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A Simple Approach for Ultraclean Multisite Selective Excitation Using Excitation Sculpting

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Recently it has been demonstrated that a double pulsed-field-gradient spin echo (DPFGE) scheme, known as excitation sculpting, constitutes a powerful tool in high-resolution NMR experiments for many different purposes, such as solvent suppression (*1, 2*), selective excitation in 1D experiments (*3–8*), band-selective selection and homonuclear decoupling in the evolution dimension of multidimensional NMR experiments (*9–13*), and isotope filtering (*14–16*).

For a single pulsed-field-gradient spin echo (SPFGE) experiment, its phase properties are closely related to the phase properties of the refocusing element. The SPFGE experiment is the gradient-enhanced version of the spin-pinging sequence (*17*). In contrast to this, the excitation profile of the DPFGE experiment depends only on the inversion profile of the refocusing pulse, while the amplitude is scaled both by the inversion profile of the refocusing pulse and by losses due to relaxation during the spin echo. The main features in favor of the use of these gradient-based spin–echo schemes as the favorite selective excitation methods are (i) ultraclean pure-absorption phase 1D spectra are simply obtained without frequency-dependent phase variations of the excited signal throughout the selected region, and without sidelobes and/or sidebands outside the effective bandwith; (ii) the full refocusing of all *J*-evolution at the end of the echo; (iii) the sequence is very tolerant to miscalibrated pulses and RF inhomogeneities; and (iv) the sequence requires no phase cycling, thus avoiding the need for difference spectroscopy.

Simultaneous selective excitation at different sites could be achieved by replacing the typical selective 180° pulse in the SPFGE or DPFGE experiments by simultaneous 180° pulses applied as, for instance, amplitude modulated pulses (*18*), interleaved sequences each with a different rate of phase ramping (*19, 20*), or phase-modulated frequency-shifted laminar pulses (SLP) (*21*). In particular, SLPs have been recently used for multiple selective excitation and multiple solvent suppression in SPFGE based experiments (*22*). We present here an alternative, simplest and more user-friendly way to achieve multiple-site selective excitation using the basis of the DPFGE experiment. The basic idea is simply to apply as many concatenated selective 180° pulses as desired instead of a single selective 180° pulse (Fig. 1). The result is the same as that obtained by applying simultaneous selective 180° pulses at different frequencies, but the significant advantage relies in its highly improved performance. The analogous SPFGE experiment gives undesirable phase distorted peaks due to its improper phase properties.

The main advantage of such an approach is that each selective 180° pulse can be individually optimized, taking into account its duration and the power level applied, its frequency, its pulse shape, and its phase. In addition, the experiment is easily implemented on any spectrometer having a single channel equipped with gradient capabilities.

It is widely known that selective excitation using the DPFGE scheme affords unsurpassable results with a single scan. We have chosen a sample of the alkaloid strychnine (**1**) in $CDCl_3$ as a model. Ultraclean pure-phase multiplet excitation of the protons H-15b and H-22 is achieved with great simplicity using a 20 ms selective 180° pulse (effective bandwidth of 50 Hz) and without any residual excitation on nearby resonances (Fig. 2B and 2C). When the refocusing element in the DPFGE sequence is composed of two consecutive selective 180° pulses, they produce the same effect as if they were applied simultaneously. The spectrum containing both protons is obtained in a single scan (Fig. 2D) and keeps all features described above for the regular DPFGE excitation. This type of excitation should be equivalent to a cosine-modulated two-site excitation, in which the two desired resonances appear with the same phase. In addition, both protons show the similar integrated intensity ratio as in the conventional proton spectrum. The only drawback is the signal intensity losses due to the unavoidable T_2 relaxation effects. In Fig. 3 we show the signal dependence of the H-22 proton as a function of the length of the selective 180° pulse in a regular DPFGE experiment. In practice we can consider that selective 180° pulses smaller than 30–40 ms cause minimal losses in the signal intensitites. As a practical guide to optimize the required selectivity, Fig. 4 shows several excitation profiles for different pulse lengths.

