ChinaRxiv 中X

AI translation · View original & related papers at
chinarxiv.org/items/chinaxiv-202602.00178

Drug-Loaded Deuterium-Tagged Liposome Nanoassembly for Intracellular Distribution Tracking via SERS Imaging

Authors: Liu, Miss Jinke, Zhang, Dr. Minglu, Liu, Dr. Jing, Zhang, Mr. Tingfeng, Wang, Miss Meng, Fang, Mr. Hao, Liang, Dr. Shanshan, Yao, Mr. Yuchi, Hu, Miss Xinyan, Wei, Mrs. Hui-Ling, Wang, Prof. Meng, Zheng, Dr. Lingna, He, Prof. Xiao, Zeng, Dr. Yiyang, Ma, Dr. Chun-Wang (Nuclear physics) 马春旺, Wang, Dr. Bing, Feng, Prof. Weiyue, Wang, Dr. Bing

Date: 2026-01-30T10:59:48+00:00

Abstract

Stable isotope labelling combined with the Raman spectroscopy microscopy is a potential analytical technique for tracing intracellular liposome. In this study, we developed a deuterium-labeled liposomal nano-assembly co-loaded with a hypocrellin-derived photosensitizer and gambogic acid (GA), designated as HB4/GA@D-Lip. Compared to free HB4, the assembled system exhibited enhanced photophysical performance, leading to substantially elevated generation of reactive oxygen species (ROS), including hydroxyl radicals ($\bullet$ OH) and superoxide anions ($O_2 \bullet^-$). By monitoring characteristic Raman shifts of deuterium-labeled liposome nano-assembly, HB4 (1220 cm^{-1}), GA (1600 cm^{-1}), and C-D (2105 cm^{-1}), we may successfully trace their intracellular spatiotemporal distribution of the liposome nanoassembly.

Full Text

Preamble

Drug-Loaded Deuterium-Tagged Liposome Nanoassembly for Intracellular Distribution Tracking via SERS Imaging∗ Jin-Ke Liu,1, 2, † Ming-Lu Zhang,2, 3, † Jing Liu,4, ‡ Ting-Feng Zhang,2 Meng Wang,2 Hao Fang,2 Shan-Shan Liang,2 Yu-Chi Yao,2 Xin-Yan Hu,2 Hui-Ling Wei,1 Meng Wang,2 Ling-Na Zheng,2 Xiao He,2 Yi-Yang Zeng,5 Chun-Wang Ma,1, 5, § Bing Wang,2, ¶ and Wei-Yue Feng2 1School of Physics, Centre for Theoretical Physics, Henan Normal University, Xinxiang 453007, China 2CAS Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, Institute of High Energy Physics, Chinese Academy of

Sciences, Beijing 100049, China 3Qingdao Central Hospital, School of Health and Life Sciences, University of Health and Rehabilitation Sciences, Qingdao 266113, China 4BSRF, Institute of High Energy Physics, Chinese Academy of Sciences, 100049 Beijing, P.R. China 5Institute of Nuclear Science and Technology, Henan Academy of Sciences, Zhengzhou 450046, China Stable isotope labelling combined with the Raman spectroscopy microscopy is a potential analytical technique for tracing intracellular liposome. In this study, we developed a deuterium-labeled liposome nanoassembly co- loaded with a hypocrellin-derived photosensitizer and gambogic acid (GA), designated as HB4/GA@D-Lip.

Compared to free HB4, the assembled system exhibited enhanced photophysical performance, leading to sub- stantially elevated generation of reactive oxygen species (ROS), including hydroxyl radicals (∙OH) and super- ∙ –). By monitoring characteristic Raman shifts of deuterium-labeled liposome nano-assembly, oxide anions (O2 HB4 (1220 cm−1), GA (1600 cm−1), and C-D (2105 cm−1), we may successfully trace the intracellular distri- bution distribution of the liposome nanoassembly.

Keywords: Deuterated Labelled Liposome, SERS, Nano-assembly, Intracellular tracing

INTRODUCTION

Liposome nanoassemblies, such as liposomes and lipid nanoparticles, are among the most promising drug delivery platforms in contemporary research [1, 2]. A great chal- lenge in their application lies in optimizing subcellular de- livery efficiency [3]. Most studies indicate that liposome nanoassemblies are primarily internalized by cells through endocytic pathways, including clathrin-mediated, caveolae- mediated, lipid raft-mediated endocytosis, macropinocytosis, phagocytosis, and membrane fusion [4, 5]. Following in- ternalization, the intracellular fate and targeting of these li- posome nanoassemblies are dictated by the therapeutic ob- jective. For instance, gene therapies require nuclear local- ization, pro-apoptotic drugs must reach mitochondria, and agents affecting enzymatic degradation need to be delivered to lysosomes. However, when internalized via endocytosis, liposome-based carriers typically traffic through the endo- lysosomal pathway. This often results in lysosomal entrap- ment and degradation by hydrolytic enzymes, regardless of the initial endocytic route [6–8]. Consequently, a detailed un- ∗ Supported the National Key R&D Program of China (2022YFA1207600), the National Natural Science Foundation of China (grant nos. 12535020, 12275302, 12275300), the Beijing Natural Science Foundation (Z230008, 1232034), the directional institutionalized scientific research platform relies on Beijing Synchrotron Radiation Facility of Chinese Academy of Sciences, and scientific and technological innovation project of Institute of High Energy Physics, Chinese Academy of Sciences (No. E4545CU2) † These authors contributed equally to this work. ‡ Corresponding author, liujing1987@ihep.ac.cn § Corresponding author, machunwang@126.com ¶ Corresponding author, wangbing@ihep.ac.cn derstanding of the intracellular fate of liposome nanoassem- bly

