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Supplementary information

Solution-state nuclear magnetic resonance spectroscopy of crystalline cellulosic materials using a direct dissolution ionic liquid electrolyte

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Supplementary Information

Solution-State Nuclear Magnetic Resonance Spectroscopy of Crystalline Cellulosic Materials using Ionic Liquids a Direct Dissolution Ionic Liquid Electrolyte

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1. Examples for NMR spectra obtained in poor quality electrolyte

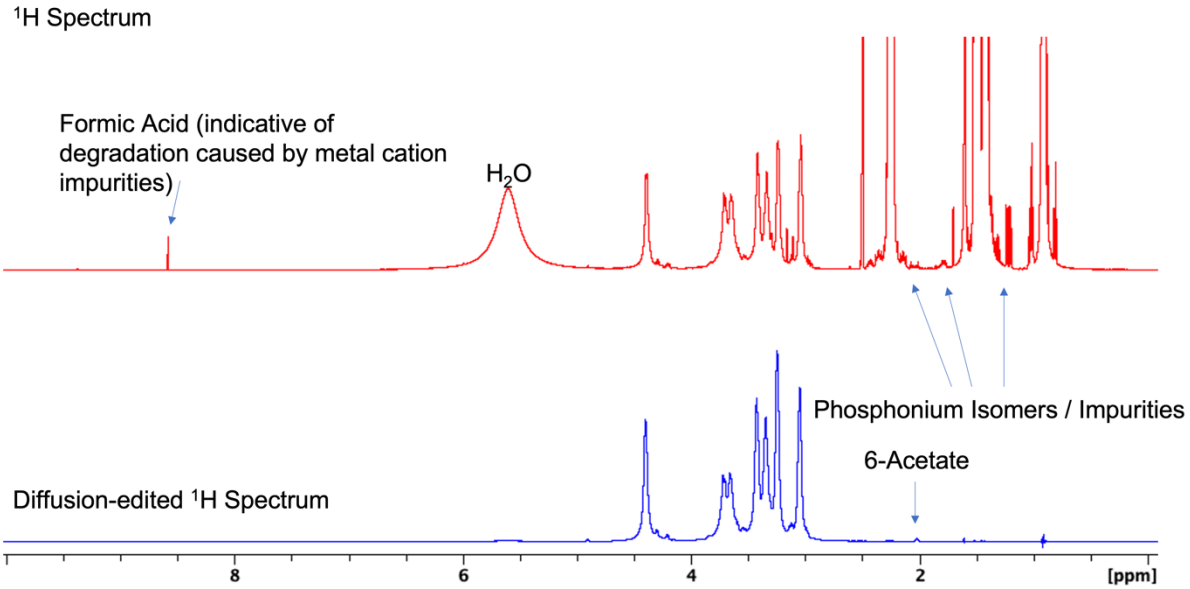

Fig. S1. ^{1}H spectrum (**top**) and diffusion-edited ^{1}H spectrum (**bottom**) of an MCC sample (Avicel pH101, 5 wt%) obtained in a poor quality (contains impurities) [P_{4444}][OAc]:DMSO-d_6 electrolyte (65 °C, 600 MHz). **Top**: In the quantitative ^{1}H experiment a strong formic acid peak (8.61 ppm) stemming from catalytic degradation reactions of cellulose with metal cation impurities becomes visible (Note: this peak is also visible in correctly prepared electrolytes, but in much smaller quantities). The broad H_2O peak and signals caused by phosphonium isomers or other impurities decrease the accessible measuring range due to peak superposition. **Bottom**: An additional indicator for electrolyte impurities in the diffusion-edited ^{1}H experiment is the appearance of a cellulose 6-acetate peak at 2.02 ppm.

2. Influence of t_1-increments on f1 resolution in HSQC

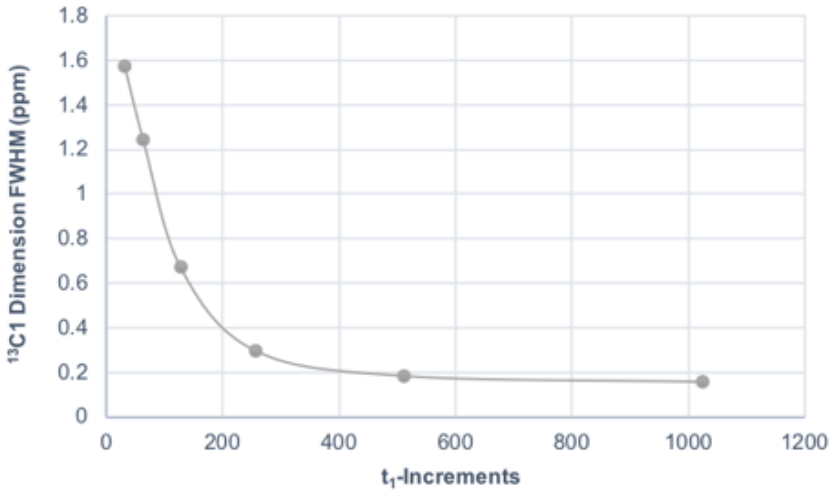

Fig. S2. Resolution in the ^{13}C (f1) dimension of HSQC for C1 in MCC (DP_N 153), with increasing number of real t_1-increments, as measured by the full-width at half-maximum (FWHM) values (in ppm) of the CH1 correlation 1D projections. The plotted t_1-increment values correspond to digital f1-resolutions of 778, 389, 194, 97, 49 and 24 Hz/pt.

3. Beginners Guide

3.1 Detailed instructions for acquisition of spectra using Bruker TopSpin®

CRITICAL: Check from your responsible NMR technician what the appropriate workflow should be for the respective available spectrometers, as these may change from system to system. Often some kind of formal training is required prior to use.

