Chapter 27

Renal pH Mapping Using Chemical Exchange Saturation Transfer (CEST) MRI: Experimental Protocol

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Abstract

Chemical exchange saturation transfer (CEST) is recognized as one of the premier methods for measuring pH with this environmental variable expected to be an excellent biomarker for kidney diseases. Here we describe step-by-step CEST MRI experimental protocols for producing pH and perfusion maps for monitoring kidney pH homeostasis in rodents after administering iopamidol as contrast agent. Several CEST techniques, acquisition protocols and ratiometric approaches are described. The impact of length of acquisition time on the quality of the maps is detailed. These methods may be useful for investigating progression in kidney disease in vivo for rodent models.

This chapter is based upon work from the COST Action PARENCHIMA, a community-driven network funded by the European Cooperation in Science and Technology (COST) program of the European Union, which aims to improve the reproducibility and standardization of renal MRI biomarkers. This experimental protocol is complemented by two separate chapters describing the basic concepts and data analysis.

Key words Magnetic resonance imaging (MRI), Kidney, Mice, Rats, Chemical exchange saturation transfer (CEST), pH imaging, Iopamidol, Contrast agent, Responsive contrast agent

1 Introduction

The kidneys are responsible for filtration of plasma in order to remove waste and toxins and for maintaining the acid–base balance of the body through regulation of systemic HCO_3^- concentrations [1]. The kidneys can either reabsorb or generate new HCO_3^- through acid excretion. Renal control of pH could be impacted by a number of factors including whether or not there are abnormalities in perfusion, filtration, amino acid metabolism or if renal tissue edema or inflammation are present. Based on a number of studies relating changes in acid production, secretion or reduced NH_4^+ with pathological conditions [2–8], pH should be a good biomarker for assessing renal function. A number of methods are

now established for determining local pH in vivo, including use of pH micro electrodes [9–11], fluorescence imaging [12, 13], ^1H , ^{31}P , ^{19}F MRS [14–21] hyperpolarized ^{13}C MRS [22], pH-dependent MR relaxometry [23], and chemical exchange saturation transfer (CEST) MRI [24, 25]. CEST is a novel MRI contrast mechanism allowing detection of low concentrations of contrast agent through the application of saturation radiofrequency (RF) pulses on their labile protons to destroy their magnetization with the resulting signal loss transferred to water through chemical exchange which has now emerged as the premier MRI method for pH imaging [7, 26–32].

Here we describe CEST MRI for monitoring of the local pH variation found in the kidney of rodents in a step-by-step experimental protocol. The rationale for the choosing acquisition parameters is given in generic terms, together with specific parameter examples.

Mapping of contrast agent renal perfusion is described as an optional component of the experiment. Contrast agent perfusion is a functional parameter that is also sensitive to kidney damage. Currently CT, SPECT and gadolinium based MR imaging are available for assessing renal perfusion in patients, each with its own limitations [33]. CEST MRI contrast enables production of high resolution images of perfusion without need of using ionizing radiation making mapping of perfusion a valuable complement to pH imaging [34].

The protocols contained in this chapter were tested on healthy control mice using Iopamidol as the contrast agent to standardize the pH mapping and tested for both an axial slice and a coronal slice for bench marking. These methods may be useful for investigating renal pH and perfusion variations found in vivo for various rodent models with acute kidney injuries or which progress to chronic kidney disease.

This experimental protocol chapter is complemented by two separate chapters describing the basic concepts and data analysis, which are part of this book.

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2 Materials

2.1 Animals

This experimental protocol is tailored for immunocompetent (typically C57BL/6 and Balb/C) or immunocompromised (nude, NOD-SCID) mice with a body mass of 15–35 g. Advice for adaptation to rats is given as *Notes* where necessary. All animal studies have to be carried out in compliance with specific legislation covering the use of animals for scientific purposes. Therefore, all experiments must be authorized under national regulations.

2.2 Lab Equipment and Chemicals

1. Mouse tail illuminator/restrainer for catheterizing the tail vein.
2. Saline solution or heparin solution, 1-ml syringe, catheter (e.g., PE 20 polyethylene tubing) of known inner diameter to hold the saline flush and for contrast agent bolus is required.
3. Anesthesia: please refer to the chapter by Kaucsar T et al. "Preparation and Monitoring of Small Animals in Renal MRI" for an in-depth description and discussion of the anesthesia. Typically, 0.5–1.5% isoflurane is used for anesthesia administered to the mice using an anesthetic gas vaporizer (Leica Biosystems, Maryland, USA). For nonrecovery experiments, urethane solution (Sigma-Aldrich, Steinheim, Germany; 20% in distilled water) can provide anesthesia for several hours with comparatively few side effects on renal physiology, which is an important issue. For intramuscular anesthesia, please refer to **Note 1**.
4. Gases: O₂, N₂, and compressed air, as well as a gas-mixing system or general inhalation anesthesia equipment, including an anesthetic vaporizer, a flow meter and an induction chamber.
5. Mouse cradle.
6. pH imaging contrast agent: typically iodinated-based contrast media are employed, as Iopamidol (Isovue[®] 370, Bracco Imaging SpA, Milano, Italy), Iopromide (300 mg iodine/ml Ultravist[®], Bayer Healthcare, Germany) or Iobitridol (Omnipaque[®], GE Healthcare, USA). Such clinically available contrast agents have stock concentrations of 0.9–1 M that can be directly used (*see Note 2*).
7. Small Animal Ventilator: animals (rats) needs to be ventilated so the CEST MRI acquisition can be gated to minimize the respiratory motion artifacts. We used a TOPO Dual Mode Rodent/Small Animal Ventilator (Kent Scientific, Torrington, CT).
8. Syringe Infusion Pump: an MR-compatible single syringe pump (Harvard Bioscience, Holliston, MA) for contrast agent administration.

