

# Optimum spin-state selection for all multiplicities in the acquisition dimension of the HSQC experiment

Pau Nolis <sup>a</sup>, Juan Félix Espinosa <sup>b</sup>, Teodor Parella <sup>a,\*</sup>

<sup>a</sup> *Servei de Resonància Magnètica Nuclear, Universitat Autònoma de Barcelona, E-08193 Bellaterra, Barcelona, Spain*

<sup>b</sup> *Centro de Investigación Lilly, Avda. de la Industria 30, E-28108 Alcobendas, Spain*

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## Abstract

Most conventional heteronuclear spin-state-selective ( $S^3$ ) NMR experiments only work for a specific multiplicity, typically IS spin systems. Here, we introduce a general and efficient IPAP strategy to achieve  $S^3$  editing simultaneously for all multiplicities in the acquisition dimension of the HSQC experiment. Complementary in-phase (HSQC-IP) and anti-phase (HSQC-AP) data are separately recorded with a simple phase exchange of two  $90^\circ$  proton pulses involved in the mixing process of the F2-coupled sensitivity-improved HSQC pulse sequence. Additive and subtractive linear combination of these IP/AP data generates simplified F2- $\alpha/\beta$ -spin-edited HSQC subspectra for all IS,  $I_2S$ , and  $I_3S$  spin systems and combines enhanced and optimized sensitivity with excellent tolerance to unwanted cross-talk contributions over a considerable range of coupling constants. Practical aspects such as pulse phase settings, transfer efficiency dependence, inter-pulse delay optimization, and percentage of cross-talk are theoretically analyzed and discussed as a function of each  $I_nS$  multiplicity. Particular emphasis on the features associated to spin-editing in diastereotopic  $I_2S$  spin systems and application to the measurement of long-range proton–carbon coupling constants are also provided.

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**Keywords:** NMR; HSQC; Spin-state selection; Coupling constants; Spin-editing

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## 1. Introduction

The heteronuclear single-quantum correlation (HSQC) experiment is one of the most important tools for high-resolution NMR studies of molecules in solution. It is the basis of many different and very important multidimensional NMR experiments developed for the study of small-to-medium sized molecules at natural abundance and also for large isotopic labeled bio-molecules, in particular proteins and nucleic acids. Thus, any modification in the regular HSQC pulse scheme can have a great impact in the design of new and improved NMR methodologies because of the easy extrapolation on many different applications. Examples on the importance of some improvements introduced into the basic HSQC pulse sequence

have been the incorporation of pulsed-field gradients for coherence selection combined with the PEP principle for sensitivity-improved versions [1,2], the use of water-flip back techniques for a proper application in bio-molecules dissolved in non-deuterated water, or the modification of the coherence-transfer mixing period to afford specific spin-state-selective ( $S^3$ ) editing, as established in the widely known TROSY experiment [3]. The principles associated to homonuclear and heteronuclear spin-editing have emerged as an important feature in modern heteronuclear NMR spectroscopy in order to simplify spectra and to avoid peak overlapping and it has been widely used to measure scalar and residual dipolar couplings or to study the relaxation properties of the different lines belonging to the same multiplet. All  $S^3$ -based NMR experiments employ two different ways to perform the addition/subtraction procedure of the in-phase (IP) and anti-phase (AP) data: (i) design of a specific pulse train that combines coherently the two

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\* Corresponding author. Fax: +34 93 5812291.

E-mail address: [teodor.parella@uab.es](mailto:teodor.parella@uab.es) (T. Parella).

