Supporting Information for

Free Base Porphyrins as CEST MRI Contrast Agents with Highly Upfield Shifted Labile Protons

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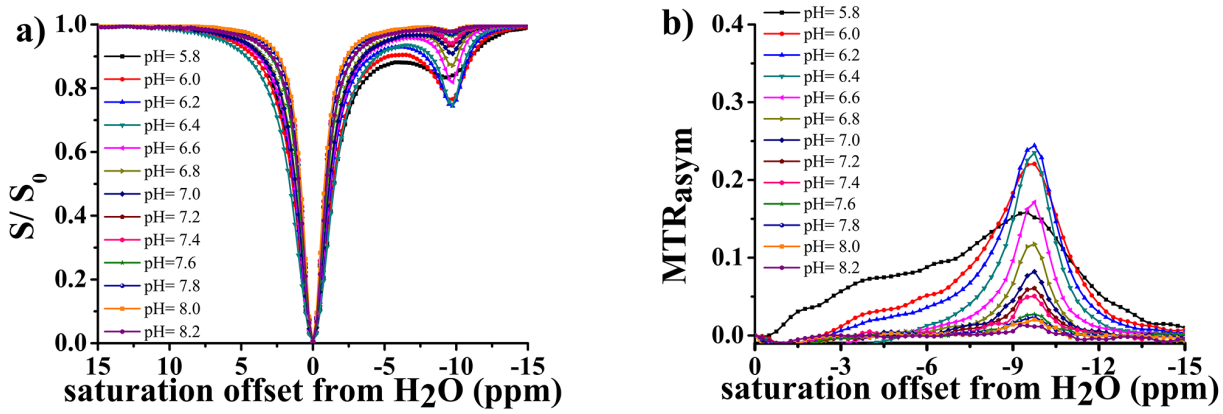
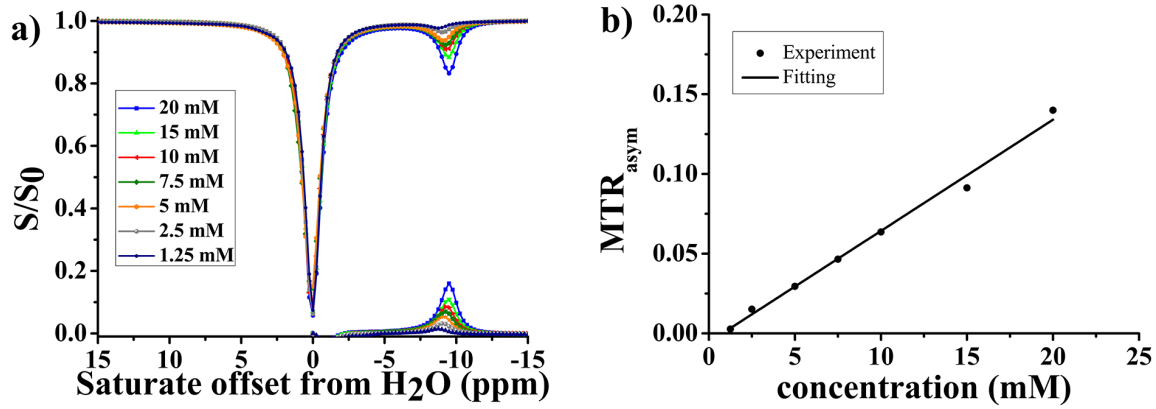
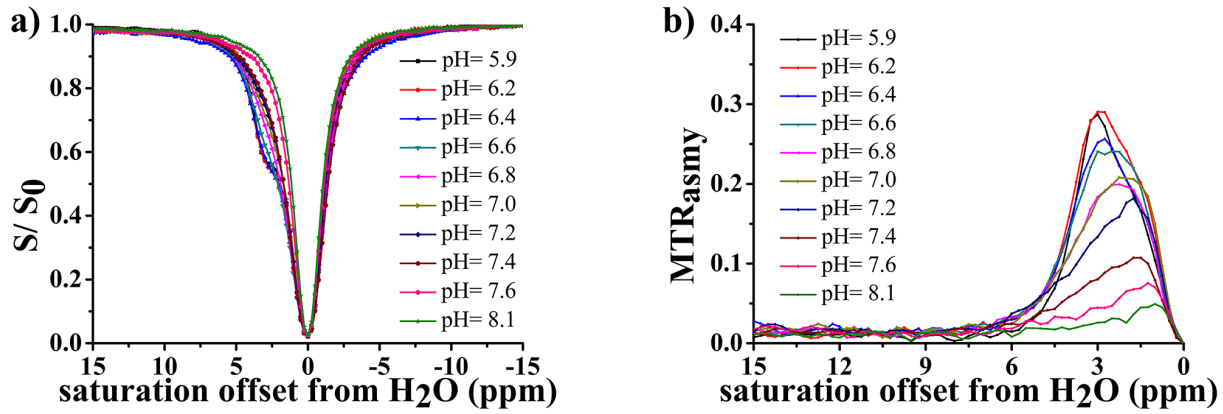


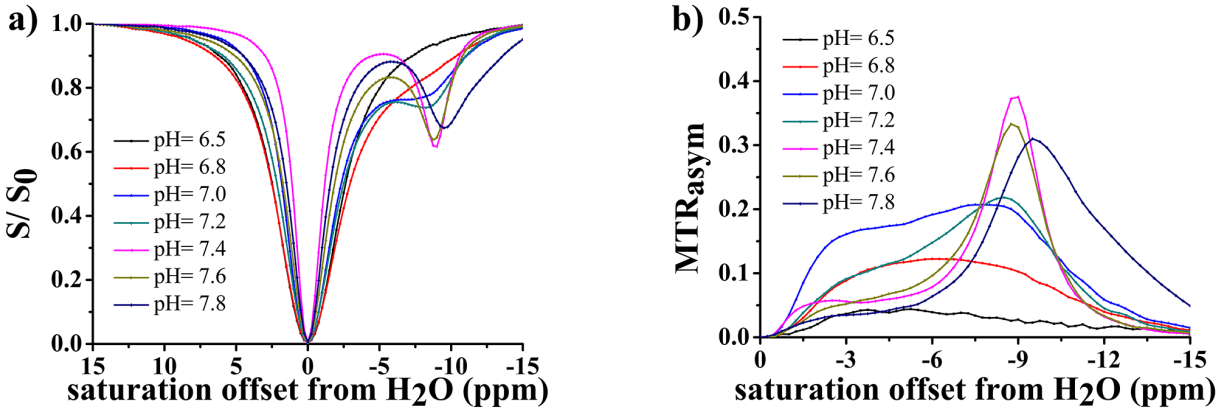
Supporting Information Figure S1 Influence of pH on the contrast of $TPPS_4$ (ω_1 = 5.4 μT, t_{sat}= 3 s, 12.5 mM).



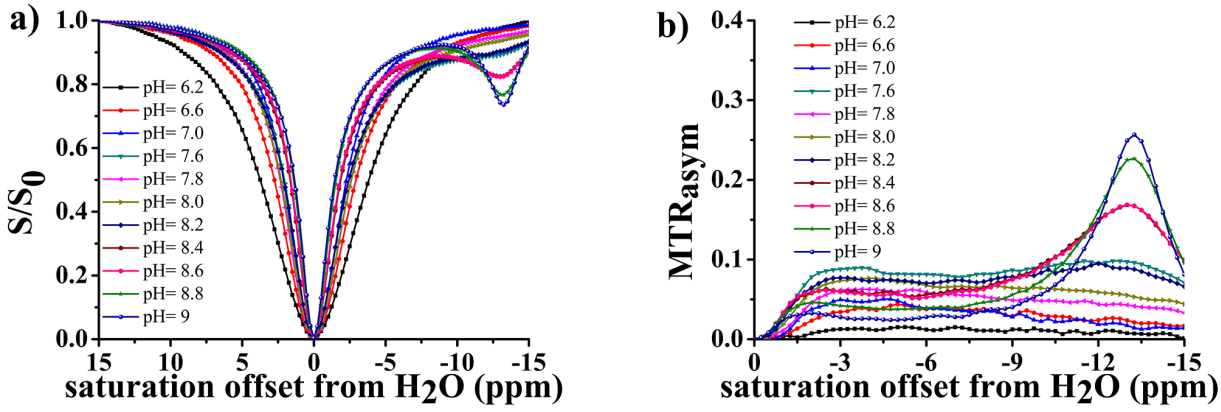
Supporting Information Figure S2 Influence of concentration on the contrast of $TPPS_4$. The concentration dependence of the contrast of $TPPS_4$ at pH 7.0-7.2 was measured at a saturation field strength (ω_1) = 5.4 μT. The Z-spectra and MTR_{asym} spectra at concentrations 0 mM,1.25 mM, 2.5 mM, 5 mM, 7.5 mM, 10 mM, 15 mM and 20 mM were collected and are shown below. 1.5% contrast was obtained at 2.5 mM.



