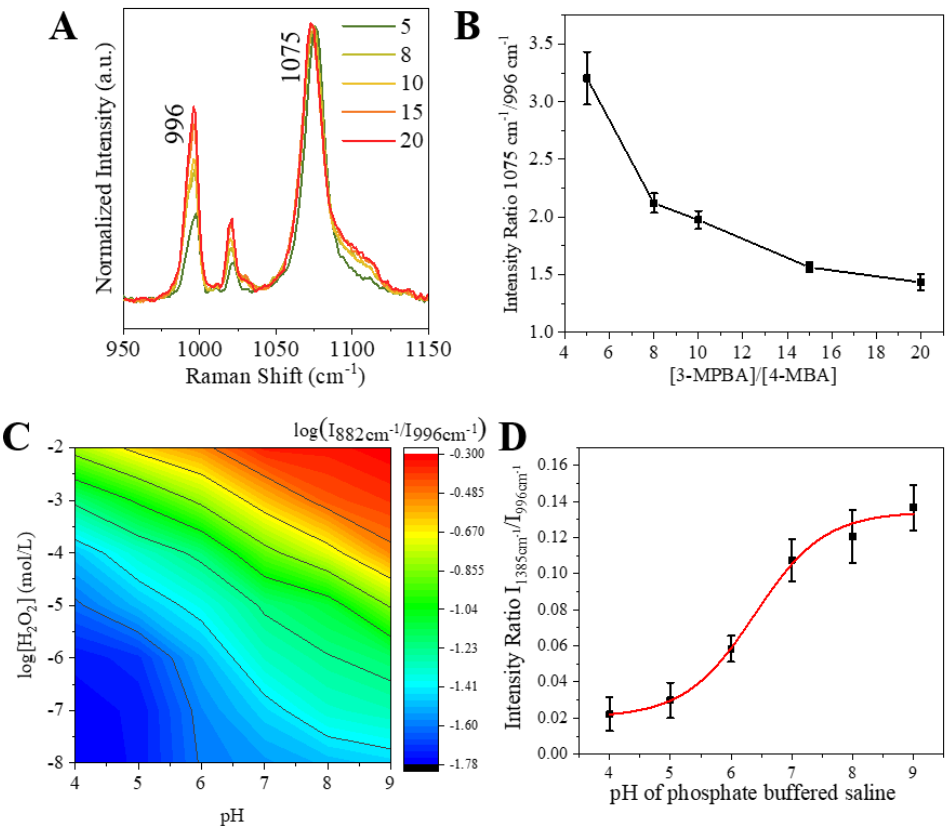
# Fe de errata

1. Page 9 line 5, “nanometrails” should be changed for “nanomaterials”.
2. Page 9 line 9, “hydrogen peroxide13” should be changed for “hydrogen peroxide”.
3. Page 12 line 15, “SESR” should be changed for “SERS”.
4. Page 13 line 25, “perticipates” should be changed for “participates”.
5. Page 15 line 20, “SESR” should be changed for “SERS”.
6. Page 15 line 21, “as the best of our knowledge” should be changed for “to the best of our knowledge”.
7. Page 15 line 23, “absorbed” should be changed for “adsorbed”.
8. Page 16 line 2, “aslo” should be changed for “also”.
9. Page 25, in abstract, line 9 and line 11, “conformation” should be changed for “formation”.
10. Page 32 line 2, “µl” should be changed for “µL”.
11. Page 37 line 4, “in briefly” should be changed for “in brief”.
12. Page 37 line 5, “co-absorption” should be changed for “co-adsorption”.
13. Page 40 line 5 and line 21, “absorption” should be changed for “adsorption”.
14. Page 40 line 22, “mental” should be changed for “metal”.
15. Page 41 line 2 and line 22, “absorbed” should be changed for “codified”.
16. Page 41 line 12, “balk” should be changed for “bulk”.
17. Page 42 line 11, “plamonic” should be changed for “plasmonic”.
18. Page 42 line 13, “absorbed” should be changed for “adsorbed”.
19. Page 43 line 1, “In comparation” should be changed for “In comparison”.
20. Page 45 line 19, “Figure 3C-F” should be changed for “Figure 3C-E”.
21. Page 46 line 2, “hided” should be changed for “hide”.
22. Page 47 last line, “SESR” should be changed for “SERS”.
23. Page 48, in the legend of Figure 4, line 2, “SESR” should be changed for “SERS”.
24. Page 49 line 16, “1075 cm-1” should be changed for “1075 $cm^{-1}$”.
25. Page 64, in the legend of Figure SI-8, line 1 and line 2, “SESR” should be changed for “SERS”.
26. Page 65, in abstract, line 18, “40-fold” should be changed for “30-fold”.
27. Page 67 line 19, delete “(ref10)”.
28. Page 69 line 3, “Moreover, none of the articles discussed the pH effect on aromatic boronates oxidation for $H_2O_2$ sensing.” should be changed for “Moreover, to the best of our knowledge, none of the articles discussed the pH effect on aromatic boronates oxidation for $H_2O_2$ sensing.”.
29. Page 71 line 9, delete “(Controlling Size and Distribution for Nano-sized Polystyrene Spheres)”.
30. Page 71 line 11, “mg/ml” should be changed for “mg/mL”.
31. Page 71 line 25, “negatively charged 3-5 nm” should be changed for “negatively charged 2-3 nm”.
32. Page 73 line 16, “Integration time was set to 10 s and a power at the sample of 5 mW. Laser power of 10 mW and exposure time 20 s used for in vitro Raman experiments.” should be changed for “Integration time was set to 11 s and a power