remains a critical challenge.

Current analytical techniques for probing the cellular entry and intracellular transport of liposome nanoassemblies com- bine advanced imaging with fluorescence labeling, isotope la- belling techniques, cryo-electron microscopy (cryo-EM), Ra- man techniques, etc. The challenges with simultaneous imag- ing of nanocarrier and payloads include the limit of image resolution and the ability to confidently distinguish the re- leased payloads from the subcellular accumulated ones. Ra- dionuclide labeling with PET or SPECT enables quantitative in vivo liposome biodistribution analysis but lacks subcellular resolution and requires specialized facilities for labeling and detection [9, 10]. Super-resolution fluorescence microscopy (SRM) holds significant promise for non-invasive tracking drug dynamics at the subcellular level [11]. However, the practical application of SRM is constrained by inherent lim- itations of fluorescent labels, including dye detachment, low contrast, and photobleaching, which can compromise data re- liability [12]. A major barrier for characterizing liposomes using cryo-EM is the difficulty of characterizing liposomes at cellular level [13]. Raman micro-spectroscopy provides sig- nificant advantages for imaging liposomes at the subcellular level [14]. Furthermore, it enables the visualization of subcel- lular structures based on their intrinsic vibrational properties and chemical composition, without the requirement for exter- nal staining [15, 16]. However, Raman spectroscopy analysis is usually limited by low Raman signals and low signal-to-noise ratios of intracellular liposome. Raman spectroscopy combined with stable isotope labelling is a potential analyti- cal technique due to the significant C-D band redshift of the C-H band in the high wavenumber range [17].

To address this, we propose a deuterated carrier strategy using stable isotope-labeled lipids (e.g., D-DPPC) to con- struct the delivery system [18]. From a nuclear science per- spective, the replacement of hydrogen with deuterium in the lipid tail introduces a unique physical tag [19]. According to the quantum harmonic oscillator model, the vibrational fre- quency is inversely proportional to the square root of the re- duced mass (µ) of the oscillating atoms. Consequently, the increased mass of the C-D bond induces a significant spec- tral redshift of its Raman scattering peak into the biologi- cal silent region (2100-2200 cm^{-1}) [20]. This strategy offers several distinct advantages over traditional methods. Unlike bulky fluorophores, deuterium labeling is minimally invasive and preserves the inherent physicochemical properties of the liposomes. Moreover, the resulting Raman signals are im- mune to photobleaching, allowing stable and long-term track- ing. Additionally, detection within the cellular 'silent region' eliminates background interference, ensuring a high signal- to-noise ratio. By combining this isotope effect with SERS amplification, we can track the delivery vehicle' s location.

The integration of Raman micro-spectroscopy with SERS and deuterium labeling provides a highly effective strategy for studying the intracellular fate of liposome nanoassembly.

In the study, HB4 and gambogic acid (GA) co-loaded li- posome nano-assembly

(HB4/GA@Lip) was synthesized and characterized. Hypocrellin, a plant-derived photosensitizing agent, has emerged as a promising candidate among vari- ous photosensitizers for photodynamic therapy (PDT) [21].

Gambogic acid (GA), the primary bioactive and caged xan- thone constituent, exhibits a broad range of biological and clinical activities against multiple cancers and may function synergistically with PDT [22]. We combined SERS with the deuterium labeling as Raman tag to develop a method for trafficking intracellular distribution of liposome-based nano- assembly. This successful tracking underscores the potential of stable isotope labelling in combination with the Raman imaging for subcellular liposome characterization [23, 24].

II. MATERIALS AND METHODS

2.1 Chemical Compounds

chemical suppliers. reagents were obtained from com- mercial 1,2-Dipalmitoyl-sn-glycero-3- phosphatidylcholine (DPPC) and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)- 2000] (DSPE-PEG2000) were purchased from Avanti Polar Lipids (USA). Cholesterol and 1,2-dipalmitoyl-d62- obtained (D-DPPC) were sn-glycero-3-phosphocholine from Aladdin Biochemical Technology Co., Ltd. (Shang- hai, China). Dimethyl sulfoxide (DMSO), high-glucose Dulbecco' s modified Eagle' s medium (DMEM), Hydrox- yphenyl fluorescein (HPF), dihydroethidium (DHE), and 1,3-diphenylisobenzofuran (DPBF) were purchased from Beyotime Biotechnology Co., Ltd. (Shanghai, China).

