LIMNOLOGY and OCEANOGRAPHY: METHODS

ASLO

Limnol. Oceanogr.: Methods 2023

doi: 10.1002/lom3.10553

A fast and efficient tool for the structural characterization of marine dissolved organic matter: Nonuniform sampling 2D COSY NMR

Sahithya Phani Babu Vemulapalli,[1]* **Christian Griesinger**,[2] **Thorsten Dittmar**[1,3]*

[1]Research Group for Marine Geochemistry, Institute for Chemistry and Biology of the Marine Environment (ICBM), University of Oldenburg, Oldenburg, Germany

[2]Department of NMR-Based Structural Biology, Max Planck Institute (MPI) for Multidisciplinary Sciences, Göttingen, Germany

[3]Helmholtz Institute for Functional Marine Biodiversity at the University of Oldenburg (HIFMB), Oldenburg, Germany

Abstract

An in-depth structural characterization of marine dissolved organic matter (DOM) is crucial for a better understanding of its connection to marine and global biogeochemical cycles. High-field nuclear magnetic resonance (NMR) spectroscopy in general and two-dimensional (2D) correlation spectroscopy (COSY) in particular are powerful tools for the molecular level structural analysis of marine DOM. These 2D NMR experiments demand prolonged experimental times of days to weeks per sample due to the requirement of a large number of experiments to record the second dimension (t_1) of the 2D NMR experiment. Herein, we demonstrate the efficacy of nonuniform sampling (NUS) in 2D COSY, which (i) reduces the measurement time by half without compromising spectral quality and (ii) enhances the signal intensity for the given experiment time. This approach can lead to substantial progress in the structural analysis of previously poorly characterized marine DOM. NUS COSY has been exemplified on two solid-phase extracted DOM samples from the surface and deep ocean at 800 MHz and 1.2 GHz instruments. A dramatic improvement in sensitivity and spectral resolution is observed in NUS COSY spectra recorded at 1.2 GHz instrument when compared to 800 MHz instrument. NUS COSY NMR is versatile and anticipated to have significant potential for uncovering the hidden molecular diversity of DOM from various aquatic environments within a reasonable timeframe. The introduction of NUS into the environmental sciences was long overdue, and our study now opens the door for a wide field of new applications of NMR in the marine and aquatic sciences.

Uncovering the thus far hidden molecular diversity of dissolved organic matter (DOM) in marine and aquatic ecosystems is an actively evolving field of research and is expected to provide further insights into the source, transportation, and millennial persistence of one of the largest exchangeable carbon reservoirs (Carlson and Hansell 2015; Dittmar 2015; Repeta 2015; Ridgwell and Arndt 2015). Marine DOM is among the most complex organic mixtures with at least hundreds of thousands of different compounds residing in each liter of seawater (Zark et al. 2017). The remarkable complexity of marine DOM and indicated low concentration of individual components in seawater (Arrieta Jesús et al. 2015) pose challenges to the structural characterization using current analytical techniques. Ultrahigh-resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) is a well-established technique for the molecular characterization of marine DOM. The molecular diversity of marine DOM across the oceanic provinces has been extensively studied on a molecular formula level using FT-ICR-MS (Koch et al. 2005; Hertkorn et al. 2013; Riedel and Dittmar 2014; Zark et al. 2017; Zark and Dittmar 2018), and ten-thousands of molecular formulae of individual DOM constituents have been identified so far via FT-ICR-MS (Riedel and Dittmar 2014). Yet, FT-ICR-MS fails to distinguish between the many isomeric structures that exist for each molecular formula (Zark et al. 2017). A single molecular formula observed in an ultrahigh-resolution FT-ICR-MS spectrum represents a multitude of distinct isomers of unknown proportion, because the

*Correspondence: sahithya.phani.babu.vemulapalli@uni-oldenburg.de; thorsten.dittmar@uni-oldenburg.de

Author Contribution Statement: S.P.B.V. conceptualized and designed the study, optimized and performed the experiments, evaluated the data, and wrote the original draft. C.G. evaluated the data and critically revised the manuscript. T.D. evaluated the data, acquired the funding, and critically revised the manuscript.

Additional Supporting Information may be found in the online version of this article.

lack of suitable reference compounds prevents the determination of ionization efficiencies in mass spectrometry (Zark et al. 2017). Thus, the structural composition of DOM from different oceanic provinces appears highly similar on a molecular formula level due to isomeric averaging in FT-ICR-MS (Zark and Dittmar 2018). In this regard, high-field NMR spectroscopy is a promising complementary tool for the molecular-level structural characterization of marine DOM. In general, NMR can discriminate between isomeric structures (except enantiomers) of a given molecular formula by exhibiting characteristic chemical shifts and *J*-coupling multiplet patterns in proton NMR, as protons in each isomer experience different chemical environment. Given the complexity of marine DOM, it is impossible to identify individual isomeric structures using NMR. But we gain detailed structural information of marine DOM, which is impossible with FT-ICR-MS. So far, aliphatic, carboxyl-rich alicyclic molecules (CRAM; Hertkorn et al. 2006), material derived from linear terpenoids (Lam et al. 2007), carotenoid degradation products (Arakawa et al. 2017), carbohydrate, olefinic, and aromatic structural-feature groups have been identified as major components of dissolved organic matter using comprehensive ultrahigh-resolution mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy.

Two-dimensional (2D) ^{1}H-^{1}H COSY (correlation spectroscopy) is a valuable NMR experiment (Mopper et al. 2007; Woods et al. 2011; Hertkorn et al. 2013; Dutta Majumdar et al. 2017; Seidel et al. 2022), providing information on the neighboring protons connected via scalar through-bond couplings (*J*-couplings; Jeener 1971; Aue et al. 1976) conveniently connecting protons that are usually not more than 3 bonds apart. The double-quantum filtered (DQF) COSY (Rance et al. 1983) is widely used for the structural elucidation of individual isolated compounds and determination of coupling constants from the multiplet structure. This comes at the expense of $\sqrt{2}$ reduced signal to noise to avoid the double dispersive in-phase diagonal peaks that would deteriorate spectral quality. For mixtures in which there is severe overlap, the antiphase patterns of different peaks overlapping tend to cancel each other out (Supporting Information Fig. S1). These limitations restrict the application of this technique for structural analysis of complex mixtures, particularly mass-limited (~1 mg) marine DOM. Therefore, the magnitude COSY method is highly preferred for complex mixtures compared to DQF COSY recorded and processed with pure phases (Supporting Information Fig. S1). Peak picking in the magnitude COSY is straightforward and can be analyzed using multivariate statistical methods (Seidel et al. 2022). Magnitude COSY provides a higher level of metabolomic informative content (Féraud et al. 2015; Feraud et al. 2019) compared to 1D ^{1}H NMR spectra, as indicated by the peak capacity (Shen and Lee 1998; Hertkorn et al. 2007).

The use of multidimensional NMR spectroscopy for the analysis of environmental samples has been limited to very few studies due to the long measurement times required (Bell et al. 2015; Bell et al. 2016; Mitschke et al. 2023). Particularly, for mass-limited DOM samples, which is usually the case for samples from remote and deep ocean locations, one has to acquire a high number of scans per t_1 increment (number of experiments to sample the second dimension of the 2D experiment) to maximize the sensitivity and resolution. This results in several days of measurement time per sample for each COSY spectrum (Hertkorn et al. 2013; Seidel et al. 2022). One of the comprehensive high-field NMR studies of marine DOM reported eight different 2D and three 1D ^{1}H and ^{13}C NMR experiments, with a total measurement time of ≈50 d per sample (Hertkorn et al. 2013). This amount of instrument time is rarely available on high-field NMR spectrometers.

Setting the stage for detailed structural analysis of marine DOM, Seidel et al. (2022) have successfully reduced the experimental time to 2 d per COSY spectrum and the sample amount to only ~1 mg solid-phase extracted DOM (SPE-DOM) by employing a high-field NMR instrument equipped with a 1.7 mm helium-cooled microcryoprobe. Although the molecular composition of SPE-DOM from different marine provinces is highly similar on a molecular formula level, 2D COSY NMR analysis revealed that DOM is very dissimilar on the molecular structural level (Seidel et al. 2022). However, the full potential of 2D COSY NMR is underutilized for the structural analysis of marine DOM, predominantly due to very limited access to the high-field NMR instruments equipped with sensitivity-improved small diameter cryogenic probes. To circumvent this issue, there is a need for the development of advanced NMR methods to enhance the sensitivity and reduce the measurement time.

