Routine 1D NMR Experiments (1H, 13C, 19F, 31P, etc)
  Procedure for acquiring 1D Proton spectrum
  Procedure for acquiring routine 1D Carbon spectrum
  Other 1D X-Nuclei Experiments: 19F, 31P, 11B, etc

Common 2D NMR Experiments (COSY, HSQC, HMBC, NOESY, etc)
  2D COSY, HSQC, HMBC – Through Bond
  2D NOESY, ROESY – Through Space

Variable Temperature NMR experiments (contact Paul Ralifo)

Sample Preparation

1. Dissolve your sample in an appropriate 0.55 to 0.7 mL of deuterated NMR solvent. Make sure there is no un-dissolved material. If there is, you will need to either centrifuge or filter your sample to remove crystals/debris. TIP: use a small amount of KimWipe in a Pasteur Pipette to quickly filter out any undissolved material.

2. Transfer your sample solution into a clean NMR tube. We recommend high-quality NMR tubes - rated 600 MHz or higher, but economy tubes will be OK for routine work. Your sample height should be about 4 - 5 cm.

Caution: do NOT remove or add your sample while the carousel is moving, and do NOT reach across the area where the sensor is located. Tripping the sensor with your hand may result in shutdown of the autosampler.
Experiment: Routine Proton NMR
Parameter Set: PROTON

Login and Startup
Log into your iconnmr user account. If a previous user is currently logged in, you will need to select Change User, then log into your account.

Set Initial Parameters
1. Identify an empty/available holder in the autosampler.
2. Place your sample into an available blue spinner, set depth using the depth gauge, and drop your sample off in the appropriate holder. **Do NOT drop off your sample while the carousel or sample-arm is moving.**
3. Identify the matching holder in IconNMR menu and double-click to add an experiment.
4. Enter your details: Data Directory, Experiment Name, Experiment Number, Solvent, Experiment/Parameter Set.

Check/Edit Acquisition Parameters
5. If desired, hit the = button to edit standard Proton acquisition parameters including number of scans, spectral width.
6. Edit the title if desired. You can add any sample/experiment description in this text box.
7. If you would like to also collect another experiment on this sample (e.g., C13CPD), you can highlight the current experiment and then hit the Add button. Another experiment will appear below with experiment number 11 and an empty experiment type. Select the appropriate experiment and edit acquisition parameters if necessary.
8. **For experiments longer than 30 mins click the ☀ to toggle to ☽ for nightQ.** You will need to reserve the nightQ on the NMR reservation calendar.
9. When you are satisfied with all experiments and are ready to collect your data, highlight the appropriate experiment or holder directory and hit the Submit icon.
Experiment: Routine 1D Carbon NMR
Parameter Set: C13CPD

Set Initial Parameters
1. Identify an empty holder in the autosampler.
2. Place your sample into an available blue spinner, set depth using the depth gauge, and drop your sample off in the appropriate holder. **Do NOT drop off your sample while the carousel or sample-arm is moving.**
3. Identify the matching holder in IconNMR menu and double-click to add an experiment.
4. Enter your details: Data Directory, Experiment Name, Experiment Number, Solvent, Experiment/Parameter Set. If you are collecting both Proton and Carbon, you can hit the Add button and select both experiments from the drop-down list.

5. Click the button to edit standard Carbon acquisition parameters including number of scans, spectral width, acquisition time, offset frequency, etc. **Note:** The default acquisition parameters for 13C are good, but if you have high sample concentration (>= 20mg) the default of 1024 scans may be overkill. Try changing to 256 scans for concentrated samples.
6. Edit the title if desired. You can add any sample/experiment description in this text box.
7. **For experiments longer than 30 mins click the ☀ to toggle to ☾ for nightQ.** You will need to reserve the nightQ on the NMR reservation calendar.
8. When you are satisfied with all experiments and are ready to collect your data, **highlight** the appropriate experiment or holder directory, and hit the Submit icon.
Experiment: 1D X-Nuclei NMR Experiments (19F, 31P, 11B, etc)

