Electronic Supplementary Material (ESI) for Biomaterials Science

Supplementary Information

Sulfobetaine-terminated PEG Improves the Qualities of an Immunosensing Surface

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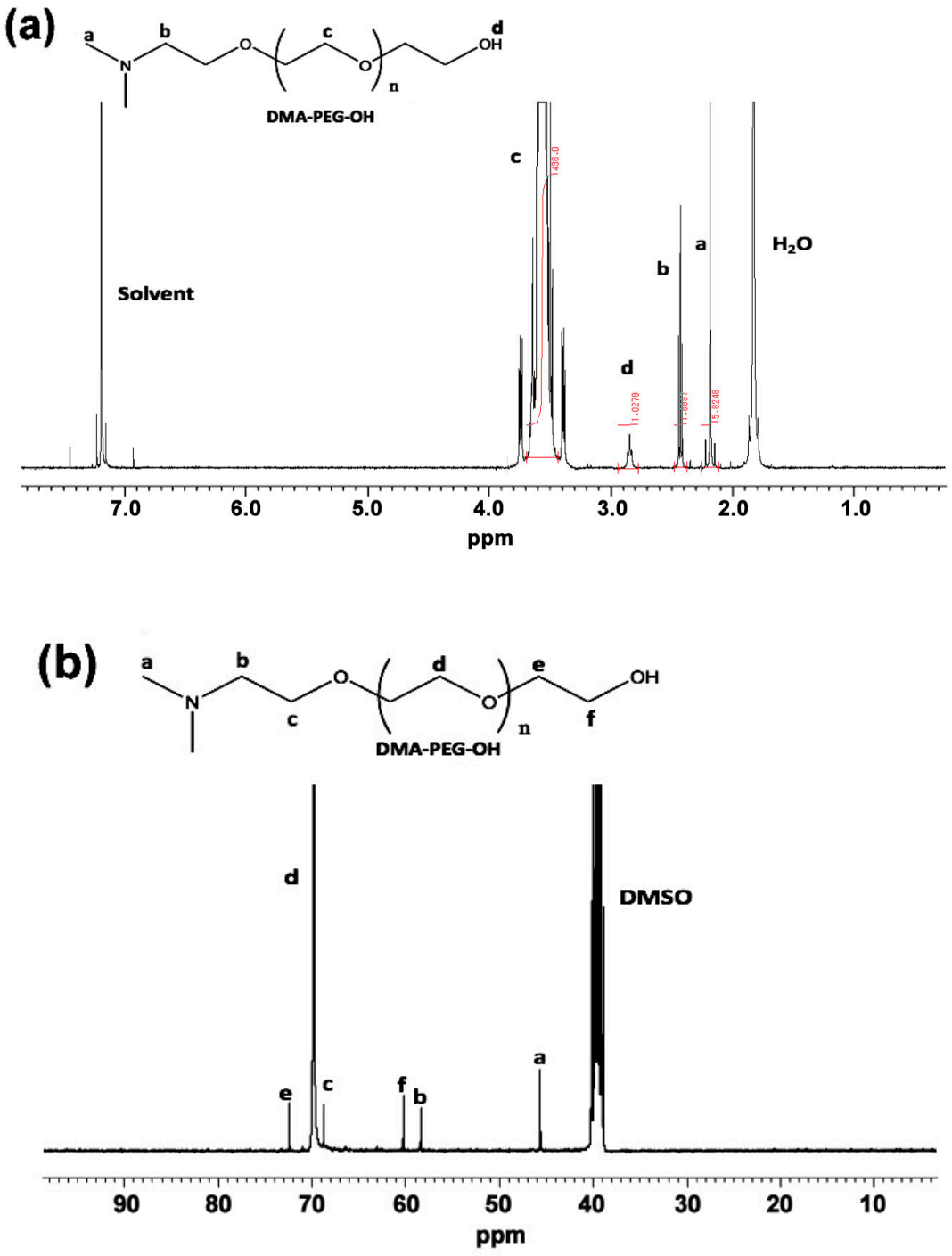


Figure S1. (a) ^{1}H-NMR spectrum of DMA-PEG-OH (400 MHz, 64 scans, $CDCl_3$, RT). (b) ^{13}C-NMR spectrum of DMA-PEG-OH (100 MHz, 8192 scans, DMSO, RT).

