Transporting mitochondrion-targeting photosensitizers into cancer cells by low-density lipoproteins for fluorescence-feedback photodynamic therapy†‡

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Low-density lipoproteins (LDLs) are an endogenous nanocarrier to transport lipids in vivo. Owing to their biocompatibility and biodegradability, reduced immunogenicity, and natural tumor-targeting capability, we, for the first time, report the reconstitution of native LDL particles with saturated fatty acids and a mitochondrion-targeting aggregation-induced emission (AIE) photosensitizer for fluorescence-feedback photodynamic therapy (PDT). In particular, a novel AIE photosensitizer (TPA-DPPy) with a donor–acceptor (D–A) structure and a pyridinium salt is designed and synthesized, which possesses typical AIE and twisted intramolecular charge transfer (TICT) characteristics as well as reactive oxygen species (ROS)-sensitizing capability. In view of its prominent photophysical and photochemical properties, TPA-DPPy is encapsulated into LDL particles for photodynamic killing of cancer cells that overexpress LDL receptors (LDLRs). The resultant LDL (rLDL) particles maintain a similar morphology and size distribution to native LDL particles, and are sufficiently ingested by cancer cells via LDLR-mediated endocytosis, followed by the release of TPA-DPPy for mitochondrion-targeting. Upon light irradiation, the produced ROS surrounding mitochondria lead to efficient and irreversible cell apoptosis. Interestingly, this process can be fluorescently monitored in a real-time fashion, as reflected by the remarkably enhanced luminescence and blue-shifted emission, indicating the increased mechanical stress during apoptosis. Quantitative cell viability analysis suggests that TPA-DPPy exhibits an outstanding phototoxicity toward LDLR-overexpressing A549 cancer cells, with a killing efficiency of ca. 88%. The rLDL particles are a class of safe and multifunctional nanophototheranostic agents, holding great promise in high-quality PDT by providing real-time fluorescence feedback on the therapeutic outcome.

1. Introduction

As an endogenous nanoparticle with a well-defined structure, a low-density lipoprotein (LDL) functions as a biological nanocarrier to transport lipids in blood.1–4 Structurally, each LDL (20–30 nm in size) contains a phospholipid shell, one apolipoprotein molecule (ApoB-100), and a hydrophobic core, which allow LDL particles to accommodate a variety of exogenous theranostic agents via established reconstitution strategies.1,3 LDLs have a plethora of prominent advantages, including absolute biocompatibility and biodegradability, reduced immunogenicity, and natural tumor-targeting capability, making them a novel class of nanocarriers for targeted cancer theranostics.1,2 Among various strategies for LDL reconstitution, core-loading has attracted much attention due to its capability of accommodating a large quantity of theranostic agents.1–3 This method involves the removal of endogenous cholesteryl esters and triglycerides in the core, followed by filling the cavity with exogenous hydrophobic materials. Taking advantage of the core-loading strategy, a variety of pioneering LDL-based systems have been developed to deliver porphyrin and its derivatives to the tumor sites for targeted photodynamic therapy (PDT).4–7 In these studies, cholesteryl esters are typically used as the matrix for core reconstitution. However, they are considered harmful to human health as they cause atherosclerosis in the arteries.8–10 In addition, porphyrin and its derivatives are a class of aggregation-caused quenching (ACQ) molecules that are prone to form aggregates in aqueous
media due to the strong intermolecular interactions (e.g. π–π stacking), which mitigates their photophysical performance and reactive oxygen species (ROS) generation.11–14 In a previous study, Zhu et al. reported a eutectic mixture of lauric acid and stearic acid to replace cholesteryl esters as the reconstitution matrix.2 These naturally-occurring fatty acids have a potential positive effect on lowering cholesterol in blood, and are thus considered as a promising matrix for core reconstitution. Considering the unmet needs in traditional ACQ photosensitizers, it is of great importance to develop an alternative class of photosensitizers to boost their applications in PDT.

