Research Article

Magnetic Resonance in Chemistry

Received: 24 June 2010 Revised: 21 July 2010 Accepted: 25 July 2010 Published online in Wiley Online Library: 5 September 2010

(wileyonlinelibrary.com) DOI 10.1002/mrc.2675

A capacitively coupled temperature-jump arrangement for high-resolution biomolecular NMR

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A simple design for performing rapid temperature jumps within a high-resolution nuclear magnetic resonance (NMR) setting is presented and exemplified. The design is based on mounting, around a conventional NMR glass tube, an inductive radiofrequency (RF) irradiation coil that is suitably tuned by a resonant circuit and is driven by one of the NMR's console high-power RF amplifiers. The electric fields generated by this coil's thin metal strips can lead to a fast and efficient heating of the sample, amounting to temperature jumps of ≈20 °C in well within a second – particularly in the presence of lossy dielectric media like those provided by physiological buffers. Moreover, when wound around a 4-mm NMR tube, the resulting device fits a conventional 5-mm inverse probe and is wholly compatible with the field homogeneities and sensitivities expected for high-resolution biomolecular NMR conditions. The performance characteristics of this new system were tested using saline solutions, as well as on a lyotropic liquid crystal capable of undergoing nematic → isotropic transitions in the neighborhood of ambient temperature. These settings were then incorporated into the performance of a new kind of single-scan 2D NMR spectroscopy acquisition, correlating the anisotropic and isotropic patterns elicited by solutes dissolved in such liquid-crystalline systems, before and after a sudden temperature jump occurring during an intervening mixing period. **Copyright © 2010 John Wiley & Sons, Ltd.**

Keywords: temperature-jump; capacitive coupling; biomolecular NMR; ultrafast 2D NMR; isotropic–anisotropic correlations

Introduction

Nuclear magnetic resonance (NMR) is one of the foremost spectroscopy tools available to analyze chemical structures and dynamics.[1,2] NMR also provides a potentially unique window to follow chemical reactions and transformations at a site-resolved level. NMR can elucidate not only equilibrium molecular structures and steady-state dynamics[3] but also inquire about transient states created upon shifting a chemical system away from its normal thermodynamic equilibrium.[4,5] Such transient dynamic studies will often require shifting a chemical environment into a transient state that is out of thermodynamic balance, as well as collecting NMR data in real-time while focusing on one or many observables such as couplings, shifts, relaxation rates or diffusivities. In order to rely on such approaches while reaping the full benefits that NMR spectroscopy can disclose about transient changes and chemical processes, several issues need to be addressed. Foremost among these is a need to minimize the 'deadtime' delay that may originate between the triggering of the off-equilibrium dynamic process, and the actual beginning of the NMR data acquisition. As many interesting chemical features may arise during this blind time delay, making it as short as possible is essential in order to fully characterize the initial stages of a reaction. One of the usual precautions taken in order to reduce such time loses to a minimum, includes initiating the chemical reaction or dynamics '*in situ*'; i.e. when the sample has already been placed within the NMR magnet and data is ready for collection. This is often a challenging issue, as the fast triggering of off-equilibrium processes is usually associated with suboptimal NMR acquisition conditions – particularly in terms of NMR's high demands on field homogeneity and sample uniformity. An additional aspect that needs to be considered in this kind of measurements concerns the actual requirements of the NMR experiment itself; particularly the timescales of the NMR protocol being used to fully characterize the reaction details. Two-dimensional (2D) NMR, in particular, presents an unusual challenge to real-time dynamic measurements: although this kind of spectroscopy can provide a high chemical site resolution and yield important insights regarding the dynamics of different sites in the targeted molecule, it also involves intrinsically time-consuming acquisitions demanding relatively long periods regardless of sensitivity considerations. This is a consequence of 2D NMR's acquisition schemes, requiring the collection of a minimal number of scans to satisfy the Nyquist criteria along the indirect domain.

