Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supplementary Information

TEMPO-CONJUGATED TOBACCO MOSAIC VIRUS AS A MAGNETIC RESONANCE IMAGING CONTRAST AGENT FOR DETECTION OF SUPEROXIDE PRODUCTION IN THE INFLAMED LIVER

Jenica L. Lumata[1*], Laurel M. Hagge[1,2*], Miguel A. Gaspar[1], Ikeda Trashi[1], Ryanne N. Ehrman[1], Shailendra Koirala[1], Alyssa C. Chiev[1], Yalini H. Wijesundara[1], Cary B. Darwin[1], Salvador Pena[2], Xiaodong Wen[2], Janaka Wansapura[2], Steven O. Nielsen[1], Zoltan Kovacs[2], Lloyd L. Lumata[2,3], and Jeremiah J. Gassensmith[1,2,4,]

[1]Department of Chemistry and Biochemistry, The University of Texas at Dallas
[2]Advanced Imaging Research Center, University of Texas Southwestern Medical Center
[3]Department of Physics, The University of Texas at Dallas
[4]Department of Bioengineering, The University of Texas at Dallas
Email: gassensmith@utdallas.edu
*Authors contributed equally

Table of Contents

1. Materials

Chemicals were procured from various sources, including Sigma-Aldrich (St. Louis, MO), ThermoFisher Scientific (Pittsburgh, PA), Agdia (Elkhart, IN), ChemImpex (Wood Dale, IL), Alfa Aesar (Ward Hill, MA), and TCI America (Portland, OR). These chemicals were used as obtained without requiring further purification.

2. Instrumentation

Liquid Chromatography-Mass Spectrometry (LC-MS) analyses were conducted using an Agilent 1100 High-Performance Liquid Chromatography (HPLC) system equipped with a PLRP-S column for separation and an AB Sciex 4000 QTRAP system for detection. Gel images were obtained using the Bio-Rad ChemiDoc MP imaging system.

3. Expression of nTMV and kTMV

Tobacco plants (*Nicotiana benthamiana*) were cultivated for 8 weeks and subsequently infected with 0.01 mg/mL TMV and incubated for 14 days. Infected leaves were stored at -80°C. Approximately 100 g of these leaves were blended with 3 volumes of cold (4 °C) extraction buffer, consisting of 0.1 M, pH 7.4, and β-mercaptoethanol (0.2% (v/v)). The filtrate was then centrifuged at 11,000 ×g (4 °C, 20 min). The obtained supernatant was collected and an equivalent volume of chloroform/1-butanol solution (in a 1:1 ratio) was added and placed in ice for 30 min. Then, the mixture was centrifuged at 4500 ×g for 10 min, and the aqueous phase was collected. This solution was then supplemented with 0.2 M NaCl, PEG 8000 (8% (w/w)), and Triton X-100 surfactant (1% (w/w)), stirred in ice for 30 min and stored at 4 °C for 1 hour. Following this, the mixture was centrifuged at

22,000 ×g (4 °C, 15 min), and the resulting pellet was collected and resuspended in KP buffer (0.1 M, pH 7.4). The suspension was gently layered onto a 40% (w/v) sucrose gradient in KP buffer (0.01 M, pH 7.4) and centrifuged using a swing bucket rotor at 96,000 ×g for 2 hours. The blue band was carefully collected with the assistance of LED light shining upwards from the bottom of the centrifuge tube. The colloidal suspension from the blue band was subjected to another centrifugation at 360,562 ×g for 1.5 hours. The resulting pellet was resuspended in KP buffer (0.01 M, pH 7.4).

4. MD modeling of TMV with nTMV-TEMPO and kTMV-TEMPO

To build the model, we began with the 2tmv.pdb entry from the RCSB Protein Data Bank, which includes a section of the RNA strand, one protein molecule, one calcium counterion, and 74 crystallographic water molecules. This structure was replicated using the provided BIOMT transformations to yield the unit cell comprising 49 proteins with a length of 69.0 Å along the TMV cylinder axis. The RNA strand is bonded to itself using 3d periodic boundary conditions, yielding an infinitely long TMV nanorod. For the nTMV-TEMPO system, all 49 protein molecules had their Y139 residue modified to incorporate the TEMPO molecule. For the kTMV-TEMPO system, all 49 protein molecules had their T158 residue modified to K and further modified to incorporate the TEMPO molecule. In both systems, the inner TMV cavity is solvated with water molecules. Water is also added on the outside of the TMV cylinder. Sodium and chloride ions are introduced into the water at an ionic strength of 0.2 M. The resulting simulation unit cell is of dimensions 210 by 210 by 69 Angstroms cubed and comprises 313321 and 312283 atoms for the nTMV-TEMPO and kTMV-TEMPO systems, respectively. These systems were simulated using the NAMD molecular dynamics software package for 40 ns. The CHARMM all-atom force

field was used for the RNA, proteins, water, and ions. The TEMPO parameters are from Sezer et al.[1, 2] were supplemented, where needed, by the CGenFF force field using the online server at https://cgenff.paramchem.org.[3] As a reference for the electric field calculation, the TEMPO molecule including the ammonium nitrogen near the TEMPO ring was simulated in water at an ionic strength of 0.2 M.

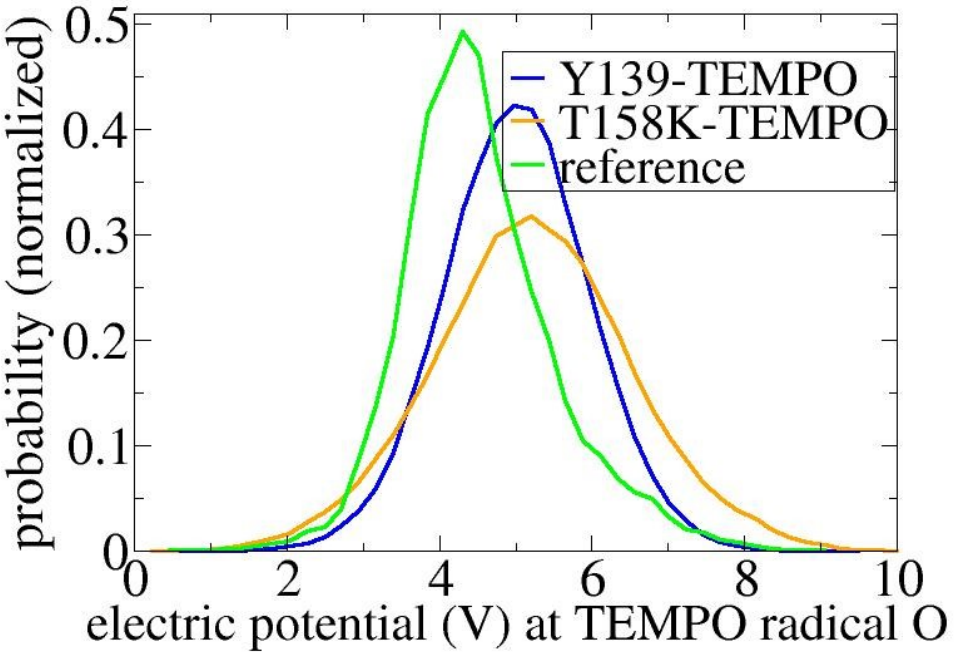


Figure S1. The distribution of the electric potential at the TEMPO radical oxygen atom position (not including this atom). This distribution includes all 40 ns of the MD simulations and all 49 TEMPO molecules in the unit cell. For the reference system there is one TEMPO molecule in water at 0.2 M ionic strength. The electric potential is converged with respect to the periodic images used in the MD simulations.