S1) Insert the NMR tube into the spinneret using the sample leveler. Ensure the sample is at the correct height for the detection window.
Prepare the spectrometer to receive the sample by ejecting any previous sample, by entering '*ej*' on the Topspin command line.
CRITICAL: This is a necessary step, whether there is a sample in the probe or not.

S2) Place the sample in the probe and insert by entering '*ij*' on the Topspin command line.

S3) Change the probe temperature to 65 °C and allow the probe and sample to equilibrate. This typically takes 5-10 min, depending on the probe.

S4) Lock the spectrometer to the DMSO-d_6 deuterium frequency by entering '*lock*' in the Topspin command line and selecting 'DMSO-d_6'. The automatic locking feature works robustly.

S5) Set up the selected experiments that you wish to run.
CRITICAL: The selection of the experiments relies on the desired information and on the available measuring time. Measuring time is usually quite costly! Consult **Table 2** or **Boxes 1-7** of the main manuscript to avoid the acquisition of redundant data. Variation of parameters can be performed *ad hoc* to improve the quality of the spectra. We listed the influence of several adjustable parameters in **Table 3**. Often it is advisory to use shorter trial collection times before performing an experiment.

S6) Enter '*atma*' on a selected experiment from the Topspin command line to auto-tune the probe.
CRITICAL: If you are running HSQC or ^{13}C then auto-tune from those experiments, so that both ^{1}H and ^{13}C coils will be tuned. Auto-tuning is typically robust but manual refinement of the tuning and matching can be made by entering '*atmm*' from the

Topspin command line. Cryoprobes typically require accurate tuning of both ^{1}H and ^{13}C.

S7) Enter '*topshim*' from the Topspin command line to auto-shim the spectrometer. Auto-shimming is typically robust.

S8) Enter '*getprosol*' from the Topspin command line for the selected experiment, to apply standard probe parameters for each experiment.

S9) Enter '*pulsecal*' from the Topspin command line for the selected experiment to automatically perform accurate ^{1}H pulse calibration for each experiment.

S10) Enter '*rga*' from the Topspin command line after the pulse calibration is completed, to automatically adjust the receiver gain.

S11) Start the measurement by entering '*zg*' from the Topspin command line.

3.2 Detailed instructions for processing of spectra using Bruker TopSpin®

CRITICAL! Initial processing (*i.e.*, Fourier transformation and phase correction) is often carried out automatically as a part of the programmed experiment. If required, alternative automatic phase correction, or manual phase correction may be necessary. Calibration and baseline correction should be carried out manually for each spectrum. In bullet points **S12** and **S13** the processing procedure using the Bruker TopSpin® (version 4.0) software is listed.

S12) Open the respective 1D NMR spectrum in topspin (**Fig. S3**) and perform the following processing steps:

A) ^{1}H spectra (**Fig. S4**)

i) Apply manual phase correction (Adjust Phase + Manual Spectrum Phase manually, 0 order and 1st order correction, safe and return).

ii) Apply automatic baseline correction – type command '*abs*', with '*absg*' (polynomial for baseline correction) set from 3 to 5.

iii) Reference the spectrum to the DMSO-d_6 signal (Calib. Axis + Manual Axis Calibration) to DMSO signal (δ ^{1}H: 2.50 ppm, **Fig. S5a**).[1]

B) 1D Diffusion-edited ^{1}H spectra

i) Apply manual phase correction and automatic baseline correction as in step **S12 A**.

ii) As signals of low molecular weight compounds are not visible in the spectrum, it cannot be referenced directly against the residual solvent peak. Choose one polymeric signal in the diffusion edited spectrum and search for the same peak in the standard ^{1}H spectrum, referenced against DMSO-d_6. Reference the peak in the diffusion edited 1H spectrum to the same ppm value. Usually, the isolated cellulose H1 region (*e.g.,* at δ ^{1}H of 4.39 ppm) is a good choice to perform the procedure.

C) ^{13}C spectra

i) Reference the spectrum to the DMSO-d_6 signal (δ ^{13}C: 39.52 ppm, **Fig. S5b**).[1]

