Supplementary Information

A simple and eco-friendly one-pot synthesis of nuclease-resistant DNA-inorganic hybrid nanoflowers

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Experimental Section

Materials: DNA oligonucleotides (**Table S1**) were purchased from Integrated DNA Technologies. The list of DNA sequences is summarized in **Table S1**. dNTPs mixture was purchased from iNtRON Biotechnology (Seongnam, Korea) and pETDuet-1 plasmid was purchased from Novagen (Darmstadt, Germany). Genomic DNA of *Salmonella typhimurium* and *Chlamydia trachomatis* was extracted using G-spin Genomic DNA Extraction Kit (iNtRON Biotechnology), and PCR products were purified using PCR quick-spin PCR product purification kit (iNtRON Biotechnology). The final concentrations of genomic DNA and PCR products were quantified by measuring the absorbance at 260 nm with Nanodrop 1000 (Thermo Fisher Scientific, Waltham, MA, USA).[1] DNase I and Exonuclease III were purchased from New England Biolabs (Ipswich, MA, USA). Phosphate-buffered saline (PBS, pH 7.4) was obtained from Thermo Fisher Scientific. All other chemicals were of analytical grade and used without further purification. Aqueous solutions were prepared using UltraPure DNase/RNase-Free Distilled Water (Thermo Fisher Scientific).

Preparation of DNA-copper nanoflowers: To prepare DNA-nanoflowers, 20 μL of aqueous $CuSO_4$ solution (120 mM) was added to 3 mL of 1X PBS (pH 7.4) containing DNAs at different concentrations, which was then incubated at RT for 3 days.

Characterization of DNA-copper nanoflowers: Scanning electron microscopy (SEM) images were obtained by using a Magellan™ 400 Field Emission Scanning Electron Microscope. The elemental composition was analyzed by using energy-dispersive spectrometer (EDS) (Bruker, Billerica, MA, USA). For the preparation of samples, the suspension of DNA-nanoflowers was filtered and dried on a membrane (pore size: 0.1 μm). For X-ray diffraction (XRD) analysis (D/MAX- 2500, Rigaku Corporation, Tokyo, Japan), the precipitate of DNA-nanoflowers was washed with deionized water, and dried at 80 °C for 1 day. The encapsulation yield of DNA in the nanoflowers was calculated by measuring DNA amount in

the supernatant with Nanodrop 1000 (Thermo Fisher Scientific). The weight percentage of DNA in nanoflowers was calculated based on the encapsulation yield and the weight of the powder.

Nuclease reaction and gel electrophoresis: Free DNA (0.1 μM) and DNA-nanoflowers (10 μM) were incubated with DNase I (100 U/mL) or Exonuclease III (1,000 U/mL) at 37 °C. After the nuclease reaction, the reaction products were analyzed on 2% (w/v) agarose gel using 1X TBE as the running buffer at a constant voltage of 135 V for 30 min. After staining with SYBR green II, the resulting image was obtained with Gel Doc Ez Imager (Bio-Rad, Hercules, CA, USA). The negatively charged DNA is released from copper nanoflowers during the electrophoresis and observed at the right position after being stained by SYBR green II.

Cytotoxicity assay of DNA-copper nanoflowers: Cytotoxicity of DNA-nanoflowers was quantitatively measured by using the Ez-Cytox Cytotoxicity assay kit (MTT assay). HeLa cells (1 x 10^4 cells/well) were seeded in a 96-well culture plate and incubated for 24 h. Then, the media of the cells were replaced with media containing various concentrations of DNA-nanoflowers (0 – 100 μg/mL). The cells were incubated for another 24 h, followed by analysis via the MTT assay, according to the manufacturer's instructions. The absorbance value from the untreated control cells is used to normalize the values obtained from the treated cells.

