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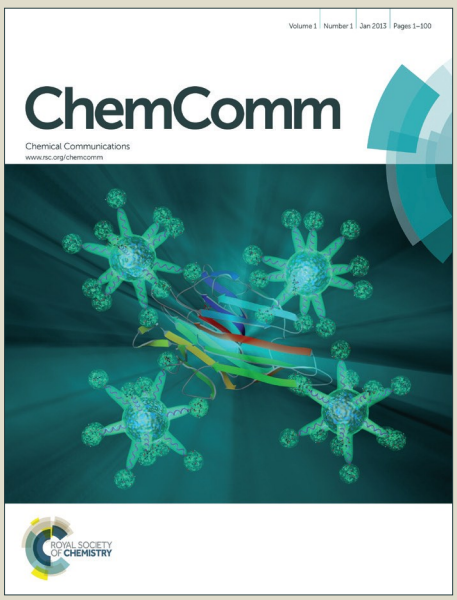

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Site-specific control of silica mineralization on DNA using a designed peptide

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We developed a site-specific method for precipitating inorganic compounds using organic compounds, DNA, and designed peptides with peptide nucleic acids (PNAs). Such a system for site-specific precipitation represents a powerful tool for use in the nanobiochemistry and the material chemistry.

Nanoscale constructs with hybrid inorganic-organic compounds[1-5] could be used in diverse applications in nanotechnology, electronics, and biotechnology. However, fabrication of such constructs using existing top-down and bottom-up strategies[6-8] is generally difficult. Mimicking biological systems is one of the most powerful approaches for overcoming this difficulty. For example, in biomineralization systems, some proteins can be used to precipitate various inorganic compounds.[9-13] The mechanisms of precipitation of inorganics with certain proteins have been elucidated and several researches have demonstrated that both the shape and the size of the resulting precipitate can be controlled with high reproducibility and accuracy. Although these reports provide an excellent overview of inorganic structural control, relatively few studies have examined controlled site-specific precipitation on organic compounds to create novel nano-structures. Developing such a process would enable the easy construction of hybrid inorganic-organic block constructs in nano-scale.

Consequently, we attempted to control inorganic precipitation using organic compounds. Peptides are promising compounds for use in this process because they confer several advantages:

1) Small peptides derived from the sequences of isolated proteins for biomineralization, or artificial sequence that forms nanotube can be used to precipitate inorganic compounds.[14-17]

