Electronic Supplementary Information (ESI)

Covalent immobilization of gold nanoparticles on a plastic substrate and subsequent immobilization of biomolecules

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Experimental

Materials

2-Aminoethyl methacrylate hydrochloride (AEMA□HCl) and di-*tert*-butyl dicarbonate (Boc_2O) fluorescein isothiocyanate (FITC) were purchased from Sigma-Aldrich (St Louis, MO). 3-[(2-aminoethyl)dithio]ropionic acid (AEDP) was purchased from Toronto Research Chemicals (Toronto, Canada). Tris(2-carboxyethyl)phosphine hydrochloride wase purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Tetrachloroauric(III) acid trihydrate ($HAuCl_4$) was purchased from Strem chemicals (Boston, MA). Dithiothreitol and A.B.T.S. kit, 20 × SSC were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). *N*-hydroxysuccinimide was purchased from Thermo Fisher Scientific Inc. (Waltham, MA). 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSC) was purchased from Dōjindo Laboratories (Kumamoto, Japan). Tween-20 was purchased from Merck (Darmstadt, Germany). Thiolated and (FITC)-modified DNA oligonucleotides (19-mer) were purchased from Tsukuba Oligo Service Co. Ltd. (Tsukuba, Japan). DNA sequences used in the study were thiol-5′-TTAGTTCTCCAGCTATCTT-3′ and FITC-5′-AAGATAGCTGGAGAACTAA-3′ (the complementary strand). The NAP-5 column was purchased from GE-Healthcare (Piscataway, NJ). An acrylic substrate [made of poly(methyl methacrylate), abbreviated PMMA] was purchased from Nitto Jushi Kogyo Co., Ltd. (Tokyo,

Japan). Substrates (0.5 mm thick) were cleaned with a detergent solution and cut into 1 cm × 1 cm pieces prior to use. The water used was high-quality deionized water (Milli-Q water, >15 MΩ cm), produced using a Milli-Q Integral 3 system (Millipore, Molsheim, France). Other chemicals were purchased from Wako Pure Chemical Industries and used as received.

Synthesis of 2-(tert-butoxycarbonylamino)ethyl methacrylate (Boc-AEMA)

Boc-AEMA was synthesized according to our previous report.[1] AEMA-HCl (12.1 mmol) and trimethylamine (24.2 mmol) were dissolved in chloroform (40 mL). Di-tert-butyl dicarbonate (13.3 mmol) was added to this solution and reaction was performed at 4 °C overnight. Ethyl acetate (60 mL) was added to the reaction mixture and the resulting precipitate was removed by filtration. The filtrate was evaporated and the resulting residue was washed three times with water and freeze-dried overnight. The yield was 67%. ^{1}H-NMR (300 MHz, $CDCl_3$): δ(ppm) 1.45 (s, 11H), 1.95 (t, 3H, J = 1.5 Hz), 3.44 (t, 2H, J = 6.6 Hz), 4.21 (t, 2H, J = 30.6 Hz), 4.74 (s, 1H), 5.59 (t, 1H, J = 2.1 Hz), 6.13 (s, 1H, J = 1.2 Hz). DART-TOF/MS (m/z) DART-TOF MS (m/z): $[M+H]^+$ calculated for $C_{11}H_{19}NO_4$, 230.1; found, 230.1.

Synthesis of poly(MMA-*r*-(Boc)AEMA) (termed PMBA, Fig. S1)[2]

MMA (54.8 mmol), Boc-AEMA (6.09 mmol) and AIBN (0.122 mmol) were dissolved in ethyl acetate (30.6 mL) and polymerization was performed overnight at 70 °C. An excess of *n*-hexane was added to precipitate the copolymer and the precipitate was dried under vacuum overnight. ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) 0.94 (d, 31H, J = 51.6 Hz), 1.47 (s, 9H), 2.01–1.75 (m, 15H) 3.42 (s, 2H), 3.61 (s, 29H), 4.04 (s, 1H). The weight-average molecular weight (M_w) and number-average molecular weight (M_n) were measured by size exclusion chromatography (SEC) (ChromNAV Ver.2, JASCO, Tokyo, Japan) with a 7.5 × 300 mm SEC column (GF510, Showa Denko K.K., Tokyo, Japan) and a refractive index detector (RI-8031, JASCO, Tokyo, Japan) at 40 °C using tetrahydrofuran as an eluent. Poly(methyl methacrylate) was used as a molecular weight

standards. The weight-average molecular weight (M_w) and polydispersity (M_w/M_n) of the synthesized polymer are shown in Table S1. An organic trace element analyzer (Exeter Analytical, Inc.) was used to analyze the CHN elements. The results of the elemental analysis are shown in Table S1. The monomer composition ratio (MMA:Boc-AEMA) was 9:1.

