NMR studies of metabolites and xenobiotics: From time-points to long-term metabolic regulation

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Akademisk avhandling

som med vederbörligt tillstånd av Rektor vid Umeå universitet för avläggande av filosofie doktorsexamen framläggs till offentligt försvar i Sal KB3A9, KBC Building, fredagen den 23 januari, kl. 10:00. Avhandlingen kommer att försvaras på engelska.

Fakultetsopponent: Professor Graham Farquhar, Research School of Biology, Australian National University, Canberra, Australia.
Abstract

Chemical species carry information in two dimensions, in their concentrations and their isotopic signatures. The concentrations of metabolites or synthetic compounds describe the composition of a chemical or biological system, while isotopic signatures describe processes in the system by their reaction pathways, regulation, and responses to external stimuli. Stable isotopes are unique tracers of these processes because their natural abundances are modulated by isotope effects occurring in physical processes as well as in chemical reactions. Nuclear magnetic resonance (NMR) spectroscopy is a prime technique not only for identification and quantification of small molecules in complex systems but also for measuring intramolecular distribution of stable isotopes in metabolites and other small molecules. In this thesis, we use quantitative NMR in three fields: in food science, environmental pollutant tracing, and plant-climate science.

The phospholipid (PL) composition of food samples is of high interest because of their nutritional value and technological properties. However, the analysis of PLs is difficult as they constitute only a small fraction of the total lipid contents in foods. Here, we developed a method to identify PLs and determine their composition in food samples, by combining a liquid-liquid extraction approach for enriching PLs, with specialized $^{31}$P,$^1$H-COSY NMR experiments to identify and quantify PLs.

Wide-spread pollution with synthetic compounds threatens the environment and human health. However, the fate of pollutants in the environment is often poorly understood. Using quantitative deuterium NMR spectroscopy, we showed for the nitrosamine NDMA and the pesticide DDT how intramolecular distributions (isotopomer patterns) of the heavy hydrogen isotope deuterium reveal mechanistic insight into transformation pathways of pollutants and organic compounds in general. Intramolecular isotope distributions can be used to trace a pollutant’s origin, to understand its environmental transformation pathways and to evaluate remediation approaches.

The atmospheric CO$_2$ concentration ([CO$_2$]) is currently rising at an unprecedented rate and plant responses to this increase in [CO$_2$] influence the global carbon cycle and will determine future plant productivity. To investigate long-term plant responses, we developed a method to elucidate metabolic fluxes from intramolecular deuterium distributions of metabolites that can be extracted from historic plant material. We show that the intramolecular deuterium distribution of plant glucose depends on growth [CO$_2$] and reflects the magnitude of photorespiration, an important side reaction of photosynthesis. In historic plant samples, we observe that photorespiration decreased in annual crop plants and natural vegetation over the past century, with no observable acclimation, implying that photosynthesis increased. In tree-ring samples from all continents covering the past 60 – 700 years, we detected a significantly smaller decrease in photorespiration than expected. We conclude that the expected “CO$_2$ fertilization” has occurred but was significantly less pronounced in trees, due to opposing effects.

The presented applications show that intramolecular isotope distributions not only provide information about the origin and turnover of compounds but also about metabolic regulation. By extracting isotope distributions from archives of plant material, metabolic information can be obtained retrospectively, which allows studies over decades to millennia, timescales that are inaccessible with manipulation experiments.

Keywords:
NMR spectroscopy, isotopomer, phospholipid, persistent organic pollutant, CO$_2$ fertilization, photorespiration.