Supporting Information for *Analytical Chemistry*

Real-Time Biomolecular Binding Detection Using a Sensitive Photonic Crystal Biosensor

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Supporting Information

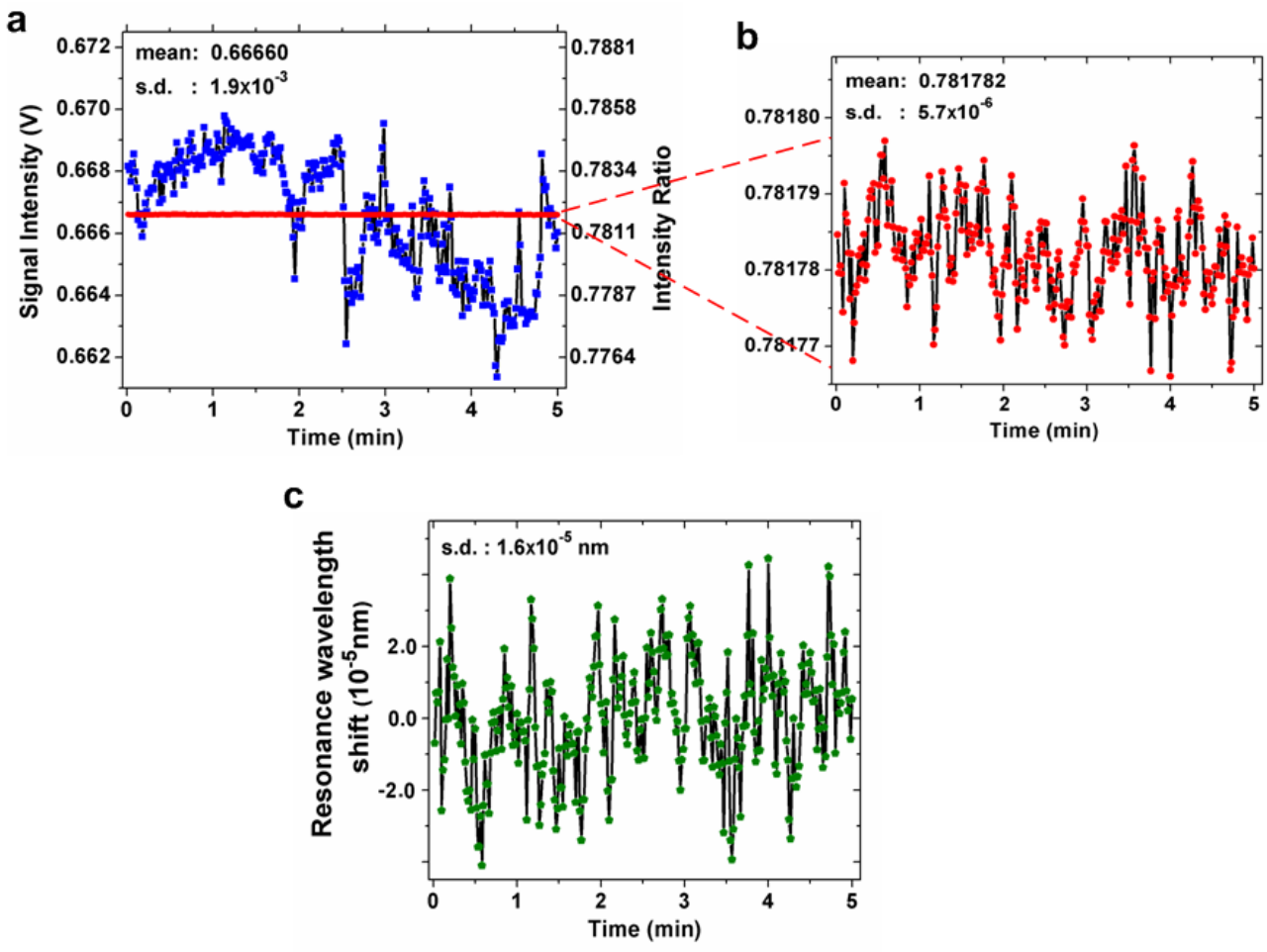


Figure S-1. Baseline noise floor of the PC-TIR sensor (a) Baseline fluctuations of the signal channel intensity and of intensity ratio between signal and reference channels; by normalization, the baseline noise floor was suppressed by nearly 400 times. (b) Baseline fluctuation of the intensity ratio (i.e. normalized standard deviation (s. d.)) was below 8×10^{-6}. The noise of the presented system could be further decreased by improving the quality and uniformity of the PC-TIR structure, controlling temperature stabilization and decreasing the laser fluctuation. (c) The resonance wavelength shift can be derived from the intensity ratio change with a Lorentz equation. From (b), we can get the baseline fluctuation of corresponding resonance wavelength shift was 1.6×10^{-5} nm, which can be considered as the minimum detectable signal of the PC-TIR sensor.

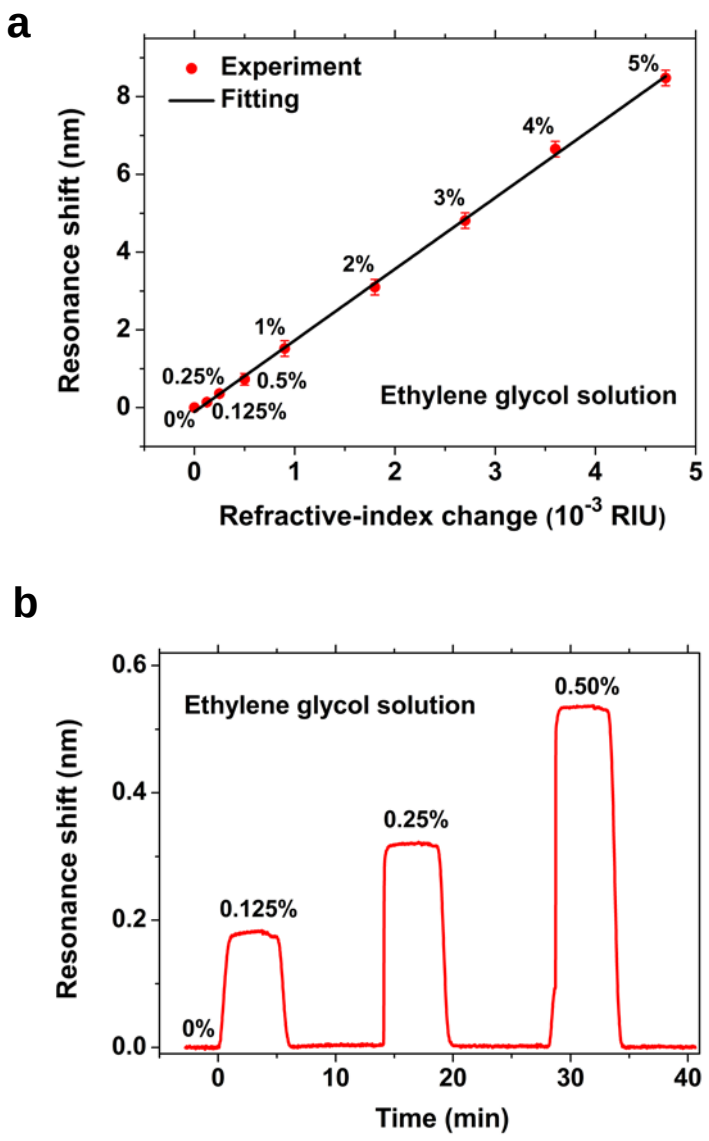


Figure S-2. Characterization of PC-TIR sensor with bulk solvent index change. (a) Spectral measurement with different refractive indices solution on the sensing surface (0 to 5% ethylene glycol solution); the sensitivity of 1840 nm/RIU was then obtained by measuring the slope of the resonance shift versus the refractive index change. (b) Real-time normalized intensity measurement of the bulk solvent refractive index. Given that 0.125% ethylene glycol solution with a larger refractive index by 1.25×10^{-4} RIU than the baseline solution yields a 0.182-nm wavelength shift, and the resolution of this PC-TIR biosensor can be 1.6×10^{-5} nm, the detection limit was determined to be 1×10^{-8} RIU.

