Supporting Information

1. Experimental Setup

The photoacoustic setup used in this work is shown in Figure S1: a frequency-doubled, Q-switched Nd:YAG laser (SL280 Spectron Laser System, 532 nm, 6 ns, 10 Hz) was employed. The laser beam was focused by a lens (100 mm) into a conventional 1-cm glass cuvette equipped with a piezoelectric transducer on one side (side-on detection).[7] The PA signals were amplified (HCA-100M-50k-C current amplifier, Femto) and recorded by a digital oscilloscope. The data recording by a digital storage oscilloscope was triggered by the laser. A fraction of the laser beam was coupled out and employed for on-line monitoring of the laser pulse energy by a pyroelectric detector (Pyroelectric J25LPMB).

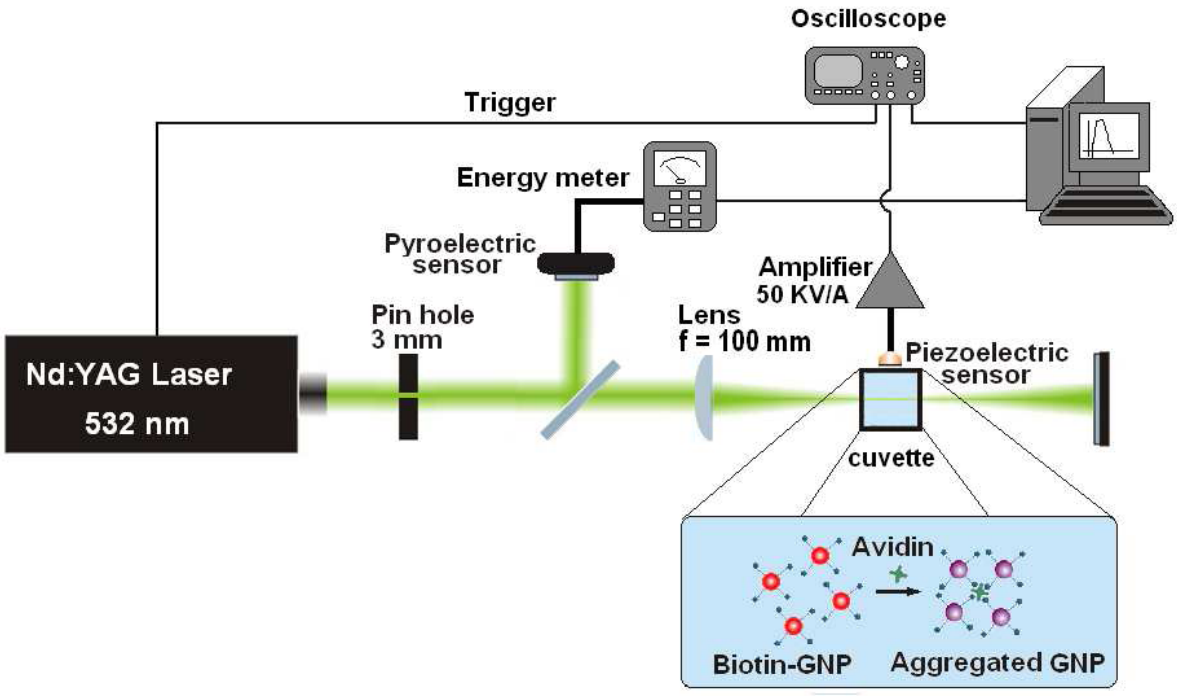


Figure S1. Scheme of the experimental setup.

2. Photoacoustic signal analysis

The origin of the abscissa is set to the instant of laser pulse generation. The noise in the first ~1 µs originates from electromagnetic emissions of the Q-switch of the Nd:YAG laser. The peak at t_a =2.25 µs is the PA signal of GNPs.

The time delay (t_a) the generated pressure pulse needs to reach the cuvette walls, where the detector is placed, depends on a distance z and the speed of the sound in the corresponding medium: $t = z/c$. According to this equation, t_a corresponds to a distance of 2.1 mm in water with a sound velocity of 1490 ms^{-1} (the distance between the inner walls and the center of the cuvette) plus 4.1 mm in glass with a sound velocity of 5000 ms^{-1} (cuvette walls thickness).[1] A detailed view of the PA signal is shown in the inset of figure S2. The p_{ac} amplitude was to represent the PA signal.