2.3 MRI Hardware

The general hardware requirements for renal ¹H MRI on mice and rats are described in the chapter by Ramos Delgado P et al. "Hardware Considerations for Preclinical Magnetic Resonance of the Kidney." The technique described in this chapter has been tailored for MR preclinical systems at magnetic fields higher than 3 T but advice for adaptation to other field strengths is given where necessary (*see Note 3*). No special or additional hardware is required, except for the following:

1. A physiological monitoring system that can track the respiration and which is connected to the MR system such that it can be used to synchronize CEST MRI with respiration. Typically, we use the MR-compatible rodent monitoring and gating system (Small Animal Instruments, New York, USA) equipped with an air-pillow to monitor breath rate.

2.4 MRI Sequences

The CEST MRI sequence includes continuous wave (CW) RF saturation followed by fast image readout such as echo planar imaging (EPI), Rapid Imaging with Refocused Echoes (RARE) and/or fast imaging with steady-state precession (FISP) [6, 35–37]. Herein we describe those based on fast refocused multiecho sequences since they provide the higher SNR in acceptable acquisition times and are without geometrical distortions. The same sequence readout is described for B_0 mapping, for full Z-spectrum acquisition and for dynamic acquisition applications.

3 Methods

3.1 MR Protocol Setup

3.1.1 Rapid Acquisition with Refocused Echoes Sequence for External B_0 Mapping Using WASSR

1. Select a 2D Rapid Acquisition with Refocused Echoes sequence in conjunction with a magnetization transfer preparation module that has a frequency offset which can be incremented within a single scan. This is a standard sequence on Bruker MRI systems, called “RARE.”
2. Switch on the magnetization transfer module. The magnetization transfer pulse parameters are then adjusted to allow detection of direct water saturation vs. excitation frequency and also the signal-to-noise $>50:1$ when the saturation pulse is 500 Hz or more away from water. Typically, we employ $N = 11,000$ ms rectangular shaped pulse with saturation power (amplitude) of 1–1.5 μT , similar to what was previously described for the WASSR Scheme [38].
3. Sample several frequency offsets around the frequency of bulk water signal (commonly set at 0 ppm, in contrast to the 4.8 ppm as in conventional NMR experiments). Usually the sampled range is between -3 and $+3$ ppm with different step size, according to the available acquisition time and expected B_0 inhomogeneity. Please see **Note 4** for an example of the frequency list used in B_0 mapping.
4. Choose a repetition time (TR) of $\sim 5\text{--}6$ s for good signal stability and signal-to-noise per time (SNR/ t) efficiency. TR will be limited by the length of the saturation pulse, length of echo train and the number of slices to be acquired, and saturation pulse(s)’s field strength.

5. Use the shortest echo time (TE) and echo spacing (ΔTE) possible. One wants to acquire as many lines of k space as possible to minimize motion distortions of the images. For that reason, it is advantageous to have ΔTE well below 10 ms. Larger ΔTE are not advisable because the SNR in the kidney will be so low that these images must be excluded. Acquisition bandwidth should be considered to shorten the ΔTE .
6. Set the rare factor as high as possible to reduce scan time. Same as the CEST acquisition scan (Subheading 3.1.2).
7. Set the acquisition bandwidth (BW) as for the CEST contrast scan (Subheading 3.1.2).
8. Switch on the fat saturation module.
9. Do not use the respiration trigger since this will increase the overall acquisition time.
10. Set the same geometry as for the CEST contrast scan (Subheading 3.1.2).
11. Set the matrix size/acceleration the same as for the CEST contrast scan (Subheading 3.1.2).
12. For an example of a specific parameter set please *see* **Note 5**.

3.1.2 Full Z-Spectrum Acquisition for CEST-pH Mapping

1. Select a 2D Rapid Acquisition with Refocused Echoes sequence with magnetization transfer preparation module. This is a standard sequence on Bruker MRI systems, called “RARE.” RARE sequence with centric encoding is used. More details on CEST sequences for Bruker scanners are provided in **Note 6**.
2. Switch on the Magnetization transfer module. The magnetization transfer pulse parameters are then adjusted to allow detection of CEST contrast while keeping the current within limits given by the RF transmit coil and the ^1H RF power amplifier. For CEST pH mapping, Z-spectra are acquired by applying a 1.5–6 μT continuous wave (CW) block shaped (bp) presaturation pulse for 2–5 seconds or by applying a pulsed saturation scheme (*see* Subheading 2.4.3.35).
3. Acquire a series of 30–50 frequencies in the range of ± 10 ppm for the whole CEST Z-spectrum acquisition. A macro file containing the MR frequencies is manually implemented by the operator in Bruker systems, by creating a list of frequencies covering the positive and negative side of CEST spectra. At the resonating frequencies of mobile protons (4.2/4.3 ppm and 5.5 ppm for Iopamidol and Iopromide, 5.6 ppm for Iobitridol) a denser sampling is usually applied (frequency resolution of 0.1 ppm). Please *see* **Note 7** for an example of the frequency list.

