Electronic Supplementary Information for

Electrostimulus Associated PD-L1 Expression on Cell Membrane Revealed by Immune SERS Nanoprobes

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1. Experimental Section

1.1 Materials

The 4-mercaptobenzoic acid (4-MBA), silver nitrate (AgNO₃), sodium citrate and PBS buffer solution (0.1 M Na₂HPO₄, 0.1 M Na₂HPO₄, pH = 7.4) were purchased from Beijing Chemical Reagent Company. The Dulbecco’s modified Eagle’s medium (DMEM), antibiotic solution and 0.25% trypsin/2.2 mM EDTA solution were purchased from Biological Industries biotech Co., Ltd. The certified fetal bovine serum (FBS) was purchased from Vivacell, Shanghai, China. Calcein-AM (2.0 μM) and propidium iodide (PI, 4.0 μM) were bought from Sigma-Aldrich. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay kit was bought from Aladdin. Ltd. PD-L1 antibody (Ab) was obtained from the Shanghai abcam Co., Ltd. HeLa (cervical cancer), MCF-7 (breast cancer), and H8 (cervical epithelial) cell lines were bought from the American Type Culture Collection (ATCC, USA).

1.2 Instruments

Ultraviolet-visible (UV-vis) absorption spectra were obtained with a Lambda 750 spectrophotometer (Perkin-Elmer). Dynamic light scattering data were measured with a Zetasizer Nano ZS90 from British Marwen Co., Ltd. The inverted microscope (Leica DMI6000B, Germany) with a fluorescence module having three excitation wavelength ranges was employed for fluorescent imaging. Pictures were collected with a Leica DFC450 C digital camera. TEM (Jeol, Tokyo, Japan, JEM-2100F) was used to take the photo of Ag NPs, and the nano measure 1.2.5 was used to measure the size of Ag NPs.

1.3 Cell Culture

HeLa, MCF-7 and H8 cells were grown in the Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 10 % fetal bovine serum (FBS), 100 U/mL penicillin,
and 100 μg/mL streptomycin at 37 °C in a humidified atmosphere containing 5% CO₂.

1.4 Preparation the Ag NPs

The Ag NPs were prepared based on the Lee’s method. ¹ AgNO₃ (0.027g) was dissolved in 150 mL of pure water to boil, then 3.0 mL of sodium citrate (1.0 wt %) aqueous solution was added. The mixed solution was boiling for 40 min and then cooled to room temperature to obtain a greyish-green AgNP colloid.

1.5 MTT assay

The MCF-7 cells were planted in the 96 -well microtiter plates for 24 h culture. Then, the cells were washed three times using a cold PBS solution (pH=7.4). Different concentrations of the prepared immune SERS tags were added to each well and incubated with cells for 24 h. After that, the cells were washed three times with PBS. 10 μL of the MTT solution (5.0 mg/mL) was added into each well and cultured at 37 °C for another 4 h in the CO₂ incubator. After removing the supernatant medium, 150 μL of DMSO was added to each well to dissolve purple formazan crystals. The absorbance values of the wells were measured by using a microplate reader at 570 nm.
2. Results

2.1 The TEM imaging of the Ag NPs

Figure S1. (a) The TEM image of the Ag NPs. The scale bar is 100 nm, (b) The size distribution of the Ag NPs.

2.2 Dark-field imaging of MCF-7 cells cultured with immune SERS tag

Figure S2. The dark-field images of MCF-7 cells incubated with the immune SERS tags for different periods. The scale bar is 75 μm.

2.3 The AM/PI fluorescent imaging

Figure S3. The fluorescent images of MCF-7 cells standing for different periods (10 to 50 min) after the ES treatment, and then they were stained with AM/PI.
2.4 Reproducibility of PD-L1 specific SERS tag in response to different cells

![SERS spectra of different cell lines](image)

**Figure S4.** The SERS spectra of different cell lines; MCF-7 (a), HeLa (b), and H8 (c) cells, respectively. (a1)-(c1) The corresponding reproducibilities of the SERS spectra recorded on 10 randomly selected cells. Relative standard deviation (RSD) values at the 1575 cm\(^{-1}\) band are 18% for MCF-7 cells, 8% for HeLa cells, and 15% for H8 cells, respectively.

The repeatability of immune SERS tag for cell lines testing was performed (as shown in Figure S5), and the results indicate that the relative standard deviations (RSD) at 1575 cm\(^{-1}\) are less than 20 %, which meets the standards of SERS detections and our immune SERS tag provides acceptable reproducibility.\(^7\)

2.5 Characterizations of the immune nanoprobes without SERS tag

![Characterizations](image)

**Figure S5.** a) The UV-vis spectra of Ag NPs and Ag NP-Ab. b) The zeta potentials of the Ag NPs and the Ag NP-Ab. c) The MTT assays for MCF-7 cells after they were incubated with different concentrations of the Ag NP-Ab for 24 h.
2.6 SERS band assignments for cell membrane

Table S1 The SERS band assignments for the cell membrane compartments

<table>
<thead>
<tr>
<th>Peak (cm⁻¹)</th>
<th>Assignment</th>
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<tbody>
<tr>
<td>415</td>
<td>Phosphatidylinositol</td>
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<tr>
<td>480-494</td>
<td>Glycogen</td>
</tr>
<tr>
<td>543</td>
<td>Cholesterol</td>
</tr>
<tr>
<td>569</td>
<td>Tryptophan</td>
</tr>
<tr>
<td>653</td>
<td>ν(C-S) gauche (aminoacid methionine)</td>
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<tr>
<td>800</td>
<td>Phosphodiester</td>
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<tr>
<td>918</td>
<td>Proline, hydroxyproline</td>
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<tr>
<td></td>
<td>Glycogen and lactic acid</td>
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<tr>
<td>925</td>
<td>C-C stretch of proline ring/glucose/lactic acid</td>
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<tr>
<td></td>
<td>C-C, praline ring (collagen assignment)</td>
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<tr>
<td></td>
<td>Phenylalanine and Proline of collagen</td>
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<tr>
<td>1043</td>
<td>Proline</td>
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<tr>
<td>1240-1265</td>
<td>C–N stretching mode of proteins, indicating mainly α-helix conformation</td>
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<td>Tryptophan</td>
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<td>C=C, olefinic stretch (protein)</td>
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<td>Phenylalanine</td>
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<td>Amide I (protein)</td>
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<td>1754</td>
<td>C=C, lipid</td>
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</table>
2.7 The size distribution of 4-MBA-Ag NP-Ab and Ag NP-Ab

Figure S6. a) The size distribution of 4-MBA-Ag NP-Ab, b) The size distribution of Ag NP-Ab. Measurement unit is nanometre.

2.8 Stability of immune SERS tags

Figure S7 The SERS spectra of the freshly prepared SERS tags and the SERS tags after 6-month storage.

3. References