Appendix 1

Average values and CNR of AREX(3.5 ppm) and AREX(2 ppm)

AREX(2 ppm) was calculated according to Eq. [2] from the main text, with reference signal as the opposite signal at –2 ppm, i.e., *Z*(–2 ppm). Supplementary *Figure S1* shows the average AREX values and contrast-to-noise ratio (CNR) of the AREX(3.5 ppm) and AREX(2 ppm) of all five animals. The left column shows the average AREX values of the ischemic and non-ischemic tissues, along with the corresponding standard deviations represented by the shaded regions. APT and amine effect were decreased in ischemic tissue, in line with previous study (28). The corresponding CNR between the ischemic and non-ischemic tissues are shown in the right column. Similar to NOE(–1.6 ppm), the AREX CNR of amide and amine were maximized at 276° flip angle (FA).

Previous studies have found the optimal saturation power for maximum NOE(–1.6) effect to be 0.5 μT in human (64) and 1.0 μT in rat brain (31) at 9.4 T. Although this may maximize the NOE(–1.6) effect observed in normal tissue, it may not necessarily produce the highest CNR in the diseased tissue, as shown in the results of the present study (*Figure 3* in the main text), equivalent average saturation powers used from approximately 0.5 to 1 μT. In the case of ischemic stroke, the optimal FA for maximum NOE(–1.6 ppm) CNR was found to be 276° (equivalent to 0.82 μT continuous wave saturation). The differences in the optimal power for the same effect may be due to the different subjects scanned, acquisition parameters and quantification methods used. During clinical investigation, clinicians usually evaluate the acquired or quantified images only, thus the CNR between the normal and ischemic tissue becomes more important than merely maximizing the signal intensity of the image. Other endogenous CEST effects—amide at 3.5 ppm and amine at 2 ppm also produced maximum AREX CNR at 276° (*Figure S1*). Thus, for the saturation scheme and quantification methods used here, the optimal FA for maximum CNR between ischemic and non-ischemic tissue for the endogenous CEST effects is around 276° (~0.82 μT). This is generally in agreement with the reported optimal power for low exchange rate protons, especially for NOE(–1.6 ppm) due to its close frequency separation with the water signal; higher power will lead to larger direct saturation and diminish the NOE signal (29,44).

Magnetization transfer ratio asymmetry at 3.5 ppm [MTRasym(3.5 ppm)]

As previously recommended (37), to ease the comparisons of existing and future studies, magnetization transfer ratio asymmetry at 3.5 ppm, MTR_{asym}(3.5 ppm) was calculated from animal CEST data as:

$$MTR_{asym}(3.5\,ppm) = Z(-3.5\,ppm) - Z(3.5\,ppm) \quad [1]$$

The relative MTR_{asym}(3.5 ppm), $rMTR_{asym}$(3.5 ppm) was then calculated as:

$$rMTR_{asym}(3.5\,\text{ppm}) = \frac{MTR_{asym}(3.5\,\text{ppm})}{\left|\text{mean}\left(MTR_{asym\_normal}(3.5\,\text{ppm})\right)\right|} \quad [2]$$

Figure S2 shows the MTR_{asym}(3.5 ppm) images of three representative animals (Animal 1–3) at the three FAs: 184°, 276°, and 366° The composite relative MTR_{asym}(3.5 ppm) within the ADC deficit and corresponding contralateral areas all five animals are shown in the bottom row. Two-tailed paired *t*-test revealed the relative MTR_{asym}(3.5 ppm) of all animals to be significantly decreased in the ADC deficit, in line with the majority of previous publications (37).

Simulation of NOE(–1.6 ppm) in ischemic and non-ischemic tissues

A six-pool CEST model consisting of water (0 ppm), amide (3.5 ppm), magnetization transfer (0 ppm), amine (2 ppm), and NOE effects at –3.5 and –1.6 ppm was simulated using the modified Bloch equations (65) in Matlabs. Field strength of 9.4 T and a saturation scheme of 50 Gaussian saturation pulses of 20 ms pulse duration and 50% duty cycle were used, in line with the experimental parameters. Four flip angles (FAs) were simulated: 92° (equivalent to 0.273 μT average power continuous wave saturation), 184°, 276°, and 366°.

