

1 A novel sample handling system for dissolution dynamic 2 nuclear polarization experiments

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8

9 Abstract

10 We present a system for facilitated sample vitrification, melting, and transfer in dissolution dynamic nuclear
11 polarization (DDNP) experiments. In DDNP, a sample is typically hyperpolarized at cryogenic temperatures
12 before dissolution with hot solvent and transfer to a nuclear magnetic resonance (NMR) spectrometer for detection
13 in the liquid state. The resulting signal enhancements can exceed four orders of magnitude. However, the sudden
14 temperature jump from cryogenic temperatures close to 1 K to ambient conditions imposes a particular challenge.
15 It is necessary to rapidly melt the sample to avoid a prohibitively fast decay of hyperpolarization. Here, we
16 demonstrate a sample dissolution method that facilitates the temperature jump by eliminating the need to open the
17 cryostat used to cool the sample. This is achieved by inserting the sample through an airlock in combination with
18 a dedicated dissolution system that is inserted through the same airlock shortly before the melting event. The
19 advantages are threefold: 1. The cryostat can be operated continuously at low temperatures. 2. The melting process
20 is rapid as no pressurization steps of the cryostat are required. 3. Blockages of the dissolution system due to
21 freezing of solvents during melting and transfer are minimized.

22 *Dedicated to Prof. Geoffrey Bodenhausen on the occasion of his 70th Birthday.*

23

24 1 Introduction

25 Dissolution dynamic nuclear polarization (DDNP)(Ardenkjaer-Larsen, Fridlund et al. 2003, Kovtunov,
26 Pokochueva et al. 2018, Jannin, Dumez et al. 2019) is a method used for hyperpolarizing nuclear spins at cryogenic
27 temperatures (Abragam and Goldman 1978) close to 1 K - typically attained in a liquid helium-cooled cryostat at
28 low pressures - coupled to subsequent temperature jump and detection at ambient conditions in a conventional
29 liquid-state nuclear magnetic resonance (NMR) spectrometer. Spin hyperpolarization is herein understood as a
30 strong increase of the population difference between the populations of two eigenstates in a magnetic field B_0 . The
31 transfer of the hyperpolarized sample from DNP conditions at low temperatures to NMR conditions at ambient
32 temperature is typically achieved with a burst of hot solvent. It rapidly dissolves the sample and pushes it through
33 a capillary to the detection spectrometer. One can thus achieve signal enhancements in liquid state NMR of four
34 orders of magnitude. (Vuichoud, Milani et al. 2015) Capitalizing on the resulting improved sensitivity, DDNP has
35 found various applications in recent years, including real-time metabolomics (Liu and Hilty 2018, Sadet,

1 Emmanuelle M. M. Weber et al. 2018), reaction monitoring (Boeg, Duus et al. 2019), structural biology (Szekely,
2 Olsen et al. 2018, Wang and Hilty 2019), and detection of long-lived spin states (Tayler, Marco-Rius et al. 2012,
3 Bornet, Ji et al. 2014). However, DDPN instrumentation is still actively being developed to improve its cost-
4 efficiency and reliability, and a need for user-friendly DDPN systems persists.

5 The sample insertion into and dissolution from the cryostat poses a challenge in designing such systems as both
6 events introduce large heat quantities and warm the instrumentation. The heat shock needs to be compensated by
7 the liquid helium bath within the cryostat (Ardenkjaer-Larsen, Bowen et al. 2019) at the expense of prolonged
8 experimental polarization times or polarization losses.

9 Indeed, upon insertion of a sample the variable temperature insert (VTI) is typically heated as the sample is warmer
10 than the helium bath and then needs to be cooled down again before efficient DNP can take place. This process
11 can significantly delay the DNP procedure if the VTI is heated too much. Upon dissolution, the VTI often needs
12 to be pressurized so that the dissolution system can be inserted, if a 'fluid-path' system is not available. During
13 this period, the sample also warms up, which might also cause loss of hyperpolarization before the dissolution
14 event due to faster longitudinal relaxation.

15 In addition, if the temperature of the capillaries used for transfer drops excessively after insertion, the liquid used
16 to dissolve the sample may freeze before exiting the cryostat, preventing the liquid containing the hyperpolarized
17 substance from reaching the NMR spectrometer for detection.