It follows from these arguments that compromise on the kind of experiments that may be assayed and processes that may be triggered, may limit NMR's ability to carry out an optimal characterization of off-equilibrium dynamic processes. The first of these challenges has led, during the last few years, to the development of a variety of alternatives capable of accomplishing multidimensional NMR acquisitions on much shorter timescales than what are usual on traditional experiments.[6–9] These variants have included proposals to shorten the recycle delays, the use

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of sparse indirect-domain sampling schemes, and non-Fourier-based methods of analysis. Among the different proposals made in this respect stand the so-called 'ultrafast' spectroscopy methods, capable of delivering full 2D (and higher dimensional) NMR spectra within a single scan, and without the need for *a priori* spectral knowledge.[10–12] Moreover, combination of the spatial encoding principles underlying this ultrafast detection with many of the other time-compressing variants, may lead to a substantial increase in the dynamic rates that can nowadays be assayed by real-time multidimensional NMR experiments.[13,14] Important obstacles, however, remain to be overcome by the second of the mentioned challenges – particularly when trying to impose fast kinetic transitions while retaining the usual advantages of NMR spectroscopy. One possible way of doing so involves rapidly mixing reactants inside the main magnet at – or nearby – the NMR coil where the experiment's detection will take place;[5] a number of designs for efficiently implementing these processes have been recently discussed.[15,16] An alternative way often used to trigger dynamic processes involves the application of sudden temperature changes or *T-jumps*.[17–23] Sudden heating (or less often, cooling) is capable of triggering substantial molecular rearrangements like the unfolding of biomolecules or the initiation of chemical reactions; potential advantages of choosing this route over mixing-triggered processes include avoiding a need to change the sample volume, of diluting the sample upon initiating the dynamics, or risking the introduction of air bubbles or other major turbulent heterogeneities which may perturb shimming. Thanks to all these factors, the probehead can be pretuned and the magnetic field preshimmed to acceptable conditions prior to the data acquisition, when the sample is already placed inside the magnet. Also, the process may be reversed and brought back to equilibrium just by cooling the chemical system after acquisition ends. Several different schemes to apply such a rapid *T*-jump to an NMR sample have been discussed in the literature. Heating with a CO_2 laser pulse was an early approach demonstrated on solid samples; this is a high-power deposition experiment that has been used in a variety of NMR test cases.[18,19,23] Still, applying this approach to the H_2O-based samples typical in most liquid-state biomolecular NMR experiments may be complicated, owing to penetration depth limitations and/or to the fine-tuning heating demands associated to this kind of samples. A different alternative, which has been proven in liquid-state biomolecular NMR settings, involves the microwave-based irradiation of the sample.[20,21] This is a very powerful and flexible technique that can also achieve high-deposition powers and uniform heating; it demands, however, the insertion of a relatively bulky (≈10-mm diameter) dielectric resonator into the NMR probe – something which is not simple in a narrow-bore high-resolution liquids setting.

A third main approach to achieve a rapid temperature jump involves relying on the inductive heating which may arise from RF irradiation – a kind of radiation whose source is naturally available in any NMR system. This approach has also been described many times in the literature;[18,22] here, we present our experience based on the development of an affordable, easy-to-build equipment designed for the sake of performing rapid *T-jumps* in biomolecular-oriented liquid-state NMR experiments. This device operates by the addition of a resonant circuit that is capacitively coupled to the sample, and is facilitated by the dielectric losses typical in aqueous-buffered solutions – an approach that is also currently under investigation by Schwalbe, Engelke and coworkers (personal communication). This paper describes some basic features of this new kind of rapid heating device for initiating physicochemical transformations *in situ*. The operation of the device is exemplified in combination with ultrafast NMR's ability to deliver 2D NMR spectra within a single transient, toward the development of novel kind of 2D NMR spectra correlating interactions in different (isotropic/anisotropic) phases.

