Electronic Supporting Information

Electrical Monitoring of Human-serum-albumin-templated Molecularly Imprinted Polymer Nanoparticles with High Affinity Based on Molecular Charges and Its Visualization

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Chemicals

Sodium tetrahydroborate ($NaBH_4$), ethanolamine, human serum albumin (HSA), glycated albumin (GA), 1X phosphate buffer saline (PBS), and glutaraldehyde were purchased from Tokyo Chemical Industry Co., Ltd. Potassium chloride (saturated solution), potassium dihydrogenphosphate, dipotassium hydrogenphosphate, 3,4-dihydroxyphenethylamine hydrochloride (dopa-HCl), sulfuric acid (H_2SO_4), sodium hydroxide (NaOH), acetone, toluene anhydrous, aminopropyltrimethoxysilane (APTMS), N-isopropylacrylamide (NIPAAm), N,N'-methylenebisacrylamide (BIS), polyoxyethylene sorbitan monolaurate (Tween 20), N-tert-butylacrylamide (TBAm), ethanol, acrylamide (AAm), N,N,N',N'-tetramethylethylenediamine (TEMED), and ammonium peroxodisdulfate (APS) were purchased from Wako Pure Chemicals Industries. Tween-20 was purchased from Kanto Chemical Co., Inc. Epoxy resin was purchased from PELNOX. Spherical glass beads with diameters ranging from 63 m to 88 m (SGMT No. 007) were purchased from Toshinriko Co., Ltd. GA, which was purified from human serum, was also supplied by PROVIGATE Inc.

Chemical modification of extended Ta_2O_5 gate electrode (Fig. S1)

Commercial planar Au substrates (Kyodo International, Inc.) with chromium as an adhesion layer were prepared. A layer of Cr was sputtered for 4 min, followed by Ta_2O_5 for 3 h using a sputtering system (ULVAC, Inc.). The substrates were then immersed in 1 mM dopa-HCl (pH 8, in PBS) and left at room temperature for 4 h under dark conditions. This process is carried out to introduce primary amine groups. The substrates were then treated with 5 mM glutaraldehyde for 1.5 h. This chemical is used to introduce aldehyde groups. Subsequently,

the substrates were treated with 0.5 mg/mL HSA (in PBS) for 1.5 h. GA was used as control and prepared in the same manner. The substrates were then treated with 1 M ethanolamine for 40 min. This process is carried out to block the unreacted aldehyde groups. The substrates were then treated with 1 mg/mL $NaBH_4$ (in PBS) for 30 min to reduce the Schiff bases. Then, epoxy resin was added to 400 μL of confined rings. These rings were then attached to the modified regions of the substrates. The substrates were then baked in an oven at 120 ˚C for 4 h to cure the epoxy resin. These rings can prevent contact between the solution and the Au region.

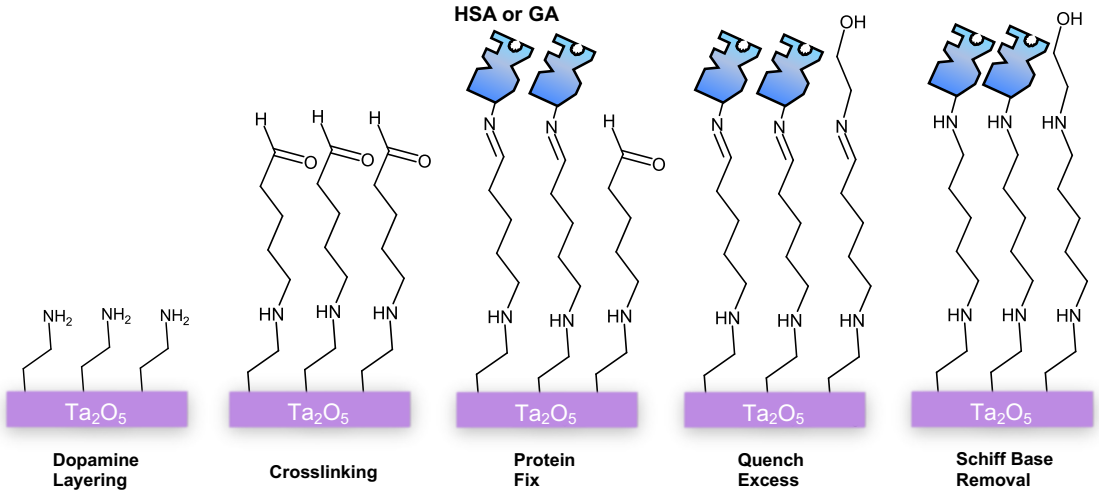

Fig. S1 Scheme of chemical modification of Ta_2O_5 gate surface.

