

Isotopic graphene–isolated-Au-nanocrystals with cellular Raman-silent signals for cancer cell pattern recognition

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Tripti Ahuja
24/03/18

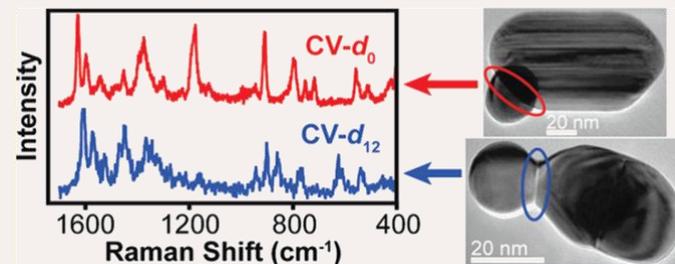
Introduction & Background Work

- Multiplexed, sensitive and specific molecular detection is highly desirable for environmental analysis, drug screening, gene and protein profiling as well as clinical diagnostics (eg. cancer)
- Several encoders like bioconjugated encoders and optical encoders (fluorescence, SERS, reflection, fluorescence lifetime) have been used for multiplexed detection and identification of attached ligand molecules.
- SERS (surface enhanced Raman spectroscopy) technologies have overcome the drawbacks of various encoders due to its high sensitivity, anti-photobleaching and ultra-narrow Raman peak.
- SERS with isotope edited approach has been widely used in various fields as:
 - (a) Biantalyte isotopologue approach serve as the strongest proof for identifying SM-SERS.
 - (b) Super-resolution imaging with isotope edited approach to understand the origin of hotspots in nanoparticle aggregates.
 - (c) Isotope labelled SERS (IL-SERS) has been utilized for analyte quantification with improved accuracy and precision.
 - (d) Recently, isotopic encoded SERS NPs seem promising for sensitive multiplexed detection.

Single-Molecule Surface-Enhanced Raman Spectroscopy of Crystal Violet Isotopologues: Theory and Experiment

Samuel L. Kleinman, Emilie Ringe, Nicholas Valley, Kristin L. Wustholz,[†] Eric Phillips,[‡] Karl A. Scheidt, George C. Schatz, and Richard P. Van Duyne*

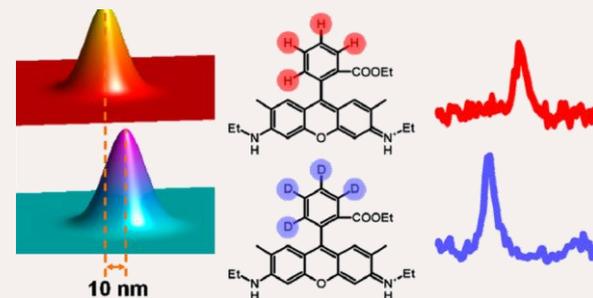
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Super-Resolution SERS Imaging beyond the Single-Molecule Limit: An Isotope-Edited Approach

Eric J. Titus, Maggie L. Weber, Sarah M. Stranahan, and Katherine A. Willets*

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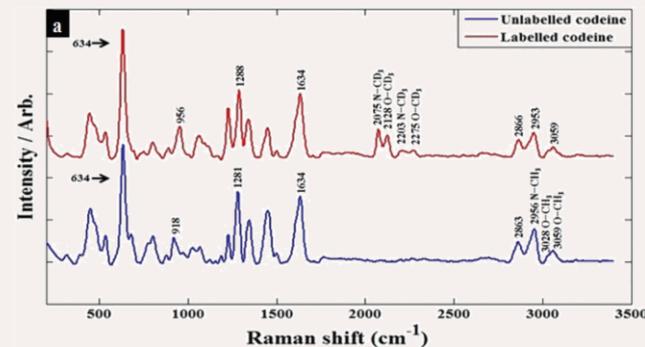
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Quantitative detection of codeine in human plasma using surface-enhanced Raman scattering *via* adaptation of the isotopic labelling principle[†]

Abdu Subaihi,^a Howbeer Muhamadali,^a Shaun T. Mutter,^{a,c} Ewan Blanch,^b David I. Ellis^a and Royston Goodacre*^a



Relevant to lab work

- IL-SERS approach to understand the ligand attached to nanoparticles at single particle level. Insights about the ligand dynamics.
- To design new cluster based SERS tags for bio-imaging and cell line detection.
- Synthesis of stable SERS tags by spray techniques.
- Understanding stable IL-SERS tags variation under polarization microscopy for different nanoparticle systems.

In this paper...

