The exchange rates of amide and arginine guanidinium CEST in the mouse brain

Kexin Wang^{1,3*}, Ran Sui^{1,3*}, Lin Chen^{1,2,4}, Yuguo Li^{1,2}, Jiadi Xu^{1,2}

1. F.M. Kirby Research Center for Functional Brain Imaging, Kennedy Krieger Research Institute, Baltimore, MD, USA

2. Russell H. Morgan Department of Radiology and Radiological Science, Johns Hopkins University School of Medicine, Baltimore, MD, USA

3. Department of Biomedical Engineering, Johns Hopkins University, Baltimore, MD, USA

4. Department of Electronic Science, Fujian Provincial Key Laboratory of Plasma and Magnetic Resonance, Xiamen University, Xiamen, China

*These authors contributed equally to this work

Corresponding Author:

Jiadi Xu, Ph.D.
Kennedy Krieger Institute
Johns Hopkins University School of Medicine
707 N. Broadway
Baltimore, MD, 21205
E-mail: jxu37@jh.edu
Tel: 443-923-9572
Fax: 443-923-9505

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Abstract:

Purpose: To develop a pipeline for measuring the exchange rates and concentrations of *in vivo* exchangeable protons, and to demonstrate this for the amide and arginine (Arg) guanidinium (Guan) protons in mobile proteins in the mouse brain.

Methods: An ultra-short echo (UTE) CEST sequence with a continuous wave presaturation (preRadCEST) was applied to acquire Z-spectra with robustness to motion and physiological fluctuations. AmideCEST and Arginine guanCEST (ArgCEST) were extracted and their proton concentrations and exchange rates obtained using a two-step multi- B_1 Bloch fitting approach that included the semisolid macromolecular background. To minimize contamination from the amine protons from creatine and phosphocreatine, ArgCEST measurements were performed on the Guanidinoacetate N-methyltransferase deficiency (GAMT^{-/-}) mouse characterized by low creatine and phosphocreatine concentrations in the brain.

Results: For the amideCEST proton pool, the exchange rate and concentrations were found to be $59.6 \pm 9.0 \text{ s}^{-1}$ and $41.7 \pm 7.0 \text{ mM}$, respectively, with the maximum signal observed at $B_1 = 0.8 \text{ } \mu\text{T}$. For the ArgCEST proton, the guanidinium exchange these were $70.1 \pm 5.5 \text{ s}^{-1}$ and $10.1 \pm 1.3 \text{ mM}$, respectively, with the maximum effect observed at $B_1 = 0.9 \text{ } \mu\text{T}$. The current study suggests that the inverse pH dependence in GuanCEST of brain is led by the CrCEST component, not ArgCEST.

Conclusion: The current pipeline is expected to have general use for *in vivo* CEST quantitation and optimization of visible CEST resonances.

Key Words:

Chemical exchange saturation transfer (CEST), amide, arginine, guanidinium, exchange rate, concentration, presaturation radial CEST (preRadCEST).

Introduction

Chemical exchange saturation transfer (CEST) MRI is a molecular imaging method that can significantly enhance the sensitivity of detecting low concentrations of proteins and metabolites through the water signal (1-3). Among many endogenous CEST contrasts, amide protons (amide proton transfer, APT, or amideCEST) in mobile proteins have been intensively studied. AmideCEST signal has a well-defined lineshape, which stands out in the crowded *in vivo* Z-spectrum (4,5). It is noteworthy that amideCEST should be distinguished from APT-weighted MRI, which also focuses on the peak at 3.5 ppm but is obtained by asymmetry analysis of the Z-spectrum and thus includes multiple other effects including those from relayed NOEs (rNOEs) and asymmetry in the semisolid magnetization transfer contrast (MTC) (6). Guanidinium (Guan) CEST was reported in creatine (Cr) phantoms (7-9), muscle (10-13), and brain (14), the Z-spectrum of which at 2 ppm also includes the phosphocreatine (PCr) and the guanidinium protons of arginine groups in endogenous mobile proteins (5,15-18). Since for proteins the only guanidinium group exists in Arginine (Arg) groups, we name the GuanCEST signal from proteins as ArgCEST, while Cr as CrCEST. AmideCEST and ArgCEST are among a few CEST contrasts that can be extracted with high confidence from the crowded *in vivo* Z-spectrum because of their distinguishable peaks at multiple MRI field strengths. The optimum acquisition and quantification of amideCEST and ArgCEST contrasts usually requires knowledge of their exchange rates. Particularly, the exchange rate of the ArgCEST proton pool is critical in the understanding of the pH and B_1 dependence of signal at 2 ppm (15,19-21). For most metabolite CEST studies, the exchange rates can be measured in phantom solution under physiological conditions, such as glutamate (22), D-glucose (23,24), Cr (9), and PCr (9,25,26). However, this method is not possible for both amideCEST and ArgCEST since these resonances reflect the composite effect of multiple unidentified proteins. An initial attempt to determine the amide proton exchange rate was carried out in the rat brain with a water-exchange filter (WEX) NMR sequence, giving a value of 28.6 s^{-1} (27). Recent studies, including frequency labeled exchange transfer MRI (28) and fingerprinting CEST method, suggested a much higher exchange rate (around $200\text{-}400 \text{ s}^{-1}$) (29), while another group reported as $34.6\text{-}47.9 \text{ s}^{-1}$ with fingerprinting (30,31). Hence, it is worth revisiting the amideCEST exchange rate with recent technology and also fill in the long-standing blank of the ArgCEST exchange rate.