Name	MW (Da)	Biotin molar ratio
Streptavidin	~52,000	4:1
Very small molecule		
D-Biotin	244.13	1:1
DNA oligonucleotides †		
Biotin-10T	3,385.3	1:1
Biotin-20T	6,427.1	1:1
Biotin-30T	9,468.9	1:1
20A	6,202.2	none
20C	5,721.6	none
Proteins		
Biotin-Protein A	~ 41,000	~ 6:1
Biotin-BSA	~ 66,000	~ 9:1
Antibodies		
Biotin-IgG	~ 150,000	unknown
IgG	~ 150,000	none

Table S-1. Detail information on molecules in our study.

† Biotin-10T means that single-strand DNA oligonucleotide with 10-T bases was biotinylated (sequence 5'-Biotin-TTT TTT TTT T-3'), same as Biotin-20T and Biotin-30T. All the DNA oligomers were used Reverse-Phase Cartridge (RP1) purification.

Method S-1: Streptavidin adsorption by specific noncovalent binding

To create a biotin-functionalized sensor for capturing streptavidin (SA), the sensing surface (silica) was first cleaned and oxidized by the piranha solution (H_2SO_4 (95%) / H_2O_2 (30%)=3:1), then silanized with 5% APTES solution in methanol/water (1:1). Next, Sulfo-NHS -LC-LC-Biotin (1 mg/mL in PBS) was slowly flowed into the flow cell for several hours so that the NHS-activated biotin reacted efficiently with the primary amino groups. After that, 500 μg/mL bovine serum albumin (BSA) in PBS was flowed to block the non-specific binding sites of the streptavidin with the silica sensing surface. Finally, 10 μg/mL streptavidin in PBS solution was flowed and specially bound to the sensing surface.

In the following experiment, a large amount of streptavidin molecules were bound on the biotin-functionalized surface that produced 5.24-nm resonance wavelength shift. Then we tested the available biotin binding sites left on the sensing surface by flowing 10 μg/mL biotinylated IgG (biotin-IgG) in PBS solution. The biotin-IgG binding only caused 0.40-nm resonance wavelength shift, which means that only few biotin-binding sites were left on sensing surface after streptavidin adsorption via specific noncovalent binding.

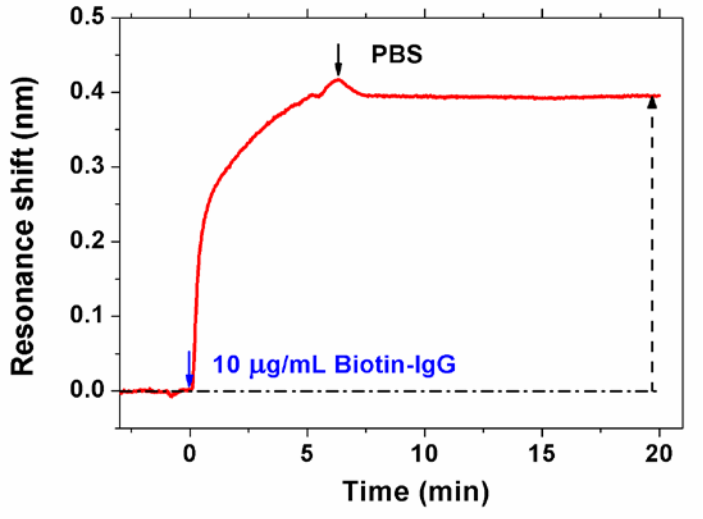


Figure S-3. Large size molecule biotin-IgG binding to streptavidin adsorbed on the surface via specific noncovalent binding.

