Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Prussian blue nanoparticles operate as a contrast agent for enhanced photoacoustic imaging

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1. Reagents

$FeCl_3 \cdot 6H_2O$, $K_4[Fe(CN)_6] \cdot 3H_2O$ and citric acid obtained from Sinopharm Chemical Reagent Co., Ltd were of analytical reagent grade and used as received. All the aqueous solutions were prepared with deionized water (18.2 MΩ cm) from a Milli-Q purification system.

2. Experimental section

All experimental procedures were performed in compliance with the relevant laws and institutional guidelines for the care and use of laboratory animals in Peking University, Beijing, China, and the experiments were also approved by the animal ethics committee of Peking University.

2.1 Fabircation of Prussian blue nanoparticles (PB NPs)

PB NPs were prepared in accordance with the previous literature.[1, 2] Briefly, 0.5 mmol citric acid was added in to 20 mL $FeCl_3$ aqueous solution (1.0 mM) under stirring at 60 ℃. Then, 20 mL $K_4[Fe(CN)_6]$ aqueous solution (1.0 mM) containing the same amount of citric acid was added dropwise into the above solution under stirring at 60℃. A clear bright blue dispersion formed immediately during the mixing process, which was allowed to cool to room temperature under stirring for another 30 min at room temperature.

2.2 Characterization of PB NPs

The morphology of PB NPs was observed by TEM (FEI Tecnai G2 Sphera Microscope) with a CCD camera operated at 100 kV. UV-vis absorption spectra of PB NPs were recorded on a Cary 4000 UV-vis spectrophotometer (Varian, USA) with a quartz cuvette of 1 cm optical path length. Zeta potential of PB NPs was measured by a 90Plus/BI-MAS instrument (Brookhaven Instruments Co., U.S.A).

2.3 Equipment for PAT

The equipment for PAT experiments was set up in accordance with previous reports.[3,4] The photoacoustic signals were excited by using a Q-switched Nd:YAG laser (LS -2137/2, LOTIS TII, Minsk, Belarus) and a pumped tunable Ti:sapphire laser (LT- 45 2211A, LOTIS TII, Minsk, Belarus). A ground glass lens was used to homogenize the laser beam and make the homogenized laser beam irradiate directly onto the test samples. The incident energy density of the laser beam on the surface of test samples (765 nm, pulse repetition rate of 2.25 Hz) was controlled to be less than 10 mJ cm^{-2}. The ultrasound signals were detected by an unfocused ultrasonic transducer with a central frequency of 2.25 MHz. The sample and transducers are both immersed in a tank filled with water which be used as medium for coupling the photoacoustic waves to the transducers. 2D scanning images were obtained by the ultrasonic transducer driven by a step motor to continuously scan horizontally along a 12-cm diameter circle around the sample for 6 min. An oscilloscope at a sampling rate of 20 MHz was used to amplify and record the photoacoustic signals detected by the ultrasonic transducer. The photoacoustic images were reconstructed through a modified back-projection algorithm and the contrast to noise ratio (CNR), signal-to-noise ratio (SNR) and noise-equivalent sensitivity was acquired according to the previously reported method.[5]

2.4 Imaging Gel Embedded under Chicken Breast Tissues

To investigate maximum tissue imaging depth, five PB NPs loaded agar gel cylinders were embedded in agar gel with the diameter of about 2.5 cm. Then，the PB NPs embedded agar gel was placed under chicken breast muscle with depths of 0, 1.2, 2.2, 3.4 and 4.3 cm, respectively. In addition, as references, two carbon rods were placed in the same plane as the agar objects, respectively. When excited by the laser pulse, the photoacoustic signals emitted from the PB NPs embedded agar gel were detected by the circularly scanning transducers and fed into the image reconstruction on the basis of a back-projection algorithm. The tissue's crosssection containing the embedded objects was imaged when blocks of chicken breast muscle were sequentially stacked to make the embedded objects at the desired depths from the

laser-illuminated tissue surface.

2.5 PA Imaging Mouse Brain Vasculature

The animal experiment was implemented in accordance with the guidelines of the Institutional Animal Care and Use Committee of Peking University. Kunming mice were used for the in vivo photoacoustic imaging experiment, and the operation was done in triplicate. Before imaging, the hair was removed from each mouse's head by using hair remover lotion. Being covered by a thin layer of ultrasonic coupling gel on head, the mouse was anesthetized and set on homemade mount. The mouse's head was protruded into the water tank filled with water through a hole in the tank's bottom. The hole was sealed with a piece of transparent thin polyethylene film, and the head of the mouse was in direct contact with the film but not with water in the tank. The laser fluence in the in-vivo study is about 11 mJ/cm^2, which is well below the laser radiation safety limit by American National Standards Institute (ANSI) at the chosen wavelength. PAT scans were performed before and after the tail intravenous injection of PB NPs (200 μL, 2 $mg\ mL^{-1}$).

