Pulseq-CEST: Towards multi-site multi-vendor compatibility and reproducibility of CEST experiments using an open-source sequence standard

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Purpose: As the field of CEST grows, various novel preparation periods using different parameters are being introduced. At the same time, large, multisite clinical studies require clearly defined protocols, especially across different vendors. Here, we propose a CEST definition standard using the open Pulseq format for a shareable, simple, and exact definition of CEST protocols.

Methods: We present the benefits of such a standard in three ways: (1) an open database on GitHub, where fully defined, human-readable CEST protocols can be shared; (2) an open-source Bloch-McConnell simulation to test and optimize CEST preparation periods in silico; and (3) a hybrid MR sequence that plays out the CEST preparation period and can be combined with any existing readout module.

Results: The exact definition of the CEST preparation period, in combination with the flexible simulation, leads to a good match between simulations and measurements. The standard allowed finding consensus on three amide proton transfer–weighted protocols that could be compared in healthy subjects and a tumor patient. In
These parameters vary significantly in the current literature, and the offset from the water resonance frequency (Δω) is crucial for an optimal CEST experiment, as the maximum effect depends not only on the tissue and solute pool of interest, but also on the efficiency of the saturation imposed by the RF pulse scheme and of its transfer during Tsat as well as on concomitant saturation effects, such as direct saturation and magnetization-transfer contrast associated with the semisolid pool. Moreover, differences in approaches for data analysis in terms of normalization or spectral regions considered can further affect the final image contrast calculated from Z-spectra.

Thus, the saturation period has to be precisely defined by such parameters as RF pulse shape (both magnitude and phase), pulse duration (tp), saturation duty cycle (DCsat), total saturation time (Tsat), saturation field strength (B1), and offset from the water resonance frequency (Δω). However, these parameters vary significantly in the current literature, and are not always provided in sufficient detail. In addition, the literature uses different definitions or terminology to describe saturation “power,” such as the flip angle of the pulse, pulse peak B1 amplitude, or continuous wave power equivalent B1cpe, potentially leading to confusion when implementing a comparable CEST MRI experiment. Therefore, it is not always possible to faithfully reproduce a CEST experiment without corresponding with the authors, and even then, the method could still be prone to errors. Thus, a common, easy-to-use format for researchers to provide and share the precise saturation parameters is desirable, especially regarding the current focus on reproducibility in MR research. Moreover, a growing number of deep learning–based evaluation approaches for large multi-site, multi-vendor data sets make a proper definition of input data even more important.

A vendor-independent, human-readable, and sharable file format for MR sequences has been introduced with the Pulseq framework. In Pulseq, all sequence parameters are defined in a text file (hereafter called pulseq-file), which can be created with various popular programming applications such as MATLAB (The MathWorks, Natick, MA) or Python. This pulseq-file is then read and played out on the scanner using a vendor-specific interpreter sequence. Although Pulseq is a great tool that enables a flexible implementation of complex sequence patterns, it is complicated to incorporate vendor-provided image reconstruction functions, which are generally proprietary. However, having the source code of a full interpreter sequence at hand, a capsulated interpreter can be included into other existing sequences for imaging readout. For example, a 3D snapshot gradient echo can be equipped with an encapsulated Pulseq interpreter that solely plays out a CEST preparation block defined in a pulseq-file. This makes the Pulseq file format a perfect candidate for sharing CEST preparation protocols. The established MRI readout following the saturation period can be used with the familiar user interface and all possibilities of adjustments and image reconstruction. This procedure enables four major advantages:

1. The CEST preparation period definition in Pulseq is complete. It is defined in a human-readable text file that is easy to interpret and allows direct comparison of different protocols. Thus, exchanging and comparing such files allows total reproducibility;
2. The definition of the RF pulses can be done in MATLAB or Python instead of implementing it in the sequence using a vendor-specific language (often C++), which, depending on the vendor, can be time-consuming and can require the compilation of a new sequence library;
3. The CEST preparation period can be used directly in simulations in the same framework (eg, MATLAB or Python), eliminating possible sources of error from transferring simulation results to the sequence source code and vice versa; and

**Conclusion:** With Pulseq-CEST, we provide a straightforward approach to standardize, share, simulate, and measure different CEST preparation schemes, which are inherently completely defined.

