Supplementary Material (ESI) for Chemical Communications

Facile Fabrication of Networked Patterns and Their Superior Application to Realize the Virus Immobilized Networked Pattern Circuit

Kyung Min Choi[a], Seok Jae Lee[b], Jung Hoon Choi[a], Tae Jung Park[c], Jong Wan Park[b], Weon Ho Shin[a], Jeung Ku Kang[a]*

[*] Prof. J. K. Kang, K. M. Choi, J. H. Choi, W. H. Shin

NanoCentury & EcoEnergy KAIST Institutes, Graduate School of EEWS (WCU),

Department of Materials Science & Engineering, KAIST, Daejeon 305-701 (Korea)

E-mail: jeungku@kaist.ac.kr

Dr. S. J. Lee, Dr. J. W. Park

NTD&I Team, National NanoFab Center, Daejeon 305-806 (Korea)

Dr. T. J. Park

BioProcess Engineering Research Center, Center for Systems & Synthetic Biotechnology, and KAIST Institute for the BioCentury, KAIST, Daejeon 305-701 (Korea)

Materials and Methods

General Procedure for pattern, immobilization and electric characterization

Nanomaterial solution preparation process

As-prepared gold-CNT hybrids (8 mg) were dispersed in 1,5-pentanediol (50 mL) by overnight bath sonication. The gold-CNT hybrids dispersed pentanediol solution was heated to 200 °C for 2 h in the presence of polyvinylpyrrolidone (PVP, Avergae MW ~55,000) (5.35g), followed by additional heating to 260 °C for 30 min. After cooling this solution to room temperature, the PVP-modified gold-CNT hybrids were precipitated using acetone, and residual polymer were removed by at least two cycles of high-speed centrifugation, decanting and redispersion in pure ethanol by mechanical agitation including mild ultrasonication to produce a stable solution of PVP wrapped gold-CNT hybrids in ethanol, uniformly dispersible up to 0.08 g/L.

Patterning process

The as-prepared gold-CNT hybrids solution was re-dispersed in a sonicator for a short time (~30 sec) while the substrates were heated on a hot plate to 60 °C. An evaporation guide was placed on the heated substrate and a droplet of the stock solution was placed onto the substrate using a micro-pipette. During the solvent evaporation, CNT networks have being patterned on the substrate *via* evaporation-directed impromptu patterning (eDIP) method (leaving behind only patterned gold-CNT hybrids). After completing evaporation-directed gold-CNT hybrids patterning process, the remaining polymers around gold-CNT hybrids were removed by dipping patterned substrate into more than 100 mL methanol for 10 min.

Immobilization process

The gold-CNT hybrid patterns were then placed into a gold binding polypeptide-fused avian influenza viral surface antigen (GBP-AIa) solution (100 μg/mL) for 1 h at 25 °C followed by washing with phosphate-buffered saline (PBS) and distilled water and dried under nitrogen gas. To prevent non-specific bindings before immobilizing GBP-AIa fusion peptide, 100 μg/ mL bovine serum albumin (BSA) was used on the gold-CNT hybrid patterns for 1 h at 25 °C. After being rinsed by deionized water and PBS, the GBP-AIa-immobilized patterns were immersed into anti-AI antibody (100 μg/mL) in PBS for 1 h at 25 °C followed by drying with nitrogen gas.

The electronic characterization process

To investigate the feasibility of eDIP method and GBP-fusion peptide immobilization into the real-time biosensors, the gold-CNT hybrid was patterned onto platinum multi-line electrodes. The platinum electrodes were fabricated by the conventional photolithography and the electron beam evaporation onto a silicon substrate with the top layer of 0.5 μm SiO_2, where the width and the space between the electrodes were designed by 3 μm all. Dosing GBP-AIa and anti-AI one after another onto the gold-CNT hybrid patterns, the electrical signals were obtained in sequence, after drying all the solvents with the nitrogen gas, using a Semiconductor Characterization System (Keithley).

Material Preparation

Gold-CNT hybrid synthesis

The synthesis of gold-CNT hybrids was based on our previous work with uniform dispersion of Au nanoparticles onto nitrogen-doped CNTs, not requiring any pretreatment and functionalization of CNTs[S1].

