

High payload delivery of optical imaging and photodynamic therapy agents to tumors using phthalocyanine-reconstituted low-density lipoprotein nanoparticles

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Abstract. To improve the labeling efficiency of a low-density lipoprotein (LDL)-based photosensitizer (PS) for achieving high probe to protein payload, a tetra-*t*-butyl silicon phthalocyanine bearing two oleate moieties at its axial positions, SiPcBOA, is designed and synthesized. Using this novel strategy, SiPcBOA reconstituted LDL (r-SiPcBOA-LDL) with a very high payload (SiPcBOA to LDL molar ratio >3000 to 35001:1) is obtained. Using electron microscopy, we find reconstituted LDL (rLDL) with such a high payload essentially retains the mean particle size of native LDL. Since acetylated LDL binds to scavenger receptors of endothelial and microglial cells instead of LDLR, SiPcBOA reconstituted acetylated LDL (r-SiPcBOA-AcLDL) is also prepared to serve as a negative control to validate the LDL receptor (LDLR) targeting specificity. Confocal microscopy studies demonstrate that the internalization of r-SiPcBOA-LDL by human hepatoblastoma G₂ (HepG₂) tumor cells is mediated by LDLR pathway. The *in vitro* photodynamic therapy (PDT) response of HepG₂ cells to r-SiPcBOA-LDL is compared to SiPcBOA (free drug control) using a clonogenic assay. The slopes of the linear regression fit to the logarithmic data for these two plots are significantly different from each other ($p = 0.0007$), indicating greatly enhanced efficacy of LDLR-targeted PDT. © 2005 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.2011429]

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1 Introduction

The low-density lipoprotein (LDL) particle is the principal carrier of cholesterol in human plasma and delivers exogenous cholesterol to cells by endocytosis via the LDL receptor (LDLR). The LDL particle is a naturally occurring nanostructure typically with a diameter of ~22 nm. It contains a lipid core of some 1500 esterified cholesterol molecules and triglycerides. A shell of phospholipids and unesterified cholesterol surrounds this highly hydrophobic core. The shell also contains a single copy of apoB-100, which is recognized by the LDLR. Due to the large amounts of cholesterol required for membrane synthesis, a number of tumor cell lines overexpress LDL receptors relative to normal liver and adrenals.¹ Therefore, LDL had been proposed as a useful discriminatory vehicle for the delivery of cytotoxic drugs, imaging probes, and photodynamic therapy agents to tumor cells.² There are two distinct advantages for using LDL and targeting LDLR. First, being endogenous carriers, LDL particles are not immu-

nogenic and escape recognition by the reticuloendothelial system (RES). Second, after binding to the LDLR, LDL is internalized and incorporated into endosomes, which deposit LDL into lysosomes for degradation. The receptors are recycled back to the plasma membrane. The round-trip time for an LDL receptor is approximately ten minutes;³ in its lifetime of about a day, it may bring many LDL particles into the cell. Therefore, the contents transported by LDL can accumulate within LDLR expressing cells.

In general, there are three ways to incorporate agents into LDL particles. The first method involves the direct conjugation of probes to the amino acid residues of apoB-100. To date, this has been done for only a few radioactive imaging agents (¹²⁵I, ¹¹¹In, or ⁶⁸Ga labeled LDL),⁴ and in most cases, the probe/LDL ratio was generally kept low to avoid disrupting its affinity to LDLR.⁵ As an alternative approach, lipid-anchored probes can be incorporated into the LDL phospholipid monolayer via an intercalation mechanism. For example, Urizzi et al.⁶ labeled the LDL with ¹¹¹In via a lipid-anchored diethylenetriaminepentaacetic acid (DTPA) chelating agent,

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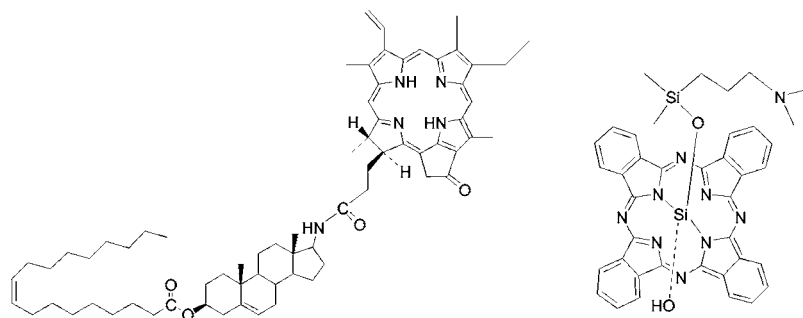


