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Sustainable and cost-effective MAS DNP-NMR at 30 K with cryogenic sample exchange

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Abstract We report here instrumental developments to achieve sustainable, cost-effective cryogenic Helium sample spinning in order to conduct dynamic nuclear polarisation (DNP) and solid-state NMR (ssNMR) at ultra-low temperatures (< 30 K). More specifically, we describe an efficient closed-loop helium system composed of a powerful heat exchanger (95% efficient), a single cryocooler and a single helium compressor to power the sample spinning and cooling. The system is integrated with a newly designed triple-channel NMR probe that minimizes thermal losses without compromising the RF performance and spinning stability ($\pm 0.05\%$). The probe is equipped with an innovative cryogenic sample exchange system that allows swapping samples

18 in minutes without introducing impurities in the close-loop system. We report that significant gain
19 in sensitivity can be obtained at 30-40 K on large micro-crystalline molecules, with unfavorable
20 relaxation timescales, making them difficult or impossible to polarize at 100 K. We also report
21 rotor-synchronized 2D experiments to demonstrate the stability of the system.

22

23 Magic angle spinning (MAS) solid-state NMR at high magnetic field has developed as a key
24 spectroscopy to study the structure and dynamics of a wide array of systems including bio-solids,
25 catalysts, drugs, and energy materials [1–5]. Nevertheless, there are still emerging applications,
26 both for biomolecules (*in vitro* and *in cell* NMR), small molecules and materials (bulk and
27 especially surfaces), where NMR still suffers from its relatively limited sensitivity[6–9].

28 Besides the access to stronger magnetic field, such limitations can be strongly attenuated by
29 cooling the electronics of the probe [10] and/or the sample to cryogenic temperatures [11,12], but
30 also by hyperpolarizing the sample by Dynamic Nuclear Polarization (DNP) through microwave
31 (μw) irradiation. The latter involves the transfer of polarization from unpaired electrons to the
32 nuclei of interest and is driven by the application of a suitable microwave irradiation [13–15]. The
33 recent renaissance of DNP and its combination with high magnetic field and sample spinning is a
34 direct consequence of the groundbreaking research undertaken by the Griffin group at
35 Massachusetts Institute of Technology (MIT) [16–18]. Since then, a growing community of DNP
36 practitioners has reported a large range of previously infeasible applications, further increasing the
37 impact of DNP and revolutionizing the scope of solid-state NMR [19–27]. These advances are
38 supported by theoretical insights [28–37], as well as the development of innovative hardware
39 [18,38–41] and biradical polarizing agents [42–45].

40 The last decade has witnessed the introduction of new polarizing agents (PAs), in particular for
41 high field and fast MAS experiments [46–55]. The last generation of PAs have improved
42 significantly the sensitivity, with ^1H DNP enhancement factors (compared to Boltzmann
43 polarization) at 100 K typically around 50 to 200 (depending on the rotor diameter) and buildup
44 times between 1s to 10 s depending on the magnetic field and the system to polarize. To improve
45 things further, the development of new PAs can be combined with performing DNP experiments

46 at temperatures much lower than 100 K. Several initiatives aiming at developing ultra-low
47 temperature MAS experiments have been reported and have recently been reviewed
48 [11,12,39,40,56]

49 Except for the system built at NIH in Tycko's group [56] that uses cold nitrogen gas (N_2) to spin
50 the sample and cold helium gas to cool the sample down to 20 K, most of the recent reports relied
51 on the use of Helium for both spinning and cooling the sample. The approach was pioneered by
52 Yannoni and coworkers, and more recently implemented in the Barnes group that showed the
53 possibility to spin the sample down to 5 K [57,58] using nitrogen-cooled helium gas and liquid He
54 blowing on the center of the rotor. In 2012 – 2013, the group of Fujiwara [59] and Levitt [12]
55 showed results down to < 25 K using pressurized LHe dewar for spinning and cooling.

56 In 2015, a milestone was achieved by the Osaka and Grenoble groups, demonstrating the use of
57 helium closed-loop systems based on the use of gas compressors and counter-flow heat exchangers
58 to achieve stable sample rotation and cooling [60,61]. The Grenoble group could demonstrate a
59 factor of ~30-50 in time-savings on self-assembled peptide nanocrystals using TOTAPOL by
60 lowering the sample temperature from 100-110 K to the 40-50 K range under DNP condition [61].
61 Also, using a 3.2 mm rotor, faster sample spinning was demonstrated with He (17.5 kHz at 50 K)
62 than N_2 (12.5 kHz at 108 K). Finally, 100 kHz 1H decoupling was reported over 50 ms acquisition
63 without arcing. This first experimental setup (called NUMOC) used LHe to cool the Bearing,
64 Drive, and VT (variable temperature) gas flows, using a single compressor and powerful heat
65 exchanger for all three lines. On the other hand, the Osaka group was able to fully remove the use
66 of LHe and to reach 4 kHz MAS at 35 K and 4.7 kHz at 40 K using two GM cryo-coolers (one for
67 the Bearing and one for the Drive) [60]. Afterwards, they were able to combine this setup with

68 DNP and access faster MAS and lower temperature (10 kHz at 22 K) by adding three additional
69 cryo-coolers [40].

70 In recent years, we have continued developing our system in Grenoble with the goal to make it
71 sustainable, in terms of Helium consumption, and cost-effective, in terms of hardware and cooling
72 power requirement, without compromising on the RF, μ w, and MAS capabilities. To reach this
73 goal, we have designed an improved cryostat that relies on the use of a single cryocooler and a
74 single compressor for all three Bearing, Drive, and VT lines, and a new generation of triple channel
75 DNP probe that truly acts as a helium-tight cryostat providing excellent thermal insulation. The
76 latter is also equipped with a cryogenic sample exchanger that allows switching samples in minutes
77 without warming up and opening of the probe. Beyond the convenience for day-to-day usage, this
78 is also an extremely important point for setting reliably the stator at the magic angle once the probe
79 is cold (30-40 K). Indeed, the angle may need to be slightly readjusted between temperature cycles,
80 although this is not always the case with the current probe. A nutation frequency of 100 kHz on
81 the ^1H channel is achieved using 76.3 W of power at 40 K, whereas 62.5 kHz ^{13}C using 50 W.
82 Combined with cAsymPol-POK, one of the latest generation of PA that we have recently
83 introduced, we can improve the signal-to-noise ratio for proton-dense methyl-containing organic
84 powdered sample [50,54] by a factor 5 to 23 by lowering the sample temperature from 100 K to
85 40 K. We also illustrate the reliability of the setup regarding RF performance and sample spinning
86 stability, by showing 2D DQ- ^{13}C - ^{13}C - ^{13}C SR26 and ^{15}N - ^{13}C TEDOR experiments performed on
87 diluted ^{13}C , ^{15}N -*f*-MLF.

88

89 **MATERIALS AND METHODS**

90 **Sample preparation for DNP**

91 The ampicillin and indomethacin samples were purchased from Sigma-Aldrich and used as is
92 for the preparation of DNP sample. 37 mg of each were ground to a fine powder and impregnated
93 with 10 μ l of 20 mM cAsymPol-POK in d8-Glycerol/D₂O/H₂O 60:30:10 (v:v:v) (GDH matrix).

