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Direct, Noncovalent Coating of a Gold Surface with Polymeric Self-Assembled Monolayers

Hojae Lee, Daewha Hong, Sangyong Jon,† and Insung S. Choi*

*Department of Chemistry, KAIST, Daejeon 305-701, Korea. *E-mail: ischoi@kaist.ac.kr*
†Department of Biological Sciences, KAIST, Daejeon 305-701, Korea
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The spatio-selective immobilization of biomolecules, such as DNAs, antibodies, or aptamers, onto a solid surface is required for the development of bioanalytical and biomedical devices that interface the immobilized probe with the target biospecifically.[1-4] Strategies for non-covalent and covalent immobilization have been developed, exemplified by biotin-streptavidin[5,6] or *N*-nitrilotriacetic acid (NTA)-histidine tag interactions[7] for non-covalent linking and *N*-hydroxysuccinimide (NHS)-mediated amide coupling[8,9] for covalent coupling. In addition to the surface-immobilized bioprobes, the surface should be non-biofouling (*i.e.*, preventing or at least minimizing the non-specific adsorption of proteins and other molecules and adherence of cells) for maximized biospecific recognition between the probe and the target and multiplexed detection of the analytes based on microarrays/patterns. In this respect, a method should be developed for providing a surface of interest with three orthogonal properties: functionalizable, non-biofouling, and surface-anchorable ones. We suggested the formation of polymeric self-assembled monolayers (pSAMs) with a random copolymer having these three properties.[10] For example, a hydrophobic cyclic olefin copolymer (COC) surface was coated with a polymer presenting the non-biofouling poly(ethylene glycol) (PEG) and NHS moieties along with a long alkyl chain (C12) for surface anchoring of the polymer via hydrophobic interactions (pPNC) (Figure 1).[10d]

On the other hand, a gold surface has widely been used for bioanalytical characterizations, including surface plasmon resonance (SPR) spectroscopy,[9,11,12] and mostly the surface coating has been achieved by utilizing the gold-thiol interactions in the form of SAMs.[13] Although there is the intense need for customized fabrication of a gold surface for biorecognition, only the SAM-based approach has been available because of the practical difficulty in the synthesis of thiol-presenting random copolymers for the pSAM formation. Instead, we have previously used the random copolymer presenting PEG and NHS, where the NHS moiety acted as both a post-functionalizable group and a surface-anchorable group to amine-terminated SAMs on gold.[14] In this paper, we report that the interactions of alkyl chains with gold are strong enough for coating the gold surface with pSAM of pPNC. Of interest, the pSAM proves fairly stable during the pattern generation of IgG/anti-IgG.

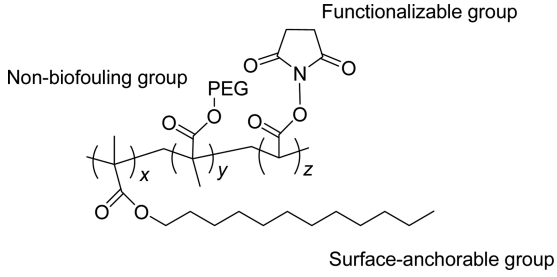

Figure 1. Structure of pPNC used in this work.

The coating of a gold substrate with the pSAM of pPNC was achieved by simply immersing the substrate in the citrate buffer solution of pPNC (10 mg/mL, pH 3.3) for 2 h at ambient temperature. We used the citrate buffer for pPNC, because the NHS group was relatively stable under acidic conditions. The typical static water contact angle of a bare gold was measured to be 59.5 ± 1.3^{o} in our case, and the water contact angle was changed to 73.3 ± 0.9^{o} after coating (Figure 2(a)). The coating was further confirmed by the ellipsometric thickness (17.1 Å), after coating. The grazing-angle Fourier transform IR (GA-FTIR) spectrum showed the peaks of pPNC at 3249 (O-H stretching), 1742 (C=O stretching, ester and imide), and 1476 cm^{-1} ($-CH_2-$ bending), indicating the formation of pSAMs (Figure 2(b)).

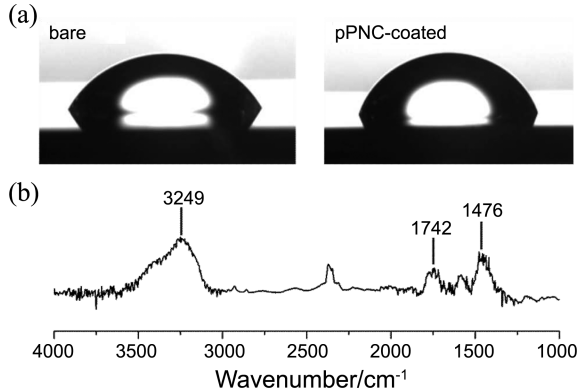

Figure 2. (a) Static water contact angles of bare and pPNC-coated gold surfaces. (b) GA-FTIR spectrum of the pPNC-coated gold surface.

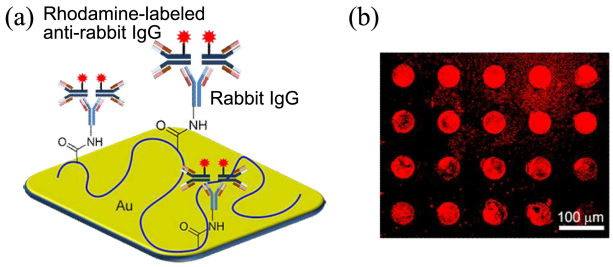

Figure 3. (a) Schematic representation of immobilization of the antibody (rabbit IgG) onto the pPNC-coated gold surface and biospecific recognition between rabbit IgG and rhodamine-labeled anti-rabbit IgG. (b) LSCM image of the rhodamine-labeled anti-rabbit IgG patterns.

After confirming the stable formation of the pSAM on gold, we tested the pSAM stability during the biorecognition event and the micropattern formation with rabbit IgG as a model protein. The poly(dimethylsiloxane) (PDMS) stamp having circular patterns (50 μm in diameter separated by 50 μm) was first immersed in a 10% sodium dodecyl sulfate solution for 10 min at ambient temperature, followed by ultra-sonication for 5 min. The stamp was then inked with the rabbit IgG solution (120 μg/mL in phosphate-buffered saline (PBS)) for 30 min and brought into contact with the pPNC-coated gold surface for 1 min. After microcontact printing, the unreacted NHS groups were reacted with 2-(2-aminoethoxy)ethanol (EG_2NH_2) to passivate the surface. The laser-scanning confocal microscopy (LSCM) image, after biospecific complexation with rhodamine-labeled anti-rabbit IgG (40 μg/mL in PBS; pH 7.4), showed the bright red circle-patterns with the black background (Figure 3). The contrast in fluorescence clearly indicated that the pSAM maintained its structural integrity during the processes employed for the pattern generation.

In summary, we demonstrated a simple but rather unexpected method for coating a gold surface with the random copolymer presenting a long alkyl chain. The poly(ethylene glycol) group in the polymer effectively minimized the unwanted adsorption of proteins onto the surface, and the *N*-hydroxysuccinimide group was utilized for covalent immobilization of the antibody. The successful generation of IgG/anti-IgG showed that the long alkyl chain-mediated coating of the gold surface led to the stable formation of the polymeric self-assembled monolayers at least in water and phosphate-buffered saline. We think that more detailed studies on the stability of the pSAMs on gold are needed for the practical applications of the method described in this work to the bioanalytical analysis, which is our next research thrust.

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