Electronic Supplementary Material (ESI) for RSC Advances.
This journal is © The Royal Society of Chemistry 2017

Supporting Information

Folate-modified silicon carbide nanoparticles as multiphoton imaging nanoprobes for cancer-cell-specific labeling

M. Boksebeld,[a] V. Kilin,[b] A. Geloën,[c] G. Ceccone,[d] A. Jaffal, [a] C. Schmidt,[b] S. Alekseev,[e] V. Lysenko,[f] J.P. Wolf, [b] L. Bonacina,[b] E. Souteyrand,[a] Y. Chevolot,[a] V. Monnier[a*]

[a] Université de Lyon, Institut des Nanotechnologies de Lyon, Ecole Centrale de Lyon, 36 Avenue Guy de Collongue, F-69134 Ecully, France

[b] Université de Genève, GAP Biophotonics, 22 Chemin de Pinchat, CH-1211 Geneva, Switzerland

[c] Université de Lyon, Laboratoire de recherche en cardiovasculaire, métabolisme, diabétologie et nutrition, INSA Lyon, 11 Avenue Jean Capelle, F-69621 Villeurbanne, France

[d] European Commission, Joint Research Centre, Directorate F: Health, Consumers and Reference Materials, I-21020 Ispra, Italy

[e] Kiev National Taras Schevchenko University, Volodymyrska St, 60, UA-01601 Kiev, Ukraine

[f] Institut des Nanotechnologies de Lyon, INSA Lyon, 7 Avenue Jean Capelle, F-6962 Villeurbanne, France

E-mail : virginie.monnier@ec-lyon.fr

Experimental section

Detailed procedure for colorimetric amine titration

An acidic buffer was prepared by mixing 85 mL of water with 10 mL of methanol and 5 mL of 99% acetic acid. Then 250 µL of nanoparticles dispersion were centrifuged (6700g, 3 min) and redispersed in the acidic buffer to protonate amines on the surface of particles. Nanoparticles were centrifuged again and redispersed in 250 µL of a 58 µM Coomassie Brilliant Blue (CBB) solution prepared in the acidic buffer. After 15 min of sonication, nanoparticles were washed three times with the acidic buffer to remove the excess of CBB. Nanoparticles were redispersed in 250µL of water. Then, 200 µL of a basic buffer prepared with 50 mL of a 1 M ammonia solution in water and 50 mL of methanol was added to the nanoparticles to deprotonate amines and release CBB immobilized onto nanoparticles. After 5 min of sonication, nanoparticles were centrifuged (10000g, 15 min) and the supernatant was analyzed with Ultraviolet-Visible absorption spectrophotometer. The number of CBB molecules (equal to the number of amine groups) can be deduced from the absorbance of the solution at 611 nm using Beer-Lambert law (molar extinction coefficient of CBB at 611 nm is equal to 87893 L mol^{-1} cm^{-1}).

Toxicity assay

Cell proliferation and/or survival was monitored with the xCELLigence Real-time Cell Analyser (RTCA) System (ACEA Biosciences, Inc., San Diego, USA), which allows label-free monitoring changes of cell number, viability, morphology and quality of cell attachment by measurement of cell to electrode responses of cells seeded in E96-well plates manufactured with integrated microelectronic sensor arrays. RTCA system measures real-time impedance variations that are then converted in cell surface occupancy, i.e. cell index, taking into account cell number, cell size, and adhesion force. When cell index reaches a sufficient value (typically between 1 and 2), silicon carbide (SiC) nanoparticles are introduced and incubated with cells during 24h using the protocol detailed in the previous section. Cell indexes are normalized at the introduction of nanoparticles in order to facilitate the comparison between the different conditions. Then SiC nanoparticles are removed by replacing the cell culture medium by a fresh one. The impedance measurements are maintened during 24h in order to study the influence of washing.

Detailed experimental conditions for X-Ray Photoelectron Spectroscopy (XPS) and Time of Flight Secondary Ion Mass Spectrometry (ToF-SIMS) analyses

For XPS and ToF-SIMS analyses, 100 µL solution of each sample was spotted on clean Si(100) substrate and let to dry overnight in air before insertion in the vacuum chamber.