94 For the *f*-MLF tripeptide, one part of ¹³C-¹⁵N isotopically labelled tripeptide (4 mg) and four
95 parts of unlabeled peptide (16 mg) were co-dissolved in isopropanol and recrystallized following
96 the protocol in Ref [62]. KBr (10 mg) was added to the sample to be used as internal thermometer
97 [63]. The sample was impregnated with 15 μ l of 10 mM cAsymPol-POK in GDH matrix.

98

99 **DNP-enhanced solid-state NMR experiments**

100 Solid-state NMR experiments were recorded using a Bruker DNP-NMR AVANCE III 400 MHz
101 (9.4 T) spectrometer equipped with a gyrotron (operating at its fundamental mode) and a
102 corrugated transmission line. For all the DNP experiments, the μ w power was about ~15-21 W,
103 measured at the probe base using a water load (before the taper). All the DNP-NMR measurements
104 were performed with a home-built 3.2 mm HXY triple-resonance MAS probe that can be operated
105 at cryogenic temperature and is suitable for Helium spinning. The field of the 9.4 T magnet was
106 slightly adjusted such that the microwave irradiation at 263.7 GHz was matching the maximum
107 intensity in the AsymPol-POK's field profile, resulting in ¹H resonating at 400.2 MHz, ¹³C at 100.6
108 MHz on the X-channel, and ¹⁵N at 40.6 MHz on the Y channel of the probe (set in triple channel
109 configuration).

110

111 **NMR parameters**

112 ***Cross Polarization experiments***

113 The 1D experiments at 100 K and 40 K were carried out at a spinning frequency (ν_r) of 8 kHz.
114 For each experiment, 15 ms of free induction decay and 128 transients were acquired. The spectra
115 were processed with Topspin without any apodisation and plotted using Nmrglue [64] and Python.
116 The referencing was done indirectly with respect to TMS using adamantane at room temperature.
117 All the experimental parameters are tabulated in Table 1 of the supplementary information.

118

119 ***DARR***

120 The DARR [65] experiment was carried out at 40 K with $\nu_r = 7.5$ kHz. 25 ms of
121 acquisition time was employed in the direct dimension with 2.6 s of recycle delay between the 4
122 transients of each indirect time increment. 256 slices (128 complex points) were collected in the
123 indirect dimension. The slices were collected using STATES-TPPI for quadrature detection, with
124 a spectral width of 250 ppm. For DARR, the mixing time was set to 20 ms with an RF field strength
125 on ^1H matching the spinning frequency. The experimental parameters are tabulated in Table 2 of
126 the supporting information.

127

128 ***z-filtered TEDOR***

129 The *z*-filtered TEDOR experiment was carried out at 40 K with $\nu_r = 7$ kHz. The REDOR
130 mixing period was set at 1.14 ms to favour polarization transfer over distances corresponding to
131 one-bond. A *z*-filter of one rotor period was used to obtain purely absorptive peaks [66]. 16
132 transients were collected with 2.6 s recycle delay for each increment. 96 slices (48 complex points)
133 were collected in the indirect dimension using STATES-TPPI with a spectral width of 40 ppm.
134 The experimental parameters are tabulated in Table 2 of the supporting information.

135

136 ***DQ-SQ correlation***

137 The experiment was carried out at 40 K at $\nu_r = 6.7$ kHz. Modified SR26 recoupling [67]
138 with STiC phase-shift [68] was used for 4.77 ms total mixing time (double-quantum excitation and
139 reconversion). During the recoupling periods, 100 kHz Lee-Goldburg continuous wave decoupling
140 was applied to remove both ^1H - ^{13}C and ^1H - ^1H couplings. For each increment, 16 scans were
141 recorded with 2.6 s recycle delay. 128 slices (64 complex points) were recorded in the indirect
142 dimension using STATES-TPPI with a spectral width 400 ppm. The experimental parameters are
143 tabulated in Table 2 of the supporting information.

144

145 **RESULTS AND DISCUSSION**

146 Compared to the results reported earlier [39,61], our 3.2 mm MAS-DNP probe (called
147 PAVLOT) was completely re-designed to make it suitable for operation at ultra-low temperature
148 in a sustainable and cost-effective fashion. With the same purpose, our closed-loop cooling system
149 was upgraded for better performances and to fully eliminate helium consumption. The key
150 modifications made to the different parts of the system as well as specifications and results
151 obtained are delineated below.

152

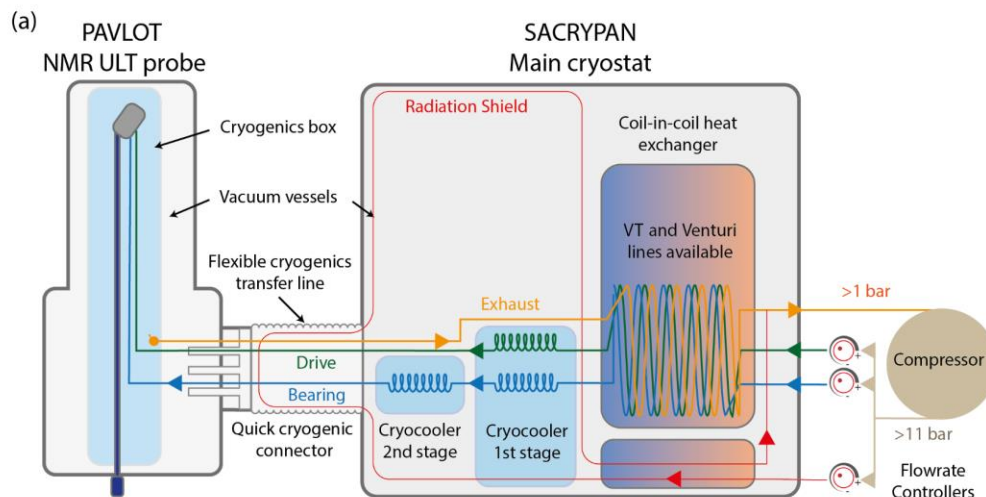
153 **Probe dewar architecture**

154 The probe was designed as a small cryostat and consists of two main parts, a probe base and a
155 cylindrical part (probe tube) that goes inside the magnet (Fig. 1). The interior of the probe tube,
156 which includes the stator, the RF capacitors and the internal waveguide, is maintained in a cold
157 helium atmosphere. The latter space is thermally isolated from the laboratory thanks to a double-
158 walled tubular envelope maintained under vacuum. To minimize thermal losses, the outer tube can

159 be clamped to the probe base housing without any contact with the inner tube, which contains the
160 cold gas. The inner tube can withstand a pressure of 4 bar on the inside while a vacuum is applied
161 on its outside (2×10^{-5} mbar), between the two walls.

162 The base of the probe is also under vacuum, to minimize thermal loss (5×10^{-6} mbar). This is
163 mandatory if one wants to minimize the number of cryocoolers needed to cool the closed-loop He
164 cycle. This is very different from a conventional 100 K DNP probe, where the probe base is
165 deliberately kept at higher temperature using flush gas to avoid freezing and condensation. This
166 latter approach is compatible with reaching 100 to 110 K at the sample using liquid nitrogen
167 cooling. This is obviously no longer suitable at helium temperatures, and for that reason we
168 designed our probe base as a small cryostat with the inner part protected from ambient temperature
169 by vacuum.