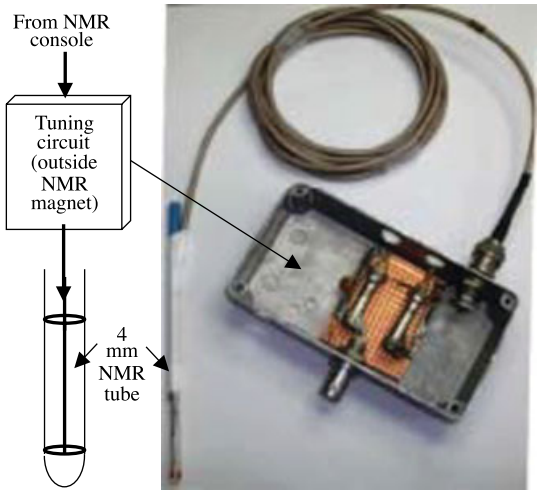

Figure 1. Scheme and illustration of a typical setup assayed and employed for the *T*-jump experiments described in this study, incorporating a matching circuit and a capacitor whose plates have been wound around a commercial 4-mm NMR glass tube.

Experimental Section

All experiments reported in this work were carried out on a Varian Inova 500 MHz NMR spectrometer. This machine was interfaced to a Bruker TXI indirect-detection probe equipped with a standard, shielded *z*-gradient, for which a variety of custom-built *T-jump* NMR inserts were constructed to execute the experiments in this report. The final design of the system that we adopted to allow a rapid heating of the sample using inductive RF field effects, is schematized and shown in Fig. 1. This setup was constructed around standard, commercially available 4-mm NMR tubes (Wilmad); around these tubes were wound a pair of thin, upper and lower copper strips, acting as the opposing plates of a capacitor. These elements were placed outside the normal observation region of the NMR coil: *ca* 2.5 cm were thus left free from the susceptibility disturbances and potential B_1 distortions arising from the copper foil, allowing the NMR observations to happen under high-resolution conditions. Such an arrangement led to a more efficient heating and to better line shapes than alternatives which were also assayed, involving the silver electroplating of 4- and 5-mm NMR tubes with different annular and cylindrical patterns. It also alleviated temperature gradients. Moreover, the copper strips illustrated in Fig. 1 were chosen thin enough to endow the overall resulting assembly with a $\leq$5-mm outer diameter, thus enabling the positioning and use of the resulting tube inside a conventional high-resolution 5-mm NMR probe.

Sudden temperature changes are facilitated by the copper strips arranged in this specific way, owing to two concurrent reasons. The main of these is that the annuli will, to a large extent, behave as plates of a capacitor. They can therefore produce an AC electric field along the sample's main axis that will, through dielectric losses and ensuing motion of ions and/or molecules,

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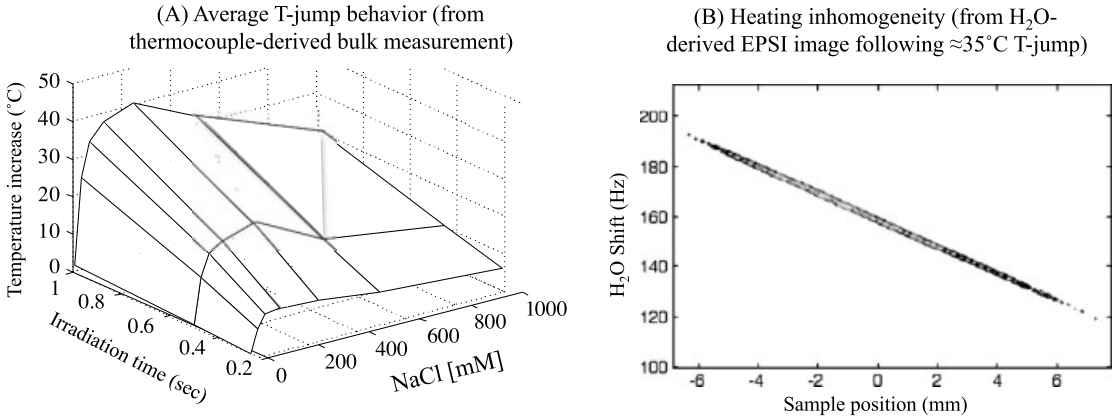