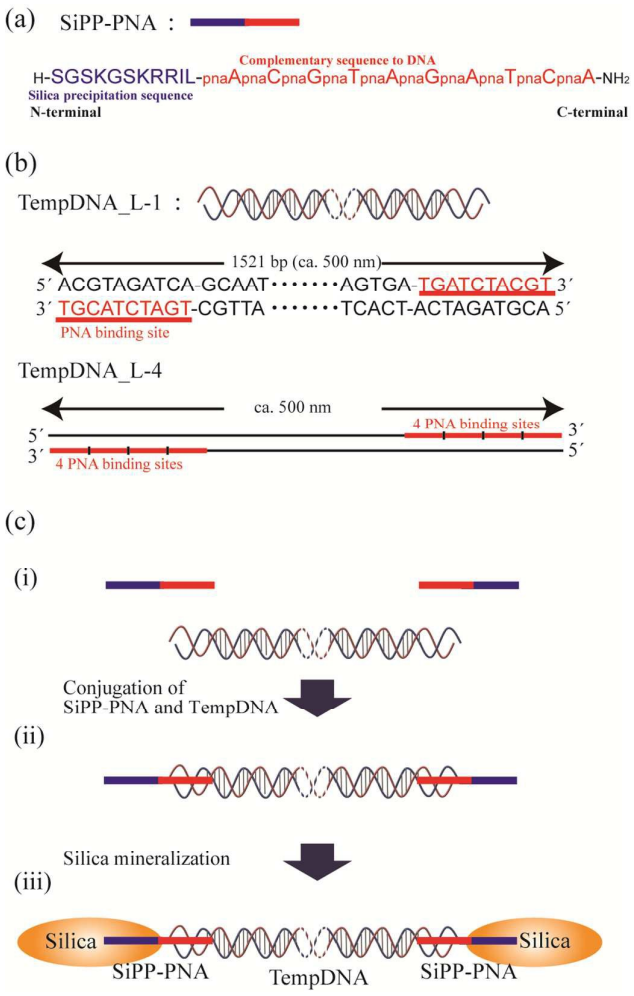

Fig. 1. (a) Sequence of the designed peptide used in this study. (b) Sequences of the target DNAs used in the study. (c) Outline of the silica site-specific mineralization using SiPP-PNA and TempDNA in this study.

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In addition, compared with proteins, these peptides are easier to handle;
2) Peptide nucleic acids (PNAs),[18-20] which can bind to DNA in a complementary manner, can be easily conjugated.

Thus, using template DNA and a designed peptide consisting of a PNA sequence and an inorganic compound–precipitating sequence, we can distribute organic compounds and inorganic compounds as a block. In this study, we focused on silica precipitation[21-26] and attempted to construct a site-specific precipitation system using DNA and a designed peptide. Using micro-scale techniques such as AFM (Atomic force microscopy), TEM (Transmission electron microscopy) and SEM (Scanning electron microscopy) as well as macro-scale techniques such as electrophoresis and DLS (Dynamic light scattering), we show that our method holds significant promise for use in constructing organic-inorganic nano-structures. Our method for site-specific precipitation thus represents a fundamental and powerful tool for use in nanobiochemistry and materials chemistry research.

We first designed a peptide consisting of two parts (Fig. 1a). One part was a previously described silica-precipitating peptide sequence (SiPP) (Fig. S1a),[23] whereas the other was a PNA sequence as a binding module for a complementary DNA sequence. The melting temperature (T_m) of the PNA/DNA duplex consisting of 10 base pairs is calculated to be 54.6 °C.[27] The resulting two-part peptide (SiPP-PNA) would thus interact with DNA (Fig. 1b) site-specifically; consequently, silica would precipitate only on the peptide bound to the DNA sites, resulting in silica-site-specific precipitation. For relatively easy detection via AFM, TEM and SEM analyses, we selected both DNA termini as site-specific peptide-binding sites. This produced dumbbell-shaped nano-structures on AFM and TEM (Fig. 1c). We prepared 2 peptide sequences and 4 DNA sequences (Figs. 1 and S1, ESI†). The peptides, SiPP-PNA and SiPP, were synthesized using Fmoc chemistry.[20,28] The following DNA sequences were designed: TempDNA_L-1, which was a ca. 500-nm-length DNA (ca. 1500 bp) with 1 PNA binding site at each terminus; TempDNA_L-4, which was a ca. 500-nm-length DNA (ca. 1500 bp) with 4 repeated PNA binding sites at each terminus; TempDNA_S-4, which was a ca. 200-nm-length DNA (ca. 600 bp) with 4 repeated PNA binding sites at each terminus; and TempDNA_L-0, which was a ca. 500-nm-length DNA (ca. 1500 bp) with no PNA binding sites. These DNAs were synthesized using PCR.

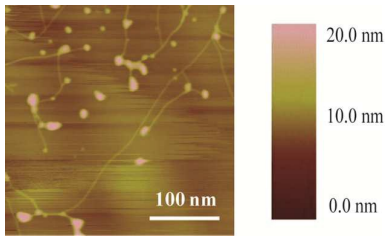

Fig. 2. Site-specific precipitation of silica using SiPP-PNA and TempDNAs. AFM image of precipitation using TempDNA_L-4.

