Supplementary Information

Thiolated 2-Methacryloyloxyethyl Phosphorylcholine for Antifouling Biosensor Platform

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1. Synthesis of MPC-SH

2-methacryloyloxyethyl phosphorylcholine (MPC) was purchased from NOF Co. (Tokyo, Japan). All the other chemicals of extra pure grade were obtained from commercial sources. MPC (14.76 g, 50 mmol) and 1,6-hexanedithiol (11.27 g, 75 mmol) were dissolved in 100 mL degassed chloroform. Michael-type addition proceeded by adding distilled diisopropylamine (278.8 μL, 2.0 mmol) for 22 h at room temperature. We collected the solid product by titrating the solution into acetone. After drying in a vacuum desiccator for overnight, the sample was dissolved in water. MPC-SH was obtained as

white powder after collection and lyophilization. MPC-SH was kept under dry nitrogen until use.

2. ^{1}H-NMR analysis

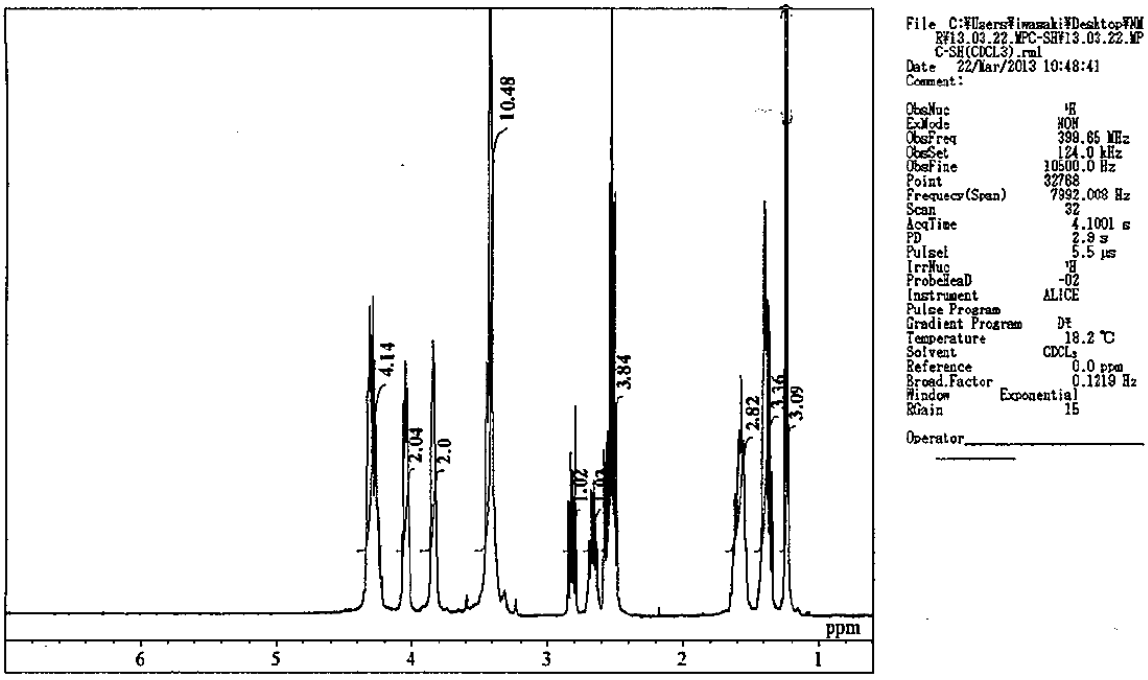

Figure S1. ^{1}H-NMR spectrum (400 MHz, in $CDCl_3$). δ = 1.17-1.28 (d; -CH_3, 3H), 1.36-1.67 (m; $CH_2CH_2CH_2CH_2$, 8H), 2.44-2.88 (m; $HSCH_2$-, 6H, m; $CH_3CH(C(=O)$-$)CH_2$, 1H), 3.23 (s; -$N^+(CH_3)_3$, 9H), 3.58-3.70 (s; -N^+CH_2, 2H), 4.01-4.13 (m; -$(C=O)CH_2CH_2OPO$, 2H), 4.23-4.36 (m; -$(C=O)CH_2CH_2OPOCH_2$-, 4H).