4. Choose the Repetition Time (TR) in the range 4–6 s for good signal stability and signal-to-noise per time (SNR/ t) efficiency, also according to the duration of the saturation pulse.
5. Use the shortest TE and echo spacing (Δ TE) possible.
6. Set the Rare factor to 64–96. Typically, a single shot acquisition (Rare Factor equal to Matrix Size) to reduce the acquisition time for each frequency. Partial Fourier acceleration should be considered to reduce the size of the matrix and improve the time efficiency of data acquisition and centric encoding to maximize SNR. Reconstruction to higher matrix size may be also considered to improve spatial resolution.
7. Use a high acquisition bandwidth (BW) to shorten Δ TE, while keeping an eye on the SNR, which decreases with increasing BW.
8. Switch on the fat saturation module. Important to avoid fat signal overlaying with the kidney due to chemical shift.
9. Set off the respiration trigger.
10. Adapt the geometry of the slice(s) so that animal fits into FOV in L-R direction (approx. 30–40 mm, according to the coil inner diameter) and use frequency encoding in H-F direction. Use a slice with the lowest thickness the SNR allows, typically around 1.5–2.0 mm. The thinner the slices the better, as this improves the resolution—which can be readily seen on the raw images.
11. Use the highest in-plane resolution that the SNR allows. A typical matrix size of 64×64 , 96×96 , or 128×128 is used for CEST images, with in-plane spatial resolution of 468, 312, or 234 μ m (for an FOV of 30 mm). This has to be balanced by the need for sufficient SNR in the images. Zero-filling or use of partial Fourier techniques in the phase encoding direction can be helpful to speed up acquisition to minimize motion artifacts and contrast agent concentration changes during the acquisition.
12. For an example of a specific parameter set for mice please *see* **Note 8** or for rats please *see* **Note 9**.

3.1.3 Rapid Acquisition with Refocused Echoes Sequence for CEST pH and Perfusion Measurements

1. Select a 2D rapid acquisition with refocused echoes sequence (RARE) with magnetization transfer preparation module.
2. Switch on the magnetization transfer module. The magnetization transfer pulse parameters are then adjusted to allow detection of CEST contrast while keeping the current within the limits given by the RF transmit coil, the ^1H RF power amplifier and the signal-to-noise $>50:1$ at the frequency of the labile protons of interest on the CEST agent. Typically, we employ signal averaging with $N = 10$, 300 ms rectangular shaped RF

pulses with an amplitude between 3 and 4 μT . The saturation frequency is alternated between 4.2 ppm, 5.5 ppm for collection of signal with saturation on resonance with the two labile protons in iopamidol and for the last ten images –100 ppm for collection of S_0 (signal with no saturation).

3. Choose a repetition time (TR) between 7 and 12 s for good signal stability and signal-to-noise per time (SNR/ t) efficiency.
4. Use the shortest TE and echo spacing (ΔTE) possible (*see* Subheading 3.1.2).
5. Use a rare factor of 20–32. Typically, 20–32 TEs are sensible and allow acquiring a 32×32 matrix slice or 48×48 with partial Fourier acceleration in a single shot. Partial Fourier acceleration should be considered to reduce the size of the matrix and improve the time efficiency of data acquisition.
6. Use a high acquisition bandwidth (BW) to shorten ΔTE , while keeping an eye on the SNR, which decreases with increasing BW. For the image with the shortest TE an SNR of at least 60 is recommended.
7. Switch on the fat saturation module. Important to avoid fat signal overlaying with the kidney due to chemical shift.
8. Use an FOV saturation of 4 mm thickness above 9–13 mm from the kidneys for coronal slices. This is not required for axial slices.
9. Set off the respiration trigger.
10. Adapt the geometry of the acquisition slices so that the animal fits into FOV in L-R direction (approx. 30–40 mm according to the coil inner diameter) and use frequency encoding in H-F direction. Use a slice with the lowest thickness the SNR allows, typically around 1.5 mm. The thinner the slices the better, as this improves the resolution—which can be readily seen on the raw images. For adaptation to rats *see* **Note 10**.
11. Use highest in-plane resolution that the SNR allows, typically around $600 \times 400 \mu\text{m}^2$ for axial slices or $200 \times 500 \mu\text{m}^2$ for coronal slices. This has to be balanced by the need for sufficient SNR in the images. Use zero-filling or partial Fourier techniques in the phase encoding direction to speed up acquisition and to minimize motion artifacts and contrast agent concentration changes during the acquisition. One may use half Fourier in read direction (asymmetric echo) to further shorten the first TE. Reducing the excitation and refocusing pulse lengths to below 1.5 ms can then also help to shorten TE.
12. For an example of a specific parameter set please *see* **Note 11**.

