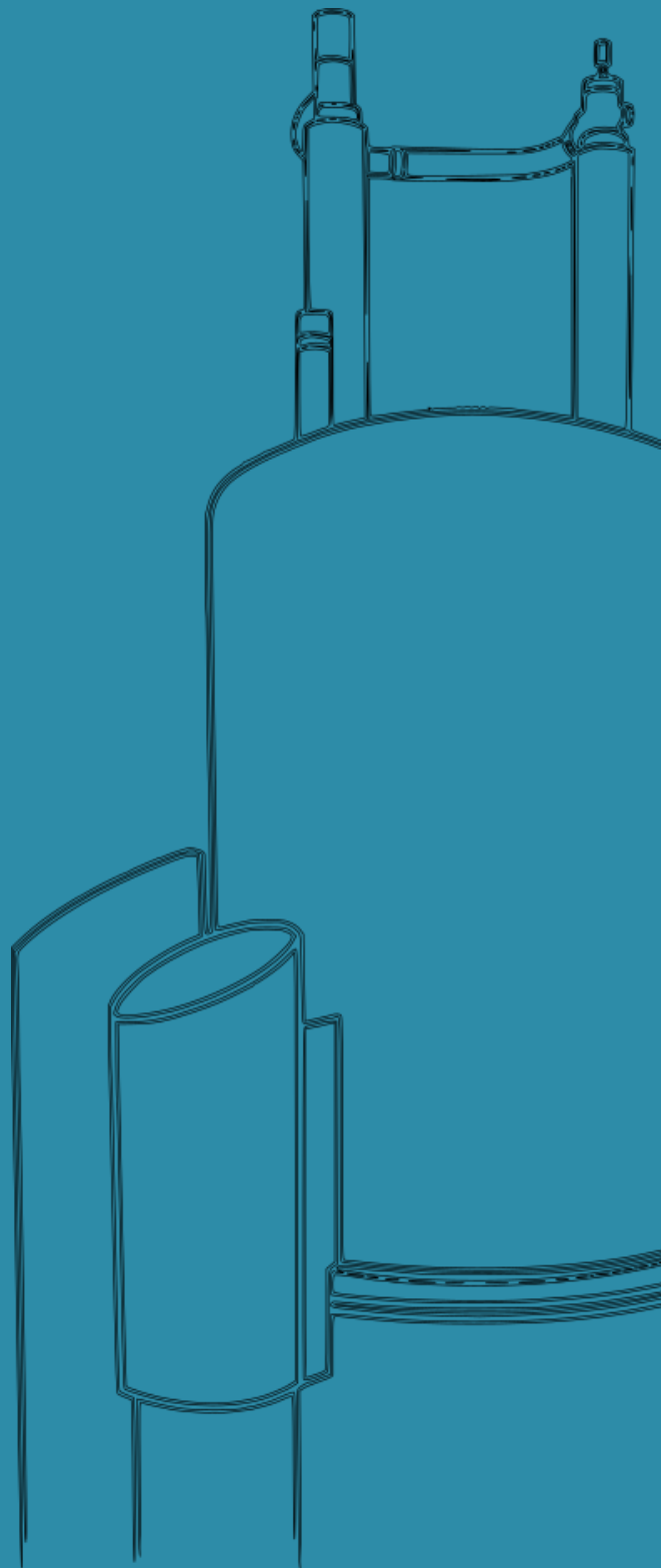


NMR Services & Information

NMR Expertise Center

Version 2.0 - March 2023





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De informatie in deze brochure is bijgewerkt tot 1 Maart 2023.

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NMR EXPERTISE CENTER

Since 2018, the NMR facilities are managed by the NMR Expertise Center, the main center of expertise for structure characterization and molecular analysis in chemical, biochemical, biomedical and materials sciences at Ghent University. The goal of the NMR Expertise Center is to make its NMR infrastructure and broad expertise accessible to the entire UGent Research Community, external academic or industry partners. To this end, it retains a close collaboration with the NMR and Structure Analysis group (NMRSTR) headed by Prof. J.C. Martins.



POINTS OF CONTACT

The NMR expertise center team is at your disposal to address all questions, concerns or provide general information concerning NMR experiments, specific service requests and training, both in-depth and for open access.

Dr. Dieter Buyst – Chief Scientific Officer (CSO)

- Point of contact
- Responsible for custom analyses and measurements
- Responsible for the day-to-day management, general organization and implementation
- Provide NMR training for dedicated NMR users



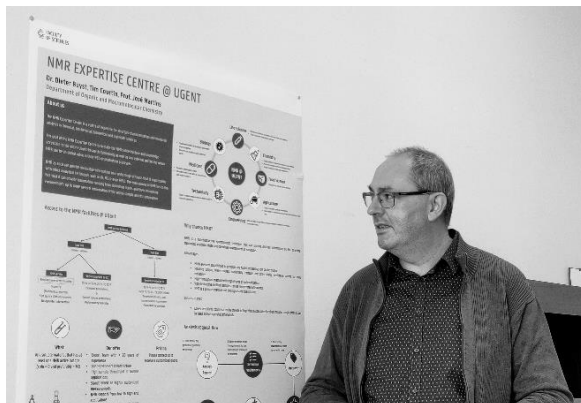
Dr. Emile Ottoy – Chief Technical Officer (CTO)

