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## ARTICLE

## Broadband ultrafast 2D NMR spectroscopy for online monitoring in continuous flow

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Flow NMR is a powerful tool to monitor chemical reactions in realistic conditions. Here we describe ultrafast (UF) 2D NMR schemes that make it possible to acquire broadband homonuclear 2D NMR spectra in 90 seconds or less for a continuously flowing sample. An interleaved acquisition strategy is used to address the spectral width limitation of UF 2D NMR. We show how, for a flowing sample, the use of a transverse axis for spatial encoding makes it possible to achieve the very high scan-to-scan stability required for interleaved acquisitions. We also describe an optimised solvent suppression strategy, that is effective for interleaved acquisition in continuous flow. These developments open the way to online monitoring with flow 2D NMR at high time resolution, as we illustrate with the monitoring of an organocatalysed condensation reaction.

### Introduction

Real-time monitoring is important to understand and control chemical reactions. Reaction monitoring can be used to collect kinetic data and observe intermediate species or side products, and this provides information on reaction mechanisms. Monitoring information is also useful to optimise reaction conditions to, e.g., increase yields or to reduce reaction times.<sup>1</sup> Nuclear magnetic resonance (NMR) spectroscopy has relevant features for monitoring applications, such as the facts that it is non-invasive and non-destructive, and that it can provide extensive structural and quantitative information. It has proven essential for an array of applications in chemical synthesis.<sup>1-3</sup>

Real-time monitoring by NMR can be implemented in different complementary ways.<sup>1-6</sup> The most straightforward approach consists of carrying out the reaction of interest in a standard NMR tube, directly in the spectrometer. However, the resulting reaction conditions are not equivalent to those found in a flask, in particular because the lack of sample stirring influences reaction kinetics significantly.<sup>7</sup> Online monitoring by flow NMR is a powerful approach to exploit the advantages of NMR spectroscopy, for reactions carried out in the conditions of interest. It consists of circulating continuously a fraction of the reaction medium between the batch vessel and the magnet. This allows for sample stirring in the flask, as well as progressive addition of the reagents, which is difficult to do inside an NMR magnet. Using a commercial or custom “flow-tube”, the temperature can be regulated over the entire system.<sup>4</sup> The relevance of flow NMR for reaction monitoring has been

demonstrated with applications in, for example, metal catalysis and photocatalysis.<sup>8-9</sup> Flow NMR also raises specific challenges due to, e.g., the effect of sample flow on quantitation or gradient-based experiments.<sup>2</sup>

The power of NMR spectroscopy lies in large part in the possibility to perform multi-dimensional experiments. 2D experiments are a major tool for the analysis of chemical mixtures, making it possible to address overlap issues that may plague 1D spectra. They also provide crucial structural information, that is useful for assignment, and is also essential for structure validation and elucidation. Conventional 2D experiments, however, typically have durations of 10 minutes or more and are hardly compatible with real-time monitoring in chemical synthesis. This is an important motivation for the development of fast 2D NMR methods.<sup>10</sup> Among these, ultrafast (UF) 2D NMR based on spatial encoding makes it possible to accelerate significantly the acquisition of 2D NMR spectra.<sup>11-12</sup> UF 2D NMR has been used for real-time reaction monitoring applications such as, e.g., the synthesis of pyrimidines, where it yielded both kinetic and structural information.<sup>13</sup> With the exception of one example using benchtop NMR,<sup>14</sup> all of these applications relied on reactions carried directly in an NMR tube. We have recently shown that the spatial encoding process on which UF 2D NMR is based can be made compatible with online monitoring by flow NMR at high field.<sup>15</sup> The resulting spectra, however, were of modest usefulness, because of the single-scan nature of the experiment. They were notably restricted to a small spectral window (a feature of single-scan UF experiments) and based on a single-line solvent suppression, which significantly limits the possible applications.

In this article, we show that broadband UF 2D COSY spectra can be acquired in continuous flow, and used for online monitoring. We use an interleaved acquisition strategy (iUF), which makes it possible to increase the spectral widths. This

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approach had never been used in flow conditions, and we show that it requires the use of a transverse gradient axis for spatial encoding. We also optimise a multi-line solvent suppression scheme for interleaved acquisitions in continuous flow. The resulting approach yields high-quality spectra, in less than 90 seconds. This is illustrated with the monitoring of an organocatalysed condensation reaction. The flow iUF NMR methodology should be broadly applicable for online monitoring.

## Experimental section

### Reagents

Reagents were all commercially available and were used as received unless otherwise noted.