at the sample of 5 mW. Laser power of 5 mW and exposure time 15-20 s were used for intracellular nanosensors."

33. Page 74 line 22, "NCs were incubated with HT29 for 24 h" should be changed for "NCs were incubated with HT29 for 48 h".
34. Page 78 line 26, "was no affected" should be changed for "was not affected".
35. Page 81 last line, "approx. 6 times" should be changed for "approx. 4 times".
36. Page 85, Figure 4 has following inconsistencies:
    - the vertical axis of Figure 4B: "Intensity Ratio $1385cm^{-1}/996cm^{-1}$" should be changed for "Intensity Ratio $1075cm^{-1}/996cm^{-1}$";
    - the horizontal axis of Figure 4B: "MPBA&MBA Concentration Ratio" should be changed for "[3-MPBA]/[4-MBA]";
    - legend in Page 86 line 5 and line 6: "Interested bands at 996 $cm^{-1}$ and 1385 $cm^{-1}$ correspond to 3-MPBA and 4-MBA relative occupations on NCs. (B) Intensity ratios between 1385 $cm^{-1}$ and 996 $cm^{-1}$ as a function for 3-MPBA and 4-MBA modification." should be changed for "Bands of interest at 996 $cm^{-1}$ and 1075 $cm^{-1}$ correspond to 3-MPBA and 4-MBA relative occupations on NCs. (B) Intensity ratios between 1075 $cm^{-1}$ and 996 $cm^{-1}$ as a function for 3-MPBA and 4-MBA modification.";
    - Update the standard deviation calculation in Figure 4D.
    - Figure 4 has been changed accordingly.




37. Page 90, "Nanosensors were incubated with cells for 24h for sufficient uptake of

NCs through endocytosis process. Bright field images in Figure 5A gotten from Raman equipment indicated that cells morphology was maintained under exposure to NCs and $H_2O_2$ and/or Bafilonycin A1." should be changed for "Nanosensors were incubated with cells after sufficient uptake of NCs through endocytosis process. Representative bright field image in Figure 5A gotten from Raman equipment indicated intracellular and extracellular NCs from where the SERS signal was collected."

38. Page 91, the SERS spectra we used in Figure 5 were collected under different irradiation time, ranging from 8 s to 50 s. As we discussed in page 94, the bonding of Raman probes, especially 4-MBA, is not stable under high irradiation time because of the heat originated by the laser irradiation. Some of the spectra were mixed and placed in the wrong conditions by mistake. Thus, we need to update Figure 5. However, the conclusion was not affected, that our multiplex sensor was able to be used for multiplex sensing extracellular and intracellular $H_2O_2$ and pH. Updated Figure 5 was shown below with corrected legend.

