Designing Polarizing Agents for Sensitive and Selective DNP-enhanced NMR

Ildefonso Marin-Montesinos,[1] Diego Gauto,[1] Frédéric Mentink-Vigier,[12] David Goyard,[3] Thomas Halbriter,[4] Olivier Renaudet,[3] Anne Imberty,[5] Snorri Th. Sigurdsson,[4] Daniel Lee,[1] Sabine Hediger,[1] Gaël De Paëpe[1]

[1] Univ. Grenoble Alpes, CEA, CNRS, MEM, Grenoble, France; [2] EMR & CIMAR/NMR National High Magnetic Field Laboratory, Tallahassee, Florida, United States; [3] Univ. Grenoble Alpes, CNRS, DCM, Grenoble, France; [4] University of Iceland, Science Institute, Reykjavik, Iceland; [5] Univ. Grenoble Alpes, CNRS, CERMAV, Grenoble, France;

MAS-DNP has revolutionized the scope of many solid-state NMR experiments by enabling new sensitivity-limited experiments to be recorded. For instance, we have recently demonstrate DNP-enabled natural abundance ^{13}C-^{13}C and ^{15}N-^{13}C correlation experiments that can be used for crystal structure determination of small molecules and to study disease-relevant protein aggregates [1,2]. Nevertheless, DNP efficiency is still very limited at high magnetic field (> 10 T) and very fast MAS (> 20 kHz), which currently impedes the application of the technique to systems requiring high-resolution conditions provided by high magnetic field. **There is thus a need to develop new polarizing agents (PAs) enabling efficient uniform hyperpolarization, as well as to invent selective hyperpolarization approaches enabling the study of interfaces and binding sites under high resolution conditions.**

In this presentation, I will first highlight our effort towards improving solid-state NMR sensitivity thanks to the development of advanced MAS-DNP simulation tools. I will notably show that such tools provide great insight into the MAS-DNP CE DNP mechanism, and can be used to help predicting DNP efficiency of known biradicals (Totapol / *b*T*b*K / Amupol / TEMTriPol [3]/ etc.) but also to guide the design of new candidates that minimize depolarization effects (off-signal losses induced by sample spinning) while improving DNP sensitivity significantly (S/N per square root of time).[4] More precisely, the potential of **advanced MAS-DNP simulations** combined with **DFT calculations and high-field EPR** to qualitatively and quantitatively predict hyperpolarization efficiency of particular PAs will be discussed and a new family of radicals (**AsymPol**) will be introduced, highlighting their excellent performance at high field and fast MAS.[4,5]

Second, I will introduce a novel method called Selective Dynamic Nuclear Polarization (**Sel-DNP**) that allows **selectively highlighting and identifying residues present in a protein binding site.**[6] This powerful site-directed approach relies on the use of localized paramagnetic relaxation enhancement (induced by a ligand-functionalized paramagnetic construct) combined with difference spectroscopy to recover high-resolution multidimensional spectra from the binding site. This approach is demonstrated on the galactophilic lectin LecA. The well resolved Sel-DNP spectra enabled the ***de novo* assignment of the binding interface residues**. Since this approach produces clean and resolved difference spectra containing resonances from a limited number of residues only, it can be applied for the study of binding sites without any size limitation of the system, and does not require any selective isotopic labelling.

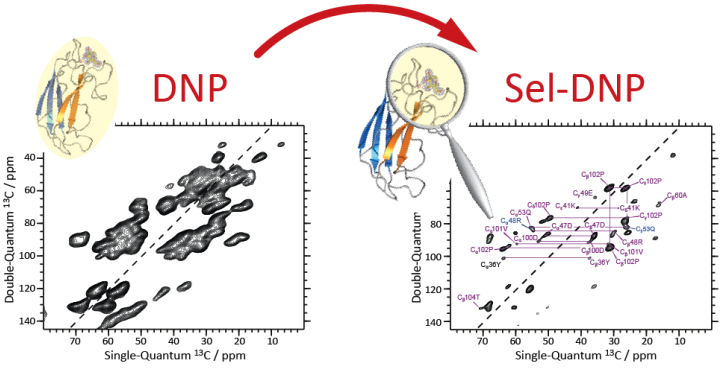

Figure 1. Unraveling protein binding site with Sel-DNP

References: 1- K. Märker *et al.*, **Chemical Science**, 8, 974-987, **2017; 2-** A. Smith *et al.*, **J. Am. Chem. Soc.,** 140 (44), 14576-14580 (**2018**); **3-** F. Mentink-Vigier *et al.*, **Chemical Science**, 12, 8150-8163 (**2017**); **4-** F. Mentink-Vigier *et al.*, **J. Am. Chem. Soc.**, 140 (35), 11013-11019 (**2018**); **5-** F. Mentink-Vigier *et al.*, **Phys. Chem. Chem. Phys.**, DOI: 10.1039/C8CP06819D, **2019; 6-** I. I. Marin-Montesinos *et al.*, ***submitted***