Electrochemical Synthesis of Gold and Protein Gradients on Particle Surfaces

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S Supporting Information

ABSTRACT: A straightforward, versatile approach to the production of protein gradients on planar and spherical particle surfaces is described. The method is based on the spatially controlled oxidation of thiolated surfaces by Au(III) ions generated via the electrochemical oxidation of a gold electrode in a phosphate-buffered saline solution (10 mM PBS, pH 7.2, 150 mM NaCl). Because the gold electrode is in direct contact with the thiolated surfaces, the released Au(III) ions, which are present as Au(III) chloride complexes, give rise to the formation of a surface gradient of Au(I)-thiolate complexes depending on the local redox potential given by the local Au(III) concentration. As is shown on the basis of the use of X-ray photoelectron spectroscopy and fluorescently labeled proteins, the Au(I)-thiolate complexes can subsequently be functionalized with thiolated proteins, yielding surface density gradients on micrometer-sized nonconducting polymer beads as well as linear Au(I)-thiolate gradients on planar silicon surfaces.

■ INTRODUCTION

Several fundamental biological processes depend on gradients of biomolecules, such as proteins, on surfaces. Surface-confined proteins stimulate human cells to migrate, proliferate, or differentiate,^{1–5} which are fundamental phenomena required for wound healing, the immune response, and tumor metastasis. Surface density gradients are also versatile tools in various biosensor^{6–8} and catalysis^{9,10} applications. At present, there are several techniques that can be used to produce molecular gradients on surfaces. Approaches such as microfluidics,^{11–14} plasma polymerization,^{15–17} and diffusion procedures through liquid droplets,¹⁸ hydrogel networks,¹⁹ and agarose²⁰ or dextran^{21,22} gels have thus been described. Photoactive molecules have likewise been used on the basis of photo-immobilization techniques, in which the light intensity and exposure times^{23–26} are controlled, or by employing photolithography.^{27,28} More automated and computerized methods such as inkjet printing²⁹ and “control dipping”³⁰ have also been described. Gradients have also been generated with various techniques based on surface-confined electrochemistry, using in-plane electrochemical potentials for the reductive desorption of thiol layers,^{31–33} corona discharge,³⁴ scanning electrochemical microscopy,^{9,35} and bipolar electrodes.^{36,37} However, the manufacturing of density gradients has so far been limited to planar surfaces.

We have recently shown³⁸ that electrochemical reactions can be used to manufacture anisotropic particles based on the site-specific oxidation of thiols present on the surfaces of polymeric particles. This method involves the oxidation of the segment of the particle in contact with a glassy carbon or gold electrode. In this letter, we show that this approach also enables the

straightforward, versatile production of surface density molecular gradients on the surfaces of nonconducting polymer particles, provided that a gold electrode is used. To the best of our knowledge, this is the first report regarding such a method that also can be used for the straightforward generation of gradients on planar thiolated surfaces.

■ EXPERIMENTAL SECTION

Thiol Functionalization of Surfaces. The reactions employed to introduce thiols onto the surfaces of polymer particles and silicon wafers as well as onto proteins have been described elsewhere.³⁸ A brief description of these reactions can also be found in the Supporting Information.

Potentiostatic Experiments. The thiolated surfaces (i.e., the particles that had an average diameter of 4.9 μm with a standard deviation of 0.2 μm (Micromod Partikeltechnologie GmbH)) or the silicon wafer was put in a Teflon beaker with an inner diameter of 0.9 cm containing 4 mL of PBS and was used in an electrochemical three-electrode setup comprising a gold working electrode, a gold counter electrode, and a platinum quasi-reference electrode. Typically, 2 mL of particles (i.e., 8.75×10^6 particles/mL), with approximately 1×10^8 thiol groups per particle, was added to the beaker and allowed to distribute on the gold surface for 10 min. The silicon surface was tilted against the gold working electrode and the inner wall of the Teflon beaker at an angle of approximately 26°. A potential of +0.9 V (vs Pt) was then applied to the gold working electrode.

