Supporting Information for

Site-specific control of silica mineralization on DNA using a designed peptide

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Experimental Section

General remarks

All chemicals and solvents were of reagent or HPLC grade and were used without further purification. Oligodeoxynucleotide PCR primers were purchased from Hokkaido System Science (Sapporo, Japan). HPLC was performed on a GL-7400 HPLC system (GL Sciences, Tokyo, Japan) using an Inertsil ODS-3 column (10 × 250 mm; GL Science) for preparative purification, with a linear acetonitrile/0.1% trifluoroacetic acid (TFA) gradient at a flow rate of 3.0 mL/min. Peptides were analyzed using MALDI-TOF MS on an Autoflex III (Bruker Daltonics, Billerica, MA, USA) mass spectrometer with 3,5-dimethoxy-4-hydroxycinnamic acid as the matrix. Amino acid analysis was carried out using an Inertsil ODS-2 column (4.6 × 200 mm; GL Science) after samples were hydrolyzed in 6 M HCl at 110°C for 24 h in a sealed tube and then labeled with phenyl isothiocyanate.

Synthesis of designed PNAs

Designed peptides were synthesized manually on Fmoc-NH-SAL-PEG resin (Watanabe Chemical Industries, Hiroshima, Japan) using Fmoc chemistry[1, 2] with Fmoc-AA-OH (4 eq., Watanabe Chemical Industries) and Fmoc PNA monomers (4 eq., Panagene, Daejeon, Korea) according to the *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU, Watanabe Chemical) method. Side-chain protection was as follows: *t*-butyl (tBu) for Ser, *t*-butyloxycarbonyl (Boc) for Lys, 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Pbf) for Arg, and benzhydryloxycarbonyl (Bhoc) for guanine, adenine and cytosine PNA monomers. Peptides were cleaved from the resins and side-chain protection was removed by incubating the peptides for 2 h in TFA (Watanabe Chemical Industries)/H_2O/triisopropylsilane (Wako Pure Chemical Industries, Tokyo, Japan) (20:1:1, v/v). Peptides were precipitated by addition of cold diethyl ether, collected by centrifugation, purified by RP-HPLC, and characterized by amino acid analysis and MALDI-TOF MS: SiPP-PNA, *m/z* 3906.0 ($[M+H]^+$ calcd. 3907.1); SiPP, *m/z* 1188.9 ($[M+H]^+$ calcd. 1188.4).

Purified peptides were dissolved in MilliQ water to about 4 mM, and their concentrations were determined by amino acid analysis. Peptides were stored at 4°C.

Synthesis of TempDNAs

TempDNAs were amplified by PCR from pBR322 (L series: 250-1750, S series: 250-850). Primers used for amplification of TempDNA_L-1 were (5'-ACGTAGATCAGCAATTTCTATGCGCACCCGTTCTC-3') and (5'-ACGTAGATCATCACTCAGGGTCAATGCCAGCGCTT-3'). Primers used for amplification of TempDNA_L-4 were (5'-ACGTAGATCAACGTAGATCAACGTAGATCAACGTAGATCAGCAATTTCTATGCGCACCCGTTCTC-3') and (5'-ACGTAGATCAACGTAGATCAACGTAGATCAACGTAGATCATCACTCAGGGTCAATGCCAGCGCTT-3'). Primers used for amplification of TempDNA_L-0 were (5'-GCAATTTCTATGCGCACCCGTTCTC-3') and (5'- TCACTCAGGGTCAATGCCAGCGCTT -3'). Primers used for amplification of TempDNA_S-4 were (5'-ACGTAGATCAACGTAGATCAACGTAGATCAACGTAGATCAGCAATTTCTATGCGCACCCGTTCTC-3') and (5'-ACGTAGATCAACGTAGATCAACGTAGATCAACGTAGATCAGAATACCGCAAGCGACAGGCC-3'). After PCR, the samples were purified using a MinElute PCR Purification Kit (QIAGEN).