Concerning XPS measurements, for each sample, a survey spectrum (0–1150 eV), from which the surface chemical composition (at. %) was determined, was recorded at pass energy of 160 eV. In addition, one set of high-resolution spectra (analyzer pass energy at 20 eV) was recorded to obtain information about the chemical bonding of the different elements. Surface charge was compensated by a magnetic charge compensation system and the energy scale was calibrated by setting the C 1s hydrocarbon peak to 285 eV. The take-off angle for the acquisitions was 90 with respect to the sample surface. The acquisition time was kept below 20 min per sample to avoid possible X-ray damage. Wide and core level spectra were acquired on at least three different sample positions. The data were processed using Vision2 software (Kratos Analytical, UK) and the analysis of the XPS peaks was carried out using a commercial software package (Casa XPS v2.3.16PR1, Casa Software Ltd., UK). The atomic percentages were calculated from the experimentally determined peak intensities and normalized by atomic sensitivity factors provided by Kratos Analytical. Peak fitting was performed without any preliminary smoothing. Symmetric Gaussian–Lorentzian (70% Gaussian and 30% Lorentzian) product functions were used to approximate the line shapes of the fitting components after a Shirley-type background subtraction.

For ToF-SIMS analyses, spectra were acquired in static mode (primary ion fluence < 10^{12} ions cm^{-2}) in order to preserve the molecular information. During analysis, charging of the surface was prevented by applying charge compensation using low-energy (20 eV) electron flood gun. Mass calibration of ToF-SIMS spectra was done by using the hydrocarbon peaks CH^+ (13 m/z), CH_3^+ (15 m/z), $C_2H_3^+$ (27 m/z), $C_3H_5^+$ (41 m/z), $C_5H_7^+$ (67 m/z), and $C_7H_7^+$ (91 m/z), and C^- (12 m/z), C_2^- (24m/z), C_3^- (36m/z), C_4^- (48m/z), C_5^- (60m/z) in order to ensure a good relative mass accuracy. Analyses were obtained from square areas of 250×250 μm^2 in high mass resolution burst mode (resolution $M/\Delta M > 6000$). Spectral interpretation was carried out using Surface Lab software v6.4 (ION-TOF GmbH, Münster, Germany).

Other characterization techniques

Transmission Electron Microscopy (TEM) images were realized with a JEOL 2100HT working at 200 kV. For TEM studies, 2 μL of the diluted dispersion of nanoparticles was deposited onto a carbon lacey grid (Ted Pella, Inc.). X-Ray diffractogram (XRD) was obtained using an X-ray diffractometer (Rigaku, Smartlab) at room temperature with Cu Kα radiation (wavelength 1.5405 Å). SHG emission spectrum was obtained using Hyper Rayleigh Scattering spectroscopy. Nanoparticles dispersed in water were put in a quartz cuvette and excited at 820 nm using the same source as the one used for multiphoton microscopy.