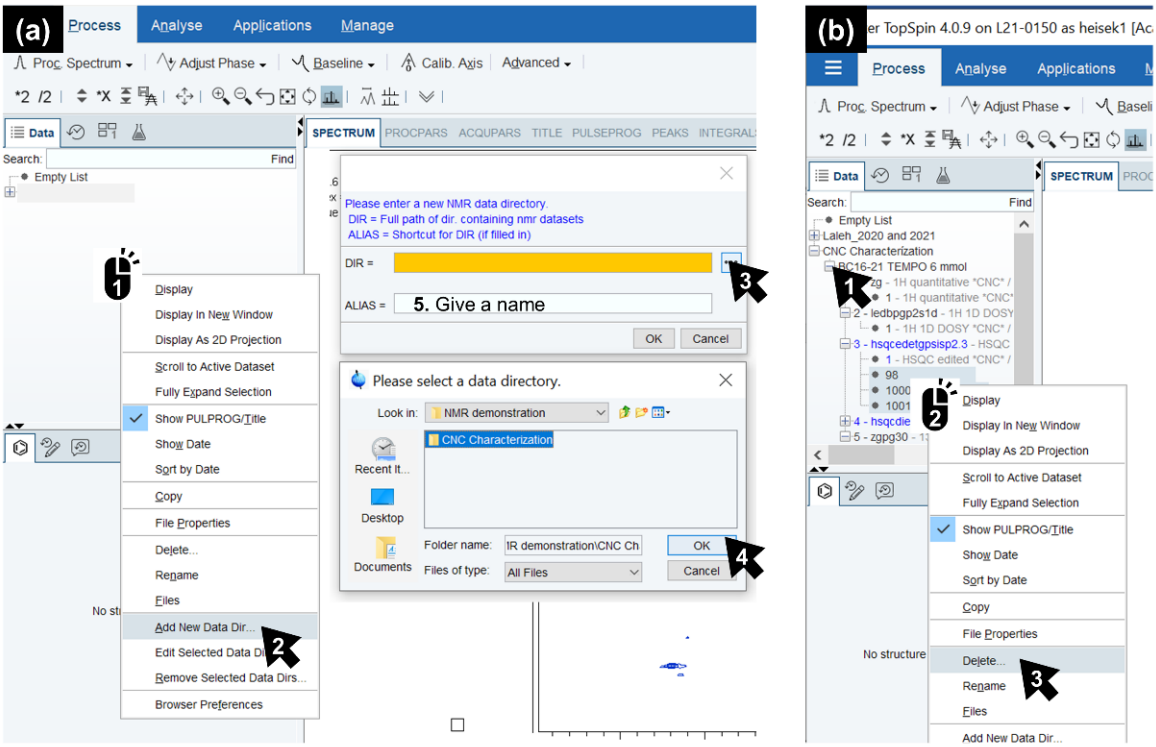

Fig. S3. Adding and opening NMR data in Bruker TopSpin® 4.0.9: **(a)** 'right-click' (1) – 'Add New Data Directory' (2) – browse '…' (3) – select data set, 'OK' (4) – rename your data set (5); **(b)** extend to open your data set and individual experiments (1) – delete unnecessary 2D experiments ('98, 1000, 1001') (2/3).

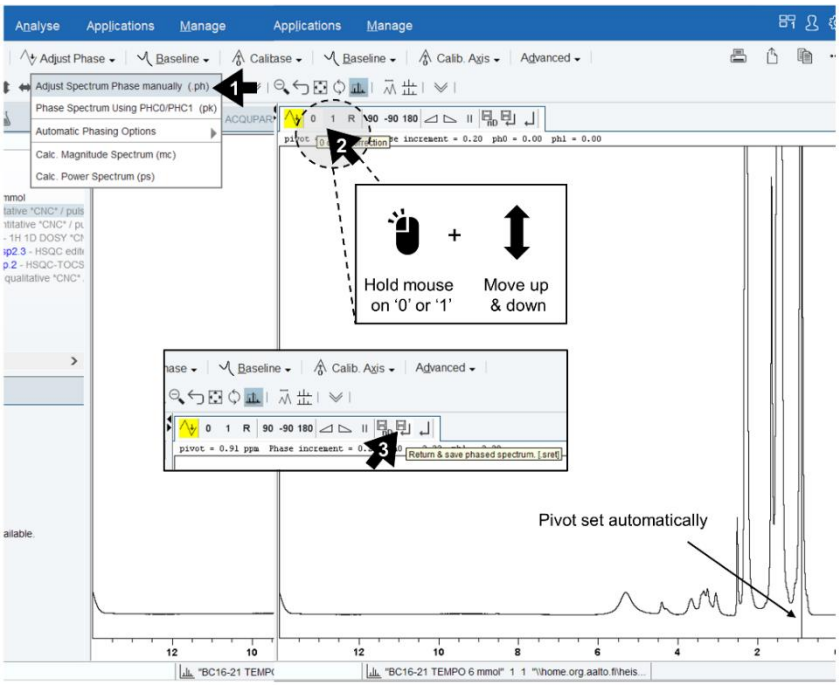

Fig. S4. Manual phase correction of 1D spectra with Bruker TopSpin® 4.0.9: in 'Process' menu - click 'Adjust Phase' – 'Adjust Spectrum Phase manually (.ph)' (1) – the pivot for the 0-order correction is set automatically – hold the mouse ('left-click') on '0' to correct the phase where the pivot is set and move the mouse up and down (2); for the rest of the spectrum (1st-order correction), hold the mouse on '1' and move up/down. The phase correction must result into single symmetric peaks. Finalize the manual phase correction by clicking 'Return and Save' (3).

