X-Pulse NMR Spectrometer

Version : QI1

X-Pulse ,an Oxford Instruments benchtop NMR spectrometer, operates with a magnetic field of 1.4 Tesla. This is equivalent to proton frequency of 60 MHz.

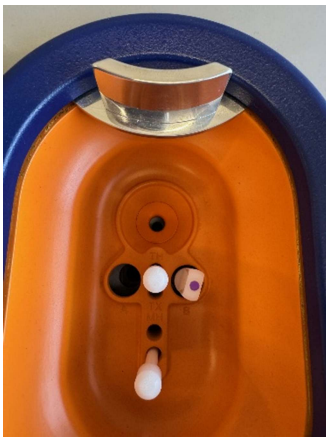
X-Pulse is a benchtop NMR system to be designed to operate over a broad range of nuclei and can be tuned to a range of operating frequencies between 60 and 9 MHz, dependent on the nucleus required for analysis

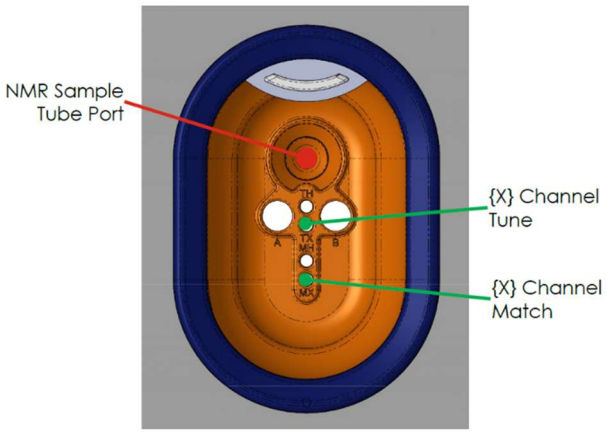
Nucleus	Gyromagnetic ratio (MHz/T)	NMR frequency @ 1.4 T (MHz)	$^{1}H/^{19}F$-only probe	Broadband probe
^{1}H	42.58	59.70	✓	✓
^{19}F	40.06	56.17	✓	✓
^{31}P	17.24	24.17		✓
^{7}Li	16.55	23.20		✓
^{11}B	13.66	19.15		✓
^{23}Na	11.26	15.79		✓
^{13}C	10.71	15.01		✓
^{29}Si	8.46	11.86		✓
^{2}H	6.54	9.16	✓ lock-channel only	✓ lock-channel only

Sample Preparation and Handling

- All sample preparation should be carried out away from the instrument.
- Samples must be prepared in 5 mm diameter, standard NMR tubes.
- Users should use NMR-grade tubes, with a minimum rating of 100 MHz.
- The recommended sample volume is 0,5 mL; greater volumes may be used. A minimum volume of 0,4 mL is required. Samples should be sealed using an NMR tube cap and appropriately labelled.

- **Care must also be taken when changing samples.** Do not force a sample tube into the spectrometer. The sample tube should insert smoothly into spectrometer. When removing the sample, lift the sample tube vertically and ensure that the bottom of the sample tube is clear of the spectrometer before moving it laterally.





Since the optimal scans for each sample are determined based on the molecular weight of the compound, the multiplicity of the signals and the quality of the spectrum required, the indications in this document are merely indicative and contemplate:

- compounds with molecular weights in the order of 200-400 g/mol
- complete solubility
- optimal volume in the NMR tube (liquid height 5 cm)

If you anticipate a high multiplicity or if you want a very good signal/noise ratio, increase the suggested scans.

Quantity (mg)	Number of scans (ns)		
	^{1}H / ^{19}F	$^{31}P\{^{1}H\}$	$^{13}C\{^{1}H\}$
15-20	**4-16 / 32**	**64-128**	**3000-4000**

