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Free-Standing Gold-Nanoparticle Monolayer Film Fabricated by Protein Self-Assembly of α-Synuclein**

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Abstract: *Free-standing nanoparticle films are of great importance for developing future nano-electronic devices. We introduce a protein-based fabrication strategy of free-standing nanoparticle monolayer films. α-Synuclein, an amyloidogenic protein, was utilized to yield a tightly packed gold-nanoparticle monolayer film interconnected by protein β-sheet interactions. Owing to the stable protein–protein interaction, the film was successfully expanded to a 4-inch diameter sheet, which has not been achieved with any other free-standing nanoparticle monolayers. The film was flexible in solution, so it formed a conformal contact, surrounding even microspheres. Additionally, the monolayer film was readily patterned at micrometer-scale and thus unprecedented double-component nanoparticle films were fabricated. Therefore, the free-floating gold-nanoparticle monolayer sheets with these properties could make the film useful for the development of bio-integrated nano-devices and high-performance sensors.*

Fabrication of nanoparticles (NPs) into hierarchical supra-structures has been pursued to maximize their chemical and physical properties.[1] In this respect, free-standing ultrathin films of NPs have garnered a great deal of interest in materials science[2] especially to make those particles used in the development of realistic devices. The free-standing nanoparticle films have been produced by spin-assisted layer-by-layer (LbL) assembly,[3] cross-linking-based interfacial assembly,[4] and drying-mediated self-assembly.[5] The NPs inside have been linked together with binders, such as polyelectrolytes,[3] ligands,[4,5b] and biomolecules.[5a] In the case of LbL assembly, a multilayered nanocomposite membrane was prepared by sandwiching a central AuNP layer with multiple polymer layers on both sides.[3] Although the AuNPs enhanced the mechanical robustness of the polymer membrane, the particles were not packed tightly enough for additional applications. Using the interfacial-assembly, densely a packed NP monolayer was produced after cross-linking them between two immiscible fluids.[4] In the drying-mediated method, well-organized AuNP arrays were also successfully prepared by using either dodecanethiol- or DNA-conjugated nanoparticles.[5] These constructs, however, could not be expanded beyond micrometer scale as they were produced over microholes. Concerning the preparation of two-dimensional (2D) nanomembranes, various substances other than NPs, such as carbon,[6] surfactant of dodecylphosphocholine,[7] organic–inorganic composite,[8] and peptoid[9] have also been employed to fabricate the functional membranes valuable for the development of optical and electronic devices and membrane systems.[10]

Herein, a bio-based strategy is introduced to produce a free-floating ultrathin AuNP sheet where AuNPs are tightly packed in a monolayer by employing a self-associative protein of α-synuclein (αS) as a binding agent. The resulting transparent 2D AuNP sheets are readily expandable, flexible and thus exhibiting conformal contact, patternable, and air/dry-stable. It is these characteristics that would allow the sheets to take crucial parts in the realistic applications. The assembler, αS, is an amyloidogenic protein comprising 140 amino acids and is a pathological component of Parkinson's disease participating in the Lewy body formation.[11] We recently reported that the αS-coated AuNPs were fabricated into hierarchical supra-structures, such as the photoconductive pea-pod type AuNP 1D nanochain[12] and the uniformly monolayered 2D AuNP array on glass capable of detecting metal ions in the presence of phthalocyanine tetrasulfonate.[13]

Our protein-based approach of preparing the free-standing AuNP single-layered sheet consists of three steps (Scheme 1). First, αS-coated AuNPs (αS-AuNPs) were prepared by incubating a mixture of wild-type αS and colloidal AuNPs for 12 h at 4 °C, and subsequent removal of the unbound αS. The αS-AuNP conjugates were then non-covalently adsorbed onto a plasma-treated polycarbonate (PC) substrate to form a closely packed monolayer at pH 4.5.

