CHAPTER III

EXPERIMENTAL

3.1 Materials

Hydrogen tetrachloroaurate ($HAuCl_4.3H_2O$), acrylic acid (AA), dimethyl formamide (DMF), 4,4-bis(4-cyanovaleic acid) (ACVA), and 4-cyano-4-(phenyl carbonothioylthio)pentanoic acid (chain transfer agent or CTA) were obtained from Aldrich (USA). Developer MFTM-321, acetone, toluene, hexane, ethanol, dichloromethane, and sulfuric acid were purchased from Merck (Germany). Dicyclohexyl carbodiimide (DCC), hydrogen peroxide (H_2O_2), dialysis bag (cut-off molecular weight of 3,500 g/mol), phosphate buffered saline pH 7.4 (PBS), 3-aminopropyltriethoxysilane (APTES), 1H,1H,2H,2H-perfluorooctyltrichlorosilane (PFOTCS) were obtained from Sigma-Aldrich (USA). Cysteine, Glutathione, bradykinin, and ICNKQDCPILE peptide were bought from Sigma (USA). Positive photoresist S1813 and glass slides were purchased from Rohm and Hass (Japan). Thermo Scientific (USA), respectively. AA was purified by vacuum distillation. Cysteine, glutathione, bradykinin, ICNKQDCPILE peptide were obtained from American peptide company. All solutions were made using ultrapure distilled water that was obtained after purification using a Millipore Milli-Q system (USA) that involves reverse osmosis, ion exchange, and a filtration step (18.2 MΩ cm resistance).

3.2 Equipments

3.2.1 Nuclear Magnetic Resonance Spectroscopy (NMR)

The ^{1}H NMR spectra were recorded in CF_3COOH/D_2O using Varian, model Mercury-400 nuclear magnetic resonance spectrometer (USA) operating at 400 MHz. Chemical shifts (δ) were reported in part per million (ppm) relative to tetramethylsilane (TMS) or using the residual protonated solvent signal as a reference.

3.2.2 Fourier Transform Infrared Spectroscopy (FT-IR)

The FT-IR spectra were recorded in KBr discs with a FT-IR spectrometer (Nicolet, USA), model Impact 410, with 32 scans at resolution 4 cm^{-1}. A frequency of 400-4000 cm^{-1} was collected by using TGS detector.

3.2.3 Transmission Electron Microscopy (TEM)

The morphology and actual size of particles were analyzed by a JEOL JEM-2010 transmission electron microscopy (Japan) operating at 200 keV. The TEM samples were prepared by dropping approximately 10 µL of AuNPs solution on the carbon-coated copper grid and dried in a dessicator before analysis. The average diameters were reported from measurements of 30 random particles for each sample using Semafore software.

3.2.4 Water Contact Angle Measurement

The dynamic advancing (θ_A) and receding (θ_R) water contact angles were measured using a contact angle goniometer (Ramé-Hart, Inc., USA, model 100-00), equipped with a Gilmont syringe and a 24-gauge flat-tipped needle. All of the

measurements were carried out in air at ambient temperature. The reported angle is an average of 5 measurements on different area of each sample.

3.2.5 X-Ray Photoelectron Spectroscopy (XPS)

The surface composition was characterized by x-ray photoelectron spectroscopy (XPS) on a Scienta ESCA 200 spectrometer (Uppsala, Sweden) with Al Kα x-rays. All the XPS data were collected at a takeoff angle of 90°.

3.2.6 Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)

Standard sample of $HAuCl_4$ solutions (0.001, 0.0005, 0.0003, and 0.0001mM) were prepared for generating the calibration curve. AuNPs on glass surface were digested by immersion of the glass substrate in aqueous solution of aqua regia ($1HNO_3$:3HCl) at room temperature for 15 min. The solution was then diluted with 100 mL with Milli-Q water before being quantified for gold composition by ICP-MS.

