SUPPORTING INFORMATION: ELUCIDATING PROTEIN- LIGAND RECOGNITION WITH COMBINED SURFACE PLASMON RESONANCE AND SURFACE ENHANCED RAMAN SPECTROSCOPY

Ju-Young Kim, Zhi-Cong Zeng, Lifu Xiao and Zachary D. Schultz*

University of Notre Dame, Department of Chemistry and Biochemistry, Notre Dame, IN 46556.

*Corresponding Author E-mail: Schultz.41@nd.edu

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Association constant measurements. Different concentrations of free streptavidin protein and streptavidin-functionalized nanoparticles in PBS solution were injected into the flow channel (flow rate 3 μL/min) onto the biotin/MUOH SAM gold film. The concentrations of the free protein assume that all streptavidin exists as tetramers. The reflectivity was monitored over time at a fixed angle to produce the sensorgram (Figure 3a). The average value was taken from the equilibrium part of sensorgram for each solution. These average values were fit to a Langmuir adsorption isotherm (Equation 1) to determine the association constant (K_A).

$$R = R_{max}\left(\frac{C\,K_A}{C\,K_A+1}\right) \qquad \text{Eq. 1}$$

In Equation 1, R is the measured reflectivity, R_{max} is the maximum value of R, C is concentration of analytes (M), and K_A is the association constant. In the same way, binding constant of STV-AuNPs and biotin were determined as well.

Simultaneous SPR- SERS detection. Streptavidin and /STV-AuNPs solution were injected into the flow channel on biotin/MUOH SAM gold film (flow rate 3 μL/min). While SPR reflectivity was recorded by photodiode, SERS spectra were simultaneously recorded by the CCD detector. The SERS acquisition time was 5 s.

SPR sensorgram measurment. Using the angle-dependent reflectance curve to identify the SPR angle, SPR sensorgrams are recorded by fixing the excitation angle at the angle of maximum slope. This angle provides the greatest change in signal associated with changes in surface coverage.[1] Sensorgrams were constructed by monitoring the change in the reflected light intensity as a function of time and analyte concentration. Increases in reflectivity are attributed to surface association, while decreases in reflectivity indicate molecules are lost from the surface.

Flow channel preparation. The flow channel mold (12 mm × 2 mm × 0.3 mm) was made using single coated adhesive scotch tape (3M, MN) on a flat glass slide. PDMS elastomer and curing agent were mixed in volume ratio of 10 to 1 (Sylgard-184 polydimethyl siloxane substrate, PDMS, Dow corning corporation, MI), poured on the mold and cured at 65 °C for one hour. The PDMS substrate was then cut into pieces (about 20 mm × 10mm) and oxidized in plasma cleaner (Harrick plasma Inc., NY) for 2 minutes. The oxidized channel was placed on the Biotin/MUOH functionalized gold film. Analyte solutions (PBS, Streptavidin, and STV-AuNPs) were injected using a syringe pump (New era pump system Inc., NY) through a O.D. 150 μm, I.D. 75.9 μm capillary (Polymicro technologies, AZ).

Figure S1.