In 2001, Tang and coworkers reported the phenomenon of aggregation-induced emission (AIE) that exhibited opposite photophysical behaviors to traditional ACQ molecules.15 Guided by the established AIE mechanism through tremendous follow-up studies, a multitude of AIE luminogens (AIEgens) have been designed and synthesized to address the issues encountered in ACQ molecules, such as fluorescence quenching upon aggregation and photobleaching upon continuous light irradiation.16–21 In general, AIEgens show no or little emission in dilute solutions but significantly enhanced emission in aggregates as a result of restricted intramolecular motions that impede the consumption of excited-state energy via nonradiative decay pathways. It is reported that a pyridinium salt and a typical donor–acceptor (D–A) structure in AIEgens facilitate mitochondrion-targeting and high photosensitization efficiency by promoting intersystem crossing from the lowest excited singlet state \( S_1 \) to the lowest excited triplet state \( T_1 \) because of the reduced energy gap \( \Delta E_{st} \).22–27 In this case, ROS production is achieved via energy transfer from \( T_1 \) to ambient oxygen \( O_2 \), allowing for efficient PDT even in the aggregated state. More importantly, the luminescence property of AIEgens with a D–A structure is concurrently sensitive to the molecular aggregation state and the polarity of their residing environment. This feature enables in-depth understanding of a spectrum of cellular processes that are impossible to achieve with conventional ACQ probes. To our knowledge, there are no studies that report LDL-mediated delivery of AIE photosensitizers into cancer cells for fluorescence-feedback PDT. As such, it is essential to develop a novel class of multifunctional phototheranostic agents to boost PDT.28–32

In this study, we, for the first time, reported the use of a novel AIE photosensitizer and naturally-occurring fatty acids to reconstitute the core of native LDL particles for fluorescence-feedback PDT (Scheme 1). We first designed and synthesized a near-infrared (NIR)-emissive AIEgen (denoted as TPA-DPPy) that was characterized by a typical D–A structure and a pyridinium salt (Scheme 2). The core of native LDL particles was then reconstituted with a eutectic mixture of lauric acid and stearic acid (melting point = 39 °C)2,33–35 and TPA-DPPy. Owing to the restricted intramolecular motions of TPA-DPPy in the hydrophobic matrix, the resultant reconstituted LDL particles (denoted as rLDL(+)) exhibited a strong emission in the NIR region. Since ApoB-100 was able to guide rLDL(+) particles to bind to the LDL receptors (LDLRs) that are typically upregulated in multiple types of cancer cells, an efficient endocytosis was triggered by A549 lung cancer cells. Upon lysosomal degradation of rLDL(+) particles and subsequent payload release, TPA-DPPy was relocated to mitochondria due to their high membrane potential. Under light irradiation, these bound AIE photosensitizers triggered the production of considerable ROS, which gave rise to irreversible cell apoptosis with a high killing efficiency. Interestingly, this process could be fluorescently monitored in a real-time manner, as reflected by the remarkably enhanced luminescence and blue-shifted emission. The significant change in the luminescence of TPA-DPPy was attributed to the apoptosis-induced increase in mechanical stress, which forced TPA-DPPy to be in a highly condensed and

**Scheme 1** Schematic illustration of an LDL particle reconstituted with saturated fatty acids and a polarity-sensitive AIE photosensitizer.
more hydrophobic microenvironment. This feature is preferred as it can provide immediate feedback on the therapeutic outcome, holding great potential in multifunctional PDT.