- Point of contact
- Main responsible for the open access facility
- Technical responsible spectrometers and IT
- Provide routine training for open access users



Prof. Dr. José C. Martins – Founder, Academic Responsible & Scientific Consultant

- Group leader NMRSTR research group
- Academic responsible expertise center
- Provides scientific support & consultancy
- Responsible long-term development



LOCATION & CONTACT DETAILS

The NMR expertise center of Ghent University is located on campus Sterre

Campus Sterre
Krijgslaan 281
Department of organic and macromolecular chemistry
Building S4bis – 1st floor
9000 Gent
Belgium



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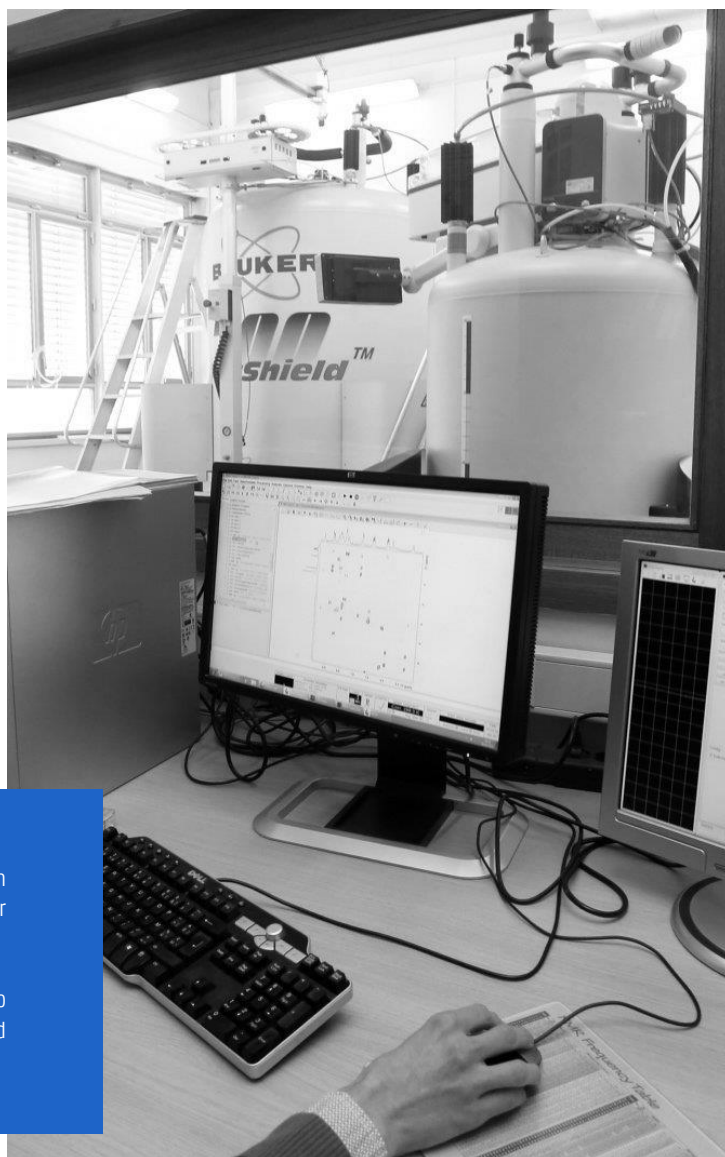
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Who can apply for access?

In principle anyone from Ghent University can apply for access to the NMR infrastructure – either through open access or as a dedicated user.

Similar services and collaborations are also offered to other academic institutes and industrial partners.



SAFETY INFORMATION

In terms of safety the presence of a strong magnet is what differentiates NMR spectrometers from most other laboratory equipment.

The magnet is potentially hazardous due to:

- The large attractive force it exerts on ferromagnetic objects.
- The large content of liquid Nitrogen and Helium.

Strong magnetic fields

Each NMR spectrometer is a strong superconducting magnet that is never switched off and is always active. This also means that the magnetic fields are always present even when the mains power is cut. Most magnets on modern spectrometers are shielded, meaning their stray magnet field is dampened sideways. However, a strong external magnetic field remains present directly above and below the magnet, even for the open access machines (300 and 400MHz) this is the case. **As a general rule**, unless you are loading or removing a sample, you should try to keep a distance of at least 2 meters from any magnet where the effects of the residual magnetic fields are minimal.

When entering a room equipped with an NMR spectrometer, the following specific safety rules must be applied in all circumstances:

- People with pacemakers and other medical implants that can be influenced by magnetic fields are not allowed to enter the NMR labs. If you are unsure, please consult your physician and/or a responsible from the center.
- Any loose metal objects are strictly forbidden in the immediate vicinity of the spectrometers. This also includes small and sharp metal objects such as paper clips, staples, spatulas, keys, etc (typically present in lab coat pockets).
- Magnetic fields may also damage personal items such as watches, mobile phones, credit cards, magnetic storage items etc.

THINK before you enter any of the magnet rooms and if unsure, please remove any objects you think may pose a problem and leave them at your desk.

