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In Aqua Structuralization of a Three-Dimensional Configuration Using Biomolecules

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ABSTRACT

Ferritin nanoparticles ornamented with a Ti-binding peptide are versatile nanoscaled building blocks. Their specific binding ability is strong enough to position them on nanopatterned Ti regions on a Pt substrate. Furthermore, the peptides mineralization activity enables the formation of titania on the outer side of the particle, and the particle's inner nanospaces can serve as a carrier for inorganic nanodots. Making use of all these properties, here we show controlled in aqua fabrication of three-dimensional nanoscale structures. The *X*–*Y* positioning obeyed the specific binding of the peptide, while fabrication in the *Z*-dimension entailed stepwise formation of titania and ferritin layers by alternately applying the binding and mineralization abilities of the Ti-binding peptide. This method paves the way for in aqua fabrication of nanodevices having complicated structures and functions.

The capacities for recognition and mineralization, nanoscale compartmentation, and self-assembly are all properties of biomolecules that have been extensively exploited in the nanobiotechnology field to establish novel bottom-up fabrication methodologies. The goal of these methodologies is the autonomous structuralization of assemblages of nanoscale building blocks in aqua. In the effort to achieve this goal, peptide evolution systems have played an important role, as they have expanded the number of target materials that can be specifically recognized by biomolecules.[1,2] A substantial number of peptide aptamers have already been created de novo as specific binders of various metals, semiconductors, and other inorganic materials.[3] Furthermore, we now know that the aptitudes of these peptide aptamers are not limited to substance recognition. They also often possess the capacity to accelerate formation of mineral deposits of their target elements.[4,5] Thus by evolving peptide aptamers and then configuring them within proper architectures, we are able to control the size, shape, arrangement, and crystallinity of metals or compound semiconductors under aqueous conditions. An illustrative example in which a peptide aptamer's capacity for mineralization was fully commanded was the construction of a lithium ion battery reported by Nam et al.[6] In this example, nanoscaled particles of cobalt oxide and gold were formed at ambient temperature in aqua on filamentous phages in which mineralizing peptides were displayed. The Au−Co_3O_4 nanowires that were formed, thanks to the self-assembling capacity of the filamentous phage, could be used as a flexible, lightweight lithium ion battery electrode.

Along with the exploration of aptamer-enabled mineralization, synthesis of inorganic nanostructures through the use of the interior nanoscaled compartmental spaces within viruses or cagelike proteins has also attracted attention.[7] For instance, the 7−8 nm diameter inner space of the 12 nm ferritin particle is naturally filled with ferrihydrite, but various functional inorganic materials, including Co_3O_4 and CdS among others, also can be loaded into the space in vitro by adjusting the reaction conditions.[8] Indeed, floating nanodot gate memory already has been constructed using Co_3O_4-containing ferritin particles.[9,10]

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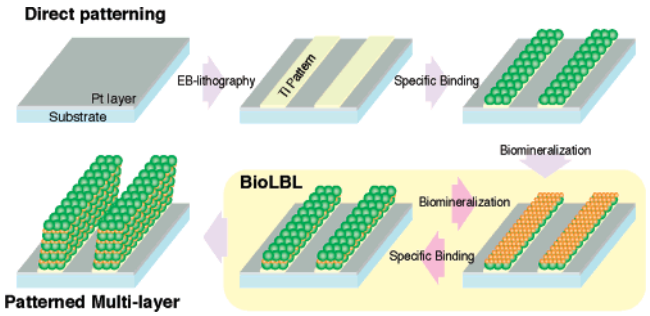

Figure 1. Schematic representation of DP-BioLBL. Initially, nanoscaled patterns of Ti film (shown as yellow lines) are drawn on a substrate coated with a Pt film using conventional direct patterning (DP). On the Ti regions, engineered ferritin (minT1-LF;[13] shown as green balls), which binds Ti but not Pt, is positioned in aqueous solution. On this first ferritin layer, the first intercalating layer of titania (orange plate) is formed, reflecting minTBP-1's capacity for mineralization. The formed titania layer then serves as a binding target for a second minT1-LF layer. By repeating these binding and mineralization cycles under aqueous conditions, three-dimensional multilayered structures are built up on the substrate.