Peroxidase mimicking activity of DNA-copper nanoflowers: DNA or Protein-nanoflowers (BSA 0.1 mg/mL) were incubated with 3,3′,5,5′-tetramethylbenzidine (TMB, 500 μM) in sodium acetate buffer (100mM, pH 4) containing H_2O_2 (10 mM). The mixture was incubated at room temperature for 15 min, which was then used to obtain images representing the progress of the reaction and UV-visible absorption spectra using a microplate reader (Synergy H1, BioTek, VT).

Table S1. DNA information used in this study.

DNA samples	**Sequences or information**
A) dNTPs	dATP, dTTP, dGTP and dCTP
B) Adenine-rich ssDNA	5'-AAA AAA AAA AAA T AAA AAA AAA AAA T AAA AAA AAA AAA T AAA AAA AAA AAA-3'
C) Thymine-rich ssDNA	5'-TTT TTT TTT TTT T TTT TTT TTT TTT T TTT TTT TTT TTT T TTT TTT TTT TTT-3'
D) Guanine-rich ssDNA	5'-GGG GGG GGG GGG T GGG GGG GGG GGG T GGG GGG GGG GGG T GGG GGG GGG GGG-3'
E) Cytosine-rich ssDNA	5'-CCC CCC CCC CCC T CC CCC CCC CCC T CC CCC CCC CCC T CC CCC CCC CCC-3'
F) ssDNA complementary to B for A-T dsDNA	5'-TTT TTT TTT TTT A TTT TTT TTT TTT A TTT TTT TTT TTT A TTT TTT TTT TTT-3'
G) ssDNA complementary to D for G-C dsDNA	5'-CCC CCC CCC CCC A CC CCC CCC CCC A CC CCC CCC CCC A CC CCC CCC CCC-3'
H) PCR amplicon (200 bp)	Sample was obtained by amplifying the genomic DNA of *Chlamydia trachomatis* using the following primers. Forward primer: 5'-CTA GGC GTT TGT ACT CCG TCA-3' Reverse primer: 5'-TCC TCA GAA GTT TAT GCA CT-3'
I) Plasmid DNA (5420 bp)	pETDuet-1
J) Genomic DNA (4857 kbp)	Sample was obtained by purifying the genomic DNA of *Salmonella typhimurium*.

Table S2. The encapsulation yield and weight percentage of DNA in the nanoflowers.

DNA samples	Initial DNA concentration (μM)	Encapsulation yield (%)	Weight Percentage (%)
Adenine-rich ssDNA	1	28	9
	0.5	48	10
	0.25*	97	10
	0.1	96	6
	0.05	100	4

*The best encapsulation yield and weight percentage were obtained from 0.25 μM initial DNA concentration.

Figure S1. TEM analysis of DNA-nanoflowers. (A) TEM image of a single DNA-nanoflower. B) High-resolution TEM image of the crystal lattice structure of the petal.

