Fabrication of Two-Dimensional Arrays of Inorganic-Binding Polypeptides for the Template-Directed Self-Assembly of Nanoparticles

Victoria Mooney, Chemistry, Viterbo University
NNIN REU Site: NanoTech User Facility, University of Washington

NNIN REU Principal Investigator: Alex Jen, Material Science and Engineering, University of Washington
NNIN REU Mentor: Melvin Zin, Material Science and Engineering, University of Washington
Contact: vlmooney@viterbo.edu, ajen@u.washinton.edu

Abstract:

Due to the unique properties exhibited by nanoparticles, there has been a surge of interest in nanotechnology for developing techniques to organize nanoparticles in a spatially resolved manner. It has been found that by combining inorganic nanoparticles with biological molecules, a high degree of organization and binding specificity can be acquired.

A septapeptide which binds to platinum was modified to form three repeat and one repeat, constrained genetically engineered polypeptides for inorganics (GEPIs). These GEPIs were patterned on two types of substrates: bare gold (Au) and a self-assembled monolayer (SAM) of (4-mercaptophenyl)-anthrylacetylene (MPAA). A high degree of binding specificity occurred on the MPAA due to Schiff base formation, particularly for the protein with three repeats of the platinum-binding sequence. On the Au substrate, the GEPI constrained with the sulfur-containing amino acids bound to the substrate better than the three repeat GEPI. This was because the two sulfurs were able to absorb onto the Au surface.

Introduction:

Nanoparticles exhibit unique optical, catalytic, magnetic, and electronic properties which could find use in a variety of nanotechnological applications [1,2]. The problems encountered regarding organizing and assembling of these nanoparticles can be resolved by using biological molecules. One of the most promising types of biological molecules to be used in conjunction with nanotechnology is proteins. Proteins are able to recognize materials based on their chemical and physical characteristics and organize these materials into miniscule structures [2]. Recently, phage display and cell-surface display were used to sort through a vast library of random amino acid sequences to identify polypeptide sequences that recognize various inorganic materials such as metals, metal oxides, and semiconductors [2,3].

Through microcontact printing, GEPIs can be physically and chemically patterned onto a surface. When physically patterned, these polypeptides are transferred directly onto a substrate; often, no covalent bonds are formed. When GEPIs are chemically patterned, the substrate has a SAM that can bond covalently with the proteins [3]. Previously, carboxylic acid terminated SAMs were used; however, additional reagents were required for reaction, thus introducing contaminants to the substrate [3]. Aldehyde terminated SAMs have been used recently because they can form Schiff base linkages (R-CHO + R'-NH_2 (R-C=N-R' + H_2O) with amine groups and only produce water as a byproduct. If a GEPI has many amine groups, then one would expect chemical patterning to be more advantageous than physical patterning.

Methods:

Substrates: Bare Au substrate and Au substrate with a SAM of MPAA were used. The Au substrate was prepared by rinsing with water and ethanol, drying with N_2, and then heating 4 min at 53°C to remove any water remaining on the surface. The SAM was formed by submerging an Au substrate in 5 mL of 0.05 mM MPAA and 25 μL NH_4OH (28-30% NH_3) under a nitrogen atmosphere for approximately 48 hr. The SAMs were sonicated in ethanol for 10 min to remove excess MPAA, rinsed with ethanol and dried with N_2 gas.

Platinum-Binding Proteins: Two GEPIs with strong peptide-substrate binding energies 4 were used: 10 μg/mL 3R SD60 ($[QSVTSTK]_3$), and 10 μg/mL 1RC SD60 ([C-QSVTSTK-C]).

Patterning of GEPIs: A poly(dimethylsiloxane) (PDMS) stamp was inked 1.5 hr with protein. To remove excess protein, the PDMS stamp was allowed to float upside down in water for 3 min, rinsed with water (x3), and rinsed with phosphate buffered saline (x3). The stamp was then placed in contact with the Au substrate for 1 min or the MPAA substrate for 2.5 hr.

