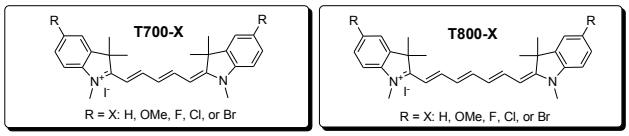
Structure-Inherent Targeted Contrast Agents for Parathyroid Glands

Hoon Hyun[1*], Min Ho Park[1,2*], Eric A. Owens[3,*], Maged Henary[3,**], and Hak Soo Choi[1,**]

[1]Center for Molecular Imaging, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA 02215
[2]Department of Surgery, Chonnam National University Medical School, Gwangju 501-746, South Korea
[3]Department of Chemistry, Georgia State University, Atlanta, GA 30303

Statement of Purpose: A fundamental problem in image-guided surgery and tissue engineering is the development of contrast agents that are targeted to specific normal or diseased tissues.[1-4] Recently, near-infrared (NIR) dyes have become the imaging technology of choice because of relatively low photon tissue attenuation/autofluorescence, and target detection depths up to 5 mm. Almost all targeted NIR fluorophores described to date require covalent conjugation of a targeting domain to an NIR fluorophore.[1] Although this strategy can work well, NIR fluorophores are relatively large molecules (500–1200 Da) to begin with, and chemical synthesis of conjugates can sometimes be time-consuming. An alternative, albeit difficult, approach is to create NIR fluorophores whose inherent chemical structure provides specific targeting to a tissue of interest.[3] The goal of this study was to create families of halogenated NIR fluorophores that exhibited specific uptake in parathyroid glands, thyroid glands, or both by virtue of their inherent chemical structure. Such molecules could provide surgeons with unambiguous guidance during head and neck surgery after a simple intravenous injection.[4]

Methods and Materials: After initial screening of a large NIR fluorophore library, the performance of a series of thyroid and parathyroid targeted NIR fluorophores were quantified in mice, rats, and pigs. Dose ranging using 0.2 – 2.0 mg/kg of fluorophores, and its imaging kinetics over a 24 h period were tested for specific thyroid and parathyroid glands targeting in each species using an imaging system.



Results and Discussion: By varying the side chains of the polymethine core, it was possible to systematically modify hydrophilicity, hydrophobicity, polarity, and electron resonance. All fluorophores exhibited max absorption and fluorescence in the NIR window, and high extinction coefficients and quantum yields, which together minimize tissue autofluorescence and maximize target signal.

To provide surgeons with unambiguous landmarks during head and neck sugary, we exploited the dual-NIR channel capability of the FLARE imaging system to highlight parathyroid and thyroid glands simultaneously and in real-time. For initial experiments we chose rat because rat possesses a single pair of parathyroid glands located on the anterior and lateral aspect of the thyroid lobes. For dual-channel imaging of parathyroid and thyroid glands, 0.2 µmol (0.35 mg/kg) of T800-F was intravenously injected into a 250 g SD rat 24 h prior to imaging, followed by 0.2 µmol of T700-F injected into the same animal 6 h before imaging. The doses and timing used were the optimal ones found during initial tests in rats (data not shown). Under these conditions, T800-F visualizes parathyroid glands unambiguously, whereas T700-F simultaneously highlights thyroid glands (Fig. 1a). The identities of resected tissues were confirmed using NIR fluorescence microscopy and consecutive H&E staining (Fig. 1b). As expected, T700-F was seen staining both thyroid and parathyroid glands, while T800-F remained only in parathyroid gland.

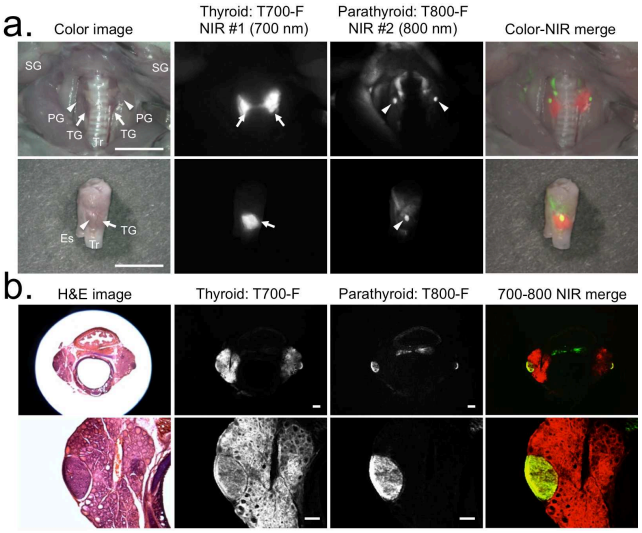


Fig 1. a) Dual-channel *in vivo* fluorescence imaging using T700-F and T800-F in the same rat. 0.35 mg/kg of T800-F was intravenously injected into a 250 g SD rat 24 h prior to imaging, followed by injection of T700-F 6 h prior to imaging. b) H&E and NIR imaging of resected parathyroid and thyroid tissues from (a). Scale bars = 300 µm.

Conclusions: In summary, the ability to highlight parathyroid and thyroid glands after simple intravenous injection, and to identify each gland simultaneously using the dual-NIR channel capability of the FLARE™ imaging system, suggests that head and neck surgery may someday be performed with increased precision and lower morbidity.

References:

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3. Phosphonated near-infrared fluorophores for biomedical imaging of bone. **Angew Chem Int Ed.** (2014).
4. Structure-inherent targeting of near-infrared fluorophores: Parathyroid and thyroid gland imaging using fluorinated polymethines. **Nat Med.** (2014).