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Dual-affinity peptides to generate dense surface coverages of nanoparticles

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ABSTRACT

Depositing gold nanoparticles is of great interest because of the many potential applications of nanoparticle films; however, generating dense surface nanoparticle coverage remains a difficult challenge. Using dual-affinity peptides we have synthesized gold nanoparticles and then pre-aggregated the particles in solution via interactions with metal ions. These nanoparticle aggregates were then deposited onto silicon dioxide surfaces using another dual-affinity peptide to control binding to the substrate. The results demonstrate that when divalent ions like Zn^{2+} or Ni^{2+} are used, densely packed gold nanoparticle monolayers are formed on the silicon dioxide substrate, which may have applications in fields like molecular electronics.

1. Introduction

Biological macromolecules provide the ability to bind, synthesize and assemble materials with a high degree of accuracy under mild, aqueous conditions [1]. Researchers in biomimetics aim to take advantage of these properties to achieve bottom-up self-assembly of nanomaterials into more complex structures using motifs found in Nature [2]. Peptides have been one sort of biological macromolecule that are of great interest because of their ability to bind and synthesize inorganic materials [3]. To date, many peptides capable of binding and participating in the synthesis of a wide array of inorganic materials including metal surfaces [4], metallic nanoparticles [5–7] and metal oxides [8–10] have been identified and isolated. These so-called affinity domains can then be further fused together to make fusion peptides that are multifunctional in nature [11]. Fusion peptides can participate in the synthesis and assembly of hybrid materials due to their multifunctional properties [11,12]. This ability to interact with several different types of materials has made fusion peptides a powerful tool in the design and synthesis of nanostructured materials [13,14].

Nanoparticle assemblies show great promise for use in molecular electronic applications [15–17], however there remain several key challenges to address in order to make such usage practical, with the most important one being control over nanoparticle density on the surface. Due to repulsive interactions between similarly charged nanoparticles, it can be difficult to generate large numbers of densely packed nanoparticles, which in turn causes breaks in the conductive network. Viral scaffolds have shown great promise to overcome this challenge [18–20], however from an experimental standpoint they can be time-consuming and difficult to work with. Fusion peptides on the other hand are available commercially [21] and are relatively simple to use, making them desirable candidates for guiding nanoparticle self-assembly.

Previous researchers have demonstrated that fusion peptides can be used to synthesize and stabilize gold nanoparticles [22] as well as control how they interact with each other [12]. Their system uses a two domain fusion peptide: one that can stabilize the gold nanoparticles and one that can interact with other metal ions to control how the nanoparticles interact with each other or other materials. These nanoparticles have been shown to deposit onto Pd substrates [11], as well as to function as a colorimetric assay for metal ions in solution [21]. To date, there have been no reports in the literature on taking advantage of peptide-driven interactions between peptide stabilized nanoparticles and metal ions to control particle density on surfaces. The ability to generate dense coverages of metal nanoparticles would resolve the issue of nanoparticle spacing in nanoparticle percolation networks, making them useful for molecular electronics, sensing, and surface enhanced Raman applications.

Here we demonstrate a facile method of synthesis for gold nanoparticles in which we can trigger weak aggregations in solution, that are also capable of binding to silicon dioxide surfaces through the use of multiple fusion peptides. Using the functional peptide domains A3, QBP1, and Flg, we can control the size, density,

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Table 1
Sequences of peptides studied.

Peptide	Sequence
Flg-A3	DYKDDDDK WYSSWAPPMPPF
A3-QBP1	WYSSWAPPMPPFPPPWLYMPPWS

and attachment of gold nanoparticles to a silicon wafer substrate surface. The nanoparticles are first synthesized using the Flg-A3 fusion peptide whose sequence is in Table 1. The Flg domain has been shown to control the plasmonic coupling between nanoparticles in solution through interactions with metal ions added to the nanoparticle solution [21], while the A3 domain serves as a monolayer capping agent to produce stable colloidal solutions of monodisperse gold nanoparticles in the presence of gold salt and reducing buffer [12]. Flg-A3 nanoparticles are then tethered to a silicon dioxide surface by use of another fusion peptide, A3-QBP1. The QBP1 domain has been shown to have a high affinity for silicon dioxide [23–25], while the A3 domain has affinity for the gold nanoparticles. The A3-QBP1 fusion thus anchors gold nanoparticles to a silicon dioxide surface to generate a high surface coverage of nanoparticles. Binding experiments were also performed using citrate-stabilized gold nanoparticles to demonstrate the usefulness of the A3-QBP1 peptide in achieving a high surface coverage of gold nanoparticles, even in the absence of peptide stabilizing ligands on the particles.

2. Experimental

2.1. Materials

Flg-A3 and A3-QBP1 peptides (≥75%) were purchased from the McGill Peptide Synthesis Facility (Montreal, QC). All other reagents were purchased from Sigma–Aldrich (Oakville, ON). Silicon dioxide wafers were obtained from Siltronic (Munich, Germany).

2.2. Gold nanoparticle synthesis

Citrate-stabilized nanoparticles were synthesized according to a procedure developed by Jana and Gearheart [26].