Evaporation of cryogens

NMR magnets are filled with large amounts of liquid nitrogen and helium in order to retain their superconductivity. The boil off process of these cryogens is a continuous process and only leads to small amounts of nitrogen and helium gas being released under normal circumstances. However, in case of a magnet quench – a sudden boil-off of these cryogens with loss of magnetic field – large amounts of gas will be vented from the top of the magnet. This results in a dense white fog vented into the room and creates a serious and asphyxiation hazard which is invisible once the initial white fog following the quench has dissipated. To address this very rare, yet real hazard O₂ sensors have been installed in each

magnet room. These will trigger both a visual and auditive alarm should a quench occur as well as start the emergency ventilation system. In this case, leave the room immediately and contact the NMR and safety staff urgently. Do not re-enter the room until NMR staff has given permission.

General safety considerations

On top of the major concerns regarding magnetic and cryogenic safety, several other rules that must be respected when entering the open access room:

- Respect the safety signs: no gloves can be worn upon entering the room
- Your NMR tubes stay closed during the measurements, meaning chemical manipulations of any kind are prohibited inside the room.
- Use the depth gauge to prepare your NMR sample before submitting, the sample should fit snugly in the spinner. If not, try another spinner
- The open access room is meant for data acquisition only, do not use the computers to further process your data
- All data transfers occur over the intranet, do not use any USB drives or other types of mobile storage
- Make sure your NMR tubes are up to standard and filled with an appropriate solvent amount (see 'NMR Tubes' section)
- Keep the room clean and safe, chemical waste and (used) gloves are strictly forbidden and do not belong in the waste bins present in the room

In case you have any questions concerning your safety or are unsure how to apply one of the rules, do not hesitate to contact the NMR team.

INSTRUMENTS

The NMR Expertise Center has a total of 5 spectrometers. Two of these are available for open access and can be used after following the open access training. The other magnets are restricted and are only available to dedicated users that have a clear need for NMR support and followed an in-depth training. In the following overview, the number in the name of each instrument refers to the corresponding ^1H frequency in MHz.

OPEN ACCESS INSTRUMENTS – SELF SERVICE

Bruker Avance 300 (Phobos)

- 5mm dual channel probe head - ^1H and BroadBand Observe (BBO-type) with automatic tuning and matching
- Running Topspin 2.1 in ICONNMR environment
- Fully automated SampleXpress sample changer for 60 samples
- Ultrashield magnet
- Used for routine 1D ^1H , ^{13}C , ^{31}P , 2D COSY, TOCSY, HSQC, HMBC, NOESY and DOSY

Bruker Avance II 400 (Deimos)

- 5mm dual channel N_2 cooled Prodigy[®] cryoprobe - ^1H and BroadBand Observe (BBO-type) with automatic tuning and matching
- Running Topspin 3.2 in ICONNMR environment
- Fully automated Bacs II sample changer for 60 samples
- Ultrashield magnet
- Used for routine 1D ^1H , ^{13}C , ^{31}P , ^{19}F (non ^1H -decoupled), 2D COSY, TOCSY, HSQC, HMBC, NOESY and DOSY

OPEN ACCESS INSTRUMENTS – LIMITED ACCESS

Bruker Avance III HD 500 (Nestor) – liquid state

- 5mm triple channel probe head - ^1H ^{13}C ^{19}F (TX0 type) with automatic tuning and matching
- Running Topspin 3.6
- Fully automated Bacs I sample changer for 60 samples
- Unshielded Oxford magnet
- Dedicated to 1D and 2D experiments involving ^{19}F with ^1H -decoupling
- Dedicated to variable temperature (-120°C to 120°C) and reaction kinetic study type measurements

Bruker Avance III 500 (Hercules) – solid state and HR-MAS

- 4mm HR-Mas probe – ^1H and ^{13}C iProbe with automatic tuning and matching, magic angle adjustment, 15kHz max spinning speed
- 4mm CP-Mas probe – ^1H and Broadband iProbe with automatic tuning, matching and magic angle adjustment, 15kHz max spinning speed
- 5mm dual channel probe head - ^1H and BroadBand Inverse (BBI-type) with automatic tuning and matching
- Running Topspin 3.6
- Automated sequential sample changer for 10 4mm MAS rotors
- Ascend magnet (shielded)
- Dedicated to soft and solid matter samples using classical high resolution and cross polarization techniques respectively

RESEARCH INSTRUMENTS

Bruker Avance II 700 (Hera)

- 5mm triple channel N_2 cooled Prodigy[®] cryoprobe - $^1\text{H}/^{19}\text{F}/-^{13}\text{C}$ ^{15}N (Prodigy TCI-type) with automatic tuning and matching
- 1mm triple channel probe head - ^1H ^{13}C ^{15}N (TXI-type)
- Running Topspin 3.2
- Fully automated Bacs II sample changer for 60 samples
- Ultrashield+ magnet

ACCESS & TRAINING

Who can use the NMR infrastructure and/or is required to follow NMR training?

In principle anyone from Ghent University & beyond can apply for access to the NMR infrastructure. In all cases, the use will be billed according to the measuring time on a quarterly basis. If you have any interest in applying NMR to your research needs, do not hesitate to contact us and a suitable usage scenario can be discussed.

