1
Introduction

1.1. NUCLEAR MAGNETIC RESONANCE: A PRESENT DAY SPECTROSCOPIC TOOL

The ability of Nuclear Magnetic Resonance (NMR) spectroscopy to offer a direct probe of the local molecular environment around atomic nuclei enabling a detailed atomistic level investigation of complex molecular systems, makes it an attractive, versatile, and widely applied scientific and diagnostic tool of the recent time. NMR has proven its usefulness in basic sciences such as Physics, Chemistry, Biology [Cobb and Murphy, 2009; Gerothanassis et al., 2002; Yu et al., 2013] and several other multidisciplinary areas such as Biochemistry [James, 2012], Biomedicine [Nikolaou et al., 2014; Ruiz-Cabello et al., 2011], Environmental Science [Simpson et al., 2018], Materials Science [Bakhmutov, 2011] as well as in various industries namely food processing [Hatzakis, 2019], pharmaceuticals [Sem and Pellecchia, 2001; Webster, 2014], agriculture [Mazzei and Piccolo, 2017], petrochemicals [Edwards, 2011; Mondal et al., 2015], materials industry [Okazoe, 2009] and many more over the years. NMR spectroscopy is the standalone technique that provides molecular structural topology in the solution-state, elucidating complex molecular networks relevant in the field of chemistry, biology, and material science.

Besides the structural determination of molecules, probing molecular interaction and associated dynamics in various chemical, physical, and biochemical systems through NMR spectroscopy has emerged as an established research area of focus over the past several decades. With the evolution of modern science, the applications of NMR have been recognized as an imperative technique in numerous *in vivo* and *in vitro* studies addressing molecular dynamics and intermolecular interactions such as bond rotation, conformational transitions, solvent exchange, molecular aggregation, recognition, association, protein dynamics, metabolomics, ligand binding, encapsulation to name a few [Dubois and Evers, 1992; Fielding, 2007; Gerig and Stock, 1975; Jenkins, 1990; Lewandowski et al., 2015; Másson et al., 2003; Modarresi-Alam et al., 2007; Šmejkalová and Piccolo, 2008a]

The most common atomic nucleus investigated to unravel molecular structure and dynamics in solution is the proton (^{1}H). However, several heteronuclear systems have been explored systematically with the development of NMR methods to analyse dynamics and interactions in the solution and in the solid-state. One dimensional (1D) and two dimensional (2D) Carbon-13 NMR experiments have become as routine methods as 1D ^{1}H NMR experiments for structural characterization of small as well as macromolecular systems [Certaines, et al., 1992; Foster et al., 2007; Vlahov, 1999]. 2D ^{1}H–^{13}C correlation spectroscopy, ^{1}H–^{13}C NOE, and ^{13}C relaxation experiments have become standard NMR experiments analysing structure-dynamics of bio-macromolecules such as proteins and nucleic acids [Foster et al., 2007; Jaipuria et al., 2012]. The other heteronuclear systems that have shown tremendous growth in past years are ^{6,7}Li, ^{29}Si, ^{15}N, ^{2}H, ^{31}P, ^{19}F, etc. These heteronuclei are in general used to analyse dynamics, for eg. ^{6,7}Li NMR in the solid-state allow determination of ion dynamics relevant for energy storage materials such as batteries [Uitz, et al., 2017], ^{7}Li NMR is particularly suitable for studying transmembrane transport [Fotopoulou and Ronconi, 2018]; Solid-state ^{29}Si NMR are widely used to characterize ceramics and silica materials [Li et al., 2019; Sanz-Pérez et al., 2019]; ^{15}N relaxation experiments are routinely used for macromolecular conformational analysis and for characterising the dynamics of the involved ions [Buck et al., 1995; Hansen, 2017]; ^{2}H NMR relaxation experiments are employed to probe fast dynamics in methyl bearing side chains of

biomolecules [Stetz et al., 2019]; ^{31}P NMR is used for studying phospholipids, understanding the dynamics of soil phosphorus and for monitoring degradation of organophosphate agrochemicals [Schiller et al., 2007; Talebpour et al., 2006; Uitz et al., 2017]; ^{19}F NMR have emerged as methods of choice to unravel molecular association and binding for molecules containing fluorine labels [Cobb and Murphy, 2009; Yu, et al., 2013]. With the advent of NMR instrumentation, more exotic heteronuclear systems such as ^{17}O, ^{23}Na, ^{67}Zn, ^{129}Xe and many others are becoming nuclei of choice to understand structure, ion dynamics, lattice movement, intracellular motions relevant in biological, bioinorganic and medicinal chemistry [Fonseca et al., 2013; Fotopoulou and Ronconi, 2018; Huang and Sutrisno, 2014; Moudrakovski et al., 2002; Moudrakovski et al., 1998; Ronconi and Sadler, 2008; Spataro et al., 2006]

Among these various aforementioned heteronuclear NMR active nuclei that are routinely or occasionally investigated to characterize the molecular structure, interaction, and dynamics of different organic, inorganic molecules and biomolecules, ^{19}F NMR has drawn specific attention over the past half a century (1970-2020). It is a resultant of an enormous advancement in NMR hardware complemented with tremendous growth in fluorine chemistry. Recent advances in NMR technology allow the acquisition of ^{19}F in the presence of ^{1}H pulsing. It has enabled the experimental realization of 2D NMR experiments involving ^{19}F. On the other hand, the unique physicochemical properties of ^{19}F offer various advantages of introducing ^{19}F in organic molecules leading to the development of effective synthetic methods generating fluorochemicals. As a result, a steady upsurge of organofluorine compounds is observed mainly in the commercial market of pharmaceuticals and agrochemicals in past decades. It has been rightly said by Professor **G. K. Surya Prakash**, holder of the George A. and Judith A. Olah Nobel Laureate Chair in Hydrocarbon Chemistry and director of the Loker Hydrocarbon Research Institute, University of Southern California that "*Fluorine is the kingpin of drug discovery and many of the modern drugs contain one or more fluorine atoms.*"

The substantial growth of commercial availability of fluorochemicals has compelled researchers to delve into the topic of molecular interaction between ^{19}F containing compounds with various targets of interest *viz.*, biomolecules, natural organic matter (NOM), supramolecular assemblies, drug delivery systems, molecular cages, etc. It has been envisaged that these sets of analyses will enable one to understand the physicochemical effect brought by ^{19}F in the field of medicine, healthcare, and the environment. The following section highlights the unique physicochemical features of ^{19}F that have generated increasing interest amongst researchers.

1.2 FLUORINE: THE KINGPIN

In general, the occurrence of organic fluorine is rarest in nature when compared to other organo-halogens. Till date, only thirty (30) naturally occurring organofluorine compounds have been recognized as against two thousand one hundred fifty (2150) organochlorine and one thousand eight hundred fifty (1850) organobromine natural compounds [Gribble, 2002; O'Hagan and Harper, 1999]. Nearly all the fluorines that exist in nature are inorganic, such as fluoride (F^-), fluorspar (CaF_2), fluorapatite ($Ca_5(PO_4)_3F$), and others [Oliver, 1997]. During the first decade of the twentieth century, fluorine chemistry was mostly concerned with the development and analysis of inorganic fluorochemicals [Tressaud, 2019a]. However, the unique as well as distinctive physical and chemical properties of fluorine has spurred enormous interest in researchers for organofluorine compounds useful in material, biological, medicinal, and environmental chemistry [Berger et al., 2011; Champagne et al., 2015; Longstaffe, 2013; Okazoe, 2009]. Figure 1.1 depicts the exploration of the chemistry of fluorine specifically at the interfaces of chemistry and biology [Berger et al., 2011; Ojima, 2013].

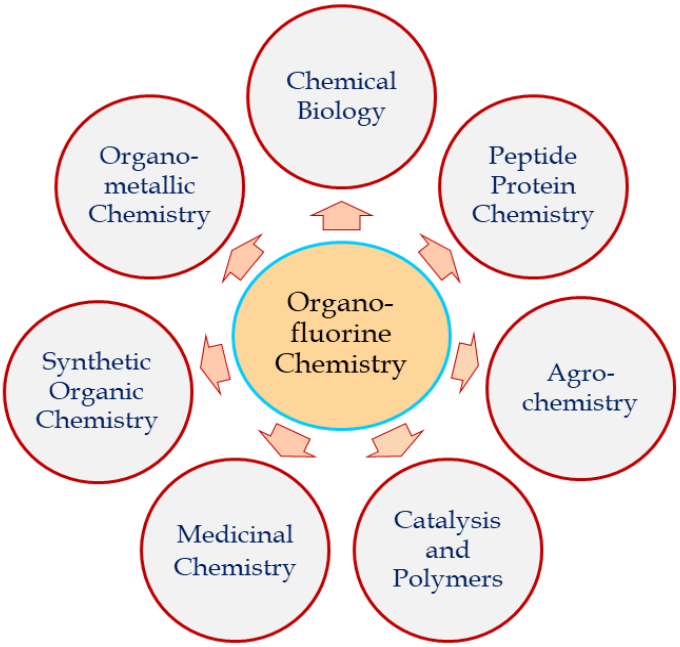

Figure 1.1: Fluorine chemistry in various multidisciplinary interfaces of chemistry and biology [Ojima, 2013].

As demonstrated in the literature, selective fluorination in potential drugs or agrochemicals can impart an incredible range of biological effects, *i.e.,* from complete metabolic inertness to highly enhanced specificity for binding at a particular site [Gerig, 1997]. Even the single fluorine substitution on a naturally occurring product will substantially alter its biological properties. Figure 1.2 schematically represents the unique physiochemical properties of fluorine and the effect observed due to the incorporation of –F substituents in organic molecules.

