Assignment due at beginning of labs July 23–29.

Use TopSpin on Callisto or Persephone for this HW.

This week you will learn how to setup and acquire 2D experiments, both “routine” and more advanced, using the TopSpin environment. This is a 2 week lab. Routine experiments — cosy and hsqc — will be done the 1st week. More advanced experiments — tocsy, hmbc, noesy and roesy — will be done the 2nd week.

1. Acquire high-quality “routine” 2D data (1st week)

Some types of 2D data can be acquired in a relatively standard fashion: few parameters changes need to be considered. cosy (COSYGPSW) and hsqc (HSQCEDETGPSISP, HSQCETGPSISP) are primary examples, where correlations of $^1$H to other $^1$H or $^{13}$C provide (often very) useful information. For these experiments, the following parameters should be checked:

$$d_1 \rightarrow \geq T_1$$ (longest of interest)

- default = 2s
- peptides and proteins can often be run faster, up to $d_1=1s$
- samples in O$_2$-free atmospheres: check $T_1$ values to avoid potentially serious artifacts, as relaxation can get significantly longer than “normal”

- $AQ[F2] \leq 0.2 \text{s}$ for hsqc; longer values can damage the cryoprobes (DCH and Prodigy).

$$TD[F1] \rightarrow 128 \text{ to } 512$$ is typical

- limits resolution overall for cosy, and in the $^{13}$C dimension $[F1]$ in hsqc
- F1 resolution $\approx SW[F1] / TD[F1]$
- linear prediction can improve the resolution; but TD[F1] remains as the limiting factor

$$ns \rightarrow$$ check the pulse sequence listing (toward the end) for minimum ns settings;
- for hsqc and hmbc, the 1st row should show proton peak intensities; otherwise ns should be increased; if the peaks are large, decrease ns (but not below the minimum)

(a) Acquire a proton spectrum of a sample of your choice, with knowledge of $T_1$ values for this sample. You can use the facility samples sucrose in D$_2$O, or thio-glucose in CDC$_3$ as alternatives.

(b) Acquire a $^{13}$C spectrum (if possible) of the same sample. This step is not required, but can be helpful when 1st working with hsqc and hmbc data. Similar with a dept-135 or -45: useful, but not required.

(c) Setup a cosy spectrum (rpar COSYGPSW) using the minimum ns (check in the pulse sequence).
(d) Apply sweepwidth optimization as follows:
   i) click **SET LIMITS**
   ii) right-click on the Proton spectrum
   iii) click **Display**
   iv) expand the spectrum (see below)
   v) click **OK**

(e) For COSY, both dimensions are set.

(f) Check **ns d1 TD[F1]**. Set **rg** the same as in the $^1$H 1D spectrum.

   **Q1:** Suggest why $\text{TD}[F2] \leq 8 \times \text{TD}[F1]$ is a condition usually met with homonuclear COSY spectra.

(g) Acquire a COSY spectrum. Process in TopSpin using **xfb** and **sym**. Plot and turn in.

$^1$H expansions ($\beta$ and $\gamma$) for sucrose.

$\alpha$. Full 1D spectrum of sucrose.

$\beta$. A good expansion, especially for the acquisition dimension, $F_2$, as larger $\text{SW}[F2]$ costs little. For the $F_1$ (indirect) dimension, the expansion could be a bit tighter (but not by much), improving the $F_1$ resolution. Leave ~10% of the spectrum on each edge of the spectrum.

$\gamma$. Too tight of an expansion. Proton multiplets at 5.4 and 3.35 ppm are too close to the edges, and will cause a variety of problems in the resulting 2D spectrum.

(h) For HSQC (rpar HSQCEDETGPSISP or HSQCETGPSISP) or HMBC (rpar HMBCTGPL3ND), perform step (d) for the $^1$H dimension [$F_2$].
(i) Repeat step (d) for the $^{13}$C dimension [F1], but now select the $^{13}$C or dept spectrum in part ii).