**KEYWORDS**

CEST, open-source, Pulseq, standardization
4. The CEST preparation period can be used directly at the scanner with different state-of-the-art readouts, bridging the gap from first publication to reproducible multi-site application not only for research, but also for clinical applications. In addition, novel developments and work-in-progress approaches can be compared much faster and more reliably than with existing approaches.

Using Pulseq for the CEST preparation part in the sequence theoretically enables a vendor-independent approach, provided that a Pulseq interpreter sequence is available for each vendor. In this work, we implemented such a hybrid Pulseq-CEST sequence for the Siemens IDEA (Integrated Development Environment for Applications; Siemens Healthineers, Erlangen, Germany) framework and tested it on three Siemens scanners at three sites, with two scanner models running very different software baselines. In addition, we present a fast and flexible open-source simulation for the same pulseq-files that are played out on the MR scanner. Moreover, we provide a platform for researchers to share and test their saturation protocols in the Pulseq format. As a first illustration of these steps, we show applications at clinically available MR scanners including adiabatic spin–lock–prepared imaging, well-defined and original-author-approved amide proton transfer–weighted (APTw) imaging, and a CEST MR fingerprinting (MRF) protocol measured at three different MR sites in Europe and the United States.

2 | METHODS

2.1 | Pulseq to standardize CEST preparation periods

Originally intended as a hardware-independent MRI sequence prototyping framework, Pulseq allows for rapid and simple sequence definitions from within MATLAB, Python, and other software programming packages, which are usually open source.23,24,28 Within these programs, RF pulse, gradient, ADC, and trigger events can easily be defined and are written to a pulseq-file, which is then read and played out by a native interpreter sequence on the scanner. Because Pulseq includes built-in functions to generate block, Gaussian, apodized sinc, and arbitrary user-defined pulse shapes, theoretically, every excitation or saturation CEST preparation scheme can be defined with only a few lines of code. Thus, defining saturation periods in Pulseq is a general and easy approach. Example codes to create different CEST preparation periods in MATLAB and Python are provided at the projects’ website (https://pulseq-cest.github.io).

The pulseq-file contains the full definition of all sequence objects, which makes it a perfectly suitable candidate for sharing protocol parameters of preparation periods for generating different types of contrast before readout, such as a CEST contrast here. Moreover, these pulseq-files are human-readable, and the aforementioned MATLAB and Python packages include plot functions to compare preparation schemes directly. The benefit of such a direct comparison becomes obvious in Figure 1. Here, RF magnitude and phase of different CEST preparation periods used in this study are shown. For instance, Figure 1A,B shows the shape of an off-resonant adiabatic spin–lock pulse scheme previously used for in vivo DGEp studies29 and phantom measurements herein. While such a pulse shape would be rather complex to describe in a publication, it is completely defined in the pulseq-file. Figure 1C-H shows three different saturation preparation protocols (APTw_3T_001, APTw_3T_002, and APTw_3T_00332) that were recently recommended for APT-weighted tumor applications.33 For comparison of the CEST preparation pulses, we use the following definitions for the $B_1$ pulse average ($B_{1,pa}$), $B_1$ average amplitude over pulse train ($B_{1,cwae}$), and $B_1$ average quadratic amplitude over pulse train ($B_{1,rms}$ or $B_{1,cwpe}$):

$$B_{1,pa} = \frac{1}{t_p} \int_0^{t_p} B_1(t) \, dt$$

$$B_{1,cwae} = \frac{1}{t_p + t_d} \int_0^{t_p + t_d} B_1(t) \, dt$$

$$B_{1,rms} = \sqrt{\frac{1}{t_p + t_d} \int_0^{t_p + t_d} (B_1(t))^2 \, dt}$$

where $t_p$ is the pulse duration, and $t_d$ is the delay between preparation pulses. For the three different APTw protocols in Figure 1C-H, $B_{1,rms}$ was set to 2 µT. The different peak amplitudes of the saturation pulses due to the different shapes and duty cycles are directly observable. For instance, in Figure 1C,E, sinc-Gauss pulses with the same shape are shown, but in Figure 1E, the peak amplitude of the pulse is higher, as this protocol uses a saturation duty cycle (DCsat) of 0.5 compared with 0.9 for the protocol in C.

Figure 1I,J shows the RF magnitude and phase over multiple repetitions of the CEST-MRF schedule.36–38 Here, the amplitude of the spin–lock saturation pulses changes over the different repetitions.

