Supporting information

Experimental Section

Fabrication of the window-type transmission electron microscopy (TEM) microchip nanopipet Semiconductor processes, including lithography and etching, were used to prepare the nanopipets from 4 inch wide, 525 μm thick wafers. Two wafers were bonded to a glass frit using screen printing, and the bonded wafer was sliced into individual nanopipets with a dimension of 1.3 mm x 1.3 mm x 1.0 mm, an electron transparent window (28 x 18 μm with two ~ 160 nm thick silicon nitride films), and a chamber width in the range of 2.0 ± 0.5 μm.

Sampling and loading processes A 1 μl sample solution was placed on a glass slide and loaded by suction into the nanopipet within 10 sec, and then the specimen was dried in a moderated vacuum chamber (~0.1 atm.) for 30 min. The dried nanopipet specimen was then loaded onto a copper grid using an epoxy resin and further dried in a vacuum for 20 min. This specimen was then placed on the TEM holder for subsequent imaging processes.

Evaluation of the distribution/aggregation of nanoparticles in the TEM images The software program *Image J* was used to measure the diameter of the particles and

the distance to neighboring particles (center to center of two particles). The distances between individual particles to the nearest particles (d) were measured. The d values of all of the observed particles were ranked in increasing order and then categorized into groups using logarithmic interval scales. Next, the particle numbers for each group were calculated in order to determine the particle number percentage vs. the d values. For each nanopipet specimen, 4-10 images (2.0 μm x 2.7 μm) were randomly taken in the image window (28 μm x 18 μm) of the nanopipet, and > 200 GNPs were calculated for the quantitative analysis.

Synthesis of the carboxyl-PEG5k gold nanoparticles (GNPs) The carboxyl-PEG5k-GNPs were synthesized through a ligand exchange process using carboxyl-PEG5k-SH, which was exchanged for citrate on 13 nm GNPs capped by citrate ions[1] that were prepared using the citrate reduction method.[2] The cPEG5k-GNPs had an overall diameter of 39.6 ± 3.0 nm and a core diameter of 13.0 ± 0.9 nm (Figure 1S). The hydrated particle size of the cPEG5k-GNPs as measured by dynamic light scattering was 39.3 nm (polydispersity index 0.12).

Blood sample preparation Male Sprague-Dawley rats (250-300 g) were anesthetized with isoflurane and their body temperature maintained at 37^{o}C with a heating pad. Blood samples were collected into heparinized tubes for spiking with the cPEG5k-GNPs to obtain 50% diluted blood. For the in vitro sampling test, the

sample was loaded into the nanopipet within 2 minutes without further incubation. For the in vivo experiments, the rats were injected intravenously through the tail vein with ~400 μl of a solution containing $3x10^{14}$/ml cPEG5k-GNPs in 5% glucose. Blood samples (600 μL) were collected into heparinized tubes via the jugular vein at the following post-injection times: 0.1, 1, 3, 7, 24, and 48 hrs. Each blood sample was immediately loaded into the nanopipet for subsequent imaging and quantification processes and analysis by inductively coupled plasma-mass spectrometry (ICP-MS). (The Institutional Animal Care and Use Committee of the National Health Research Institutes approved the experimental protocols)

Transmission electron microscopy Images were taken at an accelerating voltage of 100kV using an Hitachi TEM H-7650.

Inductively coupled plasma-mass spectrometry detected gold ion level A solution comprised of 2.25 ml nitric acid, 0.75 ml hydrochloride, and 0.5 ml hydrogen peroxide was added to the blood sample (200~500μl), and the sample was heated to 180^{o}C for 15 min. The solution was then diluted to 20 ml using doubly deionized water and subsequently subjected to ICP-MS (Agilent 7500cx, Agilent Technologies, Inc., Tokyo, Japan) for determination of the gold ion level.

(1) Love, J. C.; Estroff, L. A.; Kriebel, J. K.; Nuzzo, R. G.; Whitesides, G. M. *Chem. Rev.* **2005**, *105*, 1103-1169.