The direct measurement of exchange rates of amideCEST and ArgCEST from the brain Z-spectrum is hampered by three factors. Firstly, the acquisition of Z-spectrum needs correction for the noise and artifacts introduced by physiological motions (32,33) and/or MRI instability. A large variation is observed in the Z-spectrum when the saturation field strength in Hertz is comparable to the B_0 fluctuation in the mouse brain caused by the respiration and brain movements (34). This can be partially improved using the motion-insensitive CEST MRI methods developed recently, such as the self-gated ultra-short echo time (UTE) CEST technique (35-37), or using navigators to correct for frequency fluctuations and motion. (38-40) Secondly, the extraction of both amideCEST and ArgCEST signals from *in vivo* Z-spectrum is challenging. Multi-pool Lorentzian line-shape fitting does not function well for the amide and guanidinium resonances (41-45), due to their overlap with amineCEST, PCr amide, and amide/aromatic rNOE signals, especially for guanidinium. Fortunately, recent pH-dependent studies on animal stroke models at both high (4,15,21) and low MRI fields (18) have suggested that both amideCEST and ArgCEST show discernible peaks with smooth backgrounds in the brain Z-spectrum. These peaks can be well extracted and quantified using a recently proposed polynomial and Lorentzian line-shape fitting (PLOF) approach (17,20,25,34). Another challenge for the ArgCEST measurement is the contamination from Cr guanidinium and PCr amide peaks around 2 ppm (16,17). This problem can be solved by performing the ArgCEST assessment using a Guanidinoacetate N-methyltransferase deficiency (GAMT^{-/-}) mouse model that has low Cr and PCr concentrations in the brain (46,47). Thirdly, the quantitation of the CEST exchange rate in the brain with the QUESP (the quantification of exchange rate using varying saturation power) (48,49) and Omega plot (50-52) methods needs to account for the strong MTC and direct saturation backgrounds in tissues. To overcome all of the challenges mentioned above, we developed a two-step multi- B_1 Bloch-McConnell (BM) fitting approach for both signal extraction (background removal) and quantification. The idea is similar to that behind PLOF CEST, but strictly follows the BM equations for fitting the MTC background and the CEST peaks. The approach was first validated in a creatine (Cr) phantom mixed with cross-linked bovine serum albumin (BSA) and then applied *in vivo* for the amideCEST with wild type (WT) mouse and ArgCEST using GAMT^{-/-} mouse brains.

Methods

Presaturation Radial CEST sequence

The CEST pulse sequence with a radial readout (35-37,53) was composed of a train of interleaved Gaussian saturation pulses and 2D-UTE modules. As demonstrated in both brain (35) and liver CEST studies (36), a radial acquisition scheme is inherently less sensitive to motion artifacts. However, the steady-state UTE-CEST is difficult for CEST quantification as it is challenging to model the pulsed CEST signal. Therefore, we modified the sequence to perform continuous wave (CW) CEST labeling at the beginning followed by a train of 2D UTE readout modules as shown in Fig. 1, which is dubbed presaturation radial CEST (preRadCEST).

CEST extraction and quantification

Three pools, i.e., water, magnetization transfer contrast (MTC) and CEST, were assumed for the BM equations. Here, MTC is a combined pool from both macromolecules and other CEST components. For the macromolecular MTC proton pool, the transverse relaxation time can be neglected due to its extremely short T_2 ($\sim 10 \mu\text{s}$) (54). Then, the MTC components are determined by three parameters (supplemental material Eq. S4): the MTC exchange rate k_{MTC} , the back exchange rate from MTC to water $k_{\text{MTC}} \cdot f_{\text{MTC}}$, and the $R_{1\text{MTC}} + R_{\text{MTC}}$. $R_{1\text{MTC}}$ is typically much lower than R_{MTC} and has minimum impact on the fitting, and was set to 1 s^{-1} here. $T_{2\text{MTC}}$ usually determines the MTC lineshape in the conventional Super-Lorentzian or Gaussian assumptions for the biological tissues (55,56). In current study, a linear lineshape was used for the mixed MTC and CEST background as we fit only a small range of the Z-spectrum ($< 4 \text{ ppm}$). Then, $T_{2\text{MTC}}$ has no impact on the fitting results and was set to $10 \mu\text{s}$. The detailed BM fitting is provided in the supporting information and the Matlab code for the BM fitting will be made available at <https://github.com/jiadixu/Two-step-Bloch-Fitting> after the paper is accepted. The Z-spectra acquired with different B_1 values are fitted simultaneously, i.e., group BM fitting. Two steps of the fitting are performed as below:

1. The MTC and water background spectrum were fitted by excluding the data points for amideCEST (or ArgCEST) with varying parameters, $T_{2\text{app}}$, C_{MTC}^0 and C_{MTC}^1 , for each Z-spectrum. Here, $T_{2\text{app}}$ is a parameter including water transverse relaxation and the broadening caused by the fast-exchanging protons. The offset was fixed at 3.5 ppm (amideCEST) and 2.0 ppm (ArgCEST), while T_1 of the amide and ArgCEST pool was set to 1 s. The T_{1w} values

measured in both phantom and mouse brain were fixed for the group fitting. The fitting was achieved by minimizing the root mean square (RMS) of the difference between our fitting and experimental data. For amideCEST and ArgCEST, background fitting ranges of 2.0–5.5 ppm and 1.0–3.5 ppm were used, excluding the relevant signal ranges of [3.2, 4.0] ppm and [1.6, 2.6] ppm, respectively.

2. The extraction and fitting of amideCEST and ArgCEST signals were achieved by fixing the background signal obtained in step 1, i.e., the T_{1w} , T_{2app} , C_{MTC}^0 , C_{MTC}^1 values. The T_{2amide} , k_{amide} and f_{amide} (or T_{2Guan} , k_{Guan} and f_{Guan}) values were varied in the amideCEST (or ArgCEST) fitting to minimize the RMS of the difference between our fitting and experimental data. Either amideCEST or ArgCEST signal can be obtained by subtracting the background signal from the CEST signals at 3.5 and 2.0 ppm respectively.

Phantom Validation

The two-step BM fitting method was validated on a Cr (50 mM) solution mixed with 15% cross-linked BSA (CrCrossBSA) in phosphate buffered saline (PBS), titrated to pH 7.7. BSA crosslinking was achieved using a 25 μ L glutaraldehyde solution. The exchange rate of the CrCrossBSA phantoms was measured on a Bruker 17.6T NMR spectrometer using the selective inversion recovery method detailed in the supplemental material (13).

MRI Experiments

Animal procedures were approved by the Johns Hopkins University Animal Care and Use Committee. Six C57BL/6 mice (age: 3 months) and four GAMT^{-/-} mice (age: 3 months) were used. All MRI experiments were performed on a horizontal bore 11.7 T Bruker Biospec system (Bruker, Ettlingen, Germany). A 72 mm quadrature volume resonator was used as a transmitter, and a 2x2 phased array was used as a receiver for both phantoms and animals. Animals were anesthetized using 2% isoflurane in medical air, followed by 1% to 1.5% isoflurane for maintenance. The B_0 field over the image slice was adjusted to second order for animal using field-mapping and shimmed on the water linewidth for phantoms. T_1 -weighted maps were acquired using the same geometry and spatial resolution as CEST MRI, employing a RAREVTR sequence (RARE with variable TR = 0.5, 1, 1.5, 2, 3.5, 5, 8 s).

The pre-saturation time was set to 2 s for all CEST experiments. Seven B_1 values were collected for the CrCrossBSA phantom (0.4, 0.6, 0.8, 1.0, 1.2, 1.4 and 1.6 μT), while eight B_1 values were used for the mouse brain (0.4, 0.5, 0.6, 0.8, 1.0, 1.2, 1.4 and 1.6 μT). The frequency was swept from 2.0 to 5.5 ppm with an increment of 0.1 ppm for the amideCEST. This same frequency step size was applied for the ArgCEST from 1.0 ppm to 3.5 ppm, except for a smaller increment of 0.05 ppm between 1.4 ppm and 3.0 ppm. For phantom studies, the frequency was swept from 0.5 to 3.5 ppm with 0.1 ppm increment. The excitation pulse for the 2D-UTE radial readout was 0.4 ms with a flip angle of 10 degrees. After each saturation pulse, one radial spoke was collected with $\text{TR}_2 = 6$ ms. In the current study, single-slice CEST images were collected with a total acquisition time of 900 ms. The nominal Cartesian matrix size was set to 96×96 with the slice thickness = 2 mm. Before performing experiments on the $\text{GAMT}^{-/-}$ mice, *in vivo* MRS was performed on the mouse brain to confirm the low concentration of Cr/PCr (Supporting Information Figure S1).