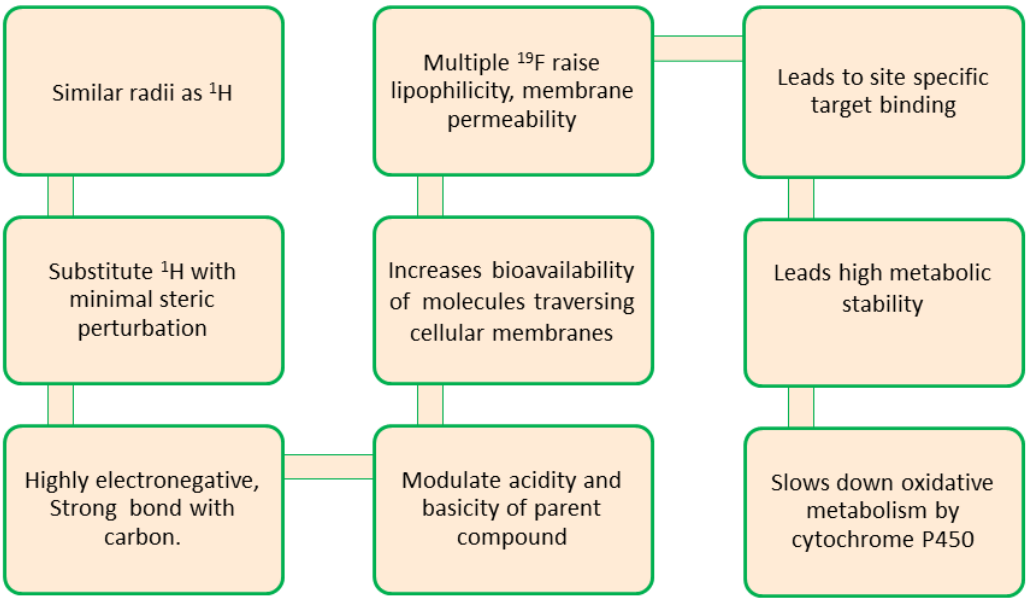

Figure 1.2: Scheme highlighting unique physicochemical properties of fluorine and the effects brought by insertion of fluorine in organic molecules.

These characteristics features of ^{19}F lead to several interesting outcomes that can be summarized as follows:

1. Van der Waal radius of ^{19}F (1.47 Å) is close to that of ^{1}H (1.2 Å), making it an isostere of hydrogen. As a result, –F can easily substitute –H in organic and biological compounds without altering the steric behaviour.
2. Due to its most electronegative character among the halogens (–X), ^{19}F gives rise to the strongest C–X bond that is even stronger than the C–H bond.
3. The C–F bond also strengthens the adjacent C–C single bonds, whereas allylic C=C double bonds are weakened by –F substitution [Müller et al., 2007].
4. The polarizability of C–F bond produces significant effect on the binding interaction of fluorinated molecules, resulting in improved target binding compared to the non-fluorinated analog.
5. The electron-withdrawing nature of –F can alter the hydrogen bond capacity, hence modulating the acidity and basicity of the parent molecule and nearby functional groups [Wang et al., 2014]. This will undoubtedly affect the pharmacokinetic properties, binding affinity, and bioavailability of the drug candidate and agrochemicals [Jeschke, 2004; Olsen et al., 2004; Tressaud, 2019b; Zhou et al., 2016].
6. The introduction of multiple fluorine substituents ($-CF_3$) in aromatic ring typically increases the bioavailability of molecules within cellular membranes by increasing the lipophilicity and membrane permeability [Fujiwara and O'Hagan, 2014].
7. The replacement of C–O and C–H bonds by C–F in biologically active compounds enhances the metabolic stability of the molecule in *in vivo* metabolism. It is because of the ability of the site-specific location of fluorine or fluorinated groups to lower the susceptibility of nearby moieties, causing cytochrome P450 enzymatic oxidation [Müller et al., 2007].

Further, it has been noted that organofluorine leads to substantial improvement in the efficacy of lead compounds by influencing their adsorption, distribution, metabolism, and excretion (ADME) within the environment and body [Cobb and Murphy, 2009; Müller et al., 2007]. Therefore, the synthetic fluorinated compounds or the fluorinated derivatives of naturally occurring compounds have been found to be extremely useful in assisting the development of lead products for various industrial applications, especially in the case of pharmaceuticals and agrochemicals industry [Zhou et al., 2016]. It was estimated that 30−40% of agrochemicals and 20% of pharmaceuticals in the commercial market contain fluorine from the industry sources [Champagne et al., 2015]. Figure 1.3 represents the continued growth of commercially available fluorinated compounds in the sector of agrochemicals and pharmaceuticals over the past five decades. As per recent literature and statistics, three out of five best-selling drugs and efficiently performing agrochemicals contain multiple –F atoms or at least a single F atom in their structures. For example, atorvastatin (Lipitor) has been registered as a best-selling fluorinated drug in 2008 and has generated a massive amount of revenue globally. A few other fluorine containing best-selling drugs are lansoprazole (Prevacid), fluoxetine (Prozac), and ciprofloxacin (Ciprobay) [Fernandez and Jahnke, 2004; O'Hagan, 2010; J. Wang et al., 2014]. Similarly, fluorinated herbicides like florasulam and flumetsulam have ruled as lead agrochemicals used for crop protection till date [Fujiwara and O'Hagan, 2014]. Undoubtedly, fluorine has risen as a key element of 21st Century [Tressaud, 2019a]. As an outcome of this colossal growth of fluorinated chemicals, especially in the pharma- and agro-industries, researchers worldwide have shown progressive inclination in analysing and monitoring interactions of these fluorochemicals with molecules of biological and environmental interest [Gerig, 1997; Sloop, 2013].

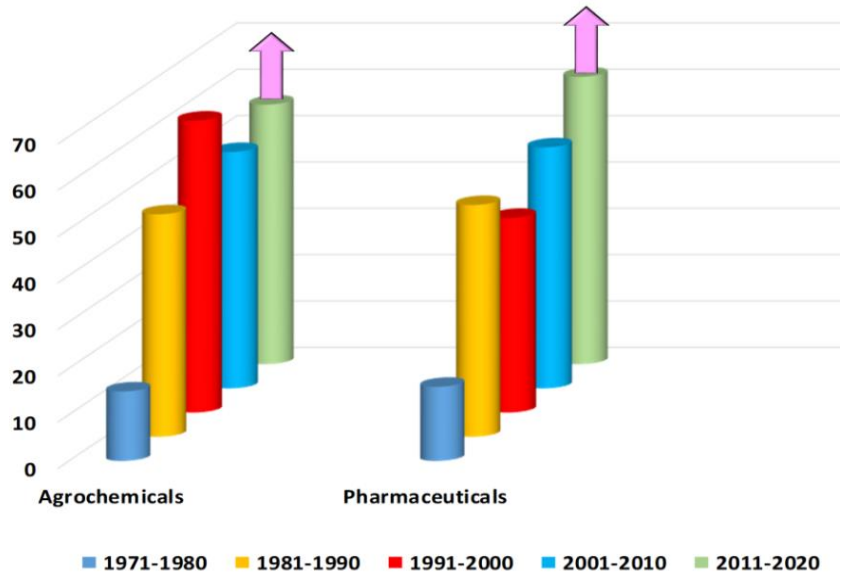

Figure 1.3: Bar graph representing the increase in fluorinated compounds in the pharmaceuticals and agrochemicals industry over the past years. Pink arrow denotes that data for 2020 will increase as it is incomplete at the time of writing. (Source: www.alanwood.net; www.prous.com, Integrity database; [Jeschke, 2010, 2017; Mei et al., 2020; Swallow, 2015])

Various analytical and spectroscopic techniques such as potentiometric ion probe, equilibrium dialysis, X-Ray Diffraction (XRD), Mass spectroscopy, FT-Infrared (IR), Fluorescence, UV-vis spectroscopy, Isothermal Titration Calorimetry (ITC), Circular Dichroism (CD) as well as theoretical calculations have been constantly applied to characterize these synthesized molecules both in terms of their structure and interaction dynamics in solution as well as in the solid-state [Baker et al., 2012; Davilas et al., 2006; Dimzon et al., 2016; Grembecka and Cierpicki, 2015; Hemalatha et al., 2016; Verbeeck et al., 1980; Welte et al,, 2020; Zhou et al., 2009]. Apart from these tools, two methods, namely i) radioactive ^{18}F Positron Emission Tomography (PET) and ii) ^{19}F Magnetic Resonance (MR) spectroscopy and imaging [Tressaud and Haufe, 2012] dedicated for fluorochemicals have been employed increasingly in the past years, enabling atomic-level analysis of these molecules. PET and MR imaging are performed in *in vivo* fashion while MR spectroscopy based techniques are mostly *in vitro* methods [Gillis et al., 2015; Tirotta et al., 2015]. These techniques characterize the –F containing systems by directly monitoring the spectroscopic behavior of their –F substituents. In the following section, the theory and applications of ^{19}F MR spectroscopy have been discussed in detail while PET and ^{19}F MR imaging are beyond the scope of the present Thesis and therefore, will not be addressed further.

1.3 FLUORINE NMR: THE PROGRESS AND THE PITFALL

Figure 1.4 demonstrates the continuous growth in the number of published articles on ^{19}F NMR over a period from 1970-2020. The data has been generated through a comprehensive literature search in PubMed using the keyword '^{19}F NMR'. It suggests an increased inclination towards ^{19}F NMR based research, which is a compound effect of amicable NMR properties exhibited by ^{19}F nucleus and the recent instrumental advancements and availability of fluorine containing materials as mentioned in previous sections.