Results:

After physically patterning 3R SD60 onto the Au substrate, it was found that the packing density of the proteins was approximately 220 proteins/μm^2 (Figure

1). The physically patterned 1RC SD60 had a packing density of approximately 250 proteins/μm^2 (Figure 2). The 3R SD60 chemically patterned onto the MPAA had a packing density of approximately 315 proteins/μm^2 (Figure 3), and the 1RC SD60 on MPAA had a packing density of approximately 120 proteins/μm^2 (Figure 4). All density values were obtained by doing a direct count of proteins in a patterned area.

Discussion:

3R SD60's greater density on the MPAA substrate can be explained by examining the amino acids' side chains. In addition to the amine group at the N terminus, 3R SD60 also has three amine groups from the lysine residues. These amine groups provide binding sites for MPAA's aldehyde group, enabling Schiff base formation. On the bare Au substrate, the 3R SD60 is only able to bind non-specifically.

Conversely, 1RC SD60's packing density is higher on the bare Au substrate due to the sulfurs in the two cysteine groups which are able to absorb directly onto the Au surface. Also, 1RC SD60 has only one lysine group, greatly decreasing the protein's ability to bind to the MPAA via Schiff base formation.

In the future, patterning of 3R SD60 and 1RC SD60 on Au (111) should be done and the spatial conformation of the proteins examined. To determine how the two patterning methods affect the proteins' platinum-binding ability, platinum nanoparticles should be assembled onto the patterned substrate and the resulting platinum nanoparticle density examined.

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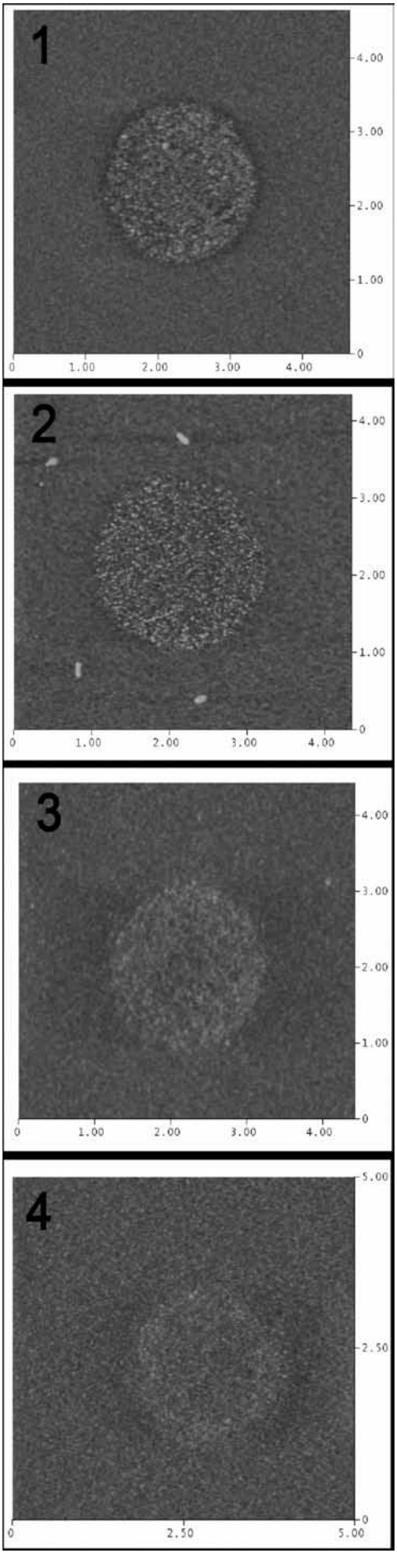

Figure 1: AFM image of 3R SD60 patterned on polycrystalline Au.

Figure 2: AFM image of 1RC SD60 patterned on polycrystalline Au.

Figure 3: AFM image of 3R SD60 patterned on a SAM of MPAA.

Figure 4: AFM image of 1RC SD60 patterned on a SAM of MPAA.