2.2 Preparation of HB4/GA@Lip and HB4/GA@D-Lip

Hypocrellin B derivatives (HB4) were synthesized through amination-based derivatization of the parent hypocrellin B structure (Fig. S1). The HB4 and gambogic acid (GA) loaded liposome nano-assembly (HB4/GA@Lip) was synthesized using a microfluidic self-assembly technique as fol- lows: (1) A lipid phase was formulated by dissolving DPPC, cholesterol, DSPE-PEG 2000, GA, and HB4 (6:2:2:1:0.1 by mass) in anhydrous ethanol. (2) The lipid phase was in- jected with a PBS aqueous phase (pH 7.4) into a staggered herringbone micromixer (SHM) at flow rates of 0.5 and 1.5 mL/min, respectively. (3) The collected liposomes were dia- lyzed (MWCO: 5 kDa) against PBS for 24 h to remove any unencapsulated HB4. (4) The final formulation was stored at 4 $\circ$C until use. For the synthesis of deuterium-labeled lipo- somes (HB4/GA@D-Lip), deuterium-labeled DPPC was em- ployed following the above same preparation procedure.

2.3 Characterization of HB4/GA@Lip

The hydrodynamic size and zeta potential of liposome and HB4/GA@Lip were measured via dynamic light scat- tering (DLS) using a Malvern Zetasizer Nano

series instru- ment. Transmission electron microscopy (TEM) was em- ployed to examine the morphology of HB4/GA@Lip, using 2% uranyl acetate as the negative staining agent. UV–Vis ab- sorption spectra were recorded using a Thermo Fisher Evo- lution 200 UV–Vis spectrophotometer. The encapsulation ef- ficiency (EE) of HB4 and GA were determined by UV–Vis spectroscopy. The encapsulation efficiency was calculated us- ing the following formula: Eq. 1 EE% = (cid:19) (cid:18) Drugloaded Drugadded × 100%

2.4 ROS Detection

The pathways of reactive oxygen species (ROS) gener- ation were characterized using specific fluorescent probes.

Singlet oxygen (1O2) production was monitored with 9,10- anthracenediyl-bis(methylene)dimalonic acid (ABDA, 50 µM). Sample solutions containing ABDA and either HB4 or HB4/GA@Lip (2 µM HB4 equivalent) were exposed to 660 nm laser irradiation (0.5 W/cm2), and the fluorescence intensity at 400 nm was recorded at different time points us- ing a HITACHI F-7000 fluorescence spectrophotometer. Hy- droxyl radicals (•OH) were detected with hydroxyphenyl flu- orescein (HPF, 40 µM). Following 660 nm laser irradiation (0.5 W/cm2), fluorescence emission at 515 nm was measured in PBS (1 mL) containing HPF and HB4 or HB4/GA@Lip (2 • –) was detected using dihy- µM each). Superoxide anion (O2 droethidium (DHE, 50 µM). A mixture of DHE and HB4 or HB4/GA@Lip (2 µM each) was added to 1 mL of aqueous solution containing calf thymus DNA (ctDNA, 250 µg/mL) and irradiated with a 660 nm laser (0.5 W/cm2). DHE inter- calates into DNA and produces red fluorescence in the pres- ence of O2 • –.

2.5 Raman Analysis for intracellular distribution of

HB4/GA@D-Lip Fig. 1 [Figure 1: see original paper]. (Color online) Characterization of HB4/GA@D-Lip. (a) Schematic illustration of HB4/GA@Lip preparation using microfluidics. (b) Transmission electron microscopy image of HB4/GA@Lip. Scale bar: 50 nm. (c) Dynamic light scattering analysis of Lip, HB4/GA@Lip and HB4/GA@D-Lip in aqueous solution. (d) Zeta potentials of Lip, HB4/GA@Lip and HB4/GA@D-Lip. (e) UV-vis absorbance spectra of Lip, HB4@Lip, GA@Lip, HB4/GA@Lip and HB4/GA@D-Lip in a mixed DMSO/H2O solution.

For Raman spectra and Raman imaging analysis, B16 melanoma cells were seeded in 6-well plates and treated with HB4/GA@D-Lip (20 nM HB4 equivalent) for 4 h. After in- cubation, the cells were collected, washed three times with PBS, and fixed with 4% paraformaldehyde. Following fixa- tion, the cells were washed three times with PBS and finally resuspended in ultrapure water before Raman imaging.

