Electronic Supplementary Information for

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

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Contents

1. Experimental Section

1.1 Materials

The 4-mercaptobenzoic acid (4-MBA), silver nitrate ($AgNO_3$), sodium citrate and PBS buffer solution (0.1 M Na_2HPO_4, 0.1 M Na_2HPO_4, 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% CO_2.

1.4 Preparation the Ag NPs

The Ag NPs were prepared based on the Lee's method.[1] $AgNO_3$ (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 CO_2 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

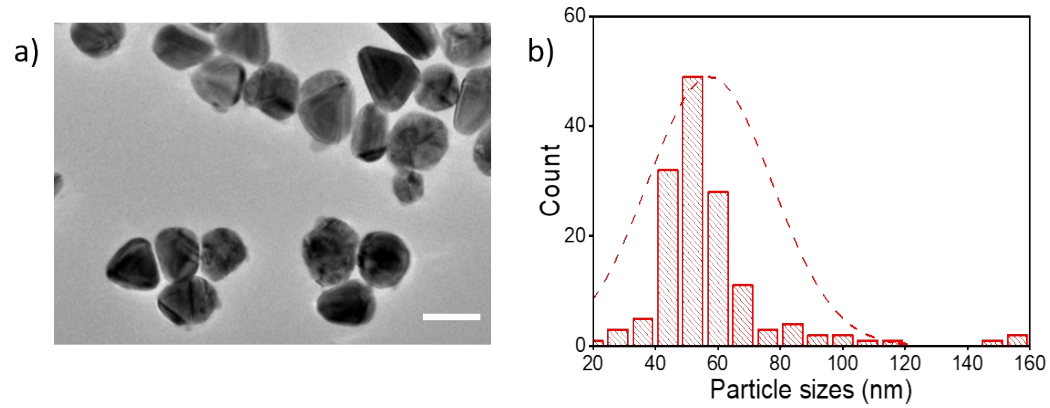

Figure S1. (a) The TEM image of the Ag NPs. The scale bar is 100 nm, (b) The size distribution of the Ag NPs.

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

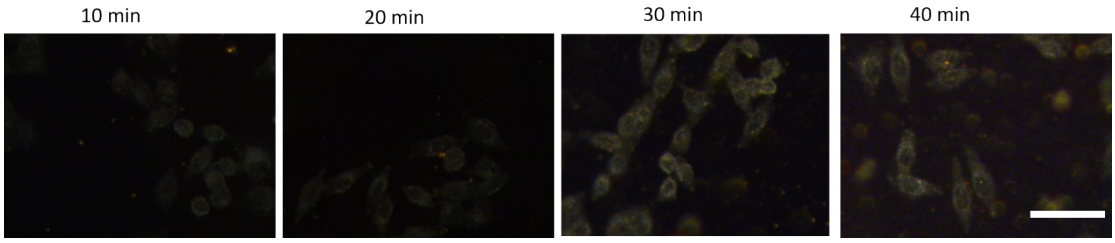

Figure S2. The dark-field images of MCF-7 cells incubated with the immune SERS tags for different periods. The scale bar is 75 μm.

2.3 The AM/PI fluorescent imaging

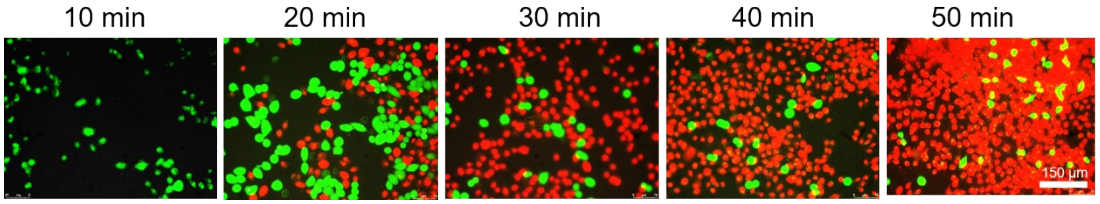

Figure S3. The fluorescent images of MCF-7 cells standing for different periods (10 to 50 min) after the ES treatment, and then they were stained with AM/PI.

