Electronic Supplementary Material (ESI) for Nanoscale.
This journal is © The Royal Society of Chemistry 2016

Supporting Information for

Site-specific control of multiple mineralizations using a designed peptide and DNA

Kenji Usui, [§*a] ***Makoto Ozaki,*** [§a] ***Aoi Yamada,*** [a] ***Yoshio Hamada,*** [a] ***Takaaki Tsuruoka,*** [a] ***Takahito Imai,*** [b] ***Kin-ya Tomizaki*** [*bc]

[a] FIRST (Faculty of Frontiers of Innovative Research in Science and Technology), Konan University, 7-1-20 Minatojima-minamimachi, Chuo-ku, Kobe 650-0047, Japan.

E-mail: kusui@center.konan-u.ac.jp; Fax: +81 78 303 1495; Tel: +81 78 303 1418

[b] Department of Materials Chemistry, Ryukoku University, 1-5 Yokotani, Seta Oe-cho, Otsu 520-2194, Japan

[c] Innovative Materials and Processing Research Center, Ryukoku University, 1-5 Yokotani, Seta Oe-cho, Otsu 520-2194, Japan.

E-mail: tomizaki@rins.ryukoku.ac.jp; Fax: +81 77 543 7483; Tel: +81 77 543 7469

[§] These authors contributed equally to this work.

Page 1: Contents
Page 2: Experimental Section
Page 6: References
Page 7: Figure S1 for the reference DNA and the reference peptide
Page 8: Figure S2 for AFM images of site-specific precipitation of calcium carbonate
Page 9: Figure S3 for AFM images of site-specific precipitation of calcium carbonate using TempDNA_L-0 and various incubation times
Page 10: Figure S4 for the blue silicomolybdic assay
Page 11: Figure S5 for standard titration with ethylenediaminetetraacetate
Page 12: Figure S6 for TEM images of silica and calcium carbonate precipitation using peptides and DNAs
Page 13: Figure S7 for TEM-histogram of core-shell sphere diameters (0.3 mM $CaHCO_3$)
Page 14: Figure S8 for TEM-histogram of core-shell sphere diameters (0.5 mM $CaHCO_3$)
Page 15: Figure S9 for HPLC and MS for purified SiPP-PNA and SiPP

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 Sciences) 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 Sciences) 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 the peptides

The 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 Industries) 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 peptide-resin for 2 h in TFA (Watanabe Chemical Industries)/H_2O/triisopropylsilane (Wako Pure Chemical Industries, Osaka, Japan) (20:1:1, v/v). Peptides were precipitated by the addition of cold diethyl ether, collected by centrifugation, purified by RP-HPLC (Fig. S9a and b), and characterized by

amino acid analysis and MALDI-TOF MS (Fig. S9c and d),: SiPP-PNA, *m/z* 3907.4 ($[M+H]^+$ calcd. 3907.1); SiPP, *m/z* 1188.9 ($[M+H]^+$ calcd. 1188.4). The peptides were dissolved in MilliQ water to about 4 mM, and the concentration was determined by amino acid analysis. The peptides were stored at 4°C.

Synthesis of TempDNAs

TempDNAs were amplified by PCR from pBR322 (250-1750). Primers used for amplification of TempDNA_L-1 were (5'-ACGTAGATCAGCAATTTCTATGCGCACCCGTTCTC-3') and (5'-ACGTAGATCATCACTCAGGGTCAATGCCAGCGCTT-3'). Primers used for amplification of TempDNA_L-0 were (5'-GCAATTTCTATGCGCACCCGTTCTC-3') and (5'-TCACTCAGGGTCAATGCCAGCGCTT-3'). After PCR, the samples were purified using a MinElute PCR Purification Kit (QIAGEN, Tokyo, Japan).

Sample preparation in single precipitation (calcium carbonate) for atomic force microscopy (AFM)

Prior to calcium carbonate precipitation, calcium carbonate (0.5 mmol) was suspended in MilliQ water (30 mL). Carbon dioxide gas was bubbled into the stirred suspension for 3 h, then the remaining solid $CaCO_3$ was removed by filtration. The concentration of calcium ions in the solution was determined by standard titration with ethylenediaminetetraacetate.[3] Calcium carbonate precipitation was conducted in a micro tube. The calcium hydrogen carbonate solution, peptide solution, and DNA solution were diluted and mixed to the desired concentration with MilliQ water and incubated for 3 h. 20-μl samples were 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, USA).