Results

Phantom validation experiments

One challenging part in the BM fitting is the great number of parameters that participate, while they are interacting with each other. We used a CrCrossBSA phantom as ground truth to validate the fitting method. The typical MRS spectrum of CrCrossBSA is plotted in the inset of Fig. 2a. The Guan exchange rate at room temperature and pH 7.7 was determined to be $106 \pm 8 \text{ s}^{-1}$ by fitting the selective inversion curves of the Guan peak (Fig. 2a).

CEST experiments were performed on the same phantom at room temperature with the preRadCEST sequence. To examine the proper selection of k_{MTC} , f_{MTC} and $T_{2\text{MTC}}$ values on the MTC background fitting. The MTC background fitting in the step one was evaluated with different k_{MTC} , $T_{2\text{MTC}}$ and f_{MTC} values while $T_{2\text{app}}$, C_{MTC}^0 and C_{MTC}^1 varied for each B_1 Z-spectrum (Figs. 2b-d). The goodness-of-fit was evaluated by the R^2 coefficient. The result suggested that the fitting is insensitive to the f_{MTC} and k_{MTC} when $f_{\text{MTC}} > 8\%$ and $k_{\text{MTC}} > 11\%$. Hence, typical *in vivo* $k_{\text{MTC}} = 20 \text{ s}^{-1}$, $f_{\text{MTC}} = 10\%$ were fixed in our study, which are representative *in vivo* MTC parameters determined by on- and off-resonance variable delay multi-pulse (VDMP) methods (57,58) as well as previous MTC studies (59-65)

The typical Z-spectrum as well as the BM fitting curves are plotted in Fig. 2e. The fitting curves matched the observed Z-spectrum for all B_1 values ($R^2 > 0.995$). From the two-step BM fitting, CrCEST exchange rate was determined to be $104.7 \pm 6.0 \text{ s}^{-1}$, concentration = $46.8 \pm 5 \text{ mM}$ and $T_{2\text{Guan}} = 18.3 \pm 2 \text{ ms}$. The exchange rate and concentration matched well with the measured rate from the inversion experiment ($106 \pm 8 \text{ s}^{-1}$) and the prepared concentration (50 mM). The fitting results for all parameters are listed in the supporting information Table S1. The CrCEST signal as a function of B_1 plotted in Fig. 2f shows typical buildup and decay pattern with the peak at $0.8 \text{ } \mu\text{T}$.

AmideCEST in the WT mice

In Fig. 3a, the averaged ($n=4$) amideCEST Z-spectrum of the WT mouse whole brain is shown for four typical B_1 values. The curve fitted using the two-step BM fitting method demonstrates the extraction of the amideCEST and the high quality of fitting. The amideCEST signals extracted by our fitting are plotted as a function of B_1 in Fig. 3b. An exchange rate of $59.6 \pm 9 \text{ s}^{-1}$, a concentration of $41.7 \pm 7 \text{ mM}$ and $T_{2s} = 5.0 \pm 0.9 \text{ ms}$ were extracted from the fitting.

ArgCEST measurement in the $\text{GAMT}^{-/-}$ mice

The averaged ($n=4$) Z-spectrum of the $\text{GAMT}^{-/-}$ mouse whole brain of is shown in Fig. 4a for the ArgCEST region. The fitted curves using the two-step group multi- B_1 BM fitting method are also displayed to demonstrate the MTC background fitting results and the extraction of the ArgCEST effect. An exchange rate of $70.1 \pm 5.5 \text{ s}^{-1}$, a concentration of $10.1 \pm 1.3 \text{ mM}$ and $T_{2s} = 3.9 \pm 0.5 \text{ ms}$ were extracted from the fitting for the *in vivo* ArgCEST. The extracted MTC background parameters T_{2app} , C_{MTC}^0 , and C_{MTC}^1 as a function of B_1^2 are plotted in Fig. 4b-d. They can be well fitted with linear functions ($R^2 > 0.83$) and are given by

$$\begin{aligned} T_{2eff} &= 0.0137B_1^2 + 0.0433 \\ C_{MTC}^0 &= 3.0B_1^2 + 0.779 \\ C_{MTC}^1 &= 78.62B_1^2 + 21.17 \end{aligned} \quad (1)$$

The above equations were used for the following B_1 dependent CrCEST and ArgCEST simulation. In Fig. 4e, the averaged ArgCEST signal extracted using two-step BM fitting with respect to the saturation power is plotted with the BM simulation curves at fixed MTC background parameters. The optimum saturation power for ArgCEST is $0.8 \text{ } \mu\text{T}$ according to Fig. 4e. The CrCEST signals with different exchange rates, i.e., 150, 400 and 1000 s^{-1} were also simulated by

fixing the concentration at 5 mM. With exchange rates between 150-400 s⁻¹, the curves show clear buildup and decay patterns with peaks at 1.0-1.4 μT, while the signal is buildup and the signal hardly decays at a high exchange rate of 1000 s⁻¹. To explain the pH dependent CEST contrasts, the ArgCEST signal as a function of exchange rate was simulated with BM equations for four typical B₁ values (0.5, 1, 2 and 3 μT) displayed in Fig. 4f by fixing the MTC background.