Nitrogen-doped CNT synthesis : The nitrogen doped CNTs were synthesized by the microwave plasma enhanced chemical vapor deposition (MPECVD) method after the deposition of an iron thin film (~120 Å) on a SiO_2/Si substrate *via* rf sputtering as catalysts. The substrate was first evacuated inside the CVD chamber and heated to 700 °C. The chamber was then charged with 12 torr of N_2 gas and at which time the substrate was treated with N_2 plasma to generate islands of iron (total duration time was 1 min). CNTs were then prepared by the catalytic decomposition of CH_4 as the carbon source *via* the MPECVD process (21 torr mixture of N_2 and CH_4 which ratio is 5:1, 20 min). The grown CNTs were nitrogen-doped and multi-wall CNTs which length and diameter were 60 μm and 20 nm, respectively.

Gold-CNT hybrid synthesis : Gold-CNT hybrid materials were synthesized by reducing Au precursor salts dissolved in ethylene glycol (EG) in the presence of NaOH as the reducing agent. 16 mL of 10 mM $HAuCl_4$ EG solution and 4 mg of NaOH were mixed with 30 mL of EG in a glass vial. Three mg of as-produced nitrogen doped CNTs were uniformly dispersed in the Au solution by bath sonication. The vial was placed in the center of a household microwave oven and was heated for 6 min. The black products were separated by centrifugation, washed with acetone, and dried under vacuums at 60 °C overnight. The obtained gold-N-doped CNTs were heat treated under the hydrogen atmosphere at 300 °C in order to remove residual EG solution and reduce Ag oxide[S1].

Prediction of Putative Antigenic Regions of H5N1 and H9N2 AI Neuraminidase.

The putative antigenic regions of the envelope protein were predicted by analyzing the primary structure of the H5N1 and H9N2 Avian Influenza neuraminidase protein obtained from the "chicken and H5N1 neuraminidase structure database" (http://protein.gsc.riken.go.jp/Research/Na/). The hydrophilicity, flexible region, antigenicity, and surface probability of the H5N1 and H9N2 AI neuraminidase protein were calculated by

Kyte-Doolittle plots, Karplus-Schulz prediction, Jameson-Wolf prediction, and Emini prediction, respectively (Fig. S1)[S2−S5].