Characterization of modified electrodes by X-ray photoelectron spectroscopy (XPS)

The modified Ta_2O_5 gate electrodes were characterized by XPS (PHI 5000 VersaProbe III, Chanhassen, MN, USA). Substrate samples were prepared at four typical phases of the chemical modification: before and after polydopamine coating and before and after HSA immobilization. All samples were characterized over 10 cycles. These cycles included five

scans of N 1s, a single scan each of C 1s and Ta 4f, and 10 scans of S 2p with a pass energy of 27 eV. Peak positions were calibrated against adventitious carbon (284.5 eV). To further investigate how these phases affect the chemical states of the samples, the peaks observed in the Ta 4f and N 1s regions were fitted using Gaussian functions.

Real-time analysis of nano-MIP-HSA binding

The device was set up as follows. The extended Ta_2O_5 gate electrode with HSA or GA was immersed in the measurement solution. The Au region of the extended electrode was connected to the gate of a metal oxide semiconductor MOSFET (Toshiba). The drain and source terminators of the MOSFET were connected to a potentiostat. A Ag/AgCl reference electrode was then immersed in a saturated KCl solution, with the other end connected to the potentiostat. Finally, the circuit was completed by placing a salt bridge, with one end in the measurement solution, and the other end in the saturated KCl solution. The nano-MIP stock solution was prepared as follows. The solvent for nano-MIPs was prepared (PBS/T): 250 mg of Tween-20 was weighed and added to 500 mL of Gibco 1X PBS to control the solubility of nano-MIPs. Before measurements, all the electrodes were conditioned by immersing them in the PBS/T solution and allowing them to stand overnight. This conditioning step stabilizes the electrodes through their interactions with the PBS/T solution, ensuring a consistent performance during experiments. Then, the nano-MIP stock solution was prepared: a tube with nano-MIP solid was prepared to which the prepared solvent was then added to prepare a 40 μg/mL nano-MIP stock solution. This nano-MIP stock solution was then sonicated in an ultrasonic bath for 30 min. Sensorgrams were then recorded with PBS/T as the initial

measurement solution. The PBS/T solution was then replaced with the nano-MIP stock solution to prepare nano-MIP solutions with concentrations of 0.625, 1.25, 2.5, 5.0, 10.0, and 20.0 μg/mL. This replacement was started only after the baseline sensorgram was obtained. Each experiment was conducted in triplicate (N=3) to ensure reliability and reproducibility, that is, three substrate samples were measured. GA-immobilized extended electrodes were also prepared in the same manner as the control. Experiments on the control electrodes were also conducted in the same manner as described in this section. The pH of the nano-MIP bulk solution was measured at 7.58, and the pH of the solution with an HSA-immobilized electrode (PBS/T) was 7.54. That is, the effect of pH on the output signal upon adding the nano-MIP solution in the measurement solution was negligible.

Visualization of nano-MIP-HSA binding using CMOS array biosensor

We used the CMOS array device, which was developed and reported in a previous paper.[S1] The details such as the device structure and the sensor readout circuit are found in that previous report. Moreover, the principle of biomolecule detection by the CMOS array device follows that by the FET biosensors, which is based on the detection of a change in the amount of molecular or ionic charges at the gate electrode surface. The CMOS array device (image sensor) with a 256 × 256 pixel array was used in this study, as shown in **Fig. S2**. The Ta_2O_5 gate electrode was chemically modified using the same procedure as described in the above section. An HSA-immobilized array (experimental) and a GA-immobilized array (control) were fabricated. The nano-MIP stock solution was also prepared using the same procedure as described in the above section. After all the sensors were conditioned, following the same

method as that for the extended gate electrodes mentioned in the previous section, the measurements were then recorded using PBS/T as the initial measurement solution. This PBS/T solution was then replaced with the nano-MIP stock solution to prepare nano-MIP concentrations of 10.0 and 20.0 μg/mL. Data were exported as voltage signals from each pixel over a total duration of 600 s. All voltage signals were normalized to start from 0 V. Heatmap images of both HSA-immobilized and GA-immobilized arrays were constructed using the voltage signals at 0, 300 (with 10 μg/mL nano-MIPs), and 600 s (with 20 μg/mL nano-MIPs).