CEST parameters used for the simulation for normal and ischemic conditions are presented in *Table S1*. The parameters were extracted from literature (57-62) with slight adjustments for field strength, except for the water longitudinal and transverse relaxations T_1 and T_2, which were the averaged values obtained from the *in vivo* experiment. The ischemic exchange rate of the NOE(–1.6) pool, *x* was varied between reduction by 30–50% of the normal value. Assumptions such as negligible changes in the proton concentrations were also made as the induced stroke

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was in the early time point (8).

From the simulated data, NOE(–1.6 ppm) was calculated according to equation (3) in the main text, where *Z*(19 ppm)/*Z*(3.5 ppm) was used as the reference, similar to the *in vivo* experiment. In addition, the contrast between the normal and ischemic NOE(–1.6 ppm) was evaluated using equation (4) in the main text.

When the exchange rate of NOE(–1.6) was decreased by 44% of the normal value (ischemic exchange rate =28 Hz) to simulate the ischemic tissue, it produced a decreased ischemic NOE(–1.6 ppm) (*Figure S3A*), in line with the experimental findings. While the simulated ischemic and non-ischemic NOE(–1.6 ppm) both increased with FA, similar to *in vivo*, the simulated values of both tissues were slightly lower than that of the experiment across all FAs. This could be due to the far away saturated offset (±300 ppm) treated as the unsaturated signal, slight differences in the water relaxation values and/or magnetization transfer parameters used. Likewise, the contrast of the simulated data was found to align well with the trend of the CNR of the experimental data, increasing from 92° to 276°, peaking at 276°, before decreasing at 366° FA (*Figure S3B*).

References

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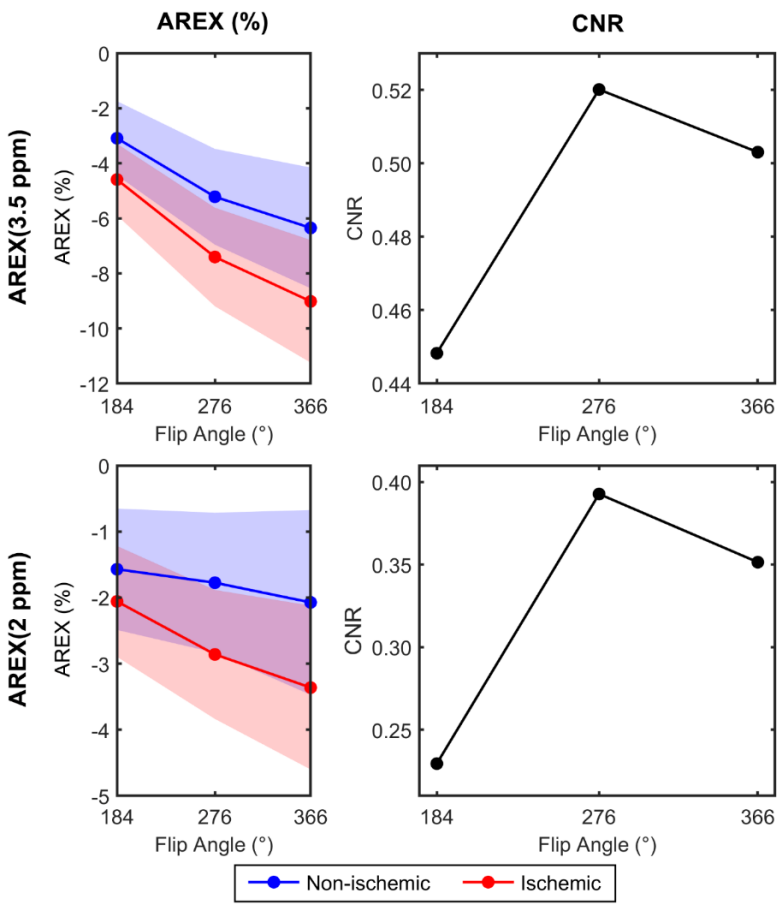

