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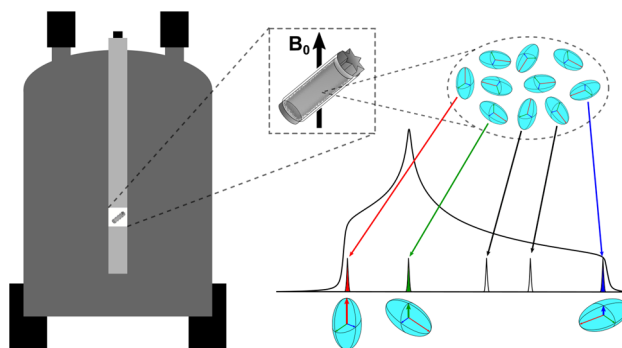
Anisotropy and NMR spectroscopy

Francesca Nardelli[1,3] · **Silvia Borsacchi**[2,3] · **Lucia Calucci**[2,3] · **Elisa Carignani**[1,3] · **Francesca Martini**[1,3] ·
Marco Geppi[1,3]

Abstract

In this paper, different aspects concerning anisotropy in Nuclear Magnetic Resonance (NMR) spectroscopy have been reviewed. In particular, the relevant theory has been presented, showing how anisotropy stems from the dependence of internal nuclear spin interactions on the molecular orientation with respect to the external magnetic field direction. The consequences of anisotropy in the use of NMR spectroscopy have been critically discussed: on one side, the availability of very detailed structural and dynamic information, and on the other side, the loss of spectral resolution. The experiments used to measure the anisotropic properties in solid and soft materials, where, in contrast to liquids, such properties are not averaged out by the molecular tumbling, have been described. Such experiments can be based either on static low-resolution techniques or on one- and two-dimensional pulse sequences exploiting Magic Angle Spinning (MAS). Examples of applications of NMR spectroscopy have been shown, which exploit anisotropy to obtain important physico-chemical information on several categories of systems, including pharmaceuticals, inorganic materials, polymers, liquid crystals, and self-assembling amphiphiles in water. Solid-state NMR spectroscopy can be considered, nowadays, one of the most powerful characterization techniques for all kinds of solid, either amorphous or crystalline, and semi-solid systems for the obtainment of both structural and dynamic properties on a molecular and supra-molecular scale.

Graphic abstract

1 Introduction

Nuclear magnetic resonance (NMR) spectroscopy is one of the most powerful techniques used for the determination of molecular structure and dynamics (Levitt 2008; Keeler

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2010). One of the most important features that makes this technique so popular is its broad applicability, due to the fact that every material contains chemical elements with NMR-active nuclei, i.e, nuclei with non-null nuclear spin I, such as, for example, ^{1}H, ^{13}C, ^{31}P, ^{29}Si, and ^{27}Al. Indeed, NMR spectroscopy exploits the interaction between suitable radio waves, typically applied for very short times (r.f. pulses) and such nuclei, consisting in stimulated transitions between different spin levels, the degeneration of which is preliminarily removed by immersion of the material within an external static magnetic field (B_0). From the standpoint of classical physics, the presence of B_0 originates an otherwise null magnetization (vector sum of all nuclear magnetic moments of a nuclear species) along the same direction of B_0. The direct interactions of the nuclear spin magnetic moments with both the external static magnetic field (Zeeman interaction) and the r.f. oscillatory magnetic field (B_1, having a direction perpendicular to that of B_0) represent the fundamentals of NMR spectroscopy. On the other hand, the nuclear spin magnetic moments can also interact with much weaker "internal" magnetic fields generated by the spins of other particles of the material (either nuclei or electrons) or by electronic currents. The "internal interactions" of a nuclear spin are strongly dependent on the chemical environment of the nucleus itself, which, for instance, makes NMR spectra very useful for the detailed determination of the chemical structure of unknown compounds. Most important in this context, the internal interactions, in contrast to the "external" ones, are intrinsically anisotropic, i.e., they depend on the orientation of a molecule (or of the molecular fragment bearing the nucleus) with respect to the direction of the external static magnetic field. We will see in the following that the anisotropy of the internal interactions is both pain and delight, bringing to tremendous line broadening (and consequent loss of spectral resolution) and, at the same time, carrying precious information on the structural and dynamic features of the material at the molecular level.

In the liquid state, the manifestations of anisotropy are canceled out by the fast isotropic tumbling of the molecules, implying the obtainment of high-resolution NMR spectra and, at the same time, a loss of structural and dynamic information. In contrast, the anisotropic interactions are completely or partially retained in solids and soft matter, where molecular motions are absent or, when present, are slow and/ or anisotropic, giving rise to considerable line broadenings, which in some cases completely prevent the distinction of different peaks in the spectrum.

In the late 70s of the past century, Magic Angle Spinning (MAS) and high-power decoupling (HPD) techniques have been devised, which for the first time allowed the obtainment of high-resolution NMR spectra also for solid samples. With this approach, a situation similar to liquids is ideally achieved, where anisotropies are nulled and lines are narrowed.

The conflict between high spectral resolution and retainment of information contained in the anisotropies was later overcome with the development of mono- and multi-dimensional pulse sequences, to be used in combination with MAS and HPD, which allow the selective determination of the anisotropy of specific internal interactions under high-resolution conditions, thus giving access to a wealth of information connected to the physico-chemical properties of molecules in the solid state.

In this paper, the theoretical basics of anisotropy in NMR spectroscopy will be presented, along with the Solid-State NMR (SSNMR) experimental techniques used to measure the anisotropy of internal interactions (Duer 2002; Apperley et al. 2012). Moreover, selected examples will be given, taken from the research activities of our group, to show how anisotropy can be exploited to investigate important structural and dynamic properties over broad length and time scales, respectively, on several categories of materials, ranging from crystalline solids to liquid crystals.

2 Theory

NMR spectroscopy is based on the study of transitions between nuclear spin levels induced by radio waves that match the Bohr frequency condition. Since the number of the nuclear spin levels for a nucleus with spin I is $2I + 1$, such transitions can only be observed for nuclei with $I \neq 0$. Moreover, the nuclear spin levels are normally degenerate; therefore, a necessary condition to observe the NMR phenomenon is that the nuclei are immersed in a static external magnetic field B_0 able to remove such degeneracy thanks to the so-called Zeeman interaction between the nuclear spin magnetic moments and B_0. The time-independent Schrödinger equation describing this interaction can be analytically solved to give the Zeeman energies for the nuclear spin levels:

$$E = -\hbar\gamma_N B_0 m_I,$$

where γ_N is the nuclear gyromagnetic ratio and m_I is the nuclear quantum spin number, which can assume $2I + 1$ different values ranging from $-I$ to $+I$ with intervals of 1.