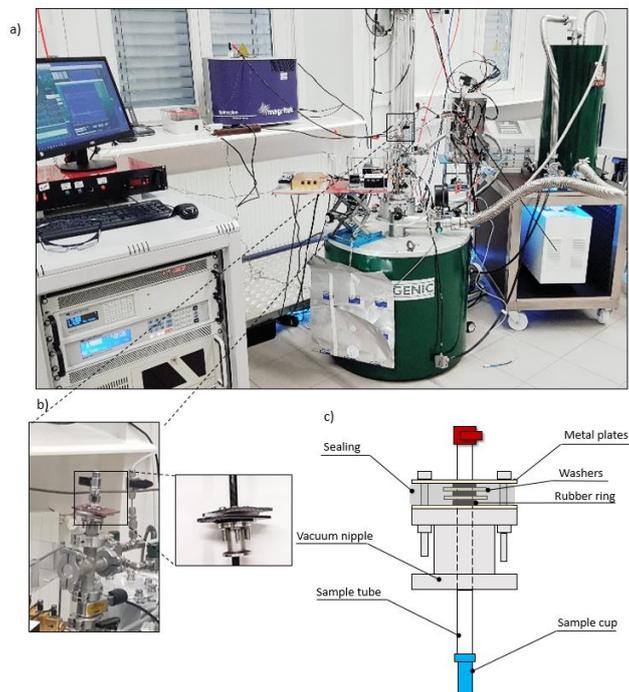
18 Two widely-used solutions to these problems have been proposed:

19 1. To minimize the heat-load, Ardenkjaer-Larsen and co-workers have developed a sample insertion and
20 dissolution system based on a 'fluid path' and a 'dynamic seal' as proposed in their original design for the SpinLab
21 DDPN system. (Malinowski, Lipso et al. 2016) The capillaries guiding the dissolution solvent are slowly inserted
22 into the cryostat together with the sample through an airlock. Thus, one can put the capillaries and sample in place
23 without breaking the cryostat's vacuum. After the DNP procedure, the sample can be dissolved through the already
24 positioned capillaries. However, as these are also held at cryogenic temperatures during the DNP build-up period,
25 the dissolution solvent might freeze if the joints between the sample holder and solvent inlet/outlet are not carefully
26 sealed to avoid liquid helium entering the sample chamber.

27 2. Alternatively, in a second approach inspired by the original 'HyperSense' apparatus, the cryostat is pressurized
28 with helium gas and opened briefly to insert the sample. Bodenhausen and co-workers successfully adapted this
29 design to recently developed DDPN systems. (Kurzbaach, Weber et al. 2016, Baudin, Vuichoud et al. 2018) After
30 completing the DNP procedure, the cryostat is pressurized and opened again to insert the capillaries needed for
31 the dissolution event. This design has the advantage of minimizing the risk of freezing the dissolution solvent as
32 the capillaries are not cooled down during the DNP period. However, this comes at the expense of increased heat
33 exchange and helium losses during sample insertion and dissolution compared to the fluid-path design.

34 To capitalize on both systems, we have developed an alternative hybrid sample handling design. Here, the sample
35 is inserted through an airlock and a vacuum seal system that enables insertion with minimal heat load, while at the
36 same time, sample dissolution can be performed with warm capillaries and without breaking the cryostat's vacuum.
37 We demonstrate this design's implementation in a cryogen consumption-free DNP system similar to that described
38 by Bodenhausen and co-workers (Baudin et al. 2018).

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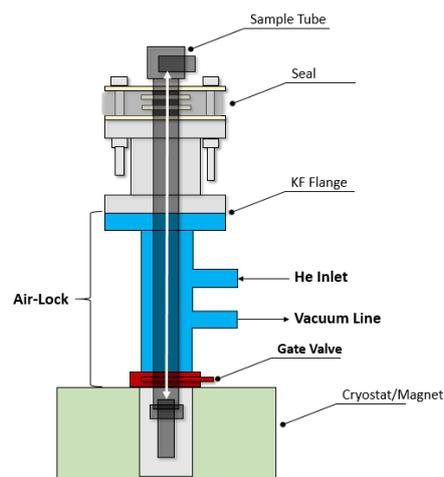


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4 **Figure 1.** a) The cryogen-consumption-free DDNP system (green magnet) used together with the proposed hybrid sample handling system.
5 The low-field spectrometer (blue magnet) used here for detection is situated in the back. b) Zoom on the airlock atop the DDNP system with
6 the vacuum seal attached. The smaller panel shows the detached seal. c) Scheme of the seal (grey), the sample chamber (blue), and the sample
7 tube (white). An array of washers, a silicon fitting, and O-rings renders the seal vacuum-tight, while the sample stick can be moved vertically.
8 The seal is attached to the airlock atop the DNP system via a vacuum nipple. (Images by K. Che and L. M. Epasto.)

