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Supporting Information

Optimized Antibody Immobilization on Natural Silica-based Nanostructure for Selective Detection of *E. coli*

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A. METHODOLOGY SECTION

1. Materials

The materials used in this study were amorphous silica precipitate as the silica nanoparticle precursor, supplied by PT Geo Dipa Energi geothermal power plant in Dieng, Central Java, Indonesia. Sodium hydroxide (NaOH) was obtained from Kanto Chemical CI., Inc., Hydrochloric acid (HCl) was obtained from J.T Baker, Rhodamine-B and Cetyltrimethylammonium Bromide (CTAB) were purchased from Merck, Toluene was obtained from Tedia company, Inc., APTES, GTA, undecylenic acid, EDC, NHS, *E. coli* antibody, and Phospate Buffer Silane (PBS) were purchased from Sigma-Aldrich. *E. coli* ATCC 25922 was obtained from National Agency of Drug and Food Control (BPOM) of Indonesia.

2. Synthesis of Fluorescent Silica Nanoparticle

The synthesis of fluorescent nanoparticle followed that of our previous work [17]. Briefly, sodium silicate was synthesized from 20 g of rinsed silica geothermal powder by the sol-gel method with the addition of 800 mL of 1.5 N NaOH. The mixture was stirred using a magnetic stirrer for 60 minutes and heated at 90 °C, then filtered to separate the solution from the solids. The resulted sodium silicate solution was added with 0.05 g of Rhodamine-B, stirred until homogeneous, then titrated with 2 N HCl until gel was formed to pH 5. Afterwards, 2% CTAB (w/w) was added, and the mixture was allowed to stand for 18 hours, rinsed with DI water to pH 7, and dried in an oven at 100 °C overnight. The dried samples were labelled as fluorescent silica nanoparticle (FSNP).

3. Modification and Characterization of Fluorescent Silica Nanoparticle

For the modification of FSNP with *E. coli* antibody, two different surface chemistry approaches namely the silanization and hydrosylilation reaction using crosslinkers APTES/GTA and EDC/NHS, respectively, were conducted. For the silanization reaction (Scheme 1), 2 g of FSNP were added to APTES in 10 mL toluene solution (w/v). The concentrations of APTES were varied *i.e.*, 1.25, 2.5, 5, 10 and 20% w/v. The mixture was shaken at room temperature for one hour, then allowed to settle. The precipitate was rinsed twice with dry toluene, then dried under compressed air. The APTES-modified FSNP (FSNP-APTES) was rinsed using distilled water, then added with 2.5 % v/v glutaraldehyde solution in PBS and shaken at room temperature for 30 minutes. The solution and precipitate were separated using centrifuge, and then the precipitate was rinsed three times by using distilled

water and dried. The dried samples were labelled as FSNP-APTES-GTA. Subsequently 2 mg of FSNP-APTES-GTA samples were added with 1 mL of 200 ng/mL of *E. coli* antibody. The mixture was incubated at 37 ºC for one hour. Finally, the antibody-modified FSNP, denominated as FSNP-Ab1, was dried, characterized, and stored at 4 ºC prior to characterization.

The hydrosylilation reaction on the FSNP surface followed Scheme 2, 1 g of FSNP was immersed in 3 ml of undecylenic acid, and the mixture was heated under reflux at 120 ºC for three hours while stirred using a 100-rpm magnetic stirrer. After the reaction, the FSNP was rinsed three times with ethanol, and then dried under compressed air. The sample was labelled as FSNP-COOH. The FSNP-COOH was added with 1 mL of PBS and sonicated for 10 minutes. The solution was added with 10 µL of EDC and 100 µL of NHS. The molar ratio of EDC to NHS was varied *i.e.*, 4:1; 2:1; 1:1; and 0.5:1, and then shaken at room temperature for 30 minutes. The mixture was centrifuged for 20 minutes at 10,000 rpm, and the precipitate was separated and labelled as FSNP-EDC-NHS. The precipitate was added with 250 µL of PBS and 2 µL of *E. coli* antibody, and shaken for 60 minutes, then subsequently added with 2.5 µL of 1% (v/v) BSA in PBS and shaked for 60 minutes. The antibody-modified FSNP samples were labelled as FSNP-Ab2 and stored at 4 ºC prior to characterization.