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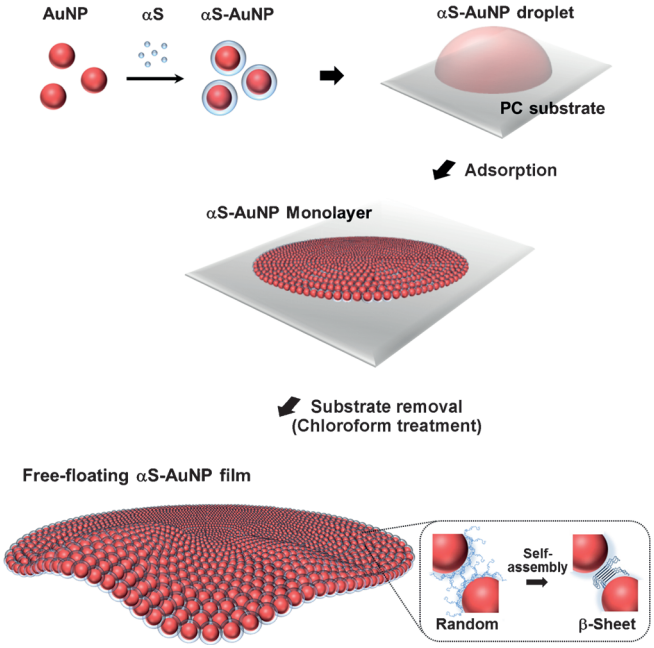

Scheme 1. The fabrication of a free-floating αS-AuNP monolayer film through the preparation of αS-coated AuNPs, adsorption of the αS-AuNPs onto the polycarbonate (PC) substrate, and subsequent free-standing film production by αS–αS self-assembly upon removal of the substrate with chloroform.

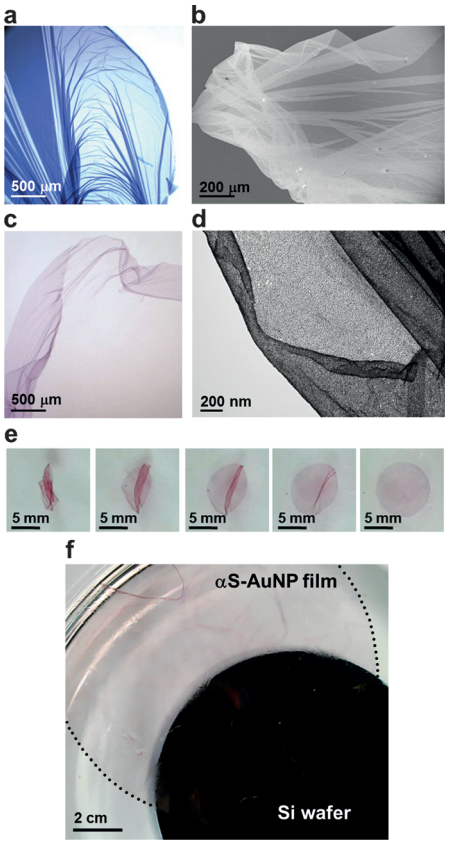

Figure 1. Flexible free-standing αS-AuNP monolayer films. a,b) Optical (a) and SEM (b) images of the αS-AuNP monolayer film prepared with 20 nm diameter AuNPs. c,d) Optical microscope image (c) and TEM image (d) of a αS-AuNP monolayer film made of 10 nm diameter AuNPs. e) A series of images of a free-standing αS-AuNP film composed of 10 nm diameter AuNPs showing the film's flexibility in chloroform. f) Optical image of a wafer-scale αS-AuNP film (indicated by dotted line) floated from the background 4-inch Si wafer.

By dissolving the PC substrate in chloroform, the AuNP monolayer was unleashed as a free-standing film from the sacrificial substrate (see Supporting Information, Movie S1). The resulting film prepared with 20 nm-diameter AuNPs was transparent and purple-blue (Figure 1 a). A scanning electron microscope (SEM) image of the film is shown in Figure 1 b. The film made of 10 nm-diameter AuNPs appeared bright pink (Figure 1 c). Its transmission electron microscope (TEM) image reveals that the AuNPs were tightly packed in monolayer (Figure 1 d). The film's flexibility was illustrated with a series of images of the film in the solvent (Figure 1 e). The crumpled film unfurled into a planar state, and it folded back. The films formed with 20 and 30 nm AuNPs exhibited the similar behavior of flexibility in the solvent (Supporting Information, Figure S1a). The free-standing αS-AuNP film was successfully grown to the size of 4-inch wafer and shown to be floating intact and bright pink (Figure 1 f).

