Progress in molecular imaging: using nanoparticles to image cellular targets
Principal Investigator: Diana Martinez, MD

Specific Aims

The specific aim of this Phase I proposal is to discuss and plan the procedures and experiments necessary to develop a new type of molecular imaging using superferromagnetic nanoparticles. The use of these nanoparticles would allow molecular targets to be imaged with magnetic resonance (MR) imaging, which would substantially advance the field of molecular imaging. MR based nanoparticle imaging has the potential to overcome the greatest barriers that now exist in the field of molecular imaging: the limited number of cellular targets that can be imaged and limited access to molecular imaging agents. Thus, the development of MR based nanoparticle imaging will allow molecular imaging to evolve into a field with greater clinical utility that would impact a number of medical fields, ranging from psychiatry and neurology to oncology or cardiology.

However, nanoparticle molecular imaging is still in the early stages of development. While research in biomedical engineering has greatly improved the design and safety of these biomaterials, very little translational research has occurred to move this technology closer to the clinical setting. Thus, the goal of the current proposal is to evaluate and design the studies needed to move forward with this transition. The Collaborative and Multidisciplinary Pilot Research Award is crucial for this process, since the collaboration of a wide range of multidisciplinary expertise will be needed to bring this goal to fruition. As a result, as described below, this group will include nanoparticle engineers, chemists, molecular scientists, and imaging and clinical researchers.

Significance of the proposed study

Molecular imaging emerged from the concept that *in vivo* imaging can be used to visualize cellular processes and function, in order to better understand and characterize disease. The ability to image molecular targets provides a method both for studying the underlying mechanism of disease and imaging biomarkers, which provides information on the progression of disease or response to treatment. Despite this potential, there are some significant barriers that arise in molecular imaging. As a result, few of these imaging agents have progressed to routine clinical use. For example, it the field of brain imaging, only the radioligand [18F] flourodeoxyglucose has made progress as widespread clinical biomarker, despite almost two decades of effort in this field. Molecular imaging has largely been dominated by positron emission tomography (PET) and Single Photon Emission Computed Tomography (SPECT) imaging. Both of these technologies use radiotracer imaging, which consists of incorporating a gamma ray emitting radionuclide into the chemical structure of a molecule, or ligand, which is specific for a particular cellular target (referred to as a radioligand). This technology has a number of advantages. For example, PET imaging uses ligands that are organic carbon based small molecules which are labeled with positron emitting carbon atoms.

The chemical structure of the radioligand is generally not affected by the incorporation of a positron emitting carbon, and its binding properties to the receptor on the neuron remain unchanged. In addition, PET (and SPECT) scanners have a very high sensitivity to the high energy photons emitted by the radiotracers, such that the injection of a very low concentration of the radioligand provides a robust signal.

Despite these advantages, there are a number of significant disadvantages to nuclide based molecular imaging. The first is that most radiotracers are labeled with [11C], which has a very short half-life of about 20 minutes. As a result, these radioligands must be synthesized on site, which requires an enormous and expensive infrastructure. This infrastructure includes both an onsite cyclotron to generate the positron emitting isotopes and a radiochemistry laboratory. Some radiotracers can be labeled with [18F], which has a longer half life (110 minutes) and can be shipped short distances. However, 18F labeling is often not possible, since the addition of the fluorine 18 may not be feasible on a number of organic molecules or it can change the polarity of the ligand. These limitations require an enormous investment in building and maintaining aPET/SPECT imaging center. As a result, the past decade has seen a decrease, rather than an increase, in the number of radioligand based imaging facilities engaged in the development of new radiotracers.

In addition to the required infrastructure, PET (and SPECT) radiotracer imaging is also limited to small molecule ligands that can be used as viable radiotracers. Also, the properties required of the small molecule are often difficult to meet. The radioligand must have a high affinity and specificity for a receptor in addition to low non-specific binding in vivo and fast kinetics. Thus, a ligand that is selective for a receptor may still fail as a radiotracer due to high nonspecific binding to other brain proteins (for example, fluoxetine, which has great clinical utility, but is a poor radioligand for this reason). Alternatively, a PET radiotracer that has high affinity and low specific binding may still be unusable for PET imaging if it does not bind to and disassociate from the receptor within the time frame of a PET scan, which is limited by the half-life of the nuclide and the patience of the person being scanned.