170



171
 172 Figure 1. (a) Schematic of the closed-loop helium spinning system built at CEA Grenoble (b) Corresponding
 173 photograph of the system, the probe being in the NMR magnet bore.

174 **Closed-loop He cycle for Bearing/Drive/VT gas powered with a single compressor**

175 As described previously [39,60,61], the rotation and cooling of the sample is achieved via a
 176 closed-loop helium cycle. In our case, the system is built around a single water-cooled screw
 177 compressor (Quantum systems – L x W x H = 0.8 x 0.6 x 1 m; Power rating = 11 kW) which can
 178 deliver a gas flow rate up to 1286 l/min (mass flow rate of 3.4 g/s) and create a pressure up to 11
 179 bar. The compressor is equipped with two 100-litre buffer gas tanks on the high- and low-pressure
 180 inlets respectively. Helium gas is compressed to a pressure of 9 bar and then fed into a cabinet that

181 controls the flow of four independent streams. The first two lines are used for sample spinning (i.e.
182 bearing and drive). The third line can be used for sample cooling (VT line) or, in the future, as a
183 Venturi line for 1.3 or 0.7 mm diameter rotor. The fourth line cools the radiation shield of the
184 cryostat, which contains the heat exchanger. We use additional 10 l buffer tanks (located before
185 the exchanger) on the bearing and drive lines to minimize fluctuations in sample spinning and to
186 act as a damper in case of compressor failure. The set up allows us to achieve a sample spinning
187 stability of 0.05 % (see Fig. 2a). The spinning detection is done using fiber optics and a home-built
188 spinning detection module. Currently the sample spinning is performed by setting manually the
189 Bearing and Drive flowmeter target values, which are regulated by feedback control. Automated
190 control of the spinning frequency using feedback loops on the drive and bearing flows will be
191 implemented soon.

192 The low-pressure tank on the compressor is maintained at 1.1 bar. Since we are working with a
193 closed-loop He cycle, this pressure corresponds also to the pressure inside the probe, which is
194 maintained above atmospheric pressure. After passing through the stator to spin the rotor, the
195 helium gas is deflected down the probe using a Kapton wrap, generating a persistent gas flow
196 around the capacitors. The high flow and high pressure inside the probe helps to prevent arcing
197 [60,61].

198

199 **Coupling the probe to the cryostat**

200 Cold He gas streams are delivered to the probe using a flexible cryogenic line, which is both
201 under vacuum (10^{-5} mbar) and thermally shielded (< 110 K). The connection ports to the probe are
202 Johnston bayonet type fittings. The five pairs of insulated tubes (bearing, drive, VT, radiation

203 shield, venturi) are equipped with a specific gasket to obtain a very good cold seal. The clamp can
204 withstand pressures up to several bars and is easily installed in less than a minute with toggle locks.

205 The initial cooling time of the entire setup, the cryostat and the probe, from RT to 30 K, measured
206 at the sample, is less than 24 h. We typically use < 500 L of He (under Normal Temperature and
207 Pressure) to flush the system before cooling. During normal system operation (i.e. under MAS),
208 there is virtually no helium consumption. The probe can be detached from the main cryostat within
209 30 minutes. While the probe is disconnected, the cryostat can be isolated and kept cold using a
210 flexible 5-line isolation loop. The probe can be reconnected and cooled down in 30 minutes due to
211 its low heat capacity and good thermal insulation. Overall, this procedure consumes < 100 L of He
212 (under Normal Temperature and Pressure). This is particularly convenient for changing the probe
213 configuration without warming up the entire system.

214

215 **Powerful heat exchanger equipped with a single cryocooler for cost-effective He spinning**

216 As previously reported, we use a counter-flow heat exchanger that has been designed to achieve
217 a perfect balance. The length of the helical coil-in-coil multi-tubes of the exchanger is calculated
218 so that the counter-flow heat exchange process takes most of the energy out of the gas entering the
219 exchanger, which implies that the out-going gas at the inlet point of the exchanger has temperature
220 close to that of the incoming gas. The efficiency of the heat exchanger can be expressed as follows:

$$221 \quad E \approx \frac{T_{1,in} - T_{1,out}}{T_{1,in} - T_{2,in}}$$

222 where, $T_{1,in}$ and $T_{2,in}$ are the temperatures of the two incoming gas flow, $T_{1,out}$ is the temperature
223 of the gas flow 1 at the outlet of the tube-in-tube architecture. When the mass flow rates of the two
224 gases are equal, the efficiency of the counter-flow heat exchange process can be conveniently
225 related to the Number of Transferrable Units (NTU):

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$$NTU = \frac{E}{1 - E}$$

NTU is a dimensionless constant which depends on the surface area available for exchange. Three configurations of heat exchanger were tested corresponding to NTU of 19, 24, and 32, which translate into 95 %, 96 %, and 97 % of transfer efficiency. The 96 % and 97 % configurations allow reducing the sample temperature by 4 K and 7 K, respectively, with respect to the 95 % one. However, they both result in a significant drop in pressure that is detrimental for fast sample spinning. Therefore, we chose to work with 95 % efficiency in this study. The design of the exchanger was guided by numerical calculations with three aims in mind: (a) to transfer most of the enthalpy from the outgoing to the incoming gas, (b) to minimize the pressure drop through the heat exchanger to favor high spinning frequencies while minimizing frictional heat inside the tubes, (c) to minimize longitudinal conduction along the tubes to produce a thermal by-pass, which prevents heat conduction from the warm to the cold end.

After passing through the heat exchanger, the He gas goes through a two-stage CRYOMECH PT cooler that can reach 10 K in one hour. The first stage produces 80 W of cooling power at 55 K while the second stage provides 22 W of cooling power at 18 K. The compressor of the PT requires 9.2 kW and can be stored easily because of the relatively small footprint (L x W x H = 0.6 x 0.6 x 0.6 m). Each of the cryocoolers used in the OSAKA setup (i.e. 3 to 5 GM) have similar performance as the unique PT cryocooler used here, highlighting both the excellent performance of our heat exchanger and the minimal losses of the system (cryostat and probe). The CRYOMECH cryocooler is mechanically connected to 2 regenerative heat exchangers, also known as capacitive heat exchangers, which consist of a single flow path inside a copper block to temper the fluid. The capacitive heat exchangers are mounted with the goal to minimize thermal resistance (0.01 K/W). Three heat exchanger configurations were tested for cryogenic Helium spinning:

- 249 1. Drive, bearing, and VT gas lines going through the first stage of the cryocooler and only
250 the VT line further through the second stage. In this configuration, we could reach about
251 50 K at the sample.
- 252 2. Drive, bearing, and VT gas lines all going through the first stage of the cryocooler and both
253 bearing and VT lines further through the second stage. With this configuration, we could
254 reach 40 K at the sample.
- 255 3. Drive and bearing gas lines going through the first stage of the cryocooler and only the
256 bearing line further through the second stage. No VT gas is used. With this configuration,
257 we could reach 30 K on the sample.