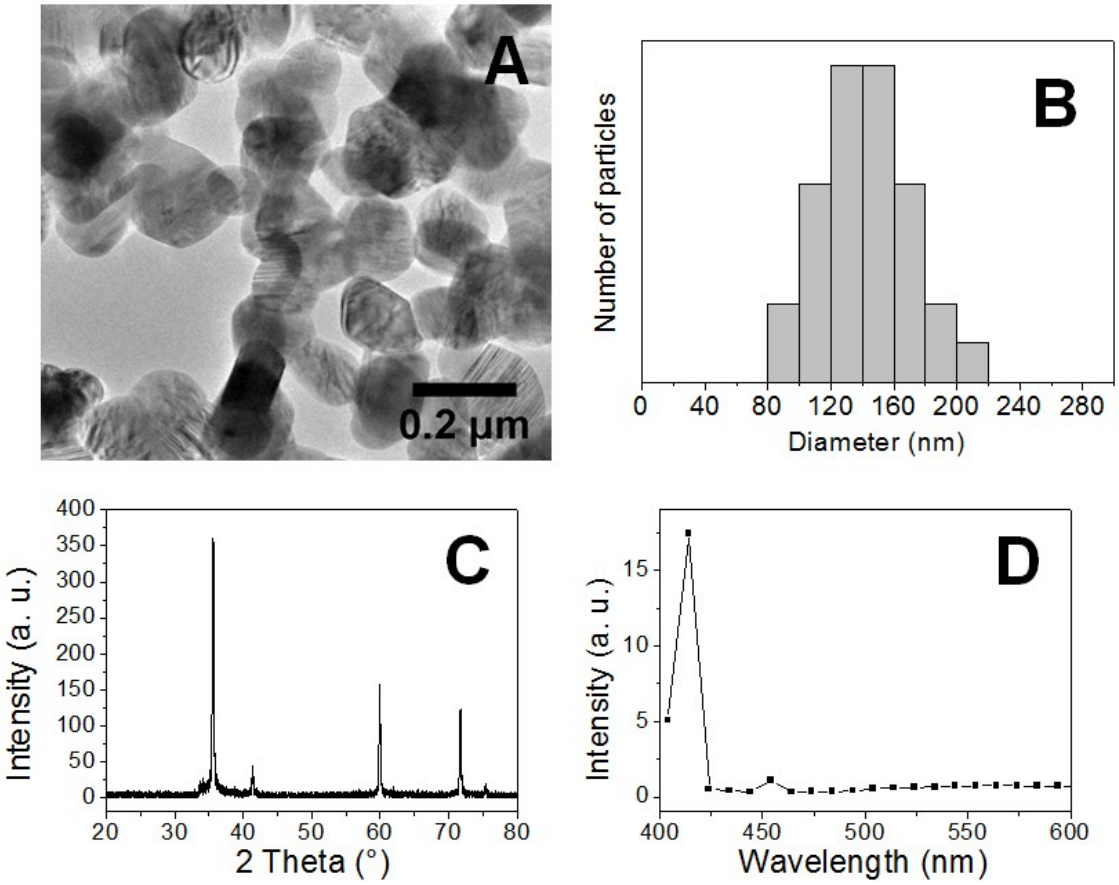

Fig. S1. TEM image (A), corresponding size histogram (B), XRD diffractogram (C) and SHG emission spectra at 820 nm excitation (D) of KOH treated SiC nanoparticles.

