Supporting information

Bromide ion-assisted nanoprobes for sensitive and reliable pH measurement by surface-enhanced Raman spectroscopy

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Methods

1. Surface coverage calculation

The surface coverage (θ) of 4-Mpy on gold nanoparticles (AuNPs) can be estimated based on the following equations:

$\theta = S_{4\text{-Mpy}}/S_{AuNPs}$ Equation 1

$S_{4\text{-Mpy}}=AC_{4\text{-Mpy}}VN_a$ Equation 2

$S_{AuNPs}=4\pi r^2C_{AuNPs}VN_a$ Equation 3

$\theta = AC_{4\text{-Mpy}}VN_a/4\pi r^2C_{AuNPs}VN_a= AC_{4\text{-Mpy}}/4\pi r^2C_{AuNPs}$ Equation 4

where S is the total surface area, A is the area that one surface ligand occupies, C is the molar concentration, V is the volume of the system, and N_a is the Avogadro number. In this study, the averaged molecular area (A) of 4-Mpy is 0.25 nm^2.[1] The average radius (r) of AuNPs is 18.2 nm. If we assume all 4-Mpy molecules ($C_{4\text{-Mpy}}$ = 0.75 μM) are adsorbed on AuNPs (C_{AuNPs} = 3.4×10^{-5} μM), the surface coverage of 4-Mpy on gold nanoparticles (AuNPs) is estimated to be 132% based on equation 4.

2. SERS detection volume calculation

For the Raman spectroscopy we used, the laser spot size is defined by lateral and axial dimensions, which can be calculated based on equation 5 and 6:

$$\delta_{lateral} = (0.61\lambda)/NA \quad \text{Equation 5}$$

$$\delta_{axial} = (2\lambda n)/NA^2 \quad \text{Equation 6}$$

where NA is numerical aperture of the objective, λ is the laser wavelength and n is a correction factor. In our case, NA=0.7, λ=780 nm, and n=1. Inputting these parameters into equation 1 and

2, we obtained the values of $\delta_{lateral}$ and δ_{axial} as 0.68 μm and 3.2 μm. If we assume the laser spot is cylindrical, the detection volume is 1.2 μm^3.

Figures

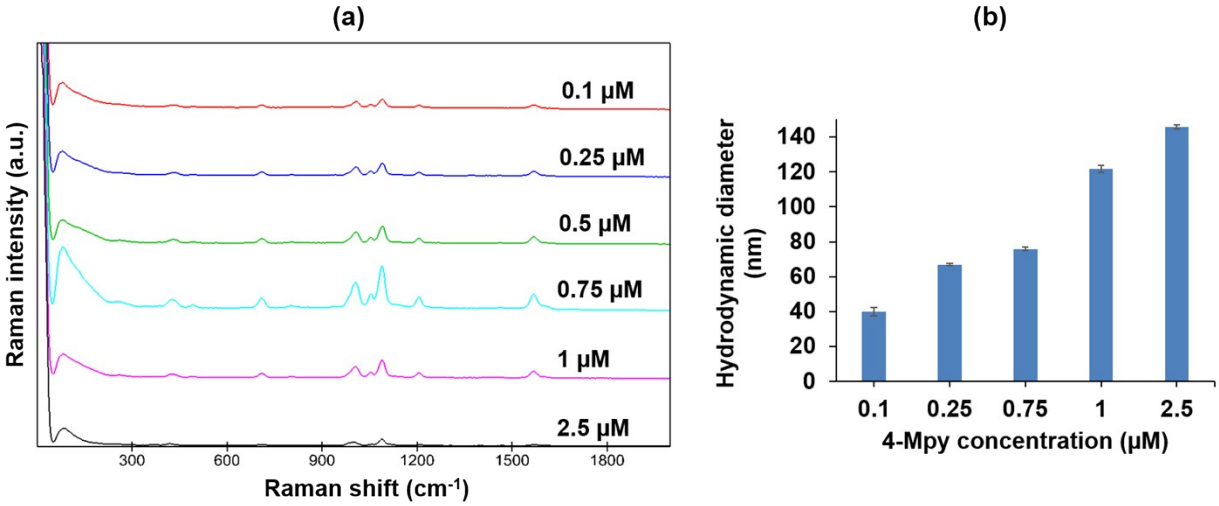

Figure S1. Selection of 4-Mpy concentration based on SERS spectra (a) and hydrodynamic diameter (b). The spectra in (a) were offset to show the differences clearly.