The αS-AuNP films were produced based on three specific interactions between αS and individual AuNPs, between αS and the polymer substrate, and between αS and αS, leading to the αS-AuNP formation, the αS-AuNP adsorption onto the substrate, and the αS–αS self-assembly, respectively. The homogeneity of the αS-AuNP conjugates was assessed with dynamic light scattering (DLS) analysis (Figure 2 a). The protein coat increased the average diameter of colloidal AuNPs from 20.6 to 38.4 nm. This 18 nm increase in the diameter is consistent with the previous studies investigating the hard corona formation of αS on AuNPs.[14] A flocculation assay indicated that a well-dispersed colloidal state was maintained for the αS-AuNPs even at a high salt concentration of 1.25 M NaCl (Figure S2). In contrast, other protein–AuNP conjugates prepared with another amyloidogenic peptide/protein of either amyloid-β (Aβ) or β2-microglobulin (β2 M) yielded non-specific agglomerates of the particles as they formed during washing steps of the conjugate preparation (Figure S3).

The adsorption process of αS-AuNPs onto the PC substrate was also specific for the protein (Figure 2 b, top) since the monodispersed array of the nanoparticles was not obtained with other soluble proteins, such as bovine serum albumin (BSA; Figure 2 b, middle), β2M (Figure 2 b, bottom), and κ-casein (Figure S4). These proteins produced rather non-specific agglomerates of AuNPs on the substrate. Therefore, the monolayer formation of αS-AuNP on the PC substrate was considered to be achieved by not only a preferential surface interaction of the αS to the substrate, but also an unaccommodating αS–αS interaction between the αS-AuNPs pre-attached on the surface and the incoming αS-AuNP conjugates. The packing density of the αS-AuNP monolayer improved as the incubation proceeded. The packing density increased from $472.0 \pm 15.7\ \mu m^{-2}$ for 5 min adsorption, $646.3 \pm 10.1\ \mu m^{-2}$ for 30 min, $706.7 \pm 10.6\ \mu m^{-2}$ for 60 min, to $1005.0 \pm 6.1\ \mu m^{-2}$ for 180 min (Figure S5). The

