ChemComm

This article is part of the

Surface Enhanced Raman Spectroscopy web themed issue

Guest editors: Professors Duncan Graham, Zhongqun Tian and Richard Van Duyne

All articles in this issue will be gathered together online at www.rsc.org/sers.

ChemComm

Dynamic Article Links

Cite this: *Chem. Commun.*, 2011, **47**, 3514–3516

www.rsc.org/chemcomm

COMMUNICATION

Multiplex cancer cell detection by SERS nanotags with cyanine and triphenylmethine Raman reporters†‡

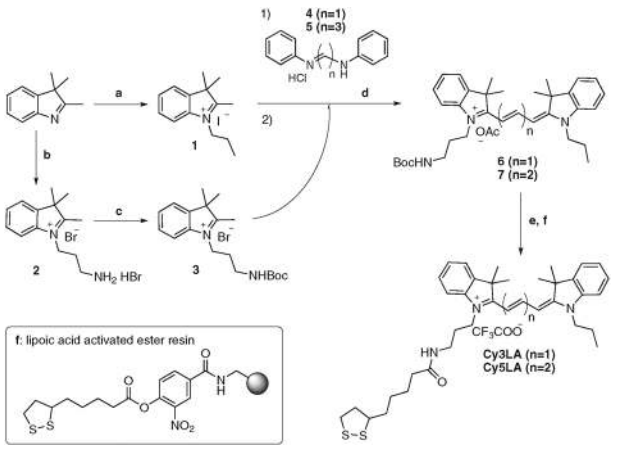
Kaustabh Kumar Maiti,[a] Animesh Samanta,[b] Marc Vendrell,[a] Kiat-Seng Soh,[a] Malini Olivo[acd] and Young-Tae Chang*[ab]

Received 30th November 2010, Accepted 15th January 2011
DOI: 10.1039/c0cc05265e

SERS nanotags have been prepared to accomplish the multiplex detection of cancer cells. Herein we evaluated the adequacy of lipoic acid-containing cyanine derivatives (Cy3LA and Cy5LA) to function as multiplex partners with a triphenylmethine Raman reporter (B2LA) under a single excitation wavelength. SERS experiments enabled the multiplex recognition of two different cancer cells with antibody-conjugated nanotags that were derivatized with optimized cyanine and triphenylmethine reporters.

In recent years surface enhanced Raman spectroscopy (SERS) has captured much attention due to the ability to provide molecular information with high sensitivity using the sharp and distinguishable spectral features of gold or silver nanoparticles (AuNPs or AgNPs).[1–4] SERS nanotags have several advantages, mainly high spectral specificity, improved contrast and multiplexing capabilities.[5,6] The multiplexing potential of SERS nanotags relies on the narrow bandwidths of the vibrational Raman spectra of the reporter molecules, and allows the simultaneous recognition of closely related targets. The concurrent detection of defined multiple targets can facilitate the development of accurate diagnostic probes, yet the identification of multiplex reporter pairs that are compatible under the same experimental conditions is difficult. Herein we prepared multiplex SERS nanotags after optimizing a new pair of Raman reporters based on triphenylmethine and cyanine compounds. After conjugation to antibodies (*e.g.* anti-EGFR and anti-HER2) that recognize different types of cancer cells (OSCC and SKBR-3, respectively), these multiplex SERS nanotags were capable to detect both cell lines using a single excitation wavelength.

Our group recently reported the excellent properties of triphenylmethines as Raman signature molecules,[7] and demonstrated the improved stability of their SERS signal upon chemisorption on AuNPs using a lipoic acid (LA) linker. A triphenylmethine derivative (**B2LA**) was identified as an outstanding reporter in terms of both SERS signal intensity and long-term stability.[8] In order to study the multiplexing capabilities of **B2LA** SERS nanotags, we evaluated their compatibility with different Raman-active chemical structures, such as cyanine molecules. **Cy3** and **Cy5** are well-known cyanine compounds with absorption properties in the red/far-red region of the visible range,[9–12] and we envisioned that they may be sensitive Raman reporters under the 633 nm laser that is required for the excitation of **B2LA** SERS nanotags. To adapt **Cy3** and **Cy5** structures for chemisorption, we designed the synthesis of lipoic acid-containing cyanine derivatives (**Cy3LA** and **Cy5LA**, respectively) so that they could be attached on AuNPs using thiol-based chemistry (Scheme 1). The chemisorption of Raman reporters is a critical step to ensure the stability and reproducibility of SERS nanotags over