3.2 Setting of the pH Calibration Curve

Prepare multiple vials of Iopamidol phosphate-buffered solution (PBS $1 \times$, 10 mM of inorganic phosphates) or human blood serum solutions, with pH titrated to physiological relevant renal pH values: 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and place them in a plastic container (e.g., 30–50 ml falcon tube) filled with water. Such a phantom calibration experiment is crucial for in vivo pH quantification at different experimental conditions such as magnetic field strength and CEST irradiation Schemes [39]. A more detailed description for pH calibration curve can be found in the chapter by Kim H et al. “Analysis Protocol for the Quantification of Renal pH Using Chemical Exchange Saturation Transfer (CEST) MRI.”

1. Acquire the CEST Z-spectrum by sweeping RF saturation to a series of frequency offsets around the bulk water resonance. Note that the magnitude of the saturation field (B_1) and duration can be adjusted serially to facilitate Z-spectral acquisition. For an example of specific parameters at 7 T please *see* **Note 12**, whereas for an example of specific parameters at 4.7 T please *see* **Note 13** and for the same at 11.7 T please *see* **Note 14**.

3.3 Preparations of the Contrast Agent Injection and of the Catheter

1. Fill the 1-ml syringe with the contrast agent stock solution.
2. Prepare the catheter by filling the PE20 tubing with the contrast agent solution, and with 20–60 μ l of a heparin solution (or a saline solution), between the needle (the animal) and the contrast agent solution to prevent clotting before the contrast agent will be injected.
3. According to the length of the catheter (usually 60–100 cm to exit from the magnet bore) the contrast agent solution can be injected directly from the syringe containing the contrast agent or by changing the syringe with one filled with a (heparin-free) saline solution for the postinjection flush.

3.4 Preparation of the Mouse

1. Place the mouse on a mouse tail illuminator restrainer for catheterizing the tail vein. Catheterize the tail vein by placing a 27/29-G needle in the animal.
2. Induce anesthesia via isoflurane in an induction box. Transfer the animal to the scanner (**Note 15**).
3. Set up the temperature monitoring (rectal probe) and respiratory monitoring (balloon on chest) unit. Keep the animal's respiration rate during imaging at 35–80 breaths per min by adjusting the dose of anesthesia (approximately 1–1.5% isoflurane with air and oxygen mixed at a 3:1 ratio administered through a nose cone attached to the animal bed and ophthalmic ointment will be applied to the eyes) (*see* **Note 16**).
4. Perform anatomical imaging as described in the chapter by Pohlmann A et al. “Essential Practical Steps for MRI of the Kidney in Experimental Research.”

Fig. 1 Series of ten saturation weighted images of a healthy mouse kidney acquired with a 2D CEST-RARE sequence at 11.7 T. Images correspond to saturation frequencies = -1.5 , -1.2 , -0.9 , -0.6 , -0.3 ppm (top row) and saturation frequencies = 0 , 0.3 , 0.6 , 0.9 , 1.2 ppm (bottom row)

5. Perform localized shimming on the kidney imaging as described in the chapter by Pohlmann A et al. “Essential Practical Steps for MRI of the Kidney in Experimental Research” (*see* **Note 17**).

3.5 B_0 Mapping

3.5.1 External B_0 Mapping

1. Load the B_0 mapping sequence and adapt the slice orientation to provide a coronal or axial view of the two kidneys based on the tripilot/scout and anatomical images collected.
2. Run the B_0 mapping scan using 42 offsets from -1.5 to $+1.5$ ppm and $B_1 = 1.5$ μ T. Example images are shown in Fig. 1.

3.5.2 Internal B_0 Mapping

1. Acquire a full Z-spectrum with some frequency offsets sampled around 0 ppm to exploit the Z-spectrum for internal measurement of B_0 shifts as described in the chapter by Kim H et al. “Analysis Protocol for the Quantification of Renal pH Using Chemical Exchange Saturation Transfer (CEST) MRI.”

3.6 CEST Acquisition for pH Mapping

1. Perform the CEST experiment by acquiring CEST images before, (during) and after injection of the contrast agent. Choose among the different pH mapping procedures by conventional ratiometric approach (Subheading 3.5.1), pH mapping with power ratiometric method (Subheading 3.5.2) or dynamic CEST perfusion and pH mapping (Subheading 3.5.3).

3.6.1 pH Mapping with Conventional Ratiometric Method (Full Z-Spectrum)

1. Load the pH mapping sequence, using the same geometry as used for anatomical and B_0 mapping. Switch off the magnetization module and use this sequence to set the receiver gain.
2. Load the pH mapping sequence, using the same geometry as used for anatomical and B_0 mapping. Set the receiver gain as calculated by the magnetization off sequence (*see* Subheading

Fig. 2 Series of saturated weighted images of a healthy mouse kidney acquired with a 2D CEST-RARE sequence at 7 T before (up panel) and after (bottom panel) the injection of iopamidol at 1.5 mg I/kg b.w. Images correspond to saturation frequencies of -10 , $+10$, 0 , $+4.2$, and $+5.5$ ppm

Fig. 3 From left to right: parametric maps of saturation transfer (ST) contrast obtained at 4.2 ppm, at 5.5 ppm, ratiometric 4.2/5.5 map and calculated pH map in kidney regions. The parametric color maps are superimposed on a T_{2w} anatomical image (gray scale)

3.5.1.15). Load the macro file containing the offset frequencies to be saturated and run the sequence for CEST preinjection scanning.