Discussion

In this paper, we used recently developed CEST acquisition, extraction, and quantification methods to determine the exchange rates and concentrations of both amide protons and guanidinium protons in arginine residues of mobile proteins in the mouse brain. Using a t_{sat} of 2 s, the maximum amideCEST signal was observed with a B₁ of 0.8 μT, comparable to the previous observed value (1 μT) with a three-point method (4). The measured amide exchange rate of 59.6 s⁻¹ was slightly higher than that measured previously by the WEX method (28.6 s⁻¹) (27), but is consistent to values measured with some of the MR fingerprinting methods (34.6-47.9 s⁻¹) (30,31). The consistent results from all three methods further confirm these methods are measuring the same protons, while other methods may have used acquisition parameters that are more sensitive to mixed effects of other proton pools possibly increasing the determined exchange rates.

Previous power dependent studies on both brain and muscle suggested an optimum B₁ of 1.0-1.4 μT for *in vivo* CrCEST (17,25,26), which indicates the *in vivo* CrCEST exchange rate is far lower than the exchange rates in solution (950 s⁻¹) by comparing with simulations in Fig. 4e. The CrCEST exchange rate in tissue is estimated between 150-400 s⁻¹ from the optimum B₁ and rapid signal decay with B₁>1.4 μT in the *in vivo* studies (17,25,26). The Cr exchange rate in solution was estimated to around 1000 s⁻¹ at room temperature and pH 7.7 (66). The CrCEST exchange rate in the cross-linked BSA is significantly lower than that of the Cr solution (106 s⁻¹ vs 1000 s⁻¹). The exact reason for the much lower Cr exchange rates in both tissues and cross-linked BSA is not clear. The exchange rate of ArgCEST (70.1 s⁻¹) found here is far smaller than that of CrCEST in the physiological pH range, which should allow the selective enhancement of the CrCEST signal in the WT mouse brain by applying high B₁ (Fig. 4e).

The measured guanidinium proton exchange rate determined from ArgCEST also reveals the reason behind the opposite pH-dependence of ArgCEST signal under different conditions of B₁ on mouse brain. It is known that the CEST signal increases with decreased exchange rates when

the exchange rates are higher than the Rabi frequency of the saturation pulses used (see Fig. 4f) (67,68). The guanidinium exchange rate in Cr ($150\text{--}400\text{ s}^{-1}$) is above the Rabi frequency (134 s^{-1}) corresponding to $B_1 = 0.5\text{ }\mu\text{T}$ and should show inverse dependence on the exchange rate, i.e., pH, as demonstrated in the Fig. 4f. On the hand, the ArgCEST (70.1 s^{-1}) is still lower than the Rabi frequency (134 s^{-1}) and should be proportional to pH for this B_1 (Fig. 4f). Therefore, the current study suggests that the inverse pH dependence in GuanCEST is led by the CrCEST component as the GuanCEST in WT mouse brain is a combined signal of CrCEST and ArgCEST (15). Although the protamine ArgCEST was also found to be inversely dependent on pH with low B_1 (15), this observation cannot be used to validate the *in vivo* pH dependence due to its extremely high exchange rates as shown in the Supporting Information Figure S2. The exchange rate of Arg guanidinium protons in protamine solution is $743 \pm 20\text{ s}^{-1}$ at pH 7.0. With high saturation RF strengths such as $2\text{ }\mu\text{T}$, both Arg and Cr guanidinium exchange rates are lower than the Rabi frequency (532 s^{-1}) and hence proportional to pH. This explains the strong pH dependence found in mouse brain with $B_1 = 2.0\text{ }\mu\text{T}$ (20,21). The slow exchange rate of ArgCEST is also consistent with the observation in eggwhite at 3T, in which a clear ArgCEST peak is visible (5).

Conclusion

We used preRadCEST acquisition, two-step group BM extraction and quantification for determination of the exchange rates and concentrations of amideCEST and ArgCEST in the mouse brain. This approach can be generalized for other CEST quantitation and optimizations as well as *in vivo* CEST ratiometric approach for pH imaging.

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Data Availability Statement

The code that supports the findings of this study will be made available at <https://github.com/jiadixu/Two-step-Bloch-Fitting>.