Sample preparation

Prior to preparation of sample solutions, TempDNAs and peptides were mixed and the solvent was completely evaporated using a centrifugal evaporator. Next, each sample (final conc. 0.125 μM) in 0.625 mM Tris-HCl buffer (pH 7.5) was heated at 90°C for 5 min and then gently cooled to 37°C at a rate of 1.0°C min^{-1}. A 1-μL volume of sample was mixed with 1 μL of phosphate buffer (1 M sodium

phosphate, pH 6.4) and 8 μL of MilliQ water. Prior to silica precipitation, 1 M silicic acid was prepared by mixing tetramethoxysilane (TMOS, Tokyo Chemical Industry, Tokyo, Japan) and 1 mM HCl and incubating for 5 min.[3] Then the solution was diluted 100 times by MilliQ. Finally, the 10-μL peptide and DNA solution was mixed with 1 μL of the 10 mM silicic acid solution and incubated for 3 h.

Atomic force microscopy (AFM)

Samples incubated for 3 h were mixed with 9 μL of MilliQ water, and the entire volume of each sample was placed on freshly cleaved mica (1 × 1 cm). After 5 min, the solvent was absorbed with filter paper. MilliQ water (20 μL) was then placed on the mica surface and immediately absorbed with filter paper. This process was repeated three times to remove salts from the sample. All samples were dried in vacuo before AFM measurements. Tapping-mode images were obtained on a MultiMode scanning probe microscope with a Nanoscope IIIa controller (Veeco, Woodbury, NY).

Transmission electron microscopy (TEM)

Samples incubated for 3 h were mixed with 9 μL of MilliQ water, and the entire volume of each sample was placed on a TEM grid (Cu 200 mesh covered with a Nisshin EM collodion membrane, Japan) for 1 min and dried with filter paper. MilliQ water (20 μL) was then placed on the grid and immediately absorbed with filter paper. This process was repeated three times to remove salts from the sample. All samples were dried in vacuo before TEM measurements, conducted at an accelerating voltage of 200 kV (JEOL JEM-2100, Japan). For immuno-TEM analyses,[4] samples incubated for 3 h were mixed with 6 μL of anti-DNA antibody (GeneTex, DNA ds & ss antibody) solution (final conc. 0.5 nM). After a 30-min incubation, the solution was mixed with 3 μL of Protein A (British BioCell International, EM Protein A: 10 nm Gold) solution (final conc. 0.5 nM). After a

30-min incubation, the entire volume of the sample was placed on a TEM grid for 1 min and dried with filter paper. The same washing process described above was then performed.

Scanning Electron Microscopy (SEM)

Sample preparation was conducted same as TEM measurement. All samples were dried in vacuo before SEM measurements were conducted at an accelerating voltage of 30 kV on a scanning electron microscope (JIB-4601F, JEOL, Japan).

Dynamic light scattering (DLS) measurements

Sample solution (10 μL) was transferred into a UV-transparent disposable cuvette S3 (Sarstedt, Germany), and DLS data were acquired on a Zetasizer ZEN3600 instrument (Sysmex) equipped with a 633-nm laser.

Gel electrophoresis

Native gel electrophoresis was performed using nondenaturing gels containing 0.7% agarose. Loading buffer (2 μL) was mixed with 10 μL of sample solution. Each sample was loaded onto the gel and electrophoresed at 100 V for 30 min at room temperature. Gels were stained with ethidium bromide and imaged using a FLA-7000 imager (Fuji Film, Tokyo, Japan). Band intensities were quantified using Multi Gauge software (V.3.2) for Windows. The Non-bound DNA percentage was calculated[5] according to the following equation: (intensity of DNA band (ca. 1500 bp) in the sample with/without peptide and/or silica precipitation) / (intensity of DNA band (ca. 1500 bp) in the sample without SiPP-PNA and silica precipitation (DNA alone)) x 100 (%).