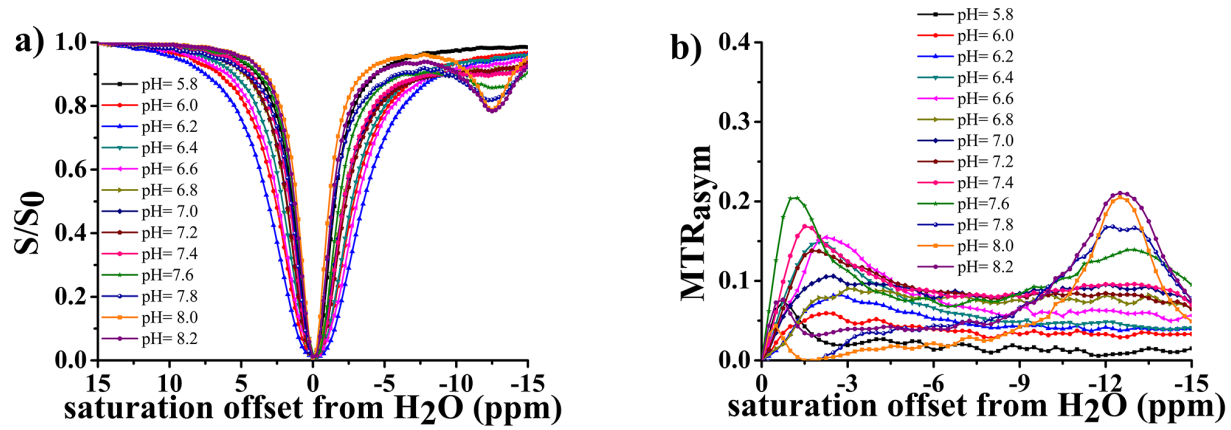
Supporting Information Figure S3 Influence of pH on the contrast of porphobilinogen (ω_1 = 5.4 µT, t_{sat}= 3 s, 12.5 mM).



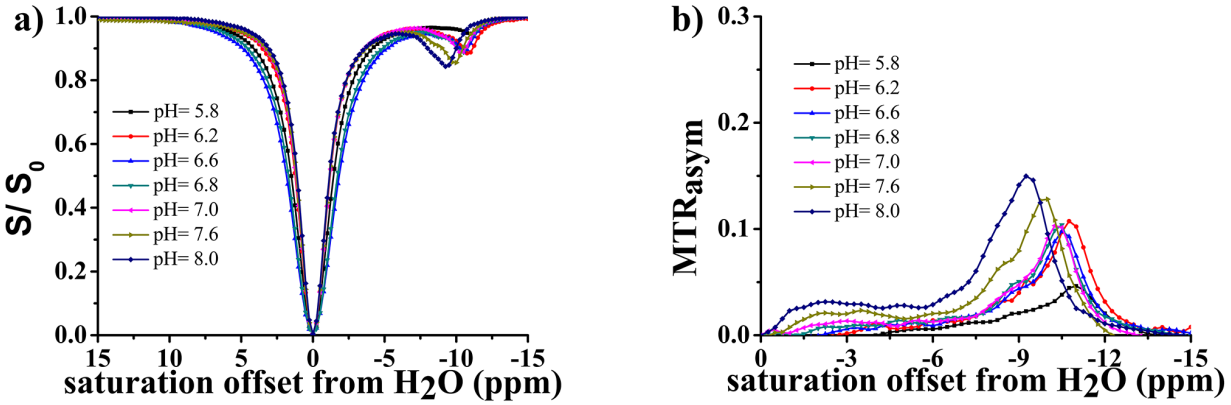
Supporting Information Figure S4 Influence of pH on the contrast of uroporphyrin I (ω_1 = 5.4 µT, t_{sat}= 3 s, 25 mM).



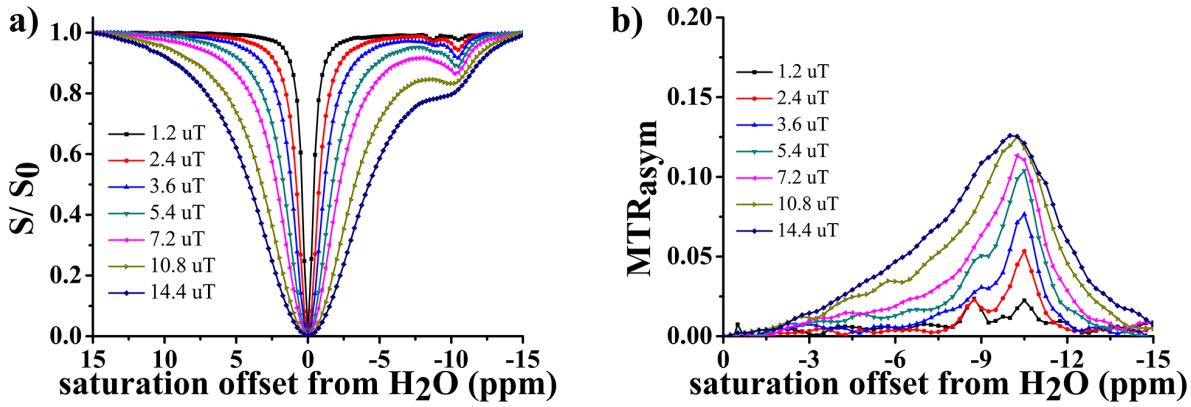
Supporting Information Figure S5 Influence of pH on the contrast of coproporphyrin I (ω_1 = 5.4 μT, t_{sat}= 3 s, 12.5 mM).



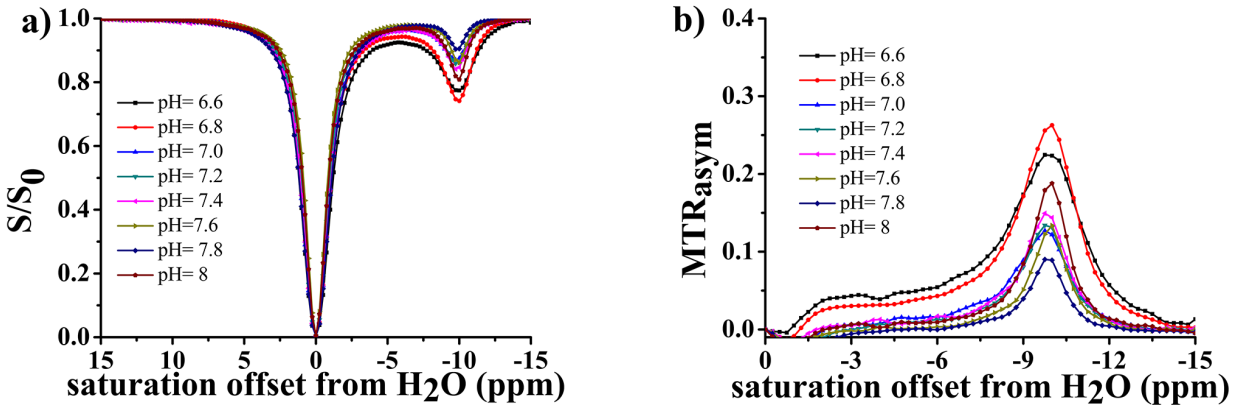
Supporting Information Figure S6 pH effect on the contrast of hematoprophyrin (ω_1 = 5.4 μT, t_{sat}= 3 s, 12.5 mM).