Raman spectra and imaging were acquired using a high- resolution confocal

Raman microscope (LabRAM Soleil, Horiba Scientific, France) equipped with a 532 nm excitation laser and an 1800 lines/mm grating. A 50× objective (NA = 0.6) was used to focus the laser onto the samples, yield- ing a spot size of approximately 1 µm. The laser power at the sample plane was maintained at 28 mW to prevent pho- todamage. Individual spectral measurements were recorded with an integration time of 20 s and 3 accumulations. In con- trast, Raman imaging was performed using a 1s integration time per pixel with a step size of 1 µm. The spectral acqui- Fig. 2 [Figure 2: see original paper]. (Color online) In Vitro photodynamic properties of HB4 and HB4/GA@Lip. (a) 1O2 generation detected using ABDA (50 µM) under 660 nm irradiation (0.5 W/cm2). (b) Hydroxyl radical (•OH) generation detected using HPF (40 µM, Ex: 490 nm, Em: 500-750 nm). (c) Superoxide anion (O2 • -) generation detected using DHE (50 µM, Ex: 490 nm, Em: 500-750 nm). sition range was set from 300 to 3500 cm−1, with a spectral resolution of approximately 1 cm−1 and a spatial resolution of 1 µm. For SERS enhancement measurement, the bare glass substrate was mounted in a DM-450A vacuum coating sys- tem and rotated at 25 rpm during the deposition of the Au layer, which was carried out under a vacuum of 7 × 10−3 Torr.

Raman imaging was conducted on cells immobilized on the Au-NIA substrate.

The acquired spectra were preprocessed by baseline cor- rection and vector normalization using the LabSpec 6 soft- For Raman imaging, hierarchical cluster analysis ware. (HCA) based on specific characteristic vibrational features was employed to separate the subcellular organelles and analyze the distribution of the HB4/GA@D-Lip liposome nanoassembly in these organelles.

III. RESULTS AND DISCUSSION

3.1 Preparation and Characterization of HB4/GA@Lip

We synthesized a liposomal nanocarrier co-encapsulating HB4 and GA (denoted as HB4/GA@D-Lip) via a microflu- idic staggered herringbone micromixer (Fig. 1a). As de- picted in Fig. 1b, the resulting HB4/GA@Lip nanoassem- bly exhibited an average hydrodynamic diameter of 126.8 nm with a low polydispersity index (PDI) of 0.2, reflecting a highly uniform size distribution. Further TEM imaging re- vealed a near-spherical vesicular morphology of the nano- assembly (Fig. 1c). The colloidal stability, a key factor in- fluencing cellular interactions, was assessed via zeta potential measurements. Both blank liposomes and HB4/GA@Lip dis- played a slightly negative surface charge, about −3 to −4 mV (Fig. 1d), attributed mainly to the presence of DSPE-PEG in the lipid bilayer [25], a typical feature known to prolong in vivo circulation time [26]. To verify that deuterium labeling did not alter the physicochemical properties of the liposomes, the UV-Vis absorption spectra, hydrodynamic diameter, and zeta potential of deuterated liposomes (HB4/GA@D-Lip) with non-deuterated controls (HB4/GA@Lip) were com- pared. As shown in Fig. 1c, 1d, 1e, comparative analy- sis of the deuterated (HB4/GA@D-Lip) and non-

deuterated (HB4/GA@Lip) formulations revealed no significant differ- ences in UV-Vis absorption spectra, hydrodynamic diameter, or zeta potential. The nearly identical size distribution and surface charge profiles confirm that structural integrity and stability are maintained. Successful co-encapsulation of the two cargo molecules was confirmed by UV-Vis spectroscopy.

The characteristic absorption peaks of HB4 (600-700 nm) and GA (300-400 nm) were clearly observed in the HB4/GA@Lip spectrum but absent in the blank liposomes (Fig. 1e). Us- ing standard curves based on the respective UV absorption maxima, the encapsulation efficiencies were determined to be 23.5 ± 2.5% for HB4 and 49.5 ± 7.42% for GA.

3.2 Photophysical properties of HB4/GA@Lip

• –), To evaluate the impact of liposomal encapsulation on the photodynamic performance of HB4, we employed three flu- orescent probes: ABDA for the detection of singlet oxy- gen (1O2), HPF for hydroxyl radicals (•OH), and DHE for superoxide anion (O2 to identify the reactive oxygen species (ROS) generated by HB4/GA@Lip. As shown in Fig. 2a, the absorption of ABDA at 380 nm decreased to 0.94 and 0.79 of its original value in the presence of HB4 and HB4/GA@Lip, respectively, under 660 nm irradiation, confirming the generation of a comparable amount of 1O2.

Furthermore, the fluorescence intensity of HPF at 515 nm increased by 7.8-fold with HB4 alone, whereas it rose by 17.6-fold with HB4/GA@Lip (Fig. 2b), indicating signifi- cantly enhanced •OH production in the liposomal formulation. Similarly, the fluorescence intensity of DHE at 575 nm was augmented by 6.3-fold for HB4 and by 14.5-fold for HB4/GA@Lip (Fig. 2c), reflecting a substantial increase in • –generation. Collectively, these results demonstrate that HB4/GA@Lip produces higher levels of ROS—including • ––than free HB4. These findings confirm that •OH, and O2 liposome encapsulation enhances the photodynamic efficacy of HB4.