Fig. 1 Structures of Pyro-CE (left)¹⁰ and Pc4 (right).¹³

as a radiopharmaceutical for tumor localization. One possible drawback of this method is that these phospholipid intercalating agents might exchange thermodynamically with similar sites on the plasma membranes of cells, thus reducing the specificity for LDLR. Following this approach, the potential of using fluorescent dye-labeled LDL as optical probes for cancer detection was also investigated.⁷ The third and perhaps most useful method is the LDL reconstitution approach. Kreiger, Goldstein, and Brown⁸ were the first to report that it is possible to remove more than 99% of core cholesteryl esters from the LDL particle by heptane extraction, and replace them with an equivalent amount of exogenous cholesteryl linoleate. The reconstituted LDL (rLDL) particle is essentially identical to native LDL in its ability to bind to LDLR, to be internalized by cells, and to be hydrolyzed in lysosomes. Moreover, the cholesterol released from the lysosomal hydrolysis of the rLDL retained its ability to modulate cholesterol metabolism. Since rLDL is internalized preferentially by LDLR, many cytotoxic compounds (e.g., doxorubicin⁹) have been delivered to cancer cells using this method and have shown good antitumor activity.

We have recently synthesized a novel chlorophyll-based photosensitizer (PS) containing anchors that render it compatible with LDL's phospholipid coat and lipophilic core.¹⁰ This new dye conjugate, pyropheophorbide cholesterol oleate (Pyro-CE) (structure shown in Fig. 1, left), contains an oleate moiety to facilitate LDL reconstitution and a cholesterol moiety to anchor the phospholipid monolayer to prevent probes from leaking. Pyro-CE was incorporated into LDL (r-Pyro-CE-LDL) with a modest PS payload (Pyro-CE:LDL molar ratio $\leq 50:1$). The reconstitution efficiency of r-Pyro-CE-LDL is 45%, which is similar to the cholesteryl linoleate LDL reconstitution efficiency.⁸ Laser scanning confocal microscopy studies demonstrated that such an r-LDL-based PS was internalized exclusively by LDLR overexpressing human hepatoblastoma G₂ (HepG₂) tumor cells.¹⁰

Although preliminary optical imaging and photodynamic therapy (PDT) studies of r-Pyro-CE-LDL appear promising,¹¹ the probe/protein ratio required for the desired imaging sensitivity and PDT efficacy is far from optimal. To reduce the dose for more efficient cancer detection and treatment, it is necessary to maximize the near infrared (NIR) optical imaging/PDT agent payload for each LDL particle. Thus, we designed a novel strategy to improve LDL's probe payload based on new NIR dyes derived from metallated phthalocyanine (Pc). Pc dyes are neutral, porphyrin-like compounds that

absorb strongly above 680 nm (within the NIR range of 650 to 900 nm). They are well-known photosensitizers for PDT,¹² and in general are much more stable photochemically and photophysically than corresponding porphyrin analogs. For our purpose, we are particularly interested in silicon phthalocyanines (SiPc) for the following reasons. 1. Pc4 (structure shown in Fig. 1, right), a SiPc analog, is currently under PDT cancer clinical trials at the National Cancer Institute.¹³ 2. The central silicon atom of SiPc allows axial coordination of two bulky ligands on each side of the Pc ring to prevent stack aggregation usually encountered in solution for the planar molecular structure.¹⁴ Such aggregation presumably is the major limiting factor for achieving high probe/LDL payload. 3. Since a bent or branched fatty acid is required for successful LDL reconstitution,¹⁵ introducing two oleate moieties via the axial coordination may improve the LDL reconstitution efficiency.

We describe the design and synthesis of tetra-*t*-butyl silicon phthalocyanine bisoleate, SiPcBOA, and detail its highly efficient LDL reconstitution. Additionally, we characterize the payload and size of the resulting LDL nanoparticles, and demonstrate the *in vitro* validation for r-SiPcBOA-LDL as a LDLR-specific optical imaging and PDT agent.

2 Materials and Methods

2.1 Materials

UV-visible and fluorescence spectra were recorded on a Perkin-Elmer Lambda 2 spectrophotometer and LS50B spectrofluorometer, respectively. ¹H NMR spectra were recorded on a Bruker 500-MHz instrument. Mass spectrometry analyses were performed at the Mass Spectrometry Facility of the Department of Chemistry, University of Pennsylvania. All chemicals and reagents were purchased from Aldrich (Milwaukee, Wisconsin). When necessary, solvents were dried before use. For TLC, EM Science TLC plates (silica gel 60 F₂₅₄) were used.