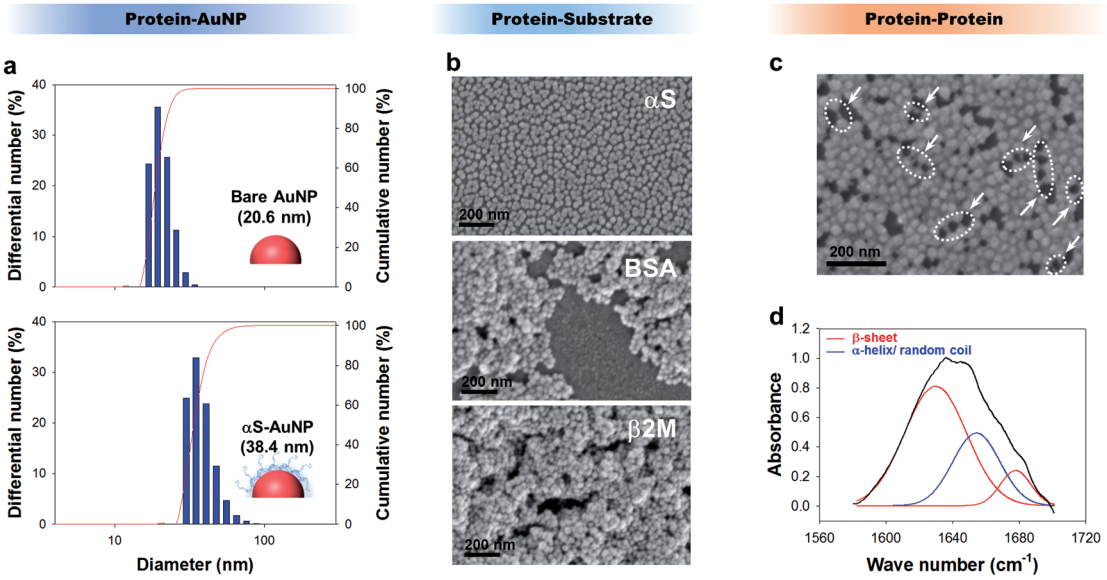

Figure 2. Specificity of αS. a) DLS histograms of bare AuNPs (top) and αS-AuNPs (bottom). b) SEM images of the protein–AuNP conjugates adsorbed on a PC substrate. The AuNPs were encapsulated with αS (top), BSA (middle), and β2M (bottom). c) SEM image of a αS-AuNP film showing the extended αS interconnections between AuNPs as indicated within the dotted line circles and arrows. d) ATR-FTIR spectra (black) for the amide I band of αS-AuNP film. Deconvoluted curves in red and pink represent the β-sheet conformation. The blue curve indicates α-helix/ random coil structure of αS.

specific adsorption of AuNPs mediated by αS was demonstrated to be dependent upon the surface properties of the PC substrate in addition to the incubation time. The packing density of αS-AuNPs on a PC substrate drastically decreased from $1022.0 \pm 9.2\ \mu m^{-2}$ to $664.7 \pm 12.3\ \mu m^{-2}$ without the O_2 plasma treatment (Figure S6). For the free-standing films, the packing density dropped from $1101.7 \pm 3.5\ \mu m^{-2}$ to $714.3 \pm 11.0\ \mu m^{-2}$ if they were prepared from the PC substrates without the plasma pre-treatment (Figure S7). In addition, a considerable amount of AuNPs agglomerates were formed on the plasma-treated substrate as the adsorption temperature increased from 60 °C to 80 °C (Figure S8).

When the underlying PC substrate was dissolved in chloroform, the AuNPs in the floating film become held together through αS–αS self-association. Under SEM, the intriguing fibril-like connections were revealed between the particles (Figure 2c). Considering αS as an amyloidogenic protein, those interconnections were presumed to be formed through the β-sheet structure in chloroform.[15] Deconvolution of an attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectrum (Figure 2d) confirmed the presence of the β-sheet structure as unraveled with the bands at 1630 and 1680 cm^{-1}.[16] As previously reported, αS forms the β-sheet structure in the organic solvent,[17] thus the use of chloroform in this study enabled us to obtain the αS-mediated nanoparticle films.

Three different size AuNPs with diameters of 10, 20, and 30 nm were used separately to create the free-standing films, and their properties were analyzed. All the protein–particle conjugates were able to produce a monolayer although the packing density appeared to decrease as the AuNP size increased (Figure S1b). Atomic force microscopy (AFM) data indicated that the thickness of each film was comparable to the size of AuNPs with which the films were prepared (Figure 3a). Optical images of those films showed transparent sheets with different colors depending on the particle size: bright pink for the 10 nm-diameter NPs, purple-blue for the 20 nm NPs, and dark blue for the 30 nm NPs (Figure 3b). Surface plasmon resonance (SPR) spectra of the films were monitored by using a micro Raman microscope[5a] (Figure S9); absorption spectra of the films made of 10, 20, and 30 nm diameter AuNPs culminated at wavelengths of 530, 570, and 620 nm, respectively, reflecting their color difference (Figure 3c). In comparison with the spectra of colloidal AuNPs, the film maxima were red-shifted from 518, 523, and 528 nm, respectively, owing to the plasmonic coupling between adjacent gold particles within the tightly packed films[18] (Figure S10). The extent of red-shift upon the film formation augmented as the particle size increased, which would be due to the enlarged area for plasmonic oscillation between adjacent particles.

These AuNP films were flexible in chloroform, and are expected to provide conformal contact (that is, shape-fitting contact) to micrometer-scale three-dimensional objects, such as spherical silica beads with a diameter of 1 μm. The film made of 10 nm AuNPs yielded conformal wrapping around a silica bead without showing any cracks (Figure 4a). In particular, the film maintained its structural fidelity at the junction between the bead and a planar surface (Figure 4b). The film also conformally overlaid multiple spheres without causing structural collapse (Figure 4c–e). In particular, the conformal-contact formation would be a crucial feature for the development of bio-compatible devices monitoring in vivo organ/tissue activities.[19] In addition, stability of the transferred film is also critical for the development of bio-electronic devices. Since the film formation was mediated by the protein of αS, the film's durability was evaluated in conditions affecting the protein's stability. Although αS