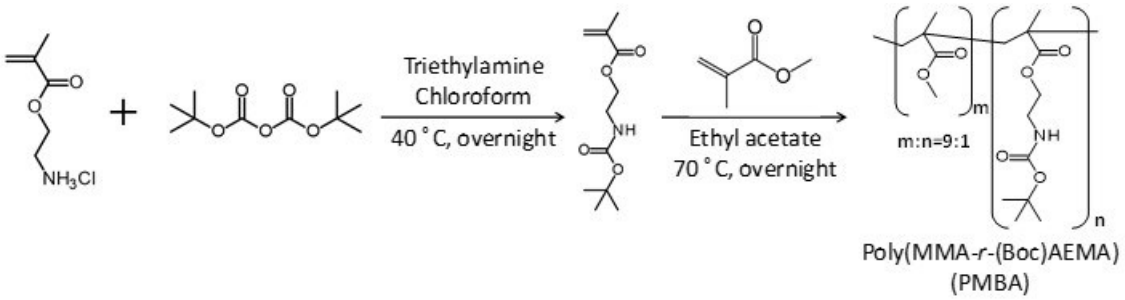

Fig. S1 Synthesis of a functional polymer (PMBA).

Table S1 M_w and M_w/M_n and results of elemental analysis of PMBA

Mn	Mw/Mn	C (%)	H (%)	N (%)
7.3×10^4	1.5	60.9	8.18	1.31

Synthesis of cleavable fluorescent compounds (FITC-S-S-COOH, Fig, S2)[2]

AEDP (0.13 mmol) was dissolved in 12 mL of a water/THF (1:1 volume ratio) mixture, which contained 2.5 vol% trimethylamine. To this solution, FITC (0.14 mmol) was added and gently mixed for 3.5 h at 25 °C. After evaporation of the solvent, a precipitate was generated by adding 1 M HCl aqueous solution. The precipitate was collected by centrifugation and washed three times with 1 mM HCl aqueous solution. After vacuum drying overnight, the yield was measured. The yield was 73%. ^{1}H-NMR (300 MHz, MeOD): δ(ppm) 3.03–2.64 (m, 8H), 3.95 (t, 2H, J = 7.8 Hz), 6.54–8.15 (m, 10H). MALDI TOF/MS ([M]+ calcd for $C_{26}H_{22}N_2O_7S_3$, 570.7; found, 570.1

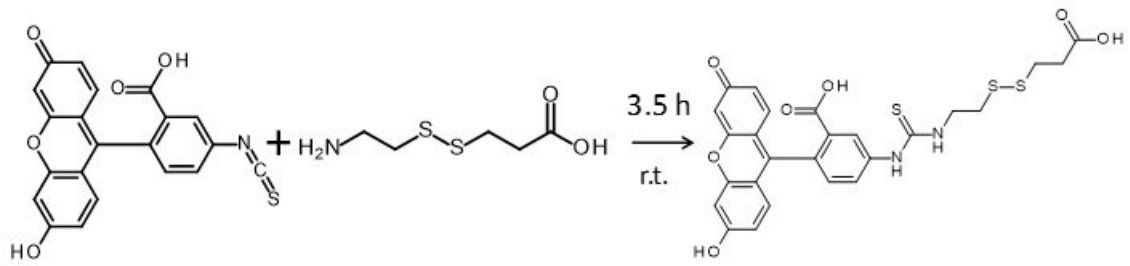

Fig. S2 Synthesis of FITC-S-S-COOH.

Synthesis of AuNPs [3]

AuNPs with a diameter of 13 nm were prepared by reducing $AuCl_4^-$ with citric acid. Aqueous $HAuCl_4$ solution (10^{-2} wt%, 200 mL) was brought to a vigorous boil with stirring and 2 mL of trisodium citrate (3 wt%) was added to the solution. The solution was boiled for another 8 min and the solution changed from pale yellow to a bright red. The solution was cooled to 25 °C with stirring. The Au-colloidal solution was used for the immobilization. The diameter of THE AuNPs was measured with a transmission electron microscope (TEM, JEM2100-M microscope, JEOL Corporation, Tokyo, Japan) at an accelerating voltage of 200 kV.