Everybody who wants to use the NMR infrastructure needs to apply for a dedicated training, be it for the open access or for in-depth NMR access.

- Open access training will allow you to use the 300 and 400MHz for standard experiments and request specific experiments on 500MHz (if applicable). In case you require high temperature, kinetic studies or other custom measurements this is also possible after contacting the NMR staff.
- In-depth training will allow you to become a user of the 500 and 700MHz in your own research activities. Only people or research groups with a clear need for in-depth NMR support can motivate a request for access to these instruments. Upon evaluation of the motivation and after the training has been completed access is provided to the online management system 'Infinity' to reserve instrument time. For more information on the latter, please visit www.ugent.be/nmr/en/equipment



CONTACT FOR TRAINING

Emile Ottoy
Emile.Ottoy@UGent.be

BEST NMR PRACTICES

OPEN ACCESS HOUSEKEEPING

The following list are a set of rules that should always be respected when using the open access facility. Any temporary deviations will be communicated via a general mail titled 'Open Access Update'.

Opening hours

- The open access (300 and 400MHz) is available between **9 AM and 7 PM during weekdays** using your personal badge after completing the training. Overnight and weekend time is for long measurements only (2D and ¹³C related measurements) with no access to the facility. Submitting any daytime samples to the queue after 7 PM will not work.
- 500MHz service is available on Monday (starting 11 AM) and Tuesday. Any samples submitted afterwards will be measured the following week.
- Periodic maintenance of the spectrometers is performed on the first working day of each week (usually Monday) meaning the infrastructure will be only available after 10.30 AM at the earliest. Safety signs posted outside the room indicate when maintenance is ongoing. During maintenance activities, access to the open access room is prohibited.

Sample considerations

- Samples should be filled with solvent to a height between 4.5 and 6cm for the 300MHz. In case of the 400MHz, the maximum filling height is 40mm or 535µL of solvent. Less will result in a failed measurement, more will waste signal-to-noise and result in bad shimming. If unsure, you can check your sample against an appropriate filled reference sample at the open access sample drop-off desk.
- Double check your sample tube before submitting it to the sample queue, any worn or damaged tubes/caps should be replaced as fast as possible before the sample is submitted to the queue.
- Use the proper tube for the job e.g. for light sensitive samples amber coloured tubes should be used, the use of silver foil is discouraged (see 'NMR Tubes' section).
- Always use the depth gauge to put your tube in the spinner. The tube should fit snugly, if it is too loose, please try another spinner and/or make sure your tube is of adequate quality (see 'NMR Tubes' section)
- When preparing a sample for 2D/¹³C measurements on 300/500MHz, fill in a sample flag with all the required details:
 - o User code and number for each experiment
 - o Solvent
 - o Research group
 - o Name + date of submission
 - o Sample amount (approximate)
 - o Choice of spectrometer (300/500 MHz)

In case of self-service measurements (day or night) on the 400MHz, also prepare a sample flag with the information mentioned above and add the sample changer position number (indicated on the back when submitting your sample). Put your flag on the whiteboard next to the appropriate sample holder number and remove it again once you come and pick-up your sample.

- When submitting a sample to the night queue or sample changer queue, follow the chronological order. Samples submitted first will be measured first! In case of 'special' samples that are e.g., labile and should be measured with priority, please contact the NMR staff first to make the proper arrangements.

General attitude

- When measuring a sample during the self-service moments, pick up your samples as fast as possible. This means either the same day (if finished before 7 PM) or the next day at the latest. Abandoned samples will be kept for one week and will result in a blocked account.
- **By no means do you remove others' samples from the sample changer – if all positions have been used for that day, contact the NMR staff for further action**

- Do not leave any used tissues around
- The waste bins in the open access facility are not meant for chemical waste of any kind nor should gloves be discarded here

EXPERIMENT TIME

The following workflow represents best practices for the majority of analyses performed in open access (300, 400 and 500MHz). Exceptions are off course always possible so use your common sense and judgement. If you are unsure do not hesitate to contact the NMR staff.

1. Always start with measuring a ^1H spectrum to assess the suitability and quality of your sample i.e., no impurities or unexpected signals and sufficient signal intensity
2. If the 1D ^1H spectrum is clean and the signal-to-noise is good, you can follow up with a COSY and ^1H - $\{^{13}\text{C}\}$ HSQC spectrum
3. If you have trouble in confirming the structure or your molecule contains a lot of quaternary carbon atoms, request a 1D ^{13}C spectrum (APT or regular 1D ^{13}C) or an ^1H - $\{^{13}\text{C}\}$ HMBC. These experiments typically require a decent amount of sample material (> 10mg) to give you decent spectra.
4. If the ^1H - $\{^{13}\text{C}\}$ HMBC and/or 1D ^{13}C fail to help you further you can request more specialized measurements such as TOCSY, NOESY, HSQC-TOCSY, etc.

Remember that measuring an HSQC and HMBC will give you the same (and more!) information as a 1D ^{13}C or APT within the same measuring time! Only request a 1D ^{13}C spectrum when you have an adequate sample amount, the resolution is crucial and/or it is required for publishing purposes. The 400MHz should be your go to spectrometer!