Full details about the protocols can be found in the pulseq-files in the Supporting Information. In addition, the RF phase evolution during saturation pulses is available, a parameter that is rarely provided in CEST literature, although it can have an influence on the experiment, as shown in more detail in Supporting Information Figures S1 and S2.
2.2 | Bloch-McConnell simulations

To be able to not only compare protocol parameters, but also simulate them, an application was written in C++ that loops through the pulseq-files, performs Bloch-McConnell simulations for the respective sequence events, and returns the current magnetization vector after preparation. The compiled code is callable as a mex function for an easy integration into a MATLAB-based pipeline. The Python implementation wraps the C++ code with SWIG (Simplified Wrapper and
Interface Generator) and is simplified by a Python parser. This setup ensures input and output compatibility between the MATLAB and Python implementation. Where not specified explicitly, the mex function was used for the simulations in this study. Due to the flexible design, it is possible to simulate an arbitrary number of CEST pools and an additional semisolid magnetization-transfer contrast pool with either a Lorentzian or super-Lorentzian line shape. In combination with the flexible Pulseq saturation period definition, any number of CEST pools can be simulated for any kind of preparation period in a relatively short amount of time, thanks to the native C++ implementation. For this study, the simulation program was compiled for a 64-bit Microsoft Windows 10 OS, using the Microsoft Visual C++ 2017 compiler. Simulations were performed on a PC with an Intel i7-7700K Kaby Lake CPU. The source code is available on the project’s website (https://pulseq-cest.github.io/).

2.3 | Pulseq sequence building block

By adapting the source code of the original Pulseq sequence for the Siemens IDEA framework, we were able to play out the pulseq-files, containing the definition of the CEST preparation period, directly on the scanner, followed by different readout sequences. Adaptions were implemented to use the code as a so-called sequence-building block (SBB). For example, (1) the FOV positioning options are removed, as this information is defined in the readout sequence; and (2) during the Pulseq building block, no data are acquired, as indicated by the ADC event; instead the ADC event is used internally as marker to interrupt the Pulseq sequence and switch to the readout sequence, which is then played out (Figure 2). Note that the Pulseq block containing an ADC event is skipped entirely, and therefore is not allowed to contain any other events. With this design of the Pulseq SBB, it can be implemented in every MR sequence where the source code is available. Because timing and specific absorption rate calculations are handled by the interpreter sequence, the workload for such implementation is small. Only a few lines of code need to be implemented in the main sequence for initialization, preparation, and running of the Pulseq SBB. For instance, to implement the SBB to an established, native EPI sequence,39,40 only 30 lines of C++ code were necessary, including code regarding communication with the user interface.

The Pulseq interpreter sequence for Siemens IDEA contains vendor-specific code, and can therefore only be obtained through the customer-to-customer partnership program (called C2P procedure). The interpreter sequence can be provided upon request to interested researchers. The current Pulseq C2P package supports multiple software and hardware platforms of Siemens, including NumarisX vb11 and vb20. For other vendors, different “interpreter” approaches need to be developed or already exist, such as the TOPPE interpreter for GE (GE Healthcare, Chicago, IL) systems.41

2.4 | Phantom preparation

A phantom was prepared using multiple 6-mL tubes with either L-arginine or D-glucose. Five tubes were filled with 50 mmol/L L-arginine (Fluka Chemie, Buchs, Switzerland) dissolved in phosphate-buffered saline (according to Cold Spring Harbor Protocols,42 but containing 2.7 mM KCl, 10 mM Na2HPO4, 1.8 mM KH2PO4, and 140 mM NaCl). The pH of the L-arginine tubes was adjusted between 4 and 6 using HCl (Sigma-Aldrich Labormchemikalien, Germany) and NaOH (Riedel-de Haën, Seelze, Germany). Two additional tubes were filled with a D-glucose solution (Glucosteril 500 mmol/L; Guerbet, Villepinte, France) was added to each tube in this phantom, yielding a final concentration of approximately 0.054 mmol/L.

2.5 | Magnetic resonance imaging measurements

The MRI measurements were performed on three clinical 3T scanners: two 3T Prismas and one 3T Trio (Siemens Healthineers). For all Prisma measurements, the 64-channel head coil for signal reception and the body coil for transmission were used. All in vivo measurements were performed under approval by the local ethics committee. Each subject gave written, informed consent before the study. Where not specified explicitly, measurements were done on a Siemens 3T Prisma scanner at the Max Planck Institute Tuebingen). Following the Pulseq SBB, a 3D gradient-echo readout,26,27 was used. A table with relevant imaging parameters for the readout sequence can be found in Supporting Information Table S1. In addition, all pulseq-files used in this study are provided in the Supporting Information.