Peptide-stabilized nanoparticles were synthesized using a modified procedure developed by Slocik et al. [12]. 10 μL of Flg-A3 peptide solution (10 mg/ml in deionized water) was added to 500 μL of HEPES buffer (100 mM, pH 7, 154 mM NaCl). 10 μL of $HAuCl_4$ solution (0.1 M) was then added. The mixture was vortexed after each addition of reagent and left to incubate for 1 h. Nanoparticles were purified by centrifugation at 13 000 rpm for 10 min and redispersed in 500 μL of HEPES buffer (100 mM, pH 7, 154 mM NaCl). For experiments in which the nanoparticle spacing was examined 2.5–10 μL of 0.1 M metal ion solution (Ag^+, Mg^{2+}, Ni^{2+} or Zn^{2+}) was added and the nanoparticle solution was left to incubate for 1 h.

2.3. Effect of ionic strength on gold nanoparticle stability

A 500 μL sample of undiluted Flg-A3 AuNPs was spiked with 1 μL aliquots of 5 M aqueous NaCl solution for a total of 7 additions. After each aliquot was added the AuNPs were left to incubate for 10 min. A UV–vis spectrum was then collected.

2.4. Gold nanoparticle binding experiments

Silicon dioxide wafers were treated with the RCA-1 and RCA-2 cleaning treatments immediately prior to usage. Two sorts of binding experiments were carried out. In the first type bare substrates were immersed in gold nanoparticle solutions overnight.

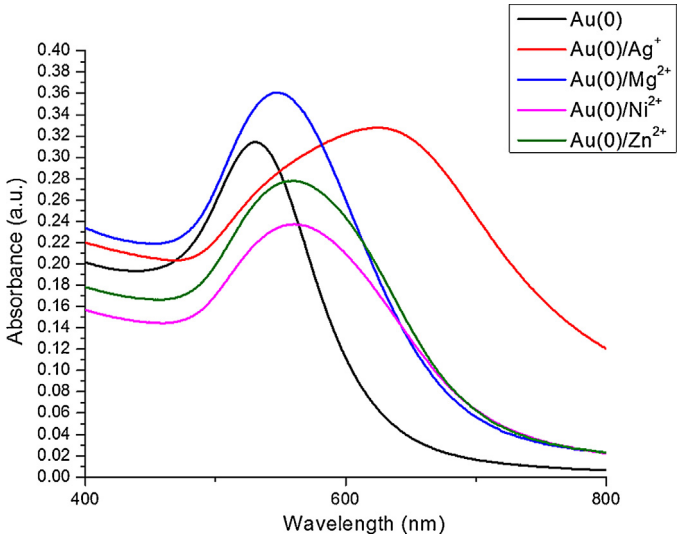

Fig. 1. UV–vis spectra for Flg-A3 AuNPs, Flg-A3 AuNPs and Ag^+, Flg-A3 AuNPs and Mg^{2+}, Flg-A3 AuNPs and Ni^{2+} and Flg-A3 AuNPs and Zn^{2+}.

Upon removal from the nanoparticle solution they were washed with DI water and dried with a gentle stream of nitrogen gas. In the second type the substrate was immersed in a 0.37 mM solution of A3-QBP1 peptide in HEPES (100 mM, pH 7, 7.5 or 8) overnight. The substrate was then removed, washed with DI water and dried with a gentle stream of nitrogen gas before being immersed in the gold nanoparticle solution overnight. The substrate was then washed with deionized water and dried with nitrogen.

2.5. Instrumentation and characterization

Gold nanoparticles were characterized with a Cary 100 Bio UV–vis (Agilent Technologies, Missisauga, ON) using quartz cuvettes that were 1 cm in pathlength (Spectrocell, Oreland, PA). Samples were diluted by a factor of 0.5 for data collection. The nanoparticle size and spacing was characterized using a Philips CM200 200 kV transmission electron microscope (TEM). Dynamic light scattering (DLS) was done using a 90Plus Particle Size Analyser (Brookhaven Instruments, Holtsville, NY). Surface characterization was done with a Hitachi S-4700 field emission scanning electron microscope (SEM) and an Asylum Cypher Scanning Probe Microscope (Asylum Research, Santa Barbara, CA).

3. Results and discussion

Flg-A3 gold nanoparticle synthesis proceeded rapidly to produce a purplish-red solution. In this particular synthesis the Au(III) is reduced by the HEPES buffer to form Au(0) while the peptide chelates the resulting nanoparticle during growth [22]. The addition of metal ions caused a noticeable change in the color of the solution to purple or blue, with the exception of Mg^{2+} which showed no significant color change (Fig. S1, Supplemental Information). Since solutions of gold nanoparticles are known to take their color from the frequency of collective electron motions known as surface plasmon resonances (SPR), changes in the color of the nanoparticle solution reflect changes in nanoparticle size, separation or both. UV–visible spectroscopy was used to determine the position of the SPR band before and after the addition of metal ions to Flg-A3 nanoparticle solutions (Fig. 1, Table 2). As expected from the observed color change, the addition of metal ions to solutions of Flg-A3 gold nanoparticles causes a measurable red shift and slight broadening of the SPR band in the UV–vis spectrum (Fig. 1). Such