9 2 Results and Discussion

10 The proposed sample handling device is described in Fig. 1. **The polarizer, that operates at 6.7 T and a nominal**
11 **base temperature down to 1.3 K (1.4 K under microwave irradiation),** and the spectrometer used for low-field
12 liquid-state detection are shown in Fig. 1a. The most crucial component of the sample handling system is a vacuum
13 seal that surrounds a hollow carbon fiber rod, which we denote as 'sample tube'. The seal is placed atop an airlock
14 *via* a vacuum nipple (Fig. 1b). The seal itself is closed vacuum-tightly around the sample tube via alternating layers
15 of washers and O-rings pressed together by two metal plates (Fig. 1c). A lateral rubber sealing additionally encloses
16 the seal.



1
 2 **Figure 2.** Scheme of the airlock system (blue/red) situated between the seal and the cryostat. The latter can be sealed with a gate valve. The
 3 sample tube can be inserted upon opening the gate valve. With the He inlet and the connection to a vacuum line, air can be purged from the
 4 airlock. The double-headed white arrow indicates the sample tube sliding path through the seal and the airlock into and out of the cryostat
 5 (Figure not to scale.)

6 When mounted on top of the cryostat, the combination of airlock and vacuum-seal allows one to slide the sample
 7 tube relative to the seal and position it inside the cryostat without the need to open the latter or break the vacuum
 8 within (cf. Fig. 2). A sample can thus be inserted without opening the DNP system to the atmosphere, thereby
 9 preventing air from condensing in the cryostat.

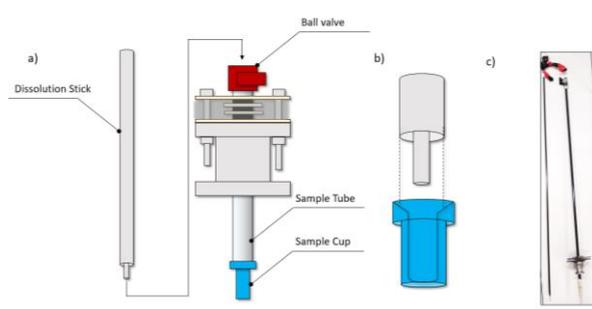
10 The sample tube is closed at its lower end by a 'sample cup' containing the substance to be hyperpolarized, and at
 11 its top by a ball valve (Fig. 3a). Hence, it constitutes a closed volume inserted into the cryostat after being flushed
 12 with helium gas. The top-end remains at room temperature above the vacuum seal (outside the cryostat), while the
 13 sample cup is pushed into the cryostat until it reaches the liquid helium bath where the sample is hyperpolarized.

14 The leakage rate of our seal been determined to $1.5 \pm 0.5 \mu\text{L/s}$ at ca. 3 mBar pressure within the probe (the VTI
 15 space is sealed from the probe space (Baudin et al. 2018). Generally, the leakage is small enough such that samples
 16 can remain in the polarizer for several days without any noticeable air contamination. To avoid ingress of air
 17 upon moving the sample tube, it needs to be inserted rather slowly, such that sample insertion takes ca. 5 min (< 5
 18 mm/s). If moved rapidly (e.g., 10 cm/s), the leakage rate rises to $> 20 \mu\text{L/s}$. A slow insertion has the further
 19 advantage to not heat the VTI excessively.

20 Once hyperpolarized, the sample can be dissolved by opening the ball valve and inserting a 'dissolution stick' that
 21 is connected via a PTFE capillary to a pressure heater that provides the hot solvent (here 5 mL of D_2O at a pressure
 22 of 1.5 MPa and a temperature of 513 K) used for dissolution. The inner capillary of the dissolution stick has a quite
 23 narrow inner diameter of 0.75 mm, such that rather high pressures are needed to dissolve the sample and push it
 24 out of the magnet. In addition, the sample has to 'climb' ca. 2 meters in our laboratory for the transfer to some of
 25 the spectrometers used for detection. We empirically determined that 1.5 MPa and a temperature of 513 K are
 26 feasible to inject the sample directly into an NMR tube waiting in the spectrometer.

