On-off switchable nanoparticles for improved detection with MRI

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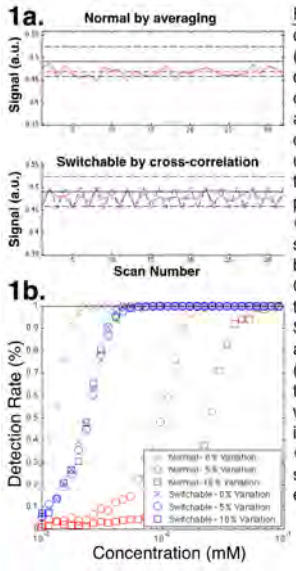
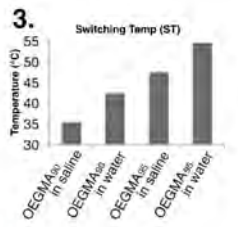
Introduction: Molecular imaging with MR relies on the ability to sensitively and accurately detect contrast agents above tissue background. In practice, both contrast agents and tissue temporal and spatial noise modulate the MRI signal, causing ambiguity that ultimately increases the amount of contrast agent required for accurate detection. This problem is similar to that observed with in vivo fMRI based on blood oxygenation. If contrast agent relaxivity could be temporally modulated from an external source, comparing the signal in a voxel containing the agent to a reference waveform, based on the time-course of switching, may reduce this ambiguity. As illustrated in Fig. 1a, in a voxel containing a switchable particle, the mean signal may fall within the background variance but the particle can still be detected by cross-correlation with a reference signal. Indeed, Menon proposed such a technique for CEST imaging[1] and Louie et al. developed a one-directional light-switchable particle[2], which is valuable when background is fixed. To understand the improved sensitivity of a switchable particle in the presence of tissue inhomogeneity, we have developed a general model comparing an on-off switchable particle to one with fixed relaxivity. To develop an on-off switchable particle, we propose coating SPIOs with the thermosensitive oligo(ethylene glycol) methacrylate (OEGMA) polymer, which transitions from hydrophilic to hydrophobic when above the switching temperature (ST). AC magnetic fields in the range of 0.1-1MHz readily heat superparamagnetic iron oxide (SPIO) nanoparticles. The OEGMA polymer has a tunable ST and may with advanced drug delivery techniques. We hypothesized that if the temperature of the particle solution were increased above the lower critical solution temperature (LCST), the polymer would collapses into a hydrophobic layer surrounding the SPIO core, thereby excluding water and increasing t_1. Externally switched relaxivity may be a route for improved detection of MRI contrast agents in vivo.

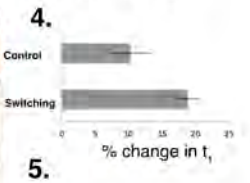
Methods: *Simulation:* We developed a simulation to calculate when an on-off switchable particle can be detected at lower concentrations that a normal particle by assuming a hypothetical particle with r2/r1=25/10 (on state) or 30/1(mM-s^{-1}, off state). We then simulated signal with normally distributed inter-voxel and intra-voxel in imaging voxels and developed detection algorithms based on cross correlations for switchable particles or signal averaging for normal particles. Detection thresholds were set such that the false positive rate was 1% in all cases. We then calculated the detection rate as a function of contrast agent concentration for different levels of inter-voxel background variance. *Synthesis:* 2-bromo-2-methyl-N-(3-(triethoxysilyl)propyl) propanamide (BTPAm) and oleic acid (OA) coated SPIOs were synthesized according to previous methods[3,4]. To exchange the OA ligand with the initiator, 50mg SPIO and 50ul BTPAm were dissolved in 20mL toluene with 0.5% triethylamine and degassed with N_2 at 80° for 30min, then 1mL 2.5% tetramethylammonium hydroxide in MeOH was injected and the particles were washed several times with toluene. To polymerize OEGMA on the surface of the initiator coated SPIOs, 50mg SPIO@BTPAm, 6.25mg 2,2'-bipyridine, and 4mmol monomer (90/10 or 95/5 oligo/di-ethylene glycol methyl ether methacrylate, MW=500/188), and 10ml DMSO were degassed in N_2 for 30min, 2.9mg CuBr in DMSO was added and the temperature was increased to 110° overnight. The particles were then dialyzed against diH_2O for 4 days. *Relaxometry:* 0.69mg/mL room temperature SPIO@OEGMA$_{90}$ 1% agar with 0.15M NaCl were placed in a Bruker mq60 relaxometer. As a control we used the exact same particle in agar without NaCl, which has a higher ST and will not switch when heated to 37°. t_1 and t_2 were measured with inversion recovery or CPMG sequences every minute for 5 minutes while the sample temperature increased to 37°.

