[Supporting Information]

# Rapid construction of an effective antifouling layer on a Au surface via electrodeposition

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#### S1. Materials and reagents

Deionized (DI) water (>18 M $\Omega$  cm) obtained from a purification system (Millipore Synergy, Millipore Co., USA) was used throughout the experiments. The phosphate buffered saline (PBS) consists of NaCl (137 mM), KCl (2.7 mM), Na<sub>2</sub>HPO<sub>4</sub> (10 mM) and KH<sub>2</sub>PO<sub>4</sub> (2 mM) in NaOH at pH 7.4. The chemicals 2amino-2-hydroxymethyl (Tris), 2-(N-morpholino) -propane-1,3-diol ethanesulfonic acid (MES), tetrahydrofuran (THF), 2-(dimethylamino)ethanol (DMAE), and N-(3- dimethylaminopropyl)-N'-ethylcarbodiimide (EDC), N,N.dimethyl-1,4- phenylene diamine (DMPD), triethylamin (TEA) tris(2-chloroethyl) phosphate (TCEP) (Sigma-Aldrich, USA), dichloromethane (DCM), 4dimethylaminopyridine (DMAP), ethyl acetate (EA) (J. T. Baker, USA), fetal bovine serum (FBS) (Gibco, USA) QCM chips (P-chip AU25, ANT Technology Co., Taiwan) were obtained from the indicated suppliers. 6,8-dithooctanate sulfobetaine (HS-SB) and 3-((4-aminophenyl) dimethyl ammonio) propane-1sulfonate (AP-SB) were synthesized in this work (the detailed synthesis protocols are described in **S7** and **S8** of ESI).

#### **S2. Thiol-SAM modification**

Gold electrodes were cleaned with a potential-sweep method<sup>1</sup> each before use. For this treatment the samples were placed in KOH solution (500 mM) and connected to a potentiostat; the electrode potential was swept from 0.2 V to -2 V (vs. Ag/AgCl) twice, at scan rate 0.1 V s<sup>-1</sup>. The samples were rinsed in water (Millipore Co., USA). A SAM was formed on the gold surface on placing it in HSoctane or HS-SB (1 mM) in PBS for 1 h to 3 days at 25 °C. The surface was thoroughly washed with propanone, IPA, and water to remove unreacted species from the surface.

### **S3. Electrodeposition**

A compound with an aminophenyl functional group (i.e. AP-C8 and AP-SB) (10 mM) was solubilized in aqueous HCl (1 M); sodium nitrite (final 10 mM) was added in adequate proportions to generate mono-aryldiazonium. The solution was then scanned with a Au electrode from 0 to -1 V (vs Ag/AgCl) at scan rate

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0.25 V s<sup>-1</sup> for 8 and 24 cycles. Electrodepositions were performed with a potentiostat/galvanostat (660E, CH Instruments Inc., USA) with a three-electrode configuration. Related processes are described in preceding reports.<sup>2, 3</sup>

#### S4. Cyclic Voltammograms (CV)

Cyclic voltammograms were recorded with a potentiostat/galvanostat (660E, CH Instruments, Inc. USA) with a three-electrode configuration. The reference electrode was Ag/AgCl (MF-2042, BASi Inc., USA), and the Pt counter electrode was cleaned in a flame before each experiment. The barrier properties of unmodified and modified gold electrodes were evaluated in a solution of ferricyanide (10 mM) ([Fe(CN)<sub>6</sub>]<sup>3-</sup>/[Fe(CN)<sub>6</sub>]<sup>4-</sup>) dissolved in 1 x PBS (phosphate buffered saline, containing NaCl (137 mM), KCl (2.7 mM), Na<sub>2</sub>HPO<sub>4 (</sub>10 mM), and KH<sub>2</sub>PO<sub>4 (</sub>2 mM), pH 7.2) at scan rate 100 mV s<sup>-1</sup>.

#### **S5. QCM Measurement**

QCM chips (P-chip AU25,  $f_0 = 9$  MHz, ANT Tech. Co., Taiwan) with various antifouling modifications were operated at the fundamental frequency with an ADS analyzer system (ANT Technology Co., Taiwan). The entire system consists of a flow cell, injection valve, peristaltic pump, and Teflon tube (1/16 in) connected to it. In the experiment on nonspecific absorption, the system is filled with the buffer at flow rate 30 mL h<sup>-1</sup>. Until the signal is balanced, a sample (200 µL) was injected into the sensor, followed by rinsing with the original buffer. We used FBS solution (10 %) at variously buffered pH for the measurements. The oscillation frequency shift ( $-\Delta f$ ) is related to the amount of adsorbed mass that was transferred to the accumulated mass change according to the Sauerbrey equation,<sup>4</sup>

$$\Delta f = -\frac{2f_0^2}{A\sqrt{\rho_q \mu_q}}\Delta m$$

in which  $f_0$  = resonant frequency/Hz,  $\mathbb{Z}f$  = frequency change/Hz,  $\mathbb{Z}m$  = mass change/g, A = area of electrodes/cm<sup>2</sup>,  $\mathbb{Z}_q$  = density of quartz, and  $\mathbb{Z}_q$  = shear modulus of quartz.