Approximate measurement times for default experiments used in open access are shown in the table below - assuming standard sample quantities as defined in the sensitivity guidelines (see 'Sensitivity Guidelines' section)

300MHz measurements

Experiment	Default experiment time	Scans or # increments	Measured during Day / Night
1D ^1H	6 -10 mins	16	Day
1D ^{31}P	6 -10 mins	16	Day
2D ^1H - ^1H COSY	30 mins	8	Night
2D ^1H - ^1H TOCSY	30 mins	8	Night
2D ^1H ^{13}C HSQC	90 mins	8	Night
2D ^1H ^{13}C HMBC	90 mins	8	Night
2D ^1H ^1H NOESY	90 mins	8	Night
1D ^{13}C / APT	180 mins	3000	Night
^1H DOSY	90 mins	16	Night

400MHz measurements

Experiment	Experiments	Default experiment time	Measured during Day / Night
1H_short_19F	1D 1H + 1D 19F	12 mins	Day
1H_long	1D 1H with 128 scans	15 mins	Day
1H_short_APT or 1H_short_13C	1D 1H and 1D 13C (APT or regular)	15 mins	Day
31P_short_APT or 31P_short_13C	1D 31P and 1D 13C (APT or regular)	15 mins	Day
2D_short	1D ^1H , 2D COSY and HSQC	20 mins	Day
2D_long	1D ^1H , 2D COSY, HSQC, HMBC and 1D ^{13}C	65 mins	Night
2D_long_noesy	1D ^1H , 2D COSY, HSQC, HMBC, 1D ^{13}C and NOESY	85 mins	Night
13C_short or APT_short	1D only (APT or regular)	6 mins	Day
13C_long or APT_long	1D only (APT or regular)	25 mins	Night
1H DOSY	1D ^1H and 2D DOSY	45 mins	Night

SENSITIVITY GUIDELINES

The routine experiment settings will give you adequate results, provided you have submitted a sample with a reasonable concentration. A good rule of thumb here is to **use at least 10mg for mid-size compounds** (200 – 600 g/mol) in **500-535 μL of deuterated solvent (400MHz)** or **550 μL – 600 μL (300MHz/500MHz)**. Adjust your sample amount accordingly for higher molecular weight compounds – assuming the solubility of your compound is not a problem. Using more solvent will only waste signal-to-noise due to a lower concentration overall. If you want to boost your signal-to-noise ratio (SNR), there are several strategies you can follow in order to achieve this:

- **Strategy I – Using higher concentrations**

The most straightforward strategy to obtain higher signal-to-noise ratio with the same number of scans, is simply using a higher concentration of your sample(s):

$$F = \frac{SNR_2}{SNR_1} = C_f * \sqrt{\frac{NS_2}{NS_1}}$$

Here F is the increase in SNR that you aim for, SNR is the signal-to-noise ratio of a spectrum, C_f is the factor indicating how much the concentration was increased and NS = number of scans used in each spectrum (assuming you are measuring both spectra on the same spectrometer). Here you can see that simply doubling your concentration (C_f= 2) you will double the signal-to-noise without having to change the number of scans! This relation is magnet independent and hence will work in any conditions and any spectrometer.

- **Strategy II – Requesting more scans**

Say boosting your concentration is not possible because of solubility issues, making more product would take too long or is simply too expensive. Then in this case you can choose to increase your number of scans. While being a sound strategy, it is not as simple as you might think. When increasing the signal to noise on the same spectrometer, the following equation applies:

$$NS_2 = F^2 * NS_1 \text{ for } SNR_2 = F * SNR_1$$

Here NS = number of scans, F is the increase you want to obtain, and SNR is the signal-to-noise ratio. Hence if you want to double the signal-to-noise of a previous measurement with the same sample (F=2) you should quadruple (F²) = 4 the number of scans for that measurement. This also means that the **time required** for this measurement will not double but **multiply with a factor of four!**

This also shows you that if you only double (F² = 2) or triple (F² = 3) your number of scans compared to the initial measurement, the corresponding increase in SNR is only $\sqrt{2}$ or $\sqrt{3}$ (1.41 and 1.73 respectively). In this light, requesting an APT or 1D ¹³C that in standard setup on 300MHz uses 3000 scans to double your scans to 6000 will give you only a minor increase in signal-to-noise ($\sqrt{2}$ or 1.41). Here, strategy I or III should be adopted instead.

- **Strategy III – Changing spectrometers**

This strategy can be more time (and cost!) efficient than strategy II although it depends of course on the availability of other spectrometers. What matters here is the relative performance of different spectrometers and their probes when measuring the same sample. For instance, **it is not because the magnetic field is higher, that it will automatically provide more signal for a specific nucleus**. This also depends to a large extent on the architecture of the probe. A fitting example here is the relative ¹H and ¹³C sensitivity of the 400 and 500MHz:

Nucleus	Relative sensitivity*	
	400MHz (Prodigy)	500MHz (Nestor TXO)
¹ H	2,7	1,0
¹³ C	4,2	1,0

