# In vivo imaging of redox state in mice using EPRI/MRI coimaging

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**Abstract**
Electron paramagnetic resonance imaging (EPRI) using nitroxide spin probes is a sensitive technique for in vivo measurement of redox state. 1D and 2D EPR imaging has been previously used to map and monitor the change in redox status of various organs in animal models. However, 3D EPR imaging of the change in redox status *in vivo* with anatomic registration is essential to understand organ specific pathology and disease. In the present work, the nitroxide 3-carbamoyl-2,2,5,5-tetramethyl-1-pyrrolidinyl-N-oxyl (3CP) was used to map and monitor the redox state of various organs in living mice using the new EPR/NMR coimaging instrumentation [1]. With rapid scan projection acquisition, we performed 3D mapping of 3CP in living mice every 8 minutes. The NMR coimaging allowed precise slice by slice measurement of the radical reduction and mapping of this metabolism in major organs such as the heart, lungs, liver, bladder and kidneys.

**Introduction**
Electron paramagnetic resonance imaging is a powerful technique that enables spatial mapping of free radicals; however, it does not in itself provide anatomic visualization of the body. Proton magnetic resonance imaging is well suited to provide anatomical visualization. A hybrid EPR/NMR coimaging instrument was constructed that utilizes the complementary capabilities of both techniques, superimposing EPR and proton-MR images to provide the precise anatomic distribution of paramagnetic species in the body. Common magnet and field gradients are utilized along with dual EPR and proton-NMR resonators, enabling coimaging without the need to move the sample [2]. Due to the design of the coimaging system there is no need for image co-registration. Such hardware co registration eliminates the need for software co registration and the inherent errors of such methods.

**Methods**
Male C57BL/6 mice were injected intravenously via carotid with 0.4 ml of 300 mM 3CP with a constant rate of 0.02 ml/min. After the free radical loading a full 3D mapping of the free radical in each mouse was acquired using EPRI every 8 minutes for approximately 64 min. After EPRI sequence showed a significant drop in the intensity signal (at least 4 times) from the initial value, the system was switched at higher field for MRI. A full 3D gradient echo sequence was used to acquire the proton distribution of the living mouse. EPRI was performed at 1.2 GHz and proton MRI at 16.18 MHz. The reconstructed volumes obtained from both modalities were then superimposed. Through this method we obtained 7-8 such fused volumes for each mouse showing the free radical distribution correlated anatomically with the whole body 3D MRI. A precise slicing of the fused volume was done and certain slices were selected and analyzed manually contouring a specific organ such as, heart, liver, bladder, kidneys etc. The mean value $I(t)$ of the intensity signal of the free radical distribution inside the contoured organ was extracted and plotted against the time $(t)$ for the EPRI acquisition from starting from the end of the loading. The result was fitted with a simple model of a an exponential decay $I(t) = I_0 e^{-t/\tau} + N$ which predicted the half life $(\tau)$ of the free radical inside each of the organs. The volumes were reconstructed, fused, and measured using in house software developed in Matlab (Mathworks, Natick, MA) and the ROI mean value was extracted using a free program for visualization of medical images, MRIcro (University of South Carolina).

**Results**
Our imaging method combined with the imaging protocol produced results which are consistent with previous studies [3]. In Fig. 1 we show an example of slices through 4 volumes (acquired at different times) at the level of heart and the corresponding contour area (green line). The free radical distribution is shown in color, and the underlying gray scale corresponds to the MRI slice. In Fig. 2 we show the fits for a representative mouse together with the mean intensity signal for slices through several organs such as heart, liver, bladder, lungs and kidneys. The exponential model shows a fairly good fit given the sparsely data (around 7-8 points). In table 1 the fitted half life in minutes is given for a mouse for several of the organs.

**Discussion**
Coimaging technique combined with a slice by slice analysis of the full 3D volumes of the free radical correlated with the 3D mapping of the proton distribution yielded for the first time, rapid high quality organ specific mapping of free radical metabolism that should be of great value in mapping alterations of redox state in disease. Our results show a very consistent half life across the whole free radical distribution volume and this could caused by the lack of the temporal resolution. Future work aims at improving temporal resolution of the EPR acquisition such that the additional parameters could be introduced in the model. Such experiments could model the distribution of free radicals and several decay rates to account for metabolism and clearance.

**References and Acknowledgements**
[1] George L. Caia et al. Magn. Reson. Med. 2007 Jul 20;58:156-166. [2] Sergey Petryakov et al. J. of Magn. Reson. 2007 Jun 9;188:68-73. [3] Murugesan Velayutham et al. Magn. Reson. Med. 2003 May 16;49:1181-1187. This work was supported by NIH Research Grants EB0890 and EB4900.

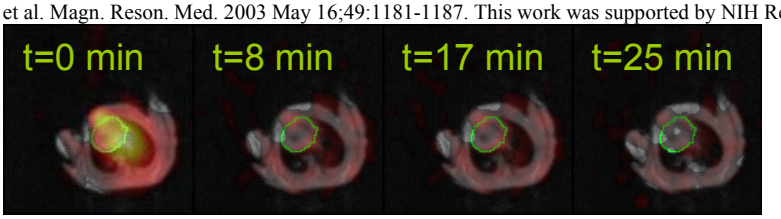


Fig. 1 EPRI superimposed over the MRI for a slice through heart (green contour).

| Organ | Slice # | Half life [min] |
|---|---|---|
| Heart | 57 | 7.402 |
| Liver | 64 | 7.837 |
| Lungs | 58 | 8.510 |
| Kidneys | 80 | 8.554 |

Table 1 Fitted half lives for the free radical in various organs.

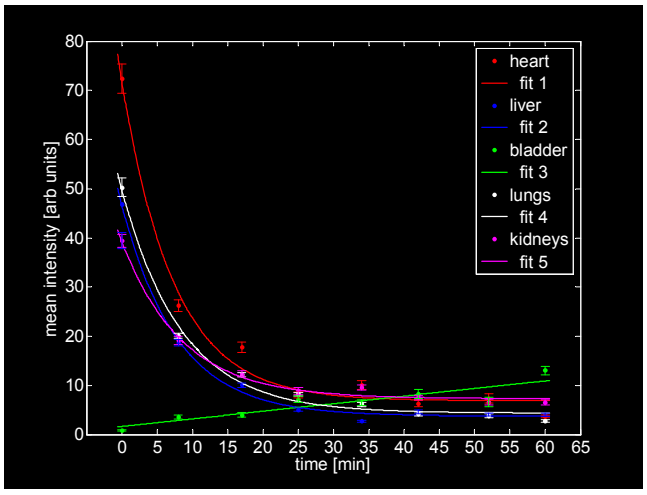


Fig. 2 Mean intensity plot extracted from the ROI for several organs shown together with the fits using an exponential model.