3.3 Raman Spectral Characteristics and Validation of

Deuterium-Labeled Probes A principal challenge in tracking liposome within cells using Raman spectroscopy is the interference from endoge- nous biomolecules. The cellular milieu is densely populated with proteins and lipids, where abundant carbon-hydrogen (C-H) bonds generate a strong and spectrally complex back- ground capable of overwhelming the subtle vibrational sig- nals from liposome nanoassembly. To overcome this limi- tation, we strategically employed deuterium labeling, lever- aging the fundamental isotope effect. The increased nuclear mass of deuterium shifts the carbon–deuterium (C-D) stretching vibration (2105 cm−1) to a significantly lower frequency compared to that of ubiquitous C-H bonds (2880 cm−1). This frequency resides within the cellular "Raman silent zone," a spectral region naturally devoid of vibrational interference from endogenous biomolecules. As a result, the nanoassem- bly

acquires a unique and unambiguous vibrational signature, resulting in the markedly enhanced signal to noise ratio [27–29]of internalized liposome.

To validate the feasibility of employing deuterium-labeled lipids as background-free Raman probes, we performed den- sity functional theory (DFT) calculations to simulate the Raman spectra of 1,2 dipalmitoyl sn glycero 3 phospho- choline (H-DPPC) and its deuterated counterpart (D-DPPC).

As shown in Fig. 3a [Figure 3: see original paper], the DFT simulated spectrum of H- DPPC displays a characteristic C-H stretching vibration at 2880 cm^{-1}. In contrast, substitution of hydrogen with deu- terium in the lipid tail induces a pronounced redshift of this vibrational mode to 2130 cm^{-1}, consistent with the kinetic isotope effect arising from the increased mass of the oscillating atoms. Notably, this shifted peak lies within the biological Raman "silent region" (1800-2800 cm^{-1}), where endogenous biomolecules contribute minimal spectral interference. Fur- ther, the acquired Raman spectra (Fig. 3b) is consistent with theoretical calculation. The C-D stretching vibration of D- DPPC appears at 2105 cm^{-1}, clearly distinct from the C-H vibration of H-DPPC at 2880 cm^{-1} [30]. Consequently, D- DPPC confers a background-free Raman fingerprint for lipo- some nanoassembly, enabling unambiguous distinction from complex biological molecules. As demonstrated in Fig. 3c, the characteristic C-D signal at 2105 cm^{-1} is clearly de- tectable for the HB4/GA@D-Lip formulation, confirming the practical utility of deuterium labeling for interference free in- tracellular tracking.

Additionally, Surface-enhanced Raman scattering (SERS) leverages the inherent advantages of Raman spectroscopy to provide exceptionally high detection sensitivity, enabling even single-molecule analysis [31, 32]. In this study, a gold nano-island array substrate (Au NIA, Fig. S2) was fabricated to serve as the SERS-active platform. As shown in Fig. 3d, the characteristic C-D vibrational peak at 2105 cm^{-1} from HB4/GA@D-Lip adsorbed on the Au-NIA substrate showed a remarkable signal enhancement. This SERS effect signif- icantly improved the detection sensitivity for the liposomes.

Crucially, the non-deuterated HB4/GA@Lip control exhib- ited no detectable signal within this spectral region, confirm- ing the exclusive specificity of the C-D vibrational tag. These results collectively demonstrate that the strategic integration of D-DPPC labeling with the Au-NIA SERS substrate enables highly specific and sensitive tracking of liposomal carriers in complex biological environments.

3.4 Intracellular Distribution of HB4/GA@D-Lip

We further validated that the developed method possessed sufficient chemical sensitivity to detect the HB4/GA@D-Lip liposome within B16 melanoma cells at subcellular level. We first performed high resolution Raman imaging of untreated cells to establish a baseline biochemical profile. Fig. 4a [Figure 4: see original paper] shows a bright field micrograph of the analyzed cells. Based on

characteristic Raman bands that serve as molecular finger- prints (Table S1) [33]. The mitochondria were identified by the prominent band at 750 cm−1, assigned to the heme group vibration of cytochrome c, as well as lipid-related bands at 1300 cm−1 (CH2 deformation) and 1430 cm−1 (C-H bend- ing vibrations of fatty acids). The nucleus was character- ized by the DNA marker at 785 cm−1, the phosphodiester groups of nucleic acids at 1090 cm−1 and the purine ring Fig. 3. (Color online) Raman spectral characteristics of deuterium-labeled DPPC. (a) DFT-simulated Raman spectra of H-DPPC (black) and D-DPPC (red). (b) Experimental Raman spectra of H-DPPC (black) and D-DPPC (red). (c) Raman spectra of HB4/GA@Lip (black) and HB4/GA@D-Lip (red). (d) Raman spectra of HB4/GA@Lip (black) in Au-NIA substrate and HB4/GA@D-Lip (red) in Au-NIA substrate.