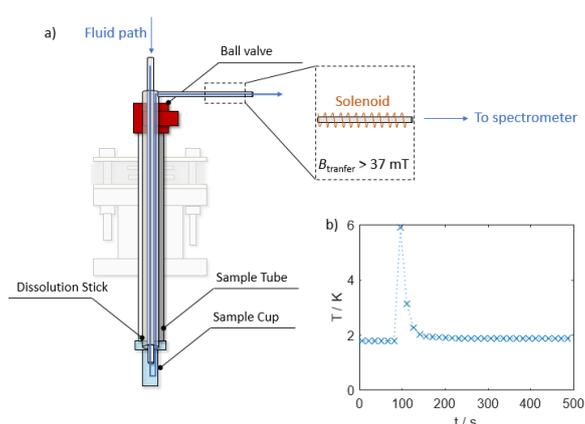
27 Fig. 3a and b show how the dissolution stick inserts into the sample tube and the cup containing the hyperpolarized
 28 substance. The inbound and outbound fluid paths are inserted with the dissolution stick such that both are at
 29 ambient temperature during the process. Upon insertion of the dissolution stick, the superheated D_2O is squirted

- 1 onto the sample via the dissolution stick's inner capillary (Fig. 3b), dissolving it and pushing it out of the cryostat.
- 2 The dissolved sample is ejected through the lumen between the inner capillary and the outer tube.



3
 4 **Figure 3.** a) Scheme of the dissolution system (airlock and polarizer omitted for clarity). For a dissolution, the ball valve (red) on top of the
 5 sample tube is opened. The sample cup is lifted 100 mm above the liquid helium bath at the bottom of the cryostat. The dissolution stick is
 6 then inserted. b) Sketch of how the dissolution stick consisting of two coaxial capillaries (grey) is inserted into the sample cup (blue). The hot
 7 solvent is squirted onto the sample through the inner capillary—the lumen between the inner and outer capillary forms the liquid-outlet. c)
 8 Image of the sample stick (left) and sample tube (right) with the seal and sample cup attached. (Image by L. M. Epasto.)

9 Fig. 4 displays the path the solvent takes upon dissolution. After melting of the hyperpolarized sample, pressurized
 10 helium gas propels the hyperpolarized liquid from the outlet at the top end of the dissolution stick to an NMR tube
 11 waiting in a spectrometer for detection. The capillary connecting the DNP and detection spectrometers is
 12 surrounded by a copper solenoid that provides a 37 mT magnetic field, as originally devised by Meier and co-
 13 workers in the context of so-called 'bullet DNP' (Kouřil, Kouřilová et al. 2018). The solenoid effectively shields
 14 the transfer path from low magnetic fields and **zero field-crossings in our laboratory** that can prohibitively
 15 accelerate the relaxation of hyperpolarization. Similar approaches based on 'magnetic tunnels' using permanent
 16 magnets have also been successful. (Milani, Vuichoud et al. 2015) **These were used here only to cover longer**
 17 **distances to other detection spectrometers (see the Supporting Information).** Upon completing the experiment, the
 18 sample tube and dissolution stick are removed from the cryostat by sliding both upward through the vacuum seal
 19 until the airlock can be closed.