Since in NMR spectroscopy the specific selection rule is $\Delta m_I = \pm 1$, the Bohr frequency condition is satisfied when:

$$\nu = -\frac{\gamma_N}{2\pi}B_0,$$

where ν is the so-called "resonance frequency" which should be observed in an NMR spectrum if only the Zeeman interaction was present.

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Although the Zeeman interaction represents the main contribution to the energy of the nuclear spin levels, the magnetic moment of each nucleus can also interact with additional weaker local fields that are generated by electronic currents or by the spins of electrons and nuclei belonging to the same or to surrounding molecules. Such "internal" interactions (shielding, direct dipolar and indirect or J-coupling, quadrupolar coupling) directly reflect the local physical and chemical environment of the investigated nucleus, thus representing an enormous source of structural and dynamic information. In particular:

- the *shielding* is the interaction between the nuclear spin and the electronic currents induced by the external magnetic field, which act reducing the effective magnetic field felt by the nucleus;
- the *dipolar coupling* is a direct through-space interaction between nuclear spins of the same or of different nuclear species; its strength is inversely proportional to the third power of the internuclear distance;
- the *indirect or J-coupling* is an interaction between nuclear spins of the same or of different nuclear species, which takes place indirectly through the bonding electrons; its strength does not depend on the spatial distance, but rather on the number of bonds connecting the coupled nuclei;
- the *quadrupolar coupling* is an electric interaction between the quadrupolar moment of the nucleus (which is non-null only for nuclei with a non-spherical charge distribution, i.e., nuclei with spin $I > 1/2$) and the electric field gradient generated by the electrons surrounding the nucleus itself.

Usually, the strength of all of these interactions is much smaller than that of the Zeeman interaction, so that, from the quantomechanical standpoint, they can be treated as perturbations of the Zeeman interaction. Therefore, an approximate solution of the complete Schrödinger equation (including both Zeeman and internal Hamiltonians) can be obtained by the first-order perturbation theory, which makes use only of the part of the internal Hamiltonians that commutes with the Zeeman Hamiltonian (secular approximation).

Importantly, the internal interactions, and therefore the energies of the nuclear spin levels obtained applying the perturbation theory, depend on the orientation of the molecule (or of the rigid molecular fragment bearing the nucleus) with respect to the direction of B_0. Consequently, such interactions are properly expressed as tensorial properties. In particular, the Hamiltonian of each internal interaction contains a rank-2 tensor C, which, in the laboratory frame, can be written as:

$$C(\text{LAB}) = \begin{pmatrix} C_{xx} & C_{xy} & C_{xz} \\ C_{yx} & C_{yy} & C_{yz} \\ C_{zx} & C_{zy} & C_{zz} \end{pmatrix}.$$

Here, each C_{ij} component of the tensor simultaneously depends on the physical and chemical environment of the nucleus and on the molecular orientation. To separate these dependences, it is useful to change the reference frame from the laboratory to the principal axes system (PAS) of the specific interaction, which is fixed on the molecule. In the PAS the C tensor assumes a diagonal form:

$$C(\text{PAS}) = \begin{pmatrix} C_{x'x'} & 0 & 0 \\ 0 & C_{y'y'} & 0 \\ 0 & 0 & C_{z'z'} \end{pmatrix}.$$

The isotropic value C_{iso}, the anisotropy ζ, and the asymmetry parameter η of the interaction can be defined as follows:

$$C_{\text{iso}} = \frac{1}{3}\left(C_{x'x'} + C_{y'y'} + C_{z'z'}\right),$$

$$\zeta = C_{z'z'} - C_{\text{iso}},$$

$$\eta = \frac{C_{y'y'} - C_{x'x'}}{\zeta}.$$

These three parameters determine the position of the center of mass, the breadth, and the shape of the signals in the NMR spectrum, respectively.

For instance, the resonance frequency due to the shielding interaction assumes the form:

$$\nu_{\text{CS}} = -\gamma_N B_0 \left[\sigma_{\text{iso}} + \frac{1}{2}\zeta\left(3\cos^2\vartheta - 1 - \eta\sin^2\vartheta\cos 2\phi\right) \right],$$

where ϑ and ϕ are the Euler angles describing the orientation of the PAS with respect to the laboratory frame, and therefore define the molecular orientation.

Similar expressions can be derived for the other internal interactions. In all of the cases, we can distinguish two different contributions to the resonance frequency: one isotropic and one anisotropic, only the latter showing a dependence on the Euler angles.
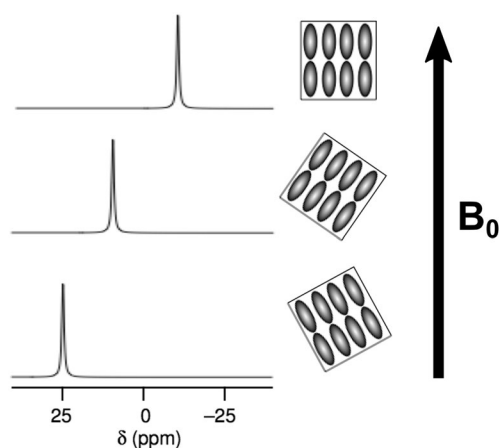
In the presence of molecular motions, the Hamiltonian assumes a time dependence, which can lead to the complete or partial average of the anisotropic term of the internal interactions. When the motion is fast and isotropic, such as the molecular tumbling occurring in liquids, all the possible orientations are assumed by the PAS with the same probability on the NMR time scale: the anisotropic contribution is completely averaged out and only the isotropic term remains. Since the isotropic value is zero for the dipolar and

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quadrupolar interactions, these interactions do not contribute to the NMR spectra of liquids, which are only determined by the shielding and J-coupling interactions. In the NMR spectra of liquids, the lines are very narrow, the resolution is very high and the spectral interpretation is simplified, allowing even slightly different chemical environments to be distinguished. These features make the NMR of liquids one of the most used techniques for the determination of chemical structures.

In contrast, when one deals with solids or soft matter, more complex NMR spectra are obtained; in fact, in these cases, the anisotropic terms of the internal interactions are fully or partially preserved, due to the absence of sufficiently fast and/or isotropic molecular motions. In the case of solids, molecular motions are often absent or too slow with respect to the NMR time scale; in such a case, the anisotropic term present in the expression of the resonance frequency is affected by the distribution of the different molecular orientations in the sample.