Herein, we present 2D nonuniform sampling (NUS) COSY as a simple and efficient tool for accelerating the molecular-level structural characterization of marine DOM. NUS (Barna et al. 1987; Hyberts et al. 2014; Delaglio et al. 2017) is an acquisition method for multidimensional NMR experiments that measures only a subset of systematically arranged t_1 increments. Thereby, NUS reduce measurement time, while keeping the desired spectral resolution and sensitivity (Mobli and Hoch 2014; Le Guennec et al. 2015; Schlippenbach et al. 2018). Due to the existing gaps in the indirect dimension, alternative reconstruction approaches must be employed instead of regular Fourier transformation for processing nonuniformly sampled NMR data (Hyberts et al. 2012*a*). Appropriate choice of NUS schedules and reconstruction approaches results in sensitivity improvement (Rovnyak et al. 2011; Hyberts et al. 2013; Palmer et al. 2015) comparing to conventionally acquired multidimensional NMR spectra. NUS can provide a sensitivity boost by adapting the sampling density to match the signal's envelope in the indirect dimension. In addition, distance-restrained samplings, such as Poisson-gap, offer advantages beyond matched sampling density. The combination of Poisson-gap sampling and compressed sensing (CS) reconstruction has been reported to outperform other methods for spectra exhibiting "clustered sparsity," as

described by Kasprzak et al. (2021). The COSY spectra of marine DOM exhibit this clustered sparsity, which may explain the success of Poisson-gap sampling and CS reconstruction in our study. Depending on the sparsity of NUS, a 2D COSY NMR spectrum of marine DOM sample might be recorded in reduced measurement time of several folds. The number of NUS points should be higher than the number of signals in the spectrum (Delaglio et al. 2017). The iterative soft thresholding (IST) method follows the CS theory. It says that the minimum number of samples required to reconstruct the 2D spectrum is proportional to $K\log(N/K)$, where K is the number of significant points in the spectrum and N is the size of a full grid. As the reconstruction of the 2D spectrum is performed as a series of 1D reconstructions, the IST algorithm requires a minimum number of 1D spectra that is proportional to $K\log(N/K)$ in order to accurately reconstruct the 2D spectrum (Shchukina et al. 2017; Monajemi and Donoho 2019). For the marine DOM 2D COSY spectra, the number of points (K) sampled in the indirect dimension is adapted to the number of significant points in the most crowded F_1 trace. Together with the theoretically improved sensitivity and resolution provided by technological developments such as high-field magnets, helium-cooled small-volume cryoprobes, and advanced radio frequency electronics, NUS could greatly facilitate the acquisition of 2D COSY NMR spectra of hundreds of DOM samples across various oceanic and freshwater ecosystems within a reasonable timeframe, thus providing better insights in DOM biogeochemistry. Here, we present a suitable 2D NUS COSY method for marine DOM and experimentally demonstrate the advances on two oceanic SPE-DOM samples from the surface and abyssal North Pacific at 800 MHz and 1.2 GHz NMR instruments. A detailed structural analysis of marine DOM is outside of the scope of this paper.

Materials and procedures

Two DOM samples (Natural Energy Laboratory of Hawaii Authority) from the surface (21 m sampling depth) and deep ocean (674 m sampling depth) were used in the current study (Green et al. 2014). The latter is North Equatorial Pacific Intermediate Water. Filtered water was desalted and concentrated with SPE using PPL cartridges following the protocol described by Dittmar et al. (2008) and Green et al. (2014). Sixty-one percent of bulk dissolved organic carbon (DOC) was recovered via SPE (Green et al. 2014). SPE-DOC concentrations were determined via high-temperature catalytic oxidation using a Shimadzu TOC-VPCH total organic carbon analyzer. A hundred milligrams of SPE-DOC for each sample was dissolved in 200 μL of 99.95% CD_3OD solvent and transferred to 3 mm NMR tubes. NMR samples were stored before and after the measurements at −21 °C.

NMR spectra were recorded on Bruker Avance Neo 800 MHz and 1.2 GHz (^{1}H resonance frequency) spectrometers equipped with 3 mm TCI (triple resonance cryogenically cooled inverse detection) cryoprobes optimized for inverse detection. All experiments were performed at 298 K. Temperature calibration was carried out using the Bruker standard 99.8% CD_3OD sample. 1D ^{1}H NMR spectra of SPE-DOM samples were acquired by employing 1D version of noesyprph (lc1pngpf2) pulse sequence for double solvent suppression during 2.4 s relaxation delay and 80ms mixing time using F_1 and F_2 channels. A 10 Hz radio frequency field was used for presaturating the protonated methanol and H_2O signals. The time-domain points, spectral width, and transmitter frequency offset were adjusted to 128 k, 19.8 ppm (parts per million), and 5.263 ppm, respectively. A 30 s total relaxation delay including acquisition time was used. Accumulation of 64 transients with 4 dummy scans resulted in an experiment time of 30 min.

A conventional uniformly sampled magnitude-mode 2D ^{1}H-^{1}H COSY was recorded using a modified Bruker pulse sequence (cosygpppqf) with off-resonance selective solvent suppression, prior to optimizing NUS 2D ^{1}H-^{1}H COSY. Key acquisition parameters for 2D conventional and NUS COSY spectra are summarized in Table 1.

NUS is not a readily available technique and if not implemented correctly, NUS results in spectra full of artifacts, making them unsuitable for further analysis. NUS optimization requires extensive experimentation, optimization, and analysis of various NUS schemes and reconstruction algorithms to employ the most effective combination for the given sample. To identify the optimal combination of NUS scheme and reconstruction algorithm for highly complex marine DOM samples, several NUS sampling schedules were carefully evaluated, including Bruker default sampling, sinusoidal-weighted Poisson-gap sampling, sine-burst sampling and sine-gap sampling schemes, and paired each scheme with two different reconstruction algorithms, namely the CS approach employing IST (CS-IST) and iterative re-weighted least squares (CS-IRLS), to evaluate the effectiveness of each individual sampling scheme (Supporting Information Fig. S2). We found that the sinusoidal-weighted Poisson-gap sampling scheme combined with the CS-IST reconstruction algorithm produced the best results for our

Table 1. Key acquisition parameters used for recording 2D ^{1}H-^{1}H COSY spectra.

Pulse sequence	TD_2	TD_1	SW_2 (ppm)	SW_1 (ppm)	o1p (ppm)	D1 (s)	NS	DS	NUS schedule
Modified-cosygpppqf	8192	1024	13.9	13.9	5.307	2	32	32	Sinusoidal-weighted Poisson-gap

TD_2 and TD_1, time-domain points in F_2 and F_1 dimensions, respectively; SW_2 and SW_1, spectral width in F_2 and F_1 dimensions, respectively; o1p, transmitter frequency offset; D1, relaxation delay; NS, number of scans per t_1 increment; DS, number of dummy scans.

experimental setup. With the protocol presented here, it is now straightforward for Environmental researchers to adopt this elegant method for the DOM analysis.

For 2D NUS COSY, FnTYPE in Topspin acquisition parameters was set to "non-uniform_sampling." Sensitivity of COSY-type NMR spectra can be improved by using NUS scheme that samples most densely around the maximum of sine-dependent time domain signal of cross peaks (Schmieder et al. 1993). But marine DOM is a complex mixture of hundreds of thousands of different molecules with varying relaxation properties and a broad range of H—H *J*-couplings. Consequently, choosing NUS scheme that matches sine modulated H—H scalar coupling evolution in conventional magnitude COSY of marine DOM is not straightforward, as it can be seen from the time domain signal (Supporting Information Fig. S3). In order to circumvent this issue, we employed sinusoidal-weighted Poisson-gap schedules (Kazimierczuk et al. 2008; Hyberts et al. 2010) in NUS magnitude COSY (Schlippenbach et al. 2018) and obtained spectra with similar sensitivity and resolution to those obtained by conventional uniform sampling, but in 2-fold decreased measurement time. Sinusoidal-weighted Poisson-gap sampling schedules were created using the Schedule Generator Version 3.0 available on nus@HMS webpage (http://gwagner.med.harvard.edu/intranet/hmsIST/gensched_new.html; Supporting Information Fig. S4). The generated Poisson-gap sampling schedule was copied to Topspin folder as a variable counter (vc) file and the default "automatic" replaced with the name of the sampling schedule, in the NUS acquisition parameters. All the spectra were processed with Topspin 4.1.3 (Bruker BioSpin, Germany) and visualized using Sparky (Goddard and Kneller 2008). The important processing parameters for 2D conventional and NUS COSY spectra are summarized in Table 2. 2D NUS COSY spectra were reconstructed with the CS (Kazimierczuk and Orekhov 2011) approach employing the IST (Hyberts et al. 2012*b*) algorithm available in Topspin 4.1.3. NMR chemical shifts are reported in parts per million (ppm). The automatic peak picking was performed inside Topspin 4.1.3. Accumulation of 32 transients per t_1 increment resulted in an experiment time of ~21 and ~10 h for 2D conventional and 50% NUS COSY spectra, respectively.

Considering all costs for an 800 MHz NMR with cryoprobes, including the original purchase (4 M€), personnel (100 k€ per year), maintenance (70 k€ per year), and a lifetime of 15 yr, 1 d at the NMR costs > 1000 €. Assuming 2 d of measurement time per sample (Seidel et al. 2022) when using conventional methods, NUS (reduce the time by half) saves about 1000 € per sample, or, a conservative estimate of 100,000 € for a medium-sized study involving 100 samples.

Assessment

1D ^{1}H NMR of marine DOM

Since DOM is composed of hundreds of thousands different compounds (Zark and Dittmar 2018), 1D ^{1}H NMR spectra of marine DOM (Fig. 1) suffer from poor spectral resolution due to the extremely overlapped *J*-coupling multiplets of protons spread over a narrow proton chemical shift range (~10 ppm), even at high magnetic fields. Consequently, the ^{1}H chemical shift regions can only be broadly categorized into key structural-feature groups (Fig. 1). We used the five chemical shift regions in ppm proposed by Hertkorn et al. (2013) for categorization: 0.0–1.9 aliphatic; 1.9–3.1 acetate and CRAM; 3.1–4.9 carbohydrate and methoxy; 5.3–6.5 olefinic and 6.5–10.0 aromatic. The integration of the aforementioned five chemical shift regions resulted in the determination of the relative quantities of respective structural-feature groups (Table 3) in the surface and deep ocean SPE-DOM.