4. Characterization of FSNP

The FSNP samples, prior and after surface modification, were characterized by using the Brunauer–Emmett–Teller (BET) method, X-Ray Diffraction (XRD), Fluorescence Spectrometer, Field Emission Scanning Electron Microscopes (FESEM) with Energy Dispersive X-Ray (EDX), Fourier Infrared (FTIR) Spectroscopy, and Ultraviolet-Visible (UV-Vis) spectrophotometer. Nitrogen adsorption-desorption isoterms were conducted in 77 K on Micrometritics Tristar II 3020 2.00 porosimeter to obtain the BET surface area, whereas the pore volumes were calculated by the amount of nitrogen adsorbed at a relative pressure of 0.99. Prior to each measurement, all samples were degassed at 110 °C and 10^{-4} Torr pressure to evacuate the physiosorbed moisture. The crystalline structure was analyzed by the XRD with a PW1710 diffractometer (Phillips, Netherlands), using CuKα radiation at 40 kV and 30 mA, and a secondary graphite monochromator. The fluorescence intensity was measured using a fluorescence spectrophotometer (Agilent, Singapore) at an excitation wavelength of 545 nm and an emission range of 550-750 nm. For FESEM-EDX measurements, the samples were prepared on silicon wafers. The samples for FESEM-EDX measurement were prepared by taking 100 µL of fluorescent nanoparticles and then dropped onto a silicon wafer and dried at

room temperature in a desiccator overnight. The FESEM-EDX images were obtained with JIB-4610F equipped with a Schottky electron gun, as well as a new FIB column capable of large current processing (maximum ion current of 90 nA) installed into one chamber. The FTIR characterization was carried out by FTIR Prestige-21 (Shimadzu, Japan) in transmittance mode at 16 cm^{-1} resolutions over the range of 400–4500 cm^{-1} with an accumulating average of 10 scans. The software used to generate spectra was IR Solution (Shimadzu, Japan).

5. Stability Test modified FSNP

A total of 5 mg of the crosslinker-modified FSNP were dissolved with 5 mL of aquadest. The mixture was placed in a cuvette and the fluorescence intensity of the samples was measured using a fluorescence spectrometer at an excitation wavelength of 545 nm. The measurements were conducted for 120 minutes and the maximum intensity at 588 nm was observed over an increment of 15 minutes.

6. *E. coli* Sensing Experiments Using Antibody-Modified FSNP

a. Bacteria preparation

E. coli ATCC 25922 culture was obtained from National Agency of Drug and Food Control (BPOM) of Indonesia. A total of 25 mL of sterile nutrient broth were added with 1 *ose* of bacteria from pure culture, and the mixture was incubated for 24 hours at 37 ºC in shaking incubator. The bacterial concentration was observed in UV-Visible Spectrophotometry (Hitachi U-2000, Japan) by using the McFarland method [39], with optical density (OD) absorbance of 0.08-0.1 in a wavelength of 625 nm.

b. Detection of *E. coli* using antibody-modified FSNP

A total of 0.5 mL of the optimized antibody-modified FSNP solution was added into 4.5 mL of *E. coli* culture. The mixture was incubated for 15 minutes in room temperature, and then the fluorescent intensity was observed using a fluorescent spectrometer at an emission wavelength range of 550-750 nm and an excitation wavelength of 545 nm. The detection of *E. coli* in the solution was carried out by calculating the decrease of the maximum intensity (%I_{loss}) of the fluorescence emission of the antibody-modified FSNP before and after the addition of bacteria. The percentage loss of intensity (%I_{loss}) was calculated by using the following equation: $I_{loss} = (I_0 - I_t) \,/\, I_0 \times 100\%$, with I_0 and I_t representing the maximum fluorescence intensity of FSNP before and after the incubation with *E. coli*, respectively after a certain incubation period. The measurements were conducted three times.

c. Analytical performance of antibody-modified FSNP

The bacterial sensitivity test was carried out following the procedure for the detection of *E. coli* mentioned above. The bacteria concentration was varied in the range of 10–10^7 CFU/mL. The percentage loss of intensity (%I_{loss}) was calculated for each concentration and measurements were conducted three times.