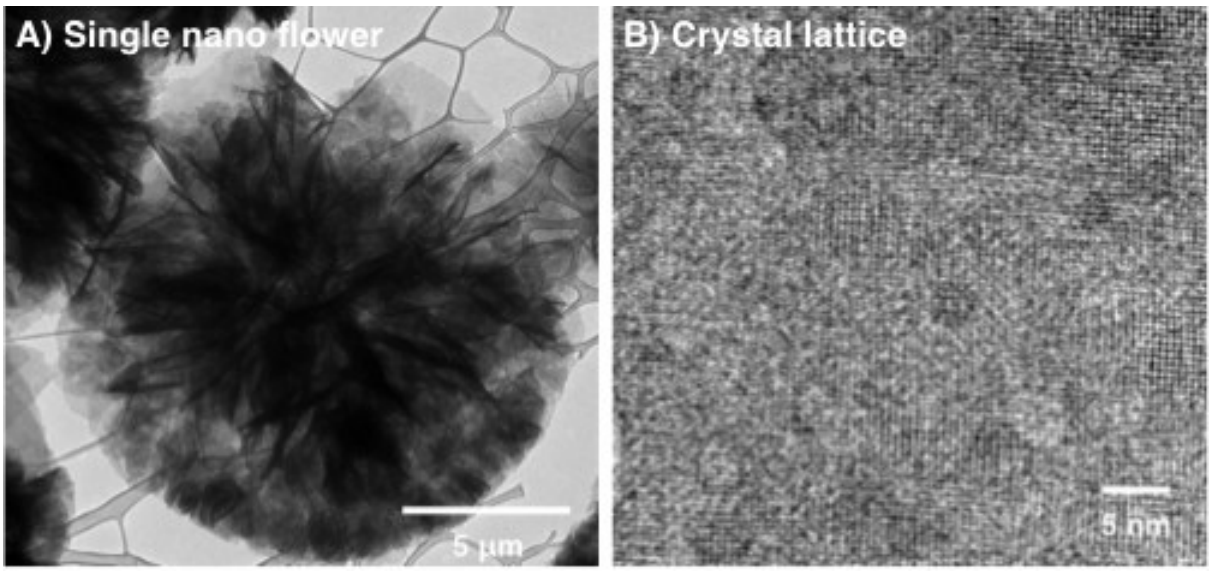


Figure S2. X-ray diffraction patterns of DNA-nanoflowers showing the peaks for $Cu_3(PO_4)_2.3H_2O$ (JCPDS 00-022-0548) and NaCl crystals (JCPDS 01-088-2300).

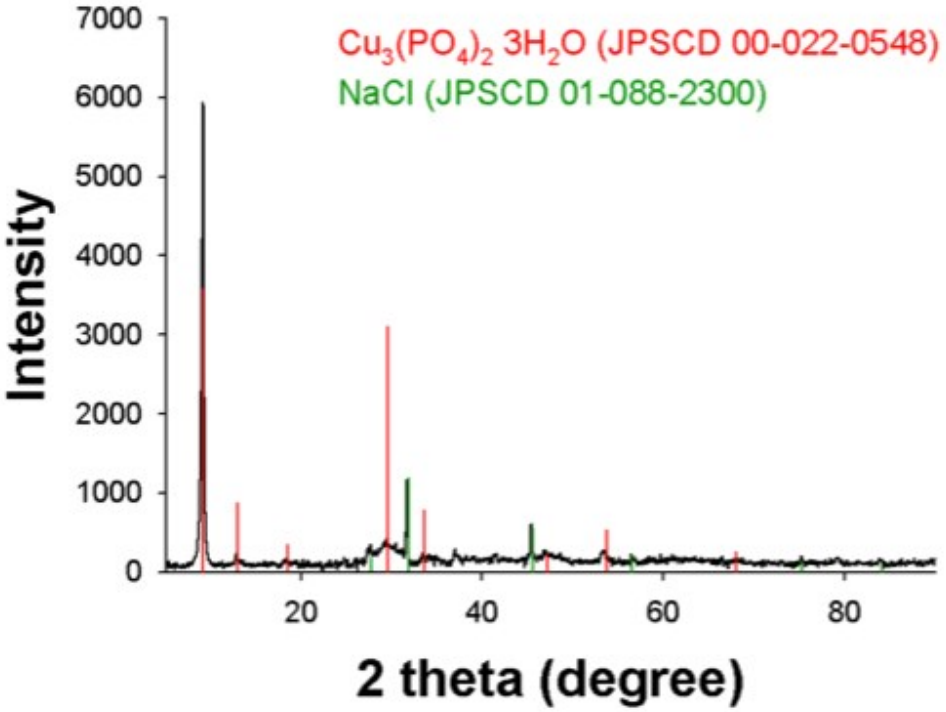


Figure S3. Element mapping of DNA-nanoflowers via EDS. DNA-nanoflowers (A) exhibit the images corresponding to copper (B), phosphate (C) and nitrogen (D).