Let us consider the ideal case of a single crystal (with a single molecule in the unit cell): all of the molecules are oriented in the same way. Therefore, each molecule gives the same contribution to the NMR spectrum, characterized by well-resolved lines, with the resonance frequencies directly reflecting the physical and chemical environment of the nuclei, as well as the molecular orientation. Consequently, by changing the macroscopic orientation of the crystal with respect to B_0, the observed lines will change their resonance frequencies (Fig. 1).

However, solid samples are typically "powders", i.e., they are formed by different crystallites (or single molecules, in the case of amorphous phases) that adopt all possible orientations with respect to B_0 with the same probability. Therefore, the NMR signal associated with one nuclear site ("powder pattern") consists of a distribution of resonance frequencies arising from the distribution of orientations of the molecules in the sample (Fig. 2). This is the main cause of the extensive *inhomogeneous* line broadening typical of solid samples, which implies a dramatic loss of spectral resolution.

It should be noted that the powder pattern generated by each nuclear site and each internal interaction presents singularities, the positions of which in the NMR spectrum correspond to the principal values $C_{x'x'}$, $C_{y'y'}$, $C_{z'z'}$ of the interaction tensor.

To recover a spectral resolution similar to that of liquids, high-resolution solid-state NMR techniques, such as MAS and HPD (see the Sect. 3) have been devised and combined, which act by eliminating the anisotropic contributions to the resonance frequencies, in a similar way to what naturally done by the tumbling motions of the molecules in liquids. As an intrinsic drawback, the application of these techniques causes the loss of the information contained in the anisotropic contributions.

In the case of abundant nuclei, such as ^1H, the line width of the signals is further broadened for each single nucleus, due to the presence of strong homonuclear dipolar couplings. In fact, in this case, there is a term of the dipolar Hamiltonian that generates a remarkable *homogeneous* line broadening. It should be noted that this is an additional source of line broadening not due to anisotropy, which is harder to suppress using standard high-resolution techniques, like MAS and HPD.

Soft materials (liquid crystals, elastomers, gels, etc.) represent a state of matter intermediate between liquids and solids, in which molecular motions are generally fast, but anisotropic (i.e., due to structural constraints the different molecular orientations with respect to B_0 are not assumed

Fig. 1 Examples of 1D spectra of a single crystal: the lines are narrow, but the resonance frequency changes by changing the orientation of the crystal with respect to B_0
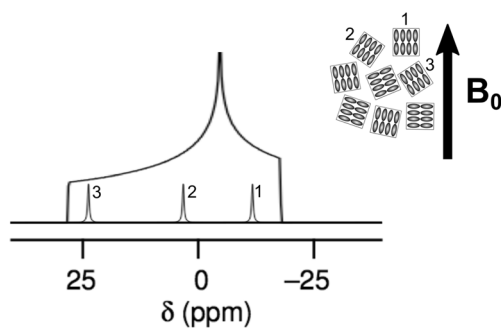

Fig. 2 1D spectrum of a powder sample due to the shielding interaction: each crystallite (or molecule) contributes to the whole spectrum with a narrow line resonating at a frequency that depends on its orientation with respect to B_0 (see Fig. 1). The isotropic distribution of the orientations generates a distribution of resonance frequencies, and the resulting powder spectrum consists of a broad pattern, which presents typical discontinuities at resonance frequencies corresponding to the principal values of the shielding interaction

with the same probability during the motion, rather some orientations are preferred), thus the averaging of the anisotropic part of the internal interactions is effective but incomplete. This still causes a line broadening in powder spectra, but to a minor extent than in solids.

A peculiar subclass of soft matter is that of *liquid crystals*; among them, rod-like mesogens can give rise to thermotropic nematic and smectic phases, stable over different temperature ranges, characterized by different degrees of orientational and positional order. In these phases, the molecules experience, like in liquids, very fast motions, which are, however, anisotropic, in contrast to the isotropic tumbling present in liquids. Indeed, the molecules tend to maintain their long axis preferentially aligned along a local *director*. Notably, within the strong magnetic field present in the NMR experiments, all the local directors are typically forced to be aligned along the same direction, which is usually that of B_0. In these conditions, the anisotropy of the different nuclear interactions is averaged by the motions to the same value for all the molecules, which, however, differs from the isotropic value. Simple 1D static spectra, therefore, provide very narrow signals, from the resonance frequencies of which the residual anisotropies of relevant interactions can be measured.

Exploiting these concepts, *aligning media*, as for instance anisotropic liquids, can be used to induce a partial alignment of molecules in solution. If the amount of the aligning medium is sufficiently low, NMR spectra remain as simple as for the isotropic solution; however, the anisotropic term of the dipolar interaction is not completely averaged out, and residual dipolar couplings (RDCs) can be detected (generally about 0.1% of the static dipolar coupling). The measurement of RDCs of specific internuclear bond vectors, such as NH bonds of a protein backbone, provides a tool to measure their relative orientation. This analysis is commonly used to refine 3D structures of biomolecules in solution, as it allows to significantly improve the quality of the structure models (Bax 2003; Chen and Tjandra 2011).

3 Methods

To gain information about the structure and dynamics of solids and soft materials by NMR spectroscopy, it is possible to choose between a variety of low-resolution (static) or high-resolution experiments, the latter being mainly based on MAS.

The use of *static techniques* is advisable when dealing with simple spin systems, for instance, when the investigated nuclear species is present in a single chemical environment or when a molecule is isotopically labeled in a selected position. In fact, this approach retains the anisotropic part of the nuclear spin interactions originating broad lines, which can

be recognized in the spectrum and interpreted only if they are present in a small number. Indeed, if the nuclear spin is affected only by a single anisotropic interaction, the characteristic shape of the signal (Fig. 2) allows the straightforward determination of the principal components of the interaction tensor, which in turn can be interpreted in terms of structural and dynamic properties of the system at a molecular level. An example is shown in Fig. 3, where in the ^{13}C static spectrum of a poly(ε-caprolactone) sample, the powder pattern of the esteric carbon due to the ^{13}C shielding interaction can be easily distinguished from those of the aliphatic carbons, thanks to their very different resonance frequencies. Unfortunately, in most real cases, the static spectrum consists of the superposition of several spectral patterns originating from distinct sites and/or from different interactions. Furthermore, if two (or more) interactions simultaneously affect the spectrum, the spectral line shape is also dependent on the relative orientations between their PASs. All this makes the analysis of 1D spectra and the subsequent extraction of physico-chemical information almost impossible.