Integration time 20 s, laser power 5.4 mW. mode at 1345 cm−1 [34]. The endoplasmic reticulum (ER) was distinguished by a combination of lipid and protein fea- tures, including the bands at 1430 cm−1 (fatty acids), 1650 cm−1 (amide I of proteins and C=C vibrations of lipids).

Lysosomes were associated with the bands at 997 (Phe ring breathing), 1072 (C-N stretching), 1162 (C-N backbone), 1279 amide III (β -sheet), 1318 (CH2 twisting), and 1564 cm−1(Trp) [35]. This approach enabled the identification of individual organelles—nucleus (red), endoplasmic reticulum (perinuclear region, magenta), mitochondria (yellow), lyso- somes (green), cell membrane (light gray), and extracellu- lar background (black) [36]—as illustrated in Fig. 4b. Raman mapping was further performed across three character- istic spectral regions of the nanoassembly: HB4 (1150-1250 cm−1), GA (1580-1680 cm−1), and C-D (2100-2200 cm−1).

The results showed uniformly low signal intensities across these bands, with no discernible spectral features detected within the cellular area (Fig. 4c). This absence of endoge- nous background provides a solid spectroscopic foundation for subsequent tracking of HB4/GA@D-Lip, ensuring that all signals within these specific ranges can be unequivocally as- signed to the internalized nanoassembly rather than intrinsic cellular constituents.

We next investigated the intracellular distribution of HB4/GA@D-Lip in B16 cells after 4 h of co-incubation.

High-resolution Raman imaging analysis and HCA were employed to assess the intracellular distribution of the nanoassembly. In contrast to the untreated control (Fig. 4c, 4e), after incubation of HB4/GA@D-Lip for 4 h, the C-D stretching vibration (2100-2200 cm−1) in the lysosome is clearly detectable, with characteristic peaks of HB4 (1220 cm−1) and GA (1600 cm−1), confirming the presence of the Fig. 4. (Color online) The microscopy image of B16 Melanoma Cells. (a) The microscopy image, Raman image of B16 cells (resolution 1.0 µm) and Raman images of specific organelles: nucleus (red), endoplasmic reticulum (magenta), mitochondria (yellow), lysosomes (green), and cell membrane (light gray) and cell environment (dark grey) (b), the average Raman spectra for all

clusters for region (c) colors of the spectra correspond to the colors of clusters; integration time 1 s, laser power 28 mW. Raman spectra of B16 cells incubated with HB4/GA@D- Lip (dose 20 nM) for 4 h (d). Raman intensity mapping of B16 cells at characteristic spectral regions (1150-1250, 1580-1680, and 2100-2200 cm−1) (e). Raman intensity mapping of B16 cells incubated with HB4/GA@D-Lip (dose 20 nM) for 4 h (f).(bar: 4 μm) drug within lysosomes (Fig. 4d, 4f, Fig. S3 and Fig. S4).

To further corroborate this, we integrated the key Raman sig- nals of the nanodrug to generate a composite intensity map (Fig. 4f), which covered the C-D vibration region (2100-2200 cm−1) and the characteristic peaks of HB4 and GA (1150- 1250 cm−1, 1580-1680 cm−1). This composite map clearly delineated the signal regions of the drug-loaded liposomes, revealing a high degree of accumulation within the lysosome.

The successful application of C-D tagging provides a method- ological paradigm for evaluating drug-organelle interactions.

The combination of stable isotope tracing and vibrational spectroscopy thus provides a powerful, non-invasive tool for probing the fate of small molecules at the organelle level.

IV. CONCLUSION In summary, we developed a traceable (designated HB4/GA@D-Lip) liposome through nanoassembly deuterium labeling and SERS, enabling highly specific and sensitive tracking of intracellular distribution of lipo- some nanoassembly. Our deuterated lipid strategy offers a distinct advantage for the investigation of liposome-based drug delivery system. This work demonstrates how stable isotope labelling technology can be integrated with nan- otechnology to investigate intracellular drug distribution and [1] S. Karmaker, P.D. Rosales, B. Tirumuruhan et al., More the evolving role of lipid-based than a delivery system: nanoparticles. Nanoscale 17, 11864–11893 (2025). doi: 10.1039/D4NR04508D [2] Y.Y. Xu, C. Jin, M. Wu et al., Carbon-based nanomaterials cause toxicity by oxidative stress to the liver and brain in Sprague–Dawley rats. Nucl. Sci. Tech. 35, 109 (2024). doi: 10.1007/s41365-024-01473-7 [3] M.L. Zhang, H.Y. Ti, P.Y. Wang et al., Intracellular transport dynamics revealed by single-particle tracking. Biophys. Rep. 7, 413–427 (2021). doi: 10.52601/bpr.2021.210035 [4] T.B. Gandek, L. van der Koog, A. Nagelkerke, A Com- parison of Cellular Uptake Mechanisms, Delivery Efficacy, and Intracellular Fate between Liposomes and Extracellular Vesicles. Adv. Healthcare Mater. 12, e2300319 (2023). doi: 10.1002/adhm.202300319 [5] Y. Zhuo, L. Zhen, Z. Zhu et al., Direct cytosolic deliv- ery of siRNA via cell membrane fusion using cholesterol- enriched exosomes. Nat. Nanotechnol. 19, 1858–1868 (2024). doi: 10.1038/s41565-024-01785-0 [6] H. Su, G. Rong, L. Li, Y et al., Subcellular targeting strategies for protein and peptide delivery. Adv. Drug Deliv. Rev. 212, 115387 (2024). doi: 10.1016/j.addr.2024.115387 [7] X. Wang, H.Y. Li, C. Chen et al., Understanding of endo/lysosomal

