Supporting Information

Label-free and reusable antibody-functionalized gold nanorod arrays for the rapid detection of *Escherichia coli* cells in a water dispersion

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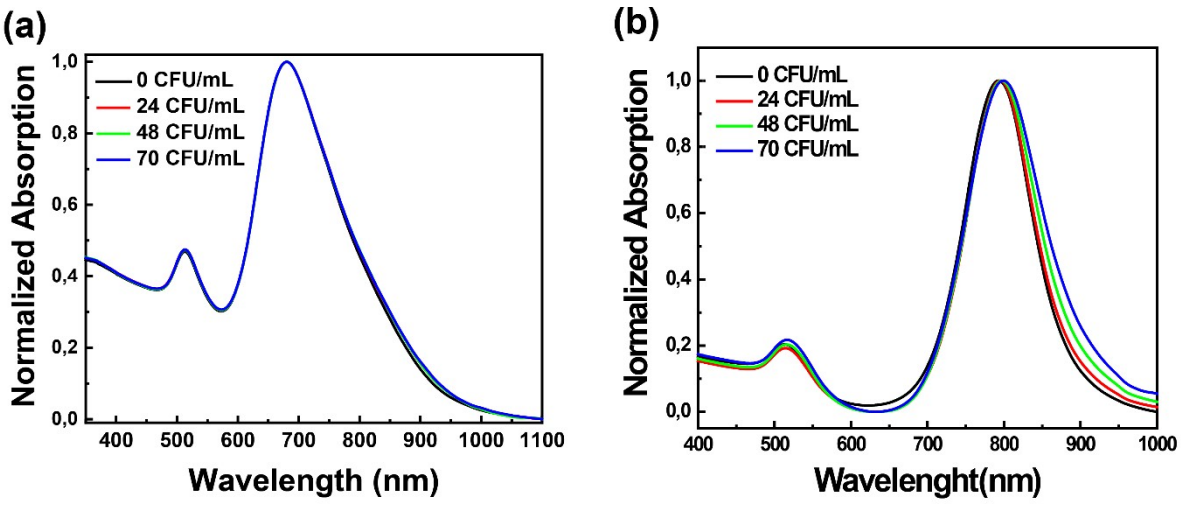

Figure SI 1 Spectroscopic investigation of Au NRs to recognize *E. coli* cells in a water dispersion in a representative concentration range from 24 to 70 CFU/mL. Absorption spectra of Au NRs 660 measured at different *E. coli* cell concentration in the wavelength range from 400 nm to 1100 nm (a); Absorption spectra of Au NRs 800 measured at different *E. coli* cell concentration in the wavelength range from 400 nm to 1100 nm (b), highlighting that the maximum wavelength of the LSPR*l* keeps unchanged as the *E. coli* cell concentration increases.