Due to these limitations, the ability to use magnetic resonance (MR) imaging for cellular targets would serve to greatly expand the field of molecular imaging. The advantages of this would be that MR based ligands, compared to radionuclides, are more stable and long-lasting (for example, the nanoparticle proposed in this application has a shelf life of 2 weeks). Thus, these could be shipped and used in any imaging facility with an MR scanner available. In addition, the structure of the nanoparticle allows a number of different ligands to be attached to it, including proteins and peptides, so that a whole new array of cellular targets can be imaged.

Despite these advantages, the transition to MR based imaging has been previously impaired by two main factors: 1) the signal required for MR imaging is much greater than that required for PET; and 2) MR detectable particles don’t enter the brain. However, recent advances in nanoparticle based MR imaging address these issues. The use of superferromagnetic nanoparticles (see figure 1) produces a detectable signal and a nanoparticle capable of entering the brain has been developed (Veiseh). These advances have been made in the realm of material science and engineering, and the goal of the present protocol is to move this technology into biological research.

The translation of nanoparticle imaging from engineering breakthroughs to clinical research can only be accomplished by combining the expertise of investigators from a wide range of fields. Importantly, it will also require communication between investigators who may know little about the each other's fields. For example, Miqin Zhang (a co-investigator from the Department of Material Science at the University of Washington) and Diana Martinez (PI of this application) first met and discussed this project a few months ago, but progress has been impaired by two major factors: 1) geographical distance; and 2) the need to better understand each other's fields of expertise. Thus the funding of this application will allow us to overcome these barriers and promote the development of this nanoparticle technology. In addition, Dr. Fei Liu, Ph.D. (chemistry), will play a key role in this project. Dr. Liu is Assistant Professor of Clinical Neurology (in Psychiatry) and is a chemist trained in radiochemistry. This project will provide Dr. Liu training in a new field of nanoparticle chemical synthesis, which could not be provided to her outside of this project. If funded, Dr. Liu would be one of very few chemists in the country with this type of expertise.

Overview of Multidisciplinary Approach

This project will allow the formation of an interdisciplinary research team with a wide range of expertise. The following investigators have agreed to participate:

Diana Martinez, M.D. (Principal Investigator)

Associate Professor of Clinical Psychiatry, Columbia University Medical Center
Dr. Martinez is a psychiatrist and clinical researcher whose work has focused on using PET radioligand imaging in drug addiction. Her prior research projects have used PET radioligand imaging to better characterize the neurochemistry of addiction, and how this can affect clinical factors, such as response to treatment. Given her years of work with PET radioligand imaging, she is very familiar with the advantages and pitfalls of nuclear molecular imaging. In addition, Dr. Martinez has done radioligand development work in non-human primates, which has expanded her imaging expertise and provided firsthand experience of the persistence required to develop new molecular imaging probes.

Fei Liu, Ph.D. (Co-investigator)
Assistant Professor of Clinical Neurology (in Psychiatry), Columbia University Medical Center
Dr. Liu is a chemist whose expertise is in the radiochemistry of PET ligands. Dr. Liu has worked to develop the chemical procedures needed to synthesize radiotracers, which requires that the synthesis be completed in a short time frame. The funding of this project would allow Dr. Liu to engage in the chemistry required to develop a new generation of molecular imaging probes. Her time and effort would be spent learning the synthetic techniques that are required to create receptor specific nanoparticles.

Dalibor Sames, Ph.D. (Co-Investigator)
Associate Professor of Chemistry, Columbia University (Morningside campus)
Dr. Sames' research focuses on developing new molecular imaging agents that can be used to investigate metabolism and neurotransmission in the brain. A key feature of his work is the development of synthetic methods that allow the development of new probes. Given his expertise in chemistry and bond functionalization, Dr. Sames will provide insight into the methods that can be used to combine novel ligands with the nanoparticles. Dr. Sames will also provide expertise with florescent imaging, which will

be used in the development stages of this technology.

