Federico De Biasi – Matrix-Assisted NMR

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Ph.D. thesis abstract

During the years, the interest of chemists towards increasingly sophisticated systems has grown exponentially, leading to a better understanding of very complex phenomena. Among the many examples in the literature, one of the biggest and still ongoing challenges in chemical complexity is the analysis of mixtures, ranging from reaction crude extracts to biological fluids (*i.e.* blood and urine). To this aim, chromatography has been – and still remains – one of the primary methods to reduce the analysis of multi-analytes systems to the study of many separate fractions comprising fewer components. Nonetheless, one intrinsic problem of the chromatographic approaches lies in their inability to identify unknown species on their own, so hyphenated techniques (mostly based on mass spectroscopy) have been developed just to overcome this stumbling block.

On the other hand, Nuclear Magnetic Resonance (NMR) spectroscopy is one of the most powerful and general techniques for the analysis of organic compounds. NMR exploits a fundamental property exhibited by some atomic nuclei – the spin – to acquire chemical and structural information through well-established experimental protocols, or "pulse sequences". In particular, solution-state NMR encompasses a vast ensemble of methods to collect detailed information about through-bond connectivities (COSY, TOCSY, HSQC,...) or through-space proximities (NOESY, ROESY,...) among the atoms in a species. Ultimately, a clever use of these techniques can often lead to the determination of the structure even for unknown compounds. However, such a wealth of information generally comes at the price of detecting many signals at once, and the interpretation of the spectra may often become a very challenging task. This occurs especially in the case of protons, as they are rather abundant in typical organic molecules and feature a small frequency dispersion of the resonances (∼12 ppm).

Not surprisingly, the situation becomes almost unmanageable when ^{1}H-NMR techniques are applied to the assay of mixtures: in this case, the superposition of signals stemming from different molecular species is the rule rather than the exception. Of course, multidimensional NMR experiments are of great help in the interpretation of crowded single-molecule spectra, but they rapidly lose all their advantages as the number of components in the sample increases. Once again, the advent of hybrid techniques like Liquid Chromatography (LC) paired with NMR has partly circumvented these difficulties, yet at the cost of expensive and dedicated instrumentations.

In the challenging scenario of mixture analysis, matrix-assisted NMR methodologies stand as an emerging alternative. Matrix-assisted NMR at large relies on the combination of NMR with an external agent, which is added to the sample to remove or label the resonances of selected species. The purpose of these additives, which can range from molecular and macromolecular species to nano and microscopic matrices, is to differentiate the signals of the various components in order to favour their detection and identification. On such premises, my Ph.D. work is organized into three Work Packages (WPs), each analysing different aspects of matrix-assisted NMR spectroscopy.

WP 1 focusses on the signal broadening phenomenon observed in matrix-assisted NMR diffusometry experiments performed in the presence of a stationary chromatographic phase (silica microspheres).[1] The purpose of "chromatographic NMR" is to overcome the limitations of plain diffusion-ordered NMR spectroscopy (DOSY), a technique designed to separate the single resonances in an NMR spectrum according to the diffusion coefficient of the parent molecules.[2] In fact, the success of DOSY depends critically on the existence of sizeable differences among the diffusion rates of the various species in

solution, a condition hardly met in ordinary samples where the analytes often have very similar hydrodynamic radii.

To this aim, the introduction of a silica matrix in the NMR sample tube perturbs the apparent diffusion rate of the analytes by virtue of the underlying partition equilibria,[3] but it also introduces some serious problems (Fig. 1). In fact, silica possesses a magnetic susceptibility which is different from that of the solvent, and a suspension of chromatographic silica generates local magnetic field inhomogeneities that cannot be corrected by the NMR spectrometer's shim system, causing a severe broadening of the signals.[4] It has been shown that hollow silica microspheres greatly help to reduce the impact of magnetic field inhomogeneities,[1] but the details of this phenomenon have so far remained unexplored. In the first part of my thesis, I have provided a description of the line broadening phenomenon for physically representative collections of hollow spheres with different geometries and filling factors. To this aim, the magnetic field distributions in model samples have been first calculated starting from the dipolar field induced by a single isolated sphere, and the resulting distributions have been compared with those obtained via numeric Finite Element Method. After this, the NMR line shape has been calculated by considering the relaxation (dephasing) of transverse magnetization induced by the random motion of the spins across the inhomogeneous field, also accounting phenomenologically for slow motions across the sample due to the presence of the porous silica matrix. The results highlight how, within the explored conditions, a proper modelling of the line broadening phenomenon should consider the enhanced relaxation of the spins during their diffusion inside the silica shells and possibly other slow motional effects. In summary, a strategy to obtain higher resolutions in "chromatographic NMR" experiments seems to be the use of hollow silica microspheres with larger pores, rather than thinner walls.