The selectivity test in this study was conducted by comparing the decrease of the maximum intensity (%I_{loss}) of the optimized antibody-modified FSNP solution when detecting *E. coli* to that when detecting *S. typhimurium* and *P. aeruginosa*. All bacteria were cultured to the same concentration of 10^7 CFU/mL. The mixture of FSNP solution with bacteria was rinsed three times by using centrifugation. The precipitate was added with PBS, and then the fluorescence intensity of the mixture was measured. The measurements were conducted three times.

B. RESULTS

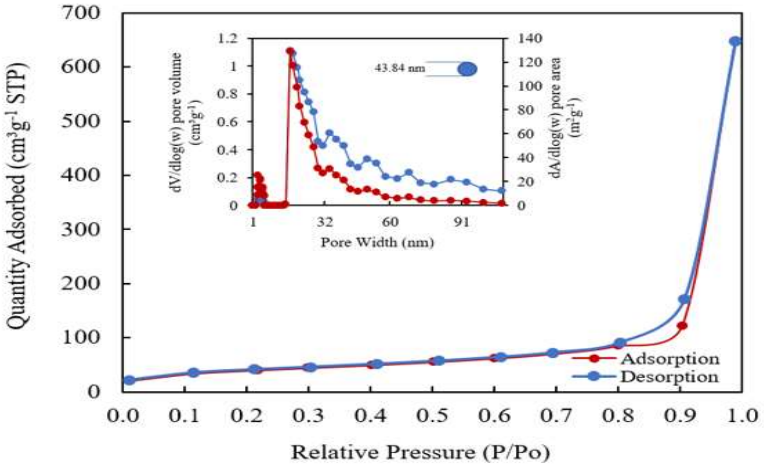


Figure S1. BET data of FSNP

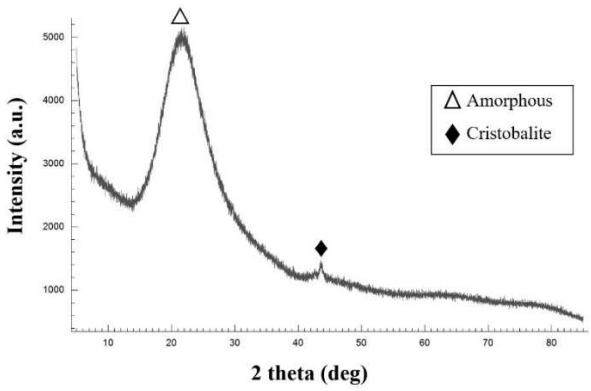


Figure S2. XRD profile of FSNP

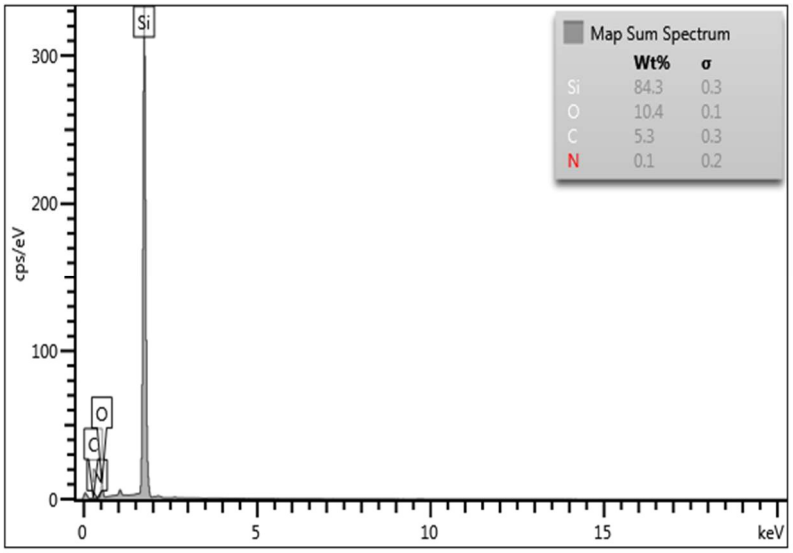
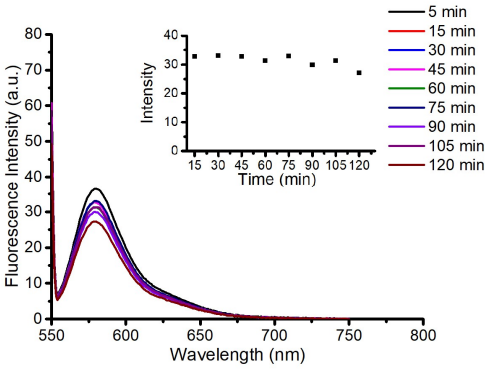
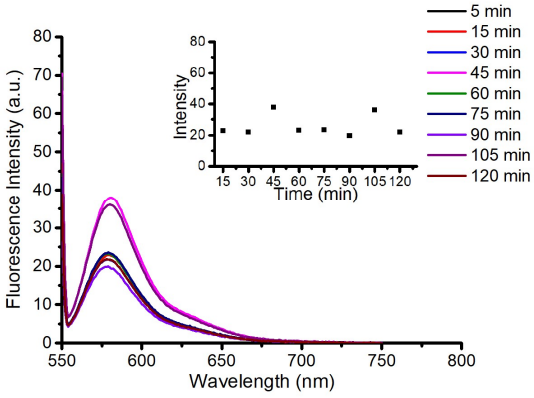


