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Electrical Visualization of Chemo-mechanical Signal Transduction Using a Smart Gelmodified Gate Field Effect Transistor

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Detailed Method

Materils. N-ispropylacrylamide (NIPAAm) (Kojin, Japan) was recrystallized in *n*-hexane. 3-(trimethoxylsilyl)propylmethacrylate and 2-(methacryloyl)ethylphosphate (MEP) were purchased from Sigma-Aldrich Japan (Japan) and used as received. N,N'-methylenebisacrylamide, 2,2'-dimethoxy-2-phenylacetophenone, 11-amino-1undecanethiol, and acryloyl chloride were all purchased from Wako (Japan) and used without further purifications. Poly(methylmethacrylate) (PMAA) micro particle (average particle size: 8 μ m) was purchased from Sekisui Plastics (Japan) and used as received. Ion-selective FETs (ISFETs) were purchased from BAS Inc., the gate insulator of which is a double layer of SiO₂ and Ta₂O₅.

Introduction of calcium-responsive gel to FET gate surface. Ion-sensitive FET (ISFET, BAS Inc.) was first plasma-cleaned in an oxygen plasma reactor (PR500, YAMATO) for 90 s at a power of 300 W under an oxygen pressure of 200 Pa. It was then immersed in a 2 wt% ethanol solution of 3-(trimethoxylsilyl)propylmethacrylate and kept overnight at room temperature. After rinsing with pure ethanol, it was dried in vacuo at 120°C for 2 h to allow the siloxane condensations. Thus, reactive vinyl groups were introduced to the Ta₂O₅ insulator surface, to which a calcium-sensitive gel layer was to be covalently attached. Each monomer - NIPAAm (0.226 mg), MEP (420 mg), N,N'- methylenebisacrylamide (12 mg), and a photo-sensitizer 2,2'-dimethoxy-2- phenylacetophenone (26 mg) - was dissolved in 1 mL ethanol and bubbled with nitrogen gas for 5 min. This solution was inserted by pipette into a space between the gate and a coverslip tightly placed on the surface. Photopolymerization was conducted by irradiating UV light through the coverslip for 150 s. After the reaction, the coverslip was carefully removed and the fabricated gel layer was thoroughly washed with ethanol and then water.

Phase diagrams of calcium-responsive gel. For this experiment, cylindrical poly(NIPAAm-co-MEP) gels were prepared via UV-irradiated radical copolymerization in the same way described above but the reactions were allowed in 10 mm-diameter glass capillaries for 24 h. Three different (NIPAAm/MEP) molar compositions, namely, 30/70, 50/50, 70/30 in feeds were prepared (see SI Figure 1). The obtained gels were taken out of the capillaries and thoroughly washed with ethanol and then water. For the temperature control, a water-flow chamber of transparent acrylic resin with cavities on top was placed on the microscope stage. The gel capillaries that were cut into 5 mm long (on shrunk state) pieces were placed in the chamber cavities filled with each experimental buffer solution. The equilibrium volume changes upon decreasing the temperature were recorded for various calcium concentrations to obtain the phase diagrams.

Capacitance measurements of NB10. For the capacitance measurement, the poly(NIPAAm-co-MEP) [50/50] gel was covalently introduced to a gold electrode surface via formation of alkane-thiol self-assembled monolayer (SAM). Gold electrode $(4 \text{ mm} \times 4 \text{ mm})$ was fabricated by sputter deposition of an adhesion layer of nickel-tungsten alloy and then a gold layer (100 nm, 99.99% purity) on a silicon substrate. The electrode was cleaned with 1 M NaOH for 10min. After brief rinses with water and ethanol, it was immersed in a 10 mM ethanol solution of 11-amino-1-undecanethiol and incubated for 24 h at room temperature. It was then rinsed and sonicated in ethanol for 5 min, again rinsed with ethanol and finally dried in vacuo. The amine-terminated SAM surface was further reacted with acryloyl chloride for 1 h at room temperature for the introduction of vinyl groups. After ethanol rinses, the gel layer was formed on the surface via an identical procedure conducted for the FET gate surface modification. Changes in the gel capacitance when inducing the Ca²⁺ induced shrinkage were measured using a precision impedance analyzer (4156C, Agilent) with an Ag/AgCl reference electrode in a saturated KCl solution.

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Preparation of micro-porous gel on gold extended FET gate. A micro-porous gel was formed on a flat gold electrode surface, which was electrically lined to an FET gate. In this configuration, the gel-modified electrode served as a so-called extended gate for the FET. Procedures for fabrication and the surface treatment of the gold electrode were identical to the methods described above. A 50-µm-thick layer of closest-packing colloidal crystals composed of PMMA sphere particles (with average particle size of 8 µm) was fabricated on the surface of a coverslip. This was utilized as a template for the fabrication of a micro-porous gel structure. A coverslip was first cleaned by sonications in an alkaline solution using ExtranR MA 01 alkaline (Merck) and then 1 M HCl, thereafter rinsed with pure water and finally dried at 120 °C. This coverslip and a 12 mm diameter silicone ring were plasma-treated, first separately and then in contact with each other leading to a tight physical attachment between the two pieces. PMMA particles were dispersed in a 50/50 (v/v) mixed solvent of water and ethanol. This suspension was added to the coverslip ring area stepwisely in portions of 100 µL to an extent obtaining the resultant 50 µm layer thickness on dried, closely packed state. The gelation was conducted in a similar manner to that described above except that it was carried out in the presence of the PMMA-templated coverslip. After the gelation, PMMA particles were thoroughly removed by immersing the electrode in toluene for 24 h. A Scanning Electron Microscopy (SEM) image showing the resultant micro-porous morphology of the gel is displayed in SI Figure 2.

Calcium detection by the gel modified FET. Each prepared FET was immersed in a 50 mM Tris buffer solution adjusted to pH7.4 (I = 0.15M). The calcium concentration of the milieu was controlled by additions of CaCl₂. All measurements were carried out at 35°C with an Ag/AgCl reference electrode in saturated KCl solution. Changes in the threshold voltage (V_T) were monitored under the conditions of constant gate voltage at 0 V, drain

voltage at 1 V, and drain-source current at 700 mA, using BioFET analyzer (GFS-301-4CH, Radiance Ware Inc.).



ESI Figure 1. Phase diagrams of poly(NIPAAm-co-MEP) gel for various compositions. With increase of the NIPAAm content, the copolymer gel tends to show more temperature-dependent volume changes due to a thermo-sensitive nature of the NIPAAm. In the present study, to minimize a potential uncertainty related to the temperature fluctuation (especially at around the range of the physiological condition) the NIPAAm molar content has been optimized at 50%.



ESI Figure 2. A SEM (Scanning Electron Microscopy) image showing a micro-porous gel structure prepared by templated gelation in the presence of PMMA sphere particles (with average particle size of 8 μm).



ESI Figure 3. **a**, Time course changes of threshold voltage (V_T) of a calcium-responsive gel modified FET (purple line) and a control FET without the gel modification (light green line) with decreased calcium concentration investigated at 35 °C (pH = 7.4, I = 0.15M). **b**, Schematic illustration of two stage model for V_T changes.



ESI Figure 4. Capacitance change of the calcium-responsive gel in the course of shrinkage on adding 100 mM calcium in a buffer solution (pH = 7.4, I = 0.15M) investigated at 35 °C.