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A Floquet description of phase alternated sequences for efficient homonuclear recoupling in solid perdeuterated systems

Sundaresan Jayanthi [a,1], Ümit Akbey [b,*,1], Boran Uluca [c], Hartmut Oschkinat [b], Shimon Vega [a,*]

[a] Department of Chemical Physics, Weizmann Institute of Science, Rehovot 76100, Israel
[b] Department NMR-supported Structural Biology, Leibniz-Institut für Molekulare Pharmakologie (FMP), Robert-Rössle-Str. 10, D 13125 Berlin, Germany
[c] Department of Chemistry, Fatih University, Istanbul, Turkey

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ABSTRACT

A Floquet description of a phase alternated homonuclear recoupling scheme for perdeuterated systems is presented. As a result, we demonstrate improvements in the recoupling efficiency of the **DO**uble **N**ucleus **E**nhanced **R**ecoupling [**DONER**; J. Am. Chem. Soc. 131 (2009) 17054] technique by utilizing **P**hase **A**lternated **R**ecoupling **I**rradiation **S**chemes [**PARIS**; Chem. Phys. Lett. 469 (2009) 342]. The effect of proton and deuterium radio frequency irradiation during recoupling has been systematically studied and theoretical observations have been verified experimentally using a deuterated model compound, L-Alanine, at 10 and 20 kHz magic angle spinning frequency. Experimental results are well in agreement with theoretical observations, thereby significantly increasing the recoupling efficiency of conventional DONER in perdeuterated systems.

1. Introduction

The recent decade has witnessed the utility of solid state NMR to determine structure and dynamics of peptides and proteins in membranes, amyloid fibrils, etc. at an atomic resolution. Various technological developments have enriched the studies of complex biological systems and their aggregates in micro crystalline and non-crystalline forms [1–6]. The interactions in solids are anisotropic which leads to severe line broadening and hence reduces resolution and sensitivity. For high spectral quality and dispersion magic angle spinning (MAS) is usually applied to remove anisotropic interactions. By MAS, interactions which carry important structural information, e.g., internuclear distances are lost in the pursuit of resolution and sensitivity. As a result, dipolar recoupling techniques that selectively reintroduce these couplings were developed and are now necessary components in structural studies of solid samples.

^{13}C–^{13}C homonuclear dipolar interactions can be recoupled in various ways, whereby the abundant protons in the system can be integrated into the recoupling realm, via the ^{1}H–^{13}C heteronuclear dipolar interaction, without or with radio frequency (RF) irradiation on the protons. Since these latter interactions can become an order of magnitude larger than the ^{13}C–^{13}C interactions, the first approach can be very effective leading to the **P**roton **D**riven **S**pin **D**iffusion (PDSD) mechanism promoting homonuclear recoupling [7]. The second approach will be the subject of this publication.

Rotational **R**esonance (R^2) [8–10] is yet another recoupling scheme that restores homonuclear dipolar couplings of interacting 'like' spin pairs, directly and without proton assistance. This scheme becomes effective when the value of spinning frequency ω_r matches the isotropic resonance frequency difference Δ^{CS} of the coupled spins, according to $\Delta^{CS} = n\omega_r$; where $n = \pm 1, \pm 2$. During R^2 recoupling of ^{13}C–^{13}C interactions the protons are in general strongly irradiated eliminating the heteronuclear dipolar couplings.

By applying simple sequences of RF pulses on coupled spin pairs synchronously with the sample spinning, their homonuclear dipole–dipole couplings can also actively be recoupled during MAS experiments. **D**ipolar **R**ecovery **A**t the **M**agic **A**ngle (DRAMA) [11,12] initiated the development of many recoupling sequences thereafter. It was shown in the early 90s that a series of rotor synchronized π-pulses can reintroduce the "flip-flop" part of the homonuclear dipolar interaction. Termed as radio-frequency driven recoupling (RFDR) [13], this sequence has been used frequently to record correlation spectra of biological systems. Several important recoupling sequences were developed utilizing RF irradiation on the coupled spins such as double quantum **HO**monuclea**R** **RO**tary **R**esonance (HORROR) [14], **D**ipolar **R**ecoupling **E**nhanced by **A**mplitude **M**odulation (DREAM) [15], symmetry based sequences such as POST-C7 [16], SPC5 [17] and stochastic recoupling [18]. Most of these have been reviewed extensively in the literature [19,20].