Quantification of amino groups on a PMMA substrate surface using FITC-S-S-COOH (Fig. S3). [4-6]

Bare and dip-coated PMMA substrates were washed three times with Milli-Q water and immersed in phosphate buffer (0.1 M, pH 8.0, 2 mL) containing 5 vol% DMSO, 0.6 mM FITC-S-S-COOH, and 5 mM 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM), and shaken for 2 h at 25 °C. This allowed FITC-S-S-COOH to bind with the amino groups on the substrate surface. To confirm the amount of non-specific adsorption of FITC-S-S-COOH on the amino group-displayed surface, samples were also prepared without addition of DMT-MM. The substrates were washed three times with phosphate buffer and shaken into an aqueous HCl solution (5 mM, 10 mL) for 1 h at 40 °C. The HCl solution was replaced with an aqueous NaOH solution (5 mM, 10 mL), and shaken for 1 h at 40 °C. The aqueous NaOH solution was then replaced with an

aqueous HCl solution (5 mM, 10 mL) and shaken at 40 °C for 1 h. The washing with the aqueous HCl solution and the aqueous NaOH solution was repeated to solubilize and remove non-specifically adsorbed FITC-S-S-COOH from the surface of the substrate. The substrates were finally washed with phosphate buffer and immersed in phosphate buffer (0.1 M, pH 8.0, 2 mL) containing 2 mM TCEP for 1 h with shaking at 40 °C. This released FITC-S-S-COOH into the solution phase. The fluorescence intensity of the phosphate buffer was measured with a fluorescence spectrophotometer (FP-8200, JASCO, Tokyo, Japan). The measurements were repeated four times and averaged data are presented with error bars (standard deviations).

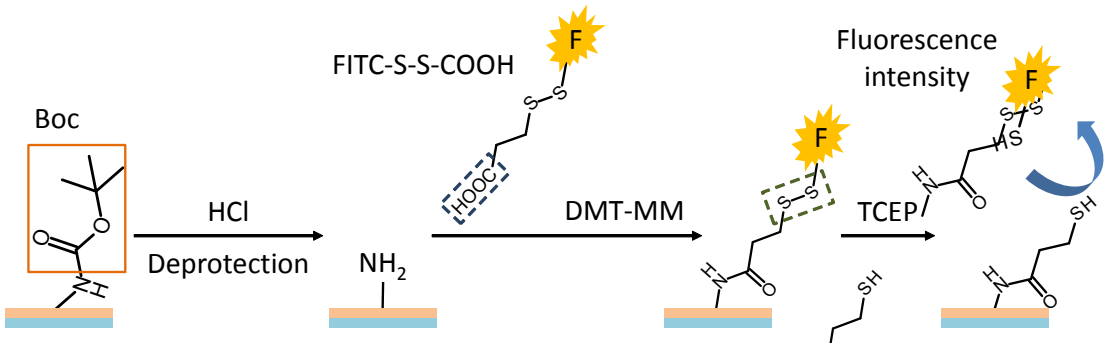

Fig. S3 Schematic illustration of the process to quantify amino groups displayed on the PMMA substrate.

Simultaneous immobilization of SH-DNA and horseradish peroxidase (Fig. 9a)

The AuNPs-immobilized substrates were washed three times with Milli-Q water and immersed in 2 mL of Milli-Q water containing 5 mM *N*-hydroxysuccinimide and 10 mM WSC and shaken for 30 min at 25 °C. The substrates were then immersed in phosphate buffer (0.1 M, pH 7.4, 2 mL) containing 0.3 mg/mL horseradish peroxidase (HRP) and 0.15 M NaCl and shaken for 2 h at 25 °C. The substrates were washed three times with phosphate buffer (0.1 M, pH 7.4) containing 0.05 vol % Tween-20 and 0.15 M NaCl, and washed twice with Milli-Q water. The enzymatic activity of HRP on the surface of the substrate was measured as described above. The acrylic substrates were washed three times with phosphate buffer (0.1 M, pH 7.4) containing 0.15 M NaCl. The substrates were then immersed in 2 mL of phosphate buffer containing 0.15 M NaCl (0.1 M, pH 7.4) and