2.6 Histology

For histological evaluation, portions of vital organs including heart, liver, spleen, lung and kidneys were collected at 15 days or 30 days after intravenous administration of PB NPs via the tail vein (200 μL, 20 $mg\ mL^{-1}$ per mouse), and then fixed in 10 % buffered formalin-saline at 4 °C overnight followed by embedded in paraffin blocks. Tissue sections of 4 μm thickness were stained with hematoxylin and eosin (H&E). Healthy mice without PB NPs injection were used as controls.

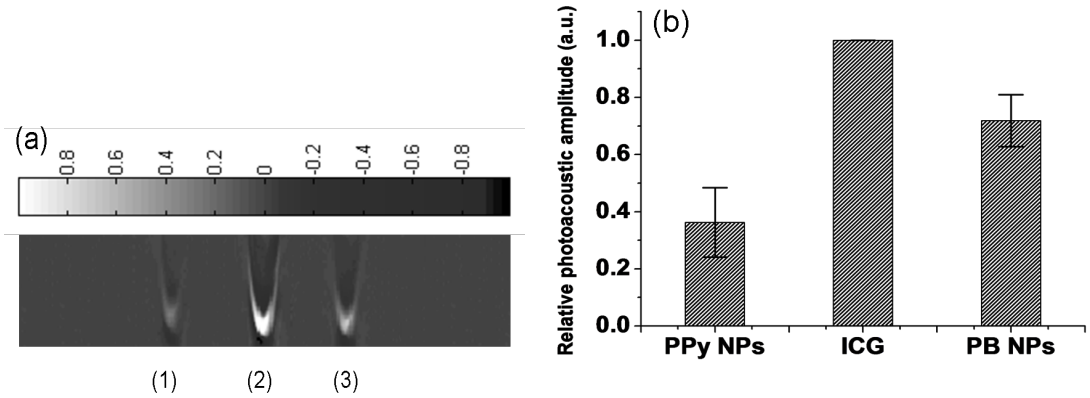

Fig. S1 (a) Photoacoustic images of the aqueous (1) PPy NPs, (2) ICG and (3) PB NPs with the same concentration of 0.2 mg/mL. (b) Quantitative analysis of the photoacoustic amplitude which were corresponding to (a) (Note that the data were normalized to ICG).

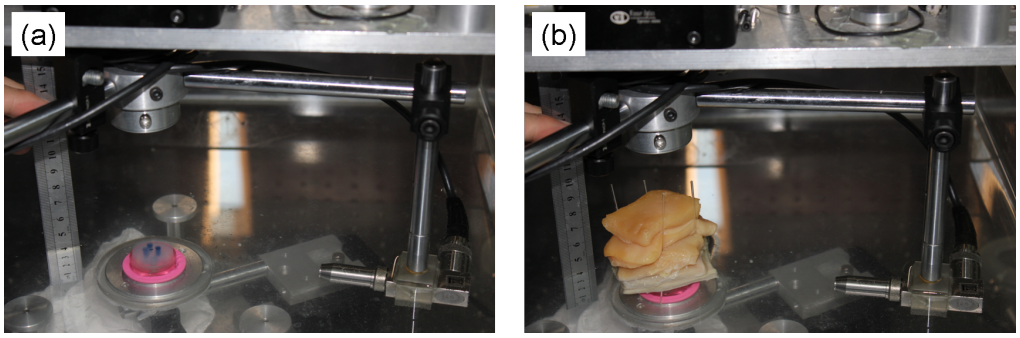

Fig. S2 Experiment setup (a) The cross section of agar gels containing PB NPs with different concentration. (b) Stacked chicken breast muscle blocks on the PB NPs contained agar gels.

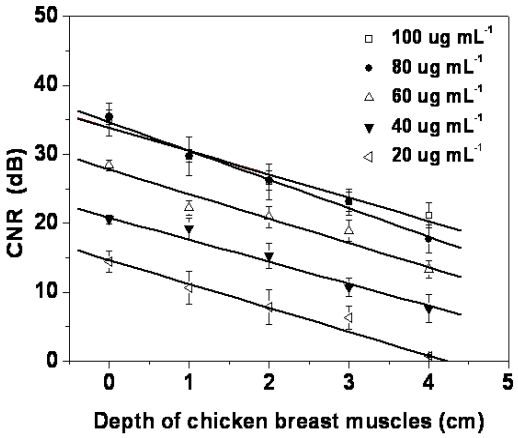

Fig. S3 Plot of CNR in decibels as a function of depth of chicken breast muscles.