We first optimized silica precipitation conditions for the following AFM, TEM and SEM observation. For the subsequent experiments, the precipitations were conducted using 0.91 mM silicic acid in 3 h incubation at RT (for detail please see the supporting information). Using AFM and SEM, we then demonstrated precipitation of silica with peptide alone (Fig. S2, ESI†). SiPP-PNA provided better control of the shape of the silica particles, compared to SiPP (a peptide containing only a silica-precipitating sequence). It was reported that the sequence consisting of positive and hydrophobic residues is important in the formation of a peptide assembly that will

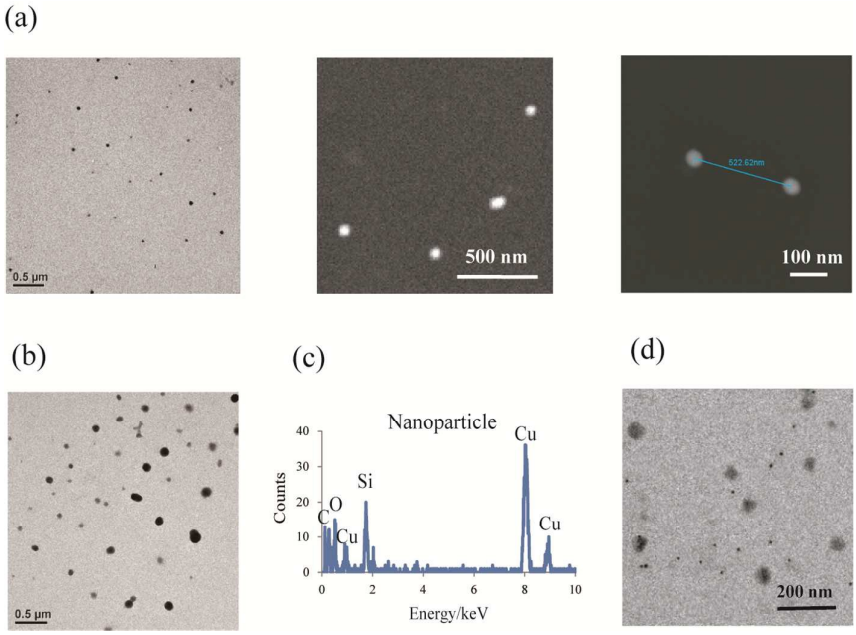

Fig. 3. Site-specific precipitation of silica using SiPP-PNA and TempDNAs. (a) TEM images of precipitation using TempDNA_L-1. (b) TEM image of precipitation using TempDNA_L-4. (c) TEM-EDX point analysis of a nanoparticle using TempDNA_L-1. (d) Immuno-TEM image using TempDNA_L-1 and SiPP-PNA. The TEM samples were not stained.

produce satisfactory shape-controlled precipitation of silica.[23] Consequently, in addition to including leucine and isoleucine in the SiPP C-terminus, the hydrophobicity of the PNA sequence also appears to be important for peptide assembly.

We then conducted site-specific precipitation of silica using SiPP-PNA and the TempDNAs described above (Figs. 2 and S3, ESI†). AFM images showed that DNA and silica precipitated as long chains and nanometer-sized spheres (Fig. 2), confirming the successful site-specific precipitation of silica on the DNA. Additionally, using SiPP-PNA and a TempDNA without the complementary sequence for the PNA (TempDNA_L-0, Fig. S1b, ESI†), AFM images showed only spheres for silica, similar to AFM images of SiPP-PNA alone (Fig. S3c, ESI†). We also confirmed that a TempDNA alone was insufficient to precipitate silica, as the sample did not show any precipitate on AFM because of poor affinity between the DNA and mica (Fig. S3b, ESI†). These results suggest that SiPP-PNA binds to DNA specifically and precipitates silica site-specifically at both DNA termini. In addition, SiPP-PNA and TempDNA_S-4 provided shorter chains with shorter distances between silica particles than did SiPP-PNA and TempDNA_L-4 (Fig. S3e, ESI†). These results also suggest that this system is capable of distributing organic and inorganic compounds as a block.