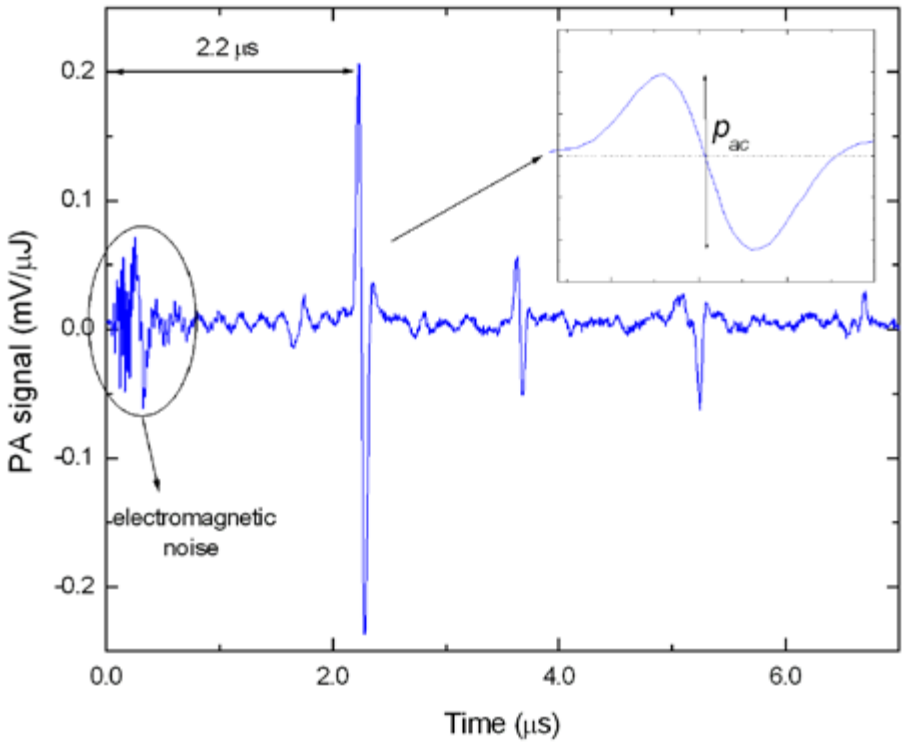


Figure S2. Normalized PA signal vs. time. The inset shows an expanded view around the main peak.

3. Synthesis and characterization Gold nanoparticles

3.1 Materials

The following reagents were used without further purification: hydrogen tetrachloroaurate(III) ($HAuCl_4$, Sigma), trisodium citrate (Fluka), hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$, Sigma-Aldrich), Albumin from bovine serum (BSA, sigma), N-(+)-Biotinyl-6-aminocaproic acid N-succinimidyl ester (Biotin-NHS, Fluka), cysteamine (Fluka), water-free DMF (Fluka). Ultrapure water (18 $M\Omega{\cdot}cm^{-1}$) was used to prepare all aqueous solutions.

3.2 Synthesis and characterization Gold nanoparticles.

Gold nanoparticles with different sizes were prepared step by step previously described in references (2), (3) and (4) with slight modifications.

GNP 1: 5 mL of 1% trisodium was quickly mixed with 1.7 mL of 1% $HAuCl_4$ in 50 mL of boiling water solution under vigorous stirring.

GNP 2: 800 µL of 1% $HAuCl_4$ was quickly adding to the solution contained 9.0 mL of 40 mM $NH_2OH{\cdot}HCl$ and 10 mL of GNP 1 and 81 mL H_2O at room temperature.

GNP 3: 600 µL of 1% HAuCl4 was quickly adding to the solution contained 2.2 mL of 40 mM $NH_2OH{\cdot}HCl$ and 40 mL of GNP 2 and 58 mL H_2O at room temperature.