References

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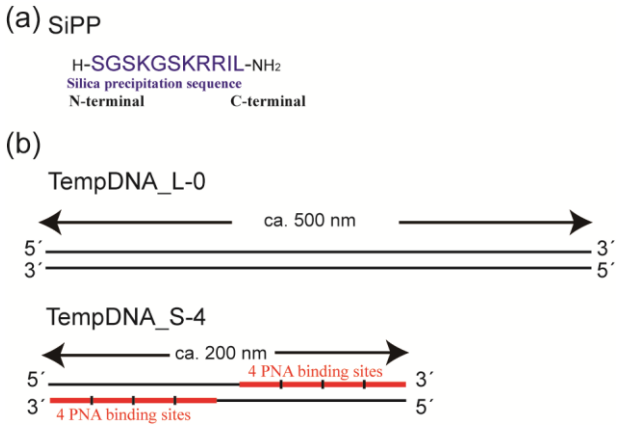

Figure S1. (a) Sequence of the silica-precipitating peptide used in this study. (b) Schematic illustrating the TempDNA_L-0 and the TempDNA_S-4.

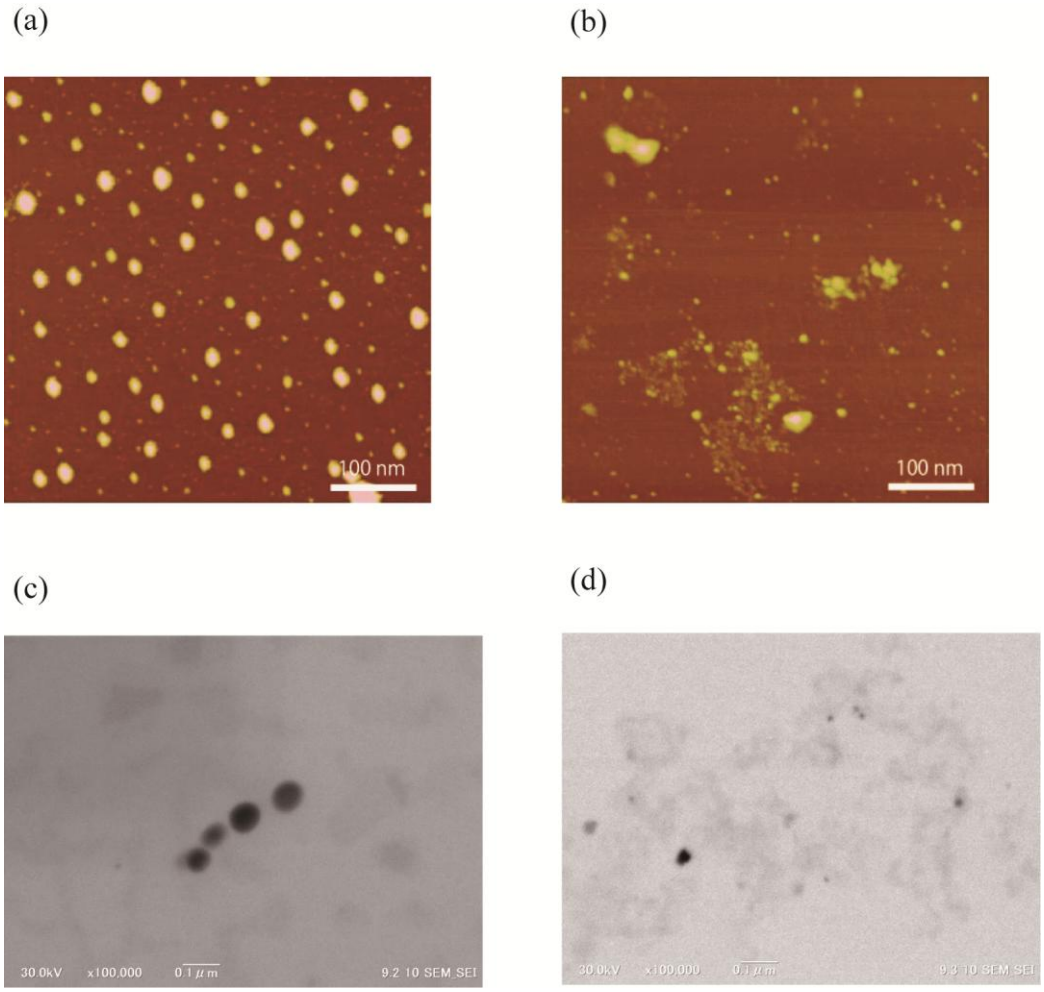

Figure S2. AFM images of silica precipitation using peptides: (a) SiPP-PNA alone, and (b) SiPP alone. SEM images of silica precipitation using peptides: (c) SiPP-PNA alone, and (d) SiPP alone.