We also conducted TEM analyses to determine whether the results would be similar to those obtained with AFM. With unstained samples, particles of ca. 45 nm were observed with SiPP-PNA and TempDNA_L-1, which agreed with AFM results (Figs. 3a, and S4ac, ESI†). We also found that SiPP-PNA and TempDNA_L-4 produced relatively lager particles (ca. 60 nm and ca. 120 nm) than SiPP-PNA and TempDNA_L-1 (Fig. 3b and S4bd, ESI†). Furthermore, the bigger particles in TEM data with SiPP-PNA and TempDNA_L-4 seemed to be oval spheres (Fig. S4e, ESI†). This result suggests that the size and the aspect ratio of the silica particles can be controlled by the number of PNA binding sites on the DNA molecule. We then determined the localization of characteristic atoms, silicon and oxygen, using TEM-EDX (Energy dispersive X-ray spectroscopy) (Figs. 3c and S5, ESI†). The particles both in TEM data with SiPP-PNA and TempDNA_L-1 and in those with SiPP-PNA and TempDNA_L-4 apparently contained silicon and oxygen, suggesting that these particles were SiO_2 (silica). Furthermore we conducted SEM analyses to determine whether the results would be similar to those obtained with TEM (Figs. S6, ESI†). The particles in SEM data with SiPP-PNA and TempDNA_L-1 apparently contained silicon and oxygen, suggesting that these particles were SiO_2 (silica), which agreed with TEM results.

Additionally, the sample was examined by immuno-TEM to ensure that the 45-nm silica particles detected were connected by DNA. Samples were applied to EM grids, and the grids were then incubated with a monoclonal anti-DNA antibody (anti ds & ss DNA antibody) followed by protein A (which binds to the antibody) conjugated to 10-nm gold particles.[29] The resulting TEM images are shown in Figs. 3d and S7, ESI†. Catenated 10-nm gold particles between ca. 45-nm silica particles were readily visible only when both SiPP-PNA and TempDNA_L-1 were present. These results also indicated that TempDNA_L-1 was certainly existed between the silica particles and implied that SiPP-PNA and TempDNA_L-1 with silica precipitation produced the dumbbell-shaped nano-structures on TEM as expected (Fig. 1).

Finally, we verified these phenomena using other methods, such as macro-observations, dynamic light scattering (DLS), and electrophoresis. TempDNA_L-4, TempDNA_L-1 and TempDNA_S-4 produced complexed structures of expected sizes upon DLS analysis (i.e., TempDNA_L-4 and TempDNA_L-1 produced complexes of more than 500 nm and TempDNA_S-4 produced complexes of more than 200 nm; Fig. 4a). These macroscopic analyses were in agreement with microscopic observations (AFM results) (Figs. 2 and S3de, ESI†). Binding of SiPP-PNA and TempDNAs before/after (with/without) silica precipitation was also assayed using electrophoresis. The TempDNA_L-4 band density at ca. 1500 bp (Fig. 1c(i)) became thin with the addition of SiPP-PNA (Fig. 1c(ii)) and with silica precipitation (Fig. 1c(iii)) (Fig. 4b, this figure showed non-bound DNA percentage calculated from the band densities,[30] for detail please see the experimental section.). In contrast,