### Instrumentation

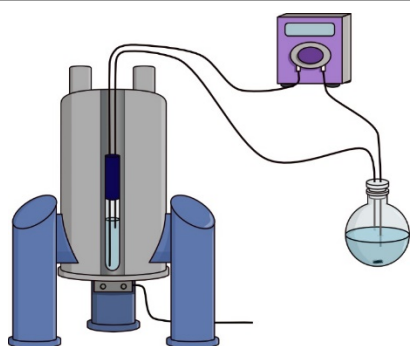


Fig. 1 Schematic representation of the used experimental set-up based on a Flow NMR device combined with a peristaltic pump.

The experimental set-up used is represented in Fig. 1. The commercial flow tube (InsightMR, Bruker) is inserted in a 500 MHz magnet and connected to a peristaltic pump (SF-10, Vapourtec) to circulate a fraction of the reaction mixture between an external flask and the NMR tube. Within the magnet, the solution flows through a modified NMR tube before returning to the reaction flask. The flow tube is based on a 5 mm NMR tube tip, and two 7 m long 0.5 mm I.D. PEEK capillaries that run to and from the tube tip through a thermostatic line. The entire volume including the flow tube, the pump and all connection tubing to connect to the reaction vessel was determined to be 5.4 mL. For each experiment, the pump was set to selected flow rates between 0.0 (static conditions) and 3.0 mL/min.

Traces of isopropanol were present in the flow tube, which is the storing solvent of the flow tube, and thus isopropanol signals were detected in most NMR spectra.

### NMR

**General.** NMR experiments were carried out with a spectrometer operating at a  $^1\text{H}$  Larmor frequency of 500.13 MHz (Bruker, Avance III), and equipped with a broadband inverse-detection probe with triple-axis gradients. NMR experiments were recorded at a temperature of 298 K for all

experiments shown in the main text and the supplementary information.

**Conventional NMR.** The reference conventional COSY spectrum of ethyl crotonate was acquired with the Bruker pulse sequence `cosygmfpqqf`, with 256  $t_1$  increments, and 4 scans on a 400 MHz Bruker Avance Neo spectrometer equipped with liquid-nitrogen-cooled cryogenic probe (5 mm CryoProbe<sup>TM</sup> Prodigy BBFO with ATMA and Z-gradient from Bruker BioSpin). The inter-scan delay was 1.5 s for conventional experiments.

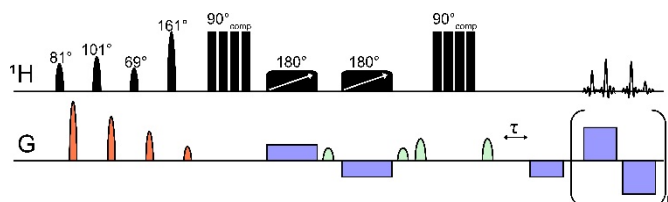


Fig. 2. Ultrafast WET COSY pulse sequence based on the ultrafast COSY combined with a solvent suppression block WET and spatially selective composite pulses. The orange gradients correspond to the WET gradients and combined with the four specific pulses, it composes the WET solvent suppression block. The purple gradients correspond to the encoding and decoding gradients which need to be on the same axis. The encoding block combined the two opposite signs gradients with the two chirp pulses, to allow the encoding of the information. For the decoding step, pairs of gradient pulses are repeated  $N$  times. The green gradients are coherence selection gradients. The  $90^\circ$  pulses used are composite pulses based on four  $90^\circ$  pulses with phases respectively on  $x$ ,  $y$ ,  $-x$ ,  $-y$ , in order to spatially select the central area of the tube where the  $B_1$  is the most homogeneous. The  $\tau$  delay correspond to the interleaving delay required for interleaved scans.

**UF NMR.** UF COSY spectra were acquired using the sequence shown in Fig. 2. The sequence incorporates a WET block and composite pulses for solvent suppression (see the “solvent suppression” section below), and a delay  $\tau$  before the start of acquisition. When  $N$  interleaved scans are acquired, this delay is incremented by  $2T_a/N$  from one scan to the next, where  $T_a$  is the duration of a readout gradient. The acquisition of a small number of “interleaved” scans makes it possible to alleviate the constraint on the spectral width of UF 2D NMR spectra.<sup>11-12</sup> While not single-scan anymore, the resulting 2D experiments are still an order of magnitude (or more) faster than conventional experiments.

As default parameters, chirp pulses with a duration of 15 ms and a sweep range of 25 kHz were applied for spatial encoding. The amplitude of the spatial encoding gradient on Y axis was 4.39 G/cm, the coherence selection gradients around the second chirp pulse were 13.71 G/cm and around the second  $90^\circ$  pulse were 22.28 G/cm. 32 pairs of gradient pulses were used during acquisition, with each gradient pulse lasting 1004  $\mu\text{s}$  with an amplitude of  $\pm 44.48$  G/cm. Eight interleaved scans were used to reach a spectral width up to 7.8 ppm in UF dimension and 8.3 ppm in conventional dimension. One dummy scan was used, for an overall duration of experiment of 64 s with an inter-scan delay of 7 seconds. For optimization, parameters were varied one by one, keeping the others to their default value.