Sample preparation in multiple precipitation for AFM and transmission electron microscopy (TEM)

Prior to preparation of the 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) and 1 mM HCl and incubating for 5 min,[4] then the solution was diluted 100 times with MilliQ water. 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.[5]

Prior to calcium carbonate precipitation, calcium carbonate (0.5 mmol) was suspended in MilliQ water (30 mL). Carbon dioxide gas was bubbled into the stirred suspension for 3 h, then the remaining solid $CaCO_3$ was removed by filtration. The concentration of calcium ions in the solution was determined by standard titration with ethylenediaminetetraacetate.[3] Calcium carbonate precipitation was conducted on a TEM grid (Cu 200 mesh covered with a Nisshin EM collodion membrane; Nisshin EM Co.,Ltd., Tokyo, Japan) or AFM mica surface. After silica mineralization and sample preparation on a TEM grid or AFM mica surface, the calcium hydrogen carbonate solution at the desired concentration was placed on the TEM grid or AFM mica surface. After the incubation, MilliQ water (20 μL) was then placed on the 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 or TEM measurements, conducted at an accelerating

voltage of 200 kV (JEM-2100; JEOL, Tokyo, Japan).

The blue silicomolybdic assay[6]

Solution A was made of 0.16 mM ammonium molybdate tetrahydrate and 0.72 mM hydrochloric acid in deionized water. Solution B was prepared by adding oxalic acid (final conc. 367 mM), 4-methylaminophenol sulfate (final conc. 32.5 mM), anhydrous sodium sulfate (46.7 mM) in 3 M sulfuric acid solution. After the silica precipitation, filtered 40 μL sample with a centrifugal filter (Durapore®-PVDF 0.22 μm Ultrafree®-MC-HV, Merck Japan, Tokyo, Japan) was diluted by 230 μL MilliQ and 70 μL 100 mM Tris-HCl buffer (pH 8.0). Then, the solution A (30 μL) was added to this sample solution. After 15 min incubation, 150 μL of solution B is added to the solution. After 2 h reaction at room temperature, the absorbance at 680 nm was measured using an UV-Vis spectrophotometer (UV-1700, Shimadzu, Kyoto, Japan). The concentration was determined by comparison to the standard curve prepared by plotting the absorbance of samples containing known concentrations.

The standard titration with ethylenediaminetetraacetate

After the calcium carbonate precipitation, 40 μL sample was filtered with a centrifugal filter (Durapore®-PVDF 0.45 μm Ultrafree®-MC-HV, Merck Japan, Tokyo, Japan). Then, 3 μL of 8 M NaOH solution was added to the filtered 20 μL sample. After 2-hydroxy-1-(2-hydroxy-4-sulfo-1-naphthylazo)-3-naphthoic acid was added to this sample solution, the titration was conducted using 6 mM EDTA (ethylenediaminetetraacetic acid disodium salt).

References

1 W. C. Chan, P. D. White, *Fmoc Solid Phase Peptide Synthesis*; Oxford University Press: New York, 2000.

2 K. Usui, A. Okada, K. Kobayashi, N. Sugimoto, *Org. Biomol. Chem*., 2015, **13**, 2022.

3 H. Kumagai, R. Matsunaga, T. Nishimura, Y. Yamamoto, S. Kajiyama, Y. Oaki, K. Akaiwa, H. Inoue, H. Nagasawa, K. Tsumoto, T. Kato, *Faraday Disscuss*., 2012, **159**, 483.

4 M. R. Knecht, D. W. Wright, *Chem. Commun.,* 2003, 3038.

5 M. Ozaki, K. Nagai, H. Nishiyama, T. Tsuruoka, S. Fujii, T. Endoh, T. Imai, K.-y. Tomizaki, K. Usui, *Chem. Commun.*, 2016, **52**, 4010.