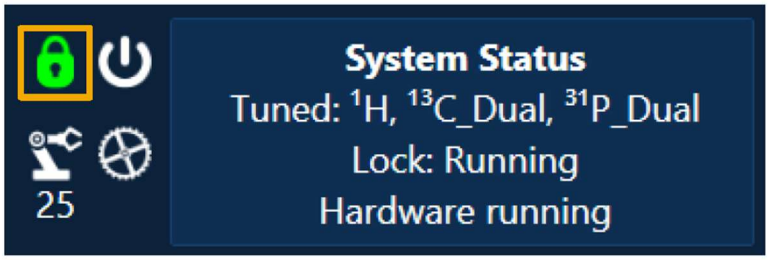
If you need to acquire two-dimensional experiments, you can only modify the scans. The experiment durations with the default accumulations, which include samples of between 15-20 mg, are as follows:

2D gCOSY	**40 min**
2D gHSQC	**1:30 min**
2D HMBC	**3 h**
2D NOESY	**4 h**

2 The Lock

2.1 External Lock

The X-Pulse external lock will automatically initialise when SpinFlow is opened. When the external lock is running the ‘Lock’ icon at the top-right of the SpinFlow window will appear **green**.



The external lock can be manually activated or deactivated by clicking on the ‘Lock’ icon; when the external lock is not running, the ‘Lock’ icon will appear red.

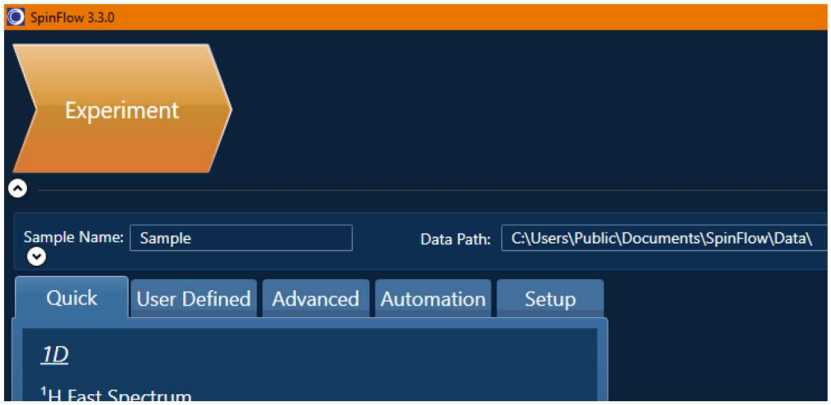
2.2 Internal Lock

To manually turn on the deuterium lock, insert an NMR tube containing a sample dissolved in deuterated solvent into the probe, and then click on the ‘Lock’ button at the top-right of the SpinFlow window.

3. Running an Experiment

3.1 Experiment Workflow overview

The Experiment workflow screen enables a user to run any experiment from any of the available experiment tabs.

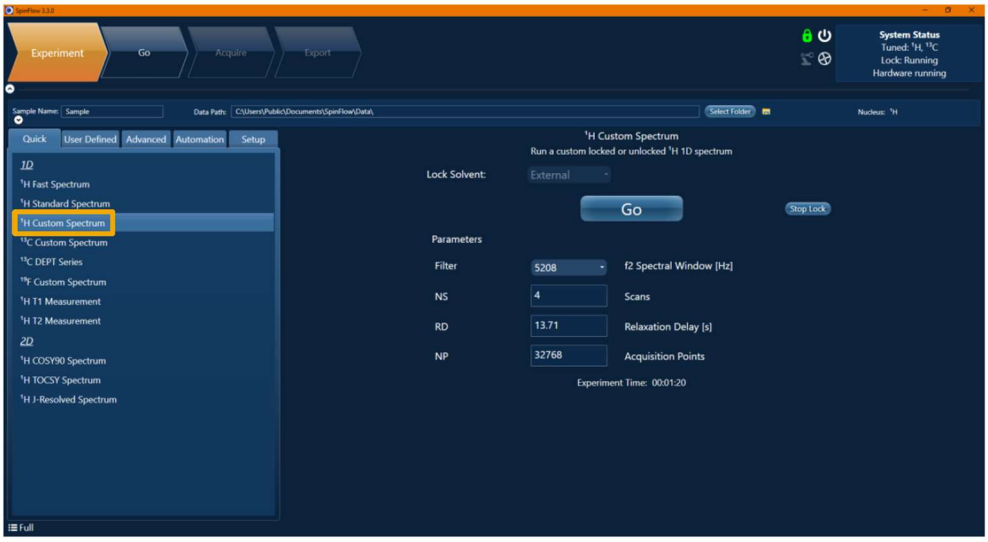


Below the ‘Experiment’ workflow button is the Sample Details panel. To expand this panel, click on the down arrow button in the lower left-hand corner of the panel to display additional information fields.