3. Clone the pH mapping scans, at least one time for acquiring one CEST postinjection scan.
4. After the acquisition of the CEST prescan, perform a bolus injection of iodine contrast agent through the tail vein catheter at a dose of 1.0–1.5 iodine/kg b.w. (*see Note 18*).
5. Run the postinjection scans.
6. A demonstration of the acquired images at different offsets and of CEST contrast that can be expected for the pH mapping scans are given in Fig. 2 and Fig. 3, respectively.

3.6.2 pH Mapping with Power Ratiometric Method (Full Z-Spectrum)

1. Load the pH mapping sequence, using the same geometry as used for anatomical and B_0 mapping. Switch off the magnetization module and use this sequence to set the receiver gain.
2. Load the pH mapping sequence, using the same geometry as used for anatomical and B_0 mapping. Set the receiver gain as calculated by the magnetization off sequence (*see* Subheading 3.6.1.15). Load the macro file containing the offset frequencies to saturate at a first selected power pulse (1.5 μ T) and run the sequence for CEST preinjection scanning.
3. Clone the scan and set the second selected saturation power pulse (6 μ T). Run the sequence for CEST preinjection scanning.
4. Clone two times the scans with 1.5 and 6 μ T saturation power pulse for acquiring the postinjection scans.
5. Perform a bolus injection of iodine contrast agent with single amide exchanging pool (e.g., iobitridol) through the tail vein catheter at a dose of 1.0–1.5 iodine/kg b.w. (*see* **Note 18**).
6. Run the postinjection scans.

3.6.3 pH and Perfusion Mapping with Two Offset Sampling

1. Load the pH mapping sequence, using the same geometry as used for B_0 mapping and setting up a collection to run for 1 h, 5 min alternating the saturation frequency between 4.2 ppm (labile proton #1 for iopamidol), 5.5 ppm (labile proton #2 for iopamidol), and for the final ten images at -100 ppm (S_0 image) (*see* **Note 19**).
2. Initiate the pH mapping scans.
3. Exactly 5 min after starting the pH mapping scans, perform a bolus injection of contrast agent: inject iopamidol through the tail vein at a dose of 1.0 g iodine/kg using ~ 100 – 150 μ L.
4. Approximately 1 h after injection, end the pH mapping scan collection.
5. A demonstration of the contrast changes that can be expected for the pH mapping scans are given in Fig. 4.

4 Notes

1. Intramuscular anesthesia can be administered as a mixture of xylazine 5 mg/kg (Rompun, Bayer, Milan, Italy) and tiletamine/zolepan 20 mg/kg (Zoletil 100, Virbac, Milano, Italy).
2. To reduce the viscosity of these solutions, hence the pressure needed to infuse these solutions, syringes can be warmed at 37°C before the injection. Please avoid the inclusion of air bubbles in the catheter filled with the saline solution or with the contrast agent solution that will be injected into the animals.

Fig. 4 Demonstration of the contrast changes that can be expected in pH mapping. CEST weighted images (from a 2D RARE acquisition, effective TE = 3.5 ms) of the kidneys throughout a pH measurement experiment, together with a final processed image of perfusion. A moving average filter was applied on the time series data to remove fluctuations in signal due to motion. The percentage of kidney pixels with >20% of maximum contrast were calculated to obtain the perfusion or filtration fraction map

3. CEST pH measurements are basically performed on Bruker MR systems as Biospec 117/20 (11.7 T), Avance 300 (7 T), Pharmascan 70/16 (7 T), or Biospec (3 T) (Bruker, Ettlingen, Germany). Standard ^1H transmit/receive coils (birdcage, quadrature) or phased-array coils (4- or 8-channel mouse body phase array receive coil) to improve SNR can be used.
4. WASSR B_0 mapping frequency list (in ppm): -1.5, -1.4, -1.3, -1.2, -1.1, -1.0, -0.9, -0.8, -0.7, -0.6, -0.5, -0.4, -0.3, -0.2, -0.1, 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, and 1.5.
5. Scan acquisition parameters for B_0 mapping: TR = 5000 ms; magnetization transfer power = 1.5 μT ; magnetization transfer module time = 3 s; MT offset mode = Sequential_Offset; effective TE = 3.49 ms; RARE Factor = 32; averages = 1;

centric encoding; slice thickness = 1.5 mm; slice orientation = axial; frequency encoding = head-feet; FOV = 28×19 mm; matrix size = 48×48 reconstructed to 128×128 ; fat suppression = on.