6 T. Coradin, D. Eglin, J. Livage, *Spectroscopy*, 2004, **18**, 567.

(a) SiPP

(b)

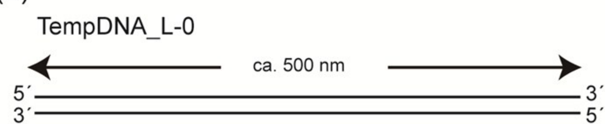

Figure S1. (a) Sequence of the silica-precipitating peptide used in this study. (b) Schematic illustrating the TempDNA_L-0.

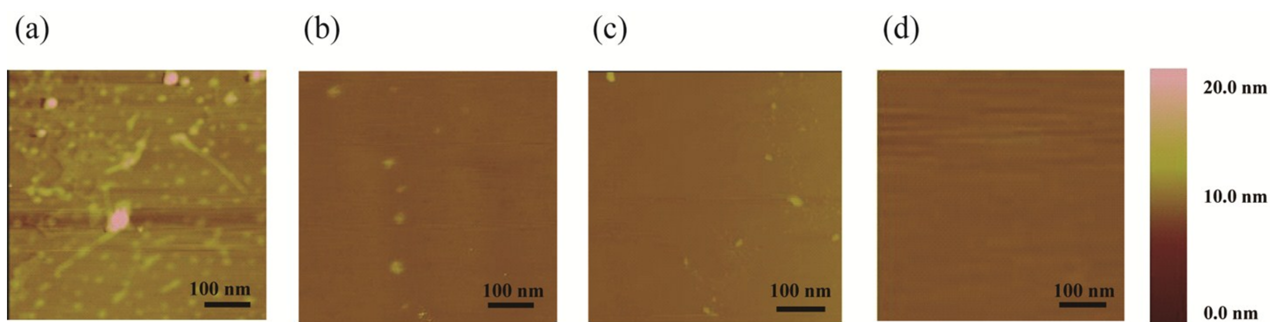

Figure S2. Site-specific precipitation of calcium carbonate or silica. (a) AFM image of calcium carbonate precipitation using TempDNA_L-0. (b) AFM image of calcium carbonate precipitation without TempDNA_L-0. (c) AFM image of calcium carbonate precipitation using SiPP-PNA. (d) AFM image of silica precipitation using TempDNA_L-0.

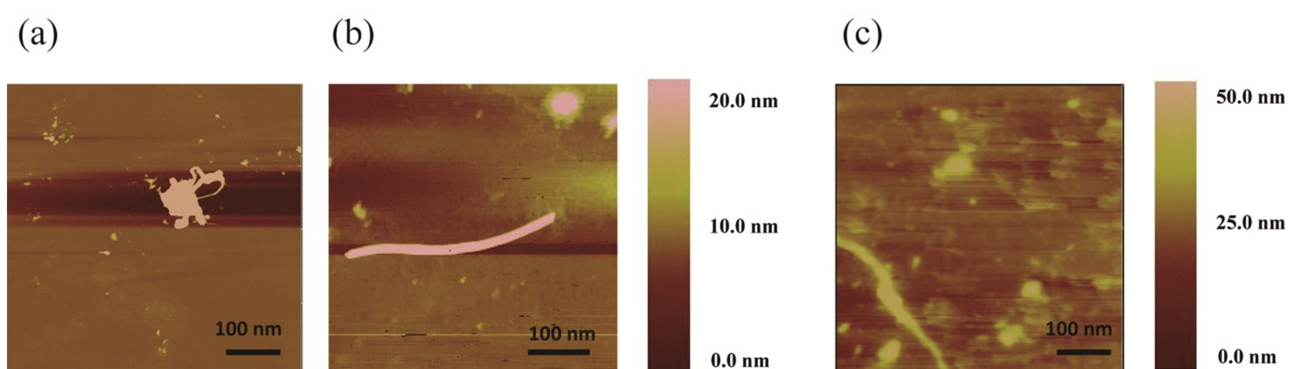

Figure S3. Site-specific precipitation of calcium carbonate using TempDNA_L-0. AFM images of calcium carbonate precipitation at (a) 3 h, (b) 6 h and (c) 24 h.