GNP 4: 295 µL of 1% HAuCl4 was quickly adding to the solution contained 2.2 mL of 40 mM $NH_2OH{\cdot}HCl$ and 25 mL of GNP 3 and 72 mL H_2O at room temperature.

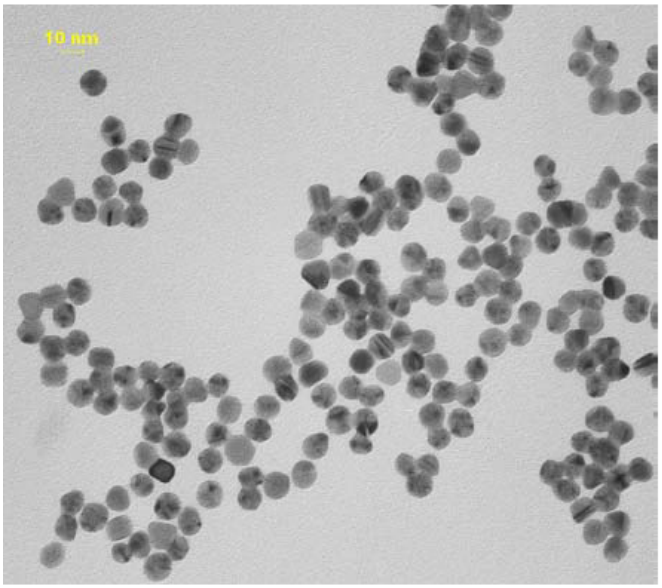
GNP 5: 220 µL of 1% HAuCl4 was quickly adding to the solution contained 2.2 mL of 40 mM $NH_2OH{\cdot}HCl$ and 20 mL of GNP 4 and 78 mL H_2O at room temperature.

GNP 6: 160 µL of 1% HAuCl4 was quickly adding to the solution contained 2.2 mL of 40 mM $NH_2OH{\cdot}HCl$ and 40 mL of GNP 5 and 58 mL H_2O at room temperature.

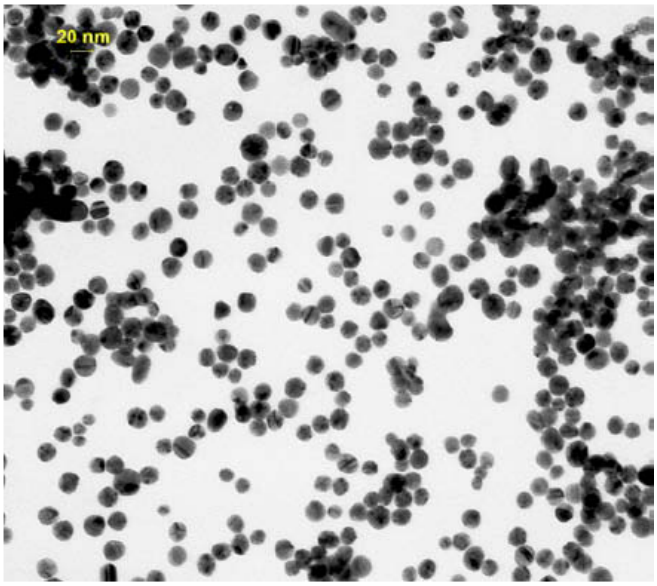
From GNP 2 to GNP 6, additional 1.0 mL of 1% trisodium citrate was added to stabilize the nanoparticles after the synthesis. Table S1. shows the detailed information of all six GNPs.

	GNP 1	GNP 2	GNP 3	GNP 4	GNP 5	GNP 6
λ_{max} (nm)	518.0	526.0	522.5	525.5	537.0	560.0
Extinction(cm^{-1})	2.50	1.07	1.04	0.49	0.39	1.07
Number concentration (10^{11} particles · mL^{-1})	150	15	6	1.5	0.3	0.12
Diameter (nm)	12.5 ± 1.0	19.1 ± 2.6	26.4 ± 3.5	44.9 ± 6.8	60.0 ± 7.9	83.3 ± 10.5