escape of nanomaterials in biomedi- cal application. Smart Mol. 3, e20240017 (2025). doi: 10.1002/smo.20240017 [8] H.M. Kong, C.X. Zheng, K. Yi et al., An antifoul- ing membrane-fusogenic liposome for effective intracellu- lar delivery in vivo. Nat. Commun. 15, 4267 (2024). doi: 10.1038/s41467-024-46533-z [9] J. Lamb, for Radi- J.P. Holland, Advanced Methods olabeling Multimodality Nanomedicines for SPECT/MRI and PET/MRI. J. Nucl. Med. 59, 382–389 (2018). doi: 10.2967/jnumed.116.187419 [10] R. Golestani, C. Wu, R.A. Tio et al., Small-animal SPECT and SPECT/CT: application in cardiovascular research. Eur.

J. Nucl. Med. Mol. Imaging 37, 1766–1777 (2010). doi: 10.1007/s00259-009-1321-8 [11] C. Zhang, Z. Tian, R. Chen et al., Advanced imaging techniques for tracking drug dynamics at the subcellular level. Adv. Drug Deliv. Rev. 199, 114978 (2023). doi: transport. Collectively, this work introduces an integrated Raman-based tracking strategy with deuterium labeling and SERS, that elucidates the nano-assembly-organelle interactions, and lysosomal retention, offering a broadly applicable framework for mapping intracellular transport pathways of liposomal nanomedicines.The SERS-based imaging strategy shows promise but also has limitations.

Currently, deuterium-labeled lipids are more expensive than conventional fluorescent dyes, which may limit large-scale routine use. Additionally, SERS signals can be affected by the stability and spatial homogeneity of the intracellular plasmonic substrates, which may lead to signal variability.

Imaging performance also depends on cellular internalization efficiency, which varies with cell types. Future work will aim to improve the substrate durability to broaden the method's applicability. 10.1016/j.addr.2023.114978 [12] M. Streit, M. Budiarta, M. Jungblut et al., Fluorescent labeling strategies for molecular bioimaging. Biophys. Rep. 5, 100200 (2025). doi: 10.1016/j.bpr.2025.100200 [13] Y. Cui, Z. Zhang, R. Sinclair et al., Cryogenic electron mi- croscopy and tomography for beam-sensitive materials. Nat.

Rev. Phys. 8, 40–54 (2026). doi: 10.1038/s42254-025-00896-4 [14] G.X. Wei, S.X. Zhang, Z. Li et al., Multi-modality measure- ment and comprehensive analysis of hepatocellular carcinoma using synchrotron-based microscopy and spectroscopy. Nucl.

Sci. Tech. 32, 102 (2021). doi: 10.1007/s41365-021-00927-6 [15] E.J. Legge, N.A. Belsey, R.T. Coones et al., Non-invasive quan- titative chemical measurements of liposomal formulations us- ing Raman spectroscopy. RSC Pharm. 2, 279-291 (2025). doi: 10.1039/d4pm00238e [16] R.R. Jones, D.C. Hooper, L. Zhang et al., Raman Techniques:

Fundamentals and Frontiers. Nanoscale Res. Lett. 14, 231 (2019). doi: 10.1186/s11671-019-3039-2 [17] S.Y. Ding, E.M. You, Z.Q. Tian et al., Electromagnetic theories of surface-enhanced Raman spectroscopy. Chem. Soc. Rev. 46, 4042–4076 (2017). doi: 10.1039/C7CS00238F [18] X.L. Chang, L.