3. ESI-TOF-MS analysis

The end product was characterized using ESI-TOF-MS (Bruker Daltonics microTOF-2 focus spectrometer) in the positive and negative ion detection modes.

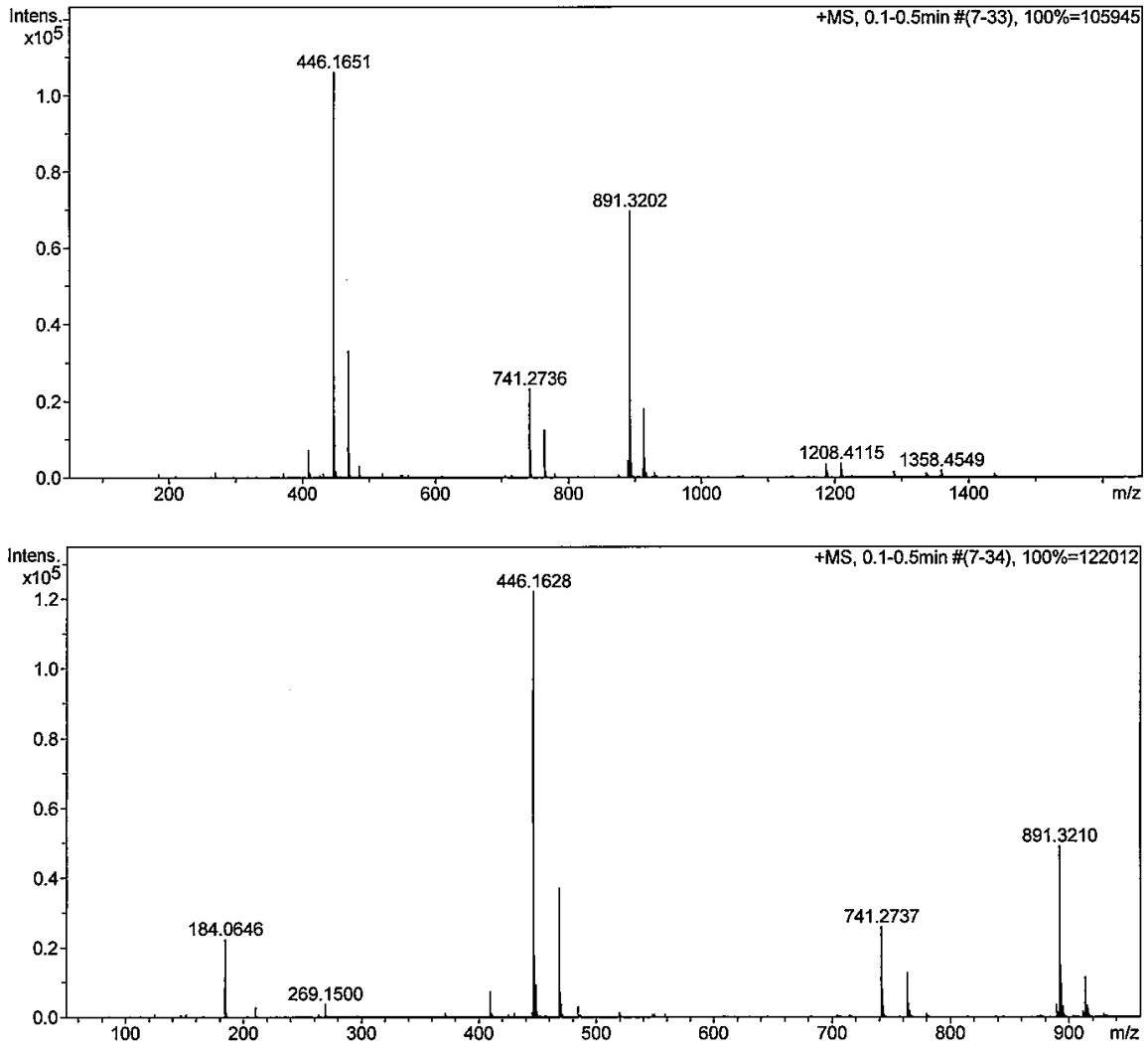

Figure S2. ESI-TOF-MS spectra (upper: negative mode, lower: positive mode) for the reaction product of MPC and 1,6-hexanedithiol. m/z = 446.16 (MPC-S-$(CH_2)_6$-SH: MPC-SH), 741.27 (MPC-S-$(CH_2)_6$-S-MPC: side product), 891.32 (MPC-S-$(CH_2)_6$-S-S-$(CH_2)_6$-S-MPC, disulfide dimer of MPC-SH).

4. SAM formation

Gold electrodes were cleaned by 1 M NaOH and 1 M HCl for 5 min each prior to use. Then, SAM was formed on the gold surface by placing in 1 mM MPC-SH in water for 24

h at 25 °C. Thereafter, the surface was washed by water thoroughly to remove unreacted species from the surface.

5. Cyclic Voltammetry (CV) for determining SAM packing density

Surface density of SAM was determined by CV using Autolab PGSTAT 302 (Eco Chemie, Utrecht, The Netherlands) with three-electrode system of platinum wire as a counter electrode and Ag/AgCl in saturated KCl solution as a reference electrode. CV was from −0.2 to −1.3 V (vs. Ag/AgCl) and then from −1.3 to −0.2 V at the scan speed of 0.5 V/s in 0.5 M KOH in