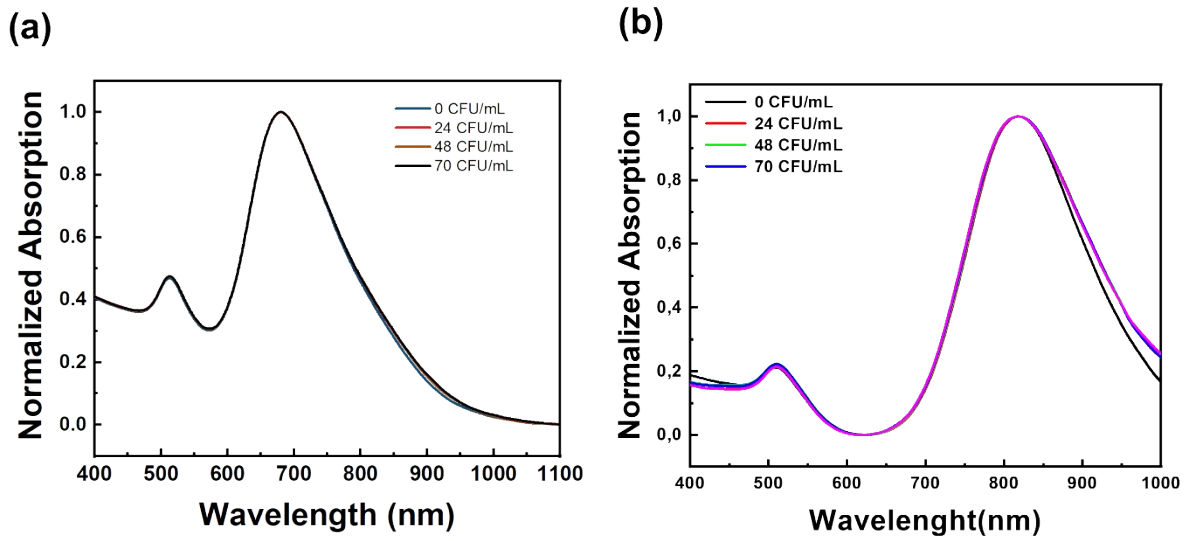

Figure SI 2 Spectroscopic investigation on the ability of Au NRs/PAH complex to recognize *E. coli* cells in a water dispersion in a representative concentration range from 24 to 70 CFU/mL. Absorption spectra of Au NRs 660/PAH measured at different *E. coli* cells concentration from 400 nm to 1100 nm (a); Absorption spectra of Au NRs 800/PAH measured at different *E. coli* cells concentration from 400 nm to 1100 nm (b). Experimental results pointed out that the maximum wavelength of the LSPR*l* of the Au NRs 660/PAH keeps unchanged as the *E. coli* cells concentration increases. The Au NRs 800/PAH produced a total optical shift of 7 nm for an *E. coli* concentration of 70 CFU/mL, lower respect to the optical shift measured for the Au NRs 800/PAH/Ab bioconjugate (16 nm), suggesting a weak electrostatic attraction between *E. coli* cells and the outer layer of Au NRs 800/PAH.

Table SI1 Parameter calculated from the four-parameter logistic interpolation of the biosensing experiments assisted by the Au NR- based bioconjugates, performed in water dispersion.

Parameter	**Au NRs 660/PAH/Ab**	**Au NRs 800/PAH/Ab**
$\Delta\lambda_{max}$(nm)	3.98 ± 0.24	18.14 ± 0.24
$\Delta\lambda_0$(nm)	-0.1 ± 0.2	0.042 ± 0.63
c (CFU/mL)	12 ± 1	11.68 ± 2.79
p	2.55 ± 0.6	1.04 ± 0.21
IC10 (LOD) (CFU/mL)	5.07	1.4

Table SI 2 Refractive indices of the *materials* used for infiltrating the glass cells fabricated with Au NRs 800 substrates.

Compound	**Refractive index**
water	1.333
Olive oil	1.467
NOA 61	1.55
E 7	1.597

Stability of the LSPR*l* absorption signal for Au NRs 800 functionalized substrates

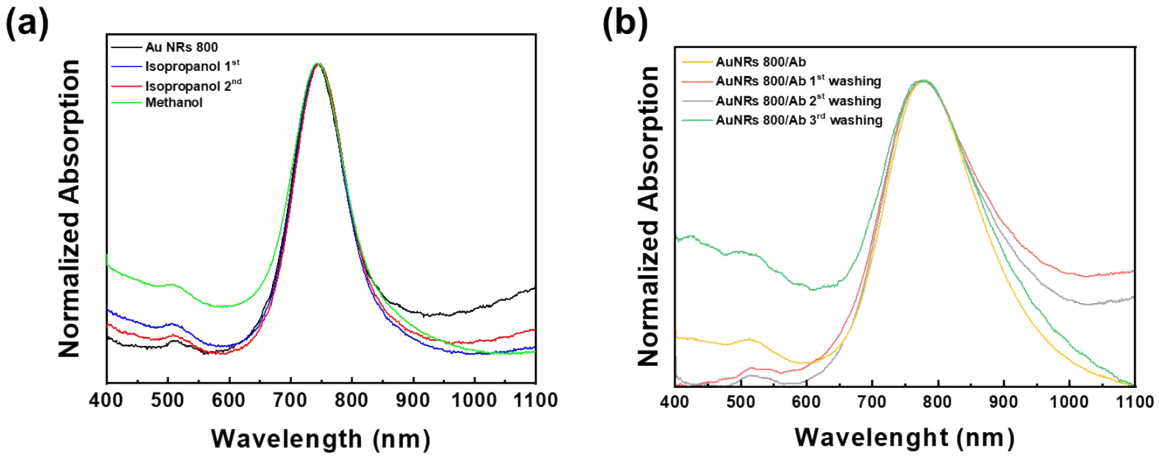

Figure SI3 Spectroscopic characterization of the Au NRs 800 substrate after washing steps performed with isopropanol and methanol for investigating the chemical stability (a).Spectroscopic characterization of the Au NRs 800/Ab substrate after several washing steps with deionized water (b) for studying the mechanical stability.