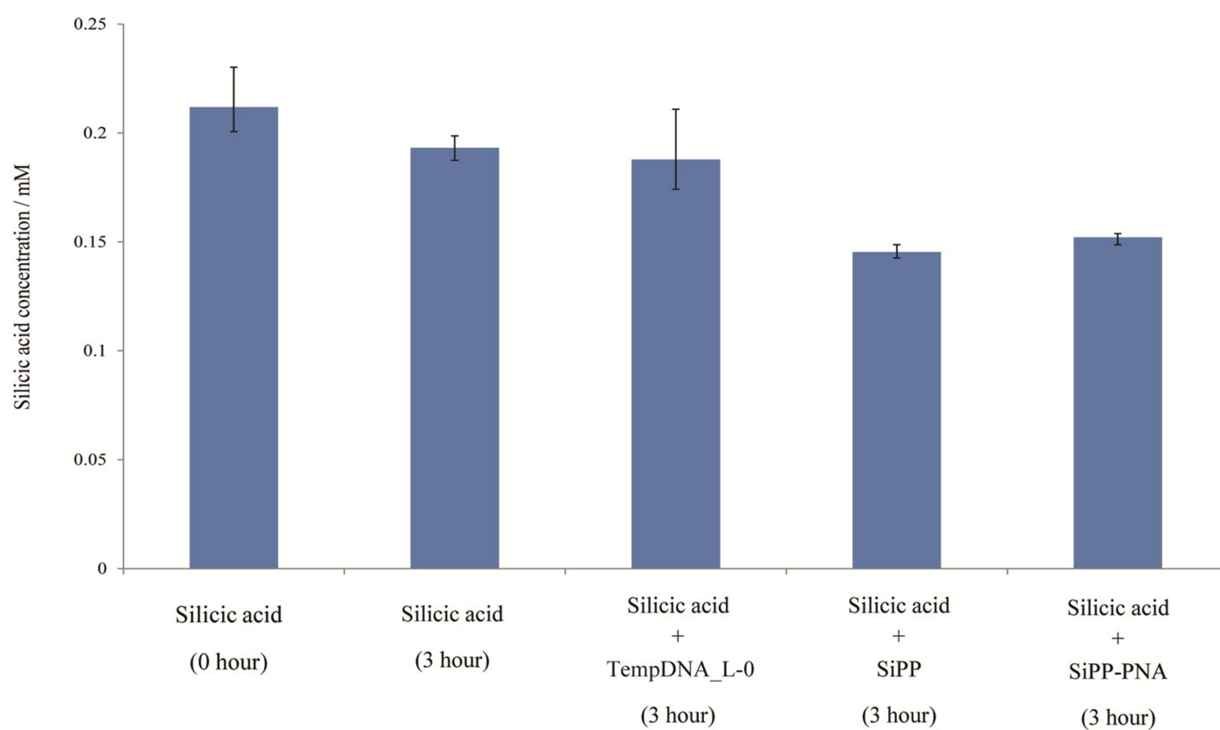

Figure S4. Residual silicic acid concentrations in various conditions by the blue silicomolybdic assay.