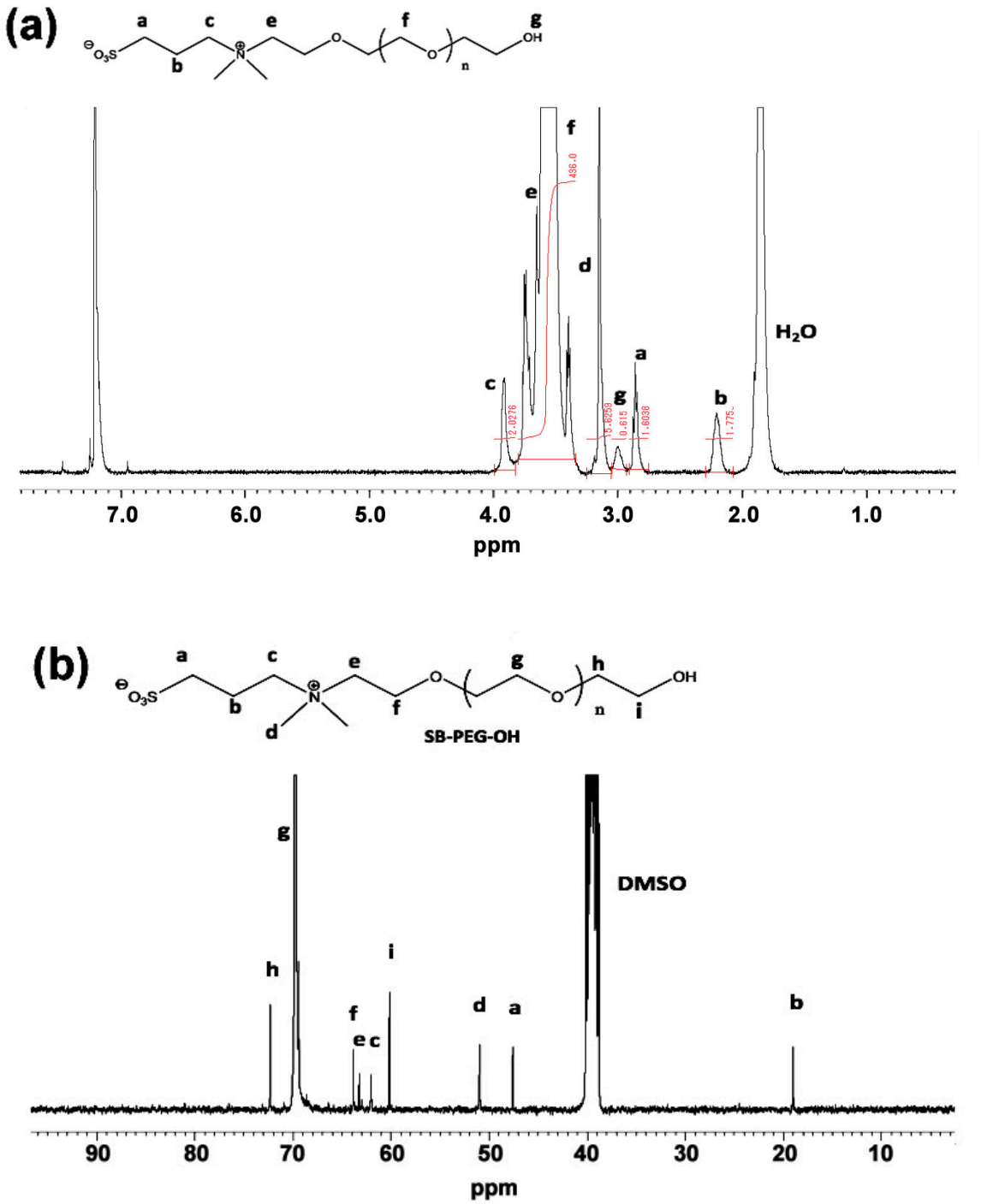


Figure S2. (a) ^{1}H-NMR spectrum of SB-PEG-OH (400 MHz, 64 scans, $CDCl_3$, RT).
(b) ^{13}C-NMR spectrum of SB-PEG-OH (100 MHz, 8192 scans, DMSO, RT).

