Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information

Photoresponsive liposomal nanohybrid cerasomes

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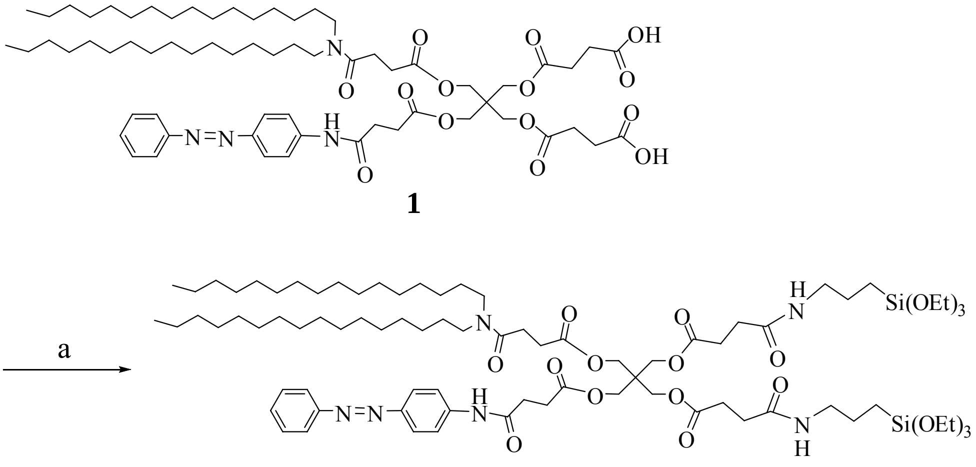
Contents

1. Instrumentation and materials

All reagents including N-(3- dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC·HCl), and 3-aminopropyltriethoxysilane are commercially available and were used without further purification. Nuclear magnetic resonance (NMR) spectra were recorded on a Brüker Avance 400 spectrometer at 25 °C. Chemical shifts were reported in parts per million (ppm) downfield from the Me_4Si resonance which was used as the internal standard when recording ^1H NMR spectra. Fourier transform infrared (FT-IR) spectra were recorded (64 scans with a resolution of 4 cm^{-1}) on a Varian FTS 3100 Fourier transform infrared spectrophotometer. Mass spectrum (MS) was tested on a API 3000 LC/MS/MS System. Photochrorism experiments were performed on a Varian 4000 UV-vis spectrophotometer. The fluorescence spectra were measured using fluorescence spectrophotometer (Varian Cary Eclipse). Scanning electron microscopy (SEM) was obtained on a Hitachi S-3400N with the operating voltage of 15 kV. Transmission electron microscopy (TEM) was obtained using an H-7650 with a tungsten filament at an accelerating voltage of 100 kV. Dynamic light scattering (DLS) measurements were determined by a 90Plus/BI-MAS instrument (Brookhaven Instruments Co., U.S.A).

2. Synthesis of 2-((4-(dihexadecylamino)-4-oxobutanoyloxy)methyl)-2-((4-oxo-4-(4-(phenyldiazenyl) phenylamino)butanoyloxy)methyl)propane-1,3-diyl bis(4-oxo-4-(3-(triethoxysilyl) propylamino) butanoate) (AZOSIL)