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Figure 1. (a) Timing diagram of the preRadCEST sequence. T_{sat} is the length of the CW saturation period, while TR_2 is the time duration between successive readout pulses.

Figure 2. (a) The inversion recovery curve of peaks at 1.92 ppm (Guan) of Cr in 15% cross-linked BSA at room temperature and pH 7.7. The curve was fitted using a single exponential curve (Supporting Information Eq. S1). Inset: typical 1D NMR spectrum of CrCrossBSA phantom. (b-d) The impact of different (b) f_{MTC} and (c) k_{MTC} on the MTC background fitting. The goodness of the fitting results was evaluated by the R^2 coefficient of determination. (b) $k_{MTC}=20$ s⁻¹ set for the simulation (c) In the k_{MTC} simulation, $f_{MTC}=10\%$. (d) Typical Z-spectra of the GuanCEST region recorded with a B_1 of 0.4, 0.8, 1.2 and 1.6 μ T and an illustration of the two-step multi- B_1 BM fitting scheme for extracting and quantifying the GuanCEST signal ($R^2=0.995$). All CrCrossBSA experiments were performed at room temperature. The black dashed lines are the background Z-spectrum, while the solid lines are fitted CrCEST curves with the fixed water and MTC background. In the whole fitting, the measured water $T_{1w} = 1.8$ s was used, while $T_{1MTC} = 1$ s and $T_{2MTC} = 10$ μ s for the MTC pool were fixed. (f) The B_1 -dependent CrCEST values for the CrCrossBSA phantom extracted with the two-step multi- B_1 BM fitting.

Figure 3. (a) Averaged Z-spectrum of the amideCEST (n=4) for the WT mice recorded using preRadCEST with a fixed saturation length of 5 s and varying B₁=0.5, 0.8, 1.2 and 1.6 μT together with the two-step B₁ group fitting curves. Dashed lines are the MTC background fitting curves, while solid lines are the difference Z-spectrum fitting. (b) The extracted amideCEST signal (ΔZ) of the WT whole brain as a function of B₁ extracted with the two-step BM fitting.

Figure 4. (a) Averaged Z-spectrum of the ArgCEST (n=4) for the GAMT^{-/-} mice recorded with four typical B₁ values together with the two-step BM fitting curves. Dashed lines are the MTC background fitting curves, while solid-lines are the difference Z-spectrum fitting. (b-c) The extracted MTC background parameters (b) T_{2app} , (c) C_{MTC}^0 , and (d) C_{MTC}^1 as a function of B_1^2 . Solid lines are the linear fitting curves. (e) The averaged saturation power-dependent ArgCEST values at 2 ppm for the whole brain of GAMT^{-/-} mouse (n=4). The solid lines are the simulated curves for both Arg and Cr GuanCEST with different exchange rates and concentrations, i.e., 70.0 s⁻¹ (10.0 mM) for Arg, 150 s⁻¹ (5 mM), 400 s⁻¹ (5 mM) and 1000 s⁻¹ (5 mM) for Cr. In the fitting, T_{2s} =3.9 ms, T_{1w} =1.8 s, T_{1MTC} =1 s, T_{2MTC} =10 μs, f_{MTC} =10%, saturation time 5 s was fixed. (f) The simulated exchange rate dependent GuanCEST signal for four typical saturation powers, i.e., 0.5 μT, 1 μT, 2 μT and 3 μT. The Rabi frequencies are labeled with red arrows, i.e. 134 s⁻¹ (0.5 μT), 268 s⁻¹ (1 μT), 538 s⁻¹ (2 μT), 806 s⁻¹ (3 μT). In the fitting, f_{guan} =11.9 mM, saturation time 5 s and T_{2guan} =15 ms were used.

Supporting Information:

Bloch-McConnell (BM) Simulations:

The observed MTC/CEST signal under influence of a continuous RF pulse can be calculated using the coupled Bloch equations for a three-pool model: water (M_{xw}, M_{yw}, M_{zw}), CEST (M_{xs}, M_{ys}, M_{zs}) and MTC (M_{zMTC})(1):MTC

$$d\vec{M} = \Lambda \vec{M} \quad (S1)$$

$$\vec{M} = \begin{bmatrix} M_{xw} \\ M_{yw} \\ M_{zw} \\ M_{xs} \\ M_{ys} \\ M_{zs} \\ M_{zMTC} \end{bmatrix} \quad (S2)$$

Here, the transverse components of MTC (M_{xMTC}, M_{yMTC}) are set to zero due to the extremely short T_2 in the semisolid.