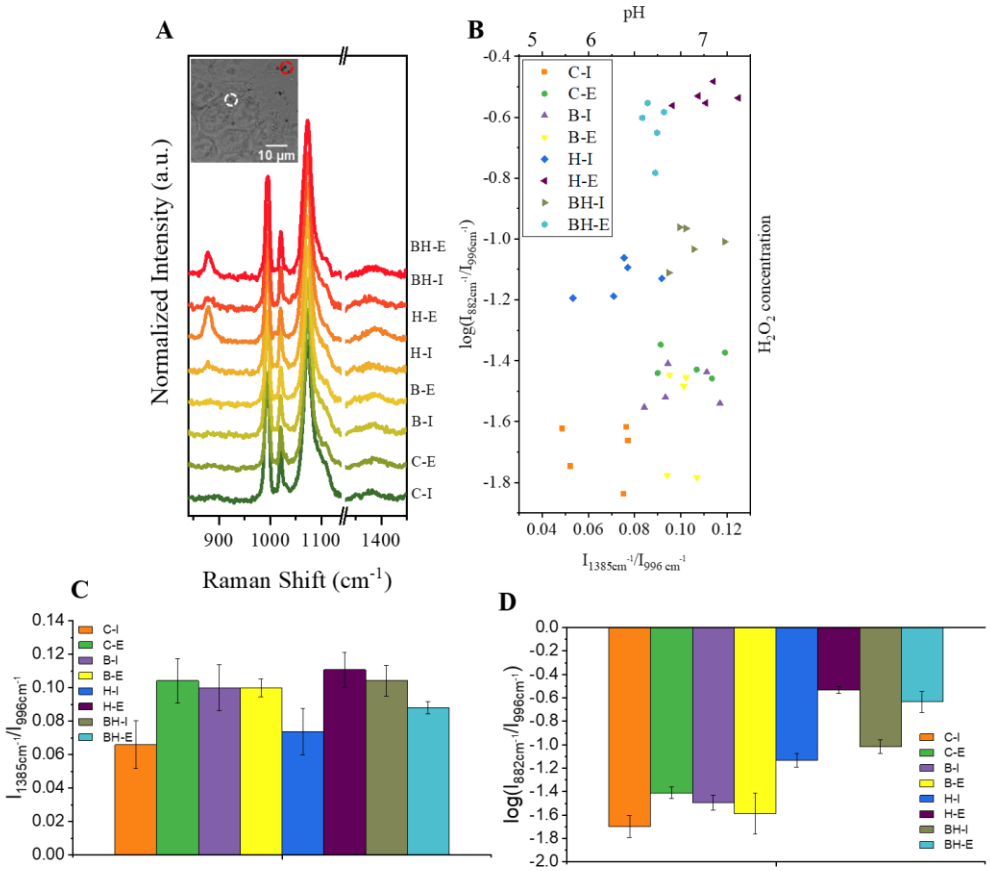



**Figure 5: Intracellular and extracellular $H_2O_2$ and pH SERS determination with NCs@3-MPBA&4-MBA.** Intracellular and extracellular SERS spectra were collected with HT29 under different treatments: control cells, Bafilomycin A1 treated, 10 mM $H_2O_2$ treated, Bafilomycin A1 and 10 mM $H_2O_2$ both treated HT29. C-I: intracellular probes of blank HT29; C-E: extracellular probes of blank HT29; B-I: intracellular probes of Bafilomycin A1 treated HT29; B-E: extracellular probes of Bafilomycin A1 treated HT29; H-I: intracellular probes of 10 mM $H_2O_2$ treated HT29; H-E: extracellular probes of 10