(2) Frens, G. *Nat. Phys. Sci.* **1973**, *241*, 20-22.

Results and discussion

Characterization of the cPEG5k-GNPs

The structure, a TEM image, and the particle size of the cPEG5k-GNPs are shown in Figure 1S. The hydrated particle size (mean diameter) was determined by dynamic light scattering (DLS) to be 39.3 ± 1.1 nm (n=5) with a polydispersity index of 0.12 ± 0.04.

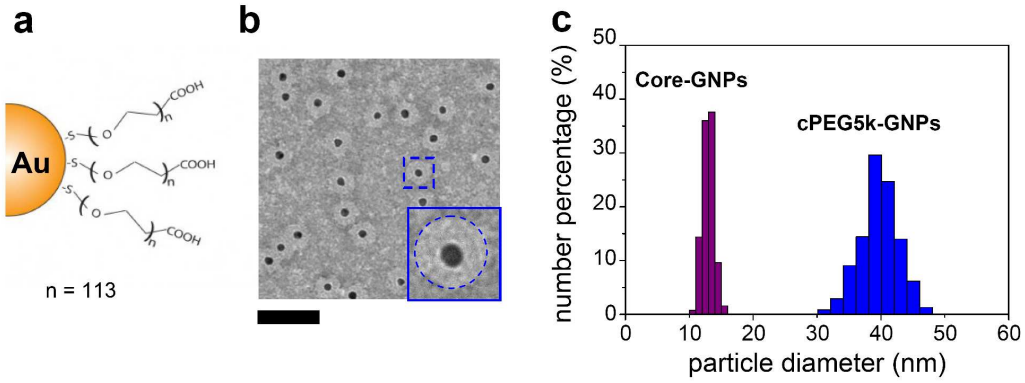

Figure 1S. The cPEG5k-GNPs: a. structure, b. TEM image (stained by 1 % uranyl acetate), c. statistical particle size of the GNPs core and cPEG5k-GNPs (n = 250). The mean diameter of the GNPs core and the cPEG5k-GNPs are 13.0 ± 0.9 nm and 39.6 ± 3.0 nm, respectively. Scale bar is 100 nm.

Prevention of the aggregation of nanoparticles due to the specific geometry of the nanopipet

A nanopipet with a 2.5 µm chamber width can fully prevent the aggregation of 300 nm polystyrene beads. However, a nanopipet with a larger 5.0 µm chamber width cannot fully suppress the aggregation of the same sample (shown ~40% particle

aggregation). On the other hand, ~97% aggregation of 300 nm PS beads was observed under the same conditions on copper grids with either a hydrophobic carbon-film or a hydrophilic SiO_x-film. These results indicate that the geometry of the nanopipet is the dominant factor for restricting movement and preventing the aggregation of the particles during the drying process, when water is gradually evaporated. The surface modification of the copper grid by the hydrophilic film cannot prevent the aggregation.