*Sensitivity relative to the 500MHz TXO setup

Relative ¹ H sensitivity		Phobos	Deimos		Nestor		Hercules	Hera		
	¹ H frequency	300 MHz	400 MHz		500 MHz		500 MHz	700 MHz		
	Probe	BBO	BBO	Prodigy	TXI	TXO (19F)	BBI	TXI	Prodigy	
SNR 0.1% (v/v) Ethylbenzene*		178	290	1040	850	380	950	1800	4430	
	¹ H frequency	Probe	Relative performance factor P from one probe to another							
Phobos	300 MHz	BBO	1,0	1,6	5,8	4,8	2,1	5,3	10,1	24,9
Deimos	400 MHz	BBO	0,6	1,0	3,6	2,9	1,3	3,3	6,2	15,3
		Prodigy	0,2	0,3	1,0	0,8	0,4	0,9	1,7	4,3
Nestor	500 MHz	TXI	0,2	0,3	1,2	1,0	0,4	1,1	2,1	5,2
		TXO (19F)	0,5	0,8	2,7	2,2	1,0	2,5	4,7	11,7
Hercules	500 MHz	BBI	0,2	0,3	1,1	0,9	0,4	1,0	1,9	4,7
Hera	700 MHz	TXI	0,1	0,2	0,6	0,5	0,2	0,5	1,0	2,5
		Prodigy	0,0	0,1	0,2	0,2	0,1	0,2	0,4	1,0

*Absolute SNR value as measured using the Bruker specification measurement

Relative performance of probes for ¹H. When read vertically the values reflect the performance of that specific probe compared to the other probes below when installed on the spectrometer in question

Generally, one would assume that given the higher magnetic field of a 500MHz versus a 400MHz, the sensitivity and resolution will automatically be higher for the 500MHz with respect to the 400MHz. Where for resolution this is indeed always true, for sensitivity it depends on the specific setup! From the table above, it is clear that for ¹H the 400MHz is 2.7 times more sensitive and even 4.2 times more sensitive for ¹³C than the 500MHz. Hence requesting a ¹³C measurement with equal signal to noise on the 500MHz will take a factor of 17.6 more time than on the 400MHz with only a limited gain in resolution!

The information collected in the table above can be used together with the following equation:

Given

$$NS_2 = \left(\frac{F}{C_f * P} \right)^2 * NS_1$$

With

$$F = \frac{SNR_2}{SNR_1}$$

C = relative concentration of one measurement versus the other and P = relative performance factor of one probe versus the other (values from Table 1). If you perform a measurement on the 300MHz with a given number of scans, how much less scans (and time!) would you need to get the same result (same SNR) if you measure on the 700MHz with the Prodigy cryoprobe? Here $C = 1$, $F = 1$ and $P = 24,9$ giving the following result:

$$NS_2 = \left(\frac{1}{1*24,9} \right)^2 * NS_1 \text{ or } NS_2 = \frac{1}{620} * NS_1$$

In principle you could measure with 620 less scans and time on the 700MHz with the Prodigy and still get the same result as you did on the 300MHz. In practice, this means that you can do a 1D ¹H experiment with 1 scan on the 700MHz and still get a better result than with 128 scans on the 300MHz!

A similar set of values can be calculated in terms of ¹³C performance:

Relative ¹³ C sensitivity			Phobos	Deimos		Nestor		Hercules	Hera	
		1H frequency	300 MHz	400 MHz		500 MHz		500 MHz	700 MHz	
		Probe	BBO	BBO	Prodigy	TXI	TXO (19F)	BBI	TXI	Prodigy
SNR 0.1% ASTM (¹³ C) *			104	170	568	150	135	180	132	959
	1H frequency	Probe	Relative performance factor P from one probe to another							
Phobos	300 MHz	BBO	1,0	1,6	5,5	1,4	1,3	1,7	1,3	9,2
Deimos	400 MHz	BBO	0,6	1,0	3,3	0,9	0,8	1,1	0,8	5,6
		Prodigy	0,2	0,3	1,0	0,3	0,2	0,3	0,2	1,7
Nestor	500 MHz	TXI	0,7	1,1	3,8	1,0	0,9	1,2	0,9	6,4
		TXO (¹⁹ F)	0,8	1,3	4,2	1,1	1,0	1,3	1,0	7,1
Hercules	500 MHz	BBI	0,6	0,9	3,2	0,8	0,8	1,0	0,7	5,3
Hera	700 MHz	TXI	0,8	1,3	4,3	1,1	1,0	1,4	1,0	7,3
		Prodigy	0,1	0,2	0,6	0,2	0,1	0,2	0,1	1,0

*Absolute SNR value as measured using the Bruker specification measurement

Relative performance of probes for ¹³C. When read vertically the values reflect the performance of that specific probe compared to the other probes below when installed on the spectrometer in question

When comparing the ¹³C sensitivity of the 400MHz BBO versus that of the same 400MHz with Prodigy, you now can calculate that for the same signal-to-noise on the 400MHz with the old BBO probe, you only need about 1/11th of the time on the 400MHz with its Prodigy. Or in other words, **a typical ¹³C measurement can be reduced from 3+ hours to about 16 minutes for the same result!**

So instead of requesting a direct 1D ¹³C measurement of about 3000 scans on the 300MHz that takes more than 3 hours, it is far more efficient to measure yourself a short or long 1D ¹³C/APT measurement on the 400MHz in open access in about 6 to 25 minutes and get an even better result in case of the latter.