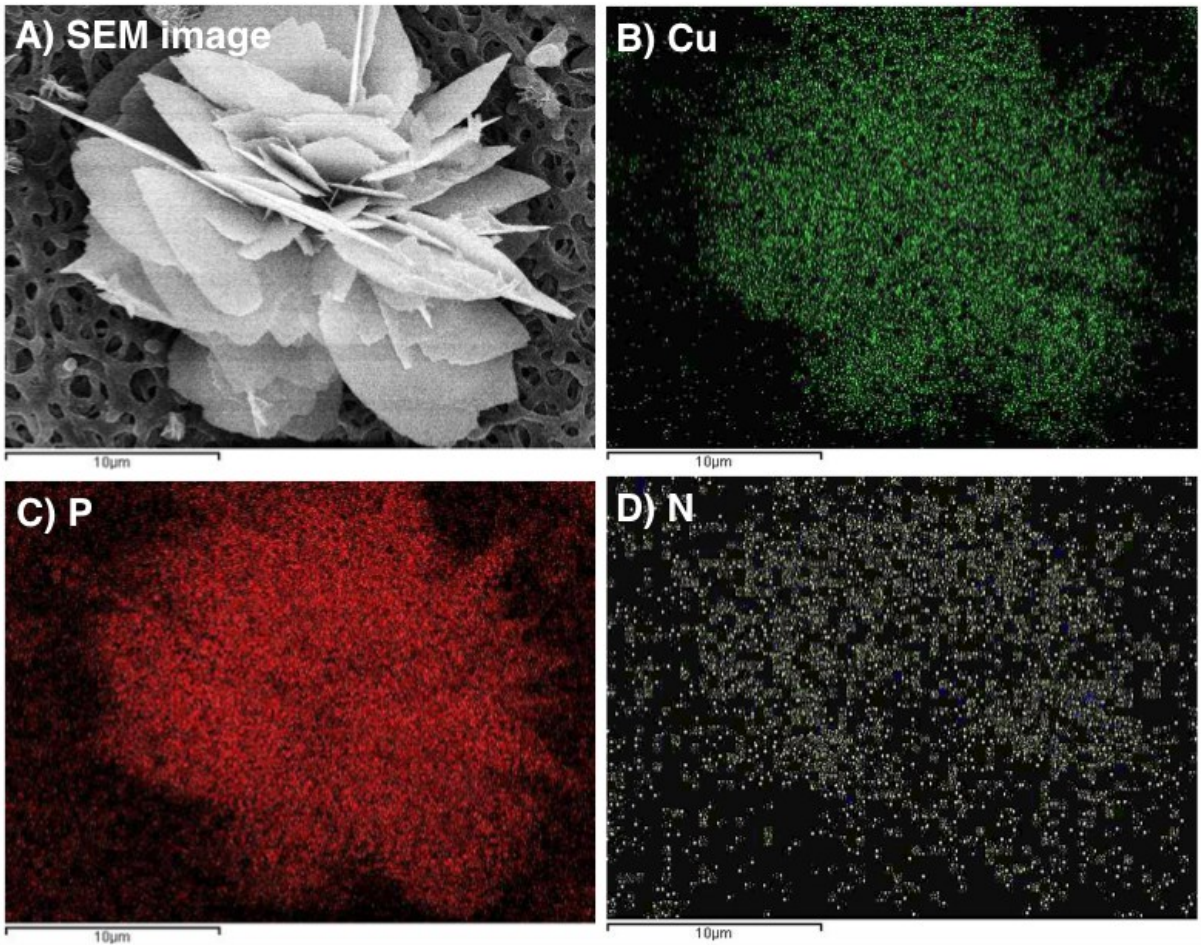
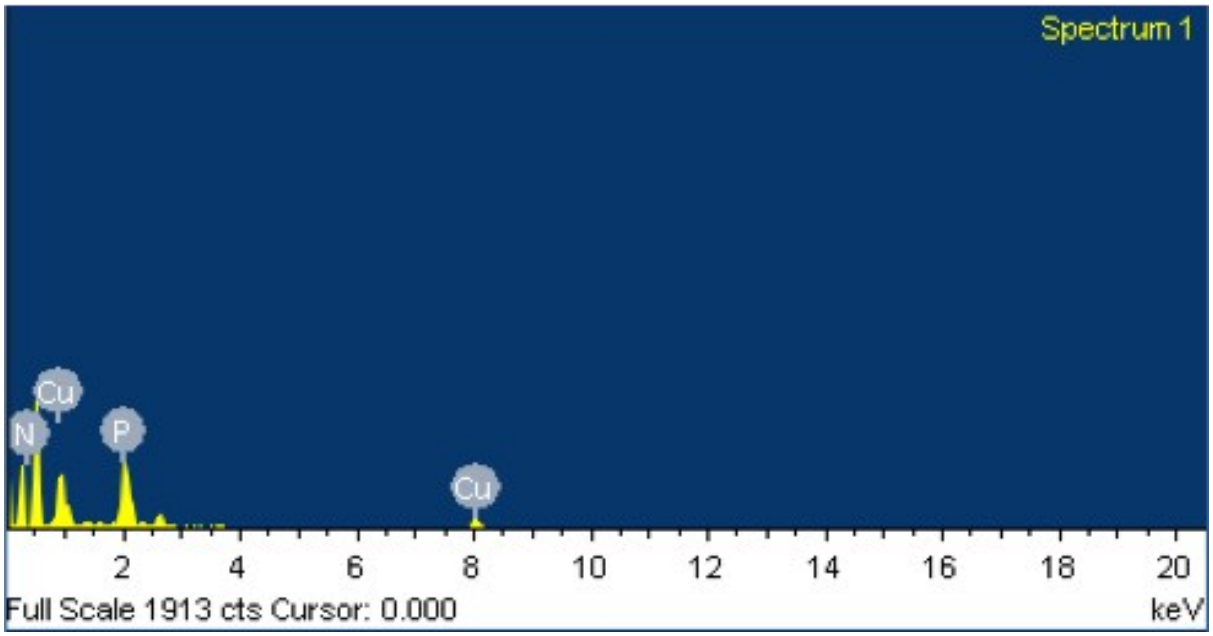