Scheme 1 Synthesis of lipoic acid cyanine derivatives. *Reagents and conditions*: (a) 1-iodopropane, CH_3CN, 80 °C, 15 h; (b) 3-bromopropylamine hydrobromide, 120 °C, 10 h; (c) di-*tert*-butyl dicarbonate, DIEA, $CHCl_3$, reflux, 4 h; (d) AcOH, acetic anhydride, pyridine, 110 °C; (e) TFA–DCM (1 : 9), r.t., 16 h; (f) lipoic acid activated ester resin, CH_2Cl_2, r.t., 16 h.

[a] *Singapore Bioimaging Consortium, Agency for Science, Technology and Research (A*STAR), 11 Biopolis Way, 138667 Singapore*
[b] *Department of Chemistry & MedChem Program of Life Sciences Institute, National University of Singapore, 117543 Singapore. E-mail: chmcyt@nus.edu.sg; Fax: 65 6478 9957; Tel: 65 6478 8761*
[c] *Division of Medical Sciences, National Cancer Centre, 169610 Singapore*
[d] *School of Physics, National University of Ireland Galway, Ireland*

† This article is part of a ChemComm web-based themed issue on Surface Enhanced Raman Spectroscopy.

‡ Electronic supplementary information (ESI) available: Synthetic procedures and characterization (NMR, MS); procedures for: nanotag labeling, antibody conjugation, cell culture, Raman spectra determination and mapping experiments; TEM images and evaluation of the SERS stability of prepared nanotags; additional data for SERS measurements in cells. See DOI: 10.1039/c0cc05265e

time.[8,13] Starting from 2,3,3-trimethylindoline, we prepared the intermediates **1** and **2** using reported procedures.[14–16] Afterwards, **1** was condensed to the commercially available bis-phenylimines **4** and **5** in acidic conditions, followed by the subsequent addition of **3**, the Boc-protected derivative of **2**. As a result, the Boc-derivatives of **Cy3** and **Cy5** (**6** and **7**, respectively) were obtained with overall yields slightly over 50%. Deprotection of **6** and **7** with an optimized TFA-DCM (1 : 9) solution afforded the corresponding free amine compounds, which were treated with a lipoic acid-containing activated ester resin[17] to obtain **Cy3LA** and **Cy5LA** in good purities and yields (see ESI‡ for characterization data).

After chemisorbing **B2LA**, **Cy3LA** and **Cy5LA** on AuNPs, we analyzed their SERS spectra under the 633 nm laser and evaluated their multiplexing compatibility. Notably, a number of peaks could be used to uniquely identify the three different nanotags: 1617, 1374, 1366, 918 and 440 cm^{-1} for **B2LA**-AuNPs, 1589, 1383 and 613 cm^{-1} for **Cy3LA**-AuNPs and 1596, 1501, 1404 and 1353 cm^{-1} for **Cy5LA**-AuNPs (Fig. 1). Whereas the discrimination between **Cy3LA** and **Cy5LA**-derivatized nanotags may require further optimization, it was apparent that the combination of triphenylmethine and cyanine Raman reporters could be used as a basis for the construction of multiplex SERS nanotags, and we explored their application for the detection of related cancer cells. Nanotags derivatized with **B2LA** and **Cy3LA** were encapsulated with a mixture of thiol polyethyleneglycol (PEG-SH) and carboxylic acid-containing PEG-SH that allowed the covalent linkage to the free amine groups of antibodies.[3]

After encapsulation, we conjugated monoclonal antibodies against two different epidermal growth factor receptors (EGFR (Erb-B1) and HER2 (Erb-B2)) to render **B2LA** anti-EGFR and **Cy3LA** anti-HER2 nanotags. EGFR is over-expressed in diverse cancer cells (*e.g.* OSCC), and HER2 is a well-known breast cancer marker with a high expression in SKBR-3 cells.[18] The antibody conjugation was verified by the appearance of protein absorption peaks at 280 nm, and the size of the fully functionalized nanotags was determined by transmission electron microscopy (TEM) (Fig. S2, ESI‡). Furthermore, we confirmed that the SERS signal intensities of both nanotags were stable for several days (Fig. S3, ESI‡).