6. Standard Bruker sequences for the software versions PV5 and PV6 include only a Magnetization Transfer module that allows the saturation of a single frequency offset. As a consequence, full Z-spectrum acquisition can only be acquired by cloning the same scan and modifying for each scan the irradiated frequency offset. Therefore, for in vivo experiments this may lead to acquisition times that are not feasible in reasonable time. Bruker sequences for PV5 and PV6 that have been modified to accept a frequency list for acquiring the full Z-spectrum in a single scan are available upon request to the authors (dario.longo@unito.it; mtmcmaho@gmail.com; pzhesun@emory.edu). Starting from PV360 a new CEST module has been implemented with different readout sequences (RARE, EPI, Spiral) that allows to fix this issue. MR Solutions vendor has already a CEST module that can be applied with an EPI-based readout scheme.
7. Frequency list (in ppm) for a full Z-spectrum acquisition (43 offsets): 0, 0.5, -0.5, 1.0, -1.0, 1.5, -1.5, 2.0, -2.0, 3.0, -3.0, 3.9, -3.9, 4.0, -4.0, 4.1, -4.1, 4.2, -4.2, 4.3, -4.3, 4.4, -4.4, 4.5, -4.5, 5.2, -5.2, 5.3, -5.3, 5.4, -5.4, 5.5, -5.5, 5.6, -5.6, 5.7, -5.7, 5.8, -5.8, 7.5, -7.5, 10.0, and -10.0.
8. Example for a 30 g mouse at 7 T: TR = 6000 ms; magnetization transfer power = $3 \mu\text{T}$; magnetization transfer module time = 5 s; MT offset mode = from file; effective TE = 4.14 ms; RARE Factor = 96; averages = 1; centric encoding; slice thickness = 1.5 mm; slice orientation = axial; frequency encoding = head-feet; FOV = 30 mm; matrix size = 96×96 reconstructed to 128×128 ; fat suppression = on.
9. Example for a 150 g rat at 4.7 T: Z-spectra are collected with moderate saturation RF power (B_1) levels of 1.0 and $2.0 \mu\text{T}$. The in vivo CEST MRI parameters were as the following: RF saturation offsets from -7.0 to 7.0 ppm with intervals of 0.125 ppm, and TR, TS and TE were 6 s, 3 s, and 18 ms, respectively, for an image matrix of 48×48 .
10. For rats increase the FOV to the body width and keep the matrix size the same or similar. The relative resolution is then identical and the SNR should also be similar, because the larger rat RF coil provides worse SNR (e.g., eight-channel rat body phase array receive coil vs eight-channel mouse body phase array receive coil).

11. Example for a 30 g mouse at 11.7 T: TR = 9000 ms; magnetization transfer power = 4 μ T; magnetization transfer pulse number = 10; magnetization transfer inter pulse delay = 10 μ s; magnetization transfer pulse length = 300 ms; magnetization transfer module time = 3 s; MT offset mode = from file; effective TE = 3.49 ms; RARE Factor = 32; averages = 1; centric encoding; slice thickness = 1.5 mm; slice orientation = axial; frequency encoding = head-feet; FOV = 28×19 mm²; matrix size = 48×48 using partial Fourier of 1.5; fat suppression = on.
12. Phantom CEST acquisition parameters at 7 T: RF saturation offsets from -10.0 to 10.0 ppm with intervals of 0.1–0.2 ppm. TR = 10s, TE = 4.8 ms, saturation = 3 μ T $\times$ 5 s, matrix size = 64×64 , FOV = 30×30 mm² with centric encoding.
13. Phantom CEST acquisition parameters at 4.7 T: RF saturation offsets from -7 to 7 ppm with intervals of 0.25 ppm. TR = 12 s, TE = 39.5 ms, saturation: 1, 1.5, 2, 2.5, 3 and 4 μ T $\times$ 5 s, matrix size = 64×64 , FOV = 48×48 mm with centric encoding.
14. Phantom CEST acquisition parameters at 11.7 T:: 25 mm transmit/receive volume coil; TR = 5000 ms; magnetization transfer power = six different saturation power (B_1) from 1 to 6 μ T; magnetization transfer module time = 3 s; MT offset mode = Sequential_Offset; 71 CEST offsets between ± 7 ppm plus one at +40 ppm for M_0 ; effective TE = 3.39 ms; RARE Factor = 16; averages = 1; centric encoding; slice thickness = 1.0 mm; slice orientation = axial; frequency encoding = head-feet; FOV = 28×19 mm²; matrix size = 64×48 .
15. Mice can be alternatively anesthetized with intramuscular anesthesia, tail veins heated with hot water/heater equipment at 37 °C and catheter placed in the animal.
16. You must monitor the respiration continuously throughout the entire experiment.
17. B_0 Shimming is particularly important, since macroscopic magnetic field inhomogeneities affect the exact resonance frequency of the labile protons in a voxel, and can impact the pH and perfusion measurements and hinder quantitative intra- and inter-subject comparisons. Shimming should be performed on a voxel enclosing both kidneys using either the default iterative shimming method or the Mapshim technique (recommended).
18. Injections can be manually or automatically performed. For manual injection, keep injection constant within 20–30 s. Automatic injection can be performed with dedicated injection pumps, by setting injection speed of contrast agent at 400 μ l/

min. For instance, a 25 g mouse would receive ca 70 μ l to achieve a dose of 1 g iodine/kg b.w. with a stock solution of 370 mg iodine/ml.

19. The exact list of frequencies used here can be adjusted based on the B_0 shimming conditions of the kidneys and the saturation power applied. For example, if the B_0 varies by more than say 0.1 ppm, additional frequencies around each labile proton should be added to allow B_0 correction at the expense of SNR due to the reduced redundancy of the data collected.