Especially for rare nuclei, for which the homonuclear dipolar coupling is negligible, MAS (Maricq and Waugh 1979) and HPD (Hodgkinson 2005) techniques are of fundamental importance to obtain *high-resolution spectra*. MAS consists of a fast rotation of the sample, contained in a cylindrical rotor, about an axis forming an angle of 54.74° (the "magic angle") with respect to the direction of B_0. If
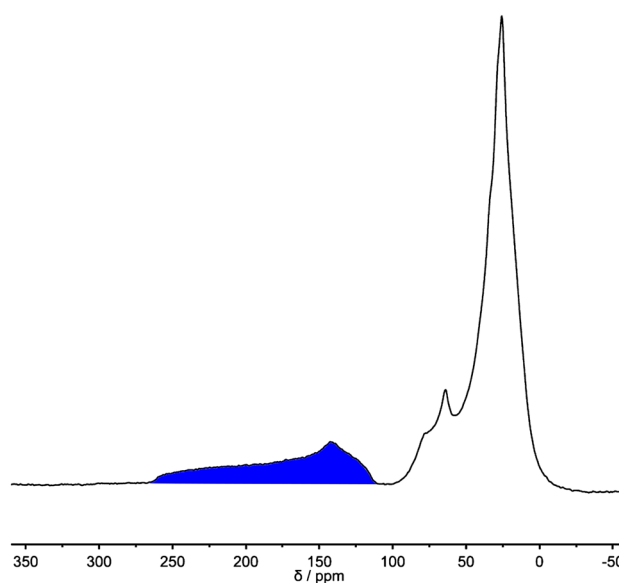
**Fig. 3** 1D ^{13}C ^1H-decoupled static spectrum of a poly(ε-caprolactone) sample. The blue area highlights the powder pattern of the esteric carbon, which is clearly distinguishable from the powder patterns of the other (aliphatic) carbons thanks to its very different isotropic chemical shift. Adapted from Borsacchi et al. (2014) (https://pubs.acs.org/doi/10.1021/ma5005716, further permissions related to this figure should be directed to the ACS)

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the spinning rate is sufficiently high, MAS averages out all the anisotropic terms of the interactions that have an orientational dependence on $(3\cos^2\theta - 1)$, where θ is the angle between the z axis of the PAS and the direction of B_0: this applies for shielding, dipolar coupling, J-coupling, and first-order quadrupolar coupling. Therefore, all the interactions are reduced to their isotropic values, with the sole exception

of the quadrupolar interaction if the second-order term (arising from the application of the perturbation theory to the second order) has to be considered. Examples of spectra recorded under MAS conditions are shown in Fig. 4.

To date, the highest MAS frequency that can be reached on commercial instruments is 110 kHz; however, standard measurements are usually performed using
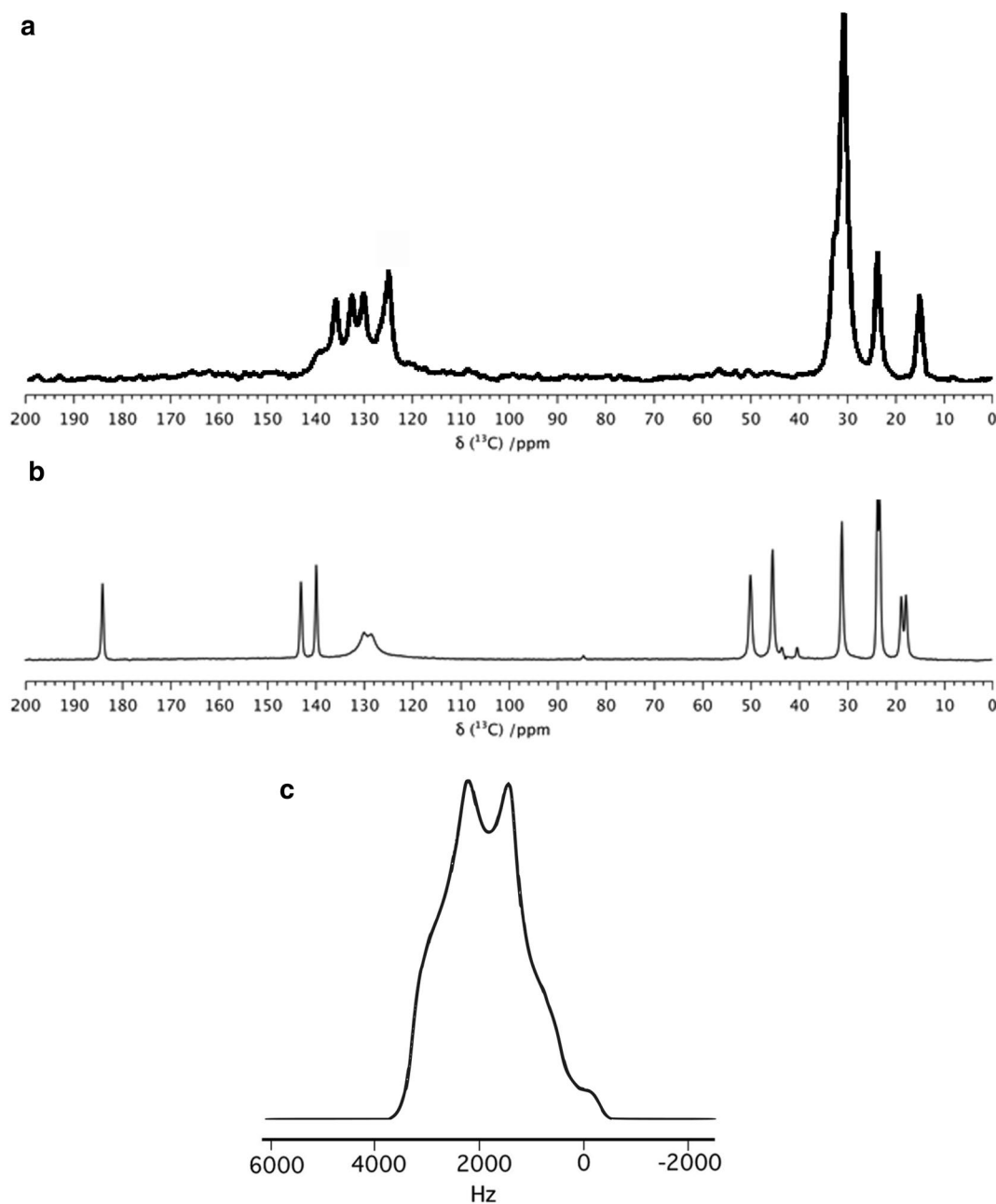
Fig. 4 Examples of 1D MAS spectra: **a** ^{13}C spectrum of poly(3-hexylthiophene), P3HT (adapted with permission from Martini et al. (2013). Copyright (2013) American Chemical Society); **b** ^{13}C spectrum of (S)-Na-ibuprofen dihydrate (adapted with permission from Rossi et al. (2014). Copyright (2014) American Chemical Society); **c** ^{11}B spectrum of the borinated liquid crystal 4DBF2 (reprinted from Borsacchi et al. (2011), Copyright (2011), with permission from Elsevier). In the first two cases, the spectra are determined by the isotropic chemical shifts only, while for ^{11}B a residual anisotropy due to the second-order quadrupolar interaction, only partially averaged by MAS, is present

frequencies between 5 and 30 kHz, which can typically reduce, but not completely average out, the anisotropic parts of the different interactions. Moreover, spectral line broadening has typically both an inhomogeneous (arising from the anisotropic interactions) and a homogeneous nature. The latter, although originating from the homonuclear dipolar interaction, is also present in the spectra of rare nuclei through their heteronuclear dipolar coupling with homonuclear dipolar coupled abundant spins. In these cases MAS is often ineffective in reducing the homogeneous line broadening and homo- or heteronuclear HPD techniques must be also used.