In order to examine the multiplex differential recognition of **B2LA** anti-EGFR and **Cy3LA** anti-HER2 nanotags in cells, we incubated an equal amount of both nanotags in OSCC cells (EGFR-positive and HER2-negative) and SKBR-3 cells (HER2-positive and EGFR-negative). After washings with PBS, the SERS measurement in OSCC cells fully resembled the SERS spectra of **B2LA** (Fig. 2a) whereas

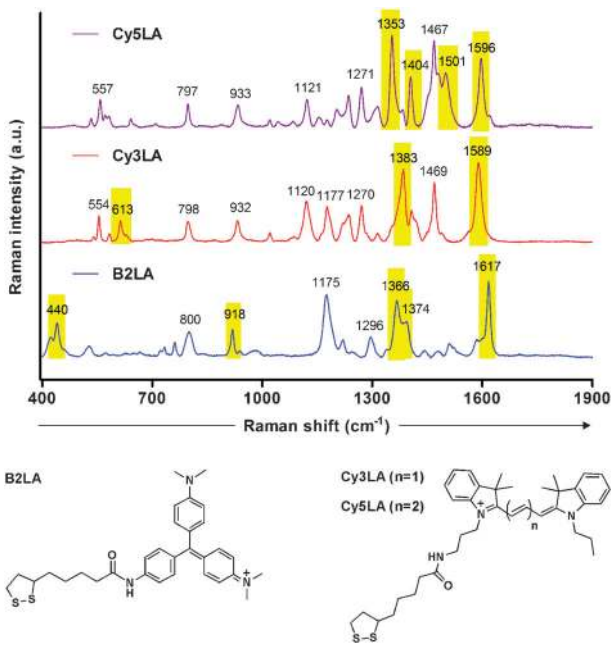


Fig. 1 Normalized SERS spectra of **B2LA**, **Cy3LA** and **Cy5LA** after chemisorption on AuNPs. Spectra were measured in a Raman microscope (633 nm laser excitation, 6.2 mW laser power, acquisition time: 10 s) and plotted as average intensities (n = 3). The most distinctive peaks from every reporter are highlighted in yellow.

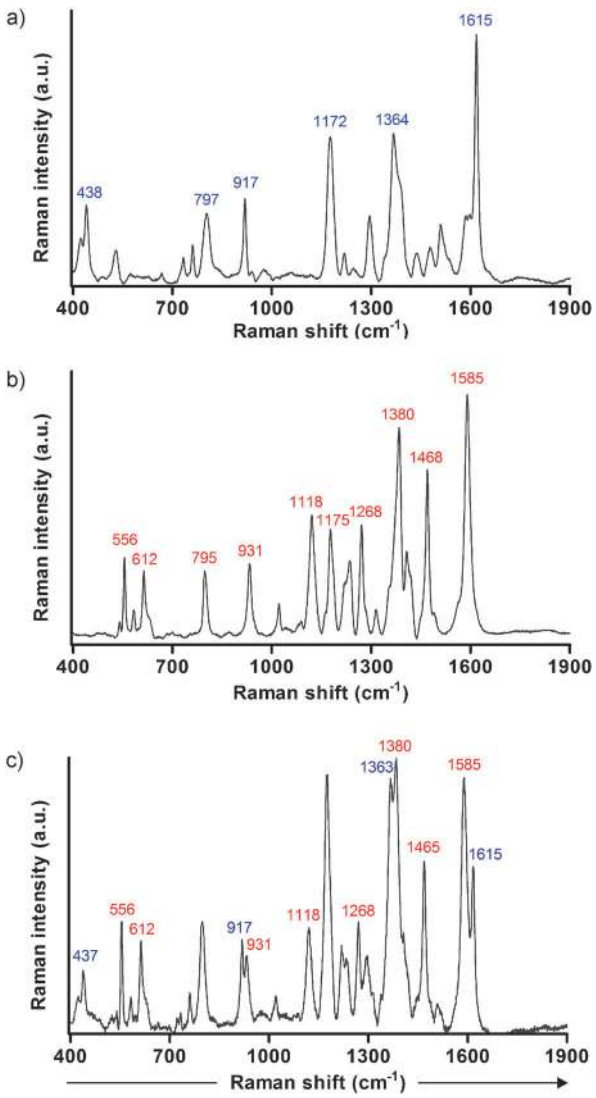


Fig. 2 Multiplex SERS spectra upon incubation of both **B2LA** anti-EGFR and **Cy3LA** anti-HER2 nanotags with: (a) OSCC cells, (b) SKBR-3 cells, (c) co-cultured cells. Spectra were measured with a Raman microscope (633 nm excitation wavelength, 6.2 mW laser power, acquisition time: 10 s) and plotted as average intensities (n = 3).