On the other hand, the relative phase of any of the two signals can be reversed by simply changing by 90° the phase of the corresponding selective 180° pulse only in the first echo (Fig. 2E), while their relative intensities remain unchanged. This should be equivalent to a sine-modulated two-site excitation in which the two desired resonances appear in opposite phase.

This same approach can be extended to further spins.

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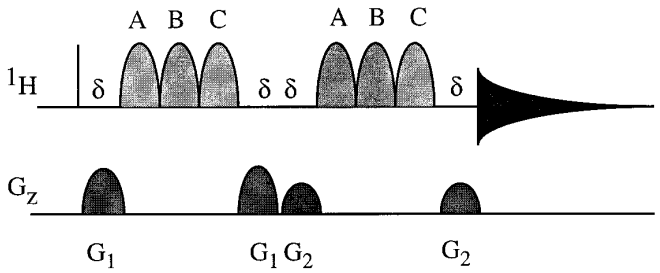
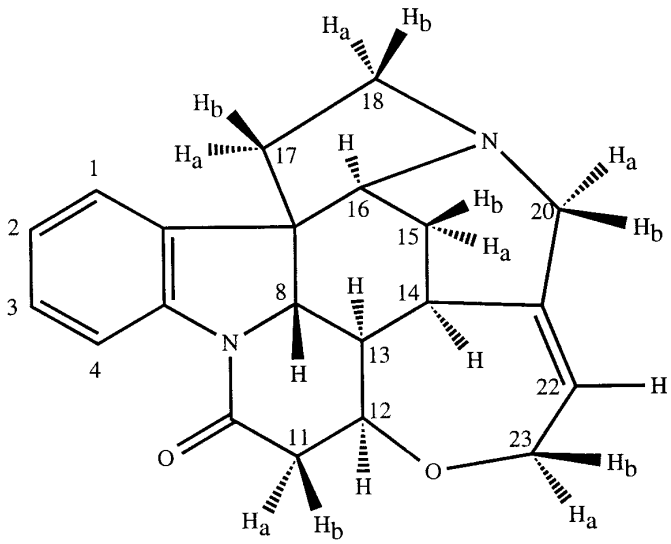


FIG. 1. General pulse sequence for multiple-site selective excitation using DPFGE. Each selective 180° pulse is applied on a specific resonance (A, B, . . .) with its particular features. All pulses are applied from *x*-axis unless otherwise indicated.

Thus, concatenating three independent selective 180° pulses we can achieve clean selection at three different sites. Figure 5B shows the effect of adding a third 30 ms selective 180° pulse applied on the H-20b proton. This should be equivalent to the application of three simultaneous selective 180° pulses. The three signals show the same phase, and the overall intensity ratio of all signals is basically governed by the length of all selective pulses. Figure 5C shows the effect of changing the phase of this third selective pulse by 90°, with the corresponding resonance inverted. Finally, in Fig. 5D a four-site selective excitation spectrum is shown in which the H-8 proton has been also excited by a selective pulse of 60 ms. As shown earlier, the phase of any of these signals can be inverted by changing the phase of the corresponding pulse in the first echo (figs. 5E and 5F).