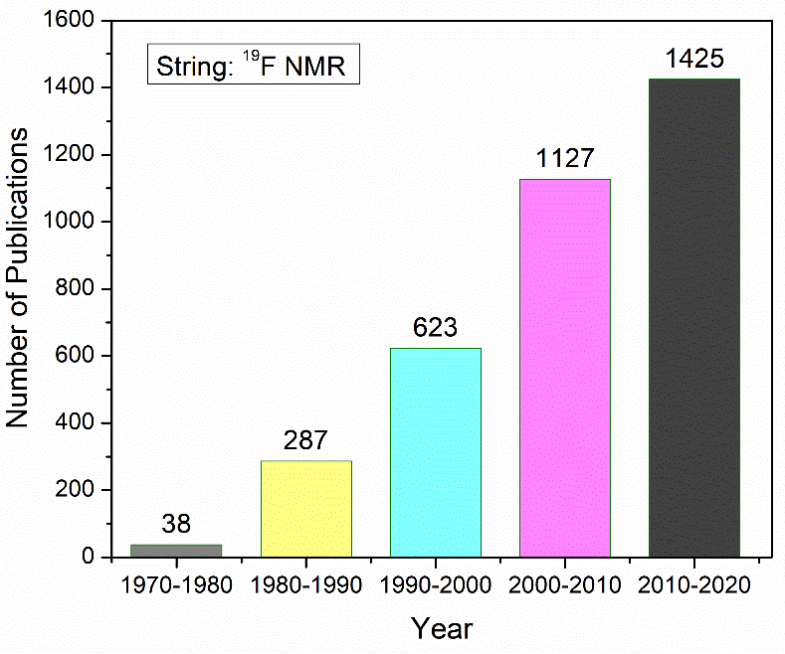

Figure 1.4: Bar graph representing the number of ^{19}F NMR publications over past decades (Source: PubMed). Data for 2011-2020 will increase as it is incomplete at the time of writing.

1.3.1 NMR properties of ^{19}F: The favourables and the unfavourables

Fluorine nucleus (^{19}F) presents several favourable nuclear spin properties. Table 1.1 documents the ^{19}F spin properties compared to the other nuclei investigated in the current Thesis.

Table 1.1: Spin properties of the nuclei investigated in the current Thesis.

Nucleus	Spin (I)	Gyromagnetic ratio (γ) (rad s^{-1} T^{-}×10^{-7})	Relative NMR Frequency (MHz) at 11.7 T	Natural Abundance	Relative sensitivity	Chemical shift range (ppm)
^{1}H	1/2	26.753	500.0	99.98	1.0	15
^{19}F	1/2	25.179	470.4	100.0	0.83	400
^{13}C	1/2	6.728	125.0	1.108	0.0159	250
^{2}H	1	4.107	76.77	0.0156	1.5×10^{-6}	15

The comparable sensitivity, high gyromagnetic ratio, and 100% natural abundance of fluorine (^{19}F) with respect to proton (^{1}H) make it a desirable atomic nucleus in NMR. In general, a very high sensitivity of ^{19}F compared to the other routinely investigated NMR active heteronuclei *viz.*, ^{13}C, ^{15}N, etc, makes it an easily detectable one. Other than sensitivity, the large gyromagnetic ratio of ^{19}F nuclei translates into strong dipolar couplings enabling strong ^{19}F–^{19}F or ^{19}F–^{1}H nuclear Overhauser effect (NOE) [Marsh and Suzuki, 2014]. This property is exploited to extract a gamut of information related to intermolecular and intramolecular distance constraints, such as internuclear distance between fluorinated ligand interacting target and solvent contacts, etc. [Aziz et al., 2002; Kitevski-LeBlanc, 2010; Loewen et al., 2001]. Also, being a spin-1/2 nucleus, the spectral analysis of fluorine NMR is considerably simplified in comparison to quadrupolar nuclei like ^{2}H that encounter contributions from the quadrupole

moment. However, there are several factors, such as chemical shift anisotropy (CSA), that do complicate the implementation and analysis of ^{19}F NMR.

Figure 1.5 summarizes the overall pros and cons associated with the implementation of ^{19}F NMR while addressing the structure and dynamics of fluorinated systems.

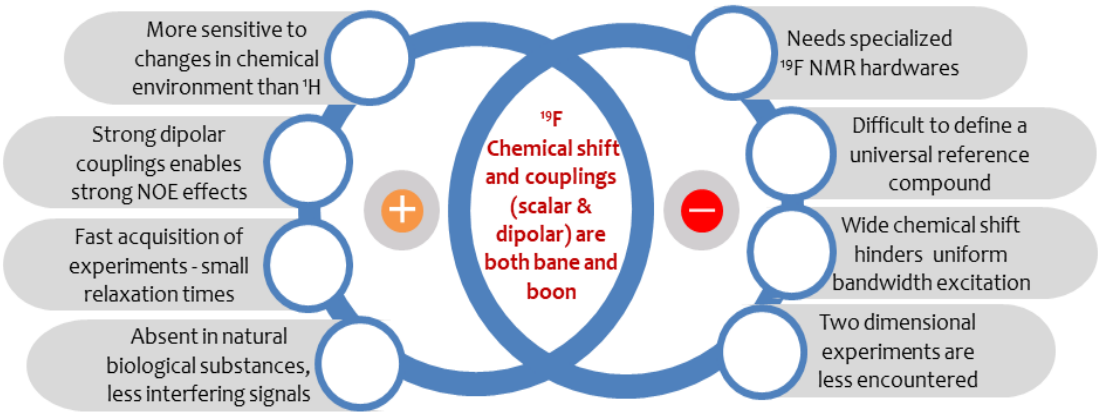

Figure 1.5: Advantages (+) and limitations (–) associated with ^{19}F NMR as a tool to probe molecular structure and dynamics in solution- and solid-state.

The specific NMR properties of ^{19}F that play a significant role during setting up of experiments and/or interpretation of NMR spectra are as follows:

(i) ^{19}F Chemical shift:

(a) Unlike the ^{1}H nucleus with a single orbiting electron responsible for creating magnetic shielding, ^{19}F nucleus experiences screening of external magnetic field due to the presence of nine electrons in its atomic orbitals resulting **a wide chemical shift range of *ca.* 600 ppm** as opposed to *ca.* 20 ppm for the protons [Becker et al., 2018; Yu et al., 2013].

(b) This property generates an **inherent sensitivity for ^{19}F nuclei towards any subtle changes in the local chemical environment**. It proves to be immensely informative in terms of the chemical properties of the fluoro-compounds.

(c) ^{19}F chemical shifts are also highly sensitive towards solvent (H_2O/D_2O) isotope effects, and this property finds application in probing structural changes and interactions, especially in protein-membrane systems.

(d) However, this property also creates **difficultly in defining a universal fluorinated compound** that is experimentally convenient to use as a chemical shift reference similar to TMS (trimethylsilane) in the case of ^{1}H and ^{13}C NMR. In general, to mitigate this problem, a compound that is chemically similar to the molecule under investigation is chosen as a ^{19}F reference compound through hit and trial exercise [Dolbier, 2009; Gerig, 1997; Togni et al., 2018].

(e) The wide dispersion of chemical shift also **hinders uniform radiofrequency pulse excitation of entire ^{19}F bandwidth** for fluorine attached carbon atoms (with narrow shift dispersion). As a consequence, 2D NMR experiments involving ^{19}F are less routinely encountered than for ^{1}H and ^{13}C. However, similar to ^{13}C NMR experiments cases, there are evidences of use of adiabatic pulses to overcome this issue in case of ^{19}F NMR experiments.

(ii) Fluorine couplings:

(a) In many cases, a **proper knowledge about ^{19}F through bond coupling constants** compared to ^{13}C and ^{1}H is missing that limits setting up of many multi-dimensional NMR experiments for ^{19}F [Battiste and Newmark, 2006; Cheatham and Groce, 2004]. However, it is to point out that it

is now possible to determine ^{19}F couplings using ab initio QM calculations. And these values are sufficiently accurate for designing pulse sequences.
(b) The molecular analysis of a number of fluorinated molecules is in general complicated because of quite an **unpredictability of ^{19}F chemical shifts and unusual ^{19}F coupling constants that often do not decrease monotonically with an increasing number of bonds** [Dabbit and Sutcliffe, 1980; Emsley et al., 1976; Yu et al., 2013].

It is thereby commented several times in literature that the strong ^{19}F scalar and dipolar couplings have found to be both boon and bane for various experimental aspects [Battiste and Newmark, 2006; Twum et al., 2015].

(iii) Relaxation:
(a) ^{19}F relaxation parameters exhibit a huge dependence on large ^{19}F CSA besides very strong ^{1}H–^{19}F dipolar interactions. ^{19}F CSA has been found to be useful for small molecules while investigating their binding with the target. On the other hand, large CSA at a higher magnetic field causes extreme broadening of ^{19}F signals of proteins and is always a matter of concern for biomolecular NMR experts [Hattori et al., 2017; Kitevski-LeBlanc and Prosser, 2012].
(b) The substantial contribution of CSA and the cross-correlation effect (arises due to CSA and dipolar interaction) sometimes results in a very small magnitude of ^{19}F transverse relaxation time (T_2 becomes very shorter) making its acquisition unreliable.
(c) Transverse relaxation rate ($R_2=1/T_2$) of a small fluorinated molecule shows a pronounced enhancement on binding with large molecule compared to the longitudinal spin relaxation rate (R_1) and is exploited well to probe binding events. But this T_2 induced line broadening and signal overlap make it challenging to apply NMR spectroscopy to large molecules. However, the applications of ^{19}F-^{13}C transverse relaxation-optimized spectroscopy have recently emerged as a novel method to investigate macromolecules structure and dynamics [Boeszoermenyi, 2019].
(d) ^{19}F nuclei in organofluorine compounds generally have relatively short spin-lattice relaxation time ($T_1=1/R_1$) that enables the fast acquisition of NMR experiments.

