

Electronic Supplementary Information

Surface-enhanced hyper Raman hyperspectral imaging and probing in animal cells

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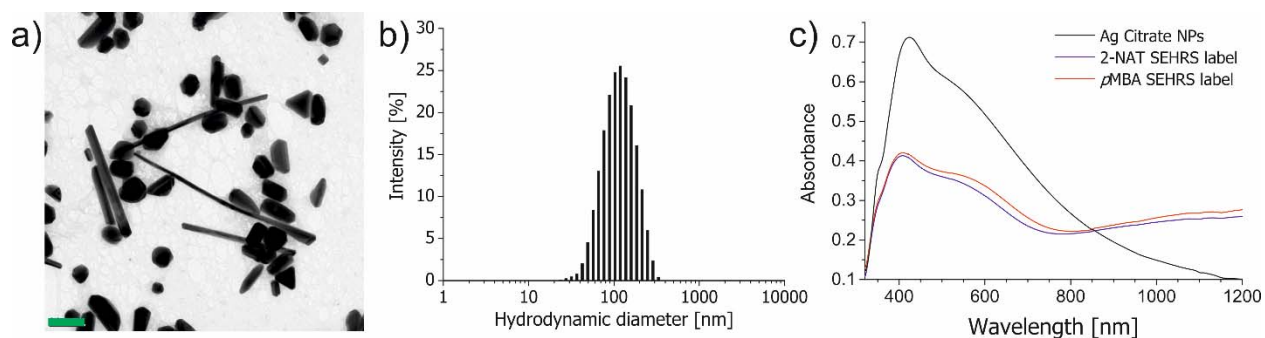


Figure S1. (a) Transmission electron micrograph of the citrate stabilized silver nanoparticles that are used as the basis of the SEHRS labels (green scale bar: 200 nm). (b) Size distribution of the silver nanoparticles determined by dynamic light scattering. (c) Absorbance spectra of the silver nanoparticles (black trace), of the 2-NAT SEHRS label (blue trace), and of the pMBA SEHRS label (red trace), diluted in the culture medium. To obtain the spectra, culture medium without the addition of the indicator phenol red was used.

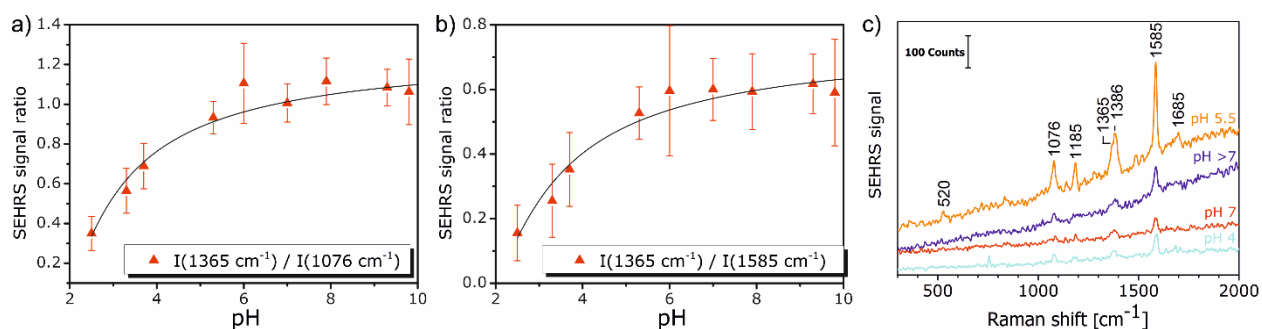


Figure S2. (a, b) Calibration curves used to reconstruct the SEHRS pH maps shown in Figure 3 of the manuscript. (a) Intensity ratio of the bands at 1365 and 1076 cm⁻¹ in the SEHRS spectra of the pMBA SEHRS label as a function of pH. (b) Intensity ratio of the bands at 1365 and 1585 cm⁻¹. All intensity ratios were calculated using an average of 30 spectra, the error bars represent the standard deviations. (c) Selected single SEHRS spectra of the pMBA labels from a live macrophage cell, which, according to the data in (a) and (b), represent different pH-values inside the endosomal system of the cell. Excitation wavelength: 1064 nm, photon flux density: $3 \cdot 10^{28}$ photons cm⁻² s⁻¹, acquisition time: 30 s.

Table S1. Band positions (cm⁻¹) tentative assignments in the SEHRS spectra of the 2-NAT SEHRS label obtained in live macrophages after 3 h incubation (see Fig. 1c). Assignments are based on the SERS data discussed in references 1-2.

Band position in cm ⁻¹	Assignment	References
360	phenyl deformation and C-S-stretching	1,2
515	ring twist	1,2
599	C-H out-of-plane deformation	1,2
638	C-H out-of-plane deformation and (CCC) out-of-plane deformation	1,2
845	C-H twist	1,2
940	S-H bending	1
1067	symmetric C-H bending	1,2
1155	C-H-in-plane-bending	1,2
1380	ring stretching	1,2
1454	ring stretching	1,2
1569	ring stretching	1,2
1584	ring stretching	1,2
1624	ring stretching	1,2

References

- [1] Alvarez-Puebla, R.A.; Dos Santos Jr, D.S.; Aroca, R.F. Surface-enhanced Raman scattering for ultrasensitive chemical analysis of 1 and 2-naphthalenethiols. *Analyst* **2004**, 129, 1251–1256.
- [2] Joo, T.H.; Kim, M.S.; Kim K. Surface-enhanced Raman scattering of benzenethiol in silver sol. *Journal of Raman Spectroscopy* **1987**, 18, 57–60.