Supplementary Information

High-efficiency immunoassay platform with controllable surface roughness and oriented antibody immobilization

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1. Experiment section

Preparation of the AAPTS- and CPBA-functionalized surfaces

The flat references and as-prepared surfaces was immersed into the [3-(2-aminoethyl) aminopropyl] trimethoxysilane (AAPTS) of anhydrous toluene solution (2%, v/v) under ambient temperature for 2 h, followed by washing several times with toluene solution and ethanol and blow-dried by air flow. The resultant samples were respectively denoted as V- AAPTS, S- AAPTS and D- AAPTS for short. Then 0.12 mmol 4-carboxyl boronic acid (CPBA) and 0.24 mmol 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) were dissolved in the mixture solution of dimethyl formamide (DMF) and 2-(N-morpholino) ethanesulfonic acid (MES) (1:1,

v/v) and incubation at room temperature for 2 h. The AAPTS -modified surfaces were immersed into the above solution for reaction about 2 h, followed by rinsed with copious DMF and ultrapure water and dried by air stream. The obtained surfaces were denoted as V-CPBA, S-CPBA and D-CPBA, respectively.

The oriented immobilization of human serum albumin antibody (HSA-Ab)

The CPBA-modified samples were firstly soaked in ultrapure water with 0.5% Tween-20 at 37 °C for 1 h, followed by rinsing with deionized water. Then the samples was incubated in carbonate buffer solution (pH = 9.4) at 37 °C for another 1 h. After being taken out, these samples were placed into different concentrations of human serum albumin antibody with FITC labeled (FITC-HSA-Ab) solution for 37 °C for another 2 h. After the immobilization, all samples were mildly rinsed with buffer solution and dried by air stream.

Recognition of Human serum albumin (HSA-Ag).

Firstly, the defined concentration of HSA-Ab (200 μg/mL) in carbonate Buffer solution (pH = 9.4) was used to prepare the antibody-immobilized samples. Then, FITC-labeled HSA-Ag solutions with different concentrations were added into the sample wells. After 2 h incubation at 37 °C, the samples were removed and rinsed with PBS buffer and ultrapure water, followed by drying with air stream and stored in the darkness for further analysis.

Surface chemical analysis

Infrared absorption spectra were obtained from a Fourier transform infrared spectroscopy (FTIR, BRUKER Vertex 70) with an ATR (attenuated total reflection) mode. For each spectrum obtained, a total of 32 scans were accumulated with a resolution of 4 cm^{-1}. Surface elemental compositions of the samples were determined via X-ray photoelectron spectroscopy with Al/Kα (hν = 1486.6 eV) anode mono-X-ray source (XPS, VG Scientific ESCA MK II Thermo Avantage V 3.20 analyzer) at the detection angle of 90◦. Spectra over a range of 0-1200 eV, and high-resolution spectra of B1sregions were collected. Atomic concentrations of the elements were calculated by their corresponding peak areas.

Surface wettability

Water static contact angles on the surfaces were measured with a drop shape analysis instrument (DSA, KRÜSS GMBH, Hamburg 100) at room temperature. For each sample, 2 μL water droplet was dropped each time and at least five contact angle measurements were performed for calculating the average value.

Fluorescence intensity scanning and data analysis

Fluorescent images of the samples were collected by a confocal laser scanning microscope (Zeiss, LSM 700). Target biomolecules labeled with FITC and RBITC were respectively excited by an argon ion laser at 488 nm. In order to obtain fluorescence intensity, original fluorescent images were analyzed using Image Pro software. Fluorescence intensity value was measured from the six different positions of each fluorescence image.

Comparison of surface roughness between a flat, a single-scale, and a dual-scale structured surface

Surface roughness can be defined as the ratio between the total surface area and the projected area (a fixed value); we focus our discussions below on the surface area for structured surfaces.

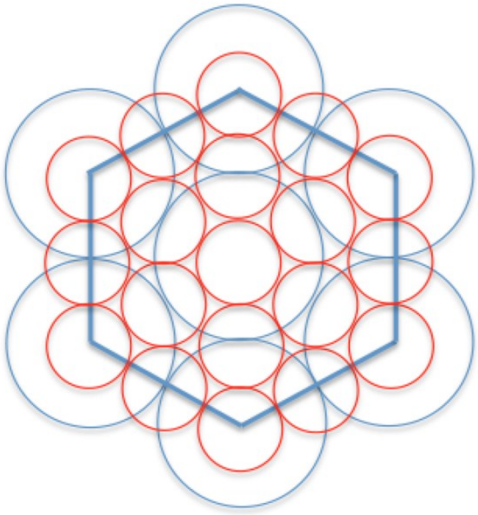

Fig. S1 Closely packed spheres on a hexagon.