Apart from these specific ^{19}F NMR parameters, instrumentation plays a major role in establishing ^{19}F NMR experiments for structural and dynamical analysis. An elaborate (radio frequency) RF-circuitry is required to prevent the interference of the RF resonant signals at ^{19}F Larmor frequency with the very close ^{1}H Larmor frequency. Modern spectrometers and dedicated ^{19}F NMR probeheads incorporate electronic modules shared between ^{1}H and ^{19}F channels through high-band RF amplifiers. It allowed highly sensitive double-resonance $^{19}F/^{1}H$ NMR experiments to be recorded, including experiments where ^{1}H decoupling is applied during the acquisition of ^{19}F signal [Chen et al., 2013; Danielson and Falke, 1996; Yu et al., 2013]. These advancements of specialized NMR hardware required for ^{19}F have made the practical implementation of ^{19}F NMR possible and convenient to investigate various systems of interest. It can be commented that ^{19}F NMR definitely invokes interest among researchers due to its interesting NMR properties and spectral effects observed during molecular interactions and dynamics.

1.3.2 Applications of ^{19}F NMR in diverse areas:

Applications of ^{19}F NMR based methods are found to be flourishing in diverse areas. In the initial period, ^{19}F NMR has been primarily confined to synthetic chemists for structure elucidations and detection of various fluorinated compounds, *i.e.*, materials, pharmaceuticals, and agrochemicals, etc. [Saunders et al., 2018]. But, the evolution of NMR hardware and the exploitation of unique physiochemical properties of ^{19}F in designing the analogues of biologically relevant molecules have increased the utility of ^{19}F NMR in the biological systems as well. ^{19}F NMR serves as an intrinsic probe to unravel the structure and function of various biological macromolecules, *i.e.*, proteins, peptides, lipids, etc., labelled with ^{19}F. The absence of ^{19}F in biological systems and naturally occurring compounds generally reduces the spectral

complexity. The dynamic range complications are also reduced for ^{19}F systems due to the lack of any interference from strong water or any other solvent signal. This virtual absence of background signals is advantageous in studying protein complexes and *in vivo* applications [Buer and Marsh, 2012; H. Chen et al., 2013]. ^{19}F NMR with fluorinated molecular probes have provided an attractive and non-invasive approach to understand biologically significant events such as protein conformational analysis, folding and unfolding, aggregation, fibrillation, protein solvation, enzymatic action, protein-protein, protein-lipid or ligand-protein interactions, nucleic acid structure, and functions, etc. [Kitevski-LeBlanc and Prosser, 2012]. Other important areas of ^{19}F NMR that have been recognized in industry are drug screening and discovery, degradation analysis, xenobiotics screening, ligand binding, assessing the environmental impact of agrochemicals, metabolism analysis, *in vivo* tracking of bioactive molecules, gene transfection reporting, and molecular imaging, to name a few [Chen et al., 2013]. Figure 1.6 schematically represents the various areas where the utility of ^{19}F NMR applications in combination with fluorinated molecular probes have been expanded in past decades.

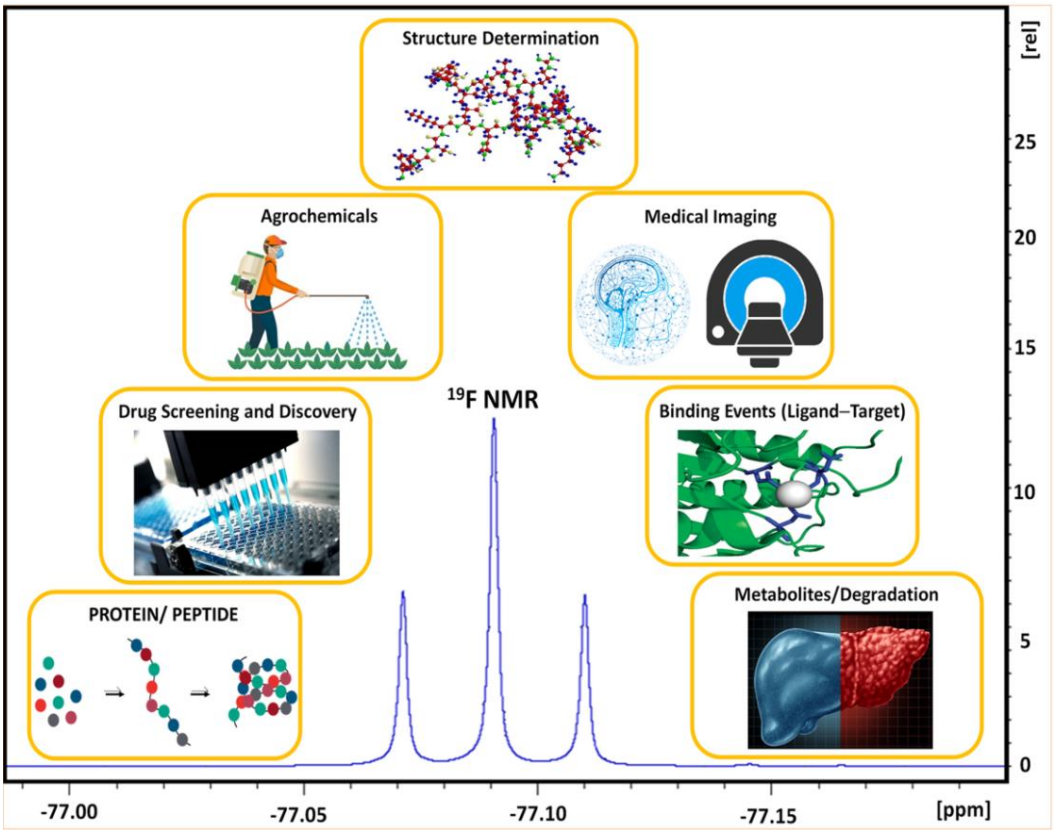

Figure 1.6: Applications of ^{19}F NMR for ^{19}F labelled molecules in diverse areas.

In the following section, a detailed literature review has been provided that highlights the development and application of NMR experiments, specifically ^{19}F NMR, in the field of molecular interaction in the solution-state.

1.4 MOLECULAR INTERACTIONS: ASSESSMENT BY NMR METHODS

1.4.1 Molecular Interactions: Definition and Occurrence

Molecular interactions are omnipresent in nature. It is correctly said that the world would be a uniform ideal gas in the absence of interactions. It is well understood that molecular interactions are resultant of various molecular forces existing in the gaseous as well as condensed phases of

matter. These are the key factors giving rise to essential phenomena relevant in the field of biology, chemistry, physics, and their interfaces. Figure 1.7 represents the most common classification of molecular interactions encountered in various fields of science. The intermolecular forces leading to molecular interactions are in general, non-covalent and are inherently electrostatic in nature. Alteration of molecular interaction due to any external factor manifests itself in a plethora of chemical and biological processes, such as binding events between ligand-macromolecules, protein denaturation via folding and unfolding, DNA-RNA transitions, solvation, adsorption-desorption and phase transitions (e.g., water), etc. These processes do not fall under chemical reactions where actual breaking and formation of bond happens [Mishra and Suryaprakash, 2017]. Hence it is significant to probe these interactions in various systems of interest to understand the underlying principles of these interactions, particularly the binding efficacy. These interactions cannot be visualized directly but can be qualitatively and quantitatively analysed in terms of kinetic parameters such as association and dissociation constants, exchange rate, binding strength, the stoichiometry of interaction, and the relevant thermodynamic parameters, *viz.*, free energy, enthalpy, and entropy of binding. Among various *in vitro* and *in vivo* biophysical techniques [Alberto, et al., 2011], NMR spectroscopy has a unique standing and is perceived as the gold standard comprising of a series of methods that covers all the aspects of truthful validation (qualitative assessment) of molecular interactions [Gossert, 2019].

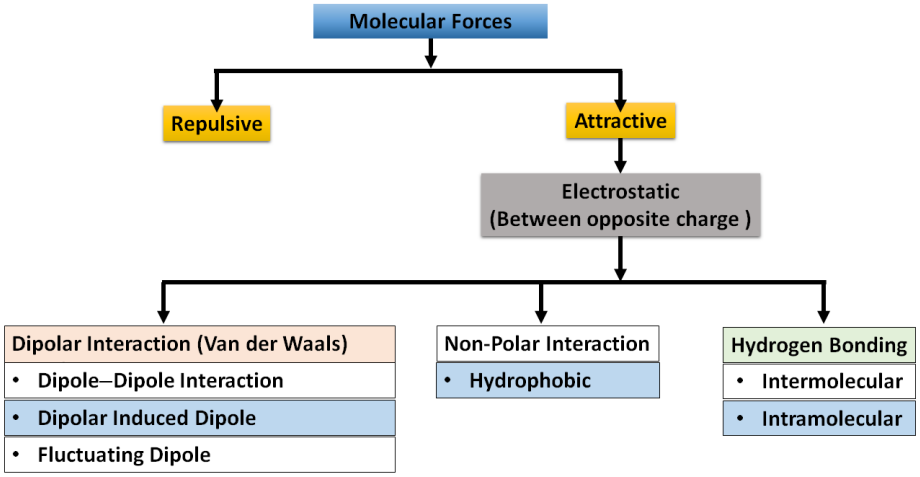

Figure 1.7: Classification of molecular interactions present in the nature [Mishra and Suryaprakash, 2017].

1.4.2 Overview of NMR methods available to probe molecular interactions

Through NMR, a detailed investigation of molecular interaction between a ligand (small) and a target (large) molecule in a system can be accomplished by monitoring the NMR spectral properties of either the ligand or the target. The ligand-target interaction processes are considered as an equilibrium process consisting of association and dissociation events. The said equilibrium process results in the modification of various physicochemical parameters such as chemical environment, mobility, diffusion, and structural conformation of both the ligand and the target. It in turn, affects the relevant NMR parameters associated with the ligand and target in their free and bound state. The binding efficacy of such a ligand-target interaction can be quantified by comparing the experimentally measured NMR parameters of the free and the complex state.