Figure 2. Heating efficiency (A) and temperature heterogeneity (B) resulting from the setup depicted in Fig. 1. For convenience, the points illustrated in (A) were gathered from thermocouple-based measurements performed outside the actual NMR magnet, which allowed us a more flexible operation of the various solutions and irradiation times assayed. Still, similar behaviors could be observed from NMR measurements based on temperature-dependent chemical shift effects on the water resonance. The frequency shifts exhibited in (B) result from an EPSI axial imaging measurement, and amount to ≈6 °C spreads over the sample volume.

lead to a rapid heating of the solvent's bulk. The magnitude of the heating arising in an aqueous sample will thus depend on a variety of factors including the amplitude of the electric field being generated, its duration as well as on the solution's conductivity. In order to maximize the first of these parameters, the impedance of the *ad hoc* copper coil was tuned and matched, using an external tank circuit based on the usual impedance tuning principles.[24] The timing and amplitude of capacitive heating pulses were fed into the resulting tune/matched circuit by a high-power amplifier using one of the spectrometer's RF channels, driven in turn by the usual commands of the spectrometer's pulse programmer. Small, nonmonotonic dependencies of the heating efficiency on the actual tuning frequency of the resulting circuit could be observed within the 20–100 MHz range assayed; given the availability of a broadband (American Microwave Technologies 1 kW) amplifier to drive this auxiliary channel and of tunable high-power filters, no special precautions were thus taken to choose a precise frequency for the heating irradiation – other than making sure it would not coincide with one of the Larmor frequencies involved in the actual NMR experiment.

A second factor influencing the sample's temperature, stemmed from the heating that the copper strips themselves will undergo when current passes through them. Though local, these heating effects may be significant and can contribute to an undesirable temperature gradient across the sample. Minimizing the latter was also a consideration upon deciding where to place the heating plates vis-à-vis the main location of the NMR detection coil.

With these considerations at hand, we turned to examine the performance of the resulting setup. All chemicals used in these tests were purchased from Sigma–Aldrich and used as received. Further descriptions of the experimental results observed are given in the following sections.

Results

As mentioned, the main mechanism contributing to sample heating in the scheme introduced in Fig. 1 stems from the sample's dielectric losses upon subjecting it to the RF field. Figure 2A summarizes this behavior, as well as the system's overall performance characteristics, in a 3D plot illustrating the changes in temperature measured for aqueous saline solutions as a function of salt concentration and sample irradiation time, for a constant power of ≈60 W. Dielectric-driven losses are clearly evidenced by the strong differences in the temperature jumps that could be read from these shift changes, as imparted by identical irradiation fields as a function of a solution's varying saline concentrations. Notice, in particular, that for the ionic strengths typical of physiological solutions (≈100 mM), temperature jumps of tens °C can be achieved using RF irradiation pulses lasting only fractions of a second, while relying on commonly available RF power levels. Despite this efficient performance, the system was found to exhibit a modest behavior regarding its thermal homogeneity. This could be deduced from the increasing line widths exhibited by the water resonance as a function of the sample's *T*-jump,[25] and was quantified from a series of single-scan echo-planar spectroscopic imaging experiments[26] reporting the water's shift displacement as a function of its position in the sample. Particularly large profiles were found upon measuring the shift's dependence along the sample's main axis (Fig. 2B); we ascribe this spatial variability to the local heating effects imparted by the copper strips themselves, as the applied currents run through them.