For reaction monitoring, chirp pulses with a duration of 15 ms and a sweep range of 25 kHz were applied for spatial encoding. The amplitude of the spatial encoding gradient on Y axis was 4.23 G/cm, the coherence selection gradients around the second chirp pulse were 13.71 G/cm and around the second

90° pulse were 22.28 G/cm. 40 pairs of gradient pulses were used during the acquisition, with each gradient pulse lasting 1080  $\mu$ s with an amplitude of  $\pm$  44.48 G/cm. Ten interleaved scans were used to reach a spectral width up to 9.3 ppm in UF dimension and 9.0 ppm in conventional dimension. One dummy scan was used, for an overall duration of experiment of 80 s with an inter-scan delay of 7 seconds.

The use of a reduce inter-scan delay limits the recycle delay, and thus imply an incomplete relaxation. This means that the proportionality constant between peak volume and concentration is peak-dependent. However, in the case of 2D experiments, peak-dependence is already present due to the various delays and pulses between excitation and detection. Therefore, calibration would be necessary to perform absolute 2D peak quantification, as it is commonly used in multi-impulsion NMR experiment. Only the relative 2D peak volume variation was studied here.

A two-line WET solvent-suppression block was used to suppress MeOH signals for reaction monitoring and optimization, and a one-line WET solvent-suppression block was used to suppress CHCl<sub>3</sub> signal for optimization. We previously optimized the WET solvent suppression on a 1D spectrum, by dephasing the two solvent signals and optimizing the power of the selective pulses – the powers of all the WET pulses are varied simultaneously to improve solvent suppression, with starting powers described in the literature.<sup>1</sup> Four selective Sinc pulses with one lobe with a duration of 15 ms were used.

#### Data processing

**1D <sup>1</sup>H and conventional COSY spectrum.** In the 1D <sup>1</sup>H, integration was performed using the *MestReNova* software, with the data analysis tool on the stacked spectra for the reaction monitoring.

**iUF 2D COSY.** All processing was performed using a custom MATLAB code. The iUF 2D COSY data were imported in MATLAB, and then rearranged into a 2D matrix. Along the spatial dimension, the data were inverse Fourier transformed, apodised with a Gaussian window, zero-filled and Fourier transformed. Along the spectral dimension, the data were apodised with a sine window, zero-filled and Fourier transformed. Magnitude spectra were used in all cases. For the build-up curves, the integration range of peaks were selected on the first spectrum and were used for integration on the following spectra. A baseline correction was done before integration.

#### Reaction monitoring

**Reaction mixture.** 1,3-Cyclohexanedione (560.5 mg, 5 mmol) was dissolved in 17 mL of MeOH. Then, this solution was flown into the flow tube which is already full of MeOH (correspond to 5.4 mL). In total, the compounds are dissolved/diluted in 22.4 mL. After 15min of stabilization a solution of ethylenediamine in MeOH was added (125  $\mu$ L, 12.5  $\mu$ moles, 0.1 M). After 10 min, citral was added (855  $\mu$ L, 5 mmol).

**General procedure.** The following procedure was used for reaction monitoring. First, as preparation steps, the solution of

1,3-cyclohexanedione in MeOH at 0.22 M was prepared. Then, on the spectrometer, the tuning, locking and shimming were done on a reference tube in MeOD. Finally, isopropanol, which is the storing solvent of the flow tube, was replaced by MeOH by using the peristaltic pump. The flow was stabilized at the targeted value of 1.5 mL/min for 15 minutes. During all this preparation procedure, the flowing solution went directly to the waste flask.

Thereafter, the flow NMR was set-up. In the first place, after removing the reference tube, the flow tube was inserted into the spectrometer. Then the tuning and shimming were done directly on the flow tube which contained MeOH. Finally, the 1,3-cyclohexanedione solution was connected to the set-up and the system was closed to have a loop.

Before running the reaction, some verifications were done on the spectrometer such as the optimization of the 90° pulse and the first recording of an iUF 2D COSY spectrum to verify the quality of the data. Then, 1D <sup>1</sup>H spectra were recorded regularly to follow the stabilization of the system.

When the system is stabilized with the solution of 1,3-cyclohexanedione, a solution of ethylenediamine, which is the catalyst, was added through a syringe into the reaction mixture. In the same time, the monitoring was initiated to record data before the addition of the last reactant. This monitoring was based on the array of experiments described in the following “Monitoring” paragraph. After 10 minutes of NMR recording, the citral solution was added to start the reaction and the monitoring was done for at least 120 minutes.

The temperature was set to 298K throughout the system.

**Monitoring.** The WET block is previously optimized on 1D then the same gradients values are used in the WET block of the iUF 2D COSY.