258 We conclude that for 3.2 mm rotor and our current setup, there is no need to use a VT line. The
259 sample temperature was measured using a rotor filled with KBr and spinning the sample at 8 kHz
260 [63]. Note that 20 W μ w irradiation (measured at the probe base) increases the sample temperature
261 by \sim 5 K for a zirconia rotor filled with KBr.

262

263 **Probe performances: spinning stability, noise figure, and RF field strengths**

264 The spinning stability of our system was monitored by following the position of the first
265 sideband of a series of ^{79}Br spectra. As each spectrum can be recorded with only one scan, it
266 provides a direct access to frequency fluctuations of our system. As shown in Fig. 2a, we monitored
267 the position of the sideband every 2 s, over a period of about 45 min. The variation in spinning
268 frequency is about 0.05% of the nominal value, indicating an excellent stability, which is important
269 for conducting experiments that demand rotor synchronization and spinning stability.

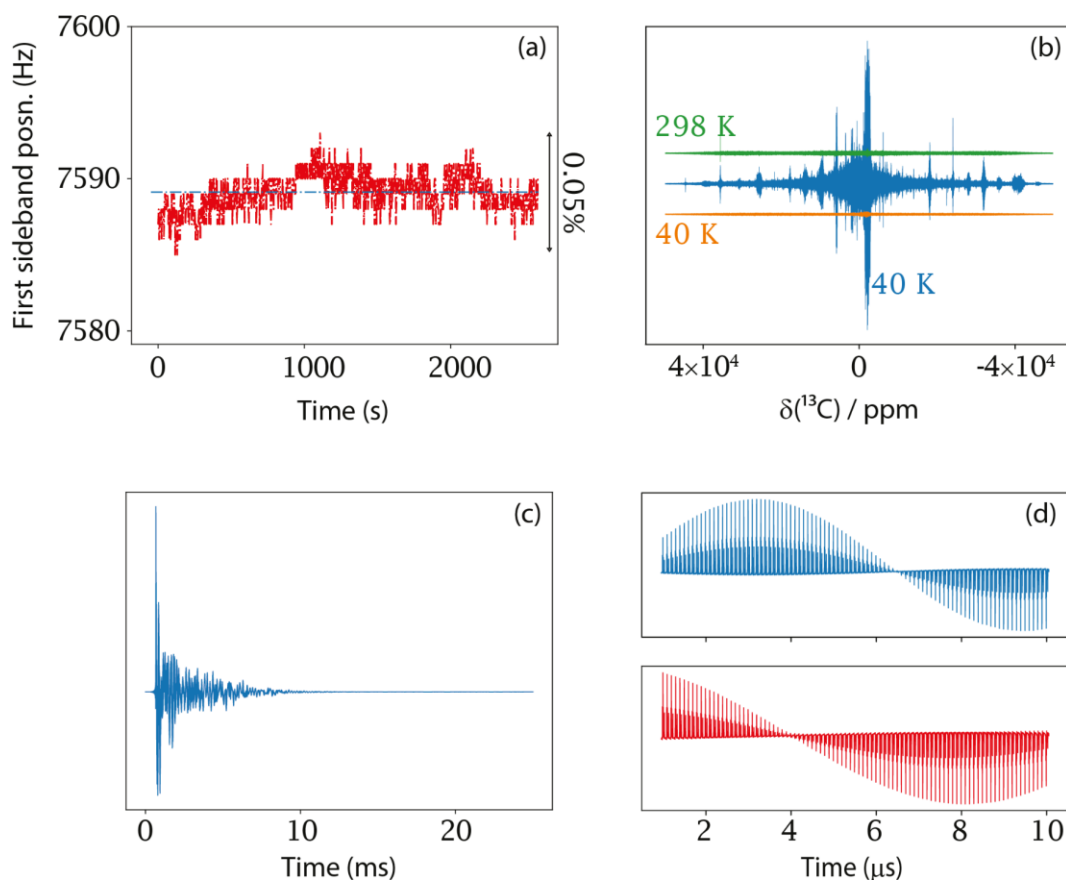
270 The current setup allows reaching 27 K at low spinning speed (\sim 5 kHz) and in absence of
271 microwave irradiation. The rotors can be spun up reliably up to 10 kHz at 30-35 K in absence of

272 microwave irradiation. The latter increases the temperature of standard samples by 5-7 K using the
273 full power of the gyrotron (about 22 W at the base of the probe).

274 In order to provide best sensitivity, it is mandatory to investigate the noise level of the probe. At
275 helium temperature, the probe circuit can act as a good receiver to pick up RF signals from
276 surrounding electromagnetic waves and from other electronic circuits in proximity (temperature
277 sensors, MAS detection, etc.). In Fig. 2b, the blue trace shows the initial noise spectrum obtained
278 for PAVLOT at 40 K with the X channel tuned to ^{13}C . It clearly demonstrates the undesirable RF
279 pickup of FM radio broadcasting. After shielding and grounding of the probe connectors and
280 internal components (mainly the Cernox sensor used for the detection of the sample temperature),
281 the noise spectrum was significantly improved at 40 K (orange trace). Considering the specific
282 frequency region of the carbon spectra (0 – 200 ppm), the noise is reduced by a factor of 1.5 going
283 from 298 K to 40 K (comparison between green and orange data). With an electronic circuit (probe
284 and preamplifier) entirely cooled, one would expect the thermal noise to improve by a factor of
285 ~ 2.8 by going from 298 to 40K. We ascribe the discrepancy to the fact that the pre-amplifier is not
286 cooled here. This strongly support the development of cold preamplifier for NMR experiments run
287 at ultra-low temperature, as recently demonstrated by Matsuki *et al.*[69].

288 As mentioned previously, we maintain high flow and pressure in the probe to avoid any arcing.
289 This approach seems so far successful as demonstrated in Fig. 2c, which shows the free induction
290 decay (FID) of a CP experiment acquired with 25 ms of ^1H decoupling at 100 kHz rf field strength.
291 Nutation curves on both ^1H and ^{13}C channels are given in Fig. 2d at RF field strengths of 78.1 kHz
292 (at 60 W) and 62.5 kHz (at 50 W), respectively. The ^1H nutation curve was recorded indirectly
293 through CP by detecting ^{13}C while increasing the length of the ^1H excitation pulse, giving
294 maximum signal intensity for a 90° pulse. The carbon nutation curve was recorded by increasing

295 the length of a flip-back pulse after the CP, resulting in a zero-crossing of the signal intensity for
296 the 90° pulse.



297
298 Figure 2 (a) Position of the 1st sideband, with respect to the centerband, in a ^{79}Br spectrum of KBr. The spectrum was
299 recorded with 1 scan every 2 s. The nominal spinning frequency was 7589 Hz, and the maximum variation observed
300 was 0.05% of the nominal frequency as indicated. (b) Noise spectra acquired with a large spectral window at 40 K
301 before shielding and grounding (blue) and at 298 K (green) / 40 K (orange) after shielding of the probe components.
302 (c) ^{13}C FID acquired for 25 ms with 100 kHz ^1H decoupling. (d) Nutation experiments performed indirectly via CP
303 on ^1H (top panel), and ^{13}C (bottom panel). The corresponding nutation frequency are 78.1 kHz at 60 W for ^1H , and
304 62.5 kHz at 50 W for ^{13}C (3.2 and 4 μs 90° pulse for ^1H and ^{13}C , respectively).