If the sample concentration is too low to acquire a $^{13}$C spectrum, the sweepwidth can be set manually. Take sucrose as an example, with its $^{13}$C spectrum as shown. It is important to keep in mind that a $^{13}$C spectrum is not needed to setup the range of $^{13}$C chemical shifts for HSQC or HMBC experiments. For sucrose, reasonable estimates (staying conservative) would be:

$$45 \text{ ppm} \leq \delta \leq 120 \text{ ppm}.$$  

To manually setup the spectra window:

- type `sw` in F1 enter: 120-45
- type `o1p` in F1 enter: $\frac{120+45}{2}$

(j) Both dimensions are now set. Check ns d1 TD[F1].

Q2: Show a calculation for the resolution in the $^{13}$C dimension? Give the answer in Hz/pt and ppm/pt.

(k) Use rga with HSQC and HMBC spectra.

(l) Acquire an HSQC spectrum. Plot and turn in.

2. Acquire a portion of two high-quality “non-routine” 2D spectra (2nd week)

2D data other than standard cosy and hsqc should only be acquired. Choose two types from hmbc, tocsy, noesy and roesy; note the parameter sets listed in the table at the end of this HW. Check and modify as needed the following parameters:

- d1 – set according to the “$T_1$ Abusability” table given in the online guide “Pulse width calibrations and $T_1$ estimates in TopSpin”

- ns – make certain ns is set to minimum value or a multiple of that value

- TD[F1] – resolution must be set according to information required

  i) some cosy-types involve J-evolution sufficient to observe small J-couplings; here we need see an unambiguous crosspeak (having sufficient intensity), and often do not need to resolve the coupling (which might not be possible in any event)

  ii) when J-couplings need to be measured in a 2D, resolution is critical; see Claridge Fig 5.50 and surrounding discussions involving anti-phase cancellation effects for J measurements

  iii) constant-time experiments may be required, especially for labeled compounds

- mix – a variety of different parameters are involved; see notes in following table
proton rf pulse lengths – a pw90 = p1 calibration may be needed, and a correction can be made:

Suppose you found:

\[ p1 = 15.4 \]
\[ plw1 = -10.3 \]

The power must be read off properly:

→ typing \( plw1 \) will not give the correct value!! rather
→ type ased ↓ or click in ACQPARS
→ read the value in the 2rd box

Now use the following command:

\[ \text{getprosol} 1H 15.4 -10.3 \]

which updates all 1H rf pulses.

(a) Choose one advanced experiment from the following table: noesy, roesy, tocsy, cosydqf
(b) Read in the parameters for the experiment, and adjust the four parameters listed above: ns d1 TD[F1] mix (e.g., = d8 in noesy types).
(c) Perform the getprosol correction if pw90/p1 is > 5% different than the default value.
(d) Do an expt or click to estimate the experiment time.
(e) Acquire at least 8 rows, but stop after 5 min (unless you need the spectrum for research purposes, and have sufficient time scheduled).
(f) Plot at least the 1st row and turn in.

3. Brief processing tips for 2D spectra in TopSpin

I (cfg) don’t like the PROCESS → PROC. SPECTRUM option available in TopSpin’s flowbar. No doubt, this antipathy has much to do with my lack of knowledge about the proc2d au routine that it runs. But in general, automated processing of 2D spectra must be balanced: ease-of-use seems useful, but knowledge about what is done is usually imperative. Improper processing often leads to significant issues with 2D data.

(a) General processing commands:

\[ \text{xfb} \] ; performs apodization (see next section), then transforms data in both dimensions
\[ \text{rser #} \] ; reads row # from the 2D serial file, and puts it in ~TEMP location; the data can now be efp ‘d, and some proton peaks should be observed (see Fig 3(a) below); if not, ns may be too small; use \[ \text{xf2m} \] ; some data need to be displayed in mixed-phase mode, such as hmbcetgpl3nd; see end comments in the pulse sequence to find out if this command is needed
Fig 3(a): rser 1; efp from an hmbc at close-to-insufficient ns (some protons are observed, but others are not). This method works well for checking ns in hsqc/hmbc type experiments. Other methods are needed to check cosy types.