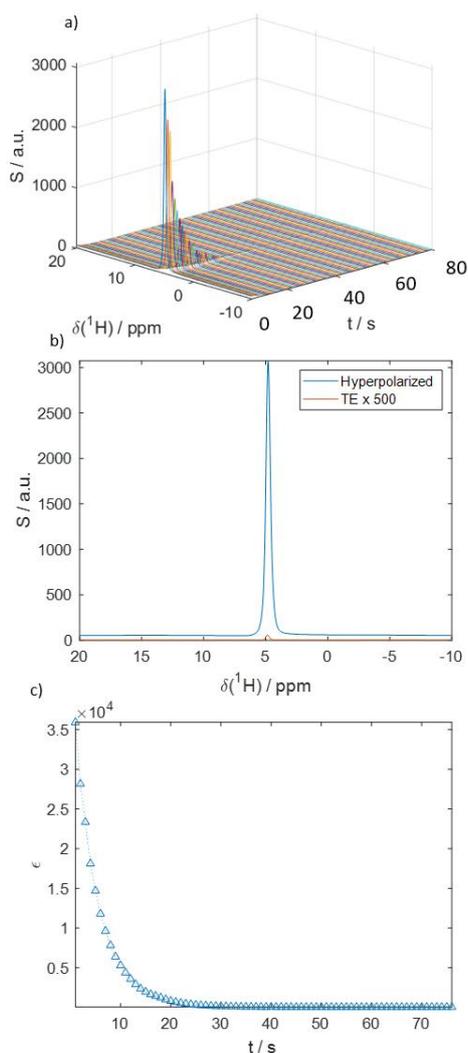


20
 21 **Figure 4.** a) Scheme of sample tube with dissolution stick inserted. The inlet for the hot D₂O and the path the fluid takes is marked with a blue
 22 arrow. The outlet capillary exiting towards the detection spectrometer is surrounded by a solenoid to maintain a constant magnetic field during
 23 the sample transfer. Note that the magnetic field within the solenoid is perpendicular to the field of the DNP apparatus and the detection
 24 spectrometer. b) Temperature profile during a dissolution experiment. During the dissolution, the temperature rises by 4 K and subsequently
 25 returns to stand-by temperature within a minute.

1 Fig. 4b displays the temperature changes observed within the cryostat during the dissolution event and
2 demonstrates the relatively low heat-load. The heating is mainly a result of the insertion of the dissolution stick
3 and the dissolution with hot solvent. The sample tube was removed relatively fast (ca. 10 s). It should be noted
4 that our polarizer is smaller than other cryogen-free systems ($\varnothing 40$ mm VTI bore size, $\varnothing 12$ mm sample space), and
5 so is its capacity to compensate the heat-shock upon dissolution. As a result, the temperature jump can be higher
6 despite a smaller heat load. Further temperature profiles for sample insertions and dissolutions can be found in the
7 Supporting Information. For sample insertions, the heat-load depends on the rate at which the sample is inserted.
8 If inserted slowly (3-5 min) the temperature jump is quite small (typically < 0.5 K). If inserted rapidly (< 1 min)
9 the VTI temperature can rise by more than 10 K.

10 The recovered volume after a dissolution experiment is typically up to 4.5 mL out of 5.05 – 5.15 mL total volume
11 (50-150 μ L sample volume + 5 mL hot solvent for dissolution) in our experiments. Ca. 500 μ L remain in the
12 capillary system and need to be flushed before the subsequent dissolution. However, only the 600 μ L of
13 hyperpolarized solution needed to fill a 5 mm NMR tubes were injected for detection.

14 Fig. 5 displays hyperpolarized HDO spectra obtained with the proposed system using a sample containing 40 mM
15 TEMPOL in a mixture of 50% glycerol- d_8 , 40% D_2O , and 10% H_2O . In this example, a series of 1D NMR signals
16 was detected at one-second intervals on a benchtop spectrometer operating at $B_0 = 1$ T, using a 10° flip angle pulse.
17 The resulting 1H signal enhancement was $\epsilon \approx 36\ 000$, corresponding to a polarization of $P(^1H) \approx 12\%$. In the solid-
18 state, a polarization of $P(^1H) = 15 \pm 3\%$ was achieved at 1.8 K, indicating that ca. 20% of the proton
19 hyperpolarization was lost during the transfer. In contrast, when the solenoid was removed, $P(^1H)$ of only ca. 7%
20 was observed, corresponding to a significantly larger ca. 53% polarization loss. Fig. 5a shows how the signal
21 intensity decays after injection into the benchtop NMR spectrometer. Fig. 5b shows the first detected signal
22 immediately after injection overlaid upon the corresponding thermal equilibrium signal detected with the same
23 pulse angle. Fig. 5c shows the decay of the signal enhancement at a 1 s sampling interval. The hyperpolarization
24 decays to naught exponentially with a relaxation rate of $R_1 = 0.21 \pm 0.03$ s^{-1} . Polarization levels obtained using the
25 hybrid system presented here are competitive with those previously reported for other dissolution systems. For
26 example, Vuichoud et al. (Vuichoud, Bornet et al. 2016) reported 6% and Ardenkjar-Larsen et al. (Lipso, Bowen
27 et al. 2017) 13% 1H water polarizations with comparable samples derived from TEMPOL/water-glycerol
28 mixtures. (Leavesley, Wilson et al. 2018) Other recent polarization approaches capable of providing polarization
29 levels of up to 70%, were also reported using samples containing UV-induced radicals. (Pinon, Capozzi et al.
30 2020)