Miqin Zhang, Ph.D. (Co-Investigator)
Professor of Material Sciences and Engineering, University of Washington
Dr. Zhang is professor of materials sciences and engineering whose work is dedicated to developing biomaterials, which are materials that can be introduced into biological systems in order to promote or suppress a biological response. Dr. Zhang's work is done in three main domains, tissue engineering, biosensor development, and nanoparticle research. Dr. Zhang's work on nanoparticles has made this new imaging technique possible. She has worked to develop a superparamagnetic nanoparticle that can be detected by MR imaging and can carry specific probes across the blood brain barrier.

Amy Newman, Ph.D. (Co-Investigator)
Senior Investigator, National Institute on Drug Addiction
Dr. Newman is a senior investigator in Medicinal Chemistry. Her work has focused on the design and synthesis of novel ligands to study the monoamine transporters and dopamine receptors of the brain. As described below, we will use the dopamine transporter as the target for nanoparticle based imaging, and Dr. Newman's expertise in transporter ligands and their binding properties is crucial for this project. Thus, the collaboration of both Dr. Newman and Dr. Sames will allow us to address the issue of how to start with the most appropriate ligands and how to attach these to the nanoparticles, without affecting their chemical properties.

Jonathan Javitch, M.D., Ph.D. (Co-Investigator)
Professor of Psychiatry and Pharmacology, Columbia University Medical Center
Dr. Javitch is the director for the Center for Molecular Recognition where his work focuses on the structural basis of agonist and antagonist binding to dopamine receptors and transporters. Thus, his input into this project will be critical for determining the feasibility of combining different agonists and antagonists to the nanoparticles. In addition, Dr. Javitch will provide input, in collaboration with Dr. Sames, into designing fluorescent imaging experiments that will be designed for the initial imaging projects with the nanoparticle.

Patrick Hof, M.D. (Co-Investigator)
Professor of Neuroscience, Mount Sinai School of Medicine
Dr. Hof is the director of the computational neurobiology and imaging center at the Mount Sinai School of Medicine. His work has focused on the organization and structure of the mammalian brain, and he has developed comparative brain atlases. Dr. Hof's expertise in MR imaging of rodents will be crucial for this project. The role of Dr. Hof in this project will be to develop the MR imaging methods of rodents, which will be a future step in development of this project.

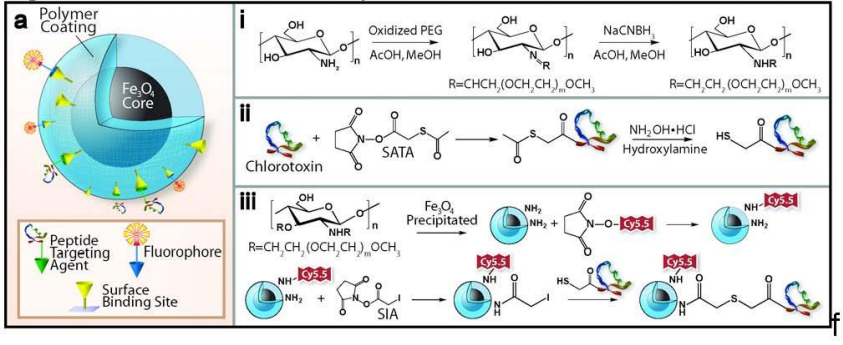
Scott Russo, Ph.D. (Co-Investigator)
Assistant Professor of Neuroscience, Mount Sinai School of Medicine
Dr. Russo was recently recruited to the department of neuroscience at Mount Sinai to continue his work in studying brain function in addiction and stress. Dr. Russo has expertise in electron and confocal microscopy, and his role will be design confirmatory microscopy studies in rodents that are planned to occur with Dr. Hof.