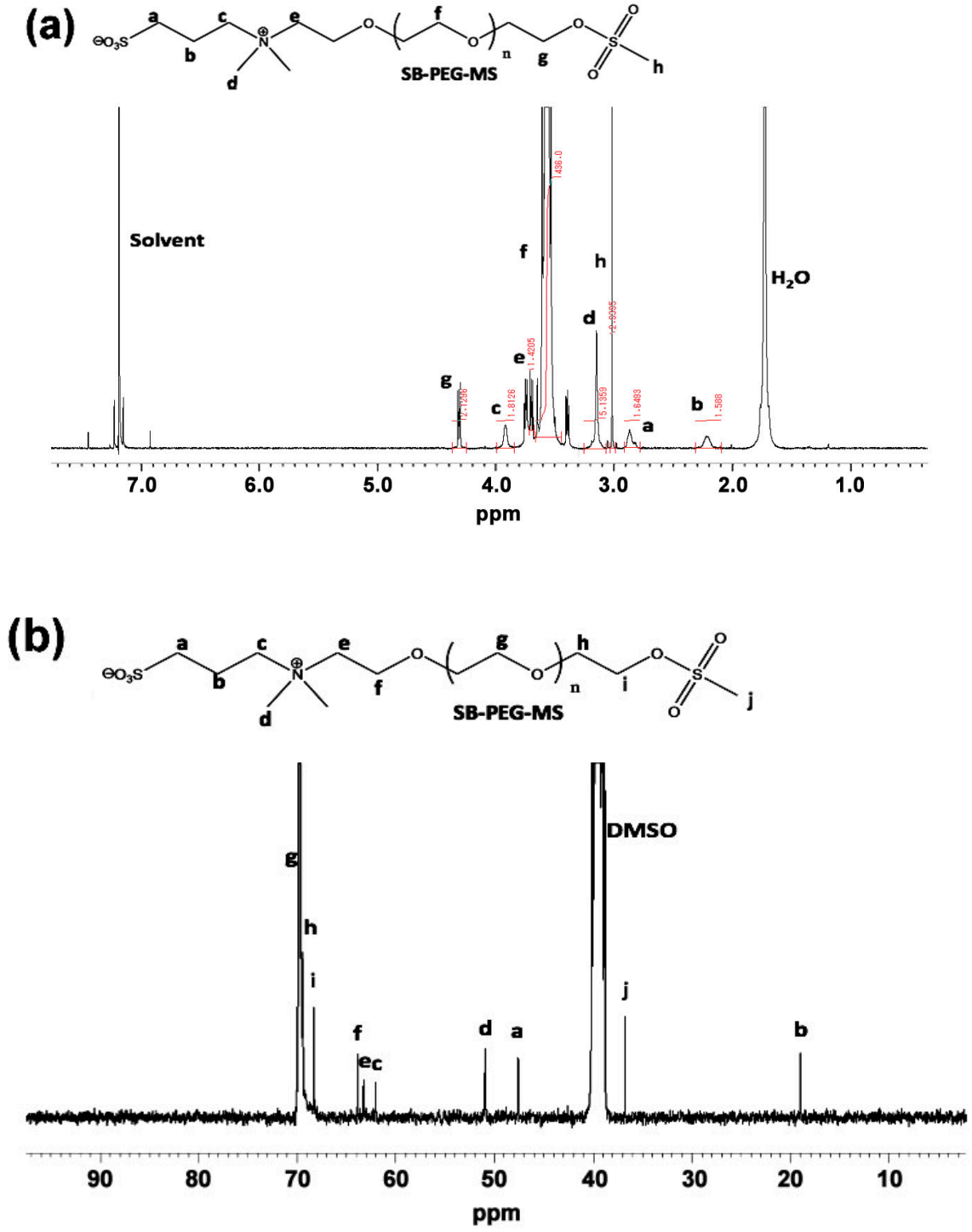


Figure S3. (a) ^{1}H-NMR spectrum of SB-PEG-MS (400 MHz, 64 scans, $CDCl_3$, RT).
(b) ^{13}C-NMR spectrum of SB-PEG-MS (100 MHz, 8192 scans, DMSO, RT).

