Strategies for Engineering High Relaxivity Biomolecular Contrast Agents for Functional Magnetic Resonance Imaging

By
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Magnetic resonance imaging (MRI) is a powerful imaging tool that allows non-invasive visualization of live and intact tissues with high spatial and temporal resolution. Used in conjunction with targeted contrast agents, MRI can facilitate molecular imaging of biological processes in their true physiological environments. Research on MRI contrast agents and their application to problems in neuroscience is burgeoning, and there is particular interest in developing MRI agents that are sensitive to time varying components of neurophysiology. Relatively recent development of biomolecular probes has demonstrated the potential and versatility of bioengineered MRI sensors for molecular imaging. These biomolecular probes impart novel functionality to contrast agents, such as target binding or ligand responsiveness. However, a major limitation of these probes is the high concentration needed for imaging, which can lead to issues such as analyte buffering and toxicity, and restrict the applicability of the sensors.

Developing higher relaxivity probes could circumvent these issues by allowing them to be applied at lower concentrations. In this thesis, we explored two approaches for increasing the relaxivity of protein-based contrast agents. First, we combined an existing suite of monoamine neurotransmitter sensors derived from the protein cytochrome P450-BM3 (BM3) with superparamagnetic iron oxide nanoparticles (SPIO). This mechanism coupled ligand sensing with the robust changes in relaxivity that accompany spatial rearrangement of SPIOs, which could allow the BM3 probes to be applied at concentrations of up to 100 times lower than the amount needed when nanoparticles are not incorporated. More broadly, this mechanism could be adapted to create high relaxivity sensors for other small molecule neurotransmitters with protein binding partners. The second strategy investigated the feasibility of increasing the relaxivity of the metalloprotein phenylalanine hydroxylase (PAH) by introducing mutations in the metal binding site that could improve gadolinium binding. Mutations were found that both improved the binding affinity for the lanthanide ion and enhanced the MRI signal, demonstrating the viability of using semi-rational protein design to engineer a high relaxivity protein-based agent.

The results of this thesis advance approaches for creating high relaxivity contrast agents which can be applied to the development of probes for other analytes, ultimately allowing the study of a wide variety of neurophysiological processes at the molecular level.

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