Supplementary Information for

Nanoprobe Implantation into Mammalian Cells by Cationic Transfection

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This document contains additional information for the following items:

- **Supporting instrumentation;**
- **Full experimental details on nanoprobe implantation in tsA201 cells, including ultrasonic implantation, transfection of DNA-coated nanoprobes, and negative Ag staining;**
- **Efficiency of nanoprobe implantation and effect on cell viability;**
- **Descendent cell lines carrying inherited nanoprobes.**

Supporting Instrumentation

An inverted fluorescence microscope with infinity-corrected optics (Olympus IX-50) was used for direct imaging of the nanoparticles, which were illuminated by a mercury arc lamp (Ushio USH-102D). Scattering was detected using an epipolarization filter (Chroma Immunogold Standard filter set 33001, including UV (GG420) and IR (KG5) filters). Images were acquired with a color digital camera (Diagnostic Instruments Spot RT), and were taken using 20× or 40× objective lenses; the final magnification was not determined.

A micromanipulator (Eppendorf Femtojet 5426/5171) was used to perfuse submicromolar quantities of Ag staining solution onto tsA201 cells to verify nanoprobe implantation. Solutions were delivered using glass micropipettes (i.d.= 0.5 µm; o.d.= 0.7 µm) mounted onto the injector, with a pressure of 100 hP and a compensation pressure of 30 hP.

Nanoprobe implantation: experimental protocols

Human tsA201 cells, a simian virus 40 T-antigen expressing derivative of the human embryonic kidney cell line HEK293, were maintained in monolayer culture in Dulbecco's modified Eagle's medium/F-12 (Invitrogen) containing 1% penicillin–streptomycin and enriched with 10% fetal bovine serum (FBS, Hyclone Laborotaries) and incubated at 37 °C in a 10% CO_2 atmosphere. After nanoprobe implantation, cells were incubated for another 24 hr as described above, but using a growth serum containing 1% FBS with a 5% CO_2 atmosphere to reduce the rate of cell proliferation.

Ultrasound-mediated implantation: A dilute suspension of cells (1 mL, ca. 2×10^5 cells) was treated with a solution of nanoprobes (10^6–10^7 particles dispersed in 10 µL) in an Eppendorf vial, then immersed for 5 min in an ultrasonic cleaning bath (Branson 200, 42 kHz) operating at 19 W. The cells were then transferred to a 35-mm plastic culture dish and diluted with 1 mL fresh medium, and incubated for 24 hr at 37 °C as described above for surface adhesion, prior to imaging (see Figure S1).

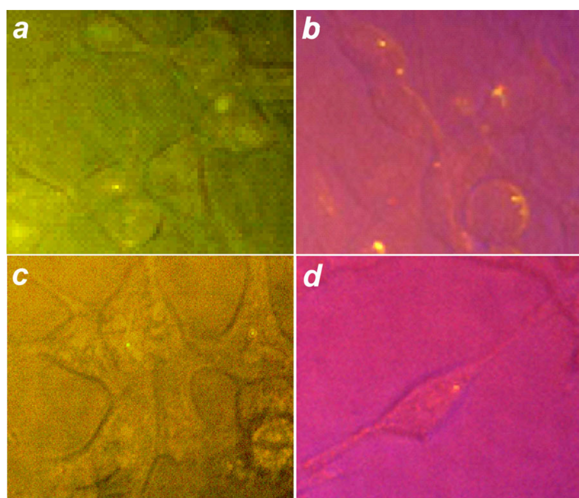

Fig. S1. Epipolarization images of Au nanoprobes (yellow) implanted by ultrasonication. tsA201 cells were exposed to Au particles of various size: (*a*) 150 nm; (*b*) 250 nm; (c) 60/550-nm Au/SiO_2 superparticles; (*d*) 40/550-nm Au/SiO_2 superparticles.

Cationic transfection: Three commercially available cationic delivery systems were tested: TransIT®-293 (Mirus), GenePORTER™ (Gene Therapy Systems), and GeneJuice® (Novagen). The first two systems employ dioleoyl phosphatidylethanolamine (DOPE)-derived liposomes; the third uses recombinant proteins mixed with a polyamine. All reagents were used as directed by the supplier for standard DNA transfection. Cells were plated one day prior to implantation experiments, and were 30–50% confluent on the day of transfection. In a typical experiment, 10 μL of transfection reagent was mixed thoroughly with 100 μL of serum-free medium, and allowed to sit at room temperature for 10 min. A suspension of DNA-coated nanoprobes (10^6–10^7 particles dispersed in 20 μL) was diluted with 100 μL of serum-free medium, then combined with the transfection reagent solution. Confluent tsA201 cells were washed and plated in 2 mL of serum-free medium, then treated with the transfection reagent–nanoprobe mixture and incubated for 4 hrs at 37 °C. The serum-free medium was then replaced by growth medium containing 1% FBS, and incubated for another 24 hrs as described above.