In order to probe the spectroscopic possibilities that could be opened by the simple setup illustrated in Fig. 1, we decided to use it for exploring real-time anisotropic/isotropic phase transitions in liquid-crystalline phases. In particular, we sought to explore a water-soluble lyotropic system, where biomolecules could be codissolved under nearly physiological conditions and for whom phase transitions between isotropic and anisotropic phases would not arise too far away from physiologically relevant temperatures. A system that can fulfill these multiple aims is provided by the disodium cromoglycate (DSCG) salts. From both chemical and biological perspectives, cromoglycates present quite an unusual array of behaviors.[27,28] These chemicals were first isolated and identified from Middle Eastern herbs, known and used since ancient times, thanks to their muscle-relaxing and bronchodilating properties.[29] Today DSCGs are commercially widely available, and commonly prescribed as nonsteroidal antiasthmatic agents. Owing to their flexible but semiflat charged structures, these molecules can arrange in a

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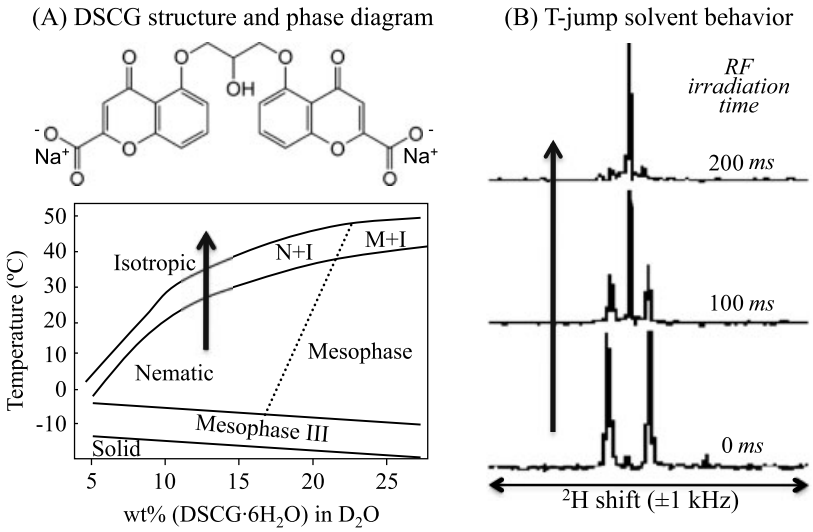

Figure 3. (A) Schematic phase diagram characterizing solutions of sodium cromoglycate (DSCG, structure on top) in water (adapted from Ref. [30]). (B) Spectral changes evidenced by the ^{2}H resonance arising from a 12.5% solution of DSCG dissolved in deuterated water, cooled to ≈10 °C and then subject to a 50-W RF capacitive heating over the indicated irradiation times, using the equipment introduced in Fig. 1. The vertical arrow indicates the approximate range of the heating, taking the sample from a purely nematic region to a region of nematic + isotropic coexistence and onward to a mostly isotropic solution.

variety of different manners when placed in solutions, including in liquid-crystalline, nematic arrangements.[28] Moreover, owing to the double charges in their molecules, sodium salts of these aggregates are highly water soluble despite their aromatic nature. Water-based DSCG liquid crystals have a well-defined phase-transition behavior, with the temperatures defining their passage from an ordered lyotropic to an isotropic phase depending on the solute's concentration. Figure 3A schematizes the phase-transition diagram of DSCG dissolved in H_2O as a function of its temperature and concentration.[30] Roughly speaking, below an 18% w/v concentration, sample heating will transform DSCG solutions from an initial nematic phase to a fully isotropic one, with a transition region of anisotropic/isotropic coexistence in the neighborhood of ambient temperature.

