



Final Summary Report

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“Development and application of new Nuclear Magnetic Resonance methodologies for structure determination of solid or semi-solid samples”

My project was aimed at the development and the application of new solid-state Nuclear Magnetic Resonance (NMR) experiments for the measurement of interatomic distances in molecular systems containing a high density of magnetic nuclei. In fact, over the past few years, solid-state NMR has rapidly progressed as a method of choice for investigating structural properties of biomolecular systems or pharmaceutical formulations. This relies on the possibility of accessing dipole-dipole couplings, which depend on the interatomic distance and therefore yield information about the tridimensional properties of the molecules (i.e., its conformation) as well as about its supramolecular spatial arrangement. In spite of the inherent difficulties related to the presence of a network of multiple nuclear spins coupled, limiting the precision of the measurement of the distance between two selected nuclei, several methods have been proposed in the last 5 years for the measurement of distances between pairs of carbon atoms. Because of the low natural abundance of the only NMR-active isotope of carbon nuclei (^{13}C , about ~1%), these techniques require a uniform isotopic labeling of the molecule, which can be labor intensive and increases the research costs. To make the research more cost-effective, I proposed to observe a magnetically active nuclear isotope with high natural abundance and largely diffuse in the majority of organic and inorganic systems: ^1H (proton). ^1H nuclei have a natural abundance of almost 100% and they are highly sensitive from an NMR point of view. However, the observation of ^1H nuclei in solid-state NMR has been traditionally limited in the past by the scarce spectral resolution achievable. This is related to the strong ^1H - ^1H dipolar couplings present, which generate very large and often unresolved signals. In the last decade several efficient *homonuclear decoupling* techniques have been developed to overcome these limitations, which allow, today, the dipolar couplings to be virtually cancelled out, providing a very good ^1H spectral resolution - especially for small solid organic molecules - using standard solid-state NMR equipment (typically, static fields of 400 MHz/9.4 Tesla, magic-angle spinning the sample at 10-20 kHz). Despite these advances, no NMR experiment was reported in the literature allowing the direct measurement of individual ^1H - ^1H distances in solids because of the difficult control of the ^1H spin dynamics. During my MC fellowship, I have been able to develop the first solid-state NMR method that enables the *direct* measure of ^1H - ^1H interatomic distances.

This method relies on the following concept. To measure a ^1H - ^1H interatomic distance by solid-state NMR, we first need to be able to acquire a ^1H spectrum in which the different peaks corresponding to the nuclei of interest look well resolved. Once the high-resolution ^1H spectrum is available, the modulation of the intensity of these peaks in function of an evolution time under a suitable pulse scheme can be quantitatively related to the distance between the corresponding atoms. As mentioned above, to get high-resolution spectra, a suitable homonuclear-decoupling sequence must be applied. Ideally, the best spectral resolution attainable using homonuclear decoupling corresponds to a complete removal of the ^1H - ^1H dipolar coupling interaction: however, it is exactly this interaction we want to

exploit to get the information about distance. Therefore, I looked for experimental conditions leading to a slight *detuning* from the optimal decoupling conditions: this would allow only a small fraction of the dipolar interaction of interest to be reintroduced, guaranteeing high spectral resolution. Inspired by a recent paper by Prof. P. K. Madhu (Mumbai, India) and Prof. M. H. Levitt (Southampton, UK), I found these conditions in the application of the homonuclear-decoupling irradiation under specific *rotor-synchronized* conditions. With this expression, we mean that the decoupling irradiation block repeats synchronously with the rotation of the sample around the sample holder (called *rotor*) rotation axis. It turns out that these conditions are particularly appropriate, since, operating under average radio-frequency field and spinning rate conditions (about 80 kHz and 15 kHz, respectively) only reintroduces a maximum of 3% of the full dipolar coupling, which is consistent with good resolution conditions. Most importantly, the slight amount of dipolar coupling which is reintroduced into the experiment is enough to modulate the signal intensity and hence to get the information about the distance. The theoretical aspects of the methodology proposed were treated using the “theory of symmetry-based sequences” developed by Prof. M. H. Levitt. This allowed the exact analytical expression of the signal generated by this experiment to be obtained. This analytical function shows a dependence on: i) the dipolar coupling scaling factor, that is a constant whose value depends on the experimental conditions used and can be calculated precisely through Levitt’s symmetry theory; ii) the dipolar coupling constant. A simple non-linear fit of the experimental data with the analytical function thus allows the dipolar coupling to be obtained, and the distance between the two selected nuclei to be *directly* measured. **This is the first experimental NMR method allowing the direct estimation of the internuclear ^1H - ^1H dipolar coupling in solid systems.** The method has been validated using two model systems, solid glycine and L-alanine, which were used as test samples for the measurement of a short (glycine, 1.76 Å) and an intermediate (alanine, 3.52 Å) ^1H - ^1H distance. I was able to measure an intramolecular ^1H - ^1H coupling in glycine, obtaining the distance between the two CH_2 protons, with a precision of ± 0.02 Å. Similarly, I used a selective pulse to isolate the intermolecular CH-CH coupling in L-alanine. This allowed this interaction to be estimated with a precision of ± 0.09 Å. In both the cases, the resolution obtained is between 10 and 50 times higher than that achievable using the currently available diffraction methods (i.e., X-ray and neutron diffraction).

The method developed during my MC fellowship has the potential of paving new ways towards the quantitative determination of the tridimensional structure of solids through the use of hydrogen atoms. This approach is in principle applicable to all molecular systems whose ^1H solid-state NMR spectrum is sufficiently resolved, without any a priori limitations to the study of systems lacking long-range order, like the amorphous ones. This technique has immediate application to the study of pharmaceutical systems as the active principle ingredients, typically constituted by small organic molecules organized in a crystalline structure. Moreover, because of the limitations displayed by X-ray and neutron diffraction methods in the exact location of hydrogen atoms in a crystalline solid, this methods opens for the first time direct access to the study of the hydrogen bond network often stabilizing the supramolecular structure of organic solids, without even requiring monocrystals.

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