

# Fast multidimensional NMR spectroscopy by spin-state selective off-resonance decoupling (SITAR)

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## PRACTICAL ASPECTS OF PREPARATION, CONFIGURATION AND ASSIGNMENT OF SITAR-SPECTRA IN CARA

Before the user can do the assignment a repository and a project with CARA must be set up. The repository is created best by using a template. The project can then be created by importing the sequence and the spectra (see <http://www.nmr.ch> or Ref. 10 for more information). Our test setup is based on Ubiquitin and contains a [<sup>15</sup>N,<sup>1</sup>H]-HMQC, SITAR-HNCA, SITAR-HNCACB and SITAR <sup>15</sup>N-resolved [<sup>1</sup>H,<sup>1</sup>H]-NOESY spectra with the SITAR-specific values  $j = 92$ ,  $cw = 102$ ,  $c = 700$  and  $dir = 'asc'$  (Table 1, see also Section on Experimental). The [<sup>15</sup>N,<sup>1</sup>H]-HMQC experiment is measured with the off-resonance decoupling applied to the SITAR experiment during the interscan delay to circumvent potential chemical shift differences between the spectra due to different sample heating.

To start the HNCA assignment procedure, the SITAR-HNCA is opened using SitarViewer (from the context menu of the CARA spectrum explorer). The contour parameters of the plane and the strips are adjusted to show a fixed threshold and positive contours only. The polarity of the projections is automatically taken into consideration by CARA when clipping off contours. Following this, the [<sup>15</sup>N,<sup>1</sup>H]-HMQC is opened in PolyScope. The SITAR-HNCA is selected for the strips. The peak width of the <sup>15</sup>N/<sup>13</sup>C strip is set to the full spectral width (~30 ppm) to display all ghost peaks within a given <sup>1</sup>H chemical shift. Ghost peaks are peaks from nonselected spin systems. This allows the user to assess whether a given intensity maximum is already assigned. In addition, the option *View/Sync to Global Cursor* and *View/Sync to Depth* is switched on in PolyScope to synchronize cursor movement with SitarViewer. The two scopes should be kept open simultaneously. The user can apply the ALT + TAB keyboard shortcut on Windows and Linux to quickly navigate between the two scopes. This may be of convenience since assignment of spin systems may be accomplished either in PolyScope by looking for <sup>1</sup>H/<sup>15</sup>N cross-peaks in the [<sup>15</sup>N,<sup>1</sup>H]-HMQC spectrum or

by the SitarViewer by looking for twin peak patterns. The first approach is identical to the procedure to identify spin systems in conventional triple-resonance experiments, whereas the latter profits from the high resolution along the coded <sup>15</sup>N dimension.

If the assignment strategy is started in PolyScope, a [<sup>15</sup>N,<sup>1</sup>H]-HMQC together with a SITAR-HNCA or SITAR-HNCACB spectrum is displayed in PolyScope (Fig. 3(A)). The cursor is placed on an unassigned intensity maximum of a cross-peak in the [<sup>15</sup>N,<sup>1</sup>H]-HMQC and a new spin system and the corresponding 3D strip is created (right side of the PolyScope shown in Fig. 3(A)). Then the <sup>13</sup>C<sup>α</sup>( $i$ ) and <sup>13</sup>C<sup>α</sup>( $i - 1$ ) peaks are identified and picked in the corresponding strip. If there is only one peak at the given <sup>1</sup>H/<sup>15</sup>N chemical shifts the assignment is usually unproblematic. Because of clipping and gradient matching (Eqn (8)) a quite good selectivity can be achieved even under noisy conditions. If there is an overlap, the use of the SitarViewer will most probably resolve the ambiguity due to its higher resolution. For using the SitarViewer, the cursor is placed on a twin peak pair with the appropriate residual scalar coupling or splitting. The offset of the split cursors in SitarViewer can be adjusted by SHIFT-dragging the mouse or by pressing the SHIFT and a cursor key at the same time. The horizontal slice display can be used to optimize position and offset of the split cursor. This procedure also identifies the <sup>1</sup>H/<sup>15</sup>N cross-peak in the [<sup>15</sup>N,<sup>1</sup>H]-HMQC and the corresponding 3D strip in PolyScope. For example, the twin peaks shown in Fig. 3(B) correspond to the spin system 38 shown in Fig. 3(A). Since SitarViewer does not yet contain peak management features, the spin system and the <sup>13</sup>C<sup>α</sup>( $i$ ) and <sup>13</sup>C<sup>α</sup>( $i - 1$ ) cross-peaks are picked and assigned in PolyScope.

If a SITAR-HNCACB or SITAR-<sup>15</sup>N-resolved [<sup>1</sup>H,<sup>1</sup>H]-NOESY is available, it is helpful to display them in a second or third SitarViewer. Since cursor movements are synchronized, the twin peaks can be simultaneously observed in the HNCA, HNCACB or NOESY.