In view of all these properties – water solubility, clearly different properties in the neighborhood of room temperature and high ionic strength – we chose to further examine the ability of the capacitively coupled device to perform rapid temperature jumps, using a ≈12% w/v DSCG aqueous solution as test case. These changes were explored by focusing on the ^{2}H NMR spectrum arising upon dissolving DSCG in heavy water, which can provide a clear reporter for the actual phase of the solution via the quadrupolar splitting that it may exhibit. Figure 3B shows a representative series of ^{2}H NMR spectra recorded upon heating this sample, starting from a temperature that has been stabilized at 10 °C using the spectrometer's variable-temperature system, and subject to different heating times and for a fixed (≈50 W) RF irradiation power. A quadrupole-derived doublet with a ≈500-Hz splitting is seen at low temperature, which clearly transitions through a nematic/isotropic coexistence region and onward to a fully isotropic phase as the temperature increases by *ca* 30 °C. This confirms that not only can rapid heating be achieved but also the availability of a certain level of temperature control associated to different RF irradiation strengths/durations.

The availability of a *T*-jump device compatible with high-resolution observations, and of a 'switchable' liquid-crystalline phase compatible with aqueous biomolecular experiments, could

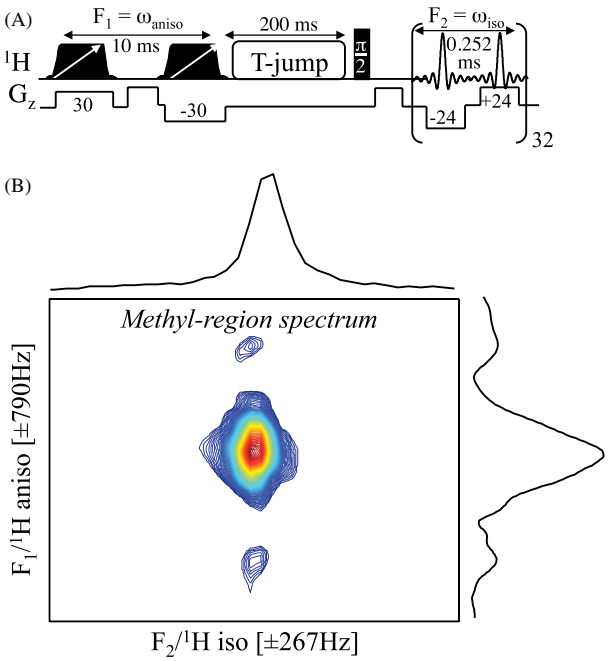

Figure 4. Single-scan 2D NMR sequence (A) and ensuing experimental results (B) used to assay the new opportunities opened by the *T*-jump device introduced in Fig. 1. The pulse sequence involved the timing (ms) and gradient (G/cm) parameters illustrated in (A). The two arrowed trapezoids acting over the F_1 indirect domain represent swept $\pi/2$ pulses (see Ref. [32] for further details); small coherence selection gradients were also applied as indicated to clean up the single-scan results. Spectral data are shown in magnitude mode.

open up new opportunities in the inquiry of molecular properties by NMR. As example of this we have began to explore new correlation experiments, where these capabilities are combined with the new opportunities opened up by single-scan 2D NMR acquisitions. In particular, we conclude this discussion with preliminary results obtained from the ultrafast experiment

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illustrated in Fig. 4A, which aims at correlating, in a 2D fashion, the NMR resonance frequencies arising when nuclei situated in a nematic phase are suddenly transported into an isotropic environment. This experiment proceeded by dissolving the targeted analyte in a DSCG solution at a temperature characteristic of the latter's nematic phase, encoding the analyte's spin evolution under these anisotropic conditions along the indirect domain of a 2D experiment, storing this spin evolution along a long-lived longitudinal axis while the sample was rapidly heated over the course of an ensuing mixing period so as to originate a rapid nematic $\rightarrow$ isotropic phase transition, and complete the measurement of the isotropic frequencies along the 2D direct domain. The result is then the characterization of informative but relatively poorly resolved dipolar couplings along the indirect spectral axis, with the high resolution typical of isotropic phases along the direct domain. The two ingredients lying at the heart of this scheme are the application of rapid heating during a relatively short mixing period during which the sample changes phases, and the use of ultrafast 2D NMR approaches which allow one to carry such experiment in a convenient single-scan fashion. This is by contrast to what would be involved in the mapping of such 2D correlations using conventional NMR, where a series of independent scans would have to be acquired and in-between each of these scans the system would have to cool down below a stable transition temperature before a renewed acquisition becomes possible.[21,31] Also important is the fact that the nematogenic molecules themselves do not yield observable signal due to their much stronger dipolar couplings, thus enabling the realization of ^{1}H-based experiments.