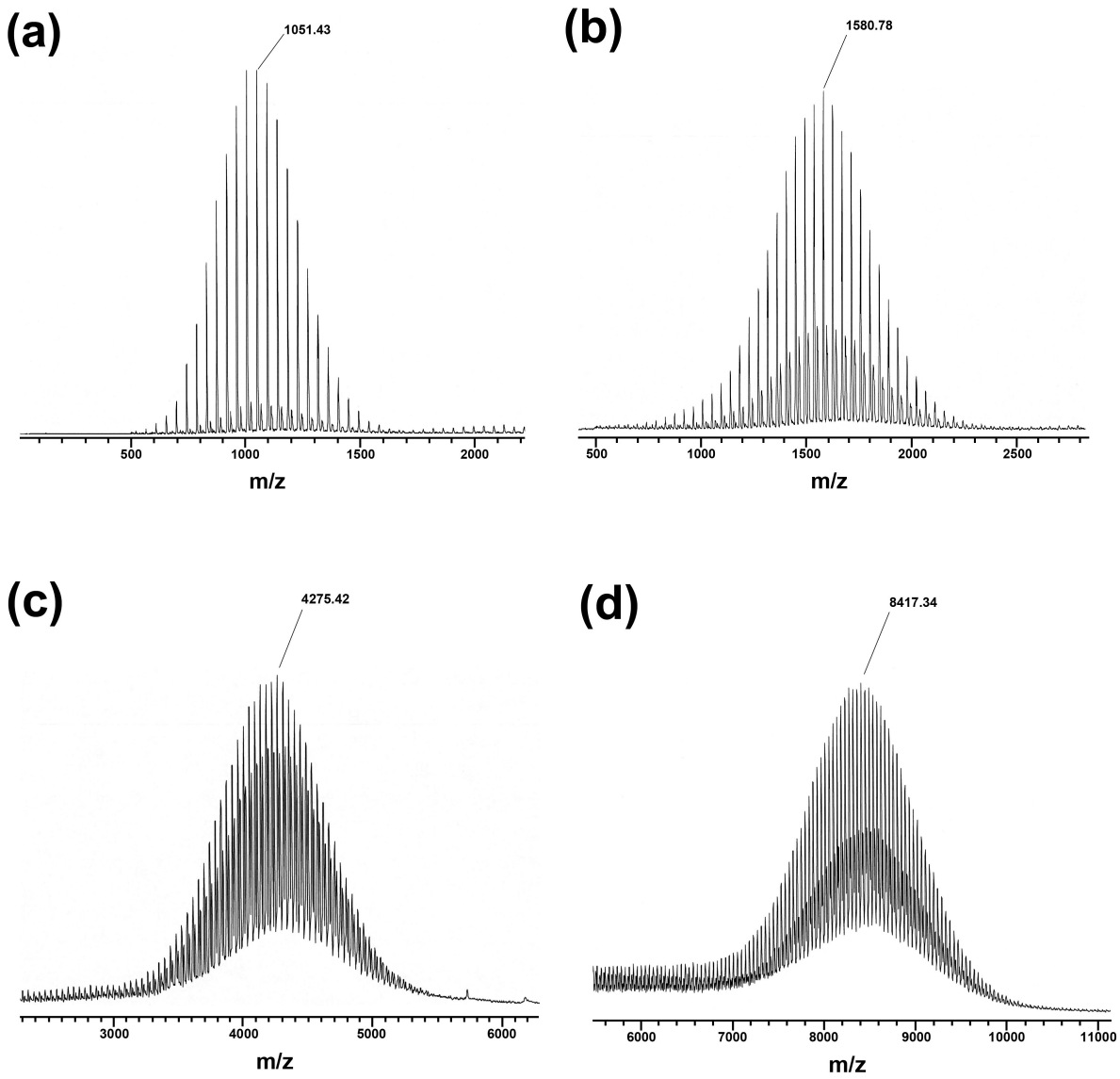


Figure S4. MALDI-TOFMS spectrum of PEG standard which molecular weight were 1,010 (a), 1,480 (b), 3870 (c) and 7,830 (d) (Agilent Technologies Inc., Shropshire, UK). The polydispersity index (M_w/M_n) of each polymer was 1.04, 1.04, 1.03 and 1.05, respectively.

