Ph. D. Thesis Project (3 years):

# Ultrahigh-sensitivity NMR spectroscopy for pharmaceuticals and vaccines

# RMN de très haute sensibilité pour l'étude des médicaments et vaccins

Host Institute: Very High Field NMR Center (CRMN), CNRS, ENSL, UCBL, University of Lyon
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Nuclear Magnetic Resonance (NMR) in the solid-state has developed as a valuable analytical technique to characterize both pure and formulated drugs and vaccines formulations. However, this spectroscopy generally suffers from low sensitivity, which often limits its applicability in pharmaceutical research. Dynamic nuclear polarization (DNP) has recently emerged as a key method to enhance the sensitivity of solid-state NMR spectroscopy experiments under Magic Angle Spinning (MAS) by several orders of magnitude. This technique has found successful applications. Notably, key developments have been achieved in the field of drug and vaccine developments, with the demonstration that DNP NMR spectroscopy could be applied to distinguish polymorphs in active pharmaceutical ingredients (APIs), detect interactions between drugs and excipients or characterize the organizational structure of virus-like assemblies. We propose a Ph.D project aiming at implementing and developing **new highly sensitive NMR methodologies** to unravel the atomic-scale structure of pharmaceutically-relevant formulated **drugs** and **vaccines** of complex composition.

More specifically, the project will develop along two concerted axes: i) the investigation of the molecular structure of **lipid nanoparticles** (LNPs) by DNP enhanced solid-state NMR spectroscopy at high magnetic field and fast MAS using a combination of advanced labelling techniques with $^{13}C$, $^{15}N$ and $^{2}H$ isotopes, state-of-the-art NMR methods and numerical approaches. In the context of the COVID-19 crisis, LNPs have emerged as effective vehicles to deliver mRNA vaccines. However, the structure of mRNA-loaded LNPs remains elusive and a number of aspects such as the interactions between their many components, the environment of the nucleic acid cargo or the lipid spatial distribution, are still open questions that we will address during this PhD; ii) the development of novel $^{19}F$ DNP solid-state NMR approaches. $^{19}F$ solid-state NMR is an extremely attractive technique in drug development, for example to detect the transformation of an **active pharmaceutical ingredient** into different polymorphs, cocrystals or salts, as today about 30% of APIs contain at least one fluorine atom. In practice the sensitivity of NMR is however often too limited to detect low amounts of unwanted polymorphs or implement multi-dimensional and multi-nuclear techniques (such as $^{19}F$ -$^{13}C$ or $^{19}F$ -$^{15}N$) needed to probe the drug structure. The combination of DNP with$^{19}F$ NMR for pharmaceutical research remains largely unexploited and sensitivity enhancement currently obtained are far below the theoretically expectations. Different $^{19}F$ DNP strategies will be developed during this PhD and applied to both neat APIs as well as drugs.

The project will take place at the Very High Field NMR Center (CRMN), a unique NMR facility affiliated with the CNRS, the ENS Lyon and the UCB Lyon-1. The center is operated by five internationally recognized groups (about 35 scientists) working on a range of interdisciplinary problems from catalysis to biology, and accommodates 6 NMR spectrometers of frequency 400, 500, 700, 800 (x 2) and 1000 MHz fully operative for solid-state NMR spectroscopy, offering exceptional capabilities, including two DNP instrumentation (at 400 and 800 MHz). The project will be developed within established collaborations with **industrial partners**. The CRMN provides excellent and **international** working atmosphere and is located in Lyon, one of the most lively French cities, recognized for its life quality.

Zhao et al, *Magn Reson Chem* **2018**, *56* (7), 583–609. https://doi.org/10.1002/mrc.4688.
Schlagnitweit et al, *ChemBioChem* **2019**, *20* (19), 2474–2478. https://doi.org/10.1002/cbic.201900297.
Viger-Gravel et al, Angew. Chem.-Int. Edit. **2019**, 58 (22), 7249–7253. https://doi.org/10.1002/anie.201814416.
Viger-Gravel et al, Chem.-Eur. J. **2020**, 26 (41), 8976–8982. https://doi.org/10.1002/chem.202001141.
Jaudzems et al, Angew. Chem.-Int. Edit. **2021**, 60 (23), 12847–12851. https://doi.org/10.1002/anie.202013189.

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