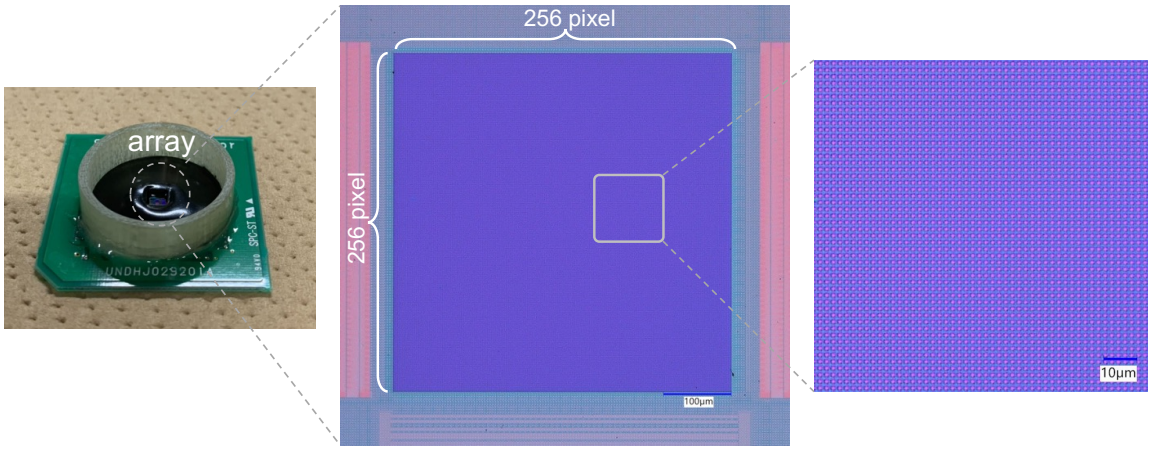

Fig. S2 Image of CMOS array biosensor with a 256 × 256 pixel array.

Contextualization of previous work[S2]

Preparation of the HSA-immobilized glass beads as solid support (Fig. S3)