Figure S3. EDX images of FSNP

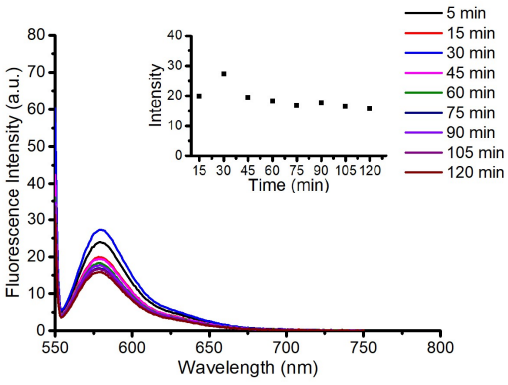
a. Silanization with APTES and GTA



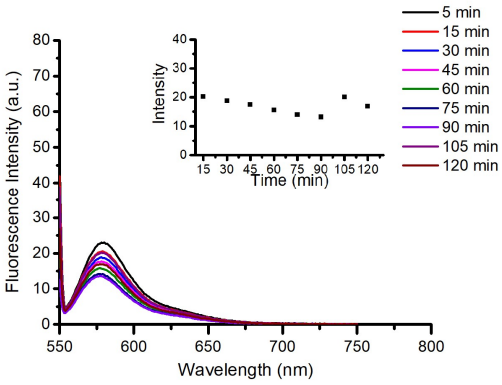
FSNP-APTES 0%



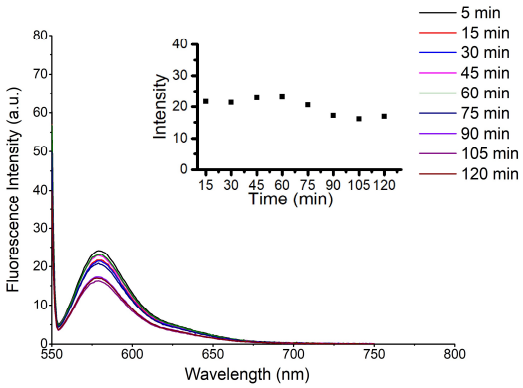
FSNP-APTES 1.25%



FSNP-APTES 2.5%

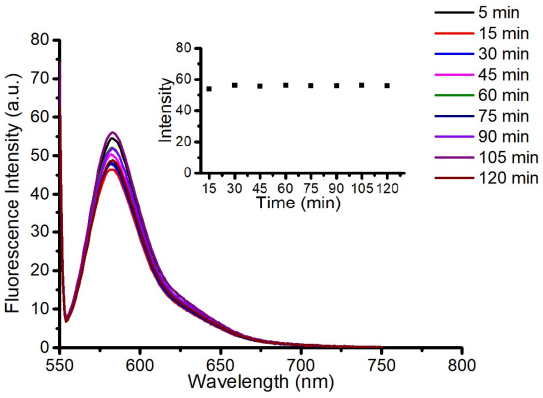


FSNP-APTES 5%

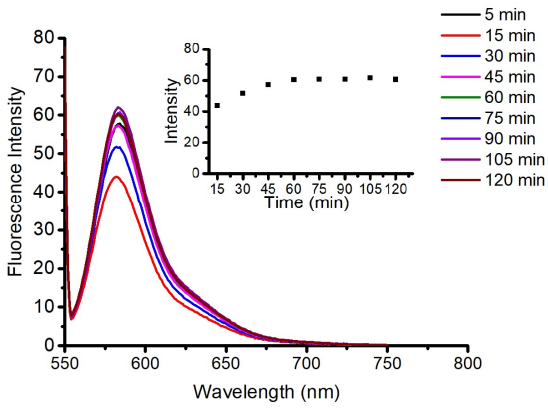


FSNP APTES 20%

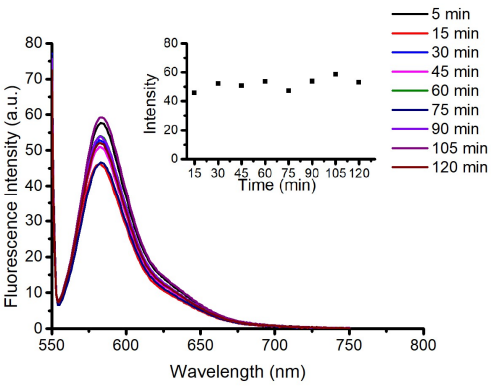
b. Hydrosylilation with undecelynic acid and EDC/NHS



FSNP- EDC/NHS 2:1



FSNP- EDC/NHS 1:1



FSNP-EDC/NHS 0.5:1

Figure S4. Fluorescence stability test of FSNP after silanization (a) and after hydrosylilation reaction (b) at various concentrations of crosslinking agents for 120 minutes in aquadest.

Yield of Immobilized Antibody on Modified FSNP

The yield of antibody immobilized on the FSNP samples was estimated through the absorption difference of the modified FSNP concentration in solution before and after the immobilization of antibody, referring to samples FSNP-EDC/NHS and FSNP-Ab2, respectively. The absorbance of the samples were measured at the same concentration of 1 mg/mL in aquadest using UV-Vis Spectrophotometer. The difference of absorption was calculated using the following equation,

$$\frac{A_{561}, before - A_{561}, after}{A_{561}, before}$$

Where A_{561} is the absorbance of FSNP at 561 nm, which is the maximum intensity of the modified FSNP as shown in Figure R1. We measured the absorbance in triplicate and the results are summarized in Table R1. Given that A_{561},before = 0.386 and A_{561}, after = 0.201, then the absorption difference was calculated at 0.479, hence a 47.9% drop of absorption was determined. This correlates to the amount of antibody coverage or the yield of antibody on the nanoparticle surface was 47.9%.