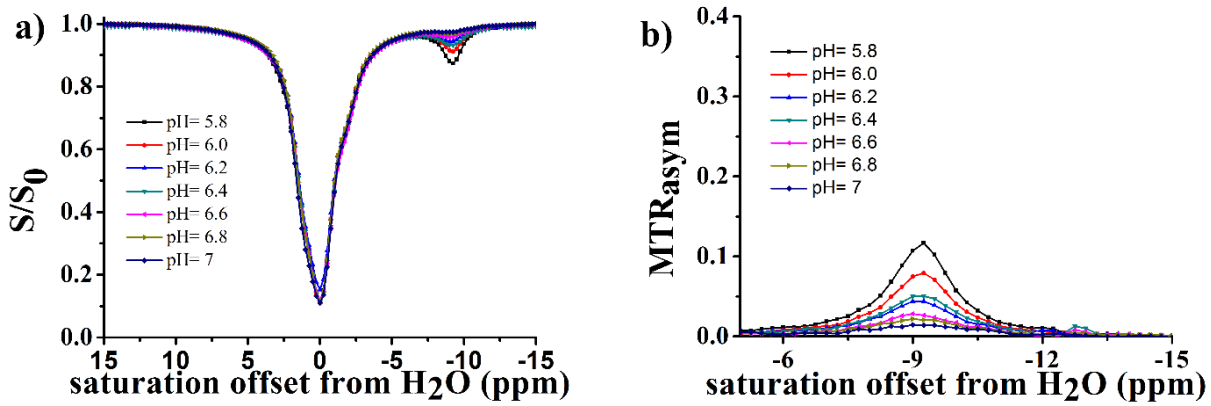
Supporting Information Figure S7 Influence of pH on the contrast of Chlorin e6 (ω_1 = 5.4 μT, t_{sat}= 3 s, 12.5 mM).



Supporting Information Figure S8 Z-spectra and MTR_{asym} of Chlorin e6 using different saturation field strengths. The saturation power dependence of the contrast of Ce6 at pH 7.0 was measured using different saturation field strength. The Z-spectra and MTR_{asym} spectra using ω_1 = 1.2 μT, 2.4 μT, 3.6 μT, 5.4 μT, 7.2 μT, 10.8 μT and 11.4 μT were collected and are shown below. Two peaks were observed at -8.75 ppm and -10.25 ppm when weak saturation power (ω_1 = 1.2 μT, 2.4 μT, 3.6 μT or 5.4 μT) was employed.



Supporting Information Figure S9 Influence of pH on the contrast of TCPP (ω_1 = 5.4 μT, t_{sat}= 3 s, 12.5 mM).



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47 **Supporting Information Figure S10** Influence of pH on the contrast of TGPP complex (ω_1 = 5.4 μT, t_{sat}=

48 3 s, 12.5 mM).

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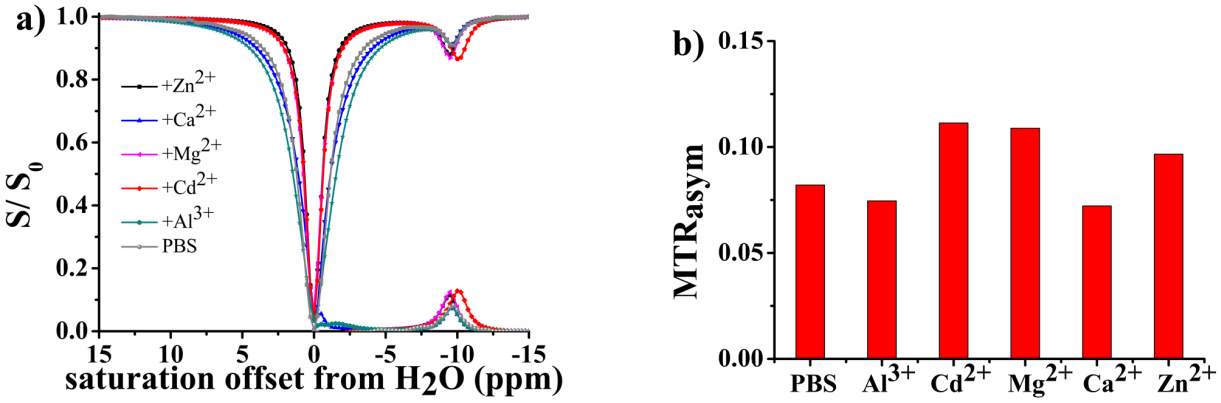

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52 **Supporting Information Figure S11** CEST contrast of $TPPS_4$ in the presence of various metal ions (1

53 eq.). The effect of metal ions on the contrast of $TPPS_4$ were tested at a concentration of 12.5 mM, pH= 7,

54 ω_1 = 5.4 μT. After simply mixed metal ions with $TPPS_4$ solution at 37 °C for 4 h under constant shaking

55 and titrated using high-concentration HCl/NaOH to neutral pH, the Z-spectra in presence of 1 eq. metal

56 salts include $ZnCl_2$, $CaCl_2$, $MgCl_2$, $CdCl_2$ and $AlCl_3$ were collected.

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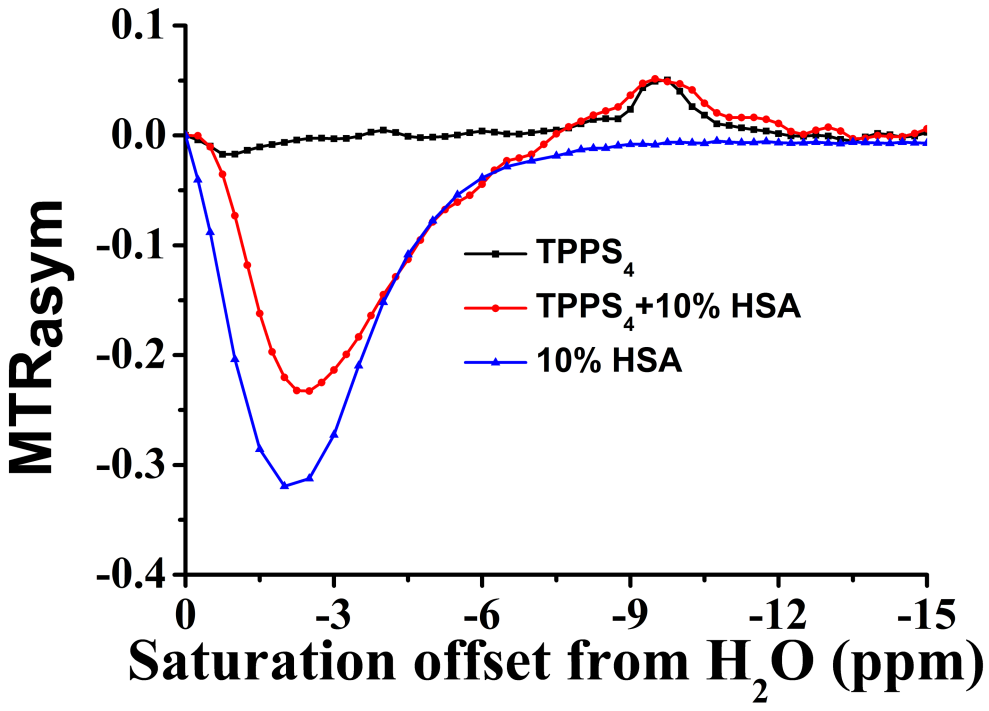

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59 **Supporting Information Figure S12** In vitro test of $TPPS_4$ in the presence of 10% HSA (pH=7.3, 37 °C,

60 5.4 μT, 3 s for saturation).