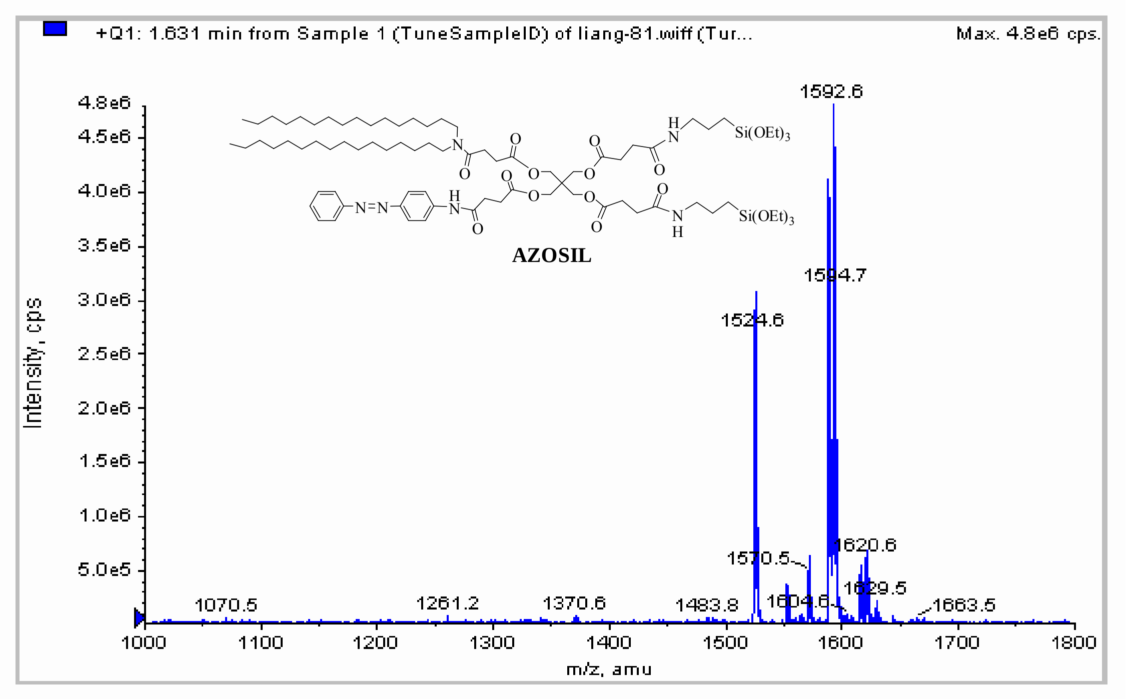


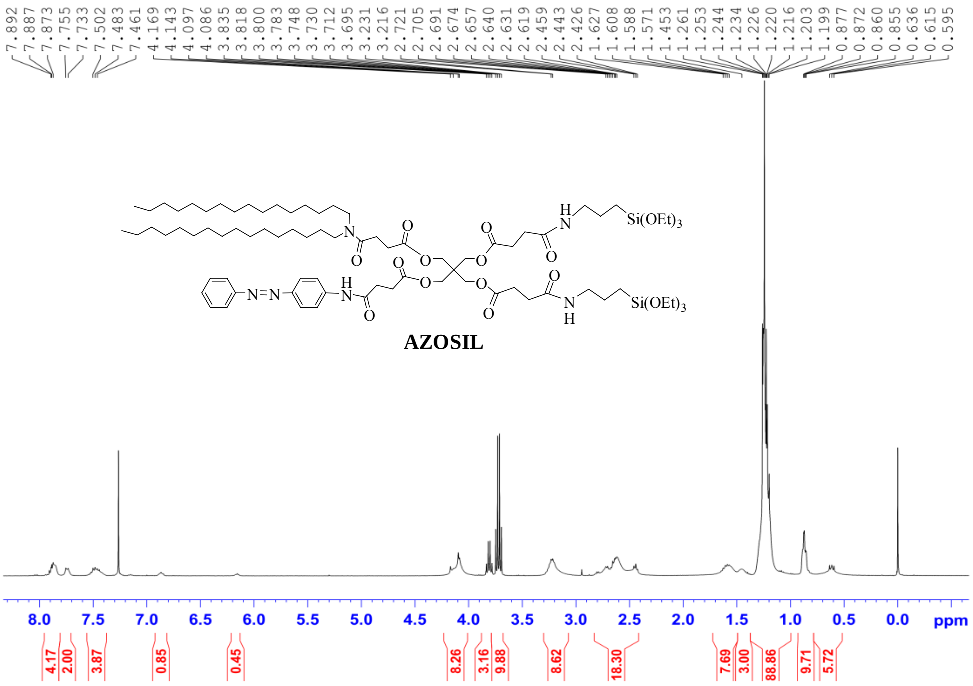
AZOSIL

Scheme 1. Synthesis of AZOSIL lipid: a) APTES, EDC, CH_2Cl_2.