2.2 Synthesis of Bisoleate Conjugate of Silicon Tetra-*t*-butyl-phthalocyanine, SiPcBOA

A suspension of silicon tetra-*t*-butyl-phthalocyanine dihydroxide (200 mg, 0.25 mmol) in 20 mL of 2-picoline was mixed with the oleoyl chloride (300 mg, 1.00 mmol) and stirred under argon for 2 h. The 4-dimethylaminopyridine (376 mg, 3.08 mmol) was added to the mixture portion wise, which

was kept well stirred under argon for an additional 36 h at 60 °C. On completion, the solvent was evaporated under reduced pressure, and the product was purified by column chromatography (silica gel-hexane: CH₂Cl₂=1:1) to yield the desired conjugate (130 mg, 0.098 mmol, 39.1%). UV-vis [nm (ϵ) in CH₂Cl₂]: 362 (1.39 × 10⁵), 620 (5.32 × 10⁴), 658 (4.58 × 10⁴), 691 (2.54 × 10⁵); emission λ_{\max} in CH₂Cl₂ (excitation wavelength 680 nm): 697 nm. ESI-MS calculated for C₈₄H₁₁₄N₈O₄Si: 1327.94, found: 1327.96 (M⁺); HRMS calculated for C₈₄H₁₁₄N₈O₄Si+Na: 1349.8630, found: 1349.8643. ¹H NMR (CDCl₃, δ ppm): 9.54–9.70 (m, 8H, Aromatic H), 8.42 (m, 4H, Aromatic H), 5.25 (2 m, each 2H, from oleoyl vinyl H), 1.67–2.01 (m, 52 H, from oleoyl chain H), 1.21–1.28 (m, 36H, Boc H), 0.90 (m, 4H, from oleoyl chain), 0.85 (t, 6H, from oleoyl chain terminal CH₃).

2.3 LDL Reconstitution and Characterization

LDL, purchased from Lund-Katz' laboratory at the Children's Hospital of Philadelphia (Philadelphia, Pennsylvania) was isolated from fresh plasma of healthy donors by sequential ultracentrifugation as described previously.¹⁶ LDL reconstitution with SiPcBOA was performed following a minor modification of the method of Krieger.¹⁵ Briefly, LDL (1.9 mg) was lyophilized with 25-mg starch, and then extracted three times with 5 mL of heptane at -5 °C. Following aspiration of the last heptane extract, 6 mg of SiPcBOA was added in 200 μ L of benzene. After 90 min at 4 °C, benzene and any residual heptane were removed under a stream of N₂ in an ice salt bath for about 45 min. The r-SiPcBOA-LDL was solubilized in 10-mM Tricine, pH 8.2, at 4 °C for 24 h. Starch was removed from the solution by a low-speed centrifugation (500 × g) followed by a 20-min centrifugation (6000 × g). The reconstituted LDL was stored under an inert gas at 4 °C. Similarly, r-SiPcBOA-AcLDL was also prepared from SiPcBOA and acetylated LDL (AcLDL, Biomedical Technologies, Incorporated, Stoughton, Massachusetts). The protein content of the specimen was determined by the Lowry method.¹⁷ The absorption spectrum of SiPc-BOA was measured after extraction with a chloroform and methanol mixture (2:1), and probe concentration was calculated based on the following formula: $C=(A/\epsilon) \times D$, where C is the concentration of the probe, A is the OD value, ϵ is the extinction coefficient, and D is the dilution fold. Probe/protein molar ratio was calculated using the molecular mass of the ApoB-100 protein (514 kDa), knowing that one LDL particle contains only one ApoB-100.

2.4 Cell Preparations

HepG₂ tumor cells, which were obtained from van Berkel's laboratory from the University of Leiden in the Netherlands, were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 10-mM HEPES, with 100-U/mL penicillin G sodium and 100- μ g/mL streptomycin sulfate. Cells were grown at 37 °C in an atmosphere of 5% CO₂ in a humidified incubator.