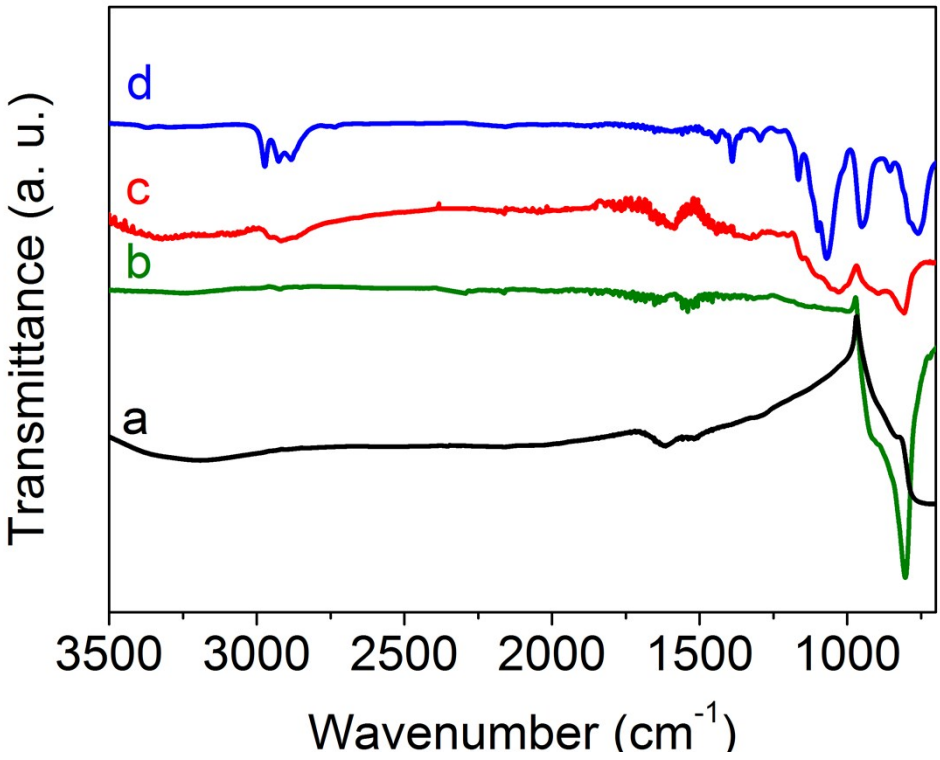

Fig. S2. Full infrared transmission spectra of KOH treated (a), APTES-1 (b), APTES-2 modified SiC nanoparticles (c) and APTES (d).

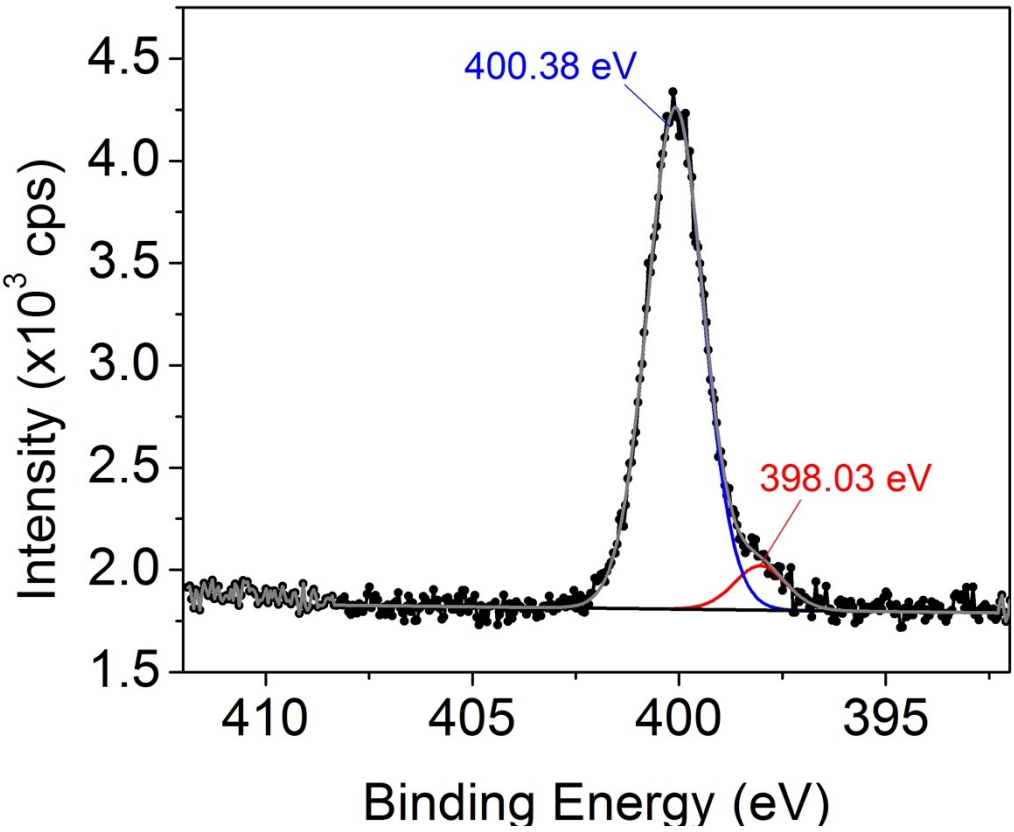

Fig. S3. N1s level XPS spectrum of APTES-2 modified SiC nanoparticles.