Chen, B. Liu et al., Stable isotope label- ing of nanomaterials for biosafety evaluation and drug de- velopment. Chin. Chem. Lett. 33, 3303–3314 (2022). doi: 10.1016/j.cclet.2022.03.057 [19] Z. Zheng, Y. Cui, B. Zhang et al.,Raman spectroscopy of deuterium-labeled macromolecules: Emerging strategies in biomedical research. Int. J. Biol. Macromol. 320, 145752 (2025). doi: 10.1016/j.ijbiomac.2025.145752 [20] S. Egoshi, K. Dodo, M. Sodeoka, Deuterium Raman imaging for lipid analysis. Curr. Opin. Chem. Biol. 70, 102181 (2022). doi: 10.1016/j.cbpa.2022.102181 [21] Y. Wu, Y. Guo, K. Feng et al., MOF-based hypocrellin com- posite nanomaterials as advanced plant-derived photosensitiz- ers for multimodal therapy. Chem. Eng. J. 520, 11864–11893 (2025). doi: 10.1016/j.cej.2025.166071 [22] Y. Yang, K. Chen, X. He et al., Nanoscale Gambogic Acid Liposomes for NIR-II Photoacoustic Imaging and Photothermal-Chemo Tumor Therapy. ACS Appl. Nano Mater. 7, 17688–17696 (2024). doi: 10.1021/acsanm.4c02918 [23] Y. Wang, W.E. Huang, L. Cui et al., Single cell stable isotope probing in microbiology using Raman microspec- troscopy. Curr. Opin. Biotechnol. 41, 34–42 (2016). doi: 10.1016/j.copbio.2016.04.018 [24] D. Berry, E. Mader, T.K. Lee et al., Tracking heavy water (D2O) incorporation for identifying and sorting active micro- bial cells. Proc. Natl. Acad. Sci. U. S. A. 112, E194–E203 (2015). doi: 10.1073/pnas.1420406112 [25] M.A. Khazaei, D. Bastani, A. Mohammadi et al., Adsorp- tion Dynamics of Surface-Modified Silica Nanoparticles at Solid–Liquid Interfaces. Langmuir 38, 12421–12431 (2022). doi: 10.1021/acs.langmuir.2c01234 [26] B.K. Barik, H.M. Mallya, R.K. Sinha et al., Molecular struc- ture and spectra of danthron and emodin studied by FTIR, Ra- man spectroscopy and DFT techniques. Lith. J. Phys. 61, 1–26 (2021). doi: 10.3952/physics.v61i1.4403 [27] X. Wang, G. Liu, M. Xu et al., Development of Weak Signal Recognition and an Extraction Algorithm for Ra- man Imaging. Anal. Chem. 91, 12909–12916 (2019). doi: 10.1021/acs.analchem.9b02887 [28] M.Z. Zhao, X. Wang, Y.K. Xing et al., DNA origami-templated assembly of plasmonic nanostructures with enhanced Raman scattering. Nucl. Sci. Tech. 29, 6 (2017). doi: 10.1007/s41365- 017-0347-z [29] M. Yuan, Y.B. Li, J. Guo et al., Solvent effects on gold nanopar- reduction of Au(III) ticle formation from photochemical by UV irradiation. Nucl. Sci. Tech. 29, 158 (2018). doi: 10.1007/s41365-018-0505-y deuterated [30] Yu.V. Zaytseva, N.V. Surovtsev, Raman scattering in protonated 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine (DPPC): Indicators of conformational and lateral orders. Spectrochim. Acta. A. Mol. Biomol. Spec- trosc. 267, 120583 (2022). doi: 10.1016/j.saa.2021.120583 [31] W.L. Syu, Y.H. Lin, A. Paliwal et al., Highly sensitive and re- producible SERS substrates of bilayer Au and Ag nano-island arrays by thermal evaporation deposition. Surf. Coat. Technol. 350, 823–830 (2018). doi: 10.1016/j.surfcoat.2018.04.043 [32] J.Q. Chen, Q.S. Huang, R.Z. Qi et al., Effects of sputtering power and annealing temperature on surface roughness of gold films for high-reflectivity synchrotron radiation mirrors. Nucl.

Sci. Tech. 30, 107 (2019). doi: 10.1007/s41365-019-0635-x [33] A.K. Kniggen-

dorf, T.W. Gaul, M. Meinhardt-Wollweber, Hier- archical Cluster Analysis (HCA) of Microorganisms: An As- sessment of Algorithms for Resonance Raman Spectra. Appl.

Spectrosc. 65, 165–173 (2011). doi: 10.1366/10-06064 [34] A.M. Nowakowska, A. Pieczara, W. Korona, A et al., The Ra- man Map of the Human Cell. Anal. Chem. 97, 16374–16382 (2025). doi: 10.1021/acs.analchem.5c02035 [35] J. Yue, Y. Shen, C. Liang et al., Investigating Lyso- somal Autophagy via Surface-Enhanced Raman Scattering Spectroscopy. Anal. Chem. 93, 13038–13044 (2021). doi: 10.1021/acs.analchem.1c02939 [36] K. Beton, B. Bro˙zek-Płuska, Biochemistry and Nanomechani- cal Properties of Human Colon Cells upon Simvastatin, Lovas- tatin, and Mevastatin Supplementations: Raman Imaging and AFM Studies. J. Phys. Chem. B 126, 7088–7103 (2022). doi: 10.1021/acs.jpcb.2c03724

Note: Figure translations are in progress. See original paper for figures.

Source: ChinaXiv —Machine translation. Verify with original.