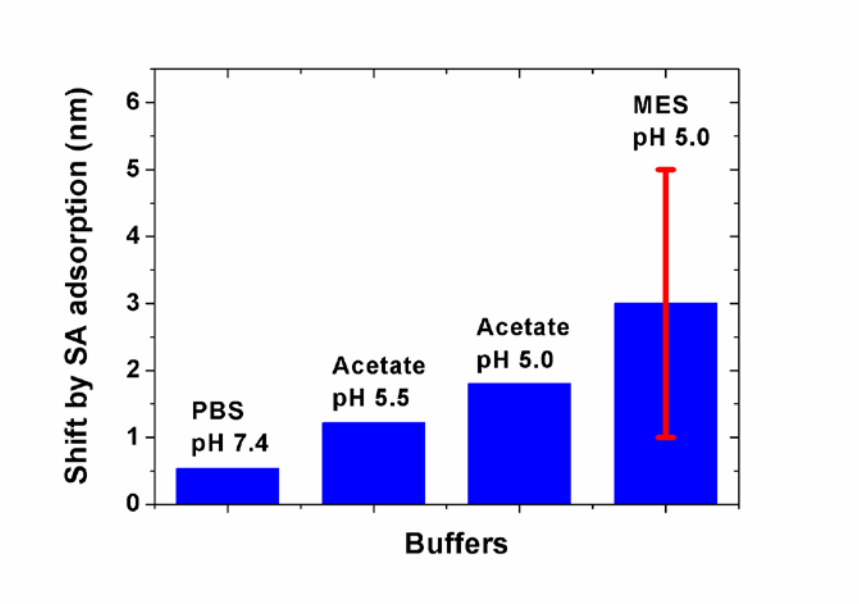
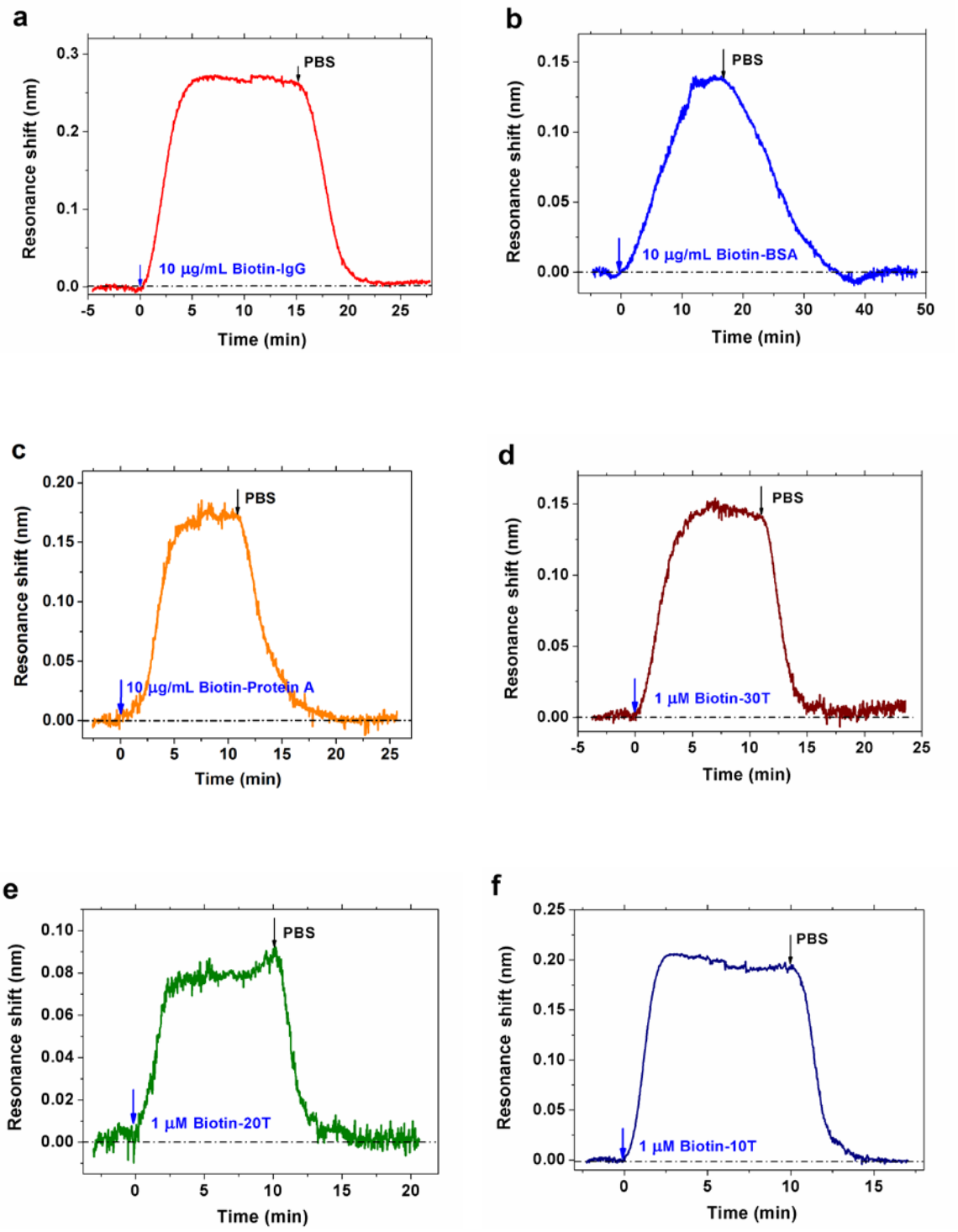