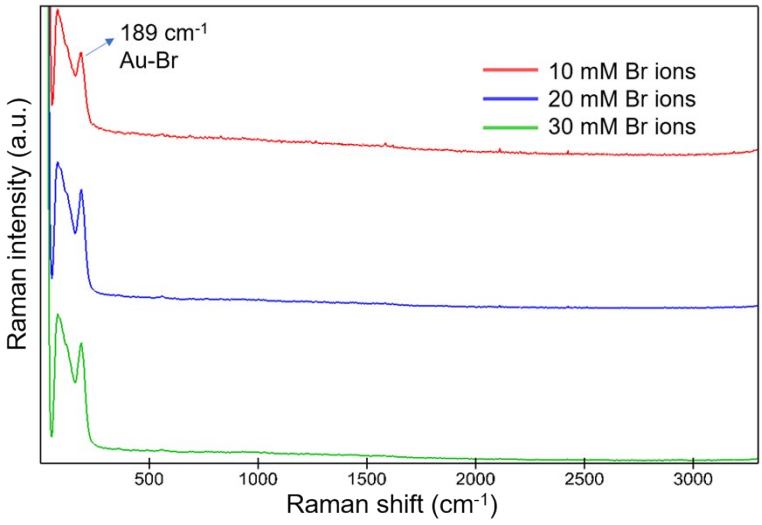

Figure S2. SERS spectra of AuNPs after modification by different concentrations of Br^- in the absence of 4-Mpy and PEG.

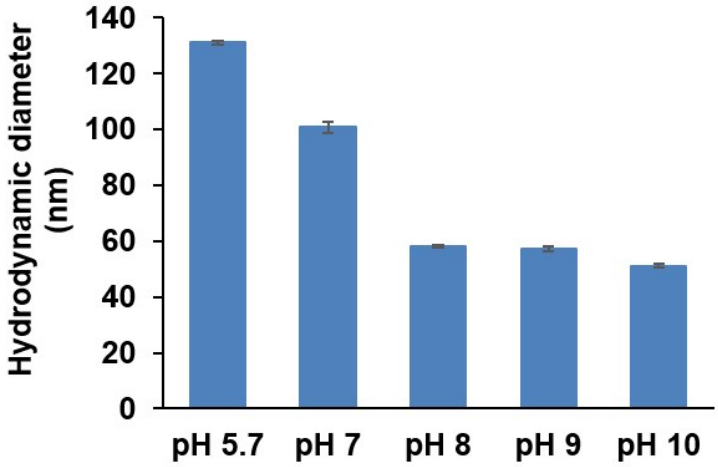

Figure S3. Hydrodynamic diameter (nm) of nanoprobes functionalized at different pHs by 0.75 μM 4-Mpy and 20 mM Br^-, and coated by 0.01 μM PEG.

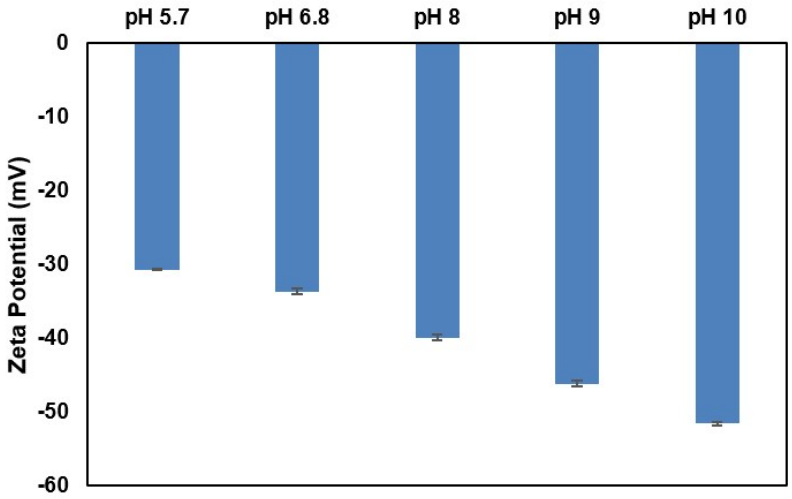

Figure S4. Zeta potential of AuNPs functionalized by 4-Mpy (0.75 μM) at different pHs. The zeta potential was measured after centrifuging (4500 rpm, 15 min) 4-Mpy-functionalized AuNPs and resuspending them in nanopure water.