For the phantom experiment, a spin-lock (SL) Z-spectrum acquisition was performed using HSExp pulses43 with the pulse shape parameters recently optimized for 3 T29 (see Figure 1A,B and SLExp_3T_Photon.seq in the Supporting Information). For each saturation, a single spin-lock pulse with a duration of 100 ms was played out after a recovery delay (Trec) of 5 seconds. For this protocol, 39 evenly distributed offsets between ±6 ppm were acquired, together with an unsaturated $S_0$ scan. To enable realistic simulations,
a WASABI (simultaneous mapping of water shift and $B_1$),\textsuperscript{44} saturation recovery, and $T_2$ magnetization-preparation\textsuperscript{45} sequence were applied to determine $B_0$, $B_1$, $T_1$, and $T_2$ maps. The phantom was scanned at a room temperature of about 25°C.

The three different APTw protocols shown in Figure 1C-\textit{H} (APTw\_3T\_001.seq, APTw\_3T\_002.seq, and APTw\_3T\_003.seq in the Supporting Information) were scanned for direct comparison in vivo in a healthy volunteer. In addition, a WASABI measurement was performed to correct the Z-spectra for $B_0$ field inhomogeneity. To generate magnetization-transfer asymmetry (MTR asym) maps with higher SNR in vivo, all APTw protocols were acquired with repeated acquisitions (three repetitions) at the offsets of interest and a dummy scan at the beginning (APTw\_3T\_001\_AVG.seq, APTw\_3T\_002\_AVG.seq, and APTw\_3T\_003\_AVG.seq in the Supporting Information). This averaged protocol was additionally measured in a patient with a glioblastoma (World Health Organization grade IV, IDH mutation, and methylation of MGMT (O(6)-methylguanine-DNA methyltransferase) promoter) at the University Hospital Erlangen under approval of the local ethics committee.

Finally, we performed a whole-brain, in vivo CEST-MRF\textsuperscript{36} measurement at three different sites. For this purpose, the Pulseq SBB was implemented into a 3D-EPI sequence.\textsuperscript{39,40} All relevant readout parameters can be found in Supporting Information Table S1. The protocol consisted of the following measurements: (1) an APTw MRF protocol with 31 scans, using spin-lock pulses with a $B_1$ varying between 0 \(\mu\)T and 4 \(\mu\)T at a constant offset of 3.5 ppm (Figure 1I,J); (2)
a semisolid magnetization-transfer contrast–weighted MRF protocol with 31 scans, using spin-lock pulses with $B_1$ varying between 0.2 µT and 4 µT at offsets varying between 6 ppm and 14 ppm; (3) a WASABI measurement for $B_0$ and $B_1$ field inhomogeneity maps; (4) a saturation-recovery measurement for $T_1$ maps; and (5) a $T_2$ preparation measurement for $T_2$ maps. All pulse-seq-files for this experiment can be found in the Supporting Information (MRF_Amide.seq, MRF_MT.seq, T1prep.seq, T2prep.seq, and WASABI.seq). The protocol was applied at three different sites for one healthy volunteer each (two Siemens 3T Prisma Scanners at MPI Tuebingen and Massachusetts General Hospital, and a Siemens 3T Trio scanner at University Hospital Erlangen). For the Trio system, a 32-channel coil was used for reception, whereas a 64-channel coil was used at both Prisma systems.

### 2.6 Postprocessing

For the phantom experiment, MTR$_{asym}$ maps were generated voxel-wise, with $MTR_{asym}(\Delta \omega) = (S(\Delta \omega) - S(\Delta \omega))/S_0$, after a $\Delta B_0$ correction using a linear interpolation between acquired offsets. Additionally, three regions of interest (ROIs) were drawn in the center slice in the tubes listed in Table 1. Z-spectra in these ROIs were simulated as described in section 2.2. All simulation parameters can be found in Supporting Information Tables S2-S4). Both simulated and measured Z-spectra were normalized by the measurement at $-6$ ppm.

For the in vivo APTw experiment, all measurements were motion-corrected using elastix.$^{46}$ The applied elastix parameter file can be found in the Supporting Information (Rigid_MMI.txt). The MTR$_{asym}$ maps were generated in the same way as for the phantom but with applying an additional principal component analysis denoising approach$^{47}$ using the Malinowski criterion and a spatial 2D in-plane Gaussian filter ($\sigma = 0.6$) to smooth the images. A white-matter ROI was generated automatically by segmenting the image at $-6$ ppm.