1

2 **Figure 5.** a) Time series of 1H -detected spectra of hyperpolarized HDO at $B_0 = 1$ T. At $t = 0$ s the hyperpolarized liquid is injected into the
 3 spectrometer. The transfer took ca. 1 s over a length of 1.5 m. b) The HDO spectrum directly after injection (blue) compared to the signal in
 4 thermal equilibrium (orange). The signal enhancement was $\epsilon \approx 36\,000$, corresponding to a 1H polarization of $P(^1H) \approx 12\%$. The detection flip
 5 angle was $\alpha = 10^\circ$. c) Decay of the signal enhancement in comparison to thermal equilibrium with time after injection. The mono-exponential
 6 decay rate constant R_1 was fitted to 0.21 ± 0.03 s $^{-1}$.

7 More dissolution-DNP results can be found on the Supporting Information. Data are shown for ^{13}C -detection of
 8 acetate and glycerol- d_3 , as well as the 1H -detection of HDO, on a 500 MHz magnet upon dissolution of a larger,
 9 150 μ L sample. For detection with the 500 MHz spectrometer, the samples were transferred through a magnetic
 10 tunnel providing a 0.9 T magnetic field, as the samples had to travel longer distances (ca. 4 m). In addition, we
 11 show DNP build-up curves for ^{13}C and 1H nuclei at 1.5 K and 3.5 K for TEMPOL concentrations of 40 and 70
 12 mM.

13 We found that sample transfer problems only occurred as the result of unexperienced operators who failed to
 14 couple the dissolution stick with the dissolution cup, leading to leaking of the dissolution solvent into the sample
 15 tube. No other modes of failure were observed so far upon dissolution.

1 The most common 'mode of failure' is the intrusion of air through the seal upon removal of the sample tube after
2 dissolution. If this process is performed too slowly, air can enter the VTI as the bottom end of the sample tube
3 shrinks in diameter during the DNP period and the seal doesn't close tightly anymore around the carbon fiber tube.

4 **3 Conclusions**

5 The proposed sample handling system for dissolution DNP has three advantages: 1. The cryostat can be maintained
6 at low temperatures, and the vacuum within is not broken at any stage of the dissolution process. In addition, the
7 heat-load introduced during dissolution is reduced as the dissolution stick does not come into contact with the
8 helium bath. 2. The melting process is very rapid as pressurization of the cryostat is eliminated, in contrast to other
9 HyperSense-inspired systems. 3. Freezing and blockage of the dissolution system are avoided as the dissolution
10 stick is not cooled down at any stage of the experiment. The novelty of our implementation lies in the independent
11 insertion of the dissolution stick while simultaneously maintaining the VTI and the sample space under low
12 pressure. It should be noted that Krajewski et al. (Krajewski, Wespi et al. 2017) also developed a device, that
13 enables to form the contact between sample and dissolution system, while keeping the VTI under low pressures.
14 In their implementation, the layout was designed for multi-sample experiments.

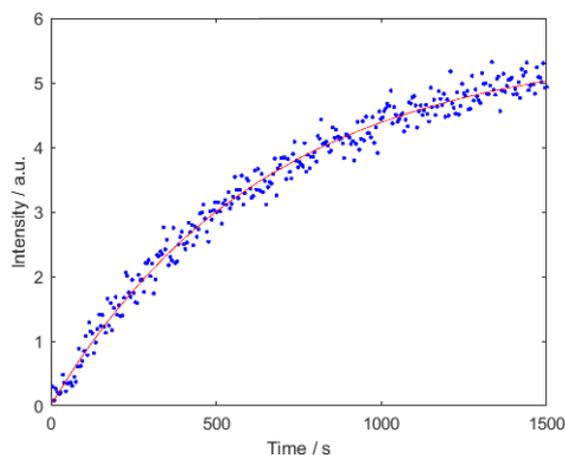
15 The system is furthermore readily adaptable to different polarizer systems as the vacuum nipple connecting the
16 seal to the DNP apparatus can be adjusted to any flange size. The system is also compatible with narrow sample
17 spaces. For example, the sample tube needs to pass through a bore as narrow as 12 mm in the system presented
18 here.

19 In conclusion, using this compact and cost-efficient sample handling system, it is possible to perform dissolution
20 DNP experiments with a cryogen consumption-free cryostat without risking quenching of the magnet or
21 introducing air contamination into the cryostat. Moreover, following dissolution, the system reliably returns to its
22 stand-by temperature within a minute, which is a promising step towards higher throughput DDNP.