(b) Phasing 2D spectra:
This is simple: click PROCESS → ADJUST PHASE (or type .ph.). Right-click on peaks and select ADD. What is not so simple is that many parameter sets have PH_mod set to no in the F1 dimension. This must be changed to pk for correcting phase in that dimension (along the columns).

(c) 2D Apodization:
SINE SSB=0 – sine curve from 0 to 180° matching TD: =0 at 0°/pts=1, =1 at 90°/pts=TD/2; =0 at 180°/pts=TD: provide resolution-enhancement, but with some loss (often considerable) in sensitivity
SSB=2 – cosine curve from 0° to 90° matching TD: =1 at 0°/pts=1, =0 at 90°/pts=TD
QSINE SSB=0 – sinebell-squared: square of the above curve; provides more resolution-enhancement with additional loss in sensitivity; standard apodization for cosy
SSB=2 – cosine-squared: square of cosine curve; provide excellent retention of signal at the beginning of the fid with a good taper to zero at the end; standard apodization for hsqc
The function is selected either directly from the PROCPARS panel (see above), or by entering `wm j`. The dialog box differs depending on whether you are in 1D or 2D mode. You can try the manual window adjustment (works only in 1D mode), which will bring up a new window after clicking OK. Bruker’s HELP is reasonably good.
## STANDARD 2D SEQUENCES

<table>
<thead>
<tr>
<th>Description</th>
<th>PARAMETER SET</th>
<th>d1&lt;sup&gt;a&lt;/sup&gt;</th>
<th>mix&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>standard</strong> (magnitude-mode) COSY <strong>&quot;routine&quot;</strong></td>
<td>¹H-¹H correlations; usually just 2- to 3-bond couplings</td>
<td>COSYGPSW cosygplrqf</td>
<td>1 to 1.5 x T&lt;sub&gt;1(loi)&lt;/sub&gt;</td>
</tr>
<tr>
<td><strong>long-range COSY</strong></td>
<td>confirm ¹H-¹H correlations with small (0.5 to 3 Hz, 2- to 5-bond) couplings</td>
<td>cosyr.UW&lt;sup&gt;c&lt;/sup&gt; cosygplrqfd</td>
<td>1 to 1.5 x T&lt;sub&gt;1(loi)&lt;/sub&gt;</td>
</tr>
<tr>
<td><strong>double-quantum filtered COSY</strong></td>
<td>strong singlets (including solvent peaks) via double-quantum filtering (DQF), and enables measurement of ¹H-¹H coupling constants; note special setup requirements in pp (?for rg?)</td>
<td>COSYGPDFPHSW cosygpmfphp</td>
<td>2 to 3 x T&lt;sub&gt;1(loi)&lt;/sub&gt;</td>
</tr>
<tr>
<td><strong>TOCSY</strong></td>
<td>¹H-¹H correlations based on couplings; 2-3 datasets differing by mix time are often required to observe &quot;relayed&quot; couplings</td>
<td>MLEVPHSW mlevphp</td>
<td>1.5 to 5 x T&lt;sub&gt;1(loi)&lt;/sub&gt;</td>
</tr>
<tr>
<td>**standard multiplicity-edited HSQC <strong>&quot;routine&quot;</strong></td>
<td>¹H-¹³C 1-bond correlations, –CH₂– inverted (dept-135 analog)</td>
<td>HSQCEDETGPSISP hsqcedetgpsisp</td>
<td>1.5 to 2 x T&lt;sub&gt;1(loi)&lt;/sub&gt;</td>
</tr>
<tr>
<td>**standard non-edited HSQC <strong>&quot;routine&quot;</strong></td>
<td>¹H-¹³C 1-bond correlations, all peaks positive (dept-45 analog)</td>
<td>HSQCETQPSISP hsqctqpsisp</td>
<td>1.5 to 2 x T&lt;sub&gt;1(loi)&lt;/sub&gt;</td>
</tr>
<tr>
<td>coupled HSQC</td>
<td>¹H-¹³C 1-bond correlations with coupling</td>
<td>HSQCETNDGPSISP hsqctndgpsisp</td>
<td>1.5 to 2 x T&lt;sub&gt;1(loi)&lt;/sub&gt;</td>
</tr>
<tr>
<td>**standard HMBC **&quot;routine&quot;</td>
<td>¹H-¹³C n-bond correlations, 2- and 3-bond (usually), with 3-fold 1-bond filter; often acquire 2&lt;sup&gt;nd&lt;/sup&gt; set with smaller cnst13</td>
<td>HMBCTETGPL3ND hmbctetgp3nd</td>
<td>1.5 to 2 x T&lt;sub&gt;1(loi)&lt;/sub&gt;</td>
</tr>
<tr>
<td><strong>NOESY</strong></td>
<td>¹H-¹H correlations based on proximity (also for exchange)</td>
<td>NOESYGP noesypgphp</td>
<td>2 to 5 x T&lt;sub&gt;1(loi)&lt;/sub&gt;</td>
</tr>
<tr>
<td><strong>ROESY</strong></td>
<td>¹H-¹H correlations based on proximity; for intermediate MW</td>
<td>ROESYPHPR roesyphr</td>
<td>2 to 5 x T&lt;sub&gt;1(loi)&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