$$\Lambda = \begin{bmatrix} & & & 0 \\ & A_w & C_w & 0 \\ & & & f_{MTC}k_{MTC} \\ & & & 0 \\ C_s & & A_s & 0 \\ & & & 0 \\ 0 & 0 & -k_{MTC} & 0 & 0 & 0 & -R_{1MTC} - R_{MTC} \end{bmatrix} \quad (S3)$$

A_w and A_s are two 3x3 matrixes that describe the evolution of the water and solute magnetizations under an RF pulse, respectively, while C_w and C_s are two 3x3 matrixes describing the chemical exchange processes from the water and from the solute pools, respectively. The exchange between solute pool and MTC pool was neglected. k_{MTC} is the exchange rate from the MTC pool to water, and f_{mtc} is the fractional concentration of the MTC protons to the water protons. R_{1MTC} is the longitudinal relaxation rate of the MTC pool. R_{MTC} is the lineshape of the macromolecular pool together with other saturation transfer components. The MTC lineshape has been reported as Gaussian for Agar and Super-Lorentzian for semi-solid biological tissues (2,3). Considering the frequency range studies in Z-spectra is far smaller than those in MTC studies and the complicated

contributions from other exchangeable protons, the MTC lineshape is assumed to be a linear function:

$$R_{MTC} = C_{MTC}^0 + 2\pi(\Delta - \Delta_{MTC}) C_{MTC}^1 \quad (S4)$$

where Δ_{MTC} is the offset of the MTC pool resonance center relative to the water signal (Hz).

A_w and A_s are given by

$$A_w = \begin{bmatrix} -R_{2w} & 2\pi\Delta_w & 0 \\ -2\pi\Delta_w & -R_{2w} & \gamma B_1 \\ 0 & -\gamma B_1 & -R_{1w} \end{bmatrix} \quad (S5)$$

$$A_s = \begin{bmatrix} -R_{2s} & 2\pi\Delta_s & 0 \\ -2\pi\Delta_s & -R_{2s} & \gamma B_1 \\ 0 & -\gamma B_1 & -R_{1s} \end{bmatrix} \quad (S6)$$

where R_{2w} and R_{2s} are the transverse relaxation rates and R_{1w} and R_{1s} the longitudinal relaxation rates for the two pools. Δ_w and Δ_s are chemical shift offsets (Hz) for the two pools relative to the water proton pool. The exchange matrixes C_w and C_s are given by

$$C_w = \begin{bmatrix} f_s & k_s & 0 & 0 \\ 0 & f_s & k_s & 0 \\ 0 & 0 & f_s & k_s \end{bmatrix} \quad (S7)$$

$$C_s = \begin{bmatrix} -k_s & 0 & 0 \\ 0 & -k_s & 0 \\ 0 & 0 & -k_s \end{bmatrix} \quad (S8)$$

Exchange rate measurement of the CrCEST:

The exchange rates of CrCEST in the cross-linked BSA phantoms were measured by the magnetization recovery of the exchangeable proton peaks after inversion by a single selective Gaussian pulse (width of 2.5 ms) assumed to have negligible effect on the semisolid. After a delay T_I , the NMR signal was detected by a 3-9-19 WATER suppression by GrAdient Tailored Excitation (WATERGATE) sequence with a total echo time of 1 ms. Repetition time was 10 s. A list of 15 inversion times from 0 ms to 200 ms were used. The offset was set to 2.0 ppm (with respect to water). The recovery rate was determined by the apparent relaxation rate, $1/T_1 + k_{sw} \approx k_{sw}$, from the fitting of the recovery curve using the following equation

$$S = A - Be^{-k_{sw}T_I} \quad (S1)$$

***In vivo* MRS on GAMT^{-/-} mice:**

The total creatine (Cr + PCr) concentrations in all GAMT^{-/-} mouse brains were obtained from *in vivo* proton MRS experiments performed on voxels of $2.5 \times 2.5 \times 2.5$ mm³ each using a stimulated echo acquisition mode (STEAM) sequence (TE = 3 ms, TM = 10 ms, TR = 2.5 s, NA = 64). These MRS spectra for the four GAMT^{-/-} are plotted below and were fitted with the LCModel as detailed previously (4), showing negligible total Cr (1.47 ± 0.68 mM):

Supporting Information Figure S1: *in vivo* ¹H NMR spectra measured from regions of the midbrain in all four GAMT^{-/-} mice. The peak at 3 ppm of all GAMT^{-/-} mice shows a residual signal due to gamma aminobutyric acid (GABA) and mobile macromolecules (MM), while LCModel fitting give negligible Cr/PCr remaining.

Bloch-McConnell (BM) Fitting results:

Table S1: The T_{2app} , C_{MTC}^0 and C_{MTC}^1 obtained by the MTC background fitting for the CrCrossBSA phantom. $T_{1w}=1.8$ s, $T_{1MTC}=1$ s, $k_{MTC}=20$ s⁻¹ and $f_{MTC}=10\%$ were fixed during the global BM fitting.