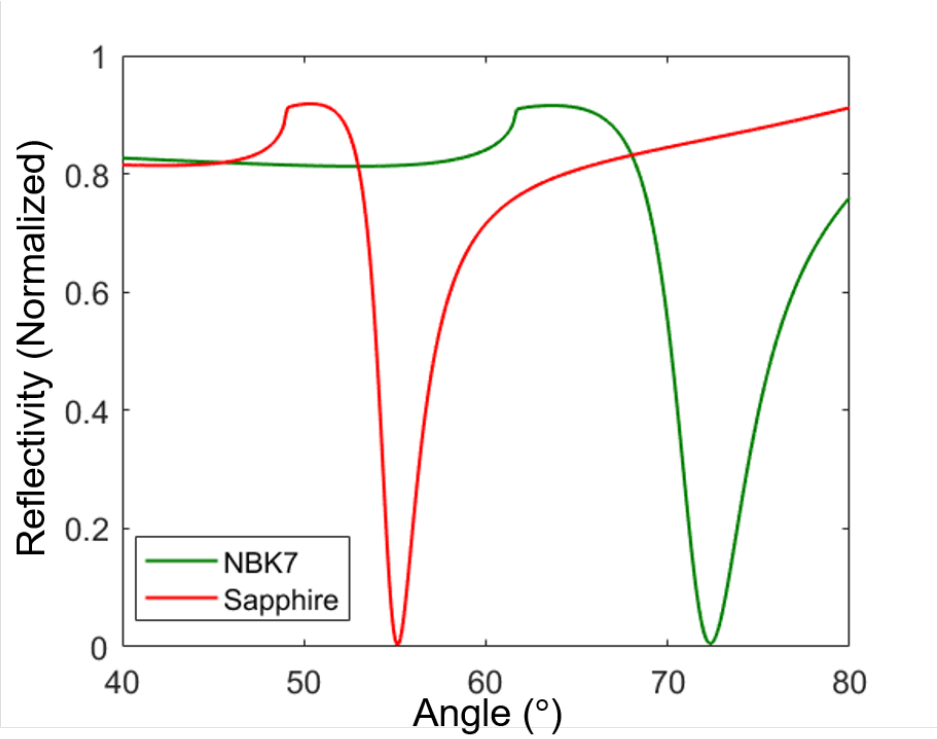


Figure S1. The theoretical SPR angle at air/gold (50nm thickness)/N-BK7 prism interface is 72.3° (green solid line) 55.1° from air/gold (50nm thickness)/sapphire prism interface (red solid line). The goniometer in our instrument enables scanning from about 30° to 60°, so we chose a sapphire prism whose SPR angle falls within the accessible range.

Figure S2.

