Contrast-enhanced molecular photoacoustic imaging of aggressive prostate cancer using fluorescence-quenching prostate-specific membrane antigen (PSMA)-targeted dye

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Background, Motivation, and Objective: PSMA is a type II integral membrane protein that can produce selective expression on aggressive PCa cell surface more than indolent. Our recent study presented a PSMA-targeted agent providing a contrast in spectroscopic photoacoustic (sPA) imaging at PSMA-expressive tumor *in vivo*. However, the preclinical study still has limited implication in human translation, because there must be far significant degradation of sensitivity due to optical/acoustic attenuation when sensing through thick human prostate tissue.

Statement of Contributions: In this paper, we investigate a contrast-enhancing sPA imaging strategy for highly-sensitive detection of PSMA-targeted agent. Currently we are developing fluorescence (FL)-quenching mechanism on multi-scaffold IRDye800CW on a dendrimer structure bound on PSMA-binding ligand. From the newly-configured PSMA-targeted dye (5D3), optical properties were characterized by spectrophotometry and spectrofluorometry as well as tubing phantom experiments in water tank. The preserved PSMA affinity of 5D3 was also validated in *in vitro* experiment using isogenic PSMA+ PC3-PIP and PSMA- PC3-flu cell cultures. The sPA data in phantom and *in vitro* experiments were collected by a tunable Nd:YAG OPO laser (700-900nm, Phocus Inline, Opotek, Inc., U.S.) and US research package (Vantage 256, Verasonics Inc., U.S.).

Results, Discussion and Conclusions: Theoretical analysis using spectrophotometry, spectrofluorometry, and tubing phantom data indicated up to 164.7-fold quantum yield decrease from the 5D3 when compared with original IRDye800CW at the same molar concentration (10μM): 0.001 vs. 0.140 for 5D3 and IRDye800CW, which corresponds to 0.999 and 0.860 in FL quenching efficiency (Figure 1a). This led up to 8.7-times reciprocal PA contrast enhancement at 780nm (Figure 1b). The PSMA targeting specificity of the complex was validated with 2.94-times higher PA intensity on the PSMA+ PC3-PIP cell suspension than that due to the suspension of PSMA- PC3-flu cells: 1.00±0.07 vs. 0.34±0.02 of normalized intensities at 780nm (Figure 1c). We will pursue *in vivo* validation of the contrast-enhanced sPA imaging of the novel PSMA-targeted agent.

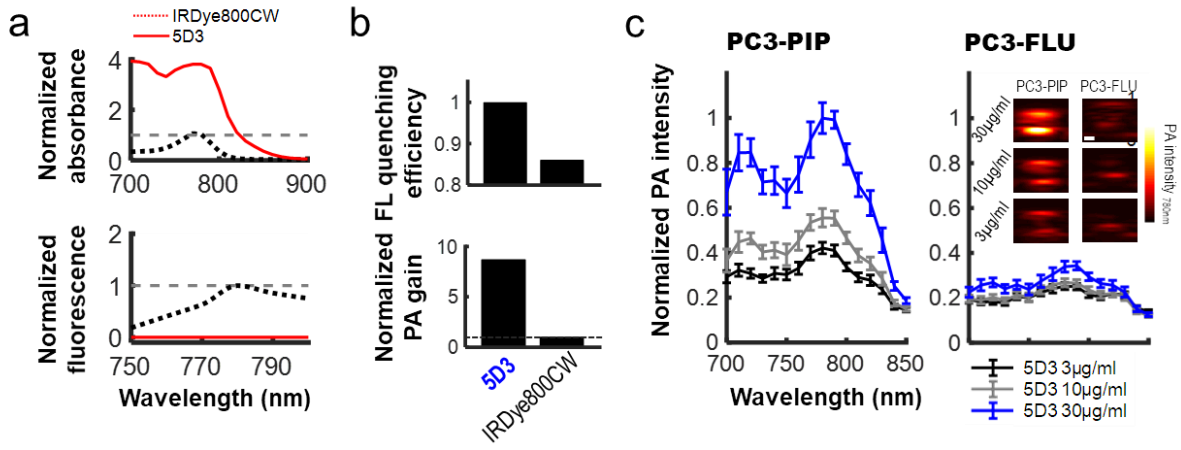

Figure 1 Fluorescence (FL)-quenched PSMA-targeted agent for spectroscopic photoacoustic (PA) imaging. (a) Normalized absorbance and FL emission of IRDye800CW dye and our PSMA-targeted dye, i.e., 5D3; (b) Quenching efficiency and normalized PA gain of 5D3 compared to IRDye800CW contrast; (c) The PSMA affinity of 5D3 to PSMA$^+$ PC3-PIP and PSMA$^-$ PC3-FLU cells at different concentration.