An array of experiments was repeated before, during and after the reaction, for at least 2 hours. This array is composed of:

1. iUF 2D COSY with WET block to suppress MeOH with spatial encoding on Y-axis
2. 1D <sup>1</sup>H with WET block. With a relaxation delay of 7 seconds. With 4 scans and 2 dummy scans.
3. 1D <sup>1</sup>H with WET block. With a relaxation delay of 45 seconds. With 1 scan.

The first repetition allows to determine the stability of the system, before the addition of the last reactant. Thanks to this array, an iUF 2D COSY spectrum is recorded every 220 seconds.

## Results and discussion

#### Acquisition strategy

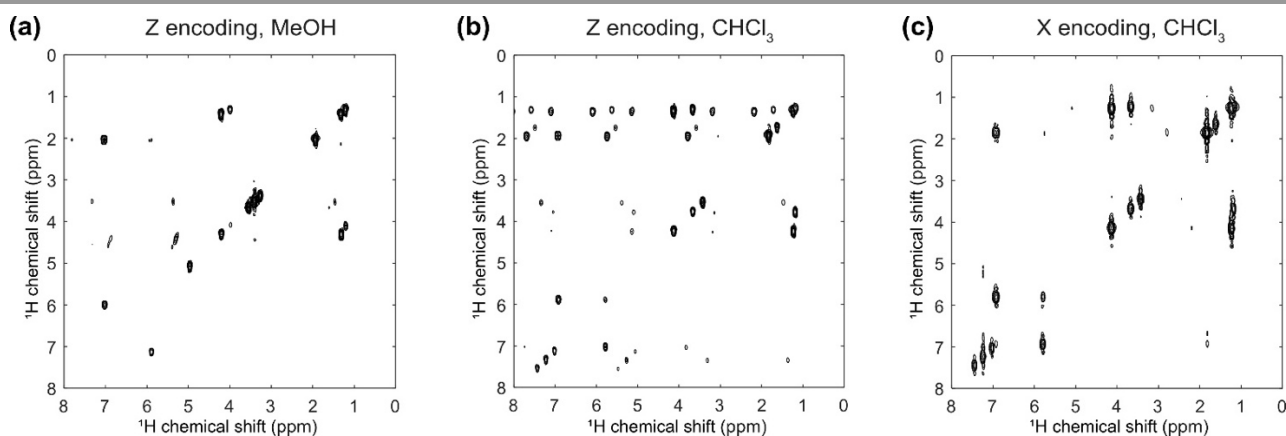
UF 2D NMR provides the remarkable possibility to record a complete 2D data set in a single scan. Such speed, however, comes at the cost of a compromise between the spectral width in each dimension, and the resolution in the indirect dimension. In order to assess the possibility to collect broadband 2D spectra with UF 2D NMR, we chose to study a sample of ethyl crotonate. For this sample, signals span 5.8 ppm, corresponding to 2900 Hz on the 500 MHz spectrometer as the one used here, and this

cannot be covered with a single-scan acquisition. The sample was studied in flow conditions, using a commercial flow tube (InsightMR, Bruker) connected to a peristaltic pump to flow the sample at a desired flowrate between 0 to 3 mL/min, and inserted in the NMR spectrometer, as shown in Fig. 1.

Sample flow, flow fluctuations, and the use of undeuterated solvent make the implementation of flow iUF 2D NMR challenging. Several iUF COSY spectra were recorded for the flowing solution of ethyl crotonate, using a triple-axis gradient probe and the sequence shown in Fig. 2. A selection of spectra is shown in Fig. 3. The spectra shown in Fig. 3a and 3b were obtained by using spatial encoding along the longitudinal (z) axis, which is the standard implementation of UF 2D NMR, in two different fully undeuterated organic solvents. Two significant issues are readily apparent in these spectra, which would make them unreliable or unusable for monitoring applications. The first issue is that signals are attenuated in a way that depends on their chemical-shift in the indirect dimension –signals with higher chemical shifts are more attenuated. For the spectrum shown in Fig. 3a, obtained in methanol, in the extreme case of the diagonal peaks at 5.9 and 7.0 ppm the peaks have simply disappeared. This attenuation is

the result of an interplay between the spatial encoding process and sample motion. This is analogous to the known effects of velocity when gradient pulses are applied,<sup>16</sup> and it is made more complex by the fact that, in UF experiments, the gradient area is effectively position dependent. For broadband spectra, the attenuation effect is even more pronounced.

The second issue is the appearance of “ghost” images of the real NMR peaks. These artefacts arise from the interleaving process when the stability of the sample and instrumentation is not sufficient.<sup>17</sup> In this respect, the spectrum shown in Fig. 3b illustrates a complete failure of the interleaving strategy with z encoding for a flowing sample. The fluctuations of the flow rate induced by the peristaltic pump generate instabilities, to the point that some ghost peaks are as intense as the main peaks. These fluctuations are difficult to predict, and the quantitative description of their effects remains to be addressed. In any case, they result in unusable spectra, as illustrated by Fig. 3b. Empirically, we find that the intensity of these ghost peaks is difficult to predict or rationalise. In this case they were found to be more present when CHCl<sub>3</sub> is the solvent.