- Fabrication of biocompatible multicolour SERS tags based on regulating Raman shift of intact graphene coated on AuNPs by the chemical vapour deposition method (CVD), defined as graphene-isolated-Au-nanocrystals, or GIANS.
- GIAN tags were ultra-stable and resistant to oxidation with a full range of pH and capacity for powerful laser irradiation. These tags possess simple Raman fingerprint region as D-band (1355 cm^{-1}), G-band (1590 cm^{-1}) and 2D- band (2706 cm^{-1}).
- The 2D-band located in the cellular Raman-silent region free from the interference of biomolecules ($1800\text{-}2800\text{ cm}^{-1}$) made it possible to realize precision bioimaging.
- Multiplexed SERS tags were designed through the introduction of isotopic carbon compositions
- Multiplexed in vivo and in vitro Raman imaging with low background interference was demonstrated with such isotopic GIAN SERS tags.
- Aptamer conjugated isotopic GIAN-encoders were synthesized which behaved as a built in pattern recognition component for simultaneous, rapid and targeted cancer cell imaging.
- Using aptamer conjugated isotopic GIAN-encoders, different SERS barcodes were designed that easily distinguished two cancer cell lines.

Results and Discussion

Advanced characterization of GIANS:

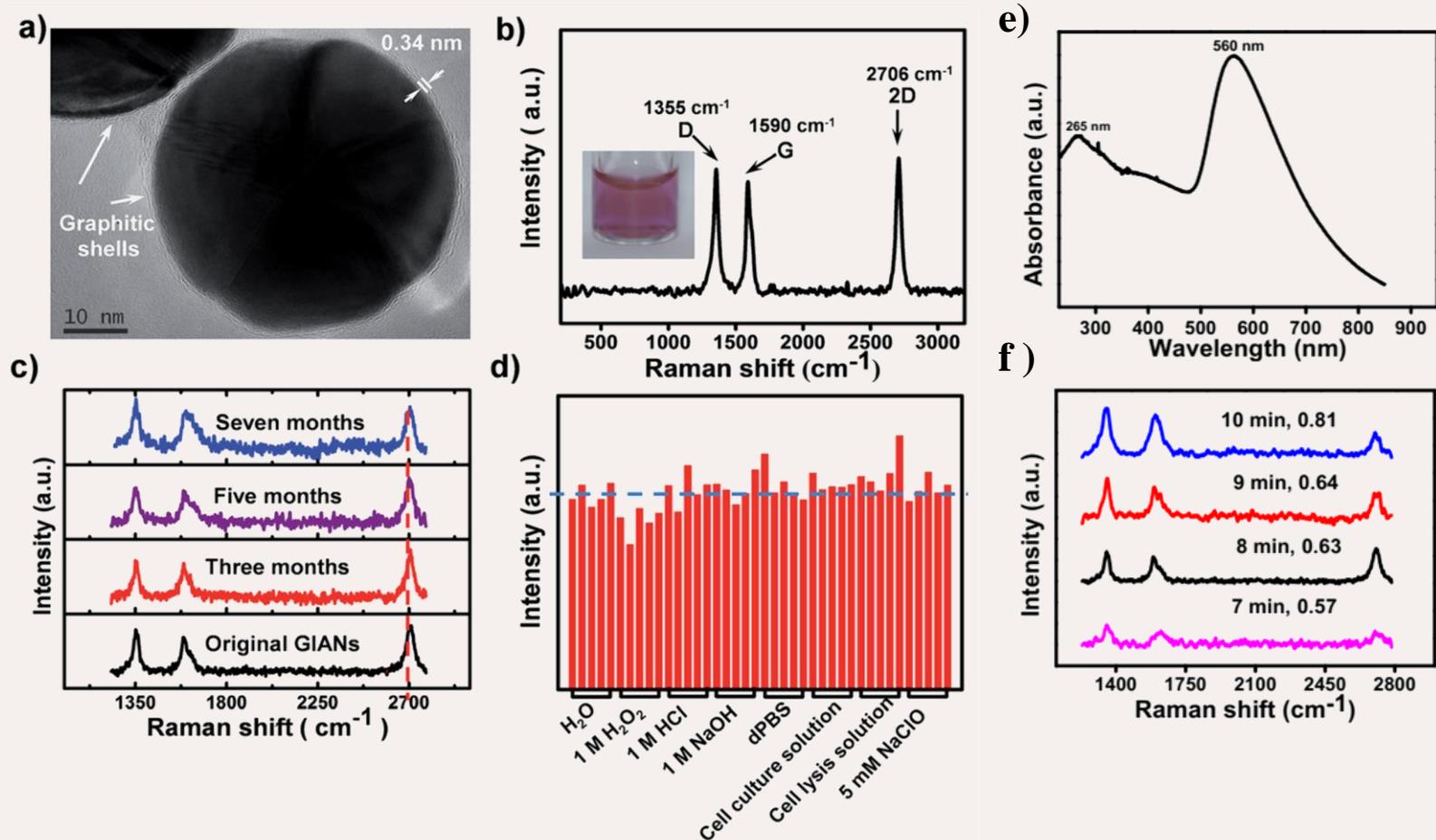


Fig. 1 (a) HR-TEM image of GIANS. (b) SERS spectrum of aqueous GIANS suspension. Inset in (b) presented the image of aqueous GIANS suspension. (c) SERS spectra of GIANS stored in water for various time. (d) SERS intensity stability of 2D-band of GIANS after incubating with H₂O, 1 M H₂O₂, 1 M HCl, 1 M NaOH, Dulbecco's phosphate buffered solution (dpBS), cell culture solution, cell lysis solution, and 5 mM NaClO for 3 hours. (e) UV-Vis spectrum of aqueous GIANS. (f) The Raman spectra of GIANS at different growth time.

Raman characterization of multiplexed isotopic GIANS:

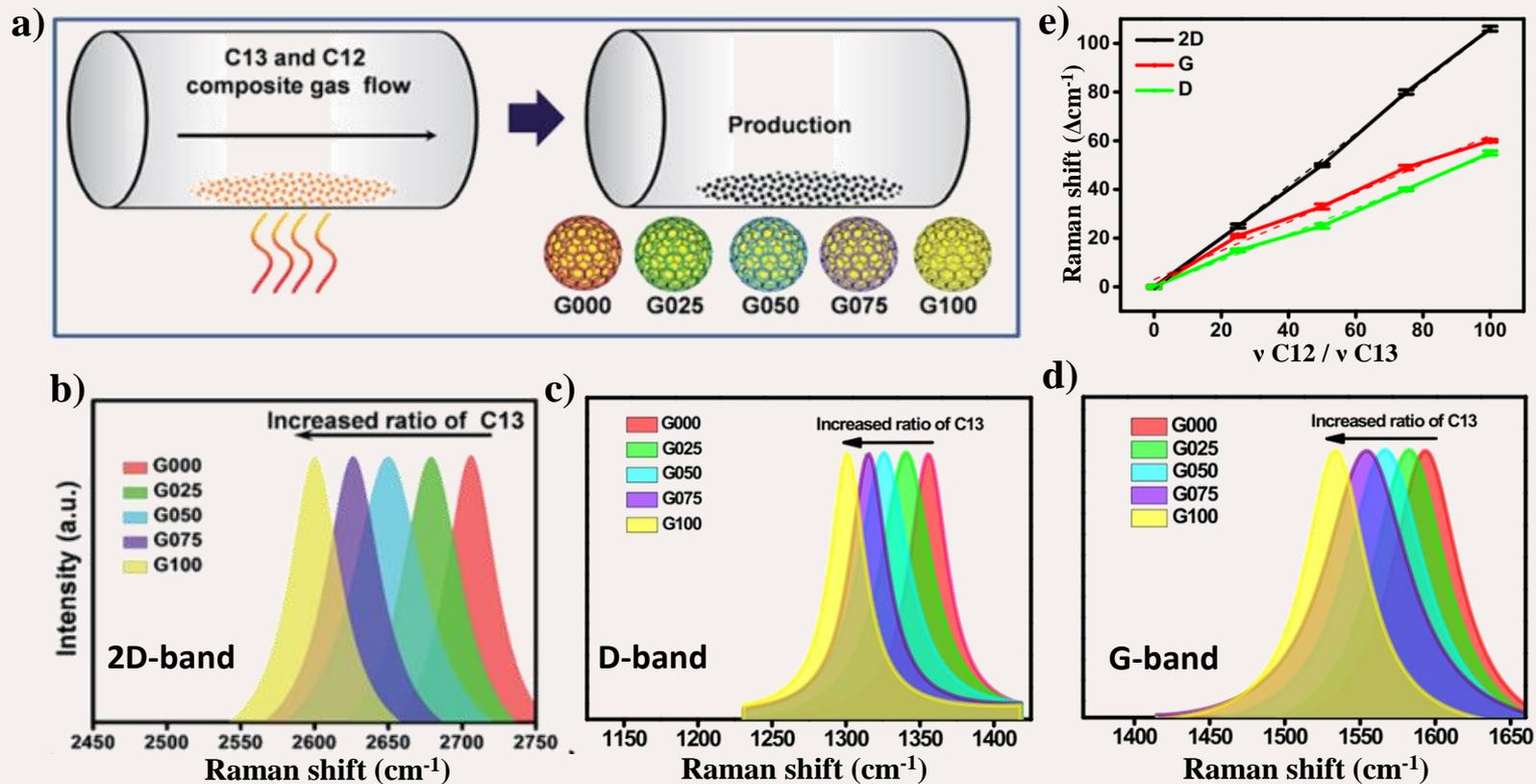


Fig. 2 (a) Schematic illustration of synthesis of multiplexed GIANS tags, defined as G100 (100% C¹³), G075 (75% C¹³), G050 (50% C¹³), G025 (25% C¹³), and G000 (0% C¹³). (b) Raman spectrum of 2D band, (c) D band and (d) G-band of GIANS with different fractions of C¹³ methane. (e) The plot line and linear fitting relationship between the fraction of C¹³ methane and the value of Raman shifts. Error bar depended on 10 spectra. Results showed that the shift of 2D-band at about twice the frequency of D-band.

Dark field microscopy imaging for precise SERS measurements:

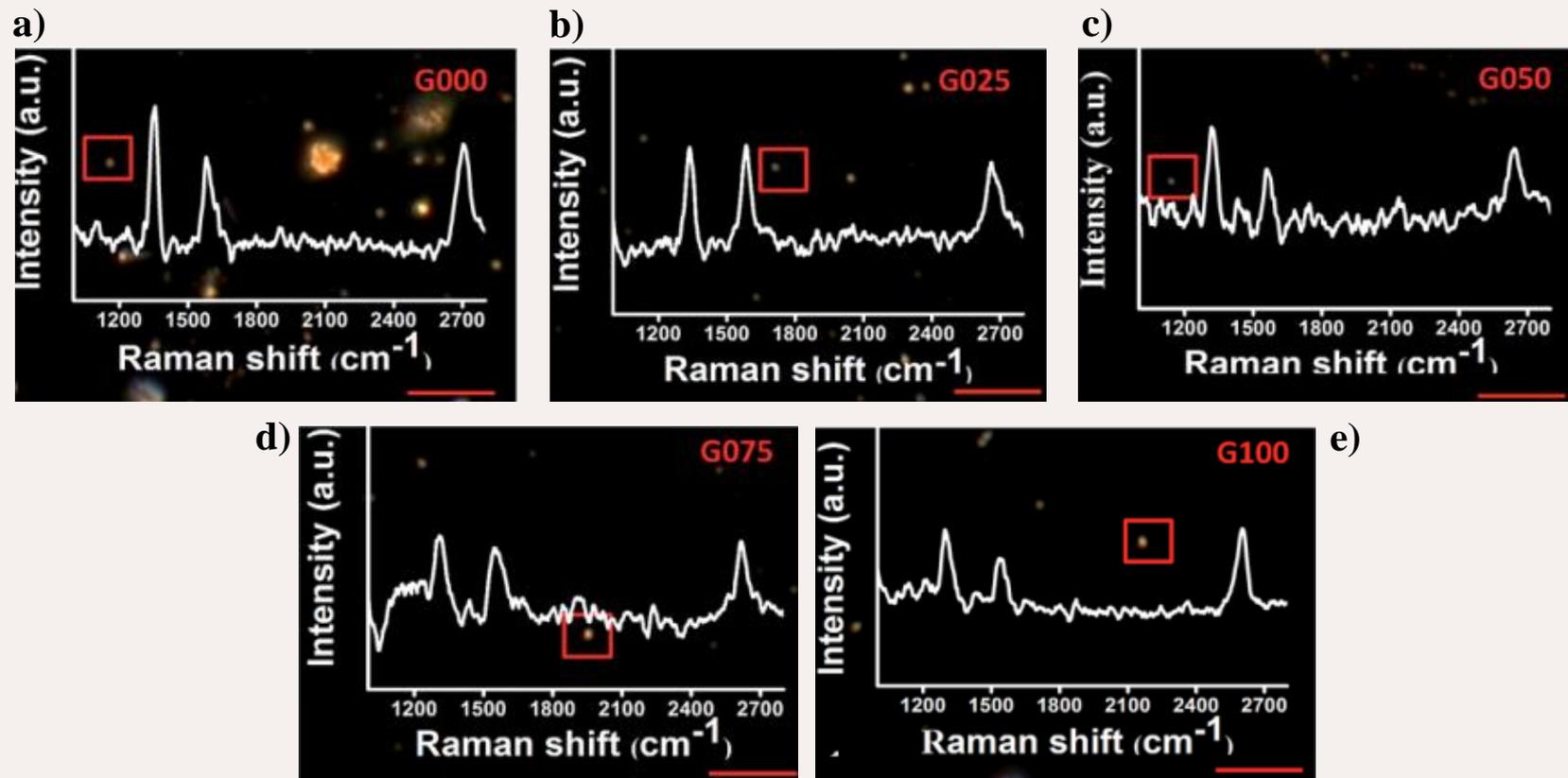
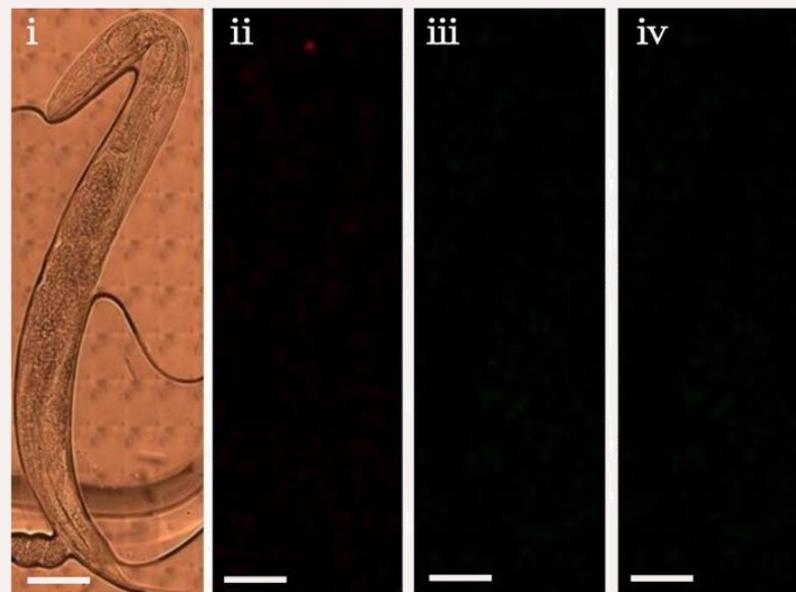
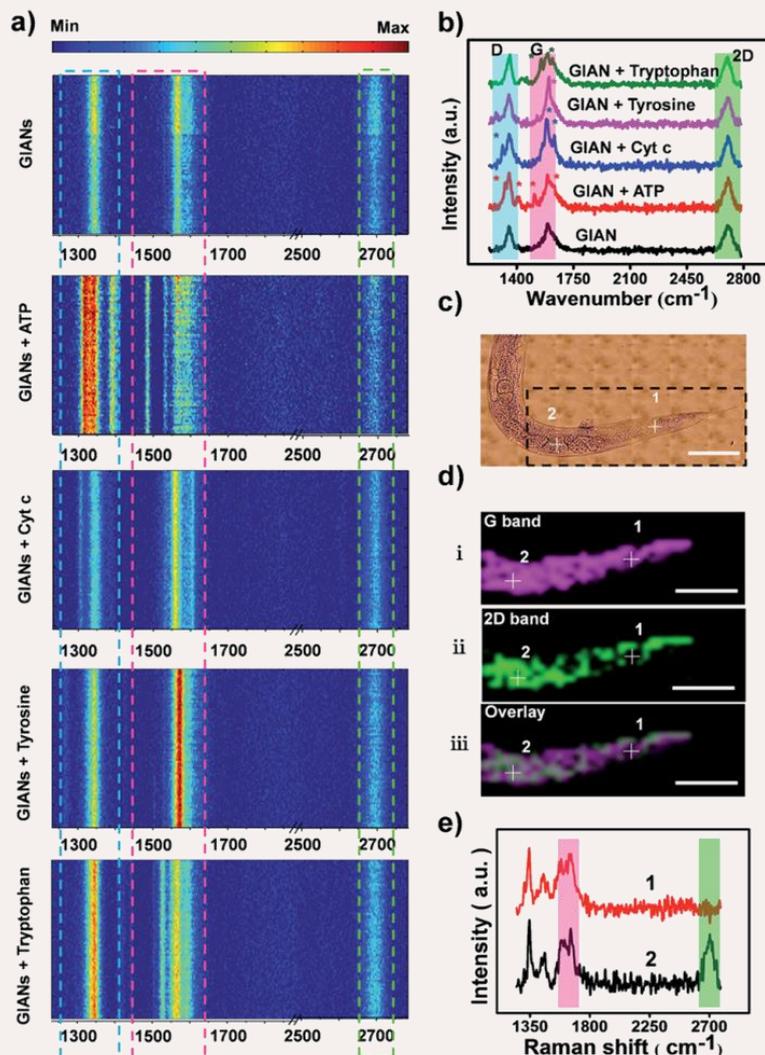


Fig. 3 SERS spectra of multiplexed GIANS suspension. Under the leading of dark-field imaging, SERS spectra on few (a) G000, (b) G025, (c) G050, (d) G075 and (e) G100 particles boxed in respect dark-field image; scale bar, 5 μm .