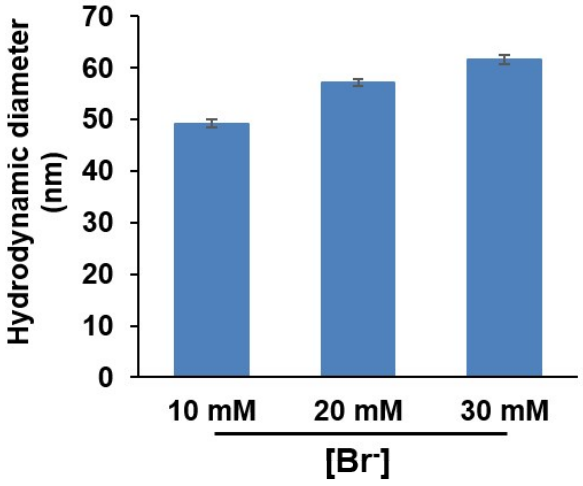

Figure S5. Hydrodynamic diameter (nm) of nanoprobes functionalized at pH 9 by 0.75 μM 4-Mpy and different [Br^-], and coated by 0.01 μM PEG.

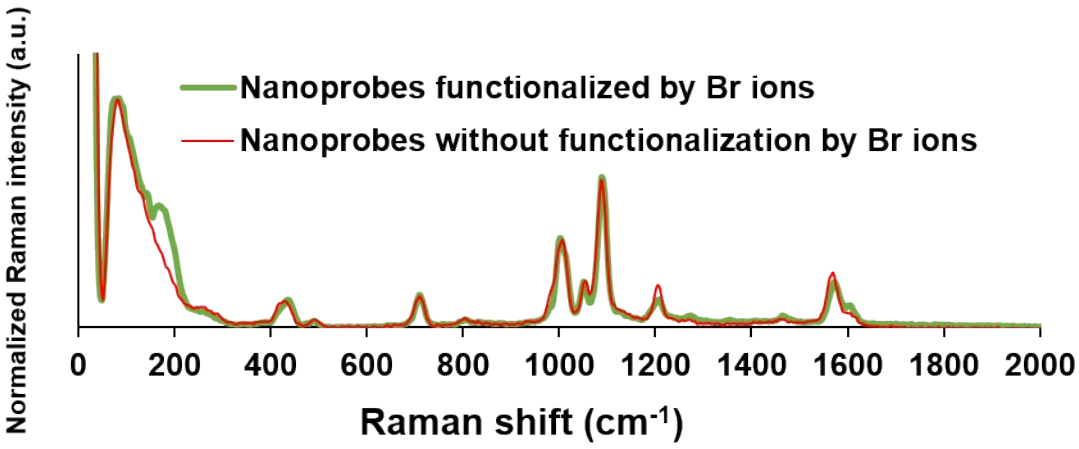

Figure S6. SERS spectra of nanoprobes without and with functionalization by bromide ions. The spectra are normalized to the elastic scattering peak at 81 cm^{-1} in order to exclude the variation caused by SERS "hotspots" and demonstrate the coverage of 4-Mpy on AuNP surface.