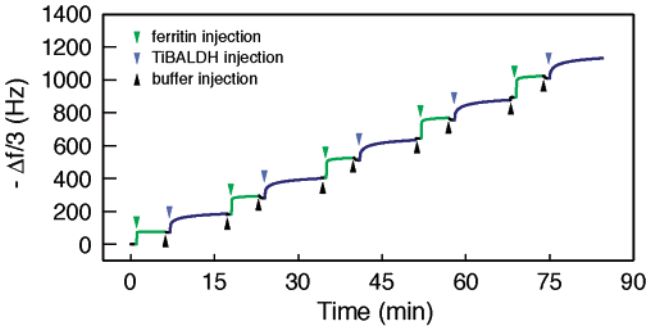

Figure 2. Time-dependent changes in the resonance frequency (f) during QCM analysis of the newly established titania-based BioLBL. The values on the y-axis represent the negative of Δf so that an ascendant line represents an increase in mass. Green arrowheads indicate points at which minT1-LF solution was infused. Black arrowheads indicate points at which buffer solution was infused. Titania mineralization was started by infusing TiBALDH at the time indicated by blue arrowheads.

While mineralization capacity and the nanospaces within biomolecules have been extensively explored in the context of nanoscale structuralization, in some cases yielding operating devices, the biomolecular property of specific binding has not yet been extensively investigated. Moreover, assembly of biomolecules that is controlled in all three dimensions has not yet been achieved. Here we introduce our in aqua fabrication of nanoscaled structures in which we took full advantage of the specific binding and mineralization capacities of a peptide aptamer and the nanospace within a cagelike protein.

TBP-1 is a peptide aptamer first isolated as a Ti binder,[11] although further studies revealed that TBP-1 also recognizes the surfaces of Ag and Si but not Au, Cr, Pt, Sn, Zn, Cu, or Fe.[12] The capacity of synthetic TBP-1 to bind Ti was rather low ($K_d \cong 13.2\ \mu M$[12]), however, which highlights the difficulty of using a peptide as the glue between inorganic materials and biomolecules. On the other hand, when the core of the TBP-1 sequence (minTBP-1) was displayed on the surface of 24-mer ferritin, the resultant modified protein (minT1-LF) showed a much greater affinity for Ti ($K_d \cong 3.8\ nM$[13]), one strong enough to position the ferritin on nanopatterned Ti regions in aqua.[14] As is often the case with peptide aptamers, TBP-1 also enhances the formation of oxides of Ti, Ag, and Si in aqua, and this capacity for mineralization is retained by the minTBP-1 component of minT1-LF (Supporting Information Figure S2). This previously enabled us to establish a novel method for the assembly of multilayered ferritin structures, which we call BioLBL.[15] With this method, we alternately utilize the binding and silicification capacities of minT1-LF to assemble multilayered nanostructures composed of metal-loaded minT1-LF layers and intercalating silica layers. To establish a three-dimensionally controllable fabrication method, we aimed to combine the $X-Y$ positioning obtained through the specific binding of minT1-LF and the controlled stacking in the Z-dimension obtained with BioLBL (Figure 1).

For this purpose, we first had to optimize solution conditions so that they would be compatible with both specific binding and BioLBL. Because our pilot tests with silica-based BioLBL showed that conditions allowing selective binding of minT1-LF to regions if Ti on a Pt substrate hinder layering (Supporting Information Figure S1), we established a new, titania-based BioLBL in which layers of ferritin and titania are alternately stacked by using the binding and mineralization capacities of minTBP-1 (Figure 2, Supporting Information Figures 3 and 4). The optimal conditions for titania-based BioLBL are also compatible with the selective binding of minTBP-1 to Ti. With this modified BioLBL method, the deposition of ferritin layers proceeded as follows: (i) The Ti substrate was incubated in minT1-LF solution for 10 min at ambient temperature, followed by washing. (ii) The substrate was incubated in Ti(IV) bis-(ammonium lactato)-dihydroxide (TiBALDH) for 15 min at ambient temperature, followed by washing. (iii) Repeat steps (i) and (ii) until the desired numbers of ferritin layers sandwiched by titania layers are built up.

With the appropriate conditions established, we next fabricated 6- and 12-layer ferritin structures on nanopatterned areas of Ti on Pt (Figure 3). In this experiment, we first lithographed Ti nanopatterns on a Si substrate coated with a thin Pt film using the conventional top-down method. Then, on the Ti regions, the first layer of ferritin was formed by soaking the substrate in minT1-LF solution. This first ferritin layer was fabricated by making use of minTBP-1's ability to specifically recognize Ti. On the minT1-LF layer, a thin layer of titania was then formed by soaking the substrate in solution of TiBALDH, a titania precursor, taking advantage of the mineralization capacity of minT1-LF. This layer then served as the target for a second minT1-LF layer. Thus, by alternately soaking the substrate in the two solutions, layers of ferritin and titania were laid down in stepwise fashion and were constrained to regions of the Ti (6 and 12 layers in Figure 3). During the BioLBL processes, the layered structures were slightly extended over the initial titanium patterns, which was discernible in the 12-layered structure

