Supporting Information

DNA Aptamer-Based Activatable Probes for Photoacoustic Imaging in Living Mice

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1. Materials and Methods

4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), and human serum were purchased from Sigma-Aldrich, Inc. (St. Louis, MO). Human alpha thrombin was obtained from Haematologic Technologies, Inc. (HTI). Agarose LE was purchased from Gold Biotechnology. Fluorinated ethylene propylene (FEP) tubing with a wall thickness of 0.01” and inner diameters measuring 0.12” or 0.08” were purchased from McMasterCarr. Other chemicals used to prepare different solutions included the following: Ethylenediaminetetraacetic acid, sodium free (EDTA) (Fluka), MgCl₂.6H₂O (Alfa Aesar). All buffers, and metal ion solutions were prepared with Milli-Q water. The pH values of the buffers were determined using a Fisher Scientific Accumet AB15 pH meter.

The following HPLC-purified oligonucleotides were purchased from Integrated DNA Technologies, Inc. (Coralville, IA): from left to right: 5’ to 3’
Thrombin aptamer (TBA):
CCT GCCACGCTCCGCT CACTGT GGTTGGTGTGGTTGG
IRDye-labeled DNA (FDNA) for Thrombin sensor:
/IRDye 800 CW/ GCGGAGCGTGGCAGG
Complementary DNA strand for DNA-induced disassembly:
CCA ACC ACA CCA ACC ACA GTG A
The following HPLC-purified oligonucleotides were purchased from TriLink BioTechnologies (San Diego, CA): from left to right: 5’ to 3’
QC-1-labeled DNA (QDNA) for thrombin sensor:
CAACCACAGT-(C6-NH) (IRDyeQC-1)

Buffers used in this work:
HEPES buffer: pH 7.4, 0.01 M HEPES, 0.1 M KCl, 0.001 M MgCl₂, 1 mM EDTA
1x PBS buffer: pH 7.4

2. Spectroscopic Methods

UV-vis spectrophotometry was recorded on a Hewlett-Packard 8453 UV-Vis spectrometer. Fluorescence measurements were carried out on a Fluoromax-2 fluorimeter (HORIBA Jobin Yvon)
inc., Edison, NJ). Photoacoustic images were recorded with an Endra Nexus 128 photoacoustic tomography system (Ann Arbor, MI, USA), and the data were analyzed using OsiriX Lite 8.5.1.

3. Preparation of Tissue Phantoms for In Vitro Test

Tissue phantoms were prepared according to previous literature. Briefly, 4.0 g of agarose was dissolved in a solution of 2% milk (8.0 mL) and deionized H₂O (72.0 mL). The suspension was heated in a microwave oven for 1 min to afford a viscous gel which was poured into a mold made from a 50 mL centrifuge tube. The gel was cooled in a refrigerator for a minimum of 5 hrs. Immediately prior to use, the phantom was removed from the mold and cut with a razor such that there was 1.0 cm of gel above and below where the FEP tubes were inserted.

4. Preparation of the DNA probe for thrombin detection

The DNA probe was prepared by mixing FDNA, QDNA, and TBA in HEPES buffer with a final ratio of 1:1:1. To obtain the thermal denaturation profile of a particular reaction mixture, the DNA solution was heated to 90 °C for 2 min, and stored at 4 °C overnight to allow full hybridization. The final concentrations of the DNA probe for fluorescence and PA measurements were 500 nM and 1.0 μM, respectively.

5. In vitro PA test

100 μL of the DNA probe were treated with thrombin in different concentrations for 30 min at room temperature. The sample was subsequently added into the FEP tube, sealed and insert into the phantom. The PA signal was acquired at 725 and 780 nm, respectively. For the PA test in human serum, different concentrations of thrombin were first spiked into human serum, and then mixed with the DNA probe.

To investigate the DNA-induced disassembly of DNA probe, 100 μL of the DNA probe were incubated with the partially complementary DNA strand of TBA in different concentrations for 30 min at room temperature. The sample was subsequently added into the FEP tube, sealed and insert into the phantom. The PA signal was acquired at 725 and 780 nm, respectively.

The selectivity test was performed by incubating the DNA probe with different protein or protein mixture under the identical experimental conditions.

6. Animals.

Six week old BALB/c mice (male) were purchased from Jackson Laboratories. Food and water were available ad libitum. All animal experiments were performed in compliance with the guidelines established by the Illinois Institutional Animal Care and Use Committee (IACUC) of University of Illinois at Urbana-Champaign. For all the animal studies, mice were randomly allocated to each group. The investigator was aware of the group allocation during the animal studies, as demanded by the experimental designs.

7. In Vivo PA Imaging of Thrombin

The mice were anesthetized using 2% isoflurane in oxygen. Their flanks were shaved and further treated with hair removal creme. The mice were placed in the Endra Nexus 128 PA imaging system and the background signal from endogenous species was determined at 725 nm and 780 nm before subcutaneous injection of the DNA probe (50 μL, 12.5 μM) in both, left and
right flank. After recording the PA signals of the DNA probe, 10 units of thrombin (50 μL, 1.5 μM) were injected subcutaneously at the same position where the DNA probe was localized in the left flank. After incubation for 45 min, the PA signal at 725 nm and 780 nm was acquired using the “continuous rotation” mode (12s rotation time) of the PA system. Data acquisition of a single set of data required <45 s. The data for every time point for each of the individual animals was obtained in triplicates and subsequently averaged. As a control, 50 μL of PBS buffer was injected on the right flank after injection of the probe, and the PA signal was collected after 45 min. Data acquisition followed the procedure that was used for the left flank.

Figure S1. Chemical structures of IRDye 800CW and the quencher IRDye QC-1.

Figure S2. Fluorescence measurements for monitoring the assembly and disassembly process of the DNA probe in response to thrombin.
Figure S3. Photoacoustic imaging of the DNA probe with the excitation wavelength of 725 nm, 780 nm, and 825 nm, respectively.

Figure S4. a) Scheme of a DNA-induced disassembly of the DNA probe using the partially complementary DNA strand of TBA. b) Photoacoustic imaging of cDNA with different concentrations. c) Normalized PA signal ratio at 780/725 nm in response to cDNA. $P < 0.005$
Figure S5. Selectivity test. a) Photoacoustic imaging of the DNA probe in response to competing protein or protein mixture. b) Normalized PA signal ratio at 780/725 nm. The concentration of competing protein is 1.0 μM, while the concentration of thrombin is 250 nM. The protein mix contains 250 nM thrombin, and 1.0 μM of BSA, Cytochrome C and streptavidin.
Figure S6. In vivo PA imaging of DNA probes in response to PBS (a) and thrombin (b). The PA images represent the subtraction signals of PA$_{725}$ from PA$_{780}$ on both right and left flanks in living mice. c) Time-dependent change of the normalized PA signal ratio at 780/725 nm in response to PBS and Thrombin.

Reference
1. Li, H.; Zhang, P.; Smaga, L. P.; Hoffman, R. A.; Chan, J. J. Am. Chem. Soc. 2015, 137, 15628.