Rational Design of ICG-based Contrast Agents for Near-infrared Photoacoustic Imaging

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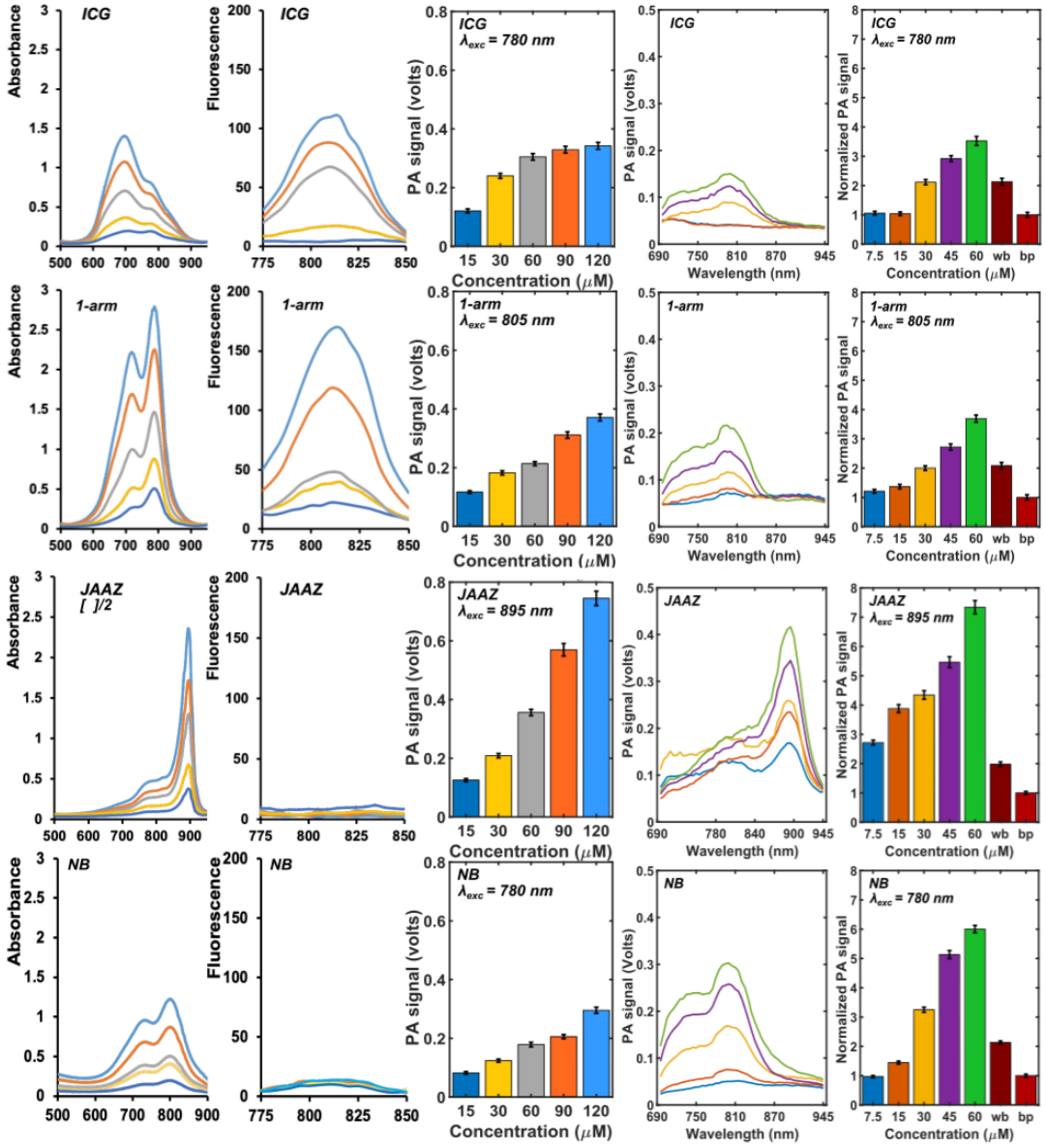
Photoacoustic imaging (PA) is a non-invasive technique that relies on the absorption of laser light and the subsequent thermoelastic expansion of biological tissues for generating ultrasound signals. To reduce photon scattering in biological tissues and enhance imaging depth, near-infrared (NIR) illumination is employed in PA imaging. However, adoption of this technology and clinical translation are hindered due to a lack of NIR-PA contrast agents (CA). Indocyanine green (ICG) dye is a viable candidate due to its NIR optical absorption, biocompatibility, and approval by the FDA, and demonstrated use in PA imaging. However, ICG has a few shortcomings, including concentration-dependent optical-absorption signature and lack of facile targeting strategies. Ideal NIR-PA CA should have the following characteristics: 1) NIR optical absorption; 2) optical and structural stability *in vivo*; 3) facile bioconjugation; and 4) strong PA signal-to-background ratio. To address this unmet need, we examined three novel ICG-based CA platforms, namely a DNA-ICG nano-construct, an ICG J-aggregate (JAAZ), and an ICG-nanobubble and compared them to free ICG. These platforms possess distinct optical and PA signatures, and can be readily conjugated with targeting molecules, antibodies, or aptamers.