mM $H_2O_2$ treated HT29; BH-I: intracellular probes of Bafilomycin A1 and 10 mM $H_2O_2$ treated HT29; BH-E: extracellular probes of Bafilomycin A1 and 10 mM $H_2O_2$ treated HT29. **(A)** Representative intracellular and extracellular NCs@3-MPBA&4-MBA SERS spectra of HT29 under different treatments. Optical HT29 image was collected with Raman microscope. White dash circle showed internalized probe, and red dash circle showed extracellular probe. **(B)** Intensity ratio between 1385 $cm^{-1}$ and 996 $cm^{-1}$ ($I_{1385}/I_{996}$) and intensity ratio between 882 $cm^{-1}$ and 996 $cm^{-1}$ ($\log(I_{882}/I_{996})$) were calculated and shown of the spectra gotten with HT29 under different treatments. $I_{1385}/I_{996}$ reflected local pH value, and $\log(I_{882}/I_{996})$ corresponded to local $H_2O_2$ concentration. **(C)** $I_{1385}/I_{996}$, for local pH determination, was calculated of intracellular and extracellular probes with HT29 under different treatment. Each column was the average of 5 different probes. **(D)** $\log(I_{882}/I_{996})$, for local $H_2O_2$ detection, was calculated of intracellular and extracellular probes with HT29 under different treatment. Each column was the average of 5 different probes.

39. Consequently to the change of Figure 5, we have updated the text in this section.

- Page 92 to 93, two paragraphs are affected:

  "SERS spectra of extracellular and intracellular NCs@3-MPBA&4-MBA of blank cells were first collected from 10 different NCs separately as showed in Figure 5A (C-I and C-E). Figure 5B showed the distribution of $I_{1385}/I_{996}$ and $\log(I_{882}/I_{996})$ obtained from spectra which corresponding to pH value and $H_2O_2$ concentration, respectively. And in Figure 5C and Figure 5D showed the average value and standard deviation of $I_{1385}/I_{996}$ and $\log(I_{882}/I_{996})$ separately. The intensity ratios $I_{1385}/I_{996}$ of those 10 spectra of extracellular NCs were around 0.1, according to pH calibration curve, pH there were around 7 which agreed with the cells growth media pH. While intracellular NCs we collected here indicated local pH ranging from 5 to close 7. The intensity ratio $\log(I_{882}/I_{996})$ for extracellular NCs had values around -2, implying the $H_2O_2$ concentration in cell growth media were lower than 0.8 μM. The intracellular signal was around our negative control signal, which means the physiological lysosomal $H_2O_2$ above our limit, since our NCs has LOD at lysosomal acidic pH (pH 5) was around $10^{-6}$ M.

  Then we mimic cellular stress by increasing the amount of $H_2O_2$ exposed to the cells. We used 1 mM $H_2O_2$ to study intracellular and extracellular $H_2O_2$ concentration changes as showed in Figure 5 (H-I and H-E). Compared with the calibration curve, extracellular pH maintained the pH of growth media which was around 7, while intracellular pH ranged from 5 to 6. Interestingly, we found that, after the one-shot addition of $H_2O_2$ to cells growth media, the extracellular $H_2O_2$ concentration was not the same with the addition concentration. Since we already demonstrated that proteins in our growth media had no or extremely low effect on $H_2O_2$ sensing, the rapid removal of extracellular $H_2O_2$ was because of the active cellular metabolism[24]. The gradient would be around 5 to 10 times extracellular concentration lower than the addition concentration depending on the metabolism of cells. Moreover, intracellular $H_2O_2$ concentration was around 40 times lower than extracellular concentration."

  should be changed for:

  "SERS spectra of extracellular and intracellular NCs@3-MPBA&4-MBA of

control cells were first collected from 5 different NCs separately as showed in Figure 5A (C-I and C-E). Figure 5B showed the distribution of $I_{1385}/I_{996}$ and $\log(I_{882}/I_{996})$ obtained from spectra which corresponded to pH value and $H_2O_2$ concentration, respectively. And in Figure 5C and Figure 5D showed the average value and standard deviation of $I_{1385}/I_{996}$ and $\log(I_{882}/I_{996})$ separately. The intensity ratios $I_{1385}/I_{996}$ of those 5 spectra of extracellular NCs of control cells were around 0.1, according to pH calibration curve, pH there were around 7 which agreed with the cells growth media pH. While intracellular NCs we collected here indicated local pH ranging from less than 6 to close 7. The intensity ratio $\log(I_{882}/I_{996})$ for extracellular NCs had values around -1.4, implying the $H_2O_2$ concentration in cell growth media were around 0.8 μM. The intracellular signal was around our negative control signal, which means the physiological lysosomal $H_2O_2$ lower than our detection limit, since our NCs has LOD at lysosomal acidic pH (pH 5 and pH 6) was around $10^{-6}$ M.