For the CEST-MRF experiment, all images were again motion-corrected and registered to the $T_1$ measurement using elastix. The $T_1$ maps were generated by fitting a monoexponential function to the data of the saturation-recovery measurement. The $T_1$ map was then used to generate a synthetic $T_1$-weighted image, which was subsequently used to generate gray-matter and white-matter segmentation masks using SPM. Dictionary generation and calculation of amide and semisolid magnetization transfer–contrast concentration and exchange-rate maps were performed according to Perlman et al.$^{38}$ A major advantage in using the Puleseq SBB in this context is that for both measurement and dictionary generation, the same pulse-seq-file describing the saturation period definition is used. This reduces possible error sources from transferring the measurement parameters to the simulation or vice versa.

### 3 RESULTS

#### 3.1 Simulation and phantom measurement

The first experiment demonstrates how the same pulse-seq-file can be used for simulation and phantom measurements. Figure 3 shows the MTR$_{asym}$ map (Figure 3A) at 2 ppm, as well as the measured Z-spectra ($Z(\Delta \omega) = S(\Delta \omega)/S(-6 ppm)$) and simulated Z-spectra $Z(\Delta \omega) = M_z(\Delta \omega)/M_x(-6 ppm)$ (Figure 3B) and the MTR$_{asym}$ (Figure 3D) curve for the three different ROIs. The residuals between these measured and simulated normalized intensities are displayed in Figure 3C. The maximum residuals were 0.009, 0.008, and 0.004 for ROIs 1, 2, and 3, respectively. Simulation of the pulse-seq-file, using 500 samples per pulse (60 000 pulse samples per Z-spectrum) took approximately 0.19 seconds for a single amide CEST pool Z-spectrum (ROIs 1 and 2) and 0.77 seconds for four different D-glucose CEST pools (ROI 3).

#### 3.2 In vivo APT-weighted measurements

The second experiment demonstrates the value of well-defined pulse-seq-files for comparison of different author-approved APTw CEST implementations. The APTw (MTR$_{asym}(3.5 ppm)$) results for the volunteer and the tumor patient can be found in Figure 4. The MTR$_{asym}$ maps for the three different protocols are shown in Figure 4A-C, and despite different saturation, they show similar contrast in the healthy volunteer. The contrast in healthy brain is very low, which is expected, as the APTw-imaging parameters are designed to yield almost no contrast in healthy tissue.$^{4,49-51}$ Corresponding Z-spectra, with $Z(\Delta \omega) = S(\Delta \omega)/S(-1560 ppm)$, and MTR$_{asym}$ spectra can be found in Figure 4H,I, respectively. Comparison with simulation can be found in Supporting Information Figure S3. While the intensity in the MTR$_{asym}$ maps of the healthy volunteer is similar for all three protocols, a clear contrast can be seen in the tumor region (Figure 4D-F) of the glioblastoma patient. These protocols are all in use at clinical scanners, but were, to our knowledge,
never compared side by side. Such a comparison can now be performed in different pathologies, to validate different protocols and their relation to previous work.

### 3.3 In vivo CEST-MRF measurements

The final experiment demonstrates that with Pulseq-CEST, sophisticated CEST sequences can be shared between sites, for both simulation (and training of reconstruction networks in this CEST-MRF example) as well as for measurement of work-in-progress developments at different systems. The spin-lock based CEST-MRF scheme with whole-brain EPI readout was applied in three healthy volunteers and at three different MR sites. The resulting maps for the semisolid proton fraction ($f_{MT}$), the semisolid proton to water proton exchange rate ($k_{MT}$), the amide proton fraction ($f_{Amide}$), and the amide proton to water proton exchange rate ($k_{Amide}$) are shown in Figure 5. The mean values for gray matter and white matter across the entire brain are shown in Table 2. A visual as well as quantitative comparison indicates reproducibility of effects across multiple sites.

