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Nanoscale Self-Assembly Mediated by Genetically Engineered Gold-Binding Polypeptide

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***Abstract* — We present a self-assembly method for guiding and positioning nano- and microscale objects onto a template mediated by a genetically engineered polypeptide. We have identified a polypeptide that can specifically recognize and bind to gold via cell surface display and biopanning. We demonstrate that this polypeptide can differentiate between various inorganic materials such as platinum, gold, and silicon dioxide. We have utilized this polypeptide to guide the assembly of nanoscale quantum dots or microscale gold spheres onto patterned microfabricated substrates. Our approach of using the material recognition polypeptides opens a new venue for bridging the organic and inorganic domains and guiding self-assembly of structures and devices from the bottom up.**

***Index Terms* — gold binding polypeptide, self-assembly, nanofabrication, cell surface display.**

I. Introduction

By evolution, nature has created an astonishing collection of organic and inorganic structures ranging across the micro and nano scales. Nature produces organic and inorganic structures with proteins as the molecular elements for synthesis and assembly [1, 2]. Inspired by biology, our group has embarked on a project to identify polypeptides that can specifically recognize and bind to inorganic materials and utilize them to generate functional systems. We aim to use these polypeptides for the bottom up self-assembly of nano- and microscale structures and functional devices. This approach can potentially enable room-temperature self-assembly of Nanoelectromechanical Systems (NEMS) and Microelectromechanical Systems (MEMS) from aqueous solutions using proteins as the basic building blocks or the mediators. Compared to other self-assembly processes at the micro- and nanoscale, which are driven by covalent chemical bonds, capillary, electrostatic or magnetic forces [3-7], this method provides sufficient driving force for assembly of objects across the nano- and microscale. In addition, this method can be used to achieve selective self-assembly based on the material recognition capability of the polypeptide.

In this paper, we describe a procedure how to use such a polypeptide to achieve selective self-assembly of nano quantum dots and micro gold (Au) particles onto a microfabricated heterogeneous substrate. In the following sections, we discuss the synthetic biology procedure used to identify a gold-binding polypeptide. We verify that the polypeptide can differentiate between various inorganic materials such as platinum (Pt), Au and silicon oxide (SiO_2), and finally we demonstrate how to use this polypeptide to guide the self-assembly of objects onto the microfabricated templates. Our results are strong demonstrations of the utility of the genetically engineered polypeptides for self-assembly across the micro- and nanoscale. This material based "selective" self-assembly is a novel extension of the existing methods for applications from the nano to the microscale. It is also a critical technology for interfacing of organic and inorganic systems.

II. Principles

The self-assembly method includes three major steps:

- Identification of the polypeptide that binds specifically to selected inorganic material;
- Immobilization of the polypeptide on the substrate;
- Self-assembly of the nanoscale (or microscale) objects mediated by the polypeptide.

As an example, we use the gold binding polypeptide (GBP) to explain the method. Following a similar procedure, polypeptides binding to other inorganic materials such as Pt and SiO_2 can also be identified and used to direct self-assembly as well. In the following sections, we describe the three steps in detail.

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A. Polypeptide Identification

The selection of a polypeptide that specifically binds to an inorganic material is performed *in vivo* by combinatorial biology protocols via different display methods, either phage display (PD) [8, 9] or cell surface display (CSD) [10, 11].

The selection of GBP is performed by cell surface display. Fig. 1 shows the schematics of the selection of the polypeptide binding to specific materials by the CSD. Fig. 1a depicts the CSD system of the bacteria, the *E. coli*. The displayed protein is fused to the major flagella protein FliC of the *E. coli*.

To identify the polypeptide with the desired binding capability, first a specific segment of a DNA sequence is randomized (Fig. 1b) and encoded in the bacterial plasmid (Fig. 1c). As the result, different polypeptide sequences are displayed within a flagella protein of the cell (Fig. 1d). By comparing the binding strengths of the polypeptides to the target inorganic surface, a better binding cell can be selected (Fig. 1e-g). The DNA sequence of cells with a strong binding affinity is extracted, and the polypeptide sequence is determined (Fig. 1h).