3.2 Quick Tab

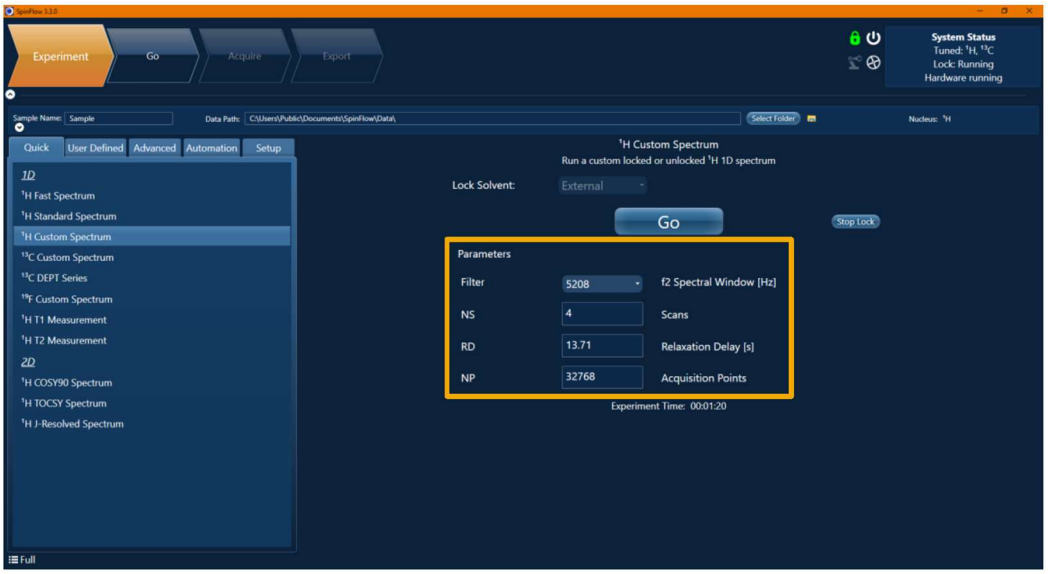
The Quick tab contains a set of pre-defined, easy-to-run experiments that have been designed with the non-expert user in mind. Depending on the Quick Experiment chosen, there are minimal or no parameters which need to be set by the user. **1D Quick Experiments can be run unlocked or locked using the deuterium lock. 2D Quick Experiments must be run using the deuterium lock.**

To run any of the quick experiments, select an experiment by clicking on it.



If the lock is not currently running, the lock will be automatically turned on before the experiment begins.

Next, adjust any of the available parameters and press ‘Go’ to run the experiment



The experiment time will be displayed under the parameters.

3.3 1D Quick Experiments

The one-dimensional (1D) quick experiments available in SpinFlow are summarised below,

Quick Experiment	Available parameters	Brief Description
¹H Fast Spectrum	*none*	The ¹H Fast Spectrum experiment allows the user to obtain a 1-scan ¹H 1D spectrum with one click. All the parameters are pre-set for this experiment to values that will work for most samples, assuming the sample is concentrated enough to produce useful data in a single scan.
¹H Standard Spectrum	RD	The ¹H Standard Spectrum experiment enables the user to obtain an 8 scan ¹H 1D spectrum either with one click (using the default parameters), or with a user-defined relaxation delay.
¹H Custom Spectrum	Filter NS RD NP	The ¹H Custom Spectrum experiment enables the user to run a quick ¹H experiment, while also allowing some parameters, including the number of scans, to be modified as desired.