2.4 Reproducibility of PD-L1 specific SERS tag in response to different cells

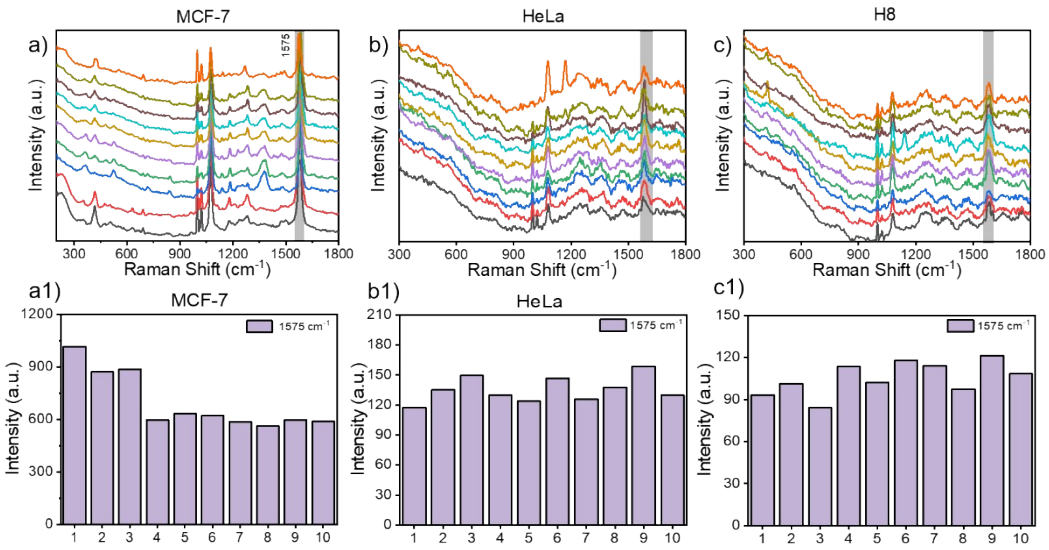

Figure S4. The SERS spectra of different cell lines; MCF-7 (a), HeLa (b), and H8 (c) cells, respectively. (a1)-(c1) The corresponding reproducibilities of the SERS spectra recorded on 10 randomly selected cells. Relative standard deviation (RSD) values at the 1575 cm^{-1} band are 18% for MCF-7 cells,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

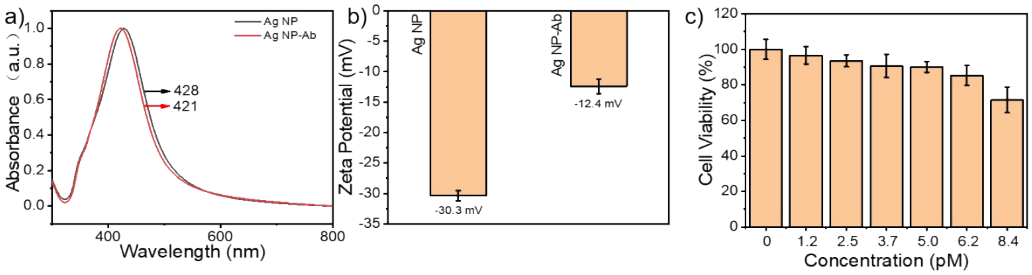

Figure S5. a) The UV-vis spectra of Ag NPs and Ag NP-Ab. b) The zeta potentials of the Ag NPs and the Ag NP-Ab. c) The MTT assays for MCF-7 cells after they were incubated with different concentrations of the Ag NP-Ab for 24 h.