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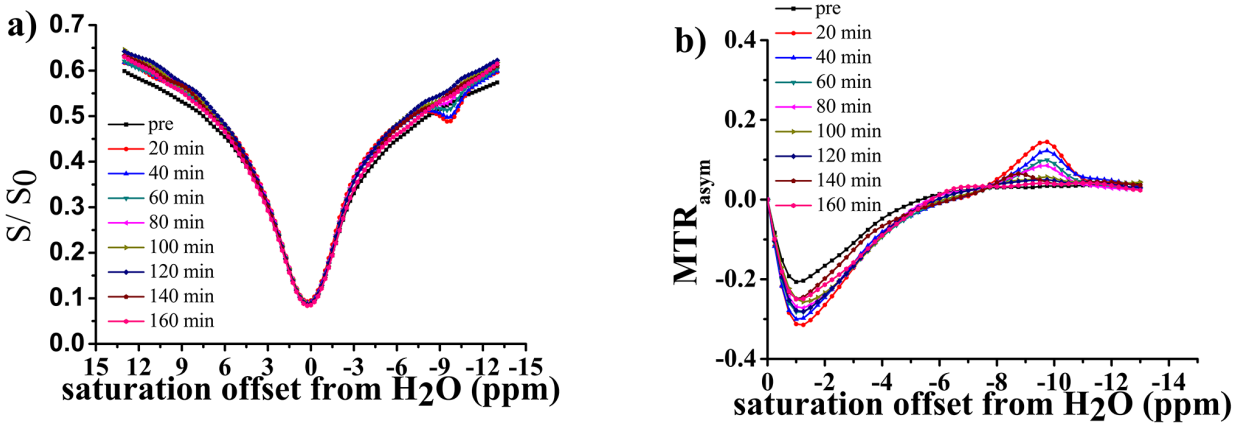

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63 **Supporting Information Figure S13** In vivo Z-spectra and MTR_{asym} spectra for the tumor of mouse 2

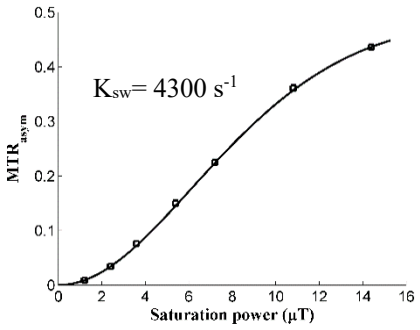
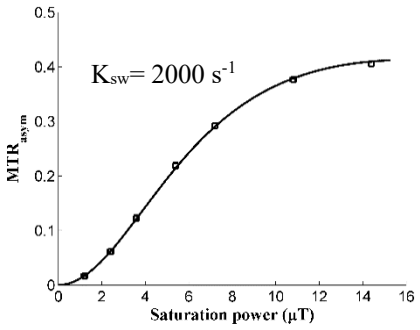
64 with data collected pre-injection and post injection. a) Z-spectra of tumor at different time points (pre, 20

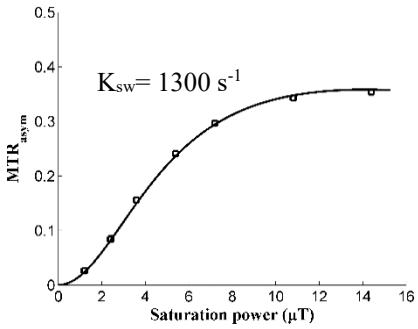
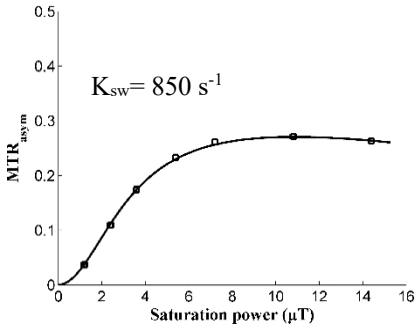
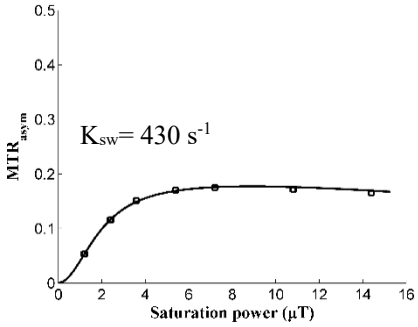
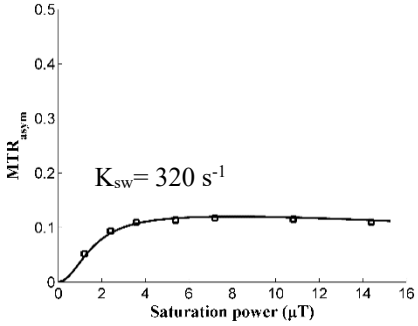
65 min, 40 min, 60 min, 80 min, 100 min, 120 min, 140 min and 160 min); b) MTR_{asym} of tumor at different

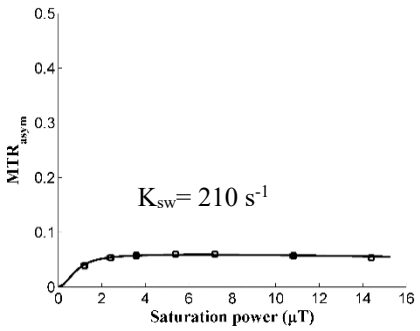
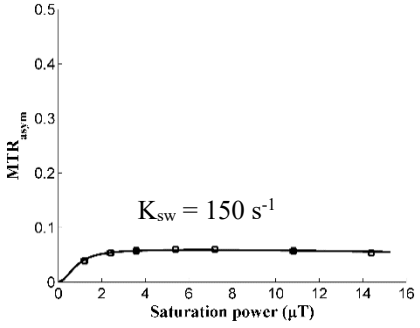
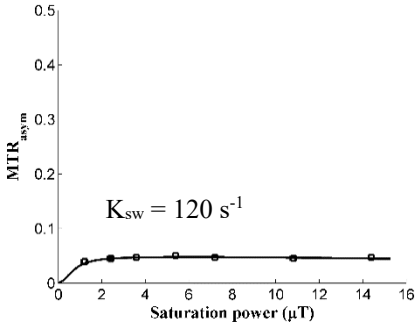
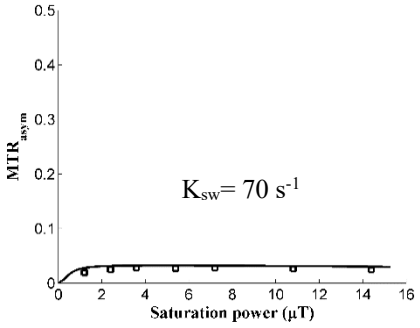
66 time points. For these experiments, 50 μl of 0.1 M $TPPS_4$ solution was injected into mouse through

Intratumoral (IT) injections. Before injection, the B_0 inhomogeneity was measured and corrected using the water saturation shift referencing approach. An 105-offset Z-spectrum (from 15 ppm to -15 ppm) was also acquired using saturation field strength of 3.6 μT. For the dynamic CEST contrast measurements, a series of whole Z-spectra and water saturation shift referencing experiments were acquired after injection. The CEST contrast map was calculated by $MTR_{asym}=[S(+\Delta\omega)–S(-\Delta\omega)]/S(+\Delta\omega)$. The images at every three adjacent time points were averaged to increase the contrast-noise-ratio. The maximum CEST contrast at -9.75 ppm was up to 14.5% after injection of $TPPS_4$ and markedly decreased over 3 hours. The contrast map before and after injection are showed in Supporting Information Table S8 and S9.

Supporting Information Table S1 Measured proton exchange rates of $TPPS_4$ at different pH values.