### SELECTIVE 1D SEQUENCES

<table>
<thead>
<tr>
<th>Description</th>
<th>PARAMETER SET</th>
<th>d1&lt;sup&gt;a&lt;/sup&gt;</th>
<th>mix&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>selective COSY-1D</td>
<td>protons 2- to 6-bonds from selected multiplet give antiphase peaks; d&lt;sub&gt;4&lt;/sub&gt;= large (≤ T&lt;sub&gt;1&lt;/sub&gt;; for small couplings) can be used; coupling will transfer through heterobonds</td>
<td>SELCOGP selcogp</td>
<td>1.5 to 3 x T&lt;sub&gt;1(loi)&lt;/sub&gt;</td>
</tr>
<tr>
<td>selective NOESY-1D</td>
<td>protons within 5Å produce NOEs; phase selected peak negative, then other peaks are positive for small MW, negative for large MW; exchange will produce negative peaks; acquire a mix time series, plot build-up curve to confirm NOE</td>
<td>SELNOGP selnogp</td>
<td>2 to 5 x T&lt;sub&gt;1(loi)&lt;/sub&gt;</td>
</tr>
<tr>
<td>selective ROESY-1D</td>
<td>protons within 5Å produce ROEs; phase selected peak negative, all other peaks are positive independent of MW; acquire a mix time series, plot build-up curve to confirm ROE</td>
<td>SELROGP selrogp</td>
<td>2 to 5 x T&lt;sub&gt;1(loi)&lt;/sub&gt;</td>
</tr>
<tr>
<td>selective TOCSY-1D</td>
<td>protons 2- to 3-bonds from selected multiplet give in-phase peaks; only couplings ≥ 3 Hz transfer; couplings will not go through heterobonds; use d&lt;sub&gt;9&lt;/sub&gt; series to see coupling &quot;relays&quot;</td>
<td>SELMLGP selmlgp</td>
<td>1.5 to 5 x T&lt;sub&gt;1(loi)&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> loi = longest of interest  
<sup>b</sup> foi = fastest of interest  
<sup>c</sup> these parameter sets are located in the par/user folder (all others are in par, in /opt/topspin3.1/exp/stan/nmr/)  
<sup>d</sup> these pulse sequences are located in the pp/user folder (all others are in pp, in /opt/topspin3.1/exp/stan/nmr/lists/)