Let us first examine the difference in total surface area between a flat surface and a single-scale structured surface based on spheres. For the sake of simplicity, let us assume that spheres of the same size are closely packed on a hexagon with the side length $S = 20$ (only the hexagonal area in Fig. S1 is considered).

Scenario 1: If the sphere diameter $D = S = 20$, then 3 blue spheres in total are needed to cover the hexagon (Fig. S1), with the total surface area (SA) of 1981.7, including 3 spheres (only the upper hemisphere is considered, $3\pi D^2/2$) and interstices between

spheres ($3\sqrt{3}S^2/2 - 3\pi D^2/4$). The total SA is significantly greater than (almost twice) that of the flat hexagon ($3\sqrt{3}S^2/2$), 1039.2.

Scenario 2: If the sphere diameter *D* becomes 10 while maintaining *S* = 20, then 12 red spheres in total (Fig. S1) are required to cover the hexagon, and the total *SA* is calculated to be 1981.7, exactly the same with Scenario 1. The difference between the two cases is that the total sphere volume (or mass) in Scenario 2 is only half of that in Scenario 1.

Despite no difference in the *SA* between the two cases, the surface wettability may differ due to the size of the interstices (especially in the case of non-close packing), which is an important factor that impacts liquid/solid interaction. In terms of antibody immobilization, Scenario 2 may allow slightly more antibody to be chemically bonded onto the structured surface, considering the slightly more accessible curvature on smaller spheres and the relatively large size of the antibody.

On a single-scale structured surface, the relationship between *SA* as a function of the sphere diameter *D* is plotted in Fig. S2 (again, assuming close packing for spheres), together with the dependence of the total sphere volume (V_S) on *D*. It becomes apparent that the total *SA* for close-packing spheres does not change as *D* becomes smaller; however, to maintain the same *SA* level, larger V_S are required for larger spheres, thus reducing the efficiency of using spheres in achieving the same *SA*.

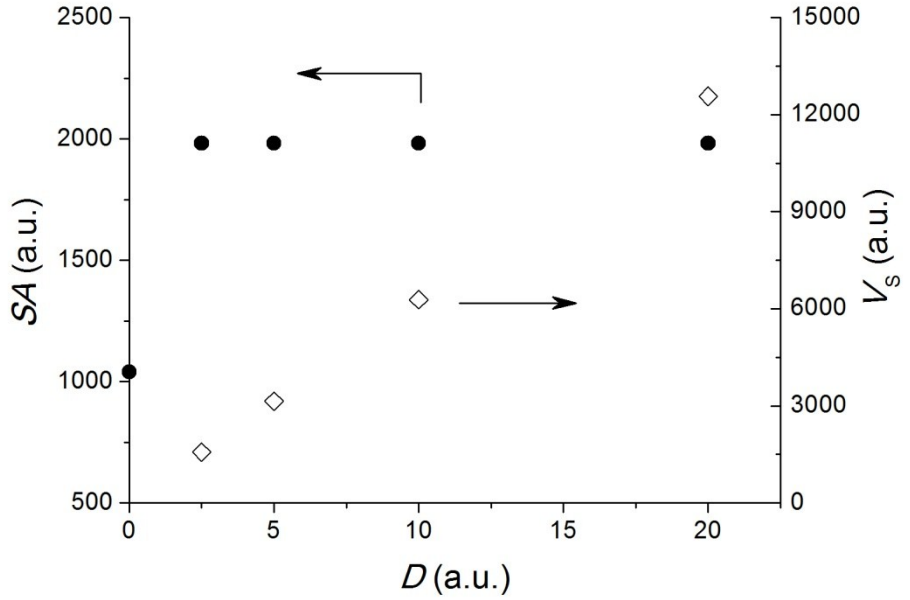

Fig. S2 Total surface area (*SA*) and total sphere volume (V_S) as a function of the sphere diameter (*D*) on single-scaled structured surfaces.

The total *SA* can be significantly increased by adding a layer of secondary, smaller spheres on top of the primary spheres (i.e., our dual-scale structured surface). It is much more complicated to calculate the *SA* on a dual-scale structured surface, since it is not straightforward to describe the packing of secondary spheres on a primary sphere due to the curvature. However, on the basis of the above calculation for single-scale surfaces, it can be estimated that the total *SA* on a dual-scale structured surface would be, at least, about twice as much as that on a single-scale structured surface.