2.5 Confocal Microscopy Studies

For confocal microscopy studies, HepG₂ cells were grown in 4-well Lab-Tek chamber slides (Naperville, Illinois) at a density of 40,000 cells/well. Experiments were started, after two

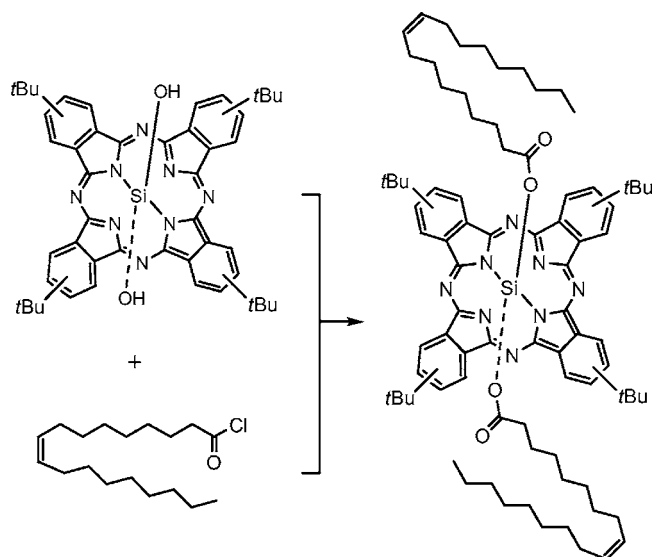


Fig. 2 Synthesis of SiPc-BOA.

quick washes with preincubation medium [medium with 0.8% (w/v) BSA instead of FBS], by the addition of preincubation medium containing the indicated amounts of r-SiPcBOA-LDL/AcLDL and/or unlabeled LDL. After a 4-h incubation at 37 °C, the cells were washed three times with ice-cold PBS and fixed for 15 min with 3% formaldehyde in PBS at room temperature. Then the chamber slides were mounted and sealed for confocal microscopy analysis. Confocal microscopy was performed with a Leica TCS SPII laser scanning confocal microscope (Heidelberg, Germany). Filter settings were 633 nm for excitation and 638 to 800 nm for emission.

2.6 Electron Microscopy Studies

Five microliters of the reconstituted LDL suspension were placed on carbon-coated 200 mesh copper grids and allowed to stand for 5 min. Excess sample was wicked off with lens paper and 2% saturated aqueous uranyl acetate was applied to the grid in 5 consecutive drops within 20 s. The stain was then drained off with filter paper and the grid was air dried. Digital images were taken using JEOL JEM 1010 electron microscope (JEOL-USA, Incorporated, Peabody, Massachusetts) at 80 kV using AMT 12-HR software aided by a Hamamatsu CCD Camera. All related supplies were purchased at Electron Microscopy Sciences (Fort Washington, Pennsylvania).

2.7 In Vitro PDT Studies Using r-SiPcBOA-LDL as a Photosensitizer

Flasks containing approximately 2×10^6 HepG₂ cells were incubated for 5 h at 37 °C in preincubation medium with no drug, 8- μ g/mL r-SiPcBOA-LDL protein (equivalent to 50- μ g/mL SiPcBOA), or 50- μ g/mL SiPcBOA. [The effective concentration of the SiPcBOA is calculated as follows: (50 μ g/mL)/(1328 g/mol)=37 μ M, where 1328 is the molecular weight of SiPcBOA.] Cells were washed with 10-mL HBSS and subsequently incubated for 30 min with fresh preincubation medium. After this incubation, cells were again washed with HBSS, collected, and resuspended at a concen-