Figure S1 Average AREX values of ischemic and non-ischemic tissue, and the corresponding CNR of AREX(3.5 ppm) and AREX(2 ppm). The shaded region represents the standard deviations of the quantified AREX values. AREX, apparent exchange-dependent relaxation; CNR, contrast-to-noise ratio.

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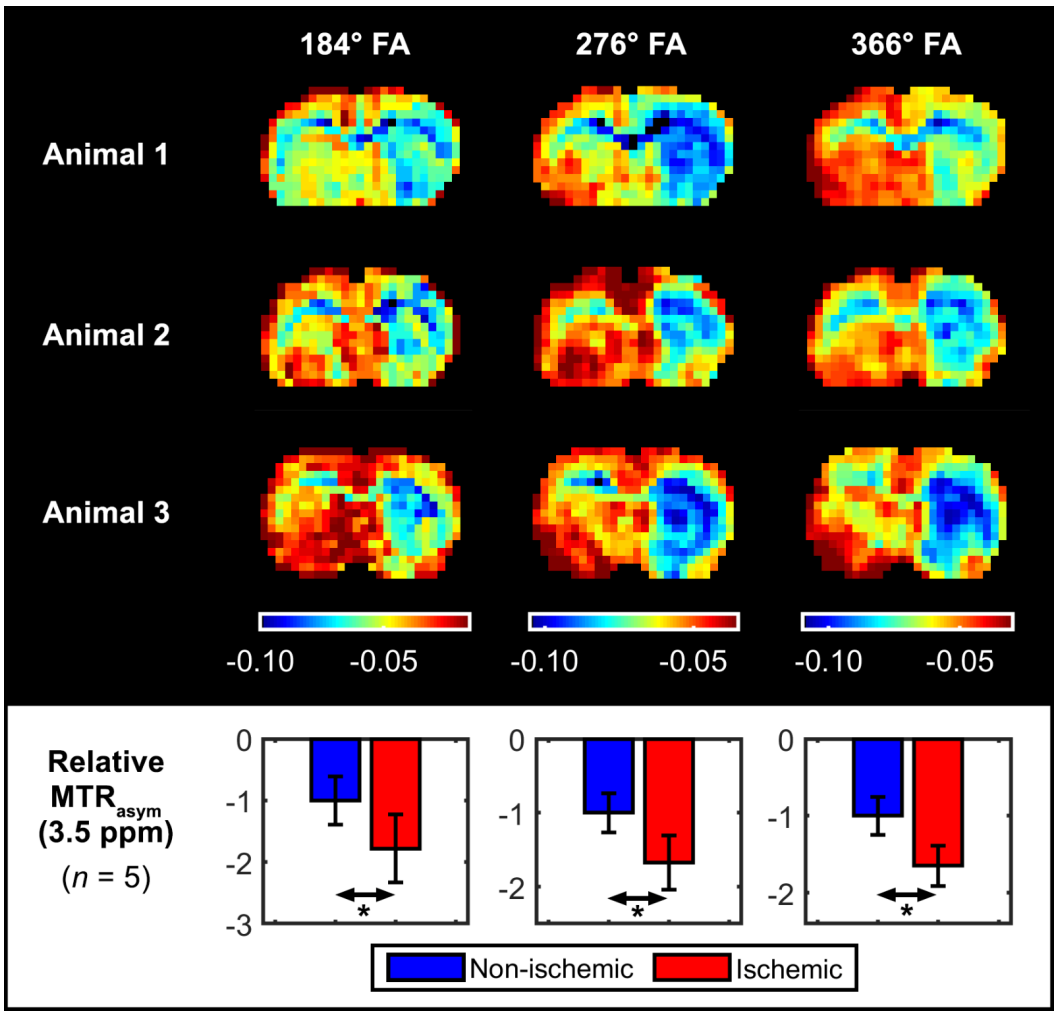

Figure S2 AThe MTRasym(3.5 ppm) maps of three representative animals (Animals 1–3) and the composite relative MTRasym(3.5 ppm) of all five animals at three saturation flip angles: 184°, 276°, and 366°. *, P<0.05. MTRasym, magnetization transfer ratio asymmetry; FA, flip angle.