The distribution of distances between the TEMPO radical oxygen atom and the closest atom in the TMV, not including the residue to which TEMPO is attached, is shown in Figure S2. This distribution includes all 40 ns of the MD simulations and all 49 TEMPO molecules in the unit cell. Both TEMPO molecules undergo repeated straightening and

bending conformational changes, exposing them to water and bringing them into contact with other TMV protein residues, respectively. However, in the kTMV-TEMPO system, the TEMPO molecules are more exposed. In particular, the TEMPO radical oxygen atom is located more than 1 nm away from the TMV surface 2% of the time for the nTMV-TEMPO system and 16% of the time for the kTMV-TEMPO system.

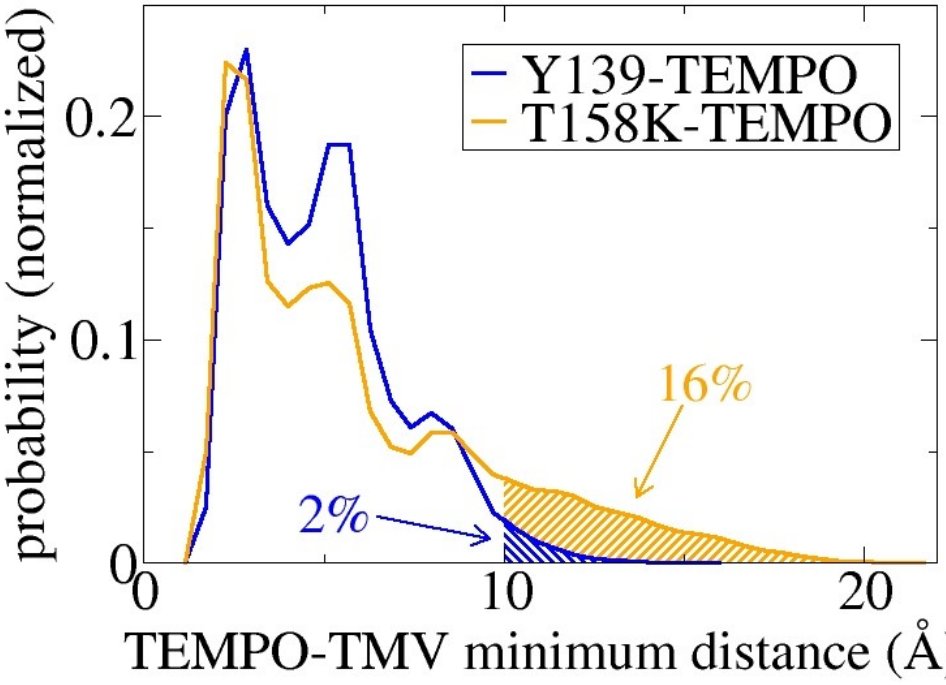
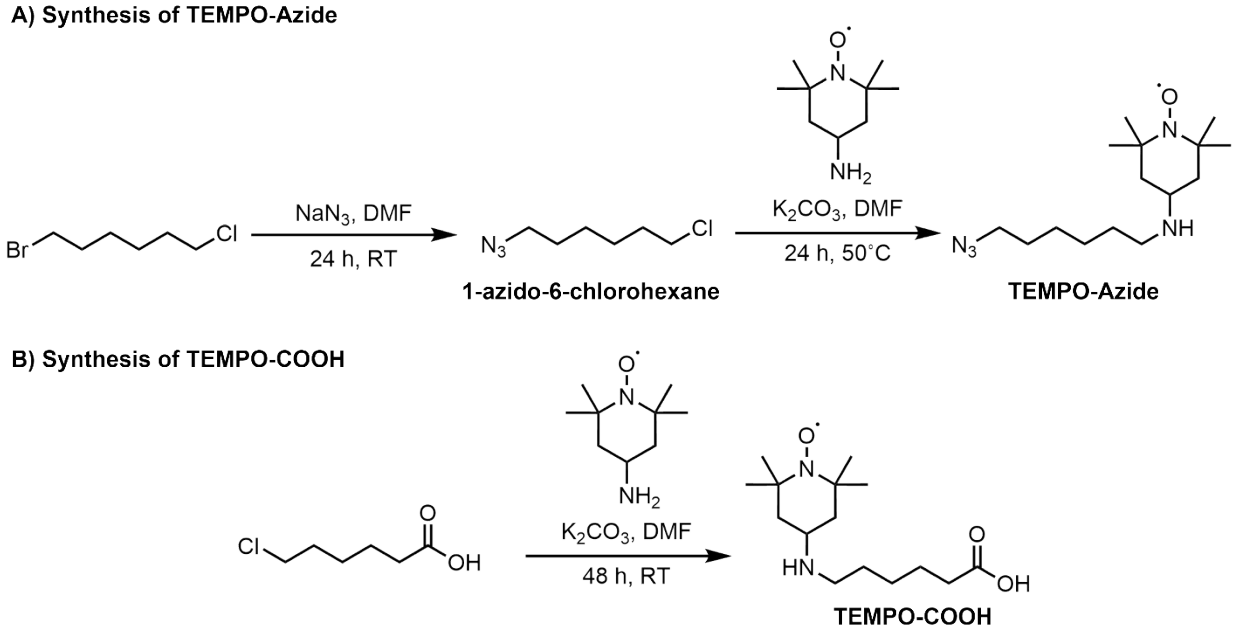


Figure S2. The normalized distribution of distances between the TEMPO radical oxygen atom and the closest atom in the TMV not including the residue to which TEMPO is attached. This distribution includes all 40 ns of the MD simulations and all 49 TEMPO molecules in the unit cell.

5. Synthetic Methods



Scheme S1 A) The synthesis of TEMPO-Azide that is required for the bioconjugation on nTMV to produce nTMV-TEMPO. B) The synthesis of TEMPO-COOH that is required for the bioconjugation on kTMV to produce kTMV-TEMPO.

5.1 SYNTHESIS OF 1-AZIDO-6-CHLOROHEXANE

Bromo-6-chlorohexane (3.99 g, 20.0 mmol) and sodium azide (1.30 g, 20.0 mmol) were dissolved in DMF (25.0 mL) and stirred for 24 hours at room temperature. Then, water (25.0 mL) was added, and the product was extracted with DCM (40.0 mL× 3). The organic phase was combined and washed with water three times. Then, the organic phase was dried with $MgSO_4$. The solvent was removed under reduced pressure. See scheme S1A. ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 1.41 (q, J = 8.4 Hz, 2H), 1.47 (q, J = 7.7 Hz, 2H), 1.61 (q, J = 7.2 Hz, 2H), 1.79 (p, J = 6.8 Hz, 2H), 3.28 (t, J = 6.9 Hz, 2H), 3.54 (t, J = 6.6 Hz, 2H).