^{13}C Custom Spectrum	Filter NS RD NP	The ^{13}C Custom Spectrum experiment allows the user to easily obtain a ^{13}C{^{1}H} 1D NMR spectrum with minimal user intervention. While the ^{13}C 1D experiment is running, the FIDs will be displayed on the Acquire screen. Note that because ^{13}C is a much less sensitive nucleus than ^{1}H, the displayed data may look like noise; this is perfectly normal. Also, because of the lower sensitivity, many more scans will generally be required for a ^{13}C 1D experiment than would be needed for a ^{1}H 1D experiment.
^{13}C DEPT Series	NS RD	The ^{13}C DEPT Series experiment enables the user to run the DEPT series of 1D experiments, DEPT-45, DEPT-90, and DEPT-135, in a single easy experiment. While the DEPT experiments are running, the FIDs will be displayed on the Acquire screen. Note that because ^{13}C is a much less sensitive nucleus than ^{1}H, the displayed data may look like noise; this is perfectly normal. Also, because of the lower sensitivity, more scans will generally be required for a DEPT experiment than would be needed for a ^{1}H 1D experiment. However, due to polarization transfer from ^{1}H to ^{13}C, fewer scans are typically needed for a DEPT experiment than for a standard ^{13}C 1D experiment.
^{19}F Custom Spectrum	Filter NS RD NP	The ^{19}F Custom Spectrum experiment enables the user to run a quick ^{19}F 1D experiment. Before running this ^{19}F experiment, the probe should be tuned to ^{19}F using the Tune and Match optimisation in the Setup tab (see the ^{19}F Tune and Match section for details).

3.4 2D Quick Experiments

The two-dimensional (2D) quick experiments available in SpinFlow are summarised below, for further details on the modifiable parameters, see the parameters section.

Quick Experiment	Available parameters	Brief Description
^{1}H COSY90 Spectrum	Filter NS RD NP2 NP1	The ^{1}H COSY90 Spectrum Quick experiment enables the user to easily obtain a ^{1}H gradient selected 2D COSY90 (**Co**rrelation **S**pectroscop**y**) experiment in as little as a few minutes.
^{1}H TOCSY Spectrum	Filter NS RD NP2 NP1 D12	The ^{1}H TOCSY Spectrum Quick experiment enables the user to obtain a ^{1}H 2D gradient selected TOCSY (**To**tal **C**orrelation **S**pectroscop**y**) spectrum quickly and easily.

^{1}H J-Resolved Spectrum	Filter NS RD NP2 NP1	The ^{1}H J-Resolved Spectrum Quick experiment enables the user to obtain a ^{1}H 2D spectrum capable of resolving overlapping multiplets and measuring J-coupling constants within a multiplet.

3.5 Parameters

- **TxPPM** (or **TxPPMX** for X-nuclei) is the frequency off-set of the radio-frequency (RF) transmitter pulse in ppm, and corresponds to the centre of the resulting NMR spectrum. For sequences which require targeting of a specific signal (*e.g.* for suppression or selective enhancement sequences), TxPPM should be set to the chemical shift of the signal of interest.
- **Filter** is the spectral window in Hz (centred around TxPPM). The spectral window in ppm may be calculated by dividing the **filter in Hz by the spectrum frequency, SF in MHz.**
  For two-dimensional sequences, the Filter, applies to the spectral window of the observed nucleus (*f*2 axis). For two-dimensional sequences, the spectral window of the indirect nucleus (*f*1 axis) is defined by the **SW1** parameter; generally this is only applicable for heteronuclear two-dimensional spectra, otherwise SW1 = Filter.
- **NP** is the number of data points obtained during acquisition (of a one-dimensional spectrum), and **should be a power of two (*i.e.* 2^{13} = 8192, 2^{14} = 16384, 2^{15} = 32768, 2^{16} = 65536).** The acquisition time in seconds (duration of the free-induction-decay) can be **calculated by dividing NP by the filter in Hz**.
  For two-dimensional sequences, **NP2** is the number of data points obtained during acquisition; while **NP1** is the number of slices along the indirect (*f*1) axis.
  For two-dimensional sequences with the option to run with non-uniform sampling (NUS), there is an additional **Sampling** parameter, which defines the percentage of the total slices in the indirect (*f*1) dimension which are observed, and hence the reduction in experiment time for an otherwise identical parameter set.7
- **NS** is the number of scans obtained during the experiments. In NMR spectroscopy the signal-to-noise ratio of the spectrum is proportional to the square-route of the number of scans. Additionally, to obtain the best possible spectra NS should be a multiple of the

phase cycle, for most sequences that means, **NS should be a multiple of four or eight.** Therefore, most NMR spectroscopists will use NS values of **4^n: 16, 64, 256, 1024, 4096.**