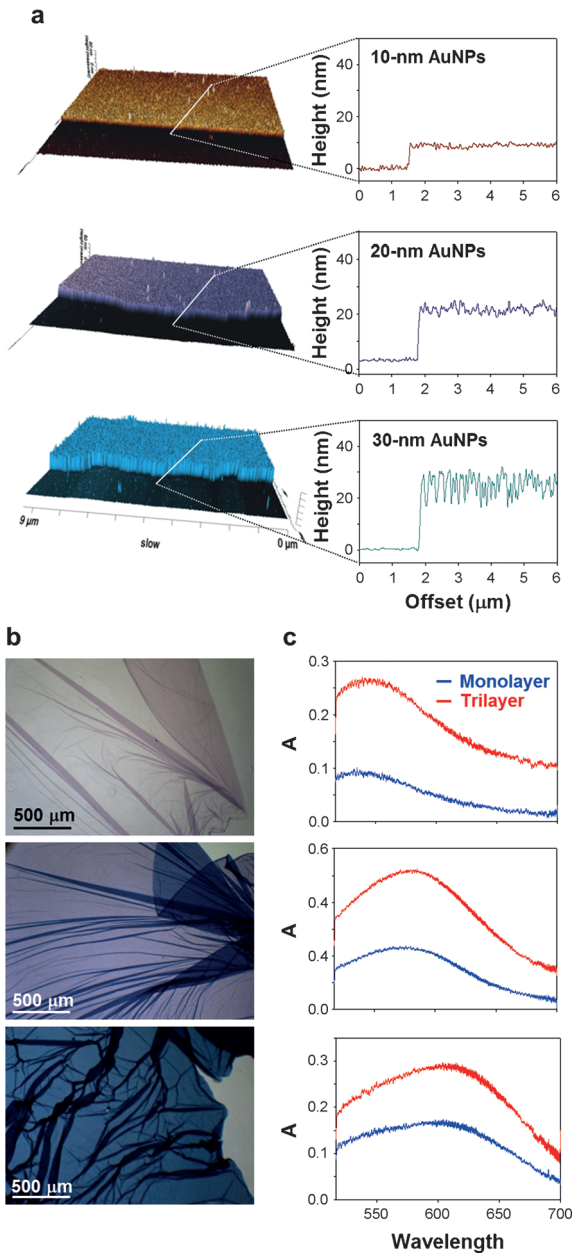

Figure 3. αS-AuNP monolayer films produced with 10 nm diameter AuNPs (top row), 20 nm diameter AuNPs (middle row), and 30 nm diameter AuNPs (bottom row). a) Three-dimensional AFM images of the αS-AuNP films showing the thickness profiles. b) Optical microscope images of the αS-AuNP films. c) SPR spectra of the films acquired by monitoring local absorbance of the films with a Reinshaw InVia Raman spectrometer. The red and blue curves indicate the adsorption spectra of the αS-AuNP films in triple layers and single layers, respectively.

molecules tend to be self-interactive to form protein aggregates (protein aggregates include oligomers, fibrils, and amorphous forms) during a prolonged in vitro incubation, the film was demonstrated to be unaffected even after 7 days of incubation in 20 mM 2-(4-morpholinyl)ethanesulfonic acid (MES; pH 6.5) at 37 °C[20] (Figure 4 f). The absorption spectra were almost unchanged and after the prolonged incubation (Figure 4 g). Moreover, thermal stability of the film was also tested at 80 °C at which most proteins undergo structural denaturation. Intriguingly, the film's morphology and its absorption spectra were hardly affected even after 7 days of incubation at the high temperature (Figure 4 h, i). Its thermal durability is possibly attributed to the inherent heat-stability of αS.[21] In addition, the film's solvent stability was also checked in ethanol, acetone, dimethyl sulfoxide (DMSO), and acetonitrile The mean absorbance of the film in the region of 565–575 nm remained virtually unaffected before and after a 1 h immersed incubation in the solvents (Figure 4 j). These experimental data suggest that the αS-AuNP film could be considered as a sustainable biocompatible material to be used in the device development.