In all the examples presented so far, there was no coupling



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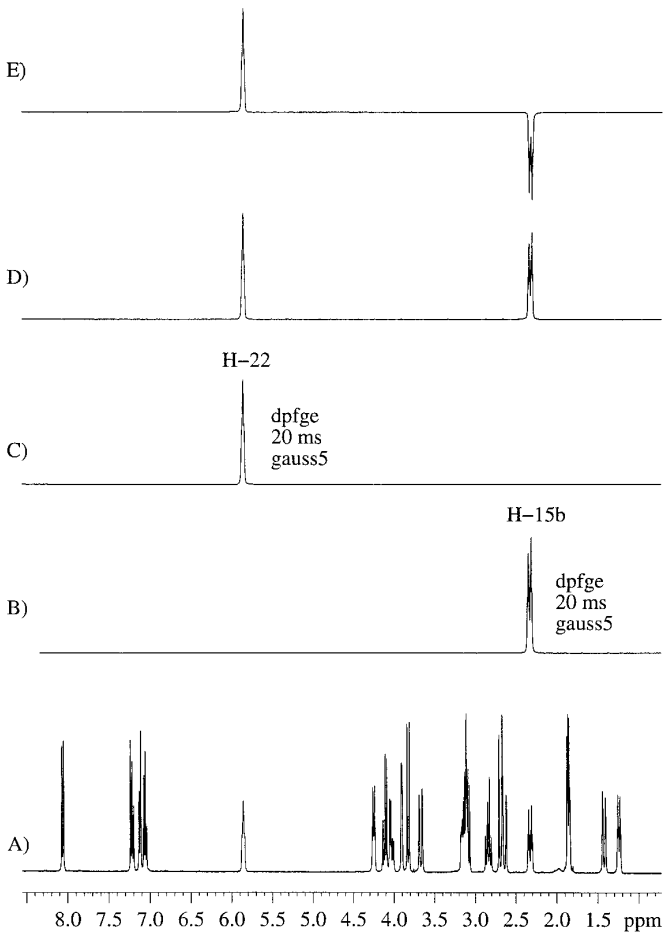

FIG. 2. (A) Conventional 400 MHz proton spectrum of strychnine. (B, C) Clean selective excitation of the H-15b and H-22 protons, respectively, achieved with a single scan using the conventional DPFGE scheme. The selective 180° pulse was 20 ms long with a Gaussian shape (5% truncation level). The strengths of the G_1 and G_2 gradients were approximately 5 and 3.5 G/cm, respectively, and their duration was 1 ms. A 100 μs recovery delay was inserted after the gradient. (D) Simultaneous clean selection of both H-15b and H-22 protons applying two independent selective 180° pulses in the middle of each echo under the same conditions as described before. (E) As in D, but changing the phase of the selective 180° pulse on the H-15b in the first echo by 90°.

between the excited spins. However, if the proposed scheme is applied to a pair of mutually coupled spins, there is evolution of this coupling during the echo because it feels both selective 180° pulses. This double resonance two-spin effect, called TSETSE experiment (*23, 24*), converts the in-phase absorption-mode magnetization into antiphase dispersion-mode magnetization. This effect has already been extensively studied by Freeman and coworkers using phase cycling (*23–28*). With our proposed scheme, we obtain the same results with a single scan (no dummy scans) and, therefore, without the need for difference spectroscopy and/or more sophisticated modes of excitation. Figure 6B shows small antiphase contributions owing to *J* evolution when applying double excitation on the pair of geminal H-15a and H-15b protons. In this case, optimum conversion to antiphase magnetization occurs by introducing an extra delay, for instance $\Delta = 10$ cm (Fig.

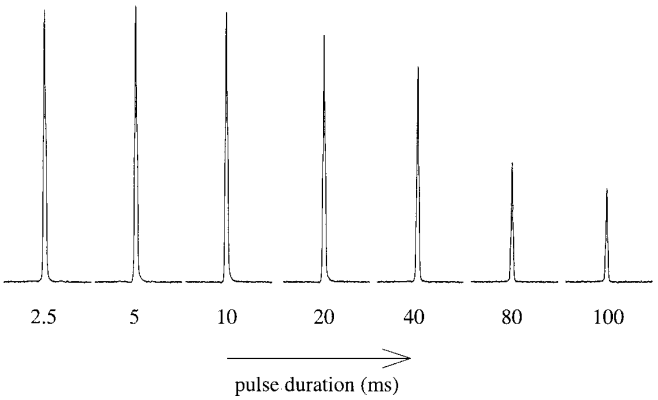

FIG. 3. Dependence of the signal intensity of the H-22 proton as a function of the duration of the selective 180° pulse in the DPFGE experiment. All experimental conditions as described in Fig. 2.