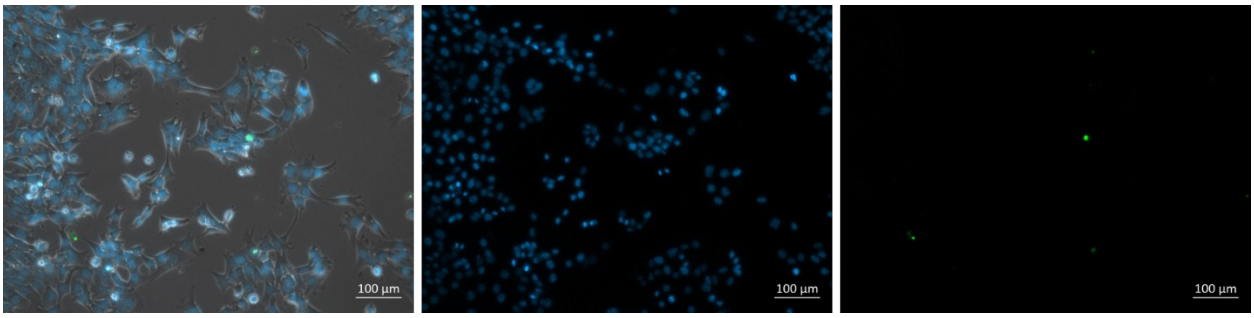

Figure S7. Cancer cell viability assay after nanoprobe internalization and SERS analysis. A composite bright-field and fluorescence microscopy image of the cells is shown on the left. The fluorescence microscopy images show live cells (blue, middle) and dead cells (green, right). The cell viability was calculated to be 99.6% based on 272 cells. All scale bars are 100 µm.

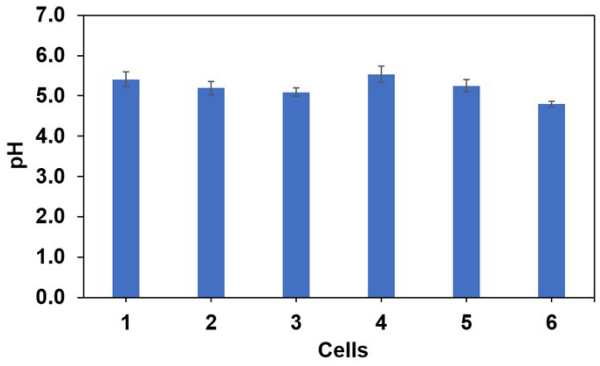

Figure S8. Average pH values in cancer cells measured by the developed nanoprobes using SERS surface mapping.

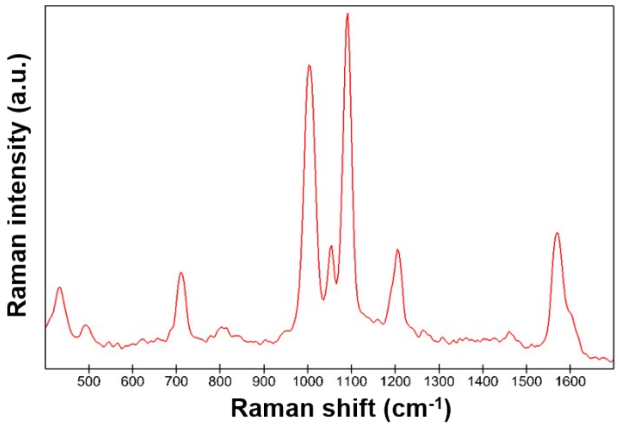

Figure S9. A representative SERS spectrum of the pH-sensing nanoprobes in cancer cells.

Table S1. SERS peak assignments of the developed nanoprobes[2–4]

Peak position (cm^{-1})	Peak intensity	Assignment
81	s	Elastic scattering
172	w	Br-Au
431	m	δ(C-S)/γ(CCC)
710	m	β(CC)/ν(C-S)
1006	s	Ring breathing
1054	m	δ(CH)
1091	s	Ring breathing/C-S
1208	m	β(CH)/δ(NH)
1572	m	$8b_2$ ring stretching with deprotonated nitrogen
1606	m	$8b_2$ ring stretching with protonated nitrogen

References

(1) Bi, X.; Du, X.; Jiang, J.; Huang, X. Facile and Sensitive Glucose Sandwich Assay Using in Situ -Generated Raman Reporters. *Anal. Chem.* **2015**, *87* (3), 2016–2021.

(2) Hu, J.; Zhao, B.; Xu, W.; Li, B.; Fan, Y. Surface-Enhanced Raman Spectroscopy Study on the Structure Changes of 4-Mercaptopyridine Adsorbed on Silver Substrates and Silver Colloids. *Spectrochim. Acta - Part A Mol. Biomol. Spectrosc.* **2002**, *58* (13), 2827–2834.

(3) Do, W. H.; Lee, C. J.; Kim, D. Y.; Jung, M. J. Adsorption of 2-Mercaptopyridine and 4-Mercaptopyridine on a Silver Surfaces Investigated by SERS Spectroscopy. *J. Ind. Eng. Chem.* **2012**, *18* (6), 2141–2146.

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