Examining the Effects of Superparamagnetic Iron Oxide Nanoparticles (SPIONs) on *Daphnia magna*

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Superparamagnetic Iron Oxide Nanoparticles (SPIONs) are becoming a contaminant of considerable interest in environmental studies.[1] With their increased introduction to aquatic systems their long-term impacts to organisms is of critical importance.[2] *Daphnia magna* (water fleas) are keystone aquatic organisms for toxicity testing due to their prevalence in freshwater world-wide, and relatively short life span, on which the effects of SPIONs have never been examined.[3] SPIONs are of particular interest due to their ability to be studied both through traditional NMR studies via metabolomics, as well as through Nuclear Magnetic Imaging as a contrast agent. When larger SPIONS (>7nm) are utilized for imaging they become negative (T2) contrast agents. By causing a strong local magnetic field inhomogeneity, T2 relaxation of the surrounding water molecules is increased. This appears as a darkening in the image.[1] The effects on the T2 relaxation time can then be numerically measured. A visual example of how the signal is affected in the proton imaging in *Daphnia* can be seen in Figure 1.

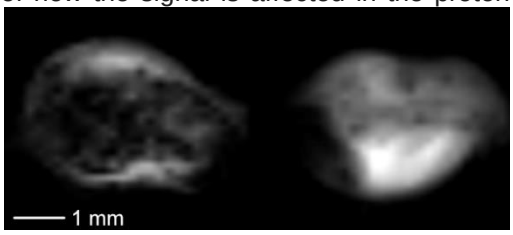

Figure 1. Proton imaging of two Daphnia: Exposed (left) to 12 nm core size and 1 KD ligand size, and control (right). Daphnia were exposed for 24 hours in a solution of 2 µM of nanoparticles. The nanoparticles cause a non-selective intensity loss in the *Daphnia* due to their increased T2 relaxation.

SPIONs utilized in this study were synthesized in three different core sizes (8 nm, 10 nm, 12 nm), and three different ligand sizes (1 KD, 5 KD, 10 KD), resulting in nine total samples. To determine whether these nanoparticles were consumed by the *Daphnia*, Nuclear Magnetic Imaging was utilized to monitor the changes in the T2 relaxation inside the organisms. In this experiment, the T2 values were used as a measure of uptake by the *Daphnia*. *Daphnia* were exposed to 2 µM concentrations of each nanoparticle core and ligand size (9 exposures) and measured in triplicate to determine which particles were actively taken up best by the organisms.

Originally, the hypothesis was that the SPIONs could be locally monitored through the imaging, and the exact location of uptake and usage of the SPIONs would be monitored. However, due to the small size of the organisms, the darkening is not selective, and does not provide local specificity (see Figure 1). Thus, the average values in the T2 changes of the whole organism were used to assess the nanoparticle uptake. Figure 2 shows impact of different ligand size on the SPION particles with 8 nm cores, which had the highest impact of the three core sizes. The 5 KD ligand size had the largest impact on the T2 relaxation time, while the 1 KD had no impact. As *Daphnia* are filter feeders, to ingest food it must become trapped in their filtering apparatus. [4] It appears the 1 KD particles are too small and pass through while the large particles (5 KD and 10 KD) become *bolus* (entrapped food particles) which are then transferred to the gut and thus have an impact on the T2 relaxation of the water inside the Daphnia.

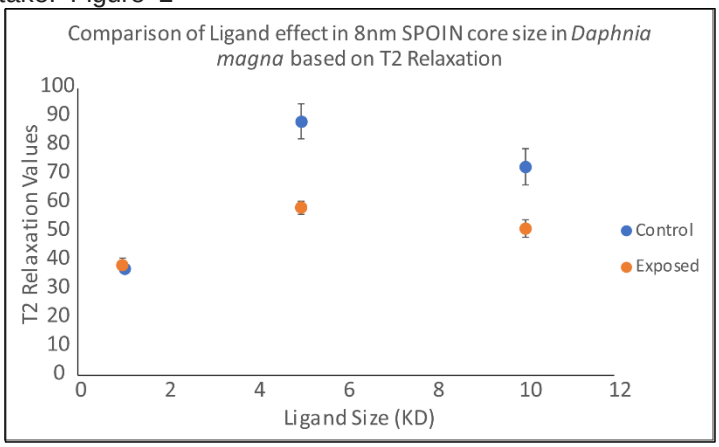

Figure 2. Plot of 8 nm nanoparticles with three different ligand sizes to examine the impacts on the T2 relaxation time. Each was conducted in triplicate and averaged. Exposure to 8 nm and 1 KD had the largest impact on the *Daphnia magna*, and will be utilized for further studies.

This study will be combined with 2D *in-vivo* NMR of ^{13}C enriched Daphnia to investigate metabolic impacts. Combining the imaging and *in-vivo* metabolomics should provide a detailed picture of SPION impacts inside environmentally relevant aquatic organisms, which will provide new insight into a new potential environmental contaminant.

References

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