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References

1. Hamm LL, Nakhoul N, Hering-Smith KS (2015) Acid-base homeostasis. *Clin J Am Soc Nephrol* 10(12):2232–2242
2. Soleimani M, Rastegar A (2016) Pathophysiology of renal tubular acidosis: core curriculum 2016. *Am J Kidney Dis* 68(3):488–498. <https://doi.org/10.1053/j.ajkd.2016.03.422>
3. Gil-Peña H, Mejía N, Santos F (2014) Renal tubular acidosis. *J Pediatr* 164(4):691–698. <https://doi.org/10.1016/j.jpeds.2013.10.085>
4. Gillies RJ, Raghunand N, Garcia-Martin ML, Gatenby RA (2004) pH imaging. *IEEE Eng Med Biol Mag* 23(5):57–64
5. Raghunand N, Howison C, Sherry AD, Zhang S, Gillies RJ (2003) Renal and systemic pH imaging by contrast-enhanced MRI. *Magn Reson Med* 49(2):249–257
6. Longo DL, Busato A, Lanzardo S, Antico F, Aime S (2013) Imaging the pH evolution of an acute kidney injury model by means of iopamidol, a MRI-CEST pH-responsive contrast agent. *Magn Reson Med* 70(3):859–864
7. Longo DL, Cutrin JC, Michelotti F, Irrera P, Aime S (2017) Noninvasive evaluation of renal pH homeostasis after ischemia reperfusion injury by CEST-MRI. *NMR Biomed* 30(7). <https://doi.org/10.1002/nbm.372>
8. Hashim AI, Zhang XM, Wojtkowiak JW, Martinez GV, Gillies RJ (2011) Imaging pH and metastasis. *NMR Biomed* 24(6):582–591. <https://doi.org/10.1002/nbm.1644>

9. Ashby BS (1966) pH studies in human malignant tumours. *Lancet* 288(7458):312–315
10. Prescott DM, Charles HC, Poulson JM, Page RL, Thrall DE, Vujaskovic Z, Dewhirst MW (2000) The relationship between intracellular and extracellular pH in spontaneous canine tumors. *Clin Cancer Res* 6(6):2501–2505
11. Wike-Hooley JL, van den Berg AP, van der Zee J, Reinhold HS (1985) Human tumour pH and its variation. *Eur J Cancer Clin Oncol* 21(7):785789–787791
12. Li C, Xia J, Wei X, Yan H, Si Z, Ju S (2010) pH-activated near-infrared fluorescence nanoprobe imaging tumors by sensing the acidic microenvironment. *Adv Funct Mater* 20(14):2222–2230
13. Hassan M, Riley J, Chernomordik V, Smith P, Pursley R, Lee SB, Capala J, Amir HG (2007) Fluorescence lifetime imaging system for in vivo studies. *Mol Imaging* 6(4):7290.2007.00019
14. García-Martín M-L, Hérigault G, Rémy C, Farion R, Ballesteros P, Coles JA, Cerdán S, Ziegler A (2001) Mapping extracellular pH in rat brain gliomas in vivo by ¹H magnetic resonance spectroscopic imaging: comparison with maps of metabolites. *Cancer Res* 61(17):6524–6531
15. Gillies R, Liu Z, Bhujwalla Z (1994) ³¹P-MRS measurements of extracellular pH of tumors using 3-aminopropylphosphonate. *Am J Phys Cell Phys* 267(1):C195–C203
16. Gillies RJ, Morse DL (2005) In vivo magnetic resonance spectroscopy in cancer. *Annu Rev Biomed Eng* 7:287–326
17. Vermathen P, Capizzano AA, Maudsley AA (2000) Administration and ¹H MRS detection of histidine in human brain: application to in vivo pH measurement. *Magn Reson Med* 43(5):665–675
18. Bhujwalla ZM, McCoy C, Glickson J, Gillies R, Stubbs M (1998) Estimations of intra- and extracellular volume and pH by ³¹P magnetic resonance spectroscopy: effect of therapy on RIF-1 tumours. *Br J Cancer* 78(5):606
19. Aoki Y, Akagi K, Tanaka Y, Kawai J, Takahashi M (1996) Measurement of intratumor pH by pH indicator used in ¹⁹F-magnetic resonance spectroscopy: measurement of extracellular pH decrease caused by hyperthermia combined with hydralazine. *Investig Radiol* 31(11):680–689
20. Zhou R, Bansal N, Leeper DB, Glickson JD (2000) Intracellular acidification of human melanoma xenografts by the respiratory inhibitor m-iodobenzylguanidine plus hyperglycemia: a ³¹P magnetic resonance spectroscopy study. *Cancer Res* 60(13):3532–3536
21. Ojugo AS, McSheehy PM, McIntyre DJ, McCoy C, Stubbs M, Leach MO, Judson IR, Griffiths JR (1999) Measurement of the extracellular pH of solid tumours in mice by magnetic resonance spectroscopy: a comparison of exogenous ¹⁹F and ³¹P probes. *NMR Biomed* 12(8):495–504
22. Gallagher FA, Kettunen MI, Day SE, Hu D-E, Ardenkjær-Larsen JH, Jensen PR, Karlsson M, Golman K, Lerche MH, Brindle KM (2008) Magnetic resonance imaging of pH in vivo using hyperpolarized ¹³C-labelled bicarbonate. *Nature* 453(7197):940–943
23. Garcia-Martin ML, Martinez GV, Raghunand N, Sherry AD, Zhang S, Gillies RJ (2006) High resolution pHe imaging of rat glioma using pH-dependent relaxivity. *Magn Reson Med* 55(2):309–315
24. McMahon MT, Gilad AA, Bulte JWM, van Zijl PCM (2017) Chemical exchange saturation transfer imaging: advances and applications, 1st edn. Pan Stanford Publishing, Singapore
25. Longo D, Aime S (2017) Iodinated contrast media as pH-responsive CEST agents. In: McMahon MT, Gilad AA, JBM B, PCM VZ (eds) Chemical exchange saturation transfer imaging. Vol. Advances and applications. Pan Stanford Publishing, Singapore, pp 447–466. <https://doi.org/10.1201/9781315364421-20>
26. Pavuluri K, McMahon MT (2017) pH imaging using chemical exchange saturation transfer (CEST) MRI. *Isr J Chem* 57(9):862–879. <https://doi.org/10.1002/ijch.201700075>
27. Longo DL, Dastrù W, Digilio G, Keupp J, Langeris S, Lanzardo S, Prestigio S, Steinbach O, Terreno E, Uggeri F (2011) Iopamidol as a responsive MRI-chemical exchange saturation transfer contrast agent for pH mapping of kidneys: In vivo studies in mice at 7 T. *Magn Reson Med* 65(1):202–211
28. Longo DL, Sun PZ, Consolino L, Michelotti FC, Uggeri F, Aime S (2014) A general MRI-CEST ratiometric approach for pH imaging: demonstration of in vivo pH mapping with iobitridol. *J Am Chem Soc* 136(41):14333–14336
29. Wu RH, Longo DL, Aime S, Sun PZ (2015) Quantitative description of radiofrequency (RF) power-based ratiometric chemical exchange saturation transfer (CEST) pH imaging. *NMR Biomed* 28(5):555–565. <https://doi.org/10.1002/nbm.3284>
30. Yang X, Song X, Ray Banerjee S, Li Y, Byun Y, Liu G, Bhujwalla ZM, Pomper MG, McMahon