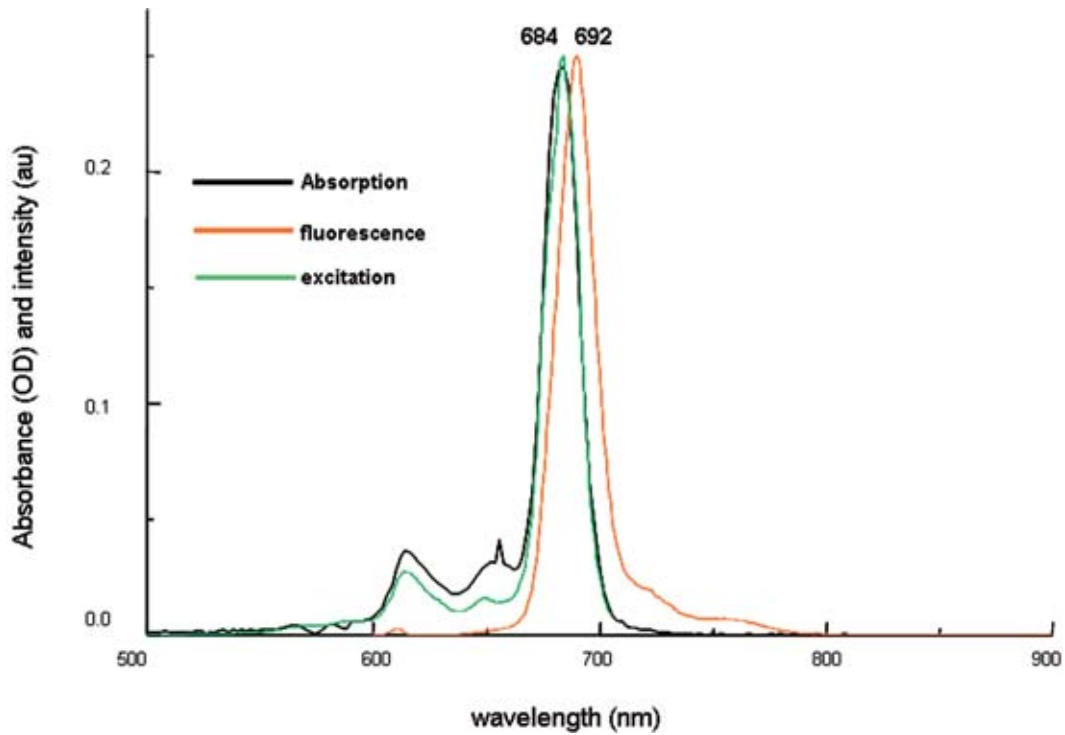


Fig. 3 Absorption (black), excitation (green), and fluorescence spectra (red) of SiPcBOA.

tration of 1×10^6 cells/mL. Aliquots of the drug-exposed cells were transferred to individual 60×15 -mm dishes and treated with PDT at 5 mW/cm^2 to a total fluence of 1 J/cm^2 (200 s), 2.5 J/cm^2 (500 s), 4 J/cm^2 (800 s), or 5 J/cm^2 (1000 s). Light at 680 nm was delivered using a KTP Yag-pumped dye module (Laser Scope, San Jose, California). Additionally, controls were prepared containing either $8 \text{ }\mu\text{g/mL}$ r-SiPcBOA-LDL protein ($50 \text{ }\mu\text{g/mL}$ SiPcBOA) or

$50 \text{ }\mu\text{g/mL}$ SiPcBOA with no light dose exposure, no drug with a 5 J/cm^2 light dose exposure, or neither drug nor light exposure. Dishes ($100 \times 20 \text{ mm}$) were plated in triplicate and placed in a $37 \text{ }^\circ\text{C}$ incubator ($5\% \text{ CO}_2$) for 11 days. Following incubation, dishes were rinsed with PBS and allowed to air dry. Subsequently, cells were fixed and stained with methylene blue, then colonies were counted. This experiment was replicated three times.