B_I (μ T)	T_{2app} (s)	C_{MTC}^0 (s ⁻¹)	C_{MTC}^1 (10 ⁻⁶ s ⁻¹ /rad)
0.4	0.0585	0.677	19.3101
0.6	0.0605	0.775	11.623
0.8	0.0648	1.366	15.504
1.0	0.0732	2.482	42.6371
1.2	0.0862	4.143	86.290
1.4	0.0994	5.461	106.096
1.6	0.1089	7.073	139.772

Table S2: The T_{2app} , C_{MTC}^0 and C_{MTC}^1 obtained by the MTC and water background fitting for *in vivo* WT mice. $T_{1w}=1.8$ s, $T_{1MTC}=1$ s, $k_{MTC}=20$ s⁻¹ and $f_{MTC}=10\%$ were fixed during the global BM fitting.

B_I (μ T)	T_{2app} (s)	C_{MTC}^0 (s ⁻¹)	C_{MTC}^1 (10 ⁻⁶ s ⁻¹ /rad) f
0.4	0.0499	0.8106	24.920
0.5	0.0173	1.118	33.726
0.6	0.0285	1.803	51.731
0.8	0.0298	2.898	86.045
1.0	0.0303	4.119	118.428
1.2	0.0310	5.173	145.000
1.4	0.0309	6.173	170.193
1.6	0.0382	7.592	207.347

Table S3: The T_{2app} , C_{MTC}^0 and C_{MTC}^1 obtained by the MTC background fitting for *in vivo* GAMT^{-/-} mice. $T_{1w}=1.8$ s, $T_{1MTC}=1$ s, $k_{MTC}=20$ s⁻¹ and $f_{MTC}=10\%$ were fixed during the global BM fitting.

B_I (μ T)	T_{2app} (s)	C_{MTC}^0 (s ⁻¹)	C_{MTC}^1 (10 ⁻⁶ s ⁻¹ /rad)
0.4	0.0380	0.836	18.992
0.5	0.0575	1.695	51.852
0.6	0.0508	2.080	61.582
0.8	0.0475	2.653	65.883
1.0	0.0560	4.032	105.768
1.2	0.0601	4.893	119.264
1.4	0.0714	6.810	178.059
1.6	0.0795	8.370	225.996

Arg and Amide exchange rates in protamine:

Protamine has been used previously as model protein for an ArgCEST study (5). Therefore, the exchange rates of guanidinium protons were measured in the protamine solution for comparison with *in vivo* results. Several 50 mg/ml protamine (1.1×10^4 mM) solutions (Sigma-Aldrich) were prepared in phosphate buffered saline (PBS) and titrated to pH 6.4 ± 0.1 , 6.7 ± 0.1 , 7.0 ± 0.1 , and 7.3 ± 0.1 . The protamine solutions were studied in 5 mm NMR glass tubes at 37 °C at 17.6T. The exchange rates of the amide and ArgCEST in protamine were measured with selective inversion recovery method detailed in the main text method section. A list of inversion times (start = 0 ms, end = 60 ms, number of samples = 15) was used for the ArgCEST exchange rate measurement in protamine. The offsets (with respect to water) were set to 3.5 ppm for amideCEST and 2.0 ppm for ArgCEST.

17.6T NMR spectra of protamine for typical pH value are presented in Fig. S2, presenting both amide (3.5 ppm) and Guan (2.0 ppm) exchanging proton groups. Due to the abundant Arg residues in protamine (6), aromatic and other overlapping protons were assumed to be negligible. The Guan peak broadens significantly at pH 7.3, due to the increased exchange rate. Typical inversion recovery curves for the amide and Guan peaks are shown in Fig. S2b, together with the curves fitted using Eq. S1. The pH dependence of the exchange rates for the amide and Guan groups are presented in Fig. S2c. At pH 7.0, $k_{\text{amide}} = 112 \pm 5 \text{ s}^{-1}$ and $k_{\text{Guan}} = 743 \pm 20 \text{ s}^{-1}$ were determined. Beyond that, the guan exchange rate is difficult to determine due to the extremely high value, since its saturation/inversion in the selective inversion method will be an obstacle.

Supporting Information Figure S2: (a) The NMR spectra of protamine solution recorded with a WATERGATE sequence (TE = 1ms) at three pH values (6.7, 7.0 and 7.3). (b) The averaged

inversion recovery curves (n=3) of peaks at 2 ppm (Guan) and 3.5 ppm (amide) in protamine solution, respectively. The curves were fitted using a single exponential curve (Eq. 1). (c) pH dependence of the exchange rates for the ArgCEST and amideCEST peaks. All protamine experiments were performed at 37 °C.

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