Ultrahigh-Resolution NMR at Full Sensitivity

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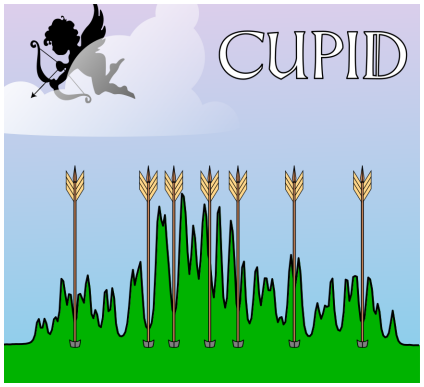
Abstract

We present CUPID (Computer-assisted Undiminished-sensitivity Protocol for Ideal Decoupling), a technique which uses parametric estimation to produce pure shift spectra from easy-to-acquire J-Resolved datasets without loss of sensitivity, and with absorption-mode lineshapes. We illustrate that it is effective even in scenarios where state-of-the-art pure shift experimental techniques are too insensitive, and where undesired solvent/impurity peaks overlap with signals of interest. The method also enables the assignment of multiplet structures based on a simple criterion involving the relationship between a particular resonance's direct- and indirect-dimension frequencies. The functionality required to use CUPID is provided by NMR Estimation in Python (NMR-EsPy), an open source package with a simple-to-use API and supporting graphical interface.



1 Introduction

2D J-resolved (2DJ) spectroscopy[1, 2] is a valuable technique for separating chemical shift and scalar coupling information. The experiment is commonly used to acquire broadband homodecoupled (pure shift) spectra, achieved by applying a 45° shear to the spectral data such that $S(f^{(1)}, f^{(2)}) \rightarrow S(f^{(1)}, f^{(2)} - f^{(1)})$. This transformation leaves chemical shifts and scalar couplings lying perfectly orthogonally to each other, along the $F^{(2)}$ and $F^{(1)}$ axes, respectively. A subsequent summation along $F^{(1)}$ yields the pure shift spectrum. Despite the simplicity of this approach, J-Resolved datasets are hypercomplex (i.e. phase-modulated with respect to both time dimensions), from which it is impossible to generate a spectrum with desirable absorption-mode Lorentzian lineshapes. In fact, summation of the spectrum, which comprises "phase-twist" lineshapes leads to positive and negative contributions cancelling each other out. It is therefore conventional to display 2DJ spectra using magnitude mode, leading to pure shift spectra with broad wings as they possess both absorption- and dispersion-mode character. Post-processing techniques such as pseudo-echo reshaping[3] and sine-bell apodisation[4] can help suppress the dispersive component, though these induce a significant hit to sensitivity, principally as they drastically reduce the magnitude of the initial points in the FID. There has been considerable development of "1D" pure shift methods, which typically involve concatenating the initial "chunks" of FIDs in a 2D experiment. Prominent examples involve the use of the Zangger-Sterk (ZS) pulse element[5, 6], Bilinear rotational Decoupling (BIRD)[7, 8], and Pure Shift Yielded by Chirp Excitation (PSYCHE)[9, 10]. These techniques all suffer from a significant reduction in sensitivity as only a subset of the available spin magnetisation contributes to the final signal, albeit for differing reasons (see [11] for an overview).

We introduce a new method to generate pure shift spectra, which utilises NMR Estimation in Python (NMR-EsPy), an open source package we recently presented[12]. The technique involves estimation of the parameters which describe the 2DJ FID. With knowledge of these parameters, it is possible to generate pure shift spectra with absorption-mode lineshapes, with the same sensitivity as a simple pulse-acquire experiment. We name the technique CUPID (Computer-assisted Undiminished-sensitivity Protocol for Ideal Decoupling). Using parametric estimation as a route to pure shift spectra also provides extra benefits, such as the ability to assign multiplet structures, and remove unwanted signals such as solvent peaks, and peaks which arise from strong coupling effects[13, 14].