To overcome signal superposition issues present in low-resolution spectra and simultaneously preserve the information contained in the anisotropic interactions, 2D separation MAS experiments have been devised, in which typically one dimension is used to distinguish each nuclear site by its isotropic chemical shift and the other to measure one selected anisotropic interaction (e.g., shielding, hetero- or homo-nuclear dipolar coupling, quadrupolar coupling). A variety of techniques has been developed through the years, exploiting different sample rotation conditions during the experiment and/or particular (often very sophisticated) pulse sequences. An example is shown in Fig. 5.

In the next section, several examples, taken from the research activity carried out in our research group, will be provided showing how the experimental determination and the analysis of the anisotropy of the nuclear interactions can provide useful information about the structure and dynamics of solid and soft materials.

4 Applications

The strength of SSNMR in providing structural information on crystalline samples has been consolidated in the last years with the emergence of *NMR crystallography* (Bryce 2017; Hodgkinson 2020). This approach consists of exploiting NMR data collected on solid samples to refine or cross-validate the structures obtained from other methods, such as X-ray diffraction (XRD) methods. Especially in the case of small molecules, this is generally done in combination with quantum mechanical (QM) calculations, which either include the NMR data as constraints for the calculation/refinement of crystal structures, or simulate the NMR spectrum and compare it with the experimental spectrum for validation purposes. This is possible since QM methods allow the calculation of the electronic structure, which can be used to predict the nuclear interaction tensors and simulate the NMR spectra. Although many applications of NMR crystallography reported in the literature are based on isotropic chemical shifts, a much larger piece of information can be accessed when dealing with both the isotropic and the anisotropic parts of the interactions. From the experimental standpoint, this means that static techniques (when applicable) or 2D separation experiments must be used, as described in the previous section. For example, a 2D experiment based on the symmetry sequence $R10_1^3$ (Carravetta et al. 2000) was applied to determine the ^{31}P chemical shift tensor of black phosphorus (Martini et al. 2019); in this experiment, the contribution arising from the chemical shift anisotropy is retained and separated from the contribution due to the homonuclear dipolar interaction, which is instead suppressed (Fig. 6a). The combination with Density

Fig. 5 Example of 2D separation experiments: **a** 2D ^{19}F spectrum containing anisotropic shielding and isotropic chemical shift along the first and second dimensions, respectively. In (**b**) the vertical slices highlighted in (**a**) are shown along with the simulation curves used to determine individual shielding principal components for ^{19}F nuclei in different chemical environments. Reprinted from Martini et al. (2015), Copyright (2015), with permission from Elsevier
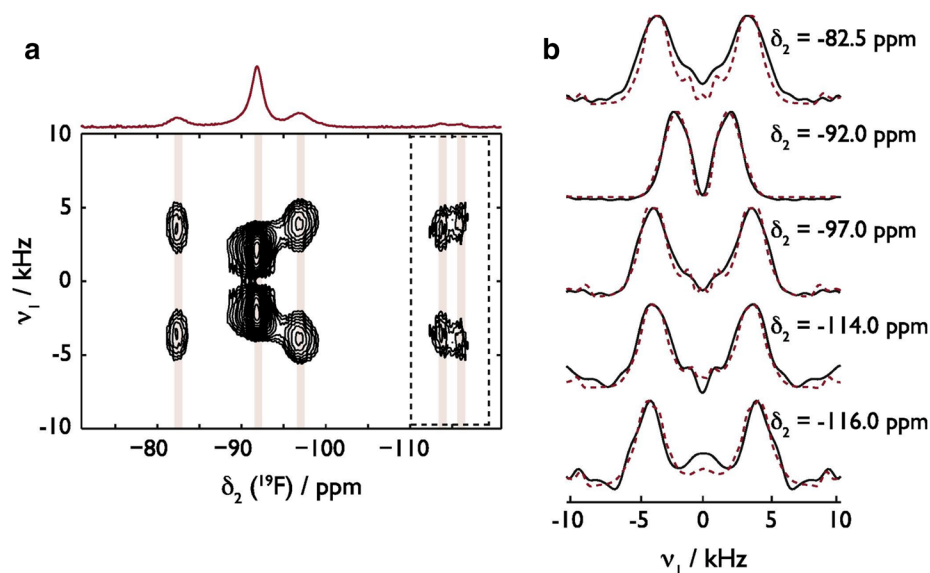
Fig. 6 ^{31}P shielding principal values were determined for black phosphorus by the 2D separation experiments reported in (**a**). The simulation of the vertical slice, also reported in (**a**), allowed the determination of the shielding principal values of the two groups of magnetically nonequivalent ^{31}P nuclei drawn in (**b**). Adapted with permission from Martini et al. (2019). Copyright (2019) American Chemical Society
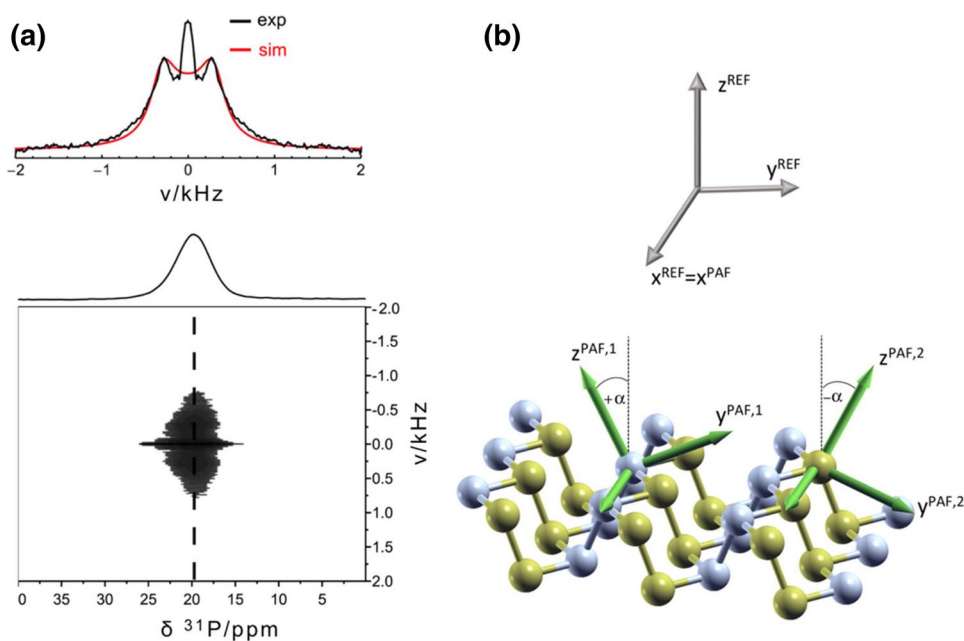
Functional Theory (DFT) calculations allowed two kinds of magnetically nonequivalent ^{31}P nuclei, characterized by the same shielding principal values, but different orientations of the principal axes, to be identified (Fig. 6b). It should be noted that in general the determination of the shielding anisotropy is particularly challenging for abundant nuclei, like ^{31}P in the previous case and, typically, ^{1}H in organic solids, due to the concomitant presence of the much stronger homonuclear dipolar interaction (Czernek and Brus 2019).

