Supporting Information

Photoacoustic Tomography of a Rat Cerebral Cortex *in vivo* with Au Nanocages as an Optical Contrast Agent

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Au Nanocage Preparation

Au nanocages with an edge length of ~50 nm were prepared in a two-step process. First, Ag nanocubes were prepared by a modified polyol process.[1] 6.0 mL of ethylene glycol (EG, J.T. Baker 9300-01 Lot C42B27) was added to a glass vial and heated at 152 °C for 1 hour while being stirred with a Teflon-coated magnetic stir bar at 260 rpm. 90 μL of a 3 mM sodium sulfide (J. T. Baker 3910)-EG solution was then injected into the vial. After 8.5 minutes, 1.5 mL of a PVP (20 mg/mL, J. T. Baker, cat. no. 3910, MW = 29,000) solution in EG was injected into the vial. Immediately thereafter, 0.50 mL of a $AgNO_3$ (48 mg/mL, Sigma-Aldrich 209139) solution in EG was injected into the vial. A series of color changes were observed over the next 12 minutes, with the reaction being stopped by cooling the vial to room temperature after the reaction media appeared opaque, green-ochre when viewed head-on and ruddy-red when viewed from the top. Plating was observed on the vial walls. The quenched reaction media was then diluted twice its volume with acetone and the Ag nanocubes were collected by centrifugation. The Ag nanocubes were resuspended in water and washed an additional 3 times then stored in 4 mL of deionized water for future use.

The Ag nanocubes were then converted into Au nanocages via the galvanic replacement reaction. 400 μL of the Ag nanocubes were dispersed in 10 mL water containing 1 mg/mL PVP in a 50 mL flask under magnetic stirring. The suspension was heated to a gentle reflux (~10 minutes). Then 5.4 mL of a 0.5 mM $HAuCl_4$ aqueous solution was added to the flask via syringe pump at a rate of 0.75 mL/min. Upon completion of $HAuCl_4$ solution addition, the suspension appeared dark blue. The suspension remained heated for an additional 10 minutes. Once cooled to room temperature, the sample was saturated with NaCl (to remove AgCl) then spun down. The supernatant was discarded and the sample resuspended in water. The sample was washed 6 additional times to remove PVP and NaCl. The Au nanocage optical properties were assessed using a UV-visible spectrophotometer. The resulting Au nanocages had a peak optical extinction (absorption and scattering) at ~820 nm, a wavelength that overlaps with the optically transparent window of biological tissues and the laser employed in the PAT experiments.

The concentration of Ag nanocubes was determined by correlating atomic emission spectroscopy with SEM, with ~10 % of the anticipated Au nanocages being lost during washing. The Au nanocages were then functionalized with poly(ethylene glycol) (PEG), which has been shown to suppress immunogenic responses and thus improve blood circulation times.[2,3] Au nanocage surfaces were functionalized with PEG by adding 1 mL of a 1 mM mPEG-SH (MW=5,000, Nektar)-water solution to a 2 nM nanocage suspension. The suspension was gently agitated and then allowed to sit undisturbed overnight. Residual mPEG-SH was removed by centrifugation.

PAT Experimental Setup

The setup for noninvasive photoacoustic tomography of rat brains is shown in Figure S1. A Q-switched Nd:YAG laser (LS-2137/2, LOTISTII)-pumped tunable Ti:sapphire laser (LT-2211A, LOTIS TII) was employed to provide laser pulses with a FWHM < 15 ns, a pulse repetition rate of 10 Hz, and a wavelength of 804 nm. The incident energy density of the laser beam was controlled to be less than 10 mJ/cm^2 on the surface of the rat head, which is well below the ANSI limit (32 mJ/cm^2 at 804 nm[4]). An unfocused ultrasonic transducer (V310, Panametrics) with a central frequency of 10 MHz and a -6dB bandwidth of about 70% was used to detect the ultrasound signals. The rat was fixed with a homemade mount so that its head protruded into the water tank through a hole in the tank's bottom. The hole was sealed with a piece of polyethylene film. Ultrasonic coupling gel was applied on the surface of the rat head. The photoacoustic signals detected by the ultrasonic transducer were received by an amplifier and then sent to an oscilloscope. A computer collected the digitized signals to reconstruct the distribution of optical absorption in the imaging plane through a modified backprojection algorithm.[5]

Sprague Dawley rats (100-150 g, Harlan Sprague Dawley, Inc., Indianapolis, Indiana) were employed for the imaging experiments. Before imaging, the hair on each rat's head was removed with hair removal lotion. A dose of 85 mg/kg Ketamine plus 15 mg/kg Xylasine was administered intramuscularly to anesthetize the rats. The subsequent anesthesia was achieved by the inhalation of a mixture of O_2 and isoflourane. The heart rate and blood oxygenation (SpO_2) level were monitored by a pulse oximeter. The heart rate of the animals was ~300 bpm and the SpO_2 level was >98% during the data acquisition.

[1]Siekkinen, A. R.; McLellan, J. M.; Chen, J. Y.; Xia, Y. Chem. Phys. Lett. 2006, 432, 491-496.

[2] Chen, A. M.; Scott, M. D. BioDrugs 2001, 15, 833-847.

[3] Harris, J. M.; Martin, N. E.; Modi, M. Clin. Pharmacokinet. 2001, 40, 539-551.

[4] Laser Institute of America, American National Standard for Safe Use of Lasers ANSI Z136.1-2000, American National Standards Institute Inc., New York, NY, 2000.

[5] Xu, M. H.; Xu, Y.; Wang, L. V. IEEE Transactions on Biomedical Engineering 2003, 50, 1086-1099.

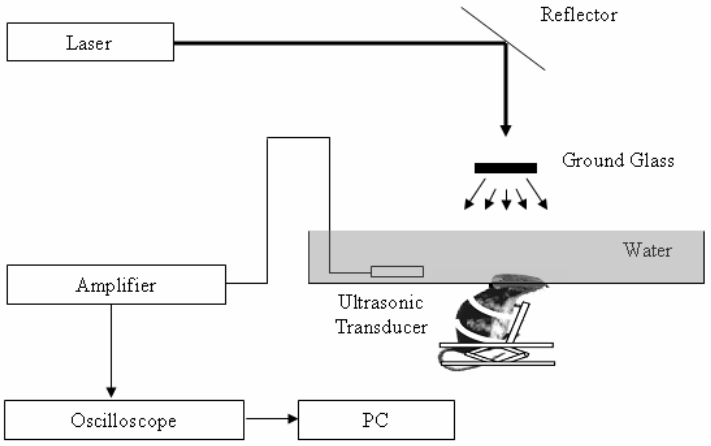

Figure S1

Figure S1 Schematic of PAT of the rat brain in vivo.