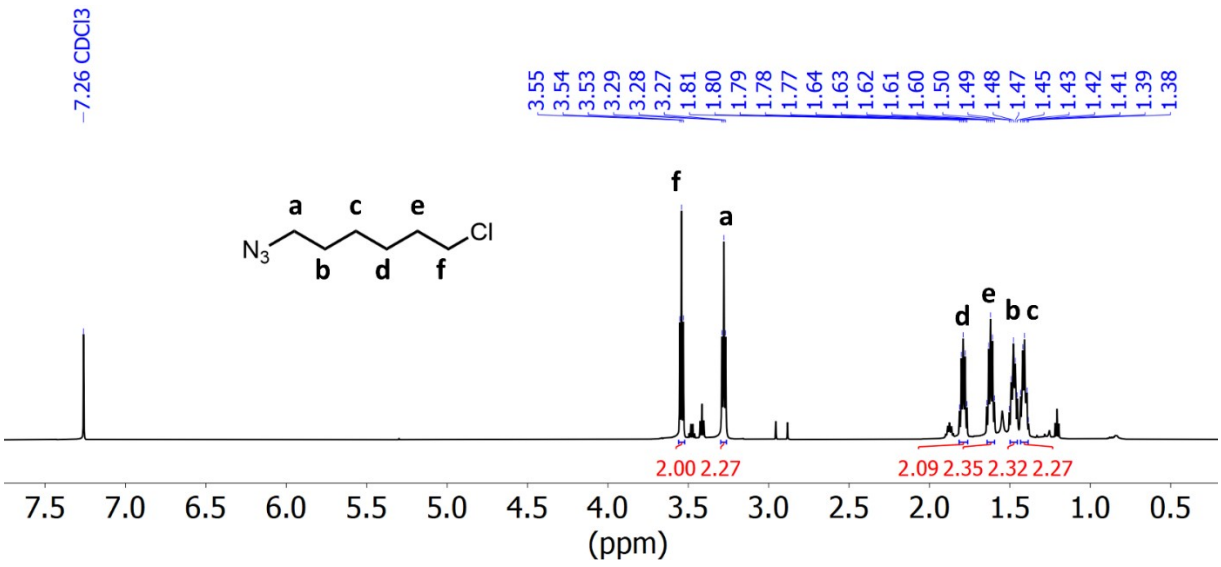


Figure S3. ^{1}H NMR spectrum of 1-azido-6-chlorohexane.

5.2 SYNTHESIS OF TEMPO-AZIDE

TEMPO-NH_2 (1.54 g, 9.00 mmol) and potassium carbonate (1.24 g, 9.00 mmol) were dissolved in DMF (40.0 mL) for 1 h at room temperature. Then, 1-azido-6-chlorohexane (1.46 g, 9.00 mmol) in solution was added dropwise for 24 hours at 50˚C. The solution was then filtered. DCM (40.0 mL) was added to the product and washed with water three times. The organic phase was combined and further washed with water three more times. Then, the organic phase was dried with $MgSO_4$. The solvent was removed under reduced pressure. See scheme S1A. ^{1}H NMR (600 MHz, $CDCl_3$, 15% v/v PhSH) δ ppm 1.18 (s, 12 H), 1.35-1.44 (m, 4 H), 1.53 (q, 2H), 1.61 (t, J = 5.6 Hz, 2H), 1.79 (d, J = 6.6 Hz, 4H), 2.63 (p, 1H), 3.06 (q, J = 3.9 Hz, 1H), 3.28 (p, J = 3.6 Hz, 2H), 3.53 (t, J = 3.8 Hz, 2H). Found *m/z* (ESI-MS): 298.4230, Calculated for $C_{15}H_{33}N_5O$ $[M+H]^+$ 298.4230

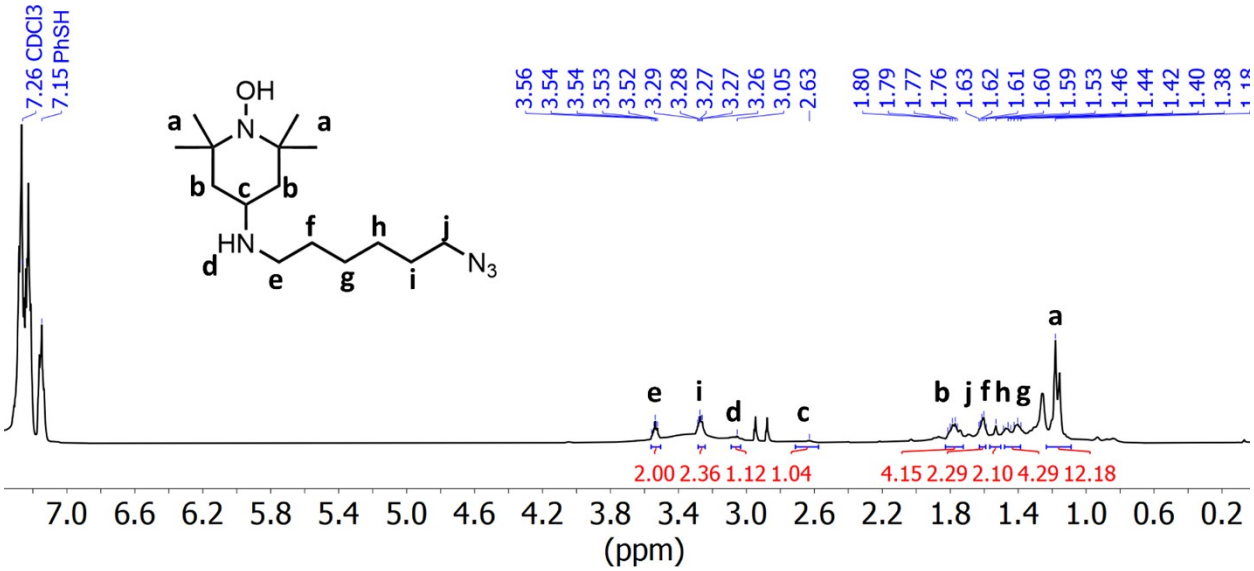


Figure S4. ^{1}H NMR spectrum of TEMPO-Azide.

5.3 SYNTHESIS OF TEMPO-COOH

TEMPO-NH_2 (0.500 g, 3.00 mmol) and potassium carbonate (0.404 g, 3.00 mmol) were dissolved in DMF (20.0 mL) for 1 hour at room temperature. 6-chlorohexanoic acid (0.440 g, 3.00 mmol) was added dropwise to the solution, and the mixture was stirred for 48 hours at room temperature. The solution was then filtered. DCM (40.0 mL) was added to the product and washed with water three times. The organic phase was combined and further washed with water three more times. Then, the organic phase was dried with $MgSO_4$. The solvent was removed under reduced pressure. See Scheme S1B. ^{1}H NMR (600 MHz, $CDCl_3$, 15% v/v PhSH) δ ppm 1.16 (s, 12H), 1.37 (q, J = 8.1 Hz, 2H), 1.46 (p, J = 6.6 Hz, 2H), 1.65 (p, 2H), 1.76 (d, 4H), 2.63 (q, 1H), 4.06 (q, 1H), 4.14 (t, 2H), 4.22 (q, 2H). Found *m/z* (ESI-MS): 286.24, Calculated for $C_{15}H_{29}N_2O_3$ $[M+H]^+$ 286.24.