1D ^{1}H NMR spectral analysis revealed that the aliphatic, acetate, and CRAM structural groups are relatively higher in deep ocean DOM, while carbohydrate and methoxy groups are more abundant in surface ocean DOM. The trend in observed differences in the relative abundance of structural-feature groups between the surface and deep ocean is consistent with the results published by Hertkorn et al. (2006, 2013) and Seidel et al. (2022). Aromatic and olefinic structural groups are similarly abundant in surface and deep ocean DOM. Because of the inferior spectral resolution, the information content (theoretical peak capacity) obtained from 1D ^{1}H NMR spectra of marine DOM is very limited, thus acquiring multidimensional NMR experiments is necessary for further structural analysis.

Fast acquisition of 2D COSY NMR spectra of marine DOM

The conventional uniformly sampled magnitude-mode 2D ^{1}H-^{1}H COSY experiment was optimized on 1.2 GHz instrument for solvent suppression and sensitivity prior to assessing the performance of NUS COSY. A conventional COSY spectrum (Fig. 2a,b) of surface SPE-DOM was recorded with 1024 t_1 increments and 32 scans per increment resulting in ~21 h of experiment time per sample. As a rule of thumb, the number of NUS points must be higher than the number of signals, to

Table 2. Key processing parameters used for 2D ^{1}H-^{1}H COSY spectra.

SI (F_2)	SI (F_1)	WDW (F_2)	WDW (F_1)	PH_mod, (F_2)	PH_mod, (F_1)	2D NUS COSY reconstruction
4096	2048	QSINE, SSB = 0	QSINE, SSB = 0	No	MC	CS-IST algorithm

SI, zero filling points; WDW and PH_mod, window function and phasing mode for 2D processing, respectively; F_2 and F_1, direct and indirect dimensions, respectively.

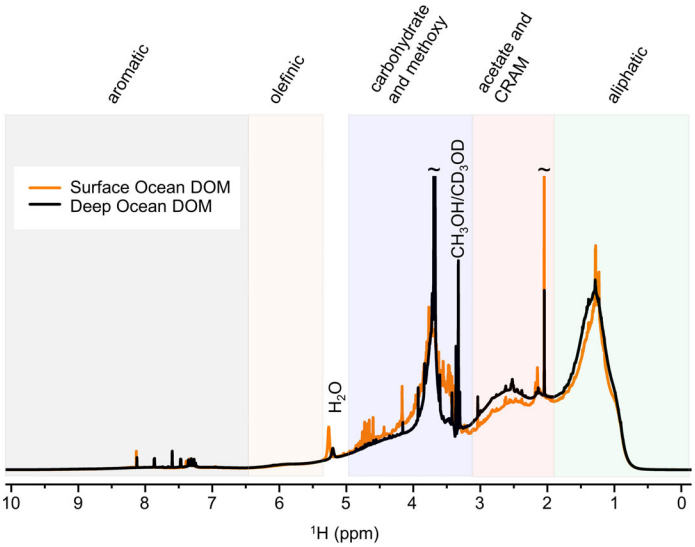

Fig. 1. Overlay of ^{1}H NMR spectra of surface ocean (orange) and deep ocean (black) SPE-DOM recorded at 1.2 GHz instrument. The assignment for the residual H_2O, CH_3OH, and CD_3OD signals is shown. Presaturation was used to suppress multiple solvent signals, using two channels F_1 and F_2. The whole spectrum is classified into five structural-feature groups as indicated by color boxes; assignments are shown on the top.

obtain desired spectral quality after NUS reconstruction (Sidebottom 2016; Delaglio et al. 2017). We tested the impact of 50%, 35%, and 25% NUS on the quality of the COSY spectrum (Supporting Information Fig. S5) of deep ocean SPE-DOM. The number of automatically picked peaks were 319, 332, 292, and 154 in conventional COSY, 50%, 35% and 25% NUS COSY spectra, respectively, clearly highlighting the better performance of 50% NUS schedule. Consequently, 50% NUS was used for recording 2D COSY NMR spectra of marine SPE-DOM samples throughout this study.

The implementation of 50% NUS in a 2D COSY (Fig. 2; Supporting Information Fig. S6) allowed a 2-fold reduction in the measurement time without compromising spectral quality. In this section, we compare the conventional 21 h experiment with 50% NUS in 10 h. Further down, we will compare the conventional with 50% NUS run with the same measurement time.

The many H—H correlations observed even in a small portion of COSY spectra (Figs. 3–5) reflect the molecular complexity of marine DOM. The application of 50% NUS in 2D COSY of surface (Fig. 3) and deep (Fig. 4) ocean samples reproduced spectra that are comparable in sensitivity and resolution to the conventional uniformly sampled COSY, while benefiting from the advantage of 2-fold reduction in experiment time. The number of automatically detected peaks was 627 and 620 in a selected region of conventional and 50% NUS COSY spectra, respectively, of surface ocean SPE-DOM (Fig. 3).

Sensitivity enhanced 50% NUS COSY NMR for marine DOM at the same magnetic field

Improving the sensitivity of 2D NMR experiments is pivotal for an in-depth structural analysis of marine DOM possessing hundreds of thousands of different molecules at very low concentrations. This is particularly advantageous for the detailed analysis of deep ocean DOM, which can often only be obtained in small quantities (~1 mg of SPE-DOM per sample) (Seidel et al. 2022). Herein, we demonstrate the advantage of NUS for enhancing the sensitivity of 2D COSY within the same measurement time as it takes for the conventional counterpart. The sensitivity of NUS cannot be directly measured as a standard signal-to-noise ratio (SNR) due to the nonlinearity of the reconstruction (Hyberts et al. 2013; Palmer et al. 2015). Therefore, the term "detection sensitivity" has been introduced by Hyberts et al. (2013) to define the probabilistic ability to detect weak peaks in the NUS spectra. Alternatively, the intrinsic SNR, as described by Palmer et al. (2015), can be used to calculate the sensitivity improvements provided by time-equivalent NUS spectra.

For this part of our study, we used a high-field 800 MHz NMR spectrometer. Recording 50% NUS COSY with twice the number of scans (NS = 64) per t_1 increment compared to conventional COSY (NS = 32) led to an enhanced peak intensity by a factor of ~2, thus significantly improving the detection sensitivity (Fig. 5). The number of automatically picked peaks was 401 (conventional) and 419 (50% NUS), clearly highlighting the superior performance of 50% NUS COSY over conventional COSY of surface ocean SPE-DOM (Fig. 5), recorded for the same total measurement time. It can be seen from the comparison of 1D slices (Fig. 5c,d) that 50% NUS COSY recorded within the given measurement time increased peak intensity by a factor of two when compared to conventional uniformly sampled COSY. Indirect dimension signal envelope-matched NUS can boost sensitivity, but at the same time, it can worsen the conditions for CS reconstruction, as described by (Kazimierczuk et al. 2014). However, despite this, the

Table 3. 1D ^{1}H NMR-derived relative abundance (% of normalized integrals) of key structural-feature groups of marine SPE-DOM samples.

Structural-feature groups (δ_H ppm)	Aliphatic (0.0–1.9 ppm)	Acetate and CRAM (1.9–3.1 ppm)	Carbohydrate and methoxy (3.1–4.9 ppm)	Olefinic (5.3–6.5 ppm)	Aromatic (6.5–10.0 ppm)
Surface	34.9	25.8	35.4	2.2	1.9
Deep	37.7	28.0	30.4	2.0	1.9