305 306 **RF circuit and matching/tuning capability**

307 The RF part of the probe is a commonly used triple resonance configuration, consisting of a
308 transmission line in the ^1H channel and lumped elements such as variable capacitors and
309 inductances for tuning, matching and providing isolation between channels. The variable

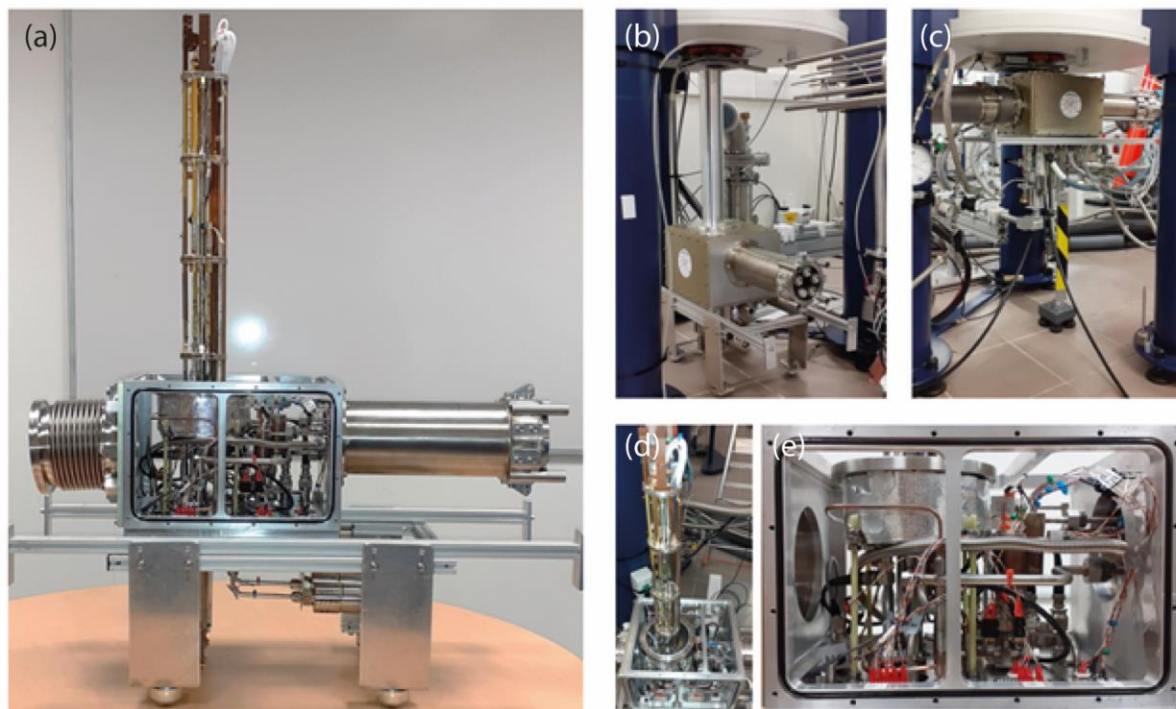
310 capacitors consist of two coaxial sapphire cylinders with variable overlap. Using the same material
311 for the inner and outer part of the capacitor allows for tuning in a wide temperature range,
312 preventing any mechanical jamming due to different thermal expansion coefficients. Hence, the
313 coil and the RF capacitors are placed in the cold He environment consistent with our previous
314 design, which also leads to an improvement in signal-to-noise ratio due to lower thermal noise.

315 The X and Y tuning capacitors feature a capacitance range of 2 to 50 pF, giving both channels
316 broadband capabilities. By adding shunt capacitors in parallel to the tuning capacitors, the range
317 can be further extended towards lower frequencies, whereas a serial capacitor can be connected to
318 increase the X and Y frequencies, and the XY stop circuit can be changed to implement various
319 triple resonance configurations. The RF circuit is not optimized for one fixed set of nuclei but is
320 kept flexible to enable tuning to multiple nuclei. A proper isolation between the different RF
321 channels is done by LC stop circuits and grounding at the zero voltage crossing along the
322 transmission line.

323 It is important to stress that the probe behaves like a small helium-tight cryostat, which imposes
324 new constraints, such as the design of helium-tight cryogenic feedthroughs that control the cold
325 RF capacitors and the magic angle setting. The probe shown in Fig. 3 is equipped with 7 cryogenic
326 feedthroughs corresponding to the tuning/matching capacitors for the three HXY channels and the
327 magic angle adjustment system.

328

329



330

331 **Figure 3.** (a) Profile of our re-designed probe. (b) and (c) Photograph of the probe being inserted in a wide-bore 9.4
332 T magnet. (d) Top view of the RF circuits with (e) zoom on the tuning and matching rod feedthroughs.

333

334 **Cryogenic sample exchange compatible with a helium closed loop cycle**

335 To prevent any pollution of the closed-loop He cycle, e.g. seeping moisture, which would cause
336 spinning problems or rotor blockage in the transfer line, we conceived an antechamber fixed at the
337 sample insert/eject point of the probe. Before inserting a sample, the rotor is placed in a home-
338 built sample-catcher acting as antechamber, and three cycles of helium gas flushing and
339 subsequent pumping (two minutes) are performed. To our knowledge, this is the first example of
340 an insert-eject system that is compatible with a closed-loop He-spinning system. This is an
341 important step towards routine use of such a technology. Indeed, the absence of cryogenic sample
342 exchange implies to warm up, disconnect, and open up the probe before the sample can be
343 exchanged. Then, the probe needs to be reconnected and cooled down again. The cooling of the
344 probe is then done while spinning the sample from RT to cryogenic temperature (20 to 40 K

345 typically) [60]. Overall, such a procedure is time-consuming, tedious and has a high probability of
346 introducing impurities into the probe and therefore into the recirculated closed-loop system. With
347 the cryogenic insert-eject system described here, the sample is inserted into the cold probe within
348 minutes, ready to be spun and measured. Overall, inserting/ejecting a sample requires about 1 L of
349 He gas (at Normal Temperature and pressure).

350 Furthermore, this insert-eject system enables refining the magic angle (if needed) at the
351 experimental cryogenic temperature prior to start experiments. Without this system, one needs to
352 warm up the entire probe after magic-angle setting, e.g. using KBr, to insert the sample of interest
353 before cooling back again. Such important temperature cycles of ~250 K can sometimes slightly
354 change the magic angle settings, making it unreliable.

355

356 **Sensitivity considerations at 100 K and below**

357 As reported in the literature, there is a clear interest in performing MAS NMR at cryogenic
358 temperatures of 100 K and below, and combining it with DNP, when possible. In addition to
359 increasing the spin polarization, cooling the sample and probe electronics also reduces noise
360 [60,61,70], both improving the signal-to-noise ratio (SNR). Additionally, cooling the sample to
361 cryogenic temperatures also lengthens nuclear coherence dephasing times, which allows for
362 significantly increased polarization transfer. This has proven particularly useful in the case of
363 quadrupolar nuclei and to record long-distance ^{13}C - ^{13}C and ^{15}N - ^{13}C transfer at natural isotopic
364 abundance [20,71–76].