Experimental results

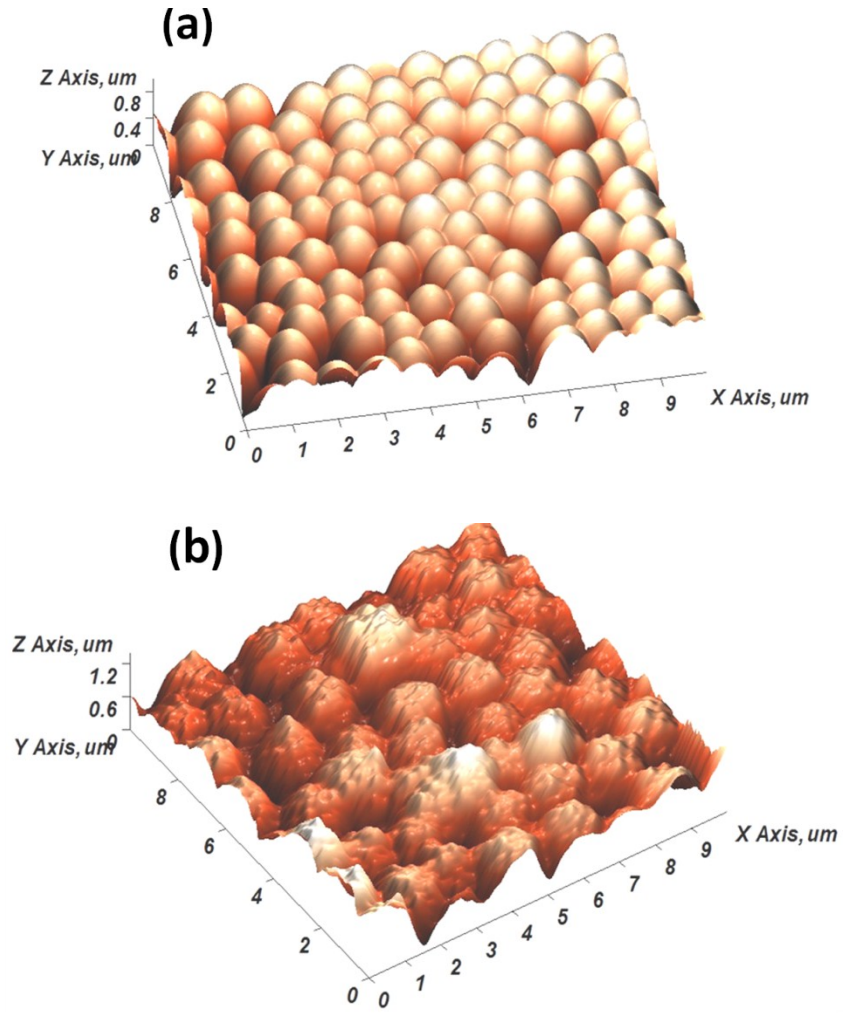

Fig. S3 AFM images of the as-prepared surfaces. (a) single-scale structured surface (RMS = 140 ± 40 nm), and (b) dual-scale structured surface (RMS = 245 ± 30 nm).