The AuNP films were patterned in two-dimensions by employing a microfluidic device prepared with poly(diemthylsiloxane) (PDMS) through soft lithography.[22] A colloidal dispersion of αS-AuNPs was injected into a channel on the patterned device tightly attached to the bottom layer of plasma-treated PC substrate[23] (Figure 5 a and Figure S11). As the PC layer was detached from the top PDMS mold, the αS-AuNPs were found to be adsorbed at the bottom in monolayer with the patterned arrays of 100 μm diameter circular holes separated by distances of 300 μm (Figure 5 b, left), 450 μm (Figure 5 b, middle), and 600 μm (Figure 5 b, right). These micrometer-scale patterns were maintained in the free-floating AuNP films following the removal of the PC substrate with chloroform (Figure 5 c). Since patterning techniques are crucial for fabricating electronic devices,[24] the patternable and flexible ultrathin αS-AuNP films would afford another potential to be applied in nano-bioelectronics.[25]

Double-component AuNP films comprising two different sizes of the particles were constructed by sequential adsorptions of 10 nm and 20 nm AuNPs (Figure 5 d). The 20 nm αS-AuNPs were adsorbed on one half of a round PC substrate by masking the other half with a paraffin film. The mask was then removed and the conjugates with 10 nm diameter NPs were adsorbed onto the newly exposed half of the substrate (Figure S12). After dissolving the underlying PC with chloroform, the flexible free-floating double-component AuNP film was obtained (Figure 5 e and Movie S2). The resulting two semi-circles were different in colors, depending on the sizes of AuNPs. Each area was confirmed to be single layered by measuring the thickness with AFM (Figure 5 f). Using the same procedure, another free-floating double component film was fabricated with the "SNU" logo embedded (Figure 5 g and Figure S13). This double-component film was also stable and flexible in chloroform (Figure S14 and Movie S3). This maneuver clearly demonstrates a feasibility of manufacturing patterned multi-component monolayer films of NPs with various functionalities using, for example, magnetic NPs or quantum dots.

We have described a facile procedure of fabricating the protein-based free-standing AuNP monolayer films over wafer-scale, which can be not only patterned using soft lithographic technique but prepared in the free-floating multi-component sheets as well. This procedure is established based on three unique interactions between αS and AuNP, between αS and PC substrate, and between αS and αS. The αS

