

Development of Absorption and Fluorescence Probes Based on Mouse Model for Molecular Optical Imaging

Lixin Ma¹ and Ping Yu²

¹Department of Radiology and International Institute of Nano and Molecular Medicine, University of Missouri, Columbia, MO 65203 Biomolecular Imaging Center, Harry S. Truman Memorial Veteran's Hospital, Columbia, MO 65203 Mal@health.missouri.edu ²Department of Physics and Astronomy, University of Missouri, Columbia, MO 65211 yuping@missouri.edu

In this work we summarize our collaborative research on a project to develop absorption and fluorescence targeting probes. Several groups from University of Missouri and Harry S. Truman Memorial Veteran's Hospital including Dr. Ma's group, Dr. Yu's group, Dr. Smith's group, Dr. Hoffman's group, and Professor Wynn Volkert have been involved in the project. Our goal is to develop probes based on mouse model for molecular optical imaging.

In vivo imaging of targeted fluorescence molecular probes, or molecular imaging, is an emerging field in biomedical imaging. During the past forty years, three dimensional biomedical imaging technologies such as CT and MRI have been extensively used in human health and diseases. However, the human body is a complex and interactive biological system. A fundamental scientific barrier in previous biomedical imaging technologies is their limited ability to study physiological processes in vivo at the cellular and molecular levels. Molecular imaging technologies can overcome this barrier. Optical imaging modalities have the highest sensitivity compared to other imaging techniques. So they are good candidates for molecular imaging. We develop probes for two biomedical optical imaging techniques. The first technique is coherence domain imaging. This technique can be used to monitor interactions between targeted peptide conjugates and cancer cells at a tissue level. It requires absorption properties of the probe for effective molecular imaging. The second technique is fluorescence mediated tomographic imaging using an image-intensified CCD camera. This technique uses fluorescence of the probe for molecular imaging.

Dye bombesin conjugates are synthesized for site-specific absorption and fluorescence imaging in human prostate and breast cancer cells. Bombesin (BBN), an amphibian analog to the endogenous ligand, binds to the gastrin releasing peptide receptors (GRPr) with high specificity and affinity. BBN conjugates have a specific significance in cancer detection and therapy due to high over-expression levels of GRPrs in human cancer cells. Previously, we have developed an Alexa Fluor 680 BBN peptide conjugate. This probe can not be used as an absorption probe in near-infrared imaging since its absorption peak is in the visible wavelength range. In addition, long wavelength fluorescence is desired because long wavelength photons can penetrate deeper into

tissue when using the conjugates as a fluorescent probe. The new absorption and fluorescent probe we developed is based on the last eight-residues of BBN and labeled with Alexa Fluor 750 through an effective linker. The developed probe, AF750-BetaAla-BBN[7-14]NH₂, exhibits optimal pharmacokinetic properties for targeting GRPr over-expressing cancer cells in mice. Absorption spectra have been measured and showed absorption peaks at 690nm, 720nm and 735nm. Fluorescent band is located at 755nm. Fluorescent microscopic imaging of the conjugates in human PC-3 prostate cancer and T-47D breast cancer cells indicated specific uptake and internalization in vitro. In vivo optical and MR imaging was performed in SCID mice bearing human breast and prostate xenografts. In vitro and in vivo studies have demonstrated the effectiveness of the fluorescent probe Alexa Fluor 750-BetaAla-BBN[7-14]NH₂ to specifically target GRPr overexpressed cancer tissues.