Then we mimic cellular stress by increasing the amount of $H_2O_2$ exposed to the cells. We used 10 mM $H_2O_2$ to study intracellular and extracellular $H_2O_2$ concentration changes as showed in Figure 5 (H-I and H-E). Compared with the calibration curve, extracellular pH maintained which was around 7, while intracellular pH was around 6. Interestingly, we found that, after the one-shot addition of $H_2O_2$ to cells growth media, the extracellular $H_2O_2$ concentration was not the same with the addition concentration. Since we already demonstrated that proteins in our growth media had no or extremely low effect on $H_2O_2$ sensing, the rapid removal of extracellular $H_2O_2$ was cause by the active cellular metabolism[24]. The gradients were around 5 to 10 times extracellular concentration lower than the addition concentration, however this value can vary upon the metabolism of cells. Moreover, intracellular $H_2O_2$ concentration was around 30 times lower than extracellular concentration."

- Page 93, line 23: "with 1mM $H_2O_2$ treatment" should be changed for "with 10 mM $H_2O_2$ treatment".
- Page 94, line 3 to line 15: "We continuously used more $H_2O_2$ concentration (10 mM and 0.5 mM) and further verified again our conclusions. Intracellular and extracellular spectra of NCs@3-MPBA&4-MBA with HT29 under different treatments: Bafilomycin A1 treated and non-treated HT29 with different amount of $H_2O_2$ addition (10 mM and 0.5 mM) and without $H_2O_2$ addition were collected and $\log(I_{882}/I_{996})$ and $I_{1385}/I_{996}$ values were summarized in Figure SI-18. While the pH and $H_2O_2$ values of each NCs were calculated based on pH and $H_2O_2$ calibration curves and shown in Table SI-4. The complete spectra showed in Figure SI-19. Interestingly, we found that even intracellular NCs located in different lysosomes or endosomes which were during endocytic pathway, even within different pHs, environmental $H_2O_2$ were consistent under same treatment." should be changed for "All the complete SERS spectra were showed in Figure SI-18. And the pH and $H_2O_2$ values of each NCs were calculated based on pH and $H_2O_2$ calibration curves and shown in Table SI-4."
- Figure SI-18 does not apply anymore.

- Figure SI-19 is now Figure SI-18 and is display as follows:

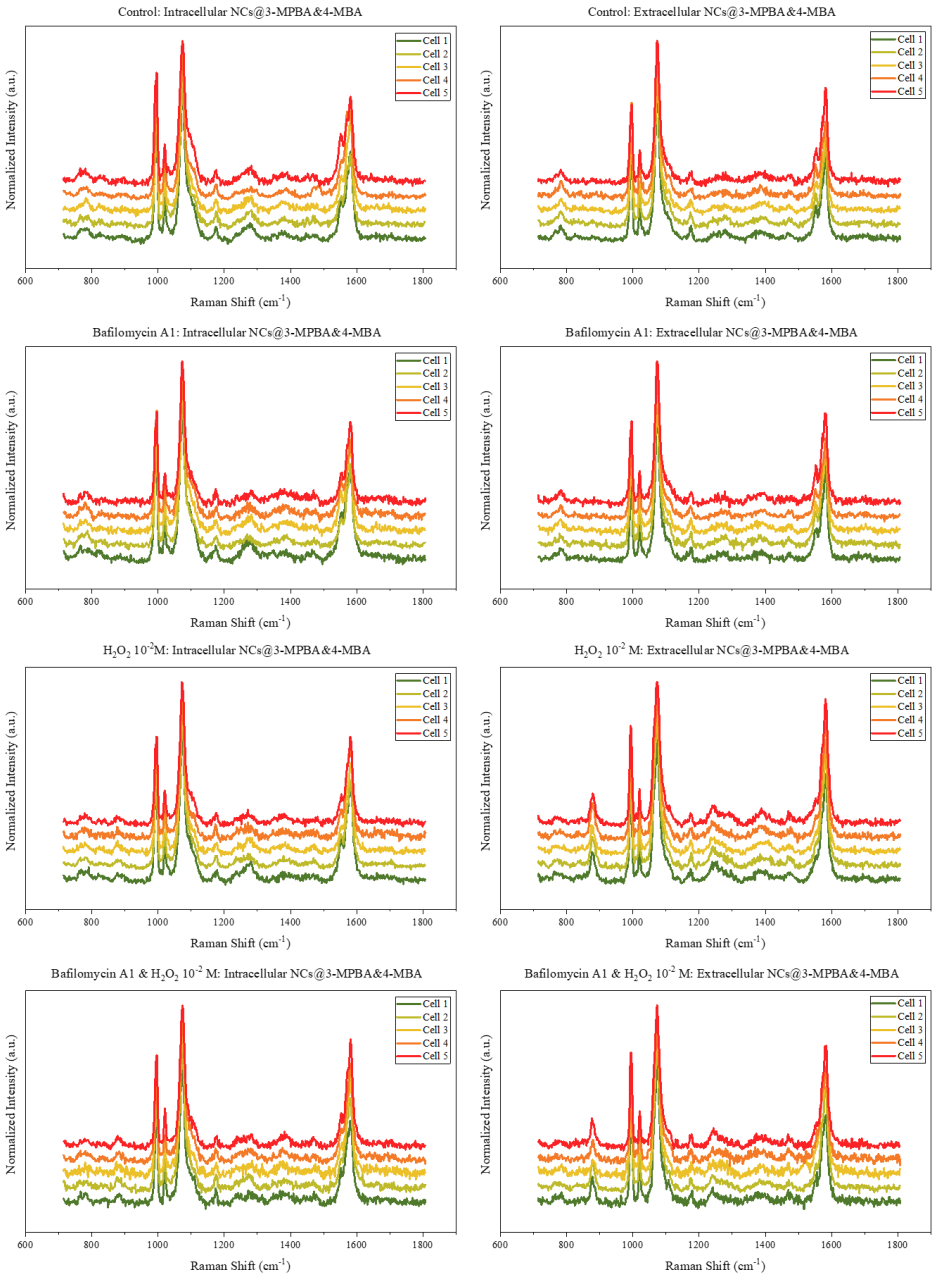


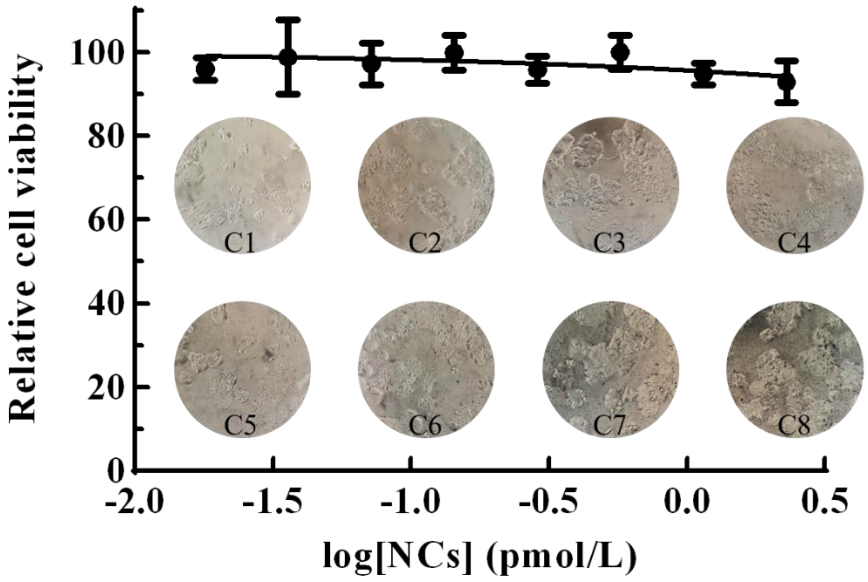

Figure SI-18: Intracellular and extracellular SERS spectra of NCs@3-MPBA&4-MBA with HT29 under different treatments: Bafilomycin A1 treated and non-treated HT29 with 10 mM $H_2O_2$ addition and without $H_2O_2$. Each spectrum was collected with one NCs@3-MPBA&4-MBA.