Although the plans for this phase 1 project do not include the acquisition of pilot data, the participation of these co-investigators is crucial for planning and troubleshooting the methods and future experiments that will be needed to accomplish this goal. The inclusion of a wide range of experts in the planning phase will help prevent future missteps in the development of nanoparticle based imaging, an advantage that is frequently lacking in this type of translational research.

Brief Description of nanoparticle based imaging

Magnetofluorescent nanoparticles can be used with high spatial resolution MR and quantitative fluorescence imaging to allow for quantitative imaging of these targets. Previously, use of these nanoparticles to image the brain has been impeded by their inability to cross the blood brain barrier (BBB) and vascular endothelium, as well as the non-specific uptake by surrounding tissues and macrophages. However, a recently developed nanoprobe by our co-investigator (Miqin Zhang, PhD) is able to cross the BBB and label brain-specific sites (Veiseh 2009). The nanoprobe (NPCPCTX-Cy5.5) is comprised of an iron oxide nanoparticle (NP) coated with a PEGylated-chitosan branched copolymer (CP), to which a targeting ligand, chlorotoxin (CTX, which binds to astrocytic tumor tissue) and a near-infrared fluorophore, Cy.5.5 were conjugated (see figure 1). The nanoparticle contains iron oxide for detection by the MR scanner. Chitosan is used as a linker and stabilizer, since its glucosamine backbone anchors the polymer to the iron oxide surface through an electrostatic interaction and physical adsorption. This alleviates the need for crosslinking agents while providing sites for subsequent conjugation of ligands. In addition, the bound chitosan also acts as a stabilizing corona, preventing particle aggregation under physiological conditions. Polyethylene glycol (PEG) is integrated into the polymer coating to reduce protein adsorption, limit immune recognition (by the reticuloendothelial system), and increase the nanoprobe serum half-life. Thus, the nanoparticle coated with the PEGylated-chitosan branched copolymer (NPCP) has the magnetic property for MR detection and also uses near-infrared fluorophore, Cy.5.5, for biophotonic imaging.

Figure 1: structure of the nanoprobe



Dr. Zhang's group has used the scorpion venom chlorotoxin (36 amino acid peptide) to bind to matrix metalloproteinases (MMP-2), an endopeptidase, which, in the brain, binds specifically to gliomas. Using a rodent model (transgenic ND2:SmoA1 mice) of medulloblastoma which does not involve compromise of the BBB, Dr. Zhang was able to show that this nanoprobe (NPCP-CTX-Cy5.5) can be detected with both MR imaging and optical fluorescent imaging. In this study, the MR imaging of the ND2:SmoA1 mice expressing cerebellar tumors (with no BBB compromise, confirmed with histology) showed significant uptake and signal detection with MR compared to the wild type mice.

Phase I Planning Activities and Timeline

The first goal of this group will be to outline the methods and experiments needed to develop proof of concept studies showing that this nanoprobe can be used to image a target in the brain. We will begin with brain imaging, since this organ is among the most difficult to image. We will also begin with a well-characterized target in the brain. For this, we chose the dopamine transporter for the following reasons: 1) the concentration of the dopamine transporter is very high in the striatum; but is low in all other brain regions, providing a circumscribed and well defined target for imaging and 2) the chemistry of the dopamine transporter is very well characterized. A number of ligands and peptides have been developed to image this transporter, and the binding site and affinity of these are well known.

Therefore, the planning phase of this grant will include the determination of the most appropriate small molecules and peptides for the dopamine transporter, with the following timeline:

In the first two months this group will complete the identification of the DAT ligands (small molecule and peptide) that will be used for the first synthesis with the following issues discussed: 1) the ligand must tolerate the addition of the bridge to the nanoparticle without affecting its binding to the transporter; 2) the particular bridge(s) to the nanoparticle must be identified; 3) the affinity and specificity for DAT binding must be known (in vitro and in vivo); 3) the time course required for imaging the DAT with nanoparticles is not known, therefore the DAT binding compounds must bind to the DAT for long periods of time (for the initial imaging studies).