DNA-based probes were synthesized using tile assembly with oligonucleotides bearing ICG dyes or targeting moieties. JAAZ were synthetized by incubating different molar ratios of ICG and ICG-azide dye at 60°C in presence of KCl. The shell-stabilized ICG-nanobubbles were produced by incorporating ICG in lipid solution followed by mechanical agitation, and size filtering. The optical properties of the CAs were characterized using a customized spectrophotometer and a fluorescence plate reader for free ICG concentrations ranging from 15 to 120 μM. The PA signal was measured in buffer and blood in tube phantoms at the same concentrations.

In PBS, the absorption peak of free ICG exhibited a shift from monomeric (780 nm) to dimeric (700 nm) form with increase in concentration. When templated on DNA nano-scaffolds, ICG remains in monomeric form with an absorption peak that appears slightly red-shifted (~805 nm), at all concentrations. The JAAZ showed a strong, red-shifted absorption peak at 895 nm. ICG-nanobubbles showed an optical absorption signature that was similar to plain ICG but with a higher absorption peak. Fluorescence spectrometry measurements revealed an increase in fluorescence intensity as a function of ICG concentration in the DNA nano-constructs. However, the JAAZ showed almost no fluorescence upon optical excitation, suggesting strong fluorescent quenching effects, which are conducive to PA-based sensing and imaging. ICG-nanobubbles also exhibited diminished fluorescence signal, similar to JAAZ, likely due to localized dye aggregation as a result of thermal effects associated with nanobubble synthesis. Consistent with absorbance profiles, the JAAZ exhibited the highest PA-signal amplitude when CAs were examined in PBS. When mixed in whole blood, JAAZ and ICG-nanobubbles produced the strongest PA signal, which

was more than six times stronger than that from whole blood. All CAs produced a PA signal that is stronger than that from whole blood at concentrations above 45 μM. Interestingly, the JAAZ showed a PA signal stronger than whole blood at a concentration as low as 7.5 μM.

These CA platforms represent a promising alternative to free ICG for biomedical applications encompassing biomarker research, drug development, and whole-brain neuroimaging. Surface plasmon resonance indicated that all three platforms are amenable for molecular targeting. Similar to ICG, DNA-ICG probes are bimodal and can be used for optical fluorescence and PA imaging. ICG-nanobubbles are acoustically active and can be used for ultrasound and PA imaging. *In vivo* imaging indicated that JAAZ construct produced a stronger PA signal in whole-body mouse imaging compared to free ICG and the signal persisted for over 90 minutes.



Optical properties, PA signals in PBS and blood, and normalized PA signals in blood for ICG-based platforms (ICG: free dye, 1-arm: 1D DNA-ICG construct, JAAZ: ICG J-aggregates, NB: ICG nanobubbles)

250 words abstract

Near-infrared photoacoustic imaging (NIR-PA) can enable deep-tissue imaging. However, broad adoption and clinical translation are hindered due to a lack of biocompatible, targeted, and optically stable contrast agents. While indocyanine green (ICG), an FDA-approved NIR dye, represents a promising candidate, its concentration-dependent optical absorption and lack of facile targeting strategies restrict its use as a NIR-PA contrast agent. To address this unmet need, we examined three novel strategies for synthesizing ICG-based contrast-agent platforms, which are DNA-ICG nanoprobes, ICG J-aggregates (JAAZ), and ICG-nanobubbles. Our results demonstrate that all contrast agents produced a PA signal stronger than that from whole blood at concentrations above 45 μM and as low as 7.5 μM for JAAZ. Surface plasmon resonance was used to confirm molecular targeting. In comparison to free ICG, DNA-ICG nanoprobes, and ICG-nanobubbles remained in monomeric form at all concentrations resulting in a predictable optical absorption signature in vivo. JAAZ showed a strong, red-shifted absorption peak with a profile distinctly different from endogenous chromophores. JAAZ and ICG-nanobubbles exhibited reduced fluorescence signals making them particularly conducive for PA-based sensing and imaging. Similar to free ICG dye, DNA-ICG probes are bimodal and can be used for optical fluorescence and PA imaging. ICG-nanobubbles are acoustically active and can be used for ultrasound and PA imaging. In vivo imaging indicated that JAAZ constructs produced a stronger PA signal in whole-body mouse imaging compared to free ICG, and the signal persisted for over 90 minutes.

100 words abstract

Near-infrared Photoacoustic imaging (NIR-PA) can enable deep-tissue imaging, yet clinical translation has been hindered by a lack of suitable NIR-PA contrast agents. The FDA-approved Indocyanine green (ICG) dye is a promising candidate, but it offers limited targeting ability and poor stability. To address this unmet need, we examined three novel ICG-based platforms in the form of DNA scaffolds, J-aggregates, and nanobubbles. We demonstrated that all three platforms yield a PA signal stronger than whole blood at concentrations as low as 45 μM and are amenable to molecular targeting.

Keywords: Indocyanine green, Photoacoustic imaging, Contrast agent platform, Near-infrared optical absorption, Fluorescent dye, Scaffold bioconjugation, Aggregation, Encapsulation,