**Introduction:** The superoxide anion ($O_2^-$) is a free radical produced in biological systems in response to normal immune system activation and during $O_2$ consumption during mitochondrial respiration. Although useful when produced as a means of host immune defense against invasion of microorganisms, it is also considered undesirable due to its ability to alter healthy cellular components and thus compromise their normal function, leading to disease. Superoxide dismutate (SOD) enzymes in the mitochondria (Mn based) and cytosol (Cu and Zn based) catalyze the dismutation of superoxide into oxygen ($O_2$) or hydrogen peroxide ($H_2O_2$), and catalase decomposes $H_2O_2$ as indicated by Eqs. 1 and 2:

$$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2 \quad \text{Eq. 1}$$

$$2H_2O_2 \rightarrow 2H_2O + O_2 \quad \text{Eq. 2}$$

M40403 (Fig. 1C) is an SOD mimic with a history of *in vivo* testing [1] whose catalytic activity rate of $O_2$ dismutation approaches that of native Mn SOD enzyme [1]. Since Mn is paramagnetic, the delivery of M40403 *in vivo* could potentially be monitored using MRI. In this study, we incorporated M40403 into long-circulating PEGylated liposomes and characterized their relaxivity properties. Furthermore, we administered the paramagnetic liposomes *in vivo* to observe perfusion in the mouse brain.

**Methods:** M40403, manganese(II)dichloro[2,5,21S]-dimethyl-(4R,9R,14R,19R)-3,10,13,20,26-pentaazatetracycl[20.3.1.0][6]hexacosa-1(26),22(23),24-triene], was synthesized as described in [2]. Liposomes containing M40403 were composed of distearoylphosphatidylcholine (DSPC), distearoylphosphatidylethanolamine (DSPE)-PEG(2000) (90:10 mol/mol). Lipids were dissolved in chloroform and a thin lipid film was created by rotary evaporation. Water solutions of M40403 were added to the film and the lipids were hydrated at 65°C. Unilamellar liposomes were formed by extruding through a Nuclepore film and the lipids were hydrated at above the phase transition temperature. Unencapsulated M40403 was removed using a Sephadex G-25m gel-filtration column eluted with DPBS buffer, pH 6.8. Quasi-elastic-light (QEL) scattering was used to determine the actual diameter of liposomes. Relaxivity quantifications of M40403 and M40403 liposomes were performed at 0.47 T. Kinetic experiments were performed in 96-well plates by measuring time-dependent absorbance at 560 nm to assess the activity of SOD and SOD mimetics (M40403 and M40403 liposomes) using xanthine and xanthine oxidase to generate superoxide, and horseradish peroxidase (HRP)/Amplex Red as a dismutation detection system in a reaction buffer (0.5M TrisHCl, 0.25M NaCl, 0.25M KCl at pH 7.5). *In vivo* MR imaging was performed in mouse brains (n=3) at 3T to observe the uptake of M40403 liposomes. T1-weighted (T1-WT) gradient-echo MRI was performed pre- and post-IV injection of M40403 liposomes at M40403 dose of 44 µmol/kg.

**Results and Discussion:** Fig. 1A shows a transmission electron microscope image of DSPC/DSPE-PEG(2000) liposomes loaded with M40403 after negative contrast (Fig. 1B). QEL scattering established the diameter of the liposomes as 170 ± 50 nm (mean ± SD). At 0.47 T, the relaxivity of M40403 was 4.44 mM$^{-1}$s$^{-1}$. Final liposome preparation contained 0.22 µmol of M40403 per milligram of lipid and the yield of M40403 encapsulation into liposomes was ~25%. $V_{int}$ (initial reaction rate) reflecting the activity of M40403 and M40403 liposomes as SOD mimetics closely related to that of SOD at low M40403 concentrations (<150 µM) (Fig. 2); however, at high M40403 concentrations (>150 µM), the SOD mimetics showed greater $V_{max}$ changes compared to SOD indicating that SOD mimetics can dismutate $O_2$ more efficiently than SOD. Liposomes loaded with paramagnetic M40403 were injected via tail vein in mice. M40403 within the liposomes shortened the proton T1 relaxation time of the water within the liposomes and allowed direct visualization of the liposomes. We observed enhancement in the mouse brain within minutes after IV-injection of M40403 liposomes which had disappeared by the fifth day (Fig. 3). This enhancement and clearance of signal indicated the entry of liposomes into the vascular bed of the brain. The sub-cortex region of the brain showed greater T1 enhancement (higher SNR) than the cortex region, which could also indicate the potential entry of M40403 liposomes or free M40403 into the cerebrospinal fluid via the choroid plexus.

**Conclusion:** We established that M40403 is a highly efficient SOD mimic that doubles as a paramagnetic contrast agent, being one of the few truly theranostic compounds useful for MR imaging. Liposomes loaded with M40403 when injected into mice did not exhibit any outward ill health effects and resulted in transient enhancement of the cortical and sub-cortical regions of the brain. Our results indicate that M40403 liposomes enable a systemic delivery route of an SOD mimic to the brain that can potentially be used not only for therapeutic treatment, but also MRI monitoring the delivery to the diseased tissues that generate high and damaging levels of $O_2^·$ (e.g., in cerebral ischemia).