The stability of the LSPR*l* signal was investigated under a chemical and a mechanical perturbation. In particular, the strength of the spectroscopic features of the Au NRs 800 sample under a chemical perturbation was investigated. To this end, the sample was consecutively washed two times, once with isopropanol and more one time with methanol. The washing steps were performed by vigorously rinsing the substrate under a flux of solvents. Results reported in Figure **SI3 a** indicate that the LSPR*l* remains almost unchanged after the chemical interferences within a range of ± 3 nm.

After the bioactivation process, the mechanical stability of the resulting Au NRs 800/Ab sample was addressed. In particular, the Au NRs 800/Ab sample was washed three times. The washing step was performed by vigorously rising the substrate under a flux of deionized water. Subsequently, the absorption spectrum was acquired to evaluate possible alterations of the position of the LSPR*l* after each washing step. Results reported in **Figure SI3b** highlight that the as-prepared Au NRs 800/Ab displays an LSPR*l* centered at 779 nm. After the washing steps, the average wavelength value of the LSPR*l* is 778 ± 2 nm, indicating excellent stability of the LSPR*l* position for the Au NRs 800/Ab upon a mechanical perturbation.

Role of the monoclonal antibody

The Au NRs 800 substrate were bioactivated with a monoclonal antibody that plays a twofold role of i) behaving as a capture antibody and ii) triggering the antigen-antibody recognition that the AuNRs 800/Ab substrate transduces in a shift of the LSPR*l* position. To further demonstrate the function of promoting the accumulation of *E. coli* cells on the Au NRs 800/Ab substrates, the number of *E. coli* cells (obtained from the microscopy images displayed in Figures 6 b, e, h, and k, by analyzing a region of interest of 90000 μm^2) are reported. Experimental results reported in **Figure SI 4** demonstrated an increase in the counted *E. coli* cells with increased *E. coli* cell concentrations.

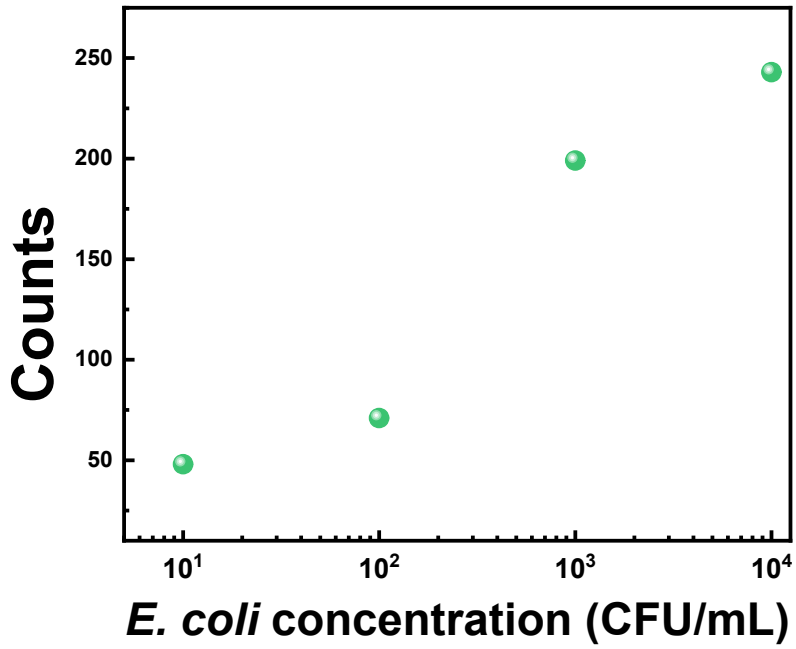

Figure SI4 Evaluation of *E. coli* cell number confined on the plasmonic bioactive substrates, determined by analyzing fluorescence microscopy images as a function of the *E.coli* cells concentration in the incubation dispersion, reported in logarithmic scale.