Figure S-4. The effect of buffer and pH on streptavidin adsorbed on the functionalized sensing surface via covalent binding method. The level of streptavidin immobilization in MES buffer changes with different EDC/NHS activation time.


a
Resonance shift (nm)
Time (min)
PBS
10 µg/mL Biotin-IgG
b
Resonance shift (nm)
Time (min)
PBS
10 µg/mL Biotin-BSA
c
Resonance shift (nm)
Time (min)
PBS
10 µg/mL Biotin-Protein A
d
Resonance shift (nm)
Time (min)
PBS
1 µM Biotin-30T
e
Resonance shift (nm)
Time (min)
PBS
1 µM Biotin-20T
f
Resonance shift (nm)
Time (min)
PBS
1 µM Biotin-10T

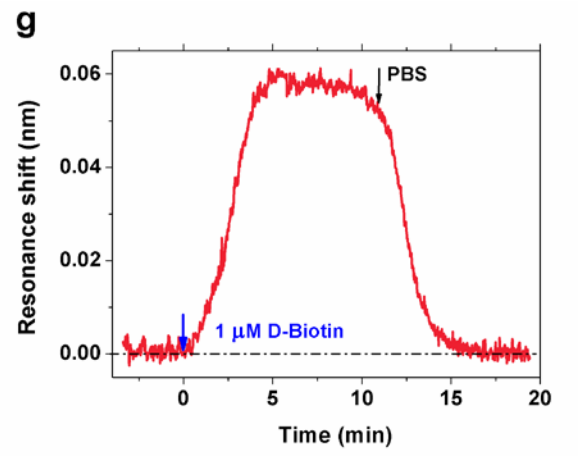


Figure S-5. Control experiments to check the nonspecific binding between biotin molecules and functionalized sensing surface. It shows that there are no observable nonspecific binding between these molecules and sensing surface. Notice that the sensor response to those biotin molecule solutions in PBS are not related to their molecular sizes, but the difference of their bulk solvent refractive indices from that of PBS buffer.