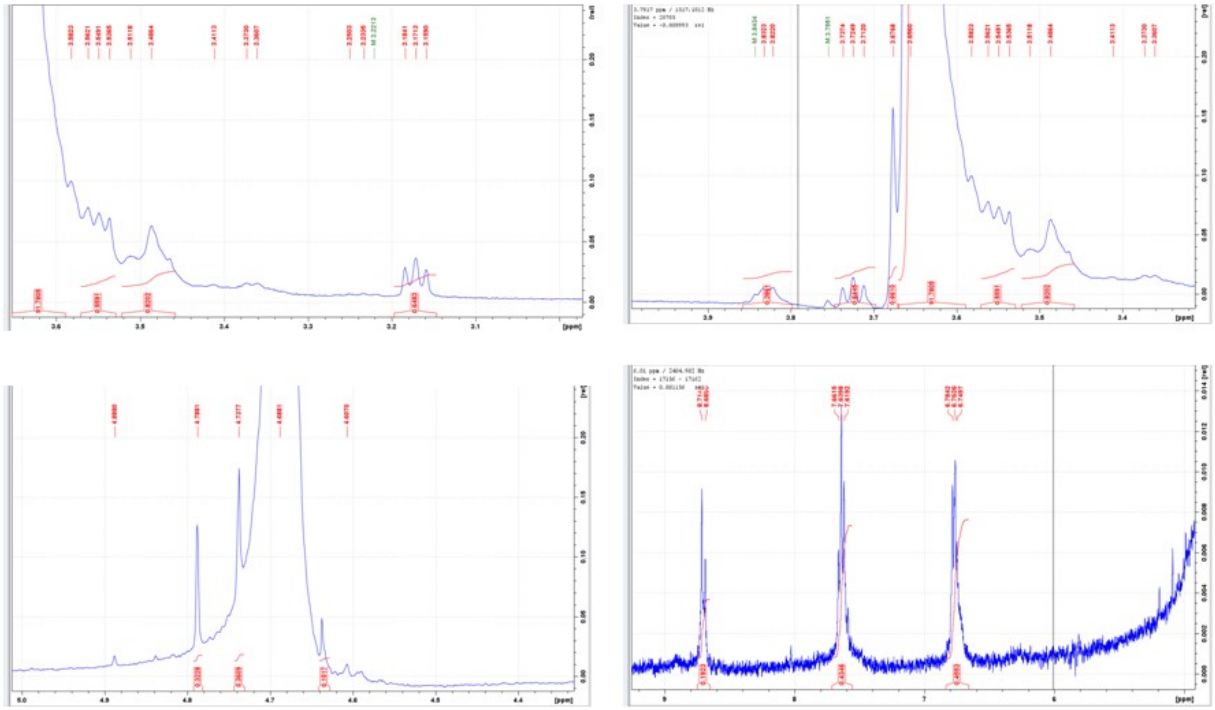

Fig. S4. ^{1}H NMR spectrum of PEG-folate.