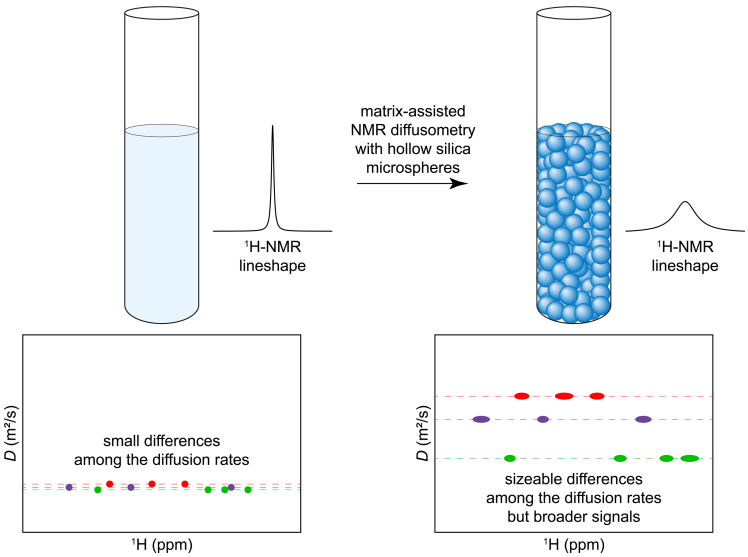

Figure 1 *The addition of chromatographic silica in a sample tube helps separating the signals in DOSY experiments, but it also induces a strong broadening of the signals in the frequency domain, reducing both the spectral resolution and the signal-to-noise ratio.*

WP 2 deals with nanoparticle-assisted NMR chemosensing, a technique where the molecular recognition properties of monolayer-protected gold nanoparticles (AuNPs) are exploited to "extract" the full ^{1}H-NMR spectrum of selected classes of analytes from that of a mixture (Fig. 2).[5] Because of their size, AuNPs are characterized by reduced translational and rotational diffusion rates. In addition, small molecules in solution are known to interact appreciably with suitable monolayers that passivate AuNPs. The combination of

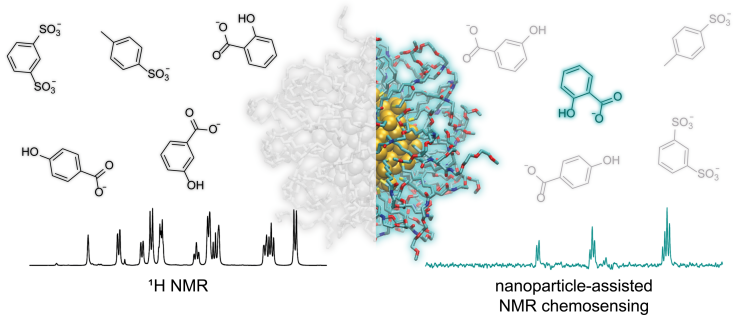

Figure 2 *Nanoparticle-assisted NMR chemosensing allows the selective acquisition of the full ^{1}H-NMR spectrum of only those species that interact with monolayer-protected AuNPs.*

these two characteristics makes AuNPs eligible for the manipulation of the magnetization of interacting species by means of particular NMR protocols such as the NOE-pumping experiment[6] or Saturation Transfer Difference (STD) spectroscopy.[7] Both these approaches rely on the nuclear Overhauser effect (NOE), which enables a selective magnetization transfer from the monolayer of the AuNPs to the interacting analytes.[5,8,9] In this way, not only are the targets unequivocally identified among many other species, but it also becomes possible to identify and assign the structures of unknown species.[10] Nonetheless, as in many NMR experiments, sensitivity remains a formidable challenge.

In this second part of the thesis, different strategies for the improvement of the limit of detection in NMR chemosensing experiments are presented. On first, it has been found that water spins in long-lived association at the nanoparticle monolayer constitute an alternative source of magnetization that can deliver a remarkable boost of sensitivity, especially when combined with saturation transfer experiments (Fig. 3). Secondly, a generalized procedure based on a joint water–nanoparticle saturation has been proposed to further upgrade the sensitivity, which ultimately endows selective analyte detection down to the micromolar range on standard instrumentation.[9] Notably, the strategy developed is very general and can be implemented also in NMR-based drug screening analysis that exploit protein-ligand interactions. Third, is has been verified how the NOE efficiency can be amplified through the pairing of charged AuNPs with larger inorganic nanoparticles via electrostatic interactions, in order to slow down their tumbling rate in solution without affecting the dynamics of the passivating monolayer which governs the binding of the analytes.

