A Dual Histology Stain/MRI Contrast Agent for Molecular Imaging of the Brain

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Introduction: In order to improve models of brain cytoarchitecture we are obtaining very high resolution magnetic resonance images of fixed and fresh *ex vivo* brains from humans, rats, and mice. The decreased signal-to-noise ratio (SNR) associated with the very high resolution images (20-100 μm isotropic) is compensated for by the use of high magnetic field strengths (7 and 14 Tesla) and the use of exogenous MRI contrast agents. In particular, we are working on developing different molecular imaging MRI "stains" for molecular imaging of the brain that can be directly correlated with histology stains. Initial studies have investigated the use of the myelin stain Luxol Fast Blue 38 (LFB-38) as a dual histology / MRI "stain" for myelinated structures in the brain. The molecular structure of LFB-38, shown in Figure 1 below, is a sulfonated copper phthalocyanine compound with a paramagnetic Cu^{2+} ion and should therefore act as an MRI relaxation agent.

Figure 1: *Chemical structure of Luxol Fast Blue 38, where X is SO_3^-, used for staining myelin. The copper ion is in the +2 oxidation state and is paramagnetic and should act as a MRI relaxation agent.*

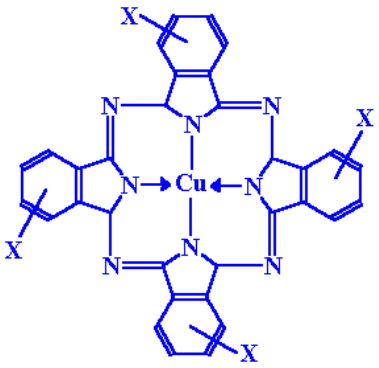
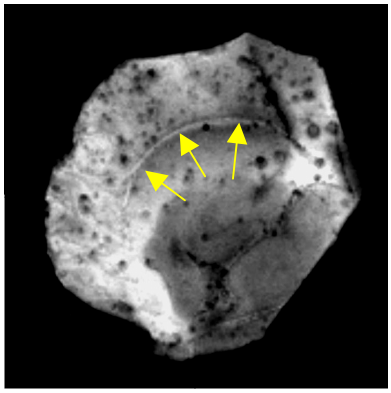


Figure 2: *14 Tesla gradient-echo image of a LFB-38 stained rat hippocampal brain slice. The MRI slice thickness was 500 μm with an in plane resolution of 31 μm. The pulse sequence parameters were TR=300 ms, TE=4.7 ms, flip angle=30 degrees, and 16 averaged acquisitions. The yellow arrows indicate the corpus callosum white matter tract.*



Materials and Methods: Average T_1 and T_2 relaxation times of 400 μm thick fixed Sprague-Dawley rat hippocampal brain slices were measured at 14 Tesla on a Bruker Avance MRI scanner. Relaxation times for both unstained and LFB-38 stained samples were measured using T_1-Inversion Recovery (for T_1) and Spin-Echo (for T_2) pulse sequences. The stained specimens were soaked in a solution of LFB-38 dissolved in approximately 10:1 ethanol/acetic acid (680 mg LFB-38 in 50 ml ethanol and 5 ml acetic acid) for approximately 12 hours. Following LFB-38 staining the stained slices were rinsed and soaked in fixation buffer (4% paraformaldehyde in phosphate buffer solution) for several days to wash away excess LFB-38 and to rehydrate the tissue slices. High resolution (31 μm in-plane) gradient-echo images were also acquired with the acquisition parameters given in Figure 2.

Results and Discussion: The use of molecular agents that act as both histology stains and MRI molecular imaging contrast agents provides a powerful tool not only for the co-registration of MRI and histology images but also for improving the MRI signal-to-noise ratio (SNR) and highlighting specific brain cytoarchitectural features. Our initial studies have involved examining the potential of the myelin stain LFB-38 to act as a myelin specific contrast agent in *ex vivo* brain studies. Experiments performed at 14 Tesla indicate that LFB-38 shortens the average T_1 of rat brain hippocampal slices from 2.0 seconds for unstained specimens to 1.5 seconds for the stained specimens. T_2 relaxation times were only slightly shortened in LFB-38 stained specimens with T_2 = 24 ms for stained specimens and T_2 = 27 ms for unstained specimens. Shown in Figure 2 is a preliminary high resolution image of an LFB-38 stained rat hippocampal slice in which the myelinated corpus callosum white matter tract is highlighted by the LFB-38. Future studies will involve attachment of Gd-DOTA MRI contrast agents to other histology stains, such as thionin used for Nissl staining.