The intermediate compound **1** was synthesized from pentaerythritol, dihexadecylamine, 4-aminoazobenzene according to the reported method[1]. To the solution of **1** (0.090 g, 0.078 mmol) in dry dichloromethane (20 mL) was added EDC (0.038 g, 0.188 mmol). After 15 min of stirring, 3-aminopropyltriethoxysilane (0.042g, 0.188 mmol) was added to the solution and the mixture was stirred for 24 hours at room temperature. Then, the solution was concentrated under vacuum and the residue was purified with a silica gel column (eluent: ethyl acetate/chloroform, 6/1, *V*/*V*) to give a pale yellow oil (0.024 g, 20%). Compound **AZOSIL**: ^1H NMR ($CDCl_3$, 300 MHz) δ: 0.62 (t, J = 8.4 Hz, 4H, $SiC\mathbf{H}_2CH_2CH_2NH$), 0.85 ~ 0.88 (m, 6H, $C\mathbf{H}_3(CH_2)_{13}CH_2CH_2N$), 1.10 ~ 1.34 (m, 70H, $NCH_2CH_2(C\mathbf{H}_2)_{13}CH_3$ and $SiOCH_2C\mathbf{H}_3$), 1.35 ~ 1.70 (m, 8H, $CH_3(CH_2)_{13}C\mathbf{H}_2CH_2N$ and $SiCH_2C\mathbf{H}_2CH_2NH$), 2.42 ~ 2.72 (m, 16H, $COC\mathbf{H}_2C\mathbf{H}_2CO$), 3.20 ~ 3.25 (m, 8H, $SiCH_2CH_2C\mathbf{H}_2NH$ and $CH_3(CH_2)_{13}CH_2C\mathbf{H}_2N$), 3.69 ~ 3.84 (m, 12H, $SiOC\mathbf{H}_2CH_3$), 4.08 ~ 4.17 (m, 8H, $COOC\mathbf{H}_2C$), 7.46 ~ 7.51 (m, 3H, Ar**H**), 7.73 ~ 7.76 (m, 2H, Ar**H**), 7.87 ~ 7.90 (m, 4H, Ar**H**). MS Calcd for $C_{83}H_{144}N_6O_{18}Si_2$: 1570.23, found $[M]^+$: 1571.7, $[M+Na]^+$: 1592.6.

3. MS and ^{1}H NMR spectra of new compounds





4. Preparation and characterization of photoresponsive cerasomes

Fabrication of photoresponsive cerasomes: 1-5 mg of AZOSIL lipid was dissolved in 2-5 mL of $CHCl_3$, which was removed by a nitrogen stream to form a thin film layer on the wall of vial. The film was then dried under vacuum overnight. Then, 2-5 mL of ultrapure water was added to the vial, followed by incubation in a water bath at 60 °C for 30 min. The mixture was vortexed for 20 min followed by a 10-15 min sonication with a probe-type sonicator until a clear and transparent solution was obtained. The resultant solution was incubated at room temperature for 24 h before measurements.

Loading of Nile Red into photoresponsive cerasomes: The Nile Red loaded cerasomes were fabricated by dissolution of AZOSIL lipid and Nile Red (molar ratio is 20:1) in $CHCl_3$ according to the same procedure. The dye loading in cerasomes was calculated according to fluorescence spectroscopy/concentration standard curve. The entrapment efficiency (EE) of Nile red in photoresponsive cerasome was determined using the following equation:

$$\mathrm{EE}(\%) = \frac{\text{weight of the drug in Nanoparticles}}{\text{weight of the feeding drug}} \times 100\%$$

and the drug loading capacity (DL) was calculated according to the following equation:

$$\mathrm{DL}(\%) = \frac{\text{weight of the drug in Nanoparticles}}{\text{weight of the Nanoparticles}} \times 100\%$$