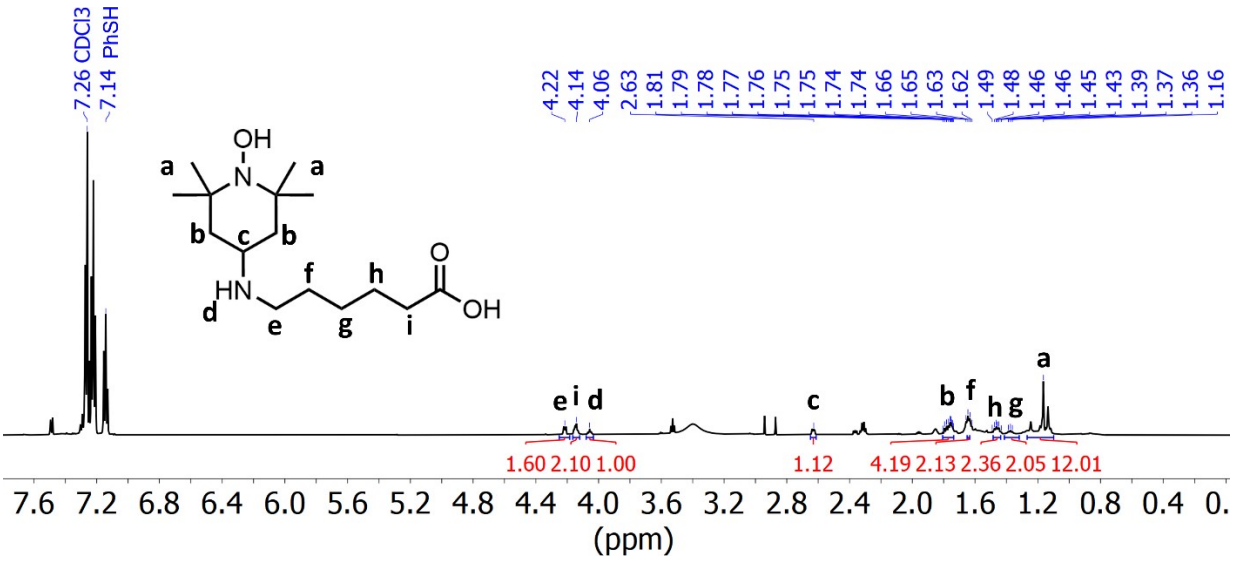


Figure S5. ^{1}H NMR spectrum of TEMPO-COOH.

5.6 TMV FUNCTIONALIZATION (NTMV-ALKYNE)

The diazonium salt was prepared by mixing p-toluenesulfonic acid monohydrate (0.20 mL, 0.30 M), 3-ethynylaniline (75 µL, 0.68 M), and sodium nitrite (25 µL, 3.0 M). The mixture was then subjected to incubation on ice for 1 h in the absence of light exposure. The resulting diazonium salt (10 µL) was subsequently introduced to a solution containing 2 mg/mL of nTMV in borate buffer (0.10 M, pH 8.8). This mixture was allowed to incubate on ice for 45 min. The solution was centrifuged at 4,303 ×g for 1 minute to separate the excess catalytic dye residue. The resulting TMV-Alkyne complex is then purified through Sephadex and concentrated through centrifuge filtration, utilizing an EMD Millipore Amicon Ultra Centrifugal Filter Unit (with a 10,000 MW Cutoff) at a centrifugal force of 4,303 ×g.

5.7 BIOCONJUGATION OF TEMPO-AZIDE TO NTMV (NTMV-TEMPO)

The following procedure was employed to synthesize nTMV-TEMPO. TEMPO-Azide (1.7 mg, 5.7 μmol) was dissolved in 1 mL of ACN. Cold (4 °C) KP buffer (0.1 M, pH 7.4) was added to the above solution, resulting in a well-mixed solution. To the resulting solution, a cold (4 °C) solution of TMV-Alkyne (20 mg/mL, 100 μL, 0.1 μmol) in KP buffer (0.10 M, pH 7.4) was added. The solution was mixed thoroughly. An aqueous solution of copper sulfate pentahydrate (0.1 M, 10 μL) was then added to the mixture, and the solution was mixed well. Subsequently, an aqueous solution of sodium ascorbate (0.2 M, 10 μL) was added to the solution and mixed thoroughly. The reaction mixture was allowed to proceed at room temperature for 24 h. After 24 h, the resulting product was purified. This purification step was carried out either through size exclusion chromatography using a GE Healthcare PD-10 Desalting Column or by centrifuge filtration using an EMD Millipore Amicon Ultra Centrifugal Filter Unit with a 10,000 MW Cutoff. The purification was performed at a centrifugal force of 4,303 ×g.

5.8 BIOCONJUGATION OF TEMPO-COOH TO KTMV (KTMV-TEMPO).

To modify the lysine of TMV with TEMPO-COOH, the following steps were carried out:
A stock TMV solution (20 mg/mL) was diluted to 2 mg/mL by adding HEPES buffer (674 μL, 0.100 M, pH 7.40) at room temperature. To this solution, TEMPO-COOH (130 μL, 0.10 M), HOBt (3.0 mg), and EDC (96 μL, 0.10 M) were added. The reaction mixture was incubated at room temperature for 24 h. The mixture was then purified using a PD

MidiTrap G-25 column, washed three times with 0.10 M KP buffer, and concentrated to 10 mg/mL using a 10K MWCO Pierce™ Protein Concentrator.

6. Electrophoretic Mobility Assays

For the electrophoretic mobility assays, two types of gels were utilized.

1% (w/v) Agarose Gels:

A 1% (w/v) agarose gel was employed for this assay. The sample was prepared by combining 3 µg of TMV with 5 µL of Thermo Scientific 6X DNA Loading Dye. From this mixture, 4 µL was carefully added to each well of the agarose gel. The gel was subjected to electrophoresis at a voltage of 100 V for a duration of 45 minutes. Following electrophoresis, the gel was stained using coomassie brilliant blue and subsequently visualized using the Bio-rad ChemiDoc MP gel imager.

