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Probing lipids in biological membranes using SERS

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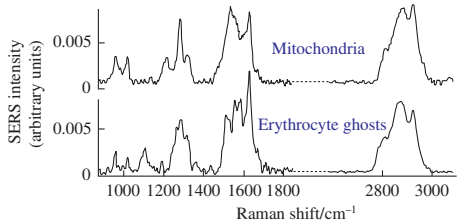
A selective and sensitive experimental approach to the examination of lipids as well as lipid–protein interactions in biological membranes using surface-enhanced Raman spectroscopy has been proposed. This approach has been used for probing the intact mitochondria, membranes of erythrocyte ghosts and liposomes.



Raman spectroscopy (RS) represents a powerful tool for exploring conformational changes in biomolecules both *in vitro* and *in vivo*.[1–6] It has been successfully applied to the investigation of biomembranes, vesicles and lipid structures in cells. In particular, the RS approach has been used for estimation of the relative amounts of saturated *vs.* unsaturated lipids as well as their population in *gauche* and *trans* conformations in droplets, which indicates the ordering in the lipid phase.[7,8] Despite the advantage of being noninvasive and label-free method, RS has definite limitations due to weak spontaneous Raman scattering. Moreover, in cell investigations the signal from membrane lipids and trans- and submembrane proteins is obscured by the overlapping scattering originated from the relatively high volume of cytoplasm and organelles. Alternatively, the method of surface-enhanced Raman spectroscopy (SERS) provides a many fold increase in the intensity of scattering from molecules in the close vicinity to nanostructures, thus making it possible to investigate conformational properties of molecules in the cellular plasma membrane and in the membranes of isolated organelles as well as in artificial vesicles, *i.e.* liposomes.[9,10] For example, it has been demonstrated that the model monolayer and bilayer lipid membranes reveal SERS spectra in the high frequency range, namely 2750–3050 cm^{-1}, if they are in the close contact with 15 nm silver islands.[11] As well, plasmonic gold nanoshells have been used to examine a lipid exchange between vesicles.[12] SERS has been also applied to gold nanoparticles for the investigation of the lipids conformational dynamics in artificial hybrid bilayer leaflets, and thus the possibility to monitor individual lipid molecules has been demonstrated.[13] Nanostructured gold surfaces in combination with streptavidin coated gold colloids have been used for the attachment of biotinylated liposomes bearing crystal violet as a SERS probe. In this case, the long distance enhancement of SERS signal from the dye inside the liposomes was achieved, while the signal from lipids was not explored.[14] To the best of our knowledge, the SERS investigations of lipids in cellular membranes have not been published, whereas lipids represent key components of the plasma membrane and other cellular compartments, moreover, their conformational changes are closely related to membrane processes, such as transmembrane transport as well as variations in the transmembrane potential, fluidity and surface charge.

In this work, we applied the SERS method to the investigation of lipids conformation in artificial and intact cellular membranes using plasmonic silver nanostructures. For this purpose, the following preparations were tested: (*1*) rat heart mitochondria isolated as described previously;[15] (*2*) erythrocyte ghosts, *i.e.* closed vesicles consisting of plasma membrane with transmembrane proteins, submembrane hemoglobin (Hb) and submembrane cytoskeleton lacking cytosolic Hb, produced as described;[16] (*3*) liposomes prepared from egg yolk phosphatidylcholine (PC) and bovine heart cardiolipin (CL); and (*4*) proteoliposomes from PC (80 wt%) and CL (20 wt%) with enclosed cytochrome *c* (cytC).[†]

Plasmonic silver nanostructured surfaces (AgNSS) were prepared according to the established protocol.[18] The SERS spectra[‡] of mitochondria and erythrocyte ghosts in the low frequency range 600–1800 cm^{-1} under 514 nm laser excitation corresponded to the SERS spectra of heme *c* in cytC and heme *b* in submembrane or membrane-bound Hb, respectively (Figure 1). These spectra contain several common peaks with maxima at 1127, 1170, 1371 and 1638 cm^{-1} as well as specific peaks at 1313 and 1300 cm^{-1}, corresponding to heme *c* and

[†] Liposomes were prepared by a standard protocol[17] from egg yolk phosphatidylcholine (Sigma–Aldrich) and bovine heart cardiolipin (Avanti Polar Lipids). The total content of cytochrome C (Sigma–Aldrich) was 6.5 μM.