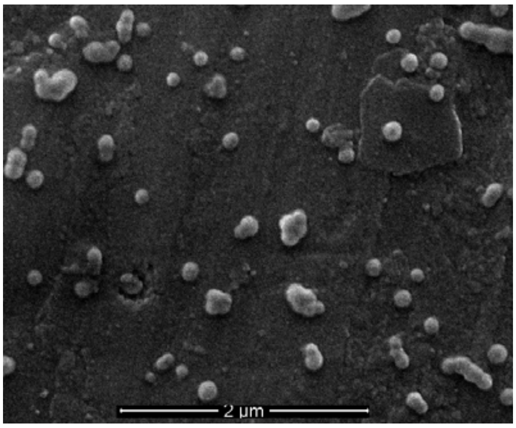


Fig. S1. Scanning electron micrograph of photoresponsive cerasomes with the scale bar of 2μm.

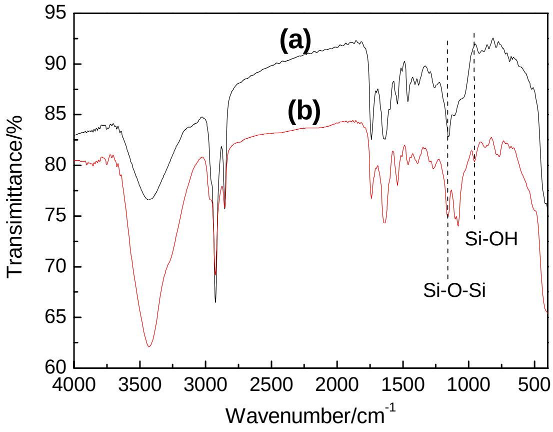


Fig S2. FT-IR spectra: (a) AZOSIL lipid; (b) photoresponsive cerasomes.

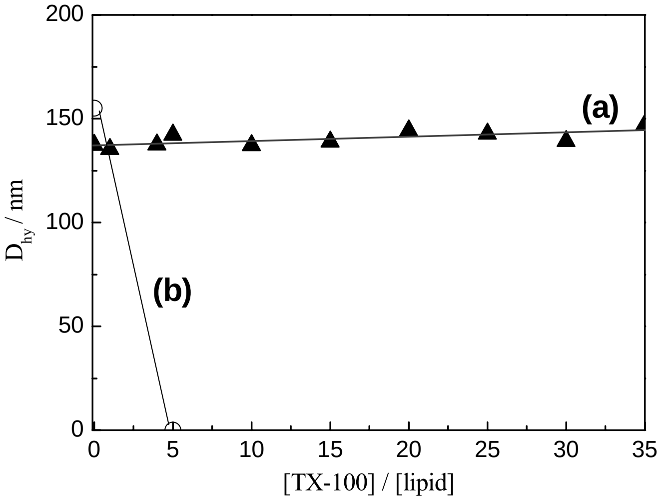


Fig. S3. Effect of the addition of Triton X-100 on the hydrodynamic diameter (D_h) of (a) photoresponsive cerasomes and (b) the DSPC liposome in water at pH 6.