Figure 4B illustrates the results arising from such anisotropic/isotropic ultrafast 2D NMR approach. The spatial encoding underlying this sequence relied on two consecutive $\pi/2$ chirp RF pulses sweeping in the presence of opposite magnetic field gradients.[32] During this real-time t_1 encoding period, the temperature was kept at $\approx$12 °C using the spectrometer's variable-temperature system, which stabilized the lyotropic below the nematic $\rightarrow$ isotropic phase-transition point. Thereafter, following the storage of the spatially encoded evolution, the capacitively coupled irradiation was applied to increase the sample's temperature by $\approx$30 °C and beyond the isotropic transition temperature on a subsecond timescale; this heating rate was fast even when compared to the protons' relatively short T_1 times. The direct-domain isotropic dimension was then monitored while under the action of oscillating magnetic field gradients, to afford the full isotropic/anisotropic 2D correlation sought. As a first trial, we focused such experiment on the ^{1}H NMR response arising from the methyl group of alanine, dissolved at a $\approx$10 mM concentration in a 12 wt% DSCG in D_2O solution. The ensuing 2D ^{1}H spectrum showed a triplet pattern appearing for the group along the indirect dimension, correlated with a narrower single peak along the direct domain. The triplet pattern reflects the intermethyl dipolar interactions among the three intervening protons; a coupling that remains only partially averaged in the ordered nematic phase. Following the rapid temperature jump, however, the fluid becomes a homogeneous isotropic environment leading to a single ^{1}H peak for the methyl moiety.

Discussion and Conclusions

The initial results described in this paper are meant to illustrate the potential that may arise from combining rapid *T*-jump devices, with emerging NMR techniques. The method here described to achieve the sudden heating is a straightforward adaptation of similar concepts described in the literature,[18–22] to the case of 5-mm high-resolution systems. Such sample sizes and probes are applicable and available for a wide variety of conditions, including for biomolecular studies where resolution and sensitivity are always at a premium. Their main technical requirement is the interfacing of a simple homemade coil to one of the spectrometer's RF channels, and, although some of its features like its thermal homogeneity or its operation under low ionic strengths are yet to be optimized, the method presents the advantage of being compatible to virtually any contemporary NMR setup. Further uses of this feature in biomolecular folding studies will be the topic of future analyses.

As part of the new approaches that could be opened by a more widespread use of such systems, this work explored applications involving residual dipolar couplings and new 2D state-correlating NMR methods. In connection to these tests a liquid-crystalline system was identified, that was particularly convenient owing to its reliance on aqueous solutions and its involvement of phase transitions that could be tuned and manipulated within the neighborhood of ambient temperature. These features were exploited in combination with the sudden temperature jump apparatus and with 2D ultrafast NMR, to monitor anisotropic/isotropic 2D ^{1}H correlations in alanine. This is naturally a very simple example, but is indicative of the new opportunities which could be opened by this new combination of approaches to extract more detailed structural and longer range information on biomolecular systems that are currently available. We trust to report further examples of these aspects based on both homo- and heteronuclear correlations in upcoming reports.

Acknowledgements

We are grateful to Prof Zeev Luz (Weizmann Institute) for fruitful discussions about liquid crystals in general and cromoglycates in particular, and to Ms Zohar Noy for assistance in the experiments. This research was supported by the Israel Science Foundation (ISF 447/09), the European Commission (EU-NMR contract No. 026145), a Helen and Kimmel Award for Innovative Investigation and the generosity of the Perlman Family Foundation.

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