Set Initial Parameters

1. Identify an empty holder in the autosampler.
2. Place your sample into an available blue spinner, set depth using the depth gauge, and drop your sample off in the appropriate holder. **Do NOT drop off your sample while the carousel or sample-arm is moving.**
3. Identify the matching holder in IconNMR menu and double-click to add an experiment.
4. Enter your details: Data Directory, Experiment Name, Experiment Number, Solvent, Experiment/Parameter Set.
5. Select relevant X-nuclei from dropdown experiment list. Parameter sets are typically named based on nucleus and the type of 1H decoupling used (i.e., no decoupling, CPD for standard decoupling, IG for inverse-gated decoupling).

Check/Edit Acquisition Parameters

6. If desired, hit the equals button to edit standard Carbon acquisition parameters including number of scans, spectral width, acquisition time, offset frequency, etc. **Note:** For each routine 1D X-Nuclei experiment you should be able to edit all important acquisition parameters using the Equals icon. Be sure to set your center / offset O1P to the expected center of your spectrum.
7. Edit the title if desired. You can add any sample/experiment description in this text box.
### Acquisition Parameters – Most Common X-Nuclei Experiments, 19F, 31P, 11B, 29Si

<table>
<thead>
<tr>
<th>Experiment Description</th>
<th>Parameter Set</th>
<th>Parameter Name</th>
<th>Parameter</th>
<th>Default Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>FID NMR with no decoupling</td>
<td>FID</td>
<td>Number of Points</td>
<td>TD</td>
<td>128k</td>
<td>This is recommended</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Number of Scans</td>
<td>NS</td>
<td>16</td>
<td>Set based on your signal intensity.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dummy Scans</td>
<td>DS</td>
<td>4</td>
<td>Default is 4.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spectral Width</td>
<td>SW</td>
<td>240 ppm</td>
<td>Feel free to change SW based on prior knowledge of your system. Be sure to set D1P based on where you expect your signals to be, and be mindful of acquisition time.</td>
</tr>
<tr>
<td>FID NMR with 1H Decoupling</td>
<td>F19CPD</td>
<td>Acquisition Time</td>
<td>AQ</td>
<td>0.7 seconds</td>
<td>Set based on TD. Be aware of how long you need to acquire data based on how long your FID rings out. If your FID dies quickly you should use fewer points.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Relaxation Delay</td>
<td>D1</td>
<td>1 second</td>
<td>You will need to increase D1 for slow-relaxing resonances. Center of spectrum. You should move this to the center of where you expect your signals to show up.</td>
</tr>
<tr>
<td>31P</td>
<td>P31</td>
<td>31P Offset</td>
<td>O1P</td>
<td>-100 ppm</td>
<td>Center of spectrum. You should move this to the center of where you expect your signals to show up.</td>
</tr>
<tr>
<td>31P with no Decoupling</td>
<td>P31CPD</td>
<td>Number of Points</td>
<td>TD</td>
<td>64k</td>
<td>This is recommended</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Number of Scans</td>
<td>NS</td>
<td>16</td>
<td>Set based on your signal intensity.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dummy Scans</td>
<td>DS</td>
<td>4</td>
<td>Default is 4.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spectral Width</td>
<td>SW</td>
<td>400 ppm</td>
<td>Feel free to change SW based on prior knowledge of your system. Be sure to set D1P based on where you expect your signals to be, and be mindful of acquisition time.</td>
</tr>
<tr>
<td>31P with 1H Decoupling during acquisition</td>
<td>P31CPD</td>
<td>Acquisition Time</td>
<td>AQ</td>
<td>0.5 seconds</td>
<td>Set based on TD. Be aware of how long you need to acquire data based on how long your FID rings out.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Relaxation Delay</td>