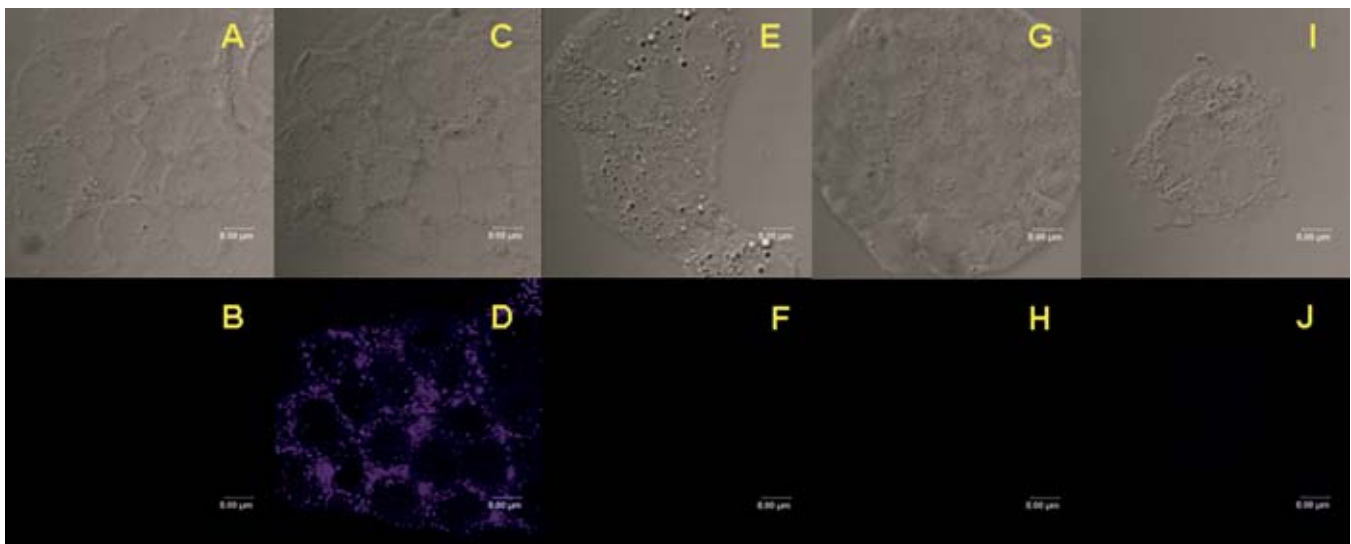


Fig. 4 Confocal fluorescence images of HepG₂ cells incubated w/wt fluorescent probes [(b), (d), (f), (h), (j)] as well as the corresponding bright field images [(a), (c), (e), (g), (i)]. (a) and (b) Cell alone control; (c) and (d) cell+ $85 \text{ }\mu\text{g/mL}$ r-SiPcBOA-LDL protein; (e) and (f) cell+ $85 \text{ }\mu\text{g/mL}$ r-SiPcBOA-LDL protein +50-fold over excess native LDL; (g) and (h) cell+ $170 \text{ }\mu\text{g/mL}$ r-SiPcBOA-AcLDL protein; (i) and (j) cell+ $570 \text{ }\mu\text{g/mL}$ SiPcBOA (same amount of SiPcBOA as in $85 \text{ }\mu\text{g/mL}$ r-SiPcBOA-LDL protein).

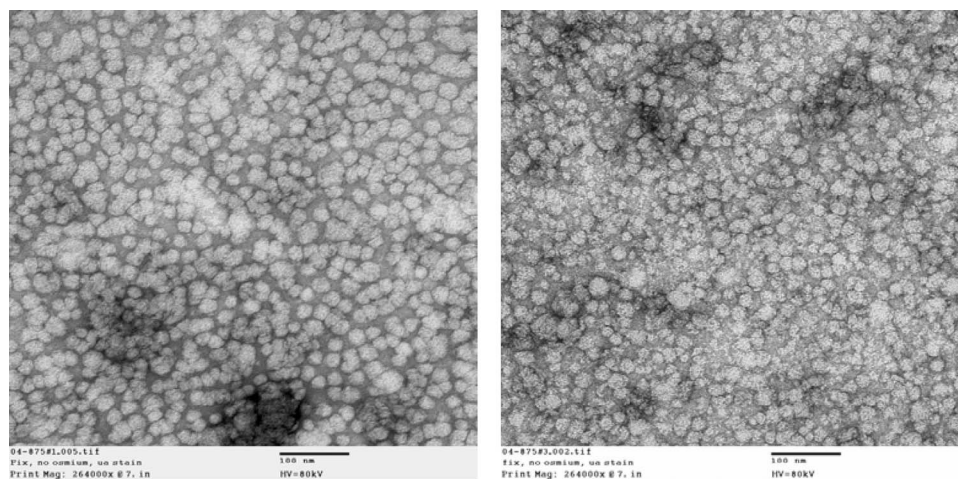


Fig. 5 EM images of native LDL (left) and r-SiPc-BOA-LDL (right).

2.8 Statistics

Analyses of the clonogenic assays were performed using STATA software (STATA Corporation, College Station, Texas) and data were plotted in SigmaPlot (SPSS, Incorporated, Chicago, Illinois). The outcome variable, surviving fraction, was log-transformed and data were fit by linear regression. The addition of a quadratic term to the model was tested, but a likelihood ratio test indicated no significant support for the square term. The outcome was analyzed using the multiple regression procedure, treating drug as a class variable and light as continuous. Significance of effects for light and drug combinations was determined by t-test. Controls for light-alone (at the highest dose tested), drug-alone (free and conjugated), and untreated controls showed no difference and were averaged to generate a control plating efficacy to which experimental data were compared.