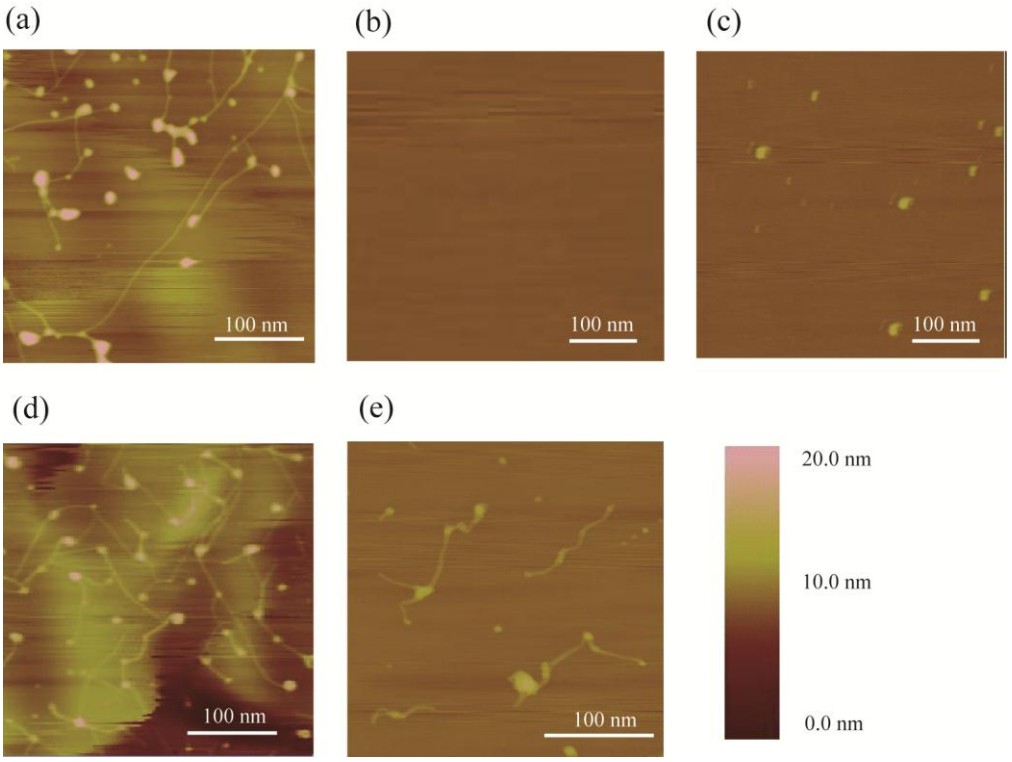

Figure S3. AFM images of silica precipitations using (a) TempDNA_L-4 with SiPP-PNA, (b) TempDNA_L-4 without SiPP-PNA, (c) TempDNA_L-0 with SiPP-PNA, (d) TempDNA_L-1 with SiPP-PNA, (e) TempDNA_S-4 with SiPP-PNA.