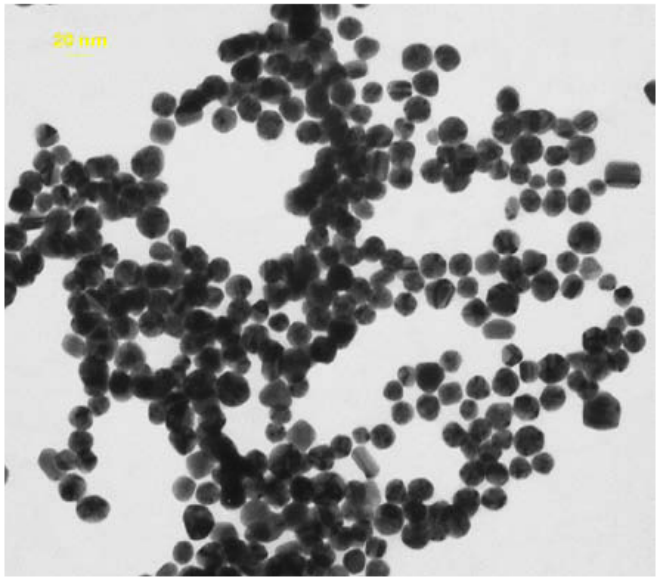
Table S1. Detailed information about the gold nanoparticles. The absorption peaks and their intensities were obtained by means of a scanning spectrophotometer (Beckman 650). The number concentration of GNP 1 (2.5×10^{-8} M) was determined according to the Beer's law by using the extinction coefficient of 10^8 $M^{-1}\cdot cm^{-1}$ for 13 nm GNP in diameter at 520 nm[5]. According to previous reports[2, 3, 6], the reduction of Au^{3+} by NH_2OH is dramatically accelerated by Au surfaces, as a result, no new particle nucleation occurs in solution during the preparation. So, form GNP 2 to GNP 6, the number concentration was calculated based on the small GNP concentration that added as seeds. The NP sizes were verified by TEM images (below) collected by a JEOL JEM 2010 instrument (at least 200 particles were measured, respectively).



