Supporting Information for

Highly specific nuclear labeling via *in situ* formation of fluorescent copper nanoparticles

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Material and methods

Materials

The DNA oligonucleotide (GGG TTT TTA TAT ATA TAT ATA TTT TTT ATA TAT ATA TAT AT) used in this study was purchased from Integrated DNA Technologies (Skokie, IL, USA). Sodium chloride (NaCl), sodium ascorbate, and calcium chloride ($CaCl_2$) were purchased from Samchun Chemical (Seoul, Korea). magnesium chloride ($MgCl_2$) was purchased from DUCKSAN GENERAL SCIENCE (Seoul, Korea), and 3-(N-morpholino)propanesulfonic acid (MOPS), copper sulfate ($CuSO_4$), RNA (from yeast; R6625), and histones (from calf thymus; Type II-A; H9250) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 4′,6-Diamidino-2-phenylindole (DAPI), SYBR Green I, and 5-dodecanoylaminofluorescein (DAF) were purchased from Thermo Fisher Scientific (Waltham, MA, USA). All chemicals of analytical grade were used without further purification.

Fluorescence signal measurement of CuNPs and DAPI in the presence of DNA and RNA

For the synthesis of CuNP, DNA oligonucleotide (126 μg/mL) or RNA (126 μg/mL) in MOPS buffer [(20 mM MOPS, 93 mM NaCl, 1 mM $CaCl_2$, and 0.5 mM $MgCl_2$ (pH 8.0)] was mixed with CuSO4 (690 μM) and sodium ascorbate (2 mM), and the fluorescence signal was immediately measured. A control sample was prepared in the same manner after excluding DNA or RNA. For DAPI staining, DNA oligonucleotide (126 μg/mL) or RNA (126 μg/mL) in MOPS buffer was mixed with DAPI (300 nM) and incubated for 10 min at room temperature before fluorescence signal measurement. A control sample was prepared in the same manner after excluding DNA or RNA. The fluorescence signals of the prepared

samples were measured at an excitation wavelength of 340 nm using a microplate reader (Spectramax iD5; Molecular Devices, Sunnyvale, CA, USA).

Fluorescence signal measurement of CuNPs and DAPI in the presence of histone-bound DNA

For the synthesis of CuNP, DNA oligonucleotide (1 μM) in the presence and absence of histones (5 mg/mL) in MOPS buffer was mixed with $CuSO_4$ (690 μM) and sodium ascorbate (2 mM), and the fluorescence signal was immediately measured. A control sample was prepared in the same manner after excluding both DNA oligonucleotide and histones. For DAPI staining, DNA oligonucleotide (1 μM) with and without histones (5 mg/mL) in MOPS buffer was mixed with DAPI (300 nM) and incubated for 10 min at room temperature before fluorescence signal measurement. A control sample was prepared in the same manner after excluding both DNA oligonucleotide and histones. Fluorescence signals were measured at an excitation wavelength of 340 nm using a microplate reader (Spectramax iD5; Molecular Devices).

Cell culture and specimen preparation

The human breast cancer cell line MCF-7 (KCLB No. 30022) was cultured in Dulbecco's modified Eagle medium (HyClone, Logan, UT, USA) supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY, USA) and 1% penicillin-streptomycin (Gibco) at 37°C and 5% CO_2. For cell imaging, 105 cells/mL were seeded in a cell culture plate and cultured for 24 h, after which the cells were fixed using fixation buffer (BioLegend, San Diego, CA, USA).

Nuclear staining

For nuclear staining with CuNPs, various concentrations of $CuSO_4$ and sodium ascorbate (30 mM) in MOPS buffer were added to the fixed cells. For nuclear staining with DAPI or SYBR Green I, various concentrations of either dye in MOPS buffer was added to fixed cells, which were incubated for 10 min. Samples stained with CuNPs were directly imaged without additional incubation or washing steps, whereas those stained with DAPI were incubated for 10 min and washed once with MOPS buffer before imaging under a fluorescence microscope (KI-2000F; Korea Lab Tech, Seongnam, Korea) with two filter cubes [cube 1 (excitation: 330–385 nm and barrier: 420 nm) and cube 2 (excitation: 450–480 nm and barrier: 515 nm)].