2. Results and discussion

2.1 Molecular design, synthesis and characterization of TPA-DPPy

The synthetic route to TPA-DPPy is shown in Scheme 2. To construct a D–A structure and concurrently red-shift its emission spectrum to the NIR region, TPA-DPPy was constructed with an electron-donating triphenylamine unit and an electron-withdrawing pyridinium salt. Specifically, 4-fluorobenzophenone and diphenylamine were reacted under a nitrogen atmosphere to give compound 1, which was used for McMurry coupling with 4-benzoylpyridine to afford compound 2. Through a reaction with iodomethane followed by ion exchange with potassium hexafluorophosphate, compound 2 was converted to TPA-DPPy. The chemical structures of all compounds were confirmed by nuclear magnetic resonance spectrometry and high-resolution mass spectrometry (Fig. S1–S6‡). We then investigated the basic photophysical property of TPA-DPPy. As shown in Fig. 1A, the UV-vis spectra of TPA-DPPy in dimethylsulfoxide (DMSO) and H2O exhibited two predominant absorption peaks, with the second one ranging from 400 to 550 nm, which overlapped well with the spectrum of white light for efficient light harvesting. The attachment of aromatic rings to the backbone was achieved via single chemical bonds, which endowed TPA-DPPy with multiple rotatable elements to trigger AIE. To verify our hypothesis, we prepared a series of DMSO solutions with different water fractions (\(f_w\)) and studied their luminescence property (Fig. 1B). When \(f_w\) was lower than 90%, no evident emission was observed. When \(f_w\) was increased to a value higher than 90%, the hydrophobic TPA-DPPy started to precipitate from the solution and formed small aggregates, resulting in exponentially enhanced emission in the range of 500–800 nm with the peak maximum at ca. 625 nm. The Stokes shift of TPA-DPPy (ca. 185 nm) was much larger than that of traditional ACQ fluorophores (less than 50 nm), which was highly desired to reduce self-absorption during fluorescence imaging. Further, the emission intensity at 625 nm \((I)\) in solutions with different \(f_w\) was compared with that in DMSO \((I_0)\) for plotting the AIE curve. As shown in Fig. 1C, there was an over 15-fold enhancement in \(I/I_0\) when \(f_w\) was increased to 99%, indicating the typical AIE feature. In view of the D–A structure of TPA-DPPy, the emission spectra in water and a series of organic solvents (tetrahydrofuran (THF), dioxane, and ethyl ether (Et2O)) were measured to explore its TICT property. The result shown in Fig. 1D demonstrated that in contrast to water, organic solvents with low polarity blue-shifted the emission peak of TPA-DPPy from 625 nm (water) to 450 nm (Et2O), indicating that TPA-DPPy was able to sense the polarity of its residing environment via TICT. Notably, although a number of solvents were tested, only those with ether groups were able to trigger a prominent AIE phenomenon by providing a less polar yet more aggregated environment for TPA-DPPy, which could be observed from the broadened peaks in the corresponding absorption spectra (Fig. S7‡). Furthermore, we conducted density functional theory (DFT) calculations to get more insight into the intramolecular charge transfer. The electron density distribution of the frontier molecular orbitals (FMOs) is presented in Fig. 1E, where HOMO and LUMO represent the highest occupied molecular orbital and the lowest unoccupied molecular orbital, respectively. The electron cloud of the HOMO was mainly localized on the 4-(diphenylamino)phenyl group, whereas the electron cloud of the LUMO was primarily located on the acceptor unit, together with an energy gap of 2.57 eV. The spatial separation of the HOMO and LUMO electron clouds favored the intramolecular charge transfer from the 4-(diphenylamino)phenyl group to the pyridinium unit and thus endowed TPA-DPPy with an intrinsic TICT property.
We then evaluated the generation of ROS by TPA-DPPy using two commercial indicators, including 2',7'-dichlorofluorescin diacetate (DCFH-DA) and 9,10-anthracenediyl-bis(methylene)-dimalonic acid (ABDA). DCFH-DA is a fluorescently active probe that responds to various ROS. First, DCFH-DA was activated to its deacetylated non-fluorescent form 2',7'-dichlorofluorescin (DCFH). In the presence of ROS, DCFH was converted to the highly fluorescent form 2',7'-dichlorofluorescein (DCF). As shown in Fig. 2A, irradiation of TPA-DPPy under white light gave rise to a significant increase in the emission of DCF, characterized by a remarkable emission at ca. 525 nm. When the irradiation time was extended to 120 s, the enhancement ratio of the emission intensity at 525 nm was as high as 47, indicating the generation of considerable ROS. As a control, DCFH was also exposed to light irradiation in the absence of TPA-DPPy (Fig. 2B). In this case, no marked increase in DCF emission was found (2.4-fold), suggesting that ROS generation was ascribed to the TPA-DPPy-mediated photochemical reactions. We then used ABDA to specifically detect the generation of singlet oxygen. In the presence of singlet oxygen, ABDA was converted to the endoperoxide form, leading to a decrease in the absorption spectra. As shown in Fig. 2C, the absorbance of ABDA with TPA-DPPy dramatically decreased under light irradiation. Taking the absorbance at 378 nm as the intensity parameter (A) and rose bengal as the reference, we calculated the decomposition rate constant of ABDA with TPA-DPPy by plotting ln(A0/A) against irradiation time (Fig. 2D). According to a method reported in previous studies, the singlet oxygen quantum yield of TPA-DPPy was determined to be 0.245.