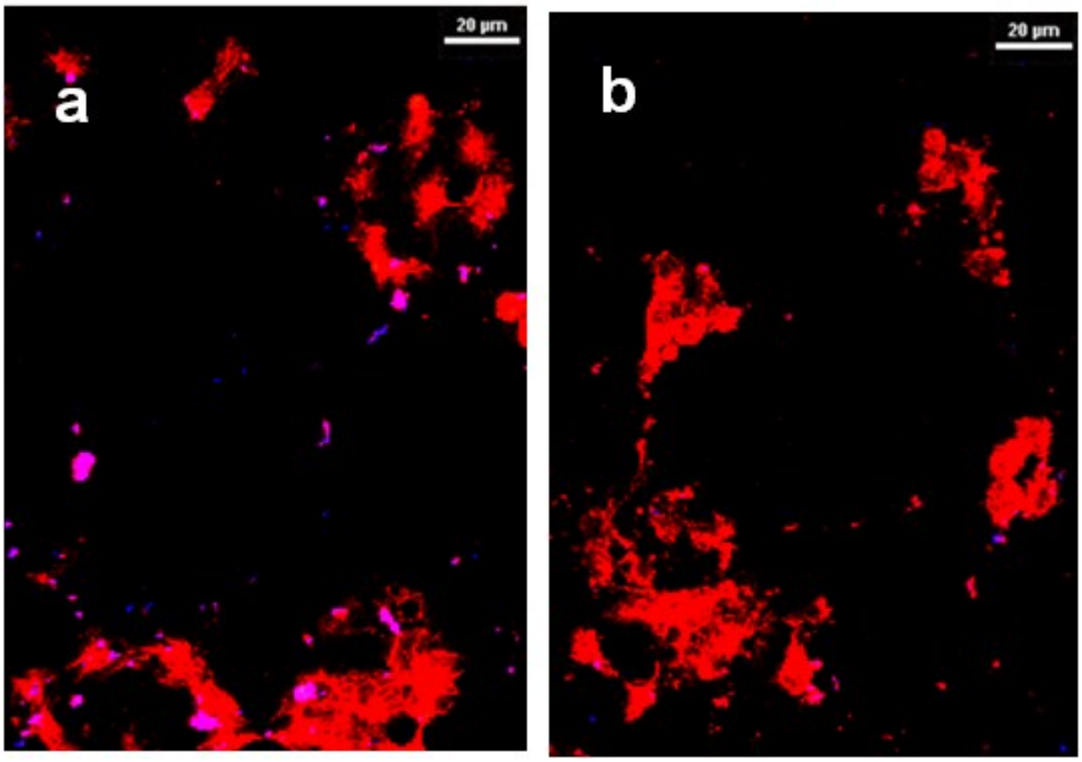

Fig. S5. Multiphoton images of HuH7 cancer cells (a) after incubation with folate-modified SiC nanoparticles in competition with free folic acid and (b) after incubation with PEG-modified SiC nanoparticles. Blue-Purple spots correspond to SHG signal measured at 395 nm and red spots correspond to TPEF signal measured at 607 nm. Excitation wavelength is 790 nm.

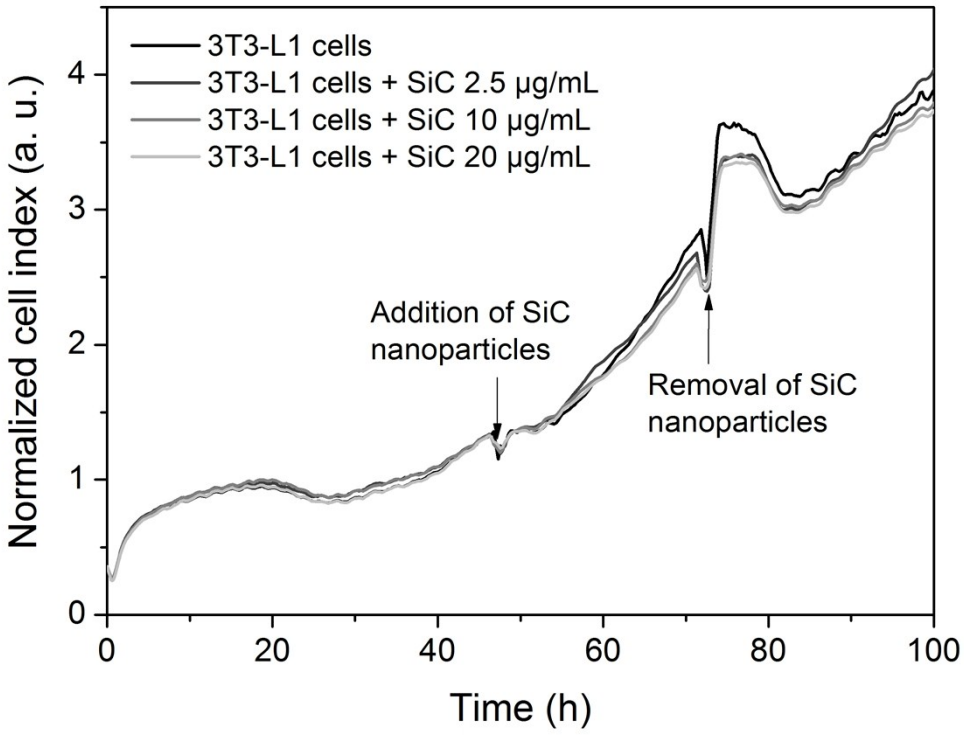

Fig. S6. Cell proliferation of 3T3-L1 healthy cells in presence of various SiC nanoparticles concentrations.