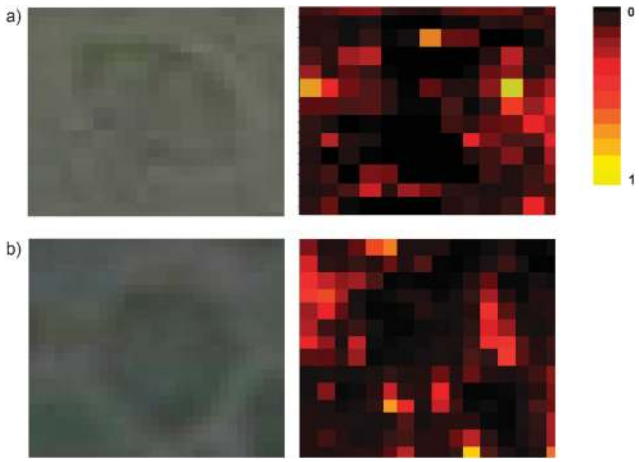


Fig. 3 Bright field and SERS mapping images of: (a) **B2LA** anti-EGFR nanotag-treated OSCC cells (1615 cm^{-1}), (b) **Cy3LA** anti-HER2 nanotag-treated SKBR-3 cells (1468 cm^{-1}). All mapping images (size: 30 × 30 μm^2) were scanned at an interval of 2 μm (633 nm excitation wavelength) and the intensities were normalized between the lowest (0) and the highest color (1) values.

the SERS signal of SKBR-3 cells coincided with the spectra of **Cy3LA** (Fig. 2b). Moreover, as negative controls, we did not observe significant SERS signals in OSCC or SKBR-3 cells after incubation with antibody-free **B2LA** and **Cy3LA**-nanotags (Fig. S4, ESI‡). Altogether, these results clearly indicated that: (1) **B2LA** anti-EGFR and **Cy3LA** anti-HER2 nanotags specifically recognized OSCC and SKBR-3 cells with non-overlapping SERS peaks, (2) the SERS signals derived from any possible non-specific binding of the two nanotags were negligible. Finally, we demonstrated that **B2LA** and **Cy3LA**-nanotags could be used as a multiplex platform by recognizing both OSCC and SKBR-3 cells after they were co-cultured in the same wells. Upon incubation with an equal proportion of both **B2LA** and **Cy3LA**-nanotags, the SERS signals of the co-cultured cells showed clearly separable peaks from the two reporter molecules: 1615, 1363, 917 and 437 cm^{-1} for **B2LA** anti-EGFR, and 1585, 1465, 1380, 1268, 1118, 931, 612 and 556 cm^{-1} for **Cy3LA**- anti-HER2 (Fig. 2c). With this data, we attested that **B2LA** and **Cy3LA** are fully compatible Raman reporters for the preparation of multiplex SERS nanotags.

To confirm the recognition properties of **B2LA** anti-EGFR and **Cy3LA** anti-HER2 nanotags and analyze their localization in OSCC and SKBR-3 cells, we performed SERS mapping experiments in both cell lines. As shown in Fig. 3, images of nanotag-treated OSCC cells and SKBR-3 cells displayed intense SERS signals at two distinguishable frequencies (*e.g.* 1615 and 1468 cm^{-1} respectively) in the cell surface region. Non-treated OSCC and SKBR-3 cells showed negligible SERS signals at both frequencies (Fig. S5, ESI‡). These mapping pictures confirmed that the interaction between **B2LA** anti-EGFR and **Cy3LA** anti-HER2 nanotags and their respective receptors was mainly localized at the cell surface, which corresponds well with the high expression of EGFR and HER2 at the plasma membrane of cancer cells.[19,20]

In summary, we developed a novel multiplex SERS platform for cancer cell detection based on the combination of triphenylmethine and cyanine Raman reporters. The SERS compatibility under a single excitation wavelength between a selected triphenylmethine and different lipoic acid-containing cyanine reporters was examined. Nanotags derivatized with a selected pair (**B2LA** and **Cy3LA**) were derivatized with anti-EGFR and anti-HER2 antibodies, and proved to specifically recognize the respective cancer cells (*e.g.* OSCC and SKBR-3) with non-overlapping SERS peaks. After confirming the performance of these nanotags in co-culture conditions and determining their localization by SERS mapping experiments, we demonstrated that **B2LA** and **Cy3LA** are fully compatible Raman reporters for the preparation of multiplex SERS nanotags and can be used for the concurrent detection of related cancer cells.