stored overnight at 4°C in the dark. The substrates were washed three times with sterile water and dried at 25 °C using a vacuum chamber. A solution of reduced 5'-thiolated DNA (2.5 μM, 100 μL) was then loaded as a droplet onto the surface of a substrate. After incubating the substrate for 2 h at 25 °C, the substrates were washed three times with phosphate buffer (50 mM containing 1 M KCl, pH 7.0). The enzymatic activity of HRP on the surface of the substrate was measured using the ELISA POD Substrate A.B.T.S. kit. A 100 μL portion of the reaction solution was periodically withdrawn and transferred to a UV/vis microplate reader to measure the absorbance at 405 nm. The activity of HRP was measured when SH-DNA was present on the surface of the substrate. After measuring the activity, the substrates were washed three times with phosphate buffer (50 mM containing 1 M KCl, pH 7.0). A solution of the complementary DNA strand conjugated to FITC (fluorescein isothiocyanate) at the 5′ end (2 μM in 8 × SSC buffer of 100 μL) was loaded onto the DNA-immobilized surface. The substrate with a fluorescent DNA solution was heated at 80 °C for 10 min and then incubated overnight in a humid chamber set at 40 °C. This procedure was used to perform DNA hybridization. After washing the substrates with excess 8 × SSC buffer, the substrates were immersed in fresh phosphate buffer (0.1 M, pH 7.6, 2.0 mL) for 10 min at 90 °C to dissociate the DNA duplexes. The fluorescent DNA liberated in the supernatant was measured using a fluorescent spectrophotometer (λ_{ex} = 495 nm, λ_{em} = 515 nm) at 25 °C. This allowed us to measure the amount of SH-DNA immobilization when the HRP was present on the substrate surface.

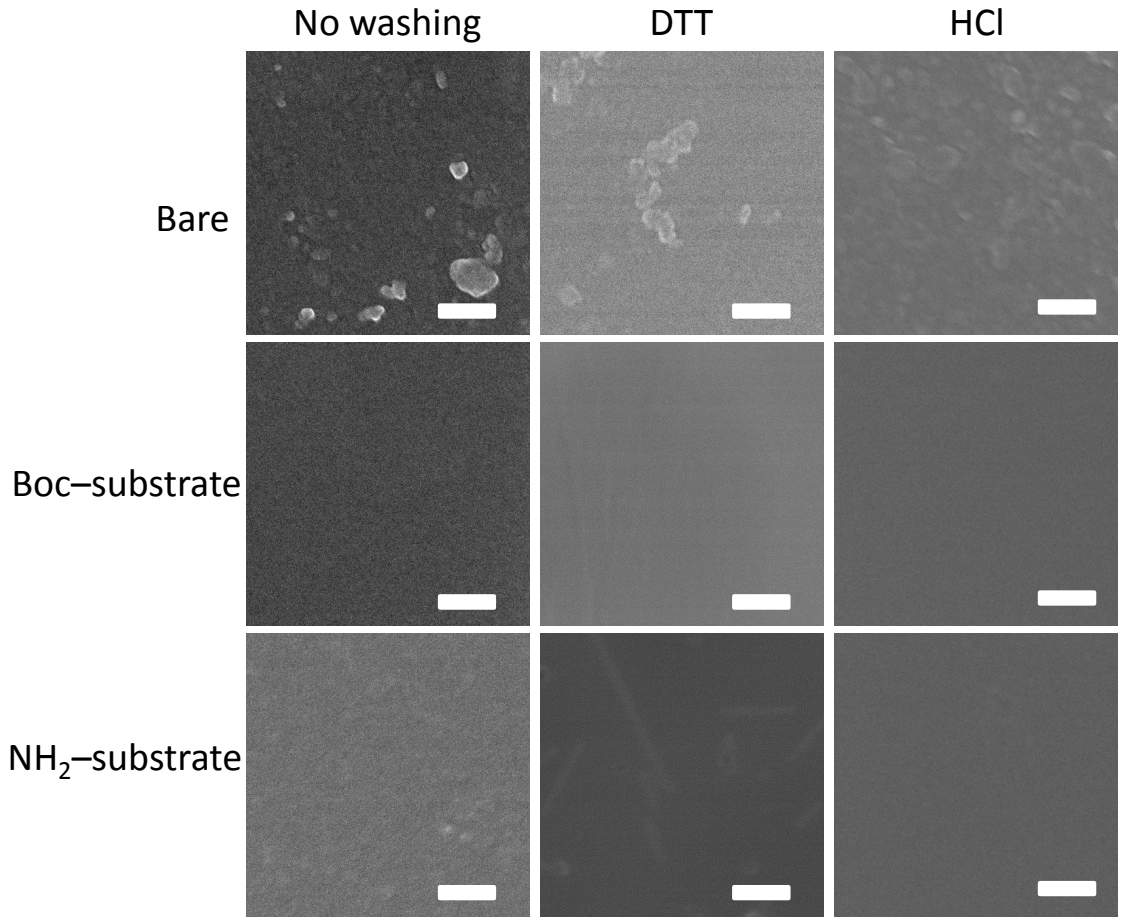

Fig. S4 SEM images of substrates. Scale bars are 200 nm.

