Comparative study of random and oriented antibody immobilization as measured by dual polarization interferometry and surface plasmon resonance spectroscopy

*Hong Yan SONG, Donna ZHOU, Jonathan HOBLEY, Xiao Di SU**

Institute of Materials Research and Engineering, A*STAR, 3 Research Link, Singapore 117602

xd-su@imre.a-star.edu.sg

Supporting Information

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Preparation of Biotinylated Ab_1 (Ab_1-b)

PSA antibody (Ab_1) was diluted to 1 mg/ml in 50 µl of PBS (with 10% 1M sodium bicarbonate in H_2O). Immediately before use, 10 mM NHS-LC-Biotin in DMSO (4.5 mg/ml) was prepared. NHS-LC-Biotin (0.2 µl, ~5 times of Ab_1 in molar ratio) was added to the Ab_1 solution, and the mixture was incubated at room temperature for 30 min. Gel filtration was used to purify the obtained Ab_1-b.

DPI measurement of repeated cycle of Ab_1 immobilization on protein G and detection of PSA

Figure S-1 shows DPI measurement of Ab_1 immobilization on protein G surface, sandwiched PSA detection and surface regeneration in real time. The measurement was illustrated in resolved mass and thickness. When the protein G layer was stabilized (curve not shown in this figure), Ab_1 was injected. At the end of 30 min rinsing, PSA (10 pg/ml) was injected, followed by secondary antibody (Ab_2). The binding of PSA/Abs can be seen from the signal increase after Ab_2 injection. Once the detection was complete, the glycine solution was flowed through. The baseline returned back to its original position before Ab_1 was immobilized, indicating that the Ab_1/PSA/Ab_2 complex was completely removed. The freshly regenerated protein G surface can immobilize the same amount of Ab_1, and detect PSA/Ab_2 with repeated results.

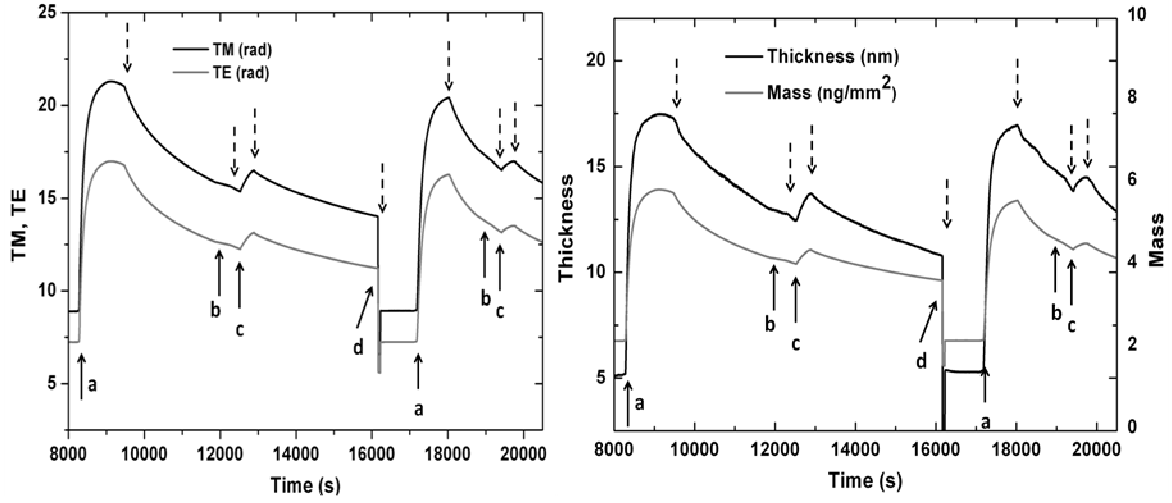

Figure S-1. Two cycles of immobilization of Ab_1 onto protein G surface followed by PSA detection using Ab_1/PSA/Ab_2 sandwich complex. Original data (TM and TE) and the resolved layer thickness and

mass were recorded. a. Ab_1 (200 nM); b, PSA (10 pg/ml); c, Ab_2 (5 μg/ml); d, Glycine (1M, pH 2). The start and end point of each injection are indicated by a solid and broken arrow.

SPR detection of PSA in ng/ml level using immobilized Ab_1 without orientation control

Figure S-2 shows SPR measurement of the Ab_2 signal following PSA addition (10 and 100 ng/ml) on the randomly immobilized Ab_1.

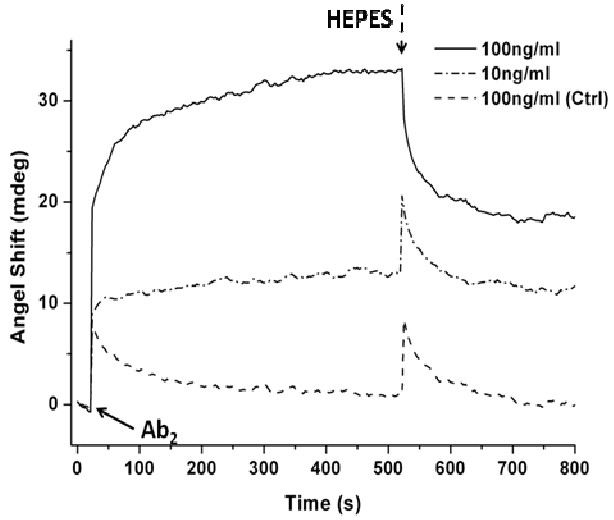

Figure S-2. SPR sensorgram of Ab_2 (10 ug/ml) following PSA injection (100 ng/ml and 10 ng/ml) on random immobilized Ab_1. Control, no injection of Ab_1 on the surface (PSA 100 ng/ml).