In addition, a further control experiment was performed, and the results are reported in **Figure SI 5**. An Au NRs 800 functionalized substrate was immersed for 20 min in an *E. coli* cell dispersion of 10^2 CFU/mL. Then 2 µL of SYTO 9™ molecules were introduced, and the Au NRs 800 was left to

incubate in the dark for an extra 10 min. After that, the sample was washed, dried under nitrogen flow, and characterized, as reported in **Figure SI 5**.

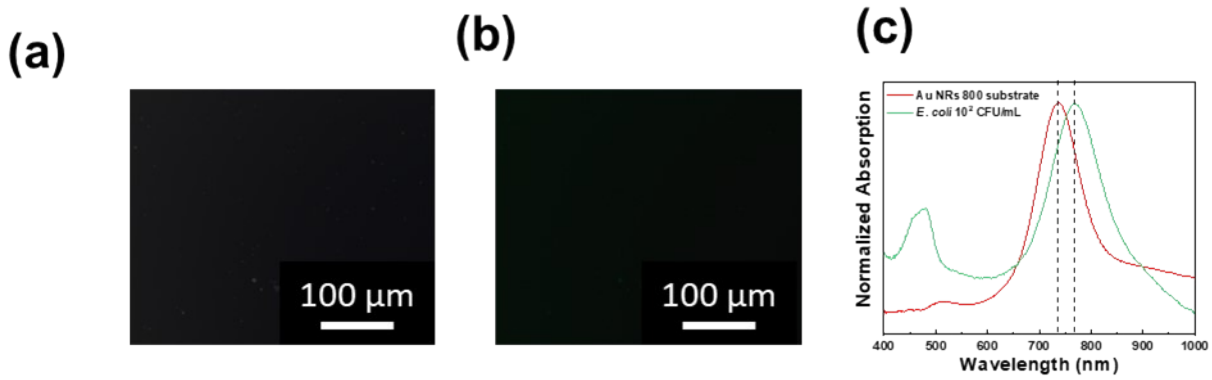

Figure SI 5 Control experiment: characterization of the plasmonic substrates (without the incorporation of the Ab) after the incubation in *E. coli* dispersion in minimal E 10^2 CFU/mL. Contrast phase microscopy image (a) fluorescence microscopy image (b) and absorption spectroscopy characterization (c). A very low amount of *E. coli* cells were accumulated on the plasmonic substrate. Therefore the LSPRl shift of 33 nm is associated with the presence of SYTO 9^{TM} molecules.

Figure SI 5 a and b demonstrated that in the absence of the Ab, the bare Au NRs 800 functionalized substrate could not accumulate *E. coli* cells, and the optical shift of **Figure SI 5 c** can not be associated with a biorecognition event likewise, a false-positive test.

Spectroscopic response of the Au NRs 800/Ab biosensor and effect of the staining agent

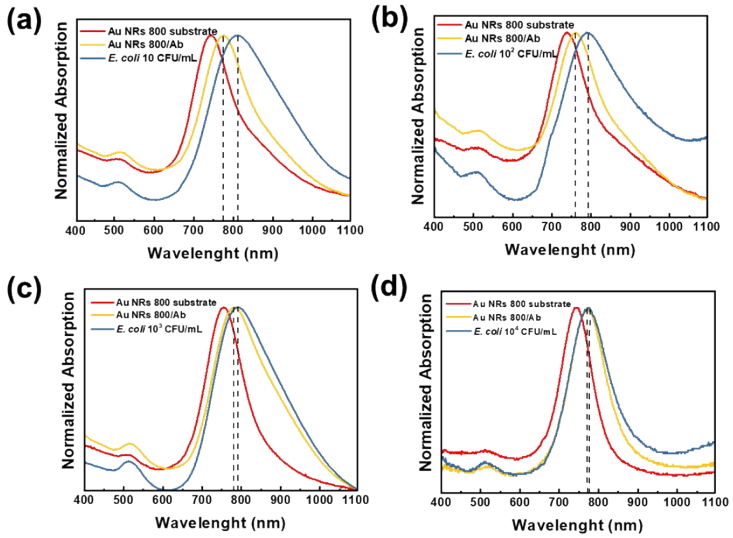

Figure SI 6 Characterization of the bioactive plasmonic substrates after incubating *E. coli* dispersion in minimal minimum E at different cell concentrations. 10 CFU/mL (a), 10^2 CFU/mL (b) , 10^3 CFU/mL (c), and 10^4 CFU/mL (d). All the experiments were performed without the staining agent.