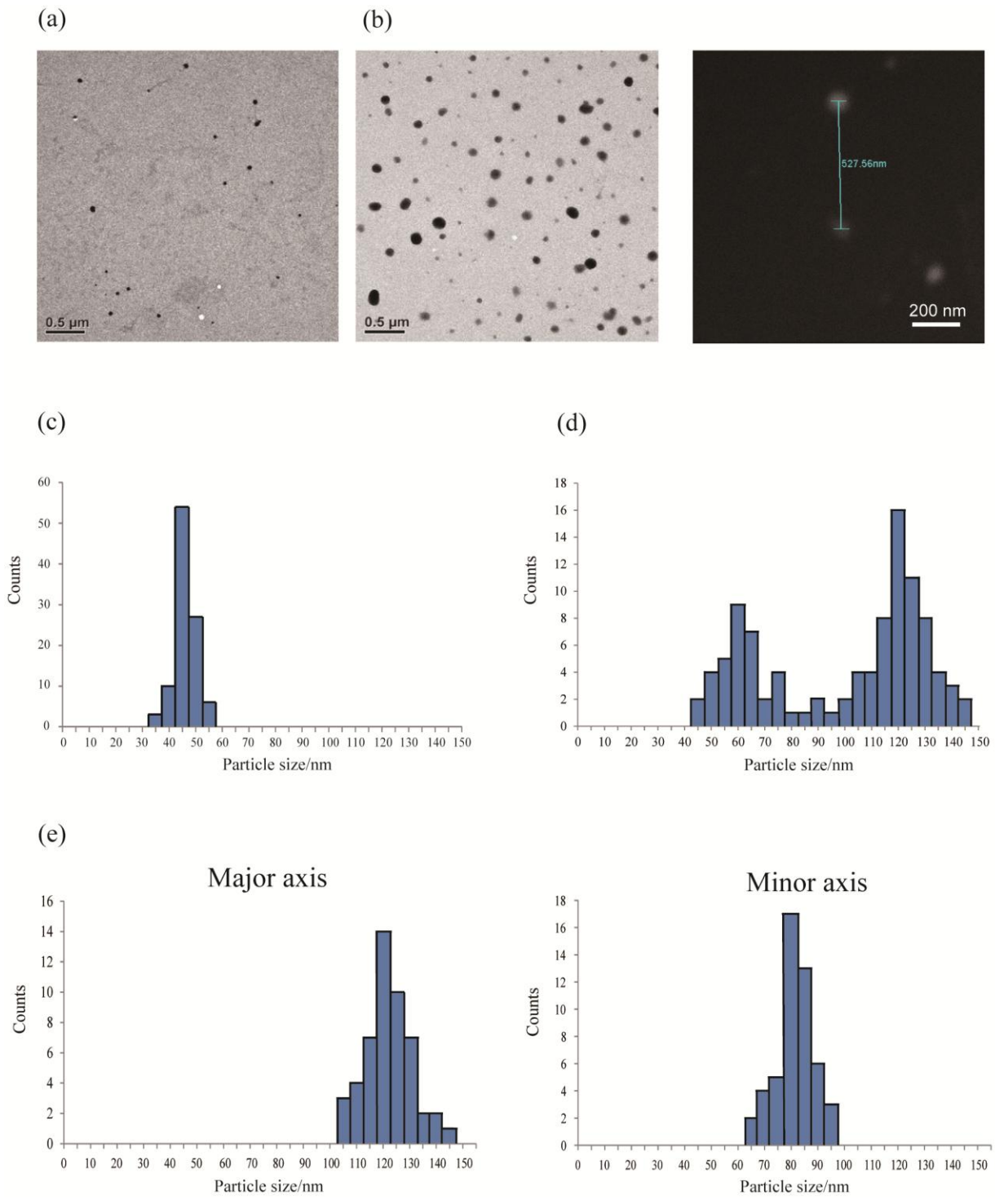

Figure S4. Site-specific precipitation of silica using SiPP-PNA and TempDNAs. (a) Additional TEM image of precipitation using TempDNA_L-1. (b) Additional TEM image of precipitation using TempDNA_L-4. (c) Histogram of silica particle sizes in TEM data with SiPP-PNA and TempDNA_L-1. (d) Histogram of silica particle sizes in TEM data with SiPP-PNA and TempDNA_L-4. (e) Histograms of major axes and minor axes of bigger silica oval spheres in TEM data with SiPP-PNA and TempDNA_L-4.