Results: *Simulations:* To estimate increased sensitivity of a switchable particle in inhomogeneous tissue, we simulated the detection rate for non-switchable and switchable particles for variable amounts of inter-voxel background variance. In an illustrative voxel (Fig 1a), the signal is reduced but the particle is not detected because the signal remains within the background variance, whereas a switchable particle is detected by cross-correlation with a reference function (Fig 1b). For a normal particle, the minimum detectable concentration increased significantly as the inter-voxel variance increased; for switchable particles that concentration was unaffected by inter-voxel variance (Fig 1b). With 10% background variance, a switchable particle was detectable in a 10x lower concentration. *SPIO@OEGMA:* We synthesized colloidally stable SPIO@OEGMA in water. When the temperature was increased above the LCST, the solution became immediately became visibly turbid (Fig 2) and became transparent again when the temperature was decreased. The ST was tunable based on the monomer ratio used in synthesis and the salt concentration of the solution (Fig 3). When the temperature was raised from 33° to 37°, the t_1 of SPIO@OEGMA$_{90}$ in saline (switching, ST=35°) increased 19±2% but the t_1 of SPIO@OEGMA$_{90}$ in water (control, ST=42°) increased only 10±3%, p<0.01 (Fig 4). This heat/cool cycle was repeated three times on the particles in saline, demonstrating that particles are reversibly switchable (Fig 5). The t_2 increased 1-3% for both particles (not shown) over this temperature range. We attribute these relaxivity changes to the OEGMA, which is hydrophobic above the ST, decreasing water access to the superparamagnetic core.

Discussion: To detect contrast agents in inhomogeneous tissue, the signal must overcome the background variance. Increasing the inter-voxel variance from 0 to 10% increased the detectable concentration of a normal contrast agent by nearly 100x, but the detectable concentration of a switchable particle is independent of the background variance. We further developed an SPIO that quickly switches between on and off states when it crosses a tunable temperature threshold. This novel mechanism may be useful for molecular imaging in organs such as the liver, kidney, or spleen, which has traditionally been difficult due to the high tissue variance. These particles may also readily be combined with drug delivery or thermal ablation technologies.







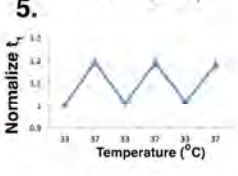


Fig. 1a: (top) Illustrative example where a contrast agent is undetectable because the signal (red) falls within the background variance (dotted black). (bottom) An on-off switchable particle can be detected by detected by cross-correlation with a reference function (blue). Fig 1b: The detection rate of normal contrast agents is highly dependent on background inter-voxel variation, whereas the detection rate of an on-off switchable particle is independent of background variance. Fig. 2: We developed a particle that may be used for on-off switching. (top) Illustration of thermosensitive SPIO@OEGMA that change relaxivity when the temperature rises above the switching temperature (ST). (bottom) photograph of SPIO-@OEGMA$_{90}$ at 37° in water (below ST) and saline (above ST). Fig. 3: ST is tunable based on the monomer ratio used in OEGMA polymerization and salt concentration. Fig. 4: Change in relaxation time of SPIO@OEGMA$_{90}$ solutions between 33° and 37°. The particles in saline solution (switching) crosses the ST in this range and t_1 increases 19%, whereas the particles in water (control) does not cross the ST and t_1 increases only 10%. Fig. 5: The t_2 of SPIO-@OEGMA$_{90}$ for three heat cool cycles showing reversible on-off switching for several heat/cool cycles.

References: 1) Jones, CK. et al. *Proc 15 ISMRM* (2007). 2) Tu, C. et al. *Tetrahedron* 2009 65;1241-1246. 3) Sun, Y. et al. *J Solid State Chem* 2007 43;762-772. 4) Massart, R. et al. *J Magnetism Mag Mater* 1995 149;1-5.