#### S6. Anti-adhesion images

Red fluorescent protein, mCherry, is produced in the lab and utilized for the antifouling test. The protein in PBS buffer (10 mg/mL) was seeded on micropatterned gold electrodes on a silica substrate. After incubation for 3 h, unattached proteins were removed on gentle washing with the buffer. The adhered proteins produce red emission images with excitation at 500 nm. The images of this excitation and white sidelights recorded at the same position were utilized to identify the relative position of the electrodes and mCherry proteins. All images were recorded with an inverted fluorescent microscope (Axio Scope.A1, Carl Zeiss Co. Germany).

#### S7. Synthesis of thiol-sulfobetaine (HS-SB), compound (III)



**Scheme S1.** Scheme of synthesis of 3-((4-(3-mercaptopropanamido)phenyl) dimethylammonio) propane-1-sulfonate. Reaction condition **(i)**: 3,3'-dithiodipropionic acid (1 eq), N,N.-dimethyl-1,4-phenylene diamine (2 eq), EDC (4 eq) in DCM/THF (1: 1), 4 °C, 12 h, yield = 92 %. Reaction condition **(ii)**: 1,3-propanesultone (2 eq) in DCM, 75 °C, 48 hr., yield =70 %. Reaction condition **(iii)**: TCEP (1.5 eq) in DMSO/H<sub>2</sub>O, 25 °C, 0.5 hr., yield =100 % (directly used without further purification)

#### Synthesis protocol

1. 3,3'-dithiodipropionic acid (5 g, 23.78 mmol), N,N.-dimethyl-1,4- phenylene diamine (DMPD) (6.47 g, 47.56 mmol) was dissolved in THF/DCM (1:1) (50

mL for each) at room temperature under nitrogen.

- After stirring for 30min, EDC (18.2 g, 95.12 mmol) was added in the reaction mixture and the reaction proceeded further under nitrogen at 4 °C for 12 h. The reaction was monitored by TLC. The resulting mixture was purified by column chromatography (60 mesh SiO<sub>2</sub>, with DCM: Methanol 10:1) to yield (92%) compound **(I)** as powder.
- Compound (I) (2 g, 4.48 mmol) and 1,3-propanesultone (1.1 g, 8.96 mmol) were added in THF/DCM (1:1) (50 mL) for further reaction under nitrogen atmosphere at 75 °C for 48 h.
- The reaction mixture was further evaporated to dryness. The resulting precipitate was washed sequentially by dichloromethane (100 mL x3) and methanol (50 mL x3) to remove the unreacted starting material. Compound (II) (2.17 g) was obtained with yield about 70 %.
- Compound (II) (0.5 g, 0.724 mmol) was dissolved in DMSO/water (10 mL for each) under sonication. *Tris*(2-carboxyethyl)phosphine (TCEP) (0.272 g, 1.09 mmol) was added to the solution with vigorously stirring at room temperature for 30 min. The product was diluted to 10 mM as stock solution. The product was directly used without further purification.
- The structure of compound (II) was confirmed by using <sup>1</sup>H NMR, <sup>13</sup>C NMR, and ESI-MS (positive mode). (<sup>1</sup>H NMR (300 MHz, DMSO): δ1.6-1.63 (t, 4H), 2.33-2.38 (t, 4H), 2.76-2.78 (t, 4H), 2.99-3.02 (t, 4H), 3.15-3.16 (m, 4H), 3.52-3.53 (m, 12H), 3.91-3.96 (t, 4H), 7.75-7.83 (m, 8H). <sup>13</sup>C NMR (75 MHz, D<sub>2</sub>O): δ20.31, 33.69, 34.23, 36.3, 36.54, 47.9, 54.38, 68.01, 120.01, 120.95, 122.35, 139.27, 140.62, 170.22 ppm. ESI-MS (*m*/*z*): [M+H]<sup>+</sup> = 691.2. (Fig. S1)



**Fig. S1.** (A) ESI-MS, (B) <sup>1</sup>H NMR, and (C) <sup>13</sup>C NMR spectra of 6,8-dithooctanate sulfobetaine.

S8. Synthesis of 3-((4-aminophenyl) dimethylammonio) propane- 1sulfonate (HS-SB), compound (VI)



**Scheme S2.** Scheme of synthesis of 3-((4-aminophenyl) dimethylammonio) propane-1 - sulfonate. Reaction conditions **(i)**: N,N.-dimethyl-1,4-phenylene diamine (1 eq), di-tert-butyl dicarbonate (1.1 eq), triethylamine (4 eq) , 0 °C ~23 °C, 12 h., 80 %. **(ii)**: 1,3-propanesultone (1 eq) in DCM, 75 °C, 48 h., 75 %. **(iii)**: trifluoroacetic acid (10 %) in DCM, RT, 8 h, 95 %.