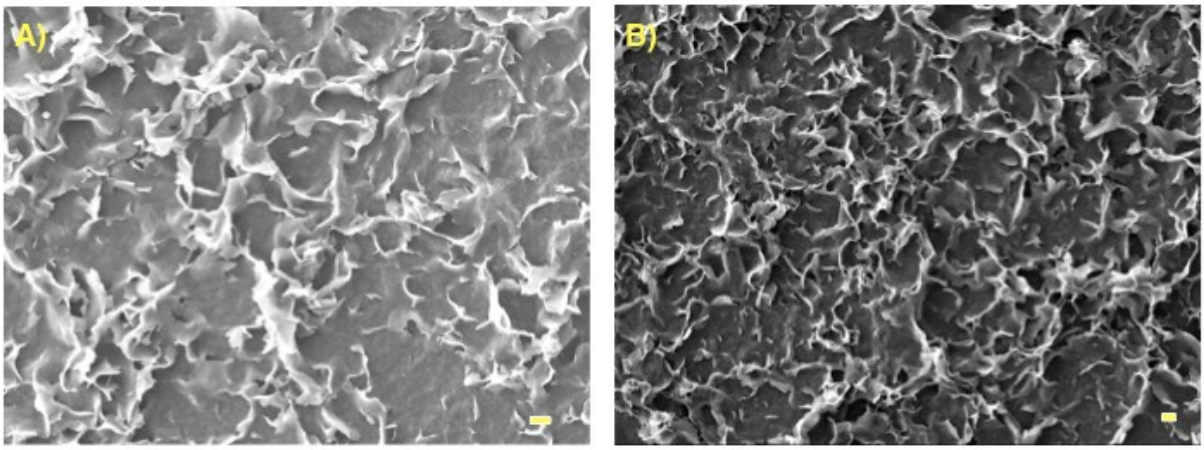