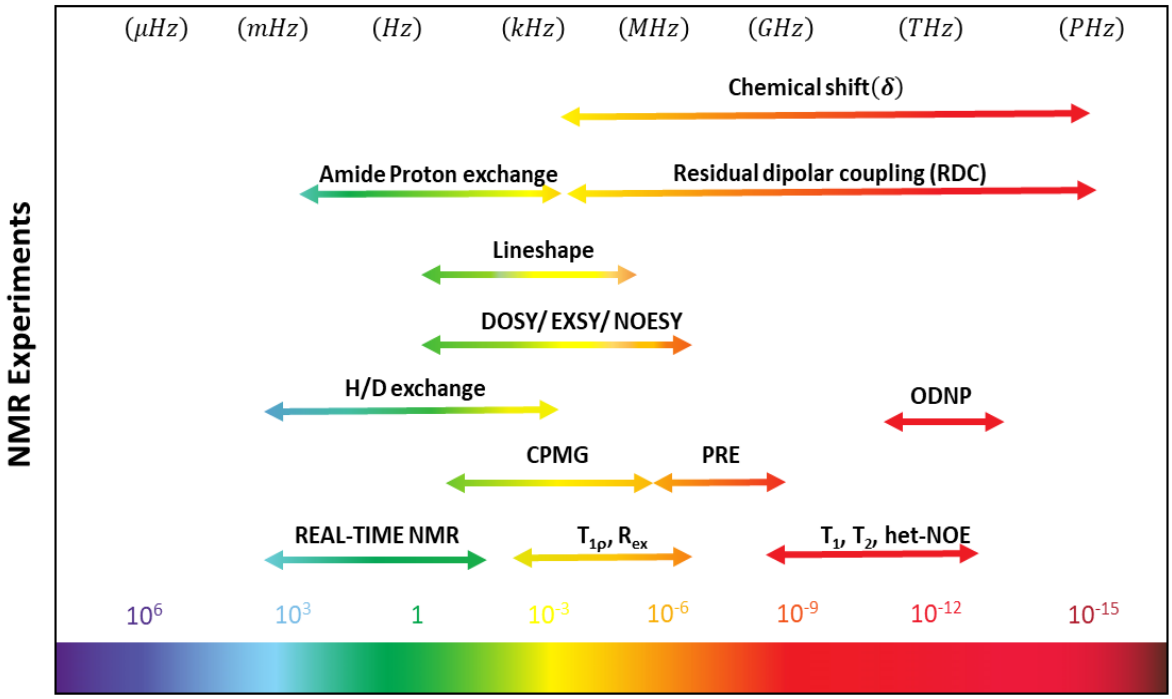

Figure 1.8: Various NMR experiments to probe the molecular dynamics at different NMR timescales.

The currently available solution-state NMR experiments classified as (a) Ligand detected and (b) Target detected NMR methods [Cala et al., 2014; Leung, 2015] offer a broad window for probing molecular interaction and dynamics at various timescales, as shown in figure 1.8 [Vazquez and Gauri, 2019]. In ligand detected methods, the spectrum of the ligand is selectively acquired and compared while titrating with the target and in case of target detected, reverse (target) is monitored. Ligand-based NMR methods are generally easy to handle when compared to protein-based methods. It is defined as an inexpensive technique as it requires a lower magnetic field, typically with micromolar concentrations of the target. On the other hand, target-based methods require the acquisition to be made at the higher magnetic field for excellent resolution of the complex spectrum. The spectral acquisition times are longer as these are generally multi-dimensional experiments. In order to get improved detection sensitivity and reduce spectral complexity, targets (*i.e.*, proteins, macro, or supramolecular systems) labeling are required with NMR active isotopes ^{19}F, ^{13}C, and ^{15}N, etc. [Danielson and Falke, 1996]. In the case of macromolecules, the monitored parameters are generally limited, typically to chemical shifts. For small molecules, the choice of NMR parameters is more diverse [Dalvit, 2007].

The present Thesis employs solution-state ligand-based NMR methods to gain useful insights about three specific molecular interactions, namely ligand-protein binding interaction, host-guest encapsulation, and solute-solvent interactions. Chemical shift, relaxation, diffusion experiments, intermolecular and intramolecular magnetization transfer (MT), namely, nuclear Overhauser effect (NOE), saturation transfer difference (STD), water LOGSY (water ligand observed through gradient spectroscopy), SALMON (solvent accessibility, ligand binding, and mapping of ligand orientation by NMR Spectroscopy), INPHARMA (inter-ligand NOE for pharmacophore mapping), etc. are the various extensively used solution-state ligand-based NMR experiments proposed in the literature to probe the aforementioned molecular interactions [Angulo et al., 2010; Cala et al., 2014; Fielding, 2003, 2007; Fisher, 2014; Krishnan, 2005; Ludwig and Guenther, 2009; Unione et al., 2014]. A representative pictorial scheme depicting the effect of ligand-target intermolecular binding interaction on a few ligand-based NMR parameters is shown in figure 1.9 [Tengel, 2008].

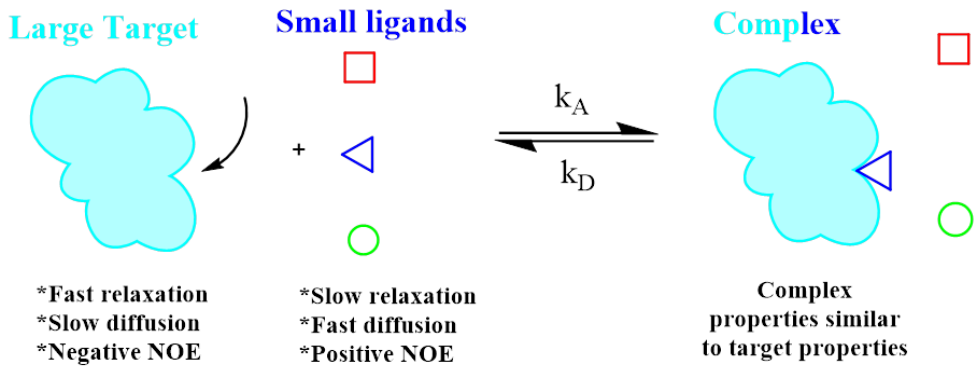

Figure 1.9: Fate of small molecule NMR parameters upon interacting with large target molecules during equilibrium binding process.

Apart from these established ligand-based NMR methods, application of Dynamic Nuclear Polarization (DNP) in combination with solid and solution-state NMR has gained attention as an emerging tool for studying interactions between different molecules in recent past [Du et al., 2008; Thankamony et al., 2017; Zhang and Hilty, 2018]. In solution-state, the enhancement offered by DNP reduces the requirement of sample concentration to sub-micromolar range, mitigating the solubility problems of ligands. These coupled experiments also overcome the long averaging time required in high-resolution NMR and enable to study fast non-equilibrium processes. Various research groups headed by *viz.*, Prof. Christain Hilty, Prof. Geoffrey Bodenhausen, Prof. Lucio Frydman and many others have employed use of Dissolution-DNP (DDNP) to investigate protein-ligand interaction, protein structure and conformational dynamics employing multidimensional high resolution NMR of protein (water soluble specifically) through hyperpolarized solvents [Chappuis et al., 2015; Kadeřávek et al., 2018; Kurzbach et al., 2017; Olsen et al., 2016; Szekely et al., 2018; Vuichoud et al., 2016]. On the other hand, Prof. Songi Han and co-workers have utilized Overhauser DNP (ODNP) methods to decipher hydration water dynamics associated with several biomolecules including proteins, polymers, poloxmers, lipid membrane vesicles, *etc.* [Armstrong and Han, 2009; Armstrong et al., 2009; Cheng et al., 2012; Franck et al., 2013a, 2013b; Kaminker et al., 2015; Lingwood, et al., 2010a, 2010b]. In India, Prof. Chandrakumar and co-workers have worked on sensitivity enhancement and characterized dynamics of ionic liquids and fluorinated solvents employing low field ODNP experiments [Banerjee et al., 2016, 2019; Dey et al., 2017; George and Chandrakumar, 2014]. All the above mentioned ligand-based NMR methods in the solid and solution-state along with the application of ODNP methods are viable tools for ^{19}F NMR spectroscopy.

^{19}F NMR has emerged as an exceptionally sensitive tool to probe molecular and microenvironmental changes for fluorinated systems both in solution and in solid-state [Yu et al., 2013]. A brief overview of the advantages and limitations of the ^{19}F ligand and target-based NMR methodologies compared to other screening techniques have already been reported in the literature [Dalvit, 2007]. Moreover, ^{19}F NMR coupled with ^{19}F hyperpolarization (DNP) offers an immensely powerful combination to understand the dynamics associated with intermolecular interactions of fluorinated systems over a time window upto 1000 ps range [Y. Kim and Hilty, 2015]. A comprehensive list of ligand-based ^{19}F MR techniques to probe intermolecular interactions relevant for the present Thesis is documented in table 1.2. Further, a detailed description of the specific ^{19}F based NMR methods employed in the current Thesis based on relaxation, diffusion, and magnetization transfer along with the effect of intermolecular interactions on these parameters have been provided in Chapter 2 highlighting the special features relevant for ^{19}F.

Table 1.2: A brief literature report on various ^{19}F NMR methods probing intermolecular interactions.