#### Synthesis protocol

- N,N.-dimethyl-1,4- phenylene diamine (DMPD) (10 g, 73.45 mmol) was dissolved in dichloromethane (50 mL). Triethylamine (TEA) (29.7 g, 29.39 mmol) was injected into the preceding solution at 0 °C with vigorous stirring.
- 2. After stirring for 10 min, prepared di-*t*-butyl dicarbonate (17.64 g, 80.82 mmol) in dichlorometane (25 mL) was added to the mixture and removed to an ice bath for 12 h. After reaction (monitored with TLC), the mixture was evaporated under vacuum; compound **(VI)** was recrystallized from EtOAc/ hexane to produce needle-like white crystals. Yield 80 %, 13.88 g.
- 3. Compound (VI) (8 g, 33.88 mmol) was following dissolved in DCM (50 mL). 1,3-propanesultone (4.13 g, 33.88 mmol) was added to the reaction and refluxed at 75 °C for 48 h. After the starting material vanished, the entire reaction was cooled to 23 °C and frozen at -20 °C to precipitate the products.
- The precipitate was then filtered and washed with dichloromethane (50 mL, three times) and dried under vacuum. Compound (V) is a pale yellow solid. Yield: 75 % (9.1 g).
- 5. Compound (V) (5 g, 13.96 mmol) was dissolved in DCM (20 mL).

Trifluoroethanoic acid (2 mL, 10 %) was injected into the reaction mixture with stirring for 8 h at 23 °C. After reaction (monitored with TLC), the reaction mixture was evaporated to remove TFA and DCM completely. To the residue was added methanol (20 mL), which was again evaporated to yield compound **(V)** product (pink solid) without purification. Yield: 95 %, (3.42 g).

6. The produced compound (VI) was confirmed with <sup>1</sup>H NMR, <sup>13</sup>C NMR, and ESI-MS. (<sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O): δ1.83-1.98 (m, 2H), 2.83-2.87(t, 2H), 3.67 (s, 6H), 4.04-4.1 (t, 2H), 7.65-7.69 (d, 2H), 7.93-7.96 (t, 2H). <sup>13</sup>C NMR (75 MHz, D<sub>2</sub>O): δ18.92, 46.86, 54.53, 67.63, 122.86, 125.45, 132.26, 143.74 ppm. ESI-MS (positive mode) (*m*/*z*): [M+H]<sup>+</sup> = 259.1.) (Fig. S1)



**Fig. S2.** (A) ESI-MS, (B) <sup>1</sup>H NMR, and (C) <sup>13</sup>C NMR spectra of 3-((4-aminophenyl) dimethylammonio) propane-1-sulfonate.



**Fig. S3.** Comparison of the ESCA spectra of the bare Au (black line) and the SBmodified Au via the electrodeposition method (red line). Differences in the signal intensities of  $Au_{4p}$ ,  $Au_{4d}$ ,  $Au_{4f}$ ,  $O_{1s}$ ,  $N_{1s}$ ,  $C_{1s}$ , and  $S_{2s}$  provide clear evidence of the successful modification of the Au surface with sulfobetaine.

# **Figure S4**



**Fig. S4** CV analyses of bare Au (black), C8- and SB-modified surfaces. Using  $[Fe(CN)_6]^{3-}$  as the redox indicator: (A) and (C). Using RuHex,  $[Ru(NH_3)_6]^{3+}$ , as the redox indicator: (B) and (D). The scan rate is 100 mV s<sup>-1</sup>. Note that C8-modified Au was prepared by electrodeposition using 4-octylaniline as reagent.

# **Figure S5**



**Fig. S5** Real-time recording of the frequency change of QCM measurement in response to FBS (10 %). SB-modified chips prepared by electrodeposition were kept at room temperature for 1 day (red), 7 days (blue), 14 days (green) before QCM analyses. Note that the analysis using bare Au was shown in black and the outcome of 1-day and 7-day chips were nearly identical.



**Fig. S6.** Real-time recording of the frequency change of QCM analyses in responses to FBS (10 %). The outcomes from bare Au, C8-modified and SB-modified chips, prepared by electrodeposition, were shown in black, green and blue, respectively.

## References

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