Figure S4. EDS spectrum of complete element distribution. The elements corresponding to Cu, P and N were observed from DNA-nanoflowers. Weight and atomic percentage of the elements were also calculated based on the EDS spectrum.



Element	Weight %	Atomic %
Cu	37.34	13.73
P	19.98	15.07
N	42.69	71.2

Figure S5. SEM images showing the structures formed by control polymer, poly(ethylene glycol) composed of carbon, oxygen and hydrogen. Poly(ethylene glycol) with MW 4000 was used at two different concentrations (A: 0.1 mg/mL and B: 1 μg/mL). Scale bar = 10 μm.



Poly(ethylene glycol) without amide and amine groups was not able to produce flower-like structures but just resulted in the formation of the amorphous bulky structures, which is very similar with the one observed from another control case where DNA was excluded (**Figure 2F**). This confirms that the nitrogen atoms in nucleic acids are critical for the formation of flower-like structures through the complexation with copper ions.

Figure S6. SEM images showing the effect of DNA concentration on the formation of DNA-nanoflowers. (A) 0.05 μM, (B) 0.1 μM, (C) 0.25 μM, (D) 0.5 μM, (E) 1 μM, and (F) 0 μM. The T-rich ssDNA at different concentrations was used as the synthetic template.

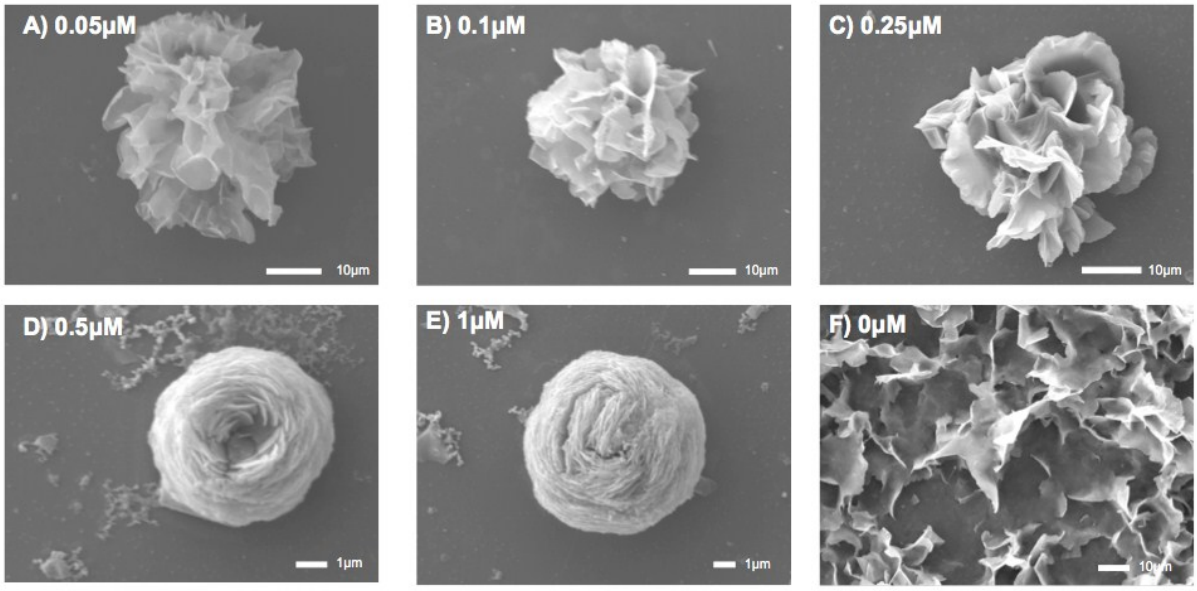


Figure S7. SEM images showing the effect of DNA concentration on the formation of DNA-nanoflowers. (A) 0.05 μM, (B) 0.1 μM, (C) 0.25 μM, (D) 0.5 μM, (E) 1 μM, and (F) 0 μM. The G-rich ssDNA at different concentrations was used as the synthetic template.