pH	QUESP fiting
pH= 5.8	
pH=6.0	

pH= 6.2	
pH= 6.4	
pH= 6.6	
pH= 6.8	

pH=7.0	
pH= 7.2	
pH= 7.4	
pH= 7.6	

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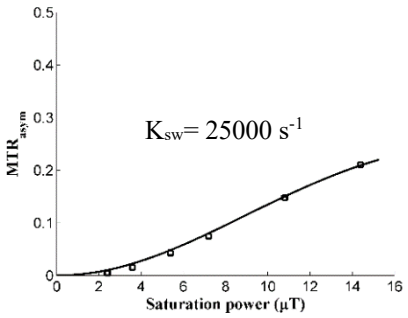
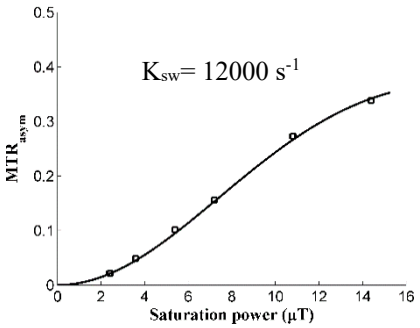
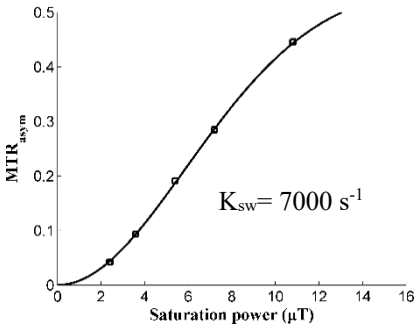
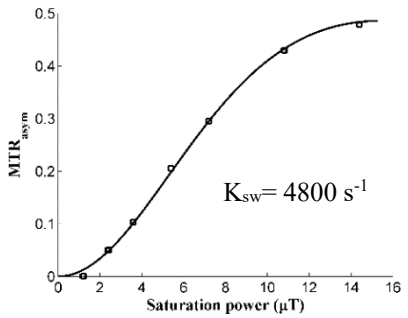
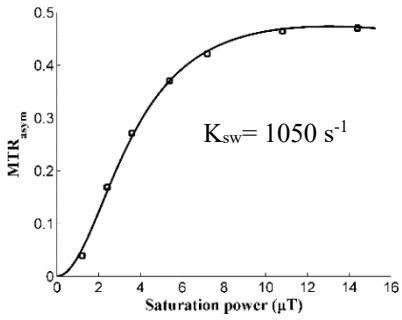
Supporting Information Table S2 Measured proton exchange rates of uroporphyrin I at different pH values.

compound	QUESP fiting
pH= 6.6	
pH=6.8	
pH= 7.0	
pH= 7.2	

pH= 7.4	

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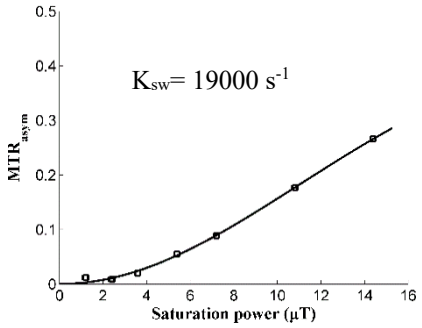
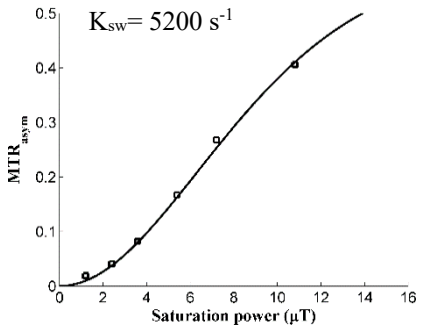
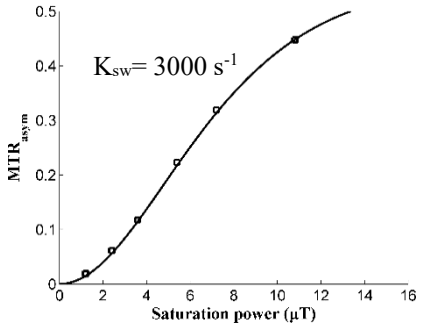
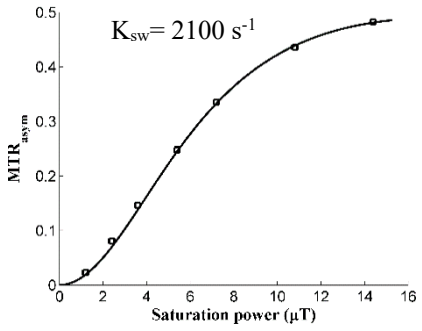
84 **Supporting Information Table S3** Measured proton exchange rates of coproporphyrin I at different pH.

pH	QUESP fiting
pH= 8.0	
pH=8.4	
pH= 8.8	

pH= 9.0	

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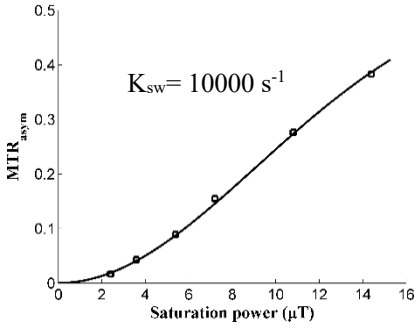
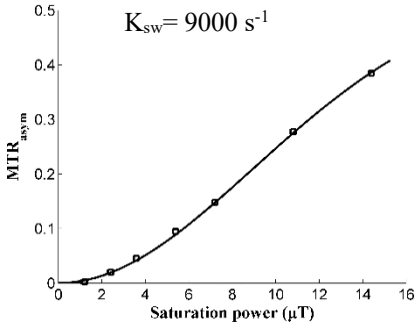
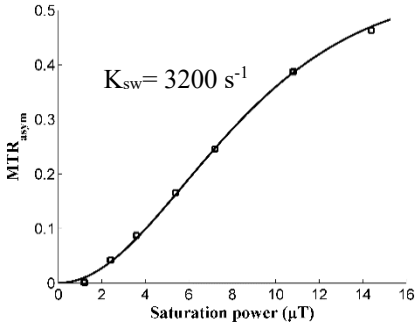
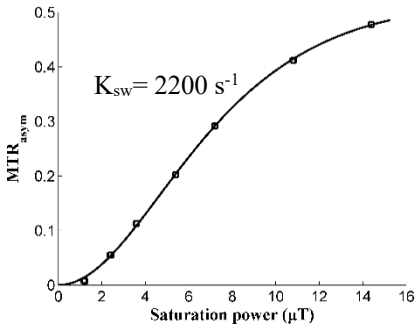
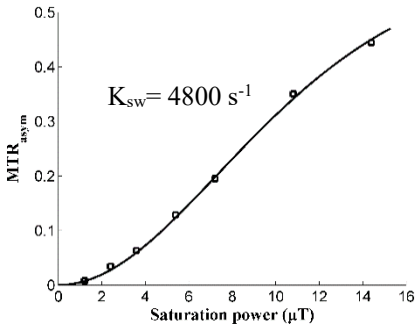
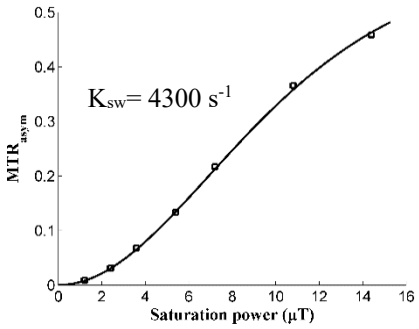
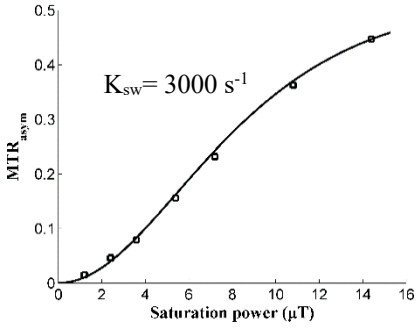
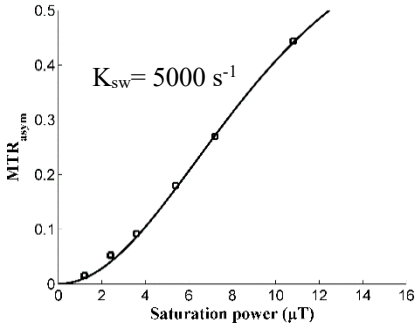
88 **Supporting Information Table S4** Measured proton exchange rates of hematoporphyrin at different pH.