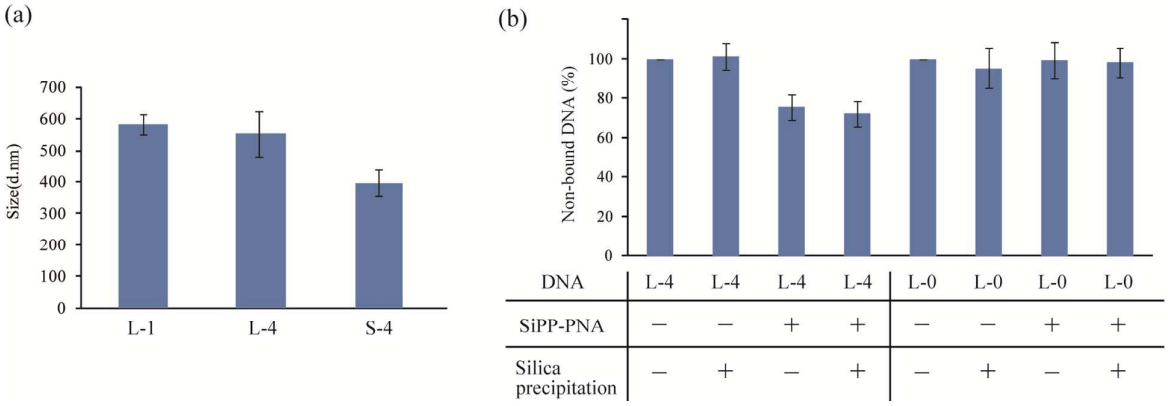

Fig. 4. (a) DLS analysis of the size of TempDNA and SiPP-PNA nano-structures after silica precipitation. (b) TempDNA band density (Non-bound DNA, DNA alone) in agarose gel electrophoresis of TempDNA alone (SiPP-PNA "-"; Silica precipitation "-", Fig. 1c(i)), TempDNA alone after (with) silica precipitation (SiPP-PNA "-"; Silica precipitation "+"), TempDNA mixed with SiPP-PNA before (without) silica precipitation (SiPP-PNA "+"; Silica precipitation "-", Fig. 1c(ii)), and TempDNA mixed with SiPP-PNA after (with) silica precipitation (SiPP-PNA "+"; Silica precipitation "+", Fig. 1c(iii)) in TAE (40 mM Tris-acetate, and 1 mM EDTA) buffer. The Non-bound DNA percentage was calculated 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 (%).

the TempDNA_L-0 band at ca. 1500 bp did not change with the addition of SiPP-PNA and silica (Fig. 4b). These results indicate that the PNA sequence bound specifically and complimentarily to the TempDNA with PNA binding sites and that these macroscopic results also agreed with microscopic results (AFM and TEM). Additionally, SiPP-PNA needs to fully bind to all the binding sites of the TempDNA in order to obtain dumbbell-shape structure with a higher probability. Single strand DNA would be substituted for duplex DNA at the PNA binding sites of the TempDNA for the improvement of this system.

In conclusion, we developed a system for the site-specific precipitation of inorganic compounds using DNA, and designed peptide-PNA conjugates. Using micro-scale techniques such as TEM, SEM and AFM as well as macro-scale techniques such as electrophoresis and DLS, we show that our method holds significant promise for use in constructing organic-inorganic nano-structures. The method described herein is not limited to producing nanostructured silica with a unique dumbbell-like morphology as described in this study. For example, because this peptide can also precipitate titania, this system could contribute to constructing photocatalytically active nanostructures. This system with substitution of silica precipitating sequence for other inorganic compound precipitating sequences could further provide a variety of well-controlled precipitation of inorganic compounds. For instance substitution of the sequence for a gold-precipitating sequence and control of numbers of binding sites in template DNA may generate gold nanorods with particular aspect ratio as desired. Our unprecedented results provide good examples of the controlled precipitation of inorganic compounds by organic compounds. Additionally, peptide/protein nanostructures or DNA/RNA nanostructures have been reported.[31-34] These nanotechnologies can be easily and directly combined through the PNA peptides. Using this system, organic and inorganic compounds could be precisely distributed as a block for various application such as electronic nanocircuits, DNA computers, solar batteries, nano-sized alloys and catalytic materials. Our method for site-specific precipitation thus represents a powerful and fundamental tool for use in nanobiochemistry and materials chemistry research.

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