^{19}F Methods	Applications	References
^{19}F Chemical shift and linewidth	Chemical structure and environment, binding site determination, competition binding, fragment based drug discovery, stoichiometry	[Dalvit and Vulpetti, 2011; Jenkins, 1990, 1991; Kitamura et al., 2004; Kitamura et al., 2007; Norton et al., 2016; Restu, 1989; Shi et al., 2011; Wilson and Verrall, 1998; Xing et al., 2007; Xu et al., 1996]
^{19}F Chemical shift Anisotropy (CSA)	Cross-correlation rate (CCR), CSA contribution, structure parameter, probing binding, fragment based drug discovery, CSA-based affinity ranking of fluorinated ligands	[Alemany et al., 2012; Crassous and Hediger, 2003; Dorai and Kumar, 2001, 2001; Elavarasi and Dorai, 2010; Grace and Kumar, 1995; Kumar et al., 2000; Norton et al., 2016; Peng, 2001; Rüdisser et al., 2020]
^{19}F Relaxation (T_1 and T_2)	Molecular mobility, molecular correlation time (τ_c), dissociation (K_D) or association constant (K_A), exchange rate (K_{ex}), residence time (τ_{res}) / lifetime of the complex	[Buratto et al., 2016; Chaubey and Pal, 2018; Dixon et al., 1999; Dubois and Evers, 1992; Kumar et al., 2003; Lee et al., 2012; Lin 2016; Shi et al., 2011; Šmejkalová et al., 2009]
^{19}F FAXS (Fluorine Chemical Shift anisotropy and exchange for fluorine) ; ^{19}F FABS (Fluorine Atoms for Biochemical Screening)	High throughput screening, lead optimization, specific hits for the target, screening for potential drugs and inhibitors for enzymes	[Dalvit, 2007; Dalvit et al., 2005; Dalvit, et al., 2003; Dalvit and Vulpetti, 2012; Fernandez and Jahnke, 2004; Lingel et al., 2020; Miller et al., 2010; Papeo et al., 2007; Veronesi and Dalvit, 2008]
^{19}F Diffusion	NMR signals are separated based on self-diffusion coefficient in mixtures, hydrodynamic radius, impurity profiling, determination of K_D, K_A, bound fraction (P_b), aggregation, disaggregation	[Dalvit and Vulpetti, 2012; Derrick et al., 2002; Longstaffe et al., 2016; Mathias et al., 2010; Mistry et al., 1999; Nyugen et al., 2016; Power et al., 2016; Zhuang et al., 2013a, 2013b]
^{19}F–^{1}H Saturation Transfer Difference (STD) NMR	K_D determination, epitope mapping, binding modes for ligand and target, mixture screening	[Furihata and Tashiro, 2018; Furihata et al., 2017; Longstaffe and Simpson, 2011; Longstaffe et al., 2010; Ribeiro et al., 2015; Sakuma et al., 2015]
^{19}F–^{1}H HOE, ^{19}F–^{19}F NOE	Preferential solvation, Cross relaxation rate, spatial Binding information, internuclear distance, chemical exchange	[Chatterjee and Gerig, 2006; Dixon et al., 1999; Dolores and Berger, 2001; Gerig 2004; Guerrero-Martínez et al., 2006; Neuman Jr and Gerig, 2009; Shikii et al., 2004; Weiss-Errico, et al., 2017]
^{19}F Hyperpolarization [Dynamic Nuclear Polarization (DNP)]	Hyperpolarization coupled with the above mentioned experiments to gain similar information with high sensitivity for a reduced ligand target concentration, water dynamics, dynamics of bonding	[Banerjee et al., 2016; Borah and Bates, 1981; Chappuis et al., 2015; George and Chandrakumar, 2014; Kim and Hilty, 2015; Kim et al., 2018; Kuhn et al., 2006; Kurzbach et al., 2017; Lee et al., 2012; Olsen et al., 2016; Zhang and Hilty, 2018]

Table 1.2 demonstrates the strength of modern ^{19}F NMR analysing molecular interactions in the solution-state. In the following section, applications of ^{19}F NMR are reviewed for the similar types of molecular systems studied in the Thesis.

1.5 ^{19}F NMR ADDRESSING SPECIFIC INTERACTIONS UNDER INVESTIGATION

A brief literature survey on the importance of assessing molecular interactions specifically for the relevant systems investigated in the current Thesis employing ^{19}F NMR methods have been presented in the subsequent sections:

1.5.1 Ligand-Protein interactions in chemical biology: Application of ^{19}F NMR

The non-covalent binding interactions between protein and low molecular weight ligand are essential to analyse as they form the molecular basis of many necessary biological processes [Bohm and Klebe, 1996]. These interactions substantially affect the ADME of the ligand and the physiological behavior of interacting proteins [Copeland, 2003]. Determination of 3D structure of proteins/oligopeptides and investigation of their structural transition are exciting fields of research. Exploring protein/peptide folding-unfolding kinetics in the presence of foreign interaction partners or as a function of various physical and chemical properties of the solution reveal information related to conformational stability and biological function of the macromolecule [Fioroni et al., 2002; Kemple et al., 1997]. These kinds of studies are essential to be taken under consideration as they can provide useful insights in understanding the effect of accumulation of such misfolded proteins in degenerative and neurodegenerative human diseases like Alzheimer's disease, Parkinson's disease, etc. that are prevalent nowadays [Anderson and Webb, 2012; Taubes, 1996]. Protein detected ^{19}F NMR had been found as one of the versatile techniques to provide detailed information about protein structure and dynamics by evaluating in terms of side chains, specific residues, or localized sites [Danielson and Falke, 1996]. While other widely used techniques such as circular dichroism and fluorescence to study protein structure probes the transition in terms of global properties of the protein [Cobb and Murphy, 2009; Enoki et al., 2004]. But this protein detected methods necessitate the incorporation of ^{19}F isotopic labels into proteins. Labeling can be successfully achieved either via biosynthetic incorporation using microorganism/ synthetic ^{19}F-labelled amino acids or by using a fluorinated reagent that can chemically modify the existing cysteine amino acids residues of the protein [Frieden et al., 2004; Hattori et al., 2017]. Several excellent review articles cover the applications of ^{19}F NMR spectroscopy employed to investigate protein-ligand interactions through labelled proteins [Dalvit, 2007].

Ligand-based NMR methods for screening of small organofluorine fragments have now been recognized as one of the most powerful and reliable tools employed for the identification of potential candidates (lead) to be used as drug and agrochemicals. These methods have high applicability in lead optimization, hit validation, and hit find. ^{19}F NMR's foremost ability to detect even weak intermolecular interactions between a ligand and a target can provide valuable suggestions for building Structure-Activity relationship. There is a large body of literature on the use of ligand detected ^{19}F NMR to study protein-ligand interactions in *in vitro* conditions [Fielding, 2007]. Dalvit et al., have worked on developing widely used ^{19}F ligand detected screening methods *i.e.*, FAXS and FABS, for high throughput screening besides theoretical analysis of ^{19}F competition based experiments quantifying and expressing relationships between equilibrium parameters [Dalvit, 2007; Dalvit et al., 2003; Papeo et al., 2007; Stockman and Dalvit, 2002]. ^{19}F detected chemical shifts [Dalvit and Vulpetti, 2011; Kitamura et al., 2004, 2007; Restu, 1989], relaxation [Buratto et al., 2016; Gerig, et al., 1977; Gerig and Stock, 1975; Lee et al., 2012], molecular diffusion [Derrick et al., 2002; Zhuang et al., 2013a, 2013b] and magnetization transfer based effects, *i.e.*, STD [Derrick et al., 2002; Longstaffe and Simpson, 2011; Ribeiro et al., 2015; Zhuang et al., 2013a, 2013b] and NOE [Shikii et al., 2004] are extensively used methods to study fluorinated ligand-protein systems for quantitative assessment of ligand

binding in terms of binding constant, understanding kinetics and exchange dynamics [Jenkins, 1990, 1991]. These parameters have proven to be a quick probe of ligand-protein interactions [Chaubey and Pal, 2018]. Cross-relaxation rate (σ_{DNP}) obtained as a fit parameter from D-DNP curves provides spatial information between the two spins and are used as one of the probes of molecular interactions [Min, 2014]. ^{19}F NMR has also gained huge attention towards quantitative *in vivo* detection of various physiological parameters and has been proved useful in *in vivo* metabolism and pharmacokinetics investigations [Cobb and Murphy, 2009; Kadayakkara, et al., 2010; Srinivas, et al., 2007; Yu et al., 2013]. There are several other applications of ^{19}F NMR related to the ligand, or biological target system can be found in the literature [Afonin et al., 2014; Didenko et al., 2014; Frutos et al., 2006; Hoeltzli and Frieden, 1995; Seitz et al., 2015; Zhu et al., 2015].

1.5.2 Host-Guest type interactions in the environment through ^{19}F NMR

The interaction of xenobiotic organic compounds with components of soil at the molecular level is central to their mobilization, transport, accumulation, toxicity, biodegradation, and bioavailability within the environment [Shirzadi et al., 2008a, 2008b; Weissenfels et al., 1992]. Humic Acid (HA) is considered as an operational alkaline soluble or acid insoluble extract of humic substances (HS) present as a principal component of natural organic matter (NOM) [Khalaf et al., 2003; Schnitzer, 1978]. The complex supramolecular nature of HA as a result of the self-assembling of small heterogeneous molecules imparts a profound multi-functionality to these superstructure [Fan et al., 2004; Guetzloff and Rice, 1994; Kögel-Knabner, 2000; Piccolo, 2001; Simpson et al., 2002; Sutton and Sposito, 2005; Varga et al., 2000; Wandruszka, 1998; Wandruszka, 1997; Wershaw, 1994; Zang et al., 2000]. The structure of soil humic fractions, their components, and humic acid domains have been accounted by various researchers employing solution-state NMR, solid-state (^{19}F & ^{13}C) NMR, and ^{1}H & ^{19}F microimaging [Albers and Hansen, 2010; Cardoza et al., 2004; Khalaf et al., 2003; Masoom et al., 2016; Mitchell and Simpson, 2013; Simpson, 2001; Simpson and Song et al., 2007; Simpson and Simpson, et al., 2007; Xu et al., 2019].