365 Recently, several groups have combined DNP at temperature much lower than 100 K. In
366 all cases, these reports have pointed towards a systematic gain in sensitivity, typically up to one
367 order of magnitude, by reducing the sample temperature from 100 K to 30 K, depending on the

368 systems and PA used [39,56,61,69,77,78]. This was also tested with our new probe and is discussed
369 in the next paragraph (see also Fig. 4).

370

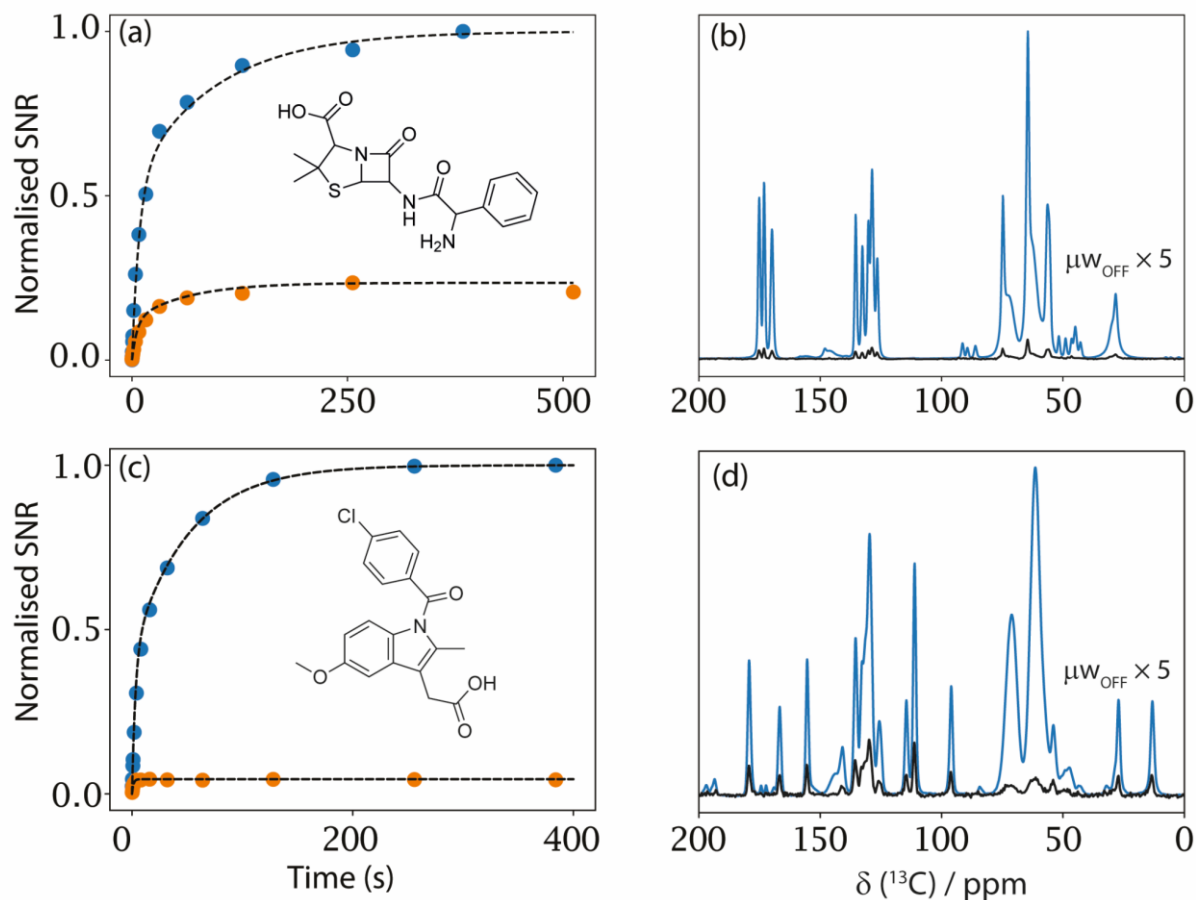
371 **Polarizing protonated microcrystals with DNP at $\ll 100$ K**

372 As recently reported by our group [54], it is important to report the performance in sensitivity of
373 polarizing agents on protonated systems and not simply on deuterated frozen solutions. Therefore,
374 we chose two proton-dense microcrystalline solids impregnated with cAsymPol-POK, one of the
375 latest polarizing agents reported so far [50]. Organic microcrystals are non-porous micrometer-
376 size particles. Polarizing microcrystals with DNP is challenging but can be facilitated if one of the
377 crystal dimensions (or more) is smaller than a micron, or if the ^1H bulk longitudinal relaxation
378 time constant T_{1n} of the undoped system is much longer than the time needed to propagate the
379 hyperpolarization from the outer shell to the core of the system [79–81].

380 To illustrate the benefits in lowering the sample temperature down to 30-40 K, we chose to
381 polarize two organic microcrystals, ampicillin and indomethacin, that have an inherent bulk ^1H T_{1n}
382 (undoped) of > 70 s and ~ 3 s, respectively, at 100 K. Since it typically takes a few seconds (or
383 more) to polarize microcrystals with DNP at 100 K and 9.4 T, the very short bulk ^1H T_{1n} of
384 indomethacin makes it extremely difficult to polarize compared to ampicillin, which has a longer
385 bulk ^1H T_{1n} of > 70 s.

386 The results are reported in Fig. 4, which shows the ^{13}C signal-to-noise (SNR) as a function of
387 the hyperpolarization time, at 100 K and 40 K. The ^{13}C signal is measured through ^1H to ^{13}C
388 CPMAS. The obtained buildup curves can be fitted using one or two exponential functions. For
389 the build ups requiring two components, the shorter of the two is directly related to the efficiency
390 of the PA to polarize the outer shell of the particles (about hundreds of nm)[79], whereas the longer

391 component can be seen as the ability to polarize the nuclei at the core which are more distant from
 392 the polarizing agent [46,54,73,73,76,81].



393
 394 Figure 4. (a) and (c) Signal to noise (SNR) ratio as a function of the hyperpolarization time (DNP build up experiments)
 395 at 100 K (orange circles) and 40 K (blue circles) of (a) the C11 resonance of ampicillin at 172 ppm and (c) the C10'
 396 of indomethacin at 155.1 ppm. SNR was measured from ^1H - ^{13}C CP spectra. The samples were impregnated with 20
 397 mM cAsymPol-POK in GDH matrix (see Materials and Methods). The polarization buildups were fitted with a sum
 398 of two exponentials as described in the text, except for the indomethacin one at 100 K, which is mono-exponential.
 399 (b) and (d) Microwave on (blue) and microwave (off) (black and scaled vertically by a factor 5) CPMAS spectra at 40
 400 K of (b) ampicillin with a recycle delay of 11.2 s and (d) indomethacin with a recycle delay of 5 s. The recycle delay
 401 was calculated in each case to obtain the best sensitivity [54]. The apparent DNP enhancement factor $\epsilon_{\text{on/off}}$ is about
 402 98 for ampicillin and 23 for indomethacin. For the CP experiments, 81 kHz of ^1H decoupling using SW_f -TPPM [82,83]
 403 was applied during detection. Ramped CP of 2 ms contact time was used to transfer the polarization from the ^1H to
 404 ^{13}C [84]. Pre-saturation pulses were used on both channels and an echo of two rotor-periods in all was applied prior
 405 to detection.