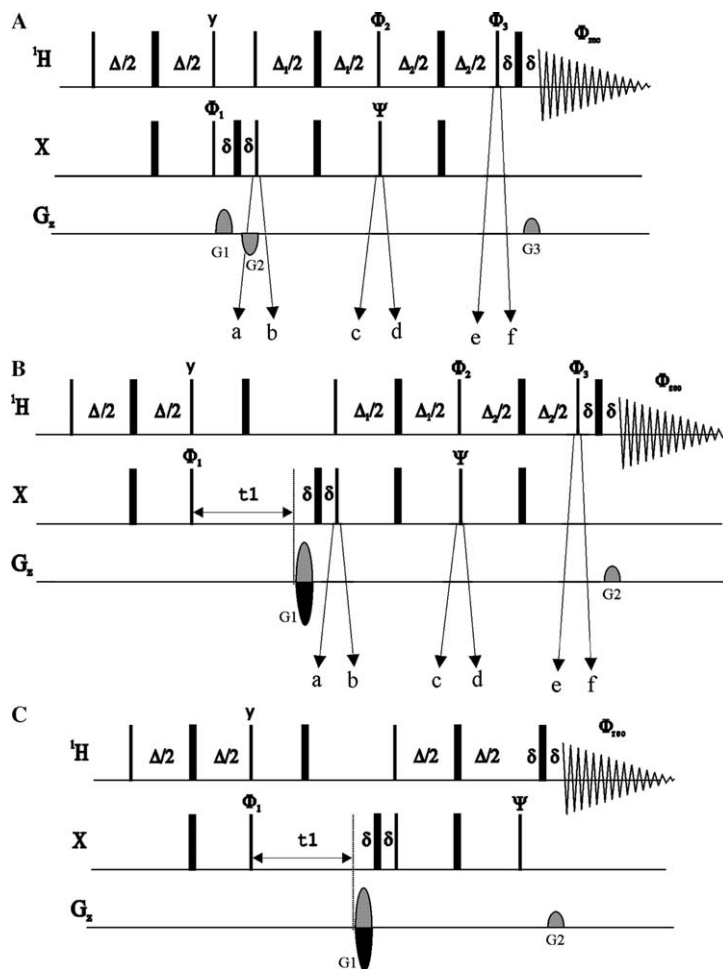


Fig. 1. Pulse sequences of the (A) 1D and (B) 2D versions of the F2-coupled  $^1\text{H}$ -X sensitivity-improved HSQC experiment to achieve spin-selected spectra simultaneously for all IS,  $\text{I}_2\text{S}$ , and  $\text{I}_3\text{S}$  spin systems: (i) HSQC-IP( $y$ ): ( $\phi_2 = y$ ,  $\phi_3 = x$ ,  $\Psi = y$ ); (ii) HSQC-AP( $y$ ): ( $\phi_2 = x$ ,  $\phi_3 = y$ ,  $\Psi = y$ ); (iii) HSQC-IP( $x$ ): ( $\phi_2 = y$ ,  $\phi_3 = x$ ,  $\Psi = x$ ); (iv) HSQC-AP( $x$ ): ( $\phi_2 = x$ ,  $\phi_3 = y$ ,  $\Psi = x$ ). (C) Original HSQC- $\alpha/\beta$  pulse train, as reported in [4–7] ( $\Psi = y$ ) and in [24] ( $\Psi = x$ ). Thick and thin rectangles represent  $90^\circ$  and  $180^\circ$  pulses, respectively. A basic two-step phase cycle was used ( $\phi_1 = \phi_{\text{rec}} = x, -x$ ). The delay  $\Delta$  is optimized to  $1/2J(\text{IS})$  whereas  $\Delta_1$  and  $\Delta_2$  are optimized as a function of the experiment and multiplicity (see Table 4 for details). See Tables 1–3 for magnetization components available at times a–f.

observable IP and AP magnetization components prior to data acquisition, or (ii) separate recording of the IP and AP components using equivalent pulse schemes followed by a post-processing mathematical protocol. In principle, this second approach should be much more attractive from the sensitivity point of view because if two spectra are added prior to acquisition, one component is lost, while both are retained when post-acquisition editing is used.

Nowadays, heteronuclear spin-editing in 2D HSQC spectra can be achieved in the directly detected F2-dimension (referred as HSQC- $\alpha/\beta$  experiment) [4–7], in the indirect F1-dimension ( $\alpha/\beta$ -HSQC or also known as IPAP-HSQC experiment) [8], or in both dimensions by E.COSY- or TROSY-type selection in F1,F2-coupled HSQC experiments ( $\alpha/\beta$ -HSQC- $\alpha/\beta$ ) [3,9,10]. These reported  $\text{S}^3$ -edited HSQC experiments only work properly for IS spin systems and they have been mainly applied to backbone NH and  $\text{C}_\alpha\text{H}_\alpha$  spin systems in labeled proteins. During the last years, a different number of methylene-specific [11–16] and methyl-specific [17–20] spin-edited HSQC-type pulse

Table 1  
Product operator components of a IS spin system present at different times of the HSQC experiment (Fig. 1B) following the evolution time  $t_1$ <sup>b,c</sup>

Phases	$t_1$ <sup>a</sup>	b	c	d	e	f
$\phi_2 = y$	$H_z S_x \cos(\Omega_S t_1)$	$H_y S_x$	$H_y S_x$	$H_y S_z$	$H_x S'$	$H_x S'$
$\phi_3 = x$	$H_z S_y \sin(\Omega_S t_1)$	$H_y S_z$	$H_x S$	$H_z S$	$H_z S$	$H_y S$
$\Psi = y$						
$\phi_2 = x$	$H_z S_x \cos(\Omega_S t_1)$	$H_y S_x$	$H_y S_x$	$H_z S_z$	$H_z S_z$	$H_x S_z$
$\phi_3 = y$	$H_z S_y \sin(\Omega_S t_1)$	$H_y S_z$	$H_x S$	$H_x S$	$H_y S_z S'$	$H_y S_z S'$
$\Psi = y$						
$\phi_2 = y$	$H_z S_x \cos(\Omega_S t_1)$	$H_y S_x$	$H_y S_x$	$H_y S_x$	$H_y S_x$	$H_y S_x$
$\phi_3 = x$	$H_z S_y \sin(\Omega_S t_1)$	$H_y S_z$	$H_x S$	$H_z S$	$H_z S$	$H_y S$
$\Psi = x$						
$\phi_2 = x$	$H_z S_x \cos(\Omega_S t_1)$	$H_y S_x$	$H_y S_x$	$H_z S_x$	$S_y S'$	$S_y S'$
$\phi_3 = y$	$H_z S_y \sin(\Omega_S t_1)$	$H_y S_z$	$H_x S$	$H_x S$	$H_y S_z S'$	$H_y S_z S'$
$\Psi = x$						

<sup>a</sup> Trigonometric factors showing chemical shift dependence are shown only on this column.

<sup>b</sup>  $\Delta = 1/(2J)$ .

<sup>c</sup> Trigonometric simplifications:  $s = \sin(\pi J_{HS} \Delta_1)$ ;  $c = \cos(\pi J_{HS} \Delta_1)$ ;  $s' = \sin(\pi J_{HS} \Delta_2)$ ;  $c' = \cos(\pi J_{HS} \Delta_2)$ .

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