3.2.7 Mass Spectrometry Measurements

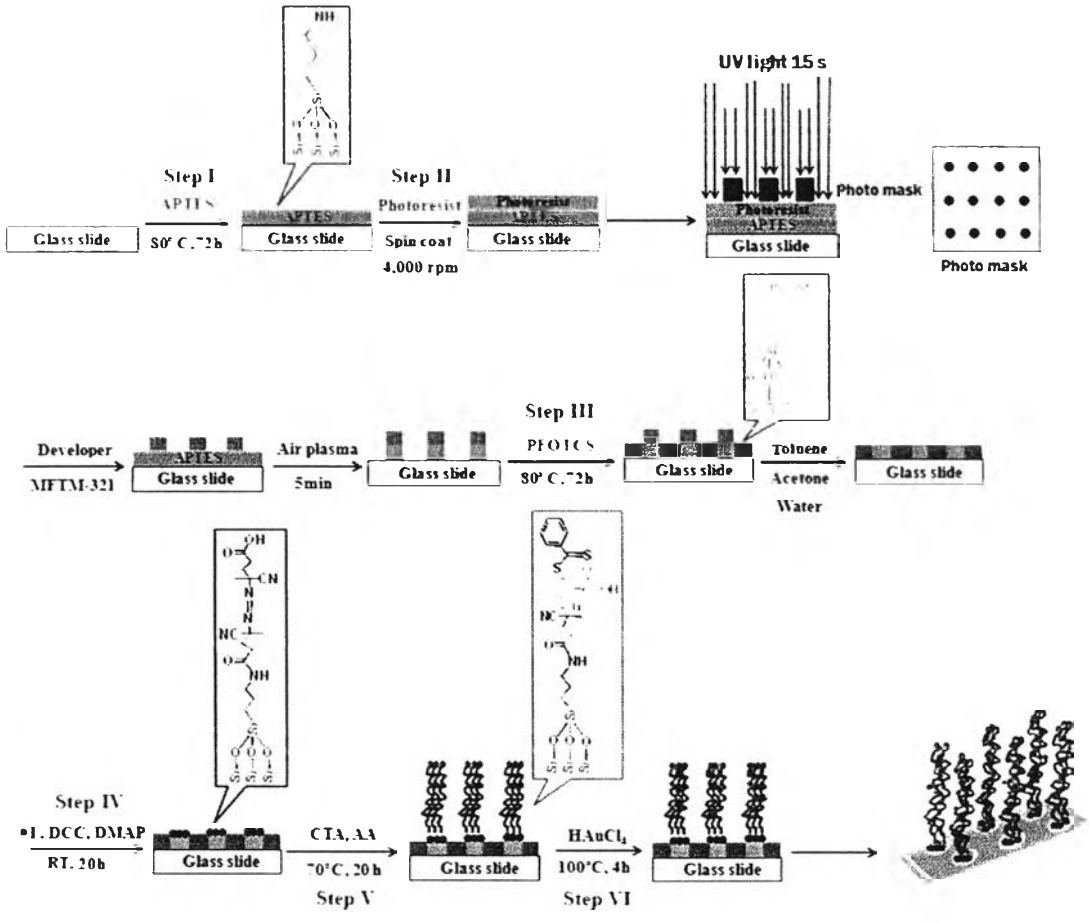
SALDI mass spectra were acquired by mass spectrometry measurements in MALDI-TOF-MS (Autoflex III) from Bruker Daltonics at 50% laser 100 shots.

3.3 Experimental Procedure

3.3.1 Surface Patterning by Photolithography

A glass slide (diameter 1.5 cm) was treated with plasma cleaner for 5 min before exposed to vapor of 100 µL APTES in a 50 cm^3 vial sealed with a cap in an oven at 80 °C for 72 h and rinsed sequentially with toluene, acetone, and deionized water to yield Si-NH_2 (Scheme 3.1, Step I). Positive UV-sensitive resist S1813 was then spin-coated at 4,000 rpm for 3 min on the surface of Si-NH_2 and cured at 115 °C for 1 min. A photomask was placed on the photoresist-coated surface before irradiation

with UV light (365 nm, 500W) for 15 sec. The irradiated pattern was treated with developer MFTM-321 for 1 min and rinsed with deionized water followed by treatment in plasma cleaner for 5 min to destroy the APTES layer and recover back the silanol groups on the unmasked area (Scheme 3.1, Step II). The obtained patterned Si-NH_2/OH was exposed to vapor of 100 µL PFOTCS in a 50 cm^3 vial sealed with a cap in an oven at 80 °C for 72 h and rinsed sequentially with toluene, acetone, and deionized water. The recovered silanol groups of the patterned Si-NH_2/OH would react with PFOTCS and yielded patterned Si-NH_2/$O(CH_2)_2(CF_2)_5CF_3$ (Scheme 1, Step III). At this point, the photoresist on the masked area was also removed so that the amino groups of APTES are readily available for initiator immobilization in the next step.