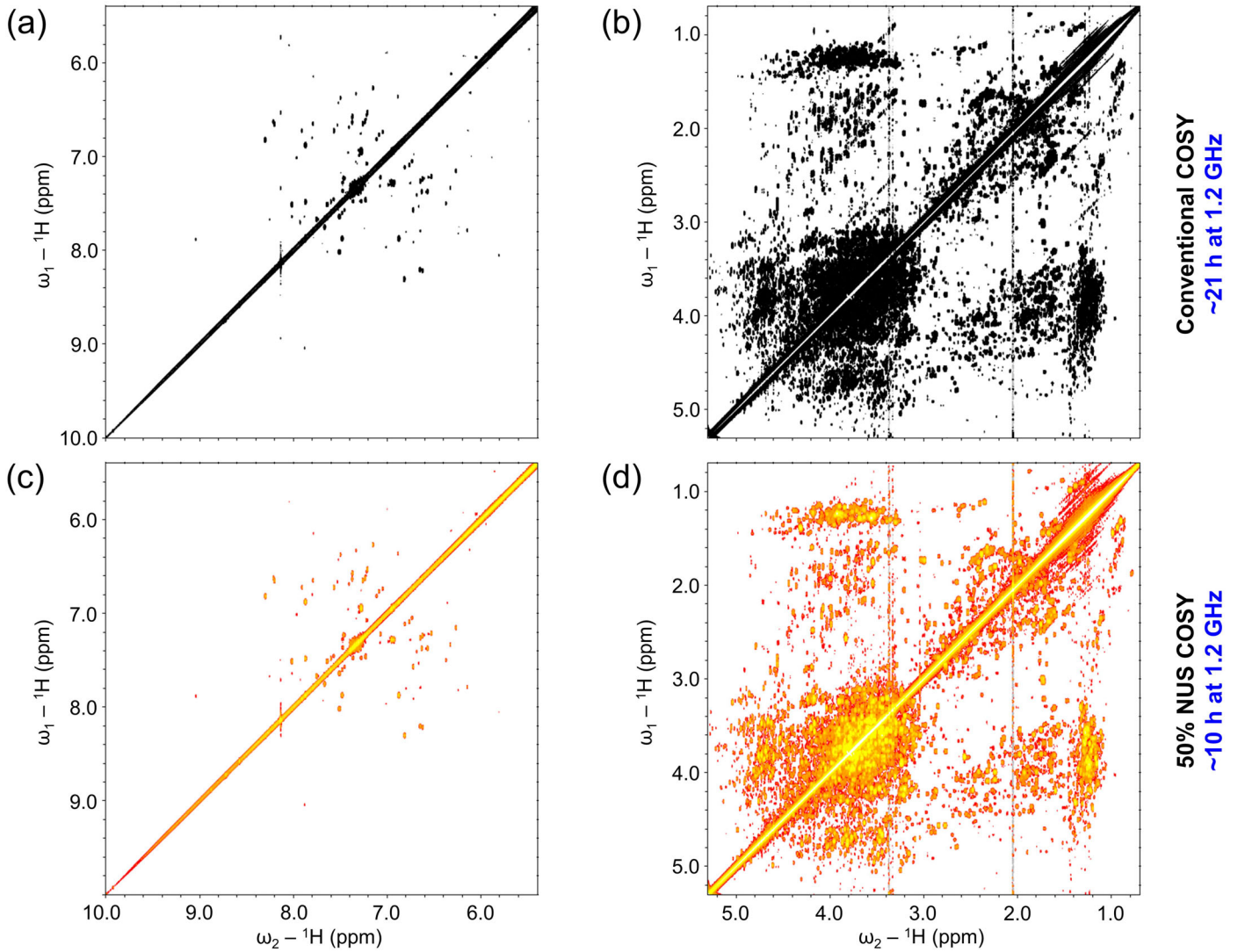

Fig. 2. Comparison between downfield (10.0–5.4 ppm) and upfield (5.3–0.7 ppm) regions of conventional uniformly sampled COSY (**a**,**b**) and 50% NUS COSY (**c**,**d**) NMR spectra of surface ocean SPE-DOM recorded at the 1.2 GHz instrument. Total experiment time was ~21 and ~10 h for conventional and 50% NUS COSY, respectively. As it can be seen, the sensitivity and spectral resolution of 50% NUS COSY recorded in 2-fold decreased measurement time is comparable to conventional uniformly sampled COSY.

sensitivity benefits are usually more significant, as confirmed by our study. This enhancement in sensitivity without further increase in measurement time greatly extends the applicability of 2D NUS COSY NMR for the structural characterization of precious mass limited (~1 mg) deep ocean DOM samples.

NUS COSY recorded in half the time is essentially undistinguishable from the conventional COSY (Figs. 2–4). This facilitates the knowledge gain on marine DOM by means of measuring twice the number of samples in an available measurement time. The NUS COSY obtained within the given experiment time clearly shows additional structural features of DOM, as evidenced by the appearance of new cross peaks in Fig. 5b. This can significantly contribute to the discovery of novel structural information of marine DOM. In addition to the methodological improvement, we present the potential of technological advancement for providing further insights into the structural composition of marine DOM. First 2D COSY NMR spectra of marine DOM recorded at 28.2 T (corresponding to 1.2 GHz ^{1}H operating frequency, which is the worldwide highest magnetic field for NMR) displayed ~2-fold increased number of DOM structural features (new cross peaks) compared to the 800 MHz instrument.

Fifty percent NUS COSY of marine DOM at the 1.2 GHz: The gain in sensitivity and resolution

Recent technological advancements made ultrahigh magnetic field strength of 28.2 T, corresponding to 1.2 GHz

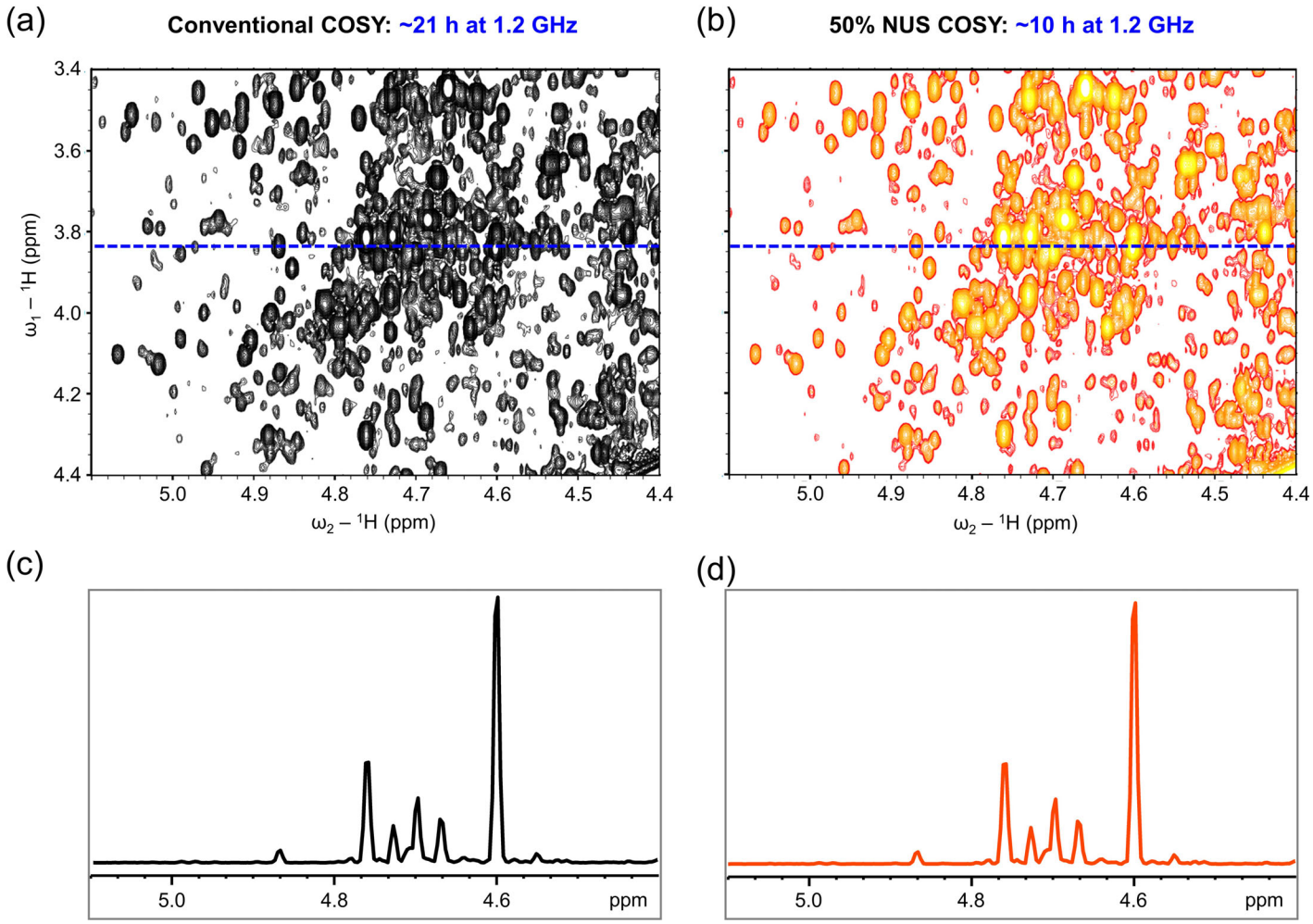

Fig. 3. Comparison of a selected region of (**a**) conventional COSY and (**b**) 50% NUS COSY NMR spectra of surface ocean SPE-DOM recorded at the 1.2 GHz instrument. Both spectra were plotted on the same contour level. Total experiment time was ~21 and ~10 h for conventional and 50% NUS COSY, respectively. (**c**,**d**) 1D slices extracted along the F_2 dimension of 2D conventional and 50% NUS COSY spectra, respectively, at the chemical shift position as indicated by blue dashed lines. The vertical axis of 1D slices is the intensity in arbitrary units. 50% NUS COSY yields peaks with similar intensity when compared to the conventional COSY.