NMR TUBES

Tube condition

It is essential that the NMR tubes you use for sample preparation are up to standards as faulty tubes may lead to serious damage to the spectrometer! Make sure to check that your tube meets the following requirements before preparing your sample:

- **Minimum** tube length is **16.7 cm/6.57 inches**
- **Maximum** tube length is **19.2 cm/7.56 inches**
- Each tube must have a fitting cap
- **Minimum filling** height of **4.5 cm/1.77 inches** or 550 μl for 300MHz and **4.0cm/1.57 inches** or 500 μl max for 400MHz
- **Maximum filling** height of **6.0 cm/2.36 inches** or 750 μl

Broken tubes or tubes with jagged edges at the end are a potential hazard and are not to be used. Simply discard it and take a new one. The bottom of the tube is to be checked for hairline cracks and if present, the tube in question should also be discarded accordingly.

Concerning the NMR caps: if the cap itself on your tube is worn out, please replace it with a new one. Caps are cheap, spectrometers are not. We expect everybody to use appropriate caps i.e., made from hard plastic that fit snugly over the opening of the tube (see fig. 1). Rubber stoppers or any other type of caps are not considered to be safe and should be avoided.



Fig. 1. Regular NMR tube caps

NMR staff will confiscate any broken NMR tubes or tubes with faulty caps – whether they contain precious samples or not!

Pre-flight check

When setting up an NMR sample at the spectrometers, make sure that the tube in question is fitting snug inside the spinner (= blue sample holder) when using the depth gauge. A spinner that is too loose can also cause serious issues and/or sample breakage. If you feel that the spinner is too loose, simply take another one. If you feel that your tube is loose in the majority of the spinners, then the tube itself is out of specifications and should be discarded. Typically, this happens with cheap tubes where the tolerances are not strict enough.

If there are any spinners that are always too loose, then report this to the NMR staff – they will replace the rubber rings accordingly.

If there is an adequate amount of solvent in your tube, you should see that the black bars on the depth gauge corresponding with 5mm should sit well below the solvent level of your sample. If not, return to your lab and add solvent until you are above this level. Sitting too close to these bars can also result in spectral artefacts (especially with water suppression experiments) or may cause the sample to be rejected by the instrument during the shimming of the magnetic field.

Tube cleaning and re-use

In general, only tubes of adequate quality should be re-used. Economy tubes are typically meant for one time use and are only to be used at room temperature! These tubes should be avoided in any case (see section 3).

Drying tubes – do not heat or store them in an oven as it is very likely to distort the tube. Similar to cracked ones, warped tubes can cause serious damage to the spectrometer and/or probe. Wash the tubes with acetone and blow them dry or dry them under vacuum.

Variable temperature experiments – depending on the solvent, there might be a pressure build-up during the experiments with a higher chance of the tube breaking. In order to minimize the chance, preferably use a new high-quality tube or a special high-pressure tube with screw cap. Do not reuse the same tubes over and over for variable temperature measurements.

Tube recommendations

Below you can find a list of the preferred NMR tubes that are known to be of high quality and can be used in various temperature conditions – they are used within the NMRSTR research group and NMR EC on a daily basis. In addition, 'special' NMR tubes for various conditions/samples are also available. Some examples include tubes for light sensitive compounds (no more aluminum foil required) or for air sensitive compounds including a screw cap top.

Norell tubes - Eurisotop

- 5mm tube – Norell 507-HP-7 standard series ultra precision (300 - 400MHz)
- 5mm tube – Norell 508-UP-7 standard series ultra precision (500MHz)



Figure 2. Sample, spinner, and depth gauge used to prepare an NMR sample

- 5mm tubes – amber colored for light sensitive compounds: Norell 507-HP-AT-7
- 5mm screw cap tube – for air sensitive compounds: Norell S-5-500-SC-7

Wilmad tubes – Merck/Sigma-Aldrich

- 5mm tubes - Wilmad economy Z562769 (600MHz)
- 5mm tubes – Wilmad precision Z272019 (500MHz)

When in doubt concerning appropriate tubes, please contact the NMR staff!

A warning for high pressure tubes, 3mm sample inserts or other more specialized tubes: these are not to be used in the regular routine measurements and require a separate appointment to be measured in all cases.

Below you can find a list of tubes that are of **suboptimal quality and should be discarded** if still present in your research group. As a rule of thumb: if the tube costs on average between 1-2€/tube then the quality is very low and these tubes must be avoided at all costs. Specific examples include:

- NMR tubes, high-throughput, thin walled, 5 mm Ø, SP Wilmad-LabGlass (SP Industries, 634-0871)
Reason: tubes will break when heated or cooled beyond room temperature; glass wall is not thick enough and will break easily
- Economy NMR tubes EP54.1 (ROTH, DKW Life Sciences)
Reason: one time use only, glass wall is not thick enough and will break easily