^{19}F NMR has emerged as one of the best techniques in assessing the environmental impact of fluorinated agrochemicals [Simpson et al., 2018; Simpson and Simpson, 2014]. Information on inclusion/adsorption of xenobiotics to HA or the degradation of these chemicals in the presence of HA are generally achieved by monitoring relevant NMR parameters. In the literature, humic matter-pollutant association specifically in solution, has been confirmed by monitoring a) the changes in NMR chemical shift and NMR linewidth [Šmejkalová et al., 2009], b) relaxation time [Chien et al., 1998], c) molecular diffusion coefficient [Šmejkalová and Piccolo, 2008b], d) magnetization transfer due to nuclear Overhauser effect such as $^{19}F–^{1}H$ NOE [Dixon et al., 1999], STD [Longstaffe and Simpson, 2011; Longstaffe et al., 2010] to name a few. NMR diffusion and relaxation analysis have allowed quantification of binding parameters. The magnetization transfer-based methods such as $^{19}F–^{1}H$ or $^{1}H–^{1}H$ NOE, and ^{19}F or ^{1}H observe STD enables identification of favorable sites of contaminant interactions unambiguously. RHSTD (reverse heteronuclear STD) has emerged as one of the interesting methods that provide information about the average molecular orientation of organofluorine molecule on interaction with HA. RHSTD talks about binding mode from the target side. Hence, it can be said as an experimental inversion of the conventional STD experiment, which gives information from the ligand side only [Longstaffe and Simpson, 2011; Longstaffe et al., 2010; Longstaffe, 2013].

Moreover, agrochemicals are generally designed in a way that they remain adhered to crops. Therefore, they are usually hydrophobic with limited water solubility. It causes the natural accumulation of these agrochemicals on the soil for long enough to affect soil's productivity due to interaction with valuable soil nutrients [Flaherty et al., 2013]. Removal of accumulated toxic pesticides from fertile topsoil is, therefore, one of the major concerns nowadays. In the recent past, apart from HA, cyclodextrins (CDs) have been successfully used to extract numerous commonly used pesticides from contaminated soil via forming inclusion

complexes with them. The formation of complex modifies the chemical and physical properties of agrochemicals [Luo et al., 2003; Morillo and Villaverde, 2017; Perez-Martınez et al., 2000; Villaverde et al., 2004]. Relevant information on the stoichiometry and association constant through Jobs Plot [Hirose, 2001] can be obtained from NMR chemical shift or linewidth measurements. Stability, affinity, and the mode of entry of the fluorinated guest into the host for these inclusion complexes are extracted through ^{19}F NMR diffusion, relaxation, and NOE measurements. These kinds of studies can help in the development of appropriate pesticide-CD formulations and can be of great interest in toxicology as it can account for the toxic effects of these agrochemicals within the environment [Lucas-Abellan et al., 2008; Weiss-Errico et al., 2017].

1.5.3 Solute-solvent interactions: Solvation dynamics using ^{19}F NMR

Solvents affect nearly all chemical, physical and biological properties of a molecular species, *i.e.*, reactivity, solubility, partitioning, structure, conformational transition and biological activity etc. Analysing the solute-solvent interactions in terms of solvation phenomena is critically important towards deciphering molecular recognition, molecular encapsulation, and affinity of complex formation in various biophysical and biochemical systems [Guerrero-Martínez et al., 2006]. Studies in a mixture of solvents (co-solvents) provide a promising tool giving new insights into solvation phenomena for various systems of interest in terms of preferential solvation [Cabot and Hunter, 2012]. Solvation phenomena have developed a significant interest among chemists and biologists as it plays a key role in many areas of biochemistry [Silva et al., 2008]. Understanding the structure, stability, function, and dynamics of biomolecules always remained an enticing subject for various research groups [Takis et al., 2013]. Besides different physical parameters of a solution (pH, temperature, viscosity), the solvent composition also plays a vital role in deciding biomolecules' biological function [Buck, 1998; Chatterjee and Gerig, 2006]. In general, biological systems are mainly studied in aqueous environments. However, several proteins/peptides do interact with lipid membranes in the case of the formation of transmembrane channels [Naumenkova et al., 2010]. Water/alcohol co-solvent mixtures are a suitable model to mimic the hydrophobic/hydrophilic character of a lipid membrane surface and simulate cellular conditions [Anderson and Webb, 2012; Fioroni et al., 2002]. Therefore, 'cosolvent engineering' involving studies in the presence of this kind of mixtures have become valuable tools for deciphering biomolecular structure, function, and dynamics [Buck, 1998; Chatterjee and Gerig, 2006; Wescott and Klibanov, 1994].

There are several ^{19}F NMR reports in the literature where interactions of the fluorinated alcohols with small proteins and peptide have been monitored to provide useful insights into solvation dynamics. $^{1}H–^{19}F$/ $^{1}H–^{1}H$ NOE is the commonly used NMR method to comment on preferential solvation of peptides (*viz.* Bombesin, Melittin, polypeptide, trp-cage peptide etc.) in the presence of fluorinated alcohols like trifluoroethanol (TFE) and hexafluoro-2-propanol [Cammers-goodwin et al., 1996; Carver and Collins, 1990; Chatterjee and Gerig, 2006]. 2D NOE experiments are used to quantify cross-relaxation rates (σ_{HF}) arising from dipolar interactions among protons of peptide and fluorine of alcohol in the solvent mixture, indicating solute-solvent interaction [Gerig, 2004; Neuman Jr and Gerig, 2009]. Extraction of hydrodynamic radius for tetrapeptide in the presence of TFE from ^{19}F diffusion experiments, probing side chain and main chain dynamics of mellitin by relaxation measurements are other reported literature on solute-solvent solvation interaction dynamics [Díaz and Berger, 2001; Fioroni et al., 2002; Kemple et al., 1997]. ^{19}F NMR solvent detected magnetic dispersion relaxation studies have been reported for the structural transition of lactoglobulin in the presence of TFE [Kumar et al., 2003; Shiraki, et al., 1995]. The coupling factor obtained from the DNP experiment has also been employed to study the solvation in terms of water dynamics in and over the surface of the proteins/peptides [Armstrong and Han, 2009]. Similar, multifield and multinuclear NMR measurements based on relaxation and intermolecular homo or heteronuclear NOE have emerged as one of the widely employed methods to investigate selective solvent-solute

interactions (also known as "solvent sorting") for carbohydrates and their derivatives (*viz*., glucose, cyclodextrin (CD) etc.) [Angulo and Berger, 2004; Gerig, 2004; Guerrero-Martínez et al., 2006; Mayer, 2002; Sabadini et al., 2008]. Cosolvents are found to play a vital role in CD solvation, significantly influencing the inclusion reaction mechanisms and stability of the complexes [Boonyarattanakalin et al., 2015; Guerrero-Martínez et al., 2006; Sabadini et al., 2008]. Previous ^{19}F NMR studies suggest that the addition of third component, *i.e.*, TFE enhances the apparent association strength between CD-host complexes as it can act as a capable spacer in the complex formed by removing water from the CD cavity [Elliott et al., 1993].

1.6 SCOPE OF THE THESIS

The potential of ^{19}F NMR methods in solution and solid-state chemistry has been foreseen long ago. However, the lack of sufficient instrumental development has always constrained the expansion of ^{19}F MR applications in diverse areas to the fullest. Modern state-of-the-art spectrometers have widened the scope of ^{19}F MR methods addressing distinct facets of solutions and solid-state chemistry. The present Thesis aims to expand the utility of ^{19}F ligand detected NMR methods in unravelling the molecular interaction between organofluorine molecules (solvents, drugs, agrochemicals, and their metabolites) with various targets of biological and environmental interest (protein, peptide, carbohydrates, cyclodextrin, and humic acid) in solutions under *in vitro* conditions. The Thesis particularly emphasizes the exploitation of 1D ^{19}F NMR methods at high and low fields to demonstrate the qualitative and quantitative picture of the intermolecular binding processes and solvation dynamics. ^{1}H NMR, ^{2}H (deuterium) NMR and ODNP experiments are employed to enable additional findings on the solvation and binding events whenever required. Circular Dichroism (CD) and UV-vis spectroscopy are also used as supplementary spectroscopic techniques revealing both qualitative and quantitative data pertaining to the specific molecular systems investigated in the current Thesis. Figure 1.10 highlights a brief schematic representation of the methods used in the present Thesis unravelling the molecular interactions of interest.

Qualitative analysis of the interaction processes, namely ligand-protein binding, host-guest encapsulation, and solute-solvent interaction, has been accomplished by monitoring changes in ^{19}F chemical shift and line width. Such qualitative preliminary data are used to predict the likelihood of interaction among the molecules under investigation. Further, ^{19}F NMR experiments based on ^{19}F spin-lattice and spin-spin relaxation, diffusion and magnetization transfer have been employed systematically to unveil the said interaction quantitatively. Both kinetic and thermodynamic parameters relevant to the specific molecular interaction under investigation have been reported; binding constant, bound fraction, association and dissociation constants, exchange rates, ligand-protein complex lifetime as kinetic parameters while free energy of interaction as thermodynamic parameters have been reported to evaluate the dynamical properties of a fluorinated ligand during such interactions. Besides the application of these established ligand-based NMR methods, the present Thesis benchmarks the applicability of low field ^{19}F relaxation data in combination with low field DNP for the first time for analysing peptide structural transition using the solvent dynamical behaviour. Low field ^{19}F ODNP experiments in the presence of paramagnetic species allowed a different independent window to access motions in the timescale of 10-1000 ps and supported the findings of ^{19}F low field relaxation while investigating solvent dynamics around peptides.