### 4 DISCUSSION

By adapting the source code of the Pulseq interpreter sequence to the SBB concept of the Siemens IDEA framework, we were able to use it as a sequence building block in established MR sequences and subsequently run CEST experiments at different clinical 3T scanners. Hence, we combined the full flexibility of Pulseq and the sophisticated readout methods from native sequences to generate an easy-to-use and flexible method for reproducible CEST measurements. For instance, it was directly possible to perform CEST-MRF experiments at three different sites on scanners with distinctly different software versions using identical CEST preparation periods defined in Pulseq. Thus, a sophisticated and still work-in-progress protocol could be reliably shared between research sites using Pulseq-CEST, without being limited by hard-coded user interface interactions. The quantitative results of the applied CEST-MRF method are consistent across sites, although the maps generated from the Trio system appear noisier. We attribute this primarily to the used 32-channel receive coil, leading to lower SNR compared with the 64-channel coil used on the Prisma system.
In addition, the actual amplitude of the RF pulses is not only determined by the defined pulse shape in the *pulseq*-file, but also by the reference voltage of the system. It is therefore possible that different reference voltages lead to different results. Such MRF differences can now be evaluated and handled. While larger, further studies using this protocol should compare and discuss results with previous CEST-MRF studies,\textsuperscript{38,52} it is beyond the scope of the work presented here, as Bloch-McConnell-based quantification is extremely challenging in vivo. Results presented here represent a work-in-progress state of a sophisticated method undergoing an active development. The *Pulseq*-CEST standard allows for efficient and active exchange between the research sites, even in such an early stage of development, accelerating generation and refinement of simulation databases and improving model training. The *Pulseq* SBB presented in this paper allows convenient though reliable multi-center collaboration to further investigate the method in detail in a larger cohort.

In addition, our software provides a Bloch-McConnell simulation tool for *pulseq*-files to simulate the exact same CEST preparation period that is played out by the interpreter on the scanner. The fast, native C++ implementation allows for pulsed CEST simulations, also with multiple isochromats, an application discussed in more detail in Supporting Information Figures S4 and S5. To ensure broad applicability, we provide implementations of the Bloch-McConnell simulation in both...
MATLAB and Python, which are the most frequently used programming languages in research. Confirmation that both implementations yield identical results is provided in Supporting Information Table S5. We hope that with this work, we provide the first version of a valuable and needed tool for the CEST community, to exchange and test CEST preparation periods for the many different types of different CEST experiments. For instance, a researcher publishing data could share a pulseq-file containing all RF pulse, gradient, and delay parameters through the Supporting Information, and it could subsequently be used by other researchers. Additionally, all pulseq-files can be made available on the project’s website (https://pulseq-cest.github.io/).

Moreover, the Pulseq sequence building block can be used to test all of these CEST-preparation blocks with a minimum workload, even with different readout sequences, such as gradient echo, EPI or RARE, which have been optimized with regard to their imaging performance beforehand. Although the SBB is so far only available for Siemens scanners, it already demonstrated its multiplatform capabilities by executing the same CEST preparation periods on scanners built on different hardware components and running different software versions. Furthermore, it is generally possible to transfer the approach to GE and Bruker (Bruker Biospin, Ettlingen, Germany) systems, where Pulseq implementations have been demonstrated. For Bruker systems, we recently proposed an initial approach to combine CEST preparation periods from pulseq-files with native Bruker readouts automatically with MATLAB. By doing so, we were able to measure the above-mentioned APTw protocols on a Bruker 14.1T scanner with ParaVision 6. This will be useful for comparison between preclinical and clinical trials, especially for pulsed CEST approaches.

A design of the interpreter software for other manufacturers (eg, Philips, United Imaging, or Canon) is needed for a universal application. However, even without actually applying pulseq-files on the scanner, the possibility to share, edit, display, and simulate saturation periods can be very insightful and beneficial for the design of CEST experiments. Because the pulseq-file guarantees a completely defined CEST preparation period inherently, which we believe is needed to improve the reproducibility of data in the CEST community, this will be a valuable tool especially when designing multisite clinical trials of technology, such as APTw MRI of brain tumors, which is being performed currently at many sites but often with different protocols.
The *Pulseq* interpreter sequence has been adapted and used as a sequence building block in native readout sequences herein, but it is also possible to use readouts implemented directly in *Pulseq*. The current version of the *Pulseq* interpreter allows for the images to be reconstructed directly on the scanner, which makes the development of novel imaging sequences even more convenient. As the presented approach is compatible with the “parent” *Pulseq* project, the previously published *Pulseq*-CEST preparation periods could be trivially integrated with these novel readout modules, implemented directly in *Pulseq*. This will allow full reproducibility, as the complete sequence (including preparation and readout) can be published in the *pulseq-file* format in the same database.