<td>D1</td>
<td>2 seconds</td>
<td>You will need to increase D1 for slowly-relaxing resonances. Center of spectrum. You should move this to the center of where you expect your signals to show up.</td>
</tr>
<tr>
<td>31P Offset</td>
<td>O1P</td>
<td>50 ppm</td>
<td></td>
<td></td>
<td>Center of spectrum. You should move this to the center of where you expect your signals to show up.</td>
</tr>
<tr>
<td>11B</td>
<td>11B2G</td>
<td>Number of Points</td>
<td>TD</td>
<td>64k</td>
<td>This is recommended</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Number of Scans</td>
<td>NS</td>
<td>128</td>
<td>Set based on your signal intensity.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dummy Scans</td>
<td>DS</td>
<td>4</td>
<td>Default is 4.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spectral Width</td>
<td>SW</td>
<td>200 ppm</td>
<td>Feel free to change SW based on prior knowledge of your system. Be sure to set D1P based on where you expect your signals to be, and be mindful of acquisition time.</td>
</tr>
<tr>
<td>11B with no decoupling</td>
<td>11B2G</td>
<td>Acquisition Time</td>
<td>AQ</td>
<td>1.3 seconds</td>
<td>Set based on TD. Be aware of how long you need to acquire data based on how long your FID rings out. If your FID dies quickly you should use fewer points.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Relaxation Delay</td>
<td>D1</td>
<td>1 second</td>
<td>You will need to increase D1 for slow-relaxing resonances. Center of spectrum. You should move this to the center of where you expect your signals to show up.</td>
</tr>
<tr>
<td>11B Offset</td>
<td>O1P</td>
<td>0 ppm</td>
<td></td>
<td></td>
<td>Center of spectrum. You should move this to the center of where you expect your signals to show up.</td>
</tr>
<tr>
<td>29Si</td>
<td>5029Si</td>
<td>Number of Points</td>
<td>TD</td>
<td>64k</td>
<td>This is recommended</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Number of Scans</td>
<td>NS</td>
<td>128</td>
<td>Set based on your signal intensity.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dummy Scans</td>
<td>DS</td>
<td>4</td>
<td>Default is 4.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spectral Width</td>
<td>SW</td>
<td>400 ppm</td>
<td>Feel free to change SW based on prior knowledge of your system. Be sure to set D1P based on where you expect your signals to be, and be mindful of acquisition time.</td>
</tr>
<tr>
<td>29Si with inverse-gated decoupling</td>
<td>5029Si</td>
<td>Acquisition Time</td>
<td>AQ</td>
<td>1 sec</td>
<td>Set based on TD. Be aware of how long you need to acquire data based on how long your FID rings out.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Relaxation Delay</td>
<td>D1</td>
<td>5 seconds</td>
<td>512 typically have long T1’s. You should try the 512 DEPT experiment if your 512 sites have couplings with 1H. Center of spectrum. You should move this to the center of where you expect your signals to show up.</td>
</tr>
<tr>
<td>29Si Offset</td>
<td>O1P</td>
<td>100 ppm</td>
<td></td>
<td></td>
<td>Center of spectrum. You should move this to the center of where you expect your signals to show up.</td>
</tr>
</tbody>
</table>
8. For experiments longer than 30 mins click the ☀ to toggle to ☽ for nightQ. You will need to reserve the nightQ on the NMR reservation calendar.

9. When you are satisfied with all experiments and are ready to collect your data, highlight the appropriate experiment or holder directory, and hit the Submit icon.

**Experiment: Routine 2D Through-Bond Correlation NMR Experiments**

Parameter Sets: COSY, HSQC, HMBC

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1. Setup a new experiment and load a routine PROTON. You will always need to collect a Proton experiment prior to any proton-detected 2D experiments.