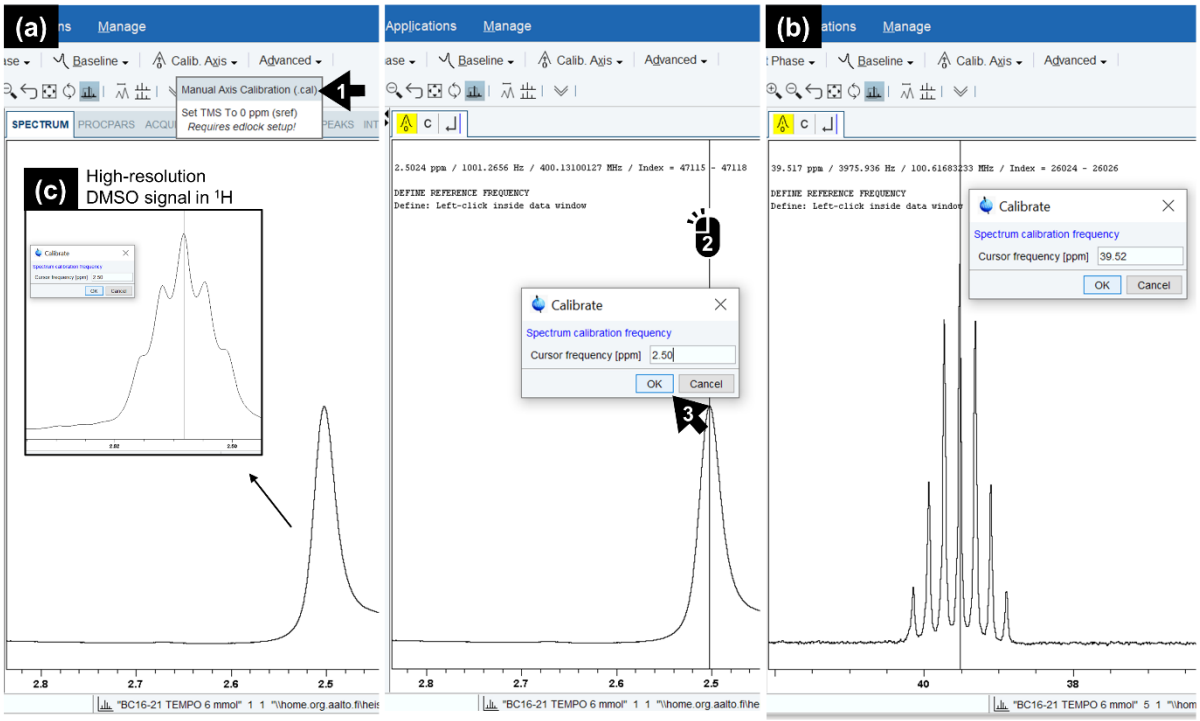

Fig. S5. Manual calibration of ^{1}H (a) and ^{13}C (b) 1D spectra with Bruker TopSpin® 4.0.9: in 'Process' menu - click 'Calib. Axis' – 'Manual Axis Calibration (.cal)' (1) – 'left-click' (2) – type reference chemical shift (here: DMSO – δ ^{1}H: 2.50, δ ^{13}C: 39.52), 'OK' (3). (c) Note: In high-resolution spectra, the 1H DMSO signal shows as a quintet. Use the center peak for calibration.

S13) Open the respective 1D NMR spectrum in topspin and perform the following processing steps:

A) Multiplicity-edited ^{1}H-^{13}C HSQC

i) Add 1D spectra (External Projections) in f1 (^{13}C) and f2 (^{1}H) direction (**Fig. S6**).

CRITICAL: It is often useful to add the diffusion-edited ^{1}H spectrum instead of the standard ^{1}H spectrum, to allow for the distinction between polymeric resonances from solvent or other impurities.

ii) Apply manual phase correction (select two to four signals in different spectral regions; 0 order and 1st order correction as described for 1D spectra) but in both dimensions, if needed (**Fig. S7**).

iii) Apply automatic baseline correction in f1 and f2, if needed – type commands "*abs1*" and "*abs2*".

iv) Reference the spectrum to the DMSO-d_6 signal as for 1D ^{13}C and ^{1}H (**Fig. S8**).

v) optional for presentation purposes: Reopen the spectrum in Mestrenova and remove t1 noise by using 'Reduce t1 noise' in the 'Processing' menu.

B) 2D ^{1}H-^{13}C HSQC-TOCSY

i) Perform the same processing steps as for the HSQC spectrum in step **S13 A**.

ii) Overlay the HSQC onto the HSQC-TOCSY spectrum, using different spectrum colours for spectral assignments and to distinguish HSQC from TOCSY correlations (**Fig. S9**).

C) ^{1}H-^{13}C HMBC

i) Perform the same processing steps as for the HSQC spectrum in step **S13 A**.

ii) optional: Overlay the HSQC onto the HMBC spectrum, using different spectrum colours. This facilitates the assignment of correlations in crowded areas (**Fig. S9**).

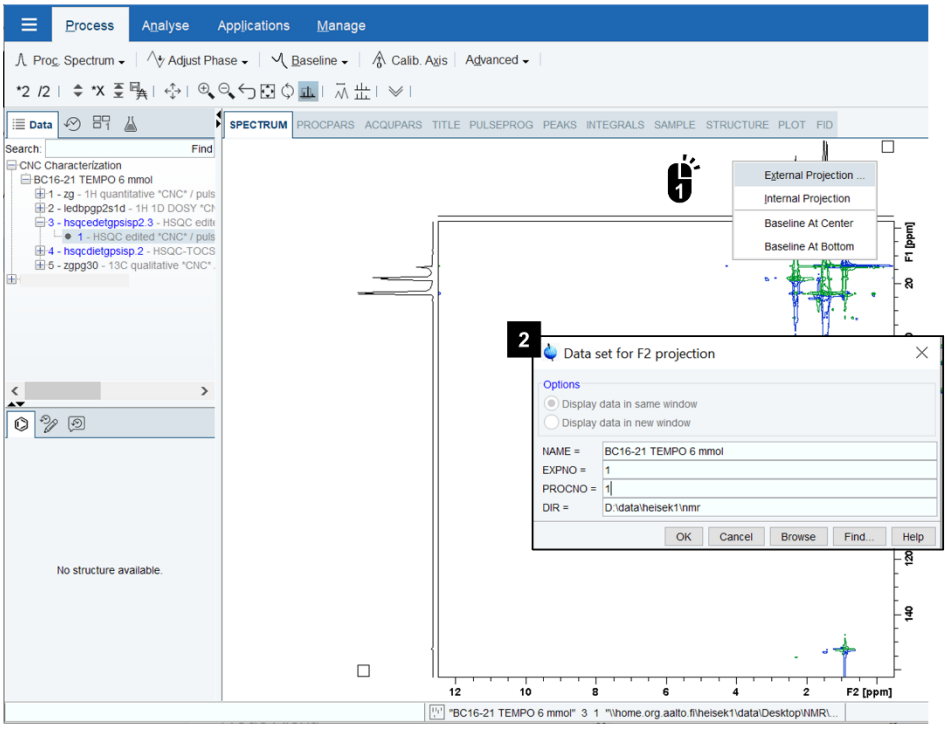

Fig. S6. Add data sets for the ^{1}H (f2) and ^{13}C (f1) projection for 2D NMR spectra with Bruker TopSpin® 4.0.9: 'right-click' in the area of the 1D projection and click 'External Projection' (1) – select the experiment number ('EXPNO') and the processing number ('PROCNO') of the external data set (2) (here: 1/1 for ^{1}H and 5/1 for the ^{13}C dimension).