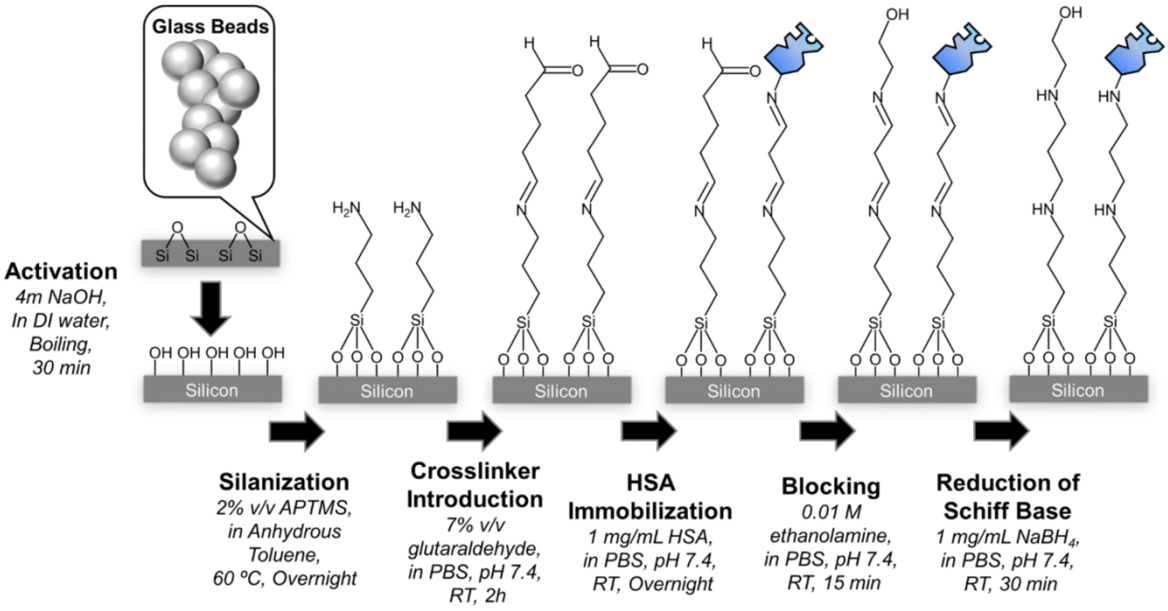

Fig. S3 Preparation of the solid support (HSA-immobilized glass beads)

Fig. S3 illustrates the process for preparing HSA-immobilized glass beads. Firstly, bare glass beads (200 g) were activated by boiling in NaOH (4 M) for 30 min in a 1000 mL Florence flask on a hotplate with temperature set up to 300 °C. After 30 min, the flask was cooled down for 15 min to avoid cracking when washing. Then the activated glass beads were washed thoroughly with Milli-Q water, each wash consisting of 400 mL and repeated eight times. Subsequently, the glass beads were incubated in 20 % (v/v) sulfuric acid solution for 60 min. This process is for converting sodium silicate (Na_2SiO_3) to silicon dioxide (SiO_2), which provides a more control and well-defined surface functionalization. The beads were then washed thoroughly with PBS (to neutralize the acid), Milli-Q water, sequentially. The beads were then transferred in a suction funnel and the water was replaced with acetone. The

beads were thoroughly washed with acetone, each wash consisting of 300 mL and repeated three times. This process is for the later drying because acetone evaporates faster. The beads were then dried in an oven at 120 °C for 30 min, placed in a 500 mL glass bottle and treated with 2 % (v/v) APTMS in anhydrous toluene (100 mL) overnight at 60 ºC with stirring (170 rpm) in a shaker. This process is for grafting primary amine groups onto the glass beads. The glass beads were washed thoroughly with acetone and dried in the oven at 120 °C for 30 min. The glass beads were then transferred into a 500 mL glass bottle with a funnel and incubated with a 100 mL solution of glutaraldehyde in PBS (7 % (v/v), pH 7.4) for 2 h at room temperature with stirring (170 rpm) in a shaker. This process is for introducing functional aldehyde groups onto the glass beads. The glass beads were then thoroughly washed with Milli-Q water, each wash consisting of 400 mL and repeated 5 times. The glass beads were then separated into two halves (each 100 g). 100 g glass beads were incubated with HSA (1 mg/mL) in PBS (100 mL) with stirring (170 rpm) overnight at room temperature (HSA (+) for experimental group). The other 100 g glass beads will be used as negative control group (HSA (-) for negative control). After the HSA (+) and HSA (-) beads were rinsed with Milli-Q water, the remaining unreacted aldehyde groups were blocked using ethanolamine in PBS (0.01 M, pH 7.4) for 15 min, followed by the reduction of the Schiff base using $NaBH_4$ in PBS (1 mg/ml, pH 7.4) for 30 min.

Synthesis of the HSA-imprinted nano-MIPs (Fig. S4)