The anti-interference ability of GIANS in vitro and in vivo:



(f) SERS images of untreated *C. elegans*. (i) Bright-field; SERS images of *C. elegans* acquired with (ii) 2600 cm^{-1} , (iii) 2650 cm^{-1} and (iv) 2706 cm^{-1} . Scale bar, 200 μm .

Fig. 3 (a) Maps of 81 SERS spectra on GIANS mixed with the biomolecules ATP, cytochrome c, tyrosine, tryptophan and the control, respectively. (b) SERS spectra of GIANS mixed with biomolecules corresponding to (a). (c) Bright-field of *C. elegans*. (d) SERS mapping images of *C. elegans* corresponding to boxed area in (c). (i) G-band, 1590 cm^{-1} (plum); (ii) 2D-band, 2706 cm^{-1} (green); (iii) overlay. Step size, 11 μm , scale bar, 200 μm . (e) SERS spectra marked in SERS mapping images of *C. elegans* (d).

Multiplexed SERS imaging with isotopic GIANS in vitro and in vivo:

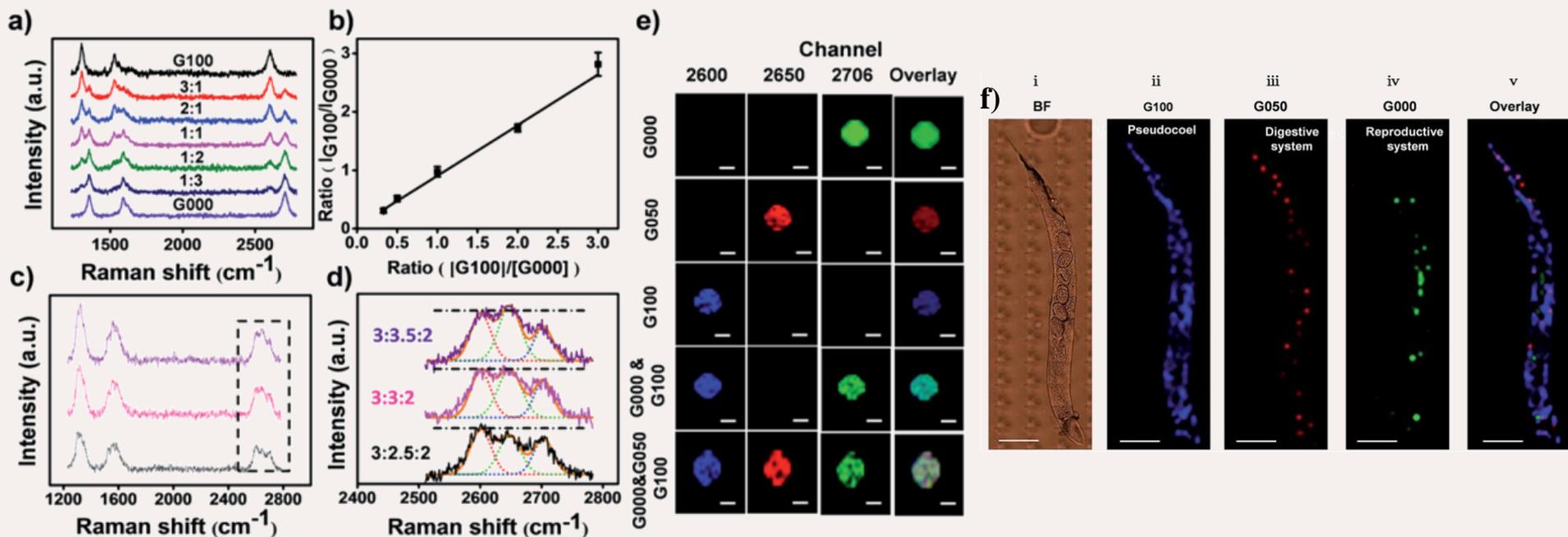


Fig. 5 (a) SERS spectra of mixed G100 and G000, from top to bottom, in varying ratios (CG100 : CG000) of 3 : 1, 2 : 1, 1 : 1, 1 : 2, and 1 : 3. (b) Linear fitting of (a). (c) SERS spectra of mixed G100, G050 and G000 and fixed concentration of G100 and G000, while decreasing the concentration of G050, as G100 : G050 : G000 of 3 : 2.5 : 2, 3 : 3 : 2 and 3 : 3.5 : 2. (d) Gaussian fitting of enlarged spectra of 2D-bands corresponding to boxed area in (c), 2D-band fitting of G100 (blue dotted line), G050 (red dotted line) and G000 (green dotted line). (e) SERS mapping images acquired in 2D-band of G100 (blue, 2600 cm⁻¹), G050 (red, 2650 cm⁻¹), G000 (green, 2706 cm⁻¹) and overlay of images. GIANS mixtures were at equal concentrations; scale bar, 10 μm. (f) (i) Bright-field (BF) of *C. elegans*. (ii–v) SERS imaging of *C. elegans* with 2D-band of (ii) G100 (blue), pseudocoel; (iii) G050 (red), digestive system; (iv) G000 (green), reproductive system; (v) overlay of G100, G050 and G000. Step size, 11 μm, scale bar, 200 μm.

Targeting strategy for cancer cells based on GIAN-aptamer complexes:

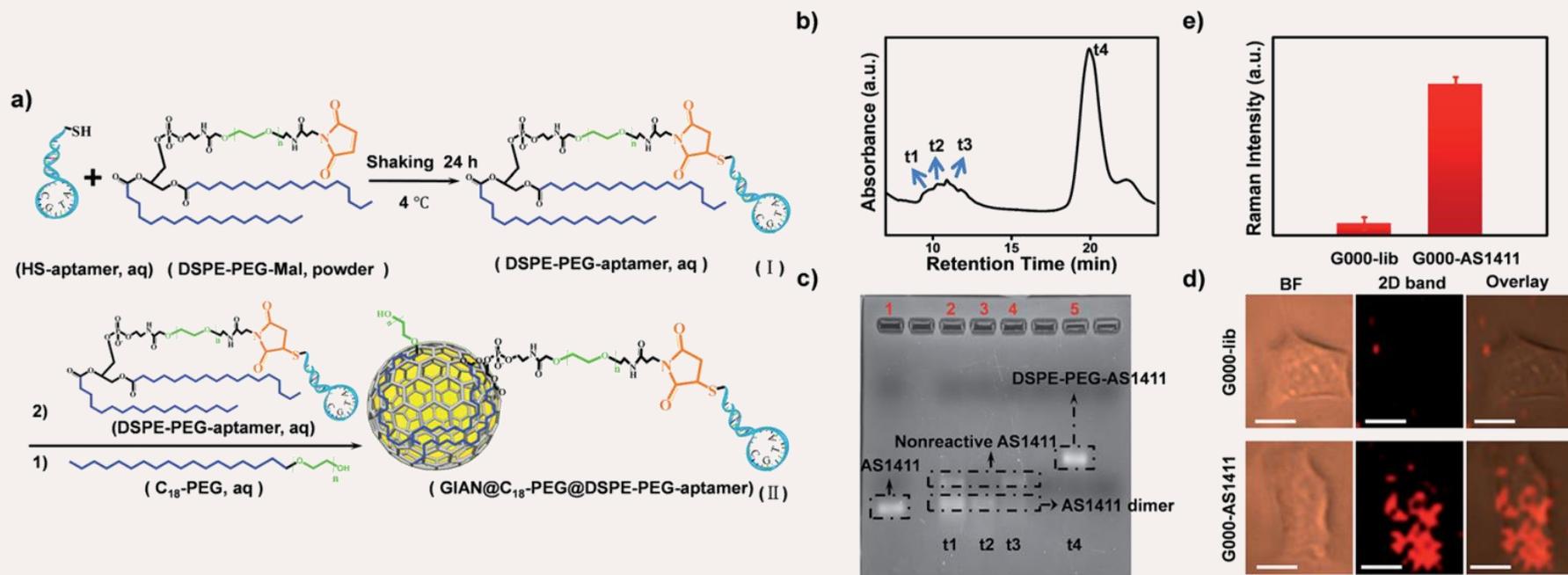


Fig. 6 (a) Schematic illustration of aptamer-functionalized GIAN. (b) DSPE-PEG-AS1411 purified by reverse phase HPLC. (c) Agarose gel electrophoresis characterized the DSPE-PEG-linked aptamers (DSPEPEG-AS1411) separated by HPLC. (d) SERS imaging of A549 cancer cells incubated with G000-lib (top) and G000-AS1411 (bottom); scale bar, 10 μm. (e) Statistics of 2D-band SERS intensity are shown in (d); the error bar came from three independent experiments.