2.2 Reconstitution of native LDL particles with saturated fatty acids and TPA-DPPy

To incorporate TPA-DPPy into native LDL particles, the Krieger method was adopted with minor modifications for core reconstitution, where a eutectic mixture of lauric acid and stearic acid was used to replace the endogenous core lipids. As a control, we also fabricated a fatty acid-reconstituted LDL particle without TPA-DPPy (denoted as rLDL(−)). We first employed UV-vis spectroscopy to characterize the resultant rLDL particles. The UV/vis spectra shown in Fig. S8A‡ suggested that the structural stability of rLDL(−) and rLDL(+) particles was greatly maintained as shown by the existence of the characteristic absorption peak of ApoB-100 at ca. 280 nm. In addition, rLDL(+) particles also exhibited an absorption peak at ca. 450 nm, indicating the successful
loading of TPA-DPPy into rLDL(+) particles. We then examined the morphology and integrity of the resultant rLDL particles using transmission electron microscopy (TEM). Both rLDL(−) and rLDL(+) particles had good dispersity and a spherical shape identical to native LDL particles (Fig. 3A–C). Further analysis of the size distributions of rLDL particles showed that all samples had a narrow size distribution in the range of 10–60 nm, with the majority falling in 20–30 nm (Fig. 3D–F). In contrast to native LDL particles, the size of rLDL particles was slightly enlarged, which was attributed to the differential packing manner between fatty acids/TPA-DPPy and endogenous lipids. Nevertheless, this result indicated that the reconstitution process had a negligible impact on the morphology and size of rLDL particles. To quantify the concentration of TPA-DPPy, rLDL(+) particles were disassembled and extracted with methanol under ultrasonication. The supernatant was then measured with a UV/vis spectrometer, and the concentration curve of TPA-DPPy was determined by referring to the calibration curve of TPA-DPPy (Fig. S9A and B†). Further quantification indicated that the average number of TPA-DPPy in each LDL particle was 27 ± 1. We also tested the emission spectrum of rLDL(+) particles (Fig. S8B†). Interestingly, in contrast to TPA-DPPy, there was a ca. 15 nm blue shift in the peak maximum, suggesting that TPA-DPPy resided in a relatively hydrophobic environment in rLDL(+) particles. As a comparison, we also characterized the production of singlet oxygen by rLDL(+) particles. However, only a slight decrease in the absorbance of ABDA for rLDL(+) particles was found, which was attributed to the protective effect of the hydrophobic core that prevented TPA-DPPy from coming into contact with dissolved oxygen in aqueous media (Fig. S10†). Further analysis revealed that the decomposition rate of ABDA for TPA-DPPy was 7.4-fold higher than that of rLDL(+) particles (Fig. S10†), and the singlet oxygen quantum yield of rLDL(+) particles was calculated to be 0.055.