In addition to structural information, NMR spectroscopy can provide very detailed characterizations of the motions of molecular systems. As already stated, the presence of fast molecular motions relatively to the NMR timescale can average (partially or completely) the anisotropic part of the internal interactions of the nuclear spins. Although this is particularly evident for reorientational motions, which change the orientation of the interaction PAS with respect to the direction of B_0, also small amplitude motions such as librations and vibrations (Fig. 7), always very fast with respect to the NMR timescale, may affect the detected anisotropies. For example, in Refs. Carignani et al. (2011, 2018), the 2D PASS sequence (Antzutkin et al. 1995, 1998) was used to determine the principal components of the ^{13}C chemical shift tensors, which were interpreted in terms of different vibrational motions of the aromatic ring of ibuprofen. In particular, the principal components were measured over a wide range of low temperatures, thanks to the use of non-standard instrumentation (Concistrè et al. 2008), allowing a linear trend of the root mean square amplitude of the vibrational motions to be observed. These studies also highlighted

how discrepancies between experimental and calculated chemical shift tensors could indeed arise from neglecting the effects of vibrations in the application of QM methods.

Nevertheless, in many cases in NMR, the use of anisotropies for dynamic investigations is devoted to the analysis of spectral lineshapes to obtain information on reorientational motions of whole molecules or molecular fragments. On the one hand, these studies can be applied to solids, where the line narrowing is usually limited, since the motions are strongly restricted, and therefore, 2D techniques are typically necessary to experimentally determine the anisotropies of selected interactions. On the other hand, in soft materials, motions are less restricted and therefore, the residual anisotropies can often be measured by means of simpler 1D static experiments.

For example, phospholipids in water (a very interesting system mimicking cell membranes) can form a variety of soft phases, such as lamellar α (L_α) and β (L_β), inverse hexagonal (H_{II}), and micellar. These can be recognized by the analysis of ^{31}P static spectra; in fact, the ^{31}P lineshapes depend on the types of motional processes occurring in these systems, which differ for each phase. This feature has been exploited to investigate the effect of the addition of 1,7-dioxaspiro[5,5]undecane (pheromone of the olive tree fly) to a biomimetic lipid bilayer formed by dioleoylphosphatidylcholine (DOPC) in D_2O (Borsacchi et al. 2016). The pheromone, when added in suitable amount, was found to induce a L_α–H_{II} phase transition of the model membrane (Fig. 8). Simultaneously, the analysis of the spectral lineshape of ^{2}H nuclei, determined by the residual anisotropy of the quadrupolar interaction, allowed reorientational motions of water molecules to be characterized in the different phases.

Fig. 7 (left) ^{13}C experimental powder patterns of the aromatic carbons of ibuprofen determined by the 2D-PASS separation experiment at different temperatures. (right) Trends of the chemical shift principal values (δ_{11}, δ_{22}, δ_{33}) as a function of temperature, which were interpreted in terms of the vibrational motions drawn at the top. Reprinted from Carignani et al. (2018), Copyright (2018), with permission from Elsevier
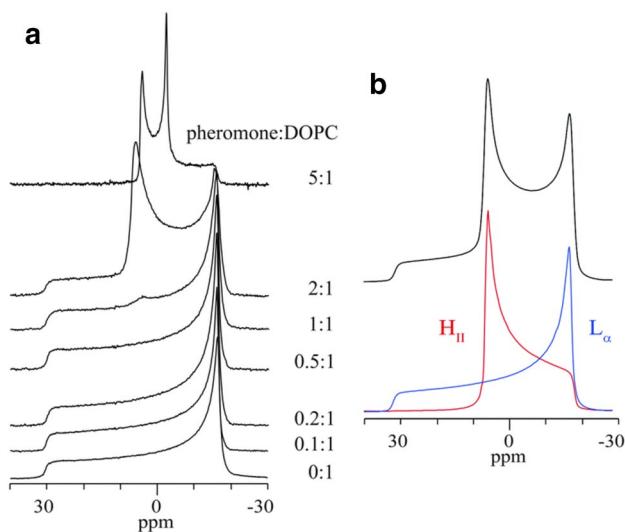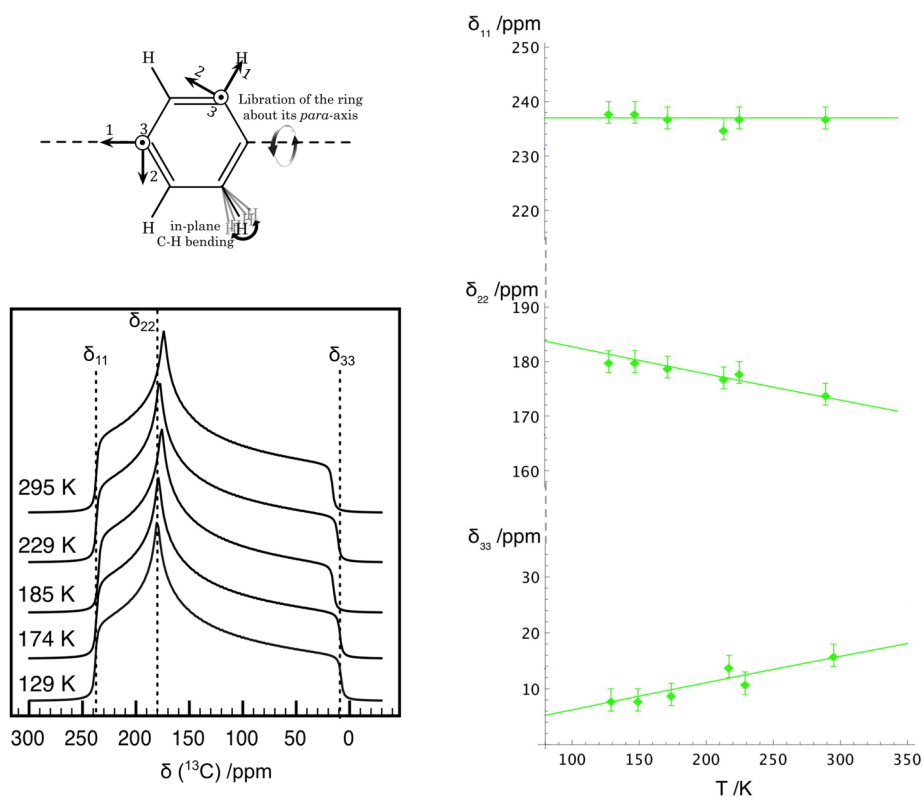
Fig. 8 **a** 1D ^{31}P static spectra of mixtures of 1,7-dioxaspiro[5,5]undecane and DOPC/water dispersions at different pheromone:DOPC molar ratios. The transition between lamellar L_α and inverse hexagonal phases is evident from the change of spectral shape at the highest pheromone:DOPC ratios. **b** simulation of the spectrum of the dispersion with 2:1 pheromone:DOPC molar ratio highlighting the contributions from lamellar L_α and inverse hexagonal phases. Adapted from Borsacchi et al. (2016) with permission from the PCCP Owner Societies