Cancer cell pattern recognition with aptamer-functionalized isotopic GIAN-encoders:

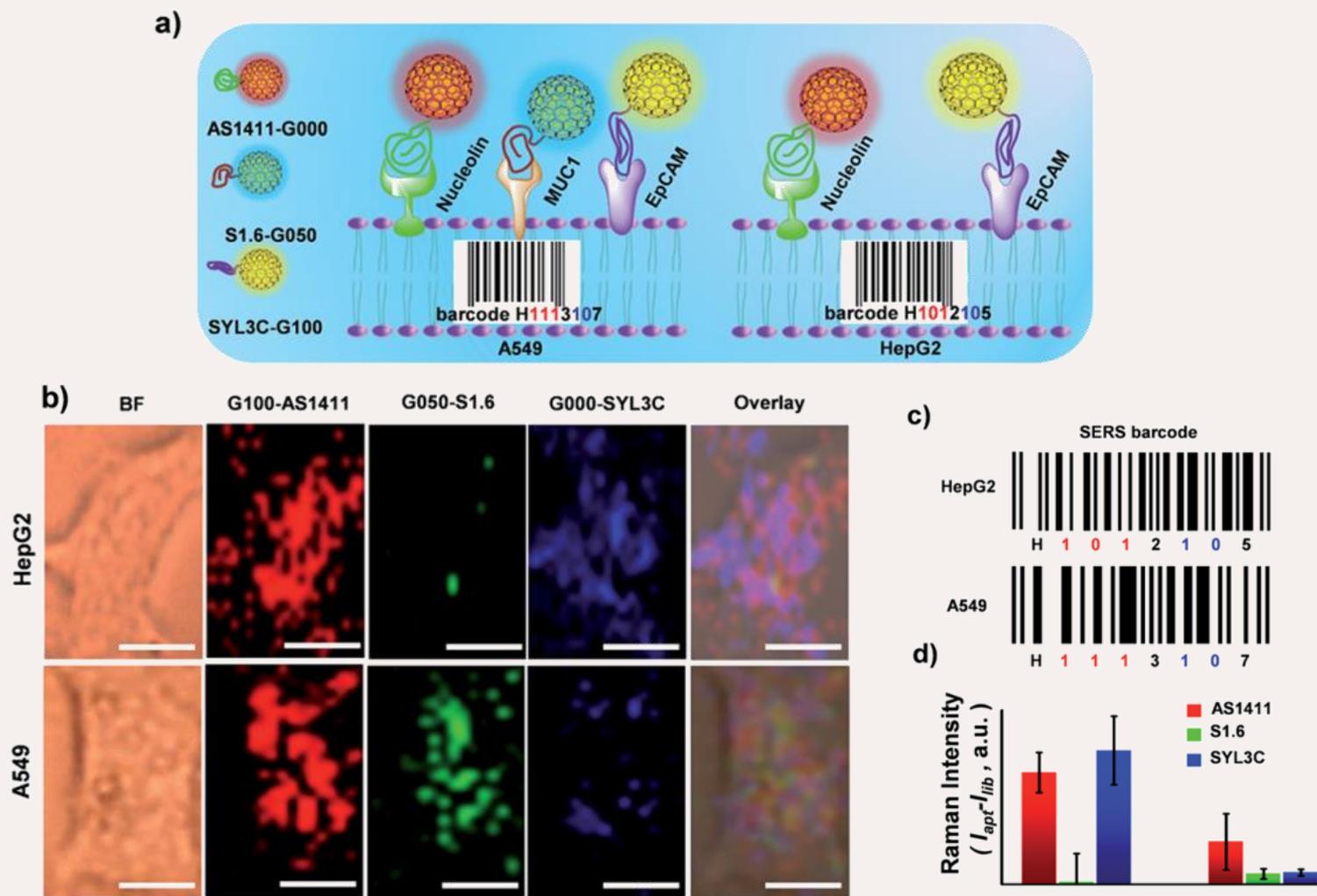


Fig. 7 (a) Schematic illustration of pattern recognition and discrimination of cancer cell lines with multiplexed GIAN-encoders. (b) SERS images of cancer cells, scale bar, 10 μ m. G100, G050 and G000 conjugated with DSPE-PEG-linked aptamer AS1411, S1.6 and SYL3C, respectively. (c) SERS barcodes of HepG2 and A549 cell lines. (d) Statistics of normalized SERS signals shown in (b).

Future Plan

- Isotope labelled citrate capped nanoparticles, single particle studies need to be performed to understand the ligand binding possibilities.

Thank you!

A blue ballpoint pen is shown in the lower right quadrant, angled towards the bottom left. The words "Thank you!" are written in a black, cursive script, appearing to be written by the pen. The pen's tip is positioned at the end of the word "you!".