2.3 LDLR-mediated endocytosis of rLDL(+) particles and their release behaviors

Considering the targeting capability of ApoB-100 toward LDLRs, we next studied the uptake of rLDL(+) particles by LDLR-overexpressing A549 cancer cells.2,45 As shown in Fig. 4A, B and Fig. S11† a time-dependent accumulation of rLDL(+) particles was found as the incubation time prolonged from 0.5 h to 6 h. In view of the strong fluorescence signal of rLDL(+) particles at 6 h, we further investigated the release behavior of rLDL(+) particles by co-staining with lysosome- and
Fig. 3  Morphology and size of rLDL particles. (A–C) TEM images of (A) native LDL particles, (B) rLDL(−) particles, and (C) rLDL(+) particles. (D–F) Particle size distributions from TEM images for (D) native LDL particles, (E) rLDL(−) particles, and (F) rLDL(+) particles (n = 100 for each group).

Fig. 4  Cell uptake of rLDL(+) particles. (A and B) A549 cells incubated with rLDL(+) particles for (A) 1 h and (B) 6 h. (C and D) Colocalization of (C) lysosomes and (D) mitochondria with rLDL(+) particles at 6 h post-incubation. (E and F) Incubation of A549 cells with rLDL(+) particles in the (E) absence and (F) presence of a 20-fold excess of native LDL particles. The nuclei were stained with Hoechst 33342 to show in blue, the lysosomes or mitochondria were stained with Lyso-Tracker or Mito-Tracker to show in green, and TPA-DPPy were shown in red.
mitochondrion-specific dyes (Lyso-Tracker and Mito-Tracker) at 6 h post-incubation. The overlay image shown in Fig. 4C indicated that although rLDL(+) particles were co-localized with lysosomes, there remained a large proportion of non-overlapped green and red signals. Since rLDL particles entered cells via an endocytic pathway, these separated fluorescence signals presumably originated from the unloaded TPA-DPPy from rLDL(+) particles. In contrast to lysosomes, the red signals were mostly co-localized with mitochondria as seen in Fig. 4D. Because the cells were supplemented with rLDL(+) particles during the entire 6 h incubation, these non-overlapped red signals should be ascribed to the newly bound rLDL(+) particles or rLDL(+) particles in lysosomes. Typically, organic molecules pendent with a pyridinium salt possess the capability to associate with mitochondria due to their high membrane potential. As such, we examined the intracellular localization of TPA-DPPy in A549 cells. As shown in Fig. S12, TPA-DPPy was able to shuttle into cells within 30 min. Further comparison showed that TPA-DPPy overlaid well with Mito-Tracker, suggesting its mitochondrion-targeting capability. To verify that ApoB-100 was biologically functional after core reconstitution and the endocytosis was specifically mediated by LDLRs, we performed a receptor-blocking experiment by incubating A549 cells with rLDL(+) particles in the presence of a 20-fold excess of native LDL particles. As shown in Fig. 4E and F, in contrast to the group without receptor blocking, the uptake of rLDL(+) particles was basically shut down, as no noticeable fluorescence was found in the cytoplasm. This result suggested that rLDL(+) particles retained the biological function of native LDL particles. Taken together, the uptake of rLDL(+) particles by A549 cells was mediated by the specific binding of ApoB-100 toward LDLRs. As the incubation time prolonged, rLDL(+) particles were disassembled and the released cargos escaped from lysosomes, which facilitated the mitochondrial targeting of TPA-DPPy.