a 3.3 M KCl. The solution was purged with nitrogen for 30 min prior to use. A clear reduction peak at −0.8 to −1.0 V in the first scan was attributed to charge transfer from the electrode to the thiol during the reductive desorption. Thus, the number of alkanethiol per unit area (γ, nm^{-2}) was calculated as follows:

$$\gamma = Q_{SAM} N_A / F \qquad \text{(S1)}$$

where Q_{SAM} is the total surface charge density determined by the peak area, scan speed, and electrode surface area and F is Faraday constant. Very small peaks were observed in the first oxidative scan and there were no significant redox peaks at the same position as the first scan in the subsequent cycles. These indicate that detachment of MPC-SH SAM from the Au electrode in the first reductive scan is irreversible in the alkaline measurement solution because MPC-SH is water-soluble, and aqueous MPC-SH did not reassemble onto the electrode to form the fully packed monolayer again. The indistinctive peaks in the second and third CV scans might represent redox reactions of some moieties in the dissolved MPC-SH molecule.

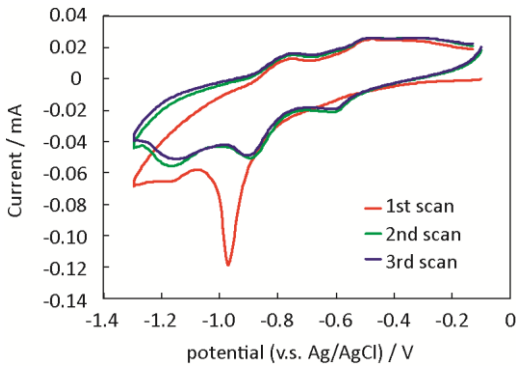

Fig. S3 Cyclic voltammograms for determining the surface density of MPC-SH SAM on gold.