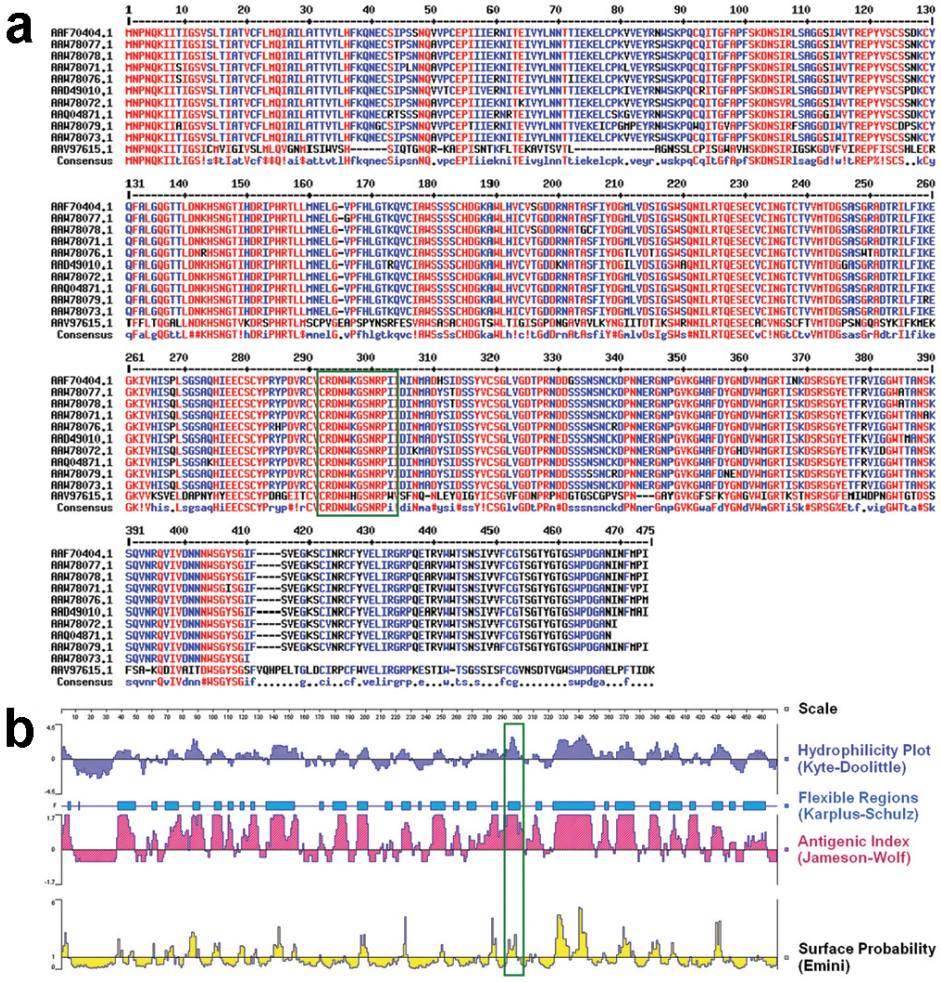

Figure S1. Amino acid sequence analysis of AI neuraminidase. (a) Multiple alignment of neuraminidase in Korean type AI. (b), The hydrophilicity, flexible region, antigenicity, and surface probability of AI neuraminidase protein were calculated by Kyte-Doolittle plots, Karplus-Schulz prediction, Jameson-Wolf prediction, and Emini prediction, respectively. The two green boxes represent the selected antigenic sequence used in this study.

Synthesis of Polyclonal Antibody against its Surface Antigen.

Polyclonal rabbit serum was produced by immunization with a peptide corresponding to the neuraminidase protein residues 291−302 (CRDNWKGSNRPI-NH_2) of H5N1 and H9N2 type AI containing cysteine for conjugation. It was synthesized and conjugated to maleimide-

activated keyhole limpet hemocyanin (KLH; Pierce Chemical) by *N*-[4-maleimidobutyryloxy] succinimide ester (GMBS) conjugation method[S6], and conjugated to ovalbumin (OVA; 45,000 M_W) which serves as a non-relevant carrier protein for enzyme-linked immunosorbent assay (ELISA). Female rabbits (age 12 to 22 weeks) were injected 3 times at 21 day intervals with 500 mg of peptide-KLH conjugate in Freund's complete adjuvant (FCA; Pierce Chemical) according to the manufacturer's protocol. Serum was screened by indirect ELISA using the peptide-KLH conjugate. Each well of a 96-microwell ELISA plate was coated with 10% (w/v) of peptide-OVA conjugate in 50 mM carbonate buffer (pH 9.0), and the plates were incubated overnight at 4°C. Without blocking, 100 μL of antiserum or hybridoma supernatant was incubated for 45 min at 37°C. Bound antibody was detected with goat anti-rabbit immunoglobulin G (IgG)-horseradish peroxidase and *O*-pheylene-diamine dihydrochloride (Sigma). The titer of the rabbit antiserum following immunization was approximately 1:100,000. This antibody was purified by using HPLC column and concentrated up to 1.6 mg/mL. Purified anti-AI antibodies were conjugated with Cy3 fluorophore using a Cy3-labelling kit (GE Healthcare). The protein concentration was determined by Bradford's method using BSA (Sigma) as a standard.

Procedures of Fusion Peptide Preparation and Fluorescence Imaging after Bindings

For the synthesis of the fusion peptide, the gold binding polypeptide (GBP, MHGKTQATSGTIQS-NH_2) and antigenic region against AI (AIa), the polypeptide encoding 26 amino acids of GBP-AIa were prepared using an automatic synthesizer by the Fmoc solid phase synthesis system[S7] according to the manufacturer's procedure (Peptron, Korea).

To visually examine the antigen-antibody interaction, the gold-CNT hybrid patterns were washed thoroughly with deionized water and then dried under nitrogen gas. The gold-N-doped CNT patterns were then placed into a GBP-AIa solution (100 μg/mL) for 1 h at 25 °C followed by washing with PBS and distilled water. They were subsequently dried under

nitrogen gas. To prevent non-specific bindings before immobilizing GBP-AIa fusion peptide, 100 μg/mL BSA was used on the gold-N-doped CNT patterns for 1 h at 25 °C. After being rinsed by deionized water and PBS, the GBP-AIa-immobilized patterns were immersed into Cy3-labelled anti-AI antibody (100 μg/mL) in PBS for 1 h at 25 °C. After washing and drying, sequential bindings of GBP-AIa and Cy3-labelled anti-AI on the gold-*N*-doped CNT patterns were observed by confocal microscopy (Carl Zeiss LSM 510 Meta, Germany) for the imaging Cy3. Samples were excited by a 488 nm argon laser, and the images were filtered by a longpass 505 nm filter.

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