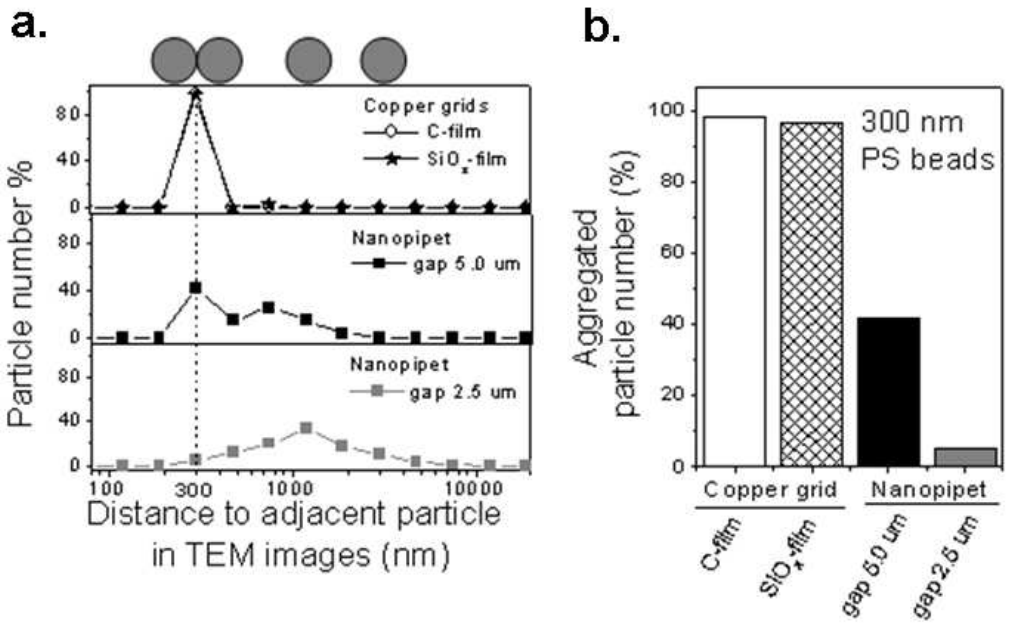

Figure 2S. The spatial distribution of 300 nm polystyrene beads in copper grids and nanopipets: (a) particle number percentage vs. the distance to neighboring particles in TEM images (more than 200 beads were measured in each specimen); (b) aggregation extent of the particles dried on copper grids (C-film & SiO_x film) and in nanopipets with chamber widths of 5.0 μm and 2.5 μm.

Aggregation extent of citrate-modified gold nanoparticles in 5% glucose and 50% diluted blood

Citrate-modified gold nanoparticles, which are known to be short-lived in circulating blood and also tend to aggregate in blood, were used as an intentionally aggregated example. The citrate-modified gold nanoparticles exhibited ~70% particle aggregation in 5% glucose with an aggregation extent of 2-10 particles in each aggregate. In the 50% diluted blood, the aggregation increased to ~87% with a higher aggregation extent (~40% particles aggregated with 11-100 particles in each aggregate). The nanopipet therefore has the potential to distinguish the aggregation extent of intentionally aggregated nanoparticles in their native environment, such as an injection buffer or blood.

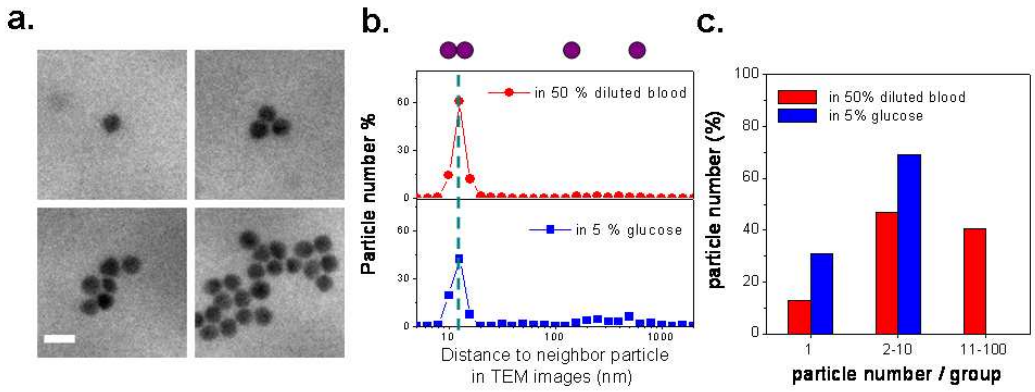

Figure 3S. The aggregation extent of citrate-modified gold nanoparticles in 5% glucose and 50% diluted blood analyzed using nanopipets: (a) TEM images of citrate-modified gold nanoparticles in 50% diluted blood; (b) measured particle number percentage vs. the distance to neighboring particles in the TEM images (calculated particle number n = 612 in 50% diluted blood in 2 devices, and n = 166 in 5% glucose) and (c) the aggregation extent of citrate-modified GNPs in 50% diluted blood and 5% glucose.