Cell membrane staining

For membrane staining, DAF solution (250 nM) in MOPS buffer was first added to fixed cells, incubated for 10 min, and washed once with MOPS buffer. For nuclear counterstaining, either a CuNP-generating solution comprising $CuSO_4$ (690 μM) and sodium ascorbate (30 mM) in MOPS buffer or DAPI (900 nM) in MOPS buffer was added to DAF-stained cells. Samples stained with CuNPs were directly imaged without additional incubation or washing, whereas those stained with DAPI were incubated for 10 min and washed once with MOPS buffer before imaging under a fluorescence microscope (KI-2000F; Korea Lab Tech) with two filter cubes [cube 1 (excitation: 330–385 nm and barrier: 420 nm) and cube 2 (excitation: 450–480 nm and barrier: 515 nm)].

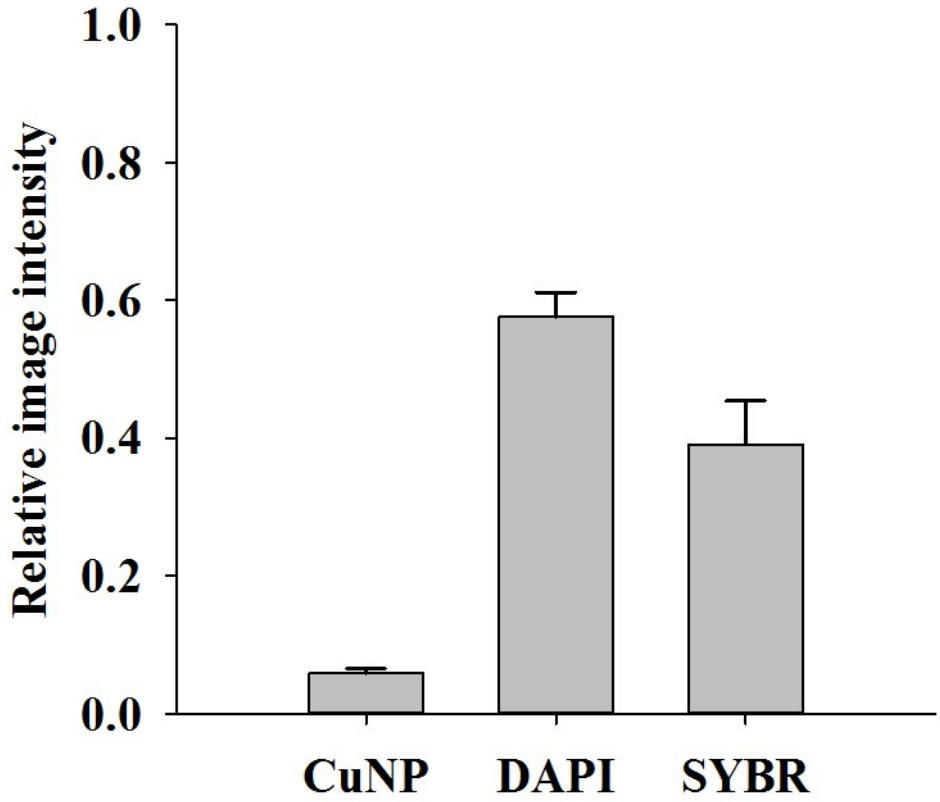

Fig. S1 Relative fluorescence intensity of CuNPs, DAPI (900 nM), and SYBR Green I (3×) in the cytoplasm as compared with the nucleus. CuNPs were prepared by the addition of $CuSO_4$ (690 μM) and sodium ascorbate (30 mM). The relative image intensities were calculated by dividing the intensity in the cytoplasm with that in the nucleus (n = 5).