**Fig. 3** iUF 2D COSY spectra comparison of (a) Z encoding axis on a sample of ethyl crotonate in MeOH, (b) Z encoding axis on a sample of ethyl crotonate in CHCl<sub>3</sub> and (c) X encoding axis on a sample of ethyl crotonate in CHCl<sub>3</sub>. These spectra were acquired in flow conditions at 1.5 mL/min with a WET solvent suppression. Composite pulses are used for both 90° pulses. The sample is ethyl crotonate in MeOH or CHCl<sub>3</sub> at 0.22 M. Fully undeuterated solvents were used. These experiments were acquired with the pulse sequence described in Fig. 2. The (c) panel correspond to the expected spectrum while panel (a) has missing peaks and panel (b) displays intense ghost peaks. A reference spectrum can be found in Fig. S2.

The spectrum shown in Fig. 3c was obtained using a transverse axis for spatial encoding. Strikingly, this change of the encoding axis provides high-quality spectra, that display all of the peaks and have much reduced (or even absent) ghost peaks. In the flow tube, the main direction of the flow is the longitudinal axis. When an orthogonal axis is used for spatial encoding, the process is much less sensitive to both velocity effects and their fluctuations. Transverse encoding thus opens the possibility to use interleaved acquisitions for a continuously flowing sample and thus record broadband spectra in a very short time. Together with earlier results, these findings illustrate the relevance of triple-axis gradient probes for flow NMR.<sup>15, 18</sup>

An alternative approach to mitigate flow effects on UF 2D spectra would consist of using stopped flow acquisitions. This approach was used recently by Bazzoni *et al.* for the in-line

monitoring of flow photochemical reactions.<sup>19</sup> In that case, stopped flow acquisitions were appropriate, because the solution does not evolve after it exits the flow reactor. However, for the online monitoring of batch reactions, stopped-flow acquisition reduces the rate at which data can be acquired, and may break the assumption that the solution in the flow cell is representative of that in the batch reactor.

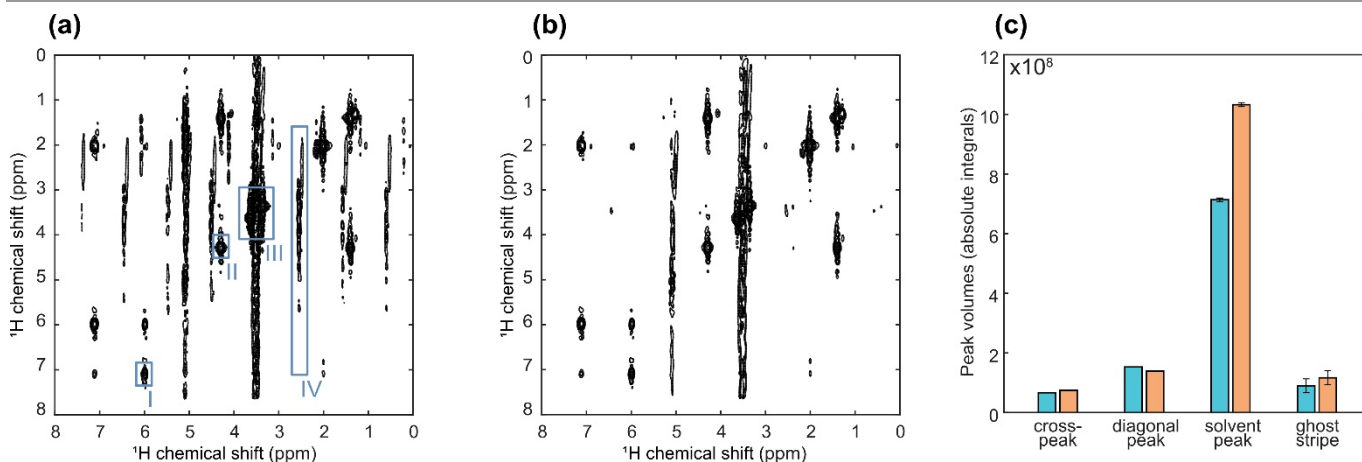
#### Solvent suppression

The results shown in Fig. 3 required high-quality solvent suppression, the development of which will be described in this section. Reaction monitoring by flow NMR is typically performed using undeuterated solvents. This is because using tens of milliliters of deuterated solvents would be prohibitively expensive, but also to keep the reaction under realistic conditions. As a result, efficient methods are needed to

