Simplifying ^{1}H-NMR Spectra in NMR Metabolic Profiling
—Applying Projections of ^{1}H homonuclear 2D *J*-resolved spectra—

Product used：Nuclear Magnetic Resonance (NMR)

Spectral overlap often hampers subsequent analyses (*ex.* multivariate analyses) in ^{1}H-NMR spectra of molecular mixtures such as in NMR metabolic profiling (NMR-MP). One of the most straightforward solution is using more higher field NMR system. However it is not always easy to address. In this note, the method simplifying ^{1}H-NMR spectra of mixture is introduced by use of projections of ^{1}H homonuclear 2D *J*-resolved spectra.

Simplifying ^{1}H-NMR spectrum by applying projections of ^{1}H homonuclear 2D *J*-resolved spectrum [1]

In the 2D *J*-resolved spectroscopy experiment, an evolution of *J*-coupling is encoded while that of chemical shift is refocused in the indirect evolution period. Therefore the proton multiplets appear at a 45 degree angle in the resultant 2D spectrum as shown in **Figure 1(a)**. Then this 2D spectrum is tilted by 45 degrees to remove *J*-couplings from x-axis. After tilting, as shown in **Figure 1(b)**, the 2D spectrum contains chemical shift and *J*-coupling alone in x- and y-axis, respectively. The projection of the x-axis is a pseudo pure shift spectrum (**Figure 1(c)**: upper). You can see how spectrum goes is simplified relative to a standard single pulse experiment.

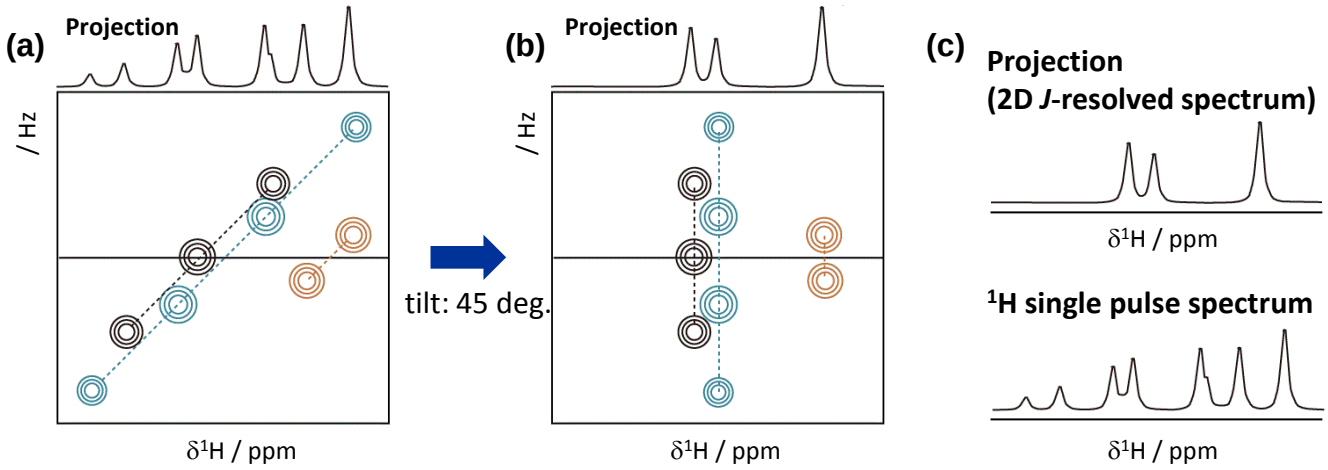

Figure 1 Simplifying ^{1}H-NMR spectrum by applying projections of ^{1}H homonuclear 2D *J*-resolved spectrum. (a) The proton multiplets appear at a 45 degree angle in original 2D spectrum. (b) After tilting, the 2D spectrum contains chemical shift and *J*-coupling in x- and y-axis, respectively. (c) The projection of (b) is more simple than the conventional ^{1}H single pulse spectrum.

Suppressing artifacts from strong couplings in *J*-resolved spectroscopy

We can record simple spectra using projections of 2D *J*-resolved spectra even though standard magnetic field NMR system such as 400 MHz system is used. However there is a drawback in *J*-resolved spectroscopy that artifacts from strong coupling can occur, especially when a lower magnetic field system is used.

As it was introduced before in our application note “Suppressing artifacts from strong couplings in 2D *J*-resolved spectroscopy” (NM050018), these artifacts can be suppressed when double spin-echo type of 2D *J*-resolved spectroscopy [2] is used.

A projection of 2D J-resolved spectrum of polar metabolites in broccoli sprout.

There are often severe spectral overlap in ^{1}H-NMR spectra when plants are used as samples for NMR-MP because they produce various kind of metabolites including plant specialized metabolites. Here, we demonstrate that using projections of 2D *J*-resolved spectra is a powerful method in actual NMR-MP studies. Polar metabolites in broccoli sprouts were extracted and used as a NMR sample. All spectra were recorded with standard a NMR system (JNM-ECZ400S spectrometer with a ROYALPROBE). The double spin-echo method was used as a 2D *J*-resolved spectroscopy experiment.