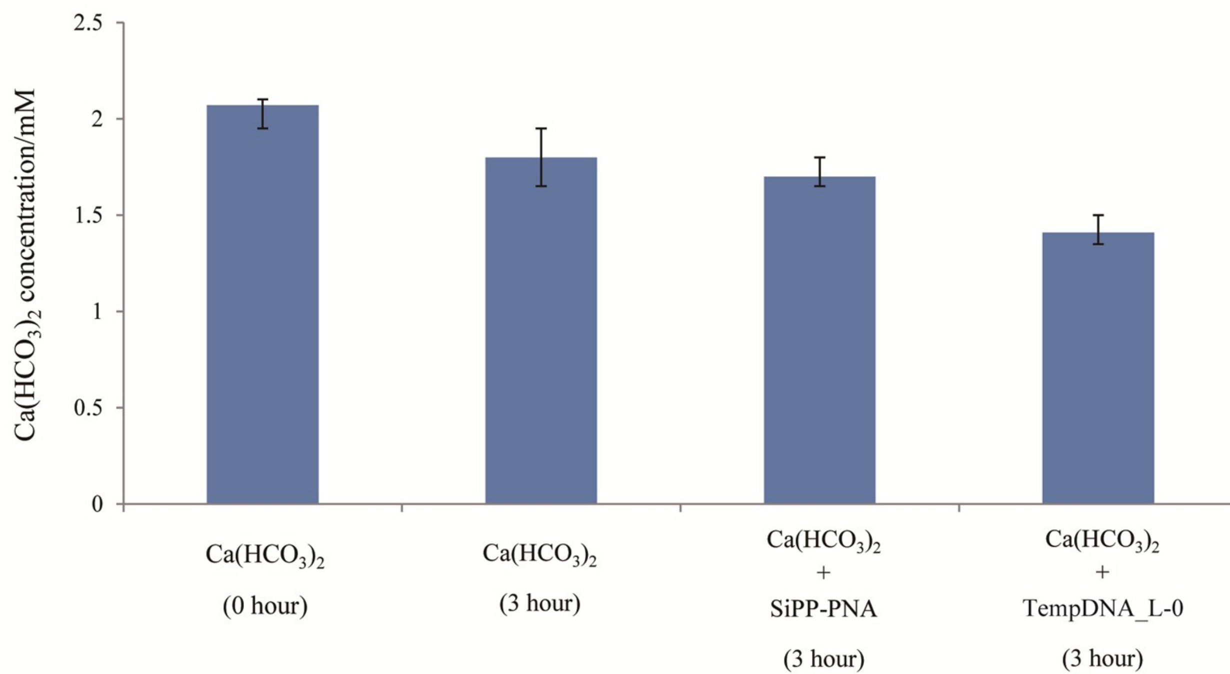

Figure S5. Residual calcium hydrogen carbonate concentrations in various conditions by standard titration with ethylenediaminetetraacetate

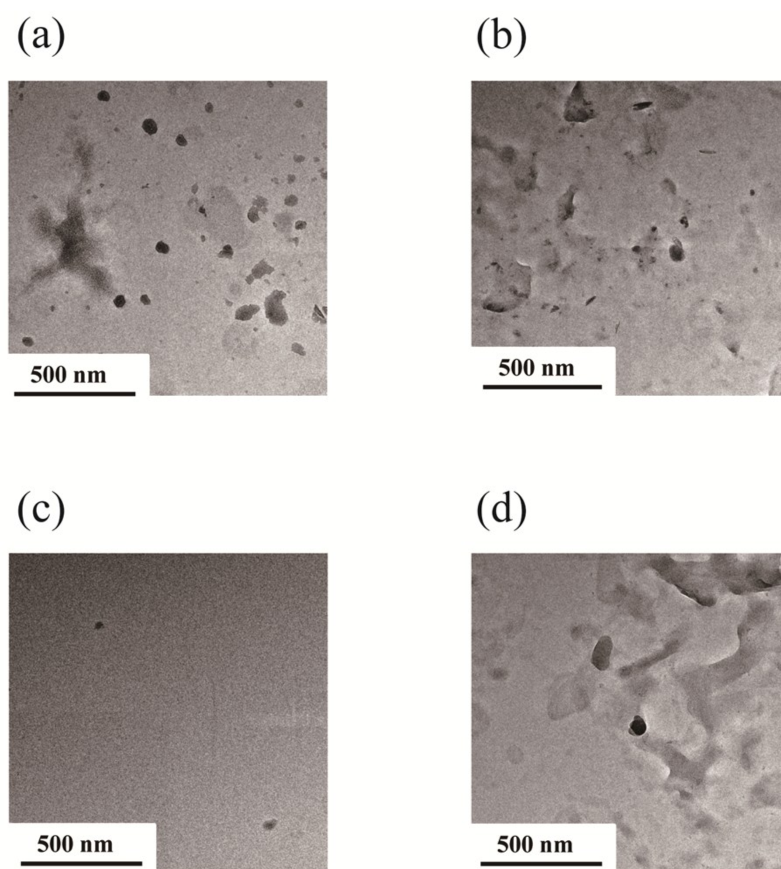

Figure S6. TEM images of silica and calcium carbonate precipitation using peptides and DNAs: (a) TempDNA_L-0 and SiPP-PNA, (b) TempDNA_L-1 and SiPP, (c) SiPP-PNA alone, (d) SiPP alone.