proton resonance frequency, in persistent superconducting magnets commercially available (Banci et al. 2019; Callon et al. 2021; Luchinat et al. 2021; Nimerovsky et al. 2021). The acquisition of multidimensional NMR spectra of complex organic mixtures such as marine DOM would greatly benefit from the superior sensitivity and resolution, when going from 18.8 T (800 MHz for ^{1}H) to 28.2 T (1.2 GHz for ^{1}H) magnetic field. Assuming negligible contribution to the linewidth from relaxation due to chemical shift anisotropy (CSA), when going from low-field (B_{0l}) to high-field (B_{0h}) NMR instruments, the chemical shift dispersion in a 1D experiment (measured in Hz) is increased by a factor of approximately "B_{0h}/B_{0l}", resulting in improved resolution in NMR spectra. Concomitantly, the linewidth, measured in ppm, decreases linearly by the same factor of approximately "B_{0h}/B_{0l}". To demonstrate this effect when increasing the magnetic field strength from 800 MHz to 1.2 GHz, we selected eight well-isolated peaks in the spectra recorded at both instruments. These eight peaks were not overlapping with other peaks, thus avoiding any signal accumulation effects in the linewidth analysis. The comparison of eight randomly selected isolated peaks in 50% NUS COSY spectra of surface SPE-DOM highlighted the linewidth narrowing on the ppm scale obtained at 1.2 GHz as compared to 800 MHz instrument in the range of 0.66–0.70, consistent with the theoretical estimate of 0.67 (ratio of the fields, 800/1200; Supporting Information Fig. S7). The observed sensitivity improvement on the order of 1.5- to 2-fold at 1.2 GHz compared to 800 MHz is consistent with the expected magnetic field-dependent $(1200/800)^{3/2}$ gain in sensitivity (Abragam 1961). The number of automatically picked peaks in the selected portion of COSY spectra of surface ocean SPE-DOM recorded at 800 MHz and 1.2 GHz instruments

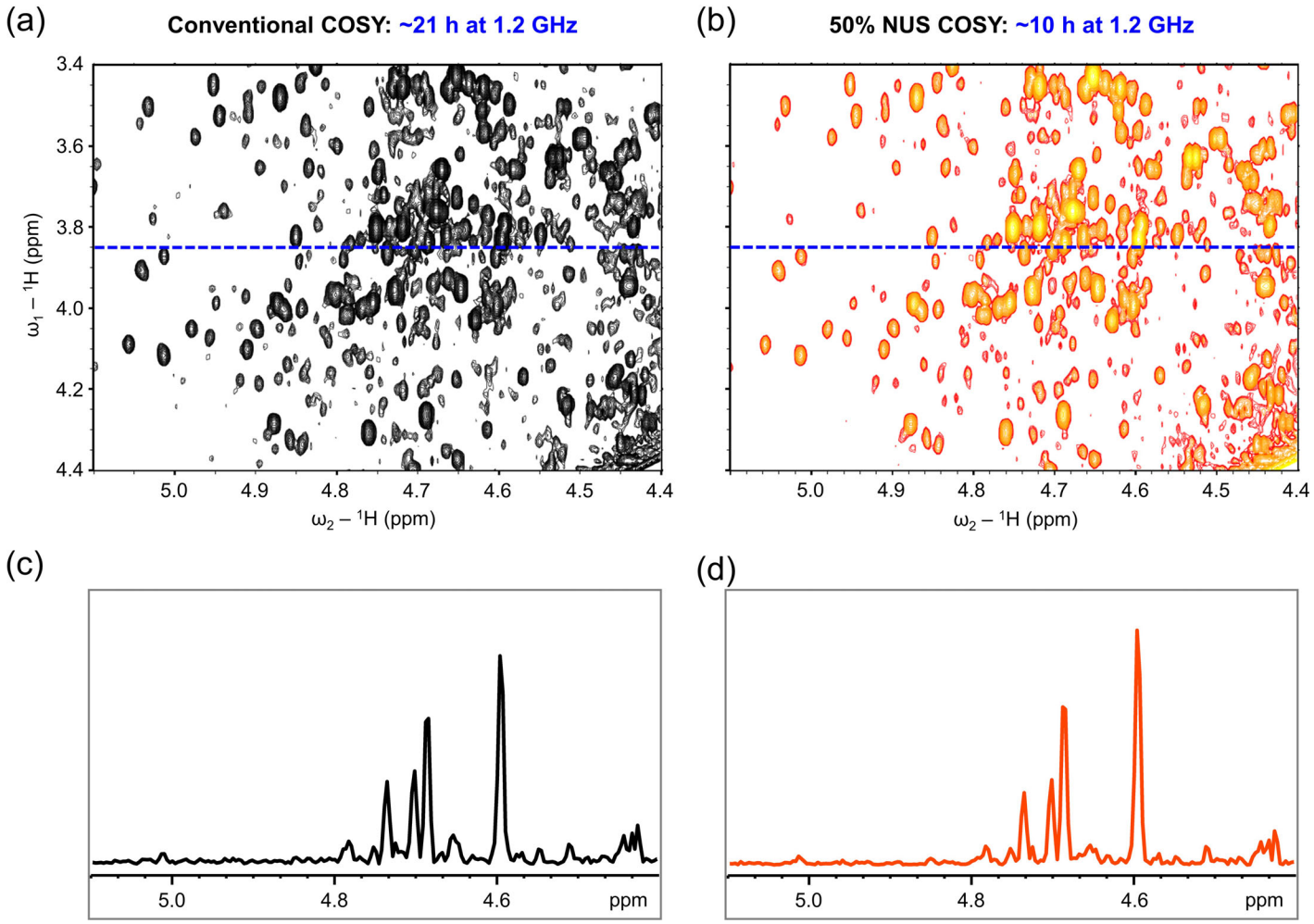

Fig. 4. Comparison of a portion of (**a**) conventional COSY and (**b**) 50% NUS COSY NMR spectra of deep ocean SPE-DOM recorded at the 1.2 GHz instrument. Both spectra were plotted on the same contour level. Total experiment time was ~21 and ~10 h for conventional and 50% NUS COSY, respectively. (**c**,**d**) 1D slices extracted along the F_2 dimension of 2D conventional and 50% NUS COSY spectra, respectively, at the chemical shift position as indicated by blue dashed lines. The vertical axis of 1D slices is the intensity in arbitrary units. The comparison of 1D slices suggest that the NUS dataset produce peaks with similar intensity when compared to the conventional COSY.

increased from 395 to 620 peaks (Fig. 6), as a result of improved sensitivity and resolution at the higher field.

The theoretical improvement in sensitivity by a factor of 2 equals 4 times decrease in measurement time at 1.2 GHz as compared to 800 MHz, if recorded at the same sensitivity. On top of this effect, a 2-fold reduction in the experiment time due to 50% NUS accounts for an overall 6-fold reduction in the measurement time at a 1.2 GHz instrument.

Discussion

In this study, we present a fast 2D NUS COSY NMR method. The significant advantages with the NUS COSY are the following: (i) 2-fold reduced measurement time (from days to hours) without losing spectral quality or (ii) enhanced signal intensity for the same total measurement time, compared to conventional uniformly sampled COSY. The method allowed us to record 2D COSY NMR spectra of two marine DOM samples in less than a day, while the conventional approach requires ~2 d of instrument time. On the other hand, 50% NUS COSY spectrum acquired for the same total experiment time as compared to conventional COSY led to an enhanced intensity of peaks by a factor of ~2. This improved sensitivity without further increase in measurement time broadens the applicability of 2D NUS COSY NMR for the structural characterization of precious mass- and volume-limited DOM samples from remote marine locations, ice cores and pore waters, which otherwise is impractical using conventional NMR methods.

Furthermore, this is the first study to demonstrate the potential of ultrahigh magnetic field strength of 28.2 T