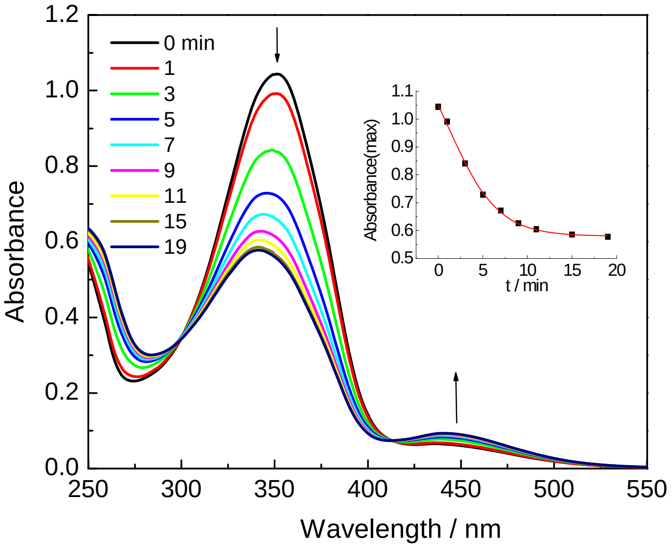


Fig. S4. UV/vis absorption spectra of AZOSIL lipid in $CHCl_3$ upon UV light irradiation (λ=365 nm) (Insertion: the relationship between maximum absorbance at about 352 nm and irradiation time).

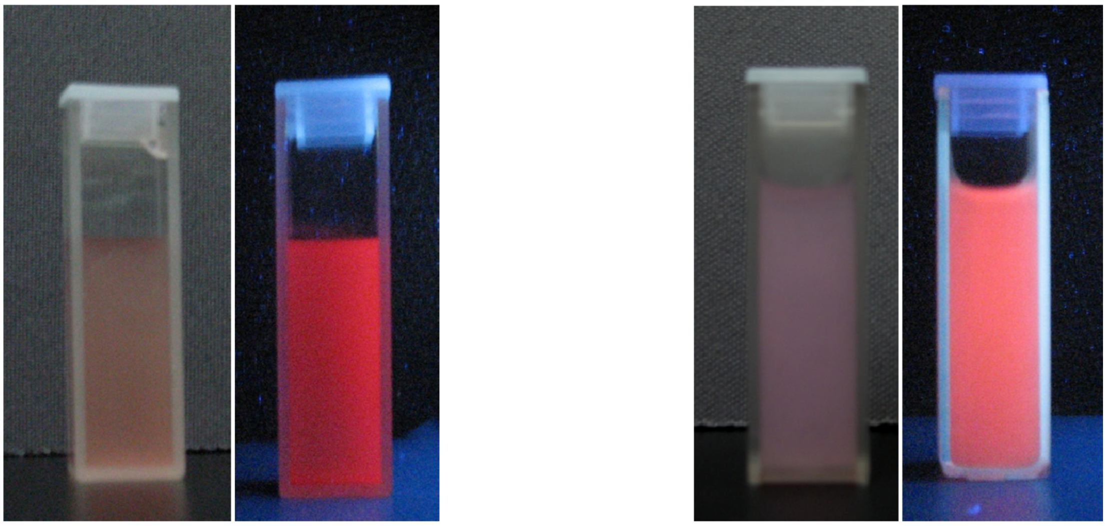
5. Release of Nile Red from photoresponsive cerasomes

Nile Red loading photoresponsive cerasomes were dispersed in phosphate buffered saline (PBS, pH = 7.4, 3 mL) (Figure S5(1)). For comparative study, another Nile Red loading liposomal hybrid nanoparticles were prepared from an organoalkoxysilylated lipid without azobenzene unit (Figure S5(2) and Figure S6). The initial fluorescence intensity was adjusting to the same value. The Nile Red release from nanoparticles was measured at the desired time points using fluorescence spectrophotometer (Varian Cary Eclipse, excitation at 553 nm, emission at 632 nm). The amount of Nile Red released (F_{rel}

/ %) from nanoparticles was calculated by the following equation:

$$F_{\mathrm{rel}} / \% = I_t / I_0$$

where I_0 is the fluorescence intensity of the nanoparticles dispersion containing Nile Red at the initial time, I_t is the fluorescence intensity with respect to time. The release experiment was proceeded at 25 ˚C.



(1)

(2)

Fig. S5. Images of Aqueous Dispersions of Nile Red loading (1) Photoresponsive cerasomes (left: under daylight; right: under UV light) and (2) Cerasomes without azobenzene unit (left: under daylight; right: under UV light).