- MT (2016) Developing imidazoles as CEST MRI pH sensors. *Contrast Media Mol Imaging* 11(4):304–312
31. McMahon MT, Gilad AA, Zhou J, Sun PZ, Bulte JW, van Zijl P (2006) Quantifying exchange rates in chemical exchange saturation transfer agents using the saturation time and saturation power dependencies of the magnetization transfer effect on the magnetic resonance imaging signal (QUEST and QUESP): pH calibration for poly-L-lysine and a starburst dendrimer. *Magn Reson Med* 55(4):836–847
 32. Anemone A, Consolino L, Arena F, Capozza M, Longo DL (2019) Imaging tumor acidosis: a survey of the available techniques for mapping in vivo tumor pH. *Cancer Metastasis Rev.* <https://doi.org/10.1007/s10555-019-09782-9>
 33. Grenier N, Cornelis F, Le Bras Y, Rigou G, Boutault JR, Bouzgarrou M (2013) Perfusion imaging in renal diseases. *Diagn Interv Imaging* 94(12):1313–1322. <https://doi.org/10.1016/j.diii.2013.08.018>
 34. Anemone A, Consolino L, Longo DL (2017) MRI-CEST assessment of tumour perfusion using X-ray iodinated agents: comparison with a conventional Gd-based agent. *Eur Radiol* 27(5):2170–2179. <https://doi.org/10.1007/s00330-016-4552-7>
 35. Sheth VR, Li Y, Chen LQ, Howison CM, Flask CA, Pagel MD (2012) Measuring in vivo tumor pHe with CEST-FISP MRI. *Magn Reson Med* 67(3):760–768. <https://doi.org/10.1002/mrm.23038>
 36. Sun PZ, Cheung JS, Wang E, Benner T, Sorensen AG (2011) Fast multislice pH-weighted chemical exchange saturation transfer (CEST) MRI with unevenly segmented RF irradiation. *Magn Reson Med* 65(2):588–594. <https://doi.org/10.1002/mrm.22628>
 37. Pavuluri K, Manoli I, Pass A, Li Y, Vernon HJ, Venditti CP, McMahon MT (2019) Noninvasive monitoring of chronic kidney disease using pH and perfusion imaging. *Sci Adv.* <https://doi.org/10.1126/sciadv.aaw8357>
 38. Kim M, Gillen J, Landman BA, Zhou J, van Zijl PC (2009) Water saturation shift referencing (WASSR) for chemical exchange saturation transfer (CEST) experiments. *Magn Reson Med* 61(6):1441–1450. <https://doi.org/10.1002/mrm.21873>
 39. Sun PZ, Longo DL, Hu W, Xiao G, Wu R (2014) Quantification of iopamidol multi-site chemical exchange properties for ratiometric chemical exchange saturation transfer (CEST) imaging of pH. *Phys Med Biol* 59(16):4493

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