pH	QUESP fiting
pH= 7.0	
pH=7.4	
pH= 7.8	

pH= 8.2	
pH= 8.6	
pH= 9.0	
pH= 9.4	

pH= 9.8	

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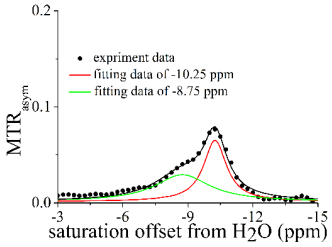
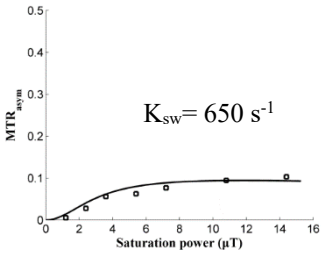
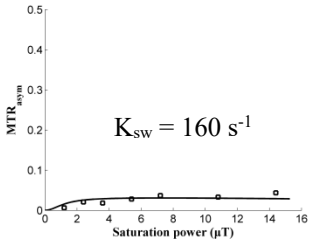
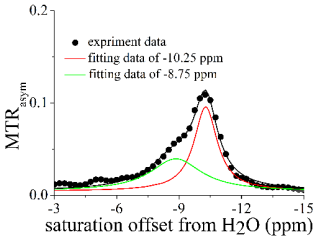
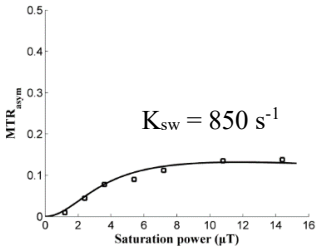
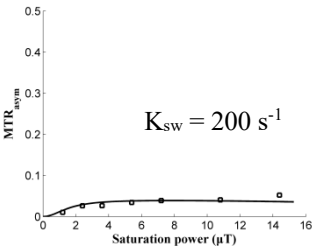
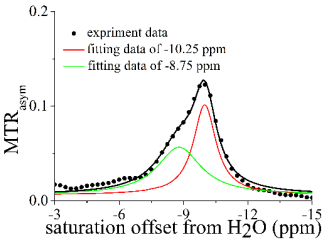
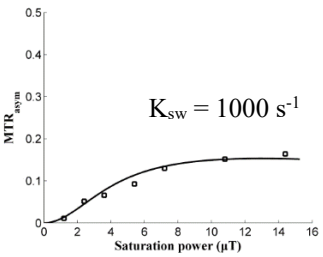
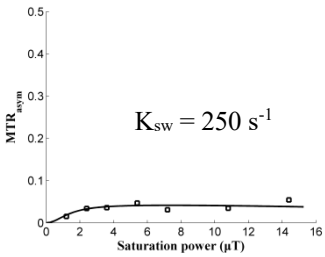
91 **Supporting Information Table S5** Measured proton exchange rates of Ce6 at different pH.

pH		Peak at -10.25 ppm	Peak at -8.75 ppm
6.6			
7			
7.4			

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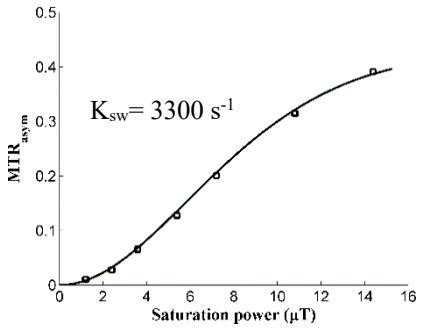
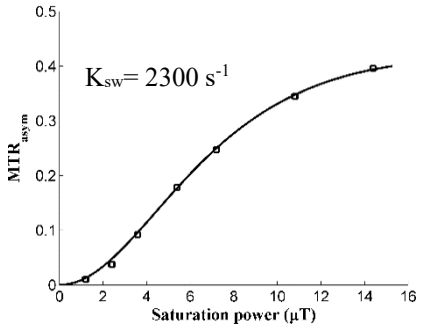
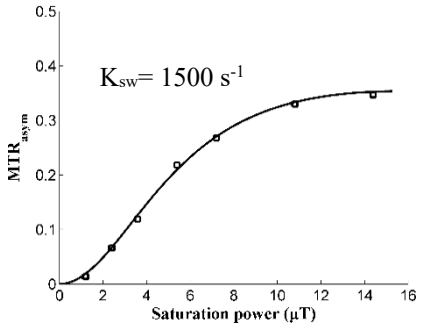
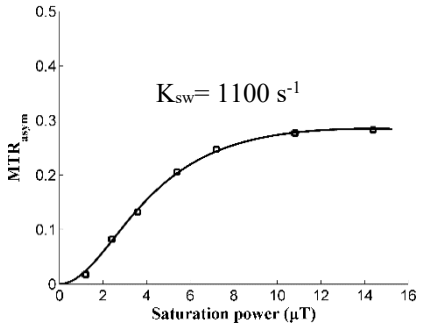
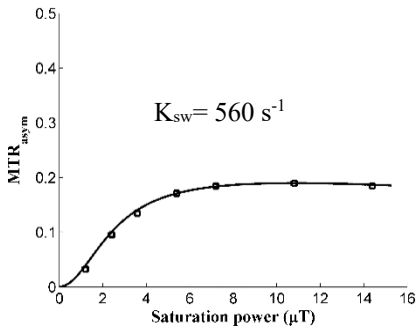
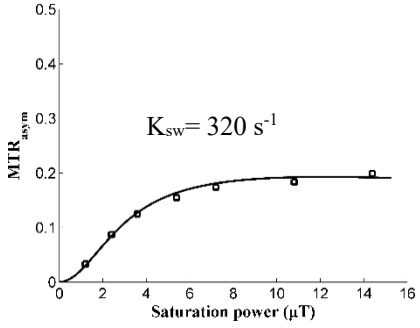
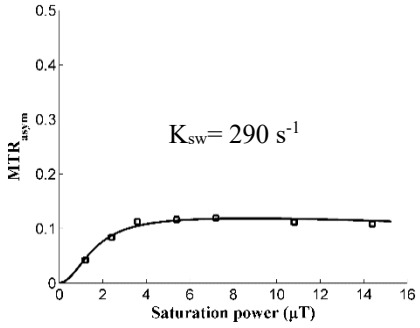
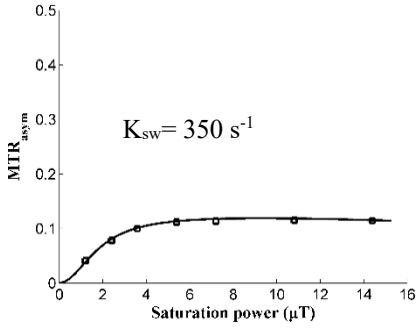
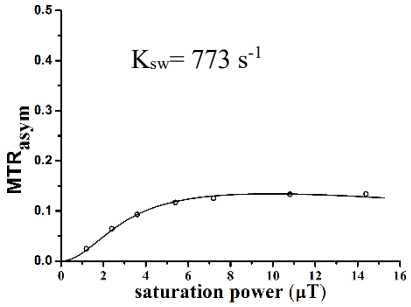
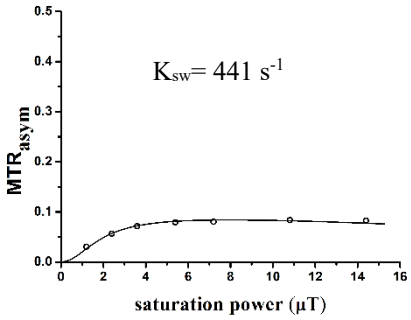
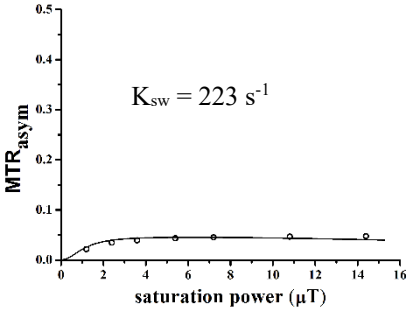
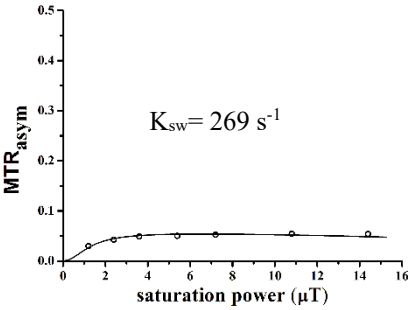
94 **Supporting Information Table S6** Measured proton exchange rates of TCPP at different pH.

