isotope ratio measurements ($^{13}C/^{12}C$) using nuclear magnetic resonance (NMR) spectrometry for purposes including tracing the origins of drugs, food products and pollutants.

KEY COLLABORATORS

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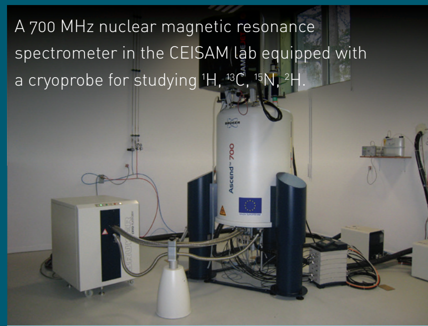
GÉRALD REMAUD, Head of the EBSI team and Professor at the University of Nantes, France, specialises in the NMR methods for isotope ratio analysis. Following his postdoctoral research in Uppsala, Sweden, he held a number of positions in industry. He recently established the start-up 'Spectromaitrise' for developing new NMR applications.



A 700 MHz nuclear magnetic resonance spectrometer in the CEISAM lab equipped with a cryoprobe for studying ^{1}H, ^{13}C, ^{15}N, ^{2}H.

current pollution issue, and show that the intramolecular information is valuable even when the molecule has a relatively small number of carbons." Thus, if the technique works for MTBE, it will be even more effective on large molecules like pesticides or pharmaceuticals.

ENVIRONMENTAL IMPACT

This project should demonstrate ^{13}C NMR to be a comprehensive tool to manage pollution, able to provide information on containment, spread and bioremediation possibilities when used alongside modelling.

The impact of this innovative project will be manifold. It will deliver a database of the isotope composition of ethers from different manufacturing processes, which could be used to identify them in future tests. From a fundamental biological standpoint, it will also contribute to understanding the processes that lead to fractionation, including biodegradation and hydrolysis, and explain whether these processes act on isotopes at specific sites. Finally, Remaud aims to develop a model to predict isotope ratios as a function of time, space and the processes acting on the contaminant.

Although the data are still being collated, the early results look promising. "The information we have is very new, and will likely change the existing models for describing remediation modes," Remaud enthuses.

ISOTOPIC ITERATION

The real benefit of this tool lies in its enhanced discriminatory ability. Isotopic ^{13}C NMR increases the number of discrimination parameters, increasing the ability to differentiate samples. For example, it can distinguish between natural and synthetic products. In developing such a tool, the EBSI group has established itself as a leader in high precision, quantitative NMR, and is also translating its expertise into the commercial landscape through a spin-off company called Spectromaitrise.

Due to the ubiquity of isotopes, this technology could have truly widespread applications. "Our approach can be applied in several domains, including identifying counterfeit drugs, the fate of a pollutant or the authentication of a natural origin," Remaud qualifies. Indeed, the team is close to developing new models of pollutant migration and remediation that could help to reduce their environmental impact.

Remaud has no plans to stop here; he is continuously seeking to improve the technique. His next steps are to develop position-specific isotope analysis for ^{15}N, a rare, stable isotope of nitrogen used in medical and agricultural research, and to further improve the sensitivity of NMR spectrometry. "This would allow us to work with much smaller amounts of sample. At present, isotopic ^{13}C NMR requires hundreds of milligrams of product. We hope to reduce it by a factor of 10," he ambitiously concludes.

COUNTERFEITERS AND CAFFEINE

The increased sensitivity of ^{13}C NMR can be used for many, and sometimes surprising, reasons:

DETECTING COUNTERFEIT DRUGS

- Stable isotope analysis can be used to detect counterfeit drugs that copy processes or infringe upon medical patents
- They can reveal the unique atomic composition of a molecule, which can be used to characterise a drug at the different stages of its history
- Using ^{13}C NMR, the isotopic profiles of two nonsteroidal anti-inflammatory drugs (NSAIDs), ibuprofen and naproxen, were obtained at high precision and quickly

THE ORIGIN OF COFFEE

- The food industry faces major challenges from product substitutions, copies and contamination. Isotope profiling of food can detect these instances
- In a recent study, Remaud applied 13C NMR to measure the isotope distributions in commercially derived caffeine
- As a result, he found that caffeine can be used as a single molecular probe to assess the geographical origin of coffee, tea and cocoa