X-ray Photoelectron Spectroscopy. The formation of Au(I)-thiolates on the surfaces of the particles and silicon wafers was investigated with X-ray photoelectron spectroscopy (XPS) employing

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a Scienta ESCA-300 spectrometer using monochromatic Al $K\alpha$ radiation with a photon energy of 1487 eV. The particles were dried onto a piece of $1 \times 1 \text{ cm}^2$ conductive glass prior to the XPS measurements. The recorded spectra for the silicon wafers were referenced to the Si 2p core level at a binding energy of 99.3 eV. For the particles, the reference was instead made to the Sn 3d core level at 486.6 eV (Sn(IV)).⁴⁴ The binding-energy scale was the Fermi level adjusted by measuring the Au 4f level of a clean Au foil. The spectra were recorded at normal photoelectron emission.

RESULTS AND DISCUSSION

Thiol-functionalized magnetic polymer particles with a diameter of $4.9 \mu\text{m}$, which were suspended in a physiological buffer containing sodium chloride (10 mM PBS, pH 7.2, 150 mM NaCl), were attracted to a gold working electrode surface by an external magnet mounted underneath the electrode as is depicted in Figure 1. A potential of +0.9 V versus a Pt quasi-

Figure 1. Fabrication of protein gradients on the surfaces of nonconducting polymer particles. (A) Thiol-functionalized particles are oxidized by released Au(III) chloride complexes to yield (B) a gradient of Au(I)-thiolate complexes on its surface. (C) Proteins are conjugated to the Au(I) thiolates to generate a surface density protein gradient.

reference electrode was then applied to the gold electrode to generate Au(III) ions in the vicinity of the gold surface. These Au(III) ions, which are present as Au(III) chloride complexes, diffuse into the electrolyte solution and react with the thiolated surfaces to yield disulfides and surface-confined Au(I)-thiolate complexes (Figure 1). Although this formation of Au(I)-thiolates as a result of the oxidation of the thiols by Au(III) ions is well known and has been utilized for the generation of thiol-supported gold nanoparticles,³⁹ we are not aware of any previous reports of its use in the oxidative generation of surface density gradients.

More explicitly, the Au(I)-thiolate gradients are formed as a result of the fact that the particles are spherical because this causes the surface-bound thiols to be located different distances from the electrode surface (i.e., at different positions in the Au(III) diffusion layer). Thiols close to the gold working electrode will thus be exposed to a higher Au(III)

concentration (i.e., a more positive redox potential) than thiols anchored on top of the particles as is depicted in Figure 1b. Finally, thiol-functionalized immunoglobulin G (IgG) was conjugated to the surface-bound gold atoms via gold–sulfur bonds (Figure 1c) to generate protein surface density gradients.

The present approach consequently relies on the generation of Au(III) chloride complexes acting as oxidation agents with respect to the surface-immobilized thiol groups. Figure 2 shows

Figure 2. Cyclic voltammogram recorded for a gold electrode in PBS buffer containing 0.15 M chloride employing a scan rate of 50 mV/s.

a cyclic voltammogram recorded for the gold electrode in 10 mM sodium phosphate buffer (pH 7.2) containing 0.15 M sodium chloride and employing a three-electrode setup with a gold counter electrode and a platinum quasi-reference electrode. (The potential of this electrode was +0.31 V vs a Ag/AgCl reference electrode.) It is seen that the oxidation of the gold electrode commenced at about +0.7 V and that the oxidation charge was significantly larger than the reduction charge, indicating the formation of soluble Au(III) chloride complexes. The release of Au(III) ions from gold electrodes in the presence of a complexing agent such as chloride is not surprising because this phenomenon has been described previously.^{40,41} More importantly, the release of the Au(III) species gives rise to an Au(III) concentration gradient in the vicinity of the gold electrode. Because the Au(III) chloride species are good oxidizing agents, the local redox potential will thus depend on the oxidation time as well as the distance between the particles and the gold electrode, indicating that the thiols on the surfaces of particles will experience different redox potentials depending on the positions of the thiols with respect to the electrode surface. This redox potential gradient (which will also depend on the particle number density) is responsible for the generation of the gradients of disulfides and Au(I)-thiolates on the surfaces of the particles. For a preset oxidation time, the obtained surface gradients will also naturally depend on the size of particles used in the experiment.