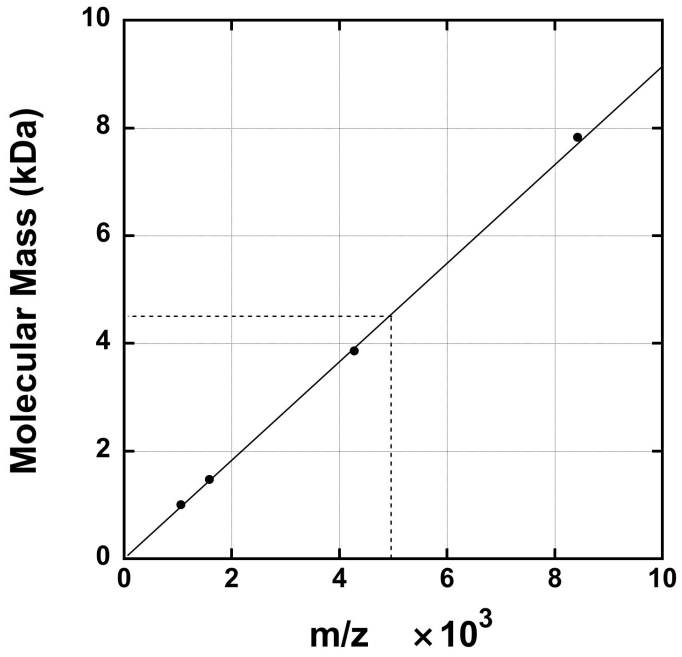


Figure S5. Standard curve of commercially available PEG standards which molecular weight were 1,010, 1,480, 3870 and 7,830 (Agilent Technologies Inc., Shropshire, UK). The molecular mass values of these standard PEG were calculated by GPC. The standard curve shows the correlation between these molecular mass and m/z value of maximum peak intensity. Each mass spectrum was shown in Figure S4. The calculated molecular mass of SB-PEG-N6 by MALDI-TOF MS was agreed well with that of SB-PEG-N6 by GPC.

Evaluation of adhesion strength of protein on gold surface using SPR

The SPR sensor chip with a plain gold surface was washed with piranha solution before modification of the surface. For protein modification of the gold surface, the sensor chip was placed in an SPR instrument (BIAcore 3000). 50 mM PBS (150 mM NaCl, pH 7.4) solution was used as running buffer and the flow rate was 5 μL/min. 0.1 mg/mL (1.52 μM) human serum albumin (HSA, Sigma-Aldrich, Steinheim, Germany) was applied for 30 min. After the preparation HSA modified gold surface, 200 μM of SB-SH was flowed for 0, 5, 10 or 30 min. 0.5 μM of anti-HSA (Sigma-Aldrich, Steinheim, Germany) containing buffer was flowed for 30 min. The amount of anti-HSA binding was evaluated by measurement of the SPR angle shift. Figure S6 shows the schematic procedure of surface modification. To confirm the protein exchange on the surface, 0.1 mg/mL of BSA solution was also used instead of SB-SH solution.

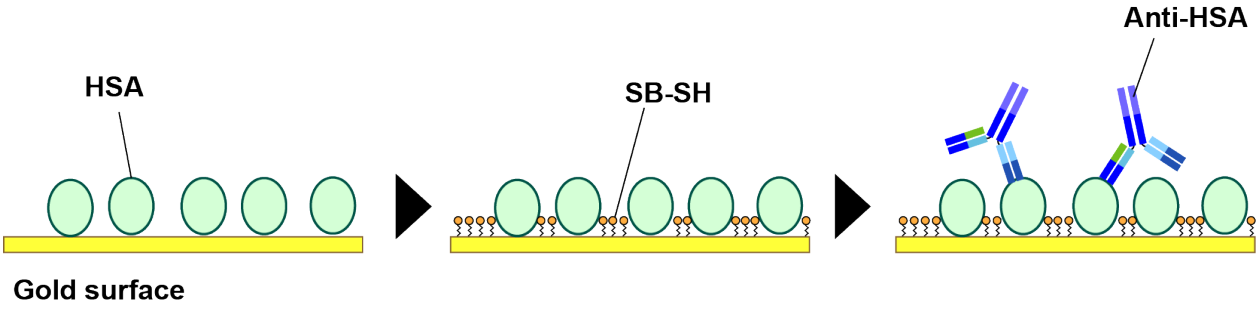


Figure S6 Schematic procedure of surface modification to confirm the protein detachment.