suppress the large solvent signals. Here we chose to use the WET (water suppression enhanced through T1 effects) scheme, as it is known to provide reliable solvent suppression in flow conditions in the 1D case,<sup>20-21</sup> and as it can be used to suppress multiple solvent lines. Since the WET block relies on pulsed-field gradients to dephase the solvent signals, this could interfere with the effect of the magnetic-field gradients used for spatial encoding. The gradient pulses of the WET block were thus applied on an axis that is orthogonal to the spatial encoding axis. In the case of transverse spatial encoding, this leaves two possibilities, that are compared in Fig. 4a-b. When a transverse gradient axis is used for solvent suppression, the undeuterated solvent peaks are poorly suppressed (Fig. 4a). In this case, the ghost images of the vertical stripes due to incomplete solvent suppression are even visible, and can hinder the analysis of the spectrum. In contrast, when the gradient pulses of the WET block are applied along the longitudinal axis, solvent

suppression is much more efficient (Fig. 4b). In this case, velocity effects can further dephase the solvent magnetisation.

The quality of solvent suppression was further improved by replacing the 90° pulses of the COSY pulse sequence by spatially selective composite pulses. These pulses are designed to amplify the effect of B1 inhomogeneity,<sup>22</sup> in order to attenuate the contribution of the edges of the detection region (along z) which are not efficiently suppressed by the WET block. The effect of using these composite pulses is summarised in Fig. 4c which shows a comparison of selected representative peak volumes with and without composite pulses. When using composite pulses, the volume of peaks of interest experience minimal changes (~10%), while the volume of the solvent peak decrease by ca. 40%. Additional parameters were also optimised, as described in the ESI, with, smaller but noticeable effects. The resulting experiment can provide high-quality broadband 2D NMR spectra in just a few scans in continuous flow.



**Fig. 4** iUF 2D COSY spectra comparison of (a) WET gradients axis along X axis and (b) WET gradients axis along Z axis. Both experiments present two composite pulses. The flowrate is 2.5 mL/min in (a) and (b). (c) Plotted peak volume extracted from selected regions (shown in panel (a)), to compare signals obtained without (in orange) and with two (in blue) composite pulses (CP), for peaks of interest (cross (I) and diagonal (II) peaks), ghost stripe (IV) and solvent signal (III). The flowrate is 1.5 mL/min in (c). All spectra were acquired with encoding axis along Y. Note that the peak volume of the ghost stripe has a large error bar, which illustrates the non-repeatability of flow effects, while the error bar for the cross and diagonal peaks are too small to be seen. The sample is ethyl crotonate in MeOH at 0.22 M. Fully undeuterated solvents were used. The experiments in a and b panels were acquired with the pulse sequence described in Fig. 2. The data described in panel c was obtained by employing the same pulse sequence with the presence of two composite pulses (in blue) or by replacing both pulses by simple 90° pulses (in orange). The spectrum of the final optimisation, with both composites and WET gradients axis along Z axis, is represented in panel (b).

### Reaction monitoring

The flow iUF COSY methodology was used to monitor the organocatalyzed condensation of citral with 1,3-cyclohexanedione. The reaction was carried out at 293 K. A 22.4 mL solution was prepared in MeOH, with a concentration of about 220 mM for 1,3-cyclohexanedione and 0.25 mol% for ethylenediamine as catalyst. To initiate the reaction, a citral solution in MeOH is added with a syringe into the medium. The citral and the 1,3-cyclohexanedione are in stoichiometric proportion at the start of the reaction. In these conditions, the initial reaction lifetime is less than 1 hour. The solution was flown at 1.5 mL/min. This leads to a “dead time” of ca 160 seconds between the reaction vessel and the detection region which is small compared to the reaction timescale. Flow iUF COSY spectra were collected using 10 interleaved scans, to achieve a spectral width of 9.0