Several control experiments were performed to get more insight into the spectroscopic response of the Au NRs-based biosensor and to elucidate further the role played by the SYTO 9™. In particular, the spectroscopic recognition experiment of *E. coli* cells (described in the section "Spectroscopic detection of *E. coli* cells on Au NRs-based glass substrates") was performed in the absence of the staining agent. The results are reported in **Figure SI6**. Experimental results in **Figure SI 6 a-d** pointed out that the interaction between the *E. coli* cells and the Au NRs 800/Ab produced a redshift of the LSPR*l*. In particular, the Δλ values are 34 nm (**Figure SI 6 a)**, 27 nm (**Figure SI 6 b)**, 13 nm (**Figure SI 6 c),** and 7 nm (**Figure SI 6 d)** for the cell concentration of 10 CFU/mL, 10^2 CFU/mL, 10^3 CFU/mL 10^4 CFU/mL respectively. Accordingly, the higher *E. coli* cell concentrations, the lower are the induced Δλ values, as reported in **Figure 7b** and discussed in the main manuscript. The role played by the SYTO 9™ in the spectroscopic behavior of the bioactive substrate Au NRs 800/Ab was deepened by performing two additional control experiments.

The first experiment aims to assess whether the bioactive Au NRs 800/Ab substrate in contact with the SYTO 9™ molecules experienced an increase in the refractive index. The investigation was carried out by incubating the Au NRs 800/Ab substrate in 500 µL of minimal E for 20 min in the dark, followed by introducing 2 µL of SYTO 9TM. After that, the Au NRs 800/Ab was left in the

dark for 10 min and subsequently raised (immersion in 600 µL of water for 1.5 min), dried under nitrogen flow, and characterized by absorption spectroscopy. The experimental results are reported in **Figure SI7 a**. The UV-Vis spectrum of the Au NRs 800/Ab substrate pre-adsorbed by SYTO 9^{TM} (Au NRs 800/Ab S) clearly shows the absorption signal at 483 nm, indicating the presence of SYTO 9^{TM} molecules on the surface of the bioactive substrate. Such an intense absorption band hinders the weak transverse plasmon band of Au NRs 800 centered at 520 nm.