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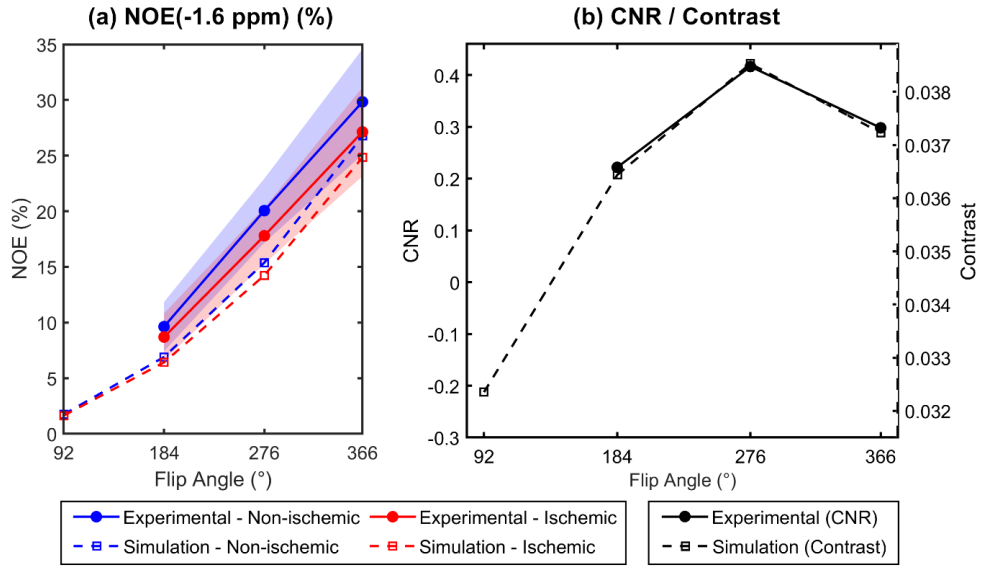

Figure S3 NOE(–1.6 ppm) and CNR/contrast of the simulated and experimental data. (A) Simulated NOE(–1.6 ppm) and average experimental NOE(–1.6 ppm) of ischemic and non-ischemic tissues; the shaded areas represents the standard deviations of the experimental NOE(–1.6 ppm). (B) CNR or contrast between the two tissues of experimental and simulated data respectively. NOE, nuclear Overhauser enhancement; CNR, contrast-to-noise ratio.

Table S1 Six-pool CEST data simulation parameters taken from literature (57-61)

CEST Pools	Water	Amide	MT	NOE	Amine	NOE
Chemical shift (ppm)	0	3.5	0	–3.5	2	–1.6
T1 (s)						
Normal	1.63	1.63	1.63	1.63	1.63	1.63
Ischemic	1.83	1.63	1.63	1.64	1.63	1.63
T2 (ms)						
Normal	40.16	20.00	0.02	0.40	38	0.4
Ischemic	38.68	20.00	0.02	0.40	38	0.4
Exchange rate (Hz)						
Normal	–	30	25	50	1,000	50
Ischemic	–	18	25	50	500	x*
Concentration (M_{0a})						
Normal	1	0.001	0.1	0.007	0.002	0.003
Ischemic	1	0.001	0.1	0.007	0.002	0.003

*, varied between 30–50% reduction from 50 Hz. CEST, chemical exchange saturation transfer; NOE, nuclear Overhauser enhancement; MT, magnetization transfer.

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