2 Methodology

2DJ FIDs can be modelled as a summation of exponentially damped hypercomplex oscillators, with six parameters that define each oscillator: amplitude (a), phase (ϕ), two frequencies ($f^{(1)}, f^{(2)}$), and two damping factors ($\eta^{(1)}, \eta^{(2)}$), such that a signal comprising M oscillators can be fully described by $6M$ parameters.

A technique has been previously presented which estimates the $4M$ parameters associated with 1D NMR signals using NMR-EsPy, which employs numerical optimisation to minimise a cost function associated with how well a given set of parameters describes the underlying data[12]. NMR-EsPy has been extended to support the estimation of hypercomplex 2D datasets, with more detail of the underlying theory provided in Section A of the supplementary material. One noteworthy feature of the technique is it typically requires the generation of frequency-filtered "sub-FIDs" in the direct dimension, as the computational requirements scale considerably with (a) the number of data points, and especially (b) the model order M.

The key principle behind CUPID is generation of a 1D FID from the estimated parameters, named the "$-45°$ signal" [1] $\boldsymbol{y}_{-45°} \in \mathbb{C}^N$:

$$\boldsymbol{y}_{-45°}[n] = \sum_{m=1}^{M} a_m e^{\mathrm{i}\phi_m} e^{\left(2\pi\mathrm{i}\left(f_m^{(2)} - f_m^{(1)}\right) - \eta_m^{(2)}\right)n\Delta t}, \tag{1}$$

$\forall n \in \{0, \cdots, N-1\}$. This signal takes the same mathematical form as a conventional FID acquired by a pulse-acquire experiment, except the usual frequencies $\{f_m^{(2)}\}$ $\forall m = 1, \cdots, M$ are replaced with $\{f_m^{(2)} - f_m^{(1)}\}$. Oscillators which belong to a particular multiplet have frequencies $f_m^{(1)} = f_\mathrm{d}$ and $f^{(2)} = f_\mathrm{c} + f_\mathrm{d}$ in 2DJ datasets, where f_c is the multiplet's central frequency, and f_d is the displacement of a given oscillator from the central frequency[2]. Rethink notation here perhaps. Hence, all oscillators belonging to the same multiplet will contribute a component with the multiplet's central frequency (f_c) in the $-45°$ signal. It is worth restating here that under the assumption of successful extraction of the 2DJ FID's parameters, Equation 1 leads to spectra with absorption Lorentzian lineshapes without any loss of sensitivity.

The assignment of multiplet structures can be achieved by grouping together oscillators in an estimation result, such that any pair of oscillators $\{i, j\}$ that are part of the same multiplet satisfy

$$\left|\left(f_i^{(2)} - f_i^{(1)}\right) - \left(f_j^{(2)} - f_j^{(1)}\right)\right| < \epsilon, \tag{2}$$

where ϵ is a threshold to account for uncertainty in the estimation. A sensible threshold to set for ϵ is the sampling rate in the more poorly resolved dimension. In practice, this can be a little too optimistic, and ϵ sometimes needs to be slightly increased to resolve multiplets effectively.

The process we describe has similarities with work from Nuzillard[15, 16], and Mutzenhardt et al.[17], in which 1D estimation is used to determine signal parameters for each slice either in $t^{(1)}$ (Nuzillard) or $t^{(2)}$ (Mutzendardt et al.). A "Full echo" is generated via propagation into negative time in estimated dimension, with Fourier Transformation producing to an absorption-mode spectrum which can be sheared and summed as described already. Use of a holistic

[1] See Section A.6 of the supplementary material for the reasoning behind the name.