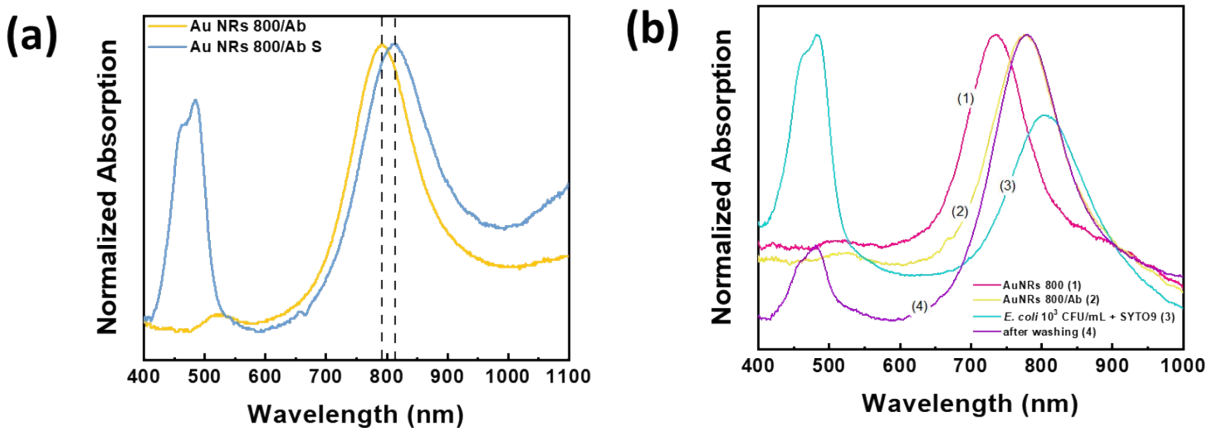

Figure SI 7 Control experiments: (a) spectroscopic characterization of the plasmonic bioactive substrates (after the incubation in 500 µL of minimal E (for 20 min) followed by the introduction of 2 µL of SYTO $^{9TM.}$. (b) Investigation of the effect of SYTO 9TM molecules on the spectroscopic behavior of Au NRs 800/Ab substrate. After the Au NRs immobilization (track/spectrum 1, red) and bioactivation (track/spectrum 2, yellow) the substrate was immersed in an E. coli cell dispersion of 10^3 CFU/mL (20 min) and stained with SYTO 9TM molecules (10 min). After this step, the substrate was dried, and the absorption spectrum was collected (track/spectrum 3, cyan). Then, the sample was rinsed and dried to remove the staining agent. The corresponding absorption spectrum (track/spectrum 4, purple) highlights a blue shift of the LSPRl from 804 nm (excess of SYTO 9TM) to 779 nm.

In the Au NRs 800/Ab-S, the intense Au NRs 800 LSPR*l* is centered at 814 nm, resulting in a red-shift (Δλ) of 23 nm to the Au NRs 800/Ab. The result indicates that the Au NRs 800/Ab, interacting with SYTO 9^{TM} molecules, came into contact with a chemical environment with a high refractive index value.

To further corroborate this experimental evidence, an additional experiment was carried out. The bioactive substrate Au NRs 800/Ab (**Figure SI7 b**, track 2) was immersed in 500 µL of an *E. coli* cell dispersion 10^3 CFU/mL (in minimal medium E), selected as a representative cell concentration. After 20 min of contact with the *E. coli* cell dispersion, the substrate was stained with SYTO 9™ (2 µL) for 10 minutes, dried, and the absorption spectrum was collected without performing the washing procedure. **Figure SI7 b** (track/spectrum 3) shows that the LSPR*l* peak is centered at 804 nm. After washing, the LSPR*l* maximum was shifted to 779 nm (**Figure SI7 b**, track 4). The reported results further confirm that the SYTO 9™ increased the refractive index of the medium surrounding the Au NRs array. However, as reported in the main manuscript, the staining agent produced a passivation layer accounting for the sensitivity decrease as demonstrated by experimental data reported in **Figure 7 b-c**.

Au NRs 800/Ab specificity

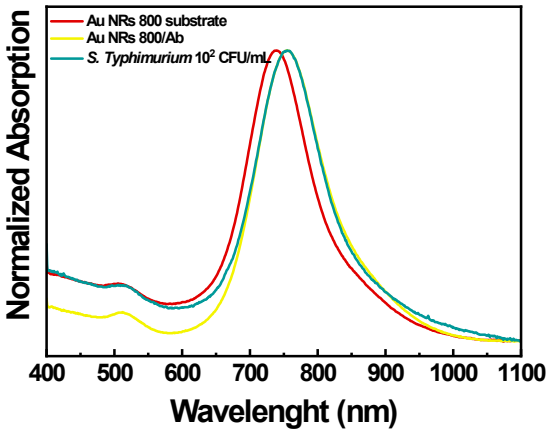

Figure SI 8 Specificity investigation was performed by incubating the Au NRs 800/Ab substrate in a dispersion of S. Typhimurium in minimal E. The S. Typhimurium induced a negligible shift of the LSPRl (±2nm), thus pointing out the specificity of the bioactive substrate for E. coli recognition.

To demonstrate that the biofunctionalization of Au NRs 800 substrates with a monoclonal antibody resulted in a specific Au NRs 800/Ab biosensing nanoplatform we performed a control experiment by testing the optical response of the Au NRs 800/Ab substrates in the presence of another coliform: the *Salmonella enterica serovar Typhimurium LT2* . In particular, a freshly prepared Au NRs 800/Ab

substrate was incubated for 30 in an *S. Thyphimurium* dispersion 10^2CFU/mL in minimal medium E. After the incubation, the substrate was rinsed with water and dried under nitrogen blow before measuring the absorption spectrum. Experimental results reported in **Figure SI8** highlighted that the *S. Thyphimurium* did not alter the LSPR*l* position of the Au NRs 800/Ab, thus providing conclusive evidence that the specificity of the biofunctionalized Au NRs 800/Ab substrates.