Results and discussion

Figure S7a shows the SPR sensorgrams from the construction of HSA/SB-SH surfaces to binding anti-HSA on the surfaces. After the immobilization of HSA, further immobilization of

SB-SH was observed because small molecule of SB-SH could fill up the gap of HSA (66 kDa) immobilized gold surfaces (Figure S6). The amount of immobilized SB-SH was increased when the flow time of SB-SH solution was extended. The significant angle shift was observed after the anti-HSA flow, which means anti-HSA interacted with HSA on the surfaces. The case of only SB-SH surface, the angle shift derived from nonspecific adsorption was observed, however, quite smaller than specific interaction. Figure S7b show the angle shift after anti-HSA flow converted from Figure S7a. When the SB-SH flow was 5 min, the amount adsorbed anti-HSA was 16% lower than 0 min (no SB-SH). The result may indicate that the nonspecific adsorption of anti-HSA was decreased after the immobilization of SB-SH. Note that the amount of adsorbed anti-HSA was not changed even though SB-SH flow time was extended to 10 or 30 min. If HSA was exchanged to SB-SH, the angle shift will be decreased. The result indicates that the amount of immobilized SB-SH was increased without exchange. We have also done the same experiment by BSA instead of SB-SH. The result indicated that significant protein exchange was not observed by BSA treatment (Figure S8a, S8b). Thus, we have confirmed that the immobilized proteins were not removed by SB-SH treatment or protein treatment during the modification process.

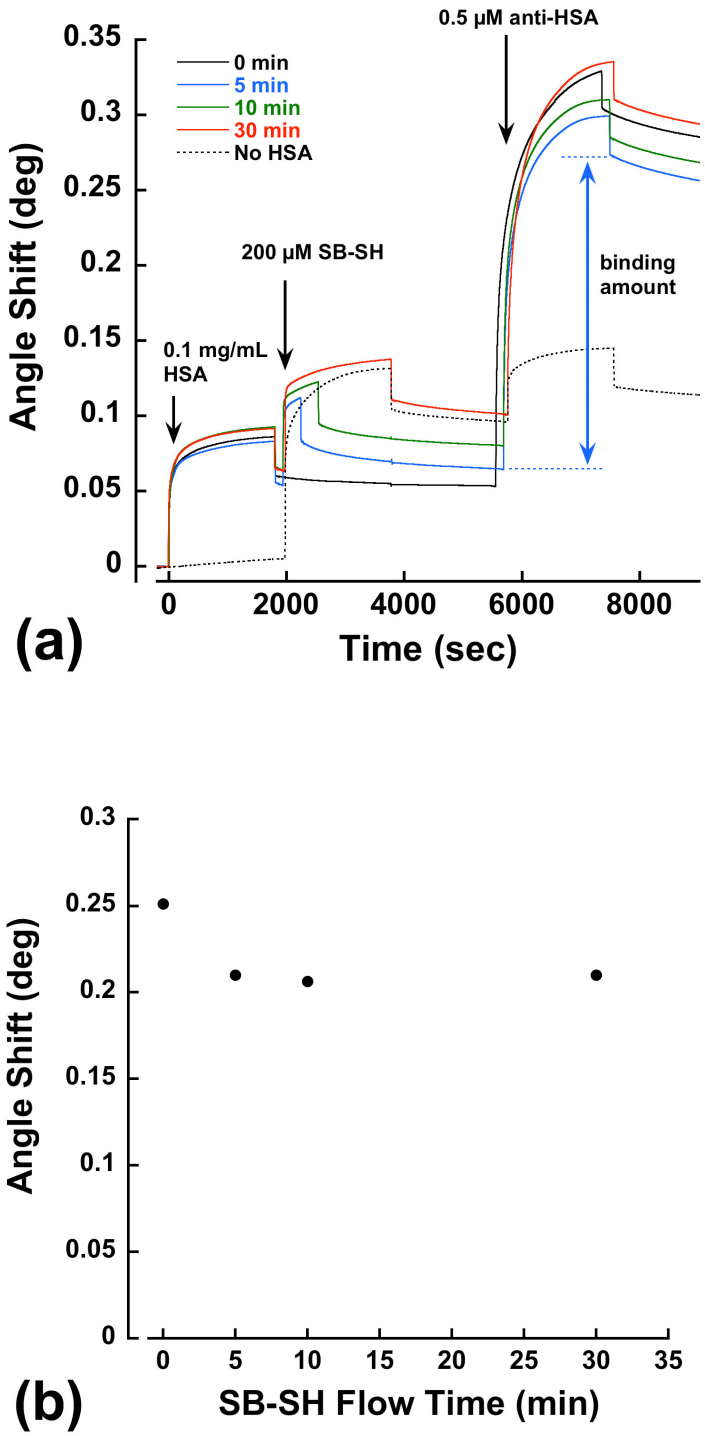
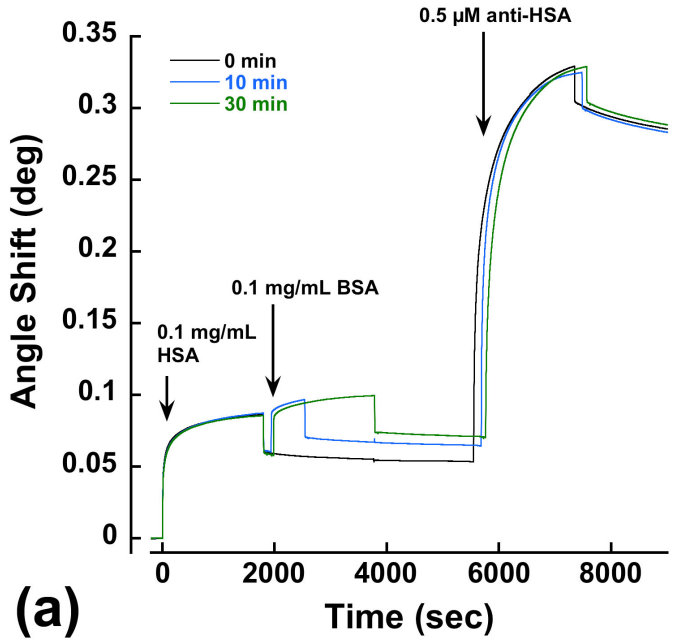