- **DS** is the number of dummy scans, which are performed prior to (and in addition to) the scans defined by NS; although aren't saved or used to generate the averaged spectrum at the end of the sequence.
- **RD** is the relaxation/recycle delay, which occurs before the start of each iteration of the pulse sequence (and hence for experiments using multiple scans, between each scan). For accurate quantitative measurements, RD is recommended to be *at least* five-times the longest spin-lattice relaxation time (*T*1), most small organic molecules have ^{1}H *T*1 durations of ≤ 5 s.

Nuclei	SF (MHz)	Filter (Hz)	TxPPM (ppm)	NP	RD (s)	NS	
^{1}H (one-dimensional)	59.70	5208	+5	32768	4 54 (qNMR)	16	
^{1}H (two-dimensional)	59.70	1289	+5	2048 (NP2)	2	128 (NP1) 4 (NS)	
^{19}F	56.17	20833	−75	65536	4 54 (qNMR)	16	
^{13}C	15.01	10417	+100	32768	2	256+	low receptivity nucleus
^{29}Si	11.86	10417	−50	32768	2	256+	low receptivity nucleus
^{31}P	24.17	20833	0	65536	2	64	

3.6 Data Export

Once the acquisition is complete, the spectrum is automatically exported to the MNova software package for processing.

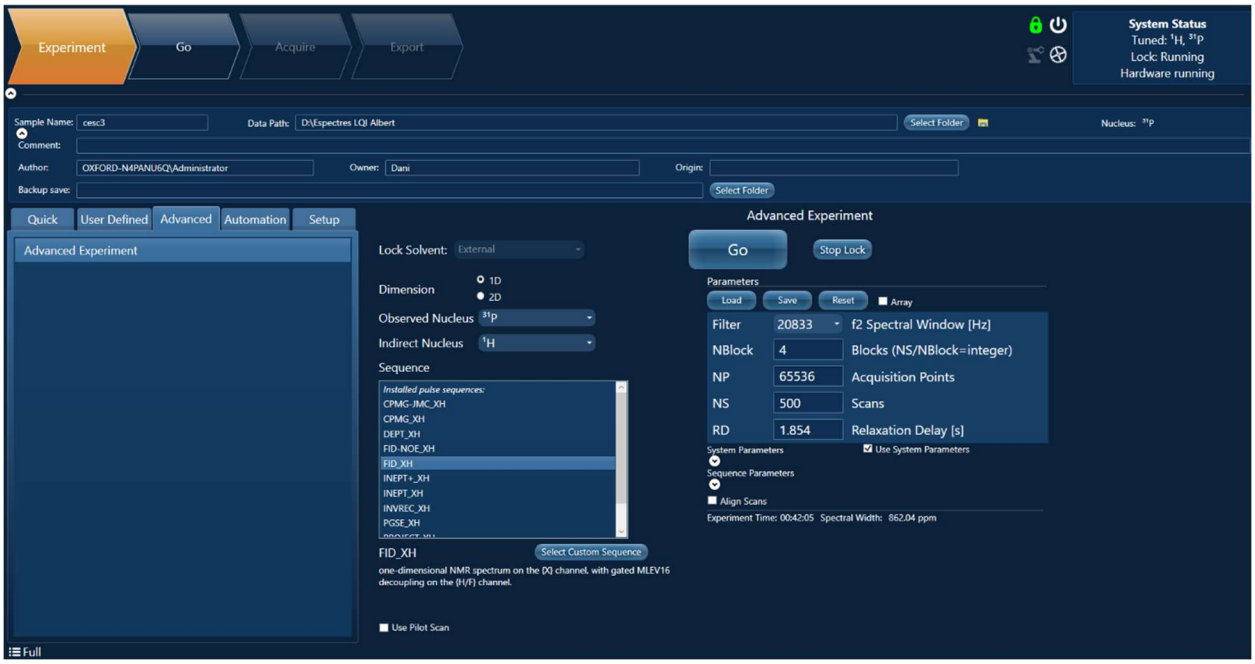
4. Advanced Experiments

Advanced tab can be used to select and run any one-dimensional (1D), 1D Arrayed, or two-dimensional (2D) experiment for any nucleus or combination of nuclei available on the system (^{1}H, ^{19}F, ^{13}C, ^{31}P). All experimental parameters are visible and customisable for Advanced experiments. 1D advanced experiments can be run either with the deuterium lock or with SoftLock (Align Scans) . 2D experiments must be run using the deuterium lock. Parameter sets and array parameter lists can be saved and loaded for later use.