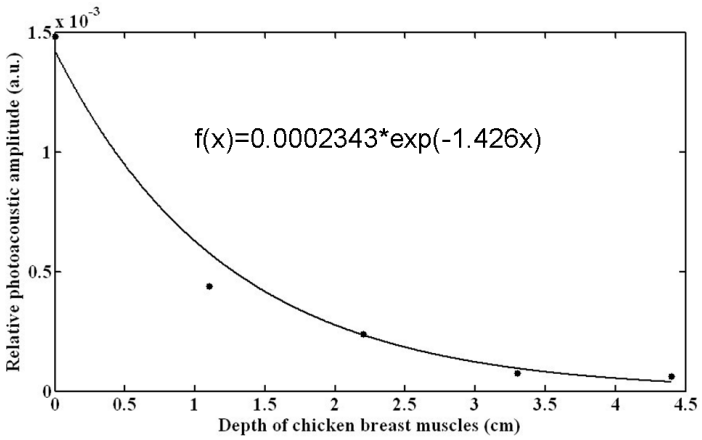

Fig. S4 Experimental photoacoustic signal intensity of 100 μg mL^{-1} PB NPs contained in agar gel embedded in chicken breast muscle measured at a wavelength of 765 nm as a function of depth from the laser-illuminated surface.

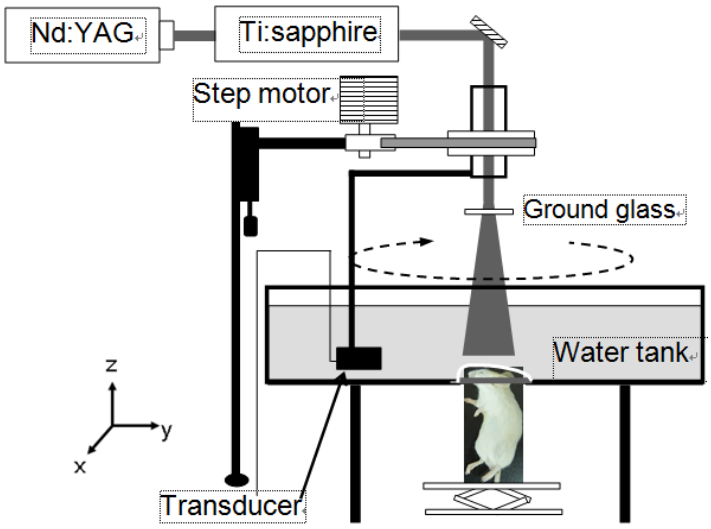

Fig. S5 Schematic illustration of the PAT system for the in vivo imaging. Laser beamform: Orthogonal PAT design, which means the laser illumination passing through a ground glass, was orthogonal to ultrasound detection plane. Scanning step: the transducer scanned the object evenly along a horizontal circle trajectory, and signals were obtained every 2.7° with averaging 16 times.

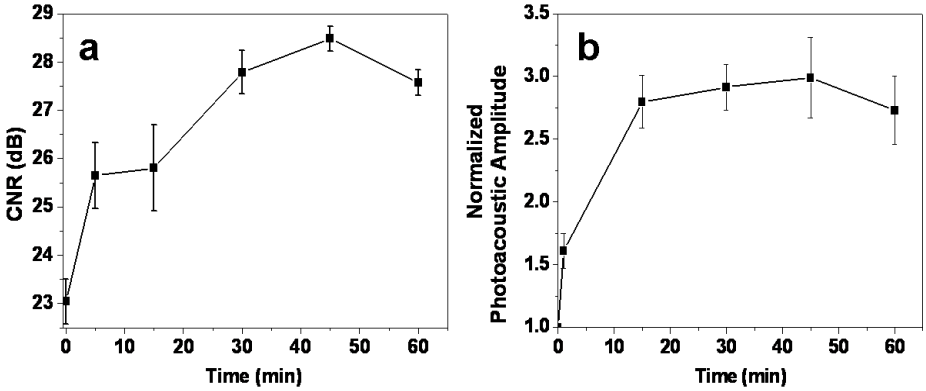

Fig. S6 (a) Plot of CNR in decibels at different times following the injection of PB NPs. (b) The integrated absorption calculated from the in vivo brain images of three mice at different times following the injection of PB NPs.

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References

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