23 **4 Experimental**

24 For DNP 50 μ L of a solution of 40 mM TEMPOL in a mixture of 50% glycerol- d_8 , 40% D_2O , and 10% H_2O was
25 hyperpolarized at 1.8 K in a magnetic field of 6.7 T for 2500 s using continuous-wave microwave irradiation at
26 188.08 GHz. DNP samples were always freshly prepared to avoid ripening effects. (Weber, Sicoli et al. 2018) Fig.
27 6 displays the build-up kinetics. A VDI microwave source was used together with a 16x frequency multiplier that
28 provided an output power for the microwave of ca. 50 mW. The magnet-cryostat combination was purchased from
29 Cryogenic Ltd. and operated as described in reference (Baudin, Vuichoud et al. 2018).

30 For detection of the solid-state polarization, a 400 MHz Bruker Avance III system was adapted to a 1H resonance
31 frequency of 285.3 MHz and a ^{13}C frequency of 71.72 MHz of by using a broad-band preamplifier for both
32 channels. The detection circuit and the external tune-and-match system were home-built, as described in reference
33 (Baudin, Vuichoud et al. 2018). To monitor the build-up, detection pulses with a flip angle of 1 degree were applied
34 every 5 s.



1
 2 **Figure 6.** Polarization build-up curve at 1.8 K. The build-up rate constant was $0.0016 \pm 0.0001 \text{ s}^{-1}$. The polarization was of $P(^1\text{H}) \approx 15 \pm$
 3 2%.

4 After DNP, the sample was dissolved with a burst of 5 mL D_2O at 1.5 MPa as described in the main text. The
 5 hyperpolarized liquid was then pushed with helium gas at 0.7 MPa to the detection spectrometer. The dissolution
 6 process employed a home-built pressure heater actuated with an Arduino micro-controller. A home-written
 7 MATLAB-based user interface controls the dissolution and injection steps.

8 **For low-field detection,** the hyperpolarized liquid was transferred to a Magritek SpinSolve Phosphorous
 9 spectrometer operating at room temperature and a magnetic field of 1 T. The transfer path was ca. 1.5 m long and
 10 the transfer took ca. 1 s. A PTFE capillary with a 1 mm inner diameter and 3.2 mm outer diameter was used. The
 11 solenoid (2 turns / mm) surrounding the transfer path provided a $>37 \text{ mT}$ magnetic field during the sample transfer
 12 at a current of 3 A and a power of 450 W. The solenoid ended ca. 500 mm before reaching the bore of the magnet.
 13 To avoid heating of the PTFE capillary path, the solenoid was only switched on during the transfer.

14 A volume of 600 μL of the hyperpolarized liquid was directly injected into an NMR tube that was treated with
 15 strongly oxidizing rinsing solutions (Helmanex III) beforehand to reduce the likelihood of gas inclusions forming
 16 upon injection into the NMR tube and disrupting NMR detection. (Dey, Charrier et al. 2020)

17 In the liquid state, ^1H single pulse acquisitions were repeated at one-second intervals, using a flip angle of 10
 18 degrees. The spectral width was 30 ppm at a carrier frequency centered at 5 ppm. The spectrometer's external lock
 19 system was used for referencing the chemical shift.

20 **For high-field detection,** the hyperpolarized sample was transferred to a Bruker NEO 500 MHz NMR spectrometer
 21 equipped with a Prodigy BBFO probe. Again, a volume of 600 μL of the hyperpolarized liquid was directly
 22 injected into an NMR tube that was treated with strongly oxidizing rinsing solutions (Helmanex III) beforehand.
 23 Pulses with 1° flip angles for ^1H detection and 5° flip angles for ^{13}C detection were applied every second for
 24 detection.

25 All data were processed with home-written scripts using the MATLAB 2019 software package or Bruker TopSpin
 26 4.0. All data were zero-filled and apodized with a Gaussian window function before Fourier transformation.

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4 innovation programme (grant agreement 801936). This project was supported by the Austrian FWF (stand-alone
5 grant no. P-33338).

6 **Author contribution**

7 TK, KC, LME, FK, MN, GO, AS and DK built the DDNP system and performed experiments. DK wrote the
8 manuscript with the help of all authors.

9 **Competing interest**

10 The authors declare no conflict of interest.

11 **Data availability**

12 All data are available under DOI: [10.5281/zenodo.4738932](https://doi.org/10.5281/zenodo.4738932).

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