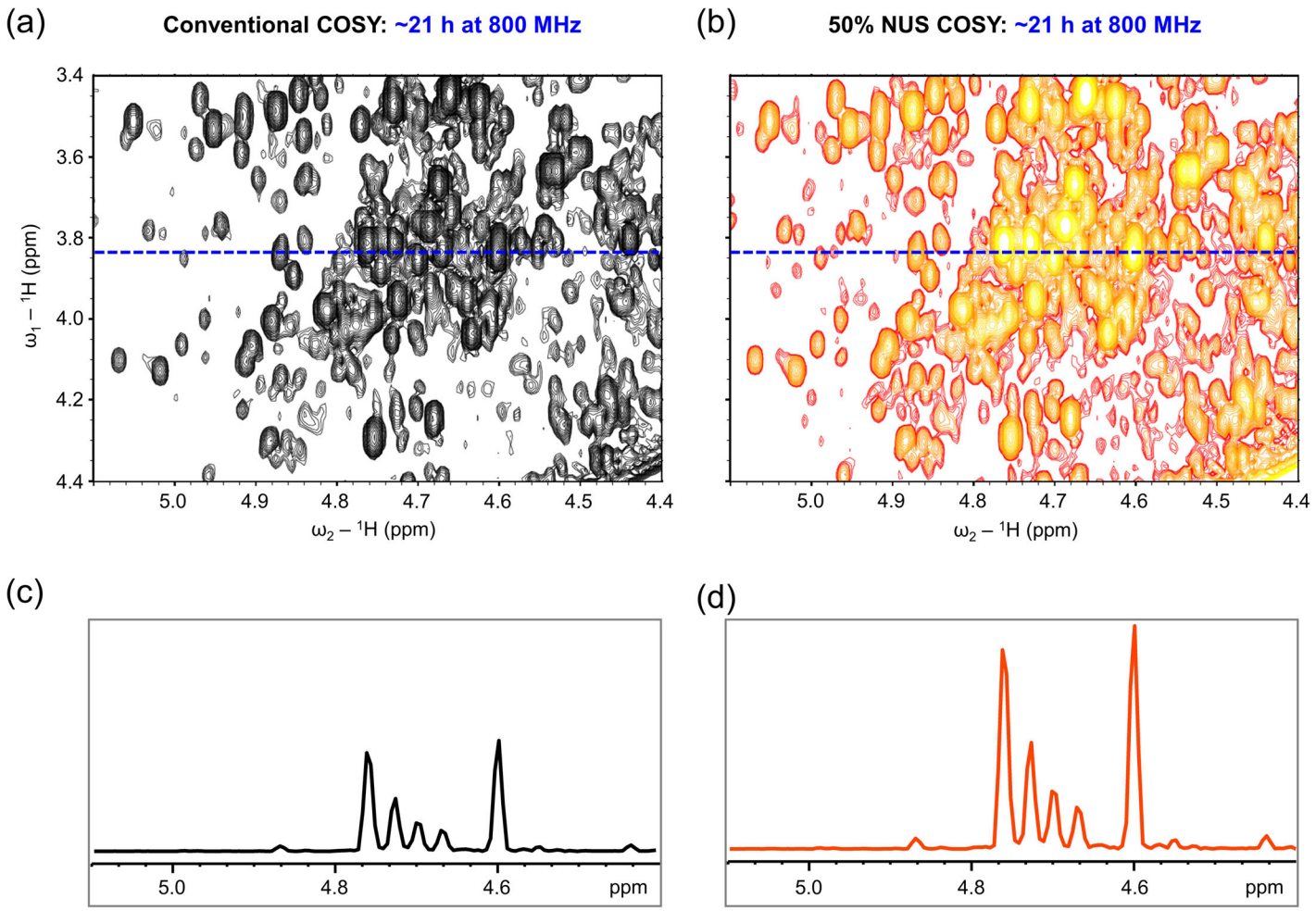

Fig. 5. Comparison of a portion of (**a**) conventional COSY and (**b**) 50% NUS COSY NMR spectra of surface ocean SPE-DOM recorded for the same total measurement time at 800 MHz instrument. Each spectrum was acquired for ~21 h. The number of scans per t_1 increment was 32 and 64 for conventional and 50% NUS COSY, respectively. (**c**,**d**) 1D slices extracted along the F_2 dimension of 2D COSY spectra (**a**,**b**), respectively, at a chemical shift position as indicated by blue dashed lines. The vertical axis of 1D slices is the intensity in arbitrary units. As can be seen from the comparison of 1D slices, 50% NUS COSY showed 2-fold increase in signal intensity when compared to conventional uniformly sampled COSY.

corresponding to 1.2 GHz proton resonance frequency for recording sensitivity-enhanced high-resolution 2D COSY NMR spectra of oceanic DOM. We compared the linewidths of eight randomly selected isolated peaks in 50% NUS COSY spectra of marine DOM recorded at both 1.2 GHz and 800 MHz. We found a substantial improvement in resolution, specifically in terms of linewidth narrowing on the ppm scale, in the range of 0.66–0.70. This improvement is consistent with the expected linewidth narrowing factor of 0.67 on the ppm scale, which is the ratio of the magnetic fields (800/1200). Furthermore, sensitivity improved about 1.5- to 2-fold at 1.2 GHz compared to 800 MHz. The increase in sensitivity observed at 1.2 GHz compared to 800 MHz, on the order of 1.5- to 2-fold, is in line with the expected magnetic field-dependent gain in sensitivity, calculated as $(1200/800)^{3/2}$ (Abragam 1961). However, when comparing the sensitivity of conductive samples measured in cryoprobes, caution should be taken as Abragam's equation was developed for non-cryoprobes where the conductive loss due to the sample was not a factor. In this study, all NMR spectra of surface and deep ocean SPE-DOM were measured in methanol, which has a much lower dielectric constant than water, and the samples contained no salts. Therefore, the sensitivity according to Abragam's formula (field ratio to the power of 3/2) is still valid, even for cryoprobes, provided that they are constructed in the same way as the ones used in this study. The number of automatically picked peaks in a selected region of COSY spectra recorded at 800 MHz and 1.2 GHz instruments increased from 395 to 620 peaks, reveals improved sensitivity and resolution at the higher field. Together with this significant improvement in sensitivity and resolution offered by ultra-high magnetic fields (1.2 GHz instrument), the reduction in the experiment time achieved by NUS in 2D COSY enables high throughput analysis of marine DOM.

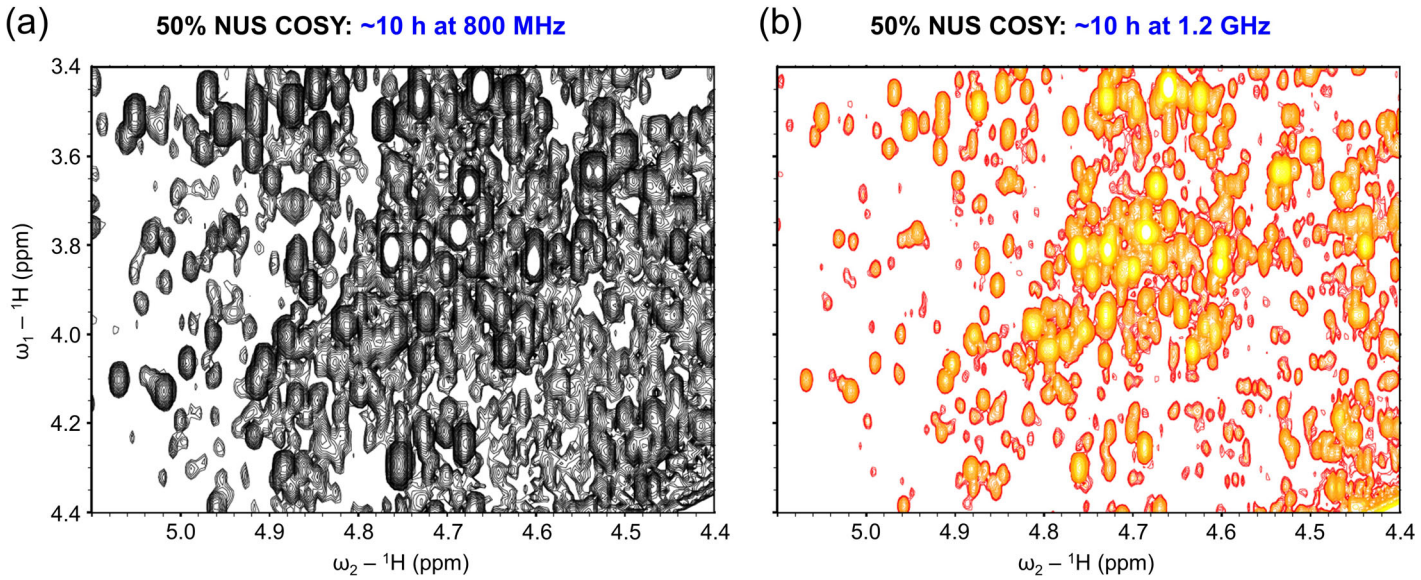

Fig. 6. Comparison of a selected region of 50% NUS COSY spectra of surface ocean SPE-DOM acquired at (**a**) 800 MHz and (**b**) 1.2 GHz instruments. The experiment time was ~10 h for each spectrum. The number of automatically picked peaks increased from 395 (800 MHz) to 620 (1.2 GHz) peaks.

The molecular composition of DOM from the surface and deep ocean appeared similar on the molecular formula level (FT-ICR-MS), but exhibited profound differences on the structural level (NMR). This difference can be attributed to the selectivity and sensitivity of the analytical techniques used. The distinct signals observed in COSY NMR spectra provide further evidence of the unique molecular characteristics of DOM in the surface and deep ocean. Therefore, the combination of NMR and FT-ICR-MS data is particularly powerful, as it provides highly complementary information on the molecular composition of DOM. Advanced multivariate statistical methods, such as canonical correlation analysis, can be used to compare complex NMR and FT-ICR-MS datasets, as described in Seidel et al. (2022). The complementary structural information obtained from this approach is expected to provide novel insights into the largely unknown molecular composition of marine DOM.

Structural diversity is a main driver for DOM bioavailability and potentially for its long-term persistence over millennia (Arrieta Jesús et al. 2015; Mentges et al. 2019). The number of H—H correlations in the 2D COSY spectra indicate a higher structural diversity of DOM at the sea surface compared to DOM from the deep sea, because far more cross peaks were detected at the surface compared to the deep. This is consistent with previous studies (Hertkorn et al. 2013; Seidel et al. 2022). However, the number of detectable features in 2D NMR is not necessarily a reflection of structural diversity, as a single compound may produce signals in the regions all the way from aliphatic to aromatic. DOM consists of at least hundreds of thousands individual compounds, each present at very low abundance (Zark et al. 2017). In a complex mixture, the cross peaks from a very dilute individual compound may appear due to the cumulative signal intensities in an overlapped spectral region. At high spectral resolution, the signals of these individual compounds in 2D NMR may not overlap and fall below the limit of detection (well-known dynamic range problem) because of their low abundance. Furthermore, the lack of cross peaks in a COSY spectrum may be indicative of shorter T_2 relaxation times of DOM in the deep sea than at the sea surface. At this point, we do not have evidence in favor of one or the other explanation. Future studies should be dedicated towards this important knowledge gap.