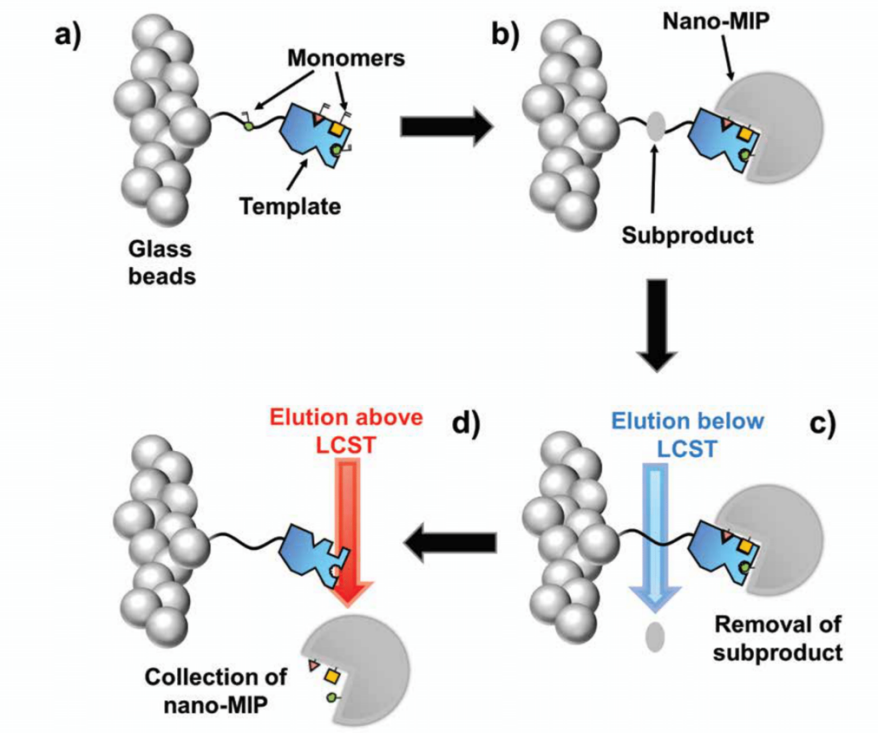

Fig. S4 Solid-phase synthesis process of the HSA-immobilized nano-MIPs.

Fig. S4 illustrates the process for HSA-imprinted nano-MIP preparation, adapted from the protocol.[S3] NIPAAm (294 mg), TBAm (248 mg), AAm (138 mg), and BIS (40 mg) were weighed and transferred to a 200 mL glass bottle. Then 95 mL Milli-Q water was added and then the bottle was placed in ultrasonic bath for 5 min to thoroughly dissolve all monomers and crosslinker. Meanwhile, 100 g HSA (+) glass beads were weighed and transferred to another 200 mL glass bottle. Then, the 95 mL monomer and crosslinker solution was mixed with the glass beads. The glass bead mixture was then sonicated for 5 min and bubbled with nitrogen gas for 30 min. Then, APS (27 mg) was weighed and dissolved in 5 mL Milli-Q water with TEMED (15 uL). Then, the 5 mL APS and TEMED solution was poured into the glass bead mixture to initiate polymerization. The polymerization was allowed to proceed at room temperature for 2 h. After the polymerization is done, the glass bead mixture was

transferred to a fritted solid-phase extraction (SPE) cartridge and washed thoroughly with Milli-Q water, each wash consisting of 300 mL and repeated three times. This process is for removing all the subproducts. Then, the SPE cartridge was fixed on a stand and placed in a water bath preset at 60 °C with Milli-Q water prepared at 60 °C as well. 25 mL of 60 °C Milli-Q water was transferred to the SPE cartridge. The glass beads were then treated with it for 15 min with the bottom of the SPE cartridge capped. This process is for harvesting the nano-MIPs from the glass beads. Then the SPE cartridge was placed on a vacuum bottle to collect the nano-MIP suspension by connecting the vacuum bottle to a vacuum pump through suction filtration. This process from treating the glass beads with Milli-Q water to suction filtration was repeated 4 times. Thus, 100 mL nano-MIP suspension was collected. The 100 mL nano-MIP suspension was then divided into five 20 mL aliquots, transferred into falcon tubes and treated with liquid nitrogen until they are thoroughly frozen. The falcon caps were then punched with holes and these tubes were put in a desiccator, connecting to a cool trap and a pump. The pumping ran for two days, and the resultant nano-MIP solid can be seen. Additionally, to prepare non-imprinted polymer nanoparticles (nano-NIPs) as control, HSA (-) glass beads were dealt with in the same manner mentioned above.

Preparation of surface plasmon resonance (SPR) sensor chip

Silicon substrates were sectioned into approximately 1 × 1.8 cm^2 pieces and subsequently cleaned using acetone and methanol. Chromium and Au layers were deposited on these substrates using a sputtering system (ULVAC, Inc.). The Au substrates were then immersed in an ethanol solution containing 5 mM cysteamine and left at room temperature for 24 h.