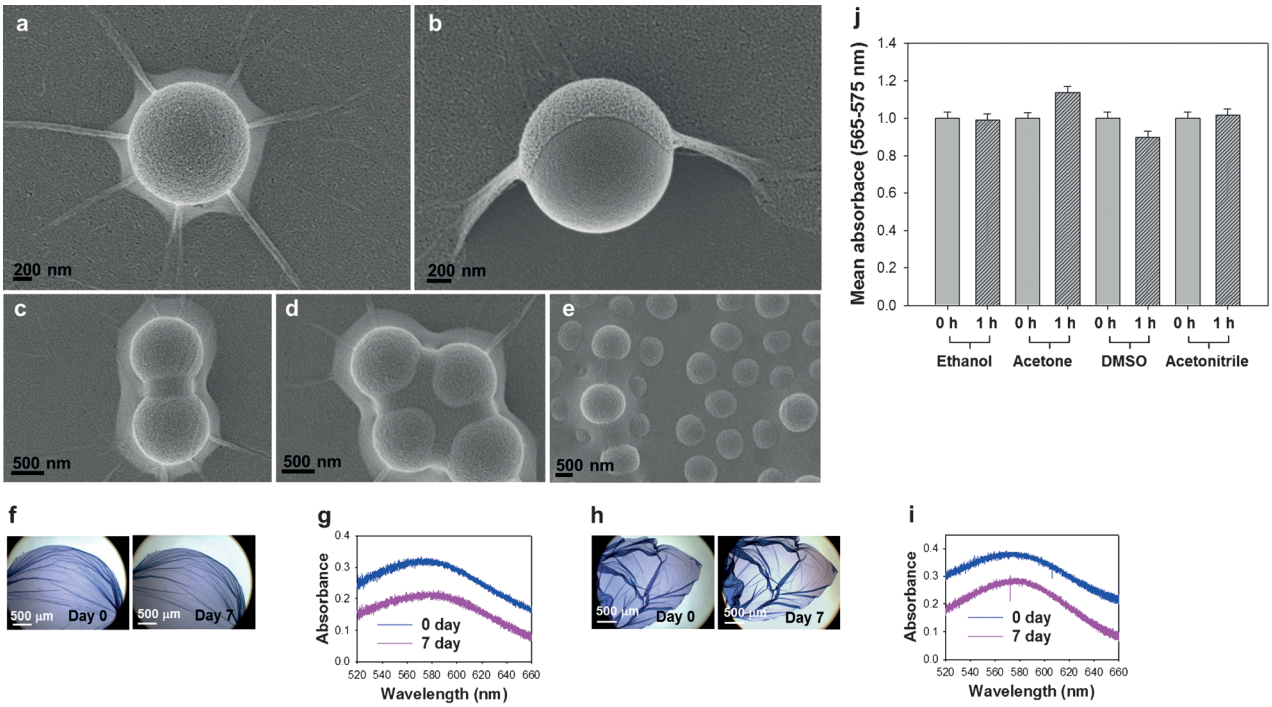

Figure 4. Conformal contact and stability of αS-AuNP film. a) SEM image showing conformal contact of the αS-AuNP film wrapping a 1 μm-diameter silica bead. b) An image showing the film partly covering a silica bead. c,d,e) SEM images of the film wrapping two (c), four (d), and numerous (e) adjacent silica beads. f) Macroscopic optical images of the transferred films before and after incubating them in aqueous environment of 20 mM MES (pH 6.5) at 37 °C for 7 days, and g) absorption spectra of the films on glass substrate monitored by Raman spectroscopy before and after the incubation. h) Morphologies of the films attached on glass substrate before and after its exposure to 80 °C for 7 days, and i) their absorption spectra. j) Absorbance of the films attached on the glass were obtained as the mean value of absorbance over the range of 645–655 nm before (gray) and after (stripes) their exposure to ethanol, acetone, DMSO, or acetonitrile for 1 h.