6. Electrochemical Impedance Spectroscopy (EIS)

The capacitance (C) and charge transfer resistance (R_{ct}) were determined by EIS (Autolab PGSTAT 302, Utrecht, the Netherlands) with the three electrode system in 150 mM Dulbecco's PBS containing 5 mM ferrocyanide/ferricyanide ($Fe(CN)_6^{3-/4-}$) or 5 mM Ruthenium hexammonium chloride ($Ru(NH_3)_6^{2+/3+}$) as a redox couple. The sweep scans ranged from 1 Hz to 10 kHz at 10 points per decade at DC bias voltage of +0.2 V (vs. Ag/AgCl) at AC amplitude of 50 mV. Bundled frequency response analyzer was used to determine each component by curve fitting using Randle's equivalent circuit model of $R_s(Q[R_{ct}W])$, where R_s, Q, and W stand for the solution resistance, constant phase element (CPE), and Warburg impedance, respectively.

The electron transfer rate (k_{et}) was calculated using the Butler−Volmer equation with the obtained R_{ct} as follows:

$$i_0 = FAk^0 C^*_O{}^{(1-\alpha)} C^*_R{}^{\alpha} \qquad \text{(S2)}$$

$$R_{ct} = RT / nFi_0 \qquad \text{(S3)}$$

where i_0 is the exchange current density (A/cm^2), A the electrode active surface area (cm^2), k_0 the standard heterogeneous rate constant (cm/s), C^*_O and C^*_R, respectively, the bulk concentration of oxidized and reduced form of the electro active species (mol/cm^3), α the transfer coefficient (estimated to be 0.5), R the universal gas constant (≈ 8.31 J K^{-1} mol^{-1}), T the absolute temperature (K) and n the number of electrons transferred in the electrochemical reaction.

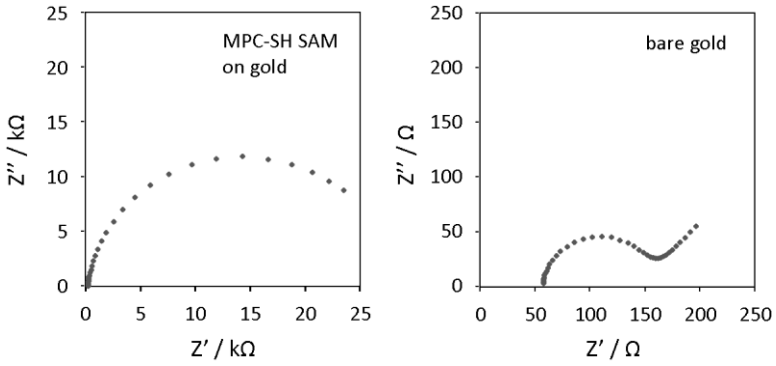

Figure S4. Nyquist plots for MPC-SH SAM on gold and bare gold electrodes using $Fe(CN)_6^{3-/4-}$.

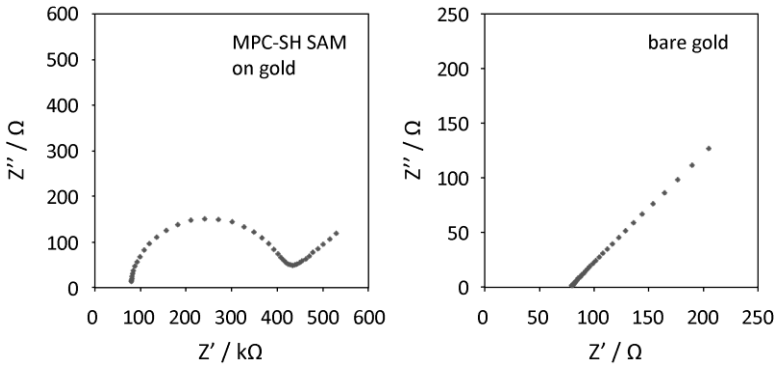

Figure S5. Nyquist plots for MPC-SH SAM on gold and bare gold electrodes using $Ru(NH_3)_6^{2+/3+}$.

Table S1. Double layer capacitance (C or CPE), R_{ct}, and k_{et} determined by the curve fitting on Nyquist plots using Randle's model (mean ±sd, n = 4).