Fig. S5 Transparency of an acrylic substrate after dip-coating and deprotection.

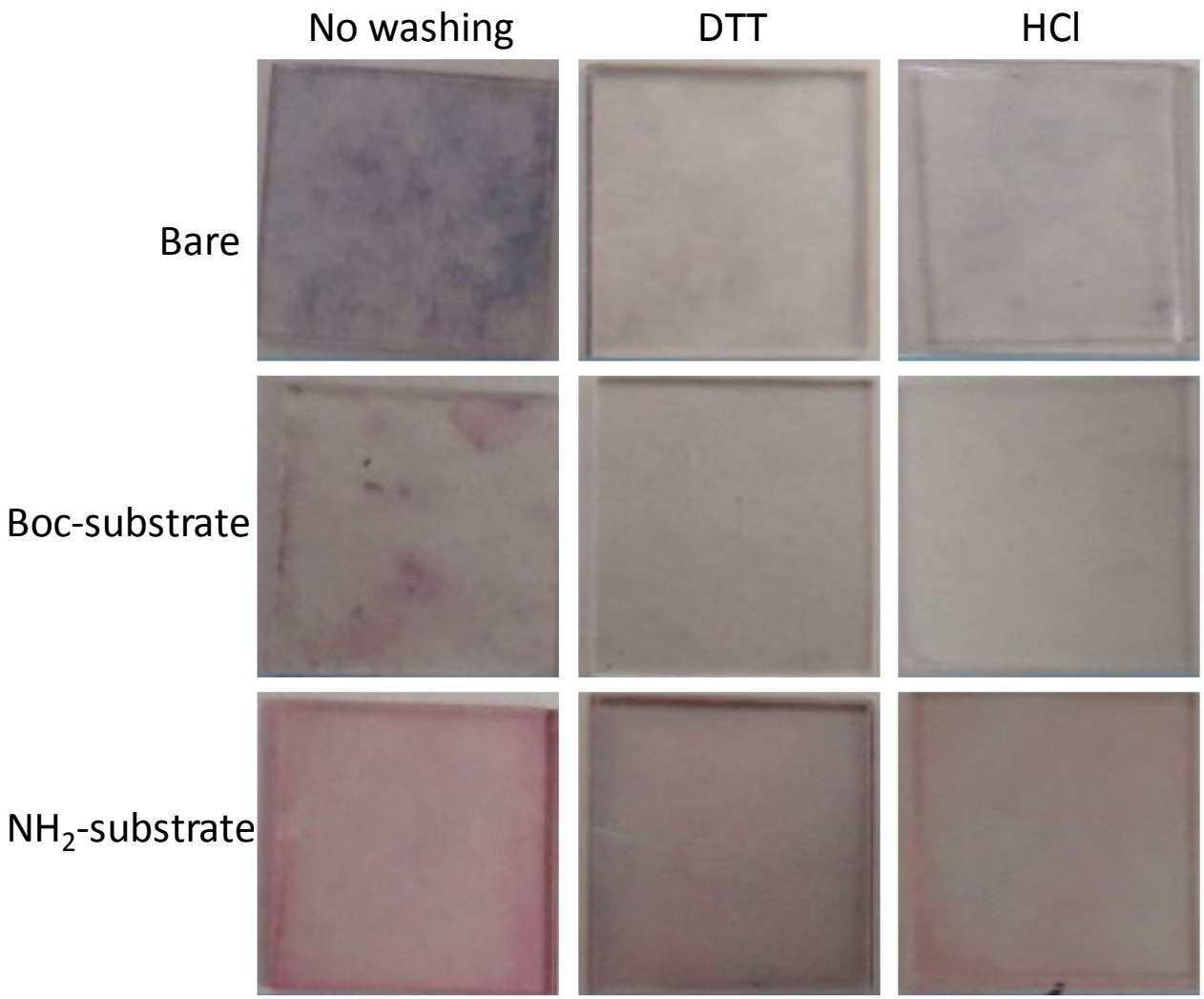

Fig. S6 Photographs of the AuNPs-immobilized substrates (1 cm × 1 cm, 0.5 mm thick). Insets of Fig. 3a.

References for ESI

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