[2] f_d will be some linear combination of the scalar couplings associated with a given spin, with all coefficients in the combination being ± 1

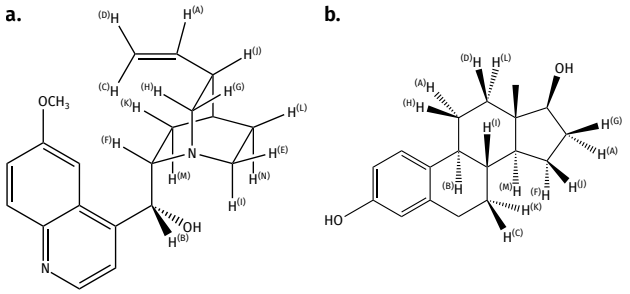


Figure 1: The structures of molecules considered. **a.** quinine, **b.** 17β-estradiol.

2D estimation routine as employed here opens up the possibility of (a) estimating more complex datasets, as individual resonances are more separated, and (b) assigning 1D multiplet structures, which requires knowledge of oscillator frequencies in both dimensions.

3 Results

We present here two examples of the application of CUPID, on the following samples (Figure 1):

- Quinine in CD_3OD (Figure 2).
- Low concentration (2 mM) 17β-estradiol in DMSO-d_6 (Figure 3). A PSYCHE spectrum was also acquired for comparison.

Extra examples are presented in Section B of the supplementary material, including results involving simulated data, which illustrates that in the limit of a "perfect" estimation result, a spectrum which agrees exactly with the expected pure-shift spectrum is achievable. After the generation of each result, certain oscillators were removed from the set of parameters. The oscillators in question were typically spurious and of low-intensity. These tend to arise in situations where a slight over-fitting of the data has occurred. Most of these could be removed automatically, based on a couple of simple criteria outlined in the supplementary material (Section A.7). The updated results re-underwent optimisation after editing. While these edits did improve the results by removing spurious oscillators, the original results still successfully generated homodecoupled spectra with only a small differences to the final spectra presented here.

The quinine example provides an example of suppressing an undesired signal from the final pure shift spectrum. An intense broad singlet arising from residual water was detected with a very similar frequency to the central frequency of the multiplet arising from proton D. The parameters describing the water resonance was simply neglected prior to construction of the pure shift spectrum, leading to a peak which is solely derived from proton D. A similar approach can also be applied to remove components arising from strong coupling artefacts from

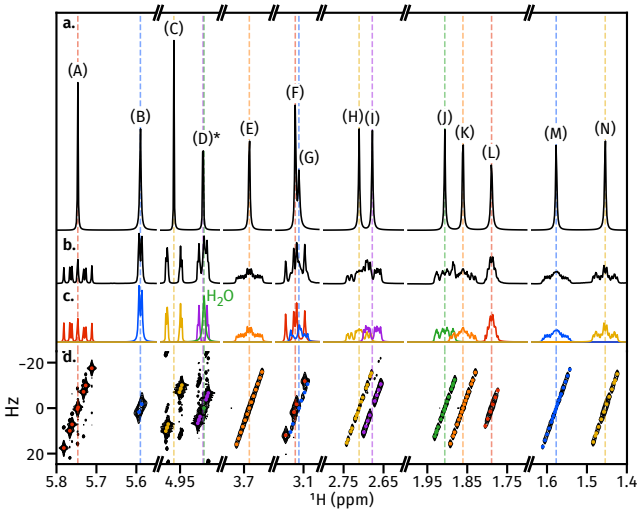


Figure 2: Application of CUPID on the non-aromatic regions of a quinine dataset. **a.** Spectrum generated using CUPID, with a signal arising from residual water, overlapping with proton (D), neglected. **b.** 1D spectrum, produced from Fourier Transformation of the first direct-dimension FID in the 2DJ dataset. **c.** Multiplets assigned, using a threshold $\epsilon = 0.92\,\mathrm{Hz}$, the digital resolution in the direct dimension. **d.** The 2DJ spectrum, displayed in magnitude mode, with positions of assigned peaks marked. Vertical lines mark the central frequencies of predicted multiplets.