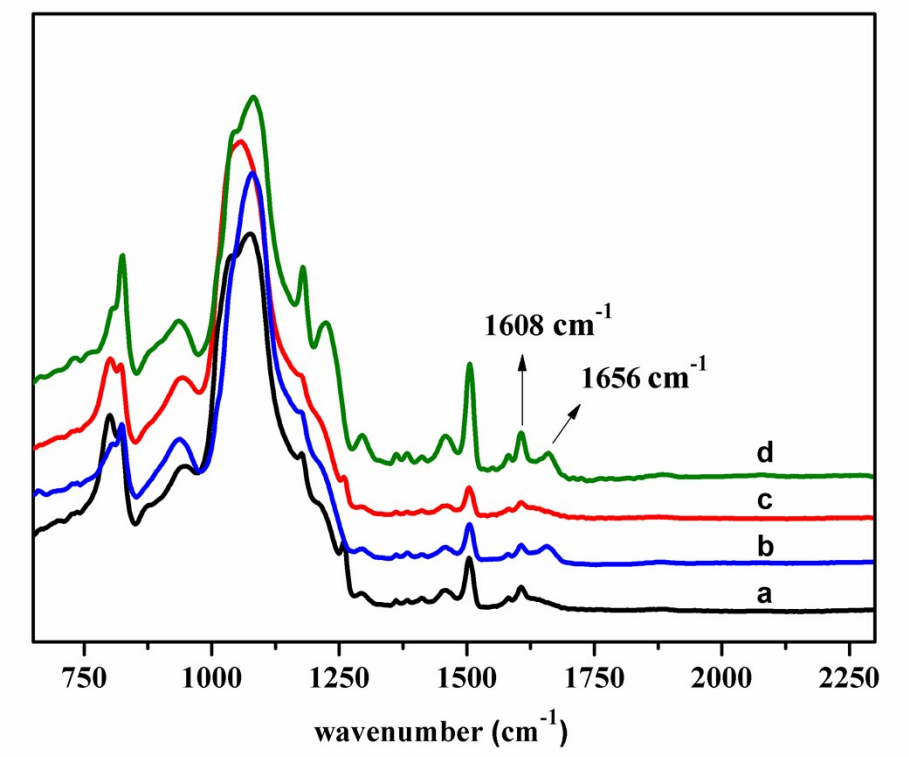

Fig. S4 FTIR-ATR spectra of the as-prepared surfaces. (a) S-AAPTS, (b) D-AAPTS, (c) S-CPBA and (d) D-CPBA.

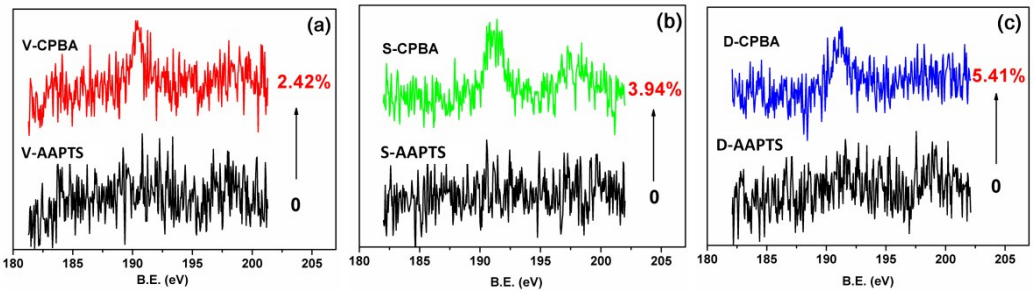

Fig S5. High-resolution B_{1S} spectra of the AAPTS-modified (bottom) and CPBA-modified (upper) samples. (a) flat reference, (b) S-CPBA surface, and (c) D-CPBA surface.

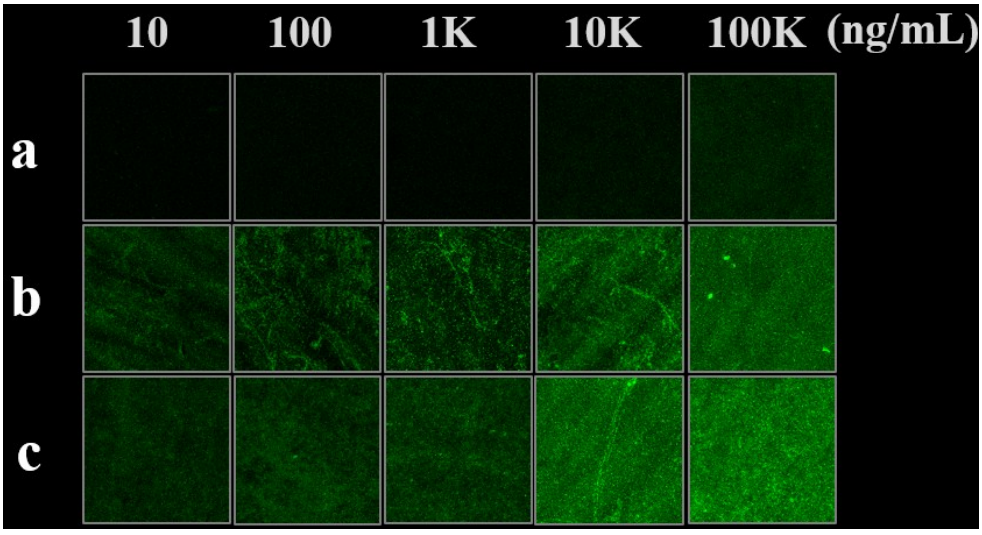

Fig S6. Representative fluorescence images of the samples after the immobilization of FITC-labeled antibodies at different antibody concentrations. (a) V-CPBA, (c) S-CPBA, (d) D-CPBA.