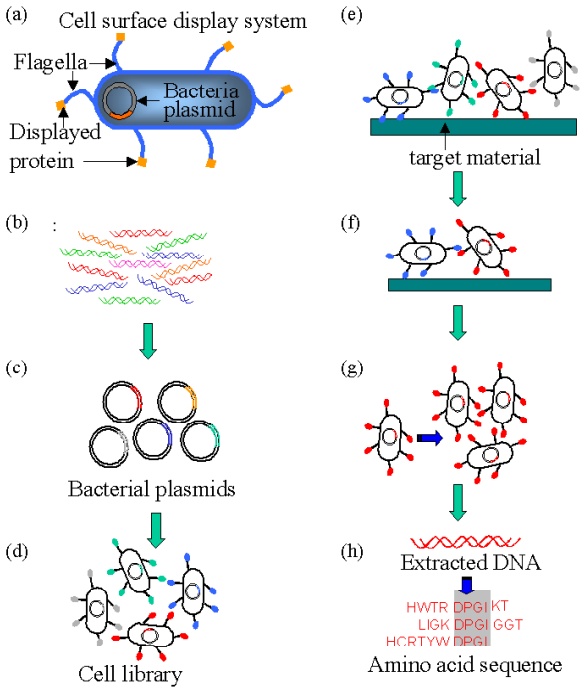

Fig. 1. Genetic selection of Au binding polypeptide by the CSD. (a) The CSD system of a cell. The displayed polypeptide is fused to the flagella protein of the cell. (b) Prior to the selection process, randomized oligonucleotides are generated. (c) They are inserted into the bacterial plasmids. (d) The cell library can be generated by the protein expression of the different plasmids. (e) The biopanning of the cell library to the target substrate is performed, followed by (f) removal of the weak binding cells by washing. (g) The biopanning process is repeated several times to find the strongest binder. Elution of the bound cell from the surface. The binder is replicated and (h) DNA sequence is extracted and the amino acid sequence is obtained.

The PD method is performed in a similar manner. Instead of using the bacteria, the phage M13 is used, and the displayed protein is fused to the P8 minor coating protein at the tip. By either the CSD or the PD method, polypeptides binding to different inorganic materials can be obtained. The amino acid sequences of the polypeptides binding to Au and Pt are listed in Table I.

TABLE I.
EXAMPLES OF POLYPEPTIDES BINDING TO DIFFERENT INORGANIC MATERIALS.

Materials	Method	Binding polypeptide
Au	CSD	MHGKTQATSGTIQS
Pt	PD	QSVTSTK

B. Polypeptide Immobilization and Self-Assembly

Fig. 2 shows the schematic flow of the polypeptide immobilization and self-assembly processes. First, the substrate is prepared with Au patterns as shown in Fig. 2a. To immobilize the GBP, we incubate the substrate in the GBP solution (Fig. 2b). To demonstrate the method, we use synthesized three-repeated GBP (42 amino acids) for the self-assembly experiment. The bottom image of Fig. 2b illustrates the binding of the GBP on Au. After the GBP immobilization, the micro Au spheres (or quantum dots) are added to the solution. As the result, the spheres can be self-assembled on the GBP immobilized on the Au patterns on the substrate (Fig. 2c).

In the following section, we describe the experiment method in detail.

III. EXPERIMENT METHOD

To prepare the substrate, Au was patterned by photolithography, followed by sputtering TiW/Au (10 nm/25 nm) and a lift-off process on a silicon substrate covered by thermally grown silicon dioxide (Fig. 2a). To clean the substrate, we sonicated the substrates in ethanol and isopropyl alcohol (IPA) for fifteen minutes each, followed by rinsing with de-ionized (DI) water and drying

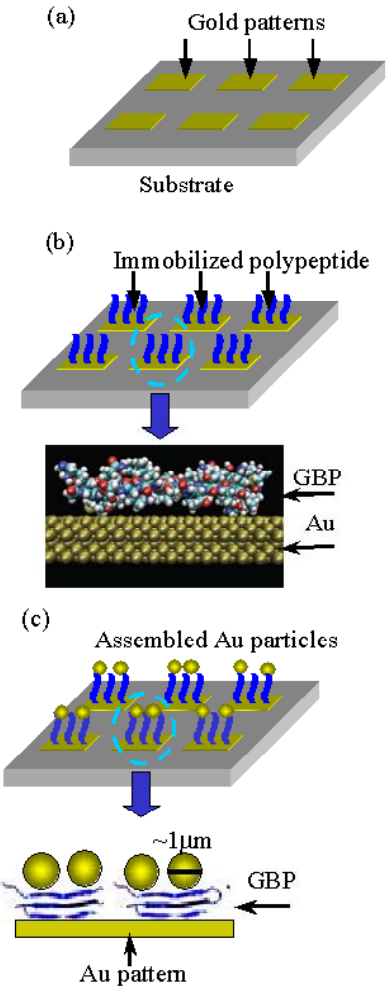

Fig. 2. Schematic plot of polypeptide immobilization and self-assembly of Au spheres (not to scale). (a) Patterning Au electrodes on the silicon substrate with an oxidation layer. (b) Immobilization of GPB on the Au patterns with the bottom image shows how the GBP binding to the Au. (c) Self-assembly of the Au spheres on the immobilized three-repeated GBP.

with nitrogen. For incubation of the GBP, the substrate was immersed in the 0.5μg/ml buffered solution of the biotinylated GPB (bio-GBP) for two hours with mild agitation provided by an orbital shaker. The buffer solution had 10 mM potassium phosphate and 0.1M KCl (pH=7.2). As the result, the bio-GBP was immobilized on the Au patterns (Fig. 2b).