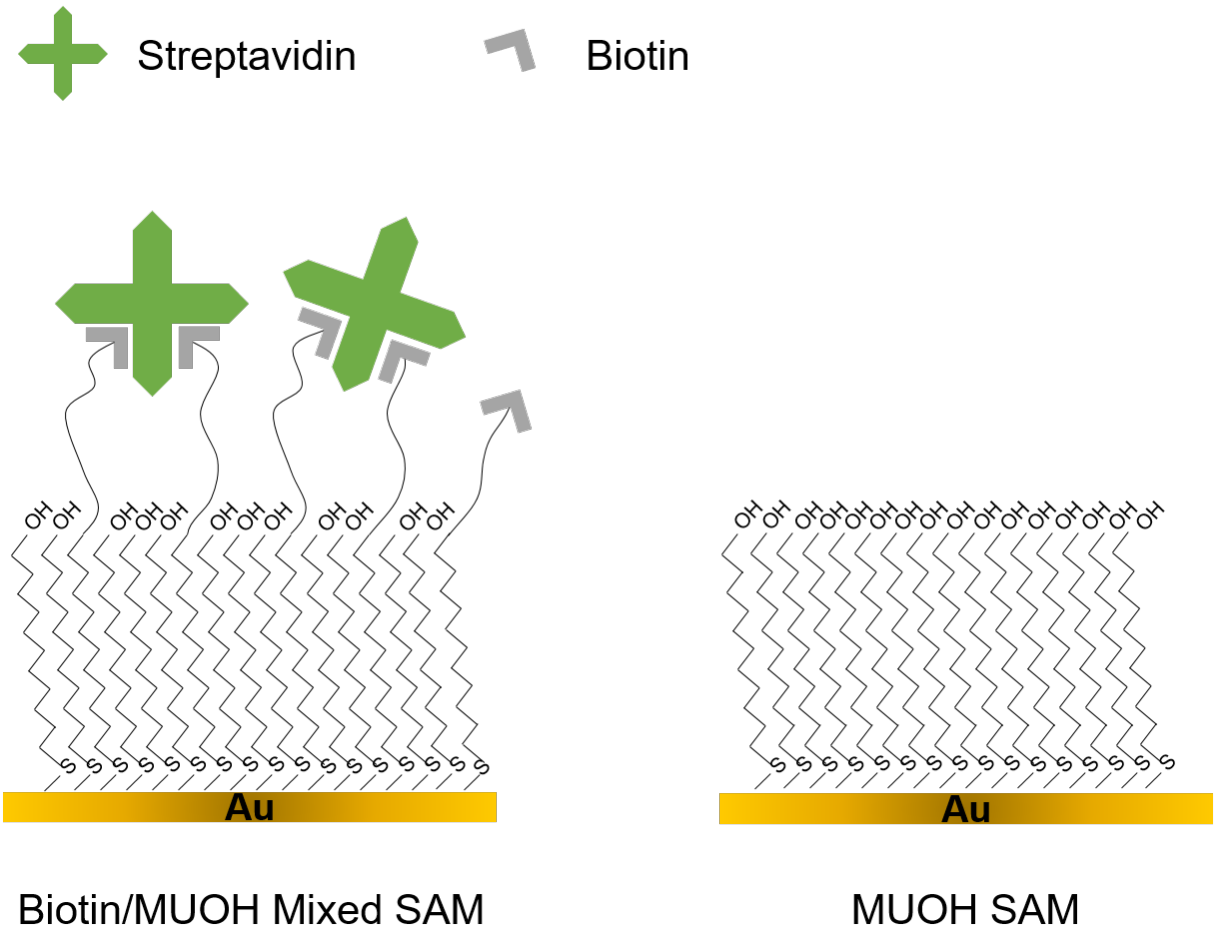
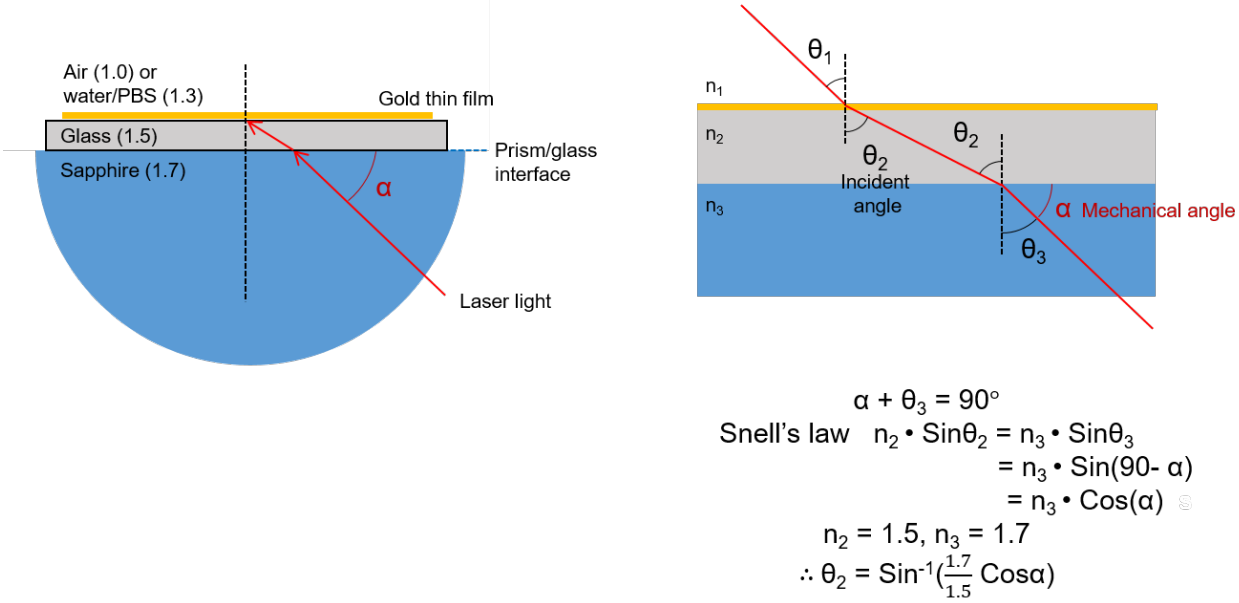


Figure S2. Biotin PEG thiol/ MUOH mixed SAM gold substrate.

(Left) Biotin/MUOH mixed SAM on gold substrate. Streptavidin forms strong covalent bonding with biotin. Gold film into 3 mL mixed solution of Biotin PEG Thiol (0.05 mM) and 11-mercapto-1-undecanol (MUOH, 0.45mM) in a 1:1 volume ratio for 24 hours, then rinsed with ethanol and dried by argon gas, and then re-immersed in ethanol and sonicated for 3 minutes and dried by argon gas.

