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Recombinant bacteriophages as gold binding bio-templates

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ABSTRACT

Bacteriophages are nano-sized virion particles infecting bacteria. In this study, it is shown that metal binding properties of filamentous fd-bacteriophages can be enhanced by genetic engineering. Quartz crystal microbalance (QCM) analyses, UV–vis absorption spectra measurements and scanning electron microscopy (SEM) imaging revealed that expression of MMM short amino acid sequence on major coat protein p8 facilitates recombinant MMM-phage binding to Au surfaces and nanoparticles (NPs) via gold–sulfur (Au—S) interaction. Electroless deposition of Au particles on phage assemblies was investigated upon chemical reduction reaction with $NaBH_4$ at different $HAuCl_4$ precursor concentrations. Energy dispersive X-ray spectroscopy (EDX) measurements confirmed the presence of Au on both AuNP decorated and chemically metallized phage structures. Further studies on patterning and controlled immobilization of recombinant bacteriophages on specific surfaces may contribute to bio-templated nanowire development field and biosensor application studies.

1. Introduction

Bottom-up manufacturing is an advantageous method in order to obtain nano-sized electronic device components in a simpler and more economical manner [1]. Top-down manufacturing, on the other hand, can bring some device integration and configuration problems [2,3]. Various bionano-structures such as DNA [4], virus particles [5] and bacterial surface layer proteins (S-layers) [6] have been used as building blocks for bottom-up production technology. For example, nucleic acid filaments were chemically modified and applied in nanoelectronics [7], catalytic research [8] and biosensor development [9] studies. Due to the ease of amplification, isolation and genetic modification of bacteriophages, different members of filamentous phages have been applied in inorganic bio-templating studies [10].

The filamentous fd-phage (~1–2 μm long, ~6 nm thick) consists of coat proteins surrounding a circular single stranded DNA (ssDNA) [11]. Major coat protein p8 (~2700–3000 copies) line up along the ssDNA [12]. Minor coat proteins p3 and p6 (5 copies) are found at one end and p7 and p9 (3–5 copies) are located at the other end of phage filament [13]. The best known application field of bacteriophages is phage display technology. With this method, one can identify short specific peptide sequences binding to surfaces [14], proteins [15] and even to human cells [16]. Recently, Lee et al. have developed high-power lithium ion battery electrodes by genetically modifying bacteriophage coat proteins to specifically bind SWNTs and $FePO_4$ [17].

Gold is a precious noble metal which has been utilized in everyday's life and in science since centuries. Colloidal gold nanoparticles (AuNPs) were first used in 4–5th centuries B.C. in ancient Egypt and China [18]. AuNPs are used in bioimaging [19], cancer therapy [20] and in electronic device fabrication [21] studies. As an anti-cancer agent, AuNPs can be functionalized with specific antibodies targeting specific cancer cells [20]. EDC/NHS (N-ethyl-N′-(3-dimethylaminopropyl) carbodiimide/N-hydroxysuccinimide) chemistry is widely applied to induce the formation of covalent bonding between Au surfaces and side chain amino acids of peptides [22]. Thiol groups (R-SH) have the highest binding affinity to Au surfaces (~200 kJ/mol) [23]. Therefore thiol modified oligonucleotides are used to be coupled with either AuNPs or Au surfaces via Au—S linkage for applications such as nanostructure fabrication, biosensor or drug delivery studies [24]. Similarly, amino acids "cysteine (Cys-C) and methionine (Met-M)" carrying sulfur (S) containing functional groups can be linked with AuNPs. Honda et al. [25] have reported on Au—S bond formation in monolayered L-cysteine on Au surfaces. Kim et al. [26] have genetically modified the immunoglobulin Fc-binding B-domain of protein G to express two Cys residues at its C-terminus. Recombinant G protein retained its IgG binding activity and provided strong Au surface binding ability due to Au—S linkage. Souza et al. [43] have recently investigated the coupling of AuNPs with wild type fd-phages via electrostatic interactions for cell targeting applications. Authors

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demonstrated that fd-phage molecules can bind AuNPs and form a network structure at pH values below pI of the major coat protein p8.

In this study, wild type fd- and genetically modified MMM-phages are investigated in terms of their gold binding affinities. Gold binding potential of engineered and wild-type phages were compared here by means of Quartz crystal microbalance (QCM), UV–vis absorption spectroscopy, scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDX) analyses. EDX analyses showed that phage assemblies were coated with either AuNPs or reduced Au clusters after metallization experiments conducted with different concentrations of AuNPs or $HAuCl_4$ precursor solutions. Enhancing material binding property of engineered phage molecules may be a promising tool for bottom-up configuration of nanoelectronic device compartments.

2. Materials and methods

2.1. Cloning and phage amplification

E. coli MC1061 (New England Biolabs) and *E. coli* K91BluKan (K91BK) cells were applied for plasmid and phage amplification. *E. coli* K91BK cells and fd-tet vector were kindly provided by Prof. Dr. Georg P Smith (University of Missouri, USA). MMM short amino acid sequence was displayed on the N-terminus of the major coat protein p8 as discussed previously [27]. For phage amplification, *E. coli* K91BK cells were first transformed with fd and p8MMM vectors by electroporation. LB agar plates supplemented with tetracycline (Roth) and kanamycin (Roth) were utilized for positive colony selection. Phage purification was carried out by PEG/NaCl precipitation method as described before [28]. Phage concentration in terms of colony forming units per milliliter (cfu/ml) was measured by phage tittering. *E. coli* K91BK cells were infected with different dilutions of purified phages at 37 °C for 30 min and overnight grown colonies on LB agar plates supplemented with tetracycline and kanamycin were counted per ml. Final phage suspensions were dialyzed against 1× PBS at 4 °C overnight and stored in the fridge.