2.4 Fluorescence-feedback cell apoptosis in response to PDT

We then evaluated the in situ PDT effect of rLDL(+) particles on cancer cells. As discussed earlier, in contrast to TPA-DPPy, rLDL(+) particles were relatively inactive in the generation of ROS. This feature was highly desired as no evident phototoxicity of rLDL(+) particles would be elicited unless they were delivered to LDLR-overexpressing cancer cells and disassembled to release the mitochondrion-targeting photosensitizer. As such, the PDT experiment was performed after a large quantity of TPA-DPPy molecules was located to mitochondria. After incubation with rLDL(+) particles for 6 h, A549 cells were exposed to in situ light irradiation for a period of 10 min. The bright-field images shown in Fig. 5A demonstrated that as the irradiation time increased, the morphology of cells exhibited a remarkable change, including blebbing, chromatin condensation, and cell shrinkage from an inflated 3D structure to a flattened 2D shape, together with a plethora of aggregated granules, which were actually the characteristic features of cell apoptosis. We also examined the changes in the corresponding fluorescence images, and two significant changes were found (Fig. 5B). On one hand, the fluorescence intensity was remarkably enhanced when the irradiation time changed from 0 min to 10 min. Further analysis of the hue (H) and lightness (L) values in an area of a representative cell showed that the L value increased from 73 at 0 min to 217 at 10 min, suggesting the strengthened aggregation of TPA-DPPy. On the other hand, the emission color exhibited a hypsochromic shift, with the H value shifting from 5 (smaller values correspond to red hue) to
36 (larger values correspond to blue hue), indicating the decreased polarity in the residing environment of TPA-DPPy. Based on the aforementioned results, we inferred that under in situ light irradiation, the mitochondrion-targeted TPA-DPPy initiated the production of considerable ROS, which disrupted the structure and biological function of mitochondria and thus triggered cell apoptosis. The irreversible cell death gave rise to cell blebbing, chromatin condensation, and shrinkage, thereby forcing the intracellular space to be in a relatively crowded state. The condensed cytoplasm elicited a mechanical stress on mitochondria and all other organelles, leading to the restricted intramolecular motions of TPA-DPPy and thus the activation of AIE. In addition, the elevated mechanical stress strengthened the molecular packing of phospholipids and made TPA-DPPy to be in a more hydrophobic environment, which shifted its emission maximum toward a shorter wavelength.

2.5 Intracellular ROS production and cell viability analysis for PDT

To provide direct evidence of intracellular ROS generation by TPA-DPPy, A549 cells were incubated with rLDL(−) and rLDL (+) particles for 6 h, respectively, and the ROS-specific probe DCFH-DA was used as an indicator for ROS detection via confocal laser scanning microscopy (CLSM). Considering the absorption peak of TPA-DPPy located at ca. 440 nm, we chose a 450 nm lamp as the light source to maximize the PDT effect. In this experiment, untreated A549 cells without or with light irradiation were taken as controls. As shown in Fig. 6, for the cells treated with rLDL(−) particles in the absence and presence of light irradiation, no fluorescence signals were found, which was similar to the negative control. When the cells were incubated with rLDL(+) particles in the dark, the fluorescence signal was extremely weak. However, when these cells were exposed to light irradiation, strong green fluorescence could be found. These comparative data demonstrated that the mitochondrion-targeted TPA-DPPy was able to trigger intracellular generation of ROS in the presence of an appropriate light source.