Yet another RF irradiation approach was introduced to recouple the interactions between heteronuclear spins. An example of this is the **R**otary **R**esonance **R**ecoupling (R^3) scheme [21], where an RF

* Corresponding authors.
E-mail addresses: akbey@fmp-berlin.de (Ü. Akbey), Shimon.Vega@weizmann.ac.il (S. Vega).

[1] These authors contributed equally to this work.

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field is applied to one of the spin types with an intensity satisfying the condition $\omega_1 = n\omega_r$ with $n = 1, 2$. This scheme has been employed to efficiently correlate isotropic chemical shifts with the chemical shift anisotropies in spinning solids [22]. Due to its narrow recoupling condition, this sequence has not been used much on biological samples at high frequency MAS conditions (high-MAS). On the other hand **R**otational **E**cho **D**ouble **R**esonance (REDOR) type of recoupling is extensively used for heteronuclear distance measurements in a large variety of samples [23–26].

Takegoshi et al. and Zilm et al. introduced a variant of the R^3 approach in order to recouple ^{13}C–^{13}C homonuclear spins via proton irradiation by exploiting heteronuclear dipolar couplings between these carbon spins and the protons in a sample. Termed as **D**ipolar **A**ssisted **R**otational **R**esonance (DARR) [27] or **R**adio frequency **A**ssisted **D**iffusion (RAD) [28] recoupling, respectively, the underlying mechanism of this recoupling scheme is basically an interference effect between the heteronuclear dipolar interactions and the applied RF field. Thus this sequence utilizes the ^{13}C–^{1}H couplings for recoupling ^{13}C–^{13}C spin pairs. Since the RF irradiation applied to the protons in this scheme is of low intensity, long recoupling mixing times, up to hundreds of milliseconds, are feasible without undesired sample heating effects, thus enabling long-range correlation measurements. With these moderate power levels, RAD/DARR has been used as a powerful tool for structural determination of proteins [29–31]. Unfortunately, the recoupling efficiency of this sequence declines at high-MAS due to narrowing of the recoupling conditions. Most of the recoupling techniques including those mentioned above become less robust at high-MAS and high magnetic fields. The reasons for this are the extensive averaging of the anisotropic dipolar interactions by MAS and the large isotropic chemical shift dispersions caused by the magnetic field. Most of the RF dependent recoupling sequences are sensitive to RF inhomogeneities as well. Various modifications of the homonuclear recoupling sequences for improving their recoupling performance, in particular in the case of 2D ^{13}C–^{13}C correlation spectroscopy, by incorporation of phase alternations at high-MAS has been demonstrated in the literature [32–36].

Weingarth et al. introduced similar phase alternation schemes during RAD/DARR irradiation for promoting efficient and uniform recoupling at high-MAS and high magnetic fields [37]. Their basic **P**hase **A**lternated **R**ecoupling **I**rradiation **S**cheme (PARIS) utilizes an RF field with moderate amplitudes, applied to the protons. The phase of the RF field is reversed from (+x) to ($-x$) after a pulse duration τ_p. They observed that such a phase alternation promotes efficient recoupling at low RF amplitudes without fulfilling any well-defined rotary resonance condition when τ_p is set either to $\tau_r/2$ or $2\tau_r$, where τ_r is the rotor period. At the above mentioned conditions, they demonstrated that PARIS performs more efficiently than RAD/DARR at sample spinning frequencies between 30 to 60 kHz. Further modifications of the sequence, utilizing a four-phase alternation scheme during two or more rotor periods were introduced later by the same group ($PARIS_{X,Y;m=1}$; $PARIS_{X,Y;m=2}$) [38]. These modified sequences achieved magnetization transfer over a broad range of chemical shifts at low RF amplitudes, high-MAS and high magnetic fields. A new four-phase modulation scheme, named SHANGHAI (**S**econd order **H**amiltonian among **A**nalogous **N**uclei **G**enerated by **H**eteronuclear **A**ssisted **I**rradiation) [39], was also introduced for ^{13}C–^{13}C recoupling at high-MAS, and its amplitude modulated supercycled version (SHA+) [40] showed better performance than the variants of PARIS. These modified sequences were developed and tested on spin-half systems as shown in the literature [41–43].