The COSY NMR analysis indicated that carbohydrate and methoxy groups are enriched in the surface compared to deep ocean, consistent with the 1D ^{1}H NMR analysis. Surprisingly, acetate, CRAM, and aliphatic region showed fewer H—H cross peaks in the COSY spectrum of deep SPE-DOM despite of their high abundance observed in 1D ^{1}H NMR spectrum compared to surface water. These results are in line with the findings reported by Hertkorn et al. (2013) and Seidel et al. (2022). The consistency between the studies showing the robustness of the experimental setup and sample preparation protocols. Signals in 1D ^{1}H NMR spectrum are due to all but exchangeable protons (—OH, —COOH, and NH_2) in the sample excited by a single 90° radio frequency pulse. The integral of a signal provides information about the number of protons contributing to that signal (quantitative). 2D ^{1}H-^{1}H COSY shows cross peaks between protons that are *J*-coupled to each other. The intensity of the cross peaks depends on the strength of the *J*-coupling and relaxation related signal losses. Unlike in 1D ^{1}H NMR spectrum, a single proton shows several cross peaks in the COSY based on the number of neighboring coupled partners and respective *J*-couplings. This complementary spectral information extracted from 1D ^{1}H and 2D ^{1}H-^{1}H COSY

spectra can be efficiently utilized for the better understanding of structural composition of oceanic SPE-DOM.

The 2D NUS COSY method described in this study allows the acquisition of high-quality 2D COSY NMR spectra of hundreds of oceanic DOM samples within a reasonable timeframe. Uncovering the molecular diversity of DOM across marine ecosystems using multivariate statistical methods will greatly benefit from the accelerated acquisition of 2D NUS COSY spectra. Following the procedure mentioned herein, 2D NUS COSY method can be easily adopted and implemented on any modern NMR spectrometers. The high-resolution and sensitivity offered by ultrahigh magnetic fields, such as 1.2 GHz NMR spectrometers, enable efficient structural characterization of marine DOM. The utilization of 2D NMR spectra recorded at 1.2 GHz instrument, such as 2D JRES (Bruch and Bruch 1982; Ludwig and Viant 2010), has the potential to provide unprecedented structural information on marine DOM. The structural information obtained from high-field multidimensional NMR spectroscopic techniques and ultrahigh-resolution FT-ICR-MS are highly complementary and will provide further insights into the molecular diversity of DOM between marine ecosystems.

Data availability statement

NMR raw data and processed data, along with the pulse sequence, acquisition parameters and processing parameters, were uploaded (https://doi.org/10.17617/3.1VEXVN) to Edmond, an open research data repository of the Max Planck Society.

References

Abragam, A. 1961. The principles of nuclear magnetism. Oxford Science Publications.

Arakawa, N., L. I. Aluwihare, A. J. Simpson, R. Soong, B. M. Stephens, and D. Lane-Coplen. 2017. Carotenoids are the likely precursor of a significant fraction of marine dissolved organic matter. Sci. Adv. **3**: e1602976. doi:10.1126/sciadv.1602976

Arrieta Jesús, M., E. Mayol, L. Hansman Roberta, J. Herndl Gerhard, T. Dittmar, and M. Duarte Carlos. 2015. Dilution limits dissolved organic carbon utilization in the deep ocean. Science **348**: 331–333. doi:10.1126/science.1258955

Aue, W. P., E. Bartholdi, and R. R. Ernst. 1976. Two-dimensional spectroscopy. Application to nuclear magnetic resonance. J. Chem. Phys. **64**: 2229–2246. doi:10.1063/1.432450

Banci, L., and others. 2019. Biomolecular NMR at 1.2 GHz. arXiv preprint arXiv. doi:10.48550/arXiv.1910.07462

Barna, J. C. J., E. D. Laue, M. R. Mayger, J. Skilling, and S. J. P. Worrall. 1987. Exponential sampling, an alternative method for sampling in two-dimensional NMR experiments. J. Magn. Reson. **1969**: 69–77. doi:10.1016/0022-2364(87)90225-3

Bell, N. G., A. A. Michalchuk, J. W. Blackburn, M. C. Graham, and D. Uhrin. 2015. Isotope-filtered 4D NMR spectroscopy for structure determination of humic substances. Angew. Chem. Int. Ed. Engl. **54**: 8382–8385. doi:10.1002/anie.201503321

Bell, N. G., M. C. Graham, and D. Uhrin. 2016. Isotope-filtered nD NMR spectroscopy of complex mixtures to unravel the molecular structures of phenolic compounds in tagged soil organic matter. Analyst **141**: 4614–4624. doi:10.1039/C6AN00999A

Bruch, R. C., and M. D. Bruch. 1982. Two-dimensional J-resolved proton NMR spectroscopy of oligomannosidic glycopeptides. J. Biol. Chem. **257**: 3409–3413. doi:10.1016/S0021-9258(18)34792-6

Callon, M., and others. 2021. Biomolecular solid-state NMR spectroscopy at 1200 MHz: The gain in resolution. J. Biomol. NMR **75**: 255–272. doi:10.1007/s10858-021-00373-x

Carlson, C. A., and D. A. Hansell. 2015. Chapter 3—DOM sources, sinks, reactivity, and budgets, p. 65–126. *In* D. A. Hansell and C. A. Carlson [eds.], Biogeochemistry of marine dissolved organic matter, 2nd ed. Academic Press. doi:10.1016/B978-0-12-405940-5.00003-0

Delaglio, F., and others. 2017. Non-uniform sampling for all: More NMR spectral quality, less measurement time. Am. Pharm. Rev. **20**: 339681.

Dittmar, T. 2015. Chapter 7—Reasons behind the long-term stability of dissolved organic matter, p. 369–388. *In* D. A. Hansell and C. A. Carlson [eds.], Biogeochemistry of marine dissolved organic matter, 2nd ed. Academic Press. doi:10.1016/B978-0-12-405940-5.00007-8

Dittmar, T., B. Koch, N. Hertkorn, and G. Kattner. 2008. A simple and efficient method for the solid-phase extraction of dissolved organic matter (SPE-DOM) from seawater. Limnol. Oceanogr. Methods **6**: 230–235. doi:10.4319/lom.2008.6.230

Dutta Majumdar, R., L. Bliumkin, D. Lane, R. Soong, M. Simpson, and A. J. Simpson. 2017. Analysis of DOM phototransformation using a looped NMR system integrated with a sunlight simulator. Water Res. **120**: 64–76. doi:10.1016/j.watres.2017.04.067

Féraud, B., B. Govaerts, M. Verleysen, and P. de Tullio. 2015. Statistical treatment of 2D NMR COSY spectra in metabolomics: data preparation, clustering-based evaluation of the metabolomic informative content and comparison with 1H-NMR. Metabolomics **11**: 1756–1768. doi:10.1007/s11306-015-0830-7

Feraud, B., J. Leenders, E. Martineau, P. Giraudeau, B. Govaerts, and P. de Tullio. 2019. Two data pre-processing workflows to facilitate the discovery of biomarkers by 2D NMR metabolomics. Metabolomics **15**: 63. doi:10.1007/s11306-019-1524-3

Goddard, T. D., and D. G. Kneller. 2008. SPARKY 3. Univ. of California, San Francisco.

Green, N. W., and others. 2014. An intercomparison of three methods for the large-scale isolation of oceanic dissolved organic matter. Mar. Chem. **161**: 14–19. doi:10.1016/j.marchem.2014.01.012

Hertkorn, N., and others. 2006. Characterization of a major refractory component of marine dissolved organic matter. Geochim. Cosmochim. Acta **70**: 2990–3010. doi:10.1016/j.gca.2006.03.021

Hertkorn, N., and others. 2007. High-precision frequency measurements: Indispensable tools at the core of the molecular-level analysis of complex systems. Anal. Bioanal. Chem. **389**: 1311–1327. doi:10.1007/s00216-007-1577-4

Hertkorn, N., M. Harir, B. P. Koch, B. Michalke, and P. Schmitt-Kopplin. 2013. High-field NMR spectroscopy and FTICR mass spectrometry: Powerful discovery tools for the molecular level characterization of marine dissolved organic matter. Biogeosciences **10**: 1583–1624. doi:10.5194/bg-10-1583-2013

Hyberts, S. G., K. Takeuchi, and G. Wagner. 2010. Poisson-gap sampling and forward maximum entropy reconstruction for enhancing the resolution and sensitivity of protein NMR data. J. Am. Chem. Soc. **132**: 2145–2147. doi:10.1021/ja908004w

Hyberts, S. G., H. Arthanari, and G. Wagner. 2012*a*. Applications of non-uniform sampling and processing. Top. Curr. Chem. **316**: 125–148. doi:10.1007/128_2011_187

Hyberts, S. G., A. G. Milbradt, A. B. Wagner, H. Arthanari, and G. Wagner. 2012*b*. Application of iterative soft thresholding for fast reconstruction of NMR data non-uniformly sampled with multidimensional Poisson gap scheduling. J. Biomol. NMR **52**: 315–327. doi:10.1007/s10858-012-9611-z

Hyberts, S. G., S. A. Robson, and G. Wagner. 2013. Exploring signal-to-noise ratio and sensitivity in non-uniformly sampled multi-dimensional NMR spectra. J. Biomol. NMR **55**: 167–178. doi:10.1007/s10858-012-9698-2

Hyberts, S. G., H. Arthanari, S. A. Robson, and G. Wagner. 2014. Perspectives in magnetic resonance: NMR in the post-FFT era. J. Magn. Reson. **241**: 60–73. doi:10.1016/j.jmr.2013.11.014

Jeener, J. 1971. Ampere International Summer School, Basko Polje, Yugoslavia. p. 197.