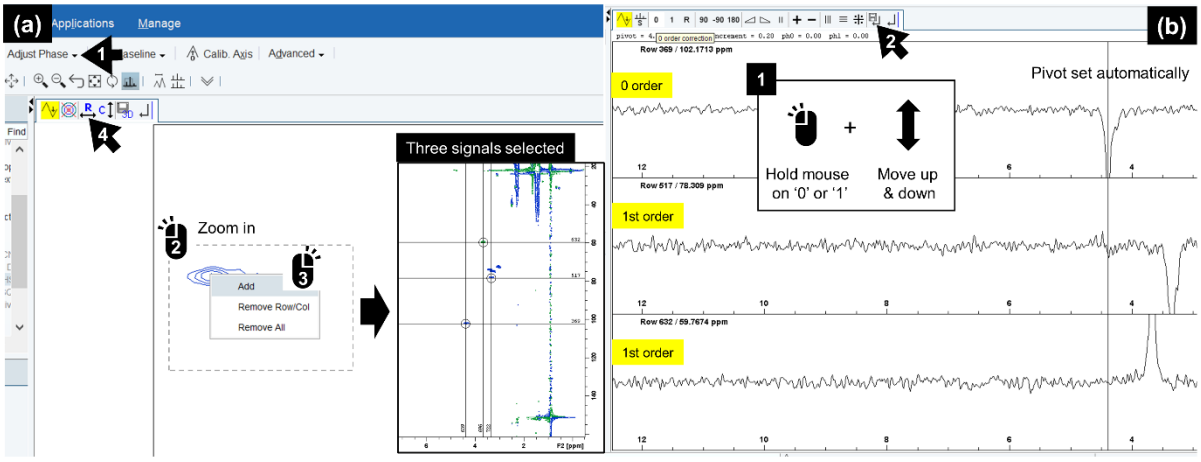

Fig. S7. Manual phase correction of 2D NMR spectra with Bruker TopSpin® 4.0.9: **(a)** in the 'Process' menu click 'Adjust Phase' and 'Manual Phase Correction (.ph)' (1). For the phase correction on rows, select three different signals in the spectrum by zooming in (with 'left mouse click'(2)) – ´right-click' in the center of the signal and 'add' (3), zoom out (type '.all' in command line) to select the other two signals and repeat steps (2) and (3) – click 'R' for phase correction in rows (4). **(b)** 0 order and 1st order correction in rows – 'right-click' and hold mouse on '0' and '1' and move up/down (1) – finalize the phase correction by clicking 'Return and Save' (2) and 'Return'.

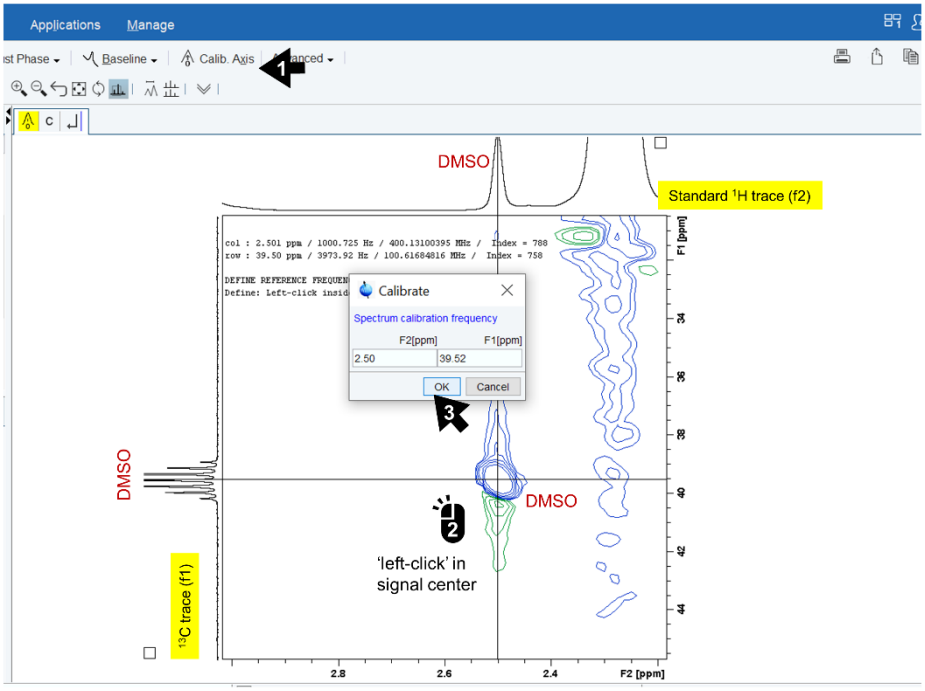

Fig. S8. Manual calibration of 2D NMR spectra with Bruker TopSpin® 4.0.9: Zoom in (left mouse) – in 'Process' menu click 'Calib. Axis' (1) – 'left-mouse-click' in selected (here: DMSO) signal center (2) – type reference chemical shift in f1 and f2 dimension (here: DMSO – δ ^{1}H: 2.50, δ ^{13}C: 39.52) and click 'OK' (3) to finalize calibration.