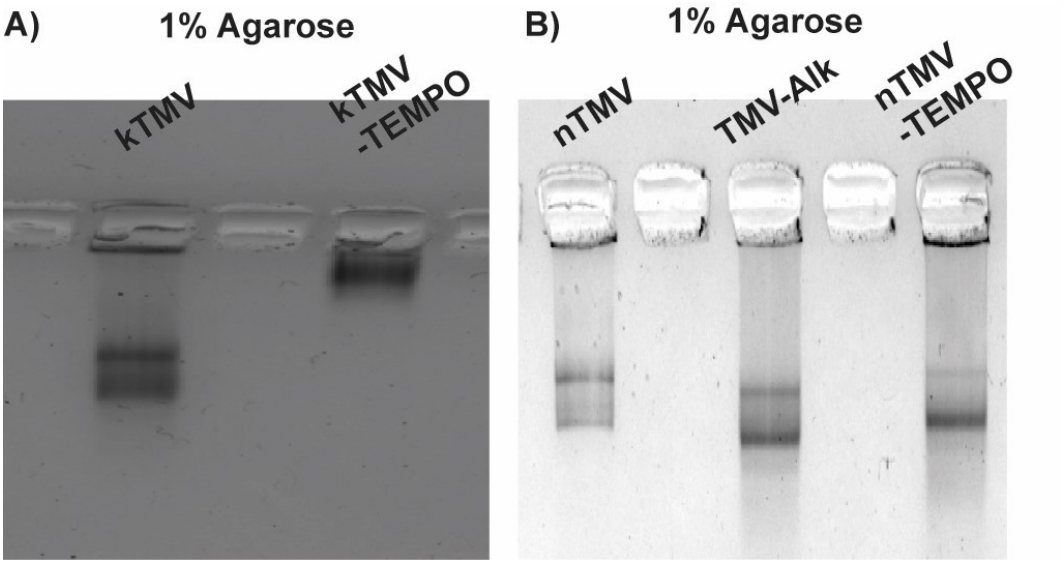


Figure S6. 1% Agarose gels of TMVs and their chemical modifications.

18% SDS-PAGE Gels:

For this assay, 18% SDS-PAGE gel was employed. The sample was prepared by mixing 3 µg of TMV with 5 µL of SDS loading dye, which consisted of β-Mercaptoethanol (5%), Bromophenol blue (0.02%), Glycerol (30%), SDS (Sodium dodecyl sulfate, 10%), and Tris-Cl (250 mM, pH 6.8). An additional 5 µL of 0.1 M dithiothreitol was added to the mixture. The resulting mixture was then boiled for 10 minutes to denature the proteins. Subsequently, 4 µL of the prepared sample was added to each well of the SDS-PAGE gel. The gel was then subjected to electrophoresis at a voltage of 100 V for 45 min. Following electrophoresis, the gel was stained with coomassie brilliant blue and visualized using the Bio-rad ChemiDoc MP gel imager.

7. EPR Spectroscopy

For the EPR spectroscopy experiments, the following procedure was carried out: A solution of the TMV-TEMPO variant was prepared with a concentration of 10 mg/mL. The solution was carefully drawn into a glass capillary tube with a diameter of 1 mm. The prepared capillary tube was then placed inside a quartz EPR tube with a diameter of 4 mm. All EPR measurements in this paper were conducted using the specified instrumental conditions.

-Microwave Power: 4.54 mW

-Microwave Frequency: 9.38 GHz

-Modulation Frequency: 100 kHz

-Modulation Amplitude: 0.4 mT (4 G)

-Temperature: 298 K

-Center Field: 334 mT (3340 G)

-Sweep Range: 8 mT (80 G)

7.1 REDOX EPR KINETICS

A 50 µL solution of TMV-TEMPO variants, each with a concentration of 10 mg/mL, was prepared. To monitor the reduction rate, 100 equivalents of sodium ascorbate were introduced to the solution. Subsequently, the solution was drawn into a 1 mm diameter glass capillary tube, which was then inserted into a 4 mm diameter quartz EPR tube. An EPR spectrum was recorded for this sample every minute over 1 h.

For monitoring the oxidation rate, a reduced TMV-TEMPO variant was first prepared by adding 100 equivalents of sodium ascorbate. A purification step was carried out through size exclusion chromatography using a GE Healthcare PD-10 Desalting Column to eliminate excess sodium ascorbate, followed by centrifugation using an EMD Millipore Amicon Ultra Centrifugal Filter Unit. The concentration was determined using the Lowry assay. Finally, a 50 µL solution of reduced TMV-TEMPO variant with a concentration of 10 mg/mL was mixed with 50 equivalents of potassium superoxide. The resulting solution was drawn into a 1 mm diameter glass capillary tube and placed inside a 4 mm diameter quartz EPR tube. An EPR spectrum was recorded for this sample every minute for 1 hour.

To calculate the k' and $t_{1/2}$ for both the first—

and post— 3 min time points, we used "one phase association" mathematical model from Graphpad Prism 8.4.2:

Equation 1.

$$Y = Y_0 + (Y_{Plateau} - Y_0) \times (1 - \exp(-K \times X))$$

where:

Y is the dependent variable (response),

Y_0 is the initial value (the response when $X = 0$),

$Y_{Plateau}$ is the plateau or maximum response,

K is a rate constant,

X is the independent variable.