4.1 One-Dimensional (1D) Experiments

Choose 1D by selecting the appropriate option from the Dimension drop-down box. Next choose the observed nucleus from the drop-down list. **For a standard ^{1}H 1D experiment, the appropriate observed nucleus would be ^{1}H**. All available nuclei for the system will be listed. Select an **indirect nucleus** if appropriate or select **'None'** to select no indirect nucleus. In the case of ^{1}H 1D experiment the indirect nucleous will be 'None'.

For a standard ^{31}P {^{1}H} 1D experiment , the observed nucleus would be ^{31}P and the indirect nucleus ^{1}H.



The next step will be to choose the **Pulse sequence (5 section)**

Once a pulse sequence is selected, the '**Go**' button becomes active and the default parameters for the nuclei and sequence are shown. These parameters can be modified prior to acquisition if required.

4.2 Two-Dimensional (2D) Experiments

To run a two-dimensional (2D) Advanced Experiment, the deuterium lock should be active

Then select '2D' as the Dimension

Then select the directly observed nucleus from the drop-down box. This is the nucleus that will be displayed in the *f*2 dimension and is the one to which the receiver will be tuned. For a homonuclear 2D experiment, such as a ^{1}H COSY, ^{1}H should be selected.

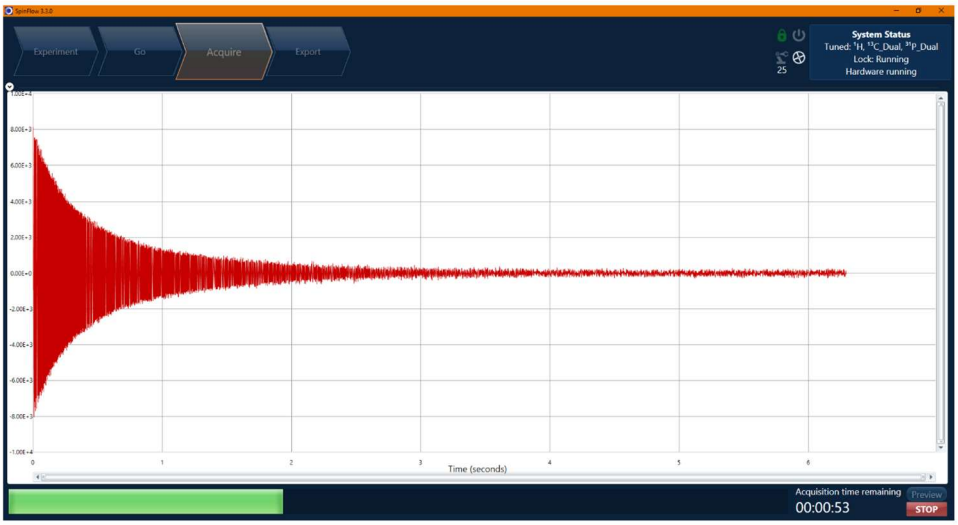
Then select the (optional) indirectly observed nucleus from the drop-down box. This is the nucleus that will be displayed in the *f*1 dimension. For most homonuclear 2D experiments, the indirect nucleus should be the same as the direct nucleus (2D J-Resolved is an exception, where the correct selection is 'None'), so for the ^{1}H COSY, the appropriate selection is ^{1}H.

Note that for a heteronuclear 2D experiment it is important to know whether it is a 'direct' experiment, such as HETCOR, or an 'indirect' experiment such as HSQC. For direct experiments, such as HETCOR, the X-nucleus (^{13}C or ^{31}P) is the directly observed nucleus (f2), and ^{1}H (or ^{19}F) is indirectly observed (f1). For indirect experiments, such as HSQC, ^{1}H (or ^{19}F) is the directly observed nucleus (f2), and the X-nucleus (^{13}C or ^{31}P) is indirectly observed (f1).