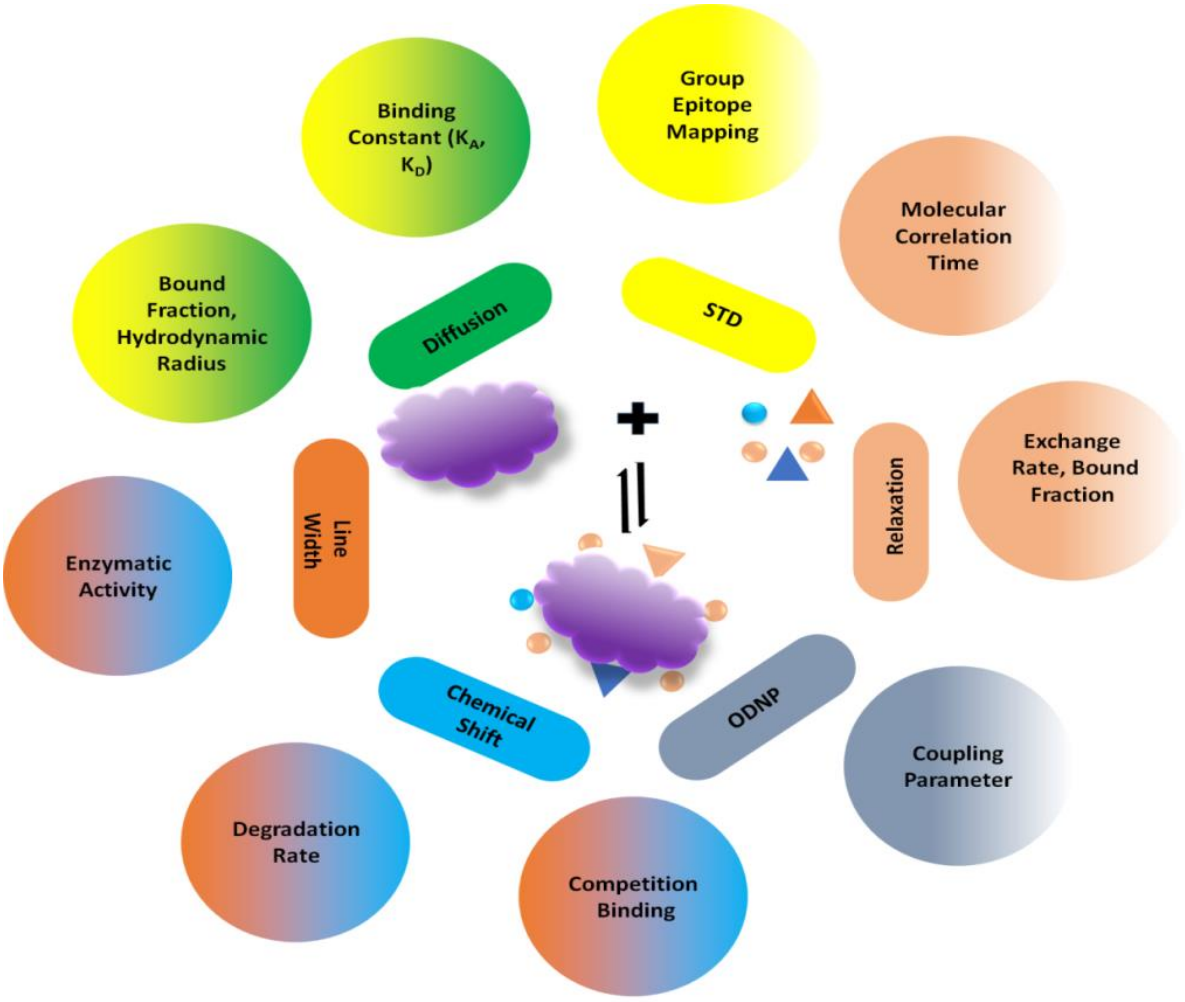

Figure 1.10: Schematic representations of the NMR methods and related parameters discussed in the Thesis to unravel intermolecular binding and solvation dynamics. (*Cloud represents the target; triangle and circle represent the binding ligand and solvent, respectively*.)

Throughout the Thesis, 1D ^{19}F NMR methods are presented with an understanding that these 1D methods are equally reliable and robust when compared to 2D experiments. Moreover, these 1D methods are easily implemented and inexpensive compared to several 2D experiments projecting their fair chance of acceptability in the area of molecular interactions involving fluorochemicals. The first part of the present Thesis highlights the pros and cons of some of the established 1D ligand based ^{19}F NMR methods exploiting relaxation and diffusion to analyse ligand-protein interaction in solution. The next part of the Thesis brings forth one of the important molecular system involving NOM where simple 1D ^{19}F NMR analysis reveals exciting information regarding the photo-degradation behaviour of a fluorinated agrochemical. In the last part of the Thesis, the focus is to benchmark a novel and more sensitive approach to decipher the behaviour of fluorinated co-solvent in terms of solvation dynamics by employing low field ^{19}F MR (relaxation and ODNP) and high field ^{2}H NMR relaxation methods directly monitoring the solvent side as a substitute of traditional NOE experiments occasionally employed to investigate the said problem. Table 1.3 lists the various molecular ligand-target systems investigated in the current Thesis.

Table 1.3: List of investigated ligand-target Pair in the current Thesis.

S.No.	Small molecules (Organofluorine ligands)	Target
(a)	**Ligand**	**Protein**
1.	2, 6 Diflourobenzoic acid (DFBA, a metabolite of pesticides like Hexaflumuron, starting fragment)	Bovine serum albumin (BSA, plasma protein)
2.	Diflunisal (DFL, Antipsychotic drug)	Human serum albumin (HSA)
3.	Flupyradifurone (FPD, Insecticide)	Trypsin (enzymatic digestive protein)
4.	Difluoroacetic acid (DFAA, metabolite of FPD)	
5.	2, 6 Diflourobenzoic acid (DFBA)	
6.	Diflunisal (DFL, Antipsychotic drug)	
(b)	***Guest***	***Host***
1.	Hexaflumuron (HFM, Insecticide)	β-Cyclodextrin (β-CD,supramolecule)
2.	2, 6 Diflourobenzoic acid (DFBA)	Humic acid (HA, natural organic matter)
3.	Benzoic acid (BA, non-fluorinated analog of DFBA)	
4.	Flupyradifurone (FPD, Insecticide)	
5.	Difluoroacetic acid (DFA, a metabolite of FPD)	
(c)	***Solvent***	***Solute***
1.	2,2,2 Trifluoroethanol (TFE, fluorinated solvent)	Melittin (Antimicrobial peptide)
2.		β-Cyclodextrin (β-CD, carbohydrate)
3.		Glucose (carbohydrate / sugar)

The Thesis is organized in the following manner:

Chapter 1 *Introduction* of the Thesis presents a brief background on unique physicochemical and NMR properties of fluorine atom that has flourished fluorine chemistry and ^{19}F NMR respectively in various multidisciplinary domains. Further, a short literature review on different ^{19}F NMR methods used over the decades to explore distinct systems has been presented. A comprehensive overview of past literature has also been discussed for the fluorinated (a) ligand-protein (b) host-guest and (c) solute-solvent systems.

Chapter 2 *Experimental Aspects* provides details of the materials used in the present Thesis and offers a brief overview of the experimental and theoretical background of the ^{19}F NMR methods employed to investigate the molecular interactions in a, b and c systems.

Chapter 3 *Quantification of organofluorine-protein interactions* exhibits that a complete set of ligand-based ^{19}F NMR methods is sufficient to provide a detailed quantitative analysis of ligand-protein complex investigated in the current Thesis, *i.e.,* (a) DFBA–BSA (b) DFL–HSA (c) DFBA–Trypsin (d) DFL–Trypsin and (e) DFA–Trypsin. For system (a) and (b), ^{19}F transverse relaxation (T_2) and translational diffusion coefficient (D) of fluorinated molecule in absence and presence of the SA have been measured to quantify the number of binding sites (n), bound fraction (P_b) and the dissociation constants (K_D). ^{19}F constant time fast pulsing CPMG experiments are performed to extract the exchange rate (K_{ex}) between the free and bound state of the fluorinated molecules employing a two-site exchange model. The lifetime/residence time of the test molecule-protein complex are further evaluated. Similar diffusion and relaxation measurements are done for system (c) to (e) to evaluate the binding.

Chapter 4 *Interactions of fluorinated agrochemicals with humic materials* covers the use of ^{19}F and ^{1}H NMR methods to investigate intermolecular interactions between organic xenobiotic compounds and HA (humic acid, NOM), *i.e.*, (a) DFBA–HA (b) BA–HA (c) FPD–HA and (d) DFA–HA. Binding strength in terms of association constant (K_A) was determined using suitable adsorption isotherms. P_b, hydrodynamic radius (r_H), molecular correlation time (τ_c), and thermodynamic parameters *viz.*, free energy ($\Delta G°$) etc. are also determined. The effect of fluorine on binding mechanism is compared for different fluorinated molecules or on corresponding non-fluorinated analog. Factors affecting the degradation of insecticide (FPD) and photo-degradation mechanism are evaluated; the corresponding photo-degradation rate and half-life are quantified. The effect of HA extracted from soil of arid region on photo-degradation of FPD has also been monitored.

Chapter 5 *Solvent detected NMR approach for the assessment of solute-solvent interactions* sheds light on the dynamical behavior of cosolvent TFE: water in the absence and presence of peptide MLT. Low field measurements enabled direct extraction of ^{19}F molecular correlation time (τ_c) from ^{19}F longitudinal relaxation time (T_1) and coupling factor from ^{19}F ODNP experiments. These low field measurements provided a simple alternative and more sensitive approach to get a better knowledge of solvent dynamics inducing conformation transitions in MLT. On a similar note, the preferential solvation of carbohydrates (β-CD and glucose) is also investigated in TFE: D_2O cosolvent mixture to benchmark a unique combination of ^{2}H NMR and ^{19}F NMR based relaxation measurements deciphering solute-solvent interactions. A direct determination of τ_c from ^{2}H T_1 and low field ^{19}F T_1 simplified the analysis of the specific problem of interest.

Chapter 6 *Summary* provides an overall brief conclusion and future aspects of the research work followed by references.

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