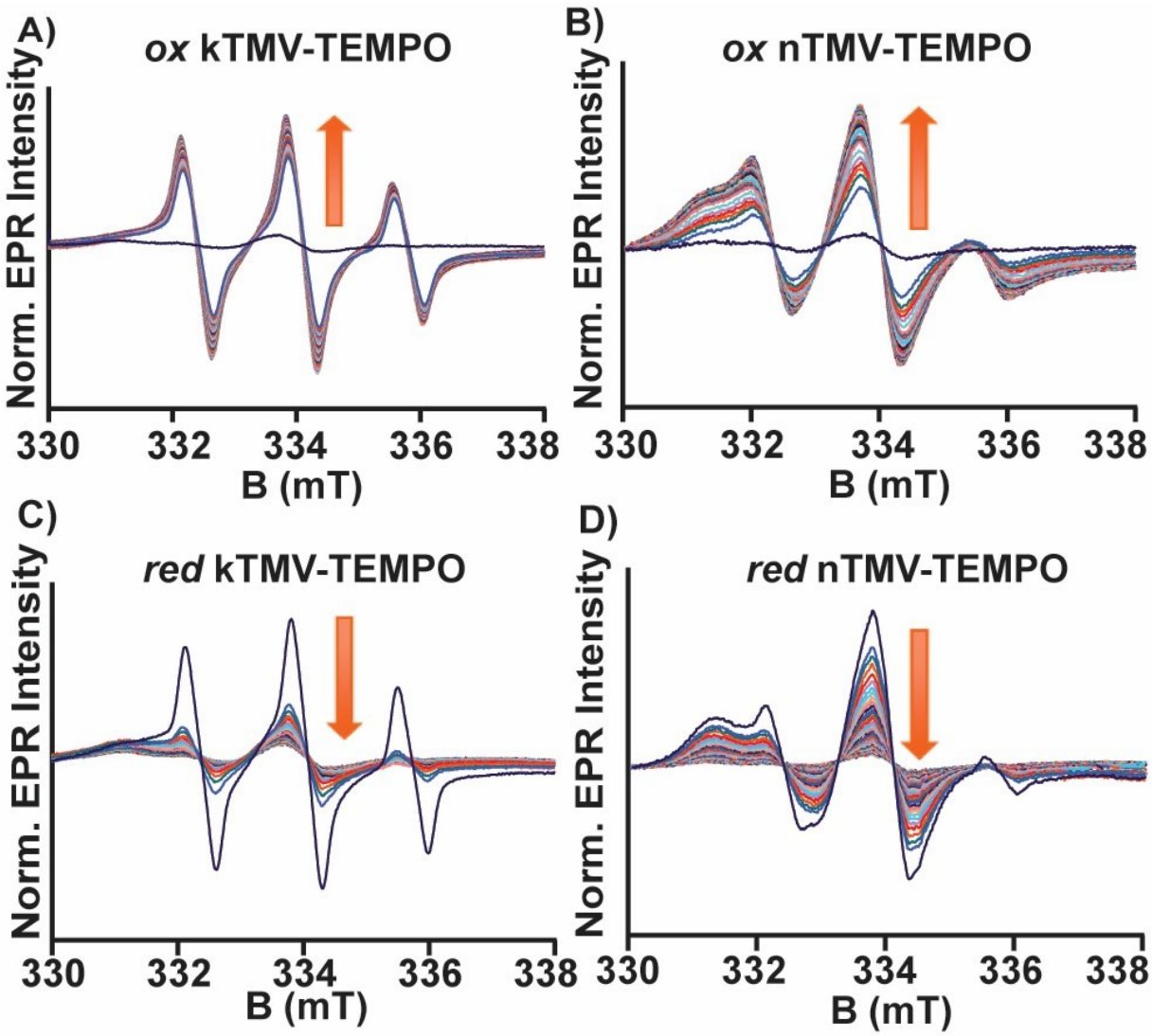


Figure S7. EPR kinetic spectrum collected every minute for an hour. A) Oxidation of kTMV-TEMPO. B) Oxidation of nTMV-TEMPO. C) Reduction of kTMV-TEMPO. D) Reduction of nTMV-TEMPO.

7.2 EPR REDOX RESPONSE TO BLOOD

To compare the REDOX behavior of the agent when injected intravenously, 200 µL of blood was collected from a healthy mouse and a mouse post 8 h after being hepatically injected with 50 µg/kg LPS to model future *in vivo* disease status.

Blood samples were collected from both healthy and diseased mice. To induce significant liver inflammation, a solution containing 100 µL of lipopolysaccharide at a concentration of 50 µg/kg in PBS was injected into the liver of the mice and allowed to incubate for 8 h before blood extraction. The samples tested included free TEMPO, nTMV-TEMPO, kTMV-TEMPO, and their respective reduced variants. Approximately 3.5 µL of a solution containing 20 mg/mL (equivalent to 1 mM per TEMPO) was added to 25 µL of blood. For control samples, only KP buffer was added instead of blood. Subsequently, each solution was drawn into a 1 mm diameter glass capillary tube, which was then placed inside a 4 mm diameter quartz EPR tube. EPR spectra were recorded for each sample at various time points, specifically at 1 min, 5 min, 10 min, and 30 min. EPR spectra were taken with similar parameters as kinetic studies.

8. Relaxometry

To determine both longitudinal and transversal relaxation times, the following steps were carried out using a Magritek Spinsolve NMR spectrometer operating at a magnetic field strength of 1 Tesla (43 MHz). For T1, TE = 1 ms, TR =1000 ms, number of scans =8 were performed; for T2, TE =4 ms, TR = 2000 ms, and number of scans = 8 were conducted. Relaxation times were measured for various solutions at 310K. Three solutions (nTMV-TEMPO, kTMV-TEMPO, free TEMPO) were prepared with concentrations from 0 to 0.5 mM TEMPO. Linear regression analysis was performed on the relaxation rates of these solutions to determine the relaxivity.

9. Phantom Imaging with nTMV-TEMPO and kTMV-TEMPO Samples

Phantom images were acquired simultaneously for nTMV-TEMPO and kTMV-TEMPO samples, along with their reduced and oxidized variants, and 18.2 MΩ DI H_2O, KP buffer, and TEMPO-NH_2. The imaging was performed on a Bruker 3T small animal MRI system using standard 5-mm diameter cell culture glass tubes and a 38-mm diameter 1H birdcage volume coil. Each sample had a volume of approximately 200 µL with a concentration of 1 mM per TEMPO in PBS buffer at pH 7.0. The sample temperature was monitored and maintained at 25°C using a heated air system from Small Animal Instruments (Stony Brook, NY). 3D T2-weighted gradient echo multi-slice scans (TE =48 ms, TR = 1506 ms, Matrix = 128 × 128 × 128) were performed

10. LDH Cell Viability Assay

Cell viability assay was conducted according to the manufacturer's protocol. RAW 264.7 murine macrophage cells were grown in DMEM supplemented with 10% FB Essence and 1% solution of penicillin–streptomycin. The cells were then seeded at 1 × 10^6 cells/mL in a 96-well plate (100 µL/well), incubated overnight, and allowed to adhere. All incubations took place in a 37 °C, 5% CO_2 incubator. The following day, the cells were treated with the nTMV, kTMV, nTMV-TEMPO, kTMV-TEMPO, or free TEMPO. Next, 20 µL of lysis buffer was added to a set of untreated cells for 30 min to create the negative control. After that, 100 µL of the working solution was added to all of the wells for 30 min in a light-protected area. Last, 50 µL of the stop solution was added to all wells before the absorbance was read at 490 nm on a BioTek Synergy H4 hybrid microplate reader.