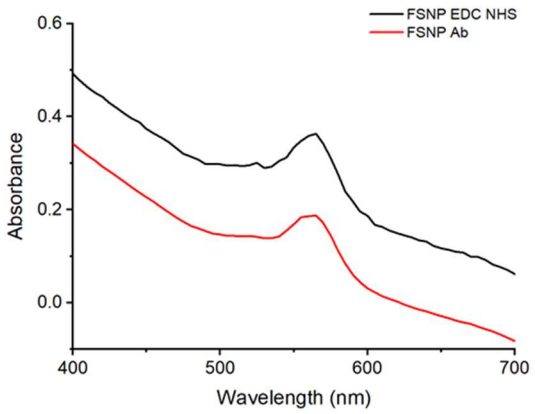


Figure S5. Absorbance of modified FSNP in aqueous solution before and after immobilization of antibody.

Table S1. Measured absorbance values used to calculate the corresponding concentration of modified FSNP in solution before and after immobilization

	Absorbance at 561 nm	Standard Deviation
Before immobilization with antibody (FSNP-EDC/NHS)	0.386	± 0.017
After immobilization with antibody (FSNP-Ab2)	0.201	± 0.011

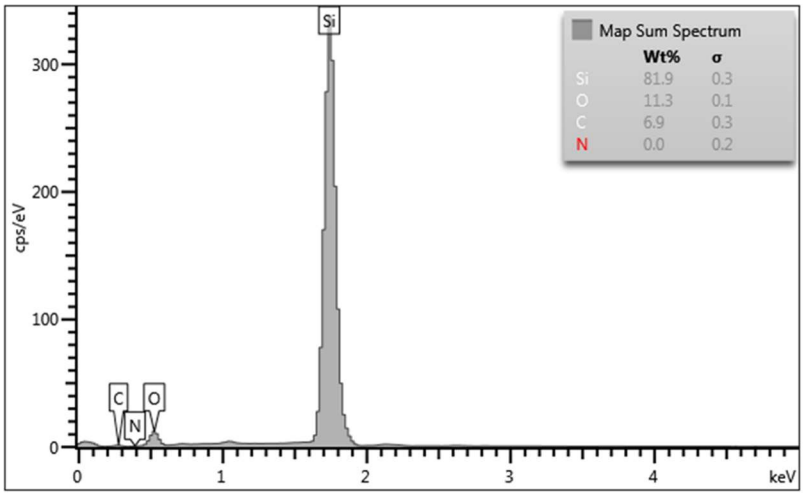


Figure S6. EDX images of FSNP-Ab2

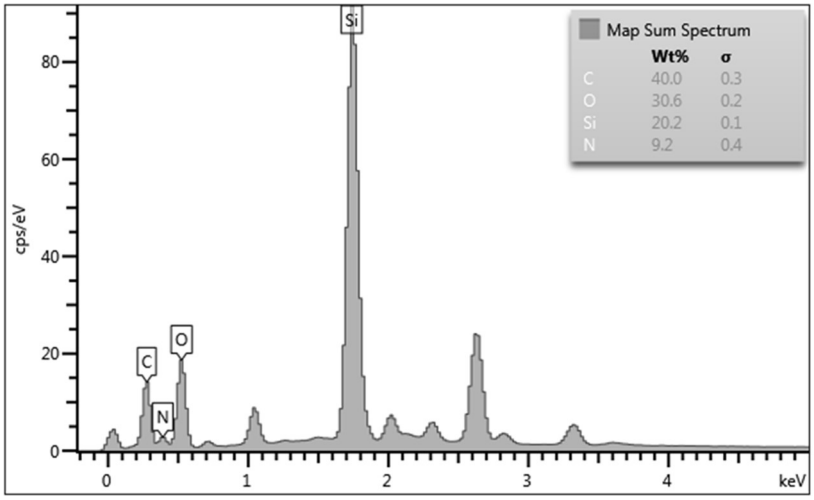


Figure S7. EDX images of FSNP-Ab2 after the incubation with *E.coli*