DNA-coated nanoprobes were prepared as follows: an excess of GFP cDNA plasmid (30 μL of a 0.78 μg/μL solution) was mixed vigorously with a suspension of nanoprobes (100 μL, 10^6–10^8 particles/mL), followed by the addition of $CaCl_2$ (10 μL of a 1 M solution). The coated nanoparticles were centrifuged at 5,000 rpm for 20 min, washed with 70% and 100% ethanol, then redispersed in 200 μL water. The conditions for isolating DNA-coated nanoprobes are critical for establishing a good correlation between GFP expression and nanoprobe implantation. Higher centrifugation speeds forces the precipitation of unadsorbed cDNA plasmids, compromising the quality of this correlation.

Negative Ag staining protocol: Cells were washed with phosphate-buffered solution (pH 7.2), then immersed in fresh medium containing 1% hydroquinone and exposed to a 5 mM $AgNO_3$ solution in 15% NH_4OH and 5% $(NH_4)_2CO_3$, which was perfused onto cell surfaces in submicroliter quantities using the microinjector–micromanipulator. Gold colloid exposed to the staining solution served as nucleation sites for the electroless deposition of Ag, and could be detected by amplification of light scattering (see Figure S2). Two minutes of exposure was sufficient to amplify the scattering of most of the particle labels within the vicinity of diffusion. Longer exposure times (10 minutes) increased the range and brightness of scattering amplification, but also produced additional scatterers near the perfusion epicenter not related to the original particles. An exposure time of 3–5 minutes was deemed to be optimal.

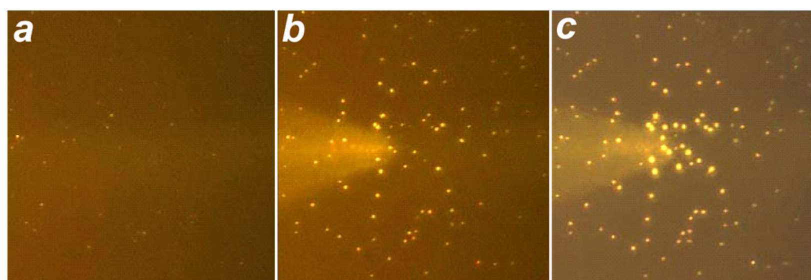

Fig. S2. Control experiment for detection of extracellular nanoprobes by amplified light scattering, using 100-nm Au particles: (*a*) before Ag addition; (*b,c*) after Ag addition (t = 2 and 10 min, respectively).

Nanoprobe Implantation Efficiency and Effect on Cell Viability

Plated tsA201 cells were implanted with 60/550-nm Au/SiO_2 superparticles coated with GFP cDNA, incubated for 24 hr, then washed several times with phosphate buffer (pH 7.2, with minimal Mg^{2+} and Ca^{2+}) prior to analysis. The implantation efficiency was quantified by fluorescence microscopy (see Table S1).

Table S1. Efficiency of nanoprobe implantation

Cationic transfection agent	GFP-expressing cells / total cell count, 24 hours after transfection	Implantation efficiency
TransIT®-293	101/1249	8.1%
GenePORTER™	71/1203	5.9%
GeneJuice®	115/1080	10.7%

A follow-up experiment was performed to compare the viability of tsA201 cells carrying nanoprobes (implanted by TransIT®-293) with that of untransfected cells grown under the same conditions (see Table S2). No significant differences in viability were observed (also see next section).

Table S2. Effect of nanoprobe implantation on cell proliferation

Time elapsed (hrs)	GFP-expressing cells / total cell count	% implantation
0	--/114	
24	15/170	8.8%
48	31/270	11.5%

Descendent cell lines after nanoprobe implantation

GFP cDNA-coated superparticles (60/550 nm) were implanted inside of tsA201 cells by cationic transfection agents. Cell growth was accelerated using culture media enriched with 10% FBS, and monitored to the fourth generation over a period of several days. Cells containing nanoprobe implants continued to proliferate to the fourth generation without any visible signs of deterioration. Images of third-generation cells are presented below (fourth-generation cells were not photographed).

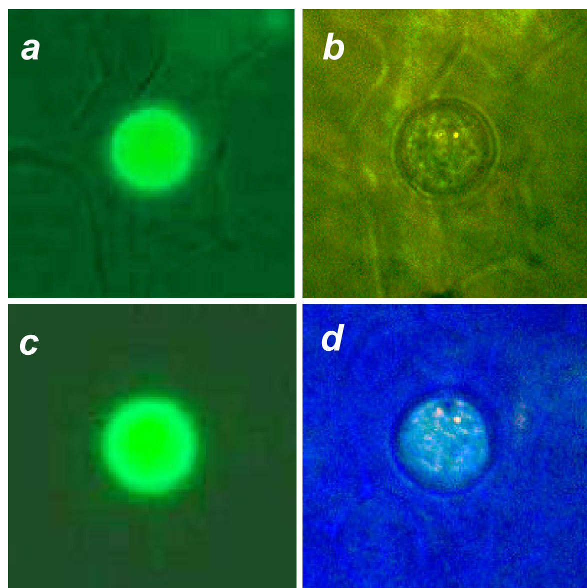

Fig. S3. Fluorescence and epipolarization images of third-generation tsA201 cells carrying 60/550-nm Au/SiO_2 superparticles, implanted originally with TransIT®-293 (*a*, *b*) or GenePORTER™ (*c*,*d*).