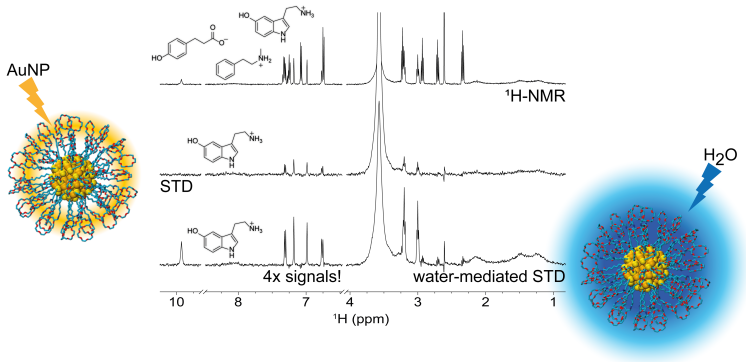

Figure 3 *Water-mediated saturation transfer difference (STD) spectroscopy allows the acquisition of much stronger signals in nanoparticle-assisted NMR chemosensing experiments compared to the "standard" version of STD.*

In close connection with the NMR of gold nanoparticles, WP 3 stems from a collaboration with the group of prof. Bürgi (Univ. of Geneve) regarding the full ^{1}H-NMR characterisation of the atomically precise and chiral $Au_{38}(SBut)_{24}$ gold nanocluster.[11] Interestingly, this cluster in the solution state actually features a mixture of four different symmetry-unique and equally populated binding sites for the grafting of the ligands that constitute its coating monolayer.[12] As a consequence, the overall ^{1}H-NMR spectrum of the cluster is the result of the superposition of four independent sub-spectra. In this case, 1D TOCSY experiments complemented with chemical shift-selective filters (CSSFs) proved to be an ideal tool to unravel the complex ^{1}H-NMR spectrum of $Au_{38}(SBut)_{24}$, since

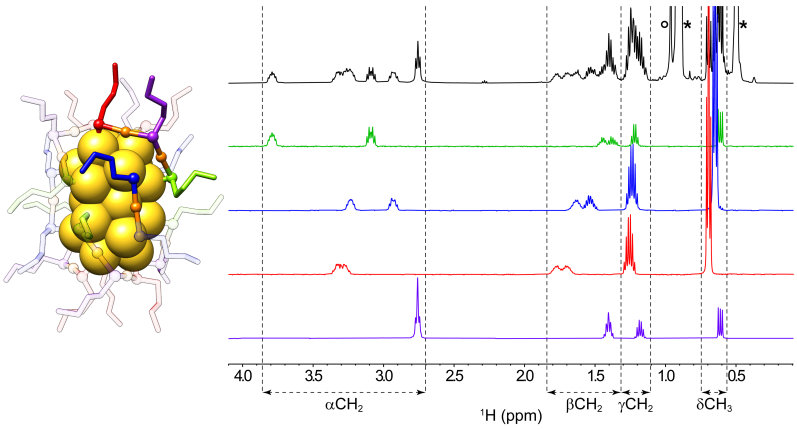

Figure 4 *The atomically precise and chiral $Au_{38}(SBut)_{24}$ gold nanocluster features four symmetry-unique and equally populated binding sites for the grafting of the ligands comprised in its monolayer. Its ^{1}H-NMR spectrum (black) is hence constitute by the superposition of four independent sub-spectra (coloured), each of which displays a different degree of diastereotopicity, even in the presence of achiral thiolate ligands.*

each (symmetry-equivalent) butylthiolate chain group represents an isolated spin system whose sub-spectrum can be conveniently extracted from the total spectrum (Fig. 4). The full assignment of the $Au_{38}(SBut)_{24}$ spectrum has been achieved through supplementary NOESY spectra, ^{13}C relaxometry and Molecular Dynamics (MD) simulations (in collaboration with the group of dr. De Vivo, IIT Genova). In particular, MD simulations have revealed largely different conformational dynamics of the ligands among the four grafting sites, explaining the distinct diastereotopicities observed for the αCH_2 and βCH_2 protons of the four butylthiolate groups. On the NMR side, the emerging picture is one wherein the diastereotopic effect observed between geminal protons is proportional (among other factors) to the unbalance in the equilibrium populations of fast interchanging "super-diastereoisomers".

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