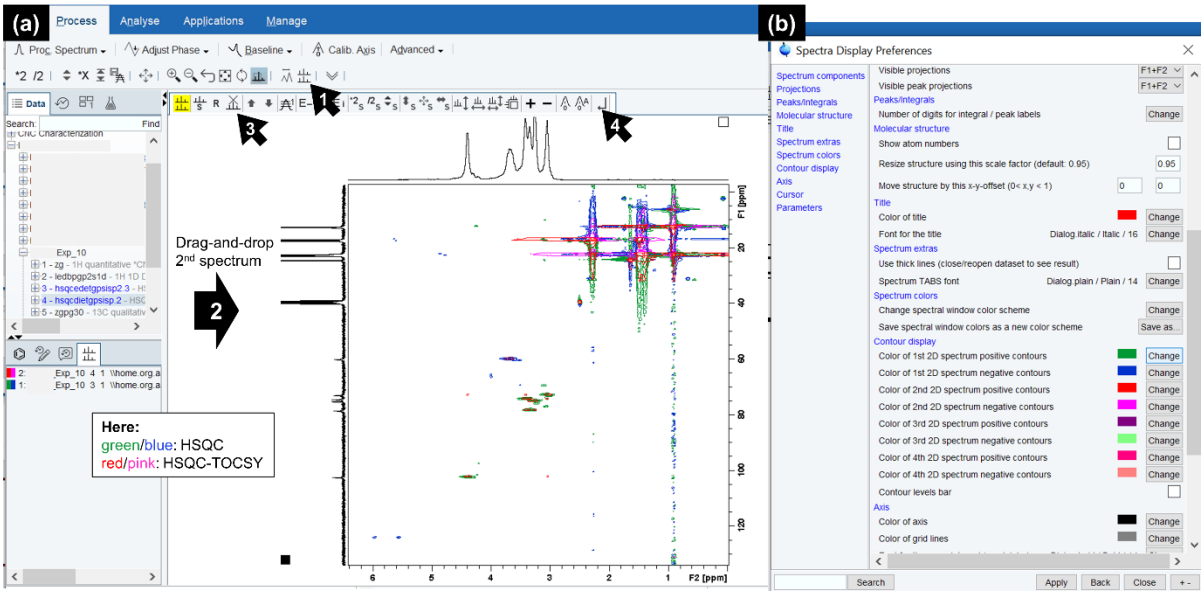

Fig. S9. Overlaying 2D NMR spectra with Bruker TopSpin® 4.0.9: **(a)** select and open 2D NMR spectrum (here: HSQC) by 'drag-and-drop' in spectrum window – in 'Process' menu click 'Multiple display' (1) (the multiple display menu is now open in the left bottom corner) – overlay the second 2D spectrum (here: HSQC-TOCSY) by drag-and-drop in the spectrum window. You can remove spectra from the display by selecting and clicking 'Remove all selected datasets from display' (3) and you can leave the overlay menu with the 'Return' button (4). **(b)** Customize the spectrum colors in the 'Spectra Display Preferences' menu which you can open with a 'left-mouse-click' in the spectrum area.

3.3 Detailed instructions for obtaining quantitative information from the ^{1}H experiment using *Fityk*

The peak-fitting process using *Fityk*[2] allows to extract quantitative information from the standard ^{1}H experiment, where there is significant peak overlap preventing simple integration. This allows to take advantage of the significantly shorter measuring times, compared to the quantitative ^{13}C experiment. Before starting the quantification, thorough peak assignment must be performed and peak superposition, with low molecular weight impurities, must be excluded! It is important to take special care with shimming, tuning, and matching prior to data collection, to obtain a high-quality spectrum with a smooth baseline. Elimination of water from the cellulose sample and electrolyte is highly preferential but can be accounted for during the peak-fitting procedure, if unavoidable. Initial processing (apodization of the FID signal, Fourier transformation, phasing and calibration) can easily be performed with TopSpin®. Baseline correction can also be performed in TopSpin® but MestreNova often offers more control for a rapid initial baseline correction, where the efficacy of the correction can be assessed prior to application. Further, more aggressive spline baseline correction can then be easily applied using *Fityk*. However, TopSpin® has excellent baseline correction functions once you learn how to use them and, in principle, spline baseline correction in *Fityk* can be applied directly to the phased and calibrated spectra, without preliminary baseline correction. However, additional background correction may be necessary as both TopSpin® and MestreNova are not able to suppress water contribution, which may be partially overlapping with the cellulose backbone signals.

In the following steps the peak-fitting process will be explained using the DS determination of a surface modified cellulose acetate sample as example.[3]

S14) Record a quantitative ^{1}H spectra (**Box 1**) of the cellulosic material with a 10 s relaxation delay (d1, 30° pulse) and typically 16 transients (ns) collected. Calibrate and phase the spectrum according to previous processing instructions (see **step S12 A**).

S15) Carefully assign all peaks in the spectral areas that will be used for the calculations.

CRITICAL: Wrong assignments or overlooked peak superpositions will inherently lead to errors in the calculation steps. This usually affords cross validation by the other available 2D NMR experiments.

S16) Perform additional spectral pre-treatment:

A) Using TopSpin®:

i) Type '*wm*' in the Topspin command line to adjust apodization (also known as '*window function*' or '*line broadening*') and increase or decrease the '*line broadening*'. **CRITICAL:** This increases or decreases spectral resolution, as needed for rapid and accurate fitting.

ii) Type '*ft*' in the Topspin command line to perform Fourier transformation and rephase the spectrum according to step **S12 A**.

iii) Type '*convbin2asc*' in the Topspin command line to create an ASCII text file (ascii-spec.txt) of the spectral data in the experiment subfolder.

iv) Open the ".txt" file and strip away any file header information, if present. Make sure that only the numerical data remains.

B) optional using Mestrenova:

i) Open the baseline correction button and apply a modest baseline correction, *e.g*., using a low order polynomial fitting.

ii) Save the spectrum as the 'NMR CSV file' format.

CRITICAL: This is not actually a comma separated file format but rather tab separated data. It is also possible to save the file straight to ".txt" format by typing the extension after the file name upon saving the spectrum. Files from MestreNova with both extensions (".txt" and ".csv") can be directly opened with '*Fityk*'.