[‡] A suspension of mitochondria (supplemented with 5 mM succinate, 2 mM pyruvate, 5 mM malate and 0.1 mM ADP), liposomes or erythrocyte ghosts (300 μl) was dropped on AgNSS in a glass bottom Petri dish. The SERS spectra were collected from several points using an inVia Raman microscope (Renishaw, UK) with a 20× objective having a numerical aperture of 0.4. Acquisition time was 20 s and the 514 nm argon laser power was 1–5 mW per registration spot, which was considered non-invasive for these biological objects.

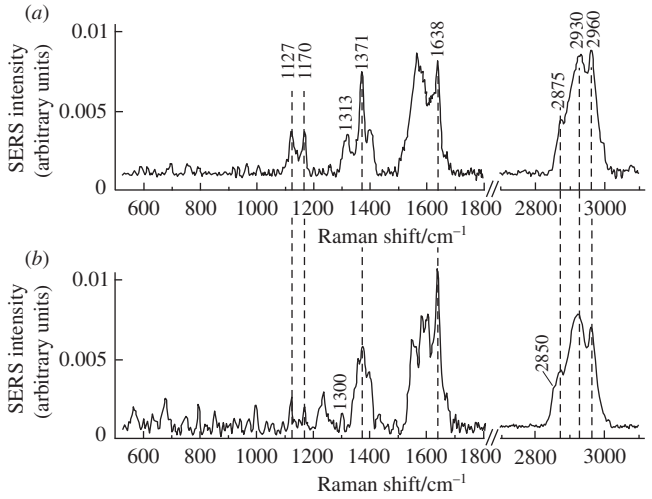


Figure 1 SERS spectra of (*a*) functionally active mitochondria and (*b*) erythrocyte ghosts on AgNSS in high and low frequency ranges, normalized by the total intensity.

heme *b*.[15,18] It is known, that using the above spectral range it is possible to explore the conformational changes in cytC heme inside the functional mitochondria with modulation of their respiratory chain activity,[15] as well as the conformation of submembrane Hb in erythrocytes.[18] In general, lipids are not detected in this spectral range due to their weak SERS signal compared with the strong signal of heme-containing proteins. However, the use of AgNSS ensured the enhancement of Raman scattering in an alternative range of 2700–3100 cm^{-1}, where the SERS spectra of mitochondria and erythrocyte ghosts contain three intensive peaks at 2875, 2930 and 2960 cm^{-1} (Figure 1).

We suppose that the SERS signal of mitochondria originates mainly from the outer membrane, because it is located closer to AgNSS than the inner mitochondrial membrane, and the enhancement of Raman scattering depends crucially on the distance between nanostructures and the analyte. According to the known data, the peaks at 2930 and 2960 cm^{-1} correspond to the symmetric and asymmetric vibrations, respectively, of methyl groups in membrane lipids and proteins, while the peak