- Table SI-4 must also be updated:

| | C-I | | C-E | | B-I | | B-E | | H-I | | H-E | | BH-I | | BH-E | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | pH | $H_2O_2$ | pH | $H_2O_2$ | pH | $H_2O_2$ | pH | $H_2O_2$ | pH | $H_2O_2$ | pH | $H_2O_2$ | pH | $H_2O_2$ | pH | $H_2O_2$ |
| Cell 1 | 6 | <4E-6 | 7 | <8E-7 | 7 | <8E-7 | 7 | <8E-7 | 6 | 6E-05 | 7 | 2E-03 | 7 | 1E-05 | 7 | 1E-03 |
| Cell 2 | 6 | <4E-6 | 7 | 1E-06 | 7 | <8E-7 | 7 | <8E-7 | 6 | 6E-05 | 7 | 2E-03 | 7 | 5E-05 | 7 | 2E-03 |
| Cell 3 | 6-7 | <4E-6 | 7 | <8E-7 | 7 | <8E-7 | 7 | <8E-7 | 6-7 | 2E-05 | 7 | 2E-03 | 7 | 4E-05 | 7 | 2E-04 |
| Cell 4 | 6-7 | <4E-6 | 7 | <8E-7 | 7 | <8E-7 | 7 | <8E-7 | 6-7 | 1E-05 | 7 | 3E-03 | 7 | 2E-05 | 7 | 8E-04 |
| Cell 5 | 6-7 | <4E-6 | 7 | 1E-06 | 7 | <8E-7 | 7 | <8E-7 | 6-7 | 1E-05 | 7 | 2E-03 | 7 | 3E-05 | 7 | 1E-03 |

Table SI-4: Intracellular and extracellular pH and $H_2O_2$ concentration calculated based on calibration curve of all the NCs@3-MPBA&4-MBA determined. Intracellular and extracellular SERS spectra were collected with HT29 under different treatments. C-I: intracellular probes of control HT29; C-E: extracellular probes of control HT29; B-I: intracellular probes of Bafilomycin A1 treated HT29; B-E: extracellular probes of Bafilomycin A1 treated HT29; H-I: intracellular probes of 10 mM $H_2O_2$ treated HT29; H-E: extracellular probes of 10 mM $H_2O_2$ treated HT29; BH-I: intracellular probes of Bafilomycin A1 and 10 mM $H_2O_2$ treated HT29; BH-E: extracellular probes of Bafilomycin A1 and 10 mM $H_2O_2$ treated HT29.

40. Page 125: Figure SI-20 is now Figure SI-19. Therefore, the sentence in page 94 line 17: “shown in Figure SI-20” should be changed for “shown in Figure SI-19”.
41. Page 94, line 15: Intracellular measurements were highly variable. We want to highlight this phenomenon by adding one paragraph “SERS measurements in eukaryotic cells exhibit high variance. Reasons that may contribute to this phenomenon can be related to differences in the laser excitation of individual capsules because, (i) the NCs locate at different depth within the cells; and (ii) the physicochemical properties of the NCs (and thus the SERS response) may have changed after 48h cellular exposure.” We refer to the publication for further clarifications and data.
42. Page 94: the paragraph related to the spectrum modification of the Raman probe depending on the laser irradiation is updated from
    “The intensities of peaks at 1385 $cm^{-1}$ and 1590 $cm^{-1}$, corresponding to symmetric carboxyl stretching mode and aromatic ring vibrations of 4-MBA, decreased under high irradiation time, indicating that we were losing 4-MBA under high irradiation time. It seems 4-MBA is more sensitive to energy than 3-MPBA. Controlling equal irradiation is critical for multiplex measurements.”
    to
    “The intensities of peaks at 1385 $cm^{-1}$ and 1590 $cm^{-1}$, corresponding to symmetric carboxyl stretching mode and aromatic ring vibrations of 4-MBA, decreased under high irradiation time, indicating a possible photosublimation appearance with 4-MBA under high irradiation time because of the thermo heating by laser[45]. We observed different photo-sensitivity of 3-MPBA and 4-MBA upon irradiation because of their nature properties. Controlling equal and moderate irradiation is critical for multiplex measurements.” We have introduced a new reference (ref-45) supporting this conclusion: 45. Álvarez-Puebla, R. A. Effects of the excitation Wavelength on the SERS spectrum. *J. Phys. Chem. Lett.* **3**, 857–866 (2012).
43. Page 95 last line: “approx. 40 times less” should be changed for “approx. 30 times less”.
44. Page 96 line 1: “the extracellular $H_2O_2$ concentration were around 5 to 10 times less than the addition concentration” should be changed for “the extracellular $H_2O_2$ concentration were less than the addition concentration”.
45. Page 106: The horizontal axis on figure SI-4 does not agree with Figure 2 in the main text (Page 80). Therefore, we have corrected it.