In biological NMR, perdeuterated proteins are often used for achieving improved resolution and sensitivity. Moreover, significant reduction in the required RF strengths for proton decoupling helps to retain sample integrity. Unfortunately, most of the proton-mediated recoupling techniques do not work efficiently in these systems due to the absence of sufficient ^{13}C–^{1}H dipolar interactions. Akbey et al. addressed this issue by introducing a novel recoupling strategy that utilizes the abundant deuterons by simultaneous irradiation of deuterium ($I = 1$) and proton spins to enhance carbon–carbon recoupling, termed as **DO**uble **N**ucleus **E**nhanced **R**ecoupling (DONER) [44]. They demonstrated that highly sensitive carbon–carbon correlation spectra of proteins can be obtained for MAS frequencies around 10 kHz. Following this, Leskes et al. systematically studied the underlying recoupling mechanism for DARR and DONER employing the Floquet theory [45]. A comparative study using various recoupling sequences for obtaining high quality homonuclear 2D correlation spectra of perdeuterated microcrystalline Ubiquitin has recently been performed by Huang et al. [46]. A recent study of Akbey et al. depicts various experimental aspects of DONER and ways to achieve efficient coherence transfer in perdeuterated proteins with different proton/deuteron ratios. They observed long range correlations between carbon spins spatially separated by about ~ 6 Å [47].

In the present work, we reconsider the DONER approach and apply phase-modulated RF schemes, as introduced by Weingarth et al., for carbon–carbon recoupling via simultaneous deuterium and proton irradiation on highly deuterated systems. In Section 2, we discuss the proton mediated homonuclear recoupling mechanism underlying the RAD/DARR experiment using Floquet theory. At first, we consider the recoupling efficiency of a cw RF irradiation on the protons and introduce a recoupling efficiency coefficient and a powder representation of the efficiency. These efficiencies are simulated for a three-spin system and the results are shown. Then this approach is extended to investigate the influence of phase-modulated RF fields on the recoupling efficiencies. A similar discussion is presented in Section 3 for deuterium mediated RAD/DARR recoupling experiments. In Section 4, the combined proton and deuterium RF irradiation is described and presented for a four-spin system. Although parts of the derivations necessary for an understanding of the DONER experiment are already published [45], we shortly repeat here some of them in order to set the stage for the understanding of the phase-modulated recoupling versions.

The theoretical descriptions are followed by experimental demonstrations in Section 5. Applications of these phase-alternated DONER sequences to partially deuterated L-Alanine [U-$^{13}C/^{15}N$/2, 3-d_4] are presented here. The new recoupling schemes employ proton and deuterium irradiation modulated according to $PARIS_{X,-X}$, $PARIS_{X,Y;m=1}$ and $PARIS_{X,Y;m=2}$.

Experiments were done at 10 and 20 kHz MAS frequencies with recoupling mixing times of 100 and 200 ms, respectively. To dissect the proton and deuteron contributions, we systematically implemented the new sequences into 2D experiments as (i) only proton mediated, (ii) only deuterium mediated and (iii) both proton and deuterium mediated ^{13}C–^{13}C recoupling. The intensities of cross peaks correlating $^{13}C_\alpha$–$^{13}C_\beta$, $^{13}C_\beta$–^{13}CO and $^{13}C_\alpha$–^{13}CO are systematically analyzed for all three phase modulation cases. These experiments show that the DONER efficiency can be increased by a factor of two by phase alternation. Section 5 concludes with detailed comparisons of the various irradiation schemes.

2. Proton mediated RAD/DARR recoupling

2.1. Continuous wave RF irradiation

2.1.1. The Floquet spin Hamiltonian

Floquet theory has been used to describe the spin dynamics and to derive theoretical insights on the mechanisms of recoupling in the present study due to its numerous merits. A variety of mag-