After the partial oxidation of the particles, the thiolated particles were investigated with X-ray photoelectron spectroscopy (XPS). The formation of Au(I)-thiolates was confirmed by detecting the presence of Au(I) species on the surfaces of the particles (Supporting Information, Figure S1). No Au(I) could be detected on the particles in the absence of the oxidation step.

As depicted in Figure 3, the Au(I)-thiolate gradients could be used to generate gradients of proteins on the surfaces of the particles. To enable fluorescence microscope studies of the protein gradients, fluorescein isothiocyanate (FITC)-labeled

Figure 3. Fluorescence microscopy images of particles with protein molecular gradients obtained after oxidation at +0.9 V (vs Pt) for (A, B) 0.1, (C) 1, and (D) 10 s. Note the protein gradients on the particles in A and B and the different fluorescence intensities in C and D. The length of the scale bar corresponds to 5 μm .

IgG molecules were employed. The particles were first oxidized at +0.9 V (vs Pt) using different oxidation times and thereafter treated with thiolated proteins. For the shortest oxidation times (i.e., 0.1 s), fluorescence intensity gradients were clearly seen on the individual particles, as is seen in Figure 3a,b. For longer oxidation times (i.e., 1 and 10 s, respectively; see Figure 3c,d), entire particles were instead found to emit fluorescence radiation of approximately the same intensity. When comparing particles oxidized for 1 and 10 s, respectively, it is immediately evident that the fluorescence intensity was significantly higher for the longer oxidation time. The latter can be explained by the fact that the longer oxidation time results in a higher Au(III) concentration in the vicinity of the particles, which should give rise to a higher degree of oxidation and thus a higher concentration of protein on the surfaces of the particles. No fluorescence intensity was seen for particles that had not undergone the oxidative treatment. The latter indicates that there was no significant immobilization of the thiolated protein as a result of disulfide formation with the thiols immobilized on the particles. It is therefore clear that the immobilization of the thiolated proteins relies on the presence of the Au(I)-thiolates generated in the oxidation of the thiols by the released Au(III) ions.

The present results also indicate that sufficiently short oxidation times are needed to obtain Au(I)-thiolate gradients on individual particles whereas longer oxidation times result in an oxidation of the entire surfaces of the particles as a result of the growth of the Au(III) diffusion layer with time. The experimental results are in fact in good agreement with estimations of the diffusion layer thickness based on the following equation: $\Delta = (2Dt)^{1/2}$ in which Δ , D , and t denote the distance, the diffusion coefficient, and the time, respectively.⁴² For an oxidation time of 0.1 s, the thickness of the diffusion layer can then be estimated to be about 4.5 μm , assuming a diffusion coefficient of $10^{-6} \text{ cm}^2/\text{s}$. Because this

value is of the same order of magnitude as the diameter of the particles, it is reasonable to expect the formation of partially oxidized surfaces under these experimental conditions. For oxidation times of 1 and 10 s, the corresponding values are 14 and 45 μm , respectively, indicating that these oxidation times should indeed give rise to homogeneous oxidation of the entire surfaces of the particles. These straightforward calculations hence demonstrate the possibility of producing batchwise surface density gradients on nonconducting polymer particles merely by controlling the length of the Au oxidation pulse. These findings are also in excellent agreement with our previous finding³⁸ that dots, rather than gradients of surface-confined Au(I) species, were obtained on single particles when an oxidation time of 0.1 s was used with a gold electrode. The present approach can hence be used to generate both surface gradients (of Au(I)-thiolate or proteins) on individual particles (i.e., patchy particles) and homogeneously coated particles with different surface concentrations of Au(I)-thiolate or proteins. In the latter case, the surface concentrations of Au(I)-thiolate or protein will depend on where in the Au(III) concentration gradient the particles were located during the oxidation step.