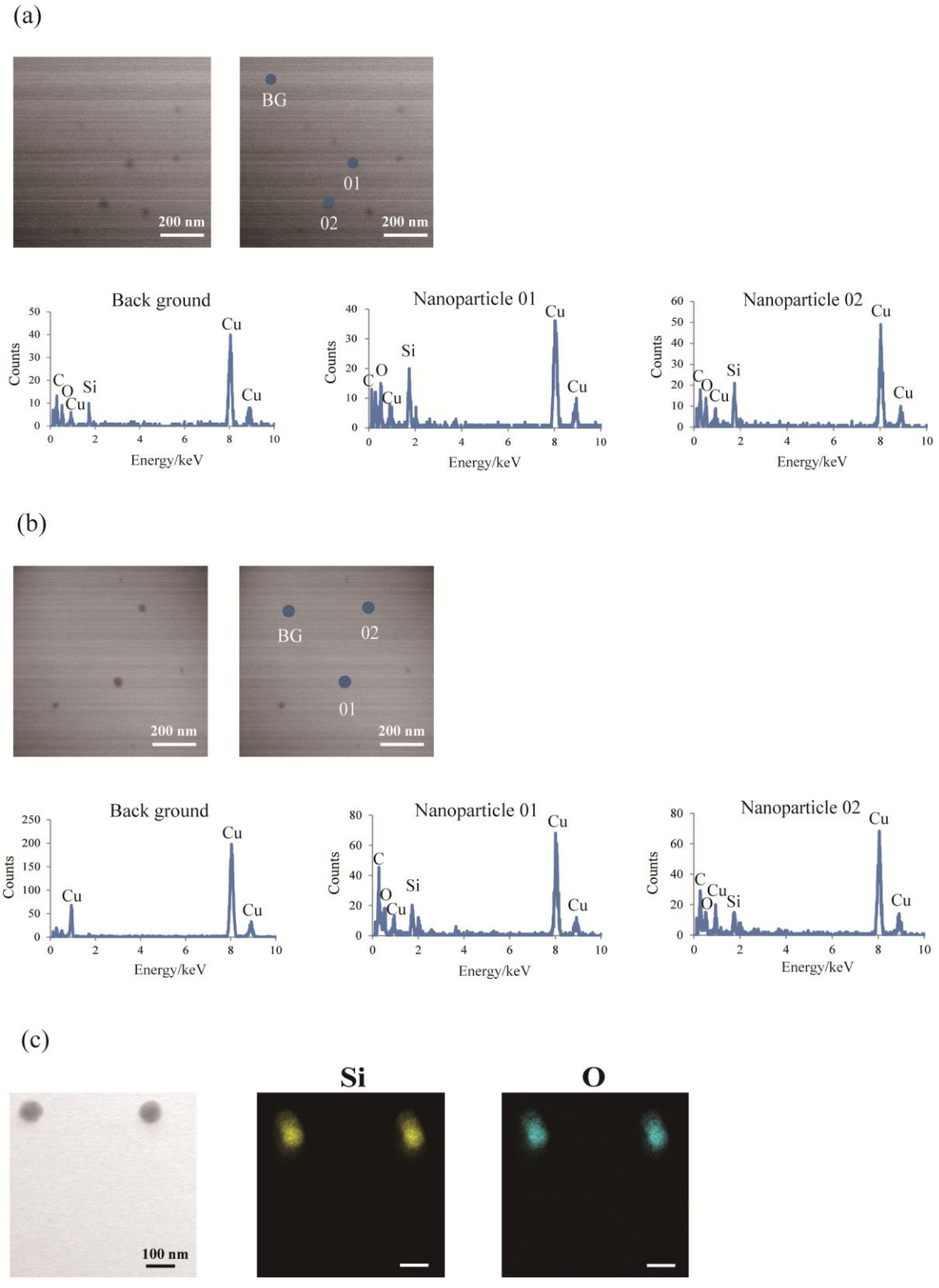

Figure S5. Site-specific precipitation of silica using SiPP-PNA and TempDNAs. (a) TEM-EDX point analyses of SiPP-PNA and TempDNA_L-1 sample. (b) TEM-EDX point analyses of SiPP-PNA and TempDNA_L-4 sample. (c) TEM-EDX mapping analyses of SiPP-PNA and TempDNA_L-1 sample.