Kasprzak, P., M. Urbanczyk, and K. Kazimierczuk. 2021. Clustered sparsity and Poisson-gap sampling. J. Biomol. NMR **75**: 401–416. doi:10.1007/s10858-021-00385-7

Kazimierczuk, K., A. Zawadzka, and W. Koźmiński. 2008. Optimization of random time domain sampling in multidimensional NMR. J. Magn. Reson. **192**: 123–130. doi:10.1016/j.jmr.2008.02.003

Kazimierczuk, K., and V. Y. Orekhov. 2011. Accelerated NMR spectroscopy by using compressed sensing. Angew. Chem. Int. Ed. **50**: 5556–5559. doi:10.1002/anie.201100370

Kazimierczuk, K., O. Lafon, and P. Lesot. 2014. Criteria for sensitivity enhancement by compressed sensing: practical application to anisotropic NAD 2D-NMR spectroscopy. Analyst **139**: 2702–2713. doi:10.1039/c4an00381k

Koch, B. P., M. Witt, R. Engbrodt, T. Dittmar, and G. Kattner. 2005. Molecular formulae of marine and terrigenous dissolved organic matter detected by electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. Geochim. Cosmochim. Acta **69**: 3299–3308. doi:10.1016/j.gca.2005.02.027

Lam, B., and others. 2007. Major structural components in freshwater dissolved organic matter. Environ. Sci. Technol. **41**: 8240–8247. doi:10.1021/es0713072

Le Guennec, A., J. N. Dumez, P. Giraudeau, and S. Caldarelli. 2015. Resolution-enhanced 2D NMR of complex mixtures by non-uniform sampling. Magn. Reson. Chem. **53**: 913–920. doi:10.1002/mrc.4258

Luchinat, E., L. Barbieri, M. Cremonini, and L. Banci. 2021. Protein in-cell NMR spectroscopy at 1.2 GHz. J. Biomol. NMR **75**: 97–107. doi:10.1007/s10858-021-00358-w

Ludwig, C., and M. R. Viant. 2010. Two-dimensional J-resolved NMR spectroscopy: Review of a key methodology in the metabolomics toolbox. Phytochem. Anal. **21**: 22–32. doi:10.1002/pca.1186

Mentges, A., C. Feenders, C. Deutsch, B. Blasius, and T. Dittmar. 2019. Long-term stability of marine dissolved organic carbon emerges from a neutral network of compounds and microbes. Sci. Rep. **9**: 17780. doi:10.1038/s41598-019-54290-z

Mitschke, N., S. P. B. Vemulapalli, and T. Dittmar. 2023. NMR spectroscopy of dissolved organic matter: A review. Environ. Chem. Lett. **21**: 689–723. doi:10.1007/s10311-022-01528-4

Mobli, M., and J. C. Hoch. 2014. Nonuniform sampling and non-Fourier signal processing methods in multidimensional NMR. Prog. Nucl. Magn. Reson. Spectrosc. **83**: 21–41. doi:10.1016/j.pnmrs.2014.09.002

Monajemi, H., and D. L. Donoho. 2019. Sparsity/undersampling tradeoffs in anisotropic undersampling, with applications in MR imaging/spectroscopy. Inf. Inference J. IMA **8**: 531–576. doi:10.1093/imaiai/iay013

Mopper, K., A. Stubbins, J. D. Ritchie, H. M. Bialk, and P. G. Hatcher. 2007. Advanced instrumental approaches for characterization of marine dissolved organic matter: Extraction techniques, mass spectrometry, and nuclear magnetic resonance spectroscopy. Chem. Rev. **107**: 419–442. doi:10.1021/cr050359b

Nimerovsky, E., and others. 2021. Proton detected solid-state NMR of membrane proteins at 28 Tesla (1.2 GHz) and 100 kHz magic-angle spinning. Biomolecules **11**: 752. doi:10.3390/biom11050752

Palmer, M. R., and others. 2015. Sensitivity of nonuniform sampling NMR. J. Phys. Chem. B **119**: 6502–6515. doi:10.1021/jp5126415

Rance, M., O. W. Sørensen, G. Bodenhausen, G. Wagner, R. R. Ernst, and K. Wüthrich. 1983. Improved spectral resolution

in COSY ^{1}H NMR spectra of proteins via double quantum filtering. Biochem. Biophys. Res. Commun. **117**: 479–485. doi:10.1016/0006-291X(83)91225-1

Repeta, D. J. 2015. Chapter 2—Chemical characterization and cycling of dissolved organic matter, p. 21–63. *In* D. A. Hansell and C. A. Carlson [eds.], Biogeochemistry of marine dissolved organic matter, 2nd ed. Academic Press. doi:10.1016/B978-0-12-405940-5.00002-9

Ridgwell, A., and S. Arndt. 2015. Chapter 1—Why dissolved organics matter: DOC in ancient oceans and past climate change, p. 1–20. *In* D. A. Hansell and C. A. Carlson [eds.], Biogeochemistry of marine dissolved organic matter, 2nd ed. Academic Press. doi:10.1016/B978-0-12-405940-5.00001-7

Riedel, T., and T. Dittmar. 2014. A method detection limit for the analysis of natural organic matter via Fourier transform ion cyclotron resonance mass spectrometry. Anal. Chem. **86**: 8376–8382. doi:10.1021/ac501946m

Rovnyak, D., M. Sarcone, and Z. Jiang. 2011. Sensitivity enhancement for maximally resolved two-dimensional NMR by nonuniform sampling. Magn. Reson. Chem. **49**: 483–491. doi:10.1002/mrc.2775

Schlippenbach, T. V., P. J. Oefner, and W. Gronwald. 2018. Systematic evaluation of non-uniform sampling parameters in the targeted analysis of urine metabolites by ^{1}H,^{1}H 2D NMR spectroscopy. Sci. Rep. **8**: 4249. doi:10.1038/s41598-018-22541-0

Schmieder, P., A. S. Stern, G. Wagner, and J. C. Hoch. 1993. Application of nonlinear sampling schemes to COSY-type spectra. J. Biomol. NMR **3**: 569–576. doi:10.1007/BF00174610

Seidel, M., S. P. B. Vemulapalli, D. Mathieu, and T. Dittmar. 2022. Marine dissolved organic matter shares thousands of molecular formulae yet differs structurally across major water masses. Environ. Sci. Technol. **56**: 3758–3769. doi:10.1021/acs.est.1c04566

Shchukina, A., P. Kasprzak, R. Dass, M. Nowakowski, and K. Kazimierczuk. 2017. Pitfalls in compressed sensing reconstruction and how to avoid them. J. Biomol. NMR **68**: 79–98. doi:10.1007/s10858-016-0068-3

Shen, Y., and M. L. Lee. 1998. General equation for peak capacity in column chromatography. Anal. Chem. **70**: 3853–3856. doi:10.1021/ac9802426

Sidebottom, P. J. 2016. A new approach to the optimisation of non-uniform sampling schedules for use in the rapid acquisition of 2D NMR spectra of small molecules. Magn. Reson. Chem. **54**: 689–694. doi:10.1002/mrc.4444

Woods, G. C., M. J. Simpson, P. J. Koerner, A. Napoli, and A. J. Simpson. 2011. HILIC-NMR: Toward the identification of individual molecular components in dissolved organic matter. Environ. Sci. Technol. **45**: 3880–3886. doi:10.1021/es103425s

Zark, M., and T. Dittmar. 2018. Universal molecular structures in natural dissolved organic matter. Nat. Commun. **9**: 3178. doi:10.1038/s41467-018-05665-9

Zark, M., J. Christoffers, and T. Dittmar. 2017. Molecular properties of deep-sea dissolved organic matter are predictable by the central limit theorem: Evidence from tandem FT-ICR-MS. Mar. Chem. **191**: 9–15. doi:10.1016/j.marchem.2017.02.005

Acknowledgments

This work was financially supported by the VolkswagenStiftung within the framework of the project: "Global Carbon Cycling and Complex Molecular Patterns in Aquatic Systems: Integrated Analyses Powered by Semantic Data Management." The 1.2 GHz instrument was funded by the Max Planck Society and the DFG and the Land Niedersachsen (76251-12-1/12) with the support of the BIN (Center of Biomolecular Imaging in Neurodegeneration) board. The authors would like to thank Heike Simon for her assistance in the laboratory. We would like to express our sincere gratitude to the Associate Editor and all the reviewers for their constructive feedback and suggestions, which greatly improved the quality of this manuscript. Open Access funding enabled and organized by Projekt DEAL.

Submitted 20 February 2023
Revised 02 May 2023
Accepted 05 May 2023

Associate editor: Isaac Santos