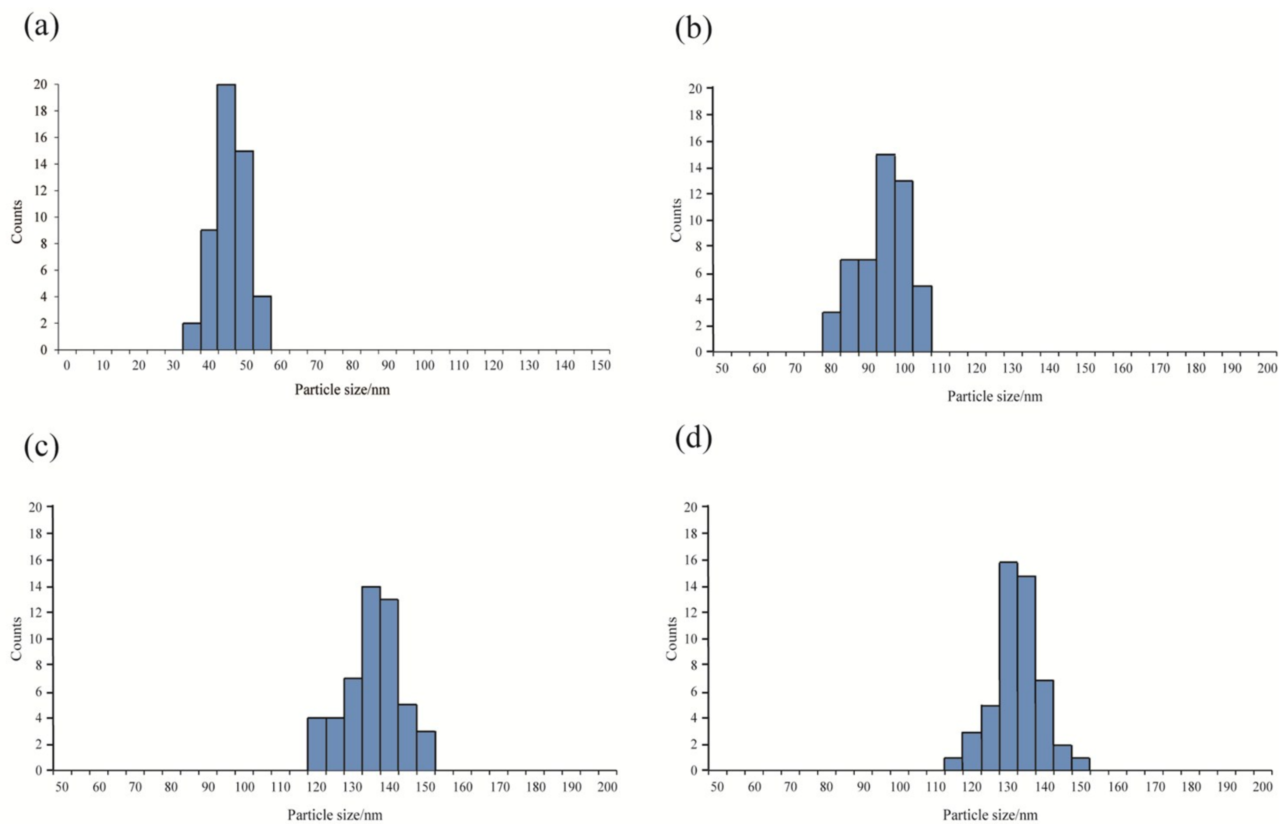

Figure S7. TEM-histograms of core-shell sphere diameters obtained using silica and calcium carbonate precipitation at (a) 0 h, (b) 3 h, (c) 6 h, and (d) 24 h with calcium hydrogen carbonate solution (0.3 mM).