3 Results and Discussion

3.1 Design and Synthesis of SiPcBOA

The aim of this work is to improve the labeling (reconstitution) efficiency of LDL-based PS for achieving high probe to protein payload and to provide targeting of PS to LDLR. To achieve this aim, PS should be neutral, highly soluble in non-polar solvent, have minimal aggregation, and contain a suitable linker for conjugation to a lipid anchor to prevent the dye from dissociating from LDL and nonspecifically binding to phospholipid bilayers on cellular membranes. For these purposes, we designed a new PS for LDL reconstitution based on SiPc. Because Si coordination allows the binding of two axial oleate ligands, these ligands will then create steric hindrance on each side of the Pc ring, thereby limiting stack aggregation. Therefore, we anticipate a large increase in the PS payload of LDL.

To synthesize bisoleate-anchored SiPc, commercially available silicon tetra-*tert*-butylphthalocyanine dihydroxide was conjugated at the axial position with oleoyl chloride in the presence of 4-dimethylaminopyridine and 2-picoline. The desired conjugate, SiPcBOA, was obtained in 40% yield. This efficient synthetic pathway is depicted in Fig. 2. The structure of this compound was confirmed by ^1H NMR and high reso-

lution mass spectroscopy analysis. Figure 3 shows the absorption, excitation, and fluorescence spectra of this new compound. It has a very intense absorption at 684 nm and emission at 692 nm, both within the NIR range.

As shown in Fig. 2, this method has several distinct advantages. First, the reaction condition is very mild. It can be carried out in weak base (picoline, dimethyliminopyridine) at warm temperatures ($<60^\circ\text{C}$) instead of at 150°C in a much stronger base (sodium alcoholate), as is commonly used for the preparation of Pc derivatives. Second, the starting material, $(\text{tBu})_4\text{SiPc}(\text{OH})_2$, is commercially available and consists of four lipophilic and bulky *t*-butyl groups at the peripheral position of the Pc macrocycle, further increasing its lipophilicity. Finally, the bisoleate anchor (BOA) is known to strongly associate with the lipid membrane, a characteristic similar to that of the cholesterol moiety. Therefore, for Pc LDL reconstitution, we expect that the bisoleate anchor will be an enhancement over the corresponding cholesterol oleate moiety.

3.2 LDL Reconstitution and Characterization

Protein recovery determined by the Lowry method¹⁷ is an excellent assay for evaluating the success of the reconstitution. 55 to 70% protein recovery was observed for both r-SiPcBOA-LDL and r-SiPcBOA-AcLDL, which is better than that observed for r-Pyro-CE-LDL.¹⁰ The absorption spectrum for the recovered SiPcBOA after LDL reconstitution is the same as it was before reconstitution, indicating that absorbance measurement can serve as the basis for calculating the SiPcBOA concentration in the reconstituted LDL (payload). It was found that ~ 3000 to 3500 SiPc-BOA molecules were reconstituted into one LDL molecule core. Compared to the 50:1 probe:protein ratio for r-Pyro-CE-LDL we prepared previously, the new probe design reported here improved probe payload on each LDL nanoparticle by 60 fold.

3.3 Confocal Microscopy Studies of the LDLR-Specific Uptake

To visualize LDLR-mediated internalization of r-SiPcBOA-LDL, we performed laser scanning confocal microscopy studies on HepG₂ tumor cells. Figure 4 shows the confocal fluo-

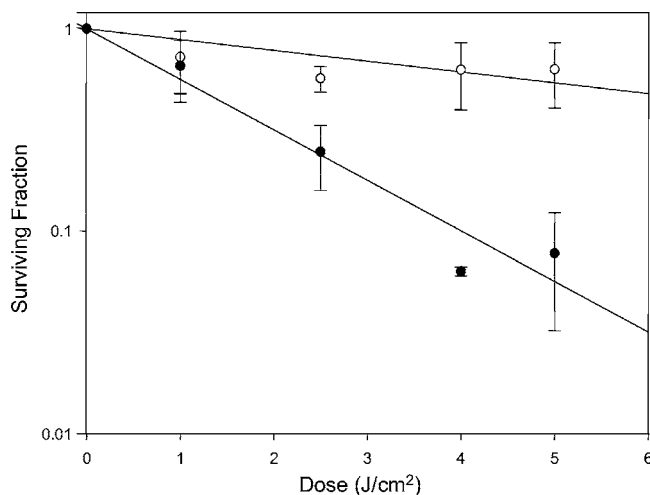


Fig. 6 *In vitro* PDT response of HepG₂ cells to r-SiPcBOA-LDL (closed circles) and SiPcBOA (open circles). SEMs were generated for the arithmetic mean.

