

Optical Imaging Probes for *in vivo* Tumor Biology

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Introduction

Optical imaging probes that fluoresce in the Near Infrared (NIR: 650-950 nm) are increasingly useful for *in vivo* studies of cellular and molecular events central to neoplasia and host response. Reactive fluorescent dyes are commonly used to label biopolymers and small molecules to produce optical probes. It is critical to label a probe to retain its targeting properties while minimizing pharmacokinetic perturbations due to the labeling agent. Multiplex detection of distinct probes can reveal simultaneous and interactive events that are the basis of pathology and treatment. Vascular imaging can be used to study vascular structure, function, and events in angiogenesis. We have developed contrast agents with transient vascular imaging properties ranging from a few minutes to a few hours. We show the determination of an optimal degree of labeling (DOL; mole fluorophore/mole probe) for targeted antibodies with an NIR Alexa Fluor® dye and demonstrate transient imaging and multiplexed imaging of vascularized tumors in nu/nu mice.

Animal Protocols, Imaging, and Image Analysis

All animal work was performed in accordance with AALAC guidelines. Athymic nude (nu/nu) mice carrying LS174-T (ATCC) human colon cancer xenografts were used for all imaging experiments. The Anti-carcinoembryonic antibody (anti-CEA; clone Col-1) was conjugated with Alexa Fluor NIR dyes using a SAIIVI Alexa Fluor 750 antibody/protein labeling kit (S30040) or a SAIIVI Alexa Fluor 680 antibody/protein labeling kit (S30039). Images were acquired with the Maestro In-Vivo Imaging System (Cambridge Research and Instrumentation), at the indicated excitation wavelength (Ex), and emission range (Em). The image cubes were spectrally processed using the Maestro software; the emission of the introduced fluorophore was isolated from the autofluorescence and quantified as indicated on the corresponding graphs

Over-labeling Increases Non-Specific Liver Fluorescence

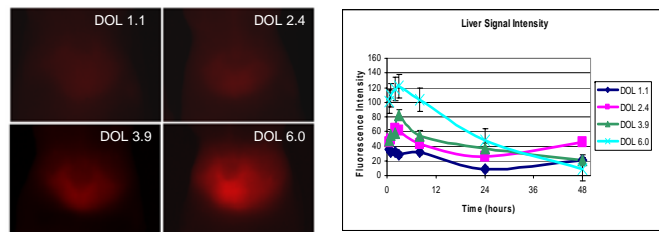


Figure 1: The intensity of conjugate accumulation in the liver *in vivo* is proportional to the number of fluorophores conjugated to the antibody. At higher than optimal DOLs, the intensity of the liver signal can overwhelm the target signal. The anti-CEA antibody was conjugated to 4 different DOL's (1.1, 2.4, 3.9, 6.0). 50 µg of the conjugates were injected and the mice imaged prone at 1, 2, 3, 8, 24, and 48 hours. Ex: 735 nm; Em: 790-950 nm.

Optimal DOL for Targeted Labeling *in vivo*

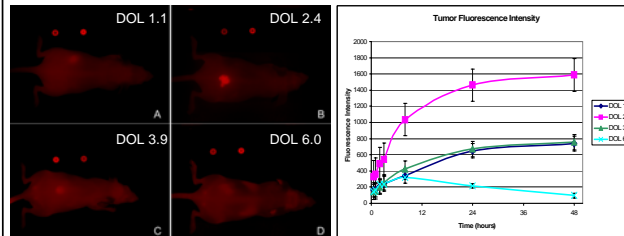


Figure 2: The intensity of target labeling *in vivo* is not proportional to the number of fluorophores conjugated to the antibody. Increasing the DOL above the optimal level causes a decrease in signal intensity. The anti-CEA antibody was conjugated to 4 different DOL's. 50 µg of the conjugates were injected and the mice imaged supine at 1, 2, 3, 8, 24, and 48 hours. Ex: 735 nm; Em: 790-950 nm.

Transient Labeling of Tumor Vasculature with Bovine Serum Albumin

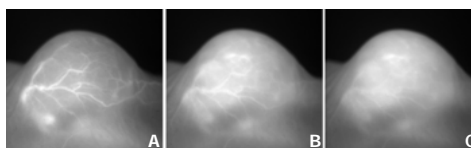


Figure 3: Tumor vasculature labeled with SAIIVI Alexa Fluor 750 Injectible Contrast Agent, bovine serum albumin (S34789). Imaged at 5 min (A), 1 hour (B) and 2 hours (C) post-injection. Ex: 735 nm; Em: 790-950 nm.

Quantum Dot Labeling of Tumor Vasculature

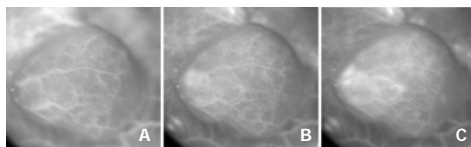


Figure 4: Tumor vasculature labeled with QTracker® 655 Non-Targeted Quantum Dots (Q21021MP) Imaged at 5 min (A), 1 hour (B) and 2 hours (C) post-injection. Ex: 465 nm; Em: 790-950 nm.

Multiplexing: Tumor Cells and Vasculature

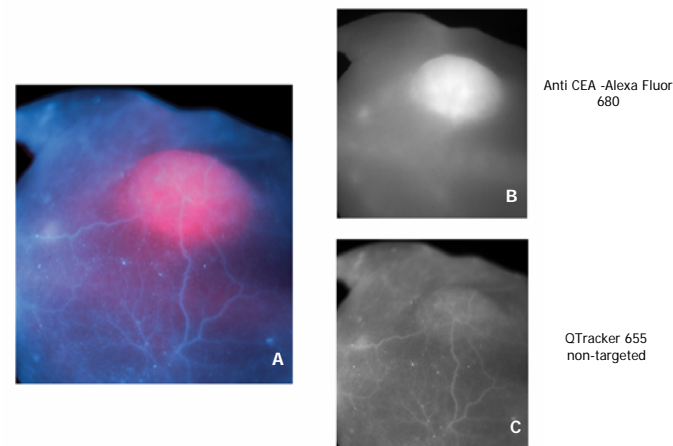


Figure 5: Panel A: Composite image of an Anti-CEA-Alexa Fluor 680 antibody conjugate (DOL: 2.6) labeling tumor mass (red) and QTracker 655 non-targeted quantum dots (Q21021MP) labeling vasculature (blue). Images B and C show the spectral and spatial separation of the two labels. The quantum dots were injected 48 hours after injection of 50 µg of antibody conjugate, and the image acquired 30 minutes later.

Results and Conclusions

- The degree of labeling (DOL) of fluorescent targeting antibodies influences the signal associated with the targeted site and clearance of the antibody from the circulation.
- Optimal fluorescent signals from targeted sites were observed with antibodies labeled with fluorescent dyes at a DOL of 3 or less. Antibodies with higher DOL have decreased targeted signal *in vivo* (Figure 2). This is likely due to clearance of the high DOL antibodies from the circulation by the liver (Figure 1).
- We demonstrate two vascular contrast agents with distinctly different behaviors. The extravasation rate of Alexa Fluor conjugated BSA is rapid in comparison to the Qtracker Non-Targeted Quantum Dots, which showed little or no extravasation up to 2 hours post-injection.
- We demonstrate multiplex imaging of a tumor-targeted antibody in a vascular milieu.