Since the produced ROS would result in irreversible cell apoptosis, we further evaluated the PDT effect of rLDL(+) particles against A549 cells. We first performed live/dead staining with calcein-AM (live indicator, green) and propidium iodide (PI, dead indicator, red) at 12 h post-irradiation to visualize cell states. Similar to ROS detection, both untreated cells and cells treated with rLDL(−) particles were used as controls. As shown in Fig. 7A, for untreated cells, light irradiation did not result in cell death. When the cells were treated with rLDL(−) particles in the dark, they remained viable but with a slightly decreased cell number, which was probably ascribed to the negative feedback by excessive ingestion of rLDL(−) particles. Meanwhile, light irradiation also did not give rise to cell death. When the cells were treated with rLDL(+) particles in the dark, they maintained an identical viability to those treated with rLDL(−) particles. However, light irradiation exerted a destructive effect on cell viability, causing most cells to detach from the Petri dish, with only a small quantity of shrunk dead cells being observed. To offer a quantitative evaluation of the phototoxicity of rLDL(+) particles, we performed a cell viability analysis based on 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). As shown in Fig. 7B, there was a dose-dependent growth inhibition of A549 cells.
both in the dark and with light irradiation when treated with different concentrations of rLDL(+) particles. The inhibitory effect in the dark was presumed to be the negative feedback by excessive ingestion of rLDL(+) particles. A similar phenomenon was also found when the cells were treated with rLDL(−) particles (Fig. S13‡). Nevertheless, the cells still maintained a ca. 70% survival rate even in the highest concentration of rLDL(+) particles. In comparison with the cells placed in the dark, light irradiation significantly decreased their viability. When the concentration of TPA-DPPy in rLDL(+) particles reached 20 μM, the inhibitory rate was as high as ca. 88%, indicating the outstanding PDT effect of TPA-DPPy. To exclude the impact of the nanocarrier, the viability of cells incubated with rLDL(−) particles was also tested. However, no obvious toxicity was found both in the dark and under light irradiation (Fig. S13‡). It should be noted that although the cells exhibited a dose-dependent decrease in survival rates, they remained viable unless light irradiation was applied (as indicated in Fig. 7A), suggesting that the cell proliferation was transiently suspended and could be recovered under appropriate culture conditions. Furthermore, to verify that the phototoxicity originated from the LDLR-mediated endocytosis, cell viability analysis was also performed in the presence of excess native LDL particles. As shown in Fig. 7C, a 20-fold excess of native LDL particles in the dark resulted in a negative feedback on cell proliferation, and light irradiation slightly strengthened this effect. For the cells treated with rLDL(+) particles in the absence and presence of light irradiation, the cell viability was identical to that
shown in Fig. 7B. Interestingly, receptor blocking with a 20-fold excess of native LDL particles rescued the cells to a healthy state by competitive binding with LDLRs, with a survival rate identical to that of excess LDLs alone. These results altogether demonstrated that rLDL(+) particles were able to achieve photodynamic killing of cancer cells that overexpress LDLRs.

3. Conclusions

In summary, we, for the first time, reported the reconstitution of native LDL particles with saturated fatty acids and a mitochondrial-targeting AIE photosensitizer for fluorescence-feedback PDT. In particular, a novel AIE photosensitizer (TPA-DPPy) with a D–A structure and a pyridinium salt was designed and synthesized, which possessed typical AIE and TICT characteristics as well as ROS-sensitizing capability. In view of its prominent photophysical and photochemical properties, TPA-DPPy was encapsulated into LDL particles for photodynamic killing of LDLR-overexpressing cancer cells. The resultant rLDL(+) particles maintained a similar morphology and size distribution to native LDL particles, and were efficiently ingested by cancer cells via LDLR-mediated endocytosis, followed by the release of TPA-DPPy for mitochondrial targeting. Upon light irradiation, the produced ROS surrounding mitochondria led to efficient and irreversible cell apoptosis. Interestingly, this process could be fluorescently monitored in a real-time fashion, as reflected by the remarkably enhanced luminescence and blue-shifted emission, indicating the increased mechanical stress during apoptosis. Quantitative cell viability analysis suggested that TPA-DPPy exhibited an outstanding phototoxicity toward LDLR-overexpressing A549 cancer cells, with a killing efficiency of ca. 88%. In contrast to a bare AIE photosensitizer, rLDL(+) particles were inactive in ROS generation due to the restricted contact of TPA-DPPy with dissolved oxygen in aqueous media. This difference enabled rLDL(+) particles as a class of safe nanophototheranostic agents, as no evident phototoxicity would be imposed until they were delivered to LDLR-overexpressing cancer cells and disassembled to release the mitochondrial-targeting photosensitizer. The rLDL(+) particles are a class of safe and multifunctional nanophototheranostic agents, holding great promise in high-quality PDT by providing real-time fluorescence feedback on the therapeutic outcome.

Conflicts of interest

There are no conflicts to declare.

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