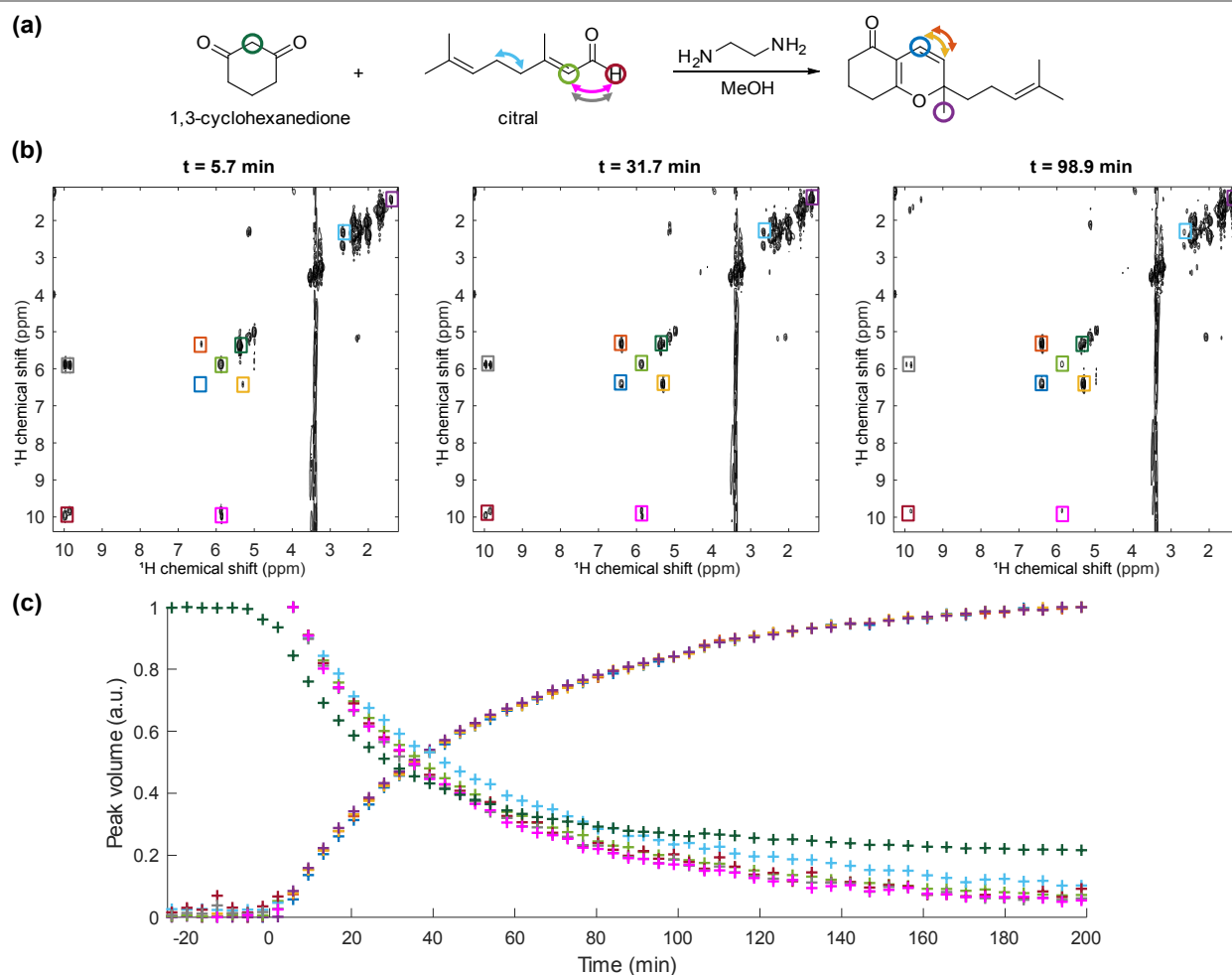
ppm in both dimensions. Each experiment lasted 80 seconds, which yielded a significantly higher time resolution than what would be obtained with conventional 2D NMR. Spectra were collected every ca 220 seconds, interlaced with 1D <sup>1</sup>H experiments. Good quality spectra were obtained and a selection is shown in Fig. 5b. Such broadband iUF spectra are unprecedented in flow conditions.

The resulting monitoring data based on flow iUF COSY is shown in Fig. 5c with the volumes of selected peaks (each normalised to their maximum value) as function of time. Two citral diagonal peaks and three citral cross-peak signals are shown, as well as two product diagonal peaks and two product cross-peaks, and one diagonal peak for 1,3-cyclohexanedione.

The monitoring was started before the addition of citral. When citral is added, the citral signals decrease while the

product signals increase and demonstrate the start of the organocatalysed reaction. The reaction was also monitored by 1D  $^1\text{H}$  experiments, that were interleaved with UF 2D experiments. The resulting spectra and peak areas, shown in Fig. S10-S12 for peaks that are well resolved in the 1D spectra, are in good agreement with the information obtained from the cross peaks of UF 2D NMR spectra (the

integral for one diagonal peak, for cyclohexanedione, suffers from partial overlap with a diagonal signal from the product). The proposed UF 2D COSY method thus makes it possible to reliably monitor the reaction, with spectra acquired in less than 90 seconds that provide extra signal dispersion and correlation information.



**Fig. 5** (a) Reaction scheme of the organocatalyzed condensation of citral with 1,3-cyclohexanedione with peak attributions, (b) 2D iUF COSY spectra measured during the reaction for three different times, at  $t=5.7$  min,  $t=31.7$  min and  $t=98.9$  min, (c) plotted peak volume of some reagents and product correlations as a function of time. The spectra were acquired using spatial encoding along a transverse axis. The flowrate was 1.5 mL/min. The time  $t = 0$  min corresponds to the addition of the last reactant which is the citral.