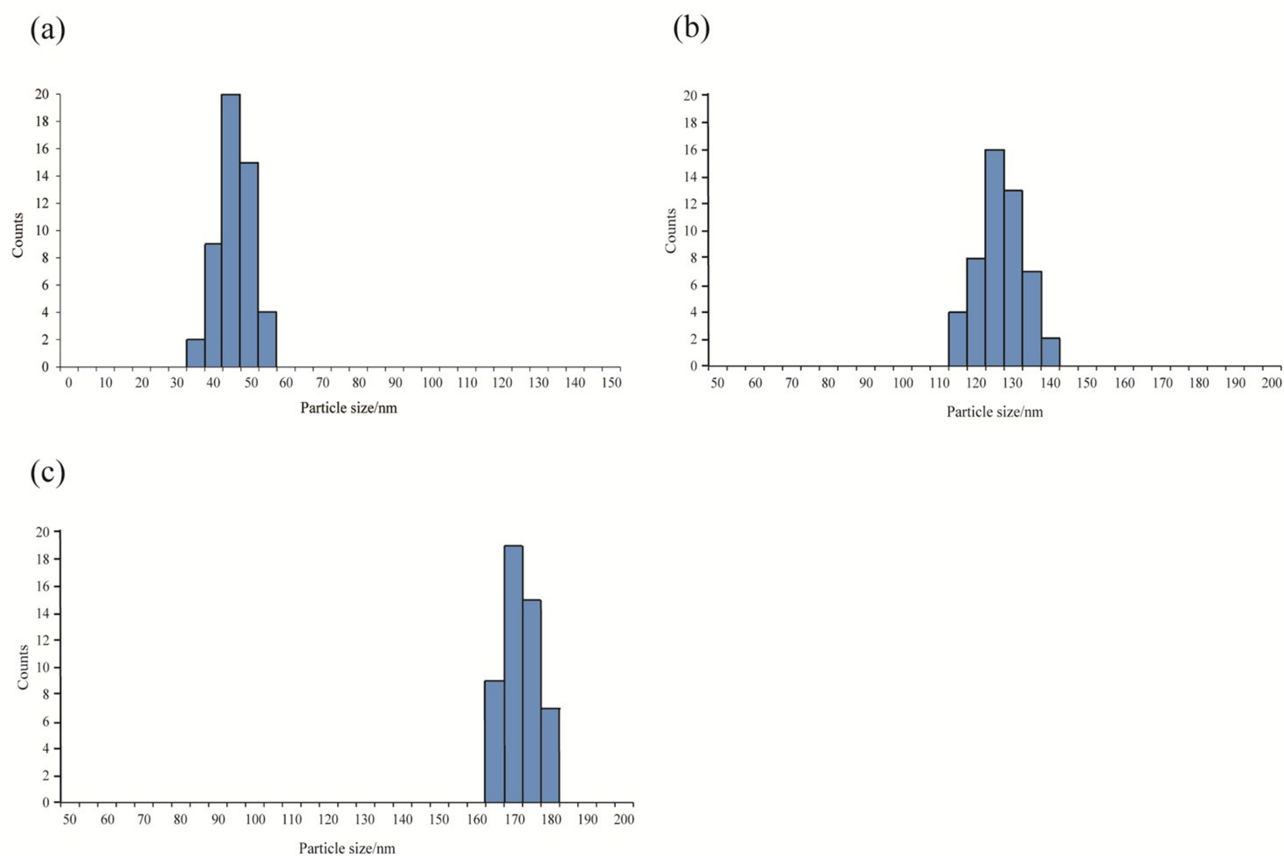

Figure S8. TEM-histograms of core-shell sphere diameters obtained using silica and calcium carbonate precipitation at (a) 0 h, (b) 3 h, and (c) 6 h with calcium hydrogen carbonate solution (0.5 mM).