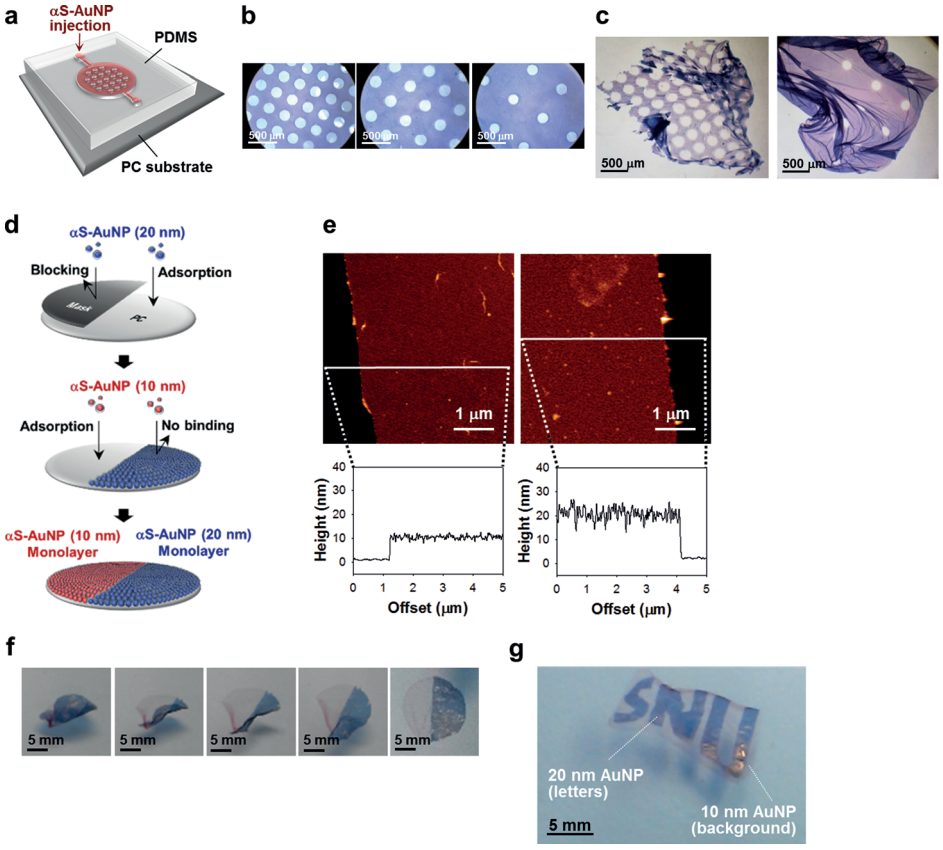

Figure 5. Patterning of αS-AuNP films and fabrication of double-component films. a) A schematic image showing the PDMS microfluidic soft lithography mold and the PC substrate. b) Optical images of the microchannels loaded with the αS-AuNPs containing 10 nm-diameter AuNP. Center-to-center distances between holes are 300 μm (left), 450 μm (middle), and 600 μm (right). c) Images of the patterned αS-AuNP free-floating films with 100 μm-diameter holes with the center-to-center spacing of 300 μm (left) and 600 μm (right). d) Schematic diagram showing the fabrication of a double-component αS-AuNP film from 10 and 20 nm diameter AuNPs. e) AFM images and height profiles of the double-component film. The areas made of 20 and 10 nm diameter AuNPs are shown in the left and right panels, respectively. f) A series of time lapse images of the double-component αS-AuNP film in chloroform. The flexible film was prepared with 10- and 20 nm-diameter AuNPs. g) Double-component film showing the "SNU" logo. The letters were prepared with the 20 nm diameter AuNPs while the background was made of 10 nm diameter AuNPs.

encapsulating AuNPs directs adsorption of the protein–NP conjugates onto the plasma-treated PC substrate to form the AuNP monolayer. Subsequent chloroform-induced αS–αS interaction allows interconnection of the αS-coated AuNPs into the free-floating monolayer film as the PC substrate is dissolved. The resulting protein–protein interaction produces the β-sheet conformation between the αS molecules, which permits the films to exert resistance to chemical degradation and favorable mechanical property[26] as witnessed with the conformal contact formation and the thermal and solvent durability of the films. Taken all these properties together, the ultrathin free-standing AuNP monolayer film has potential to be applied in a wide range of nanobiotechnology including development of nano-electronic devices. Furthermore, this type of single layered NP sheets could be used in ultrathin display and nano-membranes with electromechanical properties by incorporating various types of NPs such as quantum dots and magnetic nanoparticles. realistic

Experimental Section

The AuNP monolayer films were prepared in two steps: adsorbing the αS-AuNPs onto a 30 μm thick polycarbonate (PC) substrate (2 × 2 cm) (SEJIN TS, Korea) to form the AuNP monolayer, and then dissolving the substrate to give rise to simultaneous association between the αS-AuNPs through specific αS–αS interactions. To induce optimal adsorption of αS-AuNPs onto the substrate, the pH value of the solution containing αS-AuNPs was changed from 6.5 (20 mM MES) to 4.5 (50 mM citrate). The buffer exchange was carried out by precipitating the αS-AuNP conjugates with centrifugation at 16100 × g and re-suspending the resulting precipitates in the new citrate buffer. A 50 μL droplet of the αS-AuNP solution was then immediately placed on top of the PC substrate, which was pre-treated with oxygen plasma (CUTE-MPR, FEMTO SCIENCE, Korea) at 60 W for 1 min. After incubation in a humidified chamber at 40 °C for 3 h, the particle-loaded substrate was thoroughly washed with deionized (DI) water (Direct-Q, Millipore, Germany). The underlying PC layer was subsequently dissolved with chloroform (HPLC grade, Fisher, MA) while facilitating the self-assembly of αS-AuNPs. The resulting αS-AuNP monolayer film was freely suspended in the solvent. After replacing the PC-containing chloroform with the fresh solvent, the free-floating αS-AuNP film was transferred to the surface of slide glass (MARIENFELD, Germany) or silicon wafer (Siltron Inc., Korea).

Keywords: nanoparticles · protein–protein interactions · self-assembly · supramolecular chemistry · thin films

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