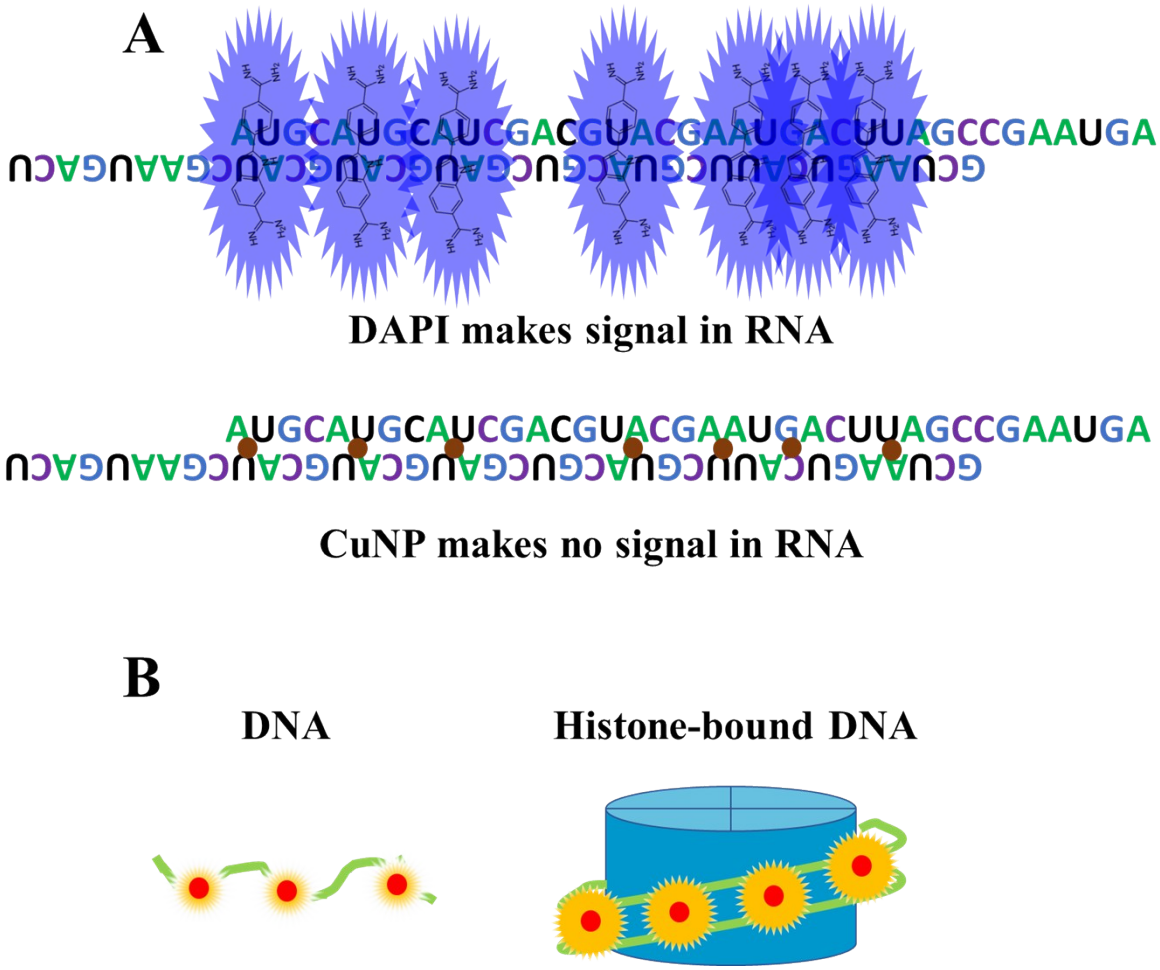

Fig. S2 Schematics describing DAPI or CuNP interaction with RNA (A) and histone-bound DNA (B).