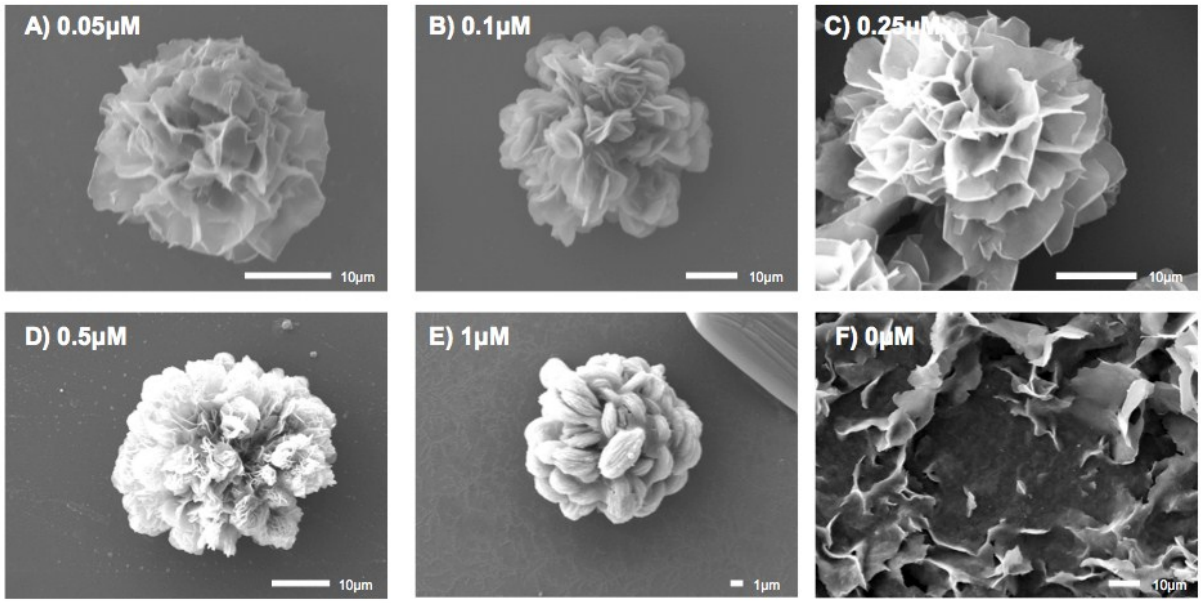


Figure S8. SEM images showing the effect of DNA concentration on the formation of DNA-nanoflowers. (A) 0.05 μM, (B) 0.1 μM, (C) 0.25 μM, (D) 0.5 μM, (E) 1 μM, and (F) 0 μM. The C-rich ssDNA at different concentrations was used as the synthetic template.