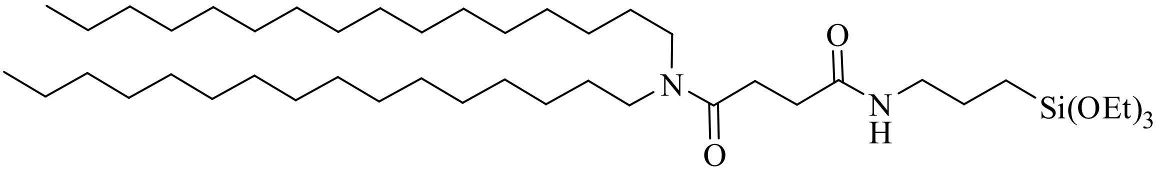


Fig. S6. Chemical structure of organoalkoxysilylated lipid without an azobenzene unit.

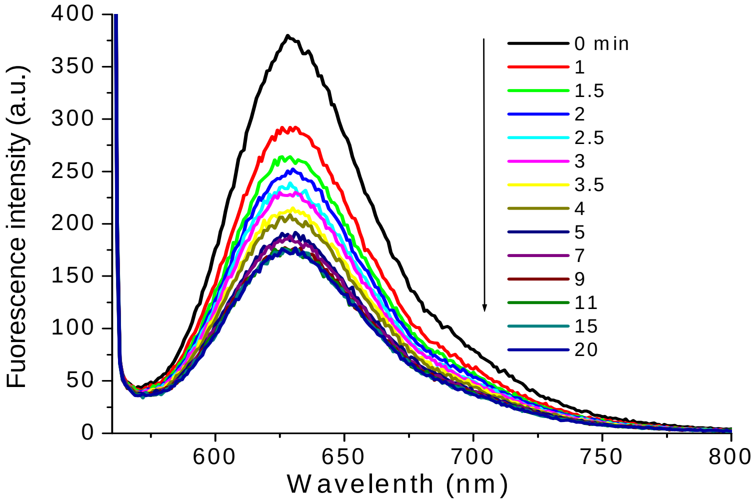


Fig. S7. Release of Nile Red from photoresponsive cerasomes result in fluorescence decrease.

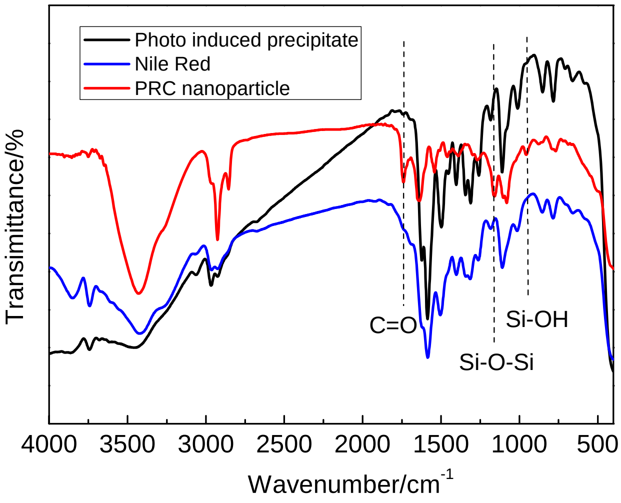


Fig. S8. FT-IR spectra of the photoinduced precipitate after UV irradiation (Fig. 4f) (black curve), Nile Red (blue curve) and PRC (red curve).

The photoinduced precipitate was characterized by Fourier transform infrared spectroscopy compared with Nile red and PRC nanoparticle (Fig. S8). The peaks of photoinduced precipitate was well consistent with Nile Red, indicating the precipitate was Nile red. While for PRC, stretching bands assigned to the Si-O-Si and Si-OH groups were observed around 1100 and 950 cm^{-1}, respectively. On the contrary, there were no stretching bands around 1100 and 950 cm^{-1} for photoinduced precipitate and Nile Red. Therefore, it provides a strong evidence that the photoinduced precipitate in Fig. 4f is actually Nile red molecules and the cerasome is not contained. The cerasomes are still stably suspended and only Nile red molecules form precipitate after photoirradiation.