S17) Open the generated file in '*Fityk*' and start performing the processing.

CRITICAL: If the files cannot be opened by the software, change the text file endings to "*.xy*" and reopen it in '*Fityk*'. If certain steps in the following processing are unclear, resort to the online manual listed in the **Materials section** in main manuscript.

S18) Apply baseline correction using the "spline baseline correction function" (**Fig. S10**)**.**

CRITICAL: Pay specific attention to the water contribution. Save the session into a separate file after the baseline correction. Saving into separate files is recommended for each major transformation. Furthermore, it should be remembered that the more aggressive correction is applied to the baseline, the more error will be introduced to the integral or peak volumes.

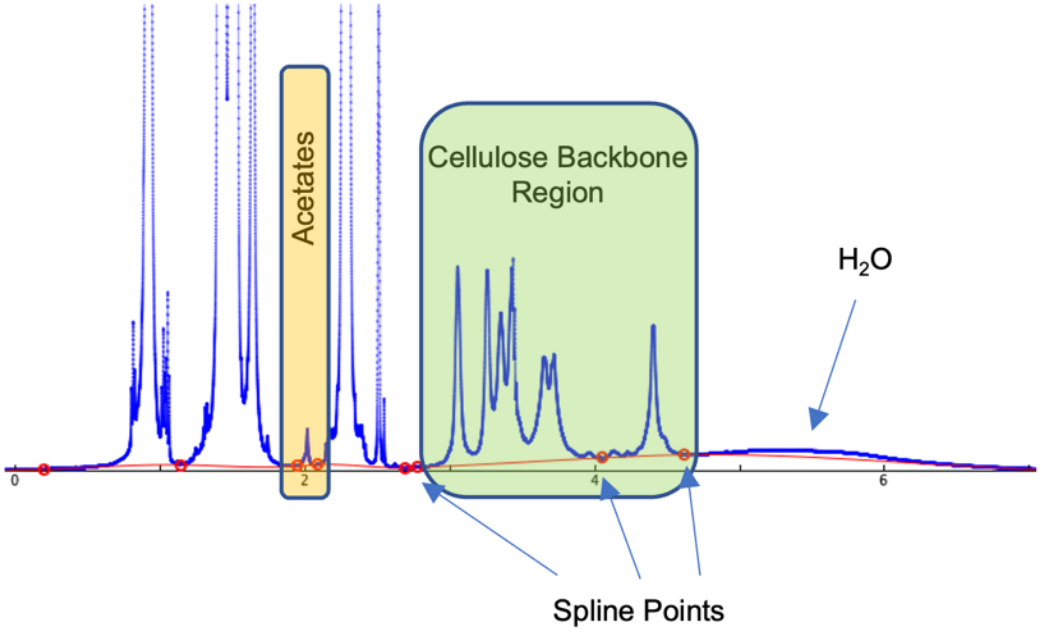

Fig. S10. ^{1}H spectrum of acetylated cellulose, viewed in *Fityk,* showing spline baseline correction using 8 spline pivot points. Note: This is absolute minimum number of points for background correction.

S19) Perform the following processing steps separately for each area selected for the subsequent calculations. In the used example the spectral areas of the cellulose backbone (2.75 – 5.3 ppm; see **Fig. S11**) and the acetate peaks (1.75 -2.1 ppm; see **Fig S12**) The processing can be performed using either the interface or the command line.

CRITICAL: Start fitting from the most complex region with many peaks, e.g., in the presented case the cellulose backbone.

A) Processing using the interface:

i) Open the '*Data-range mode*' button functions and 'Disactivate' the parts of the spectrum which are non-relevant for the area under investigation. In the presented case of the cellulose backbone 'Disactivate': -2 – 2.75 ppm & 5.3 – 15 ppm)

ii) Open the '*auto-add*' button to apply Gaussian function '*guesses*' to yield a relatively low residual baseline error.

iii) Fit the peaks using the '*Start fitting*' button.

B) For processing using the command line type the following commands:

i) To 'Disactivate' the parts of the spectrum which are non-relevant for the area under investigation, type:

"A = a and not (-2 < x and x < 2.75)" & "A = a and not (5.3 < x and x < 20)"

CRITICAL: This command is exemplary for the cellulose backbone. For adjusting the 'Disactivate' areas, change the integers according to the ppm-values of the spectral areas selected in step S**15**.

ii) To apply the Gaussian function 'guesses' type:

"guess Gaussian"

iii) To start fitting type:

"fit"