6C), as found in the TSETSE-2 experiment (*25*). This approach could be extrapolated to coupled spins in overlapped regions, thus mimicking homonuclear equivalents of the INEPT experiment, as described in refs. *26–28*.

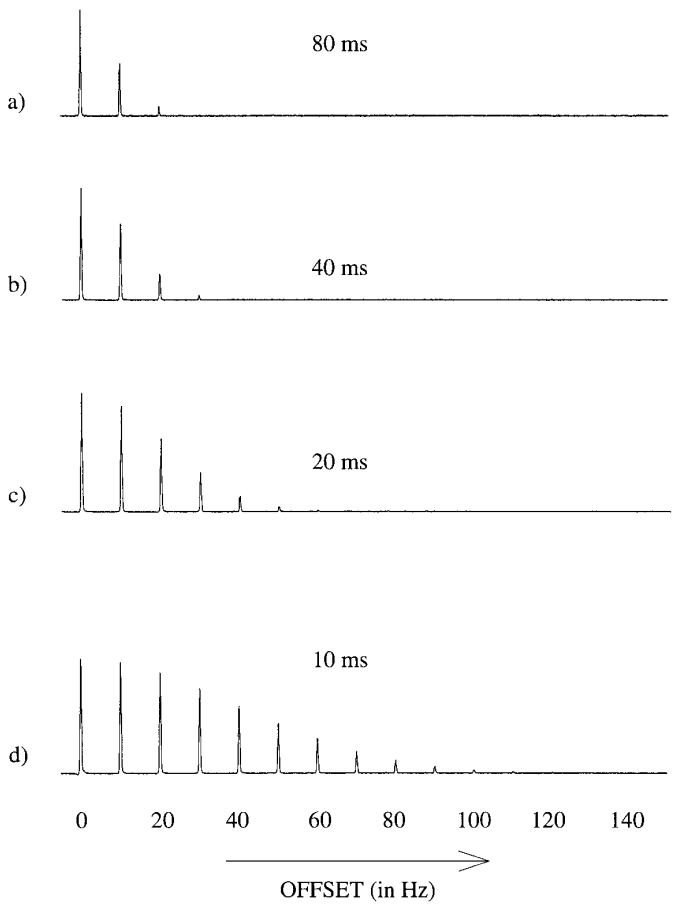

FIG. 4. Excitation profiles of the DPFGE sequence as a function of the selective 180° pulse length (Gaussian shape truncated to 5%). The well-isolated H-22 proton is the target, and the experimental conditions are the same as described in Fig. 2C with the following pulse durations: (a) 80 ms, (b) 40 ms, (c) 20 ms, and (d) 10 ms.