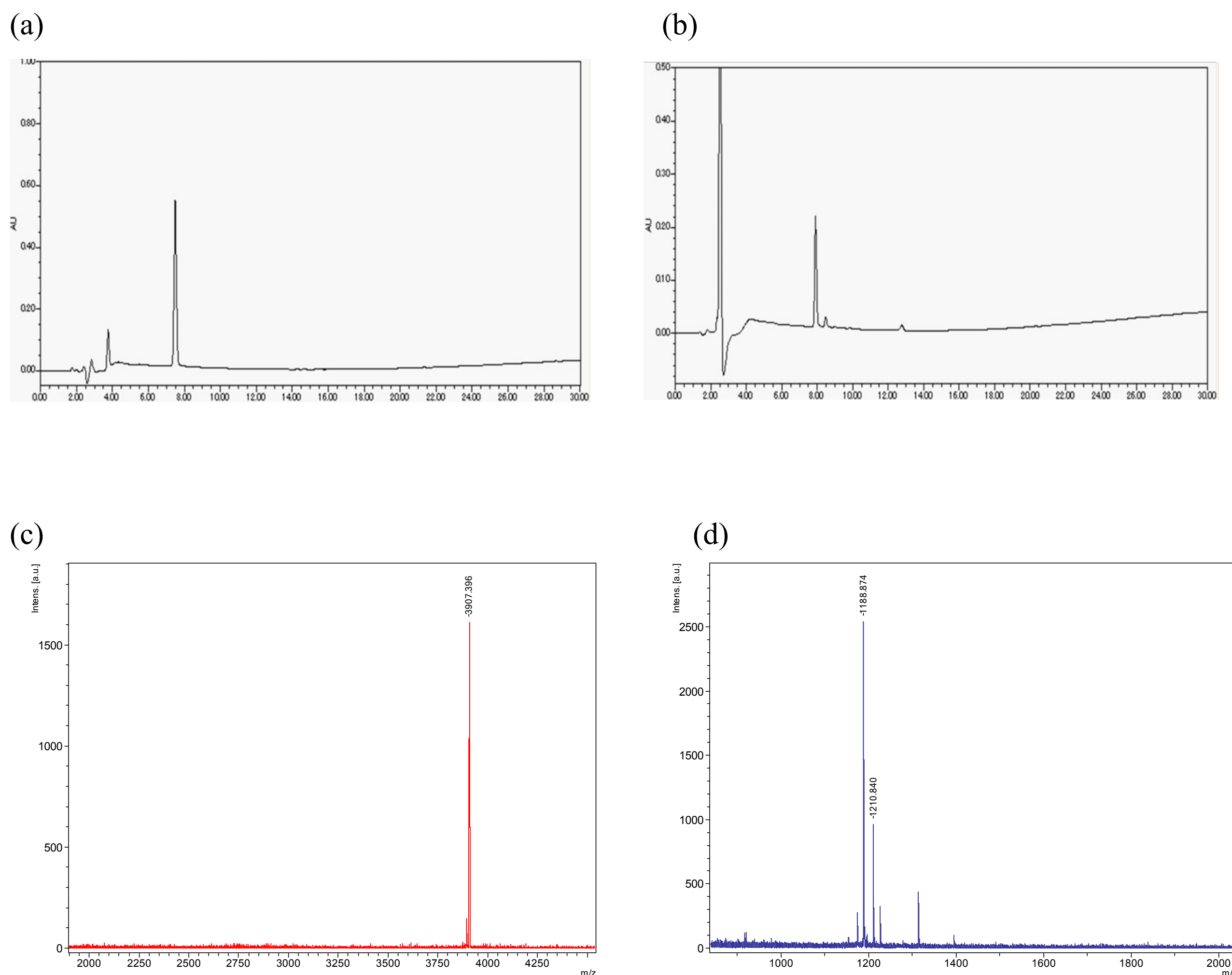

Figure S9. HPLC for purified SiPP-PNA (a) and SiPP (b) separated on an ODS column (150×4.6 mm 5 mm) with MilliQ water (containing 0.1% TFA) using a gradient from 5% to 95% acetonitrile (containing 0.08% TFA) over 30 min, 1.0 mL/min; detection at 220 nm. MALDI-TOF MS for purified SiPP-PNA (c) and SiPP (d).