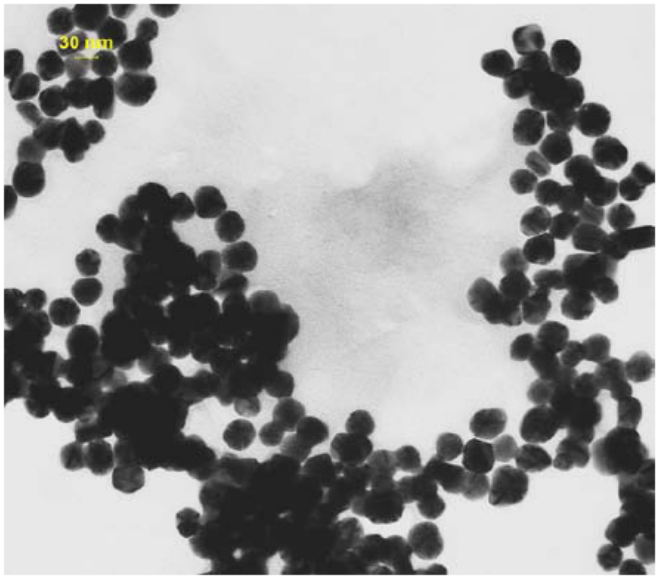
GNP 1



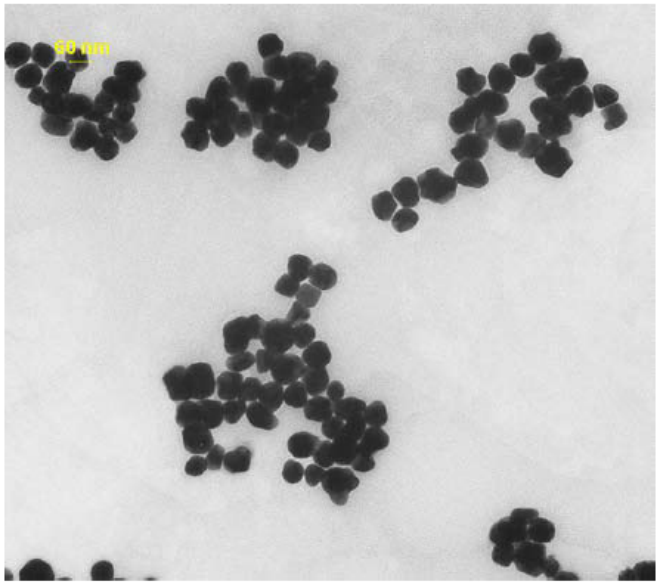
GNP 2



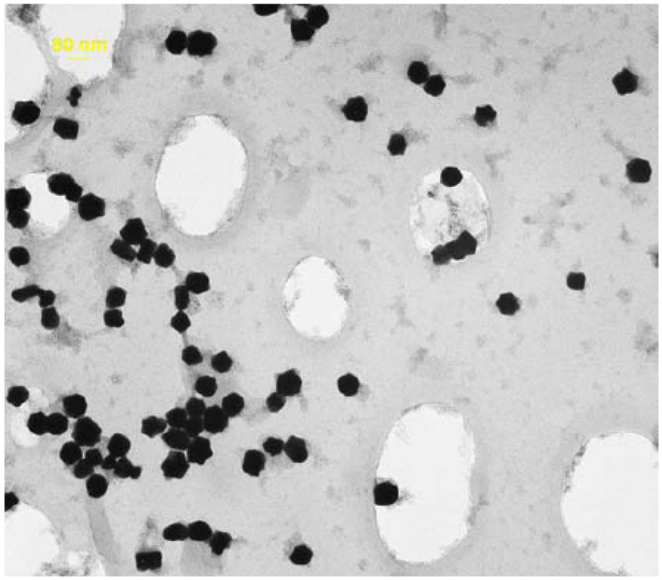
GNP 3



GNP 4



GNP 5



GNP 6

Figure S2. TEM images of GNPs.

3.3 Preparation of Biotin-conjugated Gold nanopariticles.

Gold nanoparticles with different sizes were synthesized via a hydroxylamine seeding method. Biotin-labelled GNPs were prepared by the following steps: 1.0 mg (2.2×10^{-6} mol) N-(+)-biotinyl-6-aminocaproic acid N-succinimidyl ester and 0.17 mg (2.2×10^{-6} mol) cysteamine were dissolved in 1.0 mL water-free DMF. Four hours later, 100 μL of above solution was added into 10 mL GNPs under gentle stirring at room temperature. After 10 min, 300 μL 10% Albumin from bovine serum (BSA) was added to help to stabilize the nanoparticles and incubated overnight at room temperature. Then the Biotin-GNP was separated from the reaction mixture by centrifugation and redispersed in 10 mL H_2O containing 0.1% BSA, and stored in 4 °C until use.

3.4 Aggregation of Biotin-conjugated GNPs in the presence of Avidin

Aliquots (3 mL) of solutions containing Avidin (0 - 432 nM) in the presence of Biotin-GNP were maintained at room temperature for two hours. Then, a part of above aliquots (1.5 mL) was measured by our PA setup. For comparison, the other part (1 mL) was measured by the spectrophotometer.

References

(1) Schmid, T.; Panne, U.; Niessner, R.; Haisch, C. *Anal. Chem.* **2009**, *81*, 2403-2409.
(2) Brown, K.; Walter, D.; Natan, M., *J. Chem. Mater.* **2000**, *12*, 306-313.
(3) Brown, K.; Walter, D.; Natan, M. *J. Chem. Mater.* **2000**, *12*, 306-313.
(4) Liu, X.; Huan, S.; Bu, Y.; Shen, G.; Yu, R. *Talanta 2008*, *75*, 797–803.
(5) Mucic, R.; Storhoff, J.; Mirkin, C.; Letsinger, R. *J. Am. Chem. Soc.* **1998**, *120*, 12674-12675.
(6) Stremsdoerfer, G.; Perrot, H.; Martin, J. R.; Clechet, P. *J. Electrochem. Soc.* **1988**, *135*, 2881-2885.