4.3 Acquisition

Once all parameters are set as required, click the 'Acquire' workflow tab to run the experiment.

The Acquire screen displays the free-induction decay (FID).



The FID display is a green progress bar which illustrates the progress through the experiment.

To the right of the progress bar is a timer which displays the time remaining until the data acquisition is complete.

In the bottom-right of the SpinFlow window; the **Preview** button will combine the data acquired so far and automatically open the data in Mnova; and the **STOP** button allows the user to abort the experiment, returning the user to the Parameters screen.

4.4 Data Export

Once the acquisition is complete, the spectrum is automatically exported to the MNova software package for processing.

5 Pulse sequence

5.1 One-Dimensional (1D) Sequences

Sequences ending with _H or _X are observed on the 1H/19F or X-channel respectively; while sequences ending with _HX or _XH are observed on the 1H/19F or X-channel respectively, with decoupling on the indirect channel

	_H	_HX	_X	_XH	
FID	✓	✓	✓	✓	standard one-dimensional NMR experiment, with inverse-gated decoupling (during the acquisition time only)
FID-NOE	×	×	×	✓	standard one-dimensional NMR experiment, with decoupler on for *both* the acquisition time *and* relaxation delay
InvRec	✓	✓	✓	✓	**Inv**ersion **Rec**overy
CPMG	✓	✓	✓	✓	**C**arr-**P**urcell-**M**eiboom-**G**ill
CMPG-JMC PROJECT	✓	✓	✓	✓	**CPMG** without ***J*-modulation couplings** **P**eriodic **R**efocusing **o**f ***J*** **E**volution by **C**oherence **T**ransfer [5]
PGSE	✓	✓	✓	✓	**P**ulsed field **G**radient **S**pin-**E**cho
J-PGSE	✓	✓	×	×	***J*-compensated PGSE** [6]
WET-FID WET-FID-AUTO	✓	✓	×	×	spectrum with signal suppression using '**W**ater suppression **E**nhanced through T_1 effects' with automatic selection of the highest intensity peak for suppression

WET-PRE	✓	×	×	×	spectrum with multiple signal suppression using WET & **pre**-saturation
INEPT INEPT+	×	×	×	✓	**I**nsensitive **N**uclei **E**nhancement by **P**olarisation **T**ransfer, the INEPT_XH & INEPT+_XH sequences do ***not*** decouple the $^{1}H/^{19}F$ channel
DEPT	×	×	×	✓	**D**istortionless **E**nhancement by **P**olarisation **T**ransfer

5.2 Two-Dimensional (2D) Sequences

JRES_H	***J*-Res**olved
COSY45_HH	**Co**rrelation **S**pectroscop**y** (with final 45° pulse)
COSY90_HH	**Co**rrelation **S**pectroscop**y** (with final 90° pulse)
gs-COSY45_HH	**g**radient-**s**elective **Co**rrelation **S**pectroscop**y** (with final 45° pulse)
gs-COSY90_HH	**g**radient-**s**elective **Co**rrelation **S**pectroscop**y** (with final 90° pulse), with optional non-uniform sampling
gs-DQF-COSY_HH	**g**radient-**s**elective **Co**rrelation **S**pectroscop**y** with **D**ouble **Q**uantum **F**iltering
gs-COSY_1H19F	**g**radient-**s**elective ^{1}H-^{19}F **Co**rrelation **S**pectroscop**y**
TOCSY_HH	**To**tal **C**orrelation **S**pectroscop**y**
gs-HMBC_HX	**g**radient-**s**elective **H**eteronuclear **M**ultiple-**B**ond **C**orrelation, with optional non-uniform sampling
gs-HSQC-ME_1H13C	**g**radient-**s**elective ^{1}H-^{13}C **H**eteronuclear **S**ingle-**Q**uantum **C**orrelation with **M**ultiplicity **E**diting, with optional non-uniform sampling
HETCOR_XH	**Het**eronuclear **Cor**relation