Entry	C (CPE) / μF cm^{-2}	R_{ct} / Ω cm^2	k_{et} / 10^{-5} cm s^{-1}
MPC-SH SAM on gold, $Fe(CN)_6^{3-/4-}$	11.9 ±0.5	4830 ±590	1.11 ±0.14
bare gold, $Fe(CN)_6^{3-/4-}$	153 ±46	19.8 ±8.9	356 ±266
MPC-SH SAM on gold, $Ru(NH_3)_6^{2+/3+}$	12.5 ±2.6	72.6 ±44.6	95.9 ±48.1
bare gold, $Ru(NH_3)_6^{2+/3+}$	728 ±110	0.46±0.19	16200 ±14400

7. QCM Measurement

A gold-coated quartz sensor (f_0 = 30 MHz) was operated at the fundamental frequency (no overtone) using the analyzer (Nihon Dempa Kogyo, Tokyo Japan) in an injection flow system at 25.0 ±0.02 °C. A 200 μL-protein solution were injected to the sensor at the flow rate of 50 μL/min, followed by the rinse with 1x Dulbecco's PBS (150 mM, pH 7.4). We used a 1.0 mg/mL BSA, 0.3 mg/mL fibrinogen, and 10% FBS solution in the buffer for the measurements. The QCM sensor tracks changes in the oscillation frequency of the piezoelectric crystal ($-\Delta f$), which is related to the amount of adsorbed mass per unit area (Γ) of the sensor represented by Sauerbrey equation as

$$\Gamma = (-\Delta f)(\rho_q \mu_q)^{1/2} / 2Nf_0^2 \qquad \text{(S4)}$$

where N, f_0, ρ_q, and μ_q represent the overtone number, resonance frequency, density of quartz, and shear modulus of quartz, respectively.

8. Potentiometric Measurement

We used 10-round-shape gold microelectrodes (500 μm in diameter) on a rectangular glass-epoxy chip (Towa Tech, Shizuoka, Japan). Wiring part was completely covered by insulating SU-8. Each electrode was connected to Keithley 6517B electrometer/high resistance meter (Cleveland, OH, USA) through a switching circuit. Time course of the interface potential was monitored using home-built data logger at sampling rate of 0.1 Hz under no DC bias potential versus Ag/AgCl reference electrode at 25 °C. The 100 μL-protein solutions were allowed to contact with the gold electrode. The protein concentration was 1.0 mg/mL for BSA, 0.3 mg/mL for fibrinogen, and 10% FBS in the buffer.

9. Cell Imaging

We used HepG2 cells for the cell attachment tests. The cell suspension (3 mL) were seeded on micropatterned gold electrodes on a glass substrate at 10^5 cells/mL in DMEM supplemented with 10% serum (FBS). After incubation for 6 h, unattached cells were removed by gently washing with the buffer. Thereafter, the adhered cells were stained with DAPI for imaging. Bright field and fluorescent images were taken at the same position using an upright fluorescence microscope (Eclipse, Nikon, Tokyo, Japan).

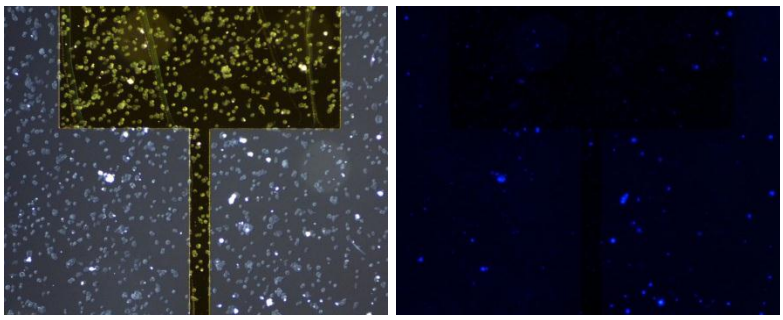

Figure S6. A direct comparison of adhesion of HepG2 cells on a glass with that on a bare gold electrode using a micropatterned gold electrode on the glass substrate; bright field (a) and nuclei-stained (b) images at the same position. No applied DC bias voltage. Magnification x4.