SHG-emitting nanoparticles	Second-order nonlinear optical coefficients from bulk crystals (pm/V)	Cytotoxicity as nanoparticles	Use as nanoparticles for SHG bio-imaging and therapy
$LiNbO_3$	17.3-25.7[1,2]	Low[1,3]	Stem cell imaging[3]
$BaTiO_3$	12.8-14.1[1,4]	Low[1,5]	Cancer cell targeting[6] Cancer cell imaging[5] Phototherapy[5]
$KNbO_3$	15.3-22.3[1,2]	Low[1]	Healthy cell imaging[7]
ZnO	1.4-2.8[1,8]	High[1,9]	Cancer cell targeting[10] Phototherapy[11]
SiC-3C	26.0-34.2[12,13]	Low[14]	Cancer cell targeting[14]

Table S1. Comparison of SHG-emitting nanoparticles used for bio-imaging and therapy from literature.

1 D. Staedler, T. Magouroux, R. Hadji, C. Joulaud, J. Extermann, S. Schwung, S. Passemard, C. Kasparian, G. Clarke, M. Gerrmann, R. Le Dantec, Y. Mugnier, D. Rytz, D. Ciepielewski, C. Galez, S. Gerber-Lemaire, L. Juillerat-Jeanneret, L. Bonacina and J. P. Wolf, *ACS Nano*, 2012, **6**, 2542.

2 I. Shoji, A. Kitamoto, T. Kondo and R. Ito, *J. Opt. Soc. Am. B*, 1997, **14**, 2268.

3 J. Li, J. Qiu, W. Guo, S. Wang, B. Ma, X. Mou, M. Tanes, H. Jiang and H. Liu, *Nanoscale*, 2016, **8**, 7416.

4 D. N. Nikogosyan, *Nonlinear Optical Crystals: A Complete Survey*, New York, Springer, 2005.

5 S. Wang, X. Zhao, J. Qian and S. He, *RSC Adv.*, 2016, **6**, 40615.

6 C. L. Hsieh, R. Grange, Y. Pu and D. Psaltis, *Biomaterials*, 2010, **31**, 2272.

7 R. Ladj, T. Magouroux, M. Eissa, M. Dubled, Y. Mugnier, R. Le Dantec, C. Galez, J. P. Valour, H. Fessi and A. Elaissari, *Colloid Surf. A*, 2013, **439**, 131.

8 G. Wang, G. K. L. Wong and J. B. Ketterson, *Appl. Opt.*, 2001, **40**, 5436.

9 H. Yang, C. Liu, D. Yang, H. Zhang and Z. Xi, *J. Appl. Toxicol.*, 2009, **29**, 69.

10 A. V Kachynski, A. N. Kuzmin, M. Nyk, I. Roy and P. N. Prasad, *J. Phys. Chem. C*, 2008, **112**, 10721.

11 B. Gu, A. Pliss, A. N. Kuzmin, A. Baev, T. Y. Ohulchanskyy, J. A. Damasco, K. T. Yong, S. Wen and P. N. Prasad, *Biomaterials*, 2016, **104**, 78.

12 J. Bouclé, A. Kassiba, M. Makowska-Janusik, J. Sanetra, N. Herlin-Boime, A. Bulou and S. Kodjikian, *Opt. Commun.*, 2005, **246**, 415.

13 I. J. Wu and G. Y. Guo, *Phys. Rev. B*, 2008, **78**, 35447.

14 Our work.