The substrates were then treated with 5 mM glutaraldehyde for 1.5 h. Afterward, nano-MIPs were prepared in a pH 6 phosphate buffer at 0.1 mg/mL and introduced to the substrates, subject to interaction for 24 h.[S4]

Evaluation of the affinity for HSA using SPR

A buffer solution was prepared by mixing 5 mM PBS (pH 7.35) with Tween 20. To evaluate the affinity of nano-MIPs for HSA, HSA solutions of various concentrations (0, 12.5, 25.0, 50.0, and 100.0 ng/mL) prepared in this buffer were continuously introduced to the sensor chip at a flow rate of 20 μL/min, each for a duration of 20 min. To remove any unbound HSA or unspecific bindings after each introduction, the sensor chip was rinsed with 4-minute injections of the buffer solution. Nano-NIPs were also evaluated using the same method.

Affinity analysis (Fig. S5)

The nano-MIP-HSA response curve exhibited increasing spikes in SPR response with HSA introductions, indicating interactions between the HSA and the sensor chip, as shown in **Fig. S5** (a). As shown in **Fig. S5** (b), the SPR responses at equilibrium in each dissociation phases during buffer washing were plotted against the corresponding concentrations and then fitted to a Langmuir isotherm model:

$$\theta = \frac{\theta_{MAX}[c]}{K_d + [c]} \quad (1)$$

where θ is the SPR reponse, θ_{MAX}, the maximum SPR reponse, K_d, the dissociation constant and [c], the concentration of HSA. The fitting of our data to the Langmuir isotherm reveals

a K_d of 2.37 x 10^{-9} M. In contrast, the nano-NIP-HSA response curve did not exhibit any significant signal change, reaffirming the affinity of the nano-MIPs for HSA.

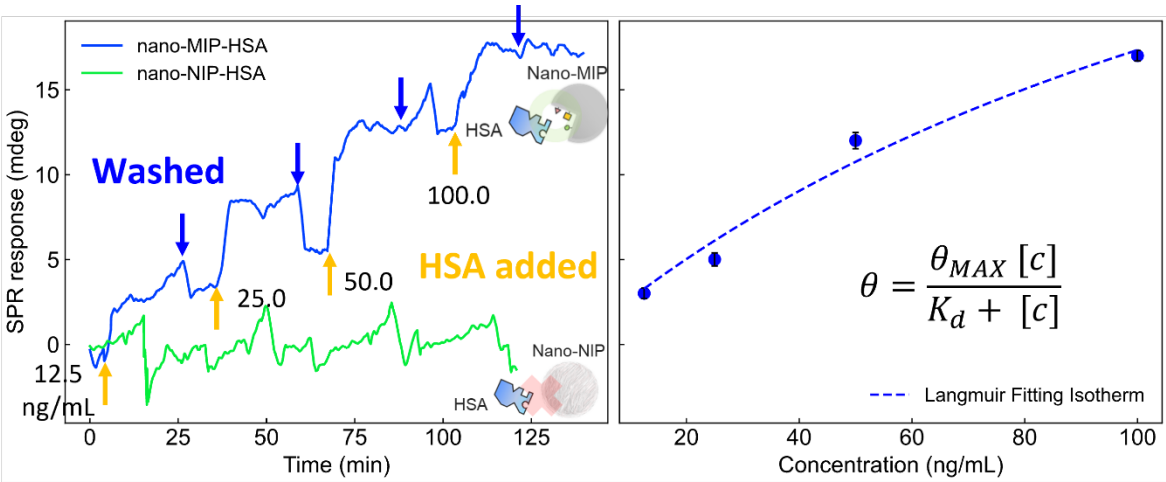

Fig. S5 Affinity analysis

References

S1 Y.-N. Lee, T. Araki, Y. Kimura, F. Dasai, T. Iwata, K. Takahashi and K. Sawada, IEEE Trans. Biomed. Circuits Syst., 2019, 13, 352–363.

S2 Y. Man, S. Nishitani and T. Sakata, Sens. Mater., 2024, accepted.

S3 F. Canfarotta, A. Poma, A. Guerreiro and S. Piletsky, Nat. Protoc., 2016, 11, 443–455.

S4 I. Dmitriev, I. Kuryndin, N. Bobrova, and M. Smirnov, Mater. Today Commun., 2015, 4, 93–100.