Figure S7. (a) SPR sensorgrams from the construction of HSA/SB-SH surfaces to binding anti-HSA on the surfaces. After the preparation of HSA modified gold surface, 200 μM of SB-SH

was flowed for 0 (black line, no SB-SH), 5 (blue line), 10 (green line) or 30 min (red line). Dashed line is SB-SH only (no HSA). (b) Correlation between SB-SH flow time and angle shift after anti-HSA flow. This figure was converted from (a).



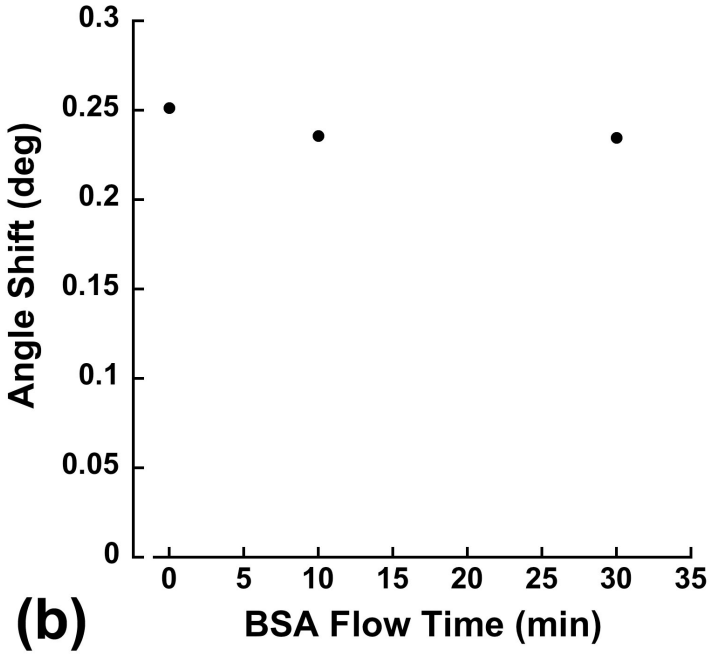


Figure S8. (a) SPR sensorgrams from the construction of HSA/BSA surfaces to binding anti-HSA on the surfaces. After the preparation of HSA modified gold surface, BSA solution was flowed for 0 (black line, no BSA), 10 (blue line) or 30 min (green line). (b) Correlation between BSA flow time and angle shift after anti-HSA flow. This figure was converted from (a).