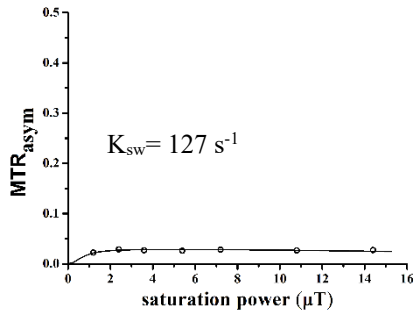
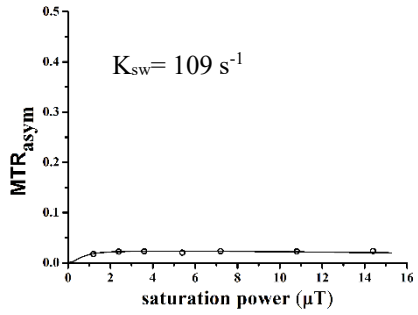
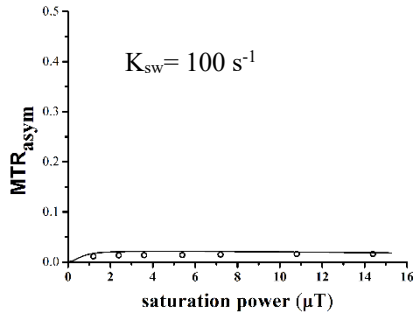
pH	QUESP fiting

pH= 6.6	
pH=6.8	
pH= 7.0	
pH= 7.2	

pH= 7.4	
pH= 7.6	
pH=7.8	
pH= 8.0	

Supporting Information Table S7 Measured proton exchange rates of TGPP at different pH.

pH	QUESP fiting
pH= 5.8	
pH=6.0	
pH= 6.2	
pH= 6.4	

pH= 6.6	
pH= 6.8	
pH= 7.0	

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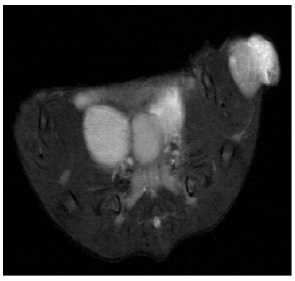
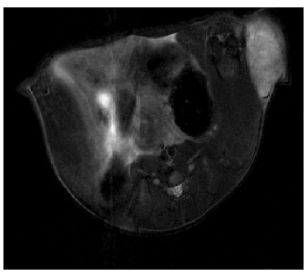
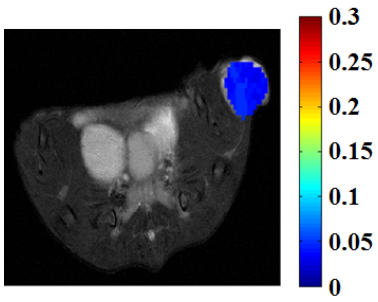
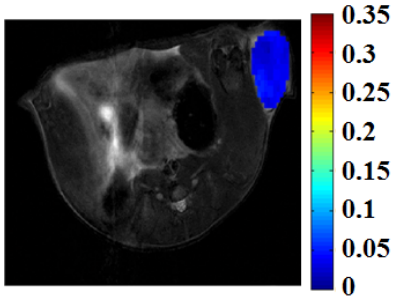
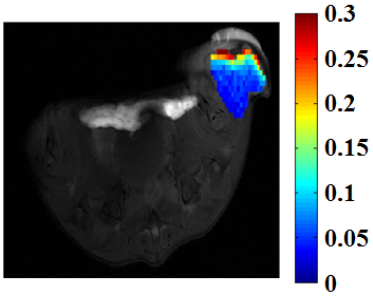
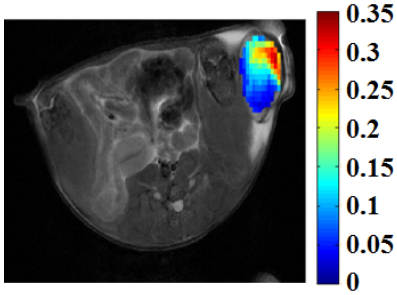
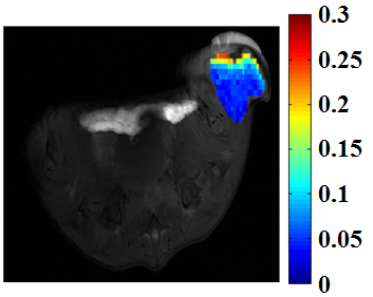
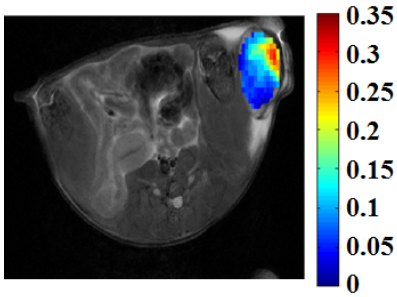
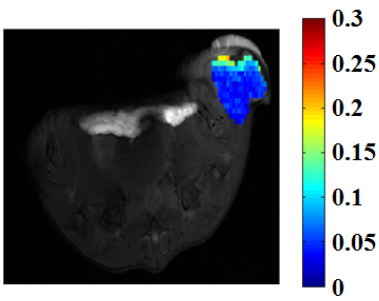
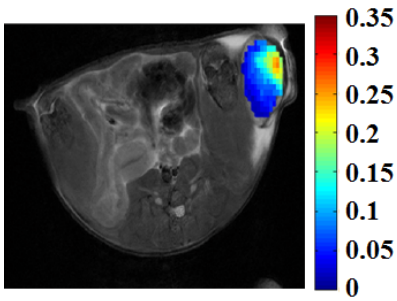
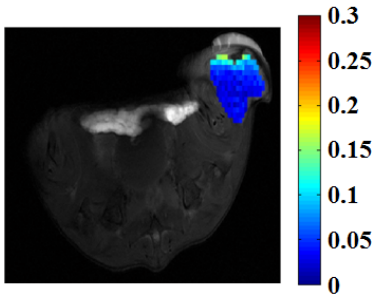
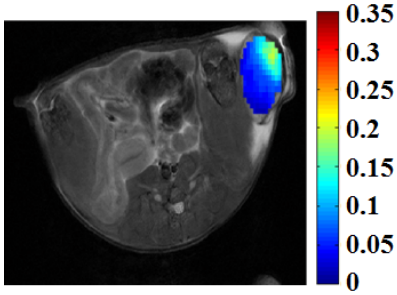
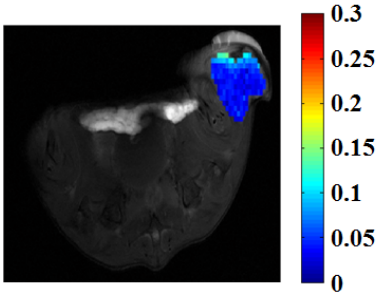
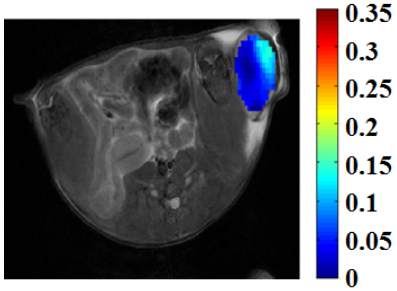
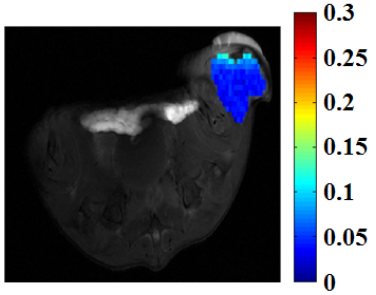
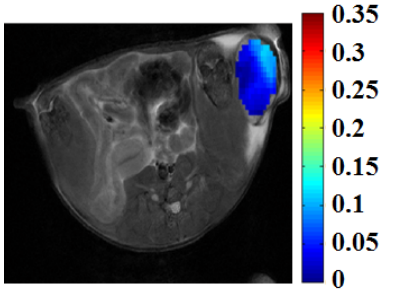
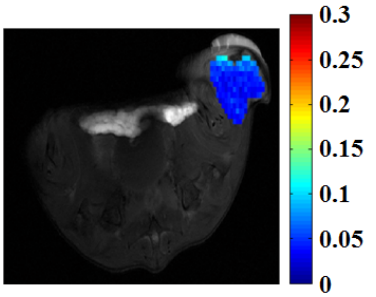
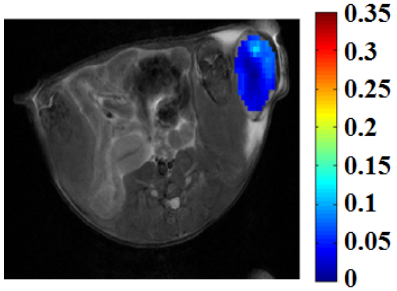
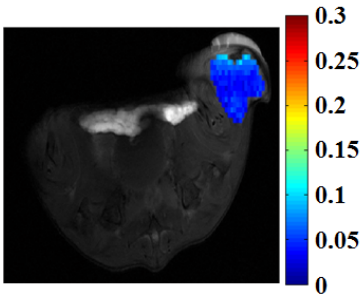
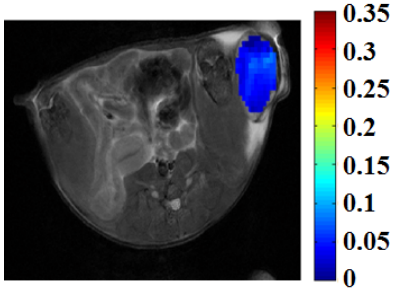
01
02

03 **Supporting Information Table S8** T_{2w} map and CEST contrast map of Mouse 1 and Mouse 2.