2.6 SERS band assignments for cell membrane

Table S1 The SERS band assignments for the cell membrane compartments.[2-6]

Peak (cm^{-1})	Assignment
415	Phosphatidylinositol
480-494	Glycogen
543	Cholesterol
569	Tryptophan
653	ν(C-S) gauche (aminoacid methionine)
800	Phosphodiester
918	Proline, hydroxyproline Glycogen and lactic acid
925	C-C stretch of proline ring/glucose/lactic acid C-C, praline ring (collagen assignment)
1032	CH_2CH_3 bending modes of collagen & phospholipids Phenylalanine and Proline of collagen
1043	Proline
1240-1265	C–N stretching mode of proteins, indicating mainly α-helix conformation
1359	Tryptophan
1361	Tryptophan
1461	δCH_2, Disaccharides, sucrose
1488	Collagen
1570	COO^-
1585	C=C, olefinic stretch (protein)
1602	Phenylalanine
1650	Amide I (protein)
1754	C=C, lipid

2.7 The size distribution of 4-MBA-Ag NP-Ab and Ag NP-Ab

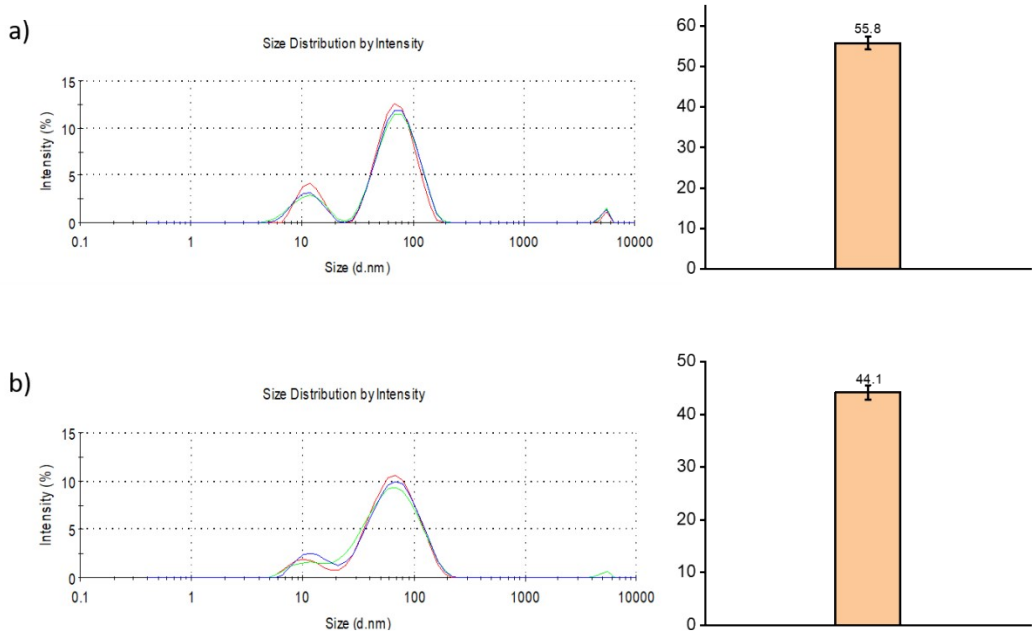

Figure S6. a) The size distribution of 4-MBA-Ag NP-Ab, b) The size distribution of Ag NP-Ab. measurement unit is nanometre.

2.8 Stability of immune SERS tags

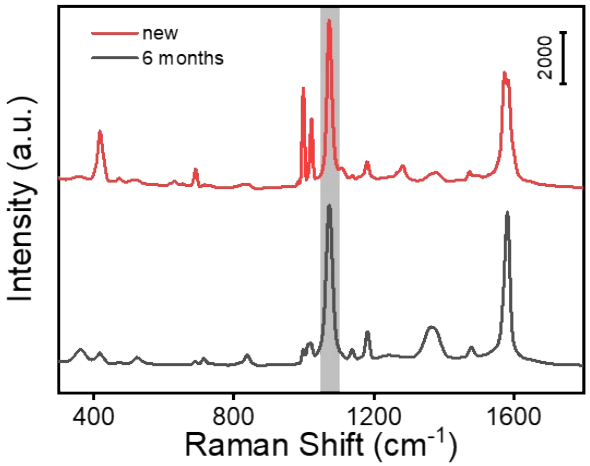

Figure S7 The SERS spectra of the freshly prepared SERS tags and the SERS tags after 6-month storage.

3. References

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