Supplementary Information

Nanoscale mapping of newly-synthesised phospholipid molecules in a biological cell using tip-enhanced Raman spectroscopy

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S1. Optical set-up

Fig. S1. Detailed schematic diagram of the optical set-up used in this work showing major optical components.

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S2. TERS system

A transmission-mode AFM-TERS system was used for this study consisting of an inverted confocal microscope (Nikon, Japan) coupled with an atomic force microscope (AFM) (AIST-NT, The Netherlands) on top. Near-field and far-field Raman spectra were measured using a Raman spectrometer (Horiba Scientific, UK) (600 lines/mm grating) with an electron-multiplying charged coupled device detector (Andor Technology, Ireland). A 532 nm laser was focused onto the sample using a 100×, 1.49 NA oil-immersion objective lens (Nikon, Japan) after being radially polarised using a liquid crystal polariser (ARCoptix, Switzerland). TERS measurements were conducted in contact-mode AFM. Confocal Raman measurements were performed with a laser power of 500 µW at the sample whereas a laser power of 350 µW at the sample was used for the TERS measurements.

S3. Tip preparation

TERS tips used in this work were prepared by first oxidising contact-mode Si AFM tips (Mikromasch, Estonia) to a thickness of 300 nm SiO$_2$ in a tube furnace at 1000° C. The oxidised tips were then cleaned using UV-ozone ((UVOCS Inc., USA) for 45 minutes to get rid of any organic contamination on the surface. Finally, the cleaned tips were coated with a nominal thickness of 100 nm Ag using thermal evaporation at $10^{-6}$ mbar pressure with a slow deposition rate of 0.05 nms$^{-1}$, which resulted in a typical tip-apex radius of $\approx 26.5$ nm as shown in the Fig. S2.

![Fig. S2.](image)

(a) Scanning electron microscopy (SEM) image of a Ag-coated TERS tip prepared in the same batch as the tip used in this work. (b) Zoomed-in region of the area marked by dotted rectangle in (a).

S4. Sample preparation

1 mM D-35 stearic acid (DSA) stock solution was prepared by dissolving 3.2 mg of DSA (Sigma-Aldrich) in 2 ml of ethanol. It was then complexed with 400 µl of 100 mM solution of NaOH in ethanol. Alcohol was evaporated with nitrogen gas obtaining fatty acid soaps, which were dissolved in 0.5 ml of hot ultrapure water and kept in a 55°C water bath for 10 minutes. 1 g of fatty acid-free bovine serum albumin (Sigma-Aldrich) was dissolved in 9.5 ml Dulbecco's Modified Eagle's Medium (DMEM) (Sigma-Aldrich), warmed to 55°C, added to the dissolved fatty acid soaps, and vortex mixed for 10 seconds, followed by a further 10 minutes incubation at 55°C. The 1 mM DSA stock solution was sterilised by filtration, aliquoted and kept at -20°C.
Mouse preadipocytes were seeded on 13 mm plastic coverslips (Thermanox) in 24-well plates. The next day, cells were treated with 10 µM deuterated stearate for 6 hours. Upon treatment completion, cells on coverslips were washed with Phosphate-buffered saline (PBS) and fixed at room temperature for 20 minutes in solution of 4% paraformaldehyde and 1% glutaraldehyde in 100 mM 1, 4-Piperazinediethanesulfonic acid (PIPES) pH 7.4 warmed up to 37°C. Secondary fixation in 2.5% glutaraldehyde in 100 mM PIPES pH 7.4 followed for 1 hour at room temperature. Cells were then transferred to 4°C and left overnight.

The next day, samples were washed 3 times for 10 minutes each with 100 mM PIPES pH 7.4, followed by osmication with 1% osmium tetroxide in 100 mM PIPES pH 7.4 for one hour and washed in deionised water for 20 minutes. The cells then went through a graded ethanol series, first at 50% ethanol for 15 minutes, then 70% ethanol overnight at 4°C, then 90% ethanol for 15 minutes, then 95% ethanol for 15 minutes, and finally 100% ethanol for 2 hours with 3 solution changes during this time. Gradual infiltration with Agar 100 epoxy resin (Agar Scientific) followed, starting with 25% resin for one hour, then 50% resin for two hours, then 75% resin for one hour, and 100% resin overnight. The next day, samples were transferred twice to fresh 100% resin for 3 hours each time. The cells were then embedded in fresh resin and left for 24 hours at 60°C for polymerisation. The plastic coverslip was removed and the specimen was cut to obtain thin sections of 1 µm using a Leica UC7 ultramicrotome with a diamond knife (Diatome). Thin cell sections were then mounted on 0.17 mm thick (thickness no. 1.5) glass coverslips for TERS measurements.

S5. Raman spectrum of 1,2-distearoyl-d70-sn-glycero-3-phosphocholine

![Raman spectrum of 1,2-distearoyl-d70-sn-glycero-3-phosphocholine](image)

**Fig. S3.** Raman spectrum of 1,2-distearoyl-d70-sn-glycero-3-phosphocholine (DSPC-d70) liposomes in an aqueous environment. Raman bands of C-D and C-H stretching from DSPC-d70 molecules and O-H stretching from water are highlighted. Adapted with permission from Matthaus et al. Mol. Pharm. 2008, 5(2), 287 – 283. Copyright 2008 American Chemical Society.
S6. TERS maps of C-D band intensity (Fig. 3) with linear smoothening

**Fig. S4.** (a) TERS maps shown in Fig. 3a with linear smoothening for easier visualisation of the distribution of newly-synthesised phospholipid (NSP) molecules. (b) TERS map shown in Fig. 3b with linear smoothening.