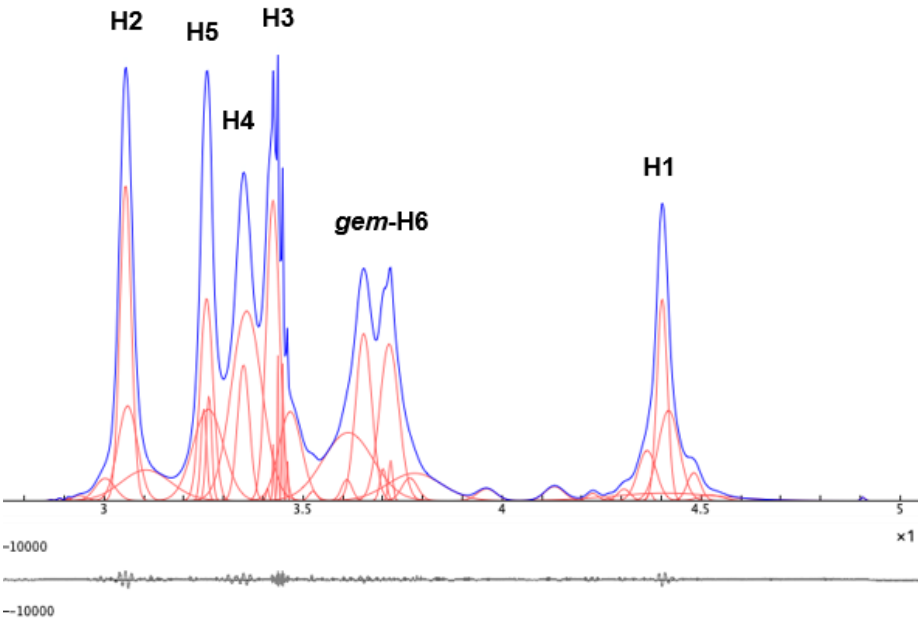

Fig. S11. ^{1}H spectrum of acetylated cellulose - cellulose backbone region (2.75 - 5.3 ppm), viewed in *Fityk,* after spline baseline correction – with peak-fitting Gaussian functions shown (low residual baseline error shown at the bottom).

S20) Save the processed data in a separate file and return to viewing the full data range using the '*View whole*' button.

S21) Repeat step **S19** for the next spectral region, in the selected sample the cellulose acetate area (1.75 -2.1 ppm, see **Fig. S12**).

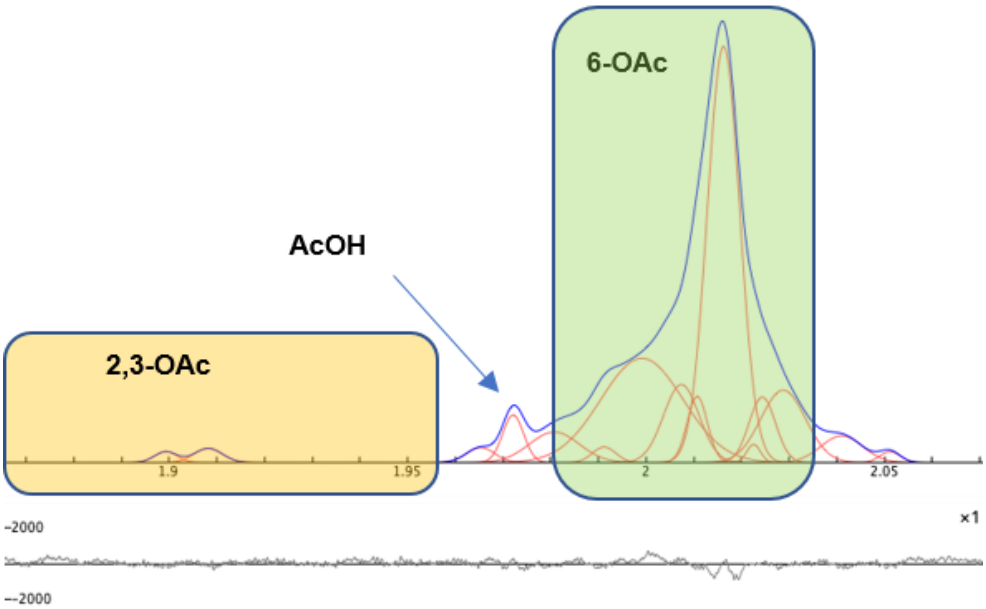

Fig. S12. ^{1}H spectrum of acetylated cellulose - cellulose acetate region (1.75 – 2.1 ppm), viewed in *Fityk*, after spline baseline correction - with peak-fitting Gaussian functions shown (low residual baseline error shown at the bottom). As can be seen a slight low molecular weight impurity of acetic acid (AcOH) is present and the peaks of cellulose acetate can be assigned to different regioisomers (2,3-OAc or 6-OAc). Both aspects can be considered in the final calculation steps.

S22) Save the session to a separate file using the '*Save session to file*' button, once all needed regions are well described by the set of Gaussian functions.

S23) Export the peak parameters to a text file by opening '*Functions*' menu and using the '*Export Peak Parameters*' command. This will create a ".*peaks*" or ".*txt*" file (ASCII) based on the manually typed file ending.

S24) Save images of the fitted regions for each correction/fitting step, with residual fitting errors.

S25) Import the data into a data management application, e.g., Microsoft Excel, while only importing the '*Center*' and '*Area*' data columns.
CRITICAL: The '*Center*' column corresponds to the ppm value of the peak maximum of the respective Gaussian Fit and the '*Area*' column corresponds to the respective integration value.

S26) Sort the data based on the '*Center*' column, from smallest to largest, for comparison with the fitting images. Check if all the data got incorporated correctly.

S27) Combine the respective spectral areas in blocks and summarize the respective peak areas, e.g., using the '*SUM*' command. The obtained values represent the relative molar compositions for the specific functionalities.

CRITICAL: As can be seen in **Fig. S12,** it is possible to adjust the data in this step. For example, in the presented sample the contribution of an acetic acid (AcOH) impurity can be subtracted, by not including its Gaussian in the summation. Furthermore, differences in the regioselectivity of the performed reaction can be assessed, provided the peaks could be assigned to different regioisomers in the qualitative analysis in **step S15**.

S28) Use the obtained relative molar compositions for the calculation of the desired quantitative information. In the presented example **Equation S1** is suitable for the determination of the DS by considering the different ratio of hydrogen atoms represented by the different peak areas:

$$DS = \frac{I_A/_3}{I_C/_7} \qquad \text{(Equation S1)}$$

CRITICAL: Here, I_A (Integral Acetate) is the sum of substituent signals Gaussian functions of the acetate region (1.75 - 2.1 ppm) and I_C (Integral Cellulose) is the sum of combined Gaussian functions for the cellulose backbone (2.75 - 5.3 ppm). "3" is the number of protons of the acetate substituent moiety and "7" is the total number of protons for the cellulose backbone fragment, respectively.

4. References

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