(Right) MUOH SAM on gold substrate for control experiment. Streptavidin only nonspecifically binds to random MUOH molecules on the gold surface. The substrate was prepared in the same way with above, but using only the 0.45 mM MUOH solution.

Figure S3.



$$\alpha + \theta_3 = 90^\circ$$

Snell's law $n_2 \cdot \sin\theta_2 = n_3 \cdot \sin\theta_3$

$= n_3 \cdot \sin(90 - \alpha)$

$= n_3 \cdot \cos(\alpha)$

$n_2 = 1.5,\ n_3 = 1.7$

$\therefore \theta_2 = \sin^{-1}\left(\frac{1.7}{1.5}\cos\alpha\right)$

Figure S3. The diagram describes the calculation of the incident angle through on a gold film/ cover glass /prism interface.

Figure S4.

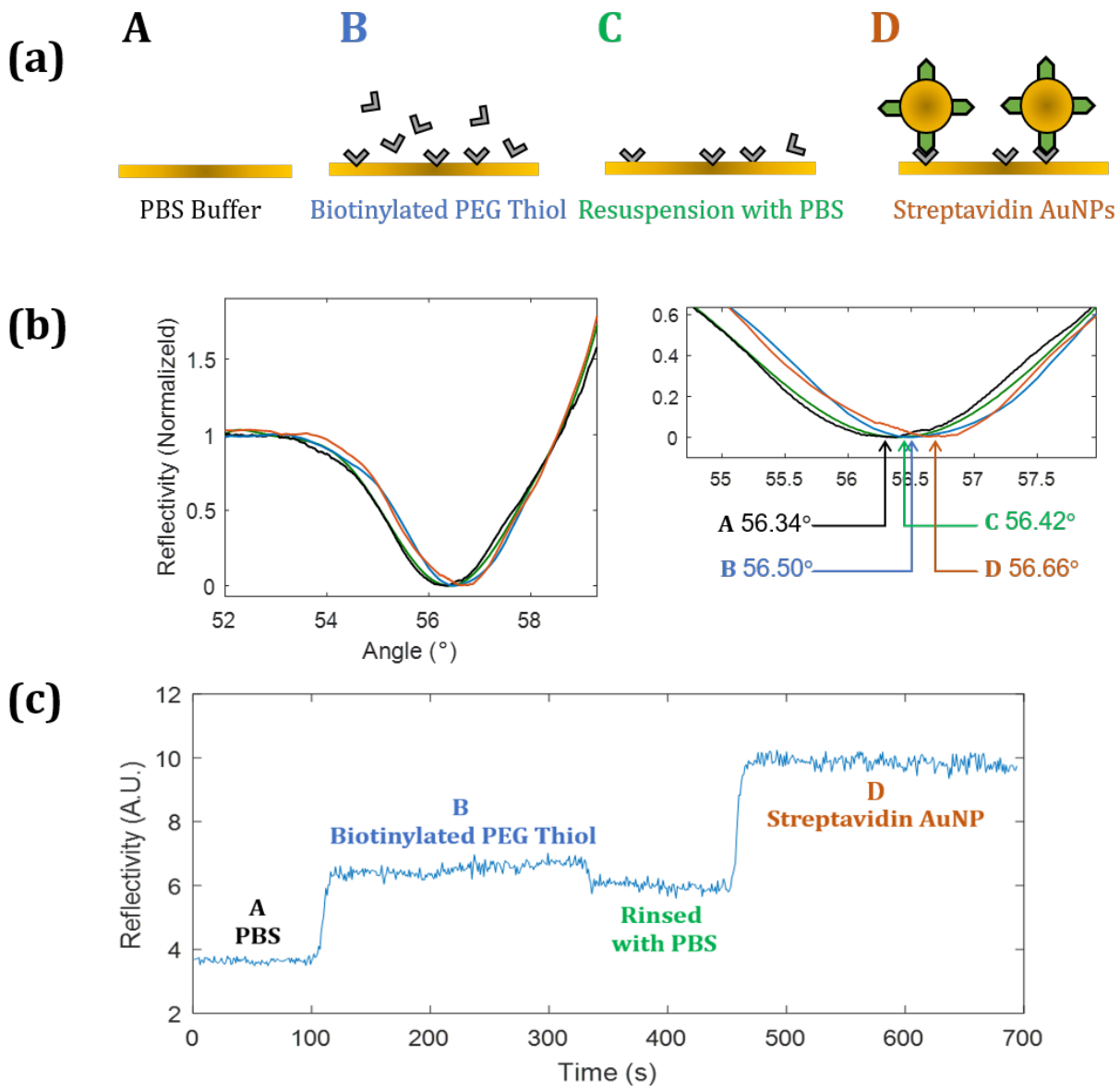


Figure S4. (a) The schematic illustrates the steps measured in the experimental data. (b) The SPR curves for each step are plotted. The inset shows a zoom of the minima observed. The SPR angles at each step are: A) 56.34° (PBS), B) 