2. After creating your initial PROTON experiment, hit Add and select the relevant 2D NMR experiment or experiments. In this example, a routine 1H-1H COSY experiment is selected. Note, there may be multiple variants of each experiment. Feel free to try other versions of these experiments. Be sure that your F2 reference has the same name and experiment number as your routine 1H experiment or your experiment will fail in iconnmr. In this example the 1H experiment number is 10 and so is the F2 reference.

3. For experiments longer than 30 mins click the ☀ to toggle to ☽ for nightQ. You will need to reserve the nightQ on the NMR reservation calendar.

4. **Edit Basic Acquisition Parameters (Optional but recommended):** Select the = button to change basic parameters including number of scans, dummy scans, number of points, etc. Note, your spectral width SW is set automatically based on your F2 reference, so no need to change this. If you want to change the resolution (number of points) in the indirect (F1) dimension, you will need to change the parameter 1TD mostly by doubling the default value. Recommended values suggested below.

5. **Submit your experiment:** Highlight ALL experiments you want to submit, or highlight the root directory, then hit Submit. Your experiments should now be added to the queue.
**Recommended parameters to edit:**

**I. COSY (COSYGPSW)**
   a. 1TD = 128 (default) for better resolution change this value to 256 or 512 if you have a crowded 1H NMR spectrum. This parameter is the size of your FID in the F1 dimension.
   b. NS = 1 (default) change the number of scans depending on your sample concentration.

**II. HSQC (HSQCGP)**
   a. 1TD = 256 (default) If you have a crowded C13 NMR spectrum, you should increase this to 400 or 512 for better resolution in your F1 dimension.
   b. NS = 2 (default) change the number of scans depending on your sample.

**III. HMBC (HMBCGP)**
   a. 1TD =128 (default) If you have a crowded C13 NMR spectrum, you should increase this to 400 or 512 for better resolution in your F1 dimension.
   b. NS = 2 (default) change the number of scans depending on your sample.

When you are done, log out of your user account.

**Experiment:** Routine 2D Through-Space Correlation NMR (NOESY and ROESY)
**Parameter Sets:** NOESYPHSW, ROESYETGP

1. Setup a new experiment and load a routine PROTON. You will always need to collect a Proton experiment prior to any proton-detected 2D experiments.
2. After creating your initial PROTON experiment, hit Add and select the relevant 2D NMR experiment or experiments. In this example, the 2D NOESY experiment is selected. Note, there may be multiple variants of each experiment. Feel free to try other versions of these experiments. Ask Paul for suggestions on which experiments will be best for your needs. Be sure that your F2 reference has the same name and experiment number as your routine 1H experiment! Otherwise IconNMR will fail because it is looking for an F2 reference.
3. For experiments longer than 30 mins click the ☀ to toggle to ☽ for nightQ. You will need to reserve the nightQ on the NMR reservation calendar.

4. Edit Basic Acquisition Parameters (Optional but recommended): Select the = button to change basic parameters including number of scans, mixing time, size of FID in the F1 dimension etc.

5. Submit your experiment: Highlight ALL experiments you want to submit, or highlight the root directory, then hit Submit. Your experiments should now be added to the queue.

6. Below are recommended parameters for both the NOESY and the ROESY:

I. 2D NOESY – (NOESYPHW)
   a. 1TD = 256 (default) is enough in most cases but for very crowded 1H NMR spectrum change to 512.
   b. D8 = 0.3 (default NOE mixing time) – 0.5s are good values for small or medium size molecules. For molecules between 1000 and 1500kDa change to 0.1 to 0.3s. For larger molecules (>1500kDa use 0.1s or consider running a ROESY instead).
   c. NS = number of scans will depend on your sample.

II. ROESY: - (ROESYETGP)
   a. 1TD = 256 (default) is enough in most cases but for very crowded 1H NMR spectrum change to 512.
   b. P15 = 0.2s (default ROESY mixing time) is generally good. Do not go longer than 0.3s. For more quantitative results try shorter spin-lock time. Note in ICON NMR this value is in µsec (200000 µsec).
   c. NS = number of scans will depend on your sample.