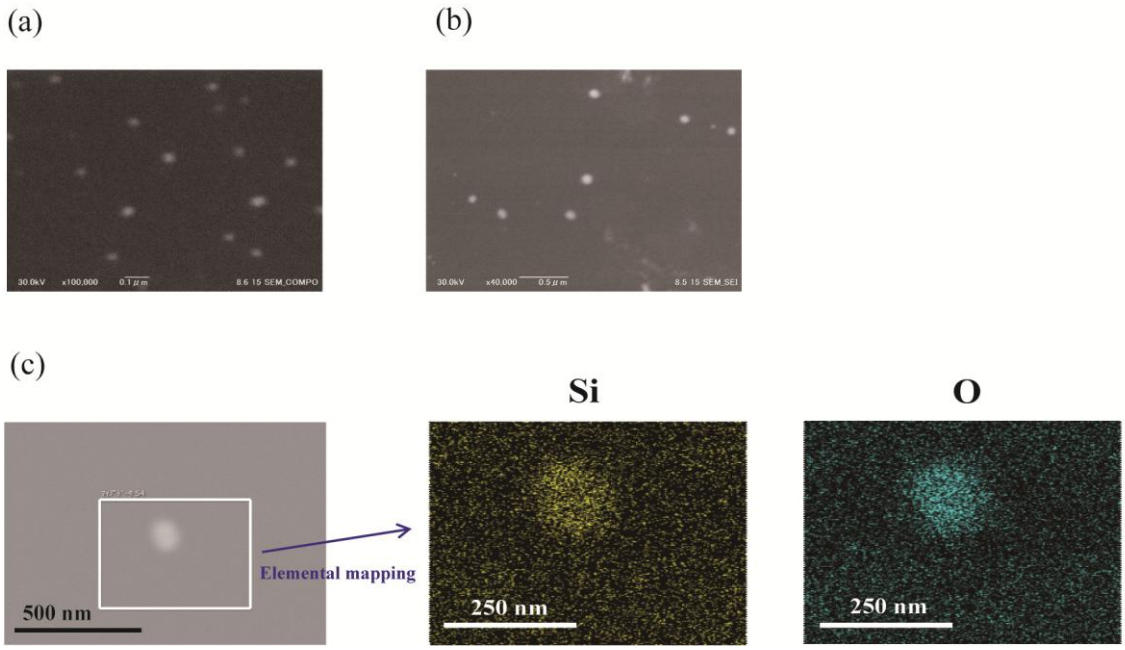

Figure S6. Site-specific precipitation of silica using SiPP-PNA and TempDNAs. (a) SEM image of silica precipitation using SiPP-PNA and TempDNA_L-1 sample. (b) SEM image of silica precipitation using SiPP-PNA and TempDNA_L-4 sample. (c) SEM-EDX mapping analyses of SiPP-PNA and TempDNA_L-1 sample.

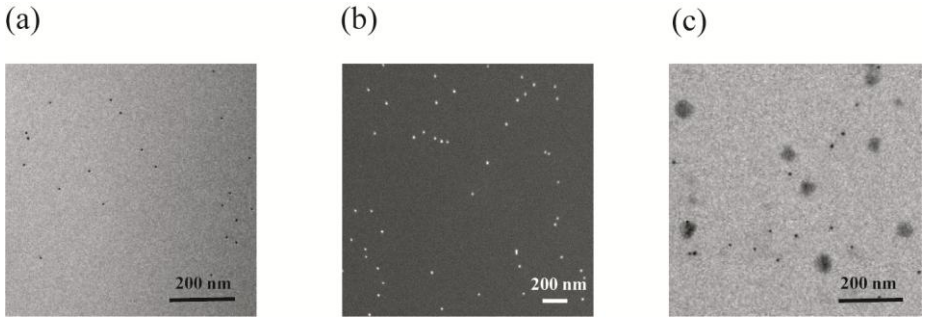

Figure S7. (a) Immuno-TEM image without TempDNA_L-1 and SiPP-PNA peptide (control sample). (b) Immuno-TEM image with TempDNA_L-1 alone. (c) Immuno-TEM image using SiPP-PNA and TempDNA_L-1. All TEM samples were unstained.