To perform the self-assembly of quantum dots (QDs), we used the streptavidin conjugated QDs (QD605, Quantum Dot Corporation). The three-repeated GBP was synthesized with biotin, which coupled with the streptavidin on the QDs, when incubated in the bio-GPB buffered solution with the concentration of 1:10 (v/v) for forty-five minutes. The bio-GPB readily directed the self-assembly of the QDs on the substrate. The QDs were imaged by fluorescent microscopy.

We used Au spheres (Alfa Aesar) to demonstrate the self-assembly of microscale objects guided by the GBP. The Au spheres with the nominal diameter of 0.8-1.5 μm were sonicated in ethanol and IPA for one hour each prior to use. To perform the self-assembly, we immersed the prepared substrate with the GBP in the Au spheres in the buffer solution for overnight (~9 hours) with mild agitation by an orbital shaker. As a result, the Au spheres were self-assembled exclusively on the Au patterns on the substrate (Fig. 2c).

IV. Results and Discussions

The results of the self-assembled QDs on bio-GBP on the Au patterns are shown in Fig. 3. Fig. 3a is a bright field image, and Fig. 3b is a florescent image. As the red QDs bind to the bio-GBP, red patterns in the florescent image indicates the existence of the bio-GBP and the directed self-assembly of the QDs. Comparing Fig. 3a and 3b, we can see that the bio-GBP binds only to the Au patterns. In both images, zoomed-in pictures are inserted at the top right corners, which show a pair of Au and Pt square patterns with the size of $100\times100\mu m^2$. We note that the binding of the bio-GBP is highly selective to Au in contrast to Pt and SiO_2.

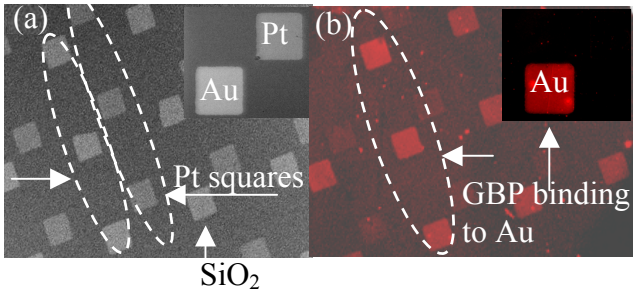

Fig. 3. Images of substrates patterned with Au and Pt squares on a SiO_2background after GBP incubation: (a) the bright field and (b) fluorescent images of a substrate with two-hour GBP incubation. The inserted images at the upper right corner are zoomed images of a pair of Au and Pt pads. The squares are $100\times100\mu m^2$. The darker background in the images is SiO_2 area.

Fig. 4 shows the self-assembly results of the microscale spheres onto the Au patterns on a substrate. As a control experiment, we incubated a patterned substrate with the buffer solution and exposed it to a collection of Au micro spheres. As Figs. 4a and 4b show, there is no any significant binding in this case. When bio-GBP was added

to the buffer solution and the patterned substrate was incubated, we observed a significant difference in the results. In this case, when the Au micro spheres were added to the patterns already covered by bio-GBP on the substrate, the spheres readily bound to the patterns. Fig. 4c and 4d show the self-assembly of the spheres on the microfabricated pads mediated by the GBP.

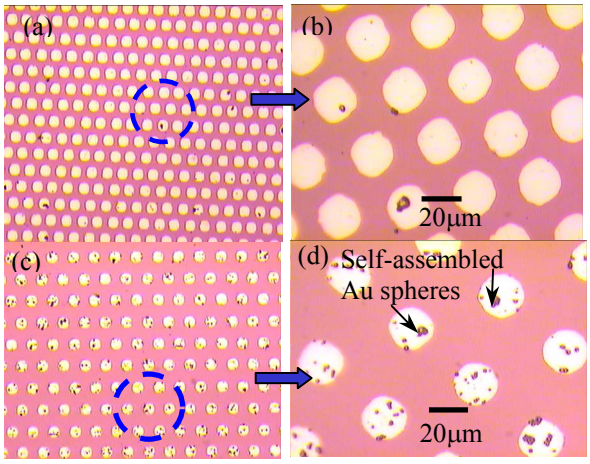

Fig. 4. Self-assembly results of micro Au spheres by GBP. (a) A control sample incubated in the buffer solution without GBP, and no significant assembled happened on the Au patterns. (b) A zoomed-in image of the circled area in (a). (c) An image of a sample with self-assembled Au spheres on GBP on the Au patterns on a substrate and (d) a zoomed in picture of the selected area in (c).

This experiment clearly verifies our ability to identify an Au recognition polypeptide GBP, and integrate GBP with the microfabricated substrates to achieve guided self-assembly of nano- and microscale structures. As an enabling technology, it can be used for low-temperature selective deposition of inorganic materials from solution phase onto microstructures. In addition, with different polypeptides selectively binding to different inorganic materials, *heterogeneous* micro and nano structures and systems, can be constructed. Integration of the genetically engineered polypeptides with the micro and nano technology will create new methods for material synthesis, structure formation and device development in the future.

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