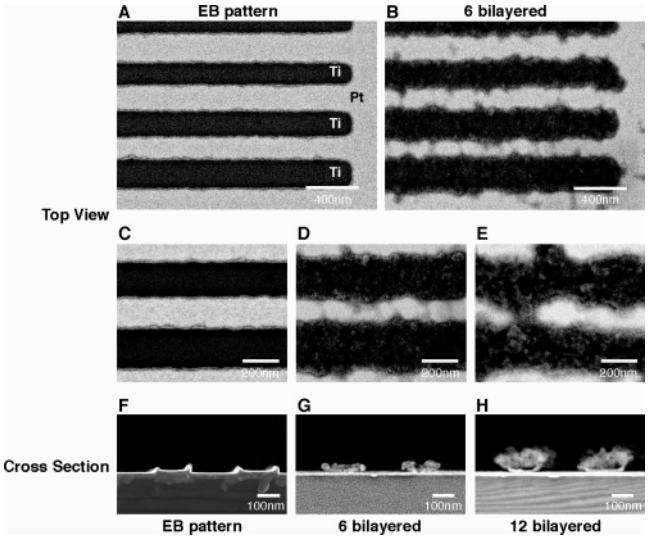

Figure 3. Field emission type scanning electron micrographs showing top (A−E) and cross-sectional (F−H) views of fabricated structures. (A) Nnoscaled patterns of Ti film (darker lines) on a Pt layer. (B) Image after six ferritin layers were built up. (C) Enlarged image of (A). (D) Enlarged image of (B). (E) Image after 12 ferritin layers were accumulated. (F) Cleaved site of the substrate. (G) Images after six ferritin layers were accumulated; (H) after twelve layers were accumulated.

(Figure 3H). We named this method DP-BioLBL (direct patterning biomimetic layer-by-layer assembly).

In conventional layer-by-layer fabrication, diffusion of stacked molecules often becomes problematical and makes it difficult to form well-separated layers in the vicinity to the base substrate. In our earlier investigation of silica-based BioLBL, we investigated a thin cross section of the manufactured heterogeneous multilayer stack and found that each ferritin layer was well separated from the others.[15] Apparently, the thin intervening mineral layers act as mortar, stabilizing the lamellar structure and preventing interlayer diffusion. Because of the excellent separation of layers, we are able to fabricate heterogeneous multilayer structures by using various metal-containing ferritins. In our earlier report,[15] we used the ion-milling method (outsourced to TOPCON, Tokyo) to prepare thin (∼40 nm) cross sections of the manufactured multilayered structures, and observed in detail the alternating composition of metal-containing ferritins and silica formed on the Au substrate. We tried to prepare similar thin cross-sectional specimens composed of alternating ferritin and titania layers on a QCM−Au sensor. However, we found that the organic layers of the manufactured structures readily detached from the inorganic portion during the course of the milling. This appeared to stem from the inherent fragility of the titania layers, and we could find no milling conditions better than the ones that yielded approximately 100 nm thick specimens with distorted layers. Figure 4 shows the cross section of the BioLBL products in which 3×Co@minT1-LF−3×Fe@minT1-LF−3×Co@minT1-LF formed a total of nine layers of ferritin on a Au substrate. Although a high resolution was not obtained from secondary X-ray observation with this thickness, elemental mapping indicated localization of Fe was limited to a region that was

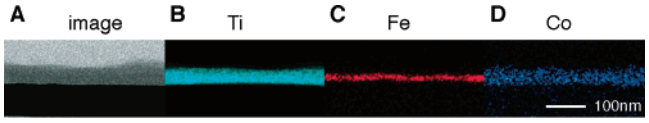

Figure 4. EDS mapping of a cross section of 3×Co@minT1-LF, 3×Fe@minT1-LF, and 3×Co@minT1-LF (from bottom to top) layers (with intervening titania layers) built up on a Au QCM sensor. (A) STEM image. (B−D) EDS mapping showing Ti, Fe, and Co.

thinner than the Ti representing titania, which showed that diffusion of ferritin particles across the titania layers did not occur. In this experiment, we did not observe the skewed localization of the Co element, which is presumably due to the low signal-to-noise ratio in the Co signal. With the new titania-based BioLBL, we also observed that there was no diffusion of ferritin molecules across layers, which paves the way for in aqua fabrication of nanodevices having complicated structures and functions: multivalued memory elements, solar batteries, and biosensors, to name a few.

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Supporting Information Available: Methods and optimization of DP-BioLBL conditions. This material is available free of charge via the Internet at http://pubs.acs.org.

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