IMPORTANT: PUBLISHING & ACKNOWLEDGEMENTS

The equipment of NMR EC represent a multi-million euro investment obtained over the years through grants awarded by the FWO (Hercules) or UGent (BOF). In its regular reports to these funding agencies all the publications where our NMR infrastructure has played a role need to be accounted for. In addition, as of 2023, grant numbers need to be associated with any published entry in the University's academic bibliography. Therefore, when you publish a paper or communication where you use results (NMR spectra or data resulting from their interpretation) that were directly obtained from use of our NMR infrastructure, this must be duly mentioned and acknowledged in the appropriate section of your paper. The author(s) are free to choose how they phrase the respective acknowledgement; an example of appropriate phrasing is listed here below:

"We thank the NMR expertise centre (Ghent University) for providing support and access to its NMR infrastructure. The 300/400/500/700MHz (select appropriate) used in this work has been funded by a grant/project of the Research Foundation Flanders (FWO) / the 'Bijzonder Onderzoeksfonds' (BOF) (project/grant code XXXXX). (select appropriate)"

One should only select those equipments and grant number relevant to the published material. The grant code(s) for each instrument is published on our website: <https://www.ugent.be/nmr/en/equipment/spectrometers.htm>. It also contains short descriptions of the equipment used.

We would also appreciate you sending us a copy of or direct link to the paper.

SOLVENTS AND REFERENCING

General information

No matter which solvent you want to use for NMR measurements, it should always contain a strong deuterium signal. This usually means a deuteration level of 99,50% or more. The presence of deuterium is required for two reasons:

- Locking field procedure required for stability and reproducibility but also for referencing your spectrum close to its ideal value
- If the solvent would be protonated, the signals would interfere or completely block the signals of interest, producing a spectrum that is of little use

Signal referencing for ^1H and ^{13}C

In case of organic solvents such as CDCl_3 , Acetone- d_6 , DMSO- d_6 , etc. You can use the known ppm values of the solvent's ^1H and ^{13}C signals to reference your ^1H and/or ^{13}C spectra. A useful solvent chart with these so-called secondary internal reference values can be found at the following address:

<https://www.uqent.be/nmr/en/collaboration/nmr-solvent-referencing-chart>

A second possibility for referencing your ^1H and ^{13}C measurements is using an internal standard. Examples of this include TetraMethylSilane (TMS) for organic solvents or TrimethylSilylPropanoic acid (TSP) and 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS) for $\text{H}_2\text{O}/\text{D}_2\text{O}$ or mixtures (cannot be removed through evaporation afterwards). In all cases a single signal can be observed around and should be set to 0 ppm. In all cases a concentration of 0,05mM is more than sufficient.

Signal referencing for other heteronuclei (^{19}F , ^{31}P , ^{15}N , ...)

Similar reference compounds exist for other nuclei outside ^1H and ^{13}C and the corresponding signals are also set to Oppm:

^{11}B	Boron trifluoride etherate	$\text{BF}_3(\text{OEt})_2$
^{15}N	Nitrate ion	NO_3^-
^{19}F	Fluoro-trichloromethane	CCl_3F
^{31}P	85% Phosphoric acid	H_3PO_4

Note that these are typically not added to your sample tube but are used either externally or added inside a capillary to the sample solution. Lastly, you can also use a referenced 1D ^1H spectrum to reference other heteronuclei spectra, ask the NMR staff if you want to know more!

General remarks

- Try to avoid mixtures of (deuterated) solvents as much as possible, not only do you risk of obtaining a 'shifted' NMR spectrum, sample shimming might also fail and produce bad spectra
- if you want to use an 'exotic' solvent that is not in the standard solvent list, please contact the NMR staff for discussing the possibilities or alternatives

Windows

Map a new network drive in the 'This PC' section

Server address: <\\shares.isilon.uqent.be/we07data>

Activate 'Reconnect at login' and 'Connect using different credentials'. In the login window use your UGent login and password. Do not forget to add 'UGENT\' before your login account ID.

Macintosh

On the Finder menu bar, click 'Go > connect to server'. Enter the network address in the server address box

Server address: <smb://shares.isilon.uqent.be/we07data>

Click 'connect'. If you are prompted to enter a password, select Registered User, and enter your UGent user name and password. Do not forget to add 'UGENT\' before your login account ID.

General pointers

- When everything is ok in your network drive configuration, you should now see the landing page with different data folders of the different experimental techniques.
- The server is 'write protected' meaning you can only copy from the server to your own laptop/pc/mac. Once the data is stored locally, you can modify and process it using Topspin, Mnova or ACD Labs.
- Lastly, self-service measurements will be stored in your own folder directly, e.g. $\text{NMR}\backslash 400\backslash \text{group}400\backslash \text{data}\backslash \text{group}+\text{user}\backslash \text{nmr}\backslash \text{user}$.

All 300MHz night and weekend measurements are stored in the 'ope300' subfolders of your research group, e.g. $\text{NMR}\backslash 300\backslash \text{group}300\backslash \text{data}\backslash \text{ope}300\backslash \text{nmr}\backslash \text{user}$

All 500MHz measurements are stored in the 'bruker' subfolders of your research group, e.g. $\text{NMR}\backslash 500\backslash \text{group}\backslash \text{data}\backslash \text{bruker}\backslash \text{nmr}\backslash \text{user}$

SOFTWARE

Topspin (Bruker) is the software we advise for processing your spectra and can be downloaded for free for academia. Please visit:

<https://www.bruker.com/en/products-and-solutions/mr/nmr-software/topspin.html>

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