## Conclusions

We have shown that high-quality broadband 2D spectra with multiple-line solvent suppression can be acquired in just a few scans in continuous flow, thanks to novel flow-compatible iUF 2D NMR pulse sequences. The resulting flow iUF NMR methodology was used to monitor an organocatalysed condensation reaction, and should be applicable to a broad range of reactions that yield complex NMR spectra.

## Author Contributions

All the authors: conceptualisation, methodology, writing – review & editing. CL, MB, JB: investigation, data curation, formal analysis, validation. CL, visualisation, writing – original draft; CL, MB, AB: resources; PG, FXF, JND: funding acquisition, supervision. JND: project administration.

## Conflicts of interest

There are no conflicts to declare.

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22 A. Bax, *J. Magn. Reson.*, 1985, **65**, 142.

## Notes and references

- 1 M. V. Gomez and A. de la Hoz, *Beilstein J Org Chem*, 2017, **13**, 285.
- 2 A. M. R. Hall, J. C. Chouler, A. Codina, P. T. Gierth, J. P. Lowe and U. Hintermair, *Catalysis Science & Technology*, 2016, **6**, 8406.
- 3 Y. Ben-Tal, P. J. Boaler, H. J. A. Dale, R. E. Dooley, N. A. Fohn, Y. Gao, A. Garcia-Dominguez, K. M. Grant, A. M. R. Hall, H. L. D. Hayes, M. M. Kucharski, R. Wei and G. C. Lloyd-Jones, *Prog. Nucl. Magn. Reson. Spectrosc.*, 2022, **129**, 28.
- 4 A. Saib, A. Bara-Estaún, O. J. Harper, D. B. G. Berry, I. A. Thomlinson, R. Broomfield-Tagg, J. P. Lowe, C. L. Lyall and U. Hintermair, *Reaction Chemistry & Engineering*, 2021, **6**, 1548.
- 5 D. A. Foley, E. Bez, A. Codina, K. L. Colson, M. Fey, R. Krull, D. Piroli, M. T. Zell and B. L. Marquez, *Anal. Chem.*, 2014, **86**, 12008.
- 6 M. Khajeh, M. A. Bernstein and G. A. Morris, *Magn. Reson. Chem.*, 2010, **48**, 516.
- 7 D. A. Foley, A. L. Dunn and M. T. Zell, *Magn. Reson. Chem.*, 2016, **54**, 451.
- 8 A. M. R. Hall, R. Broomfield-Tagg, M. Camilleri, D. R. Carbery, A. Codina, D. T. E. Whittaker, S. Coombes, J. P. Lowe and U. Hintermair, *Chem. Commun.*, 2017, **54**, 30.
- 9 A. M. R. Hall, P. Dong, A. Codina, J. P. Lowe and U. Hintermair, *ACS Catalysis*, 2019, **9**, 2079.
- 10 D. Golowicz, P. Kasprzak, V. Orekhov and K. Kazimierczuk, *Prog. Nucl. Magn. Reson. Spectrosc.*, 2020, **116**, 40.
- 11 L. Frydman, A. Lupulescu and T. Scherf, *J Am Chem Soc*, 2003, **125**, 9204.
- 12 C. Lhoste, B. Lorandel, C. Praud, A. Marchand, R. Mishra, A. Dey, A. Bernard, J.-N. Dumez and P. Giraudeau, *Prog. Nucl. Magn. Reson. Spectrosc.*, 2022, **130-131**, 1.
- 13 Z. D. Pardo, G. L. Olsen, M. E. Fernandez-Valle, L. Frydman, R. Martinez-Alvarez and A. Herrera, *J Am Chem Soc*, 2012, **134**, 2706.
- 14 B. Gouilleux, B. Charrier, S. Akoka, F.-X. Felpin, M. Rodriguez-Zubiri and P. Giraudeau, *TrAC, Trends Anal. Chem.*, 2016, **83**, 65.
- 15 C. Jacquemmoz, F. Giraud and J.-N. Dumez, *Analyst*, 2020, **145**, 478.
- 16 P. Callaghan, *Principles of nuclear magnetic resonance microscopy*, Clarendon Press, Oxford, 1993.
- 17 L. Rouger, B. Charrier, M. Pathan, S. Akoka and P. Giraudeau, *J Magn Reson*, 2014, **238**, 87.
- 18 A. Marchand, R. Mishra, A. Bernard and J. N. Dumez, *Chemistry*, 2022, e202201175.
- 19 M. Bazzoni, C. Lhoste, J. Bonnet, K. E. Konan, A. Bernard, P. Giraudeau, F. X. Felpin and J. N. Dumez, *Chem. Eur. J.*, 2023, **29**, e202203240.
- 20 P. A. Keifer, 2007, pp. 1-47.
- 21 B. Gouilleux, B. Charrier, S. Akoka and P. Giraudeau, *Magn. Reson. Chem.*, 2017, **55**, 91.