The authors gratefully acknowledge the A*STAR Cross Council Office (CCO), Singapore (Grant CCOGA02_005_2008), and the National University of Singapore (NUS) (Young Investigator Award: R-143-000-353-101) for their financial support.

Notes and references

1 C. L. Zavaleta, B. R. Smith, I. Walton, W. Doering, G. Davis, G. B. Shojaei, M. J. Natan and S. S. Gambhir, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 13511–13516.
2 X. M. Qian, X. Zhou and S. Nie, *J. Am. Chem. Soc.*, 2008, **130**, 14934–14935.
3 X. Quin, X.-H. Peng, D. O. Ansari, Q. Yin-Geon, G. Z. Chen, D. M. Shin, L. Yang, A. N. Young, M. D. Wang and S. Nie, *Nat. Biotechnol.*, 2007, **26**, 83–90.
4 S. Keren, C. Zavaleta, Z. Cheng, A. de la Zerda, O. Gheysens and S. S. Gambhir, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 5844–5849.
5 J. Kneipp, H. Kneipp, B. Wittig and K. Kneipp, *Nanomed.: Nanotechnol., Biol. Med.*, 2010, **6**, 214–226.
6 A. Matschulat, D. Drescher and J. Kneipp, *ACS Nano*, 2010, **4**, 3259–3269.
7 S. J. Cho, Y. H. Ahn, K. K. Maiti, U. S. Dinish, C. Y. Fu, P. Thoniyot, M. Olivo and Y. T. Chang, *Chem. Commun.*, 2010, **46**, 722–724.
8 K. K. Maiti, U. S. Dinish, C. Y. Fu, J. J. Lee, K. S. Soh, S. W. Yun, R. Bhuvaneswari, M. Olivo and Y. T. Chang, *Biosens. Bioelectron.*, 2010, **26**, 398–403.
9 K. Jia, Y. Wan, A. D. Xia, S. Y. Li, F. B. Gong and G. Q. Yang, *J. Phys. Chem. A*, 2007, **111**, 1593–1597.
10 A. Toutchkine, P. Nalbant and K. M. Hahn, *Bioconjugate Chem.*, 2002, **13**, 387–391.
11 H. J. Gruber, G. Kada, B. Pragl, C. Riener, C. D. Hahn, G. S. Harms, W. Ahrer, T. G. Dax, K. Hohenthanner and H. G. Knaus, *Bioconjugate Chem.*, 2000, **11**, 161–166.
12 A. Iqbal, L. Wang, K. C. Thompson, D. M. J. Lilley and D. G. Norman, *Biochemistry*, 2008, **47**, 7857–7862.
13 M. Gellner, K. Kompe and S. Schlucker, *Anal. Bioanal. Chem.*, 2009, **394**, 1839–1844.
14 B. Chipon, G. Clave, C. Bouteiller, M. Massonneau, P. Y. Renard and A. Romieu, *Tetrahedron Lett.*, 2006, **47**, 8279–8284.
15 C. Bouteiller, G. Clave, A. Bernardin, B. Chipon, M. Massonneau, P. Y. Renard and A. Romieu, *Bioconjugate Chem.*, 2007, **18**, 1303–1317.
16 A. Toutchkine, D. V. Nguyen and K. M. Hahn, *Bioconjugate Chem.*, 2007, **18**, 1344–1348.
17 J. W. Lee, Y. Q. Louie, D. P. Walsh and Y. T. Chang, *J. Comb. Chem.*, 2003, **5**, 330–335.
18 H. Zhang, A. Berezov, Q. Wang, G. Zhang, J. Drebin, R. Murali and M. I. Greene, *J. Clin. Invest.*, 2007, **117**, 2051–2058.
19 P. Monaghan, M. G. Ormerod and M. J. O'Hare, *Int. J. Cancer*, 1990, **46**, 935–943.
20 W. Ueda, S. Wang, N. Dumont, J. Y. Yi, Y. Koh and C. L. Arteaga, *J. Biol. Chem.*, 2004, **279**, 24505–24512.