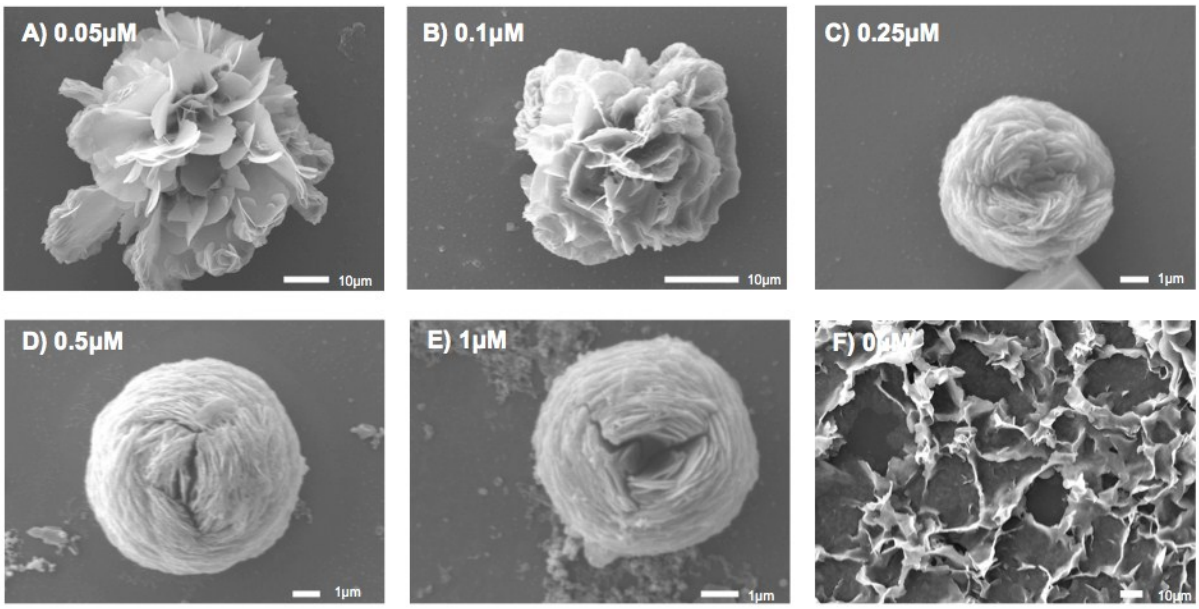
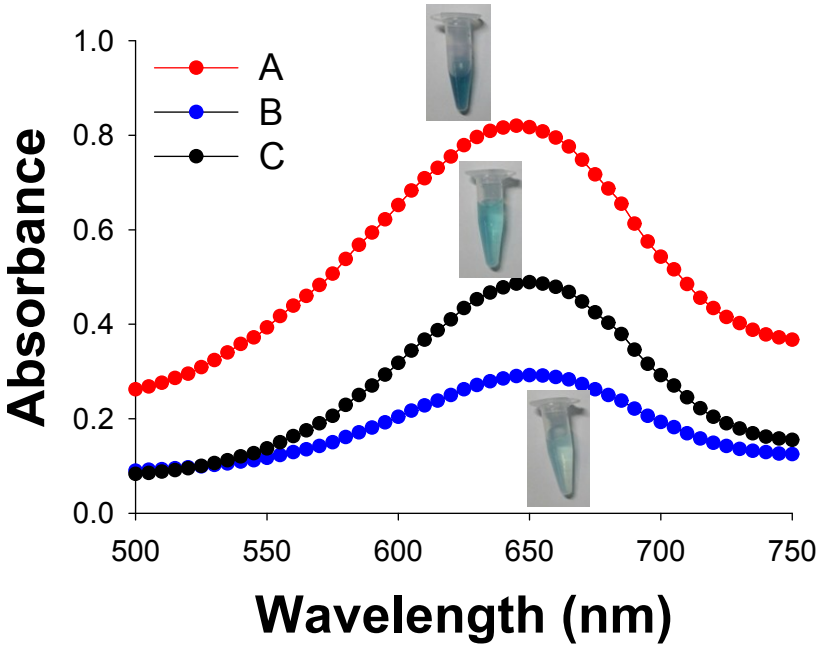


Figure S9. Peroxidase mimicking activity of DNA-copper nanoflowers. (A) DNA-copper nanoflowers formed by T-rich ssDNA, (B) Amorphous bulky structures formed in the absence of DNA, and (C) Protein-copper nanoflowers formed by bovine serum albumin. The peroxidase colorimetric substrate, TMB, was used to determine the catalytic activity of the nanoflowers.



References

1 (a) K.S. Park, C.Y. Lee, H.G. Park, *Chem. Commun.,* 2016, **52**, 4868; (b) K.S. Park, C.H. Huang, K. Lee, Y.E. Yoo, C.M. Castro, R. Weissleder, H. Lee, *Sci. Adv.,* 2016, **2,** e1600300.