Scheme 3.1 Schematic representation of stepwise method showing how to prepare patterned PAA brushes containing AuNPs supported on glass slide: (I) reaction with APTES, (II) surface patterning by photolithography, (III) attachment of perfluorooctylsilyl groups, (IV) attachment of initiator, (V) surface-initiated RAFT polymerization of AA, and (VI) *in situ* synthesis of AuNPs on PAA brushes.

3.3.2 Grafting of PAA Brushes on Patterned Surface

The ACVA (0.21g, 1 mmol), DCC (0.19g, 1 mmol), and DMAP (0.01g, 0.1 mmol) were dissolved in 20 mL of DMF. The solution was stirred for 4 h at room temperature before transferred to the glass slide having patterned Si-NH_2/$O(CH_2)_2(CF_2)_5CF_3$ in a vial. The solution was stirred under nitrogen atmosphere at

room temperature for 20 h. Then, the glass slide was rinsed with ethanol and deionized water to yield Si-I/O$(CH_2)_2(CF_2)_5CF_3$ (Scheme 3.1, Step IV). ACVA (0.014g, 0.5 mmol) and CTA (0.056g, 2 mmol) were dissolved in 20 mL of PBS buffer (pH 7.4). AA (1.37ml, 1.44 mol) was added to the mixture which was then transferred into a vial containing patterned Si-I/O$(CH_2)_2(CF_2)_5CF_3$. The surface-initiated RAFT polymerization of AA was allowed to proceed under nitrogen atmosphere at 70°C for 20 h. The resulting patterned Si-PAA/O$(CH_2)_2(CF_2)_5CF_3$ was then obtained after rinsing with ethanol and deionized water (Scheme 3.1, Step V)

3.3.3 *In situ* Synthesis of AuNPs on PAA brushes

The glass slide having patterned Si-PAA/O$(CH_2)_2(CF_2)_5CF_3$ was subjected to 5 cycles of alternate dipping in aqueous solution of $HAuCl_4$ (5ml, 1mM) and deionized water. Then, the glass slide was immersed in DI water at 100 °C for 1h followed by an aqueous solution of $HAuCl_4$ (5ml, 1mM) at 100 °C for another 4 h. The glass slide was then rinsed with deionized water and dried with stream of nitrogen to obtain the patterned PAA brushes containing AuNPs (Scheme 1, Step VI).

3.3.4 Preparation of Peptide Sample Solutions

Each peptide samples was dissolved and diluted in Milli-Q water to a final concentration of 500 nM (3 µL). Then the peptide solutions were deposited onto the MALDI target plate. For SALDI-MS analysis, the samples were directly deposited onto the glass slide modified with patterned PAA brushes containing AuNPs. The modified glass slides were attached on to MALDI target plate by a two-side conductive tape and then introduced into a Bruker Microflex/Autoflex III MALDI-TOF

mass spectrometer for SALDI-MS analyses. The N_2 laser (337 nM) with 50% laser intensities and 100 laser shots were used for all analyses.

For MALDI-MS analysis, each peptide sample was mixed with CHCA MALDI matrix (1:9 50% acetonitrile:0.1% TFA) and then deposited onto MALDI target plate. After solvent evaporation and sample crystalization, the target plate was introduced into a Bruker Microflex/Autoflex III MALDI-TOF mass spectrometer for MALDI-MS analyses.

3.3.5 Analysis of Thiol-containing Peptide in a Mixture

A mixture of 500 nM thiol-containing peptide and 500 nM non thiol-containing peptide was deposited onto the glass slide modified with patterned PAA brushes containing AuNPs. The deposited mixture was incubated on the modified target plates at room temperature for 10 min. Then the unbounded peptides were washed out from the target by using PBS buffer (pH 7.4) and Milli-Q water using a micropipette. After solvent evaporation, the target plate was introduced into a Bruker Microflex/Autoflex III MALDI-TOF mass spectrometer for both SALDI-MS and MALDI-MS analyses.