^{1}H spectra of polar metabolites in broccoli sprout are shown in **Figure 2**. **Figure 2(a)** and **(b)** show a projection of 2D *J*-resolved spectrum and ^{1}H single pulse spectrum, respectively. As many metabolites were present in the sample, there is severe spectral overlap. Using a projection of 2D *J*-resolved spectrum, we were able to record far simpler spectra than the results of a typical ^{1}H single pulse spectrum. For example, the insets in the spectra below highlight four different methyl protons in valine and isoleucine that were clearly resolved in the projection of 2D *J*-resolved spectrum **(a)** whereas they were ambiguous in ^{1}H single pulse **(b)**.

2D *J*-resolved spectroscopy is also useful to assign metabolites [3]. Please see detail of an actual NMR-MP study using projections of 2D *J*-resolved spectra in our application note “Unsupervised Multivariate Analyses for NMR-based Metabolomics” (NM170013) [4].

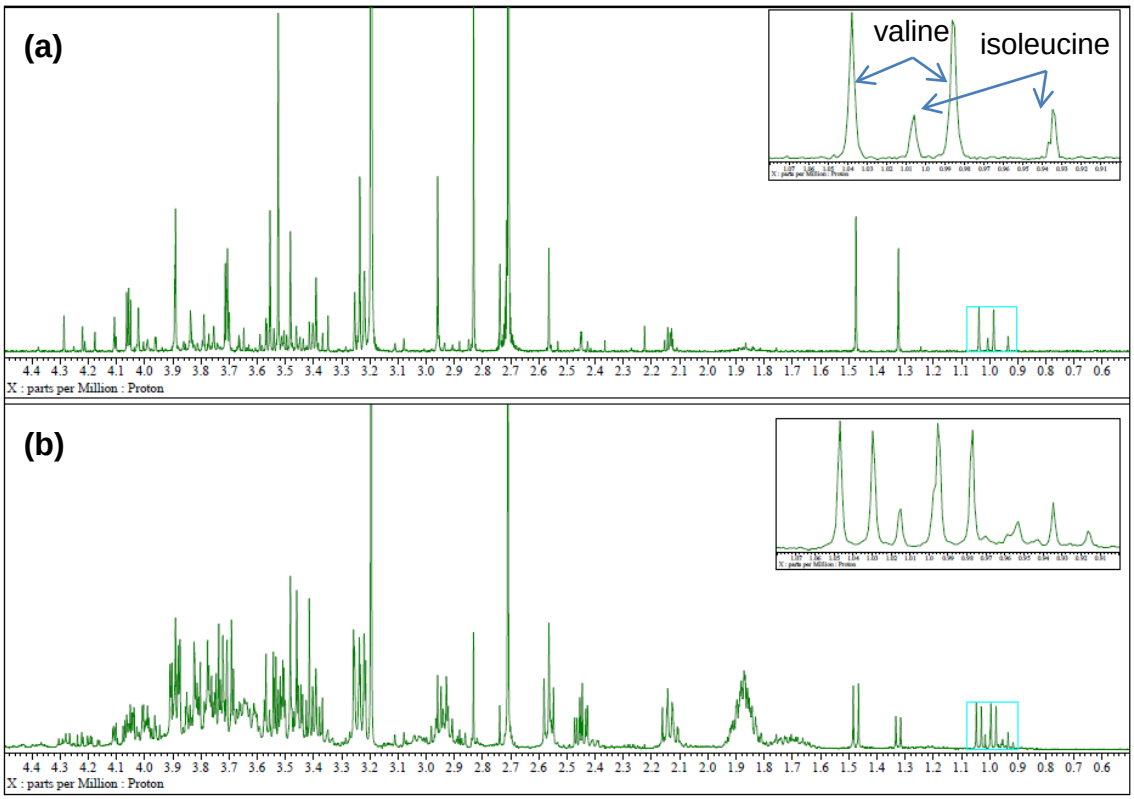

Figure 2 A comparison between a projection of 2D *J*-resolved spectrum and conventional ^{1}H single pulse spectrum of polar metabolites in broccoli sprout. (a) A projection of 2D *J*-resolved spectrum. The double spin-echo method was used. (b) ^{1}H single pulse spectrum.

References

[1] Ludwig; C., Viant; M., *Phytochem. Anal.*, **21** (2010) 22-32.

[2] Thrippleton; M. J., Edden; R. A. E., Keeler; J., *J. Magn. Reson.*, **174** (2005) 97.

[3] Kikuchi; J. et al., *Anal. Chem.*, **88** (2016) 659.

[4] Application note“*Unsupervised Multivariate Analyses for NMR-based Metabolomics*” (NM170013).

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