In months 3 and 4 the following issues will be planned and discussed: 1) the first studies will be in vitro studies, in DAT expressing cells, using fluorescent imaging; 2) imaging and microscopy studies in rodents will be designed; 3) imaging studies in non-human primates will be planned; 4) the toxicity of the NPCP-Cy5.5 nanoprobe has been investigated in rodents (who did not show toxicity); but never in another species. This issue will be discussed and the studies needed to address this will be planned.

It should be noted that the DAT binding studies are proof of concept. A number of PET and SPECT radio probes exist for the DAT, which allows us to begin with a well known target. However, once we have established the feasibility of imaging this target, we will discuss other potential targets, which will likely involve investigators in other fields. For example, we will explore the use of this technology for targets both inside the brain and outside the brain. Thus, we envision expanding this technology to research in neurology (stoke and traumatic brain disease), oncology (imaging of tumor expansion and apoptosis), and perhaps cardiology (atherosclerotic plaque).

Long-term Aims (including plans for future funding)

Following the planning phase, the next goal will be to use the NPCP-Cy5.5 nanoprobe which labels the DAT, as a proof of concept. The following steps are required:

1. Production of nanoprobes combination with DAT binding compounds. In collaboration with Amy Newman and Dalibor Sames, we use the most appropriate DAT binding agents (using the criteria described above) for this purpose. These will include both small molecules and peptides. This chemistry will be conducted by Dr. Liu.
2. Fluorescent imaging of DAT expressing cells (in collaboration with Jonathan A. Javitch, Columbia University). HEK293 cells stably expressing human dopamine transporter (DAT) will be imaged with the nanoparticles (Cy5.5 labeled) . We will detect nanoparticle binding using Cy 5.5 fluorescence in a Pherastar plate read. Parental cells that do not express DAT, an excess of ligand that binds to DAT and thereby prevents binding of the nanoparticles, as well as nanoparticle that are not coupled to the DAT ligand, will all serve as controls to establish that the binding is specific.
3. MRI and microscopy studies in rodents will be performed once the in vitro work shows feasibility, in collaboration with Drs. Hof and Russo. Multiecho multislice imaging will be performed on a 9.4 T magnet. Spin-spin relaxation time T2 maps will be generated by multi-echo images. Scans will be performed with both the DAT binding and control nanoparticles. The animals will then be sacrificed for microscopy studies, using excess of ligand that binds to DAT. Microscopic images of tissue will be acquired using an E600 upright microscope (Nikon) equipped with a CCD color camera.
4. Imaging studies in nonhuman primates. This will be done by Dr. Martinez (with imaging collaborators). The methods of this study will be determined by the results of the prior studies. Notably, to date almost no imaging studies have been performed in nonhuman primates, even though this step is crucial for using nanoparticles in clinical research. In the event that DAT binding is not seen in the studies above, we will explore other targets (both inside and outside of the brain) and go back to step one. In the event that the above studies do show specific DAT binding we will also begin with step one and develop nanoparticles for other new targets.

Ultimately, the receipt of these funds will allow the planning phase to be accomplished. If received, we will apply for the phase 2 CAMPR support, which will pay for the acquisition of pilot data. In the even that we are successful in detecting a signal, and R01 will be submitted for the development of nanoparticle imaging.

References:

1. O. Veiseh, J. W. Gunn, M. Zhang, *Advanced drug delivery reviews* **62**, 284-304 (Mar 8).
2. O. Veiseh *et al.*, *Cancer research* **69**, 6200-7 (Aug 1, 2009).

Budget requested

1. Salary support
   Salary support is being requested. This project will provide training in a new field of nanoparticle chemical synthesis, which could not be provided outside of this project. I
2. Travel and meeting funds
   Travel funds are requested for the following: 1) Travel to New York in order to meet with investigators here; and 2) Travel to Seattle Washington in order to learn more about nanoparticle technology.

The remaining co-investigators have agreed to participate without requesting support.