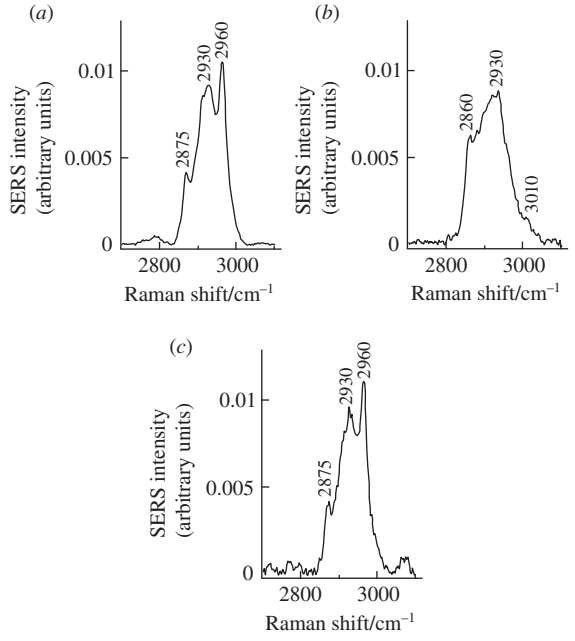


Figure 2 SERS spectra of liposomes consisting of (*a*) PC, (*b*) CL and (*c*) PC : CL + CytC in high frequency range, normalized by the total intensity.

at 2875 cm^{-1} is assigned to the stretching of CH_2 bonds.[11,19] The shoulder at 2850 cm^{-1}, corresponding to CH_2 bond vibrations, can also be observed in the SERS spectrum of erythrocyte ghosts. Since lipids are the main components of the outer mitochondrial membrane and the erythrocyte ghost membrane, we suggested that these peaks originate mainly from lipids.

To comfirm the origin of the peaks, we recorded the SERS spectra of three types of unilamellar liposomes, namely those consisting of PC, CL and PC : CL mixture with enclosed cytC. PC was chosen as the most abundant lipid in cell membranes, and CL was selected as a lipid constituting up to 20% of the inner mitochondrial membrane lipids with an important role in the membrane structural organization.[20] Figure 2 represents SERS spectra of these three types of liposomes.

In general, liposomes consisting of PC reveal the most intensive SERS signal with high signal-to-noise ratio, whereas liposomes composed of CL gave less intensive spectra with lower signal-to-noise ratio. Therefore, for better appearance, the spectra were normalized by their total intensities. We have found that the SERS spectrum of PC liposomes contains three main peaks, namely 2875, 2930 and the most intensive one at 2960 cm^{-1} [Figure 2(*a*)]. In the spectrum of CL liposomes there are also a broad peak at 2930 cm^{-1} with a shoulder at 3010 cm^{-1}, corresponding to the vibrations of C=C bonds, and a smaller peak at 2860 cm^{-1}, the peak at 2960 cm^{-1} being absent [Figure 2(*b*)]. The spectrum of liposomes containing both PC and CL with enclosed cytC [Figure 2(*c*)] is almost identical to that for pure PC liposomes, which can be interpreted by the following reasons: (i) PC with its higher SERS intensity compared to CL determines the spectrum appearance in their mixture; (ii) CL in the liposome membrane does not affect the conformation of PC and, therefore, the PC spectrum does not alter; and (iii) the molecules of cytC enclosed in PC : CL vesicles also do not affect the conformation of PC and, hence, the spectrum.

The SERS spectra of investigated mitochondria and erythrocyte ghosts in the high frequency spectral range are similar to the ones for PC liposomes and PC : CL liposomes with enclosed cytC. The only difference is that the peaks at 2930 and 2960 cm^{-1} for mitochondria have similar intensities, while for erythrocyte ghosts the peak at 2930 cm^{-1} is stronger as compared with that at 2960 cm^{-1}, whereas for PC liposomes the peak at 2960 cm^{-1} is the most intensive. The observed difference between SERS spectra of cellular membranes in mitochondria or erythrocytes relative to artificial liposomes corresponds to hampered asymmetric vibrations of methyl group bonds in lipids and proteins in the cellular membranes as compared to lipids in liposomes. We suppose that this distinction originates from the complex lipid–protein arrangement and more complicated orientation of molecules towards one another in the natural membranes of mitochondria and erythrocytes.

In summary, the SERS method allows one to perform a label-free investigation of lipids and proteins in cell membranes *via* their Raman fingerprints. We suppose that SERS based approach can be used to monitor the conformation of membrane lipids and proteins for further examination of membrane rearrangements, damage and disturbances.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2019.11.009.

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