2.2. Binding measurements

Phage binding onto the gold surface was first analyzed by QCM measurements performed with Qsense E1, Sweden, according to manufacturer's instructions. Gold coated quartz crystals were purchased from Qsense. After the sensor was mounted in the probe arm of the instrument, washing steps with MilliQ water and 1× PBS (filter sterilized) were performed until the frequency signal became steady. 1 ml fd- or MMM-phages (10^{10} cfu/ml in 1× PBS) were injected at a flow rate of 15 μl/min and the sensor was rinsed with MilliQ at the same flow rate. In order to investigate the effect of pH on Au binding affinity of phages on Au coated quartz sensors, 300 μl of phages (10^9 cfu/ml) in 1× PBS buffer at pH 5, 7 and 9 was injected to the system and the sensors were washed with the same buffer at the same flow rate. Frequency change was measured for 5 different overtone resonance frequencies (15, 25, 35, 45, 54 and 64 MHz) at room temperature. Data were analyzed by QTools software program and experiments were repeated 3–4 times.

AuNP binding on bacteriophages was then analyzed by electron microscopy and UV–vis spectroscopy measurements. Citrate stabilized AuNPs were purchased from Sigma (~20 nm diameter, $\sim 7.2 \times 10^{11}$ particles/ml). Different volumetric dilutions (DF1 = 1:1, DF2 = 1:3 and DF3 = 1:10 [AuNP:PBS]) of AuNPs were prepared in 200 μl of 1× PBS and mixed with fd- and MMM-phages at a final concentration of 10^{10} cfu/ml. Samples were incubated at room temperature (RT) for 2 h on a rolling drum (7 rpm) and 70 μl of each was analyzed by UV–vis spectroscopy. For investigating the influence of pH on AuNP binding, citrate stabilized AuNPs were diluted in 1× PBS (pH 5, 7 and 9) at a 1:10 ratio and mixed with phages at a final concentration of 10^9 cfu/ml. Experiments were performed in triplicates.

2.3. Metallization

100 μl of fd- and MMM-phage solutions (10^{10} cfu/ml) were prepared in 3 mM, 30 mM and 60 mM $HAuCl_4 \cdot 3H_2O$ (Sigma). Phage samples were first incubated at RT for 2 h on a rolling drum (7 rpm) in order to initiate the Au^{3+} binding on phage filaments. After 2 h of incubation, samples were prepared for SEM imaging before the reduction. Chemical reduction reaction was performed with $NaBH_4$ (Sigma) at a final concentration of 15 mM and SEM samples were prepared as described below.

2.4. Microscopy

SEM imaging was conducted with an FEI Quanta 250 FEG scanning electron microscope at either 5 or 30 kV in high vacuum. EDAX™ Team EDX system was applied for the elemental characterization of phage assemblies. Industrial scale standard Si wafers with a native oxide layer was used for microscopy sample preparation. SiO_2 wafers were cleaned by sonication for 15 min in acetone, ethanol and finally in sterile MilliQ water. 10–20 μl was dropped on a piece of cleaned wafer. After 15–25 min, excess solution was removed with tissue paper, and 10–20 μl of sterile MilliQ water was dropped. After 5–10 min, the excess was removed and the wafers were blow-dried by nitrogen gas.

3. Results and discussion

3.1. Phage–Au surface binding

Introducing thiol groups to noble metal surfaces is a well-known method to induce metal-peptide conjugation [23]. Recently, our research group has generated a genetically engineered fd-phage construct carrying an MMM short amino acid sequence bearing three sulfur residues at N-terminal region of major coat protein p8 subunits (Fig. 1a). MMM-phages were shown to be conjugated with the enzyme glucose oxidase and applied as a biosensor [27]. Here, binding affinity of MMM-phages to Au surfaces and AuNPs is studied (Fig. 1b). Au—S bonding is commonly utilized in various application fields ranging from electronics to biosensor studies [29]. Thiolated oligonucleotide molecules are used for covalent binding to Au surfaces or NPs [24]. Recently, Kim et al. [26] have expressed two Cys residues at C-terminus of immunoglobulin Fc-binding B-domain of protein G. Recombinant G protein preserved its IgG binding activity and provided strong Au surface binding affinity due to Au—S linkage. Similarly, in this study genetically modified MMM-phages showed a considerable affinity to Au sensor. QCM analyses conducted with Au coated quartz crystal sensors showed an initial frequency decrease of ~160 Hz upon the injection of MMM-phages (Fig. 1d). At the end of washing step, net frequency change was measured as ~70 Hz showing that weakly or partially bound phage particles were removed from the Au sensor. As the frequency decrease is correlated with increase of mass accumulated on the QCM crystal [30], final frequency decrease demonstrates phage binding on the Au coated sensor surface. QCM result implies that MMM-phages bind the Au surface due to the interaction of MMM short amino acid sequence displayed on body part of the phage with the surface. Upon fd-phage injection on the other hand, frequency first decreased showing a mass accumulation on Au sensor and then increased after the washing step with 1× PBS revealing that fd-phages did not bind the Au surface (Fig. 1c).