406
 407 For the ampicillin sample, we first notice that the SNR at the plateau is increased by a factor of
 408 4.8 by lowering the temperature from 100 K to 40 K. For a better understanding, it is interesting

409 here to rationalize this signal-to-noise improvement factor in order to disentangle the different
410 contributions. On one hand, comparing the noise level at 40 K versus 100 K, we observe a
411 reduction of about $\times 1.2$. On the other hand, lowering the temperature from 100 K to 40 K leads to
412 an increase of the electron Boltzmann polarization by ~ 2.5 . Considering these two factors, it
413 suggests that the DNP efficiency is roughly improved by a factor of ~ 1.6 in this case.

414 The polarization buildup shown in Fig. 4 (a) had to be fitted with a sum of two exponential
415 functions at both temperatures. The short and the long components are 5.7 s (50 %), and 62 s (50
416 %) at 100 K, and 8.5 s (55 %) and 96 s (45 %) at 40 K. The requirement of a two-components fit
417 is consistent with the hyperpolarization of a core-shell structure. Interestingly, both the short and
418 long components of the buildup get slightly longer upon cooling from 100 to 40 K, while their
419 respective weight is roughly preserved (about 1:1). Note that the long component is limited by
420 bulk $^1\text{H } T_{1\rho}$ (undoped). Fig. 4 (b) shows the ^{13}C CPMAS spectrum with and without microwave
421 irradiation for a recycling time that maximizes the sensitivity with microwave. The corresponding
422 DNP enhancement factor is 98 at 40 K and 62 at 100 K, with 11.5 s and 8.2 s recycling time,
423 respectively. Comparing the sensitivity, a factor of ~ 2.7 of improvement is observed when
424 lowering the temperature from 100 K to 40 K. In terms of resolution, the full width at half height
425 (FWHH) of the CO resonance is roughly identical at both temperatures. This is therefore a nice
426 example where sensitivity is improved without compromising the resolution. Another striking
427 observation is the large $\times 35$ of signal enhancement of the methyl groups at 40 K compared to 100
428 K. It is well established that the methyl rotation at 100 K makes the ^1H hyperpolarization by DNP
429 challenging, and that it can also potentially interfere with CP and ^1H decoupling spin dynamics,
430 resulting usually in low intensities for CH_3 signals. As suggested in Ref [85], the reduced methyl
431 rotation below 75 K is likely to help recovering more favorable relaxation properties, which is

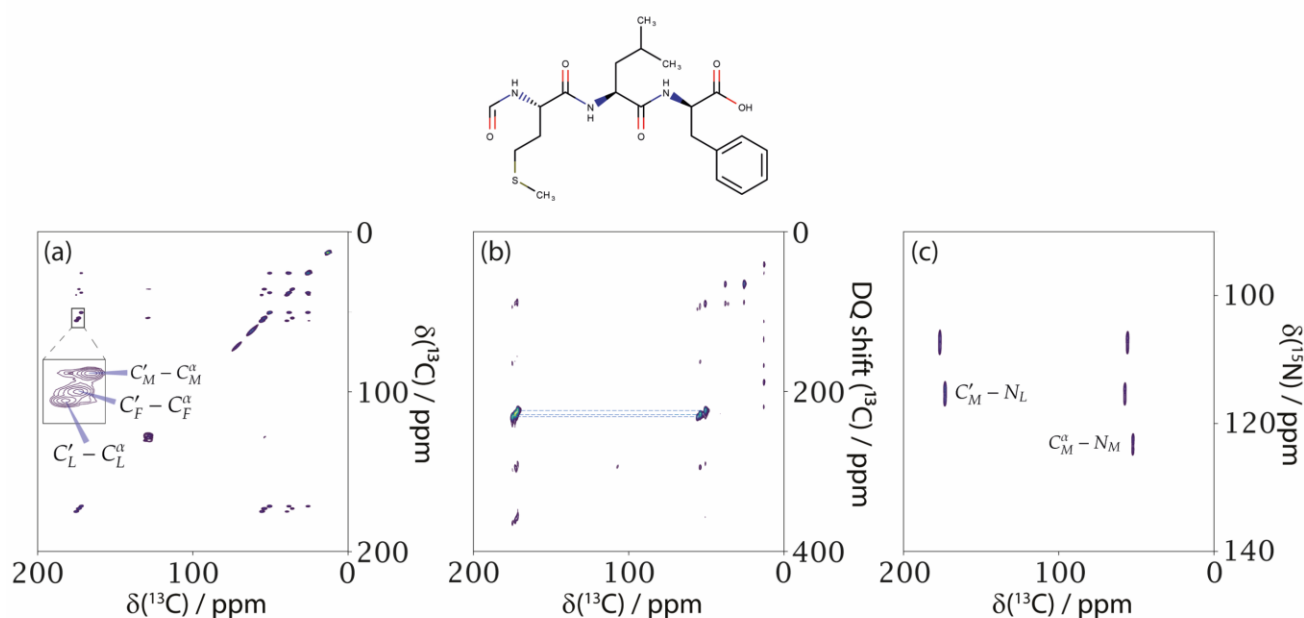
432 confirmed by our data. Lowering the sample temperature at 40 K should definitely help probing
433 methyl-methyl long-range constraints as suggested in Ref [85].

434 The case of indomethacin microcrystals is very different. Indeed, the time required to
435 hyperpolarize the particles (up to 10 s or more) is typically longer than the bulk ^1H T_{1n} of ~ 3 s at
436 100 K, making them difficult to polarize at 100 K, as shown in Fig. 4 (c). Interestingly, the ^1H
437 polarization buildup at 100 K can be fitted using only one component of about 1.3 s. This makes
438 sense considering the bulk ^1H bulk T_{1n} (undoped). In this case, we only start polarizing the outer
439 shell of the microcrystals but diffusion of the hyperpolarization within the particle is limited by
440 the bulk ^1H T_{1n} , which brings the polarization back to Boltzmann equilibrium. Remarkably, as we
441 cool the sample down to 40 K, the ^1H buildup (Fig. 4 (c)) becomes bi-exponential, with a short
442 component at 3.3 s and a longer one at 47 s (weights of 41 % and 59 %, respectively). This clearly
443 highlights that hyperpolarization can now be transferred beyond the outer shell of the particles, as
444 a direct consequence of a longer bulk ^1H T_{1n} of indomethacin microcrystals at 40 K. Finally, Fig.
445 4 (d) shows the ^{13}C CPMAS spectrum of indomethacin with and without microwave irradiation at
446 40 K for a recycling time of 5 s that maximizes the sensitivity with microwave. The corresponding
447 DNP enhancement factor is 23, to be compared to the DNP enhancement factor of 3 at 100 K with
448 1.8 s recycling. Overall, this translates into an improvement in sensitivity of x5 and or in time-
449 savings of x25 when going from 100 K to 40 K, using cAsymPol-POK as polarizing agent.