56.50° (biotin PEG thiol, 6.67×10^{-5} mM), C) 56.42° (rinsed by PBS), and D) 56.66° (0.3 nM of STV-AuNPs binding to gold film). (c) The SPR sensorgram for the sequential steps shown in (a) shows the reflectivity increases when molecules attach to the surface, and decreases when unbound molecules are rinsed away. (b) and (d) are separate trials and substrates, although all the other conditions were kept the same.

Figure S5.

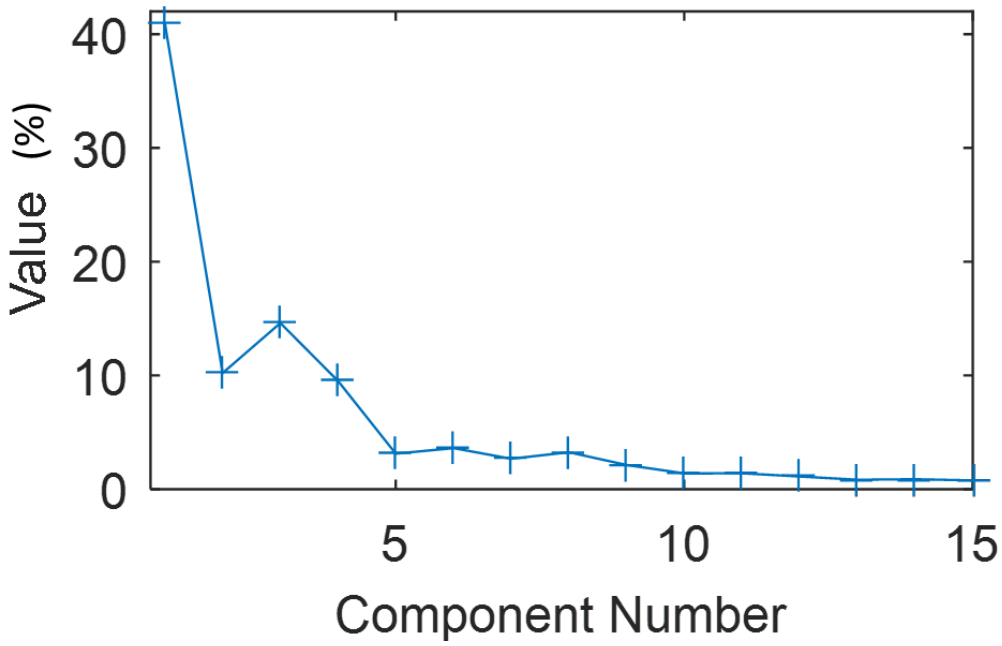


Figure S5. A scree plot of the MCR component number vs. fitting value. From component 5, the percentage of error shows low value. This justified that the 5 components of MCR can fit the data properly.

Table S-1.

Matter	Air	Water	PBS	Gold	Sapphire
Refractive index	1.000	1.332	1.334	0.173 + 3.422 i	1.766

Table 1. Refractive indices of matters at 632.8 nm wavelength.

Reference.

(1) Jang, D.; Chae, G.; Shin, S. *Sensors (Basel)* **2015**, *15*, 25385-25398.