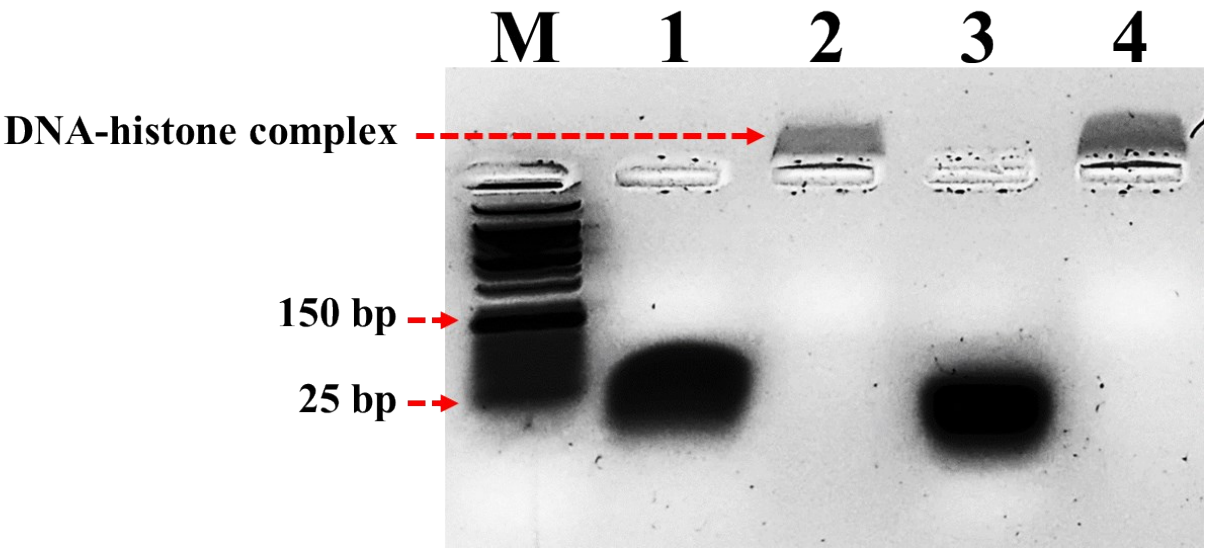

Fig. S3 Gel electrophoresis analysis to prove the complexation of DNA with histone. M: size marker, Lane 1: DNA oligonucleotide + CuNPs, Lane 2: DNA oligonucleotide + histone + CuNPs, Lane 3: DNA oligonucleotide, Lane 4: DNA oligonucleotide + histone. The samples (1-4) were resolved on a native agarose gel (3%), which were stained by Greenstar. The concentrations of DNA oligonucleotides and histone are 1 uM and 5 mg/mL, respectively.

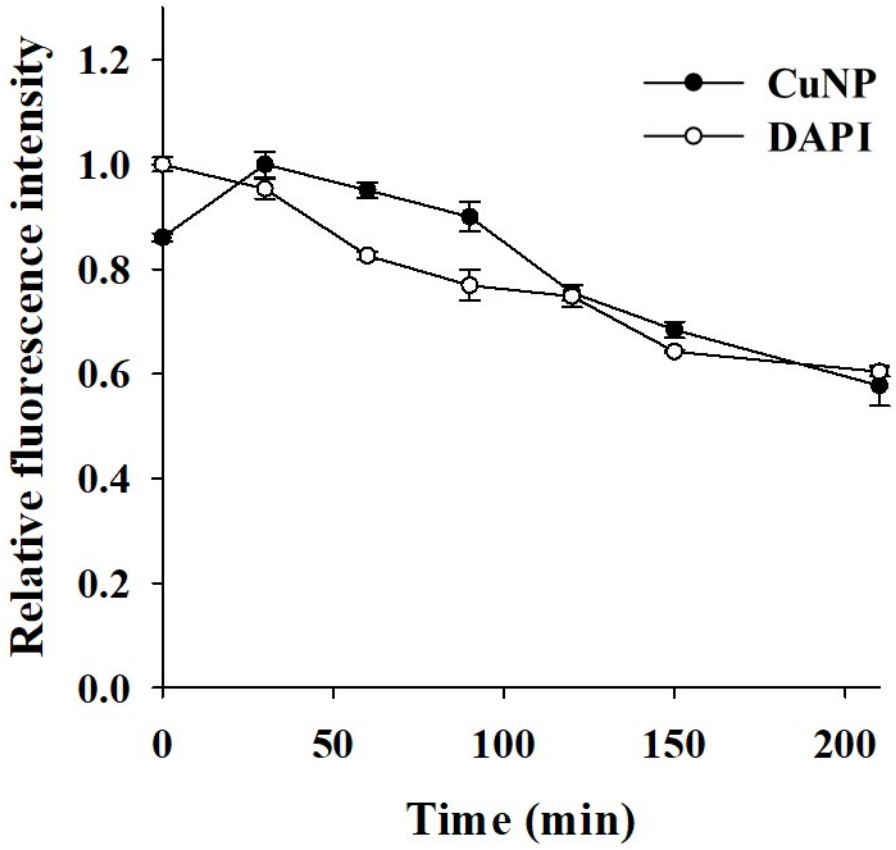

Fig. S4 Photostability of CuNPs and DAPI. The relative fluorescence intensities were calculated by dividing the maximum signal by the ones at different times. Each sample was irradiated by the light source at a wavelength of 365 nm and an intensity of 0.6 w/cm^2.

Table S1. Comparison of CuNPs with DAPI.

	CuNPs	**DAPI**
Excitation max.	340 nm	358 nm
Emission max.	600 nm	461 nm
Color	Yellow-Orange	Blue-Turquoise
Stokes shift	260 nm	103 nm
Nuclear staining	Yes	Yes
Nonspecific cytoplasmic staining	No	Yes
Incubation time	Not required	At least 5 min
Washing step	Not required	Required
Cost per mL solution	2.7×10^{-4} USD	14.9×10^{-4} USD