rescence images of HepG₂ cells incubated with/without fluorescent probe (B, D, F, H, J) as well as corresponding bright field images (A, C, E, G, I). Figures 4(a) and 4(b) depict images of the cell alone, providing values for the fluorescence of the cells. When cells were incubated with 85- $\mu\text{g}/\text{mL}$ r-SiPcBOA-LDL protein at 37 °C for 4 h, the fluorescence signal appears to be localized in the cytoplasm [Figs. 4(c) and 4(d)]. To determine the specificity of this r-SiPcBOA-LDL toward LDLR, three sets of control experiments were performed. When HepG₂ cells were incubated with 85- $\mu\text{g}/\text{mL}$ r-SiPcBOA-LDL protein plus 50-fold excess of unlabeled native LDL, complete fluorescence inhibition was observed [Figs. 4(e) and 4(f)]. When 170- $\mu\text{g}/\text{mL}$ r-SiPcBOA-AcLDL protein was incubated with HepG₂ cells, despite the fact that the fluorophore concentration doubled, no fluorescence was observed. This is consistent with the inability of Ac-LDL to target LDLR [Figs. 4(g) and 4(h)]. Figures 4(i) and 4(j) show that incubation with 570- $\mu\text{g}/\text{mL}$ SiPcBOA alone (equivalent to 85- $\mu\text{g}/\text{mL}$ r-SiPcBOA-LDL protein) did not lead to any observable fluorescence, indicating that no internalization occurred. Collectively, the previous experiments indicate that r-SiPcBOA-LDL was internalized into HepG₂ tumor cells specifically via the LDL receptor pathway.

3.4 Electron Microscopy Studies

A light scattering size scanner was originally used to measure the size of the SiPcBOA reconstituted LDL particle. However, we found that Pc absorption interferes with the laser wavelength used by the scanner; therefore, electron microscopy was used to directly visualize the LDL particles. As shown in Fig. 5, the mean particle size of r-SiPcBOA-LDL was 23.2 ± 4.6 nm ($n=30$), which is about the same size as native LDL (20 ± 2.7 nm, $n=37$).

3.5 *In Vitro* PDT Studies (Clonogenic Assay)

Figure 6 shows the *in vitro* PDT response of HepG₂ cells to r-SiPcBOA-LDL and SiPcBOA using a clonogenic assay. At a dose of 4 and 5 J/cm², there was significantly more cell kill

with 8- $\mu\text{g}/\text{mL}$ r-SiPcBOA-LDL protein (equivalent to 50- $\mu\text{g}/\text{mL}$ SiPcBOA) than with 50- $\mu\text{g}/\text{mL}$ SiPcBOA [$t(4)=3.85$, $p=0.02$, and $t(4)=2.79$, $p=0.05$, respectively]. At the drug dose used, SiPcBOA induced limited cell kill, even at the highest light dose tested. Conversely, when r-SiPcBOA-LDL was used as a photosensitizer, increasing cell kill was detected with increasing light dose. The slopes of the linear regression fit to the logarithmic data for these two plots are significantly different from each other [$F(1, 28)=14.71$, $p=0.0007$], indicating greatly enhanced efficacy of LDLR-targeted PDT in an LDLR-overexpressing cell line. Light and drug alone controls for both the free and conjugated photosensitizer show no difference compared to untreated controls. Currently, Pc4 is a leading phthalocyanine-based photosensitizer candidate for PDT.¹⁸ Compared to Pc4, our r-SiPcBOA-LDL nanoparticle requires a higher effective photosensitizer concentration for PDT-mediated cell kill. However, Pc4 is not a target-specific photosensitizer, whereas our agent is highly tumor-specific in HepG₂ cells overexpressing LDLR. Since many cancer cells overexpress LDLR, we anticipate these novel nanoparticles can serve as a useful discriminatory vehicle for the delivery of PDT agents to tumor cells.

4 Conclusion

In conclusion, a new photosensitizer, SiPcBOA, is synthesized and successfully reconstituted into the LDL lipid core with very high payloads (3000 to 3500 probe per LDL molecule), and such payload has no effect on the mean particle size of the LDL nanoparticles. It is found that r-SiPcBOA-LDL internalization into HepG₂ tumor cells is exclusively mediated by LDLR, as indicated by laser scanning confocal microscopy. Moreover, the clonogenic assay demonstrates that r-SiPcBOA-LDL is an effective PDT agent for LDLR overexpressing HepG₂ tumor cells. These data suggest that r-SiPcBOA-LDL can be used as a targeted NIR optical imaging and PDT agent for cancers overexpressing LDLR.

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