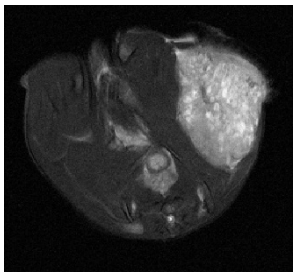
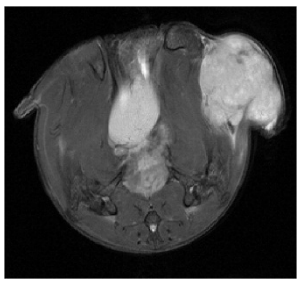
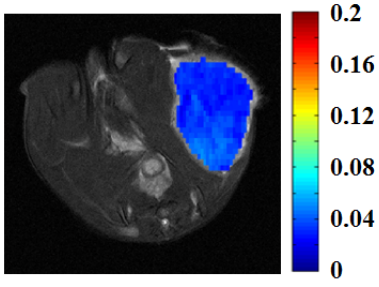
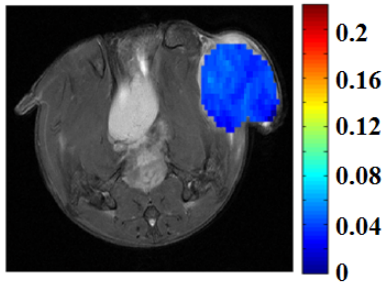
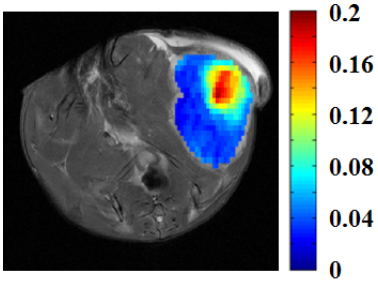
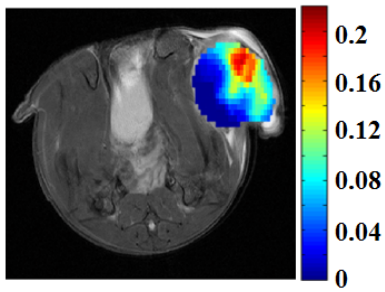
	Mouse 1	Mouse 2
T_{2w} map		

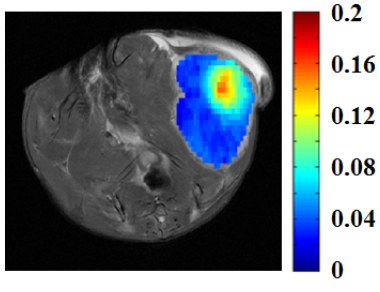
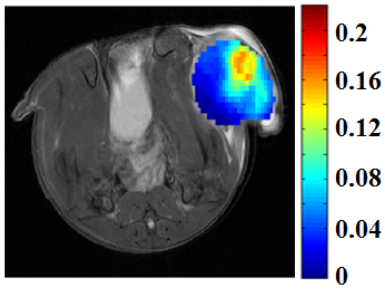
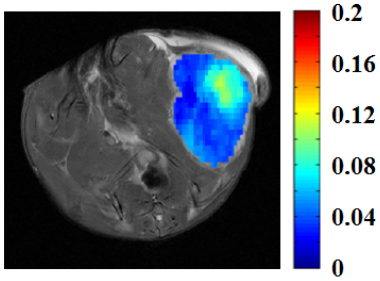
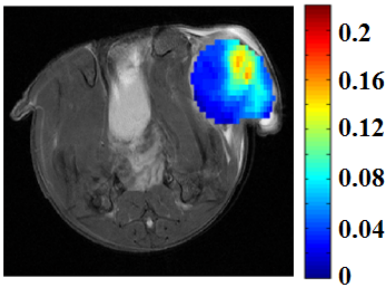
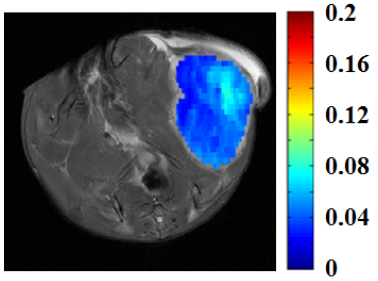
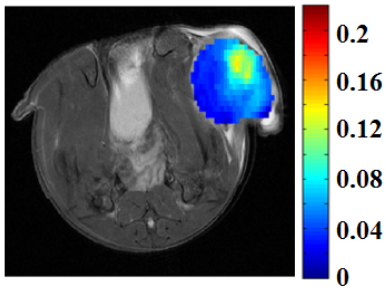
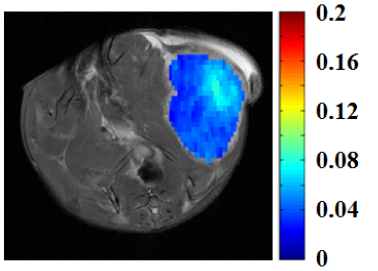
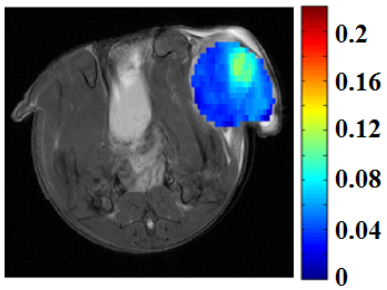
Pre-injection		
20 min		
40 min		
60 min		

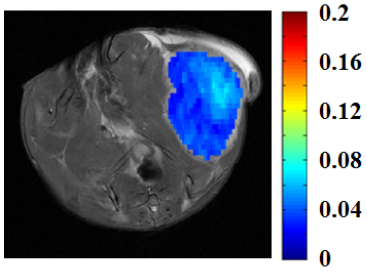
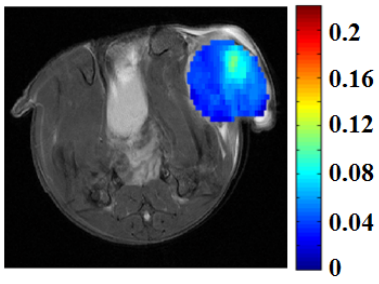
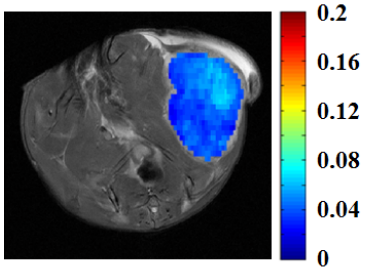
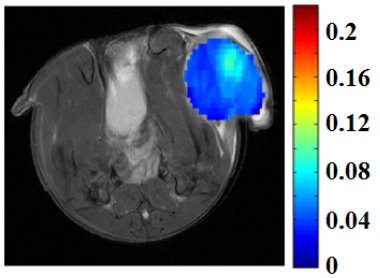
80 min		
100 min		
120 min		
140 min		

160 min		

Supporting Information Table S9 T_{2w} map and CEST contrast map of Mouse 3 and Mouse 4.

	Mouse 3	Mouse 4
T_{2w} map		
Pre-injection		
20 min		

40 min		
60 min		
80 min		
100 min		

120 min		
140 min		
160 min