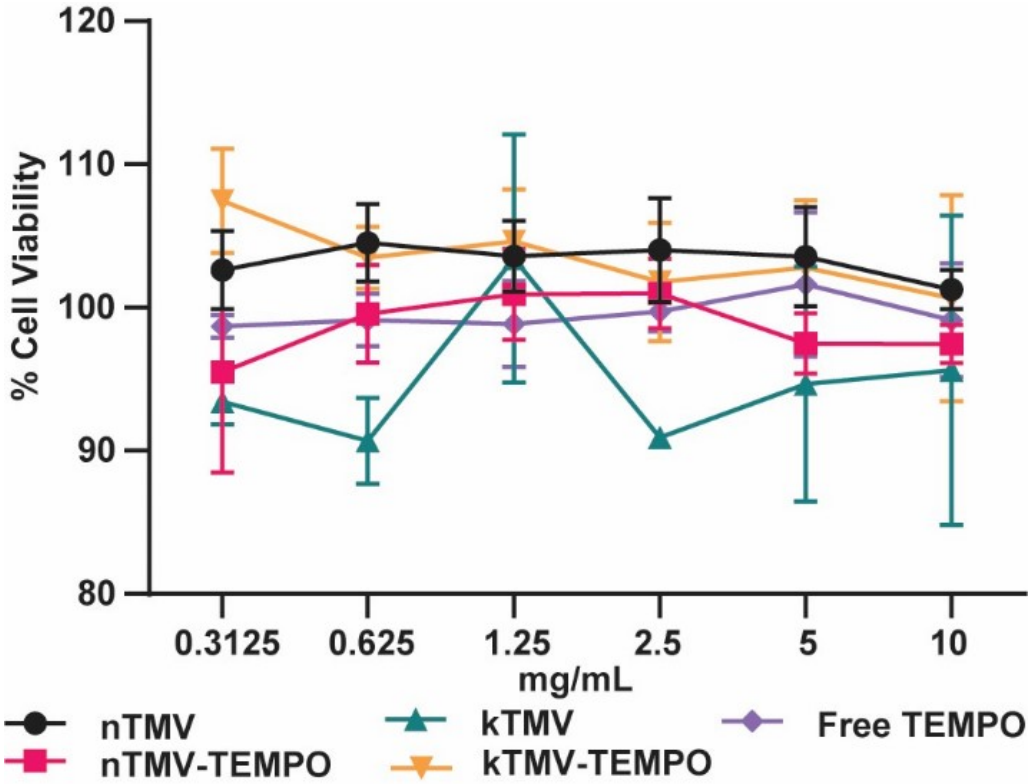


Figure S8. Cytotoxicity of TMV variants, nTMV-TEMPO, kTMV-TEMPO, and TEMPO at varying concentrations (0.3125 mg/mL, 0.625 mg/mL, 1.25 mg/mL, 2.5 mg/mL, and 10 mg/mL) using LDH (lactate dehydrogenase) assay was performed with Raw 264.7 cells.

11. MRI in vivo Studies

The *in vivo* MRI studies involving mice were conducted following the protocols approved by the UT Southwestern IACUC committee under protocol number 103077. To induce significant liver inflammation, 100 µL of 50 µg/kg lipopolysaccharide in PBS was injected into the liver of mice (N=3). After 8 hours, mice were subjected to anesthesia, and to maintain a consistent temperature throughout the study, a heater was used to maintain the ambient temperature around the mice at 30 °C. The mice were then positioned in a 3T Bruker MRI scanner. 3D T2-weighted gradient echo multi-slice scans (TE =48 ms, TR = 1506 ms, Matrix = 128 × 138 × 128) were performed, pre-injection of TMV-TEMPO formulations, post 5 min, 10 min, and 30 min after injection.

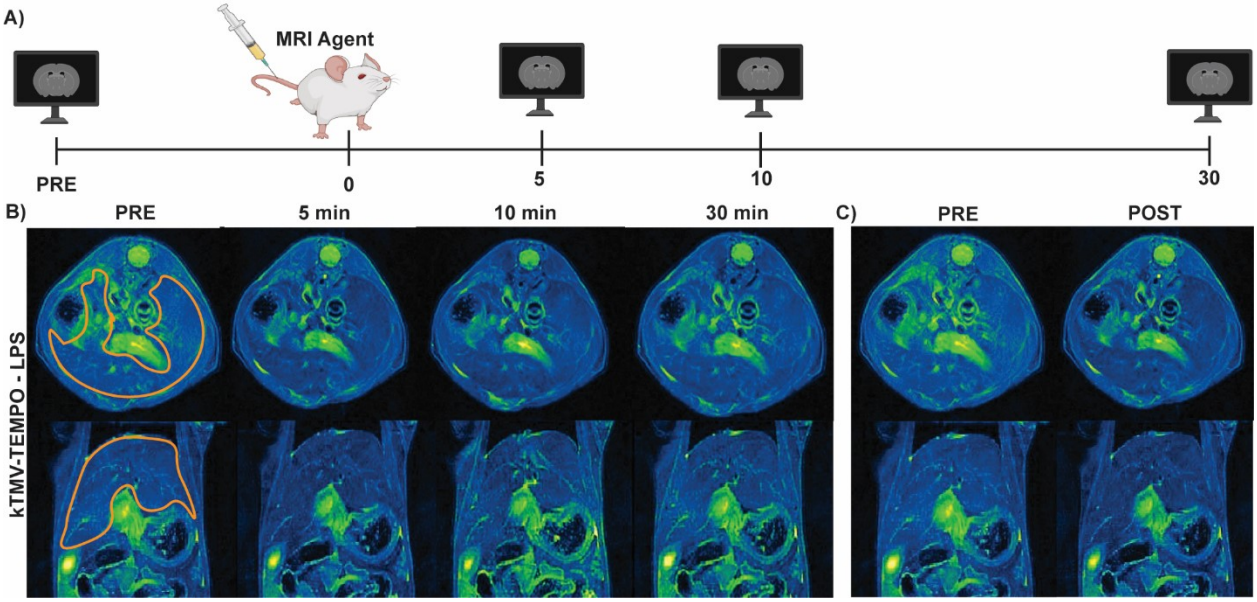


Figure S9. A) Timeline of the *in vivo* MRI study. B) Axial (top) and coronal (bottom) views of mouse liver at different time points. C) Summary images at time of most interest (POST= 5min).