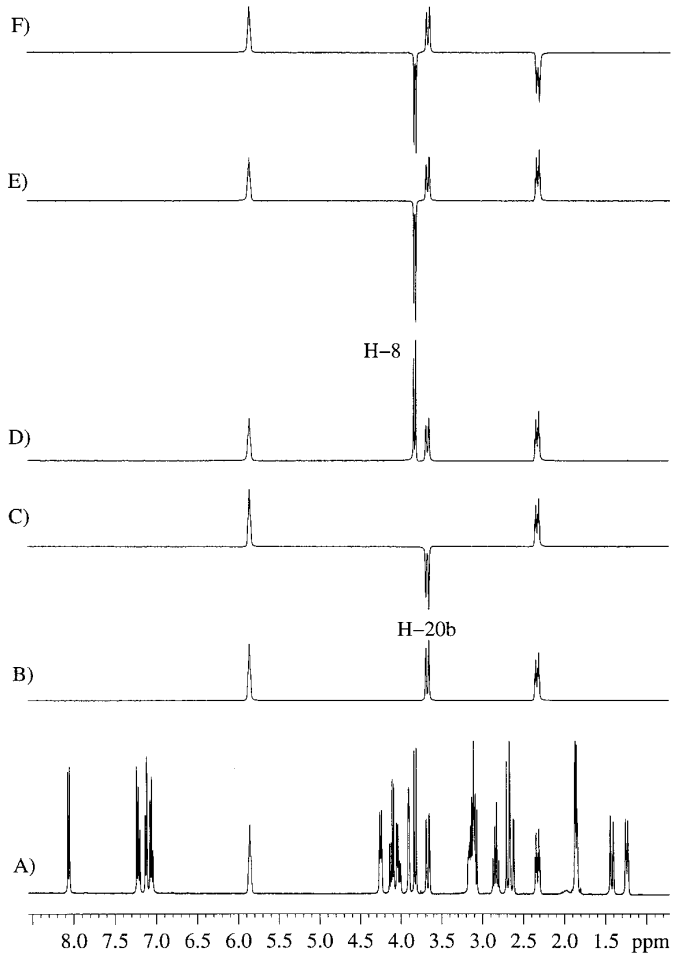

FIG. 5. (A) Conventional 400 MHz proton spectrum of strychnine. (B–F) Several examples of multiple-site selective excitation using the DPFGE scheme. (B) Three-site excitation after adding selective excitation on the H-20b proton (30 ms). (C) As in B, but only changing the phase of the selective pulse on the H-20b proton in the first echo by 90°. (D) Four-site selective excitation after adding selective excitation on the H-8 proton (60 ms). (E, F) As in D, but changing the phase of one or several selective 180° pulses by 90°. All other experimental conditions as described in Fig. 2.

In summary, we have shown that the incorporation of concatenated selective 180° pulses as the refocusing element in the conventional DPFGE sequence affords a very simple way to achieve multisite excitation without sophisticated approaches. In practice, this should be equivalent to applying all these pulses simultaneously, but the performance is highly improved because the optimization of each selective excitation block can be individualized for each site. In addition, the practical implementation is very straightforward and the excellent results obtained are highly reproducible. On the other hand, the same principles could be extrapolated to other excitation sculpting analogs. For instance, a related DPFGE scheme could be designed to efficiently suppress several intense signals as found in solutions with mixed nondeuterated solvents or to selectively excite several spectral regions using semiselective pulses. Much work is in progress.

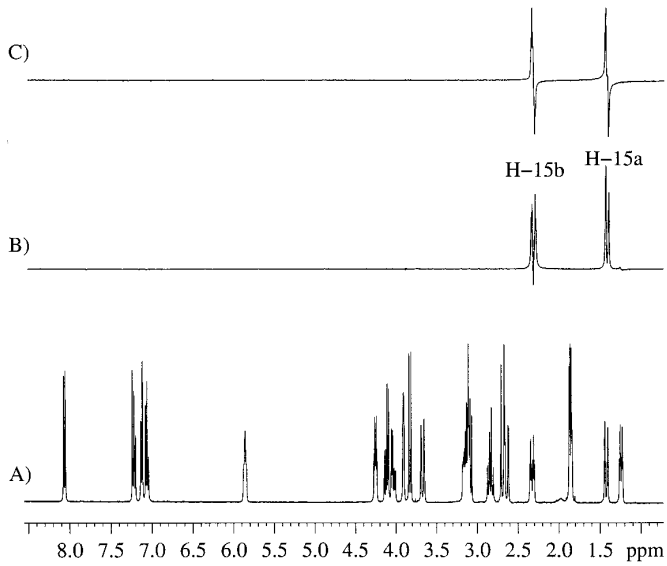

FIG. 6. Two-site excitation on mutually *J*-coupled spins. (A) Conventional 400 MHz proton spectrum of strychnine. (B) Two-site excitation on H-15b and H-15a protons using two concatenated 20 ms selective pulses. (C) As in B, but adding an evolution period Δ of 10 ms before and after each gradient echo.

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