To model the gold gradient on the particles and to estimate how the concentration of gold adsorbed along the gradient varies with distance from the electrode, experiments were also carried out with planar thiol-functionalized silicon surfaces. In this case, gradients could be generated when the silicon surface was tilted against the gold working electrode because this induced a varying distance between the gold electrode and the different positions on the silicon surface hosting the thiol groups (the tilt angle was approximately 26°). In these experiments, an oxidation potential of +0.9 V (vs Pt) was employed for 60 s and the Au(I)-thiolate density along the silicon surface was determined by probing the Au(I) intensity as a function of the position employing XPS (Figure 4a). The XPS measurements were performed at four different positions corresponding to distances of approximately (i) 0.5, (ii) 2.5, (iii) 4.0, and (iv) 4.5 mm from the gold electrode. As is seen in Figure 4a, which depicts the obtained Au 4f_{5/2} and Au 4f_{7/2} doublets, a binding energy of 85.5 eV was obtained for the Au 4f_{7/2} peak indicating the presence of surface-confined Au(I)-thiolate species.⁴³ The binding energy for metallic Au is 84.0 eV.³⁹ These results clearly show that the formation of the Au(I)-thiolates is due to the oxidation of the surface-confined thiols by the Au(III) chloride complexes generated as a result of the oxidation of the gold electrode. Because the gradient of the Au(I)-thiolate can be changed by modifying parameters such as the tilt angle, oxidation potential, hydrodynamic conditions, and chloride concentration, this approach opens up new possibilities for the straightforward generation of different protein gradients on thiolated surfaces.

The release of Au(III) during the oxidation was also verified by determining the gold concentration in the electrolyte after the oxidation experiment using inductively coupled plasma mass spectrometry (ICP-MS) measurements. It was found that approximately 80 μg of gold was released during a 60 s oxidation. As discussed previously, the Au(III) chloride complexes can readily oxidize the surface-confined thiol groups provided that the local concentration of Au(III) species is high enough. The ICP-MS results also clearly showed that there was no significant release of Au(III) when chloride was absent in the electrolyte. As a further control, Au 4f photoelectron spectra were recorded for thiol-functionalized silicon surfaces that had not been exposed to any release of Au(III). In the

Figure 4. (A) Au 4f_{5/2} and Au 4f_{7/2} doublets recorded for the thiolated silicon surface at different positions (i.e., 0.5–4.5 mm) from the gold electrode ($h\nu = 1487$ eV); (B) integrated Au 4f peak area as a function of the position along the silicon surface. The solid line represents a linear least-squares fit based on the included equation.

latter case, there were no traces of gold on the surface of the thiolated silicon surface.

On the basis of the XPS data in Figure 4a, the integrated peak area of the Au 4f_{5/2} and Au 4f_{7/2} doublet was evaluated as a function of position along the gradient. As is seen in Figure 4b, the peak area decreased linearly with increasing distance from the gold surface. This finding, which is in good agreement with expectations based on the convective mass transport of the released Au(III) species away from the electrode surface,⁴² indicates that this approach can be used for the generation of linear Au(I)-thiolate gradients that subsequently can be converted into linear protein gradients. The present approach is consequently very promising for the straightforward design of surfaces with spatially varying protein coverage.

CONCLUSIONS

A novel, straightforward electrochemical way to produce density gradients of Au(I)-thiolates on the surfaces of both nonconducting spherical particles and planar surfaces has been demonstrated on the basis of the generation of Au(III)-chloride complexes acting as spatially controlled oxidation agents. The generated gradients can be readily functionalized with proteins in a subsequent step to yield protein density gradients. With the

present technique, it should therefore be possible to fabricate surface gradients of almost any type of thiolated molecule. The density profiles of the Au(I)-thiolate or protein gradients can be controlled merely by varying the length of the oxidation potential pulse. With sufficiently short oxidation pulses, gradients on individual particles can even be obtained. For longer oxidation pulses, the protein surface concentration on different particles will differ depending on the location of the particles during the oxidation process. Moreover, the presented approach will yield not only disulfide and Au(I)-protein gradients on surfaces but also gradients of nonfunctionalized surface thiols. The latter thiols can be functionalized further to provide surfaces, planar or spherical, with two protein gradients. We believe that this approach for making protein gradients with controlled density profiles will become a very versatile research tool for studies of, for example, cell migration or proliferation. Another exciting application involves the manufacturing of density gradients of thiol-supported gold nanoparticles by reducing surface-bound gold(I)-thiolates using either an electrode or sodium borohydride (NaBH₄) rather than functionalizing them with proteins.

■ ASSOCIATED CONTENT

■ Supporting Information

Additional experimental details and the results of investigating protein gradients on particle surfaces with fluorescence microscopy and X-ray photoelectron spectra, detecting surface-confined Au(I) species on electrochemical oxidized particles. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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