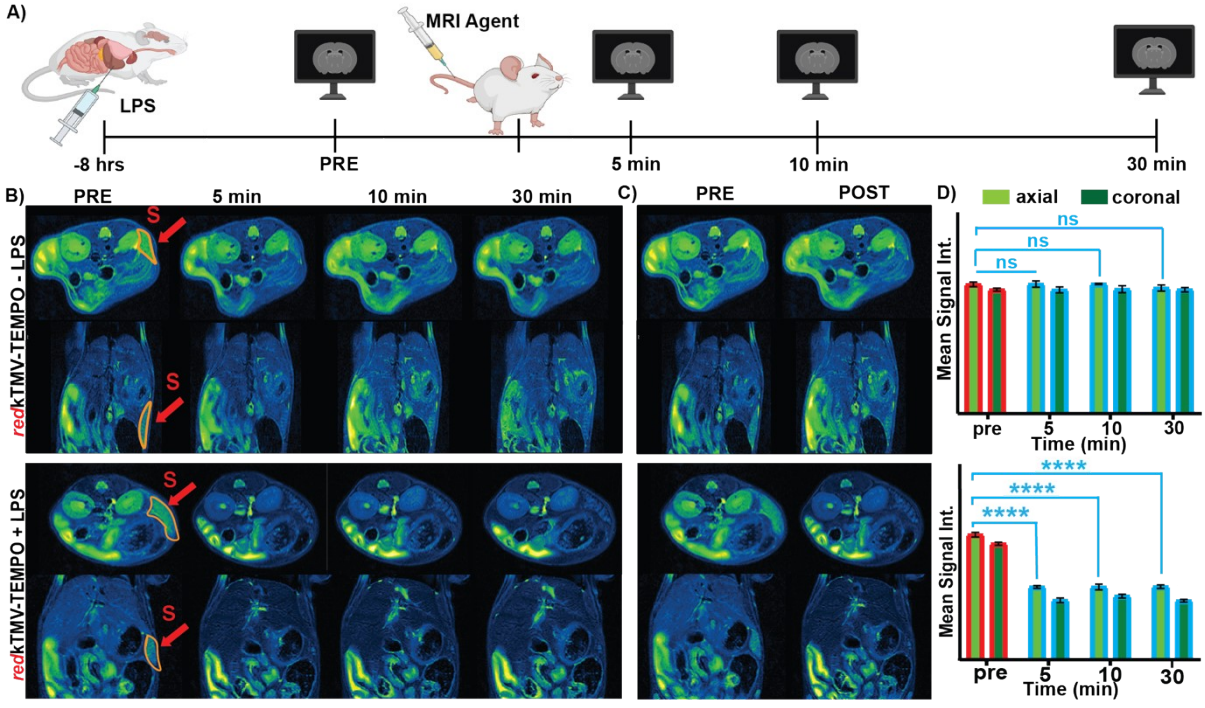


Figure S10. A) Experimental timeline: female BALB/c mice were subjected to liver inflammation through an intrahepatic LPS injection (50 µg/kg) eight hours prior to the initial MRI scan. Following the pre-scan, the mice were administered with reduced kTMV-TEMPO via tail-vein injection, with subsequent scans conducted at 5, 10, and 30 min post-injection. B) T_2- weighted images, oriented on the spleen (red arrow) of healthy and sick mice (outlined with an orange line) were administered with reduced kTMV-TEMPO. C) Summarized comparison focusing on the PRE and POST (5 min post-injection) images. D) Mean signal intensity plots at different time points of both healthy and sick mice (N=3). 3D T_2-weighted gradient echo multi-slice scans (TE =48 ms, TR = 1506 ms, Matrix = 128 × 138 × 128) (* $p < 0.05$; **, $p < 0.01$; ***, $p < 0.0005$; ****, $p < 0.0001$; ns = not significant ($p > 0.05$)).

12. LIST OF TABLES

Table S1: Kinetics of the oxidation and reduction of TMV-TEMPO variants.

Contrast Agent	First 3 minutes		Post 3 minutes	
	k' (s^{-1})	$t_{1/2}$ (sec)	k' (s^{-1})	$t_{1/2}$ (sec)
ox kTMV-TEMPO	0.063	10.94	0.0004752	1459
red kTMV-TEMPO	0.03597	19.27	0.0005759	1204
ox nTMV-TEMPO	0.021	33.78	0.001047	662
red nTMV-TEMPO	0.01527	45.38	0.001285	539.6

Table S2. Contrast Agent Relaxivity Comparison. *Comparing relaxivity rates directly at various field strengths can be challenging due to the suppression of r_1 values and

enhancement of r_2 values at higher fields. These values are provided for the sake of comprehensiveness and to recognize the efforts of other researchers in this field.

Contrast Agent (CA)	CA per Particle	r_1 per CA ($mM^{-1}·s^{-1}$)	r_2 per CA ($mM^{-1}·s^{-1}$)	r_1 per Particle (mM-1·s-1)	r_2 per Particle (mM-1·s-1)	r_2/r_1	Field (T)*	ref
kTMV-TEMPO	~2130	2.89 ± 0.1	8.97 ± 0.1	~6156	~19106	3.1	1	This work
nTMV-TEMPO	~2130	2.72 ± 0.1	8.11 ± 0.1	~5793	~17274	2.9	1	This work
TMV-6	~2130	2.8 ± 0.1	10.3 ± 0.1	~5964	~21939	3.7	1	4
TMV-6+CB[8]	~2130	1.9 ± 0.1	3.1 ± 0.1	~4047	~6603	1.6	1	4
TEMPO-NH_2	1	0.6 ± 0.1	2.0 ± 0.1	0.6 ± 0.1	2.0 ± 0.1	3.3	1	4
TEMPO-NH_2+CB[8]	1	0.2 ± 0.1	0.2 ± 0.1	0.2± 0.1	0.2 ± 0.1	1	1	4
Gd-DOTA	1	3	5	3	5	1.7	1	5
*ex*TEMPO-TMV	~2130	1.5 ± 0.1	4.7 ± 0.1	~3195	~10011	3.13	1.5	6
*in*TEMPO-TMV	~3919	0.4 ± 0.1	1.7 ± 0.1	~1567.6	~6662.3	4.25	1.5	6
3-CP	1	0.15	0.17	0.15	0.17	1.13	7	7
Chex-MM	N/A	0.21	0.3	N/A	N/A	1.42	7	8
Chex-dendrimer	N/A	0.44	0.86	N/A	N/A	1.95	7	9
Chex-bottlebrush	N/A	0.32	0.82	N/A	N/A	2.56	7	8
BASP-ORCA1	N/A	0.41	4.67	N/A	N/A	11.39	7	7
BASP-ORCA3	N/A	0.63	4.62	N/A	N/A	7.33	7	10
Dy-DTPA-PcHexPh2	1	0.11	3	0.11	3	27	7	11

13. References

1. D. Sezer, J. H. Freed and B. t. Roux, *The Journal of Physical Chemistry B*, 2008, 112, 5755-5767.
2. K. Vanommeslaeghe, E. P. Raman and A. D. MacKerell, Jr., *Journal of Chemical Information and Modeling*, 2012, 52, 3155-3168.
3. K. Vanommeslaeghe and A. D. MacKerell, Jr., *Journal of Chemical Information and Modeling*, 2012, 52, 3144-3154.
4. H. Lee, A. Shahrivarkevishahi, J. L. Lumata, M. A. Luzuriaga, L. M. Hagge, C. E. Benjamin, O. R. Brohlin, C. R. Parish, H. R. Firouzi, S. O. Nielsen, L. L. Lumata and J. J. Gassensmith, *Chemical Science*, 2020, 11, 2045-2050.
5. L. Liepold, S. Anderson, D. Willits, L. Oltrogge, J. A. Frank, T. Douglas and M. Young, *Magnetic Resonance in Medicine*, 2007, 58, 871-879.
6. M. Dharmarwardana, A. F. Martins, Z. Chen, P. M. Palacios, C. M. Nowak, R. P. Welch, S. Li, M. A. Luzuriaga, L. Bleris, B. S. Pierce, A. D. Sherry and J. J. Gassensmith, *Molecular Pharmaceutics*, 2018, 15, 2973-2983.
7. H. V. T. Nguyen, Q. Chen, J. T. Paletta, P. Harvey, Y. Jiang, H. Zhang, M. D. Boska, M. F. Ottaviani, A. Jasanoff, A. Rajca and J. A. Johnson, *ACS Central Science*, 2017, 3, 800-811.
8. M. A. Sowers, J. R. McCombs, Y. Wang, J. T. Paletta, S. W. Morton, E. C. Dreaden, M. D. Boska, M. F. Ottaviani, P. T. Hammond, A. Rajca and J. A. Johnson, *Nature Communications*, 2014, 5, 5460.
9. A. Rajca, Y. Wang, M. Boska, J. T. Paletta, A. Olankitwanit, M. A. Swanson, D. G. Mitchell, S. S. Eaton, G. R. Eaton and S. Rajca, *Journal of the American Chemical Society*, 2012, 134, 15724-15727.
10. H. V. T. Nguyen, A. Detappe, N. M. Gallagher, H. Zhang, P. Harvey, C. Yan, C. Mathieu, M. R. Golder, Y. Jiang, M. F. Ottaviani, A. Jasanoff, A. Rajca, I. Ghobrial, P. P. Ghoroghchian and J. A. Johnson, *ACS Nano*, 2018, 12, 11343-11354.
11. P. Caravan, M. T. Greenfield and J. W. M. Bulte, *Magnetic Resonance in Medicine*, 2001, 46, 917-922.