The analysis of the lineshape of ^{2}H nuclei was also exploited to reveal the dynamic properties of water in self-assembling systems made by ascorbyl dodecanoate and D_2O (Borsacchi et al. 2010). These systems give rise to two different phases: coagel and gel, stable below and above 50 °C, respectively. In the coagel phase, two overlapping signals with distinct lineshapes were found in the ^{2}H spectrum, arising from two well distinct types of water molecules: liquid-like bulk water (narrow line) and water confined within the thin interspace between two facing surfactant lamellae, experiencing very restricted and anisotropic dynamics (broad spectral pattern, Fig. 9). In the gel phase, only one signal was found, due to water with a mobility intermediate between the two types present in the coagel phase, confined in the interspaces between the surfactant lamellae, which is much thicker than in the coagel phase. Furthermore, the analysis of the residual shielding anisotropy in ^{13}C static spectra, much larger for the coagel phase than for the gel one, revealed that the surfactant aliphatic chains within the lipid bilayers experience a much more restricted mobility in the coagel phase (Fig. 9).

As previously mentioned, liquid crystalline phases, such as the nematic and some fluid smectic phases, behave in a peculiar way, as the molecules experience very fast motions as in liquids, but they assume a preferential orientation, which is usually the same for all of the molecules, giving rise to spectra characterized at the same time by a
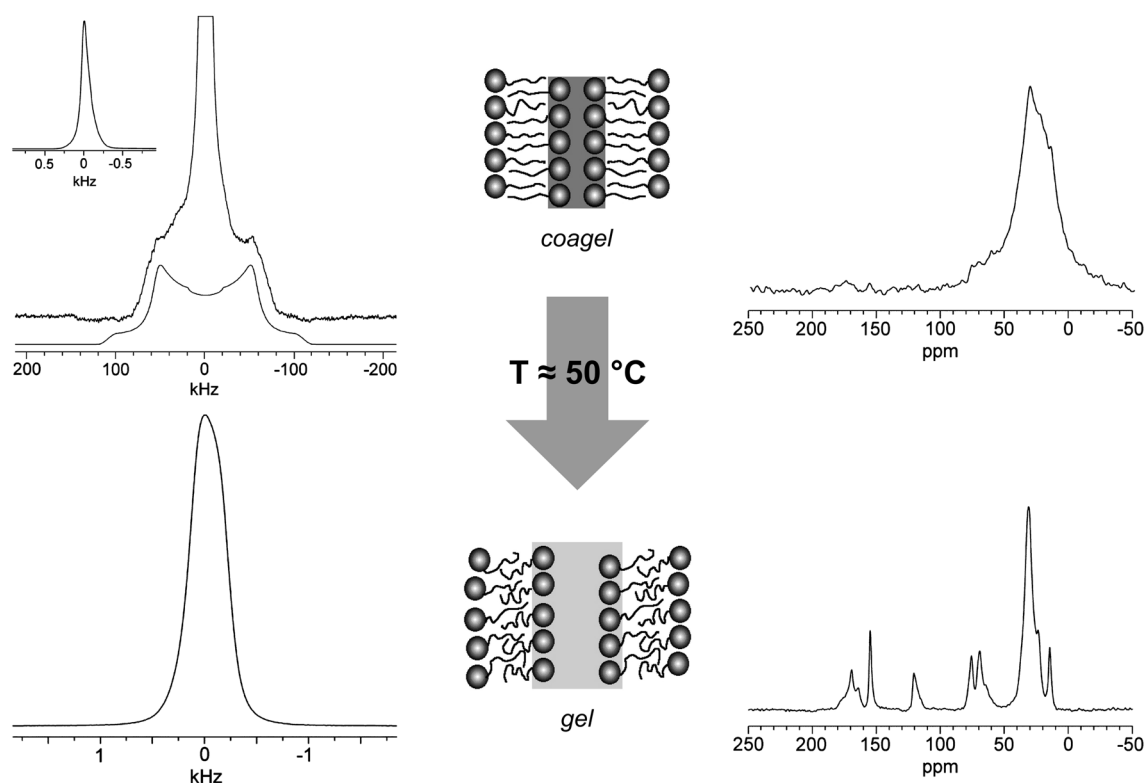
Fig. 9 Static spectra of ascorbyl dodecanoate in deuterated water in the coagel (top) and gel (bottom) phases. ^{2}H and ^{13}C spectra are shown on the left and on the right, respectively. Adapted with permission from Borsacchi et al. (2010). Copyright (2010) American Chemical Society

high-resolution and by the presence of contributions from residual anisotropic interactions. As an example, the resonance frequencies of ^{13}C signals of fluorinated liquid crystals can be used to derive the residual anisotropies of ^{13}C shielding and ^{13}C-^{19}F dipolar interactions (Catalano et al. 2007). Similarly, ^{2}H residual quadrupolar anisotropies can be measured from ^{2}H static spectra when suitably deuterated liquid crystals are available (Calucci et al. 2010). These experimental data were used to determine the orientational order parameters of the rigid molecular fragments and/or of the whole molecule for a variety of liquid crystals as a function of temperature in different phases (Fig. 10).