46. Page 108: The peak position in figure SI-6, should be 1553 $cm^{-1}$ instead of 1550 $cm^{-1}$.

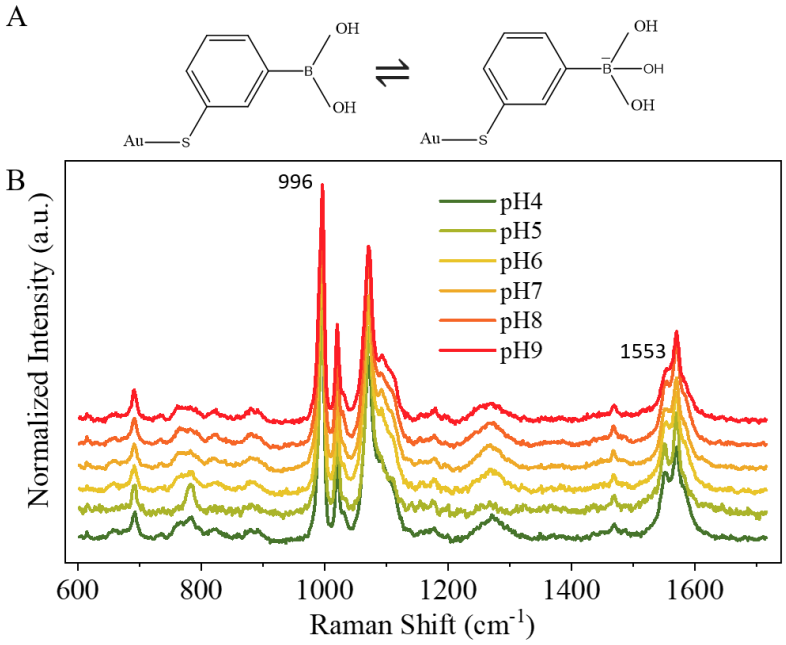



Figure SI-6: NCs@3-MPBA dispersed in different pH buffer without $H_2O_2$. (A) Scheme of 3-MPBA format in acidic pH (phenylboronic acid) and alkaline pH (boronate acid); (B) SERS spectra of NCs@3-MPBA dispersed in phosphate buffer with pH ranging from 4 to 9, showing that intensity at 1553 $cm^{-1}$ decreased with pH increasing. Each spectrum was the average of 5 spectra gotten from 5 different NCs@3-MPBA.

47. Page 129 line 1: “by adding different concentrations of hydrogen peroxide” should be changed for “by adding hydrogen peroxide”.
48. Page 129 last paragraph: “7. We found the extracellular hydrogen peroxide was not

the same as the one-shot addition concentration, because of the active metabolism of cells, and the value was around 5 to 10 times lower. Also there are gradients between intracellular and extracellular hydrogen peroxide. The gradient was estimated around 40 times lower from the intracellular to the extracellular concentrations. Meanwhile, although the nanocapsules might be located in different organelles of endocytic pathways with different luminal acidity (lysosomes, endosomes), the concentrations of hydrogen peroxide were equivalent."
should be changed for
"7. We found the extracellular hydrogen peroxide was lower than the one-shot addition concentration of $H_2O_2$, because of the active metabolism of cells. Also, there are gradients between intracellular and extracellular hydrogen peroxide. The gradient was estimated around 30 times lower from the intracellular to the extracellular concentrations for HT29."