Electronic Supplementary Material (ESI) for RSC Advances

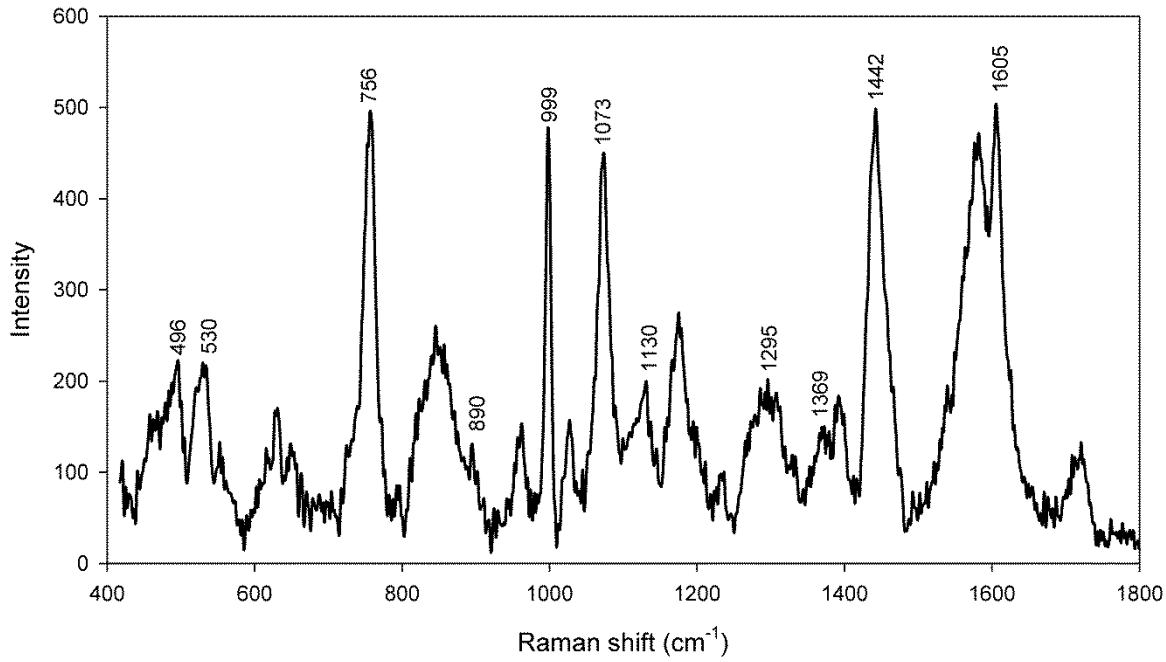
Supporting Information

Distribution of label free cationic polymercoated gold nanorods in live macrophage cells reveals formation groups of intracellular SERS signals from probe nanoparticles

Dakrong Pissuwan, Alison Hobro, Nicolas Pavillon, Nicholas Smith

SERS spectrum of PDAC-GNRs

The Raman spectrum of concentrated PDAC-GNR colloids was measured using Raman microspectroscopy (Nanophoton Raman-11). A 785 diode laser was used as the excitation source with a power of 35 mW for 10 s. The measurement was carried out in point mode and the sample was focused using a microscope objective lens (Nikon Apo 20x). The SERS signal of PDAC-GNRs shows many clear peaks at 756 cm^{-1} (possibly from O-H deformation vibration),[1] 996 cm^{-1}, 1073 cm^{-1}, 1442 cm^{-1}, and 1605 cm^{-1}. The weak peaks at 496 cm^{-1} (Au-S vibration),[2] 530 cm^{-1}, 890 cm^{-1}, 1130 cm^{-1} (PSS-PDAC complex: aromatic C-H in pane bending),[1] 1295 cm^{-1}, and 1369 cm^{-1} were detected. Unassigned peaks here are the same peaks detected in poly(sodium 4-styrenesulfonate (PSS) and poly(diallydimethylammonium chloride) (PDAC) polyelectrolytes.



SERS spectra extraction methods

Spectra of interest are extracted from the spatially-resolved data sets as measured by the line-scanning microscope through an automated method[3], and the steps involved in the spectral extraction are summarized below.

A first overall reduction of spectral data is performed by assuming that SERS measurements have a spectral energy above background noise signals. Spectra having an energy below 50% of the maximal energy measured in the field of view are then discarded. It should be noted that retaining the top 50% of spectral energy discards far more than 50% of the data since since SERS signals appear as intense signals with a sparse distribution. Spectra of interest are then selected from this reduced data set through a criterion based on counting the number of peak-like features[3], assuming that relevant SERS spectra should

contain several spectral peaks. Peak detection is performed on pre-smoothed spectra (Savitzky-Golay smoothing, with a window width of 20 data points) through a gradient-based approach. The spectra are then ranked by number of peaks, and the ones having more than 50% of the maximal amount of peaks identified in the field of view are then extracted.

The baseline of the retained spectra is then estimated through a statistical approach[3], by calculating the baseline value at several points in the spectrum with the 0.02-quantile value over a local window of 30 data points. The baseline curve is then estimated between quantile values with cubic spline interpolation. Baseline-corrected spectra are then clustered through the thresholding of their cross-correlation. If curves have a cross-correlation above 0.7, they are grouped in a cluster, and if not, they are placed in two different clusters.

The number of collected spectra and the frequencies of occurrences for different types of multiple and single occurrence spectra

The number of collected spectra of a multiple occurrence (Fig.3a-f) was shown in a table. It was found that there were 6 spectral profiles detected from measured areas in Fig.3. Each spectral profile provided different numbers of collected spectra and various numbers of frequencies of occurrences as shown in a table. In the case of a single occurrence (Fig.4a-d), it is clearly show that the detected spectral profile of each measured area provided only one occurrence. As well, the number of collected spectra of each detected spectral profile in a single occurrence is lower than that of a multiple occurrence.

Measured areas	Number of collected spectra	Frequencies of occurrences
Fig.3a	7	3
Fig.3b	10	3
Fig.3c	12	4
Fig.3d	14	6
Fig.3e	25	4
Fig.3f	6	2
Fig.4a	3	1
Fig.4b	4	1
Fig.4c	3	1
Fig.4d	1	1

References

1 A. Bragaru, M. Kusko, A. Radoi, M. Danila, M. Simion, F. Craciunoiu, R. Pascu, I. Mihalache, T. Ignat, *Cent. Eur. J. Chem.* 2013, **11**, 205.

2 W. Xie, L. Su, A. Shen, A. Materny, J. Hu, *J. Raman Spectrosc.* 2011, **42**, 1248.

3 N. Pavillon, K. Bando, K. Fujita, N.I. Smith, J. Biophotonics 2013, **6**, 587.