5 Conclusions

Although anisotropy is often seen as an undesired source of line broadening, causing a dramatic loss of resolution in the NMR spectra of solids, in this paper, we have seen how anisotropy can be positively exploited to determine precious structural and dynamic information on several types of molecular systems in solid and soft materials. The relationship between anisotropy and the nuclear spin properties measured by NMR spectroscopy has been explored from the theoretical and applicative standpoints. At first, the

theoretical dependence of the internal nuclear spin interactions on the orientation of the molecules (or the single molecular fragments) with respect to the external magnetic field direction, which is the origin of anisotropy in NMR, has been discussed. Afterwards, we have described the experimental NMR techniques that can be used in different cases to selectively detect the anisotropies of the different nuclear interactions, ranging from 1D "low-resolution" static spectra to 2D "high-resolution" separation experiments, along with their range of applicability to different nuclei and phases. In general, in solids, the inhomogeneous line broadening due to anisotropy (along with possible homogeneous line broadening due to the homonuclear dipolar coupling) prevents the direct use of 1D static spectra to measure the anisotropic part of the interactions, and therefore it is often necessary to exploit 2D separation experiments; in contrast, in soft matter the fast molecular mobility strongly reduces the line broadening effects making 1D static experiments as the method of choice. Finally, several practical applications to solids (pharmaceuticals, inorganic crystals) and soft materials (liquid crystals, amphiphilic systems self-assembling in water) have been briefly described, showing how the measured anisotropies can be exploited to derive different structural and dynamic properties. The structural properties that can be investigated from anisotropies include electronic
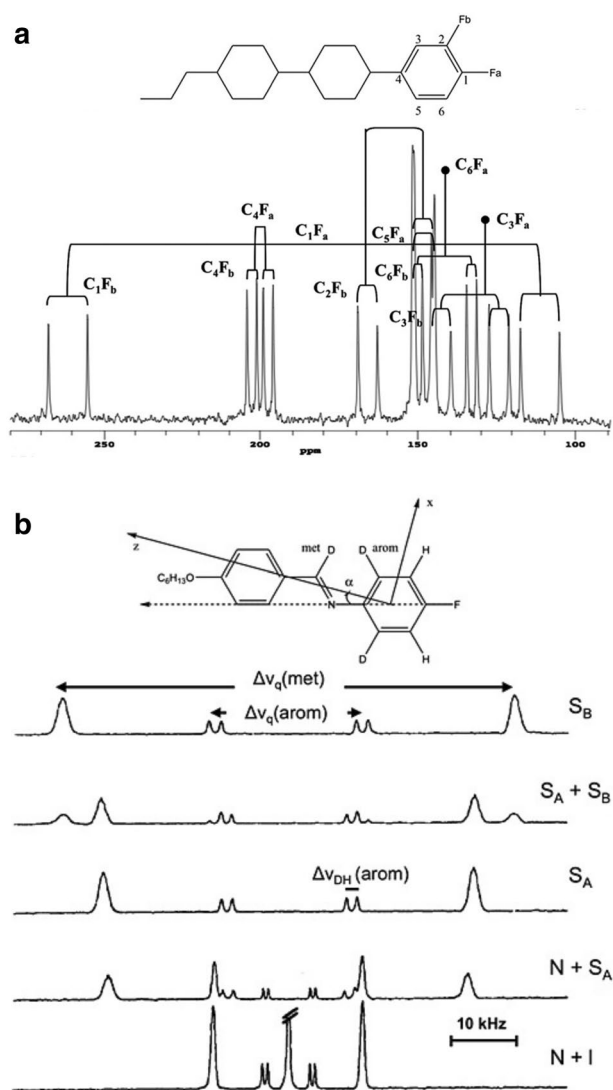
Fig. 10 **a** Expansion of the aromatic region of the ^{13}C static spectrum of the liquid crystal 3CyCyBF2 in its nematic phase: the residual ^{13}C–^{19}F dipolar couplings for the different pairs of nuclei are indicated (adapted with permission from Geppi et al. (2008). Copyright (2008) American Chemical Society). **b** ^{2}H static spectra of the liquid crystal FABOC6-d$_3$ in different mesophases: residual quadrupolar couplings for different deuterium nuclei are indicated (adapted with permission from Calucci et al. (2014). Copyright (2014) Wiley)

distribution, molecular packing, and orientational order, while, the dynamic properties mainly concern reorientations of the whole molecule or molecular fragments, occurring on the same time-scale of the NMR properties. However, although vibrational motions occur on a much shorter time-scale, it has been shown how their amplitudes can be effectively determined from the chemical shift anisotropy.

Although a variety of experimental techniques are nowadays available to determine all types of nuclear anisotropic parameters in several experimental conditions, further efforts are needed to enhance the accuracy of the experimental data.

On the other hand, upgrading the accuracy of DFT prediction techniques appears crucial to further improve the quality of the structural and dynamic information that can be eventually derived.

Summarizing, we can try to answer the big question: "is anisotropy a friend or foe for NMR spectroscopy?". We can certainly state that anisotropy is a foe, since it induces a great degree of complexity in NMR from both the theoretical and experimental standpoints. However, at the same time, anisotropy is a great friend, as it allows very detailed structural and dynamic pieces of information on solids and soft matter to be obtained, which are often inaccessible by other techniques.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This paper implies no specific bio-medical, or anthropometric action, or sampling on humans and animals. Cited research and related images refer to studies completed and published in the past.

Informed consent All authors have given approval to the final version of the manuscript.

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Affiliations

Francesca Nardelli[1,3] ⬤ · **Silvia Borsacchi**[2,3] ⬤ · **Lucia Calucci**[2,3] ⬤ · **Elisa Carignani**[1,3] · **Francesca Martini**[1,3] ⬤ · **Marco Geppi**[1,3] ⬤

✉ Marco Geppi
marco.geppi@unipi.it

[1] Dipartimento di Chimica e Chimica Industriale, Università di Pisa, via G. Moruzzi 13, 56124 Pisa, Italy

[2] Istituto di Chimica dei Composti OrganoMetallici, Consiglio Nazionale delle Ricerche, via G. Moruzzi 1, 56124 Pisa, Italy

[3] CISUP, Centro per l'Integrazione della Strumentazione dell'Università di Pisa, Lungarno Pacinotti 43, Pisa, Italy