While native *Pulseq* readout modules can be considered for measurements in the future, it is already possible to combine them with CEST preparation modules for more realistic simulations. In that case, the pseudo ADC event needs to be replaced by a full readout sequence. Various examples of possible readout sequences implemented with *Pulseq* can be found on the project’s website (https://pulseq.github.io/). By explicitly including the readout, it becomes possible to simulate the influence of the RF pulses during the readout with the provided simulation (Supporting Information Figure S6). It is also possible to use a different *Pulseq*-compatible simulation software, such as *JEMRIS*, which supports Bloch-McConnell simulations, and provides a full MRI simulation framework. In addition, if the readout module is included in the *pulseq-file*, it is possible to estimate the expected specific absorption rate value using *sar4seq* before the scanning session. In general, the open format of *Pulseq* allows for a broad development of useful applications.

With regard to the growing number of applications of neural networks to CEST data reconstruction, often trained on simulated data, a match of the sequences used in silico and in vivo becomes crucial. In fact, the CEST-MRF reconstruction network used to infer the quantitative maps of Figure 5 was trained using simulated data with exactly the same *pulseq-file* as used at the MR scanner. This is just one example showing that *Pulseq*-CEST could be valuable for many emerging machine and deep learning–based approaches.

Finally, the method is obviously not limited to CEST applications. In principle, any magnetization-preparation sequence can be realized, as exemplified in Supporting Information Figure S7, for the WASABI field-mapping approach and a $T_1$ saturation-recovery sequence.

### 5 | CONCLUSIONS

We present a *Pulseq*-based sequence framework for CEST preparation pulse sequences and an accompanying simulation tool, the use of which in combination with available MRI sequences allows for straightforward sharing, implementing,
testing, optimizing, and running of CEST MRI studies. Because the pulseq-files inherently include a complete CEST parameter definition, this fosters faster comparison and facilitates reproducibility—not only between different MR sites, but also between real and simulated environments.

Source code for the Siemens IDEA interpreter sequence is available on request. All code for creating and simulating pulseq-files is open source and can be obtained on the project’s website (https://pulseq-cest.github.io/).

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DATA AVAILABILITY STATEMENT
All source code used for pulseq-file generation and simulation in this study is openly available at https://pulseq-cest.github.io/, where links to the corresponding GitHub repositories can be found.

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**SUPPORTING INFORMATION**

Additional Supporting Information may be found online in the Supporting Information section.

**FIGURE S1** Single magnetization vector trajectory during the CEST preparation period at 3.5 ppm with phase accumulation transfer during the saturation pulses (A,B) and without (C,D). The eight different colors indicate the trajectory during the eight different pulses. Due to the phase difference
between magnetization vector and RF pulse in (C) and (D), large oscillations can occur

**FIGURE S2** Z-spectra of a single magnetization vector for the sequences from Supporting Figure 1A,B (blue) and C,D (red). Depending on the frequency offset Δω, the artifacts are more or less severe, as the accumulated phase and therefore the phase difference between magnetization vector and RF pulse is dependent on the frequency offset and the duration of the pulse

**FIGURE S3** Measured (A,B) and simulated (C,D) Z-spectra (A,C) and magnetization-transfer asymmetry (MTR asym) curves (B,D) for the three different amide proton transfer–weighted (APTw) protocols from the main paper

**FIGURE S4** Comparison of simulation results between a single (blue) and multiple isochromats (red) in different tissue environments. The red curve shows the mean Z-spectrum of all 200 isochromats, and the error bars indicate the SD between isochromats

**FIGURE S5** Trajectory during a rectangular saturation pulse of a single isochromat with short (left) and long T₂ (right)

**FIGURE S6** Evolution of the z-magnetization after different CEST-preparation periods at 3.5 ppm during a 2D gradient-echo readout

**FIGURE S7** Comparison of simulation results from the MATLAB implementation (solid blue) and the Python implementation (dashed orange) for a WASABI (A) and a T₁ saturation-recovery (B) example

**TABLE S1** Basic imaging parameters of readouts used for the presented Pulseq-hybrid sequence

**TABLE S2** Simulation parameters for first region of interest (ROI)

**TABLE S3** Simulation parameters for second ROI

**TABLE S4** Simulation parameters for third ROI

**TABLE S5** Currently available pulseq-files in the Pulseq-CEST open database with the RMS error of the spectra simulated in MATLAB and Python

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