Supporting Information

Sequential sandwich immunoassay for simultaneous detection in trace samples using single-channel surface plasmon resonance

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Figures and Results

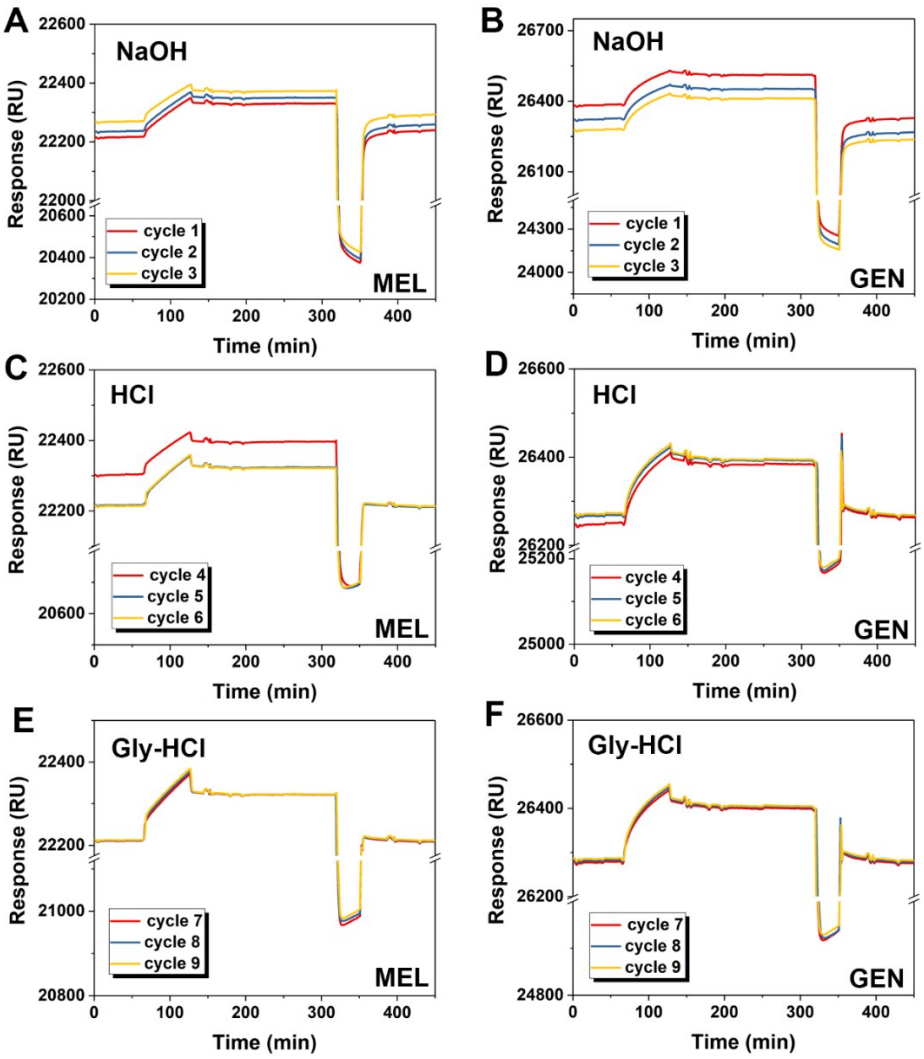

Figure. S1 SPR sensorgrams for the regeneration of MEL and GEN using NaOH, HCl, and Gly-HCl solutions. Each solution was tested for three time.

As shown in Figure S1. NaOH, HCl, and Gly-HCl were successively injected into the chamber. Each solution was performed 3 times. For the MEL, after rinsing with NaOH solution, the baseline increased and sample responses obviously decreased, which indicated that AbMEL was not completely dissociated from the chip. Due to residual antibody on chips, cycle 4 needed to elute much more antibody. Therefore, it showed a little difference from other two cycle (cycle 5 and 6). For all this, no significant change was found in the sample response and baseline. After the injection of Gly-HCl, the SPR sensorgrams of cycle 7, 8, and 9 were basically overlapped together, indicating good regeneration effect. As for the GEN, the baseline dropped and sample response increased after the cycle 1, 2 and 3, which may affect the stability of the functional surface. And similar results as that of MEL were observed during cycle 4 to cycle 9. Based on all the results, we chose Gly-HCl as the final regeneration solution in this work.