450

451 **Multidimensional experiments at 40 K**

452 After discussing the improved sensitivity that is achieved on organic microcrystals, we also show
 453 that our closed-loop He cycle can be used to record multidimensional experiments that requires
 454 stable spinning, double/triple channel irradiation and high power H/X/Y pulses. The experiments
 455 shown in Fig. 5 were performed on diluted tri-peptide U- ^{13}C , ^{15}N -N-Formylmethionyl-leucyl-
 456 phenylalanine (*f*-MLF). With a bulk ^1H $T_{1\rho}$ of ~ 3 s at 100 K, this microcrystalline system is also
 457 challenging to polarize. Similarly, to the indomethacin case, a drastic improvement in SNR of a
 458 factor of 6 is obtained by hyperpolarizing this sample at 40 K compared to 100 K, using cAsymPol-
 459 POK as polarizing agents. The polarization buildup at 40 K is bi-exponential with components at
 460 3.6 s (40 %) and 20 s (60 %).



461 Figure 5. Different 2D experiments on the *f*-MLF sample. (a) 2D ^{13}C - ^{13}C DARR spectrum obtained in 49 minutes
 462 with 20 ms of mixing time. The inset shows intra- (labelled) and inter-residue cross peaks. (b) DQ-SQ spectrum using
 463 SR26 with STiC phase shifts. It was acquired in 1 hr 42 min with 4.77 ms of total mixing time. (c) Triple-resonance
 464 z-filtered TEDOR experiment with excellent sensitivity obtained in 1 h 14 min, with a mixing time of 2.2 ms.

465
 466 Fig. 5 (a) shows a 2D ^{13}C - ^{13}C DARR correlation spectrum obtained with 20 ms of mixing time.
 467 As shown in the inset, the spectrum is dominated by intra-molecular cross peaks (e.g. $\text{C}_{\text{M}'} - \text{C}_{\text{M}}^{\alpha}$)

468 , $C_F' - C_M^\alpha$, and $C_L' - C_L^\alpha$ for the region considered in the inset), but inter-residue cross peaks (e.g.
469 $C_M' - C_L^\alpha$ and $C_L' - C_F^\alpha$) are also visible at smaller intensity because of the isotopic dilution of the
470 sample. The well-resolved data stresses two important points: (a) the magic angle can be set very
471 accurately given the resolution of the carbonyl lines, (b) high power ^1H decoupling can be applied
472 during 30 ms (or more) without any sign of arcing, enabling the good resolution achieved in both
473 dimensions.

474 To further highlight the excellent spinning stability and RF performance of the probe, we report in
475 Fig. 5 (b) a 2D DQ-SQ correlation experiment required at 6.7 kHz MAS using SR26 [67] combined
476 with STiC phase shift [68]. The latter alleviates the need to rotor-synchronize evolution in the
477 indirect dimension. This yields clean 2D data, where the spectral width in the indirect dimension
478 can be chosen sufficiently large to avoid signal folding. The very good quality of the 2D spectrum
479 and the absence of artefact are the direct consequence of the excellent spinning stability and RF
480 performance of the probe. Finally, we report a triple resonance zf-TEDOR experiments in Fig. 5
481 (c) employing 15 ms of 75 kHz ^1H decoupling. The correlations seen in the 2D correspond to N-
482 $C\alpha$ and N- C' transfer over one-bond.

483

484 **CONCLUSIONS**

485 In this work, we have described a sustainable and cost-effective approach to cryogenic Helium
486 spinning. The system is based around a new He spinning MAS-DNP probe, a powerful counter-
487 flow heat exchanger (95 % efficient), a single cryo-cooler and a single He compressor. The current
488 setup allows reaching 27 K at low spinning speed (~ 5 kHz), and about 30-35 K at 10 kHz (in
489 absence of microwave irradiation). Faster spinning frequencies can be achieved (up to 15 kHz at
490 40 K) but not reliably. Indeed, at such spinning frequencies, the drive caps often come off due to
491 the differential thermal contraction of the rotor (zirconia) and the caps (vespel), but we are

492 currently working on this problem with the design of new rotor caps for faster spinning. In addition,
493 the system is equipped with a cryogenic sample exchange capability, which allows swapping
494 samples in minutes. Tested on organic microcrystals, we report large SNR improvements by
495 decreasing the sample temperature from 100 to 40 K. The improvements are particularly
496 pronounced for systems that are difficult to polarize because of their intrinsic short $^1\text{H } T_{1\rho}$ at 100
497 K. The excellent MAS stability and RF performance of the probe are illustrated with double/triple
498 resonance 2D experiments.

499 Beyond the goal of performing NMR/DNP in the < 50 K regime, the Grenoble setup has been
500 developed with a clear commitment towards cost-effectiveness and sustainability, with
501 considerable attention paid to thermal isolation and heat transfer efficiency, to minimize cooling
502 power requirements, footprint, power consumption and maintenance cost. The Grenoble setup has
503 now an efficient sample insert-eject capability and will be upgraded in a next future with a second
504 cryocooler to reach < 15 K. Overall, the Grenoble setup paves the way to a cost-effective solution
505 for sustainable fast MAS He spinning. These advances will be combined with the development of
506 tailored polarizing agents and should prove particularly useful for the development of very high
507 field DNP combined for instance with cheaper solid-state sources.

508

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520

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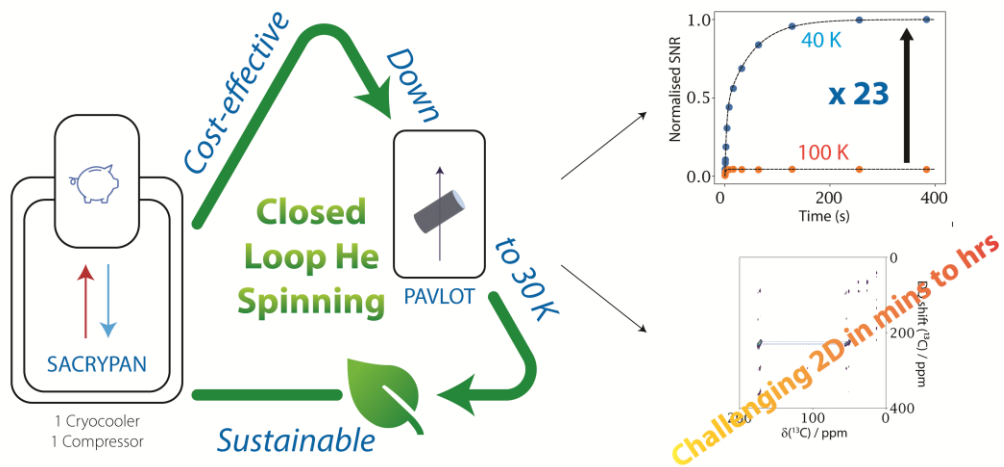
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