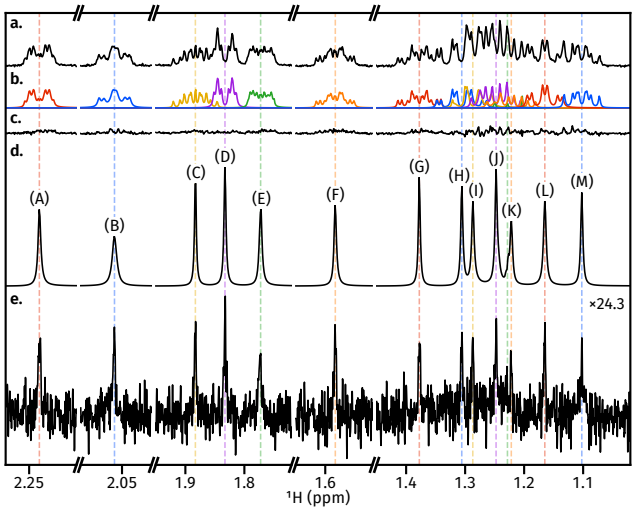


Figure 3: The result of applying CUPID on a 2DJ dataset of 2 mM estradiol in DMSO-d_6. **a.** Conventional 1D spectrum, generated from the FT of the first direct-dimension signal of the 2DJ FID. **b.** Multiplets assigned ($\epsilon = 2$ Hz). **c.** Residual for the first direct-dimension signal in the dataset, given by subtracting the sum of lines in panel b from the line in panel a. **d.** Pure-shift spectrum generated from the FT of the $-45°$ signal. **e.** PSYCHE spectrum for the same sample. The PSYCHE spectrum is scaled so that the most intense point has same magnitude as the equivalent point in the CUPID spectrum.

the final spectrum. An example of this is illustrated in the Supplementary Material, where a 2DJ dataset of camphor is considered. The application of CUPID to the estradiol example illustrates that it is able to cope with datasets with highly complex overlapping multiplet structures, in signal-to-noise regimes where PSYCHE is approaching the point of being too insensitive to generate usable spectra.

CUPID works even in scenarios where the estimation routine has under-fit a particular multiplet structure. However, the resulting pure shift peak tends to be broadened beyond the "true" linewidth in these situations. This effect can be seen for peak (B) in the estradiol result. These under-fitting phenomena do not tend to significantly perturb the integrals of the peaks however.

4 NMR-EsPy

All the results presented in this paper were generated using the NMR-EsPy Python package. The typical CUPID workflow is as follows:

1. Acquire a magnitude-mode J-Resolved dataset for the sample of interest. As a rule of thumb, it is recommended to have an indirect-dimension of

at least 64 points. If you can spare the time, or if you have particularly complex regions, such as with the estradiol example, more points are advised.

2. Import the data[3], phase the direct dimension, and (optionally) baseline correct[18].

3. Select direct-dimension regions of interest, generate filtered sub-FIDs for each region, and estimate the parameters of each sub-FID.

4. (Optional) make edits to correct spurious features in the estimation result if required (i.e. removal of unnecessary oscillators), and re-run the optimisation.

5. Construct the pure shift spectrum and, if desired, spectra for assigned multiplets, and export to the desired format[3].

From version 2.0, NMR-EsPy provides the functionality to easily achieve the last 4 points, either by writing Python scripts making use of the `Estimator2DJ` object, or with a graphical user interface, which can be loaded from both the command line or within TopSpin. The reader is directed to the documentation for details on usage, found at `https://foroozandehgroup.github.io/NMR-EsPy/`. The project's source code is hosted on GitHub at `https://github.com/foroozandehgroup/NMR-EsPy/`. We welcome queries on usage, bug reports, and suggestions of features to add/improvements.

5 Conclusions

We have presented CUPID, a procedure which generates pure shift spectra from 2DJ datasets via parametric estimation. CUPID can produce spectra with absorption-mode lineshapes, with full sensitivity, and as a complement can predict multiplet structures of crowded spectral regions. CUPID has been shown to be effective even in scenarios where state-of-art pure shift methods are too insensitive to provide useful results.

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[3]Currently, only data in Bruker format is supported

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