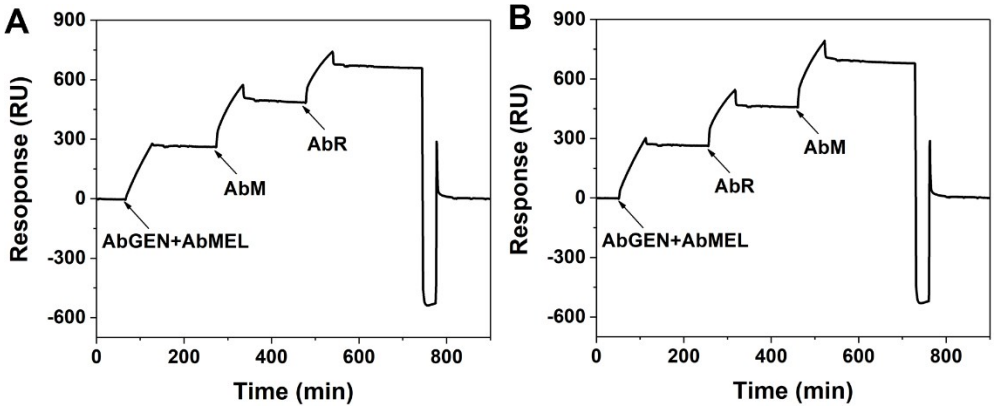

Figure S2 SPR monitoring the effect on different sequences of second antibodies during simultaneous detection.

As shown in Fig. S2, the primary antibody was firstly injected, and then AbM was used prior to AbR in the secondary antibody readout. The sample responses of AbM and AbR were 244.8 RU and 195.5 RU, respectively. On the other hand, when AbR was used prior to AbR, the sample responses of AbR and AbM were 202.6 RU and 247.5 RU, respectively. Compared with the results of different injected sequences, the sample responses of AbM and AbR presented no obvious changes. Therefore, the sequence of detection of the secondary antibobies had no effects on the adsorption of second antibodies.

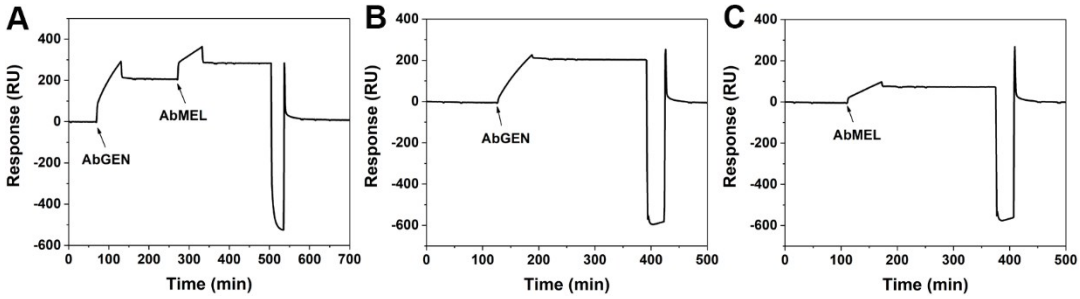

Figure S3 SPR monitoring the adsorption for the sequence test of AbGEN and AbMEL (A), single test of AbGEN (B), and single test of AbMEL (C).

As shown in Fig. S3, the sample responses of AbGEN and AbMEL were 213.1 RU and 80.8 RU during the sequencial experiments. And the results of single AbGEN and single AbMEL were 215.6 RU and 80.2 RU, respectively. Based on the comparison of these results, we can demonstrate that the crosstalk effect between AbGEN and AbMEL was almost negligible.